Horticulture Innovation Australia

Final Report

Parent Project for APRP2 program

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University of Ballarat

Project Number: PT09039

PT09039

This project has been funded by Horticulture Innovation Australia Limited using funds from the Australian Government and the following sources:

Victorian Certified Seed Potato Authority Inc
Horticulture New Zealand
AHDB Potato Council-R422
A & L Canada Laboratories Inc.
NZIPF - Andrea Bourhill - #24656
NZIPF - Barbara Waddell - #24646
Potato - Processed (R&D Levy)

ISBN 0 7341 3618 8

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
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PROJECT SUMMARY

PT09039 – Parent Project for APRP2 program

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2 November 2015

The purpose of this report is to capture the work undertaken and delivered as part of the Australian Potato Research Program Phase 2 (PT09039). This report contains final reports for individual research projects PT09004, PT09019, PT09023, PT09026 and PT09029, and also the management project PT09040, which made up the program.

FUNDING SUPPORTERS

This project was funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, A&L Canada Laboratories, the Victorian Certified Seed Potato Authority and matched funds from the Australian Government. The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, the University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority provided in-kind support.

DISCLAIMER

The following report detailed studies on compounds and use patterns not currently registered in Australia and was for research purposes only. Information on registered pesticides in Australia can be found at: https://portal.apvma.gov.au/home .

FINAL REPORT CONTENTS

This final report is made up of seven chapters with each chapter dedicated to a project within the program. Each chapter is structured as a standalone HAL final report and the chapter number is used as a prefix on page numbers, tables and figures. The following provides an overview of the content in each chapter:

- Chapter 1 - Overview of the Australian Potato Research Program – Phase 2 (this chapter)
- Chapter 2 - Control of potato psyllid with an IPM strategy
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LIST OF ABBREVIATIONS

Abbreviation	Full name
A&L	A&L Laboratories Canada
AAFC	Agriculture and Agri-food Canada
AC	Advisory Committee
ai	Active ingredient
ALS	ammonium lignosulphonate
APRP1	APRP1
APRP2	Australian Potato Research Program Phase 2
AUSVEG	Australian Vegetable Peak Industry Body
CFU	colony forming units
DEPI Vic	Department of Environment and Primary Industries Victoria (succeeded DPI)
DNA	Deoxyribonucleic acid
DPI Vic	Department of Primary Industries Victoria
HAL	Horticulture Australia Limited
HERDC	Higher Education Research Data Collection
HESDC	Higher Education Student Data Collection
HNZ	Horticulture New Zealand
IP	intellectual property
IPM	integrated pest management
LSM	liquid swine manure
MASH	Managing Advanced Soil Health
NZ	New Zealand
ON	Ontario
PCR	polymerase chain reaction
PED	Potato early dying
PEP	Potato Extension Program
PFNZ	New Zealand Institute for Plant and Food Research Limited
PPIAC	Processing Potato Industry Advisory Committee
R&D	research and development
RBK	Russett Burbank
RIBG	Research Infrastructure Block Grant scheme
SA	South Australia
SARDI	South Australian Research and Development Institute

Abbreviation	Full name
TIA	Tasmanian Institute of Agriculture (succeeded TIAR)
TIAR	Tasmanian Institute of Agricultural Research
TOC	Technical Operations Committee
TOG	Technical Oversight Groups
TRFLP	Terminal restriction fragment length polymorphism
UB	University of Ballarat
UK	United Kingdom
VC	Voluntary contribution
VFA	Volatile fatty acid
Vic	Victoria
ViCSPA	Victorian Certified Seed Potato Authority

CHAPTER 1. THE AUSTRALIAN POTATO RESEARCH PROGRAM PHASE 2 (APRP2)

MEDIA SUMMARY

The Australian Potato Research Program Phase 2 (APRP2) was the dedicated highly collaborative research and development program for the Australian processing potato industry. The five-year program commenced in 2009 and was funded by processing potato growers, manufacturers, international and national research organisations, with matched funds from the federal government. The principal focus of the program was the improvement of soil and plant health with a chief focus on major soil-borne potato diseases: common scab, powdery scab and Rhizoctonia, and a secondary focus on potato psyllid, bacterial wilt and Verticillium. A sixth project provided independent management of the program.

An APRP2 produced survey conservatively estimated that Rhizoctonia, powdery scab and common scab annually cost the commercial processing potato industry $18.4M mainly as a result of discarded diseased tubers on farm, treatment costs or plant yield impacts. The APRP2 program has delivered new tools and know-how that will allow growers to reduce their exposure to these costs of disease. New information and tools have also been developed that will facilitate future research into complex diseases like powdery scab and to more quickly and cheaply evaluate potential treatment options.

The following outlines the key work completed and the outcomes from each area of work:

NEW SOIL TESTS TO DETERMINE THE RISK OF SOILBORNE DISEASES

A DNA-based preplant soil testing service “Predicta Pt” was developed which provides growers with an estimate of the risk to a crop of powdery scab, black dot or root knot nematode. PreDicta Pt was developed over phases 1 and 2 of the APRP program. Led by the South Australian R&D Institute (SARDI), the project involved international collaboration and the service was launched in Australia in mid-2013. It is used by growers who access the service via accredited agronomists or service providers. With ongoing research the service will be expanded to include common scab, *Verticillium*, and potentially Rhizoctonia. DNA tests are currently provided for these pathogens/diseases but without risk ratings, as more work is needed to relate the DNA levels to actual risk of disease in key potato growing regions. There is also an opportunity to expand the service beyond the diseases researched in the APRP2 program.

Capability to test pathogen levels in the peel of tubers has been developed for the same suite of pathogens for which soil DNA testing is available. The ability to quantify pathogen DNA levels, both in the soil and the peel of seed tubers, in combination with field validation of disease levels conducted in this project has increased our understanding of inoculum and disease risk for these pathogens. Access to this technology has opened up new opportunities at both an on-farm practical level and to undertake research and development approaches that were previously not feasible.

BETTER UNDERSTANDING OF SEED POTATO PATHOGEN LOAD AND IMPACT ON PROGENY CROP

Research from the Tasmanian Institute of Agriculture (TIA) focussed on the role of tuber inoculum on resulting disease in progeny crops. The work showed that the current visual ratings of disease as used by seed certification authorities correlate well with DNA levels found using the Predicta Pt test on tuber peel.

Regardless of the method used to assess the pathogen load, the presence of pathogen on seed is generally associated with increased risk of disease.

The TIA team also established a clear relationship between planting diseased seed and progeny crop outcomes. When planting tubers infected with Rhizoctonia AG3 and common scab there was a clear relationship with disease in progeny roots and tubers. The relationship between powdery scab diseased tubers and progeny outcomes was less clear. Diseased seed is likely to result in greater root disease, but the relationship with progeny tuber disease was less obvious.

Other findings showed that grading of heavily diseased seed tubers represents a significant risk to the grower as graded seed can contain high levels of pathogen from the discarded diseased seed and can result in subsequent crop disease.

These studies emphasise the importance of using the highest health certified seed, particularly when planting into soil without a history of disease or possibly in the growing of elite early-generation seed. DNA based technology might develop into a viable alternative should the cost of testing become significantly reduced principally for instances where diseases are unable to be visually assessed (e.g. most potato viruses, *V. dahliae*).

Suggestions for future research includes studies to validate whether current certification disease incidence levels are robust and relevant, further studies into the grading of seed lines, and more work to understand the basic form in which pathogens exist on seed and how this influences its contribution to progeny disease.

BUILDING KNOWLEDGE ABOUT CROP ROTATIONS USED IN POTATO PRODUCTION

The impact of crop rotations on reducing or increasing soil pathogen levels was studied by another team from TIA. This work offered great insight into the fluctuations of pathogen populations over time and some insight into the impact of rotation crops on resulting disease. Poppy and potato were associated with increased Rhizoctonia AG2.1, while carrot, fallow, poppy and ryegrass with increased Rhizoctonia AG3. Potato was the biggest contributor to increased concentrations of powdery and common scab.

TIA showed that in many instances it was not until the first potato crop in a paddock was grown that Rhizoctonia, common scab and powdery scab were a problem which strongly suggests that the pathogen had been introduced on the potato seed tubers. The work also showed that once powdery scab was found, soils that grow potatoes once every 5 years are unlikely to return to a low risk category. Potato growers will need to take all necessary steps to minimise the risk of powdery scab e.g. choose less risky paddocks, grow resistant cultivars, and where possible manage soil moisture during tuber initiation.

This work further demonstrated the value in using the PreDicta Pt soil-testing service.

IDENTIFYING SOIL HEALTH INDICATORS THAT CAN BE USED TO SUPPRESS DISEASES OF POTATOES

Research carried out by Canada-based A&L Laboratories together with the Victorian Department of Environment and Primary Industries (DEPI) developed new tools to identify soils that may be have an inherent ability to suppress disease. The work showed that this ability is due to microbes in the soil that either prevent or reduce the impact of some soil pathogens, such as *Streptomyces* spp that causes common

scab. With further research these tools could result in growers being able to determine if their soils have the potential to naturally limit disease onset in potatoes.

Further, the Canadian led research successfully showed that bacteria isolated from the rhizophere of potatoes and screened for disease suppression, have been able to reduce common scab on progeny tubers when used in the field as a seed treatment. Similar research looked at whether some naturally-occurring, beneficial microbes that live inside potato plants (endophytes) could be harnessed as seed/soil treatments to enhance potato growth disease suppression. Work led by Flinders University in South Australia showed that some endophytes showed promise as potential seed treatment for disease suppression. This work by A&L Laboratories and Flinders University has provided proof-of-concept that microorganisms could be developed as inoculants.

UNDERSTANDING SOIL NUTRIENT INTERACTIONS AND THEIR IMPACT ON SOILBORNE DISEASES

Additional work led by DEPI showed that manipulation of soil chemistry proved effective for controlling powdery scab. The work showed treating soils with sulphur (S) before planting or adjusting zinc (ZN) levels with Zn EDTA may reduce the incidence and/or severity of powdery scab. Additionally three fungicide treatments (not registered for use in Australia) were used for comparison of which fluazinam was highly effective in powdery scab control. Recommendations for using S and Zn have been integrated into a powdery scab decision management tree.

Zn EDTA was also effective in reducing Rhizoctonia in the field, but manipulation of soil chemistry for control of common scab was less successful. Findings have been incorporated into Rhizoctonia and Common Scab decision management trees.

EVALUATING GREEN MANURE CROPS

A&L Laboratories in Canadian led research that showed that green manure crops grown for a specific period of time and incorporated into soil while green will significantly increase marketable yield of potatoes between 20 to 48% compared to continuous cropping of potatoes. The significant yield increases suggest that there is value in replicating this research in Australia.

MONITORING BACTERIAL WILT PATHOGEN IN IRRIGATION WATER

Bacterial wilt is known to occur in parts of Victoria, NSW and Queensland. While the disease only occurs sporadically and seasonally, the losses attributed to this disease are significant. This research focused on the validation of sampling methods and diagnostic testing of irrigation water from the Bunyip River for the presence of bacterial wilt. All of the methods and knowledge developed as a result of this project can be used in other water area catchments.

In this study, bacterial wilt was only detected once in water collected from the Bunyip River in 2012/13. This result suggests that although there is potential for the river to act as a source of inoculum other factors may be contributing to the survival of the pathogen in this catchment. The potential report of a new host for Bacterial Wilt, pending further testing, may show that the aquatic native plant Starfruit (*Damasonium minus*)

may have a role in disease development of bacterial wilt of potato. Ground keeper potatoes, unharvested from the previous season, were shown to harbour the pathogen and carryover inoculum between seasons.

CONTROL OF POTATO PSYLLID WITH AN IPM STRATEGY

The aim of this work was to deliver control methods to deal with potato psyllid that are compatible with an overall integrated pest management (IPM) strategy if this pest should arrive in Australia. The project work was undertaken in New Zealand by IPM Technologies, Plant & Food Research and Potatoes NZ.

Laboratory results showed that key insect predators that occur in NZ and Australia are able to prey on all stages of the potato psyllid. Field trials were conducted in commercial crops in Canterbury where the IPM approach was applied involving the use of biological, cultural and border sprays with insecticides also applied but selectively. The commercial crops produced had no sign of Zebra Chip. Since the conclusion of the project growers in the South Island of NZ and many more in more severely affected North Island are successfully using an IPM approach to manage psyllid.

It is reassuring for Australian growers that an IPM approach is able to be used should it arrive on our shores. For the IPM approach to be successful in managing psyllid in Australia there is a need for growers not currently using IPM to manage pests to do so.

NOVEL NON-FUNGICIDAL MATERIALS FOR CONTROL OF COMMON SCAB

There are few practical control methods in the management of common scab and none that are both reliable and effective. Earlier TIA led work showed that certain chemicals when applied to the foliage of a potato crop can suppress common scab disease in the developing tubers. This TIA led study refined the prior work, and determined optimal rates and timing of novel foliar and tuber applied chemical treatments for control of common scab. Importantly, the chemicals were not fungicides and did not kill the common scab pathogen. Rather they appeared to induce disease resistance in the potato and protect it from infection.

The study identified several alternate chemicals that provide disease suppression, and demonstrated a common mechanism of action. Treatments when applied at the optimal time and rate gave excellent disease control with no observable detrimental effects on plant growth, tuber yield and quality.

The project recommended engagement with the agrichemical industry and facilitating registration trials as the foliar sprays and/or tuber treatments could offer a cost effective disease mitigation strategy. The authors also recommend further studies are undertaken to better understand the mechanisms by which the foliar treatment induces disease resistance in the potato.

GROWING OUR KNOWLEDGE OF VERTICILLIUM WILT

Yield of potato crops in parts of south-eastern Australia are declining due to potato early dying (PED) syndrome. PED refers to the early maturation and death of a potato crop and can be caused by an interaction between different species of the soil borne fungal pathogen Verticillium and of the root lesion nematode Pratylenchus.

The University of Melbourne led work involving PhD and Masters students showed a synergistic interaction between nematode *Pratylenchus crenatus* and *Verticillium Dahliae* (*V.dahliae*) which lead to increased PED severity in potato plants. Soils with a history of PED should be analysed for the levels of the two pathogens and if the levels are high then planting into these soils should be avoided.

Although *V. dahliae* was found to be widespread in potato tubers in South East Australia another potentially more serious Verticillium species (*V. albo-atrum*) was identified in a few tuber seed lots in Tasmania and Victoria. As well, the aggressiveness of isolates of *V. dahliae* to infect and cause disease was found to vary. Further surveys and assessment of the pathogenicity of isolates of the Verticillium species needs to be undertaken across a wider area of the Australian potato producing regions.

Tubers infected with *V. dahliae* were widespread across seed lots in Victoria and Tasmania and although this infection appeared to have little impact on established plants, tuber infection may play a significant role in building up soil inoculum over several generations of planting, and may be important in transmission of the pathogen between regions. Further studies are required into the build-up of soil inoculum in field trials planted to infected tubers over several cropping generations.

Several Australian potato cultivars were identified as being resistant to *V. dahliae* in glasshouse trials. Field trials now have to be implemented in soils with a history of high incidence of PED to study resistance of commercial potato cultivars.

Soil amendments sulfur and brown coal were shown to reduce the viability of microsclerotia in laboratory assays. These soil amendments may have potential in integrated disease management programs however; further trials are needed to assess their efficacy in the field.

In conclusion a combination of resistant cultivars, avoidance of soils with high levels of *Verticillium dahliae* and nematodes, and soil amendment treatments may reduce PED syndrome in Australian potato production systems.

EXTERNAL PROGRAM MANAGEMENT

The Australian Potato Research Program phase 2 (APRP2) was managed by SED Advisory on behalf of the University of Ballarat (UB, now known as Federation University). SED was appointed to provide independent management to the program and successfully facilitated the delivery of program contractual obligations. The task was not straightforward owing to the extremely complex contracting arrangements and ongoing political challenges which often drew more attention than the valuable science delivered by the program.

Although value adding was limited owing to the onerous program administrative requirements, as a part of evaluation commitments SED was able to undertake original research to estimate the cost of the major soil borne diseases to the processing potato industry. Prior to the program these figures were not readily available and their production has been acknowledged as a valuable contribution to the industry. The SED-led work estimated that powdery scab is costing the processing potato industry approximately $13.4M per annum, common scab $1.7M and Rhizoctonia $5.4M.

These figures, and an updated benefit/cost analysis suggesting a $7.48 return for every dollar invested in just the Predicta Pt component of the program, demonstrate the value of the investment in APRP2 and also provide guidance for considerations of future investment in processing potato R&D.

FINAL WORDS

The APRP2 program was a large national and international collaboration and featured a team of passionate, dedicated and professional scientists that were strongly supported by industry representatives. The achievements of this program suggest there is value in supporting a program approach to address the future research and development needs of the processing potato industry. An alternative contracting model would need to be developed such that program managers have sufficient time and flexibility to adapt the program to industry needs.

Future programs would also benefit from the incorporation of communication and extension activities into each project.

Chapter 1 of this final report provides a collated list of communication and publication activities from the program.

EXECUTIVE SUMMARY

OVERVIEW

The Australian Potato Research Program Phase 2 (APRP2) was a national and international collaboration that formed the dedicated research and development program for the Australian processing potato industry. The five-year program commenced in 2009 and comprised five projects based around the theme of soil and plant health with a principal focus on major soil-borne potato diseases: common scab, powdery scab and Rhizoctonia, with a secondary focus on potato psyllid, bacterial wilt and Verticillium. A sixth project provided independent management of the program.

Following a process of industry consultation and research prioritisation, the program was funded based on the following research components:

Soil disease:

- International collaboration to develop molecular markers for identified soil-borne pathogens;
- The importance of tuber-borne inoculum on the health of seed potatoes; and
- Enhancing the understanding of Verticillium spp. in Australian potato production

Soil health:

- Microbial soil health and endophytes;
- Soil amendments & soil nutrients, green manure crops, research principles to be established for active compounds; and
- Bacterial wilt

Insect pests:

- potato psyllid control

The program was highly collaborative and involved 13 research organisations including four from overseas.

The following executive summary has been created by condensing executive summarises from individual project chapters.

PT09004 - CONTROL OF POTATO PSYLLID WITH AN IPM STRATEGY

Prominent Australian integrated pest management (IPM) expert Dr Paul Horne of IPM Technologies led this project, which was conducted in New Zealand (NZ) in collaboration with Plant and Food New Zealand (PFNZ) and Horticulture New Zealand (HNZ). The project was part of a push to prepare Australian producers for a possible future incursion of potato psyllid and the zebra chip disease it causes. The project aim was to assess whether the psyllid could be managed through an IPM strategy rather than relying on the use of broad spectrum insecticides.

Tomato-potato psyllid (*Bactericera cockerelli*) is a devastating pest of potatoes and related crops such as tomatoes, capsicums and tamarillos. The insect itself can significantly impact on crop yield but as a vector of *Candidatus liberibacter,* the cause of zebra chip, leads to devastating product losses. It was discovered in NZ in 2006 and spread rapidly throughout the country over the next few years. The approach taken in NZ and in the United States of America to control the psyllid has been to apply between 14 and 19 insecticides to each potato planting. Broad-spectrum insecticides that kill a wide range of insects, including beneficial species, have been predominant in most spray programs.

The project showed that three species of predatory insects (a ladybird beetle, a damsel bug and a brown lacewing) found in Australian potato crops would accept the psyllid as prey, even in the presence of alternative food sources. A draft IPM strategy was developed based on observations of the behaviour of the psyllid and knowledge of certain selective insecticides. The strategy involved a seed treatment and border spraying with certain 'soft' insecticides that would not harm the key beneficial species.

Two collaborating farmers from Canterbury in the South Island of NZ agreed to trial the proposed IPM strategy, and the crops were monitored by entomologists from PFNZ. The aim was to see if the draft IPM approach dealt with potato psyllid in a satisfactory way, as determined by the growers and the processor buying the potatoes. The end result was that the crops were grown to the satisfaction of both farmer and processor (McCain Foods).

Since the conclusion of the project all growers in the South Island of NZ and many more in the more severely affected North Island are successfully using an IPM approach to manage psyllid. It is of great reassurance to Australian growers that an IPM approach is able to be used to manage the pest should it arrive on our shores. Further, should the potato psyllid arrive, it may be possible to strengthen the IPM approach using other predators found in Australian potato crops that do not occur in NZ.

Linked to this project were two separate projects conducted by PFNZ and HNZ. This work has produced a considerable body of information to better understand psyllid, its predators and the bacterium that is vectored by the psyllid which will be valuable to the Australian industry should psyllid arrive on our shores.

With a successful strategy developed there is a need for key Australian industry stakeholders to be informed of the existence of the IPM strategy and what might occur if potato psyllid should arrive and broad spectrum insecticides are used rather than the IPM. Growers who are not currently using IPM to manage existing pests should be encouraged to do so. An initiative to upskill growers in the use of IPM would not only improve preparedness should potato psyllid arrive, but would also see a reduction in wastage through reduced insect damage and improve yields. The benefits of IPM for industry warrant further investment in this area.

PT09019 - IMPORTANCE OF TUBER-BORNE INOCULUM ON SEED POTATO HEALTH

Effective certification schemes within the potato seed industry are important in ensuring disease-free seed tubers are supplied consistently and economically. Current visual assessment strategies, for diseases that produce obvious visual symptoms on seed tubers, have been a central to these schemes but rigorous testing of current sampling strategies and testing of new technologies such as DNA testing (qPCR) are essential to ensure that current visual assessment practice is still providing the best approach to certification. Additionally, the impact of planting seed tubers carrying varying inoculum loads needs to be better understood in terms of progeny crop production, so that the benefits of planting certified seed can be clearly demonstrated to industry.

This project, designed to address the above questions, was led by Dr Ian Kirkwood and later by Dr Robert Tegg and Dr Calum Wilson of the Tasmanian Institute of Agriculture (TIA). Key pathogens and diseases targeted the project included: *Rhizoctonia solani AG3* (black scurf), *Spongospora subterranea* (powdery scab), and *Streptomyces scabiei* (common scab).

Statistically rigorous seed-sampling strategies were undertaken over a three-year period within current commercial seedlots (38 in total) grown for seed. The project tested two differential sampling strategies: 2 lots of 100-tuber samples (current practice) and 10 lots of 20-tuber samples, and compared seed health testing by qPCR detection of pathogen DNA and traditional visual disease assessments. Additionally the impact of planting diseased versus healthy seed on crop progeny disease was assessed through a series of experiments over four years. Other research investigated alternative sampling strategies for determining pathogen load in seed, the impact of seed grading on inoculum load and the movement of inoculum from diseased seed tubers into the soil environment.

The current simple sampling strategy was shown to provide a suitable measure of disease load under most circumstances. While qPCR provided better distinction of pathogens that produce similar symptoms and could detect pathogens that do not produce obvious tuber symptoms, the measurable benefits of this detection tool to the grower were not obvious in the majority of cases. The four-fold increase in costs associated with these tests being utilised for certification currently does not warrant their use under most circumstances.

The relationships established when planting diseased seed tubers that carry either *Rhizoctonia solani* AG3 (*R. solani* AG3) or *Streptomyces scabies (S. scabies)* into pathogen-free soil was clear-cut, showing a positive association with increased disease in both progeny roots and stolons (for *R. solani* AG3) and tubers (both *R. solani* AG3 and *S. scabies*). Where available, seed treatments are recommended where both these pathogens are present on the tubers.

The relationship between planting diseased seed and progeny crop outcomes were less clear for powdery scab. There was a consistently strong positive relationship between planting diseased seed and root disease, a key outcome as root disease can lead to reduced yields and soil inoculum build up. The relationship between planting diseased seed and progeny tuber disease was less obvious, although there was a trend indicating greater disease was likely. This reflects that other factors such as environmental conditions strongly influence powdery scab tuber disease.

Other findings include quantitative confirmation that diseased seed tubers can contaminate pathogen-free soils, and that subsequent potato crops grown in these contaminated soils can result in high levels of disease. Additionally, grading of heavily-diseased seed tubers represents a significant risk to the grower as

the graded seed, although visually disease-free, can contain high levels of pathogen from the discarded diseased seed, which can result in subsequent crop disease.

Recommendations from the project are that in most circumstances, current visual certification strategies are adequate to identify risk of diseased seed to growers and no change in sampling strategies or technologies is needed. In some circumstances, such as the growing of elite early-generation seed or where large tracts of pathogen-free land are to be planted then testing seed by qPCR may be worthwhile. Additionally, where diseased seed is heavily graded and for specific seed-borne pathogens that are impossible or difficult to detect visually (e.g. most potato viruses, *V. dahliae*) qPCR may identify high risk seed better than visual assessment. If the costs of qPCR are reduced over time then this technology may become a viable alternative and this project has established some useful criteria to progress this technology to commercial development.

The project also identified further work that should be undertaken. This includes studies to (i) confirm/validate whether the current disease incidence levels used for certification (e.g. 2% for powdery scab) are scientifically robust risk thresholds for progeny disease (and whether there is any cultivar susceptibility variation); (ii) clarify at what disease incidence level (10, 20%, etc.) grading of seed lines would pose a significant disease risk, so as to reduce the likelihood of growers buying pathogen-containing seed; and (iii) understanding the basic form (spore, hyphae, etc..) in which pathogens exist on seed, and how this influences its contribution to disease in progeny crops and its enumeration by qPCR.

The project also raises questions about the cost-benefit of using certified seed for growers and the role and benefit of seed treatments and dressings on management of seed. There are also questions about the impact of *Spongospora* root infection on both yield and soil inoculum build up. Finally, there may be scope for further studies of seed-borne pathogens beyond powdery scab, common scab and black scurf.

PT09023 - DIAGNOSTIC TESTS FOR SOIL-BORNE PATHOGENS – INTERNATIONAL COLLABORATION

Where project PT09019 addressed aspects of tuber-borne inoculum of the major diseases, PT09023 investigated soil-borne sources of infection. The overall aim of the project was to develop a soil pathogen diagnostic service (building on work undertaken during APRP1) with accompanying, research-based information to allow disease risk to be understood from test results. The project was led by Dr Kathy Ophel Keller and Mr Mike Rettke of South Australian Research and Development Institute (SARDI), in collaboration with TIA, Department of Environment and Primary Industries (DEPI Vic), the Potato Council UK, PFNZ and HNZ.

This project has developed a commercial DNA testing service, PreDicta Pt. This service enables growers to identify fields (or parts of fields) that are at risk of specific diseases before planting. The testing service has been delivered to growers since September 2013 and has been designed and set up to operate as a standalone commercial service post-project.

Currently the PreDicta Pt test provides an indication of the risk of powdery scab (*Spongospora subterranea)*, black dot (*Colletotrichum coccodes*) and root knot nematode (*Meloidogyne fallax*). Research test results for pathogen DNA levels in the soil of pathogenic *Streptomyces* (common scab), *Verticillium dahliae* and *Pratylenchus crenatus, P. neglectus, P. penetrans* (potato early dying), *Meloidogyne hapla*, *M. javanica/incognita/arenaria* (root knot nematodes), *Rhizoctonia solani* AG-3 and AG2-1 (black scurf and other Rhizoctonia diseases) are also reported but as research results only and without sensitivity and risk

categories as these are not available for these tests. Capability to test pathogen levels in the peel of tubers has also been developed for the same suite of pathogens.

As part of the international collaboration in this project, an inter-laboratory DNA test comparison demonstrated the ability of laboratories in Australia (SARDI), NZ and the UK to quantify pathogen DNA levels of *Colletotrichum coccodes*, *Rhizoctonia solani* AG3 and *Spongospora subterranea* with reasonable agreement.

Potato growers can access PreDicta Pt via agronomists accredited by SARDI to interpret the results and provide advice on management options to reduce the risk of disease losses. Growers may also undertake the training and to access the service directly if they wish to do so. Knowing the disease risk prior to planting can assist to mitigate disease risk by assisting pre-season decision making (choosing paddocks, matching varieties to specific disease risks, considering treatments and management strategies) and monitoring and improving practices (e.g. crop rotation strategies).

Work undertaken during the project to support the test has shown that:

- Pre-plant testing for *Spongospora subterranea* provides a useful indication of powdery scab risk to tubers, as soil inoculum is the most important source of disease risk. Under highly conducive conditions (e.g. Tasmania), seed inoculum may also need to be considered in assessing risk. Region and potato variety influence disease expression and taking these into account improves the interpretation of disease risk. *S. subterranea* is widely distributed wherever potatoes have been grown and a high proportion of paddocks are at high risk, especially in Tasmania.
- Pre-plant testing for *Meloidogyne fallax* provides a useful indication of root knot nematode damage to tubers. Inoculum in the soil is most important source of disease risk, with limited evidence of seed inoculum contributing to disease incidence. Region (particularly soil temperature) influences disease expression and taking temperature into account improves the interpretation of disease risk. *M. fallax* is widely distributed, but levels in the soil and risk to tubers are highest in the southeast of South Australia.
- Pre-plant testing for *Meloidogyne hapla* provides a useful indication of the level of nematode colonisation in harvested tubers, but colonisation of harvested tubers has not been linked with root-knot nematode damage seen on the surface of tubers. The Impact of soil inoculum of *M. hapla* on yield reduction is inconclusive, but does not appear to be a major contributor. When *M. hapla* populations in the soil are high prior to planting potatoes, population balance in the harvested tubers suggests this species may compete with *M. fallax*.
- Detection of the *Streptomyces txt*A gene DNA in the soil is infrequent. When it is detected at low levels there is a slightly increased risk of common scab compared with when not detected, but when detected at high levels (>2.5 log (DNA), there is greatly increased risk of common scab. However, these levels were rarely detected in commercial paddocks. When it is not detected in soil, there is still a risk of common scab. *Streptomyces txt*A gene DNA is detected more frequently in the peel of seed tubers than it is in the soil prior to planting. Inoculum on seed tubers can be an important contributor to common scab disease risk, with high levels of DNA of pathogenic *Streptomyces* found in the peel of tubers that have a high incidence of common scab. Environment and irrigation practices are likely to be main the driver of disease expression.
- Detection of *Rhizoctonia solani* AG3 in the soil is infrequent and this appears to be linked to the low and sporadic distribution of sclerotia in soil. *R. solani* AG3 is the main anastomosis group associated with black scurf and rhizoctonia stem canker. Pre-plant testing is a poor indicator of the risk of black scurf on tubers. When *R. solani* AG3 is not detected in the soil, there is still is a high risk of

black scurf in harvested tubers, although when it is detected in soil, there is higher risk of black scurf than in soils where the pathogen was not detected. This indicates the test may be some value to growers, but the limitations of the test will need careful communication. Inoculum is present either on the seed tubers planted or in the soil in most situations. Inoculum on seed tubers can be important contributor to black scurf disease risk. Environment and control practices such as seed and in-furrow chemical treatments are likely to be main driver of disease expression. Pre-plant soil inoculum may be a useful indicator of risk of 'burn- off' prior to emergence caused by Rhizoctonia at the location of sampling. To understand risk across a paddock a higher intensity of testing would be required than utilised for current PreDicta Pt tests.

- Pre-planting soil test results for *Rhizoctonia solani* AG2.1 are not linked to black scurf incidence on tubers. However, they may be a useful indication of the risk of Rhizoctonia 'burn-off' prior to emergence in the southeast of South Australia. They may also provide a useful indication of the risk of deformed tubers. Inoculum on seed tubers can also be important contributor to risk of deformed tubers.
- DNA assays are now available for pathogens involved in potato early dying disease complex. These include new tests for *Verticillium dahliae* and the root lesion nematode *Pratylenchus crenatus* (tests were already available for root lesion nematodes *P. neglectus* and *P. penetrans*). Pre-planting soil levels of *V. dahliae* are strongly related to DNA levels of *V. dahliae* in harvested tuber and provide a useful indication of the risk of reduced yield at a site. Where *V. dahliae* DNA was detected in the soil prior to planting, DNA of at least one of the three *Pratylenchus* spp. was detected at over 93 % of locations in South Australia.

PT09026 - SOIL HEALTH / DISEASE MITIGATION PROGRAM

OVERVIEW

PT09026 was a large project comprising five sub-projects, all contributing to the theme of identifying novel control approaches for the major disease of potatoes: powdery scab, common scab, Rhizoctonia canker and black scurf, Verticillium wilt and bacterial wilt. The project was led by Dr Ian Porter and later Dr Dolf De Boer of DEPI (Vic). Collaborating agencies were TIA, the Victorian Certified Seed Potato Authority (VicSPA), Flinders University, PFNZ, A&L Laboratories (Canada) and Agriculture and Agri-Food Canada.

The first group of sub-projects (Ai-Aii) explored disease management through the manipulation of nutrients and soil health factors. The second group (Bi-Bii) examined novel approaches (chemical and biological) to disease management and the last (C) studied bacterial wilt in a river catchment.

PT09026 AI - SOIL AMENDMENTS AND NUTRIENTS

This subproject was led by Dr Tonya Wiechel of DEPI (Vic). It built upon opportunities identified through APRP1 to use soil amendments or ameliorants, nutrient and biological manipulation to control soil-borne diseases. The work was conducted in Australia, New Zealand and Canada.

Manipulation of soil chemistry proved effective for controlling powdery scab. Fifteen treatments were tested using different rates and different methods of application in field trials conducted over five seasons in a ferrosol soil on a commercial property near Ballarat, Victoria. Powdery scab (*Spongospora subterranean)* was consistently reduced by soil incorporations of elemental sulphur (S) and zinc (Zn) EDTA. Soil S was increased from 10-30 ppm to 100-120 ppm and soil Zn from 2-3 ppm to 20-30 ppm in the effective treatments. Iron (Fe) EDTA gave small disease reductions in two out of the five seasons. Bioassays

showed that Zn and Fe directly inhibited root infection by *S. subterranea,* but none of the tested nutrients arrested the development of powdery scab galls on potato roots.

Additionally, three fungicide treatments (not registered for use in Australia) were used for comparison, of which only fluazinam was effective. In susceptible cultivar Shepody, the improved tuber quality from fluazinam, elemental S and Zn EDTA translated to an increased 14, 10 and 8 t/ha accepted by the French fry factory and 27, 23 and 11 t/ha at the crisping factory. In resistant cultivar Russet Burbank, the disease reduction was still significant but less dramatic. Recommendations for using S and Zn have been integrated into a powdery scab decision management tree developed as part of this program.

Zn EDTA was also effective in reducing Rhizoctonia disease in the field, but manipulation of soil chemistry for control of common scab was less successful. Despite five years of field trials in two different soil types, applying pH modifiers and various forms of S, calcium (Ca), magnesium (Mg) and nitrogen (N) had little effect on disease outcome. Considerable effort was directed towards manipulating the K:Mg ratio to 0.4, which reduces common scab in Canada and was correlated with disease reduction in APRP1. A survey of 160 potato fields found that the ratio varied widely from 0.1 to 2.7. Despite recommendations from soil testing laboratories, the correct balance of amendments to reach the desired target was rarely achieved and only one trial resulted in reduced common scab.

Manipulation of soil biology showed promise for controlling common scab, with potential development of a 'soil bio-health indicator'. Terminal restriction fragment length polymorphism (TRFLP) was used to profile the rhizosphere microbial communities from 28 Victorian potato fields classed as positive or negative for *Streptomyces scabies.* The TRFLP profiles appeared to group on the basis of the common scab disease potential of the different soil samples. This type of study of soil microbial diversity could serve as a more useful bio-indicator of disease potential than the pathogen DNA itself and can help to understand the factors that are responsible for disease suppression. A disease-suppressive soil from a Victorian potato farm was identified and characterized, and a glasshouse experiment proved that the suppression was transferable. Identifying the casual organisms of suppression and what factors encourage their prevalence in soil could ultimately lead to targeted biological control treatments and the possibility of manipulating the soil environment to optimise disease suppression.

During APRP1 and PT07038, thousands of bacteria were isolated from potato rhizospheres, screened for diversity of biological functions using chaperonin gene technology (cpn60) and in many cases found to have antibiotic genes or nitrogen fixing (nifH) genes. In PT09026(Ai), three of these (*Pseudomonas monteilii, P. chlororaphis, P. brassicacearum*), which inhibited *S. scabies* in vitro and significantly reduced disease in bioassays, were evaluated as seed treatments in a field trial. All three reduced common scab on progeny tubers, although *P. monteilii* also reduced tuber number and yield. The other two have been identified as potential biological control agents for seed-borne common scab.

Effective delivery and establishment is essential for the use of beneficial microorganisms. A consortium of N-fixing bacteria were established on roots of tissue cultured plantlets, which were then transplanted into field soil. Plants inoculated with eight separate N-fixing communities showed an average five-fold increase in biomass compared to uninoculated plants. The N-fixing communities were still detectable 30 days after transplanting. This provides proof-of-concept that these microorganisms could be developed as inoculants.

Green manures are crops grown for a specific period of time and incorporated into soil while green to improve the soil condition. In the final two years of a four-year trial initiated during APRP1 in Canada, incorporating millet green manure crops treated in four different ways increased marketable yields of potato between 20 to 48% compared with the continuous cropping of potatoes. These increases were not

attributed to reductions in *Verticillium wilt* or common scab but to changes in the soil chemical and microbial environment.

Outputs from this project include draft disease management decision trees for the diseases powdery scab, common scab and Rhizoctonia, which were built on the research outcomes from this program and previous research. The decision trees involve a step-wise process that identifies high risk factors that cause disease and actions available to prevent or mitigate these factors for a particular paddock/ cultivar combination. A preface for the use of the decision trees is a standard soil nutrient analysis and, for powdery scab, a soil DNA test (PreDicta Pt), to optimise soil and plant nutrition, determine a baseline for potential nutrient amendments for disease control and to determine disease risk.

Specific recommendations from this project are:

- Further development of the draft disease management decision trees into extension tools.
- Future research into the mechanisms of the common scab disease suppression found in Victoria to identify potential biological control agents and / or how to manipulate soil biology for disease suppression.
- Development of a 'soil health bio-indicator' to predict disease risk by common scab. This pathogen has proven intractable for incorporation into the PreDicta Pt risk model to date.
- Continued exploration of potato microbial communities to harness their potential for improving disease management and potato productivity.
- Evaluation of the disease reducing nutrient amendment treatments in other soil types and production regions to develop practical thresholds that can be integrated with soil testing services for nutrients and pathogen DNA.
- Further evaluation of green manure cropping in Australian production systems.
- Continued research effort into Rhizoctonia disease and common scab to enable accurate prediction of disease risk.
- A renewed look at the benefits of using disease resistance cultivars as a disease management tool and using new tools to screen potato genomes for resistance to common and powdery scab, both of which have heritable resistance traits.

PT09026 AII - IMPACT OF ROTATIONS

Since 2005, soil from 29 commercial paddocks in northern Tasmania and 15 in south east South Australia have been sampled annually to monitor changes in pathogen DNA in the soil, and to relate these changes to the year-by-year sequence of crops or pasture grown at the sites. This subproject, led by Dr Leigh Sparrow of TIA, continued this study.

Concentrations in surface soil (0-150 mm) of the DNA of the soil-borne potato pathogens *R. solani*, *S. subterranea* and *S. scabies* were monitored annually using real-time PCR assays. A non-linear model was fitted to the data to test if changes in DNA concentrations could be explained by changes in land use in the paddocks. *S. subterranea* and *R. solani* anastomosis group (AG) 2.1 were the most prevalent pathogens monitored in both states, followed by *S. scabies* and *R. solani* AG3, and *R. solani* AG4 in Tasmania. *R. solani* AG2.2 was found at very few sites, as were *R. solani* AG8 in Tasmania and AG4 in South Australia. At a number of paddocks, the DNA of *R. solani* AG3, *S. scabies* and *S. subterranea* was only found after the first potato crop in that paddock, strongly suggesting that the pathogen had been introduced on the potato seed tubers used to plant those paddocks.

From the model, half-lives for pathogen DNA concentrations were calculated and these ranged from 0.22 years for *S. scabies* DNA in Tasmania to 0.83 years for *S. subterranea* DNA in Tasmania. The fitted model also identified a number of crops that were significantly ($P<0.05$) associated with subsequent increases in pathogen DNA: poppy and potato were associated with increased *R. solani* AG2.1 DNA concentrations; carrot, fallow, poppy and ryegrass with increased *R. solani* AG3 DNA concentrations; and potato with increased concentrations of the DNA of *S. subterranea* and *S. scabies*.

Of all these increases, the effect of potato on concentrations of the DNA of *S. subterranea* was the greatest, with average increases of 3300 (South Australia) to 7300 (Tasmania) pg DNA/g soil, more than 10 times greater than the other increases. Given (1) the already high concentrations of *S. subterranea* DNA in many of the soils, (2) the large increase in *S. subterranea* DNA concentration caused by subsequent potato crops, (3) the estimated *S. subterranea* DNA half-life of about 1 year, and (4) the apparent low soil DNA threshold concentration for powdery scab risk (about 20 pg/g, established elsewhere), soils in which potato is grown once in every 5 years are unlikely to return to a low-risk category. Potato growers will need to take all necessary steps to minimise the risk of powdery scab, e.g. choose less risky paddocks, grow resistant cultivars, and where possible manage soil moisture to avoid wet soil at tuber initiation, because damp soils are likely to favour development of this disease.

In the absence of reliable relationships between concentrations of DNA of the other pathogens monitored and the risks of the diseases they cause, it was not possible to make similar judgements for the other diseases.

PT09026 BI - BACTERIAL ENDOPHYTES

This subproject was led by Professor Chris Franco of Flinders University. It aimed to capitalise on the successful development of cereal endophytes in Grains Research and Development Corporation-funded work.

Endophytic actinobacteria are capable of preventing disease as well as improving plant growth and yield. In this study a total of 452 endophytes were isolated from nine varieties of potato plants grown in South Australia and Tasmania. Putative characterisation of the isolates revealed an abundance of members of the genus *Streptomyces* (> 50% of isolates) and species of *Microbispora, Kribbella, Micromonospora* and *Sphaerisporangium.*

Pyrosequencing and TRFLP analysis was used to identify and characterise genera from two varieties (cv. Russet Burbank and Bernadette) of potato grown at two locations (South Australia and Tasmania). When eubacterial primers were used the results from the pyrosequencing showed at least 55 genera, the predominant ones being *Enterococcus, Pantoea, Teribacillus, Curtobacteria, Bacillus, Pseudomonas, Lactococcus, Exiguobacterium and Rhodococcus,* but very low levels of *Streptomyces.* Cluster analysis showed that despite minimal overlap of genera, the rhizospheres of the two South Australian-grown varieties (Russet Burbank and Bernadette) contained populations more closely related to each other than to the Russet Burbank grown in Tasmania.

Of the 452 isolates, 17 showed a strong *in vitro* activity against scab and Rhizoctonia pathogens. Spore production of these endophytes was scaled up and spore suspensions sent to the Tasmania Institute of Agriculture (TIA) for *in planta* evaluation. In each of the two seasons 2011-12 and 2012-13, two field and three pot trials tested the efficacy of several promising endophytes, coated onto seed tubers, for suppressing the key diseases common scab, powdery scab and black scurf. High disease pressure was obtained in the pot trials with low disease recorded from the field trials. Sequential assessment during early

tuber development identified stem cankering and necrosis caused by the black scurf pathogen and root galling caused by the powdery scab pathogen.

Except for moderate control of common scab by four strains in one field trial, no significant suppression of disease symptoms was observed with endophyte treatment compared with controls. Tuber assessments at plant senescence revealed that some endophyte treatments may be slightly suppressing tuber-based symptoms for both common and powdery scab.

In conclusion, whilst there were no endophyte treatments that significantly suppressed disease from a statistical perspective, some treatments did show activity in a couple of trials and may be worthy of further investigation.

PT09026 BII - NOVEL CHEMICAL TREATMENTS FOR CONTROL OF COMMON SCAB

Subproject PT09026(Bii) examined the possible application of 2,4-dichlorophenoxyacetic acid (2,4-D) to control common scab. It was led by Dr Calum Wilson of TIA. Common scab is caused by pathogenic Streptomyces spp. that produce thaxtomins, necrosis-causing phytotoxins that are essential for pathogenicity. There are few practical control methods for common scab and none that are both reliable and effective. Disease is minimised through planting resistant varieties, strategic use of irrigation, seed treatments and late planting.

Previous research conducted in the UK had found that 2,4-D, a herbicide and synthetic auxin, controlled common scab symptoms when applied to the foliage of potato, but also resulted in undesirable phytotoxic effects. It has been demonstrated that when 2,4-D is translocated to potato tubers, it suppresses thaxtomin toxicity.

This study determined optimal rates and timing of 2,4-D application for control of common scab whilst minimising phytotoxic effects of the treatments. It found that treatment of potato plants as soon as five days after emergence provided greater protection against common scab and greater suppression of thaxtomin toxicity in harvested tubers than treatments after tuber initiation. Rates much lower than had previously been tested were found to reduce disease and induce toxin tolerance to levels similar to that obtained with treatments at near herbicidal rates, suggesting that maximum toxicity suppression occurred at very low tuber 2,4-D levels. These very low rates did not induce any noticeable phytotoxic symptoms, nor affect harvested tuber yield or quality, and resulted in 2,4-D residue levels well below maximum residue limits in tubers at harvest. Additionally, it was found that if tuber seed pieces were treated prior to planting, daughter tubers would have some protection from disease and show tolerance to the toxin without an additional post-emergence treatment.

The study also tested a range of different chemicals for both disease suppression and their ability to inhibit thaxtomin A toxicity in both Desiree and Russet Burbank cultivars. The results showed different chemicals varied in their ability to suppress common scab. Tuber slice assays with thaxtomin A showed a strong correlation between the ability of the chemical to suppress common scab symptom development and also the ability of the chemical to inhibit thaxtomin A toxicity. A Bayesian measurement error linear regression model was derived for each cultivar and trial and demonstrated a clear positive relationship between disease and thaxtomin A-induced necrosis. The relationships obtained were much stronger than would have been obtained without adjustment for measurement error. This demonstrates that disease mitigation using chemical foliar sprays is strongly correlated with the ability of the chemical to inhibit thaxtomin A toxicity suggesting this mechanism as a key mode of action for understanding this novel disease control strategy.

Specific recommendations from this project are:

- Commercial development of foliar sprays and/or tuber treatments of 2,4-D or 2,5-DBB as a cost-effective disease mitigation strategy easily integrated into current production practices. This requires engaging the agrichemical industry, and facilitating registration trials.
- Further studies on tuber treatments with 2,4-D and alternate chemistries. These would reduce exposure of operators and the environment to chemical treatments, would reduce the amount of material needed, and would ensure all plants receive material at the earliest stage of crop growth.
- Further fundamental research on the mechanisms of thaxtomin suppression and disease mitigation.

PT09026 C - MONITORING THE BACTERIAL WILT PATHOGEN IN IRRIGATION WATER

This subproject was led by Dr Nigel Crump of ViCSPA. Bacterial wilt is a destructive disease of potato caused by the bacterium *Ralstonia solanacearum.* The disease is seen only in certain areas of Queensland, Victoria and South Australia but can be highly devastating where it occurs. The subproject sought to validate the role of irrigation water as a source of inoculum for the disease in potato crops grown in the catchment of the Bunyip River in Victoria.

Water was tested by culturing *R. solanacearum* on selective growth media, supported by DNA-based detection. Populations of the bacterium in the water vary according to the time of year, with detections most probable in the warmer months. In this study, the bacterium was only detected once in water collected from the Bunyip River in 2012/13. This suggests that although there is potential for the river to act as a source of inoculum other factors may be contributing to the survival of the pathogen in this catchment. The aquatic weed starfruit (*Damasonium minus*) may have a role in the epidemiology of bacterial wilt by multiplying the bacterium and increasing the inoculum load in the river water. As expected, groundkeeper potatoes (unharvested from the previous season) were shown to harbour the pathogen and to carry over inoculum between seasons.

Growers are urged to implement strict on-farm biosecurity that includes the use of disease-free certified seed potatoes to prevent the introduction of the disease. Long crop rotations and the proactive management of self-sown or groundkeeper tubers between seasons to prevent pathogen build-up are also advised.

In catchments that experience an outbreak of bacterial wilt it is possible to test irrigation water and identify the significance of water contamination in the spread of the disease. Accurate diagnosis of infected plants is critical to allow appropriate management decisions to be made, importantly to prevent further spread of the disease and restrict additional losses. In-field diagnostic test kits are available to test suspect plants.

Future research is required to enhance high throughput, rapid and cost-effective testing of water and other samples to assist in the management and prevention of bacterial wilt outbreaks.

PT09029 - ENHANCING THE UNDERSTANDING OF VERTICILLIUM SPP IN AUSTRALIAN POTATO PRODUCTION

Potato early dying (PED) is the early maturation and death of a potato crop caused by the soil-borne fungal pathogen *Verticillium.* In conjunction with the root lesion nematode *Pratylenchus*, PED causes crop losses as high as 50 per cent in North America. Very little was known about PED in Australia.

PT09029 was led by Professor Paul Taylor of the University of Melbourne. PhD and Masters students studied: i) incidence of seed tuber infection, taxonomy and pathogenicity of *Verticillium* species infecting potatoes in Australia, ii) the role of *V. dahliae* tuber infection on the development of Verticillium wilt disease, iii) the interaction between *V. dahliae* and root lesion nematode *P. crenatus* in PED, iv) identification of potato cultivars resistant to *V. dahliae*, and v) assessment of the efficacy of soil amendments to suppress Verticillium wilt disease in potatoes.

V. dahliae, *V. albo-atrum* and *V. tricorpus* were isolated from commercially-grown seed tubers from Victoria and Tasmania. Infection by *V. dahliae* within a seed lot varied greatly from 0-55%. Around 41% of seed lots tested were infected by *Verticillium* spp. Over 12% of seed lots tested ranged from 0-5% infection within the seed lots. Only one seed lot had more than 50% of seed tubers infected with *V. dahliae*. Overall percent infection of seed lots from Victoria and Tasmania were 28 (*V. dahliae*), 8 (*V. albo-atrum*) and 5 (*V. tricorpus*). Stem-end vascular discoloration of tubers was not correlated with presence of *V. dahliae*.

Pathogenicity of selected isolates of Verticillium was assessed by root dip inoculation of potato cv Shepody. All inoculated plants showed typical Verticillium wilt symptoms although several Tasmanian *V. dahliae* isolates were highly aggressive. Most *V. dahliae* and *V. albo-atrum* isolates showed the same level of aggressiveness while *V. tricorpus* was substantially lower. Glasshouse pot trials showed that the synergistic interaction between *P. crenatus* and *V. dahliae* will cause PED.

It is recommended that soils that have grown potatoes for several years be tested for levels of *V. dahliae* and *P. crenatus*, and that potatoes not be sown where these are high. Further surveys of the three Verticillium species and assessment of their pathogenicity (notably *V. albo-atrum,* which is highly virulent in cooler regions overseas) should also be undertaken on a broad range of cultivars. Further glasshouse trials to study the interaction of different concentrations of *P. crenatus* with varying inoculum levels of *V. dahliae* and *V. albo-atrum* are also needed.

V. dahliae tuber infection appears to have little impact on infection and disease severity of first-generation plants grown from these tubers. However, it may play a significant role in the build-up of soil inoculum over several generations and may be important in transmission of the pathogen between regions. It is recommended that the build-up of soil inoculum in field trials planted to infected tubers over several cropping generations be studied.

For resistance screening, an inoculum threshold of 10^4 spores/mL was required to establish infection and wilt symptoms. Cultivar Denali showed moderate to high resistance, Catani and Desiree moderate resistance. Nicola, Russet Burbank C, Shepody and Trent were susceptible. Research is needed on the mechanism of resistance. Further screening trials should be done using soil inoculated with microsclerotia, as this reproduces field conditions more closely. Field trials are also needed to assess the resistance of Denali, Catani and Desiree in soils with a history of high PED incidence.

Soil amendments atomic sulphur 1% (w/w) and brown coal 1% reduced the viability of microsclerotia in laboratory assays, but brown coal did not prevent the infection of plants inoculated with 500 or 750 CFU of microsclerotia/ g of dry soil. These soil amendments may have potential in integrated disease management programs but further glasshouse trials should be undertaken to assess their efficacy to suppress Verticillium wilt and PED before field trials are conducted.

PT09040 - PROGRAM MANAGEMENT

APRP2 was largely designed and operated in accordance with the recommendations of the Pyksis Report (2009). Key elements of the program design were:

- Independent management and administration by SED Consulting (now SED Advisory).
- Program contracting through a higher education provider to exploit an opportunity for additional funding – the University of Ballarat (UB, now Federation University). This opportunity did not eventuate but the program retained a complex contractual structure.
- A Project Leader heading each of the five research projects.
- A Technical Operations Committee (TOC), comprising the Project Leaders and other researcher team members and personnel from SED, HAL, the Processing Potato Industry Advisory Committee (PPIAC) and AUSVEG.
- An Advisory Committee (AC), comprising senior personnel from each of the funding organisations.

The cumbersome contractual arrangements meant that SED's role was more administrative and less strategic than intended, involving the production of templates, facilitation of contractual changes, milestone and payment coordination, organisation of TOC meetings and progressing a multitude of ad hoc program-related tasks. SED also undertook a range of communications activities (articles for Potatoes Australia, talks to grower groups, liaison with key stakeholders such as HAL and AUSVEG, updates to the PPIAC) and in 2011 and 2013 convened symposia to showcase the outcomes of APRP2 to a broader industry audience including growers, field advisors and agchem personnel.

An important contribution to the program by the management project was some original research to estimate the cost of the major soil-borne diseases to the industry – information not available prior to the program. The analysis estimated that powdery scab is costing the processing potato industry approximately $13.4M per annum, common scab $1.7M and Rhizoctonia $5.4M. These figures, and an updated benefit/cost analysis suggesting a $7.48 return for every dollar invested in Predicta Pt alone, demonstrate the value of the investment in APRP2 and also provide guidance for future investment in processing potato R&D.

INTRODUCTION

The Australian Potato Research Program Phase 2 (APRP2) was an international collaboration that formed the dedicated research and development program for the processing potato industry. The five-year program commenced in 2009 and was made up of five projects based around the theme of soil and plant health with a chief focus on major soil-borne potato diseases: common scab, powdery scab and Rhizoctonia, with a secondary focus on potato psyllid, bacterial wilt and Verticillium. A sixth project was dedicated to the management of the program.

The program consisted of three integrated research components managed by external Program Coordinators SED Advisory:

1. *Soil disease*: there were three parts to this subprogram:
   - International collaboration to develop molecular markers for identified soil-borne pathogens;
   - The importance of tuber-borne inoculum on the health of seed potatoes; and
   - Enhancing the understanding of Verticillium spp. in Australian potato production
2. *Soil health*: there were three parts to this subprogram:
   - Microbial soil health and endophytes;
   - Soil amendments & soil nutrients, green manure crops, research principles to be established for active compounds; and
   - Bacterial wilt
3. *Insect pests*: potato psyllid control

The program involved 13 collaborating organisations including four from overseas. Table 1-1 outlines the project numbers, project names and organisations involved in each project.

Table 1-1 – APRP2 Project number, name and contributing organisations (lead organisation is highlighted in bold)

Project Number	Project Name	Organisations
PT09004	Control of potato psyllid within an IPM strategy	**IPM Technologies** Horticulture New Zealand New Zealand Plant and Food Research
PT09019	Importance of tuber-borne inoculum on seed potato health	**Tasmanian Institute of Agriculture** South Australian Research and Development Institute

Project Number	Project Name	Organisations
PT09023	Diagnostic tests for soil-borne pathogens – international collaboration	**South Australian Research and Development Institute** Tasmanian Institute of Agriculture Department of Environment and Primary Industries Victoria Potato Council (UK) Horticulture New Zealand New Zealand Plant and Food Research
PT09026	Soil health / disease mitigation program A - Manipulation of nutrients and soil health to induce disease suppressive conditions Ai - Soil amendments and nutrients Aii - Impact of rotations B - Novel products for disease control Bi - Endophytes Bii - Novel non-fungicidal treatments for control of common scab C - Bacterial wilt in irrigation water	**Department of Environment and Primary Industries Victoria** New Zealand Plant and Food Research A&L Laboratories (Canada) Tasmanian Institute of Agriculture Flinders University Tasmanian Institute of Agriculture Victorian Certified Seed Potato Authority
PT09029	Enhancing the understanding of Verticillium spp in Australian potato production	**Melbourne University** Department of Environment and Primary Industries Victoria Victorian Certified Seed Potato Authority
PT09040	APRP2 program management	SED Advisory

The APRP2 program was the main investment in research & development for the Processed Potato levy. The program received significant assistance from Voluntary Contributions (VCs) from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, and A&L Canada Laboratories and the Victorian Certified Seed Potato Authority. The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, the University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority provided in-kind support. The levy and VC contributions were matched by the federal government with the whole of program funding totalling $13.7M over 5 years (Figure 1-1).

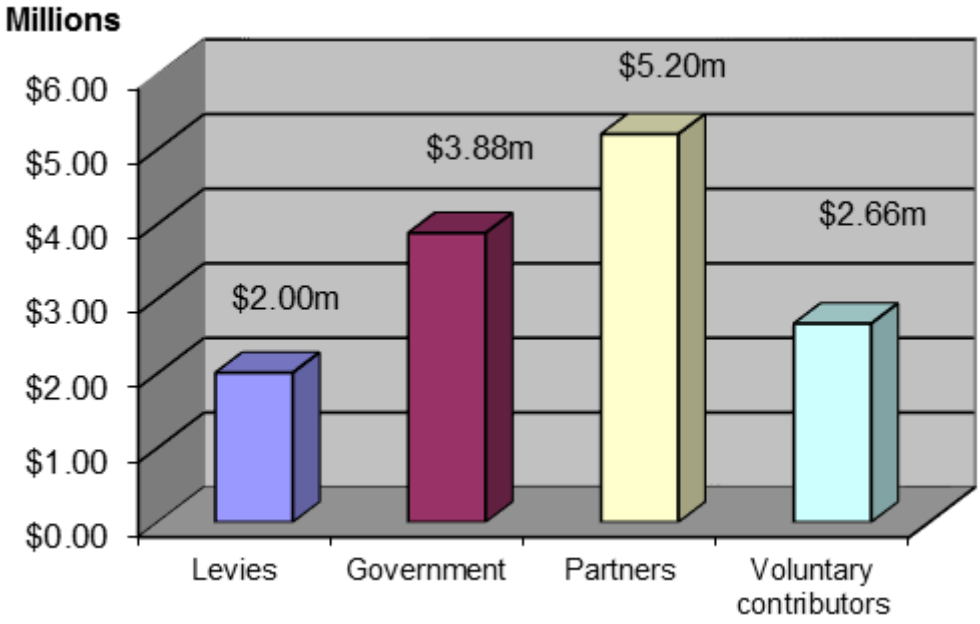

Figure 1-1 – APRP2 Funding Sources

BACKGROUND

APRP2 was created as a result of a scoping study contracted by HAL and conducted by Pkysis Pty Ltd in 2009. Pkysis reviewed the global processed potato industry and generated an independent market research report to identify priority research areas for the Australian industry. The primary goal of APRP2 was set to focus on research programs that have the potential to increase the size of the Australian processed potato market and ensure the industry continues to be competitive (Pyksis Pty Ltd, 2009).

A number of research areas were identified during the market research. The following research areas were set, as listed in order of priority:

1. Disease mitigation
2. Farming practices
3. Reduced input costs
4. Climate change
5. New cultivars

A tender process followed, and after a consultation process, 12 research project proposals with 25 subprojects were developed for funding consideration and submitted to the Processing Potato Industry Advisory Committee (PPIAC) for discussion. The PPIAC generated a short list of 11 research projects with five of these contracted within the APRP2 program, and several others contracted outside of the program. In the final selection, funded projects within APRP2 have focussed on the highest research priority area: disease mitigation.

The Pyksis report also included a recommendation for APRP2 to be managed by a provider independent of the organisations conducting the research for the program. A recommendation for an integrated communication and extension program was acknowledged by a concurrent Industry Development Needs Analysis commissioned by HAL (Figure 1-2). It was also recommended to HAL that the program be

managed in a manner that enabled it to leverage its contribution through a research institution that could attract thirty percent uplift through the Research Infrastructure Block Grant scheme (RIBG).

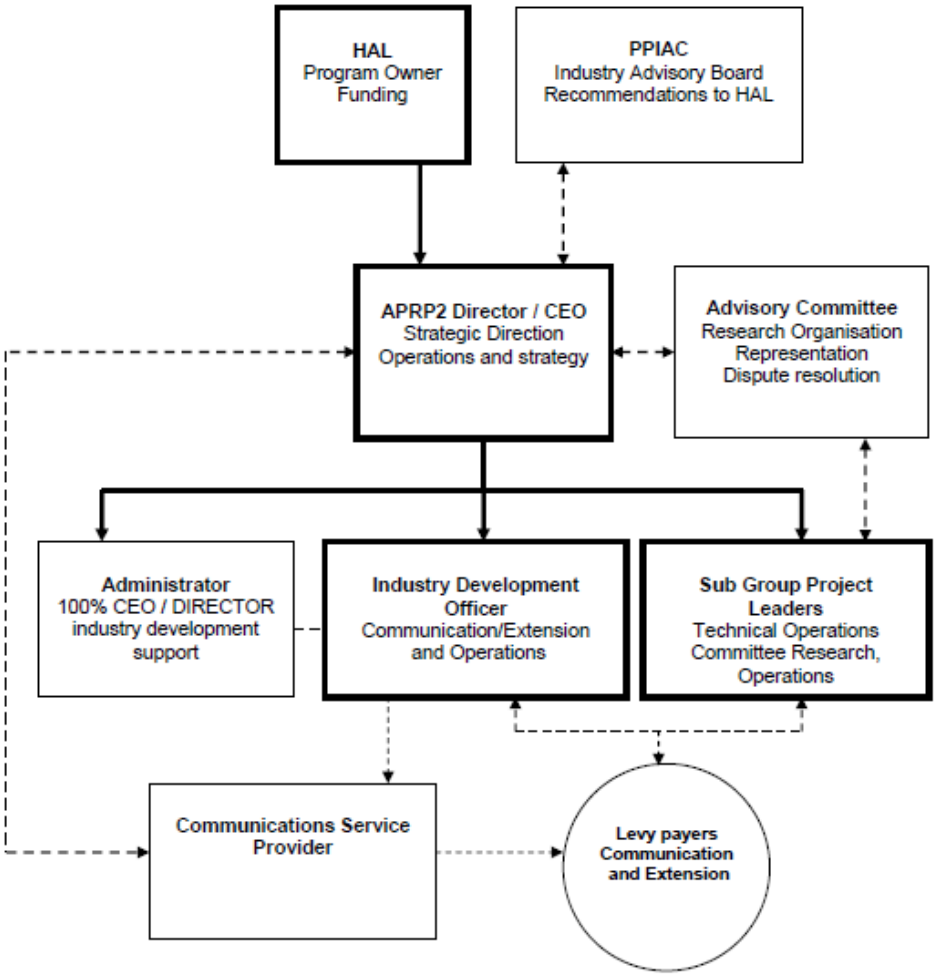

Figure 1-2 – Original proposed APRP2 structure (Source: Pyksis Pty Ltd, 2009)

SED Advisory (formerly SED Consulting) won the tender to manage APRP2 through an alliance with the University of Ballarat (UB, now Federation University) to enable access to potential RIBG funds. The University of Ballarat was contracted as the provider of the 'parent' project for the program (PT09039) responsible for the contracting of the six projects making up the program. Mr Scott Williams (formerly of SED Advisory, and subcontracted back to SED after January 2013) was appointed Program Director for APRP2 (following the Pyksis-recommended structure) with Ms Anne Ramsay (subcontractor to SED Advisory) appointed as Administrator.

The following diagram outlines the contracting structure for the APRP2 program (Figure 1-3). In this structure, the milestones applicable to each of the subcontracts between the University and the six project providers were aggregated up to a single set of milestones between the University and HAL, with a corresponding linking of payments from HAL through to providers. Thus, the contracting was an 'all-in' arrangement where all parties were required to meet all of their milestone criteria in order for the head agreement milestones to be met.

Within several months of contracting the program the RIBG was replaced by a system of block grants determined by the Higher Education Research Data Collection (HERDC) and the Higher Education Student Data Collection (HESDC). Further, the Government tightened the rules such that higher education providers were only able to claim money they were directly spending on research itself. Additional money was therefore not available to the program as the PPIAC and HAL had intended.

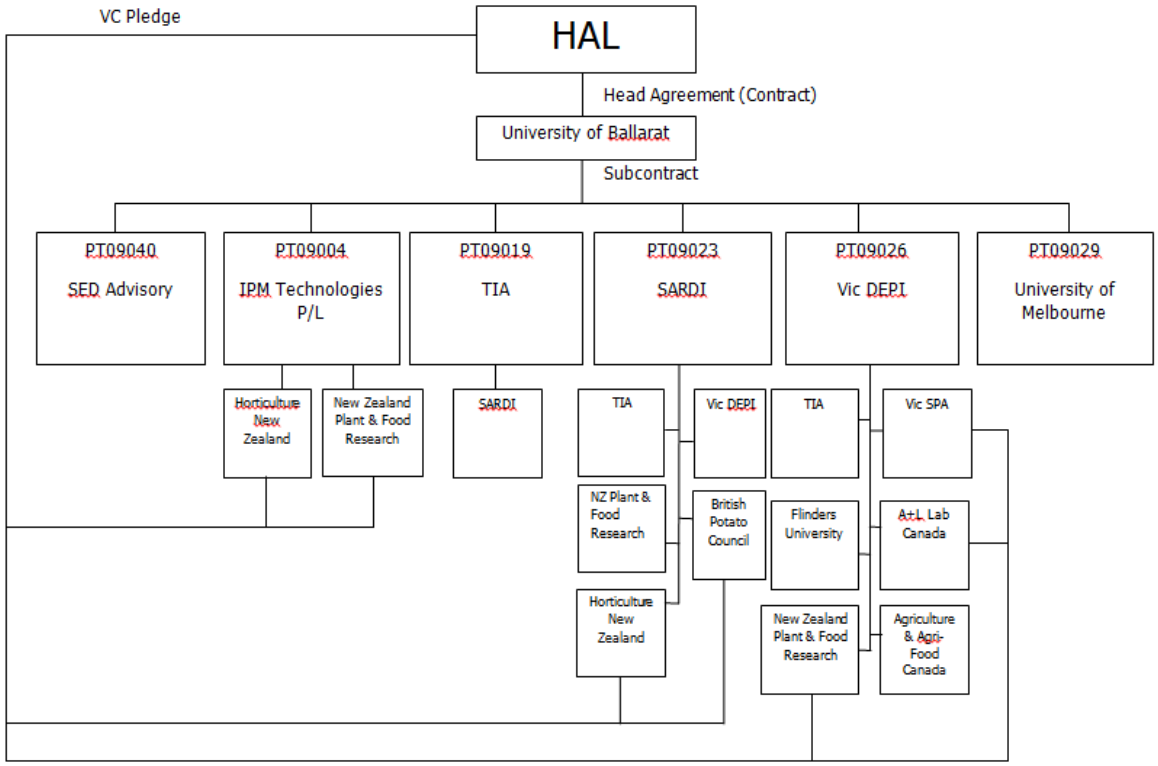

Figure 1-3 – Legal diagram for the APRP2 program

Unfortunately, this left the program with a very complicated contractual structure with little if any benefit additional to a direct arrangement between HAL and the individual providers. In light of the RIBG changes, in May 2010 SED Advisory applied to HAL to have the APRP2 contracting structure replaced with direct contracts with HAL rather than via the University of Ballarat. This would 'unshackle' each project from the next whereby if one party failed to meet milestone requirements other projects would not be impacted by associated delays and implications. This was taken to the PPIAC and endorsed. HAL researched the legal requirements for recontracting the program soon after the PPIAC endorsement, but a simple process of novating the contracts from UB to HAL was unable to be progressed. Conflicting internal legal advice and short time frames for recontracting between milestones meant that recontracting of the program was not achieved.

The legacy of the 'all-in' contractual structure was a very high administrative overhead within the program. This is explained more fully in the chapter on PT09040, the management project.

Two years after the establishment of APRP2, the PPIAC funded an extension program that sat outside of the APRP2 program. APRP2 itself therefore remained a science-based program and largely without its own resources for communication and extension, being reliant instead upon the Potato Extension Program (PEP) to drive awareness and adoption of APRP2 outcomes. APRP2 managers were not involved in the design, governance or delivery of the PEP.

INDIVIDUAL PROJECT OVERVIEW

The following provides an introduction to the six projects that make up the APRP2 program.

PT09004 - CONTROL OF POTATO PSYLLID WITHIN AN IPM STRATEGY

Service Provider – IPM Technologies Pty Ltd

Project Leader – Dr Paul Horne

The objective of this project was to trial and evaluate in New Zealand options for the control of potato psyllid and how to deal with this insect pest within an IPM strategy, rather than waiting until the pest occurs in Australia. Australia is a world leader in adoption of IPM and consequently the minimal use of insecticides. The arrival of the potato psyllid (for which there is no 'soft' insecticide) threatened this status. The potato psyllid has natural enemies such as lacewings and damsel bugs overseas but they are different species to those in Australia. This project assessed the biological control potential of species from Australia that also occur in New Zealand, as well as investigating cultural control options.

PT09019 - IMPORTANCE OF TUBER-BORNE INOCULUM ON SEED POTATO HEALTH

Service Provider –Tasmanian Institute of Agriculture

Project Leaders – Dr Calum Wilson and Dr Robert Tegg

This project aimed to determine if current certification practices of soilborne diseases (visual assessment) are adequate and looked to validate and test DNA technology as a tool for assessing disease loading in seedlots. Additionally the project aimed to assess the importance of seed-borne inoculum to disease in subsequent crops (both for root and seed disease) and for its importance in contamination of pathogen-free soils with pathogen inoculum. The project was closely linked with APRP2 project PT09023.

PT09023 - DIAGNOSTIC TESTS FOR SOIL-BORNE PATHOGENS – INTERNATIONAL COLLABORATION

Service Provider – South Australian Research and Development Institute

Project Leader – Dr Kathy Ophel Keller

The objective of this project was to improve management of key soil-borne diseases of economic importance to the processing potato industry by improving interpretation of DNA tests to quantify soil-borne inoculum so that the results can be used by growers to assess disease risk prior to planting a crop. Ultimately the project aimed to deliver a commercial pre plant soil testing service that would predict a level of disease risk based on the DNA levels found.

This project was undertaken in Australia, the United Kingdom and New Zealand. The objective of this collaborative project was to build on previous research and development and validation of diagnostic tools in the Australian Potato Research Program phase 1 (PT04016) as well as a pilot project to establish an international collaboration (PT08048) to extend the availability and interpretation of DNA tests to quantify soil-borne pathogens as risk management tools for growers.

The relative importance of soil- and seed-borne inoculum was reviewed for each of the diseases and knowledge gaps identified. In order to assess disease risk from both soil- and seed-borne sources of

inoculum, this project was closely linked to another project in APRP2, PT09019 'Importance of tuber-borne inoculum on seed potato health' which aimed to more accurately quantify seed-borne inoculum.

PT09026 - SOIL HEALTH/ DISEASE MITIGATION PROGRAM

Service Provider – Department of Environment and Primary Industries, Victoria

Project Leader – Dr Dolf de Boer

The objective of this project was to develop disease control strategies for soil-borne diseases of economic importance to the Australian processing potato industry through manipulation of nutrients and soil health factors to induce disease suppressive conditions. This project was undertaken in Australia, Canada and New Zealand. The objectives of the project were to build upon the international collaborations and research outcomes delivered from APRP1 (PT04016).

The project aimed to understand the relationships between soil factors (physical, chemical and biological) that influence pathogen populations, disease incidence and severity and develop integrated disease control strategies based upon this knowledge.

More specifically the project undertook work in the following areas:

- A. Soil amendments/ameliorants for soil health and disease suppression

  This research stream investigated the use of soil amendments/ameliorants and manipulation of nutritional factors to control soil-borne diseases of potato, enhance soil health and increase production. The work was conducted in Australia, New Zealand and Canada.

  Included in this stream was a long-term survey of the impacts of commercial rotations on potato pathogen inoculum levels.

- B. Novel approaches to disease control

  Endophyte discovery: this work identified potato endophytes and investigated their potential use and that of promising cereal endophytes for disease suppression in Australian potato production.

  Novel non-fungicidal treatments: this study evaluated whether foliar applications of sub-lethal concentrations of novel non-fungicidal foliar and tuber treatments have the potential to control common scab of potato.

- C. Monitoring bacterial wilt pathogen in irrigation water

  This subproject was initiated to develop and validate diagnostic tests that would help manage the isolated yet devastating bacterial wilt pathogen *Ralstonia solanacearum*. Primarily, the project monitored levels of bacterial wilt pathogen in irrigation water with the aim of developing effective catchment management strategies for controlling outbreaks of bacterial wilt in potato crops.

PT09029 - ENHANCING THE UNDERSTANDING OF VERTICILLIUM SPP IN AUSTRALIAN POTATO PRODUCTION

Service Provider – The University of Melbourne

Project Leader – Professor Paul Taylor

The objective of this project was to better understand potato early dying (PED) in Australian potato production and to provide fundamental knowledge regarding the causal agents involved in PED, ultimately

allowing industry to better manage this disease. This research project was undertaken by a PhD and Masters student.

The research aimed to:

- Identify the incidence of PED in Australian potato crops.
- Identify which Verticillium species and nematodes are responsible for PED in Australia and understand their interactions.
- Assess the resistance of Australian potato cultivars to Verticillium spp.
- Assess key management strategies to reduce the impact of PED in Australian potato crops.

PT09040 – APRP2 PROGRAM MANAGEMENT

Service Provider – SED Advisory

Project Leader – Mr Scott Williams

The project was established to provide independent oversight and management of the APRP2 program. Reporting directly to HAL the program managers were responsible for supporting the program in delivering high quality research and development outputs.

PROJECT TEAM CONTACT DETAILS

Each chapter of this final report provides a comprehensive list of personnel involved in the respective project. Names and contact details for project leaders and subproject leaders are also summarised below.

PT09004 - Control of potato psyllid with an IPM strategy

Dr Paul Horne
IPM Technologies Pty Ltd
Phone: 03 9710 1554
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PT09019 - Importance of tuber borne inoculum on seed potato health

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Dr Robert Tegg
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PT09023 - Diagnostic tests for soil-borne pathogens – international collaboration

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PT09026 - Soil health/disease mitigation program

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Ai - Soil amendments & nutrients

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Flinders University
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Bii – Novel products for disease control

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Tasmanian Institute of Agriculture
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Email: Calum.Wilson@utas.edu.au

C – Bacterial wilt in irrigation water

Dr Nigel Crump
Victorian Certified Seed Potato Association
Phone: 03 5962 0000
Email: nigelcrump@vicspa.org.au

PT09029 - Enhancing the understanding of Verticillium spp. In Australian potato production

Professor Paul Taylor
Melbourne School of Land and Environment
The University of Melbourne
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Email: paulwjt@unimelb.edu.au

MANAGEMENT AND OVERSIGHT

The HAL funded program was contracted through the University of Ballarat and managed by SED Advisory. The original program design (Pyksis Pty Ltd, 2009) incorporated a management arrangement for APRP2 that was to mirror a corporate structure:

- HAL fills the role of shareholder (and overall manager) with final program authority
- PPIAC fills the role of the Board, including Chair
- Program Director fills the role of the Chief Executive Officer (CEO)
- Project Leaders fill the role of the divisional managers of research units (Figure 1-2).

Further, as recommended by Pyksis, the following committees were formed:

TECHNICAL OPERATIONS COMMITTEE

The program operated with a Technical Operations Committee (TOC) that met twice yearly face to face and via phone or webinar as required. The TOC was made up of Project Leaders, Subproject Leaders, the APRP2 managers, HAL Portfolio Manager, HAL Industry Services Manager, representatives from the PPIAC and representatives from the AUSVEG extension and communication programs.

APRP2 ADVISORY COMMITTEE

A recommendation from Pyksis was the formation of an Advisory Committee (AC) to be used as a forum to resolve issues that could not be settled by the Technical Operations Committee. The AC was to be convened by the program managers and only on an as-required basis. Representatives of the AC were welcome to attend any TOC meetings as an observer.

TECHNICAL OVERSIGHT GROUP

The final year of the program saw the introduction of the Technical Oversight Groups (TOG) to each of the 6 projects making up the program. The TOG process was initiated and managed by HAL without the involvement of the APRP2 project managers. The groups were made up of one to two industry representatives from the PPIAC with representatives nominated based on proximity to the project and expertise relating to the project.

Further information, detail and discussion relating to the structure of APRP2 is found in chapter 7 of this final report.

TECHNOLOGY TRANSFER

The communication and extension activities associated with each project are reported in the respective chapters. Provided below is a higher level summary of the number of activities from across the program.

REFEREED JOURNAL ARTICLES

- 18 published papers
- 9 papers published based on associated research

SCIENTIFIC CONFERENCE PAPERS

- 59 conference papers
- 8 papers published based on associated research

INDUSTRY & MEDIA PUBLICATIONS

- 44 articles in Potatoes Australia or other rural media publications

INDUSTRY & PEER PRESENTATIONS

159 presentations delivered at grower workshops, company supplier conferences, industry conferences (not including APRP2 Technical Operations Committee presentations).

ACKNOWLEDGEMENTS

The Program Managers would like to acknowledge the pragmatic and efficient support provided by the University of Ballarat (now Federation University) that enabled the program to function despite its contractual complexity. Notably we would like to acknowledge the proficiency of Professor Frank Stagnitti, Derek White, Diane Clingin, Thembi Dube and Jane Eltringham.

SED is particularly grateful for the industry support and encouragement that was provided to the program by Frank Mulcahy of Simplot, Dave Antrobus formerly of McCain, Graeme Henman formerly of SA Fries and Kevin Clayton-Greene from the HAL-led Technical Advisory Group. We are particularly appreciative of Kevin, Frank, Dave and Graeme for promptly reviewing the various published materials produced from the program.

Thanks too to Sarah Sullivan and Ben Callaghan, the two Portfolio Managers at HAL responsible for the program over the five years. Ben's unwavering support and advice was greatly appreciated by all and especially Scott and Anne.

Special thanks also to Nigel Crump who provided technical support, program insight and corporate memory to the Program Managers, who are not potato experts.

Finally, we would like to acknowledge the passion and dedication delivered to the program by the research team. The collaborative spirit of the team prevailed despite (at times) challenging circumstances and was a credit to the professionalism of the individuals involved.

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Pyksis Pty Ltd. (2009). *Final Report: PT07037 - Scoping Phase 2 Processing Potato R&D Program.*

Horticulture Australia

PT09004 – (30 October 2015)

Control of Potato Psyllid with an IPM Strategy

Report

Paul Horne and Jessica Page

IPM Technologies Pty Ltd

PROJECT SUMMARY

PT09004 – Control of Potato Psyllid with an IPM Strategy

To develop control methods for potato psyllid that are compatible with the IPM strategy currently available and used in Australian potato production.

Project Leader: Dr Paul Horne
IPM Technologies Pty Ltd, PO Box 560, Hurstbridge, Victoria 3099
Ph: 03 97101554, Fax: 03 97101354
Email: ipmtechnologies@bigpond.com

Other personnel:

Ms Jessica Page, IPM Technologies Pty Ltd
Dr David Teulon, Plant & Food New Zealand
Dr Lindsay Fung, Horticulture New Zealand

PURPOSE OF THE REPORT:

The aim of this project was to help to prepare the Australian potato industry for the possible arrival of potato psyllid from New Zealand by identifying methods of control other than regular and heavy use of insecticides. This report describes how an IPM approach can be utilised to deal with potato psyllid as well as all other potato pests.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute of Plant & Food and Horticulture New Zealand, and matched funds from the Australian Government.

DISCLAIMER

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MEDIA SUMMARY

This project has developed a method of controlling potato psyllid (also called tomato potato psyllid) that does not rely on the regular and heavy use of broad-spectrum insecticides.

Instead, a strategy based upon the main controls being biological and cultural, with support being applied via chemicals as required, has been successfully tested in New Zealand.

Potato psyllid is a devastating pest of potatoes and related crops such as tomatoes, capsicums and tamarillos. It was discovered near Auckland, New Zealand in 2006 and it spread rapidly throughout New Zealand over the next few years. The approach taken to deal with it in potato crops was by the frequent use of insecticides and initially between 14 and 19 insecticides were applied to each planting. Broad-spectrum insecticides that kill a wide range of insects, including beneficial species, were predominant in most spray programmes.

This project was carried out in order to try and find a better method of controlling potato psyllid that did not require such use of insecticides, before the probable arrival of the pest. The main reason for the project was that such insecticide applications would destroy the biological control of a range of other pests (such as potato moth, aphids and thrips) and so destroy the IPM (integrated pest management) approach that is currently used by many growers in Australia.

There was an urgency to this work, as no-one knew, and still no-one knows, when the psyllid would be found in Australia. Therefore, the project was planned to have three stages and at the conclusion we wanted to be able to say that an alternative approach existed. The three stages were:

1. To identify predatory insects that would eat the psyllid in potato crops
2. To develop an alternative IPM strategy that would include potato psyllid
3. To field-test the new IPM strategy in commercial potato crops in New Zealand.

These three stages were completed successfully.

Bioassays with 3 species of predatory insects (a ladybird beetle, a damsel bug and brown lacewings) found in Australian potato crops demonstrated that all would accept the psyllid as prey, even in the presence of alternative food sources.

A draft IPM strategy was developed, using what we observed about the behaviour of the psyllid and what we knew of certain selective insecticides. It involved a seed treatment and then only border spraying with certain insecticides that would not harm the key beneficial species.

Two collaborating farmers from Canterbury in the South Island of New Zealand agreed to trial the proposed IPM strategy, and the crops were monitored by entomologists from Plant & Food Research. The aim was to see if the draft IPM approach dealt with potato psyllid in a satisfactory way, as determined by the growers and the processor who was supplied. The end result was that the crops were grown to the satisfaction of both farmer and processor (McCain Foods), without compromising any standards regarding quality.

Linked to this project were two separate projects conducted by the New Zealand Institute of Plant & Food Research and Horticulture New Zealand. The work was linked such that the information developed in these projects would be made available to Australia and that the Australian led IPM component would offer further management options to the New Zealand industry. The New Zealand led work has produced a considerable body of information to better understand psyllid, its predators and the bacterium that is vectored by the psyllid which will be valuable to the Australian industry should psyllid arrive on our shores.

TECHNICAL SUMMARY

Potato psyllid (*Bactericera cockerelli*), also called tomato-potato psyllid, is a devastating pest of potatoes and related crops such as tomatoes, capsicums and tamarillos. It was discovered near Auckland, New Zealand, in 2006 and it spread rapidly throughout New Zealand over the next few years. The approach taken to deal with it in potato crops there (and in the USA) was by the frequent use of insecticides and initially between 14 and 19 insecticides were applied to each planting. Broad-spectrum insecticides that kill a wide range of insects, including beneficial species, were predominant in most spray programmes.

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Linked to this project were two separate projects conducted by the New Zealand Institute of Plant & Food (Appendix 1) and Horticulture New Zealand (Appendix 2). The work was linked such that the information developed in these projects would be made available to Australia and that the Australian led IPM component would offer further management options to the New Zealand industry. The New Zealand led work has produced a considerable body of information to better understand psyllid, its predators and the bacterium that is vectored by the psyllid which will be valuable to the Australian industry should psyllid arrive on our shores.

INTRODUCTION

Potato psyllid (*Bactericera cockerelli*, also called tomato-potato psyllid or TPP) is an extremely serious pest of solanaceous crops in USA and recently in New Zealand. The psyllid vectors a serious disease of potatoes, known as "yellows", that is caused by a bacterium, *Candidatus* Liberibacter solanacearum (Lso). Significant losses (up to 50%) have been reported from potato crops in the USA and up to 80% in glasshouse tomatoes in New Zealand (NZ). Total crop losses have occurred in processing potato crops in the USA in Idaho in the last year. In processing potatoes the problem it causes is called “Zebra chip” because of change in sugars stored in the tubers. The insect and the disease pose a massive threat to the Australian potato industry.

Potato psyllid was discovered in New Zealand in a glasshouse near Auckland in 2006 and is now established throughout the country but has not yet been detected in Australia. History suggests that this pest will soon be a problem for Australian farmers and so we should be prepared for it. At the July 2012 Psyllid Conference in Auckland, New Zealand, a researcher from the USA commented that it was certain that potato psyllid would arrive in Australia, and it was just a matter of when.

In New Zealand the occurrence of potato psyllid destroyed IPM in many crops where minimal insecticide use had previously been practised. This includes glasshouse crops where highly developed IPM strategies had been implemented for many years. The approach to dealing with this pest in NZ potato crops has so far been by the regular use of a range of insecticides, including many broad-spectrum insecticides. The method of dealing with TPP in New Zealand when the problem was first detected was by applying insecticides weekly, or up to 19 insecticides per crop (Mark Heap, Simplot and Monty Spencer, Wilcox Farms, pers. comm.).

In Australia by contrast there has been a world-leading adoption of IPM and consequent minimal use of insecticides (Horne and Page 2008). The arrival of potato psyllid threatens that status. If broad-spectrum insecticides are used against potato psyllid then it will destroy the IPM control of other pests such as aphids, thrips and potato moth which are currently dealt with within IPM strategies.

Rather than wait until potato psyllid arrives in Australia before we consider how to deal with it, this project based in New Zealand aimed to identify management options using an IPM strategy. That means that when the pest arrives in Australia we are not restricted to a pesticide-based response. Rather, we will know how to deal with this pest without recourse to broad-spectrum insecticides.

The project was planned to have three stages and at the conclusion we wanted to be able to say that an alternative approach existed. The three stages were:

1. To identify predatory insects that would eat the psyllid in potato crops
2. To develop an alternative IPM strategy that would include potato psyllid
3. To field test the new IPM strategy in commercial potato crops in New Zealand.

This project was a joint collaboration between IPM Technologies P/L from Australia and Plant & Food Research in NZ.

Figure 2-1 – Adult potato psyllid

MATERIALS AND METHODS

This project consisted of three distinct phases:

1. Laboratory testing of potential biological control agents
2. Development of draft IPM strategies
3. Commercial testing of draft IPM strategies.

Phase 1 was essential as we needed to know whether or not at least some of the generalist predators that occur in Australian potato crops would recognise and accept potato psyllid as prey. If they did not eat potato psyllid then there would be no biological control component to be sure of within an IPM strategy, and that would mean total reliance on cultural and chemical components. The psyllid has escaped its natural enemies by arriving in New Zealand and this includes specific parasitoids.

Therefore the only biological control agents that are useful in this case are generalist predators that are not restricted to a single type of prey.

Three species were chosen by us as they were generalist predators that occurred in both Australia and New Zealand. These were:

1. Brown lacewing – *Micromus tasmaniae*
2. Damsel bug – *Nabis kinbergii*
3. Common spotted ladybird – *Harmonia conformis*

Brown lacewings and damsel bugs are very important predators in Australian potato crops (Horne, De Boer and Crawford 2002) and *H. conformis* is found in potato crops but is not necessarily the most important species of ladybird. These species represent some of the most important potential predators of potato psyllid. Hoverflies are also important predators, and these were already being tested by entomologists at Plant & Food Research although they are different species to those occurring in Australia.

LABORATORY TESTING

Laboratory testing was done by a team at Plant & Food Research laboratories in Auckland (Mt Albert) co-ordinated by Graham Walker. The team also included Ngaire Larsen, Frances MacDonald and Robin Garner-Gee.

PREDATOR TESTING – *HARMONIA CONFORMIS*

- Two types of tests were conducted – 'Choice' or 'No-choice'. As the name suggests, the 'No-choice' tests contained only potato psyllids as potential prey whilst the 'Choice' tests contained another, alternative food source. The number of prey eaten was recorded.
- There were 3-10 or more replicates of each set-up (which was verified by a statistician).

'CHOICE' TESTS:

Protocol:

- 25 TPP and 25 green peach aphids (GPA) were given to each predator.
- Controls only contained 25 GPA.
- All tests were conducted at 20°C ± ~2°C, at 16:8 hours Light:Dark.
- All predators were starved 24 hours prior to being tested.
- Each predator was tested for 24 hours.

All predators were tested in 9cm vented petri dishes with filter paper (see

- Figure 2-2).

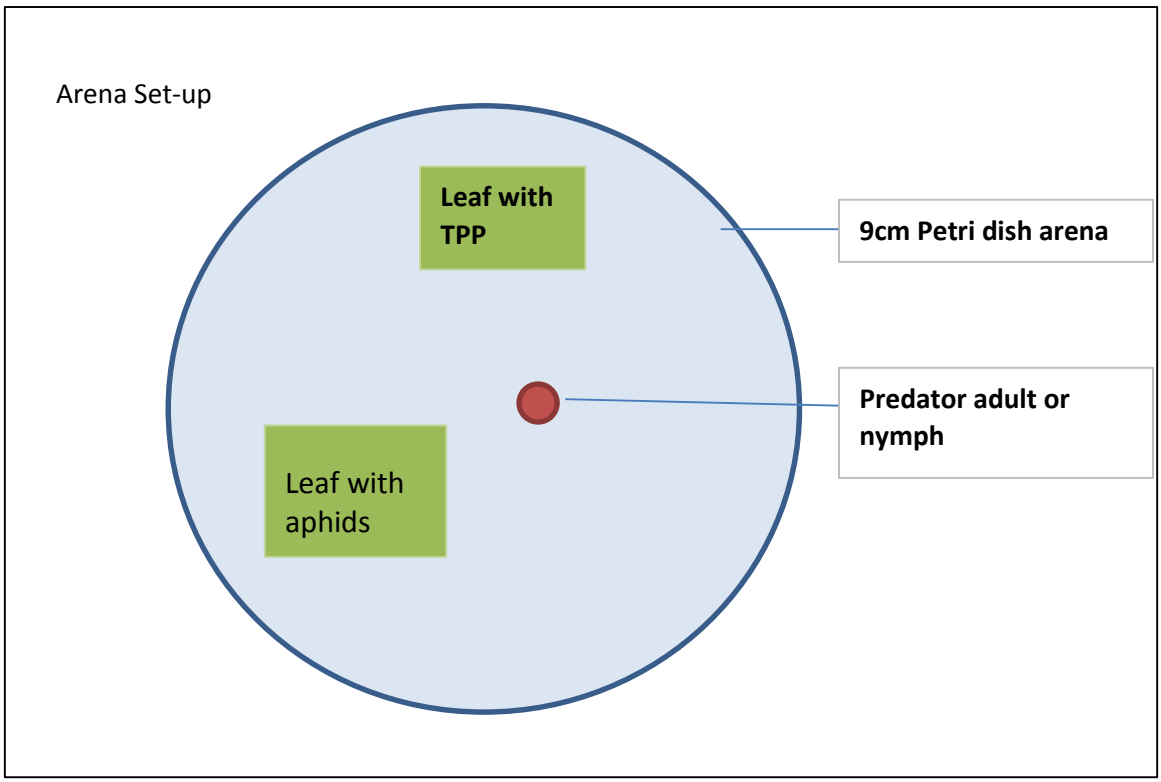


Figure 2-2 – Arena set up for 'Choice' tests

'NO-CHOICE' TESTS:

Protocols: test efficacy of predaceous life stages of the three predator species against all life stages of TPP.

- Starve predators for 24 hours before assay (some young instars died in 24 hours so these stages were fed before assay).
- The assay was run for 24 hours at 18-20^{o}C at 16:8 LD.
- A data-logger was running in controlled temperature room.

Arenas of 9 cm petri dishes with filter paper were used (see

- Figure 2-2).
- The assay used 20 eggs, 20 small and large nymphs and 10 TPP adults.

DEVELOPING AN IPM STRATEGY

When potato psyllid arrived in New Zealand, control methods were based on a heavy use of insecticides, as previously described. It was therefore essential for this project that we took a different approach for the benefit of Australian potato growers and the Australian potato industry. We took the view that the Australian potato industry could not sit and wait for New Zealand researchers to deliver us the answer that we needed regarding an IPM approach.

In this project it was essential that we looked in commercial crops and the range of control measures employed at a range of sites across New Zealand. To do this we visited sites assessed by others as highest risk (Scott Lawson, Organic, Hawkes Bay) as well as discussing control options used in high pressure areas (Pukekhoe, North Island – discussed with Bryan Hart and staff at HortiCentre), and (perceived, but not necessarily) lower pressure areas such as Canterbury (discussed with Duncan McLeod).

The task for IPM Technologies (Paul Horne and Jessica Page) was to look at the current control methods of potato psyllid in New Zealand, look at what may be possible in terms of changed methods, develop an alternative control strategy, convince NZ farmers and agronomists to trial our approach in a commercial crop and assess our proposed approach in a commercial crop in NZ. This was achieved.

A possible cultural control was to increase populations of predators at the perimeter of the crop. Planting cereals in order to attract aphids and leafhoppers, and in turn populations of predators of these species, was something that we have worked on before in other crops (HAL Project VG05008 - Development of cultural control methods for pests of leafy vegetables) with some success, and there has been research in other parts of the world along the same lines (Holland et al. 2012). Therefore, we asked the two collaborating growers to plant grassy strips or leave existing grassy strips around the perimeter of the crops. The strips needed to be easily integrated into current management and also needed to be present quickly when potatoes were just emerging. The consensus from agronomists and farmers with which this was discussed was that rye-grass was the best option and a strip approximately 2m wide was planted alongside the crop.

COMMERCIAL TESTING OF DRAFT IPM STRATEGY

To assess the draft strategy we needed sites where the entire crop was grown using the strategy. It is not possible to do the type of trial that we needed using small plots. The main measure of success or failure would be the conclusion of the collaborating farmer after harvest. In addition to that, we wanted to collect data on both beneficial and psyllid numbers at different distance from the edge of the crops.

Our observations on the potato psyllid in potato crops, and discussions with agronomists in the North and South Islands of New Zealand, led us to believe that the efforts on control should be at the perimeter of the potato paddock. Therefore we proposed using selective insecticide sprays using products such as “Chess” (pymetrozine), “Success” (spinosad) and “Movento” (spirotetramat). A seed dressing of “Actara” (thiamethoxam) at planting was also included. These treatments were not chosen for being able to kill most psyllids but because they would leave the key beneficial species largely intact. Therefore they would work together rather than as stand-alone treatments. These were applied by standard boom-spray equipment, but just with one arm of the boom used as a border spray.

TRIAL SET UP

Two potato crops in Chertsey, Canterbury were used for the trial. Both crops were Russett Burbank grown for processing by McCain Foods (NZ).

MONITORING:

Monitoring consisted of 3 parts:

1. **PLANT MONITORING:** plant monitoring was carried out weekly on a total of 31 potato plants per site, each site being a normal commercial scale paddock. The plants for monitoring were selected in a grid from 1m up to 50m into the potato crop from the grass edge. The sampling points were marked with poles and a randomly selected potato plant in the vicinity was assessed for the presence of TPP, other pest species (e.g. thrips and aphids) and beneficial insects (e.g. brown lacewings, ladybirds and hoverflies). The entire plants were assessed from emergence and up to 50 cm (first two sampling dates). After this only two main stems per plant were assessed.
2. **STICKY TRAP MONITORING:** 4 yellow sticky traps were placed around the grid (N, S, E, W) to monitor the weekly flights of TPP, other pest species and beneficial insects.
3. **SUCTION SAMPLING IN GRASS STRIPS:** fortnightly suction sampling was carried out in the grass strips to determine presence of beneficial insects and other insects that may serve as additional food source for these (e.g. thrips and aphids). The grass edges were separated into 1 primary grass strip and 2 secondary grass strips There were a total of 12 grass sampling points per site: 6 sampling points in the primary grass strip and 3 sampling points in each of the secondary grass strips. The sampling method consisted of lowering the vortis sampler for 5 x 3 sec in a circle around each sampling point. The samples were stored in 96% alcohol and sorted in the laboratory.

In the original project plans there was the scope to involve independent NZ entomologists who had local knowledge of biological control and the generation of insects via commercial means. Initial work in the project did involve planning and looking at potential biological control agents with a view to mass-rearing, but the focus of the entomologists was in glasshouse work, which had little relevance to the field environment. So although it was a very useful introduction to the pest problem for IPM Technologies, it meant that their formal involvement was not desired and so the task of formulating an IPM strategy was left solely to IPM Technologies.

RESULTS

FEEDING TRIALS

The results of the feeding trials are presented in the graphs below (Figure 2-3 – Figure 2-14). The overall result was that all three species of predators tested would eat potato psyllid. Furthermore, all active life stages of each predator tested would eat all life stages of the potato psyllid.

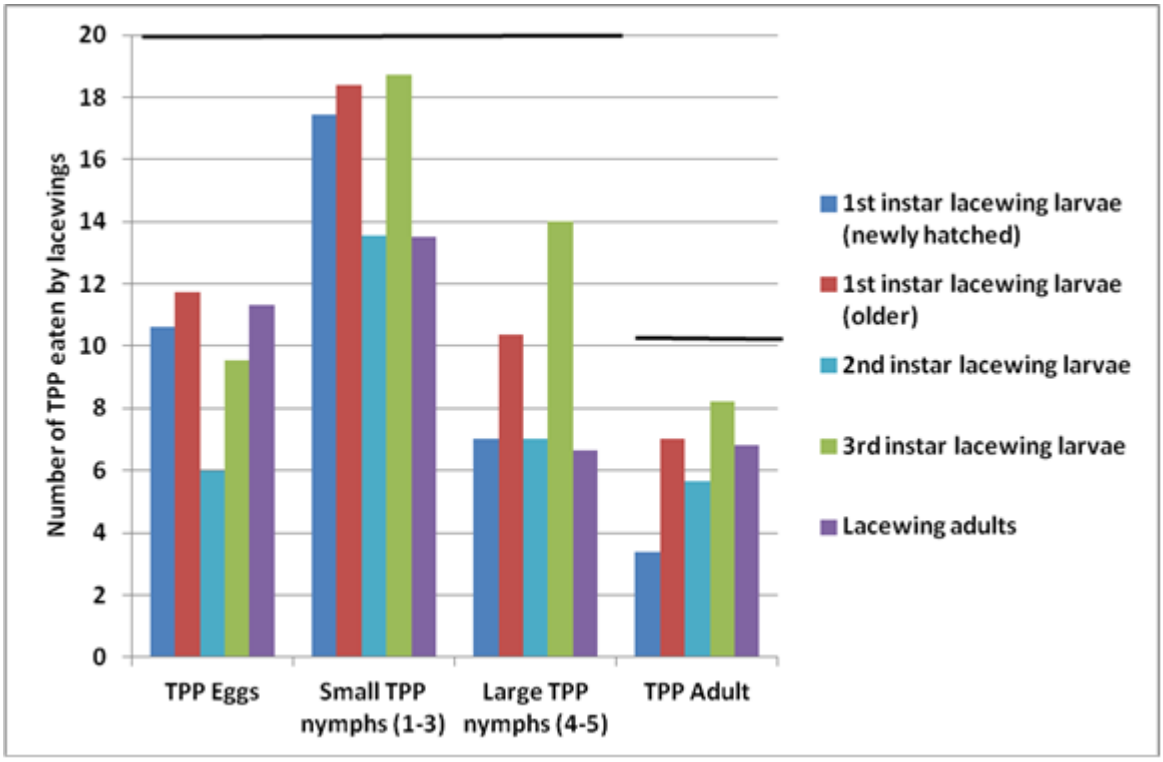


Figure 2-3 – Feeding trial results - *Micromus tasmaniae* (brown lacewing)

* black horizontal line indicates where 20 TPP or 10 TPP were supplied

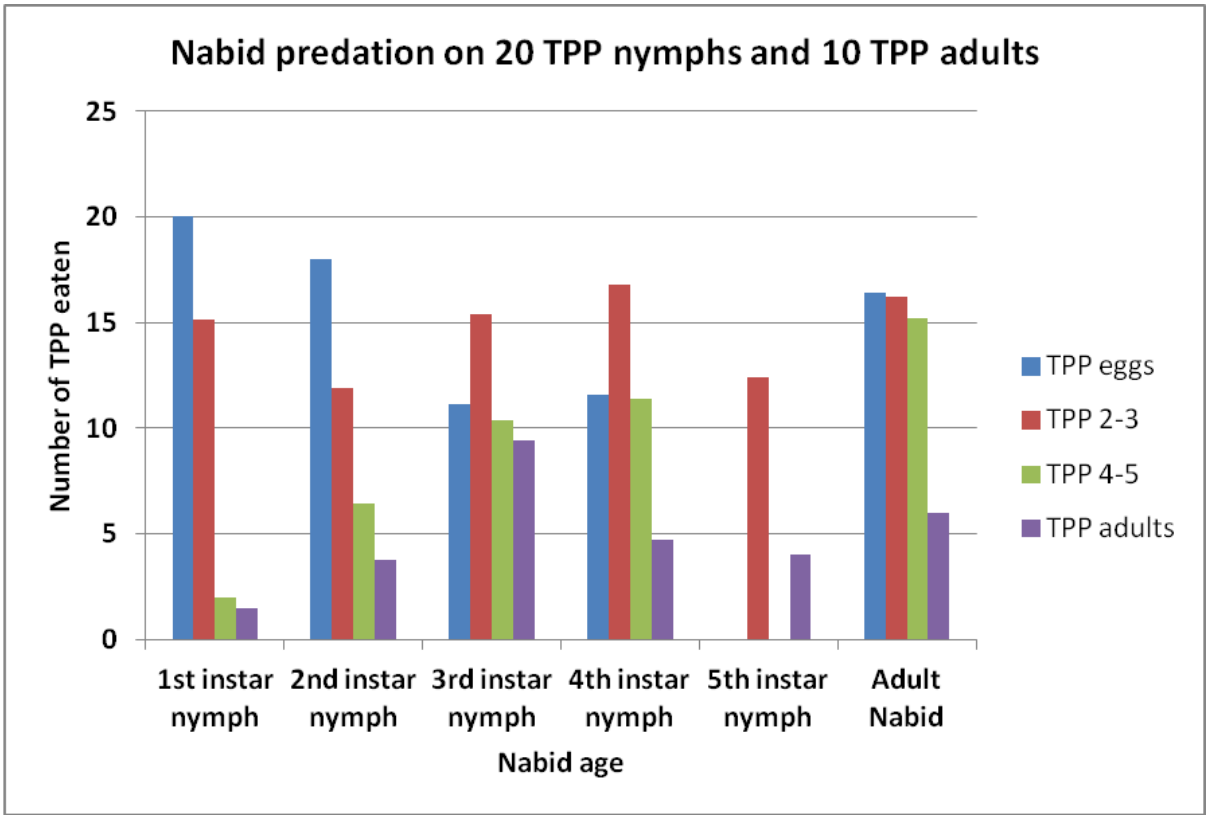


Figure 2-4 – Feeding trial results - *Nabis kinbergii* (damsel bug)

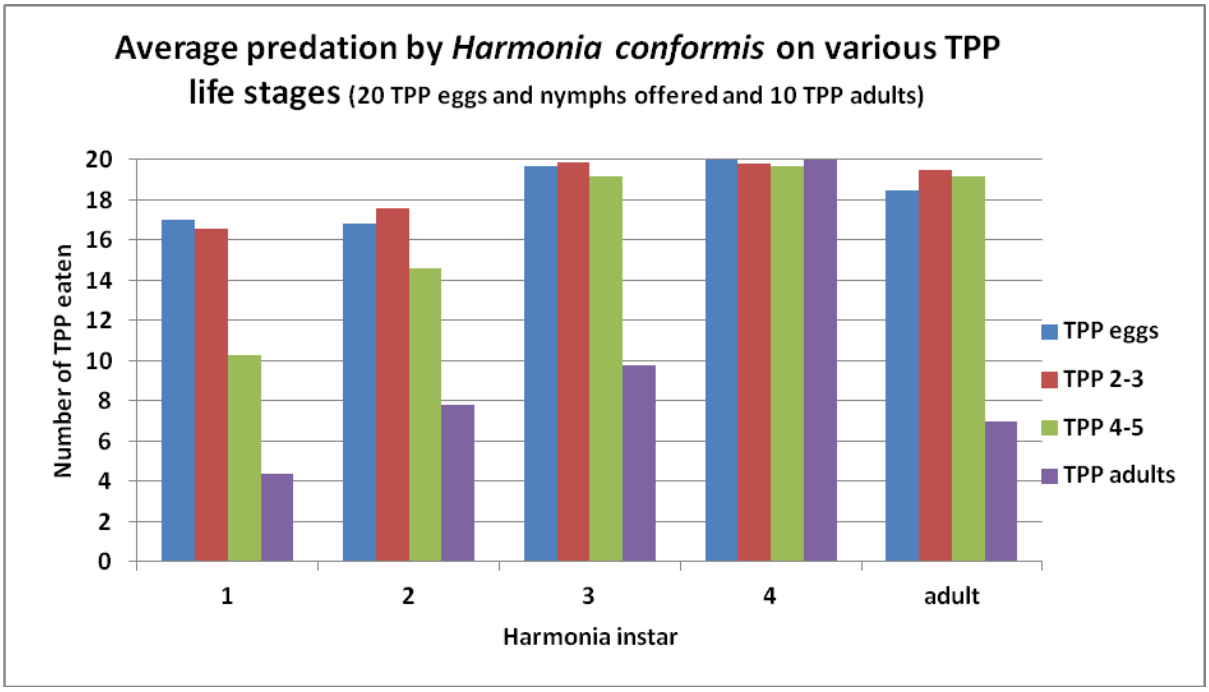


Figure 2-5 – Feeding trial results - *Harmonia conformis* (common spotted ladybird)

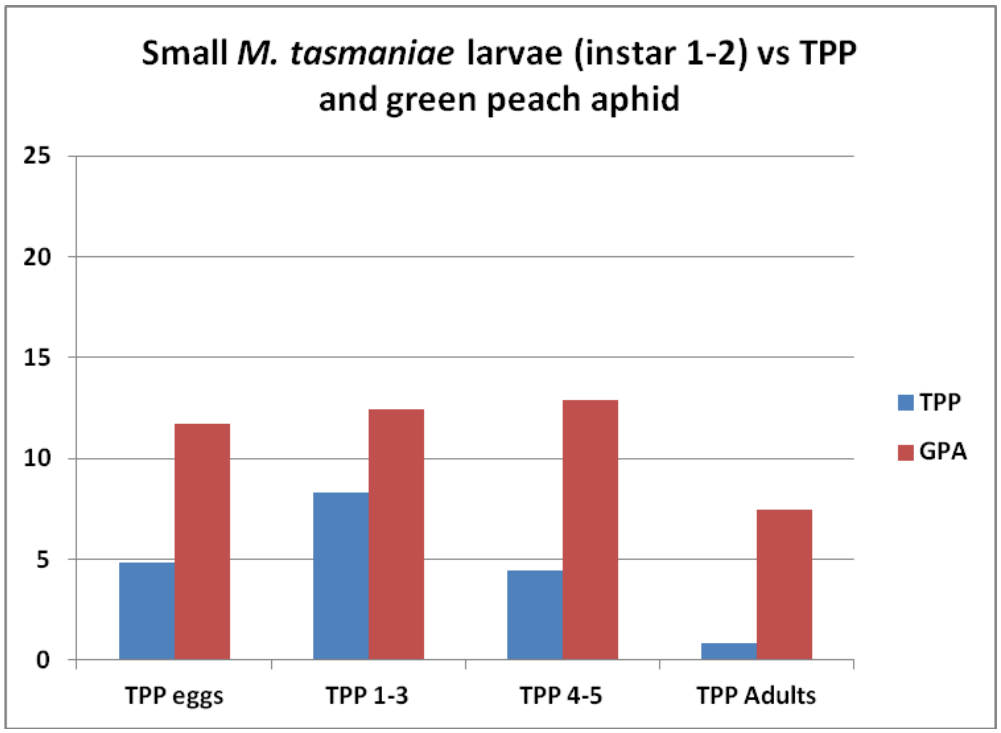


Figure 2-6 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for small *M. tasmaniae* (instars 1-2)

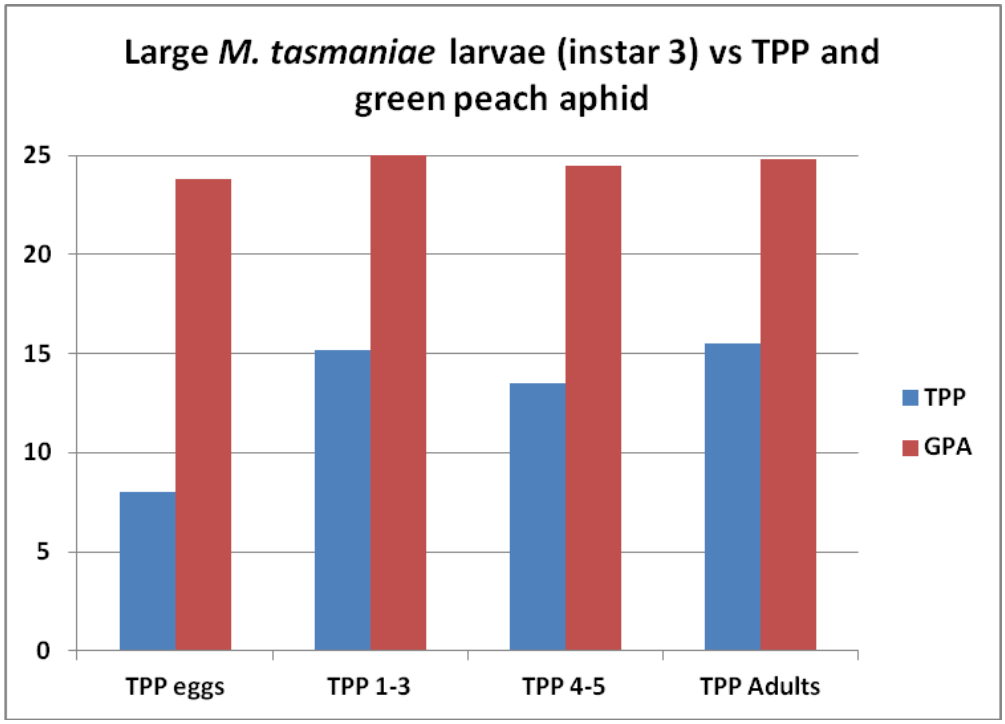


Figure 2-7 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for large *M. tasmaniae* (instar 3)

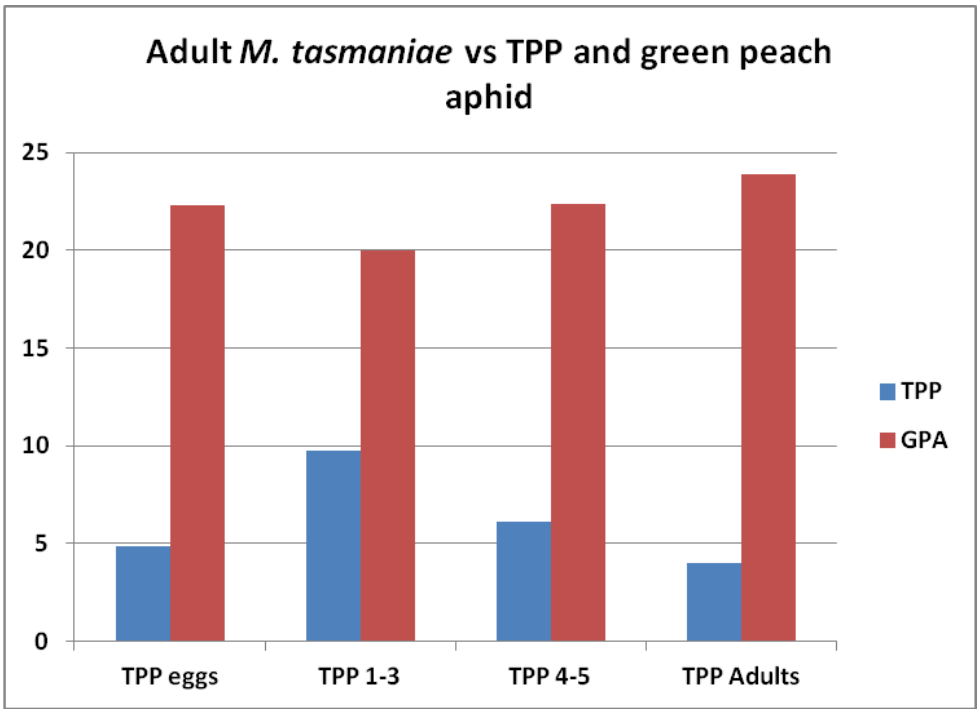


Figure 2-8 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for adult *M. tasmaniae*

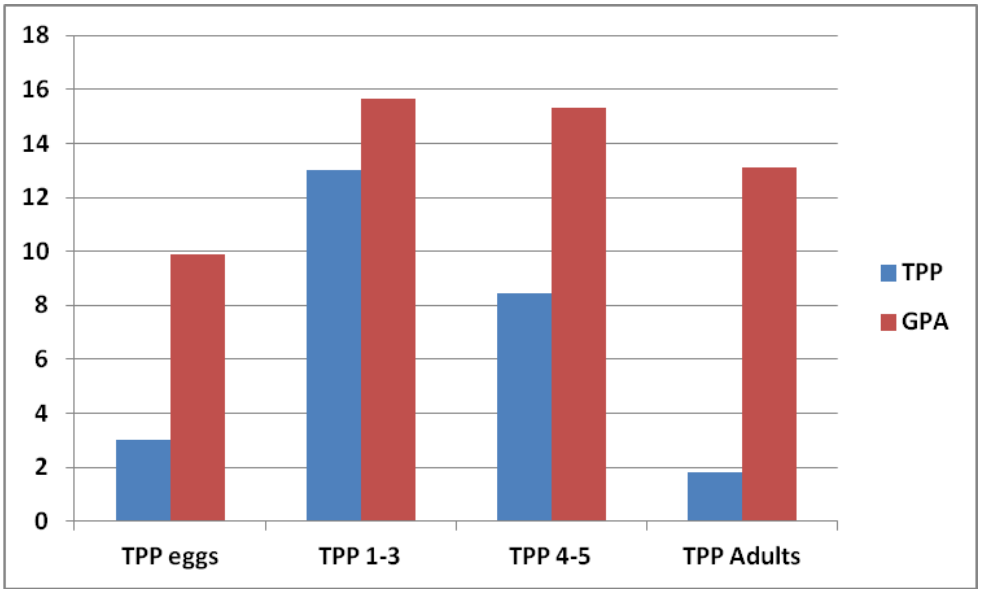


Figure 2-9 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for small *Nabis kinbergii* (instars 1-2)

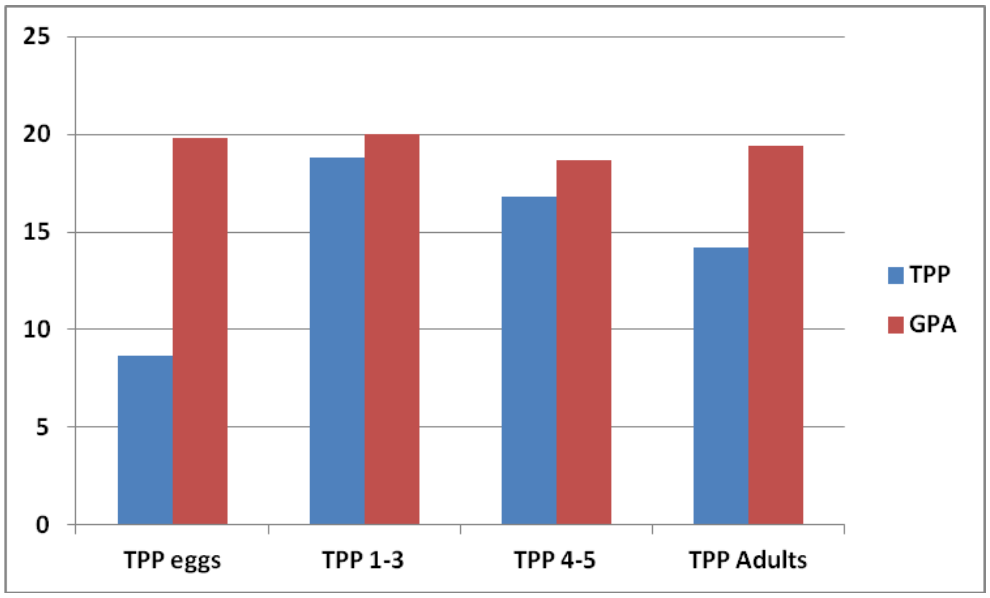


Figure 2-10 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for large *Nabis kinbergii* (instar 3)

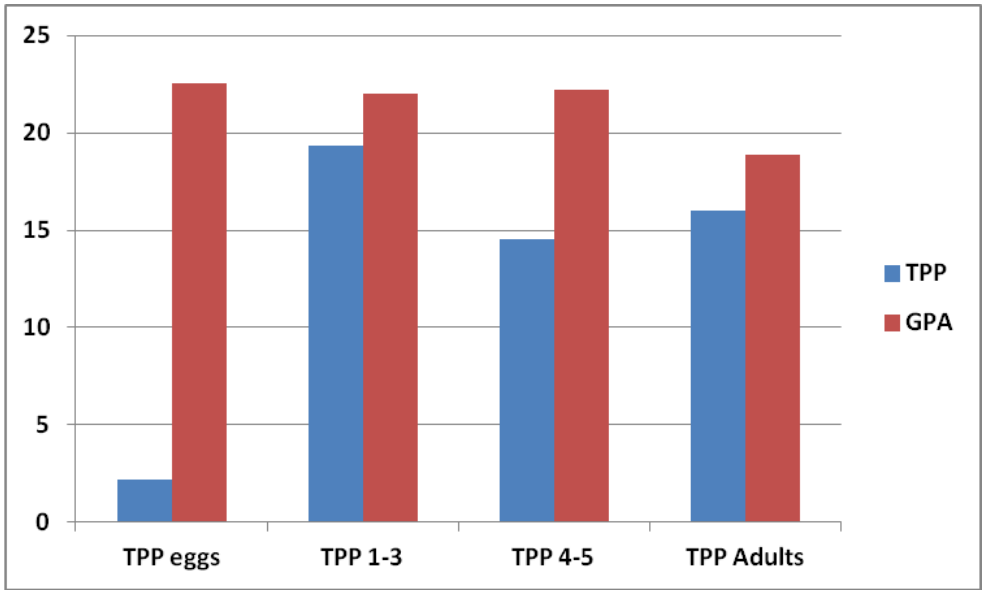


Figure 2-11 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for adult *Nabis kinbergii*

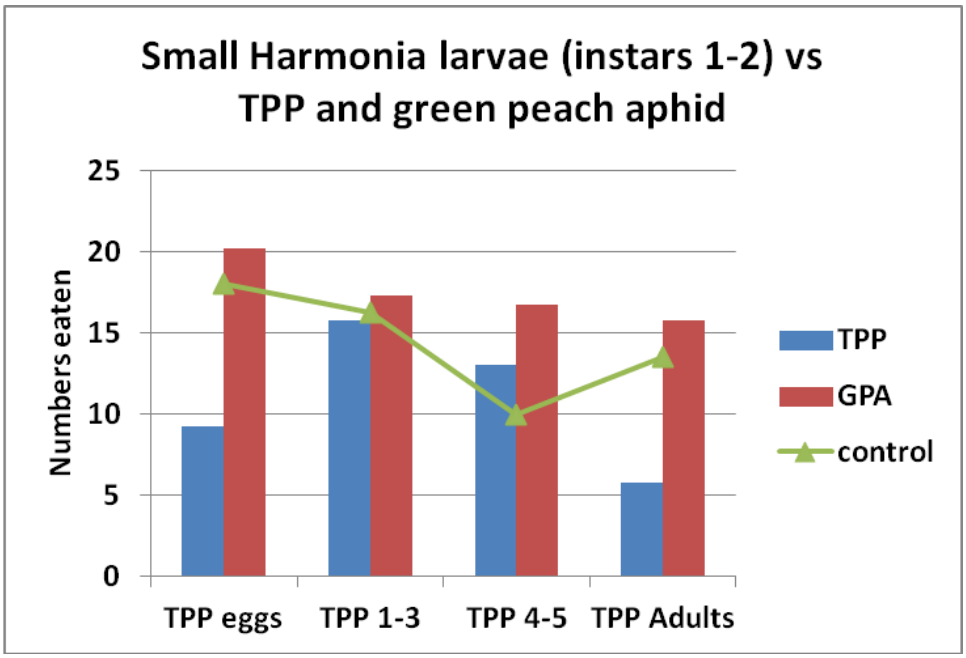


Figure 2-12 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for small *Harmonia conformis* (instars 1-2)

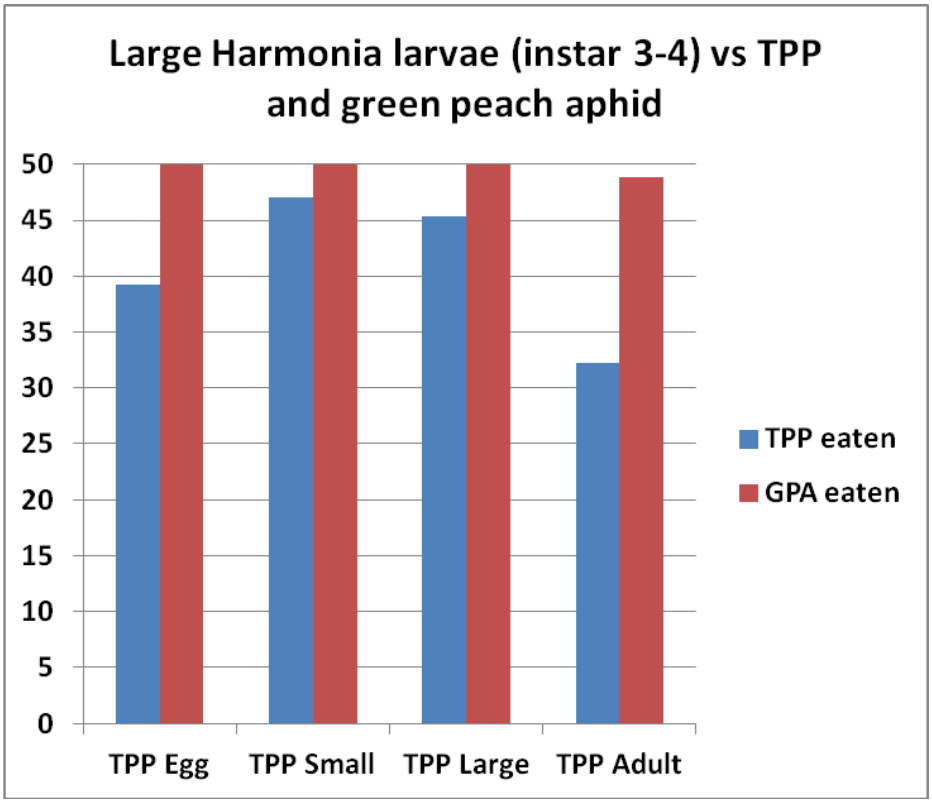


Figure 2-13 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for small *Harmonia conformis* (instars 3-4)

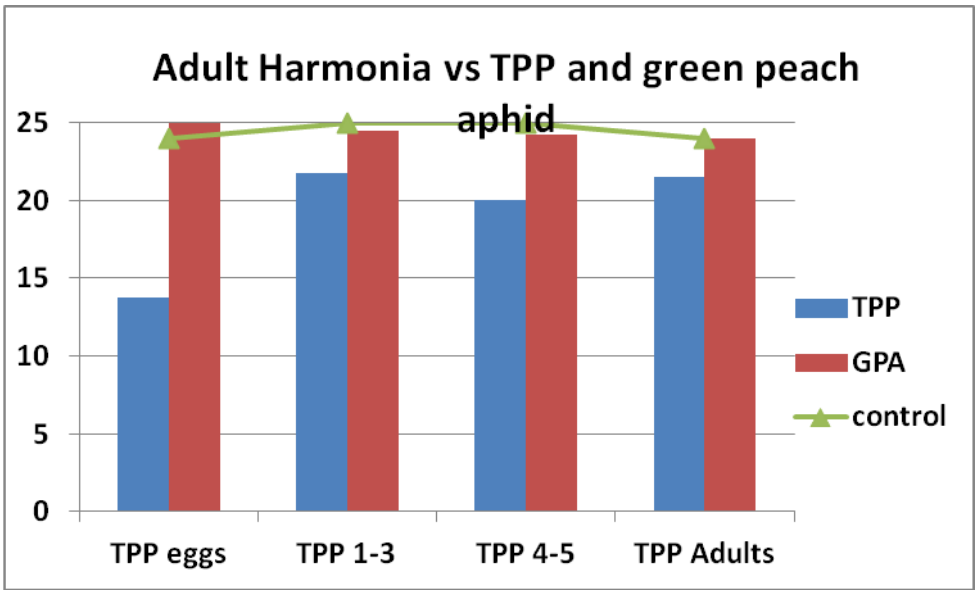


Figure 2-14 – Choice testing (tomato potato psyllid (TPP) vs. green peach aphid (GPA)) for adult *Harmonia conformis*

DRAFT IPM STRATEGY

From the laboratory trials we were confident that beneficial species were present that could eat potato psyllid. We needed to improve the cultural and chemical components as well before the biological controls could be used.

Two growers in Canterbury who agreed to the trial being run in their crops agreed to plant grass strips or else leave existing grass in place. In at least one of the sites, flowering weeds also grew, in addition to the grass. In this case the presence of flowering weeds was useful, but that may not always be so. Planting cereals alone without the presence of weeds, especially solanaceous weeds, would be a safer practice.

The chemicals used in the New Zealand trial included “Oberon”, which is a product similar to “Movento” (it is unlikely that “Oberon” will be registered in Australia for commercial reasons). Other products such as “Chess”, “Success” and “Movento” are already registered for use on potato crops in Australia, but of course there is no registration for use on potato psyllid as it has not yet been detected in Australia.

TRIALS IN ENTIRE COMMERCIAL CROPS

Brown lacewings and hoverflies were the most abundant predators in the trial sites, and often recorded as eggs (see below).

Table 2-1 – Total lacewings and hoverflies at trial sites (BLW = Brown lacewing)

Total Brown Lacewings and Hoverflies

BLW Adults	BLW Eggs	BLW Larvae	Hoverfly Adults	Hoverfly Eggs	Hoverfly larvae
8	160	1	2	558	21

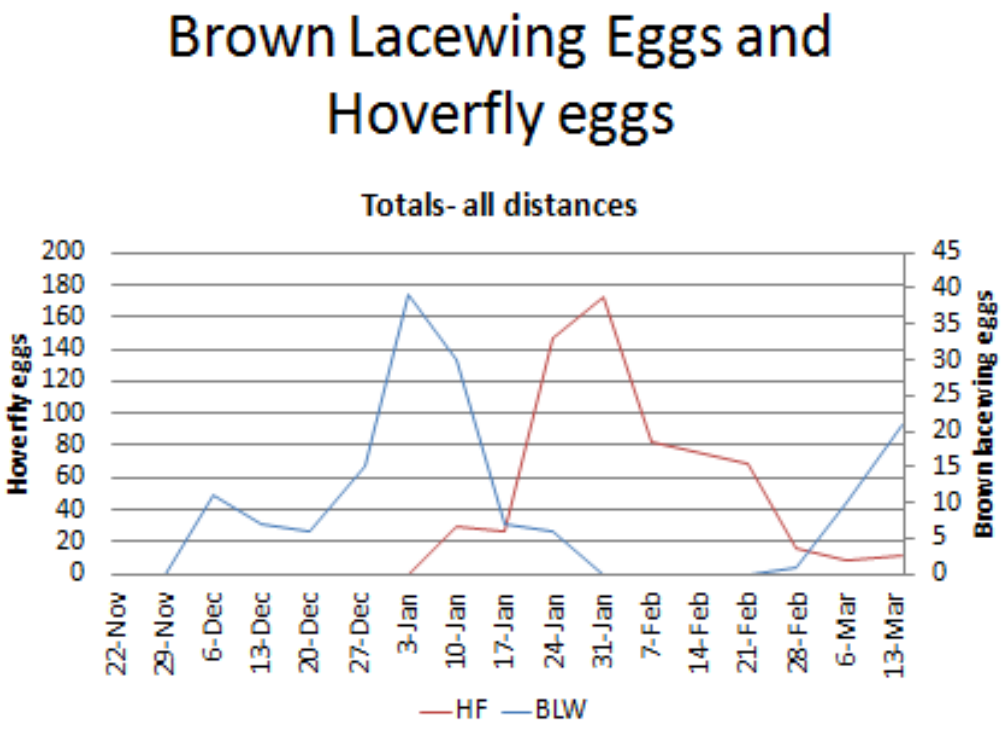
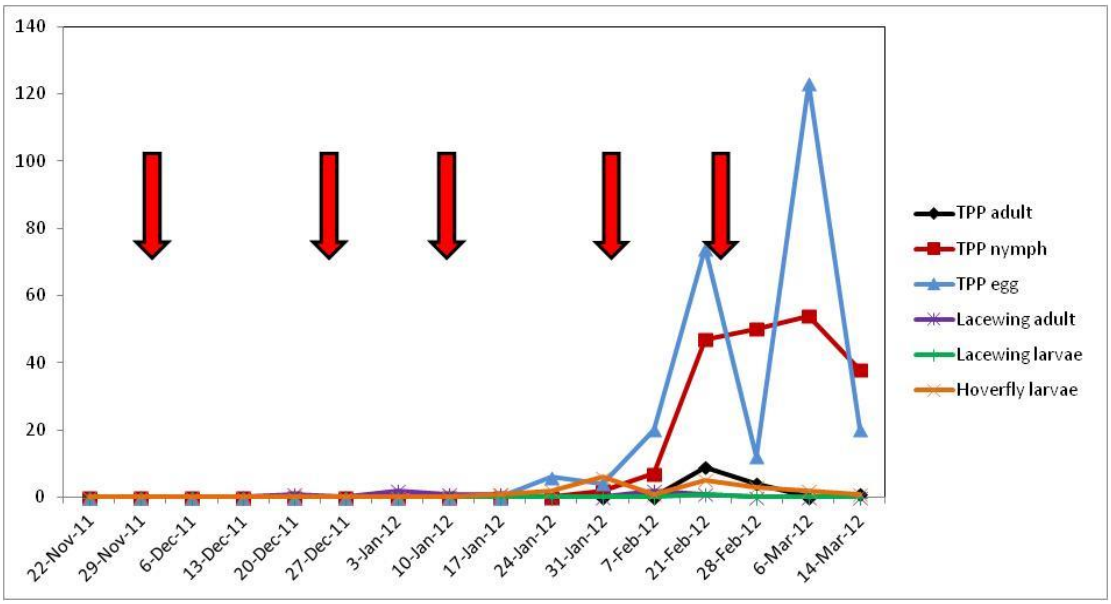


Figure 2-15 – Brown lacewing eggs and hoverfly eggs (numbers were recorded at various distances into the crop but were pooled for this analysis)

The numbers of psyllid (TPP) adults, eggs and nymphs at the two trial sites is shown in the two graphs below (Figure 2-16 and Figure 2-17), with the arrows indicating the application of border sprays of insecticide. A calendar-based spray programme was implemented using chemicals from different groups to minimise resistance, applied only as a border spray and most importantly, we selected products which would have minimal impact on the key beneficial species. They were applied when weather conditions and other practices (eg. Irrigation) permitted and were not based on any thresholds relating to potato psyllid. There was no statistical correlation with numbers of beneficials and distance from the grassy strips.

Trial 1

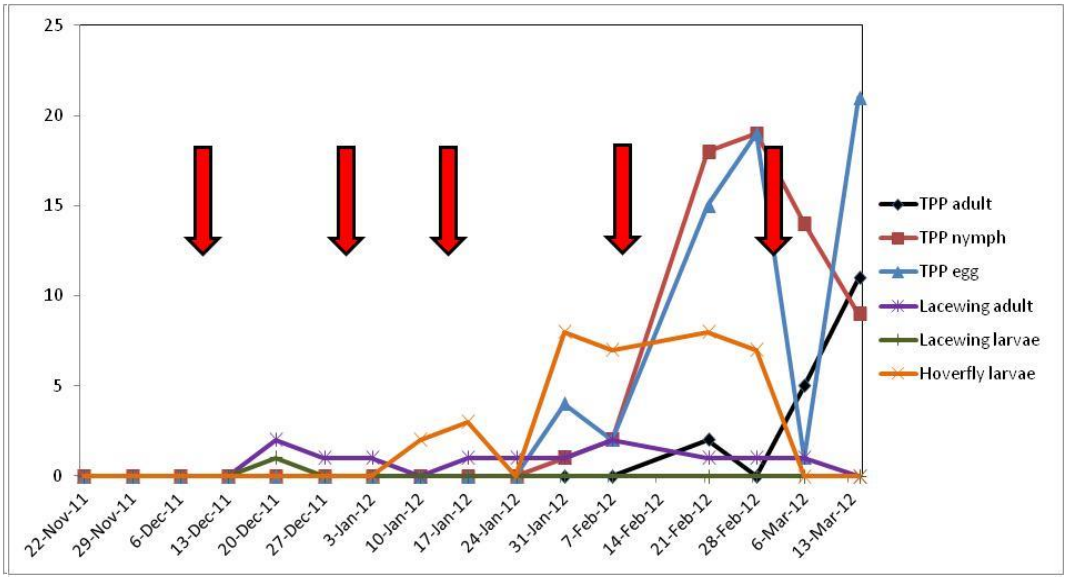


Crop data
Planting: 8 October 2011
Grass edge: 30 Oct 2011
Harvest: 25 March 2012

Spray calendar
8 Oct – Actara
1 Dec – Chess (whole field)
27 Dec – Movento (headland <18m)
14 Jan – Movento (headland <18m)
6 Feb – Oberon (headland <18m)
23 Feb – Oberon (headland <18m)

Figure 2-16 – Numbers of psyllid (TPP) adults, eggs and nymphs - trial 1. Arrows indicate the application of border sprays of insecticide.

Trial 2



Crop data
Planting: 4 October 2011
Grass edge: not cultivated from last crop
Harvest: 25 March 2012

Spray calendar
4 Oct – Actara
9 Dec – Chess (whole field)
30 Dec – Chess (whole field)
16 Jan – Movento (headland <32m)
8 Feb – Oberon (headland <32m)
3 March – Success (headland <32m)

Figure 2-17 – Numbers of psyllid (TPP) adults, eggs and nymphs - trial 2. Arrows indicate the application of border sprays of insecticide.

In the sequence of graphs below (Figure 2-18 – Figure 2-21), all have the same Y-axis (total psyllid number found) to allow comparison, and it is easy to see that although many psyllid eggs were found late in the life of the crop, these did not develop through to adults. The combination of biological and chemical controls was enough to prevent them establishing.

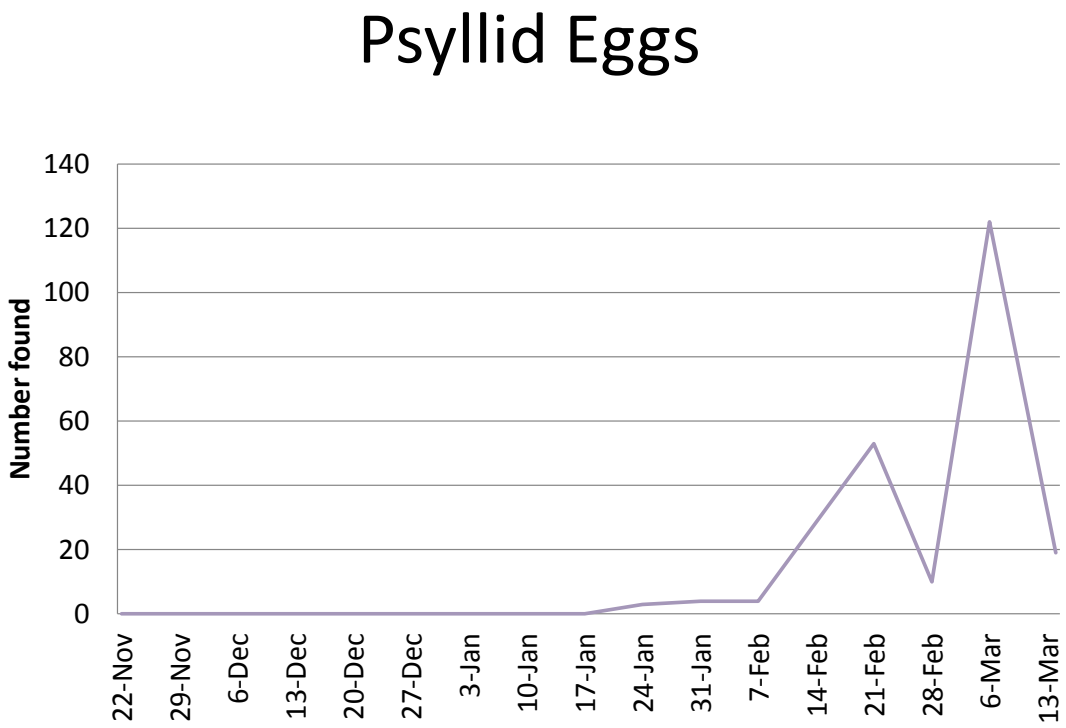


Figure 2-18 – Numbers of psyllid (TPP) eggs

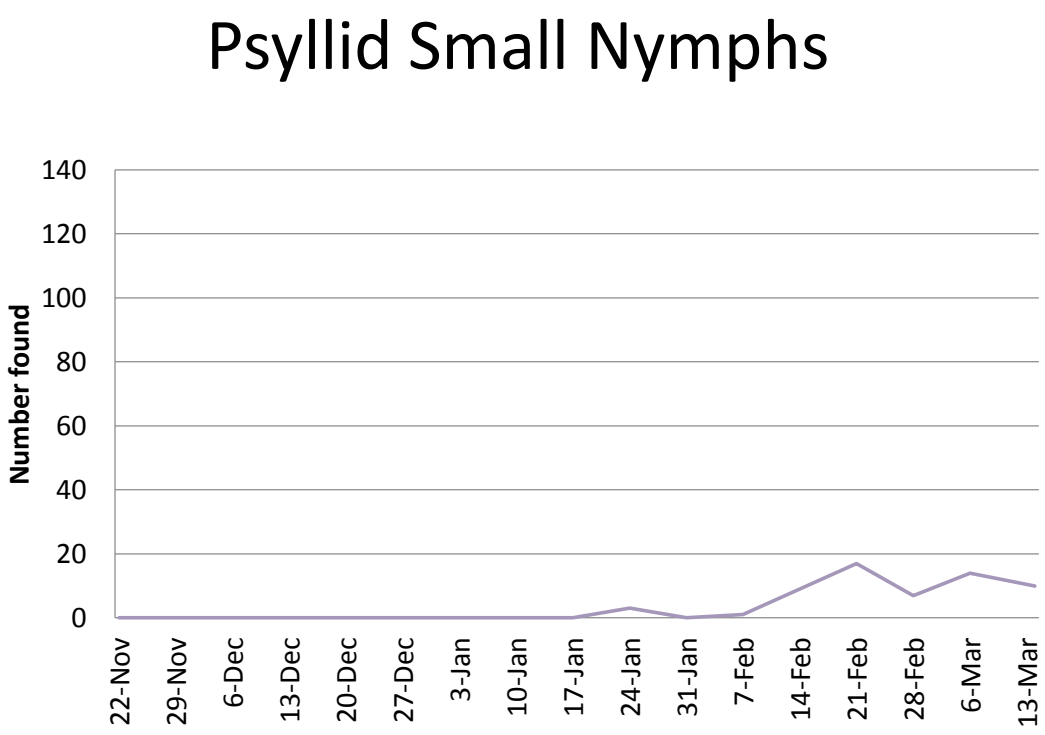


Figure 2-19 – Numbers of small psyllid (TPP) nymphs

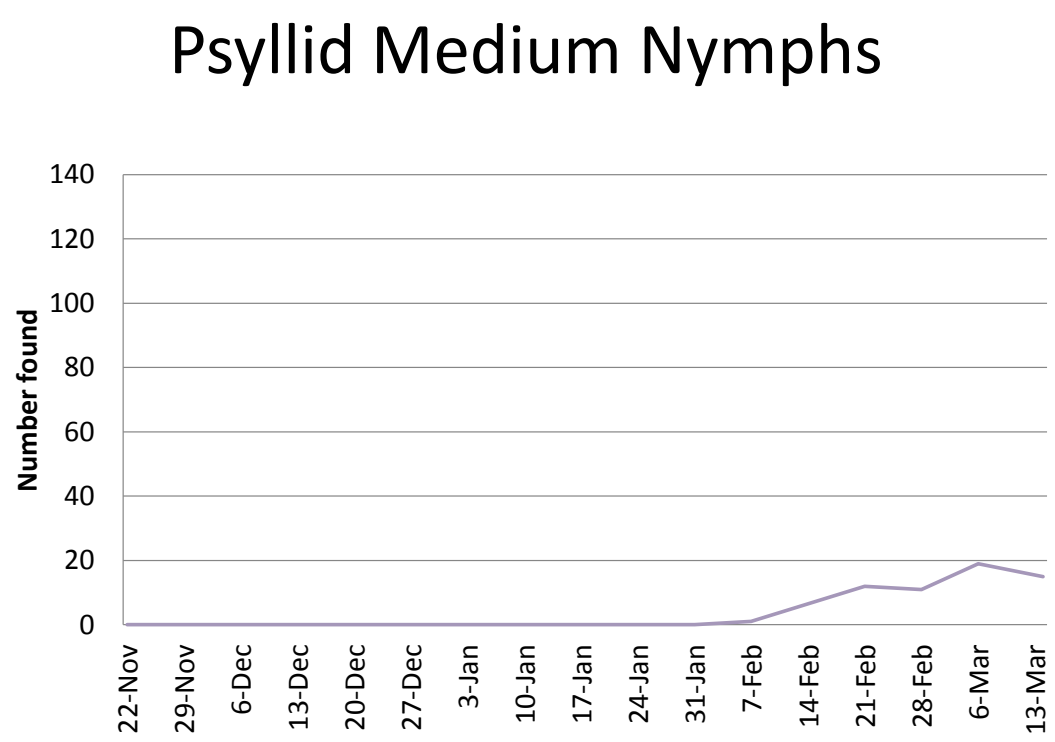


Figure 2-20 – Numbers of medium psyllid (TPP) nymphs

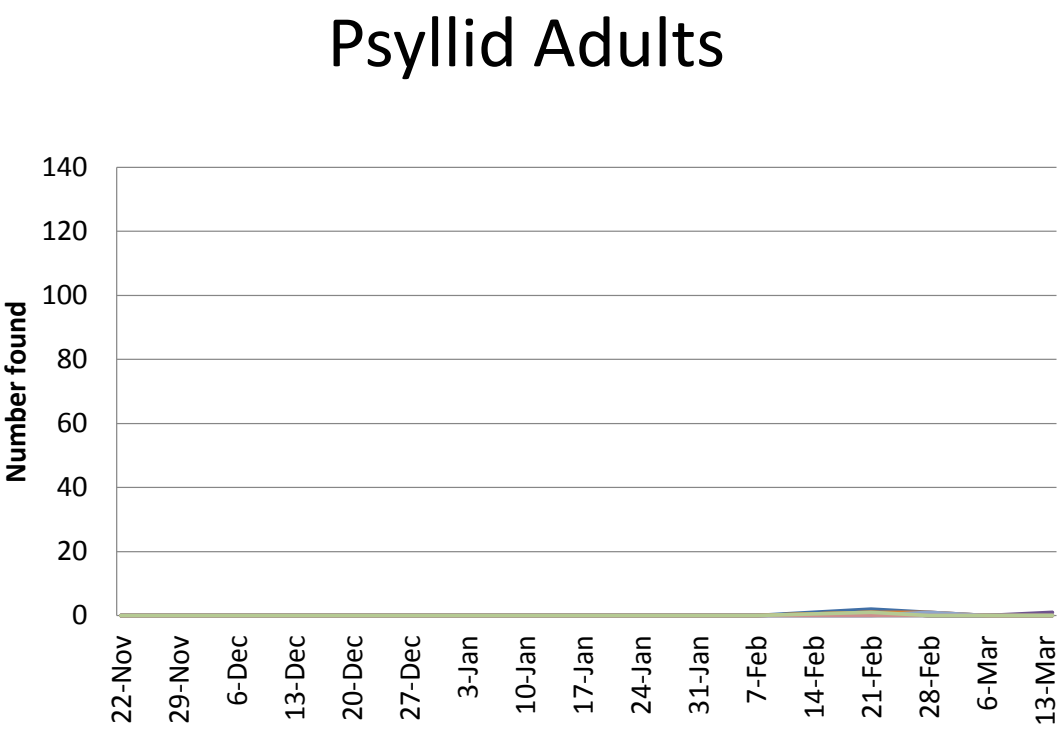


Figure 2-21 – Numbers of adult TPP

These results indicate that the total strategy (not just individual components) was effective in giving control that was satisfactory to the farmer and the processor. That is, the results achieved using this approach were equal to that by growers relying only on frequent insecticide applications.

There is a perception that the pressure from potato psyllid in the South Island is extremely low, and some believe that Liberibacter is found only in the North Island. However, Nina Jorgensen and Andy Pitman from Plant & Food Research visited sites in Canterbury near these study sites following the conclusion of this project field sampling and collected potato psyllids that were definitely carrying Liberibacter. This is locally referred to as the psyllids being "hot" with the disease.

In addition to the results presented here, Appendix 1 contains a summary of the related work conducted by Plant & Food Research and Potatoes New Zealand that comprise the basis for the voluntary contribution to this project.

DISCUSSION AND CONCLUSIONS

An IPM strategy was developed during the 1980's and 1990's in Australia that involves minimal use of insecticides, and many growers able to use no insecticides. This strategy and rates of adoption are described by Horne and Page (2008). It can be summarised as set out in the table below for most of the major pests. In the following tables, PTM = potato tuber moth; WFT = western flower thrip; TSWV = tomato spotted wilt virus.

Table 2-2 – Outline of current IPM management strategies for potato in Australia

IPM in Australian Potato Crops

Pest	Beneficial 1	Cultural 2	Chemical (3)
PTM	*Orgilus lepidus* *Apanteles Copidosoma* Nabid bugs	Overhead Irrigation Soil management	Nil (Spray after Senescence)
Aphids (Virus)	*Aphidius* Ladybirds Brown lacewings	Certified seed Weed management	Nil (Seed dressing)
Thrips (TSWV)	Predatory thrips Predatory mites	Control volunteers Variety/ Location Weed control	Nil (Seed dressing) (Spinosad)
Caterpillars	Nabid bugs Pentatomid bugs Parasitic wasps	-	BT or GemStar*

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This strategy relies on biological and cultural controls to deliver most of the pest management and selective chemicals are used very much as the support tools. This strategy has been used for over 16 years now by some farmers in Victoria (Case study – Wayne Tymensen, pp. 95 – 97 in Page and Horne 2012).

However, if potato psyllid is added to the list of pests, and the type of chemical control used (prior to this project) in New Zealand and the USA is applied here, then the situation could then be described as follows:

Table -2-3 – Impact of the heavy use of insecticides to control psyllid on current IPM management strategies in Australia*.

Impact of Potato Psyllid?

Pest	Beneficial	Cultural	Chemical
Potato Psyllid	????	???	Foliar Sprays
Potato Moth	Gone	Overhead Irrigation Soil management	Nil?? (Spray after Senescence)
Aphids	Gone	Certified seed Weed management	Nil?? (Seed dressing)
Thrips - WFT	**Gone**	Control volunteers Variety Weed control	Nil?? (Seed dressing)
Caterpillars	Gone	-	BT or GemStar*

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*Note the size of the fonts in Table -2-3 is an attempt to graphically illustrate the relative likely problems with different pests if the heavy insecticide programme used in New Zealand and USA is followed in Australia. The larger the font the larger the likely problem. So in this scenario, problems with western flower thrips, which is resistant to all insecticides and transmits tomato spotted wilt is likely to become a serious pest.

What this illustrates is that growers will have lost the biological control agents for all pests, including aphids and western flower thrips, which vector viral diseases of potatoes. At the commencement of this project we did not have much idea of the potential biological control agents or cultural control options for tomato potato psyllid.

This project aimed to develop an IPM compatible set of options and test them urgently in New Zealand, hopefully before the psyllid arrived in Australia. As a result, we developed the strategy outlined in the following table, and it was basically this strategy that was used by growers in Canterbury in the 2011-12 season.

The trial needed to be done on an entire paddock scale and could not be done in small plot trials. Although there is work done in NZ and reported on elsewhere in this report about small plot insecticide trials, we think that these trials are inappropriate for assessing the impact of beneficial species, and so we do not agree with some of the conclusions made by our NZ colleagues. The impact of the movement of beneficial species into, but not back out of, insecticide-treated areas means that there can be no meaningful conclusions made on the value of beneficial species from such trials.

Similarly, no assessment can be made where non-selective insecticides (such as abamectin) are applied in larger scale trials.

There is a commonly held perception in New Zealand that abamectin ("Vertimec" or "Avid") is safe on beneficial species. However, our own testing and field observations suggest that it is not safe and so it was not included in our trials (data not presented). There are data available from sources such as Koppert and Biobest in Europe which give relative rankings of this product, as does our own testing at IPM Technologies.

Table 2-4 – Current IPM management strategies for the potato industry including psyllid

Draft IPM Strategy

Pest	Beneficial 1	Cultural 2	Chemical (3)
Potato Psyllid	**Damsel Bugs Brown Lacewings Ladybirds Hoverflies**	**Border strip plantings?**	**Border Sprays Seed Dressing *Chess* *Movento* *Success***
PTM	*Orgilus lepidus* *Apanteles* *Copidosoma* Nabid bugs	Overhead Irrigation Soil management	Nil (Spray after Senescence)
Aphids (Virus)	*Aphidius* Ladybirds Brown lacewings	Certified seed Weed management	Nil (Seed dressing)
Thrips (TSWV)	Predatory thrips Predatory mites	Control volunteers Variety/ Location Weed control	Nil (Seed dressing) (Spinosad)
Caterpillars	Nabid bugs Pentatomid bugs Parasitic wasps	-	BT or GemStar*

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This strategy was tested with successful results on the two farms that collaborated in this trial on the South Island of NZ. Figure 2-22 shows a group of people from various sectors of the NZ potato industry attending a field day held at one of the sites used in this project. The two crops were harvested with the yield and quality required. The results have prompted the Agricultural Manager for McCain Foods (NZ) to comment that he would like to encourage all potato growers supplying McCain in New Zealand to use the IPM approach (John Jackson, personal communication).

Adoption of IPM is often regarded as slow (e.g. Bajwa and Kogan 2003), but this project has shown that by working in collaboration with farmers and other advisors in what can be called participatory research, IPM adoption can be rapid (Herbert 1995; Horne, Page and Nicholson 2008). In the course of this 3 year project we have managed to test an IPM strategy in commercial potato crops where at the commencement of the project the only recognised method of control was frequent application of insecticides. We expect that potato growers in Canterbury will be using this approach on all of their crops in the forthcoming season.

No trials were conducted in the North Island where the pest pressure from potato psyllid is much higher. This was despite offers of assistance from IPM Technologies P/L, Simplot and some private advisors. This reflects the extreme threat that is perceived by North Island growers that they were not willing to try any other method of control other than heavy use of insecticide.

The numbers of potato psyllid found in the South Island are certainly lower than areas in the North Island such as Hawkes Bay and Pukekhoe. This has led some to believe that there is little risk of damage by psyllids in Canterbury, or even that there is no Liberibacter in the South Island. Both of these points are incorrect. There are enough psyllids present, containing the disease, to cause crop losses and the presence of zebra-chip. There is also a perception by some that the work carried out in the South Island would not work in the North Island. We did not get the opportunity to test this in the field during the project, but the fact that the combination of biological, cultural and chemical controls used here were successful in controlling the pest and the disease when both were present suggests that it is certainly a potential strategy for other areas, including the North Island and Australia, if/when required.

The value of the grassy strips was not conclusive in terms of increased numbers of beneficials along the edge. We found no direct correlation between increased levels of any predatory species with distance from the strips. However, the flowering weeds that occurred as well as grass in these strips almost certainly provided a positive impact on hoverflies by supplying a nectar source for adult flies. This would have increased longevity and fecundity of individuals, but because of their mobility and the design of this trial, there was no way to accurately measure this. At this stage we think that more work will be required before it could be concluded that there were definite advantages in using such strips to assist with control of psyllids.

Figure 2-22 – A Field day held at harvest at one of the two sites used in this project.

A strategy has been developed and implemented successfully in New Zealand potato crops, which is compatible with the IPM strategy that has been developed and used by Australian potato growers for many years.

Appendix 1 of this report contains summaries of the work that has been done separately by Plant & Food Research (PFR). This work has provided a great deal of very useful information but is obviously a different approach than that taken in this project. Also, the interpretation by the PFR researchers involved of some of the results of that work, is not the same as that of IPM Technologies researchers (Paul Horne and Jessica Page) who led this project. In particular, there has been reporting at several conferences and in several publications on the basis of their field trials that the biological control of potato psyllid is insufficient in summer. While this may appear to be so from the data presented, our conclusion is that, in these trials, the impact of insecticides such as abamectin was not considered disruptive (we believe it is disruptive) to predators, and the trials were small plot trials, not whole paddocks, and so the impacts of movement of beneficial species into and out of different treatments were also not adequately considered.

The results reported in this project differ from the PFR trials in that no disruptive insecticides were applied in this project and so the full impact of beneficial species was possible. Similarly, the trials were conducted in entire paddocks and so there was no impact of disruptive insecticides on nearby, internal or adjacent areas.

CONCLUSION

The project has concluded successfully with the original aims all met. The challenge for us was to achieve practice change in another country in order to test our draft strategy, and this has been done. As can be seen by the work reported on in Appendix 1 and Appendix 2, this outcome would not have been achieved by this time if this project had not been proposed and supported by Australia. Australian potato growers can confidently expect that an IPM alternative to deal with potato psyllid is available when and if it arrives.

COMMUNICATIONS/ EXTENSION

Results of this project have been communicated to industry throughout the course of the project by way of articles in the industry newsletter, field days and talks. The following table 5 outlines the communication activities delivered through the course of the project:

Table 2-5 – IPM Technologies PT09004 communication activities

Activity	Impact for Processing Potato Industry
Talk presented: Dr Paul Horne, Zebra Chip Summit 16th December, 2009 – Melbourne	Highlight to the Australian potato industry the opportunities of using an IPM approach to control potato psyllid.
Talk presented: Dr Paul Horne, NZ Potatoes R & D Meeting, March 17, 2010, Wellington	Raise awareness of the opportunities to use IPM approach to manage psyllid in order to identify suitable commercial sites to trial an IPM approach in New Zealand.

Talk presented : Dr Paul Horne to PPIAC, 2010, Hobart	Industry provided with project update
Interview with NZ Potato Industry for their journal, 2010	Awareness that another option to full pesticide applications is possible. The field testing of our proposed strategy could only be done in New Zealand at this stage and so we needed the co-operation and collaboration of New Zealand potato growers
Potatoes Australia article: 'Pre-empting the potato psyllid through IPM strategies', August 2011	Industry provided with project update
Talk presented: Canterbury potato growers and industry. June 2011' Ashburton, NZ.	Awareness that another option to full pesticide applications is possible. The field testing of our proposed strategy could only be done in New Zealand at this stage and so we needed the co-operation and collaboration of New Zealand potato growers.
Discussions with McCain NZ agronomists June 2011, Timaru, NZ.	Awareness that another option to full pesticide applications is possible. The field testing of our proposed strategy could only be done in New Zealand at this stage and so we needed the co-operation and collaboration of New Zealand potato growers.
Discussions with Plant & Food Research entomologists, Lincoln, NZ. July 2011	Awareness that another option to full pesticide applications is possible. The field testing of our proposed strategy could only be done in New Zealand at this stage and so we needed the co-operation and collaboration of New Zealand potato growers.
Talk presented: New Zealand Plant Protection Society Conference, Rotorua (Special Session on Potato Psyllid). August 2011	Awareness that another option to full pesticide applications is possible. The field testing of our proposed strategy could only be done in New Zealand at this stage and so we needed the co-operation and collaboration of New Zealand potato growers.
Talk presented: Talk to McCain Conference (Australia and NZ) at Ballarat, Australia August 2011	Industry provided with project update
Workshop on IPM with key potato growers. Ballarat, Australia August 2011	Industry provided with project update
Field Day (NZ), Canterbury, March, 2012	Paul, Just a note to say thanks for the Field day yesterday, very interesting and informative on what has been achieved in such a short time. John Jackson Agricultural Manager McCain Foods (NZ) Limited Timaru, New Zealand
Talk presented: Seed Potatoes Victoria - Potato Industry Conference, July, 2012	Project results presented and discussed, Australian industry have greater awareness for psyllid control options

Talk presented: NZ Psyllid Conference, July 2012, Auckland.	Project results presented and discussed providing opportunity for peer discussion and raise awareness of the success of IPM to an Australian and NZ audience
Extension project PT11006 (Field Days held in Gippsland and northern Tasmania in 2012)	This project prompted another proposal to the processing potato industry which was supported by industry and HAL called "Preparing for Potato Psyllid" (Project PT11006). This project has been completed and involved increasing awareness of beneficial species in Australian potato crops.

RECOMMENDATIONS

This report and the strategy that has been developed can now form the basis for control of potato psyllid in Australia for those growers wanting an alternative to regular and heavy use of insecticides.

We also recommend that the strategy would be improved upon, when the psyllid is found in Australia, by looking at other predators found in Australian potato crops that do not occur in New Zealand.

In order to be able to use this strategy for control of potato psyllid, the industry needs to be aware of the limitations on insecticide applications that will be required (for any pest). More detailed awareness-raising workshops or training will be required for both growers and their advisors.

The next steps in preparing Australia for the potential arrival of psyllid are:

1. Make sure that the entire potato industry is aware that an IPM strategy has been developed based on the findings from project PT09004
2. Strongly recommend growers use IPM for existing pests so that they are prepared should potato psyllid arrive in Australia
3. Update Plant Health Australia, peak industry bodies and potato industry experts on the success of IPM in managing psyllid so that this research can be considered for any future review of the zebra chip/potato psyllid disease response plan.

ACKNOWLEDGEMENTS

This work would not have been possible without the support of Duncan McLeod (Seed and Field, SI) and farmers Danny Lovett and Ross Hewitt.

Many people from Plant & Food Research were involved in different aspects of the project, but we thank Nina Jorgenson in particular for her excellent work in the final year field stage. We also thank Nadine Berry, David Teulon, Graham Walker, Robin Gardner-Gee, Ngaire Larson and Frances McDonald, (Plant & Food Research).

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Horne, P.A., Ridland, P.M. and New. T.R. (2001) *Micromus tasmaniae*: a key predator on aphids in field crops in Australasia. Pp388-394, In: "*Lacewings in the crop environment*".

McEwen, P., New, T.R. and Whittington, A.E. (eds) Page, J. and Horne, P.A. (2012). *Controlling invertebrate pests in agriculture*. CSIRO Publishing. 116 pp.

Horticulture Australia

PT09004 – Appendix 1

Plant & Food Research New Zealand

PROJECT SUMMARY

PT09004 – Appendix 1

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PURPOSE OF THE REPORT:

This project has been co-funded from New Zealand and Australian sources, with the work being undertaken in New Zealand on tomato potato psyllid (TPP) and the bacterium *Candidatus* Liberibacter solanacearum (Liberibacter), that contribute to the physiological disorder of potato known as Zebra Chip. This report documents a range of areas of research, including crops other than potato. The knowledge from the project has direct benefits for Australia in assisting to better understand TPP and Liberibacter and has been included as an appendix to the IPM Technologies report as part of project PT09004 – *Control of Potato Psyllid Within an IPM Strategy* from the Australian Potato Research Program Phase 2 (APRP2).

2 November 2015

FUNDING SUPPORTERS

Contributions and funding for this program are gratefully acknowledged

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited and Potatoes New Zealand, and matched funds from the Australian Government.

DISCLAIMER

CONTENTS OF APPENDIX 1

MEDIA SUMMARY

The Plant & Food Research VC-funded research for the three years of the HAL-funded project included the following work:

- Laboratory studies on the efficacy of tomato potato psyllid (TPP) predators and parasitoids (additional to the three designated in the HAL project)
- Field studies on TPP on the efficacy of TPP predators
- Identifying host range of TPP and its associated bacterium *Candidatus* Liberibacter solanacearum (Lso)
- Presenting and publishing results from HAL and PFR funded research on TPP predators

This work has provided greater understanding of the impact of predators on TPP than the work funded in the HAL-funded research with respect to enabling additional predators to be studied in the laboratory as well as in additional field trials. Importantly, the PFR VC-funded work has enabled this work to be made available to a wider audience in New Zealand and Australia including industry, growers and scientists in the form of conference presentations (verbal and poster) and publications (abstracts and manuscripts).

TECHNICAL SUMMARY

MAIN FINDINGS FROM PUBLISHED RESEARCH PAPERS

- Berry N, Scott I, Thompson S, Beard S 2010. Detection of *Candidatus* Liberibacter solanacearum in trapped insects and non-crop plants in New Zealand. Proceedings of the 2010 Zebra Chip Annual Reporting Session: 159-163. Full paper & presentation.

  MAIN FINDING: Molecular screening of TPP DNA from sticky traps has shown that *Candidatus* Liberibacter solanacearum is present in all NZ monitoring locations over most monitoring periods. Molecular screening of non-crop plant DNA, from twelve survey sites throughout New Zealand, reveals that *Ca.* L. solanacearum has a much broader host range than previously believed. Further studies are required to determine the effect of *Ca.* L. solanacearum in TPP and non-crop host plants on acquisition, transmission and ensuing disease expression.

- Gardner-Gee, R 2011. Progress towards biological control of *Bactericera cockerelli* in covered crops in New Zealand. IOBC/WPRS Bulletin, Vol. 68, 41-45. Research paper & presentation.

  MAIN FINDING: A range of potential biological control agents for TPP has now been identified within New Zealand. More work is required however to establish which of the potential biological control agents are suitable for use within covered crops. If permission is gained to release the parasitoid *Tamarixia triozae* into New Zealand, this parasitoid will further expand control options in both field and covered crops.

- Walker GP, MacDonald FH, Fergusson H, Puketapu A, Wright PJ, Anderson J 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. New Zealand Plant Protection 65: 148-154. Research paper & presentation.

  MAIN FINDING: Tomato potato psyllid (TPP) and associated insects in the trial were monitored weekly using yellow sticky traps and sampling plants from mid-October until mid-December 2011. TPP adult catches remained very low, reaching 1.5 per trap per week in December, and egg and nymphal infestations were absent or very low. Other exotic psyllid species dominated

trap catches in December. The predator *Micromus tasmaniae* (brown lacewing) was the most common insect present throughout the trial. No damage was caused by TPP in any treatments indicating that insecticides may not be required to produce healthy 'early crop' potatoes at Pukekohe.

- Walker GP, MacDonald FH, Larsen NJ, Wallace AR 2011. Monitoring *Bactericera cockerelli* and associated insect populations in potatoes in South Auckland. New Zealand Plant Protection 64: 269-275. Research paper & presentation. Reported on in year 1 & 2.

  MAIN FINDING: Naturally occurring predators appear to be important biological control agents of aphids, small caterpillars and probably TPP on potatoes at Pukekohe.

MAIN FINDINGS FROM PUBLISHED ABSTRACTS

- Berry N, Madge D, Yen A 2011. The tomato psyllid: lessons from New Zealand for Australia. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.10. Abstract & presentation. Reported on in year 1 & 2.

  MAIN FINDING: This paper illustrates how Australia can gain valuable insights from New Zealand research on TPP, in particular: the developmental requirements of TPP life stages alternative plant, especially overwintering, hosts of the TPP and *Candidatus* Liberibacter solanacearum; and the potential impacts of a diverse Australian psyllid fauna and its associated natural enemies.

- Gardner-Gee, R 2012. "Importing answers": Options for classical biological control of TPP in New Zealand. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.21. Abstract & presentation.

  MAIN FINDING: *Tamarixia triozae* is the most common parasitoid of TPP in the USA, and its potential for use in both classical and inundative control programmes is under investigation both in New Zealand and in Mexico. Experiments have been finalised and discussion regarding its suitability in New Zealand are being held.

- Horne P, Vereijssen J and Jorgensen N 2012. Integrated control of potato psyllid. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.22. Abstract & presentation.

  MAIN FINDING: Predators were present in the potato crop when the first TPP arrived. Brown lacewings were present early in the season and they were replaced by hoverflies later in the season. Yellow sticky board seemed to better detect the first psyllids in the crop than plant assessments did.

- Horne P & Teulon D 2011. Strategic trans-Tasman collaborations enhance arable and vegetable IPM in Australia and New Zealand. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.9. Abstract & presentation. Reported on in year 1 & 2.

  MAIN FINDING: This paper emphasises the importance that strategic trans-Tasman collaborations, such as the HAL TPP project, enhances arable and vegetable Integrated Pest Management (IPM) in Australia and New Zealand.

- Jorgensen N, Taylor NM, Berry NA, Butler RC. 2011. Seasonal abundance of *Bactericera cockerelli* and beneficial insects in a potato crop in Canterbury. New Zealand Plant Protection 64: 292. Abstract & poster. Reported on in year 1 & 2.

  MAIN FINDING: In Canterbury 2010-11, the seasonal abundance of TPP and beneficial insects was monitored in a low-spray potato crop using yellow sticky traps and in-crop plant assessments. The brown lacewing (*Micromus tasmaniae*) was the most abundant beneficial

insect encountered, followed by ladybird species (*Adalia bipunctata* and *Coccinella undecimpunctata*).

- Larsen N.J., MacDonald F.H., Connolly P.G., Walker G.P. 2011. Could *Harmonia conformis* be an important predator of *Bactericera cockerelli*? New Zealand Plant Protection 64: 293. Abstract & poster. Reported on in year 1 & 2.

  MAIN FINDING: In confined no-choice cage tests in the laboratory, it was established that all life stages of *Harmonia conformis* readily eat all life stages of TPP. Additionally, larvae and adults of *H. conformis* ate all life stages of TPP, even when other food (green peach aphid) was available.

- MacDonald F.H., Walker G.P., Larsen N.J., Wallace A.R. 2010. Naturally occurring predators of *Bactericera cockerelli* in potatoes. New Zealand Plant Protection 63: 275. Abstract & poster. Reported on in year 1 & 2.

  MAIN FINDING: Together with separate data on predator incidence, these results suggest that naturally occurring predators are likely to be important biological control agents of TPP, particularly early in the season when TPP infestations are low.

- Walker GP 2012. Biological control of TPP by existing natural enemies (lab and field studies) and developing IPM tools for TPP (at Pukekohe). Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.20. Abstract & presentation.

  MAIN FINDING: Results indicate that all predatory life stages of brown lacewing, small hover fly, 11-spotted ladybird beetle, damsel bug and sheet-web spider predate on all life-stages of TPP, even in the presence of aphids, although TPP eggs are less preferred. In early crop trials, trap catches were below an economic injury level, indicating that early crops may be grown without insecticides. The 2011-12 main crop summer trial, where the use of an action threshold based on increasing trap catches of TPP led to a 50% reduction in insecticide use with 1% ZC, whereas untreated potatoes exhibited 34% ZC symptoms.

- Walker G 2011. Towards developing an IPM programme for potatoes in New Zealand; the role of natural enemies. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.11. Abstract & presentation. Reported on in year 1 & 2.

  MAIN FINDING: Weekly monitoring and spring and summer field trials show that there are existing biological control agents (BCAs) that give substantial control of all pests in potatoes. However, two summer generations of TPP from January to April cause major crop losses. Lower populations of TPP in cooler regions provide an opportunity for naturally occurring BCAs to give longer-lasting control of TPP. Laboratory choice and no-choice studies indicate that all life-stages of the common predators, brown lacewing, small hover fly, 11-spotted ladybird and nabids are capable of consuming all life stages, and that they consume large numbers of TPP.

INTRODUCTION

Here we report Plant & Food Research VC (PFR VC) funded research achievements. Tomato potato psyllid (TPP) and its associated bacterium *Candidatus* Liberibacter solanacearum (Lso) have not been detected in Australia to date and this research serves to provide a better understanding of the vector and pathogen in New Zealand. If TPP and Lso were found in Australia, this research will assist Australian growers to better understand the psyllid and bacterium and the disease it causes.

Plant & Food Research VC funded research consisted of:

- Laboratory studies on the efficacy of TPP predators and parasitoids (additional to the three designated in the HAL project)
- Field studies on TPP and on the efficacy of TPP predators
- Identifying host range of TPP and its associated bacterium Lso
- Presenting and publishing results from HAL and PFR funded research on TPP predators

MATERIAL AND METHODS

This project covered a wide range of research areas, each with a number of discrete laboratory and/or field trials. Descriptions of the trial methodologies are provided in the scientific/conference papers and client reports referenced under the Technology Transfer section.

RESULTS, DISCUSSION & RECOMMENDATIONS FOR PFR VC FUNDED RESEARCH

PUBLISHED RESEARCH PAPERS

- Berry N, Scott I, Thompson S, Beard S 2010. Detection of *Candidatus* Liberibacter solanacearum in trapped insects and non-crop plants in New Zealand. Proceedings of the 2010 Zebra Chip Annual Reporting Session: 159-163.

  Abstract. The impact of the Tomato Potato Psyllid (*Bactericera cockerelli*) and its associated pathogen, *Ca.* L. solanacearum has threatened the future production of potatoes, tomatoes, capsicums and tamarillos in New Zealand. Seasonal variation in the incidence of *Ca.* L. solanacearum in TPP and non-crop plants may provide essential information to aid in pest management decisions. In the current study, molecular screening of TPP DNA from sticky traps has shown that *Ca.* L. solanacearum is present in all NZ monitoring locations over most monitoring periods. Molecular screening of non-crop plant DNA from twelve survey sites throughout NZ, reveals that *Ca.* L. solanacearum has a much broader host range than previously believed. Further studies are required to determine the effect of *Ca.* L. solanacearum in TPP and non-crop host plants on acquisition, transmission and ensuing disease expression.

- Gardner-Gee R 2011. Progress towards biological control of *Bactericera cockerelli* in covered crops in New Zealand. IOBC/WPRS Bulletin, Vol. 68, 41-45.

  Abstract. *Bactericera cockerelli* is a North American pest species known in New Zealand as the tomato/potato psyllid (TPP). First reported in New Zealand in 2006, it has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand. Effective biological control agents are urgently needed to increase and improve control options for growers. Searches conducted within New Zealand have identified a number of psyllid predators that are potential biocontrol agents for TPP. In addition, in 2009 a North American parasitoid, *Tamarixia triozae*, was imported into quarantine facilities at Plant & Food Research, Auckland, for assessment as a biological control agent for TPP.

- Walker GP, MacDonald FH, Fergusson H, Puketapu A, Wright PJ, Anderson J 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. New Zealand Plant Protection 65: 148-154.

  Abstract. An early season potato trial at Pukekohe assessed the damage caused by *Bactericera cockerelli*, tomato-potato psyllid (TPP), and investigated the need for insecticide treatment. Four treatments were used: insecticide drench at planting; insecticide drench and weekly foliar

sprays; insecticide drench and threshold-based foliar sprays; and no insecticides. TPP and associated insects in the trial were monitored weekly using yellow sticky traps and sampling plants from mid-October until mid-December 2011. TPP adult catches remained very low, reaching 1.5 per trap per week in December, and egg and nymphal infestations were absent or very low. Other exotic psyllid species dominated trap catches in December. The predator, *Micromus tasmaniae* (brown lacewing) was the most common insect, present throughout the trial, peaking at a combined total of 6.6 eggs and adults per plant. No damage was caused by TPP in any treatments indicating that insecticides may not be required to produce healthy 'early crop' potatoes at Pukekohe.

- Walker GP, MacDonald FH, Larsen NJ, Wallace AR 2011. Monitoring *Bactericera cockerelli* and associated insect populations in potatoes in South Auckland. New Zealand Plant Protection 64: 269-275. Reported on in year 1 & 2.

  Abstract. *Bactericera cockerelli* (the tomato-potato psyllid; TPP) and associated insects were monitored weekly in unsprayed potatoes at Pukekohe by using yellow sticky traps and sampling plants from late July 2009 until mid March 2010. TPP adult catches and egg and nymphal infestations were absent or low until mid December. Other exotic and native psyllid species dominated trap catches until TPP populations increased markedly in mid January and peaked at 120 adults per trap in late February, with egg numbers reaching 520 per plant a week later. TPP nymphs peaked at 260 per plant in early February. *Micromus tasmaniae* (brown lacewing) was common in spring and summer, but *Melanostoma fasciatum* (small hover fly) became the dominant predator, peaking at 162 eggs and 35 larvae per plant in mid January. Naturally occurring predators appear to be important biological control agents of aphids, small caterpillars and probably TPP on potatoes at Pukekohe.

PUBLISHED ABSTRACTS

- Berry N, Madge D, Yen A 2011. The tomato psyllid: lessons from New Zealand for Australia. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.10. Reported on in year 1 & 2.

  Abstract. The exotic insect pest, tomato potato psyllid (TPP, *Bactericera cockerelli* (Sulc)) was first discovered in Auckland greenhouses and volunteer potatoes in 2006 and has spread rapidly throughout the North Island and into the South Island. To date the TPP has not been found in Australia, but is considered a high plant pest risk. In New Zealand the arrival of the TPP has significantly reduced the use of IPM in greenhouse crops and outdoor tomato and potato crops. In Australia, IPM practices are commonly used for the control of pests such as aphids and the potato tuber moth. The arrival of the TPP would severely impede the use of these practices. Australian researchers aim to gain a better understanding of the TPP/*Candidatus* Liberibacter solanacearum complex in New Zealand to enable development of a number of tools to combat an incursion into Australia. Australian researchers are anticipating an incursion by the TPP and have therefore developed collaborative research projects and an exchange of ideas with New Zealand researchers.

  New Zealand research to date includes: use of and mode of action of insecticides, national monitoring, diagnostic protocols and techniques, biological control, insect/pathogen/plant interactions, transmission, temperature effects on development and biology, alternative vectors and use of alternative host plant species, especially overwintering hosts. Australian research has involved preparation of diagnostic protocols for TPP and testing the effectiveness of different TPP surveillance techniques. Australia can gain valuable insights from New Zealand research, in

particular: the developmental requirements of TPP life stages alternative plant, especially overwintering, hosts of the TPP and *Ca.* L. solanacearum; and the potential impacts of a diverse Australian psyllid fauna and its associated natural enemies.

- Gardner-Gee, R 2012. "Importing answers": Options for classical biological control of TPP in New Zealand. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.21.

  Abstract. The presence of TPP in New Zealand has disrupted existing integrated pest management programmes, and necessitated an increase in insecticide use on vulnerable crops (especially tomatoes and potatoes). Effective biological control agents are urgently required to increase and improve grower options in solanaceous crops. Since 2006 Plant & Food Research has worked with the New Zealand horticultural industries to indentify potential BCAs, both within New Zealand and overseas. In this presentation, overseas research into biological control of TPP will be reviewed. Potential candidates for use in New Zealand are examined, with particular emphasis on the parasitoid *Tamarixia triozae*. This parasitoid has been found in many areas of the USA (Arizona, California, Colorado, Idaho, Kansas, Montana, New Mexico, and Washington) and more recently in Mexico. *Tamarixia triozae* is the most common parasitoid of TPP, and its potential for use in both classical and inundative control programmes is under investigation both here in New Zealand and in Mexico.

- Horne P, Vereijssen J and Jorgensen N 2012. Integrated control of potato psyllid. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.22.

  Abstract. This presentation describes the culmination of a 3-year Horticulture Australia Limited project which aimed to develop an approach to controlling tomato-potato psyllid using methods that would be compatible with the IPM approach already developed and implemented in Australian potato crops. The IPM strategy in Australia involves integrating the use of biological, cultural and compatible chemical control measures. Therefore, this project conducted in New Zealand assessed biological controls in the laboratory, cultural controls in the field, and the use of strategic, selective insecticides in commercial crops. Laboratory trials were conducted in Auckland and the final field trials were conducted in Canterbury in the 2011–12 season. We present the successful results of a commercial assessment of the total IPM strategy, involving the use of all three control components and the overall result as assessed by farmers and processors. In particular, we present preliminary results on the value of border planting of grassy strips. The project was a collaboration between IPM Technologies from Australia, The New Zealand Institute for Plant & Food Research Limited, private New Zealand agronomists and farmers in Canterbury. It could not have been conducted without all parties working together.

- Horne P & Teulon D 2011. Strategic trans-Tasman collaborations enhance arable and vegetable IPM in Australia and New Zealand. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.9. Reported on in year 1 & 2.

  Abstract. The considerable similarities in pest and beneficial arthropod complexes in Australian and New Zealand arable and vegetable crop systems represent large opportunities for co-development of IPM Strategies in a range of crops between these two countries. This paper examines how strategic trans-Tasman collaborations can enhance arable and vegetable Integrated Pest Management (IPM) in Australia and New Zealand and serves as an introduction and overview of to a series of symposium papers on the same subject. To place the subject in perspective the major arable and vegetable (hectares, $$ returns) are reviewed for Australia and New Zealand to establish the relative importance of each crop in each country and to indicate where research collaborations might be worthwhile. Successful historic research collaborations that have contributed to the development of IPM in Australia and New Zealand

are reviewed and commonalities identified. The paper summarises some current initiatives where collaboration between Australia and New Zealand researchers have proved to beneficial to the development of arable and/or vegetable IPM in either country. It identifies current and potential sources of funding to drive research collaborations between Australia and New Zealand in the future and examines what aspects of IPM would especially worthwhile from Trans-Tasman collaborations (e.g. biosecurity threats, classical biological control, diagnostics for pests and diseases, registration of pesticides).

- Jorgensen N, Taylor NM, Berry NA, Butler RC 2011. Seasonal abundance of *Bactericera cockerelli* and beneficial insects in a potato crop in Canterbury. New Zealand Plant Protection 64: 292. Reported on in year 1 & 2.

  Abstract. The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), has been regarded as a significant pest of solanaceous crops in New Zealand since its discovery in 2006. Current management of TPP relies primarily on the use of insecticides. A better understanding of the role of beneficial insects in the control of TPP is needed to guide future Integrated Pest Management (IPM) strategies. In Canterbury 2010-11, the seasonal abundance of TPP and beneficial insects was monitored in a low-spray potato crop using yellow sticky traps and in-crop plant assessments. The brown lacewing (*Micromus tasmaniae*) was the most abundant beneficial insect encountered, followed by ladybird species (*Adalia bipunctata* and *Coccinella undecimpunctata*). Numbers of adult brown lacewings and ladybirds peaked on sticky traps in December 2010, while adult TPP numbers peaked in February 2011. Brown lacewing eggs were present on potato plants throughout the season, with the highest numbers found during January/early February 2011. Numbers of all life stages of TPP were highest on potato plants from mid January to the end of the monitoring period in mid February 2011 (crop spray-off).

- Larsen NJ, MacDonald FH, Connolly PG, Walker GP 2011. Could *Harmonia conformis* be an important predator of *Bactericera cockerelli*? New Zealand Plant Protection 64: 293. Reported on in year 1 & 2.

  Abstract. The tomato-potato psyllid (TPP), *Bactericera cockerelli*, is a new major pest of solanaceous plants in New Zealand. Both nymphs and adults may cause damage to plants by feeding on leaves and stems, and by also transmitting a bacterial pathogen, *Candidatus* Liberibacter solanacearum. TPP infestations affect the quality and yield of important crops such as potatoes, tomatoes, capsicums and tamarillo. Existing natural enemies of insect pests in these crops may be important in controlling TPP, particularly in winter and spring when TPP populations are low. In confined no-choice cage tests in the laboratory, it was established that all life stages of *Harmonia conformis* readily eat all life stages of TPP. *Harmonia conformis* was tested in choice trials to assess predation of TPP when offered an alternative food source, green peach aphid (GPA), *Myzus persicae*. Predators were offered equal numbers of TPP and GPA, ranging from 25 to 50 per species. Larvae and adults of *H. conformis* ate all life stages of TPP, even when other food (GPA) was available. This study is part of a larger project, partly funded by Horticulture Australia (HAL), investigating the potential effectiveness of selected predators against TPP.

- MacDonald FH, Walker GP, Larsen NJ, Wallace AR 2010. Naturally occurring predators of *Bactericera cockerelli* in potatoes. New Zealand Plant Protection 63: 275. Reported on in year 1 & 2.

  Abstract. In recent intensive sampling of insecticide-free potatoes at Pukekohe, several insect predators and spiders have been found in association with the new pest *Bactericera cockerelli*,

tomato-potato psyllid (TPP). *Micromus tasmaniae* (brown lacewing), *Melanostoma fasciatum* (small hover fly), *Coccinella undecimpunctata* (11-spotted ladybird) and sheet web spiders (Linyphiidae) were collected from potatoes at Pukekohe Research Station and reared in the laboratory to assess their potential as predators against TPP nymphs. Individual predators were presented every 24 h to a maximum of 10 TPP nymphs of different instars ranging from 2nd to 4th instar depending on the size of the predator. All predator species fed on TPP nymphs, and all predatory life stages were capable of consuming more than five nymphs per day. Ongoing studies with these species show that all predatory life stages of all predatory species feed on all life stages of TPP. Together with separate data on predator incidence, these results suggest that naturally occurring predators are likely to be important biological control agents of TPP, particularly early in the season when TPP infestations are low.

- Walker GP 2012. Biological control of TPP by existing natural enemies (lab and field studies) and developing IPM tools for TPP (at Pukekohe). Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.20.

  Abstract. We have assessed the impacts of naturally occurring predators for control of TPP (and other pests) in potatoes in three years of weekly assessments at Pukekohe Research Station, and in laboratory choice and no-choice assays at Mt Albert Research Centre. Predator species assessed include brown lacewing, small hover fly, 11-spotted ladybird beetle, damsel bug and sheet-web spider. Results indicate that all predatory life stages of all these species predate on all life-stages of TPP, even in the presence of aphids, although TPP eggs are less preferred. Results from insecticide trials indicate that some insecticides are less harmful to predators than others. In early crop trials run over three years, trap catches were below an economic injury level, indicating that early crops may be grown without insecticides. We have also assessed action (spray) thresholds based on 1) plant infestations of TPP and 2) monitoring of TPP flights using sticky trap technology. We discuss this year's main crop summer trial, where the use of an action threshold based on increasing trap catches of TPP led to a 50% reduction in insecticide use with 1% ZC, whereas untreated potatoes exhibited 34% ZC symptoms.

- Walker G 2011. Towards developing an IPM programme for potatoes in New Zealand; the role of natural enemies. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.11. Reported on in year 1 & 2.

  Abstract. Recent initiatives to develop IPM for potato crops focussed mainly on controlling aphid pests as virus vectors and managing potato tuber moth (PTM) in the warmer regions of New Zealand. Research on PTM focussed on movement of moths, both as a crop monitoring tool, but also for resistance management. Proposals included a classical biological control (CBC) project for PTM, to assess *Orgilis lepidus* either to complement or replace the existing parasitoid, *Cotesia subandinus*. In 2008, we reported high levels of resistance in PTM populations from the Pukekohe and Waikato regions to pyrethroid and organo-phosphate insecticides, giving impetus to improving biological control and developing IPM tools, particularly for PTM. However, the arrival of tomato-potato psyllid (TPP) relegated prospects of CBC for other potato pests to a low priority compared with urgently required management tools for TPP. Research on CBC was redirected to assess existing biological control agents (BCAs) and their impact on pests, initially in the Pukekohe region. To date, two years of weekly monitoring and spring and summer field trials show that there are existing BCAs that give substantial control of all pests in potatoes. In particular, brown lacewing, small hover fly (*Melanostoma fasciatum*) and spiders can control aphids, exposed caterpillar pests and TPP for 8 months of the year. However, two summer generations of TPP from January to April cause major crop losses. Lower populations of TPP in cooler regions provide an opportunity for naturally occurring BCAs

to give longer-lasting control of TPP. Laboratory choice and no-choice studies indicate that all life-stages of the common predators, brown lacewing, small hover fly, 11-spotted ladybird and nabids are capable of consuming all life stages, and that they consume large numbers of TPP. The potato industry urgently requires sound insecticide resistance management (IRM) strategies, compatible insecticides for managing resistance, selective insecticides for maximising BCAs, plus other robust IPM tools for sustainable production of potatoes, particularly for the longer (warmer) growing regions.

TECHNOLOGY TRANSFER

PFR VC-FUNDED RESEARCH

PUBLISHED RESEARCH PAPERS

Berry N, Scott I, Thompson S, Beard S 2010. Detection of *Candidatus* Liberibacter solanacearum in trapped insects and non-crop plants in New Zealand. Proceedings of the 2010 Zebra Chip Annual Reporting Session: 159-163.

Gardner-Gee R 2011. Progress towards biological control of *Bactericera cockerelli* in covered crops in New Zealand. IOBC/WPRS Bulletin, Vol. 68, 41-45.

Walker GP, MacDonald FH, Fergusson H, Puketapu A, Wright PJ, Anderson J 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. New Zealand Plant Protection 65: 148-154.

Walker GP, MacDonald FH, Larsen NJ, Wallace AR 2011. Monitoring *Bactericera cockerelli* and associated insect populations in potatoes in South Auckland. New Zealand Plant Protection 64: 269-275.

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Berry N, Madge D, Yen A 2011. The tomato psyllid: lessons from New Zealand for Australia. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.10.

Gardner-Gee R 2012. “Importing answers”: Options for classical biological control of TPP in New Zealand. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.21.

Horne P, Vereijssen J, Jorgensen N 2012. Integrated control of potato psyllid. Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.22.

Horne P & Teulon D 2011. Strategic trans-Tasman collaborations enhance arable and vegetable IPM in Australia and New Zealand. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.9.

Jorgensen N, Taylor NM, Berry NA, Butler RC 2011. Seasonal abundance of *Bactericera cockerelli* and beneficial insects in a potato crop in Canterbury. New Zealand Plant Protection 64: 292.

Larsen NJ, MacDonald FH, Connolly PG, Walker GP 2011. Could *Harmonia conformis* be an important predator of *Bactericera cockerelli*? New Zealand Plant Protection 64: 293.

MacDonald FH, Walker GP, Larsen NJ, Wallace AR 2010. Naturally occurring predators of *Bactericera cockerelli* in potatoes. New Zealand Plant Protection 63: 275.

Walker GP 2012. Biological control of TPP by existing natural enemies (lab and field studies) and developing IPM tools for TPP (at Pukekohe). Proceedings Psyllid 2012: Tomato Potato Psyllid in New Zealand. P.20.

Walker G 2011. Towards developing an IPM programme for potatoes in New Zealand; the role of natural enemies. Proceedings of the 3rd Combined Australian and New Zealand Entomological Societies. P.11.

Scientific Presentations

Berry N, Madge D, Yen A 2011. The tomato psyllid: lessons from New Zealand for Australia. Verbal presentation. 3rd Combined Australian and New Zealand Entomological Societies. 28th August – 1 September 2011. Lincoln University, Christchurch, New Zealand.

Berry N, Scott I, Thompson S, Beard S 2010. Detection of *Candidatus* Liberibacter solanacearum in trapped insects and non-crop plants in New Zealand. Verbal presentation. 2010 Zebra Chip Annual Reporting Session. Dallas, United States, 7–10 November 2010.

Gardner-Gee R 2012. “Importing answers”: Options for classical biological control of TPP in New Zealand. Verbal presentation. *Psyllid 2012: Tomato Potato Psyllid in New Zealand*, Ellerslie Events Centre, 26-27 July 2012, Auckland, New Zealand.

Gardner-Gee R 2012. Use of “climate-limited” biological control agents in NZ glasshouses. Verbal presentation. Symposium on the potential value of generalist predators in New Zealand IPM and the science required to ensure safe use. Environmental Protection Agency, BP House, Wellington, New Zealand, 2 April 2012.

Gardner-Gee R 2011. Progress towards biological control of *Bactericera cockerelli* in covered crops in New Zealand. Verbal presentation. IOBC-WPRS Working Group "Integrated Control in Protected Crops, Temperate Climate”, Norton Park Hotel, Sutton Scotney, Nr Winchester, Hampshire, SO21 3NB, UK.

Horne P, Vereijssen J and Jorgensen N 2012. Integrated control of potato psyllid. Verbal presentation. *Psyllid 2012: Tomato Potato Psyllid in New Zealand*, Ellerslie Events Centre, 26-27 July 2012, Auckland, New Zealand.

Horne P & Teulon D 2011. Strategic trans-Tasman collaborations enhance arable and vegetable IPM in Australia and New Zealand. Verbal presentation. 3rd Combined Australian and New Zealand Entomological Societies. 28th August – 1 September 2011. Lincoln University, Christchurch, New Zealand.

Jorgensen N, Taylor NM, Berry NA, Butler RC 2011. Seasonal abundance of *Bactericera cockerelli* and beneficial insects in a potato crop in Canterbury. Poster. New Zealand Plant Protection Conference, Rotorua, New Zealand, August 2011.

Larsen NJ, MacDonald FH, Connolly PG, Walker GP 2011. Could *Harmonia conformis* be an important predator of *Bactericera cockerelli*? Poster. New Zealand Plant Protection Conference, Rotorua, New Zealand, August 2011.

MacDonald FH, Walker GP, Larsen NJ, Wallace AR 2010. Naturally occurring predators of *Bactericera cockerelli* in potatoes. Poster. New Zealand Plant Protection Conference, New Plymouth, New Zealand, August 2010.

Walker GP 2012. TPP work at Pukekohe and Auckland: potato research and milestone 3: insecticide trial. TPP SFF project no. 09/143, progress report. Verbal presentation. SFF Project Team Meeting, Ellerslie Events Centre, 26 July 2012, Auckland, New Zealand.

Walker GP 2012. Biological control of TPP by existing natural enemies (lab and field studies) and developing IPM tools for TPP (at Pukekohe). Verbal presentation. Psyllid 2012. *Psyllid 2012: Tomato Potato Psyllid in New Zealand*, Ellerslie Events Centre, 26-27 July 2012, Auckland, New Zealand.

Walker GP, MacDonald FH, Fergusson H, Puketapu A, Wright PJ, Anderson J 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. Verbal presentation. New Zealand Plant Protection Conference, Nelson, New Zealand, August 2012.

Walker G 2011. Towards developing an IPM programme for potatoes in New Zealand; the role of natural enemies. Verbal presentation. 3rd Combined Australian and New Zealand Entomological Societies, 28 August – 1 September 2011. Lincoln University, Christchurch, New Zealand.

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Horticulture Australia

PT09004 – Appendix 2

Horticulture New Zealand

PROJECT SUMMARY

PT09004 – APPENDIX 2

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PURPOSE OF THE REPORT:

This project has been co-funded from New Zealand and Australian sources, with the work being undertaken in New Zealand on tomato potato psyllid (TPP) and the bacterium *Candidatus* Liberibacter solanacearum (Liberibacter), that contribute to the physiological disorder of potato known as Zebra Chip. This report documents a range of areas of research, including crops other than potato. The knowledge from the project has direct benefits for Australia in assisting to better understand TPP and Liberibacter and has been included as an appendix to the IPM Technologies report as part of project PT09004 – *Control of Potato Psyllid Within an IPM Strategy* from the Australian Potato Research Program Phase 2 (APRP2).

This report documents research, including pesticide-based control and management strategies undertaken in New Zealand which are not registered for use in Australia. The recommendations for this report are specific for New Zealand only. The Australian Pesticides and Veterinary Medicines Authority (APVMA) website should be consulted for information regarding pesticides registered for use in Australia.

2 November 2015

FUNDING SUPPORTERS

Contributions and funding for this program are gratefully acknowledged

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant & Food Research Limited and Potatoes New Zealand, and matched funds from the Australian Government.

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CONTENTS OF APPENDIX 2

MEDIA SUMMARY

Tomato potato psyllid (TPP, *Bactericera cockerelli*), is a relatively new and significant pest of key horticultural crops in New Zealand including potatoes, tomatoes (greenhouse and outdoors), capsicums and tamarillos. In New Zealand it was first discovered in 2006, and the bacterium that is vectored by the psyllid, *Candidatus* Liberibacter solanacearum (Liberibacter) was detected in 2008. The economic impact across the potato industry in the 2010-11 growing season was estimated at $28 million.

The objective for this project was to develop sustainable management systems for tomato potato psyllid (TPP, *Bactericera cockerelli*) for the NZ potato industry. A series of research themes were identified: monitoring TPP distribution throughout New Zealand; assessment of the impacts of TPP on the potato industry; insecticide trials to assist with the development of spray programmes; biological control agent evaluation; and the development of diagnostic tools to detect Liberibacter to better understand the distribution of Liberibacter in plant tissue and in TPP.

The project has provided information that is of direct benefits to growers:

- Insecticide trial work has demonstrated that there are many agrichemicals that are effective for TPP control which can be used in conjunction with organophosphate insecticides in a resistance management programme.
- Guidelines have been produced on obtaining optimum spray coverage to make best use of these products.
- In areas such as Pukekohe where early season TPP pressure is low it is possible to reduce insecticide inputs in early potato crops.
- Having collected three years of national monitoring data the tamarillo, field tomato, and potato industries have a good knowledge of the timing of TPP population increases. This knowledge can be combined with climate data to anticipate population peaks.
- Accurate diagnostic methods are now available to confirm Liberibacter infections or for screening of potato tubers or TPP.
- The efficacy of entomopathogenic ('insect-killing') fungi for TPP control has now been verified and the greenhouse industry can make informed decisions about the use of these products for TPP control.
- The tamarillo industry now has a good base of knowledge of insecticides that are effective for TPP control in their crops.

Perhaps the biggest difference that the project has made is that, in addition to these tools, all industry participants are much better informed about TPP and Liberibacter and have the confidence to continue producing these crops while longer term solutions are developed.

TECHNICAL SUMMARY

The objective for this project was to develop sustainable management systems for tomato potato psyllid (TPP, *Bactericera cockerelli*) for the NZ potato industry. A series of research themes were identified: monitoring TPP distribution throughout New Zealand; assessment of the impacts of TPP on the potato industry; insecticide trials to assist with the development of spray programmes; biological control agent evaluation; and the development of diagnostic tools to detect Liberibacter

(*Candidatus* Liberibacter solanacearum - the bacterial pathogen vectored by TPP) to better understand the distribution of Liberibacter in plant tissue and in TPP.

Psyllid monitoring over three years found that TPP numbers were highly variable between sites and regions. Generally, the North Island had higher TPP numbers than the South Island. In general, the abundance of TPP appeared to peak between early February and late March, with declining numbers towards mid-April. This means that early potatoes in the North Island may escape TPP/*Candidatus* Liberibacter solanacearum (Lso) damage and could potentially be grown without chemical psyllid control. Greater TPP numbers were generally found on the edge of a crop rather than in the middle. This is a similar observation to that reported from TPP monitoring programmes in the USA. The sticky trap monitoring programme is most useful at the beginning of the cropping season, providing an indication of when TPP could be expected to arrive in the crop. Sticky traps recorded psyllids 1-4 weeks earlier than plant assessments did. Analysis of the Liberibacter status of psyllids collected during the 2009-2010 growing season demonstrated that Liberibacter was present at all sites at most monitoring periods.

The economic impact across the potato industry in the 2010-11 growing season was estimated at $28 million. These impacts were most severe in the North Island where process grower costs increased by up to an additional $5,600 per hectare, compared to $540 in the South Island. Costs included crop impacts (yield and quality), control costs and other costs (management, research, market access and compliance).

A 3-year main crop insecticide trial at Pukekohe Research Station tested different insecticides and action thresholds and their impact on TPP populations and damage to potato crops. Three to five treatments were used: weekly foliar sprays (i.e. ‘standard’ spray programme) and different threshold-based foliar sprays (i.e. reduced spray programmes); and no insecticides (i.e. control). The sticky trap action threshold gave promising results that deserve further investigation to assess if this method may be used as an economic action threshold, being an economic injury level (EIL) ‘trigger’. Also, the ‘standard’ sequence of blocks of Avid®, Movento® and Sparta® gave the best results of all the insecticide rotation strategies tested in these ‘insecticide’ trials.

The North American parasitoid, *Tamarixia triozae*, was imported into quarantine facilities for assessment as a biological control agent. Host-range testing has been carried out to evaluate the potential for *T. triozae* to impact negatively on non-target psyllid species in New Zealand. *Tamarixia triozae* did not oviposit on six of the eight non-target psyllid species it was exposed to in no-choice screening tests, but did oviposit on two native psyllid species, although not to the same extent as on TPP and with limited emergence or subsequent fecundity.

A DNA extraction protocol has been developed and successfully optimised for the detection of Liberibacter in psyllids and plant tissue. Ring testing against USA samples indicates that this methodology is more sensitive than the USA methodology. Testing of TPP collected from crops may provide a more practical approach than testing plant material. TPP can be processed immediately after collection and testing TPP for Lso could provide information about the infective potential of the TPP population.

INTRODUCTION

Tomato potato psyllid, *Bactericera cockerelli* (TPP), is a relatively new and significant pest of key horticultural crops in New Zealand including potatoes, tomatoes (greenhouse and outdoors), capsicums and tamarillos. In New Zealand it was first discovered in 2006, and the bacterium that is vectored by the psyllid, *Candidatus* Liberibacter solanacearum (Lso) was detected in 2008. As this is a new pest, sustainable management practices were not available for use by industry.

TPP has had a major impact on key horticultural crops. The economic impact across the potato industry in the 2010-11 growing season was estimated at $28 million. These impacts were most severe in the North Island where process grower costs increased by up to an additional $5,600 per hectare, compared to $540 in the South Island. Bacterial infections of tamarillo trees has resulted in production losses and sometimes tree death and in the absence of export market access the future of this industry is under threat. The process potato industry ($87M exports 2011-12) faces impacts on the quality of both the crisp and chip industry with the bacterium causing a physiological disorder called Zebra Chip. TPP has terminated use of the highly successful Integrated Pest Management Programs (IPM) in both greenhouse crops and process tomatoes with increased insecticide use having effects on biological control agents.

The overarching objective for this project was to develop sustainable management systems for TPP for the potato, tomato, capsicum and tamarillo industries. To achieve this, a series of research themes were identified to encompass all aspects of TPP including monitoring TPP distribution throughout NZ, assessment of the impacts of TPP on the potato industry, insecticide trials to assist with the development of spray programmes, alternative control strategies such as biological control, soft chemical trials, and entomopathogens. Additionally, the development of diagnostic tools to detect Lso (the bacterial pathogen vectored by TPP) to better understand the distribution of Lso in plant tissue and in TPP was developed through this project.

These project objectives were developed into a series of research milestones:

- MS 1-3: Insecticide field trials
- MS 4: National psyllid monitoring
- *Extension activity* (June 2010): TPP monitoring
- MS 5: Liberibacter diagnostics
- MS 6: Evaluation of soft chemicals
- MS 7: Biological control agent development
- MS 8: Entomopathogens for TPP control
- MS 9: Tamarillo insecticide trials and effects of antifeedants on TPP activity
- MS 10: Tamarillo monitoring for TPP
- MS 11: Annual literature scan of TPP research
- MS 12: Information transfer to the NZ industry
- MS 14: *Extension activity* - Assessment of economic impact of TPP in NZ

At its outset, the project identified several areas of research to specifically address the concerns of the potato, tomato, capsicum and tamarillo industries around the future management of TPP in New Zealand. In formulating the project objectives and milestones, each industry sector (potatoes,

tomatoes capsicums and tamarillos) was widely consulted to ensure that industry priorities were captured in the final work-plan. The objectives were a mixture of cross-sector and sector-specific research. Although some objectives were targeted towards providing information for a specific sector the intention was that the information resulting from the work would be available for all groups.

The approach was to undertake work that would add to the understanding of TPP and Lso biology, and develop a range of monitoring and control tools that could move the industries away from using non-selective synthetic pyrethroid or organophosphate insecticides towards more targeted agrichemical products, oils, and entomopathogenic fungi.

The project was funded from 2009 to 2012.

MATERIALS AND METHODS

This project covered a wide range of research areas, each with a number of discrete trials. Descriptions of the trial methodologies are provided in the scientific/conference papers and client reports referenced under the Technology Transfer section.

RESULTS

MILESTONES 1, 2 AND 3 - TOMATO-POTATO PSYLLID INSECTICIDE TRIALS (2009–12)

Milestones 1, 2 and 3 of this Sustainable Farming Fund (SFF) project were to undertake three years of main crop insecticide trials at Pukekohe Research Station to test different insecticides and action thresholds and their impact on TPP populations and damage to potato crops.

Three to five treatments were used: insecticide drench and weekly foliar sprays (i.e. 'standard' spray programme); insecticide drench and different threshold-based foliar sprays (i.e. reduced spray programmes); and no insecticides (i.e. control). Reduced spray programmes were based on nominal action thresholds utilising either the number of TPP nymphs per middle leaf or the mean number of TPP adults per yellow sticky trap. Thresholds based on infestations of TPP nymphs on plants led to a range of 5–8 applications of insecticides, approximately half the number applied in the weekly spray programme.

The incidence of Zebra Chip (ZC) damage in treatments triggered by nymph thresholds was unacceptable, ranging from 5 to 27% ZC. However, thresholds based on sticky trap catches led to a 50% reduction in sprays with <1% ZC when the 'standard' sequence of insecticides was applied. The sticky trap action threshold gave promising results that deserve further investigation to assess if this method may be used as an economic action threshold, being an economic injury level (EIL) 'trigger'. Also, the 'standard' sequence of blocks of Avid®, Movento® and Sparta® gave the best results of all the insecticide rotation strategies tested in these 'insecticide' trials.

MILESTONE 4 - PSYLLID NATIONAL MONITORING

The seasonal abundance and distribution of the TPP was assessed using weekly sticky trap monitoring in commercial potato, field tomato and tamarillo crops during the 2009-10, 2010–11 and

2011-12 growing seasons. At an additional two sites, weekly sticky trap and plant assessments were performed.

The most important findings and conclusions were:

- TPP numbers were highly variable between sites and regions. Generally, the North Island had higher TPP numbers than the South Island.
- In general, for both the North and South Islands, the abundance of TPP appeared to peak between early February and late March, with declining numbers towards mid-April. This means that early potatoes in the North Island may escape TPP/*Candidatus* Liberibacter solanacearum (Lso) damage and could be grown without chemical psyllid control.
- The monitoring (both sticky trap and plant assessment) was influenced by crop management and local climate.
- Greater TPP numbers were generally found on the edge of a crop rather than in the middle. This is a similar observation to that reported from TPP monitoring programmes in the USA.
- The sticky trap monitoring programme is most useful at the beginning of the cropping season, providing an indication of when TPP could be expected to arrive in the crop. Sticky trap monitoring should be accompanied by actual plant assessments throughout the growing season to give a true indication of pest infestation in the crop.
- Sticky traps recorded psyllids 1-4 weeks earlier than plant assessments did.

Further studies should include trapping and plant assessments in unsprayed crops and development of a less labour-intensive weather-based supervised control system for TPP. In addition, this study has shown that in many cases, the first one or two insecticide applications can be omitted, as they were applied too early in the season. Education of growers regarding this would increase profits and decrease TPP insecticide resistance.

MILESTONE 4 EXTENSION ACTIVITY (JUNE 2010): TPP MONITORING

Retrospective analysis of the Liberibacter status of psyllids collected during national monitoring of the 2009-2010 growing season demonstrated that Liberibacter was present at all sites at most monitoring periods. This result suggested that Liberibacter incidence is very high in the New Zealand psyllid populations. The results also suggested that there is no disease-free period when psyllids are present in a crop. Therefore the potential for infection with Liberibacter is very high whenever psyllids are present.

MILESTONE 5 - IMPROVED LIBERIBACTER DIAGNOSTICS

A DNA extraction protocol has been developed and successfully optimised for the detection of Liberibacter in psyllids and plant tissue. This method uses a single-step semi-nested SYBR Green qPCR targeting the Lso 16S rRNA gene. Ring testing against USA samples indicates that this methodology is more sensitive than the USA methodology.

A study was undertaken to characterise the distribution of '*Candidatus* Liberibacter solanacearum' (Lso) in infected potato plants over time. Results from this study showed that the distribution of Lso within plants was uneven, with spatial and temporal differences. Titre was also variable between plant tissues. These results lead to recommendations for field sampling of Lso in potato crops. First, samples should be collected from mid-point stem or stolon tissue, not foliar material or upper stems.

Second, testing should be delayed until 2-3 weeks after recognised exposure to TPP. These guidelines are designed to avoid the generation of false-negative test results. PCR testing of TPP collected from crops may provide a more practical approach than testing plant material. TPP can be processed immediately after collection and testing TPP for Lso could provide information about the infective potential of the TPP population.

MILESTONE 6: SOFT CHEMICALS IN THE GREENHOUSE INDUSTRY

The aim of this milestone was to evaluate the efficacy of soft chemicals (essential oils) for the control of TPP in the greenhouse industry. The repellency of 10 essential oils was investigated using a no-choice laboratory assay. From this testing the essential oils neem and cedarwood were found to produce the most significant response after 1 hour. After 48 hours, neem and patchouli reduced oviposition and significantly repelled adult female TPP.

A review of the Soft Chemicals milestone (Milestone 6) by the tomato and capsicum industry prior to the October 2011 project planning meeting identified that a continuation of the soft chemical research would be of little commercial benefit. As a result, the project team agreed to terminate the milestone and redirect the remaining budget to support the entomopathogen research (Milestone 8).

MILESTONE 7 - HOST RANGE TESTING OF BCA (*TAMARIXIA TRIOZAE*)

Searches conducted between 2006 and 2008 failed to identify any natural enemies within New Zealand that were likely to control TPP on tomatoes, so in 2009 a North American parasitoid, *Tamarixia triozae*, was imported into quarantine facilities at the Mt Albert Research Centre, Auckland, for assessment as a biological control agent (BCA).

Host-range testing has been carried out to evaluate the potential for *T. triozae* to impact negatively on non-target psyllid species in New Zealand. *Tamarixia triozae* did not oviposit on six of the eight non-target psyllid species it was exposed to in no-choice screening tests. *Tamarixia triozae* did oviposit on two native psyllid species, *Trioza curta* and *Trioza panacis*. However the oviposition rate on both was lower than the oviposition rate on the target pest TPP. In addition, no *T. triozae* adults emerged from parasitized *T. curta*, suggesting that *T. triozae* would not be able to maintain itself over time in situations where *T. curta* was the only host available. *Tamarixia triozae* did emerge from parasitized *T. panacis* nymphs but the first generation female parasitoids from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP).

MILESTONE 8 – ENTOMOPATHOGENS

Seventeen commercial entomopathogen products and isolates were identified as candidates for screening against TPP in New Zealand. Within the importation, legal and time constraints, 12 of the 17 candidates were successfully sourced, and 11 were evaluated, including seven entomopathogen products and four fungal isolates. These were evaluated in the laboratory and greenhouse using adult (immersion) and nymph (detached leaf) bioassays as a means of selecting suitable candidates for larger scale greenhouse trials.

- *Screening of entomopathogens:* All entomopathogens screened using a leaf bioassay showed varying degrees of activity against nymphs and adults. K4B3, BotaniGard® ES and Met52® EC

outperformed the conventional insecticide standard, Oberon®, and all other entomopathogens screened, and were therefore selected as the best potential candidates for subsequent greenhouse and host plant trials based on overall efficacy and obtaining a rapid solution for industry.

- *Impact of environmental conditions on isolate growth and germination*: All isolates had high rates of germination at 20°C and 25°C and showed consistent growth at 15°C, 20°C and 25°C. In contrast, all isolates had limited or no germination or growth at 30°C or within the greenhouse in February. Light had no effect on germination. These results are consistent with most fungal entomopathogens.
- *Effect of host plant on the susceptibility to entomopathogens:* Host plant (capsicum, tomato and potato) had no effect on the observed efficacy of selected entomopathogens against TPP.
- *Caged greenhouse trials:* These trials, performed on capsicum and tomato, confirmed the efficacy of BotaniGard® ES and Met52® EC. BotaniGard® ES demonstrated more consistent efficacy in response to changes in environmental factors.
- *Environmental stability:* Performance of the greenhouse tested entomopathogens was influenced by temperature and relative humidity.

The results presented provide an indication of product and isolate efficacy under specific environmental conditions. Both BotaniGard® ES and Met52® EC are suited to greenhouse temperatures, but the activity of these fungi will be dependent on the amount of time conditions are optimal, that is 23–25°C and 25–30°C, respectively. As such, timing spray applications to correspond to periods in the growing season when higher greenhouse temperatures are maintained is likely to maximise the effectiveness of the spray. In greenhouse environments where environmental conditions are not controlled, the use of these entomopathogens would be best suited to early spring, early and late summer and autumn.

MILESTONES 9 AND 10: TAMARILLO INSECTICIDE TRIALS AND ORCHARD MONITORING

The aim of this work was to determine the seasonal abundance of the different life stages of TPP on tamarillo trees through field monitoring (MS 10), and to relate this information to results from the potted plant insecticide trial (MS 9) to determine the optimum timing of insecticide applications to control TPP.

MILESTONE 10: ORCHARD MONITORING.

The main findings of the orchard monitoring work are as follows:

- TPP in Northland have multiple overlapping generations.
- TPP appears to overwinter as late instar nymphs in tamarillo orchards.
- There is low survival of TPP from young nymph to older nymph in tamarillos.
- There appears to be a constant migration of adult TPP into the orchards (this is based on monitoring and on grower observation).
- The new spray program has likely made an impact on the number of TPP found in the tamarillo orchards that were monitored.
- Disease symptoms progressed quickly over the summer months, with some trees showing severe symptoms within 6 weeks.

These results may be influenced by seasonal difference (e.g. dry summer (drought) vs. wet summer), and as unmanaged solananaceous crops die from TPP burden, there may be fewer sources of TPP to infest tamarillo orchards. Disease symptoms progressed quickly over the summer months, with some trees showing severe symptoms within 6 weeks. Early results have indicated that TPP were not found on plants with disease symptoms, indicating a reduced attraction of these plants for feeding or laying eggs. There is significant crop loss due to Liberibacter infection which has a detrimental effect on orchard production.

MILESTONE 9 - TAMARILLO INSECTICIDE TRIALS.

- Insecticide testing – susceptibility of different life stages of TPP to insecticides:
  - Of the 11 products tested, Avid® + oil, Talstar®, Oberon®, and Movento® gave effective control of TPP nymphs over a 6-week period.
  - Avid® + oil and Talstar® had good knockdown effect against TPP nymphs, while Oberon® and Movento® took a couple of weeks to become effective.
  - Oberon® + oil and the mineral treatment controlled TPP nymphs for up to 2 weeks.
- Testing efficacy of spray residues:
  - Laboratory trials tested the residual activity of 5 insecticides against TPP nymphs at 1, 3, 7, 17, 21 and 28 days after treatment.
  - Compared to untreated plants, Avid®, Movento® and Oberon® treated plants had significantly fewer nymph numbers with 21 day old residues.
  - 28 day old residues did not result in a significant reduction of TPP on plants.
  - The percent mortality of TPP adults exposed to residues of Sparta® (up to 28 day old residues), Avid® (17 day old residues), and Movento®, Oberon®, and Neemazal® (1 and 3 day residues), was significantly higher compared to controls.
  - Fewer eggs were laid by adults exposed to 1 and 3 day old residues of each insecticide treatment compared to controls.
- Testing antifeedants against the tomato potato psyllid (TPP) using EPG technique: Tomato plants were sprayed with one of four treatments: Neemazal® (neem oil), Surround® (kaolin clay), DC-Tron® (mineral oil) or tap water (control). Using the EPG method, the treatment of tomato plants with Neemazal®, Surround® or DC-Tron® did not deter TPP feeding enough to prevent phloem feeding and therefore probable Liberibacter transmission.

MILESTONE 14: EXTENSION ACTIVITY - ASSESSMENT OF ECONOMIC IMPACT OF TPP

A survey of the NZ potato industry was undertaken by Alan Kale (ELAK Consultants) commissioned by Potatoes NZ with funding from SFF. It covered forty-two growers from all sectors of the potato industry as well as five processors and three seed merchants. A survey of the tomato/capsicum industries for the economic impact of TPP was completed by Market Access Solutionz.

Regional grower costs were broken into three categories: crop impacts, control costs, and other costs (such as extra compliance costs, other production and management costs). This survey (Kale, 2011) reported that the economic impact across the potato industry in the 2010-11 growing season was $28 million. These impacts were most severe in the North Island where process grower costs increased by up to $5,600 per hectare, compared to $540 in the South Island. Costs included crop impacts (yield and quality), control costs and other costs (management, research, market access and

compliance). In 2011, approximately $5 million of losses was reported in the capsicum and tomato industries combined as a result of TPP (MAS, 2011). In addition to yield impacts due to TPP, these losses were incurred as a result of increased insecticide applications, additional crop monitoring and costs of compliance for export produce.

DISCUSSION

The project provided an early foundation for ongoing and improved communication and collaboration between NZ industry groups (Potatoes New Zealand, Tomatoes New Zealand, Vegetables New Zealand, and NZ Tamarillo Growers Assn.), the processing industries (Heinz Watties, Mr Chips, McCain Foods, Bluebird Foods), agrichemical companies, and the research community. By bringing these groups together, and all parties becoming aware of the magnitude of the problem and the research challenge, an integrated research programme has been developed to find answers to the underlying biological questions in the longer term.

The project has provided the following information:

- Insecticide trial work has demonstrated that there are many agrichemicals that are effective for TPP control which can be used in conjunction with organophosphate insecticides in a resistance management programme.
- Guidelines have been produced on obtaining optimum spray coverage to make best use of these products.
- In areas such as Pukekohe where early season TPP pressure is low it is possible to reduce insecticide inputs in early potato crops.
- Having collected three years of national monitoring data the tamarillo, field tomato, and potato industries have a good knowledge of the timing of TPP population increases. This knowledge can be combined with climate data to anticipate population peaks.
- Accurate diagnostic methods are now available to confirm Liberibacter infections or for screening of potato tubers or TPP.
- The efficacy of entomopathogenic fungi for TPP control has now been verified and the greenhouse industry can make informed decisions about the use of these products for TPP control.
- The tamarillo industry now has a good base of knowledge of insecticides that are effective for TPP control in their crops.

Perhaps the biggest difference that the project has made is that, in addition to these tools, all NZ industry participants are much better informed about TPP and Liberibacter and have the confidence to continue producing these crops while longer term solutions are developed.

TECHNOLOGY TRANSFER

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Psyllid 2012: Tomato Potato Psyllid in New Zealand. The conference titled 'Psyllid 2012: Tomato Potato Psyllid in New Zealand' was held over 2 days (26th and 27th July 2012) at the Ellerslie Convention Centre in Auckland.

> The aim of the conference was to focus on important new developments to manage and control psyllid and was a synthesis of all recent psyllid research that has been on-going in New Zealand and overseas. The Psyllid conference was attended by 135 people, comprising growers (from the potato, tomato and tamarillo sectors), scientists, agronomists, and agrichemical industry personnel. *Conference proceedings and selected presentations will be available on the Potatoes NZ website shortly.*

WEBSITES:

Potatoes NZ Inc. (http://www.potatoesnz.co.nz) contains regular updates of psyllid research activities, reports and grower newsletters (Psyllid News).

- Psyllid resources
- Control strategies
- Monitoring guidelines and data

RECOMMENDATIONS – SCIENTIFIC

New Zealand industry recommendations and guidelines can be found on the Potatoes New Zealand website (links provided above).

FURTHER RESEARCH RECOMMENDATIONS

MILESTONE 3: INSECTICIDE FIELD TRIALS

- The sticky trap action threshold tested in year 3 (2011/12) gave promising results that deserve further investigation. This threshold used a baseline of about 3 per trap per week.
- More research is required to better define the economic action threshold based on sticky trap catches to reduce the reliance on calendar spraying of insecticides.
- The insecticide programmes established at Pukekohe should be tested in other regions – Canterbury and Hawke's Bay.

MILESTONE 4: NATIONAL TPP MONITORING

- An important question that arises from this is how prevalent Lso is within the psyllid population that arrives in a crop.

- Incorporate degree day data and modelling developed through Luc Tran's research to better inform timing of crop monitoring.

MILESTONE 5: LIBERIBACTER DIAGNOSTICS.

- Options for commercial uptake of this screening technology will be dependent on the level of demand from the NZ industry.
- As part of technical transfer activities (Milestone 12) a guidance and information sheet on Liberibacter testing will be prepared.

MILESTONE 7: BIOLOGICAL CONTROL AGENT

- The potential for *Tamaraxia* to establish in the NZ environment – Climate modelling of *T. triozae* has been supported by a SFF (under $25K) funded project.
- Following a review of the outcomes of the climate modelling a decision will be made whether to proceed with an application to the NZ EPA for BCA release.

MILESTONE 8: ENTOMOPATHOGENS

- Fungal entomopathogens (in particular BotaniGard® ES and Met52® EC) have demonstrated efficacy against *B. cockerelli* and should be considered for inclusion in future IPM programs.
- Further work is required to optimise entomopathogen performance under a wider range of environmental conditions.
- That the effects of candidate entomopathogens on non-target organisms, such as predators and parasitoids of *B. cockerelli*, are examined prior to the inclusion within an IPM programme.
- The commercial product MBI203, manufactured by Marrone Bio Innovations (not available for this testing), should be included in future testing.

ACKNOWLEDGEMENTS

FUNDERS:

- Potatoes New Zealand, Tomatoes New Zealand, Vegetables New Zealand, and NZ Tamarillo Growers Association
- Sustainable Farming Fund (Ministry for Primary Industries), Plant & Food Research, the Australian Government through Horticulture Australia (the Australian Potato Research Program phase 2)
- Mr Chips, McCain Foods, Bluebird Foods, Heinz Watties

RESEARCH PROVIDERS:

- Plant & Food Research
- IPM Technologies
- Peracto NZ Ltd
- Agribusiness Training

INDUSTRY PARTICIPANTS:

- Growers
- Heinz Watties, Mr Chips, McCain Foods, Bluebird Foods
- Agrichemical companies
- Horticentre, FruitFed Supplies

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Horticulture Australia

PT09019 – (30/09/2014)

Importance of tuber borne inoculum on seed potato health

Final Report

Dr Robert Tegg

Dr Calum Wilson

Tasmanian Institute of Agriculture

PROJECT SUMMARY

PT09019 – Importance of tuber borne inoculum on seed potato health

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Purpose of the report: This report is the final report for the project "Importance of tuber borne inoculum on seed potato health", Project number PT09019. The usage of certified potato seed is a recommended practice that helps to reduce the likelihood of disease in planted crops. Visual assessment of key soilborne diseases at harvest is currently standard practice used in certification although new technologies such as qPCR are being developed and may have a role to play in future certification schemes. Additionally sampling strategies used to certify seed may have an impact on certification outcomes. This report details outcomes from Project PT09019 which aimed to provide potato growers and industry stakeholders with an assurance that current certification practices were providing suitable seed with a low level of disease and planting risk. Additionally, qPCR was validated as an additional technology that may be suitable for industry use. Alternative sampling technologies, the cost of qPCR in comparison to visual assessment and the merits of seed sorting practices (such as grading) are also discussed.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy and matched funds from the Australian Government. The University of Tasmania/Tasmanian Institute of Agriculture has provided in-kind support.

DISCLAIMER

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LIST OF ACRONYMS

Acronym	Definition
ANOVA	Analysis of variance
APRP	Australian Potato Research Program
AUC	Areas under the ROCs
CI	Confidence interval
CS	Common scab
CSF	Common scab forward primer
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSR	Common scab reverse primer
CTREE	Conditional Inference Trees
DAE	Days after emergence
DNA	Deoxyribonucleic acid
EU	European Union
F1	Field trial 1
F2	Field trial 2
FT	Field trial
GPS	Global Positioning System
LSD	Least squares mean
MAD	Mean absolute differences
NW	North West
PCR	Polymerase chain reaction
R&D	Research and development
RDTS	Root Disease Testing Service
ROC	Receiver operating characteristic
SARDI	South Australian Research and Development Institute
TIA	Tasmania Institute of Agriculture
UK	United Kingdom
UNECE	United Nations Economic Commission for Europe
USA	United States of America
UTAS	The University of Tasmania
VICSPA	Victorian Certified Seed Production Authority

CHAPTER 3. IMPORTANCE OF TUBER BORNE INOCULUM ON SEED POTATO HEALTH

MEDIA SUMMARY

Key components of this project included:

- Adaption of new multiplex qPCR based assays developed for soil testing for significant potato pathogens as a tool to improve potato seed tuber inoculum screening and certification practices.
- Examination of current seed tuber sampling strategies and comparing visual based disease assessment with pathogen detection by DNA based tests and discussing possible benefits or limitations of any change to a new testing regime.
- Assessment of the importance of seed-borne inoculum to disease in subsequent crops (both for root and seed disease) and for its importance in contamination of pathogen-free soils with pathogen inoculum.

This project confirmed:

- The importance to growers of utilising disease-free certified seed tubers.
- That the current seed tuber certification practices, using simplified sampling and visual assessment, were adequate to determine seed tuber health and risk for the major soil-borne pathogens that produce visual lesions on tubers, at an affordable price.

Whilst qPCR (DNA) technology provided some additional benefits in pathogen detection and showed its usefulness as a research tool, the current cost-benefit analysis suggests that assessing disease status of tuber seed is still, in most circumstances, best carried out for certification by visual means. qPCR may be useful for assessing seed in:

- elite, early generation seed-lines
- where large tracts of land are to be planted on pathogen-free soil
- situations where diseased seed has been heavily graded
- detecting specific seed-borne pathogens that are unable, or not always visually obvious on the tuber (e.g. most potato viruses, *Verticillium dahliae*).

The project also identified that pathogen detection limits used in certification schemes (e.g. 2% threshold level for powdery scab) may need further validation. Additionally, seed grading was identified as a practice that may result in the graded seed (whilst visually disease free) containing elevated levels of pathogen that may represent a disease risk to the grower. More research may be needed to address what level of diseased seed is suitable for grading whilst limiting contamination of the visually disease free seed.

Take home messages:

- Both visual and qPCR assessment technologies were validated for detection of seed tuber-borne pathogens in this project, both technologies are suitable for certifying seed.
- Seed-borne pathogens pose significant risks to potato growers.
- Growers should purchase certified seed to reduce the risk of seed-borne diseases.

TECHNICAL SUMMARY

Effective certification schemes within the potato seed industry are important in ensuring disease-free seed tubers is supplied consistently and economically. Current visual assessment strategies, for diseases that produce obvious visual symptoms on seed tubers, have been a central component of these schemes but rigorous testing of current sampling strategies and testing of new technologies such as qPCR are essential to ensure that current visual assessment practice is still providing growers with a reliable and cost-effective means of certifying seed tubers. Additionally, the impact of planting seed-tubers carrying varying inoculum loads needs to be better understood in terms of progeny crop production, so that the benefits of planting certified seed can be clearly demonstrated to industry.

Statistically rigorous sampling strategies were undertaken over a three year period within current commercial seedlots (38 seedlots in total) grown for seed. The project tested two differential sampling strategies (2 lots of 100 tubers samples – current practice, and 10 lots of 20 tuber samples), and compared seed health testing by qPCR detection of pathogen DNA and traditional visual disease assessments. Additionally the impact of planting diseased versus healthy seed on crop progeny disease was assessed through a series of experiments over four years. Other research investigated alternate sampling strategies for determining pathogen load in seed, the impact of seed grading on inoculum load and the movement of inoculum from diseased seed tubers into the soil environment.

Project findings included validation that the current simple sampling strategy (2 by 100 tubers per seedlot) provides a suitable measure of disease load under most circumstances. While qPCR provided better distinction of pathogens providing similar symptoms and could detect pathogens that do not produce obvious tuber symptoms, the measurable benefits of this detection tool to the grower were not obvious in the majority of cases. The four-fold increase in costs associated with these tests being utilized for certification currently does not warrant their use under most circumstances. The relationships established when planting diseased seed tubers that carry either *R. solani* AG3 or *S. scabies* into pathogen-free soil was clear-cut, showing a positive association with increased disease in both progeny roots and stolons (for *R. solani* AG3) and tubers (both *R. solani* AG3 and *S. scabies*). This would indicate that where available, seed treatments would be recommended where both these pathogens were present on the tubers. In the case of powdery scab, the relationship between planting diseased seed and progeny crop outcomes were less clear. A strong positive relationship between planting diseased seed and root disease was consistently shown, a key outcome as root disease can lead to reduced yields and soil inoculum build up. The relationship between planting diseased seed and progeny tuber disease was less obvious, although there was a trend indicating greater disease was likely. This reflects that other factors such as environmental conditions strongly influence powdery scab tuber disease.

Other findings include quantitative confirmation that diseased seed tubers can contaminate pathogen-free soils, and that subsequent potato crops grown in these contaminated soils can result in high levels of disease. Additionally we identified that grading of heavily diseased seed tubers represents a significant risk to the grower as the graded seed, although visually disease-free, can contain high levels of pathogen inoculum, from the discarded diseased seed, that can result in subsequent crop disease.

Recommendations are that in most circumstances, current visual certification strategies are adequate to identify risk of diseased seed to growers and no change in sampling strategies or technologies is needed. In some circumstances, such as the growing of elite early-generation seed, or where large tracts of pathogen-free land are to be planted then testing seed by qPCR may be worthwhile. Additionally, where diseased seed is heavily graded and for specific seed-borne pathogens that are impossible or difficult to detect visually (e.g. most potato viruses, *V. dahliae*) qPCR may better identify high risk seed, than visual

assessment. If the costs of qPCR are reduced over time then this technology may become a viable alternative and this project has established some useful criteria to progress this technology to commercial development. Notwithstanding, the technology as it exists is already an extremely important research tool contributing to international progress in understanding key diseases, targeted in this project.

This project identified further work that should be undertaken. This includes studies to (i) confirm/validate whether the current disease incidence levels used for certification (e.g. 2% for powdery scab) are scientifically robust risk thresholds for progeny disease; (ii) clarify at what disease incidence level (10, 20%, etc.) would grading of seed lines give significant disease risk, so as to reduce the likelihood of growers buying pathogen-containing seed; and (iii) understanding the basic form (spore, hyphae, etc..) that the pathogen exists on seed, and how this influences its contribution to disease in progeny crops and its enumeration by qPCR.

INTRODUCTION

While Australia has some of the most rigorous standards for potato seed tuber certification for disease (Tegg et al. 2014), methodologies in certification practice can always be improved. Certification for most diseases is conducted by visual assessment of a 200 tuber sample collected at two points within the seedlot, by certification officers, but the process of tuber sample selection may have the potential to bias certification results. More rigorous sampling strategies may improve reliability of certification outcomes. Also, the use of pathogen specific tests, such as quantitative PCR (qPCR), may be useful in validating the visual assessment systems, and provide an alternative tool in future certification schemes. Indeed, some of the PCR tests developed for determining soil inoculum load (Predicta Pt) may be directly applicable as seed-tuber tests.

While the importance of adequate certification schemes is well established, the role of seed tuber-borne inoculum and its effect on subsequent potato crop production is not fully understood and requires further study. High levels of stem and stolon disease (Atkinson *et al.*, 2010; Tsror and Peretz-Alon, 2005) and black scurf (Atkinson *et al.*, 2010; Errampalli and Johnston, 2001; Tsror and Peretz-Alon, 2005) in progeny has consistently been associated with planting seed-tubers containing high levels of *R. solani*. Likewise, high levels of *Meloidogyne* spp. (Hooker, 1981) corresponds with high nematode disease expression in progeny tubers. However, with common scab and powdery scab there is both evidence for increased (Falloon *et al.*, 1996; Merz and Falloon, 2009; Wang and Lazarovits, 2005; Wilson *et al.*, 1999) or unchanged levels of disease in progeny tubers following planting infected tubers (Adams and Hide, 1981; Burnett, 1991; Keiser *et al.*, 2007; Lapwood, 1972).

Whilst tuber disease has been the focus of most studies with *S. subterranea* in potato, root infection leading to gall production is another significant disease (Merz and Falloon, 2009; Shah *et al.*, 2012) resulting in poor growth and reduced yields (Falloon *et al.*, 1996). The relationship between seed tuber-borne pathogen inoculum and root gall production has not been studied in detail. Further study is required to better elucidate the role of seed tuber-borne inoculum on *S. subterranea* induced disease in both progeny roots and tubers.

Soil inoculum is generally regarded as the major source of disease as these pathogens are able to survive for extended periods in soil (Fiers *et al.*, 2012). Where planting occurs in new cropping areas free of soil contamination by these pathogens, diseased or contaminated seed-tubers also play a critical role in the introduction and establishment of pathogen inoculum at these sites. Quantifying inoculum levels brought in

on diseased seed will further establish the role of this inoculum source, as will understanding the role that seed grading plays in reducing this inoculum source.

In this study, we compared two sampling strategies (continuous vs point) for a range of potato seedlots and diseases over a three year period. We also utilised qPCR diagnostics on skin tissue to assess whether DNA based testing is a useful tool for measuring seed health, we compare this with traditional visual assessments; we also carry out a basic cost-benefit assessment of both assessment methodologies. We also tested alternate sampling strategies (wash material) for DNA based testing. Additionally we looked at the outcomes of planting tuber-seed carrying varying inoculum loads into pathogen-free soil in pot environments over a four year period; we measured disease in root, stolon and tuber material, as well as the role of seed-borne inoculum in contaminating pathogen-free soil. We also looked at the impact of seed grading and tuber treatments on pathogen load and the implications for growers. Finally, in collaboration with the soil pathogen diagnostic program, we tracked various seedlots into fields, evaluating the relative role of seed and soil inoculum.

This project aimed to confirm that current certification practices of soilborne diseases (visual assessment) are adequate and providing growers with quality seed at an affordable price. It also looked to validate and test qPCR technology as a tool for assessing disease loading in seedlots. Additionally the project aimed to demonstrate that planting seed carrying high inoculum levels may be associated with a greater disease risk, both in progeny roots and tubers.

MATERIALS AND METHODS

SAMPLING STRATEGIES IN CERTIFICATION SCHEMES

SELECTION OF SEEDLOTS

Commercial potato tuber seedlots were sampled over three years (2010: 12 seedlots; 2011: 12 seedlots; 2012: 13 seedlots). These included seedlots with and without obvious disease symptoms. Seedlots were popular French fry processing cultivars 'Ranger Russet', 'Innovator' or 'Russet Burbank'.

CONTINUOUS AND POINT SAMPLING

Each seedlot was assessed by visual inspection and by qPCR analysis for disease and pathogen incidence and abundance comparing two distinct sampling strategies. The first (point sampling), involved collecting two lots of 100 tuber samples, one at the beginning of the 20 tonne seedlot and one at the end, as per normal seed tuber certification practice and consistent with UNECE standards (UNECE, 2011). The second (continuous sampling), involved collecting ten lots of 20 tuber samples at regular intervals throughout the seedlot.

VISUAL DISEASE ASSESSMENT OF SEED TUBERS

Each tuber from all samples was visually assessed for black scurf, root knot nematode, powdery scab and common scab disease symptoms. Data collected was presence (a minimum of one lesion) or absence of disease from which a percentage of infected tubers per sample was obtained. For each seedlot, 12 samples were assessed (two samples of 100 tubers and ten of 20 tubers).

DNA ASSESSMENT

Following visual assessment, tuber peel was taken for qPCR analysis. A 2cm diameter layer of peel from bud end to stolon end from the entire circumference of each tuber was taken using a domestic kitchen peeler. Peels were pooled per sample (≈50 g) and dried at 40oC for 48 hours. The samples were then double-bagged and stored at room temperature (for up to 1 week) prior to qPCR analysis. The samples were tested for presence and abundance of DNA of *R. solani* AG2.1, *R. solani* AG3, *M. fallax*, *M. hapla*, *S. subterranea* and *S. scabies*.

QUANTITATIVE PCR (QPCR)

To quantify pathogen DNA levels from the tuber peel a TaqMan® MGB assay (qPCR) was developed based on soil DNA tests for these same pathogens (Ophel-Keller *et al.*, 2008; Ophel-Keller *et al.*, 2009). For *R. solani*AG2.1, AG3, *M. fallax*, *M. hapla* and *S. subterranea*, assays amplified fragments from the ribosomal ITS 1 region (Bulman and Marshall, 1998; Ophel-Keller *et al.*, 2009). For *S. scabies* a region from the thaxtomin A biosynthesis gene (txtA) was amplified (Ophel-Keller *et al.*, 2009; Crump, 2005). Quantitative data for each sample were determined by comparison to data from a serial dilution of pathogen DNA of known concentration.

STATISTICAL ANALYSES AND CORRELATIONS

To determine the relative precision of the two visual assessment methodologies and the pathogen DNA concentrations that correspond to critical disease levels, the data was modelled using Receiver operating curves (ROCs) (Krzanowski and Hand, 2009). All three years results were analysed as a pooled data set. The ROCs provided a graphical comparison for each pathogen of the effectiveness of each sampling method (point or continuous). In each ROC plot the estimated true positive (tp) rate is plotted on the vertical axis while the estimated false positive (fp) rate is plotted on the horizontal axis. The estimated tp rate is the proportion of samples with the pathogen DNA score being above a particular cutpoint given that the sample is in the infected population. The estimated fp rate is the proportion of samples in which the pathogen DNA score is above the same cutpoint but the sample is not in the infected population. The infected status is determined to be positive if the sample had 2% visual lesion assessments, and where there were sufficient data, the cut points 4, 5 or 10% visual lesion assessments were also used. The visual lesion assessments of 2, 4, 5 or 10% for the true disease status were selected for consistency with certification levels used in other countries/schemes, as described (Table 3-1). In an ideal test, the ROC rises rapidly to the top-left corner and then flattens to follow the top of the plot. In the case where the test is completely uninformative, the ROC follows a diagonal line from the bottom left to the top-right. The ROCR package in R (R Development Core Team, 2012; Sing *et al.*, 2005) was used to construct the ROCs. In each case 400 bootstrapped samples were used to calculate 95% CI for the ROC.

Table 3-1. Visual certification standards for disease tolerance in potato seed tubers for different countries and/or schemes.

Disease	Country and/or seed scheme								
		UNECE[a]			Australia[b]			Scotland[c]	
		% of tubers infected	Individual tolerance for surface area cover (%)		% of tubers infected	Individual tolerance for surface area cover		% of tubers infected	Individual tolerance for surface area cover
Powdery scab		3	10		2	nil		3	12.5
Black scurf		5	10		2	nil		3	12.5
Root knot nematode		nil	-		2	nil		nil	-
Common scab		5	33.3		2[d]	nil		4	25

[a] minimum tolerances used for Basic and certified seed potatoes produced in accordance with the United Nations Economic Commission for Europe (UNECE) standards (UNECE, 2011).

[b] minimum tolerances used for certified seed potatoes produced in Australia (VICSPA, 2007).

[c] minimum tolerances used for Basic seed potatoes produced in Scotland for marketing within the EU (SASA, 2010).

[d] Australian standard is 2% for common scab; Tasmanian standard is 4%.

To provide a comparison between methods, the areas under the ROCs were calculated. The areas provide an indication of the test performance regardless of the selected cut point. The areas range from 0.5 to 1.0; those tests with areas closer to 0.5 have little discriminatory power whereas those tests with areas closer to 1 are good discriminators. To obtain a DNA cutpoint with a good discrimination, a value of fp rate of 0.2 was selected since most of the informative ROCs were observed to bend around this point. At fp = 0.2 we report the corresponding DNA cutpoint and the associated tp value. To compare tp values and areas under curve means of continuous and point sampling a t-test was performed. Finally, to compare variances between groups, a bootstrap technique was used in which the mean absolute differences (MAD) for each group was calculated and then the proportions of differences of the MAD statistic were used to construct P values.

ALTERNATIVE SAMPLE SELECTION AND PROCESSING STRATEGIES

Over the three years of sample selection, collection and processing two trials were carried out to identify alternate options for assessing disease load within a seed lot. Trial 1 involved an alternate sampling strategy whereby skin/soil samples (from seedlot bin) within close proximity to where the tubers (10 lots of 20 tubers) had been taken from (bin/truck) to see if this was a good measure of disease risk. Two separate seedlots were sampled, a heavily diseased (powdery scab) seedlot and a seedlot with visually disease-free tubers. The soil/skin samples were analysed by qPCR with pathogen levels from each seedlot compared using ANOVA. Trial 2 involved the collection of tuber samples, washing these samples, collecting the washed material and filtration of this material. Both visually disease-free and diseased seedlots were selected for comparison with the filtrate collected on filter paper analysed by qPCR for pathogen levels. The levels were compared using ANOVA in GENSTAT v. 14.0 (VSN International Ltd., Hemel Hempstead, UK).

COSTS OF VISUAL ASSESSMENT VERSUS DNA ASSESSMENT FOR CERTIFICATION

A small study was conducted to establish the cost of certifying a typical seedlot, at harvest for key soil-borne diseases, using both visual and DNA assessment. For the purpose of this study a typical 25 tonne seedlot was used in this test case. A seed certification officer from the Tasmanian Seed Certification scheme was interviewed and provided the commercial costs associated with certifying a seedlot based on current practice for two lots of 100 tubers per seedlot. Staff from SARDI provided the costing for assessing two lots of tubers by qPCR, based on commercial pricing they provide to commercial clients for soil tests. A comparison of the costing and the benefits and limitations of both assessment systems are briefly discussed.

IMPACT OF SEED-TUBER INOCULUM ON PROGENY DISEASE

SELECTION OF TUBER SEEDLOTS

Seed-tubers were sourced from commercial seedlots with wide variation in levels of tuber disease. All were of equivalent physiological age and stored at 4 º C for 4-5 months prior to tuber selection and planting. Russet Burbank (2010 – 80 tubers) and Innovator (2011 – 140 tubers; 2012 – 110 tubers) cultivars were examined. Both varieties are moderately resistant to common and powdery scab and moderately susceptible to black scurf (Genet *et al.*, 2011; UNL, 2013; HZPC, 2013). Russet Burbank is moderately susceptible to *Spongospora* root galling with no published rating available for Innovator, however, one of its parents, Shepody, is regarded as extremely susceptible to root galling (Nitzan *et al.*, 2008).

VISUAL DISEASE ASSESSMENT OF SEED-TUBERS

Each tuber was visually assessed for black scurf, powdery scab and common scab symptoms prior to planting, and assigned a disease tuber surface cover score ranging from 0 to 6 (0 = no visible disease on tuber surface, 0.5 ≤ 1%, 1 ≥ 1-5%, 2 ≥ 5-10%, 3 ≥ 10-30%, 4 ≥ 30-50%, 5 ≥ 50-70%, 6 ≥ 70% tuber surface affected). The percentage tuber coverage was estimated from the mid values of these score ranges (Wilson *et al.*, 1999).

DNA QUANTIFICATION OF PATHOGENS ON SEED-TUBERS

Following visual assessment tuber peel was analysed for *R. solani* AG3, *S. subterranea* and *S. scabies* DNA content using qPCR. Each seed-tuber was cut in half, with one half retained for planting. The other tuber half was completely peeled (~5 mm peel depth) with a sterilised kitchen peeler and the peel sample (~ 5 g) dried at 40°C for 48 hours. Individual samples were double bagged and stored at room temperature (for up to 1 week) prior to qPCR analysis.

QUANTITATIVE PCR (QPCR) OF SEED-TUBERS

DNA extraction and qPCR was undertaken as a commercial service provided by Root Disease Testing Service (RDTS, SARDI, Adelaide, SA Australia; (Ophel-Keller *et al.*, 2009). qPCR used TaqMan® Minor Groove Binder (MGB) probes (Applied Biosystems, Foster City, CA, USA) performed on ABI PRISM® 7900HT as described previously (Riley *et al.*, 2010). The test for *R. solani* AG3 was designed by Diana Hartley (CSIRO Ecosystem Sciences, Canberra) using AG3F/AG3R primers (AG3F: 5´-AGTTTGGTTGTAGCTGGTCTATTTATGTA-3 ´, AG3R: 5 ´- GAGTAGACAGAAGGGTTCAATGAGTT-3 ´) and AG3 probe 5´- 6FAM-TGTCTCACAAGTTCACAGGT-3 ´. For *S. subterranea,* assays amplified fragments from the ribosomal ITS 1 region using the previously defined primers (Spo10, Spo11) and probe (Maldonado *et al.*, 2013). For *S. scabies* a region from the thaxtomin A biosynthesis gene was amplified using CSF/CSR primers (CSF: 5´- ACGTCCGTAACCTTCGTCTG-3 ´, CSR: 5 ´- ATAGCGGCTGAGTTCGTTGT-3 ´) and CS probe designed by Michael Tavaria (Applied Biosystems, Melbourne) 5´- 6FAM-CACGACTCCAGCGTC-3 ´. Quantitative data for each sample were determined by comparison to a serial dilution of pathogen DNA of known concentration.

PLANTING ENVIRONMENT, SETUP AND EXPERIMENTAL DESIGN

Planting in all three pot trials occurred in November (late spring), in New Town, Tasmania (GPS coordinates: 147º17'57.21"E, 42º51'24.55"S) of the respective year (2010, 2011, 2012) with pots placed outdoors in a randomised design, subject to natural spring/summer temperature variations. The tuber piece retained for planting was further halved creating two representative seed pieces which were each hand-planted (150-180mm depth) into separate 20cm diameter pots (2010 – 160 pots in total; 2011 – 280 pots; 2012 – 220 pots). The potting mix (1 part course sand: 1 part peat : 8 parts composted pine bark; pH 6.0) was tested by qPCR prior to planting (Ophel-Keller *et al.*, 2009) and determined free of detectable DNA of the three pathogens of interest. During plant establishment, prior to emergence, limited irrigation was applied to maintain moist soil. After emergence irrigation was applied at two-three days intervals, with additional irrigation when required. Soil moisture was managed to enable thorough wetting and drying to encourage multiple disease development (Falloon *et al.*, 1996; van de Graaf *et al.*, 2007; Wilson *et al.*, 2010). No pesticides were applied throughout the trials.

ASSESSMENT OF ROOT GALLING AND STOLON PRUNING

In the 2011 and 2012 trials at 12 weeks after planting one of the duplicate pots from each pair was assessed for root galls, symptomatic of *Sp. subterranea* root infection, and stolon pruning, symptomatic of

R. solani AG3 infection. Soil was allowed to dry so that it could be easily separated from the roots and stolons without damaging these plant parts. Any excess soil was gently removed in running water. A root galling score per plant was determined using a 0–4 visual rating scale modified from that of van de Graaf and colleagues (van de Graaf *et al.*, 2007); 0 = no galls; 1 = 1–2 galls; 2 = 3–10 galls, most <2 mm in diameter, 3 = >10 galls, some >2 mm in diameter; 4 = most major roots with galls, some or all >4 mm in diameter. The number of root galls per plant was also counted. Stolon pruning was assessed as the percentage of stolons per pot showing severe girdling and die-back and the presence of stem canker was recorded (Atkinson *et al.*, 2010).

ASSESSMENT OF TUBER DISEASE AT HARVEST

Tubers were harvested at plant senescence and stored at 4°C for approximately 1-2 weeks, prior to disease assessment. Tubers were washed and each tuber (> 4g) scored (0 -6) for the three tuber diseases (common scab, powdery scab, black scurf) as described earlier. An average score and percentage tuber coverage was obtained per pot. The proportion of tubers with no visible lesions per pot was also recorded (Wilson *et al.*, 1999).

STATISTICAL ANALYSIS AND MODELLING

For each of the diseases, a modelling strategy was developed to investigate whether an association could be found between the DNA status and/or visual score of disease on the seed tuber and disease in the progeny tubers (black scurf, common scab, powdery scab), progeny stolons (stolon pruning) or with progeny roots (root galling). Where one of DNA or visual score was a successful predictor it was dropped and the analysis repeated to see if the second predictor was significant. Further, for powdery scab relationships between galling and progeny tuber disease were established.

Initially, the CTREE function of the PARTY library in R (R Development Core Team, 2012) was used to conduct recursive partitioning (Hothorn *et al.*, 2006). This estimates a regression relationship by binary recursive partitioning in a conditional inference framework. Essentially it splits the outcome variable (e.g. tuber disease) in an optimal way using the explanatory variables (seed tuber DNA levels or visual scores). Each split identified by the CTREE function is always significant. This approach attempts to find the `best' model and was used to identify cut-points (where valid) for further modelling.

Then, receiver operating curves (ROCs) (Krzanowski and Hand, 2009) were constructed using the ROCR package in R (R Development Core Team, 2012; Sing *et al.*, 2005). In each ROC plot the estimated true positive (tp) rate is plotted on the vertical axis while the estimated false positive (fp) rate is plotted on the horizontal axis. The estimated tp rate is the proportion of samples with the pathogen DNA score being above a particular cut-point given that the sample is in the infected population. The estimated fp rate is the proportion of samples in which the pathogen DNA score is above the same cut-point but the sample is not in the infected population. In each case 100 bootstrapped samples were used to calculate 95% CI for the ROC.

The aim of the ROC plot is to examine the relationship of the cut-points to the proportion of tp's and fp's. Ideally, where a predictor is a useful one, the curve rises rapidly to the top-left corner and then flattens to follow the top of the plot. In the case where a predictor is not informative, the curve follows a diagonal line from the bottom left to the top-right. The areas under the ROCs (AUC) were calculated to provide an indication of the test performance regardless of the selected cut-point. The AUC can range from 0.5 to 1.0; those tests with areas closer to 0.5 have little discriminatory power whereas those tests with areas closer to 1 are good discriminators. The AUC can also be interpreted as the average probability of correctly predicting a positive case across all possible cut-points of the predictor. A rule of thumb is that a test with an AUC greater than 0.9 has high accuracy, while 0.7 - 0.9 indicates moderate accuracy, 0.5 - 0.7 low

accuracy, and 0.5 a chance result (Fischer *et al.*, 2003). Comparisons of AUC were done by bootstrapping the curves using the roc.test function in the pROC library in R (Robin *et al.*, 2011).

SEED AND SOIL BASED INTERACTIONS

SEED CONTRIBUTION TO SOIL INOCULUM AND IMPACT ON SUBSEQUENT CROP (POT STUDIES)

While seed-tuber inoculum can contribute to progeny disease another outcome is the build-up of pathogen inoculum in the soil that may impact subsequent crops. Over two consecutive years a range of pots that had grown potato plants in the previous year described above were kept for soil pathogen qPCR analysis and re-planted with mini tubers (qPCR confirmed negligible pathogen levels on tubers) to see the impact of the soil inoculum on subsequent disease. The pots chosen for soil analysis included those that had both produced low, moderate and high tuber disease and those that had produced disease-free tubers. After tuber harvest, fifty grams of soil was taken per pot and sent to SARDI for qPCR analysis. Trials were carried out in both the 2011-12 and 2012-13 season with 58 and 57 pots replanted respectively. Tubers were assessed at harvest (senescence) and the relationship between inoculum level in the soil and disease analysed using the Spearman correlation coefficient in GENSTAT v. 14.0 (VSN International Ltd., Hemel Hempstead, UK). This correlation coefficient (r) measures the linear association between two non-parametric continuous variables; conventionally: r of 0: indicates no linear relationship, r of 0.2-0.4: very weak relationship, r of 0.4-0.6: moderate relationship, r of 0.6-0.8: strong relationship, and r of 0.8-1.0: very strong relationship (Taylor, 1990).

FIELD STUDIES (IMPACT OF SEED AND SOIL ON DISEASE EXPRESSION)

As part of the interaction between the seed-based project and soil-based diagnostic project (PT09023) a number of studies aimed to elucidate the importance of both inoculum sources. Over two consecutive years (2012 and 2013) trials were planted on the NW coast of Tasmania with 4 different seedlots carrying varying pathogen loads planted into two separate field sites per year (4 trials in total).

Selection of potato lines was based on key diseases important to industry and key disease prevalence in the preceding years; the most prevalent being powdery scab, common scab and black scurf. Various seed lines were sourced from the TIA seed certification team and were analysed both visually and using DNA readings (from tuber peel) to ensure a range of seed carrying both low and high levels of pathogen inoculum. Two field sites were selected (Forthside, Wesley Vale) with varying soil pathogen content. At both sites, 4 different seed lines were planted. In 2012 seed included: 1) Ranger Russet having low pathogen content; 2) Ranger Russet with moderate levels of Powdery scab and *Rhizoctonia*; 3) Mac1 with very high levels of powdery scab; and 4) Mac 1 with low powdery scab and moderate-high *Rhizoctonia*. At both field sites 3 replicates of each seed line was planted with 33 plants per replicate. In 2013 seed included 1) Innovator having low pathogen content; 2) Innovator with high levels of Powdery scab and moderate *Rhizoctonia* AG3; 3) Russet Burbank with very high levels of powdery scab; and 4) Russet Burbank with low powdery scab and *Rhizoctonia*. Additionally, some disease free mini-tubers were also planted. At both field sites 3 replicates of each seed line was planted with 34 plants per replicate.

The large numbers of plants enabled sequential harvesting at key periods throughout the growing season to enable identification of particularly root and stolon infection (these criteria are regarded as essential, particularly for powdery scab gall development in roots). The remainder of the plants were grown through to senescence with tubers harvested and scored for disease. Results were analysed by ANOVA with treatment differences identified by LSDs in GENSTAT v. 14.0 (VSN International Ltd., Hemel Hempstead, UK).

Additionally some observational studies were conducted in commercial paddocks over the duration of this project. Five commercial seedlots were traced into commercial paddocks including seed with low and high pathogen content, and soil carrying varying inoculum loads. The seed and soil pathogen content was determined by seed and soil sampling at planting and subsequent qPCR at SARDI. At harvest disease was assessed to determine the importance of both seed and soilborne inoculum.

SEED TREATMENTS AND GRADING

SEED WASHING

A small trial was carried out in Year 2 to quantify whether pathogen inoculum could be washed from infected seed tubers and significantly reduce the pathogen inoculum on the tuber surface (quantified by qPCR). Two random seedlots were selected and 40 tubers taken from each. Twenty tubers were thoroughly washed, peeled and sent to SARDI for qPCR; the remaining 20 unwashed tubers were also peeled for qPCR analysis. The t-test was used to separate treatment effects.

SEED GRADING

Utilising the commercial seedlots that were used in Years 1-3 , a seed grading experiment was carried out over two years to identify whether seed grading was masking an underlying problem i.e. are visually diseased tubers or tubers with high pathogen DNA loads getting through the grading process. In both years (2011 and 2012) seedlots with a high disease load were graded heavily and tubers sampled both pre (5 samples of 20 tubers) and after-grading (10 samples of 20 tubers) and visually assessed for disease and tubers peeled and sent to SARDI for DNA analysis. Two seedlots were selected in both years for analysis.

In the final year of the project (2013/14) both a pot and 2 field trials looked in further detail at the issue of seed grading. The pot trial included three different seed lines (Innovator, Russet Burbank, and Innovator hybrid) that were heavily infested with powdery scab. Each seed line was heavily graded and separated into visually disease-free and visually diseased groups. From each group 20 tubers were selected, tubers were halved with one half peeled, and sent to SARDI for qPCR. The other half was cut in half and each seed piece placed into duplicate pots. There were a total of 240 pots (120 in duplicate) and they were placed in an outdoors environment at New Town, Tasmania, subject to natural growing conditions. Pots were treated and managed as described above for previous experiments. One of the duplicate pots was harvested at 12 weeks after planting for root gall assessment with the other pot grown through to senescence and assessed for disease. All disease assessments were as described earlier. Results were analysed by ANOVA with treatment differences identified by LSDs in GENSTAT v. 14.0 (VSN International Ltd., Hemel Hempstead, UK).

Two field trials looked both at seed grading and the impact of chemical treatment on disease development. The trials were established on commercial farms in NW Tasmania in 2013-14 on potato growing soils typical of the region; field trial 1 (F1) on a red ferrosol soil and field trial 2 (F2) on a brown dermosol soil. Both trials were located at Wesley Vale and they were planted in October 2013. At all field sites powdery scab had been previously recorded in prior potato crops with analysis of soil samples just prior to planting by qPCR (50 – 100 pg *S. subterranea* DNA/g soil). Planting material for all field trials was commercial seed of both 'Russet Burbank' and 'Innovator' with visually obvious powdery scab symptoms; each individual seed tuber having a surface cover score of 2 (i.e. surface cover percentage of c. 5 -10% powdery scab). Various seed treatments were applied at planting and included untreated seed, seed dipped in 1% formalin, seed dipped in fluazinam (7.5 g/10 kg seed) and seed dipped in mancozeb (32 g/10 kg seed). Soil treatments included in-furrow sprays of fluazinam (3.0 kg/ha i.e. 6L/ha in 1000 L of water) and mancozeb (7.5 kg/ha i.e.

18L/ha in 1000 L of water) applied with a knap sack sprayer (Matabi 18L Elegance, Goizper Spraying, Portugal). Additionally, visually disease-free seed was graded from the above lines and also planted as a treatment. Mini-tubers were also planted as an additional control

Each treatment plot contained 15 seed tubers, spaced at 30 cm, with plots arranged in a randomized split-plot design and replicated two times in each trial. Fertiliser and irrigation scheduling followed standard commercial practice with no additional seed or soil pesticides applied. In both FTs the average emergence date was 25th November 2013 with sequential harvesting of one plant per plot made at 15, 30, 45, 60 and 75 days after emergence (DAE). Plants were gently uprooted using a fork with roots kept for infection and gall assessment (stored at 4ºC for up to two days). All other plants (10 per plot) were grown until senescence. All tubers were harvested and a random sample of 20 tubers per plot selected for disease assessment. ANOVA was used for analysing treatment effects.

RESULTS

SAMPLING STRATEGIES IN CERTIFICATION SCHEMES

PREVALENCE OF SEED TUBER-BORNE DISEASE WITHIN SEEDLOTS

Over the three years of the study the diseases that presented in the samples were powdery scab, black scurf, root knot nematode and common scab. Powdery scab was the most prevalent disease in the 37 seedlots assessed, occurring in 33 (89%) of the seedlots. Black scurf occurred in 17 seedlots (46%), root knot nematode in 15 (41%), and common scab in 8 (22%) of the seedlots (

Table 3-2).

MODELLING OF VISUAL ASSESSMENTS WITH PATHOGEN DNA CONCENTRATION

The relationships between visual disease assessments and specific pathogen DNA concentrations as determined by qPCR are summarised in Table 3-3. The pathogen qPCR tests that were good discriminators of visual disease presence were *R. solani* AG3, *M. fallax* and *S. subterranea*, while the qPCR test for *S. scabies* provided adequate discrimination (Figure 3-1). The other two pathogen qPCR tests, *R. solani* AG2.1 and *M. hapla* were poorly associated with visual disease presence (Figure 3-1). The estimated area under each ROC provides the probability of the DNA score being above a cutpoint given that the sample is in the infected population (Table 3-3). Large values (at 2% visual cut-off) ranging from 0.85 – 0.92 for *R. solani* AG3, *M. fallax* and *S. subterranea* suggest the DNA tests provide good discriminators of visual assessment, with values slightly less for *S. scabies* (0.63 – 0.75). Values ranging from 0.48 – 0.57 for *R. solani* AG2.1 and *M. hapla* suggest poor discrimination.

As the curves demonstrate, obtaining a greater true positive (tp) is possible but at the cost of increasing the false positive rate (fp). The DNA cut-off levels were made at fp = 0.2 since this is a reasonably low false positive error rate and is the point at which most informative curves tended to bend. At this point the true positive rates are then reasonably high. The DNA levels of 523 pg DNA/g peel for *M. fallax*, 1,503 pg/g for *S. scabies*, 7,072 pg/g for *S. subterranea* and 18,089 pg/g for *R. solani* AG3 are the values derived from continuous sampling above which a crop would fail certification at the 2% visual cut-off (Table 3-3).

COMPARISON OF POINT AND CONTINUOUS SAMPLING

To assess the effectiveness of the two visual sampling strategies a comparison of the DNA cut offs and areas under the ROCs were made. The point and continuous sampling strategies produced similarly effective tests with no significant difference in mean tp at fp = 0.2. In addition, the areas under the ROCs were not significantly different (Table 3-3), which meant that across all possible cut points there was no difference between the methods. However, the continuous sampling strategy was more reliable as demonstrated by smaller variances leading to tighter 95% confidence intervals (Table 3-3).

ALTERNATIVE SAMPLE SELECTION AND PROCESSING STRATEGIES

Trial 1: For powdery scab, the skin/soil sample collected from the bottom of the seedlot did provide some degree of correlation with the disease status of the seedlot. The skin/soil taken from a bin of a heavily diseased seed-lot (22454 pgDNA/g sample) having significantly greater (3 times) DNA levels of *S. subterranea* than from soil/skin taken from a bin of disease-free tubers (6785 pgDNA/g sample).

Trial 2: Preparation of samples from filter paper for qPCR was time consuming requiring multiple sample handling procedures (A. McKay, pers. comm.). Once processed the pathogen levels detected in the water filtrate were consistent with the levels detected via standard peel based qPCR i.e. tubers from highly diseased seedlots had significantly greater pathogen levels (245634 pg *S. subterranea* DNA/g peel) detected than tubers from visually disease-free seedlots (9345 pg *S. subterranea* DNA/g peel).

COSTS OF VISUAL ASSESSMENT VERSUS DNA ASSESSMENT FOR CERTIFICATION

For certifying a seedlot the costs of visual assessment were $4/tonne indicating a standard 25 tonne seedlot would be $100 to visually certify, typically this would correspond with assessing two lots of 100 tubers. A comparison with DNA assessment indicated that a minimum of 2 lots of tubers would need to be assessed per 25 tonne seedlot. One DNA test and the report that comes with it is $180 (based on the commercial soil test in PredictaPt) and the costs for the peel test would be similar. Two tests would equate to $360. Additional costs for DNA sample preparation including peeling, drying (for 48h in specialised drying oven), posting sample and the labour associated with this would be $90. The total cost therefore to certify a 25 tonne seedlot by DNA would therefore be $450. This is a 4-fold greater cost than visual certification. Other key points noted was that visual certification occurs on the spot and a result is available immediately, DNA samples require processing, drying, posting and then qPCR analysis, so a result may take 1-2 weeks. Visual certification requires skilled certification officers whereas DNA samples can be prepared by anyone and sent to a lab for DNA analysis. While both these assessment methods provide a good measure of disease status of a seedlot, qPCR can be useful to differentiate some pathogens and also identify pathogens that are not visually obvious on tubers.

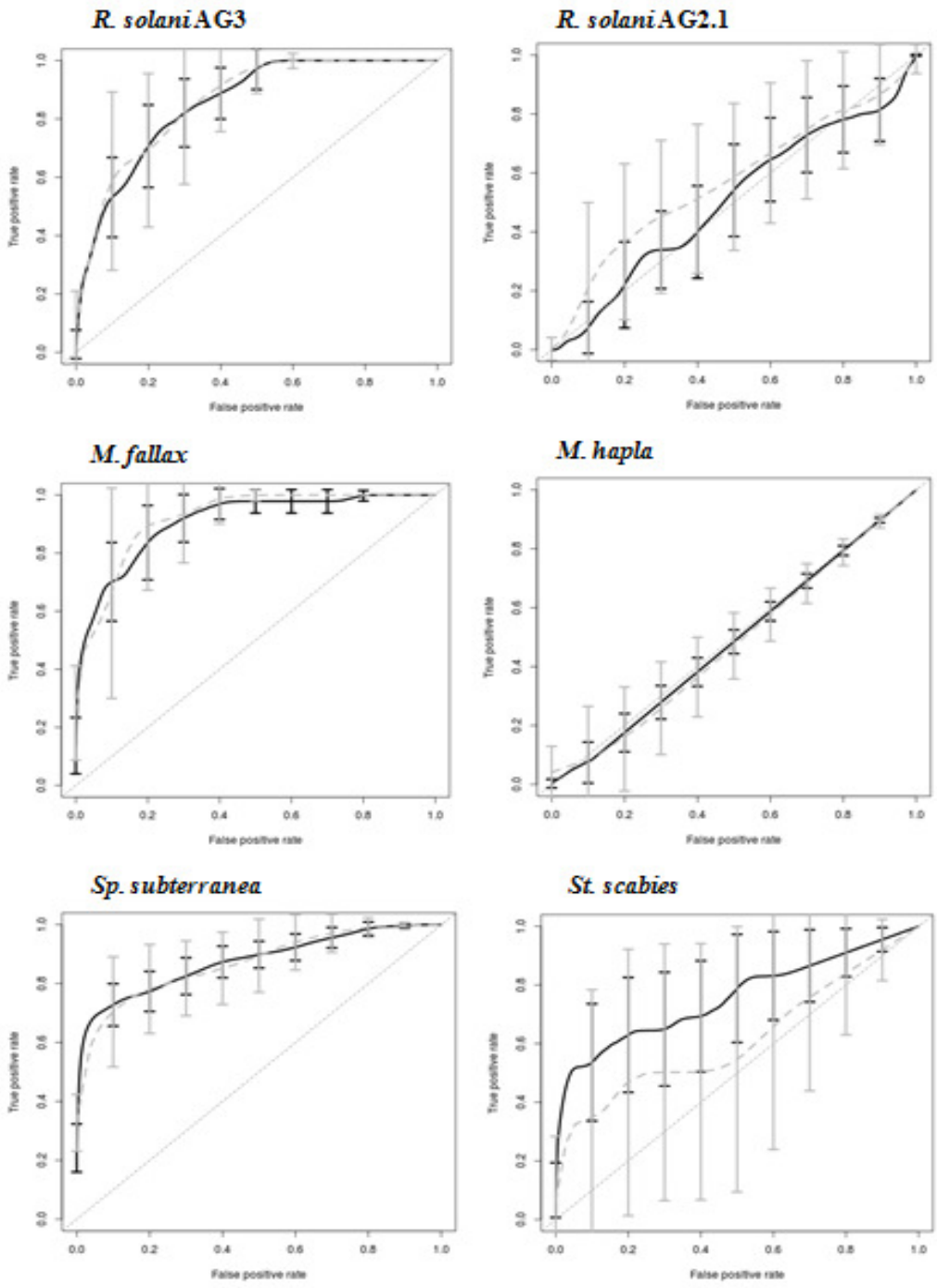


Figure 3-1. Receiver operating curves for DNA of six different seed tuber-borne potato pathogens, where a 2 % cut off on visual assessments is taken to mean infested. Continuous sampling (ten lots of 20 tuber samples) is shown by solid lines; point sampling (two lots of 100 tuber samples) is shown by dashed lines; and the chance diagonals are dotted lines. Bars indicate 95% confidence levels.

Table 3-2. Seed tuber-borne diseases visually identified in 37 Tasmanian potato seedlots sampled between 2010-2012.

Disease	Visual certification standard (% of tubers infected)[a]	2010		2011		2012	
		Number of seedlots with visual symptoms (percentage of tubers infected)[b]	Proportion of seedlots failing certification[c]	Number of seedlots with visual symptoms (percentage of tubers infected)	Proportion of seedlots failing certification	Number of seedlots with visual symptoms (percentage of tubers infected)	Proportion of seedlots failing certification
Powdery scab	2	9/12 (0 – 12.0)	6/12	11/12 (0 – 38.0)	7/12	13/13 (0.5 – 36.0)	11/13
Black scurf	2	6/12 (0 – 4.0)	4/12	9/12 (0 – 11.0)	4/12	2/13 (0 – 1.5)	0/13
Root knot nematode	2	5/12 (0 – 5.0)	2/12	5/12 (0 – 1.0)	0/12	5/13 (0 – 71.0)	3/13
Common scab	2	6/12 (0 – 6.5)	2/12	0/12	0/12	2/13 (0 – 1.5)	0/13

[a] based on Australian potato certification standards (VICSPA, 2007); the presence of one lesion indicates an infected tuber.

[b] number of seedlots per year that showed any visual presence of the diseases been assessed for; (the minimum and maximum percentage of tubers infected from the best and worst seedlots in any given year).

[c] Those seedlots not meeting Australian certification standards for the diseases been assessed.

Table 3-3. Associations of amounts of pathogen DNA (pg/g sample) from continuous (ten lots of 20 tuber samples) or point sampling (two lots of 100 tuber samples) strategies with visual disease cut-off values.

Pathogen	Visual disease assessment cut-off		P value for Comparison of tp at fp=0.2[a]		Pathogen tuber peel DNA cut-points (95% CI) corresponding to disease cut-off[b]				Areas under receiver operating curve (95% CI)[c]		
	(%)				Sampling method				Sampling method		
					continuous	point	P value variance		continuous	point	P value mean
R. solani AG2.1	2		0.80		8078 (6092 – 10389)	6155 (3576 – 7849)	<.01		0.50 (0.42 – 0.58)	0.57 (0.43 – 0.75)	0.77
	4		0.78		8088 (5968 – 10461)	6929 (5343 – 9061)	<.01		0.50 (0.42 – 0.58)	0.51 (0.26 – 0.76)	0.53
	5		0.42		8108 (6097 – 10143)	7575 (5431 – 10362)	<.01		0.50 (0.42 – 0.60)	0.38 (0.12 – 0.67)	0.21
R. solani AG3	2		0.54		18089 (12320 – 24570)	15068 (4999 – 25677)	<.01		0.85 (0.81 – 0.90)	0.86 (0.76 – 0.94)	0.54
	4		0.34		18223 (11935 – 24576)	28835 (9065 – 57570)	<.01		0.85 (0.81 – 0.90)	0.83 (0.68 – 0.94)	0.40
	5		0.31		18273 (13205 – 24820)	30917 (13343 – 55570)	<.01		0.85 (0.81 – 0.90)	0.64 (0.64 – 0.95)	0.49

Pathogen	Visual disease assessment cut-off		P value for Comparison of tp at fp=0.2[a]		Pathogen tuber peel DNA cut-points (95% CI) corresponding to disease cut-off[b]				Areas under receiver operating curve (95% CI)[c]		
M. fallax	2		0.66		523 (334 – 677)	409 (219 – 959)	<.01		0.87 (0.76 – 0.96)	0.92 (0.69 – 1.00)	0.67
	4		0.98		523 (334 – 662)	565 (250 – 1054)	<.01		0.88 (0.75 – 0.96)	1.00 (1.00 – 1.00)	0.99
	5		0.98		520 (332 – 664)	605 (277 – 1054)	<.01		0.88 (0.76 – 0.97)	1.00 (1.00 – 1.00)	0.99
M. hapla	2		0.61		1.1 (1.0 – 2.0)	2.5 (2.0 – 4.0)	<.01		0.49 (0.45 – 0.53)	0.48 (0.41 – 0.59)	0.45
	4		0.71		1.1 (1.0 – 2.0)	2.5 (2.0 – 4.0)	<.01		0.49 (0.45 – 0.53)	0.51 (0.41 – 0.66)	0.63
	5		0.72		1.0 (1.0 – 2.0)	2.6 (2.0 – 4.0)	<.01		0.49 (0.45 – 0.52)	0.51 (0.41 – 0.66)	0.63
S. subterranea	2		0.59		7072 (4245 – 9053)	5159 (585 – 11308)	<.01		0.88 (0.84 – 0.91)	0.88 (0.80 – 0.95)	0.55
	4		0.37		6914 (4558 – 9038)	17333 (6183 – 74804)	<.01		0.88 (0.84 – 0.91)	0.89 (0.80 – 0.96)	0.63

Pathogen	Visual disease assessment cut-off		P value for Comparison of tp at fp=0.2[a]		Pathogen tuber peel DNA cut-points (95% CI) corresponding to disease cut-off[b]				Areas under receiver operating curve (95% CI)[c]		
	5		0.96		7046 (4686 – 9094)	26500 (8006 – 82605)	<.01		0.84 (0.84 – 0.91)	0.97 (0.92 – 1.00)	0.99
	10		0.96		34415 (13824 – 66594)	88211 (16778 – 150190)	<.01		0.90 (0.86 – 0.94)	0.99 (0.96 – 1.00)	0.99
S. scabies	2		0.29		1503 (964 – 2075)	2459 (556 – 7157)	<.01		0.76 (0.62 – 0.88)	0.63 (0.31 – 0.93)	0.23

[a] Probability of continuous and point sampling giving different true positive rate (tp) at a false positive rate (fp) of 0.2. The estimated tp rate is the proportion of samples with the pathogen DNA score being above a particular cutpoint given that the sample is in the infected population. The estimated fp rate is the proportion of samples in which the pathogen DNA score is above the same cutpoint but the sample is not in the infected population.

[b] Pathogen tuber peel DNA content (pgDNA/g sample) cut-points corresponding to fp of 0.2; probability of continuous and point sampling giving different DNA content variances as an estimate of data precision.

[c] Area under the receiver operating curve; probability of continuous and point sampling giving different curve area means.

IMPACT OF SEED-TUBER INOCULUM ON PROGENY DISEASE

Over the three years of this case study the diseases that presented in the seed-tubers and progeny tubers were powdery scab, black scurf and common scab. Galling due to *S. subterranea* infection in roots and stolon pruning due to *R. solani* AG3 infection were also recorded. Exploratory analysis of the data for tuber disease (Figs. 3-2 – 3-4) indicated that there were no significant effects of cultivar on the disease outcomes assessed. Thus data from both Russet Burbank and Innovator were pooled, to provide a larger data set, with each of the key diseases then modelled separately. Root galling and stolon pruning (Figs. 3.5 – 3.7) was measured in the 2011 and 2012 trials with Innovator only.

Pathogen DNA and visual disease scores of seed-tubers were significantly associated with tuber disease of the progeny tubers for *Rhizoctonia solani* AG3 and black scurf ($P < 0.001$, Figure 3-2A, B) and *Streptomyces scabies* and common scab ($P = 0.004$, Figure 3-3A, $P < 0.001$, Figure 3-3B). Recursive partitioning identified two significant DNA cut points (Figure 3-2A, C and Figure 3-3A,C) and the importance of visual status of the seed-tuber (Figure 3-2B, C and Figure 3-3B, C). At higher pathogen DNA levels (>17,260 pg *R. solani* AG3 or >12,453 pg *S. scabies*) and where positive disease scores were made the resultant tuber disease was greater. The high AUC values (Figure 3-2D, Figure 3-3D) of 0.97 for both *R. solani* AG3 – black scurf and *S. scabies* – common scab, and an associated tight 95% CI, suggests the pathogen DNA tests provided a highly accurate discriminator of resultant tuber disease.

In contrast, neither amount of *S. subterranea* DNA nor visual disease status of the seed-tuber had a significant effect ($P > 0.05$) on powdery scab tuber surface cover disease in the progeny. To establish a relationship, we modelled a simplified outcome, namely, the presence/absence of tuber disease in the progeny, as a function of the visual status of the seed-tuber (Figure 3-4Figure 3-4A). This model showed a significant effect ($P < 0.001$) indicating that a seed-tuber with a visual score >0 would correspond to a greater chance of disease in the progeny tubers. Indeed 60% (110/183) of the pots produced tuber disease from visually infected seed-tubers, whereas only 24% (32/134) of the pots recorded disease from symptomless seed-tubers (Fig. 3-3A). A lack of a significant DNA cut-point (Figure 3-4B) and an AUC (Figure 3-4C) produced for *S. subterranea* – powdery scab of 0.72, with associated larger 95% CIs, suggested the pathogen DNA tests provided only a moderately accurate discriminator of resultant tuber disease.

We compared the models and ROCs for the three pathogens and tuber diseases above by using bootstrapping to compare their AUC (Robin *et al.*, 2011). Both *R. solani* AG3 and *St. scabies* DNA levels were highly accurate predictors of black scurf (Figure 3-2D) and common scab (Figure 3-3D), respectively, with no significance difference ($P = 0.99$) between their AUCs. Both these pathogens were significantly ($P < 0.001$) better predictors of disease in progeny tubers than that of *S. subterranea* DNA level in predicting powdery scab (Figure 3-4C) as determined by comparison of their AUCs.

Both visual score ($P < 0.01$, Figure 3-5A) and pathogen DNA level ($P = 0.014$, Figure 3-5B) of seed-tubers had significant effects on the production of root galls in the progeny plants. While a positive visual score on the seed-tuber led to increased root galling in the progeny, analysis detected a further significant split, whereby symptomless seed-tubers having a pathogen DNA level (>22,345 pg *S. subterranea* DNA/g peel sample) led to greater ($P < 0.001$) root galling than symptomless seed-tubers with a lower pathogen DNA level (Figure 3-5A). After excluding visual effects, recursive partitioning identified two significant pathogen DNA cut-points (>21,110 and >31,326 pg *S. subterranea* DNA/g peel sample), above which greater root galling in the progeny plants occurred (Figure 3-5B, C). The AUC (Figure 3-5D) for *S. subterranea* – root galling was 0.83 which suggested the DNA tests provided a moderately accurate prediction of root galling risk.

The number of root galls ($P < 0.001$, Figure 3-6A) was associated with powdery scab tuber disease (%) in the progeny tubers. Recursive partitioning identified a significant cut-point of seven galls per plant, if gall number exceeded that number then greater tuber powdery scab was likely to occur (Figure 3-6A, B). The

AUC (Figure 3-6C) for root galling - tuber disease of 0.76 suggested the gall number provided a moderately accurate discriminator of resultant tuber powdery scab.

Comparing the models and ROCs generated for powdery scab and root galling using their AUCs (Robin *et al.*, 2011), indicated that the association between seed-tuber pathogen DNA level and root galling production (Figure 3-5D) was significantly stronger (p=0.002) than that between seed-tuber pathogen DNA level and tuber progeny powdery scab (Figure 3-4C).

Both pathogen DNA level ($P < 0.001$, Figure 3-7A) and visual score ($P < 0.001$, Figure 3-7B) of seed-tubers had significant effects on the proportion of pruned stolons in the progeny plants. Recursive partitioning identified two significant pathogen DNA cut-points, (Figure 3-7A, C) above which greater stolon pruning occurred. Where the visual status of the seed tuber was higher (visual score >0.5, Figure 3-7B) increased stolon pruning was recorded in the progeny. The AUC (Figure 3-7D) for *R. solani* AG3 – stolon pruning was 0.92 which suggested the DNA tests provided a good prediction of resultant stolon pruning. Infrequent occurrence of stem canker due to *R. solani* infection of the potato stem was noted. These were always associated with higher pathogen DNA levels and visual scores in the seed-tuber but there were insufficient data points for analysis.

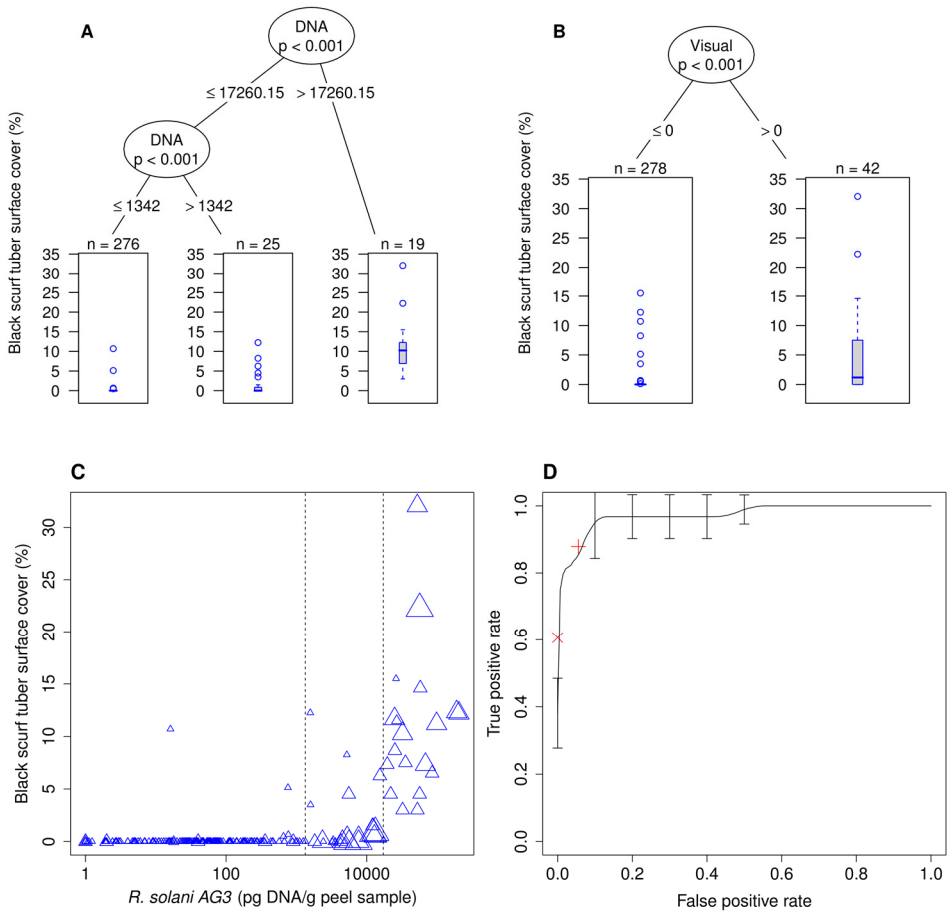


Figure 3-2. Effect of seed-tuber inoculum estimated by visual disease and *R. solani* AG3 DNA concentration on black scurf in progeny tubers. In A**, recursive partitioning shows a significant association of pathogen DNA level with the black scurf tuber surface cover %. In** B**, after excluding DNA, recursive partitioning detects a significant association of the visual status of seed-tuber with black scurf tuber cover %. In** C**, Black scurf tuber surface cover % is plotted against *R. solani* AG3 (pg DNA/g peel sample), showing cut-points from the recursive partitioning indicated by vertical dashed lines; small triangle for visual = 0, large inverted triangle for visual > 0. In** D**, Bootstrapped receiver operating curve for disease using DNA level as a predictor of disease in progeny, 95% confidence intervals, and cut-points shown as symbols: +: DNA = 1342; x: DNA = 17,260 (n = 320). The mean AUC is 0.973 with 95% CI 0.930, 0.995.**

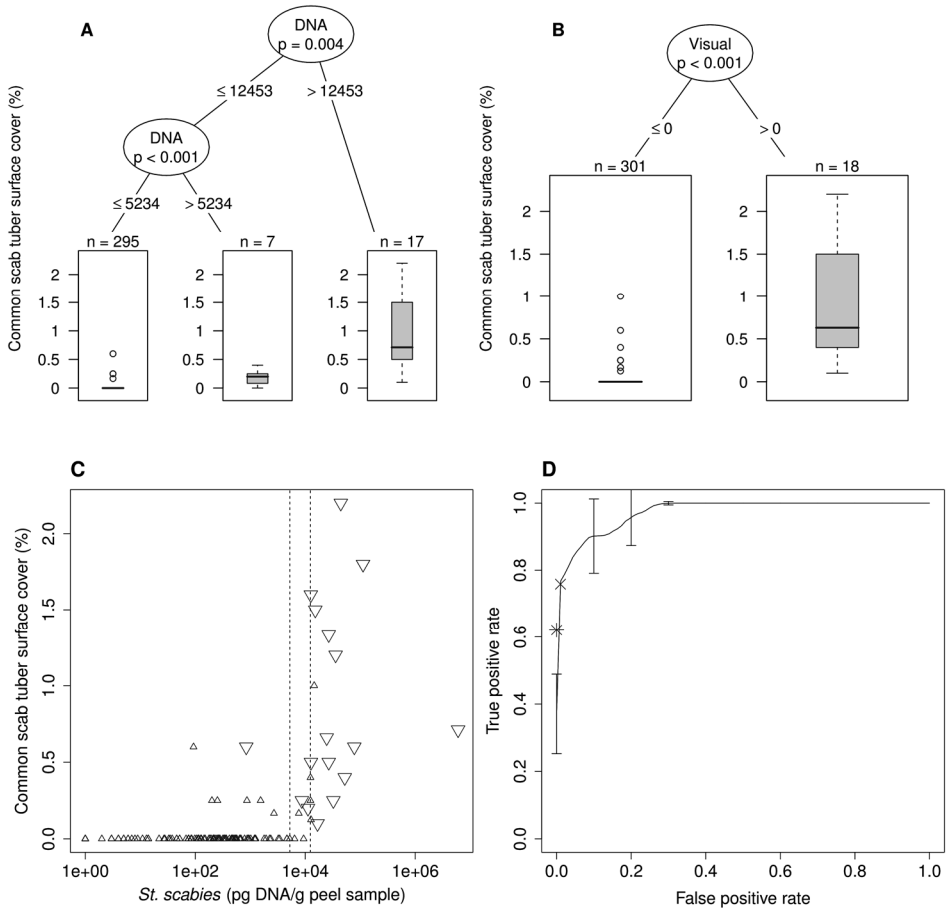


Figure 3-3. Effect of seed-tuber inoculum estimated by visual disease and *Streptomyces scabies* DNA concentration on common scab in progeny tubers. In A, recursive partitioning shows a significant association of pathogen DNA level with the common scab tuber surface cover %. In B, after excluding DNA, recursive partitioning detects a significant association of the visual status of seed-tuber with common scab tuber surface cover %. In C, Common scab tuber surface cover % is plotted against *S. scabies* (pg DNA/g peel sample), showing cut-points from the recursive partitioning indicated by vertical dashed lines; small triangle for visual = 0, large inverted triangle for visual > 0. In D, Bootstrapped receiver operating curve for disease using DNA level as a predictor of disease in progeny, 95% confidence intervals, and cut-points shown as symbols: x: DNA = 5234; *: DNA = 12,453 (n = 319). The mean AUC is 0.974 with 95% CI 0.951, 0.993.

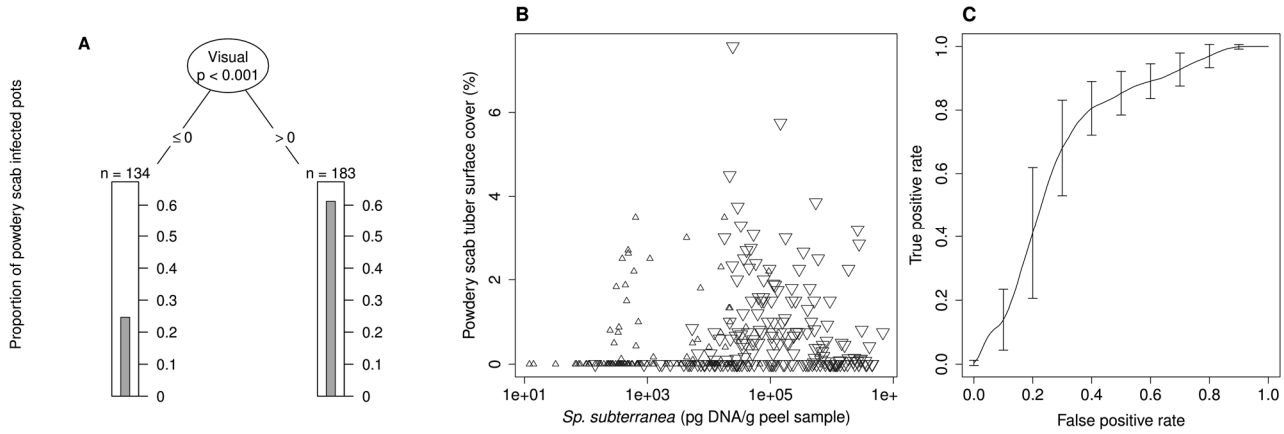


Figure 3-4. Effect of seed-tuber inoculum estimated by visual disease and *Spongospora subterranea* DNA concentration on powdery scab in progeny tubers. Recursive partitioning did not detect a significant association of pathogen DNA level or visual status of seed-tuber with powdery scab surface cover %. However, in A, simplified recursive partitioning detected a significant association of the visual status of seed-tuber on the proportion (presence/absence) of powdery scab infected pots in the progeny; i.e. progeny disease was more often present with non-zero visual scores of the seed-tuber. In B, Powdery scab tuber surface cover % is plotted against *S. subterranea* (pg DNA/g peel sample); small triangle for visual = 0, large inverted triangle for visual > 0. In C, receiver operating curve for presence/absence of disease using DNA level as a predictor of disease in progeny (n = 319). The mean AUC is 0.72 with 95% CI 0.664, 0.770.

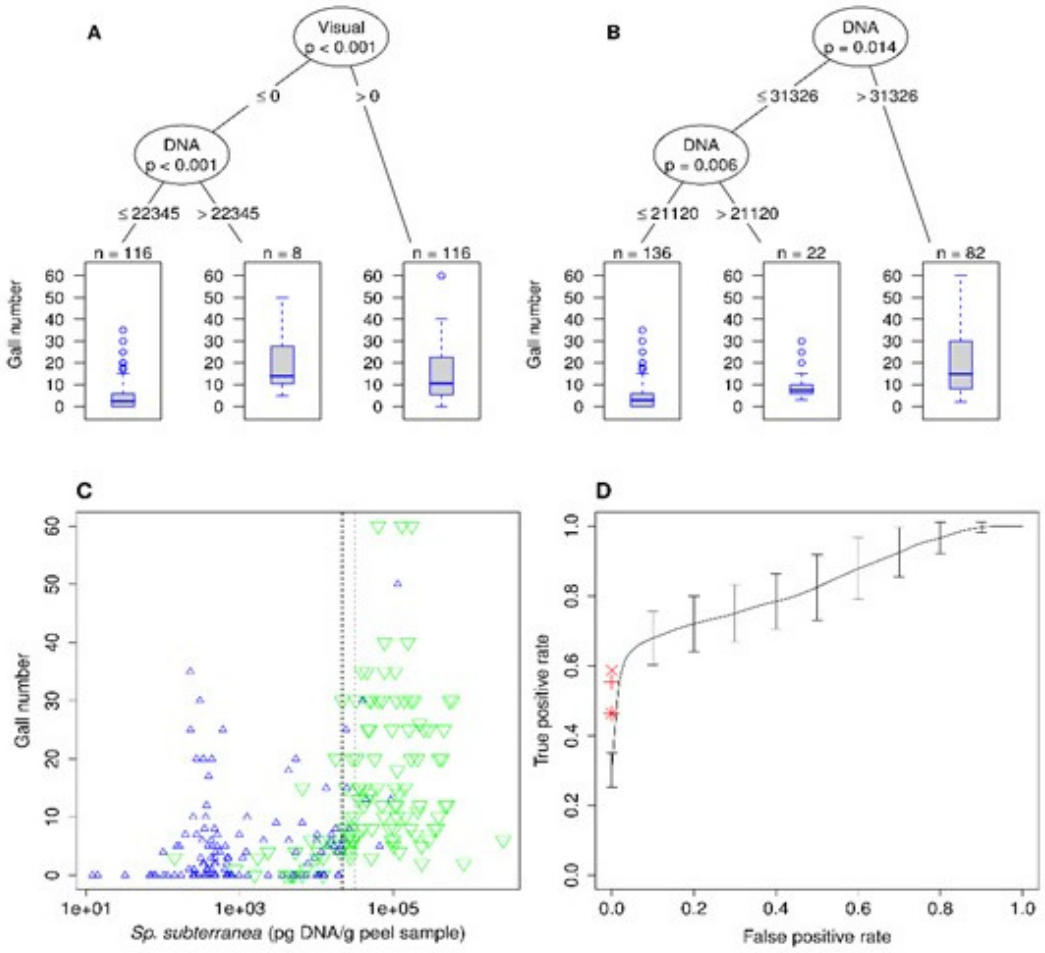


Figure 3-5. Effect of seed-tuber inoculum estimated by visual disease and *Spongospora subterranea* DNA concentration on root galling in progeny plants. In A, recursive partitioning shows a significant association of the visual status and the pathogen DNA level of the seed-tuber with the gall number. In B, that after excluding visual status, recursive partitioning detects a significant association of the DNA level of seed-tuber with root gall number. In C, gall number is plotted against *S. subterranea* (pg DNA/g peel sample), showing cut-points from the recursive partitioning indicated by vertical dashed lines; small triangle for visual = 0, large inverted triangle for visual > 0. We calculated ROCs for at least 1, 2, …, 30 galls and calculated the area under each of these curves. The maximum area was obtained from the curve predicting at least 2 galls. In D, Bootstrapped receiver operating curve for the presence of at least 2 galls using DNA as a predictor of galls, 95% confidence intervals, and cut-points shown as symbols: x: DNA = 21,120; +: DNA = 22,345; *: DNA = 31,326 (n = 240). The AUC is 0.833 with 95% CI 0.774, 0.878.

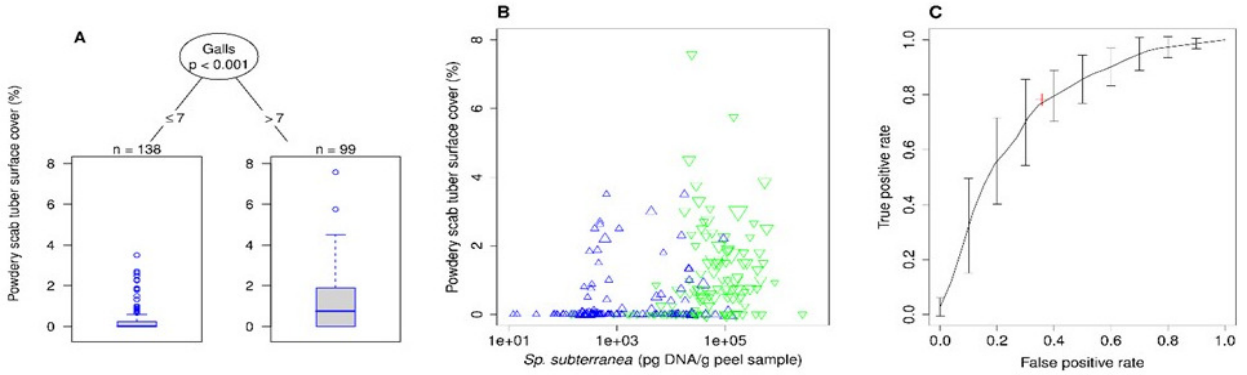


Figure 3-6. Relationship between root galling and powdery scab disease in progeny tubers. In A, recursive partitioning shows a significant association of gall number with tuber disease in the progeny. In B, Powdery scab surface cover (%) is plotted against the *S. subterranea* DNA content of the seed tuber. Symbols are sized in proportion to gall number, triangle for visual = 0, inverted triangle for visual > 0. In C, Bootstrapped receiver operating curve for the presence of tuber disease using the number of galls as a predictor, 95% confidence intervals, and the cut-point shown as a symbol: +: no. of galls = 7 (n = 240). The mean AUC is 0.758 with 95% CI 0.701, 0.811.

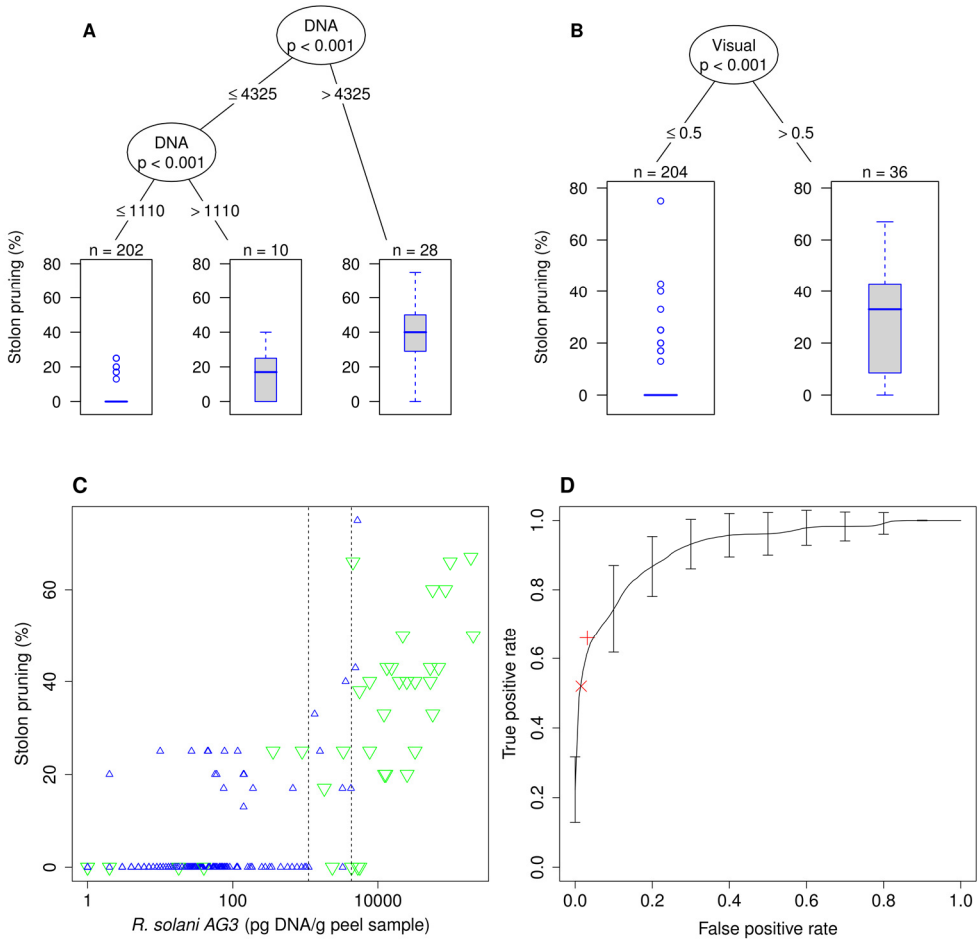


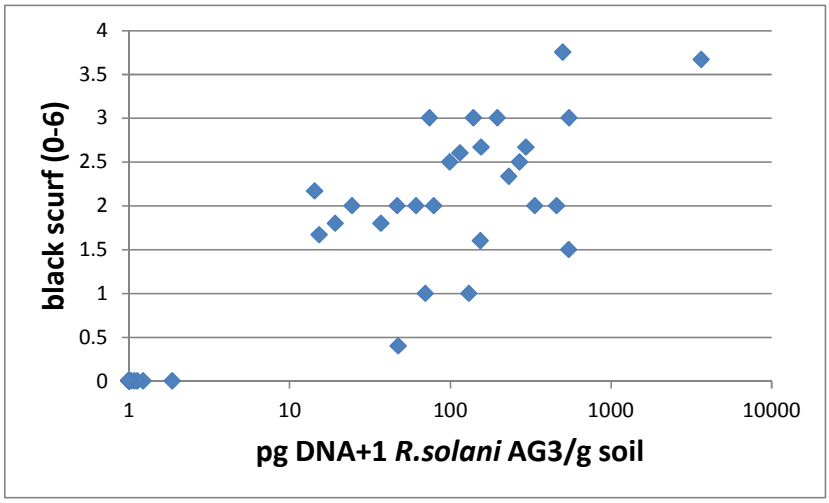
Figure 3-7. Effect of seed-tuber inoculum estimated by visual disease and *R. solani* AG3 DNA concentration on stolon pruning in progeny plants. In A, recursive partitioning shows a significant association of pathogen DNA level with the stolon pruning %. In B, after excluding DNA, recursive partitioning detects a significant association of the visual status of seed-tuber with stolon pruning %. In C, stolon pruning % is plotted against *R. solani* AG3 (pg DNA/g peel sample), showing cut-points from the recursive partitioning indicated by vertical dashed lines; small triangle for visual = 0, large inverted triangle for visual > 0. In D, Bootstrapped receiver operating curve for non-zero stolon pruning (i.e. presence/absence) using DNA level as a predictor, 95% confidence intervals, and cut-points shown as symbols: +: DNA = 1110; x: DNA = 4325 (n = 240). The mean AUC is 0.92 with 95% CI 0.871, 0.957.

SEED AND SOIL BASED INTERACTIONS

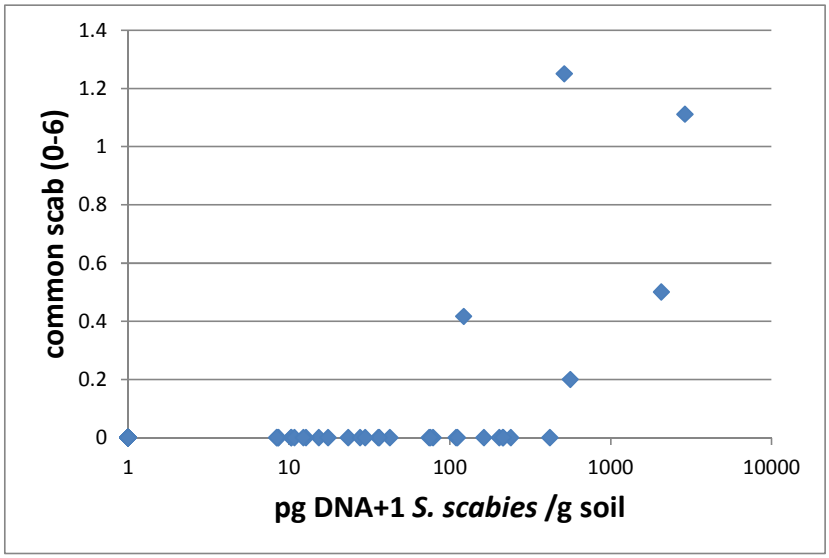
SEED CONTRIBUTION TO SOIL INOCULUM AND IMPACT ON SUBSEQUENT CROP (POT STUDIES)

Two trials were conducted in 2012 (57 pots) and 2013 (58 pots) with the key pathogens detected in the soil predominantly *R. solani* AG3, *S. subterranea* and *S. scabies*. Preliminary analysis of the data indicated that there was no significant trial or year impact on experimental outcomes so the results from both trials were pooled and analysed together (115 pots). Soil DNA content was significantly and strongly positively associated with progeny tuber disease for *R. solani* AG3 and black scurf ($r=0.734$, $P<0.001$, Figure 3-8a) and *S. scabies* and common scab ($r=0.684$, $P<0.001$, Figure 3-8b). The high r values >0.6 suggests the soil DNA tests provided a highly accurate discriminator of resultant tuber diseases. In contrast, *S. subterranea* soil DNA had a significant but weakly positive association ($r=0.395$, $P<0.001$, Figure 3-8c) on powdery scab tuber surface cover disease in the progeny. The lower r value <0.4 suggested that soil DNA tests for this pathogen provided a weak to moderately accurate discriminator of resultant tuber disease.

Where *R. solani AG3* was recorded at significant levels (>10 pg) in the soil, the daughter tubers produced showed scurf symptoms in all pots. Likewise for *S. scabies*, where significant levels were recorded (>500pg) in the soil (4/5 cases) the subsequent crop produced common scab disease. These levels of soil DNA may be useful cut-off levels that indicate an increased tuber disease risk. No such clear threshold level was available for *S. subterranea* in predicting powdery scab.



a



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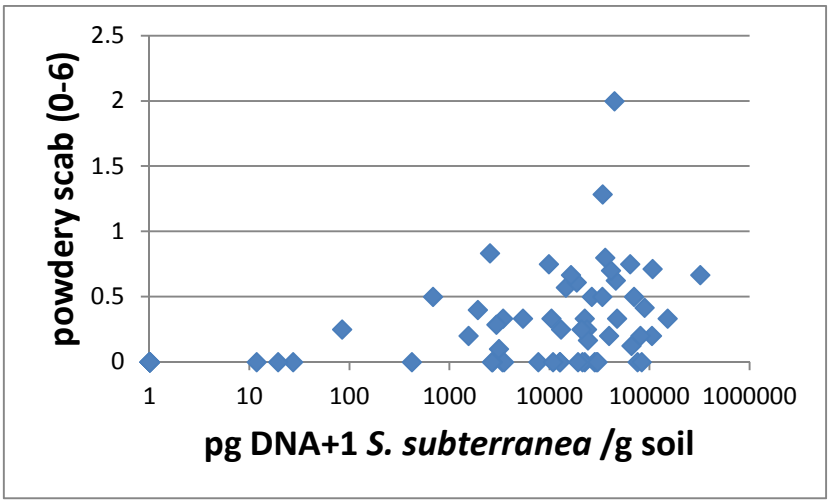

c

Figure 3-8. Presence of soilborne inoculum in potting soil (pg DNA/g soil) after growing a potato crop and subsequent disease generated (a) black scurf, (b) common scab and (c) powdery scab, after planting mini-tubers into that soil.

FIELD STUDIES (IMPACT OF SEED AND SOIL ON DISEASE EXPRESSION)

In 2011/12 field data was combined from 2 sites as there was no site effect. The key diseases observed (but in low quantities) were common scab, powdery scab and black scurf (Table 3-4). The impact of seed source was minimal, with no statistically significant effects for common scab or black scurf; it is worth noting however that seed sources with greater *R. solani AG3* inoculum (4063, 4451) did produce greater black scurf symptoms (although not significant). For powdery scab, tuber disease cover was greatest in seed line 4451 which only had low powdery scab inoculum (compare with line 501 which had high inoculum) suggesting that other factors (environmental etc..) may have been more important for powdery scab tuber infection in this experiment.

Table 3-4. Powdery scab, common scab and black scurf disease development of 'Ranger Russet' and 'Mac 1' planted with known peel tuber DNA readings: 283) Ranger Russet having low pathogen content; 4063) Ranger Russet with moderate levels of Powdery scab and *Rhizoctonia*; 501) Mac1 with very high levels of powdery scab; and 4451) Mac 1 with low powdery scab and moderate-high *Rhizoctonia*. Plants were grown in natural field conditions at 2 different sites (results pooled), and tubers were harvested at plant senescence.

Seed source	Disease					
	Powdery scab		**Common scab**		**Black scurf**	
	Surface cover (%)[a]	Infected tubers (%)[b]	Surface cover (%)[a]	Infected tubers (%)[b]	Surface cover (%)[a]	Infected tubers (%)[b]
283 (Ranger)	0.18	7.3	0.07	3.0	0.14	5.3
4063 (Ranger)	0.15	4.7	0.02	1.7	0.31	7.7
501 (Mac 1)	0.11	6.3	0.02	1.3	0.16	5.0
4451 (Mac 1)	0.38^	10.3	0.09	3.3	0.45	12
LSD (0.05)	0.09					
F prob.	0.04	0.183	0.157	0.375	0.246	0.32

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one lesion.

^significantly (P<0.05) greater disease than other seed sources for given disease.

In 2012/13 field data was combined from 2 sites (Forthside and Wesley Vale) as there was no site effect (Table 3-5). Overall, the disease outcomes from both sites were very low, with minimal galling observed during periodic assessments, this may be partly related to environmental conditions in Tasmania which were warmer than average and didn't favour powdery scab gall development. Harvested root material was also tested for *S. subterranea* DNA (at TIA); essentially, there were no significant differences in levels of DNA from the 4 variable seed sources. Where galling was seen, this corresponded with higher DNA levels. For powdery scab, tuber disease cover was greatest in seed line 1373 which had high powdery scab inoculum (compare with line 1180 which had low inoculum). This suggests that seed source is a factor in

disease incidence for Russet Burbank in this trial. There was no impact of seed source in Innovator as the cleaner seed (1370) actually had more powdery scab symptoms than the diseased seed (1001). This suggests that other factors (environmental etc..) and soil loading (both sites low-moderate powdery scab) may have been contributing factors to disease outcomes. PCR failed to identify significant differences between *S. subterranea* DNA levels in the root material.

Table 3-5. Powdery scab disease development of 'Russet Burbank' and 'Innovator' planted with known peel tuber DNA readings: 1370) Innovator having low pathogen content; 1001) Innovator with moderate levels of Powdery scab and *Rhizoctonia*; 1180) Russet Burbank with low pathogen content; and 1373) Russet Burbank with high powdery scab and moderate *Rhizoctonia*. Plants were grown in natural field conditions at 2 different sites (results pooled), and tubers were harvested at plant senescence. No common scab or black scurf recorded.

Seed source	Powdery scab	
	Surface cover (%)[a]	Infected tubers (%)[b]
1370 (Innovator)	0.13	3.7
1001 (Innovator)	0.07	2.3
1180 (R. Burbank)	0.08	3.0
1373 (R. Burbank)	0.24^	7.0
LSD (0.05)	0.10	
F prob.	0.03	0.10

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one lesion.

^significantly (P<0.05) greater disease than other seed sources for given disease.

Additionally 5 commercial seedlots were traced into commercial paddocks including seed with low and high pathogen content, and soil carrying varying inoculum loads. Data suggested that both seed and soil inoculum was important as seed carrying high levels of *S. subterranea* and planted into low-inoculum soil (low *S. subterranea* DNA readings) ended up with high disease incidence (>10 % powdery scab in 3 crops tracked). Additionally pathogen-free seed, planted into infected soil (*S. subterranea* high in soil) resulted in high disease incidence (2 crops) in the subsequent crop.

SEED TREATMENTS AND GRADING

SEED WASHING

Washing of seed tubers resulted in a significant reduction of pathogen inoculum in both seedlots for the pathogen *R. solani* AG3 (Table 3-6). In the case of this sclerotial pathogen it was visually obvious that many

of the sclerotial bodies were removed in the washing process. The other pathogens measured, including *S. subterranea*, were not significantly affected by washing treatments.

Table 3-6 – Impact of washing two separate seedlots on pathogen level (pgDNA/g sample)

Pathogen	Seedlot 1 (unwashed)	Seedlot 1 (washed)		Seedlot 2 (unwashed)	Seedlot 2 (washed)
R. solani AG2.1	1943	4973		5028	2266
R. solani AG3	1811	75*		963	1.6*
S. subterranea	1489221	924809		1283445	1645262
S. scabies	186	63		147	1626

*significantly less (P<0.05) than the unwashed treatment as determined by t test.

SEED GRADING

2011-12

The impact of grading a seedlot over the first two years of work gave mixed results (Table 3-7). If we focus on the key pathogens we can see that root knot nematode was visually very low and from a DNA perspective also had low to medium risk DNA levels. Grading in this case provided no reduction in disease risk as there was relatively low disease risk to start with. R. solani AG3 levels were low to moderate and grading in 3 of the 4 cases had no effect on DNA disease risk, in 1 case (Site No. 4) grading actually increased DNA disease risk. The visual analysis of black scurf showed no benefit due to grading; 2 crops were already certified and the other 2 crops were uncertified and remained uncertified after grading. The impact on powdery scab was quite varied. Across all 4 sites before and after grading there was high disease risk based on DNA assessment. However for visual analysis the results were different, in 2011, both crops were uncertified before and after grading, whilst in 2012, thorough grading turned a uncertified crop into a certified crop. However, it is worth noting that both certified heavily graded crops carried high powdery scab DNA levels, this may indicate that grading, whilst removing visually obvious tubers is leaving significant pathogen DNA behind in the certified crop.

2013-14

In the final year of the project a further more extensive grading project was carried out which included the growing on of tubers in both pot and field conditions. Powdery scab was the focus of this work as that was the key disease in the seed lines assessed.

Pot trial

In the pot trial, across all three cultivars assessed the visually disease-free tubers graded from the diseased line had significantly lower levels of disease both expressed as a visual score and a pathogen DNA level (Table 3-8). However, it is worth noting that pathogen levels within the visually disease-free treatments were significant (>28,000 pg *S. subterranea* DNA/g peel). These levels represent a significant risk of both root infection and tuber disease. Subsequent growing on of this seed resulted in both the visually disease-free and diseased seed producing high levels of both root and tuber infection. In terms of gall score, there was no significant difference between whether disease-free or diseased seed was planted across all three

cultivars assessed. Root gall number was only significantly higher for 'Innovator' where diseased seed was planted. While overall progeny tuber surface cover tended to be higher where 'diseased' versus 'disease-free' seed was planted it was only significant for one cultivar, 'Russet Burbank'. Progeny disease incidence was high and not affected by whether 'disease-free' or 'diseased' seed was planted.

Field trials

Both the field trials produced consistently high levels of disease with *Spongospora* induced root infection, root galling, pathogen DNA levels and tuber symptoms occurring on both cultivars assessed, Russet Burbank and Innovator. Exploratory analysis of the data indicated that there were no significant effects of cultivar on the disease outcomes assessed. Thus data from both Russet Burbank and Innovator were pooled, to provide a larger data set. The impact of using both disease free seed (mini-tubers or visually disease-free seed) and chemical treatment as both a soil furrow application and seed dip indicated that disease was being reduced in most cases, compared to untreated controls, with trends in data consistent across both field trials (Figure3-9).

The soil treatment (fluazinam) and seed dips (formalin, fluazinam and mancozeb) consistently reduced ($P < 0.05$; 2 – 6 fold decrease) mean zoosporangial observation score compared to the untreated diseased control. The mancozeb soil treatment produced a zoosporangial observation score equivalent ($P > 0.05$) to the untreated control. Using mini tubers and visually disease-free seed also significantly ($P < 0.05$) reduced zoosporangia observation score, compared to planting diseased seed (Figure 3.9a).

Root galls were only observed at 45 days after plant emergence with consistent root galling observed across all treatments at 60 and 75 days after emergence. Galling data was pooled across the two last assessment dates (60 and 75 days) as there was no significant assessment date effect. Consistent with the root infection data, similar trends were observed with the soil treatment (fluazinam) and seed dips (formalin, fluazinam and mancozeb) consistently reducing ($P < 0.05$; 2 – 8 fold decrease) root gall score compared to the untreated control (Figure 3-9b). Once again, the mancozeb soil treatment and the untreated control produced similar ($P > 0.05$) levels of moderately high galling (root gall scores of 2.3 – 2.6). The use of mini tubers and visually disease-free seed also significantly ($P < 0.05$) reduced root gall score, compared to planting diseased seed.

Levels of Spongospora subterranea DNA generally increased with time over the 5 sequential assessment dates (Figure 3-9c). Consistent with the root infection and root galling scores DNA levels were significantly higher in the diseased seed and the mancozeb soil furrow treatment compared with all other treatments, including chemical and disease-free seed treatments (visually disease-free seed and mini tubers). This was particularly obvious on the last two assessment dates at 60 and 75 days after emergence. Tuber powdery scab disease patterns were consistent with the trends for root infection. Essentially all chemical treatments on the visually diseased seed (except for Mancozeb applied in the furrow) resulted in a significant reduction ($P < 0.05$) in tuber disease symptoms (Figure 3-9d). Likewise, visually disease-free seed and mini-tubers produced less disease than visually diseased seed.

Table 3-7 – Impact of grading on visual assessment and DNA readings from various pathogens. Each result is from 100-200 tubers. The visual assessments indicate whether a given sample met certification standards: C: certified (< 2% infection) or U: uncertified (>2% infection). The DNA readings show the potential disease risk^ of a given DNA reading; classified as low (L), med (M), or high (H) disease risk. Also provided is a measure as to whether grading reduced (-), had no effect (0), or increased (+) the disease risk of a given pathogen/disease.

Site No.	job no. variety year	Tuber sampling; Same disease category (Y/N)	*R. solani AG2.1*	*R. solani AG3*	*M. fallax*	*M. hapla*	*Powdery Scab*	*Common Scab*	*V. dahliae*		*Black scurf*	*Root knot nematode*	*Powdery scab*	*Common scab*
			pgDNA/g Sample								*Visual (% infected tubers)*			
1	507 Mac 1 2011	Pre-grading After grading Disease risk (-,0,+)	1041 M 554 L - 	53058 M 32244 M 0 	513 M 761 M 0 	0 L 0 L 0 	1130585 H 299432 H 0 	13 L 6 L 0 	3 L 2 L 0 		2 (U) 2.5 (U) 0 	0 (C) 0 (C) 0 	20 (U) 13 (U) 0 	0 (C) 0 (C) 0
2	503 Mac 1 2011	Pre-grading After grading Disease risk (-,0,+)	2759 M 3424M 0 	50 L 981 L 0 	2 L 53 L 0 	0 L 0 L 0 	1164655 H 445393 H 0 	273 L 37 L 0 	0 L 1 L 0 		0 (C) 0 (C) 0 	0 (C) 0 (C) 0 	41 (U) 15 (U) 0 	0 (C) 0 (C) 0
3	1010 Mac 1 2012	Pre-grading After grading Disease risk (-,0,+)	7254 M 11877 H + 	26746 M 19160 M 0 	290 M 320 M 0 	1 L 1 L 0 	568883 H 251808 H 0 	250 L 736 M + 	2 L 37 L 0 		0 (C) 0 (C) 0 	0 (C) 0 (C) 0 	55 (U) 0 (C) - 	0 (C) 0 (C) 0
4	1200 Mac 1 2012	Pre-grading After grading Disease risk (-,0,+)	987 L 1967 M + 	3336 L 11043 M + 	168 M 188 M 0 	0 L 0 L 0 	311158 H 313539 H 0 	5584 H 1902 H 0 	0 L 1 L 0 		2 (U) 4.5 (U) 0 	1 (C) 0 (C) 0 	12 (U) 0 (C) - 	1 (C) 0 (C) 0

^ Disease categories were determined based on rating scales determined by Kirkwood:

Disease	High	Medium	Low
Rhizo AG2.1	>10,000	1000 -10,000	<1000
Rhizo AG3	>100,000	10,000 - 100,000	<10,000
M.fallax	>1000	100 - 1000	<100
Common Scab	>1000	500 - 1000	<500
Verticillium	>100	50 - 100	<50
Powdery scab	>100,000	50,000 - 100,000	<50,000

Table 3-8. Impact of heavily grading diseased seed on powdery scab visual score and *S. subterranea* DNA level (pg/g peel). Also shown is the root and tuber disease after growing on this seed in pathogen free potting soil (n = 20).

	Seed Visual disease cover (%)[a]	pgDNA *S. subterranea* /g peel	Root galling score[b]	Root gall number	Progeny disease incidence (%)[c]	Progeny visual disease cover (%)
Innovator (visually diseased)	5.2 b^	114565 b	2.7	25.3 b	90	2.4
Innovator (visually disease-free)	0 a	28246 a	2.2	13.4 a	90	1.9
LSD (0.05)	0.50	56034	ns	11.2	ns	ns
F prob	<0.001	0.02	0.15	0.04	0.76	0.32
Russet Burbank (visually diseased)	4.8 b	153432 b	2.4	18.2	85	2.9 b
Russet Burbank (visually disease-free)	0 a	32156 a	2.1	19.8	75	1.1 a
LSD (0.05)	0.50	65768	ns	ns	ns	1.5
F prob	<0.001	0.01	0.20	0.35	0.44	0.03
Innovator hybrid (visually diseased)	6.8 b	204432 b	2.5	24.2	95	3.2
Innovator hybrid (visually disease-free)	0 a	39867 a	2.2	19.4	90	2.6
LSD (0.05)	0.50	82345	ns	ns	ns	ns
F prob	<0.001	0.01	0.34	0.32	0.35	0.25

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]root galling score per plant was determined using a 0–4 visual rating scale modified from that of van de Graaf and colleagues (van de Graaf et al., 2007); 0 = no galls; 1 = 1–2 galls; 2 = 3–10 galls, most <2 mm in diameter, 3 = >10 galls, some >2 mm in diameter; 4 = most major roots with galls, some or all >4 mm in diameter.

[c]Proportion of tubers with at least one lesion.

^Means followed by same letter within the same column are not significantly different at P = 0.05 using Fisher's LSD test; ns = nonsignificant.

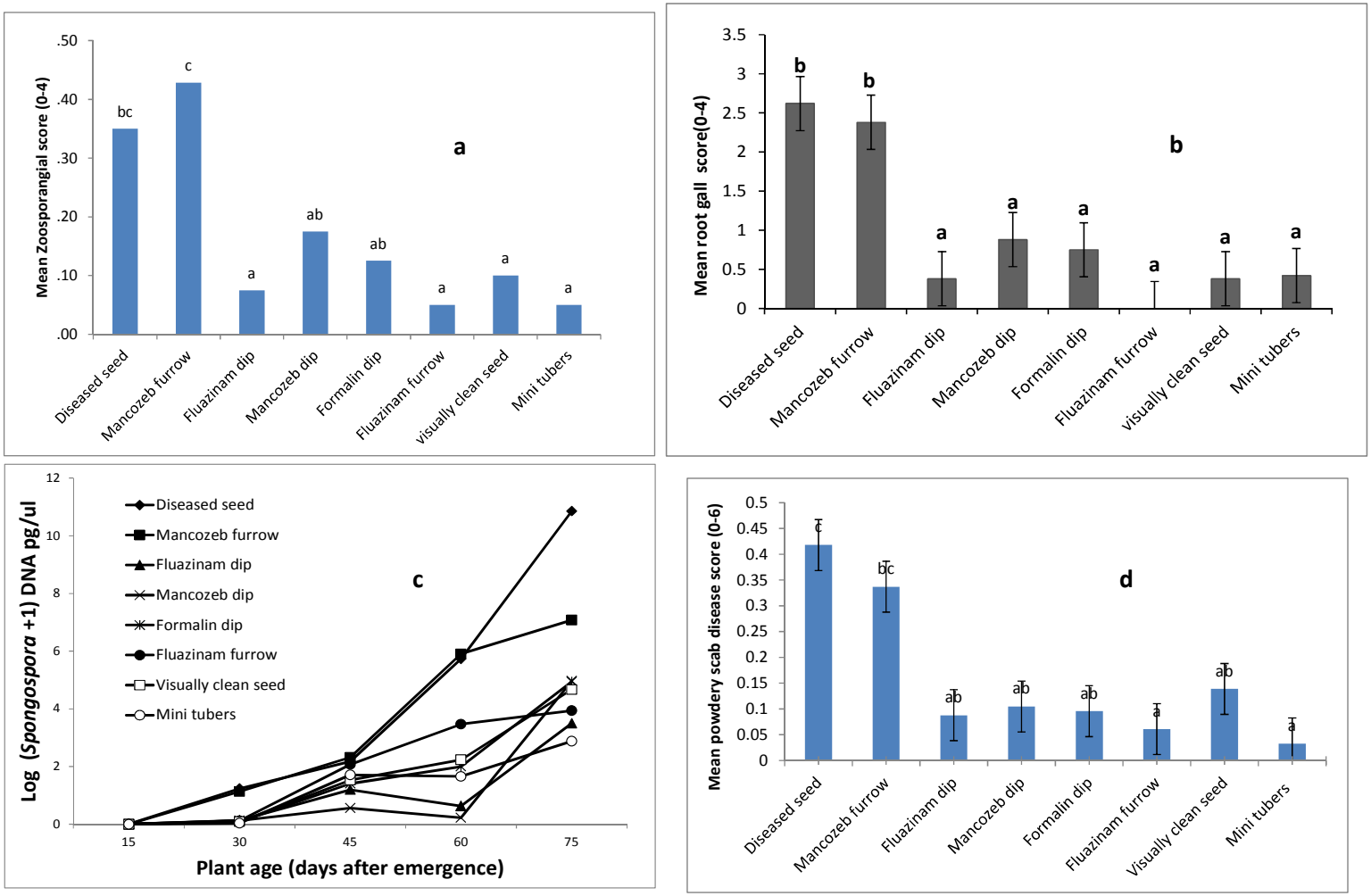

Figure 3-9. The impact of a range of chemical seed treatments and planting visually disease-free seed and mini-tubers on disease outcomes in the field. Major disease expressed was powdery scab with *Spongospora* induced a) root infection – zoosporangial score, and b) root galling shown. Additionally c) *S. subterranea* DNA levels are quantified in the root and d) tuber powdery scab disease score is shown.

DISCUSSION

SAMPLING STRATEGIES IN CERTIFICATION SCHEMES

Potato seed certification is an important disease mitigation strategy utilised to provide viable seed to growers with a maximum allowable tolerance of certain diseases (Andrade *et al.*, 2008; UNECE, 2011). For most diseases caused by nematodes, bacteria, protozoa and certain fungi a visual assessment of tubers at or just after crop harvest is the preferred method of certification. However, visual inspection relies on skilled inspectors and may result in subjective differences in assessment. There may also be misdiagnoses of diseases that produce similar symptoms to each other (Bouchek-Mechiche *et al.*, 2011). There is also the prospect that pathogen inoculum that could lead to disease in subsequent crops, may be associated with visibly healthy seed and hence escape certification inspections. This might occur where seedlots with moderate levels of disease are rigorously graded removing visually symptomatic tubers prior to inspection.

While there may be acknowledged weaknesses of visual assessment it has advantages in terms of efficiency and cost competiveness. While it is not realistic to assess every single tuber from a seedlot, sampling strategies need to be rigorously scrutinized to ensure that samples are representative of the disease incidence of whole seedlots. A 200 tuber sample, 100 taken at the start and 100 at the end of a seedlot, is current industry standard. Our studies demonstrated that taking larger less frequent samples per seedlot (point sampling) provided comparable results to smaller more frequent sampling strategies (continuous). However, the precision obtained with continuous sampling was greater than with point sampling. This greater precision may be useful when seedlots fall in categories either just inside or just outside certification thresholds, and in these cases, more rigorous sampling strategies may be justified.

qPCR improves the ability to formally identify the causal agents for the diseases present aiding diagnosis of observed symptoms. In our study the data suggests that in some circumstances, the visual symptoms of common scab may have been confused with powdery scab, leading to a reduced modelling efficiency for common scab symptoms with *S. scabies* DNA content.

PCR technology can also be valuable when dealing with pathogens that produce indistinct or unreliable symptoms. Such an example is seed-borne *Verticillium dahliae* which causes wilt symptoms and is associated with potato early dying (Fiers *et al.*, 2012). Whilst this report tended to focus on soil-borne diseases with visual symptoms and significant cost impacts, *Verticillium* in some circumstances is a significant pathogen. Preliminary work in our laboratory suggests that this pathogen may be present at moderate levels detected using PCR, while visual symptoms may not always be obvious, hence visual assessment is not an accurate assessment of inoculum load. In such cases the benefits of PCR within certification systems are obvious.

Modelling using ROCs allowed identification of which pathogen strain or species was associated with visual symptoms observed. For black scurf, it is clear that *R. solani* AG3 is responsible for sclerotia on seed tubers and not AG2.1, as has been previously reported (Tsror, 2010; Campion *et al.*, 2003; Truter and Wehner, 2004). Similarly, the tuber symptoms of root knot nematode infection were shown to be associated with *M. fallax* and not *M. hapla* in the seedlots tested in the present study.

The importance of using certified seed tubers has already been stated, particularly when planting potatoes into new ground with no soil-borne pathogen inoculum. However, pathogen inoculum can be present on tubers with no visible symptoms. This is where the benefits of more rigorous pathogen detection tools such as PCR may be useful. Recent evidence in our laboratory suggests that *S. subterranea* root galling and progeny tuber infection may occur following planting of seed tubers visually free of powdery scab and with low amounts of pathogen DNA into pasteurised soils. Others have reported similar findings following

planting of "disease-free" seed into soils with low inoculum pressures (Shah *et al.*, 2012; van de Graaf *et al.*, 2005) suggesting that accurate detection in seed tubers is essential for quantifying potential disease outbreaks.

The advantages of improving seed tuber quality assessments through improved sampling strategy and potential usage of PCR diagnostics may enable a more precise measurement of the disease loading of a given seedlot. This may enable better comparisons of seedlots allowing growers to have a greater confidence in the seed they purchase. More consistent and comparable detection systems may also enable a better comparison of seed quality coming out of different certification schemes.

However when one looks at a new technology the costs associated with it need to be considered. A basic cost analysis of certifying a typical 25 tonne seedlot indicated that DNA assessment would be more than 4-fold more expensive than visual assessment. While there are benefits of PCR diagnostics aforementioned, in most circumstances it appears that visual assessment provides an adequate means of certifying seed at an affordable price, and until the costs of PCR diagnostics are reduced, visual assessment will remain the preferred method of certifying seedlots.

Novel or alternate sampling strategies included the collection of soil and skin material from the bottom of a seedlot bin and the assessment of washings collected from varying seedlots. While pathogen levels detected were consistent with the disease loading of the individual seedlots, for both soil/skin material and filtered washing material, the benefits of utilising the alternate sampling strategies were not obvious. In the case of collecting washing material off tubers, this washing material required time-consuming filtering with the filtrates collected on filter paper also difficult to process (A. Mckay, per. comm.), making this method not very user-friendly. While the collection of soil/skin material from the bin was easily undertaken further replicated and more detailed work would be required to validate its accuracy in detecting seedlot pathogen levels. This work nonetheless has identified that this alternate sampling strategy may be useful in some cases.

IMPACT OF SEED-TUBER INOCULUM ON PROGENY DISEASE

Tracking of seed-tubers as individual units in this case study has enabled the production of validated and scientifically rigorous data that quantifies the role of seed tuber-borne inoculum, for the pathogens and cultivars used in these experiments, in inducing root, stolon and tuber disease in progeny plants. Parameters that define the health of the seed-tuber, pathogen content by qPCR and visual tuber disease score, were both associated with the production of disease in the progeny plants. This reiterates a significant role for seed health in managing these significant potato diseases.

While previous work has sought to define the relationship between seed-tuber health and subsequent disease in pathogen-free soil this present study provides new evidence and analysis techniques to complement previous studies. The statistical modelling approach provides DNA cut-points, including specific quantitative numbers that can be used (for the cultivars presented in this case study) to generate disease risk profiles for planting potato seed-tubers carrying varying pathogen loads into pathogen-free soil. For the present case study Russet Burbank and Innovator are globally important cultivars and powdery scab and root galling, common scab, black scurf and stolon pruning are economically important and common diseases worldwide.

For two of the three diseases reported here the combination of both visual disease assessment and pathogen DNA data from the seed-tuber have been strongly associated with progeny disease outcome. In the case of *R. solani* there was a strong association of both pathogen DNA and visual disease assessment of the seed-tuber with stolon pruning, stem cankering and resultant black scurf tuber disease. This strongly supports previous findings where visually infected seed resulted in increased subterranean stem and stolon

lesions, girdling and canker (Atkinson *et al.*, 2010; Tsror and Peretz-Alon, 2005) and tuber disease (Atkinson *et al.*, 2010; Errampalli and Johnston, 2001; Tsror and Peretz-Alon, 2005). The DNA associations generated by modelling (Figure 3-1A) also produced potential threshold levels that may be a useful tool in classifying the seed-tuber as suitable to plant. From a practical perspective, growers would benefit from planting tubers that had no visual disease present; if only disease-infested seed-tubers was available then a fungicide seed treatment would be highly recommended (Errampalli and Johnston, 2001; Tsror, 2010) to minimise likelihood of subsequent tuber disease.

In the case of common scab we also report a strong relationship between both high pathogen DNA and high visual disease scores of the seed-tuber with increased disease in progeny tubers. These findings support the work of some researchers (Wang and Lazarovits, 2005; Wilson *et al.*, 1999) and contradict others (Adams and Hide, 1981; Lapwood, 1972). Common scab is influenced by a range of factors, including cultivar and environmental conditions. Parameters reported in the field trials of Lapwood (Lapwood, 1972), high soil moisture and presence of high soil inoculum, were likely to have negated the role of seed-borne inoculum in some studies. The work reported by Adams (Adams and Hide, 1981), indicated extremely low tuber disease in the progeny, so differentiating a significant treatment effect (seed-borne inoculum) may have been difficult. Our results created an optimised pot environment conducive to disease, and removed the complication of soil-borne inoculum in the field, such that the role of seed tuber-borne inoculum could be studied in isolation.

In the case of powdery scab we report that only a simplified model could be generated linking the visual status of the seed-tuber with absence/presence of tuber disease in the progeny. Additionally, there was no relationship established between pathogen DNA detected on the seed-tuber and progeny tuber disease. This is reflected in the low AUC and higher variances, and larger 95% confidence intervals (Figure 3-4C), compared to the better associations developed for both black scurf (Figure 3-2D) and common scab (Figure 3-3D). Our conclusions are that powdery scab seed tuber-borne inoculum is not a good predictor of progeny tuber disease, for the cultivars and trial conditions used. This supports the findings of some researchers (Burnett, 1991; Keiser *et al.*, 2007) yet contradicts others (Falloon *et al.*, 1996; Merz and Falloon, 2009). Powdery scab is also heavily influenced by cultivar resistance (Genet *et al.*, 2011) and environmental conditions, with tuber disease favoured by wet and cool (soil temperature range: 9 – 17 ºC: (Shah *et al.*, 2012; van de Graaf *et al.*, 2005)) soil conditions. In these trials disease conducive soil moisture conditions were maintained, however, fluctuating temperatures as a result of pot culture may have led to suboptimal soil temperatures for powdery scab development. The use of cultivars with moderate resistance to powdery scab may have also influenced efficacy of transmission from seed-tuber to progeny. Future work in this could examine additional cultivars and alternate infective conditions to conclusively determine the role of seed tuber-borne inoculum in powdery scab disease.

Whilst there are a number of studies on the impact of seed tuber-borne inoculum on powdery scab disease in progeny tubers, very few have studied the impact of seed-borne inoculum on root infection and gall production. Here we show a strong significant association between seed-borne inoculum (both visual assessment and pathogen DNA content) and root gall production. This is particularly interesting given the weaker association found between seed-borne inoculum and powdery scab in tubers. *Spongospora* root infection has a wider temperature tolerance range to disease induction (soil temperature range: 11-25 ºC: (van de Graaf *et al.*, 2007; Kole, 1954)). This suggests root infection occurs more reliably than tuber infection under warmer temperatures.

Of note was the production of significant root gall numbers from symptomless (or visually disease-free) tubers, on some occasions (Figure 3-5A). Further, qPCR was able to detect the higher DNA levels (from symptomless seed-tubers) that were more likely to promote galling than symptomless seed-tubers with low DNA readings. Others have reported detection of pathogens present on seed-tubers as contaminants without obvious symptoms (van de Graaf *et al.*, 2005; Bouchek-Mechiche *et al.*, 2011) and there are reports

of powdery scab following planting of visually "disease-free" seed into soil with low inoculum pressures (Shah *et al.*, 2012; van de Graaf *et al.*, 2005) suggesting accurate pathogen inoculum detection on seed is essential for quantifying potential disease. In these cases more rigorous pathogen detection tools such as qPCR may provide a superior means of determining seed-tuber health to traditional visual assessment.

The interaction between root and tuber diseases caused by *S. subterranea* is not necessarily a simple one. Previous researchers have found that root infection was usually related to tuber infection although exceptions existed (Eraslan and Turhan, 1989; Falloon *et al.*, 2003). When studying 14 cultivars, Falloon and colleagues (Falloon *et al.*, 2003) reported that cv. Swift had very low levels of tuber infection in the field, but had high numbers of root zoosporangia and root galls in the glasshouse. Likewise, a study of 19 cultivars (Eraslan and Turhan, 1989) indicated the majority of cultivars tested showed similar levels of susceptibility to powdery scab on tubers and in roots, however cv. Russet Burbank was an exception, with low infection on tubers but with root infection levels similar to cultivars with high tuber susceptibility. Others have found no correlation between the occurrence of galls on roots and powdery scab on tubers of the same plants (van de Graaf *et al.*, 2007). Our work with Innovator suggested that root galling provided a moderately accurate association with tuber disease (Fig. 3-6C). It is quite probable that Innovator, which has reported moderate resistance to tuber infection (Genet *et al.*, 2011) may have greater susceptibility to root galling, like that reported for Russet Burbank (Nitzan *et al.*, 2008).

The use of disease-free or certified seed-tubers is a recommended practice (Andrade *et al.*, 2008) that aids the control of many important seed tuber-borne potato diseases. The standard of certified seed-tubers can vary from country to country with major differences between methodologies in accessing tubers for disease and in threshold levels of disease tolerances allowed (Tegg *et al.*, 2014). Additionally visual assessments are subjective, meaning that assessments carried out in different countries are not always directly comparable. Whilst visual assessments provide value in determining seed-tuber health quality, the addition of new technologies, such as pathogen DNA quantification using qPCR as presented here, provide extra quantitative data. If universally adopted, this may improve comparison of global results.

SEED AND SOIL BASED INTERACTIONS

The importance of using certified seed tubers has already been stated (Fiers *et al.*, 2012), particularly when planting potatoes into new ground with no soil-borne pathogen inoculum. Additionally, evidence presented in this report shows that visually diseased potato seed can result in the accumulation and contamination of 'pathogen-free' soils (potting soil) which has implications for the planting of subsequent crops. Whilst 'diseased seed' has been previously reported as a key source of contaminating pathogen-free fields we believe this is the first time that actual quantifiable levels of pathogen have been measured and attributed to seed and subsequently responsible for disease in potato crops planted thereafter.

The relationships established between pathogen inoculum levels in a soil pot environment and subsequent progeny disease were highly positively correlated for the pathogens *R. solani* AG3 and *S. scabies* and their respective tuber disease, black scurf and common scab. In contrast, levels of inoculum of *S. subterranea* were only weakly-moderately associated with progeny powdery scab disease. This is an interesting finding and partially conflicts with the findings of the soilborne inoculum project (PT09023) which has identified that *S. subterranea* inoculum is a useful predictor of tuber disease whereas the relationship for both *R. solani* AG3 and *S. scabies* and tuber disease is less useful. This may be explained by a number of reasons. Firstly our work in pots was able to exactly identify and sample from the exact region where tubers were then planted, in a commercial soil test situation the sample is a collection of multiple soil samples from various locations in the field so only provides an estimate of field pathogen levels. Additionally for pathogens like *R. solani* AG3 which exist in the soil in sclerotial forms and are unevenly distributed (associated with organic matter) the current soil sampling strategies may not be adequate to consistently

detect this pathogen in the field. The development of alternate or more efficient field detection and sampling methods may enable better correlation between pathogen levels and subsequent disease for *R. solani* AG3 and black scurf.

When we moved from a controlled pot environment into field environments we noted that relationships between soil inoculum and subsequent progeny tuber disease were not as clear-cut as we demonstrated in pots. In a number of trials where soil inoculum levels suggested a moderate disease risk to powdery scab we observed very little root galling and tuber infection and this is probably attributable to environmental conditions that prevailed through the critical stages of tuber development, where disease susceptibility is highest. In the case of powdery scab, slightly warmer weather through these critical stages (Merz and Falloon, 2009) may have been responsible for limited infection and disease. However, in some cases, particularly when we followed commercial crops through the growing season we were able to identify that disease was more likely when crops were planted into high risk sites (as identified by soil DNA tests) or when diseased seed (as identified by tuber peel DNA tests) was also planted. If a larger number of crops or sites had been tracked (beyond the scope of this specific project) it is likely that a better relationship between soil and seed inoculum in the field and progeny disease would have been established.

SEED TREATMENTS AND GRADING

There is no one effective control for powdery scab, rather an integrated approach is recommended for this recalcitrant disease (Merz and Falloon, 2009). Seed and soil furrow treatments have previously shown some degree of success in partially reducing tuber based disease (Falloon *et al.*, 1996) . Our work confirmed that previously successful treatments, including fluazinam (applied both as a seed and soil treatment) and mancozeb (seed) also played a role in reducing the impact of tuber-borne *S. subterranea*. Additional to previous work we also tracked root based disease (including infection and galling) and quantified pathogen levels, and noted that these measures of disease were all reduced with the efficacious chemicals. Demonstrating the benefits of disease-free seed we noted that mini tubers produced consistently lower levels of *Spongospora* based disease than all other commercial tubers utilised in these field experiments.

Another important component of this experimental work was the comparison of ‘disease-free’ tubers that had been graded from a heavily diseased line and a comparison of their disease expression. With a focus on powdery scab we were able to show that so called ‘disease-free’ tubers contained significant levels of *S. subterranea* DNA and that this resulted in subsequent *Spongospora* based root galling and tuber infection. This is supported by the work of others in other disease systems, common scab, where in some cases, the time-consuming practice of grading is not considered worthwhile (Pung, 2006). We believe that grading is considered worthwhile in some cases, particularly where the crop may have 4-8% disease incidence. More work is required on identifying what would be the upper limit at which seed grading would be considered non-practical (10, 15, 20% disease incidence). Additionally, this work may help validate current certification threshold levels that are used to certify current crops. There are many different certification levels used both nationally (Pung, 2006; Tegg *et al.*, 2014)and internationally (Tegg *et al.*, 2014)and we believe that qPCR may provide a useful tool in validating or developing new thresholds.

With no reliable controls for many of the soilborne diseases examined in this project alternative methods of reducing or negating tuber-borne inoculum were tested. One such hypothesis asked whether washing/brushing of tubers reduces the disease loading in a seed lot? The small piece of work indicated that seed cleaning with water was not effective in reducing tuber inoculum for powdery scab and common scab. However, significant reductions in *R. solani* AG3 tuber-borne inoculum were recorded changing a seedlot with a moderate disease risk to a low disease risk, indicating potential benefits of washing. This indicates the effects of washing were pathogen specific, with the large sclerotia of *R. solani* AG3, most likely

being removed in the washing process. It should be noted that washing tuber seed is currently not a recommended practice (N. Crump, pers. comm.).

TECHNOLOGY TRANSFER

Publications & extension activities

Refereed papers (additional manuscripts in preparation)

1. Tegg RS, Corkrey R, Wilson CR (2014). A comparison of potato seed-tuber sampling strategies using visual and DNA analyses to estimate incidence of major seed tuber-borne pathogens. *European Journal of Plant Pathology* 139(2): 359-367.
2. Tegg RS, Corkrey R, Wilson CR et al. (2014). Modelling pathogen DNA content and visual disease assessment in seed tubers to inform disease in potato progeny root, stolon and tubers. *Plant Disease* doi:10.1094/PDIS-04-14-0337-REISSN 0191-2917
3. Tegg RS and Wilson CR (2014). QPCR testing seed potato tubers for pathogens – what value for potato seed certification? *Acta Horticulturae* (submitted)

Conference papers

4. Tegg, RS and Wilson, CR, 'Does planting dirty potato seed really matter?', *Proceedings of the 7th Australasian Soilborne Diseases Symposium (ASDS)*, 17-20 September 2012, Fremantle, WA, AUS.

Industry & media publications

5. Tegg, R.S., Wilson, C.R. (2012). DNA in seed tuber diagnostics. Potatoes Australia. Feb 2012.
6. Tegg, R.S. (2012). DNA technology to be applied to humble spud. The Advocate Jan 2012.
7. Tegg, R.S. (2013) The importance of tuber-borne inoculum in seed potato health. Potatoes Australia, August/September 2013.

Grower presentations

8. Presentation at 2011 "*Growing Together*" - *McCain Agriculture Conference*, 25-25 August 2011, Ballarat, Victoria.
9. Presentation at 2011 AUSVEG Potato Summit, 17th April 2011, Brisbane, Australia
10. Presentation at the Simplot R&D day held at the Burnie campus of UTAS on 4th October 2012, attended by Simplot company personnel, other industry and university representatives
11. Presentation at the APRP2 mini conference, held in Melbourne on 6-7th October 2012 attended by APRP2 researchers and associated industry members
12. Presentation at the TIA "showcase day" held at the Sandy Bay campus of UTAS on 29th November 2012 attended by a wide range of university and industry representatives
13. Presentation at the AUSVEG sponsored Industry extension workshop in Ulverstone, 14th March 2012.
14. Presentation at the AUSVEG sponsored Industry extension workshop in Atherton, QLD, 15th Nov 2012.
15. Presentation at the APRP2 mini conference held in Melbourne on 18-19th September 2013 attended by APRP2 researchers and associated industry members.
16. Presentation at the AUSVEG sponsored Industry extension workshop in Gatton, QLD, 27th May 2014.
17. Presentation at the Potato Industry Conference held in Mt Gambier, SA on 10-12th August, 2014. Over 200 growers and industry representatives attended.

Industry & Grower tools

18. Preliminary threshold levels (on tuber peel) for some key soilborne diseases provided to assist growers who wish to use a DNA test for their seed. This will be incorporated into a section of the PreDicta Pt manual as well as advice on the benefits of utilising certified seed. Peel tests may be made available as an industry tool as part of PreDicta Pt in the near future. Agronico (a private company in NW Tasmania) have shown interest in peel tests and will provide updates on the usefulness of this tool in determining seed health for their growing operations.
19. Preliminary discussions with members of the Tasmanian Seed Potato Advisory Committee (TSPAC) about general project outcomes have occurred. We have been invited to present the practical outcomes of the work at the next TSPAC meeting.

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

Key recommendations are:

- That the current seed tuber certification practices, using simplified sampling and visual assessment, are adequate to determine seed tuber health and risk for the major soil-borne pathogens that produce visual lesions on tubers.
- Visual assessment for certification is currently approximately 4-fold cheaper than DNA assessment, so provides the most affordable seed for growers, in the majority of cases.
- Both visual assessment and DNA based diagnostics provide similar assessment of seed health, there are however some specific exceptions where DNA technology may be beneficial:
    1. For detection of pathogens where visual assessment is not possible or reliable.
    2. To distinguish pathogens producing visually similar or indistinguishable symptoms
    3. Where heavy seed grading may result in high pathogen content on visually clean seed.
    4. When planting into virgin soils.
- Use of certified seed and seed treatments are important tools, especially when planting into clean soil, to reduce:
    1. Transfer of disease into new crop
    2. Contamination of pathogen-free soils

Future recommended research and activities include:

- Validation of the current seed certification threshold levels (both visual and DNA assessment). Providing a scientific basis to the risk thresholds for disease.
- Validating the risks of seed grading (testing a greater range of infected seedlots (5, 10, 15%) to see the influence on seedlot contamination.
- Quantifying the cost:benefit of using certified seed for the grower.
- Quantifying the role and benefits of seed treatments and dressings on management of seed inoculum.

- Investment in fundamental research on the viability and form of pathogen detected by qPCR testing on tubers (determining how the number of infective units corresponds to DNA content, etc.).
- Validation of seed health risks across a wider range of cultivars that vary in susceptibility.
- Quantifying the effect of *Spongospora* root infection on both yield and soil inoculum build up.
- Further study of a diverse range of seed-borne pathogens (to date work has focussed on powdery scab, common scab and black scurf).

ACKNOWLEDGEMENTS

For project direction and critical feedback we are grateful for input from the Technical Operations Group, industry champion for our project and Horticulture Australia staff that occurred at milestone meetings and other informal gatherings. We also note the assistance in some aspects of the project from key companies, such as Simplot Australia Pty Ltd. We also appreciate the assistance provided by Anne Ramsay and Scott Williams in managing this multi-faceted research programme.

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Horticulture Australia

PT09023 – (30/10/2015)

Diagnostic tests for soilborne pathogens – International Collaboration

Final Report

Dr Kathy Ophel Keller

South Australian Research and Development Institute

PROJECT SUMMARY

PT09023 – Diagnostic tests for soilborne pathogens – International Collaboration

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The intended outcome of this project for industry is the delivery of DNA testing technology, including interpretation of tests with respect to disease risk, to potato growers.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the Potato Council-UK and Horticulture New Zealand and matched funds from the Australian Government. The South Australian Research and Development Institute, the University of Tasmania/Tasmanian Institute of Agriculture and the Department of Environment and Primary Industries Victoria have provided in-kind support.

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CHAPTER 4. DIAGNOSTIC TESTS FOR SOILBORNE PATHOGENS- INTERNATIONAL COLLABORATION

MEDIA SUMMARY

Soilborne diseases are a major production constraint to the Australian processing potato industry, impacting profitability and international competitiveness. This project has developed a commercial DNA testing service, PreDicta Pt, to allow assessment of the risk posed by key soilborne pathogens pre-planting. Since its commercial release in September 2013 the testing service for *Spongospora subterranea* (powdery scab), *Meloidogyne fallax* (root knot nematode) and *Colletotrichum coccodes* (black dot) has been utilised by potato growers in Tasmania, Victoria and South Australia. Providing an indication of disease risk prior to planting, PreDicta Pt assists growers with pre-season decision making, such as choosing paddocks, matching varieties to risk and considering treatments and management strategies. The testing service is delivered through a network of agronomists, who have completed PreDicta Pt training workshops. A manual containing comprehensive information on each pathogen and interpretation of test results has also been developed to support delivery of the service.

Research test results for pathogen DNA levels in the soil of pathogenic *Streptomyces* (common scab), *Verticillium dahliae* and *Pratylenchus crenatus, P. neglectus, P. penetrans* (potato early dying), *Meloidogyne hapla*, *M. javanica/incognita/arenaria* (root knot nematodes), *Rhizoctonia solani* AG-3 and AG2-1 (black scurf and other Rhizoctonia diseases are reported).

Capability to test pathogen levels in the peel of tubers has been developed for the same suite of pathogens for which soil DNA testing is available.

The ability to quantify pathogen DNA levels, both in the soil and the peel of seed tubers, in combination with field validation of disease levels conducted in this project has increased our understanding of inoculum and disease risk for these pathogens. Access to this technology has opened up new opportunities at both an on-farm practical level and to undertake research and development approaches that were previously not feasible.

Development and commercialisation of this DNA testing capability to quantify the risk posed by key soilborne pathogens has greatly enhanced the ability of the processing potatoes industry to manage disease risk now and into the future.

TECHNICAL SUMMARY

PREDICTA PT SERVICE

This project has developed a commercial DNA testing service, PreDicta Pt. This test enables growers to identify fields (or parts of fields) that are at risk of specific diseases before planting. The testing service has been delivered to growers since September 2013 and has been designed and set up to operate as a standalone commercial service post project.

Currently the PreDicta Pt test provides an indication of the risk of:

- Powdery scab (caused by *Spongospora subterranea*)
- Black dot (caused by *Colletotrichum coccodes*)
- Root knot nematode (caused by *Meloidogyne fallax*)

Knowing the risk prior to planting can assist to mitigate disease risk by;

- Assisting pre-season decision making
    - choosing paddocks
    - matching varieties to specific disease risks
    - considering treatments and management strategies.
- Monitoring and improving practices
    - e.g. crop rotation strategies.

As part of the PreDicta Pt testing service, DNA levels of *Streptomyces txt*A gene, *Rhizoctonia solani* AG3, *Rhizoctonia solani* AG2.1, *Meloidogyne hapla*, *Verticillium dahliae*, *Pratylenchus penetrans* and *Pratylenchus neglectus* are reported as "Tests Under Development". These research results are provided for information only as sensitivity and risk categories are not available for these tests. *Pratylenchus crenatus* is in the process of being added to the service.

Potato growers can access PreDicta Pt via agronomists accredited by SARDI to interpret the results and provide advice on management options to reduce the risk of disease losses. Growers are welcome to undertake the training and to access the service directly if they wish to do so.

Establishment of the PreDicta Pt service has been conducted under the oversight of an industry reference group.

PATHOGEN DNA RESEARCH TESTS

As a result of this project the potato industry has ongoing access to testing capability to quantify pathogen DNA levels in the soil, peel of tubers and other substrates for *Spongospora subterranea* (powdery scab),

Meloidogyne fallax (root knot nematode), *Colletotrichum coccodes* (black dot), pathogenic *Streptomyces* (common scab), *Verticillium dahliae* and *Pratylenchus crenatus*, *P. neglectus*, *P. penetrans* (potato early dying), *Meloidogyne fallax*, *M. hapla*, *M. javanica/incognita/arenaria* (root knot nematodes), *Rhizoctonia solani* AG-3 and AG2-1 (black scurf and other Rhizoctonia diseases).

Access to this technology has opened up new opportunities at both an on-farm practical level and to undertake research and development approaches that were previously not feasible.

As part of the international collaboration in this project, an inter-laboratory DNA test comparison demonstrated the ability of laboratories in Australia (SARDI), New Zealand (New Zealand Plant and Food Research) and the United Kingdom (James Hutton Institute, Scottish Agricultural College and the Food and Environment Research Agency) to quantify pathogen DNA levels of *Colletotrichum coccodes*, *Rhizoctonia solani* AG3 and *Spongospora subterranea* with reasonable agreement.

USEFULNESS OF SPECIFIC PATHOGEN DNA TESTS

SPONGOSPORA SUBTERRANEA

- Pre-plant test provides a useful indication of powdery scab risk to tubers.
- Inoculum in the soil is the most important source of disease risk.
- Under highly conducive conditions for *Spongospora subterranea*, such as may occur in Tasmania, seed inoculum may need to be considered in assessing powdery scab risk to tubers.
- Region and potato variety influence disease expression; taking them into account improves the interpretation of disease risk.
- *Spongospora subterranea* is widely distributed wherever potatoes have been grown and a high proportion of paddocks are at high risk, especially in Tasmania.

MELOIDOGYNE FALLAX

- Pre-plant test provides a useful indication of root knot nematode damage to tubers.
- Inoculum in the soil is most important source of disease risk, with limited evidence of seed inoculum contributing to disease incidence.
- Region (particularly soil temperature) influences disease expression and taking temperature into account improves the interpretation of disease risk.
- *Meloidogyne fallax* is widely distributed, but levels in the soil and risk to tubers are highest in the southeast of South Australia.

MELOIDOGYNE HAPLA

Economic loss due to Meloidogyne hapla appears to be negligible; although it is present in harvested tubers it does not appear to cause visible tuber damage or substantial yield loss

- Pre-plant test provides a useful indication of the level of nematode colonisation in harvested tubers.
- Colonisation of harvested tubers has not been linked with root-knot nematode damage seen on the surface of tubers.
- Impact of soil inoculum of *Meloidogyne hapla* on yield reduction is inconclusive, but does not appear to be a major contributor.

- When *Meloidogyne hapla* populations in the soil are high prior to planting potatoes, population balance in the harvested tubers suggests this species may compete with *Meloidogyne fallax*.
- The soil test has been included in the PreDicta Pt testing service – "Tests under development"

*STREPTOMYCES TXT*A GENE

- Detection of the *Streptomyces txt*A gene DNA in the soil is infrequent.
- When *Streptomyces txt*A gene DNA detected in the soil at low levels there is a slightly increased risk of common scab compared with when not detected, but when detected in soil at high levels (>2.5 log (DNA), there is greatly increased risk of common scab. However, these levels were rarely detected in commercial paddocks.
- When *Streptomyces txt*A gene DNA is not detected in soil, there is still a risk of common scab.
- *Streptomyces txt*A gene DNA is detected more frequently in the peel of seed tubers than it is in the soil prior to planting.
- Inoculum on seed tubers can be important contributor to common scab disease risk.
- Pathogen DNA testing detects high levels of DNA of pathogenic *Streptomyces* in the peel of tubers that have a high incidence of common scab.
- Environment and irrigation practices likely to be main driver of disease expression.
- The soil test has been included in the PreDicta Pt testing service – "Tests under development"

RHIZOCTONIA SOLANI AG3

- Detection in the soil is infrequent and this appears to be linked to the low and sporadic distribution of sclerotia in soil
- *Rhizoctonia solani* AG3 is the main anastomosis group associated with black scurf and rhizoctonia stem canker.
- Pre-plant test is a poor indicator of the risk of black scurf on tubers
    - When *Rhizoctonia solani* AG3 is not detected in the soil, there is still is a high risk of black scurf in harvested tubers
    - When *Rhizoctonia solani* AG3 is detected in soil, there is higher risk of black scurf than in soils where the pathogen was not detected. This indicates the test may be some value to growers, but the limitations of the test will need careful communication
- Inoculum is present either on the seed tubers planted or in the soil in most situations.
- Inoculum on seed tubers can be important contributor to black scurf disease risk.
- Findings in United Kingdom relating to the role of soil and seed inoculum support findings in Australia, both in controlled experiments and field validation of pathogen DNA tests.
- Environment and control practices such as seed and in furrow chemical treatments likely to be main driver of disease expression.
- Pre-plant soil inoculum may be a useful indicator of risk of 'burn- off' prior to emergence caused by Rhizoctonia at the location of sampling. To understand risk across a paddock a higher intensity of testing would be required than utilised for current PreDicta Pt tests.
- The soil test has been included in the PreDicta Pt testing service – "Tests under development"

RHIZOCTONIA SOLANI AG2.1

- Pre-plant soil test results not linked to black scurf incidence on tubers.
- Pre-plant soil test may be a useful indication of risk of Rhizoctonia 'burn-off' prior to emergence in the southeast of South Australia.
- Pre-plant soil test may be a useful indication of risk of deformed tubers. Inoculum on seed tubers can be important contributor to risk of deformed tubers.
- Pre-plant soil test has been included in the PreDicta Pt testing service – "Tests under development"

POTATO EARLY DYING

- DNA assays now available for pathogens involved in potato early dying disease complex. These include new tests for *Verticillium dahliae* and the root lesion nematode *Pratylenchus crenatus.* Tests were already available for root lesion nematodes *P. neglectus* and *P. penetrans*.
- Pre-plant soil test for *Verticillium dahliae* provides a useful indication of reduced yield potential at a site.
- Pre-plant soil levels of *Verticillium dahliae* in a paddock are strongly related to DNA levels of *Verticillium dahliae* in harvested tubers.
- Where *Verticillium dahliae* DNA was detected in the soil prior to planting, DNA of at least one of the three *Pratylenchus* spp. was detected at over 93 % of locations in South Australia.
- Pre-plant tests are included in the PreDicta Pt testing service – “Tests under development”.

INTRODUCTION

DNA tests have been developed to quantify key soilborne pathogens of potato in soil. Tests include *Spongospora subterranea* (powdery scab), *Streptomyces txt*A gene (common scab), *Meloidogyne fallax* and *Meloidogyne hapla* (root-knot nematodes) and *Rhizoctonia solani* (AG 2-1, AG 3). Test development was undertaken both within the Processing Potato R & D (APRP1) program (PT04016) and internationally, at Scottish Crops Research Institute (SCRI), now James Hutton Institute.

The APRP1 program (2004-2009) focused on development of robust quantitative tests which can determine pathogen levels in soil and seed, mapping pathogen distribution to determine sampling strategies, and defining the relationship between inoculum and disease development in bioassays. The relationship between inoculum and disease development was validated on a limited range of commercial fields.

The intended outcome of this project for industry is the delivery of DNA testing technology, including interpretation of tests with respect to disease risk, to potato growers.

In order to validate these tests for grower use, and to draw on the research being done by international groups, the following areas of research were identified.

- Grower level test validation including development of a manual to support interpretation of test results in terms of paddock history as well as risk factors (seed inoculum, environmental conditions, and varietal choice) which will impact on eventual disease development.
- Role of seed and soilborne inoculum. Relative risks from seed and soilborne inoculum have not been adequately described.
- Standardising test results between international laboratories. This would ensure that collaborating laboratories are able to relate results of DNA tests performed in different laboratories.
- Improve the usefulness of DNA tests for *Rhizoctonia solani* AG3 in soil. Sampling recommendations applicable to other pathogens may not be adequately detecting *Rhizoctonia solani* AG3 in soil. This includes a better understanding of sampling strategies to quantify inoculum in the soil, key drivers of disease development and yield loss.

During the course of the project an additional research target was identified and incorporated

- Addition of a DNA based risk assessment soil test for potato early dying, requiring the development of tests for *Verticillium dahliae* and *Pratylenchus crenatus*.

Overall, these activities contribute to improved management of key soilborne diseases of economic importance to the processing potato industry by improving interpretation of DNA tests to quantify soilborne inoculum so that these tests can be used by growers to assess disease risk prior to planting a crop.

The key outcome of this project is the establishment of the PreDicta Pt service for the pre-plant risk assessment of important soilborne diseases in processing potato crops. In addition to this use, the service provides the ability to quantify the impacts of agronomic practices, varietal differences and environmental conditions on the level of pathogens in the soil and the risk they pose.

MATERIALS AND METHODS

FIELD VALIDATION OF DNA TESTS

SOIL AND SEED PATHOGEN TESTING

In the 2010/11, 2011/12 and 2012/13 growing seasons, commercial potato paddocks in South Australia, Victoria and Tasmania were selected for a program to validate the pathogen DNA tests. Paddock selection was made in conjunction with potato processing companies and growers in each state. Both potato seed and ware crops were included.

Within each selected paddock in South Australia and Tasmania, four quadrants 1 ha in size were identified and mapped using GPS, and within each quadrant a composite soil sample comprising 40 cores using an AccuCore soil sampler (10 mm diameter to 150 mm depth) was taken prior to planting. The cores were sampled at regular spacing on a "W" sampling path across each 1 ha quadrant.

In Victoria soil sampling was tied in with that state's potato cyst nematode (PCN) monitoring program, that is a composite soil sample based on 10 x 10 m grid over 2 ha. A subsample of 500g was taken for DNA testing.

Soil samples were dried at 40°C, ground <2 mm, and analysed for pathogen DNA using the DNA probes developed as part of APRP1 (Ophel-Keller, McKay, Hartley, Herdina, & Curran, 2008). Pathogen DNA tests for *Spongospora subterranea*, *Streptomyces txt*A gene, *Rhizoctonia solani* AG2.1, *Rhizoctonia solani* AG3, *Meloidogyne fallax* and *Meloidogyne hapla* were conducted on all samples included in the field validation program. In the 2012/13 season pathogen DNA tests for *Colletotrichum coccodes* and *Verticillium dahliae* were also included. Results for pathogen DNA tests are expressed as $\log_{10}$ (picograms DNA / g air-dry soil + 1) unless otherwise stated.

In all states, samples of the potato seed planted at each site (25-100 seed pieces per seed lot) were also taken. The seed pieces were peeled (single strip from bud end to stolon end from the entire circumference of each tuber) and the peel was dried at 40°C and analysed for pathogen DNA. The cultivars planted in each quadrant were noted. Seed matched to all sampling points was not collected, often a result of changes to planting plans or a different seed lot being used.

The total number of sampling points that were assessed in each state is listed in Table 4-1. In South Australia all field validation assessments were conducted in the SE region and predominantly on the varieties Russet Burbank, Ranger Russet and Innovator. In Tasmania, sites were located in the central north, north east and midlands regions and concentrated on the Russet Burbank and Bondi varieties. In Victoria, sites were split between the Ballarat, Koo Wee Rup and Thorpdale regions and covered a wider range of varieties including Atlantic, Innovator, Snowden, Pike, Catani and Russet Burbank. Monthly mean maximum and minimum temperatures and rainfall totals at locations within production districts where field validation was conducted are tabulated in Appendix 1.

Table 4-1 – Number of sampling points assessed in each state in each year. Number in brackets represents sampling points with matched seed pathogen DNA.

State	Assessment season		
	2010-11	2011-12	2012-13
South Australia	133 (118)	152 (101)	140 (112)
Victoria	0	124 (74)	84 (72)
Tasmania	36 (32)	38 (38)	100 (92)

At crop maturity or just prior to commercial harvest, 100 potato tubers were sampled from across each of the areas from which the soil had been sampled. These tubers were washed and the incidence and severity of symptoms of powdery scab, common scab, black scurf and root-knot nematode recorded.

In South Australia and Tasmania, 25-50 potato tubers were sub-sampled from the harvested tubers. These daughter tubers were washed, then peeled (single strip from bud end to stolon end from the entire circumference of each tuber) and the peel was dried at 40°C and analysed for pathogen DNA.

In South Australia tubers were weighed so that crop yield could be estimated. The total yield of tubers, as well as the weight of tubers exceeding 100g was recorded and expressed as kg of tubers per plant. Normally 20 plants per sampling point were harvested, however if average tuber number per plant was below 8, then additional plants were harvested to ensure adequate tubers were available for assessments.

In Tasmania additional soil samples were taken after crop senescence in the 2012/13 season and analysed for pathogen DNA as per pre-plant soil samples.

Field validation data is graphically presented as disease incidence (% of assessed tubers showing symptoms) versus the DNA level of the pathogen in the soil prior to planting. Data was also collected on disease severity; however as the disease incidence and disease severity data are highly correlated, only disease incidence data has been presented. Where disease risk categories have been established and now form part of the PreDicta Pt service, these are indicated on the graphs.

The pre-plant soil DNA test is a probability based disease risk assessment tool. To assess the probability of disease, the percentage of sampling points exceeding specified levels of disease have been calculated within each risk category. Where thresholds are not yet developed or are not applicable, calculations have been made on the basis of detection /non detection or on putative threshold values.

Field validation has been conducted across a range of varieties, geographic regions and over three seasons. Where sufficient data has been gathered, the relationship between disease incidence and the DNA level of the pathogen has been broken into subsets to examine the impact of these factors.

Field validation of seed data is graphically presented as disease incidence verses the DNA level of the pathogen in the peel of seed. Depending on the pathogen, many of the data points represent situations where a high level of inoculum was also present in the soil prior to planting. For some soilborne pathogens, such as *Spongospora subterranea*, where high levels of inoculum exist in the soil they are known to outweigh the contribution of inoculum on the seed tubers (Lees, Sullivan, & Brierley, 2009). To isolate the impact of seed inoculum, data is also presented from only the sampling points where DNA of the pathogen

was not detected in the soil prior to planting. For some pathogens inoculum in the soil is widespread, which limits the size and usefulness of the remaining data set.

Incidence of disease was the main response used to validate the tests. Due to environmental factors the incidence of certain diseases can be low in some seasons, or the symptoms are not easily visually quantified as in the case of Verticillium wilt. To assist in these situations the relationship between pathogen DNA levels in the peel of daughter tubers and either pathogen levels in the soil prior to planting or in the peel of the seed tubers was investigated. Level of pathogen DNA in the peel of daughter tubers was also utilised to confirm symptoms and or the pathogen causing the symptoms.

In most cases data from the 2010/11 season has been omitted, as conditions were unseasonably wet which resulted in poor crops and high losses from water logging related tuber decay. No data was collected in this season from Victoria and the data that was collected from South Australia was impacted by the conditions. Data from the 2010/11 season is presented when comparing seasonal differences.

CROP EMERGENCE – EFFECT OF *RHIZOCTONIA SOLANI*

In 2012/13, crop emergence assessments 2-5 weeks after planting were conducted at selected sites in South Australia, Tasmania and Victoria. In each selected site, 5 rows long enough to accommodate 100 plants were assessed (i.e. 500 plant positions in each quadrant). Plants were scored as healthy, somewhat delayed but healthy, blind, suffering soft rot, sprouted and healthy or sprouted and unhealthy. At least 5 healthy, delayed but healthy and non-emerged plants were dug and examined at each site. Additionally in Tasmania at 23 sites, five plants from each of the four quadrants per site (20 plants per site) were excavated, washed gently by spraying with tap water, and scored for the presence of rhizoctonia stem canker.

In some instances, unhealthy plants infected by *Rhizoctonia solani* were removed and stored in individual bags for later DNA analysis. Samples were prepared by removing the infected portion of the plant, washing under running water, before being oven dried at 35-40°C. Healthy plants were also sampled and dried for analysis.

In 2013-14, further paddocks were selected to assess the effect of *Rhizoctonia solani* on crop emergence. Paddock selection was based on the concentrations of *Rhizoctonia solani* AG2.1 and AG3 DNA in pre-plant soil tests obtained from potato processing companies and growers. Paddocks with soil containing high concentrations of DNA of one or both of these anastomosis groups were selected so that the relative importance of these groups in affecting emergence could be assessed.

ROOT GALLING – EFFECT OF *SPONGOSPORA SUBTERRANEA*

In Tasmania in the 2012/13 season the roots and stolons were assessed at 23 sites for root galling 9-12 weeks after planting. Five plants from each of the four quadrants per site (20 plants per site) were excavated, washed gently by spraying with tap water, and scored for the presence of root galls of *Spongospora subterranea*.

ASSOCIATED INVESTIGATIONS

Field validation generated a large data set comprising pathogen DNA levels in the soil and seed prior to planting and in the peel of harvested daughter tubers along with matching data on the incidence and severity of disease. In some cases post crop pathogen DNA levels were measured.

INOCULUM LEVELS

Utilising the pathogen DNA data set generated during the field validation process the proportion of soil samples in each risk category were determined in order to benchmark levels of inoculum present in the soil in each state. In the case of *Spongospora subterranea*, *Meloidogyne fallax* and *Colletotrichum coccodes* risk categories were assigned as per the current threshold levels used in the PreDicta Pt service. For *Streptomyces txt*A gene, *Verticillium dahliae*, *Rhizoctonia solani* AG2.1 and *Meloidogyne hapla* risk categories were assigned on the basis of current putative threshold values. In the case of *Rhizoctonia solani* AG3, no risk category was assigned, with the pathogen DNA in the soil prior to planting either classified as above or below the level of detection.

Categories relating to disease risk posed by pathogen DNA levels in the peel of seed tubers are less established than for pathogen DNA levels in soil samples taken prior to planting. In order to benchmark levels of inoculum present on the peel of seed tubers, tentative high threshold values for pathogen DNA concentrations were adopted as follows. In the case of *Spongospora subterranea*, *Meloidogyne fallax, Rhizoctonia solani* AG3 and *Streptomyces txt*A gene the tentative high thresholds were adapted from findings in APRP2 - PT09019 and supported by field validation as part of this project. In the case of *Colletotrichum coccodes*, *Rhizoctonia solani* AG2.1 and *Meloidogyne hapla* indicative high thresholds were assigned on the basis of field validation data in this project.

Data on the proportion of sampling points in each risk category were determined separately for South Australia, Tasmania and Victoria.

INOCULUM BUILDUP

In the 2012/13 season soil samples were collected in Tasmania after the crop had senesced. To assess the buildup of inoculum as a result of growing the potato crop data is graphically presented on post crop pathogen DNA level in the soil verses the DNA level of the pathogen in the soil prior to planting. Similarly the contribution from seed is assessed, but only from sampling points where DNA of the relevant pathogen was not detected in the soil prior to planting. Relationships between disease incidence on the harvested tubers and post crop pathogen DNA levels were also examined.

SOIL DNA TEST DEVELOPMENT – POTATO EARLY DYING

INTRODUCTION

Potato early dying is an interaction between Verticillium wilt and root lesion nematodes. Knowledge is limited on the root lesion nematodes that interact with *Verticillium* spp. to cause potato early dying in Australia. Based on surveys conducted in the SE of South Australia, species that might be involved are *Pratylenchus crenatus*, *P. penetrans* and *P. neglectus* (Harding & Wicks, 2007). SARDI had assays for

Pratylenchus penetrans and *P. neglectus* prior to this project, but not for *P. crenatus*. Pathogens that cause Verticillium wilt of potatoes are *Verticillium dahliae* and *V. albo-atrum*. *Verticillium dahliae* was identified as the primary target pathogen involved in potato early dying in Australia and SARDI had an assay for *Verticillium dahliae* prior to this project. Surveys in South Australia and Victoria have found that *Verticillium dahliae* is the predominant species infecting potato plants.

ASSAY DESIGN

For all PreDicta Pt assays, SARDI molecular diagnostics has standardised on using TaqMan® MGB assays (real-time qPCR assays) because they are quantitative, fast and compatible with high throughput. Sequence information available in Genbank is reviewed to select a suitable diagnostic region that provides the required level of specificity. Ribosomal DNA regions, including ITS 1, ITS 2, IGS, 3D are preferred because their high copy number improves sensitivity for use on soil samples. Prospective primers and probes are then designed and evaluated on pure DNA of the target organism. In the meantime, a DNA collection which includes target and non-target organisms is assembled and used to check the specificity of the most sensitive assays.

Prior to DNA extraction, each sample is routinely spiked with a constant amount of yeast DNA, and a Yeast specific TaqMan assay is used to measure the amount recovered. This "internal control" enables any combined effect of PCR inhibition and/or DNA extraction inefficiencies to be measured. A reference DNA standard is run with each pathogen assay, which allows conversion of the cycle threshold (Ct) value from real-time qPCR into a DNA value per unit sample (e.g. DNA per gram of soil). The Ct value represents the PCR cycle number at which the fluorescence signal rises above a fixed threshold.

VALIDATION

Pathogen DNA testing for *Verticillium dahliae* was added to DNA assays included in the field validation testing program once it was available, that is commencing on the daughter peel samples in the 2011/12 season. DNA from field validation pre-plant soil samples were available from the 152 soil samples collected in South Australia in the 2011/2012 season. These sampling points had been assessed for yield and vascular browning. DNA from these sampling points was removed from storage and assayed for the pathogens levels of *Verticillium dahliae* and three *Pratylenchus* species nematodes – *Pratylenchus crenatus*, *Pratylenchus neglectus* and *Pratylenchus penetrans* to complete the data set from this year. A further 24 of the samples from Tasmania were tested; however yield and vascular browning had not been assessed at these sites.

Field validation data is graphically presented to examine relationships between the concentration of *Verticillium dahliae* DNA in the soil prior to planting and vascular browning, as well as *Verticillium dahliae* DNA level in the peel of harvested tubers. A putative threshold for disease risk has been set and yield compared from sampling points with *Verticillium dahliae* DNA levels above and below this level or not detected.

SAMPLING PROTOCOL

EFFICACY OF USING FOUR SOIL SAMPLES PER PADDOCK FOR DISEASE RISK ASSESSMENT

In October 2010, six paddocks irrigated by centre pivot irrigators (40 – 50ha) which were planted with Russet Burbank in the SE of South Australia were chosen across a range of different soil types which were thought to have a range of disease risk. Using field mapping software ArcPad® each pivot was then divided into geo referenced grids of 100m x 100m, approximately 44 square grids per pivot.

Prior to planting in these paddocks, composite soil samples of 40 cores were collected from each 1 hectare grid on a "W" sampling path using an AccuCore soil sampler (10 mm diameter to 150 mm depth).

All soil samples were analysed for pathogen DNA levels (*Spongospora subterranea*, *Rhizoctonia solani* AG2.1, *Rhizoctonia solani* AG3, *Streptomyces txt*A gene, *Meloidogyne fallax*, *Meloidogyne hapla*).

Data from an additional pivot where the same soil sampling strategy had been used in a previous project was included in the analysis for all pathogens and in the case of *Rhizoctonia solani* AG2.1, from an additional 3 pivots.

The pathogen DNA level in each grid sample was assigned a disease risk category for each pathogen based on the putative thresholds from previous research. The true level of disease risk in a pivot was then taken as the proportion of grid samples falling in to each risk category. For example, if 41 of the 45 samples in a pivot had a *Rhizoctonia solani* AG2.1 level less than 50 picogram DNA/ g soil, then the proportion in the low risk category would be 0.91.

To assess if using four 1 hectare grid samples provides a reasonable estimate of disease risk, pivots were divided into quarters (quadrants). Arithmetic means were calculated for 100 groups in each pivot, each group consisting of four samples (1 chosen at random from each quadrant of the pivot).

The data from the estimate of the true DNA concentration in each paddock were logistically transformed, and these values were regressed against the estimate obtained from the mean value of the four random samples. Fitted values from that regression were back-transformed to give predicted levels of the proportion of a paddock that would have levels exceeding the critical values for low and high risk.

A weighted regression was used where the weights were proportional to $(p + 0.01) \times (1 – (p + 0.01))$ to place more emphasis on values with an intermediate risk.

TIMING OF SOIL SAMPLING

In June 2010 four soil samples were taken in each of 16 paddocks in South Australia and 10 paddocks in Tasmania. Each sample consisted of 40 cores collected from each 1 hectare grid on a "W" sampling path using an AccuCore soil sampler (10 mm diameter to 150 mm depth). Samples were taken again at the same four sampling points in each paddock in September/ October. Regression analysis was used to fit the relationship between the paddock means of soil pathogen DNA levels at the two timings of sampling.

DISTRIBUTION OF *RHIZOCTONIA SOLANI* DNA IN THE SOIL PROFILE

High levels of black scurf incidence on tubers can occur at sampling points where the pre-planting soil test has not detected any *Rhizoctonia solani* AG3 DNA. It is possible that the level of *Rhizoctonia solani* AG3 inoculum in the soil required to cause black scurf is below the level of detection of either the field soil

sampling strategy or the laboratory assay. Sample size for pathogen DNA testing of soil samples is limited to approximately 500g. Pathogen DNA testing was conducted on sieved fractions of the soil samples to determine DNA distribution. By targeting only the sieved fraction of the soil sample, a larger initial sample could be collected which may increase the likelihood of detection of *Rhizoctonia solani* AG3. Three sites were selected, representing sites where a medium and a high level of *Rhizoctonia solani* AG3 DNA had been detected along with a site where it was not detected by the field validation sampling method. 40 cores were collected from a 1 hectare grid on a "W" sampling path using an AccuCore soil sampler (10 mm diameter to 150 mm depth). A second set of soil core samples was taken from these sites with a 20mm diameter soil corer, keeping the 0-100 mm and 100-200 mm depths separate. Cores were sampled at regular spacing on a "W" sampling path across a 1 ha area, but not from the exact location of the field validation sample. All paddocks were under continuous pasture over the time period of sampling.

Soil samples were air dried before fractionating in a sieve shaker (EFL2 Mk3) using 0.3, 0.35 and 0.5 mm screen sizes. Soil sample fractions were weighed before pathogen DNA testing. Results for pathogen DNA tests were expressed as $\log_{10}$(picograms DNA / g air-dry soil + 1) for each soil fraction. The percentage of pathogen DNA contained in each soil fraction was calculated from that fraction's proportion of the entire core sample and its pathogen DNA level expressed in pg DNA / g soil sample.

INTER-LABORATORY DNA TEST COMPARISON

INTRODUCTION

Molecular tools for detecting and quantifying a range of potato pathogens (*Rhizoctonia solani*, *Spongospora subterranea*, and *Colletotrichum coccodes*) have been developed in laboratories in Australia (SARDI), New Zealand (New Zealand Plant and Food Research; NZPFR) and the United Kingdom at both James Hutton Institute (JHI, formerly SCRI), Scottish Agricultural College (SAC)) and the Food and Environment Research Agency (Fera).

Each laboratory currently uses different protocols for DNA extraction from soil and, for some tests, different primers and probes for each of the tests. DNA extraction protocols cannot easily be standardised, for the purposes of an inter-lab comparison, identical assays were used.

The objective of this work was to calibrate tests results internationally so that there is a common understanding of what a specific tests result means across laboratories.

PREPARATION OF STANDARD MATERIAL

DNA standards

For *Rhizoctonia solani* and *Colletotrichum coccodes*, isolates Rs08JL and cc1554 respectively, were removed from long term storage and plated onto PDA and grown for 10 days. After this, hyphae were removed from the plates using a scalpel with care to avoid agar. DNA from *Rhizoctonia solani* and *Colletotrichum coccodes* was extracted to provide DNA standards. For each participant laboratory, a 200 µl aliquot of the DNA suspension was placed into a 1.5 ml tube. This was fully dried prior to dispatching to each ring test participant.

Soil samples

Soil was obtained from a permanent pasture situated in North Yorkshire where no other crops had been grown for at least 60 years. Soil was sieved < 4 mm to remove stones, large debris and organic matter before oven drying at 40°C for at least 48 hours.

Isolates Rs08JL and cc1554 were removed from long term storage and grown on PDA for six weeks. Sclerotia or conidia were removed using a scalpel and dried for two days at room temperature. For *Rhizoctonia solani*, this was added to soil to produce the following w/w, high (0.22%), medium (0.02%) and low (0.01%). For *Colletotrichum coccodes* standards were produced as follows, high (0.01% w/w) and medium (0.001% w/w). For low naturally infested soil was used (sourced by Fera from potato plots). This was because diluting at lower volumes than 0.001% produced inconsistent detection of DNA by real-time PCR.

TEST COMPARISON

Soil samples were 'spiked' with known quantities of pathogen and sent to participating laboratories. Participating laboratories used their own extraction methods. In order to determine where differences in test results occur, the same DNA assay (primers and probes) was applied to DNA extracted by each laboratory. Standard DNA was also distributed by Fera to the participating laboratories, to provide uniformity in the creation of standard curves.

Samples were sent by courier to four international ring test partners plus one set retained for blind testing at Fera. Samples were dispatched on 14 April (*Colletotrichum coccodes* set 1), 8 July (*Colletotrichum coccodes* set 2), and 20 July (*Rhizoctonia solani* AG3). On receipt of all results, a 'consensus mean' was calculated for each inoculum level. This is the arithmetic mean across labs at each inoculum level. From these data, an estimate of the degree to which the individual laboratory means deviated from the consensus mean was calculated (the z-score). These are Standard Deviations from the consensus mean.

PATHOGEN EPIDEMIOLOGY – UNDERSTANDING INOCULUM AND DISEASE DEVELOPMENT

MAIN FINDINGS FROM UNITED KINGDOM COLLABORATION - RHIZOCTONIA

Trials relating to *Rhizoctonia solani* were conducted relating to detection of inoculum in soil, impact of soil type on disease development, effect of inoculum sources and the role of tuber-borne inoculum. At the conclusion of the Potato Council UK project an extensive final report was produced. Details of methods can be accessed on request.

ROLES OF *RHIZOCTONIA SOLANI* AG2.1 AND AG3 IN CAUSING STOLON PRUNING AND STEM CANKERS

Rhizoctonia solani AG2.1 and AG3 are the most dominant anastomosis groups isolated from potato plants (Woodhall, Lees, Edwards, & Jenkinson, 2007). The aim of this controlled environment trial was to investigate whether *Rhizoctonia solani* AG2.1 and AG3 compete with each other in soil, or act synergistically to produce disease. An isolate of *Rhizoctonia solani* AG2.1 and *Rhizoctonia solani* AG3 (both

originally from potato) were inoculated into soil in combination at various rates: one plate fungal mycelium (1), half plate fungal mycelium (0.5), quarter plate fungal mycelium (0.25) per 8 kg soil.

Single minitubers of the variety Coliban were planted into pots (5 replicate pots per treatment), and grown in the glasshouse. Stolon pruning and stem canker lesions were assessed 4 times at 3-weekly intervals, beginning at 37 days after planting. Infected plant parts were sampled, before dividing into two with half being used for isolation of a culture prior to pathogen DNA analysis and the other half being used directly for pathogen DNA analysis.

VARIETY SUSCEPTIBILITY TO COMMON AND POWDERY SCAB

To better make recommendations for the management of diseases we need to know the relative resistance of varieties that are important to the industry. Two field sites were selected to evaluate a total of 20 potato varieties for resistance to powdery and common scab. Varieties evaluated (Atlantic, Catani, Chipper, Innovator, Ivory Crisp, Kennebec, Netted Gem, Pike, Ranger Russet, Russet Burbank (clones Ruen Ag, Ruen TE, Vancouver), Shepody, Simcoe, Sonic, Trent) were selected in consultation with key representatives of potato processing companies. A set of standard varieties (Coliban, Desiree, Nicola, Sebago) were included to assist when comparing with other sources of variety susceptibility information. At the assessment site for common scab, located at Cora Lynn Victoria, the pre planting level of *Streptomyces txt*A gene DNA in the soil was 2.2 log (pg DNA/ g soil +1) which confirms the presence of pathogenic *Streptomyces* and an elevated level of disease risk. Mini tubers of each variety were planted at this site on 9/12/2011 and harvested on 11/4/2012. At the assessment site for powdery scab, located at Ballarat Victoria, the pre planting level of *Spongospora subterranea* DNA in the soil was 2.3 log (pg DNA/ g soil +1) which indicates a high level of disease risk. Mini tubers of each variety were planted at this site on 6/12/2011 and harvested on 8/5/2012. At each site, a trial was established with 5 mini tubers per plot replicated five times. At harvest all tubers were assessed for disease.

DEVELOPMENT OF *SPONGOSPORA SUBTERRANEA* ROOT GALLING AND TUBER SYMPTOMS

A site known to be infested with *Spongospora subterranea* was selected at Ballarat, Victoria. The four varieties Russet Burbank, Desiree, Nicola and Kennebec were planted on 16th December 2009. Plots were 14m long with four replicates. Prior to planting soil was collected from each plot for pathogen DNA analysis, Table 4-2.

Table 4-2 – Soil levels of *Spongospora subterranea* DNA prior to planting trial root galling and tuber symptom assessment trial at Ballarat

Variety	Soil DNA concentration *Spongospora subterranea* (pg DNA/g soil)				
	Plot1	Plot2	Plot3	Plot4	Mean
Desiree	252	213	286	238	247
Kennebec	274	268	222	250	253
Nicola	135	177	125	209	161
Russet Burbank	300	272	169	219	240

The potato crop was watered by a lateral irrigator every 5 days. During the growing season plants were lifted nine times, with the first assessment at 50% emergence and the final assessment at harvest. Lifted plants were assessed for powdery scab root galling in the field using the ordinal scale (0-4) devised from the Powdery scab scoring workshop 2002 where 0 =no root galls; 1 = one or two root galls; 2 = several galls (mostly small < 2 mm in diameter); 3 = many galls (some > 2 mm in diameter); and 4 = most major roots with galls (some or all > 4 mm in diameter).

Tubers were harvested, washed and visually assessed for powdery scab symptoms, with disease incidence recorded for each plant. Harvested tubers were peeled completely and *Spongospora subterranea* DNA analysis conducted on subsample of the composite samples for each plot replicate. At harvest soil was collected from each plot for pathogen DNA analysis.

DNA from potato roots and tuber peelings was extracted using the method of (Cullen, Lees, Toth, & Duncan, 2002). In each case, 1 g of root tissue and 5 g of peel tissue was taken at random. Each sample was homogenised with 2 x w/v with CTAB extraction buffer. DNA was diluted to 40 ng/5 uL prior to real time PCR. Real time PCR was performed as described (van de Graaf, Lees, Cullen, & Duncan, 2003) using DNA extracted from samples.

RESULTS AND DISCUSSION

FIELD VALIDATION OF PRE-PLANT SOIL DNA TESTS

INTRODUCTION

Tests have been developed to quantitatively measure DNA in the soil of the pathogens *Colletotrichum coccodes*, *Rhizoctonia solani* AG2.1, *Rhizoctonia solani* AG3, *Spongospora subterranea*, *Streptomyces txt*A gene, and the nematodes *Meloidogyne fallax*, *Meloidogyne hapla*, *Pratylenchus crenatus*, *Pratylenchus neglectus*, *Pratylenchus penetrans*.

A field validation process has been conducted in commercial growing systems across South Australia, Victoria and Tasmania. The objective of this work was to determine which pre-plant soil DNA tests provide a useful indication of the risk of disease, establish or confirm risk thresholds for the tests and assess the robustness of the tests over a range of varieties, seasons and geographic locations.

Results are presented for each pathogen for which a DNA pre-plant soil test has been developed. Progress towards the commercialisation of the tests through this validation process depends on the length of time that the test has been available and the results obtained through the process. Field validation conducted within this and previous projects has enabled commercialisation of the *Spongospora subterranea*, *Colletotrichum coccodes* and *Meloidogyne fallax* tests.

SPONGOSPORA SUBTERRANEA

OVERVIEW

Soil DNA testing to assess the risk of powdery scab has been included in the current PreDicta Pt service. Disease risk is based on the level of DNA of the pathogen *Spongospora subterranea* measured in the soil prior to planting. Field validation in commercial crops grown in Tasmania, Victoria and South Australia has demonstrated the pre-plant test provides a useful indication of powdery scab risk to tubers, Figure 4-1.

Field validation has confirmed the appropriateness of previously indicated putative thresholds (APRP1 report) to describe the risk of powdery scab on tubers, when applied to varieties of moderate to low susceptibility being grown for processing, Table 4-3. At sampling points where DNA in the soil prior to planting was not detected or in the low risk category, approximately 1 in 10 sites developed visible powdery scab symptoms on more than 10% of the harvested tubers. In comparison, over half the sampling points in the high risk category resulted in a disease incidence above 10%.

Risk thresholds have been established based on the probability of powdery scab symptoms occurring on the harvested tubers. High inoculum levels in the soil do not always result in tuber symptoms. Environmental conditions and varietal susceptibility influence the incidence and severity of tuber symptoms. However, high levels of DNA of *Spongospora subterranea* detected in the soil prior to planting correspond with high levels of DNA on harvested tubers, Figure 4-2. This indicates the pathogen is still active on the peel of the tubers even when tuber symptoms are not expressed, and suggests the pathogen may still be having other impacts on the potato crop.

High pathogen levels are sometimes detected in the peel of tubers grown in soils where *Spongospora subterranea* levels prior to planting were considered low or were not detected. This suggests that conditions at these sites were conducive to rapid pathogen build-up. In some cases this resulted in visible tuber symptoms. Risk of powdery scab is higher when highly susceptible varieties are grown under conducive conditions. There is scope to refine the thresholds to reflect responses in differing regional climates, as well as differing varietal susceptibility.

Risk thresholds relate to the risk of developing powdery scab on the tubers. *Spongospora subterranea* also causes root infection and root galling. Root galling is a source of inoculum buildup and there is a possibility that root infection could cause yield reduction.

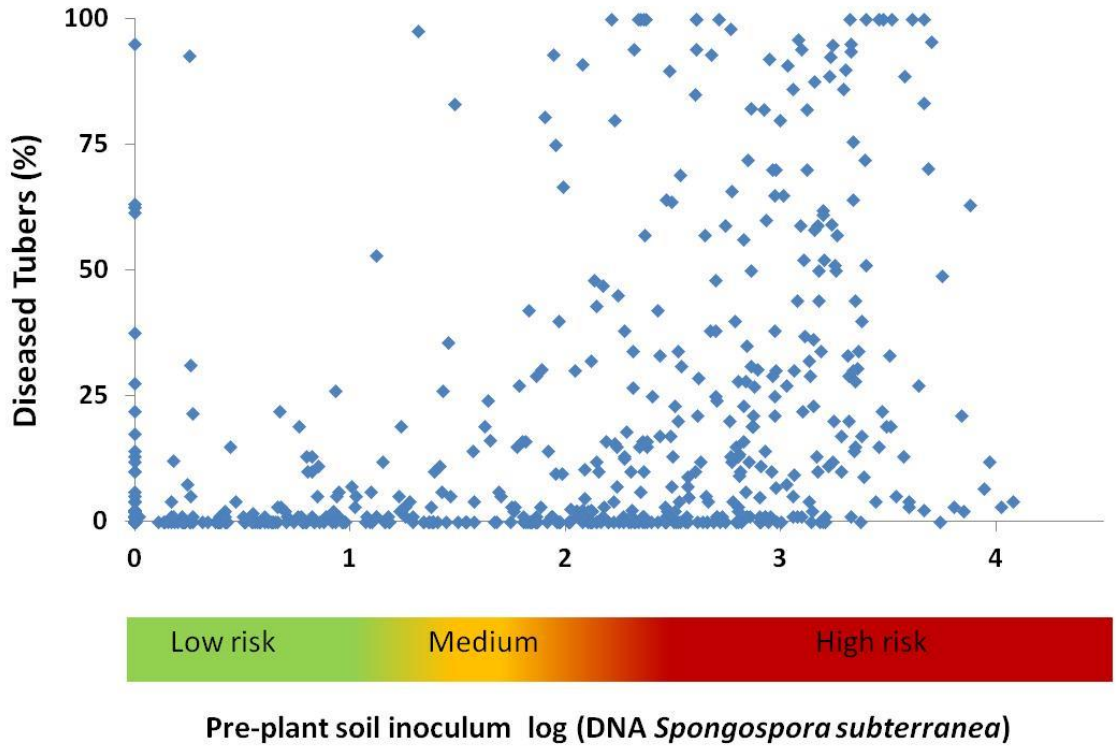

Figure 4-1 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of powdery scab on the tubers in commercially grown potato crops. (638 sampling points monitored across Tasmania, Victoria and South Australia in 2011/12 and 2012/13)

Table 4-3 – Percentage of sampling points where powdery scab incidence on tubers exceeded 10% within each PreDicta Pt risk category (638 sampling points monitored across Tasmania, Victoria and South Australia in 2011/12 and 2012/13)

PreDicta Pt Risk Category	Pre-plant soil test range log (DNA *Spongospora subterranea*)	Number of sampling points	% paddocks with tuber incidence > 10%
Below detection	0	121	9
Low	0.1 to1.3	125	11
Medium	1.4 to 1.9	57	26
High	>1.9	335	56

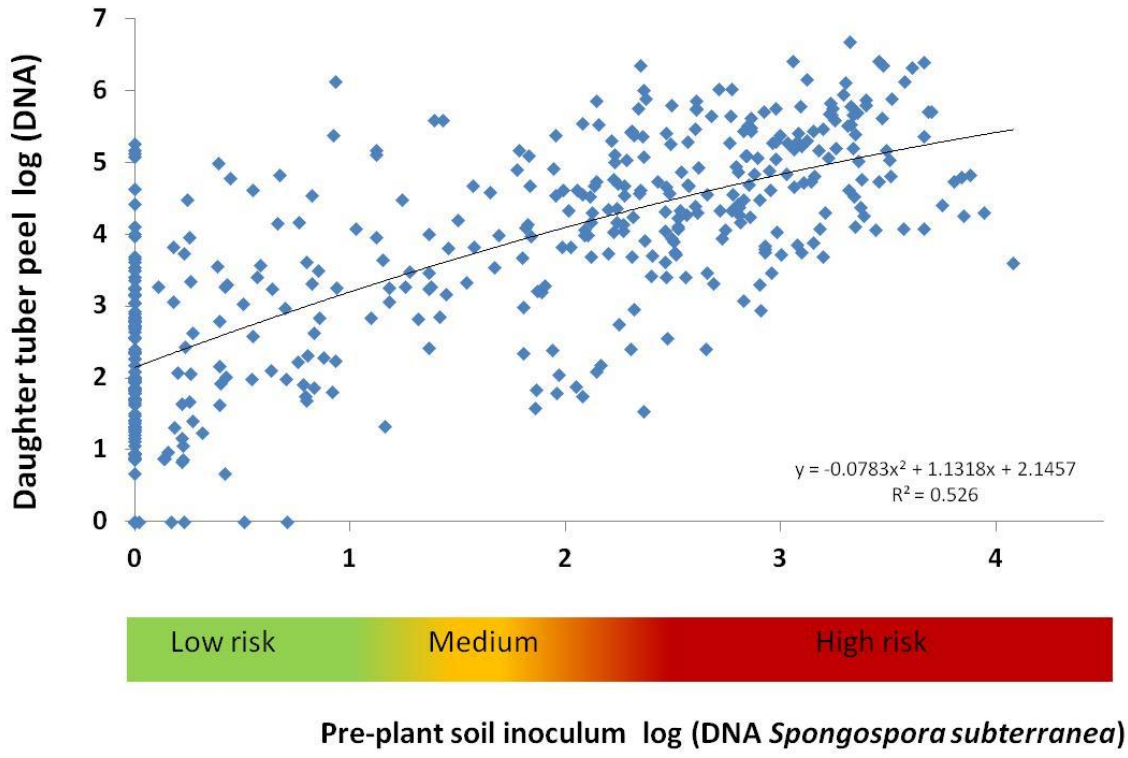

Figure 4-2 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and on the harvested tubers in commercially grown potato crops (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

IMPACT OF GEOGRAPHIC REGION

Temperature and moisture level of the soil are known to impact on the incidence and severity of powdery scab (van de Graaf, Lees, Wale, & Duncan, 2005). As a result, some growing areas are more conducive to powdery scab problems. Field validation has been conducted at multiple locations in three states. Tasmania has the most conducive climatic conditions for powdery scab, followed by Victoria. Conditions are less conducive in the Southeast region of South Australia. These differences are reflected in the incidence and severity of powdery scab on the variety Russet Burbank, a variety that is grown in all three states, Figure 4-3. When Russet Burbank potatoes are grown in soils with high levels of *Spongospora subterranea* inoculum, the incidence and severity of powdery scab is higher in the cool temperate regions of Tasmania and Victoria compared with the SE of South Australia that has warmer soil temperatures.

Within each of the states, further variation exists between growing districts. For example in South Australia a higher incidence and severity of powdery scab on Russet Burbank potatoes was evident in the Penola - Mount Gambier district compared with the Robe district, Figure 4-4. Additionally potatoes are planted earlier in the Robe district and are not generally stored in the ground for as long as crops in the Penola – Mount Gambier district. Management practices can lead to less or more conducive conditions than expected for a specific location.

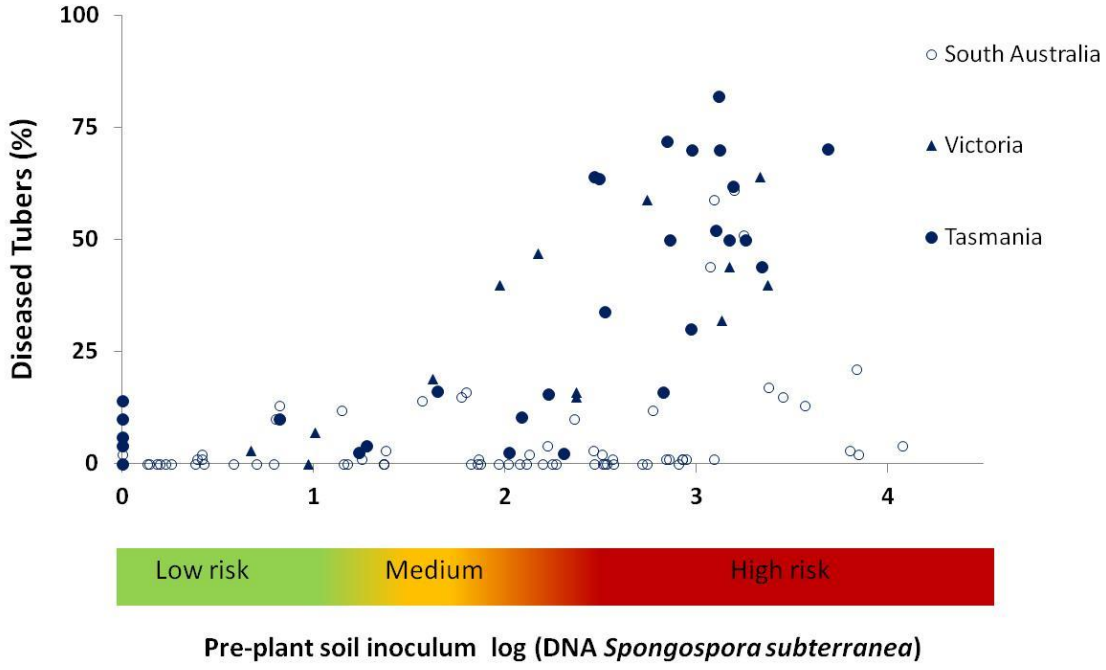

Figure 4-3 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of powdery scab depending on the location in Australia that Russet Burbank is grown. (data from 2011/12 and 2012/13 growing seasons combined).

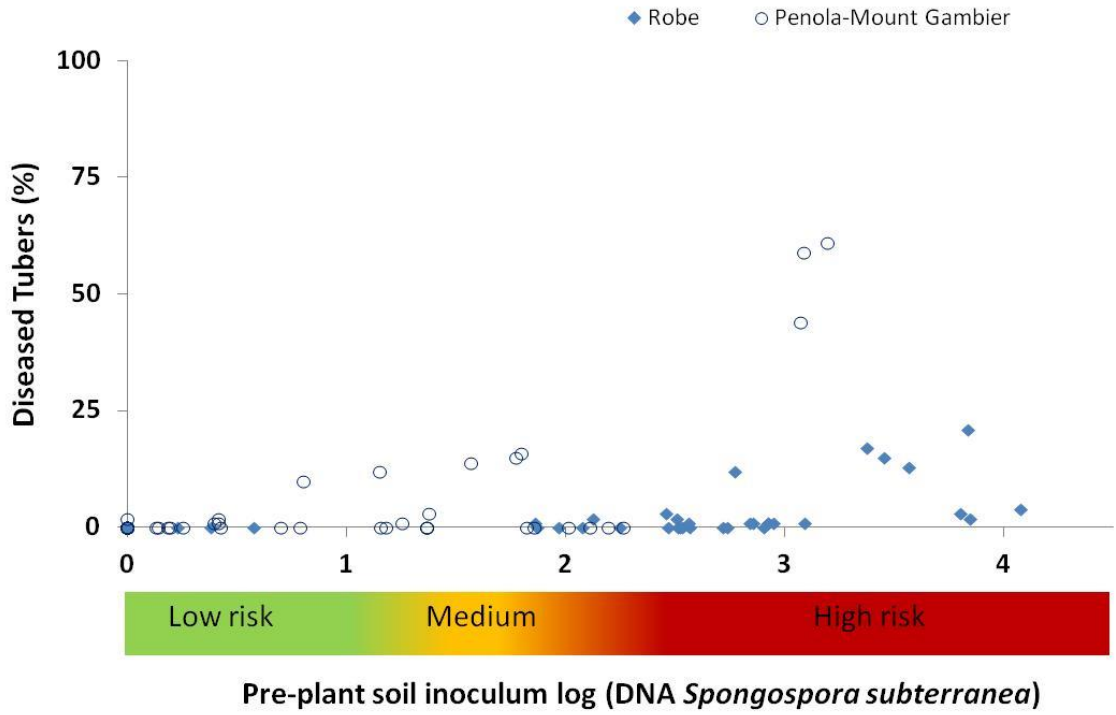

Figure 4-4 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of powdery scab on Russet Burbank depending on the growing region within SE South Australia. (Data from 2011/12 and 2012/13 growing seasons combined).

IMPACT OF SEASON

Climatic conditions are known to impact on the incidence and severity of powdery scab symptoms. Under irrigated conditions in a known high risk region such as Tasmania, seasonal variation over the three years from 2010/11 to 2012/13 did not appear to have a large impact on the incidence of powdery scab of Russet Burbank potatoes, Figure 4-5. The 2010/11 season was wetter than subsequent two seasons. This limited data over three seasons suggests that differences between growing regions have a greater influence on the risk of powdery scab than year to year variations within a region.

It is likely that the timing of planting, irrigation practices and variety grown have a greater impact on the incidence of powdery scab on tubers than variation in weather conditions between years at a given site.

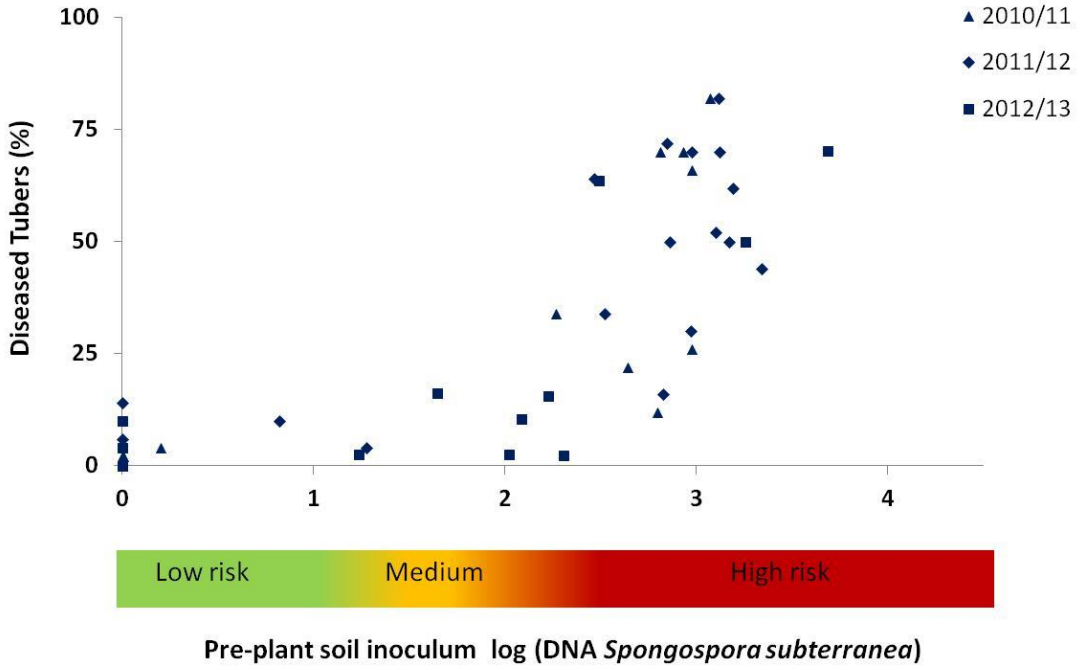

Figure 4-5 – Differences in the relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of powdery scab depending on the growing season for Russet Burbank grown in Tasmania.

IMPACT OF VARIETY

Control options for powdery scab are limited. Growing varieties that are less susceptible is one of the most cost efficient and effective ways to reduce the risk of powdery scab on the tubers (Falloon, 2008). Differences in the susceptibility of varieties are clearly evident in the field validation data, though there are variations with the region in which they are grown, Figure 4-6.

Varieties among those assessed that appeared to be least susceptible to powdery scab on the tubers were Catani, FL 1867, Pike, Snowden and Topcat. Varieties that appeared to be moderately susceptible include Atlantic, Innovator, JR2, Ranger Russet, Russet Burbank and Trent, although Innovator seemed to show a lower level of susceptibility when grown in South Australia. Varieties including Alturas, Bondi, Kennebec and Nooksack were susceptible. Though Alturas and Nooksac showed a high incidence of powdery scab, the symptoms were mostly confined to the stolon region. Nooksack is not considered to be susceptible (Falloon, Genet, Wallace, & Butler, 2003).

Even at low levels of inoculum in the soil, highly susceptible varieties can be at risk of powdery scab. Examples of varieties where this was seen are Bondi and Kennebec.

Growing highly susceptible varieties in a conducive environment and at a site with high levels of inoculum in the soil almost always results in a high incidence and severity of disease, for example Bondi grown in Tasmania.

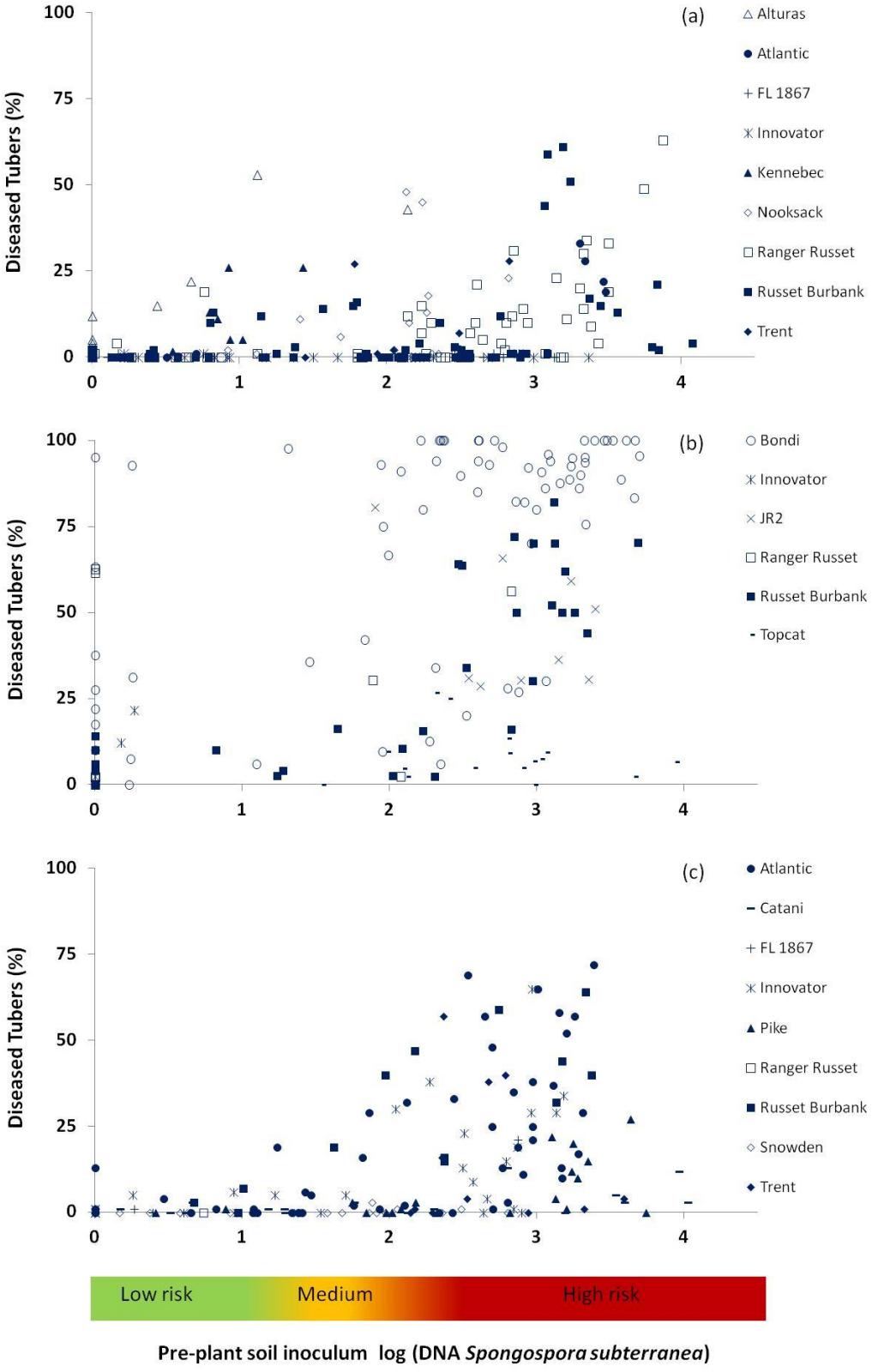

Figure 4-6 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of powdery scab on the tubers depending on the variety grown in a) South Australia, b) Tasmania and c) Victoria. (Data from 2011/12 and 2012/13 growing seasons combined).

IMPACT OF SOIL TYPE

Low levels of powdery scab occur at some sites, despite these sites being at risk. In some locations characteristics of the soil may not favour disease, for example sites in Victoria with high organic matter levels (>13%), Figure 4-7. Apart from favouring a high level of biological activity in the soil, these high organic matter soils are characterised by higher levels of zinc, iron, magnesium and sodium and lower levels of copper, manganese and sulphur than other soils tested in Victoria. High organic matter levels in the soil alone are not likely to suppress powdery scab, as disease incidence and severity can be high in pot trials using high organic matter level potting mixes.

No evidence has been found of soils that are suppressive to powdery scab in either South Australia or Tasmania during this project. In Tasmania disease expression is almost always high where a susceptible variety is grown in soils with a high inoculum level. In South Australia where low levels of disease expression are seen on sites with high soil inoculum, the lack of disease expression on tubers appears attributable to variety, growing region and management practices.

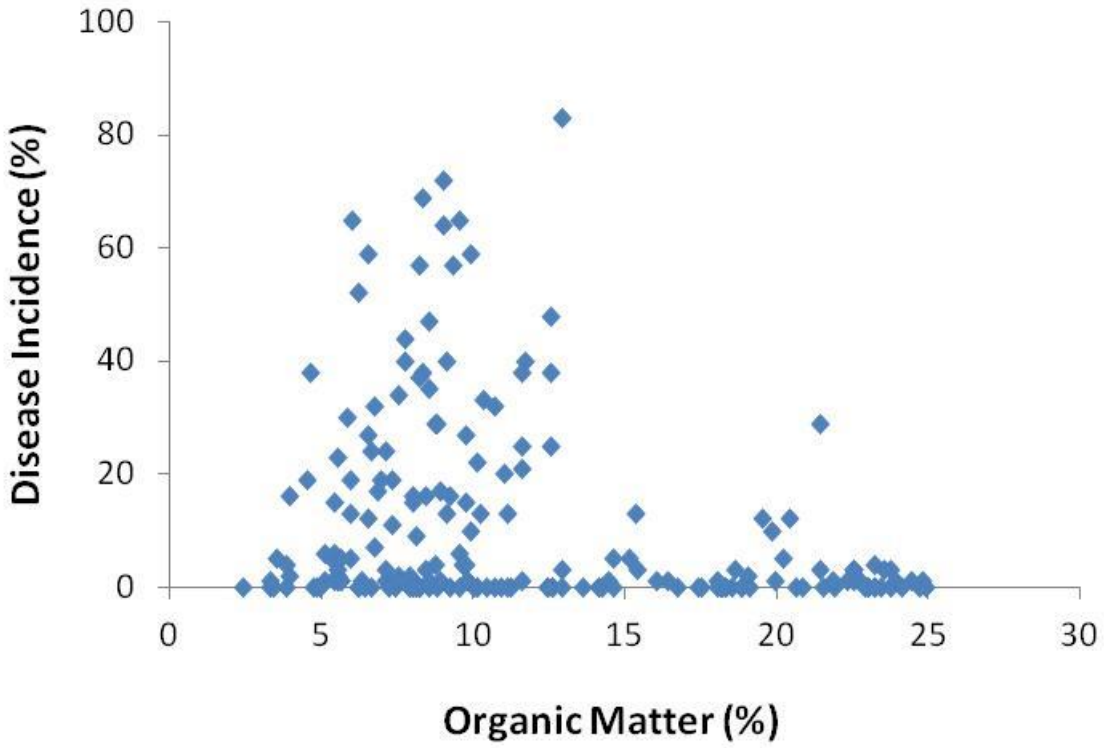

Figure 4-7 – Relationship between percent organic matter of the soil and the incidence of powdery scab on the tubers in commercially grown potato crops (198 sampling points monitored in Victoria during 2011/12 and 2012/13).

ROOT GALLING

Root galling was detected at 12 of the 92 sampling points in Tasmania examined 9 to 12 weeks after planting in the 2012/13 season. Galling would be expected to be seen under Tasmanian conditions at this

growth stage. It is possible the rate of detection might have been higher if a later timing of assessment had been used. Root galling at 9 to 12 weeks after planting was not detected at any sites where log (pg DNA / g soil + 1) concentrations in the soil prior to planting of *Spongospora subterranea* were below 2.5. These results indicate the risk of root galling being present at this early stage of crop development in the 2012/13 season was related to high levels of *Spongospora subterranea* inoculum in the soil at planting. Varieties on which root galling was detected were Russet Burbank, Topcat and JR2. Bondi was planted at over half the sites assessed. Root galling was not detected on Bondi at this stage of crop growth, despite this variety being highly susceptible to tuber symptoms, supporting the observation that susceptibility to root galling and tuber symptoms are not linked. Though root galling was not detected on Ranger Russet or Innovator, these varieties were only assessed at 7 and 4 sampling points respectively.

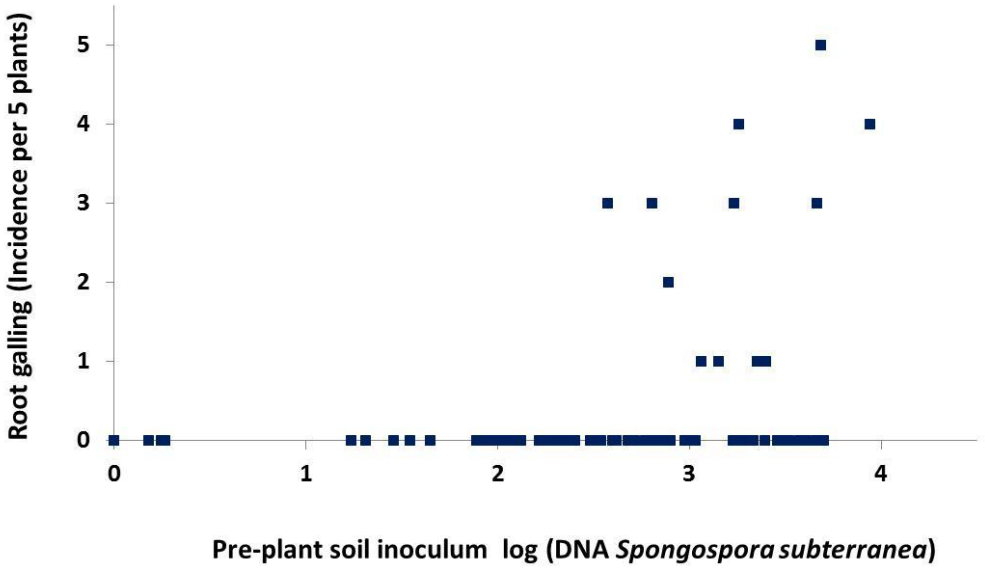

Figure 4-8 – Relationship between concentration of *Spongospora subterranea* DNA in the soil prior to planting and the incidence of root galling assessed 9-12 weeks after planting (92 sampling points in Tasmania in the 2012/13 growing season)

MELOIDOGYNE FALLAX

OVERVIEW

Soil DNA testing to assess the risk of root-knot nematode has been included in the current PreDicta Pt service. Disease risk is based on the level of DNA of the nematode *Meloidogyne fallax* measured in the soil prior to planting. Field validation in commercial crops has demonstrated that the pre-plant soil test provides a useful indication of root-knot nematode risk to tubers in South Australia, Figure 4-9. Though *Meloidogyne fallax* is present in both Tasmania and Victoria, problems on tubers are infrequent and sporadic.

Our field validation has confirmed the appropriateness of previously indicated putative thresholds (APRP1 report) to describe the risk of root-knot nematode symptoms on tubers of varieties being grown for

processing, Table 4-4. At sampling points where DNA in the soil prior to planting was not detected or in the low risk category, approximately 1 in 20 sampling points developed visible root-knot nematode symptoms on more than 5% of the harvested tubers. In comparison, half the sampling points in the high risk category resulted in a disease incidence above 5%.

Risk thresholds have been established based on the probability of root-knot nematode occurring on the harvested tubers. High inoculum levels in the soil do not always result in tuber symptoms. Environmental conditions influence the rate of multiplication of *Meloidogyne fallax*. Even though the critical threshold for visible symptoms may not be reached, high levels of DNA of *Meloidogyne fallax* detected in the soil prior to planting correspond with high levels of DNA on harvested tubers, Figure 4-10. This indicates that the pathogen is still active in the peel of the tubers even when tuber symptoms are not expressed, and suggests the pathogen may still be having other impacts on the potato crop, such as yield reduction.

There are many species of root-knot nematodes. Our data indicates that where visible root-knot nematode symptoms (bumps) are seen on the surface of tubers they are associated with high levels of *Meloidogyne fallax* in the peel, both in the SE of South Australia and in Tasmania, Figure 4-11.

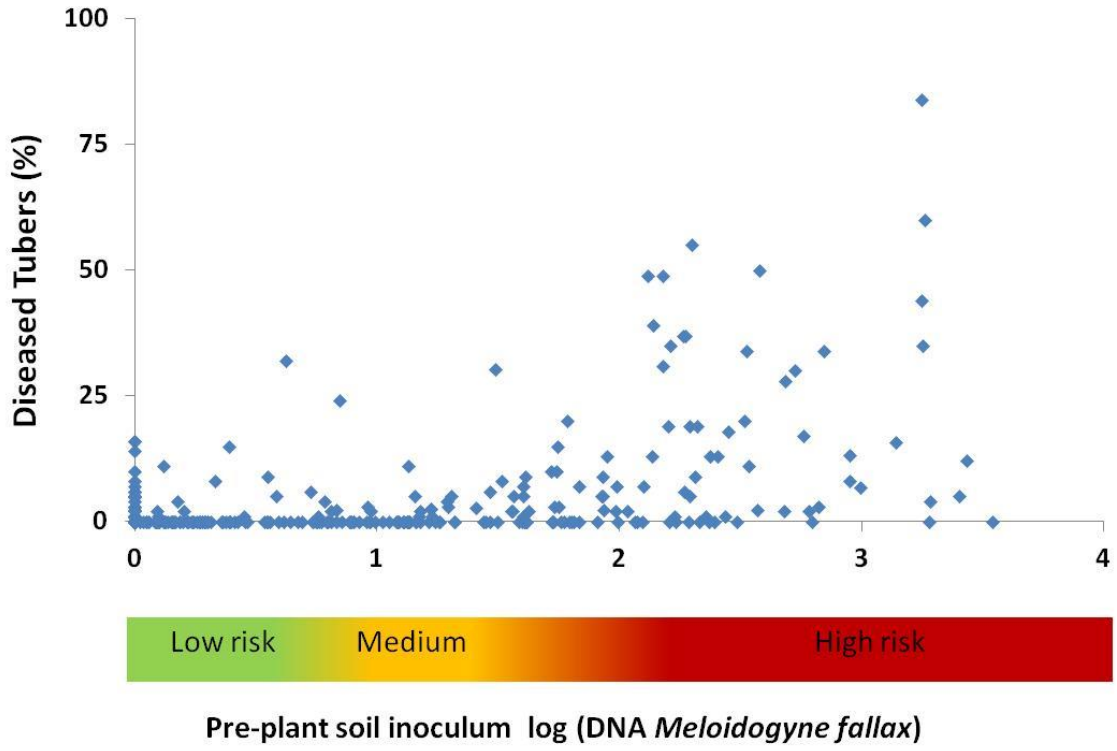

Figure 4-9 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and the incidence of root-knot nematode symptoms on the tubers in commercially grown potato crops (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

Table 4-4 – Percentage of sampling points where root-knot nematode symptoms on tubers exceeded 5% within each PreDicta Pt risk category. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

PreDicta Pt Risk Category	Pre-plant soil test range log (DNA *Meloidogyne fallax*)	Number of sampling points	% paddocks with tuber incidence > 5%
Below detection	0	187	4
Low	0.1 to 0.8	81	7
Medium	0.9 to 1.7	76	9
High	>1.7	86	52

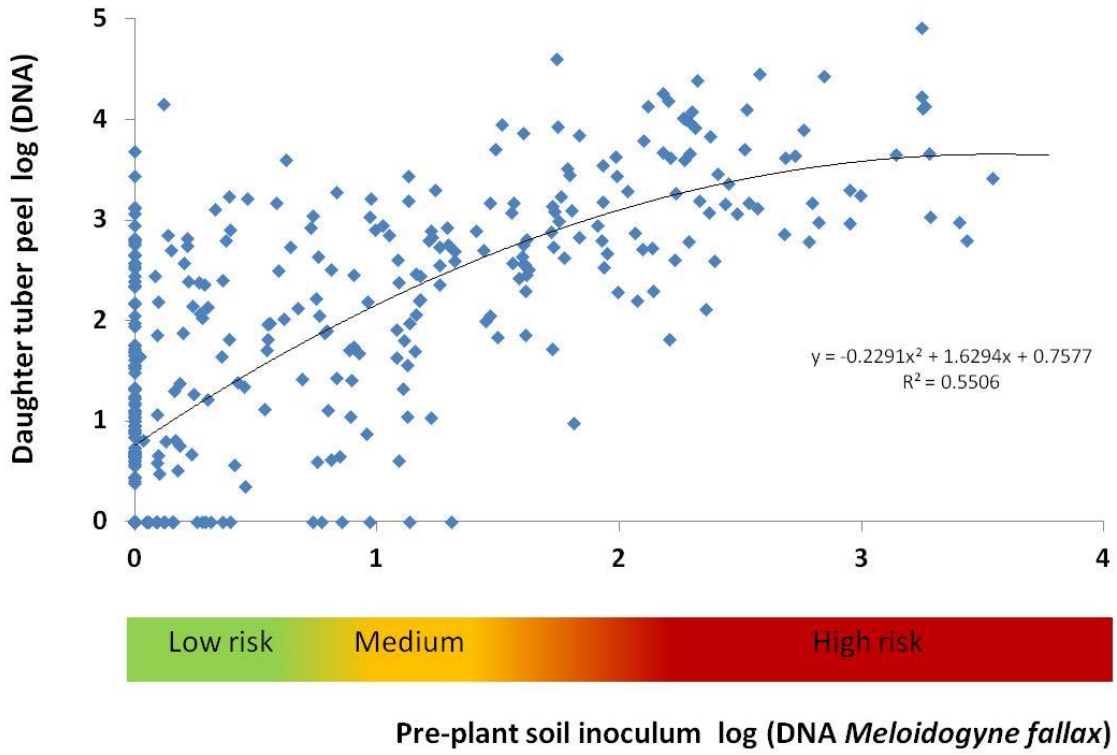

Figure 4-10 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and in the peel of harvested tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

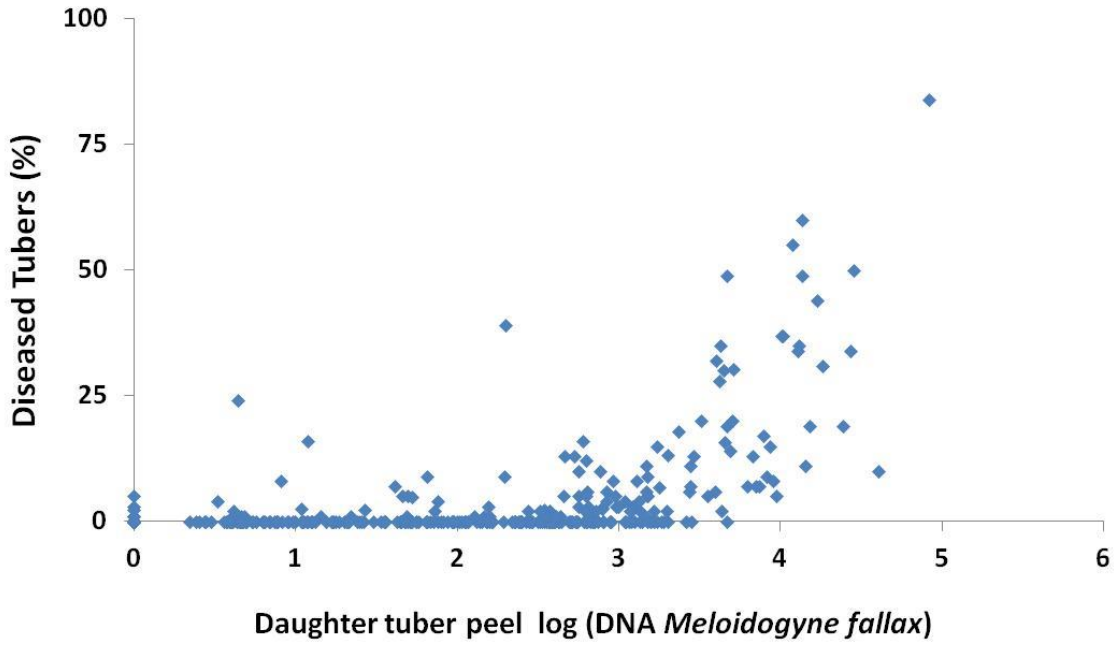

Figure 4-11 – Relationship between levels of *Meloidogyne fallax* DNA in the peel of daughter tubers and the incidence of root-knot nematode symptoms on the tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

IMPACT OF GEOGRAPHIC REGION

Field validation has been conducted at multiple locations in three states. Risk of tuber symptoms is highest where a third generation of *Meloidogyne fallax* occurs within the one growing season. Soil temperature is a key driver of the generation time of *Meloidogyne fallax* (Hay, et al., 2013). As a result, consistently warm growing areas are more conducive to root-knot nematode problems.

Of the production areas included, the SE of South Australia is the warmest. Other than soil temperature, factors that are known to increase risk of root-knot nematode are long term pasture prior to planting potatoes and sandy soils, both of which frequently occur where potatoes are grown in the SE of South Australia. Growing conditions are cooler in Tasmania, reducing the risk of *Meloidogyne fallax*. These differences are reflected in the incidence and severity of root-knot nematode on the variety Russet Burbank, a variety that is grown in both states, Figure 4-12. In paddocks tested in Tasmania, nematode levels in the soil prior to planting were rarely above the high risk threshold. An incidence of tuber symptoms above 5% was only recorded in 1 paddock of the 45 paddocks assessed in Tasmania. The very low incidence of root-knot nematode symptoms in Victoria did not warrant its assessment.

Within the SE of South Australia, further variation exists between growing districts. For example in South Australia a higher incidence and severity of root-knot nematodes on Russet Burbank potatoes was evident in the Penola - Mount Gambier district compared with the Robe district, Figure 4-13. Potatoes are planted earlier in the Robe district compared with the Penola – Mount Gambier district where potatoes are normally planted later into warmer soils. Generation time of *Meloidogyne fallax* is shorter in warm soils leading to more rapid buildup of the nematode population. Additionally in-ground storage of potatoes is more common and for longer periods in the Penola – Mount Gambier district, which increases the likelihood of a third generation of nematodes occurring. Both these factors would be impacting on differences in incidence and severity of root-knot nematode symptoms between the two districts.

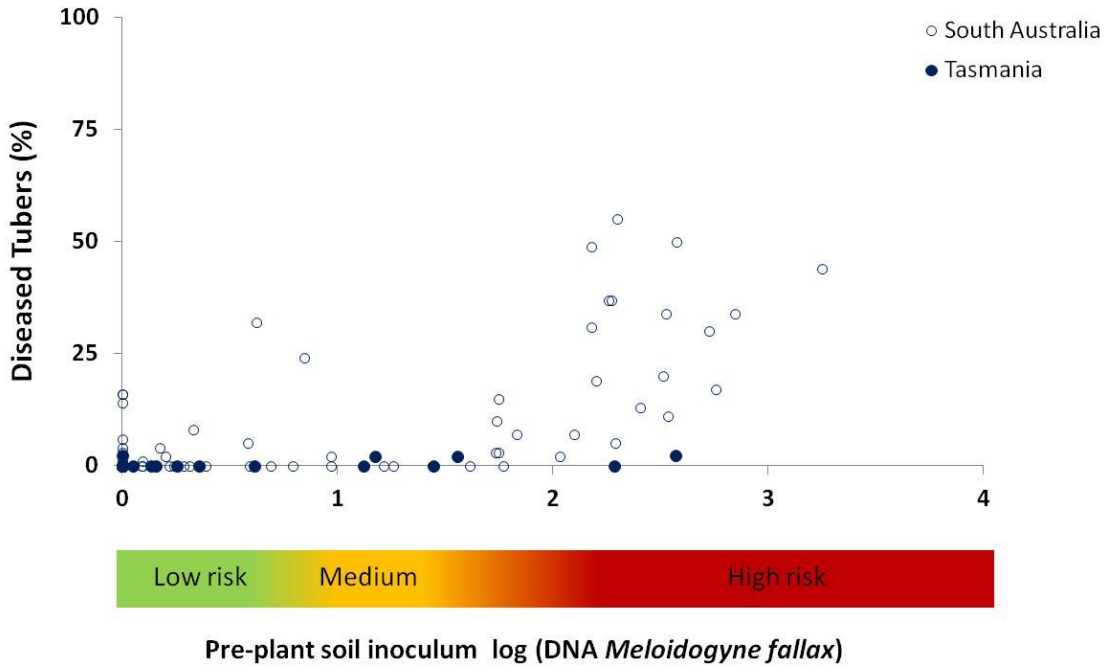

Figure 4-12 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and the incidence of root-knot nematode symptoms on tubers depending on the location in Australia that Russet Burbank is grown (Data from 2011/12 and 2012/13 growing seasons combined).

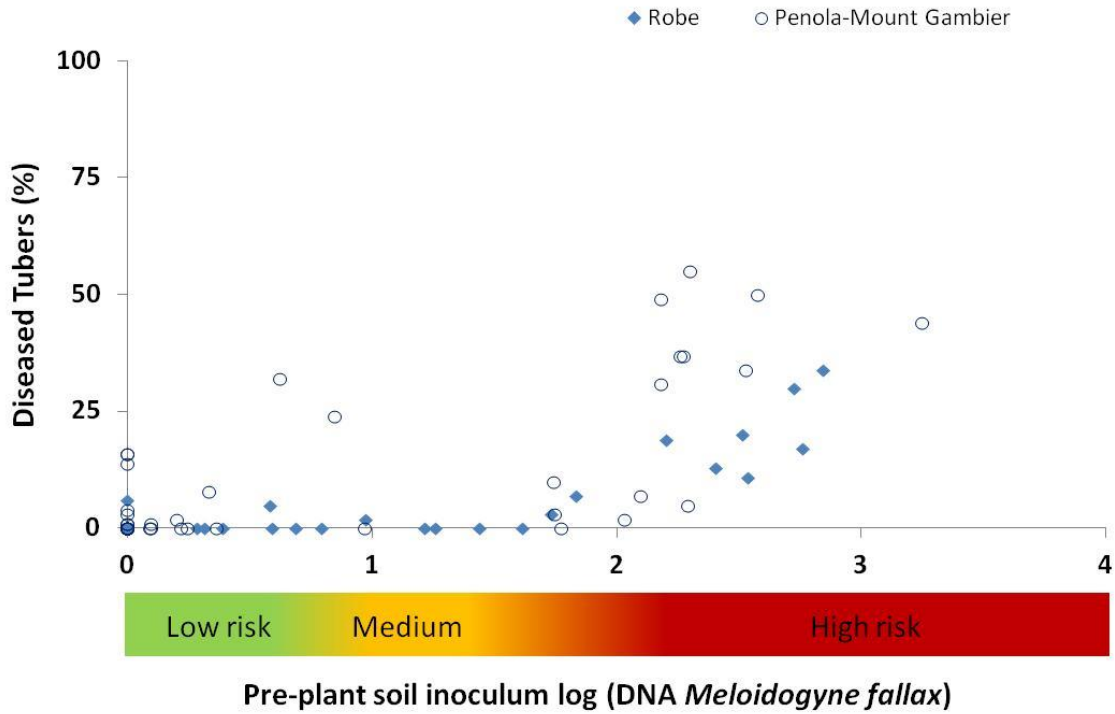

Figure 4-13 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and the incidence of root-knot nematode tuber symptoms on Russet Burbank depending on the growing region within SE South Australia. (Data from 2011/12 and 2012/13 growing seasons combined).

IMPACT OF SEASON

Climate is known to impact on the incidence and severity of root-knot nematode. Generation time is expected to decrease with optimum soil temperature (Hay, et al., 2013). Nematode damage is expected to be worse under consistently warm conditions, especially when warm conditions occur early in the growing season, such as in the 2012/13 growing season in the SE of South Australia. A higher rate of multiplication would have been expected in this season. The concentration of DNA of *Meloidogyne fallax* measured in the peel of Russet Burbank potatoes was higher in the 2012/13 season as compared with the 2010/11 and 2011/12 seasons, Figure 4-14.

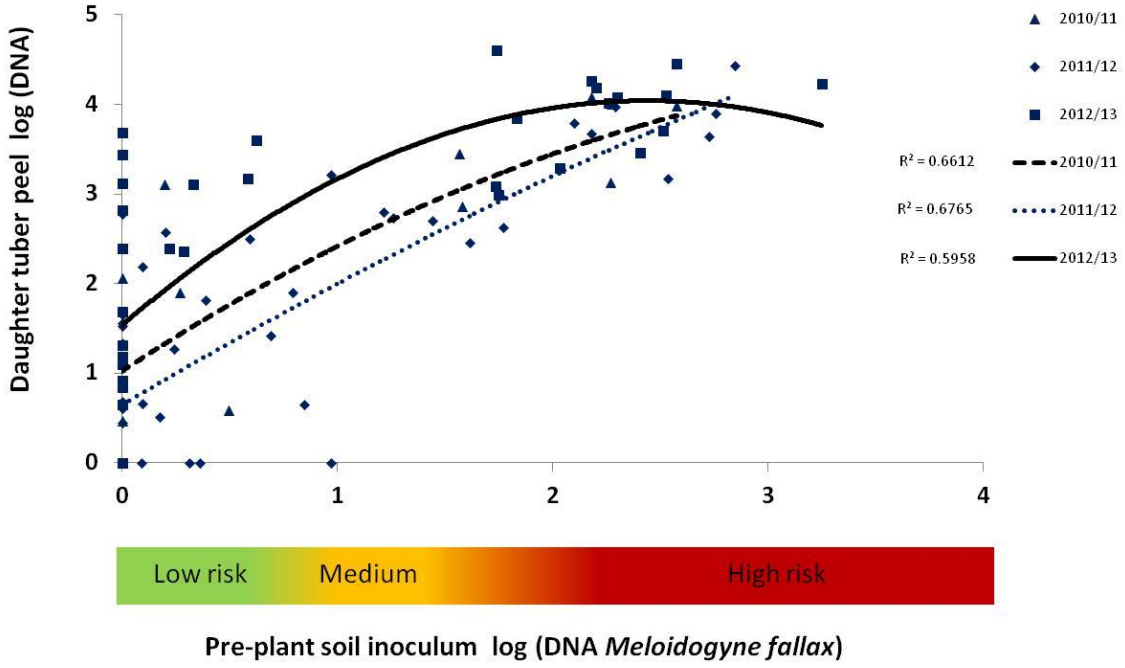

Figure 4-14 – Differences in the relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and in the peel of harvested tubers in commercially grown Russet Burbank potato crops in the South East of South Australia depending on the growing season.

IMPACT OF VARIETY

Tuber symptoms caused by root-knot nematode were recorded on all varieties assessed, Figure 4-15. Highest levels were found on Russet Burbank and Innovator. Though these varieties are clearly susceptible to damage, as is Ranger Russet, site conditions, planting times and delay to harvest would have contributed to the high incidence and severity of symptoms recorded at some sites. Based on the data collected Atlantic and FL1867 appear less susceptible to visible tuber symptoms. Supporting this finding, these varieties also tended to have lower levels of *Meloidogyne fallax* DNA in the peel of harvested tubers (data not shown). Insufficient data was collected on other varieties at sites with high levels of *Meloidogyne fallax* in the soil prior to planting to comment on their susceptibility to root knot nematode.

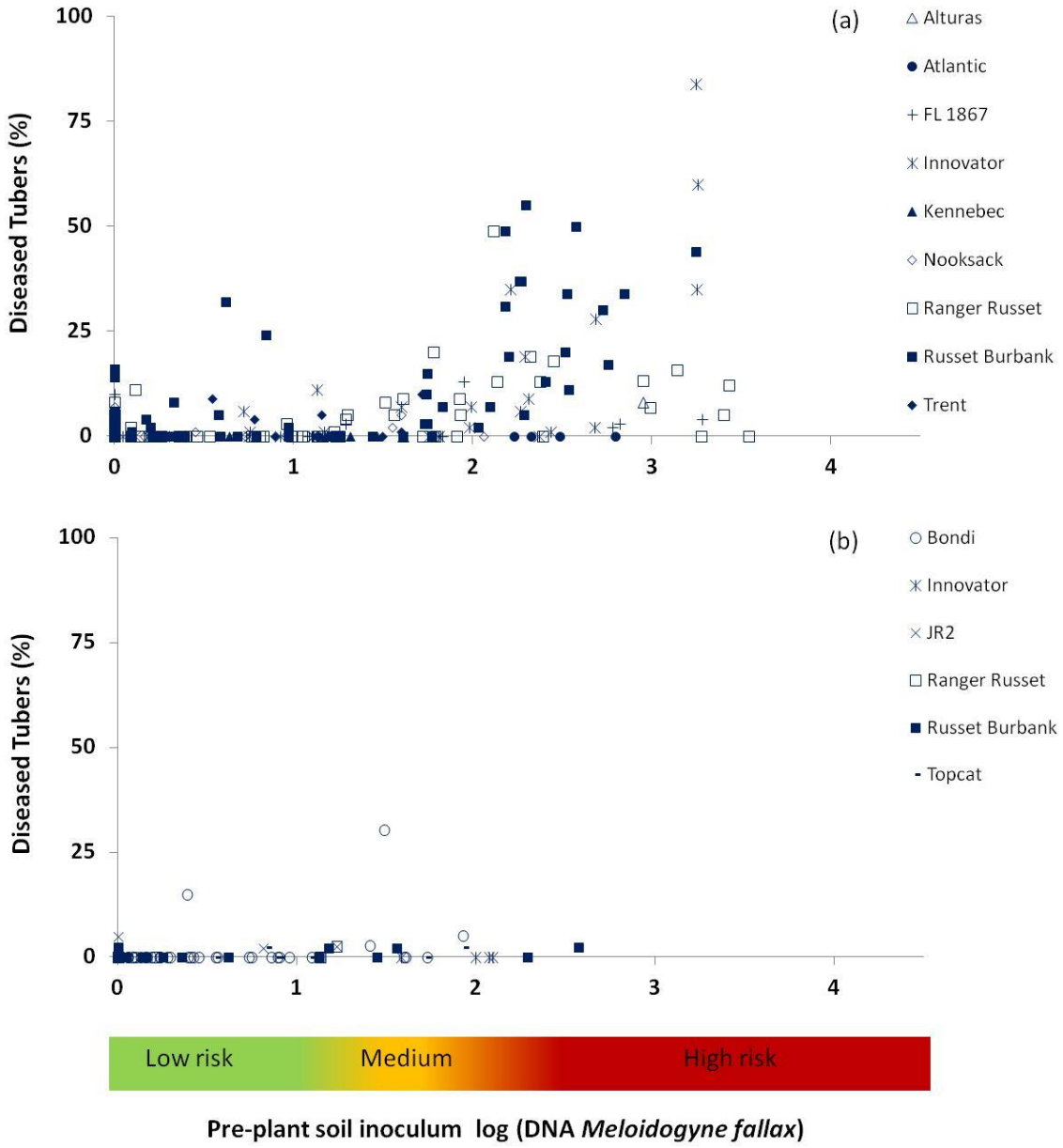

Figure 4-15 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and the incidence of root-knot nematode tuber symptoms depending on the variety grown in a) South Australia and b) Tasmania. (Data from 2011/12 and 2012/13 growing seasons combined).

MELOIDOGYNE HAPLA

OVERVIEW

Soil DNA testing to assess the risk of root-knot nematode caused by *Meloidogyne hapla* has been included on the current PreDicta Pt report as a "Test Under Development". Test results are a measure of the total level of DNA of the nematode *Meloidogyne hapla* measured in the soil prior to planting.

Field validation has concentrated on establishing a link between the level of DNA of *Meloidogyne hapla* measured in the soil prior to planting and the probability of root-knot nematode symptoms occurring on the harvested tubers. Increasing levels of *Meloidogyne hapla* DNA measured in the soil prior to planting are not associated with an increased probability of visual root-knot nematode damage on the tubers, Figure 4-16. High levels of *Meloidogyne hapla* DNA detected in the soil prior to planting do correspond with high levels of DNA in the peel of harvested tubers, Figure 4-17. However there is no evidence of a relationship between visual symptoms of root-knot nematode on the tubers and the level of *Meloidogyne hapla* DNA measured in the peel of tubers in our data, Figure 4-18. Potatoes were only assessed for the presence of bumps on the tuber surface.

Field validation in commercial crops has demonstrated that the visible symptoms of root knot nematode seen on the surface of tubers are associated with the presence of *Meloidogyne fallax* and not *Meloidogyne hapla*. There is a strong relationship between visual symptoms of root-knot nematode on the tubers and the level of *Meloidogyne fallax* DNA measured in the peel of tubers, refer back to Figure 4-11.

Results indicate that visual root knot-nematode symptoms were less likely when high levels of *Meloidogyne hapla* existed in the soil prior to planting. This may be partially explained by a possible interaction with the *Meloidogyne fallax* population in the soil. For example none of the pre-plant soil samples with a high level of *Meloidogyne hapla* DNA (>2.75) also fell in the high risk category for *Meloidogyne fallax*.

Previously indicated putative thresholds (APRP1 report) appear appropriate to describe the relationship between pre-plant soil levels of *Meloidogyne hapla* DNA and those found in the harvested tubers, but they do not apply as previously thought to the risk of root-knot nematode symptoms on tubers. Even though the critical thresholds do not apply to development of visible symptoms, the pathogen may still be having other impacts on the potato crop, such as yield reduction. Hence the relationship between pre-plant soil levels of *Meloidogyne hapla* DNA and those found in the harvested tubers may still be important to understand. Findings related to these aspects of disease development are covered elsewhere in this report.

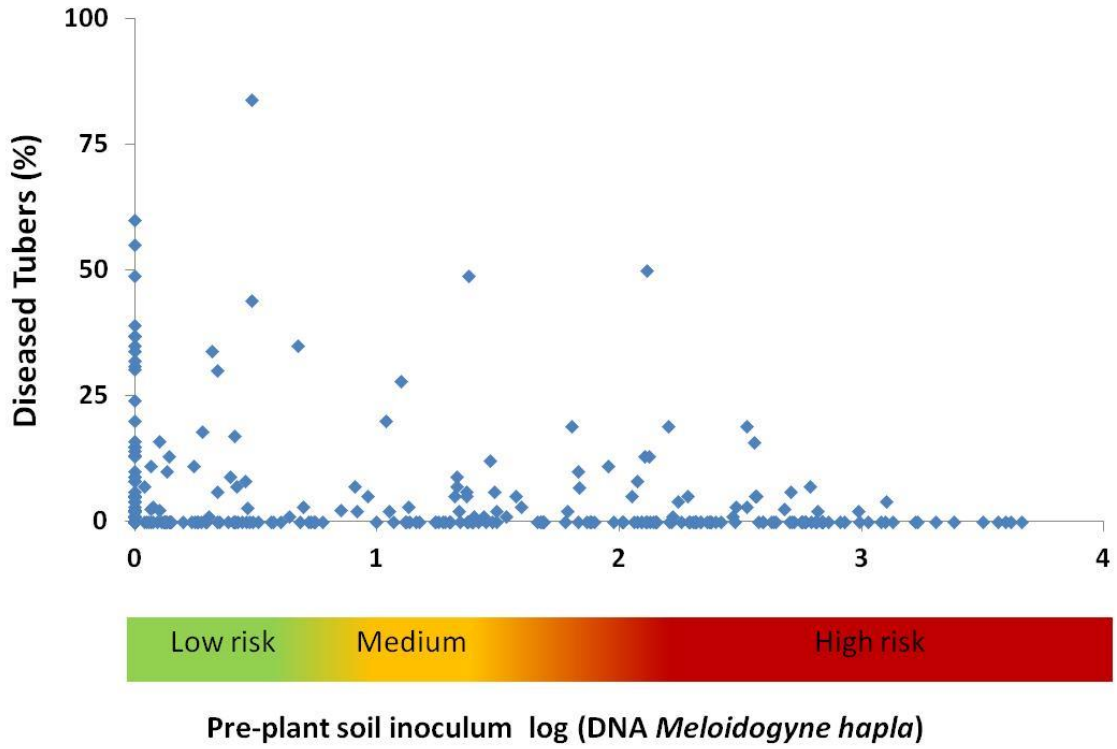

Figure 4-16 – Relationship between levels of *Meloidogyne hapla* DNA in the soil prior to planting and the incidence of root-knot nematode symptoms on the tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

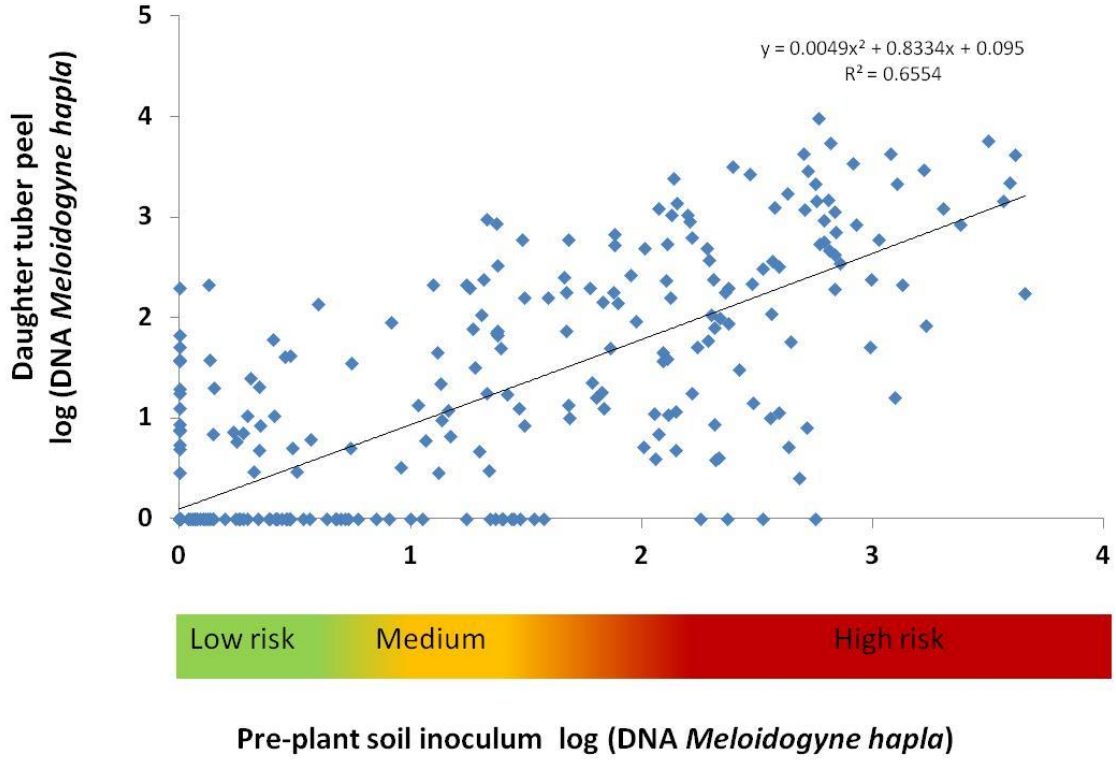

Figure 4-17 – Relationship between levels of *Meloidogyne hapla* DNA in the soil prior to planting and in the peel of harvested tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

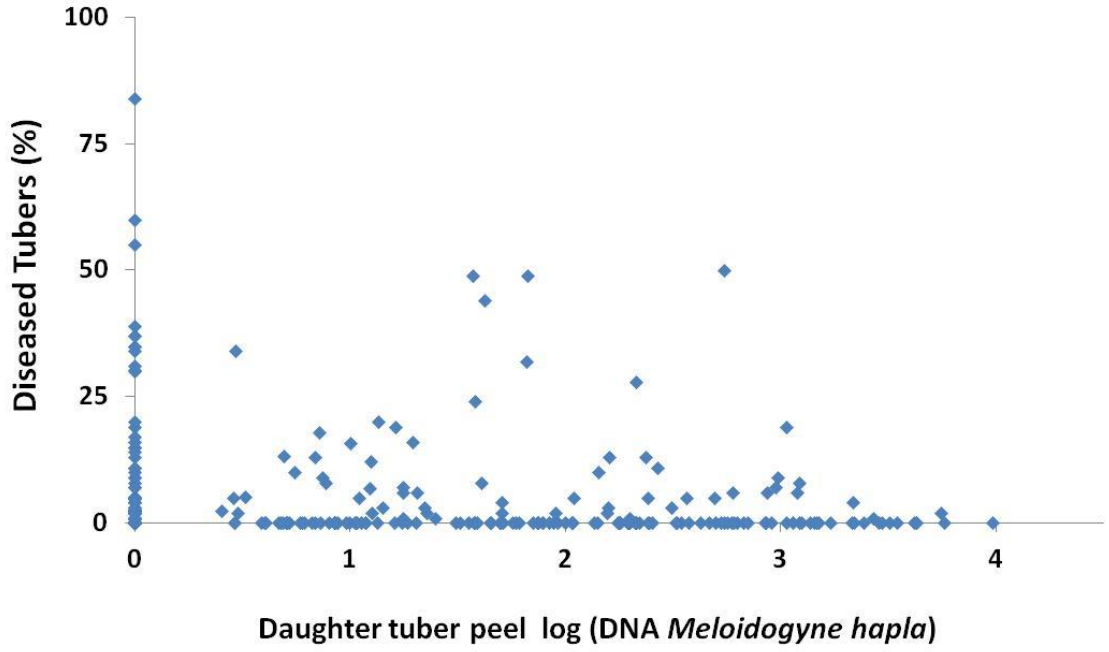

Figure 4-18 – Relationship between levels of *Meloidogyne hapla* DNA in the peel of harvested tubers and the incidence of root-knot nematode symptoms on the tubers in commercially grown potato crops (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

PATHOGENIC *STREPTOMYCES*

OVERVIEW

Soil DNA testing to assess the risk of common scab symptoms caused by pathogenic *Streptomyces* have been included on the current PreDicta Pt report as "Tests Under Development". Test results are a measure of the total level of DNA of *Streptomyces txt*A gene that is essential for disease development.

Field validation of the test for *Streptomyces txt*A gene DNA in the soil prior to planting showed the test did not provide a useful indication of the risk of common scab on tubers, Figure 4-19. When the test does detect *Streptomyces txt*A gene DNA in the soil prior to planting, then there is a high risk of the development of common scab. However, common scab occurred on harvested tubers at many sites where *Streptomyces txt*A gene DNA was not detected in the soil prior to planting. Though the risk is higher when *Streptomyces txt*A gene DNA is detected, the probability of common scab is still high when it is not detected. Based on the data presented in Figure 4-19, when *Streptomyces txt*A gene DNA was not detected the probability of significant common scab (>10% incidence on tubers) was 21%. This compares with a probability of 29% when *Streptomyces txt*A gene DNA was detected in the soil prior to planting.

A putative critical threshold of 2.5 log (*Streptomyces txt*A gene DNA + 1) for a medium level of disease risk had been previously indicated (APRP1). Detection of soil levels above this threshold was rare, occurring at only 19 of 638 sampling points. However when the log (*Streptomyces txt*A gene DNA + 1) value exceeded 2.5 in the soil prior to planting, the risk of significant common scab (>10% incidence on tubers) was much higher (47%) than when detected at lower levels (26%) or not detected (21%).

Risk thresholds have been established based on the probability of common scab symptoms occurring on the harvested tubers. However, high inoculum levels in the soil do not always result in tuber symptoms as environmental conditions and varietal susceptibility influence the incidence and severity of tuber symptoms.

Pathogen DNA testing of the peel of harvested tubers confirmed common scab on the tubers was accompanied by high levels of *Streptomyces txt*A gene DNA, Figure 4-20. However high levels of *Streptomyces txt*A gene DNA were often found in the peel of tubers where no incidence of common scab was recorded. This indicates a high level of pathogen activity on the peel of harvested tubers can occur in the absence of visible symptoms. Similar to actual incidence of common scab on the tubers, high levels of *Streptomyces txt*A gene DNA were frequently detected on tubers harvested from sampling points where *Streptomyces txt*A gene DNA had not been detected in the soil prior to planting, Figure 4-21.

There are a number of reasons why high levels of common scab incidence on tubers can occur at sampling points where the pre-planting soil test has not detected any *Streptomyces txt*A gene DNA. These include that the level of pathogenic *Streptomyces* inoculum in the soil required to cause common scab is below the level of detection of either the field soil sampling strategy or the laboratory assay. Another possible reason is that the seed is an important source of inoculum for common scab in the year of planting.

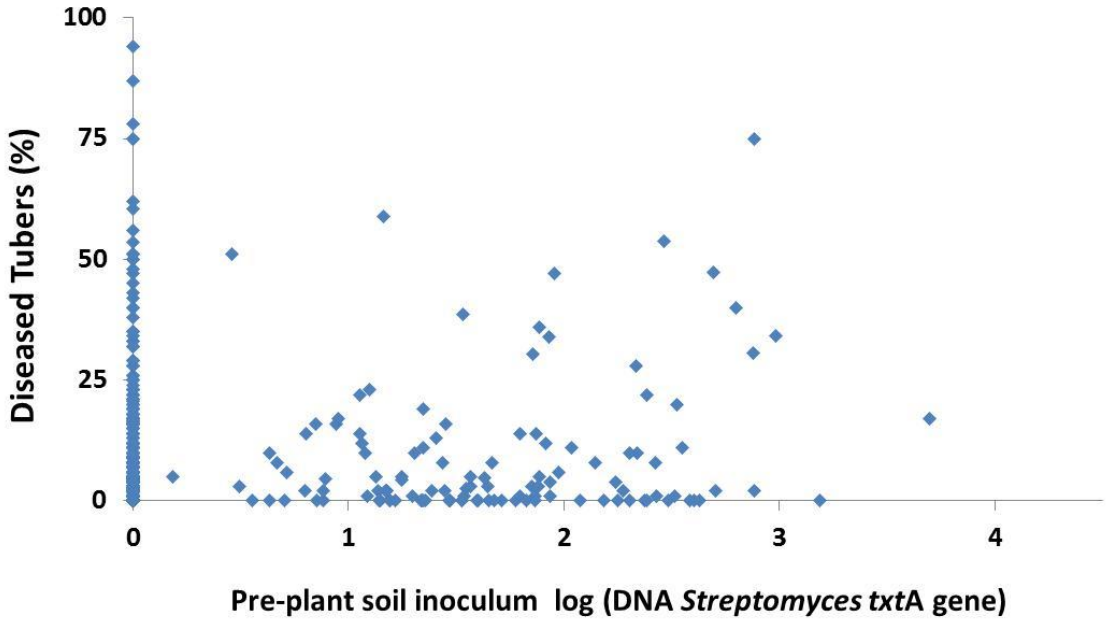

Figure 4-19 – Relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and the incidence of common scab on the tubers in commercially grown potato crops. (638 sampling points monitored across Tasmania, Victoria and South Australia in 2011/12 and 2012/13)

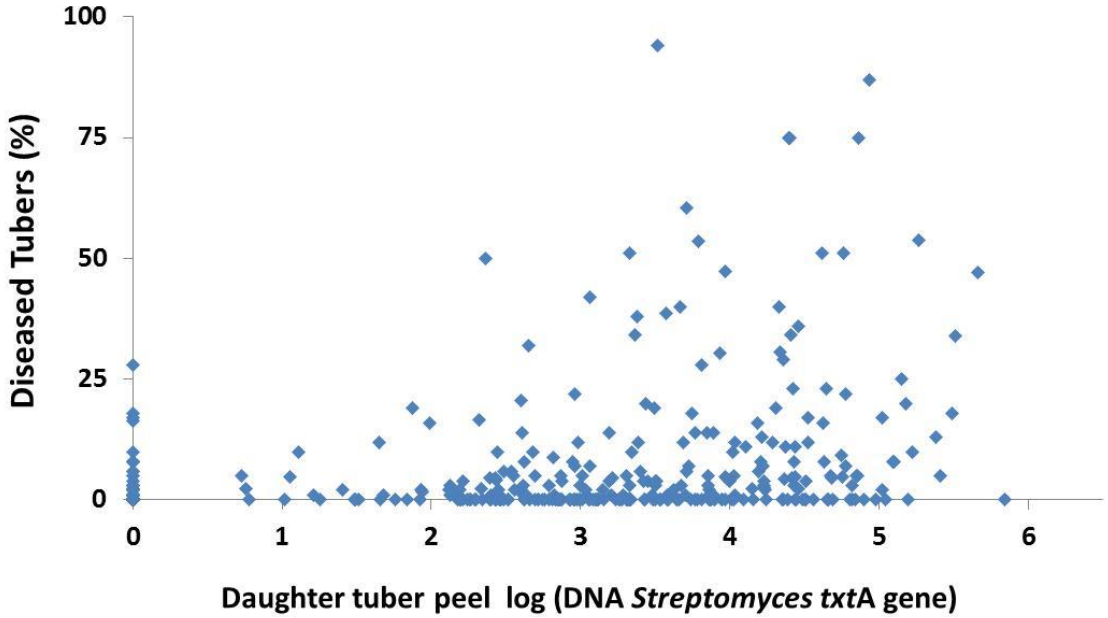

Figure 4-20 – Relationship between levels of *Streptomyces txt*A gene DNA in the peel of harvested tubers and the incidence of common scab on the tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

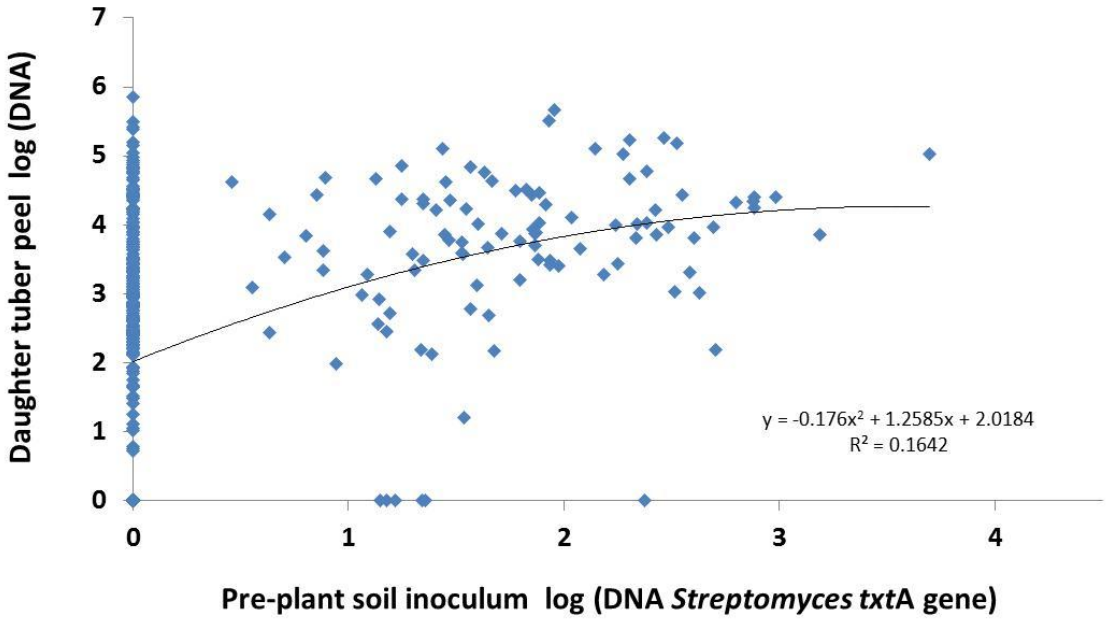

Figure 4-21 – Relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and in the peel of harvested tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

IMPACT OF GEOGRAPHIC REGION AND VARIETY

Environment and growing region are known to influence the incidence and severity of common scab (Haynes, et al., 2010). As a result, some growing areas may be more conducive to common scab problems. Field validation has been conducted at multiple locations in three states. Depending on conditions severe common scab symptoms can occur in any of the regions where assessments were made. Based on the data presented in Figure 4-22, the probability of significant common scab (>10% incidence on tubers) was highest in Victoria (36%) and similar in Tasmania (17%) and South Australia (15%).

It is likely that irrigation management and soil type in combination with temperature and rainfall at critical stages of crop development are more important than the state a paddock is located.

Control options for common scab are limited. Growing varieties that are less susceptible would be one of the most cost efficient and effective ways to reduce the risk of common scab on the tubers (Dees, 2012). Differences in the susceptibility of varieties are clearly evident in the field validation data, Figure 4-22.

Varieties among those assessed that appeared to be most susceptible to common scab on the tubers were Ranger Russet, Russet Burbank, Innovator, Bondi, Nooksack, Trent, Pike, Atlantic and Catani, Figure 4-22.

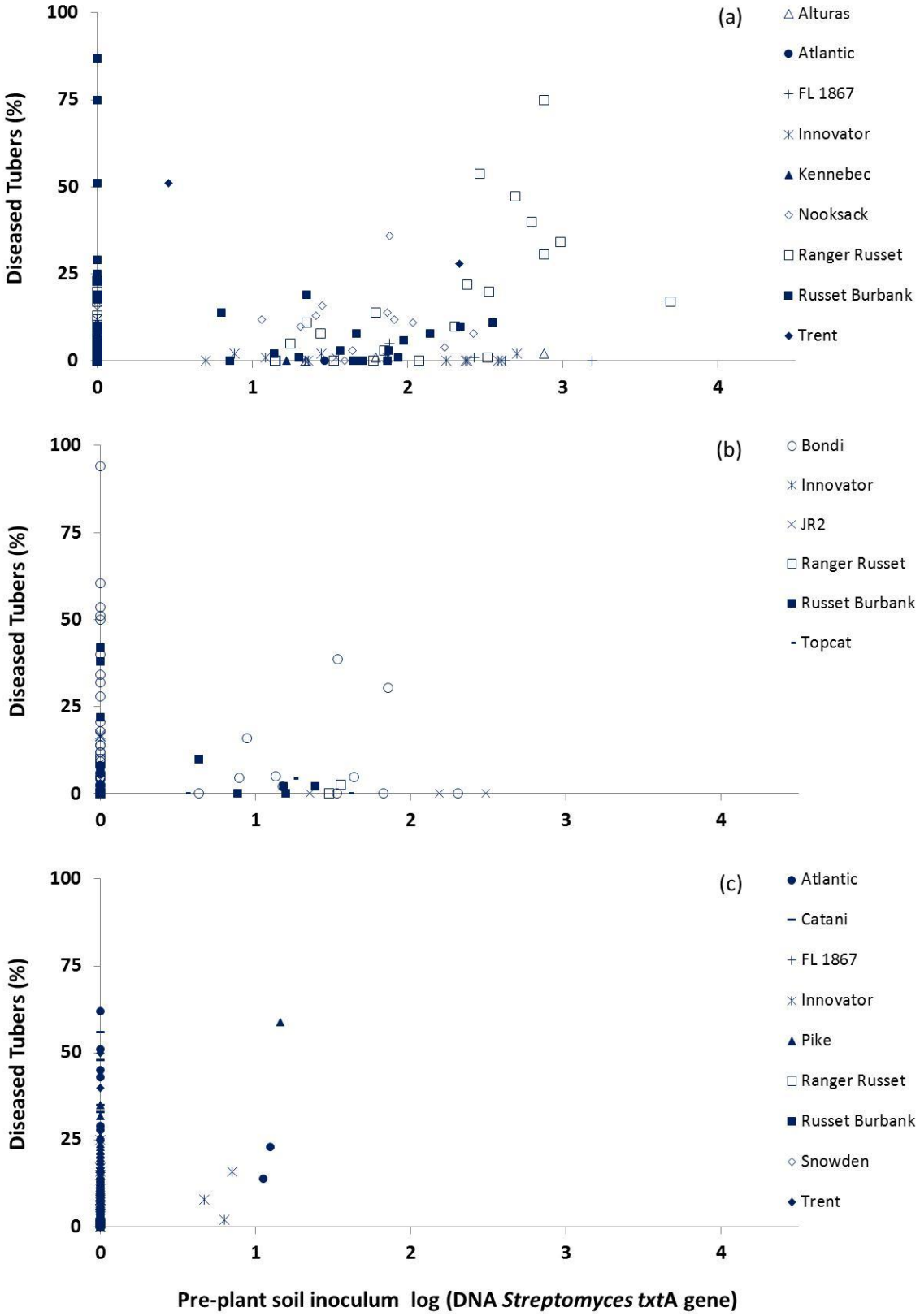

Figure 4-22 – Relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and the incidence of common scab depending on the variety grown in a) South Australia, b) Tasmania and c) Victoria. (Data from 2011/12 and 2012/13 growing seasons combined)

IMPACT OF SEASON

Climatic conditions are known to impact on the incidence and severity of common scab symptoms. It is commonly reported that increased common scab occurs during growing seasons with higher than normal temperatures. High soil moisture inhibits the activity of pathogenic *Streptomyces*, which has led to the use of irrigation for disease control in the United Kingdom (Lapwood, Wellings, & Rosser, 1970). This high level of soil moisture may also act by reducing the soil temperature.

In any of the three seasons there were examples of sampling points where a high incidence of common scab occurred. This can be seen in the data for Ranger Russet grown in South Australia, Figure 4-23. Irrigation management in individual paddocks is likely to have a greater impact than seasonal variation, though managing irrigation would be expected to be more critical when hot dry conditions coincide with the period of susceptibility to tuber infection.

When high levels of *Streptomyces txt*A gene DNA were detected in the soil prior to planting, similar levels of *Streptomyces txt*A gene DNA were detected in the peel of harvested tubers in each of the three seasons, Figure 4-24. However when *Streptomyces txt*A gene DNA was not detected in the soil prior to planting, the average levels of *Streptomyces txt*A gene DNA detected in the peel of harvested tubers was highest in the 2010/11 season and lowest in the 2011/12 season.

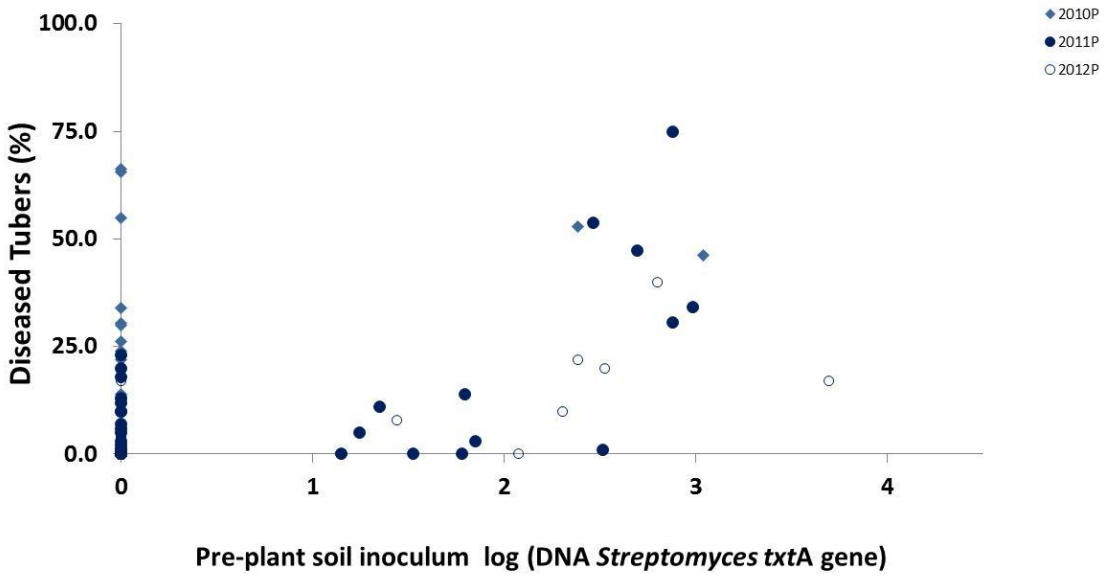

Figure 4-23 – Differences in the relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and the incidence of common scab on tubers for Ranger Russet potatoes grown in South Australia depending on the growing season a) 2010/11, b) 2011/12 and c) 2012/13.

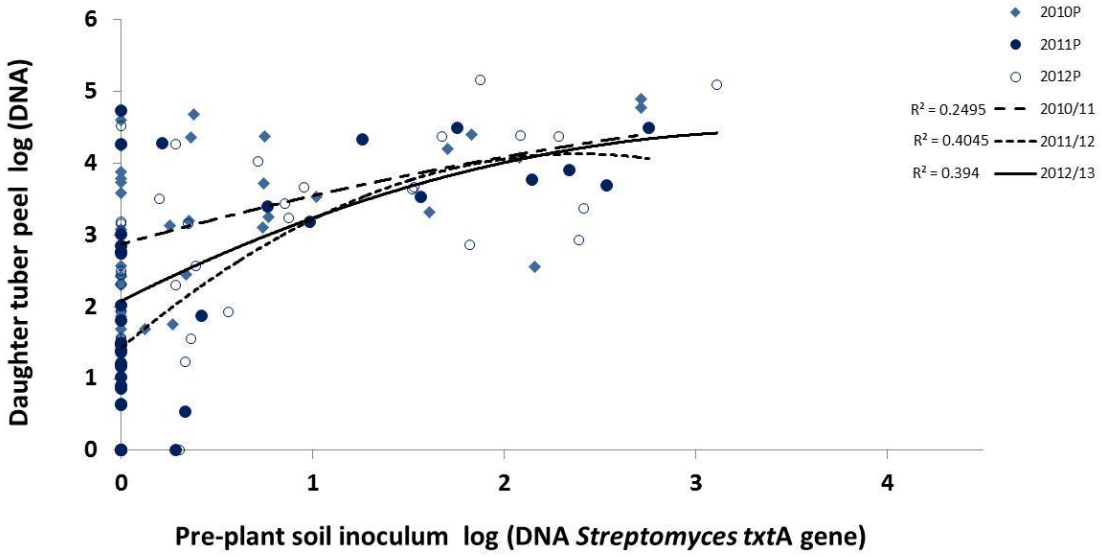

Figure 4-24 – Differences in the relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and in the peel of harvested tubers in South Australia depending on the growing season a) 2010/11, b) 2011/12 and c) 2012/13.

IMPACT OF SOIL TYPE

Soil characteristics can greatly affect the severity of common scab (Lacey & Wilson, 2001). Common scab is most severe in soils with pH 5.2 to 7.0. Across the limited range of soil pH values measured prior to planting in Victoria in the 2011/12 and 2012/13 seasons, common scab was observed to occur, Figure 4-25.

In some cases common scab control can be achieved by lowering the soil pH through the use of acidifying fertilizers or applications of sulphur, but generally pH values inhibitory to pathogenic *Streptomyces* are also unfavourable for potatoes, reducing yields. Lowering soil pH also enables more micronutrients such as zinc and manganese to become available.

It has been demonstrated that calcium fertilizers such as aglime and hotlime increase the incidence and severity of common scab, but whether this is a direct effect of pH on the pathogen or an indirect effect due to the influence of pH on other aspects of soil chemistry is unknown. High calcium levels in the absence of changes in pH may induce scab and the content of exchangeable calcium is a more reliable parameter then the soil pH (Davis, McDole, & Callihan, 1976) (Goto, 1985).

High soil moisture inhibits the activity of pathogenic *Streptomyces* (Lapwood, Wellings, & Rosser, 1970), therefore soils with a high water holding capacity should be less conducive to common scab symptom development.

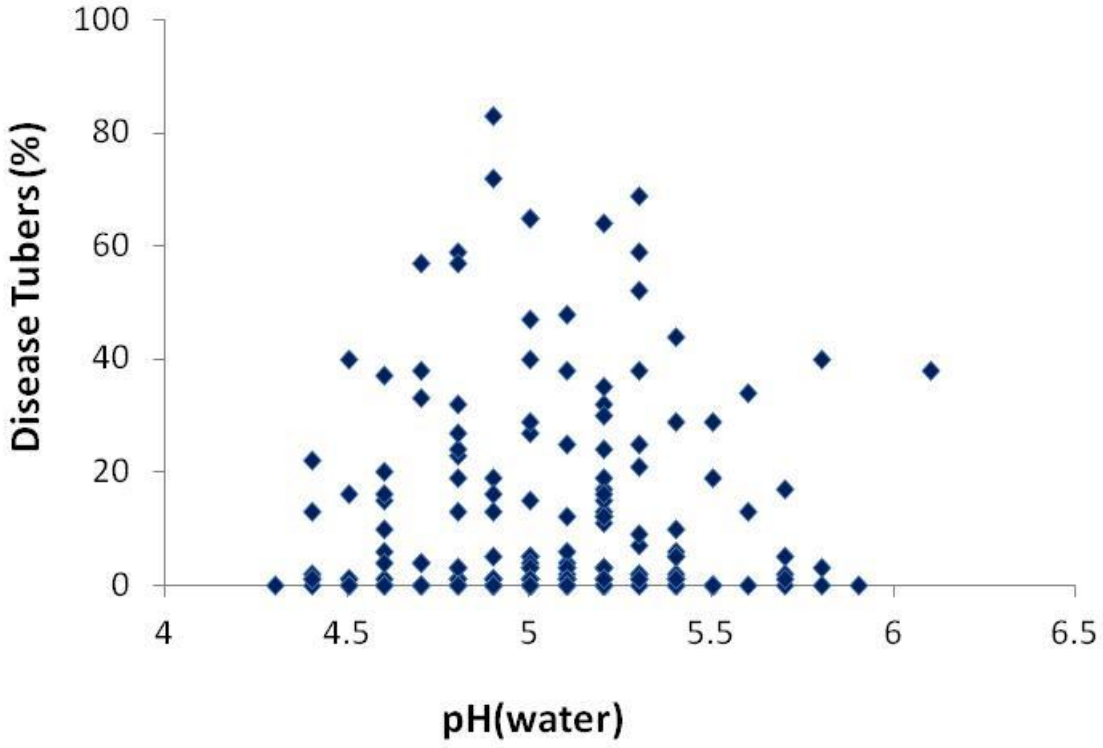

Figure 4-25 – Relationship between pH of the soil and the incidence of common scab on the tubers in commercially grown potato crops (198 sampling points monitored in Victoria during 2011/12 and 2012/13).

RHIZOCTONIA SOLANI AG3 AND AG2.1

OVERVIEW

Soil DNA testing to assess the risk of *Rhizoctonia* related symptoms caused by both *Rhizoctonia solani* AG3 and *Rhizoctonia solani* AG2.1 have been included on the current PreDicta Pt report as "Tests Under Development". Test results are a measure of the total level of DNA of *Rhizoctonia solani* AG3 or *Rhizoctonia solani* AG2.1 and are specific to each anastomosis group.

Initially field validation concentrated on establishing a link between the level of DNA of specific *Rhizoctonia solani* anastomosis groups measured in the soil prior to planting and the probability of black scurf symptoms occurring on the harvested tubers. *Rhizoctonia solani* AG3 is primarily responsible for the black scurf symptoms that are visible on the surface of tubers.

A high incidence of black scurf on the tubers is not of major concern to the processing sector. However *Rhizoctonia solani* can also cause delayed and non-emergence problems in potato plantings. In consultation with growers it was identified that a link between pre-plant test results and emergence and/or yield would be more beneficial. Additionally the incidence of deformed tubers was assessed. Results of field validation are presented separately for each of the disease symptoms evaluated.

BLACK SCURF

Field validation of the test for *Rhizoctonia solani* AG3 DNA in the soil prior to planting showed the test did not provide a useful indication of the risk of black scurf, Figure 4-26. When the test does detect *Rhizoctonia solani* AG3 in the soil prior to planting, then there is a high risk of the development of black scurf. However, black scurf occurred on harvested tubers at many sites where *Rhizoctonia solani* AG3 DNA was not detected in the soil prior to planting. Though the risk is higher when *Rhizoctonia solani* AG3 DNA is detected, the probability of black scurf is still high when it is not detected. Based on the data presented in Figure 4-26, when *Rhizoctonia solani* AG3 DNA was not detected the probability of significant black scurf (>10% incidence on tubers) was 30%. This compares with a probability of 54% when *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting.

Any level of detection of *Rhizoctonia solani* AG3 DNA in the soil prior to planting indicates a higher risk of black scurf than when it is not detected. A putative critical threshold of 0.8 log (*Rhizoctonia solani* AG3 DNA + 1) for disease risk had been previously indicated (APRP1). However, results of this field validation show that when *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting, the probability of significant black scurf (>10% incidence on tubers) was similar irrespective of whether the level was above (56%) or below (52%) this previously indicated threshold.

Testing of peel samples of the harvested tubers confirms a relationship between the level of *Rhizoctonia solani* AG3 DNA on the peel of tubers and the incidence of black scurf, Figure 4-27. A number of outliers exist, which are most likely a misdiagnosis of the visual symptoms. No relationship is evident between the level of *Rhizoctonia solani* AG2.1 DNA on the peel of tubers and the incidence of black scurf (data not presented).

There are a number of reasons why high levels of black scurf incidence on tubers can occur at sampling points where the pre-planting soil test has not detected any *Rhizoctonia solani* AG3 DNA. These include that the level of *Rhizoctonia solani* AG3 inoculum in the soil required to cause black scurf is below the level of detection of either the field soil sampling strategy or the laboratory assay. *Rhizoctonia solani* is a highly responsive pathogen to environmental conditions, which would allow small isolated points of inoculum in the soil prior to planting to cause more widespread disease at the completion of the potato growing season, when black scurf occurs on the tubers. Another possible reason is that the seed is an important source of inoculum for black scurf in the year of planting.

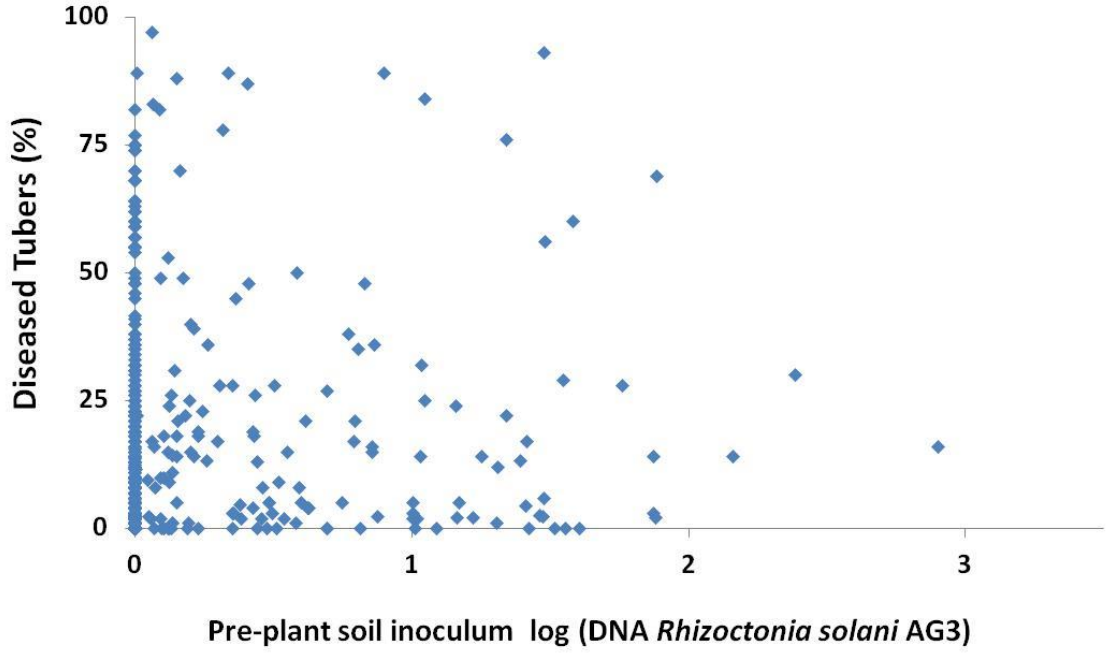

Figure 4-26 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and the incidence of black scurf on the tubers in commercially grown potato crops. (638 sampling points monitored across Tasmania, Victoria and South Australia in 2011/12 and 2012/13)

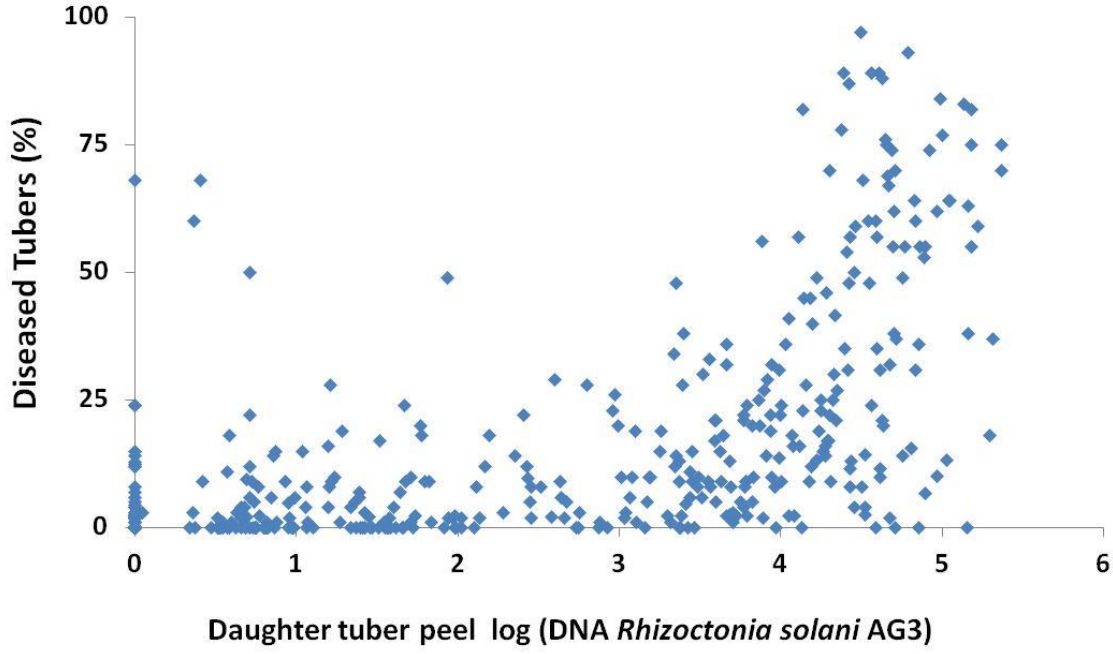

Figure 4-27 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the peel of harvested tubers and the incidence of black scurf on the tubers in commercially grown potato crops. (430 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

IMPACT OF GEOGRAPHIC REGION AND VARIETY

Field validation has been conducted at multiple locations in three states. Incidence of black scurf was higher in the SE of South Australia when compared with Victoria and Tasmania, Figure 4-28. These differences were also reflected in the level of *Rhizoctonia solani* AG3 DNA measured in the peel of harvested tubers from South Australia compared with Tasmania, Figure 4-29. Peel samples of daughter tubers were not tested from Victoria.

In South Australia, if *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting, black scurf was always present, along with a high level of *Rhizoctonia solani* AG3 DNA in the peel of tubers harvested from that sampling location. In contrast black scurf was not always found in Tasmania and Victoria when *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting and levels of *Rhizoctonia solani* AG3 DNA in the peel of harvested tubers were sometimes low or not detected in Tasmania. Differences in susceptibility to black scurf between varieties are not easily ascertained from this data, Figure 4-28 and Figure 4-29. Growing location and the control measures applied impact on the incidence of black scurf. Black scurf also increases when tubers are stored in the ground after haulm death or destruction (Tsror L. , 2010). This is probably the reason for the high incidence (up to 97%) seen on Russet Burbank at some locations, Russet Burbank generally being harvested well after haulms are dead. Varieties such as Kennebec that were assessed while haulms were still alive showed a low incidence (2.5%). Where data was collected from at least 5 sampling points, no variety was free of black scurf at all locations. For varieties that were assessed extensively (>50 sampling points) such as Atlantic, Bondi, Innovator, Ranger Russet, and Russet Burbank incidence within each variety varied from nil to greater than 70%. Results highlight the impact that location, seasonal conditions and management practices have on the incidence of black scurf.

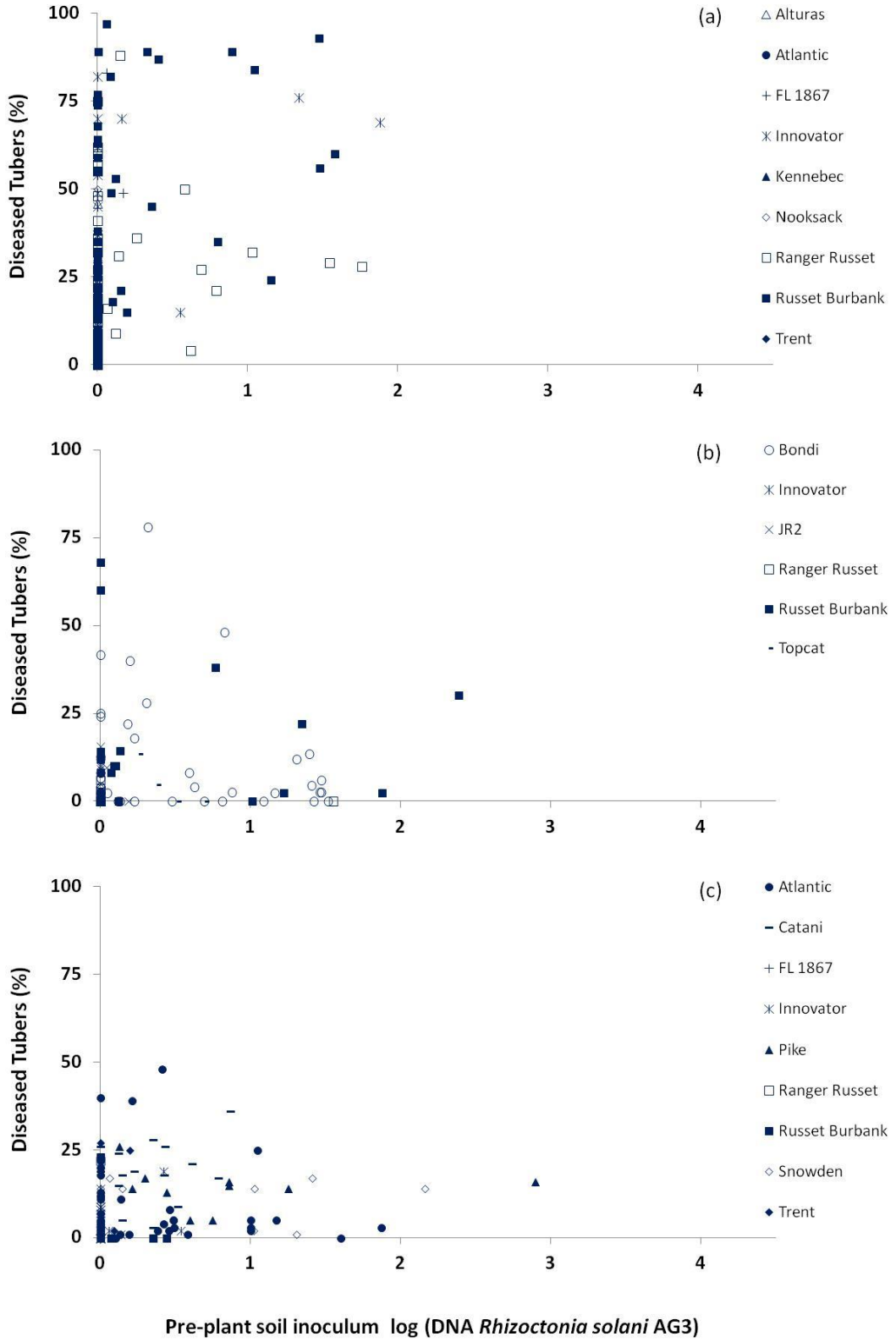

Figure 4-28 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and the incidence of black scurf tuber symptoms depending on the variety grown in a) South Australia, b) Tasmania and c) Victoria. (Data from 2011/12 and 2012/13 growing seasons combined).

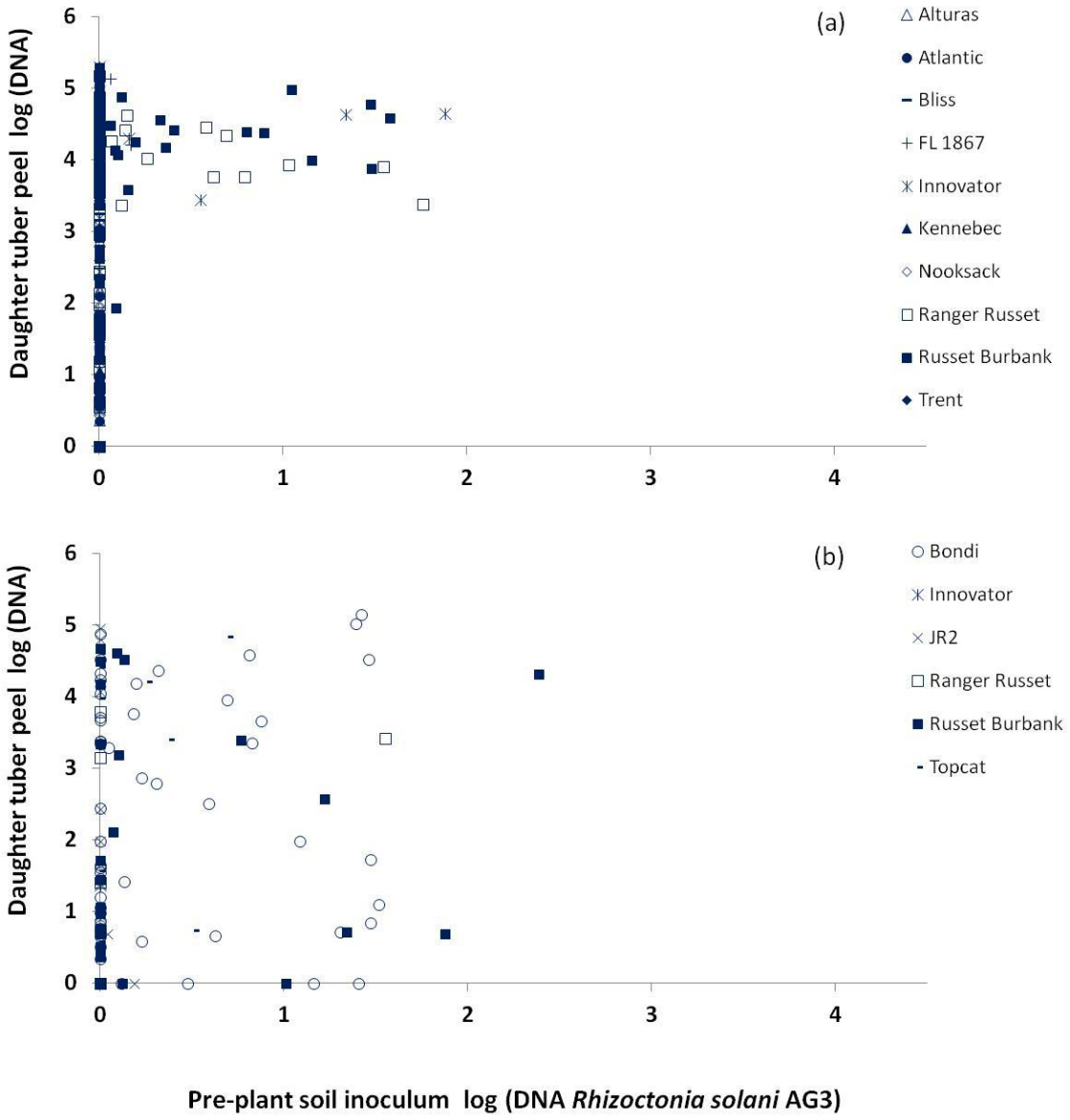

Figure 4-29 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and in the peel of harvested tubers depending on the variety grown in a) South Australia and b) Tasmania. (Data from 2011/12 and 2012/13 growing seasons combined).

IMPACT OF SEASON

Environmental conditions are known to have a substantial impact on the rate and severity of disease development caused by *Rhizoctonia solani*. This is reflected in the incidence of black scurf over the three years that disease incidence was assessed in Tasmania, Figure 4-30. Conditions appeared to be most favourable in the 2011/12 season and least favourable in drier 2012/13 season. A similar trend between seasons was seen in South Australia, though black scurf incidence was on average higher in South Australia than Tasmania in each year (data not presented).

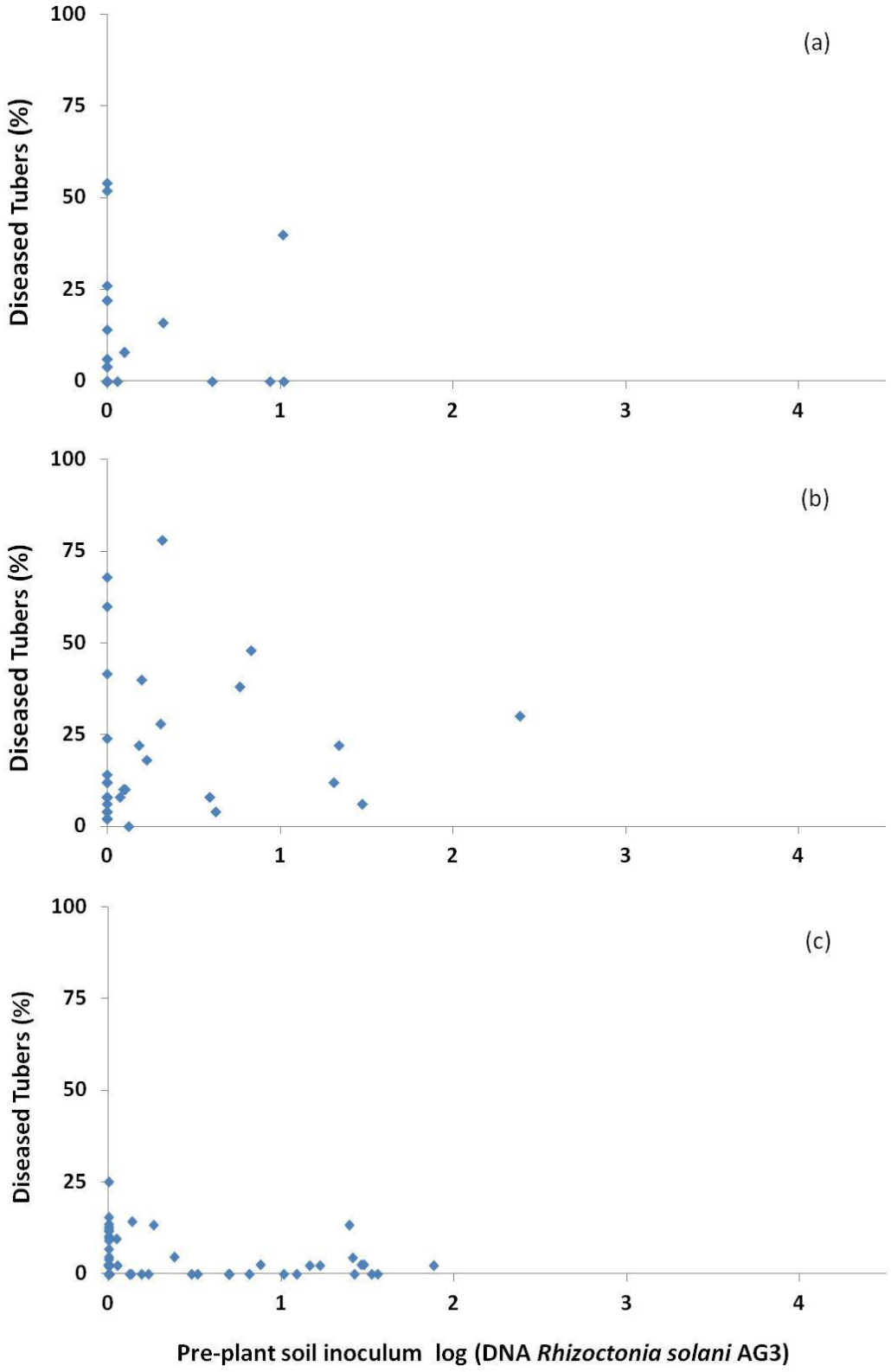

Figure 4-30 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and the incidence of black scurf tuber symptoms in Tasmania depending on the growing season a) 2010/11, b) 2011/12 and c) 2012/13.

DEFORMED TUBERS

In South Australia the percentage of deformed tubers was recorded at harvest in the 2012/13 season. The data suggests that high *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting may lead to an increase in the number of deformed tubers in the variety Russet Burbank, Figure 4-31. Deformed tubers are known to be a problem in this variety, with misshapen tubers resulting in increased wastage during processing.

Other varieties did not show a distinct response, however unlike Russet Burbank, none of the other varieties were grown at sites that recorded a high level of *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting.

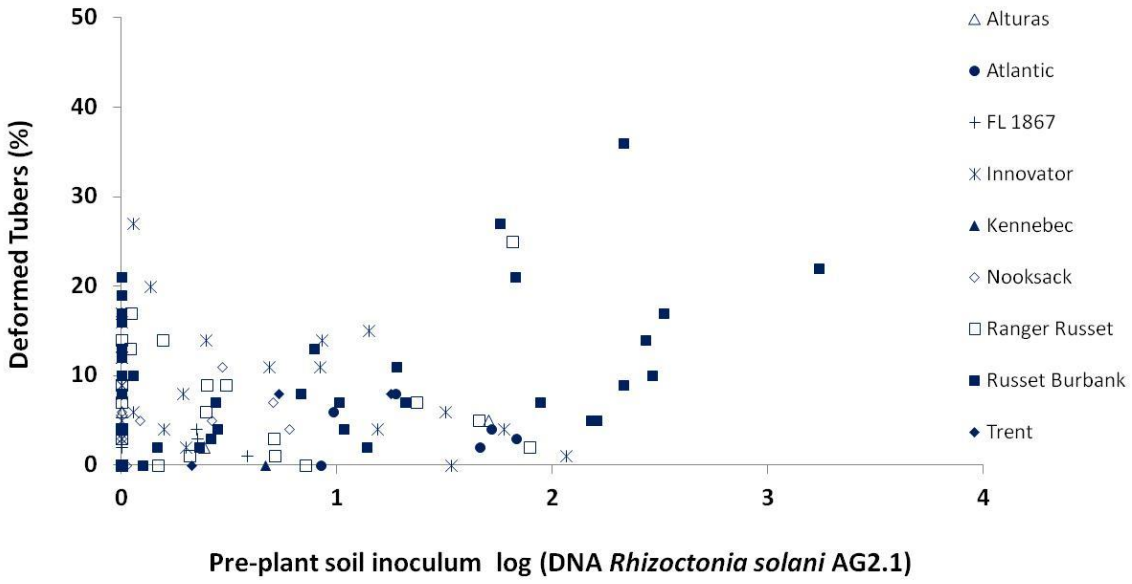

Figure 4-31 – Relationship between levels of *Rhizoctonia solani* AG2.1DNA in the soil prior to planting and the incidence of deformed tubers depending on variety grown in South Australia in the 2012/13 season

EMERGENCE

OVERVIEW

Results relating to the effects of *Rhizoctonia solani* on delayed emergence and the incidence of rhizoctonia stem canker differ between the three states.

Overall, *Rhizoctonia solani* AG3 was the main anastomosis group associated with rhizoctonia stem canker, though this was not confirmed in Tasmania, as pathogen DNA testing was not conducted on diseased samples. *Rhizoctonia solani* AG3 was isolated from over 97% of all rhizoctonia stem canker samples tested

from Victoria. Pathogen DNA testing of samples from South Australia also confirmed *Rhizoctonia solani* AG3 to be the anastomosis group most commonly associated with rhizoctonia stem canker. However, at 3 of 13 sites in South Australia *Rhizoctonia solani* AG2.1 was the main anastomosis group detected from rhizoctonia stem canker samples. *Rhizoctonia solani* AG2.1 was also detected at high levels along with *Rhizoctonia solani* AG3 at a further 3 sites in South Australia, suggesting both anastomosis groups are important in South Australia.

Lack of crop uniformity was evident at sites assessed in all three states; however factors other than rhizoctonia stem canker were most likely the main cause of the differences observed in plant height and non-emergence. Overall only a small proportion of the lack of crop uniformity was associated with rhizoctonia stem canker. Detailed investigations were not made of other causes of delayed and non-emerged plants; however potential reasons include size and vigour of seed pieces, seed piece decay and virus infection.

Pre-plant soil testing for the DNA of *Rhizoctonia solani* AG3 and AG2.1 did not provide a useful indication of the risk of rhizoctonia stem canker across a paddock. However, testing may provide a useful indication at sampling locations within a paddock, as demonstrated in South Australia. Though the pre-plant soil and seed tests together appeared to give a useful indication of risk at each site in this paddock, the results highlight the variability in risk that can exist over small distances within a paddock. Due to the patchy distribution of this pathogen in a paddock, an intensive sampling strategy across a paddock would be required to adequately assess the risk of rhizoctonia stem canker.

DNA of *Rhizoctonia solani* AG3 was detected at almost all sites in South Australia and Victoria either in the soil or more commonly in the peel of seed tubers planted, indicating that some inoculum is almost always present. This coupled with the apparent low level of inoculum required to cause disease suggest that most fields are at risk of rhizoctonia stem canker.

In most cases it is likely that environmental conditions and control measures adopted are having a greater impact on the incidence of rhizoctonia stem canker than the inoculum level in the soil or seed tubers.

The detected incidence of stem canker was low in Tasmania when compared with South Australia and Victoria. In Tasmania DNA of both *Rhizoctonia solani* AG3 and AG2.1 was detected in the soil prior to planting at levels comparable to either South Australia or Victoria. These results indicate that even though inoculum was present in many paddocks, environmental conditions and or management practices utilised on crops grown in Tasmania were not conducive to or prevented the development of rhizoctonia stem canker in most instances. Lower levels of inoculum on seed tubers may also have been a factor. *Rhizoctonia solani* AG3 was detected less frequently and at lower levels in the peel of seed tubers planted at the sites assessed in Tasmania.

Results of testing in each of the states are presented separately below.

SOUTH AUSTRALIA

Rhizoctonia solani can result in the burn off of emerging sprouts, resulting in delayed or non-emergence of plants. In all but one of the 19 sites assessed over the two seasons less than one third of the delayed or non-emerged plants appeared to result from rhizoctonia stem canker, Table 4-5. However at some sites, rhizoctonia stem canker occurred at levels that would be expected to impact on crop productivity. For example, at sites 7 and 8 rhizoctonia stem canker was responsible for 4% and 2% respectively of the crop not having emerged at the time of assessment. Rhizoctonia stem cankers were associated with 8%, 4%, 4% and 3% of the crop having been delayed at the time of assessment at sites 10, 18, 20 and 11 respectively.

Lesions tended to be detected as frequently on plants of normal height as on those that were delayed, so that the top growth of many plants appeared unaffected by the presence of rhizoctonia stem canker. The impact on final crop performance of rhizoctonia stem canker symptoms on plants that were delayed or normal height was not quantified.

Many reasons for the lack of uniformity in plant growth evident at this early stage of crop development remain unaccounted for, Table 4-5. Individual plants that were substantially delayed in their growth at this stage are likely to underperform at harvest; however this may be compensated for by neighbouring plants.

Detailed investigations were not made of other causes of delayed and non-emerged plants; however they frequently exhibited weak thin sprouts. Potential reasons, either seen or suspected, were small seed pieces, seed piece decay (particularly in the 2013/14 season), low vigour seed pieces and virus infection. Limited virus testing on plant samples that were exhibiting weak and abnormal growth in the 2013/14 season confirmed infections of Potato virus Y and Potato virus S. Conditions during the 2013/14 planting season were difficult, the southeast district of South Australia experiencing several large rainfall events during the planting period. These conditions were conducive to seed piece breakdown and high levels of black leg symptoms caused by *Erwinia* spp. were seen at several sites. It is likely that the percentage of seed piece breakdown detected underestimates the actual percentage at a number of sites, with only seed pieces that could still be found by digging up at the time of assessment being counted. This was particularly the case at sites where the number of missing plants was higher than expected from genuine missing seed drops during planting.

In the 2012/13 growing season log (pg DNA / g soil + 1) concentrations in the soil prior to planting at the sites assessed for emergence ranged from 0 to 1.9 for *Rhizoctonia solani* AG3 and 0 to 2.5 for *Rhizoctonia solani* AG2.1, while concentrations in the peel of seed tubers ranged from 0 to 4.6 for both *Rhizoctonia solani* AG3 and AG2.1, Table 4-6. In the 2013/14 growing season log (pg DNA / g soil + 1) concentrations in the soil prior to planting at the sites assessed for emergence ranged from 0 to 1.4 for *Rhizoctonia solani* AG3 and 0 to 1.9 for *Rhizoctonia solani* AG2.1, while concentrations in the peel of seed tubers ranged from 0 to 3.9 for *Rhizoctonia solani* AG3 and 0 to 2.9 for *Rhizoctonia solani* AG2.1, Table 4-6.

At the two sites (7 & 8) where burning off by rhizoctonia stem canker had the greatest impact on non-emergence, *Rhizoctonia solani* AG3 appeared to be the primary cause, Table 4-6. At both of these sites the concentration of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and in the peel of seed tubers was high.

At the two sites (2 & 6) in the 2012/13 season with the highest incidence of rhizoctonia stem canker, approximately 30% of plants having lesions, *Rhizoctonia solani* AG2.1 appeared to be the primary cause, Table 4-6. At both of these sites the concentration of *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting was high, and the variety grown was Russet Burbank in a sandy soil. At site 2 *Rhizoctonia solani* AG2.1 was not detected in the peel of seed tubers, but was present at high levels in the peel of seed tubers planted at site 6.

Stem samples with rhizoctonia stem canker collected in the 2012/13 season were also tested for the presence of *Rhizoctonia solani* anastomosis groups AG2.2, AG4 and AG8 (data not presented). Of the 43 samples tested with rhizoctonia stem canker symptoms, a low level (0.7 log(DNA)) of *Rhizoctonia solani* AG2.2 was detected in one sample, and low to moderate levels (0.7 – 1.9) of *Rhizoctonia solani* AG8 in 3 samples. *Rhizoctonia solani* AG4 was not detected in any of the 43 samples.

At most sites where rhizoctonia stem canker occurred in the 2013/14 season, *Rhizoctonia solani* AG3 appeared to be the primary cause, Table 4-6. At all sites where rhizoctonia stem canker was detected,

Rhizoctionia solani AG3 DNA was either detected in the soil prior to planting or at a high level in the peel of seed tubers. Of 42 stem samples with rhizoctonia stem canker symptoms that were DNA tested, 37 had higher concentrations of *Rhizoctonia solani* AG3 than AG2.1. *Rhizoctonia solani* AG2.1 was not detected in 33 of the samples. However at sites 10, 15 and 18 *Rhizoctonia solani* AG2.1 concentrations in 5 of the 21 stem samples tested were substantially higher than the concentration of *Rhizoctonia solani* AG3 DNA. On two of these samples *Rhizoctonia solani* AG3 was not detected, suggesting both anastomosis groups may have been important in causing disease at these sites.

At the only site (Site 5) assessed in the 2012/13 season where neither *Rhizoctonia solani* AG2.1 nor AG3 were detected in the soil prior to planting, rhizoctonia stem canker was detected on 7 % of plants, though all individual stem canker symptoms examined were minor lesions. This was a higher incidence than occurred at a number of sites where high levels of *Rhizoctonia solani* AG2.1 or AG3 were detected in the soil prior to planting. Peel of seed tubers planted at site 5 had a high concentration of *Rhizoctonia solani* AG3 DNA and mid-range level of *Rhizoctonia solani* AG2.1, which may have been the source of inoculum for the minor symptoms observed.

In the 2013/14 season 4 sites were assessed where both *Rhizoctonia solani* AG2.1 and AG3 were not detected in the soil prior to planting. Rhizoctonia stem canker was not detected at 3 of these sites. The concentration of *Rhizoctonia solani* AG3 DNA in the peel of seed tubers planted at these sites was also low. At the other site rhizoctonia stem canker was detected on 11 % of plants. Peel of seed tubers planted at this site had a high concentration of *Rhizoctonia solani* AG3 DNA.

Sites 10, 11 and 12 assessed in the 2013/14 season were located in the same paddock, approximately 100m apart from each other. DNA of both *Rhizoctonia solani* AG3 and AG2.1 was detected at sites 10 and 11, but not at site 12. Rhizoctonia stem canker occurred at the two sites where DNA of *Rhizoctonia solani* was detected, but not at the third where it was not detected. DNA of *Rhizoctonia solani* in the peel of seed tubers planted at site 12 was also lower than planted at sites 10 and 11. Though the pre-plant test soil and seed tests appeared to give a useful indication of risk at each site in this paddock, the results highlight the variability in risk that can exist within a paddock. Due to the patchy distribution of this pathogen in a paddock, an intensive sampling strategy across a paddock would be required to adequately assess the risk of rhizoctonia stem canker.

A site that was not initially assessed (site 9), but that had frequent non-emerged patches present throughout the crop was investigated in the 2012/13 season. DNA testing of tissue samples from this site indicated that *Rhizoctonia solani* AG2.1 appeared to be the primary cause. Testing had not found high concentrations of *Rhizoctonia solani* DNA in the soil prior to planting or in the peel of seed tubers. However two soil samples collected mid growing season from the affected areas had *Rhizoctonia solani* AG2.1 levels of 2.3 and 3.1 log (pg DNA / g soil + 1). Further investigation found that slow emergence of the Nooksac potatoes, a variety where seed dormancy can pose problems, favoured the disease development from a low inoculum level. This result highlights the importance of taking into account environmental and crop management factors that are important drivers of disease expression of *Rhizoctonia* when assessing the risk posed by a specific inoculum level.

Table 4-5 – Crop height and the incidence of rhizoctonia stem canker assessed 2-5 weeks after emergence in relation to concentration of *Rhizoctonia solani* DNA in the soil prior to planting and in the peel of seed tubers planted in South Australia, along with the incidence of seed piece breakdown on seed tubers that were found at the time of assessment.

Season and site number	DNA level *Rhizoctonia solani* log (DNA)				Stage of growth (% plants)				Rhizoctonia stem canker incidence (%)			Detected seed piece breakdown incidence (%)
	Pre-plant soil		Seed tuber peel									
	AG2.1	AG3	AG2.1	AG3	Normal height	Delayed	Non-emerged	Missing	Normal height	Delayed	Non-emerged	
2012/13 season												
1	0.7	0.8	0.0	2.1	92	2	1	5	0	11	29	0
2	2.4	0.0	0.0	4.6	91	5	0	4	29	33	-	1
3	1.3	0.8	0.0	4.6	93	4	0	3	0	5	0	1
4	0.2	1.2	0.0	4.6	82	15	0	3	0	16	0	3
5	0.0	0.0	2.2	4.6	74	5	3	18	7	11	12	0
6	2.5	0.0	4.6	0.0	86	9	1	4	30	22	25	0
7	1.1	1.9	2.8	3.6	73	0	18	8	6	0	22	0
8	0.9	1.3	2.8	3.6	85	0	7	9	5	0	29	0
2013/14 season												
10	0.2	0.9	1.7	3.1	70	18	5	8	25	46	0	5
11	0.7	1.4	1.4	3.6	74	17	3	6	17	19	0	2
12	0.0	0.0	2.3	1.0	67	19	6	8	0	0	0	8
13	0.0	0.2	0.0	0.9	74	18	1	7	0	0	0	9
14	0.7	0.0	0.0	3.6	43	19	0	38	4	12	0	0

Season and site number	DNA level *Rhizoctonia solani* log (DNA)				Stage of growth (% plants)				Rhizoctonia stem canker incidence (%)			Detected seed piece breakdown incidence (%)
	Pre-plant soil		Seed tuber peel									
	AG2.1	AG3	AG2.1	AG3	Normal height	Delayed	Non-emerged	Missing	Normal height	Delayed	Non-emerged	
15	1.7	0.0	2.9	3.7	74	14	4	8	9	3	17	1
16	0.0	0.0	0.0	0.0	94	2	1	3	0	0	0	1
17	1.9	0.0	0.0	0.0	20	72	3	5	0	0	0	1
18	1.9	0.0	2.7	3.9	23	19	1	57	17	19	0	7
19	0.0	0.0	0.0	2.4	62	18	5	14	0	0	0	32
20	0.0	0.0	1.3	3.2	65	12	2	21	9	28	0	27

Table 4-6 – Detection and concentration of *Rhizoctonia solani* AG3 and AG2.1 DNA in rhizoctonia stem canker lesions from individual plants in relation to the overall incidence of rhizoctonia stem canker and to the concentration of *Rhizoctoni solani* DNA in the soil prior to planting and in the peel of the seed tubers planted in South Australia.

Season and site number	DNA level *Rhizoctonia solani* log (DNA)				Site mean incidence rhizoctonia stem canker (%)	Number individual stem samples tested	DNA testing of rhizoctonia stem canker tissue samples				
	Pre-plant soil		Seed tuber peel				Number samples where DNA of *Rhizoctonia solani* detected			Range in concentration log (DNA *Rhizoctonia solani*)	
	AG2.1	AG3	AG2.1	AG3			AG2.1	AG3	AG2.1 & AG3	AG2.1	AG3
2012/13 season											
1	0.7	0.8	0.0	2.1	1	0					
2	2.4	0.0	0.0	4.6	29	8	8	5	5	2.3 - 3.9	0.5 - 1.7
3	1.3	0.8	0.0	4.6	0	0					
4	0.2	1.2	0.0	4.6	2	3	0	3	0	-	3.7 - 4.3
5	0.0	0.0	2.2	4.6	7	3	3	2	2	1.2 - 2.0	1.1 - 2.7
6	2.5	0.0	4.6	0.0	29	9	9	6	6	2.0 - 5.5	0.7 - 2.4
7	1.1	1.9	2.8	3.6	9	3	1	3	1	1.5	4.7 - 5.4
8	0.9	1.3	2.8	3.6	6	6	3	6	3	1.0 - 2.2	4.5 - 5.6
9						11	11	6	6	3.9 - 5.9	0.8 - 2.1
2013/14 season											
10	0.2	0.9	1.7	3.1	28	13	2	13	2	1.9 - 4.6	1.4 - 5.1
11	0.7	1.4	1.4	3.6	17	10	1	10	1	0.9	2.0 - 5.9
12	0.0	0.0	2.3	1.0	0	0					
13	0.0	0.2	0.0	0.9	0	0					

Season and site number	DNA level *Rhizoctonia solani* log (DNA)				Site mean incidence rhizoctonia stem canker (%)	Number individual stem samples tested	DNA testing of rhizoctonia stem canker tissue samples				
	Pre-plant soil		Seed tuber peel				Number samples where DNA of *Rhizoctonia solani* detected			Range in concentration log (DNA *Rhizoctonia solani*)	
	AG2.1	AG3	AG2.1	AG3			AG2.1	AG3	AG2.1 & AG3	AG2.1	AG3
14	0.7	0.0	0.0	3.6	6	2	0	2	0	-	5.0 - 5.7
15	1.7	0.0	2.9	3.7	8	5	4	4	3	1.0 - 4.3	1.1 - 6.0
16	0.0	0.0	0.0	0.0	0	0					
17	1.9	0.0	0.0	0.0	0	0					
18	1.9	0.0	2.7	3.9	17	4	2	3	1	2.7 - 4.0	1.1 - 5.3
19	0.0	0.0	0.0	2.4	0	0					
20	0.0	0.0	1.3	3.2	11	8	0	8	0	-	1.2 - 5.0

TASMANIA

In the 2012/13 season *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting at approximately half the 23 paddocks assessed and *Rhizoctonia solani* AG2.1 was detected at almost all paddocks, Table 4-7. When assessed from 9-12 weeks after planting rhizoctonia stem canker was detected in 4 of these 23 paddocks. Where it occurred, the incidence of rhizoctonia stem canker ranged from 25 to 50 % of the 20 plants assessed, with symptoms primarily minor lesions.

These results indicate that even though inoculum was present in many paddocks, environmental conditions and or management practices utilised on crops grown in Tasmania in the 2012/13 season were not conducive to or prevented the development of rhizoctonia stem canker in most instances.

The occurrence of rhizoctonia stem canker appeared to be more related to the paddock than to the levels of either *Rhizoctonia solani* AG3 or AG2.1 in the soil prior to planting or in the peel of the seed tubers planted. In each of the 4 paddocks where rhizoctonia stem canker was detected in the 2012/13 season, it was found in at least 3 of the 4 sampling points. In 3 of these paddocks rhizoctonia stem canker was detected on both varieties that were planted in the paddock. Only one variety was planted at the other paddock.

Pre-plant soil testing for the DNA of *Rhizoctonia solani* AG3 and AG2.1 did not provide a useful indication of the risk of rhizoctonia stem canker risk in Tasmania in the 2012/13 season. Rhizoctonia stem canker occurred in paddocks 11 and 21. In both of these paddocks DNA of *Rhizoctonia solani* AG3 was not detected in the soil prior to planting and DNA of Rhizoctonia AG2.1 was low. Similarly, levels in the peel of seed tubers were either low or not detected. No testing of rhizoctonia stem canker samples was conducted to confirm which *Rhizoctonia solani* anastomosis group was the cause of the symptoms in these paddocks.

In the 2013/14 season *Rhizoctonia solani* AG3 was detected in 8% of the 98 pre-plant soil DNA tests conducted in Tasmania, with the highest level being 0.7 log (DNA). In contrast *Rhizoctonia solani* AG2.1 was detected in 57% of soil samples, with levels up to 4.0 log (DNA). In the absence of detections of high levels of *Rhizoctonia solani* AG3 in the soil prior to planting, sites for assessment were chosen on the basis of *Rhizoctonia solani* AG2.1 levels. When assessed at approximately 3 to 5 weeks after emergence rhizoctonia stem canker was not detected at any of the 8 sites assessed, Table 4-8.

At selected sites in each of the 2012/13 and 2013/14 growing seasons assessments were made of crop height and rhizoctonia stem canker incidence, Table 4-8. Differences in crop uniformity between sites were evident, however as rhizoctonia stem canker was only detected at 1 of the 13 sites assessed; factors other than rhizoctonia stem canker are likely to be the cause of these differences in plant height and non-emergence. Detailed investigations were not made of other causes of delayed and non-emerged plants; however potential reasons include size and vigour of seed pieces, seed piece decay and virus infection.

Table 4-7 – The incidence of rhizoctonia stem canker assessed 9-12 weeks after emergence in relation to concentration of *Rhizoctonia solani* DNA in the soil prior to planting and in the peel of seed tubers planted in Tasmania in the 2012/13 growing season.

Paddock	Varieties	DNA level *Rhizoctonia solani* log (DNA)				Rhizoctonia stem canker symptoms	
		Pre-plant soil		Seed tuber peel		Number sites detected (4 sites per paddock assessed)	Number plants detected (5 plants per site = Total 20 plants assessed)
		AG2.1	AG3	AG2.1	AG3		
1	Bondi / Russet Burbank	2.2 - 2.6	0.5 - 1.2	1.7 - 4.1	0 - 3.4	4	7
2	Bondi / Russet Burbank	1.8 - 2.7	0 - 0.1	0.5 - 2.3	0 - 1.9	3	7
3	Topcat	0.3 - 2.2	0 - 0.7	1.7 - 3.9	1.3 - 2.8	0	0
4	Bondi	0.7 - 1.6	0 - 1.4	1.1 - 3.5	0 - 0.3	0	0
5	Bondi	0.8 - 2.4	0 - 1.4	1.2 - 2.9	0.1 - 4.1		
6	Bondi	0 - 1.9	0 - 0	0 - 0	0 - 0		
7	Bondi	0 - 1.1	0 - 0.9	0 - 3.2	0 - 3.0	0	0
8	Bondi	1.1 - 1.7	1.4 - 1.5	1.3 - 2.6	0.5 - 3.1	0	0
9	Bondi / Ranger Russet / RB	0.6 - 2.1	0 - 1.9	0 - 0.9	0 - 0.5	0	0
10	Bondi / Ranger Russet / JR2	0 - 0	0 - 0	0 - 1.4	0 - 0.9	0	0
11	Bondi / Ranger Russet	0 - 0.2	0 - 0	0.8 - 1.4	0 - 0.9	3	5
12	JR2	2.1 - 3.2	0 - 0.2	0 - 0.9	0 - 0	0	0
13	Bondi / Topcat	1.6 - 2.8	0 - 0	0 - 2.8	0 - 2.8	0	0
14	Bondi / Russet Burbank	1.2 - 1.4	0 - 1.1	1.8 - 3.1	0 - 4.2	0	0
15	Bondi	1.1 - 1.7	0 - 0.8	0 - 1.5	0 - 0	0	0
16	Topcat	1.2 - 2.3	0 - 0.5	1.8 - 2.8	0 - 1.5	0	0

Paddock	Varieties	DNA level *Rhizoctonia solani* log (DNA)				Rhizoctonia stem canker symptoms	
		Pre-plant soil		Seed tuber peel		Number sites detected (4 sites per paddock assessed)	Number plants detected (5 plants per site = Total 20 plants assessed)
		AG2.1	AG3	AG2.1	AG3		
17	Topcat / Russet Burbank	0 - 1.4	0 - 0.1	1.3 - 3.2	0 - 1.6	0	0
18	Bondi / Russet Burbank	0.9 - 2.7	0 - 0	2.4 - 3.0	0 - 0	0	0
19	Topcat	1.2 - 1.3	0 - 0	2.2 - 4.3	0 - 0.1	0	0
20	Bondi	0 - 0.9	0 - 0	1.0 - 1.6	0 - 1.4	0	0
21	Bondi	0 - 1.2	0 - 0	0 - 0	0 - 0	4	10
22	JR2	0 - 1.8	0 - 0	1.7 - 3.7	1.0 - 3.6	0	0
23	Bondi	2.2 - 2.8	0 - 0	2.3 - 3.6	0.9 - 2.1	0	0
24	Bondi / Ranger Russet	3.4 - 3.6	0 - 0	2.3 - 3.6	0 - 3.0	0	0
25	Innovator	1.3 - 2.8	0 - 0	0.5 - 4.6	0.6 - 3.2	0	0

Table 4-8 – Crop height and the incidence of rhizoctonia stem canker assessed 3-5 weeks after emergence in relation to concentration of *Rhizoctonia solani* DNA in the soil prior to planting and in the peel of seed tubers planted in Tasmania

Season and site number	DNA detected of *Rhizoctonia solani* log (DNA)				Stage of growth (% plants)				Rhizoctonia stem canker symptoms detected
	Pre-plant soil		Seed tuber peel						
	AG2.1	AG3	AG2.1	AG3	Normal height	Delayed	Non-emerged	Missing	
2012/13 season									
1	2.1	1.6	0	0.3	49	40	11		No
2	0	0	1.2	0	56	41	3		No
3	0.2	0.0	0.9	0	53	32	16		40 %
4	3.2	0.0	0.7	0	38	47	15		No
5	2.8	0.0	2.8	2.7	73	22	6		No
2013/14 season									
6	0	0.2	4.0	0	98	2	0	0	No
7	2.2	0	4.0	0	90	3	2	2	No
8	1.7	0	4.0	0	94	5	1	0	No
9	0	0	1.0	0.9	97	0	1	2	No
10	4.0	0			88	11	0	1	No
11	3.0	0			79	21	0	0	No
12	0	0	1.8	0	86	13	0	1	No
13	0	0	1.8	0	80	18	3	0	No

VICTORIA

In the 2012/13 season *Rhizoctonia solani* AG3 DNA was detected in the soil prior to planting in 13 out of 17 sites assessed for emergence, with levels ranging from 0 to 1.5 log (DNA), Table 4-9. *Rhizoctonia solani* AG2.1 was detected in the soil prior to planting at 14 sites, with levels ranging from 0 to 2.8 log (DNA). *Rhizoctonia solani* AG3 DNA was detected in the peel of all except one of the seed lots planted, with levels ranging from 0 to 5.2 log (DNA) and Rhizoctonia solani AG2.1 was detected in the peel of all except one of the seed lots planted, with levels ranging from 0 to 3.7 log (DNA). When plants were dug and assessed approximately 8 weeks after planting, rhizoctonia stem canker was detected in all except one of the 17 fields, Table 4-9. Where rhizoctonia stem canker occurred at a site, incidence ranged from 9 to 48 % of the plants assessed. Delayed emergence tended to be associated with the presence of typical rhizoctonia symptoms. In general, increased delayed emergence was associated with high levels of *Rhizoctonia solani* DNA in the peel of seed tubers.

Rhizoctonia cultures were collected from infected plant material. These and stem samples with rhizoctonia stem canker were tested for the presence of *Rhizoctonia solani* anastomosis groups using qPCR at DEPI Victoria. Of the 184 samples tested, 96% were identified as *Rhizoctonia solani* AG3 and 1% identified as *Rhizoctonia solani* AG2.1.

Pre-plant soil testing for the DNA of *Rhizoctonia solani* AG3 did not provide a useful indication of the risk of rhizoctonia stem canker in Victoria in the 2012/13 season. DNA of *Rhizoctonia solani* AG3 was detected at all sites, either in the soil or in the peel of seed tubers planted, indicating that some inoculum was always present. However, at a number of fields with high incidence of rhizoctonia stem canker the levels of *Rhizoctonia solani* AG3 DNA detected were relatively low and vice versa.

In the 2013/14 season *Rhizoctonia solani* AG3 was detected in the soil prior to planting at 44% of the 50 sites assessed for emergence, with the highest level being 1.7 log (DNA), Table 4-10. *Rhizoctonia solani* AG3 was detected in the peel of 92% of the seed lots planted with levels up to 4.7 log (DNA). *Rhizoctonia solani* AG2.1 was detected in soil prior to planting at 82% the sites, with the highest level being 2.8 log (DNA). *Rhizoctonia solani* AG2.1 was detected in the peel of 94% of the seed lots planted with levels up to 4.1 log (DNA). When assessed at approximately 8 weeks after emergence, rhizoctonia stem canker was detected at all fields assessed with the incidence ranging from 5 to 100%, Table 4-10.

Rhizoctonia cultures isolated from infected plant material were tested for the presence of *Rhizoctonia solani* anastomosis groups using qPCR at DEPI Victoria. Of the 92 samples tested all were identified as *Rhizoctonia solani* AG3.

DNA of *Rhizoctonia solani* AG3 was detected at all sites except one, either in the soil or in the peel of seed tubers planted, indicating that some inoculum is almost always present. The field where *Rhizoctonia solani* AG3 DNA was not detected in the soil prior to planting or in the peel of the seed tubers had the lowest incidence of rhizoctonia stem canker symptom of any field (5%). However, data from sites 35, 38 and 41 suggest that under growing conditions in Victoria in the 2013/14 season, a relatively low level of *Rhizoctonia solani* AG3 inoculum may cause a high incidence (90-95%) of rhizoctonia stem canker by 8 weeks after planting. The apparent low level of inoculum required to cause disease coupled with its widespread detection suggest that most fields are at risk of rhizoctonia stem canker. In most cases it is likely that environmental conditions and control measures adopted are having a greater impact on the incidence of rhizoctonia stem canker than the inoculum level in the soil or seed tubers.

Table 4-9 – Crop height and the incidence of rhizoctonia stem canker assessed 8 weeks after emergence in relation to concentration of *Rhizoctonia solani* DNA in the soil prior to planting and in the peel of seed tubers planted in Victoria in the 2012/13 growing season.

Site number	Variety	DNA level *Rhizoctonia solani* log (DNA)				Stage of growth (% plants)			Rhizoctonia stem canker incidence (%)
		Pre-plant soil		Seed tuber peel					
		AG2.1	AG3	AG2.1	AG3	Normal height	Delayed	Non-emerged	
1	Atlantic	0.0	0.0	0.8	4.3	84	0	16	0
2	Snowden	1.2	0.0	1.9	5.1	45	31	24	9
3	Pike	2.8	0.8	2.8	0.7	69	18	13	9
4	Atlantic	1.4	1.0	3.7	0.6	74	16	10	13
5	Snowden	1.0	1.0	2.7	4.3	64	21	15	13
6	Atlantic	0.3	1.5	3.7	0.6	69	13	18	13
7	Snowden	0.8	0.0	1.9	5.1	54	32	14	14
8	Snowden	0.7	0.0	0.0	2.4	78	20	2	17
9	Snowden	2.8	1.3	2.7	4.3	51	32	17	23
10	Atlantic	1.4	1.0	2.4	2.7	61	26	13	24
11	Atlantic	2.7	0.5	2.4	2.7	54	24	22	25
12	Atlantic	1.4	1.0	1.1	0.0	51	29	20	26
13	Catani	0.0	0.5	2.9	0.3	38	30	32	26
14	Catani	2.3	0.3	2.7	5.2	43	31	26	26
15	Atlantic	2.1	0.8	3.0	4.4	29	59	13	28
16	Catani	0.0	0.5	2.9	0.3	54	29	17	29
17	Pike	1.6	0.8	2.8	3.1	41	46	13	48

Table 4-10 – Incidence of rhizoctonia stem canker assessed 8 weeks after emergence in relation to concentration of *Rhizoctonia solani* DNA in the soil prior to planting and in the peel of seed tubers planted in Victoria.

Site number	Variety	DNA level *Rhizoctonia solani* log (DNA)				Rhizoctonia stem canker incidence (%)
		Pre-plant soil		Seed tuber peel		
		AG2.1	AG3	AG2.1	AG3	
1	Sapro Mira	0.0	0.0	4.1	0.6	5
2	Atlantic	1.1	0.0	2.3	0.0	5
3	Pike	1.8	0.0	2.4	3.2	15
4	Ranger Russet	0.8	0.3	3.4	2.8	15
5	Atlantic	1.1	0.0	3.7	2.5	25
6	Pike	2.4	1.1	2.4	3.2	30
7	Atlantic	1.4	0.0	0.7	4.7	30
8	Innovator	2.4	0.3	1.7	4.1	40
9	Snowden	1.4	0.5	0.0	3.5	40
10	Simcoe	1.2	0.7	0.8	0.0	40
11	Simcoe	1.3	0.3	0.8	0.0	40
12	Pike	2.3	1.0	2.4	3.2	40
13	Pike	1.5	0.2	2.4	3.2	45
14	Catani	1.3	0.0	3.4	0.8	45
15	Ranger Russet	0.0	0.0	3.4	2.8	47
16	Atlantic	2.2	0.0	2.7	1.4	50
17	Atlantic	2.3	1.0	3.2	4.3	50
18	Innovator	0.0	0.2	1.7	4.1	60
19	Atlantic	1.0	0.0	0.7	4.7	65
20	Atlantic	1.9	0.0	2.7	1.4	65
21	Atlantic	0.3	0.7	0.7	4.7	67
22	Snowden	0.8	0.0	0.0	3.5	70
23	Atlantic	2.2	0.0	3.7	2.5	70
24	Atlantic	2.7	1.1	0.8	1.2	75
25	Atlantic	2.0	1.7	0.7	4.7	75
26	Catani	0.3	0.0	3.1	1.7	80
27	Trent	2.8	0.3	3.0	2.6	80
28	Russet Burbank	0.0	0.0	3.7	2.3	80

29	Atlantic	0.5	0.0	3.7	2.5	80
30	Atlantic	2.3	1.5	0.8	1.2	84
31	Atlantic	2.0	0.0	3.7	2.5	85
32	Innovator	0.0	0.0	4.0	3.1	85
33	Catani	0.0	0.0	0.0	1.8	86
34	Russet Burbank	2.1	0.0	3.7	2.3	90
35	Atlantic	0.0	0.0	0.8	1.2	90
36	Atlantic	2.5	0.0	0.5	3.9	90
37	Atlantic	1.9	0.0	0.5	3.9	90
38	Atlantic	0.5	0.0	2.7	1.4	90
39	Innovator	2.0	0.0	3.6	2.4	95
40	Innovator	0.3	0.6	3.4	3.7	95
41	Atlantic	0.3	0.0	0.8	1.2	95
42	Innovator	2.0	0.0	3.5	4.3	100
43	Innovator	0.0	0.1	3.3	3.8	100
44	Snowden	1.5	0.0	1.9	4.7	100
45	Atlantic	2.1	0.1	3.7	2.5	100
46	Russet Burbank	2.8	0.0	3.1	3.1	100
47	Innovator	0.5	0.2	4.1	4.2	100
48	Innovator	0.0	1.2	3.8	0.0	100
49	Atlantic	2.0	1.7	0.8	1.2	100
50	Innovator	0.3	0.3	4.0	3.1	100

FIELD VALIDATION OF SEED PEEL DNA TESTS

The assays developed for the pre-plant DNA testing of soil samples can be utilised for testing of other sample types including the peel of seed tubers.

Inoculum in the soil is generally regarded to be the major source of disease risk for the soilborne pathogens included in the PreDicta Pt testing service. However seed tubers are also a source of inoculum that may contribute to disease risk in potato crops. In conjunction with the field validation process conducted in commercial growing systems across South Australia, Victoria and Tasmania for the soil test, where possible peel samples from seed planted at these locations has been DNA tested. Objective of this work was to assess if inoculum on seed tubers is contributing to the risk of disease in commercially grown crops, and to determine if the seed tuber DNA test provides useful additional information on the risk of disease.

SPONGOSPORA SUBTERRANEA

Field validation in commercial crops grown in Tasmania, Victoria and South Australia has demonstrated that the pre-plant soil test for *Spongospora subterranea* DNA provides a useful indication of powdery scab risk to tubers. This data ignores the possible contribution to disease risk from inoculum on seed tubers. Strength of the relationship suggests inoculum in the soil is the main inoculum source contributing to disease risk on the harvested tubers. In contrast the level of *Spongospora subterranea* DNA in the peel of seed tubers on its own does not provide a useful indication of powdery scab risk to harvested tubers, Figure 4-32. This does not mean that seed tubers are not contributing, but it does highlight that any contribution is overshadowed by contribution of inoculum in the soil.

Seed carrying *Spongospora subterranea* inoculum is sometimes planted into sites where *Spongospora subterranea* inoculum is not known to be present. In Victoria and South Australia, where *Spongospora subterranea* DNA was not detected by pre-plant soil testing, incidence of powdery scab on the harvested tubers was usually 2% or less, most often nil, Figure 4-33. This was irrespective of the *Spongospora subterranea* DNA level measured in the peel of seed tubers planted. Two notable exceptions occurred where powdery scab incidence reached 5 and 12%, in both cases the variety was Alturas grown in South Australia. These results suggest that under the growing conditions experienced in Victoria and South Australia during the 2011/12 and 2012/13 seasons, seed of the quality planted did not contribute substantially to the risk of powdery scab on tubers when varieties of low to moderate susceptibility are grown, but may have for the variety Alturas.

In Tasmania, inoculum on the seed may be a contributor to disease risk, Figure 4-33. Climatic conditions are more conducive to powdery scab in Tasmania, than in Victoria and South Australia. Incidence of powdery scab on tubers exceeded 60% at two sampling points for each of the varieties Bondi and Ranger Russet from locations where *Spongospora subterranea* DNA was not detected in the soil. Seed was a possible source of the inoculum based on the level of *Spongospora subterranea* DNA in the peel of the seed planted. Though these four samples were assessed as having a high incidence of powdery scab (> 60%) on the tubers, symptoms were not as severe as those often seen on tubers grown in soils having a high *Spongospora subterranea* DNA level prior to planting. When powdery scab on the tubers occurred at sampling points where *Spongospora subterranea* DNA was not detected in the soil prior to planting, the level of *Spongospora subterranea* DNA was lower than expected for the level of incidence recorded, Figure 4-34.

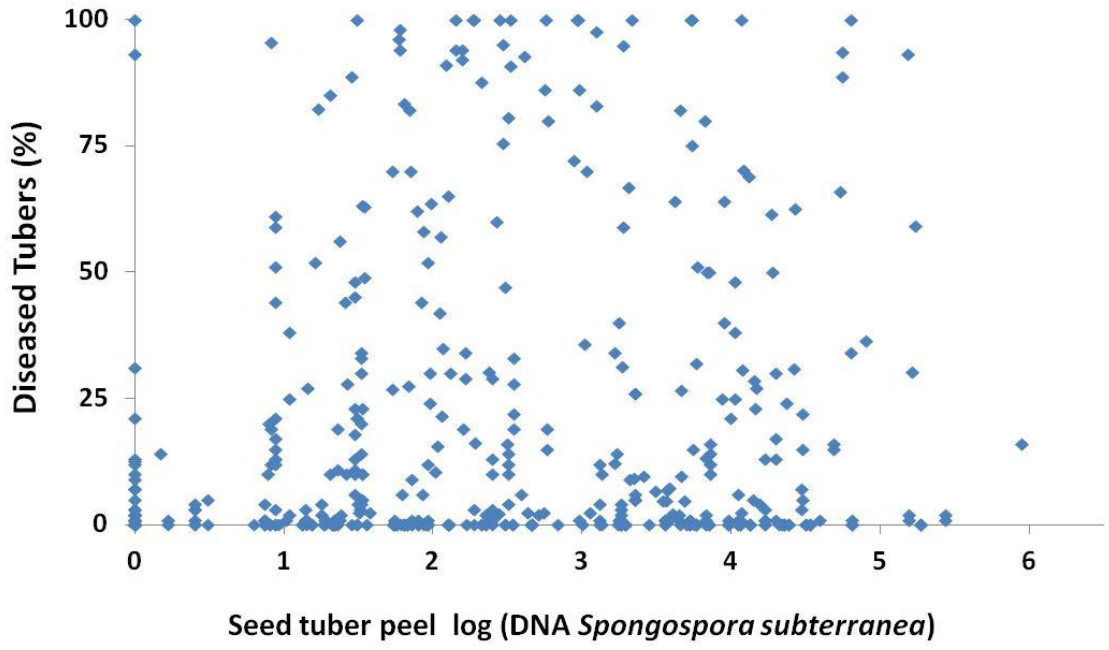

Figure 4-32 – Relationship between levels of *Spongospora subterranea* DNA in the peel of seed tubers and the incidence of powdery scab on the tubers in commercially grown potato crops (489 sampling points monitored across Tasmania, Victoria and South Australia in 2011/12 and 2012/13)

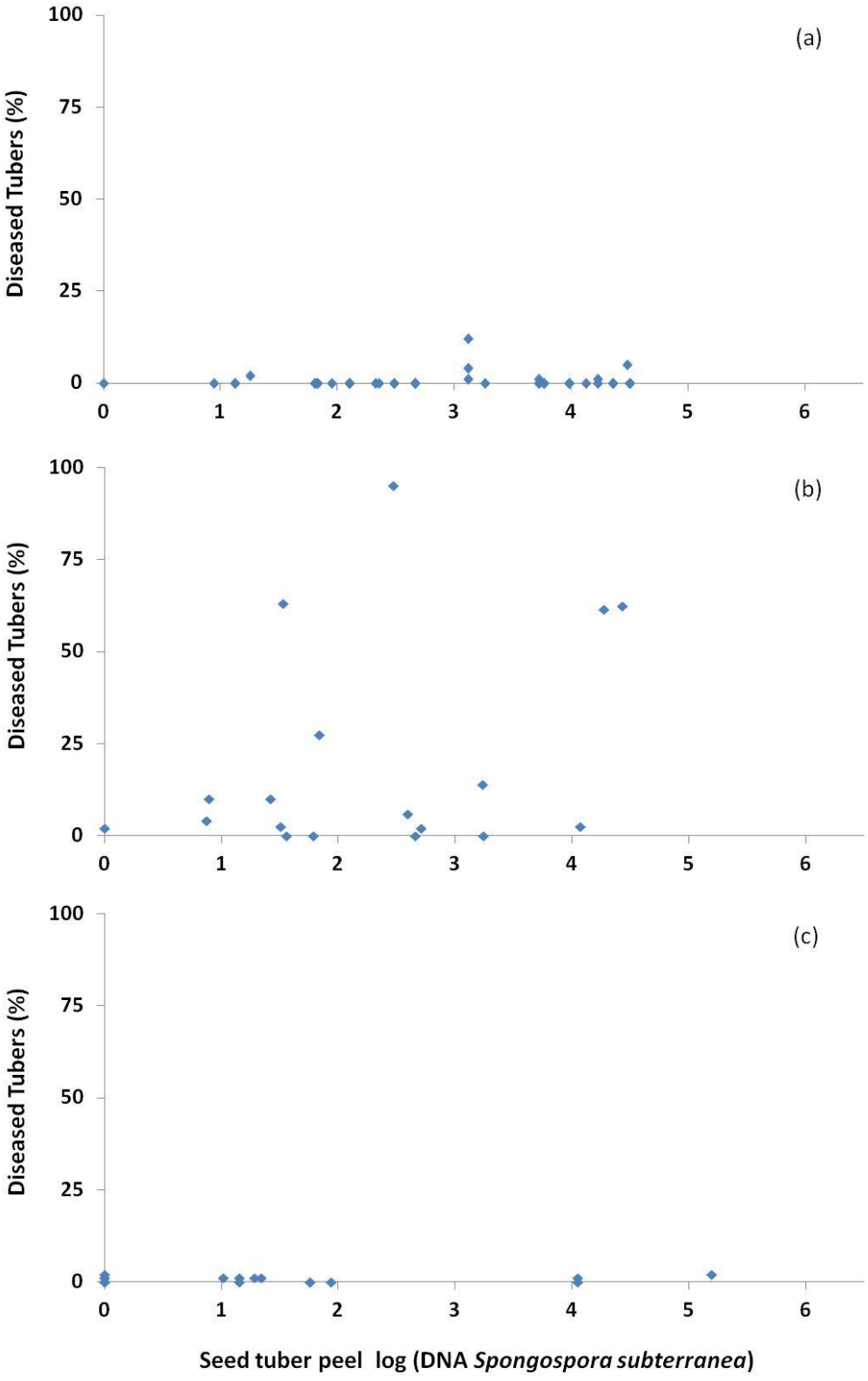

Figure 4-33 – Relationship between levels of *Spongospora subterranea* DNA in peel of seed tubers and the incidence of powdery scab on the harvested tubers growing in soils where *Spongospora subterranea* DNA was not detected prior to planting at sampling points in a) South Australia, b) Tasmania and c) Victoria (data from 2011/12 and 2012/13 growing seasons combined).

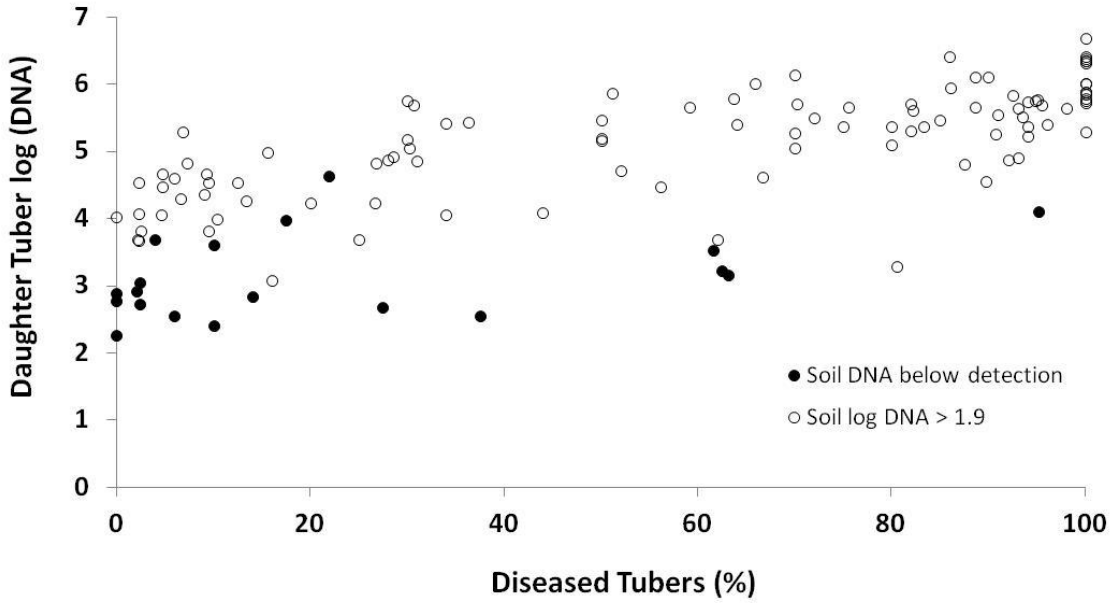

Figure 4-34 – Relationship between incidence of powdery scab on tubers and the level of *Spongospora subterranea* DNA in their peel depending on whether grown at sampling points with a non-detectable or a high level of *Spongospora subterranea* DNA measured in the soil prior to planting.

MELOIDOGYNE FALLAX

Field validation in commercial crops grown in Tasmania, Victoria and South Australia has demonstrated that the pre-plant soil test for *Meloidogyne fallax* DNA provides a useful indication of the risk of root-knot nematode damage to tubers. This data ignores the possible contribution to disease risk from inoculum on seed tubers. Strength of the relationship suggests inoculum in the soil is the main source contributing to disease risk on the harvested tubers.

Given the strength of the relationship between disease risk and level of *Meloidogyne fallax* DNA in the soil prior to planting it is surprising that there also appears to be a relationship with level of *Meloidogyne fallax* DNA in the peel of seed tubers in the same data set, Figure 4-35. Seed is unlikely to be the major contributor to disease risk in this data, as only a low incidence of disease was recorded in the absence of any detectable inoculum in the soil, Figure 4-36. This data is limited by the variation in the seed lots that were planted in the commercially grown crops. A putative high risk threshold for the log (*Meloidogyne fallax* DNA +1) level in seed peel was previously set at 3 (APRP1). Based on this threshold value only a small number of the seed lots tested had a *Meloidogyne fallax* DNA level in the high risk category. It is possible that seed tubers having *Meloidogyne fallax* DNA levels in the peel above 3 could cause root-knot nematode symptoms on the harvested tubers from that crop.

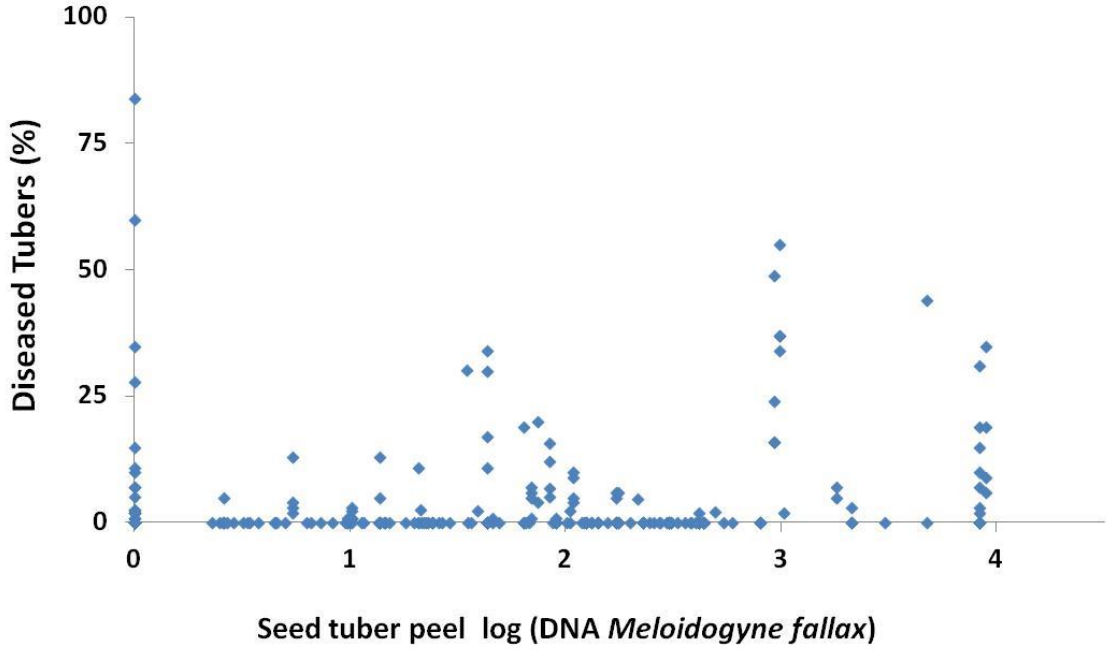

Figure 4-35 – Relationship between levels of *Meloidogyne fallax* DNA in the peel of seed tubers and the incidence of root-knot nematode symptoms on the tubers in commercially grown potato crops. (343 sampling points monitored across Tasmania and South Australia in 2011/12 and 2012/13)

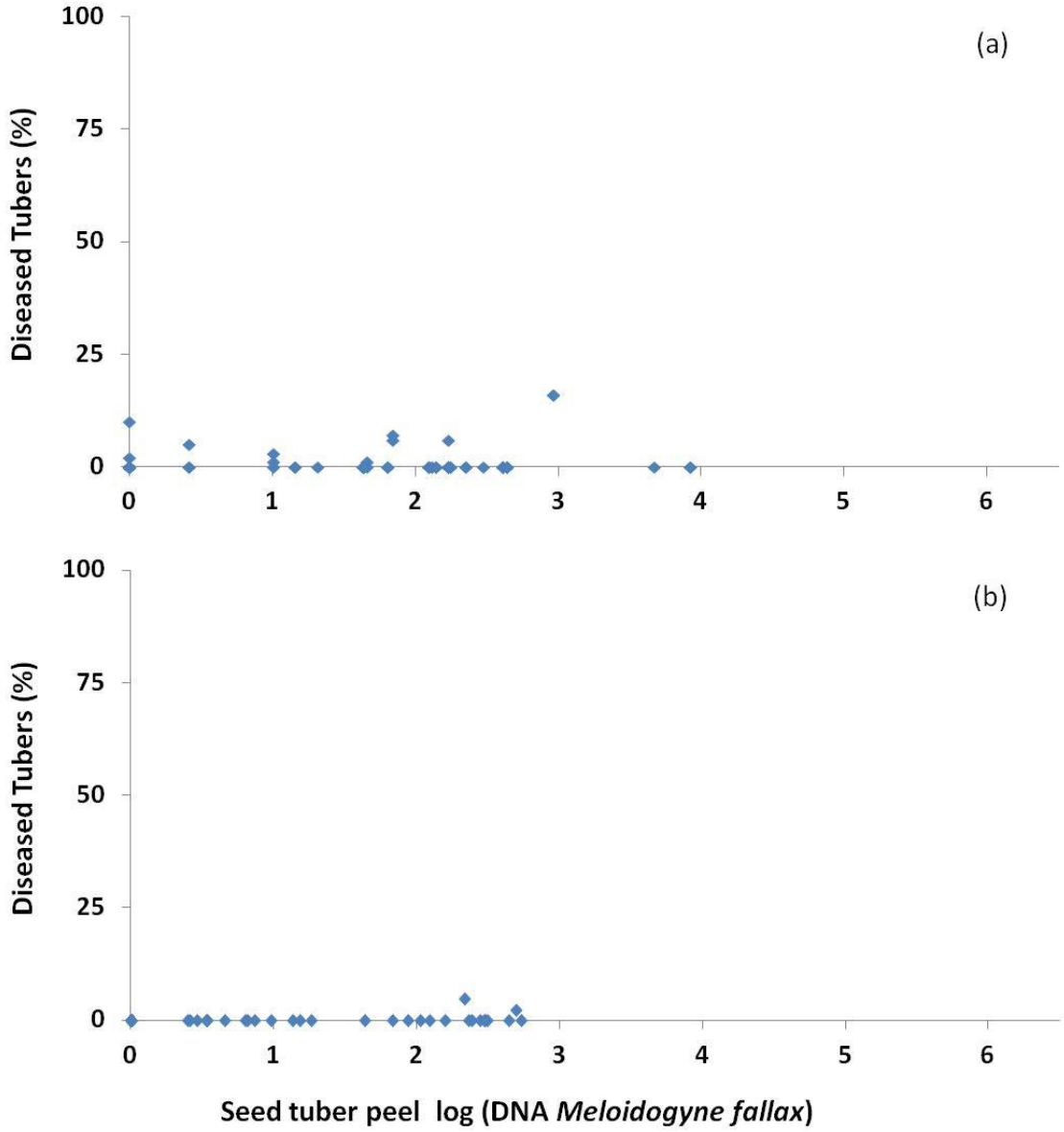

Figure 4-36 – Relationship between levels of *Meloidogyne fallax* DNA in peel of seed tubers and the incidence of root-knot nematode symptoms on the harvested tubers growing in soils where *Meloidogyne fallax* DNA was not detected prior to planting at sampling points in a) South Australia and b) Tasmania. (Data from 2011/12 and 2012/13 growing seasons combined).

MELOIDOGYNE HAPLA

Field validation in commercial crops grown in South Australia and Tasmania has demonstrated that *Meloidogyne hapla* is not the cause of visible root-knot nematode damage on the surface of tubers. However, despite not being associated with visible symptoms, *Meloidogyne hapla* DNA is frequently detected in the peel of harvested tubers. High levels of *Meloidogyne hapla* in the peel of harvested tubers are generally associated with high levels in the soil prior to planting. At sampling points where *Meloidogyne hapla* DNA was not detected in the soil prior to planting, *Meloidogyne hapla* DNA was only detected in the

peel of harvested tubers at a few sampling points in South Australia and none in Tasmania, Figure 4-37. In South Australia planting infested seed may have contributed to *Meloidogyne hapla* DNA being detected in the peel of harvested tubers at a few sampling points.

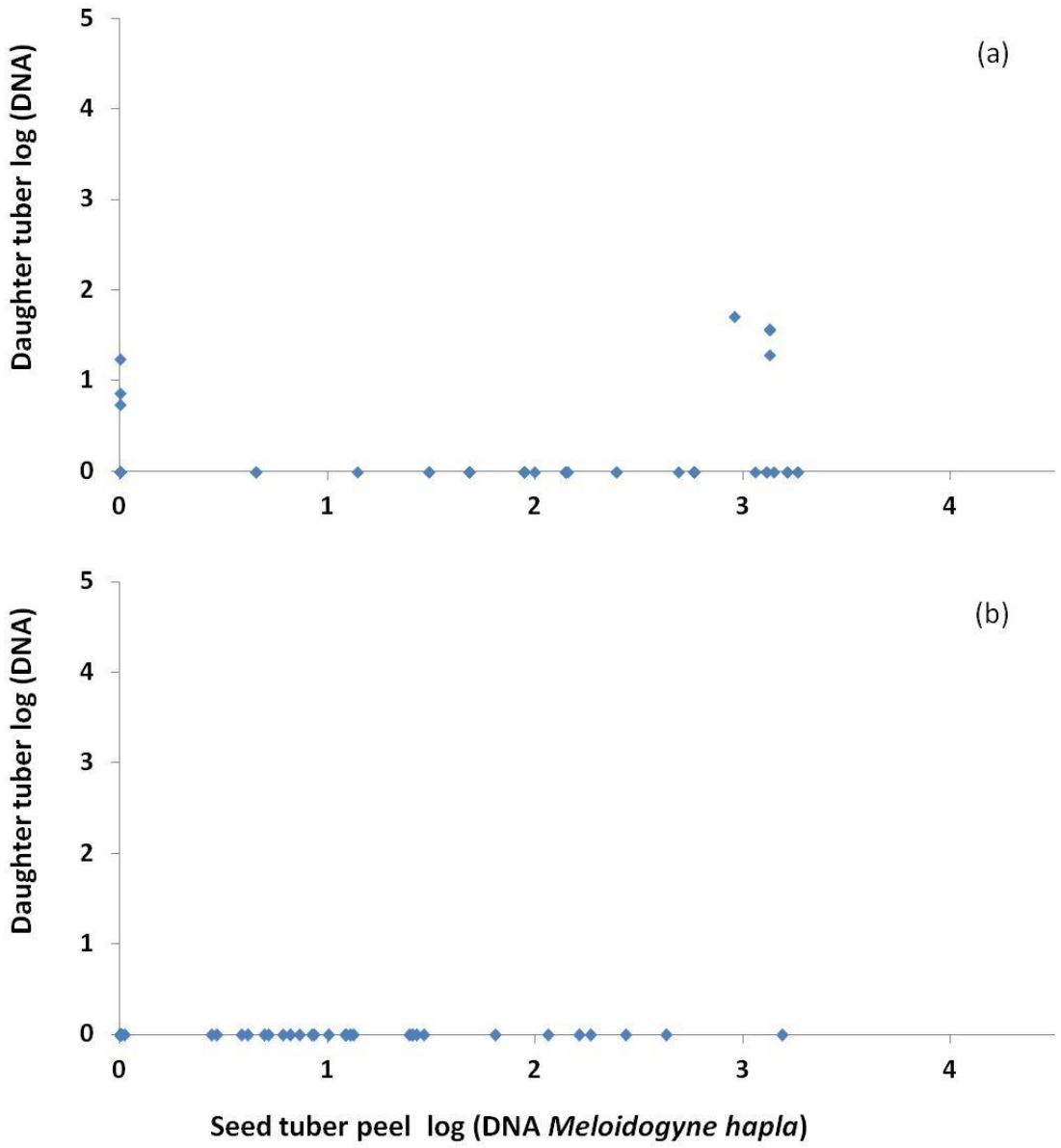

Figure 4-37 – Relationship between levels of *Meloidogyne hapla* DNA in peel of seed tubers and in the peel of harvested tubers growing in soils where *Meloidogyne hapla* DNA was not detected prior to planting at sampling points in a) South Australia and b) Tasmania. (Data from 2011/12 and 2012/13 growing seasons combined).

PATHOGENIC *STREPTOMYCES*

*Streptomyces txt*A gene DNA is detected more often on seed than in the soil prior to planting, as reflected in data collected in Victoria, Table 4-11. In the case of common scab, testing for *Streptomyces txt*A gene DNA in the soil alone has not provided a useful indication of the risk of disease. The lack of a relationship suggests inoculum in the soil prior to planting may not be the only or main inoculum source contributing to common scab disease risk on the harvested tubers. Seed tubers are a possible source of inoculum that may contribute to disease risk in potato crops.

The relationship between the level of *Streptomyces txt*A gene DNA in the peel of seed tubers and the incidence of common scab on harvested tubers was investigated by analysing data from sampling points where *Streptomyces txt*A gene DNA was not detected in the soil prior to planting, Figure 4-38. These data suggest that seed could be an important source of inoculum leading to the development of common scab. However, there are still a number of sampling points where *Streptomyces txt*A gene DNA was not detected in the soil prior to planting or in the peel of seed tubers, yet common scab occurred on the harvested tubers.

The lack of a strong relationship between the level of *Streptomyces txt*A gene DNA in the peel of seed tubers and the incidence of common scab suggests that, although pathogenic *Streptomyces* on seed is an important source of inoculum, other factors such as environmental conditions and crop management are driving disease expression.

Table 4-11 – Detection of *Streptomyces txt*A gene DNA in soil prior to planting and on seed tubers in Victoria over 3 seasons (n = number samples tested)

	Percent detections of *Streptomyces txt*A gene DNA		
	2011/2012	***2012/2013***	***2013/2014***
Soil	5 (n=378)	4 (n=182)	6 (n=160)
Seed	58 (n=71)	72 (n=61)	68 (n=73)

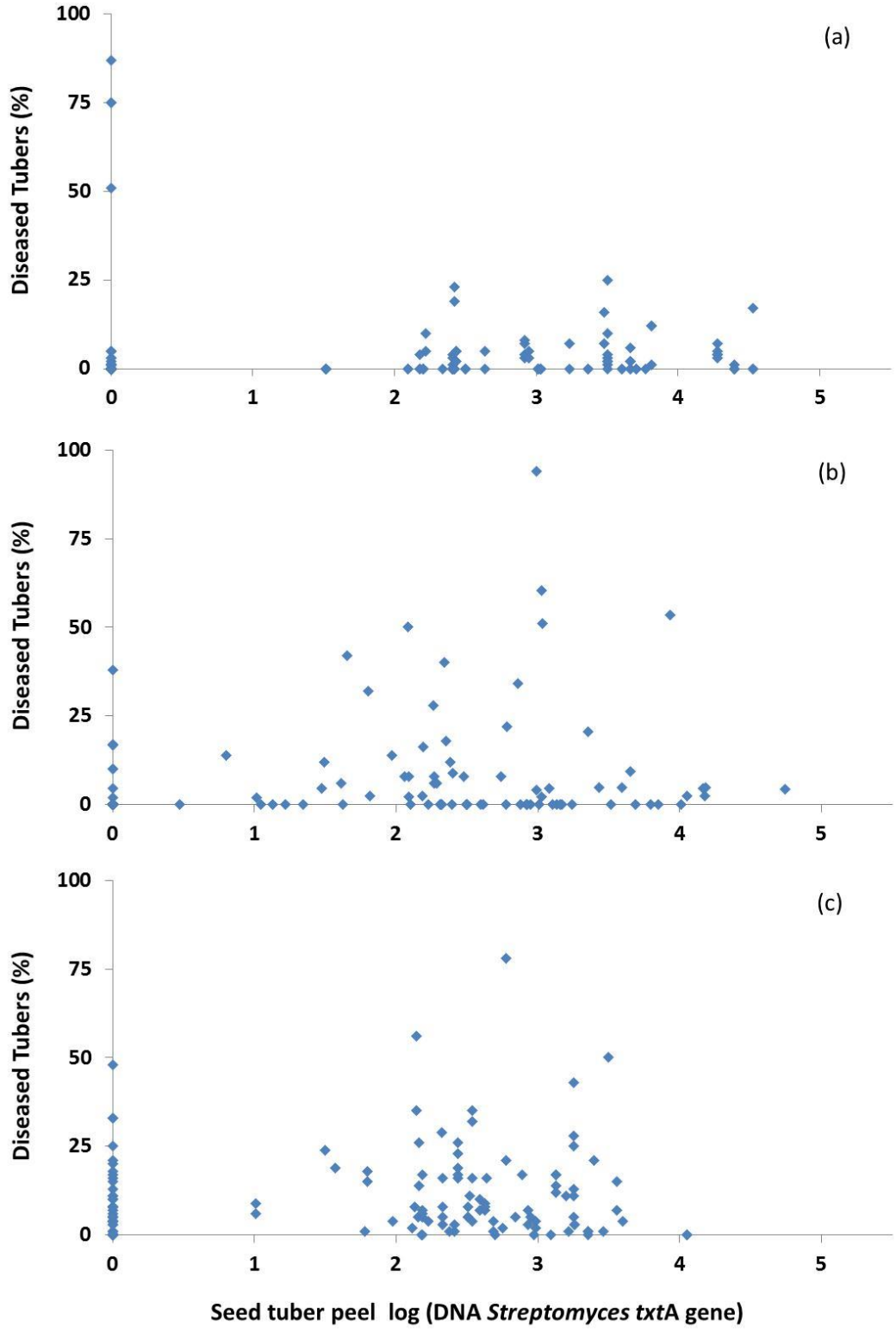

Figure 4-38 – Relationship between levels of pathogenic *Streptomyces* DNA in peel of seed tubers and the incidence of common scab on the harvested tubers growing in soils where pathogenic *Streptomyces* DNA was not detected prior to planting at sampling points in a) South Australia, b) Tasmania and c) Victoria. (Data from 2011/12 and 2012/13 growing seasons combined).

RHIZOCTONIA SOLANI AG3 AND AG2.1

BLACK SCURF

Seed tubers are a possible source of inoculum that may contribute to disease risk in potato crops. In the case of black scurf, testing for *Rhizoctonia solani* AG3 DNA in the soil alone has not provided a useful indication of the risk of disease.

Black scurf symptoms are frequently seen on seed tubers. As *Rhizoctonia solani* AG3 DNA is infrequently detected in the soil, *Rhizoctonia solani* AG3 DNA on the seed is the only measured source of inoculum at a high proportion of the sampling locations in Victoria, Table 4-12. By analysis of sampling points where *Rhizoctonia solani* AG3 DNA was not detected in the soil, the relationship between the level of *Rhizoctonia solani* AG3 DNA in the peel of seed tubers and the incidence of black scurf on harvested tubers was investigated, Figure 4-39. The relationship was not consistent between the growing regions assessed in the three states. In South Australia planting seed that had low or undetectable levels of *Rhizoctonia solani* AG3 DNA in the peel was associated with a lower probability of black scurf. Differences in chemical application at planting between sampling points in South Australia contributed to some of the response seen. When this is taken into account, the relationship found in South Australia is questionable, and this is further weakened by the lack of a relationship in Tasmania and the poor relationship in Victoria.

Table 4-12 – Detection of *Rhizoctonia solani* AG3 DNA in soil prior to planting and on seed tubers in Victoria over 3 seasons (n = number samples tested)

	Percent detections of *Rhizoctonia solani* AG3 DNA		
	2011/2012	***2012/2013***	***2013/2014***
Soil	17 (n=378)	17 (n=182)	21 (n=160)
Seed	62 (n=71)	84 (n=61)	93 (n=73)

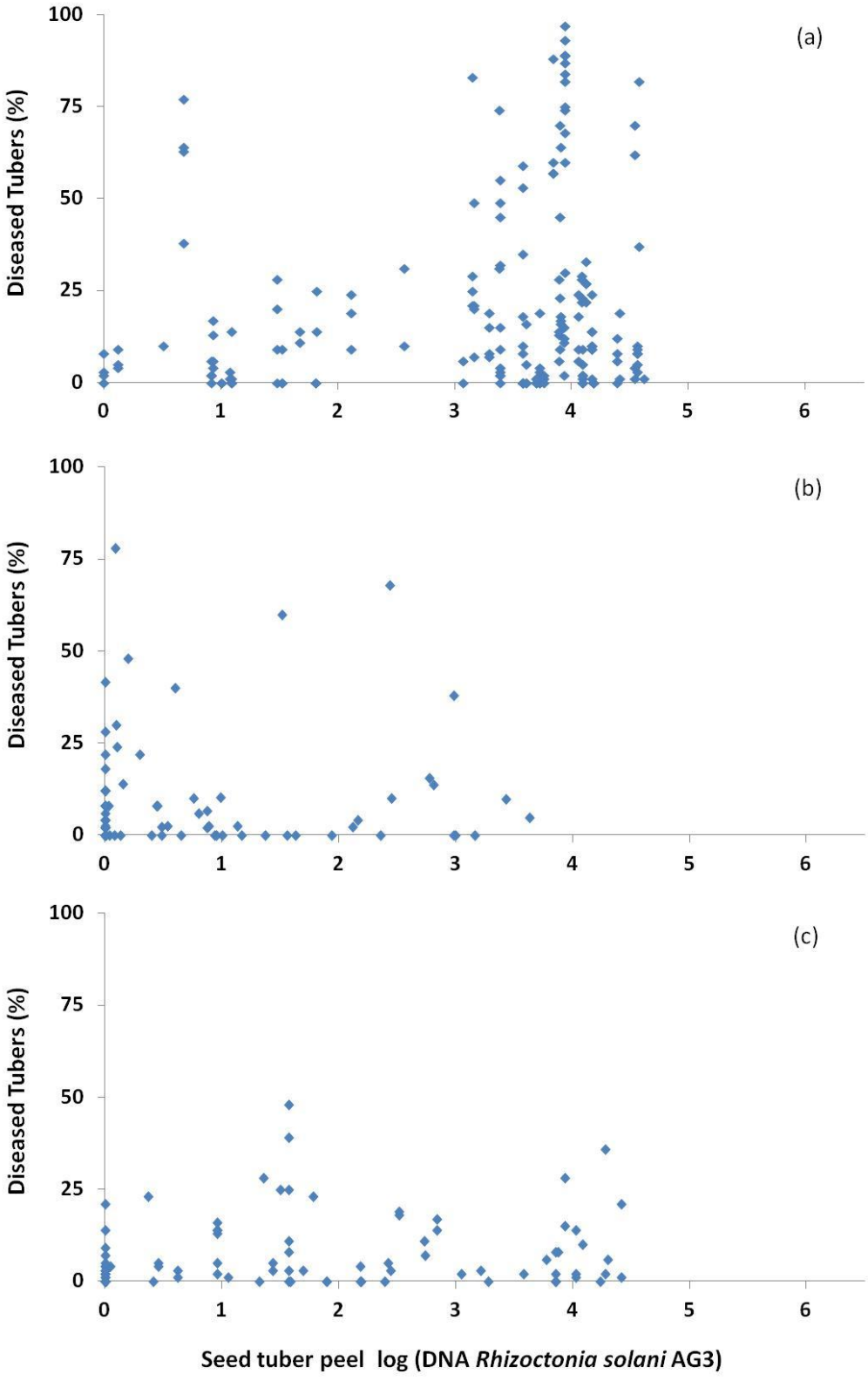

Figure 4-39 – Relationship between levels of *Rhizoctonia solani* AG3 DNA in the peel of seed tubers and the incidence of black scurf on the harvested tubers growing in soils where *Rhizoctonia solani* AG3 DNA was not detected prior to planting at sampling points in a) South Australia, b) Tasmania and c) Victoria. (Data from 2011/12 and 2012/13 growing seasons combined).

DEFORMED TUBERS

Validation data suggests that high *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting may lead to an increase in the number of deformed tubers in the variety Russet Burbank. High *Rhizoctonia solani* AG2.1 DNA on the peel of seed tubers may also contribute to an increased number of deformed tubers, Figure 4-40. At sites where *Rhizoctonia solani* AG2.1 DNA was not detected in the soil prior to planting, the number of deformed tubers trended higher with increasing levels of *Rhizoctonia solani* AG2.1 DNA on the peel of seed tubers. This data indicates that *Rhizoctonia solani* AG2.1 may be a cause of deformed tubers on other varieties than Russet Burbank, including Innovator.

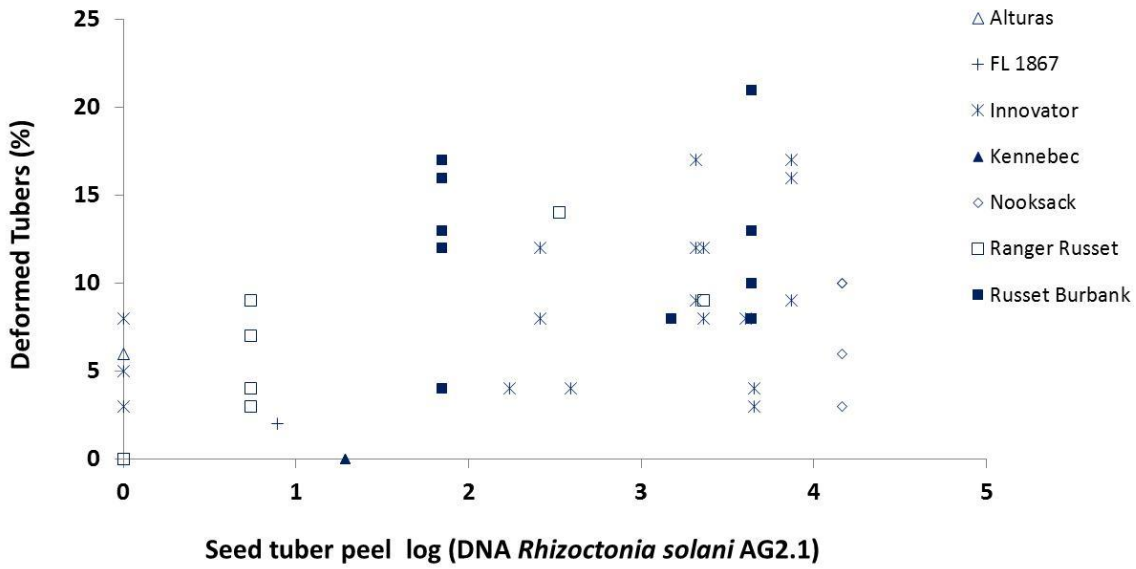

Figure 4-40 – Relationship between level of *Rhizoctonia solani* AG2.1 DNA in the peel of seed tubers and the incidence of deformed tubers grown in soils where *Rhizoctonia solani* AG2.1 DNA was not detected prior to planting depending on variety grown in South Australia. (Data from 2012/13 growing season).

PREDICTA PT SERVICE

DESCRIPTION

PreDicta Pt (Pt = potato) is a commercial soil DNA testing service to identify if certain soilborne pathogens pose a significant risk to potato crops prior to planting. PreDicta Pt has been developed in association with this project, but designed to operate as a standalone commercial service post project. The testing service has been delivered to growers since September 2013.

Currently the PreDicta Pt test provides an indication of the risk of:

- Powdery scab (caused by *Spongospora subterranea*)
- Black dot (caused by *Colletotrichum coccodes*)
- Root knot nematode (caused by *Meloidogyne fallax*)

DNA levels of *Streptomyces txt*A gene, *Rhizoctonia solani* AG3, *Rhizoctonia solani* AG2.1, *Meloidogyne hapla*, *Verticillium dahliae*, *Pratylenchus penetrans* and *Pratylenchus neglectus* are reported as "Tests Under Development". These research results are provided for information only as sensitivity and risk categories are not available for these tests.

Potato growers can access PreDicta Pt via agronomists accredited by SARDI to interpret the results and provide advice on management options to reduce the risk of disease losses. Growers are welcome to undertake the training and to access the service directly if they wish to do so.

Establishment of the PreDicta Pt service has been conducted under the oversight of an industry reference group.

COMPONENTS OF THE COMMERCIAL SERVICE

PREDICTA PT MANUAL

The PreDicta Pt e-manual was specifically prepared as a supporting resource for the PreDicta Pt testing service. It outlines the principles of soilborne disease pathology on which the testing service is based, such as the pathogen inoculum level, disease expression and disease risk. The testing service is described along with how to access the test, how to sample and how to interpret the DNA test results. Each pathogen included has a specific section covering symptoms, basic biology of the pathogen, drivers and importance of inoculum, factors conducive to disease, interpretation of PreDicta Pt results and an overview of possible management strategies. All text has been kept to a brief dot point format.

Produced in an electronic format, the manual is easy and cheap to update. As the PreDicta Pt service is expanded to include further pathogens, sections can be added seamlessly.

The PDF format chosen is compatible with most computer systems or tablet devices. Though it can be viewed on a smart phone, a larger screen size is desirable. Tablet users have fast access to the information they require via the e-manuals touch screen functionality. Thumbnail photo galleries of disease symptoms can be expanded to full screen size.

Layout enables hard copies to be printed by SARDI for use during the training workshops and for users that prefer this format.

TRAINING WORKSHOPS

Training is an important part of the roll out of the PreDicta Pt testing service. PreDicta Pt samples are only accepted and processed if they are received from an accredited agronomist with an active accreditation number. Accreditation is available to anyone involved in the potato industry or servicing the potato industry, including growers or their employees.

Training workshops were held in Ulverstone on 29 July 2013, in Ballarat on 31 July 2013, in Penola on 1 August 2013 and in Adelaide on 25 July 2014. The workshops were promoted via the AUSVEG Potato Extension newsletter. Forty participants were trained and accredited with representation from growers, processors, agribusiness, seed industry and independent consultants.

Training is mandatory to ensure that users are aware of proper and appropriate use of the testing service. Training assists the industry maximise the benefit from the testing service. It also lowers the likelihood of a loss of confidence in the service during its adoption phase. This could easily occur if users have unrealistic expectations of the test or are not familiar with probability-based test results. PreDicta Pt results are presented as risk categories. It is critical that users understand the concept of probability of certain disease outcomes in relation to a test result and risk category. Understanding the nature of the results is necessary to know how to interpret and utilise PreDicta Pt results in decision making.

Sampling is a critical part of obtaining meaningful results and the sampling strategies to use were an important module of the training. Additionally the use of the test kits, appropriate handling procedures, suitable freight options and how to submit samples were covered.

For each disease for which the PreDicta Pt test provides a risk category, a presentation by a pathologist covered symptoms, basic biology of the pathogen, drivers and importance of inoculum, factors conducive to disease, interpretation of PreDicta Pt results and an overview of possible management strategies.

Towards the end of the training workshop participants were split into groups to undertake a series of case studies. They were given scenarios including background information on paddocks, the growers' planting intentions, and PreDicta Pt test results. They were asked if the results should be expected for the provided scenario, to consider planting intentions of the grower and to make recommendations, along with advising what management would minimise disease. A representative of each group then reported back to all the participants, followed by workshop discussion.

Finally, each participant undertook a multiple choice test as part of their accreditation. The assessment process involved a one on one discussion between a member of the training group and each participant to review their responses and provide any further instruction or follow up training as required.

Workshop Evaluation

On completion of the training workshop and before leaving, each participant was asked to fill out an evaluation of the training workshop, refer Appendix 2. Survey sheets were received from 34 participants. In general the feedback and responses were highly positive. Average ratings (scale 1 = poor, 5 = excellent) for individual modules within the training course ranged from 4.2 – 4.6 with a response rate of 100%. In

relation to the e-manual, only 43% of participants provided a rating (average response 4.8), with a common response from others that they had not yet looked at it.

The suggestion of "Online training" was not popular. There was not a single participant that said they would prefer it. Participants expressed that the interaction that occurs is a key part of the training workshops. A few participants stated that online updates for already accredited agronomists may be useful.

At this stage hard copies still appear to be essential. Ninety percent of participants stated that they needed a hard copy of the manual.

Based on the feedback from participants and subsequent discussions among the presentation team, some minor adjustments to the workshop presentations were made in preparation for future workshops.

TEST KITS

To access the PreDicta Pt service accredited agronomists are provided PreDicta Pt kits for each sample. Each kit comprises the specialised soil sample bag, an instruction sheet, secondary packaging bag for biosecurity use, rubber band and a self-adhesive delivery address label.

The soil sampling bag is of a size appropriate for a 500 g sample, is made of strong puncture-resistant plastic, and has a heavy duty zip-lock seal and air-release valve to minimise air content in the bag. Each PreDicta Pt sample bag is labelled with a unique individual sample barcode. Defined entry fields are provided on the label to collect all the critical information to identify, process and report results for the sample.

These kits facilitate ease of use by the accredited agronomist, as well as streamlining sample receival, processing and reporting.

PREDICTA PT REPORTS

Reports are currently provided within a two week period from the time of sample receival at SARDI's Molecular Diagnostics Group.

The PreDicta Pt report consists of 6 main sections:

- Sample information – Reproduces data entered by the accredited agronomist on the PreDicta Pt label along with the weight and condition of the sample at receival.
- Test results – Total level of DNA of specific pathogens expressed as log (pg DNA / g soil). Currently includes test results for *Colletotrichum coccodes*, *Spongospora subterranea* and *Meloidogyne fallax*. In addition to the quantitative level, an indication of the risk of disease is provided. The risk is indicated along a band defined by the following categories; Below detection, low risk, medium risk, high risk.
- Padddock history – Critical cross check for the agronomist in considering the DNA results.
- Pathogen level comments – Provides information on the probability of disease associated with the risk category for pathogens included in the test result section. Comments also indicate some of the situations that alter risk from the category and the probabilities listed.

- Agronomist comments – Blank entry field where the agronomist may add recommendations prior to providing to their client. Other fields on the report are locked from editing.
- Tests under development – These research results are provided for information only as the sensitivity levels and risk categories are not available for these tests. Results are presented for the total level of DNA of specific pathogens expressed as log (pg DNA / g soil). Currently includes test results for *Meloidogyne hapla*, *Streptomyces txt*A gene, *Rhizoctonia solani* AG2.1, *Rhizoctonia solani* AG3 and *Verticillium dahliae*. Results for *Pratylenchus penetrans* and *Pratylenchus neglectus* are reported as nematodes / g soil.

ADOPTION

Test utilisation

In the 2013/14 planting season a total of 103 PreDicta Pt tests were conducted, with results delivered to 29 growers via the PreDicta Pt accredited agronomists. A further 1,681 Potato Research tests were performed. It is difficult to estimate how many of the research samples will translate to PreDicta Pt tests in future years.

The highest levels of penetration for PreDicta Pt in the market were for commercial processing potato crops in the SE of South Australia and for the testing of seed paddocks in Tasmania, and to a lesser extent in Victoria. Problems with root knot nematode are a driving factor for adoption of the PreDicta Pt test in the SE of South Australia. Testing in this area has shown to be a useful indicator of risk for root knot nematode. Additionally there are some options available for growers to reduce risk where it has been identified as being high. Options are more limited for the control of powdery scab in commercial crops. If better control options were available for growers to manage the risk of powdery scab, this would increase the value of the PreDicta Pt test in relation to this disease, especially in Tasmania.

Tolerance levels in seed production are much lower than in commercial crops and this was always going to be one of the first sectors to adopt testing. Some seed growers have altered their planting plans based on the test results.

FEEDBACK AND REVIEW

The PreDicta Pt service is a new test for the potato industry. Consultation and advice was sought from participants of various sectors of the potato industry during its final development leading up to release. However, it was always anticipated that further refinements would be made to the service after its release. This is expected to be an ongoing process. A number of adjustments have already been made and others are under consideration.

Test kits

The PreDicta Pt testing service is aligned with the existing PreDicta B service for cereal producers operated by SARDI. In order to manage costs it is important that the receival systems are compatible with each

other. Where appropriate, other aspects of the operation of the PreDicta B service that have proven to be reliable over time have been adopted. This is especially evident in the design of the test kits.

Consideration and consultation was carried out in the specific design of the PreDicta Pt label for the soil sampling bags. The overall aim was to minimise the effort required in filling in the labels, whilst still capturing essential information and some highly useful information for test interpretation and the potato industry.

No changes appear to be required to the current test kit for taking and submitting PreDicta Pt soil samples.

A change that has been made in both services resulting from the development process for PreDicta Pt is the addition of a specifically manufactured biosecurity bag.

Lack of adherance to sampling strategies

Guidelines published in the PreDicta Pt manual for sampling strategies advise the number of individual PreDicta Pt tests to conduct in a paddock based on its size. For example in a paddock greater than 10 hectares, at least 4 PreDicta Pt tests are advised. Some growers consider the cost per paddock of undertaking 4 tests to be prohibitive. A review of the PreDicta Pt samples received last season indicates that growers are opting to test less samples per paddock than advisable, often only 2 samples in large paddocks (>20ha).

Inadequate testing increases the likelihood of misestimating the risk in a paddock. These practices increase the likelihood of poor outcomes for the client, and therefore undermining confidence in the testing service.

It is important that the requirement for adequate sampling continues to be emphasised through the accredited agronomist network. Consideration could be given to the linking of samples from paddocks and offering a discount to encourage an appropriate level of testing in large size paddocks.

Inclusion of "TESTS UNDER DEVELOPMENT" on PreDicta Pt report

The PreDicta Pt test report was to provide the results of the 3 pathogen tests for which DNA soil testing is providing a useful indication of the risk of disease, i.e.the pathogens *Colletotrichum coccodes*, *Spongospora subterranea*, *Meloidogyne fallax*.

Tests developed for other pathogens including *Meloidogyne hapla*, *Streptomyces txt*A gene, *Rhizoctonia solani* AG2.1, *Rhizoctonia solani* AG3, *Verticillium dahliae* all offer useful information to a grower, but also have some significant limitations in their use. These tests were not going to be reported.

Feedback from growers requested access to the results for *Streptomyces txt*A gene and *Rhizoctonia solani* AG2.1 and *Rhizoctonia solani* AG8 be made available.

Savings from only running the three assays included in the PreDicta Pt service as opposed to 8-10 assays are negligible to the cost of operating the commercial service.

Subsequently, changes were made to the reporting structure, and results for these tests are now reported in a separate section of the report "TESTS UNDER DEVELOPMENT" with the qualification "Tests are

currently being developed for the following pathogens. These research results are provided for information only as the sensitivity levels and risk categories are not available for these tests".

Sample collection

Sample collection is a time consuming and costly part of the testing process. Improvements made in this area would be appreciated by clients; however the cost of an automated sampling device may outweigh the benefit for many smaller clients. SARDI has been investigating hand held mechanically assisted sampling equipment.

PreDicta Pt demand for evaluating effectiveness of control options

Control options for many soilborne diseases of potatoes are limited. Growers often rely on long rotations and increasingly are looking to bio fumigant or specific rotational crops to reduce the potential for disease. These crops generally do not provide any cash return as they are simply incorporated back in the soil. Apart from the conditioning effect on the soil, which is well known, growers are interested to know what positive or negative impact these crops are having on soil pathogens. It is difficult to assess if these strategies are providing a meaningful benefit. A number of growers have successfully utilised the PreDicta Pt test to assist them in making this assessment.

Expansion of test range

A test for the DNA of *Phytophthora erythroseptica*, the cause of pink rot, has frequently been put forward as a desirable test to develop. A primary reason for this request is that it may be able to directly offset the cost of expensive preventative treatments that are currently applied as an insurance against the disease. Pink rot occurs infrequently, but when it does the losses can be dramatic. A reliable test for the presence or absence of *Phytophthora erythroseptica* would reduce costs and provide environmental benefits from reduced chemical usage. Control of pink rot is currently reliant on the continued effectiveness of a single chemical option. Should resistance to this chemical develop, access to a reliable soil test may become even more important in the future.

There has also been mention of white mold caused by the pathogen *Sclerotinia sclerotiorum* as a possible target. Problems with this disease appear to have increased, but the concern has mainly been raised from fresh market producers.

ASSOCIATED FINDINGS

Field validation resulted in the generation of a large data set comprising pathogen DNA levels in the soil and seed prior to planting and in the peel of harvested daughter tubers along with matching data on the incidence and severity of disease. In some cases post crop pathogen DNA levels in the soil are available. This data provides a powerful resource to look at these pathogens in ways that were not previously possible, and these are presented and discussed in the following section.

INOCULUM LEVELS

SOIL

Utilising the pathogen DNA data set generated during the field validation process the proportion of soil samples in each risk category were determined, Table 4-13.

DNA of *Spongospora subterranea*, *Colletotrichum coccodes, Verticillium dahliae* and *Rhizoctonia solani* AG2.1 were the most frequently detected pathogens.

Tasmania had the highest proportion of soil samples in the high risk category for *Spongospora subterranea*, with Victoria having the highest proportion for *Colletotrichum coccodes*.

South Australia had the highest proportion of samples in the high risk category for *Meloidogyne fallax* and *Meloidogyne hapla*, with few sites at high risk in either Tasmania or Victoria.

DNA of *Rhizoctonia solani* AG3 and *Streptomyces txt*A gene were detected infrequently and generally at low levels in all states. DNA of *Rhizoctonia solani* AG2.1 was frequently detected, but generally at low to medium levels.

Table 4-13 – Percentage of pre-plant soil samples in each pathogen DNA risk category (Includes only the samples taken in South Australia, Tasmania and Victoria as part of field validation process).

Pathogen	Risk category	Soil test log (DNA)	South Australia	Tasmania	Victoria
Spongospora subterranea	Below detection	0	21	18	12
	Low	<1.3	27	9	22
	Medium	1.3 – 1.8	11	5	12
	High	>1.8	41	68	54
Meloidogyne fallax	Below detection	0	43	48	41
	Low	<0.8	17	24	33
	Medium	0.8 – 1.7	18	20	22
	High	>1.7	22	8	4
Meloidogyne hapla	Below detection	0	38	73	85
	Low	<0.8	15	17	9
	Medium	0.8 – 1.7	14	6	5
	High	>1.7	33	3	1
Rhizoctonia solani AG2.1	Below detection	0	43	18	21
	Low	<1.7	46	41	39
	Medium	1.7 – 3.0	9	39	31
	High	>3.0	2	3	10
Rhizoctonia solani AG3	Below detection	0	85	72	68
	Detected	>0	15	28	32
*Streptomyces txt*A gene	Below detection	0	72	83	94
	Low	<2.5	23	14	6
	Medium	2.5 – 3.0	4	2	0
	High	>3.0	1	0	0
Number samples tested			450	180	209

Pathogen	Risk category	Soil test log (DNA)	South Australia	Tasmania	Victoria
Colletotrichum coccodes	Below detection	0	30	16	2
	Low	<0.7	5	5	6
	Medium	0.7 – 1.6	12	4	5
	High	>1.6	53	75	87
Number samples tested			362	100	85
Verticillium dahliae	Below detection	0	32	26	39
	Low	<0.7	37	21	26
	High	>0.7	31	53	35
Number samples tested			299	100	85

PEEL OF SEED TUBERS

Utilising the pathogen DNA data set generated during the field validation process the proportion of seed tubers where DNA in the peel exceeded tentative categories for risk were determined, Table 4-14.

DNA of *Spongospora subterranea*, *Colletotrichum coccodes* and *Rhizoctonia solani* AG3 and AG2.1 were the most frequently detected pathogens in seed tubers. DNA of *Meloidogyne fallax* and *Streptomyces txt*A gene were also commonly detected.

DNA of *Spongospora subterranea* was detected at high levels in 14 to 23% of samples depending on the state.

South Australia had the highest proportion (approximately 10%) of seed peel samples with high levels of *Meloidogyne fallax* and *Meloidogyne hapla*.

DNA of *Rhizoctonia solani* AG3 was frequently detected in the peel of seed samples, particularly from South Australia. High levels were detected in the peel of approximately 20 % of seed samples collected from South Australia and Victoria, but only 5% from samples collected in Tasmania.

DNA of *Streptomyces txt*A gene was frequently detected in the peel of seed samples, and was detected at high levels in 1 to 7% of samples depending on the state.

Table 4-14 – Percentage of peel samples from seed tubers in each pathogen DNA category (Includes only the samples taken in South Australia, Tasmania and Victoria as part of field validation process).

Pathogen	DNA level	Soil test log (DNA)	South Australia	Tasmania	Victoria
Spongospora subterranea	Below detection	0	13	3	10
	Low - medium	< 4	73	80	67
	High	> 4	14	17	23
Meloidogyne fallax	Below detection	0	32	41	60
	Low - medium	< 3	57	57	39
	High	> 3	11	2	1
Meloidogyne hapla	Below detection	0	45	74	78
	Low - medium	< 3	45	25	22
	High	> 3	10	1	0
Rhizoctonia solani AG2.1	Below detection	0	17	14	14
	Low - medium	< 4	78	84	86
	High	> 4	5	2	0
Rhizoctonia solani AG3	Below detection	0	7	42	25
	Low - medium	< 4	70	53	58
	High	> 4	23	5	17
*Streptomyces txt*A gene	Below detection	0	48	31	35
	Low - medium	< 4	45	63	64
	High	> 4	7	5	1
Number samples tested			341	167	146

Pathogen	DNA level	Soil test log (DNA)	South Australia	Tasmania	Victoria
Colletotrichum coccodes	Below detection	0	0	15	0
	Low - medium	< 4	33	65	22
	High	> 4	67	20	78
Number samples tested			341	92	72

INOCULUM BUILDUP

DNA testing has enabled the quantification of pathogen levels based on the measurement of the total amount of the DNA in a sample of soil. This technology allows the build-up of pathogen levels in the soil during a potato crop to be quantified. The mechanisms of pathogen multiplication and disease expression can be quite different. Data was collected on post crop soil levels from 25 paddocks in Tasmania in the 2012/13 growing season. This limited data on the changes in DNA levels in the soil before and after a potato crop has demonstrated differences in inoculum buildup dependant on the pathogen, paddock and variety grown, along with the role of seed in pathogen introduction.

SPONGOSPORA SUBTERRANEA

Resting spores of the pathogen *Spongospora subterranea* are produced on potato crops and provide a source of inoculum to infect future crops. Resting spores may be produced from the pustules on the tubers or from galls on the roots. Varieties can differ in their susceptibility to tuber and root galling symptoms and the two though sometime related, are not always (Falloon, Genet, Wallace, & Butler, 2003). Some resting spores will also remain dormant in the soil from prior to the crop being planted.

Due to the multiple sources from which *Spongospora subterranea* resting spores can be left in the soil after a crop, the incidence of powdery scab on the tubers alone is not a good indication of the post-crop level of inoculum in the soil, Figure 4-41. For example high levels of *Spongospora subterranea* DNA were found in the soil at harvest after crops of Russet Burbank and Topcat, even though the incidence of diseased tubers on the crop was low or nil.

At sampling points where high levels of *Spongospora subterranea* DNA were measured in the soil prior to planting, levels were generally high or higher in the soil after the crop was harvested, irrespective of the variety grown, Figure 4-42. Where *Spongospora subterranea* DNA was not detected in the soil prior to planting, levels ranged from not detected to high in the soil after the crop was harvested. This data suggests that a high level of inoculum in the soil can be built up after one potato crop if the conditions are favourable, a finding that was confirmed by annual monitoring of *Spongospora subterranea* DNA in paddocks over an eight year period (see Chapter 5.A II). Inoculum may be introduced into paddocks that are free of *Spongospora subterranea* on seed tubers. *Spongospora subterranea* DNA was detected in the soil after the crop had been harvested in all but one of the sampling points. This was the only sampling point where *Spongospora subterranea* DNA was not detected either in the soil prior to planting or on the seed tubers planted. In other cases where seed was most likely the only source of inoculum, the actual

level of *Spongospora subterranea* DNA measured in the soil after the crop had been harvested appeared unrelated to the level measured on the seed, Figure 4-43. Though the number of sampling points was limited, other factors such as the variety grown, soil moisture and temperature may be more important in determining the level of buildup that occurs in one season.

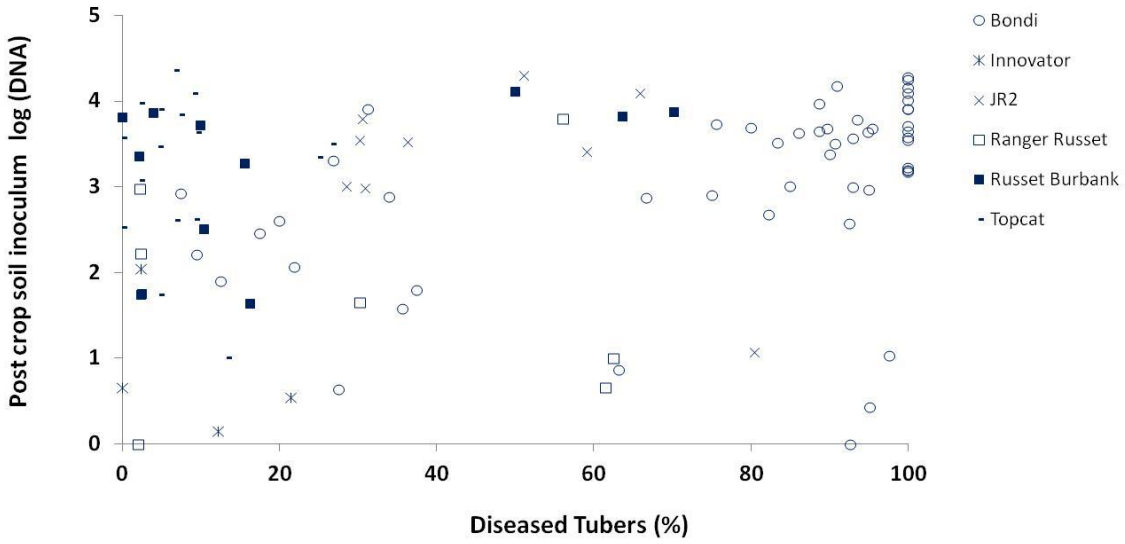

Figure 4-41 – Relationship between incidence of powdery scab on tubers and the level of *Spongospora subterranea* DNA in the soil at harvest depending on variety grown in Tasmania.

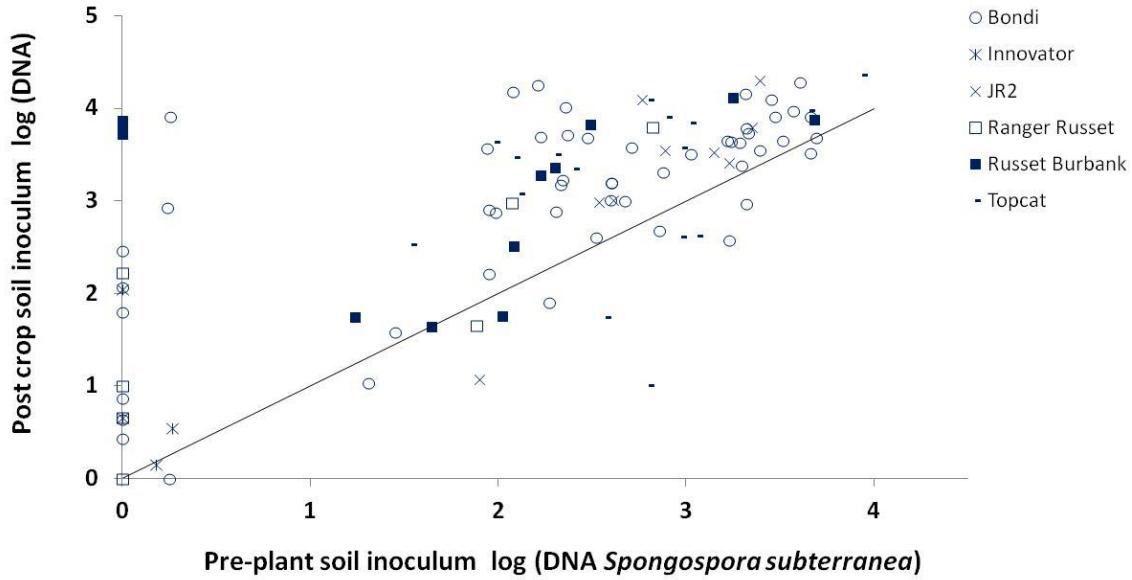

Figure 4-42 – Relationship between levels of *Spongospora subterranea* DNA in the soil prior to planting and the level of *Spongospora subterranea* DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

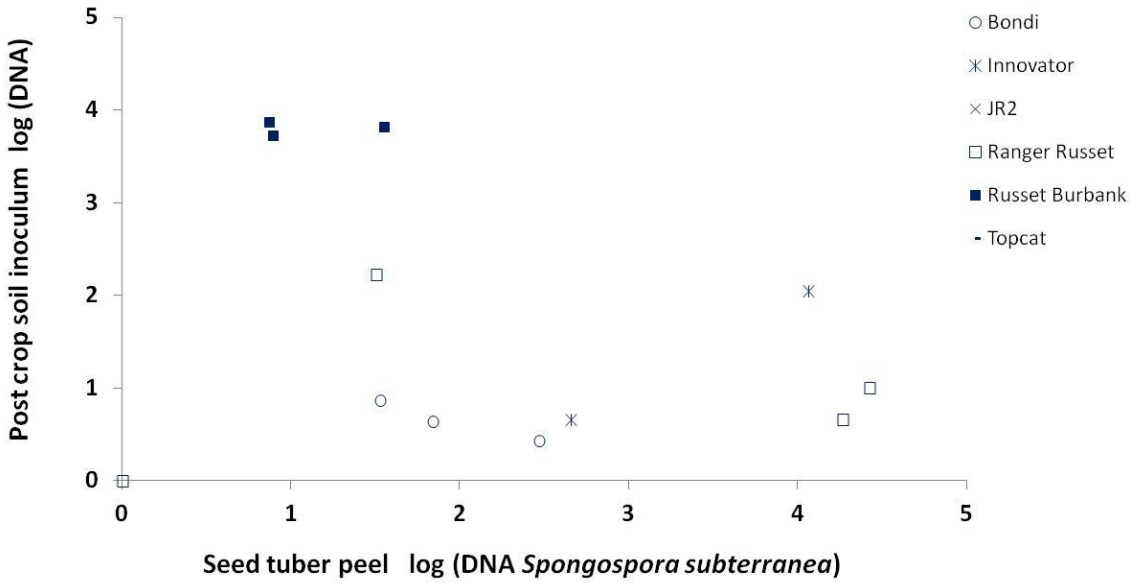

Figure 4-43 – Relationship between levels of *Spongospora subterranea* DNA in peel of seed tubers and the level of *Spongospora subterranea* DNA in the soil at harvest when potatoes grown at locations where *Spongospora subterranea* DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

MELOIDOGYNE FALLAX

Potatoes are a preferred host of *Meloidogyne fallax*, and the growing of a potato crop would be expected to result in an increased nematode population in the soil (Hay, et al., 2013). At sampling points where high levels of *Meloidogyne fallax* DNA were measured in the soil prior to planting, levels were generally as high or higher in the soil after the crop was harvested, irrespective of the variety grown, Figure 4-44. Where *Meloidogyne fallax* DNA was not detected in the soil prior to planting, levels in the soil after the crop was harvested ranged from below the level of detection to moderate. This data suggests that under Tasmanian conditions, more than one crop of potatoes is required for *Meloidogyne fallax* levels in the soil to build to those which pose a high risk of root-knot nematode damage to a subsequent crop of potatoes. This may not be the case in regions where environmental conditions are more conducive to root-knot nematode such as in the SE of South Australia.

Inoculum may be introduced into paddocks that are free of *Meloidogyne fallax* by the planting of infested seed tubers. At a number of sampling points where *Meloidogyne fallax* DNA was not detected in the soil prior to planting, *Meloidogyne fallax* DNA was detected at low to moderate levels in the soil after the crop was harvested. Unfortunately at the sampling points that showed the highest levels in the soil after the crop, the Bondi seed lots planted were not tested. Data from other sampling points did not provide any strong evidence that seed of the quality tested contributes to the buildup of high levels of *Meloidogyne fallax* DNA in the soil by the end of single potato cropping cycle in Tasmania, Figure 4-45. At two sampling points in separate paddocks Topcat potato crops left a level in the soil that posed a moderate risk to a subsequent crop. Though *Meloidogyne fallax* DNA was not detected in the soil prior to planting at either of

these sampling points, *Meloidogyne fallax* DNA was detected at low to moderate levels in other sampling points in each of these paddocks prior to planting, suggesting soil inoculum may have been the source.

Incidence of root-knot nematode damage is low in Tasmania. This lack of symptoms makes it difficult to determine if the incidence of root-knot nematode damage on tubers is a good indication of the post crop level of inoculum in the soil, Figure 4-46. However, as the level in the soil after the crop was frequently as high from sampling points that showed no symptoms as those that did, it is unlikely to be a reliable indicator of *Meloidogyne fallax* level in the soil or risk to future crops.

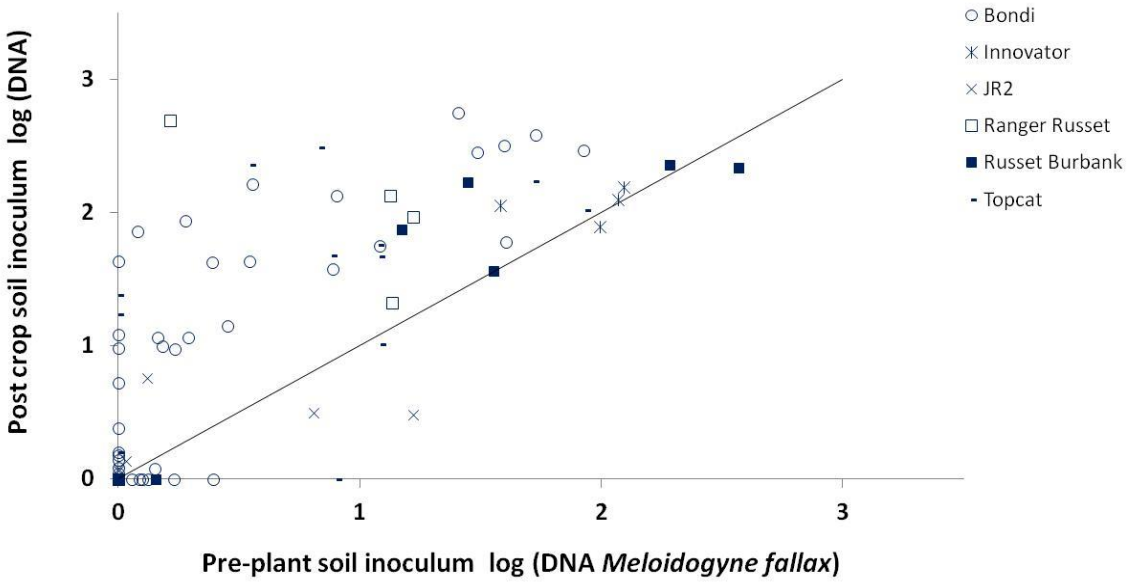

Figure 4-44 – Relationship between levels of *Meloidogyne fallax* DNA in the soil prior to planting and the level of *Meloidogyne fallax* DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

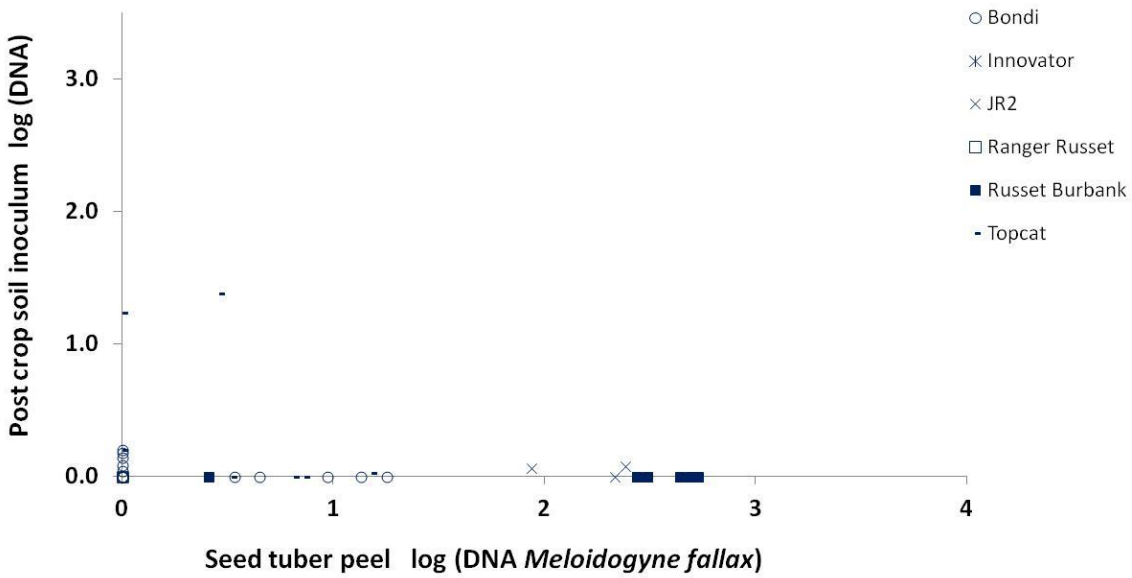

Figure 4-45 – Relationship between levels of *Meloidogyne fallax* DNA in peel of seed tubers and the level of *Meloidogyne fallax* DNA in the soil at harvest when potatoes grown at locations where *Meloidogyne fallax* DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

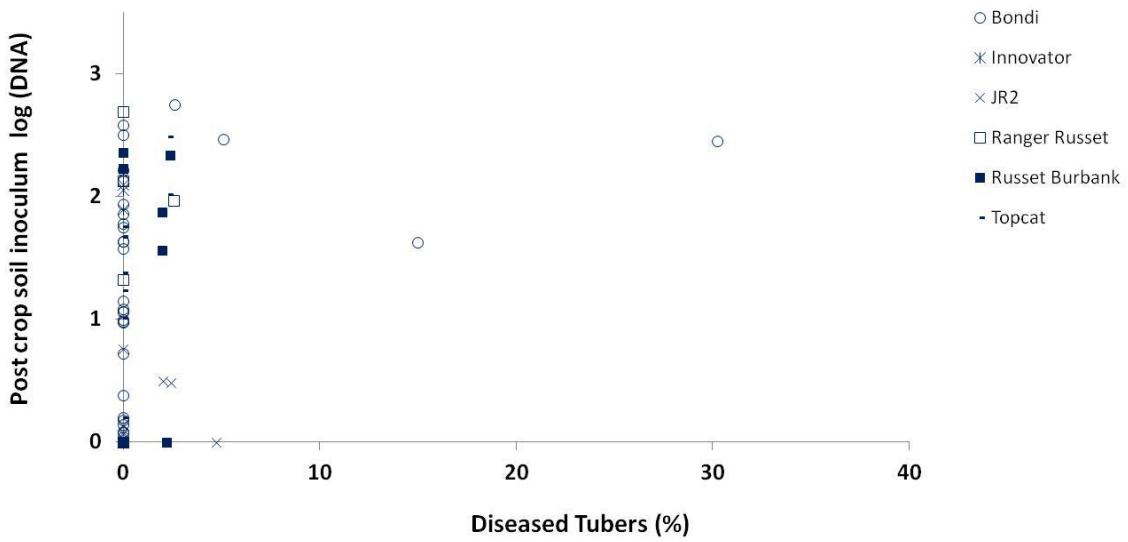

Figure 4-46 – Relationship between incidence of root-knot nematode damage on tubers and the level of *Meloidogyne fallax* DNA in the soil at harvest depending on variety grown in Tasmania.

MELOIDOGYNE HAPLA

Potatoes are a host of *Meloidogyne hapla*, and the growing of a potato crop would be expected to result in an increased nematode population in the soil (Hay, et al., 2013). In Tasmania there was not a strong

relationship between the level of *Meloidogyne hapla* DNA in the soil prior to planting and that measured after harvest, Figure 4-47. In some cases the level of *Meloidogyne hapla* DNA measured in the soil increased, but in almost as many cases the level dropped. This data suggests that factors other than the growing of the potato crop are having a substantial effect on the multiplication and survival of *Meloidogyne hapla* in the soil, and that potato may not be a strong host of *Meloidogyne hapla* under conditions in Tasmania. This is despite *Meloidogyne hapla* infesting potato tubers as evidenced by the detection of moderate levels of *Meloidogyne hapla* DNA in the peel of tubers.

Inoculum may be introduced into paddocks that are free of *Meloidogyne hapla* by the planting of infested seed tubers. At a number of sampling points where *Meloidogyne hapla* DNA was not detected in the soil prior to planting, *Meloidogyne fallax* DNA was detected at low to moderate levels in the soil after the crop was harvested. The data does not provide any strong evidence that seed of the quality tested contributes to the buildup of high levels of *Meloidogyne hapla* in the soil by the end of single potato cropping cycle in Tasmania, Figure 4-48.

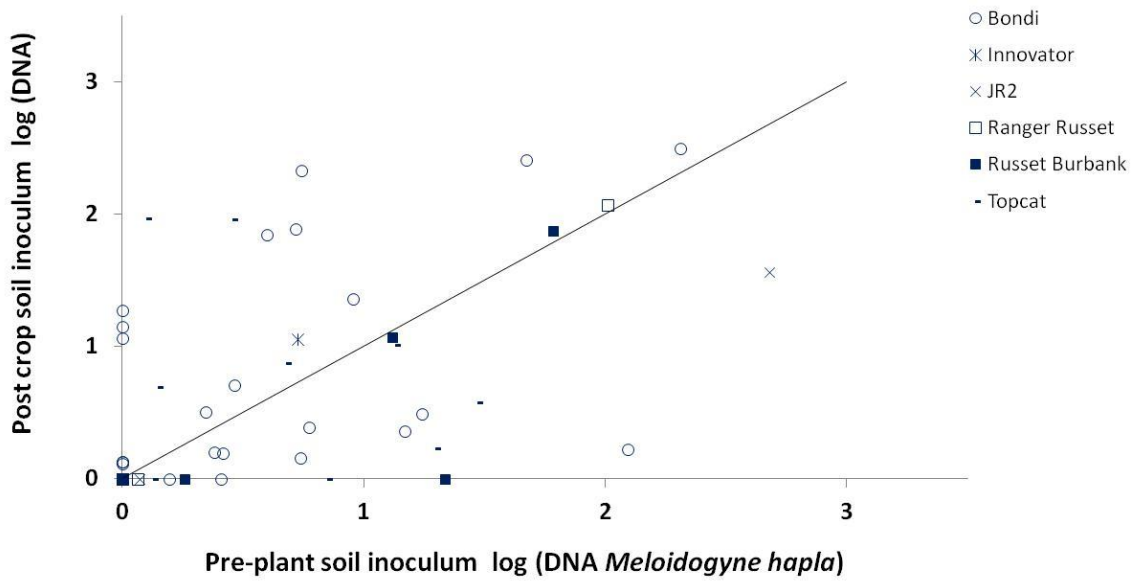

Figure 4-47 – Relationship between levels of *Meloidogyne hapla* DNA in the soil prior to planting and the level of *Meloidogyne hapla* DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

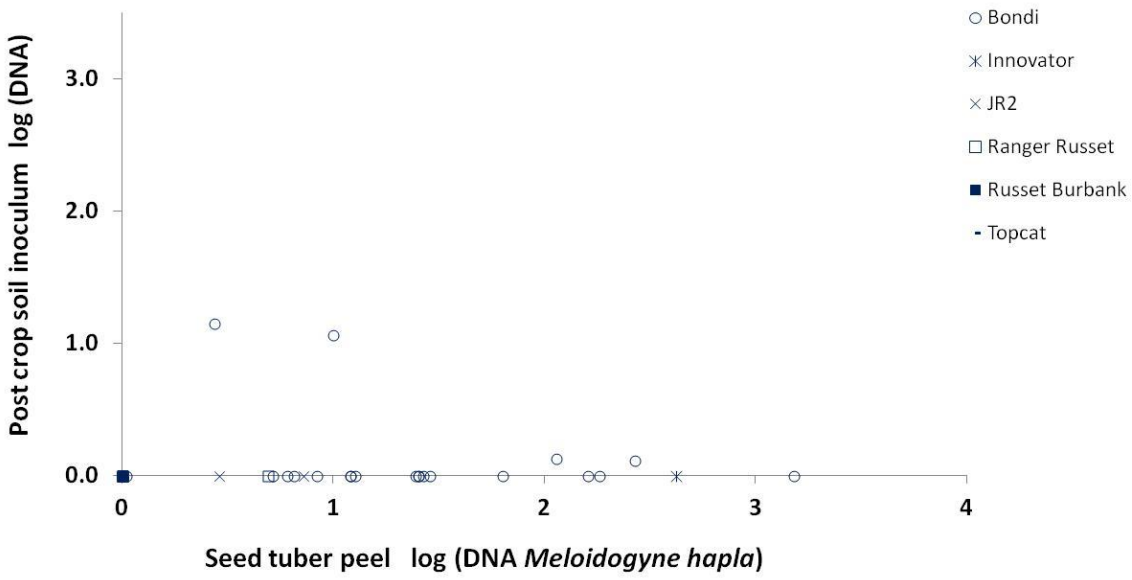

Figure 4-48 – Relationship between levels of *Meloidogyne hapla* DNA in peel of seed tubers and the level of *Meloidogyne hapla* DNA in the soil at harvest when potatoes grown at locations where *Meloidogyne hapla* DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

PATHOGENIC *STREPTOMYCES*

*Streptomyces txt*A gene DNA is only detected in the soil at a small percentage of samples taken prior to planting. In Tasmania, soils were sampled after crop senescence. There does not appear to be any relationship between the level of *Streptomyces txt*A gene DNA in the soil prior to planting and that measured after harvest, Figure 4-49. In some cases *Streptomyces txt*A gene DNA was detected in the soil prior to planting, but not in the soil at harvest. This data suggests that factors other than the growing of the potato crop are having a substantial effect on the multiplication and survival of pathogenic *Streptomyces* in the soil.

The presence of symptoms on harvested tubers was not a good indication of the level of *Streptomyces txt*A gene DNA that was detected in the soil at harvest, Figure 4-50. A high incidence of common scab frequently occurred at sampling points where *Streptomyces txt*A gene DNA was not detected in the soil at harvest and vice versa. Similarly, high levels of *Streptomyces txt*A gene DNA were frequently detected in the peel of harvested tubers where *Streptomyces txt*A gene DNA was not detected in the soil at harvest, Figure 4-51. However, when high levels of *Streptomyces txt*A gene DNA were detected in the soil at harvest, these were associated with high levels in the *Streptomyces txt*A gene DNA in the peel of harvested tubers.

As for soil, there was not a strong relationship between the level of *Streptomyces txt*A gene DNA on the seed prior to planting and that measured after harvest, Figure 4-52. In only a few cases the level of *Streptomyces txt*A gene DNA measured in the soil increased. The data does not provide any strong evidence that seed contributed to the buildup of high levels of pathogenic *Streptomyces* in the soil by the end of single potato cropping cycle in Tasmania.

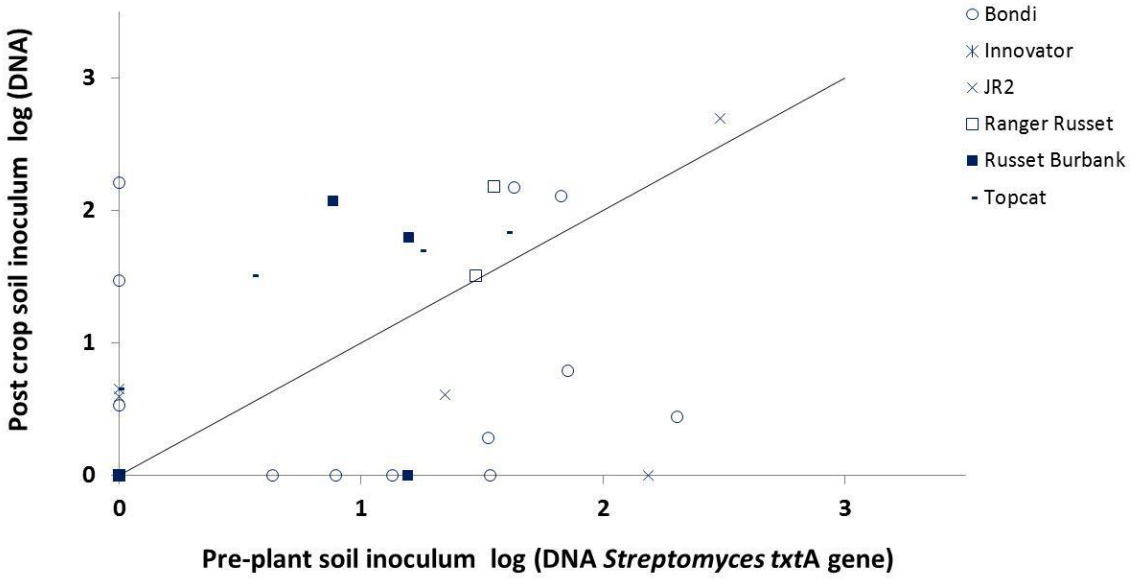

Figure 4-49 – Relationship between levels of *Streptomyces txt*A gene DNA in the soil prior to planting and the level of *Streptomyces txt*A gene DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

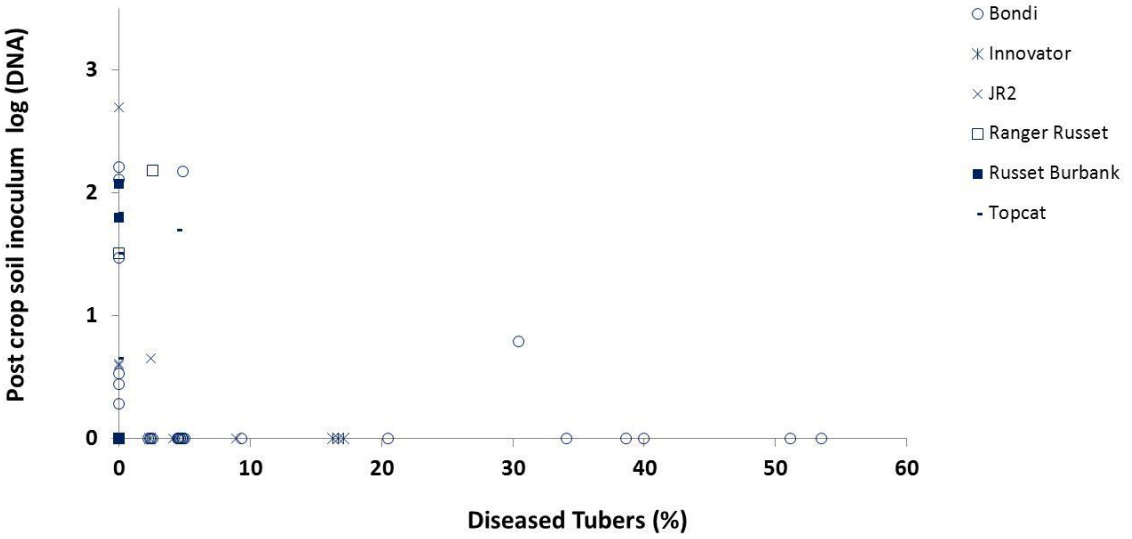

Figure 4-50 – Relationship between incidence of common scab on tubers and the level of *Streptomyces txt*A gene DNA in the soil at harvest depending on variety grown in Tasmania.

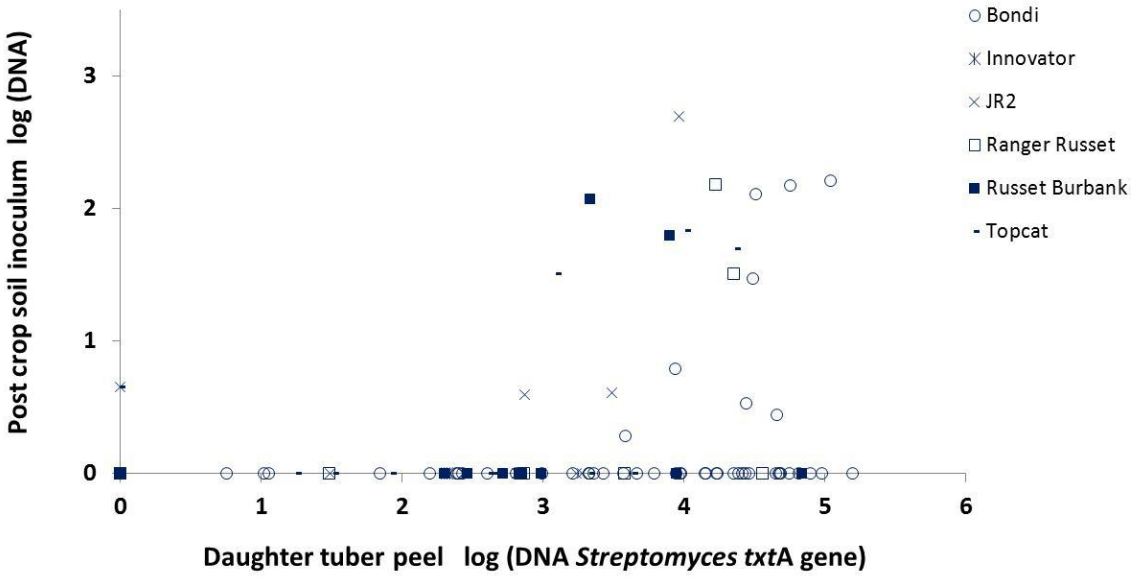

Figure 4-51 – Relationship between levels of *Streptomyces txt*A gene DNA in the peel of harvested tubers and the level of *Streptomyces txt*A gene DNA in the soil at harvest depending on variety grown in Tasmania.

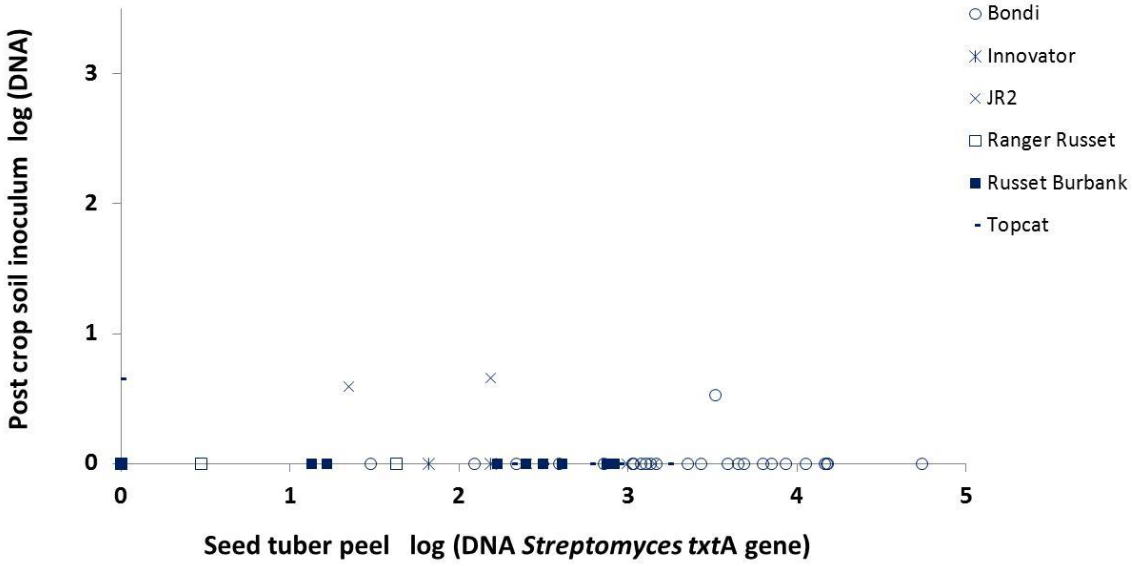

Figure 4-52 – Relationship between levels of *Streptomyces txt*A gene DNA in the peel of seed tubers and the level of *Streptomyces txt*A gene DNA in the soil at harvest when potatoes grown at locations where *Streptomyces txt*A gene DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

RHIZOCTONIA SOLANI AG3

Potatoes are a host of *Rhizoctonia solani* AG3 (Woodhall, Lees, Edwards, & Jenkinson, 2008), and the growing of a potato crop could be expected to result in an increased inoculum level in the soil. Black scurf symptoms on the surface of tubers are the melanised sclerotia of *Rhizoctonia solani*, mostly AG3, which is the long term survival phase of this pathogen in the soil (Lehtonen, Wilson, Ahvenniemi, & Valkonen, 2009). Black scurf on the harvested tubers might be expected to indicate that a high level of inoculum would be present in the soil after harvest. In Tasmania the incidence of black scurf was not a good indication of the post-crop level of *Rhizoctonia solani* AG3 DNA in the soil, Figure 4-53. In some cases DNA of *Rhizoctonia solani* was not detected in soil samples taken after harvest from sampling points where black scurf was found on the tubers. Detection of black scurf would indicate that *Rhizoctonia solani* was present. The incidence of black scurf did not exceed 16% at any of the sites assessed in Tasmania, which was much lower than in Victoria and South Australia where the incidence of black scurf frequently exceeded 50%. As high levels of black scurf did not occur at the sites assessed in Tasmania, it is unclear if higher levels of black scurf would result in a high level of *Rhizoctonia solani* AG3 DNA in the soil after harvest.

Rhizoctonia solani AG3 DNA was only detected in the soil in a small percentage of samples taken prior to planting. In Tasmania the relationship between the level of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and that measured after harvest was poor, Figure 4-54. Mostly the level of *Rhizoctonia solani* AG3 DNA measured in the soil increased as the result of growing a potato crop, but in some cases the level dropped. This data suggests that factors other than the growing of a potato crop are having a substantial effect on the level of *Rhizoctonia solani* AG3 DNA in the soil after harvest.

Detection of *Rhizoctonia solani* AG3 DNA in the soil after crop senescence at sampling points where it was not detected in the soil prior to planting suggests that it was either introduced on seed tubers or has built up from levels in the soil that were not detected by pre-plant sampling. Inoculum may be introduced into paddocks that are free of *Rhizoctonia solani* AG3 DNA by the planting of infected seed tubers (Tsror L. , 2010). Though seed tubers can introduce *Rhizoctonia solani* AG3 there does not appear to be any relationship between the level of *Rhizoctonia solani* AG3 DNA in the peel of seed tubers and the level of *Rhizoctonia solani* AG3 DNA in the soil at harvest, Figure 4-55. *Rhizoctonia solani* AG3 DNA was detected in the soil at harvest at a number of sampling points where it was not detected in the soil prior to planting or in the peel of seed tubers. It is likely that in some cases inoculum existed in the soil prior to planting that was not detected and in other cases was present on seed tubers planted at the sampling point, but not the seed tubers sampled for testing.

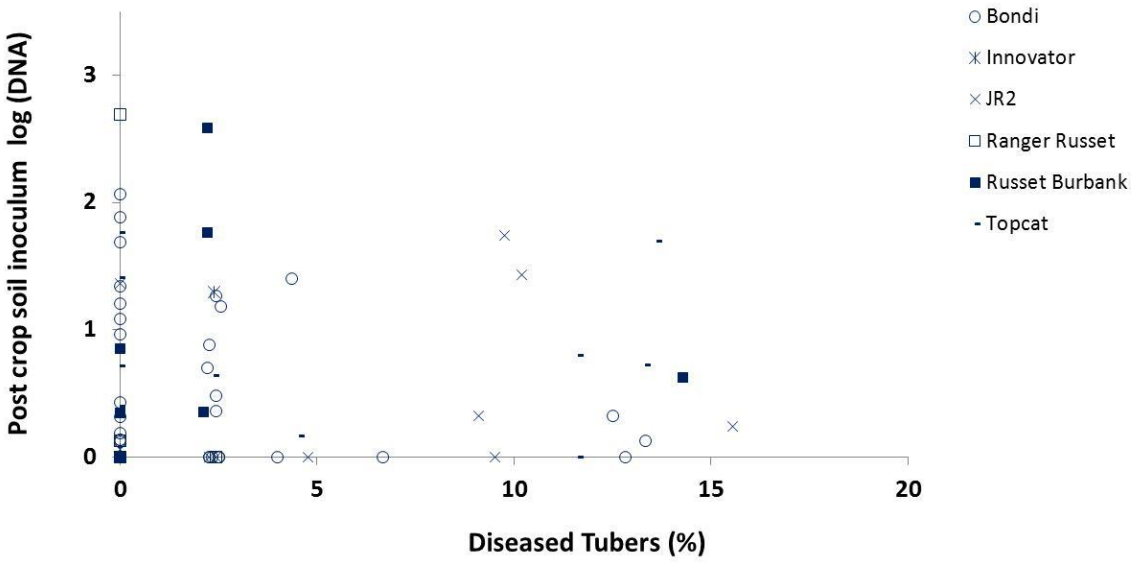

Figure 4-53 – Relationship between incidence of black scurf on tubers and the concentration of *Rhizoctonia solani* AG3 DNA in the soil at harvest depending on variety grown in Tasmania.

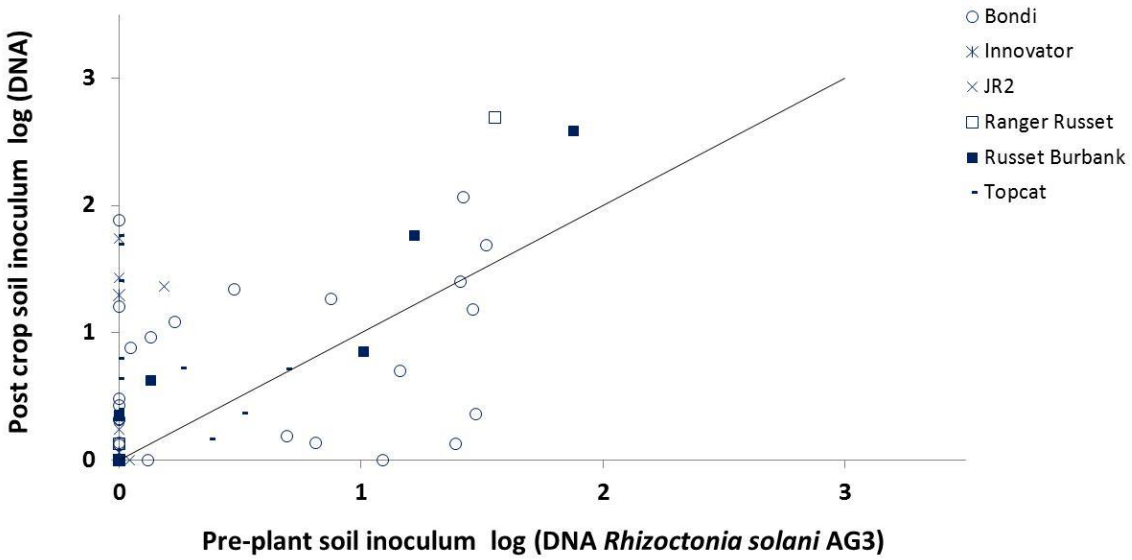

Figure 4-54 – Relationship between concentration of *Rhizoctonia solani* AG3 DNA in the soil prior to planting and the concentration of *Rhizoctonia solani* AG3 DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

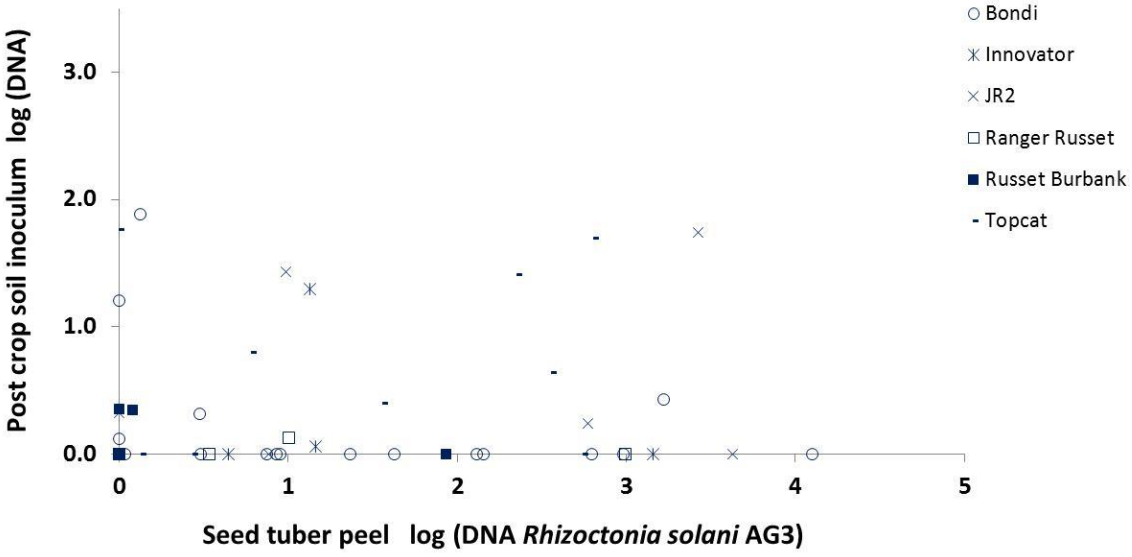

Figure 4-55 – Relationship between concentration of *Rhizoctonia solani* AG3 DNA in the peel of seed tubers and the concentration of *Rhizoctonia solani* AG3 DNA in the soil at harvest when potatoes grown at locations where *Rhizoctonia solani* AG3 DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

RHIZOCTONIA SOLANI AG2.1

In Tasmania there was not a strong relationship between the level of *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting and that measured at harvest, Figure 4-56. In some cases the level of *Rhizoctonia solani* AG2.1 in the soil was greater at harvest, but in almost as many cases the level dropped. This data suggests that factors other than the growing of a potato crop are having a substantial effect on changes in the level of *Rhizoctonia solani* AG2.1 DNA found in the soil at harvest.

At sampling points where *Rhizoctonia solani* AG2.1 DNA was not detected in the soil prior to harvest, planting seed infested with *Rhizoctonia solani* AG2.1 introduced the pathogen and frequently resulted in detectable levels of *Rhizoctonia solani* AG2.1 DNA in the soil at harvest, Figure 4-57. DNA of *Rhizoctonia solani* AG2.1 was not detected at the two sampling points where it was not detected in the soil prior to planting or in the peel of seed tubers.

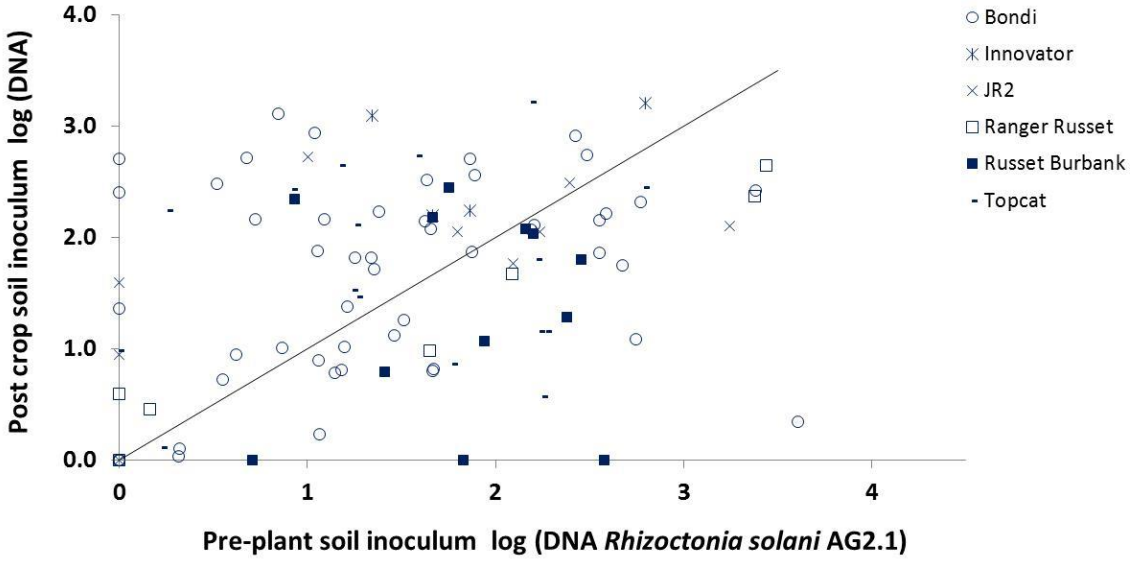

Figure 4-56 – Relationship between concentration of *Rhizoctonia solani* AG2.1 DNA in the soil prior to planting and the concentration of *Rhizoctonia solani* AG2.1 DNA in the soil at harvest depending on variety grown in Tasmania. Line represents 1:1 relationship.

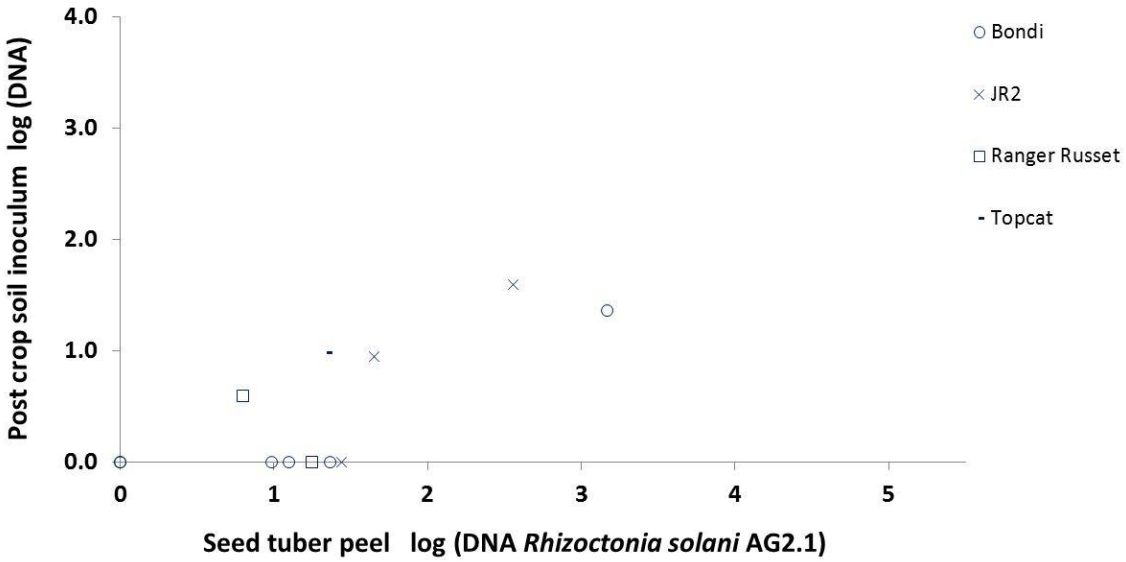

Figure 4-57 – Relationship between concentration of *Rhizoctonia solani* AG2.1 DNA in the peel of seed tubers and the concentration of *Rhizoctonia solani* AG2.1 DNA in the soil at harvest when potatoes grown at locations where *Rhizoctonia solani* AG2.1 DNA was not detected in the soil prior to planting, depending on variety grown in Tasmania.

SOIL DNA TEST DEVELOPMENT – POTATO EARLY DYING

Potato early dying is an interaction between Verticillium wilt and root lesion nematodes. *Verticillium dahliae*, *Pratylenchus crenatus*, *Pratylenchus penetrans* and *Pratylenchus neglectus* were identified as the primary target pathogens on which to base an early dying DNA pre-plant soil test to assist in the risk management of potato early dying.

ASSAY DEVELOPMENT

VERTICILLIUM DAHLIAE

The sequences used to design the primers and probe to detect *Verticillium dahliae* were generated by SARDI prior to this project, Table 4-15.

In previous work conducted by SARDI in a RIRDC funded project SAR 15-A 'Development of DNA based diagnostic probes for Verticillium', DNA was extracted from over 40 Verticillium isolates representing *Verticillium dahliae*, *V. albo-atrum*, *V. tricorpus*, *V. lecanii*, *V. nubileum*, *V. fungicola*. Genetic analysis of the intragenic transcribed spacer (ITS) region of the 18S ribosomal DNA was undertaken.

Sequence data in the 18S ribosomal gene region was obtained for 30 Verticillium isolates. The data indicated that *Verticillium dahliae* and *V. albo-atrum* are clearly distinct groups, based on genetic similarity in the intragenic transcribed spacer (ITS) region of the 18S ribosomal DNA.

DNA probe sequences have been designed which distinguish the *Verticillium dahliae* and *V. albo-atrum*. The probes were designed for use in a hybridisation assay format. In the APRP project, they were re-designed for use in the TaqMan® MGB assay (real-time qPCR) format.

Table 4-15 – Sequences of the primers and probe for the *Verticillium dahliae* assay.

Forward primer VdF	5' ATAACCCTTTGTGAACCATATTGTTG 3'
Reverse primer VdR	5' CACTCAGAAGTATCGTTGGTATAAACAG 3'
Probe (TaqMan MGB, FAM labelled)	5' AACGAGCCGCCGA 3'

PRATYLENCHUS CRENATUS

A quantitative, real-time PCR assay (TaqMan® MGB) was designed for *Pratylenchus crenatus* based on rDNA D3 domain sequences available in Genbank. New sequences were obtained from a *Pratylenchus crenatus* isolated in Ohio, USA, and added to the available sequences.

Several primer and probe sets were designed. In total, 2 forward and 3 reverse primers were tested in combination with one probe for their sensitivity to detect *Pratylenchus crenatus* DNA extracted from a single nematode. The primer/probe combination giving the best result was selected, Table 4-16.

Table 4-16 – Sequences of the primers and probe for the *Pratylenchus crenatus* assay.

Forward primer F PcF	5' AAAGTGAAGAAGGCGGCTTG 3'
Reverse primer R PcR	5' TCAGGAATAGTTCACCATCTTTCG 3'
Probe (TaqMan MGB, FAM labelled)	5' CTATCCCGATTGCTTG 3'

TEST SPECIFICITY/SENSITIVITY

VERTICILLIUM DAHLIAE

The specificity of the *Verticillium dahliae* assay was assessed against closely related *Verticillium* species including *Verticillium albo-atrum*, Table 4-17. The assay specifically and consistently detected *Verticillium dahliae*. The very low detection of *Verticillium lecanii* was shown to be due to a contamination by *Verticillium dahliae*.

Table 4-17 – Specificity assessment of the *Verticillium dahliae* qPCR assay against a collection of *Verticillium* species.

Species Name	Number of isolates tested	Detection
Verticillium albo-atrum	5	-
Verticillium dahliae	37	+
Verticillium lecanii	1	+/-
Verticillium nigrescens	2	-

The assay is sensitive enough to detect as little as 2fg/ul of pure *Verticillium dahliae* DNA and successfully detects *Verticillium dahliae* in soil samples.

PRATYLENCHUS CRENATUS

The specificity of the selected assay was checked, using a collection of DNA extracted from various nematode species, including several *Pratylenchus* species (*P. penetrans*, *P. neglectus*, *P. thornei*, *P. teres* and *P. zeae*) as well as other pathogenic and non-pathogenic nematodes, Table 4-18. The newly designed assay specifically detects *Pratylenchus crenatus* but none of the other nematode species tested, particularly none of the *Pratylenchus* species, which are the most closely related to *Pratylenchus crenatus*.

Table 4-18 – Specificity assessment of the *Pratylenchus crenatus* qPCR assay against a collection of nematode species.

Species name	Number of isolates tested	Detection
Pratylenchus penetrans	2	-
Pratylenchus neglectus	9	-
Pratylenchus thornei	2	-
Pratylenchus zeae	1	-
Pratylenchus teres	1	-
Meloidogyne fallax	1	-
Meloidogyne hapla	1	-
Meloidogyne arenaria	1	-
Meloidogyne incognita	1	-
Meloidogyne javanica	1	-
Heterodera avenae	1	-
Heterodera trifolii	1	-
Ditylenchus dipsaci	1	-

Species name	Number of isolates tested	Detection
Dorylaimids	2	-
Fungal Feeding Aphelenchs	1	-
Fungal Feeding Tylenchs	1	-
Fungal Feeding Ditylenchs	1	-
Bacterial Feeding Cephalobes	1	-
Bacterial Feeding Acrobeles	1	-
Mononchids	1	-
Pratylenchus crenatus	18	+
Ntc	NA	ND

The assay is sensitive enough to detect DNA extracted from a single *Pratylenchus crenatus* nematode. To validate the ability of the assay to detect *Pratylenchus crenatus* in soil, 14 soil samples were collected from potato fields. Samples were split, one half being examined visually for *Pratylenchus crenatus* and the other half being processed for DNA extraction and qPCR. The assay successfully detected *Pratylenchus crenatus* DNA in 32 of 34 samples in which *Pratylenchus crenatus* was visually identified. Of the 2 samples where *Pratylenchus crenatus* was not detected by qPCR one had a low visual count and *Pratylenchus penetrans* was present in the other which could have led to misidentification.

CALIBRATION

VERTICILLIUM DAHLIAE

A calibration standard was prepared using pure *Verticillium dahliae* DNA. A 10 fold-dilution range (from 200000 down to 2fg DNA/ul) was prepared and tested using the qPCR assay. This allows the correlation of Ct values with the amount of *Verticillium dahliae* DNA, Table 4-19. The assay was shown to have an efficiency of 89%.

Table 4-19 – Calibration standard for the *Verticillium dahliae* assay using pure genomic DNA

DNA concentration (fg/ul)	Ct value
2	35.62
20	31.99
200	28.35
2000	24.72
20000	21.09
200000	17.46

PRATYLENCHUS CRENATUS

It was not possible to source enough pure *Pratylenchus crenatus* DNA to prepare a calibration standard. A synthetic piece of DNA, with the exact same sequence as the qPCR product (synthetic oligonucleotide) was used instead. A 10 fold dilution range of the synthetic oligonucleotide (from 2000000 down to 2copies/ul) was used to correlate the Ct values to the number of copies of the PCR product, Table 4-20. Using this standard, the assay was shown to have an efficiency of 95.6%.

Field soil samples collected in Tasmania were tested to correlate the qPCR (copy number) results to the number of nematodes per sample determined by manual counting. Specific samples were sourced from Tasmania for the calibration as they had no detectable levels of *Pratylenchus penetrans*, *P. neglectus* or *P. thornei* as determined by DNA pathogen testing. Ten 800g soil samples were split in 4 x 200g aliquots. Two aliquots of each sample were dried for DNA extraction and PCR. The 2 other aliquots of each sample were placed in the mister. Nematodes were collected and numbers of each species were counted. The regression of the copy number vs nematode number gives a tentative equivalent of approximately 88,000 copies per *Pratylenchus crenatus*, Figure 4-58. Further testing is required to confirm this calibration.

Table 4-20 – Calibration standard for the *Pratylenchus crenatus* assay using synthetic oligonucleotide

No of copies/ul	Ct value
2	33.38
20	29.97
200	26.57
2000	23.16
20000	19.76
200000	16.35
2000000	12.92

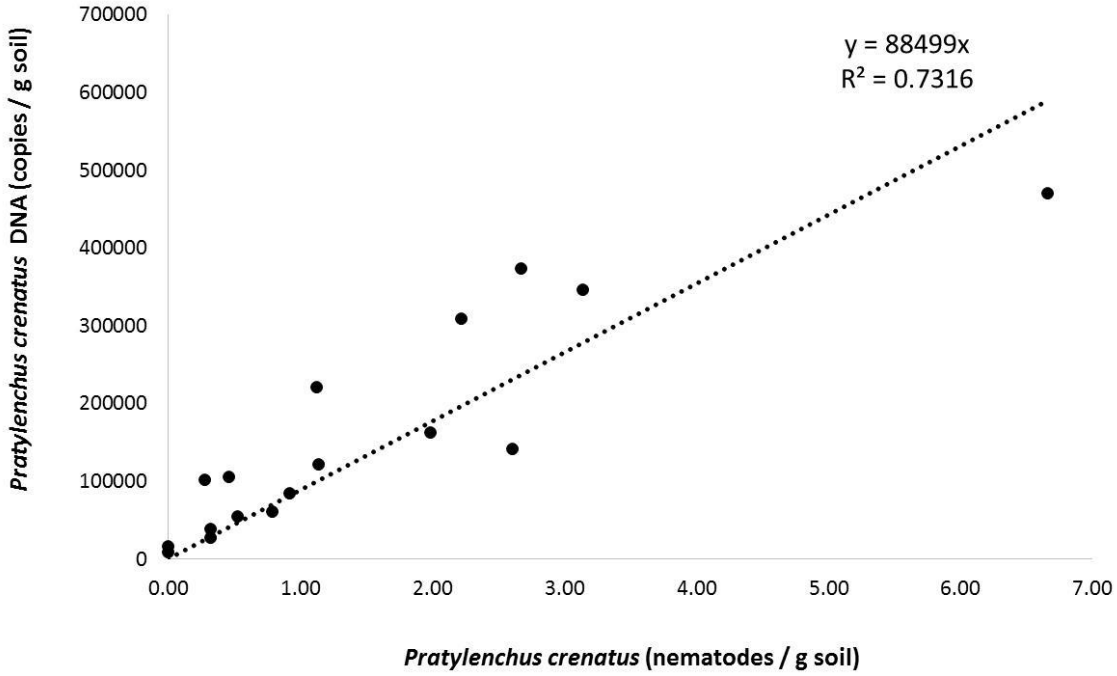

Figure 4-58 – Relationship between DNA *Pratylenchus crenatus* (copies / g soil) and manual count of *Pratylenchus crenatus* (nematodes / g soil) extracted on a mister.

FIELD VALIDATION

INTRODUCTION

Field validation to provide interpretation of soil DNA test results for *Verticillium dahliae*, *Pratylenchus crenatus*, *Pratylenchus neglectus* and *Pratylenchus penetrans* in relation to the risk of potato early dying was conducted. At this stage data has only been collected from a limited number of samples over two growing seasons. Soil DNA test results for *Verticillium dahliae* have been included on the current PreDicta Pt report as a "Test Under Development". Test results are a measure of the total level of DNA of *Verticillium dahliae* in the soil prior to planting. A putative level of 0.7 log (pg *Verticillium dahliae* DNA / g soil + 1) measured prior to planting has been established for the high risk threshold for Verticillium wilt. DNA results for the root lesion nematodes *Pratylenchus neglectus* and *Pratylenchus penetrans* have been included in the PreDicta Pt report, with *Pratylenchus crenatus* in the process of being added.

VERTICILLIUM DAHLIAE

Field validation in commercial crops grown in South Australia and Tasmania has demonstrated a good relationship between the level of *Verticillium dahliae* DNA in the soil prior to planting and in the peel of harvested tubers, Figure 4-59. In paddocks where four samples were taken, the relationship of paddocks means is stronger, Figure 4-59ab. In paddocks where *Verticillium dahliae* DNA was not detected prior to planting, levels of *Verticillium dahliae* DNA in the harvested tubers were either low or not detected. The relationship found between the level of *Verticillium dahliae* DNA in the soil prior to planting and in the peel of harvested tubers was similar in South Australia and Tasmania, Figure 4-60.

The strength of these relationships is comparable to those found for other pathogens where soil DNA testing prior to planting provides a useful indication of disease risk. However caution is required in interpreting these results as data on other pathogens has demonstrated that high levels of pathogen DNA in the peel of harvested tubers does not always result in disease expression.

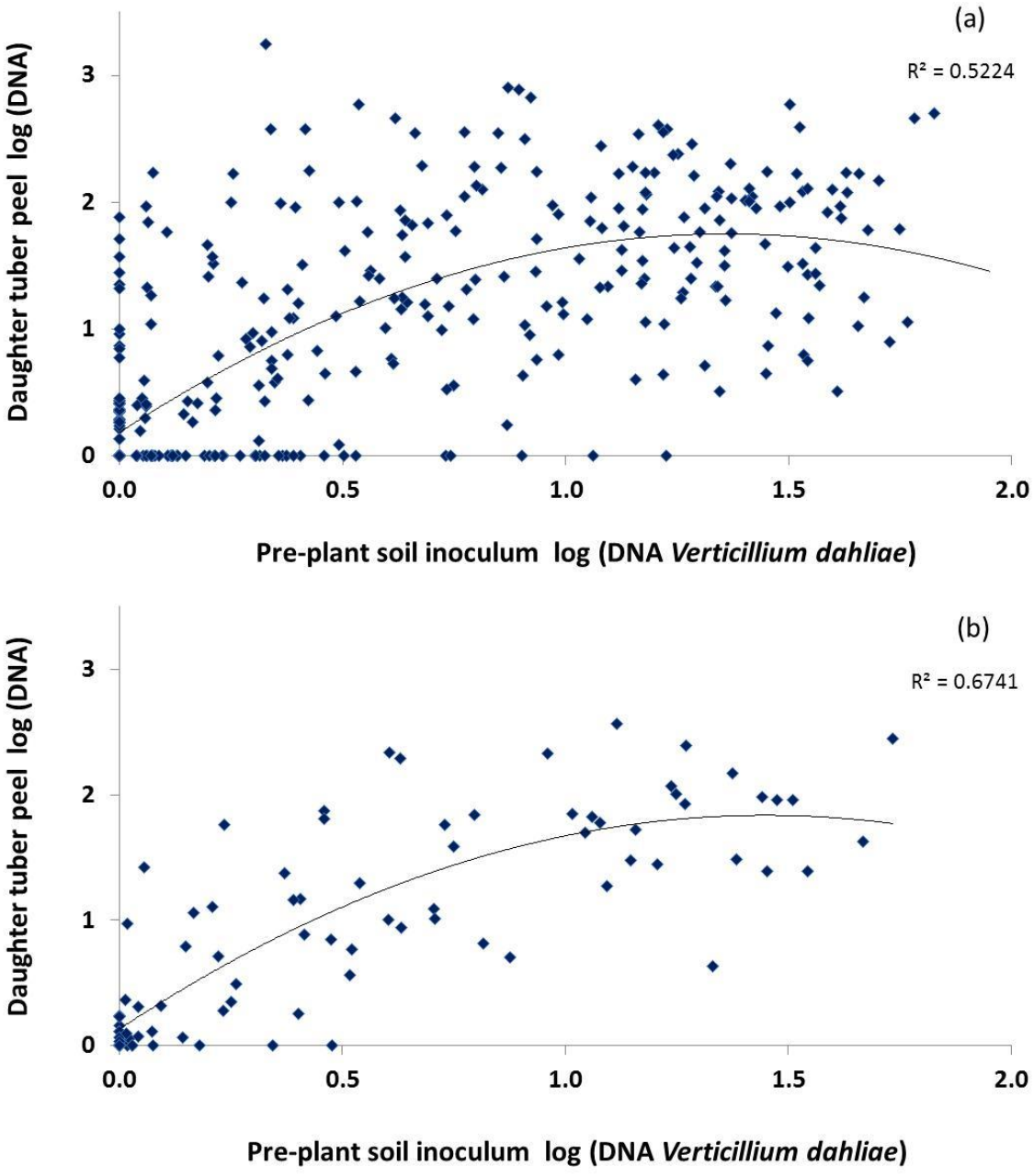

Figure 4-59 – Relationship between levels of *Verticillium dahliae* DNA in the soil prior to planting and in the peel of harvested tubers for a) Individual sampling points and b) paddock means of 4 sampling points (Combined data from South Australia in 2011/12 and 2012/13 growing seasons and Tasmania in 2012/13 growing season).

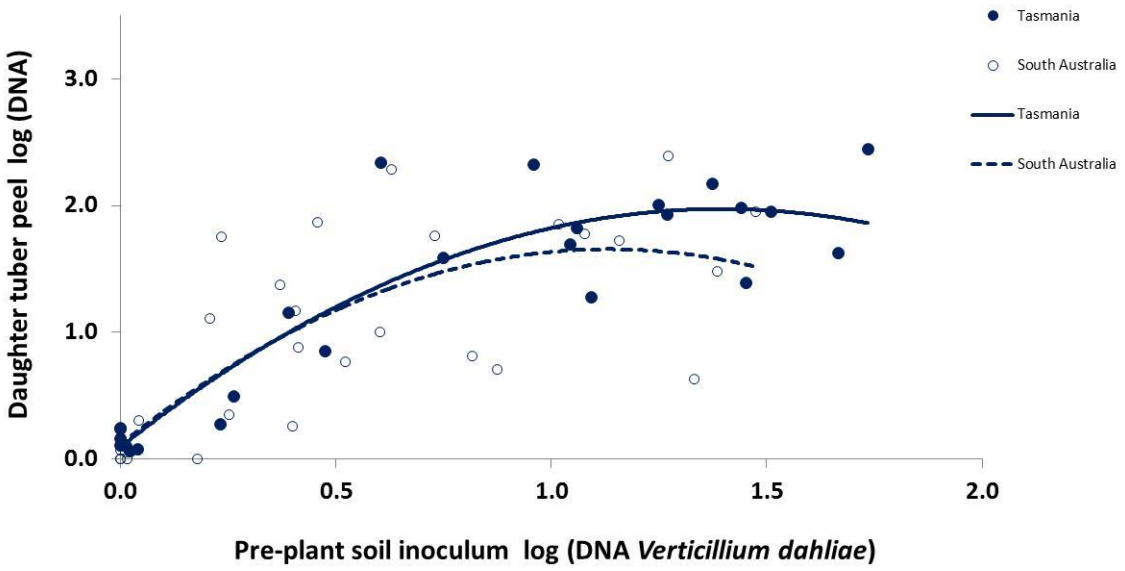

Figure 4-60 – Comparison of relationship between levels of *Verticillium dahliae* DNA in the soil prior to planting and in the peel of harvested tubers grown in either Tasmania or South Australia in the 2012/13 growing season.

Vascular browning

Verticillium wilt is known to cause browning of the vascular tissue of potato plants and tubers (Pasche, Thompson, & Gudmestad, 2013). In the 2011/12 season harvested tubers were examined for vascular staining. The relationship between vascular staining of the harvested tubers and level of *Verticillium dahliae* DNA in the soil prior to planting appears poor and would be impacted by many other possible causes of vascular staining, Figure 4-61. In a number of cases there was an unusually high incidence of vascular staining in the tubers that did not appear to be related to *Verticillium dahliae*. There are a number of other possible causes of vascular staining including other diseases of the plant vascular system, heat stress, herbicide uptake and salinity; however these other possibilities were not investigated. As poor as the relationship appears, the proportion of sampling points that had substantial vascular staining (>10% incidence vascular staining) was higher (85%) when *Verticillium dahliae* DNA level in the soil prior to planting exceeded 0.7 log (pg DNA / g soil + 1) than when *Verticillium dahliae* DNA was not detected (37%). Similarly the proportion of sampling points that had substantial vascular staining (>10% incidence vascular staining) was higher (69%) when *Verticillium dahliae* DNA was detected in the peel of harvested tubers than when it was not detected (29%), Figure 4-62.

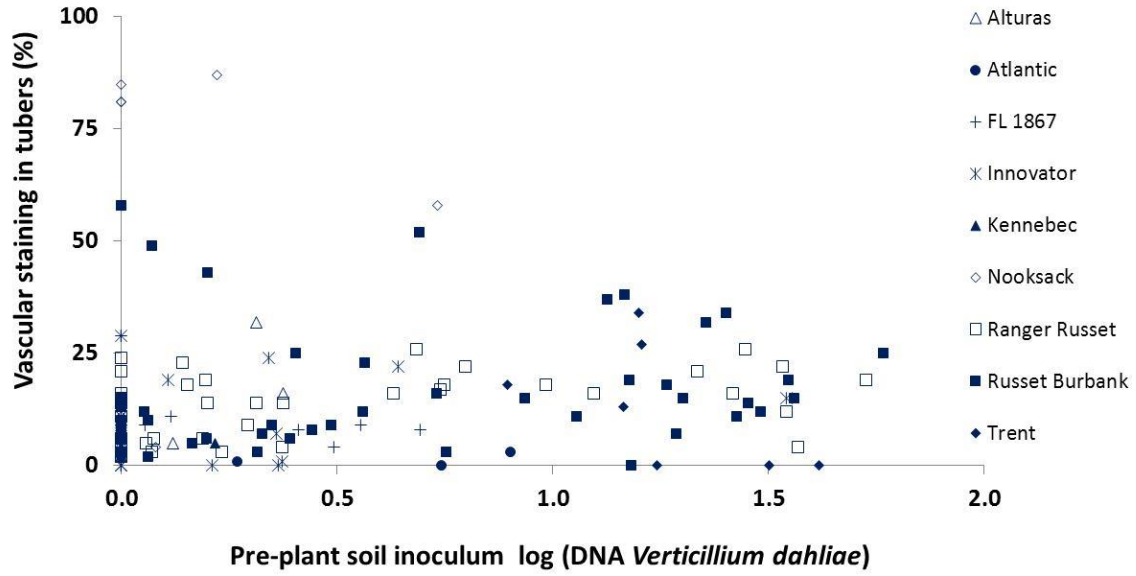

Figure 4-61 – Relationship between levels of *Verticillium dahliae* DNA in the soil prior to planting and incidence of vascular staining of harvested tubers grown in South Australia in 2011/12 season.

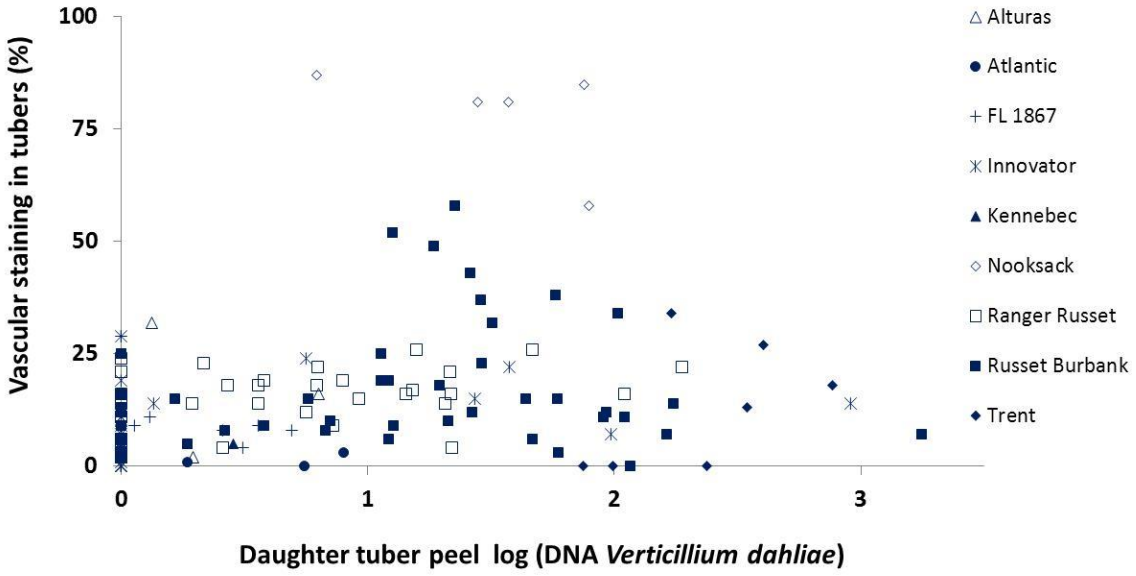

Figure 4-62 – Relationship between levels of *Verticillium dahliae* DNA in the peel of harvested tubers and incidence of vascular staining of harvested grown in South Australia in 2011/12 season.

Yield

Yield loss is the main concern of potato growers in relation to the impacts of potato early dying. However, many factors including soilborne diseases can impact the yield of potatoes in commercial paddocks. In this field validation study many of these additional yield-limiting factors are unaccounted for.

Yield data was only collected in South Australia and was examined only for the varieties Russet Burbank, Ranger Russet and Innovator, Table 4-21. Other varieties were not assessed at a sufficient number of sampling points to compare yields. Where *Verticillium dahliae* was detected in the soil yields of Russet Burbank and Innovator were lower than from sampling points where *Verticillium dahliae* was not detected. At sampling points where *Verticillium dahliae* DNA in the soil prior to planting was above 0.7 log (pg DNA / g soil + 1), yield of all varieties was reduced, Table 4-21. Innovator was only planted at 6 sites where the levels in the soil prior to planting exceeded 0.7 log (pg DNA / g soil + 1), so this yield figure should be treated with caution.

Most sites that have a high level of inoculum of one pathogen also have high levels of other pathogens as indicated by the correlation between pathogen concentrations measured in the soil prior to planting, Table 4-22, so it is difficult to attribute yield loss to specific pathogens. Other pathogens that have been demonstrated to cause yield reduction include *Colletotrichum coccodes* (Tsror, Erlich, & Hazanovsky, 1999), *Meloidogyne fallax* (Hay, et al., 2013) and *Rhizoctonia solani* (Banville, 1989), while other pathogens such as *Spongospora subterranea* have been shown to have the potential to cause yield reduction (Shah, Falloon, & Lister, 2012). These pathogens were in the soil at high levels at many of the sites where *Verticillium dahliae* DNA was detected, so yield loss cannot be completely attributed to *Verticillium dahliae*. Correlations of yield with several of the other pathogens were as strong as that found with *Verticillium dahliae*, Table 4-23. It is likely that yield is being reduced by many of these pathogens, and that their contribution to yield loss varies between crops. More detailed studies would have been required to quantify the contribution of each pathogen to yield loss in specific paddocks; however this data demonstrated that yield potential was reduced at sites where high levels of known yield reducing pathogens occurred.

Table 4-21 –Mean total yield per plant and associated standard errors in brackets of potato tubers harvested from sampling points with different pre-plant soil *Verticillium dahliae* DNA levels for three varieties grown in South Australia (Combined data from 2011/12 and 2012/13 growing seasons).

Pre-plant soil DNA level log (*Verticillium dahliae* DNA + 1)	Russet Burbank		Ranger Russet		Innovator	
	Number sampling points	Yield kg/plant	Number sampling points	Yield (kg/plant)	Number sampling points	Yield (kg/plant)
Not detected	16	2.06 (0.09)	17	1.96 (0.08)	30	2.08 (0.07)
<0.7	30	1.91 (0.05)	20	1.95 (0.06)	21	1.97 (0.10)
>0.7	34	1.85 (0.04)	25	1.73 (0.04)	6	1.75 (0.08)

Table 4-22 – Correlation coefficients (Pearson) between log (DNA) concentrations of pathogens in the soil prior to planting (combined data from 196 sampling points planted to Russet Burbank, Ranger Russet and Innovator in the 2011/12 and 2012/13 growing seasons)

Pre-plant soil DNA concentration	*Verticillium dahliae*	*Streptomyces txt*A gene	*Spongospora subterranea*	*Rhizoctonia solani* AG3	*Rhizoctonia solani* AG2.1	*Meloidogyne hapla*	*Meloidogyne fallax*
Colletotrichum coccodes	0.53	0.34	0.78	0.26	0.23	-0.04	0.38
Meloidogyne fallax	0.25	0.52	-0.03	0.22	0.22	-0.04	
Meloidogyne hapla	-0.12	0.06	-0.09	0.05	-0.14		
Rhizoctonia solani AG2.1	0.23	0.33	-0.00	0.03			
Rhizoctonia solani AG3	0.24	0.20	0.25				
Spongospora subterranea	0.50	0.28					
*Streptomyces txt*A gene	0.06						

Table 4-23 – Correlation coefficients between total yield (kg per plant) and log (DNA) concentrations of pathogens in the soil prior to planting (data from 80, 62, and 57 sampling points planted to Russet Burbank, Ranger Russet and Innovator respectively)

Pre-plant soil DNA concentration	Total yield per plant		
	Russet Burbank	Ranger Russet	Innovator
Colletotrichum coccodes	-0.29	-0.45	-0.08
Meloidogyne fallax	-0.39	-0.34	-0.10
Meloidogyne hapla	0.02	-0.37	-0.04
Rhizoctonia solani AG2.1	-0.09	0.07	0.02
Rhizoctonia solani AG3	-0.25	-0.42	-0.20
Spongospora subterranea	-0.38	-0.41	-0.19
*Streptomyces txt*A gene	-0.25	-0.25	-0.27
Verticillium dahliae	-0.23	-0.40	-0.17

DNA Seed test

Inoculum in the soil is considered to be the main source of disease risk caused by the pathogen *Verticillium dahliae* (Dung & Johnson, 2012). No evidence was found in this field validation study that inoculum on the seed was making a substantial contribution to disease risk. The level of *Verticillium dahliae* DNA measured in the peel of seed was not correlated with the level in the peel of harvested tubers, and *Verticillium dahliae* DNA in the peel of harvested tubers was generally low or not detected in the absence of soil inoculum, Figure 4-63. In a few cases *Verticillium dahliae* DNA was detected in the peel of harvested tubers from sampling points where *Verticillium dahliae* DNA was not detected in either the soil prior to planting or in the peel of seed tubers.

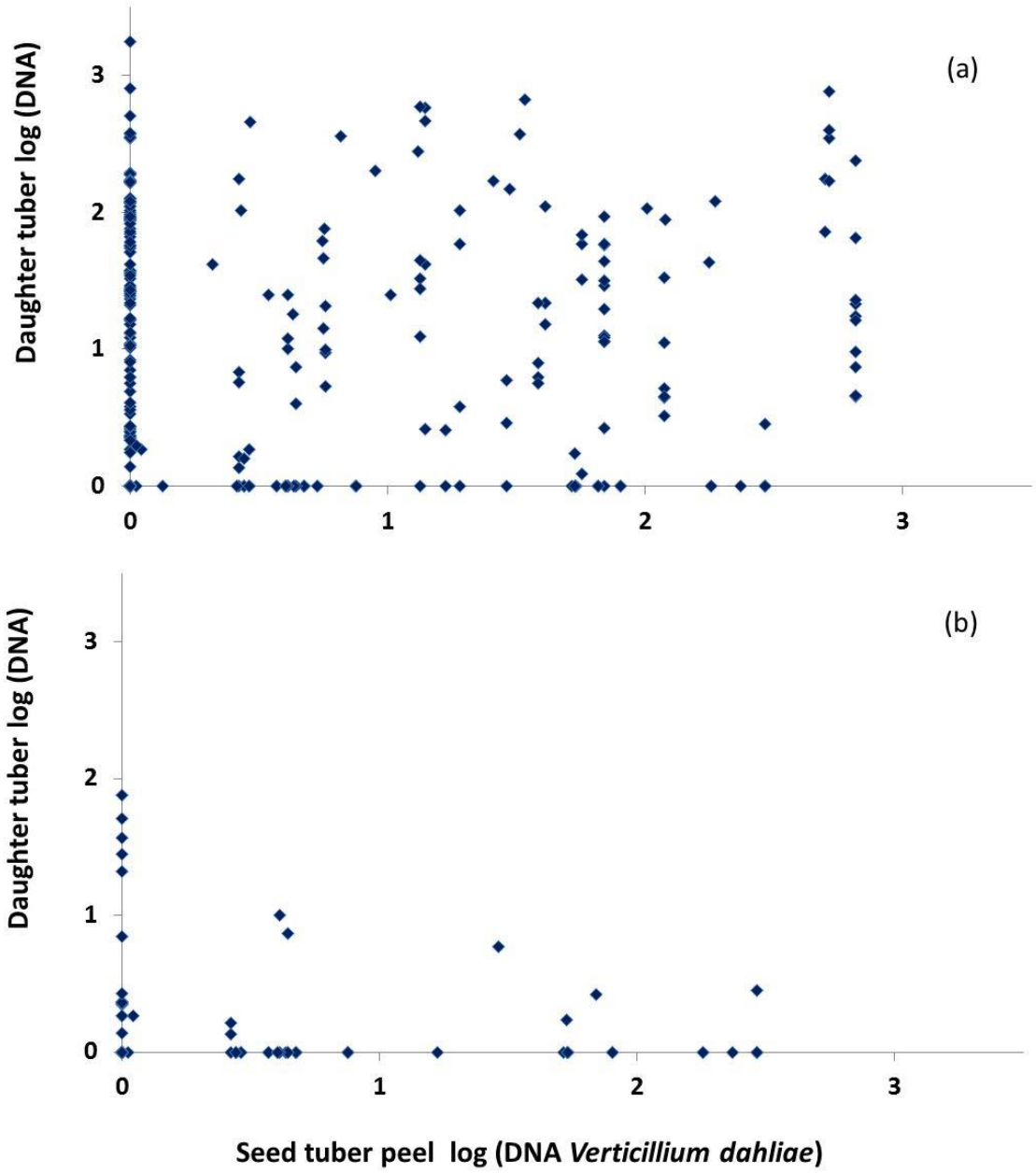

Figure 4-63 – Relationship between levels of *Verticillium dahliae* DNA in the peel of seed tubers and in the peel of harvested tubers for a) all sampling points b) sampling points where *Verticillium dahliae* DNA was not detected in the soil prior to planting. (Combined data from South Australia and Tasmania in 2011/12 growing season).

Inoculum buildup

Soil was sampled after crop senescence in Tasmania in the 2012/13 season. At sampling points where high levels of *Verticillium dahliae* DNA were measured in the soil prior to planting, levels were generally similar or higher in the soil after the crop was harvested, Figure 4-64. Growing the variety Topcat more consistently resulted in higher levels of *Verticillium dahliae* in the soil after the crop than for example Bondi

where changes varied from negligible to large increases. Where *Verticillium dahliae* DNA was not detected in the soil prior to planting, levels after the crop were generally undetectable or low.

Inoculum may be introduced into paddocks that are free of *Verticillium dahliae* by the planting of infested seed tubers. *Verticillium dahliae* DNA was not detected on most of the seed lots that were planted at sites where *Verticillium dahliae* was not detected in the soil prior to planting, Figure 4-65. At the limited number of sampling points where *Verticillium dahliae* DNA was detected on the seed, *Verticillium dahliae* DNA was either at a low concentration or was not detected in the soil at the end of a single potato cropping cycle. More sampling is required.

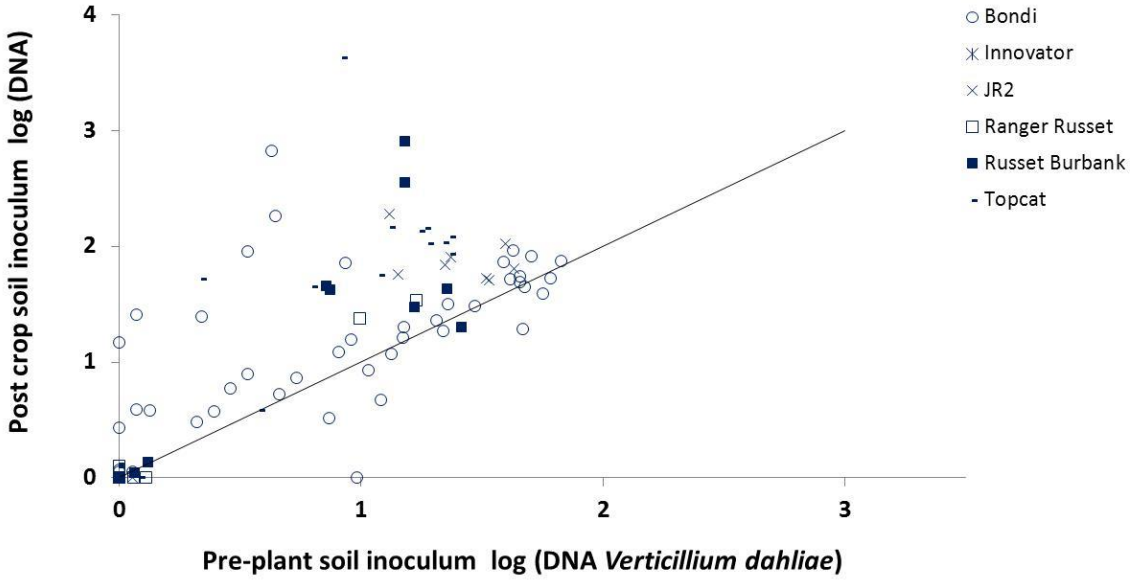

Figure 4-64 – Relationship between levels of *Verticillium dahliae* DNA in the soil prior to planting and the level of *Verticillium dahliae* DNA in the soil at harvest depending on variety grown. Line represents 1:1 relationship.

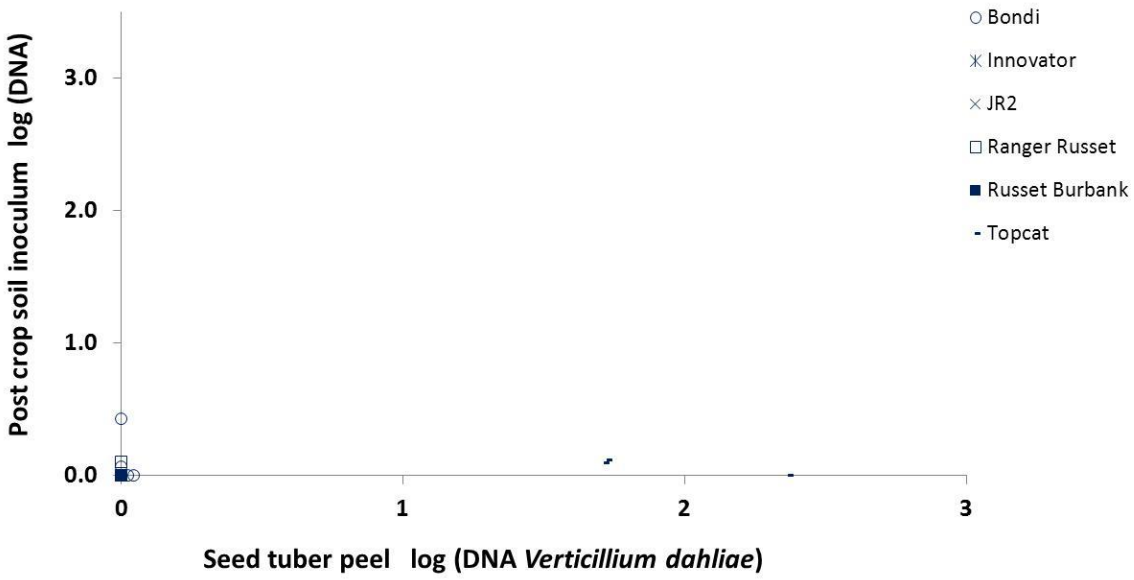

Figure 4-65 – Relationship between levels of *Verticillium dahliae* DNA in the peel of seed tubers and the level of *Verticillium dahliae* DNA in the soil at harvest when potatoes grown at locations where *Verticillium dahliae* DNA was not detected in the soil prior to planting, depending on variety grown.

PRATYLENCHUS SPP.

Root lesion nematodes are involved in potato early dying, however little is known about which species are most important in Australian production systems. DNA from South Australian field validation pre-plant soil samples were removed from storage and assayed for the pathogen levels of three *Pratylenchus* spp. nematodes – *P. crenatus*, *P. neglectus* and *P. penetrans* to complete the data set for the 2011/2012 season. A limited number of samples from Tasmania were also tested. The incidence of the three nematodes in these samples suggests *Pratylenchus* spp. were widely distributed in potato-growing soils, Table 4-24. *Pratylenchus penetrans*, which has been implicated in potato early dying in USA (Martin, Riedel, & Rowe), was not as commonly detected as the other two species tested in Australia. *Pratylenchus crenatus* is the species suspected to contribute to disease risk in Australia, but the possible involvement of *Pratylenchus neglectus* needs to be considered.

At locations where *Verticillium dahliae* DNA was detected in the soil prior to planting, DNA of at least one of the three *Pratylenchus* spp. was detected at over 93 % of locations in South Australia, suggesting that interaction between these nematodes would be possible in most situations where *Verticillium dahliae* is present, Table 4-23. At locations where *Verticillium dahliae* was not detected in the soil prior to planting the detection rate *of Pratylenchus* spp. was 66%. Detection of each of the three *Pratylenchus* spp. tended to be higher in soils where DNA of *Verticillium dahliae* was detected.

In Tasmanian the breakdown of *Pratylenchus* spp. populations in the soil were different to South Australia. *Pratylenchus crenatus* was the dominant species, being detected at 95% of locations tested. In contrast *Pratylenchus negl*ectus and *Pratylenchus penetrans* were detected at 8 and 4% sites respectively.

Table 4-24 – Percentage of soil samples that each of three *Pratylenchus* spp. were detected by DNA testing and indicated range in population density (data from 158 and 24 sampling locations in South Australia and Tasmania respectively).

Pathogen	Detection in pre-planting soil samples (%)		Range of levels detected (nematodes / g soil)
	South Australia	Tasmania	
Pratylenchus neglectus	50	8	0 - 11
Pratylenchus penetrans	12	4	0 - 6
Pratylenchus crenatus	44	95	0 - 7

Table 4-25 – Detection of *Pratylenchus* spp. nematodes associated with different pre-plant soil *Verticillium dahliae* DNA levels in South Australia (data from 158 sampling points).

Pre-plant soil DNA level log (*Verticillium dahliae* DNA + 1)	Nematodes detected (>0.1 nematodes /g soil) (% sampling locations)			
	Pratylenchus crenatus	*Pratylenchus neglectus*	*Pratylenchus penetrans*	Any of three species
Not detected	29	34	5	66
< 0.7	67	49	16	93
> 0.7	33	71	16	96

SAMPLING PROTOCOL

Sampling techniques are critical for pathogen DNA testing to provide reliable disease risk assessment prior to the planting of a potato crop.

EFFICACY OF USING FOUR SOIL SAMPLES PER PADDOCK FOR DISEASE RISK ASSESSMENT

Sampling strategies are a balance between achieving an acceptable level of accuracy and what is feasible and cost effective to commercially implement. The use of four separate pathogen DNA tests on each of four 500g composite core samples collected using a "W" sampling path across a 1 ha area has been devised for sampling large (up to 50 hectare) potato paddocks to assess the risk of disease. Log-logistic modelling on the data from intensively sampled paddocks has then been used to assess the efficacy of this sampling technique for disease risk assessment. The diagrams used to present the outputs of this analysis for each pathogen are described in more detail for *Rhizoctonia solani* AG2.1.

RHIZOCTONIA SOLANI AG2.1

A summary of the results for *Rhizoctonia solani* AG2.1 is given in Figure 4-66. The proportion of the area of a pivot that exceeds the guideline value for low risk and high risk is shown on the 'Y axis'. For each pivot there are many possible combinations of four samples, each with its own mean DNA (pg/g soil) value, as shown on the 'X axis'. For each pivot a green line of points shows the means of each possible combination of four sampling points. This line of points is positioned corresponding to the true proportion of the pivot (on 'Y axis') that exceeded the low risk criteria. There is a matching red line of points positioned corresponding to the true proportion of the pivot that exceeded the high risk criteria. As nine pivots were included in the analysis for *Rhizoctonia solani* AG2.1 there are nine pairs of red and green lines, each pair representing a pivot.

Each of the green and red points represents a possible sampling result, being the means of each combination of four sampling locations in a pivot. For any individual sampling result represented by a single point along the line, the models estimate of the proportion of the pivot exceeding the guideline value for low risk or high risk can be read from the fitted curves.

Example: If the sample mean DNA (pg/g soil) is 15 pg DNA/g soil then the model indicates that 20% of that pivot area may exceed the "Low" risk criterion (as indicated in Figure 4-66 by the black arrows). Likewise if the sample mean DNA (pg DNA/g soil) is 300 pg DNA /g soil the model indicates 10% of the pivot area may exceed the "High" risk criterion (as indicated in Figure 4-66 by the blue arrows).

By examining the spread of sample means in relation to the fitted curves the reliability of four sampling points to estimate the true proportion of a pivot at risk can be assessed.

In general it can be seen that the mean of four samples gives a good indication of *Rhizoctonia solani* AG2.1 risk. There are tails of the sampling distribution (represented by dots near the end of the lines) but in the majority of cases a useful prediction of the risk from AG2.1 is given.

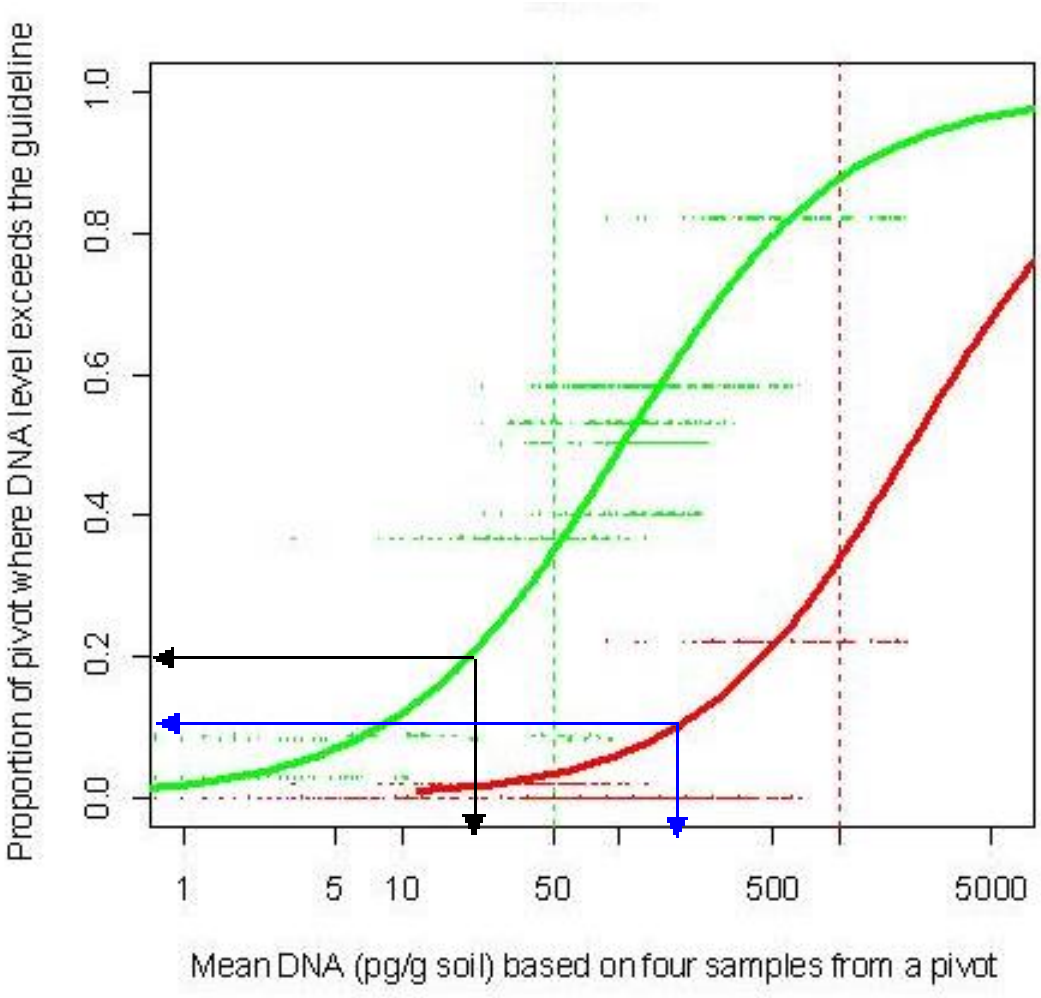

Figure 4-66 – Sample means of *Rhizoctonia solani* AG2.1 based compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show low- and high-risk DNA concentrations respectively.

RHIZOCTONIA SOLANI AG3

The results shown in Figure 4-67 for *Rhizoctonia solani* AG3 indicate that typically four samples will give a good prediction of the risk. When the sample mean was at the low risk level, the model predicts 20% of the pivot will have levels exceeding the low risk level. If the mean of the sample is at the high risk level, almost 40% of the paddock would be at high risk and over 50% at low risk.

There were 2 pivots where a wide range in means was calculated. For example in 1 pivot means ranged from 1 – 100 pg DNA/g of soil indicating large variability within the pivot. These means equate to the fraction of the pivot predicted to be at low risk ranging from 2% - 80%, whereas the true value was 30%. In these pivots there is a high probability of the mean of four samples will be misleading.

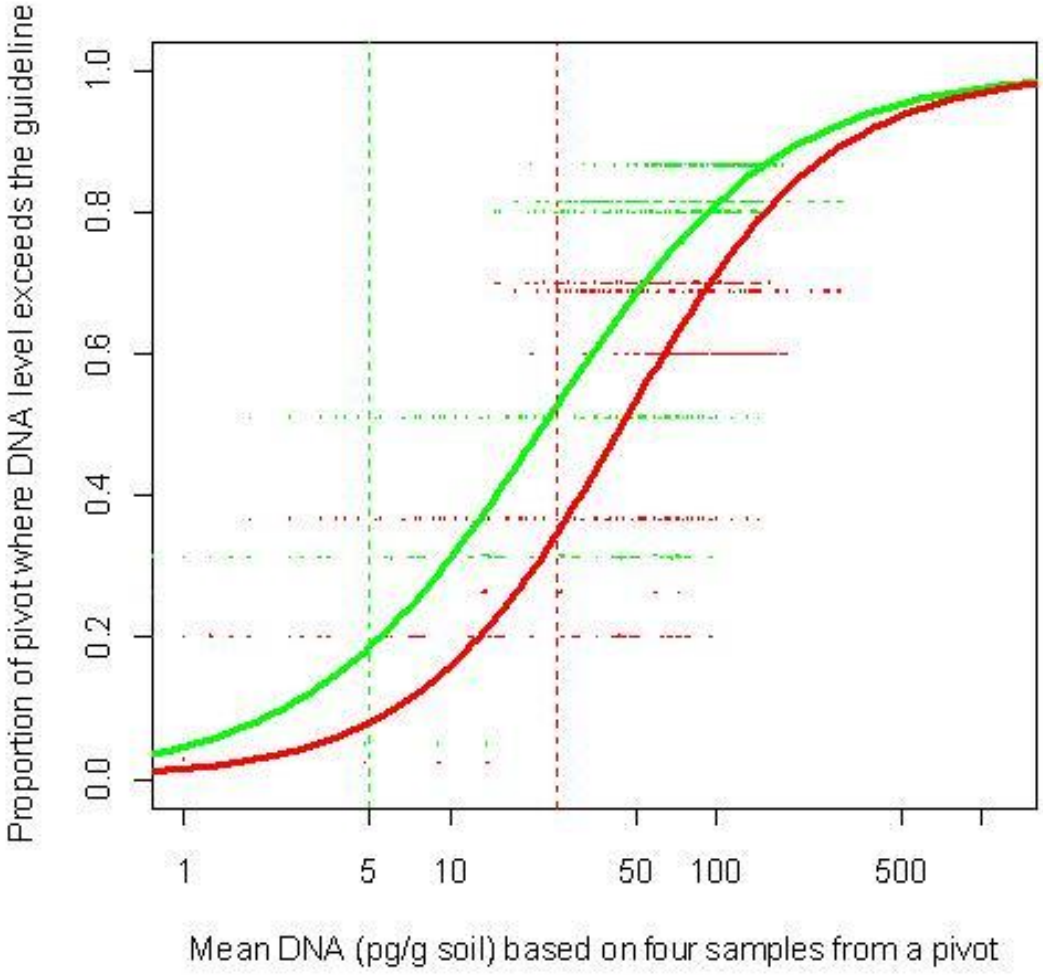

Figure 4-67 – Sample means of *Rhizoctonia solani* AG3 compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show putative low- and high-risk DNA concentrations respectively.

MELOIDOGYNE FALLAX

In general it can be seen that the mean of four samples gives a reasonable indication if a *Meloidogyne fallax* risk exists in a pivot, Figure 4-68. In most cases a reliable prediction can be obtained from the model. There are a few cases where an incorrect decision could result due to sampling error.

When the pivot mean value for *Meloidogyne fallax* DNA is low, high populations of *Meloidogyne fallax* appear to affect only a small fraction of the pivot. For example, with a mean value of 25 pg DNA/g soil less than 5% of the pivot would be at high risk. With a value of 50 pg DNA/g soil, approximately 30% of the pivot would be at high risk.

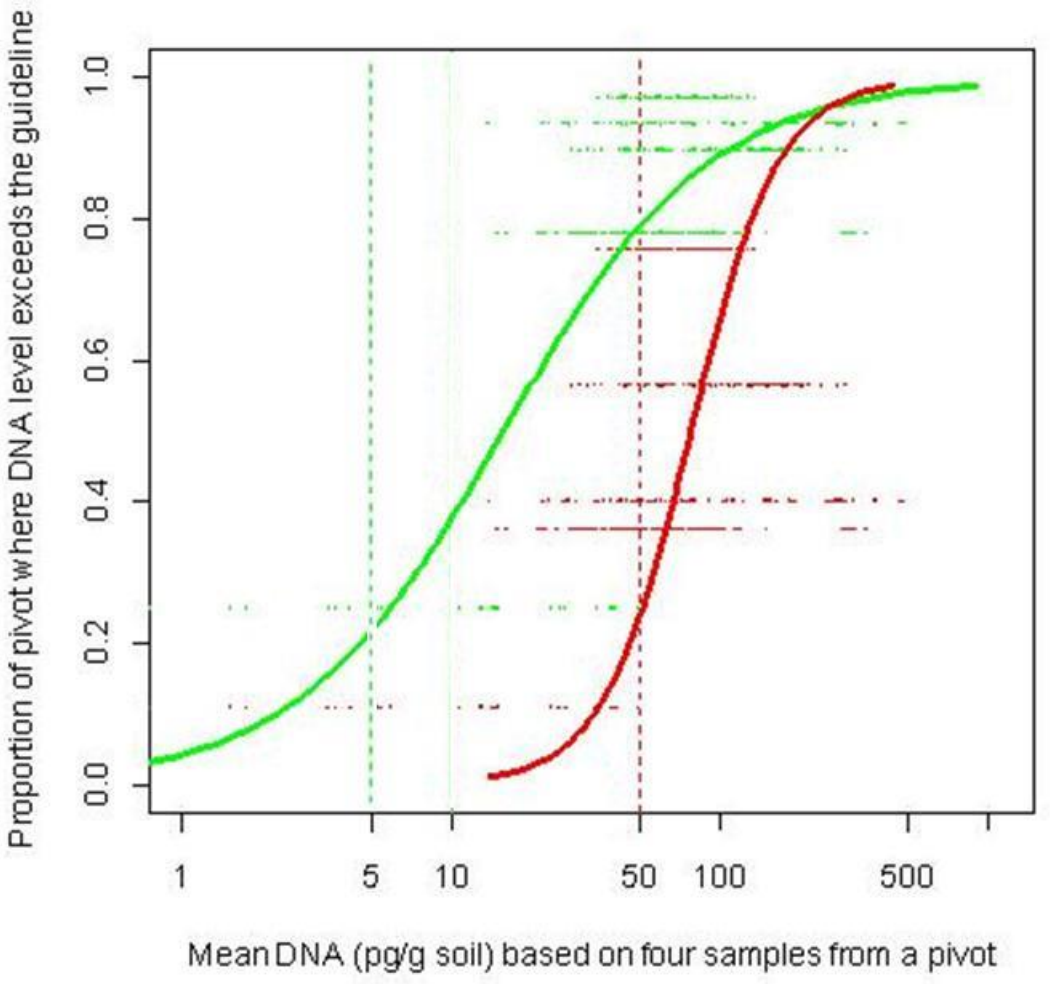

Figure 4-68 – Sample means of *Meloidogyne fallax* DNA compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show low- and high-risk DNA concentrations respectively.

MELOIDOGYNE HAPLA

In general it can be seen that the mean of four samples gives a reasonable indication if *Meloidogyne hapla* risk exists in a pivot, Figure 4-69. The shape of the curves is very similar to that of *Meloidogyne fallax*. In most cases a reliable prediction can be obtained from the model. There are cases where an incorrect decision could result due to sampling error.

At 500 pg DNA /g soil there is an anomaly where the modelled fraction of the pivot at high risk exceeds that of the fraction at low risk. This is an artefact of the modelling.

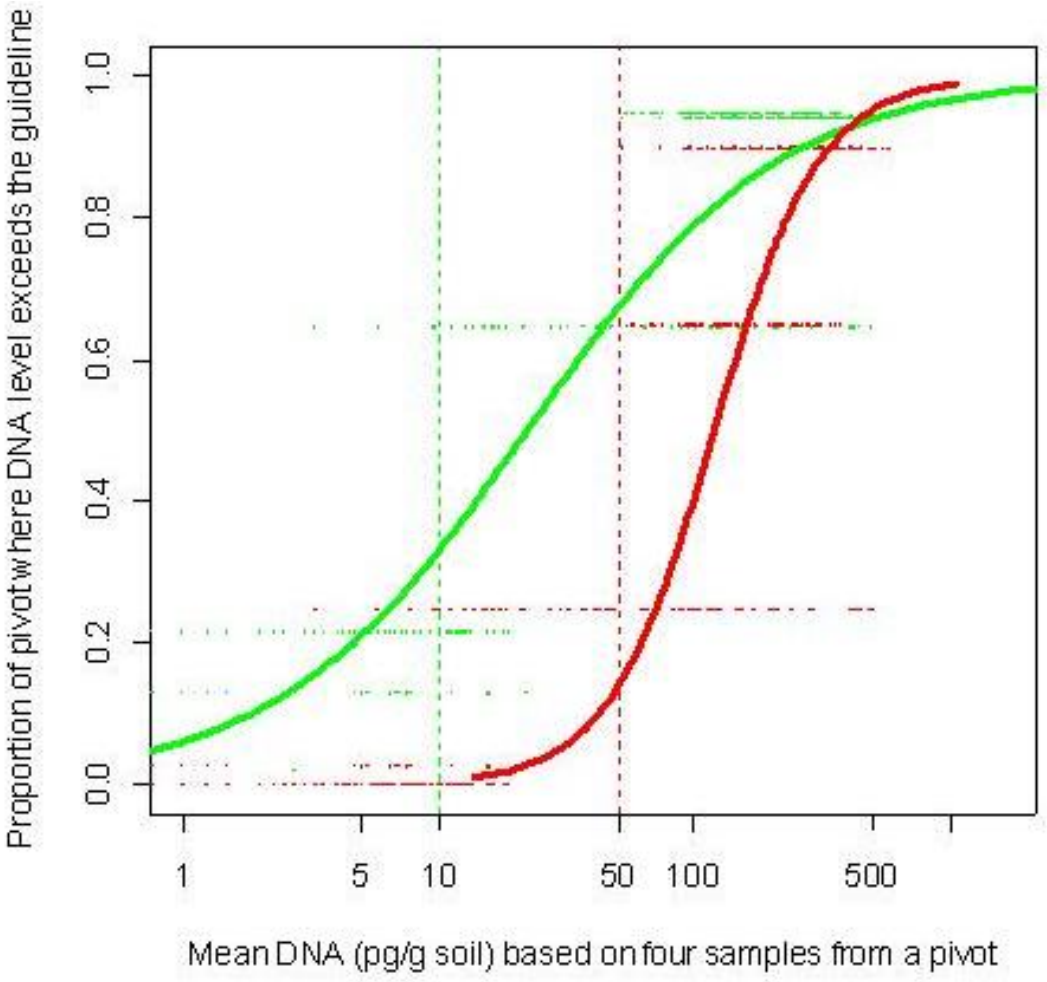

Figure 4-69 – Sample means of *Meloidogyne hapla* DNA compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show putative low- and high-risk DNA concentrations respectively.

SPONGOSPORA SUBTERRANEA

The results shown for *Spongospora subterranea* indicate that typically four samples will give a good prediction of the risk in Figure 4-70. When the sample mean was at the low risk level, the model predicts almost 30% of the pivot will have levels exceeding the low risk level. If the mean of the sample is at the

high risk threshold, approximately 20% of the pivot would be at high risk and 60% of the pivot will have levels exceeding the low risk threshold.

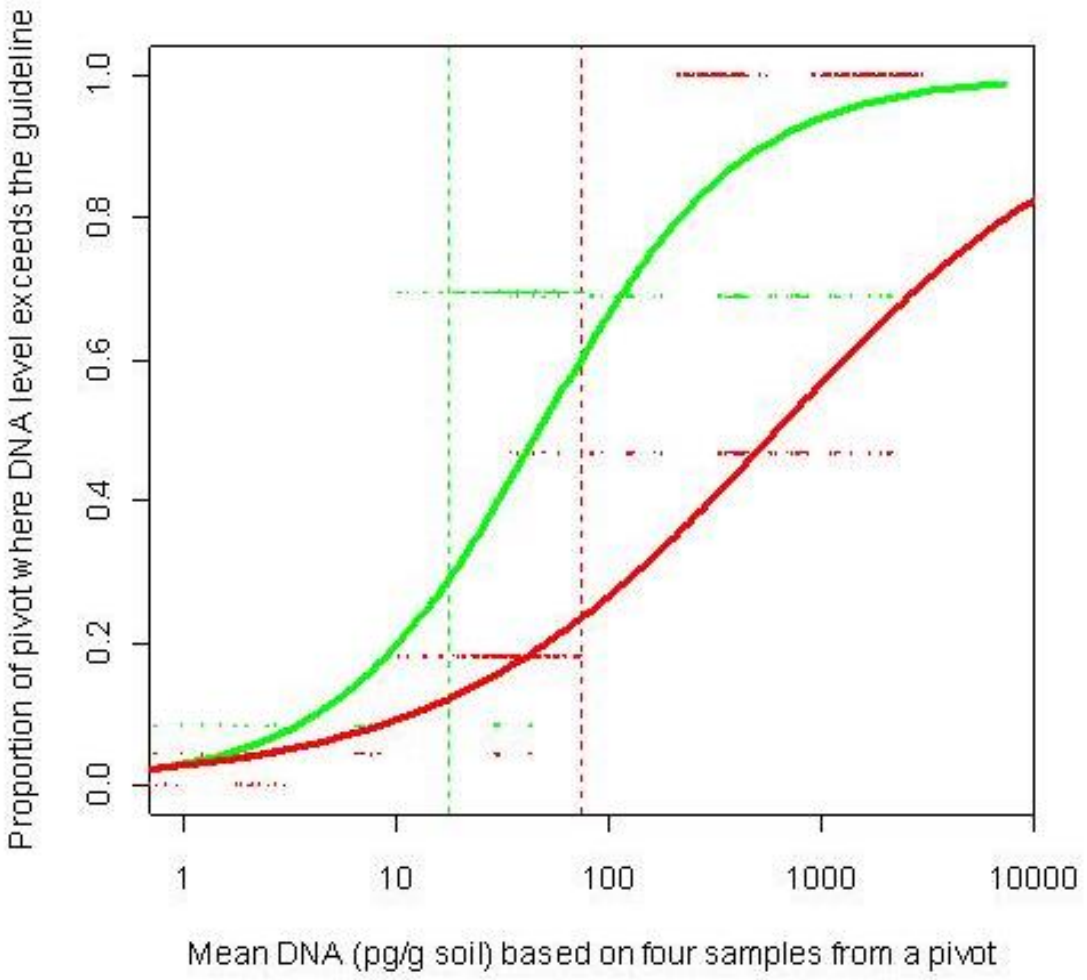

Figure 4-70 – Sample means of *Spongospora subterranea* DNA compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show low- and high-risk DNA concentrations respectively.

PATHOGENIC STREPTOMYCES

The results shown in Figure 4-71 for *Streptomyces txt*A gene indicate that typically four samples will give a good prediction of the risk.

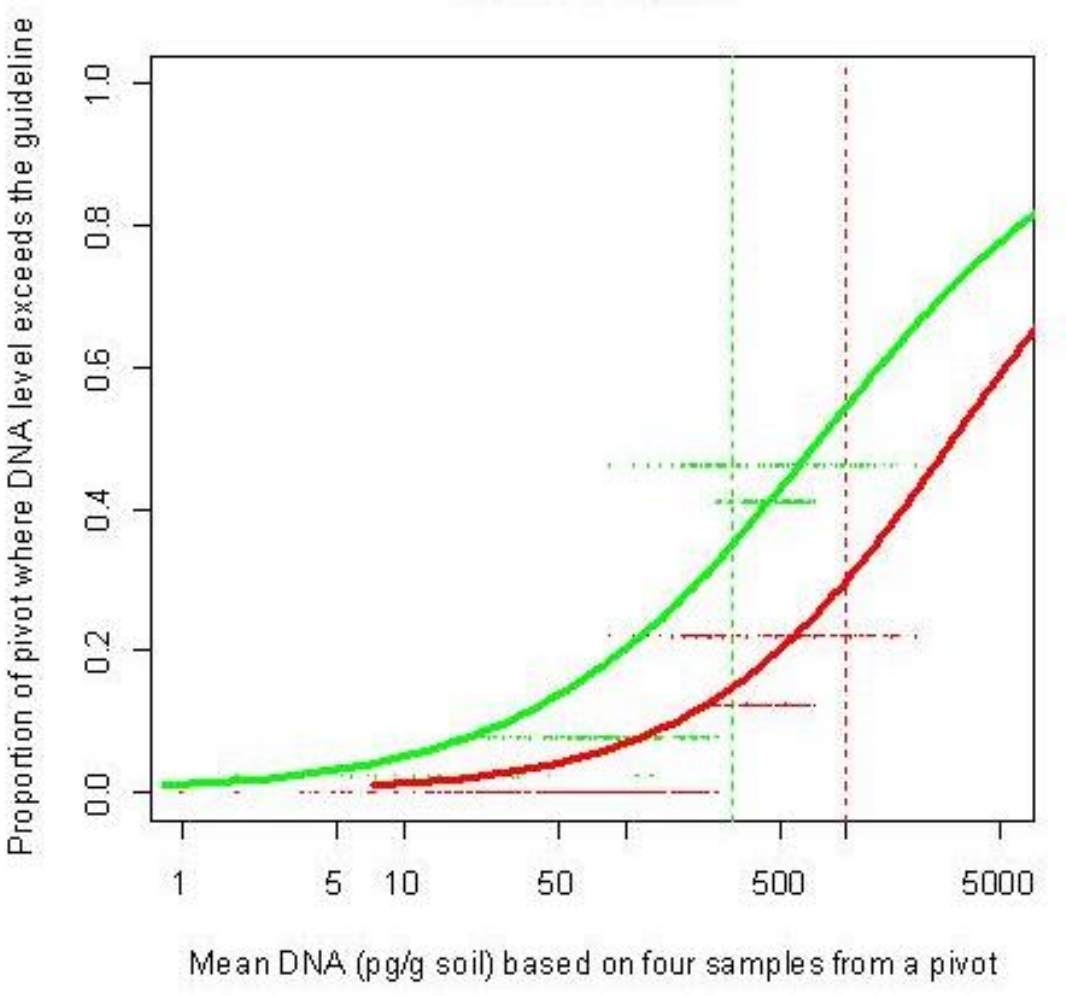

Figure 4-71 – Sample means of *Streptomyces txt*A gene DNA compared to proportion of the pivot that exceeded a critical value. The fitted lines are weighted logistic curves, while the green and red vertical lines show putative low- and high-risk DNA concentrations respectively.

TIMING OF SAMPLING

Pre-plant sampling needs to be completed in time to make decisions based on the results. Regression analysis was used to determine if samples taken in June are comparable to those taken just prior to planting in September / October. Strong relationships were found for each of the six pathogens tested, Figure 4-72. Relationships for *Spongospora subterranea*, *Meloidogyne fallax* and *Meloidogyne hapla* indicate that soil sampling in June will provide similar results to sampling in September/ October. Though the relationships are statistically significant for the other pathogens, the data reflects an inconsistency in the ability to detect DNA of these pathogens in the soil when levels are low.

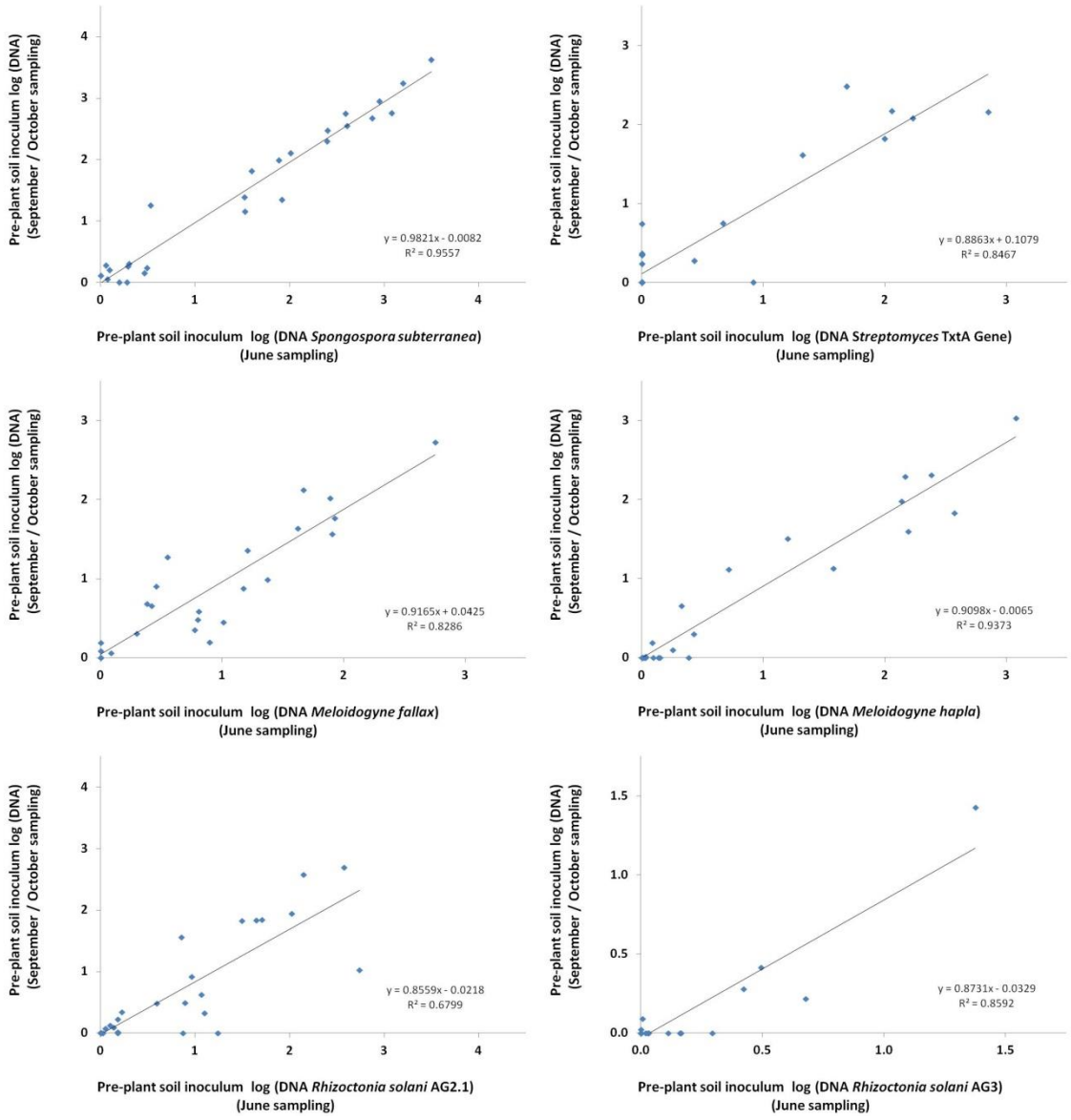

Figure 4-72 – – Relationships between soil pathogen test results from soil samples taken in June 2010 and September / October 2010 (Data from 16 paddocks in South Australia and 10 paddocks in Tasmania).

DISTRIBUTION OF *RHIZOCTONIA SOLANI* DNA IN THE SOIL PROFILE

Soil samples from three sites were fractionated and the pathogen DNA levels tested. Sites for sampling were selected where a medium and a high level of *Rhizoctonia solani* AG3 DNA had been detected along with a site where it was not detected by previous field validation sampling. All sites were under pasture and had not been cultivated for at least 2 years. Samples for fractionation were not taken from the exact location of the original field validation sample. At the site where *Rhizoctonia solani* AG2.1 and *Rhizoctonia solani* AG3 DNA were not detected in the original field validation soil sample, *Rhizoctonia solani* AG2.1 was not detected in any of the sample depths and *Rhizoctonia solani* AG3 DNA was only detected 0.2 log (pg DNA / g soil sample) in the >0.5mm fraction of the 100-200 mm depth cores, Table 4-26.

At the other two sites, results were similar, with the highest levels of DNA for both *Rhizoctonia solani* AG2.1 and *Rhizoctonia solani* AG3 detected in the >0.5 mm fraction of the 0-10 cm section of cores. At these sites 87% and 89% of the total DNA of *Rhizoctonia solani* AG3 was detected in the >0.5 mm fraction in the surface 100mm of the cores . This confirms that organic matter in the surface layers of the soil is the best target to detect *Rhizoctonia solani* AG3 in an uncultivated paddock. Similarly 82% and 39% of the total DNA of *Rhizoctonia solani* AG2.1 was found in the >0.5 mm fraction in the surface 10 cm of the cores.

Table 4-26 – Levels of *Rhizoctonia solani* AG2.1 DNA and *Rhizoctonia solani* AG3 DNA in sieved fractions of soil cores sampled from three sites in the SE of South Australia.

Sampling depth	Soil fraction (mm)	*Rhizoctonia solani* AG2.1 DNA log (pg DNA/g soil + 1)			*Rhizoctonia solani* AG3 DNA log (pg DNA/g soil + 1)		
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
0-10cm	<0.3	0.0	0.2	0.5	0.0	0.4	0.5
	0.3-0.35	0.0	0.7	0.4	0.0	0.7	1.1
	0.35-0.5	0.0	0.9	1.2	0.0	1.1	1.2
	>0.5	0.0	1.0	2.2	0.0	2.5	2.7
10-20cm	<0.3	0.0	0.0	0.0	0.0	0.2	0.2
	0.3-0.35	0.0	0.1	0.0	0.0	0.2	0.0
	0.35-0.5	0.0	0.1	0.3	0.0	0.3	0.1
	>0.5	0.0	0.0	0.5	0.2	1.3	0.1
Original sample site selected on							
0-15cm	Not applicable	0	1.9	0.8	0	1.4	1.9

INTER-LABORATORY DNA TEST COMPARISON

Molecular tools for detecting and quantifying a range of potato pathogens (*Rhizoctonia solani*, *Spongospora subterranea*, and *Colletotrichum coccodes*) have been developed in laboratories in Australia (SARDI), New Zealand (New Zealand Plant and Food Research; NZPFR) and the United Kingdom at both James Hutton Institute (JHI, formerly SCRI), Scottish Agricultural College (SAC)) and the Food and Environment Research Agency (Fera).

The objective of this work is to calibrate tests results internationally so that there is a common understanding of what a specific tests result means across laboratories.

In all four sets of inter-laboratory comparison, the range of inoculum levels provided a good range for testing the ability of laboratories to detect and quantify the target pathogen.

Coefficients of variation for 'medium' and 'high' level samples were generally within the accepted limit (<10%). However, 'low' samples always had higher than 10% CV and illustrates the difficulty in producing samples with low variation when pathogen inoculum levels are low.

In general there was reasonable agreement between all participating laboratories. Specific results for each pathogen are detailed below.

COLLETOTRICHUM COCCODES

Pre-dispatch sample testing showed that DNA did not deteriorate during one week storage at room temperature (data not shown). The coefficients of variation (cv %) were within the target limit of acceptability (<10%) for high and medium samples but variation between replicate sub-samples was higher than the target limit of acceptability in the low sample, Table 4-27. It is likely that low inoculum levels will inevitably have higher cv% than higher inoculum levels no matter how the samples are prepared.

Table 4-27 – Pre-dispatch sample coefficients of variation for *Colletotrichum coccodes* samples.

Sample	CV%
High	3.7
Medium	8.3
Low	24.4
Nil	0.0

The consensus means shown in Table 4-28 shows that the range of DNA levels in the samples were appropriate for the Low, Medium and High sample labels. All returned values from participating labs were within the 2xSD tolerance. One lab detected low levels of pathogen in the Nil sample.

Figure 4-73 shows the spread of data from individual labs around the consensus mean for *Colletotrichum coccodes* (a, set 1; and b, set 2).

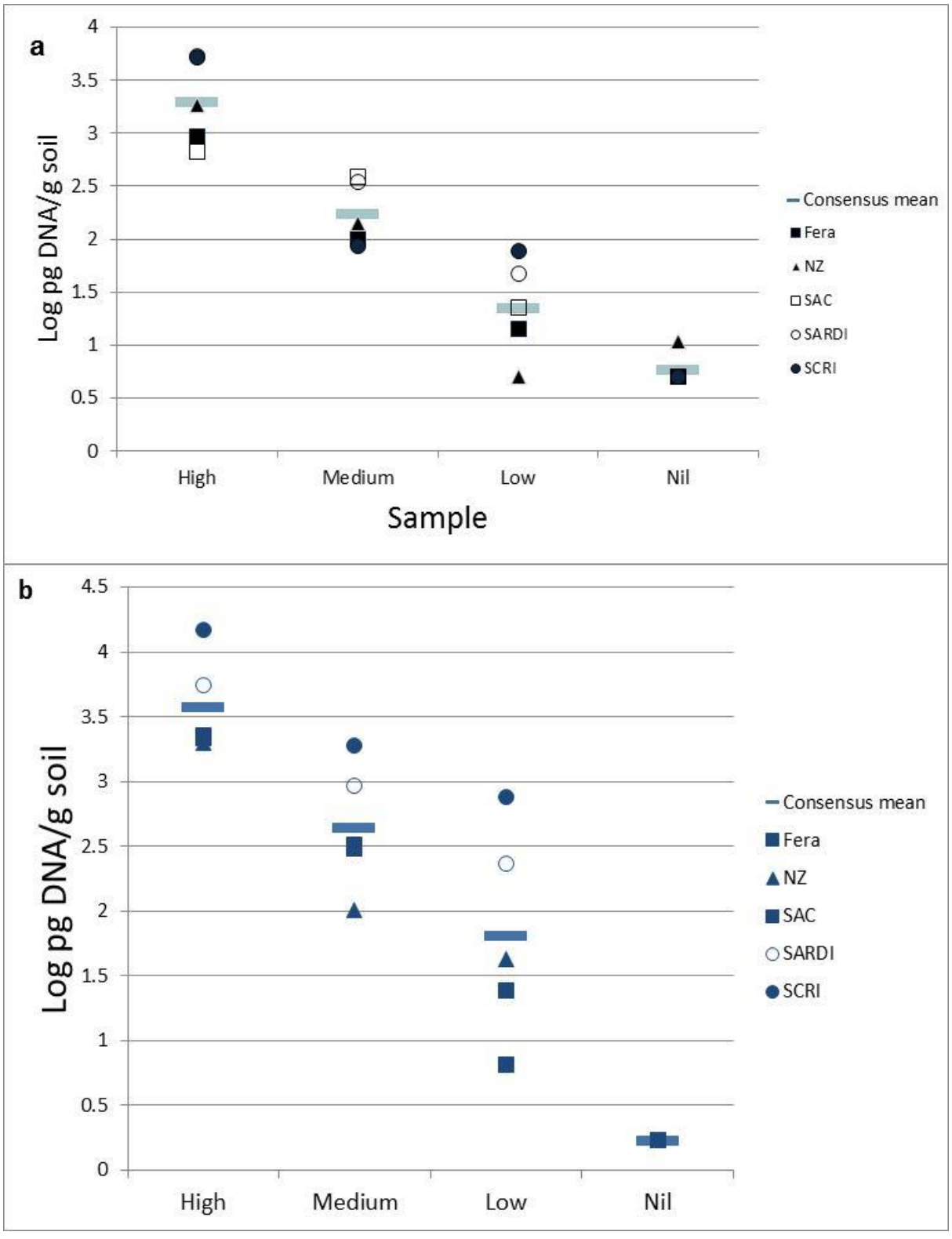

Figure 4-73 – Spread of data from individual labs around the consensus mean for *Colletotrichum coccodes* (a, set 1; and b, set 2)

Table 4-28 – Mean of logarithm of the concentration (pg/g soil) of DNA for each sample for each participating lab and associated z-scores (*Colletotrichum coccodes*).

Sample	Fera		NZ		SAC		SARDI		SCRI		Consensus mean
	Mean	z-score	Mean	z-score	Mean	z-score	Mean	z-score	Mean	z-score	
High	2.97	-0.809	3.27	-0.080	2.83	-1.148	3.73	1.036	3.72	1.002	3.30
Medium	2.01	-0.591	2.14	-0.245	2.58	0.850	2.55	0.753	1.93	-0.767	2.24
Low	1.16	-0.446	0.70nc	-1.480	1.36	-0.001	1.68	0.725	1.89	1.203	1.36
Nil	0.70†	-0.258	1.04	1.034	0.70	-0.258	0.70	-0.258	0.70	-0.258	0.77

nc = non-compliant; † 'undetected' values were treated as at the limit of detection (5 pg/g) for the purpose of the analysis.

RHIZOCTONIA SOLANI AG3

Pre-dispatch sample testing showed that DNA did not deteriorate during one week storage at room temperature (data not shown). The coefficients of variation (cv%) were within the target limit of acceptability (<10%) for High. However, the variation between replicate sub-samples for Medium and Low samples was higher than the target limit of acceptability Table 4-29.

Table 4-29 – Pre-dispatch sample coefficients of variation for *Rhizoctonia solani* AG3 samples

Sample	CV%
High	2.0
Medium	17.9
Low	50.5
Nil	0.0

The consensus means (shown in Figure 4-74) indicates that the range of DNA levels in the samples were appropriate for the Low, Medium and High sample labels. There was good agreement between participating labs for samples labelled High, but not for samples labelled Medium and Low, where Fera's extractions failed and so returned results that were out of step with those provided by the other labs.

Figure 4-74 shows the spread of data from individual labs around the consensus mean for *Rhizoctonia solani* AG3.

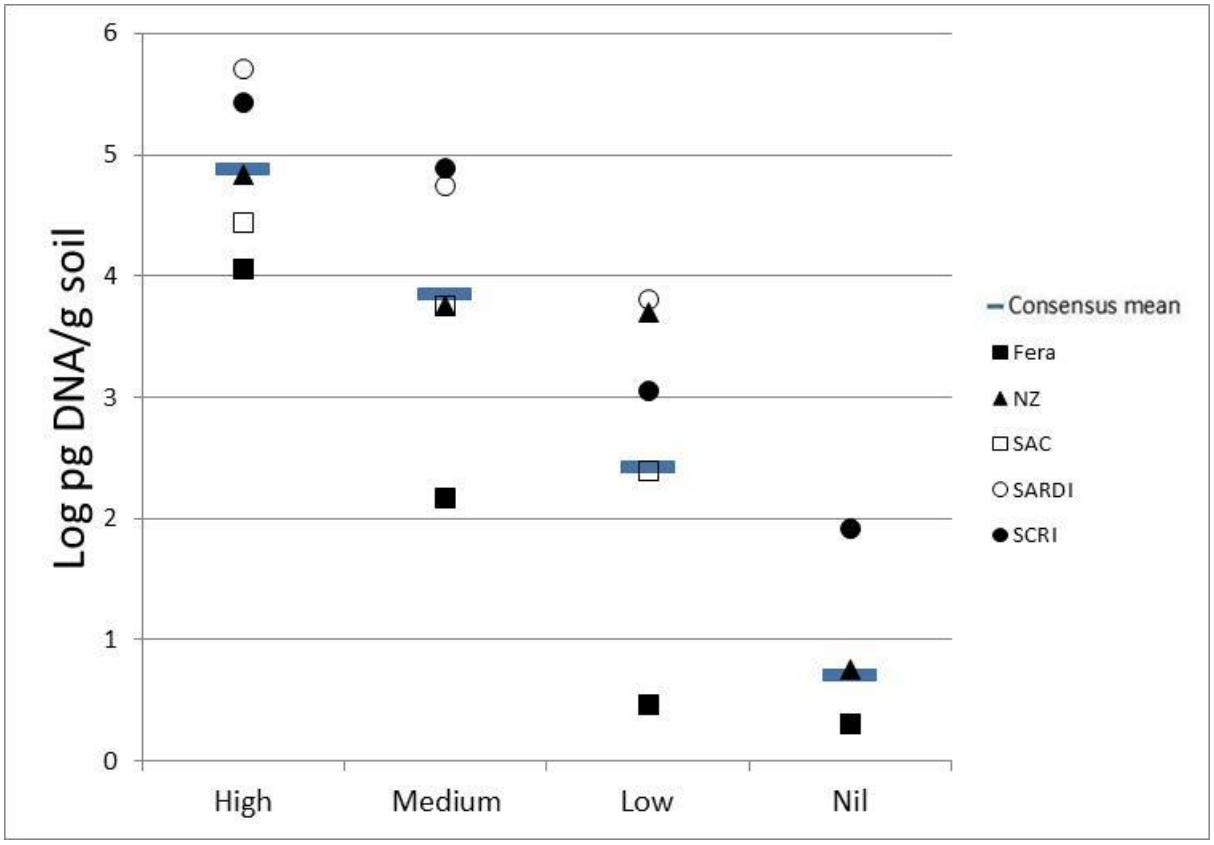

Figure 4-74 – Spread of data from individual labs around the consensus mean for *Rhizoctonia solani* AG3.

SPONGOSPORA SUBTERRANEA

Pre-dispatch sample testing showed that DNA did not deteriorate during one week storage at room temperature (data not shown). The coefficients of variation (cv%) were within the target limit of acceptability (<10%) for High and Medium samples. However, the variation between replicate sub-samples for Low samples was higher than the target limit of acceptability Table 4-30.

Table 4-30 – Pre-dispatch sample coefficients of variation for *Spongospora subterranea* samples

Sample	CV%
High	3.5
Medium	9.8
Low	27.7
Nil	0.0

The consensus means (shown in Figure 4-75) indicates that the range of DNA levels in the samples were appropriate for the Low, Medium and High sample labels. There was good agreement between all participating labs.

Figure 4-75 shows the spread of data from individual labs around the consensus mean for *Spongospora subterranea*.

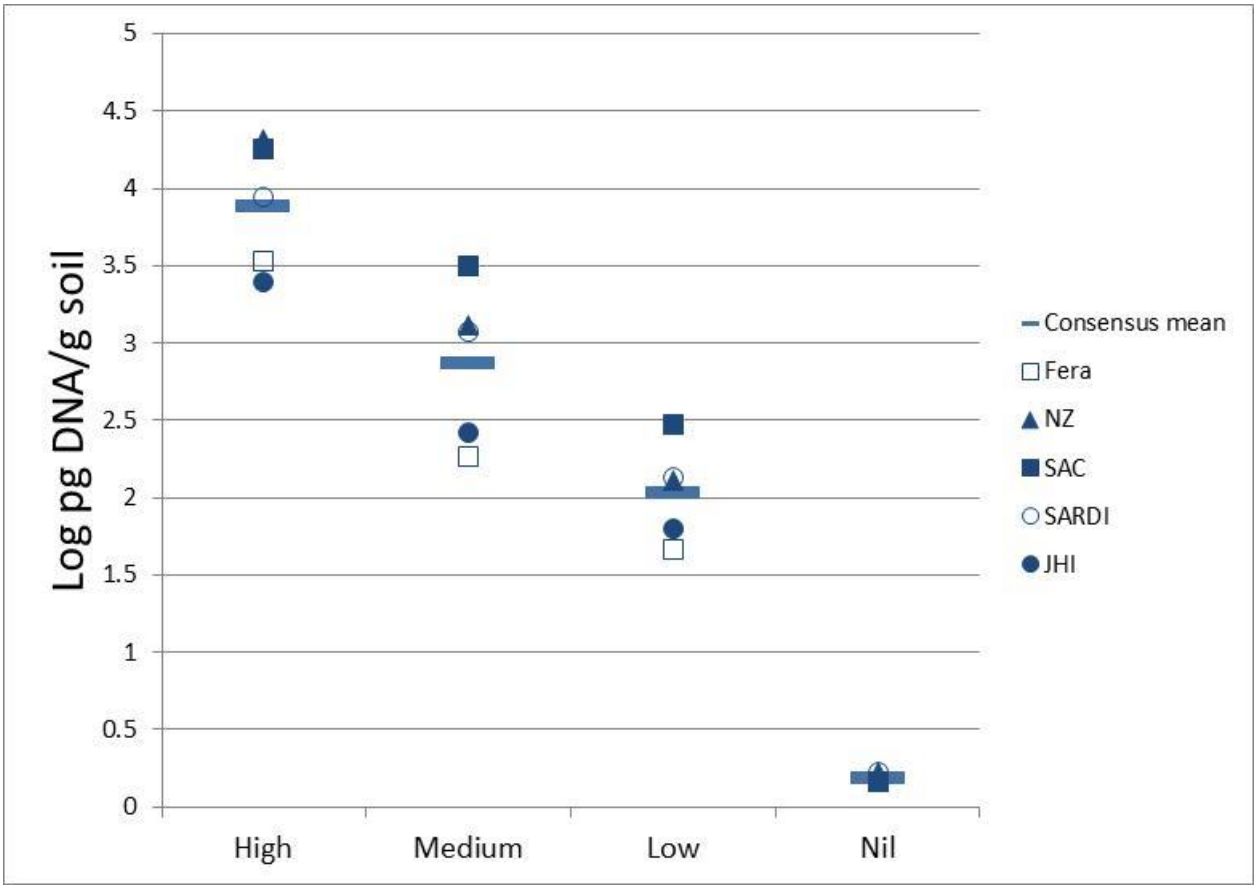

Figure 4-75 – Spread of data from individual labs around the consensus mean for *Spongospora subterranea*.

PATHOGEN EPIDEMIOLOGY – UNDERSTANDING INOCULUM AND DISEASE DEVELOPMENT

MAIN FINDINGS FROM UNITED KINGDOM COLLABORATION - *RHIZOCTONIA*

At the conclusion of the Potato Council UK project an extensive final report was produced. Below is a summary of the key findings relevant to PT09023 research on Rhizoctonia. Details of methods can be accessed on request.

DETECTION OF *RHIZOCTONIA SOLANI* INOCULUM IN SOIL

The focus of the UK project was largely on fresh market potatoes and black scurf symptoms on tubers. As in Australia, in Great Britain *Rhizoctonia solani* AG3 is found both infrequently and generally at low levels in commercial potato growing fields.

There was a poor relationship between soil inoculum levels and subsequent disease on progeny tubers in commercial potato crops. Black scurf was found on tubers in crops in which no soilborne inoculum was detected pre-planting, and the relative small number of fields in which inoculum is detected means that conclusions on the relationship between soil inoculum levels and disease are difficult to make.

Experiments were undertaken in controlled environments (Fera 2009) to determine the relative effect of sclerotia and hyphal soilborne inoculum.

Low levels of sclerotia in soil (0.01% w/w) caused nearly 100 % incidence of stem canker and black scurf. Slightly higher levels of hyphal soil inoculum (0.025% w/w) were needed to cause the same degree of disease.

Levels as low as 0.00001 g sclerotia / g soil can cause disease. Australian research shows that current detection and sampling methods cannot reliably detect levels of less than 0.002g sclerotia per g soil, and as sclerotia are likely to be unevenly distributed in field soils, we can conclude that current methods cannot reliably detect sclerotia in field soils at levels which are sufficient to cause disease, primarily due to the size of the soil sample which will not necessarily pick up low levels of unevenly distributed sclerotia.

There were 9 UK fields in which no soil inoculum was detected but there was some level of black scurf on mini-tuber progeny. In five of these fields the main crop seed had visual symptoms of black scurf and disease was also found on the main crop progeny. In the 4 fields in which black scurf was found on mini-tuber progeny but not on the main crop seed, no disease was found on the main crop progeny in 3 of these fields, but in the remaining one, the level of disease on the main crop progeny was high (41%)

IMPACT OF SOIL TYPE ON DISEASE DEVELOPMENT

A controlled environment experiment was undertaken to compare detection of *Rhizoctonia solani* AG3 DNA and subsequent disease development in different soil types.

Rhizoctonia solani AG3 (0.03 g sclerotia per 7 litre pot, equivalent to 0.0005% - 0.004 % w/w depending upon the soil type) was added to eleven field soils/composts; 4 replicates per treatment were used.

Disease expression (stem canker and black scurf on progeny tubers) was compared.

Results indicated that black scurf disease expression differed significantly between soil types, with highest disease expression in a compost, and negligible disease expression in a Yorkshire clay, Figure 4-76.

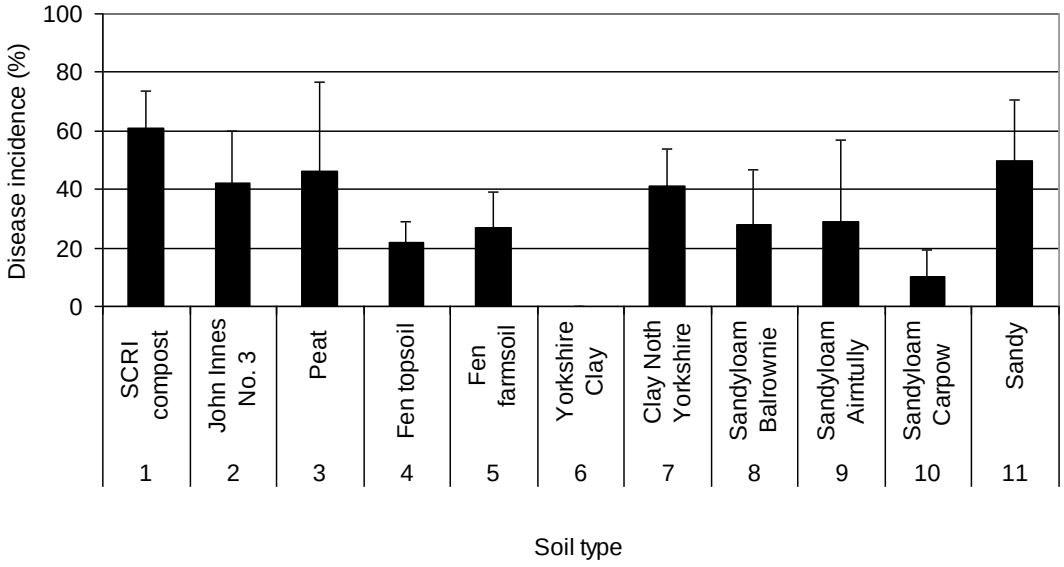

Figure 4-76 – Comparison of black scurf disease incidence on tubers grown in a range of soil types

EFFECT OF INOCULUM SOURCES

A field plot experiment was conducted to investigate the effect of seed- and soilborne *Rhizoctonia solani* AG3 inoculum (in the form of either sclerotia or mycelia) on black scurf and on tuber size distribution, and the interaction with the level of soil organic matter.

A field was selected which was naturally low in organic matter. A split plot design was created, with high and low organic matter areas of the field as main plots. A high level of soil organic matter was created with the incorporation of cow manure mixed with barley straw (approximately 35 t/ha).

Four treatments were created in each of the organic matter main-plots: Treatment A. soil inoculated with sclerotia and planted with mini-tubers; Treatment B. soil inoculated with mycelia and planted with mini-tubers; Treatment C. soil un-inoculated, planted with mini-tubers; Treatment D. soil un-inoculated but planted with mini-tubers inoculated with *Rhizoctonia solani* AG3. There were four replicates for each treatment.

Two sets of samples were taken prior to the trial being planted to ensure that no detectable inoculum was in the soil prior to the creation of the treatments. The first was taken to establish that the trial area had no detectable inoculum, the second to ensure that no inoculum was introduced with the addition of organic matter into the split plot design. After the treatments had been established and the trial planted, the area was left for one week; a soil sample was then taken from all plots, each consisting of a bulk of 25 cores per plot. Similarly, soil samples were taken from each plot at the time of the mid-season and immediately after the final harvest.

Within 5 weeks of planting the control and seed inoculated treatments in both the low- and high-organic plots had reached over 90 % emergence. Emergence was progressively delayed in the low-organic sclerotia, low-organic mycelia, high-organic mycelia and high organic sclerotia treatments respectively, with the latter taking more than 13 weeks from planting to reach over 90% emergence, Figure 4-77.

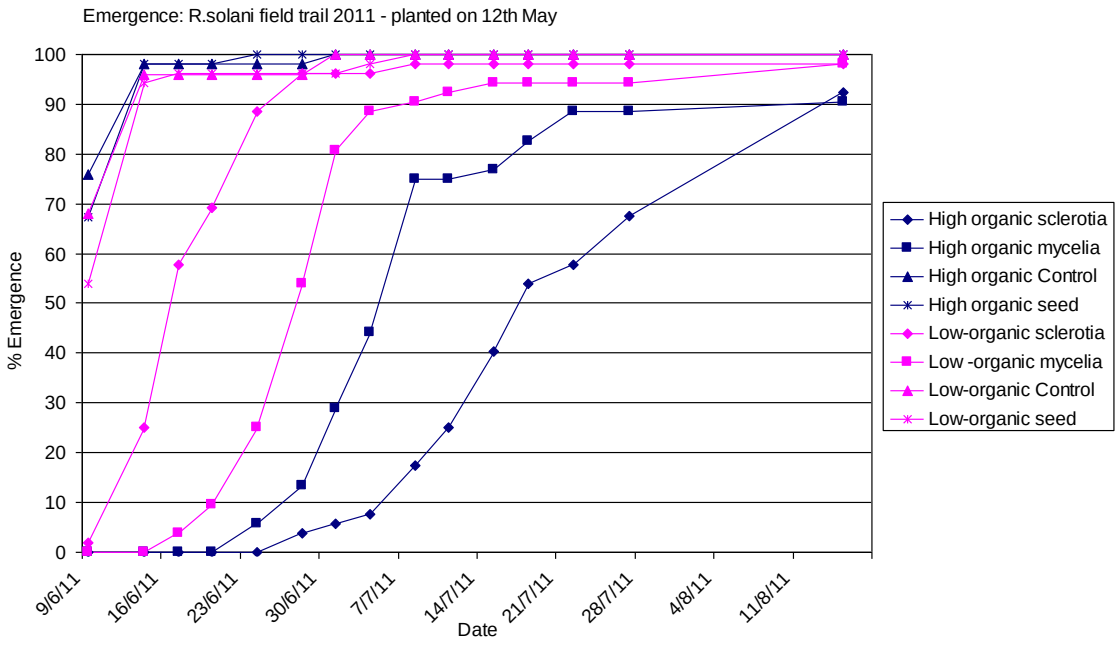

Figure 4-77 – The effect of seed- and soilborne inoculum treatments (*Rhizoctonia solani* AG3) on plant emergence in a high and low organic matter soil. Mean of four replicate plots per treatment.

Stem canker was slightly, but significantly ($P<0.05$) greater in plots with mycelia soil inoculum in high-organic soils, and both mycelial and sclerotial inoculum in low-organic soils, Figure 4-78. Both of the high-organic soil inoculum types were associated with greater numbers of pruned stolons than any other treatments ($P<0.05$), Figure 4-79.

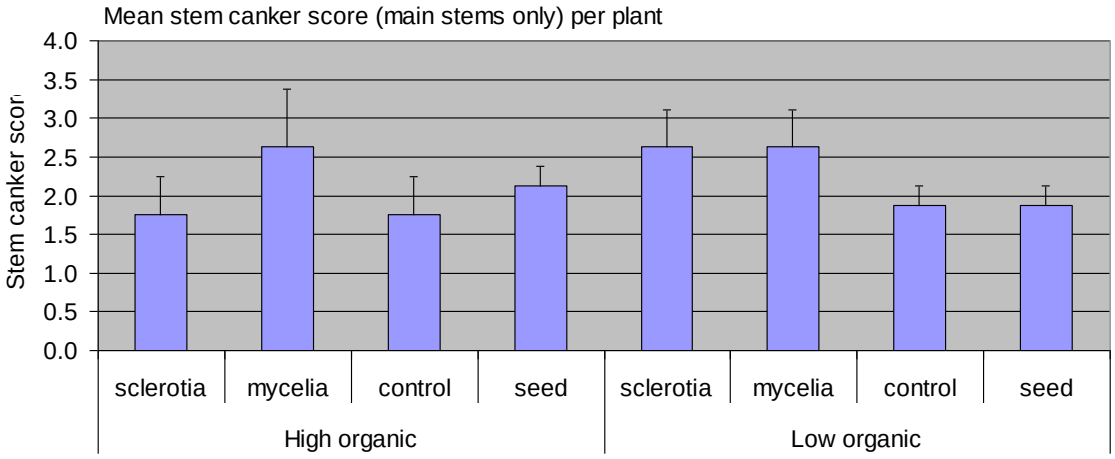

Figure 4-78 – The effect of seed- and soilborne inoculum treatments (*Rhizoctonia solani* AG3) on the mean severity of stem canker on main-stems (mean of four replicates).

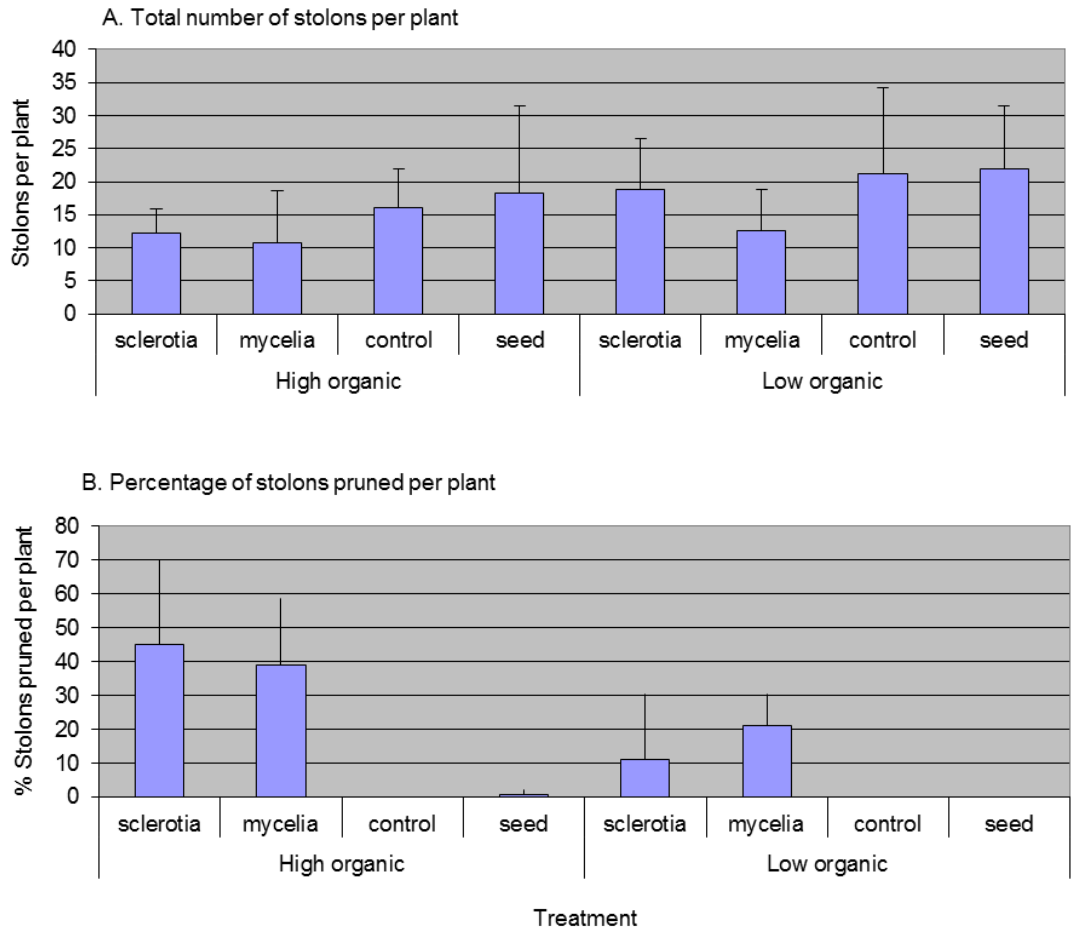

Figure 4-79 – The effect of seed- and soilborne inoculum treatments (*Rhizoctonia solani* AG3) on the total number of stolons per plant and the percentage of stolons which had been pruned per plant (mean of four replicates).

The yield of tubers was significantly lower in treatments with soil inoculum (both sclerotia and mycelia) and the effects were worse in the high-organic soils, Figure 4-80. The progressive reduction in yield from a maximum of 25kg per plot (equivalent to 52 t/ha) in low-organic sclerotia, low-organic mycelia, high-organic mycelia and high organic sclerotia reflects the length of delay in emergence between these treatments.

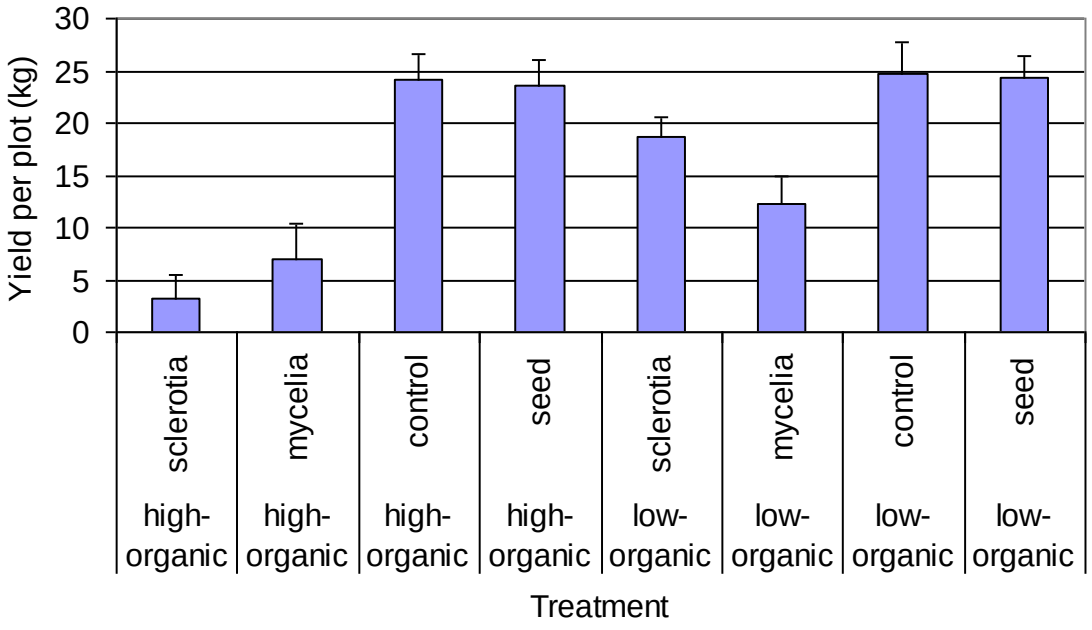

Figure 4-80 – The effect of seed- and soilborne inoculum treatments (*Rhizoctonia solani* AG3) on the yield of tubers (kg per plot) at final harvest (mean of four replicate plots).

ROLE OF TUBER-BORNE INOCULUM

Potato seed in both Great Britain and Australia is commonly infected with *Rhizoctonia solani* AG3 as determined by DNA detection as well as visual black scurf symptoms.

The results of controlled environment experiments illustrate that in the absence of soil inoculum, seed inoculum (>10 % surface area covered in sclerotia) causes high levels of both stem canker and incidence of black scurf on progeny tubers. Composts were inoculated with a mixture of mycelia and sclerotia and diluted to 1:100 or 1:50 inoculum levels. Based on the amount detected in the undiluted inoculum level (5000pg DNA / g soil) we would have expected the 1:100 dilution (50 pg DNA/ g soil) to still be detectable based on previous inoculations of field soils. As both the 1:50 and 1:100 inoculum levels caused full disease expression, the inability to detect the inoculum in the compost was identified as an issue needing more attention by the UK group.

Low levels of soil inoculum (1:100 equates to approx. 50 pg DNA/g soil) caused nearly 100 % incidence of disease, Figure 4-81.

However, in the absence of soil inoculum, seed inoculum (>10%) resulted in close to 100% incidence of black scurf on progeny tubers, Figure 4-81.

No apparent relationship between the amount of *Rhizoctonia solani* AG3 inoculum detected on seed tubers and subsequent levels of black scurf on progeny tubers has been described in commercial crops in Great Britain. However, seed inoculum undoubtedly can cause disease (both stem canker and black scurf).

Seed inoculum was significant only in the absence of soil inoculum.

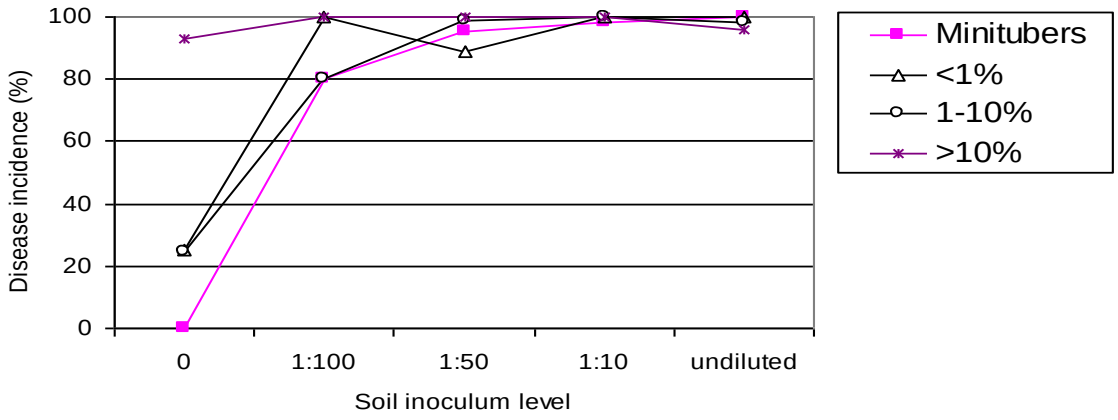

Figure 4-81 – Effect of the level of black scurf on seed tubers planted into soil inoculated with *Rhizoctonia solani* AG3.

ROLES OF *RHIZOCTONIA SOLANI* AG2.1 AND AG3 IN CAUSING STOLON PRUNING AND STEM CANKERS

A controlled environment trial was conducted in Victoria to investigate whether *Rhizoctonia solani* AG2.1 and AG3 compete with each other in soil, or act synergistically to produce disease.

SOIL DNA LEVELS

The concentrations of *Rhizoctonia solani* AG2.1 and AG3 DNA in soils inoculated with varying rates of these pathogens were measured prior to planting with minitubers (Table 4-31) and during crop growth (Figure 4-82 and Figure 4-83) below. *Rhizoctonia solani* AG2.1 DNA levels in soil tend to peak at 8 weeks and then gradually decline over time with the exception of treatment AG2.1 and 3 (1:1) where the amount of *Rhizoctonia solani* AG2.1 begins to increase after week 11. *Rhizoctonia solani* AG3 DNA levels in soil tended to increase over time with the treatment AG2.1 and 3 (1:1) being the highest at week 14.

Table 4-31 – Inoculation treatments and resulting soil pathogen DNA levels

Treatment	Inoculant level of *Rhizoctoni solani* (plates fungal mycelium per 8 kg soil)		Soil DNA concentration *Rhizoctonia solani* (pgDNA/g soil)	
	AG2.1	AG3	AG2.1	AG3
1	1	0	18,516	0
2	0	1	1	16,702
3	1	1	105,453	17,799
4	1	0.5	0	0

5	1	0.25	15,613	1,159
6	0.5	1	7,356	6,441
7	0.25	1	10,858	13,945
8	0	0.5	0	12,274
9	0.5	0	30,389	0
10	0.25	0	15,041	1
11	0	0.25	3	2,652
12	0	0	0	0

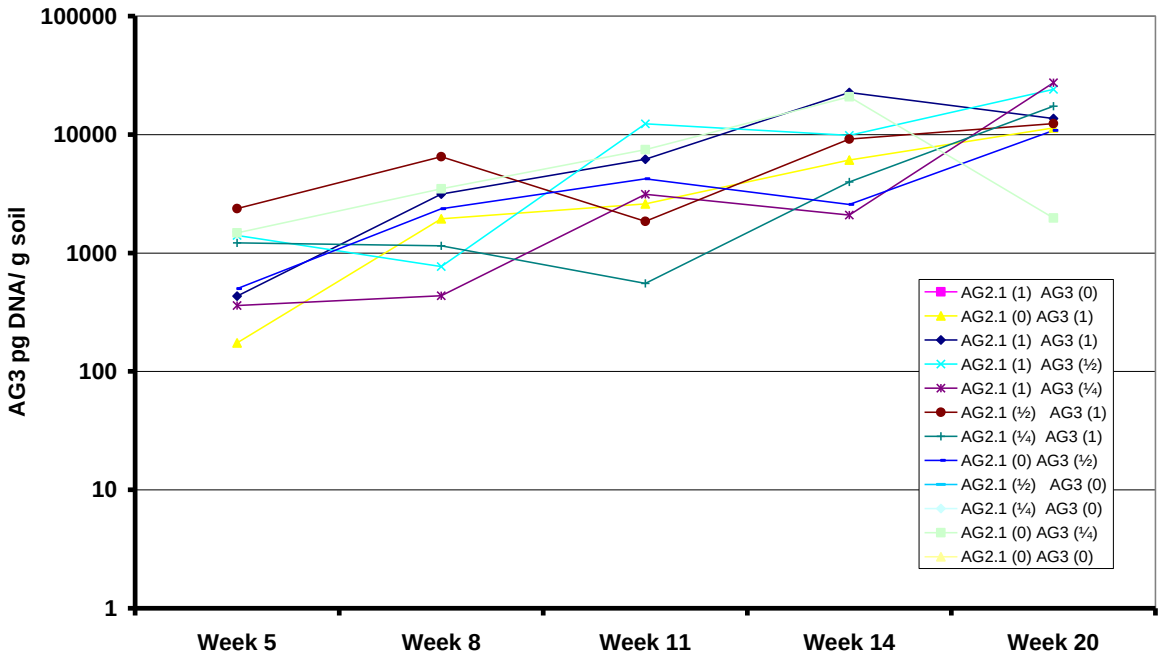

Figure 4-82 – Effect of soil inoculation treatments on change in *Rhizoctonia solani* AG3 DNA levels in potting mix over 20 weeks planted with a single potato plant.

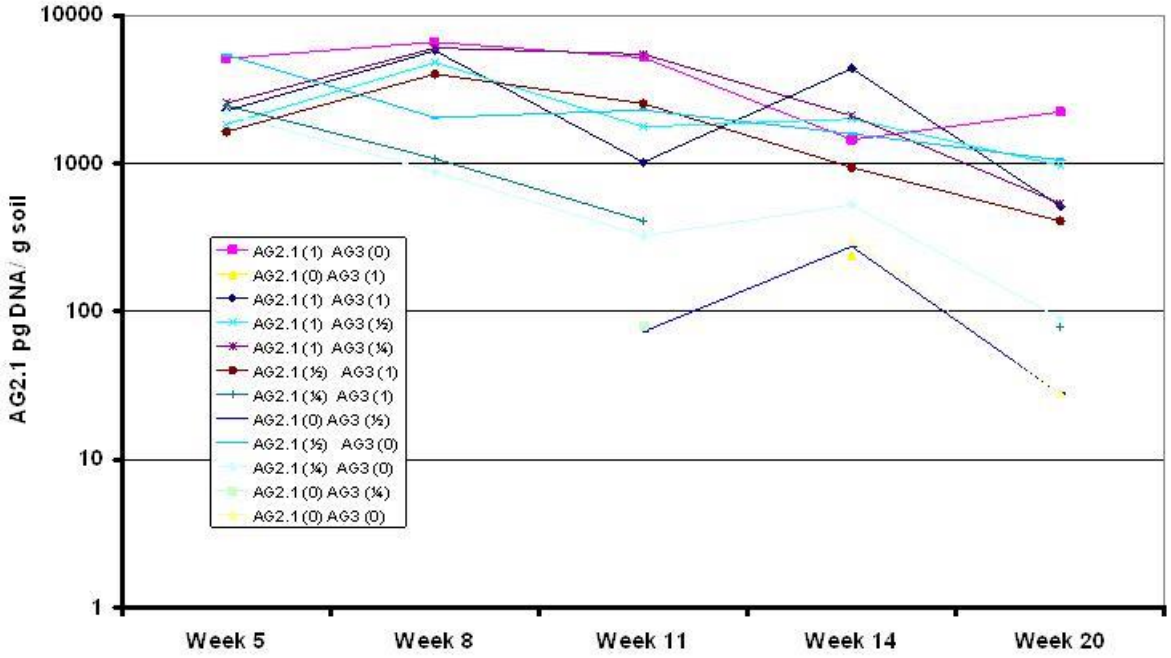

Figure 4-83 – Effect of soil inoculation treatments on change in *Rhizoctonia solani* AG2.1 DNA levels in potting mix over 20 weeks planted with a single potato plant.

STOLON PRUNING

By itself, *Rhizoctonia solani* AG2.1 produced only minor stolon pruning symptoms at each sampling date, Figure 4-84. *Rhizoctonia solani* AG3 by itself, however, caused 32-58% stolon pruning over the 4 sampling dates. When *Rhizoctonia solani* AG2.1 and AG3 were combined at the highest rate (treatment 3), stolon pruning symptoms increased from 22% to 53% over the sampling dates.

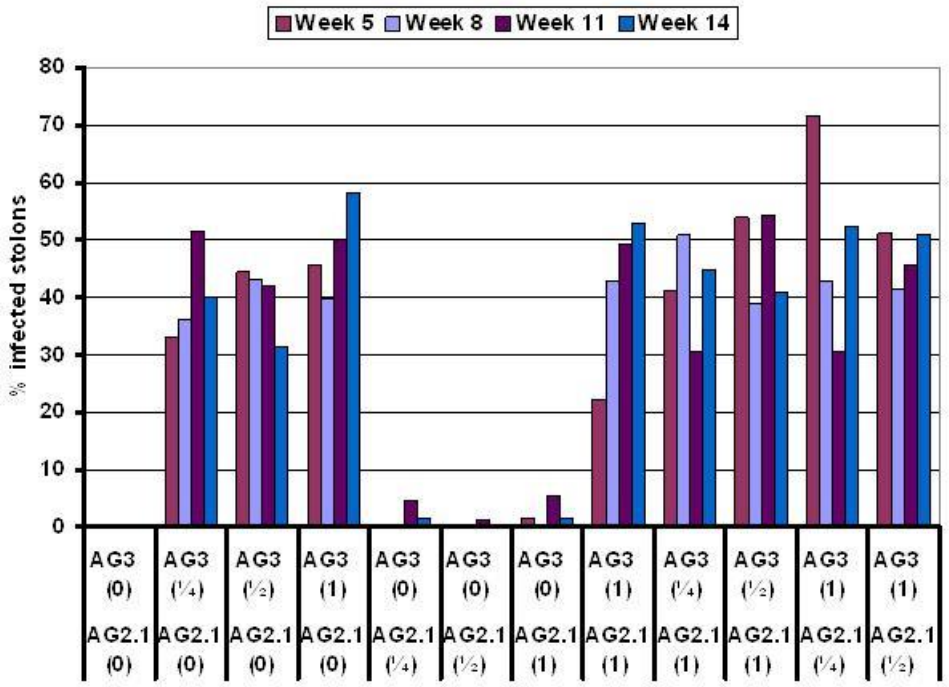

Figure 4-84 – Effect of soil inoculation treatments with *Rhizoctonia solani* AG3 and AG2.1 on stolon pruning of potato plants grown in pots at 4 sampling times.

STEM CANKER

Only the highest rate at the last sampling date of *Rhizoctonia solani* AG2.1 by itself produced stem cankers (14% stems infected), Figure 4-85. In contrast, *Rhizoctonia solani* AG3 by itself produced a minimum of 5% stem cankers by the first sampling date, increasing to up to 90% by the second sampling date. The rate of *Rhizoctonia solani* AG3 starting inoculum had no effect on the level of stolon pruning and stem canker, with the lowest rate causing as much disease as the highest rate. These results suggest that on potato, *Rhizoctonia solani* AG2.1 and AG3 do not act synergistically to produce disease symptoms.

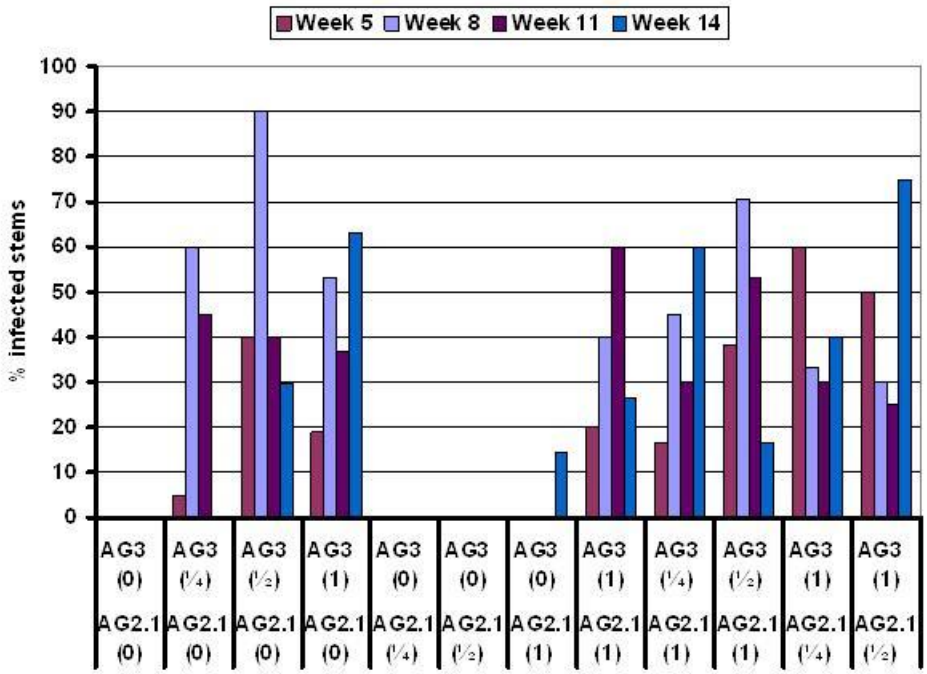

Figure 4-85 – Effect of soil inoculation treatments with *Rhizoctonia solani* AG3 and AG2.1 on stem canker of potato plants grown in pots at 4 sampling times.

IDENTIFYING ANASTOMOSIS GROUPS IN INFECTED PLANT MATERIAL

Cultivation-based analysis captures only isolates that are readily culturable or fast growing strains while cultivation-independent pathogen DNA analysis allows the detection of non culturable or slow growing strains providing a more complete picture of the infecting strains. Infected plant parts were divided into two with half being used for isolation of a culture and the other half being used directly for pathogen DNA analysis. Pathogen DNA analysis of cultures isolated from infected tissue found that *Rhizoctonia solani* AG3 is the most prevalent strain, Figure 4-86. Direct pathogen DNA analysis of infected plant material found that *Rhizoctonia solani* AG3 is the most prevalent strain, but the *Rhizoctonia solani* AG2.1 was also prevalent using this technique, Figure 4-87. Results suggest *Rhizoctonia solani* AG2.1 is not as easily isolated as *Rhizoctonia solani* AG3 and direct pathogen DNA analysis of infected tissue is a better technique to use than pathogen DNA identification from culture isolations.

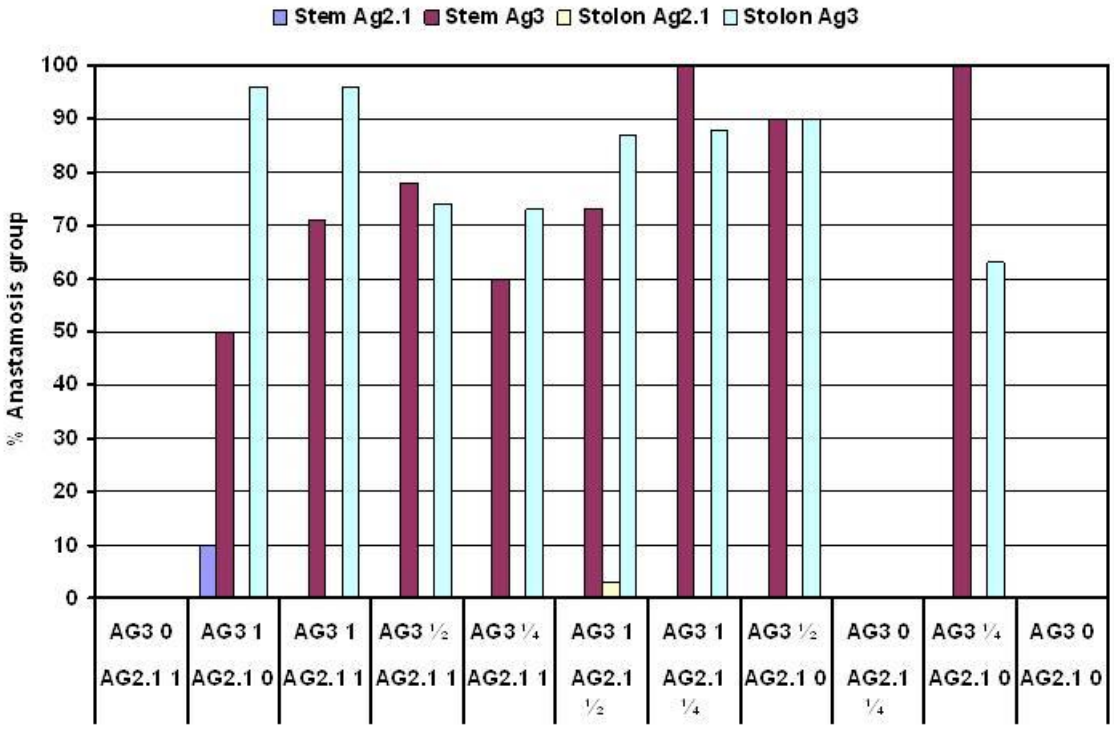

Figure 4-86 – Anastamosis group of *Rhizoctonia solani* identified by cultivation based analysis from infected plant tissue of stems and stolons (combined data from all sampling).

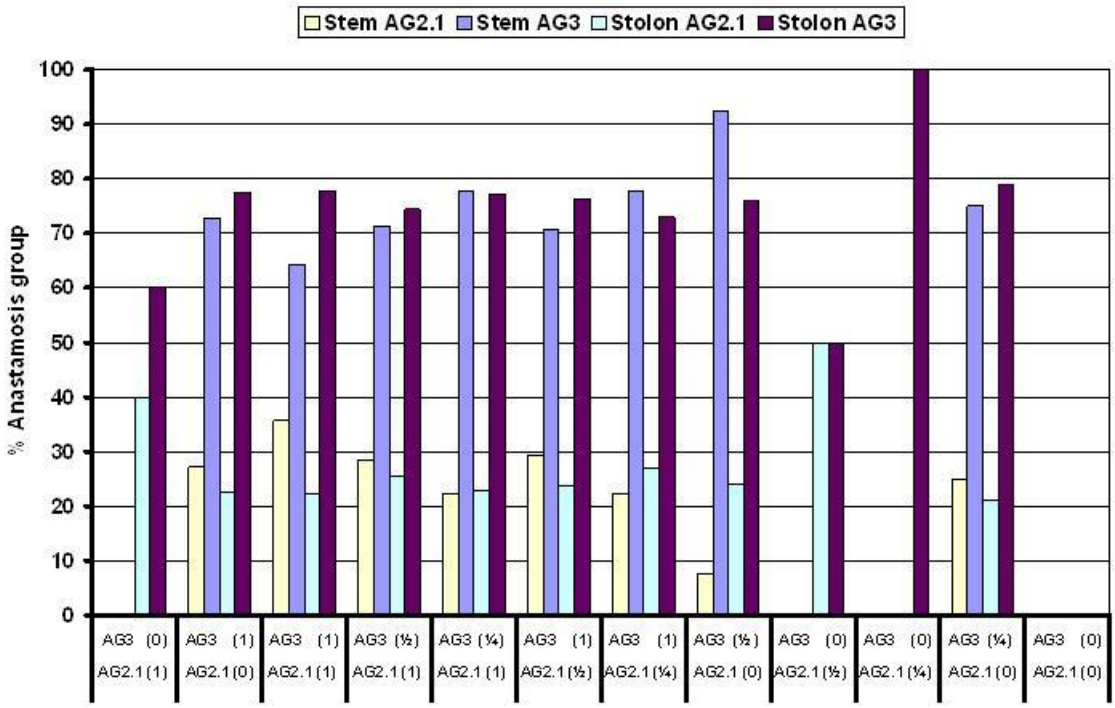

Figure 4-87 – Anastamosis group of *Rhizoctoni solani* identified by pathogen DNA testing from infected plant tissue of stems and stolons (combined data from all sampling).

VARIETY SUSCEPTIBILITY TO COMMON AND POWDERY SCAB

Powdery and common scab disease incidence and index were recorded on varieties of interest to the potato processing industry at field sites in Victoria, Table 4-32. A set of standard varieties were included to assist when comparing with other sources of variety susceptibility information.

Of the crisping varieties, Chipper, Ivory Crisp and Sonic were less susceptible to powdery scab symptoms on tubers and Chipper and Sonic were less susceptible to common scab symptoms on tubers. Of the processing varieties, Innovator, Netted Gem, Ranger Russet and Russet Burbank (clones Ruen and Vancouver) were less susceptible to powdery scab symptoms on tubers and Russet Burbank (clone Ruen) was less susceptible to common scab symptoms on tubers. Of the standard varieties, Nicola was least susceptible to both powdery scab and common scab on tubers.

Table 4-32 – Comparison of incidence and severity of powdery scab and common scab on potato varieties

Variety	Type	Powdery scab		Common scab	
		Incidence	Index	Incidence	Index
Catani	Crisping	52 ef	20 e	64 defgh	24 cdef
Chipper	Crisping	2 a	0 a	5 a	1 a
Ivory Crisp	Crisping	6 a	1 a	47 cde	15 bc
Pike	Crisping	35 cd	6 abcd	55 cdef	19 cdef
Simcoe	Crisping	11 ab	3 ab	92 j	41 h
Sonic	Crisping	4 a	1 a	24 ab	6 ab
Trent	Crisping	41 de	10 cd	87 ij	36 gh
Atlantic	Processing	27 cd	8 bcd	69 fghi	23 cdef
Innovator	Processing	2 a	0 a	68 efghi	26 Defg
Kennebec	Processing	67 g	32 f	84 hij	28 Efg
Netted Gem	Processing	3 a	1 a	63 defgh	24 Cdef
Ranger Russet	Processing	6 a	1 a	65 efgh	26 Defg
RB Ruen Ag	Processing	12 ab	2 ab	67 efghi	28 Efg
RB Ruen TE	Processing	6 a	1 a	43 bcd	14 Bc
RB Vancouver	Processing	10 ab	2 a	81 ghij	41 H
Shepody	Processing	66 fg	24 e	84 hij	36 Gh
Coliban	Standard	33 cd	12 d	61 cdefg	18 Cde
Desiree	Standard	23 bc	6 abc	70 fghi	31 Fgh
Nicola	Standard	1 a	0 A	40 bc	14 Bc
Sebago	Standard	38 de	11 cd	50 cdef	15 Bcd
p value		<.001	<.001	<.001	<.001
lsd 0.05		14	6	21	11

- RB – Russet Burbank

DEVELOPMENT OF SPONGOSPORA SUBTERRANEA ROOT GALLING AND TUBER SYMPTOMS

Differences were observed in the rate and incidence of root galling and powdery scab tuber symptoms of the varieties Russet Burbank, Desiree, Nicola and Kennebec grown at a site in Ballarat, this site had a high level of *Spongospora subterranea* DNA in the soil prior to planting. Soil temperatures, Figure 4-88, at the trial site were conducive to the development of tuber symptoms and root galling (van de Graaf, Wale, & Lees, 2007).

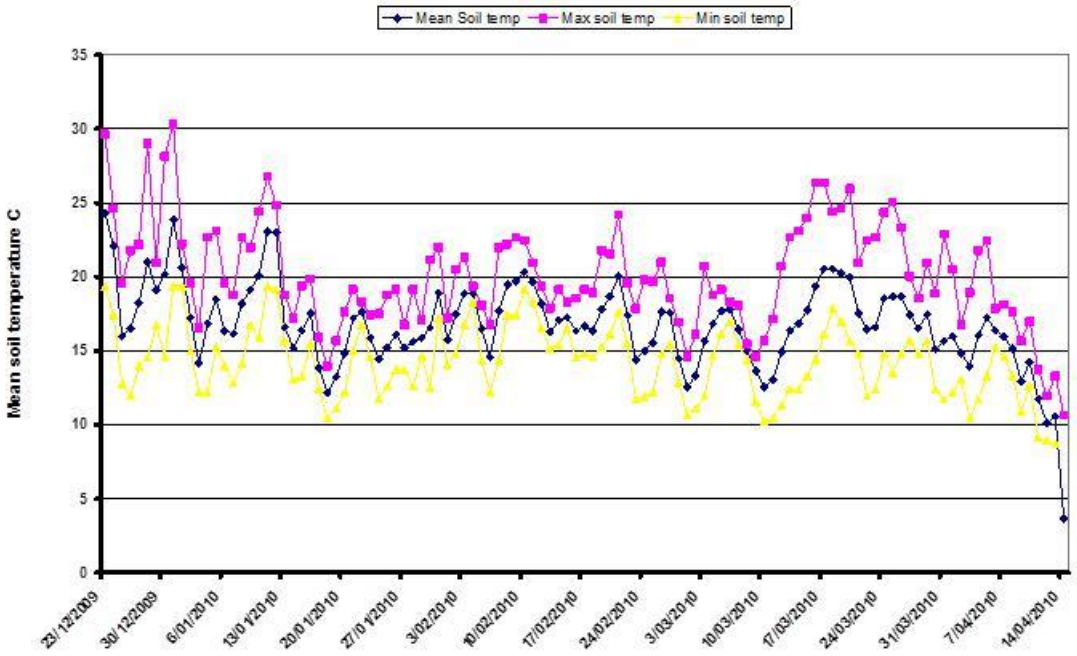

Figure 4-88 – Soil temperature at site where potatoes grown to assess *Spongospora subterranea* root galling in Ballarat

A comparison was made of root galling symptoms at nine sequential lifting dates from 50% emergence to harvest, Figure 4-89. No root galls were observed on Nicola. Root galling was first observed on the Kennebec variety 48 days after planting and on Desiree and Russet Burbank at the next assessment 55 days after planting. This timing coincided with the highest level of *Spongospora subterranea* DNA in roots of all varieties, Figure 4-90. Galling increased to its highest observed level at 70 days after planting.

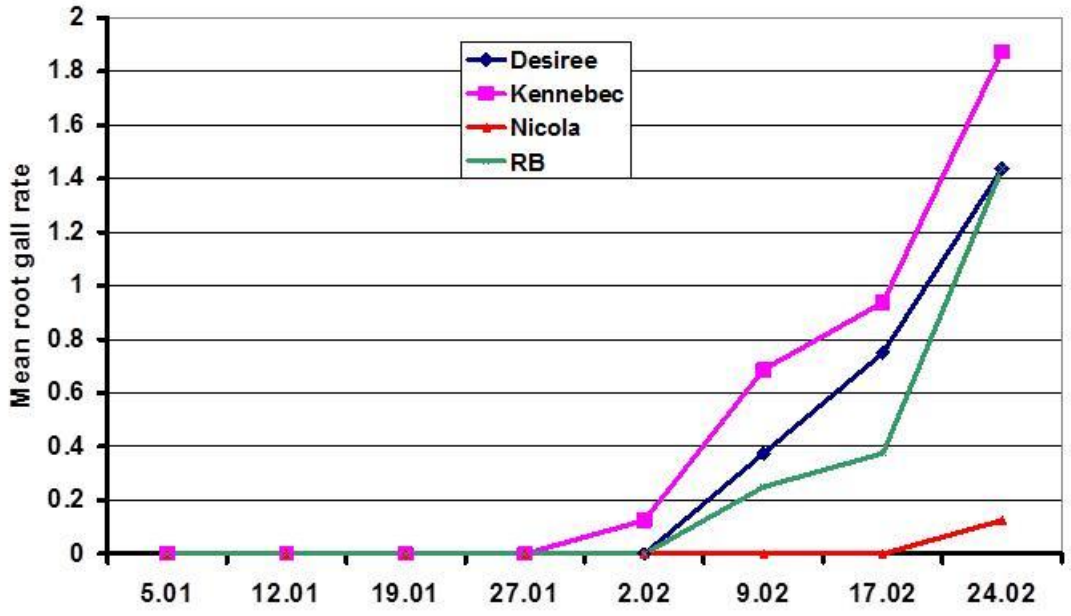

Figure 4-89 – Rate of root galling on the varieties Desiree, Kennebec, Nicola and Russet Burbank (RB) over the period from January 5 to February 24, 2010 grown at Ballarat

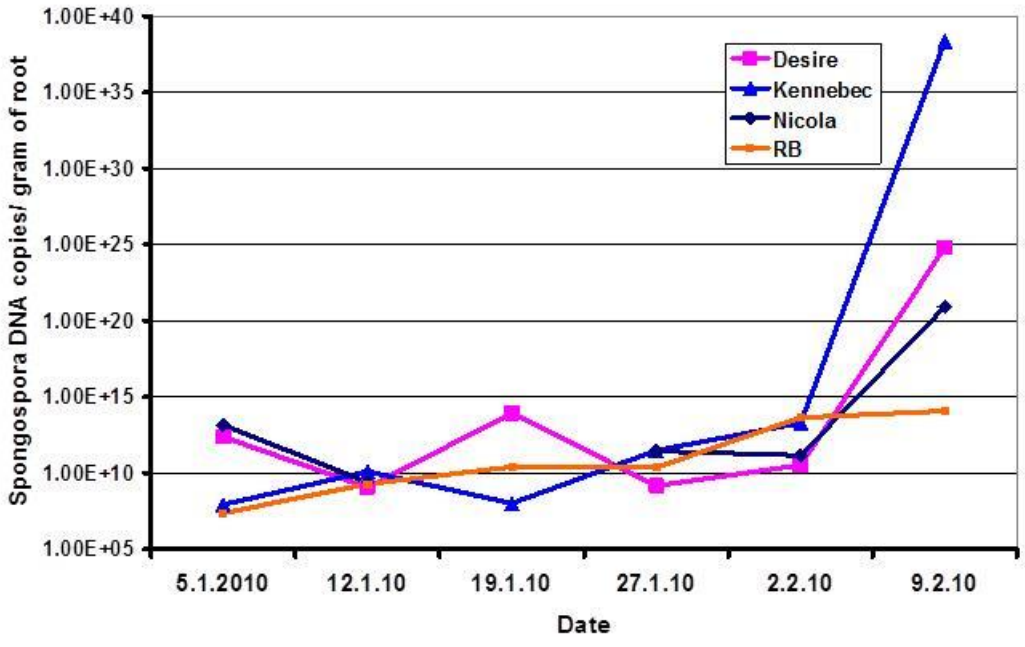

Figure 4-90 – Concentration of *Spongospora subterranea* DNA in the roots of the varieties Desiree, Kennebec, Nicola and Russet Burbank (RB) over the period from January 5 to February 9, 2010 grown at Ballarat

A comparison was made of the incidence and severity of powdery scab symptoms on tubers, Figure 4-91. Powdery scab symptoms first appeared 63 days after planting on tubers of the Kennebec and Desiree varieties and increased over the growing season with tubers having the highest incidence at harvest. Russet Burbank and Nicola only showed minor symptoms at harvest. The concentration of *Spongospora subterranea* DNA in the peel of tubers from all varieties generally increased over the sampling times, Figure 4-92. Tubers of the Kennebec and Desiree varieties consistently had more *Spongospora subterranea* DNA present than Russet Burbank and Nicola. Nicola had the least *Spongospora subterranea* DNA present in the peel which is consistent with symptom development.

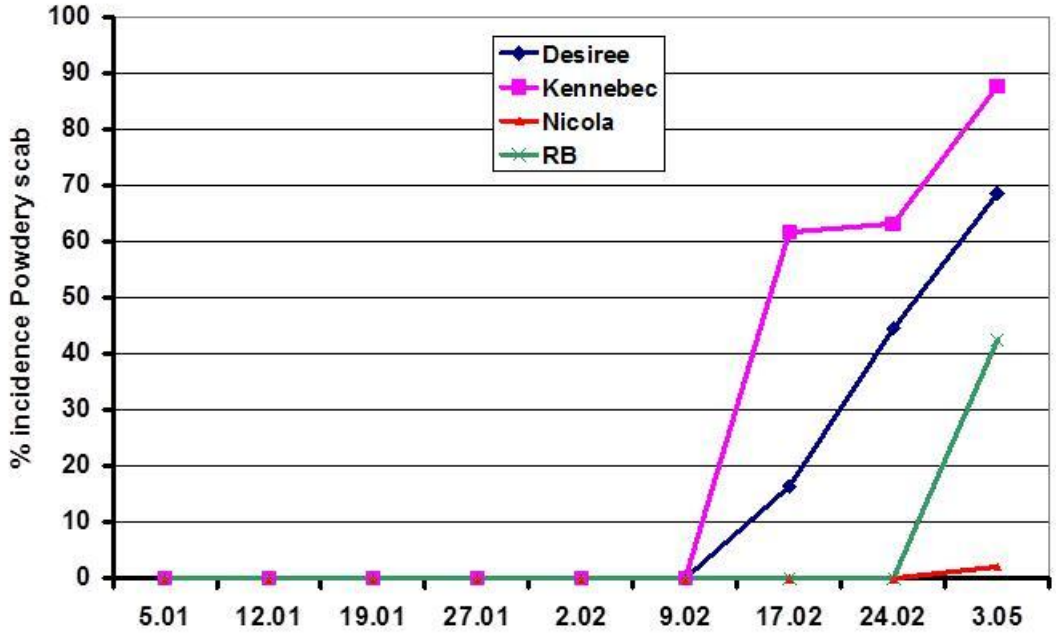

Figure 4-91 – Incidence of powdery scab symptoms on tubers on the varieties Desiree, Kennebec, Nicola and Russet Burbank (RB) over the period from January 5 to May 3, 2010 grown at Ballarat

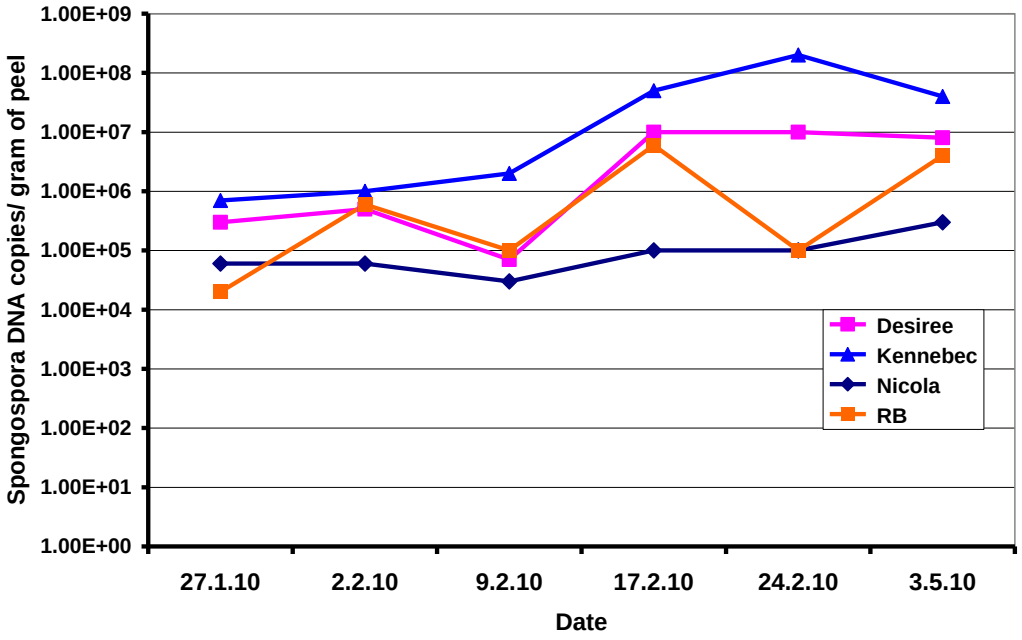

Figure 4-92 – Concentration of *Spongospora subterranea* DNA in the peel of tubers of the varieties Desiree, Kennebec, Nicola and Russet Burbank (RB) over the period from January 27 to May 3, 2010 grown at Ballarat

At harvest the concentration of *Spongospora subterranea* DNA in the soil had increased, with the trend in soil levels being consistent with varietal susceptibility to root galling and tuber symptoms, Table 4-33.

Table 4-33 – Mean concentration in the soil of *Spongospora subterranea* (pgDNA/g soil) prior to planting and after harvest depending on variety grown

Variety	Soil DNA concentration *Spongospora subterranea* (pgDNA/g soil)	
	Pre-planting	Harvest
Desiree	247	1675
Kennebec	253	3858
Nicola	161	201
Russet Burbank	240	761

TECHNOLOGY TRANSFER

The outcome of this project for industry is the delivery of DNA testing technology, including interpretation of tests with respect to disease risk, to potato growers. As part of the commercial roll-out of this service the project team has been engaged in a range of technology transfer activities. These include promotion of the service through potato processing companies, the potato industry extension network in association with AUSVEG, Potato industry conferences and local grower meetings as well as articles in Potatoes Australia magazine and via other industry bodies.

At a technical level the project has delivered accreditation training workshops to establish a network of personnel in the potato industry that understand the PreDicta Pt service, including how to use and interpret the results from the service to the potato industry. A potato soilborne disease risk management manual has been produced as a reference to assist with this process.

The field validation component of this project has been conducted in commercial fields. Many growers have been exposed to the soil DNA testing technology as part of this process and seen the results of testing and disease evaluations in their own paddocks. Approximately 66 processing potato growers have been involved in this process across South Australia, Tasmania and Victoria. In the later years of the project growers were provided with their soil DNA test results by project staff prior to planting, enabling them to utilise the information in their decision making.

Specific technology transfer activities and publications are listed below.

REFEREED JOURNAL ARTICLES

Brierley, J. L., Sullivan, L., Wale, S. J., Hilton, A. J., Kiezebrink, D. T. & Lees, A. K. (2013) Relationship between *Spongospora subterranea* f. sp. subterranea soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62:413–420.

SCIENTIFIC CONFERENCE PAPERS

Australasian Soilborne Disease Symposium, Twin Waters, Queensland, 8-11th August 2010.

Tonya Wiechel – Poster – *Rhizoctonia* in potato crops

Robin Harding - Poster – Soil sampling for pathogen DNA testing

Alan McKay - Invited talk on molecular diagnostics for decision making by growers which included the work undertaken in APRP.

Australasian Plant Pathology Society Conference, Darwin, Northern Territory, 27-30 April 2011.

Tonya Wiechel convened Diseases of potatoes workshop prior to the conference 26 April 2011. Workshop attended by 22 specialists in potato diseases, including Robin Harding.

American Phytopathological Society meeting, Hawaii, USA, 6-10 August 2011

Tonya Wiechel – Abstract- Comparison of culture based and culture independent methods for identifying *Rhizoctonia solani* AG2.1 and 3 inhabiting infected plant material of potato.

8th World Potato Congress, Edinburgh, Scotland, 27-30 May 2012

Kathy Ophel Keller – Poster – APRP program, soil pathogen DNA testing

Australasian Soilborne Disease Symposium, Fremantle, Western Australia, 17-20 September 2012.

TJ Wiechel & F Richardson – Poster - *Rhizoctonia solani* AG3 DNA levels in the presence of potato

International Congress of Plant Pathology, Beijing, China, 25-30 August 2013.

TJ Wiechel, NS Crump, A McKay & K Ophel Keller – Abstract - Preliminary validation in grower's field of soil DNA tests for 3 potato pathogens.

INDUSTRY & MEDIA PUBLICATIONS

Potatoes Australia articles

Bridging the gap. Article featuring the pilot project to PT09023. April/May 2010

International collaboration increasing R&D knowledge. April/May 2011

A collaborative project develops diagnostic tests for soilborne pathogens. Oct/November 2011

Predicting potato pathogens. August/September 2013

Diagnostic tests for soilborne pathogen – PreDicta Pt fact sheet SBD 2/June 2014.

AUSVEG Potato Extension Program

INDUSTRY & PEER PRESENTATIONS

2009 /10

Grower meetings at Cora Lynn, Ballarat and Penola

Tonya Wiechel and Robin Harding

2010/11

Potato Industry conference, Geelong, 26-27 July 2010

Robin Harding and Tonya Wiechel

Simplot Potato Futures, Ulverstone, Scottsdale, Longford

Robin Harding

2011/12

3rd European Powdery scab workshop, Boldern, Switzerland, 11-13 July 2011

Tonya Wiechel attended along with 18 international powdery scab experts

McCain Foods Agricultural Conference, Ballarat, 25 August 2011.

Tonya Wiechel and Kathy Ophel Keller.

APRP2 Review Meeting, Melbourne, 6-7 October 2011

Kathy Ophel Keller and Tonya Wiechel

Safries grower meeting, Penola, 1 December 2011

Michael Rettke and Kathy Ophel Keller.

AUSVEG Conference, Hobart, 10-12 May 2012.

Kathy Ophel Keller

2012/13

Potato Industry Extension Workshops in Ballarat, Cresswick, Warragul (Victoria) Murray Bridge, Mount Gambier (South Australia) Sassafras (Tasmania) Vasse (Western Australia) and Gatton (Queensland)

Tonya Wiechel, Kathy Ophel Keller and Michael Rettke.

Potatoes 2012 Conference, Ballarat, 29-31 July 2012

Michael Rettke.

2013/14

Potato Industry Extension Workshops in Berrigan (New South Wales) and Gatton (Queensland)

Michael Rettke.

Simplot Potato Futures, Ulverstone, Scottsdale, Longford

Michael Rettke

Safries grower meeting, Mount Gambier, 21 August 2013

Michael Rettke

APRP2 Symposium and Review Meeting, Melbourne, 18-19 September 2013

Kathy Ophel Keller, Tonya Wiechel and Michael Rettke

Snackbrands field day, Thorpedale, 7 February 2014

Tonya Wiechel

McCain Foods field day, Ballarat, 5 March 2014

Tonya Wiechel

APRP2 Symposium and future research meeting, Melbourne, 19-20 May 2014

Kathy Ophel Keller and Michael Rettke

2014/15

Simplot Research and Development day - Ulverstone, 15 July 2014

Tonya Wiechel

2014 Potato Industry Conference, Mount Gambier, 10-12 August 2014

Michael Rettke.

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

PREDICTA PT

This project has developed a commercial DNA testing service, PreDicta Pt, to allow assessment of the risk posed by key soilborne pathogens pre-planting. This is a new service and will require support, promotion and follow up to be successful in the long term.

Since its commercial release in September 2013 the testing service for powdery scab (*Spongospora subterranea*), root knot nematode (*Meloidogyne fallax*) and black dot (*Colletotrichum coccodes*) has been utilised by potato growers in Tasmania, Victoria and South Australia.

Key uses of the test are;

- Indicate disease risk before planting.
- Assist pre-season decision making:
  - choosing paddocks
  - matching varieties to risk
  - considering treatments and management strategies.
- Monitor impacts:
  - changed practices, e.g. rotations or varieties.

Suggested forward strategies to foster adoption and continue to drive industry benefit from this service are;

- Facilitate grower group program for on-farm demonstration of the use, decision making and evaluation of benefits from utilising PreDicta Pt and implementing those decisions.
- Continue to develop linkage between PreDicta Pt results and practical disease management options.
- Capture examples of where commercial use of the PreDicta Pt test has improved outcomes for promotion to the wider industry.
- Resource specific program to follow up, evaluate and assist those using the PreDicta Pt test during the establishment phase of commercial service.
- Promote, monitor and reinforce the importance of using correct soil sampling procedures.

Suggested forward strategies to further increase scope, attractiveness and benefits of the PreDicta Pt service are;

- Development of viable options to mitigate disease risk where not currently available.
  - Short term – seasonal disease management
  - Long term – managing inoculum
- Incorporate peel testing into the PreDicta Pt service.

- Progress additional pathogen DNA tests from "Tests Under Development" into the PreDicta Pt service
- Add further tests
    - *Phytophthora erythroseptica* (cause of pink rot)
    - *Fusarium oxysporum*
- Link with other tests for soil health being developed
    - Soil quality indicators, as developed in initiatives such as GRDC-funded Soil Quality Monitoring program

PATHOGEN DNA RESEARCH TESTS

As a result of this project the potato industry has ongoing access to testing capability to quantify pathogen DNA levels in the soil, peel of tubers and other substrates for powdery scab (*Spongospora subterranea*), (*Meloidogyne fallax*), black dot (*Colletotrichum coccodes*), common scab (pathogenic *Streptomyces*), potato early dying (*Verticillium dahliae* and *Pratylenchus crenatus*, *P. neglectus*, *P. penetrans*), root knot nematodes (*Meloidogyne fallax*, *M. hapla*, *M. javanica*/*incognita*/*arenaria*), black scurf and other diseases caused by *Rhizoctonia solani* AG3 and AG2.1.

Access to this technology has opened up new opportunities at both an immediate on-farm practical level and to undertake research and development approaches that were previously not feasible. Areas where this pathogen DNA testing capability should be utilised include:

- Monitoring and understanding changes in pathogen inoculum levels associated with changed on farm practices.
- Assessing the effectiveness of management strategies in reducing disease risk, such as crop rotations.
- Closing the yield gap by understanding the contribution of soil and seed borne pathogens to yield loss and how this can be better managed.
- Unlocking knowledge about pathogen interactions occurring in the soil.
- Monitoring long term trends in pathogen levels and associated disease occurrence.
- Confirming diagnosis of diseases.
- Confirming suitability of field sites for disease investigations by quantifying the level of inoculum in the soil.
- Quantifying inoculum levels in the peel of seed tubers.
- Supporting disease assessments in research trials.
- Providing another level of insight when conducting epidemiology studies.
- Providing an understanding of pathogen activity, with and without presence of symptoms.

SPECIFIC PATHOGEN TESTS - RECOMMENDATIONS FOR FUTURE WORK

The following is a list of suggestions in relation to specific pathogens for future work that would further enhance the value of this technology. Suggestions are based on the overall progress and findings made in this project, along with identified impediments to adoption and future maximisation of the value from specific pathogen DNA tests.

SPONGOSPORA SUBTERRANEA

- Refine interpretation guide for growing regions and key varieties.
- Improve variety susceptibility information (including root galling).
- Assess and quantify benefits of biofumigant crops and other available control options.
- Quantify impact of root galling (especially in Tasmania) on yield and inoculum build-up in the field
    - Determine pre-plant pathogen DNA risk thresholds specifically for symptoms associated with root infection and root galling.

MELOIDOGYNE FALLAX

- Assess and quantify benefits of improved rotations, biofumigant crops, new biologicals and other available control options (especially in the southeast of South Australia).
- Assess in season tuber peel testing as an alternative to pre-dig tuber inspection to flag risk of tuber symptoms developing.
    - Determine in-crop tuber peel DNA thresholds in relation to harvest timing and in-ground storage potential.

MELOIDOGYNE HAPLA

- Confirm if presence in tubers is causing any currently undetected problems, such as reduced seed vigour or increased wastage in storage.
- Continue to monitor potential for yield loss.

STREPTOMYCES TXTA GENE

- Develop an integrated disease risk interpretation guide. Other information is required to assess risk. In combination, DNA test results provide useful information.
- Replicated field trials to assess contribution to disease of both soil and seed inoculum in commercially grown crops.
- A year of intensive monitoring of pathogenic and non-pathogenic *Streptomyces* DNA levels in soil in a range of production systems to understand short term drivers of population dynamics.

RHIZOCTONIA SOLANI AG3

- Determine sampling intensity required within a paddock to become a useful test.
- Using higher intensity more targeted sampling strategies, complete field validation of *Rhizoctonia* 'burn- off' prior to emergence.
    - Confirm pre-plant pathogen DNA risk thresholds for burning off.

RHIZOCTONIA SOLANI AG2.1

- Complete further field validation of *Rhizoctonia* burning off prior to emergence and incidence of deformed tubers.
    - Confirmation of pathogen DNA risk thresholds for burning off and deformed tubers.

COLLETOTRICHUM COCCODES

- Assess contribution to yield loss in commercial fields including measures at an individual plant basis for yield and disease incidence.
- Confirmation of pathogen DNA risk thresholds for yield loss.

POTATO EARLY DYING (*VERTICILLIUM DAHLIAE* AND *PRATYLENCHUS* SPP.)

- Complete further validation concentrating on yield loss in commercial fields including measures at an individual plant basis for yield and disease incidence.
    - Confirmation of pathogen DNA risk thresholds for yield loss.

ACKNOWLEDGEMENTS

We would like to thank the 66 growers in South Australia, Tasmania and Victoria who allowed us access to their properties to undertake the field validation work, as well as for their contribution of time, expertise and resources. A number of growers hosted specific trials and we are especially appreciative of the assistance they provided.

We would like to acknowledge the invaluable contribution of representatives of the potato processing companies McCain Foods, Simplot Australia, Snack Brands Australia and PepsiCo Australia & New Zealand for their support, advice and assistance.

Establishment of the PreDicta Pt service has been conducted under the oversight of an industry reference group. The council provided by the members of this group played an important guiding role in the establishment and roll out of the service and we thank them for their constructive advice.

The project team would like to acknowledge the technical assistance of Dr Nigel Crump, Luke James and Joe Smith (ViCSPA).

The project team would like to acknowledge the technical assistance of Russell Burns, Geoff Fennell, Nigel Percy, Ina Dumitrescu, Tiffany Barlow and Aiden Thompson (SARDI).

We gratefully acknowledge the funding and support from the Processing Potato Industry and the Commonwealth Government through Horticulture Australia.

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APPENDIX 1 – WEATHER DATA

Table 4-34 – Mean monthly temperatures and rainfall totals for the 2010/11 to 2013/14 growing seasons sourced from selected weather stations located in processing potato growing areas in South Australia, Tasmania and Victoria where field validation was conducted.

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Robe, South Australia							
Mean daily minimum temperature (°C)	**2010/11**	7.6	8.4	9.6	9.9	12.1	13.7	14.2	14.2	12.2	11.4	10.4	9.2
	2011/12	8.6	9.3	9.7	11.1	13.1	13.3	15.5	14.6	13.5	12.9	11.2	8.7
	2012/13	8.6	8.7	9.2	10.2	11.9	13.9	14.4	16.1	16.1	12	10.8	8.8
	2013/14	9.1	10.1	11.2	11	10.7	12.2	14.6	13.8	12.2	12.5	11.5	10.2
Mean daily maximum temperature (°C)	**2010/11**	13.8	13.5	14.7	18.1	19.7	21.5	22.3	22	19.9	19	15.9	14.7
	2011/12	13.9	15.5	17	18.4	20.9	21.4	24.4	22.4	22	20.4	16.1	14.2
	2012/13	14	14.3	16.3	17.8	20.8	21.9	23.1	24.9	24.7	19.8	17.3	14.7
	2013/14	14.5	15.3	17.8	16.9	18.8	20.8	24.2	23	21.3	19.3	17.1	15.4
Monthly rainfall total (mm)	**2010/11**	89.2	129	73.8	38.8	28	86.4	41	49.6	39.2	34.2	63.2	73
	2011/12	65.2	60.4	43.8	31.4	41.4	10	9.4	12.4	45.2	37.4	82.8	127
	2012/13	64.8	119	37.2	35.8	37.8	15.2	3.6	13.9	21.1	25.9	78.3	79.6
	2013/14	126	155	57.8	76.6	21.2	28.4	20	33.8	26.4	41.6	66.7	86.5

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Penola, South Australia							
Mean daily minimum temperature (°C)	**2010/11**	4.3	5.1	6.5	7.3	9.6	10.9	12.3	12.6	10	7.8	7.1	5
	2011/12	5.1	7	6.8	8.6	9.8	10.9	13.4	13	10.7	8.4	7	4.8
	2012/13	5.7	5.4	5.7	6.2	8.9	10.2	10.8	13.1	12.7	7.7	7.2	5.4
	2013/14	6.4	7.1	8.6	8.2	7.7	9.6	12.6	11.5	9.8	8.9	8.6	7.3
Mean daily maximum temperature (°C)	**2010/11**	14.2	13.6	15.2	19.9	22.7	24.3	26.7	25.6	22.2	20	15.7	14.9
	2011/12	14	16.3	18.3	20.1	24.6	26.4	29.4	27.6	24.2	22.7	16.8	14
	2012/13	14.1	14.5	17.1	19.6	24.4	26	28.4	29.7	27.4	21.8	18.3	14.9
	2013/14	14.5	15.4	18.9	18.2	20.9	25.3	29.8	28.6	25.2	21.4	18.8	14.9
Monthly rainfall total (mm)	**2010/11**	81.6	126	85.4	43.4	32.2	97	97.2	49.1	88.3	29.4	72.4	81.6
	2011/12	98.5	93.3	33.2	39.8	46.1	33.2	7.4	2.9	39.9	27.2	45.8	126
	2012/13	71.6	109	48.6	30.2	27.7	20.9	1.8	25.8	21.8	33.9	55.1	66.6
	2013/14	138	157	107	92.8	31.7	28.7	24	18	35.8	51	62.2	116

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Mount Gambier, South Australia							
Mean daily minimum temperature (°C)	**2010/11**	4.7	5.6	7	6.9	9.5	10.8	12.1	12.3	10.9	9.2	7.8	6.2
	2011/12	6	6.9	7	8.6	10.1	10.7	13.3	12.9	10.9	9.4	7.9	5.7
	2012/13	5.6	6	6.4	6.7	9	10.8	11.1	13.7	13.2	9	7.7	6
	2013/14	6.1	7.4	8.5	8.3	8.2	9.9	12.8	11.9	10.6	9.7	9.3	7.6
Mean daily maximum temperature (°C)	**2010/11**	14	13	141	18.8	21.1	23.2	24.8	24.1	21.4	19.1	15.2	14.3
	2011/12	13.6	16.1	17.1	19	22.4	24.4	27.9	26.2	23.7	21.9	16	13.5
	2012/13	14	14	16.6	18.6	23.4	24.5	26.8	28.4	26.8	20.6	17.3	14.6
	2013/14	14.1	15	18.1	17.3	19.6	23.7	28.1	27.4	24	20.3	17.6	14.4
Monthly rainfall total (mm)	**2010/11**	98.2	163	78.2	59.8	42.6	101	122	54.8	59.6	54	82.6	89.8
	2011/12	79.2	97.2	62	44.8	72.6	29.2	13.8	7.6	50.2	45.4	83.2	126
	2012/13	78.4	114	54	33	20	26.2	7.4	17	27.2	41.8	68.8	82.2
	2013/14	159	178	58.8	117	47.4	30.8	27.4	22.2	40.4	62.8	111	143

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Devonport, Tasmania							
Mean daily minimum temperature (°C)	**2010/11**	4.3	4.7	6.4	8	10	10.4	13.3	11.9	10.8	9.5	4.9	5
	2011/12	4.7	7.4	6.4	7.4	9.9	11.1	13	13.4	10.6	9.1	6.6	4.7
	2012/13	4.2	4.5	6.4	6	9.6	10.7	11.5	14.1	12.5	8.1	6.4	5.3
	2013/14	5.5	6.3	7.7	8.1	9.2	10.9	12	12.5	12.5	9.1	7.3	5.9
Mean daily maximum temperature (°C)	**2010/11**	13.4	13.1	14.5	16.1	17.9	19	21.3	20.7	19.2	17.6	14.6	13.8
	2011/12	12.2	13.9	15.5	16	18.5	20.4	22.4	22	20.5	18.6	15.7	13.4
	2012/13	13	13.1	14.6	15.8	18.7	20.5	21.9	22.7	22	17.6	15.4	14
	2013/14	13.6	13.3	15	16.1	17.5	19.7	21.5	22.7	21.4	18.3	16.1	14.7
Monthly rainfall total (mm)	**2010/11**	69.9	84.7	87.2	77.5	66	104.2	118.4	63.6	96	81.4	32.6	67.7
	2011/12	69	100.1	51.9	63.2	113.3	19.4	31.2	66.6	66.1	35	92.4	59.9
	2012/13	43.3	89.2	100.4	10	57.6	68.7	15.3	25.5	78.4	32.4	108.5	23.1
	2013/14	135.2	214.9	112	62.2	90.2	54.1	19	0.9	60.9	59.8	77.4	49.2

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Cressy, Tasmania							
Mean daily minimum temperature (°C)	**2010/11**	0.6	2.3	4	5.9	8.5	8.2	11.2	9.2	8.5	6.4	1.7	2.1
	2011/12	2.2	5.6	4.1	4.9	7.4	9.1	10.7	11.4	8.3	6.3	4.1	1.9
	2012/13	1.7	2.1	4.4	4.2	6.7	7.4	9.9	11	9.3	4.7	3	2.1
	2013/14	2.4	3.5	6	6.6	7.7	8.3	9.6	10	9.9	5.3	3.9	1.9
Mean daily maximum temperature (°C)	**2010/11**	11.9	12.5	14.2	17.4	19.3	20.4	23.9	23	20.9	17.7	13.7	12.1
	2011/12	11.5	13.6	15.3	17	20.4	23.3	26	25	21.6	19.2	14.7	11.7
	2012/13	11.6	12.6	15	17.2	20.8	23.6	26.2	26.1	25.2	18	15.2	12.9
	2013/14	12.5	12.9	15.5	16.6	18.1	21.7	25.6	26	23.1	17.9	15.2	12.9
Monthly rainfall total (mm)	**2010/11**	42.4	68.8	55.4	57.4	80.4	87.4	70.7	43.4	119	45.5	23.4	98.6
	2011/12	31.4	65	35.4	37.8	69.4	16	19	23.8	34.6	42.6	101	70.2
	2012/13	29.2	50.6	83.8	15.2	27.6	48	20	22	72.4	12.6	56.2	16.2
	2013/14	99.8	147	86.4	69.6	115	36.8	17.8	6.8	45.7	64.2	32.8	52.6

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Scottsdale, Tasmania							
Mean daily minimum temperature (°C)	**2010/11**	3.9	3.3	4.5	6.8	8.5	8.7	12	11.1	9.9	7.6	4.2	4.2
	2011/12	3.6	6.2	4.8	5.6	8	10.2	11.7	11.9	10	8.1	5.4	4.2
	2012/13	3.6	3.8	5.1	4.8	8.1	8.7	10.1	12.8	11.1	7.7	5.9	4.5
	2013/14	5.2	4.3	6.3	5.9	7.5	9.1	10.5	11.7	11.4	7.9	6.2	5.3
Mean daily maximum temperature (°C)	**2010/11**	12.5	11.9	13.5	16.2	18.6	19.5	22.2	20.9	19.2	17.2	13.8	12.5
	2011/12	11.6	13.6	14.8	16.3	19.1	20.8	24	22.8	20.3	18.1	14.6	12
	2012/13	12.1	12.4	14.4	16.9	19.4	21.5	23.6	24.1	23.7	17	14.6	12.9
	2013/14	12.5	12.2	15	15.7	18.1	20.9						
Monthly rainfall total (mm)	**2010/11**	101	125	131	80.2	74.2	83	115	77.2	129	93.6	32	107
	2011/12	48.2	118	50.4	57.4	119	41.8	50	41.6	69.4	54.8	120	88.4
	2012/13	67.2	116	86.8	23.2	43.8	73.8	12.4	27.4	99.2	32.4	102	33.6
	2013/14	154	283	125	101	54.8	67	20	22.6	53.4	108	73.4	82.4

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Ballarat, Victoria							
Mean daily minimum temperature (°C)	**2010/11**	3.1	3.4	5	6.3	8.6	9.4	12.7	12	10	7.2	5.4	3.9
	2011/12	3.9	3.9	4.3	6.7	9.6	9.9	12.5	12.4	9.8	6.9	4.6	3.8
	2012/13	3.5	3.2	4.7	5.5	8.4	10	10.8	12.8	11.9	7.2	4.8	3.2
	2013/14	3.7	4.2	5.9	5.7	7.8	8.8	13	12.4	10.4	9.1	6.2	5.4
Mean daily maximum temperature (°C)	**2010/11**	10.4	10	12.3	16.9	19.3	21.9	24.6	23.3	20	18	12.3	11.6
	2011/12	10.5	13.1	15	17.3	21.3	23.1	26.6	25.7	20.5	19	13.6	10.8
	2012/13	10.9	11.1	14.6	17.2	21.6	23.6	27.3	27.7	24.4	19.2	14.6	11.7
	2013/14	11.4	12.3	16	16.3	18.4	23.7	28.2	28.0	23.9	17.5	15.5	11.5
Monthly rainfall total (mm)	**2010/11**	55	161	108	92.8	101	58.4	206	96.8	20.4	31.2	65.4	41
	2011/12	49.6	46.8	54.8	65.6	92.8	25.4	22.2	87.2	50.8	44.6	51.4	81.2
	2012/13	83	70	45.6	29.8	33.6	33.6	1.8	115	12.4	11	41.4	49
	2013/14	73	73.6	60.2	70.8	35.8	26	20.6	6.2	26.6	71.2	28.2	84

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Morwell, Victoria							
Mean daily minimum temperature (°C)	**2010/11**	2.6	3.9	6	6.9	9.8	11.4	14.6	13.8	11.3	8.6	6.4	4.2
	2011/12	4.5	4.1	5.8	8.2	11	11.3	13.6	13.6	11.6	9.5	5.9	4
	2012/13	3.6	4.5	6.5	7.1	9.8	11.1	12.3	13.8	11.9	8.8	5	4.4
	2013/14	4.1	6.1	7.7	7.5	9.1	11.5	13.1	12.7	12.2	9.4	6.8	5.9
						Morwell, Victoria							
Mean daily maximum temperature (°C)	**2010/11**	13.6	13.5	15.1	19.6	22.5	23.6	26.8	24.8	22.1	19.7	15	14.9
	2011/12	13.1	16.2	18.1	19.9	23.1	23.7	26.7	25.4	22	20.5	16	13.3
	2012/13	13.8	15	17.3	20	22.9	25.3	28.7	28.8	27.4	20.5	17.1	14.1
	2013/14	14.9	15.7	18.7	19	21.1	24.8	28.8	29	26.2	20.1	18.4	14.7
						Thorpedale, Victoria							
Monthly rainfall total (mm)	**2010/11**	40.6	110	78.2	121	105	66.2	43.8	175	222	103	67.2	54.6
	2011/12	78.8	45	89.2	86.4	165	6.8		72	101	70.4	116	92.6
	2012/13	56	102	94.2	63.2	48.4	80	10.6	46	49.6	37.8	73.6	142
	2013/14	68.8	139	101	36.2	20.6	59.2	45.8	27.6	58.6	81	52.6	130

Parameter	Growing season	July	August	September	October	November	December	January	February	March	April	May	June
						Koo Wee Rup, Victoria							
Monthly rainfall total (mm)	**2010/11**	52.6	128	82.2	109	129	84	77.5	131	37.5	87.2	79.9	48.4
	2011/12	56.2	34.6	70	85.4	150	49.6	46.7	71.2	71.4	96.7	116	132
	2012/13	44.4	65.4	70.7	47.3	34	59.3	3.9	57.2	58.1	18.6	89.4	132
	2013/14	62			83.1		70.4	26.5	21.8	39.2	71		118

APPENDIX 2 – TRAINING WORKSHOP EVALUATION SHEET

Evaluation PreDicta Pt Workshop Adelaide, 25th July 2014

Name _____

Module	Rating 1 - 5 (Poor – Excellent)	Comments
Introduction		
PreDicta Pt		
Powdery scab		
Root-knot nematodes		
Black dot		
Other tests		
Sampling		
Accessing technology		
Case studies		
Accreditation questions		
e-manual		
Do you need the hard copy manual?		
Would you prefer online Training?		

Comments

Horticulture Australia

PT09026 – (30/10/2015)

Soil Health/ Disease Mitigation Program

Final Report

Dr Rudolf de Boer et al

Department of Environment and Primary Industries, Victoria

PROJECT SUMMARY

PT09026 – Soil Health/ Disease Mitigation Program

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This is the Final Report for HAL Project PT09026 “Soil health/disease mitigation”, which comprises Final Reports for subprojects A(i) “Soil Amendments and Nutrients”, A(ii) “Impact of rotations”, B(i) “Bacterial Endophytes”, B(ii) “Novel Chemical Treatments for Control of Common Scab” and C “Monitoring the Bacterial Wilt Pathogen in Irrigation Water”.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, and A&L Canada Laboratories, and matched funds from the Australian Government.

The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, The University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority have provided in-kind support

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CHAPTER 5. APRP2 – SOIL HEALTH/DISEASE MITIGATION PROGRAM

MEDIA SUMMARY

The soilborne diseases powdery scab, common scab, Rhizoctonia, Verticillium wilt and bacterial wilt are the most difficult for growers to manage. This collaborative project across Australia, New Zealand and Canada built on exciting new possibilities identified in Australian Potato Research Program Phase 1 to develop and demonstrate new methods of control.

Manipulation of soil chemistry proved effective for controlling powdery scab and Rhizoctonia black scurf. Powdery scab was consistently reduced in field trials by soil incorporation of elemental sulphur and zinc. The improved tuber quality increased acceptable yields for French fry up to 10 t/ha and for crisping up to 20 t/ha.

Manipulation of soil biology was indicated as a potential key to common scab control. A disease suppressive soil was identified and the suppressive element, biological in nature, was demonstrated to be transferable. Molecular profiling of 'healthy' versus 'diseased' soils identified distinct microbial communities in each, which has potential for further development into a 'soil health bio-indicator'. Millet green manure treatments modified soil microbiology, increasing potato yields by up to 48% in Canada.

A rich diversity of promising candidate bacteria from potatoes grown in Canadian and Australian soils was examined. Several showed promise in field trials as seed treatments against common scab and others as bio-fertilisers to increase yield.

Novel plant hormone analogues were identified for controlling common scab. Foliar and tuber application rates and timing were determined that effectively controlled disease, with no detrimental effects on plant growth, tuber yield and quality. These compounds appear to protect the potato from the pathogen's toxins.

A world-first study used a soil DNA test on 42 commercial sites in south eastern Australia to monitor the influence of crop rotations on potato pathogen DNA annually over 8 years. This identified that, once powdery scab was present in a paddock, it rarely fell below disease threshold levels and rapidly increased whenever a potato crop was grown.

The Bunyip River is suspected to be a source of bacterial wilt. Regular water sampling over five seasons detected the pathogen only once, suggesting that another source exists in this river catchment. An abundant aquatic weed growing in the water courses was identified as a host of the bacterium. A brochure was produced for growers that outlines disease prevention as the most significant control measure.

Key outputs of the R&D are draft decision support trees for the diseases powdery scab, common scab and Rhizoctonia, a brochure outlining the prevention and management of bacteria wilt, a concept for a 'soil health bio-indicator' and identification of novel control compounds and biologicals.

TECHNICAL SUMMARY

This project explored a range of potential new control options for the intractable soil-borne diseases powdery scab, common scab, Rhizoctonia canker and black scurf, Verticillium wilt and Bacterial wilt. Three interrelated sub-projects explored disease management through the manipulation of nutrients and soil health factors, novel approaches (chemical and biological) and disease management in a river catchment. This collaborative study, which involved researchers from Australia, New Zealand and Canada, was built on research outcomes from the Australian Potato Research Program (APRP) Phase 1 and from related projects.

Key outputs from the R&D are draft disease management decision support trees for the powdery scab, common scab and Rhizoctonia diseases, identification of novel compounds and biologicals, a brochure outlining the prevention and management of bacteria wilt, and a concept for a 'bio-indicator" analysis of soil health.

Manipulating soil chemistry proved effective for controlling powdery scab. The disease was consistently reduced by soil incorporations of elemental sulphur and zinc in five years of trials, but not as effectively as the fungicide fluazinam (not registered in Australia) used as a control. In the susceptible cultivar Shepody, the improved tuber quality from fluazinam, elemental sulphur and zinc EDTA translated to an increased 14, 10 and 8 t/ha accepted by the French fry factory and 27, 23 and 11 t/ha at the Crisping factory. Zinc EDTA applications also reduced Rhizoctonia black scurf, but manipulation of soil chemistry for control of common scab was less successful.

Novel chemicals were investigated for control of common scab. Optimal rates and timing of foliar and tuber treatments of the synthetic auxin 2,4-D were determined. The very low rates that reduced disease had no noticeable detrimental effect on plant growth, tuber yield or quality, with 2,4-D residues in tubers well below the maximum MRL. A number of similar compounds were identified and chemical inhibition of thaxtomin-A toxicity was identified the likely mode of action. These synthetic auxin treatments will require further development before they can be recommended for growers.

Manipulation of soil biology showed promise for controlling common scab. Terminal restriction fragment length polymorphism (TRFLP) was used to profile the rhizosphere microbial communities from 28 Victorian potato fields classed as positive or negative for *Streptomyces scabies* and/or common scab. Distinct microbial community profiles were evident, providing the basis for a potential "bio-indicator" of disease risk and soil health. A disease suppressive soil was identified and the suppressive element, biological in nature, was demonstrated in pot trials to be transferable. Determining the casual organisms of suppression will help identify new options for common scab control.

During APRP1 and PT07038, screening of thousands of bacteria isolated from potato rhizospheres of cultivars grown in Canadian soils using chaperonin gene technology (*cpn60*) identified a diversity of biological functions including antibiosis or the ability to fix nitrogen (*nifH*). In a more recent study of potato endophytes, a number of growth promoting bacteria were identified. Further screening identified three species of *Pseudomonas,* which when inoculated onto diseased seed tubers in a field trial, effectively reduced common scab in the progeny. Additionally, potato plants inoculated with a consortium of free-living nitrogen fixing bacteria had a ten-fold greater biomass than un-inoculated plants when grown in a field soil.

In a related study, pyrosequencing and TRFLP analysis identified at least 55 different genera of bacteria in different potato cultivars grown in Australia soils. More than 400 bacterial endophytes, specifically actinobacteria (predominantly *Streptomyces* spp.), were isolated from potato plants. Of these, 17 showed a

strong *in-vitro* activity against *S. scabies* and *R. solani* AG3. When applied as seed-tuber inoculants in greenhouse and field trials, four demonstrated moderate control of soilborne common scab.

In the final two years of a four year trial initiated during APRP1 in Canada, different treatments of millet green manure crops increased marketable yields of potato between 20 to 48% compared with continuous cropping of potatoes. These increases were not attributed to reductions in disease but to changes in the soil chemical and microbial environment.

In a world-first study, soil DNA tests conducted annually over 8 years monitored inoculum of *Rhizoctonia solani*, *Spongospora subterranea* and *Streptomyces scabies* in response to different rotations on commercial farms in South Australia and Tasmania. *S. subterranea* and *R. solani* anastomosis group (AG) 2.1 were the most prevalent pathogens in both states. None of the rotations were associated with consistent reductions in pathogen DNA, and growing a potato crop in the rotation increased *S. subterranea* inoculum 10 times greater than any other pathogen. An analysis of the data predicted that if *S. subterranea* is detectable in soil, a cropping cycle of one in five years is unlikely to return fields to a low risk powdery scab category.

The bacterial wilt bacterium (*Ralstonia solanacearum*) was detected only once during intensive sampling of the Bunyip River in the Koo Wee Rup region of Victoria over five seasons, suggesting that, although the river is a potential source of inoculum, other factors may be contributing to the survival of the pathogen. The bacterium was detected in the aquatic weed Starfruit (*Damasonium minus*) and in potatoes from previous crops. A brochure developed for growers urges the implementation of a strict farm biosecurity program and outlines measures for prevention and mitigation of bacterial wilt.

INTRODUCTION

The soilborne potato diseases, common scab, powdery scab and Rhizoctonia, are widespread throughout the world's production areas including Australia. These diseases cause significant economic impacts through yield loss, costs of control measures, rejection of unmarketable potato tubers, both for processing and seed, as well as increased costs and reduced product recovery during processing. Current estimates of costs to the processing industry attributable to common scab are $1.6 million, $13 million to powdery scab and $5 million to Rhizoctonia. Overall industry losses are in the order of $20 million pa.

Bacterial wilt is caused by a bacterium (*Ralstonia solanacearum*) and, although not widespread in Australia, the disease can seriously reduce potato production where it occurs. Crop losses of up to 90% have been reported on farm, while additional losses in potato quality are observed in the processing of diseased tubers.

This project is an integrated research strategy focused on the concept of soil health and disease mitigation for potatoes. The main targets of the research were the diseases powdery scab (*Spongospora subterranea*), common scab (*Streptomyces scabies*) and Rhizoctonia (*Rhizoctonia solani*) (stem and stolon canker and black scurf), with a minor focus on Verticillium wilt (*Verticillium dahliae*) and Bacterial wilt. The project consisted of three interrelated components that complement each other and other research projects in Australian Potato Research Program (APRP) Phase 2. The research was undertaken in Australia (Victoria, Tasmania and South Australia), Canada and New Zealand, building on international collaborations and research outcomes delivered from the APRP Phase 1 (PT04016) (Kirkwood 2010), and from other Australian research projects. An outline of the project is presented in Figure 5-1.

A. Soil amendments/ameliorants for soil health and disease suppression – disease management strategies through improved soil health

This subproject was focused on the concept of soil health, building on the "Soil Health" subproject of APRP1 (Crump 2010).

Soil health refers to the physical, chemical and biological features of a soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. Because of the very wide diversity of natural and agricultural ecosystems, the soil also has to be "fit for purpose" within the constraints of a cropping system, for example, for potato production. Soil health can also be defined as the capacity or fitness of the soil to support crop growth without resulting in soil degradation or otherwise harming the environment. The concept is important here because there are very few simple management control options, such as fungicides, for these soil-borne diseases.

This project focused on the management of the chemical and biological properties of the soil within the constraints of the physical factors (e.g. "soil type"). Research in APRP1 had demonstrated that particular nutrients and nutrient ratios, e.g. calcium, sulphur, zinc, iron, pH, manganese and the potassium to magnesium ratio, were correlated with reduced incidence and severity of common and powdery scab.

Researchers in Canada, strongly focused on the role of microbiology in potato health and productivity, used molecular tools to characterise bacteria closely associated with the potato plant and identify groups that could be exploited for disease suppression and improved productivity, highlighting the critical importance of soil microbiology in crop management.

Crop rotation is a critical disease management tool fitting well within the concept of improving soil health, with further research focused on how rotations affect pathogen populations.

The subproject A (i) was a collaborative project involving researchers from DEPI Victoria, the Plant and Food Research Institute New Zealand and A&L Canada. The main components of the work are outlined below.

A (i) Soil amendments and Nutrients - The use of soil amendments/ameliorants and manipulation of nutritional factors to control potato diseases, improve soil health and improve productivity (DEPI Victoria, Plant and Food Research New Zealand and A&L Canada).

Manipulating soil nutrients for disease control (DEPI Victoria, Plant and Food Research NZ, A&L Canada)

Evaluation of various nutrient treatments (different forms of sulphur, zinc, iron, magnesium, potassium, manganese, pH modifiers, potassium to magnesium ratios) on the pathogen, disease and potato yield through a series of field trials in major cropping areas, complemented with glasshouse and laboratory experiments. The aim was not only to develop nutrient thresholds but to also understand possible mechanisms of disease control with nutrients and, where possible, to provide specific disease control recommendations and ultimately to enhance the interpretation of disease risk as determined by DNA diagnostic tests, linking with project PT09023.

A&L Canada provided the nutrient analysis of soil samples from field and glasshouse trials conducted in Victoria and provided prescriptions for nutrient treatments to be evaluated in field trials to achieve target concentrations of single elements or ratios of elements.

Manipulating the soil environment for disease control (A&L Canada and DEPI Victoria)

Completion of the final two years of a four-year study begun in APRP1 on the effects of different ways to grow and manage a millet green manure crop on disease (common scab and Verticillium wilt) and potato yield.

Isolating, characterising and exploiting microbes associated with potato plants for disease control and improved productivity (A&L Canada)

Building on a significant selection of bacteria collected from the potato rhizosphere and endosphere in previous APRP1 and related projects, this team evaluated groups of bacteria for their activity against potato pathogens (antagonistic bacteria) and for potato productivity (nitrogen fixing bacteria). Promising candidates were tested in the field for the control of seed-borne common scab or for their effect on potato productivity. The resilience of these bacteria in surviving on inoculated potato plant roots was also tested. A critical element of using beneficial microorganisms is their effective delivery in a production system.

Understanding disease suppression in soils (DEPI Victoria and A&L Canada)

Development of protocols for soil tests using DNA based techniques to profile the populations of the soil microbiological communities and to identify indicators of soil health and productivity. These tools were used to profile the biological communities in soil samples from Victorian fields that had common scab or were free of common scab, and a study of a common scab suppressive soil from Victoria was undertaken to determine whether this property could be transferred to other soils and determine the nature of this suppressiveness.

A (ii) Survey of the impacts of commercial rotations on pathogen inoculum levels (Tasmanian Institute of Agriculture).

This study correlated changes in the populations of key potato pathogens with particular crops or pasture in rotations over time. It continued a survey started in APRP1, involving annual sampling of commercial

sites (28 in Tasmania and 15 in South Australia) to monitor changes in populations of a suite of potato pathogens, as measured by the concentration of pathogen DNA in the top 15 cm of soil over an eight year period. Crop rotation is a common recommendation to reduce the build-up of pathogen populations but in reality, little is known about how soil-borne potato pathogen populations are affected by rotations. This study, using the soil DNA test, is a world first, and this knowledge will help inform the use of rotations for disease management.

B. Novel approaches to disease control

B (**i) "Endophyte discovery"** – microbial endophytes and their potential for disease suppression in Australian potato production (Flinders University and the Tasmanian Institute of Agriculture/University of Tasmania).

This project at Flinders University identified potato endophytes belonging to a particular group known as actinobacteria, and investigated their potential use as potato seed tuber treatments for the suppression of disease in daughter tubers, and their potential for commercial development as "inoculants". This research built on the success of an "endophyte discovery" project for soil-borne pathogens of cereals in which endophytes were discovered that suppress root disease in cereals.

This research complements the research by A&L Canada, which was focused on a much broader group of bacterial microorganisms taken from the rhizosphere (inhabiting the root surface) and the endosphere (inside the plant),with the aim of finding microbes with different functions, including disease suppression, fixing nitrogen or promoting plant growth.

B (ii) Novel chemicals for disease control – evaluation of foliar and tuber applications of sub-lethal concentrations of 2,4-D, and other synthetic auxins, as a potential management option for common scab of potato (Tasmanian Institute of Agriculture/University of Tasmania).

Research in the United Kingdom in the 1980s, and follow up research at the Tasmanian Institute of Agriculture, identified the chemical 2,4-D, a synthetic auxin used as a herbicide, as an effective control for common scab on potato tubers when sprayed on potato foliage a very low rates. The mechanism of activity for this chemical lies in the inhibition of the pathogen toxin, thaxtomin A, at the site of infection on tubers, thus preventing the disease symptom. The aim of this project was firstly to optimise a treatment for common scab control, including optimum timing, frequency and minimal rate of application, how these factors adapt to the major cultivars, and analysis of the MRLs required for commercial use. The second objective was to investigate alternative analogue materials to 2,4-D with significant disease suppressive activity. Novel chemistries were identified that inhibit the scab toxin but had no negative effects on plant growth.

C. Monitoring the bacterial wilt pathogen in irrigation water to identify effective catchment management strategies for control of bacterial wilt of potatoes (Victorian Certified Seed Potato Authority).

Bacterial wilt is a costly problem that recurs sporadically in some potato growing districts in Victoria, New South Wales and Queensland. The disease can be managed through rotations (more than two years without Solanaceous hosts), clean seed and an effective farm hygiene program. Despite this, a significant number of bacterial wilt cases are reported each year in ware crops. Irrigation water sourced from infested waterways is a well-known source of spread of this pathogen in Europe. However, this source of spread had not been tested in Australia. As this disease could be spread through water, management at a local level is best achieved through a whole-of-catchment approach, rather than by the actions of individual landholders. The aim was to investigate the levels of the bacterium in waterways through the regular

monitoring of water samples taken from strategic locations on the Bunyip River and its tributaries in the Koo Wee Rup Swamp. Data from this case study would validate UK diagnostic tests, determine if the bacterium is prevalent in irrigation water sources, identify periods when irrigation water sources are highly infested and identify potential sources of the contamination of irrigation sources that could contribute to the incidence of bacterial wilt. The information gained from this project was to be captured into best management strategies that could be employed wherever bacterial wilt occurs.

The intended outcomes for of this combined project (A(i), A(ii), B(i), B(ii) & C) include:

- An industry equipped with the best available knowledge on how to reduce soilborne diseases of potatoes by manipulation of soil factors
- An industry equipped with the best available knowledge on how to reduce inoculum of soil-borne potato pathogens sustainably through crop rotation
- Improved productivity through reduced losses to the major soilborne diseases of potato in Australia
- A whole-of-catchment approach to controlling the spread of bacterial wilt
- Identification and evaluation of novel approaches to disease management (endophytes and hormones)
- An enhanced research capacity for the potato industry through PhD training programs.

Intended key outputs from this program were draft disease management decision trees for powdery scab, common scab and Rhizoctonia disease built on the outcomes of this and the previous program. The decision trees and their supporting information are designed to help growers make more informed decisions on how to reduce disease risk and how to better manage these diseases in their fields.

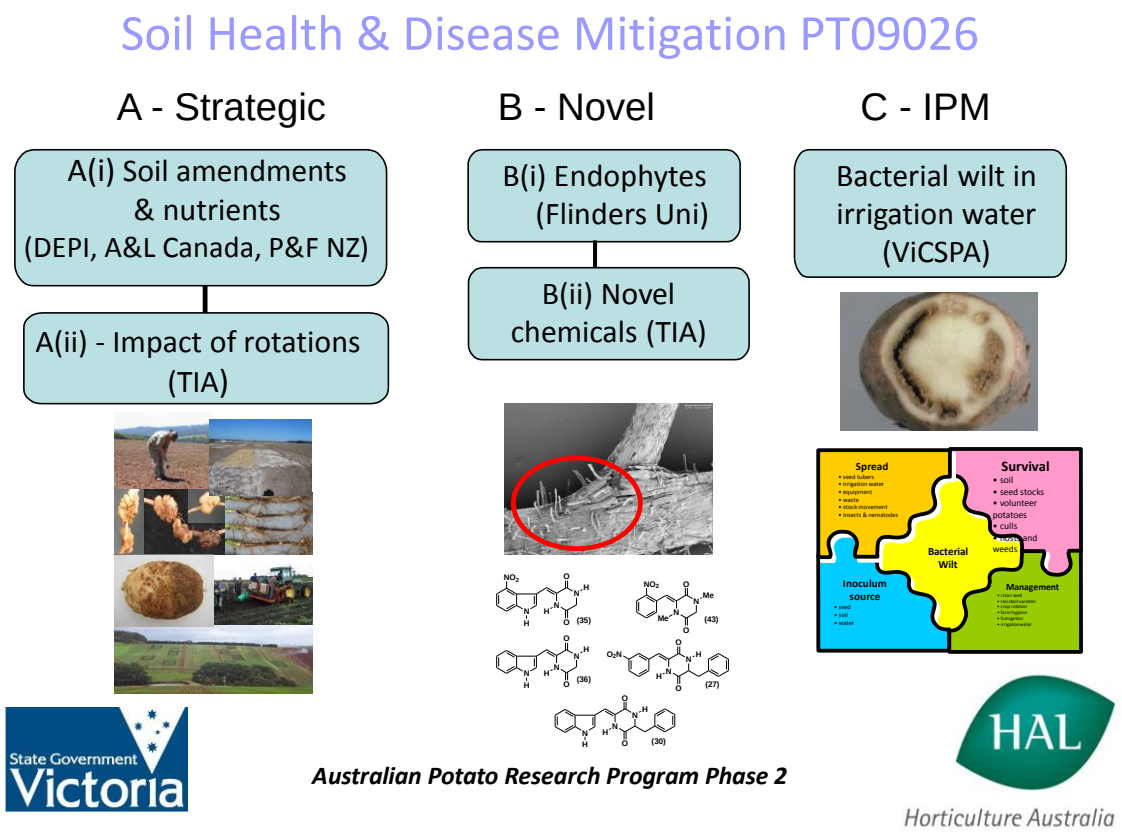

Figure 5-1 – Outline of the Soil Health and Disease Mitigation Project PT09026, which includes sub-projects under the areas of strategic research, novel research and integrated management Collaborating organisations were Department of Primary Industries (DEPI) Victoria, A&L Canada, Plant and Food Research (P&L) New Zealand, Tasmanian Institute of Agriculture (TIA)/University of Tasmania, Flinders University and the Victorian Certified Seed Potato Authority (ViCSPA)

DISCUSSION

This project explored a range of potential new control options for the intractable soil-borne diseases powdery scab, common scab, Rhizoctonia canker and black scurf, Verticillium wilt and Bacterial wilt. Three interrelated sub-project areas explored disease management through the manipulation of nutrients and soil health factors, novel approaches (chemical and biological) and disease management in a river catchment.

A significant component of this project was focused on the effects of modifying the soil environment, concerned particularly with the chemical and biological components, to improve soil health and suppress diseases.

Crop rotation is a critical disease management tool. Rotations prevent the excessive build-up of disease by breaking the disease cycle and also help modify and improve the soil condition. The eight-year study of the impact of rotations on the fate of pathogen DNA is a world first. This study showed the relative incidence of three major pathogens, *S. subterranea*, *S. scabies* and *R. solani*, including different anastomosis groups of *R. solani,* over more than 40 commercial properties in Tasmania and South Australia. There were no rotation crops associated with significant declines in pathogen DNA concentrations, and some crops, apart from potatoes, were associated with increases in pathogen DNA. The half-life of the DNA of these pathogens was also determined. The most striking results were for the powdery scab pathogen *S. subterranea*, which was the most common pathogen in both states. A potato crop in the rotation significantly increased the DNA concentration and it was determined that, because of already high concentrations in soil and the long half-life, a one-in-five year rotation (a requirement for seed production) would not reduce the pathogen DNA concentrations below the high disease risk category. This is consistent with anecdotal evidence that the "resting spore" of the pathogen survives in soil for decades without its main host, the potato. Monitoring pathogen DNA has been demonstrated as an invaluable tool to study the effects of specific rotation crops on pathogen and disease.

In studies on the effects of soil nutrient amendment on disease, applications of elemental sulphur and zinc were shown to reduce the incidence and, more so, the severity of powdery scab on potatoes. These results are consistent with similar studies conducted in the United Kingdom and New Zealand. Although not as effective as a fungicide, these treatments provide alternative options for powdery scab control on susceptible cultivars in the absence of a registered fungicide treatment in Australia. Interim recommendations for rates of applications can be made but additional testing on different soil types would provide optimum threshold rates. An application of zinc also reduced Rhizoctonia black scurf but this was only tested in one trial. Nutrient amendments for common scab were generally not successful. For the most part, treatments aimed at common scab control were evaluated on the peaty clay loams of the Koo Wee Rup crisping production area and would need to be evaluated on a broader range of soil types for a better assessment of nutrient amendments on this disease.

Optimising soil nutrients are part of the soil health equation. Modern potato production involves "prescription' farming, which includes soil and plant nutrient testing, and more recently pathogen DNA testing, which provide the basis for a number of crop management recommendations. Although the disease control through nutrient amendments appear to be modest in comparison with fungicides for example, it is important that we understand the complex interactions between nutrients and disease to ensure that recommendations provided to growers are not only designed to optimize conditions for crop growth but also for disease suppression and yield gains.

This study has identified common scab suppression in a soil from a potato field, which was identified as biological in nature. A detailed study of this suppression could identify new management options for common scab control, either beneficial microorganisms or soil management strategies that encourage the development of disease suppressive microbial communities. A study of soil microbial communities showed differences in the profiles from field soil samples with common scab and those without common scab providing a potential tool, a soil health bio-indicator, to identify the common scab disease potential of soil. This would complement the PreDicta Pt test (PT09023) which has been unable to develop an *S. scabies* DNA test into a tool to predict disease risk.

Improving soil health implies making significant changes to the soil environment over the short or long term. Incorporation of green manures and organic amendments revitalize soil microbial activities/populations, provide a reservoir of macro and micronutrients and provide plants with a buffer against pathogens. This project demonstrated significant gains in yield from a millet green manure crop in a study conducted in Canada. Green manure cropping can be particularly important in potato production systems that are heavily cropped. New tools have improved our ability to unravel the many interactions and mechanisms in soil from a green manure so that we can ultimately tailor specific green manure crop species and management for optimum disease suppression and crop production.

This project has identified a number of novel potential disease management options. The studies of the synthetic auxin 2,4-D for common scab control have identified a chemical that is effective at low concentrations, can be applied to foliage or tubers and has a mode of action that induces disease resistance in the potato tuber. Several chemicals with the same mode of action have also been identified, providing potential alternatives to the 2,4-D, which is currently registered only as a herbicide (applied at rates 10 times greater than required for common scab control). In contrast, modern fungicides are toxic to pathogens and, unless they are systemic in nature, need to be concentrated in the soil around developing tubers to be effective for control of disease in tubers.

Bioprospecting is a very novel approach to disease management in potatoes. Previous research in Canada screened more than 1500 bacteria taken from the potato root surfaces (rhizosphere) using molecular techniques and identified various functions including antibiosis (suppression of other microorganisms), the ability to fix nitrogen and plant growth promotion. In the current project, selected bacteria effectively reduced seed-borne common scab in a field trial and another group, free-living nitrogen fixing bacteria, when inoculated onto the roots of potato plants, improved growth 10 fold. The complementary research by Flinders University and TIA isolated a number of bacteria taken from inside potato plant roots and stem (endophytes), specifically actinobacteria, and demonstrated suppression of both the common scab and Rhizoctonia pathogens in laboratory. Both studies have highlighted the diversity of microorganisms associated with the potato that could be exploited for disease suppression and potato productivity in general.

Although promising in *in vitro* tests, the bacteria selected by the Flinders University team had at best only a moderate effect on soilborne disease when inoculated onto seed tubers in greenhouse and field trials. Inoculation of the seed tubers alone may not have been sufficient to allow establishment of the bacteria on the stems, stolons and developing tubers to provide protection from infection by pathogens. Recovery of these endophytes from tubers and potato roots was low. A key to the success of utilising beneficial organisms in potato production is effective delivery and establishment in the crop. The Canadian team, however, were able to demonstrate that the make-up of a consortia of nitrogen fixing bacteria established on the roots remained essentially the same after these plants were grown in a field soil. The challenge, therefore, is to find the means of delivery that will ensure effective establishment of beneficial organisms early in the life of the crop.

Despite crop rotation and the use of certified seed, bacterial wilt remains a problem in the crisping potato production region around the Bunyip River in Victoria. Anecdotal evidence suggested that the river water, used for irrigation, was a source of the bacterium. The bacterium was detected only once in an intensive five-year sampling of the river, suggesting that although, water may be one source, other factors are contributing to the disease epidemiology in production area. Further studies are needed to determine the role of the newly discovered aquatic weed host in the disease life-cycle. This research validated the water testing diagnostic protocols, providing some confidence that further tests can be done to track potential sources of serious outbreaks in the future. A draft disease management brochure produced for growers strongly outlines the need for disease prevention in this production area and has relevance to other similar production systems in Australia that are affected by this pathogen.

Key outputs from this project include the draft disease management decision trees for three main soilborne diseases which synthesize the knowledge gained in this and previous research projects. The development of these decision trees has also highlighted gaps in our knowledge of these diseases and their management which could form discussions for future research. The two pathogens *S. scabies* and *R. solani* have proved intractable for incorporation into the PreDicta Pt risk model to date. Part of the reason for this is that we do not have a good understanding of their epidemiology, making it difficult to predict disease. Although we have some knowledge of the importance of seedborne disease (Tegg et al. 2014), we know little of the relative contribution of seed and soilborne pathogen populations to disease at the field level.

TECHNOLOGY TRANSFER

Technology transfer involved a number of different communication activities, written and oral. Publications included papers in scientific journals, papers presented at scientific conferences (oral and poster presentations), scientific workshops and at industry conferences, as well as articles in various industry and media publications. Technology transfer directly to growers involved seminars/workshops in different districts organized by researchers and industry groups and, more recently, by the AUSVEG Potato Industry Extension Program. Communication within the program peer group included meetings of the relevant subproject teams, face-to-face and by teleconferencing, bi-annual APRP2 Technical Operations Committee Meetings (TOC), which included industry representatives, and the Technical Oversight Group (TOG) meetings specifically for project PT09026 Ai. The various papers, articles, extension activities and peer meetings are listed in the bibliography below.

Direct extension of information to growers and the scientific community included seminars and workshops organized by researchers and project support (SED Consulting) involving Australian and international scientists (Plant & Food Research, New Zealand; A&L Canada) (Victoria, Tasmania). Other events included industry-organized field days and meetings by VicSPA, Simplot and McCains.

The demand from industry groups for talks, field days and information, plus feedback from industry participants, demonstrates the interest in the potato-growing community for information of this kind.

An important output from this project is three draft decision trees for the management of powdery scab, common scab and Rhizoctonia canker and black scurf. These trees are the culmination of present and past research, providing growers with background information and a process of working through scenarios to minimise their disease risk, depending on the market, paddock history, soil DNA and nutrient tests, cultivar, seed health and various chemical and cultural control options. The decision trees were developed with input from the program research team, growers and industry representatives.

The disease management decision trees potentially provide a platform for a more comprehensive Technology Transfer program for the processing industry and could also be integrated with the PreDicta Pt training manual (PT09023).

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INDUSTRY AND PEER PRESENTATIONS

Potato Grower's Workshop, Warragul February 2010

Presentations by Ian Porter, Tonya Wiechel, George Lazarovits (A&L Canada) and Greg Patterson (A&L Canada)

Potato Grower's Workshop, Ballarat February 2010

Presentations by Ian Porter, Tonya Wiechel, George Lazarovits (A&L Canada), Greg Patterson (A&L Canada) and Richard Falloon (Plant & Food Research NZ)

Potato grower workshop, Cora Lynn Victoria 1 September 2010

Porter I, Wiechel TJ, Nair P (2010) Updates on APRP2 program to crisping growers from the Koo Wee Rup

ViCSPA Annual General Meeting, Potato Industry Dinner, Warragul, 3 October 2011

Wiechel TJ (2011) Effects of nutrients on common and powdery scab.

Lazarovits G (2011) Towards ecological agriculture: getting down to the rhizosphere.

Turnbull A (2011) Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Tasmanian Potato Industry Seminar, 4 October 2011, Simplot

Lazarovits G (2011) Towards ecological agriculture: getting down to the rhizosphere.

Turnbull A (2011) Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Sparrow L (2011) Tracking changes in pathogen DNA levels in different rotations

Grower Meeting, Donegan's Farm, Gordon VIC, 5th October 2011.

de Boer RF (2011) PT09026 Soil health/ disease mitigation program. Introduction.

Falloon F (2011) *Spongospora* infection of potato: root infection, effects of soil nutrients; and global population genetics.

Wiechel TJ (2011) Effects of nutrients on common and powdery scab.

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McCain Agronomy Conference, Ballarat, 25 August 2011

Wiechel TJ (2011) Overview of Soil health/Disease mitigation APRP2.

Tasmanian Institute of Agriculture "showcase day", Sandy Bay campus of UTAS on 29th November 2012 - attended by a wide range of university and industry representatives

Presentations by Calum Wilson, Robert Tegg, Leigh Sparrow

Seminar presentation to Plant & Food Research staff, Mt Albert Research Centre, NZ, 16 April, 2013.

Falloon RE, Merz U, Curtin D, Genet R, Lister R, Butler R, Shah F, Gau R, Hernandez Maldonado LM (2013). Scabs on potatoes; the more you dig the more you find. Seminar presentation to Plant & Food Research staff, Mt Albert Research Centre, NZ, 16 April, 2013.

Seminar to DEPI and La Trobe University Plant Pathologists and Soil Scientists, AGRIBIO Centre for Agri-biological Research, La Trobe University Bundoora, 23 September 2013

Lazarovits G (A&L Biologicals Canada) "Microbiology is coming to the forefront of agricultural production."

Snackbrands Field Day, Thorpdale, 7 February 2014

Wiechel TJ (2014) Discussions with growers about DNA soil testing and future commercialisation of the *PreDicta Pt* testing service

McCain Field Day, Ballarat, 5 March 2014

Wiechel TJ (2014) Discussions with grower group (*PreDicta Pt*) on DNA test results and future commercialisation and adoption of predictive tests

Simplot R&D meeting, Ulverstone, Tasmania, 16 July 2014

Wiechel TJ (2014) Presentations on management disease with nutrient amendments and the use of molecular test for indicators for soil health. Discussions with Simplot staff on future research in potatoes on 15 July 2014.

Sparrow L (2014) Long-term monitoring of soilborne pathogens in rotations

Roberts Rural Potato Industry Forum, Scottsdale, Tasmania, 28 August 2014

Sparrow L (2014) Long-term monitoring of soilborne pathogens in rotations

Radio Interviews

George Lazarovits: Radio Interview in Tasmania 4th October 2011

Leigh Sparrow: Radio Interview for ABC Rural

AUSVEG Potato Industry Extension Program

Wilson C, Tegg R (2012). Presentations at the AUSVEG sponsored Industry extension workshop in Ulverstone, 14th March 2012.

de Boer RF, Wiechel TJ (2012). Presentations to growers attending the Potato Industry Workshop, Creswick, Victoria 28 June 2012.

Wiechel TJ (2013) Presentation at the Potato Industry Extension Workshop, Creswick, Victoria June 2013 on the latest results of the Soil health/disease mitigation project

Falloon RE (2013) Collaborating scientist from Plant and Food Research NZ. Presentation of the latest research on powdery scab of potatoes at a Potato R&D Workhop, Pemberton Western Australia, September 2013 as a guest of the AUSVEG Potato Extension Program.

APRP2 Grower Evening - The American Hotel, Creswick Victoria - Presentations to growers by Ben Gallaghan HAL, Richard Fallon P&FR NZ and George Lazarovits, A&L Biologicals Canada to local growers, 19 September 2013 (associated with the APRP2 Symposium, 18-19 September, Mantra Hotel, Tullamarine Victoria)

Wiechel TJ (2012) Presentation on the effects of soil nutrients of soil-borne diseases of potatoes, Warragul meeting 2012.

*APRP2 Science Symposia 1***,** *Rydges Carlton, Melbourne, October 2011*

Agarwal A (2011) Novel molecular tests to study potato pathogens – towards improved disease management.

de Boer RF (2011) PT09026 Soil health/ disease mitigation program. Introduction.

Falloon RE (2011) *Spongospora* infection of potato: root infection, effects of soil nutrients; and global population genetics.

James L, Crump NC (2011) Monitoring of bacterial wilt in irrigation water.

Lazarovits G (2011) Towards ecological agriculture: getting down to the rhizosphere.

Sparrow L (2011). Survey of the impacts of commercial rotations on potato pathogen levels.

Thompson H (2011). Spraying away common scab.

Turnbull A (2011). Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Wiechel TJ (2011) Effects of nutrients on common and powdery scab.

Wiechel TJ (2011) Studying infection of *Spongospora subterranea* in the 3 year collaborative trial.

APRP2 Science Symposium 2, Mantra Hotel, Tullamarine, 18 & 19 September 2013

Papers presented by Dolf de Boer (overview of PT09026). Tonya Wiechel and Arati Agarwal (DEPI), Richard Falloon (P&FR NZ), George Lazarovits (A&L Biologicals Canada), Leigh Sparrow (TIA), Chris Franco (Flinders University SA), Calum Wilson (TIA), Nigel Crump and Luke James (ViCSPA).

Industry "Outputs" Sessions –Panel discussion including Dolf de Boer, Tonya Wiechel, Richard Falloon, George Lazarovits, Leigh Sparrow, Stacy Smith (Flinders University), Calum Wilson and Nigel Crump.

Lunchtime Seminar AGRIBIO Centre for Agribiological Research, La Trobe University Bundoora – George Lazarovits, A&L Biologicals Canada – "Microbiology is coming to the forefront of agricultural production." Seminar presented by Dr George Lazarovits at the AGRIBIO Centre for AgriBioscience, La Trobe University, Monday 23 September 2013 – How ecological agriculture will change farming practices.

APRP2 Powdery scab workshop, Mercure Hotel Ballarat, 1 August 2012

Meeting of researchers and key industry/grower representatives to review research on powdery scab of potatoes.(Richard Falloon, Plant and Food NZ, Tonya Wiechel, Dolf de Boer, Arati Agarwal, Calum Wilson, Robert Tegg).

Project Management Meetings

Technical Oversight Group Meetings PT09026

12 September 2013, DEPI Bundoora - Attended by Dolf de Boer, Tonya Wiechel, Arati Agarwal, Ben Callaghan, Kevin Clayton-Greene and Graham Henman

14 February 2013, HAL Melbourne Office Meeting Room – Attended by Dolf de Boer, Tonya Wiechel, Kevin Clayton Green, Graeme Henman, David Moore and Ben Callaghan.

Technical Operations Committee Meetings

Face-to-face meetings

17 February 2010, Holiday Inn, Melbourne airport

28 July 2010, DPI, Attwood, 475 Mickleham Rd, Attwood VIC

3 May 2011, DPI Attwood, 475 Mickleham Rd, Attwood VIC

7 October 2011, Rydges Carlton (plus symposium)

13 April 2012, Holiday Inn, Melbourne Airport

10 October 2012, Best Western Airport Motel and Convention Centre

19 April 2013, Holiday Inn, Melbourne Airport

18/19 September 2013 (plus symposium), Mantra, Tullamarine

Phone meetings

1 June 2010

16 Feb 2011

4 November 2011

6 March 2012

28 February 2014 (TOC wrap-up webinar)

Team meetings

George Lazarovits and Greg Patterson of A&L Canada visited Australia 14-23 Feb 2010. They took part in project team meetings to set project work plans and presented at grower workshops in Warragul and Ballarat.

Dolf de Boer, Tonya Wiechel, Arati Agarwal, Desmond PF Auer, Richard Falloon, Leigh Sparrow meeting at DEPI Bundoora (DEPI Bundoora 18 April 2013) for discussions on the Ai and Aii components of the research program.

George Lazarovits was a guest of DEPI, Bundoora, 17-23 September 2013. Research data from Canada and Australia were reviewed through team meetings.

Confidential Client Reports

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2011) Amounts of iron in plant growth medium have little effect on *Spongospora subterranea* infection in potato. *The New Zealand Institute for Plant & Food Research Ltd Report,* SPTS No. 5021: 18 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2012) The sulphate form of sulphur in plant growth medium has little effect on *Spongospora subterranea* infection in potato. *Plant & Food Research Ltd Report,* SPTS No. 6542: 14 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2013) Potassium or silicon in plant growth medium have little effect on *Spongospora subterranea* infection of potato roots: two greenhouse experiments. *Plant & Food Research Client Report No. 52561*: 18 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2014) Treatments of silicon and different irrigation regimes affect growth of potato plants in association with *Spongospora subterranea* infection. *Plant & Food Research Client Report No. 9642.*

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

A. Soil amendments/ameliorants for soil health and disease suppression

Soil amendments and nutrients

A number of the recommendations below are strongly focused on research of the soil microbial environment, an area of research that has been significantly boosted by the development of DNA-based tools. These recommended research areas are interrelated, complementary and share research tools and strategies.

- Further development of the disease management decision trees, which are in draft form, to serve as platforms on which to build more comprehensive technology transfer programs for the outputs of this and previous research.
- Integrate the powdery scab decision tree with the PreDicta Pt training module for agronomists (Project PT09023).
- Further research into the mechanisms of common scab disease suppression in a field soil with the aim of developing new management strategies based on manipulating and inducing disease suppression.
- Development of a 'soil bio-health indicator' to predict disease risk by common scab. This pathogen has proved intractable for incorporation into the PreDicta Pt risk model to date.
- Further evaluation of green manure cropping in Australian production systems.
- Continued exploration of beneficial microorganisms closely associated with the potato plant (rhizosphere and endosphere) and their utilization for disease suppression and improved growth and productivity.
- Continued research effort into Rhizoctonia disease, a widespread problem in potato production, to determine the survival and behaviour of *R. solani* in the rotation and in the cropping cycle and enable better prediction of disease risk. This pathogen has also proved intractable for incorporation into the PreDicta Pt risk model.
- Evaluation of sulphur and zinc applications for powdery scab control on a wider range of soil types to refine interim recommendations for growers, as part of a disease management extension program.
- Further studies on the effects of zinc on Rhizoctonia disease to determine whether these results are consistent and treatments economical.
- Further evaluation of the K:Mg ratio for common scab control on a wider range of soil types with a focus on a longer term approach of a number of treatments effecting a gradual change over a number of cropping cycles as part of a disease management extension program.
- A renewed look at the benefits of using disease resistant cultivars as a disease management tool and using new tools to screen potato genomes for resistance to common and powdery scab, both of which have heritable resistance traits.

Impact of rotations on pathogen DNA

- The findings of this project about *Spongospora subterranea* and the risk of powdery scab need to be incorporated into the manual and associated training for the PreDictaPt DNA testing service offered to the potato industry by SARDI.
- Findings about the other pathogens measured in this project can be incorporated into extension services when and if reliable models for the interpretation of their DNA concentrations are available.

- Consideration should be given to continuing the DNA monitoring at least at some of the sites.
- Consideration should also be given to more controlled studies of the potential impact on disease of those crops other than potato identified as "DNA-increasing" crops, i.e. poppy (*R. solani* AG2.1 and AG3) and ryegrass and carrot (*R. solani* AG3), and to studies of other crops not part of this project including so-called "trap" crops.

B. Novel approaches to disease control

Endophyte discovery

- We recommend the development of more rapid *in planta* methods of screening for the effects of beneficial bacteria against potato diseases. The reasons for this include difficulty of *in-vitro* testing against obligate pathogens such as *S. subterranea*, the late expression of disease symptoms on tubers that were infected at initiation stage (powdery scab), tracking the colonisation of plants by the inoculants. This methods also allow the observation of induced systemic resistance by actinobacterial inoculants, an alternative mode of action of endophytes (Conn et al. 2008).
- Improved methods for the monitoring of the colonisation of the added inoculum will also be required. Due to the size of the actinobacterial mycelium (~0.2-0.5 µm) and immune-gold staining procedure will be best suited to observe the *in planta* colonisation.
- Improved delivery systems need to be trialled to deliver the inoculum during tuber initiation phase when the risk of infection by the powdery scab and common scab pathogens if the greatest. However, this may require uneconomic quantities of inoculant. An alternative approach is to establish the inoculant as an endophyte in the elite tissue culture used for minituber production. If the inoculant can be established this way it may be possible to maintain efficacious endophyte concentrations at plant growth stages at highest risk of infection.
- Seek opportunities for improved collaboration between the Canadian and Australian researchers.

Novel chemicals for disease control

The efficacy of 2,4-D and other non-fungicidal chemicals for control of common scab has been conclusively demonstrated. Early applications at low concentration of 2,4-D provides excellent disease suppression and do not induce detrimental low yield and tuber distortion observed at higher rate application. Alternate non-herbicidal materials with equivalent efficacy in disease control and no toxicity even at elevated rates were also identified. The best of these being 2,5-dibromobenzoic acid (2,5-DBB).

- Commercial development of foliar sprays and/or tuber treatments with 2,4-D or 2,5-DBB as a cost effective disease mitigation strategy easily integrated into current production practices. This requires engaging the agrichemical industry, and facilitating registration trials. As 2,4-D is a registered agrichemical for use in potato cropping. Larger scale trials assessing disease mitigation efficacy and determine any impact of treatments on yield/quality under commercial growth conditions would be needed. 2,5-DBB, whilst a more attractive material in terms of safety with the crop, would require extensive toxicity and environmental testing in additional to efficacy trials.
- Further studies on tuber treatments with 2,4-D and alternate chemistries. Tuber treatment is another attractive option. It would reduce exposure of operators and the environment to chemical treatments, would reduce the amount of material needed, and would ensure all plants receive material at the earliest stage of crop growth. Further studies are required to determine if alternate materials provide a similar effect to 2,4-D, and to evaluate the optimal treatments for use on tubers. Rates may need to be higher than when applied to growing crop. We also do not know what effect tuber physiological development would have on uptake of material, and impact of material on seed quality.

- Further fundamental research on the mechanisms of thaxtomin suppression and disease mitigation. We also understand very little about how these materials provide protection against common scab. We have shown that they inhibit thaxtomin toxicity, but the mechanism of this inhibition is unknown. This information could be valuable in targeting novel disease control strategies and including possible breeding targets. For example, if a thaxtomin receptor is recognised, mutations in this receptor could be selected, or better designed blockers of the receptor developed.

C. Monitoring the bacterial wilt pathogen in irrigation water – disease management in a river catchment

- Future work should be aimed at validating real-time DNA based technologies for testing of water and plant samples with bacterial wilt. This could potentially improve the sensitivity of the test and allow a higher throughput of samples. Overseas studies have reported on the potential use of real-time DNA based technologies for detection and quantification of *R. solanacearum* in environmental samples (Stead et al. 2003) (Caruso et al. 2003). The diagnostic protocol used in this study is recognized by the EU COUNCIL DIRECTIVE 98/57/EC. A limitation of this protocol was the small number of samples that could be processed at any one time. Testing was labor intensive and needed highly skilled professionals to conduct and interpret the diagnostics.
- The survival of *R. solanacearum* in the environment is poorly understood. In addition to improved diagnostic tests for water, a DNA soil test linked to disease thresholds would be of benefit to growers in determining disease risk of (inoculum below economic thresholds) to avoid crop loss. This future research could be aligned with the platform technology developed as part of PT09023. Overseas researchers are developing such diagnostic tests (Pradhanang et al. 2000).
- While the Bunyip River catchment was used as a model catchment in this study, the importance of surface water in the spread of bacterial wilt in other catchments may be explored in future studies.
- This project found the association of *Damasonium minus* with *R. solanacearum*. The importance of *Damasonium minus* as a potential host for *R. solanacearum* requires more detailed studies.
- There is a need for a greater awareness of this disease by industry because of its potential to cause substantial crop losses and trade restrictions. The technology transfer outputs from this project can contribute to educating the industry with a very strong emphasis on the need for growers to adopt sound on-farm hygiene protocols to prevent the introduction of bacterial wilt and other pests and diseases onto their farms.

ACKNOWLEDGEMENTS

We gratefully acknowledge the participation of potato growers Ken Labbett, George Lineham, Frank Rovers, Wayne Tymensen and Tony Cummaudo, without whom the field trials on nutrient amendments would not have been possible. They willingly provided land, facilities, machinery, crop husbandry and their time, which ensured the success of the trials.

We also thank Daniel Grayling (McCain Food Australia) and Luke James and Joe Smith (ViCSPA) for organizing sampling of growers fields for crop surveys associated with the disease management decision trees, and we thank all of the growers who participated in this survey.

We thank Khagoswori Giri (Biometrician, DEPI) for his advice and assistance in conducting the REML analysis of the five years of data from the field trials evaluating the effects of nutrient amendments on powdery scab disease.

We thank the many growers who allowed us continued access to their paddocks for the long term study of the impact of rotations on pathogen DNA. Mr Robin Harding, formerly of SARDI, managed the South Australian component of this project until 2011.

Mrs Stacey Smith (Flinders) and Dr Robert Tegg (TIA) were the main contributors to the experimental work carried out in the project on microbial endophytes and their potential for disease suppression in Australian potato production. Dr Jeff Barrett (Flinders University) was responsible for setting up the T-RFLP studies and a number of visiting exchange students, notably Mr Niels Hendrickx, Caroline Vogels and Bas van Dongen contributed to the isolation and characterisation of the actinobacterial endophytes.

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Horticulture Australia

PT09026 AI – (30/10/2015)

Soil Amendments and Nutrients

Final Report

Dr Rudolf de Boer et al

Department of Environment and Primary Industries, Victoria

PROJECT SUMMARY

PT09026 AI – Soil Amendments and Nutrients

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This is the final report for Sub Project A(i) “Soil amendments and nutrients” that forms part of the “Soil health/disease mitigation” sub-Program Project No. PT09026.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, A&L Canada Laboratories and the Victorian Certified Seed Potato Authority, and matched funds from the Australian Government.

The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, The University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority have provided in-kind support.

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LIST OF AGRONYMS AND ABREVIATIONS

Acronym	Definition
APRP	Australian Potato Research Program
APRP1	Australian Potato Research Program Phase 1
APRP2	Australian Potato Research Program Phase 2
CT-value	Cycle Threshold (Real-time PCR - Number of amplification cycles required for the fluorescent signal to cross the threshold, i.e. exceeds background level)
CTAB	Cetyltrimethylammonium bromide (used in the process of DNA extraction from plant material)
DAP	Days after planting

Acronym	Definition
DAPG	2,4-Diacetylphloroglucinol (natural compound found in strains of the bacteria *Pseudomonas fluorescens* responsible for the biocontrol properties of these strains)
DEPI	Department of Environment and Primary Industries
DNA	Deoxyribonucleic acid
DW	Dry weight
EC	Electrical conductivity of a soil sample (Part of a soil nutrient analysis test)
EDTA	Ethylene diamine tetraacetic acid (Chelating agent; some mineral nutrients are in the EDTA form)
EFL	Elongation Factor-Like proteins (Elongation factors are a set of proteins that are used in protein synthesis in the process of cell cycle and elongation in some cells)
FAM™	6-carboxy-fluorescine (fluorescent dye attached to oligonucleotide primers for PCR)
FL	Frito-Lay (prefix for Frito Lay potato cultivars)
G&L	Goyer & Loria media (for the culture of *Streptomyces* species)
GHG	Greenhouse gas
HAL	Horticulture Australia Limited
IAA	Indole-3-acetic acid
ID	Identification
IPM	Integrated Pest Management
ITS	Ribosomal RNA (rRNA) gene internal transcribed spacer (sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny)
SOP	Sulphate of potash (potassium sulphate)
LB	Lysogeny Broth (“Luria Broth”, “Luria-Bertani”) media for culture of bacteria. Sometimes also referred to as “Liquid broth”
LGI	“LGI” medium used for culturing nitrogen fixing bacteria
LSD	Fishers Least Significant Difference (for the statistical comparison of the mean of one group with the mean of another in an Analysis of Variance)
MICA	A web-based tool for the analysis of microbial communities (http://mica.ibest.uidaho.edu/)
MOP	Muriate of potash (potassium chloride)
NSW	New South Wales
NZ	New Zealand
OBA	Oat Bran Agar
OM	Organic Matter content of soil
OTU	Operational taxonomic units (definition of a species or group of species used when only DNA sequence data is available)
PCA	Principal Component Analysis (statistical analysis)
PCR	Polymerase Chain Reaction (method for amplification of pieces of DNA for detection and quantification)

Acronym	Definition
PDA	Potato Dextrose Agar (for the culture of fungi)
PEI	Prince Edward Island, Canada
PGPR	Plant growth promoting rhizosphere bacteria
R&D	Research and Development
R2A	Reasoner's 2A medium (low nutrient medium for the culture of slow growing bacteria
REML	Restricted Maximum Likelihood analysis technique for linear mixed models
RNA	Ribonucleic acid
RSM	Rhizosphere growth medium (for isolation of rhizosphere bacteria)
SARDI	South Australian Research and Development Institute
SE	Standard Error of the mean (standard deviation of the sample mean which is an estimator of the population mean)
SG	Specific Gravity
TIA	Tasmanian Institute of Agriculture (formerly TIAR)
TOC	Technical Operations Committee (APRP2 program)
TOG	Technical Operations Group (APRP2 program)
TRF	Terminal Restriction Fragment (DNA fragment in a Terminal Restriction Fragment Length Polymorphism analysis)
TRFLP	Terminal Restriction Fragment Length Polymorphism (genetic fingerprinting technique for determining microbial community structure)
TS	Tuber set (early tuber initiation; begins with the swelling of stolon tips and usually ends by early flowering)
TYA	Tryptone Yeast Extract agar (general media for the culturing of bacteria)
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
VIC®	Fluorescent dye attached to oligonucleotide primers for PCR
YEM Congo Red	Yeast Extract Mannitol agar with the Congo Red die (used for the culturing of *Rhizobium* species)
YME	Yeast-Malt extract agar (used for the culturing of actinobacteria including *Streptomyces* species)

CHAPTER 5. APRP2 – SOIL HEALTH/DISEASE MITIGATION PROGRAM

5.A SOIL AMENDMENTS/AMELIORANTS FOR SOIL HEALTH AND DISEASE SUPPRESSION

5.A.I THE USE OF SOIL AMENDMENTS/AMELIORANTS AND NUTRIENT MANIPULATION TO CONTROL SOILBORNE DISEASES OF POTATO, ENHANCE SOIL HEALTH AND INCREASE PRODUCTION

MEDIA SUMMARY

This project demonstrated that control of the intractable soil-borne diseases powdery scab, common scab and Rhizoctonia diseases is possible by manipulating soil chemistry and harnessing soil biology. Soil-borne diseases are the most difficult for growers to manage. There are few, if any, registered fungicides available. In APRP1, exciting new possibilities were identified for managing these diseases by manipulating soil health. In this current project, a collaboration between Australia, New Zealand and Canada, the objective was to examine these more closely and determine whether they could be developed into practical disease control options for potato growers.

Manipulation of soil chemistry proved effective for controlling powdery scab and black scurf. In field trials conducted over five seasons, powdery scab was consistently reduced by soil incorporations of elemental sulphur and zinc EDTA. The improved tuber quality increased acceptable yields for French fry up to 10 t/ha and for crisping up to 20 t/ha. Zinc EDTA also effectively reduced Rhizoctonia black scurf.

Manipulation of soil biology was indicated as the potential key to common scab control. A disease suppressive soil was identified and the suppression characterised as biological in nature. The suppressive element (as yet unidentified) was demonstrated in pot trials to be transferable. Molecular profiling of 'healthy' versus 'diseased' soils identified distinct microbial communities in each, which has the potential for development as a 'soil bio-health indicator'. Promising candidate bacteria from the previous APRP1 were further tested for effectiveness as seed treatments against common scab and inoculants as bio-fertilisers. Additionally, millet as a green manure increased yields by up to 40%, even though common scab and Verticillium wilt were not reduced.

Key outputs of the R&D are three draft decision support trees for the diseases powdery scab, common scab and Rhizoctonia diseases, and a concept for a 'soil health bio-indicator'.

TECHNICAL SUMMARY

This project explored the potential for manipulating soil health to achieve control of the intractable soilborne diseases powdery scab, common scab, Rhizoctonia canker and black scurf and, to a lesser extent, Verticillium wilt. It was a collaborative study involving researchers from Australia, New Zealand and Canada. The focus of the research was driven from correlations between soil chemical and biological components and disease reduction that were identified during APRP1. In this current project, the objective

was to examine these correlations more closely and determine whether they could be developed into practical guidelines for disease control that can be applied by potato growers. Key outputs of the R&D are three draft decision support trees for the diseases powdery scab, common scab and Rhizoctonia diseases, and a concept for a 'soil health bio-indicator'.

Manipulation of soil chemistry proved effective for controlling powdery scab. Fifteen treatments were tested using different rates and different methods of application in field trials conducted over five seasons in a ferrosol soil on a commercial property near Ballarat, Victoria. Powdery scab was consistently reduced by soil incorporations of elemental sulphur and zinc EDTA. Soil sulphur levels were increased from 10-30 ppm to 100-120 ppm and soil zinc levels from 2-3 ppm to 20-30 ppm in the effective treatments. Iron EDTA gave small disease reductions in two out of the five seasons. Bioassays to investigate how nutrients were impacting on the host-pathogen interaction showed that zinc and iron directly inhibited root infection by the powdery scab pathogen *S. subterranea,* but none of the tested nutrients arrested the development of powdery scab galls on potato roots.

Additionally, three fungicide treatments (not registered for use in Australia) were used for comparison, of which only fluazinam was effective. In susceptible cultivar Shepody, the improved tuber quality from fluazinam, elemental sulphur and zinc EDTA translated to an increased 14, 10 and 8 t/ha accepted by the French fry factory and 27, 23 and 11 t/ha at the Crisping factory. In resistant cultivar Russet Burbank, the disease reduction was still significant but less dramatic. Recommendations for using sulphur and zinc have been integrated into a powdery scab decision management tree developed as part of this program.

Zinc EDTA was also demonstrated to be effective for reducing Rhizoctonia disease in the field, but manipulation of soil chemistry for control of common scab was less successful. Despite five years of field trials in two different soil types, applying pH modifiers and various forms of sulphur, calcium, magnesium and nitrogen had little effect on disease outcome. Considerable effort was directed towards manipulating the K:Mg ratio to 0.4, which reduces common scab in Canada and was correlated with disease reduction in APRP1. A survey of 160 potato fields found that the ratio varied widely from 0.1 to 2.7. Despite recommendations from soil testing laboratories, the correct balance of amendments to reach the desired target was rarely achieved and only one trial resulted in reduced common scab.

Manipulation of soil biology showed promise for controlling common scab, with potential development of a 'soil bio-health indicator'. Terminal restriction fragment length polymorphism (TRFLP) was used to profile the rhizosphere microbial communities from 28 Victorian potato fields classed as positive or negative for *Streptomyces scabies.* The TRFLP profiles appeared to group on the basis of the common scab disease potential of the different soil samples. This type of study of soil microbial diversity could serve as a more useful bio-indicator of disease potential than the pathogen DNA itself and can help to understand the factors that are responsible for disease suppression. A disease suppressive soil from a Victorian potato farm was identified and characterized, and a glasshouse experiment proved that the suppression was transferable. Identifying the casual organisms of suppression and what factors encourage their prevalence in soil could ultimately lead to targeted biological control treatments and the possibility of manipulating the soil environment to optimise disease suppression.

During APRP1 and PT07038, thousands of bacteria were isolated from potato rhizospheres, screened for a diversity of biological functions using chaperonin gene technology (*cpn60*) and many were found to have antibiotic genes or nitrogen fixing (*nifH*) genes. In this current project, three of these (*Pseudomonas monteilii, P. chlororaphis, P. brassicacearum*), which inhibited *S. scabies in vitro* and significantly reduced disease in bioassays, were evaluated as seed treatments in a field trial. All three species reduced common

scab on progeny tubers, although *P.monteilii* also reduced tuber number and yield. Therefore, two have been identified as potential biological control agents for seed-borne common scab.

Effective delivery and establishment is essential for the use of beneficial microorganisms. A consortium of nitrogen-fixing bacteria were established on roots of tissue cultured plantlets, which were then transplanted into field soil. Plants inoculated with eight separate nitrogen-fixing communities showed an average five-fold increase in biomass compared to un-inoculated plants. The nitrogen-fixing communities were still detectable 30 days after transplanting. This provides proof-of-concept that these microorganisms could be developed as inoculants.

Green manures are crops grown for a specific period of time and incorporated into soil while green to improve the soil condition. In the final two years of a four year trial initiated during APRP1 in Canada, incorporating millet green manure crops treated in four different ways increased marketable yields of potato between 20 to 48% compared with the continuous cropping of potatoes. These increases were not attributed to reductions in Verticillium wilt and common scab but to changes in the soil chemical and microbial environment.

Outputs from this project include draft disease management decision trees for the diseases powdery scab, common scab and Rhizoctonia, which were built on the research outcomes from this program and previous HAL sponsored research. The decision trees involve a step wise process that identifies high risk factors that cause disease and what actions to take to prevent or mitigate these factors for a particular paddock/cultivar combination. A preface for the use of the decision trees is a standard soil nutrient analysis and, and for powdery scab, a soil DNA test (PreDicta Pt, HAL Project PT09023), to optimize soil and plant nutrition, determine a baseline for potential nutrient amendments for disease control and to determine disease risk.

Specific recommendations from this project are:

- Further development of the draft disease management decision trees into extension tools.
- Future research into the mechanisms of the common scab disease suppression found in Victoria to identify potential biological control agents and / or how to manipulate soil biology for disease suppression.
- Development of a ‘soil health bio-indicator’ to predict disease risk by common scab. This pathogen has proved intractable for incorporation into the PreDicta Pt risk model to date.
- Continued exploration of potato microbial communities to harness their potential for improving disease management and potato productivity.
- Evaluation of the disease–reducing nutrient amendment treatments in other soil types and production regions to develop practical thresholds that can be integrated with soil testing services for nutrients and pathogen DNA.
- Further evaluation of green manure cropping in Australian production systems.
- Continued research effort into Rhizoctonia disease and common scab to enable accurate prediction of disease risk.
- A renewed look at the benefits of using disease resistance cultivars as a disease management tool and using new tools to screen potato genomes for resistance to common and powdery scab, both of which have heritable resistance traits.

GENERAL INTRODUCTION

The soilborne potato diseases, common scab, powdery scab and Rhizoctonia, are widespread throughout the world's production areas including Australia. These diseases cause significant economic impacts through yield loss, costs of control measures, rejection of unmarketable potato tubers, both for processing and seed, as well as increased costs and reduced product recovery during processing. Current estimates of costs to the processing industry attributable to common scab are $1.6 million, $13 million to powdery scab and $5 million to Rhizoctonia. Overall industry losses are in the order of $20 million pa.

This project is part of an integrated research strategy focused on soil health and disease mitigation for potatoes. The main targets of the research are the diseases powdery scab (*Spongospora subterranea*), common scab (*Streptomyces scabies*) and Rhizoctonia disease (stem and stolon canker and black scurf) (*Rhizoctonia solani*), with a minor focus on Verticillium wilt (*Verticillium dahliae*).

This subproject A(i) "Soil amendments/ameliorants for soil health and disease suppression" is part of a larger program in Australian Potato Research Program Phase 2 (APRP2) "Soil health/disease mitigation" Project No. PT09026. It builds on international collaborations and research outcomes delivered from the Australian Potato Research Program Phase 1 PT04016, "Soil health" subproject (Crump 2010; Kirkwood 2010).

Soil health

Soil health refers to the physical, chemical and biological features of a soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. Because of the very wide diversity of natural and agricultural ecosystems, the soil also has to be "fit for purpose" within the constraints of a cropping system, for example, for potato production. Soil health can also be defined as the capacity or fitness of the soil to support crop growth without resulting in soil degradation or otherwise harming the environment.

Soil health is a complex interaction between soil physical, chemical and biological factors (e.g. soil structure, moisture, temperature, pH, nutrients, microorganisms), all of which affect plant growth and the relationships between host plants and soilborne pathogens. It is widely accepted that soil factors greatly influence the incidence and severity of soilborne diseases. The soil health subproject of APRP1 was focused on the influence of soil amendments and nutrients that modify soil chemical and biological factors and their impact on disease.

Soil physical factors refer to the nature of the soil, the "soil type", and the associated physical properties (sand, sandy loam, clays, peaty clays), which also defines some of the chemical properties of the soil. We can influence the physical nature of the soil to some extent, ensuring its quality in terms of aggregate stability (structure), organic matter and carbon for example, but there is a much greater opportunity to manipulate soil chemistry and biology.

Soil chemistry

Various measures of soil chemistry, such as pH, electrical conductivity, cation exchange capacity, total organic carbons, are used as indicators of soil health and quality. However, soil chemistry can also influence disease. In APRP1 subproject "Soil Health" (PT04016), correlations were found between particular nutrients and nutrient ratios, e.g. calcium, sulphur, zinc, iron, pH, manganese and the potassium to magnesium ratio, and the incidence and severity of common and powdery scab. This raised the question: 'Can certain nutrient levels be manipulated to reduce these diseases, and if so, what are the thresholds required for soil

nutrients to influence disease management? Therefore, PT09026 A(i) has focused on the most promising nutrient treatments for disease control. The ultimate goal is to develop nutrient thresholds for disease control that can enhance the interpretation of disease risk as determined by DNA diagnostic tests that are being developed in PT09023 (led by Ophel-Keller).

Soil biology

The make-up of the soil microbial community strongly influences the health or quality of the soil and is the subject of considerable research worldwide. In APRP1 projects PT04016 and PT07038, our Canadian collaborators used novel molecular techniques to study the microbial communities inhabiting the soil in potato-growing fields, those on the potato root surface (rhizosphere) and those within the potato plant (endophytes). A diversity of bacteria were discovered that could fix nitrogen, promote plant growth, utilise phosphorus and produce antibiotic compounds. In APRP2, this research has been extended to examine the opportunities for exploiting the microbes with beneficial properties, of manipulating the soil environment to modify the microbial communities (i.e. with amendments and green manures), and of identifying microbial 'fingerprints' for disease suppressive soils.

The results from APRP1 have formed the foundation for studying the potential of manipulating the soil environment to provide practical disease control outcomes in APRP2. Subproject A(i) is a collaborative project involving DEPI Victoria, Plant & Food New Zealand and A&L Laboratories Canada. The main components of the work are outlined below.

Manipulating soil nutrients for disease control (DEPI Victoria, Plant and Food Research NZ, A&L Canada)

A series of field trials conducted in major cropping areas were complimented with glasshouse and laboratory experiments to evaluate the effects of different forms of sulphur, zinc, iron, magnesium, potassium, manganese, pH modifiers and potassium to magnesium ratios on the pathogen, disease outcome and potato yield. The main focus was powdery scab and common scab, drawing on the collective strengths of the teams, and was later expanded to Rhizoctonia disease. The aims were to identify nutrient thresholds for practical disease control and to understand the mechanisms by which disease control was being achieved, for example, was the pathogen being directly affected, was the potato plant's innate resistance being enhanced, or was the soil environment being altered in other ways that minimised the impact of the pathogen?

A & L Canada provided nutrient analyses of soil samples from field and glasshouse trials conducted in Victoria and provided prescriptions for nutrient treatments to be evaluated in field trials to achieve target concentrations and ratios of the nutrients under investigation.

Modifying the soil environment for disease control (A&L Canada)

During APRP1, the Canadian team demonstrated common scab and Verticillium wilt control and significant yield increases with the use of millet as a green manure crop in the first two years of trials. The trials were extended for a further two years in this current project to determine the optimum millet management techniques for using this approach.

Exploiting microbes associated with potato plants for disease control and improved productivity (A&L Canada)

Many thousands of bacteria from the potato rhizosphere and endosphere were isolated during PT07038 and related projects and maintained in a culture collection. Many of these have now been evaluated for activity against potato pathogens (antagonistic bacteria) and for boosting potato productivity (nitrogen

fixing and phosphorus utilising bacteria). Promising candidates have been further evaluated and tested in glasshouse and field conditions. A critical component of utilising beneficial microorganisms commercially is stability in an effective delivery system. The most promising bacteria have been tested for their resilience on potato roots post-inoculation and post-planting.

Identifying and manipulating disease suppressiveness in potato production soils (DEPI Victoria and A&L Canada)

During APRP1, certain paddocks were identified in both Canada and Victoria as being 'disease suppressive' to common scab i.e. although the pathogen DNA was consistently detected in the soil, disease was never seen. Protocols for DNA-based profiling of soil microbial communities were developed and used to explore this issue by profiling biological communities in soil samples from Victorian fields that had common scab or were free of common scab. Soil collected from a Victorian field identified as being suppressive to common scab was tested in glasshouse trials, which confirmed the suppressive nature of the soil and also demonstrated that the suppression could be transferred to other soils. DNA-based profiling was used to examine the biological profile of the suppressive soil for a suppressive signature.

The objectives of this project were to explore novel disease control strategies for soilborne diseases of economic importance to the Australian processing potato industry. These included soil nutrient manipulation, novel chemical treatments, utilising and modifying bacterial communities in the soil and in planta, identifying disease suppressive soils, and understanding the complex relationships influencing pathogen populations and disease expression.

Ultimately this research will provide the basis for the development of thresholds for soil nutrient concentrations to manage soil-borne diseases of potato and to enhance the interpretation of disease risk as determined by DNA diagnostic tests, linking with project PT09023.

Important outputs from this project are decision support trees for the three major diseases powdery scab, common scab and Rhizoctonia disease. These incorporate the research outcomes from APRP1 and APRP2 into a simple format that is designed to enable growers to make more informed decisions about how to manage disease risk in their fields.

For ease of reporting the experimental programs for each disease are treated under the respective headings of Powdery scab, Common scab and Rhizoctonia disease.

GENERAL MATERIALS AND METHODS

This section provides details of common materials and methods used in the Victorian component of the research, mainly in field trials and glasshouse trials. They include trial design, soil and plant sampling and analysis, harvest sampling, disease incidence and severity and yield assessments.

Apart from differences in specific treatments applied, the methodologies used in the different field trials were essentially the same. All trials were generally planted in October, November or December and harvested in April, May or June.

TRIAL LOCATION AND DESIGN

Field trials were conducted each year in two major processing potato cropping areas of Victoria. Trials directed at powdery scab control were conducted on a ferrosol soil at a site near Ballarat, in the Central

Highlands area of Victoria, a major production area for French fry processing potatoes. Powdery scab and Rhizoctonia stem canker are the predominant diseases in this production area.

Trials for common scab control were conducted on the peaty clays of the crisping potato production area of the Koo Wee Rup swampy plain, near Cora Lynn, and on a red-brown earth soil near Mirboo North in the Gippsland region of Victoria . The predominant diseases in these production area are common scab and Rhizoctonia stem canker.

Trials were generally randomized block designs with 5 replicates. Individual plots were four potato rows wide by 15 m long. For single cultivar trials, all four rows were planted to the same cultivar. In trials in which treatments were tested on two different cultivars, the four row plots were split for the two cultivars (two rows per cultivar).

Field trials were established in fields that were to be planted with commercial potato crops. This ensured that, apart from the treatments being trialed, the trial plots were managed by the grower (fertilizer, irrigation, pesticides, fungicides etc.) in the same manner as the adjacent commercial crop.

TREATMENT APPLICATION

Rows were formed with the potato planter (without fertiliser and seed potatoes) by the grower to allow marking out of plots and application of treatments before planting. The different soil treatments were broadcast onto the surface of the plots by hand and mixed into the top 30 cm of soil with a tractor-driven rotary hoe. Fungicide treatments were drenched over the surface of each plot with watering cans before incorporation with the rotary hoe.

NUTRIENT ANALYSIS

Soil, petiole and tuber peel nutrient analyses were conducted by A&L Canada Laboratories, with the exception of 2009/10 when they were performed by SWEP Pty Ltd Analytical Laboratories, Keysborough, Victoria. The different soil parameters analysed and methods of analysis are presented in Table 5.A.I-1.

http://www.alcanada.com/index.htm

Soil Basic Test (S1B): Organic Matter, Phosphorus, (Sodium Bicarbonate and Bray 1), Potassium, Magnesium, Calcium, Sodium, (Ammonium acetate), soil pH, Aluminium, Saturation of Cation Elements including Sodium, calculated C.E.C. saturation %P, %K/Mg ratio.

Petiole analysis (PT2): Nitrogen, Phosphorus, Potassium, Magnesium, Calcium, Sodium, Sulphur, Boron, Zinc, Manganese, Iron, copper, and Aluminium + Nitrate-N

Peel analysis (PT4): Nitrogen, Phosphorus, Potassium, Magnesium, Calcium, Sodium, Sulphur, Iron, Aluminium, Manganese, Boron, Copper, and Zinc

Table 5.A.I-1 – Soil tests and methods of analysis for soil and plant nutrient concentrations used by A&L Canada

Abbreviation	Parameter	Extraction Method and/or Test
OM	Organic matter	Loss of weight on ignition (105 & 360°C)
Total N	Total Nitrogen	Combustion, 950°C
$N\text{-}NO_3$	Nitrate	Lachat method, spectroscopy, 520 nm
pH (water)		1:1 ratio with water, pH meter
pH (buffer)	used if pH<6.6 only	1:1:1 soil:water:Sikora buffer
P	Phosphorus	ammonium acetate, P1 (weak Bray), ICP
P	Phosphorus	sodium bicarbonate extraction, ICP
S	Sulphate	EDTA Acetate or Melich 3, spectroscopy
B	Boron	hot water extraction, ICP
Al	Aluminium	1 M KCl, ICP*
Zn, Cu, Mn, Fe	Zinc, copper, manganese, iron	DTPA* Extraction, ICP*
all other metals	K, Na, Mo, Ca, etc	DTPA, ICP
CEC	Cation Exchange Capacity	Ammonium displacement
EC	Soluble salts (Electrical Conductivity)	1:2 or 1:5 soil:water

*ICP, Inductively Coupled Plasma (spectroscopy); DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetate

PATHOGEN DNA ANALYSIS

Pathogen DNA concentrations in soil samples from field and glasshouse trials were measured by the South Australian Research and Development Institute (SARDI) diagnostic laboratories, Urrbrae, South Australia (HAL Project No. PT09023).

SAMPLING OF SOIL AND PLANTS, HARVEST AND YIELD ASSESSMENTS

The following sampling, analyses and assessments were performed for each trial plot during the growing season :

- Pre-planting – soil samples for analyses of soil nutrient concentrations and pathogen DNA concentrations
- Approximately 8 weeks after planting – soil samples for pathogen DNA and nutrient analyses at tuber set (start of tuber initiation) and petiole samples for nutrient analyses
- Tuber harvest time - soil samples for pathogen DNA and nutrient analyses, tubers harvested, sorted and weighed for total and marketable yields. Disease incidence and severity, specific gravity and tuber peel nutrient analyses were determined on 50-tuber subsamples taken during sorting.

Soil sampling protocol

Ten samples were taken to a depth of 15 cm using a standard garden trowel. The samples were taken in a zig-zag pattern from the 2 middle rows of each four-row plot when a single cultivar was trialed, or from two rows per cultivar in the split-plots when two cultivars were trialled. The ten samples were pooled into a composite sample from which subsamples were taken for soil nutrient and pathogen DNA analyses.

Petiole sampling protocol

Plant petioles were collected at tuber set to measure the uptake of the applied nutrients into the plant. 20 petioles were randomly collected from plants in the middle 2 rows of the plot.

Harvest

Daughter tubers were mechanically harvested from 10 metre strips of the 2 middle rows per plot.

Yield assessments

Potatoes were graded into different size categories according to a standard marketable size range for either French fries or crisps. The weights of the different size categories were recorded to calculate total and marketable yields.

Ballarat French fry size grades (tuber weight): Chat <70 g, Small 71-170 g, Medium 171-340 g, Large >340 g

Cora Lynn Crisping size grades (tuber diameter): Chat <40 mm, Marketable 40-90 mm, Oversize >90 mm

The "yield to the factory" was based on the proportion of tubers exceeding disease severity standards (% tuber surface covered by scab), therefore not meeting acceptable quality for the French fry or crisping grade.

The French fry 'yield to factory' was calculated using the model of discard at the factory used by McCain (David Antrobus pers. comm).

- Any disease up to 25% coverage is docked 25% (coverage = % tuber surface covered with scab)
- Any disease between 25-50% coverage is docked by 50%
- Any disease over 50% coverage is discarded.

The Crisping 'yield to factory' was calculated using the model of discard at the factory used by Snackbrand (Michael Hicks pers. comm.)

- Any disease up to 2% coverage is docked by 2%
- Any disease up to 5% coverage is docked by 5%
- Any disease greater than 5% coverage is discarded.

Tuber quality assessments

Specific gravity is the weight of the tuber compared with the weight of the same volume of water. Ten tubers were taken at random from the sample to be measured. The tubers were weighed in air and weighed again under water.

SG = (wt in air in grams)/(wt in air in grams – wt in water in grams)

DISEASE ASSESSMENTS

Incidence (% tubers affected) and severity (% area of tuber surface affected) of powdery scab, common scab and Rhizoctonia black scurf were determined on samples of harvested tubers. In some trials the relative abundance (severity) of powdery scab galls on plant roots was also recorded. Rhizoctonia disease on plants was recorded as the incidence and severity of stems and stolons with canker symptoms 8 weeks after emergence.

Powdery scab root galling

The severity of powdery scab galls on potato root systems was determined using a relative abundance scale from 0-4, where 0 = no galls and 4 = large galls on the majority of major roots (Table 5.A.I-2).

Table 5.A.I-2 – Scoring key for the relative abundance of powdery scab root galls.

Score	Symptoms observed
0	No root galls
1	One or two root galls
2	Several galls, mostly small (<2 mm in diameter)
3	Many galls, some >2 mm in diameter
4	Most major roots with galls, some or all > 4 mm in diameter

Source powdery scab scoring workshop 2002

Rhizoctonia stem canker

Eight individual plants were harvested from each plot around eight weeks after emergence and examined for the presence of Rhizoctonia cankers (sunken lesions) on the sprouts and stolons. Disease incidence was recorded as the % of plants with cankers.

Rhizoctonia severity was assessed using a scale of 0-5 that considered the number and severity of lesions per stem and the number of stems affected per plant. The scale ranged from 0 = no symptoms to 5 = death of the plant (Table 5.A.I-3).

Table 5.A.I-3 – Scoring key for Rhizoctonia stem and stolon canker of potato.

Score	Description of symptoms
0	No symptoms
1	Very few superficial lesions not covering the whole circumference of the sprouts/stems
2	Several superficial lesions not covering the whole circumference of the sprouts/stems

Score	Description of symptoms
3	Superficial lesions covering the whole circumference of the sprouts/stems. Affected areas are not shrinking significantly, but the surface layer may be corky. A few sprouts/stems may have died.
4	Between 25-75% of the sprouts/stems have died
5	All of the sprouts/stems are dead

Tuber disease assessments

A sub-sample of 50 tubers per plot was assessed for disease incidence (% tubers with symptoms) and severity using a visual assessment scale based on the proportion of tuber surface affected (0-6 for powdery and common scab, where 0 = no disease, 6 = greater than 50% tuber surface area affected) (Figure 5.A.I-1). Symptoms of black scurf were rated on a scale of 0-4 depending on the relative quantity, width and thickness of sclerotia on the tuber surface (Figure 5.A.I-2).

Disease severity on the individual tubers was combined into a powdery scab, common scab or black scurf disease severity index for the 50-tuber sample. The disease index was calculated based on the number of tubers in each of the severity categories:

((#CAT0 x 0) + (#CAT1 x 1) + (#CAT2 x 2) + (#CAT3 x 3) + (#CAT4 x 4) + (#CAT5 x 5) + (#CAT6 x 6)) / (TOTAL OBSERVATIONS x MAX. SCORE) x 100/1

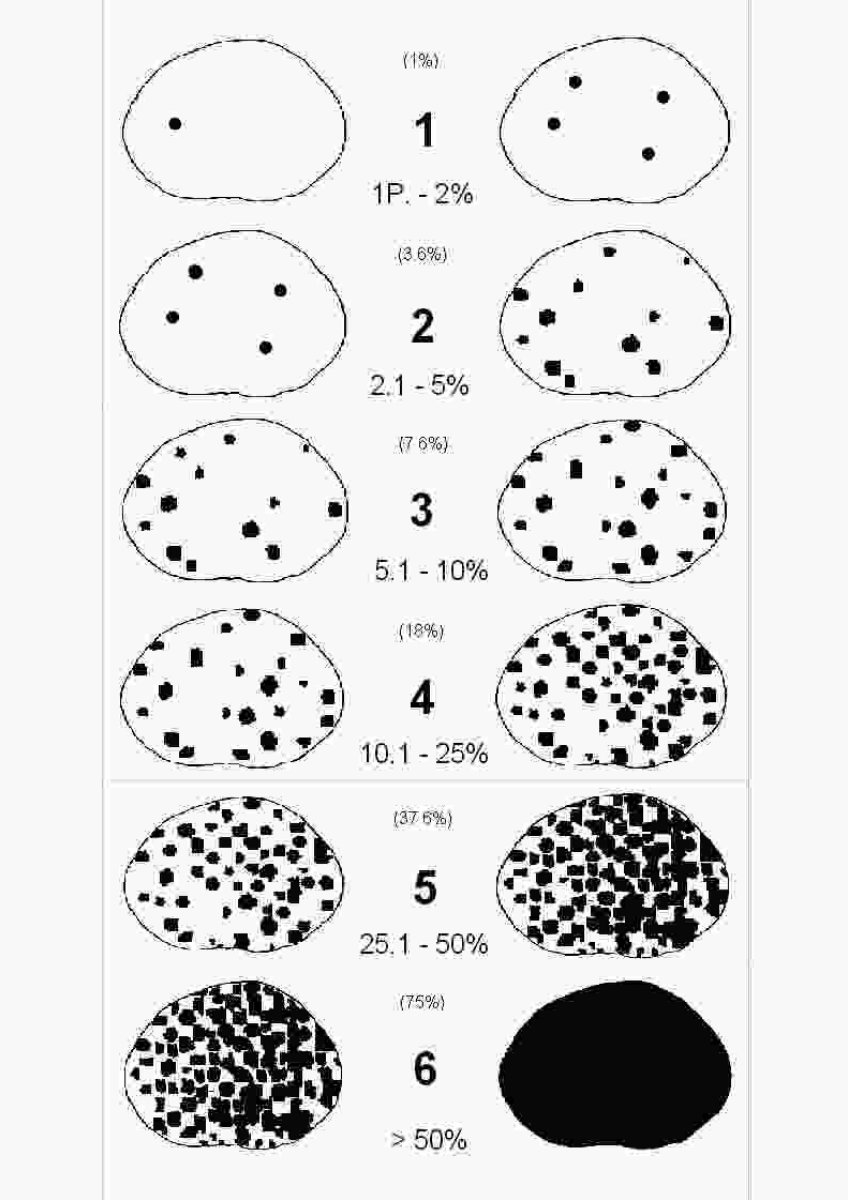


Figure 5.A.I-1 –Visual assessment key for scoring common and powdery scab disease severity based on the % tuber surface covered with symptoms

Source: Powdery scab scoring workshop 2002

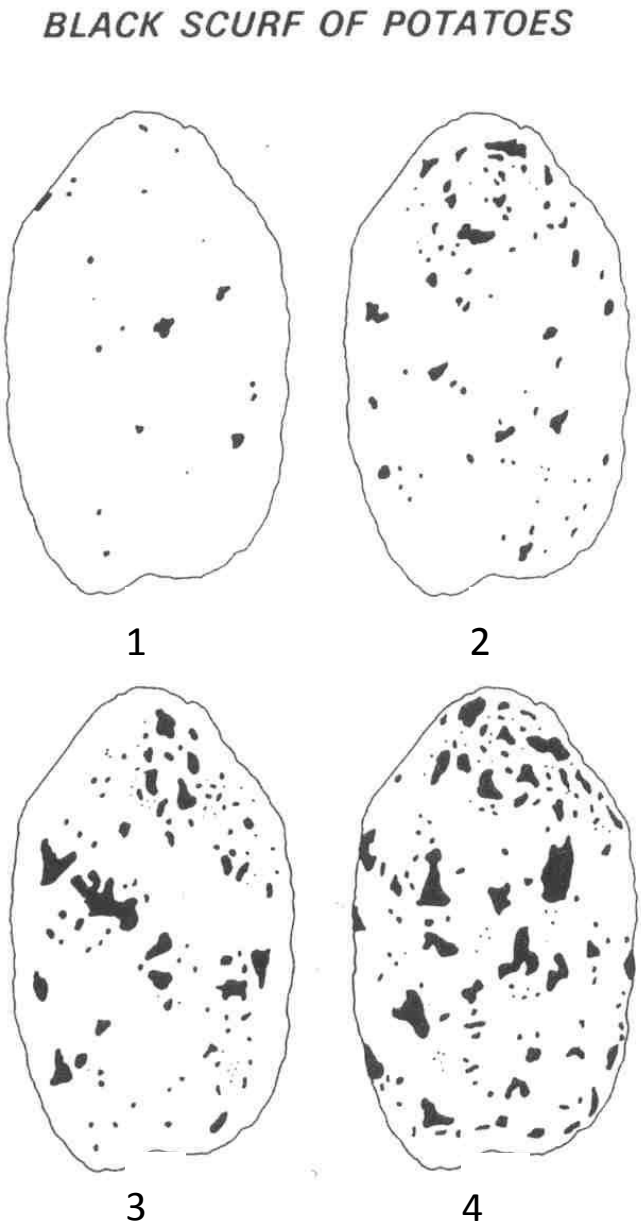


Figure 5.A.I-2 – Visual assessment key for scoring black scurf (*Rhizoctonia solani*) severity based on the % tuber surface covered with symptoms

STATISTICAL ANALYSES

Trial data was analyzed by ANOVA to determine significant differences attributable to treatments using the statistical software package GenStat®15th Edition for Windows (Payne et al. 2009) unless otherwise stated.

POWDERY SCAB DISEASE

INFLUENCE OF SOIL NUTRIENT AMENDMENTS ON POWDERY SCAB DISEASE

SUMMARY

In trials over five seasons, soil treatments of elemental sulphur and zinc consistently reduced powdery scab disease on tubers of the susceptible cultivar Shepody. Sulphur was generally more effective than zinc. In untreated soil, an average of 90% of tubers were affected with scab, with an average severity of 50% of the tuber skin affected. Sulphur incorporated into soil before planting reduced disease incidence to 58% and severity to 20%. Zinc (as EDTA) reduced disease incidence to 78% and severity to 40%.

The trials were conducted on a ferrosol soil near Ballarat. The zinc EDTA applications raised the soil zinc concentrations from a baseline of 2-3 ppm to 20-30 ppm. The EDTA form provided more consistent reductions in scab than zinc oxide or sulphate. Sulphur, applied as Atomic sulphur (99% sulphur) or Top Wettable sulphur (80% sulphur), raised soil sulphur concentrations from a baseline of 10-30 ppm to 100-120 ppm. A lower rate of Atomic sulphur (800 kg/ha) was equally effective as a higher rate in reducing powder scab (1200 kg/ha), and both these treatments were more effective than Top Wettable sulphur applied at 1000 and 1500 kg/ha. Iron EDTA applied to soil resulted in small but significant reductions in scab incidence or severity in only two of five trials.

None of the treatments adversely effected the yield of marketable tubers. When taking into account the severity of scab on tubers, which affects quality for processing, the Atomic sulphur treatments increased potential yields to the factory by an average 10 t/ha for the French fry category and 23 t/ha for the crisping category. The zinc EDTA treatment returned an average increase in yield of 11 and 8 t/ha for the French fry and the crisping categories.

Treatments used for comparison in the trials included a soil application of the fungicide fluazinam (as Shirlan) and the resistant cultivar, Russet Burbank. Both of these outperformed all of the nutrient treatments in terms of both disease and marketable yield. Fluazinam reduced disease incidence and severity on Shepody to 35% and 12%, respectively, resulting in a potential 14 and 27 t/ha increase in yield of French fry and crisping quality potatoes to the factory. However, fluazinam is not registered for use against powdery scab in potatoes.

Disease incidence in untreated Russet Burbank ranged from 4% to 17%, averaging 7% over five trials, with an average severity of 1%. Atomic sulphur at the low and high rate, and the high rate of Wettable top sulphur, reduced disease incidence and severity. The effects of zinc EDTA were variable, although a combined treatment with zinc EDTA and iron sulphate consistently reduced incidence and severity but was less effective than sulphur. The fluazinam treatments were no better than sulphur on Russet Burbank. However, because of the already very low incidence and severity, these treatments did not provide any significant additional benefits in tuber quality.

Foliar applications of different forms of iron and zinc (three applications between emergence and tuber initiation) resulted in small, but significant, reductions in scab severity in one trial but some of these treatments caused leaf burn. Iron EDTA had an effect in a second trial reducing disease incidence only. Foliar treatments were not effective at lower rates in a subsequent trial. In a glasshouse experiment, two consecutive foliar sprays of iron EDTA had no effect on the severity of root galling but reduced the incidence and severity of powdery scab on tubers, whereas foliar sprays with zinc EDTA did not affect disease.

In a potato bioassay system, concentrations of zinc at 10 ppm and iron at 15 ppm in nutrient solutions significantly reduced the quantity of *S. subterranea* DNA in roots and the severity of powdery scab root infection on Shepody plants indicating that these nutrients can have direct effects on infection at higher concentrations. In a series of experiments testing the effects of individual nutrients on powdery scab in a sand/nutrient culture system, different rates of iron had little effect on the severity of powdery scab root galls.

A new tool, a real-time quantitative reverse transcription PCR (RT-qPCR) assay was developed for *S. subterranea* using the 18S rRNA, *EFL*, actin and polyubiquitin marker genes to quantify viable spores within sporosori. This study has been successful in designing and confirming the presence of all four marker genes in *S. subterranea* sporosori and in roots infected with *S. subterranea* (zoosporangial stage).

This study shows that manipulating soil sulphur and zinc levels has the potential to be used as a disease management option in the absence of registered fungicide treatments for powdery scab in Australia, especially when integrated into a disease management program such as outlined in the powdery scab decision tree.

INTRODUCTION

Powdery scab, caused by the pathogen *Spongospora subterranea*, is a major disease of potatoes in Australia. Symptoms of disease include galls on roots and stolons and scab-like eruptions on the surface of tubers. Powdery scab is primarily a blemish disease that reduces tuber quality, but diseased tubers are prone to secondary infections in the ground and in storage. Consignments of potatoes grown for processing are downgraded in value when powdery scab is present. Grading diseased tubers is labour intensive and reduces financial returns.

In the absence of a suitable host, the pathogen survives in the soil as sporosori (sporeballs) (Jones and Harrison 1972). Root exudates from host plants stimulate the germination of the resting spores to produce primary zoospores (Kole 1954) which swim in soil water to infect stolons and roots. Secondary zoospores are released from the infected tissue and these infect surrounding tubers and roots (Kole and Gielink 1961). In time, these infections result in the development of sporosori.

The three-layered walls of the sporosori make them highly resistant to environmental stresses and enable them to survive for more than 10 years in soil (Merz 2008). This ability of S. *subterranea* to survive in soil for very long periods as resting spores and its potential to rapidly multiply as zoospores means this disease is difficult to control.

Chemical treatment of seed tubers has been promoted as a method for preventing transmission of powdery scab. Seed treatment with formaldehyde, fluazinam, flusulfamide, heat, dichlorophen-sodium, mancozeb, propineb, sulphur and compounds of copper, mercury or zinc have all been reportedly effective at reducing transmission of powdery scab (Falloon et al. 1996).

Several fungicides have been shown to reduce the incidence and severity of powdery scab when applied to the soil. Mercury- or copper-containing compounds such as mancozeb, maneb, quinotzene, fluazinam and flusulfamide have all reduced powdery scab where soil is infested with *S. subterranea* (Falloon et al. 1996). In the past, soil fumigation with methyl bromide, metham sodium applied in irrigation water and soil-applied formaldehyde reduced disease (Nachmias and Krikun 1988) but these options either have or are being withdrawn. In Australia, there are no registered chemical treatments for the control of either seed or soilborne powdery scab.

Several studies have demonstrated that modifying the soil chemical concentrations can reduce powdery scab. Soil applications of sulphur (McCreary 1967) or zinc salts (Wale 1987) have been shown to reduce disease in field, as has the application of high nitrogen amendments and fertilizers (de Boer and Crump 2005). Boron has been shown to reduce infection in laboratory or glasshouse grown potatoes (Falloon et al. 2001a).

In APRP1, multivariate analysis of survey data from across South Australia, Tasmania and Victoria showed that reduced powdery scab development on tubers at harvest was associated with increased levels of sulphur, zinc and iron in the cropping soils. The data from this survey demonstrated the potential to explore manipulating these relationships for novel disease control.

The effects of nutrients amendments on powdery scab

Zinc

Soil applications of zinc have been shown to reduce powdery scab in the field. In-furrow treatments with a mixture of zinc oxide and zinc sulphate reduced the incidence of powdery scab in UK and NZ trials (Wale 1987; Burgess et al. 1992; Falloon et al. 1996). Zinc oxide alone significantly reduced powdery scab in trials (Braithwaite et al. 1994). Seed tuber treatments, however, have produced inconsistent results, with 37% reduction (Burgess et al. 1992) or no effect (Falloon et al. 1996). Burnett and Wale (1993) investigated the effect of pathogen inoculum and found that high levels of inoculum reduced the efficacy of zinc treatments.

Sulphur

Foliar-applied elemental sulphur (S) is a very commonly-used fungicide, but additionally sulphur has been demonstrated to increase the resistance of crops to fungal diseases (Bloem et al. 2004; Haneklaus et al. 2012; Klikocka et al. 2005). Experiments to examine the nutritional effects of sulphur on disease control have been mainly carried out with sulphate salts to avoid the fungicidal impact of sulphur (Cooper and Williams 2004). In a field trial with oilseed rape infected by *Pyrenopeziza brassicae,* Schnug and co-workers showed that sulphur fertilisation using soil-applied sulphate caused a similar yield increase to fungicide application (Schnug et al. 1995). They described this as sulphur-induced resistance (Schnug 1997). Subsequently, soil-applied sulphur was found to increase resistance of different crops to a variety of fungal pathogens under glasshouse and field conditions (Bourbos et al. 2000; Wang et al. 2003; Klikocka et al. 2005). In potato field trials, sulphur applied as either sulphate or elemental S significantly reduced infection rate and severity of black scurf (*Rhizoctonia solani)* (Klikocka et al. 2005).

Iron

Iron is the second most abundant metal in the earth's crust, yet despite this, the low solubility of iron compounds in many soils prevents plant iron uptake and induces the development of iron deficiency symptoms. Iron availability in soil is related to soil pH, with decreasing soil pH increasing the availability of iron.

The importance of iron in host-pathogen interactions has been reported for various pathosystems (Expert et al. 1996). Iron EDTA reduced the aggressiveness of *Botrytis* cinerea (Brown and Swinburne 1982) and inhibited the formation of leaf necrosis induced by this pathogen on *Vicia faba* (Vedie and Le Normand 1984). Liu et al. (2007) demonstrated that pathogen infection triggers iron mobilization in plant cells, enhancing the production of reactive oxygen species, which is characteristic of an immediate host defence reaction.

Falloon et al. (2011a) found that infected potato plants were iron deficient due to the leaky nature of their infected roots, making them unable to take up and retain sufficient iron for healthy growth.

Aim and approach:

The aim of this research was to investigate whether manipulation of nutrients such as zinc, iron and sulphur could be developed as a control measure for powdery scab.

A series of field and glasshouse trials were conducted in Victoria to evaluate the practicality of manipulating these nutrients and determining the impact on powdery scab disease incidence and severity on tubers, and on the total and marketable yield of potatoes.

Greenhouse bioassays were used in New Zealand to test a range of nutrient concentrations, measuring their effects on the development of powdery scab root galls and related effects of the root galls on root function and plant growth.

Although the focus of the research was on manipulating zinc, iron and sulphur, other soil nutrients were also investigated.

FIELD TRIALS

A series of field trials were conducted on a potato property east of Ballarat in the Central Highlands region of Victoria, which had a long history of powdery scab. The trials were established within a commercial crop following the grower's normal rotation. The different treatments applied in each year are outlined in Table 5.A.I-4. All treatments were applied to two different potato cultivars, Shepody (highly susceptible) and Russet Burbank (very low susceptibility), which were grown in a split plot design. The first three years of trials included soil and foliar applied treatments and in the last two years only soil applied treatments were evaluated. A soil applied treatment of the fungicide fluazinam (Shirlan® Fungicide) was used in all trials applied at a standard rate as a reference control treatment. The trial results for the different cultivars are reported in separate tables.

Table 5.A.I-4 – Active ingredient (*a.i.*), rate of application and rate of product for all chemical treatments applied to foliage or soil in field trials on powdery scab of potatoes over five seasons, Ballarat 2009-2014

				2009/2010		2010/2011		2011/2012		2012/2013		2013/2014	
Compound or *a.i.*	Application	Product	*a.i.* %	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha
Untreated		Nil	—	—	—	—	—	—	—	—	—	—	—
Fluazinam	Soil	Shirlan® Fungicide (500 g/L fluazinam)	50	4.5	9	4.5	9	4.5	9	4.5	9	4.5	9
Sulphur	Soil	Top Wettable Sulphur®	80	1200	1500	1200	1500	1200	1500	1200	1500	1200	1500
Sulphur	Soil	Top Wettable Sulphur®	80	—	—	—	—	—	—	800	1000	800	1000
Sulphur	Foliar	Top Wettable Sulphur®	80	2	2	1	1.25	1	1	—	—	—	—
Atomic S	Soil	Atomic S	99	—	—	—	—	—	—	1200	1212	1200	1212
Atomic S	Soil	Atomic S	99	—	—	—	—	—	—	800	808	800	808
Fe EDTA	Soil	Rapisol®Fe or Tradecorp®Fe	13.2	100	758	100	758	100	758	100	758	100	758
Fe EDTA	Foliar	Rapisol®Fe or Tradecorp®Fe	13.2	1	8	2	15	0.25	2	—	—	—	—

				2009/2010		2010/2011		2011/2012		2012/2013		2013/2014	
Compound or *a.i.*	Application	Product	*a.i.* %	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha
$FeSO_4$	Soil	Iron sulphate heptahydrate	20	100	500	100	500	100	500	100	500	100	500
$FeSO_4$	Foliar	Iron sulphate heptahydrate	20	2	10	1	5	2	10	—	—	—	—
ZnO	Soil	Ultra Zinc	100	100	100	100	100	—	—	—	—	—	—
ZnO	Foliar	Ultra Zinc	100	2	2	1	1	—	—	—	—	—	—
$ZnSO_4$	Soil	Zinc sulphate heptahydrate	22.7	100	441	100	441	100	441	100	441	100	441
$ZnSO_4$	Foliar	Zinc sulphate heptahydrate	22.7	2	9	1	4	2	8	—	—	—	—
Zn EDTA	Soil	Rapisol®Zn or Tradecorp®Zn	14	100	714	100	714	100	714	100	714	100	714
Zn EDTA	Foliar	Rapisol®Zn or Tradecorp®Zn	14	2	14	1	7	2	14	—	—	—	—
Fe EDTA	Soil	Rapisol®Fe or Tradecorp®Fe	13.2	—	—	—	—	—	—	100	758	100	758
+ $ZnSO_4$		Zinc sulphate heptahydrate	22.7	—	—	—	—	—	—	100	441	100	441
Zn EDTA	Soil	Rapisol®Zn or Tradecorp®Zn	14	—	—	—	—	—	—	100	500	100	500

				2009/2010		2010/2011		2011/2012		2012/2013		2013/2014	
Compound or *a.i.*	Application	Product	*a.i.* %	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha	*a.i.* kg/ha	Product kg or L/ha
+ $FeSO_4$		Iron sulphate heptahydrate	20	—	—	—	—	—	—	100	714	100	714
Mancozeb	Soil	Penncozeb® 420 SC	42	—	—	—	—	—	—	—	—	8.5	20
Mancozeb	Seed	Dithane® M45 Fungicide	80	—	—	—	—	—	—	—	—	1.2	1.5
Mancozeb	Soil	Dithane® M45 Fungicide	80	—	—	—	—	—	—	—	—	1.2	1.5
	+ Seed	+ Penncozeb® 420 SC	42	—	—	—	—	—	—	—	—	8.5	20
Potassium phosphonate	Soil	Agri-Fos®600 (600 g/L potassium phosphonate)	60	9.6	16	9.6	16	—	—	—	—	—	—

—, not tested in that season

SEASON ONE 2009/2010

Details of the 15 soil and foliar applied nutrient and fungicide treatments are presented in Table 5.A.I-4. None of the treatments applied had any measurable effect on the rate or number of plants emerged (data not presented). At harvest, an average of 98% of the cv. Shepody (susceptible cultivar) tubers in the untreated control plots had symptoms of powdery scab, whereas only 17% of the untreated Russet Burbank (resistant cultivar) tubers were affected with the disease (Table 5.A.I-5 and Table 5.A.I-6).

Results are presented for cv. Shepody in Table 5.A.I-5. Soil application of 9 L/ha of Shirlan® fungicide (fluazinam) significantly reduced ($P<0.05$) the incidence of tubers with powdery scab to 52% and the severity of the disease on tuber skin to 19% of the untreated control, respectively. The soil sulphur treatment of 1200 kg/ha reduced the incidence and severity of powdery scab to 67% and 33%, respectively, corresponding with elevated concentrations of available sulphur in the soil and plant petioles at the time of tuber set. Foliar application of sulphur had no effect on powdery scab incidence, severity or the sulphur concentration in petioles. Agri-Fos®600 applied to the soil had no significant effect on disease incidence and severity.

All iron and zinc soil and foliar treatments reduced the severity of powdery scab, with the exception of soil-applied ZnO and foliar-applied $ZnSO_4$. These treatments were less effective in reducing scab severity than Shirlan® and sulphur soil treatments. The three different soil zinc treatments ($P<0.05$) increased the zinc concentrations in the tuber set soil (22-30 ppm) compared with the untreated control (2 ppm). Only the ZnO soil treatment significantly raised the concentrations of zinc in the petioles. Only the Fe EDTA soil treatment significantly raised the soil iron concentrations (73 ppm compared with 43 ppm in the untreated). The iron concentrations in soil were also higher ($P<0.05$) with the Zn EDTA soil treatment. Total and marketable yield were not significantly affected by any of the soil and foliar treatments.

Table 5.A.I-5 – (cv. Shepody) Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on tuber set soil and petiole nutrient concentrations, powdery scab disease, and total and marketable yield of tubers in a field trial at Ballarat, 2009/2010.

Treatment	Powdery scab		Tuber set soil (ppm)			Petiole			Yield (t/ha)	
	% tubers affected	Severity index (%)	S	Zn	Fe	%S	Zn ppm	Fe ppm	Total	Marketable
Untreated	98	69	1.80	1.65	43.0	0.197	27	56	45	34
Fe EDTA S	93	55*	1.90	1.44	73.4*	0.183	23	88	44	35
Fe EDTA F	92	49*	-	-	-	0.190	21	53	45	34
$FeSO_4$ S	92	43*	2.54	1.83	50.8	0.220	24	50	46	36
$FeSO_4$ F	95	56*	-	-	-	0.213	24	80	45	36
ZnO S	95	59	1.86	30.10*	53.2	0.200	56*	70	43	35
ZnO F	96	55*	-	-	-	0.213	40	98	45	35
$ZnSO_4$ S	88	43*	2.74	21.70*	41.0	0.223	47	17	46	38
$ZnSO_4$ F	97	60	-	-	-	0.197	37	25	47	36

Treatment	Powdery scab		Tuber set soil (ppm)			Petiole			Yield (t/ha)	
	% tubers affected	Severity index (%)	S	Zn	Fe	%S	Zn ppm	Fe ppm	Total	Marketable
Zn EDTA S	90	51*	1.84	23.9*	59.0*	0.200	45	61	46	35
Zn EDTA F	98	53*	-	-	-	0.200	27	49	43	34
Top Wettable S S	67*	33*	10.70*	1.6	51.0	0.268*	27	36	44	35
Top Wettable S F	96	62	-	-	-	0.203	23	49	45	35
Shirlan® S	52*	19*	-	-	-	-	-	-	45	37
Agri-Fos®600 S	98	61	-	-	-	-	-	-	43	33
lsd (P=0.05)	13	12	1.34	14.8	12.4	0.029	21	ns	ns	ns

*Significantly different from the untreated control at P=0.05, ns, not significant

The incidence and severity of powdery scab on the untreated control of cv. Russet Burbank tubers was 17% and 3%, respectively (Table 5.A.I-6). Soil-applied Shirlan®, sulphur, Fe EDTA, $FeSO_4$ and Zn EDTA treatments significantly reduced (P<0.05) both incidence and severity but Agri-Fos®600 had no effect. Foliar-applied $FeSO_4$, ZnO and Zn EDTA significantly reduced (P<0.05) both incidence and severity, and foliar-applied Fe EDTA significantly reduced severity but not incidence. Foliar-applied Fe EDTA was phytotoxic and burnt the leaves, reducing yield. There were no other significant effects on total and marketable yield. Soil iron and zinc concentrations at tuber set were as described previously for cv. Shepody.

Table 5.A.I-6 – (cv. Russet Burbank) Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on tuber set soil and petiole nutrient concentrations, powdery scab disease, and total and marketable yield of tubers in a field trial at Ballarat, 2009/2010.

Treatment	Powdery scab		Tuber set soil (ppm)			Petiole			Yield t/ha	
	% tubers affected	Severity index (%)	S	Zn	Fe	%S	Zn ppm	Fe ppm	Total	Marketable
Untreated	17	3.2	1.80	1.65	43.0	0.16	28.5	28	40	30
Fe EDTA S	3*	0.5*	1.90	1.44	73.4*	0.16	50.4	20	45	35
Fe EDTA F	5*	0.9	-	-	-	0.17	33.7	42	34*	25
$FeSO_4$ S	7*	1.2	2.54	1.83	50.8	0.17	31.3	54	42	30
$FeSO_4$ F	7*	1.3	-	-	-	0.15	19.3	52	41	31
ZnO S	18	3.2	1.86	30.10*	53.2	0.17	49.5	27	42	30
ZnO F	7*	1.3	-	-	-	0.16	30.7	27	41	30
$ZnSO_4$ S	11	1.9	2.74	21.70*	41.0	0.17	67.1	7	43	32
$ZnSO_4$ F	8	1.6	-	-	-	0.15	25.1	26	39	31

Treatment	Powdery scab		Tuber set soil (ppm)			Petiole			Yield t/ha	
	% tubers affected	Severity index (%)	S	Zn	Fe	%S	Zn ppm	Fe ppm	Total	Marketable
Zn EDTA S	5*	1.3	1.84	23.90*	59.0*	0.16	36.6	148	44	35
Zn EDTA F	9	1.5	-	-	-	0.15	22.6	37	38	27
Top Wettable S S	3*	0.5*	10.70*	1.60	51.0	0.18	24.8	77	41	32
Top Wettable S F	15	2.7	-	-	-	0.15	40.1	13	41	29
Shirlan® S	2*	0.3*	-	-	-	-	-	-	38	28
Agri-Fos®600 S	18	3.6	-	-	-	-	-	-	45	31
lsd (P=0.05)	9.1	1.8	1.34	14.80	12.4	ns	ns	ns	5.8	ns

*Significantly different from the untreated control at P=0.05, ns, not significant

The most promising treatments were soil-applied Top Wettable sulphur and the fungicide Shirlan®, and this was consistent for both cultivars. Foliar-applied Top Wettable sulphur and $ZnSO_4$, and soil-applied ZnO had had no significant effect on disease in either cultivar. The other zinc and iron treatments all reduced incidence and severity of disease in Russet Burbank, but only reduced severity, not incidence, in Shepody.

SEASON TWO 2010/2011

The 2009/2010 trial showed that some soil- and foliar-applied nutrient and fungicide treatments reduced the incidence and severity of powdery scab. The trial was repeated in 2010/11 using the same 15 products, but with reduced rates for the foliar applications (Table 5.A.I-4). Rates in the previous trial caused some phytotoxicity, particularly Fe EDTA which caused some leaf burn and reduced yield.

The three different soil zinc treatments significantly increased (P<0.05) the zinc concentrations in the tuber set soil (16-36 ppm) compared with the untreated control (2 ppm, Table 5.A.I-7). Iron sulphate and Top Wettable sulphur soil treatments significantly raised the soil iron concentrations compared with the untreated. The iron concentrations in cv. Shepody petioles were significantly increased by the foliar applied iron treatments. Only foliar-applied zinc treatments significantly raised the concentrations of zinc in the petioles (Table 5.A.I-8).

Table 5.A.I-7 – Effects of soil- (S) and foliage-(F) applied nutrient and fungicide treatments on the concentration of *S. subterranea* in soil at tuber set soil and on soil nutrient concentrations at tuber set and harvest in a field trial at Ballarat, 2010/2011.

Treatment	*S. subterranea* pg DNA/g soil			Tubers set soil (ppm)				Harvest soil (ppm)			
	Planting	Tuber set	Harvest	pH (water)	S	Zn	Fe	pH (water)	S	Zn	Fe
Untreated	205	182	535	5.22	11	2	55	5.22	17	3	61

Treatment	*S. subterranea* pg DNA/g soil			Tubers set soil (ppm)				Harvest soil (ppm)			
	Planting	Tuber set	Harvest	pH (water)	S	Zn	Fe	pH (water)	S	Zn	Fe
Fe EDTA S	262	203	551	5.16	10	2	57	5.16	15	3	63
Fe EDTA F	175	136	224	5.14	13	3	57	5.14	21	3	63
$FeSO_4$ S	292	177	794	5.10	22	3	60*	5.10	22	3	66*
$FeSO_4$ F	226	142	1040	5.26	15	3	55	5.26	15	3	62
ZnO S	249	156	843	5.24	14	36*	57	5.24	16	58*	62
ZnO F	285	192	686	5.20	14	3	56	5.20	15	4	62
$ZnSO_4$ S	207	165	693	5.26	15	16*	56	5.26	20	39*	61
$ZnSO_4$ F	241	153	463	5.06	17	4	56	5.06	17	4	63
Zn EDTA S	245	149	280	5.14	15	19*	54	5.14	16	37*	58
Zn EDTA F	245	186	951	5.22	13	4	58	5.22	15	4	63
Top Wettable Sulphur S	297	200	644	4.84	98*	3	59*	4.84	170*	3	67*
Top Wettable Sulphur F	201	152	631	5.20	16	2	56	5.20	21	12	62
Shirlan® S	197	157	163	5.12	14	3	52	5.12	19	3	62
Agri-Fos®600 S	283	209	1335	5.22	12	2	57	5.22	16	3	64
lsd (P=0.05)	ns	ns	ns	0.17	12	4	3.8	0.17	15	13	2.8

*Significantly different from the untreated control at P=0.05, ns, not significant

In the untreated control, disease incidence and severity on Shepody tubers were 90% and 33% respectively (Table 5.A.I-8). The soil applied Shirlan® and sulphur treatments were the most effective, reducing (P<0.05) incidence to 20% and 51% and severity to 5% and 15%, respectively. Foliar-applied sulphur and Fe EDTA treatments reduced (P<0.05) disease incidence to 65% and 68%, respectively, but had no effect on disease severity.

No other treatments had a significant effect on disease incidence and severity. Total and marketable yields were not affected by the treatments with the exception of an 8 t/ha reduction in marketable yield with the foliar application of Fe EDTA.

Table 5.A.I-8 – (cv. Shepody) Effects of soil- (S) and foliage-applied (F) nutrient and fungicide treatments on tuber set soil and petiole nutrient concentrations and powdery scab disease and total and marketable yield of tubers in a field trial at Ballarat, 2010/2011.

Treatment	Powdery Scab		Yield t/ha		Petioles			Peel		
	% tubers affected	Severity Index (%)	Total	Marketable	%S	Zn (ppm)	Fe (ppm)	%S	Zn (ppm)	Fe (ppm)
Untreated	90	33	43	38	0.46	34	184	0.241	30.5	977
Fe EDTA S	80	29	37	32	0.47	34	226	0.244	22.8	1420
Fe EDTA F	65*	25	35	30*	0.45	38	304*	0.248	27.5	1484
$FeSO_4$ S	90	36	45	40	0.50	30	184	0.236	17.5	1498
$FeSO_4$ F	87	28	42	37	0.46	40	455*	0.254	36.5	1203
ZnO S	80	26	41	38	0.46	53	211	0.249	68.4*	1665
ZnO F	92	36	41	35	0.53*	524*	167	0.253	49.7	1567
$ZnSO_4$ S	85	29	43	39	0.51	45	193	0.247	34.8	1496
$ZnSO_4$ F	86	32	45	41	0.46	227*	155	0.253	23.9	1827
Zn EDTA S	67	22	37	33	0.47	50	186	0.231	44.2	1322
Zn EDTA F	82	28	41	36	0.45	242*	255	0.237	21.3	1451
Top Wettable S S	51*	15*	45	41	0.64*	32	183	0.265	22.0	1593
Top Wettable S F	68*	21	39	34	0.67*	38	165	0.244	25.3	1094
Shirlan® S	20*	5*	45	41	0.47	36	202	0.236	19.6	1212
Agri-Fos®600 S	95	42	42	38	0.47	35	187	0.254	22.2	2983
lsd (P=0.05)	20.1	13.2	ns	6.5	0.07	129	96	ns	23	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

On untreated cv. Russet Burbank, the incidence of powdery scab was low (4.4%) and severity was negligible (0.7%, Table 5.A.I-9). None of the treatments significantly reduced incidence or severity, but soil-applied Zn EDTA significantly increased incidence to 11.6% and severity to 1.9%. Despite this, marketable yields were significantly increased by soil-applied Fe EDTA, $FeSO_4$, and Shirlan®.

Table 5.A.I-9 – (cv. Russet Burbank) Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on tuber set soil and petiole nutrient concentrations and powdery scab disease and total and marketable yield of tubers in a field trial at Ballarat, 2010/2011.

Treatment	Powdery scab		Yield t/ha		Petiole			Peel		
	% tubers affected	Severity Index	Total	Marketable	%S	Zn (ppm)	Fe (ppm)	%S	Zn (ppm)	Fe (ppm)
Untreated	4.4	0.7	43	33	0.36	32	174	0.19	28.8	1577
Fe EDTA S	6.8	1.3	44	37*	0.34	33	194	0.16*	22.7	1954
Fe EDTA F	0.4	0.1	29*	22*	0.36	35	409*	0.21	28.4	1699
$FeSO_4$ S	8	1.3	46	37*	0.35	31	158	0.18	23.7	1887
$FeSO_4$ F	0.8	0.1	37*	28	0.33	31	404*	0.17	26.9	1287
ZnO S	5.6	0.9	43	36	0.36	38	183	0.18	111*	1533
ZnO F	2.4	0.4	42	35	0.36	559*	161	0.2	35	2009
$ZnSO_4$ S	4.4	0.7	43	36	0.37	34	180	0.19	97.2*	1448
$ZnSO_4$ F	5.2	0.9	43	35	0.38	269*	163	0.19	30.2	1630
Zn EDTA S	11.6*	1.9*	44	35	0.37	44	171	0.19	77.6*	1621
Zn EDTA F	4.4	0.8	42	34	0.38	175*	183	0.18	27.3	1543
Top Wettable S S	0.4	0.1	44	35	0.38	29	177	0.23*	29.1	1551
Top Wettable S F	2.4	0.4	41	33	0.63*	29	153	0.18	23	1564
Shirlan® S	0.8	0.1	46	37*	0.36	95	220	0.17	25.3	1496
Agri-Fos®600 S	5.6	0.9	43	35	0.37	28	173	0.17	23.6	1706
lsd (P=0.05)	6.4	1.11	4	3.8	0.05	139	107	0.02	24.5	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

Once again the most promising treatments were soil-applied Top Wettable sulphur and the fungicide Shirlan®. In this season, foliar-applied Top Wettable sulphur and Fe EDTA reduced disease incidence in Shepody but not severity. The level of disease on cv. Russet Burbank was very low and the only treatment that showed an effect was soil-applied Zn EDTA which increased disease.

SEASON THREE 2011/2012

In 2011/12, the two zinc oxide treatments and Agri-Fos®600 were omitted and the remaining 12 treatments were trialled (Table 5.A.I-4). The concentration of *S. subterranea* DNA averaged 187 pg/g soil at planting time, a level considered to be high risk for powdery scab disease according tothe PreDicta Pt rating scale.

Table 5.A.I-10 – Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on the concentration of pathogen DNA, sulphur (S), zinc (Zn) and iron (Fe) concentration in soil at planting (P), tuber set (TS), and harvest (H) in a field trial at Ballarat, 2011/2012.

Treatment	*S. subterranea* pg DNA/g soil			Soil S ppm			Soil Zn ppm			Soil Fe ppm		
	P	TS	H	P	TS	H	P	TS	H	P	TS	H
Untreated	194	123	2345	22	31	25	2	2	3	60	63.2	64
Fe EDTA S	172	96	1212	22	28	26	2	2	3	60	68.2*	67
Fe EDTA F	216	100	1651	19	28	22	2	2	3	62	62.8	62
$FeSO_4$ S	163	79	2648	23	37*	37	2	2	3	61	64.6	66
$FeSO_4$ F	216	105	1830	27	29	26	2	2	3	61	63.0	65
$ZnSO_4$ S	174	95	1608	24	36*	30	2	6*	23*	59	63.2	62
$ZnSO_4$ F	139	89	1983	23	27	24	2	2	3	60	62.6	61
Zn EDTA S	173	100	1575	22	29	26	2	20*	30*	61	64.2	65
Zn EDTA F	189	123	2893	23	32	24	2	2	3	62	63.0	63
Top Wettable S S	209	93	908	21	113*	180*	2	2	3	60	64.0	67
Top Wettable S F	213	99	1828	20	29	27	2	2	2	61	61.8	63
Shirlan®	193	127	1750	21	25	24	2	2	3	61	63.8	62
lsd P=0.05	ns	ns	ns	ns	9	12	ns	3	6	ns	2.2	3

* Significantly different from the untreated control at P=0.05; ns not significant

The soil application of Zn EDTA raised increased soil zinc from 2 ppm at planting to 20 ppm at tuber set and 30 ppm at harvest (Table 5.A.I-10). The soil-applied zinc sulphate treatment raised soil zinc concentrations from 2 ppm at planting to 23 ppm at harvest. The soil-applied sulphur treatment increased soil sulphur from 21 ppm at planting to 113 at tuber set and 180 ppm at harvest. Zinc and sulphur concentrations in soil were not affected by any of the other treatments.

At harvest, the disease incidence on cv. Shepody untreated control tubers was 87% and severity was 56% (Table 5.A.I-11). Disease incidence was significantly reduced by Shirlan® (49%), soil-applied sulphur (73%) and soil-applied Zn EDTA (72%). Disease severity was significantly reduced by Shirlan® to 17%, by both sulphur treatments to 35% and by soil-applied Zn and Fe treatments (35-45%).

The soil-applied Zn EDTA treatment was the only treatment that significantly increased total and marketable yields (by 3 t/ha). Marketable yield at the factory, however, was significantly improved by several treatments for both French fry and Crisp, with the greatest increase from Shirlan® (15 t/ha).

Table 5.A.I-11 – (cv. Shepody) Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on the incidence and severity of powdery scab, total and marketable yield, French fry yield at the factory and nutrient concentrations in the plant petiole and tuber peel in a field trial at Ballarat, 2011/2012.

Treatment	Powdery Scab		Yield t/ha		Marketable yield t/ha at factory		Petiole			Peel		
	% tubers affected	Severity Index	Total	Marketable	French fry	Crisp	% S	Zn (ppm)	Fe (ppm)	S %	Zn (ppm)	Fe (ppm)
Untreated	87	56	51	48	27	16	0.25	25	49	0.27	30	2735
Fe EDTA S	83	42*	52	49	34*	27*	0.25	21	56	0.23	25	1911
Fe EDTA F	84	46	51	48	32*	22	0.25	25	78	0.24	27	2102
$FeSO_4$ S	85	44*	50	46	32*	22	0.26	21	83	0.27	29	2586
$FeSO_4$ F	82	45*	49	45	31	23	0.27	25	58	0.26	29	2253
$ZnSO_4$ S	83	43*	52	49	35*	24*	0.26	41*	77	0.26	38*	1832
$ZnSO_4$ F	84	46	50	47	32*	21	0.24	57*	86	0.28	31	1880
Zn EDTA S	72*	35*	54	51	38*	30*	0.25	45*	92	0.25	41*	1869
Zn EDTA F	87	47	51	48	33*	22	0.24	38*	113	0.26	31	2219
Top Wettable Sulphur S	73*	35*	52	49	37*	28*	0.24	18	71	0.27	27	1375
Top Wettable Sulphur F	83	38*	49	45	34*	25*	0.26	19	24	0.26	28	1496
Shirlan®	49*	17*	52	49	42*	41*	0.25	21	96	0.24	26	1080
lsd P=0.05	11	10	3	3	4	7	ns	12	ns	ns	5	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

As for the previous year, there was too little disease (7% incidence) in cv. Russet Burbank to detect treatment effects (Table 5.A.I-12). This clearly demonstrates the benefits of planting resistant varieties where possible.

Table 5.A.I-12 – (cv. Russet Burbank) Effects of soil- (S) and foliage- (F) applied nutrient and fungicide treatments on the incidence and severity of powdery scab, total and marketable yield, French fry yield at the factory and nutrient concentrations in the plant petiole and tuber peel in a field trial, Ballarat 2011/2012.

Treatment	Powdery scab		Yield t/ha		Marketable yield t/ha at factory		Petiole			Peel		
	% tubers affected	Severity Index	Total	Marketable	French fry	Crisp	S%	Zn ppm	Fe ppm	S %	Zn ppm	Fe ppm
Untreated	6	1	53	41	41	41	0.198	23	70	0.20	27	1361
Fe EDTA S	4	1	45*	40	38	39	0.194	20	85	0.20	28	1407
Fe EDTA F	5	1	49*	37	37	38	0.193	22	93	0.19	25	1238
$FeSO_4$ S	5	1	52	40	40	41	0.196	21	97	0.19	25	1050
$FeSO_4$ F	4	1	50	38	39	40	0.209	19	92	0.20	27	1202
$ZnSO_4$ S	4	1	50	40	41	40	0.213*	56*	92	0.20	82*	1132
$ZnSO_4$ F	2	0	49	41	39	39	0.215*	58*	113	0.20	34	1310
Zn EDTA S	2	0	49*	39	39	39	0.203	54*	118	0.20	80*	1329
Zn EDTA F	7	1	54	44	45	45	0.197	52*	143	0.19	28	1345
Top Wettable Sulphur S	2	0	51	42	42	42	0.195	24	61	0.23*	27	1298
Top Wettable Sulphur F	2	0	50	36*	37	37	0.215*	23	57	0.19	26	1132
Shirlan®	3	0	52	41	41	42	0.205	21	70	0.20	26	1022
lsd P=0.05	ns	ns	3	4	4	4	0.016	14	ns	0.02	18	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

For the third consecutive season, Shirlan® and soil-applied sulphur gave the best disease control in Shepody. Soil-applied Zn EDTA, however, was the only treatment that produced a yield advantage.

SEASON FOUR 2012/2013

In season four, 11 treatments were trialled (Table 5.A.I-4). All treatments were soil-applied with foliar-applied treatments no longer tested. Two different rates of two different forms of sulphur were trialled, and combination treatments of zinc and iron were added. The concentration of *S. subterranea* DNA in the trial plots at planting time averaged 112 pg/g soil, a high disease risk rating according to the PreDicta Pt rating scale.

At harvest, there was 99% incidence and 50% severity in the untreated control of cv. Shepody (Table 5.A.I-13). Shirlan® and both rates of Atomic sulphur reduced incidence and severity. Shirlan® was the most

effective with incidence at 28% and severity at 4%. The two rates of Atomic sulphur were comparable, with incidence 48-55% and severity 15-16%. Zinc EDTA alone and in combination with iron sulphate reduced incidence to 71-73% but had no significant effect on severity.

Total and marketable yields of Shepody at harvest were not significantly affected by the treatments. Shirlan® was the only treatment to significantly (P<0.05) increase the yield of the French fry category (an increase of 12 t/ha), compared with the untreated control. The crisping factory yields were increased significantly (P<0.05) by the Shirlan® treatment (27 t/ha), 1.2 t/ha of atomic sulphur (21 t/ha) and 0.8 t/ha of atomic sulphur (19 t/ha) (Table 5.A.I-13).

Table 5.A.I-13 – (cv. Shepody) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments and a soil-applied fungicide treatment on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab, total and marketable yields in field and factory yields for French fry and crisps in a field trial, Ballarat 2012/2013.

Treatment	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Untreated	114	55	2700	99	50	48	46	31	16
Fe EDTA	94	47	3510	81	45	49	47	32	19
$FeSO_4$	120	73	1899	86	44	52	50	34	23
Fe EDTA + $ZnSO_4$	129	54	697	87	46	43	40	28	17
Zn EDTA	99	57	1183	71*	36	50	48	37	28
$ZnSO_4$	99	60	1416	88	37	51	48	37	28
Zn EDTA + $FeSO_4$	104	53	841	73*	34	48	46	36	28
Atomic S 0.8	107	56	653	55*	16*	43	41	35	35*
Atomic S 1.2	89	57	1248	48*	15*	45	43	37	37*
Wettable Top S 1.0	117	57	813	82	43	41	39	28	18
Wettable Top S 1.5	131	65	934	89	46	45	42	31	17
Shirlan®	139	60	1339	28*	4*	49	46	43*	43*
lsd (P=0.05)	ns	ns	ns	23	20	ns	ns	9	14

* Significantly different from the untreated control at P=0.05; ns, not significant

Only 7% of Russet Burbank untreated tubers were affected by powdery scab (Table 5.A.I-14). Despite the low levels of disease on this variety, soil applications of 0.8 and 1.2 t/ha of Atomic sulphur, 1 t/ha Top Wettable S and 714 kg/ha of Zn EDTA plus 500 kg/ha iron sulphate significantly reduced (P<0.05) disease incidence even further, to between 0.8-1.2%. In this trial, however, the Shirlan® treatment had no significant effect on disease incidence and severity. None of the treatments significantly affected the total

and marketable yields, nor the French fry and crisping categories of marketable yields of Russet Burbank at the factory.

Table 5.A.I-14 – (cv. Russet Burbank) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments and a soil-applied fungicide treatment on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab, total and marketable yields and factory yields for French fry and crisps in a field trial, Ballarat 2012/2013.

Treatment	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable yld at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Untreated	114	61	332	7.2	1.22	54	50	49	50
Fe EDTA	94	55	79*	9.6	2.00	47	43	42	43
$FeSO_4$	120	53	332	5.2	0.86	55	49	48	49
Fe EDTA + $ZnSO_4$	129	71	82*	5.6	1.02	45	41	40	41
Zn EDTA	99	54	183	3.6	0.74	60	56	55	56
$ZnSO_4$	99	76	35*	2.4	0.40	57	52	51	52
Zn EDTA + $FeSO_4$	104	56	43*	1.2*	0.19*	52	48	48	48
Atomic S 0.8	107	41	46*	0.8*	0.12*	50	45	45	45
Atomic S 1.2	89	43	34*	1.0*	0.11*	48	44	44	44
Wettable Top S 1.0	117	53	85*	1.2*	0.19*	44	39	39	39
Wettable Top S 1.5	131	54	122*	5.2	1.14	52	48	47	47
Shirlan®	139	81	64*	2.8	0.46	56	51	51	51
lsd (P=0.05)	ns	ns	191	5.4	1.00	ns	ns	ns	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

In the plots where the susceptible Shepody was grown, pathogen DNA concentrations in the soil increased considerably from tuber set to harvest (Table 5.A.I-13). However, where resistant Russet Burbank was grown, several treatments significantly reduced pathogen DNA concentrations at harvest (Table 5.A.I-14).

The four sulphur treatments resulted in significantly raised (P<0.05) soil sulphur concentrations at tuber set and harvest, compared with the untreated control, but had no effect on sulphur concentrations in the petiole and tuber peel (Table 5.A.I-15). Atomic sulphur treatments resulted in twice (P<0.05) the sulphur concentrations in the soil than the Top S sulphur treatments.

Soil zinc concentrations at tuber set and harvest were increased ten-fold (P<0.05) following treatments containing zinc, including when combined with iron, compared with the untreated control (3 ppm zinc). Zinc EDTA, $ZnSO_4$ and Zn EDTA + $FeSO_4$ treatments significantly (P<0.05) raised zinc concentrations in the plant petiole, but zinc sulphate, however, was the only treatment to raise zinc concentrations in the peel.

Fe EDTA, Zn EDTA, Fe EDTA + $ZnSO_4$ and Zn EDTA + $FeSO_4$ elevated the soil iron concentrations at tuber set, but this effect was not evident in the harvest soil. None of the iron treatments raised iron concentrations in the plant petiole or the tuber peel.

Table 5.A.I-15 – (cv. Shepody) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments and a soil-applied fungicide treatment on the corresponding tuber set soil, harvest soil and peel S, Zn, Fe concentrations in a field trial, Ballarat 2012/2013.

Treatment	Tuber set soil (ppm)			Petiole			Harvest soil (ppm)			Peel		
	S	Zn	Fe	%S	Zn (ppm)	Fe (ppm)	S	Zn	Fe	%S	Zn (ppm)	Fe (ppm)
Untreated	26	3	59	0.20	52	328	30	3	61	0.27	29	1261
Fe EDTA	27	3	73*	0.22	33	276	25	3	62	0.26	26	2145
$FeSO_4$	38	3	59	0.22	46	297	39	3	62	0.26	27	1298
Fe EDTA + $ZnSO_4$	49	26*	73*	0.20	61	283	75	26*	62	0.28	43	1015
Zn EDTA	27	32*	68*	0.19	77*	232	21	37*	56*	0.27	52	1011
$ZnSO_4$	37	46*	58	0.23	114*	279	37	44*	58	0.26	71*	880
Zn EDTA + $FeSO_4$	38	26*	68*	0.25	80*	318	37	31*	61	0.25	50	1122
Atomic S 0.8	113*	3	63	0.22	58	256	205*	3	64	0.24	28	553
Atomic S 1.2	117*	3	61	0.21	66	290	208*	4	64	0.27	26	860
Wettable Top S 1.0	63*	3	60	0.19	45	244	132*	5	62	0.27	26	1028
Wettable Top S 1.5	86*	7	60	0.22	57	285	169*	8	64	0.28	50	1245
Shirlan®	25	2	60	0.22	50	246	28	3	60	0.25	27	932
lsd (P=0.05)	24	13	7	ns	23	ns	59	14	4	ns	24	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

The effects of the various treatments on soil nutrient concentrations in the Russet Burbank plots follow similar patterns to that of Shepody (Table 5.A.I-16). However, the two rates of Atomic sulphur and highest rate of Wettable Top S resulted in elevated iron concentrations in the soil at harvest. The two rates of Atomic sulphur and the four treatments containing zinc resulted in elevated concentrations of sulphur and zinc in the peel of the Russet Burbank tubers, respectively.

Table 5.A.I-16 – (cv. Russet Burbank) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments and a soil-applied fungicide treatment on the corresponding tuber set soil, harvest soil and peel Fe, Zn and S concentrations in a field trial, Ballarat 2012/2013.

Treatment	Tuber set soil (ppm)			Harvest soil (ppm)			Peel		
	S	Zn	Fe	S	Zn	Fe	%S	Zn (ppm)	Fe (ppm)
Untreated	34	3	58	28	2	58	0.18	26	1892
Fe EDTA	28	3	69*	25	2	62*	0.18	25	1105
$FeSO_4S$	36	4	61	28	3	59	0.17	26	1343
Fe EDTA + $ZnSO_4$	49	29*	67*	84	26*	63*	0.18	46*	1481
Zn EDTA	27	32*	64*	22	29*	57	0.21	52*	1247
$ZnSO_4$	34	42*	57	30	39*	57	0.17	79*	1186
Zn EDTA + $FeSO_4$	43	36*	65*	38	32*	61	0.18	45*	1317
Atomic S 0.8	116*	3	60	145*	3	62*	0.22*	27	1078
Atomic S 1.2	168*	3	61	195*	3	65*	0.24*	24	752
Wettable Top S 1.0	73*	4	58	113	3	61	0.18	27	1365
Wettable Top S 1.5	85*	6	59	179*	5	63*	0.18	31	1410
Shirlan®	28	3	59	25	3	60	0.18	25	1392
lsd (P=0.05)	28	15	4	87	11	4	0.04	16	ns

* Significantly different from the untreated control at P=0.05; ns, not significan. Nutrient concentrations in the plant petiole are not reported because samples were lost in transit to A&L Canada for testing.

Once again, Shirlan®, sulphur and Zn EDTA all significantly reduced powdery scab. Shirlan® was the best treatment, reducing incidence and severity in Shepody considerably. Of the two forms of sulphur tested, Atomic S gave the best results and the lower rate was just as effective as the higher rate. These three treatments all resulted in increased acceptability at the Crisp factory. Zinc EDTA alone and in combination with $FeSO_4$ significantly reduced disease incidence but not severity in Shepody.

SEASON FIVE 2013/2014

Season five (2013/2014) included the addition of the fungicide mancozeb, applied to seed tubers before planting, broadcast in the soil, or both (Table 5.A.I-4). Mancozeb has known efficacy against powdery scab (Falloon et al. 1996) and had been trialled by agronomists in Tasmania. DNA concentration of *S. subterranea* in the trial plots at planting time was uniformly high at 500 pg/g soil indicating a high risk for powdery scab.

For cv. Shepody, disease incidence was 93% and severity was 48% in the untreated control (Table 5.A.I-17). The Shirlan® fungicide treatment reduced (P<0.05) disease incidence and severity to 23% and 6%, respectively. The Atomic S treatments reduced (P<0.05) disease incidence and severity to 56% and 19-21%, and the Wettable Top S sulphur treatments reduced them to 69-77% and 27-35%, respectively. The lower rates of sulphur of both treatments were just as effective as the higher rates.

Zinc EDTA + iron sulphate and Fe EDTA + zinc sulphate reduced (P<0.05) disease incidence to 64% and 77%, and disease severity to 28% and 31%, respectively. Zinc EDTA alone reduced incidence to 76% but had no significant effect on severity. Iron sulphate, zinc sulphate, Fe EDTA and the mancozeb treatments all had no effect on reducing disease.

Marketable yields at the French fry factory were significantly increased by the high rate of Wettable Top S sulphur, both Atomic S treatments and Shirlan® (8-18 t/ha). These treatments all increased the marketable yield at the Crisp factory by 18-34 t/ha, as did the two combined iron and zinc treatments and the low rate of Wettable Top S sulphur to a lesser extent (11-14 t/ha).

None of the treatments significantly (P>0.05) affected the concentration of pathogen DNA at tuber set. At harvest, reduced (P<0.05) DNA concentrations corresponded with treatments that significantly reduced disease incidence and severity (Table 5.A.I-17).

Table 5.A.I-17 – (cv. Shepody) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments and a soil-applied fungicide treatment on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab, total and marketable yields and factory yields for French fry and crisps in a field trial, Ballarat 2013/2014.

Treatment	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Untreated	631	711	21400	93	48	56	52	34	19
Wettable Top S 1.5	438	430	5738*	69*	27*	56	52	42*	37*
Wettable Top S 1.0	552	572	9302*	77*	35*	59	55	40	30*
Atomic S 1.2	362	473	2426*	56*	19*	55	52	44*	42*
Atomic S 0.8	508	480	5121*	56*	21*	58	55	46*	43*
Shirlan®	428	427	6067*	23*	6*	59	55	52*	53*
Zn EDTA	513	509	7120*	76*	38	53	48	36	26
$ZnSO_4$	554	758	12529	84	40	53	49	37	25
$FeSO_4$	450	551	14707	88	40	56	52	38	27
Fe EDTA	447	465	7707*	89	50	46*	42*	28	16
Fe EDTA + $ZnSO_4$	516	487	7271*	77*	31*	52	47	37	30*
Zn EDTA + $FeSO_4$	574	566	7420*	64*	28*	52	48	39	33*
mancozeb (soil)	553	540	14730	86	45	53	50	35	22
mancozeb (seed)	564	519	19674	89	52	55	52	35	17
mancozeb seed + soil	451	467	14378	92	46	53	47	33	22
lsd P=0.05	ns	ns	10170	14	12	6	7	7	10

* Significantly different from the untreated control at P=0.05; ns, not significant

The effects of the different treatments on the soil and plant nutrient concentrations for cv. Shepody are presented in Table 5.A.I-18. At tuber set, soil sulphur was significantly increased by all sulphur treatments and the combined iron and zinc treatments, soil zinc was significantly increased by all zinc treatments, and soil iron by all iron treatments with the exception of iron sulphate and Zn EDTA. At harvest, all sulphur treatments, zinc treatments and combined zinc and iron treatments resulted in elevated ($P<0.05$) corresponding nutrient concentrations in the soil. Sulphur treatments also resulted in elevated iron concentrations in the soil. All zinc treatments raised ($P<0.05$) zinc concentrations in the tuber peel. Zinc sulphate was the only treatment that significantly increased zinc levels in the petioles.

Table 5.A.I-18 – (cv. Shepody) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments, two soil-applied fungicides and a seed-applied fungicide treatment on the corresponding tuber set soil, harvest soil and peel S, Zn, Fe concentrations in a field trial, Ballarat 2013/2014.

Treatment	Planting soil (ppm)			Tuber set (ppm)			Petiole			Harvest soil (ppm)			Peel		
	S	Zn	Fe	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)
Untreated	28	4	61	36	5	59	0.3	82	340	20	4	52	0.3	34	1280
Wettable Top S 1.5	27	4	60	137*	4	61	0.3	83	331	274*	4	72*	0.3	39	904
Wettable Top S 1.0	28	4	65	116*	5	61	0.3	86	346	174*	4	64*	0.3	31	925
Atomic S 1.2	28	3	62	167*	5	63	0.3	83	348	181*	4	61*	0.3	32	735
Atomic S 0.8	37	4	62	135*	5	62	0.3	99	315	147*	4	62*	0.3	43	1216
Shirlan®	30	4	66	38	5	59	0.3	85	333	33	5	59	0.3	33	1075
Zn EDTA	29	3	59	39	54*	68*	0.3	98	323	28	31*	58	0.3	73*	1016
$ZnSO_4$	35	4	60	53	36*	59	0.3	110*	344	41	33*	57	0.3	101*	1337
$FeSO_4$	29	4	62	65*	7	62	0.3	74	286	39	4	61*	0.3	36	1444
Fe EDTA	26	4	68	35	6	74*	0.3	50	402	25	5	62*	0.3	31	988
Fe EDTA + $ZnSO_4$	28	4	66	67*	64*	77*	0.3	80	379	33	28*	61*	0.3	65*	1778
Zn EDTA + $FeSO_4$	32	4	71	73*	54*	72*	0.3	83	393	44	35*	64*	0.3	68*	495
Mancozeb soil	29	4	62	40	4	59	0.3	88	335	29	4	60*	0.3	31	1541
Mancozeb seed	30	4	67	39	5	62	0.3	100	290	32	5	59	0.3	36	1180
Mancozeb seed + soil	29	4	69	36	5	61	0.3	70	269	30	4	59	0.3	38	2600*

Treatment	Planting soil (ppm)			Tuber set (ppm)			Petiole			Harvest soil (ppm)				Peel	
	S	Zn	Fe	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)
lsd P=0.05	ns	ns	ns	25	14	5	ns	23	ns	40	6	7	ns	18	1000

* Significantly different from the untreated control at P=0.05; ns, not significant

In the untreated control of cv. Russet Burbank, disease incidence was 11% and severity was 2% (Table 5.A.I-19). Incidence and severity were significantly reduced by Shirlan®, Atomic S 1.2, Atomic S 0.8, Top S 1.5, Top S 1.0, Fe EDTA, Fe EDTA + $ZnSO_4$, Zn EDTA + $FeSO_4$ and the mancozeb seed + soil treatment. This did not translate into any marketable yield increase at the factory.

Table 5.A.I-19 – (cv. Russet Burbank) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments, two soil-applied fungicides and a seed-applied fungicide treatment on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab, total and marketable yields and factory yields for French fry and crisps in a field trial, Ballarat 2013/2014.

Treatment	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Untreated	631	473	2993	11.0	2.0	58	52	51	52
TopS 1.5	438	216	1842*	1.2*	0.2*	64	58	58	58
TopS 1.0	552	359	1952	3.2*	0.5*	64	58	58	58
Atomic S 1.2	362	299	1245*	0.4*	0.1*	61	56	56	56
Atomic S 0.8	508	310	1544*	0.8*	0.1*	64	60	60	60
Shirlan®	428	275	2111	1.6*	0.3*	61	57	57	57
Zn EDTA	513	313	1446*	8.0	1.5	62	58	57	58
$ZnSO_4$	554	327	2667	4.0	0.7	61	55	55	55
$FeSO_4$	450	272	2740	4.0*	0.7*	63	57	58	58
Fe EDTA	447	277	1346*	4.0*	0.7*	64	59	55	56
Fe EDTA + $ZnSO_4$	516	228	1275*	1.6*	0.3*	69	63	61	61
Zn EDTA + $FeSO_4$	574	437	985*	3.6*	0.6*	63	60	59	60
Mancozeb soil	553	421	3036	10.0	2.0	64	59	58	59
Mancozeb seed	564	304	1741*	6.0	1.0	61	57	56	57
Mancozeb seed + soil	451	177	1711*	4.0*	0.7*	60	54	53	54

Treatment	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
lsd P=0.05	ns	ns	1129	6.1	1.2	ns	ns	ns	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

The effects of the different treatments on the soil and plant nutrient concentrations for cv. Russet Burbank are presented in Table 5.A.I-20. At tuber set, sulphur treatments and zinc sulphate increased soil sulphur but only the sulphur treatments remained high at harvest. Zinc treatments increased soil zinc at both tuber set and harvest. Iron treatments and Zn EDTA increased soil iron levels at tuber set but these did not stay high at harvest. None of the treatments affected petiole concentrations of sulphur, zinc or iron. At harvest, soil iron was increased by both rates of Atomic sulphur and the highest rate of Wettable Top S. Sulphur and zinc treatments increased the corresponding nutrient in peel.

Pathogen DNA concentrations in soil at harvest were all much higher than those at planting, regardless of treatment. However, compared to the untreated control, the levels were lower in the Atomic S, Zn EDTA, Fe EDTA, combined zinc and iron treatments, and mancozeb seed treatments.

Table 5.A.I-20 – (cv. Russet Burbank) Effects of soil-applied iron (Fe), zinc (Zn), sulphur (S) treatments, two soil-applied fungicides and a seed-applied fungicide treatment on the corresponding tuber set soil, harvest soil and peel S, Zn, Fe concentrations in a field trial, Ballarat 2013/2014.

Treatment	Planting soil (ppm)			Tuber set (ppm)			Petiole			Harvest soil (ppm)			Peel		
	S	Zn	Fe	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)	S	Zn	Fe	S%	Zn ppm	Fe ppm
Untreated	28	4	61	30	4	59	0.302	42	2505	21	5	56	0.2384	30	773
TopS 1.5	27	4	60	143*	4	62	0.304	49	1221	190*	3	62*	0.3092*	29	752
TopS 1.0	28	4	65	107*	5	61	0.294	45	1422	175*	4	61	0.2872*	32	820
Atomic S 1.2	28	3	62	96*	12	64*	0.313	45	1690	221*	4	64*	0.3014*	33	743
Atomic S 0.8	37	4	62	122*	6	62	0.301	41	2209	158*	4	63*	0.2758*	30	626
Shirlan®	30	4	66	32	5	59	0.271	43	1755	24	4	57	0.2308	32	864
Zn EDTA	29	3	59	32	40*	64*	0.317	61	1712	22	32*	59	0.2228	47*	524
$ZnSO_4$	35	4	60	87*	32*	60	0.314	62	1416	27	32*	57	0.2344	70*	592
$FeSO_4$	29	4	62	48	6	62	0.314	52	1167	30	4	61	0.2416	37	484
Fe EDTA	26	4	68	67	5	70*	0.271	41	1478	22	4	61	0.2410	29	695

Treatment	Planting soil (ppm)			Tuber set (ppm)			Petiole			Harvest soil (ppm)			Peel		
	S	Zn	Fe	S	Zn	Fe	S%	Zn (ppm)	Fe (ppm)	S	Zn	Fe	S%	Zn ppm	Fe ppm
Fe EDTA + $ZnSO_4$	28	4	66	50	66*	68*	0.315	42	1320	28	28*	60	0.2546	66*	686
Zn EDTA + $FeSO_4$	32	4	71	45	47*	69*	0.312	48	3112	30	34*	61	0.2518	42	629
Mancozeb soil	29	4	62	32	4	59	0.275	47	1472	24	3	58	0.2352	29	724
Mancozeb seed	30	4	67	35	5	59	0.315	51	1781	21	4	61	0.2364	30	821
Mancozeb seed + soil	29	4	69	30	5	60	0.309	47	568	20	3	60	0.2236	27	508
lsd P=0.05	ns	ns	ns	46	14	4	ns	ns	ns	33	8	5	0.0278	16	ns

* Significantly different from the untreated control at P=0.05; ns, not significant

A SUMMARY ANALYSIS OF FIVE-YEARS OF POWDERY SCAB FIELD TRIALS

Field trials evaluating the potential for using nutrient manipulation for powdery scab disease control were conducted for five consecutive seasons on the same property. The opportunity to undertake such a long-term study is rare. Various forms, rates and methods of application of sulphur, zinc and iron were compared for effectiveness against the unregistered fungicides, Shirlan®, Agri-Fos®600 and mancozeb on two different cultivars, the susceptible cv. Shepody and resistant cv. Russet Burbank.

Over the five years, the most effective form of control was cultivar resistance. Russet Burbank was consistently much less affected by powdery scab than Shepody. If cultivar resistance is an option for the grower, it is highly recommended.

Of the unregistered fungicides, only Shirlan® was consistently effective. This was used as a standard to compare the relative efficacy of the nutrient treatments used in our field trials. Sulphur and Zn EDTA were both consistent in significantly reducing incidence and severity of powdery scab, but sulphur reduced them to lower levels than was achieved with Zn EDTA.

Foliar applications were abandoned after three years, as they were not effective. Soil application was the only method that consistently brought results. This suggests that the mode of action is either a direct effect on the pathogen itself or impedes its ability to infect the potato roots. There was no evidence for nutrient uptake via the root system to reduce disease.

Restricted Maximum Likelihood (REML) analysis of five years of trial data

The entire five year trial dataset of the incidence and severity of powdery scab and marketable yields of cv. Shepody and cv. Russet Burbank from the various soil- and foliar- applied nutrient treatments and different soil-applied fungicide treatments was analysed for average trends.

Data was analysed using Restricted Maximum Likelihood (REML) linear mixed models. Predicted means for powdery scab incidence and severity were obtained using REML mixed model analyses (Payne et al. 2012) that included an *a priori* treatment effect as a fixed effect, with random effects of year, replicate-within-a-year and plot-within-a-replicate-and-a-year. A separate residual variance was fitted in these REML analyses to account for differing number of treatments in each year. In all REML analyses, the experimental unit was the measurement taken from a plot within a replicate. The percentage of powdery scab was angularly transformed prior to REML analyses to reduce skewness of residuals, with the disease severity index for Russet Burbank data 'square root transformed' for the same reason. Predicted means were back-transformed to the original scale for presentation.

Using REML to re-examine the data, the effects of soil and foliar treatments on disease and yield for cvs. Shepody and Russet Burbank are presented in Table 5.A.I-21 and Table 5.A.I-22.

An average of 93% of Shepody tubers from the untreated control were affected with powdery scab, with 52% of the tuber surface covered with scab. For the soil-applied treatments, Shirlan® was the most effective treatment reducing ($P<0.05$) disease incidence and severity to 38% and 12%, respectively.

Atomic sulphur reduced ($P<0.05$) incidence and severity to 58% and 20%, but the higher rate was no better ($P<0.05$) than the lower rate. The Top S sulphur treatments were less effective than the Atomic sulphur treatments and the higher rate of Top S was more effective in reducing scab incidence and severity (71% and 30%) than the lower rate.

The combined iron and zinc treatments and Zn EDTA reduced (P<0.05) disease incidence to 78-81%, with a greater effect on severity, which was 35-37% (P<0.05). Zinc sulphate reduced (P<0.05) incidence and severity to 86% and 39%. Iron sulphate and zinc sulphate reduced disease severity to 35%. Iron EDTA and zinc oxide had no significant effect on disease, but iron sulphate reduced (P<0.05) disease severity to 42%.

For the foliar treatments, applications of Top S sulphur and Fe EDTA reduced disease incidence and severity in the order of 9% and 20%, respectively.

The soil-applied Shirlan® treatment returned yields of the crisping and French fry grades to the factory of 27 and 14 t/ha over the yields of the untreated control (P<0.05). The four sulphur treatments improved the yield of crisps by between 11-23 t/ha, although the yield improvement from the Atomic S treatments were double (P<0.05) that of the Top S treatments. The two Atomic sulphur treatments and the highest rate of Top S sulphur improved (P<0.05) yields of the French fry grade by 7-10 t/ha. The lower rate of Top S had no significant effect. The treatments with Zn EDTA (single and combined with iron sulphate) and the zinc oxide improved (P<0.05) crisp and French fry yields by between 6-11 t/ha. Of the foliar treatments, Top S sulphur improved (P<0.05) the yield of the crisp grade by 8 t/ha. The other foliar treatments had no effect on yield.

The improvements in crisp and French fry yields reflect the significant impact of various treatments on the incidence, and in particular, on the severity of powdery scab on the harvested tubers of a susceptible cultivar, and the improvements in yields (up to 23 t/ha) that could be achieved by simple nutrient manipulation of the soil.

The effects of all treatments on cv. Russet Burbank are presented in Table 5.A.I-22. On Russet Burbank, only an average of 7% of the untreated controls were affected with powdery scab with an average of 1% of the tuber surface covered with scab. Despite the very low disease levels, there were some treatment effects consistent with the effects observed for cv. Shepody. The effects on powdery scab on Russet Burbank were not observed in every year of the trials. Treatments of Shirlan®, the four sulphur treatments and combined applications of iron and zinc reduced (P<0.05) disease incidence and severity by more than half. Foliar applications of sulphur and Fe EDTA and sulphate caused small but significant reductions in disease incidence and severity. However, none of the treatments had a significant effect on the marketable tuber yields and on the crisp and French fry categories.

Neither of the other fungicides tested, mancozeb and Agri-Fos® 600, had any effect on disease or improved yields.

Table 5.A.I-21 – (cv. Shepody) Predicted means (REML analysis) of the effects of soil- (S) and foliage- (F) applied nutrient treatments and soil- and seed-applied fungicide treatments on the incidence (% tubers affected) and severity (severity index), marketable yield of tubers and the marketable yield of the French fry and crisping categories for the 5 years of field trials in Ballarat, Victoria (2009-2014).

Treatments	Powdery scab disease			Marketable yields (t/ha)		
	% tubers affected		Disease Severity Index (%)	Tuber yield	Crisps	French fries
	Back transformation	Angular transformation				
Untreated	93	75.10	52	47	17	30
Shirlan® S	38	37.84*	12*	49	44*	44*
Atomic S 0.8 S	58	49.41*	20*	49	40*	40*
Atomic S 1.2 S	55	48.09*	18*	48	40*	40*
TopS S 1.0 S	81	63.80*	36*	46	25*	34
TopS S 1.5 S	71	57.52*	30*	49	28*	37*
Fe EDTA S	88	70.04	44	48	23	33
$FeSO_4$ S	88	69.94	42*	47	23	34
Fe EDTA S + $ZnSO_4$ S	81	64.41*	35*	44	24	32
Zn EDTA S	78	62.04*	37*	50	28*	38*
Zn EDTA S + $FeSO_4$ S	69	56.05*	29*	47	30*	37*
ZnO S	89	70.98	45	-	-	-
$ZnSO_4$ S	86	67.77	39*	48	25*	36*
Mancozeb seed	91	72.07	51	49	15	33

Treatments	Powdery scab disease			Marketable yields (t/ha)		
	% tubers affected		Disease Severity Index (%)	Tuber yield	Crisps	French fries
	Back transformation	Angular transformation				
Mancozeb soil	89	70.38	47	48	19	32
Mancozeb seed + soil	93	74.91	48	45	19	30
Agri-Fos® 600 S	96	78.21	52	-	-	-
Top S® F	87	68.68	40*	44	25*	34
Fe EDTA F	85	67.48*	42*	49	22	33
$FeSO_4$ F	89	70.22	44	45	22	32
Zn EDTA F	90	71.31	44	48	22	33
ZnO F	93	74.30	46	-	-	-
$ZnSO_4$ F	89	70.26	46	46	20	33
Average lsd P=0.05		7.51	8.5	5	7.2	4.8

*Significantly different to the untreated control at P=0.05; ns, not significant

Table 5.A.I-22 – (cv. Russet Burbank) Predicted means (REML analysis) of the effects of soil- (S) and foliage- (F) applied nutrient treatments and soil- and seed-applied fungicide treatments on the incidence (% tubers affected) and severity (severity index), marketable yield of tubers and the marketable yield of the French fry and crisping categories for the 5 years of field trials in Ballarat, Victoria (2009-2014).

Treatments	Powdery Scab disease				Marketable yields (t/ha)		
	% tubers affected		Disease Severity Index (%)		Tubers	Crisps	French fries
	Back transformation	Angular transformation	Back transformation	Root squared			
Untreated	6.7	15.00	1.13	1.06	47	48	48
Shirlan®	1.0	5.78*	0.17	0.42*	49	49	50
Atomic S 0.8	0.4	3.42*	0.05	0.23*	49	50	50
Atomic S 1.2	0.3	3.14*	0.05	0.21*	47	47	47
Top S S 1.0	1.8	7.70*	0.30	0.55*	47	47	47
Top S S 1.5	1.2	6.17*	0.20	0.44*	49	50	50
Fe EDTA S	4.1	11.67	0.77	0.88	46	46	46
$FeSO_4$ S	4.6	12.45	0.79	0.89	49	49	49
Fe EDTA S + $ZnSO_4$ S	2.0	8.05*	0.34	0.58*	51	51	51
Zn EDTA S	4.6	12.36	0.83	0.91	48	49	49
Zn EDTA S + $FeSO_4$ S	1.6	7.36*	0.27	0.52*	50	51	51
ZnO S	7.8	16.24	1.27	1.13	-	-	-
$ZnSO_4$ S	3.6	10.93	0.61	0.78	48	49	49
Mancozeb seed	5.6	13.63	0.98	0.99	48	48	48

Treatments	Powdery Scab disease				Marketable yields (t/ha)		
	% tubers affected		Disease Severity Index (%)		Tubers	Crisps	French fries
	Back transformation	Angular transformation	Back transformation	Root squared			
Mancozeb soil	10.2	18.61	2.02	1.42	49	50	51
Mancozeb seed + soil	3.0	9.98	0.49	0.70	45	45	45
Agri-Fos® 600	9.3	17.74	1.65	1.29	-	-	-
Top S® F	2.8	9.63*	0.45	0.67*	44	45	45
Fe EDTA F	2.8	9.64*	0.48	0.70	45	45	45
$FeSO_4$ F	2.5	9.05*	0.42	0.65*	47	47	47
Zn EDTA F	5.1	13.07	0.86	0.93	52	53	53
ZnO F	3.6	10.87	0.61	0.78	-	-	-
$ZnSO_4$ F	3.2	10.26	0.56	0.75	47	47	47
Average lsd P=0.05		5.29		0.38	ns	ns	ns

*Significantly different to the untreated control at P=0.05; ns, not significant

Correlation of powdery scab incidence and severity with soil nutrients concentrations at tuber set.

An analysis of possible correlations between powdery scab incidence and severity and soil nutrient concentrations at tuber set, the time when tubers are most susceptible to infection by *S. subterranea*, was conducted.

To examine the relationships between powdery scab incidence and severity on cv. Shepody and nutrient concentrations at tuber set, the data was analysed using a series of REML (Payne et al. 2012) mixed model analyses that included an *a priori* treatment effect as a fixed effect and random effects of year, replicate within a year and plot within a replicate and a year. A separate residual variance is fitted in these REML analyses to account for differing number of treatments in each year. In all REML analyses, the experimental unit was the measurement taken from a plot within a replicate. The percentage of powdery scab was angularly transformed prior to REML analyses to reduce skewness of residuals. A measurement from a plot (plot 12, replicate 4 in 2010/11) with an exceptionally low disease index was removed from analyses as a statistical outlier (outliers row 50, row 15).

For each of powdery scab disease percentage and index, a parsimonious treatment model was selected by fitting a saturated model first and then sequentially dropping individual term based on Wald F-tests.

Manganese levels in soil at tuber set were strongly related ($P<0.001$) to powdery scab disease incidence and severity. Raising levels of manganese in soil from 20 ppm to 200 ppm was associated with a reduction in powdery scab incidence of more than 30%, whereas the severity of the disease reduced to 20% when manganese level reached 130 ppm, and remained stable thereafter. When manganese was included in the model, no other soil nutrient such as zinc, sulphur or iron had any significant effect on powdery scab disease. However, it is important to note that manganese and sulphur concentrations were significantly positively correlated ($P<0.001$).

Applications of elemental sulphur reduce soil pH which in turn can make manganese more available (Huber and Wilhelm 1988b). There is no evidence from our work or in the literature that manganese has a direct effect on powdery scab. The question remains, however, whether the reductions in powdery scab were as a direct effect of the sulphur treatments alone or were related to the increased manganese levels resulting from the higher sulphur.

DISCUSSION AND CONCLUSIONS

This study was aimed at evaluating the effects of manipulating soil and plant nutrient concentrations (different forms of sulphur, zinc and iron) on the incidence and severity of powdery scab and on the yield and quality of tubers. In five seasons' of trials on a ferrosol soil, pre-planting applications of elemental sulphur and zinc (as EDTA) consistently reduced disease incidence and severity. Although iron treatments reduced disease, they were not consistent over five seasons and were less effective than zinc. Foliar applications of these nutrients also reduced disease but not consistently, particularly when rates were reduced to avoid phytotoxicity. These treatments generally reduced disease severity more so than incidence, resulting in significant improvements in tuber quality, an important criterion for suitability for the processing factory. The greatest impact of these treatments was on the susceptible cultivar Shepody but they also reduced disease on Russet Burbank, which has a very low susceptibility to powdery scab.

The hierarchy of disease control from the treatments on Shepody, and including the cultivar Russet Burbank, from the most to the least effective treatment is listed below:

- Untreated Russet Burbank, average incidence and severity of 7% and 1%;
- Fungicide treatment, 38% and 12%;
- Elemental sulphur (Atomic S), 56% and 19%;
- Zinc (as EDTA), 78% and 37%;
- Untreated Shepody, 93% and 52%.

The purer form of sulphur (Atomic S) proved to be more effective than Wettable Top S at equivalent rates of sulphur applied, the former being a purer form with finer particles and more soluble. Although there was no statistical differences between the effects of different forms of zinc on disease, zinc EDTA was consistently more effective over five trials than zinc oxide or zinc sulphate. Of the three compounds, zinc sulphate is the most soluble and therefore more transient in the environment. Zinc oxide is the least soluble with the zinc likely to be slowly released over time. The complex for of zinc (EDTA) is more suited for uptake up by micro flora.

It is important to note that the first option for powdery scab control would be to choose a cultivar with a relatively low reaction (low susceptibility) to powdery scab on tubers, such as Russet Burbank. If a market imperative dictates the use of a susceptible cultivar, however, our results show that sulphur and zinc provide a degree of disease control that improve tuber quality and yield to the factory as an alternative to a fungicide treatment, which is currently not registered for control of powdery scab control in Australia.

These results are consistent with those reported in a comprehensive study in the United Kingdom (Wale et al. 2005) involving different cultivars, and fungicide, sulphur and zinc treatments. The relative efficacy of the different treatments tested in the United Kingdom and Victoria was essentially the same. Soil applications of zinc compounds reduced powdery scab on tubers in trials in New Zealand, as did soil treatments of elemental sulphur (Falloon et al. 1998). The efficacy of sulphur has also previously been reported from Queensland (Hughes 1980).

The sulphur and zinc treatments tested in Victoria resulted in significantly raised soil sulphur and zinc concentrations at the time of tuber set well above the pre-plant soil baseline concentrations. At tuber set, a period of about 3 to 4 weeks when the plants population of tubers are initiated and enlarge, the developing tubers are highly susceptible to infection by *S. subterranea*. These levels are well above optimum thresholds for crop growth and the impact of these elements on pathogen and disease is not likely to be due to the correction of nutrient deficiencies.

In our trials, the various treatments were tested on a ferrosol soil only. However, given that efficacy has been demonstrated on a range of soils in the United Kingdom, New Zealand and Australia, the effects of these treatments are likely to be universal. Furthermore, given that the fungicide and nutrient amendments reduced disease incidence and severity on the Russet Burbank, rated as being very low in susceptibility to powdery scab, it is more than likely that they will be efficacious on cultivars with lower susceptibility ratings than Shepody. Interim recommendations can be made on rates of sulphur and zinc required for disease control. However, these treatments need to be evaluated in range of soil types and on different cultivars to determine threshold levels for optimum disease control.

IN VITRO STUDIES OF NUTRIENT TREATMENTS AND CULTIVAR SUSCEPTIBILITY ON *SPONGOSPORA SUBTERRANEA* AND POWDERY SCAB USING BIOASSAYS AND MOLECULAR TOOLS.

SUMMARY

Real-time quantitative polymerase chain reaction (qPCR) was used for detecting and quantifying the pathogen DNA levels in *S. subterranea* sporosori and in infected roots (zoosporangia). The ability of this technique to distinguish between live and dead spores was tested on sporosori that were untreated, autoclaved or gamma-irradiated. There was a significant decrease in pathogen DNA levels in sporosori that had been autoclaved but not in those that were gamma-irradiated.

A real-time reverse transcription quantitative PCR (RT-qPCR) assay was developed for *S. subterranea* using the 18S rRNA, *EFL*, actin and polyubiquitin marker genes to quantify viable spores within a sporosori. This study has been successful in designing and confirming the presence of all the four marker genes in *S. subterranea* sporosori and in roots infected with *Spongospora* (zoosporangial stage).

A series of experiments were conducted using a bioassay for *S. subterranea* root infection. Micropropagated potato plants were grown in nutrient solution in controlled environment conditions. Root disease severity was recorded as relative abundance of zoosporangia and the level of pathogen DNA in roots using qPCR 24 days post inoculation.

The effects of two concentrations of zinc (as SO_4) and iron (as EDTA) were tested on severity of root infection. The higher rates of zinc (10 ppm) and iron (15 ppm) reduced pathogen DNA levels by 87% and severity of root infection by 75 and 50% respectively.

Six potato cultivars varying in susceptibility to powdery scab on tubers, Shepody, Russet Burbank, Ranger Russet, Atlantic, Simcoe and Innovator were assessed for relative susceptibility to powdery scab based on root infection. Based on pathogen DNA levels and severity of root infection, Shepody was confirmed to be most susceptible followed by Simcoe, Atlantic, Ranger Russet, Russet Burbank with Innovator being the least susceptible. In susceptible cultivars, a higher abundance of zoosporangia was present in root epidermal cells which were structurally different compared with the less susceptible cultivars.

Low levels of pathogen DNA were detected in roots inoculated with autoclaved and gamma-irradiated sporosori. These treatments killed the spores reducing zoospore release which in turn reduced severity of root infection.

Overall the potato bioassay is a good method for host-pathogen interaction studies for powdery scab disease. The results of pathogen DNA level correlated to the visual assessment of root infection severity.

DNA DETECTION QUANTIFIES VIABLE AND NON-VIABLE SPORES AND DEVELOPMENT OF A MOLECULAR TOOL TO DETECT AND QUANTIFY VIABLE RESTING SPORES OF *SPONGOSPORA SUBTERRANEA*

INTRODUCTION

Powdery scab caused by the biotrophic pathogen *Spongospora subterranea* is a significant soilborne disease of potatoes. Disease symptoms of galls on roots and stolons and scab-like eruptions on the surface

of tubers contain multicelled sporosori, conglomerations of 150-1500 dormant resting spores (Falloon et al. 2011b). It is hypothesised that sporosori of the powdery scab pathogen do not all germinate at once when exposed to potato root exudates, which explains the longevity of this organism (up to 30 years) in the soil without the potato host. Zoospores are likely to be released in progression over several years which makes it difficult to quantify viable resting spores *in vitro*. Quantifying the pathogen DNA may in fact significantly overestimate the disease risk of these sporosori at any one time. A real-time RT-qPCR assay was developed to quantify the viable resting spores of *S. subterranea,* potentially those that will germinate during the season, using the 18S rRNA, *EFL*, actin and polyubiquitin marker genes. All these four genes perform different functions in the cell. Actin gene forms microfilaments which give support to cells and support signal transduction. Polyubiquitin gene is involved in controlling a vast variety of biological functions ranging from proteolysis to DNA damage tolerance. 18S rRNA gene – ribosome is the organelle-site of protein synthesis in all living cells and in eukaryotes it is present in the small ribosomal subunit. Elongation Factor-Like (*EFL*) gene (S. Bulman, pers. comm.) is similar in function to *EF-1α* gene which is a core element of the transition apparatus and member of the GTPase protein family. Quantitative PCR (qPCR) was used for detecting and quantifying the pathogen DNA levels in *S. subterranea* sporosori and in infected roots (zoosporangia). The ability of this technique to distinguish between live and dead spores was tested on sporosori that were untreated, autoclaved or gamma-irradiated. The new assay had demonstrated proof of concept in that the technique was able to detect the target genes in viable spores.

MATERIALS AND METHODS

Primer design

Forward and reverse primers and probe sets were designed using the software tool Primer3 (version 0.4.0) (Koressaar and Remm 2007; Untergrasser et al. 2012). Primer/probe sets were designed for 4 genes (actin, polyubiquitin, elongation factor like (EFL) (Simon Bulman, New Zealand) and 18S rRNA) specific to *S. subterranea* (Table 5.A.I-23). The primers were ordered online from Sigma-Aldrich (NSW, Australia) and the TaqMan® probe from Applied Biosystems (California, USA). PCR products of actin and polyubiquitin genes were cloned into the pGEM®-T Easy Vector (Promega, USA) following the manufacturers' instructions. PCR product of each gene was sent for sequencing to Australian Genome Research Facility (Australia) and each gene sequence confirmed specific to *S. subterranea*. The copy number for standard *Spongospora* DNA molecules were determined from the plasmid DNA concentration and subsequently used for constructing a standard curve for each gene. For 18S rRNA and EFL genes the gBlocks® Gene Fragments of the full length of the amplified gene (125 base pairs) were ordered from Integrated DNA Technologies (Iowa, USA) and copy number of standard DNA molecules determined using the absolute quantification method for constructing a standard curve for each gene.

Table 5.A.I-23 – List of primers designed for the detection of mRNA in *Spongospora subterranea*

Gene name	Gene ID	Sequence size	Product size	Primer sequence (5' to 3')
Actin	AY452193.1, GI 40647181	733	142	FP, 5'-ATG AAA ATC CTC ACC GAA CG-3'
				RP, 5'-CCA CTT CTG ACG ACT GCT CA-3'
				5' 6FAM-ATT CAC CAC CAC CGC CGA GC - MGBNFQ 3'
Polyubiquitin	AY452192.1, GI 40647179	346	150	FP, 5'-TCA AAG CCA AGA TCC AGG AC-3'
				RP, 5'-CTC CGC GTA GAC GAA GAA CT-3'
				5' VIC-GGA AAC AGC TGG AAG ATG GCC - GMGBNFQ 3'
18S rRNA	AY604173.1, GI 48479027	1837	101	FP, 5'-GAGACCTCAGCCTGCTTTTG-3'
				RP-5'-ATTGCCTCAAACTTCCATCG-3'
				5' 6FAM-CGGGCCTATCCTCCGGGCTA - MGBNFQ 3'
EFL	S. Bulman	1438	105	FP, 5'-GCC TTG GTT GTC GAC TCA TT-3'
				RP,5'-GTT GCC AAC AGC GGT AAT CT-3'
				5' VIC-ACT CCG CCG TCA TGC TTG GC - MGBNFQ 3'

Sample preparation for DNA and RNA extraction from *S. subterranea* sporosori

The sporosori were collected from *S. subterranea* infected field-grown potato tubers (2009/2010) cv. Shepody by scraping the scab lesions from tubers, passaging through a sieve (53 µm mesh) and then stored at 4°C. *S. subterranea* 2009 sporosori samples were weighed (0.5 g) in five eppendorfs each and autoclaved using the dry cycle at 121°C for 25 minutes and irradiated by exposing to gamma (γ) radiation (25 kGray). This served as a control treatment for testing against untreated viable sporosori. The 1982 sporosori were collected and stored in 1982 at Department of Primary Industries, Knoxfield at 4°C. For DNA extraction, 50 mg of sporosori powder containing 1.18×10^5 sporosori was weighed in four eppendorfs each of 2009, 1981, 2009 autoclaved and 2009 γ-irradiated spores. A 10^5/mL sporosori suspension of *S. subterranea* was prepared by mixing 250 mg of sporosori powder in 50 mL of deionised water. Two to three mL aliquots were used for each RNA extraction.

DNA extraction and real-time quantitative PCR - Sporosori

DNA was extracted from four replicates of each sample, using the PowerSoil® DNA Isolation Kit (MoBio), quantified by spectrophotometry and stored at -20°C. The primer/probe set used in this study (van de Graaf et al. 2003) was designed from the internal transcribed spacer region (ITS1 and ITS2) of *S. subterranea*. PCR reactions were set up in triplicates for DNA samples and standards using the Platinum®

Quantitative PCR SuperMix-UDG (Invitrogen), 0.3 µM of each SsTQF1 (5'-CCG GCA GAC CCA AAA CC-3') and SsTQR1 (5'-CGG GCG TCA CCC TTC A-3') forward and reverse primers, 0.1 µM Taqman® probe SsTQP1 (5'-CAG ACA ATC GCA CCC AGG TTC TCA TG-3'), 5 µL of template DNA (5 ng/µL) and made up to 25 µL with RNA-free water. A non-template control (water) was included in the run to check for contamination. The following cycling parameters were used: two step denaturation – 1) 50°C for 2 min. and 95°C for 10 min., followed by 40 cycles of amplification in a twostep procedure: 15 s at 95°C and 60 s at 60°C. Ten-fold serial dilutions (10^7–10^3) of the cloned gene fragment was included as standards in each run for estimating the pathogen DNA copy number. The PCR run was repeated twice.

RNA extraction

Total RNA extraction and two step real-time quantitative RT-PCR (sporosori and tomato root)

RNA was extracted from sporosori using the Soil Total RNA Purification kit (Norgen Biotek, Canada) and roots using the RNeasy Plant mini kit (Qiagen). RNA was extracted from: 1) sporosori collected in 2009 (viable); 2) sporosori collected in 1982 (viable); 3) *Plasmodiophora brassicae* resting spores collected and stored at -20°C (a related pathogen) and from *S. subterranea*-infected and non-infected tomato roots. The quantity and the integrity of all the RNA samples were determined using a spectrophotometer (NanoDrop Technologies, Wilmington, USA). A known volume of extracted RNA from each sample was then treated with DNase1 (DNA-free™ Kit, Ambion) enzyme and 50 ng of total RNA reverse transcribed into cDNA (High Capacity cDNA Reverse Transcription kit, Applied Biosystems). PCR reactions were set up using the Platinum® Quantitative PCR SuperMix-UDG (Invitrogen), 0.3 µM of each forward and reverse primers, 0.1 µM probe, 5 µL of template cDNA (10 ng/µL) and made up to 25 µL with RNA-free water. Four individual PCR runs were performed for each four primer/probe set designed above and threshold cycle value (Ct) calculated for each sample. No standards were available at this stage, hence the copy number of individual genes could not be estimated. Results were further confirmed by resolving the PCR products on a 2% w/v ethidium bromide-stained agarose gel at 100 volts for 40 minutes.

Two step real-time quantitative RT-PCR (treated and non-treated sporosori)

RNA was extracted from four replicates of each sample from 1) sporosori collected in 1982 (viable spores); 2) sporosori collected in 2009 (viable spores); 3) 2009 sporosori that had been autoclaved (dry cycle at 121°C for 25 minutes) and 4) 2009 sporosori that had been γ-irradiated (25 kGray). PCR reactions were set up in replicates for cDNA samples and in triplicates for standards using the Rotor-Gene Multiplex PCR Master Mix (Qiagen), 0.3 µM of each forward and reverse primers, 0.1 µM probe, 5 µL of template cDNA (50 ng/µL) and made up to 25 µL with RNA-free water. A non-template control (water) was included in the run to check for contamination. Cycling conditions were 95°C for 5 minutes of initial denaturation followed by 45 cycles of amplification in a twostep procedure; 15 s at 95°C and 30 s at 60°C. Two separate PCR runs were performed one with actin and polyubiquitin primer/probe set and the other run with18S rRNA and *EFL* primer/probe sets. Ten-fold serial dilutions (10^6–10^1) of the cloned gene fragment was included as standards in each run for estimating the copy number of all four genes – 18S rRNA, *EFL*, actin and polyubiquitin.

Inoculum preparation – *S. subterranea* spore suspension

The *S. subterranea* 2009 sporosori collection was used for all the bioassays (tomato and potato). Sporosori powder (0.25 g) was weighed and imbibed in a Schott bottle containing 500 mL of Hoagland solution to obtain a final concentration of 10^4 sporosori/mL (178 sporosori/mL). The bottle was wrapped in aluminium

foil and incubated in the growth cabinet at 18/15°C, 16/8 hour day/night cycle for 3 days to be used for inoculation. Another Schott bottle containing 500 mL of Hoagland solution with no sporosori was also wrapped in aluminium foil and incubated in the same growth cabinet to be used as control inoculum. For the validation of spore viability potato bioassay, 1982, autoclaved and gamma-irradiated 2009 sporosori were prepared for inoculation.

Plant growth and inoculation of potato plants in a hydroponic system

Four-week old tissue cultured plantlets (cv. Shepody) were uprooted from vials and roots were washed gently in a container with deionised water to get rid of agar. The experiment was set up in controlled environment growth cabinets using 18/15°C, 16/8 hour day/night cycle 75% humidity and light level one (1000 lux). Each cabinet had two tubs filled with 7L of Hoagland solution (inoculated and uninoculated). The tubs were covered with perspex lid which had 24 holes with plugs for holding the seedling. Roots were trimmed to 60 cm in length and then placed in each plug with roots dipped in the Hoagland solution. The plants were left to grow and develop new root hairs for about two weeks prior to inoculation (Figure 5.A.I-3). One tub in each growth cabinet was inoculated with 125 mL/tub of Hoagland solution containing sporosori pre-imbibed for three days. The uninoculated plants were mock inoculated with 125 mL/tub of Hoagland solution. All the tubs were set up with plants and left in the growth cabinet to grow for 24 days. Roots of potato plants were washed with deionised water and transferred into fresh Hoagland solution three and ten dpi (days post-inoculation). Plants were sampled at 24 dpi from five inoculated and five uninoculated plants. Fresh shoot weight was recorded at day 24 only and dry weight recorded after drying in an oven at 40°C. The shoot material was sent to A&L Canada for nutrient analysis.

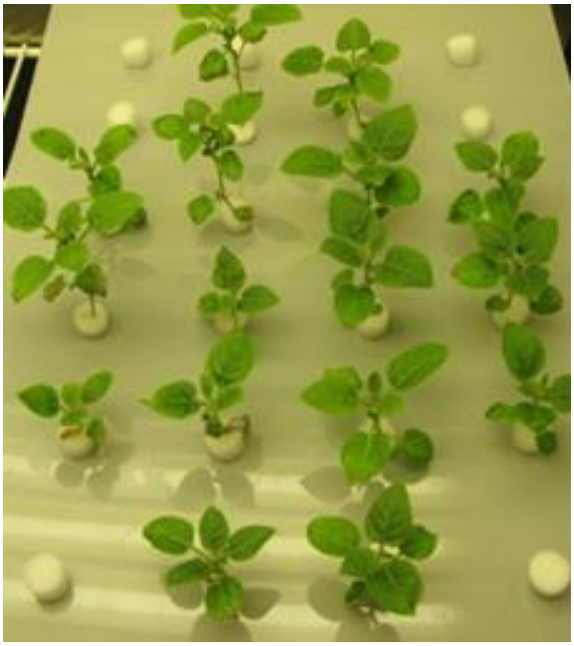


Figure 5.A.I-3 – Two-week old tissue-cultured potato plantlets cv. Shepody set up in Hoagland solution in a growth cabinet.

DNA extraction and qPCR – potato bioassay

The following DNA extraction procedure was used for all the potato bioassay experiments conducted in this study. The whole root system was washed in tap water, dried using paper towel to absorb excess water, cut just below the hypocotyl, weighed and stored in pre-labelled extraction bags (Bioreba) at –20°C until required for DNA extraction. DNA was extracted from four to five replicates of each inoculated and uninoculated root using the CTAB DNA extraction method. CTAB (with PVP 40) buffer was added to each bag twice the weight of root biomass and homogenised using the Homex grinder (Bioreba) and processed for DNA extraction. DNA was resuspended in 50 µL RNase-free water, quantified by spectrophotometry and stored at -20°C until required for PCR.

PCR reactions were set up in triplicates using the Platinum® Quantitative PCR SuperMix-UDG (Invitrogen), 0.3 µM of each forward (SsTQF1) and reverse (SsTQR1) primers, 0.1 µM Taqman® probe SsTQP1, 5 µL of template DNA (25 ng/µL) and made up to 25 µL with RNA-free water.

Microscopic examination of roots

Roots from four to five replicates of inoculated and uninoculated plants were washed in tap water, stained with 0.5% aniline blue for 10 min. and stored in Formaldehyde-Acetic acid-Alcohol (FAA) fixative for microscopic examination of *Spongospora* root infection. Four to five slides were prepared from each plant for microscopic examination by mounting 20 mm long root segments in a solution containing a mixture of lactic acid and glycerol (x 400 magnification). Rating of root infection was based on the scale used by Merz (1989): 0 = no sporangia, 1 = only a few sporangia, 2 = several roots with sporangia, 3 = sporangia regularly present, moderate infection, 4 = sporangia regularly present, heavy infection.

Statistical analysis

The statistical software GenStat™ (14th edition) was used for statistical analysis. Analysis of Variance (ANOVA) was used in all the experiments conducted in this study. All values were transformed base $\log_{10}$ as and when required prior to conducting the statistical analysis. An lsd (least significant difference) was calculated when differences were significant (P=0.05) to compare means.

RESULTS

Heat treatment and gamma-irradiation inhibits release of zoospores

Q-PCR confirmed the detection of *S. subterranea* DNA in all the samples: non-treated 1981; non-treated 2009; heat-treated (autoclaved) and gamma-irradiated 2009 sporosori collection (Table 5.A.I-24). There was a significant decrease in DNA copy number (1.7 fold) in sporosori that had been autoclaved compared with non-treated 2009 sporosori collection. DNA copy number was similar for both non-treated and gamma-irradiated 2009 sporosori. However, pathogen DNA level was significantly lower (1.3 fold) in 1981 sporosori than 2009 sporosori (non-treated).

The autoclaved and gamma-irradiated sporosori has been used as inoculum to compare with non-treated 1982 and 2009 sporosori in the potato bioassay for confirming their viability (Table 5.A.I-24). There was no difference in root and shoot weight between different inoculums. Zoosporangia were abundant and the severity of root infection was highest in roots infected with non-treated 2009 sporosori. No visual symptoms were observed during microscopic examination in uninoculated and in roots infected with 1982

and 2009 treated sporosori. Low levels of pathogen DNA were detected with qPCR in these samples. *S. subterranea* DNA level was significantly higher in roots infected with non-treated 2009 sporosori compared with other sporosori samples.

Table 5.A.I-24 – Validating spore viability in potato roots infected with non-treated 1982 and 2009 treated and non-treated sporosori at 24 days post inoculation with *S. subterranea*

Sporosori samples	Pathogen DNA copy number/ng of DNA in sporosori (log_{10})	Pathogen DNA copy number/ng of DNA in potato root (log_{10})	Severity of root infection scale (0-4)
1981 (non-treated)	5.82b	nd	nd
1982 (non-treated)	nd	2.48b	0
2009 (non-treated)	7.9c	5.75c	4
2009 (autoclaved)	4.64a	1.55a	0
2009 (gamma-irradiated)	7.57c	1.64a	0
Uninoculated	na	0.89a	0
lsd (P=0.05)	0.49	0.78	na

na, statistical analysis not applicable; nd, not determined; numbers followed by the same letters are not significantly different from each other

Actin, polyubiquitin, 18S rRNA and *EFL* genes detected in *S. subterranea* infected roots and sporosori

Real-time PCR analysis confirmed the amplification of all the four genes in the infected roots (zoosporangial stage) and in the *S. subterranea* 2009 resting spores. As expected all four genes were amplified in 2009 sporosori (more viable) earlier than 1982 sporosori collection (less viable) (Table 5.A.I-25). Differences in the Ct value for 2009 and 1982 *S. subterranea* sporosori samples were approximately similar for 18S rRNA (3.8) and *EFL* (4) and higher for polyubiquitin (4.7) (Table 5.A.I-25). All the genes except actin amplified in the *S. subterranea* 1982 sporosori. No PCR product amplified in *P. brassicae* spores in either non-infected roots or in non-template control using any of the primer/probe sets, confirming the specificity of the primer and probe sets to *S. subterranea*.

Agarose gel electrophoresis confirmed the amplification of one PCR product as revealed by the presence of one band only for each of the four genes tested in this study (Figure 5.A.I-4B). The presence or absence of bands on the gel image corresponds with the results displayed in the table below. 18S rRNA primer and probe set amplified *P. brassicae* cDNA (band present on the gel image) but did not fluoresce on the real-time cycling curve (Figure 5.A.I-4A). This is because the 18S rRNA sequence of *S. subterranea* is very similar to the *P. brassicae* sequence, the only difference being in the probe sequence.

Table 5.A.I-25 – Estimated threshold cycle (Ct) value for the detection of actin, polyubiquitin, 18S rRNA and *EFL* genes in *S. subterranea* sporosori and infected root

Genes	**Ct value**					
	1982	2009	*P. brassicae*	Non-infected	Infected	Non-template
Actin	NA	34.7	NA	NA	26.2	NA
Polyubiquitin	29.5	24.8	NA	NA	26.9	NA
18S rRNA	15.2	11.4	NA	NA	17.9	NA
EFL	28.8	24.8	NA	NA	25.2	NA

NA, not amplified

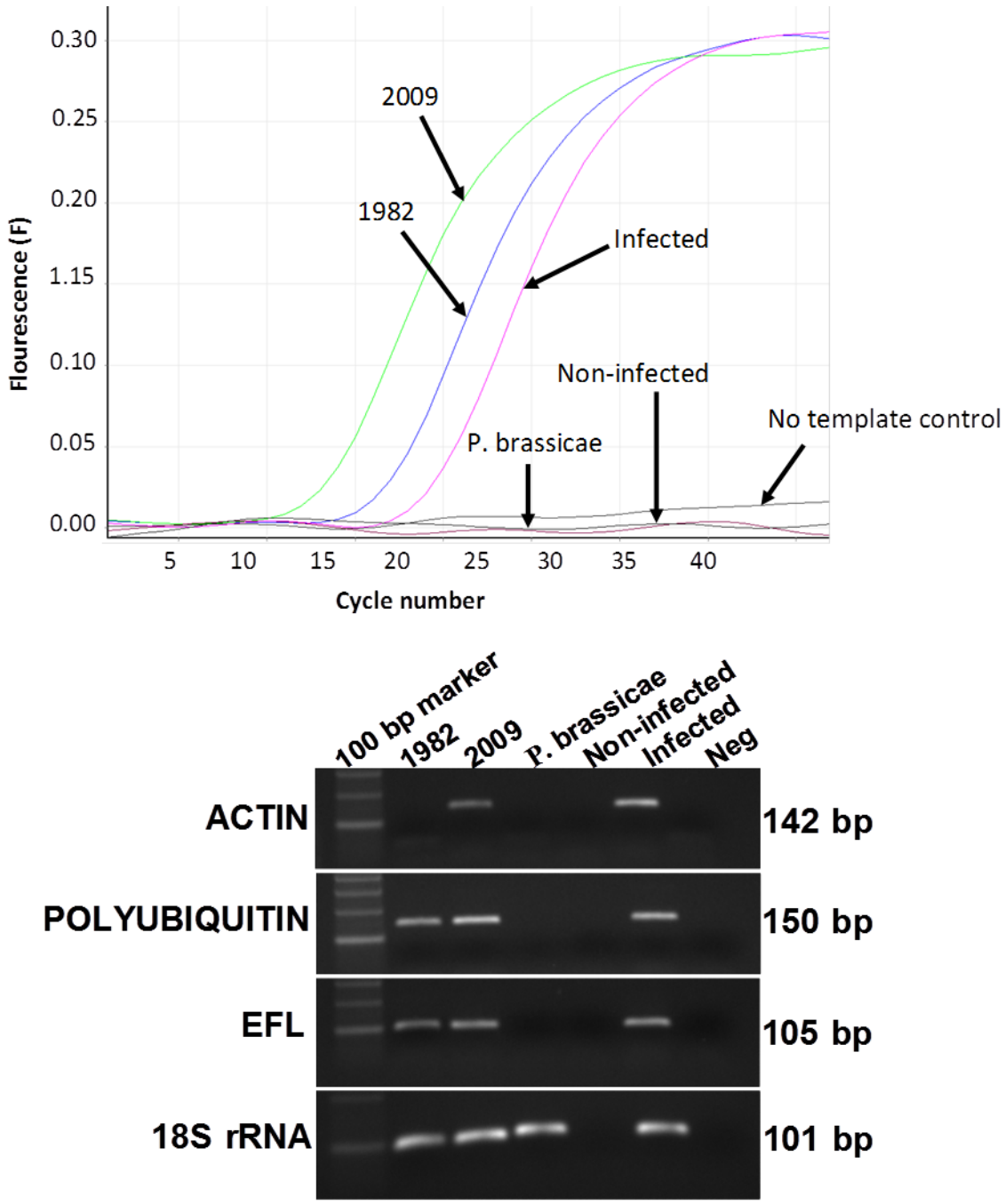


Figure 5.A.I-4 – A (Top) Cycling curve generated from real-time PCR analysis using 18S rRNA primer and probe set specific to *Spongospora* (fluorescence vs cycle number). No PCR product amplified in the *P. brassicae* or in non-infected and in non-template control. B (Bottom) PCR products of four genes run on a 2% agarose gel with 100 bp DNA marker. Note the presence of band on the gel with 18S rRNA primer and probe set with *P. brassicae*.

Heat treatment affected spore viability - RNA

All four genes were expressed in 2009 non-treated sporosori and were significantly higher than the other samples as would be expected (Table 5.A.I-26). Actin gene was not detected in the 1982 sporosori, and the expression of the other three genes was significantly lower in comparison with 2009 sporosori. In the 2009 autoclaved sporosori, only 18S rRNA was detected (significantly lower than the other three sporosori samples) and the other three genes were not detected at all. In the gamma-irradiated sporosori, all four genes were expressed; the copy number being almost similar to 1982 sporosori.

Table 5.A.I-26 – Gene copy number for 18S rRNA, *EFL*, actin and polyubiquitin genes in treated and non-treated sporosori of *S. subterranea*

Sporosori samples	Gene copy number			
	18S rRNA	EFL	Actin	Polyubiquitin
1982 non-treated	8897865	111	0	1155
2009 non-treated	119221576*	3110*	30*	35446*
2009 autoclaved dry	1039	0	0	0
2009 gamma-irradiated dry	59173911	269	12	3068
lsd (P=0.05)	32295179	587	8	8721

*Significantly different to the untreated control at P=0.05

DISCUSSION

A study conducted to distinguish between live and dead spores did not confirm that autoclaving or gamma-irradiation can destroy DNA in *Spongospora* sporosori. To reduce the risk of false positives due to the detection of DNA in dead cells qPCR studies were combined with culturing potato plants and validating these spores, as has also been suggested by Sanzani et al (2014). This validation experiment confirmed that autoclaving and gamma-irradiation can possibly destroy DNA within the spores. The low level of pathogen DNA detected in roots inoculated with autoclaved and gamma-irradiated sporosori suggests that both treatments had an effect on zoospore release which significantly reduced severity of root infection. The 1982 sporosori collection was later found to have very low sporosori count compared with 2009 sporosori.

A real-time RT-qPCR assay has been developed for *S. subterranea* using the 18S rRNA, *EFL*, actin and polyubiquitin marker genes. This study has been successful in designing and confirming the presence of all four marker genes in *S. subterranea* sporosori and in roots infected with *Spongospora* (zoosporangial stage). Autoclaving is likely to have disrupted or destroyed the RNA in the spores, whereas gamma-irradiation is more likely to have damaged, rather than destroyed, the RNA in the spores. Our results indicate that autoclaving sporosori can possibly destroy RNA as only the 18S rRNA genes could be detected.

The aim of this study was to quantify viable spores within a sporosori. RT-qPCR has been demonstrated to be useful in differentiating between active, dormant and recently dead cells Sanzani et al. (2014). Therefore we used mRNA as an indicator of living cells to exclude the detection of dead cells. The RT-qPCR study was

not consistently successful in distinguishing between viable and non-viable spores nor in quantifying viable spores in the sporosori and needs further refinement. RNA analysis is more complex than DNA analysis, since RNA has to be first reverse transcribed to be analysed and the easy degradation of RNA during sample processing could lead to false positives Sanzani et al. (2014). Other reliable methods or tools need to be explored for future studies which can quantify the presence of viable spores in the soil inoculum. This would be useful for disease prediction and for developing control strategies for powdery scab.

PLANT BIOASSAY – TOMATO BAIT BIOASSAY TO CONFIRM PATHOGEN INFECTION AND ACTIN AND POLYUBIQUITIN GENE EXPRESSION DURING PLANT INFECTION

INTRODUCTION

Molecular methods (RT-qPCR) have been developed in this study to detect and quantify pathogen infection in tomato roots using mRNA. A tomato bioassay in a hydroponic system has been developed based on the method developed by Merz (1989). In this experiment we report the successful infection of tomato plants with direct microscopic examination of the root system and correlate the infection stages with molecular tests.

Here we report two tomato bioassay experiments conducted in two different growth cabinet conditions. Light intensity and temperature used during inoculation has an effect on disease severity and rate of infection.

MATERIALS AND METHODS

Plant growth and inoculation of tomato plants in a hydroponic system

Tomato seeds were germinated within trays containing sterile river sand (BioGro) and kept in controlled environment in a growth cabinet (23^{o}C, 12 hour day/night cycle, 55% humidity and light level 1 (12000 lux). Once the seeds had germinated the light was increased to level 2 (16000 lux). The tray was kept moist and watered regularly using deionised water until the seeds germinated. After that seedlings were constantly fed with Hoagland solution. The experiment was set up in four growth cabinets (18^{o}C, 12 hour day/night cycle and light level 2 (16000 lux) each containing two tubs filled with 7L of Hoagland solution (one inoculated and one uninoculated). The tubs were covered with perspex lids which had 24 holes with plugs for holding the seedlings. Nine-day-old tomato seedlings were uprooted, roots washed gently in a container with deionised water and then placed in each plug with roots dipped in the Hoagland solution. The plants were left to grow and develop new root hairs for about a week before inoculation. Four tubs were inoculated with 125 mL/tub of Hoagland solution containing sporosori pre-imbibed for three days. The uninoculated plants were mock-inoculated with 125 mL/tub of Hoagland solution (four). All the tubs were set up with plants and left in the growth cabinet to grow for 28 days. Roots of tomato plant were washed with deionised water and transferred into fresh Hoagland solution 3, 10, 17 and 24 days post-inoculation. Plants were sampled from the same growth cabinet for up to 28 days at 3, 7, 10, 15, 21 and 28 days post-inoculation (dpi) from 10 inoculated and 10 uninoculated plants. Five of each inoculated and uninoculated roots were processed for RNA extraction and the other five roots processed for microscopic examination of pathogen root infection.

Two tomato bioassay experiments were set up to optimise growth conditions.

In the first experiment, sporosori were imbibed in the growth cabinet at 18°C for three days prior to inoculation. Tomato plants were inoculated with imbibed spores and set up in a growth cabinet (18°C, 12 hour day/night cycle) and level two light (1600 lux).

In the second experiment, sporosori were imbibed in the growth cabinet (18/15°C 16/8 hour day/night cycle) for three days prior to inoculation. Tomato plants were inoculated with imbibed spores and set up in a growth cabinet (18/15°C, 16/8 hour day/night cycle) and the light level was increased to three (2600 lux).

Total RNA extraction

The root system was washed in tap water and then dried using paper towel. For RNA extraction approximately 1 g of the root tissue was cut using a scalpel blade from the midsection of the root system (the root hair zone likely to have most infection). Each root sample was stored in a labelled tube containing 3-4 mL of RNAlater (Ambion) and left for a minimum period of 24 hrs on the bench at room temperature. Thereafter the solution was decanted and samples stored at -20°C until required for RNA extraction. Total RNA was extracted from each sample (3 inoculated and 3 uninoculated) using the RNeasy Plant mini kit (Qiagen). The root tissue was ground in a sterilised mortar and pestle using liquid nitrogen. From this step onwards RNA was extracted using the RLC buffer method following the manufacturer's protocol (Qiagen). The quantity and the integrity of all the RNA samples were determined using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Real-time quantitative RT-PCR

Extracted RNA was treated with DNase1 enzyme and 100 ng of total RNA reverse transcribed into cDNA. PCR reactions were set up in replicates for cDNA samples and in triplicates for standards using the Rotor-Gene Multiplex PCR Master Mix (Qiagen), 0.35 µM of each forward and reverse primers, 0.2 µM probe (actin and polyubiquitin primer/probe set), 5 µL of template cDNA (50 ng/µL) and made up to 25 µL with RNA-free water. Tenfold serial dilutions (10^6–10^1) of the cloned actin and polyubiquitin fragment was included as standards in each run for estimating the copy number of genes present in the unknown samples.

Microscopic examination of roots

Roots stained with aniline blue were examined under the microscope and confirmed for the presence of *S. subterranea* zoosporangia in root epidermal cells and root hairs.

RESULTS

Confirmation of pathogen infection – a microscopic study

Microscopic examination of tomato roots over time confirmed pathogen infection and disease progression in roots (Table 5.A.I-27). No infection stage could be observed in the roots 3 dpi. 60% of the root hairs containing primary zoosporangia were observed by day 7. By day 10, as the infection in the root progressed, 80% of the root hairs contained zoosporangia with incipient zoospores. At 15 dpi, 50% of the root hairs containing empty zoosporangia were observed as most of the zoospores had exited through the pore and 50% of the zoosporangia were full of incipient zoospores. These zoospores further developed into secondary zoospores and reinfected root hairs. At 21 dpi, empty zoosporangia within root hairs were observed in 80% of the root hairs. Primary zoosporangia as a result of secondary infection were observed in

only 10% of the root hairs at this time point. At 28 dpi, root hair infections were difficult to observe as the roots started browning from *Spongospora* infection. It was often quite difficult to observe the early infection stages - primary and secondary plasmodia within root hairs. It is possible that, although these infection stages are present in the root hairs, we could not distinguish them. This experiment was not a very good set up as the control uninoculated roots were also infected with the same severity of *Spongospora*.

Table 5.A.I-27 – Microscopic examination of tomato roots infected with *S. subterranea* in a hydroponic system

Days post inoculation	*S. subterranea* stage of development in tomato roots
3	No infection stage observed
7	Primary zoosporangia, 60% root hairs infected
10	Zoosporangia with incipient zoospores, 80% root hairs infected
15	50% infected root hairs contained fully developed zoosporangia, 50% empty zoosporangia (secondary zoospores exited)
21	80% root hairs contained empty zoosporangia and 10% root hairs contained secondary infection (primary zoosporangia)
28	Empty zoosporangia, root hair infection difficult to observe due to browning of roots

Confirmation of pathogen infection - Gene expression

Gene expression of the actin and polyubiquitin genes was confirmed in infected tomato roots using the real-time RT-qPCR assay. The mean (3 replicates) log copy number of actin gene in inoculated roots increased significantly from day 3 ($10^{0.5}$) to day 7 ($10^{2.1}$) and then to day 10 (10^{3}) (Figure 5.A.I-5). There was no significant increase in copy number between 10, 15 and 21 dpi (10^{3}, $10^{2.7}$ and $10^{2.9}$ respectively). However there was a significant decrease at 28 dpi ($10^{1.8}$). The uninoculated roots also expressed the gene suggesting that they were infected with *Spongospora*.

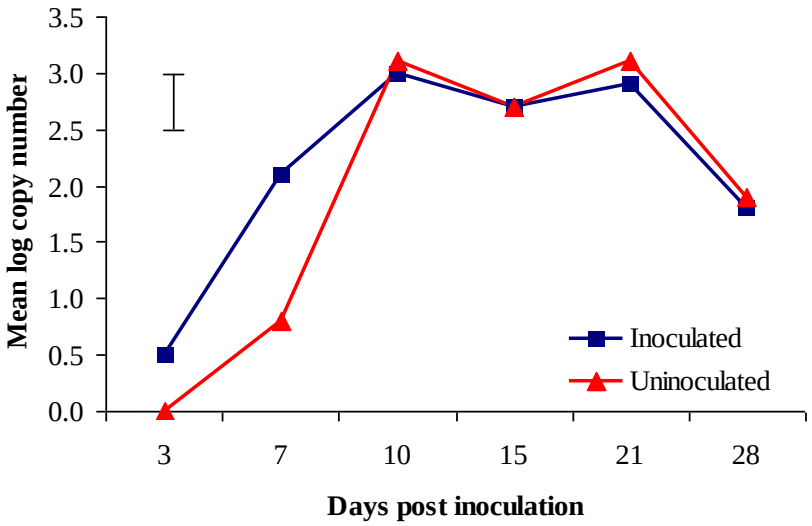


Figure 5.A.I-5 – Mean log_{10} copy number of actin gene expressed in inoculated and uninoculated tomato roots monitored over a 28 day period. lsd=0.5

The mean log copy number of polyubiquitin gene in inoculated roots increased significantly from day 3 ($10^{1.6}$) to day 7 (10^{3}) (Figure 5.A.I-6). There was no significant increase in copy number between 7, 10 and 15 dpi (10^{3}, $10^{3.3}$ and $10^{3.3}$). However there was a significant increase from day 15 ($10^{3.3}$) to day 21 ($10^{4.2}$) and then a significant decrease at 28 dpi ($10^{3.5}$). The uninoculated roots also expressed the gene, suggesting that the roots were infected with *Spongospora*.

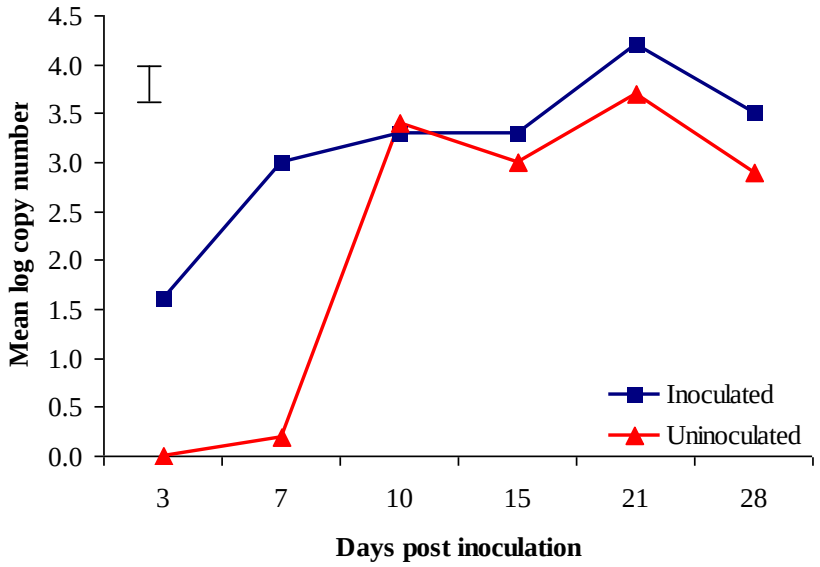


Figure 5.A.I-6 – Mean log_{10} copy number of polyubiquitin gene expressed in inoculated and uninoculated tomato roots monitored over a 28 day period. lsd=0.4

Microscopic study and gene expression study confirm infection of tomato roots by *S. subterranea* - second experiment

Microscopic examination of tomato roots from the first experiment confirmed pathogen infection and disease progression in roots (Table 5.A.I-28). No infection stage could be observed in the roots at 4, 7 and 10 dpi. At day 13 and 15 as the infection in the root progressed, very few root hairs with zoosporangia could be observed. Even at 18 dpi infection was low with some root hairs and cortical cells containing mature zoosporangia with incipient zoospores. At 21 dpi, few root hairs and cortical cells had mature zoosporangia, few contained empty zoosporangia and some root hairs had a mixture of zoosporangial stage. These zoospores further develop into secondary zoospores and reinfect root hairs. At 28 dpi, empty zoosporangia were observed in few root hairs. Small zoosporangia were present in the epidermal cells of the root possibly due to secondary zoospore infection. At 28 dpi, root hair infections were difficult to observe as the roots started browning from *Spongospora* infection. There was no *S. subterrranea* infection in the roots of uninoculated tomato seedlings.

Gene expression of the actin and polyubiquitin genes was confirmed in infected tomato roots using the real-time RT-qPCR assay (Table 5.A.I-28). At day 4 and 7 no gene was detected in infected roots. At day 10 and 13 very low copy numbers of both the genes were detected in infected roots. From day 15 onwards there was an increase in the copy number of both these genes which correlates to the severity of *S. subterranea* infection observed microscopically. Actin and polyubiquitin genes were not expressed in the uninoculated roots. Overall, the gene expression study correlated to the microscopic observation of root infection.

Under these growth conditions, the germination of primary zoospores was low which in turn slowed down the development of pathogen infection in roots. Also, it was very difficult to confirm *Spongospora* infection in roots by microscopic examination prior to 10 dpi.

Table 5.A.I-28 – Microscopic examination of tomato roots infected with *S. subterranea* in a hydroponic system confirmed with the expression of actin and polyubiquitin genes

Days post inoculation	*S. subterranea* stage of development in tomato roots	Gene expression copies/µL	
		Actin	Polyubiquitin
4	No infection stage observed	0	0
7	No infection stage observed	0	0
10	No infection stage observed	4	3
13	Primary zoosporangia developing within root hairs, very low infection	3	5
15	Primary zoosporangia developing within root hairs, very low infection	67	115
18	Mature zoosporangia with incipient zoospores within root hairs and cortical cells	118	206
21	Mature zoosporangia within root hairs and cortical cells, half full half empty zoosporangia within root hairs, empty zoosporangia (secondary zoospores exited) within root hairs and cortical cells	103	213
28	Few empty zoosporangia, small developing secondary zoosporangia mostly in the epidermal cells of the root, root hair infection difficult to observe due to browning of roots	896	6892

Microscopic examination of tomato roots from the second experiment confirmed good pathogen infection and uniform disease progression in roots (Table 5.A.I-29). No infection stage could be observed in the roots 7, 10 and 13 dpi. At day 17, root hairs and cortical cells had mature zoosporangia, some of the root hairs contained empty zoosporangia and some were mixed half-filled with incipient zoospores whilst others had exited through the pore. By day 20, roots were heavily infected. Cortical cells were filled with mature zoosporangia containing incipient zoospores. By day 24, empty zoosporangia within root hairs were obvious. There was heavy infection in root hairs and cortical cells containing mature zoosporangia most likely to have developed from secondary zoospore infection. No *S. subterrranea* infection was observed in the roots of uninoculated tomato seedlings.

Lowering the temperature to 15°C for 8 hours facilitated release of primary zoospores. Under these growth conditions infection was good and pathogen development (zoosporangia) in the roots was found to be more uniform.

Table 5.A.I-29 – Microscopic examination of tomato roots infected with *S. subterranea* in a hydroponic system

Days post inoculation	*S. subterranea* stage of development in tomato roots
7	No infection stage observed
10	No infection stage observed
13	No infection stage observed
17	Mature zoosporangia with incipient zoospores, half full half empty zoosporangia within root hairs, some root hairs with empty zoosporangia (secondary zoospores exited)
20	Roots are heavily infected. Mature zoosporangia with incipient zoospores mostly observed in cortical cells
24	Empty zoosporangia within root hairs, heavy infection in root hairs and cortical cells. Mature zoosporangia in roots due to secondary zoospore infection

DISCUSSION

The expression of both these genes in inoculated roots was similar as confirmed by molecular tests and correlates to the microscopic study of *Spongospora* infection stages. As the infection progressed in the root there was an increase in gene copy number from day 3 to 21. By day 15 the pathogen had completed all the primary infection stages and started the secondary infection stage as confirmed microscopically at day 21. Since the two genes function differently, the graph confirms that polyubiquitin gene is expressed more than the actin gene at 21 dpi. At day 28, the expression of both these genes decreased markedly as the root hairs started to die off due to infection. Both the molecular test and microscopic study confirmed the infection in inoculated roots under optimised growth conditions. Lowering the temperature to 15°C for 8 hours facilitated germination of primary zoospores. Under these growth conditions severity of root infection was good and zoosporangia development in the roots were found to be more uniform.

RNA extraction is costly and the processing of samples for RT-qPCR assay laborious and time consuming. The gene-expression study did not add much value for determining the severity of root infection. On the other hand extraction of DNA is easier, less expensive and less time consuming. Q-PCR assay is a versatile technique for the accurate, sensitive, reliable and high-throughput detection and quantification of pathogen DNA in environmental samples and in host tissue Sanzani et al. (2014). Primers and probe pairs were first designed by van de Graaf et al. (2003) from the internal transcribed spacer region (ITS1 and ITS2) of *S. subterranea* for the detection and quantification of pathogen DNA in soil, and later used for root

samples (van de Graaf et al. 2007); (Hernandez Maldonado et al. 2013). Hence, we have used q-PCR technique in future experiments for detecting and quantifying *S. subterranea* DNA in potato root samples.

PLANT BIOASSAY – REFINEMENT OF A POTATO PLANT BIOASSAY FOR DETECTION AND QUANTIFICATION OF *SPONGOSPORA SUBTERRANEA* INFECTION OF POTATOES USING QPCR DETECTION AND CORRELATING WITH SEVERITY OF ROOT INFECTION FOR *IN VITRO* DISEASE MANAGEMENT EXPERIMENTS

EFFECT OF ZINC AND IRON ON *SPONGOSPORA* ROOT INFECTION USING POTATO BIOASSAY

INTRODUCTION

We have found in field trials that elevated concentrations of zinc and iron reduce powdery scab on tubers. Previous studies have reported the effects boron , ammonium nitrogen , zinc and manganese (Falloon et al. 2001b; Falloon et al. 2009, 2010) have on root galling by *S. subterranea* in a sand/nutrient culture system. The effects of different concentratinons of iron, sulphate sulphur, potassium and silicon on root galling in the same culture system, as part of this project, is reported in a later section. The protocols developed for the tomato bioassay and potato bioassay developed by Merz (1989) and Merz et al. (2004) were slightly modified for growing tissue culture potato plantlets. All of the experiments listed below were assessed for root infection using qPCR technique, and for morphological assessment of severity of root infection.

Experiments conducted using the potato bioassay:

The effect of Zn (as SO_4) (0.025 (baseline), 1.0 and 10 ppm) on spore imbibition and severity of root infection was conducted using cv. Shepody.

A similar study on the effect of Fe (as EDTA) (3 (baseline), 6 and 15 ppm) on spore imbibition and severity of root infection was conducted using cv. Shepody.

Six potato cultivars varying in susceptibility to powdery scab on tubers, cvs Shepody, Russet Burbank, Ranger Russet, Atlantic, Simcoe, and Innovator were assessed for relative susceptibility to powdery scab based on severity of root infection.

MATERIALS AND METHODS

Plant growth and inoculation of potato plants in a hydroponic system

To test the effect of zinc on *Spongospora* root infection in potato plants, tissue-cultured plantlets were grown in six tubs containing Hoagland solution with two tubs each of 0.025 (baseline), 1.0 and 10 ppm of zinc. Sporosori were also imbibed in the same zinc concentration prior to inoculation. The uninoculated plants were mock inoculated with 125 mL/tub of Hoagland solution containing 0.025 (baseline), 1.0 and 10 ppm of zinc.

To test the effect of iron on *Spongospora* root infection in potato plants tissue culture plantlets were grown in six tubs containing Hoagland solution with two tubs each of 3 (baseline), 6 and 10 ppm of iron. Sporosori

were also imbibed in the same iron concentration prior to inoculation. The uninoculated plants were mock inoculated with 125 mL/tub of Hoagland solution containing 3, 6 and 10 ppm of iron.

To test the susceptibility of six potato cultivars to *Spongospora* root infection, tissue culture plantlets of cvs Atlantic, Innovator, Shepody, Simcoe, Russet Burbank and Ranger Russet were used for the bioassay using the protocol described previously.

RESULTS

Ten ppm zinc reduced *Spongospora* DNA levels by 87%

The effect of two concentrations of zinc, 1 and 10 ppm, was analysed at 24 dpi for root biomass, severity of root infection, concentration of zinc in shoots and pathogen DNA copy number in potato roots infected with *S. subterranea* (Table 5.A.I-30).

Both inoculated and uninoculated roots treated with 10 ppm of zinc had significantly reduced root biomass (1.11 and 0.89 g) compared with inoculated and uninoculated roots that were treated with 1 ppm and baseline zinc. There was no significant difference in root biomass between inoculated and uninoculated roots treated at the same concentration of zinc. Zoosporangia developed in all the inoculated roots. Development of zoosporangia was sparse and hard to observe in infected roots treated with 10 ppm of zinc (scale 1) compared with roots treated with 1 ppm and baseline zinc (scale 4) in which zoosporangia were abundant in the root epidermal cells. No visual symptoms were observed during microscopic examination, but low levels of pathogen DNA were detected with qPCR on uninoculated roots. There was no difference in shoot weight between treatments. Increasing zinc concentration in roots increased zinc concentration in shoots, significantly higher in inoculated and uninoculated roots treated with 10 ppm of zinc (Table 5.A.I-30).

There was a significant decrease in pathogen DNA copy number in infected roots treated with 10 ppm of zinc (1.9-fold) compared with 1 ppm and baseline zinc. When pathogen copy number was calculated for whole root biomass, pathogen DNA levels were significantly higher in baseline and 1 ppm zinc compared with 10 ppm zinc. Root biomass did not have an effect on pathogen DNA levels. Ten ppm of zinc reduced pathogen DNA levels by 87% and severity of root infection by 75% compared with baseline zinc.

Table 5.A.I-30 – Effect of zinc on root biomass, severity of root infection (microscopic examination), concentration of zinc in shoots and pathogen DNA copy number in potato roots at 24 days post-inoculation with *S. subterranea*.

Zinc treatment	Root biomass (g)	Severity of root infection scale (0-4)	Concentration of zinc in shoot (ppm)	Pathogen DNA copy number/ng of DNA (log_{10})	Pathogen DNA copy number/root
Inoculated 0.025 ppm (baseline)	1.97bc	4	77a	6.44c	1.02E+16b
Inoculated 1 ppm	2.43c	4	276ab	6.36c	9.82E+15b

Zinc treatment	Root biomass (g)	Severity of root infection scale (0-4)	Concentration of zinc in shoot (ppm)	Pathogen DNA copy number/ng of DNA (log_{10})	Pathogen DNA copy number/root
Inoculated 10 ppm	1.11ab	1	1486c	3.40b	2.70E+13a
Uninoculated 0.025 ppm	2.084c	0	56a	0.52a	1.27E+10a
Uninoculated 1 ppm	1.62abc	0	273ab	0.66a	2.12E+09a
Uninoculated 10 ppm	0.89a	0	1313c	0.65a	2.76E+10a
lsd (P=0.05)	0.92	na	223	0.72	6.85E+15

na, statistical analysis not applicable; numbers followed by the same letter are not statistically significant at P<0.05

Six and 15 ppm of iron reduced *Spongospora* DNA levels by 79 and 87%

The effect of two concentrations of iron, 6 and 15 ppm was analysed at 24 dpi for root biomass, severity of root infection, concentration of iron in shoots and pathogen DNA copy number in potato roots infected with *S. subterranea* (Table 5.A.I-31).

Overall, root biomass in inoculated roots was lower than the uninoculated roots. Inoculated roots treated with 15 ppm of iron had reduced root biomass (2.27 g) compared with 6 and 3 ppm of iron. Six ppm of iron promoted root growth significantly in uninoculated roots (6.36 g). Plants were healthy and there was no difference in shoot weight between treatments. Iron concentration in shoots of uninoculated plants was significantly higher than inoculated plants except in uninoculated plants treated with 15 ppm of iron. Zoosporangia developed in all the inoculated roots. Development of zoosporangia was much less (sparse) and hard to observe in infected roots treated with 15 ppm of iron compared with roots treated with 3 ppm of iron in which zoosporangia were abundant in the root epidermal cells. No visual symptoms were observed during microscopic examination, but low levels of pathogen DNA were detected with qPCR in uninoculated roots.

There was a significant decrease in pathogen DNA copy number in infected roots treated with 6 and 15 ppm of iron (6.05 and 5.80) compared with 3 ppm (baseline) of iron (6.70). However there was no significant difference in pathogen DNA levels between infected roots treated with 6 and 15 ppm of iron. When pathogen copy number was calculated for whole root biomass, pathogen DNA levels were significantly higher in baseline iron compared with higher iron concentrations. Root biomass did not have any effect on pathogen DNA levels. Six and 15 ppm of iron reduced pathogen DNA levels by 79 and 87% and severity of root infection by 25 and 50% respectively compared with 3 ppm of iron.

Table 5.A.I-31 – Effect of iron on root biomass, pathogen DNA copy number, severity of root infection (microscopic examination) and concentration of iron in shoots in potato roots at 24 days post-inoculation with *S. subterranea.*

Fe EDTA treatment	Root biomass (g)	Severity of root infection scale (0-4)	Concentration of iron in shoot (ppm)	Pathogen DNA copy number/ng of DNA (log_{10})	Pathogen DNA copy number/root
Inoculated 3 ppm (baseline)	3.33ab	4	100a	6.70c	4.00E+14b
Inoculated 6 ppm	3.74b	3	100a	6.05b	1.00E+14a
Inoculated 15 ppm	2.27a	2	134b	5.80b	1.00E+13a
Uninoculated 3 ppm	4.23b	0	183c	1.70a	1.00E+09a
Uninoculated 6 ppm	6.36c	0	250d	2.02a	3.00E+09a
Uninoculated 15 ppm	4.54b	0	107a	1.52a	7.00E+08a
lsd (P=0.05)	1.30	na	17.7	0.50	2.20E+14

na, statistical analysis not applicable; numbers followed by the same letter are not statistically significant at P<0.05

Comparison of the relative susceptibility to powdery scab in six potato cultivars

Six potato cultivars varying in susceptibility to powdery scab on tubers, cvs Shepody, Russet Burbank, Ranger Russet, Atlantic, Simcoe, and Innovator were assessed for relative susceptibility to powdery scab based on root infection (Table 5.A.I-32).

Root fresh weight was significantly lower in both uninoculated and inoculated roots of cv. Innovator (1.47 and 1.44). At 24 days post-inoculation, zoosporangia developed in the roots of each cultivar. Shepody had the highest severity of root infection (4). In susceptible cultivars, a higher abundance of zoosporangia was present in root epidermal cells which appeared structurally different compared with the less susceptible cultivars. In the more susceptible cultivars (Shepody, Simcoe and Atlantic) many tetrads of subunits were present in a single zoosporangium, whereas much fewer tetrads comparatively roundish in shape were present in the less susceptible cultivars (Ranger Russet, Russet Burbank and Innovator) (Figure 5.A.I-7).

DNA copy number was highest (2.2-fold) in roots of Shepody and least in Innovator. When pathogen copy number was calculated for whole root biomass, pathogen DNA levels were significantly higher in cv. Shepody and Simcoe. Root biomass did not have an effect on pathogen DNA levels. Based on pathogen DNA copy number and severity of root infection, Shepody was confirmed to be most susceptible followed by Simcoe, Atlantic, Ranger Russet, Russet Burbank with Innovator being the least susceptible.

Table 5.A.I-32 – Comparison of relative susceptibility of six potato cultivars based on root biomass, pathogen DNA copy number and severity of root infection at 24 days post-inoculation with *S. subterranea*

	Potato cultivars	Root biomass (g)	Severity of root infection scale (0-4)	Pathogen DNA copy number/ng of DNA (log_{10})	Pathogen DNA copy number/root
Inoculated	Atlantic	3.76cd	2	4.40h	1.00E+12a
Inoculated	Innovator	1.44a	1	2.76f	5.00E+10a
Inoculated	Russet Burbank	2.44ab	1	2.94fg	5.00E+10a
Inoculated	Ranger Russet	4.30cd	1	2.99g	4.00E+10a
Inoculated	Shepody	4.05cd	4	6.03j	5.00E+13b
Inoculated	Simcoe	3.17bc	1	5.36i	1.00E+13b
Uninoculated	Atlantic	3.76cd	0	0.81d	8.00E+07a
Uninoculated	Innovator	1.47a	0	0.00a	1.00E+08a
Uninoculated	Russet Burbank	3.53bc	0	0.23b	2.00E+08a
Uninoculated	Ranger Russet	3.37bc	0	0.00a	-1.00E-03a
Uninoculated	Shepody	4.89cd	0	2.51e	7.00E+09a
Uninoculated	Simcoe	3.68c	0	0.49c	1.00E+08a
lsd (P=0.05)		1.17	na	0.20	8.62E+12a

na, statistical analysis not applicable; numbers followed by the same letter are not statistically significant at P<0.05

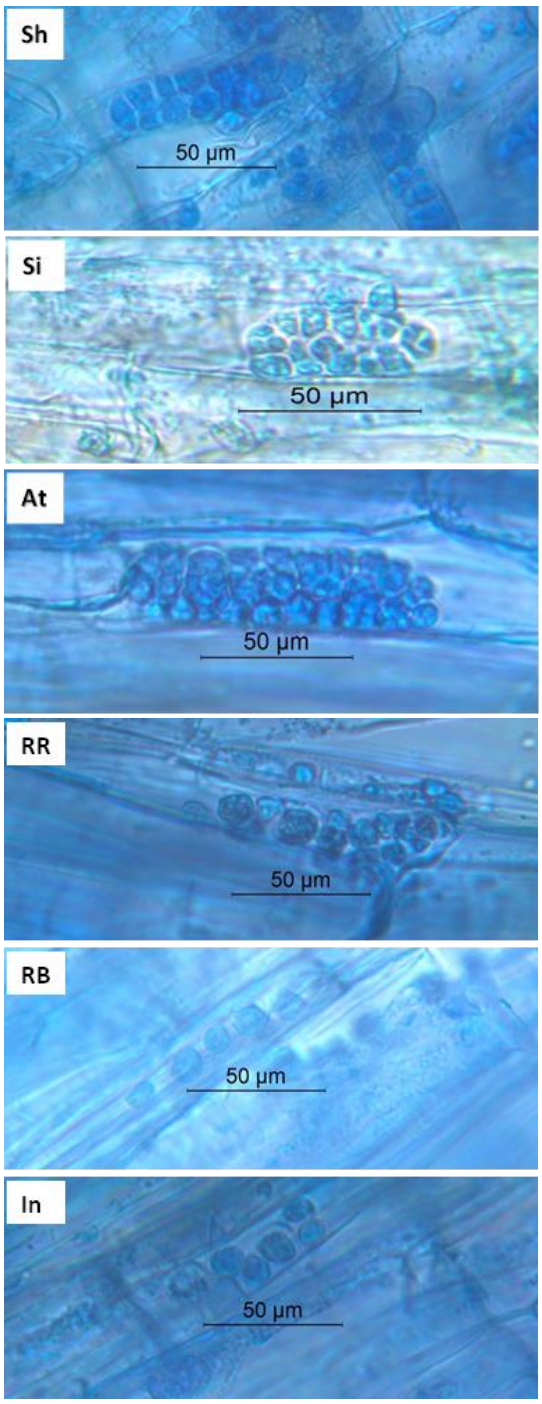


Figure 5.A.I-7 – Micrographs of zoosporangia in stained roots of six potato cultivars at 24 days post-inoculation with *S. subterranea* sporosori grown in nutrient solution. In the more susceptible cultivars (Shepody (Sh), Simcoe (Si) and Atlantic (At)) many tetrads of subunits are present in a single zoosporangium, whereas much fewer tetrads comparatively roundish in shape are present in the less susceptible cultivars (Ranger Russet (RR), Russet Burbank (RB) and Innovator (I)).

DISCUSSION

There are three phases of the powdery scab disease – root infection (zoosporangia), root gall and tuber symptom. Root infection enables the build of inoculum in soil. There is very little information on effect of treatments on root infection (zoosporangial stage). A series of experiments have been conducted in this study using the potato bioassay for validating various experiments and field results. Micro-propagated potato plants were grown in nutrient solution in controlled environment conditions within a growth cabinet. Plants were harvested at 24 days post-inoculation to visually assess the roots for abundance of zoosporangia and determine the severity of root infection using q-PCR technique. Similar experiments with potato plants grown in sand/nutrient solution in controlled glasshouse conditions were assessed at 42 dpi for abundance of powdery scab root galls as reported in detail in the following section.

In this study we report the effect of two concentrations of zinc and iron in nutrient solution compared with standard nutrient solution using both visual assessment and qPCR technique on severity of root infection. Ten ppm of zinc reduced pathogen DNA levels by 87% and severity of root infection by 75% compared with baseline zinc but had an adverse effect on root biomass. Testing a lower concentration of zinc (5 ppm) might reduce root infection without compromising on root biomass. Effect of six different amounts of zinc (as SO_4) ranging from 0.01 to 2.0 µg/mL were tested on infection of potato roots by *S. subterranea* (Falloon et al. 2010). These amounts of zinc were below, similar and above field soil levels. They did not observe any significant difference between treatments as root galls developed even at the greatest amounts of zinc.

Both six and 15 ppm of iron reduced pathogen DNA levels by 79 and 87% respectively and severity of root infection by 25 and 50%. In this experiment, none of the iron concentrations tested were detrimental to root or shoot growth. This indicates that zinc and iron treatment can possibly reduce severity of zoosporangia in infected roots which could stop secondary multiplication in root, the only soil inoculum causing infection. They also suggest that sporosori and roots treated with high concentration of zinc and iron could be inhibiting either zoospore release or both zoospore release and zoospore infection of potato roots.

These results reported here are in contradiction to the results reported next, where the effect of seven different amounts of iron (as EDTA) ranging from 2.5 to 160 ppm were tested in sand/nutrient culture (to simulate low to high soil amounts of iron) on plant growth and severity of root gall and severity of tuber powdery scab. According to those results, the different rates of iron had little effect on severity of root galls and the galls increased with increasing iron content in the nutrient media.

Growing resistant varieties based on tuber symptoms is a principle component of powdery scab management. However, there is little knowledge on the varietal differences of root infection despite indications that root infection could be an important factor in root epidemiology (Falloon et al. 2003). Both the visual examination of zoosporangia and quantification of the pathogen DNA enabled the detection of minor differences in host susceptibility which confirms the accuracy of qPCR technique in determining differences in susceptibility between cultivars. Based on pathogen DNA levels and severity of root infection, cv. Shepody was confirmed to be most susceptible cultivar, followed by Simcoe, Atlantic, Ranger Russet, Russet Burbank with Innovator being the least susceptible. In susceptible cultivars a higher abundance of zoosporangia was present in root epidermal cells which were structurally different compared with the less susceptible cultivars which has previously been observed (de Boer, pers. comm.). This has also been reported by Falloon et al. (2003) where resistant cultivars had fewer zoosporangia in their roots and fewer

root galls than susceptible cultivars. Based on this study, the relative susceptibility to root infection (zoosporangia) for these cultivars correlates to previously known susceptibility of tubers to powdery scab.

The potato bioassay developed in this study is a good method for growing plants and obtaining clean roots for studying root infection in hosts. This method can also be used for the study of other host-pathogen interactions (root pathogens). This method enabled uniform development of disease in roots which was extremely useful for accurately detecting and quantifying pathogen DNA using molecular tools (qPCR). In our studies, results of pathogen DNA level correlated to the visual assessment of root infection severity.

ASSESSMENTS OF INDIVIDUAL SOIL ELEMENTS AS FACTORS INFLUENCING *SPONGOSPORA SUBTERRANEA* INFECTION AND POWDERY SCAB DISEASE IN A GREENHOUSE SAND/NUTRIENT SYSTEM

This section reports on a series of greenhouse experiments conducted by the team led by Dr Richard Falloon of Plant and Food Research NZ. This research compliments the field and glasshouse studies conducted in Australia. The experiments examine the effects of individual nutrient elements on powdery scab root galling in sand/culture solutions, and complete a series that was started in APRP1. This work provides fundamental information on the direct effects of these nutrient solutions on infection and disease in an environment that is very favourable to *Spongospora subterranea* and the development of powdery scab.

SUMMARY

A series of glasshouse based experiments were conducted in which potato plants were grown in sand/nutrient solutions to test the effects of different concentrations of iron (as EDTA), sulphate sulphur, potassium (nitrate) and silicon (sodium metasilicate) on the infection of roots by the powdery scab pathogen *S. subterranea*. This was measured by the relative abundance of powdery scab root galls and the effects of galling on plant water uptake and subsequent plant growth in inoculated and un-inoculated treatments. The concentrations of the various nutrient amendments generally spanned the range likely to occur in the field.

In all experiments, inoculation of the roots with *S. subterranea* significantly reduced plant growth (dry shoot weight) and the numbers of tubers per plant, indicating that the pathogen can harm host-plant growth and productivity.

The different rates of iron had little effect on the severity of root galling. The infection of potato plants by the pathogen was not substantially affected by the different amounts of sulphur (as sulphate), potassium and silicon. The addition of silicon to the plant growth medium generally increased the shoot dry weights, both for inoculated and un-inoculated plants treated with 24 mg L^{-1} silicon or greater. The higher concentrations of silicon offset the deleterious effects caused by *S. subterranea* in the inoculated plants.

INTRODUCTION

An on-going, glasshouse-based research project is examining effects of different soil chemical factors on infection of potato (*Solanum tuberosum*) by *Spongospora subterranea* f. sp. *subterranea*, the plasmodiophorid pathogen which causes powdery scab of potato tubers. The overall aim of the project is to determine which soil chemical factors may reduce host infection by the pathogen and, from this knowledge, examine the feasibility of manipulating soil nutrients as a strategy for management of powdery scab. The soil factors investigated in the project included pH (Falloon et al. 2005), and the plant nutrients boron (Falloon et al. 2001a; Falloon et al. 2001b), zinc and manganese (Falloon et al. 2010), and ammonium and nitrate nitrogen (Falloon et al. 2009). Soil nutrient manipulation may have potential as part of integrated management of powdery scab, alongside the other disease management strategies (cultural, host resistance, pesticides) which have been shown to reduce severity and incidence of this economically important disease (Falloon 2008; Merz and Falloon 2009).

A correlative study carried out in APRP1, involving a large number of commercial potato fields, examined the relationships between pre-planting soil chemical factors (indicated from detailed soil chemical analyses) and the incidence and severity of tuber diseases (including powdery scab) in harvested potatoes. Results from the study suggested that soils with high iron and sulphate sulphur content tended to result in less powdery scab on harvested tubers than soils with low iron and sulphate levels and soils with high levels of potassium tended to produce more powdery scab on harvested potato tubers than soils with low potassium levels. Silicon was not evaluated but is thought to enhance host physical barriers to pathogen penetration and/or induction of biochemical plant defences.

The overall aim of the project is to determine which soil chemical factors may reduce host infection by the pathogen and, from this knowledge, examine the feasibility of manipulating soil nutrients as a strategy for management of powdery scab. The soil factors investigated in this project included iron, sulphate sulphur, potassium and silicon. The materials and methods section below details the make-up of the chemical solutions used for these nutrient factors.

This report describes the experiments and outlines effects of these treatments on root disease caused by *S. subterranea* as measured by relative differences in the daily uptake of water, growth and abundance of root galls on inoculated and uninoculated plants. The experiments used a standard procedure for growing potato plants in a sand/nutrient solution culture, a cultivar (cv. Iwa) known to be very susceptible to *S. subterranea*, and environmental conditions that give good infection of host plants by the pathogen.

MATERIALS AND METHODS

The glasshouse experiments were established in a single glasshouse cell maintained at 16 (± 2)°C, with supplementary lighting to give a 16 h light/8 h dark diurnal cycle. The experiments ran for a total of 56 days.

Four different experiments were conducted. They were different rates of iron, sulphate sulphur, potassium and silicon, and silicon at different irrigation regimes.

Potato plants and irrigation with nutrient solution

White plastic pots (680 mL capacity, without drain holes) were filled with 600 mL of sand (1–2 mm grade) Figure 5.A.I-8. The pots were irrigated (by weight) to 90% of the sand water holding capacity with nutrient solutions Figure 5.A.I-8. Each pot was then planted with a plantlet (from tissue culture) of the potato cv. Iwa, known to be very susceptible to powdery scab (Falloon et al. 2003). Throughout the experiment, the pots were regularly irrigated (at 0830–1000 h each Monday, Wednesday and Friday) with nutrient solution, by weighing each pot and adding the appropriate nutrient solution (by weight) to 90% of sand pot water holding capacity.

The standard nutrient solutions used in four different experiments were prepared by adding the listed chemicals at the indicated rates, to reverse osmosis purified water Table 5.A.I-33. The experimental designs and the quantity of sporosori added to each pot for each experiment are also listed.

Figure 5.A.I-8 – Left. A potato plant in a pot containing sand, and irrigated (by weight) with nutrient solution, the experimental unit used in the greenhouse experiment. Right. General view of greenhouse pot experiment.

Table 5.A.I-33 – A list of standard nutrient solutions, experimental designs and quantity of sporosori added to each pot in each of the four different experiments.

Salt	Iron	Sulphate sulphur	Potassium or silicon	Silicon and irrigation
		mg/L		
Calcium nitrate ($Ca(NO_3)_2.4H_2O$	722	479	361	361
Potassium nitrate (KNO_3)	253	253	253	253
Magnesium sulphate ($MgSO_4.7H_2O$)	246	-	-	-
Magnesium nitrate ($MgNO_3.6H_2O$)		246	246	246
Ammonium nitrate (NH_4NO_3)	40	40	40	40
Potassium chloride (KCl)	19	19	3.7	3.7
Potassium dihydrogen orthophosphate (KH_2PO_4)	116	116	23.1	23.1
Boric acid (H_3BO_4)	7	7	1.2	1.2
Manganese sulphate ($MnSO_4.7H_2O$)	4	4	0.85	0.85
Zinc sulphate ($ZnSO_4.7H_2O$)	0.12	0.12	0.12	0.12
Cupric sulphate ($CuSO_4.5H_2O$)	0.05	0.05	0.05	0.05

Salt	Iron	Sulphate sulphur	Potassium or silicon	Silicon and irrigation
		mg/L		
Molybdic acid (H_2MoO_4)	0.02	0.02	0.02	0.02
Design	Latinised resolvable block	Latinised resolvable block	Latinised resolvable block	Latinised resolvable row-column
No. of replicates	15	7	7	6
S. subterranea inoculum/pot	30,000 or 3,000 sporosori	30,000 sporosori	30,000 sporosori	30,000 sporosori

***Spongospora* inoculation**

Fourteen days after planting, half of the pots were inoculated with suspensions of sporosori of *S. subterranea* in the respective nutrient solutions, applying 30,000 sporosori per pot in 20 ml of nutrient solution. This amount of inoculum was equivalent to 1.42×10^7 resting spores per pot (determined using the method of Falloon et al. (2011a). The other half of the pots were left uninoculated. After inoculation, all of the pots were irrigated with the respective nutrient solutions to bring them to a standard weight (90% water holding capacity).

Plant harvest and disease assessment

At 56 days after planting (42 days after inoculation), the plants were harvested. The shoot of each plant was separated by cutting at the sand surface. Roots and tubers of each plant were carefully washed free of sand, and the numbers of tubers were counted and weighed. The severity of powdery scab on tubers was assessed as nil, light, moderate or heavy. The numbers of root galls on each plant were counted. Shoot and root dry weights for each plant were determined after drying at 75°C for 24 h.

IRON TREATMENTS

For the different iron rate treatments used in the experiment (Table 5.A.I-34), separate nutrient solutions were prepared using the standard solution (above) to which different rates of ethylene diaminetetraacetic acid ferric monosodium salt (Fe EDTA) were added to give the respective iron concentrations. The rate of 20 mg/L iron EDTA has been used previously (Falloon et al. 2003) to provide nutrient conditions adequate for plant growth. The different solutions were added to the respective treatment pots in the experiment at each of the regular irrigations. The seven rates of iron applied in the experiment were chosen to span a broad range, from low to excess, including the normal rate used for previous experiments. The lowest rate of iron tested (0.375 mg/L) was one eighth of that used in a standard nutrient solution (3 mg/L iron, (Falloon et al. 2003)), while the highest rate (24 mg/L) was eight times greater than the standard rate.

Table 5.A.I-34 – Different rates of iron (Fe) in nutrient solution applied in the glasshouse pot trial.

Iron rates	
Fe (mg/L nutrient solution)	Fe EDTA (mg/L nutrient solution)
0.375	2.5
0.75	5
1.5	10
3.0	20
6.0	40
12.0	80
24.0	160

Statistical analyses of data

All analyses were carried out with Genstat (Payne et al. 2009) unless otherwise stated. A level of P=0.05 was used throughout to determine statistical significance.

Four plants treated with the highest rate of iron (160 mg/L Fe EDTA) were either very small or dead, and these were excluded from statistical analysis. Initial analyses showed that no statistically significant spatial trends for any of the data occurred across the trial that were not associated with the main replicates. All data were analyzed with analysis of variance, with data of all measured parameters transformed to stabilize variances. The transformations used were:

- for plant shoot and root dry weights, ln (data)
- for weight of tubers/plant, ln (data + 1)
- for numbers of tubers/plant, numbers of root galls/plant and numbers of root galls/g root dry weight, √(data).

SULPHUR TREATMENTS

Separate nutrient solutions were prepared for the different sulphur treatments used in the experiment. Different rates of concentrations of calcium sulphate were added to the standard solution to give the respective sulphur concentrations used as experimental treatments (Table 5.A.I-35). The lowest sulphur rate (2 mg/L nutrient solution) would correspond to a soil sulphur test of less than 1 mg/kg (extremely low), while the highest rate (150 mg/L) would translate to a soil test of approximately 50 mg/kg (very high sulphur status). These treatments also included the rate which has been previously shown to support good plant growth. The lowest rate of sulphur tested (2 mg/L) was 7% of that used in a standard nutrient solution (30 mg/L; (Falloon et al. 2003)), while the highest rate (150 mg/L sulphur) was five times greater than the standard rate.

Table 5.A.I-35 – Different rates of sulphur applied (as calcium sulphate) in nutrient solutions to potato plants in the greenhouse pot trial.

Sulphur rates	
Sulphur (mg/L nutrient solution)	$CaSO_4.2H_2O$ (mg/L nutrient solution)
2	11
8	43
15	81
30	162
50	269
75	404
100	538
150	807

Statistical analyses of data

Initial analyses showed that spatial trends for the data across the trial not associated with the replicates were negligible, so no adjustments for these were required in the statistical analyses. Corrected plant daily water use data were explored graphically, and data for two selected days (28 and 56 days after planting; respectively 14 and 42 days after inoculation) were analysed separately with analysis of variance. Plant shoot and root dry weights were also analysed with analysis of variance. Numbers of root galls per plant and numbers of root galls/g root dry weight were analysed using a Poisson generalised linear model (McCullagh and Nelder 1989).

POTASSIUM EXPERIMENT

Separate nutrient solutions were prepared for the different treatments used in the potassium experiment, as modifications of the standard nutrient solution described above. Treatments ranging from about one-fifth to 10 times the normal potassium concentration were included in the present study to ensure that the effects of both very low (deficiency) and excessive potassium levels were evaluated. Previous work in our laboratory has shown that the standard rate of potassium (106 mg/L) supports good plant growth, and gives heavy infection of potato roots by *S. subterranea* in susceptible potato cultivars. The lower potassium concentrations (20 and 50 mg/L) were achieved by reducing the amounts of potassium nitrate added. To compensate for the concomitant decrease in the nitrogen, additional amounts of calcium nitrate were added to ensure that the nitrogen concentration was constant across treatments. The higher potassium concentrations (250, 500, 1000 mg/L) were achieved by adding potassium chloride at the appropriate rates.

SILICON EXPERIMENT

Separate nutrient solutions were also prepared for the different silicon treatments used in this experiment. Different rates of sodium metasilicate (Na_2SiO_3) were added to the standard solution (Table 5.A.I-33), to

give the respective silicon concentrations applied as experimental treatments (Table 5.A.I-36). As the sodium metasilicate is alkaline, hydrochloric acid was added at different amounts to adjust each solution to pH 6.

Table 5.A.I-36 – Different rate treatments of silicon (as sodium metasilicate), and amounts of hydrochloric acid used to balance pH, applied in different nutrient solutions to potato plants in a greenhouse pot trial.

Silicon treatment (mg/L)	Sodium metasilicate (mg/L)	1M Hydrochloric acid (mL/L)
0	0	0
7	31	0.6
14	61	1.5
28	122	2.3
42	183	3.5
56	244	4.6

Statistical analyses of data

Corrected daily water use for all assessments was explored graphically. Data for the final assessment (56 days after planting) were analysed formally with a mixed model analysis fitted with REML (Payne et al. 2012). This allowed adjustment for spatial trends in water use. Differences between treatments, and between particular treatment contrasts, were assessed using F-tests of Wald statistics, with degrees of freedom estimated using the method of Kenward and Roger (1997).

Shoot and root weights were analysed with analysis of variance, while a Poisson generalized linear model (McCullagh and Nelder 1989) was used for analyses of numbers of galls and numbers of galls/g root dry weight.

SILICON AND IRRIGATION FREQUENCY EXPERIMENT

Irrigation with nutrient solutions

For the first 28 d of the experiment all the pots were regularly irrigated (at 0800–0900 h each Monday, Wednesday and Friday) with respective nutrient solutions (see below). From that time, half the pots continued to be irrigated in this regime (at 2- to 3-d intervals), and this irrigation treatment was designated “infrequent” (I). The other pots were each irrigated with respective nutrient solutions on Monday, Wednesday and Friday, and were also irrigated with reverse osmosis water at 0800-0900 h each Tuesday, Thursday, Saturday and Sunday, and this irrigation treatment was designated “frequent” (F). The two irrigation treatments aimed to apply the same inputs of nutrients (from nutrient solution applied each Monday, Wednesday and Friday), while the “frequent” treatment received additional water on the other days each week. The weight of each pot was recorded prior to the respective irrigations, and the

appropriate nutrient solution was added to standard weight (equivalent to 90% pot water holding capacity). Pots treated with the "infrequent" or "frequent" irrigation treatments (12 of each), containing sand and nutrient solution but without plants, were included in the experiment (Figure 5.A.I-8). Fibreglass rods, each weighing 11 g (Figure 5.A.I-8) were added to all pots (including those without plants) at 24 d after planting to support plant shoots, and the target weight for pots was appropriately adjusted.

SILICON TREATMENTS

Different rates of sodium metasilicate (Na_2SiO_3) were added to the standard solution (Table 5.A.I-33), to give the respective silicon concentrations applied as experimental treatments (Table 5.A.I-37). As sodium metasilicate is alkaline, hydrochloric acid was added at different amounts to adjust each solution to pH 6.

Table 5.A.I-37 – Different silicon rate treatments (as sodium metasilicate), and amounts of hydrochloric acid to balance pH, applied in different nutrient solutions to potato plants in a greenhouse pot trial.

Silicon treatment (mg/L)	Sodium metasilicate (mg/L)	1M Hydrochloric acid (mL/L)
0	0	0
14	61	1.5
28	122	2.3
42	183	3.5
56	244	4.6
84	366	7.0

Determination of daily water use

Weight losses for the pots containing plants and those without plants were used to calculate the daily amount of water used by each plant in the experiment. This was estimated by subtracting the weight lost by each respective plant-less pot in the replicate from those of pots with plants in the same replicate with the same irrigation treatment and adjusting for the numbers of days between the respective irrigations.

Statistical analyses of data

Daily water use data were explored graphically. For selected days (5 d after planting and 14, 28 or 42 d after inoculation), the daily water use data were also analysed separately. Initial analyses indicated that there were spatial trends in water use within the replicates and across the columns of pots. Mixed model analyses fitted with restricted maximum likelihood (Payne et al. 2012) were therefore used to adjust for these trends, with replicates and columns included as random effects. Differences between treatments (main effects and interactions) were assessed using F-tests of Wald statistics, with degrees of freedom estimated using the method of Kenward and Roger (1997). Plant shoot and root dry weight data were analysed with analysis of variance, as initial analyses indicated that there were no strong spatial trends for these parameters in the experiment. Numbers of root galls were analysed with a Poisson generalised linear

model (McCullagh and Nelder 1989), with a logarithmic link. For these analyses, means and associated confidence limits were obtained on the transformed (link) scale, and then back-transformed.

RESULTS

IRON EXPERIMENT

Plant parameters

Shoot and root dry weights

Inoculation effect

The inoculation of plants with *S. subterranea* sporosori reduced shoot dry weights. Uninoculated plants had a mean shoot dry weight (back transformed) of 2.43 g, while the overall mean shoot dry weight for the *S. subterranea* inoculated plants was 1.98 g (a 19% reduction) (Figure 5.A.I-9). Shoot dry weights also tended (P=0.060) to be less for plants inoculated with 30,000 sporosori (mean [back-transformed] dry weight = 2.09 g) than for plants inoculated with 3,000 sporosori (mean=1.87 g). Root dry weights were not affected by inoculation.

Iron rate effect

Root and shoot weights decreased noticeably with the highest rates of Fe EDTA (P<0.001 for the iron rate effect), with a similar rate response for both inoculation rates (0.08 <P< 1) for the iron by inoculation rate interaction). Four of the 12 plants from the highest Fe EDTA treatment were either dead or very small (hence excluded from the statistical analyses), and the surviving plants had greatly reduced shoot and root dry weights compared with the other Fe EDTA treatments.

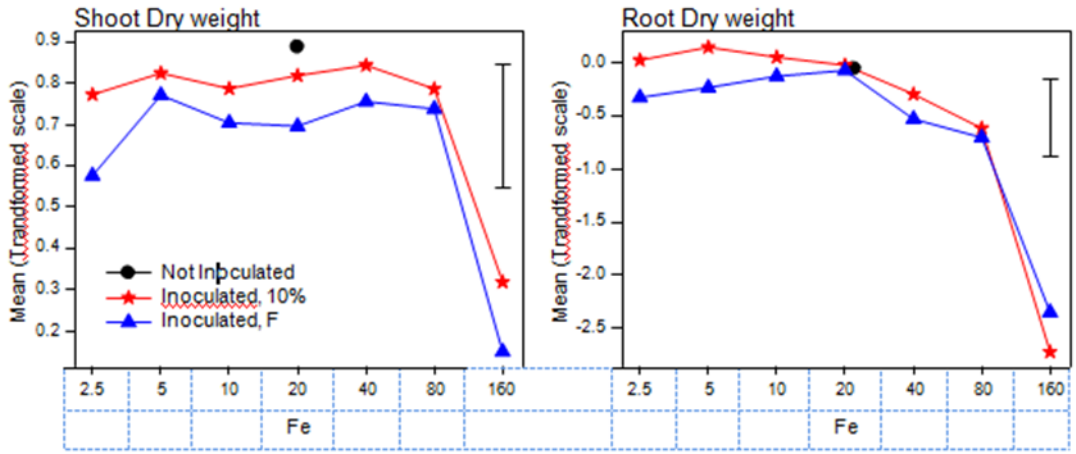


Figure 5.A.I-9 – Mean shoot and root dry weights (in transformed data) for potato plants (cv. "Iwa") which were grown in sand/nutrient solution culture to which different rates of iron EDTA (Fe) were applied in the nutrient solution, and were either uninoculated or inoculated with 30,000 or 3,000 *S. subterranea* sporosori. The plants were harvested 56 days after establishment (42 days after inoculation).

Number and weight of tubers per plant

Inoculation effect

Numbers of tubers/plant were greater for uninoculated plants (mean [back transformed] = 1.7) than for those inoculated with *S. subterranea* sporosori (mean=0.7; P=0.039). This was equivalent to a 58% reduction as a result of inoculation. Similarly, uninoculated plants produced a greater weight of tubers/plant (mean [back transformed] = 14.1 g) than inoculated plants (mean=3.5 g; P=0.005), equivalent to a 75% reduction as a result of inoculation.

Iron rate effect

There were trends for numbers of tubers/plant (P=0.005) and weight of tubers/plant (P=0.011) to be least from the lowest and highest rates of iron than for intermediate rates. This effect was very similar for both of the *S. subterranea* sporosorus inoculation rates, as indicated by non-significant inoculation × iron rate interactions for numbers of tubers/plant (P=0.788) and weight of tubers/plant (P=0.681). Mean number of tubers/plant was 0.1 at the highest rate of iron EDTA, and mean weight of tubers/plant from this treatment was 0.7 g.

Severity of* Spongospora *infection

Powdery scab on tubers

None of the uninoculated plants produced tubers with powdery scab, while 14 (17%) of the 82 inoculated plants had powdery scab symptoms on tubers. The severity of tuber infection on affected plants ranged from light to severe. There were no relationships between either the inoculation rate or the iron rate and powdery scab on tubers. Eight plants from the 3,000 sporosori/plant inoculation rate had powdery scab on tubers and seven plants from the 30,000 rate were similarly affected. Tuber powdery scab occurred across all of the iron rate treatments.

Spongospora root galling

Inoculation effects

No root galls occurred on any of the uninoculated plants (Figure 5.A.I-10). Numbers of root galls per plant were similar (P=0.238) for the two inoculation rates (30,000 and 3,000 sporosori per plant), with the inoculated plants having an overall mean (back transformed) of 52.1 galls per plant. Numbers of root galls/g root dry weight were also similar (P=0.439) for the two inoculation rates (other than at the highest of iron EDTA), with an overall back-transformed mean of 92.2 galls/g.

Iron rate effects

On average, numbers of root galls per plant tended to be greater at the highest rate of Fe EDTA (P<0.001), but were similar across the other six Fe EDTA treatments. This effect was similar for the two inoculation rates (P=0.351 for the inoculation rate by Fe EDTA interaction). Numbers of root galls/g root dry weight tended to increase with increasing rate of Fe EDTA (P=0.002 for the overall Fe EDTA rate effect on this parameter). This effect was similar for both inoculation rates, except for the highest rate of Fe EDTA. For this treatment, mean number of galls/g root dry weight was much greater with the lower inoculation rate than with the higher rate (P=0.032 for the inoculation rates by Fe EDTA interaction).

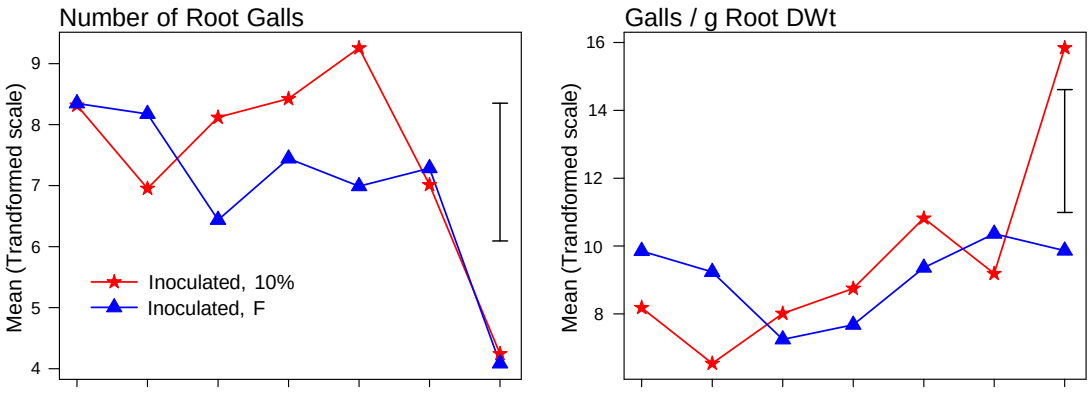


Figure 5.A.I-10 – Mean numbers of *Spongospora* root galls per plant and numbers of root galls/g root dry weight (square root transformed data) for potato plants (cv. "Iwa") which were grown in sand/nutrient solution culture to which different rates of iron EDTA (Fe) were applied in the nutrient solution, and were either uninoculated or inoculated with 30,000 or 3,000 *S. subterranea* sporosori per plant. Counts of root galls were made 6 weeks after inoculation.

SULPHATE EXPERIMENT

Water use

Neither inoculation nor sulphur rate affected daily water use at 28 days after planting (14 days after inoculation) ($P>0.10$) (Figure 5.A.I-11). However, at 56 days after planting (42 days after inoculation), water use varied strongly ($P\leq0.005$), both with inoculation and sulphur rate treatments (Figure 5.A.I-12).

Inoculation effect

At the end of the experiment (42 days after inoculation) plants inoculated with *S. subterranea* were using, on average, 58% less water per day than uninoculated plants.

Sulphur rate effects

At the end of the experiment, the inoculated plants receiving the least and greatest rates of sulphur were using 44% (2 mg/L sulphur) and 35% (150 mg/L) less water per day than uninoculated plants. There was a greater effect on plants receiving the intermediate sulphur rate treatments. Plants in the 15 mg/L sulphur treatment were the most affected by inoculation, with inoculated plants using 78% less water per day than uninoculated plants at 42 days after inoculation.

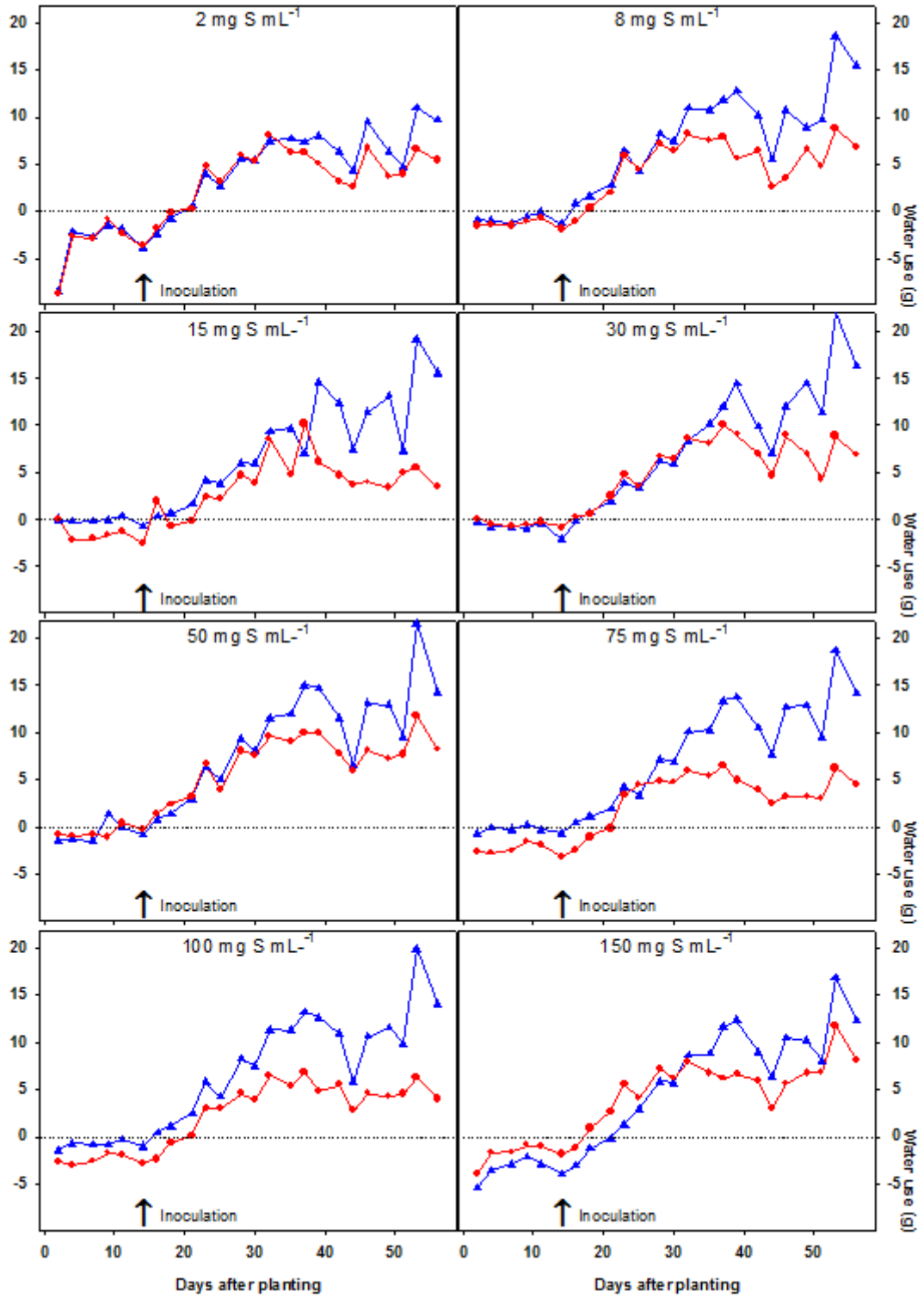


Figure 5.A.I-11 – Estimated mean daily water use (g/day) for potato plants grown in sand/nutrient solution culture with different rates of sulphur (2–150 mg/L), either uninoculated (blue symbols) or inoculated with *S. subterranea* (red symbols). Inoculation date (14 days after planting) is indicated.

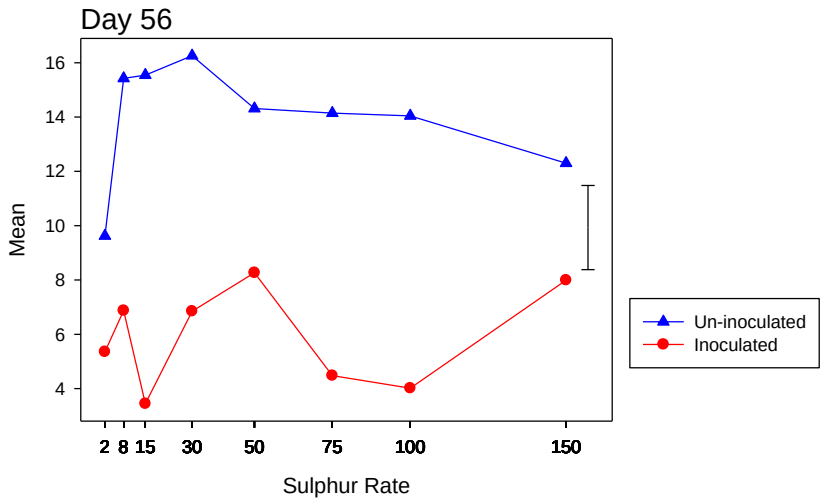


Figure 5.A.I-12 – Mean daily water use (g/day at 56 days after planting) for potato plants grown in sand/nutrient solution culture with different rates (mg/L) of sulphur, either uninoculated or inoculated with *S. subterranea*. Bar indicates least significant difference between two means (P=0.05).

Plant parameters

Shoot and root dry weights

Inoculation effects

Inoculation of plants with *S. subterranea* sporosori reduced shoot dry weights compared with uninoculated plants (Figure 5.A.I-13). Mean shoot dry weight for the uninoculated plants was 2.66 g, while that for the inoculated plants was 1.64 g (a 38% reduction). Root dry weights were similarly reduced by the inoculation treatment. Uninoculated plants had a mean root dry weight of 1.18 g, while that for inoculated plants was 0.69 g (a 41% reduction).

Sulphur rate effects

Shoot dry weights were not substantively affected (P=0.327) by the different sulphur rate treatments. However, the effect of sulphur rate on root dry weights was statistically significant (P=0.021), although the changes in root dry weight with sulphur rate were inconsistent (Figure 5.A.I-13).

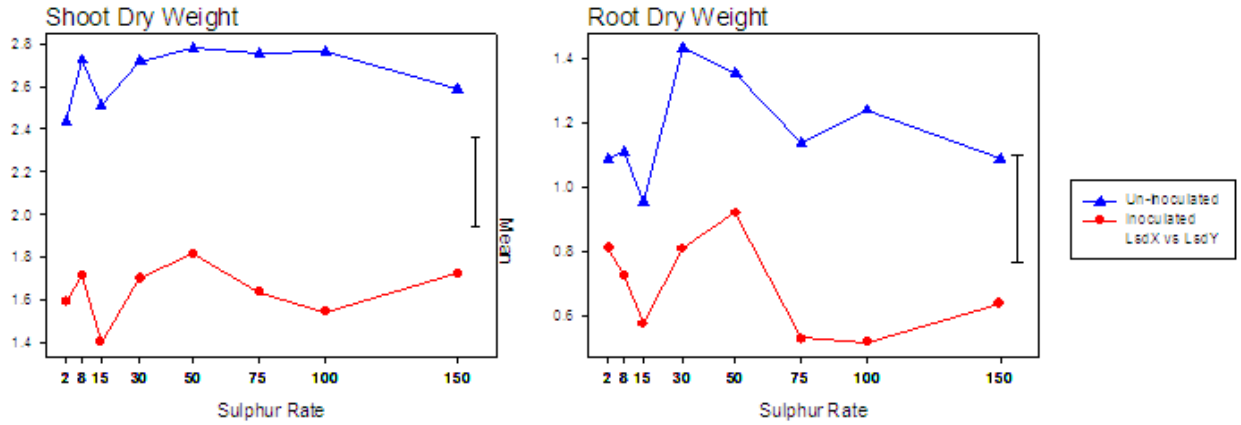


Figure 5.A.I-13 – Mean shoot and root dry weights (g) for potato plants grown in sand/nutrient solution culture at different rates of sulphur that were either uninoculated or inoculated with *S. subterranea* sporosori. The plants were harvested 56 days after establishment (42 days after inoculation). Bars indicate least significant differences between two means (P=0.05).

Numbers of tubers per plant, weight of tubers per plant and weight per tuber

Inoculation effects

The mean number of tubers per plant was slightly greater for uninoculated plants (mean=0.6) than for inoculated plants (mean=0.4; P=0.072) (Figure 5.A.I-14). Similarly, mean weight of tubers per plant (Figure 5.A.I-14a) was greater for uninoculated plants (6.5 g) than for inoculated plants (4.2 g; P=0.018), equivalent to a 35% reduction in tuber yield. Mean weight per tuber (Figure 5.A.I-14b) was also greater for uninoculated plants (5.6 g) than for *S. subterranea*-inoculated plants (3.9 g; P=0.049), a 30% reduction.

Sulphur rate effects

There were only minimal effects of the different rates of sulphur on the tuber yield parameters. For mean number of tubers per plant this effect was not statistically significant (P>0.3). Similarly, the effects of different sulphur rates on mean weight of tubers per plant were not strong (P>0.3), although the effect of inoculation was greatest at the two highest rates of sulphur (Figure 5.A.I-14a). Similarly, the effect of inoculation on mean weight per tuber was greatest at these two high rates of sulphur (Figure 5.A.I-14b).

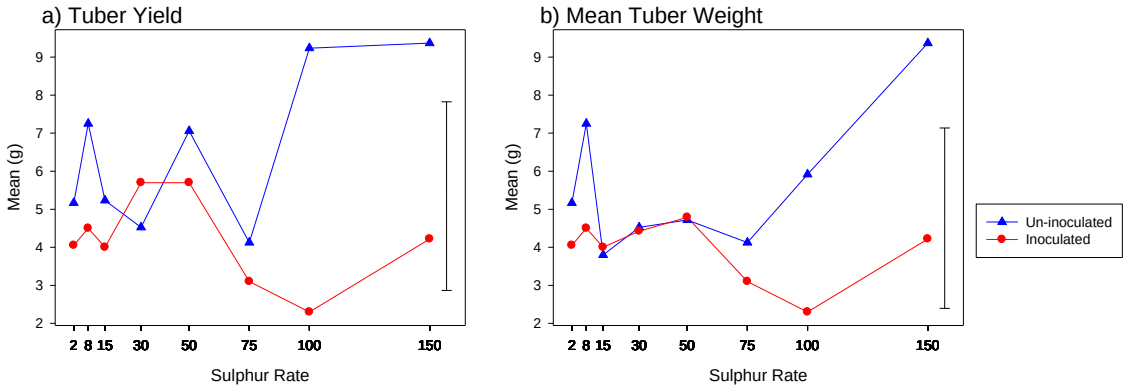


Figure 5.A.I-14 – Mean weight (g) of tubers per plant (a) and mean weight (g) per tuber (b) from potato plants grown in sand/nutrient solution culture at different rates of sulphur that were either uninoculated or inoculated with *S. subterranea* sporosori. The plants were harvested 56 days after establishment (42 days after inoculation). Bars indicate least significant differences between two means (P=0.05).

Severity of* Spongospora *infection

Inoculation effects

No root galls occurred on the uninoculated plants, while the plants that were inoculated had an average of 49 galls/plant (Figure 5.A.I-15). Also, the roots of the uninoculated plants were creamy white ("healthy"), while roots of the inoculated plants were brown.

Sulphur rate effects

There was a trend for plants grown at the greater rates of sulphur to have fewer root galls per plant. However, these effects were not statistically significant, both for mean numbers of galls per plant (P=0.663; Figure 5.A.I-15a) and for numbers of galls/g root dry weight (P=0.071; Figure 5.A.I-15b).

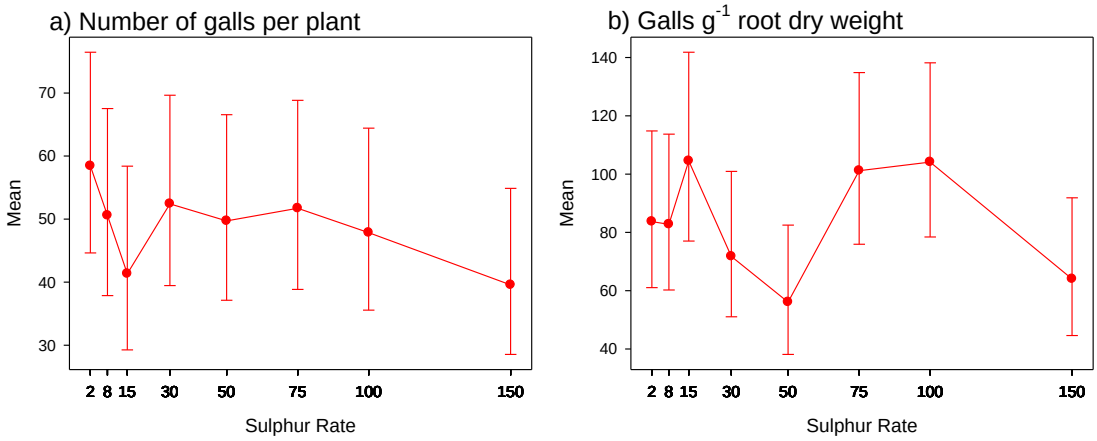


Figure 5.A.I-15 – Mean numbers of *Spongospora* root galls/plant (a) and numbers of root galls/g root dry weight (b) for potato plants grown in sand/nutrient solution culture to which different rates of sulphur (mg/L) were applied in the nutrient solution, and were inoculated with *Spongospora subterranea* sporosori. Counts of root galls were made 6 weeks after inoculation. Confidence limits (95%) for each mean are indicated.

POTASSIUM EXPERIMENT

Water use

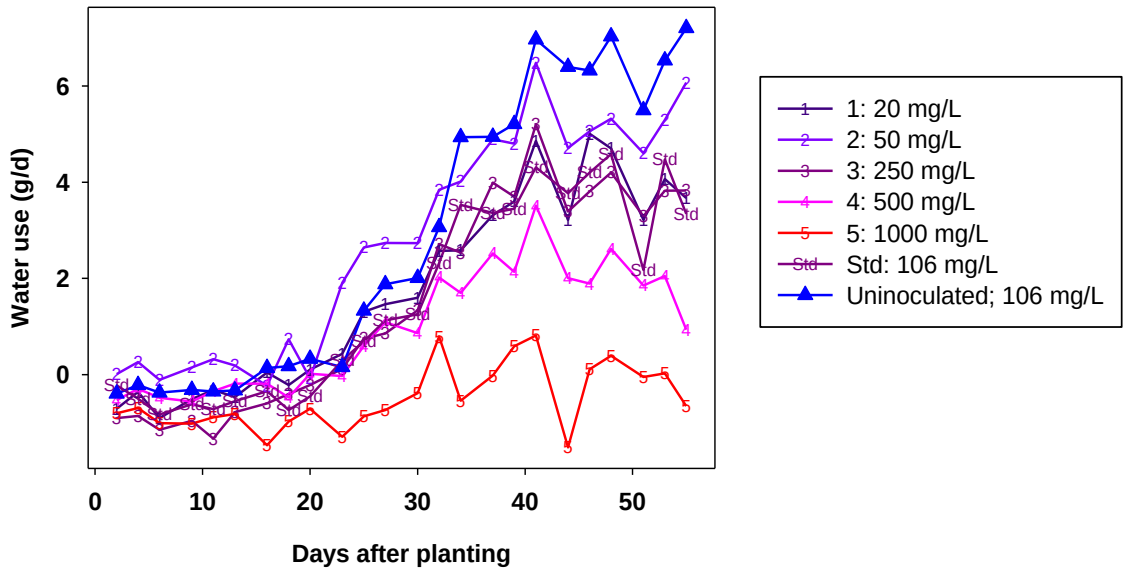


Figure 5.A.I-16 – Mean daily water use for potato plants that were either uninoculated (▲), or inoculated with *Spongospora subterranea* sporosori at 14 d after planting (all other treatments), and grown with different rates of potassium, applied in nutrient solutions.

At 56 d after planting (Figure 5.A.I-17), mean water use by the *S. subterranea* inoculated plants at the standard potassium rate (106 mg/L) was 3.58 g/d, 50% less (P=0.003) than for the uninoculated plants (7.21 g/d) receiving the same amount of potassium. Water use for the inoculated plants at 56 d after planting was similar for the four lowest rates of potassium tested, but was markedly reduced by the two treatments with the greatest rates of potassium (Figure 5.A.I-16).

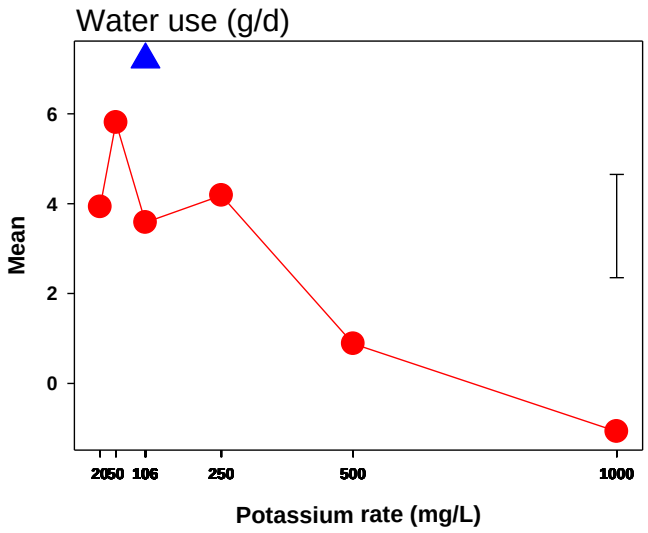


Figure 5.A.I-17 – Mean daily water use 56 d after planting, for potato plants that were either uninoculated (▲), or inoculated with *S. subterranea* sporosori (●), and grown with different rates of potassium, applied in nutrient solutions.

Shoot and root weights

For plants grown at the "standard" rate of potassium (106 mg/L), uninoculated plants had a mean shoot dry weight of 1.45 g, while that for plants inoculated with *S. subterranea* sporosori was 0.94 g, a 35% reduction due to inoculation (P<0.001) **(**Figure 5.A.I-18**)**. Mean root dry weights were similarly affected by inoculation, with mean root dry weight of 0.75 g for the uninoculated plants and 0.46 g for the *S. subterranea*-inoculated plants, a 39% reduction in root dry weight due to inoculation (P<0.001).

For the *S. subterranea*-inoculated plants, mean shoot dry weights were similar across the potassium rate treatments up to 500 mg/L, but drastically reduced (P<0.001) at the greatest potassium rate of (1000 mg/L). A similar effect occurred for mean root dry weights (Figure 5.A.I-18).

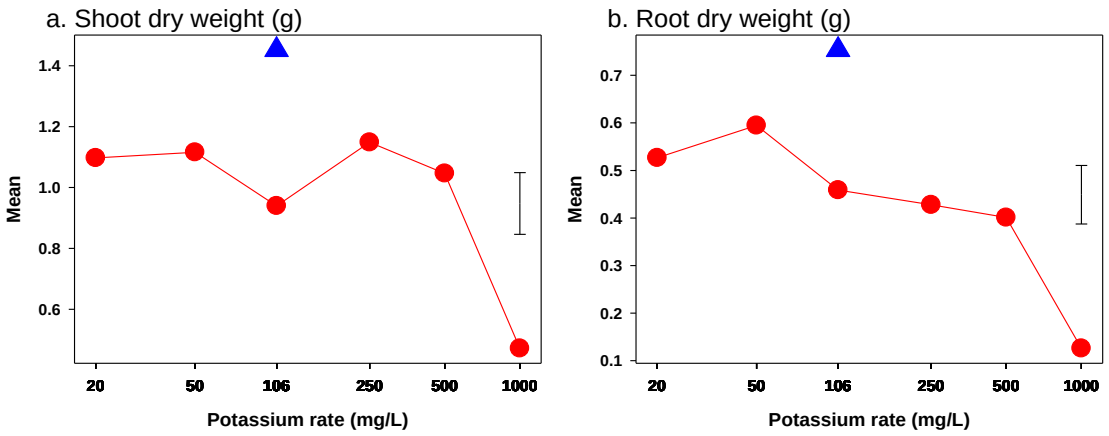


Figure 5.A.I-18 – Mean shoot (a) and root (b) dry weights for potato plants 56 d after planting, that were either uninoculated (▲) or inoculated with *S. subterranea* sporosori (●), and grown with different rates of potassium, applied in nutrient solutions. Bars indicate lsds (P=0.05; df=28 for shoots, 27 for roots).

Numbers of galls/plant (P<0.001) and numbers of galls/g root dry weight (P=0.012) varied substantially between the potassium rates, with fewer galls developing on plants grown at the two greatest rates of potassium than for the other rates (Figure 5.A.I-19**)**. However, the greatest rate of potassium (1000 mg/L) gave very considerable reductions in plant growth (including root dry weights), indicating that this treatment was very toxic to the plants.

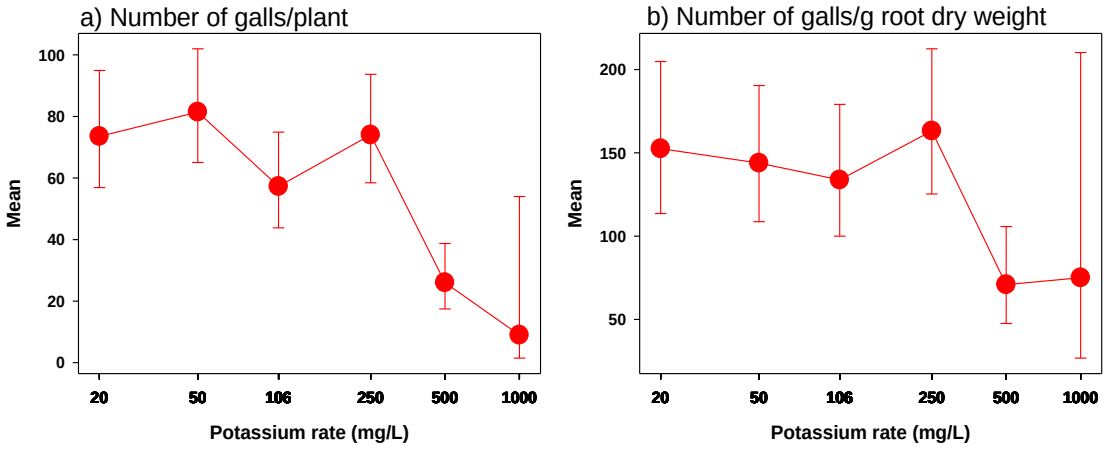


Figure 5.A.I-19 – Mean numbers of galls (a) and mean numbers of galls/g root dry weight (b) on root systems for potato plants (at 56 d after planting), that were inoculated with *S. subterranea* sporosori 14 d after planting and grown in sand with different rates of potassium, applied in nutrient solutions. Bars indicate 95% confidence limits for each mean.

SILICON EXPERIMENT

Water use

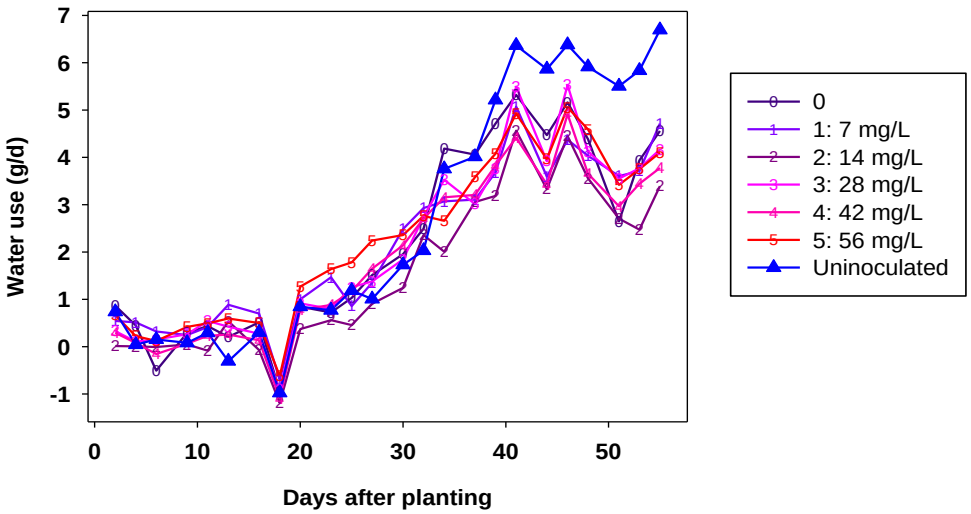


Figure 5.A.I-20 – Mean daily water use for potato plants that were either uninoculated (▲), or inoculated with *S. subterranea* sporosori at 14 d after planting (all other treatments), and grown with different rates of silicon, applied in nutrient solutions.

At 56 d after planting (Figure 5.A.I-21), mean daily water use for the uninoculated plants was 6.62 g/d, while that for the plants inoculated with *S. subterranea* where no silicon was applied was 4.51 g/d, a 32% reduction due to inoculation (P=0.017). Water use at this time for the inoculated plants treated with different rates of silicon was similar (P=0.702) for all six silicon rate treatments (including no added silicon) (Figure 5.A.I-20**)**.

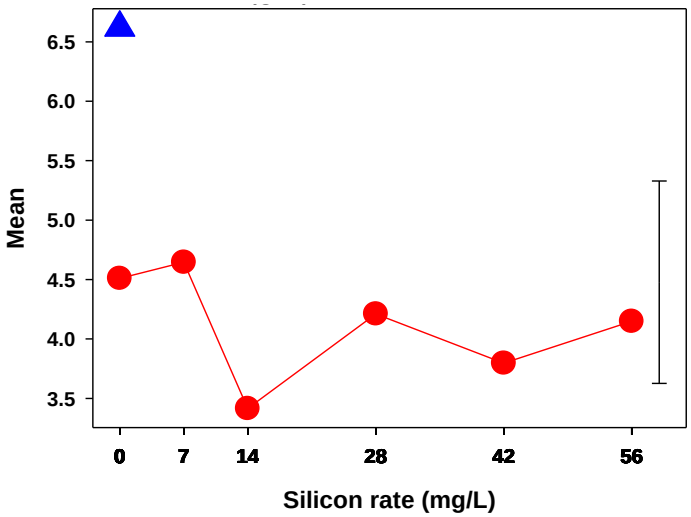


Figure 5.A.I-21 – Mean daily water use 56 d after planting, for potato plants that were either uninoculated (▲), or inoculated with *S. subterranea* sporosori (●), and grown with different rates of silicon, applied in nutrient solutions. Bar indicate lsd.

Shoot and root dry weights

For plants grown without added silicon, uninoculated plants had a mean shoot dry weight of 1.19 g, while that for *S. subterranea*-inoculated plants without added silicon was 0.86 g, a 28% reduction due to inoculation (P<0.001, Figure 5.A.I-22). Mean root dry weights were similarly affected by inoculation, with a mean root dry weight 0.63 g for the uninoculated plants, and 0.40 g for *S. subterranea*-inoculated plants, a 36% reduction due to inoculation (P=0.002).

For the *S. subterranea*-inoculated plants, there was a trend for mean shoot dry weights to increase with increasing rates of silicon. Mean shoot dry weight was greater from the highest rates of silicon than from the low rate treatments (P=0.025). However, mean root dry weights of inoculated plants were not affected (P=0.602) by the different silicon rate treatments (Figure 5.A.I-22).

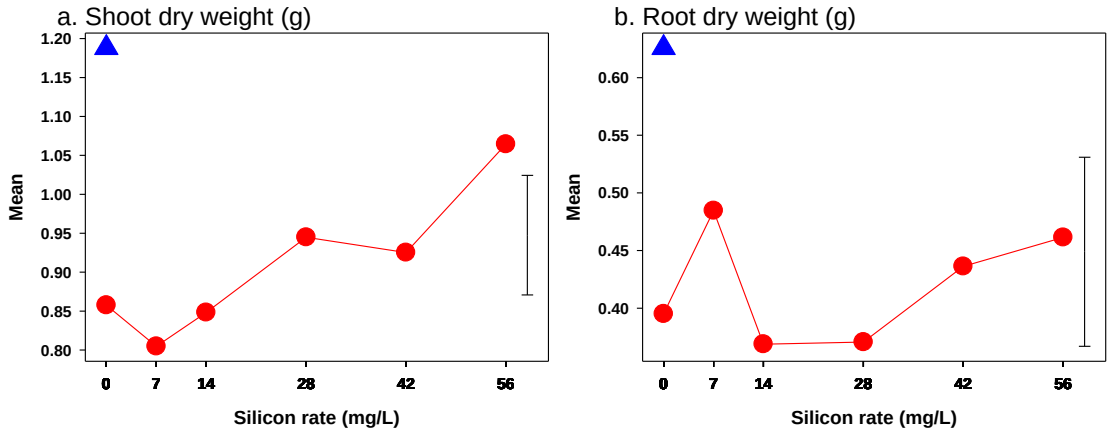


Figure 5.A.I-22 – Mean shoot (a) and root (b) dry weights for potato plants 56 d after planting, that were either uninoculated (▲) or inoculated with *Spongospora subterranea* sporosori (●), and grown with different rates of silicon, applied in nutrient solutions. Bars indicate lsds (P = 0.05; df = 29).

Intensity of *Spongospora* infection

No galls were observed on the roots of any of the uninoculated plants, and the roots of these plants were creamy white in colour. In contrast, the roots of the plants inoculated with *S. subterranea* sporosori were light brown, and roots of these plants had many creamy white to brown root galls. Microscopic examination of galls from representative plants revealed that brown galls were filled with sporosori, confirming them to be caused by *S. subterranea*.

Neither numbers of galls per plant (P=0.783) nor numbers of galls/g root dry weight (P=0.088) varied significantly between the different silicon rate treatments (Figure 5.A.I-23**)**.

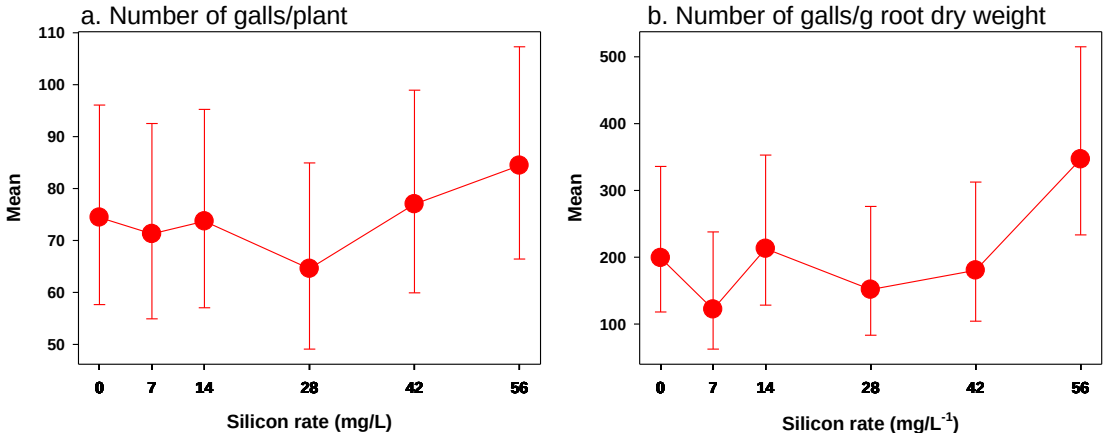


Figure 5.A.I-23 – Mean number of galls and mean number of galls/g root dry weight on root systems for potato plants (at 56 d after planting), that were inoculated with *S. subterranea* sporosori 14 d after planting, grown in sand with different rates of silicon, applied in nutrient solutions. Bars indicate 95% confidence limits for each mean.

SILICON AND IRRIGATION EXPERIMENT

Daily water use

From approx. 2 weeks after inoculation, the inoculated plants used progressively less water than the uninoculated plants, and this was true both for the frequently and the infrequently irrigated plants (Figure 5.A.I-24**)**. Furthermore, the infrequently irrigated plants generally used more water each day than the frequently irrigated plants.

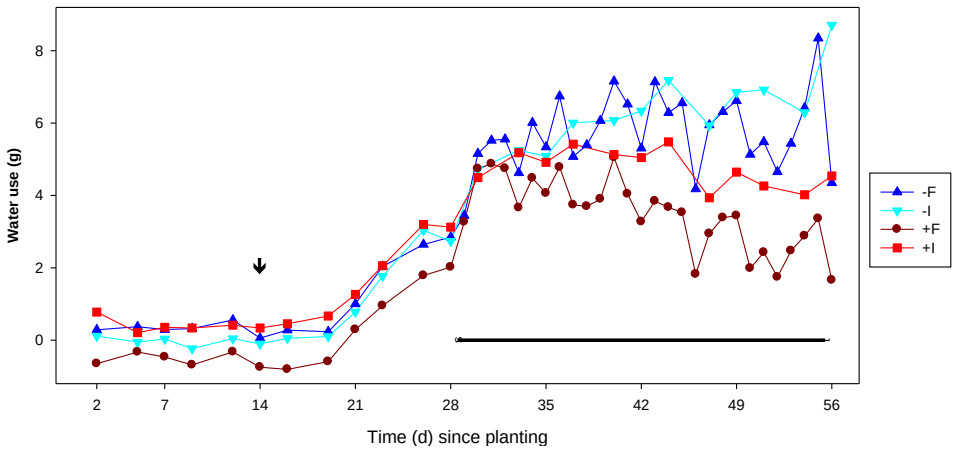


Figure 5.A.I-24 – Mean daily water use for potato plants that were either uninoculated (-), or inoculated with *S. subterranea* sporosori (+) at 14 d (↓) after planting, and grown with either "frequent" (F) or "infrequent" (I) irrigation treatments applied from 28 d after planting until the end of the experiment (horizontal line).

At 5 d after planting, well before the inoculation or irrigation treatments were applied, the plants were using very similar amounts of water (Figure 5.A.I-25**)**. At 28 d after planting (14 d after inoculation), at the time that the different irrigation treatments were first applied, the uninoculated and inoculated plants were still using similar amounts of water.

At 42 and 56 d after planting (respectively, 28 and 42 d after inoculation), the inoculated plants were using progressively less water than the uninoculated plants. Furthermore, at each of these time points the frequently irrigated plants were using less water than the infrequently irrigated plants. At 56 d, the inoculation and irrigation treatment effects were marked (P<0.001) for the inoculation and irrigation main effects, and P=0.034 for the inoculation × irrigation interaction).

The inoculation effect was greater for the plants irrigated at 2- to 3-d intervals than for those irrigated each day. On average at the end of the experiment, the uninoculated plants were using a mean of 6.50 g water per day, while those inoculated with *S. subterranea* were using 3.10 g per day, representing greater than 50% reduction in water use due to inoculation. For the uninoculated plants, those irrigated frequently were using a mean of 4.30 g of water per day, while the infrequently irrigated plants were using 8.69 g per day (double that for the “infrequent” irrigation treatment). For the *S. subterranea*-inoculated plants, those irrigated each day were using 1.65 g per day, while those irrigated every 2-3 days were using 4.56 g per d (176% more for the “infrequent” irrigation treatment).

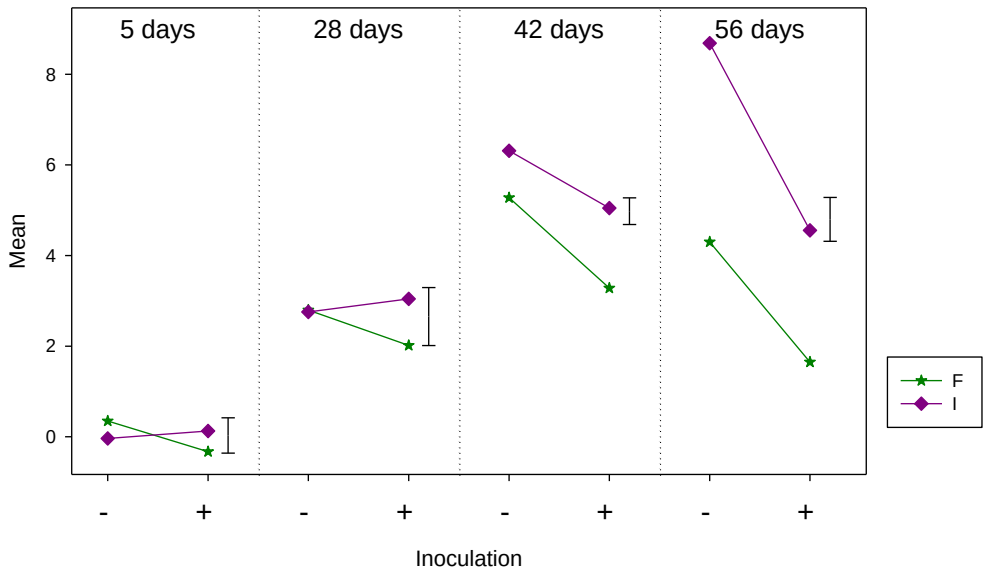


Figure 5.A.I-25 – Mean daily water use at 5, 28, 42 or 56 d after planting, for potato plants that were either uninoculated (-), or inoculated with *S. subterranea* sporosori (+) at 24 d after planting, and were received either “frequent” (F) or “infrequent” (I) irrigation treatments.

The smaller amounts of water used by the frequently irrigated plants than for those irrigated infrequently resulted in unequal amounts of nutrient solution being added to the two groups of plants. Throughout the experiment the frequently irrigated plants each received (on average) 607 mL of nutrient solution, while those irrigated less frequently received an average 886 mL (46% more).

At the end of the experiment, while water use varied with the inoculation and irrigation treatments (see above), water use was similar for plants in each of these treatments across all the different silicon treatments (P>0.1 for all effects involving silicon; Figure 5.A.I-26**)**.

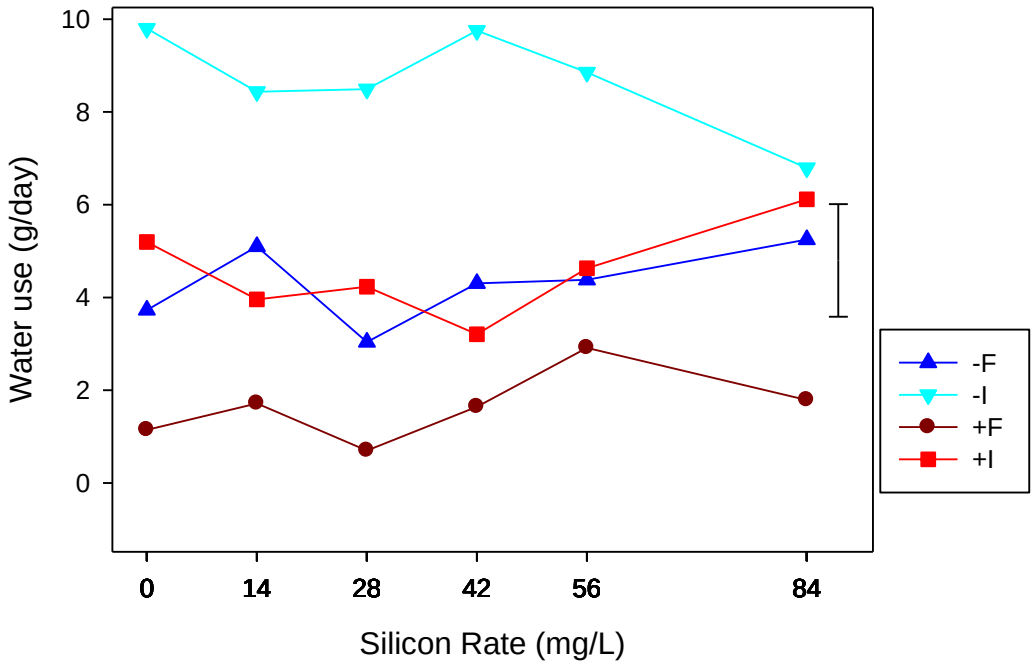


Figure 5.A.I-26 – Mean daily water use at 5, 28, 42 or 56 d after planting, for potato plants that were either uninoculated (-), or inoculated with *S. subterranea* sporosori (+) at 24 d after planting, and were received either "frequent" (F) or "infrequent" (I) irrigation treatments.

Condition and symptoms on harvested roots

Roots harvested from the uninoculated plants were creamy white in colour, and no root galls were observed on these plants. In contrast, the roots of all the inoculated plants were light brown, and many root galls were present on these plants. The galls were usually creamy white in colour, but occasionally a few dark brown galls were noted. Some of dark brown galls were mounted on microscope slides and observed with a light microscope. These all contained very many sporosori typical of *S. subterranea*, confirming that the observed root hyperplasia was caused by the pathogen.

Intensity of root hyperplasia

Numbers of galls/plant (Figure 5.A.I-27a) were greater (P<0.001) on plants irrigated at 2- to 3-day intervals than for plants irrigated each day (an overall 47% increase for the "infrequent" irrigation treatment compared with the "frequent" treatment) **(**Figure 5.A.I-27**)**. However, numbers of galls/plant did not vary substantially because of the different silicon treatments (P>0.40 for the silicon main effect and the irrigation × silicon interaction).

When adjustment was made for the differences in root dry weights between the two irrigation treatments (numbers of galls/g root dry weight; Figure 5.A.I-27b), the differences between the irrigation treatments became relatively smaller. Numbers of root galls/g root dry weight did not vary substantially as a result of either the different irrigation or the different silicon treatments (P>0.10 for the main irrigation and silicon effects, and the irrigation × silicon interaction).

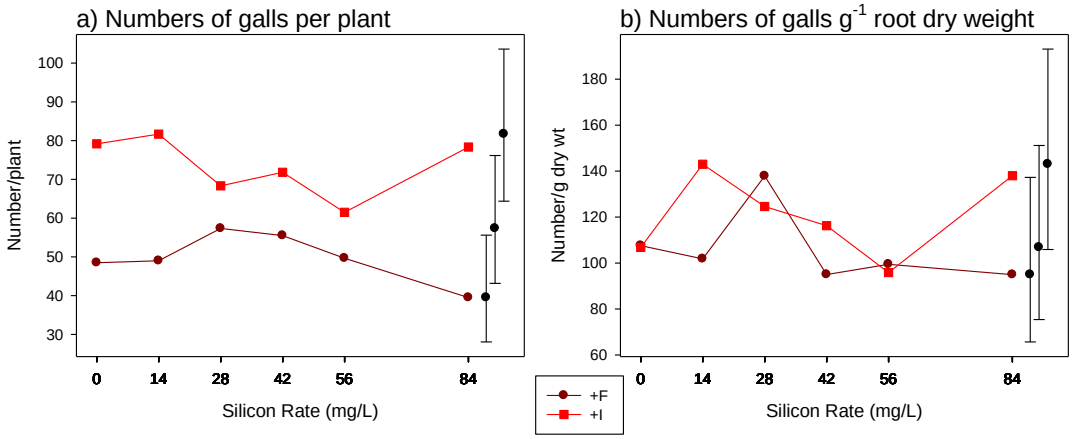


Figure 5.A.I-27 – Mean numbers *of Spongospora* galls per plant (a) and galls/g root dry weight (b) at 56 d after planting, on root systems of potato plants grown with different amounts of silicon applied in nutrient solutions, and inoculated with *S. subterranea* sporosori (at 14 d after planting), and that received either "frequent" (F) or "infrequent" (I) irrigation treatments. Bars indicate 95% confidence limits for the smallest, mid-range and largest means for each parameter.

Plant shoot and root weights

Shoot dry weights (Figure 5.A.I-28a) were affected by the inoculation, irrigation frequency and silicon treatments, with these effects acting independently of each other (0.16<P<0.81 for all treatment interactions). On average, shoot dry weights from the *S. subterranea*-inoculated plants (mean=1.03 g) were 24% less (P<0.001) than from the uninoculated plants (1.36 g). The frequently irrigated plants produced 19% less shoot dry matter (mean=1.07 g) than the infrequently irrigated plants (1.32 g; P<0.001). Mean shoot dry weights generally increased with increasing amounts of silicon in nutrient solutions (P<0.001) from 0 to 28 or 42 mg/L of silicon, and were then similar at the two greatest rates (56 and 84 mg/L). Mean shoot dry weights were greatest from the 42 mg/L silicon treatment (mean=1.29 g), 28% more than from the nil silicon treatment (1.01 g). Furthermore, mean shoot dry weights for the uninoculated plants with no

added silicon (1.18 g) were similar to those for the *S. subterranea*-inoculated plants grown with 48 mg/L silicon (mean=1.12 g).

Root dry weights (Figure 5.A.I-28b) were affected by the inoculation and irrigation frequency treatments. Root dry weights for the uninoculated plants were generally greater (mean=1.06 g) than for plants inoculated with *S. subterranea* (0.59 g; P<0.001), representing a 44% reduction due to inoculation. The inoculation effect varied with irrigation regime (P<0.001 for the inoculation × irrigation interaction). Root weights were generally less for the frequently irrigated plants than for those irrigated less frequently. For plants irrigated each day, inoculation reduced root dry weight by 54%, but for plants irrigated at 2- to 3-d intervals the reduction due to inoculation was less, at 35%. Root dry weights were not strongly affected by the different silicon treatments (0.10<P<0.90 for the main treatment effects and all treatment interactions).

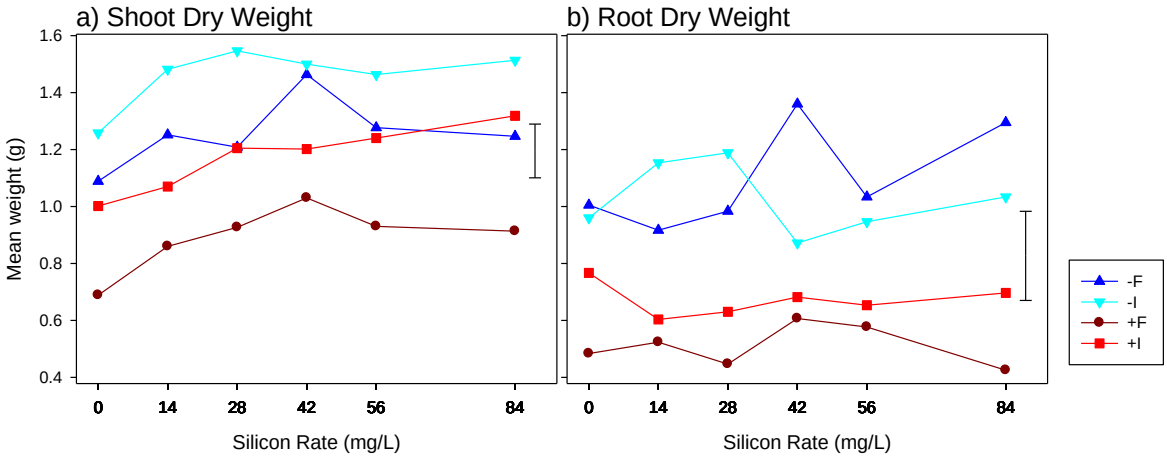


Figure 5.A.I-28 – Mean shoot (a) and root (b) dry weights for potato plants 56 d after planting, for plants that were grown with different amounts of silicon applied in nutrient solutions, and were either uninoculated (-) or inoculated with *S. subterranea* sporosori (+), and were given either "frequent" (F) or "infrequent" (I) irrigation treatments. Bars indicate lsds (P=0.05; df=115).

DISCUSSION

Iron experiment

Under the conditions applied in this experiment, infection of potato plants by *S. subterranea* was not affected by different amounts of iron in the plant growth medium. The amounts of iron applied in the experiment spanned a range likely to occur in soils. The concentration of soluble iron present in well-drained soils is commonly less than 1 mg/L (Curtin and Smille 1983), although much higher concentrations can occur in wetland soils, such as those used for rice production (Baruah et al. 2007).

The experiment demonstrated that inoculation of potato plants with *S. subterranea* reduced host plant shoot weights, and also reduced numbers and weight of tubers/plants. This is further confirmation that the pathogen can harm host plant growth and productivity (Merz and Falloon 2009).

The experiment also measured fewer tubers/plant and lower weights of tubers/plant at the lowest and highest rates of iron EDTA applied. These results suggest that the lowest rates were deficient for optimum tuber production, while at the highest rate the iron EDTA was toxic to the plants. Maximum vegetative growth (shoot and root dry weights, numbers and weight of tubers/plant) occurred at the intermediate rates of iron EDTA. There have been very few reports of iron toxicity in agricultural crops (Fageria et al. 2002). Toxicity observed in the present study may have been related to high concentrations of chelate (ethylene diaminetetraacetic acid), rather than to iron *per se*.

The two different rates of inoculation applied in the experiment were used to examine if low disease pressure could affect host infection responses to varying iron content, testing whether low levels of inhibition of the pathogen could be masked in situations of high disease pressure. While the low inoculum rate used in the experiment had a slightly lesser effect on plant growth than the higher inoculum rate, the two rates gave similar intensities of *Spongospora* galling on roots, and powdery scab on tubers occurred similarly from both inoculum rates. This suggests that even at the lower inoculum rate, disease pressure was high. Therefore, the experiment may not have applied disease pressure low enough to detect mild suppression of the pathogen by added iron.

This experiment indicated that intensity of root infection by *S. subterranea* (as indicated by numbers of root galls) tended to increase with increasing levels of iron. Furthermore, although tuber powdery scab occurred in only 17% of plants inoculated with *S. subterranea* sporosori, the incidence of tuber infection occurred across all of the different iron rate treatments. These results suggest that manipulation of soil iron content is unlikely to be a useful strategy for reducing *S. subterranea* infection of potato plants or tubers. Therefore, the previously observed relationship in the field between high soil iron and low powdery scab incidence and/or severity has not been confirmed in the present glasshouse study.

The experiment tested for effects of iron content in isolation from other soil factors, without testing for interactions between iron and other soil chemical or environmental factors that may affect *S. subterranea* infection of host plants. Furthermore, the effects of iron at very low amounts of *S. subterranea* inoculum were not fully examined. These more complex situations may be worth exploring in future investigations, particularly if further field observations or controlled trials suggest that reductions in tuber powdery scab can result from elevated iron content in soil.

Sulphur experiment

Under the conditions applied in this experiment, infection of potato plants by *S. subterranea* was not substantively affected by different amounts of sulphur (as sulphate) in the plant growth medium. The amounts of sulphate sulphur applied in the experiment spanned a range likely to occur in field soils, from "very low" to "very high" (see Hill Laboratories, www.hill-laboratories.com).

Several previous studies have indicated that treatment of soil with elemental sulphur or with sulphate salts of heavy metals (such as copper or zinc) can reduce powdery scab on harvested tubers and a positive correlation has been observed in the field between soil sulphate and powdery scab incidence and/or severity. One possible explanation for the lack of any effect of sulphate sulphur on *Spongospora* infection is that the inoculation rate used in the present study was too great to allow detection of a disease reduction

as a result of the sulphur treatments. This is unlikely, however, as very similar experiments (Falloon et al. 2009, 2010; Falloon et al. 2001b) have shown that other plant nutrients and elements, when applied at high rates in the standard sand/nutrient system used in these studies, can affect intensity of infection of potato plants by the pathogen.

The form of sulphur is likely to be very important. Elemental sulphur is well-recognised as having strong antimicrobial effects. On the other hand, sulphate sulphur, the form of the element that occurs in soil, is generally non-toxic to either micro-organisms or plants unless present at very high concentrations, when osmotic effects can occur.

The experiment in the present study tested for effects of sulphate sulphur in isolation from other soil factors, without testing for interactions between the element and other soil chemical or environmental factors that may affect *S. subterranea* infection, or for host factors such as different susceptibilities in potato cultivars. Furthermore, the effects of sulphur at very low amounts of *S. subterranea* inoculum were not examined. These more complex situations may be worth exploring in future investigations, particularly if further field observations or controlled trials suggest that reductions in tuber powdery scab can result from elevated sulphate sulphur concentrations in soils.

The experiment in this study has demonstrated that inoculation of potato plants with *S. subterranea* reduced host plant shoot and root weights, and also reduced numbers of tubers/plant, weight of tubers/plants and weight/tuber. This is further confirmation that the pathogen can be deleterious to host plant growth and productivity (Merz and Falloon 2009).

Potassium experiment

The amounts of potassium applied in this experiment spanned a range from low to excessively high. The large decrease in shoot and root mass recorded from the highest potassium concentration is likely to be the result of osmotic stress to the plants associated with elevated ion concentrations, rather than to a specific effect of elemental potassium.

Under the conditions applied in this experiment, infection of potato plants by *S. subterranea* was not substantially affected by different amounts of potassium in the plant growth medium, although at high rates of this element there was a slight reduction in intensity of *Spongospora* gall development on roots of inoculated plants. The results of the APRP1 field study suggesting that powdery scab was more severe in soils with greater potassium content compared with those containing less of the element have not been confirmed. Similarly, the suggestion that elevated potassium increased severity of clubroot in Brassicas (Karling 1968; Prabhu et al. 2007) has not been confirmed for *S. subterranea* on potato.

Silicon experiment

The experiment with silicon, applied as a soluble form (sodium metasilicate), spanned a range of silicon rate treatments from nil to excess. None of the silicon treatments tested affected intensity of *Spongospora* root galling, indicating that this element had no effect on infection of potato plants by the pathogen. The highest rate of silicon increased shoot dry weight, but the similarly severe root galling across all of the rate treatments indicated that this increase was not due to an effect on root infection by *S. subterranea*. These results are in contrast with other reports linking silicon with reduced disease susceptibility (Datnoff et al. 2007; Deliopoulos et al. 2010). Plasmodiophorid zoospores encyst on host root epidermis cells, and penetrate host tissue directly through root walls. The present results therefore indicate that effects of

silicon as an impediment to pathogen penetration of cell walls are unlikely to be operating in the *S. subterranea*/potato pathosystem.

Shoot growth in inoculated plants tended to increase as the silicon application rate increased, but was always less than that measured in the uninoculated treatment (Figure 5.A.I-28). The beneficial effect of silicon can probably be attributed to improvement in water use efficiency, which enabled silicon-treated plants to partially mitigate the adverse effect of root damage on water uptake, resulting from *S. subterranea* inoculation. Several studies have shown that transpiration from plant leaves can be reduced by application of silicon (possibly due to thickening of epidermal cell walls by silica (Walker and Lance 1991; Gao et al. 2004).

Silicon and irrigation experiment

This experiment confirms numerous other sets of experiment results which have recorded deleterious effects of *S. subterranea* inoculation on growth of potato plants (Merz and Falloon 2009), and provides further evidence of the potential harmful effects of the pathogen on potato plant productivity.

Frequent irrigation of plants in this experiment gave increased numbers of *Spongospora* root galls per plant. However, when the effect of the irrigation treatments on root dry weight were removed (numbers of galls/g root dry weight), the intensity of root hyperplasia was similar on frequently and infrequently irrigated plants. The different irrigation frequency treatments were not applied in the experiment until 14 d after inoculation. *S. subterranea* rapidly infects host plants after inoculation in conditions similar to those applied here (Hernandez Maldonado et al. 2013), and infection probably occurred before the different irrigation treatments were applied in the present experiment. *Spongospora* infection (powdery scab) of potato tubers is generally associated with moist soils, which allow zoospores of the pathogen to move from resting spores or zoosporangia to infect host stolons (Merz and Falloon 2009). The present results indicate that moisture conditions in the sand/nutrient solution medium used in the greenhouse experiment were adequate for primary infection during the 2 weeks after inoculation, and the subsequently applied irrigation frequency treatments did not affect intensity of root hyperplasia. This is further evidence that early stages of host growth (root or stolon development) are likely to be key periods for development of *Spongospora* diseases of potato (root galls and tuber powdery scab).

Silicon was applied in this experiment as a soluble form (sodium metasilicate), at a range of rates from nil to excess. None of these silicon treatments affected the intensity of *Spongospora* root hyperplasia, in a potato cultivar that is very susceptible both to this stage of disease caused by the pathogen, and to powdery scab on tubers (Falloon et al. 2003). These results confirm those obtained from a previous experiment (Falloon et al. 2013).

The greatest rates of silicon applied in this experiment (28 mg/L Si and greater) increased shoot dry weight. However, the similarity in severe root galling for inoculated plants across all the silicon treatments indicates that this increase was not due to a direct effect on root infection by *S. subterranea*. This is in contrast to reports linking silicon with reduced susceptibility to diseases in several host/pathogen systems (Datnoff et al. 2007; Deliopoulos et al. 2010). Plasmodiophorid zoospores encyst on host root epidermis cells, and penetrate host tissue directly through the epidermis cell walls. The present results therefore indicate that effects of silicon as an impediment to pathogen penetration are not operating in the *S. subterranea*/potato pathosystem.

The plants irrigated infrequently produced greater amounts of shoot and root dry matter than those irrigated more frequently. This was probably because the plants irrigated at 2- to 3-d intervals received greater amounts of plant nutrients in nutrient solution (on average 46% more) than those irrigated at daily intervals.

Shoot growth in the *S. subterranea*-inoculated plants tended to increase as the silicon application rate increased, although shoot dry weights were always less than those measured from the uninoculated treatment at the respective rates of silicon. This response meant that plants inoculated with the pathogen and treated with 28-42 (or greater) mg/L Si produced equivalent amounts of shoot dry matter to uninoculated plants grown without added silicon. This occurred in both frequently and infrequently irrigated plants. The silicon treatments therefore partially mitigated the adverse effects of root damage on water and/or nutrient uptake, resulting from *S. subterranea* infection. The beneficial effects of silicon in plants infected by *S. subterranea* can probably be attributed to improvement in water use efficiency, as has been previously suggested (Falloon et al. 2013). These positive effects of silicon were observed within the concentration range of the element commonly found in soil solutions (2-20 mg/L; Epstein (1994)).

Several studies have shown that transpiration from plant leaves can be reduced by application of silicon, possibly because of thickening of leaf epidermis cell walls by silica (Walker and Lance 1991; Gao et al. 2004). Furthermore, beneficial effects of silicon on growth of water stressed plants of wheat (Gong et al. 2003) and maize (Kaya et al. 2006) have been shown to manifest as increased shoot growth (weight), but not as increased root mass. A similar response was recorded in the present study with potato.

CONCLUSIONS

The experiments in this study have demonstrated that inoculation of potato plants with *S. subterranea* reduced host plant shoot and root weights, which is further confirmation that the pathogen can be deleterious to host plant growth and productivity (Merz and Falloon 2009).

Different rates of iron (up to eight times the standard rate) had little effect on the severity of root galling caused by *S. subterranean* infection, although there was a trend of increased root galling with increasing iron content in the media. Different rates of sulphate sulphur had no substantive effects of the severity of root galling. Very high rates of potassium (close to that causing phytotoxicity) reduced severity of root galling but at lesser rates root galling was severe.

Silicon (as sodium metasilicate) had no effect on the severity of root galling, even at the highest rate of application. In a second experiment, which tested different rates of silicon under two different irrigation regimes, high rates of silicon in growth medium had no direct effect on potato root infection by *S. subterranea*. However, this study confirmed that silicon treatments can offset the deleterious effects of the pathogen, probably through increasing water use efficiency in plants with root systems damaged by the pathogen. Although the effects of silicon on tuber infection had not been considered, the results of these two studies suggest that the manipulation of silicon could be useful as an augmenting procedure, to be included in integrated strategies for management of potato diseases caused by *S. subterranea*.

Because the experiments were carried out in controlled greenhouse conditions, these conclusions remain to be verified by field evaluations, particularly where *S. subterranea* inoculum levels in soil, and disease pressure, are likely to be far less than in the inoculated experiments described here. Furthermore, the experiments did not consider effects of potassium, sulphate sulphur or silicon on powdery scab on tubers,

although previous studies have shown that the cultivar used is very susceptible both to *Spongospora* root galling and to tuber symptoms. Although a low incidence of tubers in the iron experiment developed powdery scab, tuber infection occurred across the entire range of rates. Nevertheless, the experiments indicate that manipulations of the elements tested here are unlikely to be worthwhile as management strategies for potato diseases caused by *S. subterranea*.

Table 5.A.I-38 summarises results from all glasshouse experiments performed for this project. Responses are indicated from susceptible potato plants inoculated with *S. subterranea* and treated with different rates of the respective soil chemical factors.

These experiments have provided information on effects on a selection of individual soil constituents on development of *Spongospora* diseases. They give knowledge which can be used as the basis for development of field-based studies on manipulation of soil factors for reducing *Spongospora* diseases, particularly powdery scab of potato tubers and reduced potato yields. From these, fertiliser strategies or nutrient amendments may be developed as practical tools to assist with effective management of the yield and quality-limiting effects of *S. subterranea* in potato crops.

Table 5.A.I-38 – Responses from potato plants (cv. Iwa; very susceptible to *Spongospora subterranea* infection) grown in sand/nutrient solution culture in glasshouse experiments. The plants were treated with different rates of the respective soil chemical factors and inoculated with *S. subterranea*

Response after inoculation with, compared with uninoculated plants	Chemical factor
Markedly reduced root galling, large increases in plant growth	Elemental sulphur
	Boron
	Ammonium nitrogen
Slightly reduced root galling, small increases in plant growth	Manganese
	Zinc
	Nitrate nitrogen
	Potassium
	Silicon
No effects on disease (severe root galling), no positive effects on plant growth (markedly reduced root and shoot mass)	Sulphate sulphur
	Iron
	pH

DISCUSSION

The efficacy of applications of different forms of sulphur, zinc and iron on the incidence and severity of powdery scab and on the yield and quality of tubers in the field has been demonstrated and referenced against a cultivar with a low susceptibility rating and a fungicide treatment in a sand/nutrient culture system.

Powdery scab has three different phases of disease; infection of root hairs to produce zoosporangia, which serves as a multiplication phase but can disrupt root function, root galls (hyperplasia) producing sporosori, and tuber lesions, also producing sporosori. It has been hypothesised that the different phases can be viewed as three different diseases, possibly under different genetic control (Falloon and Merz 2013). Little is known however, how disease control options such as sulphur and zinc may impact on the different phases of the disease.

Elemental sulphur is one of the oldest known fungicides. It is oxidised to sulphate in the soil. In the studies reported here, increasing concentrations of sulphate sulphur in a sand/nutrient culture system had no significant effect on root galling severity. This suggests that elemental sulphur has a direct effect on the pathogen and/or the infection process. However, a direct effect on sporosori of *S. subterranea* is less likely because of the very resistant and long-lived nature of this resting spore.

Applications of elemental sulphur drive other interactions in the soil. Soil acidification from sulphur increases the solubility of cations such as manganese, for example. In our field trials, sulphur concentrations were positively correlated with soil manganese concentrations, which in turn was negatively correlated with powdery scab incidence and severity. Falloon et al. (2010) reported a mild reduction in the severity of powdery scab root galling with increasing concentrations of manganese (up to 10 ppm) on potato plants grown in a sand/nutrient system. Apart from this, there is no other evidence in the literature that manganese directly affects powdery scab. However, the possible link between reduced scab and higher manganese concentrations cannot be discounted without further research.

Our *in vitro* potato bioassay experiment reported in the previous section demonstrated that 10 ppm of zinc in a hydroponic solution significantly reduced the infection of roots by *S. subterranea* compared to 1 ppm, perhaps due to a direct effect on zoospores or on the infection process. There is evidence that zinc was toxic to zoospores of another form of *S. subterranea* that affects water cress (Tomlinson 1960; Walsh 2004), although in an aquatic environment and at low concentrations (up to 0.1 ppm). Falloon et al. (2010) found that increasing concentrations of zinc up to 2 ppm resulted in only a mild inhibition of root galling on potato plants grown in a sand/nutrient culture system. However, efficacious soil zinc concentrations in our field experiments were around 20-30 ppm, suggesting a more direct effect on the pathogen. However, little is known about the nature of the interaction between high zinc concentrations, the pathogen, infection and disease. It is possible that other interactions are at play, including the effects of zinc on plant physiology, disease resistance and soil microbiology, as well as on the pathogen.

The effect of iron treatments on powdery scab was surprising given the relatively high iron concentrations already present in the ferrosol soils in which the trials were conducted. Foliar applications of iron also reduced powdery scab disease and this was verified in a glasshouse trial and by an *in vitro* study in which concentrations of 15 ppm iron significantly reduced root infection. It is clear that much needs to be learnt about the complex interactions between nutrients, the pathogen and the disease. The mechanism by which the foliar application reduced disease needs further investigation.

In the absence of a registered fungicide treatment, sulphur and zinc are potential alternative management options for powdery scab for susceptible varieties. Based on our research, it is possible to provide interim recommendations on the rates of sulphur and zinc, pending further trials to determine thresholds in a wider range of soil types. Given that efficacy of sulphur and zinc has been demonstrated in a range of soil

types (Australia, United Kingdom and New Zealand), and that they do reduce disease on cultivars with high and low susceptibility, they are likely to be efficacious in a range of soil types and on cultivars with differing susceptibilities. Since most processing growers undertake soil nutrient testing, recommendations on nutrient management for optimisation of crop growth could ultimately include recommendations for disease control. With some refinement, the sulphur and zinc treatments could easily be integrated into the powdery scab management decision trees.

It is important to note that these treatments were broadcast on the soil surface and incorporated into the soil with a rotary hoe. This ensured that the nutrients were present in the soil surrounding initiating tubers i.e in the tuber zone. This would not be the case for nutrient treatments applied in-furrow during the planting operation, which places nutrients well below the zone in which tubers are initiated. This is a critical consideration for the practical application of nutrient treatments.

EFFECTS OF FUNGICIDES AND OTHER CHEMICAL TREATMENTS ON SOIL-BORNE AND SEED-BORNE POWDERY SCAB DISEASE AND ON YIELD OF POTATOES

SUMMARY

Field trials were conducted over four seasons on a site infested with the powdery scab pathogen to evaluate the effects of seed tuber treatments, in-furrow treatments and broadcast treatments of the fungicides fluazinam and flusulfamide on powdery scab disease.

Seed tuber treatments had no effect on the development of powdery scab in the progeny tubers probably because of the masking effect of a high level of soil inoculum of the pathogen. In-furrow treatments of both fungicides at different rates did not significantly reduced disease incidence or severity in two trials. However, the highest rates of both fluazinam and flusulfamide applied in-furrow resulted in small but significant reductions in total tuber yields.

The broadcast (drenched on the soil surface and rotary hoe in before planting) fluazinam treatments however, applied at three different rates reduced the incidence of powdery scab on cv. Shepody by up to 40% and severity by to 60% of the untreated control in two trials. The highest rate of the fungicide was no more effective in reducing disease than the lowest rate. These treatments had no effect on tuber yields but improved yields suitable for factory of up to 9 t/ha for the French fry category and up to 20 t/ha for the crisping category. The flusulfamide treatment only reduced disease at the highest rate tested, affecting disease severity but not incidence.

A broadcast formic acid soil treatment had no significant effect on powdery scab disease in two trials.

INTRODUCTION

Powdery scab is both a seed- and soil-borne disease. A number of studies conducted over the past four or more decades have demonstrated the efficacy of several chemical treatments, including fumigants, conventional fungicides and disinfectant chemicals, on powdery scab disease of potatoes (de Boer 2003; de Boer and Theodore 1997; Falloon et al. 1996; Wale et al. 2005). These treatments were generally applied to the soil before planting as in-furrow treatments or broadcast and incorporated into soil. It has been demonstrated that the efficacy of some fungicide treatments could be significantly improved by applying treatments at the start of tuber initiation, when developing tubers are most at risk of infection by *S. subterranea* (de Boer and Brown 1983), although this treatment is difficult to apply on a commercial crop. A number of studies have also demonstrated that chemical treatment (fungicides and disinfectant chemicals) of seed potatoes with symptoms of powdery scab can reduce the incidence and severity of the disease in daughter tubers (de Boer 2003; de Boer and Theodore 1997).

Studies conducted during the 1990's singled out the fungicide fluazinam, applied to soil as one of the more effective control measures for powdery scab (de Boer 2003; de Boer and Theodore 1997; Falloon et al. 1996). These treatments were applied in-furrow or broadcast and incorporated into the soil surface at, or prior to planting. The same fungicide also proved to be effective against a related pathogen, *Plasmodiophora brassicae,* the cause of clubroot of Brassica (Donald et al. 2001; Porter et al. 1998) and was registered as a seedling drench in Australia. Despite a good body of evidence on efficacy against powdery scab disease, this fungicide has not been registered as a soil treatment for powdery scab control in

Australia and New Zealand. In the United Kingdom the fungicide is registered for use only in seed potato crops.

Another compound, flusulphamide, also proved to be effective against powdery scab in trials conducted in New Zealand (Young 2008).However, trials in Australia with this fungicide had limited success (de Boer and Theodore 1997).

Both fluazinam and flusulfamide were trialled in this project in Victoria to provide additional data to support the possible registration of these fungicide as powdery scab control treatments in Australia. A number of seed potato treatments to reduce the carry-over of the powdery scab pathogen from seed to daughter tubers were also tested. Trials were conducted in the field and in the glasshouse. Fluazinam was also used in our nutrient amendment trials over five seasons as a reference control against various soil nutrient treatments.

FIELD TRIALS

Trials were conducted over four seasons near Ballarat on a property with a known history of powdery scab. Trials conducted in the first two seasons (2009/10, 2010/11) evaluated seed tuber treatments and in-furrow applications of fungicides on powdery scab disease and yield. Trials in the two following seasons (2011/12, 2012/13) evaluated broadcast, rather than in-furrow treatments, because of the lack of success with the in-furrow method of application.

MATERIALS AND METHODS

All trials were conducted on a potato grower's property at Ballarat with a history of powdery scab disease. Trials were arranged as randomised block designs replicated five times. Soil sampling, planting, harvests, disease and yield assessments were as described in the general Materials and methods section.

The two main fungicide products tested were Shirlan®Fungicide, *a.i.* 500 g/L fluazinam (Emblem®Fungicide, Crop Care Australasia Pty Ltd) and Nebijin®5SC, *a.i.* 50 g/L flusulfamide (Elliott Technologies Limited NZ) (Table 5.A.I-39). The 2009/2010 trial was planted with Russet Burbank and subsequent trials were planted the the susceptible cultivar Shepody.

Table 5.A.I-39 – Rates and method of application (seed-applied and in-furrow of the fungicides flusulfamide and fluazinam for the control powdery scab in two field trials at Ballarat 2009/2010 and 2010/2011.

Treatments	Rate of product
Untreated	Nil
Nebijin® Seed	4 mL/100 kg seed
Nebijin® 75	75mL/100m row in-furrow
Nebijin®150	150 mL/100m row in-furrow
Nebijin®300	300 mL/100m row in-furrow
Nebijin® Seed + Nebijin®75 IF	4 mL/100 kg seed & 75 mL/100m row in-furrow

Treatments	Rate of product
Nebijin® Seed + Nebijin®150 IF	4 mL/100 kg seed & 150ml/100m row in-furrow
Shirlan® IF	45mL/100m row in-furrow

RESULTS

BALLARAT 2009/10 FIELD TRIALS

Effects of the flusulfamide fungicide applied to seed tubers, in-furrow or combined, and a fluazinam fungicide applied in-furrow on powdery scab disease and yield on cv. Russet Burbank.

The effects of treatments on disease and yield are presented in (Table 5.A.I-40). At harvest, an average of 13% of the untreated Russet Burbank tubers developed powdery scab. None of the treatments significantly reduced the incidence and severity of the disease. Powdery scab root galls were evident on the roots of mature plants. However, none of the treatments had a significant effect on the severity of root galling. Total and marketable yield of the the untreated plots averaged 46 and 34 t/ha, respectively. None of the treatments significantly affected total, marketable yields or tuber specific gravity.

Table 5.A.I-40 – Effects of different rates of flusulfamide (Nebijin®5SC) applied as a seed tuber treatment, in-furrow (IF) treatment, or a combination of both, and fluazinam (Shirlan®) applied in-furrow, on powdery scab disease and yield of cv. Russet Burbank in a field trial, Ballarat 2009/2010.

Treatments	Rate	Powdery scab			Yield t/ha	
		Powdery scab root galling score (0-3)[A]	% tubers affected	Severity Index (0-6)	Total	Marketable
Untreated control	Nil	1	13	3	46	34
Nebijin®5SC Seed	4 mL/100 kg seed	2	14	2	45	31
Nebijin®5SC 75 IF	75mL/100m row	1	10	2	48	35
Nebijin®5SC 150 IF	150 mL/100m row	1	8	1	49	37
Nebijin®5SC 300 IF	300 mL/100m row	1	11	2	46	34
Nebijin®5SC Seed + Nebijin®5SC 150 IF	4 mL/100 kg seed + 150mL/100m row	1	13	2	45	32
Shirlan® Fungicide IF	45mL/100m row	1	12	2	49	36
lsd P=0.05		ns	ns	ns	ns	ns

[A] Severity of root galling: 0, no galls; 3 galls very abundant; ns, not statistically significant at P<0.05

2010/11 FIELD TRIALS BALLARAT

Effects of the flusulfamide fungicide applied to seed tubers, in-furrow or in combination, and a fluazinam fungicide applied in-furrow on powdery scab disease and yield on cv. Shepody.

In the 2009/10 season trial, fungicide treatments were applied to cv. Russet Burbank, the tubers of which have a relatively low susceptibility to powdery scab. The 2010/11 season trial was planted with the powdery scab susceptible cultivar Shepody.

The effects of treatments on powdery scab inoculum, disease and yield are presented in Table 5.A.I-41. The level of powdery scab inoculum in trial plots was uniformly high, averaging 142 pg DNA/g of soil (PreDicta Pt risk of "high"). None of the treatments signficantly affected plant emergence (data not presented) or the level of powdery scab DNA in soil at planting, tuber set or at harvest time. The incidence of powdery scab in the trial at harvest time was relatively high, with an average of 67% of tubers in the untreated control affected. However, none of the flusulfamide seed treatments, in furrow treatments or combined treatments had a significant (P>0.05) effect on the incidence and severity of powdery scab. Similarly, the in-furrow application of fluazinam did not reduce powdery scab incidence and severity.

The highest rate of flusulfamide applied in-furrow and the fluazinam in-furrow treatment reduced total yield by 8% and 11%, respectively, compared with the untreated control. However, these treatments did not significantly affect marketable yield. Specific gravity of tubers from the Nebijin® 75 mL/100 m row were less (P<0.05) than that of the untreated control.

Table 5.A.I-41 – Effects of different rates of flusulfamide (Nebijin®5SC) applied as a seed treatment, in-furrow (IF) treatment or a combination of both, and fluazinam applied in-furrow, on powdery scab disease and yield of cv. Shepody in a field trial, Ballarat 2009/2010.

	***S. subterranea* DNA (pg/g soil)**			**Powdery scab**		**Yield t/ha**		
Treatment	Planting	Tuber Set	Harvest	% tubers affected	Severity Index (%)	Total	Marketable	Specific Gravity
Untreated	124	106	1241	67	23	45	38	1.083
Nebijin® Seed	141	104	1166	75	24	45	40	1.080
Nebijin® 75 IF	154	121	1200	66	23	43	39	1.079*
Nebijin® 150 IF	124	91	521	53	15	44	39	1.084
Nebijin® 300 IF	144	123	954	67	23	41*	36	1.083
Nebijin® Seed + Nebijin® 75 IF	154	133	230	58	17	42	37	1.083
Nebijin® Seed + Nebijin® 150 IF	162	119	1396	70	24	43	38	1.082

	***S. subterranea* DNA (pg/g soil)**			**Powdery scab**		**Yield t/ha**		
Treatment	Planting	Tuber Set	Harvest	% tubers affected	Severity Index (%)	Total	Marketable	Specific Gravity
Shirlan® IF	129	113	493	62	21	40*	35	1.082
lsd P=0.05	ns	ns	ns	ns	ns	3.5	ns	0.003

*significantly different from the untreated control at P=0.05; ns, not statistically significant at P<0.05

BALLARAT 2011/12 FIELD TRIALS

Effects of the fungicides flusulphamide, fluazinam and the chemical formic acid soil applied as broadcast treatments on powdery scab disease and yield of cv. Shepody potatoes

The trial conducted in the previous season on the powdery scab susceptible cultivar Shepody failed to demonstrate efficacy of in-furrow treatments of fluazinam (Shirlan® Fungicide) and flusulfamide (Nebijin®5SC). The 2011/12 season trial evaluated the effects of broadcast treatments (fungicide drenched onto the soil surface followed by incorporation by rotary hoe) on powdery scab. An additional treatment was formic acid, a component of fish emulsion, which was shown to have efficacy against common scab disease when applied to soil (Abbasi et al. 2009).

The different treatments applied in this trial are presented in Table 5.A.I-42 as rates of product. The concentration of *S. subterranea* DNA prior to planting averaged 168 pg/g soil, a level equating to a high risk of powdery scab developing in a potato crop grown on that site based on PreDicta Pt testing.

Results are presented in Table 5.A.I-42. None of the treatments significantly affected the concentration of pathogen DNA at planting, tuber set or harvest. DNA concentration at harvest was an order of magnitude higher than at the other two sampling times.

At harvest, an average of 86% of tubers from the untreated control treatments were affected with powdery scab. Only the Shirlan® treatments (3, 6 and 9 L/ha) significantly reduced the incidence and severity (72%, 67% and 52% tubers affected and severity of 30%, 25% and 18%, respectively). Nebijin® applied at 9 L/ha reduced the severity of scab on the tubers surface from 49% in the untreated control to 37% but had no significant effect on the incidence of tubers with scab. No other Nebijin® treatment, nor the three formic acid treatments significantly affected the incidence and severity of powdery scab. The effect of the Shirlan® treatments is reflected in improvements in the yields at the factory. Shirlan® at 3, 6 and 9 L/ha resulted in improvements in French fry yields at the factory of 7, 9 and 11 t/ha, respectively. Similarly, the three rates of Shirlan® and the highest rate of Nebijin® improved the crisp yields at the factory by 10, 15 and 20 t/ha and 8/t/ha, respectively, compared with the untreated control.

Table 5.A.I-42 – Effects of different rates of fungicide treatments on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab and on potato yield (total and marketable in the field and marketable French fry and crisping at the factory) of cv. Shepody in a field trial, Ballarat 2011/2012.

Treatment	Rate L/ha	*S. subterranea* pg DNA/g soil			Powdery scab		Tuber yield (t/ha)		Marketable to factory (t/ha)	
		Planting	Tuber set	Harvest	% tubers affected	Severity Index (%)	Total	Market-able	French fry	Crisping
Untreated	0	139	99	5573	86	49	47	43	28	18
Shirlan®	3	193	106	4531	72*	30*	49	44	35*	28*
Shirlan®	6	164	106	2723	67*	25*	49	44	36*	33*
Shirlan®	9	166	76	1388	52*	18*	50	45	39*	38*
Nebijin®5SC	3	159	133	8549	87	51	47	43	27	18
Nebijin®5SC	6	158	103	2854	81	41	50	45	32	23
Nebijin®5SC	9	138	88	1958	80	37*	47	43	32	26*
Formic acid	3	171	104	6982	89	50	47	44	29	18
Formic acid	6	189	130	5782	87	47	48	44	30	20
Formic acid	9	202	120	6101	90	51	48	44	30	17
lsd P=0.05		189	130	5782	89	50	ns	ns	4	6

*Significantly different to the untreated control at P=0.05

BALLARAT 2012/13 FIELD TRIALS

Effects of the fungicides flusulphamide, fluazinam and the chemical formic acid soil applied as broadcast treatments on powdery scab disease and yield of potatoes

In the 11/12 season field trial, all rates (3, 6 & 9L/ha) of Shirlan® (fluazinam) significantly reduced the incidence and severity of powdery scab on harvested tubers. However, only the highest rate (9L/ha) of Nebijin® 5SC (flusulphamide) had any effect, reducing the severity of scab on tubers but not the incidence of tubers with scab.

A field trial was established in in the 2012/13 season to further evaluate the three control treatments at three different rates applied as broadcast treatments. The different treatments were Shirlan® (500 g/L fluazinam) at 3, 6, 9 L/ha of product, Nebijin®5SC (50g/L flusulphamide) at 3, 6, 9 L/ha of product and Formic acid at 3, 6, 9 L/ha of product. The concentration of *S. subterranea* DNA at the trial site averaged 99 pg/g soil prior to planting, a level equating to a high risk of powdery scab developing in a potato crop grown on that site, according to PreDicta Pt.

The results are presented in Table 5.A.I-43. None of the treatments significantly affected the pathogen DNA concentrations. At harvest, an average of 97% of tubers from the untreated control treatments were affected with powdery scab. Only the Shirlan® treatments (3, 6 and 9 L/ha) significantly reduced the incidence and severity of powdery scab (72%, 73% and 65% tubers affected and severity of 39%, 33% and 30%, respectively). Nebijin® applied at 3, 6 and 9 L/ha had no significant effect on the incidence of tubers with scab but reduced the severity of scab (49%, 43% and 49% compared with 64% in the untreated control). None of the formic acid treatments significantly reduced the incidence or severity of powdery scab.

Some of the Shirlan® treatments improved the marketable yields at the factory when both the disease incidence and severity was taken into account. Shirlan® at 3, 6 and 9 L/ha resulted in improvements in French fry yields at the factory of 5, 7 and 8 t/ha, respectively. Similarly, the three rates of Shirlan® improved the crisp yields at the factory by 13, 16 and 18 t/ha and the 6 and 9L/ha treatments of Nebijin® by 11 and 8 t/ha compared with the untreated control.

Table 5.A.I-43 – Effects of different rates of fungicide soil treatments on the concentration of *S. subterranea* DNA in soil, the incidence and severity of powdery scab and on potato yield (total and marketable in the field and marketable French fry and crisping at the factory) of cv. Shepody in a field trial, Ballarat 2012/2013.

Treatment	Rate L/ha	*S. subterranea* DNA pg/g soil			Powdery scab		Yield in field (t/ha)		Marketable to factory (t/ha)	
		Planting	Tuber Set	Harvest	% tubers affected	Severity Index (%)	Total	Marketable	French fry	Crisping
Untreated	-	89	59	30	97	64	48	45	26	10
Shirlan®	3	96	58	38	72*	39*	46	43	31*	23*
Shirlan®	6	86	58	27	73*	33*	46	42	33*	26*
Shirlan®	9	89	49	40	65*	30*	45	43	34*	28*
Nebijin®5SC	3	136	64	73	90	49*	50	44	29	16
Nebijin®5SC	6	95	60	35	81*	43*	41*	42	29	21*
Nebijin®5SC	9	87	47	40	92	49*	45	42	28	18*
Formic acid	3	101	62	39	94	64	52	49	27	11
Formic acid	6	104	56	49	95	60	48	47	29	12
Formic acid	9	103	58	46	96	60	48	46	27	12
lsd P=0.05		ns	ns	ns	11	14	6	ns	4	7

*Significantly different to the untreated control at *P*=0.05; ns, no significant treatment effects

GLASSHOUSE TRIALS

EFFECTS OF DIFFERENT FUNGICIDE AND CHEMICAL TREATMENTS ON SEEDBORNE POWDERY SCAB

Effect of formic acid as a seed treatment on powdery scab

In APRP1 we demonstrated that formic acid as a seed dip was effective at reducing the tuber-borne Rhizoctonia disease. Therefore, a replicated glasshouse trial was established to investigate the effectiveness of formic acid as a seed dip to reduce seed-borne powdery scab. The treatments included 5 concentrations of formic acid: 0, 0.5, 1, 2 and 4 % (v/v) and 3 time intervals for seed dipping: 5, 10 and 15 minutes. These treatments were applied to two different lots of cv. Shepody tubers, one, with low and one with high powdery scab symptoms. Seed tubers were planted in pots (one per pot) in a glasshouse. Sprout emergence was monitored and after plant senescence tubers were harvested and assessed for the incidence and severity of powdery scab and for yield.

Results showed that seed tuber sprouting was reduced by the high level of inoculum on seed and with increasing concentrations of formic acid. Formic acid concentrations of 1, 2 and 4% significantly reduced powdery scab symptoms regardless of the severity of initial scab symptoms on the seed tubers. Increasing the time of dipping significantly reduced powdery scab symptoms. Dipping for 15 mins in 4% formic acid significantly reduced tuber yield due to a phytotoxic effect of the chemical reducing the number of viable sprouts.

Nebijin®5SC seed dip to reduce the effect of seedborne powdery scab

In New Zealand, Nebijin® (flusulfamide) effectively controls powdery scab when used as a seed dip. Shirlan® (fluazinam) controlled powdery scab when applied as an in-furrow treatment in previous Victorian field trials. A replicated glasshouse trial was therefore established to investigate the effect of Nebijin® and Shirlan® as seed dips for the management of powdery scab. The following treatments were applied: untreated, Shirlan® 1%, Shirlan® 2%, Nebijin® 1.25%, Nebijin® 2.5%, Nebijin® 5% and Nebijin® 10%. Two weeks after seed dipping the tubers were assessed for the number of open eyes and potted into potting mix for symptom development. All plants emerged two weeks after potting up. During tuber initiation pots were watered with chilled water to enhance infection. At harvest tubers were assessed for the incidence and severity of powdery scab.

Results showed that Nebijin® as a seed dip at the different rates tested significantly reduced the incidence of powdery scab in the progeny tubers compared with the untreated control (Figure 5.A.I-29). The lowest rate was no less effective than the highest rate. The Nebijin® treatments were more effective than Shirlan® treatments. None of the treatments proved to be phytotoxic to the emerging sprouts.

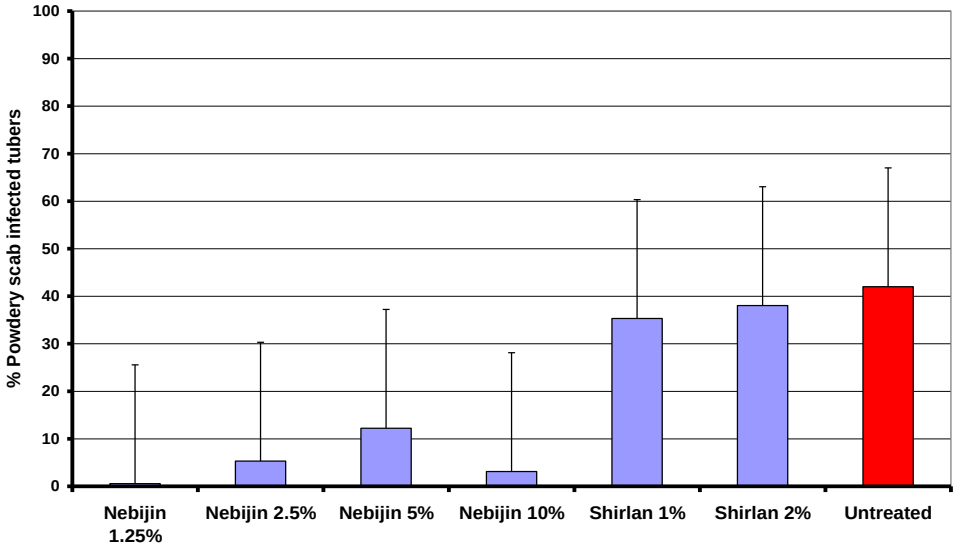


Figure 5.A.I-29 – Effects of the fungicides Nebijin® (flusulphamide) and Shirlan® (fluazinam) as dip treatments on seed-tubers affected with powdery scab on the resulting incidence of powdery scab in the progeny tubers

DISCUSSION

The efficacy of the fungicides fluazinam and flusulfamide to powdery scab disease on tubers of the susceptible cultivar Shepody has been demonstrated in the field trials at Ballarat confirming results of others (de Boer and Theodore 1997; de Boer 2003; Falloon et al. 1996; Wale et al. 2005). However, these fungicides were ineffective when applied in-furrow, even at the highest rates. The broadcast application proved to be the better option. A broadcast treatment is likely to result in a more even distribution of the fungicide in the soil profile, which ensures that there is fungicide present in the zone where tubers are initiated. The in-furrow treatment concentrates the fungicide in a band around the seed piece, with the result that the concentration in soil around the initiating tubers may be relatively low. The highest rates of both fluazinam and flusulfamide applied in-furrow resulted in small but significant reductions in total tuber yields.

Fluazinam, applied as a broadcast treatment was more generally more efficacious than a broadcast flusulfamide treatment, the former reducing both the incidence and severity of scab on tubers. However, the fungicide had a greater effect in reducing disease severity than incidence. A dose response was also apparent. Wale et al. (2005) reported similar results for field trials in the United Kingdom. They also reported that flusulfamide treatments reduced disease severity, but not incidence. In our trials, flusulfamide only had a significant effect on powdery scab at the highest rate tested and then only on disease severity.

There was no evidence of efficacy of formic acid against powdery scab in these trials, despite evidence of efficacy against common scab in trials conducted in Canada. Formic acid would act as a disinfectant treatment, having a direct toxic effect on the pathogen in the soil. The relatively resistant sporosori of *S.*

subterranea may not have been susceptible to formic acid at the concentrations applied to the soil in these trials. It is likely that germinating zoospores would be susceptible, but at significantly higher concentrations of the chemical than the residual left in the soil after application.

Although there is evidence that both fluazinam and flusulfamide can be efficacious as seed tuber treatments for control of tuber-borne scab, the very high soil inoculum levels soil in the trials would mask any effect of the seed treatments on the carry-over of disease to the progeny tubers. Certified grade seed potatoes were planted, which meet the very stringent standard of no more than 2% of the consignment with any visible scab (less than 1% of tuber surface affected), so tuber inoculum levels are relatively low compared that in the soil surrounding initiating tubers.

Fluazinam broadcast treatments were applied at rates of 3, 6 and 9 L/ha of product (Shirlan®). This fungicide has been granted a UK specific off-label approval in for use in seed potato production only at a rate of 3 L/ha (1.5 L fluazinam/ha). It can only be applied as a tractor mounted downward directed drench prior to bed tilling. If registration for powdery scab control is ever granted in Australia, the fungicide is not likely to be registered at rate higher than 3 L/ha. Our results showed that, although there was some improvement in efficacy at the higher rates, the returns to the factory did not differ significantly between the different rates.

The glasshouse trials demonstrated the efficacy of formic acid, which has a disinfectant mode of action, and flusulphamide, a protectant fungicide, on seed-borne powdery scab. The fungicide fluazinam was less effective than flusulfamide at the rates tested. The former was registered as a seed treatment in New Zealand but proved to be phytotoxic at the recommended rates and registration has been withdrawn. Our results suggest that flusulfamide may be better suited as a seed-tuber treatment for seed-borne powdery scab than as a soil treatment for soil-borne powdery scab.

COMMON SCAB DISEASE

GENERAL INTRODUCTION

Common scab of potato has been reported wherever potatoes are grown, including wet and dry soils, and in soils ranging from pH 4.0 to above 9.0 (Doering-Saad et al. 1992); (Goyer et al. 1996); (Lambert and Loria 1989); (Lindholm et al. 1997); (Park et al. 2003). The occurrence of common scab is largely determined by variety susceptibility, locality, and/or isolate virulence of pathogen species (Loria et al. 1997).

Common scab is caused by several species of actinomycetes belonging to the genus *Streptomyces*. The disease symptoms are lesions on the surface of the mature tuber and these can vary widely, being raised, netted, shallow or deep-pitted. Common scab is the result of host response to infection by the production of suberised cork layer at the site of infection that limits further penetration of the pathogen (Loria et al. 1997).

Pathogenic *Streptomyces* can be soil- or tuber-borne and is ubiquitous in potato production soils. In Australia, common scab is generally considered a disease of warm and dry seasons. The optimum temperature for pathogen growth is 30°C, and it is commonly reported that increased potato scab severity occurs during growing seasons with higher than normal temperatures. Infection of potato tubers is primarily through immature lenticels, therefore tubers are most susceptible to infection during the six week period of rapid tuber growth that commences when the tuber diameter is twice that of the stolon.

Streptomycetes species are abundant in soils, but only a small percentage of these cause common scab and they all carry genes for biosynthesis of a phytotoxin known as thaxtomin A(Loria et al. 2006). The txtA synthase gene is required for thaxtomin A biosynthesis and for pathogenicity (Healy et al. 2000). Thaxtomins inhibit cellulose biosynthesis and induce scab symptoms through necrosis and plant cell hypertrophy (King et al. 1992); (Healy et al. 2000); (Scheible et al. 2003). Genes for biosynthesis of thaxtomin are carried on a distinct chromosomal region with the hallmarks of a pathogenicity island (PAI) that can be transferred horizontally among *Streptomycetes* (Loria et al. 2006). Since the mechanism of pathogenicity based on thaxtomin seems to be conserved, the biological basis for variation in aggressiveness is assumed to be differences in the production of thaxtomin, and genetically distinct *Streptomyces* isolates differing in thaxtomin production *in vitro* have been described (Beausejour et al. 1999); (Kinkel et al. 1998); (Healy et al. 2000), (Loria et al. 1995).

Traditional management strategies have focused on host resistance, chemical control, maintaining soil moisture during and after tuber initiation, and maintaining acid soil pH (Lambert and Loria 1989); (Powelson and Rowe 1993), but these strategies are inadequate for controlling scab in the varied environments in which it occurs.

This section develops a number of different approaches to common scab control. They include:

- Manipulating soil conditions through the use of a millet green manure crop – the completion of the final two years of a four-year field study in Canada
- Determining differences in the soil microbial communities from Victorian fields that have common scab or are free of common scab to identify a ‘fingerprint’ for common scab suppressive soils

- Manipulating concentrations of nutrients, including various forms of soil pH modifiers (lime), different forms of sulphur, the potassium to magnesium ratio, manganese sulphate and ammonium sulphate. In-vitro studies investigated the direct effects of individual elements on expression of the pathogenicity gene in the common scab pathogen
- Evaluation of micro-organisms associated with the potato plant identified during PT07038 for disease management and improved potato productivity

MODIFYING THE SOIL ENVIRONMENT FOR IMPROVED SOIL HEALTH – EVALUATION OF DIFFERENT METHODS OF MANAGING A MILLET GREEN MANURE CROP FOR DISEASE CONTROL AND IMPROVED POTATO PRODUCTIVITY

Soil health refers to the biological, chemical and physical features of a soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. A main objective of the work done at A & L Biologicals (Canada) is to identify what comprises a “healthy soil” and to develop technologies to identify a “healthy soil”. The ecology of soil is one of the most complex on the planet and the area of research has been vastly overlooked since the publication by (Waksman 1927) and (Waksman and Starkey 1931) of the books *Principles of soil Microbiology*, and *Soil and the Microbe*, respectively. Plants in nature can generate enormous volumes of biomass without extraneous fertilizers. This is because virtually all land plants have evolved intimate associations with microbes that inhabit their roots and that provide them with the nutrients they need to complete their life cycle. Yet the scientific community knows little about how these systems can be used for crop production.

SUMMARY

The results of the final two years of a four-year millet-potato rotation trial at two different sites in Canada are presented here. The millet crop served as a green manure treated in 4 different ways (incorporated into soil either cut or uncut or trash left on the surface) compared with continuously cropped potatoes or a wheat-potato rotation. The trials showed that millet green manure treatments increased total and marketable yields in the order of 20-40%, although this depended on the site and the year. Generally, these treatments did not significantly reduce the incidence of plants with Verticillium wilt. The effect of treatments on common scab disease varied, with some treatments reducing and others increasing disease severity.

This research suggests that green manure treatments can be cost effective delivering significant returns to growers due to the significant yield increases achieved in the trial.

INTRODUCTION

The role of green manure crops in potato production

As environmental awareness increases there is a need for a drastic reduction in pesticide use while keeping crop pest and disease damage under control. One alternative is the implementation of agroecological practices such as biological control, intercropping, resource and biodiversity conservation practices, cover crops and green manures (Wezel et al. 2013).

Green manures refers to crops grown for a specific period of time, and incorporated into the soil while green for the purpose of soil improvement (Kumar et al. 2013). Green manures can improve chemical properties of a soil by increasing nutrient and organic matter levels, soil physical characteristics such as soil structure, water infiltration rate, water holding capacity, and aeration, and the biological characteristics of a soil, such as nitrogen production, microbial activity, and biodiversity (Abawi and Widmer 2000; Kumar et al. 2013; Sullivan 2003). Pathogenic fungi and bacteria, nematodes, and weeds can all be reduced by using a green manure crop.

Potato crops can suffer more than 40 pests and diseases caused by insects, nematodes, viruses, bacteria, and fungi (Fiers et al. 2012). The relatively shallow root system of potatoes requires significant nutrient inputs to maintain tuber productivity and quality (Tein et al. 2014). The changes provided by green manures in the soil's biology offer the short term economic incentive to use green manure crops in potato cropping systems, especially for nitrogen production and soil-borne disease management.

There are hundreds of reports on the use of green manures to control soil-borne diseases in potato production systems. In 2010, (Davis et al. 2010) reported a 60% to 70% suppression of Verticillium wilt and a 22% yield increase by the use of corn as a green manure for 2 or 3 seasons. This occurred even though the inoculum levels of *V. dahliae* and other fungi were maintained or increased (Davis et al. 2010). The same group had previously studied the used of different green manures (sudangrass, Austrian winter pea, rape, rye, oat and corn) to control Verticillium wilt in potato. Their results showed that sudangrass and corn were the best treatments for controlling Verticillium wilt and increasing potato yields (Davis et al. 1996).

In 2011, (Larkin et al. 2011b) reported the use of green manures (mustard blend and sorghum-sudangrass) to manage Verticillium wilt on potato over 3 growing seasons. Green manures significantly reduced (by 18-25%) Verticillium wilt during the first season, with the mustard blend also reducing black scurf and common scab and increasing tuber yield by 12%. However, by the second season, disease levels were high in all rotations, indicating that results with green manures can vary from season to season and from site to site. The same group compared the effects of different potato cropping systems on soilborne disease and soil microbiology communities over 3 potato seasons. Their results showed that the use of a disease suppressive cropping system (*Brassica* and sudangrass green manure crops, fall cover crops, and high crop diversity) resulted in the greatest reductions in stem and stolon canker, (20-30%), black scurf (18-58%), and common scab (25-40%) relative to the other rotations. This cropping system also produced significant shifts in soil microbial community characteristics, different from all other rotations (Larkin et al. 2011a).

Green manure cropping offers the opportunity to change soil conditions, potentially improving soil physical, chemical and biological characteristics. However, much research needs to be done before we can effectively manage a green manure crop to our own ends. This section presents the results of the final two years of a four year trial on the effects of four different methods of managing a millet green manure on disease and yield.

Effects of different treatments of a millet green manure crop on disease and yield of potato

These experiments were initiated in 2007 before the start of APRP2 to examine effect of various green manure soil treatments on potato yield, common scab severity and Verticillium wilt incidence. These experiments were undertaken as a result of numerous requests from growers as to how they could minimize disease problems using the lowest possible cost processes. The results for the early years have been reported previously (Crump 2010).

MATERIALS AND METHODS

Two sites with histories of both common scab and Verticillium wilt were selected for use in field experiments. One of the locations was the Agriculture and Agrifood site at Delhi whereas the other was a commercial field, the Blizman site. At both locations, whole seed of cultivar Yukon Gold purchased from a seed grower was planted. This variety is highly susceptible to both common scab and Verticillium wilt. Commercial rates of fertilizers and chemical treatments were used during the growing season.

Duplicate field plots were established at both locations with rotation crops starting in 2007 on half of the field plot area and in 2008 on the other half. With this field plot design, starting in 2008, data has been collected on both rotation crop treatments and on the subsequent potato crop each year for 3 years.

Both soils are luvisols. The Delhi soil is more sandy than the Blizman soil, but both are considered to be a sandy loam. The Delhi soil had an organic matter content ranging from 1.2-1.6 and a pH of 6.1 - 6.9. The Blizman soil organic matter ranged from 1.8 -3.2 and the pH 6.8 - 7.4.

Green manure treatments applied from 2007 to 2011:

Control #1: Continuous Potato Control (continuous cropping to Yukon Gold potatoes)

Control #2: Wheat-Potato Control (Winter Wheat-Potato rotation)

Millet-Potato Rotation #1: Millet-Multi-seed

- Millet seeded in 18 cm rows in late May of rotation crop year
- Late July: millet worked down, ploughed under, seedbed prepared, and re-seeded to millet
- Mid-September: millet worked down, ploughed under, seedbed prepared and reseeded to oats as a winter cover
- Disc oat cover in Spring and plant potatoes (last week of May-first week of June)

Millet-Potato Rotation #2: Millet-Strip Spread

- Millet seeded in 75 cm rows in late May of rotation crop year
- When millet about 1.5 m tall, cut with forage harvester dropping the chopped material between the rows and leaving a 30 cm stubble for re-growth
- Incorporate the cut forage into the soil between the rows with a strip tiller to a depth of 15 cm
- Repeat above procedure as the crop develops
- Make the last millet cut in late September as low as possible
- Disc tops lightly, leaving sufficient surface trash to prevent winter wind erosion
- Plough in Spring and then plant potatoes (last week of May-first week of June)

Millet-Potato Rotation #3 - Millet-Multi-cut

- Millet seeded in 75 cm rows in late May of rotation crop year
- When millet about 1.5 m tall, cut with flail mower dropping cut material where it falls and leaving a 30 cm stubble for re-growth

- Repeat above procedures as the crop develops
- Make the last millet cut in late September as low as possible
- Disc tops lightly, leaving sufficient surface trash to prevent winter wind erosion
- Plough in spring, and plant potatoes (last week of May-first week of June)

Millet-Potato Rotation #4 – Millet-Single mow

- Millet seeded in 75 cm rows in late May (Spring) of rotation crop year
- In early August (Winter), when millet about 1.8 m tall, cut down with flail mower, disc the stubble, plough under, and prepare a seedbed
- In mid-September (Autumn), seed oats as a winter cover
- Disc oat cover in Spring and plant potatoes (last week of May-first week of June)

Collection and isolation of *Verticillium dahliae* from petioles

Twenty-five petioles were collected from each plot in early August. The petioles were from one leaf collected per plant from the third or fourth leaf from the top of plants in the middle two rows. Petioles were surface sterilized and two thin sections were cut from each petiole and plated on Soil Pectate Tergitol Agar (SPT) (Hawke and Lazarovits 1994), which is semi-selective for *Verticillium* species. The petioles were incubated for 10 days at 24°C in an incubator and the presence of *Verticillium* colonies emerging from either petiole cutting was considered an indication of infection.

Tuber yield and common scab severity

The middle two rows were harvested from each plot in mid-September with a one row digger. The tubers were washed and graded over a chain for sorting marketable from unmarketable. Tubers less than 1.25 inches (3 cm) in diameter were not marketable. The weights of the marketable and unmarketable tubers were determined. One hundred tubers were randomly selected from each plot and each tuber was assessed on a 1-6 scale for scab area coverage of the skin with 0 = no scab, 1 = trace to 5%, 2 = 6-15%, 3 = 16-25%, 4= 26-35%, 5 = 36- 60%, 6 = 61-100%. In general, tubers with greater than a rating of 1 are not marketable.

RESULTS

The 3rd year of the field trial (2010)

Incidence of Verticillium dahliae recovered from petioles

The incidence of *V. dahliae* recovered from petioles was generally higher at the Delhi field than at the Blizman field (Table 5.A.I-44). The different methods of soil incorporation of millet did not significantly reduce *V. dahliae* incidence when compared to the control. In both fields, the lowest observed incidence of *Verticillium* was when millet was incorporated as multi-seed and the highest observed incidence in millet multicut treatment.

Common scab severity

Common scab disease pressure was higher in the Delhi field than in Blizman as indicated by the higher disease severity rating (Table 5.A.I-44). A significantly higher scab severity was found in all millet-incorporated plots compared with the control plot, or the wheat-incorporated plots in both fields, with the exception of the millet multi-seeded treatment in the Delhi field.

Total tuber yields

For the 2010 harvest, in both fields, incorporation of millet increased potato tuber yield between 20% and 47% compared to the continuous cultivation of potato (Table 5.A.I-44). The highest total yield in both fields was found in millet multi-seeded treatment followed by millet strip spread treatment in Delhi and single-mowed treatment in the Blizman field.

Table 5.A.I-44 – Effects of millet green manure treatments on incidence of *V. dahliae* in petioles, severity of common scab and the change in marketable yield of cv. Yukon Gold in field trials at the Blizman and Delhi sites, 2010 season.

Treatments	***V.dahliae* in petioles** (% Incidence)		**Common Scab Rating (0-6)**		**Marketable Potato yields** (% of Control 1)	
	Blizman Site	Delhi Site	Blizman Site	Delhi Site	Blizman Site	Delhi Site
Continuous Potato Control (1)	17	35	2.2	3.2	0	0
Wheat Potato Control (2)	9	34	2.0	3.2	+10	-5.0
Millet multi seed	4	26	2.9*	3.2	+42*	+48*
Millet strip spread	10	38	2.9*	4.1*	+30	+45*
Millet multi cut	18	41	2.9*	4.0*	+20	+35*
Millet single mow	11	34	2.9*	5.0*	+40*	+27*

* Indicates a significant difference from 'Continuous Potato Control (1)' using Duncan's Multiple Range Test for potato yield data and a significant difference from both 'Control (1)' and 'Control (2)' for common scab disease rating. This rating is based on a 0-6 scale with '0' being free of scab.

The 4th year of the field trial (2011)

Incidence of Verticillium dahliae recovered from petioles

The incidence of petioles with *V. dahliae* overall was lower at Blizman compared to the Delhi field (Table 5.A.I-45). For both fields, there was a trend of a lower incidence of the pathogen in the millet-incorporated plots compared with the control plots. In the Delhi plots, the incidence of *V. dahliae* was significantly less in the millet single mow treatments compared with the controls. At the Blizman site, the incidence of *V. dahliae* was less in the millet strip spread and the millet single mow treatments than in the continuous potato. The reduced levels of *Verticillium* resulted in the plants living much longer in the green manure-treated plots.

Yield and common scab assessments

At the Blizman site, millet multi-cut significantly improved marketable yields (Table 5.A.I-45). All treatments improved yields compared to the continuous potato control. Scab pressure at this site was low, so the impact of the green manure treatments was slight.

At the Delhi site, all millet treatments improved marketable yields compared to both control treatments (Table 5.A.I-45). Millet multi cut and millet strip spread treatments showed the greatest increases in yields. Scab pressure was greater at this site. Millet multi seed and millet strip spread both significantly reduced scab compared to the control, while millet multi cut treatment significantly increased scab incidence on the tubers.

Table 5.A.I-45 – Effects of millet green manure treatments on incidence of *V. dahliae* in petioles, severity of common scab and the change in marketable yield of cv. Yukon Gold in field trials at the Blizman and Delhi sites, 2011 season.

Treatments	***V.dahliae* in petioles (% Incidence)**		**Common Scab Rating** (0-6)		**Marketable Potato yields** (% of Control 1)	
	Blizman Site	Delhi Site	Blizman Site	Delhi Site	Blizman Site	Delhi Site
Continuous Potato Control (1)	23	36	0.8	1.7	0	0
Wheat Potato Control (2)	21	43	0.5	1.9	+25	+8.0
Millet multi seed	15	20	0.3*	1.4*	+17	+45*
Millet strip spread	4*	20	0.5	0.8*	+28	+51*
Millet multi cut	20	21	0.4*	2.4*	+42*	+47*
Millet single mow	8*	13	0.4*	1.7	+32	+30*

* Indicates a significant difference from 'Continuous Potato Control (1)' using Duncan's Multiple Range Test for potato yield data and a significant difference from both 'Control (1)' and 'Control (2)' for common scab disease rating. This rating is based on a 0-6 scale with '0' being free of scab.

DISCUSSION

Our results of a four-year millet-potato rotation trial provided data that is consistent with what has been described in most previous studies. Although results depended on the site and the year, millet green manure treatments significantly increased total and marketable yields by between 20-47%. The increased yields were achieved without reducing the incidence of Verticillium wilt. In one year at one site, the four millet treatments improved yields. However, one treatment had no effect on scab severity, two reduced scab and the fourth increased scab, highlighting the complexity of soil environment. It is clear that

utilization of such a green manure crop can provide economically beneficial results but the potential to enhance sustainability of potato cropping systems through improved soil health has to be evaluated for each specific site.

Variations in the results between sites, years and type of green manure used, point out the necessity of a greater research effort required to understand the mechanisms of action of green manures to determine why and how these materials influence plant growth, productivity and control disease. The biggest limitation for the use of green manures, particularly in commercial production, is that they take land out of production. While the costs involved in growing the green manures is very inexpensive, we need to better understand the benefits and potential alternative uses of these crop to offset these costs. There needs to be a more intense effort to establish which crops are best for controlling specific diseases, for specific soil types and climate conditions, how to manage these crops for the most effective and economical results, and how to best implement these crops into a potato production system.

A comprehensive study by Larkin and Halloran (2014) evaluated disease-suppressive crops (mustards, rapeseed and sudangrass) in potato rotation field trials, and their effects on disease, yield, and economic viability. Using four different types of production management (as a cover crop, green manure, harvested crop-residue incorporated, and harvested crop-residue not incorporated) they showed that all rotation crops managed as green manures produced lower disease (by 15–26%) and higher yields (by 6–13%) than other management practices. However, the best economic return was obtained when mustard or rapeseed were grown as a harvestable cash crop and then the residues incorporated, suggesting this as a good management approach for using disease-suppressive crops in potato production (Larkin and Halloran 2014).

Different factors have been associated with increasing tuber yields, including increasing total soil carbon and nitrogen, as well as providing organic matter inputs which improve soil structure and water-holding capacity (Stenberg 1998). Green manures can affect all of these soil properties, including changes in microbial community composition.

The majority of the studies aiming to control disease and increase potato yields have focused on single strategies. Based on our results, we think it is critical to conduct studies to assess the effects of multiple management approaches, not only as individual treatments, but their combinations, on soilborne diseases and potato yields. A good starting point will be to study the use of green manure rotation crops and biological control organisms (e.g. Pseudomonas spp. tested by Basahi, M.Sc. Thesis).

One of the most exciting aspects of green manures is that they offer a means of revitalizing soil microbial activities and providing plants with a means to buffer against pathogens, as well as a reservoir of macro- and micronutrients. In order to better utilise green manures we need to:

- develop better tools to unravel the multitude of mechanisms impacting on the soil physical, chemical and biological environment and their interaction (identify what is being added to soil, how it decomposes and how this impacts on the soil environment);
- what mechanisms drive potential suppressive effects on pathogens and disease (gaining a better understanding of the underlying mechanism of organic-matter mediated disease suppression is a prerequisite for the development of practices that more consistently achieve the desired disease suppression);

- develop tools to understand the microbial ecology processes involved in suppression. This knowledge would provide directions for possible manipulations of the community leading to a reproducible suppressive amendment.

INVESTIGATING A POTENTIALLY COMMON SCAB-SUPPRESSIVE SOIL FROM VICTORIA

SUMMARY

Soil suspected of being suppressive to common scab was collected from a potato grower's field, near Ballarat in Victoria, Australia. The suppressive properties of the soil was shown to be eliminated by exposure of the soil to gamma (γ)-radiation or to heat treatment, indicating that the mechanisms are associated with living organisms, not chemical components. In order to identify which microbes may be involved, we used the molecular technique (terminal restriction fragment length polymorphism, TRFLP) to determine if there is a correlation between bacterial or fungal communities and the suppressive nature of the soil. This study was able to show that significant differences in bacterial and fungal populations can be discerned in this soil after treatment with gamma irradiation and significant differences in fungal populations exist between gamma-irradiated and heat-treated soil. Plants grown in gamma-irradiated soil and heat treated soil were found to have dramatically different soil microbial populations compared to the untreated controls. The implications are that heat treatment and gamma irradiation significantly alter bacterial and fungal populations in this soil and the changes in resulting rhizosphere communities can be related to increased scab incidence. The techniques used can narrow down which group of organisms may be involved. When we examined the TRFLP data for major signals that are reduced in both bulk soil and rhizosphere soil none of the peaks were identified as a probable bio-control strains, although other smaller peaks putatively represent bacteria like *Pseudomonas fluorescens*, *Stenotrophomonas maltophilia* and *Lysobacter antibioticus*, which are known to be useful in the biocontrol of soil diseases. Further work cloning and sequencing these fragments will allow more accurate identification of these microbes and focus our efforts on culture-based isolation of these bacteria for further evaluation on their role in scab disease suppression.

INTRODUCTION

Common scab suppressive soil was first described by (Menzies 1959). Suppressive soil has a low level of disease in spite of the presence of the pathogen, a susceptible host and conditions favourable for disease expression. There have been several reports over the last 20 years of soils specifically suppressive against common scab, (Faucher et al. 1992; Keinath and Loria 1989b; Tashiro et al. 1990). Developing disease suppressive soils for common scab represents the most ideal means to manage the disease and it has become a highly desirable goal. If we identify the mechanisms then we could design production systems that bring about this phenomenon.

We have identified a farm where the incidence of common scab has been negligible over many decades. DNA of *S. scabies* has not been detectable in soil tests taken over several years, and there is no evidence of soil nutrient concentrations that could suppress common scab. We were interested in understanding why this farm does not grow common scab. To prove biological suppression the soil was treated three ways, left natural (maintain biological activity), heat treated to 80°C (only spore forming bacteria remain) and gamma irradiated (remove biological activity).

MATERIALS AND METHODS

SOIL COLLECTION & PREPARATION

Five hundred kilograms of soil (10–30 cm depth) was removed and stored at 4°C until ready to be used for pot trial experiments. Before treatment, the soil was sieved to remove particles less than 10 mm in size. The treatments for this glasshouse pot trial were: untreated/native soil; heat-treated soil (80°C dry heat, 48 hrs., HT) and gamma-irradiated (γ)-soil (25 kGrays).

INOCULATION OF SOILS

A positive inoculum/infection control was used in the experiment to confirm tuber symptoms and plant growth. A pathogenic *S. scabies* isolate (S546) was grown on yeast-malt extract (YME) plates for 7 days at room temperature before the agar plate was divided into 3 cm squares and inoculated into sterile vermiculite in 1L tubs. These tubs of vermiculite were then incubated in the dark for at least one month before use. The control plants were grown in a pine bark potting mix (Debco®). All treatments also had an uninoculated control consisting of sterile vermiculite only with all treatments inoculated with 10% v/w of either inoculated or untreated vermiculite.

After inoculation, a portion of each soil (and potting mix) was sent to Canada for nutrient analysis and TRFLP analysis to determine if there were any differences in the microbial populations in the treatments. Samples were also sent to SARDI to determine the concentration of pathogenic *Streptomyces* in the pots prior to planting.

PLANTING, MAINTENANCE, HARVESTING & DISEASE ASSESSMENT

Tissue culture plants of a common scab-susceptible cultivar, Shepody, were planted at one per pot two weeks after inoculation with the pathogenic *S. scabies* S546. Pots were watered twice per day with individual drippers and were supplemented with Aquasol™ (Yates Australia) once per week once the plants had become established. The trial ran for 16 weeks.

Tubers were harvested after plant senescence with any plant material left removed prior to harvesting. The assessments included tuber number per pot, total weight and each tuber was scored for common scab (incidence, severity) according to the standard scale. Portions of soil from all pots were sent to both SARDI (pathogenic *Streptomyces* DNA levels) and A&L Canada (TRFLP analysis).

TRFLP ANALYSIS

We wanted to profile microbes in soil before (bulk soil) and after a bioassay experiment (Rhizosphere soil). For a bioassay experiment, 10 g of dry soil was added to glass culture tubes with 4 mL of water and Murashige-Skoog basal salt mixture (for micronutrients). Tissue-cultured potato cuttings were then planted in these substrates. Four plants were grown in each treated soil for one month. Excess soil was removed from the roots by shaking and soil that remained adhering to it was considered as rhizosphere soil. This was removed by washing the roots and collecting the soil by centrifugation of the muddy water. DNA was extracted from this soil using a Norgen soil DNA extraction kit from two separate trials. DNA was also extracted from the original bulk soil samples received. PCR was performed on the bacterial 16S rDNA gene using primers 63f and 1389r, while fungal PCR was performed using the primers ITS1F and ITS4. Each

primer had a different fluorescent dye label. All PCR products were digested with the enzyme *Hha*I. Fragment analysis was conducted at the University of Guelph, Guelph, Ontario.

RESULTS

Results for the first trial are presented in Table 5.A.I-46 and initial pathogenic *Streptomyces* DNA levels ranged from 46998–89708 pg/g of soil, far in excess of levels found in the field to be conducive for common scab disease. Nutrient analysis found there were significant differences in N-NO_3 (HT & γ, decrease), N-NH_4 (γ, increase) and manganese (HT & γ, increase) compared to the untreated field soil. DNA levels of the three soil treatments at harvest ranged from 679–1282 pg/g of soil, with no significant difference in DNA levels between treatments. However, average common scab DNA levels in the inoculated potting mix at harvest were far higher (19158 pg/g of soil).

Table 5.A.I-46 – Incidence and severity of common scab on tubers and the concentration of pathogenic *Streptomyces* DNA at planting and harvest from treated and untreated soils inoculated with pathogenic *Streptomyces*.

Treatment	Common Scab		Pathogenic *Streptomyces* DNA pg/g soil	
	% tubers affected	Severity	Planting	Harvest
Natural/untreated	0	0	89708	679
Heat-treated	18*	11	98739	886
Gamma (γ)-irradiated	30*	14*	46998*	1282
Potting media (+ve control)	72*	53*	49206*	19158*
lsd (*P*=0.05)	16	12	24663	6673

* Significantly different to the natural soil at P>0.05

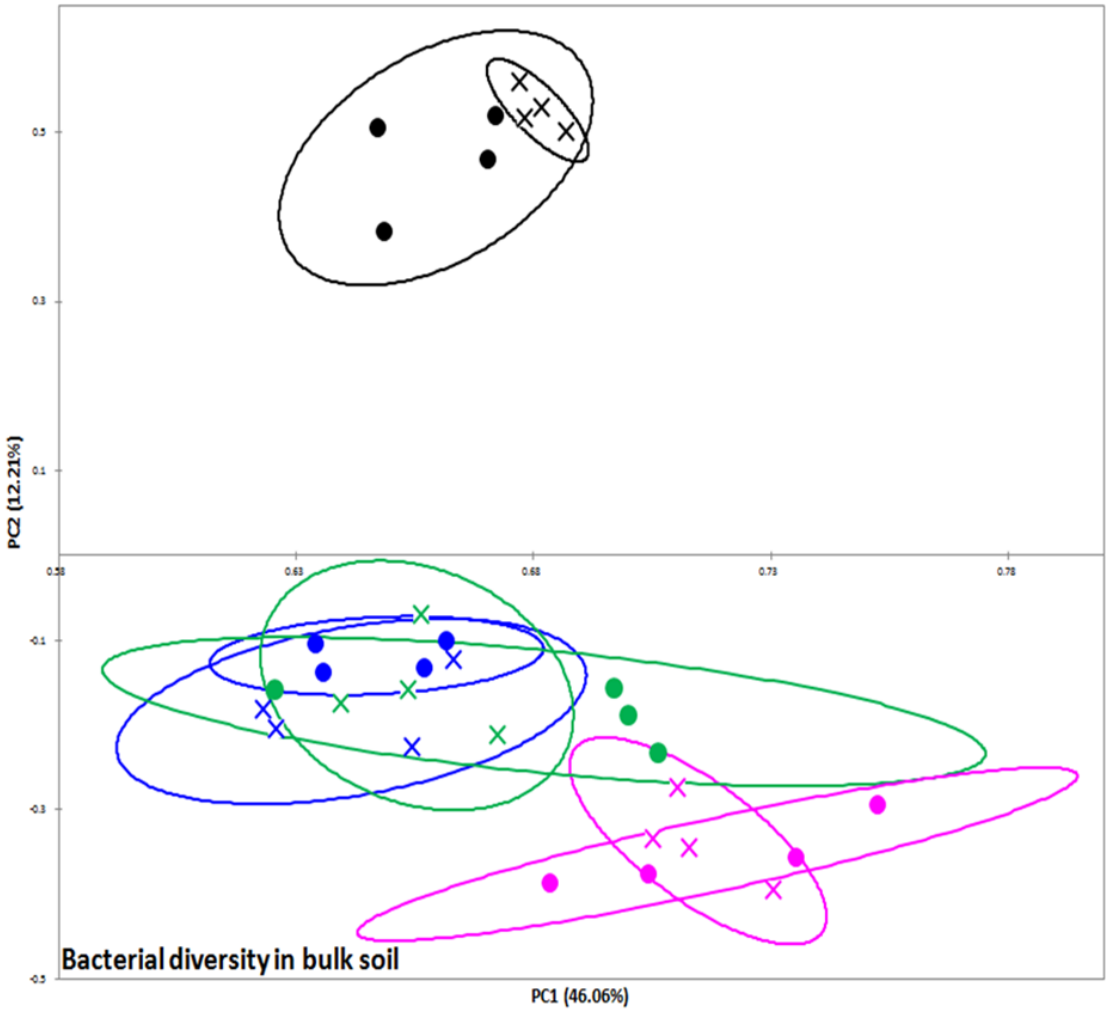
Common scab did not develop on any progeny tubers grown in the inoculated untreated/natural field soil. In contrast, 72% of tubers grown in the inoculated potting mix developed common scab, significantly more than in the inoculated heat-treated and the γ-irradiated soil (18 and 30% tubers affected, respectively). The incidence and severity of common scab did not differ significantly between the heat-treated (HT) and the γ-irradiated soils. *S. scabies* S546 DNA concentrations at harvest were very low in all three soils (native, HT, γ-irradiated) compared to that of the potting mix and in stark contrast to the DNA starting levels, consistent with the levels and severity of common scab disease in all four inoculated treatments.

UNRAVELLING THE BIOLOGICAL COMPONENT OF SUPPRESSION USING TRFLP ANALYSIS

To confirm whether we could differentiate between soil treatments and to test whether inoculation with pathogenic *Streptomyces* bacteria would result in a detectable shift in microbial populations, principal component analysis (PCA) was conducted on bacterial and fungal community DNA fingerprints (TRFLP) from

the soil that was untreated, heat-treated or gamma-irradiated. Gamma radiation is a well-known method to sterilise matter without substantially altering its chemistry. Tests with this soil have shown that it has lost most of its disease-suppressive properties. Heat treatment, while a relatively milder soil treatment than radiation, can change not only microorganism communities, but also its chemical properties. Potting mix, which is conducive to scab infection, was also included in this study as a positive inoculum control. To generate the PCA, normalised peak intensity data measured by Genemarker™ was pooled for replicates of the same sample, transformed into presence or absence data (0 or 1), and analysed in XLStat™ software. PCA data is displayed as scatter plots with 95% confidence intervals drawn around individual groups; if confidence intervals do not overlap, the two groups can be considered to be significantly different from each other. Group circles which strongly overlap can be considered equal in the community structure.

Figure 5.A.I-30 shows the PCA of bacterial 16S TRFLP comparing shifts in populations by the various treatments in comparison to the untreated suppressive control soil, in bulk soil. Inoculation with pathogenic *Streptomyces* did not significantly change the multivariate statistical results as bacterial populations in inoculated or un-inoculated γ-irradiated soil look statistically identical to each other with both groups strongly overlapping each other. This statistical similarity was observed in all PCA of bacteria and fungi profiles found in bulk and rhizosphere soils where pathogenic *Streptomyces* did not dramatically change bacterial or fungal population diversity. The PCA analysis of TRFLP data was being used to see if we could identify any significant shifts in either bacterial or fungal populations which might coincide with the changes in disease suppression. The untreated soil groups, shown as pink at the bottom right of Figure 5.A.I-30, were somewhat different from bacterial populations in heat-treated soils, with some overlap between un-inoculated untreated and inoculated heat-treated. Gamma-irradiated soil was significantly different to the untreated groups, with no overlapping of groups at all. Bacterial diversity in potting mix was significantly different compared to untreated soil, with no overlap and a large distance between groups. The changes in bacterial populations that resulted from heat treatment and γ-irradiation did result in reductions in disease suppression and suggested that our detection methods are sufficiently sensitive to identify at least some of the microbes which may be involved in this disease suppression.

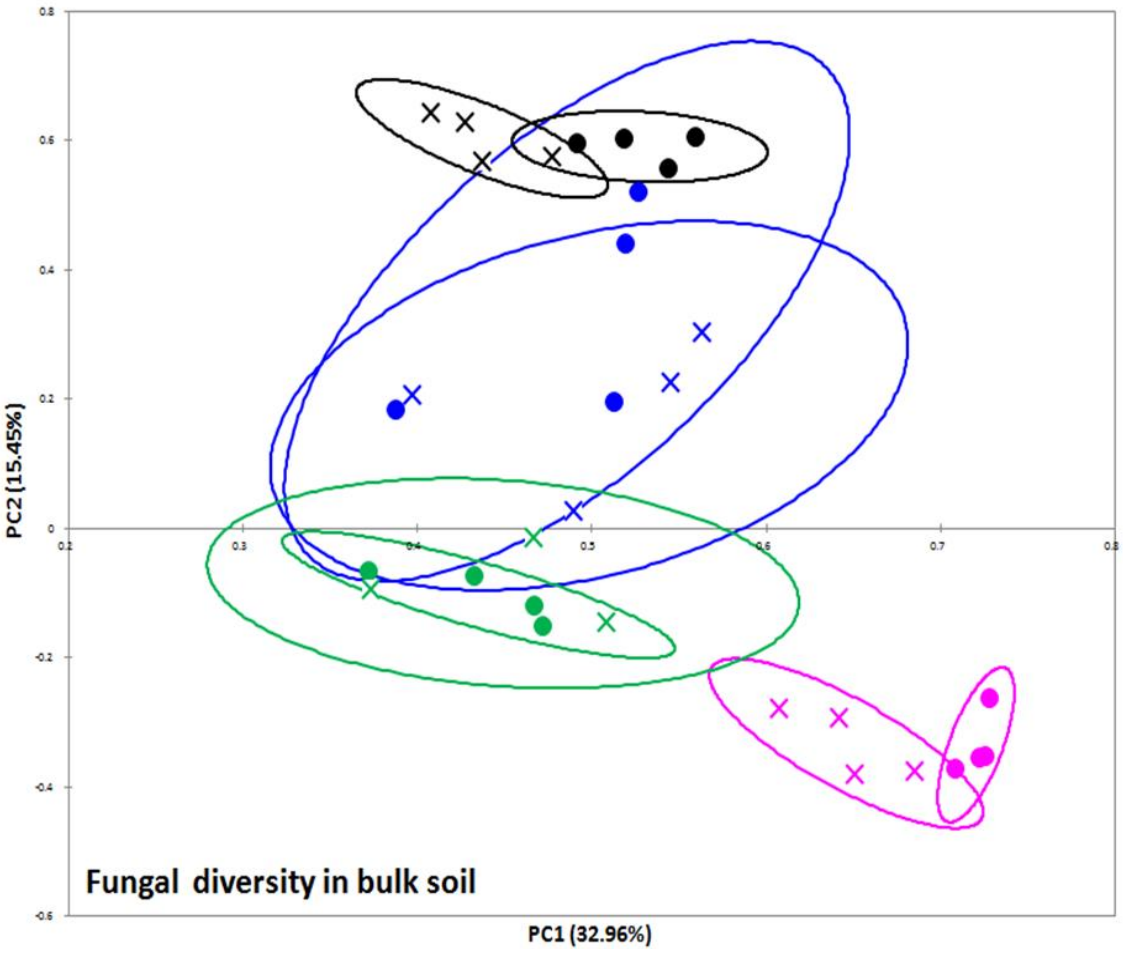


Untreated Ballarat soil (×,•), Green, heat-treated Ballarat soil (×,•), Blue, γ-irradiated Ballarat soil (×,•); Black, potting mix (×,•); × , uninoculated with *S. scabies*; • , inoculated with *S. scabies*

Figure 5.A.I-30 – Scatterplot display of PCA of bacterial 16S TRFLP data from bulk soil DNA that had been untreated, heat-treated, gamma-irradiated and/or inoculated with pathogenic *Streptomyces*.

Fungal diversity would likewise have been expected to shift significantly in response to treatments, and Figure 5.A.I-31 did show a similar segregation pattern to Figure 5.A.I-30. Again, untreated samples were somewhat different to heat-treated soil, significantly different from γ-irradiated soil, and significantly different from potting mix. Unlike bacterial diversity in γ-irradiated soil, fungal diversity appears to be much more varied, suggesting that fungal populations recolonizing the sterilized soil are more stochastic than bacterial ones. As TRFLP with PCA is a method to compare diversity amongst microbial populations (hundreds of species in the case of soil bacterial populations), the addition of pathogenic *Streptomyces* would only be detectable through its broad impact on the diversity of fungi in the soils (i.e. secreting antifungal compounds and killing susceptible fungal species). The interaction of pathogenic *Streptomyces*

with the soil microbial community is unknown, but it did not appear to interact significantly with fungal populations.



Untreated Ballarat soil (×,•), Green, heat-treated Ballarat soil (×,•), Blue, γ-irradiated Ballarat soil (×,•); Black, potting mix (×,•); × , uninoculated with *S. scabies*; • , inoculated with *S. scabies*

Figure 5.A.I-31 –Scatterplot display of PCA of fungal 16S TRFLP data from bulk soil DNA that had been untreated, heat-treated, gamma-irradiated and/or inoculated with pathogenic *Streptomyces.*

The second set of experiments were done to test whether rhizosphere soil from potato plantlets grown on differently-treated suppressive soil would also reflect differences in bacterial or fungal populations. It was hoped that the bacteria or fungi involved in this disease suppression would be enriched at the root/soil interface. Plants were grown for a month in tubes containing different soils, and DNA was extracted from the soil washed off of the root surfaces. Plant growth was estimated by looking at photographs (Figure 5.A.I-32). Plant growth was similar in all soils, although plants in “gamma-irradiated/inoculated” soil were

slightly smaller and slightly bigger in potting mix soil. The growth response is attributed to possibly fertility or the ability of microbes other than scab to affect the growth of potatoes.

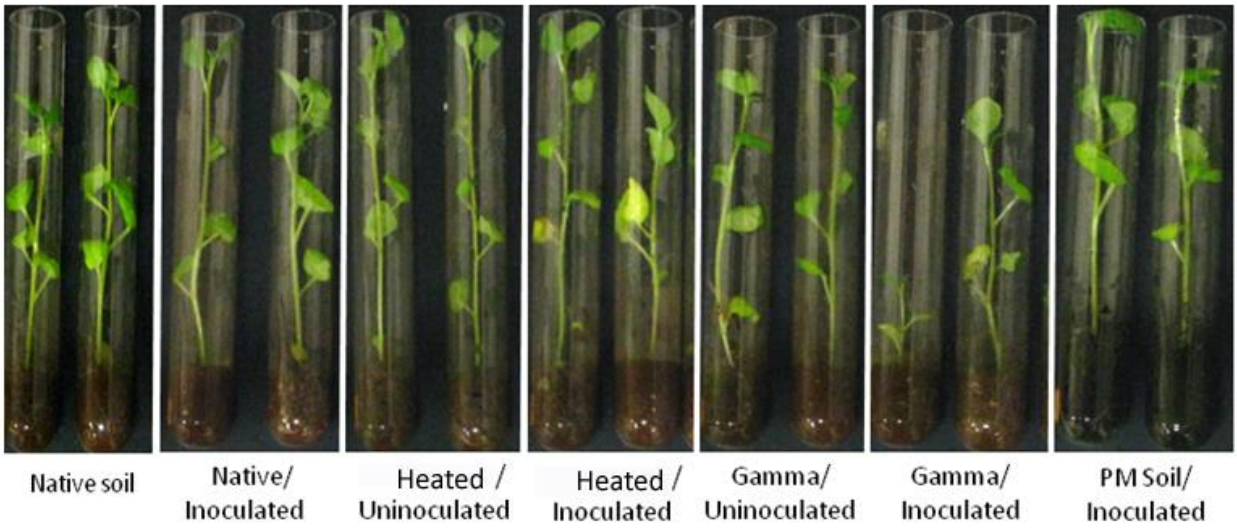


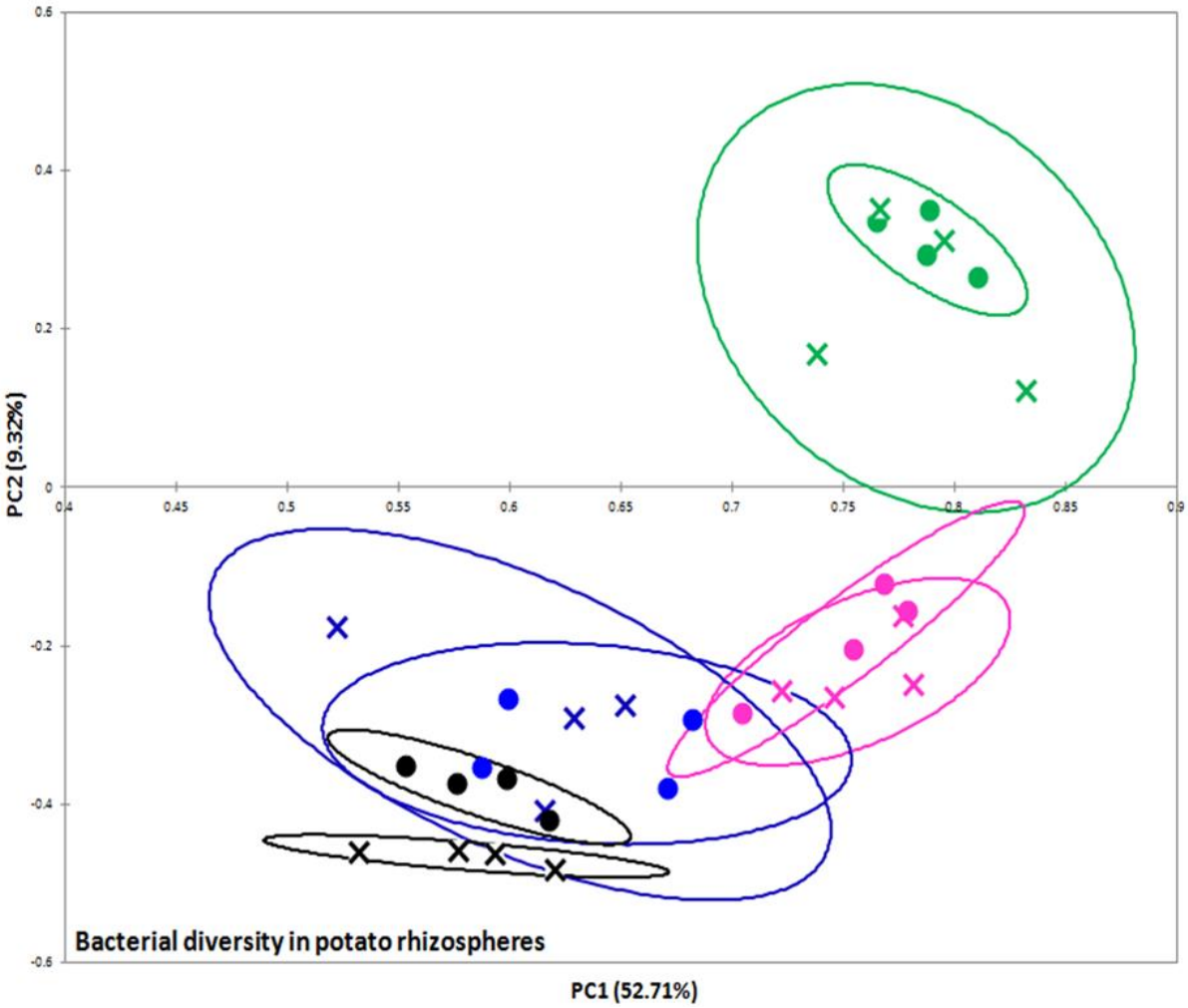
Figure 5.A.I-32 – Tissue cultured Kennebec potato plantlets grown for a month in a growth chamber in soils that had been untreated (native) heat-treated, γ-irradiated and/or were inoculated with pathogenic *Streptomyces*.

Bacterial populations in rhizospheres developed in an unexpected way, with heat-treated groups being significantly different from untreated, γ-irradiated and potting mix samples (Figure 5.A.I-33). Because heat treatment does not sterilise the sample, nor does it affect all bacterial groups equally (i.e. heat-resistant spores would survive heating, but not radiation), perhaps what we have seen here is regrowth of a subset of the original bacterial flora which was completely killed in γ-irradiated samples. Since potting mix and γ-irradiated soils were more conducive to common scab and were both overlapping in this analysis, the bacterial populations that developed in these rhizospheres may possibly be missing the same bacterial species that made this soil suppressive to common scab.

Figure 5.A.I-34 suggests that treatment effects which resulted in significant differences in fungal populations of bulk soil samples (Figure 5.A.I-31) were diminished in magnitude after a month of potato plants growing in them. Perhaps as plants grew in the heat-treated and γ-irradiated soils, some remnants of the original fungal population recolonized the soil resulting in somewhat similar species diversity – gamma-irradiation killed many more fungal species than heat treatment, resulting in greater difference to untreated controls. Again, potting mix (a dramatically different soil type) had a significantly and dramatically different pattern of fungal diversity when compared to untreated rhizospheres.

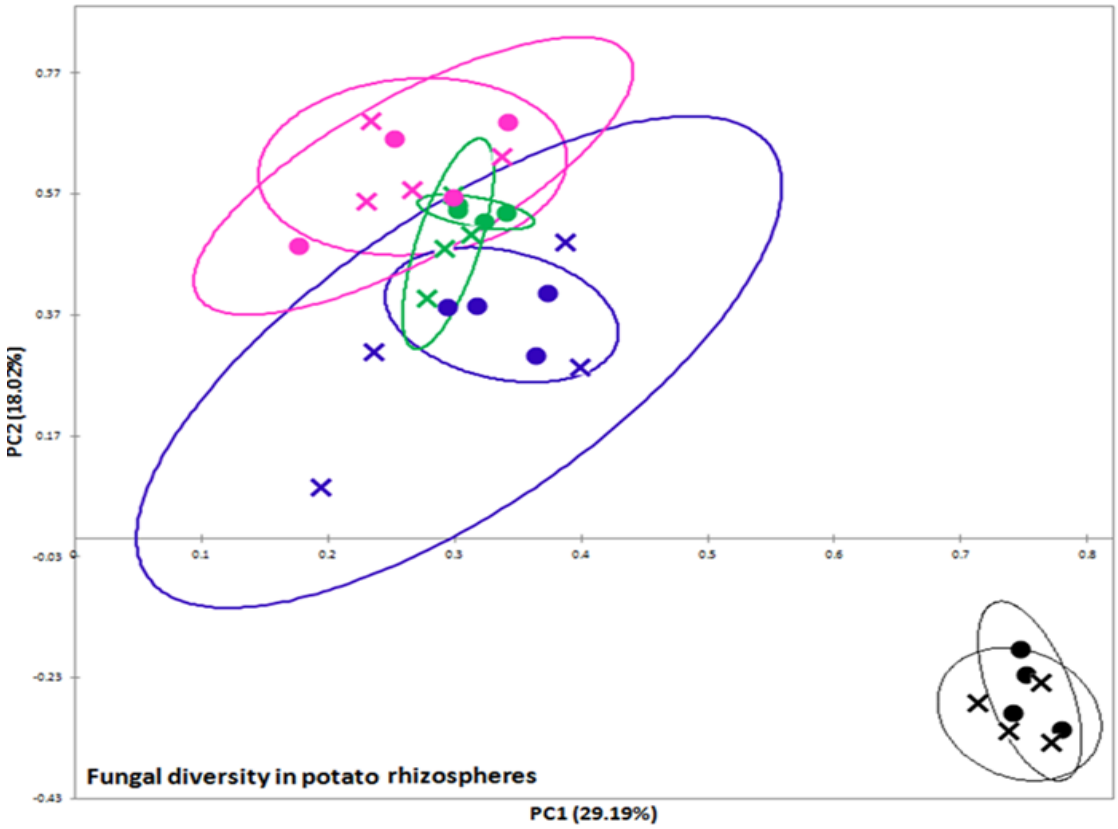
In summary, these experiments worked well showing that the treatments (heat treatment and γ-irradiation) are strong enough to cause significant shifts in bacterial and fungal differences in soils which correlated with increased disease incidence. These differences are maintained in rhizospheres over a period of one month; however they become less pronounced as the plants grow. Also of importance, these

results suggest that the disease-suppressing organisms we were hoping to discover could be being detected by the TRFLP method we are using.



Pink, untreated Ballarat soil (×,•), Green, heat-treated Ballarat soil (×,•), Blue, γ-irradiated Ballarat soil (×,•); Black, potting mix (×,•); × , uninoculated with *S. scabies*; • , inoculated with *S. scabies*

Figure 5.A.I-33 – Scatterplot display of PCA of bacterial 16S TRFLP data from rhizosphere soil DNA from roots grown in untreated, heat-treated or γ-irradiated soil and potting mix positive control with or without pathogenic *Streptomyces*.



Pink, untreated Ballarat soil (×,•), Green, heat-treated Ballarat soil (×,•), Blue, γ-irradiated Ballarat soil (×,•); Black, potting mix (×,•); × , uninoculated with *S. scabies*; • , inoculated with *S. scabies*

Figure 5.A.I-34 –Scatterplot display of PCA of fungal 16S TRFLP data from rhizosphere soil DNA from roots grown in untreated, heat-treated or γ-irradiated soil and potting mix positive control with or without *S. scabies*.

Multivariate statistics show whether entire microbial populations shifted in response to treatments, but in order to track down which microbes might be causing the disease-suppressive effect seen in this soil, we had to identify changes in individual species from the communities. The tools we have been using for bacterial TRFLP do not appear to be able to directly detect pathogenic *Streptomyces* DNA in these experiments, there was no common peak in all inoculated bulk soils and rhizospheres that could be explained as the predicted 433 bp *S. scabies* fragment. However, what we have found is that heat treatment, γ-irradiation and potting mix have very distinct bacterial and fungal populations in these substrates and rhizospheres, suggesting we may be detecting the disease-suppressive organisms, even if we don't detect pathogenic *Streptomyces*. In order to obtain statistically significant differences in TRFLP profiles, tens or even hundreds of fragment peaks have to shift in a similar pattern among groups, meaning that a lot of candidate signals need to be considered in the analysis. One way to select out specific signals is to compare TRFLP profiles from treated and control samples and pick out only those which become smaller (and theoretically less abundant) in heat-treated and γ-irradiated soil. To do this, we took averages of

normalised TRFLP profiles from untreated, heat-treated and γ-irradiated soil or the generated rhizosphere and compared them to the corresponding peak sizes from the untreated control samples. The controls were then subtracted. Because we suspect that the scab-suppressive effect is being caused by bacterial antibiotic production, we have only conducted this analysis for bacteria. Figure 5.A.I-35 shows results from bulk soil DNA samples and Figure 5.A.I-36 shows results from our rhizosphere DNA samples. Although bacteria from the rhizosphere of plants grown in the heat-treated soil appeared to be significantly different from untreated rhizosphere, it is important to remember that they were also different from γ-irradiated rhizospheres, making it difficult to understand how this was influencing their disease-suppressive abilities.

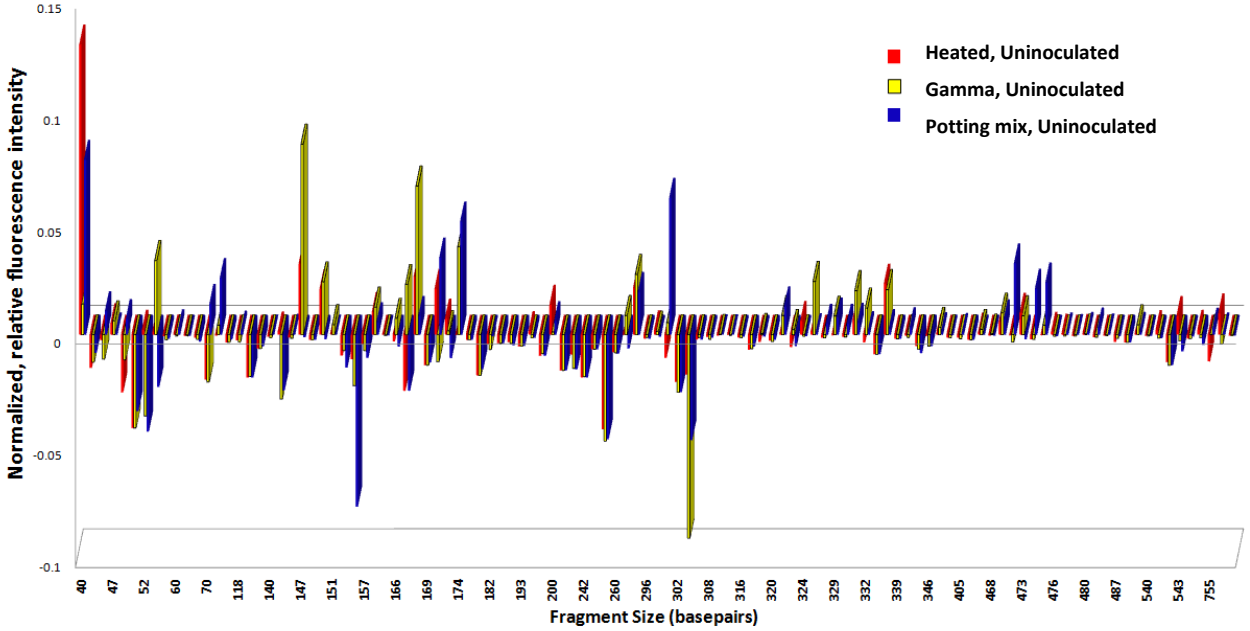


Figure 5.A.I-35 – Relativized, normalised fragment fluorescence intensity of bacterial 16S TRFLP data from potting mix, heat-treated or γ-irradiated bulk soil DNA relative to untreated putative suppressive soil.

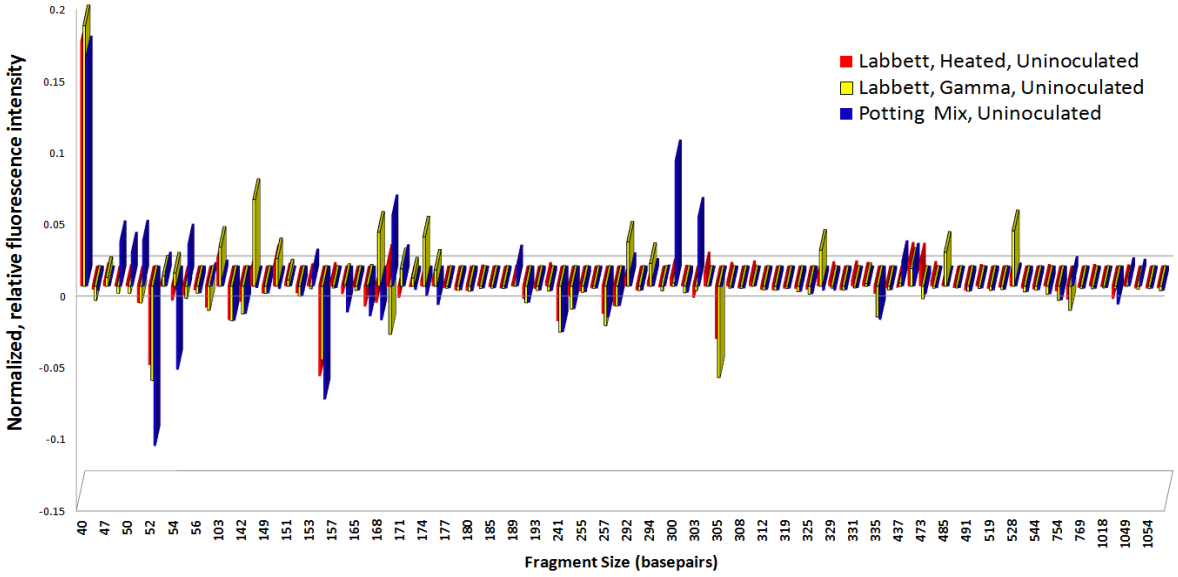


Figure 5.A.I-36 – Relativized, normalised fragment fluorescence intensity of bacterial 16S TRFLP data from untreated, heat-treated, or γ-irradiated rhizosphere soil DNA relative to untreated putative suppressive soil.

In bulk soil that was heat-treated or γ-irradiated, major peaks (drop of more than 1%) that decreased in fluorescence intensity included fragment sizes 41, 50, 51, 70, 131, 142, 155, 169, 180, 240, 241, 242, 256, 302, 305 and 542 bp (Figure 5.A.I-35). The most dramatic differences can be observed in 51 bp which had a fluorescence intensity which was about 4% lower in both heat-treated and γ-irradiated soil, the 155 bp peak which decreased by 1% and 2%, a 256 bp peak which went down by about 4% and 5% and a 305 bp peak which went down about 2% and 9% in heat-treated and γ-irradiated soil respectively. These reductions in fluorescence intensity of specific size fragments should indicate a relative reduction in DNA of that particular species or group of species and thus offer potential clues that can be used to focus on the organisms responsible for disease suppression in these soils.

Surveying a list of 37379, 63F-1389R amplified, *Hha*I-cut fragments from the website MICA (a website that allows virtual TRFLP from millions of 16S bacterial ribosome sequences) suggests potential identities for these unidentified fragments. A species of *Bacteroidetes* (FJ516921) has a 63F-*Hha*I fragment size of 51 bp; 305 bp could represent *Beijerinckia indica* (AB119196), *Chelatococcus* spp. (GQ871852) or *Methylocella silvestris* (CP001280). The other major peaks that were reduced in γ-irradiated or heat-treated bulk soil relative to the control in Table 5.A.I-47 did not have matches in the database.

In rhizosphere soil that was heat-treated or γ-irradiated, major peaks (drop of more than 1%) that decreased in fluorescence intensity included 51, 52, 70, 132, 142, 155, 241, 256, 258, 305, and 335 bp sizes. Interestingly, fragments 51, 70, 131, 142, 155, 241, 256, and 305 were also observed as dramatically reduced in heat-treated and γ-irradiated bulk soil, suggesting we are observing non-random variation – these bacteria were reduced in bulk soil and their population stayed lowered in rhizospheres a month later.

The most dramatic differences in relative fluorescence intensity in rhizosphere peaks can be observed again in 52 bp which had fluorescence intensity which was about 6% lower in heat-treated and 7% in γ-irradiated rhizospheres, in 155 bp which went down by about 6% and 5% in heat-treated and γ-irradiated soils, and in 241 bp which went down by about 2% in heat-treated and 3% in γ-irradiated rhizospheres. 305 bp went down by about 4% in heat-treated and 6% in γ-irradiated rhizospheres. These reductions in fluorescence intensity of a certain size fragment should indicate a relative reduction in DNA of that particular species and thus are potential clues we can use to zoom in on the organisms responsible for disease suppression in these soils.

Surveying the same list of 37379 *Hha*I cut fragments from the website MICA, potential fragment identities can be speculated on. The 52 bp appears to represent a type of *Bacteroidetes* (FJ516921). 70 bp could represent another species of *Bacteroidetes* (FN377775) and 142 bp could represent a species of *Aeromonas* (FJ940820). Peak sizes not mentioned either had no reasonable match in the library (i.e. only matched with Antarctic marine microbes) or were already mentioned in the section on bulk soil microbes. These results and fragment identities are summarised in Table 5.A.I-47, where unidentifiable bacterial species associated with specific base pair fragments were removed from the table.

Table 5.A.I-47 – 63F bacterial 16S fragments going down in intensity in either bulk soil or rhizospheres and their most probable matches as predicted by the TRFLP website MICA.

63f Fragment size (bp)	Reduced in heated or gamma-irradiated soil	Reduced in heated or gamma-irradiated rhizosphere	Possible Bacterial matches
42	Yes	Yes	*Pseudomonas fluorescens* Mst8.2 (DQ996132)
51	Yes	Yes	*Bacteroidetes* sp. (FJ516921)
52	Yes	Yes	*Bacteroidetes* sp. (FJ516921)
58	Yes	No	*Cytophagales* sp. (AF539753), *Pseudomonas* sp. (AM158919),
			Bacteroides sp. (A8522117)
60	Yes	No	*Cytophagales* sp. (DQ205192), *Sphingobacterium* sp. (GU474874),
			Bacteroides sp.(AF354617)
62	Yes	No	*Cytophaga* (HQ439515), *Pantoea* (EU215444), *Bacteroides* sp. (AF354617)
63	Yes	No	*Flexibacter* sp. (FN668170), *Poludibacter proplonkigenes* (A8078842),
			Bacteroides sp. (AY162087)
70	Yes	Yes	*Bacteroides* sp. (FJN377775)
118	Yes	No	*Ps. aeruginosa* (AY486361)
142	Yes	Yes	*Aeromonas veronii* (FJ940820)
165	No	Yes	*Ps. halophila* (AB021383)
169	Yes	No	*Ps. alcaligenes* (EF199996)
170	Yes	Yes	*Ps. putida* (X93997)
177	No	Yes	*Lysobacterantibioticus* (FJ930928), *Luteimonas* (FR691433), *Pseudomonas* sp. (AB021405),
			Stenotrophomonas moltophillia (EF620464), *Xanthomonas retroflexus* (DQ337602)
178	Yes	No	*Aeromonas caviae* (FJ544406), *Ps. pictorum* (AJ131116), *Xanthomonas campestris* (AM920689),
			Stenotrophomonas maltphilia (EF620464)
179	No	Yes	*Aeromonas veronii* (X60414), *Ps. pictorum* (AJ131116), *Xanthomonas campestris* (AM920689),
			Stenotrophomonas maltphilia (EF620464)
180	Yes	Yes	*Aeromonas veronii* (X60414), *Ps. pictorum* (AJ131116), *Xanthomonas campestris* (AM920689),
			Stenotrophomonas maltphilia (EF620464)
182	Yes	Yes	*Ps. fluorescens* (GQ496662)
193	Yes	Yes	*Beijerinckia mobilis* (AJ563932), *Methylosinus sporium* (AJ458495),
			Pseudomonas sp. VET-4 (EU781732)
296	Yes	No	*Brevundimonas diminuta* (DQ979376)
303	No	Yes	Beijerinckiaceae sp. (EU586037), Chelatococcus sp. (GQ871652)
			Methylocelia silvestris (CP001280)
316	Yes	No	*Mesorhizobium* sp. (DQ917826), uncultured
330	Yes	Yes	*Bacteroidetes* sp. (AF351234)
331	No	Yes	*Pseudomonas* sp. KC-EP-513(FJ711216), *Xanthomonas* sp. (FR774560)
334	Yes	No	*Enterobacter hormaechei* (HM172500), *Pantoea agglomerans* (AF290417),
			E. coli (GH594312), *Klebsiella* sp. (AF221602)
335	No	Yes	*Citrobacter, Enterobacter, Erwinia, Escherichia* sp.,
			Pantoea, Klebsiella, Serratia sp.
341	Yes	No	*Pseudoxanthomonas spadix* (AM41838)
478	Yes	No	*Beijerinckiaceae* (FR686344)
526	No	Yes	*B. subtilis* (AM110939), *Rhizobium* sp. (FJ911604)

Note: those bp fragments which have no identified bacteria have been left out of Table 5.A.I-47.

Selective screening of treatment-induced decline in bacterial TRFLP peaks yielded a number of candidate fragments which may represent disease-suppressive microbes (Table 5.A.I-47). It is interesting to recall that heat treatment and γ-irradiation resulted in higher scab incidence when potatoes are grown in that soil – a rhizosphere phenomenon which occurs over a month after the soil treatment. This suggests that only microbes which are reduced by the soil treatment and do not grow back over this long period of time are real candidates for explaining the disease-suppression ability of this soil. Peaks that fit this criteria are few – only 51, 70, 131, 142, 155, 241, 256, and 305 bp fragments are dramatically lower than controls in both γ-

and heat treatments with only 51, 70 and 142 bp fragments tentatively identified (Table 5.A.I-47). Among these peaks, several have possible identities of *Bacteriodetes* and *Beijerinkii*. However these are not well known microbes with antagonism towards other bacteria.

Some of the other fragments, however, although smaller in magnitude, were tentatively identified in the experiment and are known biocontrol microbes, such as *Pseudomonas fluorescens* (42 and 182 bp fragments), *Stenotrophomonas maltophilia* (179 and 180 bp) and *Lysobacter antibioticus* (177 bp) and will certainly require further attention.

DISCUSSION

We have demonstrated the biological nature of common scab suppression of a potato field soil from Ballarat. Using TRFLP analysis we have shown that soil microbial communities change with treatments that correlate with common scab suppression. We have tentatively identified soil microbial organisms that may be responsible for common scab suppression.

This glasshouse experiment has shown that this particular field soil is suppressive towards common scab, not allowing accumulation of pathogen DNA. Gamma irradiation eliminates the disease suppressiveness of this soil and heat treatment also reduces it significantly, suggesting that a biological process is involved in this suppression.

The absence of common scab symptoms on tubers in the native untreated soil, and the high level and severity in the inoculum control lend credence to the premise that this soil is suppressive against common scab. The incidence and severity of common scab did not differ significantly between the heat-treated (HT) and the γ-irradiated soils, indicating the possibility that non-spore-forming bacteria could be the biological factor driving the suppression. Gamma irradiation kills all organisms and the heat treatment would remove the proportion that are heat sensitive (i.e. fungi, non-spore-forming bacteria; e.g. *Pseudomonas* sp.). Fluorescent pseudomonads have been targeted and suggested as possible biological agents in a wide range of disease-suppressive soils (Duffy 1999; Haggag 2010), although a wide range of other potential biocontrol microbes have also been suggested (Kinkel et al. 2012). However, more detailed experiments need to be conducted to take into account other potentially confounding factors, such as differences in the physical conditions in the soils in pots, which may affect infection of tubers and disease development.

The differences in several key nutrients is consistent with the effects of γ-irradiation on soil chemical composition (McNamara et al. 2003) but these differences cannot account for the suppression. Pathogenic *S. scabies* DNA concentrations of the three soil treatments at harvest ranged from 320–3380 pg/g of soil, with the native soil and the γ-irradiated + 10% native soil having significantly less pathogen DNA than the γ-irradiated soil only. The fact that pathogenic *S. scabies* DNA concentrations were very low post-harvest in native soils indicates that the soil was not allowing the pathogen to establish itself, which is one of the benchmarks in describing suppressive soils (Mizuno et al. 1998).

One potential mechanism by which scab severity is reduced was identified by St-Onge et al. (2011) and Arseneault et al. (2013) who showed that *Pseudomonas* sp. that produce antibiotics such as phenazine-1-carboxylic acid (PCA) that could be a primary mechanism for disease reduction. The *Pseudomonas* strain they tested was antagonistic to the growth of *S. scabies* under *in vitro* conditions. However, when they added this bacterium to soil, the *S. scabies* populations actually increased in the geocaulosphere, but only the PCA-producing strain reduced scab and not a mutant lacking this trait. This suggests that PCA

production may not reduce pathogen populations, but rather somehow suppress the production of thaxtomin A, a compound recognized as the primary virulence factor of *S. scabies*. When tested on agar media, thaxtomin production was found to be reduced by almost half of that found with the untreated control.

Rosenzweig et al. (2012) carried out detailed microbial studies of a field with naturally-occurring common scab suppression in Michigan. They confirmed the disease suppressiveness was biologically based. Using pyrosequencing, they analysed the microbial profiles of the suppressive site and compared it to a nearby soil that was conducive to the disease. They identified 1,124 operational taxonomic units (OTUs) and of these, 565 OTUs (10% dissimilarity) were identified in the disease-conducive soil and 859 in the disease-suppressive soil. Three hundred were shared between both sites. Disease suppressive soils had significantly higher sequence frequencies of *Lysobacter*, as were sequences of group 4 and group 6 *Acidobacteria*. In disease conducive soils, sequences of the genus *Bacillus* were significantly higher by an order of magnitude. They conclude that disease suppression is likely associated with a group of microorganisms, including bacilli, florescent pseudomonads, and non-pathogenic streptomycetes.

Further work will have to be done on all of these peaks to properly identify them and, furthermore, to selectively culture, isolate and test these bacteria for their ability to kill or inhibit *S. scabies* in soil, either as single microbial biocontrol treatments (De La Fuente et al. 2006; Park et al. 2006), or as a consortia (Grosch et al. 2005; Selva Kumar et al. 2013). The next steps in this direction will include direct sequencing of PCR products instead of running them through the TRFLP protocol and a new technique called physical TRFLP which allows for direct sequencing of peaks of interest which are separated on a gel by electrophoresis, then extracted and sequenced to understand the specific identity of that particular band.

CONFIRMATION OF SUPPRESSION AND DEMONSTRATION OF PROOF-OF-CONCEPT OF TRANSFER OF COMMON SCAB SUPPRESSION

SUMMARY

In the previous study, a field soil from a potato field near Ballarat was tentatively identified as being suppressive to common scab, the most likely cause of suppression being a biological component of the soil. In this follow-up experiment, common scab failed to develop on potatoes grown in gamma-irradiated field soil that had been mixed with 10% (w/w) of the untreated field soil and inoculated with *S. subterranea*, but developed in inoculated unamended gamma-irradiated field soil. This demonstrated that the suppressive properties of the field soil are able to be transferred to other soils. A TRFLP analysis of the different soils found that the bacterial communities of the field soil and gamma irradiated field soil were very different from each other. Adding 10% of the untreated field soil to the irradiated soil broadened the make-up of the bacterial communities to more resemble those in both the treated and untreated soils but remaining unique. The implication of this study is that, once the suppressive agents are isolated and identified, they could be exploited for the management of common scab. A better understanding of the differences between microbial communities in common scab conducive and suppressive soil, and what controls these differences, may make it possible to ultimately manipulate the soil environment for suppressiveness to common scab.

INTRODUCTION

This experiment was initiated to confirm common scab suppression and to prove the transferrable nature of suppression. In the absence of a suitable soil found to be conducive for common scab disease (i.e. of similar type and chemical composition), the transfer experiment was performed on freshly γ-irradiated native soil. Note that for this experiment, the original field soil had been kept in cold storage (4°C) for the entire period between the first experiment and the second experiment (approximately 2 years).

MATERIALS AND METHODS

A similar protocol to the first trial was followed, but with the following amendments: Removal of common scab suppression from the Ballarat soil was by γ-irradiation (25 kGrays) only, since the first experiment showed no significant differences between the heat-treated and the γ-irradiated soil in terms of common scab disease. All treatments, including the potting mix, were taken out of cold storage and placed in the laboratory in sealed containers at room temperature for two weeks before inoculating the soils with either sterile vermiculite or vermiculite inoculated with our standard pathogenic isolate of *S. scabies* S546 (10% v/w). For the suppression transfer, untreated field soil was used for our test soil and as an inoculum for the γ-irradiated field soil and was added at 10 % (w/w). This was incubated at room temperature for two weeks in a sealed container prior to challenging with the pathogenic *S. scabies*. All treatments were then dispensed into individual pots (1.8 kg per pot, 2.8L capacity), laid out in a randomised block arrangement and left under glasshouse conditions for a further two weeks to allow the microbial population and the *S. scabies* of all pots to stabilise and grow before planting with a susceptible potato cultivar Shepody.

The common scab-susceptible cultivar, Shepody, was supplied as 4-week-old tissue culture plants and planted at one per pot. A positive inoculum control was used, but the medium (also 'potting mix') used was supplied by Biogro® Australia ("Professional Growing Medium") not Debco®. Pots were watered at least once every two days and were supplemented with Aquasol™ once per week until the plants were established. From week 10, pots were supplemented with 2 doses of Aquasol™ per week to saturation until harvest, and hand-watered daily. Pots were watered to saturation throughout the experiment.

Sterile potting mix was added to all pots 70 days after planting, due to the presence of tubers breaking through to the surface in some pots and the uneven nature of the material in the pots. All pots were filled to capacity to cover all roots and aerial tubers. The potting mix used was sterilised twice at 121°C for 60 minutes to kill any microbial presence in the matrix and cooled completely before filling the pots completely for uniformity.

At harvest, the surface potting mix was carefully removed from the testing soil and discarded, both tubers and any residual root system were freed from the soil were harvested and placed in plastic ziplock bags and stored at 4°C for later disease assessment. Tubers were separated into 'above' and 'soil' and assessed for weight, number and disease. No tubers in the covering potting mix showed any sign of disease.

Samples of both pre-plant and harvest soils were also sent to A&L Canada Biologicals (George Lazarovits) for TRFLP analysis and SARDI (pathogenic *Streptomyces* DNA levels).

Bulk soil bacterial and fungal for TRFLP analysis at Planting and harvest

Analysis for each soil sample received was carried out with 3 biological replications and each experiment was repeated three times for a total of 9 replicates for each soil treatment. The following are the treatments of the soil samples and names assigned:

KSSG: native soil freshly gamma (γ)-irradiated

KSSGY: γ-irradiation and inoculated with *S. scabies*

KSS10GY: γ-irradiation and inoculated with 10% **KSS** and *S. scabies*

PMY: potting mix inoculated with *S. scabies*

KSSY: native soil inoculated with *S. scabies*

KSSN: native soil uninoculated

Soil DNA was extracted from the samples using a DNA extraction kit (Norgen Biotek) and following the manufacturer's recommendation. PCR was performed on the bacterial 16S rRNA gene using primers 63f and 1389r. Fungal PCR was performed using the primers ITS1F and ITS4. PCR products were digested with restriction enzyme *Hha*I. Digested samples were sent to the University of Guelph, Guelph, ON, Canada for TRFLP fragment analysis. TRFLP data analysis was conducted with Genemarker™ (SoftGenetics®). TRFLP data was analyzed by principle component analysis (PCA) which is based on presence or absence of specific DNA fragments of the digested amplicons.

RESULTS

Tubers from the native soil displayed no common scab symptoms. Both the γ-irradiated and the positive inoculum control displayed high levels of both incidence and disease severity, although the positive control disease levels were lower than that of the first experiment(61% compared to 72%). In the critical treatment where the γ-irradiated soil was re-inoculated with 10% of the untreated native soil, none of the tubers displayed common scab symptoms, thus suppression transfer was verified and shown to be biological.

Common scab DNA was variable at planting and ranged from 9910–68700 pg/g of soil (P=0.05) (Table 5.A.I-48). Even though there was a significant difference in the common scab DNA levels in between treatments, the DNA levels far exceeded any field DNA samples seen in this project, so common scab disease was expected. The harvest levels of common scab DNA in the γ-irradiated soil that had been inoculated with 10% of the native soil was only 320 pg DNA/g soil, comparable to the levels present in the native soil alone with a reduction of 90% compared to the γ-irradiated soil.

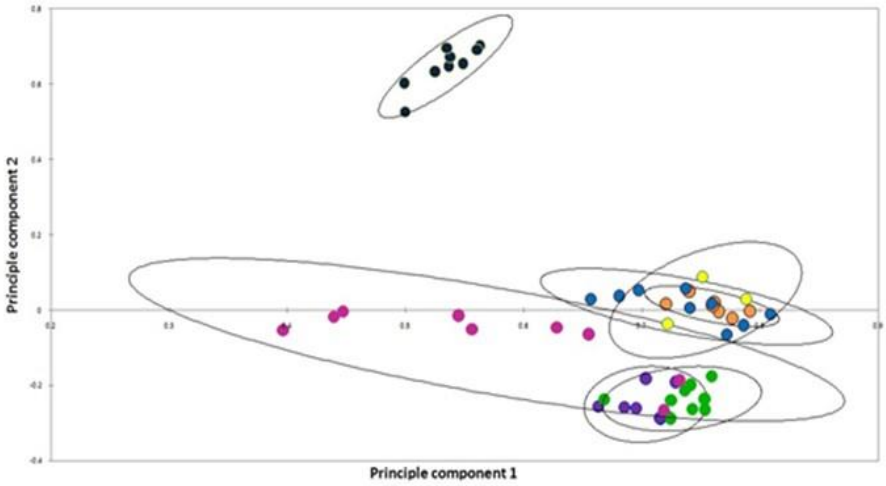
Table 5.A.I-48 – Incidence and severity of common scab on tubers and the concentration of pathogenic *Streptomyces* DNA at planting and harvest from treated and untreated soils inoculated with pathogenic *Streptomyces*.

Treatment	Common Scab		Pathogenic *Streptomyces* DNA pg/g soil	
	(% tubers affected)	Severity	Planting	Harvest
Native/Field Soil	0	0	56000*	500
γ-Irradiated	57*	19*	68700*	3380*
γ-Irradiated + 10% field soil (w/w)	0	0	9910	320
Potting mix (+ve inoculum control)	61*	26*	nd	15750*
lsd P=0.05	16	10	23000	2930

* Significantly different to the untreated control at P<0.05

Analysis of differentially treated soil bacterial communities in bulk soil at planting

The relationships of bacterial communities from the planting soil are shown inFigure 5.A.I-37. The microbial profiles of original soil from the Ballarat soil sampled in 2011/2012 and stored at 4°C (KSS, orange dots), inoculated with common scab before potting (KSSY, blue dots) and KSSN (to make sure common scab is not contributing to the profile; yellow dots) had very similar bacterial communities and were statistically different when compared to soils that were γ-irradiated (KSSG, purple dots and KSSGY, green dots). KSS10GY (pink dots) where the original native soil γ-irradiated, then mixed with 10% of the native soil. The bacterial profile of this soil had a more diversified community that slightly overlapped the γ-irradiated soil as well as the native soil. However, if anything, it more closely resembled the γ-irradiated soil. This likely resulted from the transfer of some of the dominant bacterial species present in the native soil that were able to re-establish in the γ irradiated soil even after just two weeks incubation, and thus alter its diversity. KSS is the original soil obtained from Ballarat and kept at 4°C and KSSY and KSSN are the same soils with or without *S. scabies* inoculation. The finding that KSSY (blue dots) and KSSN (yellow dots) are similar indicates that the addition of *S. scabies* is not altering the microbial profile. The potting mix soil amended with *S. scabies* (PMY, black dots) as in all previous analyses, had a completely distinct bacterial community when compared to the soils from Ballarat. This suggests that it may be possible to identify the basis for suppressiveness by creating such mixtures or adding specific organisms into γ-irradiated soils and testing them for disease establishment.

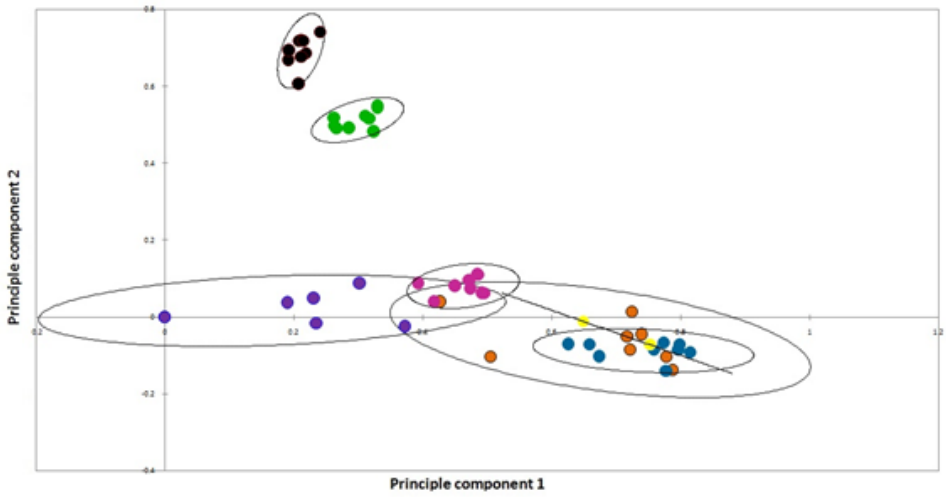


KSS • ; original field soil from 2011/12 and kept at 4°C
KSSG • ; comparison against first experiment's gamma-irradiated profile
KSSGY • ; kept at room temperature for 2 weeks after irradiation
KSS10GY • ; kept at room temperature for 2 weeks after irradiation and inoculation with KSS
PMY • ; different potting mix formulation to first experiment
KSSY • ; inoculated with *S. scabies* before potting
KSSN • ; comparison with KSSY to ensure CS is not contributing to the profile

Figure 5.A.I-37 – Principal Component Analysis (PCA) of the bacterial TRFLP profile derived from differently treated scab suppressive soils. Different soil samples are grouped by a circle and distinguished by colors. Dots represent the replicated number of the samples analysed. PMY was used as control to ensure that the TRFLP method completely separated different soil formulations.

Analysis of differentially treated soil fungal communities in bulk soil at planting

Fungal TRFLP analysis of planting soil showed that the KSS fungal community overlaps with KSSY and KSSN, but is different from the γ-irradiated soils (KSSG, KSSGY, and KSS10GY,Figure 5.A.I-38). Interestingly, soils stored at room temperature for 2 weeks after γ-irradiation and inoculated with pathogenic *Streptomyces* (KSSGY) had a fungal community that was significantly different from freshly γ-irradiated soil (KSSG) that had not been inoculated. KSSG10Y soil also showed a change in its fungal community after it was supplemented with 10% KSS soil, incubated and inoculated with pathogenic *Streptomyces*. KSSG had broader community diversity compared to KSSGY and KSS10GY. Here again the fungal community of the potting mix (PMY) is significantly different than all other samples.



KSS • ; original field soil from 2011/12 and kept at 4°C
KSSG • ; comparison against first experiment's gamma-irradiated profile
KSSGY • ; kept at room temperature for 2 weeks after irradiation
KSS10GY • ; kept at room temperature for 2 weeks after irradiation and inoculation with KSS
PMY • ; different potting mix formulation to first experiment
KSSY • ; inoculated with *S. scabies* before potting
KSSN • ; comparison with KSSY to ensure CS is not contributing to the profile

Figure 5.A.I-38 – PCA of the fungal TRFLP profile derived from scab-suppressive soils with and without γ-irradiation and with or without *S. scabies* inoculation. The soil samples grouped within a circle are not significantly different from each other. PMY is a potting mix used as control to ensure TRFLP method completely separates different soil formulations and treatments.

Rhizosphere bacterial community of planting soil

TRFLP analysis (Figure 5.A.I-39) showed consistent patterns for rhizosphere bacterial communities in each treatment. Plants grown in PMY showed significant differences in their rhizosphere bacterial communities compared to all the other soil samples. The rhizosphere of plants grown in KSS10GY (pink) were different from that of plants grown in soil used to compare against original gamma-irradiated soil KSSG (purple). Although the rhizosphere communities of plants grown in the other soils were not significantly different from each other, there were clear trends showing KSS10GY (pink) and KSSGY (green) to have some similarities. Rhizospheres of plants grown in suppressive soil; KSS (orange), KSSY (blue) and KSSN (yellow) all showed very close clustering and were most removed along principle components 1 and 2 from the rhizospheres of other plants grown in γ-irradiated soils.

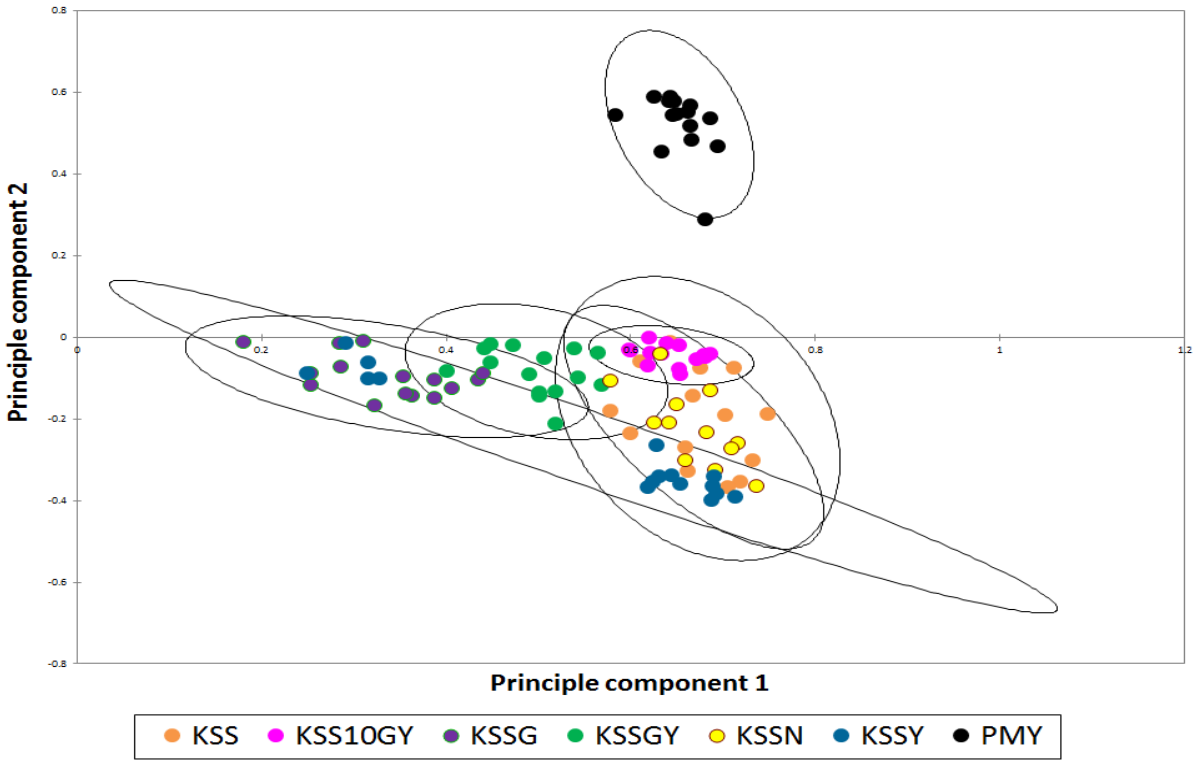


Figure 5.A.I-39 – PCA of rhizosphere bacterial communities in untreated or γ-irradiated soils from the second suppressive experiment based on TRFLP.

While PCA analysis can provide a broad sense of bacterial community patterns, an alternative way to look at differences in bacterial types among different soil samples is to examine intensity signals for each DNA fragment. A stronger signal can indicate that a specific fragment size is present at a greater frequency. The very large fragment sizes however, often show less intensity even though they may be present at high numbers. Figure 5.A.I-40 shows signals of different DNA fragment sizes among soil samples. There was a strong signal peak found between 173 and 179 bp for KSSGY and KSS10GY samples. These data suggest that specific bacterial species were present in rhizosphere soils of these treatments. It suggested that specific bacterial species can be found only if soils were γ-irradiated and inoculated with common scab.

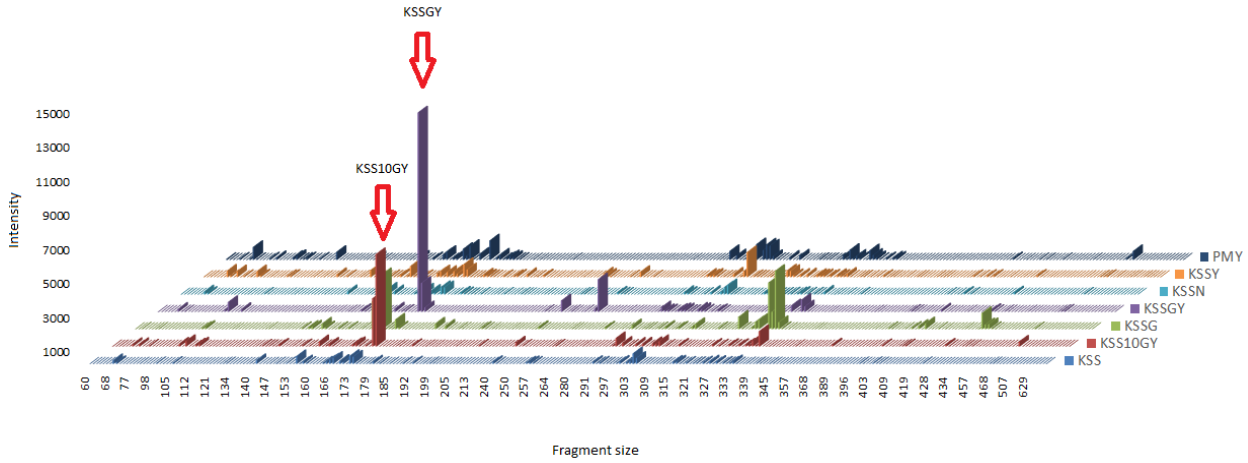


Figure 5.A.I-40 – Peak intensity of TRFLP fragments generated from TRFLP profiles of bacteria DNA extract from various soil samples from the second suppressive experiment.

Rhizosphere fungal community of planting soil

The fungal rhizosphere communities of plants grown in the various soils are shown in Figure 5.A.I-41. The rhizospheres of plants grown in potting mix (PMY, black dots) are again significantly different from all other treatments. Rhizosphere fungal communities of plants grown in soils KSS, KSSY and KSSGY10 were very similar to each other. This suggest that fungal community of the untreated farm soil rapidly colonized the γ-irradiated soil even when it is added at only 10%. Also of interest is that soils inoculated with pathogenic *Streptomyces* resulted in a measurable shift in the fungal populations of the rhizosphere of plants grown in such soils. It is possible that the inoculum added to these soils contributed some nutrients that certain fungi could exploit for growth. The rhizospheres of plants grown in soils that were γ-irradiated (KSSG and KSSGY) had differences in fungal communities from each other as well the other treatments (Figure 5.A.I-41) except for KSSN.

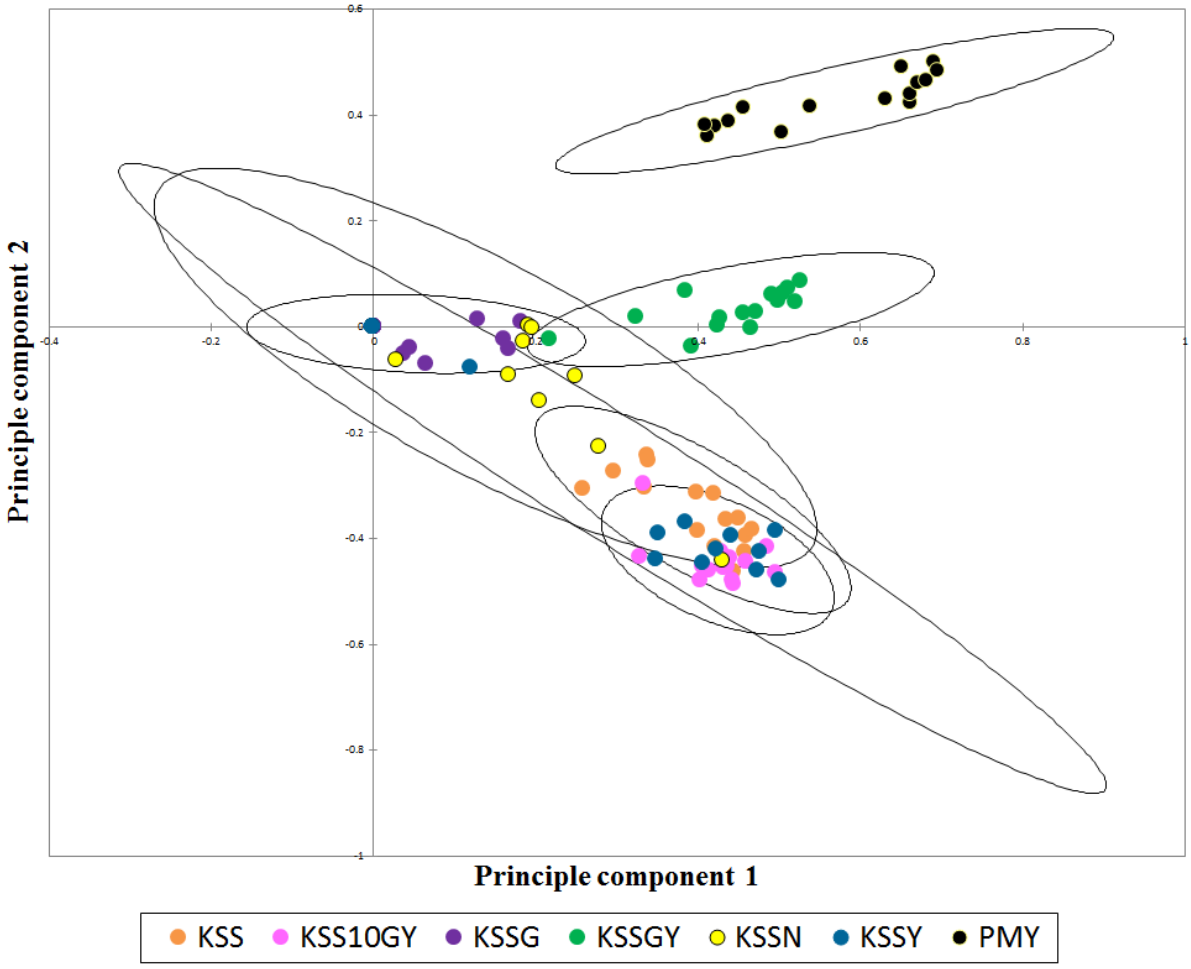


Figure 5.A.I-41 – PCA of the TRFLP results showing the rhizosphere fungal communities of potato plants grown in a scab-suppressive soil before and after γ-irradiation.

The largest signal peaks were observed from root samples grown in soils KSSGY and KSS10GY (Figure 5.A.I-42). Strong peaks were detected at a fragment size between 85 and 96 bp and a fragment size of 190 bp for KSSGY, while KSS10GY had strong peaks at fragment size between 354 and 376 bp (Figure 5.A.I-42). This again suggest that those peaks can be found after soils were γ-irradiated and inoculated with pathogenic *Streptomyces*. PMY had many strong peaks at fragment sizes from 251 to 330 bp, from 509 to 578 bp, and at 672 bp (Figure 5.A.I-42). The other soil samples did not show any intense peaks that corresponded with any of the fragment sizes in these samples.

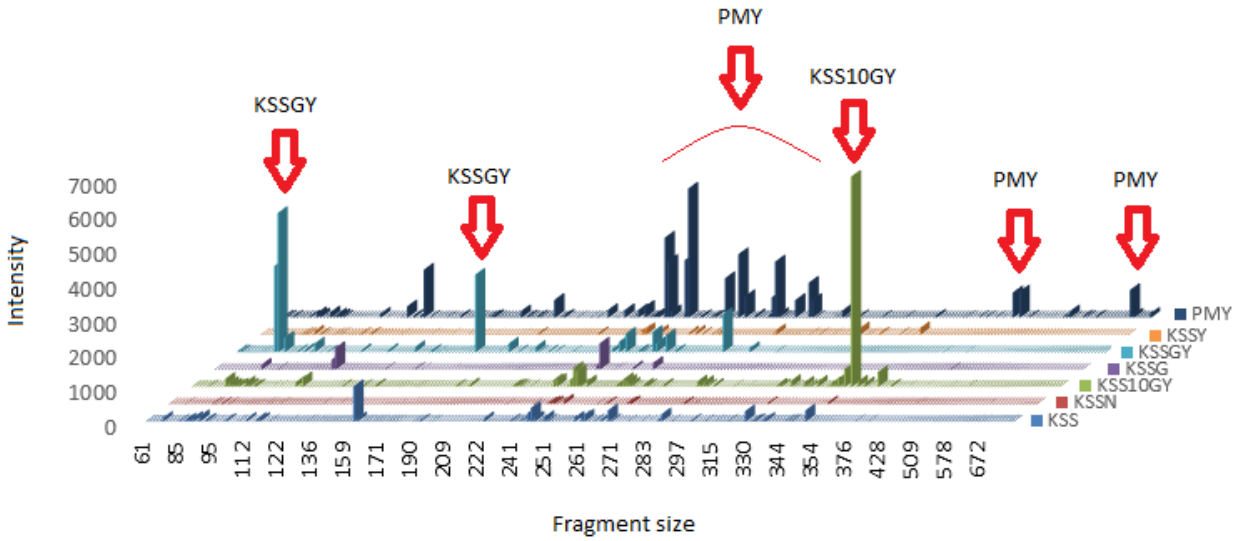


Figure 5.A.I-42 – Fungal abundance peak intensity size comparison between each treatment of each fragment size in the rhizosphere.

Bulk soil bacterial and fungal TRFLP (Harvested soil samples)

Bacterial communities in bulk soil at harvest

Analysis of harvest soil by PCA showed that the only significant differences occurred between potting mix soils PMY and PMN, and all other treatments of the soil (KSSGN, KSSGN10, KSSGY, KSSGY10, KSSN, and KSSY; Figure 5.A.I-43). This indicates that the γ-irradiation may have been insufficient to impact the bacterial communities of the bulk soil over the period of the 16 week experiment or plant endophytes colonise the soil during this time.

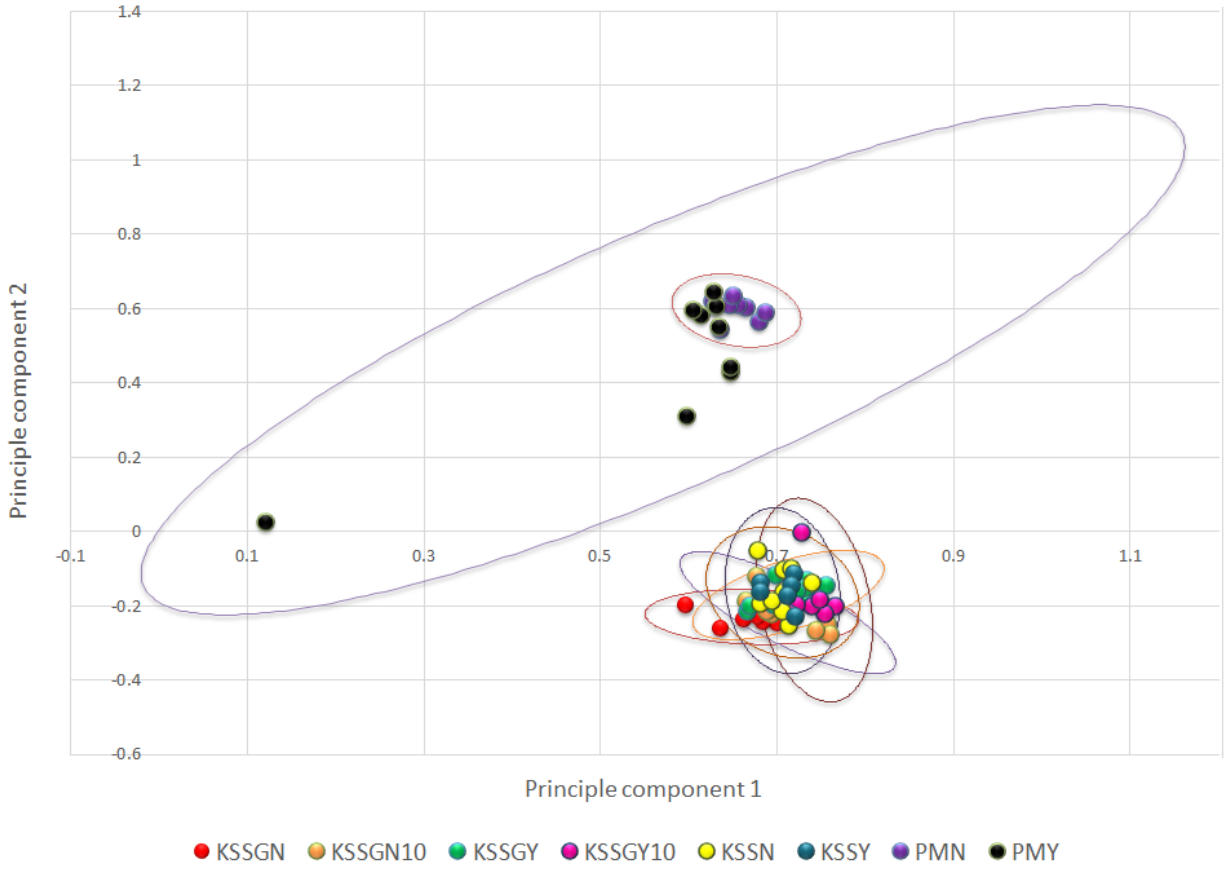


Figure 5.A.I-43 – PCA of TRFLP data for bacterial communities found in various treatments of scab-suppressive soil.

Analysis of fragment sizes from bacteria TRFLP data is shown in Figure 5.A.I-44. Here again we see that there are no clear reductions is specific peaks due to γ-irradiation over the 16-week period of the glasshouse experiment.

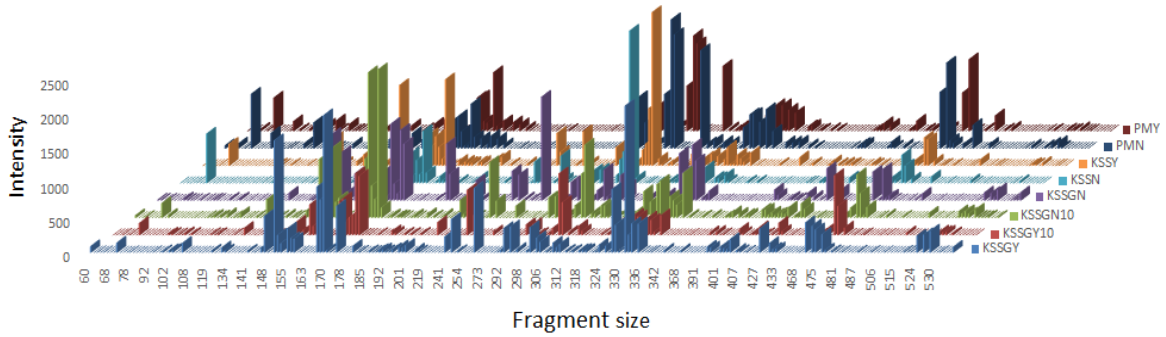


Figure 5.A.I-44 – Bacterial abundance peak intensity size comparison between each treatment (bulk soil) of each fragment size.

Fungal community in bulk soil at harvest

As found in planting soil, the fungi in the potting mix soil (PMN and PMY) differed from that found in all soils except KSSGY (γ-irradiated soil inoculated with common scab; Figure 5.A.I-45). The fungal communities in soil KSSGN (γ-irradiated soil, not inoculated with common scab) was different from all other soil samples including KKSGN10, KSSGY10, KSSGY, KSSN and KSSY. KSSGN10 (γ-irradiated Ballarat soil, inoculated with common scab and seeded with 10% KSS) also show significant differences from all other treatments (Figure 5.A.I-45). KSSN, KSSY and KSSGY10 showed very similar and overlapping community profiles.

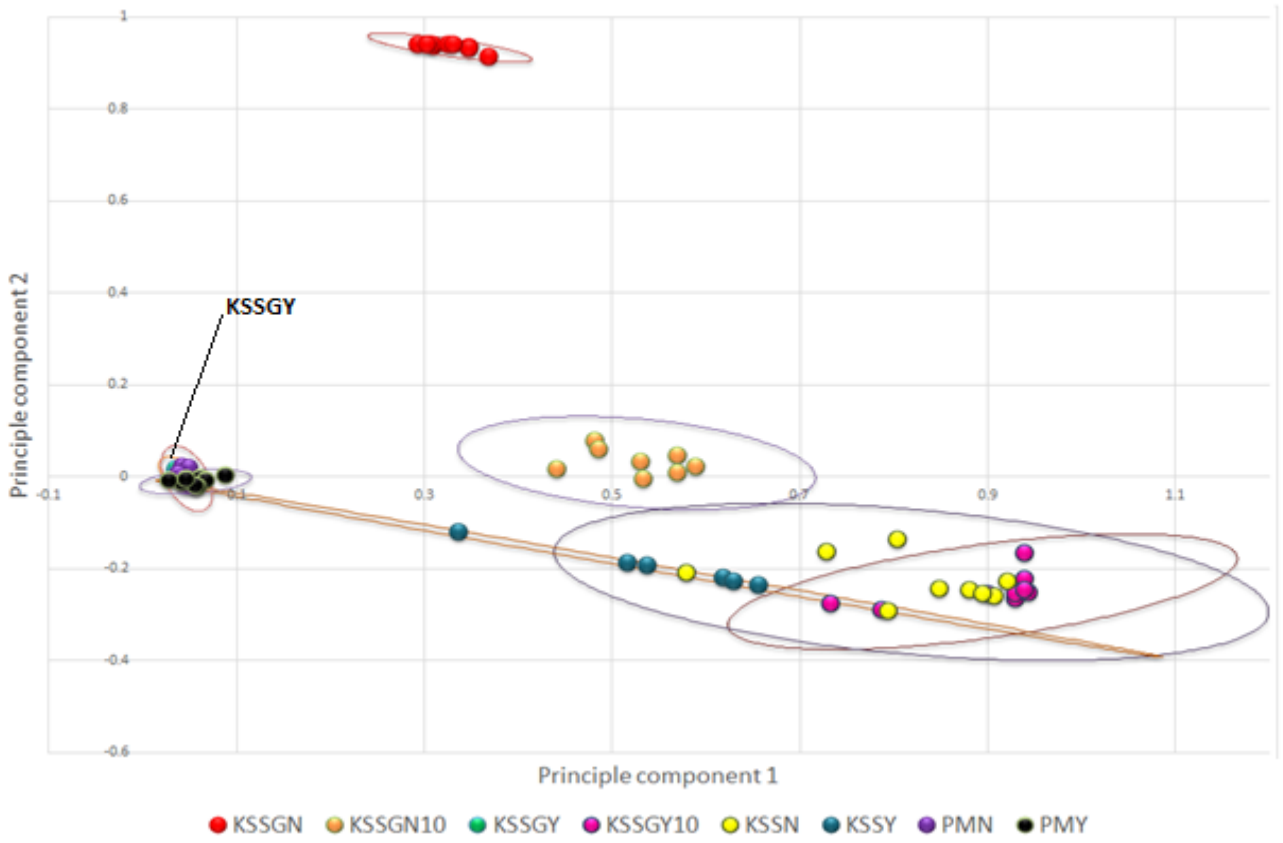


Figure 5.A.I-45 – PCA of fungal communities found in each different soil treatment (bulk soil), based on TRFLP.

A prominent peak at 92bp fragment was found in soil sample KSSGN and this may be what is most contributing to the significantly different community structure of this from all other samples (Figure 5.A.I-46). KSSGN10 (γ-irradiated soil seeded with 10% KSS not inoculated with common scab) showed a different fungal community from KSSGN, KSSGY10, KSSGY and KSSY. This may be influenced by the large peak found at 353 bp fragment (Figure 5.A.I-46). The fungal peaks in the potting mixes are clearly distinct in their bases pair configurations than that seen with all other samples.

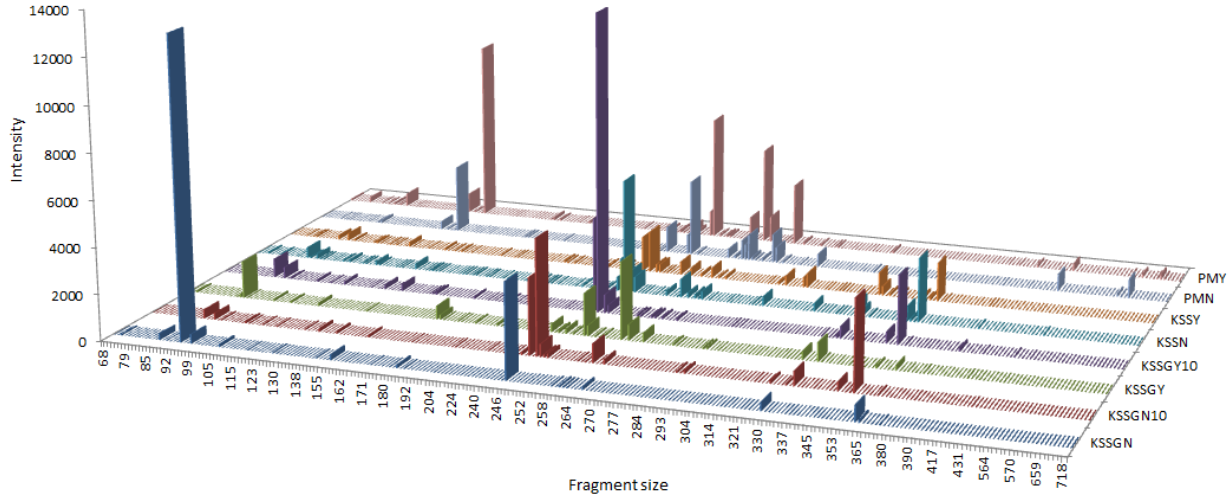


Figure 5.A.I-46 – Bacterial abundance peak intensity size comparison between each treatment of each fragment size.

DISCUSSION

We have demonstrated the transfer of the biological nature of common scab suppression to a sterile soil. Tubers from untreated native soil did not have common scab and this suppression was removed by γ-irradiation. Crucially, however, the γ-irradiated soil that had been re-seeded with native untreated soil (10% w/w) had tubers which were free completely from disease. We were able to confirm the suppressive nature of this soil to common scab and successfully transfer that suppression to a soil that was conducive for common scab disease, thus proving the biological nature of the suppression. The reduction in common scab DNA levels in both the native soil and the γ-irradiated soil supplemented with 10% of the untreated farm soil clearly showed that there was a biological component of the soil which was preventing the establishment of common scab in the soil and as a consequence, the tubers were free of common scab.

Using TRFLP analysis we showed that γ-irradiated planting soil samples have significantly different communities from that found in the untreated soil. When γ-irradiated soil (KSSGY) is mixed with 10% of KSS (original untreated soil) it widened the bacterial community pattern such that it more resembled both the treated and untreated soils but remained unique. Gamma-irradiation also changed the fungal community in the planting soil samples when compared to the fungal community in untreated soils. Interestingly, when this γ-irradiated soil (KSSGY) was mixed with 10% of KSS (original soil), (KSS10GY), the fungal community was found to be completely different from the gamma-irradiated soil (KSSGY).

Liu et al. (1995) identified two *Streptomyces* which produced antibiotics against *S. scabies*. Over a four-year period, both of these non-pathogenic *Streptomyces* survived in the soil and demonstrated significant reductions in both incidence and severity of common scab disease in a susceptible cultivar. Bowers et al. (1996) confirmed this suppression and demonstrated that there was a significant decrease in the pathogenic *S. scabies* population over time in the presence of these two *Streptomyces* strains. Ryan and Kinkel (1997) attempted to locate these common-scab suppressive *Streptomyces* and concluded that they

resided in the rhizosphere, and that there was clear evidence of competition between pathogenic and non-pathogenic strains, since an increase in pathogenic *S. scabies* reduced non-pathogenic *Streptomyces* and vice-versa. They concluded that active competition for nutrients in the rhizosphere as well as antibiotic production were key players in these suppressive soils. This competition was also observed by (Neeno-Eckwall et al. 2001) where they managed to isolate a non-pathogenic mutant of *S. scabies* which did not produce antibiotics, but was still capable of significantly reducing common scab disease when it was co-inoculated with its pathogenic parent strain. St-Onge et al. (2010) isolated at least one stable *Pseudomonas* strain that was able to stop thaxtomin biosynthesis, the cause of the common scab disease symptoms on potato tubers, by actively inhibiting genes in the thaxtomin synthesis pathway. Most studies described here have been *in vitro* plate or glasshouse studies, but most recently, Wanner et al. (2014) demonstrated that several non-pathogenic *Streptomyces* were been successful in significantly reducing both the incidence and severity of common scab in several fields over a three-year period, but not consistently, and not in the same fields.

Thus, in the case of the suppression of common scab on potatoes, there seems to be several factors at work: the effectiveness of non-pathogenic and pathogenic *Streptomyces* to colonise the soil; competition for pathogenic sites between non-pathogenic and pathogenic strains; competition for nutrients in the rhizosphere and/or direct inhibition of the pathogenic *Streptomyces* through antibiotic production, as well as a role in this suppression for other bacterial species, such as fluorescent pseudomonads.

Both of the glasshouse experiments described here have shown that this particular field soil is suppressive towards *S. scabies*, not allowing accumulation of pathogen DNA. Gamma irradiation eliminates the disease suppressiveness of this soil and heat treatment also reduces it significantly, suggesting that a biological process is involved in this suppression. The second glasshouse experiment also confirmed that this suppression to common scab suppression could be transferred, added weight to this supposition. Whether the mechanism is associated with bacteria or fungi, or another type of microbe is not clear.

In summary, these experiments showed that heat treatment and γ-irradiation are strong enough to cause significant shifts in bacterial and fungal differences in soils which correlated with increased disease incidence. These differences are maintained in rhizospheres over a period of one month; however they become less pronounced as the plants grow. These results suggest that the disease-suppressing organisms could be being detected by the TRFLP method.

Common scab suppression likely resulted from the transfer of some of the dominant bacterial species present in the native soil that were able to re-establish in the irradiated soil even after just two weeks incubation, and thus alter its diversity.

The results suggest that γ-irradiation of soils which removes the suppressive effect can provide an excellent model system for identifying the biological factors that may be playing a role in reducing the common scab disease. This can be done by adding back isolated and identified microbes, either singly or as consortia from the bulk soil back into the γ-irradiated soil to observe if common scab suppressiveness is restored.

A SOIL BIO-HEALTH INDICATOR TEST FOR DISTINGUISHING HEALTHY FROM DISEASED SOILS – A COMMON SCAB CASE STUDY

SUMMARY

To better understand why some potato fields get common scab and others do not, we sought to validate a DNA-based microbial community typing method terminal restriction fragment length polymorphism (TRFLP), on 28 soils. Soils in this experiment came from potato fields showing no disease (healthy) or a high incidence of disease (unhealthy). These were further divided into groups based on the presence or absence of pathogen DNA. These soils were used to generate fresh rhizosphere samples from tissue culture plantlets of cv Kennebec. TRFLP analysis was done on DNA extracted from these laboratory grown rhizospheres. Principle component analysis (PCA) was used to differentiate between soils that produced disease symptoms or were symptomless. No peaks for the pathogen were detected in the unhealthy soils suggesting the pathogen is either a minor component of the bacterial rhizosphere community or is not being sufficiently amplified in comparison to other bacterial species when soil inoculum is present. TRFLP profiles of soil bacterial communities appear to group based on common scab disease potential. Since non-pathogen DNA signals are able to distinguish between unhealthy and healthy soils, microbial diversity in general might serve as a more useful bio-indicator of disease than pathogen DNA itself. Efforts are underway to identify key players or factors that may be responsible for the reduced disease seen in the healthy sites. The implications are that there are potential bio-indicators of soil health and that the soil microbial community may in future be manipulated to improve soil health.

INTRODUCTION

In PT09023, the risk of common scab is not consistently predicted based on soil DNA detection of pathogenic *Streptomyces*. When pathogenic *Streptomyces* where not detected the probability of common scab was 21% compared to 29% when pathogenic *Streptomyces* was detected. The probability of predicted risk of common scab is improved when both soil and seed DNA detections are combined. However, there are still a number of cases where pathogenic *Streptomyces* were not detected in the soil or on the peel of seed tubers yet common scab occurred on the harvested tubers. This suggests that other soil microbial factors may be contributing to common scab expression.

Terminal restriction fragment length polymorphism was developed for the analysis of complex microbial communities (Liu et al. 1997; Liu et al. 1998) and early results indicated that the method was reproducible (Osborn et al. 2000). There are four parts to the TRFLP assay and each requires an objective methodology. In Stage 1, species-specific regions of DNA (e.g. the ITS regions of ribosomal DNA) are polymerase chain reaction (PCR) amplified with fluorescently-labelled primers and subsequently digested with restriction enzymes. In Stage 2, the terminal restriction fragments (TRFs) are thus tagged and their sizes are measured with extreme precision by instruments such as capillary electrophoresis DNA analysers. Variables of the fragment sizing programs are size-calling algorithm, sizing accuracy, peak detection, split peak correction, peak smoothing options, minimum peak half width, baseline setting, and analysis range. Stage 3 is a profile editing step; the process of fragment size calling with the use of programs such as GeneScan or Peak Scanner to develop a community profile or fingerprint applied to ensure that the integrity between subsequent peaks along a fingerprint has a minimum of one base-pair resolution. Stage 4 is called profile alignment; a step that requires an alignment of fingerprints for their comparison.

The TRFLP method has been one of the most widely used methods for analysis of microbial communities since its first use with soil samples (Osborn et al. 2000). For most bacterial community applications and soil studies, the16S ribosomal genes were amplified from community DNA. For soil, it is particularly useful because the method enables unknown populations, as communities, to be defined with a value of abundance, based on the digital information of peak area generated by the fragment analysis software. The fundamental principle of the TRFLP method, when applied to a community, must rely on accurate fragment size calling, which has as its basis the use of a standard curve of known fragment sizes.

TRFLP was initially developed as a community profiling technique with the number of TRFs used as an indication of diversity, and shifts in the patterns of TRFs used to describe community fingerprints (Anderson and Cairney 2004). It has subsequently been modified to permit the identification of species by comparison of multiple digestion products and/or multiple different tagged primers with a database of known species (Dickie et al. 2002). This latter approach is particularly useful for fungi, where diversity is low relative to bacteria and a database of TRF profiles of well-identified fungi can be readily obtained from sporocarps or developed virtually via databank mining (Edwards and Turco 2005).

Many studies have been conducted on agricultural soils to investigate the change in community fingerprints after cultural practices (Edel-Hermann et al. 2004).

M-TRFLP analysis is a molecular tool for simultaneous investigation of multiple taxa, which allows more complete and higher resolution of microbial communities to be obtained (Singh et al. 2006). M-TRFLP analysis is as reproducible and consistent as TRFLP analysis but is a cheaper, less labour-intensive, and more rapid method for analysis of microbial communities when information for more than one taxon is required as it allows simultaneous characterization of up to four different targets in one reaction mixture (Singh et al. 2006).

To better understand why some potato fields get common scab and others do not, we sought to validate a DNA based microbial community typing method terminal restriction fragment length polymorphism (TRFLP) on 28 soils by the following methodology.

MATERIALS AND METHODS

Dry rhizosphere soil was derived from 28 soil samples provided from Victoria, Australia, from a multitude of sites and paddocks (Table 5.A.I-49) by adding the bulk soil sample to sterile sand (10% w/w). Tissue-culture leaf cuttings of Kennebec were grown in this mixture with nutrients coming from Murashige-Skoog mixture with no added carbon. Plants were grown for one month in an incubator. Rhizosphere soil was collected by shaking uprooted plants and DNA was extracted (Norgen soil DNA extraction kit) from the soil. PCR was performed on the 16S rDNA gene using primers 63f and 1389r. Each primer had a fluorescent dye label. PCR products were digested with the enzyme *Hha*I. Fragment analysis was conducted at Western University, London, Ontario. Due to sample failure multiple times, samples 25 and 26 are not represented. Data analysis was conducted with XLStat.

RESULTS

TRFLP (TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM) DATA ANALYSIS

To confirm that we could differentiate between healthy and diseased samples, principal component analysis (PCA) was conducted on the samples with no scab incidence or scab pathogen detected (healthy, samples 15-21) and samples with scab incidence and scab pathogen detected (diseased, samples 22-28) (Table 5.A.I-49, Figure 5.A.I-47). To generate the PCA, normalized peak area data were pooled for replicates of the same sample (small peak areas were disregarded from this analysis). From this, it is evident that healthy rhizospheres are distinct from diseased rhizospheres for most samples. The same pattern emerges when binary data (peak presence/absence) are used (data not shown). Interestingly, the TRFLP profiles for samples 16 and 17 (peak area data), both from the same field, appear to be more similar to those from the diseased samples, despite no scab disease or pathogen being detected in the sample (Figure 5.A.I-47).

Table 5.A.I-49 – Soils received from Australia were grouped based on common scab incidence and the quantification of pathogenic *Streptomyces* DNA in a soil; Group 1 (pink, no detectable scab, low level DNA), Group 2 (blue, detectable common scab, no detectable DNA), Group 3 (green, no common scab or detectable DNA), Group 4 (yellow, detectable common scab and DNA).

Sample No.	Common scab incidence (% plants affected)	*S. scabies* pg DNA/g soil
1	0	119
2	0	67
3	0	63
4	0	48
5	0	32
6	0	28
7	0	26
8	53	0
9	46	0
10	19	0
11	17	0
12	15	0
13	13	0
14	11	0
15	0	0
16	0	0
17	0	0

Sample No.	Common scab incidence (% plants affected)	*S. scabies* pg DNA/g soil
18	0	0
19	0	0
20	0	0
21	0	0
22	75	20021
23	100	18781
24	65	8481
25	70	5698
26	86	5670
27	89	4863
28	30	4518

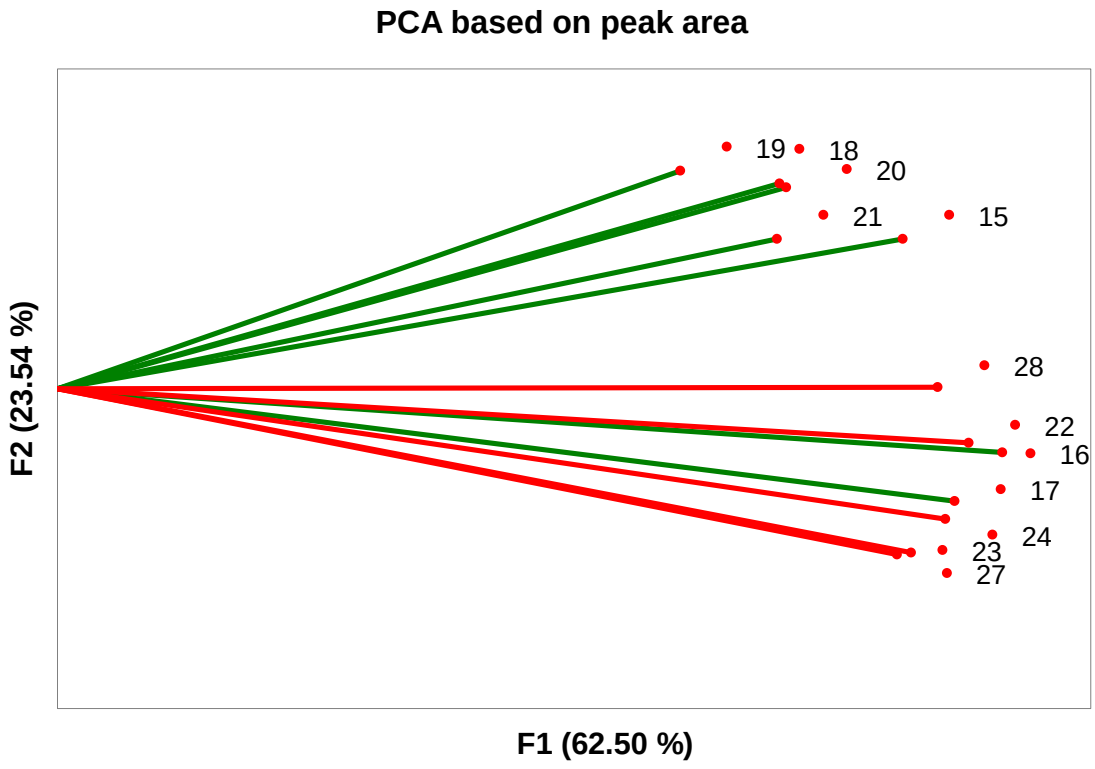


Figure 5.A.I-47 – Principal Component Analysis (PCA) (covariance) of TRFLP profiles from healthy samples (green) and diseased samples (red). F1 and F2 are measures of variation, the two most important principle components in the analysis accounting for 62.5% and 23.5% of the variation, respectively.

To confirm the statistical significance of these groupings, we drew 95% confidence intervals around the samples F1 and F2 components generated in the above PCA (Figure 5.A.I-48). Since samples 16 and 17 clustered with the diseased samples, yet were labelled as healthy, they were removed from this analysis. It is possible that these two outliers may be different in the diversity of their major bacterial groups. Their inclusion would significantly affect the analysis.

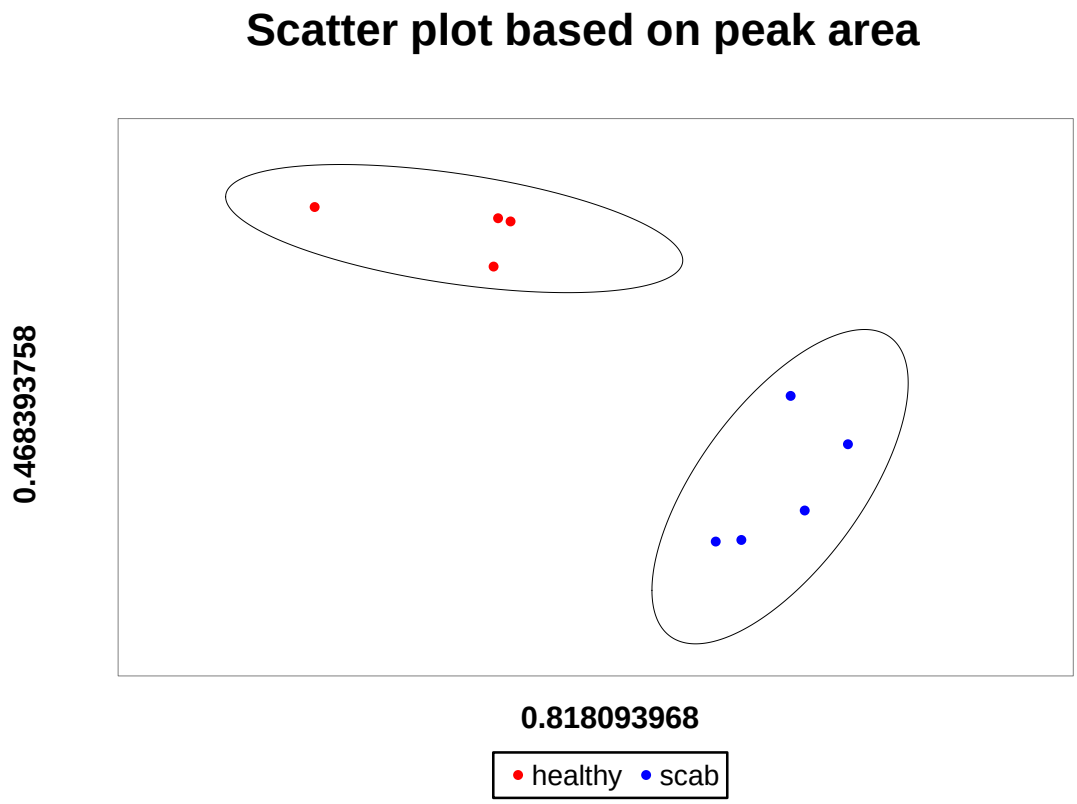


Figure 5.A.I-48 – Scatter plot showing confidence intervals of F1 and the F2 components generated in the PCA of the healthy (green) and diseased (red) samples. The outlier samples 16 and 17 were removed from this analysis. The X and the Y axis are correlation coefficients derived from the PCA analysis of the healthy and diseased samples in Table 5.A.I-49. The healthy and diseased samples have confidence intervals which do not overlap, indicating the rhizospheres from these groups are statistically different.

To observe the relatedness of the healthy and diseased samples, hierarchical clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA). To do this, binary data is used. These results agree with the pattern generated during PCA; however, sample 21 appears as an outlier in this cluster (Figure 5.A.I-49). The values on the Y axis are Jaccard's similarity coefficient. The closer this number is to 1, the more similar the two samples are. This analysis was done with peak area data that eliminated 50% of detectable signals (those which were weak and did not meet certain arbitrary cut-offs.

This analysis indicates that the samples 16, 17 and 27 have bacterial populations that are dominated by different microbes than those found in the groupings of either scab infested or scab free soils.

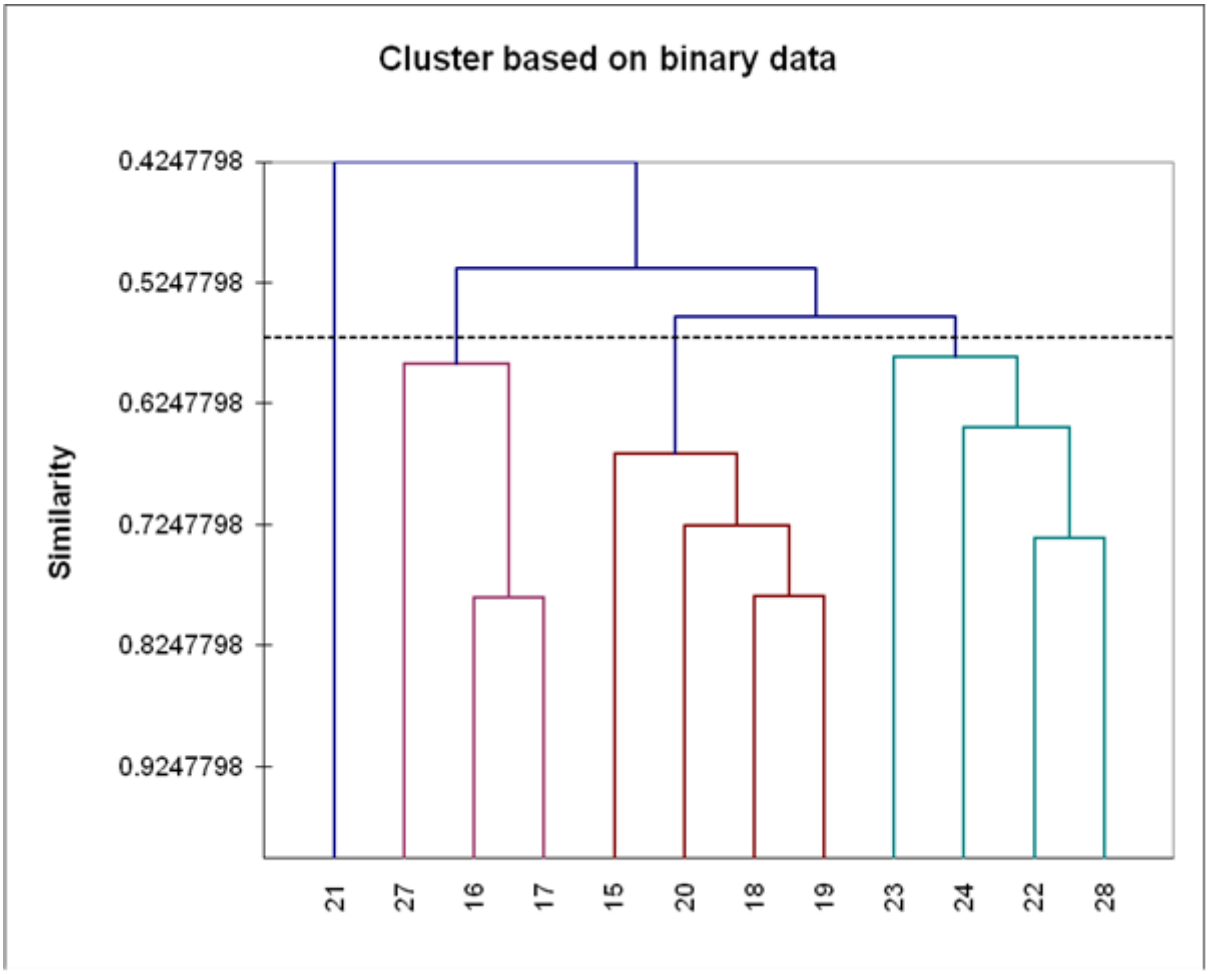


Figure 5.A.I-49 – Hierarchical cluster of binary data showing clustering of healthy (15–21) and diseased samples (22–28).

The remaining samples were added to the analysis. These samples have conflicting scab incidence and scab pathogen detection data. In group 1 (samples 1–7), the pathogen was detected (albeit in smaller quantities than the diseased plants) but no disease incidence was recorded, while group 2 (samples 8–14) exhibited scab symptoms in the absence of detectable pathogen. The data appear to fall into two categories as shown previously (Figure 5.A.I-50). The top groups contain all the diseased samples (22–28) and the two healthy samples that grouped previously with disease (samples 16 and 17). Of the group 1 samples, only two grouped with the diseased samples (samples 4 and 7) although both are on the edge of this group. Of group 2 samples, around half fell into the disease group (11, 12, 13 and 14) and just over half into the healthy group (8, 9, and 10). Samples 11 and 12 group tightly with the diseased samples and are potentially

diseased, while sample 13 groups tightly with the healthy and is likely healthy. The remaining samples in this group (8, 9, 10, and 14) fall into a middle ground. Similarly, five of seven samples from group 1 clustered with the healthy samples, indicating these samples may not have been diseased. The data indicate that samples 16, 17 and 27 (healthy, healthy and diseased samples, respectively), which together in Figure 5.A.I-49 have bacterial populations that are dominated by different microbes than those found in groupings of either scab-infested or scab-free soils.

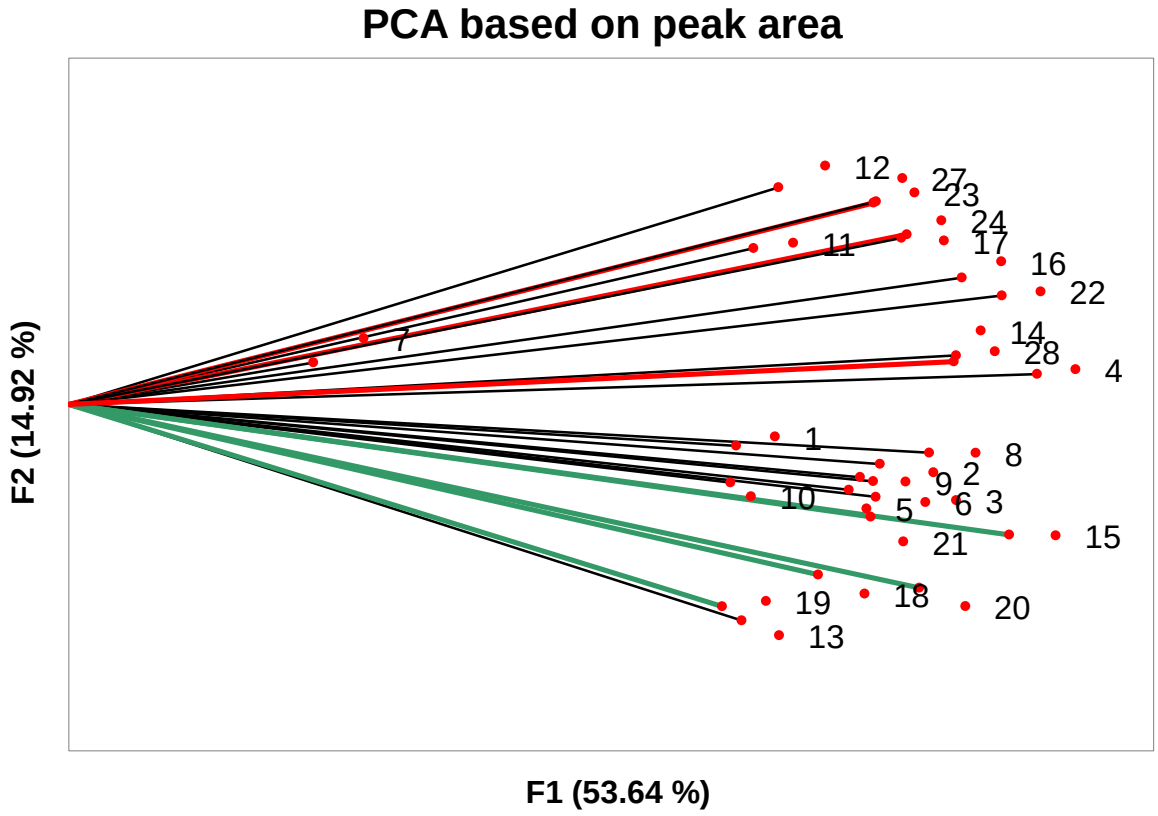


Figure 5.A.I-50 – PCA using pooled, normalized peak area data from the TRFLP analysis; healthy samples (green) and diseased samples (red), unknown samples from groups 1 and 2 (black). F1 and F2 are measures of variation, the two most important principle components in the analysis accounting for 53.64% and 14.92% of the variation, respectively

The data analysis reported here was focused on potential TRFLP relationships based strictly on peak area and disregarding any small peaks as an indicator of profile similarities. This eliminated 50% of detectable signals, essentially weak signals that did not meet a certain arbitrary cut-off. We used Peakscanner™ software and only included signals that had peak areas greater than 100 (or over 1% of the total peak area detected). This is a conservative approach whereby false positive data is excluded, i.e. peaks that were artefacts due to noise.

Further data analysis with Genemarker® allowed for a better comparison of groups that included all the peaks providing a comparison of profiles based on presence/absence data instead of peak area and size. This has resulted in a much more refined analysis providing better segregation of the different pathogen/disease groups and outliers now fitting into expected clusters. It is possible to increase signal detection by reducing the detection threshold to just above background noise levels: in our methodologies, background noise is normally around 15, any peak with intensity of 30 or above is clearly a signal.

To confirm that we could differentiate between bacterial communities that are conducive to scab or suppressive to scab, we used principal component analysis (PCA) on bacterial DNA fingerprints (terminal restriction fragment length polymorphism or TRFLP) from potato rhizospheres of plantlets grown in the soils sent to us from Australia. These soils were identified as producing tubers that had no incidence of common scab or detectable levels of scab pathogen (healthy, samples 15–21) and samples that yielded tubers with both scab incidence and detectable levels of scab pathogen (diseased, samples 22–28, Figure 5.A.I-51). Samples 7, 25 and 26 amplified poorly, perhaps because of PCR inhibitor carryover during the DNA extraction process and were thus not included in the analysis. To generate the PCA, normalised peak intensity data measured by Genemarker® were pooled for replicates of the same sample, the data were then transformed into presence or absence data (0 or 1), and analysed in XLStat. Figure 5.A.I-51A displays this PCA data as a biplot, with clear sample segregation by healthy (green) or diseased (yellow). For illustrative purposes, relative intensities of TRFLP signals from samples 17 and 27 are shown side by side with reverse fragments first, followed by forward fragments (Figure 5.A.I-51B). It is obvious in this figure that most of the differences in the two samples are not in the large peaks, but rather in the small ones, representing the more rare bacteria which are present in the samples. A notable exception is reverse fragments of sizes 101 and 102 base pairs (bp), which are exceptionally large in the healthy sample but absent in the diseased sample. We expected to find peaks associated with the amplicons from the pathogen *S. scabies* in the diseased soil. Virtual TRFLP profiles generated by scanning *S. scabies* 16S DNA sequence for the same enzyme cut sites used in this study (*Hha*I) results in predicted peaks of 307 bp (reverse with FAM label) and 433 bp (forward with a VIC label). Peaks of this size were not observed in any samples, however, strongly suggesting that relative to other bacterial species, *S. scabies* is relatively a minor component of the Kennebec potato rhizosphere bacterial community even if it is present in the soil inoculum. Nevertheless its presence can be detected through measurement of other bacterial species which obviously appear to act as bio-indicators of soil ecology where this pathogen can establish.

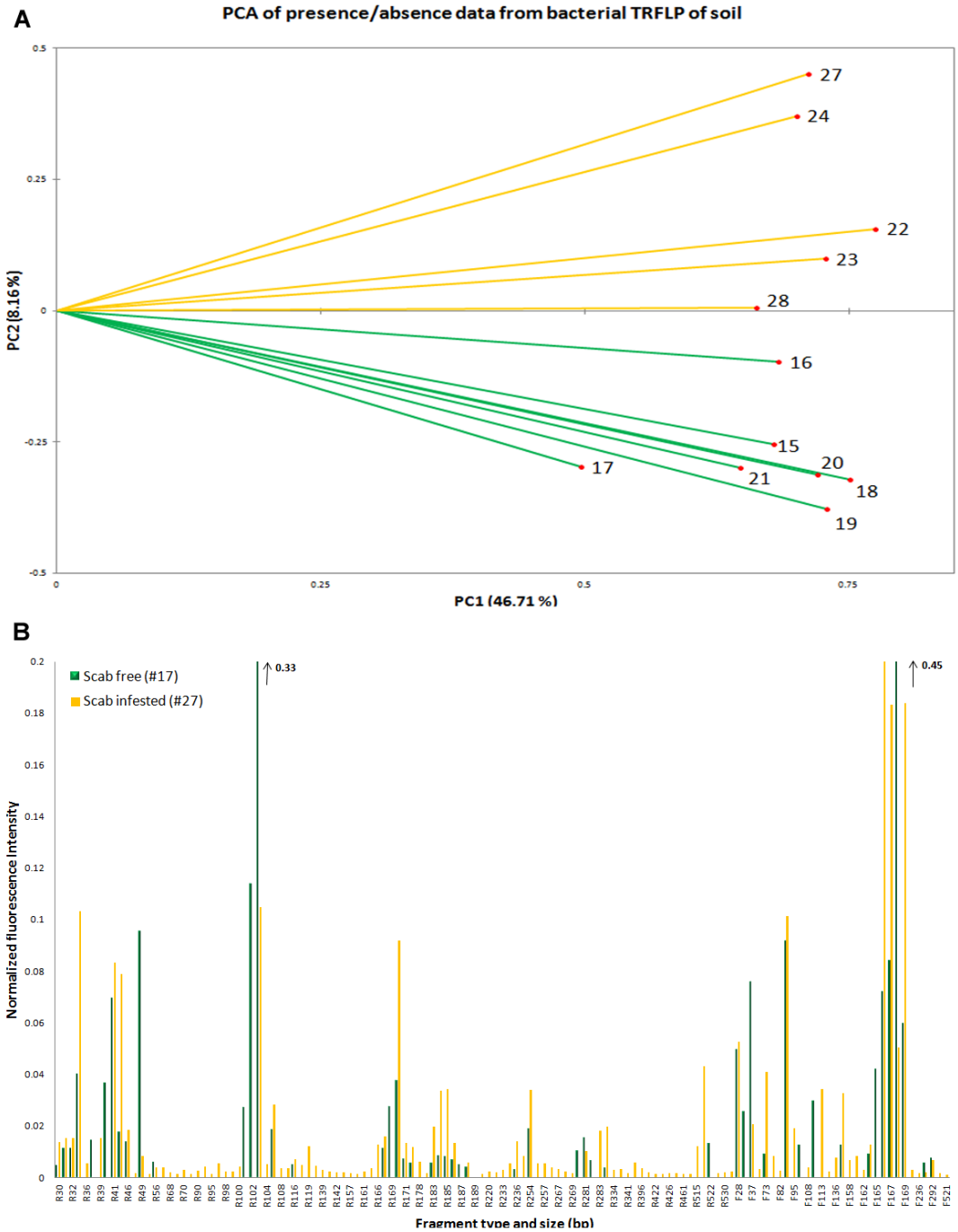


Figure 5.A.I-51 – A) Biplot display of PCA of bacterial 16S TRFLP data of DNA extracted from rhizospheres inoculated with soil having no evidence of common scab incidence (healthy - green) and soils where scab severity and DNA was high (diseased - yellow). B) A virtual TRFLP displaying normalized signal intensity values from soil samples 17 (healthy) and sample 27 (scab-infested). Peaks labelled as R, are reverse (labelled with VIC) while peaks labelled with F are forward (labelled with 6FAM).

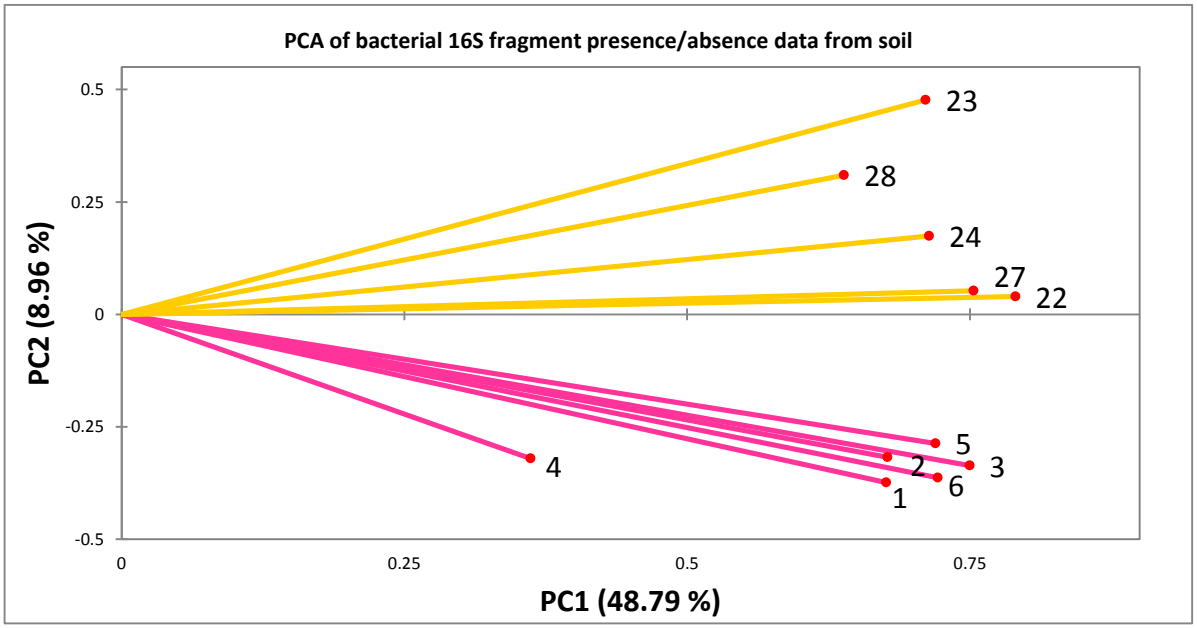


Figure 5.A.I-52 – A biplot of PCA of bacterial TRFLP from rhizospheres of potatoes inoculated with soils having DNA from the common scab bacteria but no tuber symptoms (pink) and a soil heavily infested and tubers showing scab symptoms (yellow). Sample 7, 25 & 26 omitted.

DNA extracts from rhizosphere soil samples 15–21, which produced tubers free of common scab and where no detectable levels of DNA were present would be considered 'healthy' soils for potato production (green). Similarly, soils 1–6 (pink) which also produced disease free tubers although they had low levels of pathogenic *Streptomyces* DNA present could also be considered 'healthy' as from growers' perspective there would be no difference in the quality of tubers harvested. Comparison of samples 1–6 to samples 22–28 (with heavy disease and scab DNA, Figure 5.A.I-52) indicated that the microbial communities were very clearly distinct. However, the microbiomes of soils 1–6 and soils 15–21 were quite similar to that shown in Figure 5.A.I-53. Soil 17 and 4 were comparable to each other, but were somewhat distinct from the profiles of other soils, and it is unclear what factor may have caused their bacterial profiles to be diverse from others. Sample 7 amplified poorly, perhaps because of PCR inhibitor carryover during the DNA extraction process and was thus not included in the analysis.

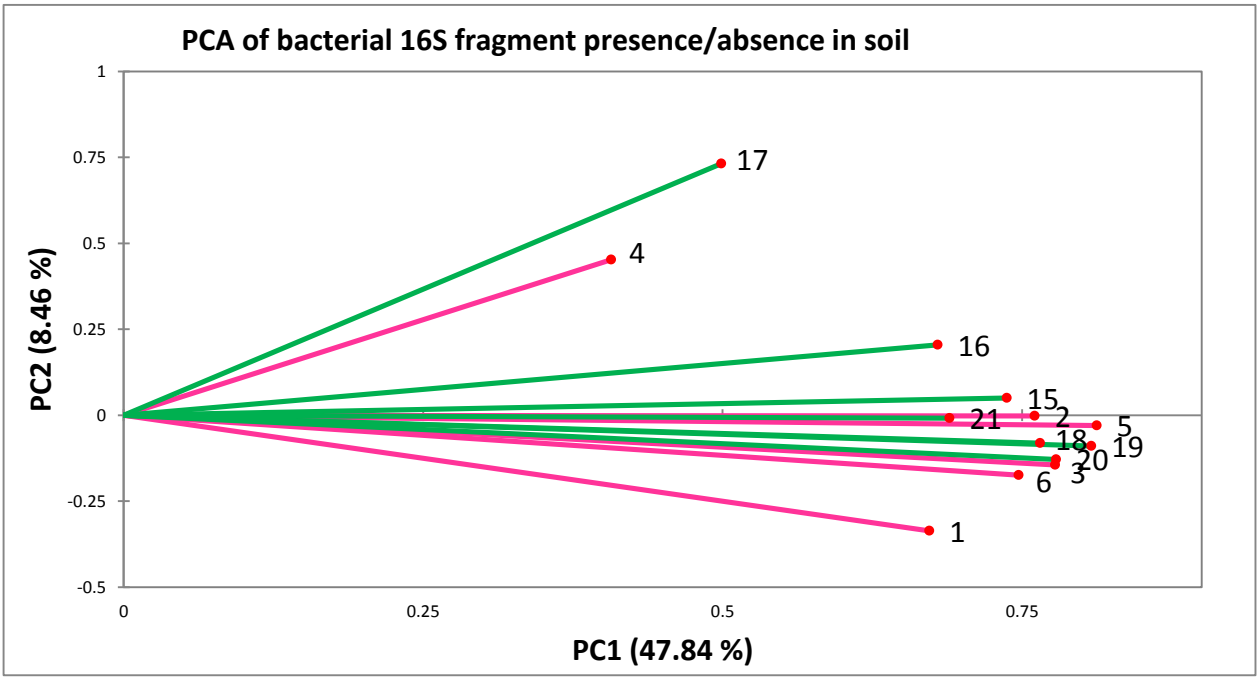


Figure 5.A.I-53 – A biplot of PCA of bacterial TRFLP from rhizospheres having low levels of DNA from the common scab bacteria but no tuber symptoms (pink) and soil were neither scab DNA or tuber symptoms were observed (green). Sample 7 omitted.

Rhizospheres from plants inoculated with soil samples 8–14 where tubers had quite significant levels of scab but no detectable levels of DNA (blue) were also expected to segregate from rhizospheres inoculated with samples 22–28 (heavy disease and Ss DNA present) (yellow). Interestingly, they appear similar in the diversity of their bacterial communities (Figure 5.A.I-54). This would suggest either that scab in this soil is being caused by another type of organism that is not pathogenic *Streptomyces*, that pathogenic *Streptomyces* and associated bacterial species are present at levels high enough to cause disease but low enough to avoid detection, or that there was some type of experimental problem in the assays used for identification of the bacterial DNA in these samples. Here again it is noted that soils 8 and 9 may have different bacterial communities from that found in the other samples.

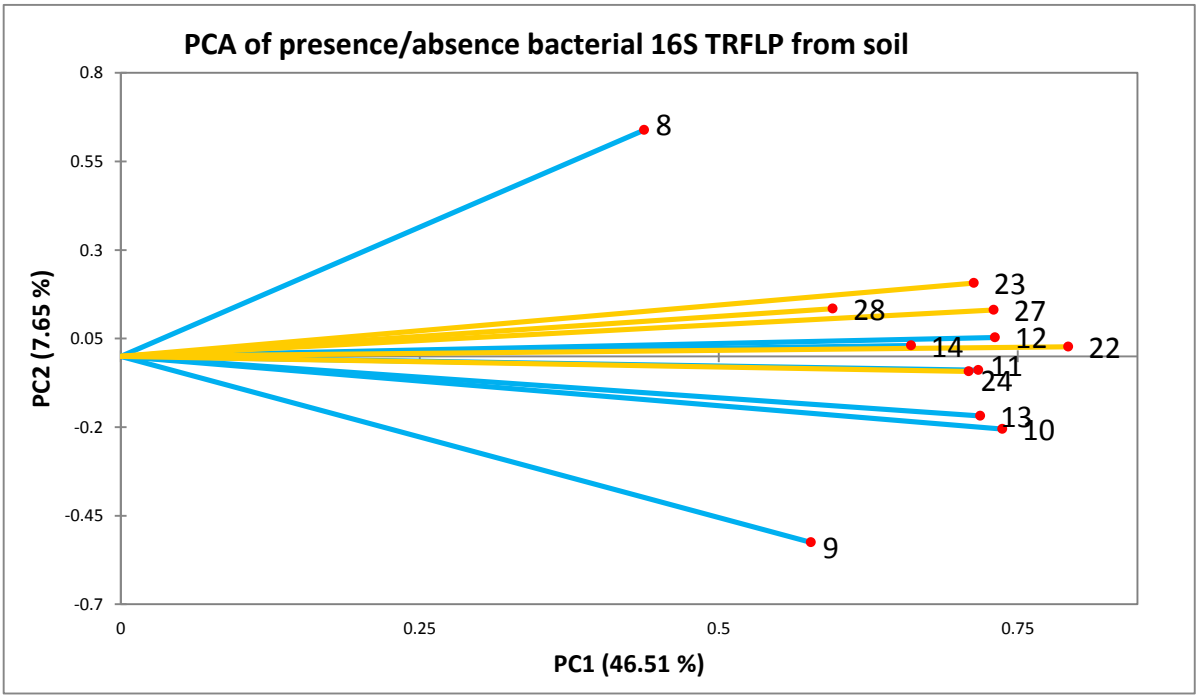


Figure 5.A.I-54 – A biplot of PCA of bacterial TRFLP from rhizospheres of potatoes inoculated with soils having moderate levels of scab symptoms but no scab DNA (blue) and soil with high DNA levels and tubers with scab (yellow). Samples 25 & 26 omitted.

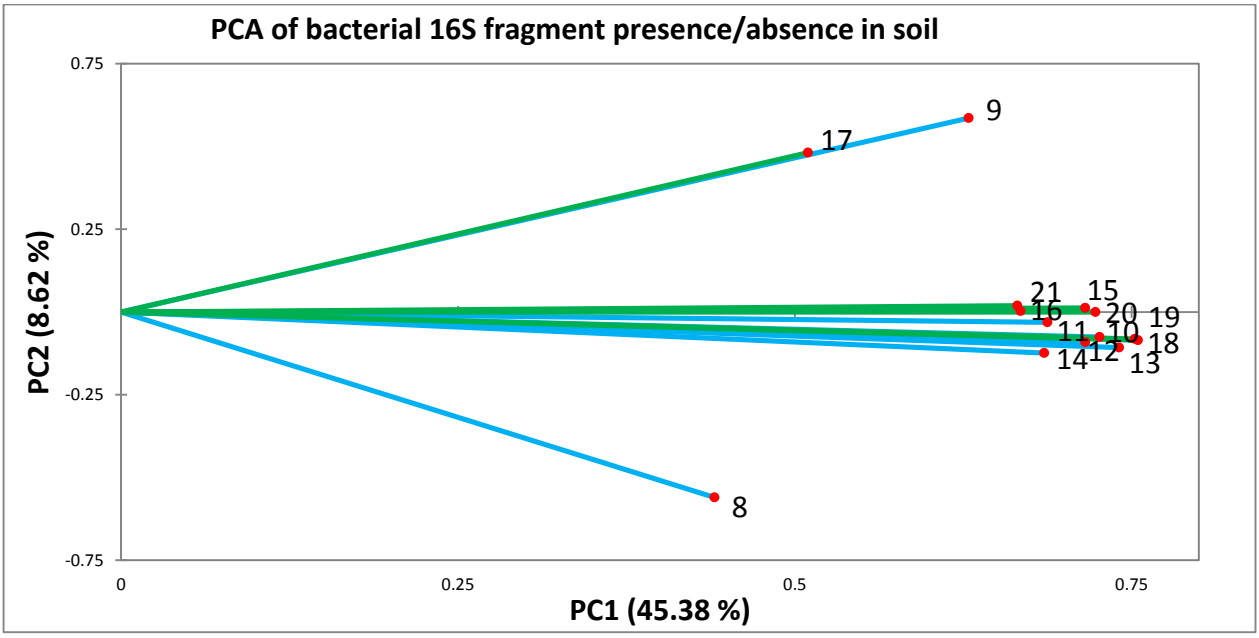


Figure 5.A.I-55 – A biplot of PCA of bacterial TRFLP from rhizospheres of potatoes inoculated with soils having moderate levels of scab symptoms but no scab DNA (blue) and soil were neither scab DNA or tuber symptoms were observed (green).

Surprisingly, when the bacterial community of rhizospheres from plants inoculated with samples 8–14 (blue; where incidence of scab was found but no DNA was detected) were compared to rhizospheres 15–21 (where no scab was present; green), the species diversity in bacterial communities was found to be similar (Figure 5.A.I-55). Once again soils 8 and 9 behaved as outliers relative to the rest of the clustered samples, in addition to sample 17, which was unlike the other green samples.

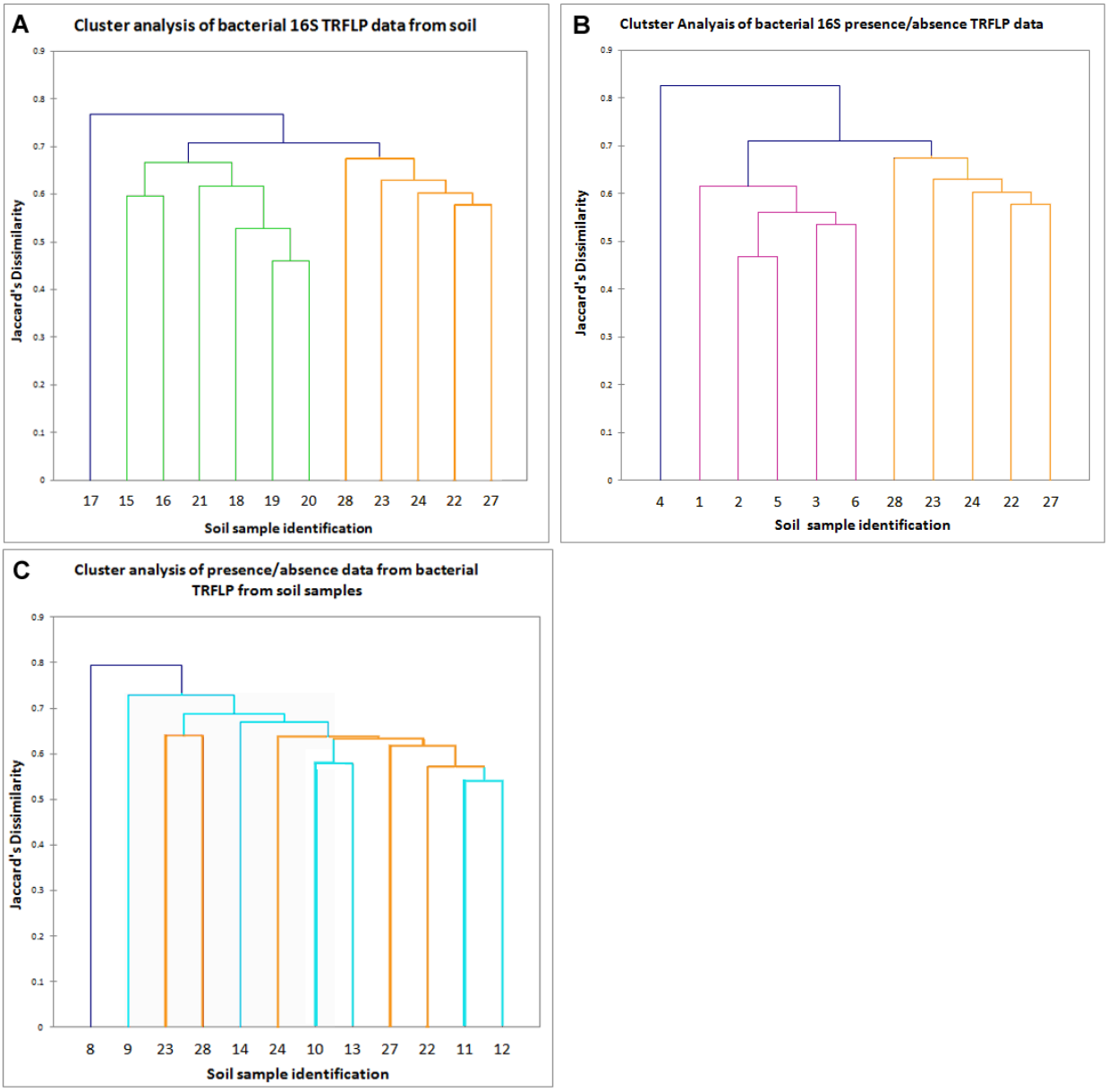


Figure 5.A.I-56 – Hierarchical clustering of binary data showing clustering of DNA from soils with high scab severity and high levels of *S. scabies* DNA (22–28) compared to scab-free and DNA-free soils (15–21)(A), disease-free and DNA-containing soils (1–7)(B), and moderately-diseased and DNA-free soils (8–14)(C). Note that soils 17, 4 and 8 are outside the common cluster groups.

DISCUSSION

In this proof-of-concept study, we could distinguish between healthy and common scab-diseased soils using TRFLP analysis. TRFLP analysis is a technique suited to measuring microbial diversity, rather than individual strains. It can be difficult to relate the TRFLP information directly to disease thresholds or pathogen DNA, and it can be criticised for not being sensitive enough or not directly yielding microbial identities. Although

it cannot be as sensitive as direct sequencing of thousands of DNA sequences, reducing detection thresholds can increase the assay's sensitivity. Using sample 17 as an example, 31 peaks were detected above the 100 cut-off (old technique), whilst 74 peaks were detected when the detection threshold was reduced to 30. Likewise, bacterial identities can be elucidated through sequencing of PCR products instead of running them through the TRFLP protocol, or a new technique called physical TRFLP (not described here, and outside the scope of the current project) which allows for direct sequencing of peaks of interest.

Although it would be good to directly detect pathogenic DNA through TRFLP, this experiment suggests that it is not necessarily at high enough levels for detection even when it is abundant and able to cause disease. The fact that other non-pathogenic DNA signals were able to predict common scab incidence suggests that microbial diversity in general (and perhaps other specific but unknown bacteria) might serve as useful, even more accurate bio-indicators than pathogenic *Streptomyces* DNA itself. This is consistent with the findings In PT09023, the risk of common scab is not consistently predicted based on soil DNA detection of pathogenic *Streptomyces*. When pathogenic *Streptomyces* where not detected the probability of common scab was 21% compared to 29% when pathogenic *Streptomyces* was detected.

These analyses suggest that to develop a diagnostic method for scab conduciveness we should be using bacterial profiles of soils known to generate severe scab in tubers (soils 22–28, for example) as reference samples to compare with test soils. Tests comparing unknown samples to soils where scab is never seen on tubers (soils 15–21) or where DNA may be present in very small amounts (soils 1–6) would not allow one to observe differences between bacterial communities, and thus draw any conclusions about the disease potential in the microbial community. Soils such as samples 8–14 for now appear anomalous and will have to be further analysed before their ecological details become clear.

There is a clear indication that soils have particular ecological conditions that permit the establishment and growth of the scab pathogen (and similar or related species of bacteria). Thus the entire bacterial community in a particular soil may serve as a bio-indicator of scab disease presence and disease potential. Common scab-free soil may occur under any number of chemical, physical or environmental conditions which suppress the population of scab pathogen in soil, resulting in any number of different types of bacterial populations that require much greater characterisation—these cannot be used directly for diagnosis of scab-causing soil. For example, Figure 5.A.I-55 shows comparison of scab-free to scab-causing, but DNA-free soil. One might have concluded that the blue samples were also scab-free because they clustered with the scab-free green samples, but this would have been erroneous.

An alternative statistical analysis (hierarchical clustering) was performed using the unweighted pair group method with arithmetic mean of all three comparisons and the results are shown in Figure 5.A.I-56. Nearly identical patterns were found to that observed with PCA in that scab-free soils (green) which segregate completely from soil with high scab (yellow) where the soils within each group are similar to each other. Similar agreements to the biplot analysis are seen with the other two comparisons made i.e. the soil with scab DNA but no symptoms and soil with tuber symptoms but no DNA and soil with high disease. Soils 8 and 9 have very different characteristics in microbial profiles to the other soils in this grouping. Since we are sure that *S. scabies* DNA is not a part of the TRFLP profiles, the soil bacterial communities appear to group by disease potential. It is evident that unknown members of the bacterial community are able to accurately serve as bio-indicators of scab disease potential in soil.

The potential benefits for growers are that these tests could provide an indication of the potential of potato fields to develop disease based on the soil microbial ecology as distinct from tests for pathogen DNA which indicates risk only on the basis of the amount of inoculum in the field.

INFLUENCE OF SOIL CHEMISTRY ON COMMON SCAB DISEASE

SUMMARY

Trials were conducted over five seasons near Cora Lynn in the peaty clay loams of the Koo Wee Rup district, a major crisping production region of Victoria to evaluate the oxide, carbonate and sulphate forms of calcium and magnesium, as well as an elemental sulphur treatment for common scab control. None of the calcium and magnesium treatments had a significant effect on the incidence and severity of common scab in three trials, despite changes in soil pH and associated soil and plant chemistry. However, hot lime (calcium oxide) increased scab incidence in one trial by nearly 50%.

Different forms and rates of elemental sulphur lowered pH and raised soil sulphur concentrations, they had no effect on common scab incidence or severity. In one case elemental sulphur at 1.5t/ha reduced common scab but had a detrimental effect on yield.

The outcome of these trials indicates that growers should be cautious when using pH modifiers. With the exception of hot lime, treatments that raise pH generally do not increase the risk of common scab on the peaty clay loams of the Koo Wee Rup region. Applications of elemental sulphur in this soil type cannot be relied on as a control for common scab.

Canadians found evidence that optimising the soil potassium to magnesium ratio (K:Mg) to 0.4 reduced common scab disease. In this project, we tried to implement this in Victoria for common scab control in five trials on two different soil types. We demonstrated that it is possible to have some impact on common scab disease by manipulating the K:Mg ratio in one field trial, but no recommendation can be made at this stage without further trials in a range of soils types. The take-home message is that it has proved difficult to determine the the correct balance of amendments to reach the desired target of 0.4.

Historically, manganese and ammonium sulphate were identified as a potential control measures for common scab. Different rates of both of these compounds tested in a field trial had no effect on common scab incidence or severity. Greenhouse gas nitrous oxide from soil from the ammonium sulphate treatments were higher than from the untreated plots, including plots treated with the stabiliser.

INTRODUCTION

This study investigated the effects of different soil pH modifiers, manipulating the soil K:Mg ratio, different forms and rates of elemental sulphur, different rates of manganese sulphate and a nitrogenous fertiliser in the form of ammonium sulphate on common scab disease, soil and plant nutrients and potato yields. Several trials were conducted near Cora Lynn on the peaty clay loams of the Koo Wee Rup swampy plain and on a red-brown earth soil near Mirboo North in Central Gippsland.

A useful and important component of common scab management is the use of resistant varieties (Loria 2001; Powelson et al. 1993, Pasco et al., 2005). Throughout this study, cultivars chosen were typical of those used by the co-operating growers. Four cultivars were used: Simcoe, Atlantic, Catani and Wilwash, in order to investigate the impact of cultivar resistance/susceptibility.

DEVELOPMENT OF MOLECULAR TOOLS TO STUDY OF THE EFFECTS OF NUTRIENT MANAGEMENT OPTIONS ON THE COMMON SCAB PATHOGEN STREPTOMYCES SCABIES IN-VITRO

SUMMARY

The real-time reverse transcription quantitative PCR (RT-qPCR) technique was developed to measure *txtA* gene expression (thaxtomin production) in pathogenic *Streptomyces* strains. A comparison of yeast malt extract (YME) and Goyer & Loria (G&L) liquid media found that the strength of both media has a direct effect on the expression of the *txtA* gene. G&L media was used at half-strength to grow *Streptomyces* spp. *in vitro* determining the expression of *txtA* gene without compromising biomass production. This technique was then used to determine the effect of manipulating the K:Mg ratio and different concentrations of manganese on the expression of *txtA* gene of *Streptomyces* in liquid culture. Only one rate of the K:Mg treatment (0.4) increased *txtA* expression in *in vitro* culture. None of the manganese treatments had an effect on *txtA* gene expression.

EVALUATING GROWTH MEDIA FOR OPTIMUM GROWTH OF STREPTOMYCES SCABIES CULTURES AND OF TXTA GENE EXPRESSION

INTRODUCTION

Thaxtomin A (*txtA*) is the predominant phytotoxin produced by pathogenic *Streptomyces* in tuber tissue, which induce scab symptoms through necrosis and plant cell hypertrophy (Loria et al. 1995). Since the mechanism of pathogenicity based on thaxtomin seems to be conserved, the biological basis for variation in aggressiveness is assumed to be differences in the production of thaxtomin, and genetically distinct *Streptomyces* isolates differing in thaxtomin production *in vitro* have been described (Loria et al. 1995; Kinkel et al. 1998; Beausejour et al. 1999; Healy et al. 2000).

Management of common scab is difficult and often fails. It has been demonstrated that some nutrient amendments applied in the field such as potassium, magnesium and manganese can reduce disease severity on potatoes. Little knowledge is available on the effect of management strategies on thaxtomin production. In this study we have developed a RT-qPCR assay to test for thaxtomin production by measuring the expression of the *txtA* gene. This technique was used to investigate the effect of cations associated with control of common scab on thaxtomin production by measuring the expression of *txtA* gene in *in vitro* studies.

We also compared two different media YME and G&L (also known as Say solution) (Goyer and Beaulieu 1997) to test the effect of media on biomass production of pathogenic and non-pathogenic *Streptomyces* strains in four dilutions of each media. Subsequently the biomass produced in each dilution was used to measure the amount of *txtA* gene expressed in the pathogenic strain.

MATERIALS AND METHODS

Primer design

Forward and reverse primer/probe sets were designed using the software tool Primer3 (version 0.4.0) (Koressaar and Remm 2007; Untergrasser et al. 2012). Primer/probe sets were designed for two genes -

thaxtomin synthetase A (*txtA*) (gene of interest) and glyceraldehyde-3-phosphate dehydrogenase (*gap*) (reference gene) specific to *S. scabiei* (Table 5.A.I-50). The primers were ordered online from Sigma-Aldrich (NSW, Australia) and the TaqMan® probe from Applied Biosystems (California, USA). PCR products of *txtA* and *gap* genes were cloned into the pGEM® - T Easy Vector (Promega, USA) following the manufacturers' instructions. PCR product of each gene was sent for sequencing to Australian Genome Research Facility (Australia) and each gene sequence confirmed specific to *S. scabies*. The copy number for standard Streptomyces DNA molecules were determined from the plasmid DNA concentration and subsequently used for constructing a standard curve for each gene.

Table 5.A.I-50 – Primer and probe pair designed for the detection of mRNA in *Streptomyces scabies.*

Gene name	Gene ID	Sequence size	Product size	Primer sequence (5' to 3')
Gap	YP_003492503, 8843710	1011	126	FP, 5'-CCGCATCGGTCGTAACTACT-3'
				RP, 5'-CCCAGGATGGTGTCGTACTT-3'
				5' VIC-ACCTGGGTGACACCGCGACC-MGBNFQ 3'
TxtA	SCAB_31791	4377	122	FP, 5'-ATAGCGGCTGAGTTCGTTGT-3'
				RP, 5'-AGGAGAAGGAACTGCTCGTG-3'
				5' 6FAM-GCGTGCACGACTCCAGCGTC-MGBNFQ 3'

Streptomyces culture

Different dilutions of YME and G&L media

Spores were collected from both pathogenic (S546) and non-pathogenic (S249) *Streptomyces* strains, and incubated at 27°C in full, half, one-quarter and one-eighth strength each of Yeast Malt Extract (YME) (4 g yeast extract, 10 g malt extract, 4 g bacteriological glucose per litre, pH 7.2) and Goyer & Loria media (G&L) (20 g sucrose, 1.2 g L-aspargine, 0.6 g KH_2PO_4, and 10 g yeast extract per litre, pH 7.2). Four replicates were set up. Two replicates of bacterial biomass, consisting of both mycelium and spores were collected 14 days after incubation by spinning them down for 25 minutes at 4000 rpm. The supernatant was tipped off and a spatula used to collect the biomass into eppendorf tubes. Bacterial biomass (approximately 0.5 gm) and five samples per dilution was then exposed to RNA Protect (Qiagen) and stored at -20°C until required for RNA extraction. The biomass from the other two replicates in each dilution of G&L and YME media were collected using the same method described previously and the weight of biomass determined. One mil of each broth treatment was pipetted in Eppendorf tubes and serially diluted with 100 µL of 0.05% NaCl. One hundred microliters of 10^{-2} *Streptomyces* biomass dilution was plated onto petri plates containing YME media in triplicate and incubated at 27°C. After one week the number of colonies were counted and colony forming units (cfu) determined.

Different concentrations of potassium and magnesium

Spores were collected from pathogenic (S546) *Streptomyces* strain, and incubated at 27°C in half strength G & L media containing varied rates of potassium (as SO_4) and magnesium (as SO_4). The rates used for potassium ion were 60 and 300 ppm, for magnesium ion were 300 and 1500 ppm, for potassium + magnesium (K:Mg) ratios were 1:5 (0.2) and 1:1 (1.0), 3:5 (0.6) (reduced rate), 2:5 (0.4) and control with no addition of potassium or magnesium ion. Four replicates were set up in conical flasks.

Different concentrations of manganese

A similar experiment was conducted as described for K:Mg to determine the effect of varied concentration of manganese (as SO_4) ion on *txtA* gene expression. The rates used for manganese ion were 10 ppm, 50 ppm, 100 ppm, 200 ppm and control with no addition of manganese ion.

Total RNA extraction and two-step real-time quantitative RT-PCR

Different dilutions of YME and G&L media

RNA was extracted from bacterial biomass using an RNeasy plant mini kit (Qiagen). The quantity and the integrity of all the RNA samples were determined using a spectrophotometer (NanoDrop Technologies, Wilmington, USA). A known volume of extracted RNA from each sample was then treated with DNase1 (DNA-*free*™ Kit, Ambion) enzyme and 100 ng of total RNA reverse transcribed into cDNA (High Capacity cDNA Reverse Transcription kit, Applied Biosystems). PCR reactions were set up in replicates for cDNA samples and in triplicates for standards using 12.5 µL Platinum® Quantitative PCR SuperMix-UDG (Invitrogen), 0.3 µM of each forward and reverse *txtA* primer, 0.1 µM probe, 5 µL of template cDNA (50 ng μL^{-1}) made up to 25 µL with RNA-free water. A non-template control (water) was included in the run to check for contamination. Cycling conditions were 2 step hold 50°C for 2 min followed by 95°C for 10 min, followed by 45 cycles of amplification in a two-step procedure: 15 s at 95°C and 30 s at 60°C. Ten-fold serial dilutions (10^6 to 10^3) of the cloned *txtA* fragment was included as standards in each run for estimating the copy number of *txtA* gene present in the unknown samples. The run was repeated twice.

For potassium, magnesium and manganese experiments RNA was extracted from six biomass samples per treatment and processed for RT-qPCR using the method described above.

Statistical analysis

The statistical software GenStat (14th edition) was used for statistical analysis. Analysis of Variance was used for all statistical analysis conducted in this study. An LSD (least significant difference) was calculated when differences were significant (P=0.05) to compare means.

RESULTS

Comparison of expression of *txtA* gene in YME and G&L media

PCR amplification in all the samples of the pathogenic strain confirmed the expression of *txtA* gene, whereas *txtA* gene was not detected in the non-pathogenic strain and in the non-template control. *Streptomyces* biomass grown in G&L media regardless of strength amplified within a difference of 2 Ct cycles (Figure 5.A.I-57A) whereas samples grown in YME media showed a greater variation in amplification with a difference of 5 Ct cycles (Figure 5.A.I-57B).

Overall more bacterial biomass was produced in G&L than in YME media and the amount of biomass decreased significantly in subsequent dilutions from full strength to one-eighth strength (Table 5.A.I-51). The expression of *txtA* gene in pathogenic *Streptomyces* strain grown in half-strength G&L media showed least variation between the two replicates without compromising on biomass production. The expression of *txtA* gene was significantly higher in one-eighth dilution G&L and quarter-strength YME than in the other dilutions even though less biomass was produced at these lower dilutions (Table 5.A.I-51). These results confirm that the strength of both YME and G&L media has a direct effect on the expression of *txtA* gene.

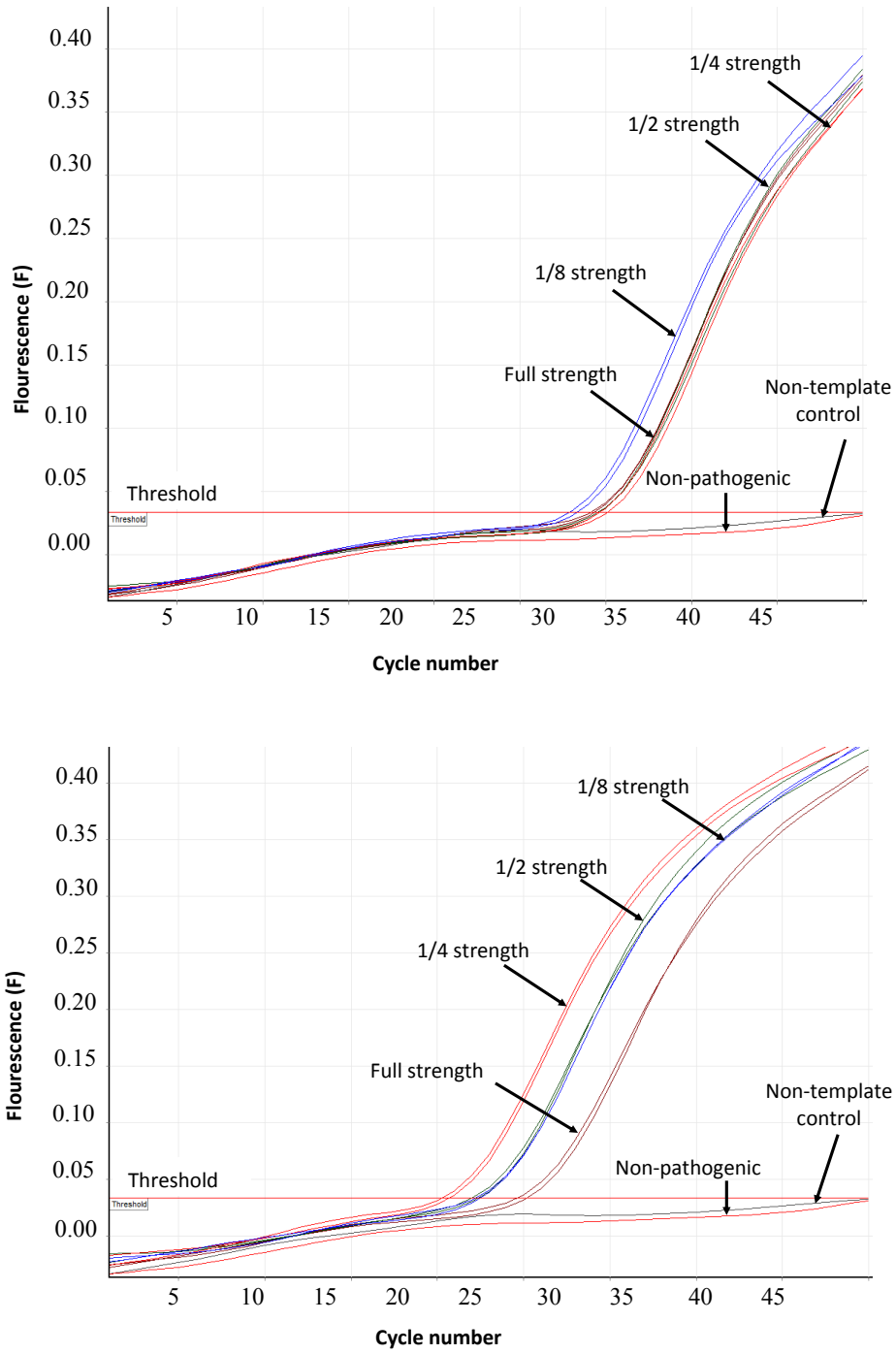


Figure 5.A.I-57 – Cycling curve generated from real-time PCR analysis using *txtA* primer and probe set specific to *S. scabies* (fluorescence vs cycle number). A (Top) G&L media showing highest amplification in one-eighth dilution and B (Bottom) YME media showing highest amplification in one-quarter dilution.

Table 5.A.I-51 – Effect of different concentrations of G&L and YME media on amount of biomass and *txtA* gene expressed in the pathogenic *Streptomyces* strain

Media concentration	G&L		YME	
	Biomass (g)	*TxtA* (copies/µL)	Biomass (g)	*TxtA* (copies/µL)
Full	0.70b	11613	0.60c	151213
Half	0.63a	12891	0.58b	945785
One-quarter	0.60a	15049	0.55a	2715943*
One-eighth	0.57a	27098*	0.55a	650564
lsd (P=0.05)	0.04	4175	0.01	502771

*significantly different from the all other values at P<0.05; numbers followed by the same letters are not significantly different from each other

Evaluating the effects of different nutrient amendment treatments on *txtA* gene expression in broth culture

The effect of various concentrations of potassium and magnesium using half strength G&L media on the production of bacterial biomass, colony-forming units (cfu) and amount of *txtA* gene from the pathogenic *Streptomyces* strain was undertaken. The rates used for potassium ion were 60 and 300 ppm, for magnesium ion were 300 and 1500 ppm, for K:Mg ratio were 1:5 (0.2), 1:1 (1.0), 3:5 (0.6) (reduced rate) and 2:5 (0.4) and control with no addition of potassium or magnesium ion.

The amount of *txtA* gene expression determined in pathogenic *Streptomyces* biomass from cultures grown in 60 and 300 ppm K and 0.6 ppm K:Mg was deleted from this table as the results within two replicates were highly variable. There was a significant increase in the expression of *txtA* gene in 0.4 K:Mg treatment compared with control (Table 5.A.I-52). No *txtA* gene was expressed in 0.2 K:Mg and in 300 ppm Mg treatment. The amount of biomass produced in two concentrations of potassium, two concentrations of magnesium and four concentrations of potassium + magnesium were not significantly different to the amount of biomass produced in control culture. There was a significant reduction in the number of viable spore colonies produced on petri plates with 0.2 K:Mg, 1.0 K:Mg and 1500 Mg treatments. However, there was significantly higher number of colony-forming units produced both in control and 300 Mg treatment.

Table 5.A.I-52 – Effect of potassium and magnesium on amount of biomass, colony-forming units (cfu) and *txtA* gene expressed in the pathogenic *Streptomyces* strain cultured in half strength G&L media.

K and Mg treatment	Biomass (g)	cfu/mL	*TxtA* (copies/µL)
0.2 K:Mg	1.13	13a	0
0.4 K:Mg	1.13	206b	16284*
1.0 K:Mg	1.12	17a	155

K and Mg treatment	Biomass (g)	cfu/mL	*TxtA* (copies/µL)
1500 ppm Mg	1.12	21a	589
300 ppm Mg	1.14	363c	0
Control	1.14	233bc	142
lsd (P=0.05)	ns	145	9473

*Significantly different from all other values at P<0.05; ns, not significant at P<0.05; numbers followed by the same letter do not differ significantly from each other

No effect of manganese treatment on *txtA* expression in *in vitro* culture

The effect of various concentrations of manganese using half strength G&L media on the production of bacterial biomass, colony-forming units (cfu) and amount of *txtA* gene from the pathogenic *Streptomyces* strain was undertaken. The rates used for manganese ion were 10 ppm, 50 ppm, 100 ppm, 200 ppm and control with no addition of manganese ion.

The amount of biomass produced in all the four concentrations of manganese were not significantly different to the amount of biomass produced in control culture (Table 5.A.I-53). There was no significant difference in the number of viable spore colonies produced on petri plates with or without the addition of manganese. Overall the amount of *txtA* gene determined in pathogenic *Streptomyces* biomass from all the cultures was low. There was no significant difference on the expression of *txtA* gene between control and manganese treatments.

Table 5.A.I-53 – Effect of manganese on amount of biomass, colony-forming units (cfu) and *txtA* gene expressed in the pathogenic *Streptomyces* strain cultured in half strength G&L media.

Mn ppm	Biomass (g)	cfu/mL	*TxtA* (copies/µL)
10	1.12	178	98
50	1.12	240	66
100	1.16	34	97
200	1.17	28	133
Control	1.15	5	103
lsd (P=0.05)	ns	ns	ns

ns, not statistically signficant at P<0.05

DISCUSSION

A comparison of YME and G&L media found that the strength of both media has a direct effect on the expression of the *txtA* gene. G&L media was used at half-strength to grow *Streptomyces* spp. *in vitro* for measuring the expression of *txtA* gene using quantitative RT-PCR without compromising on biomass production. This method was applied to determine the effect of nutrient manipulation (K, Mg and Mn) on the expression of *txtA* gene in *in vitro Streptomyces* culture. There was high variation in *txtA* gene expression between the two replicates in the potassium and magnesium experiment but 0.4 K:Mg treatment increased *txtA* expression in *in vitro* culture. None of the manganese treatments had an effect on *txtA* gene expression.

MANIPULATING THE SOIL K:MG RATIO FOR COMMON SCAB CONTROL IN THE FIELD

INTRODUCTION

In APRP1, multivariate analysis of data from a survey of 116 fields conducted over 2 years South Australia, Tasmania and Victoria correlated the ratio of K:Mg in soil with common scab development on tubers at harvest. Independently, this association was also reported in Canada. Lazarovits et al. (2007), reported that a K:Mg ratio of 0.4 was associated with a reduction of common scab on tubers in Ontario. The aims of the current project were to investigate whether manipulation of the K:Mg ratio could be developed as a practical control measure in the field for common scab, and to determine whether the activity is a direct effect on the pathogen or an indirect effect that enhances the resistance of the potato. The studies reported here examined the effects of manipulating the K:Mg ratio on common scab disease and yield at Cora Lynn and Mirboo North, crisping production regions of Victoria. The approach used was to decrease or increase the ratio from the baseline ratio in the soil in which trials were conducted. The goal was to be able to provide recommendations on an optimum ratio for common scab control.

RESULTS

2009/2010 FIELD TRIALS, CORA LYNN

Two replicated trials in fields with a history of common scab were conducted to modify the K:Mg ratio to give three target ratios of 0.2, 0.4 and 0.6 and compare the effect on common scab disease on Simcoe (susceptible) and Atlantic (tolerant). The treatments for the cv. Simcoe trial and the cv. Atlantic trial are outlined in Table 5.A.I-54 and Table 5.A.I-55.

Table 5.A.I-54 – (cv. Simcoe) Soil chemical treatments used to change K:Mg ratios in field trials at Cora Lynn, 2009/2010.

Target K:Mg	Product	Active Compound	t/ha	K kg/ha	Mg kg/ha
0.4 (Untreated)	-	-	-	-	-
0.2	Dolomite	$MgSO_4$	2	Nil	440
0.6	Sulphate of potash	K_2SO_4	0.4	398	Nil

Table 5.A.I-55 – (cv. Atlantic) Soil chemical treatments used to change K:Mg ratios in field trials at Cora Lynn, 2009/2010.

Target K:Mg	Product	Active	kg/ha
0.2 (Untreated)			
0.4	Sulphate of potash	K_2SO_4	576
0.6	Sulphate of potash	K_2SO_4	1142

In the cv. Simcoe trial, the actual baseline ratio and the ratios achieved, as measured at tuber set time, were 0.35 (expected 0.4), 0.29 (target 0.2) and 0.59 (target 0.6) (Table 5.A.I-56). The two treatments, magnesium sulphate and potassium sulphate, significantly increased petiole magnesium and potassium respectively, and the K:Mg ratios in the petiole were 0.25 and 0.6, respectively. These treatment had no significant effect on nutrient concentrations in the tuber peel.

Table 5.A.I-56 – (cv. Simcoe) Effects of soil amendments on soil and plant potassium and magnesium concentrations and on the predicted and actual K:Mg ratios in a field trial at Cora Lynn 2009/2010.

Target	Achieved	Tuber set soil (ppm)		Petiole (%)			Peel (%)		
K:Mg ratio	K:Mg ratio	K	Mg	K	Mg	K:Mg ratio	K	Mg	K:Mg ratio
Untreated (0.4)	0.35	315	274.8	2.12	1.64	0.40	4.05	0.13	9.59
0.2	0.29	316	347.2*	1.59*	1.98*	0.25	4.26	0.14	9.36
0.6	0.59*	512*	266	2.62*	1.36*	0.60	4.52	0.14	9.93
lsd (P=0.05)	0.14	125.1	53.42	0.36	0.15		ns	ns	

* Significantly different from the untreated control at P<0.05; ns, not significant; Harvest soil nutrient levels were not determined.

Table 5.A.I-57 – (cv. Simcoe) Effects of different K:Mg ratios on the concentration of *S. scabies* DNA in soil, the incidence and severity of common scab disease, total and marketable yields and crisp yields at the factory in a field trial at Cora Lynn, 2009/2010.

Target	Achieved	*S. scabies* DNA pg/g soil	Common scab		Yield (t/ha) in field		Marketable (t/ha) at factory
K:Mg ratio	K:Mg ratio	Tuber Set	% tubers affected	Severity Index (%)	Total	Marketable	Crisping
Untreated (0.4)	0.35	22.7	81	99.8	32	30	26
0.2	0.29	0	93*	98.9	35	33	22
0.6	0.59*	24.5	69*	98.4	35	33	28
lsd (P=0.05)	0.14	ns	9.9	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

Pathogen DNA concentrations at tuber set were not influenced by K:Mg ratio (Table 5.A.I-57). Common scab incidence was 81% and severity 100% at the baseline ratio of 0.35 K:Mg. While severity remained high, incidence was significantly reduced to 69% at 0.59 K:Mg and increased to 93% at 0.29 K:Mg. Yields were not affected.

In the trial with cv. Atlantic, the baseline K:Mg ratio was 0.18 at tuber set (Table 5.A.I-58). Actual K:Mg ratios were 0.31 and 0.37 for the targets of 0.4 and 0. 6, respectively. Both the magnesium sulphate and potassium treatments significantly increased (P<0.05) the potassium concentration in the soil and both potassium and magnesium concentrations in the petioles.

Table 5.A.I-58 – (cv. Atlantic) Effects of soil amendments on soil and plant potassium and magnesium concentrations and on the predicted and actual K:Mg ratios in a field trial at Cora Lynn 2009/2010.

Target	Achieved	Tuber set soil (ppm)		Petiole (%)			Peel (%)		
K:Mg ratio	K:Mg ratio	K	Mg	K	Mg	K:Mg ratio	K	Mg	K:Mg ratio
Untreated (0.14)	0.18	273	454	4	3.0	0.41	4.88	0.16	9.62
0.4	0.31*	445*	442	8*	2.3*	1.07	5.52	0.18	9.43
0.6	0.37*	516*	435	9*	1.5*	2.00	6.02	0.18	10.29
lsd (P=0.05)	0.09	88	ns	2	0.5		ns	ns	1.6

* Significantly different from the untreated control at P<0.05; ns, not significant; Harvest soil nutrient levels were not determined.

Although no *S. scabies* DNA was detected at tuber set, the Atlantic growing in the baseline soil developed 46% disease incidence and 100% severity (Table 5.A.I-59). However, an average of 46% of tubers were affected with common scab in the untreated plots. Neither treatment significantly affected common scab incidence or severity, although both total and marketable yields were significantly increased by 4 t/ha.

Table 5.A.I-59 – (cv. Atlantic) Effects different K:Mg ratios on the concentration of *S. scabies* DNA in soil, the incidence and severity of common scab disease, total and marketable yields and crisp yields at the factory in a field trial at Cora Lynn, 2009/2010.

Target	Achieved	*S. scabies* DNA pg g^{-1} soil	Common scab		Yield (t/ha)	
K:Mg ratio	K:Mg ratio	Tuber set	% tubers infected	Severity	Total	Marketable
Untreated 0.14	0.18	0	46	100	38	34
0.4	0.31*	0	46	99	42*	38*
0.6	0.37*	0	50	98	42*	38*
lsd (P=0.05)	0.09	ns	ns	ns	3	3

* Significantly different from the untreated control at P<0.05; ns, not significant

In these trials, we found that manipulating the soil K:Mg ratio to 0.6 reduced common scab incidence on the susceptible cv. Simcoe compared with the untreated soil which had a baseline ratio of 0.4. In the trial using cultivar Atlantic, no disease reduction occurred, but the baseline ratio had been approximately 0.2, rather than the expected 0.4, and the amendments were only successful at raising the ratio to below 0.4. In the potato soils around Ontario, Canada, 0.4 is the target ratio for common scab control, but in Koo-Wee-Rup soil, perhaps 0.6 is the effective ratio. This was further tested in the subsequent trials described below.

2011/2012 FIELD TRIALS, CORA LYNN

In order to generate the target K:Mg ratios of 0.4 and 0.6, different amendments from the previous trials were applied in this trial based on recommendations from A&L Canada Laboratories and SWEP Laboratories (Victoria) following soil analyses. The baseline ratio was 0.2. The cultivar used was Catani. Muriate of potash and sulphate of potash were applied at different rates to try and achieve the target K:Mg ratios (Table 5.A.I-60).

Table 5.A.I-60 – (cv. Catani) Rates of potash applied to achieve target K:Mg ratios (0.4 and 0.6) based on A&L Canada Laboratories (AL) and SWEP Laboratory recommendations in a field trial at Cora Lynn, 2011/2012.

Target K:Mg Ratio	Product	Active compound	Rate t/ha
Untreated (0.2)	Nil	Nil	Nil
0.4 MOP	Muriate of potash	KCl	1
0.6 MOP	Muriate of potash	KCl	5
0.4 SOP	Sulphate of potash	K_2SO_4	1
0.6 SOP	Sulphate of potash	K_2SO_4	5

At tuber set, 5t/ha of muriate (MOP) and sulphate (SOP) of potash applied to achieve a K:Mg ratio of 0.6 significantly overshot the target, resulting in ratios of 1.7 and 1.9 (Table 5.A.I-61). The 1 t/ha rates were more successful, resulting in target ratios of 0.6 (MOP) and 0.5 (SOP). The baseline ratio was 0.2. No treatment had come close to the target of 0.4. By harvest, the ratio of 1 t/ha MOP had reduced to 0.5, but the others were unchanged.

Table 5.A.I-61 – (cv. Catani) The effects of the treatments that change K:Mg ratios in soil on petiole and harvest soil nutrient variables at Cora Lynn, 2011/2012.

Treatment	Tuber set soil (ppm)			Petiole (%)		Harvest soil (ppm)			Peel (%)	
	K	Mg	K:Mg ratio	K	Mg	K	Mg	K:Mg ratio	K	Mg
Untreated	273	362	0.23	9.07	1.126	250	405	0.19	4.38	0.17
MOP 1 t/ha	648*	343	0.57*	10.56*	0.83*	629*	413	0.47*	4.85*	0.19*
MOP 5 t/ha	1883*	336	1.70*	11.79*	0.60*	1976*	376	1.67*	5.28*	0.19*
SOP 1 t/ha	624*	353	0.53*	10.38*	0.84*	571*	389	0.46*	4.48	0.17
SOP 5 t/ha	2261*	367	1.86*	10.19	0.57*	2178*	408	1.70*	4.71	0.17
lsd (P=0.05)	200	ns	0.19	1.28	0.21	144	ns	0.24	0.43	0.015

* Significantly different from the untreated control at P<0.05; ns, not significant

Table 5.A.I-62 – (cv. Catani) The effects of treatments that change K:Mg ratios in soil on concentration of DNA of *S. scabies*, the incidence and severity of common scab and total and marketable yield of potatoes at Cora Lynn, 2011/2012.

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field t/ha		Marketable yield to factory t/ha	
	Planting	Tuber set	Harvest	% tubers affected	Severity Index (%)	Total	Marketable	French Fry	Crisping
Untreated	67	13	0	61	17	29.9	24.6	21	21
MOP 1 t/ha	53	9	23	51	15	30.5	25.7	22	23
MOP 5 t/ha	111	3	9	55	16	21.5*	18.2*	16*	17*
SOP 1 t/ha	71	94	6	68	21	30.0	25.0	21	21
SOP 5 t/ha	30	38	10	56	18	27.1	22.3	19	20
lsd (P=0.05)	ns	ns	ns	ns	ns	3.5	3.5	4	4

* Significantly different from the untreated control at P<0.05; ns, not significant

The DNA concentration of *S. scabies* in the soil at planting ranged from 30-111 pg/g, which is considered medium to high risk by the PreDicta Pt test (Table 5.A.I-62). The DNA concentration dropped to 3-94 pg/g at tuber set and to 0-23 pg/g at harvest. At the baseline K:Mg ratio, disease incidence was 61% and severity was 17%. This was not significantly altered by any of the treatments. The 5 t/ha MOP, however, significantly reduced total and marketable tuber yield by 8 and 7 t/ha, and the yield of the French fry and Crisping categories by 5 and 4 t/ha.

In this trial, although we were able to raise the K:Mg ratio to near the target of 0.6 we did not observe the disease reduction in cv. Catani that we had observed in Simcoe in the earlier trial. We were unable to achieve the target 0.4 for comparison.

2012/2013 FIELD TRIALS, CORA LYNN

The trial was repeated in 2012-13, again using cv. Catani and the amendments MOP and SOP to adjust K:Mg ratio. This time, different rates were applied to attempt to reach the target ratios of 0.2, 0.4 and 0.6 (Table 5.A.I-63).

Table 5.A.I-63 – (cv. Catani) Rates of potash applied to soil to achieve target K:Mg ratios (0.4 and 0.6) in a field trial at Cora Lynn, 2012/2013.

Target K:Mg ratio	Product	Active Compound	t/ha	K kg/ha	S kg/ha
Untreated	Nil	-	-	-	-
0.4 MOP	Muriate of potash	KCl	0.5	250	-
0.4 SOP	Sulphate of potash	K_2SO_4	0.6	250	110
0.6 MOP	Muriate of potash	KCl	1.0	500	-
0.6 SOP	Sulphate of potash	K_2SO_4	1.2	500	220

In 2012-13, the baseline ratio was uniform across the trial plots with a K:Mg ratio of 0.3 (data not shown). The low rates of MOP and SOP both failed to increase the K:Mg ratio above the baseline (Table 5.A.I-64). The higher rates of MOP and SOP increased (P<0.5) the ratio to 0.5, which was reflected in significant increases in potassium in soil at tuber set and harvest. Plant uptake of potassium was higher and magnesium lower in these treatments, as measured by petiole concentrations. None of the treatments affected potassium and magnesium levels in tuber peel.

Table 5.A.I-64 – (cv. Catani) Effects of potash treatments that change K:Mg ratios in soil on corresponding soil and petiole at tuber set, harvest soil and peel potassium (K) and magnesium (Mg) concentrations and K:Mg ratios in a field trial at Cora Lynn, 2012/2013.

Target	Tuber set soil (ppm)			Petiole (%)		Harvest soil (ppm)			Peel (%)	
K:Mg ratio	K	Mg	K:Mg ratio	K	Mg	K	Mg	K:Mg ratio	K	Mg
Untreated	311	380	0.3	7.6	1.35	255	386	0.2	3.67	0.14
0.4 MOP	413	368	0.3	9.2	1.11	383*	369	0.3	4.107	0.15
0.4 SOP	323	367	0.3	8.5	1.41	255	393	0.2	3.53	0.14
0.6 MOP	553*	373	0.5*	10.9*	1.02*	518*	378	0.4*	4.00	0.14
0.6 SOP	531*	382	0.5*	10.04*	1.09*	458*	369	0.4*	4.01	0.15
lsd (P=0.05)	102	ns	0.1	2.1	0.24	108	ns	0.1	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

The DNA concentration of *S. scabies* in the soil at planting ranged from 2 - 44 pg/g, which is considered medium risk by the PreDicta Pt test, and remained constant through tuber set and at harvest (Table 5.A.I-65).

The incidence and severity of common scab was low for all treatments (incidence < 20%, severity <4%) and there were no impacts on yield or quality.

Table 5.A.I-65 – (cv. Catani) Effects of potash treatments that change K:Mg ratios in soil on the concentration of pathogenic *S. scabies* DNA in soil at planting, tuber set and harvest, the incidence and severity of common scab, total and marketable yield in field and factory yields for French fry and crisps in a field trial at Cora Lynn, 2012/2013.

Target	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
K:Mg ratio	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Untreated	32	4	28	13	2.5	46	43	42	43
0.4 MOP	17	10	16	17	3.1	47	45	43	44
0.4 SOP	2	5	14	18	3.7	45	42	40	42
0.6 MOP	15	3	2	16	3.0	49	47	45	46
0.6 SOP	44	7	57	18	3.3	47	44	42	44
lsd (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

Once again, the K:Mg proved difficult to manipulate and no effects of treatments were observed. In summary, the trials at Cora Lynn conducted over three seasons showed a desired effect only once in the first season. Advisors suggested that the peaty clay loams of the Koo Wee Rup swampy plain contain high organic matter and are highly buffered, preventing us from manipulating the K:Mg ratio at this site. On this basis, it was decided to change districts to a less organic soil type.

2013/2014 FIELD TRIALS, MIRBOO NORTH

In 2013/2014, a trial was established on a red-brown earth at Mirboo North in Central Gippsland. In this trial, cv. Wilwash was grown and different rates of magnesium sulphate and sulphate of potash were applied to achieve target ratios of 0.2 and 0.6 from a baseline of 0.4 (Table 5.A.I-66).

Table 5.A.I-66 – (cv. Wilwash) Pre-planting soil treatments to achieve different K:Mg ratios in a field trial at Mirboo North, 2013/2014.

Target K:Mg ratio	Compound	Product	Active %	Rate of active (*a.i.*) kg/ha	Rate of product kg/ha
0.4	Nil	Nil	-	-	-
0.2	$MgSO_4$	Epsom salts	9	515	5722
0.6	K_2SO_4	Sulfate of potash	41	1576	3844

Soil nutrient analysis prior to planting found that variables were not significantly different between plots and the DNA concentration of *S. scabies* ranged from 3 – 16 pg/g soil. Although preliminary soil samples prior to trial establishment had indicated that the baseline K:Mg ratio was approximately 0.4, by the time of planting the ratio was determined to be between 0.6 and 0.7 (Table 5.A.I-67). By tuber set, the target 0.2 K:Mg had been achieved, but the target 0.6 K:Mg ratio was again too high, at 2.2. This did not significantly influence the amount of potassium or magnesium in the petioles or peel.

Disease outcome was not influenced by the treatments, with incidence at 60-62% and severity 19% (Table 5.A.I-68). The treatments had no significant effect on total, marketable and factory yields with the exception of a reduction 6 t/ha in the marketable tuber yield resulting from the magnesium sulphate treatment (Table 5.A.I-68).

Table 5.A.I-67 – (cv. Wilwash) Effects of treatments that change K:Mg ratios in soil on corresponding soil and petiole at tuber set, harvest soil and peel potassium (K) and magnesium (Mg) concentrations and K:Mg ratios in a field trial at Mirboo North, 2013/2014.

Target	Planting soil (ppm)			Tuber set soil (ppm)			Petiole (%)		Harvest soil (ppm)			Peel (%)		
K:Mg ratio	K	Mg	K:Mg ratio	K	Mg	K:Mg ratio	K	Mg	K	Mg	K:Mg ratio	K	Mg	K:Mg
0.4	353	161	0.67	401	183	0.65	13.4	0.23	297	130	0.69	4.43	0.20	21.8
0.2	344	157	0.66	583	454	0.20	13.1	0.24	327	254*	0.40	4.24	0.21	20.6
0.6	367	175	0.64	1266*	315	2.20*	13.3	0.23	756*	148	1.59*	4.63	0.20	22.9
lsd (P=0.05)	ns	ns	ns	549	ns	0.66	ns	ns	133	62	0.32	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

Table 5.A.I-68 – (cv. Wilwash) Effects of treatments that change K:Mg ratios in soil on the concentration of pathogenic *S. scabies* DNA in soil at planting, tuber set and harvest, the incidence and severity of common scab, total and marketable yield in field and factory yields for French fry and crisps in a field trial at Mirboo North, 2013/2014.

Target	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable at factory (t/ha)	
K:Mg ratio	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
0.4	3	44	5	62	19	36	32	27	27
0.2	3	7	3	60	19	30	26*	21	22
0.6	16	31	30	60	19	33	29	24	25
lsd (P=0.05)	ns	ns	ns	ns	ns	ns	5	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

DISCUSSION

In two of the four trials we were successful in increasing the K:Mg ratio to 0.6. Of these trials only the one on susceptible variety Simcoe was successful at reducing disease when common scab incidence and severity was high on the untreated control. The other trial was using the resistant variety Catani which produced a moderate level of disease. In one trial we only managed to elevate the K:Mg ratio of tuber set soil to 0.4 and this had no effect on common scab when the untreated control had a moderate incidence that presented on resistant variety Atlantic. In the final trial, we reduced the K:Mg to 0.2 and increased it to 2.2. However, there was no effect on disease at harvest with all treatments producing a moderate incidence and low severity of common scab.

Manipulating K:Mg ratios in soil was effective at reducing common scab in one out of four trials. The trial in 2009/2010 was successful using susceptible variety Simcoe where common scab incidence and severity was high. In the unsuccessful trials the severity of disease was low even though in 2011/2012 and 2013 and 2014 the incidence of common scab was high. This suggests that soil nutrient manipulation has a greater impact on common scab severity than incidence, with (Huber 1980) postulating as such. So in fields with high disease potential (DNA) and under conducive conditions for disease expression manipulating soil K:Mg could be an insurance policy.

INFLUENCE OF SOIL PH MODIFIERS ON COMMON SCAB DISEASE

INTRODUCTION

Managing soil pH is an important agricultural practice. It is widely thought that pH is the main driver of common scab expression, and that increasing pH increases the risk of common scab and reducing pH does the opposite. There is also evidence that there is a higher risk of common scab disease following applications of lime to raise soil pH.

Soil pH is well-recognised as influencing common scab development, and disease is most severe in soils with pH 5.2–7. In some cases, control can be achieved by reducing the pH further through the use of acidifying fertilizers or applications of sulphur, but pH values inhibitory to *S. scabies* are also unfavourable for potatoes, reducing yields. It has been demonstrated that calcium-based fertilizers such as aglime and hotlime increase the incidence and severity of common scab, but whether this is a direct effect of pH on the pathogen or an indirect effect due to the influence of pH on other aspects of soil chemistry is unknown (Keinath and Loria 1989a). High calcium levels in the absence of changes in pH may induce scab and the content of exchangeable calcium is a more reliable parameter than the soil pH ((Davis et al. 1976), (Goto 1985)).

Certain organic and nutrient amendment additions to soil have been shown to reduce the incidence and severity of common scab. Sulphur was the first nutrient to be used to control common scab and its suppressive effect was thought to be due to a reduction in soil pH. However, Davis et al. (1974) demonstrated reductions in common scab severity by the application of sulphur and gypsum even though the treatments did not significantly reduce soil pH in a highly buffered soil. Manganese has been shown to reduce common scab in some trials but not others (McGregor and Wilson 1964; McGregor and Wilson 1966), (Gilmour et al. 1968). Low pH soils tend to have higher levels of manganese.

High nitrogenous amendments incorporated into soil significantly reduced common scab incidence in Canadian potato crops (Tenuta and Lazarovits 1999). The effectiveness of these treatments depends on the pH of the soil. In acidic soils (low pH), the nitrogen forms nitrous acid which is toxic to pathogens. In alkaline soils (high pH), the nitrogen accumulates as ammonium, also toxic to pathogens. In neutral soils, the nitrogen is present as harmless salts.

In APRP1 we found that the pH modifiers hotlime, magnesium oxide, dolomite and gypsum significantly increased soil pH, but the effect on common scab development appeared to be seasonal i.e. in some seasons, these amendments increased common scab, but in others they did not. These trials developed in APRP2 addressed the influence of the cations associated with the calcium and magnesium oxides to investigate the confounding effects of calcium and magnesium cations, in contrast to pH changes. These trials were established in common scab prone fields.

FIELD TRIALS

2009/2010 CORA LYNN

Two replicated field trials were conducted in heavily infested fields to determine the effect of eight different pH modifying treatments on common scab disease. One trial was planted with Simcoe (susceptible) and the second with Atlantic (resistant). The trials were sown in November 2009 and harvested in April 2010 and graded for marketable yield and assessed for common scab disease on tubers. The different treatments are Simcoe and Atlantic are outlined in Table 5.A.I-69 and Table 5.A.I-70.

Table 5.A.I-69 – (cv. Simcoe) Application rates of soil applied treatments to change soil pH in a field trial at Cora Lynn, 2009/2010.

Treatment	Active Compound	t/ha	% Ca	% Mg	% S
Untreated	-	-	-	-	-
Hotlime	CaO	1.0	72	-	-
Buchan lime	$CaCO_3$	1.9	40	-	-
Gypsum	$CaSO_4$	3.6	21	-	-
Magnesium oxide	MgO	1.0	-	55	-
Magnesite	$MgCO_3$	2.1	-	28	-
Epsom salts	$MgSO_4$	6.1	-	10	-
Top Wettable Sulphur	S	1.5	-		80

Table 5.A.I-70 – (cv. Atlantic) Application rates of soil applied treatments to change soil pH in a field trial at Cora Lynn, 2009/2010.

Treatment	Active compound	t/ha	% Ca	% Mg	% S
Untreated					
Hot lime	CaO	4.0	72		
Buchan lime	$CaCO_3$	7.2	40		
Gypsum	$CaSO_4$	13.7	21		
Magnesium oxide	MgO	4.0		55	
Magnesite	$MgCO_3$	7.9		28	
Epsom salts	$MgSO_4$	22.9		10	
Top Wettable Sulphur	S	5.0			80

The effects of the different pH treatments on soil pH and soil and plant nutrient concentrations for cv. Simcoe are presented in Table 5.A.I-71. The sulphur treatment significantly increased ($P<0.05$) sulphur in the soil at tuber set from a baseline of 7 to 146 ppm, and reduced pH from 5.43 to 3.97. Epsom salts and gypsum significantly increased ($P<0.05$) soil sulphur to 22 ppm but had no significant effect on pH. Hotlime, in contrast increased soil pH at tuber set to 5.67. The sulphur, gypsum and hotlime treatments significantly raised soil calcium concentrations.

Table 5.A.I-71 – (cv. Simcoe) Effects of pH modifiers on soil pH, tuber set plant and soil nutrients and peel nutrients at harvest in a field trial at Cora Lynn, 2009/2010.

Treatment	Tuber set soil (ppm)				Petiole (%)			Peel (%)		
	pH	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg
Untreated	5.43	6.33	1657	293	0.28	2.82	1.84	0.186	0.122	0.141
Hotlime	5.67*	3.37	1935*	317	0.30	2.66	1.74	0.177	0.124	0.136
Buchan lime	5.40	7.33	1783	288	0.30	2.77	1.74	0.182	0.128	0.134
Gypsum	5.20	22.57*	2119*	291	0.30	2.93	1.81	0.192	0.135	0.133
Magnesium oxide	5.57	3.47	1695	408*	0.28	2.33*	1.58	0.183	0.121	0.152
Magnesite	5.37	6.20	1627	293	0.29	2.77	1.88	0.176	0.110	0.128
Epsom salts	5.33	21.93*	1641	497*	0.31	2.06*	1.82	0.213*	0.108	0.162
Top Wettable S	3.97*	146*	2052*	330	0.44*	2.33*	1.78	0.250*	0.073*	0.128
lsd (P=0.05)	0.23	7.95	197.1	93.2	0.076	0.46	ns	0.025	0.023	0.024

* Significantly different from the untreated control at P<0.05; ns, not significant

Top Wettable S was the only treatment that had any effect on disease, reducing incidence from 84% to 43% and severity from 29% to 12% (Table 5.A.I-72). Soil pH was reduced to pH 4 with this treatment, however, and marketable yield was significantly reduced from 32 to 21 t/ha. Magnesium oxide also significantly reduced marketable yield to 23 t/ha, but without any corresponding reduction in disease.

Table 5.A.I-72 – (cv. Simcoe) Effects of pH modifiers on *S. scabies* DNA concentrations, common scab disease and yields in a field trial at Cora Lynn, 2009/2010.

Treatment	*S. scabies* pg DNA g soil	Common scab		Yield (t/ha) in field	Marketable (t/ha) at factory	
	Tuber Set	% tubers afected	Severity Index (%)	Total	Marketable	Crisping
Untreated	13	84	29	34	32	23
Hotlime	36	88	34	34	32	22
Buchan lime	84	85	27	30	28	22
Gypsum	129	74	23	35	33	26
Magnesium oxide	0	90	34	24	23*	17
Magnesite	38	82	27	33	31	25
Epsom salts	24	81	25	28	27	21

Treatment	*S. scabies* pg DNA g soil	Common scab		Yield (t/ha) in field	Marketable (t/ha) at factory	
	Tuber Set	% tubers afected	Severity Index (%)	Total	Marketable	Crisping
Top Wettable S	11	43*	12*	24	21*	20
lsd (P=0.05)	ns	10	8	ns	8	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

The effects of the different pH treatments on pH and soil and plant nutrient concentrations for cv. Atlantic are presented in Table 5.A.I-73. Sulphur significantly reduced pH at tuber set to pH 3.6, and hotlime and magnesium oxide significantly raised pH but not to above pH 7.As expected, magnesium oxide raised soil magnesium and gypsum raised soil calcium. Magnesite increased (P<0.05) calcium in the petioles. Concentrations of sulphur in the tuber peel were increased by sulphur and epsom salts, calcium by hotlime and gypsum, and magnesium by epsom salts.

Table 5.A.I-73 – (cv. Atlantic) Effects of pH modifiers on soil pH, tuber set plant and soil nutrients and peel nutrients at harvest in a field trial at Cora Lynn, 2009/2010.

Treatment	Tuber set soil (ppm)					Petiole (%)			Peel (%)		
	pH water	pH buffer	Ca	Mg	S	S	Ca	Mg	S	Ca	Mg
Untreated	4.8	4.2	1394	490	7	0.14	0.72	3.24	0.28	0.10	0.17
Hotlime	5.5*	4.9*	2115	574	6	0.14	0.89	3.24	0.26	0.13*	0.19
Buchan lime	4.9	4.3	1215	392	10	0.15	0.78	3.46	0.28	0.11	0.18
Gypsum	4.5	3.9	3592*	446	125*	0.16	0.81	3.34	0.30	0.14*	0.17
Magnesium oxide	5.3*	4.7*	1079	754*	7	0.15	0.69	3.85	0.27	0.11	0.20
Magnesite	4.9	4.3	1552	563	7	0.18	1.01*	3.16	0.27	0.11	0.17
Epsom salts	4.5	3.9	1471	1273	106*	0.18	0.49	3.31	0.45*	0.10	0.31*
Top Wettable S	3.6*	3.0*	1764	525	150*	0.16	0.58	3.19	0.36*	0.11	0.17
lsd (P=0.05)	0.2	0.2	1030	155	35	ns	0.29	ns	0.05	0.02	0.03

* Significantly different from the untreated control at P<0.05; ns, not significant

In the more resistant cv. Atlantic, disease incidence was 52% tubers affected with an average of 14% of the tuber surface affected (Table 5.A.I-74). Hotlime was the only treatment to affect disease outcome, significantly increasing (P<0.05) incidence by 32% and severity by 44%. Sulphur significantly reduced (P<0.05) total and marketable yield by 9 and 10 t/ha, respectively.

Table 5.A.I-74 – (cv. Atlantic) Effect of pH modifiers on *S. scabies* DNA concentrations, common scab disease and yields in a field trial at Cora Lynn, 2009/2010.

Treatment	*Streptomyces* pg DNA g^{-1} soil	Common scab		Yield t/ha in field	
	Tuber set	% tubers afected	Severity Index (%)	Total	Marketable
Untreated	0	52	14	39	35
Hotlime	76	77*	25*	41	38
Buchan lime	168	58	15	40	37
Gypsum	0	43	13	39	35
Magnesium oxide	18	61	17	38	34
Magnesite	84	52	13	39	36
Epsom salts	0	67	20	35	32
Top Wettable Sulphur	50	51	15	30*	25*
lsd (P=0.05)	ns	18	7	4	5

* Significantly different from the untreated control at P<0.05; ns, not significant

In these trials, sulphur lowered the soil pH to below the range considered conducive to common scab (ie pH 5.2-7). This resulted in a significant reduction in disease in the susceptible cultivar, Simcoe, but also negatively impacted on yield. With the more resistant Atlantic, there was no significant disease reduction, but there was still a negative impact on yield. Although hotlime and magnesium oxide raised pH, it was within the 5.2-7 range and there was no effect on disease outcome.

2011/2012 CORA LYNN

In 2011/2012 the trial was repeated at Cora Lynn using cv. Catani. Treatments applied in this trial are presented in Table 5.A.I-75. The *S. scabies* DNA concentration was 147 pg/g soil at planting time, a level considered by the PreDicta Pt test to be high risk for common scab.

Table 5.A.I-75 – (cv. Catani) Application rates of soil applied treatments to change soil pH at Cora Lynn, 2011/2012.

Treatment	Active compound	Rate t/ha
Untreated	-	-
Hot lime	Calcium oxide	1.0
Buchan lime	Calcium carbonate	1.8
Gypsum	Calcium sulphate	3.4
Magnesium oxide	Magnesium oxide	1.0
Magnesite	Magnesium carbonate	2.0
Epsom salts	Magnesium sulphate	5.7
Top Wettable S	Elemental sulphur	1.5
Manganese oxide	Manganese oxide	1.0
Manganese sulphate	Manganese sulphate	3.2

None of the treatment significantly increased or reduced soil pH measured at tuber set (Table 5.A.I-76). Soil sulphur was significantly increased by Epsom salts, Top wettable sulphur and manganese sulphate. Magnesium was significantly increased by magnesium oxide and epsom salts and manganese was significantly increased by manganese sulphate. In the petioles at tuber set; calcium was significantly increased by magnesite; magnesium was significantly increased by Buchan lime, magnesium oxide and magnesite; manganese was significantly increased by manganese sulphate. The different treatments did not have a significant effect on nutrient concentrations in the tuber peel.

Table 5.A.I-76 – (cv. Catani) Effect of soil-applied pH modifier treatments on nutrient concentrations in soil and petioles at tuber set and soil and peel at harvest in a field trial at Cora Lynn, 2011/2012.

Treatment	Tuber set soil (ppm)					Petiole				Harvest soil (ppm)					Peel			
	pH buffer	S	Ca	Mg	Mn	% S	% Ca	% Mg	Mn ppm	pH buffer	S	Ca	Mg	Mn	% S	% Ca	% Mg	Mn ppm
Untreated	5.40	45	1202	388	8	0.16	0.68	1.20	135	5.64	65	1448	412	9	0.18	0.12	0.17	12
Hot lime	5.56	49	1390	408	8	0.15	0.66	1.06	108	5.64	79	1610	441	9	0.18	0.12	0.17	12
Buchan lime	5.44	46	1318	392	9	0.17	0.77	1.44*	153	5.60	82	1626	404	10	0.19	0.14	0.19	14
Gypsum	5.58	114	1366	380	8	0.17	0.78	1.33	174	5.75	154	1628	448	9	0.19	0.13	0.16	34
Magnesium oxide	5.42	46	1166	450*	7	0.17	0.72	1.46*	121	5.58	58	1284	558*	8	0.20	0.12	0.19	64
Magnesite	5.52	46	1276	411	7	0.17	0.80*	1.49*	172	5.66	64	1508	435	8	0.18	0.13	0.17	13
Epsom salts	5.54	299*	1196	690*	18	0.17	0.60	1.35	176	5.60	345*	1624	686*	15	0.18	0.12	0.17	54
Top wettable S	5.28	351*	1206	402	10	0.17	0.59	1.22	158	5.36*	433*	1440	442	11	0.19	0.12	0.17	63
Manganese oxide	5.44	46	1200	370	23	0.17	0.73	1.31	199	5.72	54	1502	436	19	0.18	0.12	0.17	53
Manganese sulphate	5.50	236*	1310	395	296*	0.17	0.68	1.11	1202*	5.64	281*	1556	452	292*	0.18	0.13	0.17	13
lsd (P=0.05)	ns	88	ns	49	40	ns	0.10	0.21	127	0.18	105	ns	72	32	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

The incidence of common scab in the untreated control treatment averaged 56% and severity 15% (Table 5.A.I-77). The treatments had no significant effect on any measure except total yield, which was was significantly reduced (P<0.05) by 3, 6 and 9 t/ha respectively by the three treatments that increased soil sulphur: Epsom salts, top wettable sulphur and manganese sulphate.

Table 5.A.I-77 – (cv. Catani) Effects of pH modifier treatments on the concentration of pathogen DNA, incidence and severity of common scab disease and total and marketable yields of potatoes at Cora Lynn, 2011/2012.

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field t/ha		Marketable yield to factory t/ha	
	Planting	Tuber set	Harvest	% tubers affected	Severity Index (%)	Total	Marketable	French Fry	Crisping
Untreated	223	64	52	56	15	33	27	23	24
Hot lime	124	31	48	60	19	31	25	21	21
Buchan lime	52	24	8	57	15	31	24	20	22
Gypsum	254	53	14	58	18	31	25	22	22
Magnesium oxide	117	106	23	59	18	32	25	22	23
Magnesite	109	29	42	64	19	31	24	20	21
Epsom salts	232	42	26	64	19	30*	24	20	21
Top wettable S	147	65	55	61	19	27*	22	18	19
Manganese oxide	197	29	9	57	16	32	25	22	23
Manganese sulfate	20	14	0	62	18	29*	24	20	21
lsd (P=0.05)	ns	ns	ns	ns	ns	3	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

DISCUSSION

In APRP1 we found that the pH modifiers hotlime, magnesium oxide, dolomite and gypsum significantly increased soil pH, but the effect on common scab development appeared to be seasonal i.e. in some seasons, these amendments increased common scab, but in others they did not. These trials developed in APRP2 addressed the influence of the cations associated with the calcium and magnesium oxides to investigate the confounding effects of calcium and magnesium cations, in contrast to pH changes. These trials were established in common scab prone fields.

These field trials demonstrated that as long as soil pH remained within the range 5.2-7, common scab disease outcome was severe, regardless of treatment. Lowering the pH below 5 with sulphur significantly reduced disease but there was also a significant yield penalty accompanying this.

In three trials established over two seasons, we found that elemental sulphur consistently reduced pH by increasing soil sulphur at tuber set. Elemental sulphur reduced the incidence and severity of common scab in one trial where the disease expression at harvest was high. In the other two trials, the level of disease expression was moderate, 52 and 56% affected tubers in the untreated controls and a reduction in disease was not seen at harvest even though pH was reduced and sulphur increased at tuber set when tubers are most susceptible to infection.

In 2009/2010 when common scab was reduced by soil-applied elemental sulphur, so was the field marketable yield; but at the factory when the reduction in disease was taken into account, the payable yield was no different to the untreated. Therefore, in this season the option to do nothing would have been best. The unamended soil in these field trials was pH 5.4.

In three trials established over two seasons we found that hotlime increased pH in two of the three trials. If there was high disease in the untreated control, hotlime did not make common scab worse but if there was moderate disease in the untreated control, then hotlime increased common scab incidence. When pH was not increased by hotlime, neither was disease.

Cultivar demonstrated the biggest effect on disease outcome. Simcoe had high levels of both incidence and severity, Atlantic had lower incidence but still with high severity, and Catani had lower disease incidence and much lower severity.

INFLUENCE OF NUTRIENT AMENDMENTS OF ELEMENTAL SULPHUR, MANGANESE SULPHATE AND AMMONIUM SULPHATE ON COMMON SCAB DISEASE

INTRODUCTION

Sulphur

It is widely thought that soil pH strongly influences the development of common scab. In the literature, sulphur treatments have generally shown to lower soil pH and reduce common scab. There are two possible mechanisms for the effect of sulphur on common scab disease (Pavlista 1995). Applications of high amounts of sulphur reduce soil pH to below the optimal range for the common scab pathogen to grow or results in the production of hydrogen sulphide (H_2S) and possibly other volatile sulphur compounds that may affect the pathogen.

In a trial that evaluated pH modifiers in the 2011/2012 season elemental sulphur significantly reduced soil pH at tuber set and harvest but had no effect on common scab disease. In general, we have had success with 1200 kg/ha of S using Top Wettable Sulphur in reducing common scab.

Manganese

Numerous studies have demonstrated a reduction in common scab with applications of Mn, while other field studies have shown no effect. The rate of manganese application and its placement in the planting furrow appears to influence efficacy. Several studies have indicated that manganese has a direct inhibitory

effect on pathogen growth but not on sporulation (Mortvedt et al. 1961; Mortvedt et al. 1963). Studies have also shown the increased manganese content of the tuber correlates with disease reduction (McGregor and Wilson 1964; McGregor and Wilson 1966), (Mortvedt et al. 1961; Mortvedt et al. 1963). However, Barnes and McAllister (1972) observed a significant increase in the manganese content of tuber peelings without a corresponding decrease in scab. In most instances, when changes in soil pH were monitored, there was no effect on soil pH. (Keinath and Loria 1989a) suggested the application of manganese sulphate would achieve a reduction in soil pH and the inhibition of the pathogen by manganese toxicity. An alternative theory is that abundant manganese improves host resistance to tuber infection. Manganese is involved in lignin biosynthesis associated with defence of plants against pathogens.

This section further explores elemental sulphur treatments, manganese sulphate and ammonium sulphate, with or without a stabiliser for the control of common scab. The effects of the latter treatment on the greenhouse gas nitrous oxide is also examined.

RESULTS

FIELD TRIAL, CORA LYNN 2012/2013

Effects of different forms and rates of elemental sulphur on common scab disease

Trials were established in common scab-infested fields to evaluate the effect of two forms of sulphur at two different rates on common scab disease and on yield of the variety Catani. The treatments applied are listed in Table 5.A.I-78.

Soil testing prior to amendment application found that the site was uniform for soil nutrient variables with the exception of organic matter. The concentration of pathogenic *Streptomyces* DNA averaged 18 pg/g soil at planting time, a level that is considered to equate to a high risk of common scab disease developing in potatoes grown in that field and did not vary significantly between plots. The level of sulphur was 25 ppm and the soil pH 5.2. The trial was planted with the variety Catani. The concentration of pathogenic *Streptomyces* DNA in the peel of samples of seed tubers was 1340 pg/g peel. Seed was planted on the day of nutrient application.

None of the treatments had a detrimental effect on emergence. Soil samples taken after tuber set to determine the concentration of soil nutrients and the concentration of pathogenic *Streptomyces* found there was no significant treatment effect on soil DNA (Table 5.A.I-79). Plant petioles were sampled after tuber set to determine the uptake of nutrients.

The two highest rates of sulphur of 1200 kg/ha significantly reduced ($P<0.05$) soil pH at tuber set (Table 5.A.I-78). All sulphur treatments increased the concentration of sulphur in soil at tuber set. However, the highest rate of Top Wettable Sulphur (1200 kg/ha) resulted in a significantly lower sulphur concentration in petioles at tuber set. At harvest time, the pH of soils treated with sulphur were significantly lower than the pH of the untreated soil.

Table 5.A.I-78 – (cv. Catani) Effects of formulations and rates of elemental sulphur treatments in soil on corresponding soil and petiole at tuber set and harvest soil and peel sulphur (S) concentrations in a field trial at Cora Lynn, 2012/2013.

Product	Application rate t/ha	S kg/ha	Tuber Set soil		Petiole	Harvest soil			Peel	
			S ppm	pH	%S	S ppm	pH	NO_3 ppm	%S	Mn ppm
Nil untreated	-	-	35	4.9	0.17	34	4.9	52	0.14	11
Top Wettable Sulphur®	1.5	1200	296*	4.5*	0.13*	513*	4.4*	32	0.16	14*
Top Wettable Sulphur®	1	800	241*	4.9	0.19	469*	4.5*	41	0.16	12
Atomic Sulphur	1.22	1200	350*	4.5*	0.14	511*	4.4*	26	0.15	13
Atomic Sulphur	0.82	800	244*	4.8	0.17	408*	4.4*	44	0.16	14*
lsd (P=0.05)	-	-	93	0.2	0.04	142	0.1	15	ns	2

* Significantly different from the untreated control at P<0.05; ns, not significant

The incidence of common scab in the untreated control treatment averaged 35% (Table 5.A.I-79). However, none of the treatments had a significant effect on the incidence and severity of common scab compared with the untreated control, despite evidence of changes in soil and plant chemistry. Total yields in field averaged 39 t/ha for the untreated control. Yields were not affected by the sulphur treatments.

Table 5.A.I-79 – (cv. Catani) Effects of different formulations and rates of elemental sulphur treatments applied to soil on the concentration of pathogenic *S. scabies* DNA in soil, the incidence and severity of common scab, total and marketable yields in field and factory yields for French fry and crisping in a field trial at Cora Lynn, 2012/2013.

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Nil	37	20	159	35	7	39	36	33	36
Top S 1.5	5	14	54	27	5	42	39	37	39
Top S 1.0	29	29	88	21	4	40	37	35	37
Atomic S 1.2	9	13	26	26	5	42	39	36	38
Atomic S 0.8	9	5	0	22	4	41	39	37	39

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
lsd (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

The effects of different rates of manganese (Mn) on common scab disease

Two different rates of manganese were applied as manganese sulphate were applied in the trial. Soil testing prior to amendment application found the site was uniform for soil variables with the exception of organic matter. The level of *S. scabies* DNA in test plots at planting was uniformly high at 18 pg/g soil. At planting the level of manganese was 6 ppm and sulphur was 25 ppm. None of the treatments had a detrimental effect on emergence. This trial was planted with cv. Catani with a seed peel pathogenic *Streptomyces* DNA of 1340 pg/g peel. Soil samples were taken after tuber set to determine the concentration of soil nutrients and the concentration of *S. scabies* DNA. Plant petioles were sampled after tuber set to determine the uptake of nutrients by plants.

Both rates of manganese sulphate ($MnSO_4$) applied to soil prior to planting increased (P<0.05) the level of manganese and sulphur in soil at tuber set and the level of manganese in plant petioles (Table 5.A.I-80). However, they did not affect the sulphur content of petioles. The high soil levels of manganese and sulphur were maintained until harvest and there was a significant increase in manganese in the peel of harvest tubers but not sulphur.

Table 5.A.I-80 – (cv. Catani) Effects of different rates of manganese sulphate ($MnSO_4$) applied to soil prior to planting and their effect on corresponding soil and petiole at tuber set and harvest soil and peel at harvest manganese (Mn) and sulphur (S) concentrations in a field trial at Cora Lynn, 2012/2013.

Application rate $MnSO_4$ kg/ha	Mn kg/ha	S kg/ha	Tuber set soil (ppm)		Petiole		Harvest soil (ppm)		Peel	
			Mn	S	Mn ppm	%S	Mn	S	Mn ppm	%S
Nil			8	35	98	0.17	7	34	11	0.14
336	124	72	42*	66*	234*	0.16	37*	58*	21*	0.14
504	184	106	62*	82*	343*	0.15	53*	69*	29*	0.13
lsd P=0.05			16	17	120	ns	9	12	7	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

Streptomyces DNA was not detected in planting or harvest in plots with the low rate of manganese. Likewise, the concentration of *Streptomyces* DNA did not different significantly between treatments (Table 5.A.I-81). This is not likely to be due to a treatment effect, rather a pathogen distribution effect. Plots had not yet been treated when the planting sample was taken.

Total yields in field averaged 39 t/ha for the untreated control (Table 5.A.I-81). Yields were not affected by treatments. The incidence of common scab in the untreated control treatment averaged 35% of tubers affected. However, none of the treatments had a significant effect on the incidence and severity of common scab compared with the untreated control, despite evidence of changes in soil and plant chemistry.

Table 5.A.I-81 – (cv. Catani) Effects of different rates of manganese sulphate ($MnSO_4$) applied to soil prior to planting on the concentration of pathogenic *S. scabies* DNA in soil, the incidence and severity of common scab, total and marketable yields in the field and factory yields for French fry and crisps in a field trial at Cora Lynn, 2012/13

Application rate $MnSO_4$ kg/ha	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Nil	37	20	159	35	7	39	36	33	36
336	0*	1	0	26	5	41	38	34	38
504	18*	20	23	24	4	38	36	34	36
lsd *P*=0.05	23	ns	ns	ns	ns	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, no significant treatment effects

Effects of the form and rate of soil-applications of ammonium sulphate on common scab disease and yield of potatoes, and on soil greenhouse gas emissions

Nitrogen potentially influences common scab disease indirectly through its effect on soil pH. Ammonium sulphate can acidify the soil through nitrification (hydrogen ions released during the conversion of ammonium to nitrate). Applications of ammonium sulphate have reduced both the soil pH and common scab in trials, although not consistently (Stuart Wale pers comm).

Based on the literature, a potential control strategy for common scab is to adjust the soil pH to a level at which the activity of the pathogen is reduced or inhibited. Ammonia forms of nitrogen may be the most effective way of obtaining this pH reduction without creating an unfavourable pH for rotation crops.

Ammonium stabilisers (e.g. Incitec Pivot Entec®, *a.i.* 3,4-dimethylpyrazole) have been developed to help farmers reduce the frequency of their nitrogen applications. These products inhibit the activity of nitrifying bacteria thereby delaying the nitrification process. This reduces losses in nitrogen through leaching of nitrate nitrogen, reduces the spikes in nitrogen and retains nitrogen in the ammonium form in the soil for longer. This also allows for the preferential uptake of ammonium nitrogen instead of nitrate nitrogen by the growing plants. Two different rates of ammonium sulphate were applied as Gran Am® with and without the ammonium stabiliser in our trial (Table 5.A.I-82).

Soil testing prior to amendment application found the site was uniform for soil variables with the exception of organic matter. The level of common scab DNA in test plots at planting was uniformly high at 36 pg/g soil. At planting the level of sulphur was 26 ppm. None of the treatments had a detrimental effect on emergence. This trial was planted with the variety Catani which had a seed peel *S. scabies* DNA concentration of of 1340 pg/g peel. Soil samples were taken after tuber set to determine the concentration of soil nutrients and the concentration of *S. scabies*. Plant petioles were sampled after tuber set to determine the uptake of nutrients by plants. The trial was harvested in April and tubers graded for size and yield and assessed for the incidence and severity of common scab.

Table 5.A.I-82 – (cv. Catani) Rates of soil-applied ammonium sulphate fertiliser $(NH_4)_2SO_4$, with or without the ammonium stabiliser, and corresponding rates of soil nitrogen (N) and sulphur (S) in a field trial at Cora Lynn, 2012/2013.

Treatment	Product	$(NH_4)_2SO_4$ kg/ha	N kg/ha	S kg/ha
Untreated	Nil	-	-	-
Ammonium sulphate	Gran Am®	300	63	72
Ammonium sulphate	Gran Am®	500	105	120
Ammonium sulphate + ammonium stabiliser	Gran Am® + Entec®	300	63	72
Ammonium sulphate + ammonium stabiliser	Gran Am® + Entec®	500	105	120

All treatments increased sulphur at tuber set and with similar concentrations at harvest (Table 5.A.I-83). However, the 300 kg/ha Gran Am® and Gran Am® & Entec® significantly reduced the sulphur in the petioles. At harvest, soil nitrate was increased by the 500 kg/ha rates of both Gran Am® and Gran Am® & Entec®. None of the treatments significantly affected the nutrient status of the peel of harvest tubers.

Table 5.A.I-83 – (cv. Catani) Effects of different rates of ammonium sulphate fertiliser $(NH_4)_2SO_4$, with or without the ammonium stabiliser, applied to soil prior to planting, and their effects on soil pH and concentrations of soil sulphur (S) and nitrate (NO_3), and concentrations of sulphur, nitrogen (N) and nitrate in the petiole and potato peel in a field trial at Cora Lynn, 2012/2013.

Treatment	Tuber set soil (ppm)		Petiole (%)			Harvest soil (ppm)			Peel %		
	S	pH	S	Total N	NO_3	S	pH	NO_3	S	Total N	NO_3
Nil	35	4.9	0.17	3.36	0.67	34	4.9	52	0.14	3.00	0.04
Gran Am®300	66*	4.9	0.11*	3.53	1.14	70*	4.8	61	0.15	2.94	0.03
Gran Am® + Entec®300	59*	4.8	0.13*	3.69	1.21	60*	4.8	80	0.14	3.00	0.04
Gran Am®500	75*	5.0	0.14	3.84	1.26	77*	4.7	146*	0.14	2.96	0.03
Gran Am® + Entec®500	77*	4.7	0.18	3.67	0.85	99*	4.7*	145*	0.14	3.13	0.05
lsd P=0.05	9	0.2	0.04	ns	ns	12	0.1	32	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, not significant

The concentration of *S. scabies* DNA was generally low at tuber set and at harvest and did not vary significantly with treatments (Table 5.A.I-84). The incidence of common scab in the untreated control treatment averaged 35% tubers affected. However, none of the treatments had a significant effect on the incidence and severity of common scab compared with the untreated control, despite evidence of changes in soil and plant chemistry. Total yields in field were not significantly affected by the treatments.

Table 5.A.I-84 – (cv. Catani) Effects of different rates ammonium sulphate fertiliser (NH_4SO_4), with or without the ammonium stabiliser applied to soil prior to planting on the concentration of pathogenic *S. scabies* DNA in soil, the incidence and severity of common scab, total and marketable yields in field and factory yields for French fry and crisps in a field trial at Cora Lynn, 2012/2013.

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Nil	37	20	159	35	7	40	37	33	35
Gran Am®300	46	0	7	24	4	49	46	12	15

Treatment	*S. scabies* DNA pg/g soil			Common scab		Yield in field (t/ha)		Marketable yield at factory (t/ha)	
	Planting	Tuber Set	Harvest	% tubers affected	Severity Index	Total	Marketable	French fry	Crisping
Gran Am® + Entec®300	56	4	34	27	4	47	45	13	14
Gran Am®500	25	6	61	34	7	48	45	35	24
Gran Am® + Entec®500	17	14	205	24	5	41	38	23	20
lsd P=0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significantly different from the untreated control at P<0.05; ns, no significant treatment effects

Effects of ammonium sulphate treatments and ammonium stabiliser treatments on greenhouse gas emissions

As well as affecting crop growth, the amendment of soil with various nutrients can have significant effects, either directly or indirectly (affecting soil biology and respiration), on the greenhouse gas emissions from soil. In a preliminary study, greenhouse gas emissions were sampled from planting to full emergence from untreated plots and from the Gran Am 500 treatments with and without Entec. These samples were analysed for concentrations of nitrous oxide because of the additional ammonium sulphate applied to the soil in these experiments. The method of collection and analysis is described below.

Collection and analysis of on-farm greenhouse gas emissions

Measurements of on-farm greenhouse gas (GHG) emissions were made using manual (static) chambers. The static chambers consisted of a section of threaded PVC pipe (15 cm internal diameter, 16 cm high) buried in the ground to a depth of 8 cm. Prior to sample collection, lids containing a rubber O-ring were screwed onto the top of the chambers to obtain an airtight seal. Gas samples were collected into a 12mL evacuated glass vial (Exetainer, Labco UK) using a double ended needle to pierce a rubber septum in the lid of the chamber. Gas samples were usually collected 60 minutes after chambers were closed. Sampling was conducted at regular intervals, initially from planting time seven times over the first nine days (starting 15/11/2013) and weekly from 23/11/2013 to 21/12/2013. The nitrous oxide (N_2O) concentration was determined by gas chromatography. Soil temperature and moisture were measured at approximately the same time as gas sample collection. Five replicate samples were collected at each time point.

Cumulative emissions of nitrous oxide

The cumulative emissions of nitrous oxide over time is presented in Figure 5.A.I-58. The graph shows an increase in gas emissions over time with higher rate of increase in the ammonium sulphate treatments. The emission were slow to start with due to dry conditions from planting time. The rate of emissions increased with increasing soil moisture from rain. The data suggests that emission were greater from plots not

treated with Entec stabiliser than from the ammonium sulphate alone. However a rigorous analysis of the data is not possible since only one gas sample was taken on each plot at each sampling event.

The emissions from this soil, a peaty clay, are much higher than those normally observed from vegetable production areas (sandy loams) (Ian J Porter and David Riches pers. comm.) possibly due to the high organic matter content of the soils in the Koo Wee Rup swampy plain. This is the first time this type of data has been collected from Australian potato fields and the behaviour of the ammonium stabiliser in this environment may possibly differ to that in other soils and may account for these high emissions.

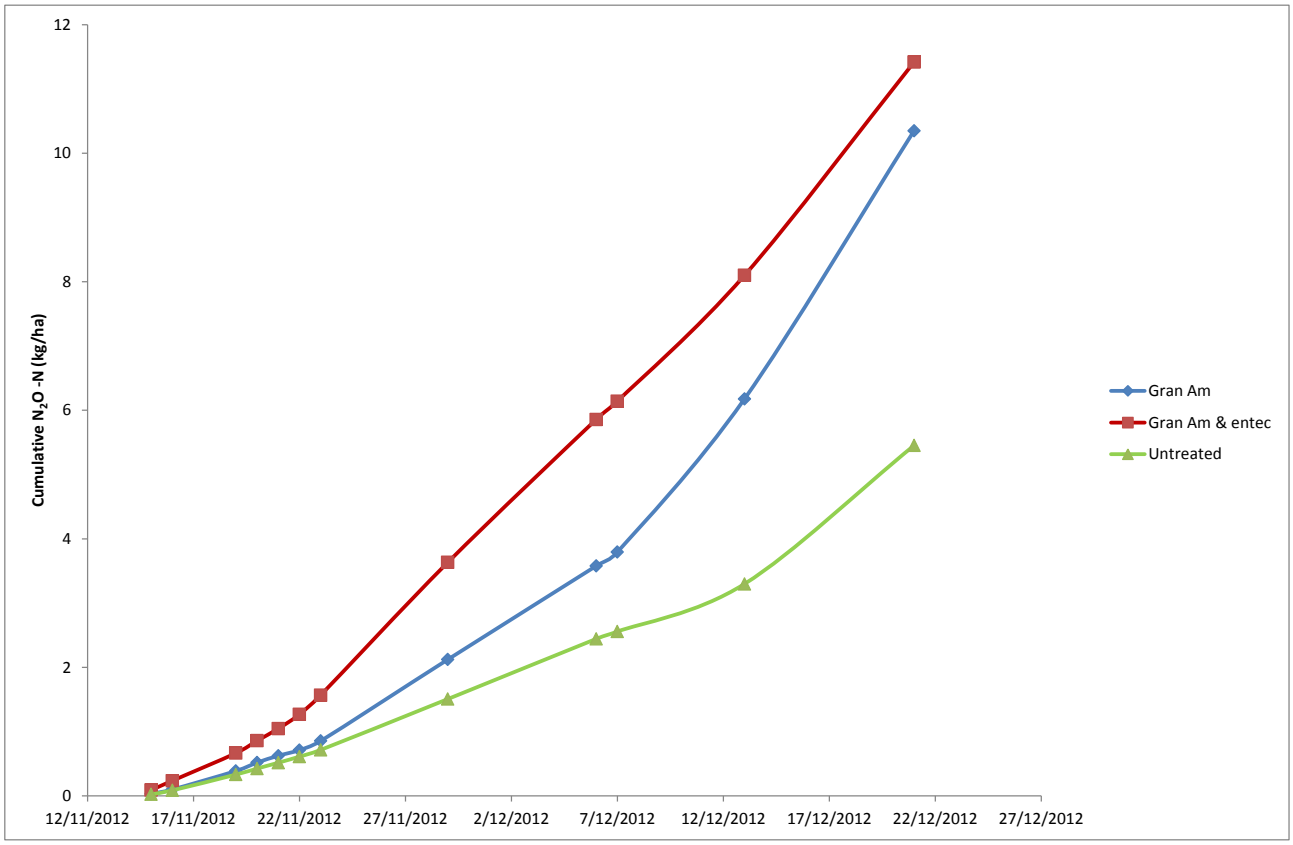


Figure 5.A.I-58 – Cumulative nitrous oxide gas emissions from planting to full crop emergence from untreated plots and plots treated with ammonium sulphate (Gran Am®, 500 kg ammonium sulphate/ha), with or without an ammonium stabiliser (Entec®) in a field trial at Cora Lynn, Victoria, 2012/13.

DISCUSSION

Variety had a large effect on disease expression at harvest. The treatments were more effective on the susceptible variety Simcoe than resistant varieties Pike and Atlantic. However, no treatments were effective on susceptible cv. Wilwash. These results suggest that greater returns could be provided to growers if they applied treatments to relatively susceptible varieties rather than resistant ones.

Sulphur was the first nutrient to be used to control common scab and its suppressive effect was thought to be due to a reduction in soil pH. In one of our field trials, elemental sulphur effectively reduced common scab on Simcoe tubers at harvest by reducing soil pH at tuber set but this adversely affected yield. As a consequence the payable yield at the factory was the same as the untreated control. Therefore, no greater returns eventuated with application of elemental sulphur. Decreasing soil pH also increases the availability of biologically toxic elements such as aluminium which may have resulted in the yield reduction.

We did not see a reduction in common scab unless soil pH was lowered, in contrast to Davis et al. (1974) who found that elemental sulphur and gypsum reduced common scab without an effect on soil pH in highly buffered soils.

It is widely thought that pH is the main driver of common scab expression, and increasing pH increases common scab and decreasing pH reduces it. Potato growers frequently use various forms of lime to condition the soil. There is circumstantial evidence that lime increases the risk of common scab. Trials were conducted over two seasons near Cora Lynn in the peaty clay loams of the Koo Wee Rup district, an major crisping production region of Victoria. Treatments included the oxide, carbonate and sulphate forms of calcium and magnesium, as well as an elemental sulphur treatment. With one exception, none of the calcium and magnesium treatments had a significant effect on the incidence and severity of common scab in three trials, despite changes in soil pH and associated soil and plant chemistry. However, hot lime (calcium oxide) increased scab incidence in one trial by nearly 50%.

The use of elemental sulphur at 1.5t/ha reduced yield and gave inconsistent results on disease. In one trial, the treatment reduced common scab incidence and severity by 50% and 25%, respectively, but in had no effect in a second trial. In both trials, yields were significantly reduced by sulphur. Magnesium sulphate and manganese sulphate treatments had no effect on disease but also caused significant reductions in total yield. In an additional trial on the same soil type, two different forms of sulphur, Atomic® Sulphur (99% sulphur) and Top Wettable Sulphur®, were applied to soil at two different rates (800 and 1200 kg/ha of sulphur) in a field trial at Cora Lynn in 2012/13. None of these treatment were detrimental to yield. Although they lowered pH and raised soil sulphur concentrations, they had no effect on common scab incidence or severity.

The outcome of these trials indicates that growers should be cautious when using pH modifiers. With the exception of hot lime, treatments that raise pH generally do not increase the risk of common scab on the peaty clay loams of the Koo Wee Rup region. Applications of elemental sulphur in this soil type cannot be relied on as a control for common scab.

In our trials, soil manganese was increased by manganese sulphate at 336, 506 and 3200 kg/ha which in turn increased petiole manganese but this was not associated with a reduction in common scab incidence, although the resulting common scab severity was low. The soils in these trial were pH 5.4 and low pH soils tend to have higher levels of manganese. However, the manganese level was only 8 ppm in the untreated controls. Others have found manganese has been linked to a reduction in common scab in some trials but not in others ((McGregor and Wilson 1966), (Gilmour et al. 1968)).

The research showed that nutrient amendments were taken up by the plant into the petiole and peel, but this did not translate into reduced disease. However, in the trials where common scab control was not effective common scab severity was low.

In Ontario, Canada, optimising the soil potassium to magnesium ratio (K:Mg) to 0.4 reduces common scab disease. In this project, we tried to implement this in Victoria for common scab control in five trials on two different soil types. The target ratio of 0.4, adjusted with different concentrations of sulphate or muriate of potash, proved difficult to achieve and sometimes significantly overshot the mark. Disease incidence and severity were only significantly reduced in one of the trials, in which the untreated control had a very high level of disease. In the other trials, disease was low in the untreated controls and no treatment effect was demonstrated.

Raising the ratio from the baseline to 0.6 reduced scab by 14%. However, disease severity remained very high. Lowering the ratio from a baseline of 0.4 to 0.3 increased the incidence of common scab by 15%. We demonstrated that it is possible to have some impact on common scab disease by manipulating the K:Mg ratio, but no recommendation can be made at this stage without further trials in a range of soils types. The take-home message is that it has proved difficult to determine the correct balance of amendments to reach the desired target of 0.4. *In vitro* studies demonstrated that *Streptomyces* thaxtomin gene expression was increased with a K:Mg ratio of 0.4 along with colony forming units.

Historically, manganese sulphate and nitrogenous fertilisers such as ammonium sulphate were identified as a potential control measures for common scab. Different rates of both of these compounds tested in a field trial had no effect on common scab incidence or severity. Greenhouse gas nitrous oxide from soil from the ammonium sulphate treatments were high than from the untreated plots, including plots treated with the stabiliser.

BENEFICIAL MICROORGANISMS - THE ISOLATION, CHARACTERISATION AND UTILISATION OF MICRO-ORGANISMS FROM THE POTATO PLANT RHIZOPHORES/RHIZOPLANE FOR DISEASE MANAGEMENT AND IMPROVED POTATO PRODUCTIVITY

SUMMARY

A previous study (PT07039) identified 1540 bacteria isolated from the rhizosphere of two different potato cultivars grown in two different soils. Using chaperonin gene technology (*cpn60*) to screen for a diversity of biological functions, 81 isolates were found to have antibiotic genes and 58 with nitrogen fixing (*nifH*) genes. These groups were further characterised to determine their diversity and putative identity. In an additional study, 96 isolates of endophytic bacteria were isolated from six genotypes of potato or wild potato relatives from Argentina and Mexico. Of these, 32 showed strong growth on nitrogen-free media and a different group of 21 isolates were found to increase either the root or shoot growth of aseptically produced potato plantlets and were identified through genetic sequencing. These microbes could potentially be used as bio fertilisers for low nutrient potato agriculture.

Three species of *Pseudomonas* (*P. monteilii, P. chlororaphis, P. brassicacearum*), which inhibited *S. scabies in vitro* and significantly reduced the number of pathogenic *Streptomyces* on treated common scab affected seed tubers were evaluated for their effect on seed-borne common scab in a field trial. Seed tubers treated with one or all three species were planted in scab-free soil. Generally, all three species reduced disease incidence, severity, lesion size and the number of pathogenic *Streptomyces* on the clean skin and in scab lesions on the progeny tubers. These effects tended to be greater on cv. Prospect than on Russet Burbank. The consortium of three species was generally less effective than individual species. *P.monteilii* reduced tuber number and yield of both cultivars, probably because of the production of phytotoxic secondary metabolites. This study illustrates the potential of biological control agents for the seed-borne common scab.

A essential criteria for the use of beneficial microorganisms is their effective delivery and establishment on potato tubers and plant root systems. A consortium of nitrogen-fixing bacteria obtained from the roots of field grown Russet Burbank were allowed to establish on the roots of Shepody plantlets under tissue culture conditions, which were then transplanted into a field soil. Plants inoculated with eight separate nitrogen-fixing communities showed an average fivefold increase in dry biomass when compared to un inoculated plants. The microbial profiles, as measured by TRFLP, remained distinct at 30 days after transplantation demonstrating that the plant rhizosphere is a resilient community and that the first bacterial community that becomes established on the root remains with the plant even when the plant is placed into soil with vastly different microbiota.

INTRODUCTION

A major focus of the research done by A&L Canada is to identify microorganisms that could be of benefit in potato production, in particular, those microorganisms that are closely associated with the potato root surface, the rhizosphere, and those found within the plant, the endosphere (“endophytes”). Previous studies in APRP1 and a follow-up project (PT07038) (Crump, Turnbull and Lazarovits) had identified 1540 bacteria from the rhizosphere of two different potato varieties grown in two different soils. The research reported here was to identify key microorganisms that reside on potato roots with the objective of using these to ultimately increase root health and reduce the input costs to growers.

The process involved:

- Isolation of the microorganisms from the potato plant rhizosphere
- Characterisation of rhizosphere bacteria through various molecular techniques
- Screening selected microorganisms for biological activity, e.g. disease suppression or promoting plant growth
- Demonstrating efficacy of these organisms in suppressing disease or improving plant productivity through glasshouse and in the field experiments.
- Determining whether these organisms can establish communities on potato seed tubers and potato plant roots, which is critical in ensuring effective delivery of these organisms into a production system.

In an additional study, bacteria endophytes (found inside the potato plant) were isolated from commercial potato or wild potato relatives, specifically looking for those that could promote potato growth or fix nitrogen.

Besides providing a suite of beneficial organisms for use in potato production, this study compliments the work reported in the previous two sections on identifying “bio indicators” of soil health for soil health tests and those causing disease suppression. Understanding who the beneficial organisms are in a potato production system will help better target the soil health tests.

The future challenge of this research is to determine methods of introducing or stimulating microbial communities to achieve disease suppression and growth promotion in the field. This will ultimately provide growers with a low cost, non-chemical disease management strategy for soilborne diseases.

The overall objectives of this work is to provide tests for soil health and to deliver formulations of beneficial microorganisms (biological control and biofertilisers) as possible seed treatments for improved potato health and productivity.

We have isolates now that can fix nitrogen, solubilize phosphate and antagonize the most serious soilborne pathogens of potatoes. These microorganisms will be tested as biofertilisers using seed inoculation as a delivery method. Furthermore, by knowing who the beneficial microorganisms are we can use their presence as bioindicators of soil health. This allows for more rapid methods of measuring the impacts of various soil treatments on soil health.

CHARACTERISING RHIZOSPHERE MICROORGANISMS

CHARACTERIZATION OF FREE-LIVING, NITROGEN-FIXING AND ANTIBIOTIC-PRODUCING BACTERIA ASSOCIATED WITH TWO CULTIVARS OF POTATO GROWN IN TWO DIFFERENT SOILS

In APRP1 and continued as PT07038, chaperonin gene (*cpn60*) (which is highly conserved) technology used in medical research was utilised to characterise the consortia of soil microbes associated with two potato cultivars (Shepody and Yukon Gold) growing in two different soils from Prince Edward Island and Ontario, Canada (Turnbull et al. 2012). The chaperonin gene was chosen because it is a single copy gene which lacks potential for artefacts which are encountered with multi copy genes, 16S rRNA and thus makes it more amenable for use in quantitative PCR assays. Bacteria with growth promoting or disease suppressive potential were sought for future development of biocontrol agents and biofertilisers for potato. The *cpn60*

technology was used to determine where best to look for microorganisms of importance to plants, and showed that microbes directly associated with roots, which also encompass the endophytic community in the roots, harbour unique concentration of potentially useful bacteria for enhancing disease suppression and promoting plant growth. The microorganism communities associated with potato roots were screened for bacteria harbouring genes involved in nitrogen-fixation and biosynthesis of the major antifungal antibiotics produced by disease suppressive fluorescent pseudomonads. The genes were detected using PCR. 1540 bacteria were isolated, of which 81 contained antibiotic genes and 58 contained nitrogen-fixing (*nifH*) genes. Further studies were conducted to identify these different groups of bacteria and their beneficial properties.

Screening of potential nitrogen-fixing and/or biocontrol isolates for possible mechanisms of activity; cell wall degrading enzymes (protease, glucanase, chitinase), phosphate solubilizing enzyme, siderophores and plant growth promoting hormones (Indole acetic acid)

A number of promising isolates (81 contained antibiotic genes and 58 contained nitrogen-fixing (*nifH*) genes) were subjected to further tests for evidence of inhibitory activity or growth promotion effects. The results of these studies included:

- Noticeable production of siderophores (62%), indole acetic acid (56%) phosphatase (43%) and protease (41%) among bacterial isolates harbouring antibiotic genes.
- For *nifH*+ bacteria (i.e. containing a nitrogen-fixing gene), other traits were observed at lower proportion than found among antibiotic positive bacteria (siderophores, 29%; phosphatase, 24%; and protease, 2%), with the exception of IAA acid production, which was found in 59% of the isolates.
- Glucanase activity was observed in 14 out of 81 antibiotic positive isolates and in 5 out of 58 *nifH* isolates.
- Degradation of chitinase was not detected in any isolate.

A assessment of diversity among isolates and identification by 16S rRNA gene sequencing

To determine the diversity and putative identity of the bacterial isolates, cluster analysis was generated from BOX-PCR fingerprinting of amplified genomic DNA obtained from the isolates.

- Separation of 81 antibiotic isolates at 70% similarity BOX pattern identified 48 different groups.
- Sixteen out of the groups contained at least one bacterium with antagonistic activity.
- Partial 16S *rRNA* gene sequencing of eighteen of the antagonistic bacteria showed affiliation to nine bacterial species with previous history of biocontrol activity, namely *Pseudomonas fluorescens, P. brassicacearum, P. chlororaphis, P. putida, Xanthomonas retroflexus, Bacillus subtilis, Enterobacter amnigenus, Arthrobacter niigatensis* and *Paenibacillus polymyxa.*
- Isolates carrying *nifH* gene were also quite diverse, 13 bacteria genera could be identified from the 27 isolates selected for 16S *rRNA* gene sequencing.
- These *nifH* isolates also belong to various bacterial genera whose members have been reported to fix nitrogen such as *Bradyrhizobium, Azospirillum, Xanthobacter, Stenotrophomonas, Paenibacillus, Rhizobium, Achromobacter, Mycobacterium* and *Ensifer*. In addition, bacterial genera (*Brevundimonas, Chitinophaga, Variovorax, Obesumbacterium)* with no known nitrogen fixing potential were also found.

Some of these bacteria were tested for growth promotion using bacterized minitubers planted into soil collected from poorly producing soils to examine if it is possible to create a biofertilzier/biocontrol formulation for use in the potato industry (see next Section).

Characterisation of populations and determination of the ecological niches of *Agrobacterium* species isolated from potato rhizosphere and roots.

Our previous study on the microorganisms residing on potato roots showed the presence of high numbers of *Agrobacterium* and closely related *Rhizobium* sp. on potato roots harvested from two vastly different Canadian soils. We were therefore interested to learn more about the relationships of the rhizobia in different soils and their potential role in rhizosphere ecology.

We used the universal primer BOX on DNA extracts of a large number of isolates of the bacteria we have in our collection to unravel their genetic lineage and relationships. The BOX PCR results show that the isolates cluster into six groups based on the type of soil in which the plants were grown in (Figure 5.A.I-59). This agrees with the cpn60 sequencing, which also clustered isolates based on soil type. Using 16S sequencing, all Agrobacteria isolates had >98% identity with *Agrobacterium tumefaciens*.

Most of the bacteria are plant pathogens that cause the disease known as crown gall. We wanted to determine if the isolates we are obtaining from potato roots were indeed potential parasites. To determine this we used PCR to identify genes associated with virulence (virB-virG). The results, however, showed that none of the isolates contained the Ti plasmid, a prerequisite for causing disease. To ensure we had correctly identified the bacteria we sequenced the 16S DNA region and this showed that the majority of isolates had 100% identity to type I *A. tumefaciens* which is pathogenic. Additionally, preliminary results using the tomato gall assay revealed that none of our isolates induced gall formation but authentic *A. tumefaciens* strains were able to do so. Future research would be aimed at determining if such microorganisms can act as biocontrol agents against more virulent bacteria by competition or cross protection.

The effect of production of quorum sensing hormones by *A. tumefaciens* in the rhizosphere

Bacteria communicate with each other in soil and on root systems by producing a family of compounds known as n-acyl homoserine lactones (AHL). When the concentration of these compounds reaches a sufficient level, it can activate a wide diversity of bacterial regulatory genes that regulate such cell functions as virulence, reproduction rate, dormancy, antibiotic production, etc. In a previous study we found that there was an increase in the number of quorum sensing strains of the *Agrobacterium* strains isolated from the rhizosphere compared to the endosphere from which we also isolated bacteria. We wanted to know what role the *Agrobacterium* AHL (3o-C8-HSL) may play in the rhizosphere. We made one community comprised of 10 species using the isolates that we found to be present in the highest numbers on potato root as identified by cpn60 sequencing. Into this community we swapped isolates of *A. tumefaciens* that were competent or incompetent in producing AHL. The sets of producers and non-producers were selected based on having the most similar genetic profiles. Potato plants were grown in sand to which the bacteria were added. At the outset of the trials half the tubes received a supplement of 25 µM AHL and half received only the carrier. After two weeks' growth we evaluated the populations using three media to differentiate the various bacteria: Medium 1 - YEM Congo Red (identifies *Arthrobacter*, *Sphingopyxis*, *Xanthomonas*), Medium 2 - R2A (identifies *Sphingobium* and *Massilia*), and Medium 3 - RSM (identifies *Agrobacterium* and *Ensifer*).

Based on the results of experiments replicated twice, both AHL producers and non-producers increased in relative numbers in the presence of AHL regardless of whether the strains can quorum sense or not, which was unexpected. Only the *Agrobacterium* populations were radically altered by AHL treatment although all other strains also tended to increase but less so. The largest increase in the Agrobacterium population was in the order of 10-40 fold, which indicates that this is unlikely to be a nutrient response. Future tests aim to determine if quorum sensing confers an advantage in the rhizosphere by directly competing sets of strains. Understanding how we can modify the dominant species in a rhizosphere in a requirement for formulation of a biofertiliser which we hope to produce.

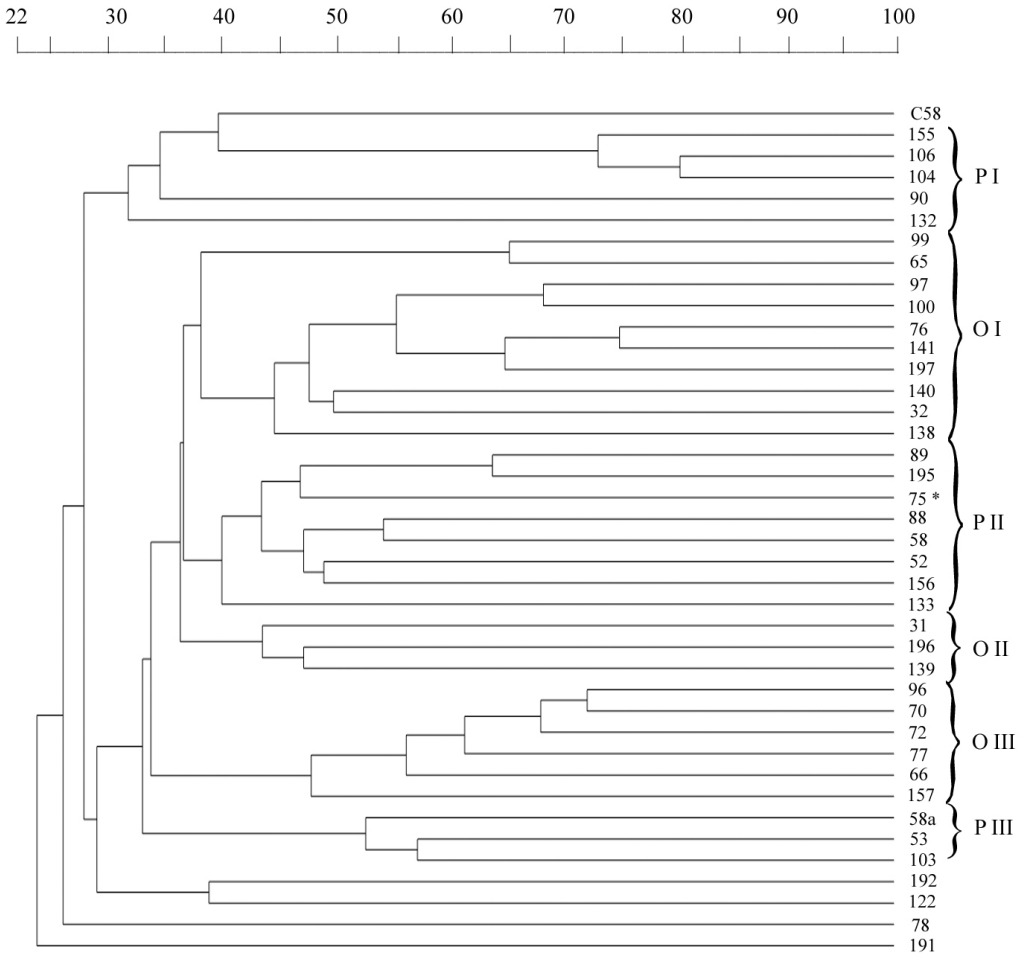


Figure 5.A.I-59 – Dendrogram of BOX PCR band profiles made using GelCompar II. Data were normalised using a median filter and least squares filter. Jaccard's similarity matrix for binary data was calculated with an optimization value of 1% and tolerance of 1%. Hierarchical clustering was then performed using Topscore unweighted pair group method with arithmetic mean (UPGMA) using the Jaccard's similarity matrix. The cophenetic correlation coefficient for the entire dendrogram was 83%. Scale bar at the top represents percent similarity. Asterisk (*) beside isolate 75 denotes it came from Ontario soil while it grouped with Prince Edward Island soil isolates. Group O III represents a new *Rhizobium* species, while isolate C58 is the type strain of *Agrobacterium tumefaciens.*

SCREENING MIROORGANISMS FOR BIOCONTROL AND NITROGEN-FIXING POTENTIAL

IN VITRO TESTING OF A COLLECTION OF BIOCONTROL AGENTS AGAINST *VERTICILLIUM DAHLIAE* AND *STREPTOMYCES SCABIES*

Bacterial biocontrol agents were isolated during a previous study using semi-selective media. Bacteria were selected based on the ability to suppress fungi on Petri plates in a dual culture (potato pathogen and bacteria). This collection was largely composed of Pseudomonads and Xanthomonads. These studies are aimed at identifying micro-organisms with the potential to suppress the common scab and Verticillium wilt pathogens for further testing in plant systems.

Methods

A suspension of *Verticillium dahliae* microsclerotia or *S. scabies* spores was spread onto agar plates. Test bacteria were streaked in a line. The control plate had only the microsclerotia/spore suspension spread. These plates were incubated at 23°C for 16 days and were read at days 3, 10, and 16. Test bacteria were scored on the basis of inhibiting pathogen growth, or delaying pathogen development (no spore production in *S. scabies* or no microsclerotia production in *V. dahliae*).

Results and Discussion

The library consisted of 48 strains. Of these, 8 showed activity by inhibiting pathogen growth or delaying maturation compared to the control on at least two time points. Five isolates delayed *V. dahlia* microsclerotia production while two different isolates inhibited vegetative growth. Two isolates inhibited the vegetative growth of *S. scabies*. One isolate 5-20 (not yet identified) inhibited growth of both pathogens, while another 2-28, a *Stenotrophomonas*, delayed or inhibited formation of pathogen resting structures (microsclerotia and spores).

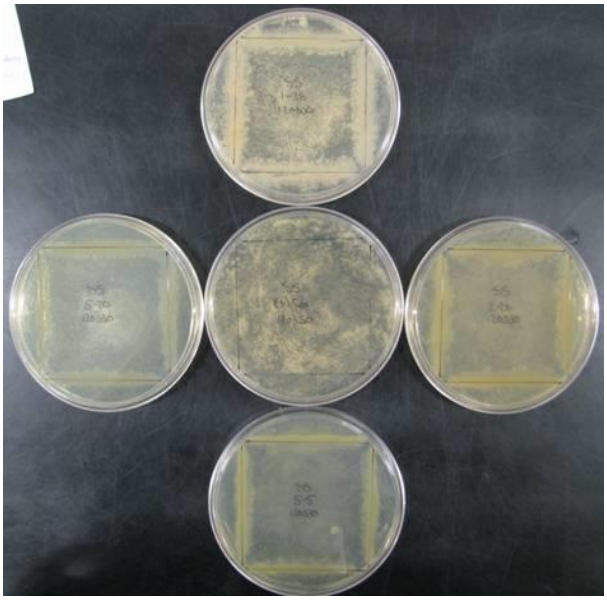


Figure 5.A.I-60 – Isolates (from top, clockwise): 1-28, 2-28, 5-5, and 5-20. The *Streptomyces scabies*-only control is in the centre.

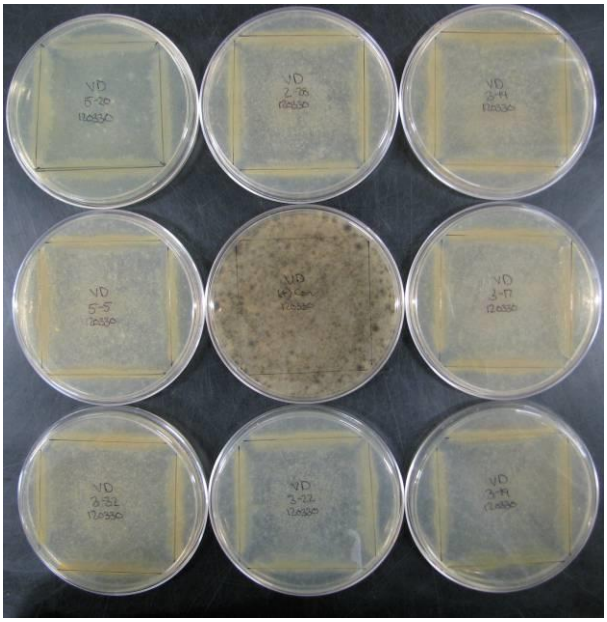


Figure 5.A.I-61 – Isolates from top left (clockwise): 5-20, 2-28, 3-14, 3-17, 3-19, 3-22, 3-32, and 5-5. The *Verticillium dahliae*-only control is in the centre.

GREENHOUSE EXPERIMENTS TO DETERMINE THE BIOCONTROL POTENTIAL OF FIVE SELECTED ANTIBIOTIC-PRODUCING ISOLATES IN SOIL NATURALLY INFECTED WITH *S. SCABIES* CULTIVATED TO POTATO

Five of the antibiotic-positive isolates, which showed strong *in vitro* activity in dual culture assays against *Streptomyces scabies*, were screened for their ability to suppress common scab of potato in two soils (DA – Delhi, continuous potato and DF – Delhi, planted with green manures) that were naturally infected with the pathogen.

The result of the first trial showed between 14% and 41% reduction in scab severity in the DA soil and 18% and 65% in DF soil with four of the inoculants (Figure 5.A.I-62). In both soils, one of the inoculant (BC+90) seems to enhance scab severity. Reproducibility of these results in being determined in another on-going greenhouse experiment (Trial 2).

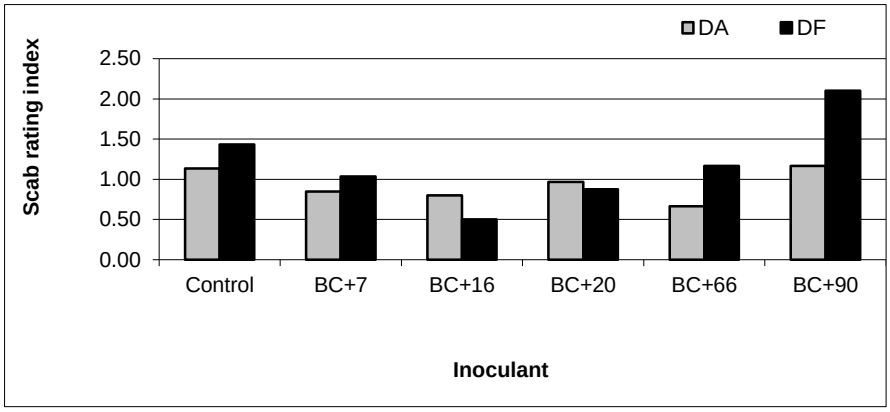


Figure 5.A.I-62 – Scab severity in potato tubers inoculated with five antibiotic positive isolates cultivated in the DA (Delhi site, continuous potato) and DF (Delhi, planted with green manures) soils.

The results, while promising, require much more rigorous trials to determine how to best deliver such bacteria to the potato rhizosphere. The following study reports on the effects of four biological control candidates, three species of the *Pseudomonas* bacteria on tuber-borne common scab.

SEED TUBER TREATMENTS WITH PSEUDOMONAS SPP. (*PSEUDOMONAS MONTEILII* A25, *PSEUDOMONAS CHLORORAPHIS* A145, *PSEUDOMONAS BRASSICACEARUM* A153) TO REDUCE COMMON SCAB (*STREPTOMYCES* SPP.) INCIDENCE AND SEVERITY.

Extracts of the Master's research by Muna Basahi, supervised by George Lazarovits (submitted mid 2014) is presented in this section

SUMMARY

Potato common scab is a tuber- or soil-borne disease caused by several species in the genus *Streptomyces*. The objective of this study was to study the treatment of seed tubers with *Pseudomonas* spp. as a means to reduce common scab incidence and severity, and improve seed tuber quality and marketability.

All three *Pseudomonas* spp. tested inhibited pathogen *Streptomyces* strains SS-1; PEI-1 and AL-1 *in vitro*. In laboratory trials, treatments of seed tubers with Pseudomonas strains A25, A145, and A153, significantly reduced in the number of pathogenic *Streptomyces* in comparison to untreated tubers within five weeks ($P<0.05$). Seed tuber treatments with A25 and A153 significantly reduced scab incidence and severity in the progeny of Russet Burbank and Prospect cultivars, respectively.

This study also demonstrated the use of TaqMan qPCR detecting the txtAB gene analysis as a novel and practical method to quantify pathogenic *Streptomyces* in tissues of scabby and apparently clean potato seed tubers.

Evaluating the effects of bacterial inoculants in reducing *Streptomyces scabies* populations on scabby seed potato tubers – a preliminary trial

We wanted to develop seed treatments with microorganisms that we have isolated and shown to be highly inhibitory to *S. scabies*. A real time PCR method was demonstrated to be able to quantitate the number of bacteria associated with scab pathogen infesting tuber surfaces. We have successfully used this to observe a reduction of *S. scabies* DNA on tubers inoculated with different biocontrol strains of *Pseudomonas* species.

Hundreds of bacteria had been screened for antagonistic activity to *S. scabies*. A number of extremely bioactive strains have been isolated and identified by sequencing. Of these, four are exceptional in the antibiotics they produce and are novel species. We are testing these strains as means to reduce the populations of *S. scabies* on the tubers to be used for seed that had been grown in scab-infested fields.

Methods

The Pseudomonas strains A25, A33, A145, and A153 were grown in LB broth for 5 days to promote antibiotic production. These strains were also tested for their ability to antagonise *S. scabies* by co-incubating these bacteria with the pathogen on petri dishes of potato dextrose agar. Scab-infected tubers of varieties Prospect, Yukon Gold and Russet Burbank were provided from PEI for field experiments. Prospect was the variety used in the laboratory assay reported on here. Tubers were dipped into bacterial culture for 30 seconds and left overnight to dry at room temperature. After a week of incubation at room temperature, DNA was extracted from tuber skins and real time PCR performed to estimate levels of *S. scabies* present.

Results and Discussion

A qPCR assay using primer pairs StrepF/R and probe Strep2Q was optimised initially using pure culture of Streptomyces scabies isolated previously at A&L Biologicals, then further refined by spiking scab DNA into Russet Burbank tuber DNA samples and compared to DNA levels extracted from known numbers of bacteria (estimated by counting number of colony forming units in a dilution before extraction of DNA).

Lab experiments to evaluate the ability of different control strains of *Pseudomonas* to reduce levels of scab were then attempted on scabby tubers of the Prospect variety, delivered by partner organisations in Prince Edward Island, Canada. Figure 5.A.I-63 shows that one of the control strains, A145, was able to significantly reduce scab population levels on tubers after a week of incubation. A153 produced a very clear zone of scab growth inhibition on the petri dish in Figure 5.A.I-63,however, results for the incubation experiment were not ready at the time of writing. A further experiment was conducted with the above bacteria treatments on seed tubers that were planted in the field trials in London, Ontario to evaluate the ability of tuber inoculations with these bacteria to reduce scab incidence of daughter tubers.

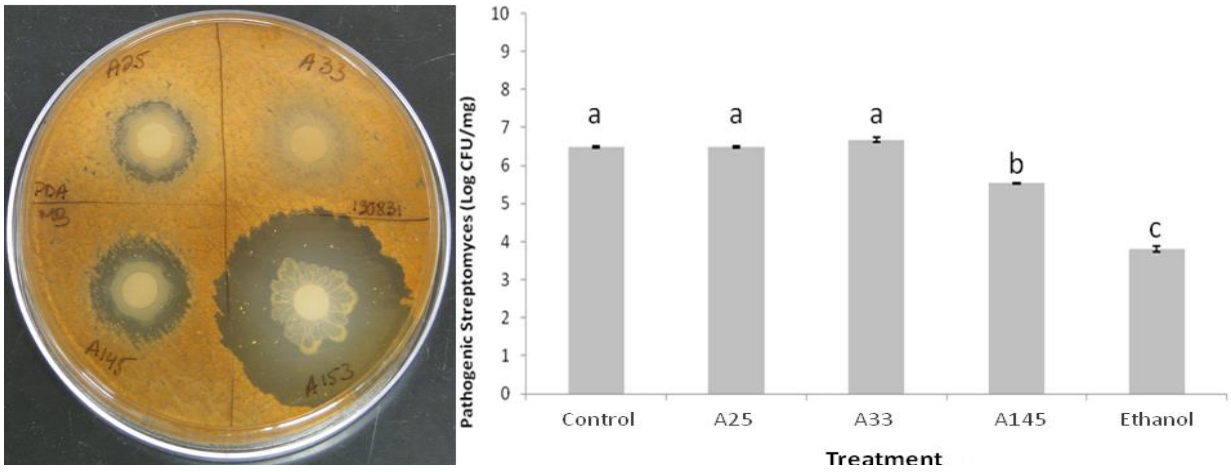


Figure 5.A.I-63 – Zones of *S. scabies* inhibition caused by antibiotic secretion by different *Pseudomonas* strains and reduction of scab populations on infected Prospect tubers after 1 week of incubation with *Pseudomonas* strains A25, A33, or A145. Ethanol-treated tubers were soaked in ethanol for 3 hours to kill surface populations of *S. scabies*. Two hundred milligrams of scabby tissue was excised and analysed using RT-PCR. Results presented are means±SE log CFU/mg after one week incubation in sterile conditions at room temperature. Significant differences are shown with different letters (P<0.05).

The effects of seed-tuber treatments with *Pseudomonas* spp. on *S. scabies* populations on tubers and on the incidence and severity of common scab in the progeny.

Introduction

The objective of this work was to assess the use of *Pseudomonas* species as biocontrol agents of common scab. Three *Pseudomonas* strains, previously shown to inhibit growth and sporulation of *S. scabies* in bioassays on agar plates, were used to treat scabby tubers of three different potato cultivars: Yukon Gold, Prospect and Russet Burbank. Field trials were conducted at a research field where potatoes had never been previously planted. The objective was to prevent the spread of the pathogen from the infected tubers to the daughter tubers. Reductions of pathogen load was assessed on the seed tubers and on the progeny after field trials were completed, by measuring scab incidence and index, and scab lesion size. We also quantified the amount of pathogenic *Streptomyces* present per milligram of tissue using quantitative PCR as a way to measure disease decline.

Material and Methods

Seed preparation: Scabby seed tubers of three different potato cultivars (Prospect, Russet Burbank, and Yukon Gold) were obtained from Prince Edward Island for use in a field trial. Tubers were graded for uniformity of scab incidence. The Yukon Gold and Prospect seed tubers had 50-60%; 30-40% scab coverage, respectively. The Russet Burbank seed tubers were visually clean but had been harvested from a field where scab was a problem with other cultivars. Tubers were initially washed with distilled water to remove adhering soil and left overnight to dry at room temperature. We were only able to obtain a limited number of scabby tubers and thus we were very limited in the number of replications that could be done. To increase the numbers, we cut the seed in half and allowed them to cure for 24 hours. The seed tubers were sorted according to scab incidence and size and fifteen tubers of each cultivar were place into paper bags (totalling 21 bags) for use in seed treatments.

Preparation of inoculum for seed treatments: Five hundred millilitres of bacterial culture was prepared for each seed treatment. *Pseudomonas monteilii* A25, *Pseudomonas chlororaphis* A145, *Pseudomonas brassicacearum* A153 were grown in liquid broth for 5 days to a concentration of 10^7 CFU/mL in order to promote antibiotic production. The three strains were also mixed in 1:1:1 ratio and tested as a seed treatment. Carboxymethyl cellulose (CMC) was mixed with the bacterial cultures to obtain a final concentration of 1%. Seed tubers were dipped for five minutes into the 500 mL bacterial suspensions in surface sterilized plastic containers then left to dry over night at room temperature. Control treatments included seed tubers dipped into liquid broth or into autoclaved water for five minutes.

Field trial

The field experiment was conducted at the Ecologistics Research Services Centre farm, London, Ontario, with planting on 4 June 2013. The plot was 19.8 x 8.2 m in size and divided into seven rows where each row represented one treatment. Each row was then divided into three sections 4.6 x 0.9 m in size. Fifteen tubers from one cultivar were planted 0.3m apart in each section. For each seed treatment one tuber represented one replicate. Plots were fertilized with N:P:K by the grower according to Ontario recommendations. Plants were treated with fungicides as required for control of late blight. Emergence readings were taken at 19 July 2013. After four months the plants had died down and the middle ten plants were harvested separately and used to compute the number of tubers per plant, weight of tubers per plant, scab lesion size, and the number of pathogenic *Streptomyces* per mg of tissue.

Treatments applied to the tubers of three different potato cultivars were: 1) No treatment of seed tubers; 2) 80% ethanol surface sterilisation; 3) liquid broth treatment (no bacteria); 4) *Pseudomonas monteilii* A25 in liquid broth; 5) *Pseudomonas chlororaphis* A145 in liquid broth; 6) *Pseudomonas brassicacearum* A153 in liquid broth and 6) a mixture of the three bacteria in liquid broth.

Scab incidence and index, scab lesion size data collection

Ten plants were dug for use in evaluating the effect of seed treatments. Harvested tubers from each cultivar and treatment were sorted into bags for analysis. Tubers from each cultivar and treatments were scored according to surface area with scab. Scores were then used to calculate scab incidence and index (Table 5.A.I-85) according to(Wang and Lazarovits 2005). Tubers with scab coverage of less than 5% were considered visually clean. Scab lesion sizes of three lesions per tuber were measured with a 10 cm ruler

$$Scab\ incidence = \frac{Total\ number\ of\ scabby\ tubers}{Total\ number\ of\ tubers\ rated}$$

$$Scab\ index = \frac{\Sigma(rating\ level\ x\ number\ of\ tubers)}{Total\ number\ of\ tubers\ rated}$$

Table 5.A.I-85 – Scab index value used to assess potato marketability.

Scab index	Marketability
0-10	Marketable
11-20	Least marketable
>30	Rejected

Detection and quantification of pathogenic *Streptomyces*

Ten tubers with similar scab score, index, and scab lesions were selected from each cultivar and treatment for quantifying the number of pathogenic *Streptomyces*. From each tuber three visually clean and three scabby tissue samples (0.5-1 mm) were excised from the surface using 1.2 cm core borer. DNA was then extracted from each tissue sample and analysed for quantification of the *txtA* gene using TaqMan real-time PCR. The number of pathogenic *Streptomyces* was calculated using a standard curve generated by plotting the CT-value against log CFU/mg.

Results

Field trials: The spring was cold and extremely wet. Germination of the cultivars was slow and plants remained quite small over the season as shown in Figure 5.A.I-64.

Figure 5.A.I-64 – Effect of seed treatments with *P. monteilii* A25 on plant growth and flowering. Pictures were taken one month after planting Prospect seed tubers. A Similar effect was found on treated Russet Burbank, with Yukon Gold not sprouting.

Treatment effects of on seed germination

Germination of seeds was measured one and four months after planting. Yukon Gold seeds completely failed to germinate likely due to seed decay of the cut pieces in the cold moist soil (Table 5.A.I-86). Russet Burbank seed tubers treated with *P. monteilii* A25, *P. chlororaphis* A145, *P. brassicacearum* A153 and the consortia had 67%, 73%, 73% and 93% of seeds germinated within the first 30 days respectively. Seed treated with 80% (v/v) ethanol had 93% germination.

Germination of Prospect one month after planting was better than of Russet Burbank and 80%, 80%, 87% of the seed tubers treated with *P. monteilii* A25, *P. chlororaphis* A145, *P. brassicacearum* A153, produced plants while seeds treated with the consortia, the liquid broth and untreated seeds had 100% germination. In this case, Prospect seed treated with ethanol failed to germinate. By season's end all seeds pieces, except for those treated with *P. monteilii* A25 or ethanol had 100% germination.

Table 5.A.I-86 – Germination of seed tubers of cvs Russet Burbank and Prospect in the field after one and four months; average number of progeny tubers per plant and yield per plant for seed tubers treated with three potential biocontrol agents and a consortium of those biocontrol agents

Seed tuber treatments	% Germination at 1 and 4 months				Tubers/plant		Yield/plant (g)	
	Russet Burbank		Prospect		Russet Burbank	Prospect	Russet Burbank	Prospect
	1 m	4 m	1 m	4 m				
Liquid broth (LB)	88	85	85	85	4.5b	7.0b	325a	450abc
LB + *Ps. monteilii* A25	80	85	88	87	3.5c	2.0d	100b	180d

Seed tuber treatments	% Germination at 1 and 4 months				Tubers/plant		Yield/plant (g)	
	Russet Burbank		Prospect		Russet Burbank	Prospect	Russet Burbank	Prospect
LB + *Ps. chlororaphis* A145	89	85	88	85	4.5b	5.1c	240a	380c
LB + *Ps. brassicacearum* A153	89	87	87	85	3.5b	7.0b	240a	400bc
1:1:1 consortium (A25, A145, A153)	86	86	85	85	5.0a	7.2a	320a	450ab
80% v/v ethanol	86	85	0	0	3.7b	ND	300a	ND
No Treatment	85	85	85	85	5.5a	6.9ab	280a	600a

Notes: Controls include liquid broth alone; no treatment (seed tubers dipped in sterile water) and a negative control of dipping in 80% v/v ethanol. Significant differences are indicated with different letters following the numbers according to either Duncan's Multiple Range Test or the T-test (P<0.05). Note 80% v/v ethanol killed Prospect plants before harvest (ND).

Effect of seed treatments on number of daughter tubers produced per plant

Russet Burbank seed tubers treated with *P. monteilii* A25 resulted in lowest numbers of tubers produced per plant, followed by *P. chlororaphis* A145, *P. brassicacearum* A153, liquid broth and 80% (v/v) ethanol, whereas those treated with the consortia of all three and untreated tubers yielded nearly 5 tubers per plant, respectively (P<0.05) (Table 5.A.I-86).

Prospect tubers untreated, treated with liquid broth, *P. brassicacearum* A153 and in consortia yielded approximately 7 tubers per plant. Contrarily, those treated with *P. chlororaphis* A145 yielded 5 tubers per plant, and those treated with *P. monteilii* A25 only yielded an average of 2 tubers per plant. However, surface sterilization of symptomatic Prospect seed tubers killed all plants ($F_{5,54}$=83.44; P<0.001) (Table 5.A.I-86).

Effect of seed treatments on yield

Seed treatments of Russet Burbank with *P. monteilii* A25 resulted in a decrease in yield by 61% in comparison to untreated tubers ($F_{5,54}$ =13.78, P<0.001; Table 5.A.I-86). None of the other treatments adversely affected yield.

In comparison to untreated Prospect seed tubers, *P. monteilii* A25 significantly decreased yield by 68% followed by those treated with *P. chlororaphis* A145 and *P. brassicacearum* A153 where yield was lower by 37%, and 29%, respectively ($F_{5,54}$=7.14, P<0.001, Table 5.A.I-86).

Effect of seed treatments on scab incidence and index in progeny tubers

Treating Russet Burbank seed tubers with *P. chlororaphis* A145 and in consortia resulted in 12.7±1% and 26.1±4% scab incidence whereas untreated seed tubers had 78.7±3% scab incidence ($F_{6,63}$=21.13, P<0.001;

Table 5.A.I-87). Progeny tubers of *P. monteilii* A25 treatment had scab incidence of less than 1% and would be considered marketable. Surface sterilization resulted in 57.2±4% scab incidence. However, *P. brassicacearum* A153 showed no effect (Table 5.A.I-87). In addition, the scab index severity on the progeny tubers of *P. monteilii* A25, *P. chlororaphis* A145 and in consortia treated seeds was <5, significantly less than the other treatments (Table 5.A.I-87).

Table 5.A.I-87 – Incidence, severity and lesion size of common scab on progeny tubers of cvs Russet Burbank and Prospect from seed tubers treated with three potential biocontrol agents and a consortium of those biocontrol agents

Seed tuber treatments	% tubers infected		Severity Index (%)		Lesion size (mm)	
	Russet Burbank	Prospect	Russet Burbank	Prospect	Russet Burbank	Prospect
Liquid broth (LB)	93a	40b	25a	6.5b	3.7a	5.5b
LB + *Ps. monteilii* A25	1.0c	10e	1.0c	1.0e	1.0c	2.3c
LB + *Ps. chlororaphis* A145	13c	18d	2.0d*	2.3c	2.0b	0.1d*
LB + *Ps. brassicacearum* A153	90a	28c	15b	3.0d	3.5a	0.1d*
1:1:1 consortium (A25, A145, A153)	27bc	30c	3.0c*	3.5d	3.5a	0.1d*
80% v/v ethanol	57ab	ND	12b	ND	2.0b	ND
No Treatment	79a	59a	12b	9.0a	2.1b	8.3a

Notes: Controls include liquid broth alone; no treatment (seed tubers dipped in sterile water) and a negative control of dipping in 80% v/v ethanol. Significant differences are indicated with different letters following the numbers according to the the LSD-test ($P<0.05$). Note that 80% v/v ethanol killed Prospect plants before harvest (ND). * indicates progeny tubers where scab coverage was less than 10% and considered marketable

Treatment of Prospect seed tubers with *P. monteilii* A25, *P. chlororaphis* A145, in consortia and *P. brassicacearum*, A153 resulted in 10 %, 18%, 28% and 29% scab incidence in comparison to 59% scab incidence in progeny tubers from untreated seeds (Table 5.A.I-87). Similarly, the above treatments showed significantly lower scab index in comparison to progeny tubers of untreated seeds (Table 5.A.I-87).

Effect of seed treatments on lesion size (mm)

Liquid broth, *P. brassicacearum* A153 and the consortia of all three *Pseudomonas* strains significantly increased scab lesion size by 1.4 mm in comparison to untreated Russet Burbank progeny tubers. However, *P. monteilii* A25 progeny tubers had average lesions size of 1 mm in comparison to untreated Russet Burbank 0tubers ($F_{2,87}=24.13$, $P<0.001$, Table 5.A.I-87).

Prospect seed tubers treated with liquid broth and *P. monteilii* A25, resulted in a significant decrease in mean lesion size from 8.3±0.3 mm to 8.3±0.12 and 2.3±0.1 mm, respectively ($F_{2,87}$=27.6; P<0.01, Table 5.A.I-87). In addition, *P. chlororaphis* A145, *P. brassicacearum* A153 and the consortia of all three strains resulted in visually clean progeny tubers.

Effect of seed treatments on the number of pathogenic *Streptomyces* per milligram of tissue

On Russet Burbank, all treatments excluding *P. monteilii* A25 treated tubers and untreated tubers, had significantly higher number of pathogenic *Streptomyces* in scabby tissue than visually clean tissue ($F_{1,86}$=24, P<0.001, Table 5.A.I-88). *P. monteilii* A25 treated tubers significantly decreased the number of pathogenic *Streptomyces* from 125 to 16 CFU/mg of scabby tissue ($F_{6,48}$=2.54, P<0.05, Table 5.A.I-88).

Table 5.A.I-88 – Number of pathogenic *Streptomyces* (log CFU/mg) quantified from excised visually clean (C) and scabby (S) tissue of progeny tubers of cvs Russet Burbank and Prospect from seed tubers treated with three potential biocontrol agents and a consortium of those biocontrol agents

Seed tuber treatments	Russet Burbank (C)	Russet Burbank (S)	Prospect (C)	Prospect (S)
Liquid broth (LB)	1.0b	2.1a*	4.1a	6.5a*
LB + *Ps. monteilii* A25	1.2bc	1.1b	3.8a	5.0ab*
LB + *Ps. chlororaphis* A145	0.7c	1.7a*	3.5ab	5.0ab*
LB + *Ps. brassicacearum* A153	0.7bc	2.3a*	1.0c	0.8c
1:1:1 consortium (A25, 145, 153)	1.5abc	2.0a*	2.0b	4.0b*
80% v/v ethanol	1.8a	2.5a*	ND	ND
No Treatment	1.6bc	2.1a	3.8ab	6.1a*

Notes: Controls include liquid broth alone; no treatment (seed tubers dipped in sterile water) and a negative control of dipping in 80% v/v ethanol. Significant differences are indicated with different letters following the numbers according to a 2-way ANOVA or the LSD-test (P<0.05). Note 80% v/v ethanol killed Prospect plants before harvest (ND). An asterisk (*) following the letters indicates significant interaction between seed treatments and tissue type (visually clean or scabby, P<0.05).

On Prospect tubers, all seed treatments except *P. brassicacearum* A153, had significantly higher number of pathogenic Streptomyces in scabby tissue samples (F1,125=67, P<0.001, Table 5.A.I-88). *P. brassicacearum* A153 showed no significant differences in the number of pathogenic *Streptomyces* between visually clean and scabby tissue samples, however, samples taken from both visually clean and scabby tissue, had approximately 84% less log CFU/mg pathogenic *Streptomyces* than those excised from untreated tubers (P<0.001, Table 5.A.I-88).

Discussion

Yukon Gold treated with all four different treatments resulted in no germination within the first month in comparison to untreated controls. This could be due to the wet spring and the quality of the seed. On the other hand, more than 50% of the Russet Burbank and Prospect seeds germinated within the first month.

P. monteilii A25 drastically decreased the number of tubers produced and yield by 55% and 61%, respectively, when applied to Russet Burbank seed tubers. Prospect tubers treated with *P. monteilii* A25 decreased in yield by 70% in comparison to untreated tubers. This effect could be due to the production of secondary metabolites that may have a deleterious effect on plant growth and tuber formation.(Blom et al. 2011) found that *P. fluorescens* CHA0, *P. chlororaphis* subsp. *aureofaciens* and *P. aeruginosa* showed high quantities of hydrogen cyanide (HCN) production that led to plant death or drastic growth reduction.

At harvest potatoes were assessed for lesion size, scab incidence and index, surface area covered with scab lesions, and the number of pathogenic Streptomyces present per milligram of tissue. Russet Burbank seed tubers were visually clean when seed tubers were planted. This cultivar is known to be moderately resistant to common scab, however, at harvest progeny of untreated tubers had scab lesions that varied in size ranging from 1-4 mm in length. *P. monteilii* A25 followed by *P. chlororaphis* A145 reduced to ≤2 mm.

Prospect seed tubers had 30-40% surface covered with scab. Progeny tubers harvested from untreated seeds had raised scab lesions that were circular in shape ranging between 4-6 mm in diameter. Seed treatments *P. chlororaphis* A145, *P. brassicacearum* A153, and the three in consortia reduced the average size of lesions to almost negligible. Reduction in lesion size may be due to the ability of *Pseudomonas* spp. to protect plants from necrotizing pathogens by strengthening the epidermal and cortical cell walls by depositing newly formed barriers such as callose (Singhai et al. 2011).

Variation in biocontrol activity between both cultivars may be due to Russet Burbank or Prospect having greater capacity in sustaining interactions with *Pseudomonas* spp. (Meyer et al. 2010). In addition, root morphology and root exudates may vary between cultivars and may have affected successful root-colonization by *P. monteilii* A25, *P. chlororaphis* A145, and *P. brassicacearum* A153 (Meyer et al. 2010). In addition, the consortia of all three had reduction in lesions size only when applied to Prospect seed tubers. (Meyer et al. 2010) found that the size and composition of DAPG-producing *Pseudomonas* spp. in wheat rhizosphere and the amount of DAPG produced may vary substantially between different cultivars (Meyer et al. 2010). However, more information is needed to better understand which cultivars promote beneficial interactions with *Pseudomonas* spp. Therefore, this might be the case in the different results found when Russet Burbank and Prospect tubers were treated with the consortium of all three *Pseudomonas* strains.

Preliminary laboratory experiments showed that within the first week, only the 80% (v/v) ethanol reduced the number of pathogenic *Streptomyces* in comparison to untreated tubers. In contrast, liquid broth increased the number of pathogenic *Streptomyces* by tenfold. Treatment with *P. chlororaphis* A145 had less CFU/mg in comparison to liquid broth, but showed similar number to those from untreated tubers. However, after two weeks *P. monteilii* A25 showed 17% reduction in the number of pathogenic *Streptomyces* compared to those treated with liquid broth but showed similar number to that of untreated tubers. These results suggest that some inhibition is occurring due to antimicrobial activity.

When the experiment was repeated with the addition of industrial sand mixed with 5% (w/w) peat moss, treatments with *P. monteilii* A25, *P. chlororaphis* A145 and *P. brassicacearum* A153 had 98%, 84 and 100 lower number of pathogenic *Streptomyces* compared to untreated tubers. The increase in inhibition is due

to increased biological activity of the *Pseudomonas* strains. Samples excised from tubers treated with liquid broth also showed 95% less number of pathogenic *Streptomyces* suggesting an increase in the activity of a naturally co-occurring microbe on the potato peel. Hiltunen et al. (2009) isolated both pathogenic and non-pathogenic *Streptomyces* from scabby lesions and tested their biological activity against each other. They found that *S. turgidiscabies* inhibited the growth *of S. scabies in vitro* and had the potential to displace it under potato growing conditions. Similarly, they found that the non-pathogenic *Streptomyces* strain 346 also inhibited *S. scabies*.

Russet Burbank treated with *P. monteilii* A25 resulted in 88% less pathogenic *Streptomyces* detected from scabby tissue of progeny tubers. On the other hand, Prospect tubers treated with *P. brassicacearum* A153 showed similar effect in the field where only 9 and 7 CFU/mg were detected from visually clean and scabby tissue, respectively. This lethal effect could be the result of direct killing of pathogenic *Streptomyces* or inhibiting the expression of the *txtA* gene. St-Onge et al. (2011) illustrated that *Pseudomonas* LBUM 223 and *Pseudomonas* LBUM 300 inhibited *Streptomyces* growth on OBA medium. However, the former lowered the transcription level of *txtA* by approximately 46%.

Conclusion

Seed treatments with *P. monteilii* A25 showed a trade-off between yield and disease control when applied to Russet Burbank and Prospect seed tubers. It was effective in minimizing the number of pathogenic *Streptomyces* to significantly low levels, but it greatly decreased yield and the number of tubers produced per plant. The aim of this study was is to minimize transmission of common scab to non-infested soils via seeds, by reducing the number of pathogenic Streptomyces found on potato surface. Seed treatments with *P. chlororaphis* and the consortium of all three strains showed better potential as seed treatments. Both produced similar yield to that of untreated seed tubers and reduced scab incidence to a degree that progeny tubers are considered marketable.

ISOLATING BACTERIAL ENDOPHYTES FROM WILD POTATOES AND TESTING THEM FOR THEIR BIOFERTILISER POTENTIAL

We have isolated 96 potato bacterial endophytes from commercial Canadian potato varieties, late blight-tolerant wild potatoes from Mexico, and nitrogen-efficient wild potatoes from Argentina. We have identified all these strains and characterised their endophyte-potato interaction on potatoes in tissue culture.

Methods

Four genotypes of potato were ordered as true potato seed from the USDA-ARS on the basis of novel phenotype. *Solanum chacoense* accessions HHR 4039 and OKA 4907 originate from Argentina, and were selected for their high nutrient use efficiency. *Solanum verrucosum* accessions HAW 1528 and HAW 1658 originate from Mexico, and were selected for their high resistance to late blight. As controls, these will be compared to two genotypes of commercial, North American potatoes grown from mini-tubers of the varieties Kennebec (KEEC W-K1) and Shepody (Shdy BB2-W). These were planted and grown on non-agricultural soil from Delhi, Ontario receiving minimal fertilisation and incubated with daily watering in a growth room at A&L Biologicals. Two-month-old plants were uprooted, cut into root and shoot, surface-sterilised with bleach and ground into a homogenate that was diluted 1000X and plated on R2A, PDA, TYA, and LGI media. Colonies of different colours and characteristics were then selected from each plate and

were screened for ability to grow on nitrogen-free media, identified by sequencing of the 16S ribosomal gene, and assayed for the ability to promote Kennebec potato plantlet growth in a germ-free environment.

Results and discussion

A library of 96 strains was generated representing a comprehensive diversity of endophytic bacteria isolated from six genotypes of potato or wild potato relatives from Argentina and Mexico (Figure 5.A.I-65). Of these, 32 showed strong growth on nitrogen-free LGI media suggesting the potential to fix atmospheric nitrogen. A somewhat different group of 21 isolates were found to increase either root or shoot weight of germ-free potatoes grown in tubes in a growth room for 30 days (Figure 5.A.I-66). The top performing bacterium in this assay was a slow growing pink strain identified as a type of *Methylobacterium fujisawaense* and was isolated from roots of a nitrogen efficient variety (HAW1528) of *Solanum verrucosum* which increased root weights an average of 225% and shoot weights by an average of 111% relative to un-inoculated plants. *Methylobacteria* belong to the Rhizobiales order of bacteria, are able to fix nitrogen (although they did not grow on LGI) and are known to promote plant growth in other species of plant. This growth assay will be repeated to reproduce growth promotion results, and a non-sterile potato growth assay developed to aid in testing these microbes as biofertiliser for low nutrient potato agriculture.

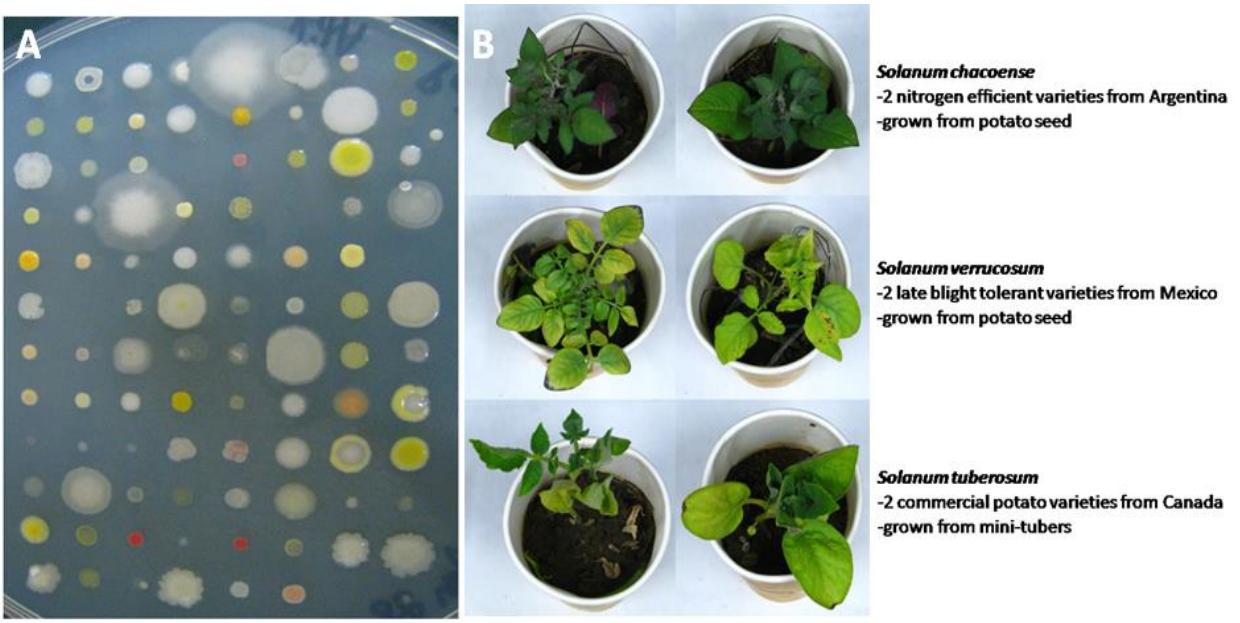


Figure 5.A.I-65 Endophyte isolates from 6 varieties of potato. A) All 96 isolates were put into a 96 well plate and are shown here growing on R2A media. B) Potato plants that were surface sterilised before bacteria were isolated by culturing.

Host Genotype	Closest Genbank Sequence Match	Growth on N Free Media	% Increase Root Weight	% Increase Shoot Weight
Kennebec	Microbacterium trichothecenolyticum, NR044937	Yes	31.8	43.4
Kennebec	Mycobacterium smegmatis, NR074726	Yes	9.1	19.4
Shepody	Paenibacillus sp. YO4-17, AM162351	No	-13.6	26.9
Shepody	Cohnella sp. AR92, FJ976043	No	-45.5	16.0
Shepody	Bacillus drentensis, AB681783	No	-4.5	17.4
Shepody	Methylobacterium rhodesianum, AB698692	No	40.9	-1.1
Shepody	Agrobacterium tumefaciens, AJ012209	No	4.5	14.9
Shepody	Microbacterium phyllosphaerae, KC355287	No	58.3	11.6
Shepody	Mycobacterium smegmatis, NR074726	Yes	0.0	19.2
Shepody	Mycobacterium frederiksbergense, AF544628	Yes	33.3	40.4
Shepody	Curtobacterium sp. 2340, AY688358	No	75.0	80.1
Shepody	Mesorhizobium ciceri, JX868851	No	91.7	-20.5
Hhr	Williamsia sp. 0713C5-1, HM222683	Yes	0.0	23.3
Hhr	Mycobacterium cosmeticum, AY449729	No	33.3	37.0
OKA	Micrococcus sp. LI-2, EU889240	No	66.7	10.3
Haw1528	Methylobacterium fujisawaense, EU379312	No	225.0	111.0
Haw1528	Bacillus thuringiensis, GU143904	No	58.3	13.7
Haw1528	Arthrobacter globiformis, KC414683	Yes	33.3	32.9
Haw1528	Microbacterium phyllosphaerae, KC355287	No	50.0	20.5
Haw1528	Agrobacterium tumefaciens, AJ012209	No	-8.3	20.5
Haw1528	Leifsonia shinshuensis, AB244485	No	-25.0	20.5

Figure 5.A.I-66 Growth-promoting endophyte isolates from 6 varieties of potato. All 96 isolates were cultured in 96 well plates containing nitrogen free LGI media and assayed for growth. All isolates were also inoculated in triplicate onto germ-free potato plantlets and incubated in a growth room for 30 days

before measuring root and shoot weights. Shown here are the 21 isolates which resulted in an average increase of either roots or shoot weights.

THE 'PIONEER EFFECT' OF RHIZOSPHERE BACTERIA – ESTABLISHING COMMUNITIES OF BENEFICIAL MICROORGANISMS ON POTATO ROOTS

The "pioneer" or "founder" effect of rhizosphere bacteria: an investigation using TRFLP

A critical consideration when using using beneficial microoganisms is their effective delivery and establishment in the potato production system. A major objectives is to establish beneficial microorganisms in the rhizosphere of potato. The question is how to ensure that the beneficial population we wish to introduce is the one that colonizes the seed tubers and the emerging roots. Potato production utilizes large seed pieces which, if we can establish a desired microbial population, would be an ideal method of delivery. However, it is not known if microorganisms establish on and in the seed tuber and in the and root zone.

The soil surrounding the root, termed the rhizosphere, is populated by bacteria that differ in numbers and types from the bulk soil. The very large populations found in this soil layer are influenced by the exudates derived from roots in the form of carbohydrates, amino acids, and organic acids. The biological activities of these bacteria can impact plant growth and performance in either a beneficial, detrimental, or neutral manner. Which of millions of bacteria in the soil come to colonize the rhizosphere is still under debate. It is likely that some bacteria have developed special adaptations to take advantage of the nutrient-rich status of the root environment or, as *Streptomyces scabies*, have become parasitic on the plant surface. This would suggest that roots would be colonized by similar bacteria from most soils. Alternatively, bacteria residing on the seed coat, or populations residing in internal layers, become the first colonizers again indicating similar populations regardless of the soils the plants are growing in.

A study by Turnbull et al. (2012) investigated the primary source for the rhizosphere community colonization, development, and maintenance using reciprocal transplant experimentation. The objective was to come to understand how to deploy biological control agents against the bacterium causing common scab as well as root parasitic organisms. Potato plants were planted into two vastly different soils and the microorganism communities were allowed to establish. After a several weeks of growth some plants were removed from their home soils and transplanted into the reciprocal foreign soil where growth was allowed to continue for several weeks. The rhizosphere bacterial communities on roots of the various treatments was determined using terminal restriction fragment length polymorphism assays (TRFLP). The results revealed evidence for what we term as a "pioneer effect" in the development of rhizosphere bacterial communities. The bacterial community on plants transplanted to a foreign soil remained very similar to that of the home soil despite the fact that the communities of the two soils were very different. This was true also for plants where all traces of soil were washed off prior to transplantation into the foreign soil. The results suggest that once a bacterial community is established in the rhizosphere, a foundation population is created and this remains fairly stable even after transplantation to a foreign environment.

This concept was futher tested in the establishment of a community of free-living nitrogen fixing bacteria on potato plant roots systems. The principles apply equally to biological control bacteria.

THE "FOUNDER EFFECT" ON POPULATIONS OF MICROORGANISMS OF POTATO - TREATMENT WITH NITROGEN FIXING CONSORTIA

Summary

There is a major gap in knowledge on how to deliver and establish beneficial microrganisms on the tuber tuber and root surfaces. An earlier study had shown that once a microbial community had established on potato roots it remained stable for a prolonged period even if the roots are transplanted into soil with a very different microbial community. An experiment was conducted to test whether it is possible to establish a functional microbiome and deliver it to an agricultural environment. A consortium of nitrogen-fixing bacteria obtained from the roots of field grown Russet Burbank were allowed to establish on the roots of Shepody plantlets under tissue culture conditions. The plants were then transplanted into a field soil. Plants inoculated with eight separate nitrogen-fixing communities showed an average fivefold increase in dry biomass when compared to mock-inoculated plants. The microbial profiles, as measured by TRFLP, remained distinct at 30 days after transplantation. These results demonstrate that the plant rhizosphere is a resilient community and that the first bacterial community that becomes established on the root remains with the plant even when the plant is placed into soil with vastly different microbiota.

Introduction

The rhizosphere is represented by the soil adjacent to the plant roots that is affected by root exudates. This zone has been shown to harbour a bacterial community that is distinct from the adjacent bulk soil or from the microorganisms that reside inside the plant root (endosphere). Bacteria are at least ten times more abundant and diverse in the rhizosphere than in bulk soil or the endosphere. However, the bacteria found in the rhizosphere have more related species than found in bulk soil. Rhizosphere ecology is greatly impacted by the plant which in turn impacts what resident populations get selected. There is a lot of evidence now that the plant selects out the similar functional groups from the soil but these may differ in their taxonomy.

Studies we carried out and reported on previously on reciprocal transplants provided clear results as to how soils influence bacterial ecology. Using terminal restriction fragment length polymorphism (TRFLP) allowed us to show that bacterial communities in the rhizosphere of potato plants grown in a forest soil are significantly different from those of plants grown in a soil used for potato production. When plants were reciprocally transferred from one soil to another, the vast proportion of the bacteria that had established, i.e. the founder colonizers, were retained in the new soil habitat. This finding was of significance as one of our main objectives is to establish a rhizosphere on potato roots composed of a consortium designed to provide beneficial effects to plant growth and productivity. If we can establish such consortia as the founder agents the tubers can be planted into any soil with relatively little chance that they will be displaced. However we will still need to determine how the microbial community shifts over time. The current experiments were a preliminary study to examine if a selected group of nitrogen fixing bacteria, isolated from potato rhizosphere, can be established as a founder population.

Materials and methods: Nitrogen-fixing community transplant

Field-grown Russet Burbank potato plants from Prince Edward Island were used as a source of nitrogen-fixing bacterial communities. Plants were grown in the field soil with a single application of 448 kg·ha^{-1} of 17-17-17 fertilizer. Roots were washed in an equal volume of sterile water (w/v) to remove rhizosphere soil. The rhizosphere soil was used as an inoculum, 100 µl of 10^{-3} dilution into 125 ml of nitrogen-free

combined carbon medium (Nf-CCM, (Rennie 1981). The medium contained, per 500 mL: sucrose, 2.5 g, 60% w/w sodium lactate, 0.5 ml; mannitol, 2.5 g; glucose, 2.5 g; pyruvate, 100 mg; K_2HPO_4, 0.25 g; $MgSO_4.7H_2O$, 0.123 g; $CaCO_3$, 0.1 g; NaCl, 0.125 g, $FeSO_4$.7H2O, 5 mg; $MnSO_4$.4H2O, 5 mg; Na2MoO_4.2H_2O, 5 mg. The pH was adjusted to 7.5. This was solidified after autoclaving 500 ml with 1.8 g agar. The medium was incubated at room temperature for two weeks without agitation. A band of bacteria became visible growing near the middle of the vessel. These bacteria were selected and used to inoculate plantlets as they likely represented nitrogen-fixing bacteria (Döbereiner 1995). In total, eight nitrogen fixing communities were cultured in two separate trials.

Plantlets of the variety Shepody were inoculated with 100 µl per plant of the above bacterial suspensions and placed in sterile sand (Reptisand) wetted with 20% w/v potato nodal cutting medium containing 0.75% sucrose and 35% of the nitrogen in the original medium. The reduction of nitrogen was met by not including NH_4NO_3 in the potato nodal cutting medium. Ten plantlets were grown per nitrogen-fixing community in a sterilized glass vessels for 15 days. After 15 days, five plants were harvested and five were transplanted into 10% w/w field soil from a potato field in Delhi ON in Reptisand and grown aseptically in a growth chamber. The plants were grown for 30 days then harvested. Dry shoot mass was determined and the rhizosphere community was profiled by TRFLP. The control was plants grown in the same conditions as above without the addition of a bacterial community.

To apply these findings to an agro-ecological setting, we sought to establish a beneficial bacterial community on potato plants and monitor the persistence of that community and measure its effects on plant growth promotion in agricultural field soil. Rhizosphere soil from a field grown potato plants in a low fertility field regimen was used to inoculate nitrogen-free semi-solid medium. The nitrogen-fixing band of organisms within this medium was used to inoculate gnotobiotic potato plants. These plants were grown for 15 days in sterile sand prior to planting in field soil. Terminal Restriction Fragment Length Polymorphism (TRFLP) technique was used to track colonization of the inoculum on plants.

Results

The nitrogen-fixing inoculum had on average 28 forward Terminal Restriction Fragments (TRFs) and 9 reverse TRFs. TRFs that were present on 45 day old plants inoculated with nitrogen-fixing communities and absent from mock-inoculated control plants were determined. On average, 8 forward TRFs and 5 reverse TRFs were observed on the majority of inoculated 45 day old plants that were also present in the inoculum (Table 5.A.I-89). There was poor agreement between TRFs across experiments, although the number of peaks carrying through the inoculum to 45 day old plants remained constant. In the first and second trials, respectively, 26% and 45% of the peaks in the 45 day old plants were absent from the control (Table 5.A.I-90). The difference in bacterial community structure in inoculated compared to control plants was also evident from principle components analysis (Figure 5.A.I-67**)**. The nitrogen-fixing bacterial communities improved plant growth, as evidenced by increase dry mass of inoculated plants compared to control plants (Figure 5.A.I-68**)**.

Table 5.A.I-89 – Detection of an inoculated nitrogen-fixing community Terminal Restriction Fragments (TRFs) after 30 days in field soil.

TRF (bp)	Inoculum community TRFs[a]				Number of potato plants with TRF[b]			
Inoculum community	1	2	3	4	1	2	3	4
Forward TRFs (bp)[c]								
40	x	x	x		4	4	2	
54	x	x			4			
168	x	x	x		4	4	4	
300	x	x	x		4	3	3	3
303	x	x			4	3	3	
469	x	x	x	x		4	4	
527	x	x	x		3	2	4	
1055	x	x	x		4	3	3	
Total forward TRFs[d]	12	8	10	7	29	23	33	21
Reverse TRFs (bp)								
186	x		x	x	4	4	4	
290	x	x	x	x	4	3	1	2
293	x	x		x	4	3	1	2
299	x	x	x			2	1	3
815	x		x	x	4	4	4	3
Total reverse TRFs[d]	13	9	10	8	25	43	31	26

[a] 'x' indicates the presence of the TRF in the semi-solid nitrogen-free medium used as inoculum;
[b] Peaks are shown in plants grown in field soil for 30 days only if present in inoculated plants and absent from all control plants.
[c] This is representative data from trial II
[d] Total TRFs in plants represents the average number of unique TRFs in four plants inoculated with one inoculum.

Table 5.A.I-90 – Comparison of Terminal Restriction Fragment (TRF) profiles between plants inoculated with a nitrogen-fixing bacterial community and control plants.

Inoculum community	Number of TRFs differentially present[a] (% of total)	Average Total TRFs per plant[b]
Trial 1		
1	22 (27%)	82

Inoculum community	Number of TRFs differentially present[a] (% of total)	Average Total TRFs per plant[b]
2	22 (30%)	74
3	19 (25%)	76
4	15 (21%)	71
Trial 2		
1	33 (59%)	56
2	24 (46%)	52
3	27 (43%)	63
4	15 (32%)	47

[a] This represents TRFs present in at least half of all plant replicates for one inoculum community and absent from all control plants, after growth for 30 days in field soil.
[b] This represents both the forward and reverse TRFs

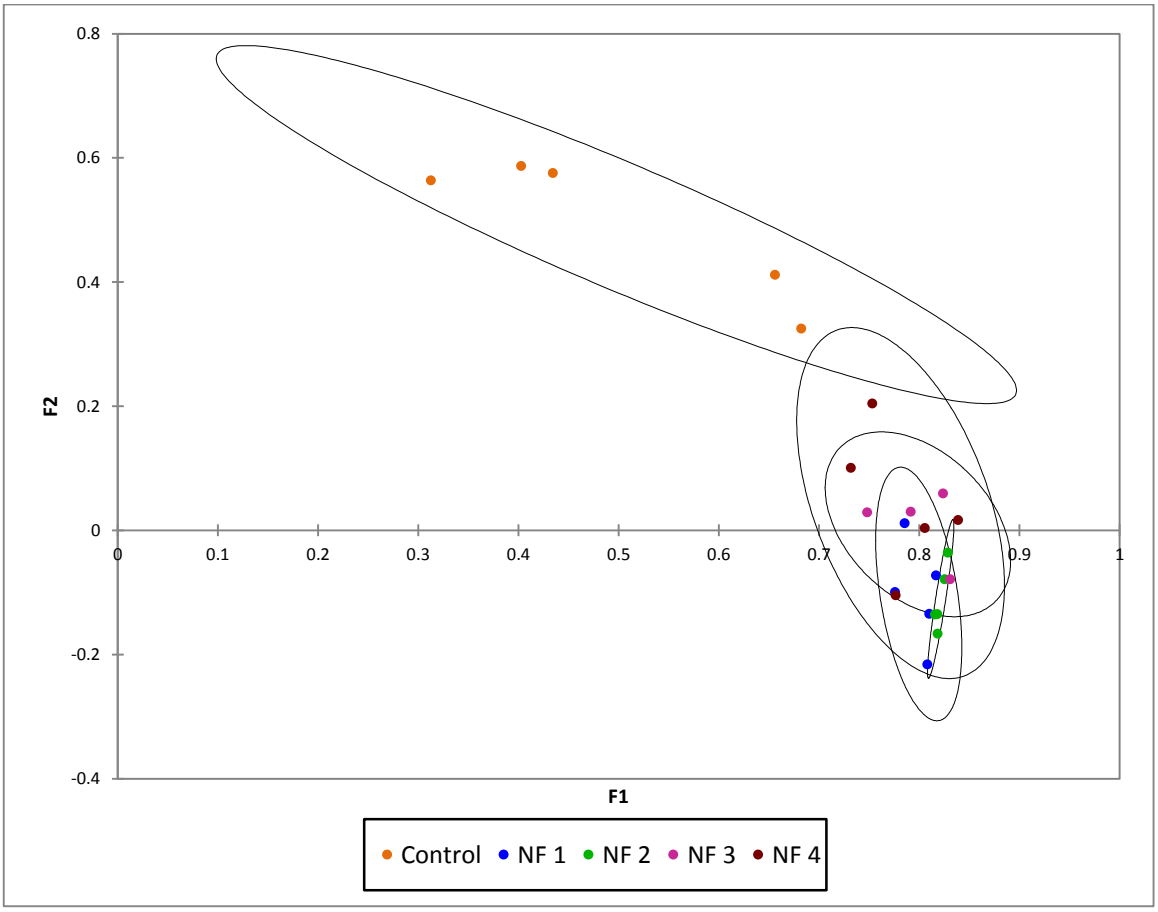


Figure 5.A.I-67 – Principal components analysis on 45 day old potato rhizosphere bacterial communities. Control plants (orange circles) were inoculated with sterile medium. Test plants (blue, green, magenta, red circles) were inoculated with nitrogen-fixing communities. Closed circles represent TRFLP profiles from one plant; each colour represents a different nitrogen-fixing inoculum treatment. Circled data points indicate 95% confidence intervals.

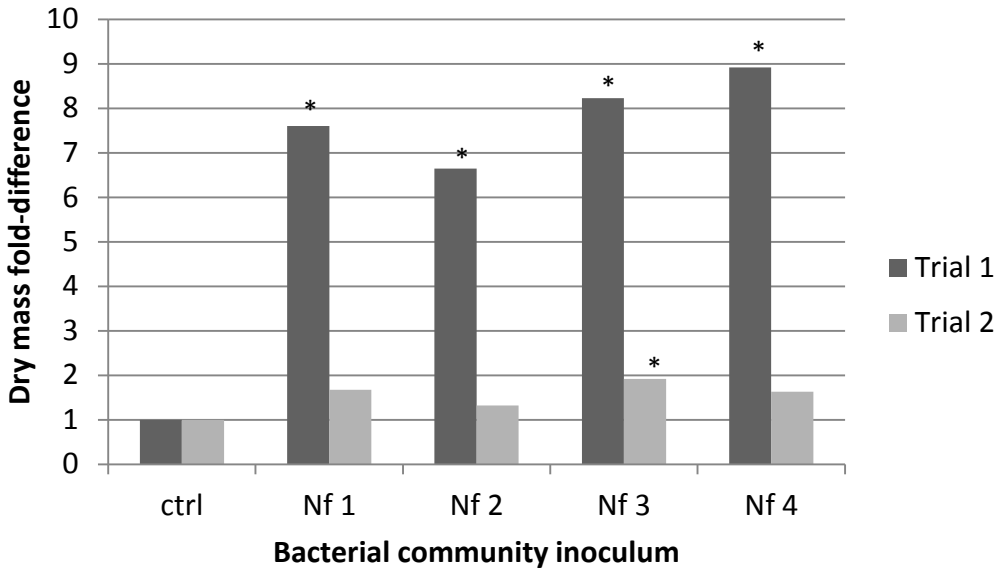


Figure 5.A.I-68 – Dry mass differences between plants inoculated with a nitrogen-fixing community (Nf) and the uninoculated control (ctrl). The mass of mock-inoculated control plants were normalized to one. Bars show the average increase from the control. Asterisk indicates statistically significant mass increase compared to the control (P<0.05).

Previous studies on potato plants have shown that soil has a larger effect on bacterial community structure than the cultivar. In this study, we expanded on these findings by demonstrating that the first soil a plant is grown in has the greatest effect on community composition. Importantly, the application of these findings is that since the founder effect occurs in the rhizosphere, by modulating the first community established on the roots, plant growth can be improved through the use of biofertilizer and biocontrol bacterial communities. Nitrogen-fixing bacteria have been shown to improve non-legume plant growth. Our method of culturing nitrogen-fixing bacteria using semi-solid media has been used previously to isolate bacteria from non-leguminous plants.

The most significant application of these results is that it would be very possible to establish beneficial microbial communities if we introduced them en masse to young plants prior to planting into a field soil. Seed tubers being quite large would be able to accommodate significant populations prior to planting. We intend to work on how we can better establish such communities on seed tubers by working out when to apply them and how to ensure that only the applied bacteria become dominant. If we can enhance the nutrient acquisition of potatoes it may reduce not only the amount required to be added but perhaps the stress levels of the plants when they are lacking adequate nutrients. In order for agriculture to meet the demands of feeding 9 billion people, yield gains must be met without degrading the environment. Notably, most current synthetic fertilizers are made using non-renewable resources. Beneficial bacteria represent an under-utilized resource for improving plant yields. A recurrent challenge in this area is the establishment of plant growth promoting rhizosphere (PGPR) bacteria in crops. This is thought to be due to difficulty of one introduced PGPR strain in invading a stable and diverse pre-existing soil microbial community. Currently, typical application methods of nitrogen-fixing bacteria to non-legume plants include inoculating seedlings

in field soil, dipping roots in inoculum at the time of transplantation, and application of inoculum to field soil. An alternative methods termed biotization, which involves the introduction of beneficial bacteria to tissue culture plants, has been shown to have significant benefits to plant growth when these plants are taken to the field. The results presented here indicate that by growing gnotobiotic plants (tissue culture plants) first in a medium containing the beneficial community, a resilient ecosystem can develop which then persists after transplantation into field soil. By using a community of bacteria rather than one strain, the community is more stable and robust to perturbations, and synergistic responses have been observed on plant growth.

DISCUSSION

The management of common scab disease was approached in a number of different ways within the context of soil health and the project title of "soil amendments and ameliorants for soil health and disease suppression". The focus was not just on nutrient amendments but on learning to understand the soil biological environment in relation to disease suppression and in identifying and utilizing the microbial community that is closely associated with the potato plant, namely those in the rhizosphere and endosphere. It is important to note, that unlike the obligate pathogen *S. subterreanea* causing powdery scab, which has longed-lived and resistant resting sporosori, the common scab pathogen *S. scabies* survives as a free-living organism and is, therefore, very much exposed to the soil chemical and biological environment.

A four-year study of a millet green manure-potato rotation demonstrated yield increases of between 20-48% compared with a control treatment of continuous cropping of potatoes. Continuous potato cropping was past practice in the Koo Wee Rup region, although growers recognised the importance of a green manure crop, in this case rye corn (*Secale cereale*), which was grown between each successive potato crop to improve the soil condition. Although the millet green manure did not have a major impact of disease incidence and severity (Verticillium wilt and common scab) in our study, it illustrates the dramatic impact this method of cropping can have on potato productivity overall. Importantly, this study highlights the importance of green manure cropping as a tool to manipulate the soil environment, impacting on the physical, chemical and biological components of the soil. Much more research needs to be done to understand mechanisms of green manure cropping. The effects on the biological component of the soil is perhaps the most exciting component of this work. The challenge is develop tools to study this process so that ultimately we can predict which crops work best as green manures and how to manage them for improved soil health and disease suppression. A bio-indicator test for soil health could be one of these tools.

The concept of a "bio-indicator" test for soil health was examined using soil samples taken from Victorian fields that had common scab or no common scab("healthy"). This was to better understand why some potato fields support common scab and others do not. A proof-of –concept study with the DNA based method of "fingerprinting" microbial populations in soil, TRFLP, was able to distinguish differences in the make-up of microbial communities between the healthy soils and soil with common scab. This study indicated that soils have particular ecological conditions that permit the establishment and growth of the common scab pathogen and similar or related species of bacteria. Thus, the entire bacterial community in a particular soil may serve as a bio-indicator of common scab disease presence or disease potential. In each soil however, these communities will require a much greater level of characterisation because common

scab free soil can occur under any number of chemical, physical or environmental conditions that may suppress the pathogen population. With further development, these type of tests could provide an indication of the potential of potato fields to develop disease based on the soil microbial ecology as distinct from pathogen DNA tests, which indicate disease risk based on the amount of inoculum only. Ultimately these other tests could be developed to identify which microbial communities are associated with a field's overall health and productivity.

An exciting development in relation to disease suppression is the discovery of a common scab suppressive soil in a potato field near Ballarat. In a glasshouse study, heat treatment and gamma-irradiation removed the suppressive properties of this soil. Common scab developed in the treated soils after inoculation with *S. scabies* but not in the untreated field soil, pointing to a biological suppression. In a follow-up experiment, seeding the irradiated field soil with untreated field soil rendered the latter suppressive to common scab. Future research should be aimed at determining the organisms responsible for this suppression with the aim of developing new management practices for common scab.

In the course of this project we have shown that by using TRFLP we can differentiate microorganism communities found associated with common scab suppressive soil from those that are conducive to the disease. Furthermore, the technique allowed us to follow the component organisms that change within the communities in response to a variety of storage and radiation treatments used to alter the communities in order to reduce the suppressive nature of the soil. The capacity of TRFLP to differentiate microorganism shifts found after various treatments makes it possible to develop a model system for more detailed studies that can better identify the specific communities being impacted. This can be done using high throughput molecular tools which are slower and more expensive but have better resolution of specific components. As such techniques generate millions of pieces of data having the ability to focus on a less complex microbial ecosystems will make it much easier to identify the key microbial elements involved in disease suppression.

As a next step we consider it important not only to further examine the microbiology of the bulk soil but far more important to examine the microbiology associated with the geocaulosphere, (the soil surrounding tubers). This the critical area where disease suppressiveness is being expressed. In previous studies funded by Horticulture Australia (Lazarovits et al. 2013) it was shown that the microbial ecosystem of the geocaulosphere differs from that of bulk soil. Future research should focus harvesting tubers at regular times during their development from both a scab conducive and scab suppressive soils and determine difference in the microbial populations. There are already published results of a list of microorganisms suspected to be involved in scab suppression and it should be possible to develop methods that immediately target these organisms as a first priority.

The study of the isolation, characterisation and utilisation of microorganisms associated with the rhizosphere of potato plants has highlighted the wide diversity of type and function of these organisms. Their functions include antibiosis, nitrogen fixing and plant growth promotion. This work has demonstrated efficacy of selected bacteria against seed-borne common scab and growth enhancement of potatoes in field soils. Furthermore the resilience of beneficial communities established on potato roots after transplanting into a field soil with a different microbial community has been demonstrated. Further studies would be focused on specifically identifying beneficial microorganisms in the bulk soil, as well as those associated with the plant rhizosphere and conducting more in-depth analysis of their function and mode of action. A gap in our knowledge is how best to deliver and establish beneficial microorganisms to the tubers

and roots. Ultimately the aim is to establish a functional microbiome with various properties and deliver it to an agricultural environment.

RHIZOCTONIA STEM AND STOLON CANKER AND BLACK SCURF

EFFECTS OF SOIL NUTRIENT AMENDMENT TREATMENTS ON RHIZOCTONIA DISEASE AND YIELD OF POTATOES

SUMMARY

Both zinc and manganese cations inhibited *Rhizoctonia solani* (*Rs*) AG2.1 and AG3 growth *in vitro*. Growth was completely inhibited by zinc concentrations of 500 ppm, whereas manganese inhibited growth by 30-60% or 80–93% at 300 ppm, depending on the salt form. The EDTA forms of zinc and manganese were far less effective than chloride and sulphate salts.

In a sand/nutrient culture system in the glasshouse, 25 ppm zinc reduced the incidence of Rhizoctonia stolon canker by 70%, and at higher doses of 50 and 75 ppm development of stolon canker was completely inhibited. Concurrently, the amount of *R. solani* AG3 DNA present was negatively correlated with increasing concentrations of zinc, and at harvest, black scurf symptoms on tubers had only developed on the control treatment (0.6 ppm).

When trialled in the field at Cora Lynn (Koo Wee Rup region), applications of manganese sulphate had no significant effect on Rhizoctonia disease. An application of zinc EDTA at 100 kg zinc/ha, however, reduced the incidence of black scurf at harvest from 16% to 2%, and severity from 5% to 1% ($P<0.1$) but had no effect on the incidence of plants with stem canker early in the season. An application of the more soluble zinc sulphate, at an equivalent rate of zinc, had no significant effect on stem canker or black scurf, although both treatments had similarly-raised soil, petiole and tuber peel zinc concentrations.

INTRODUCTION

This project has focused on the effects of several nutrient amendments, including zinc and manganese, on powdery scab and common scab, respectively. There have been few studies on the effects of either zinc (Zn) or manganese (Mn) on disease caused by *Rhizoctonia solani*, but there have been studies showing that that both Zn (Babich and Stotzky 1978; Thongbai et al. 1993b) and Mn (Kalim et al. 2003) have a direct detrimental effect on the pathogen itself. Kalim et al. (2003) determined that 10 ppm Mn significantly decreased *Rhizoctonia* root rot by 43% in cowpea grown in glasshouse trials. Babich and Stotzky (1978) demonstrated that as little as 10 mM zinc (as sulphate) completely inhibited *R.solani* growth *in vitro,* and Thongbai et al. (1993a) demonstrated that as little as 0.1 ppm zinc applied in glasshouse trials reduced *Rhizoctonia* root rot in wheat by 50%. Todd (2009), on the other hand, found no relationship between *R. solani* disease of potatoes and zinc levels in either soil or plant tissue, although zinc concentrations used were low (up to 3 ppm).

This study examines the effects of different concentrations of zinc and manganese on the growth of *R. solani in vitro*, the effects of different concentrations of Zn on Rhizoctonia disease in a sand/nutrient culture system in a glasshouse trial and the effects of soil applications of Zn and Mn on the disease in one field trial.

Two additional glasshouse trials were conducted to evaluate the effects of different concentrations of manganese applied as a nutrient solution in a sand/nutrient culture system (Dosatron®) or incorporated into potting media. Unfortunately, Rhizoctonia disease symptoms did not develop in either of these trials, so they have been omitted from this report.

THE EFFECTS OF MANGANESE AND ZINC ON THE RADIAL GROWTH OF *RHIZOCTONIA SOLANI IN VITRO*

Different concentrations of zinc (Zn) and manganese (Mn) ions were tested for their effect on the *in vitro* growth of two isolates of *R. solani* AG3 (*Rs* AG3 153, *Rs* AG3 422) and two of *R.solani* AG2.1 (*Rs* AG2.1 14, *Rs* AG2.1 394) to validate the concentrations required for testing in subsequent glasshouse and field trials.

MATERIALS AND METHODS

Preparation of zinc and manganese solutions and incorporation into agar plates

Sterile stock solutions were prepared of $ZnCl_2$, $MnCl_2.4H_2O$, $MnSO_4.2H_2O$, $ZnSO_4.7H_2O$, Zn EDTA and Mn EDTA. Potato dextrose agar was amended with the various stock solutions prior to pouring to deliver the required concentrations of Zn or Mn. Aliquots of non-sterilised PDA solution containing these metal salts were centrifuged at 3000 g to remove any particulate matter (including agar and precipitates) and the supernatant was analysed by Atomic Absorption Spectroscopy (AAS) to measure the bioavailable concentration of Zn^{2+} and Mn^{2+} ions in the media.

Inoculation, measurement of growth inhibition

Cultures of the four isolates were grown at room temperature on PDA (potato dextrose agar, Oxoid) for 7 days. Five (5) mm agar plugs from the leading edge of all isolates were aseptically transferred to the centre of Zn- and Mn-amended PDA plates, plus unamended PDA as a zero-ion control. All treatments were in triplicate. The cultures were grown at room temperature for 7 days and the diameter of *Rs* AG2.1 or *Rs* AG3 growth (i.e. radial growth) was measured, compared to the control and expressed as % growth inhibition.

RESULTS

The effects of the different forms of manganese and zinc on the inhibition of radial growth of four different isolates of *R. solani* are presented in Table 5.A.I-91.

When comparing the sulphate and chloride salts, zinc generally had a greater effect on radial growth of the four isolates than manganese. The highest concentrations of the zinc compounds (200 ppm zinc sulphate and 500 ppm zinc chloride) caused 100% inhibition of growth compared with the manganese which caused 30–90% growth inhibition (300 ppm of both salts). In general, the EDTA forms of both manganese and zinc were far less inhibitory than the sulphate and chloride, with the zinc EDTA being slightly more inhibitory than the manganese EDTA.

The two isolates of *Rs*AG2.1 tended to respond similarly to the different manganese salts at the different concentrations. *Rs*AG3 422 was more sensitive to the manganese sulphate at the highest concentration but, in general 153 was more sensitive to the manganese salts than 422. Manganese chloride was generally more inhibitory than the sulphate form.

The two isolates of AG2.1 had similar responses to the zinc compounds. The two isolates of AG3 tended to be slightly more sensitive to zinc than the AG3 isolates. The different isolates tended to be more sensitive to zinc chloride than zinc sulphate at the higher rates.

Table 5.A.I-91 – Effects of different concentrations of manganese and zinc salts on the inhibition of the radial growth (% of control with zinc or manganese) of different isolates of *R.solani* (*Rs*) AG2.1 or AG3 on PDA media

Treatment	Metal ion (ppm)	*Rs* AG2.1 isolates		*Rs* AG3 isolates	
		#14	#394	#153	#422
$MnSO_4$	0.3	7	-3	-6	-1
$MnSO_4$	3	2	-5	-1	-1
$MnSO_4$	30	2	5	7	0
$MnSO_4$	300	60	58	29	58
$MnCl_2$	0.3	-2	1	1	1
$MnCl_2$	3	0	2	-3	-10
$MnCl_2$	30	2	4	16	8
$MnCl_2$	300	86	93	80	73
Mn EDTA	0.2	-4	-1	-3	-133
Mn EDTA	2	12	-4	9	-128
Mn EDTA	20	7	-7	14	-126
Mn EDTA	200	4	-2	14	-129
$ZnSO_4$	0.2	-4	-5	0	5
$ZnSO_4$	2	5	-9	0	6
$ZnSO_4$	20	10	1	23	24
$ZnSO_4$	200	100	100	100	100
$ZnCl_2$	0.5	-1	-1	-4	-1
$ZnCl_2$	5	-5	-4	2	0
$ZnCl_2$	50	39	32	85	60
$ZnCl_2$	500	100	100	100	100
Zn EDTA	0.2	3	2	1	2
Zn EDTA	2	3	9	1	-3
Zn EDTA	20	9	10	31	5
Zn EDTA	200	13	5	32	9

THE EFFECTS OF DIFFERENT CONCENTRATIONS OF ZINC ON RHIZOCTONIA DISEASE IN A GLASSHOUSE TRIAL

The aim of this experiment was to investigate the effect of different zinc concentrations at tuber set on the development of Rhizoctonia disease on potato tubers (i.e. formation of sclerotia).

MATERIALS AND METHODS

In the experiment reported here we used a hydroponic formulation supplying concentrations of 0.6, 25, 50 and 75 ppm zinc as zinc sulphate. The trial commenced on 20/1/2012 and finished 16/4/2012 with two harvests at 53 DAP (days after planting) and 90 DAP (i.e. approximately 8 and 13 weeks). Sand (medium particle size 0.5–2 mm) was sourced from Biogro (Dandenong South, Victoria), pasteurised in bulk at 70°C for 4 hours and allowed to cool completely prior to use.

Inoculation with Rhizoctonia solani AG3

Rs AG3 422 was grown at room temperature on potato dextrose agar (PDA) until microsclerotia were clearly observable (approximately 4 weeks). Two plates of colonised agar were homogenised in 800 mL tap water and added to 16 kg sand, then thoroughly mixed in a cement mixer for 5 minutes before being placed in plastic bags and sealed until use. The control treatment consisted of 16 kg sand treated in the same manner using uninoculated PDA plates. Aliquots were taken and submitted to SARDI to determine the preplant *Rs* AG3 DNA concentrations. The inoculated sand was dispensed into 2.8 L plastic pots @ approximately 2 kg/pot.

Hydroponics formulation and delivery

The hydroponics formulation used in this trial was adapted from Corrêa et al. (2009). The basic nutrient solution was as follows: KNO_3 (253 ppm); $Ca(NO_3)_2.4H_2O$ (722 ppm); KH_2PO_4 (pH 6, 2.3 ppm); $MgSO_4.7H_2O$ (246 ppm); NH_4NO_3 (40 ppm); H_3BO_3 (4.5 ppm); $MnSO_4.H_2O$ (4.7 ppm); $ZnSO_4.7H_2O$ (2.6 ppm); $CuSO_4.5H_2O$ (940 ppb); KCl (400 ppb); $Na_2MoO_4.2H_2O$ (325 ppb). When the experimental treatments were started, the zinc solution was excluded from the mix.

A series of Dosatrons® (Dosatron Australia, Ringwood, Victoria) were set to deliver a 2% (v/v) solution in an automatic watering regime to ensure consistent nutrient delivery, connected via a timer pumping system to both a 200L tank containing the hydroponic solution and the mains water supply. Separate zinc solutions were used to supply 0.6 (baseline zinc control), 25, 50 or 75 ppm of Zn^{2+} in the form of $ZnSO_4.7H_2O$ by drippers to individual standard pots (2.8L). The Dosatron® setup delivered approximately 100 mL of solution per pot (i.e. 5% v/w, the field capacity of the sand) on a needs basis. Dosatron® outputs were monitored and recorded daily and zinc levels delivered by the Dosatron® system were measured by Atomic Absorption Spectroscopy (AAS). All pots were pre-dosed with their respective zinc concentrations for one week prior to planting.

Planting, harvesting and disease assessment

Sprouted minitubers of the susceptible cultivar Russet Burbank were planted, one per pot, 1 cm below the surface of the sand. Pots were randomised in a replicated block design consisting of 12 replicates and 8 treatments (0.6, 25, 50 or 75 ppm zinc; inoculated and uninoculated), on two separate benches. Six plants per treatment were harvested at 53 DAP for an assessment of disease and growth progress (e.g. stem and

stolon canker, tuber formation) and at 90 DAP, a full assessment of incidence of stolons with lesions including stolon pruning, tuber yield, plant and root dry weights and severity of black scurf symptoms was done. Plant, root and sand samples (both 53 and 90 DAP) were sent to A&L Canada for nutrient analysis, and *Rs* AG3 422 DNA concentrations were determined for each inoculated pot by SARDI.

RESULTS

Effect of zinc on RsAG3 422 DNA concentrations, disease severity and plant development

At 53 DAP, Rhizoctonia disease levels in the 0.6 ppm zinc inoculated treatments were very low and therefore not recorded. By 90 DAP, disease was fully established and the results are reported in Table 5.A.I-92. No uninoculated plants showed any symptoms associated with Rhizoctonia disease, nor was *Rs*AG3 422 isolated from these plants.

At planting, *RsAG3* 422 DNA concentrations were in the range 120–380 pg/g of sand. These concentrations decreased significantly ($P<0.001$) with increasing rates of zinc at both 53 and 90 DAP (Figure 5.A.I-69). No *RsAG3* DNA was found in any uninoculated pots.

Table 5.A.I-92 – The effects of different levels of Zn on *RsAG3* DNA concentrations, incidence of disease on stolons, plant and root Zn concentrations and plant and root dry weights (DW) at 90 DAP

Zinc (ppm)	Infected stolons (%)	DNA pg/g	Plant Zn (ppm)	Root Zn (ppm)	Plant DW (g)	Root DW (g)
0.6	27.8	292	115	200	4.4	0.6
25	8.1*	25*	2201*	5789*	2.4*	0.9*
50	0*	2*	3045*	7006*	0.8*	0.3*
75	0*	0.9*	5364*	10470*	0.8*	0.2*
lsd (P=0.05)	16.4	62.5	993.3	2019	0.6	0.2

*Significantly different from the control (0.6 ppm) at P=0.05

At 90 DAP, sclerotia were only present on tubers and roots at 0.6 ppm Zn (27% of tubers), with low levels of stolon pruning evident at 25 ppm Zn (8%) and no visual evidence of any infection at either 50 or 75 ppm zinc (Table 5.A.I-92). *R.solani* was re-isolated from both sclerotia and diseased stolons at 0.5 and 25 ppm and was confirmed as *Rs*AG3. Increased zinc levels significantly decreased *RsAG3* 422 DNA concentrations and correspondingly, disease severity. However, the high levels of zinc also had a detrimental effect on plant growth.

There was no significant difference in the reduction of plant dry weights or zinc levels in the presence or absence of the pathogen, showing that the differences observed were due entirely to the zinc levels. The higher zinc levels (50 and 75 ppm) resulted in a significant decrease (P=0.05) in plant and root dry weights at 90 DAP, with a significant accumulation of zinc in both plant and root tissue (3050 and 5400 ppm; 7000

and 10500 ppm, respectively) even though zinc levels in the sand matrix remained comparable to the supplied zinc levels. Actual zinc concentrations delivered by the dosatron system over the course of the 90 days were close to the calculated concentration with little variation, apart from 0.6 ppm (3, 27, 57, 79 ppm, respectively).

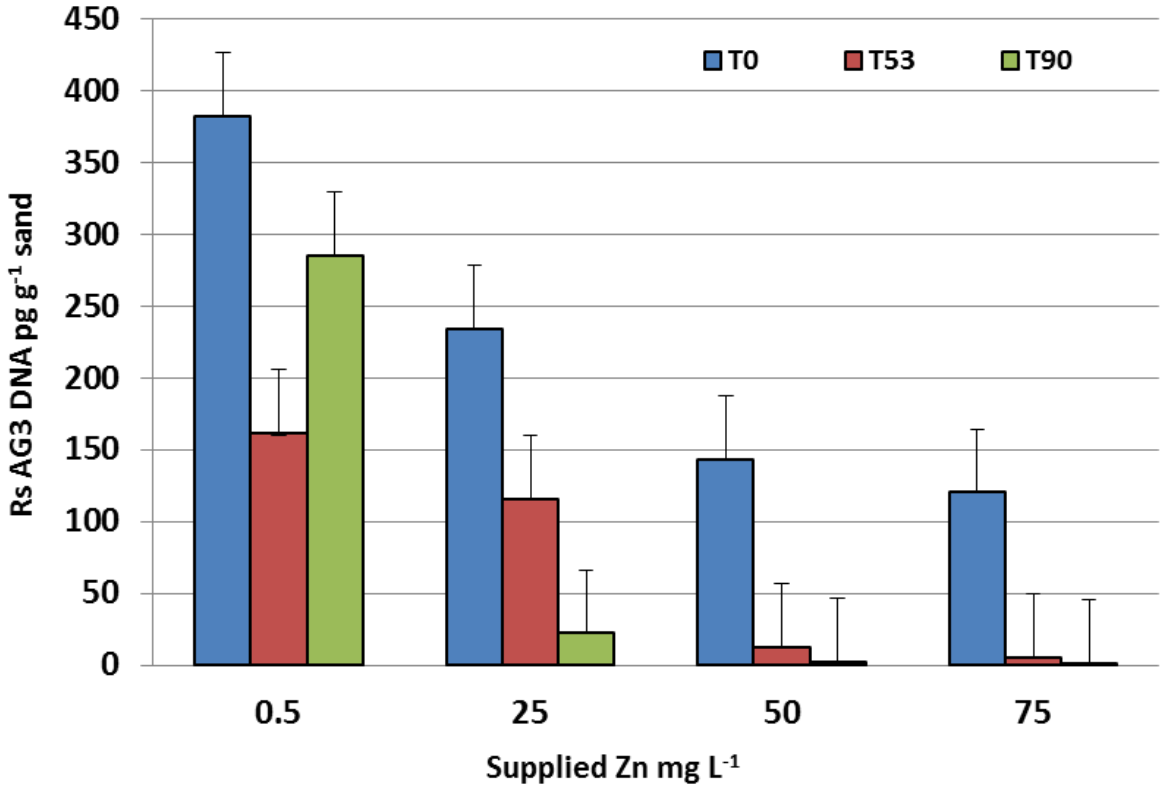


Figure 5.A.I-69 – *R.solani* AG3 DNA concentrations at planting (T_0), 53 days after planting (DAP) and 90 DAP (Bars indicate the lsd at P=0.05)

This experiment proved that high levels of zinc were detrimental to both the host plant and the pathogen. There were significant reductions in *RsAG3* DNA concentrations as zinc concentrations increased, which is not consistent with studies by Streeter et al. (2001). However, the increasing zinc concentrations resulted in significant accumulation of zinc in both plant and root tissue, reaching levels that have been reported to cause zinc toxicity (Sanderson and Gupta 1990; Broadley et al. 2007). This was observed to reduce plant growth, as measured by reduced shoot and root dry weights. This study provided some guidelines for testing in-field zinc applications for Rhizoctonia disease control. The aim would be to obtain levels of soil zinc in the range of 10–25 ppm, which demonstrated some efficacy against *Rs*AG3, but was below the level that caused plant toxicity.

A similar study was conducted to test the efficacy of manganese but we were unable to achieve significant disease in the inoculated control treatment and therefore, results were not reported.

EFFECTS OF SOIL APPLICATIONS OF ZINC AND MANAGANESE ON RHIZOCTONIA DISEASE IN A FIELD TRIAL AT CORA LYNN, 2012/13

A field trial was conducted to investigate different concentrations of Mn and Zn applied to soil at planting on the development of Rhizoctonia disease symptoms. These treatments were part of a larger trial that also evaluated the effects of various nutrient treatments on common scab disease. Trial design was a randomised block replicated five times. The trial was planted with the susceptible cultivar Catani that had a seed peel *R. solani* AG3 DNA concentration of 163516 pg/g peel

Effects of soil-applied manganese (Mn) on disease caused by *R. solani* AG3

Manganese has been suggested as a possible control for Rhizoctonia disease. McGregor and Wilson (1966) reported that 62 kg/ha Mn reduced the incidence of black scurf on tubers from 25% to 11%. Treatments used in the field trial are outlined in Table 5.A.I-93.

Soil testing prior to amendment application found that the site was uniform for soil variables with the exception of organic matter. The level of *R. solani* AG3 DNA in test plots at planting was uniformly high at 18 pg/g soil. At planting, the level of Mn was 6 ppm and S was 25 ppm. None of the treatments had a detrimental effect on emergence. Soil samples were taken after tuber set to determine the concentration of soil nutrients and the concentration of *R. solani* AG3. Plant petioles were sampled after tuber set to determine the uptake of nutrients by plants. The trial was harvested in April and tubers graded for size and yield and assessed for the incidence and severity of black scurf.

Table 5.A.I-93 – (cv. Catani) Effects of different rates of manganese sulphate ($MnSO_4$) applied to soil prior to planting and their effect on corresponding soil and petiole at tuber set (TS) and harvest soil and potato peel manganese (Mn) and sulphur (S) concentrations in a field trial, Cora Lynn 2012/13

Application rate $MnSO_4$ kg/ha	Mn kg/ha	S kg/ha	Tuber set soil		Petiole		Harvest soil		Peel	
			Mn ppm	S ppm	Mn ppm	%S	Mn ppm	S ppm	Mn ppm	%S
Nil			8	35	98	0.17	7	34	11	0.14
336	124	72	42*	66*	234*	0.16	37*	58*	21*	0.14
504	184	106	62*	82*	343*	0.15	53*	69*	29*	0.13
lsd P=0.05			16	17	120	ns	9	12	7	ns

*Significantly different to the untreated control at P=0.05

Both rates of manganese sulphate increased (P<0.05) soil manganese levels at tuber set and petioles while sulphur was only increased in soil (Table 5.A.I-93). This level of manganese and sulphur was maintained through to harvest where the manganese in the tuber peel was significantly increased, but the sulphur was

not. However, none of the rates of manganese sulphate significantly reduced symptoms of Rhizoctonia disease on the stems or tubers (Table 5.A.I-94).

Table 5.A.I-94 – (cv. Catani) Effects of different rates of manganese sulphate ($MnSO_4$) applied to soil prior to planting on the level of stem canker, the incidence and severity of black scurf and total and marketable yields in a field trial, Cora Lynn 2012/13.

Application rate $MnSO_4$ kg/ha	Stem Canker				Black scurf		Yield (t/ha) in field	
	Mean stems assessed	Mean infected stems	Mean % infected	Mean Disease severity score (0-5)	% tubers affected	Severity Index	Total	Marketable
Nil	20	17	83	1.8	16	5	39	36
336	nd	nd	nd	nd	12	3	41	38
504	22	20	92	1.5	11	4	38	36
lsd *P*=0.05	ns	ns	ns	ns	ns	ns	ns	ns

nd – not determined; ns – not significant; Severity score, 0- no stems affected, 5 all sprouts pruned

Effects of soil-applied zinc (Zn) on disease caused by *R. solani* AG3

The in vitro experiment found that 200 ppm of ZnSO4 inhibited the growth of R. solani AG3. The glasshouse pot trial found that only 25 ppm of Zn was required in hydroponic solution to significantly reduce stolon pruning and black scurf on tubers of cv. Russet Burbank and the level of *R. solani* AG3 DNA 90 days after planting.

The field trial established at Cora Lynn included soil-applied treatments of two forms of zinc: zinc sulphate and zinc EDTA for the control of Rhizoctonia disease (Table 5.A.I-95). Damage caused by *R. solani* was evident throughout this trial and disease incidence and severity were assessed on eight samples of plants from each plot taken approximately 8 weeks after emergence.

Table 5.A.I-95 – (cv. Catani) Different form and rates of zinc applied to soil and soil concentrations of zinc and sulphur at tuber set (TS) in a field trial, Cora Lynn 2012/13

Treatment	Product	Application rate kg/ha	Zn kg/ha	S kg/ha
Untreated	Nil			
$ZnSO_4$	Zinc sulphate	233	50	26

Treatment	Product	Application rate kg/ha	Zn kg/ha	S kg/ha
$ZnSO_4$	Zinc sulphate	465	100	51
Zn EDTA	Tradecorp® Zn	357	50	-
Zn EDTA	Tradecorp® Zn	714	100	-

Soil testing prior to amendment application found the site was uniform for soil variables with the exception of organic matter. The level of *R. solani* AG3 DNA in test plots at planting was uniformly high at 34 pg/g soil. At planting the level of Zn was 4 ppm and S was 26 ppm. None of the treatments had a detrimental effect on emergence. Soil samples were taken after tuber set to determine the concentration of soil nutrients and the concentration of *R. solani* AG3. Plant petioles were sampled after tuber set to determine the uptake of nutrients by plants. The trial was harvested in April and tubers graded for size and yield and assessed for the incidence and severity of black scurf.

Table 5.A.I-96 – (cv. Catani) Effects of form and rates of zinc applied to soil prior to planting and their effect on corresponding petiole, tuber set (TS) and harvest soil zinc (Zn) and sulphur (S) concentrations in a field trial, Cora Lynn 2012/13.

Treatment	Tuber set soil		Petiole		Harvest soil		Peel	
	Zn ppm	S ppm	Zn ppm	%S	Zn ppm	S ppm	Zn ppm	%S
Untreated	6	35	54	0.17	5	34	39	0.14
$ZnSO_4$ 233	24*	45*	176*	0.16	26*	41	53*	0.14
$ZnSO_4$ 465	45*	58*	320*	0.16	52*	52*	70*	0.15
Zn EDTA 357	29*	39	115	0.17	25*	38	52	0.15
Zn EDTA 714	37*	44	129	0.15	43*	43*	63*	0.16
lsd P=0.05	12	9	89	ns	9	9	13	ns

*Significantly different to the untreated control at P=0.05

At tuber set, all of the zinc treatments increased the level of zinc in the soil significantly, and these raised levels were still evident in soil at harvest time (Table 5.A.I-96). Both rates of zinc sulphate increased plant petiole Zn after tuber set. Peel Zn concentrations were significantly increased by both rates of Zn sulphate and the highest rate of Zn EDTA. None of the treatments significantly reduced stem canker, but the higher rate of Zn EDTA tended to reduce (P<0.1) black scurf incidence to 2% and severity to 1% (Table 5.A.I-97). None of the treatments had a significant effect on total and marketable tuber yields.

Table 5.A.I-97 – (cv. Catani) Effects of form and rates of zinc applied to soil prior to planting on the concentration of *R. solani* AG3 DNA in soil, the level of stem canker, the incidence and severity of black scurf and total and marketable yields in a field trial, Cora Lynn 2012/13.

Treatment	Stem Canker				Black scurf		Yield in field (t/ha)	
	Mean stems assessed	Mean infected stems	Mean % infected	Mean Disease Rating	% tubers affected	Severity Index	Total	Marketable
Untreated	20	17	83	1.8	16	5	39	36
$ZnSO_4$ 233	nd	nd	nd	nd	18	5	42	39
$ZnSO_4$ 465	21	19	87	1.4	17	5	45	43
Zn EDTA 357	nd	nd	nd	nd	11	3	35	33
Zn EDTA 714	21	14	67	1.3	2*	1*	38	37
lsd P=0.05	ns	ns	ns	ns	ns	ns	ns	ns

ns – not significant at P=0.05 but see discussion section, nd – not determined, *less than the untreated control at P<0.1.

DISCUSSION

Although both *in vitro* plate experiments and glasshouse pot trials demonstrated that zinc, and to a lesser extent, manganese reduced *R. solani* growth and infection, only the highest rate of soil-applied zinc EDTA (@ 100 kg Zn/ha) reduced disease outcome in the field. There was no significant reduction in stem canker, but the incidence of black scurf at harvest was reduced from 16% to 2%, and severity reduced from 5% to 1%, both of which were significant at P<0.1. Other plant systems involving Rhizoctonia have also demonstrated a clear reduction in disease, by directly affecting the pathogen (Kalim et al. (2003).

We were able to clearly establish that zinc and manganese had a direct effect on *R. solani* growth in plate/*in vitro* experiments. Babich and Stotzky (1978) found similar levels of zinc to be inhibitory to *R. solani*. They noted the enhanced toxicity of chloride salts, and in our experiments, the chloride salts were more inhibitory than the other salts.

Our glasshouse trial confirmed that high levels of zinc had a detrimental effect on the potato pathogen *R. solani* AG3, but unfortunately also on the host plant. Elevated zinc levels resulted in significant accumulation of zinc in both plant and root tissue, reaching levels of reported zinc toxicity in tissues (Sanderson and Gupta (1990). Sanderson and Gupta (1990) showed that in potatoes, toxic levels of zinc in leaves ranged from 97 to 224 ppm. These are much lower levels than we observed, with toxic effects began to manifest when tissue levels ranged from 3000–5400 ppm. Although Streeter et al. (2001) did not establish direct zinc inhibition of *R. solani* pathogencity on wheat, they showed that zinc significantly increased root growth enabling the wheat plant overcome the impact of *R. solani*. Our results, however, clearly showed significant reductions in *R. solani* AG3 DNA concentrations and Rhizoctonia disease levels

with increased zinc, and suggested that a soil target range of 10–25 ppm zinc should be further tested in the field.

In the field, the higher rate of zinc EDTA at 100 kg Zn/ha significantly reduced disease outcome (as black scurf on tubers) yet zinc sulphate had no effect. This result was not expected, as Zn EDTA was the least inhibitory form of zinc in the *R. solani in vitro* assays, and we did not include it in the pot trials. Zn EDTA is the chelated form of zinc and is the most plant-available of the compounds tested. On reflection, it would appear that the zinc influences tuber susceptibility. It is of note that Zn EDTA also reduced the development of powdery scab in our other field trials (see earlier powdery scab section), so perhaps influences tuber maturity in a way that reduces susceptibility to pathogen infection.

Glasshouse studies involving manganese were not as successful, since there was little disease and therefore could not be analysed. *Rhizoctonia solani* AG3 DNA was recovered even at 40 ppm, the highest level used (data not shown), demonstrating that *R. solani* can survive this level of manganese. Nutrient data from field trials have indicated that levels at both our Ballarat and Cora Lynn sites were usually well in excess of this level (up to 750 ppm), with the growers' reporting plenty of Rhizoctonia disease, both stem canker and black scurf, indicating that manganese soil amendments would not be an effective treatment. This agrees with (Huber and Wilhelm 1988a), who claim that soil-borne diseases in a wide range of plants are encouraged by manganese deficiencies in soil, and that where manganese levels are adequate, the addition of more manganese will have little effect.

MANAGING THE RISK OF SOILBORNE DISEASES – DRAFT DISEASE MANAGEMENT DECISION TREES

One of the challenges of a research program is to ensure that the findings of the research is communicated to the relevant audience and ultimately adopted by industry. The most up-to-date information on a particular disease problem is likely to be found in a number of different research reports, scientific papers and various industry media.

This information is seldom compiled for easy access by industry. Thus, Disease management "decision trees" for the three major diseases in this research project, namely powdery scab, common scab and Rhizoctonia disease (stem canker and black scurf), were developed as decision support tools for industry as part of the APRP2 project. These decision trees and accompanying information are based on research from this program (APRP2) and builds on APRP1 and other HAL sponsored research projects, including research conducted by current and previous collaborators in Australia, New Zealand, Canada and the United Kingdom.

The decision trees are accompanied with essential background information to help growers and agronomists understand the pathogen, the disease and management options. The information is in a format that identifies the high risk factors that result in disease and what action to take to prevent or mitigate these factors. The information for powdery scab and common scab is similar to that provided in publications from the British Potato Council, and is in keeping with the concept of managing disease "risk".

Members of the APRP2 project team, growers, the APRP2 Technical Operations Committee, the project Technical Oversight Group and members of the Processing Potato Industry Advisory Committee provided feedback on early drafts of the decision trees and this feedback has been incorporated into the material presented here.

The decision trees are a starting point in providing synthesised information in a package to growers and industry. They are not intended to be used on their own but should be linked with other sources of information. In the case of powdery scab, the decision tree compliments the PreDicta Pt module provided by SARDI on powdery scab, which has been designed as an information and training manual for crop consultants. Ultimately the information from both should be integrated.

Powdery scab: "Managing the risk of powdery scab" is the most comprehensive of these decision trees because we have a significant body knowledge on the pathogen and the disease. A commercial soil DNA test (PreDicta Pt, Project PT09023), which gives a measure of the amount of the pathogen in the soil, gives some surety of a prediction of disease "risk" (low, medium or high) for a particular field and there are a number of different management options suggested and readily available that can minimise risk.

Common scab: "Managing the risk of common scab" is the least developed of the decision trees. There is no reliable commercial soil DNA test for disease prediction in a field. Significant levels of disease can develop from very low soil populations, because inoculum can build significantly around the time of tuber initiation, making it difficult to predict disease risk based on a pre-plant soil test. Apart from cultivar resistance, options for managing disease are limited, hence there are fewer decision points in this tree. The importance of irrigation as a management tool is described and is based on recent research from the UK. Paddock history is the key indicator of disease risk for common scab.

Rhizoctonia stem and stolon canker and black scurf: "Managing the risk of Rhizoctonia disease" has a number of disease management options based on disease prevention and chemical control of seed-tuber and soil-borne inoculum. There is no reliable commercial soil DNA test available for prediction of disease risk in a field. The results of the DNA tests are not a reliable predictor of disease risk for this pathogen at present. An assessment of disease risk must be based on paddock history. There is a major gap in our knowledge of the behaviour and development of the inoculum of *R. solani* between the time of planting and infection of the stems, and stolons and tubers. This gap in knowledge must be addressed as a matter of urgency because of the significant economic and yield losses associated with this disease. However, there are still practical and effective chemical control options for managing disease from both the tuber-borne and soil-borne inoculum of the pathogen.

DRAFT POWDERY SCAB MANAGEMENT DECISION TREE

Powdery scab (*Spongospora subterranea*) management decision tree

"Managing the risk of powdery scab"

Dolf de Boer, Tonya Wiechel and Jacky Edwards

How to use the decision tree

Powdery scab affects seed, fresh market and processing potatoes. The disease is both seed tuber-and soil-borne. The incidence and severity of powdery scab can vary widely depending on a range of factors, including inoculum (amount of the pathogen in the soil or on seed tubers), climatic conditions and cultivar susceptibility.

This powdery scab management decision tree was developed to help growers, industry and researchers to work through a decision process to reduce the risk of a serious disease outbreak in a particular field. The focus is on information relevant to the processing potato industry, although this information is equally relevant to the seed and fresh potato sectors.

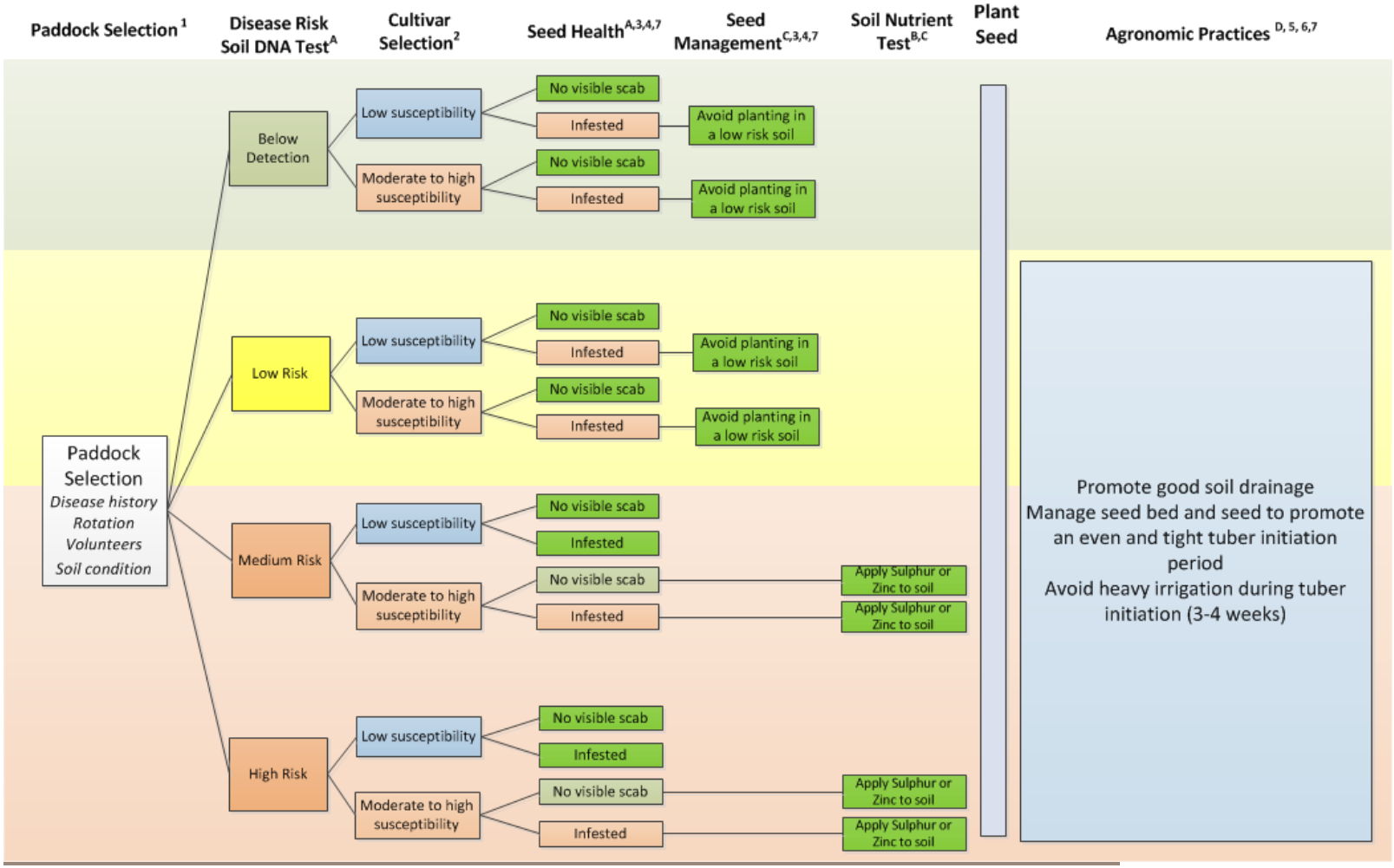
The decision tree is in two parts

Part 1. A stepwise process from left to right under the headings of; paddock selection, soil DNA testing, cultivar selection, seed 'health', seed management, soil nutrient status and agronomic practices. Each step involves a decision with different options, depending on paddock history, soil and nutrient DNA tests, choice of variety and seed health status. Each of the columns has links to background information and various decision support tools (marked with superscripts) in Part 2.

Part 2. High risk factors that contribute to powdery scab and actions to mitigate these risks are outlined. This section also explains the importance of soil nutrient testing and the DNA testing of soils in the decision making process, as well as cultural disease control options.

Growers are faced with a number of key decisions leading up to establishment of each crop. The major factor is the market for which the potatoes are grown. The market dictates choice of cultivar and end use of that cultivar, e.g. early harvest and fresh into the factory or later harvest and storage on farm or factory. Other major decisions include choice of paddock and matching cultivars with the paddock, based on the market and disease risk for that field.

Part 1 The decision tree – managing the risk of powdery scab



An example of a disease management scenario

The cultivar 'Atlantic' is susceptible to powdery scab. If a paddock soil test gives a high risk for powdery scab, this variety could still be planted for early harvest fresh to the factory. As long as conditions remain cool, it takes several weeks after infection of tubers during tuber initiation before the symptoms of powdery scab become visible on the skin. These tubers could be harvested and processed in the factory before symptoms develop. Field trials in Victoria have shown that elemental sulphur applied at 1 t/ha before planting can reduce the risk of the disease developing in some soils. As the paddock has been assessed as high risk, seed tuber-borne inoculum will not have any significant impact on the overall disease risk. Ensuring good drainage of the soil and managing irrigation during the tuber initiation period to avoid prolonged wet conditions will also minimise risk.

The worst case scenario if the tree is not used

High risk of powdery scab: paddock with a history of powdery scab; short rotations between potato crops; significant numbers of volunteer potatoes evident during the rotation period; parts of the paddock prone to water logging; soil DNA test indicates a high disease risk; paddock planted with a highly susceptible cultivar; cold, wet weather conditions around the time of tuber initiation and/or heavy irrigation at the time of tuber initiation.

Part 2 Accompanying information on managing the risk of powdery scab

Powdery scab affects seed, ware and processing crops. The disease occurs throughout Australian potato-growing regions, but some production areas have a greater risk of the disease than others. This is often associated with prevailing cool, wet weather conditions during the tuber initiation period. These conditions can also occur through irrigation of crops during the tuber initiation period.

The disease is caused by the pathogen *Spongospora subterranea*, which produces very resistant resting spores in galls on the roots and stolons of potato plants, as well as in the powdery scab pustules on the tubers. These spores, which occur as clusters of several hundred known as "spore balls", can survive for more than 13 years in soil. These resting spores, the 'inoculum', can be seed tuber- and soil-borne.

The incidence and severity of powdery scab symptoms depend on factors such as inoculum level, cultivar susceptibility and climatic conditions.

A. Assessing disease risk

Paddock history: paddock history is a good indicator of disease risk. The powdery scab pathogen has a long survival time. This means that the pathogen will still be present in a field after several years break from potatoes.

Soil DNA test: A commercial DNA soil test is available that quantifies the pathogen inoculum as the amount of DNA of the pathogen in a soil sample and provides a risk rating for a particular paddock (below detection, low, medium or high risk). This test is available from SARDI through accredited agronomists (Appendix 1).

Seed testing: Growers can determine if powdery scab exists on their seed tuber stocks by examining a washed sample of tubers. Tubers that do not have scabs may still be contaminated with spore balls of the

pathogen during the grading process if those tubers were part of a consignment containing scabby tubers or were harvested from infested soil.

(*Seed tuber peel test:* For a more accurate assessment of seed tuber inoculum levels, a DNA test can be done on samples of peel from a seed tuber lot. However, this test is not yet commercially available and there are no defined "thresholds" linking DNA levels with disease risk from seed tuber lines.)

B. Soil nutrient testing and soil treatments

Elemental sulphur – Trials conducted in Victoria have demonstrated that incorporating elemental sulphur (1 t/ha) into soil prior to planting significantly reduced powdery scab in high risk soils with histories of powdery scab (average 50% reduction in proportion of tubers affected, 70% reduction in severity of scab on tubers).

Zinc – Trials conducted in Victoria demonstrated that raising soil zinc concentrations with an application of Zn EDTA or sulphate salts before planting consistently reduced severity of powdery scab disease in high risk soils (20-30% reduction in the severity of scab on the tubers). This treatment was less effective than the sulphur treatment. Soil nutrient testing can provide baseline zinc concentrations. Zn EDTA and sulphate applications raised available soil Zn concentrations in a range of 20-30 ppm (baseline 2-3 ppm).

NB. It should be noted that these treatments have only been tested in ferrasol soils around the Ballarat region.

C. Chemical control of powdery scab

At present, there are no registered seed tuber or soil pesticide treatments for control of powdery scab in Australia.

D. Irrigation scheduling

Powdery scab is favoured by cool (12-15°C), wet conditions (periods of saturation of the soil) around the the time of tuber initiation. The tuber initiation period (when the ends of the stolons begin to swell) occurs over a period of about three to four weeks. This is the time when the developing tubers are highly susceptible to infection by the powdery scab pathogen. Trials in Victoria have shown that withholding irrigation over this period results in significantly less powdery scab on tubers of a susceptible cultivar at harvest than with normal scheduled irrigation over this period. The practice of irrigating the crop at this time, or periods of rain at this time, increases the risk of powdery scab. However, the effects of frequency of irrigation on disease risk and the Soil Moisture Deficits required to increase disease risk have not been determined.

NB The opposite is true for common scab. Disease risk is reduced by maintaining adequate Soil Moisture Deficits that ensure soils are wet during the tuber initiation period.

Managing the risk of powdery scab

High Risk Factors	What action to take	Comments
1. Paddock Selection • **Paddock with previous history of powdery scab.** • **Volunteer potatoes.** • **Solanaceous weeds (e.g. nightshade).** • **Poor drainage.**	Consider paddock history when developing a cropping plan. Avoid tight rotations and manage volunteer potatoes to keep the build-up of inoculum to a minimum. Conduct a pre-season soil DNA test to check the disease risk of selected fields (Appendix 1).	Paddock history is a good guide to the risk of powdery scab developing in a potato crop planted in that paddock. Consider planting cultivars with a low susceptibility rating in high risk paddocks. Plant susceptible varieties into uncontaminated land with uninfected seed. Practice longer rotations, control volunteer potatoes and use uninfected seed to minimise the build-up of soil inoculum.
2. Cultivar **Susceptible cultivars**	Guides on varietal susceptibility to powdery scab are available.	Link to cultivar list with disease susceptibility ratings (Appendix 2)
3. Seed Health • **Infected seed potatoes, particularly when planting in uncontaminated land or land with a very low risk of powdery scab**	Use certified seed potatoes. Use disease-free seed (sourced from a non-powdery scab area). Avoid planting clean seed stocks in fields with a high disease risk.	Seed-borne inoculum can cause disease in the resultant crop and can be a source of contamination of low risk soils. However, seed-borne inoculum has little influence on disease outcomes of crops planted in soils with high inoculum levels.

4. Grading/Hygiene • **Grading uninfected seed stocks without cleaning and disinfection of grading equipment and storage boxes/bins after diseased ware and seed stocks**	Wash and evaluate samples of all seed stocks intended for grading, or get seed peel DNA tests done to determine the level of contamination with the powdery scab pathogen. Grade seed stocks before grading ware stocks and prioritise high grade seed. Wash/disinfect grading lines between different stocks with different health status. Fully wash down and disinfect grading lines and storage equipment (boxes/bins) at the end of each season.	Resting spores can contaminate grading lines and be transmitted onto healthy seed stocks. Knowing which seed stocks carry powdery scab and grading higher grade seed stocks first, then cleaning the grading line and storage facilities, will minimise the spread of seed-borne inoculum.
5. Soil Conditions • **Cold wet soils, particularly over the tuber initiation period.** • **Soil compaction.**	Ensure good soil structure to maintain good drainage with minimal compaction. Ensure a good seed bed to promote rapid and uniform emergence and a tight tuber initiation period (reduced duration of tuber initiation). Avoid excessive tilling of the seed-bed which can degrade soil structure. Plant later in the season to avoid cold, wet soils during the tuber initiation period.	Powdery scab is favoured by cool, wet soil conditions, particularly at tuber set. Soil conditions that increase retention of water in the soil profile, such as poor soil structure or compaction layers, will increase the risk of disease. Generally, powdery scab is worse in cool and wet areas.

6. Crop Growth • **Prolonged tuber initiation period in cold soils with either seed or soil inoculum.** • **Excessive irrigation during the tuber initiation period**	Use good quality seed and ensure a good seed bed is prepared, to promote rapid and uniform emergence of the crop and a tight tuber initiation period. Avoid over-irrigation during tuber initiation.	Good seed and soil management promotes uniform crop emergence and development. A uniform tuber initiation throughout the crop minimises the period at which the population of new tubers are at risk of infection. A prolonged tuber initiation period can increase overall disease risk. Schedule irrigation to avoid waterlogging. Be aware that the risk of disease increases significantly after excessive rainfall during tuber initiation.
7. Farm Hygiene • **Dirty/dusty seed stores and storage equipment, unwashed cultivation and harvesting machinery used in clean paddocks.**	Ensure that a farm hygiene policy is in place, and rigorously applied.	A farm hygiene policy is an essential disease prevention strategy for the farm. The focus is to avoid the contamination of healthy seed stocks, facilities and equipment that could result in contamination of new ground or fields with low risk powdery scab.

Appendix 1 – Accredited agronomists for DNA soil testing in Victoria

PreDicta Pt - Soil DNA testing service			
Pre-planting risk assessment for soil-borne diseases of potatoes **(Powdery scab, Black dot, Root-knot nematode)**			
Accredited Agronomist List - VICTORIA (2014)			
Company	Accredited agronomist	Region	Phone
Ag-Challenge Consulting P/L	Russell Bell	Gippsland	(03) 5623 4788
	Stuart Jennings		
ViCSPA	Nigel Crump		(03) 5962 000
	Joe Smith		
	Luke James		
McCain Foods (Aust) Pty Ltd	Daniel Grayling	Ballarat	0403 174 765
Davies and Rose Rural and Hardware	Andrew Powell	Ballarat, Colac/Otway, Portland	0429 452 722
Elders	Simon Nowell		0439 369 006
Independent Agronomy Services P/L	David Ryan	Ballarat, Colac	0458 346 883
General enquiries on PreDicta Pt testing service can be made to Michael Rettke, SARDI (08) 8303 9414			

Appendix 2 – Relative susceptibility of potato varieties to powdery scab disease. Tubers of different varieties can vary in their susceptibility to powdery scab from highly susceptible (most tubers affected, and most of the tuber surface covered with scab) to very low susceptibility (occasional tuber affected with only a few scab pustules evident) (NB Requires input from other sources).

	Powdery scab susceptibility score (1-9)	Cultivar
Highly susceptible	1	
	2	
	3	
	4	Bliss, Denali, Kennebec, Shepody, MacRusset, Sonic
	5	Atlantic, Riverina Russet, FL 1953, FL 2027, McCain 4
	6	Catani, Pike. Russet Burbank, Simcoe, Trent
	7	FL 1867, Innovator, Umatilla
	8	Ranger Russet
Low susceptibility	9	

DRAFT COMMON SCAB MANAGEMENT DECISION TREE

Common scab (*Streptomyces scabies*) management decision tree

"Managing the risk of common scab"

Dolf de Boer, Tonya Wiechel, Jacky Edwards

How to use the decision tree

Common scab affects seed, fresh market and processing potatoes. The disease is both seed-tuber and soil-borne. Although wide spread in Australia, the disease tends to be common only in some cropping areas. The incidence and severity of the disease can vary widely depending on a range of factors, including inoculum (amount of the pathogen in the soil or on seed tubers) and climatic conditions.

The common scab pathogen is often not detected at planting time but can multiply significantly in number on and around developing tubers in the period after tuber initiation, the time at which tubers are most susceptible to infection.

Prevention and management is based on

- Avoiding high risk fields
- Matching cultivar to field according to susceptibility and disease risk
- A crop rotation strategy
- An irrigation regime
- Prioritising land according to the crop end use

This common scab management decision tree was developed to help growers, industry and researchers work through a decision process to reduce the risk of a serious disease outbreak in a particular field. The focus is on information relevant to the processing potato industry, although the information is equally relevant to the seed and fresh potato sectors.

The decision tree is in two parts

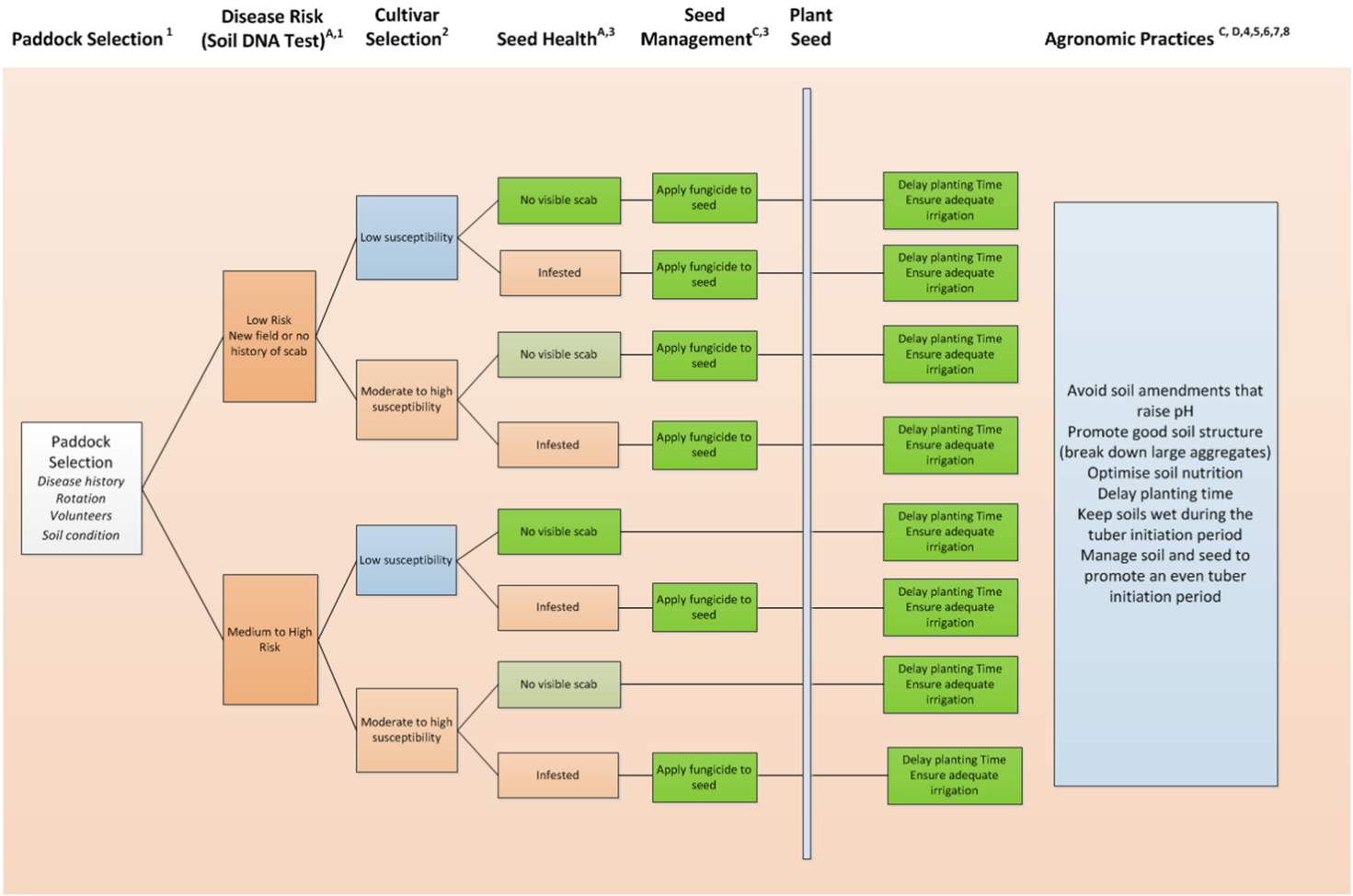
Part 1. A stepwise process from left to right under the headings of paddock selection, variety selection, seed management, soil management and agronomic practices. Each step involves a decision with different options depending on paddock history, choice of variety and seed health status. Each of these columns has links to background information and various decision support tools (marked with superscripts) in Part 2.

Part 2. High risk factors that contribute to common scab and actions to mitigate these risks, including paddock history, cultivar susceptibility, the health status of seed tubers and current chemical and cultural control options.

Growers are faced with a number of key decisions leading up to each crop. The major driver is the market for which the potatoes are grown. The market dictates choice of variety and end use of that variety, e.g. early harvest and fresh into the factory or later harvest and storage on farm or factory. Other major

decisions include choice of paddock and matching varieties with the paddock depending on the market for that cultivar and the disease risk of that field.

Part 1 The decision tree – managing the risk of common scab



An example of a disease management scenario

Paddock with a known history of common scab: Plant a cultivar with a relatively low susceptibility to common scab if possible. Treat seed tubers with a registered fungicide if there is evidence of common scab lesions in the seed consignment, or if seed-tubers were grown in soil with a known history of common scab. Plant the crop relatively late so that tuber initiation coincides with New Year (avoid the Christmas holiday period). Schedule irrigation to ensure that soils around the developing tubers are wet during the tuber initiation period (period of 3-4 weeks). This scenario should be applied to susceptible cultivars and cultivars that are rated low to moderately susceptible.

Worst case scenario if the tree is not used

High risk of common scab: paddock with a history of common scab, lime applied to soil before planting (a risk in some soil-types), a moderate to highly susceptible variety planted, seed tubers with obvious symptoms of common scab and warm, dry conditions during the tuber initiation period.

Part 2 Accompanying information on managing the risk of common scab

Common scab affects seed, ware and processing crops. The disease occurs Australia wide but some production areas have a higher risk of the disease than others. The disease is often associated with warm, dry weather conditions. Although widespread in Australia, the disease tends to occur frequently in only some cropping areas.

The disease is caused by the bacterial pathogen *Streptomyces scabies* that survives in soil on potato tubers, on potato plant debris and in the organic debris in general.

The incidence and severity of common scab symptoms depends on factors such as inoculum level, cultivar susceptibility and climatic conditions.

A. Assessing Disease Risk

Paddock history: paddock disease history is the best indicator of disease risk at present. A consistent history of common scab in a particular field indicates a relatively high risk. Significant disease can develop in paddocks that have relatively low amounts of bacteria in the soil, because the pathogen can multiply significantly in number on and around developing tubers in the period after tuber initiation, the time when the developing tubers are most susceptible to infection.

(*Soil DNA test:* A DNA soil test for the amount of inoculum of the common scab pathogen is currently not commercially available. The test needs to be further developed in order to better relate DNA concentrations with disease risk ratings).

Seed testing: Growers can determine if common scab occurs on their seed stocks by examining a washed sample of tubers for symptoms of the disease.

(Seed peel test: A DNA-based test of common scab inoculum on the samples of peel is not yet commercially available and there are no defined “thresholds” linking DNA levels with disease risk from the seed.)

B. Chemical control of common scab

There is no registered fungicide treatment for the control of soil-borne common scab at present. However, there are a number of registered treatments for the control of tuber-borne scab. These treatments would be best applied on seed stocks that have evidence of scab, particularly if the seed is to be planted in soils with a low risk of common scab (no previous history or new ground).

C. Planting time and irrigation

Delay planting time (7-10 days) to ensure that tuber initiation occurs under warmer, drier conditions when there is a greater diligence in ensuring adequate irrigation during the tuber initiation period.

Common scab is favoured by relatively warm, dry conditions. Research in the United Kingdom has shown that irrigation around the time of tuber initiation can reduce the risk of common scab. For irrigation to be effective, tubers must be covered in a film of water to prevent the multiplication of the common scab bacteria on the tuber skin. This requires a fine soil condition that ensures good contact between soil and tuber with no air pockets. This may be difficult to achieve in “cloddy” seed-beds that are likely to result in air pockets over the developing tubers.

NB. Powdery scab is favoured by cool, wet conditions during the tuber initiation period, the opposite of the conditions that favour common scab. The most appropriate management option will depend on the relative risks of each disease in a paddock, the susceptibility of the variety being used and the market end use of that variety. If the risk of powdery scab is high then heavy irrigation during tuber initiation is not advisable.

D. Seed-bed management

Good management of the seed bed (soil structure) and the management of the condition of the seed (pre-sprouted and a uniform physiological age) promotes the rapid and even emergence of the crop and tightens the tuber initiation period. A prolonged tuber initiation period will result in a longer period in which tubers are susceptible to infection.

Managing the risk of common scab

High Risk Factors	What action to take	Comments
1. Paddock Selection • **Paddock with previous history of common scab.** • **Volunteer potatoes.** • **Rotation with susceptible crops**	Consider paddock history when developing a cropping plan. Avoid short rotations and manage volunteer potatoes to keep the build-up of inoculum to a minimum. Use rotations of at least one in four years. Radish, beet, carrots and red clover are susceptible to common scab	Paddocks with a consistent history of common scab may be considered to be high risk. However, scab forming *Streptomyces* are often not detected at planting time but can multiply significantly in number on and around developing tubers in the period after tuber initiation.
2. Variety • **Susceptible varieties**	Guides on varietal susceptibility to powdery scab are available.	Potato cultivars vary in their susceptibility to common scab. However, some varieties rated as moderately susceptible to the disease can develop severe symptoms under some conditions of very high disease risk. (Appendix 1)
3. Seed Health • **Infected seed potatoes, particularly when planting in uncontaminated land or land with a very low risk of common scab**	Use certified seed potatoes. Plant seed with levels of scab as low as possible	There is evidence that tuber-borne scab contributes to disease in progeny tubers. However, there are no defined thresholds for the carry-over of the pathogen from infested seed tubers to the progeny tubers. Tuber-borne *Streptomyces* can be a source of infestation of new ground.

4. Planting time • **Early planting**	Plant the crop later rather than early	This ensures that tuber initiation occurs under warmer, dryer conditions when there is greater diligence in ensuring adequate irrigation of the crop during tuber initiation (3-4 weeks after first tubers begin to develop). A later planting date may result in slightly lower yields.
5. Dry Soil (SMD) • **Dry soils during the tuber initiation period**	Maintain adequate Soil Moisture Deficits (SMD) during the tuber initiation period.	It is essential that soils are wet at the tuber initiation for adequate control of common scab. Populations of pathogenic *Streptomyces* on the surface of tubers increase rapidly after tuber initiation, with the increase being faster in dry soils than in wet. Changes in soil water content during the first 2-3 weeks after initiation are critical in changing populations of *Streptomyces*, with little difference or change between irrigated and unirrigated after 4 weeks.
6. Water demand (Irrigation) • **Inadequate soil moisture during early tuber initiation**	If soil is dry, start irrigation when plants first begin to initiate tubers to allow time to complete irrigating the field before 50% have initiated.	

7. Soil Conditions • **Cloddy soil conditions.** • **Soil pH**	A relatively fine soil structure to ensure good contact with the surface of developing tubers. Avoid over cultivating resulting in slumping and poor drainage. Avoid the application of materials that increase the alkalinity of soil (e.g. lime).	Good soil structure is crucial to maintaining films of water round tubers to suppress *Streptomyces* populations. Clods leave air spaces, which drain rapidly producing a dry environment for the organism to thrive. Liming of soil has been associated high disease risk. Good seed-bed conditions promote rapid and even emergence of plants and a tight tuber initiation period.
8. Farm Hygiene • **Dirty/dusty seed stores, unwashed machinery used in clean paddocks.**	Ensure that a farm hygiene policy is in place.	A farm hygiene policy is an essential disease prevention strategy for the farm.

Appendix 1 – Relative susceptibility of potato varieties to common scab disease. Tubers of different varieties can vary in their susceptibility to common scab from highly susceptible (most tubers affected, and most of the tuber surface covered with scab) to very low susceptibility (occasional tuber affected with only a few scab pustules evident) (NB Requires input from other sources).

	Common scab susceptibility score (1-9)	Cultivar
Highly susceptible	1	
	2	
	3	
	4	Ranger Russet, Trent
	5	Atlantic, Bliss, Catani, Denali, Kennebec, MacRusset, Innovator, Riverina Russet, Russet Burbank, Shepody, Simcoe, Sonic
	6	FL 1876, FL 1953, Umatilla
	7	
	8	
Low susceptibility	9	Pike

DRAFT RHIZOCTONIA DISEASE MANAGEMENT DECISION TREE

Rhizoctonia stem and stolon canker and black scurf (*Rhizoctonia solani*) management decision tree

"Managing the risk of Rhizoctonia disease"

Dolf de Boer, Tonya Wiechel, Jacky Edwards

How to use the decision tree

Rhizoctonia canker (disease on stems and stolons), also called "stem and stolon canker" and black scurf (disease on tubers), caused by the soil inhabiting fungus *Rhizoctonia solani*, affects seed, fresh market and processing potatoes. The disease is both tuber-borne and soil-borne and occurs in all potato cropping areas of Australia.

The fungus damages stems, stolons and tubers. The pruning of the new sprouts and stems by the fungus results in a patchy crop emergence and patches of stunted plants. The pruning of the stolons reduces the number of marketable tubers. One or more unmarketable tubers are produced from plants where most of the stolons have been pruned off. Damage to the eyes and the skin of new tubers by the fungus can result in tubers with poor shape ("dimples") and skin defects (russeting of the skin). The formation of black sclerotia on the tuber skin as tubers mature at the end of the season is the "black scurf" symptom.

The incidence and severity of the disease can vary widely depending on a range of factors, including inoculum (the amount of the pathogen in the soil or on seed tubers) and a number of soil and climatic conditions.

The Rhizoctonia management decision tree was developed to help growers, industry and researchers work through a decision process to reduce the risk of a serious disease outbreak in a particular field. The focus is on information relevant to the processing potato industry, although the information is equally relevant to the seed and fresh potato sectors.

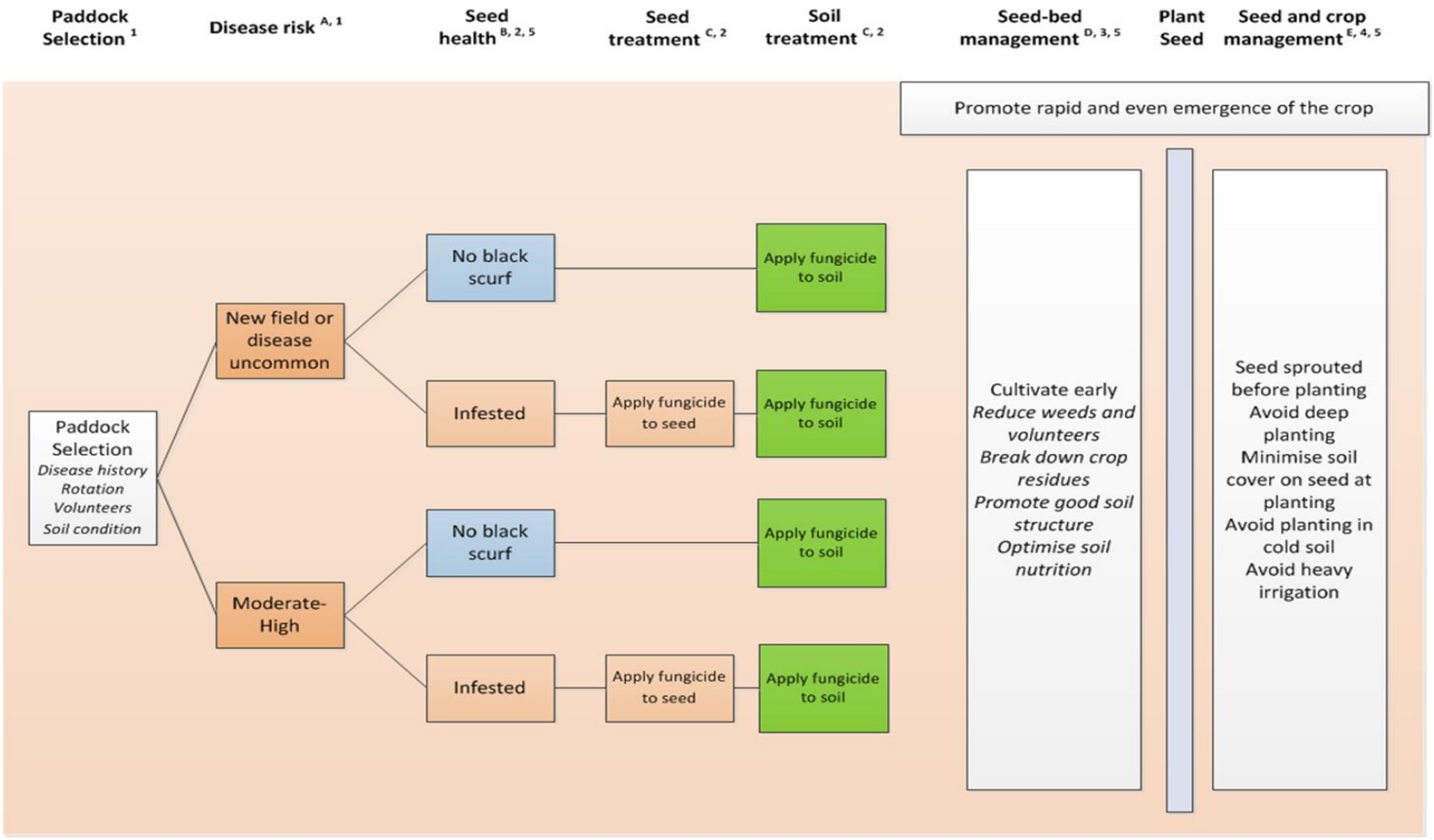
The decision tree is in two parts

Part 1. A stepwise process from left to right under the headings of paddock selection, disease risk, seed health status, seed and soil treatments, seed management and seed and crop management. Each step involves a decision with different options depending on paddock history and seed tuber health status. Each of the columns has links to background information and various decision support tools (marked with superscripts) in Part 2.

Part 2. Description of high risk factors that contribute to Rhizoctonia disease and actions to mitigate these risks. Key factors to consider for Rhizoctonia disease risk are paddock history, seed-bed preparation and the management of seed potatoes coming out of cool storage in readiness for planting and during the planting operation.

Growers are faced with a number of key decisions leading up to the establishment of each crop. The major factor is the market for which the potatoes are grown. The market dictates choice of cultivar and end use of that cultivar, e.g. early harvest and fresh into the factory or later harvest and storage on farm or factory. The other major decision is the choice of paddock and then matching cultivars with the paddock depending on the market and the disease risk for that field.

Part 1 The decision tree – managing the risk of Rhizoctonia disease



An example of a disease management scenario

Paddock with a history of Rhizoctonia disease – Field cultivated as early as possible to promote the breakdown of organic residues from the previous crop or pasture and to provide a soil structure that does not impede sprout emergence; seed tubers removed from the cool store weeks before planting to promote eye movement; seed treated with a registered fungicide before planting because of evidence of sclerotia ("black scurf") on the skin; soil treated with a registered fungicide and seed planted to a depth that minimises soil cover and planted when soil temperatures are above 12°C to avoid a slow sprout emergence (Planted deep = a longer period to grow through the soil, low temperature = slower sprout growth).

Worst case scenario if the tree is not used

High risk of Rhizoctonia disease - paddocks with a known history of the disease on short rotations and with a relatively high number of volunteer potatoes. Seed-bed cultivated close to planting resulting in a significant amount of "green" organic matter in the soil when the crop is planted. Seed planted in the dormant state (not given sufficient time to activate sprout movement once removed from the cool store) and planted relatively deep in cold ground. Evidence of Rhizoctonia sclerotia (black scurf) on the seed. Seed and soil not treated with fungicides.

Part 2 Accompanying information on managing the risk of Rhizoctonia disease

Rhizoctonia canker and black scurf affects seed, ware and processing crops and occurs Australia wide. Most potato crops will have a low level of damage due to Rhizoctonia that often goes unnoticed. Some growers have a significant and persistent Rhizoctonia disease problem. Often, however, serious outbreaks that reduce sprout emergence, cause stunting of plants and yield loss can be very seasonal and unpredictable. The disease is often associated with unseasonably cool conditions during crop emergence and crop senescence. Rhizoctonia disease occurs in patches, some as small as a dinner plate and up to a meter or larger, throughout the field.

The Rhizoctonia fungus survives in soil in infected potato debris and as the thick black structures found on tubers called sclerotia. The fungus can grow between soil particles to colonise organic matter, the underground parts of potato plants, as well the underground parts of other plant species. Damage to the stems, stolons and tubers general occurs early in the season from the time of sprout emergence. Severe infection of fleshy stems and stolons can cause complete girdling and "pruning" of the affected parts. Plants that survive stem infection, which affects the flow of nutrients, and are able to grow to maturity to produce tubers but often senesce much later than healthy plants. These plants are identified by the clusters of small tubers at their base and the presence of "aerial" tubers in the leaf axils above ground. Distortion of tuber shape (e.g. "dimples") and patches of Russeting on the tuber skin are also symptoms of Rhizoctonia damage. The sclerotia (black scurf) develop on the tuber skin as tubers begin to senesce late in the season. These sclerotia are a source of infection of new sprouts when tubers are planted as seed. Disease in the potato crop can be from Rhizoctonia in the soil and from infested tubers.

A. Assessing disease risk and diagnostic testing

Paddock history: Knowing the history of Rhizoctonia disease in a paddock, including frequency, severity and predictability over several seasons, is critical in assessing the disease risk of that paddock.

(*Soil DNA test:* There is no commercial DNA soil test to quantify the amount of the Rhizoctonia fungus ("inoculum") in a soil sample from a paddock, which gives an indicator of disease risk. The test needs to be further developed in order to better relate DNA concentrations with disease risk ratings).

B. Seed health

Seed testing: Growers can determine the level of infestation of their seed potatoes with Rhizoctonia black scurf by examining a washed sample of tubers. The tubers are obviously infested if the typical brown/black sclerotia can be seen on the skin. However, tubers that do not have sclerotia may still be infested with the fungus because it inhabit tuber skin as fine microscopic red-brown fungal threads and are, therefore, not visible to the naked eye. These Rhizoctonia hyphae can also be concentrated in the tuber lenticels (breathing pores). Trials have shown that seed tuber-borne Rhizoctonia is a source of the disease the potato crop, even with relatively low levels of infestation of the tuber. However, there are no defined thresholds for level of infestation on seed tubers and subsequent disease in a crop. Disease risk is likely to increase with increasing severity of coverage of sclerotia on the seed tuber.

(Seed peel test: There is no commercial DNA test available at present to quantify the amount of the fungus on seed potato tubers and there are no defined "thresholds" linking DNA levels with disease risk from the seed).

C. Chemical control of Rhizoctonia canker and black scurf

Infested seed tubers are a significant source of Rhizoctonia inoculum in the potato crop. The fungus grows from germinating sclerotia onto stems and stolons after the seed tubers are planted. Several registered fungicide seed treatments are available that can significantly reduce the spread of the Rhizoctonia fungus from the seed tubers and into the crop.

Soil-borne Rhizoctonia is also an important source of infection of the crop, particularly where there is a history of Rhizoctonia disease. A registered fungicide treatment can be applied to the soil at planting time that reduces the risk of soil-borne Rhizoctonia. (Check the APVMA website).

D. Seed bed management

Early knockdown of pastures, or other rotation crops, and the cultivation of the seed-bed several weeks, rather than days, before planting will help breakdown organic matter that may harbour the Rhizoctonia pathogen. This practice will also improve the condition of the seedbed to promote the rapid emergence of sprouts. Compacted soil could potentially slow sprout growth resulting in a greater risk of infection by the Rhizoctonia fungus.

E. Seed and crop management

There are several factors that can slow sprout emergence from the soil. Slow growth makes the sprout vulnerable to infection by the Rhizoctonia pathogen. Once the sprout has emerged from the soil and produces a green top, the underground parts are less susceptible to attack. Factors that reduce disease risk include: removing seed potatoes from the cool store a few weeks before planting to promote spouting, not planting in cold soils, which slows sprout emergence, and not planting too deep because it takes longer for the sprouts to emerge from the soil. Minimising the cover of soil over seed pieces after planting helps to ensure rapid emergence. Where possible, harvest tubers as early as practicable to reduce the risk of

developing black scurf. “Sprouting” of seed in this case means swelling of the buds in the eyes of the tuber, which indicates that dormancy is broken and that sprouts will soon emerge from the eyes.

Managing the risk of Rhizoctonia canker and black scurf

High Risk Factors	What action to take	Comments
1. Paddock selection • **Planting a crop in soil that is infested with the Rhizoctonia pathogen.** • **Short rotations** • **High numbers of volunteer potatoes.**	Paddock history is a good guide to Rhizoctonia disease risk. Crops grown in short rotations with a history of Rhizoctonia disease and with high numbers of volunteers may be high risk. The presence of brown lesions on the white underground stems of young volunteer potatoes can be a rough guide to the amount and distribution of the Rhizoctonia fungus in the soil.	The potato strain of Rhizoctonia does not have a wide host range. Potato is its preferred host on which is able to maintain its population in soil. However, as an active soil coloniser, the fungus is able to colonise the root systems of many other plant species allowing it to survive in the period between potato crops.
2. Seed health • **Infested seed tubers, particularly when planting in uncontaminated land or land with a very low Rhizoctonia risk**	Use certified seed potatoes Wash a sample of seed tubers from each consignment to check for the presence of Rhizoctonia sclerotia (black scurf symptom). There is also a risk that skin of the seed tubers are infested with hyphae (microscopic threads) of the fungus (i.e. not visible). Treat seed tubers with a registered fungicide before planting.	Seed tuber-borne Rhizoctonia is an important source of inoculum causing disease in the crop. Tuber-borne inoculum is also the source of contamination of “new” ground or paddocks with a low risk of Rhizoctonia disease.

3. Seed-bed conditions • **Late cultivation of seed beds resulting in undecomposed organic matter** • **Cold wet soils, particularly over the tuber initiation period.** • **Soil compaction.**	Prepare the seed bed earlier rather than later to promote the breakdown of "green" organic matter before planting. Ensure good soil structure to promote the rapid and even emergence of the crop.	Rhizoctonia survives in debris of potato crops and as sclerotia on the skin of tubers. The fungus is also able to colonise the root systems of other plant species in the rotation. Cultivating soil to promote the breakdown of organic matter may reduce the risk of disease.
4. Crop establishment • **Planting of dormant seed** • **Planting into cold soil** • **Planting seed too deep**	Use good quality seed to promote rapid and uniform emergence of the crop and a tight tuber initiation period. Acclimatise seed out of the cool store to promote sprout movement (swelling of the meristematic bud in the eyes) before planting. Plant seed tubers into warm soil (greater than 12°C if possible) and cover with as little soil as possible to promote the rapid emergence of sprouts.	Good seed and soil management promotes the rapid and uniform emergence of sprouts from the ground reducing the risk of infection by the Rhizoctonia fungus.
5. Farm hygiene • **Dirty/dusty seed stores, unwashed machinery used in clean paddocks.**	Ensure that a farm hygiene policy is in place.	A farm hygiene policy is an essential disease prevention strategy for the farm. It's focus should be avoiding the contamination of machinery, new ground and low risk paddocks with the Rhizoctonia fungus.

MONITORING POTATO FIELDS TO BETTER UNDERSTAND THE PROFILES OF SOIL AND SEED DNA, NUTRIENTS AND DISEASE IN GROWERS' FIELDS

SUMMARY

In a survey of potato fields, 160 soil samples were collected from 18 French fry (Ballarat region) and crisping growers (Koo Wee Rup/Thorpdale regions) for pathogen DNA and soil nutrient analyses. Eleven different potato cultivars were planted in these fields. Different seed lots were tested for pathogen DNA and the incidence and severity of powdery scab, common scab and Rhizoctonia black scurf on harvested tubers recorded.

The data provides a snap-shot of a typical season in two major districts of variability in soil nutrients, pathogen DNA on seed and in soil and disease incidence and severity. It provides a reference against which to validate the draft disease management decision trees.

INTRODUCTION

Subproject A(i) has focused on evaluating the effects of soil amendments on disease, particularly soil nutrients, with the aim of including nutrient management options in disease control recommendations. At this stage 'best bet' recommendations can be made for elemental sulphur and zinc treatments for powdery scab control. Efficacy of these two elements appears to be universal, having been demonstrated in Australia, New Zealand and the United Kingdom.

Data on soil nutrient concentrations in potato fields is limited to measurements taken at field trial sites. A much broader dataset would be useful as a means of benchmarking on-farm information relating to paddock choice, cultivars, pathogen DNA (seed and soil), soil nutrients and disease by providing baseline information on nutrient concentrations in growers fields for reference against nutrient thresholds for disease control. This section starts to collect such a dataset and reports on the results of a survey of potato fields planted with French fry or crisping processing potato cultivars during the 2013/14 season in Victoria.

Measurements taken included:

- Pre-planting soil nutrient analysis;
- Pre-planting pathogen DNA concentrations in soil and on samples of seed-tubers;
- Disease incidence and severity on plant (Rhizoctonia canker)and tuber samples (powdery scab, common scab, black scurf);
- Tuber yields (where practicable).

This information will help validate the disease management trees described in the previous section. The additional benefit of such a survey was the two-way communication and feed-back between project scientists and the growers involved.

MATERIALS AND METHODS

The soil sampling strategy prescribed for the PreDicta Pt service (Project No. PT09023) was used to survey potato fields. One hundred and sixty pre-plant soil samples were taken from 34 growers in the Ballarat (28), Koo Wee Rup and Thorpdale potato growing regions (6) (French fry and crisping) and tested for nutrient profiles and DNA concentrations of *S. subterranea*, *R. solani* AG2.1 and AG3 and *S. scabies*.

Eleven different potato cultivars were planted in the fields sampled. Pathogen DNA analysis was done on samples of 100 seed tubers taken from each of 73 different seed lots (23 Ballarat, 4 Koo Wee Rup/ Thorpdale) destined for planting in the survey fields. A strip of peel was removed from the heel to the rose end of each seed piece, dried at 40°C for several days and sent to SARDI for DNA extraction and quantification.

After harvest, a total of 79 tuber samples (50 tubers per sample) were collected (15 Ballarat growers and 3 Koo Wee Rup/ Thorpdale), which included 33 samples from the Ballarat region and 46 from Koo Wee Rup/ Thorpdale regions, for assessments of the incidence and severity of powdery scab, common scab and Rhizoctonia black scurf.

The results are presented in a prescriptive manner to demonstrate mean, median and range of the data.

RESULTS

Pre-plant soil nutrient analysis

A summary of the nutrient analysis of 160 pre-plant soil samples is presented in Table 5.A.I-98. The concentrations of different nutrients varied considerably from field to field. Soils were acidic, with pH ranging from 4-6 (water) or 5-7 (buffer). Soil zinc concentrations ranged from 0-7 ppm, averaging 2 ppm, with little variation between the sample sites. Soil potassium concentrations varied between 45-602 ppm and magnesium 45-525 ppm, resulting in considerable variation in the K:Mg ratio from 0.1-2.7.

Table 5.A.I-98 – Summary of nutrient analysis of pre-plant soil taken from 160 potato fields from 34 processing growers in Victoria in the 2013/14 season

Nutrient variables	Mean	Median	Min-Max
Organic Matter (OM)	9	8	3-27
Cation exchange capacity (CEC) meq/100g	22	22	9-35
pH water	5	5	4-6
pH buffer	6	6	5-7
P ppm (total)	61	52	2-195
P ppm (bioavailable)	29	28	2-67
K ppm	254	256	45-602
Ca ppm	1241	1195	520-2910

Nutrient variables	Mean	Median	Min-Max
Mg ppm	167	145	45-525
S ppm	22	17	8-121
Na ppm	51	34	15-312
Zn ppm	2	2	0-7
Mn ppm	47	29	3-246
Fe ppm	89	76	45-196
Cu ppm	1	1	0-3
B ppm	<1	<1	0-1
NO_3 N ppm	28	14	0-143
Al ppm	1510	1526	722-2310
K:Mg	0.54	0.47	0.10-2.68

Pathogen DNA concentrations on seed and in soil

The DNA concentrations of four pathogens and their relative frequency of detection in 160 soil samples is summarised in Table 5.A.I-99. The DNA of *S. subterranea* and *R. solani* AG2.1 was detected more frequently than either *R. solani* AG3 or *S. scabies*, consistent with the relative frequency of detection of these pathogens from many different sites in Victoria, Tasmanian and South Australia over several years as part of project PT09023.

Table 5.A.I-99 – Frequency of detection of the DNA concentrations (log (pg DNA/g soil+1)) of four potato pathogens in 160 soil samples from grower fields in Victoria in the 2013/14 seasons

	Rhizoctonia solani		***Spongospora subterranea***	***Streptomyces scabies***
	AG2.1	*AG3*		
Mean	2.0	0.4	2.7	0.3
Median	1.0	0.0	2.0	0.0
Minimum	0	0	0	0
Maximum	3.6	1.7	3.7	1.9
Incidence of detection of pathogens (%)	73	21	86	6

Pathogen DNA concentration in the seed peel and the relative frequency of detection is shown in Table 5.A.I-100. The incidence of detection of the four pathogens in the seed peel was relatively high, with a similar frequency of detection between the different pathogens.

The pathogens *R. solani* AG3 and *S. scabies* were detected less often in the soil than on seed tubers (Table 5.A.I-101), and less often than *R. solani* AG2.1 and *S. subterranea* in soil. This is consistent with data from the PreDicta Pt testing and may reflect real differences in concentrations in the soil between the difference pathogens. Pathogen concentrations on seed tubers were generally much higher than concentrations detected in soil samples.

Table 5.A.I-100 – DNA concentrations (log (pg DNA/g peel+1)) of four potato pathogens on the peel of 73 samples of seed potato tubers destined for planting in grower fields in Victoria in the 2013/14 seasons

	Rhizoctonia solani		***Spongospora subterranea***	***Streptomyces scabies***
	AG2.1	*AG3*		
Mean	3.7	4.0	3.2	4.2
Median	3.0	2.5	2.0	2.9
Minimum	0	0	0	0
Maximum	4.8	5.0	4.3	5.4
Incidence of detection of pathogen (%)	93	85	79	68

Table 5.A.I-101 – A comparison of the incidence of detection (%) of the DNA of four potato pathogens in soil samples and seed tuber peel samples from different fields in Victoria in the 2013/14 season

Substrate	***Total no. samples***	***R. solani AG2.1***	***R. solani AG3***	***Spongospora subterrenaea***	***Streptomyces scabies***
Soil	160	73	21	86	6
Seed	73	93	85	79	68

A simple Restricted Maximum Likelihood (REML) variance component analysis showed a strong ($P<0.001$) relationship (data not shown) between the incidence of powdery scab on tubers and soil pathogen DNA concentrations. No such relationship was apparent for either common scab or black scurf.

Tuber disease assessments

The incidence and severity of three different diseases on tuber samples from growers fields is presented in Table 5.A.I-102. Symptoms of powdery scab, common scab and black scurf were found in 51%, 67% and 71% of the tuber samples, respectively. Although disease incidence and severity in the samples was generally low, some individual samples had very high incidence (89%, 42% and 52%) and severity (20%, 11% and 25%) for powdery scab, common scab and black scurf, respectively. All three diseases occurred in the Koo Wee Rup and the Ballarat regions.

The potential impact of these diseases on the quality of material acceptable to the factory, in the French fry or crisping categories, is presented in Table 5.A.I-103. This is based on the incidence of affected tubers and, in particular, the percentage of the tuber surface affected, which reduces tuber quality. The greater the coverage of disease on tubers the less suitable the tuber is for processing. Powdery scab had the greatest impact with 1% of samples resulting in >20% loss at the factory and 5% with >10% loss. The remaining 94% of samples were acceptable by the factory (<10% affected).

Table 5.A.I-102 –Incidence and severity of powdery scab, common scab and black scurf (*R. solani* AG3) symptoms in 79 samples of tubers taken from grower fields in Victoria in the 2013/14 season.

	Powdery scab		Common scab		Black scurf	
	% tubers affected	Severity index (%)	% tubers affected	Severity index (%)	% tubers affected	Severity index (%)
Mean	9	2	6	1	11	4
Median	1	0	4	1	5	2
Minimum	0	0	0	0	0	0
Maximum	89	20	42	11	52	25
Incidence of disease in samples (%)	51		67		71	

Table 5.A.I-103 – Return to the factory (%) of the marketable French fry and crisping yield categories of 79 samples after adjustments for disease incidence and severity based on samples for tubers harvested from growers fields in Victoria in the 2013/14 season.

	% yield at French fry factory			% yield at Crisp factory		
	Powdery scab	Common scab	Black scurf	Powdery scab	Common scab	Black scurf
Mean	98	98	97	99	99	98
Median	100	99	99	100	100	100
Minimum	78	90	87	83	89	87
Maximum	100	100	100	100	100	100

	% yield at French fry factory			% yield at Crisp factory		
	Powdery scab	Common scab	Black scurf	Powdery scab	Common scab	Black scurf
% with < 10% yield loss	94	99	97	99	100	96
% with > 10% yield loss	5	1	2	1	0	4
% with > 20% yield loss	1	0	0	0	0	0

Table 5.A.I-104 shows the average incidence and severity of diseases on tubers by cultivar of eleven different potato cultivars planted by the growers surveyed. Atlantic (crisping) and Innovator (French fry) were the two most common cultivars grown. All cultivars were affected by one or more of the three diseases recorded.

Table 5.A.I-104 – Average incidence and severity of potato diseases on potato tubers of eleven different varieties in 79 samples from processing growers in Victoria in the 2013/14 season

Potato cultivar	No. samples	Powdery scab		Common scab		Black scurf	
Variety		% tubers affected	Severity index (%)	% tubers affected	Severity index (%)	% tubers affected	Severity index (%)
Atlantic	24	5	1	6	1	9	4
Catani	12	5	1	4	1	14	6
Innovator	22	18	4	4	1	7	3
Pike	1	0	0	28	7	0	0
Ranger Russet	2	1	0.3	2	0.5	22	9
Russet Burbank	6	7	1	1	0.3	7	3
Sapro Mira	1	0	0	0	0	11	5
Simcoe	3	2	0.5	35	9	17	8
Snowden	5	0	0	6	1	24	9
Sonic	2	30	11	8	2	13	6
Trent	1	0	0	25	5	5	3

DISCUSSION

This survey provides a snap-shot of information from 160 fields planted with crisping and French fry potato cultivars in two major production areas. It provides an insight into the variability of data from different fields.

The most comprehensive data collected was that of soil nutrients and pathogen DNA concentrations. There was very little variation in the soil zinc concentrations, which were well below the thresholds obtained in field trials where soil zinc application significantly reduced powdery scab incidence and severity. There was a very wide variation in the K:Mg ratio between sample fields. This suggests that there is potential to manage soil potassium and magnesium concentrations to obtain a ratio nearer the optimum (0.4) to minimise the risk of common scab. Disease risk is higher on either side of this optimum ratio. However, in our field trials, the buffering capacity of the soils made it difficult to correctly determine the amount of amendment required to reach this ratio.

In the soil, *S. subterranea* DNA was more common than that of *S. scabies* and *R. solani* AG3, consistent with that reported in PT09023. The strong relationship between increasing concentration of *S. subterranea* DNA and disease on tubers is also consistent with PT09023.

The information gathered here is largely descriptive. A thorough analysis of the data is confounded by the complexity of the relationships between the environment, pathogen and disease. Nevertheless, this information can help validate the draft disease management decision trees by providing many different scenarios against which to reference various disease management options.

GENERAL DISCUSSION

The overall aim of project PT09026 was to develop disease control strategies for soilborne diseases through the manipulation of nutrients and soil health factors to induce disease suppressive conditions. Three main areas of research were nutrient amendments, changing the soil condition with a green manure crop and a study of disease suppression relating to soil and plant microbiology.

One of the most exciting aspects of this research program was the work focused on the soil and plant microbiology and the link between microbes and soil health, disease suppression and crop productivity, a research area which will see significant improvements in the future. This work has been made possible by significant advances in DNA-based technologies that are rapidly evolving, which allow an analysis of the soil and plant microbiology. The key aspects of our work included:

- The development DNA based tools to characterize microorganisms that are closely associated with the potato plant (rhizosphere and endosphere) and identifying the function of these bacteria (e.g. antibiosis, nitrogen fixing).
- The development of tools, such as TRFLP, that have been used to show differences in soil microbial communities of soils from Victoria with and without common scab, providing a “bio-indicator’ of disease potential. These tools have also been used to show shifts in microbial communities in studies of a common scab suppressive soil from Victoria.
- Demonstration of common scab disease suppression in a field soil from Ballarat Victoria.
- Demonstration of efficacy of control of seed-borne common scab by three species of *Pseudomonas* and demonstration of enhanced growth of potato plants inoculated with free-living nitrogen fixing bacteria in the field.
- Demonstrated resilience of communities of beneficial bacteria inoculated onto potato roots when planted in a field soil, a critical aspect of ensuring effective delivery of beneficial organisms into the potato production system.

This work is building a better understanding of the function of the soil microbiological community, which is key to crop productivity, disease suppression and soil health overall. The ultimate aim is to manipulate the soil environment to control disease and improve yields, as well as utilizing selected beneficial microbes in the potato production system.

We have identified a common scab suppressive soil from a potato field near Ballarat in Victoria, and components of the soil microbiological community are the most likely candidates for this suppression. Further research is required to identify the specific microorganisms, or group of organisms that are responsible for disease suppression, with the aim of identifying novel common scab management options. It will also be valuable to analyse grower practice with reference to adjacent properties which do have common scab, which may also provide clues on disease management.

APRP1 identified a number of correlations between soil nutrient concentrations and reduced incidence and severity of powdery and common scab based on surveys and trials done in Victoria, Tasmania, South Australia and Canada and from studies conducted in New Zealand in a model greenhouse system for powdery scab. The key nutrient elements identified were tested in this project with the aim of developing specific recommendations for growers.

Applications of elemental sulphur and zinc EDTA to soil, tested over five seasons, were shown to consistently reduce the incidence of powdery scab, and more so, the severity of scab on the susceptible cultivar Shepody. Applications of iron resulted in small but significant reductions in powdery scab incidence and severity but not in all trials. Although not as effective as the fungicide treatment fluazinam (not registered in Australia), sulphur and zinc can provide alternative options for powdery scab control. These treatments were not detrimental to yield, even though they were efficacious at concentration well above the optimum required for optimum crop nutrition and returned significant yields to the French fry and crisping factories.

Although our trials were done in one soil type only, the relative effects of cultivar, fungicide and nutrient amendments on powdery scab are consistent with results of research in United Kingdom and in New Zealand. This suggests that these treatments are likely to be efficacious in a range of soil types. Based on our research, we can make interim recommendations on the rates of sulphur and zinc required for disease control. These will be relevant to different cultivars with different powdery scab susceptibility ratings, although there will be a threshold rating below which these treatments will not be economically feasible.

It is recommended that these treatments be evaluated on a wide range of soil types at different rates to obtain optimum thresholds that could be incorporated into crop nutrient management program. Such evaluations could be done as part of an extension program on powdery scab management.

Elevated levels of zinc applied in the EDTA form reduced Rhizoctonia black scurf in a trial on a peaty clay loam (Koo Wee Rup), although the more soluble zinc sulphate did not have the same effect. This research needs to be repeated to determine if this result can be obtained consistently.

Manipulating the K:Mg ratio had an effect on common scab disease in only one of five trials in conducted on two soil types in Victoria. In this trial common scab incidence was around 10% lower at a ratio of 0.29 and 10% higher at a ratio of 0.59, from a baseline of 0.35, with the susceptible cultivar Simcoe. In a survey of growers, fields the K:Mg ratios ranged from 0.1 to 2.7. Target ratios were difficult to achieve in our trials and often exceeded the target with single applications of sulphate or muriate of potash just before planting. It may be better to build up this ratio over a number of cropping cycles. This one positive result shows that it is possible to have some impact on common scab disease by manipulating the K:Mg ratio, but no recommendation can be made at this stage without further trials in a range of soils types.

Conditioning soils with pH modifiers is common practice, particularly in acidic soils, but there is evidence that applications of lime that increase pH can increase the risk of both common and powdery scab. Baseline soil pHs were typically in the acidic range of 4.2-5.4. Treatments only marginally shifted soil pH (up to 5.7 and down to 3.0). With the exception of calcium oxide, liming treatments did not increase the risk of common scab on the peaty clay soils of the Koo Wee Rup region. However, for broader recommendations to be made, trials need to be conducted on a range of different soil types. The effects of lime treatments on powdery scab in ferrosols, for example, were not tested in this project. A number of other nutrient amendment treatments, manganese sulphate, ammonium sulphate, zinc sulphate and zinc EDTA had no effect on common scab on the peaty, highly buffered soils of Koo Wee Rup region in which they were tested.

Optimising soil nutrients are part of the soil health equation. Modern potato production involves “prescription’ farming, which includes soil and plant nutrient testing, and more recently pathogen DNA testing, which provide the basis for a number of crop management recommendations. Although the

disease control through nutrient amendments appear to be modest in comparison with fungicides for example, it is important that we understand the complex interactions between nutrients and disease to ensure that recommendations provided to growers are not only designed to optimize conditions for crop growth but also for disease suppression and yield gains.

Improving soil health implies making significant changes to the soil environment over the short or long term. Green manures and organic amendments are seen as a means to revitalize soil microbial activities/populations, provide a reservoir of macro and micronutrients and provide plants with a means to buffer against pathogens. This project demonstrated significant gains in yield from a millet green manure crop in a study conducted in Canada. Green manure cropping can be particularly important in potato production systems that are heavily cropped. New tools have improved our ability to unravel the many interactions and mechanisms in soil from a green manure so that we can ultimately tailor specific green manure crop species and management for optimum disease suppression and crop production.

Research on Rhizoctonia disease formed a minor part of this project. However, the diseases by *R. solani* are widespread through production districts. Our ability to predict this disease is very limited because of a lack of knowledge of the survival and behaviour of the pathogen in the production cycle, particularly at the time of planting and early crop development. Targeted research on this issue would better inform PreDictata Pt disease risk assessments and the decision management trees. Also, although *R. solani* AG3 is considered to be the main cause of stem canker, and particularly black scurf, the role of *R. solani* AG2 in Australian potato cropping systems is still poorly understood.

There is also a significant gap in our knowledge of the common scab pathogen. Results of PreDicta Pt soil tests for *S. scabies* generally indicate very low or 'not detectable populations before planting, although subsequent disease inicidence and severity may be high. Recent research has shown that populations of pathogenic *Streptomyces* increased between three and four weeks after tuber initiation in dry soils, whereas levels were lower in irrigated soils (Thwaites and Stalham 2010). This information has renewed interest in irrigation scheduling as a control option for common scab. It shows, however, that there is still much to be learnt about behaviour of this pathogen in the soil environment and what factors control its population. The techniques used by (Thwaites and Stalham 2010) (pyrosequencing) could be used to study the effects of different environmental factors on *S.* scabies.

This work has highlighted the value of growing resistant varieties. The cultivar Shepody proved to be highly susceptible to powdery scab with 80-99% tubers affected in trials. In contrast, less than 15% of Russet Burbank tubers were affected in the same trials. Our trials also highlighted differences in the susceptibility of a number of crisping varieties to common scab. This is an often overlooked aspect of control of soil-borne diseases. The cost-benefit of growing "resistant' cultivars can be very significant and should feature in any discussion on disease management and future research. The draft disease management decision trees are one way of making this information more accessible to the grower.

Key outputs from this project are the draft disease management decision trees for three main soilborne diseases which are built on the knowledge gained in this and previous research projects. The development of these decision trees has also highlighted gaps in our knowledge of these diseases and their management which could form discussions for future research.

TECHNOLOGY TRANSFER

Technology transfer involved a number of different communication activities, written and oral. Publications included papers in scientific journals, papers presented at scientific conferences (oral or poster presentations), scientific workshops and at industry conferences, as well as articles in various industry and media publications. Technology transfer directly to growers involved seminars/workshops in different districts. These were organized by researchers or industry groups and, more recently, by the AUSVEG Potato Industry Extension Program. Communication within the program peer group included team meetings of the relevant subprojects, either face-to-face or by teleconferencing, twice yearly APRP2 Technical Operations Committee Meetings (TOC), which included relevant industry representatives, and Technical Oversight Group (TOG) meetings specifically for project PT09026 Ai. The various papers, articles, extension activities and peer meetings are listed in the bibliography below.

Direct extension of information to growers included seminars/workshops organized by researchers and project support (SED Consulting) involving Australian and international scientists (Plant & Food Research, New Zealand; A&L Canada) (Victoria, Tasmania). Other seminars were part of industry organized events (e.g. ViCSPA, Simplot, McCain). In the past two years of the project, most extension activities were conducted through the AUSVEG Potato Industry Extension Program.

An evaluation of the effectiveness of the various technology transfer activities is not within the scope of this project. However, it is fair to say that the activities involving growers and industry, such as seminars and workshops in the various districts and media (e.g. Potatoes Australia) served to make growers much more aware of the activities of the APRP2 program, including the breadth and the scope of the work being done in Australia, New Zealand and Canada.

An important output from this project are three draft decision trees for the management of powdery scab, common scab and Rhizoctonia canker and black scurf. These trees are the culmination of past and present research providing growers with background information and a process of working through scenarios to minimise this risk of disease, depending on the market, paddock history, soil DNA and nutrient tests, cultivar, seed health and various chemical and cultural control options. The decision trees were developed with input from the program research team, growers and industry representatives.

The draft disease management decision trees potentially provide a platform for a more comprehensive Technology Transfer program for the processing industry building on information from this and past research programs and projects and could also be integrated with the PreDicta Pt training manual (PT09023).

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Presentations by Ian Porter, Tonya Wiechel, George Lazarovits (A&L Canada) and Greg Patterson (A&L Canada)

Potato Grower's Workshop, Ballarat February 2010

Presentations by Ian Porter, Tonya Wiechel, George Lazarovits (A&L Canada), Greg Patterson (A&L Canada) and Richard Falloon (Plant & Food Research NZ)

Potato grower workshop, Cora Lynn Victoria 1 September 2010

Ian Porter, Tonya Wiechel and Prakash Nair provided updates on APRP2 program to crisping growers from the Koo Wee Rup

ViCSPA Annual General Meeting, Potato Industry Dinner, Warragul, 3 October 2011

Tonya Wiechel. Effects of nutrients on common and powdery scab.

George Lazarovits. Towards ecological agriculture: getting down to the rhizosphere.

Amy Turnbull. Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Tasmanian Potato Industry Seminar, 4 October 2011, Simplot

George Lazarovits. Towards ecological agriculture: getting down to the rhizosphere.

Amy Turnbull. Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Grower Meeting, Donegan's Farm, Gordon VIC, 5th October 2011.

Dolf de Boer. PT09026 Soil health/ disease mitigation program. Introduction.

Richard Falloon. *Spongospora* infection of potato: root infection, effects of soil nutrients; and global population genetics.

Tonya Wiechel. Effects of nutrients on common and powdery scab.

George Lazarovits. Towards ecological agriculture: getting down to the rhizosphere.

Amy Turnbull. Investigating the ecological role of potato rhizosphere and endosphere bacteria.

McCain Agronomy Conference, Ballarat, 25 August 2011

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Seminar presentation to Plant & Food Research staff, Mt Albert Research Centre, NZ, 16 April, 2013.

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Seminar to DEPI and La Trobe University Plant Pathologists and Soil Scientists, AGRIBIO Centre for Agri-biological Research, La Trobe University Bundoora, 23 September 2013

Lazarovits G (A&L Biologicals Canada) "Microbiology is coming to the forefront of agricultural production."

Snackbrands Field Day, Thorpdale, 7 February 2014

Wiechel TJ (2014) Discussions with growers about DNA soil testing and future commercialisation of the *PreDicta Pt* testing service

McCain Field Day, Ballarat, 5 March 2014

Wiechel TJ (2014) Discussions with grower group (*PreDicta Pt*) on DNA test results and future commercialisation and adoption of predictive tests

Simplot R&D meeting, Ulverstone, Tasmania, 16 July 2014

Wiechel TJ (2014) Presentations on disease management with nutrient amendments and the use of molecular test for indicators for soil health. Discussions with Simplot staff on future research in potatoes on 15 July 2014.

George Lazarovits Radio Interview in Tasmania 4th October 2011

AUSVEG Potato Industry Extension Program

Dolf de Boer and Tonya Wiechel – presentations to growers attending the Potato Industry Workshop, Creswick, Victoria 28 June 2012.

Tonya Wiechel, Presentation at the Potato Industry Extension Workshop, Creswick, Victoria June 2013 on the latest results of the Soil health/disease mitigation project

Richard Falloon, collaborating scientist from Plant and Food Research NZ – presented the latest research on powdery scab of potatoes at a Potato R&D Workhop, Pemberton Western Australia, September 2013 as a guest of the AUSVEG Potato Extension Program.

APRP2 Grower Evening - The American Hotel, Creswick Victoria - Presentations to growers by Ben Callaghan HAL, Richard Fallon P&FR NZ and George Lazarovits, A&L Biologicals Canada to local growers, 19 September 2013 (associated with the APRP2 Symposium, 18-19 September, Mantra Hotel, Tullamarine Victoria)

Tonya Wiechel presentation on the effects of soil nutrients of soil-borne diseases of potatoes, Warragul meeting 2012

*APRP2 Science Symposia 1***,** *Rydges Carlton, Melbourne, October 2011*

Arati Agarwal. Novel molecular tests to study potato pathogens – towards improved disease management.

Tonya Wiechel. Studying infection of *Spongospora subterranea* in the 3 year collaborative trial.

Tonya Wiechel. Effects of nutrients on common and powdery scab.

Dolf de Boer. PT09026 Soil health/ disease mitigation program. Introduction.

Richard Falloon. *Spongospora* infection of potato: root infection, effects of soil nutrients; and global population genetics.

George Lazarovits. Towards ecological agriculture: getting down to the rhizosphere.

Amy Turnbull. Investigating the ecological role of potato rhizosphere and endosphere bacteria.

Leigh Sparrow. Survey of the impacts of commercial rotations on potato pathogen levels.

Hannah Thompson. Spraying away common scab.

Luke James & Nigel Crump. Monitoring of bacterial wilt in irrigation water.

APRP2 Science Symposium 2, Mantra Hotel, Tullamarine, 18 & 19 September 2013

Papers presented by Dolf de Boer (overview of PT09026). Tonya Wiechel and Arati Agarwal (DEPI), Richard Falloon (P&FR NZ), George Lazarovits (A&L Biologicals Canada), Leigh Sparrow (TIA), Chris Franco (Flinders University SA), Calum Wilson (TIA), Nigel Crump and Luke James (ViCSPA).

Industry "Outputs" Sessions –Panel discussion including Dolf de Boer, Tonya Wiechel, Richard Falloon, George Lazarovits, Leigh Sparrow, Stacy Smith (Flinders University), Calum Wilson and Nigel Crump.

Lunchtime Seminar – George Lazarovits, A&L Biologicals Canada – "Microbiology is coming to the forefront of agricultural production."

Seminar presented by Dr George Lazarovits at the AGRIBIO Centre for AgriBioscience, La Trobe University, Monday 23 September 2013 – How ecological agriculture will change farming practices.

APRP2 Powdery scab workshop, Mercure Hotel Ballarat, 1 August 2012

Meeting of researchers and key industry/grower representatives to review research on powdery scab of potatoes.(Richard Falloon, Plant and Food NZ, Tonya Wiechel, Dolf de Boer, Arati Agarwal, Calum Wilson, Robert Tegg).

Project Management Meetings

Technical Oversight Group Meetings PT09026

12 September 2013, DEPI Bundoora - Attended by Dolf de Boer, Tonya Wiechel, Arati Agarwal, Ben Callaghan, Kevin Clayton-Greene and Graham Henman

14 February 2013, HAL Melbourne Office Meeting Room – Attended by Dolf de Boer, Tonya Wiechel, Kevin Clayton Greene, Graeme Henman, David Moore and Ben Callaghan.

Technical Operations Committee Meetings

Face-to-face meetings

17 February 2010, Holiday Inn, Melbourne airport

28 July 2010, DPI, Attwood, Vic

3 May 2011, DPI Attwood , 475 Mickleham Rd, Attwood VIC

7 October 2011, Rydges Carlton (plus symposium)

13 April 2012, Holiday Inn, Melbourne Airport

10 October 2012, Best Western Airport Motel and Convention Centre

19 April 2013, Holiday Inn, Melbourne Airport

18/19 September 2013 (plus symposium), Mantra, Tullamarine

Phone meetings

1 June 2010

16 Feb 2011

4 November 2011

6 March 2012

28 February 2014

26 September 2014 (TOC wrap-up webinar)

Team meetings

George Lazarovits and Greg Patterson of A&L Canada visited Australia 14-23 Feb 2010. They took part in project team meetings to set project work plans and presented at grower workshops in Warragul and Ballarat.

Dolf de Boer, Tonya Wiechel, Arati Agarwal, Desmond Auer, Richard Falloon, Leigh Sparrow meeting at DEPI Bundoora (DEPI Bundoora 18 April 2013) – discussions on the Ai and Aii components of the research program

George Lazarovits was guest of DEPI, Bundoora, 17-23 September 2013. Team meetings held to discuss research data from Canada and Australia.

Confidential Client Reports

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I. (2011) Amounts of iron in plant growth medium have little effect on *Spongospora subterranea* infection in potato. *The New Zealand Institute for Plant & Food Research Ltd Report,* SPTS No. 5021: 18 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2012) The sulphate form of sulphur in plant growth medium has little effect on *Spongospora subterranea* infection in potato. *Plant & Food Research Ltd Report,* SPTS No. 6542: 14 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2013) Potassium or silicon in plant growth medium have little effect on *Spongospora subterranea* infection of potato roots: two greenhouse experiments. *Plant & Food Research Client Report* No. 52561: 18 p.

Falloon RE, Curtin D, Butler RC, Shah FA, Khan I (2014) Treatments of silicon and different irrigation regimes affect growth of potato plants in association with *Spongospora subterranea* infection. *Plant & Food Research Client Report No. 9642*

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

A number of the recommendations below are strongly focused on research of the soil microbial environment, an area of research that has been significantly boosted by the development of DNA-based tools. These recommended research areas are interrelated, complementary and share research tools and strategies.

- Further development of the draft disease management decision trees, which are in draft form, to serve as platforms on which to build more comprehensive technology transfer programs for the outputs of this and previous research.
- Integrate the powdery scab decision tree with the PreDicta Pt training module for agronomists (Project PT09023).
- Further research into the mechanisms of common scab disease suppression found in Victoria with the aim of developing new management strategies based on manipulating and inducing disease suppression.
- Development of a 'soil bio-health indicator' to predict disease risk by common scab and for other pathogens. Common scab has proved intractable for incorporation into the PreDicta Pt risk model to date.
- Further evaluation of green manure cropping in Australian production systems.
- Continued exploration of beneficial microorganisms closely associated with the potato plant (rhizosphere and endophere) and their utilization for disease suppression and improved growth and productivity.
- Continued research effort into Rhizoctonia disease, a widespread problem in potato production, and into common scab disease. Both have proved to be intractable for incorporation into the PreDicta Pt risk model to date. Better knowledge of the survival and behaviour of both *R. solani* and *S. scabies* in the rotation and in the cropping cycle would enable better prediction of disease risk.
- Evaluation of sulphur and zinc applications for powdery scab control on a wider range of soil types to refine interim recommendations for growers, as part of a disease management extension program.
- Further studies on the effects of zinc on Rhizoctonia disease to determine whether these results are consistent and treatments economical.

- Further evaluation of the K:Mg ratio for common scab control on a wider range of soil types with a focus on a longer term approach of a number of treatments effecting a gradual change over an number of cropping cycles as part of a disease management extension program.
- A renewed look at the benefits of using disease resistant cultivars as a disease management tool and using new tools to screen potato genomes for resistance to common and powdery scab, both of which have heritable resistance traits.

ACKNOWLEDGEMENTS

We gratefully acknowledge the participation of potato growers Ken Labbett, George Lineham, Frank Rovers, Wayne Tymensen and Tony Cummaudo, without whom the field trials will not have been possible. They willingly provided land, facilities, machinery, crop husbandry and their time, which ensured the success of the trials.

We also thank Daniel Grayling (McCain Food Australia) and Luke James and Joe Smith (ViCSPA) for organizing sampling of growers fields for crop surveys, and we thank all of the growers who participated in this survey.

We thank Khagoswori Giri (Biometrician, DEPI) for his advice and assistance in conducting the REML analysis of the data from the powdery scab nutrient amendment field trials.

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Horticulture Australia

PT09026 AII – (30/10/2015)

Survey the Impacts of Commercial Rotations on Pathogen Inoculum Levels

Final Report

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Tasmanian Institute of Agriculture

PROJECT SUMMARY

PT09026 AII – Survey the Impacts of Commercial Rotations on Pathogen Inoculum Levels

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2 November 2015

FUNDING SUPPORTERS

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Government of South Australia

DISCLAIMER

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MEDIA SUMMARY

One important reason why farmers use crop rotations is to minimize the build-up of crop diseases. Powdery scab, common scab, black scurf and stem canker are soil-borne diseases of concern to Australian potato growers. We monitored the concentrations of the pathogens that cause these diseases by analyzing the topsoil from 28 farm paddocks in Tasmania and 15 in South Australia each year from 2005 to 2013. DNA fingerprinting tests specific to each organism were used. We assessed how these DNA concentrations changed in relation to the crops that were grown in the monitored paddocks, to see if we could identify crops that tended either to increase or to decrease the amount of disease-causing organisms in the soil. Our monitoring showed that all of the organisms assessed were present in at least some paddocks. None of the crops grown in the paddocks consistently caused concentrations of disease organisms to fall. The organism that causes powdery scab was present in every paddock in almost every year, and its concentration greatly increased each time potatoes were grown. Of equal concern was the fact that between one potato crop and the next the concentration of the powdery scab organism did not fall enough for the risk of this disease to change. All of the assessed paddocks remained at high risk. If potatoes continue to be grown in these paddocks, even with 5 years between each crop, growers will probably continue to face a high risk of powdery scab. They will need to choose less risky paddocks if available, or choose tolerant cultivars. Further, they should also keep their soils from becoming too wet at the time of tuber initiation, as damp conditions are known to favour this disease.

TECHNICAL SUMMARY

Concentrations in surface soil (0-150 mm) of the DNA of the soil-borne potato pathogens *Rhizoctonia solani*, *Spongospora subterranea* and *Streptomyces scabies* were monitored annually for 8 years in commercial potato paddocks in South Australia and Tasmania, using real-time PCR assays. A non-linear model was fitted to these data to test if changes in DNA concentrations could be explained by changes in land use in the paddocks. *Spongospora subterranea* and *R. solani* anastomosis group (AG) 2.1 were the most prevalent pathogens monitored in both states, followed by *St. scabies* and *R. solani* AG3, and *R. solani* AG4 in Tasmania. *Rhizoctonia solani* AG2.2 was found at very few sites, as were *R. solani* AG8 in Tasmania and AG4 in South Australia. At a number of paddocks, the DNA of *R. solani* AG3, *St. scabies* and *Sp. subterranea* was only found after the first potato crop in that paddock, strongly suggesting that the pathogen had been introduced on the potato seed tubers used to plant those paddocks. From the model, half-lives for pathogen DNA concentrations were calculated and these ranged from 0.22 years for *St. scabies* DNA in Tasmania to 0.83 years for *Sp. subterranea* DNA in Tasmania. The fitted model also identified a number of crops which were significantly ($P<0.05$) associated with subsequent increases in

pathogen DNA: poppy and potato were associated with increased *R. solani* AG2.1 DNA concentrations, carrot, fallow, poppy and ryegrass with increased *R. solani* AG3 DNA concentrations, and potato was associated with increased concentrations of the DNA of *Sp. subterranea* and *St. scabies*. Of all these increases, the effect of potato on concentrations of the DNA of *Sp. subterranea* was the greatest, with average increases of 3300 (South Australia) to 7300 (Tasmania) pg DNA/g soil, more than 10 times greater than the other increases. Given (1) the already high concentrations of *Sp. subterranea* DNA in many of the soils, (2) the large increase in *Sp. subterranea* DNA concentration caused by subsequent potato crops, (3) the estimated *Sp. subterranea* DNA half-life of about 1 year, and (4) the apparent low soil DNA threshold concentration for powdery scab risk (about 20 pg/g, established elsewhere), soils in which potato is grown once in every 5 years are unlikely to return to a low-risk category. In the absence of reliable relationships between concentrations of DNA of the other pathogens monitored and the risks of the diseases they cause, it is not possible to make similar judgements for the other diseases. Potato growers will need to take all necessary steps to minimize the risk of powdery scab, e.g. choose less risky paddocks, grow tolerant cultivars, and where possible manage soil moisture to avoid wet soil at tuber initiation, because damp soils are likely to favour development of this disease.

INTRODUCTION

Crop rotation, the growing of different crops in repeated sequence in the same field, has been practiced for centuries. One of the many potential benefits of this practice is the alleviation of soil-borne plant diseases, by reducing the effects of the pathogens that cause them (Karlen, et al., 1994). Two conditions need to be satisfied for disease control to be achieved: the different crops in rotation should not all be hosts for the pathogens of concern, and the pathogens should not survive for long periods without living hosts (Karlen, et al., 1994). In areas where potato (*Solanum tuberosum* L.) is grown, the soil-borne potato pathogens *Rhizoctonia solani*, the cause of black scurf and stem and stolon canker, *Streptomyces scabies*, the cause of common scab, and *Spongospora subterranea*, the cause of powdery scab, are widespread (Wale, et al., 2008), (Larkin, et al., 2011), (Sparrow & Wilson, 2012). *Rhizoctonia solani* is a complex of 13 anastomosis groups (AGs), of which AG3 is considered the main cause of disease in potato (Carling & Leiner, 1990), but AG2.1 and AG4 can also cause disease (Woodhall, et al., 2007), (Woodhall, et al., 2012) (Das, et al., 2014) while AG8 has been shown to infect potato roots but not stems (Hide & Firmager, 1990).

A number of studies have shown that, compared to longer rotations, rotations with fewer years between successive potato crops in the same field increased the incidence of one or more of these soil-borne potato diseases (Hide & Read, 1991), (Gilligan, et al., 1996), (Carter, et al., 2009), (Larkin, et al., 2010), (Larkin, et al., 2011), (Sparrow, in press). This is probably because short rotations provide more chances for the pathogens to thrive on the potato host plants and less time for inoculum to decrease between successive potato crops. However, Scholte (1992) found that while longer rotations reduced the incidence of *Rhizoctonia* black scurf and stem canker, they did not reduce the incidence of common scab.

A number of crop and weed species have been identified as alternate hosts of the above potato pathogens (Carling, et al., 1986), (Sturz, et al., 1995), (de Klerk, et al., 1997), (Qu & Christ, 2006), (Shah, et al., 2010). There is only limited evidence, however, that specific crops apart from potato directly influence amounts of potato pathogen inoculum in soils or disease severity in subsequent potato crops. Johnston et al. (1994) found that, compared to clover or wheat, growing ryegrass before potato decreased the number of *R. solani* sclerotia in the potato soil, but there was no corresponding decrease in the recovery of the pathogen from the stems and roots of plants in the subsequent potato crop. Tuber disease was not measured. In Maine, USA, Larkin and Honeycutt (2006) compared the severity of soil-borne disease of potato grown in

eight different 3-year rotations. Rotations with either sweet corn, canola or barley immediately before potato gave better control of black scurf and stem and stolon canker than rotations with either red clover or soybean as the preceding crops. No powdery scab and little common scab were observed in their study. While there is little evidence that specific cash crops have influenced subsequent soil-borne diseases in potato, cover crops (also known as green manures), some with known biofumigant properties, have been shown to reduce disease (Larkin & Griffin, 2007), (Larkin, et al., 2011).

DNA probes for a number of soil-borne potato pathogens, including *Spongospora subterranea*, *Streptomyces scabies* and *Rhizoctonia solani*, have been developed and evaluated as key components of Phases 1 and 2 of the Australian Potato Research Program (APRP) (Ophel Keller, et al., 2009); (Ophel Keller, 2014). While the primary application of the probes has been for disease diagnosis and prediction of disease risk, they are also potentially useful tools for monitoring changes in pathogen populations from season to season. In Phase 1 of APRP, 4 years of annual monitoring of 28 commercial paddocks in Tasmania and 15 in South Australia were reported (Sparrow, 2009). Records of land use at each of the monitoring sites were kept, and changes in pathogen DNA concentrations were assessed in relation to changes in land use at the sites. The aim was to see if any land uses were associated with increases or decreases in pathogen inoculum amounts in the soils, information which might justify the conduct of controlled experiments to confirm such observations and identify phases in potato farming systems which promote or retard the build-up of inoculum. In a survey of Tasmanian potato farming systems (Sparrow, 2009) 30% of growers reported growing three or more other crops in their rotations, with poppy (*Papaver somniferum*, 60% of growers) and cereals (40%) the most popular other crops, and green bean, onion, carrot, pea and brassica vegetables each grown by more than 10% of those surveyed. The potential of such crops to influence potato disease inoculum in either positive or negative ways needs to be examined.

The data for the first 4 years of monitoring reported in Sparrow (2009) showed, not surprisingly, that growing potato had the biggest effect on pathogen DNA concentrations in the soils, particularly for DNA of *Sp. subterranea* and *R. solani* AG3. However, there was no strong evidence that other land uses affected DNA concentrations, a finding which may have been at least partly because most of the land uses were replicated across only a few sites. The monitoring was continued for a further 5 years, in APRP2. This chapter reports on the full 8 years of monitoring, comprising nine annual measurements from 2005 to 2013. To the author's knowledge, no other data of this type and duration has been published.

MATERIALS AND METHODS

Twenty eight farm paddocks in north west Tasmania and 15 in south east South Australia were monitored (Figure 5.A.II-1). The sites in South Australia were all on sandy soils. In Tasmania, five sites were on sandy soils in the midlands or Fingal Valley, while the remaining sites were on clay loam Ferrosols in the state's north. All sites were originally selected as part of a 2005 survey of soil health in APRP1 (Crump, 2009), and all were planted with potato in that year. In the soil health survey, a 20 m row in each of the commercial potato crops was marked, and composite samples of forty soil cores (0-150 mm) were taken from each 20 m strip, for pathogen DNA determination (Ophel Keller, et al., 2008) and chemical analysis (Crump, 2009). The location of each sample strip was fixed in 2005 either by global positioning system or relative to pegs on boundary fences, so that it could reliably be relocated. Each year from 2006 to 2013, in July-August in Tasmania and September-October in South Australia, and another 40-core soil sample was taken from the same 20 m strip at each site. Land use at each site at the time of soil sampling was noted and these records were subsequently verified by land owners. Land use is defined as the main cash crop planted each year. Pasture and other perennial fodder crops including pure ryegrass swards and lucerne were also considered

as cash crops, but mixed pasture sites were not further classified on the basis of botanical composition. Double cropping is rare in the farming systems we studied. Cover crops are sometimes grown between cash crops (Sparrow, in press), but information on these crops was not collected.

The collected soil samples were dried at 40°C, ground <2 mm, and analysed for pathogen DNA by SARDI using the probes developed as part of APRP1 (Ophel Keller, et al., 2008). While it is recognised that the DNA extracted from soil may not all be viable (Hirsch, et al., 2010), it is still a strong indicator of functioning organisms. In contrast to DNA, RNA is produced only by active cells but it is short-lived (Hirsch, et al., 2010) and therefore not suitable for a commercial diagnostic test. Amounts of pathogen DNA in the samples were expressed as picograms of DNA per gram of air-dry soil. The test for DNA of *S. scabies* targets a gene (the TxtA gene) involved in the production of thaxtomin A, the main compound synthesised by pathogenic *Streptomyces* which attacks the surface of potato tubers and results in common scab (Loria, et al., 1997). Because this gene is present in some species of *Streptomyces* other than *S. scabies*, results for the DNA test are expressed as pg TxtA gene /g soil. Data were $\log_{10}(x+1)$- or $\log_e(x+1)$-transformed prior to conducting ANOVA and regression analyses to compare annual changes in pathogen DNA in each region. To relate these changes to changes in land use, a nonlinear model was fitted to the raw DNA data, using the following formula:

$$Y_t = B_{\text{site}} + B_{\text{crop}_{t-1}} + Y_{t-1} \times \exp(-A_N);$$

A_N is a parameter describing the decline in the concentration of DNA of the pathogen from the previous occasion (Y_{t-1}) to the current occasion (Y_t). Increasing the value of A_N increases the rate of decline.

$B_{\text{crop}_{t-1}}$ represents the outcome induced by the crop grown in the previous period , which can be a positive or negative result. There is a different $B_{\text{crop}_{t-1}}$ parameter for each type of crop. $B_{\text{crop}_{t-1}}$ can be interpreted as the change in DNA concentration that results from growing that crop on the previous occasion.

The crop is one of: carrot, fallow, lucerne, lupin, onion, poppy, potato, pyrethrum, ryegrass, trees, brassica (cauliflower or canola), cereal (wheat or barley, oat, or triticale), legume (green pea, green bean or broad bean), or pasture. The three crop groups (cereal, legume, brassica) were formed to increase replication within each group. Where possible, an additive random effect per site (B_{site}) was also fitted. The model was fitted using PROC NLMIXED in SAS version 9.3 and the South Australian and Tasmanian data were analysed separately. Summaries of fitted parameters including standard error, *P*-value and 95% confidence intervals were produced, and where model convergence was obtained, plots of the fitted models were also produced. Half-lives were calculated as $T = \log(2)/A_N$ to describe the overall decline in the pathogen in the absence of any influence of particular crops. Half-lives are in years and are estimates of the time needed for the absolute concentration of DNA (not the log-transformed concentration) to halve. Data were not available for all crops for all pathogens.

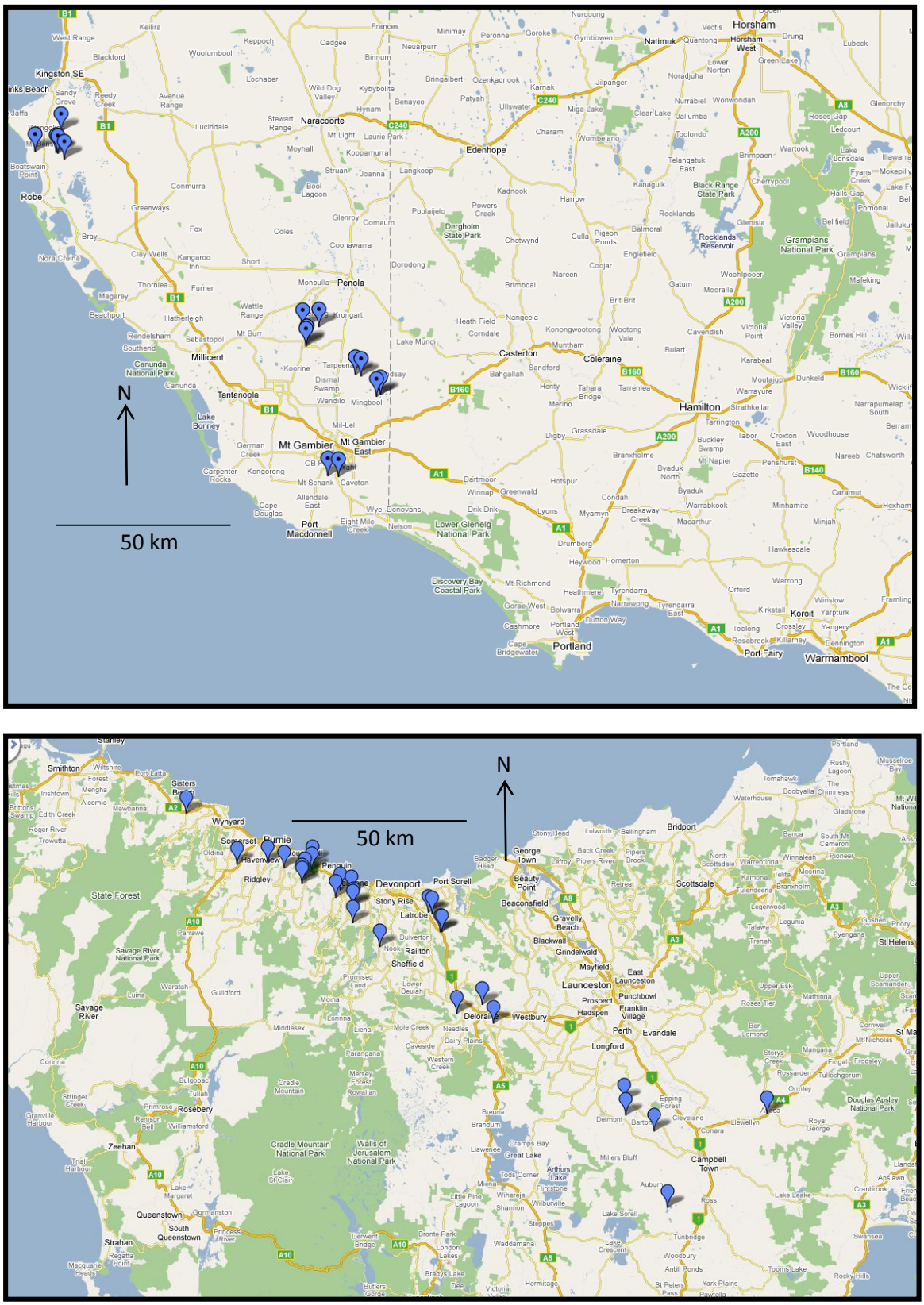

Figure 5.A.II-1. Location of sites in South Australia (top) and Tasmania (bottom), where potato fields were sampled for analyses of soil DNA and crop sequences were monitored each year from 2005 to 2013.

RESULTS

LAND USE AT THE SITES

In Tasmania, all sites grew at least one potato crop (planted in 2005), and half of the 28 sites were planted with a second potato crop later in the study (Table 5.A.II-1). Fourteen other land uses were recorded on at least one occasion, with poppy, pasture, carrot, ryegrass and cereals (mainly wheat and barley) being the most popular crops. The perennial crops pyrethrum and lucerne were grown at only a few sites.

In South Australia (Table 5.A.II-2), all of the 15 sites were planted with potato in 2005, six were planted with a second potato crop during the monitoring period and one was planted three times in potato. Pasture was by far the dominant land use apart from potato. Other crops grown once at some sites included lucerne, lupin, canola, and broad bean. One site was converted to a *Eucalyptus* plantation in 2009.

PATHOGEN DNA IN SOIL - PREVALENCE AND AVERAGE ANNUAL CONCENTRATIONS

Spongospora subterranea and *R. solani* AG2.1 were the most prevalent pathogens in both states (Table 5.A.II-3 and Table 5.A.II-4), with their DNA being detected in soil samples taken in almost every year at every site in Tasmania. *Spongospora subterranea* was similarly commonly detected in SA, as was *R. solani* AG2.1 until 2009, after which its prevalence decreased to about two-thirds of sites (Table 5.A.II-4). In contrast, *R. solani* AG2.2 was found at relatively few sites in either state, as was *R. solani* AG8 in Tasmania and *R. solani* AG4 in SA (Table 5.A.II-3 and Table 5.A.II-4). *St. scabies* and *R. solani* AG3 were intermediate in prevalence in both states, as was *R. solani* AG4 in Tasmania (Table 5.A.II-3 and Table 5.A.II-4). Averages for *R. solani* AG8 in SA are not presented because DNA of this pathogen was only measured in 4 of the 9 years (see Appendix 1).

The consistency with which DNA of the less commonly occurring pathogens was found at any one site varied with the pathogen and state. For example, DNA of *St. scabies* was found at least once at 13/15 sites in SA, but only 12/28 sites in Tasmania. *St. scabies* DNA was found every year at one SA and two Tasmanian sites (see Appendix 1 for full data). DNA of *R. solani* AG3 was found at least once at all but one site in both SA and Tasmania, but this DNA was found each year at only three sites, all in Tasmania. In SA, the DNA of *R. solani* AG4 was only ever found at two sites, but it was found at least once at 18 Tasmanian sites although never in every year at any site. At a total of 21 sites across both states, the DNA of these less prevalent pathogens was found only once in the 9 years of measurement, but there was no consistency about such observations between pathogens, sites or years.

In both states, average concentrations of DNA of *Sp. subterranea* and *R. solani* AG3 increased from 2005 to 2006 but decreased after this time (Table 5.A.II-3 and Table 5.A.II-4). However, the average concentration of *Sp. subterranea* DNA in 2013 was still greater than the starting concentration in both states. Average concentrations of DNA of *R. solani* AG3 followed a similar pattern, but the maximum average concentration in both states occurred in 2007 and not 2006 as was the case for *Sp. subterranea* (Table 5.A.II-3 and Table 5.A.II-4). The greatest concentration of *R. solani* AG3 DNA coincided with the greatest prevalence of this pathogen in both states (22/28 sites in Tasmania and 13/15 sites in SA). There was no statistically significant difference in the annual average concentration of DNA of *R. solani* AG2.2 or AG4 in either state (Table 5.A.II-3 and Table 5.A.II-4), nor of *R. solani* AG2.1 or *St. scabies* in Tasmania (Table 5.A.II-3).

However, in SA (Table 5.A.II-4) statistically significant differences in the concentration of DNA of *R. solani* AG2.1 and *St. scabies* were observed, with greatest concentrations of *St. scabies* DNA in 2006 and 2012, and for *R. solani* AG2.1 in 2007. The 2006 peak for *St. scabies* DNA in SA was preceded by the 2005 sampling when no DNA of this pathogen was detected (Table 5.A.II-4).

Table 5.A.II-1 – Crops and pasture grown at the Tasmanian sites surveyed for potato pathogens each year from 2005 to 2013

Crop	Number of sites where each crop was grown	Total site-years per crop
Potato	28	42
Poppy	18	28
Pasture	16	56
Carrot	8	10
Ryegrass	8	16
Wheat	7	10
Pea	7	9
Onion	6	7
Pyrethrum	6	17
Barley	5	7
Oat	4	4
Fallow	4	4
Lucerne	2	12
Cereal	1	1
Triticale	1	1

Table 5.A.II-2 – Crops and pasture grown at the South Australian sites surveyed for potato pathogens each year from 2005 to 2013

Crop	Number of sites where each crop was grown	Total site-years per crop
Pasture	15	78
Potato	15	23
Lucerne	3	8
Fallow	2	3
Broad bean	2	2
Canola	1	1
Tree plantation	1	4
Lupin	1	1

Table 5.A.II-3 – Average pathogen DNA concentrations (pg/g soil, log_{10} transformed) (and number of sites where pathogens were detected in each year of sampling) in Tasmania

Year	*Sp. subterranea*	*St. scabies*	*R. solani AG2.1*	*R. solani AG2.2*	*R. solani AG3*	*R. solani AG4*	*R. solani AG8*
2005	1.78 (27)	0.30 (3)	1.73 (24)	0.09 (3)	0.43 (7)	0.43 (5)	0.13 (3)
2006	3.39 (28)	0.39 (5)	1.82 (27)	0.08 (3)	0.82 (17)	0.73 (7)	0.03 (1)
2007	3.02 (28)	0.39 (7)	1.69 (25)	0.10 (2)	1.34 (22)	0.85 (9)	0.04 (2)
2008	3.10 (27)	0.38 (6)	1.38 (26)	0.08 (1)	1.04 (17)	0.78 (7)	0.32 (7)
2009	3.04 (28)	0.48 (6)	1.29 (27)	0.08 (2)	0.99 (21)	0.25 (3)	0.09 (4)
2010	3.05 (28)	0.37 (7)	1.25 (28)	0.07 (2)	0.58 (16)	0.43 (6)	0.02 (2)
2011	3.07 (28)	0.28 (5)	1.29 (26)	0.10 (2)	0.39 (12)	0.56 (7)	ND
2012	3.02 (28)	0.41 (8)	1.66 (27)	0.07 (1)	0.47 (14)	0.36 (6)	0.04 (1)
2013	2.91 (28)	0.31 (5)	1.50 (28)	0.08 (1)	0.38 (13)	0.38 (8)	0.08 (2)
$LSD_{0.05}$	0.24	NS	NS	NS	0.40	NS	0.16

NS = not significant, P>0.05; ND = not determined in that year at any site; only 27 sites were sampled in 2008 and 2009, otherwise all sites were sampled.

Table 5.A.II-4 – Average pathogen DNA concentrations (pg/g soil, log_{10} transformed) (and number of sites where pathogens were detected in each year of sampling) in South Australia

Year	*Sp. subterranea*	*St. scabies*	*R. solani AG2.1*	*R. solani AG2.2*	*R. solani AG3*	*R. solani AG4*
2005	1.60 (12)	0.00 (0)	1.23 (15)	0.57 (4)	0.53 (3)	0.00 (0)
2006	3.08 (15)	1.18 (8)	1.28 (15)	0.45 (3)	1.22 (12)	0.12 (1)
2007	2.59 (14)	0.51 (8)	1.78 (14)	0.28 (3)	1.44 (13)	0.00 (0)
2008	2.58 (15)	0.54 (3)	1.66 (11)	0.09 (1)	1.00 (11)	ND
2009	2.44 (15)	0.44 (3)	1.31 (14)	0.35 (3)	0.37 (5)	ND
2010	2.06 (14)	0.48 (5)	0.55 (10)	ND	0.29 (2)	ND
2011	2.44 (14)	0.87 (6)	1.00 (10)	0.31 (3)	0.52 (5)	0.16 (2)
2012	2.40 (13)	1.16 (7)	1.01 (9)	0.29 (4)	0.65 (5)	0.00 (0)
2013	2.31 (13)	0.63 (4)	0.79 (10)	0.24 (3)	0.34 (3)	0.00 (0)
LSD $_{0.05}$	0.44	0.37	0.38	NS	0.32	NS

NS = not significant, $P>0.05$; ND = not determined in that year at any site; only 14 sites were sampled in 2007, 2011, 2012 and 2013, otherwise all sites were sampled.

EFFECTS OF PRIOR CROP ON PATHOGEN DNA CONCENTRATIONS

The fitted model identified those crops which were associated with subsequent statistically significant changes in the concentration of the DNA of certain pathogens in soil in either one or both states (Table 5.A.II-5). The estimates shown in Table 5.A.II-5 are the average change in DNA concentration (pg/g soil) immediately following the growing of the particular preceding crops. These correspond to the $B_{\mathrm{crop}_{t-1}}$ term in the model. All of these estimates were positive, i.e. the concentration of DNA increased as a result of growing the crop. For *R. solani* AG2.1, in Tasmania only, poppy exerted the greatest influence, increasing DNA concentrations in the following soil sampling by an average of 418 pg/g, compared to 203 pg/g following potato. Fallow was the most influential land use on *R. solani* AG3 DNA concentrations in Tasmania, and the only land use affecting this pathogen in South Australia (Table 5.A.II-5), while ryegrass, poppy and carrot were also significant influences on *R. solani* AG3 DNA in Tasmania. The estimates for fallow sites, however, were generated from data from only four sites in Tasmania and two in South Australia (Table 5.A.II-1 and Table 5.A.II-2), and must therefore be viewed with caution.

The most significant ($P<0.0001$) prior crop influence in either state was potato on *Sp. subterranea* DNA (Table 5.A.II-5), with average increases of over 7000 pg/g soil in Tasmania and almost 4000 pg/g in South Australia. These contrasted with the marginally significant effects of potato (40 pg/g, $P=0.02$) and pyrethrum (50 pg/g, $P=0.05$) on subsequent concentrations of DNA of *St. scabies* in Tasmania (Table 5.A.II-5). There were no statistically significant effects of prior crops on concentrations of DNA of *R. solani* AG2.1 in South Australia, of *R. solani* AG2.2, AG4 and AG8 in either state, nor of *St. scabies* in South Australia (data not shown). In many of these cases, this was probably because of insufficient data either because the pathogen DNA was not measured or was not detected in the soil samples. Plots of the fitted

models and observed data for those pathogens listed in Table 5.A.II-5 are shown in Figure 5.A.II-2 to Figure 5.A.II-7.

Half lives (log(2)/A_N, in years) for pathogen DNA concentrations and their 95% confidence intervals were calculated for those pathogens with values of A_N which were statistically significant (P<0.05). These are shown in Table 5.A.II-6. Estimates of half-life ranged from 0.22 years for *St. scabies* in Tasmania to 0.83 years for *Sp. subterranea* in Tasmania.

Table 5.A.II-5 – Statistically significant (P<0.05) estimates and associated standard errors and t-values of B_{crop}, the parameter which describes the average increase in DNA concentration immediately following the preceding crop in the non-linear exponential decline model fitted to data from analyses of soil samples taken from Tasmania or South Australia from 2005 to 2013 (*non-significant parameter estimates have been omitted)*

Preceding crop	B_{crop} estimate	Standard error	DF	t-value	P value
		***R. solani* AG2.1 in Tasmania**			
Poppy	418	94.6	27	4.42	0.0001
Potato	203	77.4	27	2.62	0.0142
		***R. solani* AG3 in Tasmania**			
Carrot	212	99.7	27	2.13	0.0428
Fallow	626	157.5	27	3.98	0.0005
Poppy	123	59.6	27	2.07	0.0484
Ryegrass	238	78.7	27	3.02	0.0055
		***R. solani* AG3 in South Australia**			
Fallow	431	110.7	14	3.89	0.0016
		***Sp. subterranea* in Tasmania**			
Potato	7310	450.7	27	16.22	<0.0001
		***Sp. subterranea* in South Australia**			
Potato	3332	668.9	14	4.98	0.0002
		***St. scabies* in Tasmania**			
Potato	40	16.6	27	2.39	0.0244
Pyrethrum	50	23.7	27	2.09	0.0458

Table 5.A.II-6 – Calculated half-lives for DNA of different potato pathogens in soil, from analyses of soil samples taken from Tasmania or South Australia from 2005 to 2013

State	Pathogen	Half-life (years)	Lower 95% CI	Upper 95% CI
Tasmania	*R. solani* AG2.1	0.47	0.31	1.02
Tasmania	*R. solani* AG2.2	0.36	0.23	0.84
Tasmania	*R. solani* AG3	0.39	0.28	0.70
South Australia	*R. solani* AG3	0.68	0.42	1.67
Tasmania	*R. solani* AG4	0.51	0.37	0.83
Tasmania	*Sp. subterranea*	0.83	0.57	1.55
South Australia	*Sp. subterranea*	0.62	0.38	1.65
Tasmania	*St. scabies*	0.22	0.16	0.35
South Australia	*St. scabies*	0.35	0.18	9.29

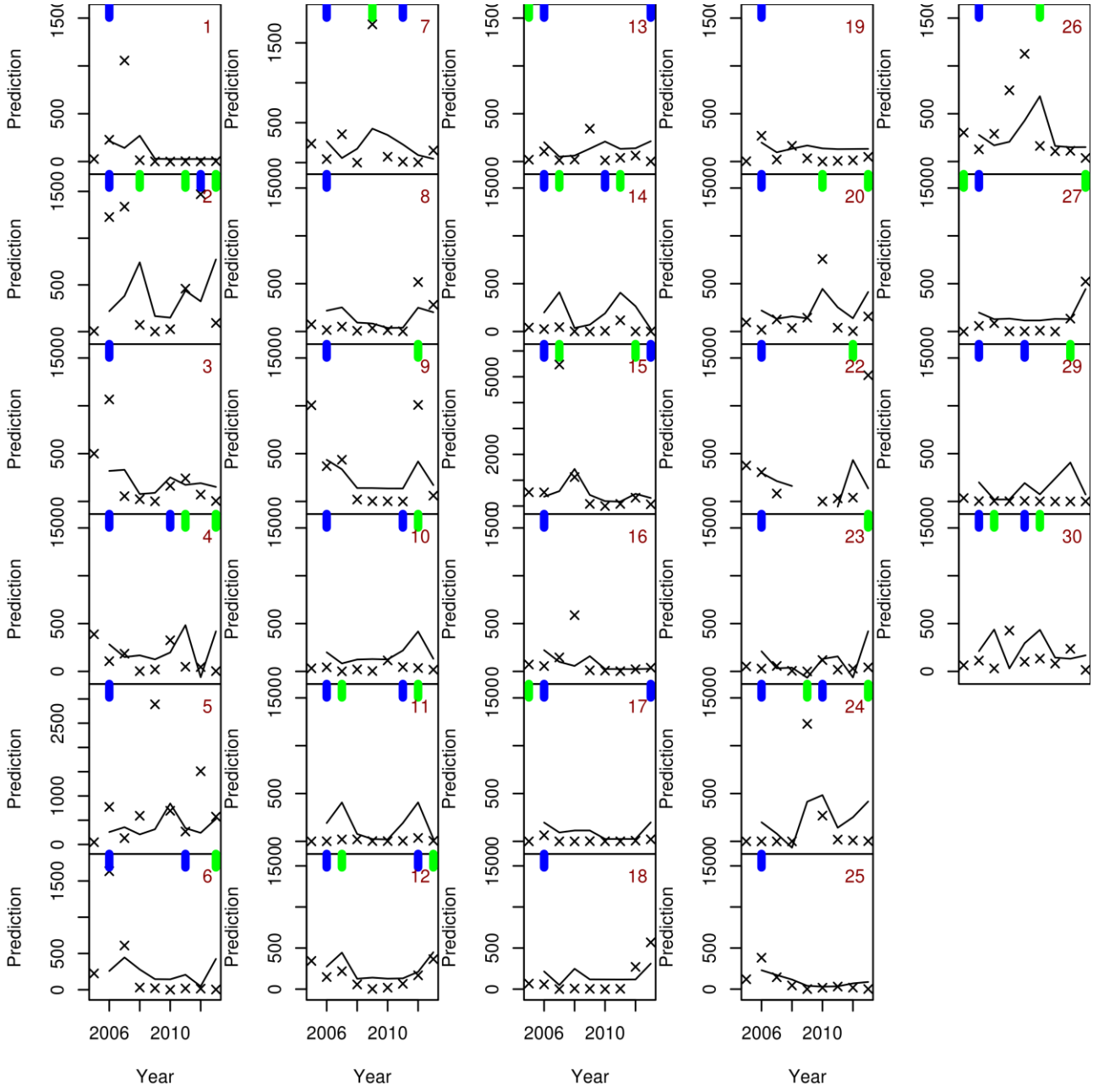

Figure 5.A.II-2 – Predicted concentration of *R. solani* AG2.1 DNA in 28 different Tasmanian soils sampled each year from 2005 to 2013. Tickmarks indicate preceding crops; blue = potato, green = poppy. Predicted responses are shown as solid lines and observed data as crosses. Site numbers are in the top right corner of each box.

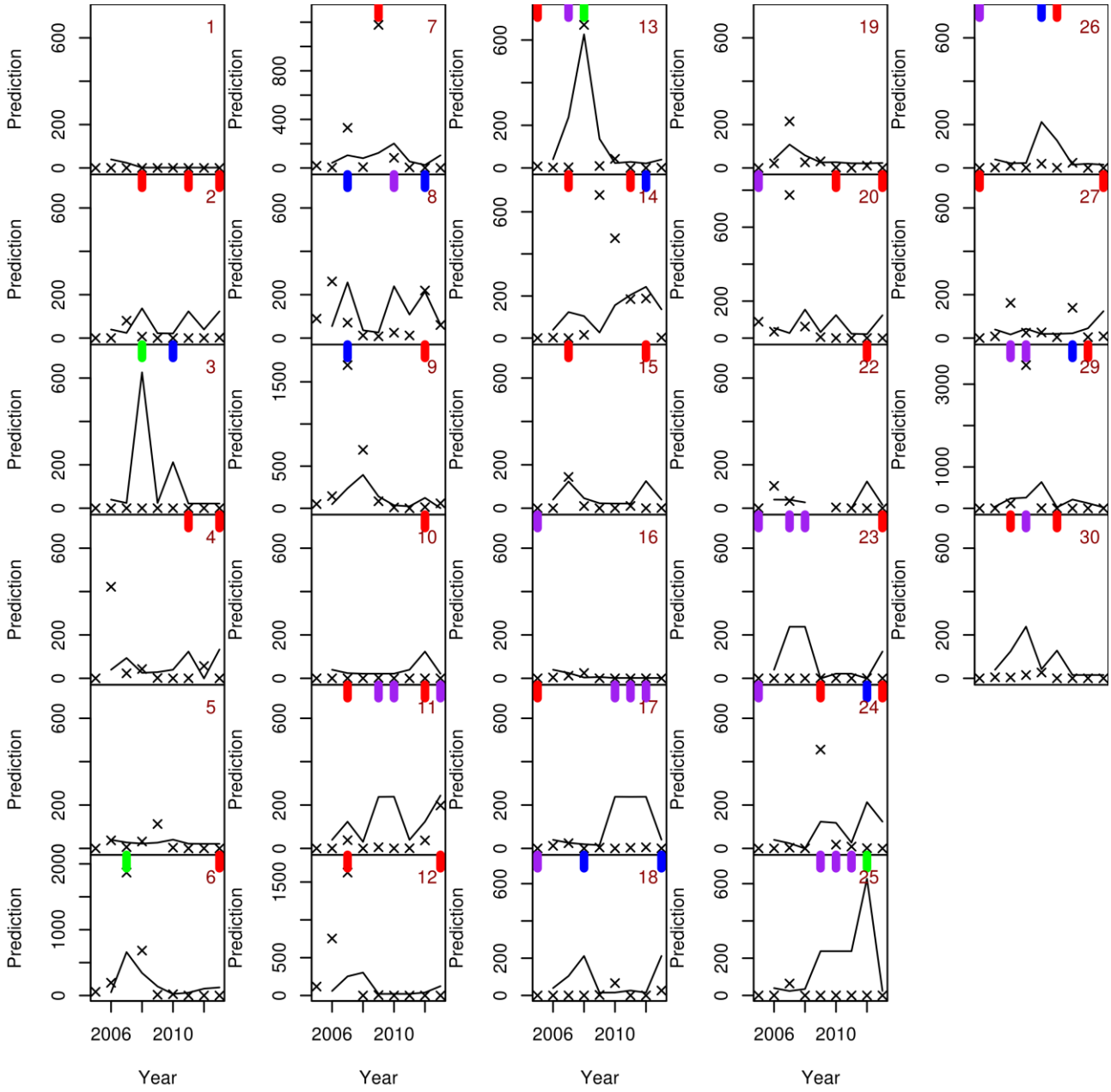

Figure 5.A.II-3 – Predicted concentration of *R. solani* AG3 DNA in 28 different Tasmanian soils, sampled each year from 2005 to 2013. Tickmarks indicate preceding crops; blue = carrot, green = fallow, red = poppy, purple = ryegrass. Predicted responses are shown as solid lines and observed data as crosses. Site numbers are in the top right corner of each box.

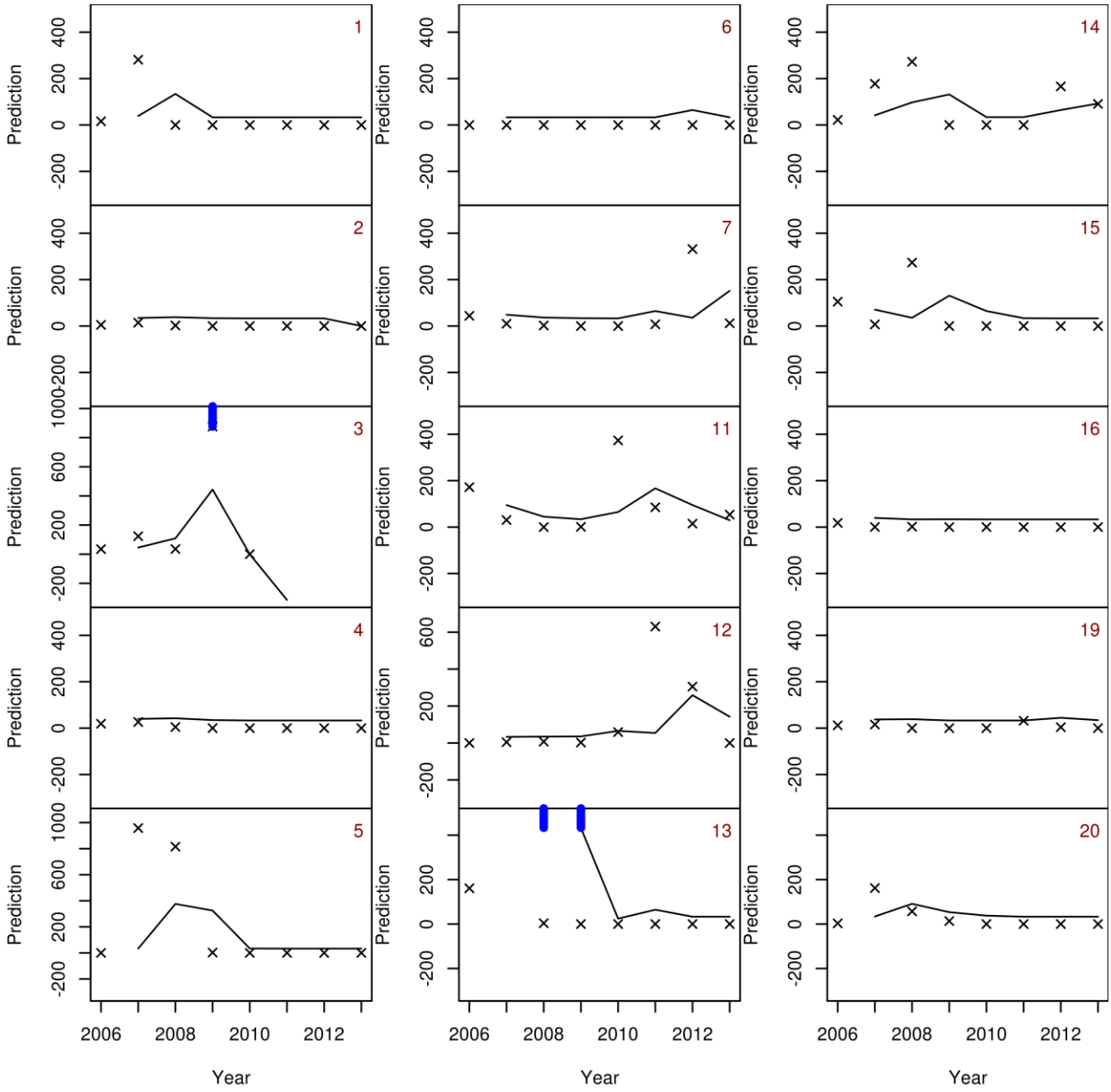

Figure 5.A.II-4 – Predicted concentration of *R. solani* AG3 DNA in 15 different South Australian soils sampled each year from 2005 to 2013. Blue tickmarks indicate a preceding fallow crop. Predicted responses are shown as solid lines and observed data as crosses. Site numbers are in the top right corner of each box.

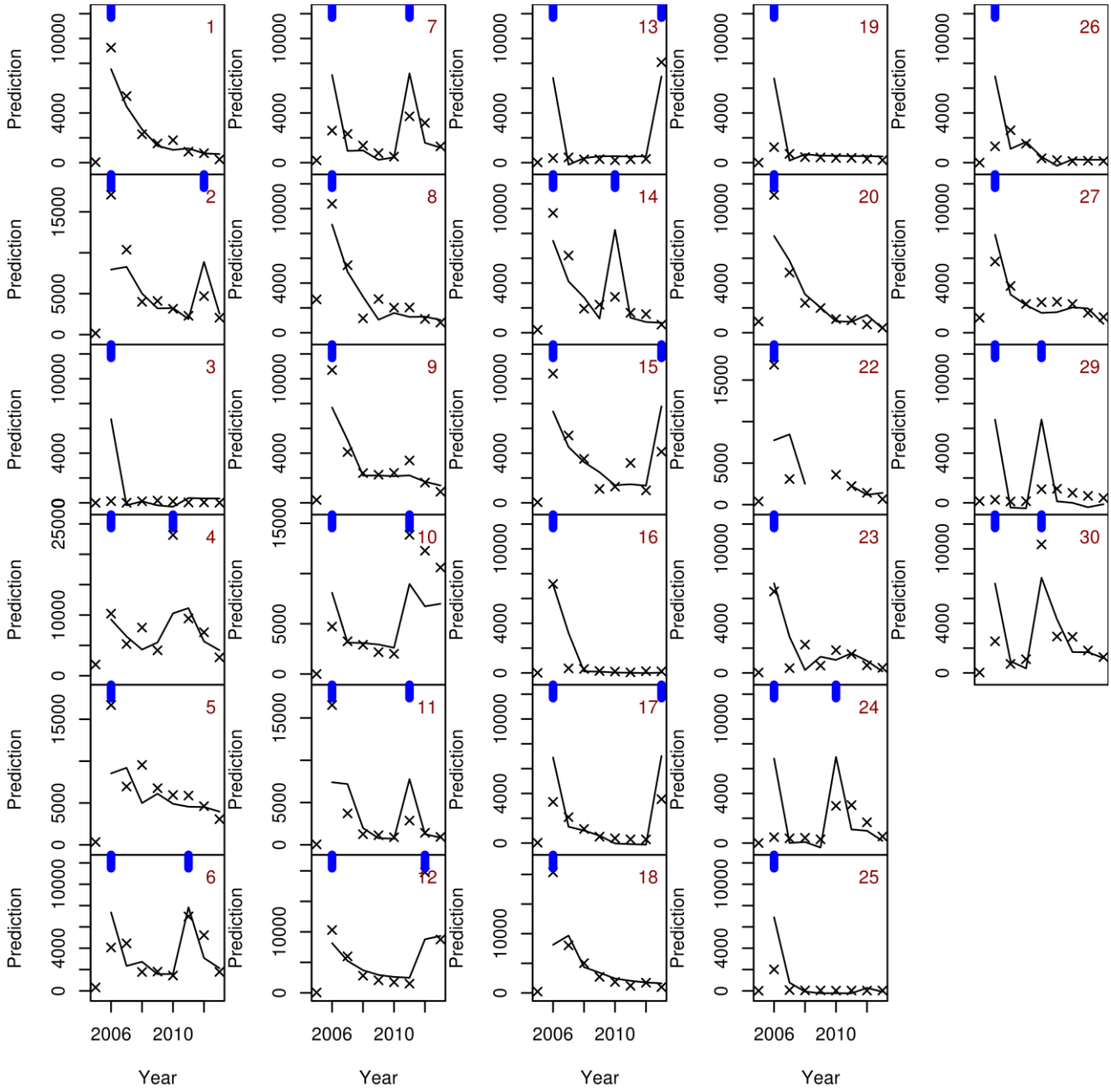

Figure 5.A.II-5 – Predicted concentration of *Sp. subterranea* DNA in 28 different Tasmanian soils. Blue tickmarks indicate preceding potato crops. Predicted responses are shown as solid lines and observed data as crosses. Site numbers are in the top right corner of each box.

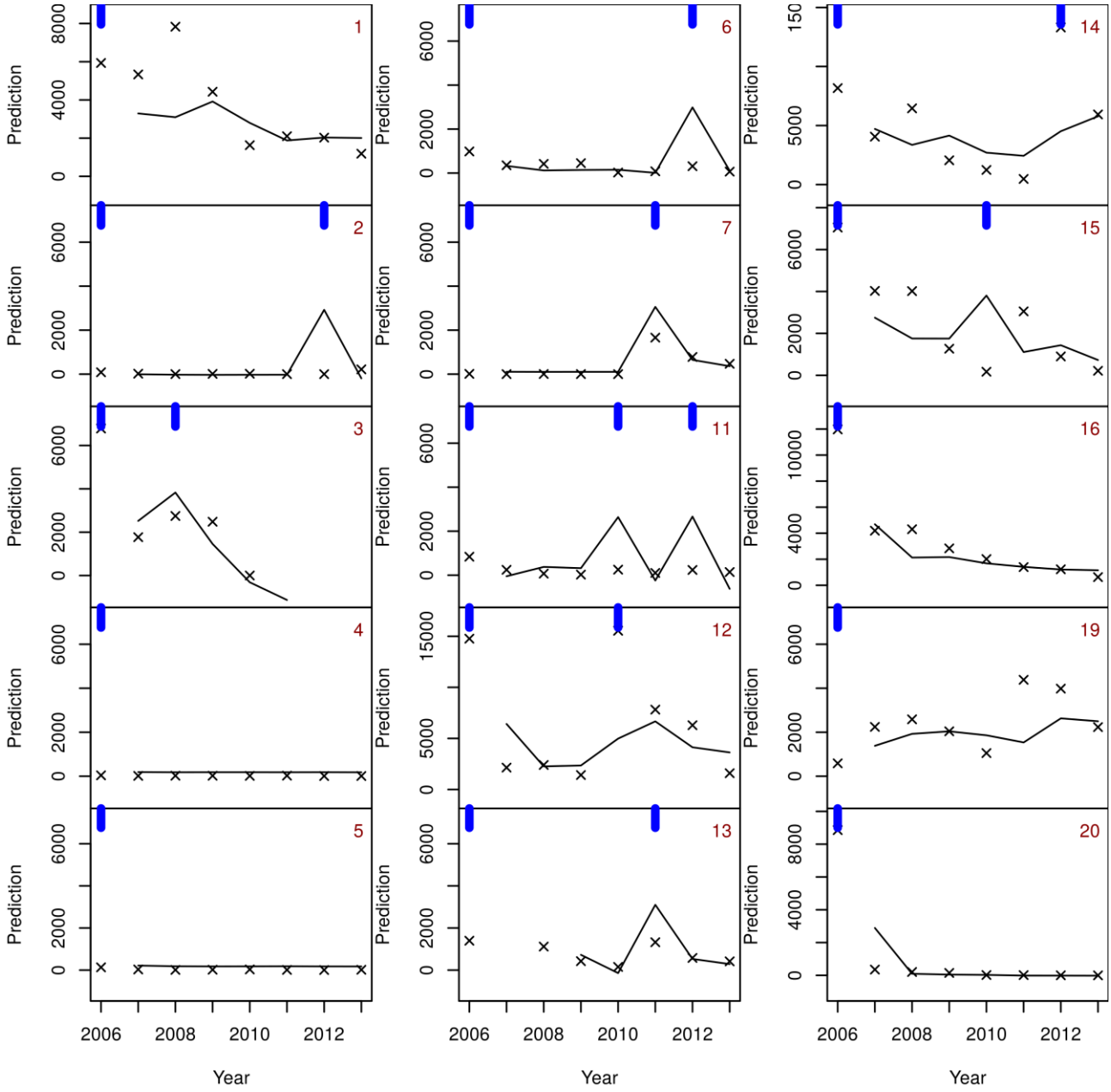

Figure 5.A.II-6 – Predicted concentration of *Sp. subterranea* DNA in 15 different South Australian soils. Blue tickmarks indicate preceding potato crops. Predicted responses are shown as solid lines and observed data as crosses. Site numbers are in the top right corner of each box.

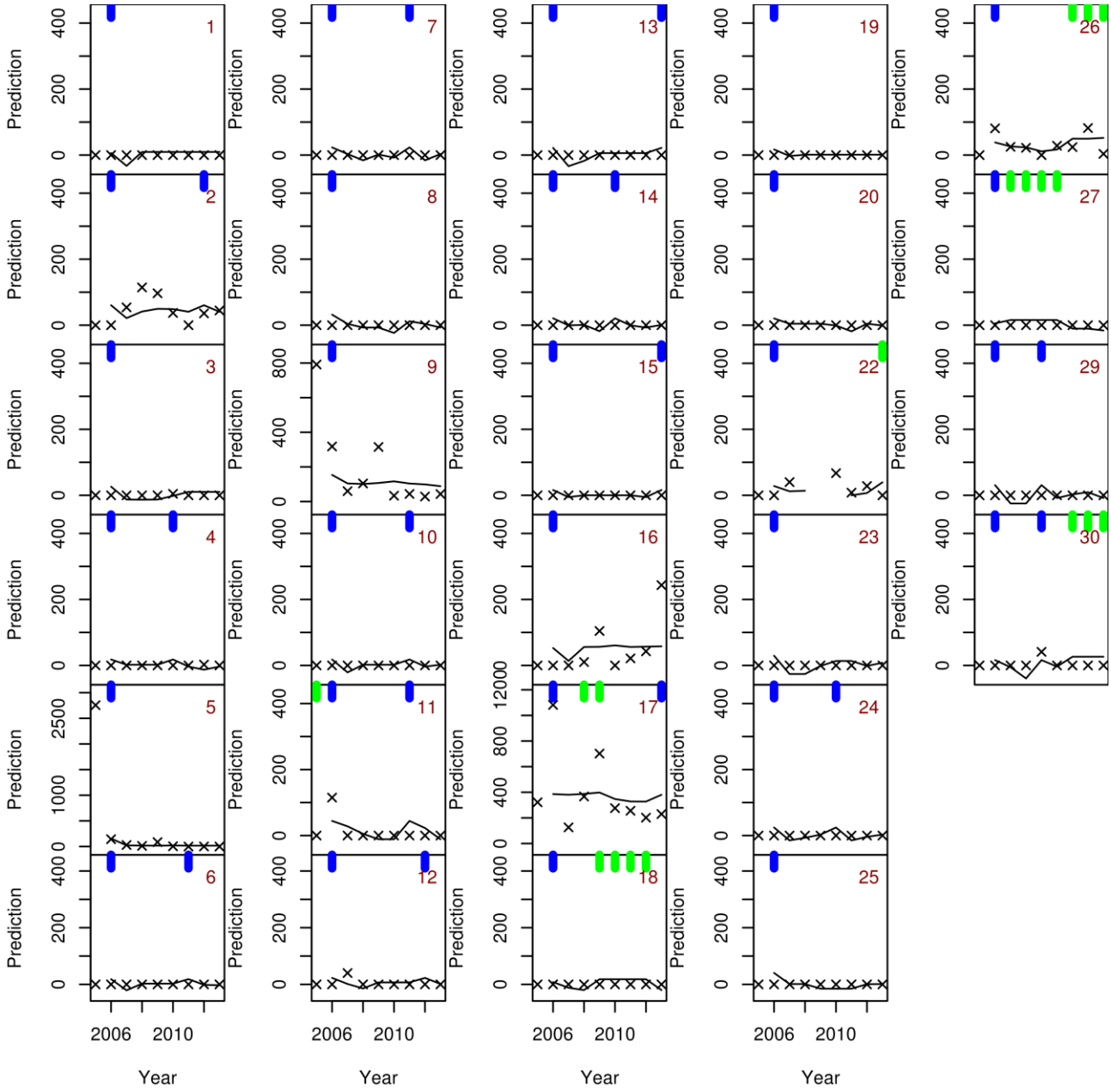

Figure 5.A.II-7 – Predicted concentration of *St. scabies* DNA in 28 different Tasmanian soils. Tickmarks indicate preceding crops; blue = potato, green = pyrethrum. Predicted responses are shown as solid lines and observed data as crosses.

DISCUSSION

PREVALENCE OF PATHOGEN DNA

Of the pathogens measured in this study, *R. solani* AG2.1 and *St. scabies* are generally considered to be widespread in agricultural soils because of their wide host ranges and saprophytic habits (Loria, et al., 1997). *Rhizoctonia solani* AG8 has been commonly found in cereal soils (Mazzola, et al., 1996). *Rhizoctonia solani* AG3 and AG4, and *Sp. subterranea,* are usually recognized to be present in soils where potato has been grown, but their persistence in the absence of a potato host has not been documented. *Rhizoctonia solani* AG4 has also been isolated from plants of the Chenopodiaceae and Fabaceae (Ogoshi, 1987). Little is known about the distribution of *R. solani* AG2.2 in agricultural soils.

The widespread, continued presence of *Sp. subterranea* DNA in soil in both states (Table 5.A.II-3, Table 5.A.II-4) confirms the already common view (Falloon, 2008) that this pathogen is very persistent, and can survive in both the temperate Tasmanian climate and the warmer Mediterranean climate of South Australia. This persistence is further emphasised by the fact that no crops other than potato were found to increase concentrations of the DNA of this pathogen (Table 5.A.II-5), i.e. potato appeared to be the only host of significance in the farming systems we encountered. Qu and Christ (2006) showed that root galls and sporosori of *Sp. subterranea* were produced on oat in solution culture, but oat was only a minor crop in the Australian rotations monitored in the present study (Table 5.A.II-1, Table 5.A.II-2).

The behavior of *Sp. subterranea* can be contrasted with that of *R. solani* AG3, which increased in prevalence in soils in both states after the 2005-06 when potato was grown at all sites (Table 5.A.II-3, Table 5.A.II-4) but became less prevalent towards the end of the study, particularly in South Australia. This suggests that, in the absence of potato, *R. solani* AG3 does not persist as strongly as *Sp subterranea*. The common use, in Tasmania (Table 5.A.II-1), of fallow and other rotation crops which were found to increase *R. solani* AG3 DNA (Table 5.A.II-5) probably helped to maintain inoculum of this pathogen to a greater degree than occurred in South Australia. The reasons for diminishing amounts of *R. solani* AG2.1 DNA in South Australia, but not in Tasmania, are not clear. This may relate to the warmer and drier summers in South Australia compared with Tasmania, in combination with the sandy soils that are common in the south east of South Australia where our sites were located. The reasons for the relatively low prevalence of *St. scabies* and *R. solani* AG4 and AG2.2 DNA in both states are also unclear. The increased prevalence of *St. scabies* and *R. solani* AG3 DNA in 2006 compared to 2005 suggests that these pathogens may have been introduced to the soil via the seed used for the 2005-06 potato crops. The importance of seed-borne inoculum for common scab has previously been shown (Wilson, et al., 1999) (Wang & Lazarovits, 2005). Those of the sites in the present survey where no *Sp. subterranea* DNA was detected in 2005 but where DNA was detected thereafter (one site in Tasmania, three in South Australia) may also have had this pathogen introduced via potato seed. Evidence for seed tuber transmission of this pathogen has been reviewed by Merz and Falloon (2009).

MODEL OUTPUTS: POTENTIAL ALTERNATIVE HOST CROPS

No measures of tuber diseases are reported here, even though potato crops were grown more than once at many sites monitored during the study. Disease incidence and severity data for the 2005-06 potato crops across all sites are reported in Crump (2009), and show that powdery scab was widespread and locally severe across the sites because of generally wet conditions at the time of tuber initiation. The average incidence (proportion of tubers at each site showing any symptoms) of powdery scab in cv. Desiree in this season was 32% across the South Australian sites and 42% across the Tasmanian sites (Crump, 2009).

Relationships between pathogen DNA concentrations and tuber disease are the subject of the APRP Soil Diagnostics project (Ophel Keller, 2014).

Of the crops grown in rotation with potato in the current study, only a few have previously been shown to be hosts for some of the pathogens we monitored. In addition, Carling et al. (1986) found that *R. solani* AG3 had an epiphytic relationship with oat, cauliflower, carrot and wheat. That is, these crops may help *R. solani* AG3 survive in the absence of potato. However, of these crops only carrot in Tasmania was associated with an increase in the concentration of the DNA of *R. solani* AG3 (Table 5.A.II-5). Grisham and Anderson (1983) also found that *R. solani* AG4 and AG2.2 caused seedling damping off and damage including cankers to mature roots of carrot, but the prevalence of these AGs was too low for any such effects to show at the sites monitored in the present study. Bairns *et al*. (2002) reported that neither barley, green pea nor wheat were infected by potato isolates of *R. solani* from Alberta, Canada. The only previously published data about the comparative effects of poppy on soil-borne potato diseases (Sparrow & Wilson, 2012) showed that in potato grown in 11 paddocks immediately after poppy there were no more or less tuber diseases than in potato grown after pasture in 11 different paddocks.

We were not able to account for any possible effects of site management, including tillage, water, pest and disease management, on pathogen DNA concentrations. The effects of weeds, especially weed potatoes (ground keepers),could be significant, given that ground keepers are difficult to control, and that many solanaceous weeds and other species shown to be alternate hosts for the potato pathogens examined here, are common in the monitored regions. These include *Chenopodium album* (fat hen), *Raphanus raphanistrum* (wild radish), *Trifolium pratense* (red clover), *Trifolium repens* (white clover) and *Solanum* sp. (nightshade), from which *R. solani* AG3 was recovered on Prince Edward Island, Canada (Sturz, et al., 1995), and *Dactylis glomerata* (cocksfoot) and nightshade (Shah, et al., 2010) which produced zoosporangia and spore balls when infected with *Sp. subterranea* (Qu & Christ, 2006). We did inspect the sites for ground keepers when sampling soil in the surveyed paddocks, and some were noted at some South Australian sites. Sites may also have been infested at times outside the sampling periods.

PATHOGEN HALF-LIVES AND IMPLICATIONS FOR ROTATIONS

The half-lives for pathogen DNA reported in Table 5.A.II-6 are all generally of the same order, i.e. a few months to almost 1 year. The associated confidence limits suggest that for the same pathogen there is no significant difference in the half-lives between states. Better estimates of half-lives may have resulted if the sites could have been sampled more often than once per year because we would have generated more data per unit time for model fitting. The significance of decay rates needs to be assessed alongside other characteristics of particular pathogens, particularly the absolute concentration of pathogen DNA in soil, the relationships of those concentrations to potato disease risk, and the potential responses of pathogens to the presence of the potato host. The halving of a DNA concentration greatly in excess of that which may pose a high disease risk will still leave that soil at high risk, while the continued decay of DNA concentrations after they have reached near-background levels is of little practical importance. For the pathogens monitored in the present study, the project of Ophel Keller (2014) has suggested a reliable relationship between DNA concentration and disease risk only for *Sp. subterranea*. Soils at high risk of powdery scab, defined as a 20-50% chance of the incidence of powdery scab exceeding 10%, are said to be those with pre-plant DNA concentrations greater than about 20 pg/g of soil ($\log_{10}$ = 1.28). The average concentrations of *Sp. subterranea* DNA at our sites were 10- to 100-fold greater than this (Table 5.A.II-3 and Table 5.A.II-4), indicating that many soils in these states are at high risk of powdery scab when potato is grown. Given this risk status, and that the model predicts increases in *Sp. subterranea* DNA concentration of *a few thousand* pg/g in both states when potato is grown (Table 5.A.II-5), it is very likely that many South

Australian and Tasmanian soils with average potato rotation lengths of 6 years (Sparrow, 2009) would not return to a low-risk category. Using a starting DNA concentration of 1000 pg/g, and average values of the estimated *Sp. subterranea* DNA half-lives for South Australia and Tasmania, and of the corresponding B_{crop} parameters for potato, the model predicts that the *Sp. subterranea* DNA concentration will never fall below the high-risk threshold when potato is grown at least once in every five years, and the concentration only falls below the threshold for the final 3 years of a recurring 10-year rotation (Figure 5.A.II-8). For sufficiently long rotations the model predicts that the DNA concentration will subtend to zero between potato crops, and therefore that the maximum concentration after the next potato crop approaches B_{crop}. While the model is necessarily an imperfect representation of reality, the raw data (Appendix 1) show that 9 out of 15 South Australian sites and 27 of the 28 Tasmanian paddocks maintained *Sp. subterranea* DNA concentrations greater than the threshold (log_{10} = 1.28) from 2006 until 2013, supporting the view that current rotation lengths may be too short to allow *Sp. subterranea* inoculum to decay to a safe concentration. In the absence of accurate DNA vs. disease risk relationships it is not possible to make judgements of this kind about the other pathogens and the diseases they cause.

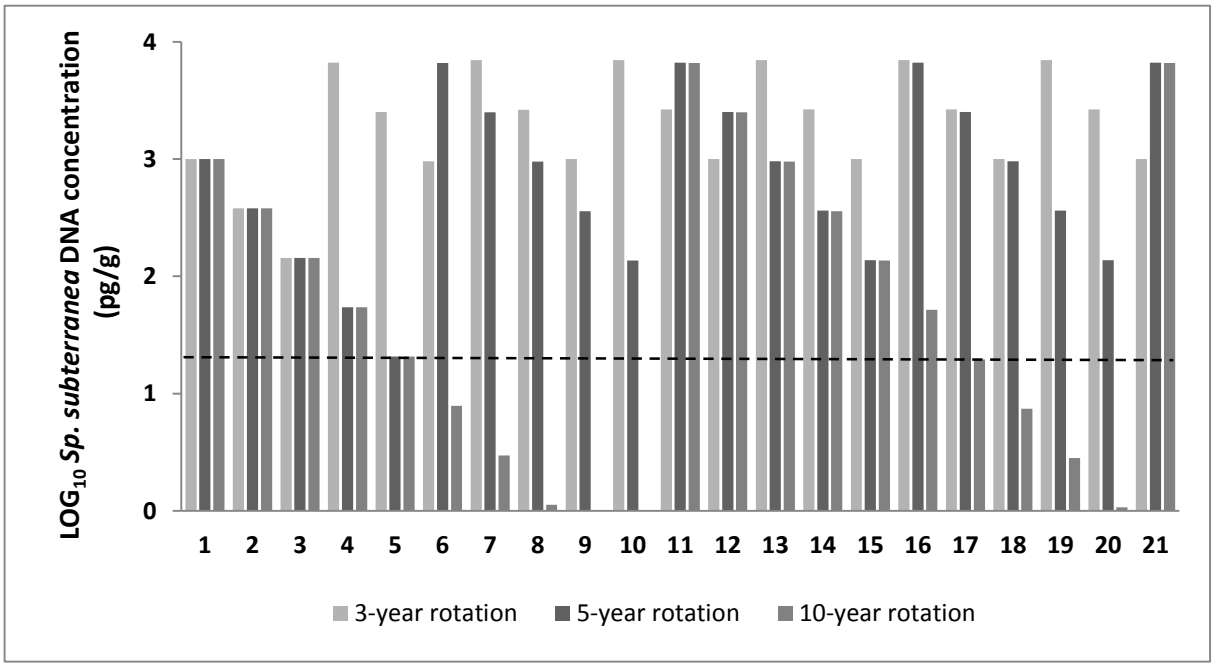

Figure 5.A.II-8 – Model predictions of *Sp. subterranea* DNA concentrations for 3-, 5- and 10-year potato rotations, assuming a starting concentration of 1000 pg/g and values of A_N and B_{crop} for potato which are the average of these estimates for South Australia and Tasmania (*the dashed line shows the threshold LOG_{10} DNA concentration of 1.28 for high risk of powdery scab*).

TECHNOLOGY TRANSFER

Sparrow, L & de Boer, R (2012). Mitigating soil health issues. *Potatoes Australia* April/May 2012, pp. 23-24.

Sparrow, L (2013). Cropping trial yields surprising results. Article in *Primary Producer* section of The Devonport Advocate, 20 June 2013.

Sparrow, L & Rettke, M (2014). Long-term monitoring of soil-borne potato pathogens in South Australia and Tasmania. *Potatoes Australia* in press at time of writing.

Sparrow, L (2014). Long-term monitoring of soil-borne potato pathogens. Article in *Primary Producer* section of The Devonport Advocate, August 2014.

A presentation on this project and other APRP2 work was given by Anne Ramsay to the 2014 Potato Industry Conference, Mt Gambier SA, 10-12 August 2014. Presentations were also given by Leigh Sparrow to the Simplot R&D Meeting held in Ulverstone Tasmania on 16 July 2014, and to the Roberts Rural Potato Industry Forum held in Scottsdale Tasmania on 28 August 2014.

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

The findings of this project about *Spongospora subterranea* and the risk of powdery scab need to be incorporated into the manual and associated training for the PreDictaPt DNA testing service offered to the potato industry by SARDI.

Findings about the other pathogens measured in this project can be incorporated into extension services when and if reliable models for the interpretation of their DNA concentrations are available.

Consideration should be given to continuing the DNA monitoring at least at some of the sites.

Consideration should also be given to more controlled studies of the potential impact on disease of those crops other than potato identified as “DNA-increasing” crops, i.e. poppy (*R. solani* AG2.1 and AG3) and ryegrass and carrot (*R. solani* AG3), and to studies of other crops not part of this project including so-called “trap” crops.

ACKNOWLEDGEMENTS

We thank the many growers who allowed us continued access to their paddocks. Mr Robin Harding, formerly of SARDI, managed the South Australian component of this project until 2011.

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APPENDIX 1 – RAW DATA

Table 5.A.II-7 –Pathogen DNA concentrations (pg/g, log_{10} transformed) in soils sampled from 30 paddocks in Tasmania each year from 2005 to 2013

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG2.1				
1	1.43	2.36	3.02	1.18	0.36	0.07	0.65	0.40	0.70
2	0.85	3.09	3.13	1.87	0.46	1.44	2.66	3.17	1.97
3	2.70	3.03	1.75	1.39	0.45	2.21	2.38	1.86	0.75
4	2.59	2.03	2.27	0.55	1.32	2.51	1.70	1.67	0.60
5	1.68	2.89	2.12	2.77	3.46	2.84	2.43	3.18	2.76
6	2.35	3.21	2.79	1.50	1.35	0.14	1.29	1.21	0.63
7	2.38	1.65	2.55	0.00	3.24	1.87	1.09	0.73	2.18
8	1.88	1.23	1.72	0.92	1.55	1.15	0.44	2.71	2.45
9	3.00	2.57	2.64	1.33	0.62	0.47	0.00	3.00	1.79
10	1.53	1.64	0.30	1.35	0.68	2.06	1.67	1.56	1.28
11	0.00	0.30	1.32	1.33	0.58	0.56	0.72	1.57	0.82
12	2.54	2.17	2.34	1.76	0.53	1.27	1.82	2.23	2.56
13	1.31	2.03	1.20	1.34	2.54	1.13	1.61	1.80	0.41
14	1.63	1.41	1.67	0.43	0.00	0.85	2.07	0.44	0.31
15	2.73	2.73	3.74	3.05	1.93	0.29	1.93	2.51	1.84
16	1.86	1.76	2.17	2.77	1.09	0.96	0.54	1.37	1.60
17	0.00	1.81	0.00	0.00	0.48	0.28	0.18	0.89	1.34
18	1.83	1.77	0.00	0.91	0.67	0.02	0.71	2.43	2.76
19	0.60	2.43	1.34	2.22	1.54	0.28	0.90	1.15	1.70
20	1.98	1.28	2.10	1.57	2.16	2.88	1.61	0.69	2.20
21	2.64	*	*	*	*	*	*	*	*
22	2.58	2.49	1.92	*	*	0.22	1.52	1.61	3.12
23	1.72	1.48	1.76	0.85	0.00	2.08	1.29	1.48	1.64
24	0.00	0.00	0.00	0.00	3.09	2.43	1.30	1.04	0.55
25	2.08	2.58	2.16	1.66	0.23	1.25	1.50	1.28	0.36
26	2.48	2.11	2.46	2.87	3.05	2.22	2.04	2.05	1.58
27	0.00	1.78	1.94	0.53	0.56	1.01	0.14	2.13	2.72
28	2.17	*	*	*	*	*	*	*	*
29	1.56	0.60	0.78	0.48	0.79	0.30	0.00	0.00	0.17
30	1.81	2.06	1.51	2.63	2.01	2.13	1.92	2.37	1.23

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG2.2				
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.98	0.70	0.00	0.00	0.00	0.73	0.80	0.00	0.00
3	1.10	1.49	2.30	2.12	1.89	1.12	2.11	1.99	2.21
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	0.00	*	*	*	*	*	*	*	*
22	0.00	0.00	0.00	*	*	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.54	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	*	*	*	*	*	*	*	*
29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG3				
1	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00
2	0.00	0.00	1.91	0.89	0.00	0.00	0.00	0.00	0.49
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	2.63	1.38	1.64	0.38	0.00	0.00	1.76	0.00
5	0.00	1.58	0.85	1.52	2.05	0.65	0.00	0.00	0.00
6	1.76	2.29	3.27	2.84	1.10	1.31	0.21	0.04	0.00
7	1.30	0.70	2.52	0.92	3.07	1.93	0.78	0.45	0.32
8	1.96	2.42	1.86	1.11	0.96	1.41	1.12	2.34	1.79
9	1.71	2.16	3.23	2.84	1.93	1.05	0.12	1.18	1.78
10	0.00	0.00	0.00	0.00	0.45	0.00	0.00	0.00	0.00
11	0.00	0.00	1.59	0.00	0.80	0.09	0.26	1.58	2.30
12	2.08	2.88	3.21	0.00	0.79	0.14	0.00	0.05	0.20
13	0.93	0.48	0.60	2.83	1.01	1.64	0.00	0.00	0.29
14	0.00	0.48	0.00	1.22	2.83	2.68	2.27	2.28	0.55
15	0.00	0.00	2.16	1.03	0.00	0.00	1.06	0.00	0.27
16	0.00	0.70	1.11	1.40	0.00	0.00	0.00	0.08	0.00
17	0.00	1.11	1.40	0.00	0.70	0.00	0.62	0.78	0.12
18	0.00	0.00	0.00	0.00	0.58	1.83	0.00	0.00	1.44
19	0.00	1.34	2.33	1.43	1.50	0.51	0.00	1.06	0.00
20	1.95	1.56	2.89	1.80	0.80	0.00	0.00	0.13	0.00
21	1.34	*	*	*	*	*	*	*	*
22	0.00	2.02	1.53	*	*	0.66	0.00	0.00	0.05
23	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.26	0.00
24	0.00	0.00	0.60	0.00	2.66	1.27	0.97	0.35	0.00
25	0.00	0.00	1.81	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.48	1.00	0.33	1.31	0.26	1.40	0.00	0.00
27	0.00	0.95	2.21	1.41	1.43	0.70	2.15	0.71	0.97
28	0.00	*	*	*	*	*	*	*	*
29	0.00	0.00	2.07	3.54	0.69	0.00	0.00	0.00	0.00
30	0.00	0.78	0.70	1.20	1.44	0.00	0.05	0.00	0.00

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG4				
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.56
2	2.97	3.58	2.85	0.00	0.00	0.00	2.80	1.29	0.47
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53
5	0.00	3.82	2.52	2.94	0.00	2.79	1.23	0.53	0.00
6	1.75	2.86	2.67	0.00	0.00	2.37	0.00	0.90	0.49
7	0.00	0.00	1.34	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	3.23	3.66	3.24	2.35	0.00	0.00	2.55	3.26	2.15
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	1.59	0.00	0.00
12	2.05	2.67	2.95	2.31	1.47	1.84	3.54	0.00	1.89
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	2.76	0.00	2.76	2.69	2.47	0.00
15	0.00	0.00	4.30	4.87	3.34	1.24	1.40	1.58	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	3.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	2.56	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	2.13	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	3.57	1.94	0.00	0.00	0.00	2.74
21	0.00	*	*	*	*	*	*	*	*
22	2.78	2.38	3.17	*	*	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	*	*	*	*	*	*	*	*
29	0.00	0.00	0.00	0.00	0.00	1.09	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG8				
1	1.58	0.00	0.30	0.00	0.15	0.45	*	1.22	2.15
2	0.90	0.00	0.00	2.10	1.36	0.00	*	0.00	0.00
3	0.00	0.00	0.00	0.30	0.00	0.00	*	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
5	0.00	0.00	0.00	0.00	0.11	0.00	*	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
12	0.00	0.00	0.00	1.96	0.00	0.00	*	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
16	1.53	0.90	0.78	1.81	0.92	0.10	*	0.00	0.00
17	0.00	0.00	0.00	0.30	0.00	0.00	*	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
21	0.00	*	*	*	*	*	*	*	*
22	0.00	0.00	0.00	*	*	0.00	*	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.17
26	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00
27	0.00	0.00	0.00	1.97	0.00	0.00	*	0.00	0.00
28	0.00	*	*	*	*	*	*	*	*
29	0.00	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
30	0.00	0.00	0.00	0.30	0.00	0.00	*	0.00	0.00
					Streptomyces scabies				
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	1.74	2.06	1.99	1.58	0.00	1.57	1.66
3	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00
5	3.44	2.16	1.49	1.13	1.94	0.78	0.00	0.47	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	2.90	2.50	1.79	2.02	2.50	1.54	1.65	1.47	1.64
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	1.61	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	1.05	2.02	0.00	1.35	1.64	2.39
17	2.51	3.03	2.10	2.57	2.85	2.44	2.41	2.31	2.36
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	0.00	*	*	*	*	*	*	*	*
22	0.00	0.00	1.61	*	*	1.83	0.95	1.46	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	1.91	1.41	1.37	0.00	1.47	1.40	1.92	0.67
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	*	*	*	*	*	*	*	*

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	1.62	0.00	0.00	0.00	0.00
					Spongospora subterranea				
1	1.58	3.97	3.73	3.36	3.19	3.26	2.95	2.88	2.42
2	2.20	4.23	4.02	3.60	3.61	3.50	3.36	3.67	3.32
3	0.00	2.14	1.10	2.12	2.27	2.10	1.53	1.56	1.15
4	3.27	4.01	3.72	3.90	3.62	4.36	3.97	3.86	3.48
5	2.53	4.22	3.84	3.98	3.83	3.78	3.77	3.66	3.49
6	2.51	3.61	3.65	3.25	3.26	3.16	3.84	3.72	3.25
7	2.27	3.41	3.36	3.14	2.88	2.69	3.57	3.51	3.12
8	3.43	4.02	3.74	3.07	3.43	3.31	3.31	3.05	2.91
9	2.37	4.03	3.61	3.38	3.36	3.38	3.53	3.21	2.95
10	0.32	3.67	3.51	3.46	3.34	3.30	4.14	4.09	4.03
11	1.71	4.22	3.57	3.10	3.06	2.96	3.46	3.15	2.97
12	1.79	4.01	3.78	3.45	3.32	3.24	3.18	4.30	3.94
13	1.36	2.57	2.63	2.42	2.45	2.29	2.43	2.48	3.91
14	2.37	3.98	3.79	3.29	3.35	3.46	3.21	3.18	2.82
15	1.71	4.02	3.73	3.55	3.05	3.12	3.51	3.00	3.62
16	1.16	3.86	2.57	2.52	2.20	2.07	1.70	2.16	2.11
17	1.52	3.52	3.32	3.06	2.72	2.59	2.48	2.48	3.55
18	2.36	4.31	3.90	3.70	3.43	3.27	3.08	3.24	3.00
19	0.85	3.10	2.85	2.65	2.62	2.57	2.58	2.48	2.30
20	2.96	4.05	3.69	3.38	3.30	3.04	3.00	2.83	2.60
21	1.22	*	*	*	*	*	*	*	*
22	2.60	4.23	3.49	*	*	3.56	3.35	3.16	2.82
23	1.63	3.82	2.59	3.36	2.77	3.26	3.18	2.79	2.62
24	0.80	2.68	2.59	2.63	2.48	3.48	3.49	3.22	2.73
25	1.04	3.30	1.89	1.66	1.63	1.45	1.52	1.48	1.55
26	0.46	3.12	3.42	3.18	2.54	2.33	2.09	2.20	2.09
27	3.08	3.76	3.58	3.37	3.39	3.40	3.36	3.20	3.10

Tas site	2005	2006	2007	2008	2009	2010	2011	2012	2013
28	0.69	*	*	*	*	*	*	*	*
29	2.13	2.40	2.04	2.13	3.04	3.06	2.91	2.75	2.59
30	1.61	3.41	2.87	3.04	4.02	3.47	3.46	3.26	3.11

*no measurement made in this year

Table 5.A.II-8 –Pathogen DNA concentrations (pg/g, log_{10} transformed) in soils sampled from 15 paddocks in South Australia each year from 2005 to 2013

SA Site	2005	2006	2007	2008	2009	2010	2011	2012	2013
					R. solani AG2.1				
1	2.35	1.82	3.49	2.63	3.41	1.30	2.03	0.00	0.22
2	1.96	1.43	1.46	0.95	0.38	0.06	0.44	0.90	2.24
3	0.78	0.60	1.15	2.12	1.16	0.27	*	*	*
4	1.56	1.78	1.38	2.92	1.46	1.23	2.31	1.91	2.76
5	2.28	2.32	2.92	2.15	2.20	0.24	1.66	0.96	1.13
6	1.52	1.74	1.90	1.46	1.66	0.00	0.83	3.10	0.78
7	1.44	1.48	2.43	0.00	1.12	0.00	0.64	1.36	0.29
11	0.30	2.13	2.01	2.66	0.22	1.34	0.50	0.76	0.77
12	0.65	0.30	0.81	0.00	1.42	0.41	0.00	0.53	0.39
13	0.60	1.11	*	1.21	0.65	0.14	0.10	0.00	0.00
14	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.30	0.30	0.00	0.00	0.50	0.00	0.00	0.00	0.00
16	2.04	1.40	2.67	4.40	2.47	3.08	2.73	2.76	1.49
19	0.30	0.48	3.33	3.31	2.67	0.20	2.75	1.82	1.02
20	2.13	1.97	1.43	1.15	0.31	0.00	0.00	0.00	0.00
					R. solani AG2.2				
1	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	*	0.00	0.15	0.00
3	0.40	0.00	0.00	0.00	0.00	*	*	*	*
4	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
6	2.44	0.48	0.30	0.00	1.31	*	1.95	1.07	0.07
7	1.27	1.49	1.49	1.30	2.41	*	1.36	0.80	1.83
11	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
13	0.00	0.00	*	0.00	0.00	*	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00
19	1.15	1.23	0.00	0.00	0.00	*	1.04	1.97	1.47

SA Site	2005	2006	2007	2008	2009	2010	2011	2012	2013
20	0.00	0.00	2.14	0.00	1.60	*	0.00	0.00	0.00
					R. solani AG3				
1	2.24	1.23	2.45	0.00	0.00	0.00	0.00	0.00	0.00
2	0.70	0.78	1.19	0.52	0.00	0.00	0.00	0.00	0.00
3	0.00	1.56	2.10	1.56	2.94	0.00	*	*	*
4	0.00	1.30	1.44	0.76	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	2.98	2.91	0.57	0.00	0.09	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	1.65	1.04	0.53	0.00	0.00	0.93	2.52	1.11
11	0.00	2.24	1.50	0.00	0.30	2.57	1.94	1.20	1.74
12	0.00	0.00	0.74	0.92	0.54	1.78	2.80	2.49	0.00
13	0.00	2.21	*	0.67	0.00	0.00	0.00	0.00	0.00
14	0.00	1.36	2.25	2.44	0.00	0.00	0.00	2.22	1.96
15	0.00	2.03	0.90	2.44	0.00	0.00	0.00	0.00	0.00
16	1.39	1.28	0.18	0.45	0.00	0.00	0.00	0.00	0.00
19	0.00	1.11	1.22	0.00	0.00	0.00	1.52	0.69	0.00
20	0.00	0.60	2.21	1.76	1.16	0.00	0.00	0.00	0.00
					R. solani AG4				
1	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
2	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
3	0.00	0.00	0.00	*	*	*	*	*	*
4	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
5	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
6	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
7	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
11	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
12	0.00	1.75	0.00	*	*	*	1.52	0.00	0.00
13	0.00	0.00	*	*	*	*	0.00	0.00	0.00
14	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
15	0.00	0.00	0.00	*	*	*	0.64	0.00	0.00
16	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
19	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00

SA Site	2005	2006	2007	2008	2009	2010	2011	2012	2013
20	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00
					R. solani AG8				
1	0.00	0.00	0.00	*	*	*	*	*	0.00
2	0.00	0.00	0.00	*	*	*	*	*	0.25
3	0.00	0.00	0.00	*	*	*	*	*	*
4	0.00	0.00	0.00	*	*	*	*	*	0.00
5	0.48	2.12	1.80	*	*	*	*	*	1.62
6	0.00	0.00	0.00	*	*	*	*	*	0.00
7	0.00	0.00	0.00	*	*	*	*	*	0.00
11	0.78	1.68	1.33	*	*	*	*	*	1.38
12	0.74	1.32	0.30	*	*	*	*	*	1.34
13	1.39	0.70	*	*	*	*	*	*	2.33
14	1.27	0.00	2.14	*	*	*	*	*	0.99
15	1.63	0.78	1.29	*	*	*	*	*	0.54
16	0.00	0.00	0.00	*	*	*	*	*	0.00
19	0.00	0.00	0.00	*	*	*	*	*	0.00
20	0.00	0.00	0.60	*	*	*	*	*	0.00
					Spongospora subterranea				
1	3.11	3.77	3.73	3.89	3.65	3.21	3.32	3.31	3.07
2	0.47	1.93	1.33	0.45	1.09	1.27	0.07	0.00	2.34
3	3.41	3.83	3.25	3.44	3.39	0.51	*	*	*
4	1.51	1.61	1.22	1.38	1.36	1.22	1.42	0.98	1.02
5	0.00	2.12	1.50	0.99	1.27	1.51	0.99	0.67	1.21
6	2.03	2.99	2.55	2.62	2.66	1.41	1.92	2.50	1.83
7	0.00	1.11	0.17	0.82	0.62	0.00	3.22	2.89	2.67
11	0.00	2.92	2.39	1.85	1.43	2.40	2.02	2.38	2.16
12	0.12	4.17	3.33	3.38	3.15	4.19	3.89	3.80	3.20
13	1.21	3.15	*	3.05	2.63	2.19	3.12	2.76	2.62
14	1.54	3.91	3.61	3.81	3.31	3.09	2.68	4.12	3.77
15	3.06	3.85	3.61	3.60	3.10	2.23	3.48	2.95	2.34
16	3.18	4.08	3.62	3.63	3.45	3.30	3.15	3.09	2.80

SA Site	2005	2006	2007	2008	2009	2010	2011	2012	2013
19	0.85	2.77	3.35	3.41	3.31	3.02	3.64	3.60	3.35
20	3.52	3.95	2.55	2.32	2.17	1.33	1.22	0.59	0.00
					Streptomyces scabies				
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	1.87	1.11	3.09	0.00	0.00	*	*	*
4	0.00	2.25	1.06	2.83	2.97	2.04	2.35	3.77	3.07
5	0.00	0.00	0.85	2.21	2.69	0.00	2.65	3.61	2.74
6	0.00	1.72	0.54	0.00	0.00	1.10	0.00	1.85	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.00
11	0.00	0.00	0.00	0.00	0.00	1.68	1.80	2.70	2.62
12	0.00	2.71	0.70	0.00	0.87	0.00	2.49	0.86	0.43
13	0.00	1.93	*	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	1.62	0.00	0.00
16	0.00	1.76	0.00	0.00	0.00	1.38	1.20	1.76	0.00
19	0.00	1.36	1.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	1.95	1.16	0.00	0.00	1.01	0.00	0.00	0.00

*no measurement made in this year

Table 5.A.II-9 – Land use history of the 28 Tasmanian sites sampled each year from 2005 to 2013

Site	2005	2006	2007	2008	2009	2010	2011	2012
1	Potato	Oat	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne
2	Potato	Barley	Poppy	Pasture	Pasture	Poppy	Potato	Poppy
3	Potato	Cereal	Fallow	Oat	Carrot	Pasture	Pasture	Pasture
4	Potato	Pasture	Pasture	Pasture	Potato	Poppy	Onion	Poppy
5	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
6	Potato	Fallow	Pasture	Pasture	Pasture	Potato	Pea	Poppy
7	Potato	Pea	Wheat	Poppy	Onion	Potato	Wheat	Pea
8	Potato	Carrot	Barley	Oat	Ryegrass	Pea	Carrot	Barley
9	Potato	Carrot	Pea	Pasture	Pasture	Pasture	Poppy	Onion
10	Potato	Wheat	Pasture	Pasture	Pasture	Potato	Poppy	Pasture
11	Potato	Poppy	Oat	Ryegrass	Ryegrass	Potato	Poppy	Ryegrass
12	Potato	Poppy	Wheat	Pasture	Pasture	Pasture	Potato	Poppy
13	Potato	Ryegrass	Fallow	Pasture	Pasture	Pasture	Pasture	Potato
14	Potato	Poppy	Pea	Wheat	Potato	Poppy	Carrot	Cauliflower
15	Potato	Poppy	Pasture	Pasture	Pasture	Pasture	Poppy	Potato
16	Potato	Triticale	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne
17	Potato	Wheat	Pyrethrum	Pyrethrum	Ryegrass	Ryegrass	Ryegrass	Potato
18	Potato	Pea	Carrot	Pyrethrum	Pyrethrum	Pyrethrum	Pyrethrum	Carrot
19	Potato	Pea	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
20	Potato	Pasture	Pasture	Pasture	Poppy	Barley	Pasture	Poppy

Site	2005	2006	2007	2008	2009	2010	2011	2012
22	Potato	Pasture	Pasture	Pasture	Pasture	Onion	Poppy	Pyrethrum
23	Potato	Ryegrass	Ryegrass	Onion	Pasture	Pasture	Onion	Poppy
24	Potato	Barley	Onion	Poppy	Potato	Barley	Carrot	Poppy
25	Potato	Wheat	Wheat	Ryegrass	Ryegrass	Ryegrass	Fallow	Barley
26	Potato	Pasture	Pasture	Carrot	Poppy	Pyrethrum	Pyrethrum	Pyrethrum
27	Potato	Pyrethrum	Pyrethrum	Pyrethrum	Pyrethrum	Pasture	Pasture	Poppy
29	Potato	Ryegrass	Ryegrass	Potato	Wheat	Carrot	Poppy	Wheat
30	Potato	Poppy	Ryegrass	Potato	Poppy	Pyrethrum	Pyrethrum	Pyrethrum

Table 5.A.II-10 – Land use history of the 15 South Australian sites sampled each year from 2005 to 2013

Site	2005	2006	2007	2008	2009	2010	2011	2012
1	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
2	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Potato	Canola
3	Potato	Pasture	Potato	Fallow	Trees	Trees	Trees	Trees
4	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
5	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
6	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Potato	Pasture
7	Potato	Pasture	Pasture	Pasture	Pasture	Potato	Pasture	Pasture
11	Potato	Pasture	Lucerne	Lucerne	Potato	Pasture	Potato	Broad bean
12	Potato	Pasture	Pasture	Pasture	Potato	Pasture	Pasture	Pasture
13	Potato	Lupin	Fallow	Fallow	Broad bean	Potato	Pasture	Pasture
14	Potato	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	Potato	Pasture
15	Potato	Pasture	Pasture	Pasture	Potato	Lucerne	Pasture	Pasture
16	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
19	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture
20	Potato	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture	Pasture

Horticulture Australia

PT09026BI – (30/10/2015)

Actinobacterial endophytes and their potential for disease suppression in potato production

Final Report

Professor Chris Franco

Flinders University

PROJECT SUMMARY

PT09026BI – Actinobacterial endophytes and their potential for disease suppression in potato production

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FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the A&L Canada Laboratories and the New Zealand Institute for Plant and Food Research Limited and matched funds from the Australian Government. The Department of Environment and Primary Industries Victoria, Flinders University, the University of Tasmania/Tasmanian Institute of Agriculture, the Victorian Certified Seed Potato Authority and Novozymes Biologicals Australia Pty Ltd, have provided in-kind support.

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LIST OF ACRONYMS

Acronym	Definition
AA	Amino acid mixture (containing 17 amino acids; combined 0.06 % (w/v)) (Hudson JA, 1989)
ANOVA	Analysis of variance
BD	Flinders code for potato endophytes
BLAST	Basic Local Alignment Search Tool
CFU	Colony forming unit counts
CMC	Carboxy methylcellulose
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
FEMS	Federation of European Microbiology Societies
FP	Flinders code for potato endophytes
FS	Flinders code for potato endophytes
GC	GC-rich sequence, used for the DGGE process
GGY	GGY Medium; Galactose (15 g L-1), Glutamic Acid (1 g L-1), Yeast Extract (Oxoid) (5 g L-1), K2HPO4 (0.2 g L-1), FeSO4.7H2O (0.001 g L-1), MgSO4.7H2O (0.25 g L-1), pH 7.3 (using NaOH)
H_2O	Chemical symbol for water
HPDA	Half-strength Potato Dextrose agar
HVA	Humic acid vitamin B agar
LSD	Least squares mean
MS	Mannitol-Soya Agar
ND	NanoDrop spectrophotometer
NPK	Nitrogen phosphorus and potassium fertilizer
NSW	New South Wales
NW	North West
PCR	Polymerase chain reaction
QLD	Queensland
RFLP	Terminal Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RO	Reverse osmosis
SA	South Australia

SARDI	South Australian Research and Development Institute
SAY	20 g sucrose, 1.2 g L-asparagine, 0.6 g K2HP04, 10 g yeast extract per litre RO water
TAS	Tasmania
TIA	Tasmania Institute of Agriculture
TRF	Terminal restriction fragment length
TWYE	Tap water yeast extract
TX	Texas
UK	United Kingdom
USA	United States of America

CHAPTER 5. APRP 2 - SOIL HEALTH/DISEASE MITIGATION PROGRAM

5.B NOVEL APPROACHES TO DISEASE CONTROL

5.B.I MICROBIAL ENDOPHYTES AND THEIR POTENTIAL FOR DISEASE SUPPRESSION IN AUSTRALIAN POTATO PRODUCTION

MEDIA SUMMARY

The use of pesticides and fungicides for crop production presents both an environmental and health hazard due to their toxicity. Therefore farmers are interested in using safer products and there has been an increase in research into bio control agents to replace agrochemicals. Actinobacteria are a class of microorganism that have been effective as bio control agents due to their ability to produce antibiotic compounds. However, actinobacteria isolated from soils are less effective because they have to compete with the microbes in the rhizosphere. Endophytic actinobacteria are found within the tissue of plants, including potatoes where they have been isolated from roots, leaves/stems, flowers and tubers. Some of these endophytes that are capable of preventing disease as well as improving plant growth/yield are expected to be more effective because they are able to recolonise the plant host and by doing this overcome the adverse effects of the rhizosphere. In this study a total of 452 actinobacterial endophytes were isolated from nine varieties of potato plants grown in South Australia and Tasmania. We performed a series of identification, characterization and activity tests and selected 17 endophytes for glass house and field testing. There were two endophytes that produced promising results in terms of reducing common scab and powdery scab diseases. Further testing of these two endophytes found that they were stable over repeated sub-culture, are able to sporulate in liquid culture (required for large scale application) and are compatible with a range of currently used agrochemicals.

The results of these studies show that some strains have displayed a weak activity for disease control indicating that the method of delivery and time of delivery is important for their successful application.

TECHNICAL SUMMARY

Endophytic actinobacteria are capable of preventing disease as well as improving plant growth and yield. In this study a total of 452 endophytes were isolated from nine varieties of potato plants grown in South Australia and Tasmania. Putative characterization of the isolates, revealed an abundance of members of the genus *Streptomyces* (> 50% of isolates) and species of *Microbispora, Kribbella, Micromonospora* and *Sphaerisporangium.*

Pyrosequencing and Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to identify and characterise genera from two varieties (cv. Russet Burbank and Bernadette) of potato grown at two locations (South Australia and Tasmania). When eubacterial primers were used the results from the pyrosequencing showed at least 55 genera, the predominant ones being *Enterococcus, Pantoea, Teribacillus, Curtobacteria, Bacillus, Pseudomonas, Lactococcus, Exiguobacterium, Rhodococcus,* but very low levels of *Streptomyces.* Cluster analysis showed that despite minimal overlap of genera, the

rhizospheres of the two South Australian-grown varieties (Russet Burbank and Bernadette) contained populations more closely related to each other than to the Russet Burbank grown in Tasmania.

Of the 452 isolates, 17 showed a strong *in vitro* activity against scab and rhizoctonia pathogens. Spore production of these endophytes was scaled up and spore suspensions sent to the Tasmania Institute of Agriculture (TIA) for *in planta* evaluation. In each of two seasons 2011-12 and 2012-13, two field and three pot trials tested the efficacy of several promising endophytes (coated onto seed tubers) for suppressing the key diseases common scab, powdery scab and black scurf. High disease pressure was obtained in the pot trials with low disease recorded from the field trials. Sequential assessment during early tuber development identified stem cankering and necrosis caused by the black scurf pathogen and root galling caused by the powdery scab pathogen. Except for moderate control of common scab by four strains in one field trial, no significant suppression of disease symptoms was observed with endophyte treatment compared with controls. Tuber assessments at plant senescence revealed that some endophyte treatments may be slightly suppressing tuber-based symptoms for both common and powdery scab.

INTRODUCTION

Plants provide the perfect environment for colonisation by a vast array of microbes in their phyllosphere (the above-ground surfaces of a plant as a habitat for microorganisms) and rhizospheres (soil that is directly influenced by roots and associated soil microorganisms) and as endophytes. Endophytic bacteria, defined as bacteria that can be isolated from visually asymptomatic, surface-sterilized internal plant tissues (Hallmann, 1997) are extremely diverse, as each individual plant is considered to host one or more type of endophyte (Qin et al., 2011; Strobel and Daisy, 2003). Studying plant bacterial endophytes is important for understanding ecological interactions and for developing biotechnological applications (Ryan et al., 2008). Studies of agricultural and native plants show that endophytic bacteria have the capacity to control plant pathogens, and positively contribute to plant nutrient levels and promote plant growth (Compant et al., 2010; Hardoim et al., 2008; Reinhold-Hurek and Hurek, 2011). Investigating the diversity of bacterial endophytes may lead to identification of novel natural bio control agents. Molecular-based approaches (e.g. rRNA cloning, sequencing, T-RFLP and pyrosequencing) to the study of microbial ecology generally reveal a broader microbial diversity than can be obtained by traditional cultivation methods (Conn and Franco, 2004a, b).

Actinobacteria that have been isolated from within healthy plant tissue (i.e. endophytes) can be used as inoculants to control soil borne fungal diseases. These endophytes provide a clear advantage as inoculants since they colonise the inside of plants and can deliver their function more effectively. The use of these versatile endophytic micro-organisms that produce a range of natural chemicals such as antimicrobials and plant growth promoters as well as induce systemic resistance can provide a sustainable, reliable, natural solution to improve crop yields (Franco, 2007). In our laboratory, we have isolated over 800 actinobacterial endophytes from wheat plants and have screened them in pot assays for their ability to protect wheat plants against fungal root pathogens such as Take-All, *Rhizoctonia, Fusarium* and *Pythium.* Five of these strains – one *Microbispora* spp., one *Nocardioides* spp. and three *Streptomyces* spp. were effective in controlling these fungal diseases in field trials set up across the wheat belt in Southern Australia.

This project sought to identify whether similar disease suppression could be obtained for key soil-borne diseases of potato. Diseases that have impacted the Australian potato industry over recent years include powdery scab caused by *Spongospora subterranea* (*S. subterranea*) (Tegg et al., 2013), common scab caused by *Streptomyces scabies* (*S. scabies*) (Tegg et al., 2008; Wilson et al., 2010), and black scurf caused by *Rhizoctonia solani* (*R. solani*) (Kirkwood, 2010; Sparrow and Wilson, 2012).

In this project the aim is to isolate and identify actinobacterial endophytes from potato plants that can control important soil-borne diseases of potato crops. The success achieved in controlling a range of fungal root pathogens via the addition of an inoculum of spores of endophytic actinobacteria to wheat seed in multiple field trials provided the confidence that endophytic actinobacteria can offer a sustainable solution to controlling root diseases of plants. The perceived advantage of employing endophytes is that they can colonise the internal tissue of roots and in doing so overcome the competition in the rhizosphere. The strains obtained from wheat appear to have a broad host range and were evaluated initially for efficacy. Actinobacteria that are endophytes of potato plants, including tubers, were also evaluated in these studies. The major differences between microbial pathogens of cereals and potato scab is that the former manifest during early plant growth whereas the latter develop after tubers are formed.

MATERIALS AND METHODS

ENDOPHYTE DISCOVERY - COLLECTION, CULTURING AND CHARACTERISATION OF BACTERIAL ENDOPHYTES FROM POTATO

COLLECTION OF POTATO MATERIAL AND RHIZOSPHERE

Nine varieties of potato plants, including tubers and their associated soil were collected 10-12 weeks post-planting from two areas in Australia: Peebinga, Eastern South Australia; Virginia, commercial growing area north of Adelaide. At a later date, based on a request from industry for inclusion of Russet Burbank, plants were collected from commercial growing areas Penola, South Australia and Forthside, Tasmania. The potato varieties included cv. Bernadette, Coliban, Desiree, Kestral, Maranca, White Lady, Nicola, Red Ruby and Russet Burbank (all collected from South Australian commercial fields).

Tasmanian samples (cv. Russet Burbank) were collected from typical cropping, ferrosol soils of the North West coast from Forthside research farm with tuber and soil samples randomly taken from research plots.

Plants collected were at a tuber-producing stage so that we could get tubers at different stages of maturity.

All of the soils tested were rhizosphere soils.

PREPARATION OF POTATO MATERIAL FOR CULTURING AND DNA ANALYSIS OF BACTERIAL ENDOPHYTES

SURFACE STERILISATION OF POTATO PLANTS:

The premise of this study is that endophytes offer advantages in enhancing plant growth by colonising the internal tissues of plants. Their presence within the plant places the endophyte in an optimum position to influence pathogen control either via antibiosis, induction of systemic resistance or blockage of entry points.

Potato plants (roots, stems, leaves and flowers) and tubers were washed by gently scrubbing under running water to remove loose soil and debris. The plants were then cut into two sections: roots and leaves/main stems. The roots, leaves/stems and tubers were surface sterilised to remove surface bacteria and thus only permit detection of endophytic bacteria. Plant parts were surface sterilised aseptically by a series of treatments (100% ethanol for one minute, followed by 4% sodium hypochlorite for six minutes, followed by

100% ethanol for 30 seconds, then followed by 5% sodium thiosulphate for five minutes and finally a series of 5 final rinses in sterile reverse osmosis (RO) water to remove all traces of chemicals). This material was used in one of two ways; for isolation of endophytic bacteria by direct culturing or direct detection of endophytic microbial populations using DNA analysis techniques as described below.

CULTURING AND IDENTIFICATION OF BACTERIAL ENDOPHYTES

CULTURE-DEPENDENT ENDOPHYTE ISOLATION:

Initially we used media listed in Coombs and Franco paper (Coombs and Franco, 2003), and after a study in our laboratory on improving the isolation of the less common types of actinomycetes, the media described in (Kaewkla and Franco, 2013) were used (for the detailed list, see Appendix). These media were found to yield a range of actinobacterial genera.

Using methods that have been previously published by our laboratory (Coombs and Franco, 2003), endophytic actinobacteria were isolated from the potato plant sections (tuber, root, leaf/main stem) of all nine varieties. Putative characterization of the isolates was based primarily on colony morphology.

DIRECT DETECTION OF BACTERIAL ENDOPHYTE COMMUNITIES IN POTATO TISSUE USING DNA ANALYSIS TECHNIQUES

For Culture-independent analysis the plant parts were then allowed to partially dry, cut into smaller pieces, frozen at -20^{o}C, freeze-dried and then stored in vacuum-sealed bags at 4^{o}C. Both plant and rhizosphere samples were sent to the South Australian Research and Development Institute (SARDI, Urrbrae, South Australia) for plant (endophytic) and bacterial (rhizosphere) genomic DNA extraction.

The actinobacteria isolated as endophytes can be present in soils. Our hypothesis is that a subset of soil microorganisms can colonise plants as endophytes. The aim was to find the diversity in rhizosphere soil in relation to what's in the plant. This study did not include any isolation from soil (rhizosphere or bulk) as we were not interested in soil microbes (this was specifically an endophyte project). In addition, for rhizosphere analysis, DNA methods give more complete data.

GENOMIC PREPARATION AND DETERMINATION OF YIELD AND QUALITY;

Freeze-dried potato samples were ground to a fine powder using a mortar and pestle, and then suspended in enzyme solution (0.1% macerozyme, 1.0% cellulase, 0.7 mol L^{-1} mannitol, 5 mmol L^{-1} N-morpholinoethanesulfonic acid, 9 mmol L^{-1} $CaCl_2$, 65 µmol L^{-1} KH_2PO_4, pH5.7) (Jiao et al., 2006). The suspensions were incubated for 16 hours with gentle agitation at 28^{o}C before subjected to differential centrifugation. Following Method II from Jiao and coworkers 2006, first, the mixture was centrifuged at 200 g for 5 min, the supernatant was collected and the pellet (i.e. 200 g-pellet) discarded. This was repeated three times and the supernatants pooled. Second, the above supernatant was centrifuged at 3000 g for 20 min and the pellet (i.e. 3000 g-pellet) was collected. The 3000 g-pellet was the only one where DNA was extracted as it was most likely to contain microorganisms, whereas the 200 g-pellet contained predominately plant material. Microbial DNA was extracted from the 3000 g-pellet only using the MoBio PowerFood® DNA extraction kit. At SARDI, plant samples were frozen at -20^{o}C and then freeze-dried, whilst soil was dried in a 40^{o}C oven. Genomic DNA extraction was performed on these dried samples using a proprietary method developed by SARDI.

DNA yield was determined using a PicoGreen® assay and also by using a NanoDrop spectrophotometer (ND-8000; NanoDrop, Wilmington, DE, USA). Genomic DNA samples were subjected to 0.8% agarose gel electrophoresis at 100 volts for 40 minutes and bands were detected using a LAS-4000 and ImageQuant™ software (Fujifilm Life Science, Brookvale, NSW, Australia).

TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP) ANALYSIS OF SOIL AND PLANT SAMPLES:

Using methods described in previous reports, T-RFLP analysis was performed to determine actinobacterial diversity within the plant and rhizosphere soil samples (Conn and Franco, 2004a). Briefly, The 16S ribosomal RNA (rRNA) gene was amplified from the genomic DNA by polymerase chain reaction (PCR) using the actinobacterial-biased primers 9F (5' 6-FAM-CGCGGCCTATCAGCTTGTTG 3') and 928R (5' CCGTACTCCCCAGGCGGGG 3'). Each sample (200 ng) were digested using three different restriction enzymes *Hha*1, *Rsa*1 and *Hinf*1 (New England Biolabs/Genesearch Pty Ltd, Arundel, QLD, Australia) with each restriction enzyme used individually, then heat inactivated according to the manufacturer's instructions. Digested samples were sent to Macrogen, Inc (Geumchen-gu, Seoul, Korea) for full sequencing analysis via capillary electrophoresis using their Genescan service.

Of the varieties and samples collected, together with the rhizosphere, DNA from the plant sections (tuber, root, leaf/main stem) were amplified via 16S rRNA PCR: cv. Russet Burbank from both South Australia and Tasmania, and cv. Bernadette from Virginia, South Australia. Each sample was done in duplicate and were digested with restriction enzymes as detailed above and sent to Macrogen, Inc for analysis.

These samples were chosen as representatives of varieties growing at both sampling sites.

PYROSEQUENCING ANALYSIS OF SOIL AND PLANT SAMPLES;

Pyrosequencing analysis was performed to determine actinobacterial diversity within the plant and rhizosphere soil samples. The comparison with rhizosphere soils was to determine the similarities and differences in microbial diversity between soil/rhizosphere and the endorhizosphere.

The 16S ribosomal RNA (rRNA) gene was first amplified from the genomic DNA by polymerase chain reaction (PCR) using the actinobacterial-biased Pyrosequencing primers 28F (5' GAGTTTGATCNTGGCTCAG 3') and 519R (5' GTNTTACNGCGGCKGCTG 3'). The following cycling parameters were used: 94°C for 2 min, 25 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, and then 72°C for 10 min. PCR products were analysed by 0.8% agarose gel electrophoresis at 100 volts for 40 min.

The following DNA samples (Table 5.B.I-1) were sent to Research and Testing (Lubbock, TX, USA) for Pyrosequencing analysis.

Table 5.B.I-1 – Potato samples sent for Pyrosequencing to Research and Testing Laboratory, Lubbock, Texas, USA

Sample #	Sample name	Concentration (ng μL^{-1})
1	Russet Burbank SA root	33.75
2	Russet Burbank SA leaf	12
3	Russet Burbank SA tuber	12
4	Russet Burbank SA soil	20
5	Russet Burbank TAS root	25.35
6	Russet Burbank TAS leaf	20
7	Russet Burbank TAS tuber	6.5
8	Russet Burbank TAS soil	20
9	Bernadette root SA	3.5
10	Bernadette leaf SA	20
11	Bernadette tuber SA	23.10
12	Bernadette soil SA	20

SA=South Australia; TAS=Tasmania

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) ANALYSIS;

DGGE was done by first PCR amplifying a ~300 base pair (bp) fragment of the genomic DNA samples. The primers used were 5'**CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCGCGGGGGGC**AAACTCAAAGGAATTGACGG 3' (with the addition of a GC clamp (bold) on the 5'end of the forward primer) and 5' CTTCCTCCGAGTTGACCC 3'determined to have high specificity for actinobacteria. The following cycling parameters were used: 95°C for five minutes, 35 cycles of 94°C for one minute, 51°C for one minute, 72°C for 30 seconds, and then 72°C for 10 minutes. PCR products were run on a 2% agarose gel at 100 volts for 40 minutes and quantitated using a NanoDrop. PCR products were then subjected to denaturing gradient gel electrophoresis (DCode System, Bio-Rad Laboratories Pty Ltd, Gladesville, NSW, Australia) using a denaturing gradient acrylamide gel containing 6-12% acrylamide and either 45-60% or 50-70% denaturant (formamide plus urea). Gel electrophoresis was run at 60 volts for 18-20 hours at 60°C. Gels were then silver-stained using standard methods and then scanned using an Epson scanner. DNA gels were stored at room temperature in a preservative solution (25% ethanol; 10% glycerol) for later cutting out of bands for sequencing.

IN VITRO SCREENING OF ISOLATED ENDOPHYTES AS BIOLOGICAL CONTROLS AGAINST SOIL-BORNE PATHOGENS OF POTATOES

EFFECTS OF ENDOPHYTES ON THE GROWTH OF THREE SPECIES OF PATHOGENIC *STREPTOMYCES* IN CULTURE

Endophyte and endophyte candidates were assessed for their ability as bio control agents against two of the three soil-borne potato pathogens of interest. Most of the isolates tested were actinomycetes (*Streptomyces* spp.). Artificial bioassays of this nature were not possible for *S. subterranea*, the causal agent of powdery scab, since it is an obligate pathogen that requires living tissue to propagate. The common scab pathogens *Streptomyces acidiscabies* (*S. acidiscabies*), *S. scabies* and *Streptomyces stelliscabiei* (*S. stelliscabiei*) were grown for seven days on Mannitol-Soya Agar (MS) at 27°C. The spores were harvested and enumerated using colony forming unit counts (CFU mL^{-1}). Each isolate was diluted to 10^7 to 10^8 CFU mL^{-1} with 0.85% sterile saline and one mL was applied to each Half-strength Potato Dextrose agar (HPDA) plate. Plates were then allowed to dry completely. The endophytic actinobacterial isolates were also grown for at least 10 days on this medium at their optimum growth temperatures 27°C or 37°C. Agar plugs (six mm diameter) were cut out from the endophyte isolate plates and placed on the HPDA plates containing the pathogen with spores on the top surface of the plug. The plates were then incubated for three days at 27°C and the diameter of the zone of inhibition measured.

EFFECTS OF SELECTED ENDOPHYTES ON THE GROWTH OF *RHIZOCTONIA SOLANI* AG3 IN CULTURE

For this bioassay, 311 different actinobacterial endophyte isolates were tested by growing them on a HPDA plate for seven days at 27°C. After seven days of growth of the actinobacterial endophytes, a plug taken from the leading edge of growth of *R. solani AG3* (original source provided by the South Australian Research and Development Institute (SARDI) and grown on HPDA for three days at 27°C) was placed in the middle of the plate and the plate was again incubated for seven days at 27°C . After seven days the zone of inhibition was measured.

TESTING SELECTED ENDOPHYTES FOR THE *TXT* GENE AND FOR THAXTOMIN PRODUCTION

PCR ASSAY FOR THE *TXTA* GENE:

The pathogenicity of the scab forming *Streptomyces* is via the formation of thaxtomin and the genes involved in the production of this toxin, *nec1, txtA* and *txtB* can be detected by Polymerase Chain Reaction (PCR) (Healy et al., 2000; Loria et al., 2008)

A PCR assay for the *txtA* gene (Qu et al., 2008) was also set up and used. The primers for the PCR assay are as follows: Forward primer (5'-3' Strep2F :GCAGGACGCTCACCAGGTAGT), reverse primer (3'-5' Strep2R: ACTTCGACACCGTTGTCCTCAA).

SCREENING FOR THAXTOMIN PRODUCTION

BIOASSAY TESTING OF BIOACTIVE ACTINOBACTERIAL ISOLATES:

Testing for pathogenicity determinants was carried out by PCR for the presence of the *txtA* genes and the production of thaxtomin on potato slices as described earlier. The endophytic isolates were grown on oatmeal agar at 27°C for seven days. A pathogenic strain of *S. scabies* was used as a positive control grown

under the same conditions to compare with the endophytes that were tested and an uninoculated agar plug was the negative control (Figure 5.B.I-1). The potato tubers were peeled and sterilized in 0.5% (v/v) sodium hypochlorite (NaOCl) for 10 minutes and rinsed with sterile reverse osmosis (RO) water. The tubers were sliced into disks around two cm^2, 0.5 cm thick with a sterile knife and placed in sterile petri plates. The agar plugs of the actinobacteria grown on oatmeal medium were placed culture-side down in the middle of the potato slice. The petri plates were incubated at 22°C (optimal temperature for thaxtomin production) for three days to ensure thaxtomin production and thus a positive reaction.

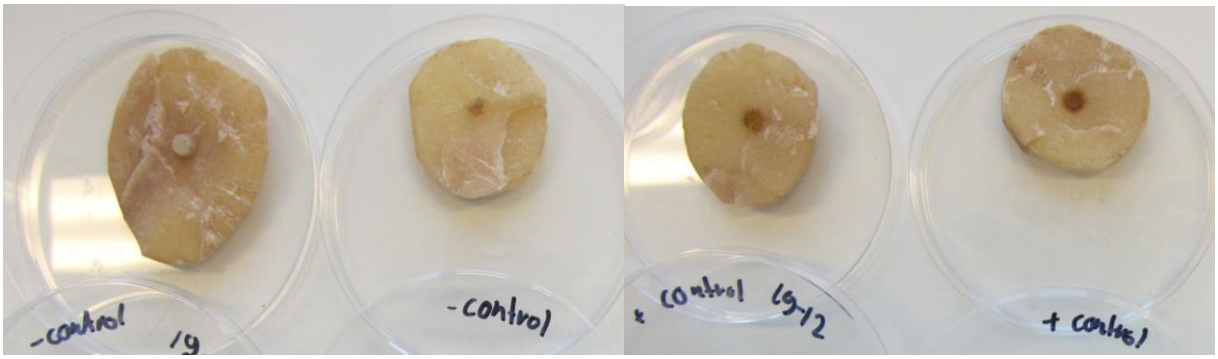

Figure 5.B.I-1 – Left: Tuber slice assay for detection of thaxtomin production by endophytic-Positive control *(Streptomyces scabies)* showing necrosis around the agar plug, Right: Tuber slice assay for detection of thaxtomin production by endophytic -Negative control showing no necrosis around the agar plug

A total of 557 endophytic actinobacteria were isolated from potato plant material. Based on the ability to sporulate effectively, 370 isolates were tested for antibacterial and antifungal activity, as well as pathogenicity by presence of *txt* genes and thaxtomin production. A total of 14 endophytes were selected for further testing in glass house and field trials in 2011-12 and were sent as spore solutions to Robert Tegg and Calum Wilson at TIA.

EVALUATING THE EFFECTIVENESS OF SELECTED ENDOPHYTIC BACTERIA IN CONTROLLING POTATO DISEASES IN THE GREENHOUSE AND THE FIELD

2011-2012 SEASON TRIALS

Fourteen isolates of endophytic bacteria with known concentration (CFU mL^{-1}) were supplied by Flinders University (Flinders University). These endophytes had previously been identified by Flinders University as possessing disease suppressive activity against cereal pathogens *in vivo* and other pathogens *in vitro*. TIA tested these endophytes under greenhouse and field conditions for disease suppression against key soil-borne diseases that impact the processing potato industry within Australia. Greenhouse trials were all undertaken in Hobart, Tasmania with field trials performed at multiple sites on the NW coast of Tasmania.

Endophyte preparation

Each isolate of endophytic inoculum was supplied with varying concentrations but all at a range greater than 10^{10} CFU mL^{-1}. Variation in settling of spores after centrifugation and different isolate characteristics meant that serial dilutions were not carried out, rather all solutions were diluted 1/100 to obtain the working concentrations that were applied to the seed tuber piece. These concentrations were all in the reported efficacious range >10^7 CFU mL^{-1}) with the endophytic isolates suspended in sterilised 0.3% v/v Xanthan gum.

Seed treatment

Potato seed was removed from cool storage approximately 1 week prior to planting, it was cut four days later and planted another three days later after endophyte treatment. Tuber pieces were physiologically at a stage where visible green sprouts (<0.3 mm) were observed in the eyes. Endophyte treatment involved pipetting approximately one mL of endophyte suspension solution per seed piece into a plastic tray and turning the tubers and gently shaking to ensure complete tuber surface coverage of the applied inoculum. After air-drying tubers were stored in a paper bag (two-12 hours) prior to planting.

Pot trials

The endophyte treatments listed in Table 5.B.I-2 were evaluated for disease suppression in two separate pot trials; one in the greenhouse against *S. scabiei* that causes common scab, the other in outdoor pots against *S. subterranea* that causes powdery scab.

Each trial had 60 separate pots that had been inoculated with either *S. scabiei* or *S. subterranea* just prior to planting. There were four replicates (pots) for each endophyte treatment and two tubers of Russet Burbank coated with the endophyte treatments and planted at ~15 cm depth in each pot. This enabled for the *S. subterranea* trial a sequential harvest of one plant at 10 weeks after planting (for root disease assessment) and a final harvest at plant senescence (approx. 16 weeks after planting). For *S. scabiei* all plants were harvested at plant senescence. Planting occurred on 17th February 2012 with pots arranged in a randomized block design. Pots were plastic (20 cm diameter) and filled with pasteurized potting mix (one part course sand, one part peat, eight parts composted pine bark; pH 6.0) that had been premixed with six kg per cubic metre fertilizer (16:3.5:10 NPK).

Common scab

For the common scab pot trial inoculum was prepared from pathogenic *S. scabiei* isolate #32 obtained from diseased potato tubers from NW Tasmania and maintained on ISP2 slopes (Tegg et al. 2008). Spores were harvested from a two-week-old culture, suspended in five mL sterile water, added to a sterilized mixture of 100 g vermiculite and 500 ml SAY solution (20 g sucrose, 1.2 g L-asparagine, 0.6 g K_2HP0_4, 10 g yeast extract, in one L water: adjusted to pH 7.2) and incubated for two weeks at 24°C where profuse sporulation was observed. Inoculum (10 g per pot) was incorporated and thoroughly mixed into the potting soil by hand. Random sampling of potting soil at tuber initiation (~four weeks after planting) and subsequent qPCR confirmed presence of high levels of common scab inoculum (5000 pg *S. scabiei* DNA g^{-1} potting mix). Overhead irrigation, providing even distribution of water to all pots was supplied every second day taking care to maintain low soil moisture content and allow the soil to periodically dry. Greenhouse temperatures were maintained between 18 and 24°C and no pesticides were applied during the trial period. At plant senescence all tubers were harvested, washed, counted and weighed. Those with a mass greater than two g were assessed for common scab disease incidence and severity. Incidence involved recording the proportion of healthy tubers with no visible lesions per pot. Severity involved scoring each tuber with a visual tuber surface cover score ranging from 0 to 6 (0 = no visible disease on tuber surface, 0.5 $\leqslant$ 1%, 1 $\geqslant$

1-5%, 2 ⩾ 5-10%, 3 ⩾ 10-30%, 4 ⩾ 30-50%, 5 ⩾ 50-70%, 6 ⩾ 70% tuber surface affected). The percentage of tuber surface covered by lesions was then estimated by taking the mid values of these score ranges. Another measure of disease severity was made by measuring the depth of the deepest lesion per tuber (excluding disease free tubers) using a 1-4 rating scale; 1 ≤ 1 mm deep, 2 = 1–2 mm deep, 3 = 2–3 mm deep, 4 ≥ 3 mm deep. All measures of disease severity were averaged across all tubers assessed per pot.

Powdery scab

For the powdery scab pot trial *S. subterranea* inoculum was obtained from heavily diseased potato tubers harvested from Riana, NW Tasmania, that had been stored (for up to a maximum of three months) in ambient cool conditions (c. 10°C) until use. Tubers were scraped and peeled with a sharp knife near and around the disease lesions collecting infected skin and underlying tissues up to one cm depth. The peeled tissue from approximately six (large) to ten (medium sized tubers) was placed in a three L plastic beaker and homogenized in a small volume of sterile water using a hand held blender. The inoculum solution was made up to 2 – 2.5 L total volume with sterile water. A standard aliquot (100 ml) of the constantly agitated inoculum solution were then added to the surface of each pot just prior to planting, with the inoculum thoroughly watered in. Pots were subsequently watered by hand-irrigation when required to maintain constant wet soil conditions. Random sampling of potting soil at tuber initiation (~four weeks after planting) and subsequent qPCR confirmed presence of high levels of powdery scab inoculum (10,000 pg *S. subterranea* DNA g^{-1} potting mix). Root galling was assessed 10 weeks after planting by carefully harvesting and washing roots; a root galling score per plant was estimated according to a visual rating scale modified from van de Graff *et al.* (Van De Graaf et al., 2007) of 0 to 4 (0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls, mostly small (<2 mm in diameter), 3 = many galls >10, some >2 mm in diameter; 4: most major roots with galls, some or all >4 mm in diameter. Additionally the number of galls per plant was counted. At plant senescence (final harvest), incidence and severity (tuber surface cover) of powdery scab were assessed as per the common scab trial above.

Table 5.B.I-2 – Fourteen isolates of endophytic actinobacteria and their concentrations used as tuber coating treatments in pot and field experiments to evaluate their efficacy against selected potato diseases. The 14 Flinders Potato endophytes and control (0.3 % v/v Xanthan gum) were used in all glasshouse and field trials.

Code	Endophyte concentrations coated onto tubers (CFU mL^{-1})
Control	0
FP 11	2.25×10^{9}
FP 14	7.5×10^{11}
FP 15	2.25×10^{13}
FP 27	8.25×10^{9}
FP 37	7.75×10^{10}
FP 52	4.25×10^{9}
FP 58	5.00×10^{9}
FP 65	9.00×10^{9}
FP 71	6.50×10^{11}
FP 86	5.50×10^{9}

Code	Endophyte concentrations coated onto tubers (CFU mL^{-1})
FP 91	6.75×10^{8}
FP 103	5.75×10^{9}
FP 107	1.10×10^{11}
FP 125	5.75×10^{9}

Field trials

Two field trials were established at a research (Forthside) and commercial farm (Wesley Vale) in NW Tasmania on potato growing ferrosol soils typical of the region. The date of planting at both trial sites was the 6th January 2012. At both field sites powdery scab had been previously recorded in prior potato crops and analysis of soil prior to planting detected the causal agent. At Forthside the key pathogen detected was at high levels (121 pg DNA g^{-1} soil) of *S. subterranea*. At Wesley Vale the key pathogen detected was at moderate level (30 pg DNA g^{-1} soil) of *S. subterranea*. At both sites no seed or soil pesticides were applied and fertiliser and irrigation scheduling followed standard commercial practice.

Forthside trial: Each individual plot contained six tubers of clean certified cv. Ranger Russet (<2 x 10^3 pg *S. subterranea* DNA g^{-1} peel) that had been coated with the appropriate endophyte treatment. The set-up was a randomised block design with two replicates (blocks).

Wesley Vale: This trial included two varieties, a clean certified cv. Ranger Russet (<2 x 10^3 pg *S. subterranea* DNA g^{-1} peel) and an uncertified cv. Innovator containing high level of *S. subterranea* (2 x 10^6 pg DNA g^{-1} peel). Each individual plot contained six tubers that had been coated with the appropriate endophyte treatment. The set-up was a randomised split-plot design with two replicates.

Disease assessment: In both trials 10 weeks after planting, one plant per replicate was harvested with roots washed and kept for infection and gall assessment as described earlier. All other plants were grown until senescence. All tubers were harvested between May 18-23 2012 and a random sample of 20 tubers per plot selected for disease assessment. Tuber surface cover and incidence were assessed as described for the pot trials.

Sample collection for endophyte identification

At various stages of plant development in the pot and field trials, potato tissue, including roots and tubers of various ages from all endophyte treatments were collected and sent to Flinders University to determine extent of colonisation of potato tissue by the various endophyte treatments.

Data analysis

Disease data for all tubers per replicate in both field and glasshouse trials were averaged prior to analysis. Data were analysed by one way analysis of variance (ANOVA) using GENSTAT 12.1 software (VSN International Ltd., Hemel Hempstead, UK) after ensuring an approximate normal distribution for each variable. The estimated percentage surface cover was to illustrate disease severity and not subject to further statistical comparisons.

2012-2013 SEASON TRIALS

In the second season of disease testing a smaller selection of endophytes (10) were chosen; this included endophytes that had shown some disease suppression activity in the previous year's greenhouse and/or field trials. Additionally a small number of new endophytes were also included in the trials. As per the previous year, multiple field trials against natural field inoculum and separate pot trials against *S. scabiei* and *S. subterranea* were undertaken. Additionally separate greenhouse trials against *R. solani* AG3 were also undertaken.

Endophyte preparation and seed treatment – Consistent with the 2011/2012 trials endophytes were prepared and coated onto tubers in an identical manner. The endophytes for testing were supplied by Flinders University in two separate batches, concentrations coated onto tubers for Greenhouse and Field trials were therefore slightly different, but were all at relatively high concentrations (Table 5.B.I-3).

Table 5.B.I-3 – Isolates used in Glasshouse and Field trials (2012-13 season):

Endophyte name	Endophyte concentrations coated onto tubers (CFU mL^{-1})	
	Field	Glasshouse
FP 14*	1.155×10^{13}	5.0×10^{12}
FP 15*	6.45×10^{13}	5.38×10^{13}
FP 27*	2.67×10^{13}	6.8×10^{12}
FP 37*	4.335×10^{13}	4.1×10^{13}
FP 65*	1.545×10^{13}	7.0×10^{12}
FP 71*	5.385×10^{13}	2.45×10^{13}
FP 91*	5.79×10^{13}	2.4×10^{13}
FP 224	2.42×10^{13}	6.0×10^{12}
FP 272	4.31×10^{13}	1.0×10^{13}
FP 297	8.20×10^{12}	6.7×10^{12}

*Endophyte strains that were also tested in 2011-12 season

Pot trials

The 10 endophyte treatments listed in (Table 5.B.I-3) and a Xanthan Gum control were evaluated for disease suppression in three separate pot trials; one in the greenhouse against *S. scabiei* that causes common scab, another in the greenhouse against *R. solani* AG3 that causes black scurf, and the other in outdoor pots against *S. subterranea* that causes powdery scab.

Each trial had 66 separate pots that had been inoculated with either S*. scabiei, S. subterranea* or *R. solani* AG3 just prior to planting. There were six replicates (pots) for each endophyte treatment and two tubers of certified Russet Burbank were coated with the endophyte treatments and planted at ~15 cm depth in each pot. This enabled for *the S. subterranea* and *R. solani* AG3 trial a sequential harvest of one plant at 10 weeks after planting (for root and stem/stolon disease assessment) and a final harvest at plant senescence

(approx. 16 weeks after planting). For *S. scabiei* all plants were harvested at plant senescence. Planting occurred on 13th December 2013 with pots arranged in a randomized block design. Pot size, potting soil and fertiliser were identical to the 2011-12 season.

Common and powdery scab

Inoculum preparation and application, trial management and disease assessments were identical to that described for the 2011-12 season.

Black scurf/stem canker

For the black scurf/stem canker pot trial *R. solani* AG3 R422 was kindly supplied as pure culture (Department of Environment and Primary Industries, Victoria). To prepare inoculum for soil application, 200 gm of millet seed was placed in a conical flask and soaked in 100 ml of water for one hour. This was then autoclaved for 20 minutes (on two consecutive days) to ensure a sterile media. *R. solani* AG3 was then transferred to the conical flasks and incubated for 14 days (20-22°C) with gentle agitation every two days. Ten grams of colonized millet was incorporated into each pot just prior to planting and incorporated through the pot by gentle hand-mixing. Random sampling of potting soil at tuber initiation (~four weeks after planting) and subsequent qPCR confirmed presence of high levels of inoculum (*R. solani* AG3 DNA). Overhead irrigation, providing even distribution of water to all pots was supplied every second day with additional hand watering where required to maintain moist soil conditions. Greenhouse temperatures were maintained between 18 and 22°C and no pesticides were applied during the trial period.

The disease assessments included measures of root infection and stem canker (Atkinson et al., 2010) made at 10 weeks after planting. Stem cankering and root necrosis were assessed using a rating scale: 0 - No disease, 1 - Less than 10% of stem/root area covered with lesions/necrosis 2 - 10-25% of stem/root area covered with lesions/necrosis 3 - 26-50% of stem/root area covered with lesions/necrosis 4 - stem/root area girdled with lesions/necrosis. Tubers were assessed at plant senescence using the scale previously described for common scab, powdery scab, and black scurf.

Field trials

Two field trials were established at a research (Forthside) and commercial farm (Wesley Vale) in NW Tasmania on potato growing ferrosol soils typical of the region. The date of planting at both trial sites was the 13th November 2012. At both field sites both powdery and common scab had been previously recorded in prior potato crops and analysis of soil prior to planting detected a range of pathogens. At Forthside pathogen levels recorded were 75 pg *S. subterranea* DNA g^{-1} soil and 5 pg *S. scabiei* DNA g^{-1} soil. At Wesley Vale the pathogen level s were 25 pg *S. subterranea* DNA g^{-1} soil and 7 pg *S. scabiei* DNA g^{-1} soil. At both sites no seed or soil pesticides were applied and fertiliser and irrigation scheduling followed standard commercial practice.

Forthside and Wesley Vale trial: Trial set-ups were identical with each individual plot containing ten tubers of clean certified Innovator (<2 x 10^3 pg *S. subterranea* DNA g^{-1} peel) and five tubers of clean certified Desiree (<2 x 10^3 pg *S. subterranea* DNA g^{-1} peel) tubers that had been coated with the appropriate endophyte treatment. The set-up was a randomised block design with two replicates (blocks).

Disease assessment: In both trials 10 weeks after planting, one plant per replicate was harvested with roots washed and kept for infection and gall assessment as described earlier. All other plants were grown until senescence. All tubers were harvested between May 1-2 2013 and a random sample of 20 tubers per Innovator plot and 15 tubers per Desiree plot selected for disease assessment. Tuber surface cover and incidence were assessed as described for the pot trials.

Sample collection for endophyte identification

As per last year tuber (2011-12 season trials) and root material was supplied to Flinders University to determine extent of colonisation by the various endophyte treatments.

Data analysis

Data analysis was identical to that described for the 2011-12 season.

Figure 5.B.I-2 –Left; Pot trial to evaluate the effects of different endophyte treatments applied as tuber coating on powdery scab disease in progeny roots and tubers (February 22 2013), Right; Pot trial to evaluate the effects of different endophyte treatments applied as tuber coating on common scab (February 22 2013)

SCALE-UP, STABILITY AND COMPATIBILITY TESTING OF SELECTED BACTERIAL ENDOPHYTES

STABILITY TESTING OF SELECTED ENDOPHYTES

After assessing field and pot trials over two seasons, two promising endophytes, FP14 and FP27 were selected for further study in a scoping study for potential commercialisation.

To be considered for commercial application, the following must be considered: is the endophyte phenotypically stable; is the endophyte able to produce large numbers of spores and can the spore production be scaled up to liquid culture (and further, to small and large scale fermentation processes), without loss of efficacy. Actinobacteria cultures FP14 and FP27 were plated out onto both MS (Mannitol Soya) and HPDA plates from spore suspensions in glycerol stocks. They were examined after 7 days of growth at 27°C. The spores were scraped off the surface and put into 10 mL of saline and spore counts conducted by haemocytometer. The spore suspension was then serially diluted in sterile RO water to yield no more than 200 CFU plate^{-1}.

After colonies could be easily distinguished on the plate, single colonies (approximately 200 colonies) were taken from both plate types at random. When a possible variation was observed, they were freshly

inoculated in a grid pattern on five MS plates. These plates were then grown for a further seven days at 27ºC. The resultant grid patterns were then scored for morphological (phenotypic) variation.

Spore production of FP14 & FP27 in submerged liquid culture: A scaling assessment to assess the capabilities of these two endophytes for commercial applications, a small-scale program of spore production scale-up was studied.

Spores production in liquid culture is preferable to solid state due to the larger surface areas to culture in solid state, thus scale up is not feasible. In utilising liquid culture, there is a more effective scale up, much faster and uniform production of spore.

GGY medium (detailed in the Appendix) (Rueda et al., 2001) has been selected because it allows *Streptomyces* cultures to sporulate in submerged culture. After studies with *Streptomyces spp*. isolated in our laboratory, the medium was modified to optimise spore production. The modified GGY medium was used to test strains FP14 and FP27 for their ability to produce spores under submerged conditions. Cultures were grown for four weeks, and samples were checked weekly via microscopy (i.e. haemocytometer counts) to determine sporulation levels.

One hundred millilitres of the medium in 500 mL conical flasks were inoculated with two loopfuls of spores from glycerol stocks. Triplicate flasks were aerated by placing them on an orbital shaker at 150 rpm at 25ºC and after one week the incubator was covered in aluminium foil to prevent light activation of the germination of spores. After a further three weeks of growth, the flask(s) was/were sampled and spore counts determined.

TESTING COMPATIBILITY OF ENDOPHYTES WITH AGROCHEMICALS

In order for these endophytes to be used, they must also be tested against common agrochemicals used in potato production. To this end, FP14 & FP27 were tested against several of these (Table 5.B.I-4). Each agrochemical was diluted or concentrated according to the application rate in the field in a series (i.e. one sixteenth, one eighth, half, quarter, two times, four times and eight times). Each treatment/test was done in triplicate.

MS agar plates were inoculated with 200 µL spore suspensions at rates of 10^6 and 10^7 for each endophyte. After the spores were spread on the surface of the agar, the plates were left partially open in the laminar flow cabinet for 20 minutes for the surface to dry. Nine six mm diameter wells were made on each agar plate and 20 µL of the below agrochemical solutions were applied to each well, one dilution per treatment per well. A control treatment of sterile water was included.

The plates were incubated at 27^{o}C for four days. The presence and radius of any zones of inhibition around the wells were recorded.

Table 5.B.I-4 – Common Agrochemicals used in potato production

Trade Name	Active Constituents	Form	Company	Label Rate
Pulse®	Polyether Modified Polysiloxane (1020 g L^{-1})	Liquid	NuFarm	200 mL 100 L^{-1}
Amistar® 250 SC	Azoxystrobin	Liquid	Syngenta	50 mL 100 mL^{-1}
Amistar® Xtra	Azoxystrobin (200 g L^{-1}) + Cyproconazole (80 g L^{-1})	Liquid	Syngenta	50 mL 200 mL^{-1}
Penncozeb®	Mancozeb (750 g L^{-1})	Powder	NuFarm	8 mg mL^{-1}
Ridomil Gold®	Mancozeb (640 g Kg^{-1}) + Metalaxyl (40 g Kg^{-1})	Powder	Syngenta	8 mg mL^{-1}
Shirlan®	Fluazinam (500 g L^{-1})	Liquid	Syngenta	400 mL 400 L^{-1}
Confidor®200 SC	Imidacloprid (200 g L^{-1})	Liquid	Bayer CropScience	25 mL 100 L^{-1}
Maxim®100 FS	Fludioxonil (100 g L^{-1})	Liquid	Syngenta	250 mL tonne of $seed^{-1}$

RESULTS

ENDOPHYTE DISCOVERY – CHARACTERISATION OF ENDOPHYTIC ACTINOBACTERIA IN POTATO PLANTS

NUMBER AND IDENTIFICATION OF ACTINOBACTERIA CULTURED FROM POTATO PLANTS:

Actinobacterial endophytes were isolated from potato plants grown in Peebinga and Virginia, South Australia. Nine varieties were used to isolate actinobacterial endophyte from surface-sterilized potato tissue. The number of isolates from each variety (Table 5.B.I-5); the number of isolates from the different parts of the potato plant (Figure 5.B.I-6) and the putative characterization of the isolates, based primarily on colony morphology (

Table 5.B.I-7) are shown below. The data from Figure 5.B.I-6 and

Table 5.B.I-7 pertain to results excluding re-isolation of endophyte treatments from 2011-2012 glasshouse trials.

Table 5.B.I-5 – The number of endophytic actinobacteria isolated from nine different potato varieties from commercial crops in South Australia

Potato variety	Number of isolates
Bernadette	26
Coliban	37

Desiree	60
Kestral	50
Maranca	75
White Lady	58
Nicola	19
Red Ruby	54
Russet Burbank	73
Total	452

Table 5.B.I-6 – The number of endophytic actinobacteria isolated from different parts of the potato plants from commercial potato crops in South Australia and Tasmania

Plant part	Number isolated
Flower (Desiree only)	35
Leaf/ main stem	91
Potato	99
Root	227
Total	452

Table 5.B.I-7 – Different genera of endophytic actinobacteria isolated from commercial potato crops of different varieties grown in South Australia and Tasmania based on morphological characteristics in culture

Genus	Number of isolates
Microbispora	128
Kribbella	1
Micromonospora	7
Streptomyces	233
Sphaerisporangium	2
not yet identified	81
Total	452

CHARACTERISATION OF ENDOPHYTIC BACTERIA IN POTATO PLANTS USING MOLECULAR METHODS

TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP) ANALYSIS

All samples amplified well, and did not require re-amplification to get enough purified DNA for restriction enzyme digestion. For all samples (with the exception of Bernadette root sample), digestion with *Hha1*, *Msp1* and *Rsa1* gave multiple peaks of different sizes, and so were able to be used for the analysis.

Results were analysed using the MiCA3 T-RFLP PATT program, which generated lists of genera based on TRF peak size. Only the genera that was present in all three restriction digests for a sample was included in the table below (Table 5.B.I-8).

Table 5.B.I-8 – Genus assignment for microorganisms from potato varieties on the basis of 16S rRNA T-RFLP analysis

	Russet Burbank, SA			Russet Burbank, TAS			Bernadette, SA	
Genus	**Roots**	**Leaves/stems**	**Soil**	**Roots**	**Leaves/stems**	**Soil**	**Leaves/stems**	**Soil**
Achromobacter		+	+					
Acidiferrobacter						+		
Acinetobacter		+	+					+
Actinobacterium		+	+	+	+	+		+
Aeromonas		+	+					
Alcaligenes		+	+		+	+		+
Alteromonas	+	+	+	+	+	+	+	+
Arthrobacter						+		+
Azoarcus		+						
Bacillus		+	+	+				+
Bathymodiolus		+	+					
Beggiatoa						+	+	
Burkholderiales		+	+			+		+
Capnocytophaga		+						
Catanema						+		
Cellvibrio	+	+	+	+	+	+	+	+
Chromatium		+	+			+		+
Chromohalobacter		+						
Citrobacter		+						
Colwellia		+						

	Russet Burbank, SA			Russet Burbank, TAS			Bernadette, SA	
Comamonas	+	+	+			+		+
Crenothrix							+	
Cycloclasticus		+						
Enterobacter						+		
Erwinia		+		+	+	+		+
Francisella							+	
Frateuria		+						
Glaciecola	+	+	+	+	+	+	+	+
Haemophilus		+						
Halomonas		+	+	+	+	+	+	
Idiomarina			+			+		
Klebsiella		+		+	+	+		+
Lamprocystis						+		
Leptothrix						+		
Marinomonas								+
Massilia		+	+					
Methylomicrobium						+		
Methylophilus		+	+			+		
Methyloversatilis						+		+
Moraxella	+	+		+	+	+	+	+
Nitrosomonas		+	+					
Oxalobacteraceae		+	+			+		
Pantoea		+	+	+	+	+		
Paucibacter						+		+
Polaromonas		+						+
Proteobacterium	+	+	+	+	+	+	+	+
Pseudoalteromonas	+	+	+	+	+	+	+	
Pseudomonas	+	+	+	+	+	+	+	+
Ralstonia		+						
Robbea		+						
Saccharophagus		+	+	+	+	+		
Serratia		+		+	+	+		+
Solimonas						+		

	Russet Burbank, SA			Russet Burbank, TAS			Bernadette, SA		
uncultured Aestuariibacter	+		+	+		+			
uncultured Aquicella	+								
uncultured Curvibacter	+					+	+		+
uncultured Cytophaga	+								
uncultured Delftia						+	+		+
uncultured Herbaspirillum	+		+						
uncultured Pelomonas						+			+
uncultured Rhodoferax	+		+	+		+			+
uncultured Sulfitobacter	+		+						
uncultured Thiobacillus	+		+			+			
Vestimentiferan	+		+	+					
Vibrio	+								
Azoarcus	+								

+ = genus present

SA=South Australia; TAS=Tasmania

PYROSEQUENCING

Pyrosequencing analysis was performed on DNA samples from the different parts of the plant and rhizosphere from Russet Burbank and Bernadette potato plants. This technique produced up to 3,000-4,000 sequence readouts with BLAST results to the genus level. The percentage of each genus present is shown in Table 5.B.I-9.

Table 5.B.I-9 – Genus assignment for clones (%) from different plant tissue types and soil from potato varieties Russet Burbank and Bernadette on the basis of Pyrosequencing analysis of 16S rRNA genes

	Russet Burbank, SA		Russet Burbank, TAS			Bernadette, SA		
Genus	**Roots**	**Soil**	**Roots**	**Leaves/stems**	**Soil**	**Roots**	**Leaves/stems**	**Soil**
Acidobacterium		3			3			4
Agrobacterium				1			3	2
Arthrobacter					3		2	13

	Russet Burbank, SA		Russet Burbank, TAS			Bernadette, SA		
Bacillus		17		10	1	90	3	6
Bradyrhizobium		3			2			
Chloroflexus					1			3
Clostridium		3						
Conexibacter		4			16			3
Curtobacterium				12			3	
Enterobacter	89		55	1				
Exiguobacterium				3				
Frigoribacterium				2			3	
Gemmatimonas					2			1
Hyphomicrobium		2						
Klebsiella	4		5					
Lactococcus				5				
Marmoricola		1			1			
Massilia								2
Methylobacterium		2		4			1	2
Microbacterium							4	
Mycobacterium		2						
Nitrosovibrio		3						
Nocardioides		2			3			4
Pantoea	5		38	16				
Patulibacter		4						
Patulibacter					16			3
Pseudomonas				4			50	1
Rhizobium		3			1			
Rhodococcus							3	
Rubrobacter								2
Sanguibacter				1			8	
Sinorhizobium					2			
Solirubrobacter		2			10			2
Sphingomonas					1			2
Sporosarcina		4						
Stenotrophomonas							3	

	Russet Burbank, SA	Russet Burbank, TAS	Bernadette, SA
Streptomyces	2	2	3
Terribacillus	36	8	4
Thermomicrobium	3		3

Hierarchical clustering was performed to observe the relatedness of the genera from the parts of the plants from the two varieties (Figure 5.B.I-3) showing Jaccard's similarity coefficient. The results indicated that the populations in the rhizosphere samples from the three locations are closely related, although those from Russet Burbank, South Australia and Bernadette are more closely related to each other than from Russet Burbank, Tasmania. Interestingly, the microbial populations in the two Russet Burbank root samples are closely related to each other, but not to the Bernadette root sample. The populations in the two leaf samples analysed do not appear to be very closely related to each other.

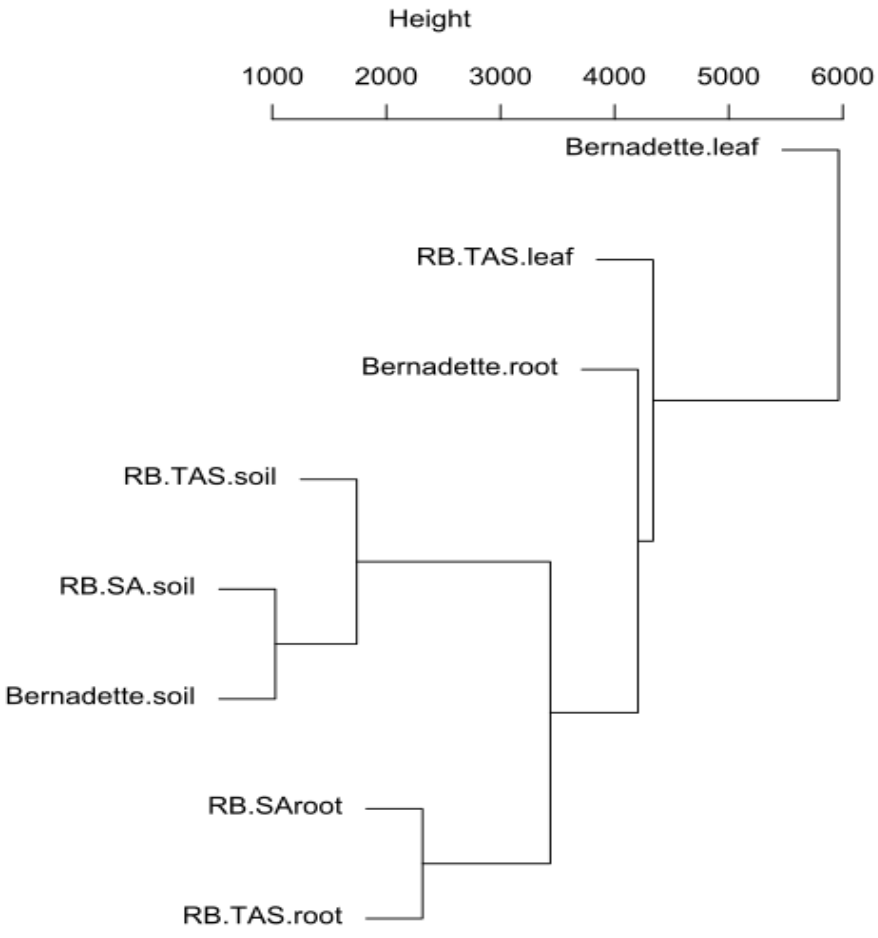

Figure 5.B.I-3 – Cluster dendogram of soil vs. root microbial populations based on Pyrosequencing analysis

SCREENING OF ENDOPHYTIC ACTINOBACTERIA FOR SUSTAINABILITY AS BIOLOGICAL CONTROL AGENTS *IN VITRO*

BIO CONTROL ACTIVITY TESTING

The results of the bio control activity of 370 of the actinobacteria isolated from potato plants are shown in Table 5.B.I-10

Table 5.B.I-10 – Summary of the *in vitro* antibiotic activity of the actinobacterial isolates from various varieties of potato plants (roots, tubers, leaves/main stem) collected from commercial crops in South Australia and Tasmania

Number of actinobacteria tested *in vivo* and number of active strains	**Number of isolates tested**
# tested vs both scab pathogens (*S. acidiscabies, S. stelliscabiei*)	370
# tested vs *R. solani AG3*	311
# active vs any *Streptomyces* spp. or *R. solani AG3*	246
# active vs *Streptomyces* spp. and *R. solani AG3*	92
# active vs both *Streptomyces* spp.	23
# active vs both *Streptomyces* spp. and *R. solani AG3*	15
Total number of isolates tested	370

SCREENING FOR PATHOGENIC DETERMINANTS

DETECTION OF THAXTOMIN PRODUCTION

Isolates which showed inhibition of common scab and *R. solani* AG3 were tested for thaxtomin A production on sterilized potato slices. Positive and negative controls (Figure 5.B.I-1) were used for evaluating the samples. The potato slices showed no clear result of necrosis caused by the endophytes, while there was a difference observed between the positive control and the negative control. The differences were not clear enough to make any reliable conclusions regarding necrosis.

DETECTION OF THE PRESENCE OF THE THAXTOMIN A OPERON

Actinobacteria were tested for the presence of the thaxomin A operon by amplifying the *txtAB* gene. The size of the *txtAB* amplification product is 71 bp. *S. scabies* was used as the positive control. The remainder of the endophyte samples were negative for the *txtAB* gene. An example of tested endophytes is shown in Figure 5.B.I-4 below (please note the prefix “BD” was early identification nomenclature, and was later replaced with “FP’).

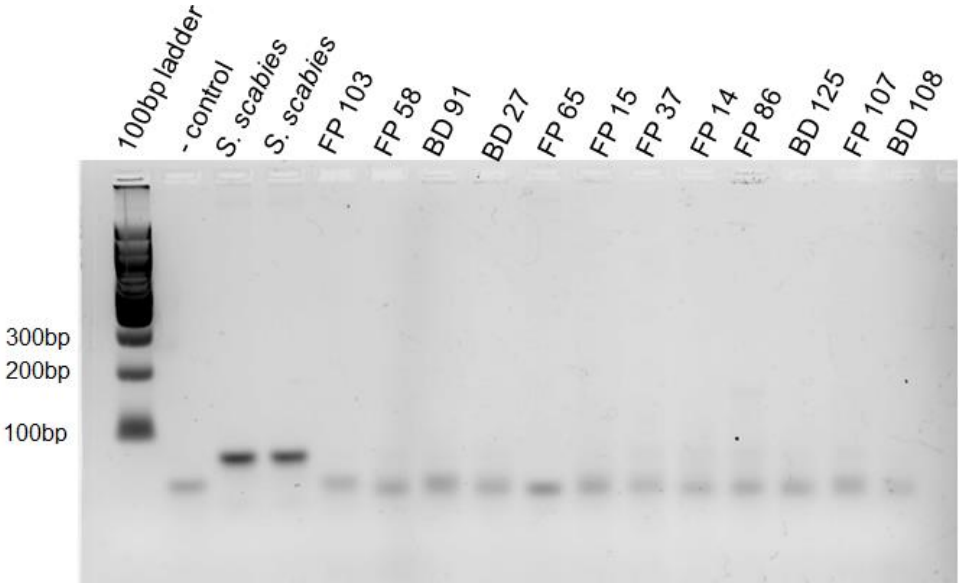

Figure 5.B.I-4 – Gel image of a portion of the endophyte DNA that was tested for amplification of the *txtAB* gene. *S. scabies* is the positive control. None of the isolate samples tested were positive for *txtAB*.

EFFICACY OF BACTERIAL ENDOPHYTES IN CONTROLLING POTATO DISEASES IN THE GREENHOUSE AND THE FIELD

2011-2012 SEASON TRIALS

POT TRIALS

Common scab

Table 5.B.I-11 shows the impact of endophyte treatment on common scab disease development. Whilst moderate disease levels were recorded across the trial (% surface covers of 1.30 – 5.02%; and infected tuber percentage of 26.7 – 64.6%); no key endophyte treatment differences were recorded at the 95% probability level, hence statistical comparison with LSDs was not valid. However, from a biological perspective, the most promising endophyte treatments at potentially reducing common scab disease were FP14, FP15, FP27 and FP52. Figure 5.B.I-5 shows typical common scab symptoms on tubers harvested from the pot trials.

Table 5.B.I-11 – The incidence and severity of common scab disease development of Russet Burbank tubers pre-treated with fourteen different endophyte treatments. Potting soil was inundated with *S. scabiei* G#32, and tubers were harvested at plant senescence

Endophyte treatment	Common scab disease			
	Tuber surface cover		Lesion depth score	Infected tubers
	score (0-6)a	(%)a	(1-4)b	(%)c
Control	0.71	2.75	1.50	58.3
FP 103	1.00	3.94	1.31	58.3
FP 107	0.88	3.10	1.77	57.5
FP 11	0.67	2.47	1.63	48.5
FP 125	0.74	2.40	1.70	62.1
FP 14	0.38	1.30^	1.33	26.7
FP 15	0.58	1.93^	1.56	47.2
FP 27	0.58	1.76^	1.33	54.2
FP 37	0.98	3.80	1.54	61.7
FP 52	0.47	1.53^	1.67	35.0
FP 58	1.06	5.02	1.65	63.8
FP 65	0.69	3.19	1.67	37.5
FP 71	0.93	3.64	1.55	63.5
FP 86	0.91	3.66	1.19	61.3
FP 91	0.95	3.79	1.58	64.6
F prob	0.849	na	0.761	0.725
lsd(p=0.05)	ns		ns	ns

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Depth of deepest lesion per tuber (excluding disease free tubers); 1 ≤ 1 mm deep, 2 = 1–2 mm deep, 3 = 2–3 mm deep, 4 ≥ 3 mm deep.

[c]Proportion of tubers with at least one common scab lesion.

^Endophyte treatments that show activity (non-significant, p>0.05) relative to the control.

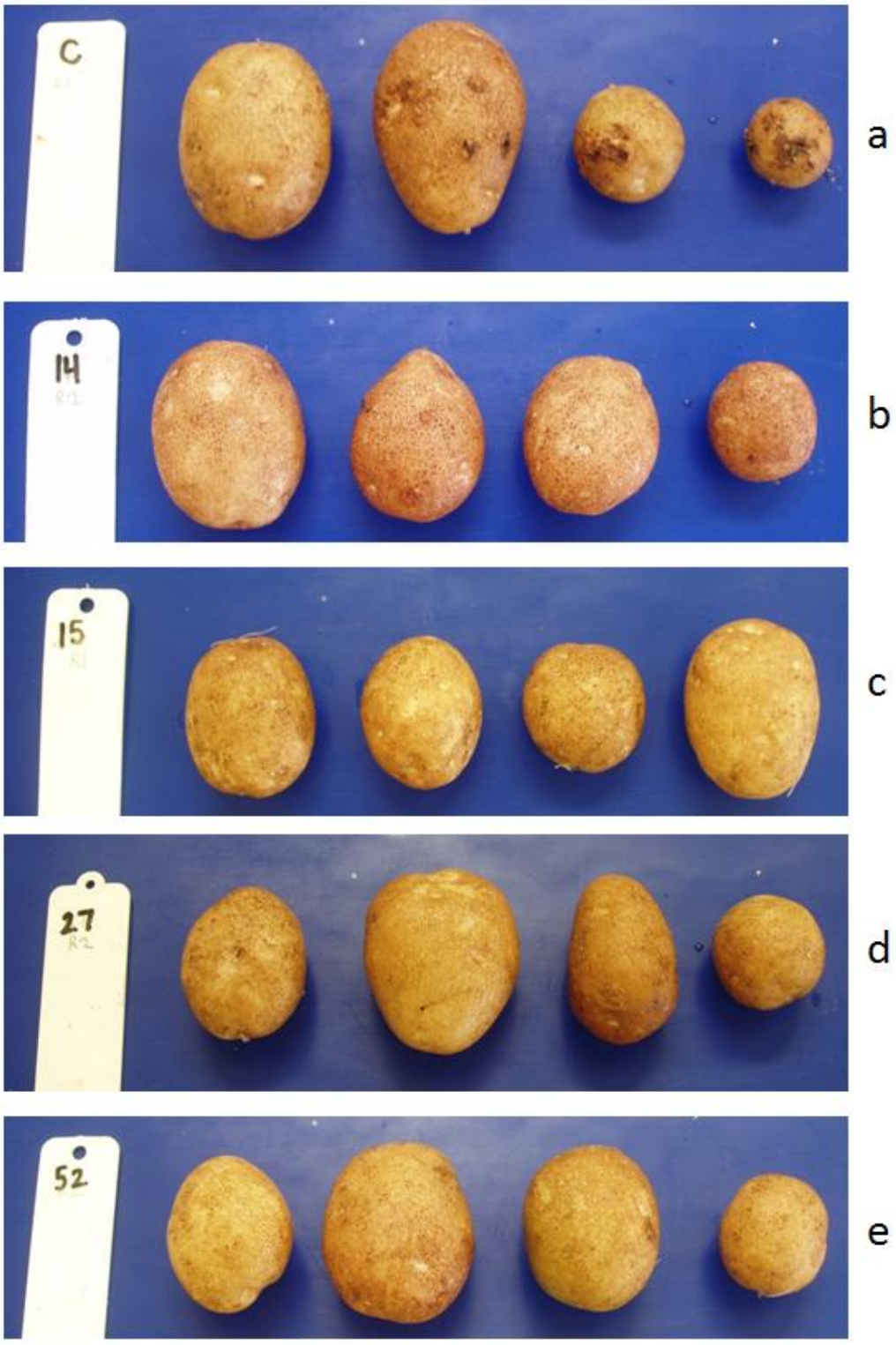

Figure 5.B.I-5 – Typical tuber symptoms on Russet Burbank tubers from the common scab pot trial. Tubers were pre-treated with endophyte treatments: a) control, b) FP14, c) FP15, d) FP27, and e) FP52. Note fewer symptoms on four of the most promising endophyte treatments.

Powdery scab

Table 5.B.I-12 shows the impact of endophyte treatment on powdery scab disease development. Whilst moderate disease levels were recorded across the trial (% surface covers of 0.94 – 3.34%; and infected tuber percentage ranged from 38.3 – 76.7%); no key endophyte treatment differences were recorded at the 95% probability level, hence statistical comparison with LSDs was not valid. However, from a biological perspective, the most promising endophyte treatments at potentially reducing powdery scab disease were FP14, FP37 and FP65.

Likewise there were no significant endophyte effects on root galling production, although significant galling was recorded across all treatments (Table 5.B.I-12, Figure 5.B.I-6). FP14 once again showed potential bio control activity in showing less galling than the control.

Table 5.B.I-12 – The effects of actinobacteria coating treatments of seed potato tubers of Russet Burbank on the incidence and severity of powdery scab disease on the roots and tubers of the progeny grown in a potting media inoculated with *S. subterranea.* Assessment of root material occurred at 13 weeks after planting whilst plants were actively growing; tubers were harvested later at plant senescence (19 weeks after planting)

Endophyte treatment	Powdery scab disease				
	Tuber symptoms			Root galling	
	Tuber surface cover		Infected tubers	Score	Number
	Severity score (0-6)a	(%)a	(%)b		
Control	0.69	2.04	66.7	2.5	12.3
FP 103	0.98	3.34	70.8	2.1	7
FP 107	0.74	2.36	62.5	2.1	7.3
FP 11	0.62	2.01	52.1	2.5	9.8
FP 125	0.70	2.24	60.8	2.5	11.3
FP 14	0.34	0.94^	38.3	1.9^	7.5
FP 15	0.60	1.74	62.5	2.6	11.3
FP 27	0.67	2.40	68.8	2	6.6
FP 37	0.50	1.50^	47.9	2	6.1
FP 52	0.63	1.71	66.7	2.3	7.8
FP 58	0.75	2.28	68.3	2.1	6.8
FP 65	0.39	1.09	41.7	2.5	12.5
FP 71	0.86	2.69	76.7	2.4	9.5
FP 86	0.71	1.98	75.0	2.9	13.3

Endophyte treatment	Powdery scab disease				
FP 91	0.75	2.69	68.3	2	7.3
F prob	0.923	na	0.7	0.628	0.501
lsd (p=0.05)	ns		ns	ns	ns

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one powdery scab lesion.

[c]Root galling score per plant was estimated according to a visual rating scale from 0 to 4 (0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls, mostly small, <2 mm in diameter, 3 = >10 galls, some >2 mm in diameter, 4 = most major roots with galls, some or all >4 mm in diameter.

^Endophyte treatments that show activity (non-significant, p>0.05) relative to the control.

Figure 5.B.I-6 – Examining potato plant roots for the presence of powdery scab root galls

FIELD TRIALS

Field trials produced low disease outcomes (with surface covers of 0-0.9% and infected tuber percentages of 0-14%) for all three diseases at both sites and across both cultivars (Ranger Russet and Innovator). The trials were pooled together to present one summary (Table 5.B.I-13). As per the previous trials there were no statistically significant differences generated by endophyte treatment. However, strain FP15 showed some activity against powdery scab; whilst FP14, FP65, FP71 and FP91 showed a reduction in black scurf symptoms. Figure 5.B.I-7 shows typical tuber symptoms of disease from tubers harvested from the field trials.

Table 5.B.I-13 – Powdery scab, common scab and black scurf disease development of Ranger Russet and Innovator tubers pre-treated with fourteen different endophyte treatments. Plants were grown in natural field conditions at two different sites (results pooled), and tubers were harvested at plant senescence

Endophyte treatment	Disease					
	Powdery scab		Common scab		Black scurf	
	Surface cover (%)a	Infected tubers (%)b	Surface cover (%)a	Infected tubers (%)b	Surface cover (%)a	Infected tubers (%)b
Control	0.06	3.95	0.02	0.80	0.25	3.57
FP 103	0.12	7.07	0.04	3.40	0.15	3.50
FP 107	0.05	3.80	0.02	0.80	0.96	14.45
FP 11	0.05	2.63	0.01	1.12	0.09	4.85
FP 125	0.05	2.50	0.01	1.12	0.20	5.68
FP 14	0.05	4.18	0.05	3.03	0.01^	1.38
FP 15	0.01^	1.05	0.07	3.13	0.35	9.45
FP 27	0.06	2.17	0.04	2.33	0.08	6.27
FP 37	0.20	9.10	0.14	5.30	0.62	7.58
FP 52	0.15	7.22	0.05	3.72	0.19	5.83
FP 58	0.04	2.45	0.06	1.98	0.72	8.88
FP 65	0.06	4.42	0.01	1.52	0.05^	2.90
FP 71	0.09	3.97	0.00	0.00	0.00^	0.00
FP 86	0.05	4.82	0.10	2.78	0.13	2.78
FP 91	0.23	11.63	0.09	4.33	0.06^	2.12
F prob.	0.301	0.305	0.898	0.887	0.821	0.924
lsd (p=0.05)	ns	ns	ns	ns	ns	ns

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one lesion.

^Endophyte treatments that show activity (non-significant, p>0.05) relative to the control.

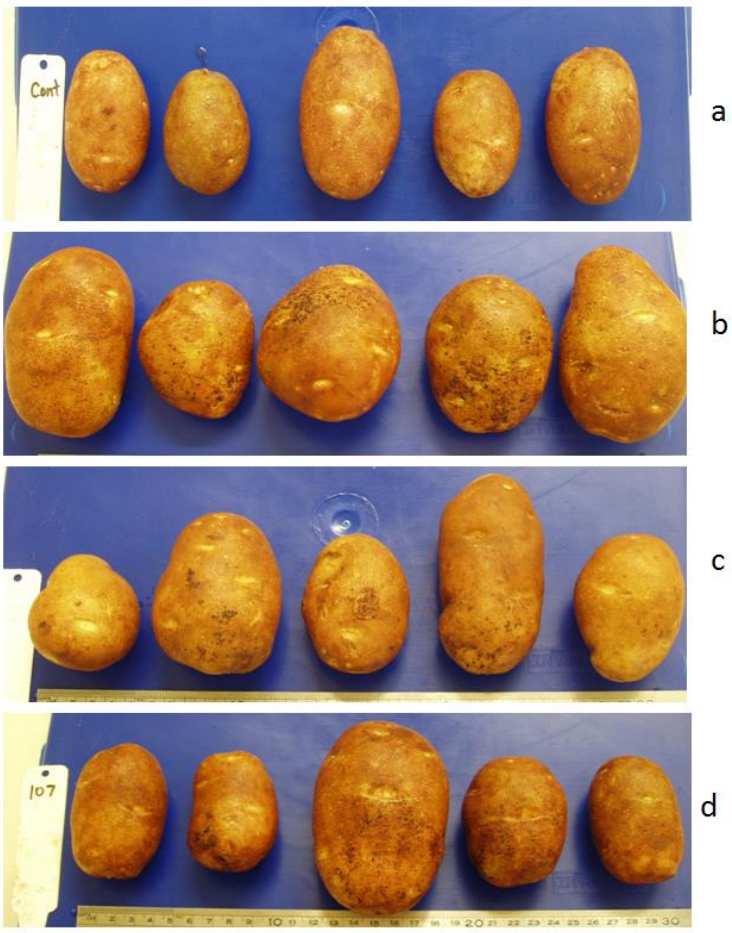

Figure 5.B.I-7 – Typical tuber symptoms on Innovator from the field trials. Tubers were pre-treated with endophyte treatments: a) control, b) FP58, c) FP86, and d) FP107. Note the control tubers show less disease whilst some of the endophyte treatments show black scurf symptoms.

ISOLATION OF ENDOPHYTES FROM 2012 GLASS HOUSE TRIALS (FLINDERS UNIVERSITY)

This part of the study was aiming to achieve two goals:

1. To determine if the particular endophyte treatment could be re-isolated, based on morphological characteristics.
2. To identify endophyte diversity once a treated plant has grown and produced tuber.

In 2012, a total of 14 endophytic isolates were sent to TIA for testing against scab diseases in glasshouse trials. Tubers were coated with the endophyte in xanthan gum and planted in pots containing potting soil and *S. scabies*. Once harvested, tubers that were scab free and tubers that exhibited surface scabs were sent to Flinders University. The aim was to re-isolate the original endophyte, as well as to isolate any other endophytes that were present in the peel and tuber.

Clean (no apparent disease) and scabby tubers from each of the following glasshouse pots inoculated with endophyte inoculants were sent to Flinders University from the glass house trials in Tasmania:

FP 14, 37 & 52, which showed initial activity in terms of disease reduction based on pot trials; and

FP 58, which showed initial increase in scab compared to pot trial control.

The tubers were surface-sterilised and the endophytes isolated as per the protocols outlined earlier. After surface sterilisation, the tubers were aseptically peeled and endophytes isolated from the peel and tuber. The tuber and peel tissues were plated onto various isolation media and incubated at either 27°C or 37°C. The number of isolates from the various treatments and their putative genera, based on morphology (Table 5.B.I-14 and Table 5.B.I-15) is shown below.

Table 5.B.I-14 – Number of endophytic isolates based primarily on morphological features

Genus	Number of isolates
Microbispora	37
Micromonospora	15
Streptomyces	12
not yet identified	2
Total	66

Table 5.B.I-15 – Isolation and characterisation of endophytes from various endophyte treatments, based primarily on morphological features in culture

Tuber + treatment	Disease state	Tissue used	Microbispora	Micromonospora	Streptomyces	not yet identified
FP14	Clean	Tuber	4	1	1	0
	Clean	Peel	2	0	1	0
	Scabby	Tuber	6	1	0	0
	Scabby	Peel	1	3	0	0
FP37	Clean	Tuber	2	2	0	0
	Clean	Peel	1	0	0	0

FP52	Scabby	Tuber	0	0	1	0
	Scabby	Peel	1	1	0	0
	Clean	Tuber	5	0	0	0
	Clean	Peel	3	2	1	0
	Scabby	Tuber	8	2	3	0
	Scabby	Peel	0	0	0	0
FP58	Clean	Tuber	1	2	0	0
	Clean	Peel	3	0	3	0
	Scabby	Tuber	0	1	2	1
	Scabby	Peel	0	0	0	1

This was to determine if the original inoculants (the four FP isolates) could be recovered from tubers, as well as isolating additional endophytes that may be specific to the Tasmanian environment.

2012-2013 SEASON TRIALS

POT TRIALS

Common scab

Table 5.B.I-16 shows the impact of endophyte treatment on common scab disease development. Whilst moderate-high disease levels were recorded across the trial (% surface covers of 2.16 – 10.90%; and infected tuber percentage of 51.4 – 95.0%); no endophyte treatment differences were recorded at the 95% probability level, hence statistical comparison with LSDs was not valid. However, the most promising endophyte treatments that showed potential in reducing common scab disease were FP14, FP224, FP27, FP272 and FP91.

Table 5.B.I-16 – Common scab disease development of Russet Burbank tubers pre-treated with ten different endophyte strains. Potting soil was inundated with *S. scabiei* G#32, and tubers were harvested at plant senescence (n = 6)

Endophyte treatment	Common scab disease			
	Tuber surface cover		Lesion depth score	Infected tubers
	score (0-6)[a]	(%)[a]	(1-4)[b]	(%)[c]
14*	0.79	2.52^	1	51.4
15*	1.14	9.34	1.40	66.6
224	0.68	2.24^	1.17	54.8

Endophyte treatment	Common scab disease			
27*	0.87	2.98^	1.07	60
272	0.84	2.73^	1.15	70
297	1.22	5.57	1.30	80.2
37	1.43	6.96	1.48	78.4
65	1.16	4.92	1.36	74.6
71	1.84	10.90	1.34	80
91	0.69	2.16^	1.20	60.6
control	1.66	7.63	1.52	95
F prob	0.126	na	0.676	0.148
lsd (p=0.05)	ns		ns	ns

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Depth of deepest lesion per tuber (excluding disease free tubers); 1 ≤ 1 mm deep, 2 = 1–2 mm deep, 3 = 2–3 mm deep, 4 ≥ 3 mm deep.

[c]Proportion of tubers with at least one common scab lesion.

* Endophyte treatments that reduced common scab disease (but not statistically significantly) in the 2011-12 season

^Endophyte treatments that show biological activity relative to the control in the current season.

Powdery scab

Table 5.B.I-17 shows the impact of endophyte treatment on powdery scab disease development. Whilst moderate disease levels were recorded across the trial (% surface covers of 1.17 – 2.71%; and infected tuber percentage of 36.0 – 69.5%); no key endophyte treatment differences were recorded at the 95% probability level, hence statistical comparison with LSDs was not valid. However, the most promising endophyte treatments with potential to reduce powdery scab disease were FP15 and FP297.

Table 5.B.I-17 – Powdery scab disease development of Russet Burbank tubers pre-treated with ten different endophyte treatments. Potting soil was inundated with field-collected *S. subterranea* inoculum. Assessment of root material occurred at 12 weeks after planting whilst plants were actively growing; tubers were harvested later at plant senescence - 19 weeks after planting (n=6)

Endophyte treatment	Powdery scab disease				
	Tuber symptoms			Root galling	
	Tuber surface cover		Infected tubers	Score[c]	Number
	score (0-6)[a]	(%)[a]	(%)[b]		
14*	0.42	1.35	36.0	2.2	7.0
15	0.41	1.22^	41.0	2.5	13.0
224	0.59	1.83	53.5	2.2	7.7
27	0.77	2.54	61.3	2.3	6.7
272	0.45	1.31	47.3	2.3	10.7
297	0.39	1.17^	37.2	2.2	6.3
37*	0.84	2.71	69.5	2.7	16.0
65	0.43	1.36	36.8	2.2	6.7
71	0.48	1.52	42.7	2.3	9.3
91	0.72	2.52	46.0	2.0	3.7
control	0.64	1.85	56.5	2.5	9.3
F prob	0.32	na	0.3	0.734	0.144
lsd (p=0.05)	ns		ns	ns	ns

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one powdery scab lesion.

[c] Root galling score per plant was estimated according to a visual rating scale from 0 to 4 (0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls, mostly small, <2mm in diameter, 3 = >10 galls, some >2mm in diameter, 4 = most major roots with galls, some or all >4mm in diameter.

* Endophyte treatments that reduced powdery scab disease (but not statistically significantly) in the 2011-12 season

^Endophyte treatments that show biological activity relative to the control in the current season.

Likewise there were no significant endophyte effects on root galling production, although significant galling was recorded across all treatments (Table 5.B.I-17).

Black scurf

Table 5.B.I-18 shows the impact of endophyte treatment on black scurf disease development. Whilst very high disease levels were recorded across the trial (% surface covers of 5.88 – 15.60%; and infected tuber percentage of 100%); no key endophyte treatment differences were recorded at the 95% probability level, hence statistical comparison with LSDs was not valid. However, the most promising endophyte treatments that potentially reduce black scurf disease were FP27 and FP37.

Table 5.B.I-18 – Black scurf disease development of Russet Burbank tubers pre-treated with ten different endophyte treatments. Potting soil was inundated with *R. solani* AG3 inoculum. Assessment of root material for necrosis and stem cankering occurred at 10 weeks after planting whilst plants were actively growing; tubers were harvested later at plant senescence - 19 weeks after planting (n=6)

Endophyte treatment	Black scurf disease			
	Tuber surface cover		Infected tubers	Root necrosis score(0-4)[c]
	score (0-6)[a]	(%)[a]	(%)[b]	
14	2.35	15.60	100	1.1
15	1.65	7.63	100	1.1
224	1.64	7.62	100	1.0
27	1.33	5.88^	100	1.3
272	2.20	13.90	100	1.1
297	2.15	13.80	100	0.9
37	1.37	6.56^	100	0.8
65	1.55	7.46	100	1.2
71	1.97	11.90	100	1.2
91	1.78	7.85	100	1.2
control	1.91	11.43	100	1.0
lsd (p=0.05)	ns			ns
F prob	0.244	na	na	0.729

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with black scurf present.

[c]Root necrosis:0-No disease, 1- Less than 10% of root area showing necrosis 2-10-25% of root area showing necrosis 3-26-50% of root area showing necrosis 4- stem/root area girdled with lesions/necrosis.

^Endophyte treatments that show biological activity relative to the control in the current season.

Likewise there were no significant endophyte effects on root necrosis score (Table 5.B.I-18). Stem cankering was also observed and recorded with every stem from every treatment showing canker development approximately 10% up the stem from the base; no discernable endophyte treatment differences could be made on canker development.

FIELD TRIALS

Field trials produced low disease outcomes (with surface covers of 0-0.49% and infected tuber percentages of 0-12.5%) for the two diseases identified at both sites and across both cultivars (Innovator and Desiree). The trials were pooled together from both sites to present one summary for each cultivar (Table 5.B.I-19). For Innovator, there was no significant impact of endophyte treatment on powdery scab, compared to the control. For common scab there was a significant impact of endophyte treatment compared to the control with FP14, FP27, FP65 and FP71 producing less disease; although surface covers were very low anyway. Of note both FP14 and FP27 showed promise in the pot trial against common scab. In Desiree there were no endophyte treatments that performed statistically better than the control. Samples of tubers harvested from the field trials are shown in Figure 5.B.I-8, Figure 5.B.I-9 and Figure 5.B.I-10.

Sequential harvesting through the growing season provided material to Flinders University for endophyte extraction. At the same time root and stems were examined for root galling and stem cankering, but were not present.

Table 5.B.I-19 – The incidence and severity of powdery scab, common scab and black scurf disease on the progeny tubers of Innovator and Desiree seed tubers treated with ten different endophyte treatments in the field at two different sites (results pooled).

	Endophyte treatment	Disease					
		Powdery scab		Common scab		Black scurf	
Cultivar		Surface cover (%)[a]	Infected tubers (%)[b]	Surface cover (%)[a]	Infected tubers (%)[b]	Surface cover (%)[a]	Infected tubers (%)[b]
Innovator	14	0.13	2.5	0.04*	1.25*	0	0
	15	0	0	0.18	4.18	0	0
	224	0.05	1.68	0.41	10.83	0	0
	27	0	0	0*	0*	0	0
	272	0.04	1.25	0.18	4.18	0	0
	297	0.08	2.5	0.13	2.5	0	0
	37	0.05	1.68	0.08	2.5	0	0
	65	0.18	5.85	0*	0*	0	0

	71	0.04	1.25	0*	0*	0	0
	91	0.00	2.93	0.13	2.5	0	0
	control	0.18	5.83	0.32	6.68	0	0
	lsd (p=0.05)	ns	ns	0.248	5.28		
	F prob.	0.481	0.31	0.03	0.006	na	na
	14	0	0	0.15	5	0	0
	15	0.08	2.5	0.08	2.5	0	0
	224	0	0	0	0	0	0
	27	0	0	0	0	0	0
	272	0.08	2.5	0.08	2.5	0	0
	297	0.08	2.5	0.6	12.5	0	0
Desiree	37	0.08	2.5	0.08	2.5	0	0
	65	0.08	2.5	0	0	0	0
	71	0.08	2.5	0	0	0	0
	91	0.15	5	0.49	12.5	0	0
	control	0.15	5	0	0	0	0
	lsd (p=0.05)	ns	ns	ns	ns		
	F prob.	0.723	0.723	0.071	0.108	na	na

[a]Estimated tuber surface coverage is calculated from disease cover score (0 = no disease, 0·5 = 0–1%, 1 = 1–5%, 2 = 5–10%, 3 = 10–30%, 4 = 30–50%, 5 = 50–70%, 6 ≥ 70%) using median percentile scores within the allocated range.

[b]Proportion of tubers with at least one powdery or common scab lesion, presence of black scurf lesions.

*Endophyte treatments that show less disease relative ($P < 0.05$) to the control, for the given disease tested.

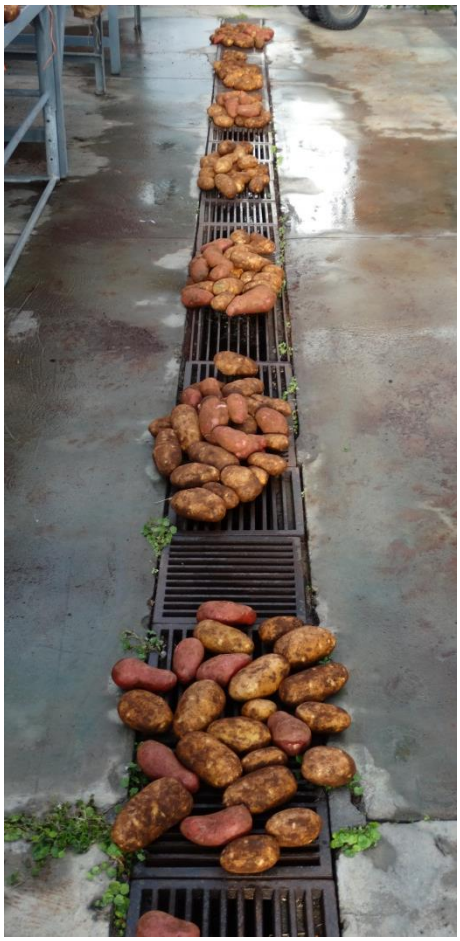

Figure 5.B.I-8 – Selection of field tuber samples after washing

Figure 5.B.I-9 – One replicate of an endophyte treatment showing both Innovator and Desiree tubers

Figure 5.B.I-10 – Close-up of a Desiree tuber showing common scab disease symptoms

SCALE-UP, STABILITY AND COMPATIBILITY TESTING OF SELECTED BACTERIAL ENDOPHYTES

STABILITY TESTING OF ACTIVE STRAINS

Both FP 14 and FP27 cultures are highly sporulating, producing 10^{13} spores per 9 cm agar plate.

Both cultures can produce spores in liquid culture. FP 14 produced approximately 8 x10^{9} CFU mL^{-1} and FP 27 produced approximately 6 x10^{8} CFU mL^{-1}. Figure 5.B.I-11 and Figure 5.B.I-12 show the set up and sporulation of cultures FP14 and FP27 in GGY liquid medium.

Figure 5.B.I-11 – FP14 cultured in liquid medium

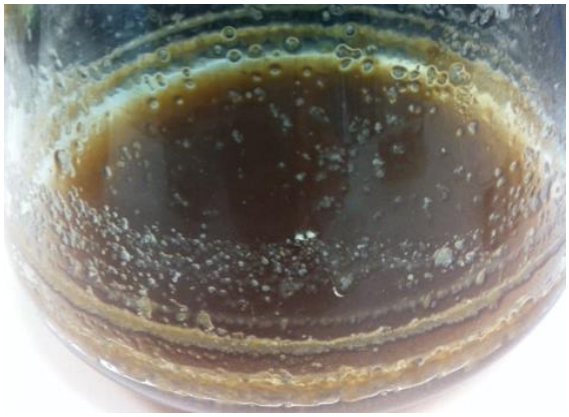

Figure 5.B.I-12 – FP27 cultured in liquid medium

SCALE UP OF SPORULATION IN LIQUID MEDIUM

No phenotypic variations were observed for either strain FP14 or FP27 as single colonies.

The colonies that were transferred and streaked out in grids showed good sporulation and no apparent change in appearance when compared to the parent culture.

COMPATIBILITY WITH AGROCHEMICALS

Pulse® (Polyether Modified Polysiloxane)
FP27: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells
FP14: no clear zones at any rate of application

Amistar® 250 SC (Azoxystrobin)
FP27: no clear zones at any rate of application
FP14: no clear zones at any rate of application

Amistar® Xtra (Azoxystrobin + Cyproconazole)
FP27: no clear zones at any rate of application
FP14: no clear zones at any rate of application

Penncozeb® (Mancozeb)
FP27: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells
FP14: complete inhibition of actinobacterial growth at all rates

Ridomil Gold® (Mancozeb + Metalaxyl)
FP27: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells
FP14: complete inhibition of actinobacterial growth at all rates

Shirlan® (Fluazinam)
FP27: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells
FP14: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells

Confidor® 200 SC (Imidacloprid)
FP27: no clear zones at any rate of application
FP14: no clear zones at any rate of application

Maxim® 100 FS (Fludioxonil)
FP27: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells
FP14: complete inhibition of actinobacterial growth at all rates; growth observed around H_2O control and empty wells

DISCUSSION

Endophytic actinobacteria have been found to control fungal root diseases that manifest early in the season in cereal crops. Their effectiveness is due to their ability to colonise internal plant tissues, thereby overcoming competition with other microorganisms in the rhizosphere. When tested against potato diseases in this study, they have shown to be more variable.

For black scurf, which occurs early in the season, two strains showed a significant reduction in disease levels in pot trials even though the disease rating was high. The field trials with this pathogen did not result in any disease. For common scab and powdery scab, which are diseases that occur on the tuber, there was no significant reduction in disease as a result of endophyte coating of tubers either in pot trials, where disease levels were high, or in field trials, which had a lower disease incidence.

Analysis of the bacterial population at the tuber stage revealed a large number of bacteria with very low levels of distinguishable inoculant. The implication is that improved delivery systems are required to maintain a high level of inoculum through the stages of plant growth to be effective at controlling these late emerging diseases. The efficacy of the biocontrol microorganism may be improved by introducing it into the plant at the callus stage so that colonisation is established within the plant in higher numbers than by using the current methods which introduce the inoculant around the roots of the young plant. There is still no guarantee that an inoculant added at the early stages of plant growth will necessarily remain within the plant roots as the tuber is forming. In other plant systems the endophytic actinobacterium inoculant induces systemic resistance to fungal and bacterial pathogens. However the actinobacterium is a very poor inducer of systemic resistance on its own, and was found to prime the plant when the bacterial or fungal pathogens were added as a challenge. It is possible that the scab pathogens which belong to other species of actinobacterium, may not elicit a systemic defence response as they share a number of morphological and physiological traits as the inoculant, and therefore not recognised as a pathogen.

Molecular methods used in this study show that the dominant non-pathogenic bacteria found in tubers are Methylobacterium and Sphingomonas spp. Therefore, isolation and screening this group of bacterial endophytes may lead to the development of inoculants that could maintain higher levels in tubers of a beneficial microorganism

Bacterial diversity in potato plants assessed by culture dependent and molecular techniques

This study used a combination of culture-dependant and -independent methods to isolate and characterise endophytic and other bacterial populations from various potato plants, grown in South Australia and Tasmania, Australia. In general, actinobacterial endophytes could be isolated readily from all the parts of the potato plant. Each of the varieties provided sufficient isolates, though it was noted that there were differences in numbers of isolates obtained between the varieties. This is not necessarily due to a lower number of endophytes being present in the particular variety, but could also be the result of the presence of fungal or bacterial endophytes, even though antifungals and antibacterial antibiotics were used.

Of the potato varieties tested, 50% of actinobacterial isolates were found in roots, which support other findings that this is an endophyte-rich organ (Manter et al., 2010). These results have shown that the endophytic community is diverse and that the most abundant genus recovered was *Streptomyces*, which is consistent with other reports from a variety of hosts (Qin et al., 2011).

Next generation sequencing analysis, such as pyrosequencing, has identified numerous new endophytic bacterial species, from many plant and rhizosphere types, including potatoes (Inceoglu et al., 2011). Our study aimed at identifying and characterising genera from two varieties of potato, grown at two locations, by 16S rRNA sequencing. The genera of bacteria present in plant roots grown in the two soils (South Australia and Tasmania) were unique to each location and only in a few cases did the same genera occur in both soils for all three samples

The abundance of any one genus in any of the three rhizosphere samples did not exceed 17%, which supports other findings that this is a diverse and bacteria-enriched environment (Pisa et al., 2011). The roots of the two Russet Burbank consisted predominately of *Enterobacter* (89% for South Australian grown and 55% for Tasmanian grown), whereas for the Bernadette variety, *Bacillus* was 90% abundant and not present in either cv. Russet Burbank samples. This finding suggests that there are different requirements for nutrient levels and growth between potato varieties, which has been shown previously (Inceoglu et al., 2011). The endophytic populations of the leaf samples were diverse, with *Terribacillus* (36%) being the most abundant genus for Russet Burbank (Tasmania), and *Pseudomonas* the most abundant for Bernadette (50%). Cluster analysis showed that, despite minimal overlap of genera, the rhizospheres of the two South Australia grown varieties (Russet Burbank and Bernadette) contained populations more closely related to each other than to Russet Burbank (Tasmania), which supports studies that found that diversity is determined by soil type and field locations (Weinert et al., 2010). The genera in the roots of the two Russet Burbank varieties were more closely related to each other than to microorganisms in Bernadette, supporting other work that investigated differences between cultivars (Inceoglu et al., 2011).

The use of pyrosequencing has provided a baseline for characterisation of endophytic bacteria in two potato varieties at two field locations. These results show that environmental factors such as soil type or climate have a stronger influence than plant genotype on the constituents of the endophytic microbial populations. Further studies using a limited number of common cultivars grown in different soil types under controlled conditions (glasshouse) are required to confirm these results and to reveal the influence both of soil type and soil microbial populations on the composition of the endophytic community.

In this study, two molecular methods were applied: pyrosequencing and T-RFLP. Both methods resulted in a large amount of data, with little cross-over of genera identified. Such discrepancy between molecular methods is not uncommon. In this scenario, the pyrosequencing data is used primarily, as this technique

gave us up to 3,000-4,000 sequence readouts with BLAST results to the genus level. It has increased the ability to characterize populations by orders of magnitude and provided more definitive results. On the other hand accuracy of the T-RFLP method is limited by the multiple genera identified for each TRF. However as the cost of T-RFLP is at least one magnitude lower than pyrosequencing, it can still be used to monitor changes in populations that have been identified by pyrosequencing. The pyrosequencing data can be used to set up T-RFLP identification tables which will allow the accurate identification of TRFs for the specific experiment.

Molecular methods have always revealed a broader microbial diversity when compared to cultivation based methods, including endophytes with potatoes (Manter et al, 2010) and often the most dominant members of the isolated populations are found to be only a small percentage of the population revealed by non-cultivation based approaches.

In our study where the focus has been on actinobacterial endophytes, we have confirmed that by using selective media, endophytic actinobacteria can be isolated from individual plant parts of nine potato varieties grown in Australia, with *Streptomyces* being the predominant species.

***In vitro* screening of actinobacterial isolates**

The actinobacterial endophyte isolates were tested for their ability to control the growth of common scab and *R. solani* AG3 pathogens *in vitro*. A similar *in vitro* test for the powdery scab pathogen *S. subterranea* could not be done as this pathogen requires living plant tissue to grow.

Of the over 300 isolates that were tested against scab pathogens *S. acidiscabies* and *S. stelliscabies* and *R. solani* AG3, 15 isolates showed activity against all 3 pathogens. As these active strains belonged to the genus *Streptomyces* they were screened further for the presence of pathogenic determinants such as the *txtA* and *txtB* genes in addition to the production of thaxtomin on potato slices. None of the 15 strains contained any pathogenic traits so they were cultivated for spore production. The spores were harvested and enumerated before they were sent for glasshouse and field testing by the team at the Tasmanian Institute for Agriculture.

2011-2012 and 2012-2013 Glasshouse and Field Trials of selected endophytes

The 14 Flinders Potato isolates (or a subset of 10 of these for the 2013/2013 trials) and control (containing the 'sticker' Xanthan gum) were used in all glasshouse and field trials.

Two pot trials and two field trials were conducted throughout the 2011-12 growing season in Tasmania to test the performance of 14 selected endophytes, which were coated onto potato seed. Seed included Russet Burbank, Ranger Russet and Innovator, three key processing cultivars. The pot trials tested endophyte performance with the potting media inundated separately with either *S. scabiei* or *S. subterranea* inoculum. The two field trials had various levels of naturally occurring inoculum including *S. scabiei*, *S. subterranea* and *R. solani* AG3. Commercial planting and other trials adjacent to this trial plot that were planted at the same time had moderate disease levels.

Both pot trials during 2011/2012 targeting powdery and common scab produced moderate disease levels (surface covers of approx. 1 – 5%, and infected tuber percentages of 26-75%), however no significant effects were noted due to endophyte treatment. Some of the endophyte treatments showed potential bio control activity and included FP14, FP15, FP27 and FP52 for common scab symptom reduction. FP14 and FP37 showed potential bio control activity for powdery scab tuber symptom reduction whilst one

endophyte FP14 once again showed potential bio control activity as it produced less galling than the control.

Field trials during 2011/2012 revealed low disease levels of the three key diseases identified (common scab, powdery scab and black scurf) with surface covers of 0-0.9% and infected tuber percentages of 0-14%. No significant differences from endophyte treatments were recorded. However, potential bio control activity was displayed by strain FP15 against powdery scab; whilst FP14, FP65, FP71 and FP91 showed a reduction in black scurf symptoms.

In conclusion, whilst there were no absolute standout endophyte treatments that significantly suppressed disease from a statistical perspective, some treatments did show potential bio control activity in a couple of trials. Both FP14 and 15 were definitely worthy of further disease screening against these three pathogens, while the other three endophyte strains were also tested further.

From the 2011-2012 trials, seven of the strains tested had shown disease suppressive activity. Three new strains were included as they showed *in vitro* suppression of three key soil borne diseases, common scab, powdery scab and black scurf, caused by the pathogens *S. scabiei*, *S. subterranea* and *R. solani* AG3, respectively.

Three glasshouse trials and two field trials were conducted over the 2012-13 growing season in Tasmania to test the performance of 10 selected endophytes, which were coated onto potato seed. Seed cultivars included Russet Burbank, Innovator and the disease susceptible Desiree. The pot trials tested endophyte performance with the potting media inundated separately with either *S. scabiei*, *S. subterranea* or *R. solani* AG3 inoculum. The two field trials had various levels of naturally occurring inoculum including *S. scabiei*, *S. subterranea* and *R. solani* AG3.

Three glasshouse trials targeting powdery scab, common scab and black scurf produced moderate to very high disease levels (surface covers of approx. 1.2 – 15.6%, and infected tuber percentages of 36-100%). At these levels, however, no significant effects were noted due to endophyte treatment.

The endophyte treatments that showed some biological activity in glasshouse trials against 1. Common scab included FP14, FP224, FP27, FP272 and FP91; 2. Powdery scab were FP15 and FP297; and 3. Black scurf included FP27 and FP37. No endophyte treatment showed significant disease suppression across more than one pathogen type.

Field trials revealed very low disease levels and whilst four endophyte treatments (FP14, FP27, FP65 & FP71) showed significant reductions against common scab, compared to the control, this was under very low disease pressures.

In conclusion, whilst there were no endophyte treatments that significantly suppressed disease from a statistical perspective, some treatments did show activity in a couple of trials and may be worthy of further investigation.

On the basis of their 16S rRNA gene sequences the active strains were characterised as:

FP 14: 99% similarity ***Streptomyces rubrogriseus*** ATCC 43691 = DSM 41477

FP15: 98% similarity ***Streptomyces variabilis*** ATCC 19815 = DSM 40179

FP 27: 99% similarity ***Streptomyces violaceorubidus*** ATCC 43697 = DSM 41478

FP37: 99% similarity ***Streptomyces variabilis*** ATCC 19815 =DSM 40179

FP71: 99% similarity ***Streptomyces pseudogriseolus*** ATCC 12770 = DSM 40026

FP91: 98% similarity ***Streptomyces thermocarboxydus*** DSM 44293 = JCM 10368.

FP 297: 98% similarity ***Streptomyces glomeratus*** strain DSM 41457 = JCM 9091

Scale-up, stability and compatibility studies

The results of the scale up and stability tests with the two most effective actinobacterial isolates *Streptomyces* spp. FP14 and FP27 show that both strains are capable of transfer to an industry partner. Both isolates are stable with subculture and do not show the presence of variants, and both have the ability to produce abundant spores on agar media as well as in submerged liquid media. The latter is an important feature for technology transfer because the production of spores on solid surfaces is a limiting step. Production of spores in a well aerated liquid medium allows the scale-up to larger volumes in a shorter time.

Compatibility studies with a limited number of agrochemicals available at the time have shown the effect of specific chemicals on the actinobacteria *Streptomyces spp*. FP14 and FP27. This information can be used to select the treatments that may be used when the microbial inoculants are applied. The microbial agents are also likely to replace some of these toxic agents leading to a safer and more sustainable production of potatoes.

Our study has shown that the agrochemicals Amistar® (Azoxystrobin), Amistar® Xtra (Azoxystrobin + Cyproconazole) and Confidor® (Imidacloprid) had no effect on the growth or sporulation of either FP14 or FP27.

In contrast, the agrochemicals Penncozeb® (Mancozeb), Ridomil Gold® (Mancozeb +Metalaxyl), Shirlan® (Fluazinam) and Maxim® (Fludioxonil) all restricted growth of FP14 and FP27. Interestingly, the application rate appeared to have little impact on the growth of either endophytes tested. The surfactant Pulse® was the only agrochemical tested that showed a marked difference in the growth of the endophytes; FP14 growth was not impacted and produced spores effectively, while FP27 growth was completely inhibited.

TECHNOLOGY TRANSFER

Publications

1. The abstract below is of the following poster presented at the symposium Plant Protection and Plant Health in Europe 2013 - Endophytes for Plant Protection: The State of the Art. Berlin 27-30 May, 2013.

ENDOPHYTIC BACTERIAL POPULATIONS OF POTATO PLANTS

Christopher Franco, Stacey Smith

ABSTRACT

The endophytic actinobacterial population of roots, leaves and rhizosphere of potato plants was examined using culture dependant methods and compared to molecular characterisation based on total Pyrosequencing analysis of bacterial 16S rRNA genes. The predominant genera isolated from surface-sterilised roots and leaves were *Streptomyces* and *Microbispora*, together with lower numbers of *Micromonospora*. When eubacterial primers were used the results from the Pyrosequencing showed at least 55 genera, the predominant ones being *Enterococcus, Pantoea, Teribacillus, Curtobacteria, Bacillus, Pseudomonas, Lactococcus, Exiguobacterium, Rhodococcus,* but very low levels of *Streptomyces*. Cluster analysis revealed that the populations in roots were similar to soil populations, but not to the leaf populations.

2. A peer-reviewed paper has been published as:

Franco C; Smith S, Endophytic Bacterial Populations of Potato Plants. In: Schneider C, Leifert C, Feldmann F (Eds), Endophytes for plant protection: the state of the art, pp. 19-26. © 2013 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-11-2.

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

The results of the project have shown that actinobacterial endophytes with disease suppression properties are present in potato plants and can be isolated and tested for use in the potato industry.

In order to obtain isolates which can be used for the potato industry, the actinobacteria were tested *in vitro* for their ability to suppress pathogenic *Streptomyces* causing common scab as well as to suppress *R. solani* AG3. There was no *in vitro* assay to evaluate the effectiveness against the causal agent of powdery scab *S. subterranea.* Actinobacteria which displayed an *in vitro* activity versus the three scab isolates as well as against *R. solani* AG3 were selected for glasshouse studies and field trials. As the diseases manifest late in the growth stages the resources and time requirements for *in planta* evaluation of bio control are higher than with many other crops where the root diseases occur within the first few weeks after sowing.

In general, while *in vitro* assays are useful indicators of the ability of the actinobacteria to produce antibiotics that can inhibit pathogens they are not the only factor that predict efficacy in controlling plant pathogens. Induction of systemic resistance by actinobacterial colonisation is another mechanism used for suppression of disease (Conn et al., 2008) which can be observed only by *in planta* testing.

Therefore, more rapid *in planta* screening methods are required for these potato diseases.

These studies have found that two strains have been effective in reducing symptoms of common scab in field trials when disease pressure is low, but not in glasshouse trials which contain artificially high levels of pathogen. The late emergence of disease symptoms for common scab and powdery scab requires the presence and persistence of the inoculant. Identification of the added bio control strain from the actinobacterial isolates reisolated from the treated plants was not possible due to large numbers of morphologically similar isolates. However, molecular methods reveal very low levels of colonisation of actinobacteria of the tuber and also the potato plant roots.

Improved methods for the monitoring of the colonisation of the added inoculum will also be required. Due to the size of the actinobacterial mycelium (~0.2-0.5 µm) and immune-gold staining procedure will be best suited to observe the *in planta* colonisation.

Improved delivery systems need to be trialled to deliver the inoculum during tuber development. Addition of inoculant at this stage will mean that large amounts will be required with low possibility that it will reach the sites in or around the plant where it is required. This is likely to be an uneconomic proposition.

An alternative approach is to establish the inoculant as an endophyte in the elite tissue culture used for minituber production. If this establishment can be achieved with a long term presence in the tissue it may be possible to maintain endophyte levels through the stages of plant growth at levels that will maintain efficacy of disease suppression when required.

ACKNOWLEDGEMENTS

Mrs Stacey Smith (Flinders) and Dr Robert Tegg (TIA) have been the main contributors to the experimental work carried out in this project. Dr Jeff Barrett (Flinders) was responsible for setting up the T-RFLP studies and a number of visiting exchange students, notably Mr Niels Hendrickx, Caroline Vogels and Bas van Dongen have contributed to the isolation and characterisation of the actinobacterial endophytes.

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APPENDIX

The isolation media used were:

1. Tap water yeast extract (TWYE; containing 0.25g yeast extract (Oxoid), 0.5g K2HPO4 and 18g agar (Oxoid) per 1L RO H2O)

2. Mannitol-Soya agar (MS; containing 20g Mannitol, 20g Soya Flour and 20g agar (all from Oxoid) per 1L RO H2O)

3. Humic acid vitamin B agar (HVA) (Hayakawa, 1987)

4. VL70 Starch agar (VL70 S)

5. VL70 with amino acid mixture (containing 17 amino acids; combined 0.06 % (w/v)) (Hudson JA, 1989)

(VL70 AA)

6. VL70 Carboxy methylcellulose (VL70 CMC)

The composition of VL70 medium was taken from Joseph et al. (Joseph et al., 2003) and Schoenborn et al. (Schoenborn et al., 2004). The pH of all media was adjusted to 7.2. Benomyl (DuPont) was added to all of the agar media at 50 µg ml^{-1} to control (endophytic) fungal growth.

The media used for purifying actinobacterial cultures were:

1. MS agar

2. Half strength potato agar (HPDA; containing 19.5 g potato Dextrose agar and 7.5 g agar (both form Oxoid)

The medium used for liquid culture testing was:

1. GGY Medium; Galactose (15 g L^{-1}), Glutamic Acid (1 g L^{-1}), Yeast Extract (Oxoid) (5 g L^{-1}), K2HPO4 (0.2 g L^{-1}), FeSO4.7H2O (0.001 g L^{-1}), MgSO4.7H2O (0.25 g L^{-1}), pH 7.3 (using NaOH).

Horticulture Australia

PT09026 – (30 October 2015)

Sub Program B(ii) "Optimisation of 2,4-D and novel chemical foliar treatments for control of Common Scab"

Final Report

Dr Calum Wilson et al.

Tasmanian Institute of Agriculture
University of Tasmania

PROJECT SUMMARY

PT09026 – Sub Program B(ii) "Optimisation of 2,4-D and novel chemical foliar treatments for control of Common Scab"

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This is the final report for Sub Program B(ii) "Optimisation of 2,4-D and novel chemical foliar treatments for control of Common Scab". Part of the "APRP2: Soil health – Disease Mitigation Program, Project Number PT09026.

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FUNDING SUPPORT

The wider project to which this sub program belongs has been funded by HAL using the processed potato industry levy, voluntary contributions from the A&L Canada Laboratories and the New Zealand Institute of Plant and Food and matched funds from the Australian Government. The Department of Primary Industries Victoria, Flinders University, the University of Tasmania/Tasmanian Institute of Agriculture, the Victorian Seed Potato Authority and Novozymes Biologicals Australia Pty Ltd, have provided in-kind support.

Additionally for this sub-program, we acknowledge financial contribution of the University of Tasmania, and the Thomas Crawford memorial Fund for PhD scholarships.

DISCLAIMER

The following report detailed studies on compounds and use patterns not currently registered in Australia and was for research purposes only.

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MEDIA SUMMARY

Common scab is an economically important disease of potato found in most growing regions of the world. Although not directly affecting tuber yields, disease lesions markedly reduce tuber value and require extra processing steps during French fry production. As a result, crops with severe disease are often rejected by processing companies and seed crops with moderate levels of disease will be downgraded to ware quality resulting in substantial losses to the producers.

There are few practical control methods and none that are both reliable and effective. Disease may be minimised through planting resistant varieties, strategic use of irrigation, seed treatments and delayed planting.

In earlier work, we showed that certain chemicals when applied to the foliage of a potato crop can suppress common scab disease in the developing tubers. This study refined this prior work, and determined optimal rates and timing of novel foliar and tuber applied chemical treatments for control of common scab. Importantly, the chemicals are not fungicides and do not kill the common scab pathogen. Rather they appear to induce disease resistance in the potato and protect it from infection.

The study identified several alternate chemicals that provide disease suppression, and demonstrated a common mechanism of action. Treatments when applied at the optimal time and rate gave excellent disease control with no observable detrimental effects on plant growth, tuber yield and quality.

Specific recommendations from this project are:

a) Commercial development of foliar sprays and/or tuber treatments as a cost effective disease mitigation strategy easily integrated into current production practices. This requires engaging the agrichemical industry, and facilitating registration trials
b) Further studies on tuber treatments. These would reduce exposure of operators and the environment to chemical treatments, would reduce the amount of material needed, and would ensure all plants receive material at the earliest stage of crop growth.
c) Further fundamental research on the mechanisms of thaxtomin suppression and disease mitigation.

TECHNICAL SUMMARY

Common scab is an economically important disease of potato found in most growing regions of the world. Although not directly affecting tuber yields, disease lesions markedly reduce tuber value and require extra processing steps during French fry production. As a result, crops with severe disease are often rejected by processing companies and seed crops with moderate levels of disease will be downgraded to ware quality resulting in substantial losses to the producers. Conservative estimates of losses due to this disease in Tasmania alone are in excess of A$3.5 million per annum. There are few practical control methods and none that are both reliable and effective. Disease is minimised through planting resistant varieties, strategic use of irrigation, seed treatments and late planting.

Common scab is caused by pathogenic Streptomyces spp. that produce thaxtomins, necrosis-causing phytotoxins that are essential for pathogenicity. Previous research had found that 2,4-Dichlorophenoxyacedic acid (2,4-D), a herbicide and synthetic auxin, controlled common scab symptoms when applied to the foliage of potato, but also resulted in undesirable phytotoxic effects. It has been demonstrated that when 2,4-D is translocated to potato tubers, it suppresses thaxtomin toxicity.

This study determined optimal rates and timing of 2,4-D application for control of common scab whilst minimising phytotoxic effects of the treatments. It found that treatment of potato plants as soon as 5 days after emergence provided greater protection against common scab and greater suppression of thaxtomin toxicity in harvested tubers than treatments after tuber initiation. Rates much lower than had previously been tested were found to reduce disease and induce toxin tolerance to levels similar to that obtained with treatments at near herbicidal rates, suggesting that maximum toxicity suppression occurred at very low tuber 2,4-D levels. These very low rates did not induce any noticeable phytotoxic symptoms, nor affect harvested tuber yield or quality, and resulted in 2,4-D residue levels well below maximum residue limits in tubers at harvest. Additionally, it was found that if tuber seed pieces were treated prior to planting, daughter tubers would have some protection from disease and show tolerance to the toxin without an additional post emergence treatment.

In this study we also tested a range of different chemicals for both disease suppression and their ability to inhibit thaxtomin A toxicity in both Desiree and Russet Burbank cultivars. Our results showed different chemicals varied in their ability to suppress common scab. Our tuber slice assays with thaxtomin A showed a strong correlation between the ability of the chemical to suppress common scab symptom development and also the ability of the chemical to inhibit thaxtomin A toxicity. A Bayesian measurement error linear regression model was derived for each cultivar and trial and demonstrated a clear positive relationship between disease and thaxtomin A-induced necrosis. The relationships obtained were much stronger than would have been obtained without adjustment for measurement error. This demonstrates that disease mitigation using chemical foliar sprays is strongly correlated with the ability of the chemical to inhibit thaxtomin A toxicity suggesting this mechanism as a key mode of action for understanding this novel disease control strategy.

Specific recommendations from this project are:

a) Commercial development of foliar sprays and/or tuber treatments of 2,4-D or 2,5-DBB as a cost effective disease mitigation strategy easily integrated into current production practices. This requires engaging the agrichemical industry, and facilitating registration trials
b) Further studies on tuber treatments with 2,4-D and alternate chemistries. These would reduce exposure of operators and the environment to chemical treatments, would reduce the amount of material needed, and would ensure all plants receive material at the earliest stage of crop growth.

Further fundamental research on the mechanisms of thaxtomin suppression and disease mitigation.

CHAPTER 1: OPTIMISATION OF 2,4-D FOLIAR TREATMENT RATES

INTRODUCTION

Common scab is a globally important soil-borne disease of potatoes characterised by scab-like lesions on the tuber surface (Loria et al. 2006). It is caused by a number of pathogenic *Streptomyces* spp., most notably *S. scabies*. Pathogenic *Streptomcyes* spp. produce a number of phytotoxins, called thaxtomins (King et al. 1989) which are essential for disease development. Common scab symptoms can be reproduced through the direct application of thaxtomins (Lawrence et al. 1990; Leiner et al. 1996). The capacity of *Streptomcyes* spp. to produce thaxtomin A is correlated with their pathogenicity (Loria et al. 1995; Goyer et al. 1998; King et al. 2001; Kers et al. 2005). Thaxtomin production is therefore an important pathogenicity factor, and the reduction in its phytotoxic effect may be one method of controlling common scab.

There is no single method for effectively controlling common scab. Disease control is currently attempted through a combination of strategies, including the management of planting dates (Waterer 2002), the planting of moderately resistant varieties(Wilson et al. 2009), and strategic use of irrigation during early tuber development (Lapwood et al. 1973), the latter used extensively in the United Kingdom. Irrigation has not been as effective in Australia for management of common scab, and increasingly scarce and expensive water resources worldwide require other management strategies to be sought. Despite various adoptions of these practices, common scab occurs frequently and results in substantial losses around the world.

Treatment of potato seed pieces with fungicides or other biocides can be useful in some circumstances (Wilson et al. 1999), but there are no currently available effective soil-applied chemical treatments. Pentachloronitrobenzene (PCNB) was a soil-applied fungicide that did provide reasonable disease suppression and was widely used, however, the material is a known carcinogen and its use has been withdrawn from most potato production regions around the world. After the banning of PCNB in the United Kingdom, a series of trials were run between 1973 and 1978 to test a range of alternate chemicals for their ability to control common scab of potato in order to find a PCNB substitute. The trials initially focussed on materials incorporated into the soil, with a number of chemicals demonstrated to be as effective as PCNB, but they displayed varying degrees of toxic effects on the plant (McIntosh 1973;1976). Later trials evaluated foliar applied chemical treatments, and found 2,4-dichlorophenoxyacetic acid (2,4-D) and 3,5-dichlorophenoxyacetic acid (3,5-D) to be highly effective in controlling disease in glasshouse trials. However both materials produced significant toxic effects on treated plants, including reduced yield, an increased numbers of small tubers, and tuber deformation. Further to these toxic effects, the level of control of common scab provided by 3,5-D treatment dropped dramatically in field testing. As such, it was not considered to be a viable control method by the authors (McIntosh et al. 1981; McIntosh et al. 1982).

The mechanism by which 2,4-D, a synthetic auxin and potent herbicide, controls common scab was not determined by McIntosh and his colleagues. Effective levels of 2,4-D did not affect the growth of the pathogen, or its ability to produce thaxtomin (McIntosh et al. 1981; Tegg et al. 2008). Studies by Tegg et al. (2008; 2012) demonstrated a strong relationship between disease levels and the tolerance of tuber tissues to thaxtomin induced toxicity, in plants that have been treated with 2,4-D. Furthermore, Tegg et al (2012) found that a range of other foliar applied chemicals which also reduce common scab similarly increase tuber thaxtomin tolerance. They showed a significant negative correlation between the extent of disease expressed and the reduction in necrosis following thaxtomin treatment in tubers harvested from treated plants. This suggested 2,4-D and these other materials may operate by reducing sensitivity of potato tubers to damage by thaxtomin, and by this reducing the invasive capacity of the pathogen.

McIntosh and colleagues (1981) found that 200mg/L applications of 2,4-D and 2,5-D reduced the development of common scab symptoms by 50% and 90% respectively, compared to control treatments, but at the rates applied caused an increased number of deformed tubers, increased overall tuber numbers, and decreased tuber size. Additionally, McIntosh et al (1982) found 3,5-D to be less effective as a control against common scab in field conditions, with reductions of only 30%. 2,4-D is a phytotoxic, synthetic auxin that is most commonly used as a herbicide. It is systemic, with 2,4-D applied to foliage being translocated through the plant. When applied to the leaves of potato plants, it is translocated to the tubers where it is metabolised (Burrell 1982). Recommended herbicidal application rates range from a minimum concentration of approximately 680mg/L applied until runoff for lawns (Nufarm Australia Limited 2011).

Early Australian research (Tegg 2006) into 2,4-D induced resistance of common scab of potato used a rate of application the same as that used by McIntosh et. al (1981), of 200mg/L 2,4-D, applied up to three times to the one crop. It was found to be effective but still resulted in an increase in tuber deformation and yield reduction. Further research (Tegg et al. 2008) found that a lower application rate of 50mg/L 2,4-D, a quarter of the rate used previously, still provided similar levels of induced toxin tolerance to those observed from the 200mg/L rate. Additionally, this rate was still effective from a single application, a reduction of up to a sixth of the original amount of 2,4-D applied in previous trials. However, at these rates, levels of 2,4-D in the tuber six weeks after application were still above the Australian maximum residue level (MRL), of 100ng/g of tuber fresh weight (Commonwealth of Australia 2011), with residue levels increasing with application rate.

This chapter described experiments to determine the relationship between the rate of 2,4-D application and resultant disease control; to determine the level at which 2,4-D is no longer effective at controlling disease; to determine the effect of rate on the toxicity of 2,4-D; and to determine the optimal rate that achieves the maximum possible disease control with the lowest, yet still practical, spray concentration.

MATERIALS AND METHODS

Two glasshouse trials and one field evaluated varying rates of 2,4-D applied as a foliar treatment to potato plants for control of common scab.

PLANTS AND INOCULUM

Planting material:

A fortnight prior to planting, potato tubers of the varieties Russet Burbank and Desiree were removed from cold storage, where they had been stored since harvesting approximately 6 months before.

For the glasshouse trials, tubers were disease free mini-tubers harvested from glasshouse grown tissue culture plantlets. For glasshouse trial #1 the tubers were cut into approximately 10g pieces and left to suberise before planting. For glasshouse trial #2, 10g tubers were used and were planted whole.

For the field trial tubers were commercial certified seed free of visible disease without any seed fungicide treatments.

Inoculum:

Pathogenic *Streptomyces scabies* strain G#20, initially isolated from a common scab infected tuber harvested from North West Tasmanian in 1990, was used as inoculum in the glasshouse experiments. *S. scabies* G#20 was grown on 10mL ISP2 (Shirling & Gottlieb 1966) agar slopes (10g/L malt extract, 4g/L yeast extract, 4g/L glucose, 12g/L agar, pH 7.3) until sporulation. Colonised agar slopes were then aseptically transferred to a sterilised mixture of 120g vermiculite and 500mL SAY solution (20g/L sucrose, 1.2g/L L-asparagine, 0.6g/L K2HPO4, 10g/L yeast extract, pH 7.2) (Labruyère 1971). The inoculum was incubated in the dark at 24°C until sporulation was observed at around 14 days.

Approximately 1L of colonised vermiculite inoculum was added to 25L of potting mix containing sand, peat and composted pine bark at a ratio of 10:10:80, at pH 6.0, premixed with Osmocote 16:3.5:10 N:P:K resin coated fertiliser (Scotts Australia Pty Ltd.) at the rate of 6kg/m3, and thoroughly mixed using a cement mixer. The inoculum containing potting mix was then used to fill plastic planter bags of 5L capacity (200 x 200mm, Botany Horticultural, Queensland, Australia).

PREPARATION OF 2,4-D TREATMENTS

Glasshouse trials:

2,4-D treatment solutions were prepared by dissolving 400mg of crystalline 2,4-D (Sigma Aldrich, St. Louis, Missouri, USA) in approximately 10mL of 70% ethanol over heat. This solution was then mixed with 1L of warm water to produce a 400mg/L solution. Half of this 400mg/L solution was retained as the most concentrated treatment, and the other half (0.5L) added to 0.5L of warm water to produce a 200mg/L solution. This two-fold dilution series was repeated until four concentrations with the lowest at 6.25mg/L solution were produced.

A control treatment of warm water only was used. Warm water was used for all dilutions to assist in keeping the 2,4-D in solution. Tween-80 was added at a rate of 0.5g/L to all 2,4-D and control treatments as a wetting agent to assist foliar application.

Field trial:

As the spray treatments would be applied from a backpack spraying rig in close proximity to other crops, and in accordance with the APVMA license, the less volatile amine form of 2,4-D was used to minimise the chance of damage to nearby horticultural crops. The commercial herbicide Amicide 625 (Nufarm Ltd) was chosen, and it was ensured that an equivalent amount of the active 2,4-D molecule was present compared with that used in the pot trials.

To prepare the spray treatments, 4.4mL of Amicide 625 was dissolved in 1L of water, with 0.5g of Tween-80 as a wetting agent. This 4.4mL/L Amicide solution was then mixed with 4L of water within the backpack spraying rig to make a 880µL/L solution. This was determined to be the equivalent of a 400mg/L 2,4-D solution.

All spray concentrations were prepared in this manner, according to the following dilution table:

Table 1. 2,4-D Amicide dilution schedule

Amicide 625	Water	Conc.	2,4-D Conc.
4.4mL	5L	880µL/L	400mg/L
1.1mL	5L	220µL/L	100mg/L
275µL	5L	55µL/L	25mg/L
68.75µL	5L	13.75µL/L	6.25mg/L
17.19µL	5L	3.44µL/L	1.625mg/L

The spray rig was washed with water between the applications of different treatments. Treatments were applied from the lowest rate to highest. The control treatment was applied with a clean handheld sprayer. The spray was applied to the foliage until run off occurred.

GLASSHOUSE TRIAL #1 (2008/09)

The first glasshouse trial was planted on 11th December, 2008. The two potato varieties used possessed moderate resistance (Russet Burbank) and moderate susceptibility (Desiree) to common scab. Plants emerged on 21st December, 2008. There were eight spray treatments of varying concentrations of 2,4-D: 400mg/L 2,4-D, 200mg/L 2,4-D, 100mg/L 2,4-D, 50mg/L 2,4-D, 25mg/L 2,4-D, 12.5mg/L 2,4-D, 6.25mg/L 2,4-D and a control treatment with no 2,4-D. The treatments were applied as single sprays. Each treatment was applied to five pots of each variety (80 pots in total), with pots arranged in a completely randomised design.

The spray treatments were timed to coincide with the critical infection period, which occurs 1 to 2.5 weeks after tuber initiation (Adams & Lapwood 1978). The spray treatments were applied 38 days after emergence (DAE) on 27th January, 2009. Prior to application of each treatment, plants were removed to a separate area to prevent spray drift onto plants of different treatments. The sprays were applied to the foliage as a fine mist until run-off occurred. The spray was allowed to dry before the plants were returned to their position in the glasshouse.

All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease (Lapwood & Hering 1970). No other pesticides were applied. Plants were grown under glasshouse conditions, with the temperature maintained at 25-30ºC. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to each pot.

Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 1st April, 2009. Tubers were stored at 4ºC in plastic netted bags prior to assessment.

GLASSHOUSE TRIAL #2 (2009/10)

The second glasshouse trial was planted on 23rd December 2009. Treatments repeated those used in the trial #1. Plants emerged on 2nd January 2010. Plants were maintained as per trial #1, but were grown outside on a concrete slab and subject to natural weather events and temperatures. Irrigation was applied as required ensuring soil dried between watering events. Pots had additional inoculated vermiculate and potting mix added on 22DAE. Tubers were harvested, following plant senescence, on 13th May 2010, and stored as for the previous trial.

FIELD TRIAL

One field trial further tested varied rates of foliar treatments of 2,4-D. The trial site was located at Waterhouse in North East Tasmania. The soil was predominately sandy. The immediate area had been sown with a trial potato crop in the previous season and a commercial processing potato crop in the season prior to that. The surrounding area had been sown with an oil seed crop in the previous season and a lucerne feed crop this season.

The trial was planted on 27th October, 2010. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants of the same variety, and plots consisting of two subplots, one each variety. To ease planting the subplots were not randomised within each plot. The trial was replicated four times.

Six different spray concentration treatments were applied: 1.625mg/L, 6.25mg/L, 25mg/L, 100mg/L, and 400mg/L 2,4-D equivalence, and a control spray containing no 2,4-D. All treatments were single sprays applied to run-off, and all plants were treated on the same date.

The average emergence date was estimated to be 20th November 2010. Plants were treated on 9th December 2010 at 20 DAE and tubers were harvested on 4th April, 2011.

The trial was watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required.

After senescence the trial was sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4ºC prior to assessment.

TUBER DISEASE AND YIELD ASSESSMENT

Tubers were washed or brushed to remove soil. The number of tubers produced (per plant for the glasshouse trials or per five plant plot for the field trial) and weight of individual tubers was recorded. Any tuber disfigurements present were noted.

Each tuber was scored for presence of any disease lesions (disease incidence) and for two measures of disease severity: mean tuber surface coverage (DCS) with lesions and depth of the deepest lesion (LDS). The following methods were used. For the field trial only, powdery scab severity (DCS) was also determined with the same scale used for common scab assessment.

Using the chart prepared by Richardson & Heeg (1954), tubers were assessed visually, and the percentage surface area covered by disease scored using the following scale and recorded as a disease cover score (DCS). Disease severity was also determined as the depth of the deepest lesion on each tuber, using the following scale modified from Bjor & Roer (1980) and recorded as a lesion depth score (LDS):

Disease cover score (DCS)	
0	no disease
0.5	0-1%
1	1-5%
2	5-10%
3	10-30%
4	30-50%
5	50-70%
6	70-100%

Lesion Depth score (LDS)	
0	no disease
1	superficial/slightly raised
2	1-2mm deep
3	2-3mm deep
4	>3mm deep

TUBER THAXTOMIN SENSITIVITY

Tuber thaxtomin sensitivity was measured using the following method of Tegg *et al.* (2008). Using a hole-punch, disks of 6mm diameter were cut from filter paper (Whatman No. 1) and then autoclaved for 20 minutes. The disks were soaked in a solution of purified thaxtomin A (Wilson *et al.* 1999) (14µM for Desiree, 7µM for Russet Burbank) for 1 hour and then air dried in a laminar flow cabinet.

Tubers without obvious lesions were chosen for the assay. Selected tubers were harvested from replicates 1-3 of the glasshouse trials or from replicates 1 and 2 of the field trial and stored for approximately 2 weeks at 4°C. Tubers were surface sterilised in 0.5% sodium hypochlorite for 10 minutes and then, under aseptic conditions, cut into 0.5cm thick slices, with the slices from each end of the tuber being discarded. The tuber slices were then placed into 90mm Petri dishes, lined with filter paper (Whatman No. 1) moistened with 2mL sterile, distilled water.

The tubers from each glasshouse trial replicate were placed in a single Petri dish (10 disks), and assessed as a single unit. Each treatment was replicated three times. For the field trial, two tubers were assayed from each replicate with one tuber per Petri dish.

Depending on tuber size, 2-4 (glasshouse trials) or 8 (field trial) filter paper disks were placed onto the cut surface of each tuber slice. The discs were moistened with 10µL sterile distilled water to ensure their attachment to the surface of the tuber slice. The tuber slices were then incubated in the dark at 24°C for 7 days.

After 7 days the filter paper discs were removed from the cut surface of the tuber and visible necrosis underneath was scored using the following scale:

Score	**Criteria**
0	no necrosis
0.5	very sparse flecks
1	few light brown flecks
1.5	few dark brown flecks
2	light brown flecks in a circle
2.5	dark brown flecks in a circle
3	light brown necrosis
3.5	dark brown necrosis
4	black necrosis

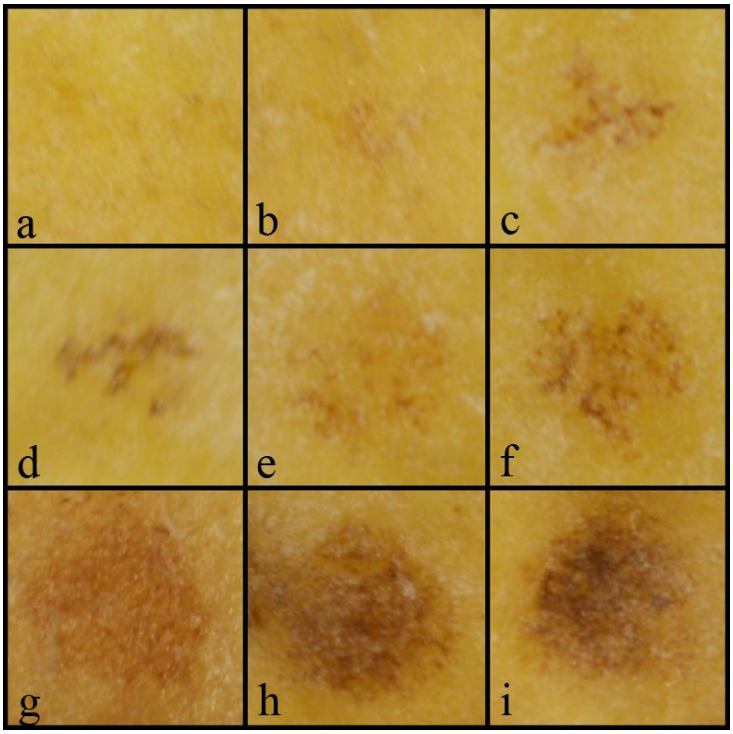


Figure 1. Examples of necrosis scored using the scoring scale:

a. 0 = no necrosis

b. 0.5 = very sparse flecks

c. 1 = few light brown specks

d. 1.5 = few dark brown specks

e. 2 = light brown flecks in a circle

f. 2.5 = dark brown flecks in a circle

g. 3 = light brown necrosis

h. 3.5 = dark brown necrosis

i. 4 = black necrosis.

TUBER 2,4-D QUANTIFICATION

The levels of 2,4-D within selected tubers from glasshouse trial #2 at harvest, were quantified by selecting 2-3 representative tubers from each treatment. All tubers were selected from the same replicate. Tuber tissue (10G) was cut into approximately 1cm2 pieces and stored in cold 80% methanol with butylated hydroxytoluene (BHT) at -20°C. Samples were homogenised with a stick blender with additional 80% methanol and left overnight at 4°C, after which they were filtered by vacuum filtration through Whatman No.1 filter paper to remove insoluble material. 90ng of 2,4-D internal standard was added to 10% aliquots of each sample, which were then stored at -20°C. The methanol was removed from the samples in a sample concentrator and then prepared for analysis using Sep Pak cartridges. The cartridge was prepared by washing with 100% methanol, then by 0.4% acetic acid. Two 1mL volumes of 0.4% acetic acid were used to transfer the samples from the vial to the Sep Pak cartridge. The samples were then eluted from the cartridge with 3mL of acetonitrile and stored at -20°C. The acetonitrile was removed in a sample concentrator, and 100µL of 0.4% acetic acid was used to transfer the sample to a microcentrifuge tube. The samples were centrifuged at 13,000rpm for 3 minutes to remove any remaining particulate matter, and 70µL was transferred to the final sample tube for analysis. Samples were kept in the dark at room temperature prior to analysis.

DATA ANALYSES

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009). Multivariate Analysis of Variance was used to determine significant effects from, and interactions between treatment factors. Probabilities less than 0.05 were considered to be significant and Fischer's least significant difference (LSD) test was used for comparison of treatment means. Linear Regressions were used to examine the relationship between powdery scab and common scab severity from the field trial.

RESULTS

DISEASE CONTROL

In glasshouse trial #1 there was no significant effect found from spray rate on either measure of disease severity. However, though not significant, all treatments did result in lower disease coverage than the control treatment, except the Desiree treatment of 200mg/L. In Russet Burbank, all treatments of 25mg/L 2,4-D and above had no diseased tubers. There was a significant effect found from spray rate on the proportion of diseased tubers per pot, with all treatments resulting in a significantly lower disease incidence than the control (Table 2).

In glasshouse trial #2 all 2,4-D treatments had significantly lower disease cover scores than the control treatment. The lowest (6.25mg/L 2,4-D) and highest (400mg/L 2,4-D) treatments had significantly lower DCS than the 12.5mg/L treatment, and there were no other significant differences between treatments. All treatments except the 12.5mg/L and 50mg/L treatments had significantly lower lesion depth scores than the control treatment. The 6.25mg/L and 400mg/L treatments had significantly lower LDS than these treatments, and the 6.25mg/L treatment also had significantly lower LDS than the 25mg/L treatment. There were no other significant differences between treatments (Table 2).

There was a significant effect found from spray rate on the proportion of diseased tubers (disease incidence). All treatments resulted in significantly less disease tubers than the control. The 6.25mg/L and 400mg/L treatments had the lowest proportion of diseased tubers, and were significantly lower than the 12.5mg/L treatment. All other treatments were not significantly different (Table 2).

For all trials and all measures of disease severity and incidence there was a significant effect found from variety. Russet Burbank had lower disease cover and proportion of disease tubers than Desiree in both trials, and mean number of lesions per tuber in glasshouse trial #2 (this was not measured in pot trial #1). Russet Burbank had significantly deeper lesions than Desiree in glasshouse trial #1, but significantly shallower lesions than Desiree in glasshouse trial #2. There were no interactions between spray rate and variety.

In the field trial, there was no significant effect found from spray rate on disease severity measured either as disease cover or lesion depth. However, when Russet Burbank was analysed separately the spray rate was found to have a significant effect on disease cover, with the 6.25mg/L, 100mg/L and 400mg/L treatments resulting in lower tuber disease cover than the control treatment. The 1.65mg/L and 25mg/L treatments were not significantly different from the control or any other treatments (Table 3).

Severity of Powdery scab was positively correlated with severity of common scab (Figure 2). Russet Burbank had significantly lower incidence of both common scab and powdery scab disease than Desiree (Tables 3& 4). For Russet Burbank (but not Desiree) there was a trend for reduced powdery scab severity with increasing 2,4-D treatment rate but these differences were not significant.

Table 2. The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank (RBK) and Desiree (DES) plants in glasshouse trials, on common scab severity and incidence.

Trial	Spray Rate (mg/L 2,4-D)	Disease Cover Score (DCS) (and Cover (%))			Lesion Depth Score (LDS)			Prop. of Diseased Tubers/Pot (%)		
		RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Pot Trial #1	**0**	0.6 (1.8%)	1.4 (6.9%)	1.1	0.6	2.0	1.3	40.0	58.7	49.3 c
	6.25	0.8 (4.6%)	0.8 (2.7%)	0.8	0.8	0.9	0.9	9.0	16.7	14.3 ab
	12.5	0.4 (1.5%)	0.4 (1.5%)	0.4	0.4	0.8	0.6	6.7	10.0	8.3 ab
	25	0.0	1.0 (5.5%)	0.5	0.0	1.0	0.5	0.0	8.3	4.2 ab
	50	0.0	1.3 (5.2%)	0.7	0.0	1.4	0.7	0.0	43.3	21.7 b
	100	0.0	0.8 (4.3%)	0.4	0.0	1.1	0.5	0.0	32.0	16.0 ab
	200	0.0	1.8 (6.6%)	0.9	0.0	2.4	1.2	0.0	29.7	14.8 ab
	400	0.0	0.4 (1.5%)	0.2	0.0	0.4	0.2	0.0	5.0	2.5 a
	Mean (variety)	0.2 a	1.0 b		1.0 a	0.2 b		7.0 a	25.8 b	
Pot Trial #2	**0**	0.9 (2.6%)	1.2 (3.9%)	1.0 c	0.9	1.4	1.2 d	29.7	24.5	27.1 c
	6.25	0.1 (0.1%)	0.0	0.1 a	0.2	0.0	0.1 a	4.0	0.0	2.0 a
	12.5	0.7 (2.2%)	0.7 (2.2%)	0.7 bc	0.6	0.8	0.7 cd	15.2	13.3	14.3 b
	25	0.1 (0.1%)	0.8 (2.7%)	0.5 ab	0.2	1.0	0.6 bc	5.0	7.6	6.3 ab
	50	0.1 (0.1%)	0.7 (1.5%)	0.4 ab	0.2	1.4	0.8 cd	3.3	17.2	10.3 ab
	100	0.2 (0.6%)	0.4 (0.8%)	0.3 ab	0.2	0.6	0.5 abc	2.5	15.4	8.9 ab
	200	0.0	0.8 (4.1%)	0.4 ab	0.0	0.9	0.4 abc	0.0	23.2	11.6 ab
	400	0.0	0.4 (1.2%)	0.2 a	0.0	0.4	0.2 ab	0.0	5.5	2.8 a
	Mean (variety)	0.3 a	0.6 b		0.3 a	0.8 b		7.5 a	13.3 b	

Disease Cover Score (DCS) estimates tuber surface coverage with lesions: 0 = no disease, 0.5 = 0-1%, 1 = 1-5%, 2 = 5-10%, 3 = 10-30%, 4 = 30-50%, 5 = 50-70%, 6 = 70-100%)

Cover (%) is derived from the DCS using mid values within each score band (this data is not statistically analysed)

Lesion Depth Score (LDS) scores deepest lesion present on each tuber: 0 = no lesion, 0.5 = superficial, 1 = >1mm deep, 2 = 1-2mm deep, 3 = 2-3mm deep, 4 = >4mm deep).

Prop. Of Diseased Tubers/Pot (%) is the number of tubers with visible lesions as a proportion of total tubers per pot

Pot trial #1 There was a significant effect found on proportion of diseased tubers from spray rate ($p < 0.001$, SED = 9.1, LSD = 18.21). There was no significant effect found from spray rate on DCS or LDS. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.172, LSD = 0.344), LDS ($p < 0.001$, SED = 0.211, LSD = 0.421) and proportion of diseased tubers from spray rate ($p < 0.001$, SED = 9.1, LSD = 18.21) and variety ($p < 0.001$, SED = 4.55, LSD = 9.11).

Pot trial #2 There was a significant effect found from spray rate on DCS ($p = 0.001$, SED = 0.215, LSD = 0.43), LDS ($p = 0.002$, SED = 0.25, LSD = 0.499) and proportion of diseased tubers ($p = 0.001$, SED= 5.66, LSD = 11.31). There was a significant effect found from variety on DCS ($p = 0.001$, SED = 0.108, LSD = 0.215), LDS ($p < 0.001$, SED = 0.125, LSD = 0.25), and proportion of diseased tubers ($p = 0.042$, SED = 2.83, LSD = 5.66). There were no significant interactions between factors.

Treatments with the same letter, in the same column and trial, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Table 3. The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank (RBK) and Desiree (DES) plants in a field trial, on common scab severity

Spray Rate (mg/L 2,4-D)	Disease Cover Score - DCS (Cover %)			Lesion Depth Score - LDS		
	RBK	DES	Mean (Rate)	RBK	DES	Mean (Rate)
0	1.21 (4.29%)	1.55 (6.15%)	1.32	1.11	1.58	1.27
1.625	0.90 (2.44%)	1.94 (10.68%)	1.42	1.17	2.21	1.69
6.25	0.63 (1.16%)	1.74 (9.13%)	1.19	1.00	2.07	1.53
25	0.72 (1.57%)	2.09 (10.53%)	1.40	1.03	2.24	1.63
100	0.63 (1.17%)	0.94 (2.65%)	0.76	1.02	1.31	1.15
400	0.60 (1.06%)	2.15 (12.21%)	1.26	1.07	2.22	1.56
Mean (variety)	0.78 a	1.75 b		1.07 a	1.98 b	

Disease Cover Score (DCS) estimates tuber surface coverage with lesions: 0 = no disease, 0.5 = 0-1%, 1 = 1-5%, 2 = 5-10%, 3 = 10-30%, 4 = 30-50%, 5 = 50-70%, 6 = 70-100%)

Percentage cover is derived from the DCS using mid values within each score band (this data is not statistically analysed)

Lesion Depth Score (LDS) scores deepest lesion present on each tuber: 0 = no lesion, 0.5 = superficial, 1 = >1mm deep, 2 = 1-2mm deep, 3 = 2-3mm deep, 4 = >4mm deep).

There was no significant effect found from spray rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.0805, LSD = 0.1610) and LDS ($p < 0.001$, SED = 0.211, LSD = 0.421). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Table 4. The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank (RBK) and Desiree (DES) plants in a field trial, on powdery scab severity

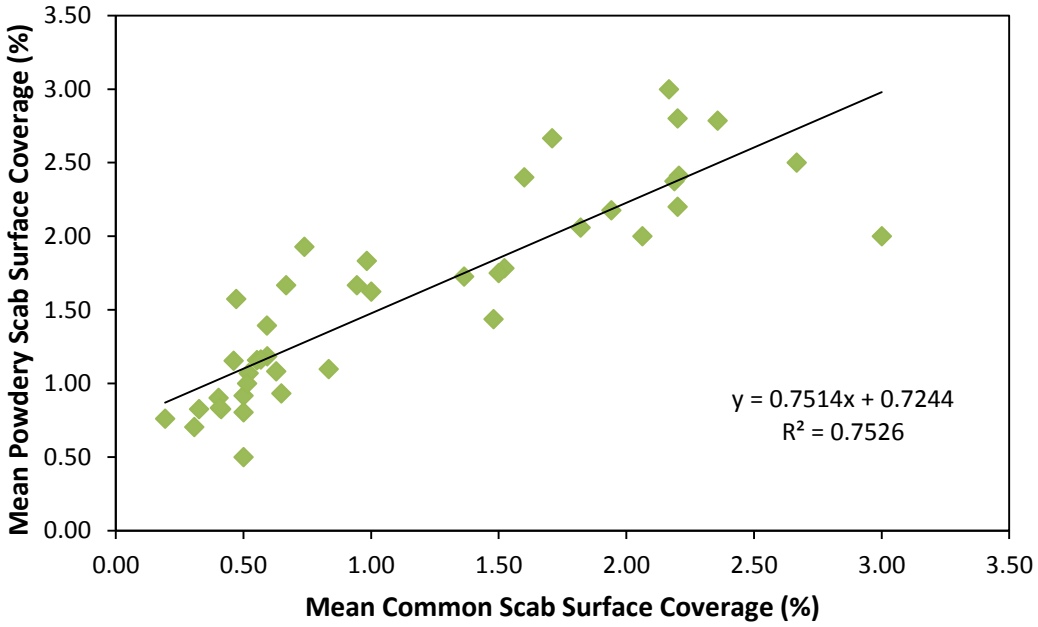
Spray Rate (mg/L 2,4-D)	Powdery Scab Disease Disease Cover Score – DCS (Cover %)		
	RBK	DES	Mean (Rate)
0	1.54 (6.57%)	1.77 (7.13%)	1.62
1.625	1.26 (4.56%)	2.21 (11.34%)	1.74
6.25	1.12 (4.04%)	1.90 (10.92%)	1.51
25	1.33 (5.33%)	2.75 (17.95%)	2.04
100	0.90 (2.50%)	1.77 (8.16%)	1.28
400	1.10 (3.84%)	2.26 (11.91%)	1.60
Mean (variety)	1.21 a	2.12 b	

Disease Cover Score (DCS) estimates tuber surface coverage with lesions: 0 = no disease, 0.5 = 0-1%, 1 = 1-5%, 2 = 5-10%, 3 = 10-30%, 4 = 30-50%, 5 = 50-70%, 6 = 70-100%)

Cover % is derived from the DCS using mid values within each score band (this data is not statistically analysed)

There was no significant effect found from spray rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.121, LSD = 0.248). There was no significant interaction between factors. Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Figure 2. Linear regression between the mean surface coverage of common scab and powdery scab, on tubers harvested from Russet Burbank and Desiree plants treated with 2,4-D at various rates.



AGRONOMIC EFFECTS

There was no significant effect found from spray rate on total tuber mass, mean mass per tuber, or number of tubers in either glasshouse trial. However, in glasshouse trial #1 the control treatment did have the greatest mean tuber mass of all the treatments, and in glasshouse trial #2 the control treatment had one of the greatest tuber masses of all the treatments, and the greatest mass per tuber of all the treatments. There was no trend apparent for number of tubers in either trial (Table 5).

Russet Burbank had a significantly lower mean total tuber mass and mean number of tubers than Desiree in both trials, and a significantly higher mean mass per tuber in glasshouse trial #2. Variety was not found to have a significant effect on mean mass per tuber in glasshouse trial #1 (Table 5).

In the field trial spray rate was not found to have a significant effect on total tuber mass, mean mass per tuber, or number of tubers in the field trial, and there was no apparent trend in any of these measures of agronomic performance. Russet Burbank had a significantly higher total tuber mass and number of tubers than Desiree, and a significantly lower mean mass per tuber (Table 6).

Table 5. Effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants in glasshouse trials on mean total tuber mass, mean mass per tuber, and mean number of tubers.

	Spray Rate (mg/L 2,4-D)	Mean Total Tuber Mass (g)			Mean Mass/Tuber (g)			Mean Number of Tubers		
		RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Pot Trial #1	**0**	27.8	54.9	41.3	17.1	23.8	20.5	2.6	3.6	3.1
	6.25	24	31.2	27.6	10.6	9.2	9.9	3.4	3.6	3.5
	12.5	23	56.8	39.9	9.6	21.9	15.7	2.8	3.4	3.1
	25	21.1	47.6	34.3	7.3	10.1	8.7	3.2	4.8	4
	50	25.1	48	36.6	11.5	14	12.8	2.4	3.4	2.9
	100	27.6	44.3	36	21.4	25.1	23.3	1.8	2.4	2.1
	200	29.7	44.2	36.9	12.1	13.9	13	2.6	3.8	3.2
	400	25.1	30.8	27.9	14.5	13.3	13.9	2.6	3.6	3.1
	Mean	25.4 a	44.7 b					2.7 a	3.6 b	
Pot Trial #2	**0**	57.0	75.8	66.4	17.71	9.28	13.5	4.60	8.60	6.6
	6.25	59.9	68.3	64.1	13.10	10.76	11.93	5.20	7.60	6.4
	12.5	64.3	69.1	66.7	14.57	8.88	11.73	5.2	8.2	6.7
	25	63.5	69.0	66.3	13.85	9.14	11.5	4.8	8.6	6.7
	50	51.9	69.9	60.9	10.09	11.48	10.78	5.2	6.4	5.8
	100	57.0	68.0	62.5	9.1	9.97	9.54	6.8	6.8	6.8
	200	59.6	60.7	60.1	11.75	7.59	9.66	5.4	8.4	6.9
	400	51.0	59.7	55.3	9.69	6.64	8.16	6	9.6	7.8
	Mean	58.0 a	67.6 b		12.5 a	9.2 b		5.4 a	8.0 b	

Pot trial #1 There was a significant effect found from variety on mean total mass ($p < 0.001$, SED = 2.83, LSD = 5.67), and mean number of tubers ($p = 0.011$, SED = 0.342, LSD = 0.683)

Pot trial #2 There was a significant effect found from variety on mean total mass ($p = 0.002$, SED = 2.87, LSD = 5.75), mean mass per tuber ($p = 0.004$, SED = 1.083, LSD = 2.166) and mean number of tubers ($p = 0.004$, SED = 0.536, LSD = 1.072). There was no significant effect found from spray rate. There were no significant interactions between factors.

Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Table 6. Effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank (RBK) and Desiree (DES) plants in a field trial on mean total tuber mass, mean mass per tuber, and mean number of tubers.

Spray Rate (mg/L 2,4-D)	Mean Total Tuber Mass (g)			Mean Mass/Tuber (g)			Mean Number of Tubers		
	RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
0	3130	1275	2512	74.3	90.0	79.6	42.0	6.8	24.4
1.625	2022	798	1410	76.2	143.4	109.8	30.8	6.8	18.8
6.25	3301	939	2120	75.1	109.5	92.3	44.5	7.0	25.8
25	3742	1107	2424	80.7	97.7	89.2	33.3	7.8	20.5
100	2755	1310	2136	79.8	100.5	88.7	34.5	9.5	22.0
400	2961	1450	2314	69.5	132.6	96.5	44.8	8.3	26.5
Mean (variety)	2952 a	1110 b		75.7 a	114.9 b		38.3 a	7.7 b	

There was a significant effect found from variety on mean total mass ($p < 0.001$, SED = 280.6, LSD = 575.8), mean mass per tuber ($p < 0.001$, SED = 8.57, LSD = 17.59) and mean number of tubers ($p < 0.011$, SED = 3.64, LSD = 7.41) There was no significant effect found from spray rate. There were no significant interactions between factors.

Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

TOXIN TOLERANCE

In glasshouse trial #1, tubers from treated Russet Burbank plants had significantly higher toxin tolerance (lower necrosis scores) than from the untreated control. The 12.5mg/L treatment had significantly higher necrosis scores than the 200mg/L and 400mg/L treatments, and the 6.25mg/L treatment had significantly higher necrosis scores than the 200mg/L treatment. All other treatments were not significantly different from each other (Table 7).

In Desiree all treatments except the 6.25mg/L treatment resulted in significantly higher toxin tolerance than the control. The 6.25mg/L treatment had significantly higher necrosis scores than all the other treatments, except the control treatment, which it was not significantly different to. The 50mg/L treatment had significantly higher necrosis scores than the 200mg/L treatment. All other treatments were not significantly different from each other (Table 7).

In glasshouse trial #2, tubers from treated Russet Burbank plants again had significantly higher toxin tolerance (lower necrosis scores) than from the untreated control. The 6.25mg/L treatment had significantly higher necrosis scores than all other 2,4-D treatments. The 12.5mg/L treatment was not significantly different to the 25mg/L, 50mg/L and 100mg/L treatments, but had a significantly mean higher necrosis score than the 200mg/L and 400mg/L treatments. The 25mg/L through to 200mg/L treatments were not significantly different from each other. The 400mg/L treatment had a significantly lower mean necrosis score than all other treatments (Table 7).

In Desiree all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control, except the 6.25mg/L treatment, which it was not significantly different from. The 100mg/L treatment was not significantly different to the 400mg/L treatment, and had significantly lower necrosis scores than all other treatments, except the 200mg/L treatment, which it was significantly higher than. The 400mg/L treatment was not significantly different to the 25mg/L treatment, and the 12.5mg/L, 25mg/L and 50mg/L treatments were not significantly different to each other. The 6.25mg/L 12.5mg/L and 50mg/L treatments were not significantly different to each other (Table 7).

In the field trial, all tubers from treated plants had significantly higher toxin tolerance (lower mean necrosis scores) than the control treatment.

In Russet Burbank the 1.625mg/L treatment had significantly higher mean necrosis scores than the 6.25mg/L, 100mg/L, and 400mg/L treatments. The 25mg/L treatment had significantly lower mean necrosis scores than all other treatments. In Desiree the 1.625mg/L treatment had significantly higher mean necrosis scores than the 6.25mg/L treatment, and was not significantly different from the 25mg/L, 100mg/L and 400mg/L treatment. The 6.25mg/L treatment had significantly lower necrosis scores than the 1.625mg/L and 100mg/L treatments, but was not significantly different to the 25mg/L and 400mg/L treatments (Table 7).

Table 7. The effect of 2,4-D foliar sprays on mean necrosis score following treatment of tuber slices with thaxtomin A.

Spray Rate (mg/L 2,4-D)	Pot Trial #1				Pot Trial #2				Field Trial			
	RBK		DES		RBK		DES		RBK		DES	
0	2.73	a	2.50	a	2.38	a	1.70	a	1.92	a	1.80	a
1.625									1.66	b	1.25	b
6.25	2.04	bc	2.35	a	1.88	b	1.54	ab	1.38	c	0.95	c
12.5	2.10	b	1.63	bc	1.66	c	1.42	bc				
25	1.96	bcd	1.48	bc	1.50	cd	1.28	cd	1.06	d	1.05	bc
50	1.90	bcd	1.69	b	1.55	cd	1.43	bc				
100	1.85	bcd	1.60	bc	1.60	cd	1.10	ef	1.33	c	1.27	b
200	1.71	d	1.42	c	1.42	d	1.03	f				
400	1.81	cd	1.73	b	1.17	e	1.21	de	1.41	c	1.08	bc

Necrosis score: 0 = no necrosis; 0.5 = very sparse flecks; 1 = few light brown flecks; 1.5 = few dark brown flecks; 2 = light brown flecks in a circle; 2.5 = dark brown flecks in a circle; 3 = light brown necrosis; 3.5 = dark brown necrosis; 4 = black necrosis

There was an interaction found between spray rate and variety in Pot trial #1 ($p = 0.001$, SED = 0.1357, LSD = 0.2669), Pot trial #2 ($p < 0.001$, SED = 0.089, LSD = 0.1745) and Field trial ($p = 0.027$, SED = 0.1125, LSD = 0.2213).

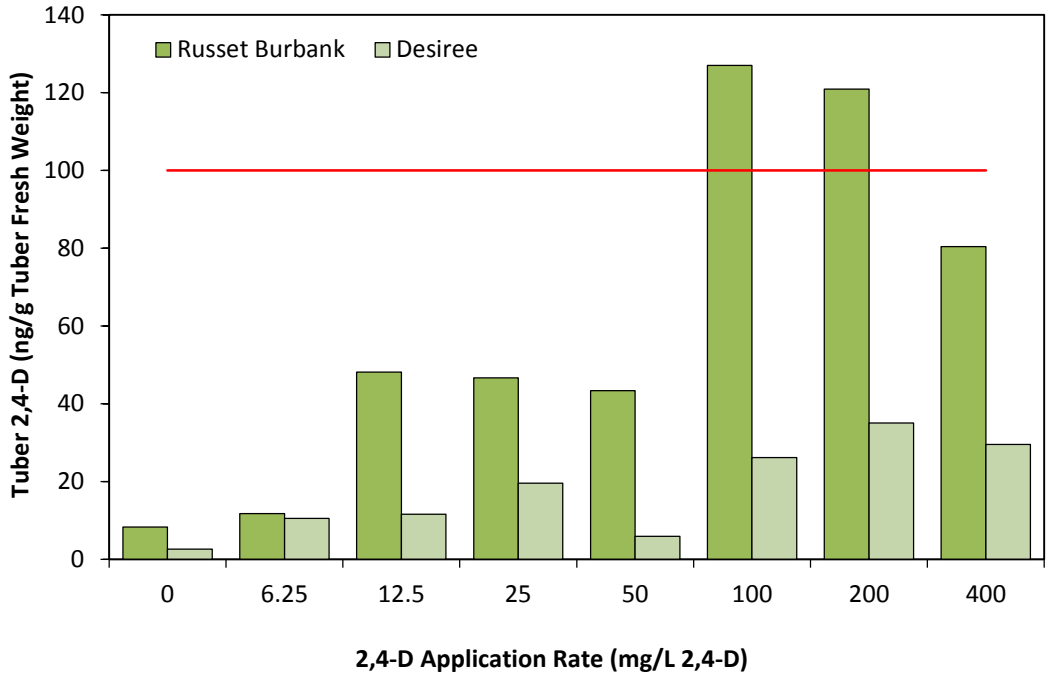
Treatments with the same letter, within each variety and trial, are not significantly different at $p = 0.05$ using Fischer's LSD test.

2,4-D QUANTIFICATION

In tubers harvested from glasshouse trial #2, Russet Burbank had a significantly greater amount of 2,4-D in tubers at harvest than Desiree. In both varieties there is a trend towards increase levels of 2,4D in tubers at harvest with increasing application rates. In both Russet Burbank and Desiree the control has the lowest levels of 2,4-D. In Desiree the 50mg/L treatment has the lowest level of 2,4-D of all the treatments, but otherwise there is an increasing level of 2,4-D with increase application rate until the highest rate, 400mg/L, which resulted in slightly less 2,4-D than the 200mg/L treatment. In Russet Burbank the 6.25mg/L treatment has the lowest level of all the treatments, the 12.5mg/L, 25mgL and 50mg/L treatments have similar levels, and the 100mg/L and 200mg/L treatment have similar levels. As in Desiree, the Russet Burbank 400mg/L treatment is lower than the 200mg/L treatment.

The 100mg/L and 200mg/L treatments on Russet Burbank resulted in 2,4-D levels above the Australian maximum residue level (MRL) of 100ng/g of tuber fresh weight (Commonwealth of Australia 2011). This data lacks replication and as such has only been analysed statistically for varietal differences.

Figure 3. Quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at rates to the foliage of Russet Burbank and Desiree plants. Red line indicates the maximum residue limit (MRL) of 2,4-D in potatoes.



DISCUSSION

MUCH LOWER RATES THAN STUDIED PREVIOUSLY MAY CONTROL DISEASE

The combined disease and tuber sensitivity data obtained in this study suggests that much lower rates of 2,4-D than have previously been studied (McIntosh et al. 1981; Tegg et al. 2008) may be as effective in controlling common scab. Initial trials (McIntosh et al. 1981) focussed on an application rate of 200mg/L, which greatly reduced scab in glasshouse trials. In field trials, rates of both 100mg/L and 200mg/L of 3,5-dichlorophenoxyacetic (3,5-D) were found to be similarly effective in suppressing disease, but no effect on disease was found from 50mg/L applications (McIntosh et al. 1982). Later research (Tegg et al. 2008) found that 50mg/L applications of 2,4-D resulted in the same increased suppression of thaxtomin A toxicity in tubers as had been achieved by 200mg/L applications. Waterer (Waterer 2010) found that much higher rates also suppressed disease, with a rate of approximately 647mg/L and 1294mg/L reducing common scab by similar amounts. The research presented in this chapter suggests that even lower rates of 2,4-D could be effective in controlling disease, with as little as 1.625mg/L 2,4-D reducing disease cover in comparison to the control treatment in the variety Russet Burbank in the field trial. In pot trials, 6.25mg/L 2,4-D resulted in similar disease cover to that achieved by the previously published effective rates of 100mg/L and 200mg/L (McIntosh et al. 1981; McIntosh et al. 1982; Tegg et al. 2008).

That lower rates of 2,4-D appear to be able to control common scab disease is important due to the herbicidal properties of 2,4-D. The phytotoxic effects of 2,4-D (as well as the previously mentioned reduced effectiveness in field trials) meant that McIntosh and colleagues (1981) abandoned it as a possible control method. The lowest label rate for 2,4-D as a herbicide is approximately 680mg/L for use on lawns (Nufarm Australia Limited 2011). The rates tested in these trials and later studies (Tegg et al. 2008), particularly when applied multiple times, resulted in near herbicidal levels of 2,4-D being applied to the plant over a period of 2-3 weeks during tuber development. This resulted in reduced yield, increased tuber number, and increased tuber deformity. Data presented in both chapter 1 and chapter 2 suggest that multiple sprays to "top up" tubers with 2,4-D are unnecessary, and that single sprays result in effective disease control. However, The minimal amount of 2,4-D required for disease control is in contrast with the amount of material required by other chemicals that have been shown to suppress common scab. 2,5-dibromobenzoic acid (2,5-DBB) has been demonstrated to control common scab with minimal phytotoxic effects (McIntosh et al. 1988). Like 2,4-D, it appears to suppress the disease by reducing the sensitivity of the tuber to thaxtomin (Tegg et al. 2012). However, results obtained by Tegg et al. (2012) suggest that higher rates of 2,5-DBB are required to achieve similar disease control as 2,4-D.

INDUCED TOXIN TOLERANCE THRESHOLD REACHED BY LOW RATES OF APPLIED AUXIN

These results suggest that very low rates of 2,4-D may be as effective in controlling scab as previously published higher rates, and this is further strengthened by the toxin tolerance of the harvested tubers. The foliar application of 2,4-D resulted in increased toxin tolerance of tubers in all three trials. This was also observed in the trials presented in chapter 2. Treatments as low as 1.625mg/L in the field trial and 6.25mg/L in the pot trials resulted in increased toxin tolerance, suggesting that very low levels of 2,4-D are required within the tuber for some degree of induced resistance. Quantification of the 2,4-D within tubers at harvest in pot trial #2 shows that, while much higher application rates resulted in higher 2,4-D levels within the tuber, the tolerance of these tubers to the toxin isn't increased to a similar extent. This suggests that there is a maximum level of toxin tolerance that can be induced by 2,4-D, and that this is reached by the foliar application of 2,4-D at rates much lower than had been previously tested. Application rates as low

as 12.5mg/L provide sufficient 2,4-D to the tuber to induce a level of tolerance similar to that reached by 200mg/L, as used in previous studies (McIntosh et al. 1981; Tegg et al. 2008).

Quantification of 2,4-D from one pot trial found that applications of 100mg/L and 200mg/L to Russet Burbank plants resulted in levels of 2,4-D in the tubers at harvest above the maximum residue level (MRL) for potato in Australia, of 100ng 2,4-D/g tuber fresh weight (Commonwealth of Australia 2009). This is consistent with previous research (Tegg et al. 2008), which found rates as low as 50mg/L resulted in tuber 2,4-D at six weeks after treatment of twice the MRL. This suggests that these rates result in too much material being translocated to the tuber for it to be metabolised to consistently safe levels before harvest. Toxin tolerance data suggests that this level of 2,4-D within the tuber is also unnecessary, with much lower amounts of tuber 2,4-D decreasing tuber toxin sensitivity to a similar level.

Levels of 2,4-D within Desiree tubers were significantly lower than in Russet Burbank tubers. While there is no published data on varietal differences in 2,4-D translocation to tubers, carbohydrate flow, photosynthetic activity, growth rate and IAA levels influence the rate of translocation of phenoxyacetic acids (Burrell 1982). Varietal differences in these, or differences in plant development at the time of application may explain the varietal effect on tuber 2,4-D levels.

LESION DEPTH REDUCED BY 2,4-D TREATMENTS

While only statistically significant in one pot trial, this study further suggests that 2,4-D can reduce lesion depth as well as lesion cover. This was found in previous studies that measured lesion depth (Tegg et al. 2008), but strong evidence of a reduction in lesion depth was not found in the trials presented in chapter 2. While a reduction in disease cover is a more visually impressive result, a reduction in lesion depth may be a more useful outcome. Common scab does not significantly reduce crop yield (Loria et al. 1997), but rather decreases quality and increases processing costs and waste. Deep lesions, a common symptom in Australia (Wilson et al. 1999) may still be present after normal steam peeling processes, while superficial lesions may not require any extra processing (Wilson et al. 2009). Therefore, a control that reduced lesion depth sufficiently so that lesions were removed by initial peeling, yet didn't significantly decrease lesion cover, may be of use in processing potato crops.

EVIDENCE FOR REDUCTION IN DISEASE INCIDENCE

Previous studies on auxin induced resistance to common scab have focussed on a reduction in disease severity (McIntosh et al. 1981; McIntosh et al. 1982; Tegg et al. 2008). The low levels of disease in the pot trials allowed for the calculation of disease incidence, measured by the proportion of diseased tubers, and in pot trial #2, the number of lesions per diseased tuber. The reduction in disease incidence from 2,4-D treatments at rates as low as 6.25mg/L was observed in both pot trials. This is the first demonstration that foliar 2,4-D treatments may also reduce disease incidence within a crop, as well as disease severity on individual tubers. In both trials Desiree had a higher proportion of diseased tubers than Russet Burbank, which is consistent with previous research that finds Desiree, while more tolerant to the toxin, to be more susceptible to the disease (Wilson et al. 2010). The proportion of tubers with any lesions within a crop can be used as a determinant of crop health and quality, and as such reducing disease incidence could be beneficial.

TUBER SLICE ASSAYS

Desiree tubers were treated with twice the concentration of thaxtomin A compared to Russet Burbank, as it is already a toxin resistant variety (Tegg & Wilson 2010), and therefore direct comparisons between the tolerance of the two varieties cannot be made. In both pot trials, all 2,4-D treatments, including the lowest rate of 6.25mg/L, suppressed thaxtomin toxicity in Russet Burbank, while in Desiree all 2,4-D treatments except the lowest rate had this effect. Lower rates therefore seem to be more effective in suppressing thaxtomin toxicity in Russet Burbank than in Desiree. This is consistent with the 2,4-D quantification data, which showed that more 2,4-D is translocated to tubers in Russet Burbank than in Desiree, and suggests that higher application rates of 2,4-D are required in Desiree to provide sufficient 2,4-D to tubers. However, all treatments in the field trial, including the lowest 1.625mg/L treatment resulted in increased toxin tolerance in both varieties, and in fact this reduction was more pronounced in Desiree than in Russet Burbank, despite Russet Burbank tubers having higher 2,4-D levels.

AGRONOMIC EFFECTS

While there were no significant effects on yield or tuber number in any trials, the trend seen in the pot trials of decreasing total tuber mass, and in pot trial #2 of decreasing mass per tuber and increasing mean number of tubers with increasing rate is consistent with previously published results. McIntosh et al. (1981; 1982) found that rates of 2,4-D and 3,5-D which were effective in controlling commons scab resulted in phytotoxic effects on the plant, observing an increased number of tubers, reduced tuber mass, and increased deformity. Tegg et al. (2008) observed similar phytotoxic effects from equivalent rates of 2,4-D. Waterer (2010), using a rate of approximately 647mg/L, the label rate for 2,4-D use to enhance colouration of red skinned varieties of potatoes in the USA, and more than triple of that used by McIntosh et al. (1981) and Tegg et al. (2008) resulted in a decrease in tuber size and an increase in tuber number, and rates double this also resulted in a decrease in yield. However, the effects varied with season and variety, and 2,4-D was considered to have little impact on yield at the 647mg/L rate, but did decrease yield at higher rates (Waterer 2010). The phytotoxic effects of 2,4-D on potatoes therefore appear to increase with application rate, but can be negligible with low rates that still suppress common scab.

CONTROLLING COMMON SCAB MAY RESULT IN REDUCED POWDERY SCAB

The correlation between the tuber surface coverage by lesions of common scab and powdery scab found in the field trial is an interesting result that suggests there may be some interaction between the two diseases. A similar correlation was also found between common scab and powdery scab severity in one of the field trials detailed in chapter 2, which focussed on timing of 2,4-D application. While the treatments tended to reduce powdery scab, this was not significant in this trial possibly due to relatively low disease level present reducing variability.

While 2,4-D appears to reduce common scab through a thaxtomin specific response, which would be unlikely to have an effect on the powdery scab pathogen, it is also possible that 2,4-D treatments may induce a SAR response, such as the production of reactive oxygen species, which may also provide resistance to powdery scab. Treatments of IAA in potato have been shown to result in an increased transcription of auxin related stress related proteins, which also increase in response to fungal infection, and may have a role in plant defence responses (Zanetti et al. 2003).

By controlling common scab, and therefore preventing the development of lesions, the size and number of entry sites for the powdery scab pathogen to enter may be reduced. This could result in a reduction of both the number of powdery scab lesions and the amount of powdery scab colonisation on the tuber. There has

been little research to date on the interaction between common and powdery scab, despite importance to potato production, as they tend to be studied in isolation. Indeed one common scab control method, targeted irrigation, may encourage the powdery scab pathogen through the creation of a more suitable environment, and its use for controlling common scab may increase powdery scab. Delayed planting, another control method of common scab, has also been found to reduce powdery scab incidence, if the later planting date allows soils to have warmed beyond temperatures acceptable to the pathogen (Falloon 2008).

Controlling common scab through the application of 2,4-D has not been shown to create a change in the pathogen environment outside of the tuber, and it is possible that it is a control method for common scab that either does not increase the risk of, or possibly reduces the risk of powdery scab. If further data on the interaction between the two pathogens supported this hypothesis, 2,4-D (or similar chemicals) may indeed be a possible control method for both diseases, if common scab is present. Waterer (2010) found the opposite effect on tubers sprayed with 2,4-D, observing that 2,4-D treatments tended to decrease common scab but slightly increase powdery scab disease levels, and suggested that this may be a result of reduced competition for entry sites.

It is worth noting that this observed correlation may simply be a result of the visual estimation of common scab symptoms being conflated by the presence of powdery scab lesions. Care was taken by assessors to identify features of the different diseases, such as sporosori inside powdery scab lesions (Harrison et al. 1997). However, due to the inaccurate nature of visual symptom scoring, and the similarity between the lesions of the two diseases, higher levels of one disease may have led to higher levels of the other disease being estimated. The use of molecular diagnostic techniques would be able to provide more accurate results.

FUTURE DEVELOPMENT

The low rates required to induce the resistance to common scab of potato may mean that it will be difficult to adopt this research by the agrichemical industry. As such a low amount of active ingredient is required, very little of the currently available commercial 2,4-D herbicides is required to spray entire crops with sufficient 2,4-D to provide disease protection. There is therefore less benefit to companies who produce and sell 2,4-D to go through the rigorous and expensive trials required to allow 2,4-D to be sprayed onto potato for common scab disease control, despite the interest in this control method from the potato processing industry in Australia. 2,4-D is currently registered for application on to potato crops in the USA, at a rate of approximately 647mg/L, and is being considered for registration in Canada, for the enhancement of the desirable red skin colour for fresh market potatoes (Waterer 2010). This rate is considerably higher than this study suggests is necessary for controlling common scab while minimising both phytotoxic effects and residue levels.

CONCLUSION

Based on toxin tolerance, disease and agronomic data obtained from the three trials detailed in this chapter, one application of 2,4-D applied at a rate as low as 25mg/L and sprayed until runoff, may be effective in reducing common scab disease of potato without phytotoxic effects.

Disclaimer: 2,4-D is not currently registered for use for the purpose of disease control in potato. As such commercial use is neither recommended nor permitted.

CHAPTER 2: OPTIMISATION OF 2,4-D FOLIAR TREATMENT TIMING AND FREQUENCY

INTRODUCTION

In attempts to optimise the application of 3,5-D to obtain the greatest disease suppression, McIntosh et al. (1981) found that earlier applications of 3,5-D in the plants development increased the efficacy of disease control, but it also increased the material's phytotoxic effects on the potato plant, further decreasing yield and tuber quality. Through the use of strategically applied irrigation treatments, which were known to suppress common scab, Lapwood et al. (1973) were able to confirm that tubers were only susceptible to infection by pathogenic Streptomcyes spp. for a defined period, coinciding with tuber elongation, and presence of immature (non-suberised) lenticels (Lapwood et al. 1973; Khatri et al. 2011). This occurs approximately four to six weeks after tuber initiation, when stomata transform into lenticels (Lapwood & Hering 1970). Initial Australian studies examining the use of 2,4-D and other chemicals for the control of common scab targeted this critical infection period with treatment applications, scheduling treatments for 2 weeks after tuber initiation (Tegg 2006; Tegg et al. 2008). However, as tuber initiation was determined by visually checking a selection of plants for hooks developing on underground stolons, and tuber initiation for any potato plant is asynchronous with tubers initiating periodically during plant development, accurate timing of treatments to cover this "infection window" was difficult. Thus, there was a possibility that treatments may have been applied after some (or perhaps many) developing tubers had already entered the critical infection period, and would not have protected these tubers from infection. As these treatments have no biocidal effect against the pathogen, it is not believed that they have any effect on disease development once infection has occurred. Attempted precision of the timing of treatments to the period of tuber susceptibility did not take into account the potential for variation in rate or efficiency in the translocation of the applied chemicals to the tubers.

Previous studies (Tegg 2006; Tegg et al. 2008) used multiple applications staggered throughout the infection period in order to increase the duration of treatment to protect the tuber for the entire period it is susceptible to infection. Tegg et al (2008) found that multiple applications provided greater control against common scab than single applications, when the initial application was targeted to the beginning of the infection window.

This chapter describes studies that aimed to determine the optimal period of application of 2,4-D foliar treatments to provide the greatest common scab control; to determine the effect of these treatments on the levels of 2,4-D in tubers at harvest; and to further examine the effect of single and multiple treatments on disease control when applied at various stages of plant development.

MATERIALS AND METHODS

Two glasshouse and two field trials testing varied timing and frequency of 2,4-D foliar treatments were conducted.

PLANTS AND INOCULUM

Planting material:

The varieties Desiree and Russet Burbank were again used in these trials. Seed tubers were removed from cold storage a fortnight before planting.

The source of potato tubers for glasshouse trials were disease-free mini-tubers harvested from glasshouse grown tissue-culture plantlets. For the field trials, these were commercial certified seed visually free of disease, without seed fungicide treatments.

Inoculum:

The *S. scabies* isolate and methodologies used to produce inoculum for the glasshouse trials was the same as in the previous chapter.

PREPARATION OF 2,4-D TREATMENTS

Glasshouse trials:

Solutions of 2,4-D for use in glasshouse trials were prepared as described in the previous chapter. For the timing and frequency trials 2,4-D solutions of 200mg/L and 100mg/L in warm water were prepared. A control treatment of warm water only was used. Tween-80 was added at a rate of 0.5g/L to all 2,4-D and control treatments as a wetting agent to assist foliar application.

Field trials:

As with the field trial described in the previous chapter to meet the requirements of our APVMA license, the less volatile amine form of 2,4-D was used. The herbicide Amicide 625 (Nufarm Ltd) was used, ensuring that an equivalent amount of the active 2,4-D molecule was present compared with that used in the glasshouse trials with 2.2mL or 550µL of Amicide 625 dissolved in 1L of water, with 0.5g of Tween-80 as a wetting agent. This solution was then mixed with 9L of water within the backpack spraying rig to make either a 220µL/L solution or 55µL/L solution. This was determined to be the equivalent of a 100mg/L 2,4-D or 25mg/L 2,4-D solution respectively.

The spray rig was washed with water between the applications of different treatments. Where applicable, treatments were applied from the lowest rate to highest. The control treatment was applied with a clean handheld sprayer. The spray was applied to the foliage until run off occurred.

GLASSHOUSE TRIAL #1 (2009)

The first glasshouse trial was planted on 30th January, 2009. Two potato varieties were used, Russet Burbank and Desiree. Plants emerged on 8th February 2009. There were 15 different 2,4-D spray treatments, grouped in three distinct application strategies.

1. "Single" – a single treatment of 200mg/L 2,4-D applied at 10, 20, 30, 40 or 50 days after emergence (DAE).
2. "Double" – two treatments, each of 200mg/L 2,4-D, the first applied at 10, 20, 30, 40 or 50 DAE, the second applied 10 days after the first application (i.e. at 20, 30, 40, 50 and 60 DAE respectively), with a total of 400mg/L 2,4-D applied.
3. "Double (half rate)" – two treatments, each of 100mg/L 2,4-D, the first applied at 10, 20, 30, 40 or 50 DAE, the second applied 10 days after the first application (i.e. at 20, 30, 40, 50 and 60 DAE respectively), with a total of 200mg/L 2,4-D applied.

There were three control treatments, one applied as a single spray at 10 DAE, and two applied as double sprays at 10 and 20 DAE. Each treatment was applied to five pots of each variety (180 pots in total), with pots arranged in a completely randomised design.

Prior to application, plants to be treated were removed to a separate area to prevent spray drift onto plants of different treatments. The sprays were applied to the foliage as a fine mist until run-off occurred. The spray was allowed to dry before the plants were returned to their position in the glasshouse.

All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease. No other pesticides were applied. Plants were grown under glasshouse conditions, with the temperature maintained at 25-30°C. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to each pot.

Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 25th May, 2009. Tubers were stored at 4°C in plastic netted bags.

GLASSHOUSE TRIAL #2 (2010)

The second glasshouse trial was planted on 19th January, 2010. Treatments and application times repeated those used in trial #1. Plants emerged on 1st February, 2010. Plants were maintained as per trial #1, but were grown outside on a concrete slab and subject to natural weather events and temperatures. Irrigation was applied as required ensuring soil dried between watering events. Tubers were harvested, following plant senescence, on 1st May, 2010.

FIELD TRIAL #1 (2008/09)

The trial site was located at Bishopsbourne in North West Tasmania. It had a brown clay soil and had been sown with a processing potato crop in the previous season. The surrounding area was sown with a processing green pea crop.

The trial was planted on 21st October, 2008. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants of the same variety, and plots consisting of two subplots, one of each variety. To ease planting the subplots were not randomised within each plot. There were four replicates.

There were 15 2,4-D spray treatments, grouped in three distinct application strategies:

1. "Single" – a single treatment of 100mg/L 2,4-D applied at 10, 20, 30, 40, 50, or 60 DAE.
2. "Double" – two treatments, each of 100mg/L 2,4-D were applied, the first at 10, 20, 30, 40, or 50 DAE, and the second 10 days after the first treatment (i.e. at 20, 30, 40, 50, and 60 DAE respectively).
3. "Triple" – three treatments, each of 100mg/L 2,4-D were applied, the first at 10, 20, 30, or 40 DAE, the second 10 days after the first treatment (i.e. at 20, 30, 40, and 50 DAE respectively), and the third 20 days after the first treatment (i.e. at 30, 40, 50, and 60 DAE respectively).

There was one control treatment for each application strategy, applied at 10 DAE for the single treatment, at 10 and 20 DAE for the double treatment, and at 10, 20 and 30 DAE for the triple treatments.

The average emergence date was estimated to be November 30, 2008. Tubers were harvested on 2nd April, 2009. The trial was watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required. After senescence the trial was sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4°C prior to assessment.

FIELD TRIAL #2 (2009/10)

The trial site was located at Waterhouse in North East Tasmania and had been sown with a processing potato crop in the previous season. The soil was predominately sandy. The surrounding area was sown with an oil seed crop.

The trial was planted on 17th November, 2009. The potato varieties, arrangement and replication repeated that used in trial #1.

There were 11 2,4-D spray treatments, grouped in two distinct application strategies:

1. "Single" – a single treatment of either 25mg/L or 100mg/L 2,4-D, each applied at 5, 10, 20, 30, 40, or 50 DAE
2. "Double" – two treatments of either 25mg/L or 100mg/L 2,4-D, each applied at 5, 10, 20, 30, 40, or 50 DAE, with a second application at 10, 20, 30, 40, 50, and 60 DAE respectively.

There were two control treatments for each application strategy; two applied at 10 DAE for the single treatment, and two applied at 10 and 20 DAE for the double treatment.

The average emergence date was estimated to be 10th December, 2009. Tubers were harvested on 8th April, 2010. Trial irrigation and harvest were as described for field trial #1

TUBER DISEASE AND YIELD ASSESSMENT

Harvested tubers were brushed or washed free from soil. The number of tubers per glasshouse pot (one plant) or field plot (five plants) was determined and individual tubers were weighed. Tubers with visual disfigurements were recorded. Each tuber was scored for common scab incidence and severity (disease cover score and lesion depth score) as described in the previous chapter. Tubers harvested from the field trials were also assessed for powdery scab severity as previously described.

TUBER THAXTOMIN SENSITIVITY

Tuber thaxtomin sensitivity was measured using the same methodologies and scoring procedures as described in the previous chapter. Tubers used for the tuber slice assays were sampled from treatments from replicates 1-3 of both glasshouse trials, and replicates 1, 2 and 3 of field trial # 2.

TUBER 2,4-D QUANTIFICATION

The levels of 2,4-D within selected tubers from glasshouse trial #2 at harvest, were quantified by selecting 2-3 representative tubers from each treatment. All tubers were selected from the same replicate. The procedures were as described in the previous chapter.

DATA ANALYSES

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009) using multivariate Analysis of Variance to determine significant effects from, and interactions between treatment factors. Probabilities of less than 0.05 were considered to be significant and least significant difference (LSD) was used for comparison of treatment means. Linear Regressions were used to examine relationships between powdery scab and common scab severity in field trial #2.

RESULTS

DISEASE CONTROL

In glasshouse trial #1 there was no significant effect found from any of the 2,4-D treatments on common scab incidence or severity. Variety was found to have a significant effect on all measures of disease incidence and severity. Desiree had a greater proportion of diseased tubers, and these had significantly greater mean disease cover scores (DCS) and lesion depth scores (LDS) than those in Russet Burbank (Table 8).

In glasshouse trial #2 there was a significant effect found from the date of the first spray on DCS, LDS, and the proportion of disease tubers to healthy tubers. The control and 50 DAE treatments were not significantly different in DCS or LDS, and all other treatments resulted in lower DCS and LDS than the control treatment (with the exception of the 20 DAE treatment, which was not significantly different in LDS to the control and 50 DAE treatments). The control and 50 DAE treatments were not significantly different in the proportion of diseased tubers, and all treatments resulted in significantly lower proportions of diseased tubers than the control treatment, and all except the 20 DAE treatment resulted in significantly lower proportion of disease than the 50 DAE treatment. All treatments including the control resulted in low disease severity and incidence in both pot trials (Table 8).

Generally earlier treatments resulted in lower DCS than later and control treatments, and in lower numbers of tubers with visible lesions than later treatments, except for treatments with a first spray date of 40 DAE, which were not significantly different from the earliest treatments. In glasshouse trial #2 there was no effect found from variety found for any measures of disease severity, but the proportion of diseased tubers was significantly higher in Russet Burbank than in Desiree, the opposite of the effect found in glasshouse trial #1 (Table 9).

In field trial #1 there was a significant interaction found from the date of the first 2,4-D application, the number of applications, and variety on both measures of disease severity (disease incidence was not recorded in the field trials; Table 10).

In Russet Burbank mean disease cover increased with increasing first spray date for single, double, and triple sprays. Treatments with the same first application date were not significantly different from each other, except those with an initial application at 30 DAE, which had a lower surface coverage in the single spray treatment than the double and triple. All treatments applied with an initial spray date of 30 DAE or earlier resulted in significantly lower DCS than the control treatment, and all those with a first spray date of 40-60 DAE were not significantly different from the control. In the single and double sprays the 30 DAE treatments were not significantly different to the later applications, while in the triple spray treatments the 30DAE initial spray treatment resulted in significantly lower DCS than the 40 DAE initial spray treatment (Table 10).

In Desiree mean disease cover increased with increasing first spray date for single sprays, but this trend was less obvious in the double and triple spray treatments. All treatments resulted in a lower DCS than the control treatment. In the double spray treatments the 10 DAE initial spray was significantly lower than the 50 DAE initial spray, but there were no other significant difference between the treatments. In the triple spray treatments the 40 DAE initial spray was significantly lower than the 30 DAE initial spray treatment, but there were no other significant differences between the treatments. Treatments with the same first spray date of 10, 20 and 50 DAE were not significantly different from each other. The single spray treatment applied at 30 DAE had a significantly higher DCS than the corresponding double spray treatment,

and the single spray treatment applied at 40 DAE had a significantly higher DCS than the corresponding triple spray treatment (Table 10).

Lesion Depth Score (LDS) showed a similar pattern as DCS in both varieties. In Russet Burbank all treatments resulted in lower LDS than the control treatment except the 40 DAE and 60 DAE single spray, and the triple spray with an initial application date of 40 DAE. There was a trend towards increasing LDS with increasing initial spray dates for single, double and triple sprays. All treatments with the same initial spray date were not significantly different from each other, except the 30 DAE single spray treatment, which resulted in significantly lower LDS than the corresponding double and triple spray treatments (Table 10).

In Desiree, all treatments resulted in lower LDS than the control treatment. In the single spray treatments there was a trend towards increasing LDS with increasing spray date, although the 30-60 DAE sprays were not significantly different from each other. In the double spray treatments, the 10 DAE treatment had significantly lower LDS than the 50 DAE initial spray treatment, and all other treatments were not significantly different from each other. In the triple spray treatments, the 30 DAE treatment had a significantly higher LDS than the 40 DAE initial spray treatment, and all other treatments were not significantly different from each other (Table 10).

In both varieties all the 2,4-D sprays resulted in lower powdery scab DCS than the control treatments. In Russet Burbank using a single rate 10-40 DAE application resulted in lower powdery scab tuber surface coverage than the control. In Desiree using a single rate all 2,4-D treatments (10-60 DAE) gave lower powdery scab DCS than the control (Table 11).

There was a moderate positive correlation ($r^2 = 0.49$) between the mean DCS of common scab and powdery scab (Figure 4).

In field trial # 2 there was a significant interaction found between spray rate, number of sprays, and date of first spray on mean disease cover measured using the DCS. For the 25mg/L spray group the control treatment was not significantly different to any treatments except the 10 DAE single spray, which had significantly lower DCS. The 30 and 40 DAE single sprays had significantly higher DCS than the other 25mg/L single sprays. In the 100mg/L spray group, the control treatment was had significantly higher DCS than the 5 DAE single spray treatment and the 20 DAE double spray treatment. The 30 and 40 DAE double spray treatments had significantly higher DCS than the 5, 10 and 20 DAE double spray treatments, and the 5 and 10 DAE single spray treatments. The 40 DAE treatment is also significantly higher than the 20 DAE single spray treatment. All other 100mg/L treatments are not significantly different from each other. Treatments with the same first spray date and number of sprays are not significantly different, across spray rates. Desiree had significantly higher DCS than Russet Burbank (Table 12).

There was a significant interaction found between spray rate, number of sprays and date of first spray on mean LDS. The control treatment had significantly lower LDS than the 30 DAE single spray 25mg/L treatment, and the 40 DAE double spray 100mg/L treatment. All other treatments were not significantly different to the controls. The 5 and 10 DAE single spray 25mg/L treatments had the lowest LDS of all treatments, which were significantly lower than the 30 and 40 DAE single spray treatments and 5, 10, and 30 DAE double spray treatments in the 25mg/L treatment group, and then the 30 DAE single spray and 5, 30 and 40 DAE double spray treatments in the 100mg/L treatment group. All other treatments were not significantly different from each other. Desiree had significantly greater LDS than Russet Burbank (Table12).

Table 8. Glasshouse trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on common scab severity

Frequency	Rate	Date (DAE)	Disease Cover Score (Percentage Cover %)			Lesion Depth Score			Proportion Diseased/Pot (%)		
			RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Single	200mg/L	10	0.00	0.40 (1.20%)	0.20	0.00	0.60	0.30	0.0	10.7	5.3
		20	0.20 (0.60%)	0.00	0.10	0.40	0.00	0.20	5.0	0.0	2.5
		30	0.40 (1.50%)	0.00	0.20	0.40	0.00	0.20	10.0	0.0	5.0
		40	0.00	0.50 (2.75%)	0.25	0.00	0.50	0.30	0.0	4.0	2.0
		50	0.00	0.40 (1.50%)	0.20	0.00	0.20	0.10	0.0	3.3	1.7
		Control	0.00	1.00 (5.50%)	0.50	0.00	1.00	0.50	0.0	25.0	12.5
Double	200mg/L	10	0.00	1.10 (4.85%)	0.55	0.20	1.20	0.70	0.0	21.2	10.6
		20	0.00	0.80 (3.00%)	0.40	0.00	0.60	0.30	0.0	10.0	5.0
		30	0.20 (0.60%)	1.00 (5.50%)	0.60	0.40	1.00	0.70	6.7	10.0	8.3
		40	0.40 (1.50%)	0.40 (1.50%)	0.40	0.40	0.20	0.30	4.0	4.0	4.0
		50	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0
		Control	0.30 (0.70%)	0.87 (3.83%)	0.58	0.20	0.60	0.40	10.0	16.0	13.0
Double	100mg/L	10	0.00	0.50 (1.60%)	0.25	0.00	0.60	0.30	0.0	5.1	2.5
		20	0.00	0.00	0.00	0.00	0.20	0.10	0.0	0.0	0.0
		30	0.30 (0.70%)	0.80 (3.00%)	0.55	0.40	1.00	0.70	9.0	15.0	12.0
		40	0.40 (1.50%)	1.10 (3.70%)	0.75	0.40	1.30	0.90	3.3	21.4	12.4
		50	0.20 (0.60%)	1.10 (4.05%)	0.65	0.20	1.10	0.70	4.0	25.0	14.5
		Control	0.20 (0.60%)	0.60 (2.10%)	0.40	0.20	1.20	0.70	4.0	5.3	4.7
Mean		10	0.00	0.67	0.33	0.07	0.80	0.43	0.0	12.3	6.1
		20	0.07	0.27	0.17	0.13	0.27	0.20	1.7	3.3	2.5
		30	0.30	0.60	0.45	0.40	0.67	0.53	8.6	8.3	8.4
		40	0.27	0.67	0.47	0.27	0.67	0.47	2.4	9.8	6.1
		50	0.07	0.50	0.28	0.07	0.43	0.25	1.3	9.4	5.4
		Control	0.17	0.82	0.49	0.13	0.93	0.53	4.7	15.4	10.1
	Mean (variety)		0.14 a	0.59 b		0.18 a	0.63 b		3.1 a	9.8 b	

There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.1084, LSD = 0.2143), LDS ($p < 0.2343$, SED = 0.1185, LSD = 0.085) and proportion of diseased tubers ($p = 0.003$, SED = 2.22, LSD = 4.4). There was no significant effect found from date of first spray or number of sprays, or interactions between factors.

Treatments with the same letter in the same variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test

Table 9. Pot trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on common scab severity

Frequency	Rate	Date (DAE)	Disease Cover Score (Percentage Cover %)			Lesion Depth Score			Proportion Diseased/Pot (%)		
			RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Single	**200mg/L**	**10**	0.15 (0.35%)	0.10 (0.10%)	0.13	0.20	0.20	0.20	13.3	5.0	9.2
		20	0.20 (0.60%)	0.00	0.10	0.60	0.20	0.40	10.0	0.0	5.0
		30	0.20 (0.20%)	0.35 (0.95%)	0.28	0.40	0.60	0.50	15.3	8.2	11.8
		40	0.10 (0.10%)	0.20 (0.60%)	0.15	0.20	0.40	0.30	2.9	5.0	3.9
		50	0.55 (1.55%)	0.80 (2.30%)	0.68	1.00	1.20	1.10	23.3	15.2	19.3
		Control	0.70 (1.50%)	0.75 (2.45%)	0.73	1.10	0.80	0.95	38.0	11.2	24.6
Double	**200mg/L**	**10**	0.10 (0.10%)	0.00	0.05	0.40	0.00	0.20	4.0	0.0	2.0
		20	0.10 (0.10%)	0.50 (1.25%)	0.30	0.40	0.70	0.55	8.0	10.3	9.2
		30	0.10 (0.10%)	0.50 (1.60%)	0.30	0.20	0.40	0.30	6.7	5.3	6.0
		40	0.10 (0.10%)	0.70 (2.20%)	0.40	0.40	0.80	0.60	5.0	8.1	6.5
		50	0.40 (1.20%)	0.30 (0.30%)	0.35	0.40	0.60	0.50	16.7	8.5	12.6
		Control	0.30 (0.70%)	0.55 (1.55%)	0.43	0.40	0.80	0.60	23.3	12.9	18.1
Double	**100mg/L**	**10**	0.20 (0.20%)	0.30 (0.70%)	0.25	0.40	0.80	0.60	14.0	6.9	10.4
		20	0.30 (0.70%)	0.30 (0.30%)	0.30	0.40	1.00	0.70	26.7	10.6	18.7
		30	0.10 (0.10%)	0.10 (0.10%)	0.10	0.40	0.20	0.30	10.0	1.8	5.9
		40	0.00	0.25 (0.80%)	0.13	0.20	0.40	0.30	0.0	6.7	3.3
		50	0.60 (1.00%)	0.30 (0.70%)	0.45	1.00	0.60	0.80	32.5	6.2	19.3
		Control	0.60 (1.80%)	0.60 (1.80%)	0.60	0.60	0.70	0.65	23.0	11.9	17.5
Mean		**10**	0.15	0.13	0.14 a	0.33	0.33	0.33 a	10.4	4.0	7.2 a
		20	0.20	0.27	0.23 a	0.47	0.63	0.55 ab	14.9	7.0	10.9 ab
		30	0.13	0.32	0.23 a	0.33	0.40	0.37 a	10.7	5.1	7.9 a
		40	0.07	0.38	0.23 a	0.27	0.53	0.40 a	2.6	6.6	4.6 a
		50	0.52	0.47	0.49 b	0.80	0.80	0.80 b	24.2	10.0	17.1 bc
		Control	0.53	0.63	0.58 b	0.70	0.77	0.73 b	28.1	12.0	20 c
		Mean	0.27	0.37		0.48	0.58		15.1 a	7.4 b	

There was a significant effect found from date of first spray on DCS ($p < 0.001$, SED = 0.1146, LSD = 0.2266), LDS ($p = 0.003$, SED = 0.1448, LSD = 0.2864) and from date of first spray ($p = 0.003$, SED = 4.41, LSD = 8.73) and variety ($p = 0.003$, SED = 2.25, LSD = 5.04) on proportion of diseased tubers. There were no other significant effects on variables, or interactions between factors.

Treatments with the same letter in the same variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test

Table 10. Field trial #1 The effect of 2,4-D applied at various times, and as single, double and triple sprays to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on common scab severity

Frequency	Rate	Date (DAE)	Disease Cover Score (and Percentage Cover %)							Lesion Depth Score				
			RBK			DES			Mean	RBK		DES		Mean
Single	100mg/L	10	1.57	a	(5.83%)	2.28	ab	(12.16%)	1.92	1.80	abc	2.49	abc	2.15
		20	1.66	ab	(6.52%)	2.44	abc	(14.78%)	2.05	2.12	bc	2.65	abcd	2.39
		30	2.08	b	(10.81%)	2.79	cde	(19.25%)	2.43	2.16	cd	3.03	def	2.60
		40	2.78	cd	(18.38%)	2.91	de	(20.82%)	2.84	2.95	efg	2.89	ef	2.92
		50	2.79	cd	(19.54%)	3.00	e	(23.11%)	2.89	2.79	ef	2.95	f	2.87
		60	2.95	cd	(21.27%)	3.03	e	(22.85%)	2.99	3.04	fg	3.00	f	3.02
Double	200mg/L	10	1.48	a	(5.35%)	2.14	a	(10.91%)	1.81	1.70	ab	2.52	a	2.11
		20	1.68	ab	(7.17%)	2.51	abcd	(14.81%)	2.09	1.89	abc	2.78	abcde	2.33
		30	2.55	c	(15.44%)	2.34	ab	(13.54%)	2.45	2.64	ef	2.82	abc	2.73
		40	2.79	cd	(19.00%)	2.50	abcd	(16.34%)	2.64	2.60	de	2.68	abcde	2.64
		50	2.77	cd	(18.38%)	2.66	bcde	(18.00%)	2.71	2.71	ef	2.92	cdef	2.83
Triple	300mg/L	10	1.35	a	(4.56%)	2.25	ab	(12.16%)	1.80	1.58	a	2.73	abc	2.15
		20	1.78	ab	(8.00%)	2.21	ab	(12.42%)	1.99	2.01	bc	2.71	ab	2.36
		30	2.61	c	(16.68%)	2.61	bcde	(16.26%)	2.61	2.76	ef	2.68	bcdef	2.72
		40	3.21	d	(20.98%)	2.15	a	(12.28%)	2.68	2.94	efg	2.46	a	2.70
	Control		3.20	d	(25.20%)	3.58	f	(32.04%)	3.39	3.27	g	3.32	g	3.30
Mean		10	1.47			2.22			1.84	1.69		2.58		2.14
		20	1.71			2.39			2.05	2.01		2.71		2.36
		30	2.41			2.58			2.50	2.52		2.84		2.68
		40	2.93			2.52			2.72	2.83		2.68		2.75
		50	2.78			2.83			2.80	2.75		2.93		2.84
		60	2.95			3.03			2.99	3.04		3.00		3.02
		Control	3.20			3.58			3.39	3.27		3.32		3.30
		Mean	2.33			2.59				2.43		2.79		

There was a significant interaction between date of first spray, number of sprays and variety on DCS ($p = 0.027$, SED = 0.2286, LSD = 0.4539) and LDS ($p = 0.024$, SED = 0.2193, LSD = 0.4355), and between date of first spray and variety ($p < 0.001$, SED = 2.027, LSD = 4.026) and number of sprays and variety ($p = 0.002$, SED = 1.896, LSD = 3.766) on percentage cover.

Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test

Table 11. Field trial #1 The effect of 2,4-D applied at various times, and as single, double and triple sprays to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on powdery scab severity.

Frequency	Rate	Date (DAE)	Powdery Scab Disease Cover Score (Percentage Cover %) RBK			DES			Mean
Single	**100mg/L**	**10**	2.79	ab	(20.11%)	2.68	bcde	(17.32%)	2.74
		20	2.78	ab	(18.40%)	3.06	efg	(23.32%)	2.92
		30	2.96	abc	(21.99%)	2.43	ab	(15.07%)	2.70
		40	3.27	cd	(26.77%)	3.24	g	(26.14%)	3.25
		50	3.33	cde	(28.11%)	3.13	efg	(25.72%)	3.23
		60	3.29	cde	(28.11%)	3.08	efg	(23.24%)	3.18
Double	**200mg/L**	**10**	2.70	ab	(17.59%)	2.57	abcd	(15.81%)	2.64
		20	2.67	ab	(17.29%)	3.16	fg	(25.21%)	2.91
		30	3.06	abcd	(23.11%)	2.59	abcd	(17.93%)	2.82
		40	3.34	cde	(28.20%)	3.02	defg	(22.76%)	3.18
		50	3.35	cde	(27.97%)	2.97	cdefg	(22.98%)	3.16
Triple	**300mg/L**	**10**	2.63	a	(16.76%)	2.73	bcdef	(18.61%)	2.68
		20	2.70	ab	(18.26%)	2.53	abc	(16.74%)	2.62
		30	3.11	bcd	(24.08%)	2.81	bcdef	(20.15%)	2.96
		40	3.49	de	(30.22%)	2.18	a	(13.31%)	2.83
	Control		3.74	e	(25.30%)	3.73	h	(34.98%)	3.73
Mean		**10**	2.71			2.66			2.68
		20	2.72			2.92			2.82
		30	3.03			2.61			2.83
		40	3.37			2.81			3.09
		50	3.34			3.05			3.20
		60	3.29			3.08			3.18
		Control	3.74			3.73			3.73
	Mean (variety)		3.08			2.87			

There was a significant interaction between date of first spray, number of sprays and variety on DCS (p = 0.014, SED = 0.2343, LSD = 0.2654) and LDS (p = 0.02, SED = 3.223, LSD = 6.401.

Treatments with the same letter in the same column are not significantly different at p = 0.05 using Fischer's LSD test

Figure 4. Field trial #1 Linear regression between the mean surface coverage of common scab and powdery scab, on tubers harvested from Russet Burbank and Desiree plants treated with 2,4-D at various rates, times, and frequencies.

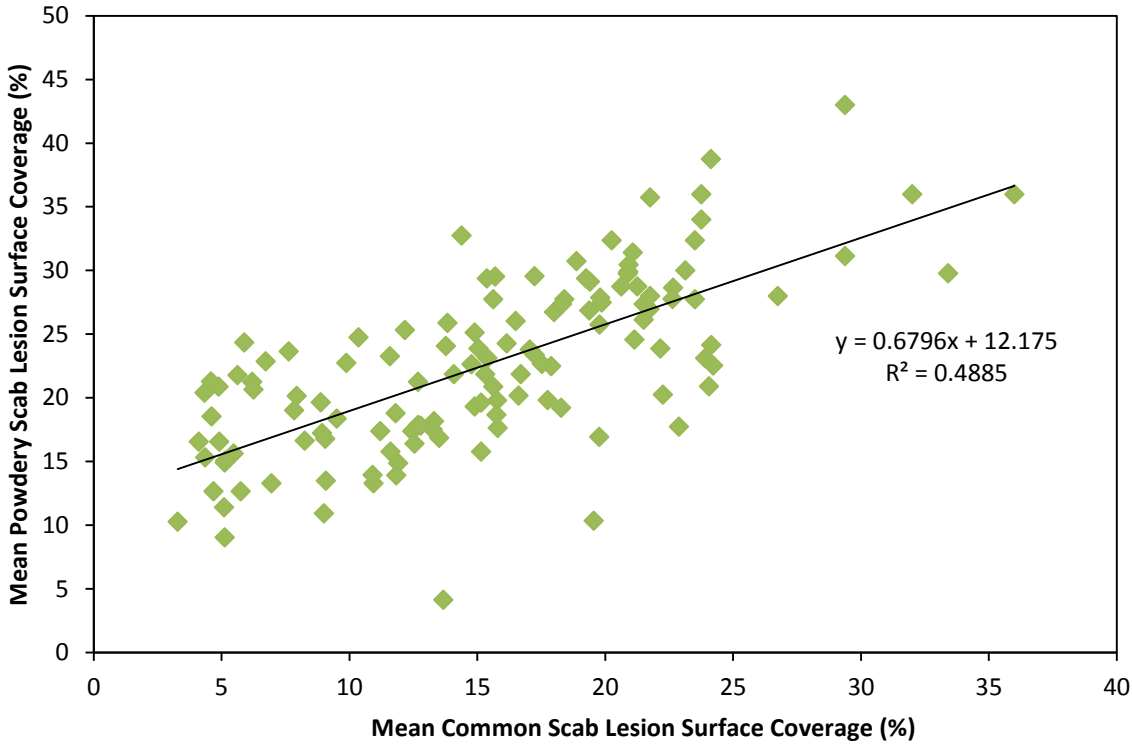


Table 12. Field trial #2 The effect of 2,4-D applied at various times, rates and as single and double sprays to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on common scab severity

Spray Rate	Frequency	Date (DAE)	Disease Cover Score - DCS (Percentage Cover %) RBK		DES		Mean		Lesion Depth Score - LDS RBK	DES	Mean	
25mg/L	Single	5	1.58	(7.54%)	1.15	(3.61%)	1.36	ab	1.02	1.06	1.04	a
		10	1.00	(3.18%)	1.70	(6.99%)	1.35	a	1.09	1.06	1.07	a
		20	1.19	(4.45%)	2.00	(10.17%)	1.60	abcde	1.04	1.29	1.17	abc
		30	1.59	(7.47%)	3.00	(21.43%)	2.30	gh	1.29	2.25	1.77	ef
		40	1.82	(9.31%)	2.45	(15.22%)	2.14	fgh	1.38	1.64	1.51	cde
		50	1.20	(4.30%)	1.86	(9.53%)	1.48	abc	1.08	1.50	1.26	abcd
	Double	5	1.56	(7.12%)	2.70	(18.25%)	2.13	fgh	1.36	1.80	1.58	de
		10	1.47	(6.44%)	2.58	(19.17%)	2.02	defgh	1.13	1.80	1.47	cde
		20	1.45	(6.16%)	2.23	(11.59%)	1.84	abcdefg	1.18	1.44	1.31	abcd
		30	1.52	(6.09%)	2.65	(16.68%)	2.08	efgh	1.21	1.69	1.45	bcde
		40	1.67	(7.68%)	2.62	(15.95%)	2.08	efgh	1.20	1.67	1.40	abcde
		Control	1.17	(4.18%)	2.58	(16.05%)	1.87	bcdefg	1.11	1.58	1.34	abcd
100mg/L	Single	5	1.34	(5.43%)	1.77	(8.63%)	1.56	abcd	1.21	1.54	1.37	abcd
		10	0.82	(2.05%)	2.67	(16.83%)	1.74	abcdef	1.06	1.63	1.34	abcd
		20	1.50	(8.34%)	2.15	(11.96%)	1.82	abcdefg	1.15	1.29	1.22	abcd
		30	1.40	(5.55%)	2.46	(16.65%)	1.93	cdefgh	1.04	1.91	1.48	cde
		40	1.54	(6.41%)	2.44	(13.06%)	1.93	cdefgh	1.15	1.67	1.37	abcd
		50	1.22	(4.56%)	2.67	(17.08%)	1.94	cdefgh	1.10	1.67	1.38	abcd
	Double	5	1.42	(7.00%)	2.30	(12.63%)	1.71	abcdef	1.27	2.05	1.53	cde
		10	1.05	(4.08%)	2.25	(12.42%)	1.65	abcdef	1.17	1.68	1.43	abcde
		20	1.05	(3.37%)	1.94	(8.14%)	1.50	abc	1.11	1.44	1.28	abcd
		30	1.67	(8.71%)	3.00	(22.95%)	2.34	gh	1.23	1.93	1.58	de
		40	1.70	(7.40%)	3.33	(26.67%)	2.40	h	1.32	2.83	1.97	f
		Control	1.67	(7.37%)	2.62	(17.43%)	2.15	fgh	1.22	1.42	1.32	abcd
Mean		5	1.47		1.93		1.69		1.21	1.55	1.37	
		10	1.08		2.30		1.69		1.11	1.54	1.33	
		20	1.31		2.08		1.69		1.12	1.36	1.24	
		30	1.56		2.80		2.18		1.20	1.95	1.57	
		40	1.68		2.69		2.14		1.26	1.93	1.56	
		50	1.21		2.32		1.73		1.09	1.60	1.33	
		Control	1.42		2.60		2.01		1.16	1.50	1.33	
	Mean (variety)		1.40 a		2.38 b				1.17 a	1.64 b		

There was a significant interaction between date of first spray, spray rate and number of sprays (DCS - $p = 0.007$, SED = 0.26, LSD = 0.5143; LDS - $p = 0.011$, SED = 0.1955, LSD = 0.3868) and a significant effect from variety (DCS - $p < 0.001$, SED = 0.0721, LSD = 0.1426; LDS - $p < 0.001$, SED = 0.1073, LSD = 0.1517).

AGRONOMIC EFFECTS

In pot trial #1 there was a significant effect found from the date of first treatment on the total mass of tubers per pot. The control and treatments with a first application date of 40 DAE resulted in the highest total tuber mass, but were not significantly different from 10 DAE treatments, Treatments with a first application date of 20, 30 and 50 DAE had tuber masses significantly lower than the control treatment. Treatments with a first application date of 50 DAE were not significantly different from any of the 2,4-D treatments except the treatment applied at 40 DAE, which it was significantly lower than. The treatments with first spray dates of 20 and 30 DAE had the lowest tuber masses. There was no significant effect found from first spray date on mean mass per tuber or number of tubers per pot, and there was no significant effect found from variety or spray rate and frequency on any agronomic features recorded (Table 13).

In pot trial #2 there was a significant interaction between first spray date, and rate and frequency of application, on the total tuber mass. In the single application treatment category the control treatment had a significantly higher total tuber mass then all other treatments, except the 50 DAE treatment. This control treatment was also significantly higher than the other two control treatments. There was a general trend for the tuber masses to increase with increasing first application date in the single and double higher rate treatment categories. In the single spray rate category the 10 DAE treatment was not significantly different to the 30 or 40DAE treatment, and the 20 DAE treatment was not significantly different to any treatments except the control, which it was lower than, and the 10 DAE treatment, which it was higher than. In the double higher rate category the 10 DAE treatment was significantly lower than the 30 and 50 DAE treatments and the control treatments, but not significantly different to all other treatments. There was a similar trend in the double lower rate treatment category, but there was no significant difference between application date treatments within this category. All treatments across all categories, with the same first spray date were not significantly different from each other, with the exception of the 30 DAE initial spray date, which had significantly higher total tuber masses in the double, higher spray rate treatment than the other two spray rate and frequency treatments. Variety was found to have an effect on all three agronomic features recorded, with Desiree having a higher total tuber mass and number of tubers per pot, while Russet Burbank had a higher mean tuber mass. There was no significant effect found from first spray date or application rate and frequency on the other two agronomic features (Table 14).

In field trial #1 there were no significant effects found from any factors on total tuber mass. There was a significant effect found from variety on mean mass per tuber and number of tubers. Russet Burbank had significantly greater number of tubers than Desiree, but Desiree had a significantly higher mean mass per tuber than Russet Burbank (Table 15).

In field trial #2 there was a significant interactive effect from spray rate, number of sprays, and date of first spray on mean total mass. However, there is no trend apparent. In the 25mg/L spray group, none of the treatments are significantly different from the control. The 5 DAE single spray has a significantly lower mean total mass than the 40 DAE single spray and 5, 10 and 30 DAE double sprays. In the 100mg/L spray group, the 30 DAE single and double sprays had significantly higher mean total masses than the control treatments, but were not significantly different to the 5 and 20 DAE single sprays, and the 5, 10 and 30 DAE double sprays. There was a significant effect found from date of first spray on mean tuber number. No treatments were significantly different from the control. The 50 DAE treatment had significantly fewer tubers than the 5, 10 and 30 DAE treatments. All other treatments were not significantly different from each other. There was a significant effect found from variety on mean total mass, mean mass per tuber, and number of tubers. Russet Burbank had a significantly greater total tuber mass and number of tubers than Desiree, but Desiree had significantly higher mean mass per tuber than Russet Burbank (Table 16).

Table 13. Pot trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on total tuber mass per pot, mean mass per tuber and number of tubers per pot.

Frequency	Rate	Date (DAE)	Total Tuber Mass/Pot (g)			Mean Tuber Mass (g)			Number of Tubers/Pot		
			RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Single	200mg/L	10	25.57	28.15	36.02	8.97	7.16	8.07	5.2	5.2	5.2
		20	27.71	23.08	23.41	4.70	5.62	5.16	6.8	4.0	5.4
		30	22.85	21.15	27.70	7.01	5.69	6.35	5.0	4.8	4.9
		40	33.72	30.18	28.08	4.76	6.16	5.46	4.6	7.2	5.9
		50	21.15	28.14	25.20	6.53	5.37	5.95	4.2	5.8	5.0
		Control	31.96	36.67	33.16	7.01	9.17	8.09	4.6	5.4	5.0
Double	200mg/L	10	28.44	19.23	23.83	6.07	3.9	4.98	6.2	5.6	5.9
		20	20.55	23.48	22.09	5.64	5.28	5.46	4.4	5.2	4.8
		30	18.68	19.23	18.96	5.01	4.13	4.57	4.0	5.0	4.5
		40	29.95	29.31	29.63	5.76	8.33	7.04	6.0	4.0	5.0
		50	22.36	24.51	23.43	5.42	4.94	5.18	4.8	5.4	5.1
		Control	27.43	37.69	32.56	3.47	7.33	5.40	8.2	5.4	6.8
Double	100mg/L	10	38.45	33.60	26.86	4.22	5.02	4.62	6.4	6.2	6.3
		20	25.27	21.54	25.40	5.74	3.66	4.70	5.6	6.6	6.1
		30	27.50	27.90	22.00	4.97	8.7	6.83	5.0	4.8	4.9
		40	20.80	35.36	31.95	8.85	6.36	7.60	4.4	4.8	4.6
		50	27.16	23.24	24.64	5.77	5.39	5.58	4.6	6.0	5.3
		Control	28.97	37.34	34.32	7.16	8.88	8.02	4.8	5.4	5.1
Mean		10	30.82	26.99	28.91 bc	6.42	5.36	5.89	5.9	5.7	5.8
		20	24.51	22.70	23.61 a	5.36	4.85	5.11	5.6	5.3	5.4
		30	23.01	22.76	22.89 a	5.66	6.17	5.92	4.7	4.9	4.8
		40	28.16	31.62	29.89 c	6.45	6.95	6.70	5.0	5.3	5.2
		50	23.56	25.29	24.43 ab	5.91	5.23	5.57	4.5	5.7	5.1
		Control	29.46	37.24	33.35 c	5.88	8.46	7.17	5.9	5.4	5.6
	Mean (variety)		26.59	27.77		5.95	6.17		5.3	5.4	

There was a significant effect found from the date of the first spray on total tuber mass ($p < 0.001$, SED = 2.3577, LSD = 4.6612). There was no significant effect found from number of sprays or variety on total tuber mass. There was no significant effect found from date of first spray, number of sprays, or variety on mean tuber mass or number of tubers per pot. There were no significant interactions between factors. Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test

Table 14: Pot trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on total tuber mass per pot, mean mass per tuber and number of tubers per pot.

Frequency	Rate	Date (DAE)	Total Tuber Mass/Pot (g)			Mean Tuber Mass (g)			Number of Tubers/Pot		
			RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Single	200mg/L	10	23.48	29.96	26.72 a	6.86	7.60	7.23	3.8	5.8	4.8
		20	34.08	40.70	37.39 bcd	9.62	7.30	8.46	4.2	6.6	5.4
		30	29.47	30.74	30.11 ab	5.60	5.56	5.58	5.6	6.0	5.8
		40	36.84	29.43	33.13 abc	9.07	5.94	7.51	4.2	5.4	4.8
		50	41.33	47.65	44.49 de	13.23	7.30	10.26	4.0	6.8	5.4
		Control	44.83	55.10	49.97 e	12.65	8.36	10.50	3.8	7.2	5.5
Double	200mg/L	10	25.22	28.76	26.99 a	7.52	3.67	5.60	3.6	7.2	5.4
		20	29.21	29.02	29.11 ab	12.36	6.58	9.47	3.2	6.0	4.6
		30	41.57	39.39	40.48 cd	14.87	6.47	10.67	3.2	7.6	5.4
		40	28.31	35.81	32.06 abc	11.03	5.60	8.31	3.4	6.6	5.0
		50	33.46	41.51	37.49 bcd	12.36	6.36	9.36	3.0	7.8	5.4
		Control	38.72	33.64	36.18 bcd	15.85	8.10	11.98	2.8	6.0	4.4
Double	100mg/L	10	29.31	39.06	34.19 abc	9.97	6.62	8.30	3.4	5.8	4.6
		20	32.23	30.86	31.55 abc	10.79	5.60	8.20	3.8	6.4	5.1
		30	36.85	38.72	37.78 bcd	14.21	6.81	10.51	3.0	6.8	4.9
		40	32.96	39.93	36.45 bcd	12.44	6.85	9.64	3.6	5.8	4.7
		50	39.08	36.38	37.73 bcd	10.16	5.26	7.71	4.4	7.2	5.8
		Control	32.45	42.81	37.63 bcd	9.64	6.35	7.99	3.8	7.4	5.6
Mean		10	26.00	32.59	29.30	8.12	5.97	7.04	3.6	6.3	4.9
		20	31.84	33.53	32.68	10.92	6.50	8.71	3.7	6.3	5.0
		30	35.96	36.28	36.12	11.56	6.28	8.92	3.9	6.8	5.4
		40	32.7	35.06	33.88	10.85	6.13	8.49	3.7	5.9	4.8
		50	37.96	41.84	39.90	11.92	6.31	9.11	3.8	7.3	5.5
		Control	38.67	43.85	41.26	12.71	7.60	10.16	3.5	6.9	5.2
	Varietal Mean		33.86 a	37.19 b		11.01 a	6.46 b		3.7 a	6.6 b	

There was a significant effect found from variety on total tuber mass per pot ($p < 0.033$, SED = 1.549, LSD = 3.062), mean mass per tuber ($p < 0.001$, SED = 0.724, LSD = 1.432) and number of tubers ($p < 0.001$, SED = 0.3, LSD = 0.593). There was a significant interaction found between date of first spray and number of sprays on total tuber mass per pot ($p = 0.013$, SED = 4.646, LSD = 9.185). There was no significant effect found from number of sprays or variety on total tuber mass. There was no significant effect found from date of first spray or number of sprays, or interactions between factors, on mean mass per tuber or number of tubers.

Table 15: Field trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank (RBK) and Desiree (DES) plants, on total tuber mass, mean mass per tuber and number of tubers.

Frequency	Rate	Date (DAE)	Mean Total Mass (g)			Mean Mass per Tuber (g)			Number of Tubers		
			RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Single	**100mg/L**	**10**	3134	4152	3643	93.7	145.0	119.3	33.5	28.5	31.0
		20	3419	3148	3283	107.5	172.7	140.1	33.3	18.8	26.0
		30	3854	3131	3492	111.7	166.0	138.8	35.3	19.0	27.1
		40	2956	2694	2825	91.0	135.9	113.4	32.3	18.5	25.3
		50	3131	3506	3319	125.3	175.2	150.3	25.5	20.3	22.9
		60	3192	3718	3455	102.7	176.5	139.6	30.0	21.3	25.6
Double	**200mg/L**	**10**	3161	3250	3206	70.4	153.8	112.1	44.8	20.8	32.8
		20	3285	3656	3471	97.0	148.0	122.5	34.0	25.3	29.6
		30	3675	2508	3091	112.7	159.1	135.9	34.0	15.5	24.8
		40	4081	3734	3908	115.1	178.0	146.5	35.3	21.5	28.8
		50	3312	2990	3151	111.4	156.6	134.0	33.3	20.0	26.6
Triple	**300mg/L**	**10**	3029	3902	3466	70.7	144.2	107.4	42.8	27.3	35.0
		20	3775	3841	3808	104.9	163.1	134.0	35.8	23.5	29.6
		30	2610	2952	2781	107.1	158.6	132.8	24.0	19.0	21.5
		40	2891	3188	3039	80.6	170.0	125.3	35.8	18.8	27.3
	Control		2332	3761	3047	111.1	198.5	154.8	22.8	19.5	21.1
Mean		**10**	3108	3768	3438	78.2	147.6	112.9	40.3	25.5	32.9
		20	3493	3548	3521	103.1	161.3	132.2	34.3	22.5	28.4
		30	3380	2864	3122	110.5	161.2	135.8	31.1	17.8	24.5
		40	3310	3205	3257	95.6	161.3	128.4	34.4	19.6	27.0
		50	3222	3248	3235	118.3	165.9	142.1	29.4	20.1	24.8
		60	3192	3718	3455	102.7	176.5	139.6	30.0	21.3	25.6
		Control	2332	3761	3047	111.1	198.5	154.8	22.8	19.5	21.1
	Mean (variety)		3240	3383		100.8 a	162.6 b		33.3 a	21.1 b	

There was a significant effect found from variety on mean tuber mass ($p < 0.001$, SED = 4.92, LSD = 9.76) and number of tubers ($p < 0.001$, SED = 1.684, LSD = 3.345). There were no significant effects found from number of sprays, or date of first spray on mean tuber mass, or interactions between factors, on number of tubers. There was no significant effect found from any factors on total tuber mass.

Treatments with the same letter in the same row and factor are not significantly different at $p = 0.05$ using Fischer's LSD test.

Table16: Field trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on total tuber mass, mean mass per tuber and number of tubers.

Spray rate	Frequency	Effective Rate	Date (DAE)	Mean total mass (g)				Mean mass per tuber (g)			Number of tubers		
				RBK	DES	Mean		RBK	DES	Mean	RBK	DES	Mean
25mg/L	Single	25mg/L	5	151.2	75.0	113.1	a	13.7	23.8	18.7	8.3	3.0	5.6
			10	291.2	178.8	235.0	abc	26.8	23.4	25.1	10.8	7.8	9.3
			20	327.5	70.0	198.8	ab	26.4	32.5	29.5	10.5	2.0	6.3
			30	455.0	300.0	377.5	abcdefg	26.3	52.3	39.3	13.5	5.5	9.5
			40	632.5	292.5	462.5	bcdefg	29.4	27.2	28.3	16.8	7.5	12.1
			50	247.5	163.3	211.4	abc	24.0	42.8	32.1	8.0	3.0	5.5
	Double	50mg/L	5	677.5	497.5	587.5	defg	33.7	44.2	39.0	14.5	11.5	13.0
			10	737.5	252.5	495.0	bcdefg	44.3	37.5	40.9	13.8	6.0	9.9
			20	320.0	460.0	390.0	abcdefg	24.7	26.4	25.6	8.5	7.8	8.1
			30	822.5	442.5	632.5	fg	38.6	53.0	45.8	15.0	5.8	10.4
			40	358.8	110.0	252.1	abcde	29.6	24.1	27.2	11.3	4.0	7.6
		Control		327.5	311.2	319.4	abcdef	31.6	30.8	31.2	8.3	6.5	7.4
100mg/L	Single	100mg/L	5	656.2	536.2	596.3	efg	37.1	38.7	37.9	15.0	10.5	12.8
			10	308.8	178.8	243.8	abcd	20.8	35.6	28.2	13.5	4.8	9.1
			20	586.2	237.5	411.9	abcdefg	41.5	27.8	34.7	12.0	5.8	8.9
			30	716.7	713.3	715.0	g	34.7	38.2	36.5	12.5	12.5	12.5
			40	408.3	91.7	250.0	abcde	33.5	29.4	31.5	10.0	2.8	6.4
			50	280.0	311.2	295.6	abcdef	37.7	74.2	55.9	6.8	4.0	5.4
	Double	200mg/L	5	672.5	317.5	554.2	cdefg	27.5	50.1	35.0	20.3	3.3	11.8
			10	465.0	328.8	396.9	abcdefg	29.7	32.4	31.1	15.5	10.0	12.8
			20	271.7	91.7	181.7	ab	26.2	21.4	23.8	7.0	2.5	4.8
			30	625.0	761.2	693.1	g	29.2	41.0	35.1	16.0	12.5	14.3
			40	626.2	140.0	417.9	abcdefg	40.3	72.5	54.1	12.3	1.3	6.8
		Control		432.5	206.2	319.4	abcdef	28.5	36.2	32.4	12.5	6.0	9.3
			5	539.4	362.1	456.7		28.0	37.7	32.5	14.5	7.1	10.8 c
			10	450.6	234.7	342.7		30.4	32.2	31.3	13.4	7.1	10.3 bc
			20	387.9	206.1	297.0		30.3	27.5	28.9	9.5	4.5	7.0 ab
			30	650.7	543.7	597.2		32.0	46.7	39.4	14.3	9.1	11.7 c
			40	513.0	168.8	353.2		33.2	37.4	35.2	12.6	3.9	8.2 abc
			50	263.8	247.9	256.3		30.8	60.7	44.8	7.4	3.5	5.4 a
		Control		380.0	258.8	319.4		30.1	33.5	31.8	10.4	6.3	8.3 abc
		Mean (variety)		476.9 a	297.3 b			30.7 a	38.0 b		12.2 a	6.1 b	

There was a significant effect found from variety on total tuber mass ($p < 0.001$, SED = 48.7, LSD = 96.4), mean tuber mass ($p < 0.001$, SED = 2.68, LSD = 5.31) and number of tubers ($p < 0.001$, SED = 0.809, LSD = 1.599). There was a significant interaction between spray rate, number of sprays and date of first spray on total tuber mass ($p = 0.027$, SED = 175.6, LSD = 347.4) and a significant effect found from date of first spray on number of tubers ($p < 0.001$, SED = 1.786, LSD = 3.53). Treatments with the same letter in the same row and factor are not significantly different at $p = 0.05$ using Fischer's LSD test

TOXIN TOLERANCE

In pot trial #1, while there was a significant interactive effect from variety, timing of sprays and number of sprays on tuber toxin tolerance after harvest, there is no particular trend apparent (Table 17).

In pot trial #2 there was no significant effect from treatment apparent in Desiree. In Russet Burbank all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control treatment, except the earliest treatment applied at 10 DAE. The 10, 20, 40 and 50 DAE treatments were not significantly different from each other. The 30 DAE treatment resulted in a necrosis score significantly lower (or significantly higher toxin tolerance) than all other treatments (Table 17).

Toxin tolerance was only determined for tubers harvested from **field trial #2**. There was a significant interactive effect found on toxin tolerance between date of first application, application rate, and variety. In Russet Burbank, the control treatment had significantly higher necrosis scores than all 100mg/L treatments, and all 25mg/L treatments except treatments with a first spray date of 20 DAE. Of the 100mg/L treatments, all the scores were not significantly different from each other, except the 10 and 20 DAE treatments, which had significantly higher necrosis scores than all other treatments, except the 40 DAE treatment, which was not significantly different to any 100mg/L treatment. Of the 25mg/L treatments, the 5, 10 and 20 DAE treatments were not significantly different to each other, and the 5 and 20 DAE treatments had significantly higher necrosis scores than all other treatments. All other treatments were not significantly different to each other (Table 18).

In Desiree, the control treatment had a higher necrosis score than all 100mg/L treatments except those with a first spray date of 10 and 20 DAE, and all 25mg/L treatments, except those with a first spray date of 10 DAE. Of the 25mg/L 10 DAE treatment also had higher necrosis scores than all treatments except for 20 DAE, and all other 25mg/L treatments were not significantly different from each other. Of the 100mg/L treatments, the 10 and 20 DAE treatments were not significantly different from each other, and both had significantly higher necrosis scores than all the other treatments, which were not significantly different from each other (Table 18).

2,4-D QUANTIFICATION

Russet Burbank had a significantly greater amount of 2,4-D in tubers at harvest than Desiree (Figures 5 & 6). In both varieties there is a trend towards increasing levels of 2,4-D in tubers at harvest with later application dates. For most application dates the 100mg/L application rate treatment had higher levels of 2,4-D in the tuber at harvest than the 25mg/L treatment, except the 40 DAE treatment in Russet Burbank and the 10 and 50 DAE treatments in Desiree. The sample taken from Russet Burbank treated at 50 DAE with 100mg/L is the only sample to have greater than Australian maximum residue level (MRL) of 100ng/g of tuber fresh weight (Commonwealth of Australia 2009; Figure 5).

For treatments applied at 20 DAE, the samples taken from Desiree tubers have similar levels of 2,4-D at harvest for both applications rates and for single and double applications. The sample taken from Russet Burbank tubers have similar levels to the Desiree samples for the 25mg/L single treatment, and much greater levels for the other treatments. The 100mg/L single and 25mg/L double treatments are similar, and the 100mg/L double treatment is slightly higher. All treatments resulted in levels at harvest below the MRL. This data lacks replication and as such has only been analysed statistically for varietal differences (Figure 6).

Table 17 Glasshouse trials: The effect of 2,4-D foliar sprays on mean necrosis levels from the treatment of harvested tuber slices with thaxtomin A.

Frequency	Rate	Date (DAE)	Trial #1			Trial #2		
			RBK	DES	Mean	RBK	DES	Mean
Single	**200mg/L**	**10**	2.042 cd	1.625 a	1.833	2.219 j	1.427 abc	1.823
		20	2.062 cd	1.875 bcde	1.969	1.667 ef	1.594 bcdef	1.63
		30	1.729 ab	1.854 abcd	1.792	1.188 a	1.688 defg	1.438
		40	2.646 g	2.083 def	2.365	2.000 hi	1.26 a	1.63
		50	2.417 fg	1.771 abc	2.094	1.792 fgh	1.521 bcd	1.656
		Control	1.979 cd	2.063 def	2.021	2.167 ij	1.688 defg	1.927
Double	**200mg/L**	**10**	1.688 ab	1.708 abc	1.698	1.594 def	1.385 ab	1.49
		20	2.354 ef	1.875 bcde	2.115	1.542 cde	1.865 g	1.703
		30	2.062 cd	1.875 bcde	1.969	1.448 bcd	1.896 g	1.672
		40	1.521 a	1.812 abc	1.667	1.250 ab	1.625 cdef	1.438
		50	2.062 cd	2.063 def	2.062	1.938 gh	1.573 bcde	1.755
		Control	2.167 de	2.250 f	2.208	1.927 gh	1.854 g	1.891
Double	**100mg/L**	**10**	1.875 bc	1.667 ab	1.771	1.688 ef	1.76 efg	1.724
		20	2.167 de	1.729 abc	1.948	1.917 gh	1.708 defg	1.813
		30	2.521 fg	1.917 cde	2.219	1.354 abc	1.604 cdef	1.479
		40	1.979 cd	1.938 cde	1.958	1.729 efg	1.802 fg	1.766
		50	2.042 cd	1.771 abc	1.906	1.521 cde	1.635 cdef	1.578
		Control	1.979 cd	2.104 ef	2.042	2.208 ij	1.708 defg	1.958
Mean		**10**	1.868	1.667	1.767	1.833	1.524	1.679
		20	2.194	1.826	2.010	1.708	1.722	1.715
		30	2.104	1.882	1.993	1.330	1.729	1.53
		40	2.049	1.944	1.997	1.660	1.563	1.611
		50	2.174	1.868	2.021	1.750	1.576	1.663
		Control	2.042	2.139	2.090	2.101	1.75	1.925

Necrosis was rated using visual scale (0 = no necrosis to 4 = black necrosis)

There was a significant interaction between first spray date, number of sprays and variety in pot trial #1 ($p < 0.001$, SED = 0.1273, LSD = 0.2498) and pot trial #2 ($p < 0.001$, SED = 0.1074, LSD = 0.2107).

Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test.

Table 18 Field trial #2 The effect of 2,4-D foliar sprays on mean necrosis levels from the treatment of tuber slices with thaxtomin A.

Number of Sprays	Spray Rate	Date of First Spray (DAE)	Mean Necrosis Score				
			Russet Burbank		Desiree		Mean
Single	**25**	**5**	1.903		1.646		1.775
		10	1.451		1.708		1.580
		20	1.660		1.438		1.549
		30	1.368		1.479		1.424
		40	1.632		1.479		1.556
		50	1.542		1.299		1.421
	100	**5**	1.236		1.444		1.340
		10	1.562		1.632		1.597
		20	1.528		1.806		1.667
		30	1.333		1.146		1.240
		40	1.285		1.562		1.424
		50	1.306		1.222		1.264
	Control		2.007		1.847		1.927
Double	**25**	**5**	1.479		1.340		1.410
		10	1.514		1.715		1.615
		20	1.792		1.583		1.688
		30	1.396		1.347		1.372
		40	1.472		1.458		1.465
	100	**5**	1.347		1.177		1.262
		10	1.521		1.625		1.573
		20	1.493		1.469		1.481
		30	1.458		1.201		1.330
		40	1.264		1.229		1.247
	Control		1.854		1.708		1.781
Mean	**25**	**5**	1.691	b	1.462	cde	1.577
		10	1.483	bcde	1.712	ab	1.598
		20	1.713	ab	1.510	bcde	1.612
		30	1.382	cde	1.413	def	1.398
		40	1.552	bc	1.471	cde	1.512
		50	1.542	bc	1.299	ef	1.421
	100	**5**	1.292	de	1.337	ef	1.315
		10	1.542	bc	1.628	abcd	1.585
		20	1.510	bcd	1.671	abc	1.591
		30	1.396	cde	1.179	f	1.288
		40	1.274	e	1.363	ef	1.319
		50	1.306	de	1.222	f	1.264
	Control		1.931	a	1.778	a	1.778

Necrosis score: 0 = no necrosis; 0.5 = very sparse flecks; 1 = few light brown flecks; 1.5 = few dark brown flecks; 2 = light brown flecks in a circle; 2.5 = dark brown flecks in a circle; 3 = light brown necrosis; 3.5 = dark brown necrosis; 4 = black necrosis

There was a significant interaction between spray rate, first spray date and variety (p = 0.045, SED = 0.1196, LSD = 0.2352).

Treatments with the same letter in the same column are not significantly different at p = 0.05 using Fischer's LSD test.

Figure 5 Field trial #2 The quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at various times (5-50 DAE) and two rates (25-100 mg/L) to the foliage of Russet Burbank and Desiree plants. Red line indicates the maximum residue limit (MRL) of 2,4-D in potatoes.

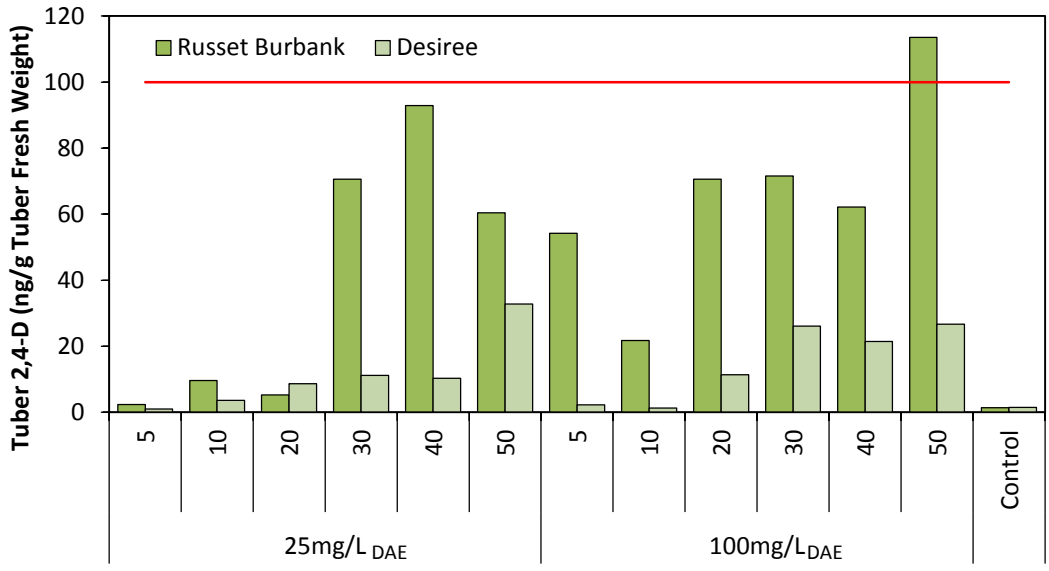
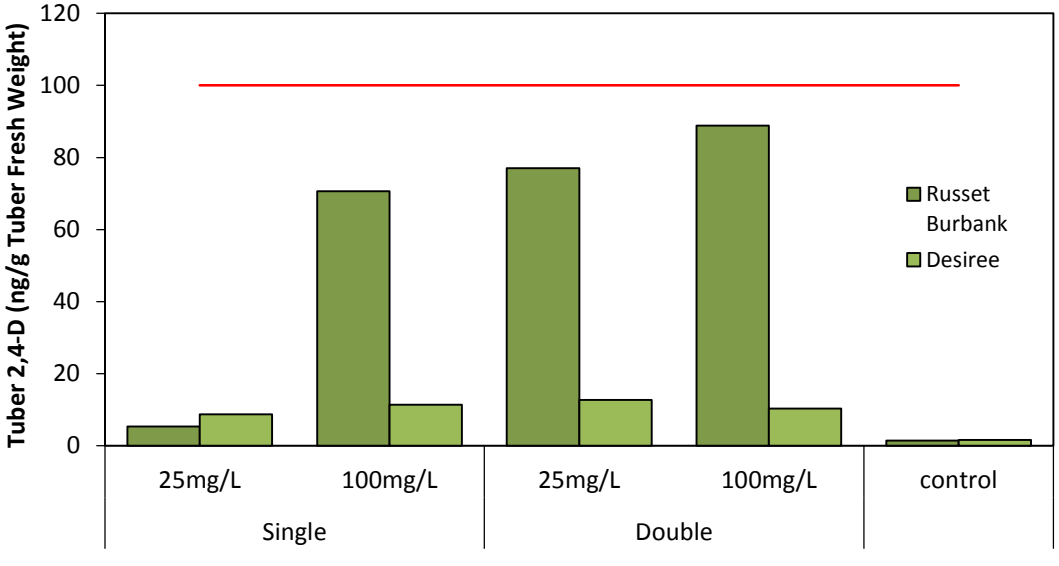


Figure 6 Field trial #2 The quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at 20DAE, at a low and high rate and as single and double sprays to the foliage of Russet Burbank and Desiree plants. Red line indicates the maximum residue limit (MRL) of 2,4-D in potatoes.



DISCUSSION

TREATMENT PRIOR TO TUBER INITIATION PROVIDES GREATEST CONTROL

A discrete window of susceptibility of potato tubers to infection with the causal agents of common scab occurs during the rapid growth phase of tuber development. Tuber internodes are susceptible to infection by S. scabies after lenticels have been formed from stomata but have not yet completely suberised (Adams 1975). Lapwood & Adams (1973) determined that only when internodes are in the 3rd and 4th position from the apical bud are their lenticels in this susceptible phase. Internodes are susceptible to infection for around 10 days, beginning 1-2.5 weeks after formation (Adams & Lapwood 1978), and individual lenticels remain susceptible for around one week under normal tuber growth rates (Adams 1975).

More recent research has found that earlier formed lenticels are more susceptible to infection than those formed later (Khatri et al. 2011), suggesting that tubers will be more susceptible to infection at the beginning of the infection window, making it the most crucial period for protecting tubers against the disease. Khatri (2011) observed that inoculating tubers 14 days after tuber initiation resulted in 68% of tubers becoming infected, while only 4% of tubers were infected as a result of inoculations 8 weeks after tuber initiation. That the majority of infection appears to occur at the beginning of the infection window, and that the infection window opens soon after tubers have initiated, suggests that controlling early infection around the time of tuber initiation will be important in controlling subsequent disease. This corresponds with both previous research (Tegg et al. 2008), in which the timing of the 2,4-D treatments appeared to be a critical factor in the efficacy of this control method, and the results of this study, in which later treatments were found to be less effective in controlling disease than those applied before or at the commencement of the initiation of the majority of tubers within the trials.

Tegg et al (2008) found that treatments applied well after tuber initiation gave little disease control when compared to treatments with a targeted application of less than 2 weeks after first evidence of tuber initiation. However, this observation had only been made through comparisons between discrete trials, rather than through the comparison of differently timed treatments. McIntosh et al. (1981) found that earlier applications (at 21 DAE) had increased efficacy but also increased the phytotoxic effects, and this led to them not recommending 2,4-D as a practical control method. This earlier application date was similar to the timing adopted by Tegg et al. (2008), but may still be after the beginning of the infection window, which changes depending on a variety of physiological and environmental factors. The current study confirmed the observations of an increased efficacy of earlier treatments found by McIntosh (McIntosh et al. 1981) and the importance of timing that were made by Tegg et al. (2008). It also provides further evidence that treatment with 2,4-D during the infection window suppresses common scab (McIntosh et al. 1981; McIntosh et al. 1982; Tegg 2006; Tegg et al. 2008; Tegg et al. 2012), and that treatments applied after the infection window has closed provide little control, if any (Tegg et al. 2008). However, it suggests that treatments aimed to be applied at the beginning of the infection window may inadvertently miss the start of this period, that these treatments may be too late to provide control for all tubers, and that treatment made prior to tubers entering the period of susceptibility gives a better result. This is presumably due to additional protection of those tubers initiation earlier than anticipated.

Potato plants produce tubers asynchronously (Vreugdenhil & Struik 1989), which means that the time of both tuber initiation and the disease infection window varies for each developing tuber. Moreover, first tuber initiation is influenced by emergence date rather than planting date (Struik et al. 1999), as well as cultivar genetics (Celis-Gamboa et al. 2003) and environmental factors such as day length. As such, the

infection window of a crop will extend for a significant period of time, and may vary with date of emergence and other factors. It can also be difficult to determine precisely when first tuber initiation occurs, as tuber growth is not easily monitored and the time at which tuber initiation occurs doesn't relate to changes in plant physiology above ground (such as the number of leaves or flower development) (Celis-Gamboa et al. 2003). Field trials by McIntosh et al. of 3,5-D (1982) resulted in much lower disease control than previous glasshouse trials (McIntosh et al. 1981), which was suggested by the authors to be the result of extended tuber initiation in field conditions compared to in glasshouse grown plants, and a lack of persistence within the tuber of the 3,5-D. However, the successful disease control in one pot trial and two field trials in this study suggest that early applications are able to both cover this extended period of tuber initiation in the field, and that reduced disease cover in field situations is likely a result of early initiating tubers not being protected. The timing of treatments to coincide with, or shortly after, first tuber initiation may be a risky practice given treatments applied after the onset of the infection window are ineffective in controlling common scab disease.

While the mechanism by which 2,4-D increases resistance in potato to common scab is as yet unknown, a relationship between auxin and thaxtomin has been demonstrated (Tegg et al. 2005; Tegg et al. 2008; Tegg et al. 2012). Tegg et al. (2005) found that an auxin sensitive mutant of Arabidopsis thaliana was more sensitive to thaxtomin A than the wild type, while Tegg et al. (2008) observed that chlorosis and death induced by thaxtomin A was suppressed with the addition of the auxins 2,4-D and IAA. Tuber sensitivity to thaxtomin A has been shown in this and previous studies (Tegg et al. 2008; Tegg et al. 2012) to be decreased with treatments of 2,4-D. Tegg et al. (2012) demonstrated a strong relationship between the suppression of thaxtomin A toxicity and disease control.

That 2,4-D treatments are more effective when applied prior to infection is consistent with examples of chemically induced SAR responses. For examples, SAR against fire blight in pears and apples induced by prohexadione-calcium, and SAR against late blight of potato induced by fosetyl-Al are both more effective when the treatments are applied earlier in the growing season. In both cases treatments are ineffective in inducing the SAR response if they are applied either late in the season, for control of late blight, or, in the case of fire blight, after symptom development has begun. (Cooke & Little 2001; Schupp et al. 2002; Andreu et al. 2006). Other research has also suggested that the induction of SAR in potatoes against tuber diseases occurs more effectively when treatments are applied earlier in the growth stages (Bokshi et al. 2003; Tegg et al. 2008; Cooke & Little 2001). While the suppression of common scab through 2,4-D treatments has been demonstrated to likely be the result of a thaxtomin specific response, the additional induction of a SAR response by 2,4-D treatments may also be possible.

TREATMENT PRIOR TO TUBER INITIATION PROTECTS PLANTS THROUGH INFECTION WINDOW

The earliest spray treatments were included in this study to determine if sprays applied prior to tuber initiation would be effective in controlling disease by ensuring that the entire infection window was completely covered. It was expected that treating plants even with low rates of a herbicide very early on in their development would be detrimental to the plant, and it wasn't known whether the 2,4-D would still be translocated to the tubers if it had been applied before they had developed. However, the results show that the 2,4-D applied soon after plant emergence (between 5-10 DAE) is retained within the plant until tuber initiation, translocated to the tubers once they have initiated and gives good disease control in comparison to sprays applied later (after tuber initiation has already occurred). Quantification of 2,4-D within tubers at harvest shows that plants treated with 2,4-D prior to tuber initiation had small amounts of 2,4-D within the tubers at harvest, This confirms that the 2,4-D is translocated to the tubers after they have

initiated, and that sufficient 2,4-D stays within the tuber to provide control against disease through the infection window.

As well as ensuring active material is present in the plant prior to tuber initiation and in doing so providing improved disease control, treatments prior to tuber initiation allow for a longer period in which the 2,4-D can be metabolised within the plant, resulting in less 2,4-D within tubers at harvest when it is not needed and unwanted. The current maximum residue limit (MRL) for 2,4-D in potatoes is 100ng/g of fresh weight (FW) tubers. Quantification of 2,4-D in tubers from field trial #2 at harvest suggests that, when treated early with a low rate of 2,4-D, tubers would have a level of 2,4-D considered safe for human consumption, reaching levels of around a 1/10th of the MRL. However, treatments of the same rate of 2,4-D applied later in the growing period, after tuber initiation, result in some tubers having 2,4-D levels near the MRL at harvest. This agrees with previous research that had shown that treatments of 2,4-D (at higher rates than those used in this study) targeted to the infection window resulted in levels of 2,4-D in tubers at harvest near or above the MRL. (Tegg et al. 2008) The research presented in chapter two of this thesis demonstrated that foliar treatments of as little as 6.25mg/L 2,4-D are required for sufficient material to reach the developing tubers and control disease, and an increasing amount of 2,4-D within the tuber does not increase the toxin tolerance of the tuber past a certain level. Therefore, earlier treatments that result in these low levels of 2,4-D within the tubers during the period of susceptibility will protect the tubers through the infection window, and with natural metabolism of 2,4-D allow for tuber 2,4-D at harvest to reach levels well below the MRL. Increased levels of IAA have been shown to decrease the amount of 2,4-D translocated to tubers (Burrell 1982), and as such the natural levels of IAA within the plant during different periods of growth may result in differing rates of translocation of 2,4-D from foliage to tubers, and therefore different levels of 2,4-D within tubers.

The success of these very early sprays led to the hypothesis that the 2,4-D could be applied even earlier, before planting, as a treatment applied directly to the seed piece. This is discussed in Chapter 5.

SINGLE SPRAYS AS EFFECTIVE AS MULTIPLE SPRAYS IN CONTROLLING DISEASE

The current study suggests that single applications are as equally effective in controlling disease as double or triple spray treatments. This is in contrast with previous work by Tegg et al. (2008), which found that multiple sprays resulted in lower disease severity than single sprays. Chemically induced resistances can require multiple applications throughout the growing season to achieve disease control. The resistance of potato to late blight induced by foliar applied phosphorous acid was found to be three times more effective from double and triple applications applied at 14 day intervals than single applications (Taylor et al. 2011). However, the induced control can be achieved with a single targeted application, such as the control achieved by a single application of benzothiadiazole, which induces resistance against post-harvest melon diseases and achieves similar levels of disease control as four applications made throughout the growing season (Bokshi et al. 2006). That single applications early in the growing season resulted in excellent disease control and low levels of 2,4-D in the tuber at harvest further suggests that one targeted application provided enough material to the developing tubers to effectively control common scab, and that multiple sprays spread across the growing season are not required to "top-up" active materials. Additional sprays would likely be applied after tubers are susceptible, and this research has suggested they would be less effective.

AGRONOMIC EFFECTS

The main aim of this study was to determine the efficacy of 2,4-D as a disease control tool, and these trials were conducted to maximise disease levels. As a result some agronomic outcomes, such as tuber size, tuber number, and yield are not indicative of what would occur in a commercial crop, grown to maximise yield and productivity. Pot trials can be poor indicators of agronomic performance in potato crops, due to restrictions on root and tuber development, and care must be taken in the interpretation of this data (Wilson et al. 2010). To determine more accurately the agronomic effects of these treatments, further trials would need to be undertaken using commercial growing practices, with standard irrigation and other management practices in place.

The 2,4-D treatments in this study were not shown to have a significant effect on the mean number of tubers or mean mass of tubers in any trials. There was an effect found from treatments on total tuber mass in one field trial and both pot trials, however the effect was not consistent between trials, with treatments resulting in both higher and lower total tuber masses when compared with controls across the trials. In both pot trials there was a trend towards earlier sprays having a lower total tuber mass, and treatments applied around tuber initiation in pot trial #2 resulted in the lowest tuber masses. This is a similar result that of McIntosh et al (1981), who observed that treatments applied at 21 DAE resulted in increased phytotoxic effects. Treatments prior to tuber initiation were not included in the McIntosh et al (1981) trials. Tegg et al. (2008) also observed reduced total tuber weight in pot trials from treatments applied at a similar date. It may be that 2,4-D applications targeted towards the beginning of the infection window, when tubers are initiating, has a greater effect on the total tuber mass of a plant than treatments applied prior to tuber initiation, or after tubers have finished rapidly growing, possibly as a result of changes in the hormone balance (Struik et al. 1999).

CONTROLLING COMMON SCAB MAY RESULT IN REDUCED POWDERY SCAB

The association between common scab and powdery scab severity found in the field trial in chapter 1 (rates) was repeated in field trial #2 (timing). In this trial, the treatment did significantly reduce powdery scab severity (lesion coverage) but not incidence

This repeated finding of diminished powdery scab in two field trials following 2,4-D treatment suggests that there is an interaction between the development of the two diseases and that control of common scab by 2,4-D may have the subsequent effect of reducing powdery scab, that 2,4-D treatments also act to reduce common scab disease, or possibly a combination of both of these.

This is in contrast to Waterer (2010), who showed 2,4-D decreased common scab disease but increased powdery scab disease (at rates higher than presented in this study). Waterer suggested this increase in powdery scab to be an opportunistic invasion by the powdery scab pathogen as a result of less competition at pathogen entry sites created by the reduction in common scab disease. There is no published evidence that 2,4-D treatments reduce the extent of colonisation of the tuber surface by the common scab pathogen, rather, the levels of 2,4-D present in the tuber after treatment have been shown to have no effect on pathogen growth or survival (McIntosh et al. 1981; Tegg et al. 2008).

Martínez-Noël (2001) observed that IAA treatments in potatoes decreased the severity of late blight, and found that the pathogen, *Phytophthora infestans*, was inhibited by the auxin, at and below rates at which 2,4-D has been shown to not be inhibitory to *Streptomyces scabies* (Tegg et al. 2008). This suggests at the applied rates, 2,4-D treatments may be directly inhibitory to other potato pathogens.

TUBER SLICE ASSAYS

Tegg et al. (2012) found a consistent relationship between the suppression of common scab disease and the thaxtomin A sensitivity of the tuber in plants that had been treated with a range of chemicals, including 2,4-D, demonstrating that the suppression of thaxtomin toxicity resulted in disease control. The authors concluded that the tuber slice assay was a good determinant of the level of disease control that is induced by chemicals that affect thaxtomin A toxicity. However, as 2,4-D is naturally metabolised within the tuber (Burrell 1982), which decreased tuber 2,4-D levels over time, the toxin-tolerance of the tuber is lower at harvest than both after treatment with 2,4-D and when the tuber is susceptible to disease. Tuber slice assays of harvested tubers therefore give a less accurate indication how toxin-tolerant the tuber was during the period of susceptibility to infection. For example, a high application rate of 2,4-D resulting in a large amount of 2,4-D in the tuber at harvest will give a lower necrosis rating in the tuber slice assay, while a late treatment, which leaves less time for the 2,4-D in the tuber to be metabolised, may result in a similarly high level of 2,4-D in the tuber at harvest, and give a similarly low necrosis scores in the tuber slice assay. However, these two application strategies will result in very different disease control efficacies. Inversely, a smaller amount of 2,4-D applied early in the season will result in lower levels of 2,4-D within the tuber at harvest, and give higher necrosis scores in the tuber slice assay, yet this treatment may provide a similar level of disease control a higher application rate, and a greater level of disease control than a later treatment. Both previous research (Tegg et al. 2008) and the results presented in this thesis suggest that higher levels of 2,4-D within the tuber do not necessarily give greater disease control, and late sprays can often give no disease control if applied after the infection window. The tuber slice assay, therefore, needs to be interpreted with rate and timing of the treatment, and date of harvest in mind.

The tuber slice assay for assessing toxin tolerance is a good indication of the levels of 2,4-D in the tubers at harvest, and is a valid and useful test (Tegg et al. 2012), but delayed tuber slice assays are not necessarily a good determinate of the levels of 2,4-D in the tuber at the time of infection or an indication of whether the tuber is protected during the infection window. Ideally the tuber slice assay should either be undertaken on tubers harvested while they are still susceptible to infection, or in conjunction with disease assessments and 2,4-D quantification. However, accurate quantification of 2,4-D within tubers can be both time-consuming and expensive. The tuber slice assay method may be used as a more efficient and cost effective method of screening tubers when a comparative indication of disease resistance is required.

CONCLUSION

This research demonstrated the importance of timing of applications in the effectiveness of 2,4-D induced resistance to common scab of potato. It has shown that foliar treatments of 2,4-D applied as early as 5 days after crop emergence can provide effective control in potatoes against common scab disease, and suggests that applications prior to tuber initiation are more effective in controlling disease than applications timed to coincide with, or after tuber initiation. These early treatments appear to provide sufficient 2,4-D to the tuber to control disease through the infection window, while also allowing sufficient time for the amount of 2,4-D within the tuber to reach acceptable levels well below the MRL by harvest. Additionally, this research has demonstrated that single, early applications are as effective in controlling disease as multiple applications.

CHAPTER 3: 2,4-D AS A SEED TUBER TREATMENT

INTRODUCTION

Common scab is an economically important potato disease caused by pathogenic *Streptomyces* spp. (Loria et al., 2006). The control of common scab is primarily achieved through a combination of management strategies, including targeted irrigation (Lapwood et al. 1973) and the delayed planting (Waterer 2002) of disease free seed of resistant varieties (Wilson et al. 2009). Seed treatments are used to a lesser extent, with mancozeb and cement dusting used infrequently in Australia (Wilson et al. 1999). The importance of seed- and soil-borne inoculum has varied between studies, with finding seed-borne inoculum to have no significant effect (Lapwood 1972; Adams & Hide 1981), while others found it to be as important as soil-borne inoculum (Wilson et al. 1999; Wang & Lazarovits 2005). The relative importance of seed- and soil-borne inoculum on disease severity is likely to be dependent on local factors such as soil type, the pathogen population, climate and agronomic practice (Wilson et al. 1999).

The majority of seed treatments against common scab work through direct action against the pathogen either on the seed tuber surface, thus reducing seed borne inoculum, or within the immediate soil surrounding the seed piece once it has been planted. Seed treatments that have been demonstrated to be effective include fumigants such as mustard meal (Al-Mughrabi 2010), biological controls such as antibiotic producing or competition introducing bacteria (Al-Mughrabi 2010) and bacteriophages (McKenna et al. 2001), and biocides such as pentachloronitrobenzene (PCNB), fluazinam, flusulfamide, fenpiclonil and mancozeb (Hooker 1981; Wilson et al. 1999).

When applied to potato foliage, 2,4-D is translocated to tubers (Burrell 1982) and has been shown to be an effective control of common scab when applied as foliar treatments (McIntosh et al. 1981; McIntosh et al. 1982; Tegg et al. 2008; Waterer 2010; Chapters 1 & 2). Tegg (2008) determined that 2,4-D suppressed the toxicity of thaxtomin A, a phytotoxin produced by the common scab pathogen (King et al. 1989) essential to pathogenicity (King et al. 1991; Loria et al. 1995; Goyer et al. 1998; Kers et al. 2005). Further research (see chapter 2) demonstrated that foliar 2,4-D treatments applied soon after tuber emergence were more effective in controlling common scab than treatments applied near tuber initiation, as had previously been tested. This suggested early sprays provide protection to tubers prior to and throughout the phase of susceptibility to infection. The success of these early treatments and issues surrounding uneven emergence led to the hypothesis that plants could be treatment prior to emergence, in the form of a seed treatment.

Chapters 1 & 2 show 2,4-D is present at low levels in harvested tubers following foliar treatment of the growing crop. Carryover of 2,4-D in vegetative propagules into the subsequent crop could have detrimental effects on the performance of the crop if present in high enough concentration. Alternatively, residual 2,4-D may provide protection of the subsequent crop from infection by the common scab pathogen.

This chapter describes a preliminary study on a) the use of 2,4-D as a seed treatment to determine if 2,4-D applied to seed pieces can effectively control common scab and to determine the most effective application method and rate; and b) to determine if foliar treatment of prior seed crops with 2,4-D provides sufficient carryover of 2,4-D to give control in tubers grown from the resultant seed.

MATERIALS AND METHODS

PLANTS AND INOCULUM

Planting material: The varieties Desiree and Russet Burbank were used in these trials. Seed tubers were removed from cold storage a fortnight before planting.

For the 2,4-D tuber treatment glasshouse experiment, tubers were disease free mini tubers from glasshouse grown tissue culture plantlets, and were cut into approximately 10g pieces and left to suberise before treatment and planting. For the 2,4-D tuber treatment field trial, tubers were visibly clean seed commercially grown seed. For the 2,4-D carryover glasshouse trial, tubers were visually disease free tubers from a field trial (2009/10 season) that had been sprayed with various rates of 2,4-D. Tubers were cut into approximately 10g pieces and left to suberise before planting.

Inoculum: The *S. scabies* isolate and methodologies used to produce and apply inoculum for the glasshouse trials was the same as described in Chapter 1.

TUBER TREATMENT EXPERIMENTS

The ability of tuber applied 2,4-D to control common scab was evaluated in a glasshouse and field trial

Glasshouse trials:

Solutions of 2,4-D for use in glasshouse trials were prepared as described in the chapter 1.

The dipping treatments were applied on the day of planting. Seed tubers were immersed completely in the dipping solution for 30 minutes. Tubers were then removed and laid in a single layer to air dry for 15 minutes before planting.

There were five dipping treatments of varying concentrations of 2,4-D: 400mg/L, 100mg/L, 25mg/L, 6.25mg/L 2,4-D and a water control treatment with no 2,4-D. Each treatment was applied to five tubers of each variety, and one tuber was planted per pot (50 pots in total), with pots arranged in a completely randomised block design.

All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease (Lapwood & Hering 1970). No other pesticides were applied. Plants were grown outside on a concrete slab and subject to natural weather events and temperatures. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to each pot.

Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested. Tubers were stored at 4°C in plastic netted bags. Soil was brushed from tubers, which were then assessed for common scab disease severity using the methods described in chapter 1. Tuber number and mass (fresh weight) were measured. Any tuber disfigurements were noted.

Sensitivity to thaxtomin A was assessed in two tubers from each pot. Tubers without obvious lesions were chosen and assayed following the method described in chapter 1.

Field trial:

In this trial two different application methods for treating seed with 2,4-D were used, dipping and spraying. The commercial herbicide Amicide 625 (Nufarm Ltd) with 625 g/L 2,4-D present as dimethylamine and diethanolamine salts was used, and it was ensured that an equivalent amount of the active 2,4-D molecule was present compared with that used in the pot trials. To prepare the 2,4-D solutions, 8.8mL Amicide 625 was dissolved in 1L of water, with 0.5g of Tween-80 added as a wetting agent. This 8.8mL/L Amicide solution was then mixed with 9L of water within a container to make a 880µL/L solution. This was determined to be the equivalent of a 400mg/L 2,4-D solution. This was repeated using 1.1mL of Amicide 625 to create a 50mg/L solution. The control solution was water and Tween-80.

For the spraying treatment the seed pieces were spread on the ground in a single layer and the visible side was sprayed, using a handheld sprayer, with the 2,4-D solution until the seed was fully covered with the solution. The seed was air dried for 10 minutes before being planted. For the dipping treatment the seed pieces were immersed in the 2,4-D solution for 20 minutes. The seed pieces were then removed and laid in a single layer to air dried for 5 minutes before being planted.

The trial site was located at Waterhouse in North East Tasmania. The soil was predominately sandy. The immediate area had been sown with a trial potato crop in the previous season (see Field Trial #2 in chapter 2) and a commercial processing potato crop in the season prior to that. The surrounding area had been sown with an oil seed crop in the previous season and a lucerne feed crop in the trial season.

The trial was planted on 27th October, 2010. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants of the same variety, and plots consisting of two subplots, one of each variety. To ease planting the subplots were not randomised within each plot. The treatments were replicated four times. Three treatment rates were applied: 400mg/L, 50mg/L equivalence, and a control spray containing no 2,4-D. Two treatment methods were used dipping and spraying. All treatments were applied on the date of planting.

The average emergence date was estimated to be 20th November 2010. Tubers were harvested on 4th April, 2011. The trial was watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required. After senescence the trial was sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4ºC prior to assessment. Before assessment the tubers were washed to remove soil and then weighed to determine yield for each plot (the combined tubers from five plants).

2,4-D CARRYOVER GLASSHOUSE TRIAL

The two potato varieties used possessed moderate resistance (Russet Burbank) and moderate susceptibility (Desiree) to common scab. Daughter tubers from potato plants that had been treated once with one of four different rates of 2,4-D: 400mg/L, 100mg/L, 50mg/L, control (water + Tween-80 only) 2,4-D were planted in pots. No further treatments were applied to the tubers. Each treatment of each variety was replicated five times (40 pots in total), with pots arranged in a completely randomised block design.

Pots were maintained as described in the tuber treatment glasshouse trial. Plants were grown to senescence, tubers harvested and assessed as for the tuber treatment trials.

TUBER DISEASE AND AGRONOMIC PERFORMANCE

Harvested tubers were assess for tuber mass, number and disease following methods in chapter 1.

TUBER THAXTOMIN SENSITIVITY

Thaxtomin sensitivity of tubers at harvest from glasshouse and field trials was measured using the methods described in chapter 1. The tubers used for the tuber slice assay were taken from replicates 1-3 of the pot trials and replicates 1 and 2 of the field trial. For the glasshouse trials the tubers from each replicate were placed in a single Petri dish (10 disks), and assessed as a single unit. Each treatment was replicated three times (three Petri dishes with 30 disks in total). For the field trial two tubers were assayed from each replicate (one tuber per Petri dish), with 8 filter paper discs distributed over the slices from each tuber. A total of 32 filter paper discs were spread over four tubers from two replicates in four Petri dishes, for each treatment.

DATA ANALYSES

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009). Multivariate Analysis of Variance was used to determine significant effects from, and interactions between treatment factors. Probabilities less than 0.05 were considered to be significant and Fischer's least significant difference (LSD) test was used for comparison of treatment means. Linear Regressions were used to examine the relationship between powdery scab and common scab severity.

RESULTS

DISEASE CONTROL

Tuber treatment experiments

Glasshouse trial: There was no significant effect found from treatment rate on DCS, mean number of lesions or LSD. Desiree had a significantly higher DCS and LDS than Russet Burbank. There was low emergence within the control treatment (Table 19).

Field trial: Both rates of 2,4-D treatment resulted in lower DCS than the control treatment. The 400mg/L treatment resulted in lower LDS than the control treatment. The 50mg/L treatment was not significantly different from either the 400mg/L or control treatments. There was no effect found from application method on DCS or LDS. Russet Burbank had lower DCS and LDS than Desiree (Table 20).

Two outlying points were removed from analyses. There was a strong positive linear regression between common scab and powdery scab DCS. However, there was no significant effect found from the seed treatments on powdery scab disease cover. Russet Burbank had a lower DCS than Desiree (Figure 7; Table 21).

2,4-D carryover trial

There was a significant interaction found between spray rate and variety on DCS. In Russet Burbank the 400mg/L treatment resulted in significantly lower DCS than the control treatment. The 50mg/L treatment and the control were not significantly different from each other. In Desiree the 50mg/L treatment had significantly lower DCS than the 400mg/L treatment and the control, which were not significantly different from each other. There was also a significant interaction between spray rate and variety on mean number of lesions per tuber. In Russet Burbank the 400mg/L treatment resulted in significantly fewer lesions than the control treatment, and the 50mg/L and control treatments were not significantly different from each other. In Desiree the 50mg/L treatment resulted in significantly fewer lesions than the 400mg/L treatment. Neither 2,4-D treatment was significantly different from the control treatment. There was no significant effect found from spray rate on LDS, but Russet Burbank had significantly shallower lesions than Desiree (Table 22).

Table 19. The effect of 2,4-D seed treatments applied as a dip to Russet Burbank (RBK) and Desiree (DES) seed tubers prior to planting in glasshouse, on common scab severity

Rate (mg/L 2,4-D)	Disease Cover Score - DCS (Cover %)			Number of Lesions			Lesion Depth Score - LDS		
	RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
0	0.76 (1.62%)	1.25 (4.01%)	1.00	1.78	1.94	1.86	0.93	1.10	1.01
6.25	1.10 (3.18%)	1.46 (7.2%)	1.28	1.9	4	2.95	1.11	1.56	1.34
25	1.30 (6.42%)	1.64 (7.92)	1.47	3.9	3.05	3.47	1.22	1.86	1.54
100	0.89 (2.71%)	1.31 (5.24%)	1.10	2.17	2.42	2.29	1.07	1.42	1.25
400	1.10 (3.78%)	1.65 (8.07%)	1.37	2.99	5.06	4.02	1.13	1.91	1.52
Mean (variety)	1.06 a	1.48 b		2.63	3.44		1.11 a	1.62 b	

There was no significant effect found from rate. There was a significant effect found from variety on DCS ($p = 0.001$, SED = 0.1237, LSD = 0.2488) and LDS ($p < 0.001$, SED = 0.1117, LSD = 0.2248). There were no significant interactions between factors.

Treatments with the same letter in the same row and variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Table 20. The effect of 2,4-D seed treatments applied as a dip or spray to Russet Burbank (RBK) and Desiree (DES) seed tubers prior to planting in the field, on common scab severity

Application Method	Rate (mg/L 2,4-D)	Disease Cover Score - DCS (Cover %)			Lesion Depth Score - LDS		
		RBK	DES	Mean	RBK	DES	Mean
Dip	**0**	1.38 (4.73%)	2.46 (15.06%)	1.92	1.4	2.2	1.8
	50	0.93 (2.35%)	2.37 (13.36%)	1.65	1.2	2.2	1.7
	400	0.90 (2.47%)	1.86 (8.96%)	1.38	1.2	1.8	1.5
Spray	**0**	1.57 (5.92%)	2.33 (14.35%)	1.95	1.3	2.3	1.8
	50	0.86 (1.76%)	1.72 (7.99%)	1.29	1.1	2.0	1.5
	400	0.78 (1.68%)	1.62 (6.53%)	1.2	1.1	1.8	1.5
Mean (rate)	**0**	1.48	2.39	1.93 a	1.3	2.3	1.8 a
	50	0.9	2.04	1.47 b	1.1	2.1	1.6 ab
	400	0.84	1.74	1.29 b	1.2	1.8	1.5 b
	Mean (variety)	1.07 a	2.06 b		1.2 a	2.0 b	

There was no significant effect found from method of application. There was a significant effect found from rate on DCS ($p < 0.001$, SED = 0.131, LSD = 0.278) and LDS ($p = 0.043$, SED = 0.104, LSD = 0.221). There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.115, LSD = 0.242) and LDS ($p < 0.001$, SED = 0.089, LSD = 0.186). There were no significant interactions between factors.

Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Table 21. The effect of 2,4-D seed treatments applied as a dip or spray at various rates to Russet Burbank (RBK) and Desiree (DES) seed tubers prior to planting in the field, on powdery scab severity

Application Method	Rate (mg/L 2,4-D)	Powdery Scab Disease Cover Score - DCS (Cover %)		
		RBK	DES	Mean
Dip	**0**	1.52 (6.18%)	2.46 (13.95%)	1.99
	50	1.41 (5.58%)	2.22 (13.11%)	1.83
	400	1.43 (5.42%)	2.23 (13.31%)	1.81
Spray	**0**	1.83 (8.1%)	2.51 (14.75%)	2.17
	50	1.26 (4.25%)	2.26 (12.53%)	1.91
	400	1.32 (5.08%)	2.49 (15.67%)	1.76
Mean of Rate	**0**	1.68	2.49	2.08
	50	1.34	2.24	1.79
	400	1.38	2.36	1.87
	Varietal Mean	1.46 a	2.36 b	

There was no significant found from method of application or rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.097, LSD = 0.204). There were no significant interactions found.

Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Figure 7. Linear regression between common scab and powdery scab severity on tubers harvested from plants treated with 2,4-D at various rates. (Two outlying points were removed from analysis.)

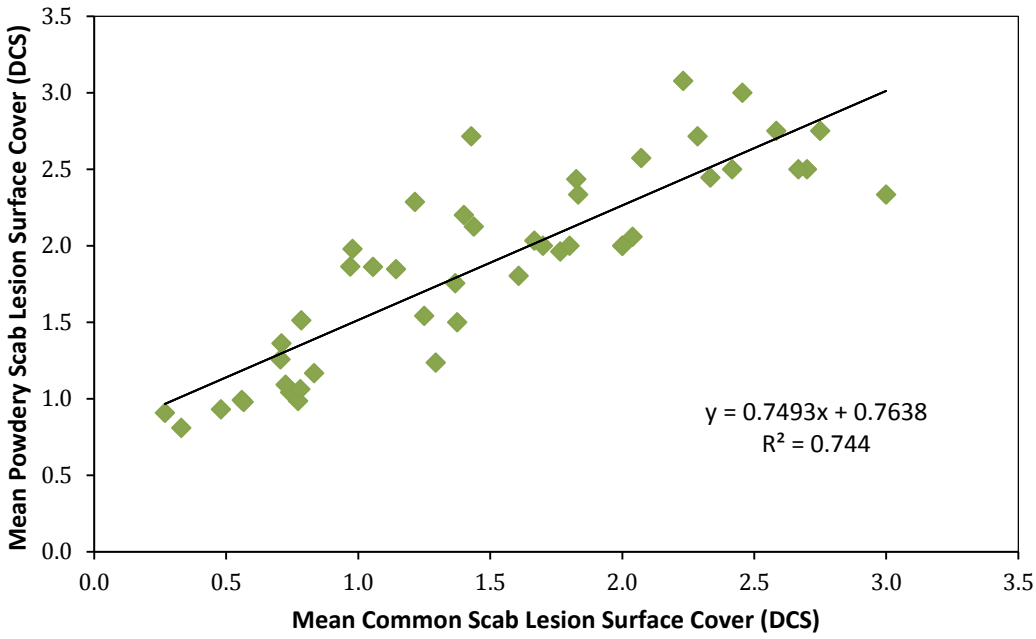


Table 22. The effect of carryover of 2,4-D in seed tubers on common scab severity in the subsequent crop.

Spray Rate (mg/L 2,4-D)	Disease Cover Score - DCS (Cover %)			Number of Lesions			Lesion Depth Score - LDS)		
	RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
0	1.53 a (6.58%)	1.71 a (8.99%)	1.62	5.4 a	3.7 ab	4.6	1.35	2.07	1.71
50	1.18 ab (6.03%)	0.82 b (2.05%)	1.00	4.3 ab	1.8 b	3.1	1.20	1.22	1.21
400	0.80 b (1.93%)	1.86 a (9.17%)	1.33	2.0 b	5.3 a	3.6	1.33	1.97	1.65
Mean (variety)	1.17	1.46		3.9	3.6		1.29 a	1.75 b	

There was a significant interaction found between rate and variety on DCS ($p < 0.001$, SED = 0.131, LSD = 0.278) and mean number of lesions ($p = 0.043$, SED = 0.104, LSD = 0.221).

Treatments with the same letter in the same row and variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

AGRONOMIC ASSESSMENTS

Tuber treatment experiments

Glasshouse trial: There was a significant effect found from treatment rate on total mass, mean mass per tuber, and mean number of tubers. The 6.25mg/L treatment had the highest total mass. The 100mg/L and 400mg/L treatments were not significantly different from any other 2,4-D treatment. All treatments had greater total mass, mean mass per tuber and mean number of tubers than the control treatment. However, there was very low emergence within the control treatments, and as such the agronomic data is not reliable. Desiree was significantly greater for all factors than Russet Burbank (Table 23).

Field trial: There was no significant effect found from the seed treatments on mean total mass, mean mass per tuber, or mean number of tubers. However, there was a trend towards a lower total and per tuber mass, and number of tubers in both 2,4-D dipping treatments compared to the control treatment, and in the 400mg/L spray treatment compared to both the 50mg/L spray treatment and the control, which were similar (Table 24).

Russet Burbank had a significantly greater total tuber mass and number of tubers than Desiree. There was no significant effect found from variety in mean mass per tuber (Table 24).

2,4-D carryover trial

There was a significant interaction between spray rate and variety on total tuber mass. In Russet Burbank the 50mg/L treatment resulted in significantly lower total tuber mass than the control treatment. The 400mg/L treatment was not significantly different from either the 50mg/L treatment or the control. In Desiree the 400mg/L treatment resulted in significantly lower total tuber mass than both the 50mg/L treatment and the control, which were not significantly different from each other (Table 25).

There was no significant effect found from the 2,4-D treatments on either mean mass per tuber or number of tubers. There was no significant effect found from variety on total tuber mass or number of tubers. Desiree had significantly higher mean mass per tuber than Russet Burbank (Table 25).

Table 23. The effect of 2,4-D seed treatments applied as a dip at various rates to Russet Burbank (RBK) and Desiree (DES) seed tubers prior to planting, on mean total tuber mass, mean mass per tuber, and mean number of tubers in glasshouse grown plants.

Rate (mg/L 2,4-D)	Total Mass (g)			Mean Mass per Tuber (g)			Number of Tubers		
	RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
0	3.2	4.3	3.7 a	1.3	1.0	1.2 a	0.6	1.2	0.9 a
6.25	29.3	51.1	40.2 c	4.1	6.9	5.5 b	4.5	6.8	5.7 b
25	19.3	32.0	25.7 b	3.0	7.0	5.0 b	4.0	4.9	4.5 b
100	13.7	42.7	28.2 bc	2.5	8.2	5.3 b	2.4	5.9	4.2 b
400	26.5	40.5	33.5 bc	4.5	8.7	6.6 b	3.4	4.1	3.8 b
Mean (variety)	20.1 a	37.5 b		3.3 a	7.0 b		3.2 a	5.0 b	

There was a significant effect of rate on total mass ($p < 0.001$, SED = 6.12, LSD = 12.3), mean mass per tuber ($p < 0.001$, SED = 0.987, LSD = 1.984) and mean tuber number ($p = 0.019$, SED = 1.359, LSD = 2.707). There was a significant effect of variety on total mass ($p < 0.001$, SED = 3.33, LSD = 6.7), mean mass per tuber ($p < 0.001$, SED = 0.537, LSD = 1.08) and mean tuber number ($p = 0.023$, SED = 0.74, LSD = 1.473). There were no significant interactions between variables. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different ($p = 0.05$) using Fischer's LSD test.

Table 24. The effect of 2,4-D seed treatments applied as a dip or spray at various rates to Russet Burbank (RBK) and Desiree (DES) seed tubers prior to planting, mean total tuber mass, mean mass per tuber, and mean number of tubers.

Method	Rate (mg/L 2,4-D)	Total Mass (g)			Mass per Tuber (g)			Number of Tubers		
		RBK	DES	Mean	RBK	DES	Mean	RBK	DES	Mean
Dip	**0**	4085	1564	2824	95.1	129.5	112.3	43.8	13	28.4
	50	3861	914	2388	87.4	96.4	91.9	45	7.8	26.4
	400	3620	836	2228	80.4	102.3	91.3	45.5	8	26.8
Spray	**0**	3951	1538	2744	87.4	94.9	91.1	47.2	13.3	30.2
	50	4161	1314	2738	87.8	92.4	90.1	48.2	13.5	30.9
	400	3230	652	1941	81.5	73.1	77.3	39.2	7.5	23.4
Mean (rate)	**0**	4018	1551	2784	91.3	112.2	101.7	45.5	13.1	29.3
	50	4011	1114	2562	87.6	94.4	91.0	46.6	10.6	28.6
	400	3425	744	2085	80.9	87.7	84.3	42.4	7.8	25.1
Mean (variety)		3818 a	1136 b		86.6	98.1		44.8 a	10.5 b	

There was no significant effect of method of application or rate. There was a significant effect of variety on total mass ($p < 0.001$, SED = 211.1, LSD = 653.6) and number of tubers ($p < 0.001$, SED = 2.75, LSD = 5.77). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different ($p = 0.05$) using Fischer's LSD test.

Table 25. The effect from replanting tubers harvested from Russet Burbank (RBK) and Desiree (DES) plants that had previously been treated with foliar applications of various rates of 2,4-D, on mean total tuber mass, mean mass per tuber, and mean number of tubers.

Spray Rate	Total Mass (g)			Mass per Tuber (g)			Number of tubers		
(mg/L 2,4-D)	RBK	DES	Mean (rate)	RBK	DES	Mean (rate)	RBK	DES	Mean (rate)
0	59.0 a	101.8 a	80.4	10.7	13.9	12.3	5.2	7.4	6.3
50	39.4 b	104.9 a	72.1	7.6	14.2	10.9	5.6	8.2	6.9
400	77.4 ab	62.1 b	69.7	13.4	14.3	13.9	6.6	4.6	5.6
Mean (variety)	58.6	89.6		10.5 a	14.1 b		5.8	6.7	

There was a significant interaction found between rate and variety on total mass ($p = 0.001$, SED = 7.4, LSD = 16.15). There was a significant effect found from variety on mean mass per tuber ($p = 0.032$, SED = 1.909, LSD = 3.252). Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

TUBER TOXIN TOLERANCE

Tuber treatment experiments

Glasshouse trial: There was a significant interaction found between spray rate and variety on mean necrosis score. In Russet Burbank, the 6.25mg/L and 400mg/L treatments had significantly lower necrosis than the 25mg/L treatment. The 100mg/L treatment was not significantly different to any 2,4-D treatment. All treatments increased toxin suppression compared to the control. In Desiree, all treatments had similarly increased toxin suppression compared to the control (Table 26).

Field trial: There was a significant effect found from application rate on mean necrosis score. The 400mg/L treatment had significantly lower necrosis than both the 50mg/L treatment and the control treatment. The 50mg/L treatment also had significantly lower necrosis than the control treatment (Table 27).

2,4-D carryover trial

There was a significant effect found from spray rate on mean necrosis score. Both 2,4-D treatments had a similarly increased toxin suppression compared to the control (Table 28).

Table 26. The effect of 2,4-D seed treatment on mean necrosis levels from the treatment of tuber slices with thaxtomin A. Tubers harvested from plants grown in the glasshouse from seed treated with various rates of 2,4-D.

Rate (mg/L 2,4-D)	Mean Necrosis Score		
	RBK	DES	Mean
0	1.72 a	1.94 a	1.83
6.25	1.23 c	1.18 b	1.21
25	1.45 b	1.18 b	1.31
100	1.28 bc	1.09 b	1.18
400	1.12 c	1.26 b	1.19
Mean (variety)	1.32	1.26	

There was a significant interaction found between rate and variety (p = 0.001, SED = 0.0652, LSD = 0.1286).

Treatments with the same letter, in the same column, are not significantly different at p = 0.05 using Fischer's LSD test.

Table 27. The effect of 2,4-D seed treatment on mean necrosis levels from the treatment of tuber slices with thaxtomin A. Tubers harvested from plants that had been grown in the field from seed treated with 2,4-D dips and sprays.

Application Method	Rate (mg/L 2,4-D)	Mean Necrosis Score		
		Russet Burbank	Desiree	Mean
Dip	**0**	2.56	1.58	2.07
	50	2.23	1.05	1.64
	400	1.64	1.03	1.34
Spray	**0**	2.48	1.41	1.95
	50	1.72	1.48	1.60
	400	1.28	0.84	0.06
Mean of Rate	**0**	2.52	1.49	2.01 a
	50	1.98	1.27	1.62 b
	400	1.46	0.94	1.20 c
	Mean of Variety	1.99 a	1.23 b	

There was a significant effect found from rate ($p = 0.005$, SED = 0.0652, LSD = 0.3501) and variety ($p = 0.006$, SED = 0.1809, LSD = 0.4428). There was no significant effect found from application method. There were no significant interactions between factors.

Treatments with the same letter, in the same column or row are not significantly different at $p = 0.05$ using Fischer's LSD test

Table 28. The effect of 2,4-D foliar spray carryover on mean necrosis levels from the treatment of tubers slices with thaxtomin A. Tubers harvested from plants treated with foliar applications of various rates of 2,4-D.

Spray Rate (mg/L 2,4-D)	Mean Necrosis Score		
	RBK	DES	Mean (rate)
0	2.19	1.97	2.08 a
50	1.31	1.17	1.24 b
400	1.30	1.02	1.16 b
Mean (variety)	1.60 a	1.39 b	

There was a significant effect found from rate ($p < 0.001$, SED = 0.0798, LSD = 0.1575) and variety ($p = 0.001$, SED = 0.0652, LSD = 0.1286).

Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at p = 0.05 using Fischer's LSD test.

DISCUSSION

This study suggests that treatment of the seed with 2,4-D does control common scab in the resultant crop. Unlike most other seed treatments, which reduce seed-borne inoculum through biocidal activity against the pathogen (Wilson et al. 1999; McKenna et al. 2001; Al-Mughrabi 2010), this seed treatment likely acts in a similar way to 2,4-D foliar treatments, with material being translocated through the plant to the tuber, and suppressing thaxtomin toxicity, thereby controlling disease (McIntosh et al. 1981; Tegg et al. 2008; Waterer 2010). Tuber slice assays suggest that 2,4-D seed treatments do result in material being translocated to daughter tubers, and corresponds with previous work that demonstrated that foliar treatments of 2,4-D shortly after emergence, and prior to tuber initiation, resulted in the translocation of 2,4-D to tubers as they are formed, thus providing protection against infection.

It was unknown prior to this work whether the direct application of 2,4-D to the seed tuber would result in sufficient levels of material within the daughter tuber for disease control. The results of this study demonstrated that seed tubers soaked for 20 minutes within a 50mg/L solution of 2,4-D absorb sufficient material that is then translocated to daughter tubers at levels capable of controlling disease. Chapter 1 demonstrated that very low rates of foliar applied 2,4-D resulted in toxin suppressive levels of material within the tuber at harvest. These results again suggest that only low levels of 2,4-D need to enter the plant (either as foliar sprays or through seed treatment) for sufficient material to be translocated to daughter tubers to both control disease and suppress thaxtomin toxicity. Quantitation of 2,4-D within both the treated seed and the harvested tubers were unavailable in these trials due to the expensive and time-consuming nature of the quantification method, and the preliminary nature of this study. We recommend in future detailed studies the 2,4-D content of treated and harvested tubers be determined.

While the 2,4-D appears to have been translocated to daughter tubers from the treated seed tubers, it is unknown how the 2,4-D passed into the seed. The seed was sprouting at the time of treatment, and it is likely that 2,4-D would have been taken up by the tuber through these actively growing shoots, as it is taken in through foliage (McIntosh et al. 1981; Tegg et al. 2008; Waterer 2010). However, the 2,4-D may have also been absorbed through the skin of the tuber during the period of soaking. Burrell (1984) found that tuber discs absorbed sufficient 2,4-D through soaking to supress thaxtomin toxicity. The treatment may also have stayed on the outside of the tuber as a coating, and was slowly absorbed by the plant. It is not known how effective 2,4-D tuber treatments may be on dormant tubers. Future studies examining tuber treatment at differing physiological development stages are warranted. The rate at which the 2,4-D was translocated from the seed tuber to the daughter tuber is also unknown, but that disease control was achieved suggests that it was translocated to the tuber before or around the time of initiation. In future studies 2,4-D quantitation in daughter tubers should be done to confirm translocation.

As foliar treatments of 2,4-D result in thaxtomin suppressive levels of 2,4-D within tubers at harvest, it was possible that, if planted as seed, this 2,4-D could be translocated to daughter tubers of the resultant crop providing multigenerational disease suppression. While the exact amount of 2,4-D within the tubers used in this study at planting was unknown, tubers from similar treatments harvested at the same time had between 10 and 100ng/g FW 2,4-D at harvest. While these results are only from a single pot trial, they suggest that plants grown from tubers that contain residual 2,4-D from foliar treatments can have reduced disease severity. The data from all these trials suggests tuber 2,4-D treatment or accumulation could provide novel and efficient disease control. We strongly recommend this be pursued in future research.

Low rates of foliar applied 2,4-D were not found to negatively effect yield, tuber number or shape, nor were phytotoxic effects observed in this study from tuber treatments or carryover 2,4-D, and as such treatments of seed crops with 2,4-D would be unlikely to negatively affect the crop, or the seed. Emergence did not appear to be detrimentally affected by the 2,4-D treatments.

Wilson et al. (1999) found that the treatment with the biocides PCNB, fluazinam, flusulfamide, fenpiclonil and mancozeb of seed with visible lesions gave effective control, while the treatment of visibly clean seed was not effective, suggesting that these chemicals control common scab through a reduction in seed borne inoculum, but have little effect on the ability of the pathogen already present within the soil to induce disease on daughter tubers. Neither the application rates used in this study, nor the levels of 2,4-D present in the tubers from foliar treated plants affect the growth of *S. scabies* (McIntosh et al. 1981; Tegg et al. 2008). Therefore, tuber treatments of 2,4-D are unlikely to control disease through a reduction in inoculum levels either on the seed piece, or in the surrounding soil, and control is likely a result of the suppression of thaxtomin toxicity.

CONCLUSION

Treatment of tubers with 2,4-D either by dipping or spraying seed pieces prior to planting will provide some control of common scab in the subsequent crop. This provides a highly efficient means to treat all plants at the earliest stage of cropping with minimal material. Further work is required to optimise treatment rate, determine influence of seed physiological age and development on 2,4-D uptake, and develop most effective treatment methodologies, and determined 2,4-D levels in treated and harvested tubers.

Carry-over of 2,4-D in tubers treated in the prior season can also provide some control of common scab in the next season's crop. No evidence for detrimental effects of 2,4-D at the rates and concentrations used in these experiments were found. This shows multigenerational disease control.

CHAPTER 4: NOVEL CHEMICALS DERIVED FROM THAXTOMINS THAT MAY COMPETE WITH THAXTOMINS, AMELIORATE TOXICITY AND POSSIBLY PROVIDE DISEASE PROTECTION

INTRODUCTION

If a novel compound can be found that can inhibit the progression or reduce the severity of the disease, it would have great commercial potential. Two possible ways this could be achieved are either to develop an antibiotic that targets the pathogen, or develop a non-toxic competitive binder for the thaxtomin target sites, which induces resistance to the disease by reducing the effectiveness of the thaxtomin phytotoxins.

Prior to the discovery of thaxtomin A and its mode of action in causing potato scab, a great deal of work was undertaken to try and develop a treatment for the disease. The majority of approaches undertaken involved the testing of compounds with known fungitoxic action. Of particular note is work by McIntosh co-workers who studied hundreds of compounds in both glasshouse and field trials and is summarised below. Several classes of compounds have showed promise is glasshouse trials. Extensive studies on the effect of daminozide were undertaken (McIntosh, 1979). Whilst the compound showed good results in reducing the incidence of scab by up to 50% in the glasshouse trials it was shown to be less effective in the field (McIntosh & Bateman, 1979). One benefit of this foliar spray was that it resulted in no obvious distortion in the tuber or loss of yield. However, the compound which had been a common foliar spray for many years was later withdrawn for use on food crops when evidence of carcinogenic properties came to light.

The second class of compound that have been extensively studied (McIntosh et al., 1981) have been the phenoxyacetic acids. A selection of substituent's (eg, Me, Cl, Br) and substitution patterns (i.e. mono – penta substituted) have been tested. Interestingly this class of synthetic auxins showed a range of activities against potato scab. Some showed increases in the incidence of scab (e.g. 2-methyl, 2,5-dichloro, 2,3,6 trichloro), whilst the majority resulted in a decrease. The most effective was 3,5- dichlorophenoxyacetic acid, which resulted in an 87% reduction in scab in glasshouse trials. (Other notable results included, 2,4-dichlorophenoxyacetic acid [2,4-D] and 2,3,4,5-tetrachlorophenoxyacetic acid). 3,5- Dichlorophenoxyacetic acid was then further tested in field trials (McIntosh et al., 1982) where it only achieved a 30% reduction in incidence of scab. The major drawback in the use of the phenoxyacetic acid herbicides to reduce the incidence of potato scab is that in both field and glasshouse trials a significant reduction in yield, mean tuber weight and number, as well as increase in the number of tuber defects present per plant was observed. These side effects coupled with the decrease in effectiveness of the treatment in field trial means that commercial application of phenoxyacetic acids for the treatment of scab was not progressed.

Later work (McIntosh et al., 1988) tested an extensive range of substituted benzoic acids including, hydroxy, nitro, and halogen substituents, and picolinic acids most of which showed promising decreases in the incidence and severity of scab. The best results were seen for 2,5-dibromobenzoic acid, 71% and 3,6-dichloropicolinic acid, 73%. The advantage of both systems is that the treatments were applied as dilute foliar sprays and resulted in little effect on the tuber yield or the rate of tuber defects, with only slight foliar distortions being observed.

The four classes tested have all shown little outright toxicity to the *Streptomyces scabiei* pathogen particularly at the concentrations used in the glasshouse and field trials. Instead they have been shown to bio-accumulate in the potato tubers, through translocation of the sprayed compounds (Burrell, 1982). It is thought that rather than preventing the disease through outright toxicity to the pathogen, the compounds act either by altering the host’s response to infection (McIntosh et al., 1988) or by competing for the active sites targeted by thaxtomin A. These results and the biological testing using the *A. thaliana*, tomato pollen and potato pot trials / tuber slice assays will provide the basis for three methods to test nontoxic thaxtomin analogues for potential as a foliar treatment for scab, which can ameliorate the effect of thaxtomin A on potato tubers.

This chapter describes preliminary studies that test synthesised non-toxic compounds derived from thaxtomin chemical structures for their ability to ameliorate thaxtomin A toxicity.

MATERIALS AND METHODS

TEST COMPOUND PREPARATION

The test compounds used in this study were synthesised within our laboratory and were prepared as stock solutions in DMSO at either 1 or 10 mM. Thaxtomin A was prepared as a 57 µM solution in acetone / water (3 : 97). All stock solutions and DMSO for controls were cold filter sterilised using 0.2 µM Acrodisc Syringe filter super membranes (PALL, life sciences), protected from light and stored at 4°C until use.

ARABIDOPSIS THALIANA AMELIORATION ASSAYS

Arabidopsis thaliana (wild type Columbia) seeds were surface sterilised by the following method: To 32 mg of seed in a 2 mL eppendorf was added 1 mL of 30% bleach solution (available chlorine: 1.5% m/v), and the mixture shaken occasionally over a period of 15 minutes. The seed were centrifuged for 30 seconds, and the bleach solution was removed. The seeds were washed with sterile de-ionised water (4 x 1 mL). After the last wash, seeds were suspended in sterile agarose solution (0.01%, 1 mL) and allowed to vernalise for a minimum of two days at 4°C before use.

Agar plates were prepared by the following method: To a pH adjusted (pH 5.8) solution of sucrose (10 g L-1) and Murashige and Skoog (MS) basal medium (4.40 g L-1), was added agar (8 g L-1). The medium was sterilised and allowed to cool to 50 - 60°C before supplementing with the test compound. After thorough mixing the medium was poured into sterile Petri dishes (25 mL per plate) and allowed to cool.

Compounds were tested for their ability to ameliorate the effect of thaxtomin A on *Arabidopsis thaliana* seedlings. All treatments, except for a healthy control, contained 0.1 µM thaxtomin A. The five test compounds were trialled at four concentrations (0.1 µM, 1.0 µM, 10.0 µM, 50.0 µM), against controls containing the same volume of DMSO and 0.1 µM Thaxtomin A. The prepared plates were then used and analysed as described for compound toxicity in chapter 2.

TOMATO POLLEN AMELIORATION ASSAYS

Pollen was collected from glasshouse-grown tomato plants (40-60 days old), which had had old flowers removed 5 days before harvest. The pollen was allowed to dry overnight at room temperature.

To 0.225 mL of growth solution containing 15% sucrose, 0.003% H3BO3, 1 mM CaCl2, 0.2 mM KCl, 0.5% DSMO (unless otherwise stated) plus the ameliorating compound place in a 96-well micro-titre plate (Selby, Biolab, Australia) and tomato pollen added. The wells were shaken to achieve a vortex motion for 1 min and homogenous mixing of the pollen. The plate was inverted, protected from light and the pollen grains allowed to germinate at 30°C for 3 - 4.5 h (The hanging drops method; Melian & Balashova, 1994). The germinated pollen was then measured using a microscope (Olympus CH biological microscope, Tokyo, Japan), and averaged as detailed below.

For amelioration screening with compounds **49, 109, 105, 120, 122**: For each supplement, concentrations of 0 µM, 1 µM, 5 µM, 10 µM, 25 µM and 50 µM also containing thaxtomin A 0.1 µM, and a healthy control, four wells were prepared. After 3.5 h growth, germinated pollen was measured, taking a total of 15 random measurements for each well, resulting in four replicates per treatment.

For extended amelioration testing with compound **120,** concentrations of 0 µM, 0.01 µM, 0.05 µM, 0.10 µM, 0.25 µM, 0.50 µM, 1 µM, 5 µM, 10 µM, 25 µM and 50 µM also containing thaxtomin A 0.1 µM, and a healthy control, four wells were prepared. After 3.5 h growth, germinated pollen was measured, taking a total of 15 random measurements for each well, resulting in four replicates per treatment.

PATHOGENICITY POT TRIALS

Two potato varieties were used, Russet Burbank and Desiree. Planting material was disease-free mini-tubers harvested from glasshouse grown tissue culture plantlets.

All pots were amended with pathogen (*Steptomyces scabies* isolate #32*)* and treatments were replicated (*n* = 5) in a randomised block design with 30 pots per cultivar.

The six spray treatments (compounds **49, 109, 105, 120, 122 (see Figure 8)** and a DMSO Control) were prepared as 0.9 mM solution in 0.1% agar from 90 mM stock solutions in DMSO.

Foliar sprays were applied 14, 24 and 34 days into the tuber setting window and harvested 8 weeks later at senescence. After harvest all tubers were washed, weighed and counted. Any tuber with a mass >4 g was assessed for common scab using the rating scales described in previous chapters.

TUBER THAXTOMIN SENSITIVITY

Potatoes from these pot trials were assayed for sensitivity to thaxtomin A using the tuber slice assays described in previous chapters.

RESULTS

Five non-toxic compounds chemically derived from thaxtomin A were chosen for further testing (Fig 8).

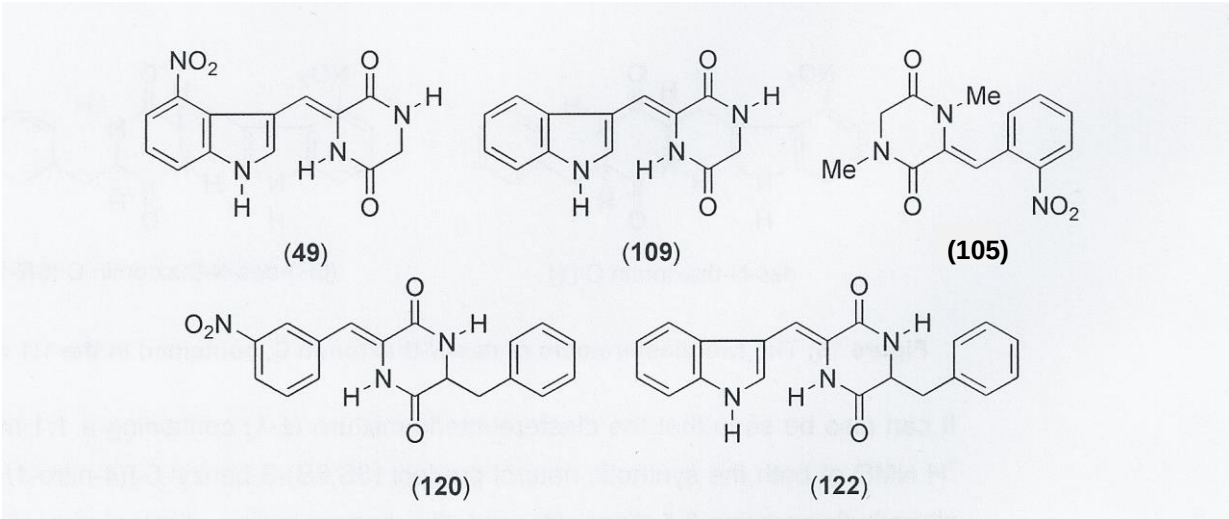


Figure 8: The five compounds tested for amelioration activity.

AMELIORATION OF THAXTOMIN A – USING ARABIDOPSIS THALIANA.

Of the five compounds tested using *Arabidopsis thaliana*, three showed promise of potential ameliorants of thaxtomin A. Compounds **49**, **105** and **120** all showed a significant improvement in the root length of the seedlings, up to a 23% for compound **120** at 50 µM. However, even at high concentration of ameliorating compounds the root length was still 13% shorter than the healthy control. Compound **120** showed most promise, as there is a clear improvement in root length as concentration of the ameliorating compounds increases. By contrast compound **109** shows no amelioration effect and compound **122** appears to have a synergistic effect on with thaxtomin A, resulting in a slight reduction in the overall root length of the seedlings versus the thaxtomin A control.

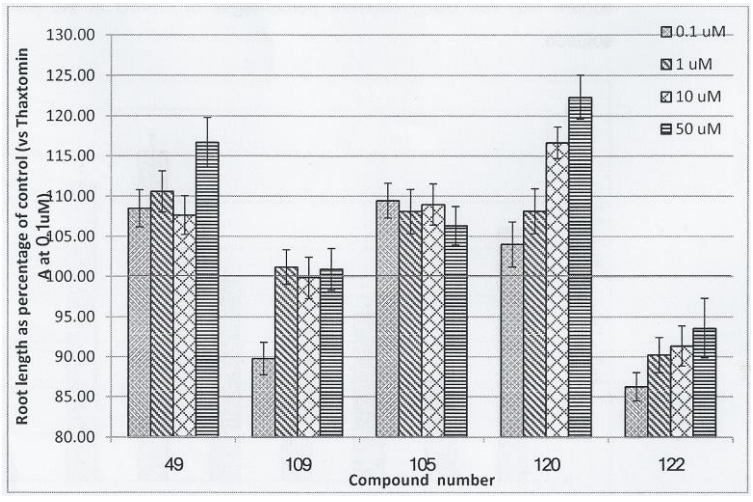


Figure 9. Results of *A. thaliana* amelioration test for five selected novel compounds

AMELIORATION OF THAXTOMIN A – USING TOMATO POLLEN.

Using the tomato pollen tube assay ameliorating effect of compounds **49, 105, 109** and **120** were less obvious. Compounds 109 and 120 significantly ameliorated thaxtomin a toxic affects at all concentrations but the extent of growth recovery was much less than seen with the Arabidopsis assay. Compounds 49 and 105 significantly ameliorated the toxic effects of thaxtomin A at 10 and 25 uM only or 10, 25 and 50 uM only respectively (Figure 10).

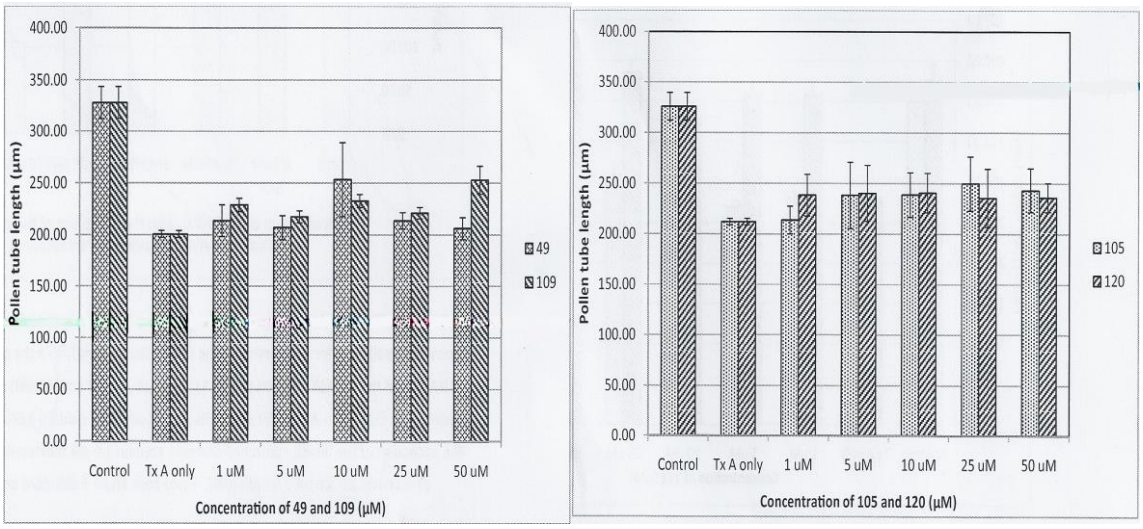


Figure 10. Results of tomato pollen amelioration tests for four selected novel compounds

In contrast to the Arabidopsis assay, compound **122** appears to significantly improve pollen tube growth to lengths comparable to the control for concentrations of 25 and 50 µM in contrast to prior results where this compound appeared to enhance inhibition. The initial result was repeated with similar significant effects although with variation in the extent of amelioration. The best result was to restore tube length to 75% of the healthy control (Figure 11).

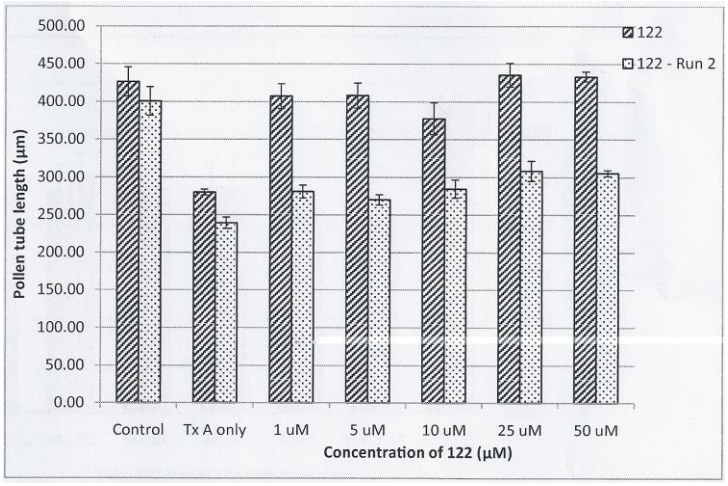


Figure 11. Results of tomato pollen amelioration tests for compound 122

Further testing of compound **120** over a wider concentration range (0.01 – 50 µM) showed a steady improvement in tube length growth increasing from 61% to 77% of the healthy control at 25 µM. This result

was consistent with the substantial improvement seen for compound **120** in the *Arabidopsis thaliana* tests (Figure 12).

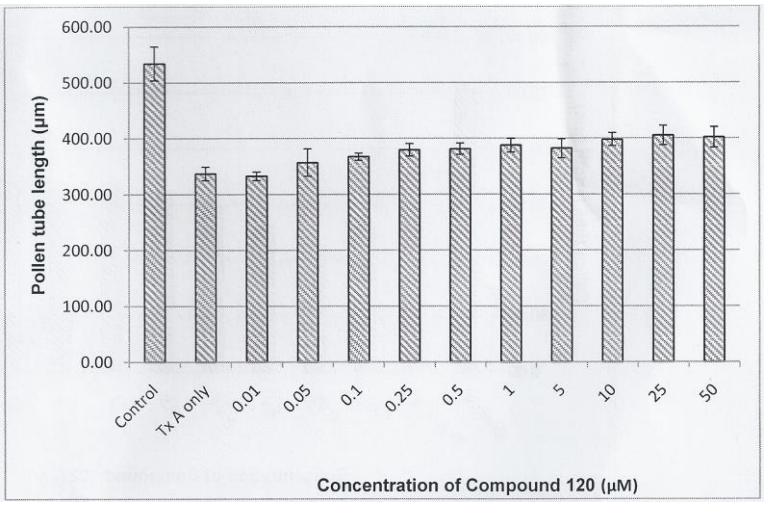


Figure 12. Results of tomato pollen amelioration test for compound 120 over wider concentration range

PATHOGENICITY POTATO POT TRIALS.

These preliminary pathogenicity assays failed to demonstrate any significant effects of these novel chemicals on reducing common scab. Of the five tested compounds, **122** showed a substantial increase in the mean tuber number and weight, but by contrast also showed an increase (double of the control) in the percentage of infected tubers (60%) for Russet Burbank. It also showed a slight decrease in disease coverage and with no change in the severity. In contrast the same material showed reduced tuber numbers and weight, and decreased disease in Desiree (Table 29).

Table 29. Agronomic and pathogencity results following foliar treatment of potato plants with novel chemicals in a glasshouse trial.

Test compound	Mean tuber number /pot	Mean tuber weight/pot (g)	Surface cover score (0-6)	Surface Cover (%)	Lesion depth score (1-4)	Infected tubers (%)
Russet Burbank						
Control	4.20	46.0	0.93	2.57	1.43	29.4
49	5.80	52.0	0.88	2.38	1.38	21.1
109	5.80	60.6	0.96	2.79	1.75	50.3
105	4.40	52.2	0.83	2.17	1.33	31.0
120	5.80	45.5	0.83	2.17	1.58	53.0
122	6.40	56.2	0.86	2.29	1.43	63.6
Desiree						
Control	3.2	57.7	1.0	3.0	2.5	16.7
49	2.0	53.0	1.0	3.0	1.0	10.0
109	2.0	46.0	1.0	3.0	1.0	20.0

105	2.8	57.3	1.0	3.0	2.3	48.3
120	1.2	31.6	1.0	3.0	1.0	20.0
122	1.8	35.1	0.0	0.0	0.0	0.0

All the compounds appeared to induce some increased tolerance towards thaxtomin A, although these differences were not significant. Compound **49** showing the greatest reduction in necrosis rating of 33% (Russet Burbank), however much less difference was observed with Desiree (Table 30). This is comparable to results seen by Tegg *et at.*(2008) for 2,4-D in which a 28 % improvement was seen. This result appears to suggest that translocation of compound **49** to the tuber may have occurred, but to confirm this result quantification of levels in the tubers would be required.

Table 30. Influence of foliar treatment with novel chemicals on sensitivity to thaxtomin A treatment of tuber tissues at harvest.

	Necrosis Rating from Tuber slice assay		
Compound	**RBK**	**DES**	**Combined**
Control	3.00	3.00	3.00
49	1.96	2.76	2.36
109	2.19	2.78	2.48
105	2.13	2.76	2.44
120	2.58	2.80	2.69
122	2.31	2.66	2.49

DISCUSSION & CONCLUSIONS

Whilst some success was observed with testing for the amelioration of thaxtomin A, with compounds **49**, **105**, **120** showing promise in *Arabidopsis* assays. Contrasting results were seen in the tomato pollen assays, with compounds **120** and **122** performing best even though **122** showed synergistic toxicity with thaxtomin A in the *Arabidopsis* trial.

Both the tomato pollen and *Arabidopsis* assays were a useful indication of the value of the compunds, but in each case the test compound is applied directly to the test subject either in the agar, or as a growth solution.

For a compound to successfully reduce disease in potato it has to be active as a foliar spray and be able to translocate through the plant to the tuber and accumulate in sufficient quantities to provide protection again thaxtomin A.

These preliminary tests indicate little if any of these novel materials translocated to the tubers. Problems occurred with limited solubility of these compounds in aqueous solutions making preparation of sprays and adsorption by potato plants difficult.

Future work could examine modification of these compounds to allow application as an aqueous spray (e.g. without use of toxic DMSO), and possibilities include introduction of phenolic groups which could be used to make the corresponding phenoxy acetic acid, which could then be applied as a sodium salt as common herbicides such as 2,4 dichlorophenoxy acetic acid are. If these modifications improved solubility and capacity for systemic translocation *in planta* further studies on foliar treatment and seed dips should be done.

CHAPTER 5: NOVEL CHEMISTRIES - RELATIONSHIP BETWEEN THE EFFICACY OF FOLIAR CHEMICALS TO REDUCE COMMON SCAB AND THAXTOMIN A TOXICITY

INTRODUCTION

Common scab caused by infection with pathogenic *Streptomyces* spp. is one of the most important diseases of the potato (*Solanum tuberosum* L.) worldwide (Loria et al,. 2006). Annual losses in Tasmania, Australia alone are estimated at approximately 4% of the industry value (Wilson et al., 2009). All pathogenic *Streptomyces* spp. produce the phytotoxin thaxtomin A, whilst non-pathogenic strains do not (King et al., 1991; Babcock et al., 1993), demonstrating its essential role in disease induction (Goyer et al., 1998; Kers et al., 2005).

There is no single effective management option for this disease, rather an integrated approach is required. Foliar sprays provide a novel strategy that has previously been shown to be highly effective in suppressing this recalcitrant soil-borne disease (McIntosh et al., 1982; 1985; 1988; Tegg et al., 2008) but other undesirable side effects (tuber distortion, yield reduction) has led to limited uptake of this technology (McIntosh et al., 1985; 1988). Our research group has studied the mechanisms by which 2,4-dichlorophenoxyacetic acid (2,4-D) may reduce this disease. We found that foliar applied 2,4-D (and auxin analogues) migrated to the tuber tissue and inhibited thaxtomin A induced necrosis. Our studies and others also confirmed that 2,4-D, at efficacious concentrations, does not affect *S. scabiei* growth (McIntosh et al., 1985; Tegg et al., 2008) or thaxtomin A production *in vitro* (Tegg et al., 2008), confirming that the novel disease control is through inhibition of thaxtomin A by the applied chemical. Also, 2,4-D as a powerful synthetic auxin has an impact on potato tuber anatomy and physiology, however it is not conclusively known whether these changes have any role in affecting common scab disease development (Tegg et al., 2008). Whilst 2,4-D is primarily known as a herbicide it is also used commercially in potato production at low concentrations to enhance red skin coloration, without apparent tuber distortion or yield effect Waterer, 2010).

McIntosh and others (McIntosh et al., 1982; 1985; 1988) tested over 100 compounds for disease control and found chemicals that either decreased, increased or had no effect on common scab disease development. Many showed potential, some without negative side-effects on tuber quality and yield, but the research was not carried forward to commercial adoption; this may be partly attributable to the usage of irrigation management as a viable alternative control for common scab in the UK. However, in Australia and other production regions of the world, where water is a scarce resource and irrigation management has provided relatively poor disease control, improved disease management with a simple and cheap foliar spray would be highly sought after by industry (Tegg et al. 2008).

A relationship between necrosis and disease has been established for 2,4-D such that the amount of reduction in thaxtomin A-induced necrosis measured in vitro (in tuber slice assays) corresponds with the amount of reduction of disease observed in the tubers Tegg et al., 2008). However, it has not been demonstrated whether other chemicals that have the capacity to inhibit or promote common scab have an effect on thaxtomin A-induced toxicity. If a relationship was established this would provide insight into a broader mechanism for chemically induced resistance to common scab. In this study we chose to utilise Bayesian measurement-error modelling approach to enable relationships to be modelled linking thaxtomin A toxicity and disease response.

A measurement-error model allows for adjustment due to errors in both explanatory and response variables unlike a conventional regression model that assumes the explanatory variables are precisely known. The use of Bayesian methods allow complex models to be constructed with greater ease than with classical approaches (Brooks, 2003). Unlike classical methods Bayesian statistics involves the use of prior distributions (Gelman et al., 2000), usually in the form of probability distributions. The results of a Bayesian analysis provide a direct description of the probabilities of the magnitude of parameters of interest.

Bayesian models may enable the identification of one parameter to aid in the prediction of another related parameter. Thaxtomin A resistance is a key mechanism postulated for resistance to common scab (Acuna et al., 2001; Hiltunen et al., 2006; 2011; Wilson et al., 2009; 2010) and its toxicity can be easily measured in a quick and rapid tuber slice assay (Tegg et al., 2010). Its measurement and modelling in response to various chemical treatments may enable the efficaciousness of those treatments to be accurately assessed, without the need for expensive and time consuming disease trials. In the case of common scab, sporadic disease intensities resulting from varied environmental influences can make assessing the effectiveness of given controls unpredictable (Tarn et al., 2004; Hiltunen et al., 2011). An initial assay could negate the need for assessment of disease (Hiltunen et al., 2011) for assessing the likely suitability of a given control, this may improve the ability to identify and rapidly screen a range of different chemical treatments.

The aim of this study was to assess a selection of compounds applied as a foliar spray to young potato plants for their relative ability to reduce common scab and to determine the association of this with their relative ability to inhibit thaxtomin A toxicity.

MATERIALS AND METHODS

PATHOGEN CULTURE AND PLANT ESTABLISHMENT.

Pathogenic *S. scabiei* strains #20 or G#32, were initially isolated in 1990 from common scab diseased potato tubers from Tasmania and Victoria, Australia, respectively. Both *S. scabiei* isolates are highly pathogenic, producing high levels of thaxtomin A (R. King, AgriFood Canada, *pers. comm.*) and contain the *nec1* gene (unpublished data). Inoculum was prepared by suspending spores harvested from a 2-week -old culture grown on ISP2 medium (Shirling & Gottlieb, 1966) in 5 ml of sterile water, and adding this to a sterilized mixture of 100 g vermiculite and 500 ml SAY solution (20 g/ litre sucrose, 1.2 g/litre *L*-asparagine, 0.6 g/litre K_2HPO_4, 10 g/litre yeast extract, pH 7.2). Inoculum was incubated at 24°C in the dark for 14 days, where upon profuse sporulation was observed.

Plastic pots (20 cm diameter, 20 cm height, 4.7 litre) were filled with a potting mix containing sand, peat and composted pine bark (10:10:80; pH 6.0) premixed with Osmocote 16-3.5-10 NPK resin coated fertilizer (Scotts Australia Pty Ltd. Baulkham Hills, Australia) at the rate of 6 kg/m^3. In trials where pathogen amendments were included, inoculum (*c*. 20 g) was mixed to the depth of the pot (inoculum concentration not determined). Potato plants (*S. tuberosum* cvs. 'Russet Burbank' – moderately resistant to common scab, and 'Desiree' – susceptible to common scab were provided as visually disease free certified seed (Department of Primary Industries, Devonport, Tasmania), with tubers planted into individual pots. Pots were placed outside on benches (replicates) exposed to the ambient environment in Hobart, Tasmania. Irrigation water was applied to each pot every third day. This regime allowed the potting mix to dry substantially between watering. Additional plants were grown alongside the trial to allow periodic monitoring for onset of tuber initiation without disturbing the trial plants. No pesticides were applied during the trial period. A soluble fertilizer was applied monthly (Miracle-Gro® Water Soluble all-purpose plant food, 15-13.1-12.4 NPK, Scotts Australia Pty Ltd.).

SPRAY TREATMENTS.

All spray treatments were timed to coincide with the critical infection period, which occurs 2-6 weeks after tuber initiation (ati) (Lapwood et al., 1973; 1975), determined by observations of additional (non-trial) plants. All treatments were applied as sequential triple sprays applied at 10, 20 and 30 days ati. Spray treatments were applied as a fine mist to leaves until run-off. Absorbent cotton towels were placed on the soil surface during spraying, to ensure soil was protected from direct spray contact, and towels were removed immediately after treatment.

Spray treatments included water controls and nine other compounds (Table 29). These compounds and concentrations utilised were based on prior published results (McIntosh et al., 1982; 1985; 1988; Tegg et al., 2008), and were a representation of chemicals that had either increased, reduced or had no effect on common scab disease (Table 29). All chemicals were sourced from Sigma Aldrich (St. Louis, Missouri, USA) except for 2,4-D (am) i.e. Amicide®625 obtained from Nufarm Pty Ltd. (Victoria, Australia), 3,6-DCP from AK Scientific (California, USA), and 2,5-D and 3,5-D which were obtained by direct synthesis, through the condensation of their dichlorophenol form with monochloroacetic acid (Que Hee & Sutherlan, 1981). All spray treatments, including controls, contained 0.2% ethanol (for dissolution of the chemicals) and 0.2 g/litre Tween 20 (wetting agent).

Table 31. Chemicals tested and rates used in this study as foliar sprays for common scab control and thaxtomin A toxicity suppression.

Active Constituent	Abbreviation	Concentration (active ingredient)	Previous reported performance of selected chemicals against common scab		
			% scab reduction[a]	Side-effects	References
4-chloro-phenoxyacetic acid	4-CPA	0.9 mM	4		McIntosh *et al.* 1981
2,4-dichloro-phenoxyacetic acid	2,4-D	0.9 mM	45 - 92	Yield reduction, Tuber and foliage distortion	McIntosh *et al.* 1981; Tegg *et al.* 2008
2,4-dichloro-phenoxyacetic acid (amide form)	2,4-D (am)	0.9 mM	not tested		
2,5-dichloro-phenoxyacetic acid	2,5-D	0.9 mM	Increase		McIntosh *et al.* 1981
3,5-dichloro-phenoxyacetic acid	3,5-D	0.9 mM	87	Yield reduction, tuber distortion	McIntosh *et al.* 1981
5-chloro-2-nitrobenzoic acid	CNB	1.6 mM	7 - 73		McIntosh *et al.* 1988; Tegg *et al.* 2008
2,5-dibromobenzoic acid	2,5-DBB	1.6 mM	71	Slight foliage distortion	McIntosh *et al.* 1988
2,5-dihydroxybenzoic acid	2,5-DHB	1.6 mM	Increase		McIntosh *et al.* 1988
3,6-dichloropicolinic acid	3,6-DCP	50 µM	73	Slight foliage distortion	McIntosh *et al.* 1988

[a] Variation in disease reduction is both a function of different environmental conditions and disease pressures in different experiments.

POT TRIAL DESIGN AND TREATMENTS.

The effects of novel foliar sprays on scab development and subsequent tuber sensitivity to thaxtomin A were studied in three pot trials in 2009 and 2010. In all three trials the same nine chemicals were tested (Table 31) on both cultivars 'Desiree and 'Russet Burbank'. In pot trials 1 and 2, *S. scabiei* isolate #20 was used, trial 3 used *S. scabiei* isolate G#32. In all trials, treatments were replicated (*n*=5: trial 1; *n*=3: trials 2 and 3) and pots arranged in a randomized block design. The first foliar sprays were applied on 13 Feb 2009 (trial 1), 1 Mar 2010 (trial 2), and 15 Mar 2010 (trial 3), with all tubers harvested at senescence 10 weeks later (equivalent to 16 weeks after planting) and scored for disease and tuber yield.

DISEASE AND TUBER YIELD ASSESSMENT.

After plants had fully senesced, tubers were harvested, rinsed gently under cold running water and air dried. Tubers were assessed immediately, with the number and individual weight of each tuber per pot recorded. Tubers weighing more than 4.0 g were assessed for disease. Both disease incidence (% of tubers with disease lesions) and severity (the mean surface area covered by lesions and the deepest lesion per tuber) assessments were done using methods described previously (Wilson 2001; Tegg et al., 2010; Khatri et al., 2011). Both disease incidence and the mean surface area covered by lesions are important measures used for certification in Australia and the United Kingdom respectively. The other severity measurement (deepest lesion per tuber) is extremely important within the Australian processing sector, with deep-lesioned tubers resulting in rejection of crops at processing factories (P. Hardman, Simplot Australia, *pers. comm.*). After assessment tubers were placed into storage at 4°C, for 2 weeks, prior to undertaking the tuber slice assay.

SENSITIVITY OF TUBERS TO THAXTOMIN A USING A TUBER SLICE ASSAY.

Tubers from each chemical spray treatment and cultivar were tested for thaxtomin A tolerance using a tuber slice bioassay refined by Tegg and co-workers Tegg et al., 2008). The tuber slice assay is the most suitable method for accurately quantifying tuber tissue response to thaxtomin A, providing a more consistent response than other tissue types (e.g. leaf tissue, outer tuber periderm) (Tegg et al., 2010). Production and purification of the toxin thaxtomin A was as previously described (Wilson et al., 2009). A selection of representative visually disease-free whole tubers were surface sterilized with 0.5% sodium hypochlorite for 10 min and air-dried before being cut into 0.5-cm-thick slices and placed in 90-mm petri dishes with moist sterile filter paper (Whatman No. 1). There were a total of three Petri dishes (replicates) per treatment. In each replicate, three tuber slices from one single tuber were used for each tested treatment. Filter paper disks of 6 mm diameter (Whatman No. 1) were immersed in thaxtomin A solution (14 µM for 'Desiree', 7 µM for 'Russet Burbank' – all dissolved in ~1% acetone) for 1 hour, air-dried and placed on the potato slices (2-3 disks per slice) with disks immersed in ~1% acetone solution and air-dried used as controls. In each replicate (Petri dish) each treatment had a total of seven individually-scored disks. After placement on the potato slices, 10 µl of sterile distilled water was applied to each disk (1) to ensure stable contact between tuber and disk. Plates were incubated at 24°C in the dark and evaluated after 7 days for necrosis in the defined area under the filter paper disk: 0= no necrosis, 0.5= very sparse flecks, 1= few light brown flecks, 1.5= few dark brown flecks, 2= light brown flecks in circle, 2.5= dark brown flecks in circle, 3= light brown necrosis, 3.5= dark brown necrosis, and 4= black necrosis.

DATA ANALYSIS.

Data were subjected to analysis of variance using Genstat 9 (Rothamsted Experimental Station, Harpenden, Hertfordshire, UK). Data using scale ratings were derived from an interval-based scale, with a continuous, ordinal, ascending nature. Significance was calculated at $P = 0.05$ as noted, and least significant difference (LSD) was used for comparison of treatment means. All data were tested for normality and homogeneity of variance and no transformation was necessary.

To investigate the relationship of disease with thaxtomin A sensitivity (necrosis) a Bayesian measurement-error model was developed. Standard regression models assume that the explanatory variables are precisely known with zero error. In practice this assumption is sometimes not the case and was known to be untrue with these data. Not allowing for such uncertainty can lead to inconsistent estimates. We assumed that the sizes of the errors do not depend on the magnitude of the measurements, but it should be noted that in general errors can behave otherwise. We assume that the explanatory variable, necrosis, is observed with an additive error, also called a classical error model Dellaportas & Stephens, 1995). While other approaches have been used (Stefanski & Carroll, 1985; Carroll et al., 1999), by adopting a Bayesian approach we aim to avoid the computational and statistical difficulties associated with such models Fuller, 1987).

The model consists of a simple linear regression of the disease rating (tuber surface cover score) with necrosis as the explanatory variable. Tuber surface cover score provides the best interpretation of average disease across a pot (Wilson 2001; Wilson et al., 2009) and was the parameter modelled instead of lesion depth or percentage of tubers infected. We initially assume that the relationship may differ for each chemical requiring individual intercepts and slopes. Below we use the subscripts *i* to refer to individual observation and *t* to refer to chemicals 1– 10. The observed disease rating is assumed to be normally distributed with mean μ_i and precision τ (reciprocal variance). The mean is defined as shown in equation (a) in which α_t is the intercept, β_t is the slope, and v_i is the unobserved true value for necrosis.

$$\mu_i = \alpha_t + \beta_t v_i \qquad \text{(a)}$$

Since this is a measurement error model we let the observed necrosis be given by a normal distribution with mean given by the unobserved true value for necrosis as shown in equation (b). We estimated the value of the precision σ_i from the standard deviation of the necrosis ratings of the seven individually-scored disks.

$$N_i \sim N(v_i, \sigma_i) \qquad \text{(b)}$$

Last, we assume that the intercepts, slopes and unobserved necrosis parameters are normally distributed with a common means, $\alpha_t \sim N(\alpha_0, \lambda)$, $\beta_t \sim N(\beta_0, \rho)$, and $v_i \sim N(v_0, \varphi)$. Normal distributions for explanatory and dependent variables are acceptable here since the range of data was relatively small compared to their possible range.

Having defined the model we further define three simpler models in which the chemicals share a common slope, a common intercept, or both. The six analyses (three trials by two cultivars) were analysed

separately and for each we choose one of the four possible models that obtained the minimum overall deviance.

The α_0 , β_0 and v_0 are assigned normal distributions with zero means and precisions 0.001. The precisions, λ , ρ , φ , and τ are all assigned vague gamma distributions with parameters 0.001, and 0.001. When fitting the model we zero-centred the v_i to improve model convergence.

Sensitivity studies and standard diagnostic techniques were used Brooks & Roberts (1998) to assess model validity. We fitted the model using Markov chain Monte Carlo (MCMC) methods (Gilks et al., 1998) by means of JAGS software Plummer, 2006). The model was run for 10,000 iterations and a 10% burn-in was used.

RESULTS

DISEASE PERFORMANCE.

Across three pot trials there were variable levels of disease incidence and severity (surface coverage and lesion depth) (Table 32). Pathogenic isolate *S. scabiei* G#32 (trial 3) produced significantly higher disease than *S. scabiei* #20 (trials 1 and 2) and this is best demonstrated by comparing control tubers from each trial. In trial 3, Desiree had an infection percentage of 100%, common scab surface coverage of 18.77% and an average lesion depth score of 2.60 whilst in trials 1 and 2 the highest levels recorded for each parameter were 47.2%, 1.89% and 2.05 respectively (Table 32).

The response to the application of a range of different chemicals showed similar trends (in terms of disease) across all three trials and across both varieties Desiree and Russet Burbank. The treatments 2,5-D, CNB, 3,6-DCP and 2,5-DHB consistently produced comparable disease (tuber surface cover) as the water-treated control (Table 32). One chemical, 4-CPA produced comparable disease to the control in five analyses, however in trial 3 with the Desiree variety it produced significantly ($P < 0.05$) less disease.

Other treatments (2,4-D, 2,4-D (am) and 2,5-DBB) showed a consistent and significant ($P < 0.05$) ability to reduce disease (tuber surface cover) across both varieties, when compared to the water treated control (Table 32; Fig 13). One additional treatment 3,5-D significantly suppressed disease, compared to the water control, in five analyses, however in trial 1 with Russet Burbank, disease suppression was not significant (Table 32).

Other measures of disease were less affected by the chemical treatment applied. The mean percentage of tubers infected showed similar trends to the tuber surface cover responses although in trial 1 and 2 these effects were not statistically significant ($P > 0.05$). This may be partly attributable to the low disease pressure recorded in both these trials where *S. scabiei* #20 was used (infection percentages below 50%). Where disease pressure was higher in trial 3 (*S. scabiei* G#32) and infection percentages reached 100%, significant treatment differences were identified and in both cultivars the four treatments: 2,4-D, 2,4-D (am), 3,5-D and 2,5-DBB produced significantly lower percentages ($P < 0.05$) of infected tubers than the water-treated control (Table 32).

The impact of chemical treatment on mean greatest lesion depth was variable (Table 30). In trial 1, Desiree recorded moderate lesion depth (ratings of ~2.0) and treatment effects were observed with 2,4-D, 2,4-D (am), 3,5-D and 2,5-DBB having less ($P < 0.05$) mean lesion depth than the controls. In Russet Burbank, generally superficial lesions were recorded (ratings up to 1.40), but significant differences obtained; in addition to the chemicals that suppressed lesion depth in Desiree, 2,5-D, CNB and 3,6-DCP also recorded significantly ($P < 0.05$) lower lesion depth ratings than the control. In trial 2, where generally only superficial lesions were recorded (ratings < 1.4) there were no significant chemical treatment effects observed across both varieties. Trial 3 recorded deeper lesion depths and in both varieties significant treatment effects were identified; the chemical treatments: 2,4-D, 3,5-D and 2,5-DBB produced significantly shallower lesions ($P < 0.05$) than the control tubers in Desiree with 3,5-D treatment also producing shallower lesions in Russet Burbank.

Tuber distortion (mainly tuber elongation) was visually observed in foliar spray treatments that included dichloro phenoxyacetic acids i.e. 2,4-D, 2,4-D (am), 2,5-D and 3,5-D. This distortion was observed in both cultivars tested and across all three trials.

Table 32. Impact of various foliar spray treatments on common scab disease of potato *cvs.* Desiree and Russet Burbank inoculated with *Streptomyces scabiei* strains #20 (trials 1 and 2) and G#32 (trial 3).

		Common scab disease rating								Thaxtomin A sensitivity	
		Tuber surface cover				Lesion depth score		Infected tubers		Mean necrosis rating	
		score (0-6)[c,d]		(%)[d]		(1-4)[c,d]		(%)[c,d]		(0-4)[c,d]	
Pot trial	Treatment[a,b]	Desiree	Russet Burbank	Desiree	Russet Burbank	Desiree	Russet Burbank	Desiree	Russet Burbank	Desiree	Russet Burbank
1	control	0.61 a	0.26 a	1.89	0.58	2.05 a	1.35 a	47.2	23.4	2.84 a	2.79 a
	2,4-D	0.13 b	0.00 bc	0.40	0.00	1.40 bc	-	25.2	0.0	2.48 de	2.16 de
	2,4-D (am)	0.13 b	0.03 bc	0.40	0.10	1.35 bc	1.00 b	23.6	6.0	2.56 de	1.98 e
	2,5-D	0.62 a	0.27 a	2.04	0.49	1.78 ab	1.00 b	43.4	20.0	2.80 abc	2.70 ab
	3,5-D	0.20 b	0.10 abc	0.37	0.15	1.00 c	1.00 b	18.7	12.5	2.58 cde	2.39 cd
	4-CPA	0.57 a	0.21 ab	1.92	0.63	2.10 a	1.35 a	42.5	25.5	2.85 a	2.68 abc
	CNB	0.40 ab	0.18 abc	1.20	0.39	1.90 a	1.00 b	35.0	12.2	2.60 bcde	2.65 abc
	2,5-DBB	0.19 b	0.03 bc	0.49	0.03	1.25 c	1.00 b	24.3	5.0	2.40 e	1.64 f
	2,5-DHB	0.60 a	0.30 a	1.72	0.63	2.00 a	1.40 a	40.0	28.0	2.69 abcd	2.69 ab
	3,6-DCP	0.61 a	0.18 abc	1.70	0.28	1.92 a	1.00 b	41.0	12.0	2.81 ab	2.42 bcd
	P	0.002	0.015			0.02	0.031	0.254	0.094	0.001	<0.001
	LSD (0.05)	0.32	0.20			0.45	0.25	ns	ns	0.220	0.288
2	control	0.38 a	0.37 a	1.02	0.90	1.11	1.00	43.7	38.1	2.75 a	2.67 a
	2,4-D	0.15 cd	0.12 d	0.20	0.15	1.00	1.00	28.1	23.7	2.32 bc	1.95 c
	2,4-D (am)	0.13 d	0.14 cd	0.20	0.14	1.00	1.00	33.2	28.9	2.35 bc	2.10 bc
	2,5-D	0.44 a	0.30 abc	1.42	0.60	1.28	1.00	46.7	40.3	2.80 a	2.81 a
	3,5-D	0.21 bcd	0.17 bcd	0.46	0.27	1.00	1.00	26.3	29.3	2.40 b	2.34 b
	4-CPA	0.32 ab	0.34 a	0.75	0.90	1.31	1.08	47.2	39.8	2.82 a	2.66 a
	CNB	0.30 abc	0.31 ab	0.70	0.69	1.22	1.00	37.0	42.5	2.66 a	2.64 a
	2,5-DBB	0.10 d	0.17 bcd	0.10	0.25	1.00	1.00	23.1	23.7	2.10 c	2.03 c
	2,5-DHB	0.34 ab	0.31 ab	0.90	0.55	1.39	1.00	37.1	37.9	2.68 a	2.72 a

	3,6-DCP	0.42 a	0.31 ab	1.21	0.60	1.22	1.00	47.1	39.6	2.76 a	2.63 a
	P	0.01	0.015			0.162	0.474	0.311	0.342	<0.001	0.003
	LSD (0.05)	0.165	0.162			ns	ns	ns	ns	0.255	0.265
3	control	2.66 a	2.28 ab	18.77	13.13	2.60 ab	1.97 ab	100.0 a	100.0 a	2.62 a	2.71 ab
	2,4-D	1.33 cde	0.34 c	4.94	1.35	1.78 bc	1.11 c	71.1 bcd	33.7 d	2.14 cd	2.11 d
	2,4-D (am)	0.99 e	0.76 c	4.11	2.31	1.86 bc	1.36 bc	70.8 bcd	52.8 c	2.16 bcd	2.05 d
	2,5-D	2.14 abc	1.77 ab	14.09	8.85	2.61 ab	1.75 abc	89.2 ab	89.7 ab	2.55 a	2.71 ab
	3,5-D	1.23 de	0.33 c	4.00	0.97	1.00 c	1.22 c	45.4 d	35.2 d	2.35 abc	2.33 cd
	4-CPA	1.73 bcde	1.63 b	9.89	6.95	2.35 ab	1.51 abc	82.2 abc	78.0 b	2.50 ab	2.62 abc
	CNB	1.99 abcd	2.04 ab	10.10	10.75	2.33 ab	2.02 a	89.7 ab	92.6 ab	2.49 ab	2.62 abc
	2,5-DBB	0.98 e	0.48 c	3.10	1.30	1.89 bc	1.27 c	60.5 cd	43.5 cd	1.98d	1.71 e
	2,5-DHB	2.58 ab	2.45 a	17.94	17.53	2.58 ab	2.15 a	96.7 ab	100.0 a	2.64 a	2.79 a
	3,6-DCP	2.68 a	1.64 b	18.08	6.83	2.88 a	1.66 ab	100.0 a	83.3 ab	2.67 a	2.52 abc
	P	0.001	<0.001			0.01	0.014	0.014	<0.001	0.006	<0.001
	LSD (0.05)	0.872	0.737			0.966	0.644	28.64	17.28	0.349	0.329

[a] Spray treatments included triple sequential sprays of 0.9 mM 4-CPA, 2,4-D, 2,4-D (am), 2,5-D and 3,5-D; or 1.6 mM CNB, 2,5-DBB and 2,5-DHB; or 50 µM 3,6-DCP; or a control water treatment, with initial applications made 10 days after tuber initiation, and subsequent applications at 10-day intervals.

[b] Total tuber numbers assessed per treatment were ≥25 in pot trial 1 and ≥15 in pot trials 2 and 3.

[c] Means followed by same letter within the same column are not significantly different at *P* = 0.05 using Fisher's LSD test; ns = nonsignificant.

[d] Common scab disease measures (tuber surface cover, lesion depth score, infected tuber %) and thaxtomin A toxicity was determined following previous methods.

SENSITIVITY OF TUBERS TO THAXTOMIN A USING A TUBER SLICE ASSAY.

Across all three pot trials and both varieties (six separate analyses) significant decreases ($P < 0.05$) in thaxtomin A-induced necrosis scores were recorded for tubers harvested from plants treated with triple foliar sprays of 2,4-D, 2,4-D (am) and 2,5-DBB, compared to tubers from water control plants (Table 32, Fig. 14. One chemical, 3,5-D suppressed thaxtomin toxicity in all six trial-variety combinations, though suppression was significant in only 5 of the 6 analyses.

The other chemicals generally had no significant impact on thaxtomin A-induced necrosis. Indeed tubers treated with foliar sprays of 2,5-D, 4-CPA, CNB and 2,5-DHB showed an analogous response to control (water-treated) tubers (Table 32) in all six analyses (three trials across two varieties). One chemical, 3,6-DCP produced comparable toxin necrosis responses to the control in five analyses, however in trial 1 with Russet Burbank it produced significantly ($P < 0.05$) less necrosis.

RELATIONSHIP OF COMMON SCAB DISEASE WITH THAXTOMIN A SENSITIVITY (NECROSIS).

For the case of Russet Burbank in trial 1 the preferred model, with the minimal deviance, was one in which there were different slopes and intercepts for the chemicals. For all other cases the simplest model was preferred in which all chemicals shared the same intercept and slope. The coefficients and their 95% credible intervals are shown in Table 33. The observed data and mean posterior predicted fits are shown in Figure 15 and 16. For Russet Burbank in trial 1 there was an increasing positive slope showing that as necrosis increases so too does disease (Fig. 15). It is of interest that two distinct groups of linear regressions were observed. The first group (control; 2,5-DHB; 2,5-D; CNB; 4-CPA; 3,6-DCP) all have positive slopes with posterior probability 1 (Table 33) and corresponds with chemicals that do not provide significant reduction in tuber disease, nor do they reduce toxin necrosis ratings. The second group (2,4-D (am); 3,5-D; 2,5-DBB; 2,4-D) have lower slopes. The posterior probabilities that they have positive slopes range between 0.76 and 0.90 indicating that they are all still likely to be increasing relationships (Table 33, Fig. 15). These correspond with chemicals that provide a significant reduction in tuber disease and an associated reduction in toxin necrosis ratings.

For the five other analyses similar trends were obtained showing that as necrosis increases so too does disease, (Fig. 16a-e). Each has positive slope with posterior probabilities of at least 0.95. The magnitudes of the positive slopes for each graph are trial dependant (Table 33) and are a function of the relative levels of disease across each trial. The third trial with much higher disease, where the highly pathogenic *S. scabiei* G#32 was used (Table 32), was characterised by having slopes of 6.81 and 5.36 for Desiree and Russet Burbank, whilst the highest recorded slopes from either trials 1 or 2 (both with low disease) were 2.37 and 1.66, respectively. Visual interpretation shows that certain treatments cluster into various parts of the linear regression. Treatments that reduced disease and toxin necrosis clustered towards the bottom left of the regression in all five analyses and included 2,5-DBB; 2,4-D; 2,4-D (am); 3,5-D (Fig. 16a-e). The other six treatments (control; 4-CPA; 2,5-D; CNB; 2,5-DHB; 3,6-DCP) that gave little or no significant reduction in either tuber disease or toxin sensitivity clustered from the middle towards the top right of the regressions in all five analyses. This clustering results from the varying efficaciousness of each chemical, but there remains an overall trend in each trial between disease severity and necrosis measure, thus providing a linkage between the two measures. We show in Figure 16 using dashed lines the simple linear regressions that would be obtained if no adjustment had been made for measurement error. In each case the slopes were smaller. This was the result of the measurements with smaller precision being given equal weight to more precise measurements.

Table 33. Summary of the relationship between common scab disease (tuber surface cover score) and thaxtomin A-induced necrosis for each trial and cultivar.

Trial\cultivar	Treatment	Intercept (α)	Slope (β)	P(slope>0)
1 (Russet Burbank)	control	-3.99 (-6.93, -1.84)	1.66 (0.81, 2.83)	1.00
	2,4-D	-0.53 (-2.51, 0.66)	0.23 (-0.26, 1.07)	0.76
	2,4-D (am)	-1.33 (-4.00, 1.15)	0.57 (-0.45, 1.67)	0.93
	2,5-D	-3.29 (-6.48, -1.34)	1.39 (0.61, 2.68)	1.00
	3,5-D	-0.92 (-3.05, 1.51)	0.42 (-0.55, 1.30)	0.87
	4-CPA	-2.83 (-5.37, -0.95)	1.20 (0.46, 2.21)	1.00
	CNB	-3.14 (-5.95, -1.13)	1.32 (0.50, 2.42)	1.00
	2,5-DBB	-0.83 (-2.70, 0.73)	0.36 (-0.29, 1.13)	0.90
	2,5-DHB	-3.35 (-6.21, -1.25)	1.42 (0.54, 2.50)	1.00
	3,6-DCP	-2.74 (-5.28, -0.87)	1.17 (0.40, 2.16)	1.00
1 (Desiree)	–	-5.94 (-12.93, -2.67)	2.37 (1.10, 4.92)	0.95
2 (Desiree)	–	-3.34 (-10.13, -0.84)	1.39 (0.40, 3.98)	0.99
3 (Desiree)	–	-14.69 (-50.23, -2.89)	6.81 (1.96, 21.46)	0.99
2 (Russet Burbank)	–	-1.97 (-5.96, -0.08)	0.89 (0.12, 2.48)	0.99
3 (Russet Burbank)	–	-12.05 (-35.87, -2.80)	5.36 (1.66, 14.82)	0.99

Shown are the posterior mean intercept and slope (95% credible interval) and the posterior probability that the slope is positive.
For trial 1 (Russet Burbank) separate regressions are provided for each chemical treatment, whilst only a single regression was required for all other trials.

Fig. 13. Desiree tubers grown in soil amended with *Streptomyces scabiei* strain G#32 (pot trial 3) and treated with 2,5-dibromobenzoic acid (2,5-DBB) foliar sprays. Spray treatments included (top) no 2,5-DBB, and (bottom) 2,5-DBB three sequential sprays at 1.60 mM. Initial sprays were made 10 days after tuber initiation, and subsequent sprays at 10-day intervals.

Fig. 14. Tuber slice assay for thaxtomin A tolerance. Typical necrotic lesions on *cv.* Desiree tuber slices after 7 days exposure to 14 µM thaxtomin A (pot trial 3). The tubers on the left plate was from an unsprayed control plant (necrosis rating of 3.0); the tubers on the right plate was from a plant that received three spray treatments of 1.60 mM 2,5-dibromobenzoic acid (2,5-DBB) (necrosis rating of 1.5).

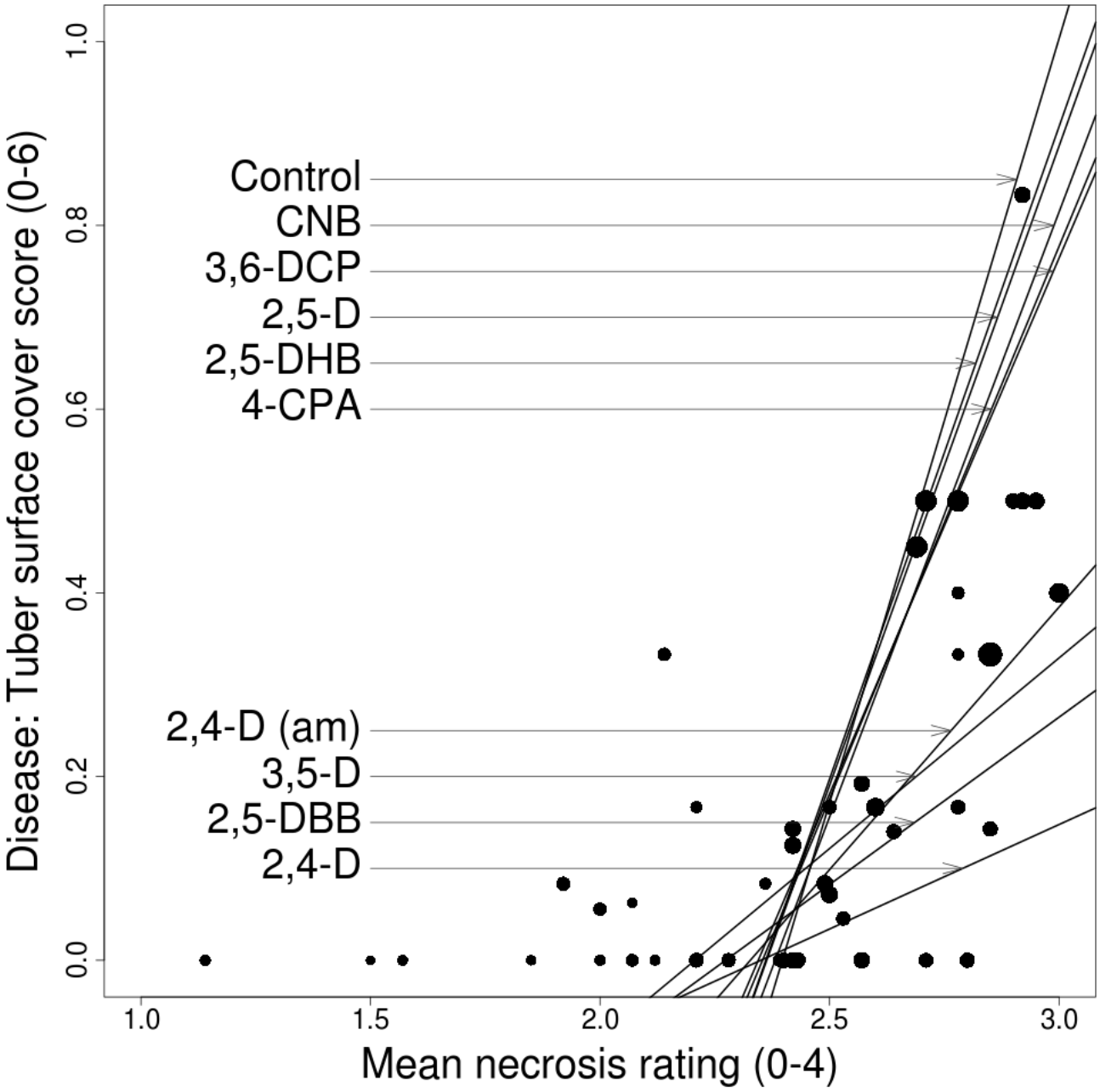


Fig. 15. Relationship between common scab disease (tuber surface cover score) and thaxtomin A-induced necrosis for Russet Burbank (trial 1)

The lines represent the mean posterior predicted fits for each chemical treatment. The observed data are shown as dots with the size of each proportional to the precision of the necrosis data.

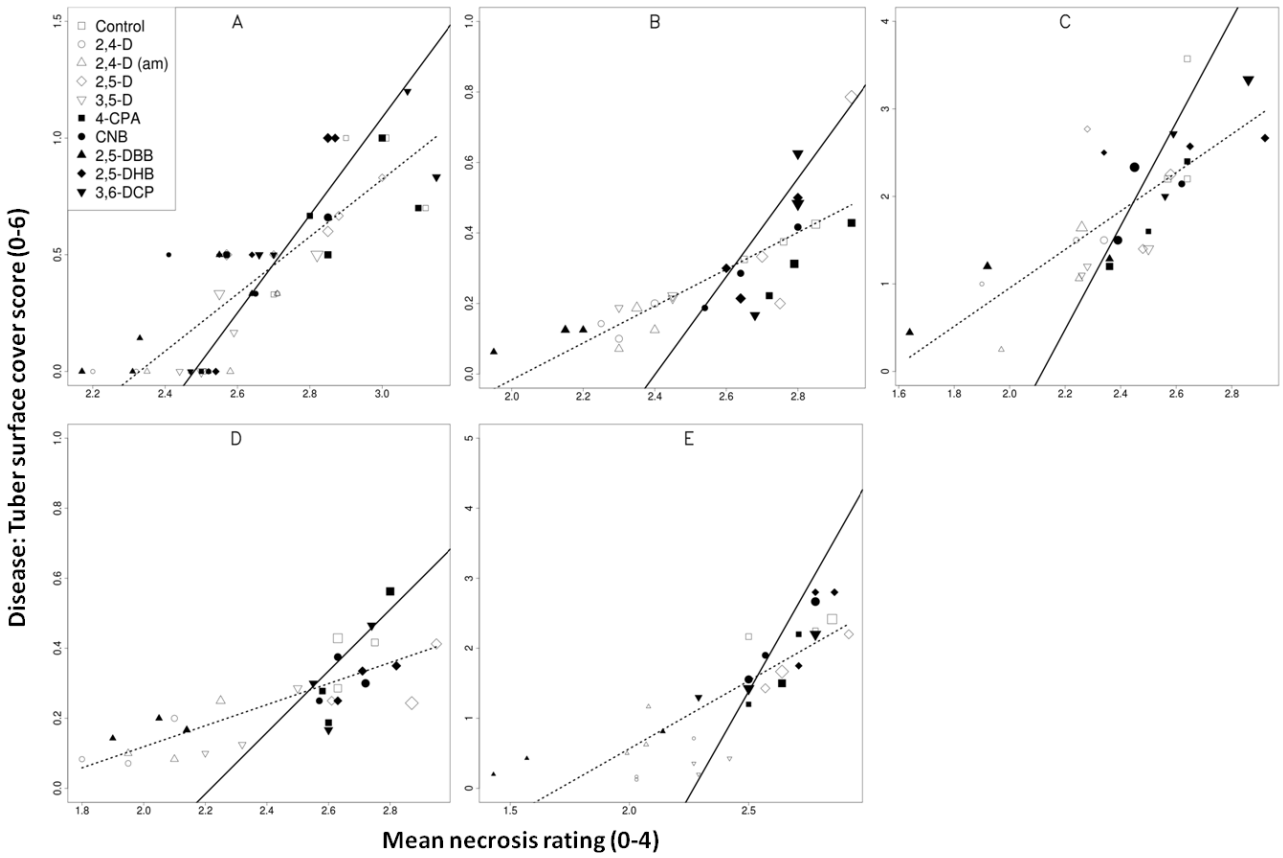


Fig. 16. Relationship between common scab disease (tuber surface cover score) and thaxtomin A-induced necrosis for respective trials A) Trial 1 Desiree, B) Trial 2 Desiree, C) Trial 3 Desiree, D) Trial 2 Russet Burbank, and E) Trial 3 Russet Burbank.

The solid line represents the mean posterior predicted fit. Also shown as a dashed line is the fitted least squares simple linear regression that does not take into account the errors in measurement. The observed data are shown as symbols with the size of each proportional to the precision of the necrosis data.

DISCUSSION

The suppression of common scab symptom development by various compounds has been previously demonstrated in a series of glasshouse and field trials (McIntosh et al., 1981, 1982, 1985, 1988; Tegg et al., 2008). Our three replicated pot trials generally support previous findings and confirm a range of compounds as able to significantly suppress disease. In this study the two dichlorophenoxyacetic acids (2,4-D and 3,5-D) consistently negated disease development, by an average of 73.7% and 61.2% respectively and we also confirm the disease negating impact of 2,4-D (am). These compounds however, when used at concentrations as in prior studies (McIntosh et al., 1981, 1982, 1985) and this study reported here have phytotoxic effects with yield reduction and tuber distortion (elongated shape) which has previously precluded the usage of these chemicals in commercial operations. Whilst the work presented in this study used concentrations of the dichloro phenoxyacetic acids that were expected to be phytotoxic, current work in our laboratory suggests that 2,4-D can be applied at significantly lower concentrations, reducing phytotoxic effects, but still providing substantial disease suppression, and may be a control option in the future with further work on rates and timing of application on-going.

As an alternative to the dichlorophenoxyacetic acids other less phytotoxic chemicals have been identified that suppress common scab without yield or the shape of the tuber being significantly affected (20). Such chemicals include the di-substituted benzoic and picolinic acids tested in this work. Confirmatory of previous work (Tegg et al., 2008) we showed that CNB did not significantly and consistently suppress common scab. Whilst this contradicts the work of McIntosh and co-workers, (McIntosh et al., 1988) who found substantial disease suppression with this compound in one trial (73% scab reduction), our results have included four separate pot trials and supporting thaxtomin A toxicity data (discussed below), suggest that CNB is not a compound which will suppress this disease. Likewise we found that the disease suppression obtained by McIntosh for 3,6-DCP in one trial (73% scab reduction) was not obtained in our three pot trials and is supported by thaxtomin A toxicity data (discussed below). On the contrary, results obtained with two other di-benzoic acids strongly support the work of McIntosh and co-workers (McIntosh et al., 1988). This includes 2,5-DHB which did not suppress scab and 2,5-DBB which produced significant disease suppressive effects.

Overall, our modelling of disease suppression and thaxtomin A sensitivity suggested that for all ten treatments there was a consistent relationship between these two variables on both potato varieties. This model may enable the development of a screening technique in which future collection of disease-based data could be minimized. These findings are supported by recent potato breeding work where thaxtomin A was successfully used as a selective screening agent to identify common scab sensitive progeny (Hiltunen et al., 2011).

The modelling presented in this paper conclusively links disease suppression and toxin sensitivity for the given cultivar being tested and provides an added tool for future studies where there is a need to assess chemical efficacy in controlling common scab disease. From a practical perspective, disease based trials are highly useful in determining applicability of chemical controls but many common scab disease trials are often compromised with low or no disease outcomes (Tarn et al. 2004; Hiltunen et al., 2011), so it is difficult to predict effectiveness of treatments. Evaluating thaxtomin sensitivities, using the tuber slice bioassay, is an easy and highly repeatable assay (Tegg et al., 2010) that serves to provide an accurate measure of potential to suppress disease. It must be stated that there are other chemical products that act via different modes of action to induce resistance or control this disease that don't specifically target thaxtomin A. Such chemicals include various fungicides that directly target the pathogen (Loria et al., 2006;

McIntosh et al., 1981), and in such cases evaluating thaxtomin sensitivity will not provide a measure of the potential to suppress disease.

Despite measurement errors, our modelling approach allowed us to extract the trends between disease (tuber surface cover score) and toxin sensitivity. A Bayesian framework for measurement error model can be more flexible than conventional approaches and more easily allow for the integration of various sources of measurement error (Richardson, 1998). The modelling approach used illustrates that the use of regression may be considerably misleading unless the existence of error in explanatory variables is taken into account. In regression models with a single predictor variable that is measured with independent random error the slope will be underestimated, an effect termed regression dilution (Frost & Thompson, 2000). This would be equivalent to reducing the significance of the regression slope and therefore both statistically and biologically significant effects would be missed. Although we estimated the error in the explanatory variables from other data we could have estimated it within the model if that had been necessary. The Bayesian context also permits the construction of more complex models with easily available software.

The above studies provide evidence that extent of disease suppression induced by the application of certain chemicals is intimately linked to the inherent susceptibility of the tuber tissue (containing that chemical, (Tegg et al., 2008) to thaxtomin A. Indeed the efficaciousness of the chemical treatment may potentially be estimated using appropriate Bayesian models with future collection of disease-based data minimized. This work provides increased knowledge of the disease and potential rapid assays for identifying suitable chemical controls that work through foliar application and systemic movement into the tuber tissue. Whilst thaxtomin A resistance is only one factor that may determine common scab resistance (Tegg et al., 2010) in this case of chemical induced resistance it appears a central component to better understanding the unique mechanisms by which these treatments control this disease.

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TECHNOLOGY TRANSFER

Note: Following concerns over possible premature experimentation with 2,4-D foliar sprays by the industry members of the TOC, industry articles and presentations were discouraged unless product names etc were not provided.

REFEREED JOURNAL ARTICLES

Three refereed journal articles relating to these studies have been published. We anticipate 3-4 further publications will be submitted in the next 12 months.

1. Tegg RS Corkery R & Wilson CR. (2012). Relationship between the application of foliar chemicals to reduce common scab disease of potato and correlation with thaxtomin A toxicity. *Plant Disease* 96: 97-103
2. Molesworth PP Gardiner MG Jones RC Smith JA Tegg RS & Wilson CR (2010). Synthesis and Phytotoxicity of Structural Analogues of Thaxtomin Natural Products. *Australian Journal of Chemistry* 68: 813-820.
3. Tegg RS Gill WM Thompson HK, Davies NW, Ross JJ & Wilson CR. (2008). Auxin-induced resistance to common scab disease of potato linked to inhibition of thaxtomin A toxicity. *Plant Disease* 92: 1321-1328

SCIENTIFIC CONFERENCE PAPERS

Four papers have been presented at scientific or industry conferences on this work.

1. Tegg RS, Thompson H, Wilson CR (2011). Optimisation of rates, timing and assessment of new compounds for control of common scab disease of potato through foliar applied treatments" Proceedings of the 18th Biennial Conference of the Australasian Plant Pathology Society, Darwin, Australia
2. Wilson CR (2011). "Common Scab – Science to cure". Key note address at 2011 AUSVEG Potato Summit, 17th April 2011, Brisbane, Australia
3. Wilson CR, Tegg RS, Thompson H (2011). Management of common scab disease through simple foliar applied treatments" Potato Workshop, 18th Biennial Conference of the Australasian Plant Pathology Society, Darwin, Australia
4. Thompson HK Tegg RS & Wilson CR (2010). 2,4-Dichlorophenoxyacetic acid induced resistance to common scab of potato. *Proceedings of the 6th Australasian Soil-borne Disease Symposium*. 9-11th August 2010, Twin Waters, Queensland.

INDUSTRY & MEDIA PUBLICATIONS

One article has been published in Potato Australia and another in the Northern Tasmanian Advocate newspaper.

1. Wilson CR & Tegg RS (2011). Spray away common scab disease of potato: research toward an alternative for control of this recalcitrant disease. Potatoes Australia Potato Australia February/March2011:20-21
2. Article published in Advocate newspaper on foliar sprays for common scab disease mitigation

INDUSTRY & PEER PRESENTATIONS

Various presentations have been made at TOC meetings. In addition, one oral paper at an industry meeting hosted by Simplot Australia and presentations at the APRP#2 mini-conference, the 2012 TIA showcase day, and the AUSVEG industry extension workshop.

1. An oral paper presented at the Simplot R&D day held at the Burnie campus of UTAS on 4th October 2012, attended by Simplot company personnel, other industry and university representatives

2. Oral paper presented at the APRP#2 mini conference, held in Melbourne on 6-7th October 2012 attended by APRP#2 researchers and associated industry members
3. An oral paper was presented at the TIA "showcase day" held at the Sandy Bay campus of UTAS on 29th November 2012 attended by a wide range of university and industry representatives
4. An oral paper was presented at the AUSVEG sponsored Industry extension workshop in Ulverstone, 14th March 2012.

ACTIVITIES UNDERTAKEN TO ENABLE ADOPTION OF PROJECT OUTCOMES

Discussions have and are continuing to be held with agrichemical companies to facilitate commercial adoption of the spray technologies by industry.

These include evaluation of the business case for registration and likely sales of 2,4-D and novel chemicals such as 2,5-DBB for common scab control. It is probable that this will require expansion to the North American market to make it economically feasible for local agrichemical companies to pursue.

PROJECT RECOMENDATIONS

The efficacy of 2,4-D and other non-fungicidal chemicals for control of common scab has been conclusively demonstrated. Early applications at low concentration of 2,4-D provide excellent disease suppression and do not induce detrimental yield low and tuber distortion observed at higher rate application. Alternate non-herbicidal materials with equivalent efficacy in disease control and no toxicity even at elevated rates were also identified. The best of these being 2,5-dibromobenzoic acid (2,5-DBB).

We recommend commercial development of foliar sprays and/or tuber treatments with 2,4-D or 2,5-DBB as a cost effective disease mitigation strategy easily integrated into current production practices. This requires engaging the agrichemical industry, and facilitating registration trials. As 2,4-D is a registered agrichemical for use in potato cropping, relatively little registration data required. Larger scale trials assessing disease mitigation efficacy and determine any impact of treatments on yield/quality under commercial growth conditions would be needed. 2,5-DBB, whilst a more attractive material in terms of safety with the crop, would require extensive toxicity and environmental testing in additional to efficacy trials.

We recommend further studies on tuber treatments with 2,4-D and alternate chemistries. Tuber treatment is another attractive option. It would reduce exposure of operators and the environment to chemical treatments, would reduce the amount of material needed, and would ensure all plants receive material at the earliest stage of crop growth. Further studies are required to determine if alternate materials provide a similar effect to 2,4-D, and to evaluate the optimal treatments for use on tubers. Rates may need to be higher than when applied to growing crop. We also do not know what effect tuber physiological development would have on uptake of material, and impact of material on seed quality.

We recommend further fundamental research on the mechanisms of thaxtomin suppression and disease mitigation. We also understand very little about how these materials provide protection against common scab. We have shown that they inhibit thaxtomin toxicity, but the mechanism of this inhibition is unknown. This information could be valuable in targeting new novel disease control strategies and including possible breeding targets. For example, if a thaxtomin receptor is recognised, mutations in this receptor could be selected, or better designed blockers of the receptor developed.

Horticulture Australia

PT09026 C – (30/10/2015)

Monitoring the bacterial wilt pathogen in irrigation water

Final Report

Dr Nigel Crump

Victorian Certified Seed Potato Authority

PROJECT SUMMARY

PT09026 C – Monitoring the bacterial wilt pathogen in irrigation water

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This is the final report for Sub Project C "Monitoring the bacterial wilt pathogen in irrigation water" that forms part of the "Soil health/disease mitigation" sub-Program Project No. PT09026.

This research focused on the validation of sampling methods and diagnostic testing of irrigation water for the presence of *R. solanacearum*. All of the methods and knowledge developed as a result of this project can be used in other areas and catchments. Critically the knowledge gained from this project means that greater emphasis can be placed on the importance of irrigation water for the spread and movement of *R. solanacearum* and the subsequent impact on potato production.

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, A&L Canada Laboratories and the Victorian Certified Seed Potato Authority, and matched funds from the Australian Government.

The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, The University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority have provided in-kind support.

DISCLAIMER

Any recommendations contained in this publication do not necessarily represent current HAL Limited policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.

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5.C MONITORING THE BACTERIAL WILT PATHOGEN (RALSTONIA SOLANACEARUM) IN IRRIGATION WATER TO IDENTIFY EFFECTIVE CATCHMENT MANAGEMENT STRATEGIES FOR CONTROL OF BACTERIAL WILT OF POTATOES

MEDIA SUMMARY

Bacterial wilt of potato is a bacterial disease caused by the pathogen *Ralstonia solanacearum*. This disease of potatoes is known to occur in parts of Victoria, NSW and Queensland. While the disease only occurs sporadically and seasonally, the losses attributed to this disease are significant with direct crop losses of up to 90% and there are further indirect losses associated with phyto-sanitary restrictions on interstate and export trade.

Overseas experience has shown the significance of *Ralstonia*-infested waterways being used for irrigation leading to the disease outbreaks of bacterial wilt in potato crops.

This research focused on the validation of sampling methods and diagnostic testing of irrigation water from the Bunyip River for the presence of *R. solanacearum*. All of the methods and knowledge developed as a result of this project can be used in other water area catchments. The knowledge gained from this project means that greater emphasis can be placed on the importance of irrigation water for the spread and movement of *R. solanacearum* and the subsequent impact on potato production.

Water was tested by culturing the bacterial wilt bacterium on selective growth media in the laboratory. This was supported by DNA based detection. Populations of the bacteria in the water vary according to the time of year, with detections most probable in the warmer months. In this study, *R. solanacearum* was only detected once in water collected from the Bunyip River in 2012/13. This result suggests that although there is potential for the river to act as a source of inoculum other factors may be contributing to the survival of the pathogen in this catchment. The potential report of a new host for *Ralstonia*, pending further testing, may show that the aquatic native plant Starfruit (*Damasonium minus*) may have a role in disease development of bacterial wilt of potato. Ground keeper potatoes, unharvested from the previous season, were shown to harbour the pathogen and carryover inoculum between seasons.

TECHNICAL SUMMARY

Bacterial wilt is a destructive disease that attacks potato it is also known as brown rot, southern wilt, sore eye or jammy eye. The bacterium that causes Bacterial wilt of potatoes is called *Ralstonia solanacearum* (previously known as *Pseudomonas solanacearum).* Bacterial wilt of potatoes is not a widespread disease within Australia, occurring only in isolated areas of Queensland, Victoria and South Australia.

Water was tested by culturing the bacterial wilt bacterium on selective growth media in the laboratory. This was supported by DNA based detection. Populations of the bacteria in the water vary according to the time

of year, with detections most probable in the warmer months. In this study, *R. solanacearum* was only detected once in water collected from the Bunyip River in 2012/13. This result suggests that although there is potential for the river to act as a source of inoculum other factors may be contributing to the survival of the pathogen in this catchment. The potential report of a new host for *Ralstonia*, pending further testing, may show that the aquatic weed Starfruit (*Damasonium minus*) may have a role in the epidemiology of bacterial wilt of potato. This putative alternative host may be multiplying the bacterium and increasing the inoculum load in the river water. As expected, ground keeper potatoes, unharvested from the previous season, were shown to harbour the pathogen and carryover inoculum between seasons.

Growers are urged to implement strict on farm biosecurity that includes the use of disease free certified seed potatoes to prevent the introduction of the disease. It is advisable, particularly for areas that have a history of the disease, to adopt long crop rotations and the proactive management of self-sown or groundkeeper tubers between seasons to prevent the build-up of the pathogen. In catchments that experience an outbreak of bacterial wilt it is possible to test irrigation water and identify the significance of water contamination in the spread of the disease.

It is essential that accurate diagnosis of infected plants occurs so that appropriate management decisions can be made, importantly to prevent further spread of the disease and restrict additional losses. In field diagnostic test kits are available that growers and advisors can use on testing suspect plants.

Future research is required to enhance the capacity for high throughput testing of water and other samples to enable cost effective and rapid testing of samples enabling growers to make informed decisions as to managing/preventing potential bacterial wilt outbreaks.

INTRODUCTION

Bacterial wilt is one of the most destructive diseases that attack potatoes. The disease of potatoes is also known as brown rot, southern wilt, sore eye or jammy eye. The bacterium that causes Bacterial wilt of potatoes is called *Ralstonia solanacearum* (earlier known as *Pseudomonas solanacearum).* Bacterial wilt of potatoes is not a widespread disease within Australia, occurring only in very isolated areas of Queensland, Victoria and South Australia.

Bacterial Wilt is also a bacterial disease of tomatoes, tobacco and some weeds related to the family of the Solanaceous group of plants. Potato crop losses of up to 90% have been reported on farm due to bacterial wilt, while additional losses in potato quality are observed in the processing of infected tubers. Further loss is attributed to restrictions on trade through phyto-sanitary measures preventing the spread of bacterial wilt.

Bacterial wilt of potato is a disease that can restrict trade, both export and interstate, through phyto-sanitary trade measures. In some States of Australia, the disease is still regulated by domestic quarantine.

Strains of *R. solanacearum* have been divided into five host specific races and five biovars based on biochemical properties. This study has focused on Race 2 Biovar 3 from potatoes.

BACTERIAL WILT SYMPTOMS

Bacterial wilt symptoms are browning and necrosis of the vascular tissue within the tuber Figure 5.C-1 and Figure 5.C-2. The pathogen can also cause severe wilting of the plant, similar to that of water stress, leading to total collapse and desiccation of the foliage (Figure 5.C-3). A milky exudate oozes from the cut surfaces of

infected tubers, stems or from the eyes of whole tubers (Figure 5.C-4). Symptomless or latent symptoms can occur at low temperatures.

Figure 5.C-1 – Symptoms of Bacterial wilt caused by *Ralstonia solanacearum* in the vascular system of cut potato tubers

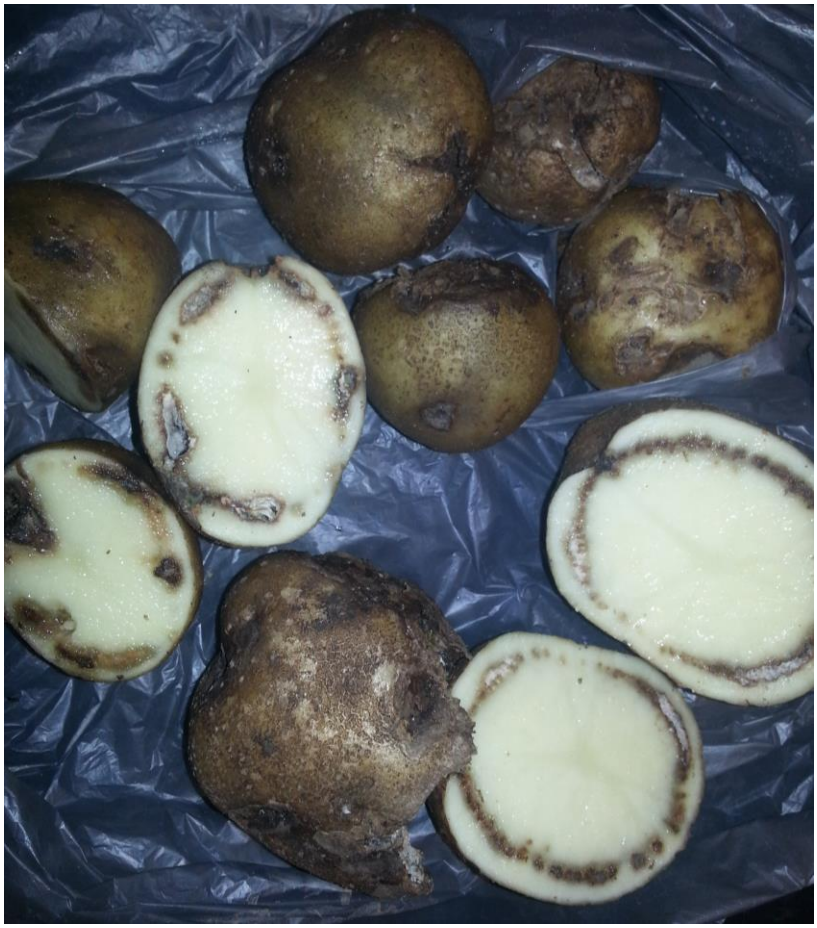

Figure 5.C-2 – Symptoms of Bacterial wilt caused by Ralstonia solanacearum in the vascular system of cut potato tubers

Figure 5.C-3 – Potato plants, one showing wilting symptoms due to infection by Ralstonia solanacearum. Image: The International Potato Center, Lima, Peru, Source http://www.aphis.usda.gov/

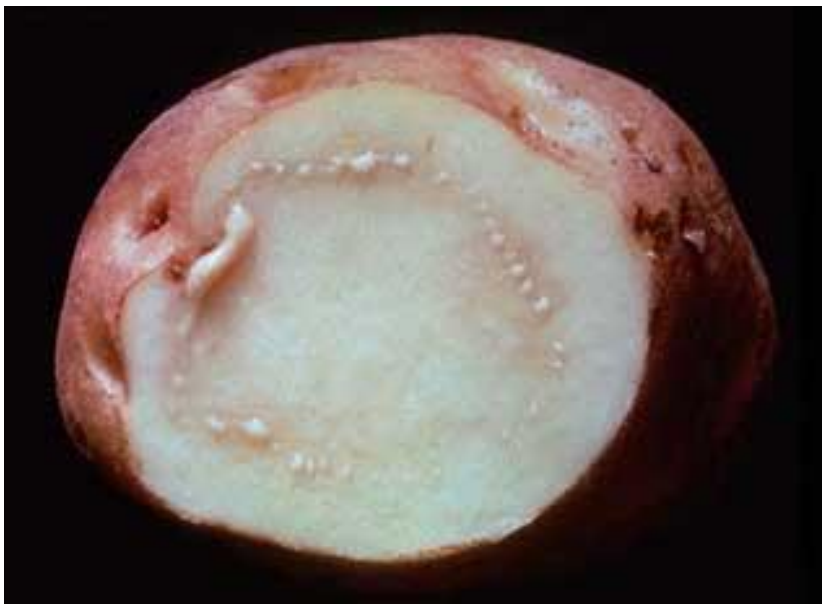

Figure 5.C-4 – Cross section showing symptoms of bacterial ooze caused by *Ralstonia solanacearum*. Image: The International Potato Center, Lima, Peru Source http://www.aphis.usda.gov/

METHODS OF SPREAD AND SURVIVAL

The most effective means of spread of bacterial wilt is through the movement and planting of infected seed potatoes; particularly via symptomless latently infected seed potato tubers (Hayward, 1991). All certified seed Schemes in Australia adopt a zero tolerance for bacterial wilt, and therefore, the use of certified seed provides an effective component of any management strategy. The movement of latently infected seed potatoes has resulted in outbreaks of the disease where it has not previously occurred (Elphinstone, 1996). In addition, to potatoes other crops, such as ornamental nursery Geranium stocks, have been implicated with the potential spread of the bacterial wilt (Williamson et al., 2002). Farm machinery carrying infested soil can provide a means of spreading bacterial wilt emphasising the importance of adopting good on farm hygiene practices.

The survival of *R. solanacearum* in the environment is poorly understood. *R. solanacearum* tends to persist in the wet but well drained soil, in deep soil layers (>75 cm) protected from antagonism by other micro-organism or in the presence of crops, weed hosts or ground keepers (unharvested potatoes from the previous crop) (Kabeil et al., 2008). Previous research has shown that the bacteria can survive in the soil for up to 2 years (Faggian and Marshall, 2004).

The very extensive host range of *R. solanacearum* includes several hundred species representing 44 families of plants (Nesmith and Jenkins Jr., 1985). The most susceptible crops being potato, tomato, eggplant, pepper, banana and groundnut.

Biovar 2 Race 3 of *R. solanacearum* has a limited host range and is found mainly on potato and sometimes on tomato and a few weed hosts; many strains are adapted to growth and pathogenesis at low temperatures (Hayward, 1991) and several studies suggest a more limited capacity for survival in fallow soil than for biovar 3 (Hayward, 1991).

RALSTONIA AND SURFACE WATER

Ralstonia solanacearum can survive in water and this has important implications for potato production areas. Temperature, pH, salt level, particulate matter and the presence of competing antagonistic and parasitic organisms are potential factors influencing the pathogens ability to survive in aquatic habitats (van Elsas et al., 2001). Temperature strongly influences the survival of *R. solanacearum* in water which may be indirectly related to the changes in water biota. *R. solanacearum* survival was shown to be enhanced in sterile water indicating the bacterium does not compete well with other microorganisms (van Elsas et al., 2001).

In the UK, there have been 5 outbreaks of Bacterial wilt, all of which have been associated with the use of infested waterways as an irrigation source (Elphinstone et al., 1998). As a result, widespread surveys of UK water ways have been conducted and have identified infested waterways which appear to be related to upstream sewage outfalls. UK waterways infested with *R. solanacearum* have the alternative host *Solanum dulcamara* (bittersweet or woody nightshade) which can rapidly multiply the bacteria increasing the pathogen levels in the water. The host *Solanum dulcamara* is not known to occur in Australia and, therefore, is not associated with the incidence of bacterial wilt of potatoes in Australia.

PROJECT RESEARCH AIMS

This research aimed to investigate the level of the bacterial wilt bacterium present in the Bunyip River and its tributaries as a case study. The information gained from this project will be captured into best management strategies for this disease that can be employed wherever bacterial wilt occurs. Ultimately, the project aimed to validate the UK diagnostic tests in Australia, determine if the bacterial wilt pathogen is prevalent in irrigation water sources, identify periods when irrigation water sources are highly infested, and identify potential sources of contamination to irrigation water sources that contribute to the incidence of bacterial wilt of potatoes.

MATERIALS AND METHODS

METHODS USED FOR WATER SAMPLING AND TESTING

As reported in EU COUNCIL DIRECTIVE 98/57/EC (see below for summary).

PRINCIPLE

The validated detection scheme, described in this section, is applicable for pathogen detection in samples of surface water and can also be applied for testing samples of potato processing or sewage effluent.

It is important to note that the expected sensitivity of detection will vary with the substrate. Sensitivity of the isolation test is affected by populations of competing saprophytic bacteria which are generally much higher in potato processing and sewage effluents than in surface water. Whereas the scheme is expected to detect as few as 10^3 cells L^{-1} in surface water, the sensitivity of detection in potato processing or sewage effluents is likely to be significantly lower due to the influence of other microorganisms.

For this reason, it is recommended to test effluents after purification treatments (e.g. sedimentation or filtration) during which saprophytic bacterial populations are reduced. The limitations in sensitivity of the test scheme should be considered when assessing the reliability of any negative results obtained. Whereas this scheme has been successfully used in survey work to determine presence or absence of the pathogen in surface water, its limitations should be realised when used in similar surveys of potato processing or sewage effluents.

SAMPLE PREPARATION

Detection of *R. solanacearum* in surface water is most reliable during late spring, summer and autumn seasons when water temperatures exceed 15°C.

Repeated sampling at different times at designated sampling points will increase the reliability of detection by reducing the effects of climatic variation.

Sampling points should take into account the effects of heavy rainfall and the geography of the watercourse to avoid extensive dilution effects that may obscure presence of the pathogen.

Surface water samples should be collected in the vicinity of host plants if these hosts are present.

At selected sampling points, collect water samples by filling disposable sterile tubes or bottles at a depth if possible below 30 cm and within 2 m from the bank. Sample sizes up to 500 mL per sampling point are

recommended. If smaller samples are preferred, it is advisable to take samples on at least three occasions per sampling point, each sample consisting of two replicated sub-samples of at least 30 mL.

Transport samples in cool dark conditions (4–10°C) and test within 24 hours.

METHODOLOGY AS APPLIED TO BUNYIP RIVER

In the development of this project, growers from the Koo-Wee-Rup district were asked at a grower meeting to anonymously indicate on a regional map the occurrence of bacterial wilt in their crops. It was interesting to find growers that irrigated from the Main Drain (Bunyip River) had reported bacterial wilt. While those growers who used bore water for irrigation reported no bacterial wilt in their potato crops. Growers in the district with farms with a history of bacterial wilt have instigated many measures to reduce the occurrence of the disease including hygiene programs, use of clean seed, no use of retained “farm saved” seed, and extended rotations. Yet despite the adoption of these appropriate measures, bacterial wilt remains as a significant disease impacting on yield and quality. It was hypothesised that the surface water of the Bunyip River may act as an inoculum reservoir for the introduction and spread of Bacterial wilt in potato crops. Furthermore, runoff and sub-surface drainage from potato fields in the district is returned to the Main drain (Bunyip River), in doing so, growers irrigating from downstream may be exposed to bacterial wilt contamination. Therefore, this project was established to determine if the water from the Bunyip River was acting as an inoculum source for bacterial wilt.

As per the EU protocol, a total of 4 sampling points were established at every 3 kilometers of the Bunyip River. The first site was located upstream of any potato production and the last below all potato producing fields (Figure 5.C-5).

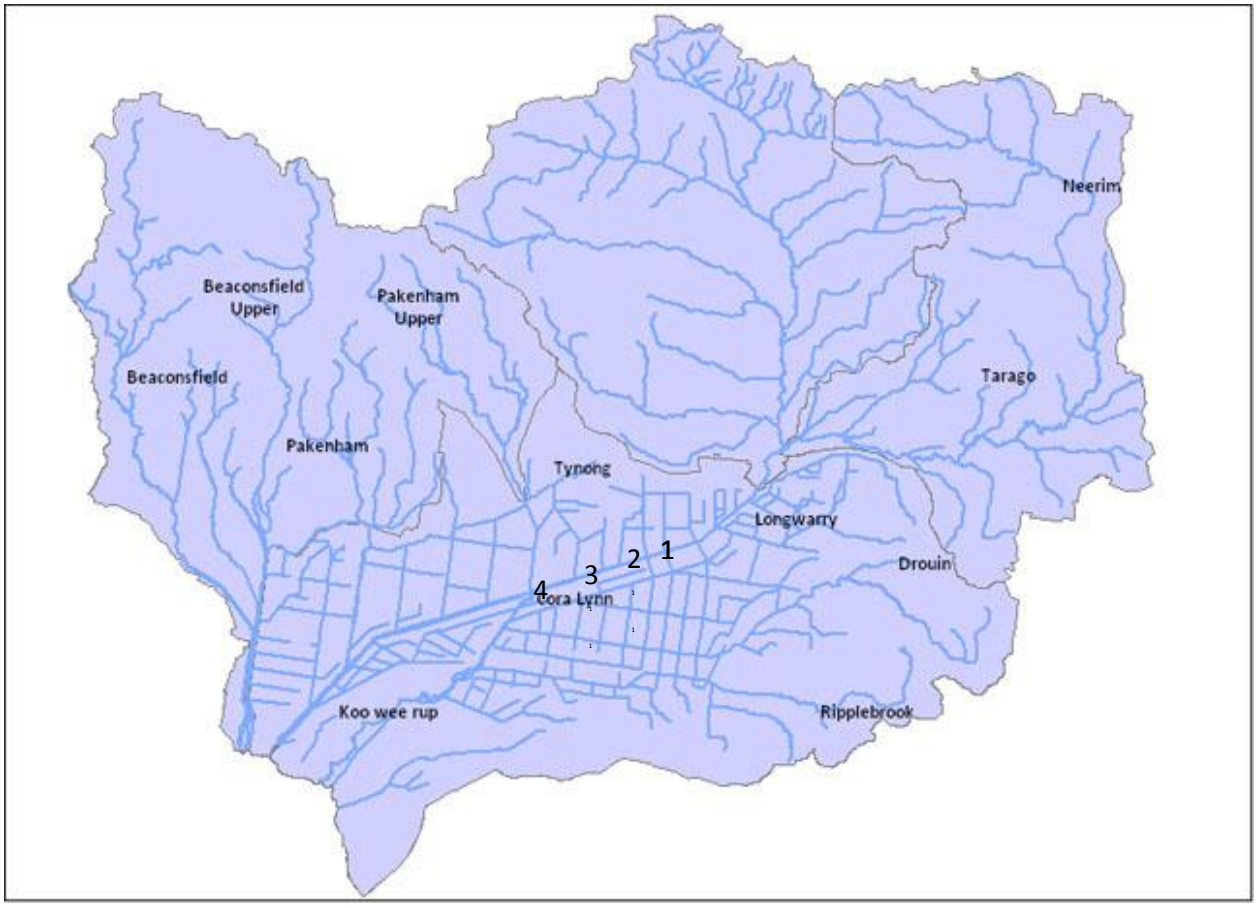

Figure 5.C-5 – Map of the catchment of the Bunyip River showing sampling points

Water samples for analysis were collected into 750 mL brown plastic bottles. This was an inexpensive option which provided sample security from UV light and ease of transport. At the time of collection water temperature and pH was recorded (Figure 5.C-6).

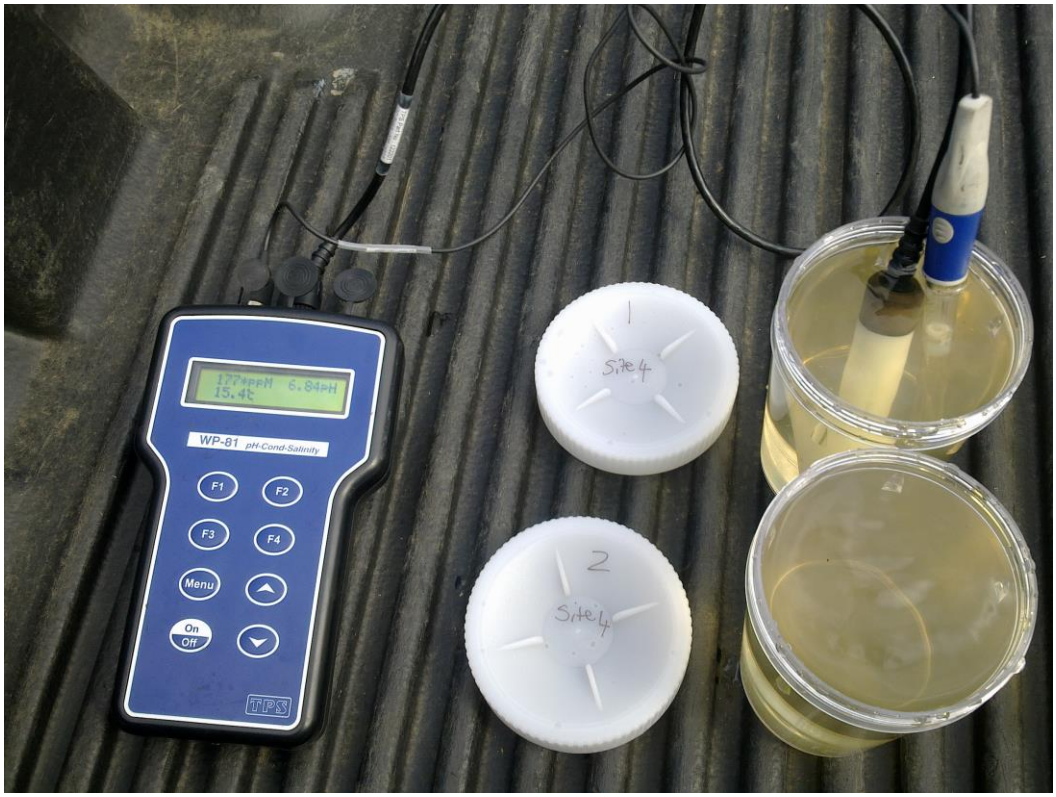

Figure 5.C-6 – All water samples have pH and temperature measurements taken at the time of collection

LABORATORY TESTING

All laboratory testing was done using third party laboratories including Crop Health Services Victoria and DPI NSW. Various modifications were tried and the final methodology is described below.

CONFIRMATION OF TARGET (CAUSAL) AGENT

Samples of tubers with symptoms of bacterial wilt were collected and the causal pathogen isolated using semi selective media SMSA (See below). To determine the species of *Ralstonia* isolated, PCR was used to amplify the 16S region of selected bacterial colonies which was sequenced and compared to published sequences.

SMSA Selective medium as modified by Elphinstone (1996)

Basal medium
Casamino acids (Difco) 1 g
Bacto peptone (Difco) 10 g
Glycerol 5 ml
Agar (Difco) 15 g
Distilled water 1 litre
Prepare 1/2 litre volumes of medium in one litre flasks.
Dissolve the ingredients and check pH. Adjust pH, if necessary, to 6.5 before autoclaving. *Ralstonia solanacearum* will not grow well on the medium at pH 7.0
Sterilise by autoclaving at 121 °C for 15 minutes.

Cool to 50 °C.
Add the following ingredients (all from Sigma) to obtain the specified final concentrations:

- Crystal violet 5 mg per litre
- Polymixin B sulphate 100 mg per litre (approximately 600 000 units) Sigma P-1004
- Bacitracin 25 mg per litre (approximately 1 250 units) Sigma B-0125
- Chloramphenicol 5 mg per litre Sigma C-3175
- Penicillin-G 0.5 mg per litre (approximately 825 units) Sigma P-3032
- Tetrazolium salts 50 mg per litre

Dissolve the ingredients in 70 % ethanol to the given concentrations for the volume of medium prepared. Some ingredients, *viz.* polymixin B and chloramphenicol require slight warming and shaking.

DIAGNOSTIC TESTING OF WATER FOR RALSTONIA

Water testing involved pelleting (100 mL) of the total water sample received at 4220 x RCF for 30 minutes. The supernatant is discarded and the pellet is resuspended in 1.5mL of pellet buffer. DNA from an aliquot of the bacterial pellet is then extracted.

A separate aliquot of the bacterial pellet suspension is spread plated as a dilution series onto SMSA media. Sterile water is added to a culture growing a range of bacteria and mixed. Water and bacteria are removed and the DNA is extracted from the bacteria by heating to 95° for 10 minutes. The mixed bacterial suspension is also tested using an Agdia ELISA kit according to the Agdia instructions.

PCR was carried out on DNA extracts from the cores taken from tubers and the bacterial suspensions from the 'selective' media plates. PCR methods are based on Pastrik et al. (2002) using primers RS-1-F and RS-1-R to detect *Ralstonia* Division II (Biovars 1 and 2). Extracts are also tested with the housekeeping primers to ensure successful extraction using NS-5-F and NS-6-R primers (Pastrik et al., 2002), and bacterial 16S primers (Marchesi et al., 1998) for the extracts from the mixed bacterial cultures.

ALTERNATIVE HOSTS

In autumn 2014, a project was initiated to identify possible aquatic and riparian host plants of *R. solanacearum* (Table 5.C-1). A field with known infestation of bacterial wilt in the 2013/14 potato season was selected as a test site. Following the crop of potatoes, the field was planted with rye corn (*Secale cereal*) as a green manure crop. Weed species were randomly collected across the field. For each species collected a total of 5 individual plants were subsequently tested using the commercial available ELISA test kit (Pocket Diagnostic™) in accordance with the directions.

In addition to the field site, a common aquatic weed Starfruit (*Damasonium minus)* was collected from a side channel/drain near the Site 4 alongside the Bunyip River. This is a significant result, in that the weed may be acting as an inoculum reservoir carrying over the pathogen between seasons. Starfruit appears to only be in channels and side drains that have slow moving water flow and not the Main drain (Bunyip River). This could explain why there were few positive detections of *R. solanacearum* in the water samples collected from the Bunyip River. The side drains do flow into the Bunyip river, however it would be assumed that inoculum is diluted in the main river. It is suggested from this study, there is a need to associate the hydrology of the catchment with the distribution of *Damasonium minus* to appreciate the impact on potato production. *Damasonium minus* is widely distributed in Australia and the relevance of the weed in the epidemiology of Bacterial wilt of potato in other catchments will need to be examined in future studies.

USE OF COMMERCIAL LATERAL FLOW KIT (POCKET DIAGNOSTIC™)

The commercial kit Pocket Diagnostic™ (Figure 5.C-7) was used to confirm field specimens of both plant and tubers following the instructions (outlined below).

1. Select sample (tuber or stem)

2. Cut or tear sample into small pieces and put into bottle.

3. Shake firmly for 30-40 seconds (30-60 seconds for more resistant samples).

4. Draw liquid into pipette.

5. Add 2 drops into sample well of test strip.

6. Read result after 3-5 minutes. One line is negative result, two lines show positive result.

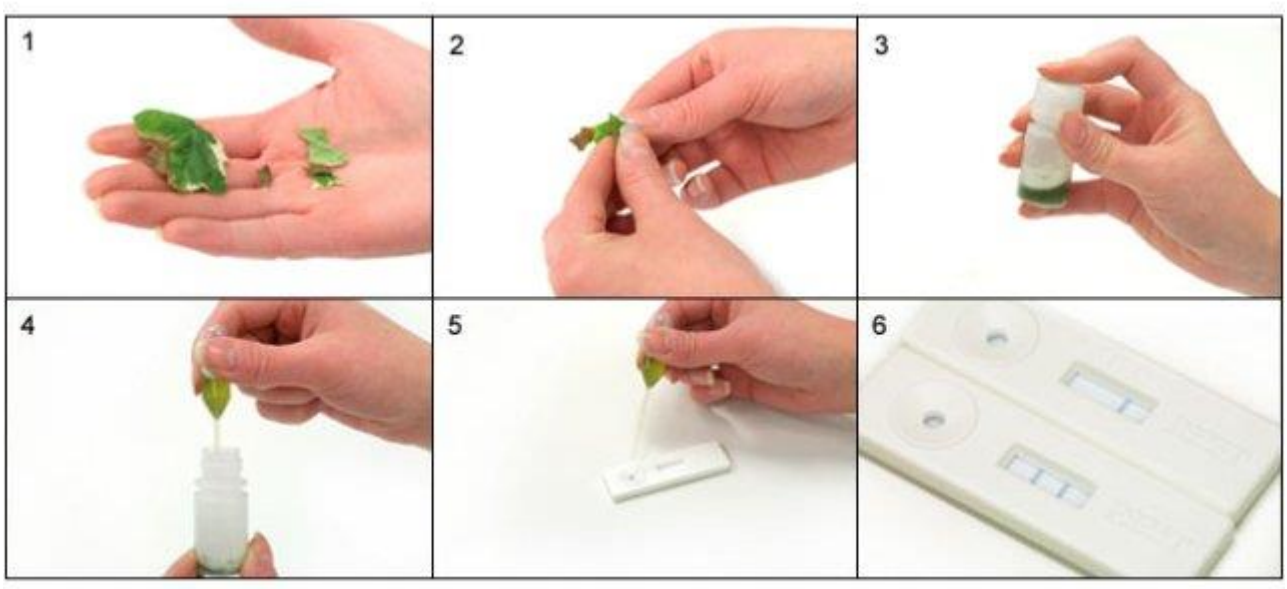

Figure 5.C-7 – The steps involved in the commercial diagnostic for *Ralstonia solanacearum* from http://www.pocketdiagnostic.com/using_the_test

RESULTS AND DISCUSSION

CONFIRMATION OF TARGET (CAUSAL) AGENT

Ralstonia solanacearum was isolated from the putatively infected tuber samples. Subsequent sequencing of the 16S region showed 100% similarity to *R. solanacearum* accession JQ323520 in GenBank which is Biovar 2 Race 3. This confirms the pathogen and the strain of *Ralstonia solanacearum* previously known to occur in the Bunyip River region. Therefore, the current diagnostics tests should be appropriate for the detection of *R. solanacearum* in water.

RESULTS FROM WATER TESTING

In the first two years (2010 and 2011) of this project, the Bunyip River experienced intensive rainfall and intensive flooding (Figure 5.C-8). These events may have adversely affected the detection of *Ralstonia* in the water samples collected from the Bunyip River. It is hypothesized that the large rainfall events may have diluted the concentration *of R. solanacearum* below detectable levels or inversely may have increased the population of other microorganisms thereby restricting the sensitivity of the diagnostic test.

It was an important observation that fields, with no previous history of bacterial wilt, that were affected by flooding of the Bunyip River were subsequently reported with infestation of bacterial wilt in potato crops.

Figure 5.C-8 – Extensive flooding in 2010, these events were repeated in 2011

Water temperature measurements over this period showed water temperatures exceeding 15 degrees which is ideal for the multiplication of *Ralstonia solanacearum* (Figure 5.C-9). Despite these conditions no *R. solanacearum* was detected in any water sample.

In April 2012, *Ralstonia solanacearum w*as first detected at the sample site 2 on the Bunyip River. This was the first detection since sampling began and indicates that the irrigation water taken from the river is a possible source of the bacterium in potato crops.

In season 2012/13 crops in the Koo-Wee-Rup swamp region were affected with bacterial wilt. The incidence of bacterial wilt appears to be associated with the areas affected by extensive flooding in the region over the past two seasons (Crump pers. observation).

In 2013/14 sampling of the Bunyip River showed no detections of *R. solanacearum*. Included in the survey were sub-surface runoff collection points which were draining from fields growing potatoes. No *R. solanacearum* was detected in any of the subsurface water samples.

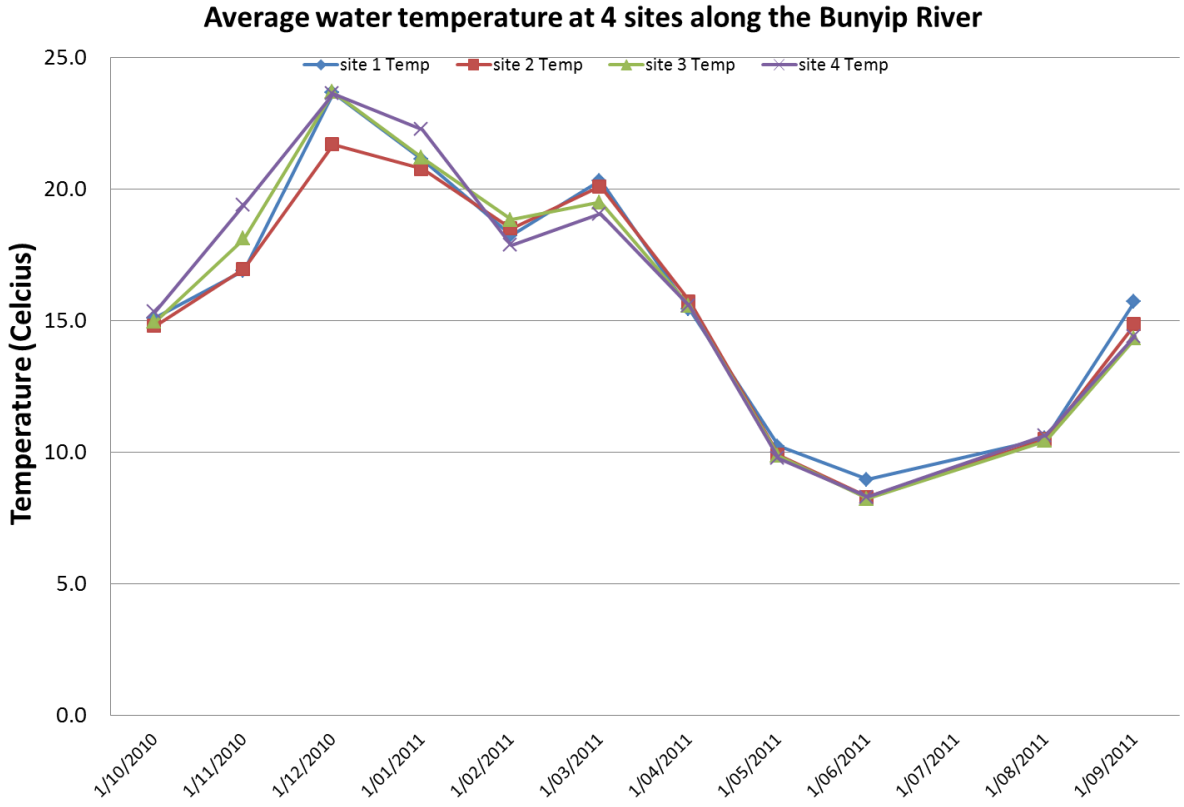

Figure 5.C-9 – Water temperatures at 4 sites along the Bunyip River 2010-11

ALTERNATIVE HOSTS

The weed plants collected from the known infected field were common to the production area (Table 5.C-1). Four of the five ground keeper potato tubers were shown to be infected with *R. solanacearum* using the commercial field testing kit, with only one of the tubers showing symptoms of disease (Figure 5.C-10). The samples of the aquatic weed *Damasonium minus* collected from nearby site 4 had 3 plants tested positive for *R. solanacearum* using the commercial field testing kit. Additional tests conducted on the *D. minus* samples indicated that both the basal stem and root of the *D. minus* samples tested positive using the commercial field testing kit. Stem and root samples of the *D. minus* were sent off for further analysis which confirmed the presence of *R. solanacearum.* There have been no previous reports of *D. minus* acting as a host for *R. solanacearum. D. minus* is an Australian native described as an *emergent aquatic, annual or*

perennial herb with 1.5-10 cm long, narrowly ovate to ovate, leaves on slender leaf stalks, 0.5-1 cm long with semi-transparent, membranous wings at their bases Figure 5.C-1.

Table 5.C-1 Plant species collected for assessment as potential host for *Ralstonia solanacearum*

Common Name	Botanical Name	Number of positives
Star fruit	*Damasonium minus*	3/5
Capeweed	*Arctotheca calendula*	0/5
Wire weed	*Polygonum aviculare*	0/5
Chickweed	*Stellaria pallida*	0/5
Rye Corn	*Secale cereal*	0/5
Groundkeeper potato	*Solanum tuberosum*	4/5
Wild radish	*Raphanus raphanistrum*	0/5
Dock	*Rumex* spp.	0/5

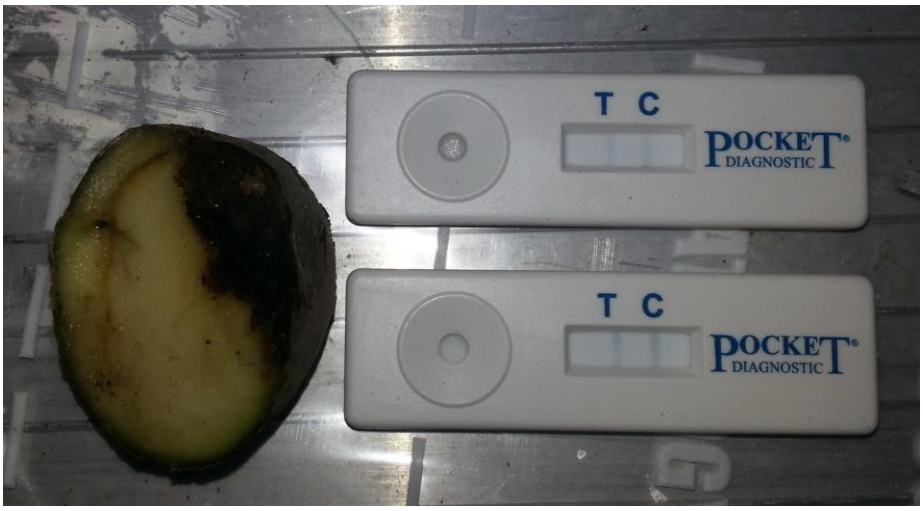

Figure 5.C-10 – Groundkeeper potato tuber collected from field with positive test for *Ralstonia solanacearum*

Figure 5.C-11 – Star fruit *Damasonium minus* source http://vro.depi.vic.gov.au/dpi/vro/vrosite.nsf/pages/sip_star_fruit

It is essential that accurate diagnosis of infected plants so that appropriate management decisions can be made, importantly to prevent further spread of the disease and restrict additional losses. In field diagnostic test kits are available that growers and advisors can use on testing suspect plants.

TECHNOLOGY TRANSFER

The original project proposal suggested that several grower events would be conducted. The first four years of the project offered little that could be solely featured in a grower event. Despite this the concept of the project, and outcomes were presented at industry events including the ViCSPA AGM's, local grower meetings and industry discussions. The following outlines the technology transfer that was delivered by the project.

Potatoes Australia Article

December, 2009 - The facts on bacterial wilt of potato. Nigel Crump and Rene de Jong.

McCain Agricultural Conference: 25th of August 2011 presentation by Nigel Crump.

APRP2 Symposia

APRP2 Science Symposia 1, Rydges Carlton, Melbourne, October 2011

APRP2 Science Symposium 2, Mantra Hotel, Tullamarine, 18 & 19 September 2013

Development of technical notes

- Bacterial wilt fact sheet (as attachment).
- Catchment management plan for bacterial wilt (as attachment).

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

1. The diagnostic protocol used in this study is recognized by the EU COUNCIL DIRECTIVE 98/57/EC, a limitation of the protocol used was the small number of samples that could be processed at any one time. This was largely due to the labor intensive and need for highly skilled professionals to conduct and interpret the diagnostics. New technology has been developed that could improve the sensitivity and high throughput capacity for testing samples for *Ralstonia solanacearum*. Future work should be aimed at validating real-time DNA based technologies for application in testing water and plant samples for bacterial wilt. Overseas studies have reported on the potential use of real-time DNA based technologies for *Ralstonia* detection and quantification in environmental samples (Stead et al., 2003, Caruso et al., 2003).
2. The survival of *R. solanacearum* in the environment is poorly understood. In developing high throughput diagnostic tests, in addition to water testing, the soil inoculum should be assessed to determine fields that may be of high risk to bacterial wilt and to determine when levels of the bacterium are below economic thresholds so that growers can avoid losses associated with this disease. This future research could be aligned with the platform technology developed as part of PT09023. Overseas studies such as (Pradhanang et al., 2000) are working on diagnostic testing of soil.
3. While the Bunyip River catchment was used as a model catchment in this study, the importance of surface water in the spread of bacterial wilt in other catchments may be explored in future studies.
4. This project found the association of *Damasonium minus* with *R. solanacearum*. The importance of *Damasonium minus* as a potential host for *R. solanacearum* requires more detailed studies.
5. The industry needs to increase its awareness of bacterial wilt as a significant disease that can cause substantial crop losses and trade restrictions. The technology transfer outputs of this project contribute to educating the industry of this disease. Importantly, this disease highlights the need for growers to adopt sound on-farm hygiene protocols to prevent the introduction of bacterial wilt and other pests and diseases to their farm.

ACKNOWLEDGEMENTS

ViCSPA is a not-for-profit, industry-based association. ViCSPA, an independent provider of Seed Certification and other services to the Australian Potato Industry.

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Attachment 1 - Bacterial Wilt Factsheet

Potato facts

ViCSPA

vicspa@vicspa.org.au www.vicspa.org.au

Bacterial Wilt of Potato

Bacterial wilt is one of the most destructive diseases that attack potatoes. The disease of potatoes is also known as brown rot, southern wilt, sore eye or jammy eye.

Although not widespread in Australia, this disease is controlled through awareness, prompt action in seeking an accurate diagnosis and through responsive cooperative attitudes.

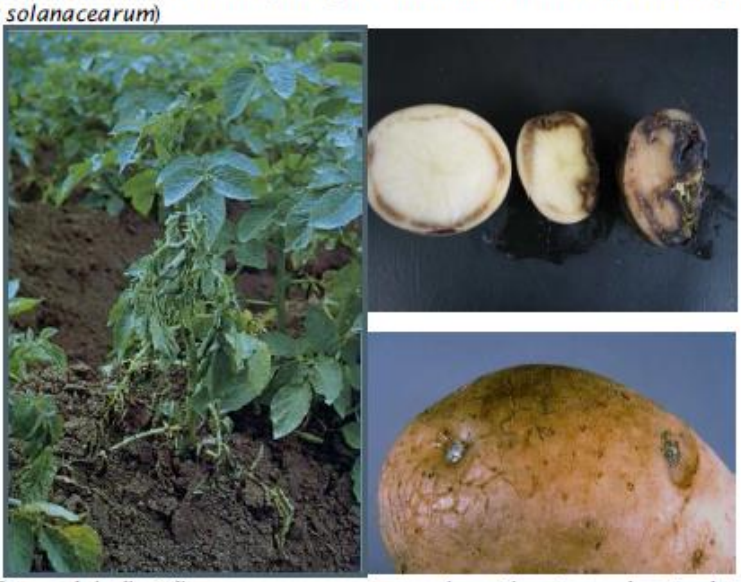
Certified seed potatoes in Australia have a zero tolerance to bacterial wilt and the use of certified seed potatoes provide the main strategy in preventing the spread of this disease.

What is Bacterial wilt of potatoes?

Bacterial Wilt is a bacterial disease of potatoes, tomatoes, tobacco and some weeds related to the family of the Solanaceous group of plants. The bacterium that causes Bacterial wilt of potatoes is called *Ralstonia solanacearum*. In previous cases of infection, crop losses of up to 90% have been reported on farm due to bacterial wilt, while additional losses in potato quality are observed in the processing of infected tubers. Bacterial wilt of potatoes is not a widespread disease within Australia, occurring only in very isolated areas of Queensland, Victoria and South Australia.

Casual agent

Bacterial wilt is caused by the bacterial pathogen *Ralstonia solanacearum* (formerly called *Psuedomonas solanacearum*)



Symptoms of Bacterial wilt. Wilt symptoms on a potato plant. Plant Protection Service, Wageningen (NL): internal rotting in the vascular ring of tubers ViCPSA, Australia; "sore eye – bacterial ooze from eye of infected tuber Central Science Laboratory, York

How is the Bacterial wilt spread?

ViCSPA –Private Bag 1 Healesville 3777 Page 1

From world wide experience, the most effective means of the spread of Bacterial Wilt is through the:

(i) planting of infected seed tubers
(ii) contaminated irrigation sources
(iii) the movement of infested soil on machinery

Bacterial wilt can survive between seasons on volunteer potato plants and weed hosts.

The spread of Bacterial Wilt can be multiplied by seed cutting if a potato being cut is infected – the spread in this case may be multiplied greater than tenfold depending on the severity of the infected tubers.

How is Bacterial wilt managed?

1. ***Prompt diagnosis***. It is extremely important that a prompt and accurate diagnosis is sought on any plant or tuber suspected to have bacterial wilt. If diagnosis is confirmed then control strategies can be put in place to prevent a carryover of the disease.

2. ***Prevention***. If you do not already have bacterial wilt - then keep it that way! On-farm hygiene ensuring that equipment, potato boxes, vehicles etc. do not bring infested soil or tubers onto your farm.
3. ***Use certified seed that is free of disease***. The National Seed Certification Rules have a nil tolerance for bacterial wilt. The use of non-certified seed, particularly from regions that are known to have bacterial wilt, is high risk for spreading bacterial wilt. Infected seed tubers are the main means of spreading of *R. solanacearum*. In cool conditions, infected but symptomless plants may harbor the bacterium and transmit it to progeny tubers as latent infection, leading to severe disease outbreaks when grown at warmer locations.

   The ViCSPA certified seed scheme, based on pathogen tested stocks, has been very successful in controlling this disease. It is therefore recommended that certified seed be planted in ground that has no history of bacterial wilt.

4. ***Hygiene***. Strict hygiene measures should be maintained at all times, not just for bacterial wilt but for a range of potato diseases. Disinfecting equipment bins, machinery etc between farms should be disinfected. Disinfecting knives on seed cutters or avoid seed cutting all together can also reduce spread of the disease.

5. ***Irrigation source***. Identify whether local water sources are a source of infection and manage appropriately.

6. ***Fumigation.*** There is no effective chemical control for bacterial wilt. Therefore, its incidence can only be reduced if various control components are combined. This involves mainly the planting of healthy seed in clean soil and the planting of tolerant varieties, in rotation with non-susceptible crops, as well as the application of various sanitation and cultivation practices. Such an integrated disease management approach can lead to significant reduction, or even eradication of bacterial wilt.

   Soil fumigation can be done but is not widely used due to high costs.

7. ***Rotations.*** Good control of volunteer potatoes and rotation with non host crops such as grasses (maize, cereals, pasture) can reduce to occurrence of the disease. Avoid continual cropping of potatoes if disease is present. Practice long rotations (greater than 5 years clear of potatoes). There is evidence to such that root knot nematode can enhance the occurrence of bacterial wilt, management options to reduce RKN populations can reduce the severity of bacterial wilt.

   It is strongly recommended that any infected paddocks should not be planted with potatoes or other host crops for at least five years. Sowing paddocks down to a pasture or cereal cropping program and effectively managing any self sown potatoes will significantly aid in the management of the disease.

For further information Contact Dr Nigel Crump 03 5962 0000 email nigelcrump@vicspa.org.au

Version 1.0 2010

Attachment 2 - Bacterial Wilt Catchment Management Plan

Waging War on Wilt

A catchment management plan for bacterial wilt

What is bacterial wilt?

- Bacterial wilt also known as brown rot can cause significant yield loss of potatoes.
- Bacterial wilt is primarily spread by the planting of infected seed potatoes, but can also spread in soil and in irrigation water.

ACCURATE DIAGNOSIS OF SUSPECTED PLANTS IS ESSENTIAL
DEAL WITH THE FACTS TO MAKE INFORMED DECISIONS
Rapid in field test kits are available for preliminary diagnosis

Hygiene – Farm Biosecurity

- **PREVENTION IS THE BEST CURE**
- Hygiene and sanitation measures are commonly used to manage a range of potato diseases and pests.

Clean seed

- **DO NOT PLANT A PROBLEM USE CLEAN SEED STOCKS**
- The certified seed scheme has a zero tolerance for Bacterial wilt. All potato seed within the Australian certification scheme is derived from pathogen tested material.
- Infected seed is the primary means of spreading bacterial wilt. If using farm saved seed (uncertified) from an area known to have bacterial wilt get a diagnostic test done to ensure clean seed stocks.

Field freedom

- BW can survive in field soil for a minimum of 2 years without a host. It is recommended that infested fields be taken out of potato production for at least 3-5 years ensuring there is no self sown potatoes. No seed production should occur on infested land.
- Ensure waste potatoes and soil from shed is not returned to fields.
- **USE NON HOST CROPS IN THE ROTATION**. Rotation with cereals and grass pastures decreases soil inoculum potential.
- In high risk fields, minimise post-emergence cultivation to avoid wounding roots.

Manage hosts

- The bacteria that causes bacterial wilt *Ralstonia solanacerum* has a wide host range including many crops with the family Solanaceae such as tomato, eggplant and potato, weeds such as nightshades, and thorn apple are hosts.
- **CONTROLLING SELF SOWN POTATO PLANTS IS CRITICAL** as the pathogen can carry over on these plants.

Irrigation source

- It is known that the pathogen causing bacterial wilt can spread in surface water. Diagnostic laboratory tests are available to have water sources tested for the presence of the pathogen. The bacteria are most likely to be detected at periods when water is warmer (above 15° C). Knowing if surface water is contaminated allows for informed decision in using water for irrigation and determining periods when it may be feasible to use a water source as an irrigation source.
- In infested areas, irrigation by well water is preferred over surface water from rivers or irrigation canals.

Nematode management

- Nematodes feed on plant roots which cause root injuries creating a path for the bacterium to enter the plant. Nematodes levels in soil must be controlled to avoid their interaction with *R. solanacearum*. Soil diagnostic tests are available to detect and quantify the level of common soil nematodes affecting potato.

For those areas without bacterial wilt – keep it that way!
Only use certified seed and ensure on farm biosecurity measures to prevent introduction
Be especially vigilant during field and storage inspections for symptoms of bacterial wilt

Horticulture Australia

PT09029 – (31/03/2014)

Enhancing the understanding of Verticillium spp in Australian potato production

Final Report

Prof Paul Taylor

The University of Melbourne

PROJECT SUMMARY

PT09029 – *Enhancing the understanding of Verticillium spp in Australian potato production*

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2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy, voluntary contributions from the New Zealand Institute for Plant and Food Research Limited, Horticulture New Zealand, the Potato Council-UK, and A&L Canada Laboratories, and matched funds from the Australian Government.

The South Australian Research and Development Institute, the Department of Environment and Primary Industries Victoria, the University of Tasmania/Tasmanian Institute of Agriculture, the University of Melbourne, Flinders University, Novozymes Biologicals Australia Pty Ltd and the Victorian Certified Seed Potato Authority have provided in-kind support.

DISCLAIMER

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CHAPTER 6. ENHANCING THE UNDERSTANDING OF VERTICILLIUM SPP IN AUSTRALIAN POTATO PRODUCTION

MEDIA SUMMARY

Yield of potato crops in parts of south-eastern Australia are declining due to potato early dying (PED) syndrome. PED refers to the early maturation and death of a potato crop and can be caused by an interaction between different species of the soil borne fungal pathogen Verticillium and of the root lesion nematode *Pratylenchus*. The fungal pathogen can establish in a field through the use of infected seed tubers or by movement of infected soil. The pathogen can also survive in the field for over 10 years through the production of durable resting structures called microsclerotia. The root lesion nematode interacts with the fungus to enhance the development of visual PED syndrome symptoms and to reduce tuber yield when population levels of the two pathogens are too low to cause disease alone.

The interaction between *V. dahliae* and *P. crenatus* in PED syndrome has not been investigated in the Australian potato production system but based on experiences in North America, PED has the potential to cause losses as high as 50 per cent. The key outcomes and recommendations from this project are summarized as follows:

A synergistic interaction was shown to occur between *P. crenatus* and *V. dahliae* which lead to increased PED severity in potato plants. Soils with a history of PED should be analysed for the levels of the two pathogens and if the levels are high then planting into these soils should be avoided.

Although *V. dahliae* was found to be widespread in potato tubers in South East Australia another potentially more serious *Verticillium* species (*V. albo-atrum*) was identified in a few tuber seed lots in Tasmania and Victoria. As well, the aggressiveness of isolates of *V. dahliae* to infect and cause disease was found to vary with some isolates being very aggressive. Further surveys and assessment of the pathogenicity of isolates of the *Verticillium* species needs to be undertaken across a wider area of the Australian potato producing regions.

Tubers infected with *V. dahliae* were widespread across seed lots in Victoria and Tasmania and although this infection appeared to have little impact on established plants, tuber infection may play a significant role in building up soil inoculum over several generations of planting, and may be important in transmission of the pathogen between regions. Further studies are required into the build-up of soil inoculum in field trials planted to infected tubers over several cropping generations.

Several Australian potato cultivars were identified as being resistant to *V. dahliae* in glasshouse trials. Field trials now have to be implemented in soils with a history of high incidence of PED to study resistance of commercial potato cultivars.

Soil amendments sulphur and brown coal were shown to reduce the viability of microsclerotia in laboratory assays. These soil amendments may have potential in integrated disease management programs however, further trials are needed to assess their efficacy in the field.

In conclusion a combination of resistant cultivars, avoidance of soils with high levels of *Verticillium dahliae* and nematodes, and soil amendment treatments may reduce PED syndrome in Australian potato production systems.

TECHNICAL SUMMARY

Verticillium wilt, caused by the soilborne fungi *Verticillium dahliae* and *Verticillium albo-atrum*, is a serious disease of potato as well as many other crops. An interaction between *Verticillium dahliae* and the root lesion nematode *Pratylenchus penetrans* has been shown to result in early maturation and death of potato crops, which is referred to as potato early dying (PED) syndrome. However, in Australian potato production systems very little is known about the role of *Verticillium* spp and *Pratylenchus* spp in causing PED syndrome. Resistance to *Verticillium* spp in commercial Australian potato cultivars is also unknown.

This project focused on several aspects; i) incidence of seed tuber infection, taxonomy and pathogenicity of *Verticillium* species infecting potatoes in Australia, ii) the role of *V. dahliae* tuber infection on the development of Verticillium wilt disease, iii) the interaction between *V. dahliae* and root lesion nematode *P. crenatus* in PED in Australia, iv) identification of Australian potato cultivars resistant to *V. dahliae*, and v) assessment of the efficacy of soil amendments to suppress Verticillium wilt disease in potatoes.

Seed tubers that had been produced by commercial seed growers in Victorian and Tasmanian potato production areas were obtained from 2010 to 2012. A total of 83 seed lots (20 tubers/lot) containing tubers from 20 cultivars were assessed for the presence of *Verticillium* species. *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* were isolated from the stem end vascular tissue of seed tubers and species identification was confirmed by multigene sequence analysis. Infection by *Verticillium* spp. within a seed lot varied greatly and ranged from 0-55%. Around 41% of seed lots tested were infected by *Verticillium* spp. Over 12% of seed lots tested ranged from 0-5% infection within the seed lots. Only one seed lot had more than 50% of seed tubers infected with *V. dahliae*. Overall percent infection of seed lots from Victoria and Tasmania were 28 (*V. dahliae*), 8 (*V. albo-atrum*) and 5 (*V. tricorpus*). Stem-end vascular discoloration of tubers was not correlated with presence of *V. dahliae*.

This was the first report in 45 years of *V. albo-atrum* infecting potato plants in Australia. Pathogenicity of selected isolates of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* were assessed by root dip inoculation with potato cv Shepody. All the inoculated plants showed typical Verticillium wilt symptoms however, several Tasmanian *V. dahliae* isolates were highly aggressive. Most *V. dahliae* and *V. albo-atrum* isolates showed the same level of aggressiveness; with *V. tricorpus* being substantially lower. Although *V. tricorpus* was pathogenic on potato the severity of infection was quite low. This was the first record of the pathogenicity of *V. tricorpus* on potatoes in Australia. Further surveys are needed within the Australian potato producing regions to determine the occurrence of the three Verticillium species and assess their level of pathogenicity on a broad range of commercial cultivars.

Internal seed tuber infection by *V. dahliae* and *V. tricorpus* of 7 potato cultivars did not significantly contribute to disease development and progeny tuber infection although the pathogen was isolated from symptomless plants. Nevertheless, decomposition of the infected tubers was shown to increase the inoculum level in the soil.

Glasshouse pot trials proved the synergistic interaction between *Pratylenchus crenatus* and *Verticillium dahliae* to cause PED syndrome. Potato cv Shepody was grown in field soil infested with a high level of the nematode (3 *P. crenatus*/g of soil) and various concentrations of *V. dahliae* inoculum. The level of *V. dahliae* inoculum was quantified in the soil using molecular techniques to assess the threshold level for infection. After 8 weeks, potato plants growing in the field soil containing nematodes and 4000 pg DNA/g of soil of *V. dahliae* inoculum had increased incidence of PED syndrome and significantly reduced tuber yield. There was a threshold level of *V. dahliae* inoculum of between 300 and 1000 pg *V. dahliae* DNA/g soil required before infection and colonization occurred in potato plants however, at these inoculum levels there was no

visible disease symptoms and no effect on tuber yield. Further glasshouse trials are required to study the interaction of different concentrations of *P. crenatus* with varying inoculum levels of *V. dahliae* and *V. albo-atrum.*

For cultivar resistance screening trials, an inoculum threshold level of 10^4 spores/mL was required to establish infection and wilt symptoms in potato plants, even though *V. dahliae* was isolated from petiole and crown root tissue of plants inoculated with as low as 10^2 spores/mL. In three glasshouse trials using 10^4 or 5 x 10^4 spores/mL, cultivar Denali showed moderate to high resistance and Catani and Desiree moderate resistance to infection by *V. dahliae*. Cultivars Nicola, Russet Burbank C, Shepody and Trent showed susceptible host reactions. Research is needed to study the mechanism of resistance to *V. dahliae* in these potato cultivars. Further screening trials need to be carried out using soil inoculated with microsclerotia to assess cultivar reaction as this is more close to reproducing field conditions. As well, field trials need to be implemented to assess the resistance of Denali, Catani and Desiree in soils with a history of high incidence of PED syndrome.

Soil amendments of 1% (w/w) atomic sulphur , 1% (w/w) brown coal and 1% (w/w) blood and bone meal mixed with air dried soil from Ballarat were shown to significantly reduce the viability of *V. dahliae* microsclerotia in laboratory microcosm (micro ecosytem) assays over a 8 week incubation period. Atomic sulphur was the most effective in inhibiting microsclerotia germination which may have been a result of a direct fungicidal activity on the microsclerotia. Although the viability of microsclerotia in the untreated controls also decreased rapidly the reduction in germination was much higher in the soils with the amendments.

There was no apparent impact of 1% brown coal on preventing the infection of plants inoculated with 500 and 750 CFU of microsclerotia/ g of dry soil. Interestingly the level of microsclerotia, as determined by CFU/ g soil, decreased significantly from the beginning of the trial to the end after 12 weeks which mirrored the results of the microcosm trials. Presumably infection of the plants must take place in the first 1 to 2 weeks of planting when the roots are actively growing and the viability of the inoculum is high. These soil amendments may have potential in integrated disease management programs however further glasshouse trials are required to assess their efficacy to suppress Verticillium wilt and PED syndrome before assessing in field trials.

INTRODUCTION

In Australian potato production, the significance and epidemiology of potato early dying (PED) syndrome is poorly understood. Yield of potato crops in parts of south-eastern Australia are declining due to PED. Affected potato crops dieback earlier than unaffected crops resulting in reduced tuber size and marketable yield. An Australian potato industry funded survey conducted in 2005 showed that around one third of all commercial processing potato crops were infested with *Verticillium dahliae* (Powney *et al.*, 2005). No reports of *V. albo-atrum* were identified in this survey.

Extensive studies have been conducted overseas, in particular the USA where PED is considered to be one of the most important diseases of potato. In North America, the potato yield losses are 10-15% in moderately affected fields and can be up to 50% in severe cases (Rowe *et al.*, 1987). In temperate regions of the US, PED is primarily caused by *Verticillium dahliae* while in the cooler areas, such as northern US and southern Canada, *Verticillium albo-atrum* is more dominant. In Australia, the distribution of the two species of *Verticillium* is relatively unknown. The significance of determining the species involved in PED is important to the development of appropriate management strategies.

Verticillium dahliae produces microsclerotia which consist of clusters of melanised, thick walled cells which are able to persist in soil and can remain viable for more than 10 years. *Verticillium dahliae* can be introduced to new fields through infested soil carried on machinery and equipment and also through infected seed. A survey of certified potato seed in the US showed up to one-third of seed lots tested was infected with *V. dahliae* (Rowe and Powelson, 2002). A smaller study in Australia showed that up to 12% of certified seed in Australia was infected with *V. dahliae (*Wicks *et al.,* 1997*)*. However, a larger survey is required to validate the levels of *Verticillium* spp. in certified seed. *V. albo-atrum* does not produce microsclerotia, but produces dark resting mycelium which does not survive in soil for more than two years. The spread of this pathogen is predominately through seed and infested soil that is carried on machinery and equipment.

In the USA, co-infection of potatoes with *V. dahliae* and the root lesion nematode *Pratylenchus penetrans* has been shown to result in the earlier onset of disease symptoms (Rowe *et al.*, 1985). In addition, the inoculum level required to initiate disease is lower than that required if the pathogens were acting individually (Botseas and Rowe, 1994). The mechanism(s) of this synergy are unknown. However, in Australian potato production areas *P. crenatus* has been shown to be the major root lesion nematode (Harding and Wicks, 2007). *Pratylenchus crenatus* has not been studied extensively because this species does not interact with *V. dahliae* to cause PED in North America (Riedel *et al.*, 1985).

Overseas, the management of PED syndrome has relied heavily on the use of broad-spectrum fumigants, which are expensive, and are not an environmentally acceptable or sustainable option for the long-term management of this disease complex. Therefore, the focus of this research is to find alternatives to soil fumigants. The use of host resistance offers promise in developing a long-term management strategy for reducing losses from PED. Resistance to *Verticillium* spp. in *Solanum* species is polygenic and complex (Jansky, 2008) and in potato the resistance to PED varies with cultivar. Host resistance remains the most economical, environmental and efficient management for this disease (Powelson and Rowe, 1993). For effective control, the goal is to reduce the disease severity as well as the amount of the inoculum that is returned to the soil (Davis, 1994). Most of the potato cultivars around the world are susceptible, only few that are resistant to Verticillium wilt (Jansky, 2009). Host resistance was not widely practiced in the past to control *Verticillium* wilt due to the past decision to manage this disease with fungicides that had a broad-spectrum activity (Johnson and Dung, 2010; Powelson and Rowe, 1993).

Resistance is expressed when the development of *Verticillium* wilt symptoms is restricted on the host. Resistant and partial resistant cultivars of *V. dahliae* may show less vascular colonization and microsclerotia formation. The expression of resistance is highly depended on environmental factors. Resistance cultivars may show symptoms of infection if planted at high inoculums level (Johnson and Dung, 2010). By planting resistant cultivars, the quantity of inoculum returned to soil will be less for the next season crop as the disease severity is reduced (Johnson and Dung, 2010). Few resistant cultivars have been found in United States, but none in Australia that were mainly distinguished based on disease symptom expression. According to cultivar screening studies in the USA, some cultivars are known to be resistant with low level of symptom expression (Jansky, 2009). To-date the potato cultivars developed in the Australian breeding program have not been indexed for their resistance to PED.

Organic soil amendments and green manure crops may be feasible and durable options to suppress PED. The incorporation of these organic products is thought to suppress *Verticillium* indirectly by either an increase in soil microbial activity or the release of toxic compounds (Quilty and Cattle, 2011; Lazarovits *et al.*, 2001; Tenuta and Lazarovits, 2004). Research in Canada has shown the incorporation of nitrogenous organic products such as meat and bone meal can kill the microsclerotia of *Verticillium dahliae* in soil. This control is the result of the accumulation of ammonia and/or nitrous acid in the soil which are both lethal to

the microsclerotia (Tenuta and Lazarovits, 2002; Lazarovits *et al.*, 2001). Another study which used mushroom compost also reduced early dying severity by delaying foliar symptoms and increased yield (LaMondia *et al.*, 1999). A study by Davis *et al.* (1996) concluded that the use of green manures, such as Sudan grass and corn residues were able to reduce soil population of *V. dahliae* in potato fields.

Atomic sulphur has also been shown to suppress soil borne pathogens and has been reported to lower soil pH, have fungicidal activity against soil pathogenic fungi, and to interact with the plant host defence mechanisms. Brown coal has been assessed as a soil biofertiliser by Monash University, Australia at 1 to 2 tonnes per hectare, and may also suppress soil borne diseases through an increase in soil microbial activity.

At present, the cultural practice of incorporating amendments into the soil is not widely integrated into most potato farming systems as long term trials are needed to confirm and quantify the effectiveness and economic value of soil amendments (Johnson and Dung, 2010). Further, to fully exploit the suppression of disease using amendments and green manures in a reliable and effective PED management strategy, a better understanding of the mechanisms involved in suppressing pathogenic fungi is required.

This research aims to:

- Identify the incidence of Verticillium species in Victorian and Tasmanian potato crops
- Validate the taxonomy of Verticillium species infecting potato and identify pathotypes
- Determine the role of *V. dahliae* tuber infection on the development of Verticillium wilt disease
- Assess the interaction between root lesion nematode *P. crenatus* and *V. dahliae* in the cause of PED syndrome
- Identify Australian potato cultivars resistant to *V. dahliae*
- Assess the efficacy of soil amendments to suppress Verticillium wilt disease in potatoes

MATERIALS AND METHODS

INCIDENCE OF *VERTICILLIUM* SPECIES IN POTATO SEED LOTS IN VICTORIA AND TASMANIA

SEED SOURCE

Certified seed tubers that had been produced by commercial seed growers in Victorian and Tasmanian potato production areas were obtained from 2010 to 2012. In 2010, 33 seed lots from Victoria; in 2011, 16 lots from Victoria and 10 lots from Tasmania, and in 2012, 11 lots from Victoria and 13 from Tasmania were selected. A total of 83 seed lots (20 tubers/lot) were obtained.

ISOLATION

Tubers were washed with high pressure water to remove surface soil, surface sterilized in 0.5% ai NaOCl for 5 min. and then rinsed in sterile distilled water. The tubers were numbered individually, transverse cut at the stolon end 3-5 mm beneath the stem end of each tuber and vascular discolouration recorded. Four pieces of vascular tissue (approx 5 mm diameter) were excised aseptically from the vascular tissue (Omer *et al.*, 2000) then placed on either Soil Pectate Tergitol (SPT) (Hawke and Lazarovits, 1994) or Ethanol Potassium Amoxicillin Agar medium (EPAA) (Mansoori, 2011) and incubated at 23^0 C in the dark for 15 days.

IDENTIFICATION OF SEED TUBER ISOLATES

MORPHOLOGICAL IDENTIFICATION

Verticillium spp were identified by sub-culturing colonies onto potato dextrose agar (PDA; DIFCO Laboratories). Conidia were collected from each isolate with a sterile loop and streaked onto water agar. After one day colonies grown from single spores were transferred onto PDA in 90 mm Petri dishes to ensure genetic uniformity, and were incubated in darkness at 23°C for 4 weeks. Cultures were examined under the stereo and compound microscope for the presence of conidiophores, microsclerotia, dark resting mycelia and chlamydospores characteristics of *Verticillium* species and identified based on published descriptions (Hawksworth, 1970; Hawksworth and Talboys, 1970a; Hawksworth and Talboys, 1970b; Zare *et al.*, 2007; Inderbitzin *et al.*, 2011a). All the single spored cultures were stored on PDA slants in 10 mL plastic tubes at 4^0C and were used for all subsequent studies.

MOLECULAR IDENTIFICATION

Isolates (18) that were difficult to identify based on morphological characteristics were identified based on Internal Transcribed Spacer (ITS) gene sequences.

GENOMIC DNA EXTRACTION AND ASSESSMENT

Isolates were grown in 10 mL V8 broth or Czapek Dox broth in McCartney bottles and incubated at room temperature for 2 weeks or until a mycelial mat was formed on top of the broth. DNA extractions were performed with 100 mg of mycelia of each mono-conidial isolate using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. The integrity of the extracted genomic DNA was assessed on 1% (w/v) agarose gel and the quantity was estimated based on a known amount of λDNA*Hind*III (Invitrogen, Australia) marker loaded next to the genomic DNA. A Beckman DU 530 spectrophotometer (Beckman Coulter, Inc., USA) was also used to cross check the amount of genomic DNA at optical density (OD) of 260 nm. 260/280 nm was used to check for the quality of DNA. The rDNA of the 18S, 28S and 5.8S ITS regions were amplified using primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990).

POLYMERASE CHAIN REACTION (PCR)

PCR was routinely performed either on a MJ Research PTC-100 Thermal Cycler or Eppendorf Thermalcycler. The PCR reactions were performed in a total volume of 25 µl including 50 ng genomic DNA, 5 µl of 10× PCR buffer containing 25 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM of each primer and 1 unit of Taq DNA polymerase (Bioline Australia).

The cycling conditions consisted of 95^0C for 3 min, followed by 45 cycles of denaturation at 95^0C for 30 sec, annealing at 50^0C for 30 sec, 72^0C for 30 sec and elongation at 72^0C for 6 min. PCR product were visualised by staining with ethidium bromide on 1.5% agarose electrophoresis gels. PCR products were also purified using QIAquick® PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The PCR products were sequenced twice at both forward and reverse directions at the Australian Genome Research Facility (AGRF, Melbourne).

SEQUENCE ANALYSIS

All sequences were checked manually and edited when necessary using MEGA version 5 (Tamura *et al.*, 2011). To identify sequences, they were compared against those sequences already found in the databases using the Basic BLAST search option of BLAST 2.2.27 (http://www.ncbi.nlm.nih.gov/BLAST). This search made use of the NCBI GenBank data base, and was able to report the species that had the greatest similarity and the percentage of similarity with ITS sequence.

TAXONOMY OF *VERTICILLIUM* SPECIES

FUNGAL ISOLATES AND MORPHOLOGY DESCRIPTION

The taxonomy of three *Verticillium* species isolated from potato was studied using both morphological characters and molecular analysis. The isolates included four of *V. albo-atrum* and two of *V. tricorpus* that had been isolated from the vascular tissue of seed tubers from the 2012 seed potato survey from Victoria and Tasmania (Table 6-1). Seventeen isolates of *V. dahliae*; and one of *V. tricorpus* isolated from either potato tubers or petiole tissue in 2005 were obtained from the culture collection of Department of Primary Industries, Knoxfield, Victoria. Two isolates of *V. dahliae* from tomato and strawberry were also included in this study as alternate hosts to potato. An isolate of *Gibellulopsis nigrescens* (formerly *Verticillium nigrescens* (Zare *et al.*, 2007) from potato petiole tissue was also included for comparison to the other potato *Verticillium* species.

For morphological description, the fungal isolates were grown on PDA for four weeks before being examined under stereo and compound microscopes. For compound microscope examination, the structures were mounted on glass slides in water and photographs were taken with a Leica DFC295 camera, using Leica application software.

MOLECULAR TAXONOMY

Genomic DNA extraction and assessment were described above. Three primer pairs used to amplify *Verticillium* species were ITS 1 and ITS 4 (White *et al.*, 1990), Bt 2A (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt 2B (5'- ACCCTCAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson, 1995) and EF1-983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and EF1-1567R (5'-ACHGTRCCRATACCACCRATCTT-3') (designed by S. Rehner and available from http://www.aftol.org/pdfs/EF1primer.pdf) respectively. The cycling condition for ITS was described above. For β-tubulin; 3 min at 95^{0}C, 30 cycles of 1 min at 95^{0}C, 50 sec at 57^{0}C, 1 min at 72^{0}C, final extension step of 10 min at 72^{0}C. The EF-1 alpha was amplified using 30 sec at 95^{0}C, 45 cycles of 30 sec at 95^{0}C, 30 sec at 60^{0}C, 30 sec at 72^{0}C, final extension step of 10 min at 72^{0}C. PCR product visualisation, purification and sequencing were described above.

SEQUENCE ANALYSIS AND PHYLOGENETIC TREE CONSTRUCTION

Sequences were compared against those sequences already found in the databases using the Basic BLAST search option of BLAST 2.2.27. All the sequences were aligned in MEGA 5 (Tamura *et al.*, 2011) using ClustalW and phylogenetic trees constructed. For each and combined gene sequences, Kimura 2-parameter model was used for phylogenetic relationships of *Verticillium* species (Kimura, 1980). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches (Felsenstein, 1985). Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selected the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

All consensus sequence data were deposited in GenBank® under accession numbers KC592062 to KC592090 (ITS), KC592091 to KC592119 (β-tubulin) and KC592120 to KC592148 (EF1-α). Sequence alignments and multigene phylogenetic trees were submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S13906).

Table 6-1 – Hosts, geographic locations, year of isolation, *Verticillium* species and the GenBank accession numbers of sequences used for taxonomic study

Isolate code	Host	Location	Year of isolation	Species	GenBank Accession		
					ITS	BT	EF
VICVd36*	Potato petiole	Cora Lynn, Victoria	2005	*V. dahliae*	KC592062	KC592091	KC592120
VICVd54*	Potato petiole	Ballarat, Victoria	2005	*V. dahliae*	KC592063	KC592092	KC592121
VICVd69	Potato petiole	Ballarat, Victoria	2005	*V. dahliae*	KC592064	KC592093	KC592122
VICVd72	Potato petiole	Ballarat, Victoria	2005	*V. dahliae*	KC592065	KC592094	KC592123
VICVd74	Potato petiole	Ballarat, Victoria	2005	*V. dahliae*	KC592066	KC592095	KC592124
VICVd85*	Potato petiole	Colac-Otway, Victoria	2005	*V. dahliae*	KC592067	KC592096	KC592125
SAVd7*	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592068	KC592097	KC592126
SAVd8	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592069	KC592098	KC592127
SAVd10	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592070	KC592099	KC592128
SAVd15	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592071	KC592100	KC592129
SAVd16*	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592072	KC592101	KC592130
SAVd20	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592073	KC592102	KC592131
SAVd21	Potato petiole	Mt Gambier, South Australia	2005	*V. dahliae*	KC592074	KC592103	KC592132
SAVd12*	Potato petiole	Robe, South Australia	2005	*V. dahliae*	KC592075	KC592104	KC592133
SAVd13	Potato petiole	Robe, South Australia	2005	*V. dahliae*	KC592076	KC592105	KC592134

Isolate code	Host	Location	Year of isolation	Species	GenBank Accession		
					ITS	BT	EF
SAVd14	Potato petiole	Robe, South Australia	2005	*V. dahliae*	KC592077	KC592106	KC592135
TASVd24*	Potato petiole	Devonport, Tasmania	2005	*V. dahliae*	KC592078	KC592107	KC592136
TASVd25*	Potato petiole	Devonport, Tasmania	2005	*V. dahliae*	KC592079	KC592108	KC592137
TASVd27*	Potato petiole	Devonport, Tasmania	2005	*V. dahliae*	KC592080	KC592109	KC592138
TOVd1	Tomato	Unknown	2005	*V. dahliae*	KC592081	KC592110	KC592139
SBVd2	Strawberry	Unknown	2005	*V. dahliae*	KC592082	KC592111	KC592140
VICVaa1*	Potato tuber	Ballarat, Victoria	2012	*V. albo-atrum*	KC592083	KC592112	KC592141
VICVaa2	Potato tuber	Ballarat, Victoria	2012	*V. albo-atrum*	KC592084	KC592113	KC592142
TASVaa1*	Potato tuber	Tasmania	2012	*V. albo-atrum*	KC592085	KC592114	KC592143
TASVaa2	Potato tuber	Tasmania	2012	*V. albo-atrum*	KC592086	KC592115	KC592144
TASVt1*	Potato tuber	Tasmania	2012	*V. tricorpus*	KC592087	KC592116	KC592145
TASVt2*	Potato tuber	Tasmania	2012	*V. tricorpus*	KC592088	KC592117	KC592146
VICVt143	Potato tuber	Cora Lynn, Victoria	2004	*V. tricorpus*	KC592089	KC592118	KC592147
VICVn126	Potato petiole	Ballarat, Victoria	2004	*G. nigrescens*	KC592090	KC592119	KC592148

*Isolates used for pathogenicity study

Reference sequences for published *Verticillium* species used to construct the ITS phylogenetic tree were obtained from GenBank® (Table 6-2) and were published by Inderbitzin et al (2011a, 2011b). The trees were rooted with *G. nigrescens*.

Table 6-2 – GenBank accession numbers of reference sequences used in the ITS phylogenetic tree

Species	GenBank accession	Species	GenBank accession
V. dahliae	HQ206718	*V. alfalfae*	JN187971
V. albo-atrum	JN188016	*V. nonalfalfae*	JN187973
V. tricorpus	JN187993	*V. nibilum*	JN188011
V. zaregamsianum	JN188005	*V. isaacii*	HQ206873

Species	GenBank accession	Species	GenBank accession
V. klebahnii	JN187967	*V. longisporum*	HQ206832

PATHOGENICITY OF *VERTICILLIUM* SPECIES ISOLATES

PLANT MATERIALS

Tissue culture plantlets of potato cv Shepody, susceptible to *V. dahliae* (Arbogast *et al.*, 1999), and moderately resistant cv Ranger Russet (Whitworth and Davidson, 2008) were planted in pasteurized sand for 15 days to establish the root system. Eggplant seedlings (cv Black Beauty) were grown in seedling mix (Yates, NSW, Australia) from seed and maintained on benches in a glasshouse until plants reached the 4-leaf stage (4-week-old).

FUNGAL ISOLATES

A) PATHOGENICITY OF *V. DAHLIAE* ISOLATES

Nine *V. dahliae* isolates were tested for pathogenicity in potato and eggplant using three isolates each from Victoria (VICVd36, VICVd54, VICVd85), South Australia (SAVd7, SAVd16, SAVd12) and Tasmania (TASVd24, TASVd25, TASVd27) in August to November 2011 and experiments were repeated in April to July 2012.

B) PATHOGENICITY OF *V. DAHLIAE*, *V. ALBO-ATRUM* AND *V. TRICORPUS* ISOLATES

The pathogenicity of the high (VICVd25) and low (VICVd54) aggressive isolates of *V. dahliae* isolates were compared to the *V. albo-atrum* (VICVaa1 and TASVaa1) and *V. tricorpus* (TASVt1 and TASVt2) isolates from potato. The isolates of *V. albo-atrum* and *V. tricorpus* were isolated from the vascular tissue of seed tubers from Victoria and Tasmania in 2012.

INOCULUM PRODUCTION AND INOCULATION

Inoculum was prepared by growing isolates on PDA in 90 mm Petri plates at 23^0C for four weeks. Spore suspensions were prepared by adding 10 mL of sterile distilled water (SDW) to each plate and scraping the cultures with a spatula. The resulting spore suspensions were filtered through double layers of cheesecloth, and then adjusted to 10^5 conidia/mL using a haemocytometer.

Tissue culture seedlings of cv Shepody and Ranger Russet, and eggplant seedlings, were gently removed from sand or soil, roots washed under running tap water, then dipped for at least five min into the spore suspension of each isolate. For uniform distribution of conidia, the spore suspension was thoroughly shaken every minute. Roots were dipped in SDW as a control. The inoculated seedlings were transplanted immediately to 2.8 L pots for potatoes and 0.5 L pots for eggplants containing pasteurised potting mix and then placed on glasshouse benches. Natural light was supplemented with incandescent lamps for a photoperiod of 16 h. Plants were watered when needed and fertilised weekly with Aquasol™ (N:P:K = 2.8:1:4.5). All the treatments were arranged in the glasshouse as a randomized complete block design with four replicates per isolate.

DISEASE ASSESSMENT

Symptom severity on potatoes and eggplants were recorded at weekly intervals starting from 2 weeks after inoculation on a scale of 0-5, where 0= no symptoms, 1= chlorosis of lower leaves, 2= moderate (30-50% of leaves) wilt with severe chlorosis, 3= moderate wilt and necrosis, 4= severe (>50% of leaves) wilt and necrosis, and 5= death of plant(Tsror and Hazanovsky, 2001).

PETIOLE ISOLATION

Potato and eggplant leaflets were collected 2-weeks after planting and the petioles were surface sterilized in 0.5% ai NaOCl for 1 min and rinsed twice with sterile distilled water. Four, 4 mm wide cross sections were cut at equal intervals from each petiole and plated onto EPAA medium (Mansoori, 2011) and incubated at 23^0C in dark. After 15 days, the resulting fungal colonies were observed using stereo and compound microscopes and identified based on morphological characters such as verticillate conidiophores and resting structures.

PLANT HEIGHT, AERIAL BIOMASS IN EGGPLANT

Eggplants were destructively sampled 7-weeks after planting and height, aerial biomass (dry weight) and dry weight of roots were measured and expressed in grams.

STATISTICAL ANALYSIS

One-way ANOVA was performed on plant height, aerial biomass and root weight in eggplant. Tests for significant differences among isolates were analysed using the statistical package Minitab 16.

ROLE OF *V. DAHLIAE* INFECTED TUBERS IN CAUSING VERTICILLIUM WILT DISEASE

SELECTION OF NATURALLY INFECTED TUBERS AND TREATMENTS

Naturally infected potato tubers grown in Victorian and Tasmanian seed potato production regions were selected for glasshouse experiments in 2011 and 2012. Based on the identification of *V. dahliae* from cultured stem end vascular tissue, infected and uninfected tubers were selected for glasshouse experiments. Care was taken to use tubers in which other fungal pathogens (*Colletotrichum coccodes*, *Plectosphaerella cucumerina* and *Fusarium* species) were not present.

Various cultivars which showed difference in the susceptibility to Verticillium wilt were used in the experiment (Table 6-3**Error! Reference source not found.**). All the treatments were arranged in the glasshouse as a randomized complete block design with replicates. Natural light was supplemented with incandescent lamps for a photoperiod of 16 h. Plants were watered when needed and fertilised weekly with Aquasol™ (N:P:K = 2.8:1:4.5).

DISEASE ASSESSMENT

For all glasshouse experiments, symptom severity on foliage was observed 45, 55 and 65 days after planting (DAP), on a scale of 0-5, where 0= no symptoms, 1= chlorosis of lower leaves, 2= moderate (30-50% of leaves) wilt with severe chlorosis, 3= moderate wilt and necrosis, 4= severe (>50% of leaves) wilt and necrosis, and 5= death of plant (Tsror and Hazanovsky, 2001).

ISOLATION OF VERTICILLIUM SPECIES FROM PETIOLE

Leaflets were collected at 45, 55 and 65 DAP and the petioles were surface sterilized in 0.5% ai NaOCl for 1 min and rinsed twice with sterile distilled water. Four, 4 mm wide cross sections were cut at equal intervals from each petiole and plated onto either SPT (Hawke and Lazarovits, 1994) or EPAA medium (Mansoori, 2011) and incubated at 23^0C in dark. After 15 days, the resulting fungal colonies were observed using stereo and compound microscopes and identified based on morphological characters such as verticillate conidiophores and resting structures.

YIELD, PROGENY TUBER INFECTION AND QUANTIFICATION OF *V. DAHLIAE* IN THE SOIL

At harvest the soil was removed from each pot and all tubers were weighed. Three randomly selected progeny tubers from each plant were assayed for *V. dahliae* infection by culturing vascular tissue on EPAA medium (Mansoori, 2011) and incubating cultures at 23^0 C in the dark for 15 days. Identification of *Verticillium* species was based on morphological characters.

After harvest, all the soil from each pot was separately packed and sent to SARDI for DNA quantification of *V. dahliae*. The quantity of *V. dahliae* was expressed as pg *V. dahliae* DNA/g of soil.

STATISTICAL ANALYSIS

One-way ANOVA was performed on DNA quantification as well as yield results. Tests for significant differences among isolates were analysed using the statistical package Minitab 16.

EXPERIMENTS

Four sets of glasshouse experiments and one field oriented study were conducted (Figure 6-1).

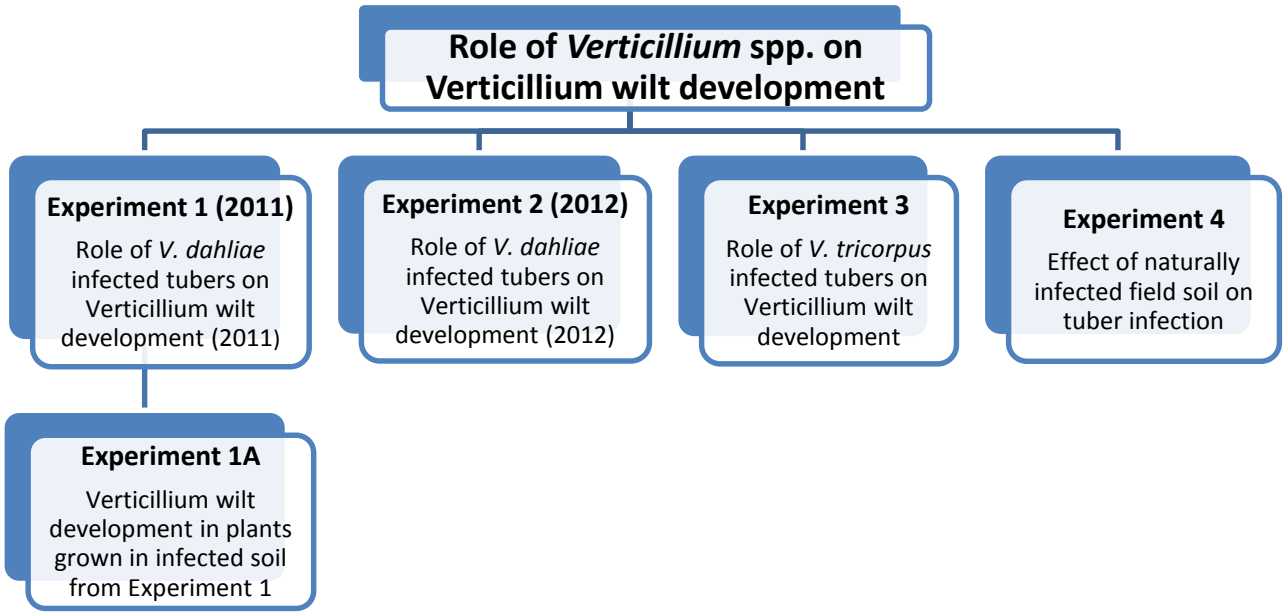

Figure 6-1 – Summary Flow Chart of Experiments

EXPERIMENTS 1 AND 2: ROLE OF *V. DAHLIAE* INFECTED TUBERS ON VERTICILLIUM WILT DEVELOPMENT (2011 AND 2012)

In 2011, cultivars Russet Burbank, Atlantic, Trent, Ranger Russet, and Nooksack (Table 6-3); with five replications each for *V. dahliae* infected and uninfected tubers were planted in 5 L pot containing pasteurized potting mix and then placed in the glasshouse. Symptom severity, petiole isolation (45, 55 and 65 DAP), yield and progeny tuber infections were assessed. After harvest, soil was mixed and then 500 g of sub samples from each pot were sent to SARDI for *V. dahliae* DNA quantification. Remaining soil was used to study the role of *V. dahliae* infested soil on subsequent Verticillium wilt development (Experiment 1A).

In 2012, cv Atlantic, Sebago, Innovator and Russet Burbank (Table 6-3) with four replications each for *V. dahliae* infected and uninfected tubers were planted in 2.8 L pots containing pasteurized potting mix and then placed in the glasshouse. Symptom severity, petiole isolation, yield and progeny tuber infection were assessed. Soil samples were sent to SARDI for *V. dahliae* DNA quantification after harvest.

Table 6-3 – Cultivars and reaction to Verticillium wilt used in the glasshouse experiment

Cultivars	Reaction to Verticillium wilt	References
Russet Burbank	Moderately susceptible	Whitworth and Davidson (2008)
Atlantic	Moderately susceptible	Whitworth and Davidson (2008)
Trent	Not known	
Ranger Russet	Moderately resistant	Whitworth and Davidson (2008)
Nooksack	Resistant	Anonomous (2009)
Sebago	Susceptible	Anonomous (2010)
Innovator	Not known	

EXPERIMENT 1A: VERTICILLIUM WILT DEVELOPMENT IN PLANTS GROWN IN INFESTED SOIL FROM EXPERIMENT 1 (2011)

Infested soil from Experiment 1 was used for assessing subsequent disease development. Soil was mixed, divided into two halves and placed in 2.8 L pots. Mini tubers of moderately susceptible Russet Burbank and very susceptible Shepody cultivars provided by ViCSPA were planted. Petiole isolation and disease evaluation were assessed 45, 55 and 65 DAP. Progeny tuber infection was assessed by culturing vascular tissue, and soil samples were again sent to SARDI for *V. dahliae* quantification.

EXPERIMENT 3: ROLE OF *V. TRICORPUS* INFECTED TUBERS ON VERTICILLIUM WILT DEVELOPMENT

In 2012, tubers of cv Nicola with 8 replications each for *V. tricorpus* infected and uninfected tubers were planted in 2.8 L pots containing pasteurized potting mix and then placed in the glasshouse. Petiole isolation and disease severity were assessed at 45, 55 and 65 DAP. After harvest, yield and progeny tuber infection were assessed.

Quantification of *V. tricorpus* in the soil was determined as colony forming units (CFU) per g of soil. After harvest, soil samples from each pot were air-dried at room temperature for four weeks to eliminate short-lived propagules such as conidia and mycelial fragments. The soil was then homogenised and passed

through 850, 500, 250 and 45 µm sieves. A 50 mg sample of soil from the final sieve was plated onto EPAA medium (Mansoori, 2011) using an Andersen Sampler (Butterfield and DeVay, 1977). Ten plates from each sample were incubated at 23^0 C for 15 days. After incubation, plates were washed with tap water to remove soil particles and aerial mycelia from the agar surface and colonies of *V. tricorpus* were identified using the stereomicroscope. Colonies of *V. tricorpus* were identified based on morphology of microsclerotia colonies formed on EPAA medium. The number of CFUs per gram of soil was calculated based on the amount of soil plated and the number of colonies observed.

EXPERIMENT 4: EFFECT OF NATURALLY INFESTED FIELD SOIL ON TUBER INFECTION

An experiment was conducted to assess the inoculum level of *V. dahliae* in field soil on subsequent progeny tuber infection on a commercial farm at Cora Lynn region of Victoria. A field was selected from a potato growing area where levels of *V. dahliae* were high. Before planting, the two hectare plot was divided into 8 subplots and 20 soil samples were collected from each plot at regular intervals along a "W" pattern from 10-15 cm depth of top soil in the field using a soil corer. Collected soil samples were mixed well and 500 g of subsamples from each plot were sent to SARDI for *V. dahliae* quantification. The soil corer was cleaned between sampling each plot. The plots were commercially planted with a range of cultivars (Table 6-18). After harvest, 20 tubers from each plot were randomly selected for isolation and the number of tubers infected with *V. dahliae* were recorded.

INTERACTION BETWEEN *V. DAHLIAE* AND ROOT LESION NEMATODE *P. CRENATUS* TO CAUSE PED SYNDROME IN AUSTRALIA

Two experiments were undertaken to determine the interaction between *V. dahliae* and *P. crenatus* in the development of PED.

EXPERIMENT 1: THE EFFECTS OF NATURAL FIELD SOIL INOCULUM LEVELS OF *V. DAHLIAE* AND *P. CRENATUS* ON THE DEVELOPMENT OF PED

COLLECTION AND PREPARATION OF SOIL

A field was selected at Cora Lynn, Victoria where levels of *V. dahliae* and *P. crenatus* had previously been observed to cause PED syndrome. Soil samples were collected from a 2 hectare plot along a "W" pattern in regular intervals from 10-30 cm of the top soil in the field using a soil corer. Soil samples were collected in 60 L plastic round bin containers. The soil was passed through a 2 cm sieve then mixed in a concrete mixer. Four samples of 500 g each were sent to SARDI for *V. dahliae* DNA quantification. Three replicates each of 200 g soil samples were sent to Plant Health Services, DPI, Knoxfield, Victoria for identification and quantification of the root lesion nematodes.

PLANT MATERIAL

Eggplant seedlings of cv Black Beauty and mini tubers of potato cv. Russet Burbank provided by Victorian Certified Seed Potato Authority (ViCSPA) were used for glasshouse experiments.

PREPLANT QUANTITY OF *V. DAHLIAE* AND ROOT LESION NEMATODES

The quantity of DNA of *V. dahliae* in the field soil was 85 pg *V. dahliae* DNA/g of field soil. *P. crenatus* was the only root lesion nematode species found in the field soil at 0.3 *P. crenatus*/g of soil.

TREATMENTS AND GLASSHOUSE CONDITION

The treatments included (i) field soil (containing 85 pg *V. dahliae* DNA/g of field soil + 0.3 *P. crenatus*/g of soil), (ii) nematicide treated soil (soil drenching with Vydate® 2 mL/L to kill nematodes), and (iii) pasteurized soil (70^0C for 30 min to kill *V. dahliae* and nematodes).

Eggplant seedlings were transplanted to 0.5 L pots and potato mini tubers were planted in 1.5 L pots containing various treatments of the field soil and then placed on glasshouse benches. Treatments were arranged in the greenhouse as a randomized complete block design. Eggplant seedlings were planted in the soil with five replications and two harvest times (6^{th} and 10^{th} week after planting) and potato cv Russet Burbank with 10 replications (destructively sampled after 10 weeks). Natural light was supplemented with incandescent lamps for a photoperiod of 16 h. Plants were watered when needed and fertilised weekly with Aquasol™ (N:P:K = 2.8:1:4.5).

DISEASE ASSESSMENT

Symptom severity on foliage was observed on a scale of 0-5, where 0= no symptoms, 1= chlorosis of lower leaves, 2= moderate (30-50% of leaves) wilt with severe chlorosis, 3= moderate wilt and necrosis, 4= severe (>50% of leaves) wilt and necrosis, and 5= death of plant (Tsror and Hazanovsky, 2001).

FUNGAL ISOLATION FROM LEAF PETIOLES

Leaf petioles were sampled at weekly intervals up to 10 weeks after planting for potato and eggplant. Tissue was surface sterilized in 0.5% ai NaOCl for 1 min and then rinsed twice with sterile distilled water. Four, 4 mm wide cross sections were cut at equal intervals from each petiole, plated onto EPAA medium (Mansoori, 2011) and incubated at 23^0C in dark. After 15 days, the resulting fungal colonies were observed using dissecting and compound microscopes and *V. dahliae* identified by morphological characters such as verticillate conidiophores and resting structures.

PLANT HEIGHT, AERIAL BIOMASS AND ROOT WEIGHT IN EGGPLANT

Eggplants were destructively sampled 10 weeks after planting and plant heights, aerial biomass (fresh and dry weight) and fresh weight of roots were measured.

POST HARVEST DNA QUANTIFICATION OF *V. DAHLIAE* AND QUANTIFICATION OF *P. CRENATUS* IN SOIL AND PLANT ROOTS

Eggplant seedlings were destructively sampled 6 and 10 weeks after planting while potato plants were destructively sampled 10 weeks after planting. 200 g of soil from each pot in the eggplant trial and 500 g of soil from each plot in the potato trial were separately packed and sent to SARDI for DNA quantification of *V. dahliae*. The level of DNA was expressed as pg DNA/g of soil. Another 200 g of soil and washed plant roots from each pot in both trials were sent to Plant Health Service, DPI, Knoxfield for *P. crenatus* quantification.

STATISTICAL ANALYSIS

Statistical analyses were performed for all parameters except disease severity, using ANOVA. Tests for significant differences among treatments were analysed using the statistical package Minitab 16.

EXPERIMENT 2: THE EFFECT OF DIFFERENT CONCENTRATIONS OF *V. DAHLIAE* ALONE OR IN COMBINATION WITH NATURAL FIELD SOIL LEVEL OF *P. CRENATUS* ON THE DEVELOPMENT OF PED.

COLLECTION OF SOIL AND QUANTIFICATION OF *V. DAHLIAE* AND *P. CRENATUS* IN FIELD SOIL

Soil samples were collected from potato growing field at Cora Lyn, Victoria. The soil was collected in four 60 L plastic round bin containers, sieved, homogenised and sent to A & L Canada Laboratories Inc., Ontario, Canada for nutrient analysis. The soil was prepared and samples sent to determine the quantity of *V. dahliae* and *P. crenatus* in the soil as described in Experiment 1.

PRODUCTION OF *V. DAHLIAE* MICROSCLEROTIA INOCULUM

Artificial production of *V. dahliae* microsclerotia was based on the method described by Xiao *et al* (1998) with slight modifications. Briefly, 125 mL Erlenmeyer flasks, each containing 50 mL of potato dextrose broth, were inoculated with two 4 mm diameter culture disks taken from the very aggressive 4-week-old *V. dahliae* isolate (TASVd25) growing on potato dextrose agar. Flasks were incubated on a horizontal shaker for two weeks at room temperature (22 to 24°C). The cultures were washed with 20 mL of sterile distilled water to dislodge the spores. The conidial suspension was filtered through cheesecloth, and then the density of conidia was adjusted to 10^7 conidia/mL in 500 mL of water. Dried field soil collected from Cora Lynn was passed through a 0.425 mm sieve then sterilized by autoclaving at 121°C for 1 h twice, on 2 successive days. Glass Petri dishes (185 mm) containing 250 g of sterilized soil were each amended with 180 mL of conidial suspension and incubated on a laboratory bench at room temperature (22 to 24°C). After 21 days of incubation, microsclerotia developed in the soil colonised by the mycelium (Figure 6-2). The colonised soil was air-dried for four weeks to kill mycelia and conidia. Dried inoculum (1 g) from each culture was spread on EPAA medium to confirm the viability of microsclerotia by assessing germination (Figure 6-3). Samples of soil containing microsclerotia produced in the laboratory were sent to SARDI for *V. dahliae* quantification (pg DNA/g of soil) and used as the inoculum carrier for soil infestation to establish inoculum densities for glasshouse experiments.

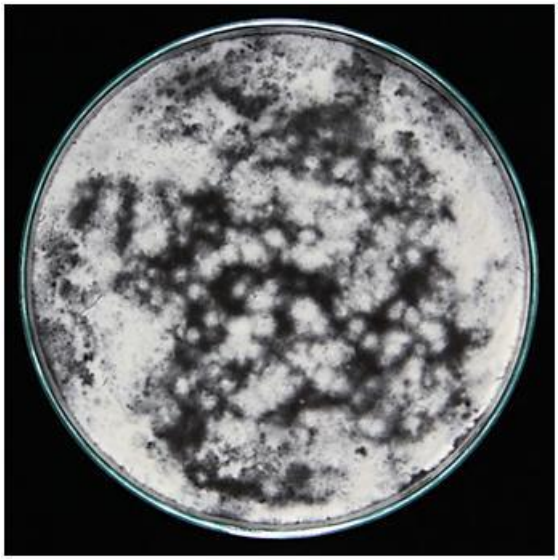

Figure 6-2 – *V. dahliae* colonization of soil 21 days after incubation

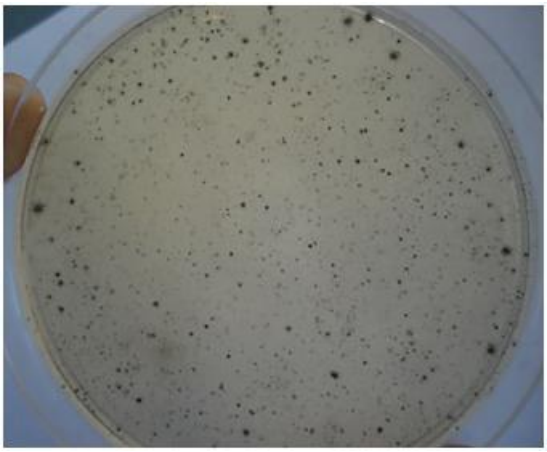

Figure 6-3 – Colonies of *V. dahliae* on EPAA medium

TREATMENTS

Quantity of *V. dahliae* in naturally infested field soil based on SARDI results showed a mean of 90 pg DNA/g of soil (range 80-100 pg DNA/g of soil). The nematode quantity was 3 *P. crenatus*/g of soil. Other nematodes in the soil included *Heterodera trifolii* cyste larvae (1.1/g soil) and *Meloidogyne* spp. (0.2/g soil). Pots (1.5 L) for potato and 0.5 L for eggplants were filled with soil and then each pot was separately inoculated with prepared inoculum of *V. dahliae* in each pot and made up to an estimated concentration of 4000, 2,000, 1000, 500, 300 and 100 pg *V. dahliae* DNA/g of soil. Then 3 samples, each of 500 g, from each treatment were sent to SARDI to confirm the final preplant quantity of *V. dahliae*. The final expected treatment concentration and actual ranges in concentration of *V. dahliae* in the treatments were as follows:

4000 pg *V. dahliae* DNA/g of soil (range 3900-4100 pg DNA/g of soil), 2000 pg *V. dahliae* DNA/g of soil (range 1850-2150 pg DNA/g of soil), 1000 pg *V. dahliae* DNA/g of soil (range 900-1100 pg DNA/g of soil), 500 pg *V. dahliae* DNA/g of soil (range 500-550 pg DNA/g of soil), 300 pg *V. dahliae* DNA/g of soil (range 250-350 pg DNA/g of soil), 100 pg *V. dahliae* DNA/g of soil (range 75-130 pg DNA/g of soil) and 0 pg DNA/g of soil from pasteurized treatment.

After filling up each pot with soil, nematicide treatment was established using nematicide Vydate 2 mL/L as soil drenching. The experimental design was a randomized complete block with six replications for potatoes and four replicates for eggplants as follows.

Potato

1. 4000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
2. 4000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
3. 2000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
4. 2000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
5. 1000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
6. 1000 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
7. 500 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
8. 500 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
9. 300 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
10. 300 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
11. 100 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil

12. 100 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
13. Control (Pasteurized soil)

Eggplant

1. 300 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
2. 300 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
3. 100 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil
4. 100 pg *V. dahliae* DNA/g of soil + 3 *P. crenatus*/g of soil (Nematicide treated)
5. Control (Pasteurized soil)

GLASSHOUSE CONDITIONS

Eggplant seedlings of cv Black Beauty and potato tissue culture seedlings of cv. Shepody were used for glasshouse experiments. Natural light was supplemented with incandescent lamps for a photoperiod of 16h. Plants were watered when needed and fertilised weekly with Aquasol™ (N:P:K = 2.8:1:4.5). Disease assessment and petiole isolations were as described in Experiment 1. Eggplants were destructively sampled at 8 weeks after planting.

POST HARVEST QUANTIFICATION OF *V. DAHLIAE* DNA IN SOIL AND *P. CRENATUS* IN SOIL AND PLANT ROOTS

At harvest, 200 g and 500 g of soil from each pot of eggplants and potatoes respectively, were separately packed and sent to SARDI for DNA quantification of *V. dahliae.* The quantity of *V. dahliae* was expressed as pg DNA/g of soil.

For eggplant and potato plants 200 g each of soil from each pot and washed plant roots of eggplants were sent to SARDI for *P. crenatus* quantification.

At harvest for the potato trial the soil was removed from each pot, potato tubers recovered, weighed and yield expressed in grams (g).

STATISTICAL ANALYSIS

Statistical analyses were performed for all parameters except disease severity, using ANOVA. Tests for significant differences among treatments were analysed using the statistical package Minitab 16.

AUSTRALIAN POTATO CULTIVARS RESISTANT TO *V. DAHLIAE*

OPTIMISATION OF INOCULUM CONCENTRATION FOR THE GLASSHOUSE BIOASSAY

Two glasshouse pot trials were undertaken to optimise the inoculum density that would differentiate resistant cultivars in a glasshouse screening trial. Preliminary trials showed that inoculum density was important in symptom expression as high inoculum level (10^6 spores/mL) applied to roots of potato plants resulted in rapid plant death. Eggplants were used to optimise the level of inoculum since these plants were easy to infect and were more amenable to measure the impact of the inoculum concentrations on plant growth.

INOCULUM PREPARATION

A single spored isolate of the virulent isolate of *V. dahliae* (TASVd27) was used for all experiments. The isolate was grown on potato dextrose agar (PDA) in 90 mm Petri dishes and incubated in the dark at 24°C for 4 weeks. Spore suspension was made by adding 10 mL of sterile distilled water to the cultures and then spores were scraped with a sterile spatula. The concentrated spore suspension liquid was filtered using sterilised cheesecloth. Using haemocytometer and dilution, the spore concentration was adjusted to the required concentration.

PLANT MATERIALS

Potato

Fifteen, 1-month-old potato tissue culture seedlings (cv Russet Burbank C) were removed from Murishige and Skoog (MS) medium and transferred to pasteurised rough sand in 950 mL pots. The inner side of inverted clear plastic cups were misted and placed over each plant for 7 days. This provided a humid environment similar to inside the container of tissue culture plants to help them adapt to the transfer to sand. Tips of the plastic cups were then cut to allow air movement for a further 3 days. Then, the cups were removed and the plants were left for several weeks until they produced new foliage. Once new foliage was produced, granular fertilizer could be applied. The seedlings were grown in glasshouse at 22-25 °C and 12-14 hours photoperiod prior to inoculation.

Eggplants

Eggplant (cv Black Beauty) seeds were surface sterilised with bleach (available chlorine 1% ai) and sown on MS medium. These seeds were incubated (16 hours of photoperiod at 24°C; 8 hours of darkness at 18°C) for one month and transferred to pasteurised rough sand in 950 mL pots. They were treated similarly as potato tissue cultures seedlings. These seedlings were grown for another month in glasshouse at 22-25°C and 12-14 hours photoperiod prior to inoculation.

EXPERIMENT 1

SPORE INOCULUM CONCENTRATION

Potato

Potato seedlings were gently removed from sands and roots were washed under running tap water. These seedlings were inoculated with 4 different inoculum concentrations, 10^5, 10^4, 10^3, and 10^2 spores/mL and run from August to November 2011. Control plants were inoculated using sterile distilled water. Plants were inoculated by dipping the roots into 50 mL of suspension for 5 minutes. There were 3 replicates for each treatment and both inoculated and uninoculated control seedlings were arranged in a randomised design, grown under glasshouse condition and watered once a day. Granular fertilizer was added once during transplantation into bigger pots (2.8 L).

Eggplant

Eggplant seedlings (5 replicates, 5 treatments) were inoculated using the same technique and spore concentrations as for potatoes.

DISEASE ASSESSMENT

As the plants grew, foliar symptom severity was assessed and determined using a 0-5 qualitative visual scale that was developed by Tsror and Hazanovsky (2001), where 0 = no symptoms (i.e. no chlorosis or necrosis), 1 = chlorosis of lower leaves, 2 = moderate (30-50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (more than 50%) wilt and necrosis, 5 = death of plant.

For disease confirmation, leaves petioles were cultured on selected agar Ethanol Potassium Amoxillin Agar (EPAA). Once the fungus grew out of the vascular tissue, small piece of EPAA containing hyphae was transferred into Potato Dextrose Agar (PDA). Once the plant senesced, potato tubers, crown roots, underground stems and stems could be cultured onto EPAA and then on PDA to detect the presence of *V. dahliae*. Fungal isolates were examined under light microscope to confirm the presence of *V. dahliae*.

EXPERIMENT 2

The experimental design was similar to Experiment 1 with the following spore concentration treatments being used in the potato trial - 10^5, 10^4, $5x10^3$, 10^3 and 10^2 spores/mL; and run from September to December 2012. For eggplants the trial was run from May to July 2013 with the spore concentrations of 10^6, 10^5, 10^4, 10^3 and 10^2 spores/mL. Plants were assessed for disease severity using the 0 to 5 scale of Tsror and Hazanovsky (2001) for up to 12 and 9 weeks for potato and eggplant respectively. At harvest the crown and petiole tissue from each plant was cultured to detect presence of *V. dahliae*.

POTATO CULTIVAR SCREENING

Three glasshouse screening trials were conducted using 10^4 spores/mL (first trial) and $5x10^4$ spores/mL (second and third trials) with inoculum prepared as described above.

PLANT MATERIALS

Thirteen cultivars were assessed for resistance over three trials. Each trial consisted of 10, 8 and 10 cultivars respectively (Table 6-33,Table 6-34, Table 6-35) with a summary of the three trials in Table 6-35. Potato tissue culture plants (3 to 5 per cultivar; 33-39 days old) were established from *in vitro* grown seedlings as described above.

PATHOGENICITY TEST

Seedlings were gently removed from sand in pots and roots washed under running tap water. The roots were non-trimmed and dipped into 50 mL of inoculum suspension for 5 minutes. Uninoculated controls consisted of plants being dipped in water for 5 min. After transplanting into 2.8 L pots, the control and inoculated seedlings were grown under glasshouse conditions at 22-25 °C and 12-14 hours photoperiod in randomised position for 12 weeks. Granular fertilizer was applied once during the transplantation. These seedlings were watered regularly.

DISEASE ASSESSMENT AND CLASSIFICATION OF RESISTANCE

For diseases assessment, foliar symptom severity was assessed at weekly intervals using the 0 to 5 qualitative scale as described above. Resistance was assigned to those genotypes that had a rating of 0 or 1, 10 weeks after inoculation (WAI).

At 12 WAI, mature leaves from each plant were sampled and one cm long pieces of petiole tissue from each leaf were aseptically cultured onto PDA medium. Crown tissue of each plant was also cultured on PDA to determine infection by *V. dahliae*.

EFFICACY OF SOIL AMENDMENTS TO SUPPRESS VERTICILLIUM WILT DISEASE IN POTATOES

Microcosm assays were established to assess the efficacy of brown coal, atomic sulphur and blood and bone meal to inhibit microsclerotia germination of *V. dahliae* isolate TASVd27; and then the efficacy of brown coal to suppress Verticillium wilt disease was assessed in pot trials in the glasshouse. Microsclerotia were artificially produced using different methods specific for the two bioassays.

MICROCOSM ASSAY TO ASSESS THE EFFICACY OF BROWN COAL, ATOMIC SULPHUR AND BLOOD AND BONE MEAL ON VIABILITY OF *V. DAHLIAE* MICROSCLEROTIA

MICROSCLEROTIA PREPARATION FOR MICROCOSM ASSAYS

Verticillium dahliae cultures were grown in semi- solid Czapek Dox medium in Petri dishes for 4 to 6 weeks in the dark at 23°C until abundant microsclerotia developed. These were then harvested by blending the cultures for a few seconds to liquefy the medium, sieved through 74 µm nylon sieve and then mixed with sterile sand. The microsclerotia/sand mix was dried at room temperature over night, and finally sieved using the vibratory shaker into different size fractions (500, 250, 125, 80, 53, and 45 µm).

SOIL AMENDMENTS

a) Brown coal. Milled brown coal from Morwell, Victoria was composed of (based on dry matter weight): C 66.4%; H 4.84%; N 0.66%; O 26.9%; S 0.21%; 1% ash content (mostly Mg 0.24%; Ca 0.49%; Fe 0.2%, and <0.005% P).

b) Atomic sulphur. 98% sulphur, Incitec Pivot, Australia.

c) Blood and bone meal. Commercial product Grow Better®, Aust: N as animal blood, bone and flesh 6.0%; Citrate soluble 3.0%, Citrate insoluble 2.0%, Total P 5.0%

MICROCOSM ASSAY

Brown coal, atomic sulphur and blood and bone meal were added to 100 g of clean air dried soil from Ballarat, Victoria; to a concentration of 0, 1 and 10% and thoroughly mixed, then added to 50 mL Falcon tubes. Microsclerotia/sand mix (50 mg = ~60 microsclerotia of 125 µm size fraction) were inserted into nylon mesh (18 x 30 mm; Unirich mesh; mesh opening: 44 µm) bags and each bag buried in the soil in the Falcon tubes. The tubes were closed loosely to allow for air exchange. Water content of each soil subsample was adjusted to 10% to ensure the activity of soil microorganism would not be limited by low soil moisture. The tubes were then incubated in the dark at 23°C.

For the 1% amendment treatments, the nylon bags were removed from the tubes at weekly intervals for 2 months whereas for the 10% amendment treatments the bags were sampled at only 4 and 8 weeks after incubation. At each sampling time microsclerotia were assessed for germination, and the pH of the soil in each tube measured.

PH MEASUREMENT

On the day of analysis, after removal of the mesh bags, the soil in the tubes was mixed thoroughly with forceps. A subsample of soil (8 g) from each tube was added to 40 mL of cold distilled water, placed inside plastic bags then sealed. The sealed bags were mechanically disrupted with a Stomacher® 400 circulator (Seward International Pty Ltd) for 30 sec at normal speed. The bags were then shaken on an orbital shaker at 4°C for 1 hour. The slurry was then mechanically disrupted again and allowed to stand at room temperature before the pH was measured.

VIABILITY OF MICROSCLEROTIA

At each sampling time the recovered mesh bags were rinsed with sterile distilled water and then dried at room temperature for 3 hours. The microsclerotia from each bag were then dispersed on to EPAA agar using an Andersen sampler. The plates were then incubated for 2 weeks in the dark at 23°C. Microsclerotia were examined under low magnification at 20 X. The viability (%) was determined for 50 microsclerotia per sample.

STATISTICAL ANALYSIS

Statistical analyses were performed for soil pH and microsclerotia viability at weekly samplings, using ANOVA. Tests for significant differences among treatments were analysed using the statistical package Minitab 16. The interaction between soil pH and amendment treatments at weekly intervals was not significant. However, results analysed at 0, 4 and 8 weeks were significantly different at $p < 0.05$ and were thus presented separately.

GLASSHOUSE TRIAL WITH BROWN COAL

MICROSCLEROTIA PREPARATION FOR GLASSHOUSE POT TRIAL BIOASSAYS

Artificial production of microsclerotia was prepared differently as to those used in the microcosm bioassays. Clean, high carbon content soil was collected from Cora Lynn, Victoria and air dried. The soil was sieved through 500 µm sifter then 10 g of soil was placed inside 100 mm glass Petri dishes. The Petri dishes were then autoclaved at 121°C for 1 hour on two successive days. Two 5 mm diameter culture disks taken from 4-week-old *V. dahliae* isolate TASVd27 were placed into 100 mL sterile Czapek dox broth in 125 mL Erlenmeyer flask. These suspensions were incubated on an orbital shaker (400 rpm) for two weeks at room temperature (20-24 °C) in the dark. The spore suspensions were then filtered and the concentration adjusted to 10^6 spores/mL. The sterile soil was inoculated by pouring 12 mL of spore suspension into the soil under sterile conditions. After four weeks of incubation at 23°C, the inoculated soil was air-dried on the laboratory bench for 15 days to kill mycelia. The microsclerotia soil (MS) was sieved through different sizes of mesh (45 µm, 53 µm, 80 µm, 125 µm, and 250 µm) using a vibratory sieve shaker Fritsch Analysette 3 Spartan for 15 minutes. A subsample (25 mg of the 125 µm fraction, 5 replicates) of MS soil was evenly spread using Andersen sampler on EPAA media which then incubated in the dark at 23°C for 10 days to assess for microsclerotia germination. The number of viable microsclerotia that germinated were determined and expressed as colony forming units (CFU)/g of soil.

OPTIMISATION OF INOCULUM CONCENTRATION

Before a pot trial could be undertaken to assess the efficacy of brown coal to suppress Verticillium wilt, two glasshouse experiments were set up to assess the level of microsclerotia inoculum required to cause

disease. One trial at DPI (Knoxfield) consisted of 0, 50 and 500 CFU/g of potting mix; and trial 2 at the University of Melbourne (UM), Parkville, consisted of 0, 50, 250 and 500 CFU/g of potting mix. The size fraction of microsclerotia in both trials was 125 µm.

Before planting, inoculum was spread evenly onto the pasteurised potting mix (about 1.5 kg for each treatment) and thoroughly mixed. After incorporation of microsclerotia into the soil, subsamples for each treatment were taken before planting for microsclerotia enumeration (50 g). The microsclerotia enumeration at pre-planting was done to confirm inoculum concentration. The subsample that was taken for microsclerotia enumeration was air dried for 3 weeks, sieved through 500 µm sieve, and spread on EPAA using the Andersen sampler. The number of germinated microsclerotia was recorded after 10 days incubation.

Potato seedlings were removed from sand, washed and one plant was transplanted into each 1.5 L pot containing potting mix with inoculum. After transplanting, the plants were grown under glasshouse conditions at 22-25°C and 12-14 hours photoperiod in a randomised block design. The plants were regularly watered and fertilised with liquid Aquasol at the recommended rates as previously described. As the plants grew, foliar symptom severity was assessed weekly and determined using the 0-5 qualitative visual scale (Tsror and Hazanovsky, 2001). For confirmation of infection by *V. dahliae*, leaf petioles were cultured at two week intervals on EPAA. Once the plants began to senesce, potato tubers, crown roots, underground stems and stems were sampled and cultured onto EPAA to detect for the presence of *V. dahliae*. Fungal isolates were examined under the microscope to confirm the presence of *V. dahliae*.

EFFICACY OF 1% BROWN COAL TO SUPPRESS VERTICILLIUM WILT

A glasshouse pot trial with five replicates per treatment was established to assess the efficacy of 1% brown coal to suppress Verticillium wilt disease in potatoes. Different concentrations of microsclerotia of 125 µm sieve size (500 and 750 CFU/g of dry soil) were incorporated into pasteurized potting mix containing 1 % brown coal in 1.5 L pots. Treatments also consisted of soil with just 1% brown coal and soil with just microsclerotia at 500 and 700 CFU/g of dry soil. Controls consisted of pots without brown coal and microsclerotia.

Before planting, inoculum and/or brown coal were spread evenly onto the pasteurised potting mix and thoroughly mixed. After incorporation of brown coal and microsclerotia into the soil, subsamples for each treatment were taken before planting for microsclerotia enumeration (50 g). The microsclerotia enumeration at pre-planting was done to confirm inoculum concentration. The subsample that was taken for microsclerotia enumeration was air dried for 3 weeks, sieved through 500 µm sieve, and spread on EPAA using the Andersen sampler. The number of germinated microsclerotia was recorded after 10 days incubation.

Thirty potato seedlings were removed from sand, washed and one plant was transplanted into each 1.5 L pots containing potting mix with inoculum and/or brown coal. After transplanting, the plants were grown at Parkville under glasshouse conditions at 22-25°C and 12-14 hours photoperiod in a randomised block design. The plants were regularly watered and fertilised with liquid Aquasol at the recommended rates. As the plants grew, foliar symptom severity was assessed weekly and determined using the 0-5 qualitative visual scale (Tsror and Hazanovsky, 2001). For confirmation of infection by *V. dahliae*, leaf petioles were cultured on EPAA. At senescence the above ground biomass of each plant was dried in an oven at 60^{o}C for 24 hours then weighed. Potato tubers, crown roots and underground stems were sampled and cultured onto EPAA to detect for the presence of V. *dahliae*. Fungal isolates were examined under the microscope to

confirm the presence of *V. dahliae*. The postharvest inoculum level was quantified by DNA analysis at SARDI.

RESULTS

INCIDENCE OF VERTICILLIUM SPECIES IN POTATO SEED LOTS IN VICTORIA AND TASMANIA

The majority of *Verticillium* isolates were identified based on morphological characteristics on SPT and EPAA medium. The 8 isolates of *V. albo-atrum*, and 4 *V. tricorpus* were confirmed by the ITS gene sequence analysis. Overall, based on the molecular identification and formation of resting structures and colour of colonies, three *Verticillium* species (*V. dahliae*, *V. albo-atrum* and *V. tricorpus*) were identified and compared with published descriptions (Hawksworth, 1970; Hawksworth and Talboys, 1970a; Hawksworth and Talboys, 1970b). On both media, *V. dahliae* microsclerotia were dark brown to black and regularly or irregularly distributed throughout the colonies which were sharply differentiated from the hyaline mycelium. The hyphae of *V. albo-atrum* differentiated into thick-walled melanised cells that became dark resting mycelia. *Verticillium tricorpus* formed large and irregular shaped microsclerotia and resting mycelium after 15 days of incubation. Most of the *V. tricorpus* isolates produced a yellow to orange pigment that diffused into the culture medium. *Colletotrichum coccodes*, *Fusarium* species and *Plectospaerella cucumerina* were also frequently isolated from the vascular tissue of seed tuber.

In 2010, 33% of seed lots collected from Ballarat and Cora Lynn region of Victoria were infected with *V. dahliae*, which was the only species isolated from the vascular tissue of seed tubers. In 2011, 31% of seed lots were infected with *V. dahliae* while 6% of seed lot were infected with *V. albo-atrum*. *V. tricorpus* was not identified in 2010 or 2011. In 2012, *V. dahliae* (18%), *V. albo-atrum* (9%) and *V. tricorpus* (9%) were isolated from 11 seed lots in Victoria (Table 6-4). From the Tasmanian seed tuber survey in 2011, *V. dahliae* (10%), *V. albo-atrum* (20%) and *V. tricorpus* (20%) were isolated from the vascular tissue of seed tubers from 10 seed lots. In 2012, infection by *V. dahliae* was comparatively higher with 31% seed lots infected from 13 seed lots. A greater percentage of seed lots in Tasmania were infected by *V. albo-atrum* (23%) compared to Victoria (Table 6-4).

Table 6-4 – Proportion of seed lots infected by *Verticillium* species in Victoria and Tasmania (2010-2012)

	2010		2011				2012			
	No of seed lots tested	***V. dahliae***	**No of seed lots tested**	***V. dahliae***	***V. albo-atrum***	***V. tricorpus***	**No of seed lots tested**	***V. dahliae***	***V. albo-atrum***	***V. tricorpus***
Victoria	33	11 (33%)	16	5 (31%)	1 (6%)	0	11	2 (18%)	1 (9%)	1 (9%)
Tasmania	0	0	10	1 (10%)	2 (20%)	2 (20%)	13	4 (31%)	3 (23%)	1 (7%)

In Victoria, overall percent infections of seed lots from 2010 to 2012 were 30% for *V. dahliae*, 3% for *V. albo-atrum*, 2% for *V. tricorpus* out of 60 seed lots tested. Overall infection of *V. albo-atrum* (22%) and *V. tricorpus* (13%) was higher in Tasmanian seed potatoes. In general, Tasmania had a higher level of seed lot infection than Victoria (Table 6-5).

Table 6-5 – Overall proportion of seed lots infected by *Verticillium* species in Victoria and Tasmania (2010 to 2012)

Location	No of seed lots tested	Percent seed lots from which *Verticillium* species isolated		
		V. dahliae	*V. albo-atrum*	*V. tricorpus*
Victoria	60	30	3	2
Tasmania	23	22	22	13

Infection by *Verticillium* spp within a seed lot varied greatly and ranged from 0–55%, while overall infection by *Verticillium* spp totalled 41% (*V.dahliae* 28%, *V.ablo-atrum* 8% and *V.tricorpus* 5%). Over 12% of seed lots tested ranged from 0-5% infection within the seed lots. Only one seed lot had 50% of seed tubers infected with *V. dahliae* (Table 6-6).

Table 6-6 – Detection of Verticillium spp within the seed lots

Percent of tubers infected within the seed lot	*V. dahliae*		*V. albo-atrum*		*V. tricorpus*	
	Number of seed lots	Percent of total tested	Number of seed lots	Percent of total tested	Number of seed lots	Percent of total tested
0-5	10	12	0	0	0	0
6-10	4	5	5	6	1	1
11-20	3	4	1	1	2	2
21-30	2	2	1	1	0	0
31-40	3	4	0	0	1	1
41-60	1	1	0	0	0	0

VASCULAR DISCOLOURATION AND *V. DAHLIAE* RECOVERY

Stem-end vascular discoloration was not correlated with presence or absence of the *V. dahliae*. Of 416 vascular discoloured tubers tested, 11% were infected with *V. dahliae* (Table 6-7). Of 1244 tubers without vascular discolouration 3.3% were infected with *V. dahliae* (Table 6-8). In general, the percent recovery rate of *V. dahliae* from vascular discoloured tubers was 0-35.7% (Table 6-7) and non vascular discoloured tubers was 0-11.7% (Table 6-8).

Table 6-7 – *Verticillium dahliae* isolation from tuber stem ends showing brown vascular discolouration

Cultivars	Number of vascular discoloured tubers	*V. dahliae* recovered	Per cent of recovery
Russet Burbank	126	16	12.6
Innovator	56	7	12.5
Ranger Russet	46	6	13
Atlantic	39	8	20.5
Catani	25	0	0

Cultivars	Number of vascular discoloured tubers	*V. dahliae* recovered	Per cent of recovery
Trent	24	4	16.6
Pike	18	0	0
Nooksack	14	5	35.7
Desiree	13	0	0
Nadine	11	0	0
Simcoe	11	0	0
Kennebec	9	0	0
Shepody	7	0	0
Sebago	6	0	0
Bondi	4	0	0
Nicola	2	0	0
Topcat	2	0	0
Harmony	2	0	0
Wont Scab	1	0	0
Moonlight	0	0	0
Total	416	46	11

Table 6-8 – *Verticillium dahliae* isolation from tuber stem ends without brown vascular discolouration

Cultivars	Number of tubers without vascular discolouration	*V. dahliae* recovered	Per cent of recovery
Russet Burbank	334	14	4.1
Innovator	224	9	4
Atlantic	181	9	4.9
Ranger Russet	94	0	0
Pike	62	0	0
Trent	56	6	10.7
Nadine	49	0	0
Sebago	34	4	11.7
Kennebec	31	0	0
Simcoe	29	0	0
Moonlight	20	0	0
Wont Scab	19	0	0

Cultivars	Number of tubers without vascular discolouration	*V. dahliae* recovered	Per cent of recovery
Nicola	18	0	0
Topcat	18	0	0
Harmony	18	0	0
Bondi	16	0	0
Catani	15	0	0
Shepody	13	0	0
Desiree	7	0	0
Nooksack	6	0	0
Total	1244	42	3.3

TAXONOMY OF *VERTICILLIUM* SPECIES

MORPHOLOGY

All *Verticillium* isolates produced flocculose colonies with conidiophores which were more or less erect with verticillate branches. Conidia were borne in small clusters at the tip of each phialide. Based on the size of conidia, formation of resting structures, and colour of colonies on PDA, three *Verticillium* species (*V. dahliae*, *V. albo-atrum*, *V. tricorpus*) were isolated from potatoes in Australia (Figure 6-4).

SEQUENCING AND PHYLOGENETIC ANALYSIS

Sequences for the ITS, Bt and EF1-α gene regions for all 29 isolates were deposited in GenBank® (Table 6-2). Sequences of the three genes were aligned and analysed separately by Kimura 2-parameter model.

The dendogram based on the ITS gene sequences showed the separation of the *Verticillium* species into clades with high boot strap values with *V. tricorpus* and *V. albo-atrum* being more closely related than to *V. dahliae*. The phylogenetic tree based on ITS provided differentiated 9 of the 10 *Verticillium* species with the exception being *V. longisporum* which was closely related to *V. dahliae*. Australian isolates of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* were similar to the ex-type strains with high boot strap values. *G. nigrescens* was phylogenetically more distinct to the other species (Figure 6-5).

All three genes were combined for increased phylogenetic resolution. The aligned data set for the combined three gene regions consisted of 1176 characters. The phylogenetic tree revealed the three monophyletic groups corresponding to the three *Verticillium* species with *V. albo-atrum* being more closely related to *V. dahliae* than to *V. tricorpus*. Isolates of each species showed no variation within the clades (Figure 6-6). The isolates of *V. dahliae* from tomato and strawberry were genetically similar to isolates from potato.

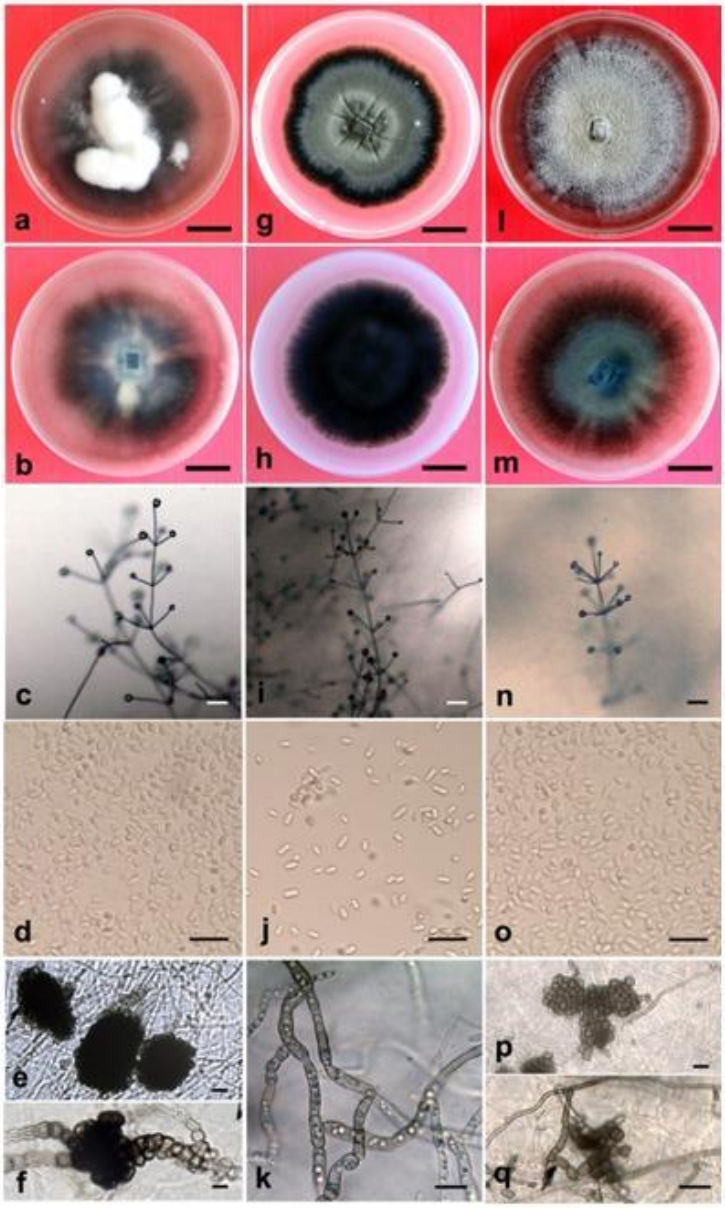

Figure 6-4 – Morphological features of *Verticillium* species. a. Colony of *V. dahliae* isolate TASVd25 after 4 weeks on PDA, frontal view. b. Reverse view. c. Conidiophores. d. Conidia. e. Microsclerotia. f. Short pigmented hyphae atteched to microsclerotia. g. Colony of *V. albo-atrum* isolate VICVaa1 after 4 weeks on PDA, frontal view. h. Reverse view. i. Conidiophores. j. Conidia. k. Dark resting mycelium forming a 'knot' in culture. l. Colony of *V. tricorpus* isolate TASVt1 after 4 weeks on PDA, frontal view. m. Reverse view. n. Conidiophores. o. Conidia. p. Microsclerotia. q. Dark resting mycelium and chlamydospores. Scale bar: a, b, g, h, l, m = 2cm; c, i, n = 40 µm; d, e, f, j, k, o, p, q = 20 µm.

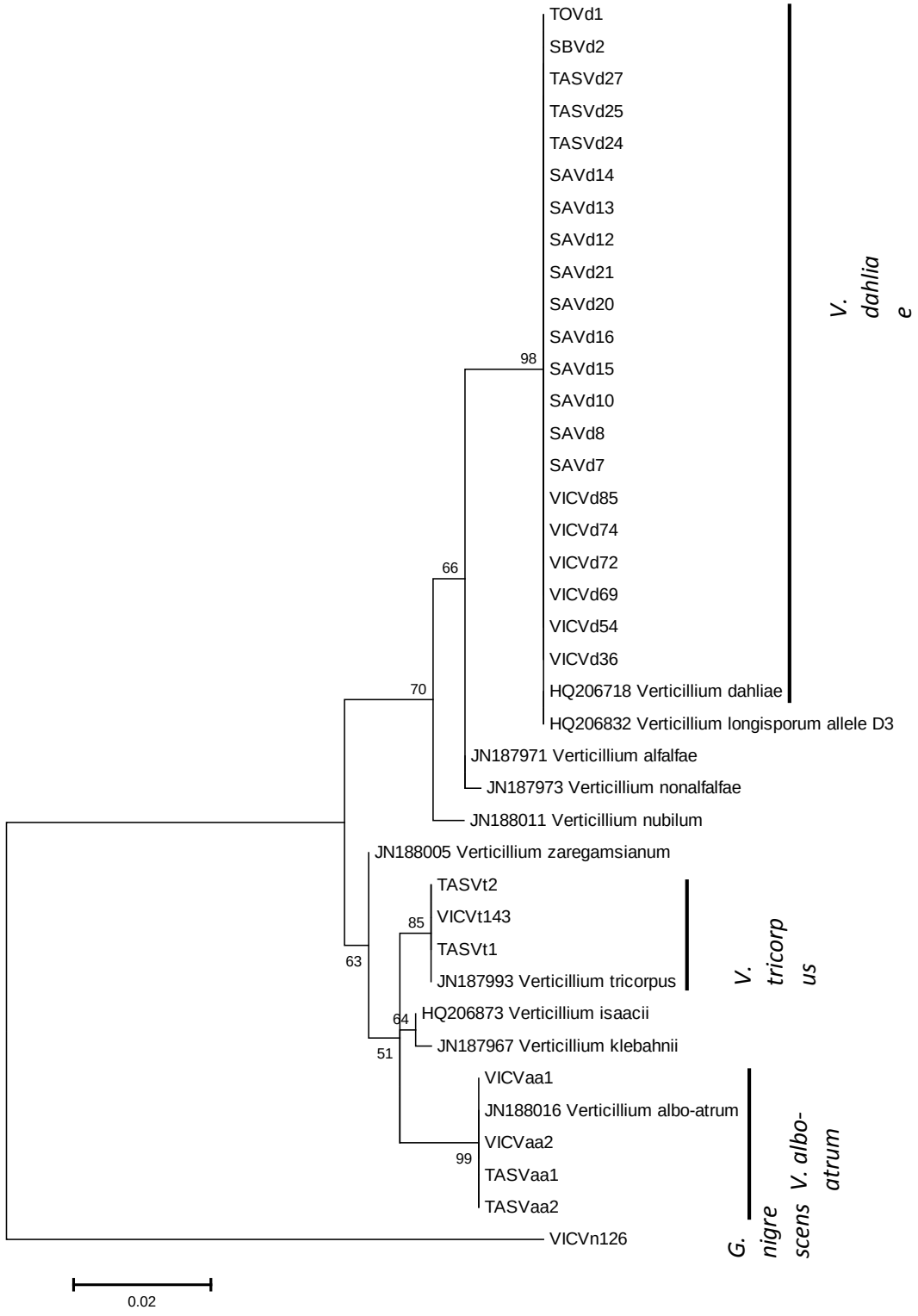

Figure 6-5 – Dendogram based on ITS sequences of Australian *Verticillium* isolates and comparison of type and ex-type strains of 10 *Verticillium* species (Inderbitzin *et al.*, 2011a; Inderbitzin *et al.*, 2011b) using the Maximum likelihood method with 1000 boot strap replicates. The tree was rooted with *G. nigrescens*

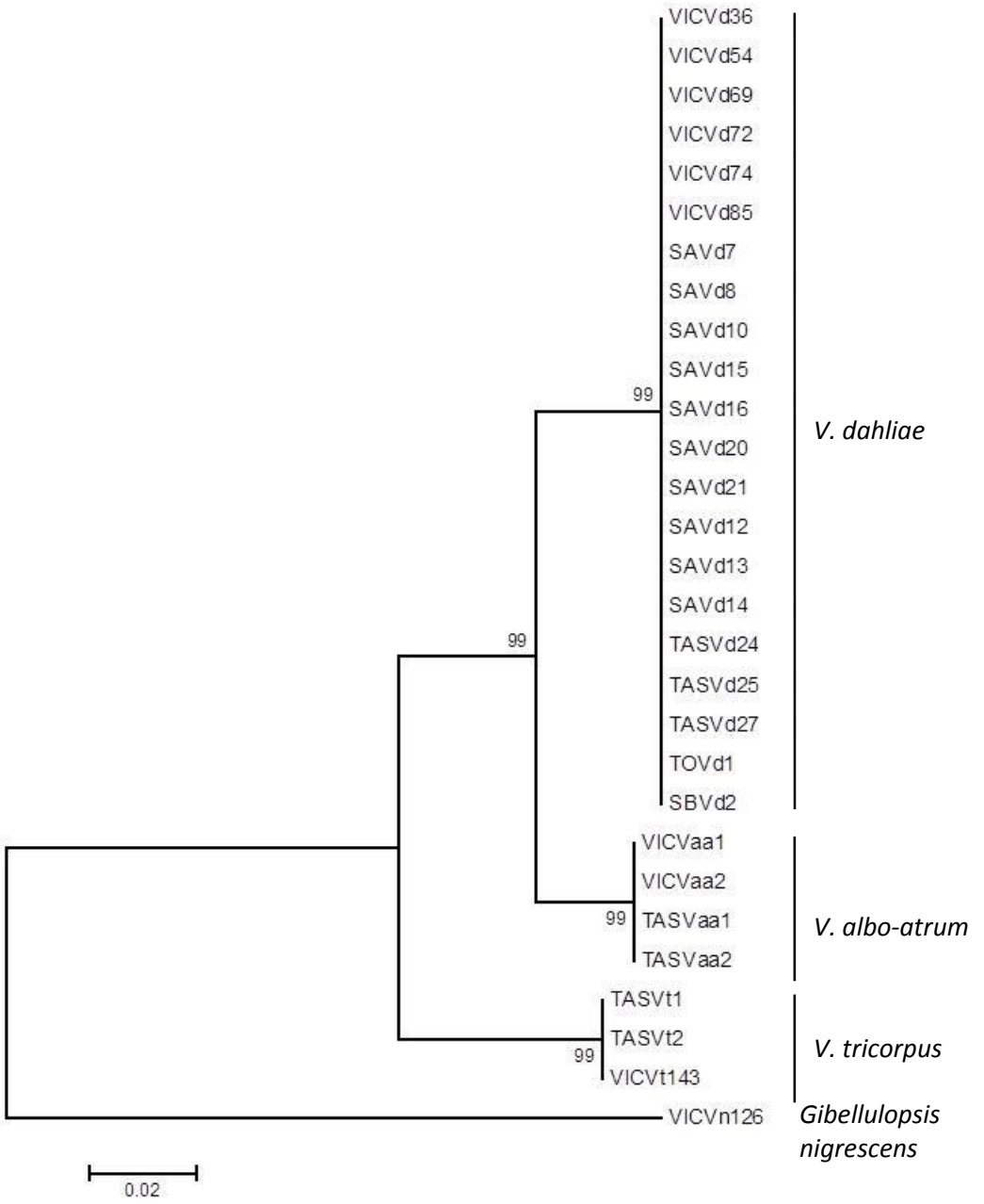

Figure 6-6 – Dendogram based on combined sequence of ITS, partial Bt and EF1-α of *Verticillium* isolates using the Maximum Likelihood method based on the Kimura 2-parameter model.

PATHOGENICITY OF POTATO *V. DAHLIAE* ISOLATES

PATHOGENICITY OF POTATO *V. DAHLIAE* ISOLATES ON POTATO CULTIVARS

The two potato cultivars inoculated with *V. dahliae* exhibited typical Verticillium wilt symptoms ranging from chlorosis to necrosis which started from the margin of the leaf then along the main vein followed by wilting (Figure 6-7). The disease symptoms were first observed on the lower leaves and appeared earlier on the susceptible cv. Shepody, 2 weeks after inoculation (wai), compared to 3 wai on the moderately resistant cv. Ranger Russet. The disease symptoms progressed faster at 4 wai on cv Shepody than on cv

Ranger Russet. At 6 wai, in the 2011 trial, disease severity was high for the cv Shepody inoculated with TASVd24, 25 and 27 isolates with death of the plants (rating 5). In the 2012 trial, the disease severity was not as high with no plant death (Table 6-9) with infection by isolate TASVd25 and 27 being the most virulent producing a disease severity rating of 4 in Shepody plants. On Ranger Russet, similar trends were observed as in the 2011 trial with TASVd25 being the most virulent causing the plants to die (5) and TAS Vd24 producing a rating of 4.

In the 2012 trial, TASVd25 was the most virulent on Ranger Russet with a rating of 4, while all other isolates, except VICVd54, only producing a disease severity rating of 2. VICVd54 was less virulent in Ranger Russset only showing moderate infection (1). The isolate VICVd54 caused mild symptoms on infected plants in both trials. No Verticillium wilt symptoms were observed in non-inoculated control plants, nor was the pathogen reisolated.

Figure 6-7 – Comparison between inoculated (TASVd25 left) and non-inoculated control plant (cv Shepody) five weeks after planting in 2011.

Table 6-9 – Verticillium wilt disease severity on potatoes cvs Shepody and Ranger Russet (6 wai) inoculated with different *V. dahliae* isolates compared to a non- inoculated control

Treatment	Disease severity*			
	2011		2012	
	Shepody	Ranger Russet	Shepody	Ranger Russet
VICVd36	4	2	3	2
VICVd54	2	1	2	1
VICVd85	4	3	2	2
SAVd7	4	3	2	2
SAVd16	3	3	2	2
SAVd12	4	2	3	2
TASVd24	5	4	3	2
TASVd25	5	5	4	4
TASVd27	5	3	4	2
Control	0	0	0	0

*Mean disease severity of 4 plants rounded to the nearest whole number. Disease severity index was rated on each plant with the following scale: 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30-50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (more than 50% of leaves) wilt and necrosis, and 5 = death of plant (Tsror and Hazanovsky, 2001). Isolates that caused a disease severity of ≥2 were considered pathogenic.

PATHOGENICITY OF POTATO *V. DAHLIAE* ISOLATES ON EGGPLANTS

In 2011 all isolates, with the exception of VICVd54, induced Verticillium wilt symptoms 2 wai in eggplant seedlings. The disease symptoms appeared on the lower leaves first and then symptoms progressed in the subsequent weeks. At 7 wai, the disease severity of plants inoculated with TASVd25 isolate was high with an incidence severity scale of 4 (severe wilt and necrosis). At 7 wai, in both trials, TASVd25 inoculated plants induced higher disease severity compared to other treatments. In 2012, disease severity was less (rating of 2) in plants inoculated with VICVd36, SAVd12 and TASVd24 isolates; and a rating of 1 for plants inoculated with VICVd54. However, TASVd25 was the most virulent producing a disease severity rating of 4, similar to the 2011 trial. *V. dahliae* was recovered and morphologically identified from petioles of infected plants. The pathogen was not isolated from un-inoculated control plants (Table 6-10).

All isolates of *V. dahliae* (including the least virulent isolate VICVd54) in both trials significantly reduced eggplant height, aerial biomass and below ground root mass compared to non-inoculated control. In 2011, TASVd25 was the most virulent isolate resulting in significant ($p \leq 0.05$) reduction of plant height and aerial biomass at 7 wai (Table 6-11 and Figure 6-8). Although, the root weight was significantly reduced compared to most other isolates, there was no significant difference between TASVd25, TASVd24 and SAVd12. In contrast in 2012, TASVd25 and TASVd27 significantly reduced the height but no significant difference was detected between most isolates for aerial biomass and root weight (Table 6-11). Nevertheless, aerial biomass of plants inoculated with TASVd25 was significantly lower compared to isolates VICVd54 and SAVd7.

Table 6-10 – Verticillium wilt severity recorded on eggplant cv Black Beauty (7 wai) inoculated with different *V. dahliae* isolates compared to a non- inoculated control

Treatment	Disease severity*	
	2011	2012
VICVd36	3	2
VICVd54	2	1
VICVd85	2	2
SAVd7	2	2
SAVd16	2	2
SAVd12	3	2
TASVd24	3	2
TASVd25	4	4
TASVd27	2	2
Control	0	0

*Mean disease severity of 4 plants rounded to the nearest whole number. Disease severity index was rated on 0-5 scale (Tsror and Hazanovsky, 2001). Isolates that caused a disease severity of ≥2 were considered pathogenic.

Table 6-11 – Effect of *V. dahliae* isolates on plant height, aerial biomass and root weight in eggplant cv Black Beauty

Treatment	Plant height (cm)		Dry weight (g)			
			Aerial biomass		Root	
	2011	2012	2011	2012	2011	2012
VICVd36	9.0 b*	13.7 d	4.23 bc	1.40 bc	1.25 b	0.65 bc
VICVd54	9.2 b	15.7 bc	3.86 bcd	1.90 b	1.26 b	0.85 b
VICVd85	10.1 b	13.7 d	2.90 bcd	1.50 bc	0.74 bcd	0.65 bc
SAVd7	9.5 b	14.2 cd	3.12 bcd	1.80 b	0.78 bcd	0.80 b
SAVd16	10.0 b	16.2 b	4.70 b	1.40 bc	1.21 bc	0.67 bc
SAVd12	6.6 b	14.7 bcd	2.20 d	1.70 bc	0.55 de	0.75 b
TASVd24	9.8 b	13.5 de	2.50 cd	1.40 bc	0.61 cde	0.75 b
TASVd25	4.1 c	10.0 f	0.28 e	0.80 c	0.08 e	0.35 c
TASVd27	10.2 b	11.7 ef	3.88 bcd	1.40 bc	1.06 bcd	0.57 bc
Control	14.2 a	18.2 a	8.17 a	4.10 a	2.65 a	1.50 a

*Means with the same letter within a column are not significantly different

Figure 6-8 – Comparison between highly aggressive isolate (TASVd25 left) treated and non-inoculated control plant seven weeks after planting in 2011.

PATHOGENICITY OF *V. DAHLIAE, V. ALBO-ATRUM* AND *V. TRICORPUS* ON POTATO

Plants inoculated with the high and low aggressive isolates of *V. dahliae* showed typical Verticillium wilt symptoms 2 wai however at 6 wai the highly aggressive isolate (TASVd25) of *V. dahliae* showed disease severity of 4 compared to the less aggressive isolate (VICVd54) which had a rating of 2. Both isolates of *V.*

albo-atrum also induced wilt symptoms at 2 wai, and at 6 wai symptoms of yellowing of lower leaves, necrosis and wilting of plants, were similar to the symptoms produced by *V. dahliae* inoculated plants but with a disease severity scale of 3 (moderate wilt and necrosis). Plants inoculated with *V. tricorpus* isolates showed mild symptoms on potato 3 wai, then at six weeks, plants showed moderate wilt with severe chlorosis (score 2) (Figure 6-9). All the *Verticillium* species were pathogenic to potato and were re-isolated from the petioles of plant. The pathogen was not reisolated from non-inoculated control plants.

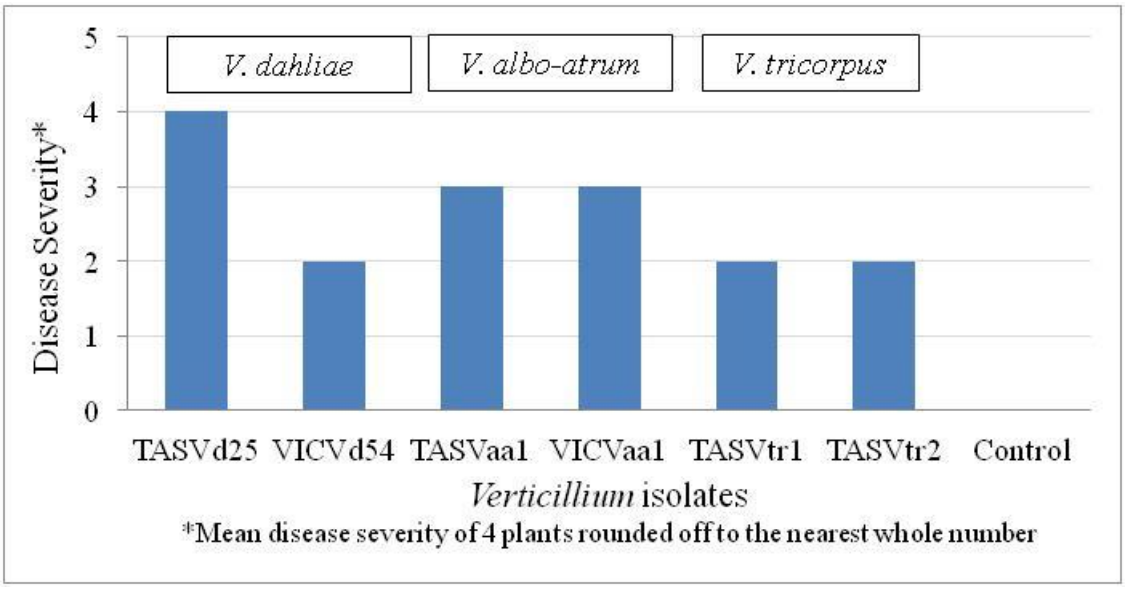

Figure 6-9 – Verticillium wilt severity (0-5 scale)* (Tsror and Hazanovsky, 2001) recorded on potatoes cv Shepody with different *V. dahliae* (TASVd25 and VICVd54), *V. albo-atrum* (VICVaa1 and TASVaa1) and *V. tricorpus* (TASVt1 and TASVt2) isolates compared to a non- inoculated control 6 wai.

ROLE OF *V. DAHLIAE* INFECTED TUBERS IN CAUSING VERTICILLIUM WILT DISEASE

EXPERIMENTS 1 AND 2: ROLE OF *V. DAHLIAE* INFECTED TUBERS ON VERTICILLIUM WILT DEVELOPMENT (2011 AND 2012)

In 2011, plants that grew from infected tubers into pasteurized soil developed very mild Verticillium wilt symptoms of chlorosis of lower leaves (rating of 1). Symptoms appeared in the lower leaves at later stages (65 DAP) and were very difficult to distinguish from normal senescence of plants. *V. dahliae* was present in the petiole of symptomless plants of all cultivars. The most susceptible cultivars Atlantic and Trent had the highest disease incidence (3 plants out of 5 infected) and the least from the moderate resistant cv Ranger Russet (1/4) at 65 DAP (Table 6-12). The fungus was not isolated from uninfected seed tuber treatments (data not shown).

Progeny tuber infection in cultivars after harvest ranged from 0 to 13%. The highest percent infection was observed in cv. Atlantic (13%) followed by cv. Nooksack (10%). The cv. Ranger Russet did not have any progeny tubers infected (Table 6-12). The planted infected tubers in each pot contributed to the *V. dahliae* inoculum in the soil. The mean pg *V. dahliae* DNA/g of soil was higher in cv. Atlantic planted soil (63 pg *V. dahliae* DNA/g of soil) ranging from 1-177 pg *V. dahliae* DNA/g of soil. The least was 11 pg DNA/g of soil (range 3-31 pg DNA/g of soil) in cv Ranger Russet planted soil (Table 6-12). There appeared to be a correlation between incidence of infection in the petioles and progeny tuber infection, and the level of *V. dahliae* in the soil. The highest number of petiole isolations, progeny tuber infection and level of *V. dahliae*

in the soil were observed in cv Atlantic. There was no significant difference in the yield between infected and non infected plants (Table 6-13).

Table 6-12 – *V. dahliae* isolation, progeny tuber infection and post harvest quantity of *V. dahliae* in five cultivars (infected tubers in healthy soil in 2011)

Cultivars	Disease severity 65 DAP (0-5 scale)[A]	Incidence of *V. dahliae* (infected number of plants from 5 replicates)			Incidence of *V. dahliae* (infected number of tubers from 15 replicates)[B]	*V. dahliae* pg DNA/g of soil[C]	
		45 DAP	55 DAP	65 DAP		Mean	Range
Russet Burbank	1	1	1	2	1 (6.6%)	34 [a]	1 - 119
Atlantic	1	1	2	3	2 (13.3%)	63 [a]	4 - 177
Trent	1	2	2	3	1 (6.6%)	43 [a]	10 - 74
Ranger Russet	1	0[D]	0	1	0	11 [a]	3 - 31
Nooksack	1	1	2	2	1 (6.6%)	23 [a]	1 - 71

Means with the same letter within a column are not significantly different

[A]Mean disease severity rounded off to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Haznovsky (2001)

[B]Three tubers from each plants were randomly selected for isolation at harvest

[C]Mean and range of pg DNA/g of soil of 5 plants rounded to the nearest whole number

[D]Incidence from 4 replicates for Ranger Russet

Table 6-13 – Effect of infected and non infected tubers on yield (g) in 2011

Tubers	Russet Burbank	Atlantic	Trent	Ranger Russet	Nooksack
Infected	234 [a]	215 [a]	162 [a]	149 [a]	146 [a]
None infected	215 [a]	231 [a]	155 [a]	156 [a]	156 [a]

Means with the same letter within a column are not significantly different

In the 2012 trial, very low disease severity was observed in plants grown from infected tubers planted in the pasteurized soil. Symptom expression was very difficult to distinguish from normal senescence of plants and rated as 1 at 65 DAP. This was similar to the observation in the 2011 experiment regarding symptom severity. The fungus was present in non wilted plants of all cultivars. Two plants from cvs Atlantic and Russet Burbank were infected with *V. dahliae* which was confirmed by re-isolation of the pathogen from the petiole (Table 6-14). The pathogen was not isolated from the petiole of uninfected seed tuber treatments (data not shown).

Progeny tuber infection in cultivars after harvest ranged from 0 to 8.3%. The cvs Atlantic and Russet Burbank were infected with *V. dahliae,* 8.3% each of progeny tubers (Table 6-14). The planted infected tubers contributed to the *V. dahliae* inoculum in the soil. The mean pg *V. dahliae* DNA/g of soil was higher in cv. Russet Burbank planted soil (70 pg *V. dahliae* DNA/g of soil) followed by cv Atlantic (67 pg *V. dahliae* DNA/g of soil) with a range of 6-149 and 1-144 pg *V. dahliae* DNA/g of soil respectively (Table 6-14). There appeared to be a correlation between petiole infection, progeny tuber infection and the level of *V. dahliae* in the soil, which was similar to the experiment in 2011. There was no significant difference in the yield between infected and non infected plants (Table 6-15).

Table 6-14 – *V. dahliae* isolation, progeny tuber infection and quantification in four cultivars (infected tubers in healthy soil in 2012)

Cultivars	Disease severity 65 DAP (0-5 scale)[A]	Incidence of *V. dahliae* (infected number of plants from 4 replicates)			Incidence of *V. dahliae* (infected number of tubers from 12 replicates)[B]	*V. dahliae* pg DNA/g of soil[C]	
		45 DAP	55 DAP	65 DAP		Mean	Range
Atlantic	1	0	1	2	1 (8.3%)	67 [a]	1 - 144
Sebago	1	0	0	1	0	21 [a]	13 - 31
Innovator	1	0	1	1	0	33 [a]	6 - 51
Russet Burbank	1	0	1	2	1 (8.3%)	70 [a]	6 - 149

Means with the same letter within a column are not significantly different

[A]Mean disease severity rounded off to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2011)

[B]Three tubers from each plants were randomly selected for isolation at harvest

[C]Mean and range of pg DNA/g of soil of 4 plants rounded off to the nearest whole number

Table 6-15 – Effect of infected and non infected tubers on yield (g) in 2012

Tubers	Atlantic	Sebago	Innovator	Russet Burbank
Infected	229[a]	230 [a]	227 [a]	234 [a]
None infected	249[a]	228 [a]	229 [a]	244 [a]

Means with the same letter within a column are not significantly different

EXPERIMENT 1A: VERTICILLIUM WILT DEVELOPMENT IN PLANTS GROWN IN INFESTED SOIL FROM EXPERIMENT 1 (2011)

Based on molecular quantification, a large difference in the preplant quantity of *V. dahliae* was observed in the soil of the various cultivars from experiment 1 (Table 6-16). Soil inoculum originating from naturally infected individual tubers did not contribute to subsequent disease development in the moderately susceptible Russet Burbank and very susceptible Shepody cultivars. However, there appeared to be a correlation between soil inoculum level and petiole infection. Out of 24 plants of cv. Russet Burbank and

Shepody in various levels of *V. dahliae* infested soil, only 2 and 4 plants respectively were infected with *V. dahliae*. However, disease symptoms and progeny tuber infection did not occur in all these plants. After harvest the soil samples showed a reduction of quantity of *V. dahliae* (pg *V. dahliae* DNA/g of soil) in the majority of soils (Table 6-16).

Table 6-16 – The effect of soil inoculum on subsequent Verticillium wilt development

Cultivars	Preplant level (pg DNA/g of soil)	Russet Burbank		Shepody	
		Petiole isolation (65 DAP)	Postharvest *V. dahliae* (pg DNA/g of soil)	Petiole isolation (65 DAP)	Postharvest *V. dahliae* (pg DNA/g of soil)
Russet Burbank	119	+	20	+	22
	25	-	12	-	8
	22	-	4	-	3
	1	-	0	-	0
	5	-	0	-	1
Atlantic	177	-	22	+	6
	41	-	1	-	1
	14	-	4	-	7
	4	-	27	-	3
	80	+	5	-	7
Trent	74	-	11	-	59
	48	-	8	-	18
	10	-	1	-	0
	69	-	5	+	2
	13	-	3	-	7
Ranger Russet	31	-	46	-	202
	6	-	0	-	0
	4	-	0	-	0
	3	-	0	-	0
Nooksack	36	-	16	-	15
	71	-	8	+	7
	0	-	0	-	0
	9	-	7	-	28
	1	-	2	-	1

+ = *V. dahliae* isolated

- = *V. dahliae* not isolated

DAP = Days after planting

EXPERIMENT 3: ROLE OF *V. TRICORPUS* INFECTED TUBERS ON VERTICILLIUM WILT DEVELOPMENT

Plants grown from the infected *V. tricorpus* tubers (cv Nicola) planted in pasteurized soil produced very few Verticillium wilt symptoms. Symptoms appeared in later stages (65 DAP) and were very difficult to distinguish from normal senescence of plants. The overall disease rating was 1. *V. tricorpus* was re-isolated from the petiole of symptomless plants (6 out of 8 plants). One out of 24 progeny tuber was infected by the pathogen. Four out of 8 infected tubers contributed to the *V. tricorpus* inoculum into the soil. The mean soil population density of *V. tricorpus* was 1 CFU/g of dry soil. There was no significant difference in the yield between infected and non infected plants (Table 6-17). The pathogen was not isolated from uninfected seed tubers.

Table 6-17 – Verticillium wilt disease incidence and severity of *V. tricorpus* infection in plants grown from infected tubers of cv Nicola, progeny tuber infection and quantification of *V. tricorpus* in soil at harvest

Tubers	No. of plants	*V. tricorpus* isolated	Disease severity 65 DAP (0-5 scale)[A]	Incidence of *V. dahliae* (infected number of tubers from 24 replicates)	Replications from which *V. tricorpus* was recovered from the soil	Mean CFU/g of soil	Yield (g)
Infected	8	6	1	1	4	1	243[a]
None infected	8	0	0	0	0	0	251[a]

Means with the same letter within a column are not significantly different

[A]Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001)

EXPERIMENT 4: EFFECT OF NATURALLY INFESTED FIELD SOIL ON TUBER INFECTION

Based on molecular detection, preplant quantity of *V. dahliae* in the plots within the study areas ranged from 35 to 954 pg DNA/g of soil. Progeny tuber infection based on isolation, resulted in a recovery of *V. dahliae* from 9 out of 20 progeny tubers from cv Pike followed by 4 out of 20 for cv Atlantic (Table 6-18). There appeared to be an association between high soil inoculum and *V. dahliae* recovery from the vascular tissue of progeny tubers.

Table 6-18 – Isolation of *V. dahliae* from potato tubers and *V. dahliae* DNA quantification in soil from a commercial plot at Cora Lynn in 2010

Cultivar	Preplant *V. dahliae* (pg *V. dahliae* DNA/g of soil)	Incidence of *V. dahliae* (infected number of tubers from 20 replicates)
Pike	954	9
Atlantic	216	4
Pike	161	0

Cultivar	Preplant *V. dahliae* (pg *V. dahliae* DNA/g of soil)	Incidence of *V. dahliae* (infected number of tubers from 20 replicates)
Pike	91	1
Pike	66	2
Pike	57	1
Catani	41	0
Pike	35	0

INTERACTION BETWEEN *V. DAHLIAE* AND ROOT LESION NEMATODE *P. CRENATUS* TO CAUSE PED SYNDROME IN AUSTRALIA

EXPERIMENT 1: THE EFFECTS OF NATURAL FIELD SOIL INOCULUM LEVELS OF *V. DAHLIAE* AND *P. CRENATUS* ON THE DEVELOPMENT OF PED

In the potato trial, PED symptoms were not observed and *V. dahliae* was not isolated from any of the leaf petioles at 10 weeks after planting.

In contrast, the eggplants in the field soil showed typical symptoms of Verticillium wilt 4 weeks after planting. After 10 weeks, the disease severity was less (2, moderate wilt with severe chlorosis of leaves) in nematicide treated soil compared to field soil (3, moderate wilt and leaf necrosis). No disease symptoms or infection occurred in plants grown in pasteurized soil. Nematicide and pasteurized soil treatments not only significantly reduced disease severity but also increased plant height compared to field soil; and as a result a significant increase in the aerial biomass (fresh and dry weight) and fresh weight of roots was observed after 10 weeks (Table 6-19)

Table 6-19 – Verticillium wilt disease incidence and severity in eggplant cv Black Beauty, 10 weeks after planting seedlings into natural infected field soil

Treatment	Incidence of *V. dahliae* (infected number of plants from 5 replicates)	Disease severity*	Plant height (cm)	Aerial biomass		Fresh weight of roots (g)
				Fresh weight (g)	Dry weight (g)	
Field soil	5	3	7.3[b]	4.6[b]	0.76[b]	0.92[b]
Nematicide treated soil	4	2	10.2[a]	8.7[a]	3.20[a]	1.41[a]
Pasteurized soil	0	0	10.1[a]	11.0[a]	3.70[a]	1.80[a]

Means in columns with the same letter are not significantly different

*Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

POSTHARVEST QUANTITY OF *P. CRENATUS* AND *V. DAHLIAE* IN FIELD SOIL PLANTED WITH POTATO MINI TUBERS

At harvest (10 weeks after planting) in the potato trial, there was an increase from 85 pg *V. dahliae* DNA/g of soil and 0.3 *P. crenatus*/g of soil to 117 pg *V. dahliae* DNA/g of soil and 1.31 *P. crenatus*/g of soil, respectively. The number of nematodes per g of roots increased dramatically to 158 *P. crenatus*/g of roots (Table 6-20).

The number of *P. crenatus* in the soil and roots were also significantly higher in the field soil treatment compared to nematicide treated soil. Nematicide significantly reduced the quantity of *P. crenatus* in the soil and roots. There was no significant difference in the quantity of *V. dahliae* in the soil between field soil and nematicide treated soil (Table 6-20). Pasteurization completely removed *V. dahliae* and *P. crenatus* from the soil.

Table 6-20 – Number of nematodes in soil and plant roots, and quantity of *V. dahliae* in soil 10 weeks after planting potato mini tubers into natural infected field soil

Treatment	Number of *P. crenatus*		*V. dahliae* pg DNA/g of soil
	Per gram of soil	Per gram of root	
Field soil	1.31[a]	158[a]	117[a]
Nematicide treated soil	0.17[b]	8[b]	116[a]
Pasteurized soil	0	0	0

Means in columns with the same letter are not significantly different

POSTHARVEST QUANTITY OF *P. CRENATUS* AND *V. DAHLIAE* IN FIELD SOIL PLANTED WITH EGGPLANT SEEDLINGS

At harvest (10 weeks after planting) in the eggplant trial, the number of nematodes did not increase from the preplant quantity in the field soil but the numbers in the roots increased (not to the same extent as in potatoes;Table 6-21). However, there was a large increase in the quantity of *V. dahliae* to 322 pg DNA/g of soil, compared to preplant quantity of 85 pg DNA/g of soil, which was again different to the potato trial.

There was a considerable decrease in the number of nematodes in the soil and roots observed 6 and 10 weeks after planting in the nematode treated soil however, the nematicide did not significantly affect the amount of *V. dahliae* in the soil (Table 6-21).

Table 6-21 – Number of nematodes in soil and plant roots, and quantity of *V. dahliae* in soil 6 and 10 weeks after planting eggplant seedlings into natural infected field soil

Treatment	Number of *P. crenatus*				*V. dahliae* pg DNA/g of soil	
	Per gram of soil		Per gram of root			
	6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks
Field soil	0.28[a]	0.43[a]	11[a]	24[a]	141[a]	322[a]
Nematicide treated soil	0.03[b]	0.06[b]	0.2[b]	0.3[b]	142[a]	282[a]
Pasteurized soil	0	0	0	0	0	0

Means in columns with the same letter are not significantly different

EXPERIMENT 2: THE EFFECT OF DIFFERENT CONCENTRATIONS OF *V. DAHLIAE* ALONE OR IN COMBINATION WITH NATURAL FIELD SOIL LEVEL OF *P. CRENATUS* ON THE DEVELOPMENT OF PED

Potato cv Shepody plants grown in field soil containing 3 *P. crenatus*/g of soil and inoculated with 4000 pg *V. dahliae* DNA/g of soil started to exhibit PED symptoms 4 weeks after planting. *V. dahliae* was isolated from the petiole of these infected plants. As well, between 4 to 7 weeks after planting there was a higher incidence of infection in *V. dahliae* and *P. crenatus* infested soil compared to nematicide treated soil. By 8 weeks after planting the symptoms on infected plants were severe (rating of 4) with more than 50% of the leaves wilting and necrotic. Based on the petiole isolation after 8 weeks, all the plants in both infected and nematicide treated soil were infected with *V. dahliae*. Compared to the nematicide treated soil where the severity of infection was much less at a rating of 2 although all plants were infected with *V. dahliae (*Table 6-22)

At 8 weeks after planting 2000 pg *V. dahliae* DNA/g of soil only 50% of the plants were infected with mild symptoms of Verticillium wilt being produced (rating of 1;Table 6-22). Inoculum levels between 1000 to 300 pg *V. dahliae* DNA/g of soil resulted in a third of the plants being infected in both the field soil and nematicide treated soils without any symptoms showing in the plants. At 100 pg *V. dahliae* DNA/g of soil and pasteurized soil treatments there was no infection of plants and no disease symptoms.

Only with the higher concentration of *V. dahliae* (4000 pg *V. dahliae* DNA/g of soil) was there a significant decrease in the yield of potatoes compared to the controls grown in pasteurized soil. The nematicide treated soil also had a significant reduction in yield but was significantly less than that of the inoculated field soil treatment (Table 6-23).

Table 6-22 – Verticillium wilt disease incidence and severity in potato seedlings from 4 to 8 weeks after planting in field soil containing various quantities of *V. dahliae* and 3 *P. crenatus*/g soil

Treatments (*V. dahliae* pg DNA/g of soil NT = nematicide treatment[A]	Incidence of *V. dahliae* (infected number of plants from 6 replicates)					Disease severity at 8 weeks[B]
	Weeks after planting					
	4	5	6	7	8	
4000	3	5	6	6	6	4
4000 NT	1	2	2	4	6	2
2000	0	1	1	2	3	1
2000 NT	0	0	1	2	3	1
1000	0	1	1	2	2	0
1000 NT	0	0	0	1	2	0
500	0	1	1	1	2	0
500 NT	0	0	0	1	1	0
300	0	0	1	1	2	0

Treatments (*V. dahliae* pg DNA/g of soil NT = nematicide treatment[A]	Incidence of *V. dahliae* (infected number of plants from 6 replicates)					Disease severity at 8 weeks[B]
	Weeks after planting					
	4	5	6	7	8	
300 NT	0	0	1	1	2	0
100	0	0	0	0	0	0
100 NT	0	0	0	0	0	0
Pasteurized soil	0	0	0	0	0	0

[A]Nematicide treated soil = Soil drenching with nematicide Vydate 2 mL/L.

[B]Mean disease severity rounded off to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

Table 6-23 – Effect of *V. dahliae* on yield (g) of potatoes 8 weeks after planting in field soil containing various quantities of *V. dahliae* and 3 *P. crenatus*/g soil

Treatment	*V. dahliae* pg DNA/g of soil					
	4000	2000	1000	500	300	100
Field Soil	114.5[c]	223.8[a]	222.8[a]	222.1[a]	232.6[a]	233.5[a]
Nematicide treated	194.8[b]	225.8[a]	226.5[a]	231.6[a]	228.8[a]	235.5[a]
Pasteurized	236.5[a]	236.5[a]	236.5[a]	236.5[a]	236.5[a]	236.5[a]

Means with the same letter within a column are not significantly different

POST HARVEST QUANTITY OF *V. DAHLIAE* AND *P. CRENATUS* IN SOIL AND PLANT ROOTS IN POTS PLANTED WITH POTATO SEEDLINGS

There was a considerable reduction in the level of *V. dahliae* in the soil from preplant to post harvest for all treatments except at 100 pg *V. dahliae* DNA/g of soil were the reduction to 82 pg was minimal. No significant difference in the quantity of *V. dahliae* was observed between nematicide treatment and field soil treatment, except for *V. dahliae* at 100 pg DNA/g of soil (Table 6-24). A significant difference was observed between the pasteurized and all other treatments. Nematicide had no effect on the inoculum of *V. dahliae* (Table 6-24).

There also was a reduction in the number of *P. crenatus* nematodes in the soil at post harvest compared to preplant. The number of *P. crenatus* in the soil was higher in the field soil compared to nematicide treated soil. Nematicide significantly reduced the quantity of *P. crenatus* in the soil compared to field soil (Table 6-24). *P. crenatus* was not found in the post harvest pasteurized soil treatment.

Table 6-24 – Post harvest levels of *V. dahliae* (*Vd*) and *P. crenatus* (*Pc*) in field soil preplanted with various quantities of *V. dahliae* and 3 *P. crenatus*/g soil and planted with potato seedlings

Treatment	*V. dahliae* pg DNA/g of soil											
	4000		2000		1000		500		300		100	
	Vd[A]	*Pc*[B]	*Vd*	*Pc*	*Vd*	*Pc*	*Vd*	*Pc*	*Vd*	*Pc*	*Vd*	*Pc*
Field Soil	115[a]	0.39	103[a]	1.1	47[a]	0.59	90[a]	0.38	87[a]	1.5	82[a]	0.74
Nematicide treated	76[a]	0	172[a]	0	42[a]	0.03	41[a]	0.01	58[a]	0	39[b]	0
Pasteurized	0.75[b]	0	0.75[b]	0	0.75[b]	0	0.75[b]	0	0.75[b]	0	0.75[c]	0

Means with the same letter within a column are not significantly different

[A]*V. dahliae* pg DNA/g of soil

[B]*P. crenatus*/g of soil

EFFECT OF *V. DAHLIAE* ON PRODUCTION OF VERTICILLIUM WILT IN EGGPLANT

The concentrations of *V. dahliae* inoculum, 300 and 100 pg *V. dahliae* DNA/g of soil containing 3 *P. crenatus*/g of soil, resulted in all plants being infected in both field soil and nematicide treated soil, 8 weeks after planting. The disease severity was lower in all plants grown in soil treated with nematicide despite plants being infected by *V. dahliae* (Table 6-25). No infection occurred in pasteurized soil.

Table 6-25 – Verticillium wilt disease incidence and severity in eggplant seedlings 8 weeks after planting in field soil containing various quantities of *V. dahliae* and 3 *P. crenatus*/g of soil.

Treatment (*V. dahliae* pg DNA/g of soil)	Incidence of *V. dahliae* (infected number of plants from 4 replicates)		Disease Severity[B]	
	Field Soil	Nematicide treated soil[A]	Field Soil	Nematicide treated soil
300	4	4	3	2
100	4	4	3	2
0 (Pasteurized soil)	0	0	0	0

[A]Nematicide treated soil = Soil drenching with nematicide Vydate 2mL/L.

[B]Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

EFFECT OF *V. DAHLIAE* ON PLANT HEIGHT, AERIAL BIOMASS AND ROOT WEIGHT IN EGGPLANT

The *V. dahliae* inoculum concentration of 300 and 100 pg *V. dahliae* DNA/g of soil in field soil containing 3 *P. crenatus*/g of soil, resulted in significant reduction in plant height, aerial biomass, and root weight of eggplants. There appeared to be similar reduction at either 300 or 100 pg *V. dahliae* DNA/g of soil (Table 6-26). Plant growth (height and aerial biomass) was also significantly reduced in the nematicide treatment

(similar at both 300 and 100 pg *V. dahliae* DNA/g of soil) of field soil but was significantly higher than in the non treated field soil.

Table 6-26 – Effect of *V. dahliae* on plant height, aerial biomass and root weight of eggplant 8 weeks after planting in field soil containing various quantities of *V. dahliae* and 3 *P. crenatus*/g soil

Treatment	*V. dahliae* pg DNA/g of soil					
	300			100		
	Plant height (cm)	Aerial biomass (g dwt)	Fresh weight of root (g)	Plant height (cm)	Aerial biomass (g dwt)	Fresh weight of root (g)
Field Soil	7.2[c]	2.3[c]	1.4[b]	7[c]	2.3[c]	1.3[a]
Nematicide treated	10[b]	3.1[b]	2.1[a]	9.8[b]	3[b]	2.2[b]
Pasteurized	15.2[a]	4.2[a]	2.5[a]	15.2[a]	4.2[a]	2.5[b]

Means with the same letter within a column are not significantly different

POST HARVEST QUANTITY OF *V. DAHLIAE* AND *P. CRENATUS* IN SOIL AND PLANT ROOTS IN POTS PLANTED WITH EGGPLANT SEEDLINGS

In eggplant, there was a considerable reduction in the level of *V. dahliae* in the soil from preplant to post harvest. The nematicide treatment did not significantly reduce the quantity of *V. dahliae* in the soil. *V. dahliae* was not found in the pasteurized soil treatments (Table 6-27).

Although there was a large reduction in the number of *P. crenatus* nematodes in the soil at post harvest compared to preplant, the number of nematodes in the roots of eggplants was approximately 12 and 9 times higher than the initial field soil concentration at preplant in 300 and 100 pg *V. dahliae* DNA/g of soil treatments, respectively. The number of *P. crenatus* in the soil and roots were high in the field soil treatment compared to nematicide treated soil which completely removed the nematodes from the soil. Pasteurization was highly effective to remove nematodes and *V. dahliae* inoculum in the soil (Table 6-27).

Table 6-27 – Post harvest levels of *V. dahliae* (*Vd*) and *P. crenatus* (*Pc*) in field soil preplanted with various quantities of *V. dahliae* and 3 *P. crenatus*/g of soil and planted with eggplant seedlings

Treatment	*V. dahliae* pg DNA/g of soil					
	300			100		
	Vd[A]	*Pc*[B] Soil	*Pc* Roots	*Vd*	*Pc* Soil	*Pc* Roots
Field Soil	88[a]	0.25	38	44[a]	0.08	26
Nematicide treated	82[a]	0	0	55[a]	0	0
Pasteurized	0	0	0	0	0	0

Means with the same letter within a column are not significantly different

[A] *V. dahliae* pg DNA/g of soil

[B] *P. crenatus*/g of soil or roots

AUSTRALIAN POTATO CULTIVARS RESISTANT TO *V. DAHLIAE*

OPTIMISATION OF INOCULUM CONCENTRATION FOR THE GLASSHOUSE BIOASSAY

POTATO TRIALS

Plants that were inoculated at 10^5 and 10^4 spores/mL showed symptoms and had petiole infection, while all plants inoculated at 10^3 and 10^2 spores/mL did not show symptoms or petiole infection with the exception of one replicate at 10^2 spores/mL that showed symptoms and had petiole infection (Table 6-28).

Table 6-28 – Verticillium wilt disease severity based on 0- 5 scale on cv Russet Burbank at various inoculum concentrations; and petiole and crown root infection at 9 wai.

Spore concentration (spores/mL)	Weeks after inoculation							Infected plant tissue 9 wai
	3	4	5	6	7	8	9	
10^5	1	1 - 2	1 - 3	2 - 3	2 - 4	2 - 5	3 - 5	Y
10^4	0	1	1	1	1 - 2	1 - 2	1 - 3	Y
10^3	0	0	0	0	0	0	0	N
10^2	0	0	0	0	0	0	0	N

In the second inoculum optimisation trial plants, that were inoculated at 10^6 and 10^5 spores/mL showed mild to moderate wilt symptoms and had petiole infection. There were mild wilt symptoms at all other spore concentrations except at 10^2 where none of the plants showed Verticillium wilt symptoms (Table 6-29).

Table 6-29 – Verticillium wilt disease severity based on 0- 5 scale on cv Russet Burbank at various inoculum concentrations; and petiole and crown root infection at 12 wai.

Spore concentration (spores/mL)	Weeks after inoculation											Infected plant tissue 12 wai
	2	3	4	5	6	7	8	9	10	11	12	
10^6	1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2	2	2 - 3	2 - 4	Y
10^5	1	1	1	1	1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 3	Y
10^4	0	0	0	1	1	1	1	1	1	1	1	Y
$5x10^3$	0	0	1	1	1	1	1	1	1	1	1	Y
10^3	0	0	0	1	1	1	1	1	1	1	1	Y
10^2	0	0	0	0	0	0	0	0	0	0	0	N

In summary there was an inoculum threshold of between 10^3 and 10^4 spores/mL where a specific concentration of inoculum was required to cause infection and symptoms.

EGGPLANT TRIALS

In eggplants, plants that were inoculated with inoculum concentrations of 10^4, 10^5 and 10^6 spores/mL showed moderate to mild levels of disease severity while at 10^3 no symptoms of verticillium wilt were produced although all the plants were infected by *V. dahliae* (Table 6-30).

Table 6-30 – Verticillium wilt disease severity based on 0- 5 scale on eggplant cv Black beauty at various inoculum concentrations; and plant infection at 10 wai trial 1

Spore concentration (spores/mL)	Weeks after inoculation										Infected plant tissue
	1	2	3	4	5	6	7	8	9	10	
10^6	1	1 - 2	2 - 3	3 - 4	3 - 4	3 - 4	3 - 4	3 - 4	3 - 4	3 - 4	Y
10^5	0	1	2 - 3	3	3	3	3	3	3	3	Y
10^4	0	0	1	1	2	2 - 3	2 - 3	2 - 3	2 -3	2 - 3	Y
10^3	0	0	0	0	0	0	0	0	0	0	Y

In the second eggplant trial, inoculum concentrations of 10^4, 10^5 and 10^6 spores/mL showed moderate to mild levels of disease severity with all plants being infected. While, at 10^2 and 10^3 spores/mL only 2 out of 5 replicate plants showed moderate symptoms after 9 weeks and were infected (Table 6-31). The results on disease severity appeared to correlate with the effect on *V. dahliae* inoculum concentration on growth of the plants with concentrations from 10^4 upwards having significant decrease in plant height, aerial biomass and below ground biomass (Table 6-32). There was no significant difference in growth parameters between the uninoculated controls and those inoculated with 10^2 or 10^3 spores/mL, although the inoculated plants generally had less growth to the controls. Therefore, the inoculum threshold for infection and symptoms to occur appeared to be at 10^4 spores/mL.

Table 6-31 – Verticillium wilt disease severity based on 0- 5 scale on eggplant cv Black beauty at various inoculum concentrations; and plant infection at 9 wai trial 2

Spore concentration (spores/mL)	Weeks after inoculation									Infected plant tissue
	1	2	3	4	5	6	7	8	9	
10^6	0	1	2	3	4	4	4	4	4-5	Y
10^5	0	1	1-2	2-3	3-4	3-4	4	4	4	Y
10^4	0	1	1-2	1-3	2-3	3	3	3	3	Y
10^3	0	0	0-1*	1-2*	1-3*	1-3*	1-3*	0-2*	0-3*	N/Y*
10^2	0	0	0	0	0	0	0-1*	0-2*	0-3*	N/Y*

*Only 2/5 replicates showed symptoms and were infected

Table 6-32 – Effect of *V. dahliae* inoculum on plant height, above and below ground plant biomass for eggplant cv Black beauty

Spores concentration (spores/mL)	Plant height (cm)	Fresh weight (g)		Dry weight (g)	
		Above ground	Below ground	Above ground	Below ground
10^6	18.50[a]	5.62[a]	2.65[a]	0.75[a]	0.43[a]
10^5	24.60[ab]	11.07[ab]	5.18[ab]	1.50[a]	0.89[ab]
10^4	28.64[ab]	14.79[ab]	6.43[ab]	1.85[a]	1.12[ab]
10^3	37.94[bc]	39.09[bc]	18.26[bc]	5.12[ab]	3.82[bc]
10^2	44.40[c]	50.40[c]	19.89[bc]	6.57[b]	3.28[abc]
Control	45.90[c]	65.16[c]	26.05[c]	8.35[b]	4.15[c]

Means with the same letter within a column are not significantly different (p>0.05)

POTATO CULTIVAR SCREENING

EXPERIMENT 1

At 10 weeks after inoculation (WAI), all potato cultivars, except Pike A, Kennebec 2 - Line B and Denali exhibited typical *V. dahliae* symptoms, of interveinal chlorosis. The disease progressed faster in the early stage of growth on the susceptible cultivars than on the resistance ones. The first cultivar to show secondary symptoms of wilting occurred in Shepody B at 5 WAI (Table 6-33). At 12 WAI, *V. dahliae* was isolated from petioles and crown roots from all cultivars, excluding Pike A, Kennebec 2 - Line B and Denali where no *V. dahliae* was detected and hence these genotypes were considered to be resistant. In Catani, although *V. dahliae* was isolated from the petiole and crown tissue, there was a disease severity rating of only 2, thus this cultivar was considered as moderately resistant.

Table 6-33 – First screening trial. Verticillium wilt disease severity based on 0- 5 scale for 10 cultivars; cultivar ratings at 10 weeks after inoculation (wai); and petiole and crown root infection at 12 wai.

Cultivar	Weeks after inoculation[A]									Cultivar rating at 10 wai[B]	Infected plant tissue 12 wai[C]
	4	5	6	7	8	9	10	11	12		
Coliban C	0	0	0	0	1	2	3	4	4	S	Y
Catani	0	0	0	0	0	1	2	1 - 3	1 - 4	MR	Y
Denali	0	0	0	0	0	0	0	0	0	R	N
Kennebec 2 - Line B	0	0	0	0	0	0	0	0	0	R	N
Nicola	0	0	1	1	1 - 3	2 - 3	3	3 - 5	4 - 5	S	Y
Pike A	0	0	0	0	0	0	0	0	0	R	N

Cultivar	Weeks after inoculation[A]									Cultivar rating at 10 wai[B]	Infected plant tissue 12 wai[C]
	4	5	6	7	8	9	10	11	12		
Russet Burbank C	0	1	1	1 to 2	1 to 3	1 to 3	3	4	5	S	Y
Ranger Russet	0	0	0	1	2	3	4	4	5	S	Y
Shepody B	1	2	3	3	4	4	5	5	5	S	Y
Simcoe C	0	1	1	2	3	4	5	5	5	S	Y

[A]Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

[B]Ratings - R= resistant (0 and 1), MR= moderately resistant (2), S= susceptible (3, 4 and 5);

[C]Infection - Y= *V. dahliae* present, N= *V. dahliae* absent

EXPERIMENT 2

At 10 weeks after inoculation (wai) all potato cultivars except Desiree exhibited typical *V. dahliae* symptoms of interveinal chlorosis (Table 6-34). Desiree showed strong resistance and had no Verticillium isolated from the petiole and crown roots, whereas, Atlantic C was very susceptible.

Table 6-34 – Second screening trial. Verticillium wilt disease severity based on 0- 5 scale for 8 cultivars; cultivar ratings at 10 weeks after inoculation (wai); and petiole and crown root infection at 12 wai.

Cultivar	Weeks after inoculation[A]									Cultivar rating at 10 wai[B]	Infected plant tissue 12 wai[C]
	4	5	6	7	8	9	10	11	12		
Coliban C	1	1	1	1	1	1	1	1	1	R	Y
Catani	0	0	0	0	1	1	1 - 2	1 - 2	1 - 2	MR	Y
Denali	0	0	0	0	1	1	1	1	1	R	Y
Nicola	2	2 - 3	3 - 4	4 - 5	5	5	5	5	5	S	Y
Pike A	1 - 2	2 - 3	3 - 4	4 - 5	5	5	5	5	5	S	Y
Russet Burbank C	2 - 3	2 - 4	2 - 5	3 - 5	3 - 5	4 - 5	5	5	5	S	Y
Atlantic C	1 - 2	2	3	4	5	5	5	5	5	S	Y
Desiree	0	0	0	0	0	0	0	0	0	R	N

[A]Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

[B]Ratings - R= resistant (0 and 1), MR= moderately resistant (2), S= susceptible (3, 4 and 5);

[C]Infection - Y= *V. dahliae* present, N= *V. dahliae* absent

EXPERIMENT 3

At 10 weeks after inoculation all cultivars exhibited moderate to severe symptoms of Verticillium wilt with Denali being the most resistant with a rating of 1 to 2 (Table 6-35). The cultivar Desiree that showed a resistant reaction in the previous trial was moderately resistant.

Table 6-35 – Third screening trial. Verticillium wilt disease severity based on 0- 5 scale for 10 cultivars; cultivar ratings at 10 weeks after inoculation (wai); and petiole and crown root infection at 14 wai.

Cultivar	Weeks after inoculation[A]											Cultivar rating at 10 wai[B]	Infected Plant tissue 14 wai[C]
	4	5	6	7	8	9	10	11	12	13	14		
Atlantic C	1	1	1-2	1-2	1-2	2	2	2	2-3	2-3	3	MR	Y
Coliban C	1	1	2	2	2	2-3	2-4	2-4	2-4	2-4	4	S	Y
Desiree	1	1	1	1	2	2	2	2	3	3	3	MR	Y
Denali	1	1	1	1	1	1	1-2	1-2	2	3	3	MR	Y
Kennebec 2 Line B	1	1	2	2	3	4	4	4	4	4	4	S	Y
Pike A	1	1	1	1	2	2	2-3	2-3	3	3	3-4	S	Y
Ranger Russet B	1^	1^	1^	1^	2^	2^	2^	2^	3^	3^	3-4^	MR	Y^
Shepody B	1	1	2	2	3	3	3	3	3	3-4	3-4	S	Y
Simcoe B	1	1	1	1	1	1	1	2	2	2	2-3	MR	Y
Trent B	1	1	2	2	2	3	3	3	3-4	4	4	S	Y

[A]Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

[B]Ratings - R= resistant (0 and 1), MR= moderately resistant (2), S= susceptible (3, 4 and 5);

[C]Infection - Y= *V. dahliae* present, N= *V. dahliae* absent. ^4/5 plants were infected and produced symptoms

SUMMARY OF THE THREE SCREENING TRIALS

Summarising the three trials (Table 6-36) resulted in cultivar **Denali** showing moderate to high resistance and **Catani** and **Desiree** moderate resistance. Cultivars Nicola, Russet Burbank C, Shepody and Trent showed susceptible host reactions. The cultivars Coliban C and Pike A showed different host reactions in

the three trials. In the first trial at 10 weeks Coliban C was susceptible (rating of 3) but was resistant (1) in the second trial and moderately resistant to susceptible (2-4) in the third trial.

Table 6-36 – Summary of the three screening trials for resistance screening to *Verticillium dahliae*

Cultivars	Use	2011	2012	2013
		Spores /mL		
		10^4	5 x 10^4	5 x 10^4
Atlantic	Crisp/Fresh		S	MR
Catani	Crisp	MR[A]	R - MR	
Coliban	Fresh	S	R	MR - S
Denali	Crisp	R	R	R - MR
Desiree	Fresh		R	MR
Kennebec	Fry	R		S
Nicola	Fresh	S	S	
Pike	Crisp	R	S	MR - S
Ranger Russet	Fry	S		MR
Russet Burbank	Fry	S	S	
Shepody	Fry	S		S
Simcoe	Crisp	S		MR
Trent	Crisp			S

[A]Cultivar ratings at 10 weeks after inoculation. Resistance (R): Low disease (scale 0-1) with/without infection; Moderate resistance (MR): Low to moderate disease (scale 2) with infection; Susceptible (S): Moderate to severe (or death) disease (scale 3 -5) with infection.

SOIL AMENDMENTS THAT SUPPRESS VERTICILLIUM WILT DISEASE IN POTATOES

MICROCOSM ASSAY

The viability of microsclerotia incubated in 1% atomic sulphur rapidly decreased from 84% to 1% after 8 weeks (Table 6-37, Figure 6-10). Although the viability of microsclerotia in the untreated controls also decreased the percent germination was higher than in the sulphur treatment (84 to 8%). The decrease in viability of microsclerotia in the controls was surprising with microsclerotia expected to survive in soil in the field for 10 years. At each weekly sampling the microsclerotia that were incubated in soil amended with 1% sulphur had reduction in viability ranging from 51% (week 1), to over 90% at weeks 5 and 7 compared to the control. A 10% sulphur treatment completely inhibited viability of microsclerotia at both 4 and 8 weeks after incubation (Figure 6-10). Although there was no significant ($p < 0.05$) change in the pH there was a general decrease over the 8 weeks from 5.27 to 5.06 (1% sulphur). However, analysis of the pH at 4 and 8 weeks showed a significant ($p < 0.05$) reduction for the 1% (5.27 to 5.06) and 10% (5.29 to 5.01) sulphur treatments compared to the controls (5.34 to 5.19) (Figure 6-11). There was a significant increase in pH of the untreated soil (control) at 4 weeks before decreasing.

Table 6-37 – The effect of 1% atomic sulphur on soil pH and microsclerotia viability

wai	Soil pH		MS viability (%)[A]		% reduction of germination
	Control	1% atomic sulphur	Control	1% atomic sulphur	
0	5.34	5.27	84[a]		
1	5.36	5.25	69[b]	34[c]	**51**
2	5.35	5.18	63[b]	14[df]	**78**
3	5.22	5.23	64[b]	9[fg]	**86**
4	5.39	5.21	24[cd]	4[fg]	**83**
5	5.34	5.12	13[defg]	1[g]	**92**
6	5.26	5.06	11[efg]	2[fg]	**82**
7	5.33	5.10	12[defg]	0	**100**
8	5.19	5.06	8[fg]	1[g]	**88**

[A]Means for microsclerotia viability with the same letter are not significantly different. LSD = 12.0%, P < 0.05.

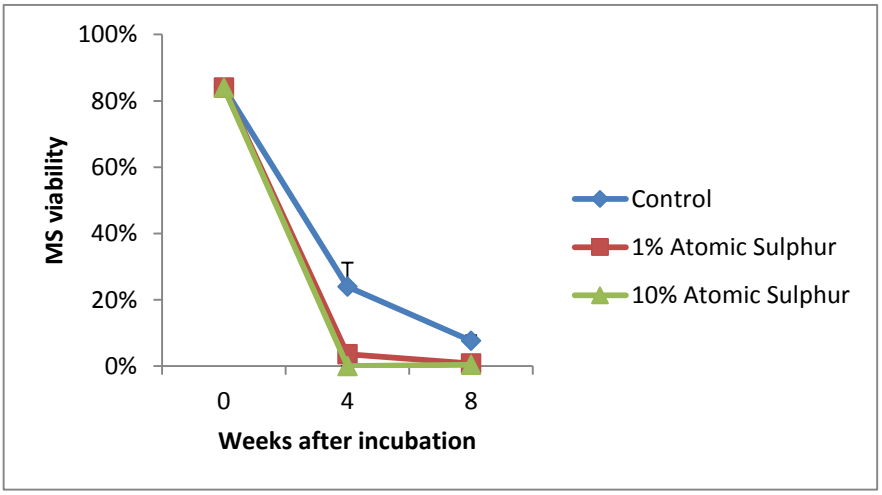

Figure 6-10 – Effect of 1% and 10 % atomic sulphur on microsclerotia viability after 8 weeks incubation. Bars indicate the standard error of the mean. LSD = 8.2%, p < 0.05.

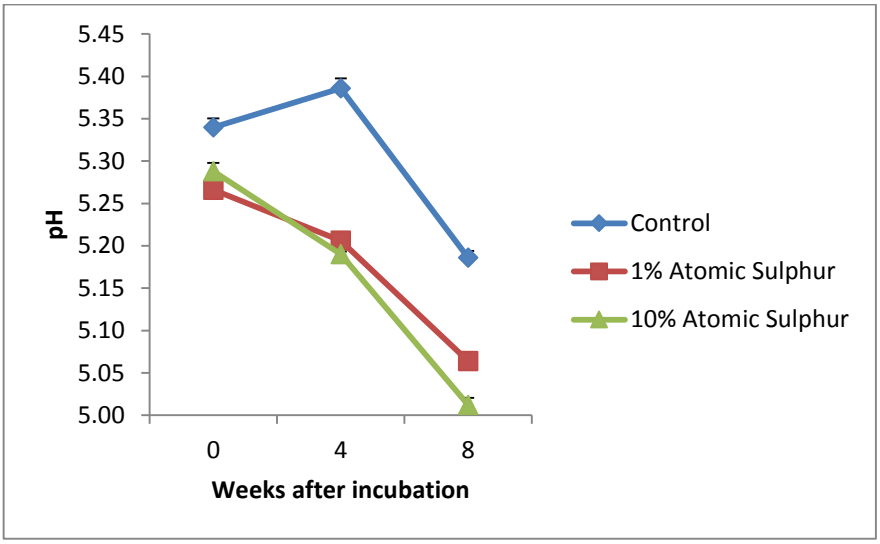

Figure 6-11 – Effect of 1% and 10% atomic sulphur on soil pH after 8 weeks incubation. Bars indicate the standard error of the mean. LSD = 0.03, p < 0.05.

The viability of microsclerotia incubated in 1% brown coal rapidly decreased from 79% to 15% after 8 weeks (Table 6-38, Figure 6-12). The viability of microsclerotia in the untreated controls decreased to 36% and 58 % at weeks 7 and 8 respectively which were much higher than in the sulphur experiment. At each weekly sampling the microsclerotia that were incubated in soil amended with 1% brown coal had reduction in viability ranging from 43% (week 1) to over 80% from weeks 4 and 6. This reduction in viability was less than for the weekly reductions in the sulphur experiments. The 10% brown coal treatment was significantly ($p < 0.05$) less severe on microsclerotia viability then the 1% brown coal treatment (Figure 6-12). The pH remained fairly constant in the controls at between 5.29 to 5.28 over 8 weeks however, there was a peak in pH of 5.41 at 4 weeks (Table 6-38). However, analysis of the pH at 4 weeks showed a significant ($p < 0.05$) reduction for the 1% (5.34) and 10% (5.30) brown coal treatments compared to the controls (5.41) (Figure 6-13).

Table 6-38 – The effect of brown coal on soil pH and microsclerotia viability

wai	Soil pH		MS viability (%)[A]		% reduction of germination
	Control	1% brown coal	Control	1% brown coal	
0	5.29	5.22	79[a]		
1	5.24	5.20	63[abc]	36[de]	**43**
2	5.27	5.28	72[ab]	52[cd]	**28**
3	5.37	5.30	45[cd]	21[ef]	**53**
4	5.41	5.34	58[bc]	8[f]	**86**
5	5.35	5.30	14[f]	7[f]	**50**
6	5.24	5.21	38[de]	6[f]	**84**
7	5.30	5.27	36[de]	16[f]	**56**
8	5.28	5.27	58[bc]	15[f]	**74**

[A]Means for microsclerotia viability with the same letter are not significantly different LSD = 18.0%, P < 0.05.

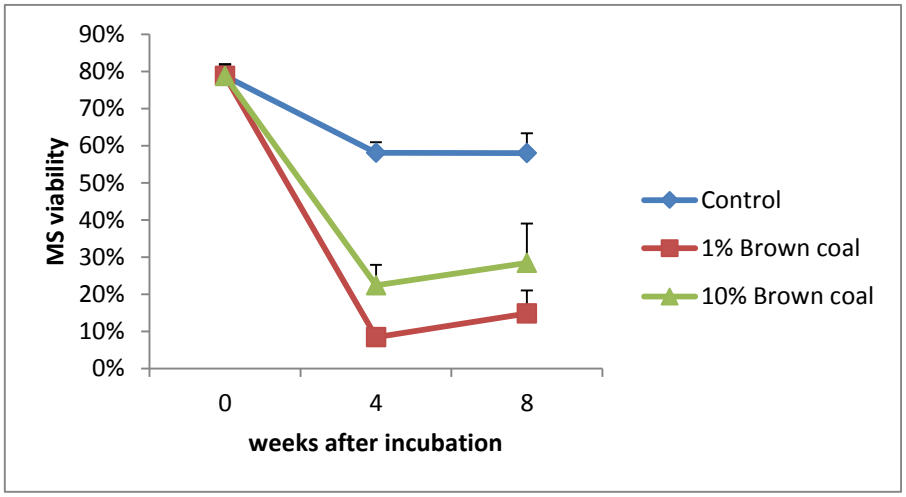

Figure 6-12 – Effect of 1% and 10% brown coal on microsclerotia viability after 8 weeks incubation. Bars indicate the standard error of the mean. LSD = 15.3, p > 0.05.

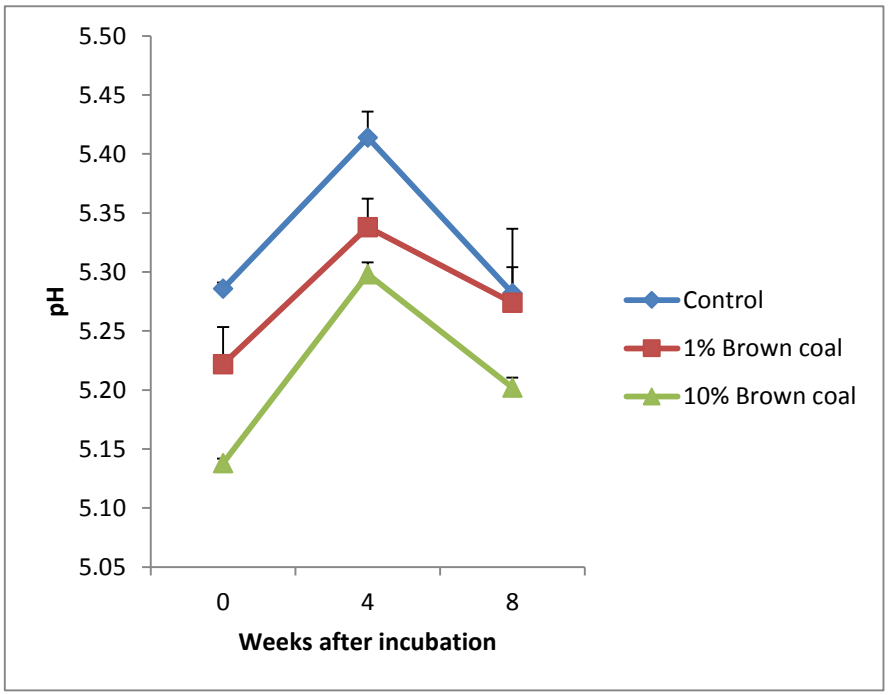

Figure 6-13 – Effect of 1% and 10% brown coal on soil pH after 8 weeks incubation. Bars indicate the standard error of the mean. LSD = 0.01, p > 0.05.

The viability of microsclerotia incubated in 1% and 10% blood and bone meal treatments decreased significantly ($p < 0.05$) from 73% to 18% and 9% respectively after 8 weeks of incubation (Table 6-39, Figure 6-14). There was no significant difference between 1% and 10% blood and bone meal treatments at 4 weeks but at 8 weeks viability was significantly less in the 10% treatment. The viability of microsclerotia in the untreated controls decreased to 56% at week 8 but unlike in the controls in the other experiments this was not significantly different to the viability at the commencement of the experiment. At weeks 4 and 8 sampling times the reduction in microsclerotia viability for the 1% blood and bone meal treatment was 58 and 68% respectively. The pH of the controls remained constant at around 5.3 over 8 weeks and was

significantly higher ($p < 0.05$) than for the 1% treatment. For 10% blood and bone meal the pH was significantly higher than the 1% treatment and had a sharp increase to 6.04 at 8 weeks (Figure 6-15).

Table 6-39 – The effect of blood and bone meal on soil pH and microsclerotia viability

wai	Soil pH[A]			MS viability (%)[B]			% reduction of germination
	Control	1% blood and bone	10% blood and bone	Control	1% blood and bone	10% blood and bone	1% blood and bone
0	5.33[b]	5.00[f]	5.29[c]	73[a]			
4	5.30[bcd]	5.02[f]	5.19[e]	62[a]	26[b]	29[b]	58
8	5.28[d]	5.01[f]	6.04[a]	56[a]	18[bc]	9[c]	68

[A]Means for pH with the same letter are not significantly different. LSD = 0.03, $P < 0.05$.

[B]Means for microsclerotia viability with the same letter are not significantly different. LSD = 19.6%, $P < 0.05$.

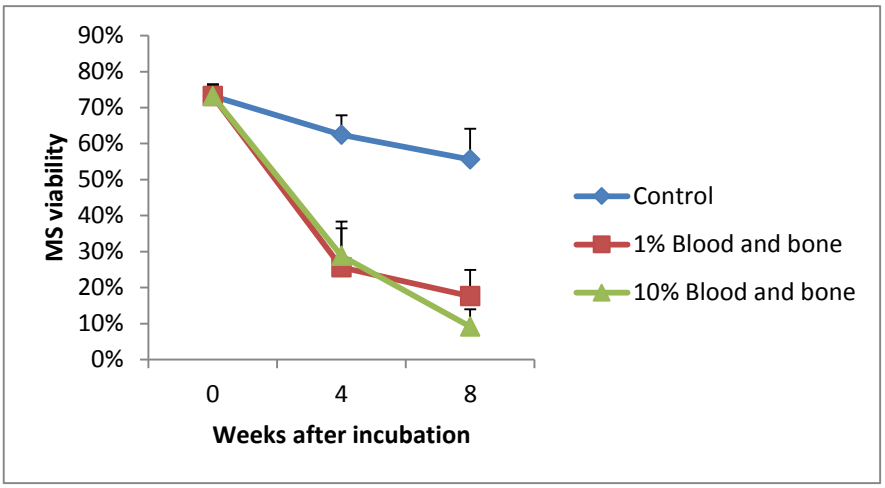

Figure 6-14 – Effect of 1% and 10% blood and bone meal on microsclerotia viability after 8 weeks incubation. Bars indicate the standard error of the mean.

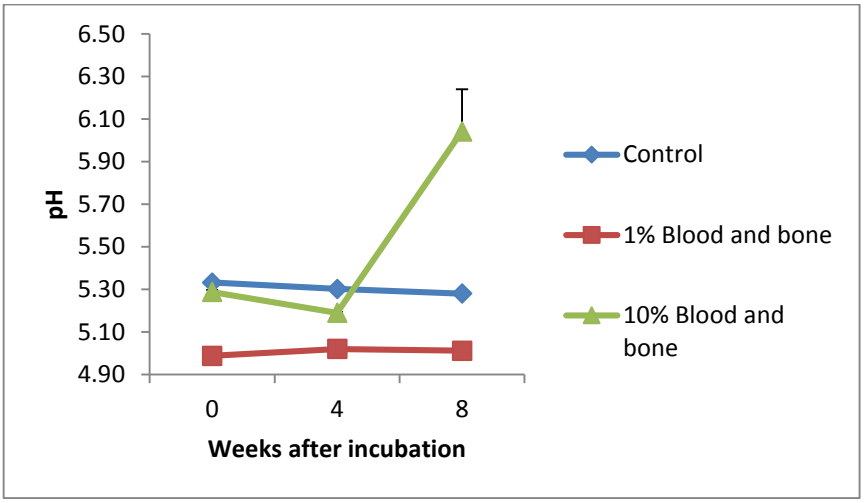

Figure 6-15 – Effect of 1% and 10% blood and bone meal on soil pH after 8 weeks incubation. Bars indicate the standard error of the mean.

GLASSHOUSE TRIAL WITH BROWN COAL

OPTIMISATION OF INOCULUM CONCENTRATION

Before a pot trial could be undertaken to assess the efficacy of brown coal to suppress Verticillium wilt, two glasshouse experiments were set up to assess the level of microsclerotia inoculum required to cause disease.

In Trial 1, at the low concentration (50 CFU/g soil) of microsclerotia in the soil, plants started to become infected 5 weeks after planting however, at the higher concentrations (250 and 500 CFU/g soil) plants started to become infected after 3 weeks in the two trials (Table 6-40, Table 6-41). In trial 1, at 10 weeks at 50 CFU all plants were infected with a disease severity of only 2 (moderate wilt and severe chlorosis) but at 500 CFU all plants were infected with a disease severity of 3 (moderate wilt with necrosis of leaves). In trial 2 at 10 weeks disease incidence was higher than in Trial 1 with the severity of infection at all treatments from 3 to 4 (severe wilt and necrosis). Based on these results, the high concentration of microsclerotia was selected for the brown coal pot trial to ensure all plants would be infected.

Table 6-40 – Verticillium wilt disease severity based on 0- 5 scale on cv Russet Burbank at various microsclerotia inoculum (CFU/g of dry potting soil) concentrations; and plant infection at 14 wai – trial 1 at Knoxfield, Victoria

Treatments	Trial 1 – Knoxfield													
	Weeks after inoculation													Infected plant tissue
	2	3	4	5	6	7	8	9	10	11	12	13	14	
50 CFU/g dry potting mix	0	0	0	1	1	1	1	1	2	2	3	4	4	Y
500 CFU/g dry potting mix	0	1	1-2	1-2	1-2	3	3	3	3	3	4	4	5	Y
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	N

Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

Table 6-41 – Verticillium wilt disease severity based on 0- 5 scale on cv Russet Burbank at various microsclerotia inoculum (CFU/g of dry potting soil) concentrations; and plant infection at 14 wai – trial 2 at Parkville, Victoria

Treatments	Trial 2 – Parkville													
	Weeks after inoculation													Infected plant tissue
	2	3	4	5	6	7	8	9	10	11	12	13	14	
50 CFU/g dry potting mix	0	0	0	1	1	2	3	3	3	3	3	4	4	Y
250 CFU/g dry potting mix	0	1	1	1	2	3	4	4	4	4	4	4	4	Y
500 CFU/g dry potting mix	0	1	1	2	3	3	3	4	4	4	4	4	4-5	Y
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	N

Mean disease severity rounded to the nearest whole number. Disease severity index was rated on each plant according to the scale of Tsror and Hazanovsky (2001).

In both trials the actual number of microsclerotia (CFU/ g of soil) in the soil at planting was much higher (over nine fold increase) than the estimated number of microsclerotia in the inoculum (Table 6-42, Table 6-43). In Trial 1, there was significant decrease (96%) in microsclerotia inoculum from the initial inoculum concentration added to the soil at planting (500 CFU/g) to the level of microsclerotia in the soil at harvest (Table 6-42). However, there was no significant change in the concentration of inoculum of 50 CFU/g from planting to harvest although there was an overall slight decrease.

Table 6-42 – Concentration of *V. dahliae* inoculum (CFU/g soil) in pots at planting and at harvest for Trial 1

Treatment	Planting	Harvest
	CFU/g soil	CFU/g soil
Control	0	0
MS 50 CFU/g	460[b]	80[b]
MS 500 CFU/g	4680[a]	175[b]

Means with the same letter are not significantly different. LSD = 1300, $p < 0.05$.

In Trial 2, there was significant decrease (99%) in inoculum from microsclerotia that was added to the soil at planting (250 CFU/g of soil and 500 CFU/g of soil) of microsclerotia at harvest (Table 6-43). In contrast to Trial 1, there was a significant change in inoculum of MS 50 CU/g from planting to harvest. Interestingly there was a small level (60 CFU/g of soil) of *V. dahliae* microsclerotia inoculum detected in the uninoculated controls at planting. This may have been due to the presence of *V. dahliae* microsclerotia in the potting mix used to set up this trial or more likely due to contamination of the uninoculated soil with inoculated soil during sampling and analysis.

For both trials, the CFUs at planting were consistently higher by a magnitude of 10 compared to the CFUs of the quantity of inoculum incorporated into the soil in each pot. Nevertheless, the quantity of inoculum (CFU) in each treatment was proportional to the inoculum that was added to the soil in each treatment. In both trials there were large variations in the number of microsclerotia (CFU) in the soil between replicates of each treatment.

Table 6-43 – Concentration of *V. dahliae* inoculum (CFU/g soil) in pots at planting and at harvest for Trial 2

Treatment	Planting	Harvest
	CFU/g soil	CFU/g soil
Control	60	0
MS 50 CFU/g	700[c]	60[d]
MS 250 CFU/g	3380[b]	40 [d]
MS 500 CFU/g	4760[a]	40[d]

Means with the same letter are not significantly different. LSD = 603, $p < 0.05$.

EFFICACY OF 1% BROWN COAL TO SUPPRESS VERTICILLIUM WILT

In the glasshouse pot trial there was no apparent impact of 1% brown coal on preventing the infection of plants inoculated with 500 and 750 CFU of microsclerotia/ g of dry soil (Table 6-44). In the lower inoculum treatment two replicate plants failed to become infected. There was no delay in establishing infection with all plants starting to show symptoms as early as 3 weeks after inoculation. Twelve weeks after planting, the severity of infection in all treatments was between 3 to 4, which indicated severe infection of all plants.

Table 6-44 – Verticillium wilt disease severity based on 0- 5 scale on cv Russet Burbank grown in soil amended with 1% brown coal with various microsclerotia inoculum (CFU/g of dry potting soil) concentrations; and plant infection at 12 wai

Treatments	Weeks after inoculation											Infected plant tissue
	2	3	4	5	6	7	8	9	10	11	12	
1% brown coal & microsclerotia 500 CFU/g dry potting mix	0	1*	1*	2*	2*	2*	3*	3*	3*	3*	3*	Y*
1% brown coal & microsclerotia 750 CFU/g dry potting mix	0	1	2	3	3	3	3	3	3	4	4	Y
Microsclerotia 500 CFU/g dry potting mix	0	1	2	2	3	3	3	3	3	3-4	3-4	Y
Microsclerotia 750 CFU/g dry potting mix	1	1	2	3	3	3	4	4	4	4	4	Y
1% brown coal	0	0	0	0	0	0	0	0	0	0	0	N
Control	0	0	0	0	0	0	0	0	0	0	0	N

*3/5 replicates confirmed to be infected thus the disease rating was based on only the three infected plants.

The actual number of microsclerotia (CFU/ g of soil) in the soil at planting was much higher (over four fold increase) than the estimated number of microsclerotia in the inoculum. As well, there was a small increase in the actual number of microsclerotia between the 500 and 750 CFU/ g soil treatments at planting. At harvest there was a significant reduction of inoculum for all treatments (ranging from 99% to 100%) with no detectable microsclerotia at harvest in all treatments except at 500 CFU/g soil (Table 6-45).

Table 6-45 – Concentration of *V. dahliae* inoculum (CFU/g soil) in pots at planting with and without 1% brown coal and at harvest; and the percent inoculum reduction

Treatment	Planting	Harvest	Inoculum reduction
	CFU/g soil	CFU/g soil	
Control	0	0	
MS 500 CFU/g soil	2000	20	99%
MS 750 CFU/g soil	3360	0	100%
1% brown coal + MS 500 CFU/g soil	2800	0	100%
1% brown coal + MS 750 CFU/g soil	3280	0	100%

Although there was a significant difference of the dry weight of biomass of plants between the control and microsclerotia inoculum at 500 CFU/g, there were no other significant differences of the dry weight between control and treatments (Table 6-46).

Table 6-46 – Above ground biomass dry weight (g) of potato plants harvested at 12 wai.

Treatment	Dry weight (g)
Control	16.328 [A]
MS 500 CFU/g	8.730 [B]
MS 750 CFU/g	12.614 [AB]
1% BC	14.17 [AB]
1% BC + MS 500 CFU/g	12.55 [AB]
1% BC + MS 750 CFU/g	11.154 [AB]

Means with the same letter are not significantly different. LSD = 6.6, $p < 0.05$.

DISCUSSION

Although Verticillium wilt of potato was reported in Australia in 1967 (Harrison, 1967), very little work had been conducted on the causal agent and its interaction with root lesion nematode to cause potato early dying (PED) syndrome in Australian potato production systems. The main goal of the research described in this report was to investigate the role of the pathogen associated with Verticillium wilt in Australia with a

focus on five main aspects; i) incidence of seed tuber infection, taxonomy and pathogenicity of *Verticillium* species infecting potatoes in Australia, ii) determining the role of *V. dahliae* tuber infection on the development of Verticillium wilt disease, iii) determining the interaction between *V. dahliae* and the root lesion nematode *P. crenatus* in causing PED syndrome in Australia, iv) identification of resistance in Australian potato cultivars to *V. dahliae*, and v) assessment of soil amendments to suppress Verticillium wilt disease in potatoes. Detailed knowledge about Verticillium wilt may lead to new prospects in the management of disease in Australian potato production systems.

SEED TUBER INFECTION, TAXONOMY AND PATHOGENICITY OF *VERTICILLIUM* SPECIES

A seed tuber survey showed that *Verticillium* was widespread throughout Victorian and Tasmania seed potato production regions. Based on morphological characters and molecular analysis of fungal genes, three *Verticillium* species were identified viz., *V. dahliae*, *V. albo-atrum* and *V. tricorpus*.

Verticillium dahliae was the predominant species within the seed lot (around 28%) which was similar to the reports in North America where 30% of seed lots were infected with *V. dahliae* (Omer *et al.*, 2000). The survey was conducted from Victoria and Tasmania where potato production was more than 50% of Australia's total production (ABS, 2012). The high percentage rate in the seed tuber lots indicated that *V. dahliae* may have been widely distributed across potato growing areas. Overseas studies reported that infected certified seed tubers can readily transmit significant levels of *Verticillium* species into potato growing regions to establish new infection (Easton *et al.*, 1972; Krikun and Orion, 1979). In Australia, *Verticillium* species have been recorded in potato seed tubers previously (Wicks *et al.*, 1997), but their presence within a large number of seed lots was not investigated.

Although *V. dahliae* was found to be widespread in potato tubers in the survey, the potentially more serious pathogen, *V. albo-atrum* was identified in a few tuber seed lots in both Tasmania and Victoria. This was the first report of *V. albo-atrum* detected in potato in Australia for 45 years. Walker (1990) reported that *V. albo-atrum* was introduced into Australia many years ago possibly with infected potatoes. However, surveys conducted in 1996-1997 and 2005 reported that *V. dahliae* was the predominant species isolated from the petiole of infected potato plants in South East Australia, but failed to detect *V. albo-atrum* (Powney *et al.*, 2005; Harding and Wicks, 2007). The prevalence and incidence of *V. albo-atrum* is of particular concern to the Australian potato industry. In USA and Canada, *V. albo-atrum* has been shown to be a major pathogen of potatoes (Uppal *et al.*, 2007; Pegg and Brady, 2002). The Verticillium pathogen has a wide host range and causes wilt disease in many other crops (Jabnoun-Khiareddine *et al.*, 2006; Kim *et al.*, 2001; Burgess *et al.*, 2008). Walker (1990) reported that earlier identifications may have failed to distinguish between *V. dahliae* and *V. albo-atrum*, hence assumed that diseases reported as Verticillium wilt were caused by *V. albo-atrum*. The taxonomy of *Verticillium* species in Australia was validated based on detailed morphological studies and confirmed by multigene phylogenetic analysis. *V. tricorpus* was also identified from the vascular tissue of seed tubers from Victoria and Tasmania. Similar observations on *V. tricorpus* were recorded from the vascular tissue of tubers in UK and Japan (MacGarvie and Hide, 1966; Ebihara *et al.*, 2003).

Stem-end vascular discoloration of the tuber was not correlated with presence of *V. dahliae*, suggesting that these symptoms were not a reliable indication of Verticillium wilt infection. This was in agreement with an earlier study on the recovery of *V. dahliae* from tubers containing vascular discolouration (McKay, 1926). Recently, Wang and Bethke (2013) reported that stem-end discolouration may cause stem-end chip defect, which is a serious quality concern for the USA potato chip industry (Wang and Bethke, 2013). Further studies are needed on the effect of vascular discolouration to cause stem-end chip defect in Australia.

Plectosphaerella cucumerina was frequently isolated from the vascular tissue of seed tubers. Recent reports suggested that *P. cucumerina* was an emerging pathogen of several plant species that caused wilt, leaf spot, fruit, root and collar rot, and collapse (Carlucci *et al.*, 2012; Ayodele and Ilondu, 2008; Gilardi *et al.*, 2013; Garibaldi *et al.*, 2013). More recently, a national survey in a population of the New Zealand pasture *Cirsium arvense* frequently recovered *P. cucumerina* in the subterranean section of stems (Skippa *et al.*, 2013). However, *P. cucumerina* has also been identified as a potential bio-control agent against Potato Cyst Nematode (Atkins *et al.*, 2003). Further research on the pathogenicity of *P. cucumerina* and its potential to cause disease in potatoes is needed.

In Australia the seed certification scheme does not require testing for *Verticillium* species. The reason for not testing for *Verticillium* in the seed tuber might have been due to the difficulties of isolating and identifying the pathogen from the internal vascular region of seed tubers on a large scale (Harding and Wicks, 2007). Infected vascular tissue of tubers may be the reason for the movement of Verticillium throughout commercial production areas in Australia. Other possibilities for the spread of the pathogen from infected fields to clean potato production areas maybe through infested soil adhering to the surface of seed tubers, shared harvester and cultivation equipment, irrigation water or even wind dispersal. Dung *et al.* (2013) reported the possibility of spreading *V. dahliae* from seed potato producing states to commercial production areas across the United States with soil that had adhered to the surface of tubers. Further large scale surveys are recommended across all potato growing areas of Australia to elucidate the level of Verticillium in soil adhering to the seed tubers and in tuber vascular tissue that may be involved in the dissemination of the pathogen to clean potato production areas and to assess whether certification standards for seed potato need to be revised.

Australian *V. dahliae* isolates varied in pathogenicity on potato cultivars and eggplant. The Tasmanian *V. dahliae* isolate TASVd25 was the most aggressive of all the isolates assessed in both potato and eggplant. These results were in agreement with other studies reporting pathogenic variability within *V. dahliae* isolates on a range of hosts (Strausbaugh, 1993; Resende *et al.*, 1994; Daayf *et al.*, 1995; Dobinson *et al.*, 2000; Uppal *et al.*, 2007; Negahi *et al.*, 2013). The disease severity caused by *V. dahliae* was higher in the susceptible cv Shepody than the moderately resistant cv Ranger Russet which was consistent with results of Arbogast *et al*. (1999) and Jansky (2009) who showed variation in cultivar resistance to Verticillium wilt. Compared to the symptom severity in potatoes, eggplants showed less disease severity in both trials. The highly aggressive isolate (TASVd25) will be used for future Verticillium wilt resistant screening programs in Australia.

Verticillium dahliae, *V. albo-atrum* and *V. tricorpus* were all found to be pathogenic on potato cv Shepody. Disease severity was generally higher for *V. dahliae* and *V. albo-atrum* compared to *V. tricorpus* isolates which were less aggressive in infected plants. This was the first record of the pathogenicity of *V. tricorpus* on potatoes in Australia. Previous studies in Canada and USA showed that *V. tricorpus* was a minor pathogen in potato that produced weak Verticillium wilt symptoms (Heinz and Platt, 2000; Robinson *et al.*, 2006). Ebihara *et al* (2003) and Robinson *et al* (2006) reported that *V. tricorpus* infected and colonized potato plants and caused disease. In contrast, Mahuku *et al.* (1999) reported that *V. tricorpus* was unsuccessful in colonizing potato stems. Further research is needed into the effect of *V. tricorpus* on plant growth and its ability to reduce yield loss in potatoes. All these Verticillium species are known to have wide host range and may infect other crops in Australia. Cross-pathogenicity studies of the isolates on different host plants in Australia need to be conducted. Such information would be valuable in developing efficient and effective management strategies for Verticillium wilt in Australian crop production systems.

In eggplant, the highly pathogenic isolate (TASVd25) caused severe disease symptoms; and reduced plant height, aerial biomass and root weight. Bhat and Subbarao (1999) reported that disease scoring in

combination with measuring growth parameters could provide an accurate evaluation of the pathogenicity of *V. dahliae* in various host plants. The eggplant seedling bioassay was less time consuming and more cost effective compared to using tissue culture generated potato plants in a pathogenicity screening trial. Eggplant has been shown to be a preferential host for *V. dahliae* (Resende *et al.*, 1994; Mansoori *et al.*, 1995; Jabnoun-Khiareddine *et al.*, 2006) and eggplant seedlings have been used in pathogenicity trials for *Verticillium* species (Korolev *et al.*, 2000) because disease development was rapid in this host.

In *V. dahliae*, pathogenic variability has been shown to be related to pathogenic factors such as toxins which induced the symptoms in the host plants (Mansoori *et al.*, 1995; Meyer *et al.*, 1994; Palmer *et al.*, 2005). Pathogenicity was also reported to be dependent on the vegetative compatibility groups (VCGs). Overseas studies reported that *V. dahliae* isolates collected from potatoes belonged to VCG 4A and VCG 4B, with VCG 4A being highly aggressive on potatoes compared with isolates of VCG 4B and other VCGs (Joaquim and Rowe, 1991; Omer *et al.*, 2000). It is possible that pathotypes belonging to different VCGs exist in Australian populations of *V. dahliae*. Recent studies found M1 and M2 molecular markers for the accurate determination of major VCGs and suggested that in future, these novel molecular markers can be used for large population genetic analysis (Papaioannou *et al.*, 2013). However, further research is needed to identify the VCGs and mating type determination of *V. dahliae* in Australia.

ROLE OF *V. DAHLIAE* INFECTED SEED TUBERS IN THE DEVELOPMENT OF VERTICILLIUM WILT DISEASE

Infection of the vascular tissue of tubers did not lead to severe wilt symptoms of the leaves of potatoes however, the pathogen was isolated from symptomless plants which may have indicated a low level of infection and colonisation of the stem, petiole and leaf tissue. A low level of infection in symptomless plants may result in a slow build-up of inoculum over several cropping seasons until a threshold level of inoculum is reached that results in severe infection and symptom development. Dung and Johnson (2012) reported that *V. dahliae* in the tubers may be compartmentalized in the vascular region of infected tubers which might slow down or completely prevent subsequent colonization of the growing plants. There were cultivar differences to *V. dahliae* with moderate resistant cv. Ranger Russet having a lower level of infection, transmission and resulting soil inoculum compared to the susceptible cv. Shepody. This was similar to previous studies where there were differences in the vascular infection between resistant and susceptible potato cultivars (Atallah *et al.*, 2007; Bae *et al.*, 2007; Davis *et al.*, 1983). Further field trial studies are required into the mechanism of the build-up of soil inoculum and the significance of soil inoculum levels on disease development over several cropping generations.

This was the first molecular study that quantified the level of inoculum of *V. dahliae* in the soil that originated from naturally infected tubers. In glasshouse trials there were large variations in the level of inoculum of *V. dahliae* (pg DNA/g of soil) in the soil of each pot at the conclusion of the trial. This variation might have been due to the difference in initial inoculum levels in the vascular tissue of the planted seed tubers. As well, when the plants were harvested at maturity the infected plant debris was discarded thus preventing inoculum from this infected plant material being incorporated into the soil. Hence the end result was lowered quantities of inoculum in the soil as detected using the molecular bioassay. Under field conditions, after senescence, the infected above ground plant parts probably contributed significantly to increasing the inoculum in the soil. Incorporation of infected potato plant tissues into soil at harvest may lead to resting structures (microsclerotia) being slowly released as the tissue decays. Microsclerotia in the plant debris has been shown to retain viability for a longer time than those in free soil and are thus able to germinate over many years (Evans *et al.*, 1966; Farley *et al.*, 1971).

The level of soil inoculum originating from the naturally infected individual tubers was too low to significantly contribute to subsequent disease development in plants grown from healthy tubers planted into the infested soil. There appeared to be an inoculum threshold level required before newly planted tubers were able to become infected. *Verticillium* infection and symptom expression in plants has been reported to be mainly influenced by the environmental conditions and quantity of inoculum present in the soil (Cappaert *et al.*, 1992; Mol *et al.*, 1995; Nagtzaam *et al.*, 1997; Bejarano-Alcazar *et al.*, 1996; Wheeler *et al.*, 1994). Further molecular and electron microscopic studies are needed to assess the infection process of *V. dahliae* from the soil into germinating tubers and to determine the threshold level required for sustained infection.

Verticillium tricorpus infected seed tubers resulted in the infection and colonization of potato plants however, very little Verticillium wilt symptoms were observed. Similarly, Ebihara *et al* (2003) isolated *V. tricorpus* from symptomless plants and Robinson *et al.* (2006) found the pathogen in the progeny tubers. The infected tubers contributed to inoculum in the soil at a very low level as detected using an Anderson sampler to quantify the inoculum in the soil. The detection of inoculum may have been greater if quantification was carried out using PCR-based molecular techniques. However, PCR-based diagnostic techniques have yet to be developed in Australia for *V. tricorpus*. Molecular based quantification has been shown to be more specific to the target *Verticillium* species (Bilodeau *et al.*, 2012) whereas the traditional plating method (Anderson sampler) has been shown to be less sensitive, as well as more time consuming (required 4 to 8 weeks) with an increase in misidentification.

INTERACTION BETWEEN *P. CRENATUS* AND *V. DAHLIAE* TO CAUSE PED SYNDROME

A synergistic interaction occurred between *V. dahliae* and *P. crenatus* resulting in increased disease severity and decreased yield of potato plants. This was the first report demonstrating the importance of *P. crenatus* nematodes and *V. dahliae* in the development of PED syndrome. In the USA *Pratylenchus penetrans* has been recorded as the major nematode involved in PED syndrome with the interaction between *V. dahliae* and *P. penetrans* resulting in early onset of disease symptoms and thus lower yields (Martin *et al.*, 1982; Rowe *et al.*, 1985; Rowe and Powelson, 2002; Rotenberg *et al.*, 2004).

The *Verticillium*/nematode interaction experiments differed from most previous experiments in that they were performed with different levels of *V. dahliae* inoculum which was quantified in the soil using molecular techniques to assess the threshold level for infection. At the high quantity of inoculum (4,000 pg *V. dahliae* DNA/g of soil) there appeared to be a synergistic interaction between *V. dahliae* and *P. crenatus* resulting in quite high disease severity. The use of a nematicide to remove the influence of nematode infection in the trials resulted in reduced disease severity (from a rating of 4 to 2) although the incidence of infection was not affected. *P. crenatus* was the only root lesion nematode species found in the field soil. *P. penetrans* has not been shown to be prevalent in potato growing regions of Australia whereas *P. crenatus* was detected from 60% of fields sampled (Harding and Wicks, 2007). These results differ from other studies in USA where *V. dahliae* and *P. crenatus* did not synergistically interact and cause disease in potatoes (Riedel *et al.*, 1985; Bowers *et al.*, 1996). Rowe *et al*. (1987) also reported that *Verticillium* species had no interaction with *P. crenatus* and thus caused little crop loss in a susceptible cv Superior.

For potato plants there was a threshold level of *V. dahliae* inoculum between 300 and 1000 pg *V. dahliae* DNA/g soil required before infection and colonization occurred in plants however, at these inoculum levels there was no visible disease symptoms and no effect on tuber yield. The maximum quantity of nematodes assessed was 3 *P. crenatus*/g of soil. This suggested that the pathogen could infect and colonize the vascular region of potato plants and serve as an inoculum source for future plantings. In contrast, Rowe *et al.* (1985) reported that yield reduction occurred in the field without infected plants showing symptoms.

Based on the petiole isolation, earlier infection of plants occurred where the inoculum consisted of both *V. dahliae* and *P. crenatus*, than from plants grown in the soil with *V. dahliae* alone (nematicide treated soil). These results were supported by the findings of Powelson and Rowe (1993) who reported that minimum quantities of *V. dahliae* and *P. penetrans* were required to establish infection and disease in potatoes. Similarly, Jacobsen *et al.* (1979) observed earlier infection in plants inoculated with *V. dahliae* and *Meloidogyne hapla* than with *V. dahliae* alone. It is difficult to determine threshold values between studies due to different non molecular techniques used by other researchers to measure the economic threshold levels of infection (Francl *et al.*, 1988; Macguidwin and Rouse, 1990; Nicot and Rouse, 1987). Further studies are required to determine *V. dahliae* inoculum threshold levels required to establish infection using molecular quantification and the interaction with higher quantities of *P. crenatus* in the soil, on development of PED.

In contrast, the susceptible eggplant seedlings were infected when inoculated with *V. dahliae* and *P. crenatus* at quantities as low as 85 pg *V. dahliae* DNA/g of soil and 0.3 *P. crenatus*/g of soil, respectively. However there was no additional effect on infection severity and plant biomass at higher inoculum quantities (at 100 and 300 pg *V. dahliae* DNA/g of soil). Saeed *et al.* (1998) reported similar findings when potato cv Russet Burbank was inoculated with *P. penetrans* and *V. dahliae*.

Nematicide treatment of the soil did not prevent infection of the plants by *V. dahliae* but reduced the severity and impact of the disease. This showed the synergistic interaction between *V. dahliae* and *P. crenatus* to cause severe wilt in eggplants. This was the first report of the synergistic interaction between *V. dahliae* and *P. crenatus* to cause severe wilt disease in eggplant. Earlier studies reported that *V. albo-atrum* and *P. penetrans* had a synergistic relationship to cause disease in eggplant (McKeen and Mountain, 1960).

Eggplant has been shown to be a preferential host for *V. dahliae* (Resende *et al.*, 1994; Mansoori, 1995; Jabnoun-Khiareddine *et al.*, 2006)because disease development is rapid on this host. Eggplants can be used as bait for Verticillium wilt from the naturally infested field soil. However, relatively high inoculum is needed to cause disease in potatoes. Further studies are needed on the comparison of infection processes in eggplant and potatoes when low quantities of *V. dahliae* and *P. crenatus* are present in the soil.

Interestingly the quantity of inoculum (both *V. dahliae* and *P. crenatus*) at harvest had decreased substantially in both potato and eggplant pot trial experiments. The lower number of *P. crenatus* in the postharvest soil might have been due to the soil type, biotic and abiotic factors that influenced nematode biology (Umesh and Ferris, 1992; Florini *et al.*, 1987). Environmental factors, such as soil type, moisture, temperature, host resistance and aggressiveness of strains may influence the quantity of *V. dahliae* microsclerotia in the postharvest soil. Similar results were observed in Artichoke planted fields at the end of the growing season (Berbegal *et al.*, 2007). Evans *et al.* (1967) found a seasonal fluctuation and a decrease in the level of microsclerotia throughout the growing season in a cotton field. They also found that an increase in the microsclerotia in the soil at harvest correlated with the release of microsclerotia from the infected plant tissue. However in the glasshouse experiments at harvest, plant debris had been removed which may have reduced the level of *V. dahliae* inoculum in the soil.

There are several other reasons that might have influenced the postharvest quantity of *V. dahliae* inoculum in the soil. Microsclerotia have been reported to be the main survival structure of *V. dahliae* and have served as inoculum for subsequent crop plantings. Microsclerotia largely formed in the shoot tissue of senescent plants following root infection and vascular colonization in plants (Soesanto, 2000). The number of microsclerotia in the plant tissue has been shown to be mainly affected by the quantity of inoculum in the soil and environmental conditions (Soesanto and Termorshuizen, 2001). From a single infected stem, more than 90,000 microsclerotia can be introduced into soil (Slattery, 1981). Soesanto (2000) studied the

dynamics of microsclerotia in the soil during the 1-2 years in the absence of host and found a low recovery one day after incorporation of inoculum into the soil, followed by a delayed increase in recovery.

Production of *V. dahliae* microsclerotia inoculum prepared in soil medium was used for various treatments in glasshouse experiments. The size of the microsclerotia in the soil was not measured because under field conditions various sizes of microsclerotia have been observed (Rowe *et al.*, 1987; Hawke and Lazarovits, 1994). Another reason for the reduction of postharvest quantity of inoculum in soil might have been due to the various sizes of microsclerotia and small sized microsclerotia may have died very quickly. Similarly, Hawke and Lazarovits (1994) reported that smaller microsclerotia exhibited lower germination percentage than larger microsclerotia (>75 µm) when stored fro long periods of time.

There were high concentrations of nutrients and soil organic matter in the soil used in the glasshouse experiments which may have reduced infection in the treatments with low quantity of *V. dahliae* inoculum. Davis *et al.* (2001) reported that improved organic matter and nutrient availability were closely related to Verticillium wilt suppression compared to reduced nutrient availability. However further studies are needed to compare the survivability and pathogenicity of artificially prepared microsclerotia compared to microsclerotia formed in the infected plant tissue. Also, further work using traditional plating methods as well as molecular based quantification needs to be done to determine the critical period of propagule release from dead plant tissue into the soil. These studies would determine the accuracy of sampling methods and assessments. This may provide growers with a better idea of the risk of PED in the upcoming growing season and could reduce the cost of crop loss and improve crop quality.

POTATO CULTIVARS RESISTANT TO *V. DAHLIAE*

Apart from soil moisture and temperature, inoculum density plays an important role in symptom expression as too high an inoculum level has been shown to break genotype resistance. Glasshouse bioassays using roots dipped in spore suspensions as the inoculation method were optimised prior to the cultivar screening experiments so as to achieve an appropriate spore concentration to inoculate the plants that would differentiate host reactions without over-killing the plants. Eggplants were also used to optimise the level of inoculum using the root dip inoculation method since these plants were easy to infect and were more amenable than potatoes to accurately measure the impact of inoculum concentration on plant growth.

Inoculum concentration experiments whereby plant roots were dipped in spore suspension cultures of various concentrations showed that for potatoes there was an inoculum threshold where a specific concentration was required to cause infection and symptoms. The inoculum threshold appeared to be between 10^3 and 10^4 spores/mL in the first trial, and 10^2 and 10^3 spores/mL in the second trial. For eggplant the inoculum threshold for infection and symptoms to occur was similar to potatoes at 10^4 spores/mL. At this inoculum concentration there was a significant reduction in growth parameters such as plant height and above ground biomass.

Glasshouse screening trials revealed several cultivars in the national potato collection (maintained by ViCSPA) with high resistance to *Verticillium dahliae*. Comparing the results of the three screening trials, cultivar Denali showed moderate to high resistance and Catani and Desiree moderate resistance host reactions in pot trials. Cultivars Nicola and Russet Burbank C showed susceptible host reactions. The cultivars Coliban C and Pike A showed different host reactions in the two trials. In the first trial at 10 weeks Coliban C was moderately susceptible (rating of 3) but was resistant (1) in the second trial. This difference may have been due to Coliban C senescing earlier in the first trial which allowed the Verticillium wilt pathogen that had already infected the plants to become more aggressive, hence the rapid increase in

ratings at 10 wai. On the other hand, cultivar Pike A which was resistant in the first trial showed a susceptible host reaction in the second trial. The change in host susceptibility of Pike A may have been due to ineffectual infection of the plants in the first trial, or the increased inoculum concentration in the second trial (from 10^4 to 5 X 10^4 spores/mL) which was above the threshold level to cause infection in this cultivar.

Resistance to Verticillium wilt has been identified in several potato clones in the USA potato breeding program using a complex method involving assessing symptom expression in the field, colonisation of stem sap by the pathogen, and numbers of propagules in senescent stems (colony forming units) (Jansky, 2009). The problem with this method for determining resistance was that it involved field planting where the level of *Verticillium* and nematodes in the soil were unknown. Host resistance is also likely to be overcome when high inoculum levels occur in the soil (Johnson and Dung, 2010) thus it is important to have a screening trial with constant level of inoculum. Understanding the mechanism of resistance would enable a more accurate screening trial to be developed since genetic resistance is likely to include restriction of root infection and colonisation of the vascular system in the plant

It is important to be certain that the resistance identified in certain cultivars is in fact due to inheritable resistance genes and not subject to changes in environmental conditions, hence further field trials for assessing resistance in these cultivars needs to be undertaken.

EFFICACY OF SOIL AMENDMENTS TO SUPPRESS VERTICILLIUM WILT DISEASE IN POTATOES

In the microcosm assay atomic sulphur severely affected the viability of the microsclerotia over 8 weeks of incubation. Although the brown coal and blood and bone meal reduced viability of the microsclerotia they were less effective over the same time period. Sulphur may have had a direct fungicidal activity on the microsclerotia. However, the severity of the sulphur treatment on microsclerotia viability may have also correlated with a decrease over time of the soil moisture in the sulphur microcosm assay (more so than in the brown coal and blood and bone meal experiments), where perhaps the drying of the soil affected the viability of the microsclerotia. This was supported by the rapid decline in microsclerotia viability in the controls of the sulphur experiment. For the blood and bone meal treatments there was no significant difference in microsclerotia viability between the 1% and 10% at 4 weeks after incubation. Blood and bone meal at 1% was sufficient to significantly reduce the viability of microsclerotia by 50 to 60% after 4 weeks. In contrast, Lazarovits *et al* (1999) reported that meat and bone meal reduced the viability of microsclerotia by about 80% in 2 weeks after application.

The pH of the untreated soil in two of the experiments actually increased after 4 weeks of incubation in the microcosm system before decreasing. This may have been due to an interaction between the soil micronutrients, water content and changes in the soil microbes in these experiments however, this change in pH did not occur in the third experiment. The significant decrease in soil pH in the sulphur treatment may not have been great enough to affect microsclerotia directly but may have altered the microbiology of the microcosm. The rapid increase in pH in the 10% blood and bone treatment at 8 weeks may be a reflection of a major shift in the composition of the soil microbe population in the microcosm due to the high levels of organic matter. Further experiments are required to assess changes in microbe populations in the microcosm environments, with and without soil amendments.

Organic soil amendments such as liquid swine manure have been shown to control soil borne pathogens of potatoes including *Verticillium dahliae* through the release of ammonia and nitrous acid following degradation of the amendment by microbes (Lazarovits *et al.*, 2001). Tenuata and Lazarovits (2002) reported that nitrous acid and ammonia were released from meat and bone meal however, the levels to which the toxins accumulated in the soil and their effectiveness depended on the composition of the soil.

Brown coal as a fertiliser has also been shown to improve the structure of soils characterised by low organic matter and increase the level of humic acid (Kwiatkowska *et al.*, 2008).

The large decrease in microsclerotia viability in the controls of all microcosm experiments, especially within the first few weeks, would appear to indicate that the artificially produced microsclerotia used in these experiments were not durable. This may have been due to the physiological properties of the *in vitro* produced microsclerotia that may have resulted in a deficiency of metabolites or melanin being incorporated into the microsclerotia. Melanin is an important component of microsclerotia required to enhance survivability under natural soil conditions. These results were in contrast to the findings of Hawke and Lazarovits (1994) that produced microsclerotia in liquid medium and reported that these microsclerotia had high viability for over 12 months. Furthermore, microsclerotia that were buried in soil for 3 wks did not lose their viability.

Perhaps microsclerotia that develop in senescing plant tissue absorb specific chemicals from the host substrate that enables them to produce secondary metabolites, such as melanin, that protects the internal fungal tissue from the stresses of the soil abiotic and biotic (microbial) environment. On the other hand, there probably only needs to be a very small percent of long-term surviving microsclerotia in field soil that can then germinate on contact with root exudates and infect roots. In the field situation, most microsclerotia probably die within weeks or months of being produced. More studies are needed into the physiology of microsclerotia production.

In the two glasshouse pot trials to optimise inoculum concentration (number of microsclerotia – CFU) required to infect potato seedlings there was a large discrepancy between the estimated number of microsclerotia in the prepared inoculum and the actual number of microsclerotia in the soil prepared with the inoculum. The reason why the quantity of inoculum (CFU) determined after planting was 10 fold higher than the estimated quantity of inoculum before planting was probably due to the microsclerotia dissociating into smaller size fragments during the initial incorporation and mixing of the microsclerotia into the soil, which resulted in an increased number of CFUs in each treatment. Nevertheless, the amount of inoculum in the soil (CFU) in each treatment was roughly proportional to the initial quantity of inoculum. In trials 1 and 2, there was a large variation in the number of microsclerotia between replicates of each treatment which may have been due to sampling error. It was most likely that after setting up the inoculated pots, the inoculum was not evenly distributed throughout the soil in the pot, hence the 50 g samples taken for microsclerotia numeration did not contain an evenly distributed quantity of inoculum.

There was no apparent impact of 1% brown coal on preventing the infection of plants inoculated with 500 and 750 CFU of microsclerotia/ g of dry soil. The failure of several plants to become infected in the trial may have been due to the roots of the plants not making contact with the low concentration of microsclerotia in the soil. However, the plants in the similar inoculum treatment, but without the brown coal, all became infected. There was a significant reduction of microsclerotia inoculum from planting to harvest with microsclerotia generally not being detectable at harvest. The rapid reduction in inoculum was reflected in the microcosm trials where the viability of microsclerotia rapidly decreased within 1 to 2 weeks of incubation in the soil. Therefore, the plants that did not become infected may have simply not come in contact with microsclerotia because their root systems were not as healthy and growing as fast as other plants within the first 2 weeks. At harvest, although there was a reduction of the dry weight of biomass of plants between the control and treatments, this was not significant ($p < 0.05$) except in the treatment with microsclerotia at 500 CFU/g which had significant reduction in biomass. Interestingly, 1% brown coal did not act as a biofertiliser and increase biomass.

TECHNOLOGY TRANSFER

REFEREED JOURNAL ARTICLES

Nair, P. V. R., Wiechel, T. J.,·Crump, N. S. & Taylor, P. W. J. (2014). Seed tuber infection, validation of taxonomy and pathogenicity of *Verticillium* species infecting potatoes in South East Australia. (in preparation).

SCIENTIFIC CONFERENCE PAPERS

Prakash, V. R., Wiechel, T. J.,·Crump, N. S. & Taylor, P. W. J. (2013). Seed tuber incidence and pathogenicity of *Verticillium* species infecting potatoes in Australia. 19th Australasian Plant Pathology Society Conference, November 2013, Auckland, New Zealand.

Dharjono, V. N., Taylor, P. W. J., Wiechel, T. J. &·Crump, N .S. (2013). Protecting commercial Australian potato genotypes from Verticillium wilt through identification of sources of resistance. 19th Australasian Plant Pathology Society Conference, November 2013, Auckland, New Zealand.

Dharjono, V. N., Taylor, P. W. J., Wiechel, T. & Crump, N. (2013). Protecting commercial Australian potato cultivars from Verticillium wilt through identification of sources of resistance. Workshop Potato Disease – where are we at, and what happens next? 25 November 2013 Auckland, New Zealand.

Dharjono, V. N., & Taylor, P. W. J. (2012). Evaluating resistance to *Verticillium* wilt in commercial Australian potato genotypes. 10th Annual MSLE Graduate Research Conference, November 2012, Dookie, Australia.

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Prakash, V. R., Wiechel, T. J., Crump, N. S., Porter, I. J. & Taylor, P. W. J. (2011). Interaction between *Verticillium dahliae* and root lesion nematodes in potato and eggplant. 9th Annual MSLE Graduate Research Conference, November 2011, Dookie, Australia; p 53.

Prakash, V. R., Wiechel, T. J., Crump, N. S., Porter, I. J. & Taylor, P. W. J. (2011). Understanding the significance of internal seed-borne inoculum in Verticillium wilt of potatoes. Workshop on diseases of potatoes, April, 2011, Northern Territory, Australia.

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INDUSTRY & MEDIA PUBLICATIONS

Potato early dying syndrome in Australia. Potatoes Australia; August/September 2012, pp. 14-15.

Fighting the fungi. Potatoes Australia; June/July 2011, pp. 28-29.

Verticillium's wake-up call. Potatoes Australia; February 2010, pp. 20-21.

INDUSTRY & PEER PRESENTATIONS

Taylor, P. W. J. (2013). Enhancing the understanding of *Verticillium* spp. in Australian potato production. Australian Potato Research Program Science Symposium & Industry Session Program, Tullamarine, Victoria.

Dharjono, V. N. (2013). Protecting commercial Australian potato varieties from Verticillium wilt through identification of sources of resistance. Australian Potato Research Program Science Symposium & Industry Session Program, Tullamarine, Victoria.

Prakash, V. R. (2013). What significance does planting *V. dahliae* infected potato seed tuber contribute to inoculum potential/carryover in the soil? Australian Potato Research Program Science Symposium & Industry Session Program, Tullamarine, Victoria.

Jiang, C. (2013). Population Genetic Diversity and Pathogenicity of *Colletotrichum* coccodes - Causal agent of black dot disease of potatoes in Australia. Australian Potato Research Program Science Symposium & Industry Session Program, Tullamarine, Victoria.

Prakash, V. R., Wiechel, T. J., Crump, N. S. & Taylor P. W. J. (2012). Influence of root lesion nematode, *Pratylenchus crenatus* on Potato Early Dying syndrome in Australia. 5th Biennial Seed Potato Conference, July 2012, Ballarat, Victoria; p21.

In addition, in 2010, there was a presentation on Verticillium wilt of potato delivered to 25 farmers at a farmers meeting at Cora Lynn, Victoria, which was followed by a discussion on the impact and control of PED. On-farm visits to potato farms in Ballarat were undertaken in 2010 to discuss the impact of seed borne Verticillium spp on incidence of PED and disease development.

At the completion of the project fundamental knowledge will have been provided regarding Verticillium wilt and PED in Australia potato production. The information generated will have been transferred to industry through industry publications such as Potatoes Australia and industry/grower workshops; and presented at scientific conferences to advance understanding of the research issues. The participative involvement of industry personnel in collecting samples (eg seed certification officers, company agronomists) has already fostered interest and knowledge transfer of project progress and results. Through this project PhD and MPhil students were trained with skills relevant to the industry sector, with specific deliverables to be achieved of at least 5 refereed scientific papers, and at least 3 articles in industry journals (Potatoes Australia). The students will also have presented at least 5 scientific conferences and 2 industry conferences/workshops.

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

The key outcomes and recommendations from this project are summarized as follows:

Potato plants infected with root lesion nematode *P. crenatus* and *V. dahliae* showed increased disease severity of Verticillium wilt disease which is related to the symptoms of PED syndrome. Therefore, it is **recommended** not to plant tubers into fields with high levels of these pathogens. In fact it is **recommended** to have soil in fields that have grown potatoes for several years to be tested for levels of *V. dahliae* and *P. crenatus*.

V. dahliae was found to be the main Verticillium species associated with potato tubers in Victorian and Tasmanian potato crops and although other species of *V. albo-atrum* and *V. tricorpus* were isolated from infected tubers at a low incidence they were less pathogenic. Differences in the level of pathogenicity of *V.*

dahliae isolates were identified with several isolates being highly aggressive and causing greater disease severity in the potato plants than the less aggressive isolates. It is **recommended** that further surveys within the Australian potato producing regions be undertaken to isolate these three Verticillium species and assess their level of pathogenicity on a broad range of commercial cultivars. Of particular concern was the identification of *V. albo-atrum* in tubers in Tasmania and Victoria as this species is known to be highly virulent in potatoes in cooler regions of overseas potato production areas. It is **recommended** that the pathogenicity of isolates of this species be assessed for their potential to cause high disease severity in genotypes grown under cooler temperatures similar to environmental conditions in the southern potato producing areas of Australia.

V. dahliae tuber infection appears to have little impact on infection and disease severity of first generation plants grown from these tubers however, may play a significant role in building up soil inoculum over several generations of planting, and may be important in transmission of the pathogen between regions. It is **recommended** to study the build-up of soil inoculum in field trials planted to infected tubers over several cropping generations.

The synergistic interaction of *V. dahliae* and *P. crenatus* to produce PED was determined by varying the inoculum levels of *V. dahliae* in nematode infested field soil. It is **recommended** that further glasshouse trials are undertaken to study the interaction of different concentrations of *P. crenatus* with varying inoculum levels of *V. dahliae* and *V. albo-atrum*.

Several Australian potato cultivars were identified as being resistant to *V. dahliae* in glasshouse trials. The bioassay to identify resistance used an inoculation method of dipping roots in a spore suspension of the pathogen. Although this provided a reproducible method to assess cultivar reaction, it is **recommended** that further screening trials be carried out using soil inoculated with microsclerotia to assess cultivar reaction as this is more close to reproducing field conditions. It is also **recommended** that field trials be implemented to assess the resistance of Denali, Catani and Desiree in soils with a history of high incidence of PED syndrome. Yield loss assessments could then be more accurately determined.

Soil amendments sulphur and brown coal were shown to reduce the viability of microsclerotia in laboratory assays. These soil amendments may have potential in integrated disease management programs however, it is **recommended** that further glasshouse trials are undertaken to assess their efficacy to suppress Verticillium wilt and PED syndrome before assessing in field trials.

It is **recommended** that the next phase of the PED project post APRP2 will need to involve the following experimental areas that should be undertaken to enhance the adoption of the outcomes of the project.

- Glasshouse studies to assess efficacy of soil amendments, atomic sulphur and brown coal and blood and bone meal against both *V. dahliae* and *P. crenatus* nematodes.
- Field trials to demonstrate the results to industry (efficacy of soil amendment to prevent PED).
- Glasshouse pot trials to screen cultivars for resistance using microsclerotia inoculum.
- Identification of the mechanism of resistance of cultivars to *V. dahliae*.
- Field trial assessment of cultivars identified in glasshouse screening to have resistance to *V. dahliae*.
- Further glasshouse trials to evaluate the interactions of *V. dahliae* and *V. albo-atrum* with *P. crenatus* in producing PED syndrome.
- Evaluating the different threshold levels of inoculum of *V. dahliae* and nematodes required to cause PED in glasshouse and field trials.

ACKNOWLEDGEMENTS

This project has been funded by HAL using the processed potato industry levy and matched funds from the Australian Government. The University of Melbourne, Department of Environment and Primary Industry, and the Victorian Seed Potato Authority have provided in-kind support.

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Horticulture Australia

PT09040 – (30/10/2015)

APRP2 program management

Final Report

Scott Williams and Anne Ramsay

SED Advisory

PROJECT SUMMARY

PT09040 – APRP2 program management

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This report is the final report for project PT09040 APRP2 program management which was the sixth project within the Australian Potato Research Program (PT09039).

2 November 2015

FUNDING SUPPORTERS

This project has been funded by HAL using the processed potato industry levy and matched funds from the Australian Government.

DISCLAIMER

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CHAPTER 7. APRP2 PROGRAM MANAGEMENT

MEDIA SUMMARY

The Australian Potato Research Program phase 2 (APRP2) was managed by SED Advisory on behalf of the University of Ballarat (UB, now known as Federation University). SED was appointed to provide independent management to the program and successfully facilitated the delivery of program contractual obligations. The task was not straightforward owing to the extremely complex contracting arrangements and ongoing political challenges which often drew more attention than the valuable science delivered by the program.

SED had a good relationship with HAL and the researchers, and attributes the successful delivery of the program to the strong communication and collaboration within the program. Credit must also be given to UB for their practical approach to the contractual and financial management of the extremely complex contracted program.

SED's role was heavily focussed on administration and saw the production of templates, facilitation of contractual changes, milestone and payment coordination, organisation of regular technical meetings and biennial research symposia, regular articles for Potatoes Australia, and the progressing of a multitude of ad hoc program-related tasks.

Although value adding was limited owing to the onerous program administrative requirements, as a part of evaluation commitments SED was able to undertake original research to estimate the cost of the major soil borne diseases to the processing potato industry. Prior to the program these figures were not readily available and their production has been acknowledged as a valuable contribution to the industry. The SED-led work estimated that powdery scab is costing the processing potato industry approximately $13.4M per annum, common scab $1.7M and Rhizoctonia $5.4M.

These figures, and an updated benefit/cost analysis suggesting a $7.48 return for every dollar invested in just the PreDicta Pt component of the program, demonstrate the value of the investment in APRP2 and also provide guidance for considerations of future investment in processing potato R&D.

INTRODUCTION AND OVERVIEW

This chapter focuses on the management of the Australian Potato Research Program phase 2 (APRP2). The management project was developed as a result of recommendations provided by Pyksis (HAL project PT07037) to HAL in its scoping of the second phase of the APRP program. The original APRP had been coordinated by one of the program's principal research providers. Accurately or otherwise, there was a perception among some stakeholders that that management and governance would be improved and conflict of interest avoided by appointing an independent program manager.

SED Advisory (formerly SED Consulting) won the tender to manage the APRP2 program in alliance with the University of Ballarat (UB, now Federation University). The reason for seeking involvement of a Higher Education institution is described in the first chapter. Effectively, UB assumed the lead agency role and outsourced the management functions to SED.

Mr Scott Williams was appointed Program Director for APRP2 with Ms Anne Ramsay (subcontractor to SED Advisory) appointed Program Administrator.

STRUCTURAL DESIGN

MODEL

As introduced in Chapter 1, the Pyksis review (HAL project PT07037) recommended a governance model for APRP2 that mirrored to some extent a corporate structure (Figure 7-1). The Processing Potato Industry Advisory Committee (PPIAC) would be the 'Board'; the Principal Investigator of the management organisation would function (albeit partly) as the 'Chief Executive Officer'; and Sub Program Leaders[1] would be the 'Divisional Heads'. HAL, like the shareholders of a company, would have the final authority, although through more direct mechanisms than those afforded to normal company shareholders.

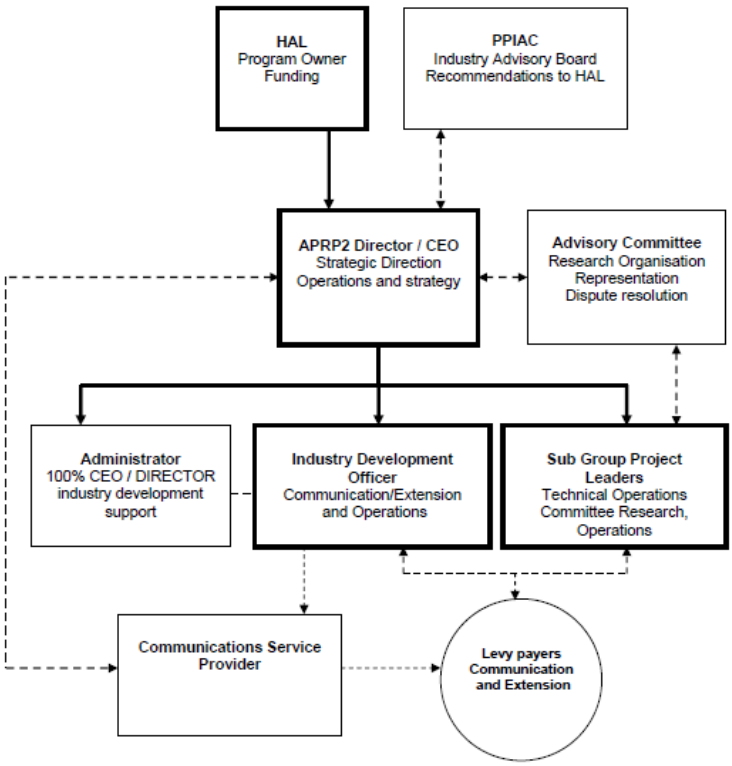

Figure 7-1 – Original proposed APRP2 structure

As shown in Figure 7-1, the Pyksis model envisaged that the program management role would function at both strategic and administrative levels. It would also provide oversight of both the research program

[1] For the purposes of clarity, areas of activity under a single contract with UB/HAL and carrying a HAL code (for example, PT09040) have been defined as 'projects' in preference to the Pyksis terminology of 'sub programs' and have been led by Project Leaders. Areas of activity within projects (for example, the bacterial wilt work within PT09026) have been termed 'sub projects'.

(APRP2 itself) and the extension of the program's outcomes to the grower community – providing important continuity across the research, development and extension (RD&E) continuum.

POSITION DESCRIPTIONS

The specific roles of each of the organisational elements in Figure 7-1, as prescribed by Pyksis and as set out in the contract with SED, are reproduced below.

MANAGEMENT ORGANISATION

- *Responsible for the development and management of the contract, including equity split and Best Practice processes with the research providers. Ensure the handover from previous APRP1 management organisation occurs.*
- *Report to HAL and the PPIAC on financial status of projects and overall program performance in the required formats and time periods.*
- *The Management Organisations will be responsible for ensuring that the contractual obligations for the sub programs are achieved.*
- *Sub Program Leaders (SPLs) are to be made accountable for meeting the contractual obligations to the overall goals of APRP2.*
- *SPLs should have a reporting obligation to the Director in the required format, in relation to the HAL obligations, in addition to reporting to the Research Organisations.*
- *All SPLs should establish in collaboration with the Director, target KPIs incorporating fiscal reporting and responsibility.*
- *Proposals for project variations should be submitted to the Director for review and proposal to the PPIAC for recommendation to HAL.*

PROGRAM DIRECTOR

- *An individual capable of championing the APRP2 program and with demonstrated management and leadership at a senior level to provide strategic direction and business expertise.*
- *Time will be split between strategic/funding activities and operations.*
- *Reports directly to HAL and the PPIAC.*
- *Develop effective reports that meet the needs of all stakeholders in an efficient and effective manner. This includes reporting timelines and requirements of the international collaborators are met.*
- *With HAL, develop templates for the reporting of contracts, international R&D contracts, Intellectual Property management, Confidentiality Disclosure Agreement, Material Transfer Agreements and R&D program variations.*
- *Develop role descriptions for the Sub-program leaders.*
- *Actively manage the program administration role.*
- *Work collaboratively with the industry development officer and communications service provider to ensure that the program delivery is achieved.*
- *Provide the formal reporting link between HAL / PPIAC and the research organisations and other associated committees.*

- *Be responsible for dispute resolution between subprograms and research organisations and consult with HAL in a timely manner.*
- *Report to HAL on the timely achievement of Key Performance Indicators and where necessary recommend required corrective action.*
- *Develop mechanism to evaluate the economic returns of project activities and work with researchers to implement these methods.*
- *Work with the recommended industry development structure, including the communications service provider.*

TECHNICAL OPERATIONS COMMITTEE (TOC)

- *The Technical Operations Committee (TOC) to remain a quarterly progress meeting between all the Subproject Leaders (SPLs), the APRP2 Program Director, industry development and communications, and nominated representatives of the PPIAC.*
- *Organisation of the TOC to be conducted and managed by the APRP2 management staff (secretariat services including agendas, minutes and facilities) and run by the Program Director.*
- *Agendas and minutes of meetings to be circulated to SPLs, Advisory Committee members and PPIAC members for review. Any comments are to be managed by the Program Director.*
- *Agenda items are to be noted via the Program Director before the meeting.*

ADVISORY COMMITTEE (AC)

- *Used as a forum to resolve issues that cannot otherwise be settled by the Technical Operations Committee.*
- *Meetings are convened by Program Director and only convened on an as required basis.*
- *Representatives are welcome to attend any TOC meeting as an observer.*

THE ROLE DESIGN IN PRACTICE

While some of the intent of the Pyksis design was realised in the execution of the program, several important elements were not.

CLEAR AND APPROPRIATE LINES OF AUTHORITY

The PPIAC and SED did not maintain a relationship comparable to that of a Board and CEO. SED attended two PPIAC meetings and presented updates in the initial stages of the program. SED was not invited to attend any PPIAC meetings after April 2011 despite repeatedly offering to do so. The HAL Portfolio Manager did not appear to have any greater access to the PPIAC than the program managers.

This was unfortunate. There was no opportunity for the PPIAC to become apprised first-hand of the latest developments in the program nor to discuss important program matters. This disconnection ruled out a strategic role for the program manager as intended in the Pyksis model. The major impact, though, was a compounding of the problems created by the contractual structure which necessitated repeated contract variations, many of which required PPIAC approval. The process of seeking contract variations was difficult, unwieldy and very slow because of the lack of a direct link to the PPIAC.

Similarly, while relationships between the program managers and the Project Leaders (PLs) were very positive throughout the program, there was never any sense that the program managers occupied a higher order in the hierarchy (as a 'CEO') than the PLs (as 'Divisional Managers'). Such a positioning was never going to be possible in retrospect. There was no codification through contractual or other mechanisms of any formal authority for the Program Director, and PLs already had existing lines of accountability within their organisation.

Thus, appropriately, PLs only worked towards one set of key performance indicators (KPIs) – that is, contract milestones. The program managers worked with PLs and/or project teams where there appeared to be a need; for example, where the direction of the R&D did not appear to be well-focused. On these occasions, though, SED played the role of independent advisor and bridge to other program stakeholders rather than hierarchically-superior manager.

The fact that the program managers did not possess clearly defined authority meant that matters requiring decisions (such as altered payment schedules or milestone criteria) had to be referred to the Portfolio Manager at HAL, regardless of how minor they were. Furthermore, it appeared that the Portfolio Manager's authority to approve changes to a project were also ill-defined. A number of decisions became multiple approvals between the Portfolio Manager, the General Manager and the PPIAC.

The lack of clear lines of authority meant that the program managers were limited in the usefulness they could offer the HAL Portfolio Manager. Ideally, external program or project managers should greatly reduce the workload of HAL staff, and this appears to have been partially achieved in APRP2 – but to nowhere near the extent possible because even seemingly trivial matters had to be dealt with by the Portfolio Manager.

RECOMMENDATION: Future programs should have clearly-defined lines of authority that devolve, to the maximum extent possible without compromising HAL's good governance, decision-making capacity to external managers.

EFFICIENT CONTRACTING THROUGH A SINGLE AGENCY

It may have been envisaged at the outset of the program that contracting all of the projects through a single agency (the University of Ballarat) would reduce transaction costs to HAL – in addition to increasing the funding available through the Research Infrastructure Block Grants (RIBG) scheme, as described in the opening chapter.

It is unlikely that this was the case in practice. While much of the contract development was effectively outsourced from HAL to SED, which may have reduced HAL's costs, a great deal of unnecessary work was created by the need to constantly implement contract variations in order to avoid situations in which none of the project agencies could be paid because one or two milestone criteria were not met on time.

The program managers became very largely focussed on administration at the expense of other possible value-adding activities. Unavoidably, the HAL Portfolio Manager and Contract Managers, as well as personnel at UB, were also required to contribute time towards implementing these variations. It must be acknowledged here that the University of Ballarat processed all contractual, legal, financial and other program matters thoroughly and expeditiously despite its being rendered largely redundant in the governance structure early in the life of the program.

The focus on administration also meant that the program was very much locked into its research objectives with little flexibility to easily alter the course of research or address some minor industry needs.

STREAMLINED ADMINISTRATION THROUGH A DEDICATED FUNCTION

It may also have been envisaged that the administration associated with the program would be streamlined by its centralisation to one dedicated resource. It is also questionable whether this was the case in practice.

There was some feedback to the program managers from PLs during the course of the program that they were overburdened with program administrative requirements. This criticism appears to have arisen, to some degree, from what seems to have been quite a lax reporting regime prior to APRP2. For example, SED was advised that there was no requirement for an annual financial acquittal from each of the projects. SED introduced such a requirement as part of good governance.

The addition of SED to the program structure as an apparent 'additional layer' of administration did undoubtedly complicate life for the teams and resentment was understandable. An example was milestone dates. Originally-agreed milestone dates for sub-projects were, effectively, brought forward during the contracting process with the introduction of a requirement to submit draft reports to SED one month ahead of the milestone due date. This was needed to allow time for the program managers to review the reports, seek changes and manage the collation process. The earlier submission was problematic for some teams in particular because the dates had been selected to coincide with significant points in the potato growing cycle. Ultimately, a compromise was struck where reports were due to SED two weeks before the original date, and in some cases later submissions were accepted on negotiation.

The charge that program administration was excessive is also attributable to the additional work created by the complex contractual structure (see above).

The program managers tried as hard as possible to keep the administrative impost on PLs to a minimum. For example, milestone templates were intended to provide a structure that would facilitate completion and when the templates were sent out ahead of each milestone they were pre-populated with milestone achievement criteria (see below).

SEAMLESS PLANNING AND DELIVERY OF RD&E

The integration of RD&E under a common structure as envisaged in the Pyksis model did not eventuate. The Potato Extension Program (PEP) started later than anticipated, two years after the commencement of APRP2. From the outset, the PEP was quite separate to APRP2. The APRP2 managers had no role in the planning or conduct of the PEP and were not invited to sit upon the reference group attached to the PEP. Where APRP2 projects were showcased during PEP events, contact was made directly with the relevant research leader and without reference to the program managers.

The only forum for exchange between APRP2 and the PEP was APRP2's Technical Operations Committee, meetings of which were attended by the deliverer of the PEP. However, TOC meetings were only ever used to present the intentions or activities of the PEP, not to seek input from other TOC participants.

This discontinuity between 'R&D' on the one hand and 'E' on the other was a serious flaw in processing potato innovation model during the life of APRP2. RD&E must be part of a cycle in which extension both *is continuously informed by* but also *continuously informs* the research and development phases. Fortunately, the research teams were generally well-connected to producers. There were also some (relatively minor) extension elements in certain of the APRP2 projects which helped the research teams to test their research with the target audience. But the opportunity for much greater interaction between the research groups and growers through the PEP was largely unrealised.

RECOMMENDATION: Future research programs should be structured with a continuous innovation cycle in mind, that is, with research, development and extension phases seamlessly integrated and informing each other.

PROGRAM ADMINISTRATION

TEMPLATES AND TOOLS

The Pyksis report emphasised the need for efficiency of process in the management of APRP2, to ensure that the synergies offered by a multi-party program were not outweighed by a heavy administrative burden. The report stated:

- Standardise and simplify processes across the APRP2 organisations, standard processes/templates should be established and used by the participants e.g. Research Organisation contracts, IP management, CDA, MTA, R&D program variations, milestones, workplans etc.
- Some of these documents and processes exist within HAL processes/templates and may be used. However, the Director should review the current systems and templates, develop new processes where appropriate and establish a procedures manual for the Program.

These recommendations were followed in the establishment of the program. The following items were developed by SED and updated on an as needed basis throughout the life of the program. A number of organisations changed staff, their name and/or their logo during the life of the program, and these changes were all reflected in the program material.

- A detailed Procedures Manual.
- Program milestone templates (that included annual project workplans and six monthly budget status).
- Project Leader role descriptions.
- A contact list.
- Powerpoint templates for presentations associated with the program, containing the correct logos and acknowledgements.
- A contract database to manage the updates to each project.

Eighteen months prior to the conclusion of the program, a final report template was commissioned from an independent Microsoft Word® expert, Chrysalis Design. The template was designed to ensure that there would be consistent formatting and numbering for all the final reports and that the process of merging them would be streamlined as far as possible. Chrysalis Design provided training and ongoing support to users of the template.

RECOMMENDATION: HAL should consider retaining a template designer to streamline the process of collating the final reports of all major, multi-party projects.

An internal password-protected website (http://aprp2.sedconsulting.com.au) was also created to capture useful documents for the program. All templates, logos, contacts, approved meeting minutes, milestones, presentations and other program-related materials were placed on the website when finalised. The site also offered a forum for discussions between users. The website was infrequently accessed, and the forum for discussions was not used. Discussions with some PLs suggested that they preferred to access information via email rather than visit the website.

CONTRACT MANAGEMENT

CONTRACT DEVELOPMENT

SED was appointed to manage the APRP2 program once all projects had been selected and proposals fully developed (milestones and deliverables established). The first task for program managers was to contract the previously agreed-to projects.

The APRP2 program operated as a whole-of-program approach rather than on a project-by-project basis, and as such projects were unable to officially commence until the completion of all contracting. The program was initially developed with a September 2009 start date, however delays in contracting the head agreement (between HAL and UB) and the subcontracts meant that contracting took over four months and was not completed until mid-March 2010. The significant delay to the start date had some significant implications for the program:

- There were delays in developing contracts between subcontractors and their collaborative parties ('sub-subcontractors'). As a result some voluntary contributions were delayed which resulted in a whole-of-program delay in payment and even further deferral of project commencement.
- A variation was required from HAL to the head agreement to move the March 2010 milestone deliverables until May.
- Some parties were unable to finalise University grant applications and missed out on needed funding to meet project obligations.
- In some cases a whole potato season was lost to commence project trials.

The first four contracts were executed quickly, particularly considering that negotiations took place over the Christmas period. However, there were two contracts that took considerably longer to finalise. The Tasmanian Institute of Agriculture (TIA) TIA insisted on some changes to the standard HAL contract conditions which required legal exchanges between TIA, UB and HAL. Signature of the South Australian Research and Development Institute (SARDI) contract was also delayed owing to the need for the State Minister's execution and the proximity to the state election.

Despite these delays some larger projects commenced work prior to the official program commencement and were able to deliver on most of their milestone requirements. Smaller projects and subprojects that were unable to commence work without funds were most affected and required contract variations to accommodate changes to future milestone deliverables.

SED was also involved in facilitating subproject contracts between major contracted parties and subcontractors. There were some initial challenges in establishing subcontracts which saw some very long and drawn out negotiations between parties. In particular:

- The Department of Primary Industries Victoria (now Department of Primary Industries and Environment, DEPI) and Flinders University had trouble reaching consensus on background IP and the availability of PhD candidates. Legal advice was required from HAL to resolve the outstanding IP issue and owing to changes in IP management this issue took 12 months to resolve. The PhD student was replaced by casual staff.
- International parties had some concerns about managing exchange rate risk.

- SED was also required to invest significant time in establishing sub-subcontracts for project PT09004, between the lead agency IPM Technologies and both Plant and Food New Zealand (PFNZ) and Horticulture New Zealand (HNZ). IPM Technologies' work was funded via matched voluntary contributions (VCs) from PFNZ and HNZ through associated projects. As a small business, IPM Technologies was neither equipped, resourced nor inclined to be responsible for two much larger organisations. SED was obliged to take full responsibility for obtaining milestone reports from PFNZ and HNZ as the program progressed.

INTELLECTUAL PROPERTY NEGOTIATION

Within the first six months of the commencement of the program SED worked with the five projects to define the equity positions associated with each. The program agreed to progress the development of equity positions using spreadsheet templates that had been developed in APRP1. Generally, intellectual property (IP) positions were agreed quickly and finalised positions provided to HAL by June in 2010.

There was one notable exception in the development of an equity position for Flinders University related to PT09026Bi (Novel approaches to disease control – endophytes). An issue with background IP associated with the commercial partner Novozymes required input from HAL. The HAL IP manager was made redundant within months of the program commencing. With only *ad hoc* IP support available from HAL the issue took well over 12 months to resolve and resulted in a considerable delay to the commencement of the Flinders work. Once the matter was resolved, several contract variations were required to accommodate the delays to the start of the program. Flinders was able to progress some aspects of the work before the issues were resolved but some aspects had to be delayed until funding was forthcoming.

'UNHITCHING'

The difficult experience of having all contracts signed before the program could be initiated was followed by the challenge of fulfilling the March 2010 milestone. The contractual structure meant that every criterion of every corresponding milestone for five different providers (and their subcontractors) had to be met before a milestone in the head agreement between UB and HAL could be considered to be completed. In some cases, this amounted to more than 20 separate, specific achievements (for example, 'PhD student appointed', 'paper published in peer-reviewed journal').

In May 2010 when the benefits of having the program contracted through a higher education provider ceased to exist (further explained in Chapter 1), approval was given by HAL and the PPIAC for the various APRP2 contracts to be 'unhitched' from each other, with the University of Ballarat ceasing to be part of the structure and contracts instead being directly between the six lead research providers and HAL. The University of Ballarat was agreeable to this new arrangement. The intention was to have the new structure in place before the September 2010 milestone.

Over the course of the program HAL attempted to enact these changes with recontracting of the program set to commence soon after the PPIAC endorsement. HAL researched the legal requirements for recontracting the program however a simple process of novating contracts from UB to HAL was unable to be progressed. Conflicting internal legal advice within HAL and short time frames for recontracting between milestones meant that recontracting of the program was not achieved.

A pathway to 'unhitching' was eventually identified and the contract managers of provider organisations were notified. However, for reasons that are not entirely clear, the initial structure was never dismantled.

The result of the failure to 'unhitch' was that the program managers became very largely focussed on administration at the expense of other possible value-adding activities, as described above.

It is possible to structure programs in way that allows synergies to be realised (for example cross-fertilisation of ideas, sharing of resources) without harnessing all parties within what is, effectively, a single contract – because this can quickly erode any synergies achieved.

RECOMMENDATION: HAL should avoid complex contractual structures for programs that cause some providers to be adversely impacted by underperforming counterparts.

PROCESSING CONTRACT VARIATIONS

The number of variations required in the program was considerable and extremely time consuming. Approximately 19 extension requests were developed and facilitated during the course of the program. Some of these were minor changes such as adjusting the wording of a milestone deliverable, whilst others were more complex changes requiring a change in project scope. In very minor circumstances the HAL Portfolio Manager was able to approve these changes but more often than not changes were deferred to the PPIAC for consideration.

The motivation for contract variations was varied:

- Some were not the fault of the researchers but a product of the complex structure of the program (delay in start of program impacted on several projects requiring variation, notably projects PT09026Bi, PT09026C and PT09029).
- Stop/go points were contained in four projects: PT09004, PT09019, PT09023 and PT09026Bi. In all instances the budget was allocated and simply required approval to continue with agreed milestones or the development of new milestones.
- Publications – often there was a requirement for publications that was not well aligned with the capture and analysis of data. In some cases, papers were submitted but had not been published, for reasons beyond the control of the authors, and this created problems when the milestone specified 'publication' rather than 'submission'.
- PT09019 had some reallocation of funding from one part of the project plan to conduct additional testing.
- Adjustments to methodology were made to better suit the experimental conditions for PT09026C.
- Two projects successfully gained additional funding (PT09004 and PT09026C).
- In some instances the wording of milestones was very rigid, providing very little room for common-sense interpretation consistent with circumstances. For example, some milestones required a PhD student to be appointed so the appointment of a Masters student, while considered an acceptable alternative, required a milestone variation; or a certain number of pot or field trials to have been completed.

RECOMMENDATION: Milestone criteria should always be worded to strike a suitable balance between flexibility (both for HAL and the provider) and accountability. This is a responsibility of both HAL and providers.

In a great number of instances extension requests were developed with sufficient time to be given reasonable consideration for approval prior to the related milestone. However, in the early stages of the program the HAL Portfolio Manager appeared overburdened with work and extension requests were unable to be reviewed in a timely fashion and milestone approval delays ensued.

MILESTONE PROCESS

COLLATION AND SUBMISSION

On the whole milestones were largely delivered on time or within a two-week window of their due date. SED followed a multi-step process in managing the submission of each milestone:

- **A template with prepopulated deliverables** specific to the project was provided to each PL six weeks prior to the submission date.
- **PLs were prompted** via email and phone prior to the submission date, starting with the sending of the template. In the second half of the program, PLs were specifically asked by e-mail if there was any reason why they would not fulfil all milestone criteria on time. An email response was required from the PLs.
- Draft milestone reports were submitted to SED for **editing and review** one month prior to milestone submission (with some parties organising to have this reduced to two weeks). Reports would typically require one or two re-drafts before being ready for submission to HAL.
- The various reports were **collated** and converted to pdf format. This could be a quite complex exercise, as the collated report contained five constituent reports and often many attachments, and usually ran to several hundred pages.
- The collated report was **submitted** to HAL where it was reviewed by the Portfolio Manager.
- Any **questions or comments** made by the Portfolio Manager were addressed in conjunction with the respective PL.
- The report was **revised and resubmitted** to HAL.

In the first year-and-a-half of the program the standard of milestone reports was generally low and possibly reflected the general poor morale in the program, which in turn appeared to be due to the unwieldy program structure and the reluctance of some projects to be grouped under the management of others. Many reports were substandard and others incomplete. SED spent a huge amount of time reviewing reports, editing and requesting more information and a higher standard of reporting. The ongoing challenges were discussed with HAL managers who admitted that in the past the standard of reporting may not have been as high. SED continued to work with the program to improve the standard of reporting although this took ongoing management and program reminders.

By the end of the program, PLs were generally very prompt in responding the requests and delivering milestones in a fairly timely fashion.

As noted above, the milestones agreed between HAL and UB for the performance management of the head agreement were composites of milestones from all of the parties below the head agreement. Most of the milestones in the PT09039 contract included obligations on every one of the sub-contractors and sub-sub-contractors. The head agreement milestone would not be considered complete unless every one of these obligations had been fulfilled.

When any one party failed to achieve each and every criterion of its milestone this required approval for a variation to the milestones from HAL (or IAC if needed), and, if approved, adjustment to the head agreement, adjustment to the project and in some instances adjustments to sub-projects. This arrangement was extremely time consuming for project leaders to manage, and it also meant that some

parties were reluctant to highlight any needed changes to work plans or project deliverables resulting in some milestone delays and some opportunities being missed to positively change project scope.

Some steps were taken by SED and HAL early in the program to help to manage the difficulties in milestones being shackled together. A clause ('Where any subproject milestone is not achieved, payment and criteria for that subproject will be moved to the next program milestone') was inserted in each milestone such that if the milestone was not achieved then the provider concerned would be the only party affected and the remainder of the program could progress unhindered. This worked in the case of standalone projects, but could not be applied to project PT09026 which comprised five sub-projects with linked milestones and payments.

In an effort to manage delays in milestone submission and review, SED implemented a further mechanism to streamline processes. It was agreed with HAL in November 2010 that the TOC would meet face-to-face within a few weeks after the submission of each major milestone (i.e. in April and October). At this meeting, each PL would present their report, allowing any questions or concerns from HAL, PPIAC or other TOC members to be raised and resolved at that time. The intention was that the milestone report would be marked as 'approved' or 'failed' by the end of the meeting or, at worst, approved subject to minor revisions to be completed within a week or similar timeframe. This process worked well in most instances to encourage faster milestone turn around, and to obtain good feedback from industry and peers on the project progress.

Providing sufficient notice that a milestone was not going to be achieved was an ongoing challenge in the program. The program managers repeatedly emphasised the need for PLs to inform SED of any potential delays as far in advance of the due date as possible because of the difficulty of making contractual adjustments. Research by nature is not always predictable, and in some cases the researchers were unable to foresee delays, but in other instances there was some inattention on behalf or project leaders to progress trials or experiments in a timely fashion.

MILESTONE PAYMENT MANAGEMENT

SED was responsible for following up the payment of voluntary contribution (VC) payments associated with twice-yearly milestone payments (in the first few years of the payments VC payments were invoiced quarterly for one particular party). In most cases parties promptly paid their invoices, which were sent out approximately 5 weeks prior to the milestone payment due date. However, these parties faced the risk that the HAL payments back to them would not be paid for several months because these HAL payments were contingent upon the associated milestone being fulfilled. In some cases this took several months (and in one case six months) owing to delays in milestone approval.

These delays caused understandable concern to contributing parties for reasons of both cash flow and exchange rate risk. Several parties who had cash flow limitations then moved to paying the invoice only upon approval of the milestone. This in itself then caused delays as the milestone payment was unable to be made to the program without the receipt of all VC payments. Most of the VC payments were from overseas parties and would take a week to arrive and to be cleared. This situation was another unfortunate consequence of the contractual structure of the program and led to some very strained relationships with VC contributors.

SED also facilitated the payment of all parties via UB. UB staff were reliant on SED for instruction regarding payment. They were extremely helpful and accommodating to work with, which made the management of payments and contracts slightly easier, and in most cases UB staff would hasten payments when long days had been experienced. In most instances payments were very straightforward. Some minor delays in

payments to providers were experienced when staff or details changed at various institutions and these had not been updated with UB.

INTERNAL COMMUNICATIONS

INTERNAL WEBSITE

As discussed previously, an internal password-protected website was created to capture useful documents for the program.

All templates, logos, contacts, approved meeting minutes, milestones, presentations and other program materials were placed on the website when finalised. The site also offered a forum for discussions between users. The website was infrequently accessed, and the forum for discussions was not used with most team members reliant on email for their information.

TECHNICAL OPERATIONS COMMITTEE

Technical Operations Committee (TOC) meetings were organised twice-yearly as face-to-face meetings and were held near the Melbourne Airport as a central location for those travelling from Queensland, Sydney, South Australia, Tasmania, New Zealand and Victoria. TOC meetings brought together Project Leaders, Subproject Leaders, representatives from the IAC, AUSVEG extension staff, a Potato Industry technical advisory group nominee and HAL management.

It was an administration feat to find at date that suited most parties. Agendas were drawn up, adjusted based on feedback and minutes and actions captured and followed up. Minutes were circulated and were uploaded onto the internal APRP2 website.

The focus of the TOC meetings was for Project and Sub-project Leaders to provide an overview of work to date and discuss the next steps. As discussed above, the TOC meetings in the latter part of the program were essentially for the approval of milestone reports. The meetings featured robust discussions and identification of opportunities for projects to work collaboratively. They were also an opportunity to discuss administration or housekeeping items with the program. Each TOC meeting generated ideas and many items for administrative follow up. Teams were encouraged to provide article suggestions based on their project work for Potatoes Australia. SED maintained a spread sheet with potential story ideas and their likely timing of completion.

Although there was a requirement for quarterly catch-ups via phone or web conferences these were organised between face-to-face meetings on an as-required basis.

FACE-TO-FACE TOC MEETINGS

Two face-to-face APRP2 meetings were held per year:

- 17 February 2010, Holiday Inn, Melbourne Airport
- 28 July 2010, DPI, Attwood, Vic
- 3 May 2011, DPI Attwood , Vic
- 6-7 October 2011, Rydges Carlton (plus symposium)
- 13 April 2012, Holiday Inn, Melbourne Airport

- 10 October 2012, Best Western Airport Motel and Convention Centre
- 19 April 2013, Holiday Inn, Melbourne Airport
- 18-19 September 2013 (plus symposium), Mantra, Tullamarine, Vic
- 19-20 May 2014, Mantra, Tullamarine, Vic

TELEPHONE AND WEBINAR TOC MEETINGS

TOC catch-ups between the face-to-face meetings were held on an as-needs basis.

- 1 June 2010
- 16 Feb 2011
- 4 November 2011
- 6 March 2012
- 28 February 2014
- 26 September 2014

TECHNICAL OPERATIONS GROUPS

Early in the program, the idea of appointing industry 'champions' to each project was raised and attracted general support. The intention was for each of the projects to have one or two industry (growing or processing) members of the PPIAC appointed to it as mentors. This would allow the PPIAC to have, within its ranks, someone with an intimate knowledge of any given project and for the projects themselves to benefit from industry input and advice and direct access to the PPIAC.

Unfortunately, and for various reasons, the 'champion' concept was not instituted until twelve months before the end of the program when 'Technical Operations Groups' were appointed to each project. The TOGs also included the HAL Portfolio Manager. At least one round of meetings was held between the TOGs and the respective project teams (including SED) and these were well regarded on both sides.

RECOMMENDATION: HAL and Industry Advisory Committees should consider appointing 'champions' from among the membership of the relevant IAC to each project in large multi-party programs similar to APRP2, to provide better direct communication between industry and research teams. These should be appointed at the outset of the program.

ADVISORY COMMITTEE

The advisory committee held an initial meeting combined with the first TOC meeting during February 2010. Most AC members were able to attend this meeting, although one member had a separate meeting with SED later in that year due to her unavailability for that meeting.

The remit for the AC was to be used as a forum to resolve significant issues if they arose. The Program did indeed faced a number of challenges but the blurred lines of authority (between program managers, HAL, the PPIAC and other parties) described above meant that there was no occasion on which the convening of the AC was considered to be appropriate. Advisory Committee members were invited to attend the two APRP2 symposia and also had an open invitation to attend TOC meetings. However, more could and should have been done throughout the program to engage with this key stakeholder group.

PPIAC/AUSVEG

In-person briefings were provided to the PPIAC on the following occasions:

- May 2010, Gold Coast, Qld
- April 2011, Brisbane , Qld

As noted above, no further invitations were extended to the program managers to present to the PPIAC throughout the life of the program.

RECOMMENDATION: Programs such as APRP2 should have regular interaction with their corresponding IAC in order to increase industry input into and understanding of their significant investment.

AUSVEG was provided with a bimonthly two-page program update for the purpose of informing the PPIAC of the program's progress. The involvement of two PPIAC representatives on the APRP2 TOC also supported communication with the PPIAC. The bimonthly update featured a summary of the latest research outcomes and a management update. Updates were provided to AUSVEG on the following dates:

- 2011- February, March, June, November
- 2012 - February, May, September, December
- 2013 - May, July, October, December
- 2014 - February, April/June

Acknowledgement of these reports or feedback on their content was never received by SED from AUSVEG or the PPIAC.

OTHER COMMUNICATION

Program managers maintained regular telephone communication with the HAL Portfolio Manager and the HAL Industry Services Manager. Several face-to-face catch-ups were held, generally around once per year in addition to TOC and other meetings.

SED also maintained regular contact with each PL and visited each research site at least once during the life of the program. Visits were made to SARDI, DEPI, TIA (Newtown and Prospect), ViCSPA, the University of Melbourne and IPM Technologies.

An internal newsletter was created in the early stages of the program which was well received but was abandoned owing to administration pressure.

EXTERNAL COMMUNICATIONS AND EXTENSION

During the development of the program, projects were specifically instructed by HAL not to include resources or budget for communication and extension activities as these were to be covered by a separate project. AUSVEG was later awarded the contracts for communication and extension in the potato industries (the Potato Extension Program, PEP) and this was the primary vehicle for communication and extension for the program.

The May 2011 TOC meeting developed a system for approving the publication of articles for the public domain. The process was developed to ensure that article content was technically correct and industry-appropriate. The new system involved sending the completed article to two IAC members, plus the Potato Industry Technical Advisory Group chair. The reviewers had one week to comment with a response from two of the three on the review team sufficient for action. Although this added an additional administrative step within the program the feedback from the reviewers added considerable value to the publications.

The program was in a position to engage externally with stakeholders as outlined in the following section.

SYMPOSIA

Two highlights during the APRP2 program were the symposia that were held in years 2 and 4 of the program.

OCTOBER 2011

The inaugural APRP2 symposium was held on the 6th and 7th of October 2011 with overwhelmingly positive feedback from research and industry. Thirty-five people met to share their learnings and to seek input from peers and industry to ensure future outcomes would be relevant and valuable. The symposium focused on the APRP2 program but also involved three other active potato research projects that fell outside the program addressing breeding, development of controlled traffic techniques and managing pathogenic nematodes.

Research findings were presented from all five core research projects. Project discussions were enhanced by input from the grower, processing and research communities, as well as representatives from agricultural chemical companies and international contributors to the program who were able to bring their own experience and perspective when reviewing results to date and suggesting steps for the future.

Several opportunities for collaboration were identified within the program but also for the external potato projects, with steps put in place to share results and also activities – for example, by projects providing testing services for each other.

A post-symposium survey yielded 14 responses (13 researchers and one processor) from 35 attendees. The feedback was very positive – for example:

- 93% found the Review to be enjoyable, valuable, informative and 100% found the Review relevant
- 79% felt there were the right number of presentations
- 64% were happy with the time allocated to discuss their project
- 73% were able to identify collaborative opportunities
- 100% valued having a hardcopy of the presentations
- 93% would have liked the Review to be conducted annually

Participants also provided some good suggestions for the format of future symposia. Overwhelmingly, participants identified greater grower involvement as a key to enhancing future symposia.

Only one post-symposium survey was returned by a processing company but an email was circulated to the program following the gathering by the Chair of the PPAA, who praised the efforts of the researchers and their research outcomes and indicated he would support the symposium becoming an annual event. A

representative of the grower community also circulated an email praising the researchers' efforts and suggesting the program was producing excellent returns on investment.

SEPTEMBER 2013

The second of the APRP2 symposia was held on the 18th to the 19th of September 2013. The symposium attracted a group of 53 with 16 of those APRP2 researchers, 7 processing company staff, 6 growers, 7 industry service providers (agronomists, agrichemical companies, etc), 5 students, 5 representatives of industry bodies, associated researchers and managers plus HAL staff. Each project provided an overview of work that had been carried out to date and results that reflected the outputs from the entire duration of the program.

The SED team used various key contacts to promote the event. Support was given to the research teams to guide them in the development of presentations for the scientific community and for a more industry-focussed afternoon.

Feedback from the symposium was again extremely positive with 97% of attendees considering the symposium to be relevant, informative, valuable and enjoyable. Specific feedback was also very positive but suggestions for enhancements included more question-and-answer sessions and even greater grower involvement.

A half-day industry 'outputs' afternoon followed the 1.5-day science symposium whereby projects were encouraged to answer four simple questions:

- What do we know now, that we didn't know before the program started?
- What is the benefit of this new knowledge?
- What has come out of your work that is ready for transfer? and
- What more needs to be done in this area?

The session also involved a presentation from AUSVEG outlining the potato extension program. The outputs afternoon was filmed and is available for viewing online.

http://goo.gl/BLCN5V

The outputs session was a little repetitive for those who had sat through the science symposium, however a late change to the format of this session saw a Q&A type approach used to outline the outcomes from the large, multi-strand 'Soil Health and Disease Mitigation' project (PT09026). Feedback suggested that this format was welcomed by participants who preferred the simplified approach rather than Powerpoint presentations.

During the science symposium, students undertaking research into potato pathogens were invited to present an overview of their work. The standard of presentation was exceptional as reflected in the encouraging comments made by key industry figures during and following the symposium, including those in a note of congratulations sent to the students by Frank Mulcahy.

At the suggestion of AUSVEG a grower evening was organised and held at Creswick following the symposium to capitalise on the visit of Dr George Lazarovits from Canada and Prof Richard Falloon from New Zealand. The workshop was facilitated by Dr Nigel Crump and was attended by 11 growers plus McCain personnel, AUSVEG personnel, industry service providers (chemical companies) and APRP2

researchers and management. The workshop also featured presentations from Anne Ramsay on the cost of diseases to industry and an overview of the APRP2 program outputs to date delivered by Dr Ben Callaghan.

The workshop featured strong group interaction and feedback from attendees. The need for a fact sheet to help the identification of the symptoms of Verticillium wilt was recognised. Further, whilst the growers could see value in the current APRP2 program they advocated for solutions to the more immediate perceived problems of pink rot and Rhizoctonia. This supported feedback on the impact of disease provided to SED through the collection of baseline cost-of-disease figures (discussed later).

RECOMMENDATION: For programs of similar or greater size that APRP2, consideration should be given to budgeting for an annual whole-of-program technical symposium and review every 12 months.

POTATOES AUSTRALIA

Every effort was made to have an article from the program available for each edition of Potatoes Australia. Over the period of the program SED liaised with a succession of six writer/editors for Potatoes Australia. A list of the general (as distinct from project-specific) program articles published during the life of APRP2 is provided in Table 7-1.

Table 7-1 – General APRP2 stories in Potatoes Australia

Potatoes Australia Issue	Story title and content
February 2010	On the brink of phase 2. An outline of the APRP2 program.
August/September 2010	Pinpointing the potato industry's biggest issues. Outline of the six projects making up APRP2.
October/November 2010	APRP2 kicks into gear. Introduction of the PT09026 project within APRP2.
October/November 2010	Researchers meet to enhance the processed potato industry. A complimentary opinion piece written by former PPIAC chair Mr John Rich.
April/May 2011	Research program making steady progress. An overall update on the program, written by Scott Williams
December/January 2012	APRP2 review reveals research preliminaries to industry. Report on the APRP2 Review. Anne Ramsay, Scott Williams.
December/January 2013	Research and development for the processing industry. Report on the third year of the APRP2 program. Written by Anne Ramsay and Scott Williams
April/May 2013	The young faces of training and education in the Australian potato industry. A story supplied by Anne Ramsay written in conjunction with the students involved in research. University of Melbourne. Part 1.
June/July 2013	The young faces of training and education in the Australian potato industry. A story supplied by Anne Ramsay written in conjunction with the students involved in research. University of Tasmania. Part 2.
October/November 2013	Showcasing potato science: APRP2 Symposium brings together horticulture's brightest minds. Produced by AUSVEG, this article provided an overview of the symposium and the grower evening that followed.

Potatoes Australia Issue	Story title and content
April/May 2014	Practical research outcomes for the Australian seed potato industry. A story supplied by Anne Ramsay and Scott Williams focusing on outputs across the APRP2 program of particular pertinence to the seed industry.

At the time of writing four articles have been submitted to Potatoes Australia that have not yet been published:

- A summary of the key findings from the work led by IPM Technologies which successfully developed an IPM strategy to manage potato psyllid.
- A summary of the cost of disease to the commercial industry.
- A summary of the findings from the work led by Flinders University that isolated and then tested potato endophytes for use as a disease-suppressive seed treatment (PT09026Bi).
- A summary of the findings from the TIA-led work that examined the role of rotations on pathogen populations over time (PT09026Aii).

EXTERNAL WEBSITE

SED was part of a meeting with AUSVEG and HAL managers in mid-January 2011 to discuss the improvement of communication with stakeholders, including the concept of a dedicated extension program. SED suggested that there was scope for SED to carry out some additional activities, in conjunction with AUSVEG, which could include the development and maintenance of a public web site about the program.

In March 2012, it was agreed that an external public website would be a good tool to ensure program information was readily available. After consultation with AUSVEG, SED initiated the development of a site but later received instruction via HAL that AUSVEG would take on this activity.

POTATO INDUSTRY ANNUAL REPORT – PROGRAM UPDATE

A program report of approximately 800 words was submitted to HAL on request in July/August annually to contribute to the potato industry annual report.

POWDERY SCAB WORKSHOP

A recommendation was made at the October 2011 APRP2 Review to run a workshop to collate current knowledge about powdery scab, to identify knowledge gaps and opportunities to fill those gaps. A workshop was organised with ViCSPA to follow the 2012 Ballarat Potato Seed Conference.

A group of experts from within the program was assembled including international authorities Professor Richard Falloon of Plant and Food New Zealand and Dr Stuart Wale from the Scottish Agricultural College. Several recommendations were generated (Table 7-2) and researchers were challenged to look at current and existing data that could offer some insights into the impact and management of the disease.

Whist some recommendations were able to be worked into existing projects many of the recommendations could not be addressed within the existing program budget. These recommendations (shown in Table 7-2) may be of interest for the industry in the setting of future R&D priorities.

Table 7-2 – Recommendations from 2012 powdery scab workshop

Workshop recommendations
Using symptomless tubers that have significant *Spongospora* DNA levels, determine what form the *Spongospora* is in (histology) and if it is viable i.e. can it produce symptoms.
Treatment with Shirlan produces consistent reduction of powdery scab.
Shirlan – clarify why application rejected with Cropcare – potentially implement a study to address barriers (minor use permit for seed crops – Peter Del Santo).
What role does root architecture play in infection?
Relating to *Spongospora* root infection, undertake a study that aims to stratify the root system – what is happening at each level or across the whole root system?
This would be a significant area of study. Need to assess mechanisms available to assist with root architecture research. This may be a useful suggestion for APRP3.
Field studies in Tasmania to understand the impact of temperature, moisture, inoculum level and timing of galling? A small study.
Determine if there is a commercial yield impact from root infection – using a plot with nil or low inoculum – apply varying rates of inoculum and determine significance.
Using existing data look for relationships between yield and DNA in soil. *Post script – Mike Rettke of SARDI will investigate this.*
Data mining of company records to establish relationship between yield and rejection rates for scab (economic impact of powdery scab).
Where known preplant DNA data is available obtain yield and rejection data from factories. *Post script – Mike Rettke of SARDI will investigate this*.
Create a powdery scab resistance/susceptibility ranking for Australian potato cultivars (possibly look at the impact on both the tuber - and root (*need to develop a scoring system for root infection – and galling*)) (start at one site and soil type to determine relative differences, then introduce soil type variables etc) .
Could possibly just start with a literature review of what is already known globally and from within Aust research, Tony Slater (Dolf to contact), Tonya Wiechel, etc. Ensure that standard variety is present in dataset as a control.
Potential for James Hutton bioassay root data to be compared to cultivar tolerance (NZ tuber work).
Pool UK and Australian soil DNA data pre and post cropping and look at multiplication under different crops.
Tonya Wiechel – from MASH work find old soil samples with powdery scab that can be sent to Canada for biological profiling (using new APRP2 tools).
Nutrient work – look at long term approaches to nutrient management and disease management.

Dr Nigel Crump, the workshop facilitator also pulled together a summary of the workshop as outlined in Table 7-3.

Table 7-3 – 2012 powdery scab workshop summary

Facilitator's summary of powdery scab workshop outcomes
Australian-based research funded by HAL is providing world leading information on powdery scab disease epidemiology and management. This is strengthened by the international collaboration of the Australian Potato Research Program (Phase 2). Areas of expertise include the development of DNA diagnostics for powdery scab and identification of potential soil nutrient interactions that can be manipulated to suppress disease.
The pathogen that causes powdery scab is an obligate pathogen, meaning that it only grows in the presence of a host and cannot be grown on artificial lab based media such as agar. This makes working with the pathogen somewhat difficult and research tedious. New technology is being utilised that can detect and quantify the pathogen in greenhouse bioassays. These research tools, which detect RNA of the pathogen, will enhance the ability for researchers to work with the pathogen and better understand mechanisms of disease management.
Research has shown that the roots of the potato plant when infected by *Spongospora* have impaired function, which may cause yield loss in crops. This is a poorly understood area of powdery scab which requires greater attention.
There are key messages for powdery scab management that can be effectively communicated to industry. A grower fact sheet could be developed covering known aspects of powdery scab management.
Various fungicides have been shown to be effective at reducing powdery scab and have been registered overseas. Constraints as to why such fungicides are not registered in Australia need to be identified and proposed solutions to overcome such constraints identified.
A fundamental gap in Australia is the lack of understanding of cultivars' relative susceptibility or resistance to powdery scab. Some data is available but it is limited or dated. It was suggested that the development of a list of potato cultivars with their known disease rating would be valuable to the industry.
The exchange of knowledge from researchers and key industry people during the discussions developed new areas and ideas for potential research and potential collaboration across the international community.

EXTERNAL CONFERENCES AND EVENTS

SED attended, and in some instances presented at, a number of industry forums over the life of the program.

SEED POTATO CONFERENCE

- Seed Potato Conference, July 2010, Marysville, Vic, presentation by Scott Williams
- Seed Potato Conference, July 2012, Ballarat, Vic, presentation by Scott Williams
- Seed Potato Conference, August 2014, Mt Gambier, SA, presentation by Anne Ramsay

AUSVEG NATIONAL CONVENTION

- May 2010, Gold Coast, Qld, attendance by Scott Williams
- April 2011, Brisbane 17 April 2011, National Potato Summit, attendance by Scott Williams

- May 2013, Gold Coast, Qld, attendance by Scott Williams

AUSVEG GROWER WORKSHOPS

- March 2012 - Devonport, Tas, attendance by Scott Williams
- August 2012 - Mt Gambier, SA, attendance by Scott Williams
- February 2013 - Bungaree, Vic, attendance by Anne Ramsay
- March 2013 - Sassafras, Tas, attendance by Anne Ramsay
- June 2013 - Creswick, Vic, attendance by Anne Ramsay

OTHERS

- December 2009 - Zebra Chip Summit, Melbourne, Vic, attendance by Scott Williams and Anne Ramsay
- September 2010 - DEPI trial field days at Cora Lyn and Ballarat, attendance by Scott Williams
- March 2011 - AUSVEG PCN management workshop, attendance by Scott Williams
- August, 2011 - McCain Agricultural conference, attendance by Anne Ramsay
- August 2012 - Powdery scab workshop, Ballarat, Vic, attendance by Anne Ramsay and Scott Williams
- February 2013 - Snack Brands supplier event, Toolangi (ViCSPA) and Warragul, presentation to group on APRP2 by Scott Williams
- September 2013 – Creswick APRP2 grower evening, presentation and attendance by Anne Ramsay

FUNDING ACKNOWLEDGEMENT

A standard form of funding acknowledgement was developed in conjunction with HAL early in the program and was provided to the project teams in the Procedures Manual and in the Powerpoint template. This acknowledgement was long and unwieldy because it included every contributing agency, and a result it was probably not used in every situation where it should have been used.

No specific recommendation is made in this respect, as there is no simple way to succinctly acknowledge the multiple parties associated with a large program, but HAL should continue to look for ways to do this effectively and consistently.

EVALUATION

An evaluation plan was developed for APRP2 early in the life of the program. As the program was largely science-based, the main focus of the evaluation plan was on the quality of scientific outputs rather than their uptake by industry because adoption was not factored into APRP2.

HAL facilitated an external review of the program early in 2014. The external review yielded HAL and relevant stakeholders with significant feedback on the program achievements, limitations and recommendations for future programs. Further, in mid-2014 Dr Kevin Clayton Greene was commissioned to review potato research including APRP1 and 2 and identify outputs and knowledge gaps which will also provide some measure of the program achievements.

Chapter 1 features a summary of the various technology transfer related activities that the program was involved in or generated. These are considerable considering the limited internal program resources available for communication and extension. The number of scientific papers produced from each project was not proportional to the value of each project. Some projects had better planned for time and resources devoted to the production of scientific publications which greatly assists the wider scientific community to have access to key findings and discussion.

RECOMMENDATION: During project planning stage ensure that there are sufficient resources devoted within the project to the development of scientifically peer reviewed papers.

SED undertook two major initiatives in order to estimate the economic benefit delivered by APRP2: an original study of the cost of the major potato diseases (on which APRP2 focused) and, building on this study, an estimation of the benefit/cost ratio for two components of the program, the PreDicta Pt service (PT09023) and ready for transfer elements of the soil health and disease mitigation program (PT09026).

BASELINE COST OF DISEASE TO INDUSTRY

The original intent of this work was to understand current grower knowledge / attitudes / skills / aspirations and actual practices in respect to the diseases under consideration in APRP2, and to gather best estimates of the current incidence and impact of those diseases on the industry.

SED had limited knowledge of what data might be available, so the assistance of the PPIAC and TOC was requested. It was proposed that if there were gaps then surveys would be used to gather missing information.

SED compiled the information provided in project proposals and from the feedback received. Much of this information came from Nigel Crump (ViCSPA), David Antrobus (McCain) and Frank Mulcahy (Simplot). SED worked through the various references and discussed the findings with the TOC but in May 2011 it was decided that more reliable data needed to be generated. ViCSPA was surveying the industry regarding seed certification. It was hoped that some additional questions could be added to this survey to gather disease impact data, but despite best efforts it was concluded that this approach would not be feasible.

In September 2011 SED proposed to work with the collective intelligence of the PPIAC and apply a Delphi method (which harnesses expert wisdom) to reach a consensus on the cost of disease to industry. The specific costs to be explored were those arising from current disease control practices, chemical costs, quality downgrades and losses in yield. The first step was to derive these costs for the commercial processing potato industry.

A questionnaire for the PPIAC was developed and was distributed in November 2011 to representatives of four major processing companies (Snackbrands, Simplot, McCain and Pepsico). Responses to the questionnaire were not forthcoming so SED contacted representatives of these companies directly and either met face-to-face to discuss responses or worked through the questionnaire over the phone. By March 2012 SED had worked with each processing company and developed estimates of the cost of disease to their growers. These were provided back to the processing companies for comment and review.

The processing companies then provided a list of representative growers or grower coordinators with whom SED could ‘pressure test’ the figures the processing companies had provided. SED spoke directly to or surveyed by a paper-based questionnaire 18 growers or grower coordinators. Generally there was a very high level of agreement between the processors and their growers, although growers were better able to

describe the on-farm discard rates (pickout) due to disease and the labour costs involved in removing the discards.

The companies contacted collectively process 650,000t of potatoes. The growers/service providers surveyed were growing/managing 239,770t of these commercial potatoes (37% of production). The total value of the proportion of industry surveyed was estimated to be close to $200M.

The following describes the process and outcomes for the commercial growing sector of the processing potato industry. A summary of the cost of disease to the seed industry follows this summary.

METHODOLOGY: COST OF DISEASE TO THE COMMERCIAL PROCESSING POTATO INDUSTRY

Diseases of potatoes impose a variety of costs on the industry. These costs can be grouped into two broad categories:

1. 'Direct' costs, including chemical treatments, on-farm discards ('pickout' or 'packout'), labour for on-farm discards, estimated yield loss beyond on-farm discards and factory grade-outs. It was possible to obtain reasonable estimates of direct costs in most cases – with the possible exception of yield loss.

2. 'Indirect' costs, such as changes to irrigation practices, variety choices and keeping fields out of production. These are real costs, but much more difficult to estimate and to attribute to particular diseases.

After considerable discussion it was concluded that the present study should focus only on the annual, **direct** costs associated with the diseases under research in the APRP2 program: common scab, powdery scab, Rhizoctonia, Verticillium and bacterial wilt. This would provide a broad, if somewhat conservative, indication of the impact of these diseases.

Those interviewed were asked to estimate **annual costs** of the each disease but to consider these costs over the past three or more years rather than providing only a single estimate based on the most recent year. By taking this approach, some of the impact of seasonal variation was removed from the estimate.

The costs were generated by asking each of the key staff at the processing companies to estimate the cost of disease across all of their growers. These figures were then cross-checked through interviews with growers or field staff nominated by the processors. Where there were significant differences between the processor and their growers, the data from the growers was collated and extrapolated across the growing group for that processor. In most cases there was a very close alignment between figures reported by the processor and the costs incurred by the growers. The growers were able to provide a more accurate account of on-farm discards and the labour involved to extract the diseased tubers. Plus, the extent of chemical treatment was updated based on grower feedback and extrapolated where possible across the growing group.

In the summaries below, the following terms have been used to describe the costs:

Table 7-4 – Terms used to describe the cost of disease

Labour for on-farm discards	*cost of labour to remove affected tubers prior to leaving the farm*
On-farm discards	*value of the product that is removed prior to leaving the farm*
Treatment	*cost of chemical and application costs*
Yield	*the impact on the overall yield excluding the on-farm discards*
Factory grade out	*volume of product that was graded out due to scab at the factory prior to processing was available from one processor*

Note that two total cost estimates are provided for most of the diseases:

- A total without an overall yield impact estimate; and
- A total including the yield estimate.

The yield impacts were very hard to estimate in a commercial situation as there are no comparative controls.

COST OF DISEASE TO THE PROCESSING POTATO INDUSTRY RESULTS

COMMON SCAB

Table 7-5 – Annual estimated cost of common scab to the Australian processing potato industry

Labour for on-farm discards	$47,485
On-farm discards	$1,173,192
Treatment	$75,800
Yield - gross	$0
Factory grade out	$110,270
Total (national)	**$1,406,748**

The incidence of common scab in growing districts was extremely variable, with some particular areas and some specific growers having significant tuber discards. Whilst the majority indicated that common scab was not a significant problem (or was well-managed through variety and management practices) or that less than 1% of tubers were discarded prior to leaving the farm, several growers reported that on average 10-15% of their crop could have up to 50% of tubers thrown out. The variety grown, the years of production, the season and paddock history were cited as factors that influenced the occurrence of common scab.

There were several recurring comments:

- It was often difficult to differentiate common scab from powdery scab.
- Improvement in irrigation practices and shifting planting dates have certainly helped in managing the problem.

- Common scab is not a big issue with proper management, but a very dry season or complacency in management may lead to a large problem at times.
- The seed industry is often the most affected owing to certification requirements.

POWDERY SCAB

Table 7-6 – Annual estimated cost of powdery scab to the Australian processing potato industry

Labour for on-farm discards	$87,806
On-farm discards	$2,721,668
Treatment	$0
Yield - gross	$9,042,615
Factory grade out	$118,560
Total (national)	**$2,928,034**
Total with yield	**$11,970,649**

The incidence of powdery scab damage to tubers appeared to be more widespread than that of common scab. Further, there was frequent comment by growers and industry personnel suggesting that powdery scab root infection has a significant impact on yield. Of the 29 people interviewed, 14 believed that a powdery scab root infection was having a significant impact on yield. Some offered rough estimates of this impact, with 10% being the most common suggestion, but many were reluctant to guess as they felt it was extremely difficult to quantify. The stated premise was that anything infecting the roots would impact on the uptake of nutrition and water and hence impact on yield. Further, some work had been done to estimate the impact of yield with 10% impact provided as a conservative figure.

The overall yield impact figure of 10% was applied to some production areas where those interviewed held a strong belief that this quantum of yield reduction was realistic, whether that was based on their own trial work or years of experience.

The largest concerns about the impact of powdery scab on root health and hence yield originated in Tasmania although similar concerns were raised by many in other states.

RHIZOCTONIA

Table 7-7 – Annual estimated cost of Rhizoctonia to the Australian processing potato industry

Labour for on-farm discards	$62,308
On-farm discards	$2,123,846
Treatment	$1,237,771
Yield - gross	$1,648,154
Factory grade out	$0
Total (national)	**$3,423,925**
Total with yield	**$5,072,079**

Overall it was estimated that 70% of growers use some kind of chemical to help to control Rhizoctonia. It was reported that the use of in-furrow treatments has risen significantly in the past few years which is hoped to reduce the volume of discarded product on-farm and increase overall yields. The figure for the on-farm discard rate was at times a figure prior to the introduction of chemical control, so in future seasons the on-farm discard figure may reduce with chemical costs increasing. There was a noteworthy concern that even with chemical control options Rhizoctonia was having a significant impact on production.

POTATO EARLY DYING

The impact of early dying as a result of Verticillium wilt appeared variable and often not well understood (which is, after all, the rationale for conducting the PT09029 project). As a part of this review experts have estimated that early dying might reduce overall yield by up to 1% and some considered that this figure may be significantly higher. Several growers indicated that yield losses as high as 20% might be attributable to early dying with Verticillium possibly playing a large role in this.

A table of costs has not been generated for early dying. Some areas did not report early dying as a problem and in other areas the cause of the early dying was often hard to attribute. South Australia, Queensland, New South Wales and some parts of Victoria reported a presence and impact of early dying associated with Verticillium wilt.

The impact on overall yield at a very conservative estimate of 0.5% equates to around a loss of over $1M/pa which does not take into consideration the losses from planting and growing the crop. Once DNA diagnostics for Verticillium are available this figure may be revisited.

Some of the statements made by processors and growers are captured here:

Snackbrands

- We see crops often go down early, some crops just don't hang in there and there is no obvious reason for the rate of senescence – often hard to know for sure its Verticillium wilt but suspect it may be a factor more often than we realise.
- We see it on the Atherton Tableland and Koo Wee Rup – can identify by looking at the petioles but would often go undiagnosed.
- Concerned of the unknown impact of Verticillium with other diseases such as PVY, Rhizoctonia, early blight etc.

Smiths

- Outcome is increased on-farm discards due to smalls.
- Approximately 5% of growers impacted?
- Rough estimate could be 1% yield impact across the board.

McCain Tasmania

- Not an issue in Tasmania.

Safries

- SA – issue for some growers using Russet Burbank – limited only to a few growers.

- Some think that AMISTAR might help.
- Potential loss of possibly 10% in yield.

McCain Ballarat

- Some incidence but often hard to know if the cause is heat, moisture, nutrition, crop stress – hard to attribute.
- Nematodes not a problem in Ballarat.

Simplot

- Verticillium may also be an issue when linked with powdery scab and possibly salinity, high rates of chloride-based fertiliser (potassium chloride) and also burn root tips allowing Verticillium to enter.
- Tasmanian temperatures don't push the disease to fully display symptoms.
- Disease may be present to some degree and will mostly be seen in a dry, hotter year.

BACTERIAL WILT

Discussions with experts and growers indicated that in some parts of the processing potato growing regions bacterial wilt is known as a serious and currently devastating problem. In 2013, in the South East of Victoria, growers reported some total crop losses that will result in land out of production for many years.

Bacterial wilt was reported to be present in the South East of Victoria, the Riverina region of Victoria and on the Atherton Tableland of Queensland.

A table of costs has not been reported for bacterial wilt at this time owing to the sensitivities around the presence of the disease. Experts in affected areas suggested that up to 30 growers were affected by bacterial wilt in Victoria alone in the 2012/2013 growing season. One grower reported the loss of 50% of his crop at a cost of $7,500 per hectare and a total loss to his business of $1,200,000. This places the possible cost of bacterial wilt at higher than some of the other diseases previously reported in this review.

RATING OTHER DISEASE THREATS

Processing companies and individuals were asked to rank various diseases by size of the impact on current production. Table 7-8 lists the diseases nominated by individual growers and some field coordinators as their **number one disease problem** in terms of production impact.

Table 7-8 – The number one disease problem nominated by growers and field coordinators/agronomists, ranked on the volume produced or managed

Rank	Disease	Tonnage	% of total volume surveyed	Responses from
1	Powdery scab	60237	25.5	4 states
2	Pink rot	60000	25.4	1 state
3	Potato virus Y	30200	12.8	3 states
4	Sclerotinia	23000	9.8	1 state
5	Tomato spotted wilt virus	21667	9.2	2 states
6	Zebra chip	11200	4.8	2 states
7	Root knot nematode	7400	3.1	1 state
8	Rhizoctonia	6600	2.8	2 states
9	Verticillium	5600	2.4	2 states
10	Common scab	3667	1.6	2 states

The following diseases were also nominated as number one diseases but only by growers representing a small proportion (less than 1%) of production: *Erwinia carotovora*, late leaf spot from PNG, target spot and nematodes.

Only a portion surveyed also offered a second-ranked disease with 31% of volume produced suggesting that powdery scab was their second largest disease concern followed by potato virus Y at 9% of volume produced.

Table 7-9 collates the disease rankings provided by each processing company.

Table 7-9 – Processing company disease impact ranking across all diseases

Rating	Snackbrands	Smiths	McCain SA	McCain Ballarat	McCain Tas	Simplot
1	Potato virus Y	Potato Virus Y	Nematode (Root knot)	Rhizoctonia	Pink rot	Powdery scab
2	Rhizoctonia	Rhizoctonia	Rhizoctonia (chemical, loss in yield)	Pink rot (treatment cost)	Target spot	Target spot
3	Common & powdery scab	Powdery scab	Target spot (fungicide cost)	Target spot (fungicide cost)	Rhizoctonia	Pink rot
4	Verticillium		Common & powdery scab	Common & powdery scab		Late blight
5	Tomato spotted wilt virus		Tomato spotted wilt virus	Potato virus Y		Fusarium

METHODOLOGY: COST OF DISEASE TO THE SEED PROCESSING POTATO INDUSTRY

Following the development of estimates of the cost of disease for the commercial processing potato industry SED was asked by the APRP2 TOC meeting (April 2013) to extend these costs to the seed industry to derive a whole-of-industry cost.

Seed certification programs operate in Western Australia, South Australia, Tasmania and Victoria. These areas became the focus of the work on the cost of disease to the processing potato seed industry.

The industry was mainly asked about common scab, powdery scab and Rhizoctonia although bacterial wilt and Verticillium were also discussed. The latter two diseases were not considered a current problem to seed production although they were acknowledged as potential threats. Some growers in Western Australia were suspicious that Verticillium was playing a role in some cases of early dying but were not confident it was the cause.

TASMANIA

Tasmania seed production for the processing potato industry is coordinated by Simplot and McCain representatives. After harvest all seed goes through a cool store for storage and grading. By working with the company representatives, the Tasmanian Institute of Agriculture's Seed Certification officer and the cool store operators, the impact of each disease was estimated.

The cost included the crops not certified in the paddock, the volume graded out, the cost of being downgraded and an estimate of any reduction of yield as a result of disease.

WESTERN AUSTRALIA

The approach taken in the small Western Australia seed industry was to telephone all processing potato seed growers to estimate their costs of disease. In total, 14 seed growers representing the entire industry that grew processing varieties were interviewed. Some of these growers sold a varying portion as an export product.

The costs associated with grade out, chemical costs, downgrades and potential yield losses were estimated.

VICTORIA AND SOUTH AUSTRALIA

A paper survey was posted out to certified seed growers in South Australia and Victoria. In total 30% of processing growers responded to the survey. The findings of the survey were validated by checking them with five larger growers in each major seed-growing region who were interviewed by telephone. There was reasonable agreement between survey results and the reports of the larger growers contacted. The figure estimated for each disease was then extrapolated to be representative of the Victorian and South Australian production.

COST OF DISEASE TO THE SEED PROCESSING POTATO INDUSTRY RESULTS

To maintain the confidentiality of survey respondents, the cost of disease has been aggregated across states. The breakdown of the costs for each disease differs to the categories used for the commercial industry. A description of each category is provided in Table 7-10 where the cost of disease is expressed with and without the impact of yield.

Table 7-10 – Summary of the cost of disease to the processing potato seed industry

	Common scab	Powdery scab	Rhizoctonia
Volume discarded or not certified on farm	$90,192	$475,399	$50,315
Chemical treatment costs	$24,301	$100,989	$279,581
Possible impact on yield	$57,928	$519,406	$20,675
Volumes graded out	$26,394	$59,340	$5,544
Downgraded in certification	$42,181	$241,440	$-
Total	**$183,067**	**$877,168**	**$290,568**
Total with yield (national)	**$240,995**	**$1,396,574**	**$356,115**

Like commercial growers, seed producers were asked to rank diseases based on the impact on their business. Table 7-11 summarises the top three diseases that impact on the various growing areas in Australia.

Table 7-11 – Processing potato seed grower disease impact ranking (top three)

Western Australia	Tasmania	Victoria and South Australia
Potato Leaf Roll Virus	Powdery scab	Potato virus Y
Potato virus Y	Insect damage	Viruses
Erwinia, blight, Rhizoctonia	Pink rot	

SEED AND COMMERCIAL PROCESSING POTATO INDUSTRY – COMBINED COST OF DISEASE

Table 7-12 shows the combined cost of disease to the seed and commercial growing sectors of the processed potato industry, with or without the impact of yield being considered.

Table 7-12 – (Conservative) estimates of the cost of diseases to the processing potato industry

	Common scab	Powdery scab	Rhizoctonia
Commercial	$1,406,748	$2,928,034	$3,423,925
Seed	$205,930	$877,168	$290,568
Total (without yield)	**$1,612,678**	**$3,805,202**	**$3,714,493**
Commercial with yield	$1,406,748	$11,970,649	$5,072,079
Seed costs with yield	$240,995	$1,396,574	$356,115
Total including yield	**$1,647,743**	**$13,367,223**	**$5,428,194**

The figures in Table 7-12 highlight the significant cost of soil-borne diseases to the processing potato industry and provide justification for the investment in the APRP2 program. These figures may also provide

the basis to estimate the impact of the APRP2 program following a reasonable period of extension and adoption – or to assist with the prioritisation of future research.

BENEFIT/COST ANALYSIS

Prior to the funding of the program a benefit/cost analysis (BCA) summary was completed by HAL for the two largest projects within the program, PT09023 and PT09026. The BCA for those projects have been revisited based on what can be implemented at this point in time.

PT09023 – DIAGNOSTIC TESTS FOR SOILBORNE PATHOGENS

The original BCA work for PT09023 the development of the pre-plant soil test PreDicta Pt estimated a return of $8.20 for every dollar invested for the commercial and seed potato growing sectors. This was on the basis that there would be 50% adoption of PreDicta Pt in the processing sector and 20% in the fresh sector after 6-7 years.

The original assumptions for this work are outlined here:

- The project was seeking to provide techniques to improve management of key soil-borne diseases of economic importance to the processing potato industry, which would be achieved by providing interpretation of DNA tests to quantify soil-borne inoculum so that these tests could be used by growers to assess disease risk prior to planting a crop.
- A 20-year planning period (i.e. from 2004-2005 to 2025-26) was considered.
- Costs of the previous HAL project (PT04016-subprogram 1) in APRP1 were included in the analysis, on the basis that without this the prior research from that project the technology would not have been developed within the time frame outlined.
- In the final years of the project, an extra $100,000 of “in-kind” contributions was included to account for product packaging and training for potato processing firms.
- An allowance of $100,000 a year of extension activity from public sector rural organisations was made for three years after termination of the project (i.e. 2014-2015 to 2016-17).
- Based on industry statistics, it was assumed that the processing industry covers an area 18,000ha producing 740,000t with a gross value of $207 million, while the fresh potato industry covers 17,000ha producing 490,000t with a gross value of $220 million.
- Actual adoption was expected to begin by 2014-15, with processed potato at 10%, increasing annually at an increment of 10% with maximum adoption of 50%; fresh potato would begin at 5%, increasing by 5% every year to a maximum adoption of 40%.
- A yield improvement of 10% was assumed.
- Adoption would involve a treatment cost of $350 per test or $35 per ha (seed growers are doing a number of tests for smaller areas, while commercial growers do the same testing over larger areas), plus there was assumed to be a 60% chance of each test indicating that something needed to be done to avoid or control disease. Forty percent of growers were assumed to move to another area, 40% to use a disease-resistant but lower yielding cultivar (equating to a 3% reduction in yield) and 20% to control the disease with treatments/cultural practices (at a cost of $180 per ha).

The modelling was rerun, using the same BCA model and most of the original assumptions but updating some of these to reflect the outputs of the project, current pricing and industry production. The following parameters were revised:

- Based on industry statistics, it is assumed that the processing industry covers an area of 13,368ha producing 648,000t of potatoes with a gross value of $197 million (referring to the cost of disease work published earlier). The fresh potato industry covers 18,349ha and produces 513,774t (which includes the seed industry) (Stride Consulting Pty Ltd, 2012) with a gross value of $231 million.
- Adoption is expected to begin in 2014-15, with processed potato at 10% which would increase annually at an increment of 10% with maximum adoption of 50%; fresh potato would begin at 5%, increase by 5% every year with maximum adoption of 40%.
- Adoption costs $180 per test or $70 per ha.

The BCA now estimates a net present value (NPV) of $7.48 returned for each dollar invested (for the commercial and seed sector) which demonstrates a very reasonable return on investment. The reduction in this figure from the pre-project BCA is a result of shifts in the size of processing potato production and an increase in cost of testing. If the assumed 10% improvement in yield is reduced to 5%, the benefit/cost ratio is reduced to a $2.78 return on every dollar invested.

PreDicta Pt as a research tool offers tremendous benefit to the potato industry which is not considered in this model. Within the APRP2 program alone the tool was used by four other projects in multifaceted ways – for example, as a means of monitoring pathogen levels in the soil over time (rotation work), by allowing insight into the role of tuber inoculum in disease expression by quantifying soil and tuber pathogen DNA, and as a means of determining the impact of treatment options on soil pathogen levels.

With some relatively minor additional investment in PreDicta Pt the service will be able to expand to include tests for pink rot and Verticillium which are likely to offer further considerable benefit to the industry. Equally the development of the tool for use in quantifying pathogen volumes on seed tubers is likely to see further benefits as highlighted in project PT09019.

PT09026 – SOIL HEALTH/DISEASE MITIGATION PROGRAM

PT09026 aimed to develop disease control strategies for soilborne diseases of economic importance to the Australian processing potato industry through manipulation of nutrients and soil health factors to induce disease suppressive conditions. New management practices were to be in three forms: a) soil amendments and manipulation of nutrients, b) novel approaches to control e.g. hormone application and endophytes, and c) management through clean irrigation sources (for bacterial wilt specifically).

The *ex-ante* BCA estimate for PT09026 was a return of $10.41 for every dollar invested and a more conservative estimate to be $3.73 per dollar invested. The higher return was based on an assumed 50% reduction of the impact of powdery scab, Rhizoctonia and common scab and an increase in yield of 10%. The more conservative estimate was based on a 10% decline in pathogen pressure and 5% increase in yield.

Assumptions for the original work were:

- A 10 year project period (2009-10 to 2018-19) was considered for analysis.
- Adoption of the project outputs will result in decline in losses caused by common scab, powdery scab, Rhizoctonia and bacterial wilt of 50% each, and lead to an overall yield increase in the processing crop of 10%.

- The novel treatments will be hormones and endophytes which will cost $10 and $10 per ha, respectively.
- The adoption of the hormone treatment will commence from year 4 onwards at 1% adoption (2012-13, year 4), and gradually increase to 50% by year 10 (2018-19), while that of endophytes will commence in year 5.
- Adoption of clean irrigation will not involve any monetary costs, and the adoption of different nutrient and soil amendment regimes will impose no net cost on growers.

Revisiting the BCA - 2014

Many of the findings from the PT09026 project require ongoing research and evaluation before they are able to be trialled on farm. The focus of the revisited BCA has been narrowed to what is able to be trialled on farm now or in the next few years. There is an assumption that there is ongoing extension of treatment options to the grower community. Treatments available are outlined in Table 7-13, Table 7-14, Table 7-15, and Table 7-16 .

The new calculation shows a return of $7.80 for every dollar invested with a technical risk of approximately 50% likelihood of success, which leaves a BCA of $3.90. This figure should be revisited in a number of (five) years to assess the penetration of the treatments into grower practice. Figures can be adjusted based on adoption rates and the impact observed. The calculation includes the research costs associated with each treatment. The research costs stretch from 2009 – 2014/2015, the benefits commence in 2014/2015 and are estimated until 2023/2024. The size of the industry is assumed to be 13,368ha producing 648,000 t of potatoes as determined in the national survey by SED discussed previously in this chapter. The value of the industry is assumed to be $197,000,000. Note "FF" denotes French fry, and "crisp" is crisping.

Table 7-13 – Powdery scab management strategies

Treatment	Results	Adoption/Costs
Sulphur	On ferrosol soils in the Ballarat region, increasing normal sulphur levels by three-fold (susceptible varieties only) resulted in increased yield (reduction in tuber scab) to factory by 25% for FF, 50% for crisp. It is assumed that these results are applicable across the industry where powdery scab is a problem*. It is also assumed that the reduction in root infection/galling is approximately 10%**. **It is recommended that sulphur be trialled in other states and soil types and the impact assessed and the BCA adjusted in several years' time.* ***If research into the impact of sulphur on root galling become available these figures should be adjusted.*	Adoption rate of 1% is assumed in 2014/15, 5% in 2015/2016 with increases of 5% adoption each year to a maximum adoption of 40% by 2024-25. **Assume reduction in pickout/packout on farm:** FF industry by 25% (current pickout 5242 t/yr) Crisp by 50% (current pickout 3282 t/yr) **Assume that root infection is also reduced:** FF industry by 10% (currently 28,200 t/yr) Crisp by 10% (currently 1665 t/yr) **Cost of treatment, atomic sulphur, at $808/ha.**

Treatment	Results	Adoption/Costs
Ongoing potato paddock DNA monitoring, rotations, (TIA)	Research demonstrated that seed was the cause of disease introduction into clean paddocks. Once infected it was very hard to return to low risk status. Hence increase awareness to keep paddocks clean and use certified tubers. Encourages those affected to: use longer rotations with greater than 5 years to reduce disease risk adopt better water management during tuber initiation. Overall this work will increase uptake of PreDicta Pt technologies with better outcomes for growers.	Adoption rate of best practices of 1% in 2014/2015, 5% in 2015/2016, with 5% increases each year until 20% national adoption. Assume outcome is a 0.5% increase in yield.

Table 7-14 – Bacterial wilt management strategies

Treatment	Result	Adoption/Costs
Water and tuber testing, awareness raising material	Lateral flow kit has been validated as a means for identifying infected plant material. Testing protocols have been validated for water detection. Awareness raising material has been produced.	Assume benefits are isolated to Victoria. Adoption of clean irrigation would not involve any monetary costs. Testing with the rapid diagnostic test costs $11 per test, with possibly 20 tests per annum conducted (resulting in mitigation of potentially millions dollars of establishment costs and lost crops). Cost of disease to Victoria approximately $2,000,000 pa (conservative estimate). Assume 50% mitigation of bacterial wilt impact in Victoria.

Table 7-15 – Rhizoctonia management strategies

Treatment	Result	Adoption/Costs
Ongoing potato paddock DNA monitoring, rotations, (TIA)	Clearly demonstrated that seed was the cause of disease introduction into clean paddocks. Hence increase awareness to keep paddocks clean. Poppies associated with AG2.1. Carrot, fallow, poppy, ryegrass associated with AG3.	Avoid rotations with poppies, carrots, fallow and ryegrass. Greater awareness to keep new paddocks disease free (use certified seed). Assume increase in yields of 0.5%.

Table 7-16 – Yield-increasing strategies

Treatment	Result	Adoption/Costs
Use of millet as green manure crop	Work in Canada showed yield increases of 20%-48% through incorporation of green manure crop (millet) into continuous potato cropping environments.	The work was completed in Canada – thus requires several years of field trials in Australia. Assume adoption takes place in 2016/2017 at 1% with 5% in 2017/2018 and increases of 5% thereafter to a maximum of 20% adoption. Cost of $12 per hectare for treatment. **Assume conservative 20% increase in yield.**

RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

The APRP2 program management was able to offer independence from the research providers, although the time and efforts of the management could have been much better directed. The cumbersome structure and systems of APRP2 necessitated a lot of work on administrative matters such as shepherding contract variations through legal teams and collating complex, often unwieldy, milestone reports. The greater burden of administration also impacted on the culture of the program with Project Leaders often frustrated at the time involved in undertaking administration rather than research. .

While it is easy to dwell on negatives, many things beside the research outcomes themselves worked well in the program:

- The program approach has reduced the duplication of research work in Australia, which is a saving to industry.
- The program was greatly enhanced by international collaboration which allowed access to the world's best expertise and to two growing seasons in each year. The extent of the international collaboration by the teams was exceptional and provided considerable assurance that the best thinking in the world was being brought to bear on solving the disease problems of Australian potato growers.
- The Technical Operations Committee worked well together. Attendance at the meetings was high and the interaction was of a high standard.
- Generally, the teams collaborated well at project and program level.
- The symposia provided opportunities for teams to share their project outcomes with a greater audience. They were well received and the program morale was boosted significantly following both of these events.
- The external management of the program relieved some of the workload of the HAL Portfolio Manager. Additionally, the employment of managers from outside the potato industry hopefully provided the industry with some fresh thinking – although it is acknowledged that the program managers' lack of potato technical expertise may have been a constraint at times.

Several recommendations for future potato R&D programs and/or similar large programs within HAL have been made throughout this chapter. They are collated below:

1. Future programs should have clearly-defined lines of authority that devolve, to the maximum extent possible without compromising HAL's good governance, decision-making capacity to external managers.
2. Future research programs should be structured with a continuous innovation cycle in mind, that is, with research, development and extension phases seamlessly integrated and informing each other.
3. HAL should consider retaining a template designer to streamline the process of collating the final reports of all major, multi-party projects.
4. HAL should avoid complex contractual structures for programs that cause some providers to be adversely impacted by underperforming counterparts.
5. Milestone criteria should always be worded to strike a suitable balance between flexibility (both for HAL and the provider) and accountability. This is a responsibility of both HAL and providers.
6. HAL and Industry Advisory Committees should consider appointing 'champions' from among the membership of the relevant IAC to each project in large multi-party programs similar to APRP2, to

provide better direct communication between industry and research teams. These should be appointed at the outset of the program.

7. Programs such as APRP2 should have regular interaction with their corresponding IAC in order to increase industry input into and understanding of their significant investment.
8. For programs of similar or greater size that APRP2, consideration should be given to budgeting for an annual whole-of-program technical symposium and review every 12 months.
9. During project planning stage ensure that there are sufficient project resources devoted to the development of scientifically peer reviewed papers.

ACKNOWLEDGEMENTS

Many of the people who assisted the APRP2 program have been acknowledged in Chapter 1, but Scott Williams and Anne Ramsay of SED wish to acknowledge some individuals who provided particular assistance to the program management role:

- Ben Callaghan, HAL Portfolio Manager, who provided great support and advice throughout the program
- Frank Mulcahy (Simplot) and Dave Antrobus (formerly McCain), both on the PPIAC and TOC, for their ongoing encouragement of SED and the entire program team as well as their technical advice
- Nigel Crump (ViCSPA), who generously provided his technical advice as well as facilitation expertise (often at short notice!)
- The Project Leaders and teams, who often exceeded the obligations originally agreed in their contracts in acceding to requests from SED and others
- All of the UB staff with whom SED interacted – Frank Stagnitti, Thembi Dube, Jane Eltringham, Di Clingin (now moved on) and Derek White, all of whom were extremely helpful and collaborative.

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