Nursery Environmental and Technical Research, Development and Extension

Dr Anthony Kachenko
Nursery & Garden Industry Australia (NGIA)

Project Number: NY11000

HAL
Horticulture Australia

NY11000

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Level 7
179 Elizabeth Street
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Telephone: (02) 8295 2300
Fax: (02) 8295 2399

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Nursery & Garden Industry Australia

Final Report
HAL Project: NY11000
Completion Date: 31 August 2012

Nursery Environmental & Technical Research, Development and Extension 11/12

Project Leaders: **Dr. Anthony Kachenko**
Nursery & Garden Industry Australia

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Project Leaders: **Dr. Anthony Kachenko**
National Environmental and Technical
Policy Manager
Nursery and Garden Industry Australia
PO Box 7129
BAULKHAM HILLS BC NSW 2153
Ph: 02 8861 5106
Fax: 02 9659 3446
Email: anthony.kachenko@ngia.com.au

Key Personnel: **Jennifer Nesini** (Administrator)
Kay Reid (Administrator)
Robert Prince (Project Team Member)
John McDonald (Project Team Member)
Michael Danelon (Project Team Member)
Megan Connelly (Project Team Member)
Grant Dalwood (Project Team Member)

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~ Disclaimer ~

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MEDIA SUMMARY

This project *NY11000 – Nursery Environmental & Technical Research, Development and Extension 11/12* consists of several environmental and technical research and development sub projects that address key environmental issues that impact on the sustainability of the Australian nursery and garden industry (NGI).

The development of each sub project involved consultation from whole of industry which involved all State/Territory Associations to ensure relevance of issues in relation to industry needs and expectations as well as alignment with the Nursery Industry 2010–2015 Strategic Plan.

The national Environment Committee funded through this project met twice (4 November 2011 and 28 June 2012) to oversee the direction of this project as well as provide input on future research, development and extension opportunities.

A key sub project of this project was developing and utilising the skill and knowledge base of the Industry Development Officer (IDO) network in representing the Australian nursery industry at a regional level on key national environmental and technical issues such as biosecurity and quarantine. This representation supported the Nursery & Garden Industry Australia National Environmental & Technical Policy Manager (NETPM) to ensure the NGI was adequately represented in areas that may have impacted on its long term sustainability.

Several outcomes relating to other sub projects within this project include:

- Completion of three university student projects that addressed key industry issues whilst educating tomorrow's future industry leaders.
- Submission of six minor use permit applications to the Australian Pesticide and Veterinary Medicines Authority (APVMA) for industry access of key pesticides.
- Inclusion of four new calculators in the online Water Management Toolbox calculator - http://watertoolbox.ngi.org.au.
- Development of resources and tools that sit behind the industry on-farm BioSecure *HACCP* program to provide industry a possible third legal instrument for market access.
- Completion of the Nursery Production Farm Management System Cost/Benefit Analysis and ROI report.

TECHNICAL SUMMARY

The project *NY11000 – Nursery Environmental & Technical Research, Development and Extension 11/12* provides the Australian nursery industry with the capacity to address several key environmental and technical issues in a holistic manner through a centralised project managed by the National Environmental and Technical Policy Manager (NETPM). The project is aligned with the strategic direction of the Australian nursery and garden industry (NGI) Strategic Plan 2010-2015 and builds on the successful achievements of earlier NGI Environmental and Technical RD&E projects (namely NY06014, NY07006, NY08002, NY09016 and NY10005). These projects have delivered industry with a suite of tools, resources as well as representation to ensure the NGI maintains a proactive and coordinated approach in addressing environmental issues across whole of industry.

This project and all sub projects were developed through a rigorous process involving the whole of industry through a consultation process which involved all State/Territory Associations to ensure relevance of issues in relation to industry needs and expectations. This also ensured the project was in alignment with the Nursery Industry 2010–2015 Strategic Plan.

The Environment Committee funded through this project provided independent direction to support the activities of the NETPM and met twice during the project (4 November 2011 and 28 June 2012). The Committee also provided input on future research, development and extension opportunities whilst discussing key challenges which included how to grow the urban forest, the impact of rising input costs, the impact of the carbon tax on industry and the need for continued investment in biosecurity to safeguard the NGI. These discussions were mindful of the boundaries and direction outlined within the Nursery Industry 2010–2015 Strategic Plan. Minutes and recommendations arising from these meetings were circulated to all stakeholders.

The IDO network was a key conduit of the extension of research and development outputs arising from this project and also represented industry on key issues at a regional level including biosecurity and quarantine. This representation supports activities of the NETPM to ensure the NGI is adequately represented in key areas that may impact on the industry's long term sustainability.

Seven sub projects were investigated as part of this centralised umbrella project. This report details the outcomes relating to these with the following having been achieved:

- Completion of three university student projects that addressed key industry issues whilst educating tomorrow's future industry leaders. The projects included:
    - **Small-scale food growing in Canberra: opportunities and obstacles** by Walter Steensby, Research Assistant, Faculty of Arts & Design University of Canberra.
    - **Tree canopy cover in residential areas of Australian cities** by Stephen Livesley and Melissa Fedrigo, University of Melbourne.
    - **The efficacy of IAA-producing PGPR in different formulations as plant growth regulators** by Apriwi Zulfitri, Faculty of Agriculture and Environment, The University of Sydney.
- Submission of six minor use permit applications to the Australian Pesticide and Veterinary Medicines Authority for industry access of key pesticides.
- Inclusion of four new calculators in the online Water Management Toolbox calculator - http://watertoolbox.ngi.org.au. The calculators were:
    - Alkalinity calculator;
    - Fertilizer calculator;

    - Plant growth regulator calculator; and
    - Liming calculator.

- Development of resources and tools that sit behind the industry on-farm BioSecure *HACCP* program to provide industry a possible third legal instrument for market access.
- Nursery Production Farm Management System Cost/Benefit Analysis and ROI report.

INTRODUCTION

The Australian nursery industry is valued at over 5.5 billion dollars and employs over 45,000 FTE in more than 25,000 small to medium sized businesses annually. The industry is closely linked with others in the supply chain providing plants for key sectors including forestry, revegetation/landcare, landscapers, fruit orchardists, cut flower and vegetable sectors. Businesses are located in urban, peri-urban and regional locations across Australia and vary in size, scale, location, end user and supply chain characteristics.

Owing to the diversity that exists with the industry, as well as a varying degree in maturity between businesses, the industry is subject to technical and environmental issues that impact on its productivity and profitability. Biosecurity has been a key issue that has received focus by industry as many of the 10,000 crop lines grown in Australia are at risk of the consequences borne by exotic plant pest incursions. In addition, there has been a heightened degree in regulatory restrictions for producers, often impacting on the logistics with plant consignments intrastate and interstate. The Australian NGI continues to focus investment into biosecurity through the development of resources, tools and extension activities by the Industry Development Officer (IDO) network and all nursery and garden industry staff.

On July 1 2012, the new carbon price policy – Clean Energy Future – was introduced by the Australian Government. While the nursery industry will not be directly involved in the carbon price mechanism, it is expected that the carbon price will result in cost increases for key agricultural inputs with the most significant costs relating to energy and energy intensive inputs such as fertilisers, chemicals and machinery. A recent report titled "The impacts of the carbon price on Australian horticulture" authored by Lene Knudsen et al. (2012) suggests that electricity costs will increase by 9.8 per cent at a carbon price of $23 and by 13.1 per cent at a carbon price of $30.82 which is the projected carbon price in 2020. Urgent and continuing investments need to be made in research and development to ensure the industry can sustain the rise in input costs including energy.

Access to safe, reliable and efficacious pesticides to meet grower needs whilst being mindful of the environment is an issue that continues to impact on the sustainability of the nursery industry. Very few pesticides are registered for use by the nursery industry and in order to facilitate access to these pesticides, investment in minor use permits (MUPs) is required. In some cases, the access to MUPs assists the industry in meeting regulatory requirements relating to chemical treatment for plant consignments across borders. The industry has invested in MUPs for several years as it is seen as a key area of focus by growers across Australia.

A channel for extension of industry technical research is through the delivery of the Nursery Production Farm Management System (FMS). This system provides a framework endorsed by industry and government to ensure a sustainable future for nursery producers. The Nursery Production FMS is aimed at guiding change and technology adoption through the following three programs:

1. Nursery Industry Accreditation Scheme Australia (NIASA) – a Best Management Practice program to improve business efficiency whilst being mindful of the environment.

2. EcoHort – an Environmental Management System which offers risk assessment, a continuous improvement pathway and opportunity to demonstrate sound environmental stewardship.

3. BioSecure *HACCP* – a biosecurity program which helps business assess their pest, disease and weed risks for both imported and exported material.

Although there are over 270 businesses engaged with the program across Australia, the industry has expressed growing concern that the cost of complying/implementing the program is potentially disproportional to the benefit; however there is a lack of data to support this assumption. The actual costs involved in implementation, maintenance and audit of the Nursery Production FMS is largely unknown and have not been quantified.

The IDO network is instrumental in delivering the Nursery Production FMS and acts as the main vehicle to extend RD&E outcomes. The skills and knowledge base of the IDO network is also used to represent the industry at a regional level on key national environmental issues and supports the National Environmental & Technical Policy Manager (NETPM) to ensure the industry is across all key environmental issues. The network also utilises Nursery Papers, Clippings, Facebook and the NGIA website to communicate RD&E outcomes to whole of industry.

The issues detailed above have been identified in the Nursery Industry 2010–2015 Strategic Plan to ensure the Australian nursery industry has the capacity to respond to growth opportunities and challenges that impact on its growth and sustainable development. They have also been identified in the National Rural Research and Development Priorities outlined by Department of Agriculture, Fisheries and Forestry (DAFF) and align with the four objectives of the Horticulture Australia Limited (HAL) Strategic Plan 2010–2015. This project aims to address these issues in alignment with the aforementioned plans and strategies.

MATERIALS AND METHODS

This project consisted of several environmental and technical research, development and extension (RD&E) sub projects and followed the approach taken over the past six years due to the breadth of interconnected environmental issues requiring a centrally coordinated response.

This project was managed by the National Environmental & Technical Policy Manager (NETPM; NY10001) with collaboration from the Industry Development Officer (IDO) network (NY09010). Independent direction was provided by the national Environment Committee funded through this project and supported the NETPM in facilitating these sub projects. The Environment Committee provided leadership and independent direction in addressing all components of this project, evaluating current environmental and technical issues and monitored the future R&D direction of the Australian Industry in relation to the NGI 2010 – 2015 Strategic Plan. The Environment Committee consisted of 4 industry representatives and NGIA representatives (Dr Anthony Kachenko and Robert Prince, NGIA CEO). The committee was chaired by NGIA Board Director Simon Smith who took over from Glenn Fenton in October 2012.

At the time of the report, the Committee had the following members:

- Simon Smith (Chairman), Managing Director, The Plantsmith Nursery, NGIA Board Member, , Northern Territory;
- John Bunker, Managing Director, Redlands Nursery Pty Ltd, Queensland;
- Steve Burdette, Business Development & Nursery Manager, Agriexchange, Renmark, South Australia; and
- Sean O'Brien, Horticultural Manager, Hunter Valley Gardens, New South Wales.

During the life of this project, two national Environment Committee meetings were facilitated (4 November 2011 and 28 June 2012). Sub projects within this project were submitted by the IDO network through State Associations to this Committee prior to the November 2010 lodgement date for RD&E projects to HAL. Each potential project was ranked through a thorough process whereby each State/Territory Association ranked projects for relevance to industry needs based on the Nursery Industry 2010–2015 Strategic Plan. The highest ranked projects were then put forward to the Industry Advisory Committee (IAC) for approval and those successful were undertaken under this project. This process proved a valuable vehicle to ensure whole of industry had input into the industry RD&E direction. The Committee was also tasked with developing a policy position based on biosecurity and quarantine owing to the need for a national position on this important industry issues.

A total of $17,000 was allocated to facilitate these meetings and develop the biosecurity and quarantine.

To assist with the extension of outcomes from this project, and to ensure industry was suitably represented at a national level, the skills and knowledge base of the IDO network was utilised. This provided industry with an opportunity to enhance the industries representation with key environmental issues, most notably in the area of biosecurity. Where an IDO represented industry at an event (e.g. meetings, conferences, workshops etc.), a meeting report was submitted to the NETPM summarising the nature of the event, outputs arising from the event and future direction which were then circulated to all State and Territory Associations. A total of $20,000 was allocated to this project cover conference/meeting attendance costs including travel and accommodation for the IDO network. An additional $30,000 was allocated to Nursery & Garden Industry Queensland (NGIQ) to fund human resource support for the QLD Industry Development Manager (IDM). This funding recognised the added responsibility the Queensland IDM had in relation to maintaining the NGIs biosecurity commitments which includes attendance to all meetings associated with the EPPRD.

The remaining sub projects are discussed below:

1. Nursery and garden industry affiliate and research linkage program

This sub project provided funding for several research projects in Australian Universities to address key industry research issues whilst enhancing research linkages between industry and academia. The project also enhanced industry capacity for innovation and made a positive contribution towards the education of tomorrows industry professionals and leaders.

All projects funded through this sub project were developed in consultation with whole of industry. In addition, the relevant academic institution had to show progress of innovation relevant to industry needs in alignment with the NGI 2010 – 2015 Strategic Plan. This approach continued the direction of the NGI over the past three years where funds allocated to this project were used to exclusively support the costs associated with research overheads to enable successful fulfilment of the research objectives. This sub project was undertaken by three universities across Australia and valued at $50,000.

The following research projects were undertaken and successfully completed during this project:

1. **Small-scale food growing in Canberra: opportunities and obstacles** by Walter Steensby, Research Assistant, Faculty of Arts & Design University of Canberra. Walter is completing his Masters of Arts in Communication (research) under the supervision of Dr David Pearson.
2. **Tree canopy cover in residential areas of Australian cities** by Stephen Livesley and Melissa Fedrigo, University of Melbourne. Melissa Fedrigo is completing her PhD in Forest Ecology under the supervision of Dr Stephen Livesley.
3. **The efficacy of IAA-producing PGPR in different formulations as plant growth regulators** by Apriwi Zulfitri, Faculty of Agriculture and Environment, The University of Sydney. Apriwi is completing her Masters of Science in Agriculture under the supervision of Dr Meredith Wilkes.

Funding from this project will also be utilised to support activities undertaken during a three year full-time PhD on 'Designed Green Wall Systems' at The University of Melbourne Burnley Campus in Richmond, Melbourne, Victoria. The project work will be undertaken by Annie Hunter-Block.

2. Provide access to seven Minor Use Permits (MUP) for industry

This sub project invested in the development of six MUP following consultation with growers for priority products that were submitted to Australian Pesticide and Veterinary Medicines Authority (APVMA) under the MUP program for nursery stock. This sub project has been in operation for three years to enable the registration of MUP required by the NGI with APVMA. A total of $20,000 was allocated to this project to facilitate this process. The following products were submitted to APVMA as part of this sub project by Peter Del Santo, AgAware consulting.

Product (active)	Target Pest
Chlorfenapyr	Lepidoptera & two-spotted mites
Petroleum oil	Aphids, mites, leafhoppers, thrips and scales
Thiamethoxam/chlorantraniliprole	Lepidoptera including DBM, cabbage white, Helicoverpa, loopers, leafhoppers, aphids, whitefly, bugs, thrips & leafrollers
Metiram/pyraclostrobin	Alternaria, Phtophthora, Colletotrichum, powdery mildew & downy mildew
Potassium bicarbonate	Powdery mildew
Copper hydroxide	Various foliage diseases and surface disinfestation

3. Develop nutrient budgeting calculators for plant production

This sub project updated the Water Management Toolbox http://watertoolbox.ngi.org.au with new nutrient budgeting calculators. An alkalinity calculator that provided recommendations for the amount of acid to add to irrigation water in order to modify the pH and alkalinity levels was developed. In addition, a fertilizer calculator was developed that will give growers the ability to calculate fertilizer formulations for water soluble fertilizer. A plant growth regulator calculator was also developed that calculated plant growth regulator (PGR) mixing rates. This calculator also calculated the final solution costs. A forth calculator to assist producers manage pH drift and lime application in containers was also incorporated into the Toolbox. These calculators are aimed to assist growers better manage fertiliser and PGR inputs in an environment where costs are increasing. This project was undertaken by National Centre for Engineering in Agriculture (NCEA) Queensland and valued at $20,000.

4. Progressing BioSecure *HACCP* towards a third market access instrument

This project aimed to develop the resources and tools that sit behind the industry on-farm BioSecure *HACCP* program to provide industry a possible third legal instrument for market access. This aims to provide an instrument that gives growers greater ability to self-certify as it proposes that the IDO network would audit on-farm compliance (as currently occurs through NIASA, EcoHort and BioSecure *HACCP* during regular accreditation audits instead of Plant Health Inspectors) and then industry through the IDO network would be audited (Technical Officers) by government. The tools and resources developed through this project will provide growers with lower costs and greater flexibility when seeking markets with their products.

In order to facilitate this, this project funded the development of both the administration and governance protocols to underpin BioSecure *HACCP* for it to meet regulatory compliance and competence including updates to the BioSecure *HACCP* manual as well as the development of an electronic Audit Management System (AMS). Blackwood and Kemp Pty Ltd were engaged to develop the administration and governance protocols and Cira Solutions were engaged to develop the AMS. This project was valued at $120,000.

5. Nursery Production Farm Management System Cost/Benefit Analysis and ROI

The project was delivered using benefit cost analysis techniques described in the Council of Rural Research and Development Corporation (CRRDC) Evaluation Guidelines (updated 2009). Three benefit cost analyses were completed as part of this project.

1. The first analysis addressed the value of Nursery Production FMS to an individual business that had implemented the system.
2. The second analysis quantified the farm management system's value to the whole nursery industry since inception.
3. The third analysis assessed benefits to the broader Australian community across the economic, social and environmental 'triple bottom line'.

The Nursery Production FMS was analysed as a 'whole' inclusive program rather than attempting separate evaluations for each of the NIASA, EcoHort and BioSecure *HACCP* programs. This project was undertaken by AgEcon Plus and valued at $60,000.

RESULTS AND DISCUSSION

Two meetings of the Environment Committee were held (4 November 2011 and 28 June 2012). The minutes arising from these meetings were circulated to whole of industry through the IDO network and State/Territory Associations to enable awareness of key issues discussed. A copy of the minutes from both meetings is provided in Appendix 1.

Some key outputs arising from the two committee meetings included:

- Review and development of the NGI Environmental Risk Matrix that was used at each meeting to assist in prioritising future RD&E activities. The current version of the Environment Risk Matrix is provided in Appendix 2. Key priorities identified in this matrix were water, biosecurity and the impact of climate change.

- Oversaw the delivery of RIRDC (Rural Industries Research and Development Corporation) under the National Weeds and Productivity Research Program to undertake a research project titled '*Weed Risk Assessment for Australian Nursery & Garden Industries'*. This external project was valued at $212,059 with the final report submitted to RIRDC in May 2012.

- Oversaw the development of 'Reducing the Pest Risk - The Australian Nursery & Garden Industry's Policy Position on Quarantine and Biosecurity'. The Policy Position was launched by John McDonald during the 2012 Nursery and Garden Industry National Conference on the Gold Coast. A copy was included in the Conference handbooks. A copy of this policy position is provided as Appendix 3. A copy is also available at http://www.ngia.com.au/Category?Action=View&Category_id=139

During the reporting period, there were no meetings that required Industry Development Officer (IDO) representation on key industry environmental and technical issues. Indeed, the network faced change with the resignation of Victorian IDO Robert Chin and Western Australian IDO, Peter Gwynn. A replacement for Peter Gwynn has been announced with Trevor Winter appointed on 1 March 2012. Trevor is a qualified horticulturist with 19 years wholesale nursery experience, three of which involved landscape consulting. A replacement for Robert Chin was announced on 1 July with the Appointmnet of David Reid who came to industry from the Victorian Department of Primary Industry. The Industry Development Officer (IDO) network (NY09010) project will cease in September 2012 and a new project proposal NY12006 will commence from 1 September to be managed by the NETPM.

John McDonald (Queensland IDO responsible for biosecurity) continued to represent NGIA on all Consultative Committee on Emergency Plant Pests and Emergency Plant Pest Categorisation Group Meetings. During the reporting period, he drafted and released version 2 of the Myrtle Rust Management Plan to assist the NGI manage Myrtle Rust on ALL plants from the Myrtaceae family. The plan is provided as Appendix 4 and is free to download from the NGIA website http://www.ngia.com.au/Section?Action=View&Section_id=527

The IDO network was involved in identifying and assessing areas of possible RD&E for NGI to present to IAC at the September 2012 meeting. All projects received by IDOs will be discussed and ranked by the entire network prior to submission for funding in 2013/14. The ranking template is provided in Appendix 5. During this process, the NETPM will provide guidance with each project evaluated based on linkages with the Nursery Industry 2010-2015 Strategic Plan and the 2012-2016 Strategic Investment Plan.

1. Nursery and garden industry affiliate and research linkage program

Three research projects were undertaken across Australian universities and research institutions during this project. Each project was developed in consultation with the NGI and the relevant academic institution and coordinated through the NETPM. Each project had to show progress of innovation relevant to industry needs in alignment with the NGI 2010 – 2015 Strategic Plan. The following projects were successfully commissioned and completed:

1. **Small-scale food growing in Canberra: opportunities and obstacles** by Walter Steensby, Research Assistant, Faculty of Arts & Design University of Canberra. Walter is completing his Masters of Arts in Communication (research) under the supervision of Dr David Pearson. A copy of this report is provided in Appendix 6.

2. **Tree canopy cover in residential areas of Australian cities** by Stephen Livesley and Melissa Fedrigo, University of Melbourne. Melissa Fedrigo is completing her PhD in Forest Ecology under the supervision of Dr Stephen Livesley. A copy of this report is provided in Appendix 7.

3. **The efficacy of IAA-producing PGPR in different formulations as plant growth regulators** by Apriwi Zulfitri, Faculty of Agriculture and Environment, The University of Sydney. Apriwi is completing her Masters of Science in Agriculture under the supervision of Dr Meredith Wilkes. A copy of this report is provided in Appendix 8.

The full reports will be written up as technical Nursery Papers over the next 12 months for extension to industry. Aspects of these reports have been posted on the NGI Facebook page and written up as Clippings articles.

2. Provide access to seven Minor Use Permits (MUP) for industry

The paperwork associated with the six MUP was lodged with APVMA. At the time of this report, the following permit was issued:

Permit number	Permit Description	Date Issued	Expiry Date	Permit holder	States
PER13328	Copper Hydroxide / Nursery stock (non-food) / Specified diseases	28-May-12	31-May-15	NGIA / AgAware	All states (excl. Vic).

This permit has been circulated through the IDO network for extension to industry. The NETPM will receive notification from Peter Del Santo upon the issuing of the remaining five permits. A copy of the aforementioned permit is provided in Appendix 9.

3. Develop nutrient budgeting calculators for plant production

This project saw the nursery industry Water Management Toolbox updated to include the following new calculators:

- Alkalinity calculator;
- Fertilizer calculator;
- Plant growth regulator calculator; and
- Liming calculator.

The updated toolbox can be found here http://watertoolbox.ngi.org.au. The new calculators are provided in

Excel format and can be saved individually on the user's computer. Alternatively, the entire Water Management Toolbox can be downloaded as a zip file and accessed offline.

4. Progressing BioSecure *HACCP* towards a third market access instrument

Blackwood and Kemp Pty Ltd have been engaged to develop the administration and governance protocols with the final guidelines to be provided to the NETPM on 22 October 2012. The guidelines will incorporate the following elements:

BioSecure *HACCP* Administration & Governance development & documentation:

- Develop clear administration guidelines and processes for managing the BioSecure *HACCP* program
    - Terms & Conditions
    - Heads of Agreement
- Develop governance processes that underpin the program integrity to meet regulatory confidence
    - Document development of program procedures for governing all aspects
        - Auditor skill sets
        - Alignment to biosecurity agencies (training)
        - On-farm audit process
        - Program third party audit (regulator) process
    - Regulator access
    - Data management
    - Compliance and non-compliance processes and notification

Cira Solutions have been engaged to develop the AMS with the delivery of the completed AMS due on 30 November 2012.

Updates to the BioSecure HACCP guidelines are currently being undertaken by John McDonald and the NETPM. An updated version of these guidelines will be released in early 2013. This release will include:

- General content update (e.g. requirement for property plans, etc.)
- On-farm surveillance methodology & procedures
- Development of quarantine pest management templates
- Document management on-farm record/BioSecure *HACCP* record matrix

A detailed overview of progress relating to this sub project is provided in Appendix 10.

5. Nursery Production Farm Management System Cost/Benefit Analysis and ROI

AgEcon Plus provided a final report detailing the outcomes of a series of cost benefit analyses on Nursery and Garden Industry Australia's (NGIA) Nursery Production Farm Management System (FMS). It was prepared to provide an evidence base for communication to industry and Horticulture Australia Limited.

Three benefit cost analyses were completed. The first addressed the value of the FMS to individual businesses. The second analysis quantified the FMS's value to the whole nursery industry while the third identified benefits to the broader Australian community. A total of 27 businesses engaged with the Nursery Production FMS were surveyed as part of this project.

Since the inception of the Nursery Production FMS, NGIA and Horticulture Australia Limited (HAL) have supported twenty two levy funded projects totalling almost $1.3 million. Contributions have also been made by various state governments. Ongoing costs include annual administration and the IDO network.

On average, businesses engaged with components of the Nursery Production FMS received between $75,000 to $85,000 net revenue increases with a payback period for investments between 5-7 years. The Nursery Production FMS has delivered a strong industry benefit – net present value of $71.22 million with a benefit cost ratio of 8.01 and a return on investment of 40.5%.

Sensitivity analysis completed on industry returns demonstrated that even with only 25% of adopters receiving a financial benefit from FMS implementation, additional industry revenue more than covered industry investment costs.

Benefits to the Australian community from the nursery industry's investment in the Nursery Production FMS were identified and analysed across the environmental, social and economic 'triple bottom line'. The most important environmental benefits realised by the Australian community were improved biosecurity (less chance of invasive weeds, pests and diseases) and improved chemical management. Community social benefits included increased demand for gardening with associated positive spin offs for health, social and visual amenity. Community economic benefits included employment and regional development.

The report will be tabled for discussion at the September IAC and Consultation meetings. A summary Nursery Paper detailing these outcomes will also be prepared.

TECHNOLOGY TRANSFER

This project was developed following full consultation with the nursery industry. All sub projects undertaken in NY11000 had been ranked as high priority projects through several levels of consultation to ensure the direction of this project was in line with industry expectations.

With much of the project work having recently been completed, or still underway, the next 12 months will see a concerted effort by the NETPM to ensure that outcomes of all sub-projects are successfully communicated to whole of industry. The NETPM will consolidate outcomes into resources that can be used by the IDO network and State Associations such as media releases for publications.

The industry Nursery Papers will also detail key outcomes of projects including the outcomes of the Minor Use Permits (MUP) sub project scheduled for the December 2012 edition. A Nursery Paper schedule will be developed in December 2012 and will provide each IDO and the NETPM an opportunity to develop a Nursery Paper on a key project outcome.

This Final Project report will also be uploaded on the NGIA National Research & Development Database http://ngia.com.au/Section?Action=View&Section_id=473 once accepted. At present, there is 342 Final Reports available for download through this database. Targeted technical communications will also be developed for whole of industry through existing NGIA communications including NGIA website (www.ngia.com.au) and NGIA Facebook page (http://www.facebook.com/nurseryandgardenindustry) which currently has 741 likes. The 'Your Levy at Work' blog which replaced the hard copy 'Clippings' will also be used as a regular communication tool direct to growers (http://yourlevyatwork.com.au).

The updated calculators have been incorporated into the Water Management Toolbox and can be found here http://watertoolbox.ngi.org.au and the Minor Use Permits once approved will be available on the Australian Pesticides and Veterinary Medicines Authority website (www.apvma.gov.au).

RECOMMENDATIONS

The holistic approach undertaken by this project to group several technical and environmental sub projects under one all-inclusive project has proven successful in terms of delivering key outcomes for industry in an efficient and timely manner. Furthermore, the consultation process utilised in scoping sub projects enabled industry to provide input into priority research areas. This approach should continue in the immediate future in scoping new projects.

As noted under Technology Transfer, the communication of outcomes will continue following the approval of this Final Report. A variety of communication channels will be utilised including websites and NGI social media. Other modes of communication will be investigated under NY12001 including the use of videos in a 'You Tube' format that can be readily posted on social media sites.

Several of the sub projects undertaken in this project should continue including funding to facilitate research activities through Australian universities. Over the past three years, this project has proven useful in generating interest for nursery industry research within academic institutions. A formalised approach will be developed to better engage with Universities in early 2013. Ongoing funding is also warranted to support industry efforts in the area of biosecurity. This includes market access related activities relating to BioSecure *HACCP* as well as resource support for the QLD Industry Development Manager (IDM). Ongoing investment into the minor use permit program is also required to ensure industry is not disadvantaged in accessing safe, reliable and environmentally sound chemistries.

Research to project the Australian urban forest as a solution to climate change and the health and wellbeing of the Australian community will also require future investment. Possible research areas may include the development of:

- Educational resources;
- A database that identified optimum trees for street conditions; and
- Best Management Practices for planting trees in landscape situation.

APPENDICES

The following appendices relate to the outcomes of the project:

1 Minutes of NGIA Environmental Committee meetings held 4/11/11 and 28/06/12.

2 Environmental Risk Matrix 2012.

3 Reducing the Pest Risk - The Australian Nursery & Garden Industry's Policy Position on Quarantine and Biosecurity.

4 NGI Myrtle Rust Management version 2.0 – Released 2012.

5 IDO Ranking Template.

6 Small-scale food growing in Canberra: opportunities and obstacles report.

7 Tree canopy cover in residential areas of Australian cities report.

8 The efficacy of IAA-producing PGPR in different formulations as plant growth regulators report.

9 Minor Use Permit 13328.

10 BioSecure *HACCP* Project Update – July 2012.

11 Nursery Production Farm Management System Cost/Benefit Analysis and ROI Report.

Appendix 1

MINUTES

Nursery & Garden Industry Australia

Environment Committee Meeting

Date:	Friday 4 November 2011
Time:	9.30 am – 2.00 pm
Location:	NGIA Office, 16-18 Cambridge Street, Epping 2121
Attendees	Glenn Fenton (Chair), Steve Burdette (9:30 am – 10:00 -10:30 am via telelink), Sean O'Brien (from 10:15 am), Robert Prince, Anthony Kachenko
Apologies	John Bunker

ITEM	TOPIC
1	WELCOME AND APOLOGIES

G Fenton formally declared the Environment Committee Meeting open at 9:30 am and extended a warm welcome to Committee Members. G Fenton noted that an apology for absence had been received from J Bunker due to prior commitments. S Burdette indicated that he had commitments from 10:30am and asked to commence the meeting by providing an overview of the 24 projects submitted to NGIA for 2012-2013 funding. The Committee concurred and G Fenton suggested that items under Agenda Item 8.0 would be covered. S Burdette indicated that he based his analysis of the projects based on risk to industry, industry benefit and industry awareness. The Committee discussed each of the following projects in details.

1. National Myrtle Rust Industry Liaison Officer. The Committee agreed that this project was relevant however beyond the scope of levy investment and should be addressed by Government. The Committee suggested that NGIA seek opportunity to seek funds through other areas to address this project. R Prince indicated that some Myrtle Rust research was being funded under the Federal Governments Transition to Management Plan which is currently embargoed.

2. Controlled Release Systemic Fungicide for Myrtle Rust. The Committee agreed that this project was relevant however beyond the scope of levy investment and should be addressed by Government as per the previous project.

3. Web Based Pest & Disease Management Tool. The Committee agreed that this was a sound proposal making existing data more accessible and should be included in a future research and development (R&D) project with revised costings of $15,000. Content management and hosting to be kept in house.

4. Occupational Health & Safety (OH&S). The Committee agreed that this project was relevant however beyond the scope of levy investment and should be addressed by businesses individually.

ITEM	TOPIC

5. Nutrient Management. The Committee agreed that this was a sound proposal and should be included in a future R&D project.

6. Shadehouse Structures. The Committee agreed that this project was relevant however there was a lot of information and a literature review assessing this information would be a logical starting point.

7. Business Management Software. The Committee agreed that this project was relevant and could be linked with project 23 and 24. The committee suggested a desktop review or situation analysis of existing tools and resources based on inventory management, orders, stock and sales control would be useful. The Committee agreed that this proposal should be included in a future R&D project

8. Value of Greenlife Information Sheets. The Committee agreed that this project would be covered through industry marketing under Plant Life Balance. No further investment was identified through R&D.

9. The Effects of Nursery Production Methods on the Future Success of Landscape Trees. The Committee agreed that this project could be linked with project 13 and 14. A Kachenko indicated that work on an Australian Standard for Tree Specification would go a long way to address the outcomes of this project. A Kachenko indicated that a workshop in early 2012 would be held to gauge grower feedback into developing this standard. He indicated that a workshop would also be held in Sydney and involve Ross Clarke who authored NATSPEC.

10. Investigate the availability of fastigiate trees and shrubs. The Committee agreed that this project was not suitable for levy funding as it did not address market failure. Indeed, the Committee indicated that the information was already available and the market would set the trends.

11. Optimising the selection of irrigation sprinklers for overhead sprinkler systems based on independent testing. The Committee agreed that this was a sound proposal and should be included in a future R&D project. A Kachenko indicated that much of this research had been conducted by NGIQ through funding out of South East Queensland Irrigation Futures and would look at sharing this resource nationally.

12. Development of a commercially applied diagnostic test to predict the susceptibility of Myrtle rust germplasm leading to selection of resistant plants. The Committee agreed that this project was relevant however beyond the scope of levy investment and should be addressed by Government. The Committee suggested that NGIA seek opportunity to seek funds through other areas to address this project.

13. The development of a healthy root system of trees by adoption of optimum cultural practices. The Committee agreed that this project could be linked with project 9 and 14 and should be included as a project based on the discussion under project 9.

14. Defining a set of greenlife standards for the nursery industry. The Committee agreed that this project could be linked with project 9 and 13 and should be included as a project based on the discussion under project 9.

ITEM	TOPIC

15. Industry research linkage – support for university research projects. The Committee agreed that this was a sound proposal and had delivered a number of key research outcomes since commencing two years ago. The Committee indicated that this proposal should be included in a future R&D project.

16. Update Flora for Fauna website. The Committee agreed that this was a sound proposal and should be investigated under future industry marketing campaigns.

17. Investigation lighting options to maximise energy efficiencies in undercover production. The Committee agreed that this was a sound proposal and should be linked with proposal 20.

18. Industry Minor Use Program. The Committee indicated that this proposal should be included in a future R&D project. The Committee indicated that greater communication of this project and investment to date should be reinforced over the next 12 months.

19. Pump Selection Tool. The Committee agreed that this was a sound proposal and should be linked with proposal 20 and included in a future R&D project.

20. Energy BMP for Production Nurseries. The Committee agreed that this project was of key importance and relevance to nursery production. A Kachenko indicated that he was working with the National Centre for Experimental Agriculture (NCEA) to develop a three year project relating to this proposal.

21. Pesticide Spray Diary and BMP Online. The Committee agreed that this was a sound proposal making existing data more accessible and should be included in a future R&D project. A Kachenko indicated that no individual data would be captured with the tool housed on the NGIA website and managed by NGIA. Resources including weather data would be housed on this website.

22. Innovation in extension using digital media. The Committee indicated that this project may assist in driving extension in conjunction with existing communication streams. The Committee agreed that this proposal should be investigated in a future R&D project.

23. Feasibility of establishing a business management - scenario analysis, decision making software tool to address supply chain issues within the Australian Nursery Industry. The Committee agreed that aspects of this project were relevant and should be included as a project based on the discussion under project 3.

24. Supply Chain – Industry Efficiency. The Committee agreed that aspects of this this project were relevant and should be included as a project based on the discussion under project 3.

A Kachenko indicated that he would incorporate this feedback into a single R&D proposal for submission to Horticulture Australia Limited (HAL) on 28 November 2011.

2 CONFIRMATION OF MINUTES – MEETING 28 JUNE 2011

G Fenton advised that the Minutes from the Meeting held on 28 June 2011 were provided in the meeting papers and the Committee were asked to raise any concerns regarding the accuracy of these minutes. Amendments were received from the Committee. No further amendments were received.

ITEM	TOPIC
	MOTION: The Environment Committee accept the minutes from the 28 June 2011 Environment Committee meeting as a true and accurate record. **Moved:** G Fenton **Seconded:** S O'Brien
3	**MATTERS ARISING FROM LAST MEETING**
	3.1 Review of Action List
	A Kachenko tabled an Action List arising from the last Meeting. He provided an update on the completed items. The following actions are outstanding: • A Kachenko to provide an update on funding opportunities through the mining industry and report back to the Committee at the next Environment Committee Meeting A Kachenko indicated that he would include this outstanding item on the Action List for Action prior to the next meeting.
4	**MATTERS ARISING (NOT ADDRESSED IN THIS AGENDA)**
	A Kachenko indicated that a revised Industry Development Officer project is needed for submission to HAL for 2012-2015 funding and was seeking input from the Committee. The Committee indicated that it would be discussed under Agenda Item 5.2.
5	**NATIONAL ENVIRONMENTAL PROJECT UPDATE**
	5.1 Summary and Status of Current Government Inquiries
	1. A Kachenko provided an update of current inquiries NGIA was involved in. 2. Implementation of model schedules for Commonwealth serious drug offences. A Kachenko indicated this inquiry remained open with no further correspondence from the Attorney Generals Department indicating otherwise. 3. Management of removal of fee rebate for AQIS export certification functions. A Kachenko indicated that there had been little contact from AQIS in Canberra regarding progress with the horticultural export program reforms. A consultation meeting between AQIS and industry was due on 29 November to discuss next steps. 4. Biosecurity Advisory Council (BAC) of Australia – Managing an Emergency Response. A Kachenko indicated that written submission was made to the BAC as part of the process to drive changes in handling responses. The BAC was due to submit feedback to Biosecurity Australia in early 2012. 5. Draft consultation regulation impact statement for the proposed risk treatment measures for precursor chemicals to have homemade explosives. A Kachenko attended a forum to discuss this draft which will be released in early 2012 for wider consultation. This may have impact on the purchasing and distribution patterns of industry in relation to key fertilisers that contain potassium nitrate.

ITEM	TOPIC

6. Consultation on improvement to rural R&D. R Prince indicated that he would be attending a round table meeting on 23 November to discuss key priority areas in this area. He indicated that key issues will relate to productivity, voluntary contributions and risks associated with reducing the matched R&D contribution from Government. No submissions were sought.AQIS Post Entry Quarantine Facilities. R Prince indicated that one facility will be developed for high risk nursery stock. State facilities will still operate pending review of state budgetary commitments.
7. AS 4454 Australian Standard on composts, soil conditioners and mulches. A Kachenko said that the revised standard will be released in January 2012. Currently Standards Australia is awaiting feedback following public consultation on the draft standard. A Kachenko indicated that a recent issues relating to biological contaminates in garden mi x had sparked interest in reopening the Australian Standard for garden mixes and potting mixes to explore threshold biological contaminant levels.

R prince indicated that NGIA was also engaged with the Australian Pesticide and Veterinary Medicines Authority (APVMA) regarding minor use provisions and changes to spray drift.

ACTION: A Kachenko to keep the Environment Committee updated of progress and outcomes relating to inquiries NGI are engaged with.

5.2 Nursery Production FMS 11/12 AOP

A Kachenko tabled the Nursery Production Farm Management System 11/12 AOP for discussion.

He indicated that $25,000 was allocated to facilitate two meetings as per the NIASA Heads of Agreement. The next meeting was agreed to as a fly in – fly out in Sydney on 7 December with the IDO network receiving biosecurity training on 8 December.

A Kachenko indicated that up to $10,000 was allocated to updating and maintaining the NIASA Audit Portal (NAP) this item to accommodate the Greenlife Market Checklist update.

Funds were also allocated to Smart Approved Watermark (SAWM) licensing fee for NIASA and EcoHort ($2,000).

A Kachenko indicated that the National NIASA Advisory Committee (NNAC) agreed to endorsing national conferences, including the NGIA national conference as well as IPPS. Hi indicated that up to $5,000 was allocated to this project.

A Kachenko indicated that the NNAC agreed to spend $2,000 to updating existing marketing collateral and $20,000 towards supporting regional conferences.

The NNAC agreed that $20,000 should be allocated on advertising in national media including Outdoor Design Source, Trade Register, Hortjournal and other avenues as required. A further $26,000 was allocated to developing grower marketing collateral.

General discussion continued about the need to update the Heads of Agreement to reflect government expectations of transparency with administering and facilitating the FMS on-farm. New NNAC members were discussed including David Eaton from SA and the recent resignation of Robert Chin. A Kachenko indicated that the NNAC Chair would be appointed after the NGIA AGM in November 2011.

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General discussion covered the role of the IDO and the need for greater consistency in developing a nationalised job description and mechanism to report the outcomes delivered through extension activities. A Kachenko said that this was timely considering the current IDO project was due for completion in September 2012.

A Kachenko and R prince discussed the Gains Table approach used by John McDonald in NGIQ to determine the benefits arising from IDO related extension activities on-farm. A revised checklist was also discussed including follow up surveys post workshops, timely meeting reports following attendance by IDOs to key meetings and the mandatory use of the NAP. A Kachenko said that NGIA would look at including the Gains Table approach in the project proposal, however indicated that it would need to be updated to reflect national outcomes. The Committee agreed that funds should be spent on updating the Gains Table to support the future project.

General discussion covered the focus of the IDO network in terms of the growers serviced through extension activities. The Committee suggested that greater focus should be directed to the 'early adopters'.

Professional development was also flagged as a key consideration in developing a future IDO project and should be included to ensure the capacity of the network was sustained.

5.3 RIRDC Weed Risk Assessment Project

A Kachenko provided a brief update on this project. This project started in April this year following a workshop with key weed scientists and industry identities to explore how the existing Botanic Gardens Weed Risk Assessment Program (WRAP) could be used by industry.

During the workshop, several changes were proposed and incorporated into the Botanic Gardens WRAP to ensure it met the requirements for the intended use by the Australian NGI. The next step of this project developed a short list of 1,000 common ornamental taxa that could be tested using the updated WRAP. A mix of native and exotic taxa that were currently cultivated in Australian nurseries for sale to the wider public were chosen. A Kachenko indicated that several growers participated in this process to ensure the final list was balanced and equitable. Michael Gleeson, retired NSW nurseryman, is championing this aspect of the project. He will input each of these plants into the WRAP using desktop software. Each plant will be entered 6 times into the software based on climatic boundaries following the Köppen classification scheme. These include: equatorial, tropical, subtropical, desert, grassland and temperate regions. A Kachenko explained that the reason for this, was to factor in climatic differences that can impact on the growth and 'invasiveness' of these plants.

Currently 560 plants have been entered into the system. After each plant is run through the system, a rating of low, medium or high in terms of its 'invasiveness' is allocated to each plant. A Kachenko explained that this information will be incorporated into the NGI National Plant Labelling Guidelines. This information will also be housed on a website to provide industry with a vehicle to correctly and justifiably, label 'safe plants' or those that are low risk, in terms of their invasiveness.

A Kachenko indicated that he was presenting aspects of this research at the 18th Australasian Weeds Conference in Melbourne in October 2012.

5.4 Plant Labelling and Human Health

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	A Kachenko provided an update on a meeting with key industry stakeholders in Melbourne on 14/8/11 to cover this issue. The Committee decided that a universal warning such as CAUTION - This plant might be harmful to some people should be explored for all plant labels. The Committee also suggested that a disclaimer on all labels directing people to a consumer advisory website – www.plantsafe.org.au where all key information, the full national plant labelling guidelines etc. will be housed. This would essentially forgo the tables that appear in the current guidelines with suggested wording in relation to health warnings. A Kachenko indicated that he is seeking legal advise on this through Christine Lowe as Davies Collison Cave lawyers.
	5.5 Urban Forests Research and PLB Phase 2
	A Kachenko provided an update on current research being undertaken by industry in this area. He indicated that two levy funded projects had just commenced. • NY11002: Understanding the carbon and pollution mitigation potential of Australian urban forest - USYD and MACQUARIE Universities ($139,994 over 2 years 11/12 and 12/13). • NY11013: Greening City – Mitigate Heat Stress with Urban Vegetation - CSIRO ($276,000 over 2 years 11/12 and 12/13). Both projects will build into the 2012 marketing campaign 'More Trees Please' and focus on the health and well-being aspects of urban Forests. The Committee suggested that regular communications to industry must commence alerting industry to this pivotal research. R Prince and A Kachenko discussed industry investment in iTree and indicated that it was a Gold Partner in championing iTree Australia in collaboration with Arboriculture Australia as well as several local councils across Australia (e.g. Sydney City and Melbourne City). A Kachenko indicated that this investment would Australianise the current American iTree tool and develop a series of road shows to councils, horticulturalists and arborists across Australia. A Kachenko also indicated that iTree was submitted to the Domestic Offsets Integrity Committee (DOIC) for assessment under the Carbon Farming Initiative as the method to quantify carbon under urban reforestation and revegetation strategies. A Kachenko also indicated that he was liaising with Smart Approved WaterMark to submit questions that they can incorporate into their annual NewsPoll survey. This would save costs in running a unique NewsPoll survey for industry in isolation. **ACTION:** A Kachenko to develop a suite of communications as part of More Trees Please in relation to these projects and convey these to industry.
6	**OPERATIONAL ISSUES**
	6.1 Review of R&D 11/12 AOP
	A Kachenko tabled a summary of the current 11/12 levy funded R&D projects being undertaken by NGIA and presented the 11/12 AOP. He indicated that the project was contracted in August 2011 for completion by August 2012. Projects that were tabled and discussed included: • **IDO Regional Representation.** A Kachenko indicated that there had been minimal update of these funds and suggested that this project would be merged into the new IDO project.

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- **Biosecurity Commitments.** A Kachenko indicated that this project is delivered in conjunction with Project 1 above and recognises the added responsibility that the QLD Industry Development Manager has regarding national biosecurity including Industry's commitment to the Emergency Plant Pest Response Deed. A Kachenko indicated that the project was running on track.
- **Industry Research Linkages – Support for Honours Research Project.** A Kachenko indicated this project was running on track with student projects including a study on community gardening, a study to assess canopy covers and a stipend to support a three year PhD program.
- **Progressing BioSecure *HACCP.*** A Kachenko said that this project funding must be assigned to the development of both the administration and governance protocols that will underpin BioSecure *HACCP* for it to meet regulatory compliance and competence including updates to the BioSecure *HACCP* manual and electronic system management. A Kachenko and J McDonald have met on several occasions to discuss the framework to his project. He indicated that the bulk of the project would be completed in early 2012.
- **NIASA Cost Benefit Analysis.** A Kachenko said that this project study into the econometrics of the program would be finalised after the December 7 NNAC meeting.
- **Nutrient Budgeting Calculators.** A Kachenko indicated that this project was underway through the NCEA for completion in early 2012. It would be integrated into the existing Water Management Toolbox that is freely available through the NGIA website.
- **Access to Minor Use Permits.** A Kachenko indicated that a short list of products for submission to APVMA was underway and permit application would be assessed in early 2012.
- **NGIA Environment Committee.** A Kachenko indicated that expenses including the development of resources such as the Biosecurity Policy Position (Agenda item 6.2) fell under this project. This project would also fund two workshops in early 2012 with growers to gain feedback on the development of an Australian Standard for Tree Specification.

6.2 Biosecurity Policy Update

A Kachenko tabled a detailed draft of the industry Biosecurity Policy Position. He indicated that whole of industry had been consulted and feedback incorporated into the tabled version. A Kachenko indicated that the cover page would be the executive summary and could be used individually when attending meetings etc. He indicated that the next step was the layout through a professional graphic designer before printing in the first quarter of 2012.

6.3 Progress of Levy

Fenton provided an update on the levy and the feedback from the recent industry consultation meeting in Sydney. He said that media in 20 litre bags or above would become leviable as well as bulk growing media. The potting mix only would be leviable – i.e. not fertiliser inputs etc. The ration between pot and media was yet to be determined. The next step would invite media manufacturers together to discuss the proposal and run through collection potential. General discussion followed covering issues relating to time lines and agreed to keep this item on the agenda for future meetings.

ITEM	TOPIC
7	**RESEARCH PROPOSALS AND OPPORTUNITIES**
	7.1 Environmental Risk Matrix
	A Kachenko tabled the Environmental Risk Matrix. It is based on the risks associated with a range of issues that impact on the profitability of Australian nursery and garden industry. The Committee discussed what areas needed updating to reflect the industry position in 2011 heading into 2012. **ACTION:** A Kachenko to update the Environmental Risk Matrix for the next Environment Committee meeting in 2012
	7.2 Future R&D Proposals to Review 12/13
	A Kachenko delivered a PowerPoint Presentation that detailed the process in how projects proposals were developed. The process included: • Detailed timeline circulated to IDO network in December 2010 and June 2011 • Guidance with proposal drafting circulated in December 2009, 2010 and June 2011 • 16 projects submitted to NGIA from NGINA and NGIQ • 8 Submitted by NGIA • Down 43% on 2010 • All projects evaluated by NGIA and sent to IDO network for ranking on 6 September 2011 • Projects ranked by IDO network based on 4 criteria (urgency, importance, impact and success) and rankings collated by NGIA He presented a table that detailed the top 14 projects that included: • Industry research linkage – support for university research projects • Industry Minor Use Program • Optimising the selection of irrigation sprinklers for overhead sprinkler systems based on independent testing • National Myrtle Rust Industry Liaison Officer (NMRILO) • The Effects of Nursery Production Methods on the Future Success of Landscape Trees • Defining a set of greenlife standards for the nursery industry • The development of a healthy root system of trees by adoption of optimum cultural practices • Innovation in extension using digital media • Web Based Pest & Disease Management Tool • Development of a commercially applied diagnostic test to predict the susceptibility of Myrtle Rust germplasm leading to selection of resistant plants • Energy BMP for Production Nurseries • Pesticide Spray Diary and BMP Online

ITEM	TOPIC
	General discussion followed on these projects. Discussion covered the possibility of a biosecurity contingency fund and overseas study trips to develop international strategic R&D alliances and shared knowledge. A Kachenko indicated that after submitting the proposals to HAL on 28 November 2011, IAC would endorse each proposal based on their individual merit and linkage to the industry Strategic Plan as well as the outcomes of the Industry Strategic Investment Plan. The Committee asked if A Kachenko could develop a database of projects that were not successful for consideration in future funding opportunities either through levy or external funding means. A Kachenko also presented results from a recent survey in the US that evaluated the state of the industry. The Committee agreed that a similar survey for the Australian industry should be commissioned in 2012. **ACTION:** A Kachenko to maintain a database of all projects submitted to NGIA for future consideration trough levy or external funding means. **ACTION:** A Kachenko to investigate a 'State of the Industry' survey for this Australian nursery industry using Survey Monkey in 2012.
	7.3 Industry Strategic Investment Planning
	R Prince gave an overview of this process and outlined the requirement by industry to translate the Industry's current Strategic Plan 2010-2015 into a Strategic Investment Plan as a prerequisite for industry research and marketing investment over the coming years. He indicated that a two day workshop on 29-30 November will be held to consult with key Industry levy payers on investment priorities for Marketing and R&D levies for the next 5 years He indicated that Associate Professor Richard Prince will facilitate the consultation process. A draft Strategic Investment Plan to take into account the outputs of the meeting will be developed and is anticipated to include some economic analysis where existing analyses can be drawn upon. R Prince indicated that the plan will be made available for wider levy-payer feedback and will be finalise at the February 2012 Industry Advisory Committee meeting.
8	**GENERAL BUSINESS**
	8.1 Future Meeting Format/Committee Structure
	All Committee members indicated that they were keen to continue in 2012. No further items were identified. G Fenton thanked everyone for their participation in 2011 and pending outcomes of the NGIA AGM, indicated that he looked forward to working with the Committee in 2012.
9	**NEXT MEETING - PROPOSED JUNE 28 2012**
	MEETING CLOSED 2:30 PM

MINUTES

Nursery & Garden Industry Australia

Environment Committee Meeting

Date:	Thursday 28 June 2012
Time:	9.30 am – 2.30 pm
Location:	NGIA Office, Unit 58, Quantum Corporate Park, 5 Gladstone Road, Castle Hill 2153
Attendees	Simon Smith (Chair), John Bunker, Steve Burdette, Robert Prince, Anthony Kachenko
Apologies	Sean O'Brien

ITEM	TOPIC
1	WELCOME AND APOLOGIES

S Smith formally declared the Environment Committee Meeting open at 9:30 am and extended a warm welcome to Committee Members. He provided a brief overview of The Plant Smith and detailed his involvement with industry both in Northern Territory and nationally.

A Kachenko advised the committee that S O'Brien had announced his retirement from the committee due to time pressures with work. The Committee expressed their thanks to S O'Brien for his input and requested A Kachenko to send a letter of sincere appreciation and thanks on behalf of the Committee.

R Prince advised A Kachenko to contact regional offices calling for nominations. S Smith indicated that D Mansfield would be a suitable replacement based on his background and interest in technical and environment and industry issues.

ACTION: A Kachenko to send S'O Brien a letter of thanks for his involvement on the Environment Committee.

ACTION: A Kachenko to notify the state Executive Officers of the current vacancy and seek nominations for a suitable replacement.

2	CONFIRMATION OF MINUTES – 4 NOVEMBER 2011

S Smith advised that the Minutes from the Meeting held on 4 November 2011 were provided in the meeting papers and the Committee were asked to raise any concerns regarding the accuracy of these minutes. No further changes were noted. The minutes were accepted as a true and accurate record.

3	MATTERS ARISING FROM LAST MEETING

3.1 Review of Action List

ITEM	TOPIC

A Kachenko tabled an Action List arising from the last Meeting. He provided an update on the completed items. The following actions were outstanding:

- A Kachenko to provide an update on funding opportunities through the mining industry and report back to the Committee at the next Environment Committee Meeting

- A Kachenko to investigate a 'State of the Industry' survey for this Australian nursery industry using Survey Monkey in 2012.

A Kachenko indicated that he would look at these two actions in the coming six months. With regards to the first action, he noted that it would be important to make contact with the right organisations/associations to identify key people and resources to discuss research, development and extension opportunities to value add industry programs and benefit whole of industry. One opportunity may relate to the utilisation of i-TREE to determine the environmental value of fly-in–fly-out mining communities. J Bunker advised that he would forward A Kachenko contact information from Queensland to pursue. Discussion continued on business development opportunities for the industry from a strategic positioning perspective. It was discussed that a new role surrounding market development opportunities for industry would be looked at based on needs identified through the Industry Advisory Committee (IAC). The NGIA Board also identified the need for resource support for A Kachenko to assist technical communications and policy related matters.

A Kachenko also discussed the 'State of the Industry' survey. He indicated that the survey will take a back seat as there was a priority to collect market data through the industry market research project. The project would be revisited once the Industry Market Research project got underway.

ACTION: A Kachenko to forward a copy of the State of the Industry survey to Committee members.

4 MATTERS ARISING (NOT ADDRESSED IN THIS AGENDA)

Discussion continued on future investment focus for industry levy investment. The Committee indicated that research focus should be more strategic and develop projects that address market growth opportunities for whole of industry. Best practice research should focus only on issues where there is a clear market failure with whole of industry benefit.

A Kachenko indicated that the Industry Biosecurity Plan was currently undergoing review through Plant Health Australia (PHA) and will be released in late 2012 after industry review. He noted that preliminary changes in the Industry Biosecurity Plan were forwarded to PHA in January 2012 based off consultation with the Industry Development Officer (IDO) network following the December National NIASA Advisory Committee meeting.

R Prince indicated that industry will be engaged in driving the biosecurity levy over the course of the next six months that will look at levy options including an additional levy on top of the current 5% Pot Levy. He noted that the funds may cover PHA membership whilst enabling a contingency of funds for future incursion management. Further discussions with PHA and Levies Revenue Services will be required in driving this forward.

5 NATIONAL ENVIRONMENTAL PROJECT UPDATE

5.1 Summary and Status of Current Government Inquiries

ITEM	TOPIC

A Kachenko advised the committee that Department of Agriculture Fisheries and Forestry (DAFF) Biosecurity had established new fees and charges for plant export. The information had been circulated by DAFF Biosecurity to key stakeholders including all exporters.

The new fees and charges included $6.5 million dollars transitional funding over the next 24 months to subsidise the cost of export operations. A Kachenko advised that the key impact of these changes was the high registered establishment charge that would preclude small businesses from exporting. To offset this increase, electronic lodgement of export certification fees and charges were reduced as were the on-site manual inspection fees and charges. Manual certification fees and charges will rise to encourage businesses to take up electronic certification. J Bunker asked A Kachenko to review the Coalition's position on DAFF Biosecurity fees and charges should there be a change of government.

A Kachenko updated the Committee on the Draft consultation regulation impact statement for the proposed risk treatment measures for precursor chemicals to have homemade explosives. A Kachenko indicated that he had made a submission to the Consultation Regulation Impact Statement 'Chemical Security – Precursors to homemade explosives' (March 2012). The submission outlined areas that should be considered more closely that would impact on the ability of nursery businesses to readily access key fertilisers for everyday use. He noted that the submission was available on www.ngia.com.au

R Prince indicated that DAFF Biosecurity was investigating the development of a protocol that would enable the import of *Phytophthora ramorum* budwood into Australia from countries where *Phytophthora ramorum* is endemic. R Prince advised that this approach did not have full support of industry and made a submission to DAFF Biosecurity to that effect. On a separate note, R Prince also indicated that DAFF Biosecurity were currently in the process of reforming and updating the national Biosecurity legislation/act and noted that NGIA will be making a submission. He had asked regional associations for input relating to key issues to consider in the industry submission.

ACTION: A Kachenko to keep the Environment Committee updated of progress and outcomes relating to inquiries NGI are engaged with.

ACTION: A Kachenko to table Agricultural and Biosecurity policy positions for major political parties at subsequent meetings.

5.2 RIRDC Weed Risk Assessment project

A Kachenko advised that the projects final report had been submitted to the Rural Industries Research Development Corporation (RIRDC) and accepted. A Kachenko indicated that the next phase of this project relates to the extension of the projects data. This will involve a number of communications including:

- In corporation of the weed risk assessment in a future update of the National Plant Labelling Guidelines.
- Development of a consumer website detailing the assessment results of completed weed risk assessments linked to Grow Me Instead.
- Technical communications detailing the above.

He indicated that these activities are expected to be completed over the next 12 months.

ITEM	TOPIC
	5.3 Urban Forest Research/More Trees Please Articles
	A Kachenko tabled a number of articles published in green industry magazines that detailed industry levy funded research outcomes with links to the More Trees Please campaign. He noted that these articles were useful to broaden the industry reputation and create a market for greenlife. New articles will be developed for the remaining nine months.
	5.4 Carbon Farming Initiative and National Urban Forest Alliance
	A Kachenko noted that the joint submission with Arboriculture Australia to the Domestic Offsets Integrity Committee (DOIC) for assessment under the Carbon Farming Initiative was unsuccessful. The proposal was based on the methodology for urban reforestation and revegetation and used i-Tree ECO for measurements. A Kachenko indicated that he will be working with Arboriculture Australia to make a resubmission. He also indicated that he would have to meet with key advisors and bureaucrats in Canberra to discuss the proposal further prior to the resubmission. This would include the development of case studies highlighting the benefits of i-Tree ECO such as the data industry collected at the Melbourne International Flower and Garden Show during march 2012. In relation to i-Tree ECO, A Kachenko noted that workshops to train users on i-Tree Eco will occur from August onwards. He noted that although the data behind the program had been collected for all states/territories in Australia, the United States Department of Agriculture had not yet included the data online. The next version of i-Tree ECO for release should include all Australian data and will be available in the next three months. Further to the market development initiatives undertaken through 'Plant Life Balance' and i-TREE Eco, A Kachenko indicated that he had also been working with Arboriculture Australia in driving the formation of the National Urban Forest Alliance (NUFA). A Kachenko indicated that the current Chair of the Alliance is Craig Hallam from ENSPEC and he is the Vice Chair. The Terms of Reference was tabled with the meeting papers. The Alliance involves a number of key stakeholder groups to promote a thriving sustainable and diverse Australian urban forest that is valued and cared for by all Australians as an essential environmental, economic and community asset. He indicated that former students who had utilised levy research investment were now prominent players and involved with NUFA. He suggested that the university scholarship research program required more formalised arrangements to ensure students were aware of the opportunities from working with industry. General discussion followed in relation to planning laws and the lack of opportunity for nursery businesses gaining credits for maintaining existing remnant vegetation on private land and new plantings on private land. It was noted that Kyoto does not recognise either of these areas for carbon credits. It was noted that State Governments have gained credits for this land as it hasn't been cleared thus contributing to Australian Kyoto requirements/obligations. It was also noted that despite the issue that we grow plants that capture and store carbon, there is difficulty in gaining credits at a farm gate as there is no guarantee that the plants will survive a minimum of 100 years required to account for the stored carbon. J Bunker discussed the outcomes of undertaking a Nursery Footprint carbon footprint of his business. He noted that the use of green waste (methane emission), energy consumption, plastics and fertiliser usage were identified as problem areas for carbon emissions.

ITEM	TOPIC
	5.5 Nursery Production Farm Management System Update/Issues
	A Kachenko updated the committee on the next National NIASA Advisory Committee meeting scheduled on 10-11 July 2012. He indicated that the Heads of Agreement and Terms and Conditions governing the FMS program will be reviewed during this meeting to pave the way for government acceptance of the FMS as a legitimate market access mechanism. He noted that a budget of $125,000 had been assigned to developing the FMS program in 12/13 and indicated that this would be the last year where such extensive funds would be allocated. He noted that am Annual operating Plan for 12/13 would be devised at the next meeting. He noted that Colin Groom would be the new chair of the meeting.
6	**OPERATIONAL ISSUES**
	6.1 Review of R&D 12/13 AOP
	A Kachenko tabled a summary of the proposed 12/13 Annual Operating Plan for project NY12001 – Nursery Industry Environmental and Technical Research and Extension 2012-2013. He indicated that the project valued at $280,000 will be contracted in August 2012 for completion by August 2013. Projects that were tabled and discussed included: • **IDO Regional Representation.** A Kachenko indicated that there remained minimal engagement with these funds during NY11000 with funding reduced in the following calendar year. • **NGIA Environment Committee.** A Kachenko indicated that expenses including the development of resources such as the National Plant Labelling Guidelines (Agenda item 6.2) fell under this project. This project would also fund resources relating to the development of an Australian Standard for Tree Specification. • **Biosecurity Commitments.** A Kachenko indicated that this project is delivered in conjunction with the project IDO Regional Representation and recognises the added responsibility that the QLD Industry Development Manager has regarding national biosecurity including Industry's commitment to the Emergency Plant Pest Response Deed. A Kachenko indicated that the project was running on track. • **Industry Research Linkages – Support for Honours Research Project.** A Kachenko indicated this project would continue and a formalised process in terms of applying for funding will be developed as noted previously during the meeting. • **Development of visual extension resources for NGI.** A Kachenko indicated that this sub-project will trial an array of short videos using existing footage where possible (e.g. footage shot during the Nursery Production FMS filming in project NY09017) to generate short 'how to' clips on key industry issues. He noted that preliminary discussions with PHA have highlighted that there is opportunity collaborate resources in developing on-farm biosecurity related media clips. This would include how to inspect plants prior to dispatch, surveillance and intake procedures for examining new plant stock. • **Literature review of efficacy of organic amendments used in plant production.** A Kachenko indicated that this sub-project will investigate the current international availability and efficacy of organic amendments used in plant production. The project will identify the relevant 'actives' and documented research regarding their application in plant propagation, production a management. Scientific Research that relates to claims such as how these products can increase microbial biomass, increase drought tolerance, result in healthier pest and disease free crops, enhance flowing etc. will be summarised.

ITEM	TOPIC

- **Online pesticide spray diary and best management practices toolbox.** A Kachenko indicated that this project will update and transfer the content of the Best Practice Manual for Pesticide Application in the Nursery and Garden Industry CD to a web resource for wider penetration with the production industry and possible application in the field using 'Smart Phones', iPads and Android devices. It will also include an update of key reference material and will be available online through www.ngia.com.au and will link to external websites that offer information on weather conditions (e.g. Syngenta's Agricast, Elders weather and Bureau of Meteorology). The toolbox will also provide a repository of all the industry minor use permits and provide direct linkages to the Australian Pesticides and Veterinary Medicines Authority website where the permit information is housed.
- **Web based pest and disease management tool.** A Kachenko indicated that this sub-project will invest to take the current electronic tool and install it as a web based information source accessible to industry via the internet. The concept will allow quick and easy access to current pest, disease, beneficial, emergency plant pests and weed identification. The resource will provide a platform from which updates with new images/pests can be provided and made available to industry in a cost effective and real time manner.
- Minor use pesticide program for NGI. A Kachenko advised that this project provides funding to enable the registration of six MUP with the APVMA. Products selected for this project will be sourced from suggestions forwarded to the National Environmental and Technical Policy Manager from the IDO network. This project continues the direction of previous Environmental and Technical Policy projects.

6.2 Review National Plant Labelling Guidelines

A Kachenko tabled an annotated copy of the voluntary National Plant Labelling Guidelines for discussion. He explained that they were last updated in 2007 and warranted review. It was also noted that the outcomes from the RIRDC project required inclusion in the guidelines under funding requirements as did the need to update the section on intellectual property following some comments from key growers suggesting the guidelines were obscure.

A Kachenko indicated the most significant change relates to the impact of plants on human health. Legal advice obtained indicated that a three tier approach was required. Tier one was non-toxic plants requiring no warning. Tier two indicated that potentially harmful plants (unknown, unidentified or inconclusive profiles) required a warning and Tier three was for harmful plants. Suggestion was to follow a list from a reputable source such as the NSW Department of Primary Industry list, however it was suggested that this would not be advisable due to different regulations across the country and the need for a national list.

Discussion continued on these areas and the following points were suggested:

- Guidelines should be reviewed every three years.
- Maintain a two tier approach with potentially harmful/harmful grouped to remain as identified at the moment. Include references to support the inclusion on the list.
- For the warning label relating to plants that may be harmful, the suggested wording should include further information can be obtained from your point of sales representative.

ITEM	TOPIC
	• Develop a website relating to plant safely to use as a resource to educate the wider public about potentially poisonous plants without evoking fear. This suggestion builds on from a recommendation from a meeting A Kachenko attended in Victoria where it was noted that an educational website would be a useful tool to support industry's proactive position on this issue. It was noted that the website would not be a priority until after the development of the website. • It was noted that the labelling guidelines can be used on all resources, not only labels and should include websites, catalogues etc. • Maintain the guideline as voluntary and not mandatory. • Provide some example of labels. • Provide a copy of the guidelines to all members upon completion.
	6.3 Australian Standard : Specifying Trees Proposal
	A Kachenko advised that on 1 March 2012 he submitted a proposal titled 'Specifying Trees' to Standards Australia outlining the need for an Australian Standard to address stakeholder concerns regarding the failure of newly planted trees to grow and establish in the landscape as a result of poor quality stock. The proposal was submitted to Standards Australia following support from key industry and external stakeholders. The proposal has been accepted to pursue to the development phase from 1 July 2012. A Kachenko advised that he would develop an industry steering committee to drive the process forward to ensure industry is adequately consulted. He also advised that communications relating to the development of the Standard will be circulated in the coming few weeks for consultation purposes. General discussion continued regarding how NATSPEC: Specifying Trees and the connection to the current standard will link as well as how Standards are enforced. It was also discussed that the language of the proposed standard must be clear in defining what 'shall' be required as opposed to what 'should' be required. It was discussed that a future project should look at the Standards for ground preparation of soils, planting and maintenance of trees to compliment the Specifying trees Standards, to ensure that all steps in the process are covered.
7	**RESEARCH PROPOSALS AND OPPORTUNITIES**
	7.1 Environmental Risk Matrix
	A Kachenko tabled the Environmental Risk Matrix. He explained that it is based on the risks associated with a range of issues that impact on the profitability of Australian nursery and garden industry. The Committee discussed what areas needed updating to reflect the industry position in 2012. Changes included the addition of the carbon economy and shifting the possibility of new incursions to red indicating that it has high potential to have serious impact on the profitability of the NGI throughout Australia. General discussion followed on lack of students embarking on Agriculture/Horticulture as a career path. ***ACTION: A Kachenko to update the Environmental Risk Matrix for the next Environment Committee meeting in 2012.***

ITEM	TOPIC
	7.2 Future R&D Proposals to Review 12/13
	A Kachenko tabled a copy of all the projects that had been submitted to date that did not make it to lodgement via the Industry Advisory Committee. He noted that these would be recirculated to the IDO network to consider prior to new projects being developed to avoid duplication. A Kachenko also advised that he had updated the research, development and extension timeline and background document in terms of what was required in developing proposals. This had also been circulated to the network to review. J Bunker discussed minimising waste streams was a key issue for him and other growers around the country. General discussion followed on the lack of data and understanding of waste streams across the industry aside from recycling of pots. Discussion on waste audits to identify opportunities under the carbon economy was warranted for consideration as a research proposal going forward. This would survey quantity and cost for throw outs, shrink wrap, pallets, cardboard, shade-cloth and should include logistics. ***Action: A Kachenko to draft a project proposal on survey of waste streams in nursery production based on discussion detailed above.***
	7.3 Draft Industry Strategic Plan
	A Kachenko tabled the draft Industry Strategic Investment Plan that has been submitted to Horticulture Australia Limited as a prerequisite for industry research and marketing investment over the coming years. Outstanding is the economic analysis which quantifies the returns on the proposed investment priorities. A Kachenko indicated that the priority areas for future investment would be Market Growth and Communications which link to Objectives 1 and 3 of the Nursery and Garden Industry Strategic Plan 2010-2015. He also noted that there would be less investment directed to on-farm best practice research, with greater emphasis on research aimed at developing the market. Less investment would also be directed to the marketing of the Nursery Production Farm Management System.
8	**GENERAL BUSINESS**
	8.1 Future Meeting Format/Committee Structure
	As discussed earlier, A Kachenko advised that he would contact the state Associations to seek nominations to fill the vacancy of S O'Brien. General discussion followed with regards to the costs and time associated with hosting meetings at Castle Hill verses the Airport. A Kachenko advised that he would review all costs and determine the most economical and practical location. Discussion also covered the use of Skype and it was agreed that it would also be a useful teleconferencing tool to consider. A test run would be warranted to decide if it would be feasible for the next meeting. ***Action: A Kachenko to investigate meeting venues for next meeting.*** ***Action: A Kachenko to forward details on downloading, installing and accessing Skype for the next meeting.***
9	**NEXT MEETING - PROPOSED 8 NOVEMBER 2012**
	MEETING CLOSED 2:30 PM

Appendix 2

ENVIRONMENTAL RISK ASSESSMENT FOR NGI

KEY

- Has High Potential to have serious impact on the profitability of the NGI throughout Australia.
- Issue needs to be monitored as it could move to Red classification.
- Issue is being controlled by NGI activities but requires ongoing diligence.

Aspect	Sector	Impact	Risk	NGI actions / proposals
PESTS & DISEASES / BIOSECURITY	**Industry**	New Incursions		Involvement with PHA - EPPRD
		Barriers to plant movement		NIASA/BioSecure HACCP promotion for market access
		Control methods		IPM - Training workshops
		Product choices		Need for access to new products/minor use permits Access to new chemistry
		Identification and awareness		Need for crop monitoring training Adoption of BioSecure HACCP IPM workshops
	Community	Control methods		Supplier promotion Development of "Greener Options" Nursery Production FMS
		Pest & Disease identification		Lack of resources in jursidictions Need for clear communication strategy
		Legionella - potting mix		Ongoing education re "safe use" Crisis Managemnt Planning
CLIMATE CHANGE & VARIABILITY	**Industry**	Adaption - Weather impacts- temp, rainfall		Need for regional analysis of impacts Best Management Practices/Accreditation Programs Better predict climate events Energy efficiency calculators Managing possible incursions
		Mitigation - Intensive horticultural production		NurseryFootprint and Energy Efficiency Calculators
		Transport component in distribution		Quantify emissions Cost pressures
	Community	Green beneficial impact on environment		Quantify benefits associated with green life
		Communications		Need for education of customers re benefits of green life Need for communications for growers

INVASIVE PLANTS / BIOSECURITY	**Industry**	Definition of an invasive plant		Review NG IInvasive Plants policy Industry recognised Weed Risk Assessment Need for single lists with Industry input/evidence based Adoption of labelling guidelines
		Barriers to plant movement		Focus of state organisations Difficulty in identifying key decision makers
		Testing for Invasiveness		Industry Recognised Weed Risk Assessment
		Plant disposal		Guidelines to trade Communication with stakeholders
	Community	Labelling of plants		Clear identification of invasiveness/Adoption of guidelines
		Community education		Furthering education - Grow Me Instead Weed awareness/management
		Opportunity to RetroFit gardens		Promotion of alternative plants Education on plant disposal
				Furthering education - Grow Me Instead
WATER	**Industry**	Restrictions to supply		Update NGI Policy Murry Darling Basin proposal Lobbying State based water authorities Nursery Production FMS and existing tools/resources
		Contamination		Nitrate/Phosphate run off minimisation Pesticide levels in run off minimisation Pathogen contamination Training programs - WaterWorks and NIASA Accreditation
		Use of recycled water		Supply issues-guarantee of supply, source and cost Treatment performance Health impacts Quality of treated water
		Irrigation/application rate		Upgrade to new technologies Lack of relevant crop data Best management practices - hand watering guidelines/Accreditation
	Community	Community education		Need for water to enjoy product Authorities targeting consumers Water efficiencies and savings
WASTE	**Industry**	Management of waste streams		Need for information on contamination treatment Accreditation programs
		Recycled organics		Need for alignment of industry with organic industry Quantify waste streams on production sites
		Plastic containers		Development of recycled programs Evaluation of alternative containers
	Community	Plastic containers		Develope consumer options for recycling AGCAS

Version August 2012

Appendix 3

Reducing the Pest Risk

The Australian Nursery & Garden Industry's Policy Position on Quarantine and Biosecurity

The Australian nursery and garden industry (NGI) is reliant upon robust, cost effective, efficient and reliable quarantine resources. This is due to the high volumes of plant material valued in the millions of dollars, which are imported, exported and shipped across all Australian jurisdictions on a daily basis.

The industry is a significant user of post entry quarantine (PEQ) and 'approved facilities' to import new germplasm in various forms, including tissue culture, vegetative cuttings, seed and whole plants. This supports a diverse range of crops in the food, fibre and foliage industries valued at more than fifteen billion dollars nationally.

Although the industry has traditionally had a small export focus, the richness of Australian Flora offers ample opportunity for export growth. To ensure the longevity of the industry and the protection of the Australian environment, plant industries and the wider community, it is essential pest risks are identified, prepared for and effectively managed. These biosecurity responsibilities must be shared equally between governments, industry and the community. Accordingly, the NGI has a lead role to play in the biosecurity continuum through information dissemination, grower education, on-farm management and risk reduction strategies.

The industry is strongly committed to effectively reducing the potential for incursions of exotic plant pests (EPPs) that could adversely impact domestic and international trade, regional and national economies and the Australian environment. It is committed to ensuring responses to any EPP incursions are undertaken as efficiently and effectively as possible to minimise the cost to growers, the industry, other plant industries, government and the wider community. To support these objectives, the Australian NGI requires a robust, resourced, practical and

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risk assessed biosecurity system (pre-border/border/post border), which is supported by sound science that accepts a unified appropriate level of protection (ALOP) setting a low level of risk.

Australia's biosecurity system must be transparent and retain the confidence of all stakeholders in its ability to deliver an objective outcome. The industry will work with all biosecurity agencies to add value and contribute to ongoing developments right along the biosecurity continuum.

The NGI fully supports the implementation of the National Plant Biosecurity Strategy (NPBS), which points the way for governments, plant industries and the community to work closely together to further develop Australia's plant biosecurity system.

There are significant benefits in strengthening the Australian biosecurity and quarantine system, including reduced financial and environmental imports from EPP incursions. Responding to the challenges currently facing the industry, six strategies have been formulated:

1 Leadership in policy development and investment in the area of quarantine and biosecurity – this recognises the impacts of policy decisions and investment on businesses and their customers.

2 Harmonised delivery of quarantine and biosecurity arrangements – establish a National Pest Risk Assessment Framework which delivers a world class biosecurity and quarantine system to whole of industry.

3 Investment in on-farm support to address quarantine and biosecurity – the realignment of investment and a commitment by governments to support on-farm practices, innovation and incentives to adapt, manage and respond to biosecurity and quarantine.

4 Recognition of established industry best management practice – this recognises and supports the Nursery Production Farm Management System (NPFMS) as a third market access instrument for the industry and investment in research, development and extension activities.

5 Implementation of a national greenlife producer communication and information scheme – this is designed to secure the reputation of the Australian NGI through knowledge based decision making.

6 Build greater stakeholder engagement and involvement to deliver a national communication network – this will assist in building industry confidence.

Issues facing the Australian nursery and garden industry

One of the greatest threats facing the Australian environment is the introduction of EPPs. To date Australia has remained relatively free from many pests due to its geographic isolation and a biosecurity system that has limited the introduction of high risk materials. This is changing however due to ease of travel and the freeing up of world markets.

To ensure Australia remains relatively pest free, a rigorous scientifically sound biosecurity system is required. The key elements of this include a combination of pre-border, border and post border management of pest threats. The program needs to clearly articulate the importance of maintaining Australia's plant health status and explicitly state that biosecurity is a 'whole of community' responsibility involving state and federal governments, industry and the wider public.

The Australian NGI acknowledges it plays a vital role in this biosecurity continuum and is actively engaged in several biosecurity initiatives across Australia. These include on-going investment in research, development and extension initiatives, including on-farm programs driving change from the bottom up.

Nursery & Garden Industry Australia (NGIA) is also a member of Plant Health Australia (PHA), which has demonstrated its willingness to participate and contribute in this arena. Accordingly, it is pertinent that its contribution in the biosecurity continuum is duly acknowledged and all parties maintain their responsibilities in this shared approach.

The National Nursery and Garden Industry Biosecurity Plan developed in 2005 provides a blueprint for the exclusion, eradication and control of key pests relevant to the Australian NGI. As a living document, the plan is reviewed every five years to embrace changes to industry biosecurity needs.

This plan is vital to the industry as it has the capacity to minimise pest risks and respond effectively to any pest threats. It also ensures the future sustainability and viability of the NGI is maintained.

As part of the National Nursery & Garden Industry Biosecurity Plan, NGIA has developed contingency plans for key threatening pests. These provide background information on the pest biology and available control measures to assist with preparedness in the event of an incursion. Each contingency plan provides guidelines to assist in developing a response plan to the specific pest incursion. It is vital this information is embraced and considered should there be an incursion.

In 2005, NGIA became a signatory to the Emergency Plant Pest Response Deed (EPPRD). The EPPRD is a progressive

partnership arrangement between governments and industries that sees them cooperating as equal parties in the management of EPPs. As a signatory, NGIA is at the forefront of developments in biosecurity complementing its historical investment in biosecurity related research, development and extension activities.

In recent times, there has been a consistent lack of prioritisation by all levels of government to the threats and costs associated with EPP incursions facing the industry and the wider Australian community. Nursery production has borne the brunt of almost every EPP incursion and this has cost millions of dollars in crop losses, mitigation programs, compliance protocols and restricted or closed market access. Despite this, NGIA remains committed to the EPPRD.

Over the past 15 years, the Australian NGI has dealt with a range of EPPs, with some eradicated, others under management plans and the remainder recognised as established pests and treated as a normal plant pest within the production system (controlled). Historically, the industry carries a major burden, both financially and operationally, when Australia has pre-border, border and post-border failures in excluding the incursion of EPPS.

In relation to the export of horticultural commodities, it has also been observed that biosecurity and quarantine agencies, are making the process cumbersome, difficult and costly. From January to December 2010, total plant exports amassed $18.28 million*, a figure which has been in steady decline over the past six years. To reverse this trend, production nurseries exporting plant material must be adequately supported to enable development and growth in the global market.

This export growth will require world-class biosecurity and quarantine agencies supporting and assisting Australian plant producers develop international market access.

Similar observations have been made about the importation of plant products. Over the years, the industry has seen inconsistencies in both the interpretation of inspection procedures and protocols as well as outcomes following post border assessments. This has resulted in significant delays in moving perishable plant products and in some cases, the loss of whole consignments.

Compounding this is the uncertainty surrounding the future operations of PEQ facilities. The industry supports the need for PEQ facilities in Queensland, New South Wales, Victoria and Western Australia, with each facility aligned to the Department of Agriculture, Fisheries and Forestry (DAFF) Biosecurity as either a DAFF Biosecurity managed facility, or managed by a state or territory government contracted as a DAFF Biosecurity service provider. The industry further supports 'approved facilities' for private providers excluding material designated as high risk.

Historically, the Australian NGI has had a long and close relationship with biosecurity and quarantine agencies across Australia, particularly in relation to the interstate movement of plant material. Despite this, the industry has identified support components that will be required so it can continue to maintain its role in the biosecurity continuum.

The Australian NGI has the capacity to play a key role in proactively and responsibly maintaining Australia's 'pest free' reputation. In doing so, it will also ensure a sustainable future for the industry itself.

*Source: Australian Bureau of Statistics, Horticulture Australia Limited analysis (2011)

Six strategic responses

1 Leadership in policy development and investment in the area of quarantine and biosecurity – this recognises the impacts of policy decisions and investment on businesses and their customers.

Policy development by state, territory and federal governments has significant implications for the Australian NGI. Rapid, poorly designed and orchestrated policy development has greater impacts on the industry than those governments currently associated with the delivery of quarantine and biosecurity arrangements across Australia. Therefore, the opportunity to provide input into strategies and decisions made by commonwealth, state and territory quarantine and biosecurity agencies is urgently required.

The Australian NGI expects to be consulted and given adequate time to respond to issues regarding current and future changes to plant health arrangements. This is to ensure the industry has a real opportunity to contribute meaningfully in these discussions and take ownership of decisions made.

At present, there is a distinct lack of industry confidence and assurance in quarantine and biosecurity agencies, due to numerous reasons related to process, general protocol interpretation, resource allocation and minimal consultation with industry on matters with financial ramifications on business sustainability. These include fees for service, red tape, market access and cost reviews.

Currently, several issues mar the delivery of quarantine and biosecurity arrangements in Australia.

These include:

- lack of state, territory and commonwealth targeted investment in maintaining Australia's plant quarantine and biosecurity arrangements;
- lack of comment and implementation undertaken by state, territory and commonwealth governments quarantine and biosecurity agencies based on the outcomes identified through the Beale Review in 2008;
- state, territory and commonwealth governments failing to adequately resource the plant health sections within each agency;
- lack of resources restricting the ability of these agencies to deliver appropriate responses to an EPP incursion while undertaking their normal biosecurity commitments;

- looming closure of PEQ facilities and the uncertainty surrounding importation of plant products; and
- increases in fees and charges associated with plant health programs coupled with reductions in service levels and calibre of delivery.

These issues are affecting the delivery of pro-active quarantine and biosecurity strategies and are jeopardising the pest free status of Australia by increasing the risk of future EPP incursions. The Australian NGI calls for increased investment and resource allocation to plant health to sustain our pest free status.

Additionally, several recent EPP incursions have had an impact on the Australian NGI and highlighted the severe deficiencies in quarantine and biosecurity arrangements to the point where agencies were unable to meet their statutory obligations. This is the result of the ongoing declining investment and the lack of resources right along the plant health biosecurity continuum.

System failures have occurred in the management of EPP incursions which include:

- failure to rapidly respond to the incursion and limited intent to eradicate;
- major failure to commit staff to the response;
- disjointed and incomplete response throughout the initial detection;
- failure of jurisdictions to assess the risk on its merits;
- lack of consistent positions on issues by jurisdictions;
- incomplete and piecemeal information flows to national committees;
- unwillingness to take pro-active action;
- failure to apply the recognised response system (PLANTPLAN);
- basic process failure (trace forward/ trace back);
- dysfunctional sample testing, recording and reporting systems;
- poorly conducted general site testing and surveillance;
- failure to adequately undertake delimiting surveillance;
- poor management of stock movement off infected properties; and
- no harmonisation of movement controls across Australia.

The NGI considers the Australian biosecurity system to be one that focuses on managing the risk(s) associated with EPPs under the auspices of facilitating market access through ALOP. The domestic quarantine system has, and is rapidly drifting away from this focus, with evidence indicating agencies are adopting the precautionary principle as opposed to one based on an assessed risk relevant to an ALOP.

NGIA supports a conservative approach to managing quarantine and biosecurity risks based on an Australian ALOP which sets a low level of risk. NGIA recognises that zero risk is unachievable due to the multitude of unregulated pathways into and across Australia. NGIA expects jurisdictions to accept this reality and develop risk based entry requirements that address the specific pathway and pest of concern.

Whilst on paper our biosecurity system looks robust and inclusive, in truth there are few checks and balances. This means decisions can be made by individuals (regulatory) to suite a particular policy or political position, as opposed to one based on an assessed risk. The current Australian domestic biosecurity system allows inappropriate personal, external policy and political influence to manipulate biosecurity decisions at state and territory level. These decisions are often cloaked in dubious scientific rationales that, in most cases, find no support outside the implementing jurisdiction. This is obviously not in the best interests of all stakeholders due to increased costs of compliance and lost markets.

Examples of this situation can be seen in recent decisions made by various state biosecurity agencies. It is clear a robust risk assessment framework under ALOP was not applied to a range of decisions stretching from prophylactic pesticide treatments to draconian plant movement protocols and complete market exclusions. These decisions have lacked scientific rigor and are often the result of external influence or professional incompetence. Furthermore, it has become evident movement controls are disguised restrictions on interstate trade, which is unacceptable and unconstitutional.

The Australian NGI calls for the establishment of a **national pest risk assessment framework** and the development of binding governance protocols on biosecurity decision pathways as an essential component of ongoing reform.

At present, the lack of an avenue for redress is a major concern for the Australian NGI. There is no vehicle allowing an agency to be challenged and no structure to ensure openness and transparency in the decision making process. Moreover, there is no forum in which the industry can present its case and achieve a binding decision requiring a jurisdiction to apply ALOP.

The NGI is also calling on the commonwealth government to take control of domestic quarantine with nationally consistent legislation applying sound risk based assessments under ALOP and engage state and territory agencies as service providers.

2 Harmonised delivery of quarantine and biosecurity arrangements – establish a National Pest Risk Assessment Framework which delivers a world class biosecurity and quarantine system to whole of industry.

Interstate biosecurity is a major issue for the Australian NGI production sectors with market access and cost minimisation priority areas requiring greater attention and resourcing by national and state biosecurity departments. A needs based assessment undertaken by NGIA has identified a number of criteria to be addressed by national and state biosecurity agencies. These include:

- market access driven strategies and policies;
- industry education and training;
- industry preparedness support;
- systems recognition through NPFMS;
- cost and red tape minimisation (including on-farm inspection fees);
- improved service delivery with a 'customer' focus;
- improved resource allocation for the development of pest specific certification guidelines (interstate certification assurance's or ICA's);
- national interstate movement controls database;
- adjustment support for industry to assist in transitioning; and
- upgrading of out-dated paper based tracking systems (certification/ record keeping) into an electronic documentation format.

Currently there are significant differences between states and territories in the processes used to identify pest risks. These differences drive variations in the market access risk mitigation, compliance evaluation and treatment protocols established by each state and territory.

These protocols dictate the volume of red tape and compliance costs borne by industry, which can be demonstrated by the pest Spiraling White Fly. Under current requirements one jurisdiction has a prescribed protocol requiring compliance if a business is within a 500km radius of a known detection while all other states and territories have a 10km radius. Such inconsistencies raise major questions about the science supporting such a significant difference in views between departmental experts. Clearly, nationally adopted and implemented systems and protocols mandating the uniform processes for plant biosecurity across Australia is urgently required.

The present system employed by the commonwealth, state and territory governments to assess the risk of an EPP is ad-hoc and lacks appropriate consensus amongst the various agencies. As an EPP can be viewed by different agencies of a different level of risk, a national emergency plant pest risk assessment methodology is needed to ensure the uniform application of EPP management strategies.

With interstate agencies recognising the value of on-farm self-certification for area and property freedom of plant pests, the NGI requires the development of ICA arrangements for a number of EPPs in Australia.

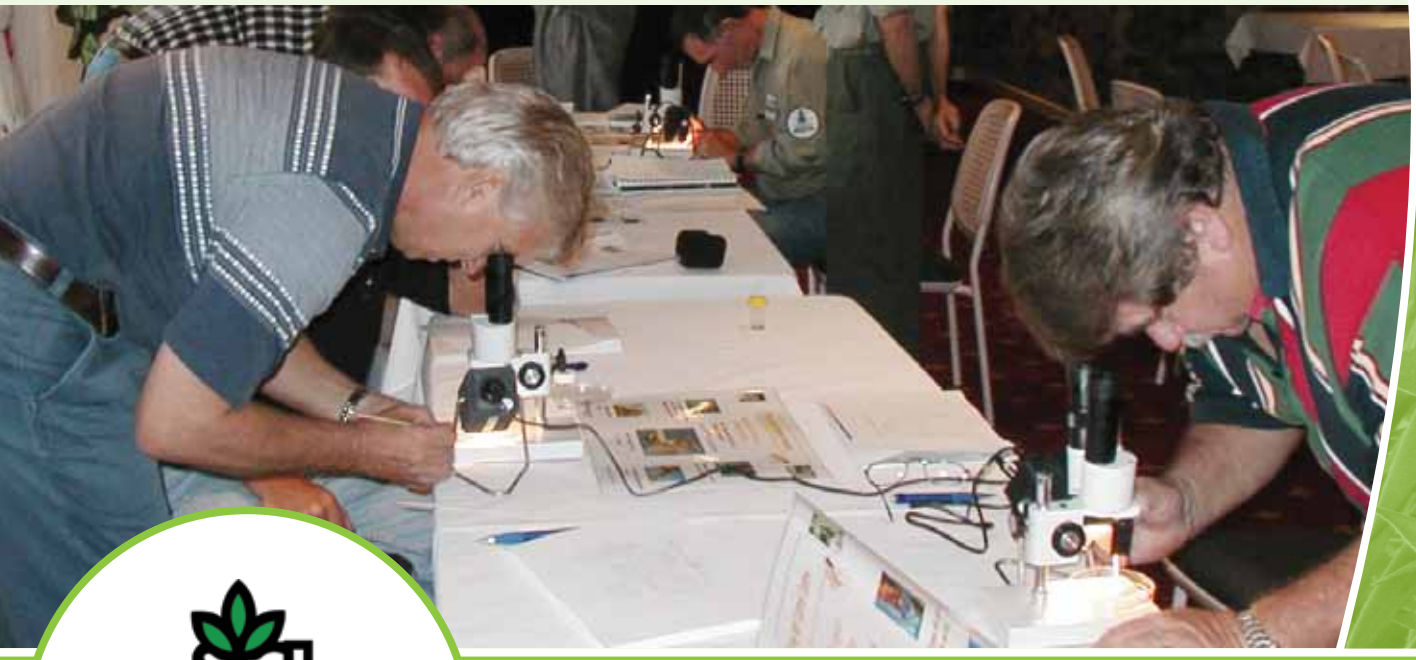
This would allow growers to be trained to detect specific pests, enhance on-farm systems and meet self-certification requirements to minimise inspection fees and give greater flexibility in product movement. It would also release departmental officers from compliance action to undertake industry education, training and support, plus participate in pest surveillance programs across the states and territories. Furthermore, this increased industry skill level will value add the participation of the NGI to the state-based plant pest surveillance.

Interstate biosecurity agencies need to address internal resourcing and customer service issues as a matter of urgency. As a service provider charging fees for service, it is unacceptable that the current service offered is delivered in an unprofessional manner and lacks value for money. As government holds a monopoly over this service, industry cannot change or seek a more competitive bid due to poor service delivery.

Electronic document creation, recordkeeping and transfer for interstate plant movement must be an immediate target for investment by state and federal agencies. The current process is paper based and costly for industry both in time and resources. With the international trade in plants fully supported by electronic documentation, it is clearly possible to implement such a system at a state and territory level to facilitate interstate trade. Further adoption of technology would allow for a web based data storage and retrieval system. This system would bring together all interstate plant movement requirements and be easily accessible to both industry and government.

As government continues to abdicate or devolve its responsibilities and reduce investment along the biosecurity continuum, industry is being expected to take over many activities previously in government hands (e.g. market access negotiations, pesticide registration and industry communication) or through increased on-farm compliance and fee for service verification services.

This shifting paradigm is happening quickly with industry struggling to keep pace. Government has not assisted industries to adjust to the new environment and in many cases is blocking industry attempts to meet new expectations. State, territory and federal governments need to provide transitional packages to assist industry in the change process. This in turn is likely to increase the rate of change and maintain the integrity of the biosecurity continuum.



3 Investment in on-farm support to address quarantine and biosecurity – the realignment of investment and a commitment by governments to support on-farm practices, innovation and incentives to adapt, manage and respond to biosecurity and quarantine.

One of the main difficulties in achieving wide-scale improvements in risk mitigation on-ground is that growers lack a meaningful and immediate incentive to improve on-farm biosecurity practices. The NGI is calling for the integration of biosecurity into existing enterprise management and quality assurance systems to provide a driver for enhanced on-ground risk management practices in nursery production across Australia.

Linking on-farm programs under the NPFMS umbrella, with potential to align to co-regulation with state, territory and federal government agencies, is also urgently required. (This initiative is discussed under Strategy 4). Without near to universal grower participation, monitoring and surveillance systems will provide an incomplete picture of Australia's pest and disease status and expenditures on communications and behavioural change programs may not penetrate as expected.

The Australian NGI supports government policy regarding on-farm practices, innovations and incentives to adapt, manage and respond to quarantine and biosecurity threats. Indeed, a critical area of preparedness for pest and disease emergencies is the need to educate key stakeholders about their roles and responsibilities in the event of an outbreak. Ongoing investment is required by the Australian Government into the DAFF National Communication Network, as this plays a critical role in terms of preparedness activities involving biosecurity education and awareness.

The industry also supports research, development and extension programs to equip production nurseries with tools and resources to support concepts such as best management practices (BMP), environmental management systems (EMS) and integrated pest management (IPM) whilst maintaining market access.

Programs that support greater grower participation in pest and disease surveillance and up-skill industry in all aspects of biosecurity (e.g. pest identification and monitoring, recordkeeping and on-farm capacity building to address biosecurity risks) are urgently required.

Further investment is also needed to develop technical guidelines to assist with this process. The industry also supports programs providing access to safer, less toxic, new and advanced pesticide chemistries through label registration and provision of minor use permits. This will ensure the application of pest management tools that fit the strategies employed by growers to meet their obligations for reduced and specific pesticide use, safe work places and environmental stewardship.

While there are provisions for owner reimbursement costs in the EPPRD, these are minimal and relate only to the actual costs of an emergency plant pest response (EPPR).

There is no provision for the recoupment of costs deemed not directly related to the EPPR, however the business has incurred these costs because of the EPP and the response. Affected growers therefore suffer a serious financial and operational impact if they are to be caught up in an EPPR, even if they are eligible for owner reimbursement payments. In past EPPR events, some affected growers have been driven out of business due to the costs incurred. The Australian NGI calls for a review of the mechanism for grower reimbursement to ensure it is equitable to all parties involved in an EPPR.

Currently, growers have no effective say in what is deemed an acceptable level of risk, even though they ultimately bear much of the cost burden in the event of an EPPR. One possible solution would be for governments to underwrite an insurance scheme to enable growers to insure against losses from exotic pest and disease incursions.

Presently, insurance of this type is not commercially available, which could be viewed as a clear case of market failure requiring government intervention. Such an insurance scheme could provide the incentive for improved on-farm biosecurity management by making grower access contingent on achieving threshold biosecurity standards. This is consistent with the philosophy of shared responsibility, and would ensure the available assistance targets enterprises which have endeavoured to manage the risks they face through investment, education and process management.

4 Recognition of established industry best management practice – this recognises and supports the Nursery Production Farm Management System (NPFMS) as a third market access instrument for the industry and investment in research, development and extension activities.

The Australian NGI seeks recognition and support of its NPFMS by all levels of government. This strategy supports Action 1.5 of the NPBS, which calls for the 'Review of domestic and international phytosanitary certification processes for the movement of plants and plant products, focusing on the national adoption of electronic systems for certification by government inspectors and by businesses accredited under approved schemes'.

The NPFMS is an industry driven best management practice program providing production nurseries, greenlife markets and growing media suppliers with a framework for sound on-farm risk management in relation to biosecurity. It is imperative businesses possess the relevant knowledge and skills to make informed management decisions and at the same time, maintain their obligation under the shared responsibility of biosecurity.

The NPFMS incorporates the nursery industry accreditation scheme Australia – best management practices (NIASA-BMP), EcoHort® (which promotes best management practices in environmental and natural resource management) and BioSecure *HACCP* (which promotes best practice in pest and disease management and biosecurity risk assessment and management). BioSecure *HACCP* is a set of protocols and procedures enabling a business to manage biosecurity risks while establishing an effective internal quarantine process for both imported and exported plant material.

The BioSecure *HACCP* risk management system encourages a business to maintain the strictest internal quarantine procedures possible while recording the actions taken at critical control points. With improved hazard analysis and control measures in place, the business is better protected in the event of a biosecurity threat or impact. Importantly, the process will support future market access both domestically and internationally. BioSecure *HACCP* is a key component of the industry wide risk mitigation strategy designed to operate at a grower level by addressing issues such as monitoring and surveillance, traceability, access restrictions, importing and treating plant material.

It is imperative these programs utilise the best available science and are regularly updated as research evolves and new findings on innovative practices and technologies become available. Investment in research and development into these best practice programs is vital to ensure these programs are relevant and in line with innovation and technological advancements in biosecurity.

To further assist in building capacity for the Australian NGI, research into issues such as pests that pose high risks of spread given new climate conditions is necessary. Climate change and variability will have a significant impact on the distribution of plant pests in Australia, with their potential temperate habitat extending into the southern regions of the continent.

This will increase the possible distribution pattern of many EPPs creating the likelihood of greater economic, social and environmental damage. Temperatures in northern Australia are also expected to increase and as a clear pathway for EPPs into Australia, this could result in EPP infestations populating at faster rates due to increased lifecycles (e.g. egg to adult). The faster development of large EPP populations will result in increased areas of rapid infestation, reducing the practicality and cost/benefit of eradication, with costs borne by industry.

To minimise the on-farm impact, NGI advocates recognition of the BioSecure *HACCP* as a third legal instrument in market access, as it provides an efficient mechanism for maintaining and/or gaining market access. By providing support services to industry, national, state and territory agencies can have an active and positive role in driving change at the farm level.

Industry programs addressing a regulatory requirement are entitled to be recognised, as the uptake by growers is generally voluntary and has a better 'fit' to the business model of that production system. The result of this 'fit' decreases the cost of implementation and is aligned to businesses productivity, profitability and sustainability, whilst also achieving the desired outcome such as enhanced biosecurity on-farm.

Ongoing investment is also required to ensure the necessary resources are available to deliver this valuable program to whole of industry through a skilled industry development officer (IDO) extension network. Extension activities will ensure businesses can apply the outcomes of the NPFMS, and implement the outcomes of government and industry research and development programs to directly address biosecurity and quarantine risks.

5 Implementation of a national greenlife producer communication and information scheme – this is designed to secure the reputation of the Australian NGI through knowledge based decision making.

Biosecurity in Australia is undergoing significant change with complete paradigm shifts in areas such as government and industry investment and participation, plus grower roles and responsibilities. NGIA has observed the increasing role peak industry bodies (state and national) are playing in the biosecurity areas of grower education, training and communication. Furthermore, these bodies are assisting government in establishing the vital details of the industry (distribution, numbers, crops, etc.) to ensure biosecurity strategies and programs are more effectively undertaken. Growers are also playing a greater role in activities relevant to their property and crop with an emphasis from government on shared responsibility, which represents a paradigm shift from total government control.

Industry cannot perform these functions under the old system and government must provide the tools (both regulatory and financially) for industry to adjust and participate in an efficient and effective manner.

If there is an incursion of an EPP, the Australian NGI does not have the ability to directly contact growers in the immediately affected region, or to quickly distribute relevant alerts to the national industry as there is no national database. The industry also lacks a national communication system to ensure biosecurity preparedness training, information and education tools are delivered to all stakeholders.

To remedy this, an opportunity exists to create a single national greenlife producer communication and information scheme. This would be based on property registration and would capture information that includes:

- contact details (name, address, phone, email);
- crops/produce (type, volume, markets);
- location (geographic locator and land tenure);
- standards (accreditation/ certification schemes); and
- business information (ABN, type).

Such a property registration scheme must focus on property types rather than individual growers. However, details on individual growers (contact details, crops and locations) are required for implementation, particularly in the event industry needs to respond quickly if an EPP is detected. The ability to identify and reach growers quickly would improve both the efficiency and effectiveness of this response.

The scheme needs to be mandatory to ensure the information is of sufficient quality to meet its intended uses and its key features should include:

- compulsory national registration for all greenlife producing properties;
- a condition of sale that all produce has greenlife property identification codes;
- annual register services (property registration and issuing of property identification codes); and
- future option of collecting greenlife property identification codes in the supply chain (e.g. at the same time as the collection of producer levies).

The scheme should be industry led and financed by an annual registration fee sufficient to maintain the scheme. Industry and government will need to provide both funds to establish the scheme and enact the necessary commonwealth and state legislation.

6 Build greater stakeholder engagement and involvement to deliver a national communication network – this will assist in building industry confidence.

To ensure the issues contained in this Policy Position are understood by all sectors of the Australian NGI, effective communication to relevant parties is important to assist with effective business management and decision-making. The NGIA will ensure growers are equipped with the tools and resources to assist them meet their on-farm obligations as part of the biosecurity continuum. It is important growers also receive information about government policies that may impact on their operations, such as changes in work plans, protocols and intake inspection procedures. This will build industry resilience and its capacity to assess opportunities and impacts.

The Australian NGI supports the Australian Government's National Communications Network as a valuable resource to address the risk of poor public communications and inconsistent messages which undermine both domestic and international confidence in an EPPR and exacerbates disease control efforts. It offers a means by which information and issues are rapidly moved between local, state and national agencies and industry.

While the network is crucial in managing a crisis situation arising from an EPP, it is vital the information flows between relevant parties in a timely and effective manner so all stakeholders are informed.

Further information

If you would like further information about Reducing the Pest Risk - The Australian Nursery & Garden Industry Position on Quarantine and Biosecurity, contact:

Dr Anthony Kachenko
Environmental and Technical
Policy Manager

Nursery & Garden Industry Australia
Unit 58, 5 Gladstone Road
Castle Hill, NSW, 2154

Mailing Address:
PO Box 7129
Baulkham Hills BC
NSW 2153

Phone: (02) 8861 5106
Fax: (02) 9659 3446

Email: anthony.kachenko@ngia.com.au
Web: www.ngia.com.au

Mr John McDonald
Nursery Industry
Development Manager

Nursery & Garden Industry Queensland
Unit 1 The Grove
Corner Orange Grove & Riawena Roads
Salisbury, QLD, 4107

Mailing Address:
PO Box 345
Salisbury QLD 4107

Phone: (07) 3277 7900
Fax: (07) 3277 7109

Email: nido@ngiq.asn.au
Web: www.ngiq.asn.au

All photography courtesy of Dr Anthony Kachenko and Mr John McDonald.

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Nursery & Garden Industry

Appendix 4

Nursery & Garden Industry

Australian Nursery Industry Myrtle Rust

(Uredo rangelii)

Management Plan 2012

Developed for the Australian Nursery Industry

Production
Wholesale
Retail
V2

Acknowledgements

This Myrtle Rust Management Plan has been developed by the Nursery & Garden Industry Queensland (John McDonald - Nursery Industry Development Manager) for the Australian Nursery Industry.

Version 02 February 2012

Photographs sourced from I&I NSW, NGIQ and Queensland DEEDI.

Various sources have contributed to the content of this plan including:

- Nursery Industry Accreditation Scheme Australia (NIASA)
- BioSecure *HACCP*
- Nursery Industry Guava Rust Plant Pest Contingency Plan
- DEEDI Queensland Myrtle Rust Fact Sheets
- I&I NSW Myrtle Rust Fact Sheets and Updates
- Biosecurity Queensland Myrtle Rust Program

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For further information contact:

John McDonald
Industry Development Manager
NGIQ
Ph: 07 3277 7900
Email: nido@ngiq.asn.au

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1. Introduction – Myrtle Rust in the Australian Nursery Industry

Myrtle rust (*Uredo rangelii*) has the potential to infect all myrtaceous plants in both our built (gardens & landscape) and natural environments plus a range of industries (nursery production, timber, cut flower, etc) more likely along the coastline of Australia due to suitable environmental conditions. Under threat from this disease, if it becomes widely established, are a number of identified threatened native plant species across Australia plus a number of endangered wildlife habitat(s) that could have a major impact on our natural biodiversity.

In April 2010 Myrtle rust was detected in Australia on the Central Coast of New South Wales (NSW). A national response was agreed to under the Emergency Plant Pest Response Deed (EPPRD) and a comprehensive surveillance and management program was initiated within NSW. By November 2010 more than 140 infected premises had been identified across NSW with the first detections outside horticultural industries being recorded in state forests and nature reserves. The initial detections of the disease in Queensland occurred on the 27th December 2010 in the south east of the state with further detections noted in Cairns, Townsville, Rockhampton, Gladstone and Hervey Bay during 2011. The most recent detections outside of NSW and Qld occurred in Victoria during the first week of January 2012 with more than 28 sites around Melbourne infected by early February 2012.

On December 22nd 2010 the Myrtle Rust National Management Group agreed the disease was not technically feasible to eradicate in New South Wales and cancelled the Myrtle Rust Response Plan previously enacted under the EPPRD. Due to the impact the disease could have across Australia it was further agreed to implement a structured management plan to limit the establishment of the pathogen within industries and the natural environment. The federal government, through the Department of Agriculture Fisheries & Forestry (DAFF), established the Myrtle Rust Coordination Group to plan the investment of $1.5 million of research funding across six key themes:

National Transition to Management Plan:

- **Theme 1: Coordination and communication**
- **Theme 2: Immediate disease management**
- **Theme 3: Taxonomy and identity of the pathogen**
- **Theme 4: Potential impact and distribution**
- **Theme 5: Chemical control options**
- **Theme 6: Resistance breeding options**

The development of this industry specific Myrtle Rust Management Plan, by the Australian Nursery Industry, is in direct response to the agreed national position in which the industry participated in developing. As a professional and responsible industry it is appropriate that all growers, wholesalers and retailers apply the relevant strategies to manage myrtle rust as described in this plan.

Myrtle rust is a notifiable pathogen in all Australian jurisdictions, where currently no positive detections have been recorded, requiring any detection of the disease be reported to the relevant state or territory biosecurity agency within 24 – 48 hours.

National Exotic Plant Pest Hotline: 1800 084 881

This **Myrtle Rust Management Plan** has been developed for use by production nurseries and retailers of greenlife including garden centres, greenlife markets (wholesalers), big box hardware, supermarkets, chain stores, etc. The plan provides a detailed framework for growers and retailers to apply on-site in the management of myrtle rust on plants of the Myrtaceae family. It is recommended that the industry apply this plan to **all** plants of the Myrtaceae family not only those that have been currently identified as hosts.

For further information on whole of property biosecurity in the nursery industry including on-farm programs such as BioSecure *HACCP* and the industry Biosecurity Manual contact your state industry peak body or go to www.ngia.com.au and follow the links.

Note: State/territory laws and requirements including interstate movement protocols over-ride this Industry Myrtle Rust Management Plan.

(Source: NGIQ – Myrtle rust on *Syzygium jambos*)

(Source: NGIQ – Myrtle rust on *Syzygium jambos*)

2. Myrtaceae Family – Genera currently found in Australia

It is possible that all genera listed may be susceptible to myrtle rust under optimum conditions in Australia. The list below may change in the future.

Myrtaceae - Genera	Myrtaceae - Genera	Myrtaceae - Genera
Acmena spp.	*Eremaea* spp.	*Paragonis* spp.
Acmenosperma spp.	*Eucalyptus* spp.	*Pericalymma* spp.
Actinodium spp.	*Eugenia* spp.	*Petraeomyrtus* spp.
Agonis spp.	*Euryomyrtus* spp.	*Phymatocarpus* spp.
Allosyncarpia spp.	*Gossia* spp.	*Pileanthus* spp.
Aluta spp.	*Harmogia* spp.	*Pilidiostigma* spp.
Anetholea anisata	*Homalocalyx* spp.	*Regelia* spp.
Angasomyrtus spp.	*Homalospermum* spp.	*Rhodamnia* spp.
Angophora spp.	*Homoranthus* spp.	*Rhodomyrtus* spp.
Archirhodomyrtus spp.	*Hypocalymma* spp.	*Rinzia* spp.
Astartea spp.	*Kardomia* spp.	*Ristantia* spp.
Asteromyrtus spp.	*Kunzea* spp.	*Scholtzia* spp.
Astus spp.	*Lamarchea* spp.	*Seorsus* spp.
Austromyrtus spp.	*Lenwebbia* spp.	*Sphaerantia* spp.
Babingtonia spp.	*Leptospermum* spp.	*Stenostegia congesta*
Backhousia spp.	*Lindsayomyrtus* spp.	*Stockwellia* spp.
Baeckea spp.	*Lithomyrtus* spp.	*Syncarpia* spp.
Balaustion spp.	*Lophomyrtus* spp.	*Syzygium* spp.
Barongia spp.	*Lophostemon* spp.	*Thaleropia* spp.
Beaufortia spp.	*Lysicarpus* spp.	*Thryptomene* spp.
Callistemon spp.	*Malleostemon* spp.	*Triplarina* spp.
Calothamnus spp.	*Melaleuca* spp.	*Tristania* spp.
Calytrix spp.	*Metrosideros* spp.	*Tristaniopsis* spp.
Chamelaucium spp.	*Micromyrtus* spp.	*Ugni* spp.
Choricarpia spp.	*Mitrantia* spp.	*Uromyrtus* spp.
Conothamnus spp.	*Myrciaria* spp.	*Verticordia* spp.
Corymbia spp.	*Myrtus* spp.	*Waterhousea* spp.
Corynanthera spp.	*Neofabricia* spp.	*Welchiodendron* spp.
Darwinia spp.	*Ochrosperma* spp.	*Xanthostemon* spp.
Decaspermum spp.	*Osbornia* spp.	

(Source: DEEDI/DERM February 2012)

Note: Genera highlighted in yellow have had species, within these genera, return positive infections in the field (natural infection) in New South Wales and Queensland between 2010 and January 2012.

3. Myrtle Rust (*Uredo rangelii*)

Myrtle rust (*Uredo rangelii*), a plant fungal disease native to South America, is a member of the fungal complex known as the guava rust (*Puccinia psidii*) group. Based on experiences in Australia between April 2010 and February 2012, information from New South Wales and Queensland, shows myrtle rust has an expanding host range currently infecting approximately 179 species from 41 genera or approximately 46% of known genera (Myrtaceae) in Australia.

The pathogen infects young, actively growing, emerging leaves, buds, flowers, green stems, fruit and shoots of plants within the Myrtaceae family. In Queensland to date the most severe infections of the disease have been recorded on:

Botanical name	Common name
Agonis flexuosa	**Willow myrtle**
Chamelaucium uncinatum	**Geraldton wax**
Decaspermum humile	**Silky myrtle**
Eugenia reinwardtiana	**Beach cherry**
Gossia inophloia **(syn. *Austromyrtus inophloia*)**	**Thready barked myrtle**
Melaleuca quinquenervia	**Broad-leaved paperbark**
Rhodamnia angustifolia	**Narrow-leaved malletwood**
Rhodamnia maideniana	**Smooth scrub turpentine**
Rhodamnia rubescens	**Scrub turpentine**
Syzygium jambos	**Rose apple**

(Source: DEEDI February 2012)

Myrtle rust may infect plants under a wide range of environmental conditions, however infection rates may be heightened when the following conditions are present:

- Soft new growth/tissue
- High humidity
- Free water on plant surfaces for 6 hours or more
- Night temperatures (optimal) within 15 - 25^{o}C however as low as 10°C (CSIRO. 2012)
- Low light conditions including darkness (minimum of 8 hours) after spore contact can increase germination success
- Life cycle can be as short as 10 – 14 days (spore to spore)

Myrtle rust has the ability to complete its entire lifecycle on a single host plant. Myrtle rust initially causes light infection on young leaves and new shoots which can appear as yellow flecks. Lesions expand radially and can coalesce (join) with age and susceptible tissue shrivels and dies. Secondary infections within the plant can occur within days of the first pustules appearing. Repeat infection may result in plant death, although this is likely to vary from species to species. The level of susceptibility of many potential and recognized hosts in Australia is unknown. As the plant drops dead leaves the pathogen will reinfect new growth limiting the plants ability to recover.

It is possible that as this disease establishes in Australia the host range may grow to include many of the internationally recorded plant species infected by guava rust. The nursery industry **must** consider **all** myrtaceous species as potential hosts of myrtle rust.

Note: Guava rust (*Puccinia psidii*) is also known as **eucalyptus rust** and has caused heavy crop losses in the Brazilian hardwood industry through the decimation of planted Eucalyptus seedlings

in the field. For identification purposes myrtle rust and guava rust are visually and symptomatically identical therefore identification tools are interchangeable.

The general symptoms of myrtle rust/guava rust include:
(Myrtle rust generally attacks soft new growth including leaf surfaces, shoots, buds, flowers, young green stems and fruit)

- **Tiny, raised spots or pustules with possible yellow flecking**
- **Small purple or red brown flecks with a faint chlorotic (yellow) halo on leaf surfaces**
- **Large purple or red/brown lesions as a result of flecks coalescing**
- **Purple or red/brown lesions and bright yellow rust pustules producing spores**
- **Bright yellow rust pustules producing spores on underside of the leaf (young infection)**
- **Bright yellow rust pustules producing spores on <u>both</u> sides of the leaf (mature infection)**
- **Small and large necrotic lesions, with possible purple margins, and leaf distortion (twisting)**
- **Older lesions can contain brown/grey rust pustules that no longer produce yellow spores on the lesions**

See images below and on pages 16, 17 and 18 of this Management Plan.
(Images sourced from I&I NSW, NGIQ and DEEDI Queensland)

Note: Myrtle rust spores are believed to remain viable (under optimal conditions) for between 3 – 6 months.

(Source: NGIQ – Myrtle rust on *Syzygium jambos*)

4. Known hosts of myrtle rust in Australia - February 2012

The species listed below have had observable myrtle rust field infections (natural infection), at some point since December 2010 in Queensland. Experienced DEEDI officers have applied the national myrtle rust susceptibility ranking to each record and given the "Ranking" as noted in Table 4.1.

Many of the species listed below have also been recorded as susceptible in New South Wales since April 2010. It can be assumed that susceptible species in Queensland or New South Wales will in all likelihood be susceptible to myrtle rust in every other like environment across Australia. NGIA recommends the industry combine tables 4.1 and 4.2 for a complete (as at February 2012) known myrtle rust susceptibility list.

4.1 Queensland host list and susceptibility rating table – February 2012.

(ES=Extremely Susceptible, HS=Highly Susceptible, MS=Moderately Susceptible, RT=Relatively Tolerant)

DEEDI susceptibility ratings are based on current observational assessments and may change over time.

Rating	Botanical name (Species)	Common name
RT	*Acmena hemilampra* (syn. *Syzygium hemilamprum*)	Blush satinash
RT	*Acmena ingens*	Red apple
MS	*Acmenosperma claviflorum*	Grey satinash
ES	*Agonis flexuosa*	Willow myrtle
HS	*Anetholea anisata* (syn. *Backhousia anisata, Syzygium anisatum*)	Aniseed myrtle
RT	*Asteromyrtus brassii*	Brass's Asteromyrtus
HS	*Austromyrtus dulcis*	Midgen berry or midyim
RT	*Austromyrtus tenuifolia*	Narrow leaf myrtle
RT	*Backhousia angustifolia*	Curry myrtle or narrow-leaved myrtle
HS	*Backhousia citriodora*	Lemon-scented myrtle
MS	*Backhousia myrtifolia*	Grey myrtle, ironwood
RT	*Backhousia oligantha* (endangered)	No common name
RT	*Backhousia sciadophora*	Shatterwood
RT	*Backhousia* sp. 'Prince Regent'	No common name
ES	*Chamelaucium uncinatum*	Geraldton wax
HS	*Choricarpia leptopetala*	Brown myrtle, rusty turpentine
RT	*Choricarpia subargentea* (near threatened)	Giant ironwood
RT	*Corymbia henryi*	Large leaved spotted gum
RT	*Corymbia torelliana*	Cadagi
RT	*Corymbia citriodora subsp. variegata*	Spotted gum
ES	*Decaspermum humile*	Silky myrtle
RT	*Eucalyptus sp.*	Red gum
MS	*Eucalyptus carnea*	Broad-leaved white mahogany
RT	*Eucalyptus cloeziana*	Gympie messmate
MS	*Eucalyptus curtisii*	Plunkett mallee
MS	*Eucalyptus grandis*	Flooded gum, rose gum
RT	*Eucalyptus planchoniana*	Bastard tallow wood
RT	*Eucalyptus tereticornis*	Blue gum, forest red gum
MS	*Eucalyptus tindaliae*	Tindale's Stringybark

ES	*Eugenia reinwardtiana*	Beach cherry
MS	*Eugenia zeyheri*	No common name
HS	*Gossia acmenoides*	Scrub ironwood
RT	*Gossia bidwillii* (syn. *Austromyrtus bidwillii*)	Scrub python tree
RT	*Gossia floribunda*	Cape ironwood
MS	*Gossia fragrantissima* (endangered)	Sweet myrtle
HS	*Gossia gonoclada* (endangered)</< TD>	Angle-stemmed myrtle
HS	*Gossia hillii*	Scaly myrtle
ES	*Gossia inophloia* (syn. *Austromyrtus inophloia*) (near threatened)	Thready barked myrtle
MS	*Gossia macilwraithensis* (near threatened)	No common name
RT	*Gossia myrsinocarpa*	Malanada ironwood, small flowered lignum
MS	*Gossia punctata*	Dotted myrtle
RT	*Lenwebbia lasioclada*	Velvet myrtle
HS	*Lenwebbia prominens* (near threatened)	Southern velvet myrtle
RT	*Lenwebbia* sp. 'Blackall Range' (endangered)	Blackall Range myrtle
MS	*Leptospermum liversidgei*	Lemon-scented tea tree, olive tea tree
RT	*Leptospermum luehmannii*	Bronze-barked tea tree
RT	*Leptospermum petersonii*	Lemon-scented tea tree
RT	*Leptospermum semibaccatum*	No common name
RT	*Lindsayomyrtus racemoides*	Daintree Penda
RT	*Lophostemon suaveolens*	Swamp box, swamp mahogany
HS	*Melaleuca fluviatilis*	Weeping tea tree
RT	*Melaleuca formosa (syn. Callistemon formosus)*	Kingaroy Bottlebrush, cliff bottlebrush
HS	*Melalecua leucadendra*	Broad-leaved paperbark
RT	*Melaleuca linariifolia*	Snow in summer
RT	*Melaleuca nesophila*	Showy honey myrtle
HS	*Melaleuca nodosa*	Prickly-leaved paperbark
RT	*Melaleuca pachyphylla*	Wallum bottlebrush
HS	*Melaleuca polandii*	No common name
ES	*Melaleuca quinquenervia*	Broad-leaved paperbark
MS	*Melaleuca saligna*	Willow bottlebrush, white bottlebrush
HS	*Melaleuca viridiflora*	Broad-leaved paperbark
MS	*Melaleuca viminalis (syn. Callistemon viminalis)*	Willow bottlebrush
RT	*Metrosideros collina*	Fiji Christmas bush
RT	*Metrosideros collina x villosa*	Fiji Christmas bush
RT	*Metrosideros kermadecensis*	Kermadec pohutukawa
RT	*Metrosideros thomasii*	New Zealand Christmas bush
RT	*Myrciaria cauliflora*	No common name
RT	*Myrtus communis*	Common myrtle
RT	*Pilidiostigma glabrum*	Plum myrtle
RT	*Rhodamnia acuminata*	Cooloola ironwood
ES	*Rhodamnia angustifolia* (endangered)	Narrow-leaved malletwood
MS	*Rhodamnia arenaria*	Cape York malletwood
MS	*Rhodamnia argentea*	Silver myrtle or malletwood
HS	*Rhodamnia costata*	Malletwood
HS	*Rhodamnia dumicola*	Rib-fruited malletwood
MS	*Rhodamnia glabrescens* (near threatened)	Smooth malletwood
ES	*Rhodamnia maideniana*	Smooth scrub turpentine

MS	*Rhodamnia pauciovulata* (near threatened)	Small-leaved malletwood
ES	*Rhodamnia rubescens*	Scrub turpentine
HS	*Rhodamnia sessiliflora*	Iron malletwood
MS	*Rhodamnia spongiosa* (syn. *R. glauca*)	Northern malletwood
MS	*Rhodomyrtus canescens*	Crater ironwood
MS	*Rhodomyrtus pervagata*	Rusty rhodomyrtus, rusty ironwood
HS	*Rhodomyrtus psidioides*	Native guava
MS	*Rhodomyrtus sericea*	Grey rhodomyrtus
HS	*Rhodomyrtus tomentosa*	Downy rose myrtle, Ceylon hill gooseberry
MS	*Rhodomyrtus trineura* subsp. *capensis*	No common name
RT	*Ristantia waterhousei* (vulnerable)	No common name
MS	*Sphaerantia discolor*	Tully Penda
MS	*Syzygium angophoroides*	Yarrabah satinash
RT	*Syzygium argyropedicum*	Silver satinash
RT	*Syzygium armstrongii*	White bush apple
RT	*Syzygium australe*	Scrub cherry
RT	*Syzygium canicortex*	Yellow satinash
RT	*Syzygium corynanthum*	Sour cherry
MS	*Syzygium cumini*	Java Plum
MS	*Syzygium eucalyptoides* subsp. *eucalyptoides*	White apple
RT	*Syzygium forte subsp. forte*	Watergum, brown satinash
RT	*Syzygium forte subsp. potamophilum*	Flaky barked satinash, white apple
ES	*Syzygium jambos*	Rose apple
RT	*Syzygium luehmannii*	Small-leaved lillypilly, riberry
RT	*Syzygium moorei*	Rose apple
RT	*Syzygium nervosum*	No comon name
HS	*Syzygium oleosum*	Blue lillypilly
RT	*Syzygium paniculatum*	Magenta cherry
RT	*Syzygium rubrimolle*	Laura apple
RT	*Syzygium tierneyanum*	River Cherry, Bamaga satinash
RT	*Syzygium wilsonii*	Powder puff lilly pilly
RT	*Syzygium wilsonii x luehmanii*	Cascade lilly pilly
MS	*Syzygium xerampelinum*	Mulgrave satinash
HS	*Tristania neriifolia*	Water gum
RT	*Tristaniopsis laurina*	Water gum, kanooka
RT	*Uromyrtus tenella*	No common name
RT	*Waterhousea floribunda* (syn. *Syzygium floribundum*)	Weeping lillypilly
RT	*Waterhousea hedraiophylla* (syn. *Syzygium hedraiophyllum*)	Gully satinash
RT	*Waterhousea mulgraveana*	No common name
MS	*Waterhousea Unipunctata*	Rolypoly satinash
RT	*Xanthostemon chrysanthus*	Golden penda
HS	*Xanthostemon oppositifolius* (vulnerable)	Southern penda
MS	*Xanthostemon youngii*	Crimson penda

(Source: DEEDI February 2012)

4.2 New South Wales additional myrtle rust host list not recorded in Queensland to date (February 2012):

Many of the species above have been recorded as susceptible in New South Wales. The list below records those species identified as susceptible in NSW and to date not observed infected in Qld.

Note: At the writing of this Management Plan there is no myrtle rust susceptibility ranking available for NSW listed species.

Botanical name	Botanical name	Botanical name
Angophora floribunda	*Melaleuca decora*	*Syzygium glenum*
Angophora subvelutina	*Melaleuca linariifolia*	*Syzygium graveolens*
Backhousia enata	*Melaleuca sieberi*	*Syzygium hodgkinsoniae*
Backhousia hughesii	*Melaleuca styphelioides*	*Syzygium maraca*
Barongia lophandra	*Melaleuca viridiflora* (purple flowered form)	*Syzygium megacarpum*
Callistemon rigidus	*Metrosideros excelsa*	*Syzygium minutuliflorum*
Callistemon salignus (not = *Melaeuca saligna*)	*Mitrantia bilocularis*	*Syzygium polyanthum*
Eucalyptus agglomerata	*Pilidiostigma rhytispermum*	*Syzygium pseudofastigiatum*
Eucalyptus deanei	*Pilidiostigma tropicum*	*Syzygium resa* (Syn. *Acmena resa*)
Eucalyptus elata	*Rhodomyrtus macrocarpa*	*Syzygium sayeri*
Eucalyptus olida	*Stockwellia quadrifida*	*Syzygium smithii* (Syn. *Acmena smithii*)
Eucalyptus pilularis	*Syncarpia glomulifera*	*Syzygium trachyphloium*
Eucalyptus siderophloia	*Syzygium alliligneum*	*Syzygium velarum*
Leptospermum rotundifolium	*Syzygium bamagense*	*Tristaniopsis collina*
Lithomyrtus obtusa	*Syzygium boonjee*	*Ugni molinae*
Lophomyrtus bullata	*Syzygium buettnerianum*	*Uromyrtus australis*
Lophomyrtus x ralphii	*Syzygium bungadinnia*	*Uromyrtus lamingtonensis*
Melaleuca alternifolia	*Syzygium cormiflorum*	*Xanthostemon chrysanthus*
Melaleuca argentea	*Syzygium dansiei*	*Xanthostemon formosus*
Melaleuca armillaris	*Syzygium erythrocalyx*	*Xanthostemon graniticus*

(Source: I&I NSW February 2012)

4.3 Victorian myrtle rust host list (February 2012)

Species identified in yellow have not been recorded as susceptible in NSW or Qld to date.

Botanical name	Common name
Acmena smithii (Syn. *Syzygium smithii*)	Lilly pilly
Agonis flexuosa	Willow myrtle
Backhousia citriodora	Lemon-scented myrtle
Lophomyrtus x ralphii	Black Stallion
Metrosideros carminea - (new species)	Red rata
Metrosideros collina	Fiji Christmas bush
Metrosideros excelsa	New Zealand Christmas bush
Myrtus communis	Common myrtle
Syzygium australe	Lilly pilly/scrub cherry
Syzygium paniculatum	Dwarf magenta cherry

5. Fungicide Treatment

For the treatment of plants (Myrtaceae family) the industry has access to an Emergency Permit (**PER12156**) that allows a range of fungicides to be applied for the management of myrtle rust. Therefore if you intend to treat plants with a fungicide you must have a copy of this permit on-site and you must use the application rates as outlined in the permit. You can download the permit by going to the APVMA website (www.apvma.gov.au) and click on 'Permits' and follow the prompts.

The **permit is a legal document** and all directions/rates/intervals must be followed as described in the document. Furthermore all relevant directions as detailed on each individual product label must also be followed by those handling and applying the fungicide(s). NGIA recommends only appropriately trained staff in pesticide handling, use and application should be applying the myrtle rust fungicide program

The table below (Table 5.1) identify's the various fungicides on the permit plus others with existing registrations and lists the **'Fungicide activity'** that will assist in selecting the appropriate product. The **'Chemical group'** is to ensure that an effective rotation program (see Table 5.2 & 5.3 with examples below) can be applied on-farm if a business intends to have a standard fungicide strategy for the management of myrtle rust. **Note**: Table 5.3 is based on medium to low risk seasonal disease pressures moving the rotation interval to 4 weeks (1 month).

5.1 Fungicide Table:

Fungicide trade name	Active constituent	Fungicide activity	Chemical group (Mode of Action)	Minimum re-treatment interval between consecutive applications
BAYFIDAN 250 EC FUNGICIDE (PER12156)	TRIADIMENOL	Systemic, curative and protectant	3	14-21 days
SAPROL FUNGICIDE (PER12156)	TRIFORINE	Systemic, slightly curative and protectant	3	7 days
IMTRADE MANCOZEB 750 DF FUNGICIDE (PER12156)	MANCOZEB	Non-systemic protectant	M3	7 days
AMISTAR 250 SC FUNGICIDE (PER12156)	AZOXYSTROBIN	Systemic, slightly curative and protectant	11	14-21 days
COPPER OXYCHLORIDE (PER12156)	COPPER OXYCHLORIDE	Non-systemic protectant	M1	7-14 days
PLANTVAX 750 WP FUNGICIDE (PER12156)	OXYCARBOXIN	Systemic, curative and protectant	7	14 days
TILT 250 EC FUNGICIDE (PER12156)	PROPICONAZOLE	Systemic, curative and protectant	3	7 days
BRAVO (Registered)	CHLOROTHALONIL	Non-systemic, slightly curative and protectant	M5	7 – 14 days

5.2 Myrtle Rust Fungicide Treatment Rotation Program (Production/Propagation)

High risk season (External environmental conditions suitable for spore production)

Crop Situation	Fungicide (Fortnight 1)	Fungicide (Fortnight 2)	Fungicide (Fortnight 3)	Fungicide (Fortnight 4)
Stock receival	Bayfidan	Plantvax	Bayfidan	Plantvax
Propagation	Bayfidan/Tilt	Mancozeb	Plantvax	Amistar
Growing on (Low level risk)	Bayfidan/Tilt /Plantvax	Mancozeb/Bravo	Copper/Bravo (use Bravo only if not used in preceding month)	Bravo/Amistar (use Bravo only if not used in preceding month)
Growing on (Medium level risk)	Bayfidan/Tilt/Saprol	Mancozeb/Copper	Plantvax	Bravo/Amistar
Growing on (High level risk)	Bayfidan + mancozeb	Copper/Bravo	Plantvax + mancozeb	Amistar + mancozeb

5.3 Myrtle Rust Fungicide Treatment Rotation Program (Production/Propagation)

Medium/low risk season (External environmental conditions not suitable for spore production)

Crop Situation	Fungicide (Month 1)	Fungicide (Month 2)	Fungicide (Month 3)	Fungicide (Month 4)
Stock receival	Bayfidan	Plantvax	Bayfidan	Amistar
Propagation	Bayfidan/Tilt	Mancozeb/Copper	Plantvax	Amistar/Bravo
Growing on (Low level risk)	Bayfidan/Tilt or Plantvax	Mancozeb/Bravo	Bravo/Amistar (use Bravo only if not used in preceding month)	Copper/Bravo (use Bravo only if not used in preceding month)
Growing on (Medium level risk)	Bayfidan/Tilt/Saprol	Mancozeb/Copper	Plantvax	Bravo/Amistar
Growing on (High level risk)	Bayfidan + mancozeb	Copper/Bravo	Plantvax + mancozeb	Amistar + mancozeb

Note: Test fungicide(s) on a sample of the crop to ensure the product is not phytotoxic to your plant species before initial batch treatment.

Note: Other APVMA Permits are available for:

- Native plant food crops – PER12746
- Home Gardener – PER12828

Fungicide rotation based on the "Chemical Group (Mode of Action)" is designed to prevent the pathogen (myrtle rust) from developing genetic resistance to a particular fungicide active ingredient due to the over use of that one product. The above (Table 5.2) gives recommendations of five product combinations (rotations) based on the degree of "risk" of infection a "process" has within a cropping system. Alternative fungicide rotations are acceptable depending on the risk profile the business faces and the results of crop inspections.

As an example from the above table (Table 5.2) **"Stock Receival"** is live host plant material grown off site and imported into the production nursery. This material has the opportunity to be mixed with other plant stock at transport depots, in vehicles, etc as it is transported to the production nursery. Therefore this plant material is a **high risk** of being infected and should be treated with a fungicide that is a systemic curative to give a high degree of confidence that any potential infections are dealt with before moving plant stock into the cropping system. The rotation plan (Table 5.2) advises producers to rotate the fungicides every two weeks from Bayfidan to Plantvax at the receival point to protect from pathogen resistance.

Defining each individual business's risk level will also be based on key aspects such as crop nutrition programs, irrigation scheduling, plant spacing, host material on the property (e.g. gardens, hedging or windbreaks), susceptibility of crops, the amount of host material across the landscape outside of business boundaries and general environmental conditions (seasonal) that are conductive to increasing spore loads such as high humidity, rainfall, prevailing winds, etc.

The three "Growing on" risk ratings can be explained in the following **example**:

Growing on - Risk	Risk explanation
Low level	Low relative humidity (<50%), outside of wet season, small number of host plants surrounding property plus not in new growth flush phase and relatively tolerant (RT) crop susceptibility
Medium level	Increased relative humidity (50% – 65%), approaching wet season, small number of host plants surrounding property in new growth flush phase and moderately susceptible (MS) crop
High level	High relative humidity (>65%), wet season, moderate to large number of host plants surrounding property in new growth flush phase and crops are either highly susceptible (HS) or extremely susceptible (ES)

5.3 Fungicide Application

Applying fungicides to manage myrtle rust will require the appropriate application equipment is available to ensure the chemical is delivered to the target crop within the acceptable parameters as defined by industry best management practice. The aim of using fungicides to manage myrtle rust is to ensure the necessary coverage is achieved that allows the fungicide to do its job.

Generally a systemic curative fungicide has some room for applicator error due to the ability of the plant to take the fungicide up in plant tissue and translocate it throughout the vegetative material. Non-systemic protectants such as Bravo, copper and mancozeb provide a "protective" film covering the plant surface which requires greater precision in the delivery technique particularly in achieving contact with the underside of vegetative material e.g. leaves.

The following list identifies the key aspects that are critical for successful fungicide treatment:

- Personnel applying fungicides appropriately trained (e.g. ChemCert/AusChem Certified)
- APVMA Permit (**PER12156**) available on-site (defines fungicide rate)

- Fungicide(s) to be applied within “best before” or “use by date”
- Applicable fungicide rotation program selected
- Appropriate Personal Protective Equipment available
- Signage advising staff not to re-enter treated areas before designated re-entry period
- Re-entry period guidelines (if not on Label) are: minimum 24hr’s, if possible 48 hr’s
- Ensure overhead irrigation is withheld for approximately 6 - 12 hours after treatment
- Application equipment is appropriate for the development of droplets that are within 150 – 250 microns such as:
    - Powered hydraulic handguns/booms fitted with either solid or hollow cone nozzles
    - Powered hydraulic application equipment rated at 600kpa or higher
    - Three point linkage/backpack powered **misters** are operated at correct speeds
    - All equipment regularly calibrated
- Use a chemical surfactant (wetter/sticker) if recommended on the product label
- Test fungicide(s) on a sample of the crop to ensure the product is not phytotoxic to your plant species before batch treatment.

Note: Knapsack sprayers powered by batteries or hand pumps are generally **not** appropriate equipment for delivering the droplet spectrum required for fungicide applications on crops.

6. On-site Biosecurity Actions

Currently (February 2012) myrtle rust is confirmed in New South Wales, Queensland and Victoria and as such it is important that businesses in **all** states and territories, production, wholesale and retail, maintain the highest plant health standards to ensure this disease is either suppressed and managed or not introduced. Any business purchasing, or has sourced, myrtle rust host plant material from an outside source **must** survey their stock to ensure freedom from the disease. Other businesses with host plants are advised to maintain a structured monitoring program (weekly) to ensure they remain free of the disease or detect infects early and apply a suitable management strategy.

Myrtle rust can move across the landscape and within a production system by:

- **Vegetative material (alive or dead)**
- **Contaminated plant containers (pots, trays, etc)**
- **Air movement of spores (dry spores can move great distances – many kilometres)**
- **Human assisted movement (spores on clothing/vehicles/containers/etc)**
- **Water splash from rain and irrigation (wet spores are difficult to move by air)**
- **Animals both native and domestic (possums, cats, birds, insects, etc)**

The following simple strategies should be applied (where possible) across all businesses growing/selling myrtle rust host material (myrtaceous species). It is further recommended to consider this program for all plants within the Myrtaceae family:

6.1 Production Nursery (including propagation)

Ensure a high standard of awareness of the disease at all staff levels

- Advise staff to avoid any plant contact prior to arriving at work & wear clean clothes
- Have on-site disease (myrtle rust/guava rust) identification information for all staff
- Train staff on disease identification & good hygiene practices (see State biosecurity websites and Nursery Paper December 2004 Issue No: 11 at www.ngia.com.au)
- Disinfest all equipment/vehicles that move off-site and return to operate within the production area

- Limit the access of people (visitors & staff) to your production areas
- Implement a hygiene protocol for essential visitors (contractors, etc) to production areas including awareness of previous work sites, inspection of clothing/tools, etc and if required provide disposable overalls while on-site
- Restrict all non-business vehicles from entry to production areas, disinfest if required on-site – APVMA Permit: PER10535
- Remove myrtaceous plants from driveways and carparks or prune to avoid possible visitor contact
- Consolidate all myrtaceous plant species within a defined area on-site away from native or landscape planted myrtaceous plant species and avoid direct exposure (buffer) to the prevailing winds of the season
- Allocate specific staff to manage all myrtaceous species
- Source myrtaceous plant material from known professional growers (e.g. NIASA Accredited)
- Request **all** suppliers of myrtaceous plant material provide evidence that they are adhering to this **Myrtle Rust Management Plan (see attached declaration page 23)**
- Maintain a quarantine area for imported nursery stock
- Inspect (at quarantine area) and treat (curative fungicide) imported myrtaceous species prior to incorporating into growing areas (7 days and re-inspect). It is recommend this be applied irrespective of the source **(see Sampling Protocol below)**
- **Inspect all myrtaceous species prior to despatch (see Sampling Protocol below)**
- Monitor all myrtaceous plant species weekly across growing areas for disease symptoms (particularly inspect areas of crop that have high humidity e.g. centre of batch and on the side exposed to prevailing winds) **(see Monitoring Protocol below)**
- Ensure growing areas remain free of all waste vegetative material
- Increase plant spacings where appropriate to reduce humidity levels within crops
- Periodically (monthly) survey myrtaceous species growing on-site or along property boundaries/roads/etc. Pay particular attention to plants located upwind based on the most common prevailing wind direction of the season
- **Implement a fortnightly fungicide treatment program across all myrtaceous plants (see recommended program(s) Section 5.2)**
- Treat with a disinfectant (e.g. copper) the growing area upon the completion of the crop growing cycle before placing a new crop down on the production bed
- Dispose of all extraneous vegetative plant material from crop management such as pruning, detailing or from natural desiccation via bulk waste, composting or deep burial
- Assess irrigation system and timing to ensure plant surfaces are dry within a short period (less than 6 hours) after irrigation. Avoid irrigating late afternoon which allows water to sit on surfaces for periods of 6 hours or more during the night. Consider installing drip/capillary or other under canopy irrigation system to myrtaceous plant species
- Access industry guidelines such as NIASA and **BioSecure *HACCP*** for guidance in developing monitoring/surveillance/inspection programs and recording templates.

6.2 Propagation (specifics)

As above plus:

- Maintain high health practices in propagation (surface/implements/equipment disinfestation, staff hygiene, etc)
- Staff to wash hands before commencing work in propagation area (start of day/after breaks/etc) using a recognised hand sanitation product

- Propagation staff to undertake any field activities at end of day and not to re-enter propagation area.
- If possible provide staff with clothing or coveralls (e.g. disposable overalls) for moving outside propagation into production areas if required
- Avoid using adsorbent surfaces such as timber, cement board, fibro, etc as propagation work surfaces unless covered with 200 micron thick black plastic (replace when cut/punctured/damaged)
- Regularly disinfest propagation surfaces throughout the day at various points such as upon returning from a break, a change of species or batch
- Disinfest all items including surfaces using a recognised industry disinfectant such as:
    - Quaternary ammonium (e.g. PathX, Sporekil, etc)
    - Combination of 70% Methylated Spirits and 30% water
- **Avoid sourcing vegetative propagation material from myrtaceous plant species off-site**
- Ensure **off-site** motherstock for **non-myrtaceous** plant species are inspected and not located within 10m of myrtaceous plants
- Prior to taking vegetative propagation material from **off-site** motherstock survey the area and inspect all myrtaceous plants for signs of Myrtle rust
- Motherstock must be monitored and inspected at weekly intervals
- **Implement a fortnightly fungicide treatment program across all myrtaceous motherstock (see recommended program(s) Section 5.2)**
- All myrtaceous vegetative cuttings should be dipped in a bath containing a recognised disinfectant prior to sticking such as diluted chlorine, a specific quaternary ammonium (PathX/Sporekil/etc) that has low phytotoxicity or an approved fungicide. **Note:** Test on a sample to ensure the product is not phytotoxic to your plant species
- Consolidate all myrtaceous plant species within propagation houses (dedicated house) and hardening off/growing areas
- Monitor and inspect struck cuttings on a weekly cycle (see Monitoring Process below)
- **Implement a fortnightly fungicide treatment program across all myrtaceous plant species in propagation houses and hardening off/growing areas (see recommended program(s) Section 5.2)**
- Treat with a fungicide (e.g. copper) the growing area upon the completion of the crop growing cycle before placing a new crop down on the propagation bed/bench and production bed

6.3 Greenlife Markets/Retailers

- Ensure a high standard of awareness of the disease at all staff levels
- Advise staff to avoid any plant contact prior to arriving at work
- Have on-site disease (myrtle rust/guava rust) identification information for all staff
- Train staff on disease identification & good hygiene practices (see State biosecurity websites and Nursery Paper December 2004 Issue No: 11 at www.ngia.com.au)
- Restrict all non-business vehicles from entry to greenlife stocking areas
- If possible remove/prune myrtaceous plant species from carparks, driveways, etc that could come into contact with staff and customers or could overhang greenlife stock
- If possible allocate specific staff to manage all myrtaceous species
- Request all suppliers of myrtaceous plant species to certify the plant material is grown under this industry **Myrtle Rust Management Plan (see declaration template page 23)**
- **Inspect <u>all</u> plant material at receival point with a close inspection of all myrtaceous plant species (see Sampling Protocol below)**

- Consolidate all myrtaceous plant species within a defined area on-site away from native or landscape planted myrtaceous plant species and avoid direct exposure (buffer) to the prevailing winds of the season
- Keep all areas stocking myrtaceous plant species free of waste vegetative material such as leaves/flowers/fruit etc dropped by plants
- Periodically, if possible, apply a recognised disinfectant treatment at monthly intervals over holding area(s) where myrtaceous plant species are stocked/placed/held
- Conduct weekly monitoring inspections of all myrtaceous plant species **(see Monitoring Protocol below)**
- Periodically (monthly) survey myrtaceous species growing on-site or along property boundaries/roads/driveways, etc. Pay particular attention to plants located upwind based on the most common prevailing wind direction of the season
- Dispose of all extraneous vegetative plant material from crop management such as pruning, detailing or from natural desiccation via bulk waste, composting or deep burial
- Have staff inspect all myrtaceous plant species at paypoint(s)
- Assess irrigation system and timing to ensure leaf surfaces are dry within short period after irrigation. Avoid irrigating late afternoon which allows water to sit on surfaces for periods of 6 hours or more during the night. Consider installing drip/capillary or other under canopy irrigation system to myrtaceous plant species
- Access industry guidelines such as NIASA and **BioSecure** ***HACCP*** for guidance in developing monitoring/surveillance/inspection programs and recording templates

Note: For home garden treatment see APVMA Permit – PER12828

6.4 Infected Crop Management

Crops found to be infected with myrtle rust can be managed by a range of options depending on part or entire batch infections and preferred treatment method. The treatments identified below are in addition to the activities and fungicide treatments being employed by the business under this plan (Sections 6.1, 6.2 & 6.3). After the below strategy is applied immediately reinstate the fungicide rotation program under the Myrtle Rust Management Plan.

6.4.1 Entire crop infected:

- Entire batch – spray with a registered fungicide (mancozeb or copper) and destroy infected crop(s) by composting on-site and treating adjacent host material with a registered fungicide (e.g. Bayfidan or Tilt or Plantvax) ; or

- Entire batch – spray with a registered fungicide (mancozeb or copper) and destroy infected plants by disposing to landfill and treating adjacent host material with a registered fungicide (e.g. Bayfidan or Tilt or Plantvax)
    - Consign plants to landfill in an enclosed vessel (bulk bin/plastic bags/etc); or

- Entire batch – spraying with a registered fungicide (Bayfidan, Tilt or Plantvax), pruning infected material and disposing of infected material as above. Remaining crop is placed in a high risk fungicide management plan for 3 consecutive fortnightly spray rotations (total of 6 weeks) using Bayfidan/Plantvax/Bayfidan in the rotation (see Table 5.2) before despatch

6.4.2 Part crop infected:

- Part batch – spray infected plants with a registered fungicide (mancozeb or copper) and treat remaining batch and adjacent host material with a registered curative fungicide (e.g. Bayfidan or Tilt or Plantvax). Destroy infected plants by composting on-site; or

- Part batch – spray infected plants with a registered fungicide (mancozeb or copper) and treat remaining batch and adjacent host material with a registered curative fungicide (e.g. Bayfidan or Tilt or Plantvax). Destroy infected plants by disposing to landfill
    - Consign plants to landfill in an enclosed vessel (bulk bin/plastic bags/etc)
    - Untreated infected plant material can be solarised in black plastic bags for three weeks before disposal; or
- Part batch – spray entire batch and adjacent host material with a registered fungicide (Bayfidan, Tilt or Plantvax), prune infected material and dispose of infected material as above. Remaining crop is placed in a high risk fungicide management plan for 3 consecutive fortnightly spray rotations (total of 6 weeks) using Bayfidan + mancozeb/Plantvax + mancozeb/Amistar + mancozeb in the rotation before despatching off-site.
- If re-using containers from infected plants disinfest by soaking in an approved sanitiser or heat treating (steam) at 60°C for 30 minutes.

Note: Follow all appropriate instructions for applying and handling plant material treated with a fungicide. Avoid handling fungicide treated plant material for a minimum 48 hours unless otherwise directed (label/APVMA permit).

7. Monitoring and Inspection Sampling Protocol

7.1 Monitoring Process

The following table provides growers with the number of plants required to complete an appropriate weekly crop monitoring plan (in-field). All aerial parts of the selected plant must be inspected including upper and lower surfaces of leaves with a keen focus on young growth, buds, flowers, shoots, green stems, etc.

Crop Monitoring Process - Myrtle rust weekly monitoring program

Enter each block or bench of plant material looking for abnormal plant symptoms
Walk at random through the area in a zigzag pattern (pay particular attention to plants lacking vigor or with obvious foliage lesions, or disease symptoms, etc)
Take at least 15 minutes to inspect 20 to 30 plants in containers or 10 – 15 tube/plug trays per 100m^2 of production area
Inspect the tops and bottoms of leaves/stems/buds/fruit looking for any direct evidence of the disease
Inspect the entire above ground area of the plant(s)
With larger plants, select leaves from all parts of the plant (upper, middle, lower) and examine them individually
Inspect the length of all stems and branches for insects, mites, and disease symptoms
Using an identification guide (images), identify any symptoms observed
Myrtle rust free state/territory - if a suspect infection is identified either leave it in-situ or place the plant in a plastic bag (if at dispatch/retail) and contact the relevant state/territory biosecurity agency

Myrtle rust infested state/territory – if a suspect infection is identified either leave it in-situ for complete batch fungicide treatment in-field or if at dispatch/retail place the plant in a plastic bag and move to a fungicide treatment area (See Section 6.4 for information on infected crop management)
Record on the 'Crop Monitoring Record' sheet (see BioSecure *HACCP*) relevant monitoring information
Observe any situational problems such as malfunctioning sprinkler heads
Routinely inspect growing areas and remove alternate hosts and reservoirs of disease and insect vectors, including weeds, crop residue, and old plants that will not be marketed.

7.2 Despatch Sampling Process

The following tables provide growers with the number of plants required to complete an appropriate sample size for consignment inspections (dispatch). All aerial parts of the selected plant must be inspected including upper and lower surfaces of leaves with a keen focus on young growth.

Despatch sampling methodology

The plants, cartons, trays or containers forming a consignment should be inspected as close as practicable and not more than 48 hours prior to the time of dispatch
Before undertaking the inspection the Nursery Manager will determine the sampling to be applied to the consignment as per below method
Depending on the size of the consignment one of the two sampling methods (below) may be used
The number of plants/ cartons/trays/containers (package) selected for inspection must be chosen at random.

Despatch sampling method

Nationally agreed sampling regime, as per **ICA 42 Nursery Freedom, Treatment and Inspection for Myrtle Rust**, can be undertaken at one of the following two points in the despatch process:

1. End-point inspection; or
2. In-line inspection.

The inspection rate (plants/packages) applied by a business for both end-point and in-line inspections is either:

- 600 units; **or**
- 2% of the number of packages.

A minimum of three (3) packages will be drawn when undertaking an inspection using the 2% sampling rate. If when applying the rate of 600 units for inspection and the total number of units is less than 600 then all units in the consignment shall be inspected.

Package means the complete outer covering or container used to transport and market the produce.

A unit means one or more plants in a growing container/unit.

An In-line inspection shall involve the selection and inspection of plants drawn from a lot and inspected during the processing and packing of the product.

The business shall sample packed product at the predetermined inspection rate from the packing line and move the packed product to the inspection area for examination.

Packed product means for in-line inspection plants within a growing container or a plant(s) that is bare rooted.

End-point inspections are only carried out on consignments that have been finalised.

The business shall sample packages at the predetermined inspection rate from the consignment and move the packages to the inspection facility ready for examination.

Packed product means for end-point inspection plants that have been packed into its final package.

Note: The Australian Nursery Industry has a complete guide for on-farm biosecurity protocols and procedures (BioSecure *HACCP*) available from state associations. Information on sterilisation, disinfestation, sanitation, quarantine, monitoring and inspecting, etc is available in this concise manual.

8. Interstate Movement Controls

Since early May 2010 there have been various movement controls put in place by a number of state and territory plant health agencies to manage the risks associated with the movement of host plant material. The following table summarises the status of current myrtle rust movement controls by jurisdiction as at February 2012:

The below table is a guide only. Prior to interstate movement of greenlife please contact your state/territory biosecurity agency (see contact details below) to receive the most up to date movement controls of the receiving jurisdiction.

Movement Controls February 2012

Jurisdiction	Myrtle Rust Movement Controls
Queensland	Must be free of myrtle rust – illegal to sell an infected plant
New South Wales	Must be free of myrtle rust – illegal to sell an infected plant
Australian Capital Territory	Must be free of myrtle rust
Victoria	Restrictions on myrtaceous plants from an infected jurisdiction
South Australia	Restrictions on myrtaceous plants from an infected jurisdiction
Northern Territory	Restrictions on myrtaceous plants from an infected jurisdiction
Western Australia	Restrictions on myrtaceous plants from **all Australian** jurisdictions
Tasmania	Restrictions on myrtaceous plants from **all Australian** jurisdictions

Note: WA will **not** accept plants of the Myrtaceae family irrespective of treatment from any jurisdiction except TAS. All species in the Family Myrtaceae are currently prohibited entry to Tasmania unless prospective importers have sought and been granted written approval to import by means of a Section 67 exemption under the *Plant Quarantine Act 1997* via the DPIPWE.

Interstate Certification Assurance (ICA) Arrangement

Biosecurity Queensland (BQ) has developed the Interstate Certification Assurance arrangement for myrtle rust (**ICA 42 Nursery Freedom, Treatment And Inspection For Myrtle Rust**) and is available to Queensland and New South Wales production nurseries for access to South Australia, Victoria and Northern Territory markets. To arrange an ICA 42 application contact Biosecurity Queensland on 13 25 23 or I&I NSW on (02) 6938 1976.

State/Territory Biosecurity Agency Contact Numbers:

Queensland – 13 25 23

South Australia - 1300 666 010

Tasmania - (03) 6233 3352

New South Wales - (02) 6938 1976

Western Australia - (08) 9334 1800

Victoria - 13 61 86

Northern Territory - (08) 8999 2118

National Exotic Plant Pest Hotline: 1800 084 881

Note: Individual jurisdiction entry conditions must be followed at all times

Myrtle Rust Management Plan Declaration

Nursery & Garden Industry

Business Name:……..

Address:……

Telephone:…… **Email**:……

Invoice Number:……**Date:**……..

I the undersigned declare that …….. has implemented the Australian Nursery Industry **Myrtle Rust Management Plan** (the plan) and are applying all relevant aspects of the plan to all myrtaceous plant species grown on-site. All myrtaceous plants in this consignment (as per Invoice) have been treated under the plan.

……. has applied the following protocols of the plan to ensure the risk of receiving/introducing and/or distributing myrtle rust is reduced and managed to the best of our ability. **Date of last fungicide application: ……../……../…….**

Note: (Insert business name in the above blank fields)

PROTOCOL IMPLEMENTED	N/A	Y/N	PROTOCOL IMPLEMENTED	N/A	Y/N
Myrtaceous plant material is imported onto this site(s)			Myrtaceous plant material propagated is sourced on-site		
All myrtaceous plant material is propagated and grown on-site(s) (no imported material)			Myrtaceous plant material propagated is sourced off-site		
All myrtaceous plant material imported is accompanied by this Declaration from suppliers			All myrtaceous plant material propagated comes from motherstock inspected and treated as per the plan		
All myrtaceous plant material imported is inspected upon receival by trained personnel			A sound hygiene system has been implemented across all aspects of myrtaceous plant production		
All myrtaceous plant material imported is treated with a curative fungicide upon receival			A sound waste disposal system for greenlife residue is in place		
All myrtaceous plant material grown is monitored & inspected at weekly intervals			Visitor vehicles are denied access to production area		
All myrtaceous plant material grown is treated at appropriate intervals as recommended under the fungicide program in the plan (see Tables 5.2 & 5.3)			A hygiene system is in place for essential visitors to the production area		

…… ……

Name **Signature**

Photographs sourced from I&I NSW and Qld DEEDI











Appendix 5

Know-how for Horticulture™

POTENTIAL INDUSTRY DEVELOPMENT ACTIVITY SCHEDULE

PROJECT DETAILS	NEEDS ASSESSMENT TEAM RANKING				ADDITIONAL COMMENTS
Description	Urgency (1–3)	Imp. (1–3))	Impact (1–3)	Success (1–3)	

Know-how for Horticulture™

PRIORITISATION RANKING GUIDE			
Urgency (in the context of the industry's national interest)	**Ranked 1 to 3 with:**		
	1. Very Urgent Must be continued (existing projects) or addressed immediately	**2. Urgent** Must be continued (existing projects) or addressed within the next three years	**3. Not so Urgent** Must be continued (existing projects) or addressed within the next five years
Importance (in the context of the industry's national interest)	**Ranked 1 to 3 with:**		
	1. Very Important Critical to the survival of the industry	**2. Important** Important for the industry's development and growth	**3. Not so Important** Would be valuable to do, funds permitting
Impact (in the context of the industry's national interest)	**Ranked 1 to 3 with:**		
	1. Greatest Impact Very significant impact on the overall industry's profitability and or future viability	**2. High Impact** Considerable beneficial impact, though not of the highest level	**3. Moderate Impact** Impact is limited or restricted to a certain sector, region or group
Success (in the context of the industry's national interest)	**Ranked 1 to 3 with:**		
	1. High Very likely to achieve the outcomes	**2. Moderate** Reasonably likely to achieve the outcomes	**3. Limited** Only a limited chance of achieving the outcomes

Appendix 6

UNIVERSITY OF CANBERRA
AUSTRALIA'S CAPITAL UNIVERSITY

Small-scale food growing in Canberra: opportunities and obstacles

Walter Steensby, Research Assistant
E: walter.steensby@canberra.edu.au
P: 02 6254 3881

David Pearson, Associate Professor
E: david.pearson@canberra.edu.au
P: 02 6201 5720

Sustainable Development and Food Security Cluster
Faculty of Arts & Design
University of Canberra
ACT 2601

Report for the Nursery and Garden Industry Australia

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DISCLAIMER
The information in this report has been collated from publicly-available sources which are identified as resources. However, little information is specific to the situation in Canberra and hence generalisations have been made. The authors take no responsibility for any actions that readers may take on the basis of the information provided in this report.

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ABSTRACT
The growing human population will place resources of food and water under great strain in the next few decades. The phenomenon of peak oil may make fuel for transport and agriculture unsustainably expensive. In Australia urban growth threatens to destroy large areas of agriculturally highly-productive land near cities. One response is to move agriculture nearer and into urban areas with the goal of producing quality foodstuffs close to their places of consumption. Little data exists on the productivity of urban agriculture. A survey of a community gardening organisation in Canberra contributes to data relating to urban food production and suggests some directions to improve gardening productivity in the region.

Contents

1 Introduction

Basic human resources essential for survival are food, water, shelter and energy. With no energy the utility of all other resources rapidly diminishes.

A growing body of literature reports that the human race is heading rapidly towards perhaps the biggest challenge it has faced in its entire history: can we find the resources to ensure our survival as human numbers grow beyond all historical levels? With the world population expected to grow from 7 billion in 2011 to peak at about 9 billion in 2050, it will be tragic to say the least if the population grows beyond the capacity of the planet to feed it.

Organisations such as the FAO are optimistic that crop yields can continue to be increased to meet demand (FAO, 2011), but on the other hand CSIRO researchers warn of an "ominous constellation of factors that now make feeding humanity sustainably our most pressing task" (Cribb, 2010: xi).

One of this "constellation of factors" is the persistent encroachment of urban development onto agricultural land; the world's urban population now exceeds the rural (UN Habitat 2010/2011), a process which is likely only to magnify the rate of agricultural land loss. While Australia is lightly populated by world standards, its agricultural sector is quite productive, feeding something like three times its own population. Since much urban encroachment is onto the best agricultural lands available — cities usually being founded in the most favourable and propitious areas — it seems objectionable in both principle and practice to destroy the agricultural potential of perfectly good food-producing land. Research is proceeding into ways to accommodate both urban expansion and agricultural production; this paper presents one contribution.

With current models of land use, a kind of terminal slash-and-burn mentality may be apparent, where the "last crop" of peri-urban agricultural land is housing or industry. Land is allocated for whatever use seems most profitable at the time, an approach based on the belief that market mechanisms if left unhindered will infallibly allocate resources in the most efficient way. A contrary opinion is that the market mechanism cannot foresee trends, and to destroy the agricultural potential of productive land needed tomorrow for the sake of financial profit today is short-sighted, unwise and a failure of planning. Both approaches can co-exist within the same political system: Bunker cites those of Texas (market driven) and Oregon (state interventionist) (Bunker, 2003: 303).

Another factor in the "constellation" is the rising price of crude oil, an essential feedstock for modern agriculture. The International Energy Agency (IEA) in its *World Energy Outlook 2010* stated that the global production of oil had reached a peak in 2006 and that for the foreseeable future production will remain on a plateau of 68–69 million barrels per day, never regaining the peak of 70 million reached in 2006 (IEA, 2010: 48).

Both factors affect the food supply and hence food security; the first factor simply by reducing the amount of land available for agriculture, the second by increasing costs of production and transport, especially from sources increasingly distant from their place of consumption. If the cost of crude oil rises then the cost of the transport fuel (mainly diesel) derived from it is likely to rise also, and one would expect the extra costs to be passed onto the consumer. It would seem wise to take steps where possible to shorten the food supply chain.

2 Urban Agriculture

One response to this situation is to grow food locally, in peri-urban and intra-urban areas. Local production of food promises enhanced security of supply, a measure of independence from remote sources, and a reduced transportation component of total food cost.

2·1 Peri-Urban Agriculture

Peri-urban agriculture has undergone great development in the past 20 years and yet measurements of its contribution have chronically been largely ignored in statistical collections (Barr, 2003: 127). To resume collecting data relating to this area of agriculture is essential to facilitate research. In 2005 an analysis of available Australian agricultural census data indicated that peri-urban regions generated about 25% of Australia's gross agricultural production from less than 3% of the agricultural land (Houston, 2005: 220–221). This is a significant contribution to the national food supply and any policies which might reduce it need careful consideration.

Significant as such contributions may be, in the context of the city peri-urban land uses do not fit in well with urban uses. Barr observes of Melbourne urban planners that they "are searching for an agricultural pastime that will fill extensive yet fragmented areas. The industry needs to be compatible with the social setting" (Barr, 2003: 127). Problems faced by peri-urban agriculture include urban and rural dwellers having quite different expectations of how land is to be used — "sheep and urban dogs are not a good mix" (Barr, 2003: 127) —, pilferage of crops and theft of tools, vandalism, and complaints made about the sights and smells of agricultural activities.

Again, detailed research is called for to assess ways of developing accommodations with land uses which presently do not comfortably co-exist.

2·2 Intra-Urban Agriculture

The term *intra-urban* is used here to designate both the suburban areas of cities and the more densely built-up areas which conventionally do not come under the heading of Suburban. These may include the city centre itself or densely-settled residential parts such as the *arrondissements* of Paris.

The contribution of intra-urban agriculture to the national food supply is little mentioned in the literature. A recent contribution is a survey of home food gardening in Toronto, Canada undertaken by Kortright and Wakefield (2010). They observe that while in the order of 600 million people worldwide work at urban agriculture in a variety of settings such vacant lots, roadside verges, allotments, backyards, balconies and verandahs, most studies have focussed on countries such as Cuba (Kortright & Wakefield, 2011: 40), seldom on the industrial West or Australia. Data relating to the output of urban agriculture from the latter countries is hard to obtain or non-existent, partly because the lands used and the output gained are private, and partly because Western town planners have historically tended to view urban agriculture as a dirty and undesirable land use, better zoned out of cities (Girardet, 1999: 59).

A fairly recent change in the character of Australian suburbia has been that of increasingly large houses occupying the same or smaller lot sizes. With the exception of the front setback, the area available for gardening or indeed any kind of planting is greatly reduced. Among other things, such densification is producing the effects of increased heat-island effects, reduced biodiversity, and greatly reduced human contact with nature (Hall, 2009: 8–10). By encouraging natural systems, even small ones, to return to urban areas, intra-urban agriculture may provide opportunities for local health improvements and community development (Wakefield *et al.*: 93). Studies indicate a strong correlation between ambient temperature and the presence of large green areas in a city (Wong & Yu, 2005). Modern data on the environmental and other social and health aspects of urban agriculture is needed to help assess its benefits and usefulness.

In countries such as Uganda, the influence of Western town planning theories and ideologies has long hindered the development of urban agriculture, and it is increasingly accepted that urban agriculture must be re-admitted to cities by being legalised and regulated. (Rutt, 2007: 61) In such places, urban agriculture is an important food source and a major contributor to dealing with rural–urban migration. Faced with a similar range of problems and challenges, Australian urban planners and managers arguably should be considering equivalent policies and practices in this country.

While interest in intra-urban agriculture is growing in Australia, the development of food production within urban areas (and the reaping of a range of concomitant benefits) remains rudimentary (Capon & Blakeley, 2007: 52). One way to promote the growth of intra-urban agriculture may be to exchange the sanitarian approach to environmental health for the ecologist. The grounds for suggesting this change are that the Victorian-era strategy of imposing technical solutions on natural systems is obsolete; a new strategy should be adopted aiming at sustainable development by means of working with the natural characteristics of ecosystems (Ashton, 1991). Such a change would accord well with the modern emphasis on respecting and conforming to ecological principles.

2·3 Functions of the Community Garden

Apart from the traditional backyard garden, another method of intra-urban food production is the community garden. Community gardens are especially interesting in terms of food security because they are "places where private and public responses to issues of food insecurity intersect" (Evers & Hodgson, 2011, 585). Further, they act in more than one role at a time: they are places where food is grown, where other gardeners and the broader public are educated about how to grow food, and where social networks are built.

The community garden is not a new development in western society. Its origins may be traced back to pre-industrial farming and as a reaction to the enclosure of the Commons in England which started at the close of the 15th century and was largely complete by the end of the 19th (Johnson, 1909; Stocker & Barnett, 1998). The community garden also may be interpreted as a response to the modern industrial food system (Evers & Hodgson, 2011) which has come under increasing criticism in recent years.

A simple definition of a community garden is that it is "[a]ny piece of land gardened by a group of people" (American Community Gardening Association, 2012). This is too simple, however; other authors observe that what distinguishes a private garden from a community garden is the latter's public character in terms of ownership, access and some degree of democratic control. As an ever-greater proportion of the world's population moves into cities, the demand for community gardens seems to be increasing (Ferris, Norman & Sempik, 2001: 560). The ACT is no exception: on 9 March 2011 the ACT government resolved *inter alia* that all new residential subdivisions must have space set aside in them for community gardens, and that resources should be provided at some level for support personnel, training and the development of policy regarding local food production (ACT, Legislative Assembly, *Debates*, 2011).

However, there is not a great deal of material analysing the contributions and effects of community gardens upon urban life: "much of the evidence used to support community gardens is anecdotal" (Wakefield *et al.*, 93). The academic literature has so far addressed mainly the social and civic benefits of community gardens, and only occasionally their health and well-being benefits (Evers & Hodgson, 2011). For example, migrants can find in a community garden a valuable and flexible environment which helps them cope with adapting to Australian culture and civilisation (Turner, 2010). Few studies have explicitly focused on the health impacts of community gardens, and a surprisingly small number of published studies have actually talked with community gardeners about their experiences.

3 Directions for Research

Empirical research is needed to investigate the feasibility and possible implementation of food production strategies in Canberra and the ACT region. There is much scope for further research into the contribution of both backyard and community-garden food-growing practices to food security issues at the community level.

Canberra is a city set in a comparatively cool, dry region with impoverished soils. Prior to the city's establishment as the national capital, land use in the area was confined mainly to sheep grazing with some cattle.

One question it seems reasonable to ask is what kinds of urban agriculture are feasible in the Canberra region, to determine what can be grown sustainably using preferably only local resources and no infrastructure such as greenhouses and/or large inputs of fertiliser and water. Does it make economic sense to undertake intra-urban and peri-urban agriculture at any level?

A second question is to identify the major components of the Canberran diet and, if the proportions of the components are altered, assess the effects upon local agriculture. For instance, if people in general were to eat more vegetables and less red meat, might local agriculture be called upon to supply the demand and what savings might accrue from importing less meat?

Little basic data exists on these topic areas nationally or at the ACT regional level. In particular, the ABS has collected no data on the non-commercial (i.e. domestic) production of fruits and vegetables since 1992, and the usefulness of this old data set has yet to be assessed. If modern data can be obtained, a simple longitudinal data set may be derived from the 1992 and 2011 observations.

4 COGS, a source of modern data

Like many towns and cities Canberra hosts a number of community gardens. Most are run by the Canberra Organic Growers' Society (COGS), a not-for-profit organisation established in 1977 to promote organic gardening practices.

In August 2011 COGS had 430 members, of which 17 were other groups or institutions and 413 persons or families (amounting to 500–600 individuals). The minimum age for membership is 18. COGS has 11 community gardens with a total area of about 1·7 hectares around Canberra. Eight are intra-urban, and three are peri-urban although located quite close to built-up areas.

Only COGS members may use a plot in a community garden, and each garden is administered along democratic lines by a Convenor elected by that garden's members. This makes each garden largely self-managing. Plot holders pay an annual levy (currently \$2 per m^2) to cover running costs such as water (the major budget item), insurance, fencing, plumbing, and so on. The gardeners are expected to use fairly strict organic methods as set out in the *National Standard for Organic and Biodynamic Produce* (Canberra Organic Growers' Society, 2009: 4). COGS has a constitution and a gardening policy setting out members' rights and responsibilities (Canberra Organic Growers' Society, 2010).

Each garden is divided into two main areas: one of all the plots allocated to individual members; the other a communal area. This latter has a garden shed for communal tools and some personal ones; it may also have among other things barbecues, pergolas, glasshouses, compost heaps, herb gardens, seed-saving plots, fruit trees and bird habitat shrubs. The communal nature of the gardens facilitates social interactions in a variety of ways: working bees, open days, recreational barbecues, morning teas, and so on.

5 Research Procedure

5·1 Background to COGS Involvement

In the 34 years since the formation of COGS the nature of organic gardening has changed, and in 2010 the COGS Management Committee (the Committee) decided to conduct a survey of its members to canvass their opinions on what goals COGS should pursue and how to run the organisation.

The opportunity was taken to gather data relating to a variety of factors relevant to research into intra- and peri-urban agriculture in the Canberra region. In particular, the Committee was aware that many COGS members garden in their backyards and other areas besides community plots, but had no further knowledge of numbers of people or land areas involved. The Survey promised to contribute data to an otherwise poorly-surveyed domain of knowledge.

Other topic areas related not only to management issues but also to key factors such as why members use a community garden, the types of fruits and vegetables grown, what grows well in the Canberra climate, who does the work, when it's better to purchase than grow, estimates of yields, numbers of people fed, contribution to the family budget, and basic demographic statistics.

5·2 Research Questions

The following two questions provide a platform for research:

Research question 1: ***what scope exists to produce fruits and vegetables locally in the urban area of Canberra?***

Research question 2: ***Is it possible to quantify the contribution of non-commercial (i.e. domestic) fruit and vegetable gardeners to Canberra's food supply?***

5·3 Survey Method and Questionnaire Design

The Survey was done by means of a self-completion questionnaire administered on the Internet-based SurveyMonkey platform. It consisted of 31 questions, a mixture of closed format for basic demographic data, some questions with fixed variable responses (e.g. Likert-style items), and open questions where the respondents could write replies however they wished.

Invitations to the Survey were sent to all personal members with a working email address: this came to 371 invitations. It had been hoped to send ordinary mail invitations to the remaining 42 members but time did not permit. A total of 135 replies was received, a statistically-significant number.

6 Results

6·1 Demographics

Gender

The COGS respondents were 40/60 male/female; the 2011 ACT population was 50/50. The COGS Membership Officer does not know the exact split but feels that numbers are about equal. For whatever reasons, more women than men seemed willing to complete the Survey.

Age Distribution

In ages COGS members are different to the ACT population. Only 7% are in the youngest (20–29) group; almost 75% of its membership is middle-aged (40 to 69). The ACT population by contrast exhibits a conventional population pyramid structure, with 18% in the 20-29 group and 35% in the middle-age group. Both populations have about 3% in the 80-and-over group.

Employment Status

COGS has more part-time and retired people than the ACT in general, very few students (a contributing factor may be the minimum age of 18), somewhat fewer full-time workers, and no-one claimed to be unemployed. This situation is reasonable: young students and full-time workers have little or no spare time for gardening. The lack of unemployed people could be explained partly by the COGS joining fee and annual levy; it could also show that some unemployed can't afford a computer or Internet access and hence did not receive a questionnaire invitation.

Income Levels

COGS members are comparatively well-off with almost five times as many people (42%) earning over $100,000 as the ACT (9%). The middle-income levels are comparable, and no COGS member is on the lowest income level.

Household Size

For families of 4, 5, or 6 & over, COGS is similar to the ACT. It has far fewer single-person households (one third) and far more two-person households (almost double) than the ACT. It may have been useful to know what *type* of dwelling the respondent lives in (flat, townhouse, detached house, etc.). This data could provide extra insights into the value people find in a community garden.

Length of COGS Membership

The shortest period is one month, the longest 24 years. The average is 3½ years and the mode is 2 years. These low numbers could indicate a fairly recent increase in membership, or a comparatively rapid level of turnover. The COGS Committee will be approached for information on this topic.

6·2 About the Gardens

Reasons for using a community garden

Note that respondents could select more than one response option, and hence percentages total more than 100%.

Almost 80% of respondents use a community garden simply because they can grow more there than in other places, such as the backyard. About 50% the respondents wish to make their food supply more certain, and another 50% to save money.

Three-quarters use it for education — of the children or of themselves — and some 15% use it to help the sick, aged, or disabled. About 20% use it for cross-cultural activities.

The desire to improve the urban environment is clear: 30% work to re-green unused or derelict land, 40% to set an example to the broader community by growing, recycling, composting, etc., and over 50% to bring gardening and some form of agriculture back into urban areas.

Reasons for growing own fruits and vegetables

This was a multiple-choice question plus an answer box for open-ended responses.

Categories attracting over 80% of the responses each are Simple Enjoyment, Enjoy being Outdoors, Better Quality Food, and Better Tasting Food. In other words, the pleasures of gardening itself are accompanied by the pleasure of the produce.

The categories attracting from 50 to 80% are basically health-related: Avoid Synthetics, Improve Health, Relaxation & Exercise, Live Greener, and Prefer Seasonal Fruit and Veg. However, the respondents are apparently looking at these as indirectly-achieved health gains, because only 9% ticked the box labelled Help Cope with Health / Medical Problems.

The lower-rating categories include Feel Independent, Reduce Food Costs, Climate Change, and Ecological Diversity.

Types of garden used — where growing is done

Most respondents (86%) use their backyard for gardening, and somewhat over half (58%) use a COGS community garden. A small number (15%) use the verandah, porch or pots.

The question did not differentiate between people using a community garden or a backyard garden or both: further research into this area would clarify supply and demand relationships.

Size of garden — actual and ideal

Actual: the average area of gardening land, community and/or backyard, is 95 m^2, with a median of 52·5 m^2 and the most common areas 40, 50, 100 and 250 m^2.

A small number of respondents reported areas of "¼ acre", "½ hectare", "2,000–3,000 msq" and "6 acres". These large areas hardly seem to qualify as backyard or community gardens and are unlikely to be within or close to the Canberra built-up area; they have been omitted from analysis.

This question did not ask the respondents to identify which particular *type* of garden (community, backyard, other) is used, although some of the open-ended responses provide some information.

Ideal: 42% of respondents are content with the amount of land they are using now. The remaining 58% would like just over 13 times as much land as they currently use. However, most of the demand comes from just 14 respondents who would like anything from 1,900 to 20,000 m^2 extra. This is a wish list, after all.

If we omit these 14, we learn that the average amount of land desired rises from 95 to 123 m^2 per respondent, not quite 30% extra and a fairly modest increase.

Considering that over half the people who are already gardening would like more land, that COGS has a 3-year waiting list for some of its community gardens and that the Committee is assessing several proposals for new community gardens, it is clear that a constant level of demand exists for more and larger garden areas in urban Canberra.

Seasonality of gardening work

When the work is done in community gardens is strongly affected by the season. In Spring most work — presumably preparatory — is done weekly (50% of garden activity) with half as much daily; in Summer daily and weekly occasions are about equal in number (40%); and in Autumn most work is done weekly (47%) — presumably harvesting and tidying. Few people work less often than weekly.

Winter is quite different. Nobody visits daily, but there is a constant low level of activity with about 10% of work visits made weekly, fortnightly and/or monthly.

When it comes to backyard gardens, the pattern is very similar except that people make almost as many daily visits as weekly. This is logical: the average backyard garden is a lot closer to home than the community garden.

Who does the work?

The respondent does most of the gardening work, everywhere.

In the community garden the respondent does not quite 60% of the work, the spouse helps 20% of the time and the children 10%. In the backyard the respondent does about 90%, the spouse 40% and the children 15%. In all places other people — extended family or friends & neighbours — help occasionally (10%).

Transport

Just over two-thirds of respondents go by car, one-fifth by bicycle, one-eighth walk; a single respondent car-pools, and nobody goes by bus. Why this latter should be the case is unclear. One may surmise that carrying tools on the bus is not easy, or that people would prefer not to travel by bus if they're hot and dirty and sweaty, or that in Autumn carrying produce home is difficult.

A next step in the research may be to analyse the location of COGS community gardens in relationship to bus routes and timetables.

The respondents' postcodes were collected, which when analysed in conjunction with the relevant community garden locations will provide origin-destination pairs and indicate travel desire lines. This phase of the analysis has yet to be completed.

Sources of difficulties in growing in Canberra

Only a 5% of respondents have no problems; the other 95% mentioned at least one concern.

The most-often mentioned difficulty (33%) is Climate: by Australian standards Canberra's is cold. Respondents repeatedly mentioned cold winters, short, hot, dry summers, and the short growing season. By way of comparison, Hobart has a similar rainfall and mean temperature range, but Canberra, owing to its inland location, has greater extremes of temperature and more clear days annually — 100 vs 41 — than Hobart (Australian Bureau of Meteorology, 2012).

The next most often-mentioned topic is Rainfall & Water. Although rainfall returned to normal about a year prior to the Survey, memories of the drought were strong and are reflected in the responses. The gardeners are conscientious about not wasting water, have trouble finding time to water adequately, wish COGS would install some kind of semi-automated watering system, and complain about the cost of water. Water prices are not set by COGS and water is COGS' chief expense, to the extent that a leaking pipe could — and one occasion nearly did — bankrupt the organisation.

Animal and Vegetable pests come next (14%), mainly insects, possums, cockatoos, weeds and couch grass. Rabbits are a problem in a couple of community gardens.

Soil Quality is not quite as bothersome (12%) an issue. Canberra is located in a broad limestone plain with impoverished clay-based soils. Prior to the city's establishment the area was used mainly for sheep and cattle grazing. One COGS principle is that the soils of community garden plots should be built up *in situ* by means of composting, green mulching and so on; soil should not be imported on the grounds that another landscape should not be denuded to benefit another. COGS' experience is that it takes 3 to 5 years to get a brand-new plot into good growing order. Some gardeners find this frustrating, and it may be a factor in the short average membership period of 3½ years.

Minor problems include Lack of Space, Lack of Time and "Other" (10%). It is interesting that Lack of Space figures so little in the responses to this question, because in responses to the question asking about actual and ideal size of garden (see above), 58% of gardeners would each like 30% more land. This may simply reflect the perception that difficulty in growing is related to environmental factors rather than to size of plot.

6·3 About the Produce

Fruits and vegetables — most desired, best performers, if-only

The respondents were asked which species they prefer to grow, which grow best for them, and which they would grow if only they could.

Dealing first with the wish list, about two-thirds of the respondents evidently wish to relocate well north and on the coast, wanting to grow avocadoes, mangoes, pineapples, bananas and so on. However, the actual numbers of mentions of a species or variety are small — e.g. avocados and mangoes with 18 each, pineapples with but one mention — and it may be concluded that most growers are realistic about growing conditions and expectations.

However, some species in the wish list are already being grown locally by others, such as apples, herbs, brassicas and strawberries. This is where the educational capacity of the community garden should come into play to share expertise in a structured manner. In fact, the Committee has em-

barked upon just such a program: training courses are being designed, and all back-issues of the newsletters and magazines have had their didactic content extracted for assembly into a set of instructional leaflets and booklets relating closely to the Canberra regional environment.

Dealing now with the other two categories, the most frequently-mentioned species are almost identical:

Prefer to Grow	*Number of Responses*	*Grow Best*	*Number of Responses*
onions	138	onions	96
brassicas	121	herbs	76
herbs	103	tomatoes	76
beans	101	beans	72
tomatoes	94	brassicas	72
stone fruit	84	spinach	71
squash	83	stone fruit	51
spinach	73	squash	50
peas	71	potatoes	46

The complete list names 63 different species or varieties. By way of comment, single-mentions include celery, chestnuts, chicory, chinese quince, guavas and passionfruit.

It appears that COGS growers are making do with what the climate permits with experience being the best teacher. The differences between what the respondents would like to grow and what they actually can and do grow suggest a level of frustration with growing conditions that may reflect an unsatisfied demand for selectively-bred climate-tolerant varieties, for suitable species or varieties yet be introduced into the Canberra region, or for small-scale engineering approaches such as greenhouses, polytunnels and the like.

As a supplementary research topic it may be useful to ask COGS members how they *use* their produce: how much is eaten at once, how much preserved and in what manner, how much shared and donated, and so on.

Numbers fed by gardening

Note that this data and information refers to the *number* of people fed, not to the *amount* of food provided.

Just over 40% of respondents grow for two people, which may be interpreted as couples gardening for their own needs. The next largest number of people fed is four (19%), perhaps representing two adults and two children, followed by three (15%), six and over (14%), one (7%), and finally five (4%) people. A pattern emerges: more even numbers of people are fed than odd numbers. This may be a function of Canberra household structure, or it may be a coincidence.

It is difficult to calculate how many people are fed by COGS gardeners because the final household size category is the open-ended “6 and over”: it is unknown how many households have 7 or more people. If the household size in this category is assumed to be 6, then the numbers fed are 123% of the numbers growing.

Hence we might deduce that the 120 respondents to this particular question feed in total almost 148 people including themselves. Extending this to the 413 non-corporate members, COGS members collectively feed around 508 people out of all gardening sites. If the COGS membership is 500–600 actual persons, then collectively these feed 615–740 people in total.

Proportion of diet own-grown

Not quite 2% of the respondents grow all of their fruit and vegetable diet; 10% grow three-quarters, 25% grow half, 56% grow one-quarter, and 6% grow none.

Analysis indicates that 120 people grow enough fruits and vegetables for 44 people, a ratio of 0·36:1. Another interpretation is that on average all the respondents produce just over one-third of their fruit and vegetable needs by their gardening work.

Money saved

Each respondent estimates average weekly savings of $15·36 or $798·57 annually. For all respondents the total annual saving is $89,440.

Extrapolating this to the 413 individual COGS members, the total annual savings are estimated to be just over $273,000. These figures need to be checked against data of fruit and vegetable sales by other outlets.

6.4 About the Benefits

Feeling better

Most respondents feel "somewhat better" or "much better" (almost 85%), 15% don't benefit one way or the other, and nobody feels "somewhat worse". One poor soul feels "much worse" — one can only wonder why. This could also be a simple data-entry error.

When to give up and go shopping

A few people (5%) say it is never better to purchase rather than grow. Apart from these dedicated gardeners, 34% of respondents will purchase if either they have "difficulties" in growing, or they want species that don't grow in Canberra.

Better value may be offered by the shops: 15% of respondents will go for it if "costs of inputs far exceeds value of crop", "if there is little difference in taste/quality between home grown and bought", "when it benefits local farmers", or "don't have the space to grow enough".

If the species is out of season then 23% will shop for it — implying that 77% will not, perhaps waiting for next season's crop. If the species is simply not being grown, 14% also will shop for it.

Other reasons (8%) mention lack of time, unreliability of supply, or when the recipe calls for something not in the garden. One respondent purchases extra vegetables throughout the year; another mentions "anything toxic in the soil, or of you just can't look after the plants."

6·5 About COGS itself

Most valued features

Percentages add to more than 100% because respondents could select more than one option.

The most voted-for items were the quarterly *Canberra Organic* magazine (73%), "access to community gardens" (69%) and "gardening advice and support" (53%). Note that the first item offers both social contact and gardening advice, and the third is purely gardening advice and support. It appears that education and knowledge-sharing are quite important to the respondents.

The other categories attracted only about half as many votes: monthly meetings (23%), the web site (21%), special interest groups (seedsavers and backyard gardens) (20%), and education (seminars, training sessions, etc) (16%). Again, the education element is strong but only education of the less structured type. Open-ended comments include "interaction with likeminded persons", "social con-

tact with other gardeners", "mixing with other people who are keen on organic gardening", and "the community garden is a unique environment for my kids to play and learn".

The social aspect of COGS evidently is valued, but the degree and extent are difficult to quantify. Further research into the social aspects of COGS' and similar gardens would be useful.

7 Summary and Conclusions

Canberra is a city set on a broad limestone plain in a cool, dry region with impoverished soils. Prior to the city's establishment as the national capital, land use in the area was mainly for sheep grazing with some cattle.

By Australian standards Canberra has cold winters, short dry summers, and a short growing season. The most comparable city is Hobart with similar rainfall and temperature ranges, but Canberra being inland has greater extremes of temperature and more clear days annually — 100 vs 41 — than Hobart.

Although rainfall returned to normal about a year prior to the Survey, memories of the drought were strong and are reflected in the responses. The gardeners are conscientious about not wasting water, have trouble finding time to water adequately, wish for some kind of semi-automated watering system, and some find the cost of water a major burden.

The differences between what the respondents would like to grow and what they actually can and do grow suggest a level of frustration with growing conditions that may reflect an unsatisfied demand for greenhouses, polytunnels and the like. Scope exists also for selectively-bred varieties which cope better with the climate than existing varieties, but the necessary technologies may be a barrier to progress.

Animal and vegetable pests in general are not a major issue. These include insects, possums, cockatoos, weeds and couch grass, with rabbits an annoyance in a couple of community gardens.

In spite of Canberra's geology, soil quality is not a major issue even though it can take 3 to 5 years to build up the soils of the community garden plots *in situ* by means of composting, green mulching and so on. Organically-based methods and products to shorten this time would be welcome.

Analysis indicates that 120 growers provide some proportion of the fruit and vegetable diet for almost 148 people including themselves. Hence COGS members collectively feed around 508 people out of all gardening sites. If the COGS membership is 500–600 actual persons, then these contribute tot the dietary intake of 615–740 people *in toto*.

The 120 growers grow the entire fruit and vegetable diet for 44 people. Another interpretation is that on average all 120 respondents produce just over one-third of their fruit and vegetable needs by their gardening work.

Respondents estimate average weekly savings of $15·36 or $798·57 annually. For all respondents the total annual saving is $89,440; extrapolated to the entire COGS non-corporate membership, the total annual savings are in the vicinity of $273,000.

In spite of the small scale of gardening activity, considerable amounts of produce are grown and large sums of money saved by own-growing efforts, although the total gardening output evidently needs supplementation from other sources of supply such as the supermarket system or farmers' markets.

These preliminary findings indicate that the community garden is an increasingly valued part of the social and cultural life of Canberra. Demand exists for more and bigger gardens, and for gardening land in general. Gardens in Canberra fulfill a variety of needs, including food production, social outlets, and educational opportunities.

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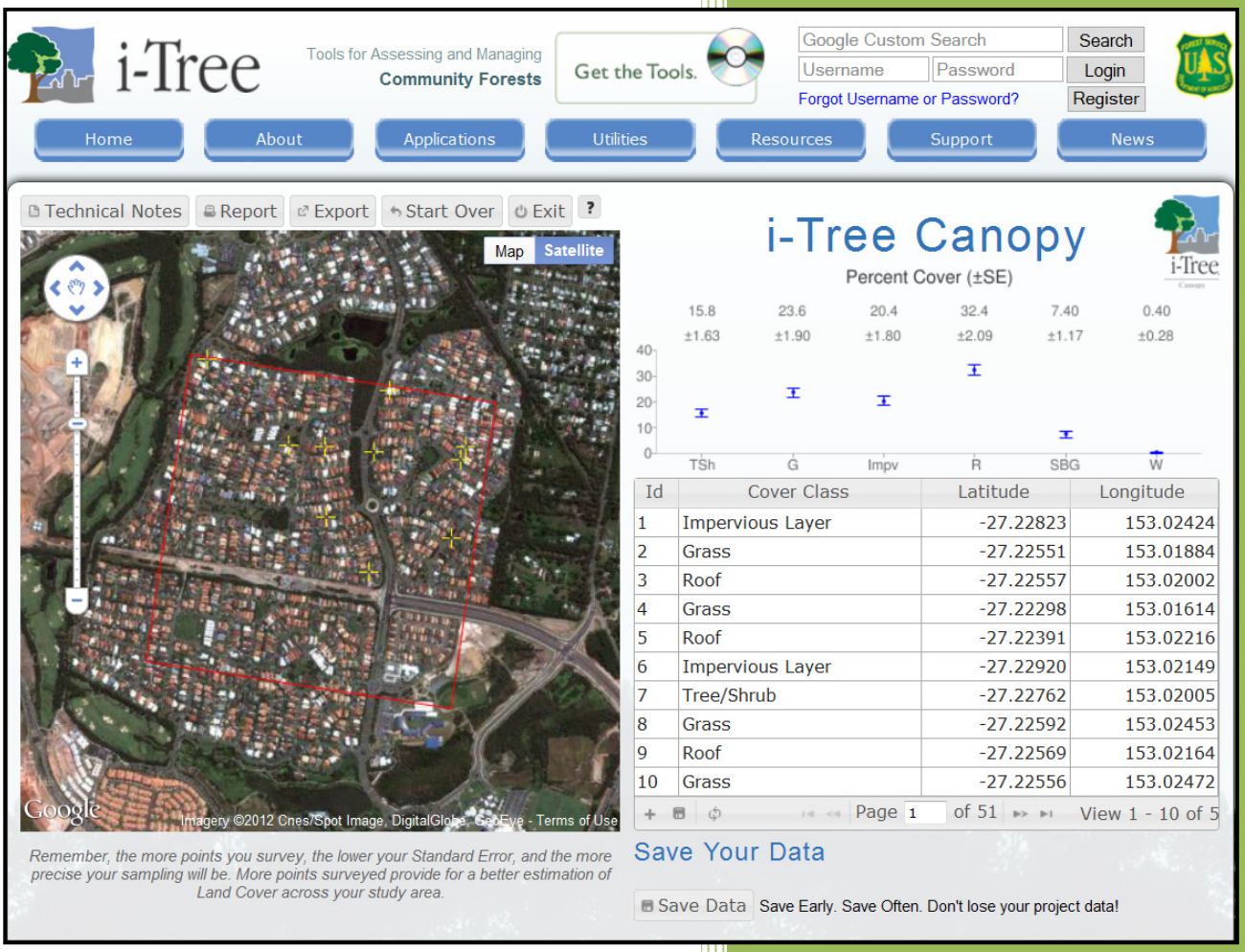
Appendix 7

THE UNIVERSITY OF MELBOURNE

2012

Tree canopy cover in residential areas of Australian cities



Stephen Livesley

The University of Melbourne

1/16/2012

Tree canopy cover in residential areas of Australian cities

Authors:

Stephen Livesley and Melissa Fedrigo

Institution:

Melbourne School of Land and Environment,

The University of Melbourne,

500 Yarra Boulevard,

Burnley campus,

Melbourne,

VIC 3121

Consultancy Report

Completed as a consultancy report by the Green Infrastructure Research Group (GIRG) of the Melbourne School of Land and Environment (MSLE) in The University of Melbourne for Nursery and Garden Industry Australia (NGIA).

Table of Contents

Executive summary

The urban forest (all the trees within a city) provide many important ecosystem services to humans, the environment and biodiversity, improved microclimate conditions and reduced urban heat island; storage and sequestration of carbon; reduced storm water run-off, cultural and heritage values and mental health benefits. In residential areas tree canopy cover can improve human thermal comfort conditions (especially during heat wave conditions) and provide an important connection to greenery and nature to children and the elderly. Owners of private residential land can introduce, increase and manage vegetation canopy cover within their own gardens, whilst local government authorities can do the same within residential streetscapes. However, at the same time tree canopy cover in residential areas may decrease because of urban densification (in-filling) and street tree removal and renewal programs.

This report quantifies the percentage canopy cover of trees and shrubs within residential areas of Melbourne, Perth and Brisbane, and aims to:

- Compare tree canopy cover in old inner suburbs with that in new, outer suburbs.
- Compare estimated tree canopy covers of Melbourne, Perth and Brisbane.

The i-Tree Canopy software package was used to systematically and accurately quantify tree and shrub canopy cover within 1 km^2 residential areas.

The tree/shrub canopy cover of residential areas within inner-city suburbs ranged between 19.2 and 30.4% and was significantly greater ($p \leq 0.01$) than that in outer suburbs where canopy cover ranged between 11.6 and 21.5%. This may be due to the younger age and canopy spread of trees in outer suburbs, as is supported by the fact that there was significantly less open grass cover within inner-city suburbs than newer outer suburbs. However, this may also simply indicate a changing preference towards open grass cover and less inclusion of tall, woody perennial vegetation.

Residential areas in Melbourne (15.4%) have significantly less tree and shrub canopy cover than in Brisbane (26.0%), and concurrently have a significantly greater area covered by impervious ground or roof surfaces (62.8% versus 46.6%). This would suggest that the residential areas of Melbourne, both inner and outer, may have a greater proportion of the land surface area covered by buildings or impervious ground surfaces and therefore less space is available for vegetation (tree, shrub or grass). Alternatively, there may simply be fewer trees and less tree canopy cover in Melbourne residential suburbs than in Brisbane or Perth.

There appears to be considerable opportunity to increase tree canopy cover of residential areas within Australian both on private residential properties and publicly managed streetscapes. In cities with less current tree canopy cover, such as Melbourne, this would provide considerable society and environmental benefits and a simple means to help adapt our cities, and the majority of our population, to future climate change conditions under global warming.

Introduction

Australia is one of the most urbanised countries in the world, with between 75% - 82% of the population estimated to live within cities or towns (ABS, 2006). The vulnerability of urban communities and urban ecology / biodiversity to extreme climate events (flood, heat wave, dust storms) and the long-term projected impacts of climate change is of increasing concern at a social, policy and scientific research level. It is widely accepted that improved management, maintenance and renewal of the vegetation canopy cover within our urban centres will help adapt our cities to climate change and ensure they are reasonable places to live in the future (Pataki *et al.*, 2011).

The urban forest (all the trees within a city) are recognised as providing many important ecosystem services to humans, the environment and biodiversity. These include: reduced particulate air pollution; reduced energy use through increased shade, reduced air conditioner use; mitigation of the urban heat island; the storage/sequestration of carbon; improved water quality and retention (Brack, 2002; McPherson *et al.*, 2005; Donovan and Butry, 2009). Urban trees also provide important cultural and heritage values (a sense of place), improved tourism and retail consumer activity, as well as mental health benefits such as stress reduction, improved work practice, improved child attention and behaviour (Ulrich *et al.*, 1991; Kaplan, 1995; Wells, 2000). Within residential areas specifically, canopy cover is important because:

1. Vegetation in residential areas, especially trees, provide the greatest opportunity for human contact with nature across all ages (children, adults and the elderly).
2. Vegetation in residential areas, especially trees, can improve human thermal comfort conditions and cool the microclimate by day, thereby reducing night temperatures, a key issue for vulnerable sections of society during a heatwave.
3. Vegetation in residential areas, especially trees, can provide direct energy saving benefits to buildings that receive shade.
4. Owners of residential land can introduce, increase and manage vegetation canopy cover within their own gardens.
5. Local government authorities can introduce, increase and manage vegetation canopy cover within streetscapes and nature-strips.

A large amount of local and state government money, as well as private household income, is spent on managing the vegetation within our urban centres, and this provides considerable business opportunities to the advanced tree and shrub nursery industries as well as the aboriculture and landscape management and maintenance industries. Regardless, there is little understanding of the status of, or change within, the urban forests of Australian cities. Whilst tree canopy cover can be increased through direct action of private property owners and local government councils, at the same time, tree canopy cover in residential areas may decrease because of urban densification (in-filling) and street tree removal and renewal programs.

The simplest indicator as to the status of an urban forest within a suburb, community of local government authority is the percentage of tree/shrub canopy cover in comparison to other land surface covers (road, building, grass, water, bare soil). The objective of this report is to quantify the percentage canopy cover of trees and shrubs within residential areas of Melbourne, Perth and Brisbane. The two aims of this report are to:

a) Compare the tree/shrub canopy cover of inner suburbs with that of outer suburbs.
b) Compare estimated tree/shrub canopy covers of Melbourne, Perth and Brisbane.

The i-Tree Canopy software package was used to systematically and accurately quantify tree and shrub canopy cover within 1 km^2 residential areas of inner and outer suburbs of Melbourne, Perth and Brisbane.

Materials and methods

Six polygons of 1 km^2 (1000 x 1000 m) were allocated within the metropolitan areas of Melbourne, Perth and Brisbane. Within each city, three of the polygons were located within inner-city residential areas and three in residential areas of newer suburbs on the city's outskirts. Polygons were selected in areas of the city where the dominant land use was residential, whilst trying to avoid areas with large green spaces (Parks, recreation fields, schools), commercial land-uses and main roads.

Each square was delineated using the line function in Google Earth. The Google Earth KML format polygon boundaries were converted to shapefile using ArcGIS and all elevation values removed for use with i-Tree Canopy. The boundaries were also converted to a geographic coordinate system (WGS 1984) to ensure the boundaries are visible in i-Tree Canopy.

For each polygon, 500 points randomly selected by i-Tree Canopy and were manually classified into one of the following categories:

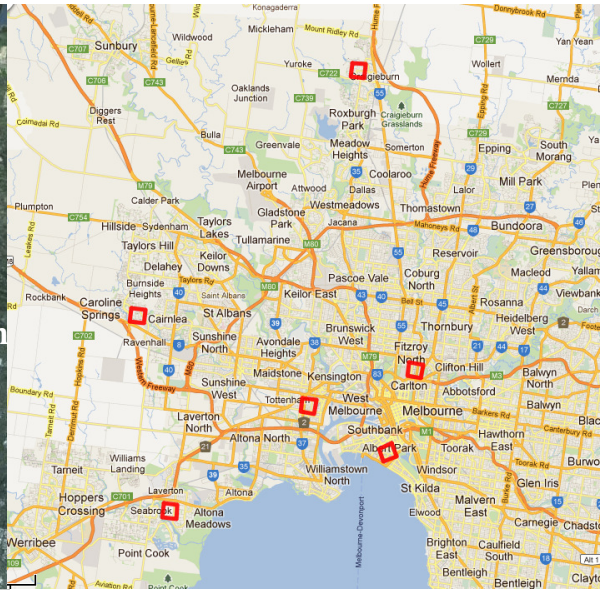
- Shrub/tree
- Grass
- Impervious ground
- Impervious roof
- Soil/Bare Ground
- Water Body

In areas where Google Earth displayed shade, the classification was based on the surrounding features and a best estimate of the land cover at the exact point.

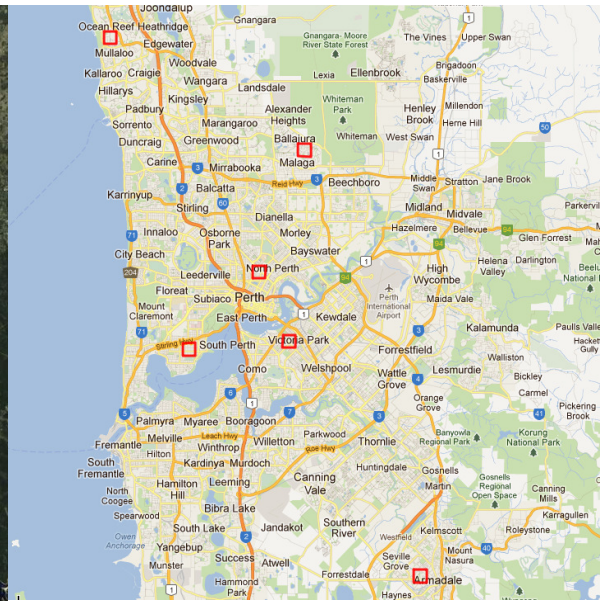
Point files were saved in .dat and .csv format by i-Tree Canopy to allow for further processing in the same or other programs. i-Tree Canopy produces reports for each analysis summarizing the statistical distribution of points for each category analysed.

Statistical differences in canopy cover between inner and outer suburbs were investigated using a student t-test. Statistical differences in percentage land suface cover types among Melbourne, Perth and Brisbane were investigated using general one-way ANOVAs (GENSTAT 14.0).


Melbourne
Craigieburn
Caroline Springs
Carlton North
Seddon
Albert park
Altona Meadows


Sunbury
Wildwood
Mickleham
Yuroke
Craigieburn
Roxburgh Park
Meadow Heights
Somerton
Epping
Bulla
Greenvale
Oaklands Junction
Melbourne Airport
Attwood
Dallas
Coolaroo
Mill Park
Plumpton
Calder Park
Westmeadows
Thomastown
Hillside
Sydenham
Taylors Lakes
Gladstone Park
Jacana
Bundoora
Taylors Hill
Tullamarine
Keilor Downs
Delahey
Reservoir
Pascoe Vale
Coburg North
Macleod
Rockbank
Burnside Heights
Keilor East
Caroline Springs
Cairnlea
St Albans
Rosanna
Viewbank
Ravenhall
Sunshine North
Avondale Heights
Brunswick West
Thornbury
Heidelberg West
Fitzroy North
Clifton Hill
Maidstone
Kensington
Carlton
Abbotsford
Balwyn North
Sunshine West
Tottenham
West Melbourne
Melbourne
Balwyn
Laverton North
Altona North
Southbank
Hawthorn East
Albert Park
Toorak
Williams Landing
Williamstown North
Windsor
Tarneit
Laverton
Altona
St Kilda
Glen Iris
Hoppers Crossing
Seabrook
Altona Meadows
Elwood
Malvern East
Carnegie
Werribee
Point Cook
Brighton East
Caulfield South
Bentleigh


Ocean Reef
Perth
Ballajura
Mount Lawley
Kensington
Nedlands
Armadale


Joondalup
Ocean Reef
Heathridge
Gnangara
Mullaloo
Edgewater
Woodvale
Kallaroo
Craigie
Wangara
Landsdale
Hillarys
Kingsley
Padbury
Alexander Heights
Sorrento
Marangaroo
Greenwood
Ballajura
Duncraig
Carine
Mirrabooka
Malaga
Karrinyup
Balcatta
Beechboro
Stirling
Dianella
Innaloo
Osborne Park
Morley
Bayswater
City Beach
Leederville
North Perth
Floreat
Subiaco
Perth
Mount Claremont
East Perth
Kewdale
Victoria Park
South Perth
Como
Welshpool
Forrestfield
Lesmurdie
Palmyra
Myaree
Booragoon
Melville
Parkwood
Willetton
Thornlie
Fremantle
Winthrop
Hilton
Kardinya
Murdoch
South Fremantle
Canning Vale
Hamilton Hill
Leeming
North Coogee
Spearwood
Bibra Lake
South Lake
Jandakot
Southern River
Yangebup
Success
Atwell
Munster
Forrestdale
Armadale
Hammond Park
Haynes


Brisbane
North Lakes
Clayfield
Paddington
Greenslopes
Inala
Crestmead


Armstrong Creek
Rothwell
Dakabin
North Lakes
Kippa-Ring
Kobble Creek
Kurwongbah
Mango Hill
Redcliffe
Whiteside
Kallangur
Clontarf
Margate
Samsonvale
Petrie
Murrumba Downs
Woody Point
Moreton Bay
Joyner
Mount Samson
Clear Mountain
Warner
Bray Park
Cashmere
Bracken Ridge
Sandgate
Closeburn
Brendale
Eatons Hill
Nudgee Beach
Yugar
Draper
Carseldine
Bridgeman Downs
Samford Valley
Bunya
Geebung
Nudgee
Camp Mountain
Chermside
Banyo
Port Of Brisbane
Wights Mountain
Ferny Hills
Everton Park
Nundah
Brisbane Airport
Keperra
Stafford
Kedron
Clayfield
The Gap
Fortitude Valley
Hemmant
Wynnum
Mount Coot-tha Forest
Milton
Murarrie
Wynnum West
Upper Brookfield
Toowong
Brisbane
Morningside
Tingalpa
Lota
Manly West
Indooroopilly
Carina
Wakerley
Thorneside
Wellington Point
Kenmore
Woolloongabba
Greenslopes
Carindale
Chandler
Capalaba West
Ormiston
Pullenvale
Jindalee
Mooroka
Capalaba
Alexandra Hills
Cleveland
Karana Downs
Anstead
Salisbury
Wishart
Seventeen Mile Rocks
Karalee
Bellbowrie
Archerfield
Coopers Plains
Sheldon
Thornlands
Sumner
Runcorn
Inala
Sunnybank Hills
Kuraby
Underwood
Collingwood Park
Forest Lake
Calamvale
Springwood
Camira
Logan Centre
Daisy Hill
Redbank Plains
Springfield Lakes
Parkinson
Shailer Park
Brookwater
Browns Plains
Meadowbrook
Loganholme
Cornubia
Hillcrest
Marsden
Springfield Central
Boronia Heights
Waterford West

(OPPOSITE)

Figure 1. The six polygons selected in the inner and outer suburb locations of Melbourne (top), Perth (middle) and Brisbane (bottom).

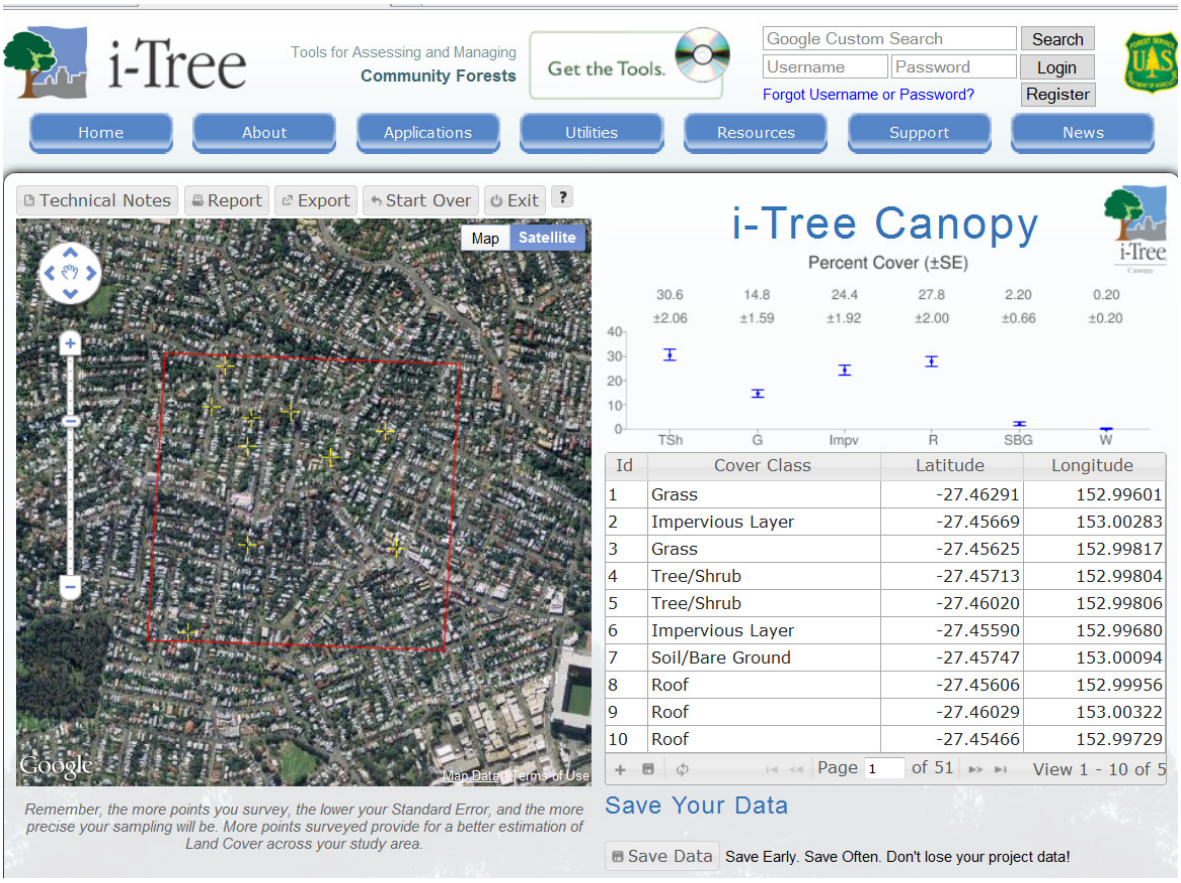


Figure 2. A screen-shot example of the i-Tree Canopy software package used to quantify tree/shrub canopy cover within residential areas. The red square (polygon) determines the 1 km2 area within which 500 random points are selected and categorised according land surface type.

Results

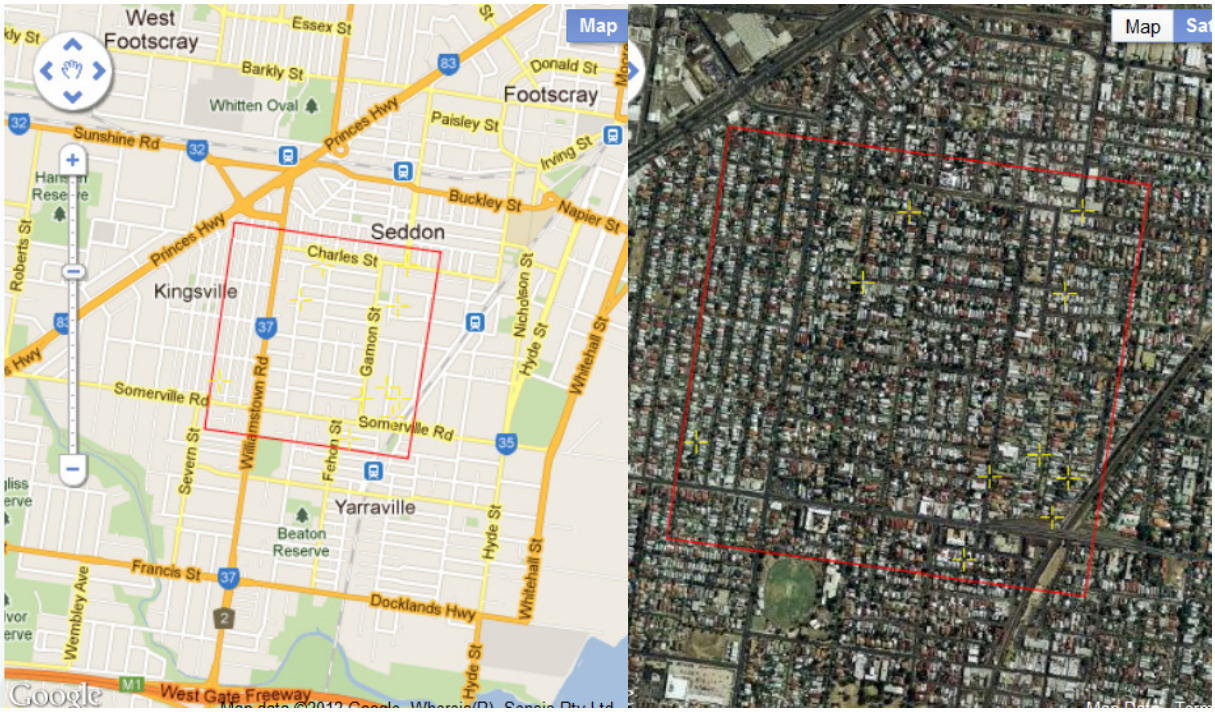
Melbourne - Inner-city

The three suburbs selected to represent the inner-city residential areas of Melbourne were Carlton North (inner north), Seddon (inner west) and Albert Park (inner south).

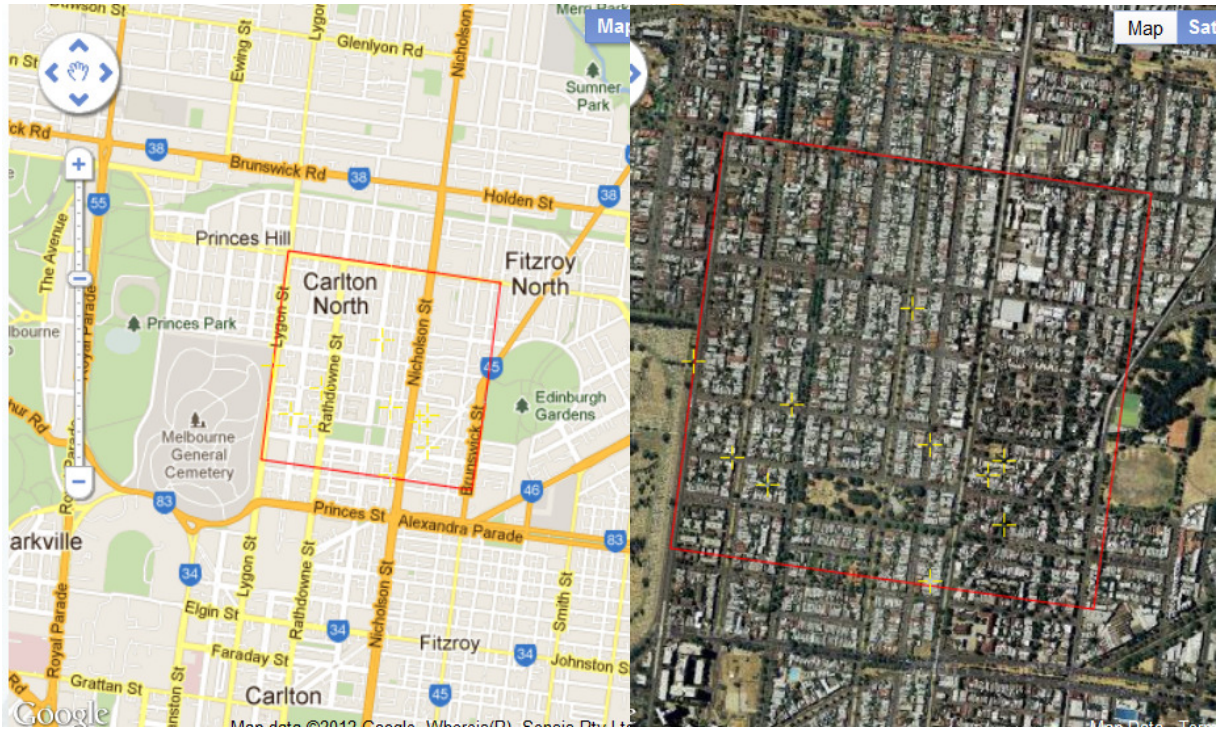
Table 1. Land surface cover types in Melbourne's inner-city residential areas

	Seddon	Carlton	Albert Pk
Tree/shrub	16.6 ±1.66	20.0 ±1.79	21.0 ±1.82
Grass	10.2 ±1.35	6.0 ±1.06	9.2 ±1.29
Imp. Ground	27.0 ±1.99	34.4 ±2.12	37.4 ±2.16
Imp. Roof	41.2 ±2.20	38.4 ±2.18	30.0 ±2.05
Soil/bare	5.0 ±0.97	1.2 ±0.49	2.4 ±0.68
Water	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00

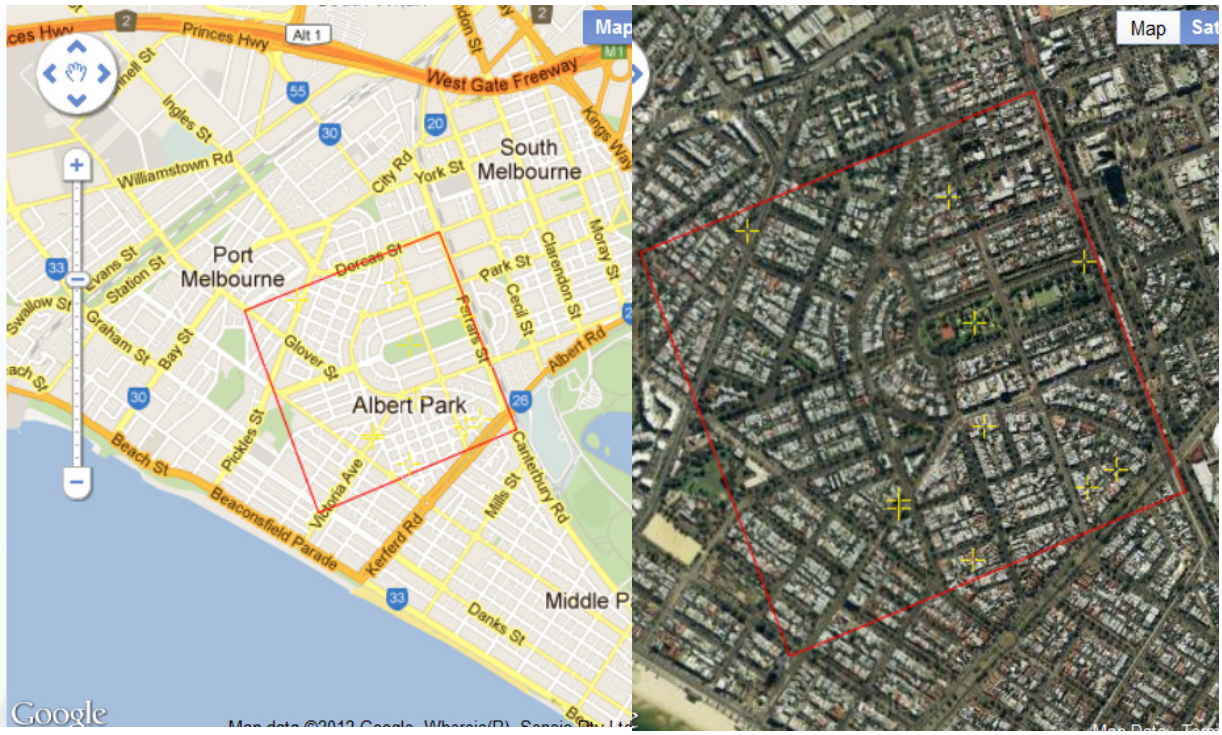
Seddon



Carlton North



Albert Park



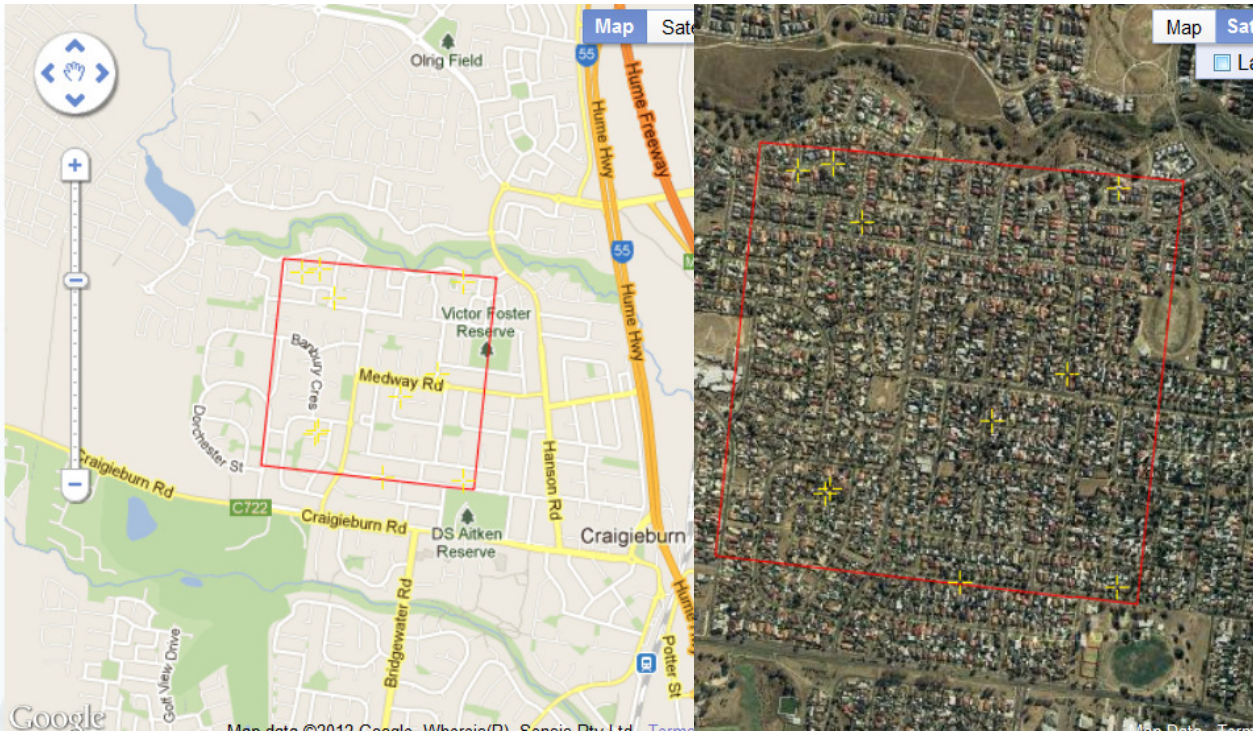
Melbourne – Outer suburbs

The three suburbs selected to represent the outer residential areas of Melbourne were Craigieburn (outer north), Caroline Springs (outer north-west) and Altona Meadows (outer west).

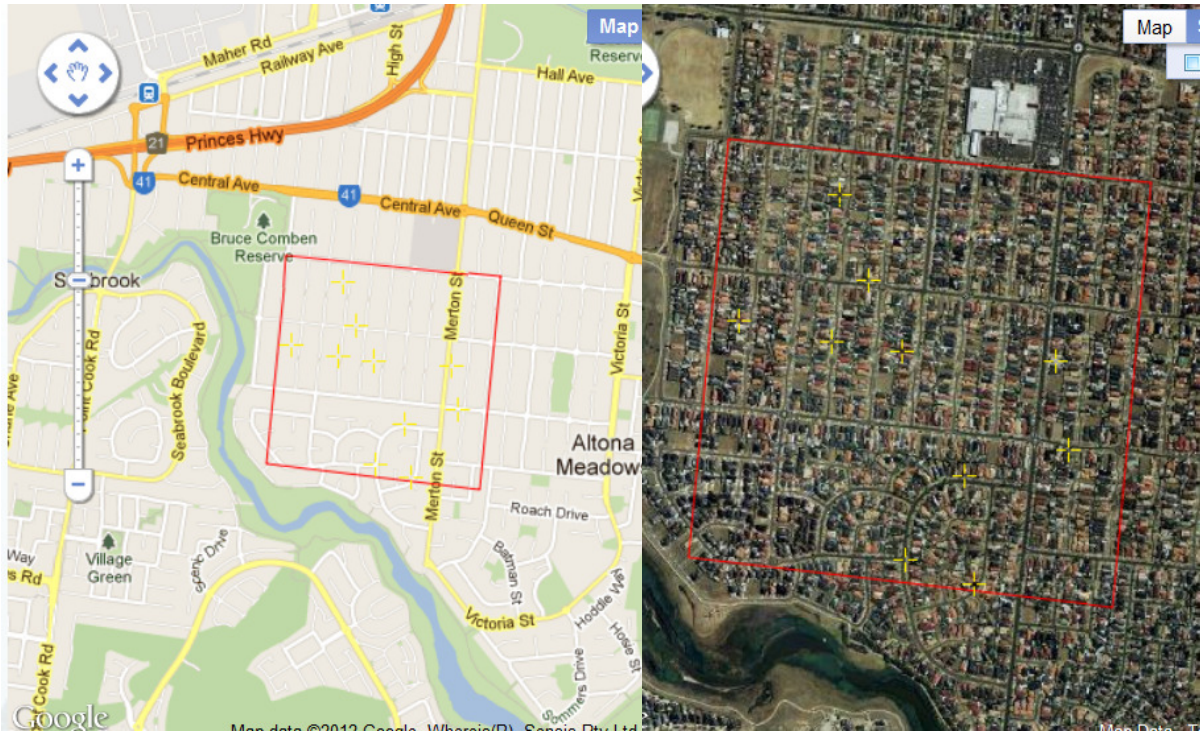
Table 2. Land surface cover types in Melbourne's outer suburb residential areas

	Craigieburn	Caroline Springs	Altona Meadows
Tree/shrub	12.8 ±1.49	13.0 ±1.50	9.0 ±1.28
Grass	27.4 ±1.99	22.2 ±1.86	22.8 ±1.88
Imp. Ground	25.4 ±1.95	26.6 ±1.98	23.2 ±1.89
Imp. Roof	28.6 ±2.02	29.0 ±2.03	35.8 ±2.14
Soil/bare	5.8 ±1.05	9.2 ±1.29	9.2 ±1.29
Water	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00

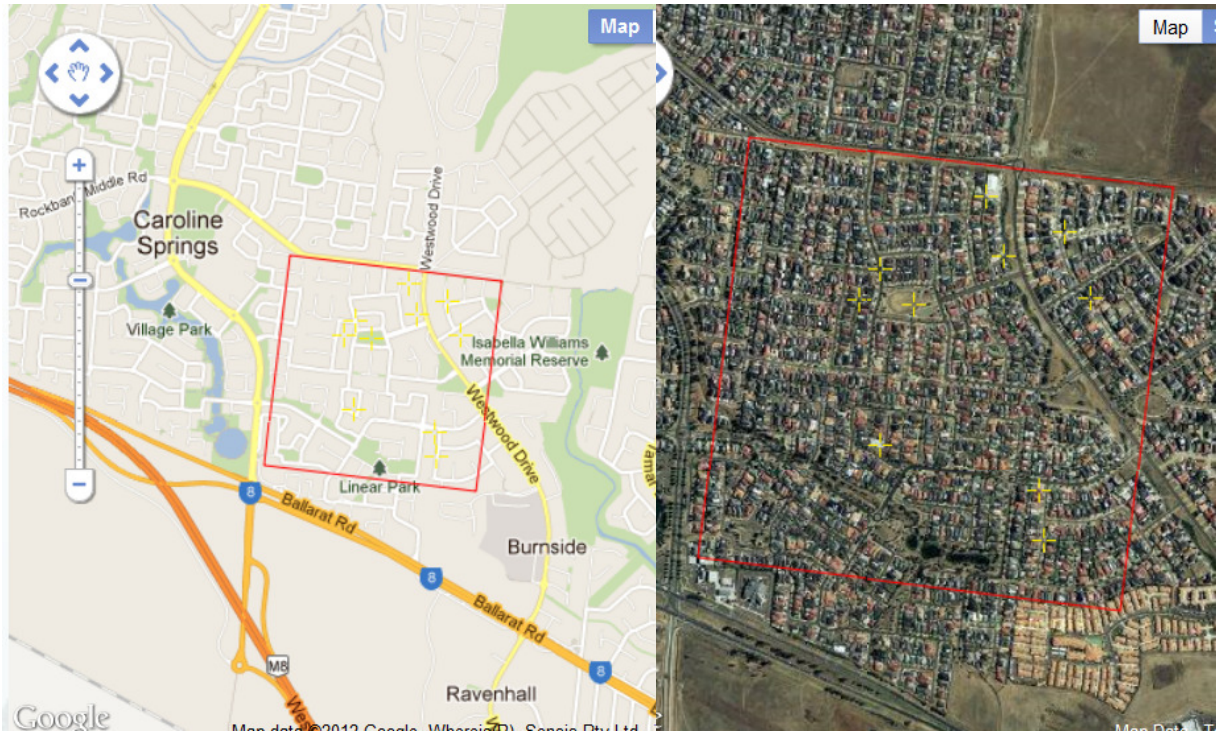
Craigieburn



Altona Meadows



Caroline Springs



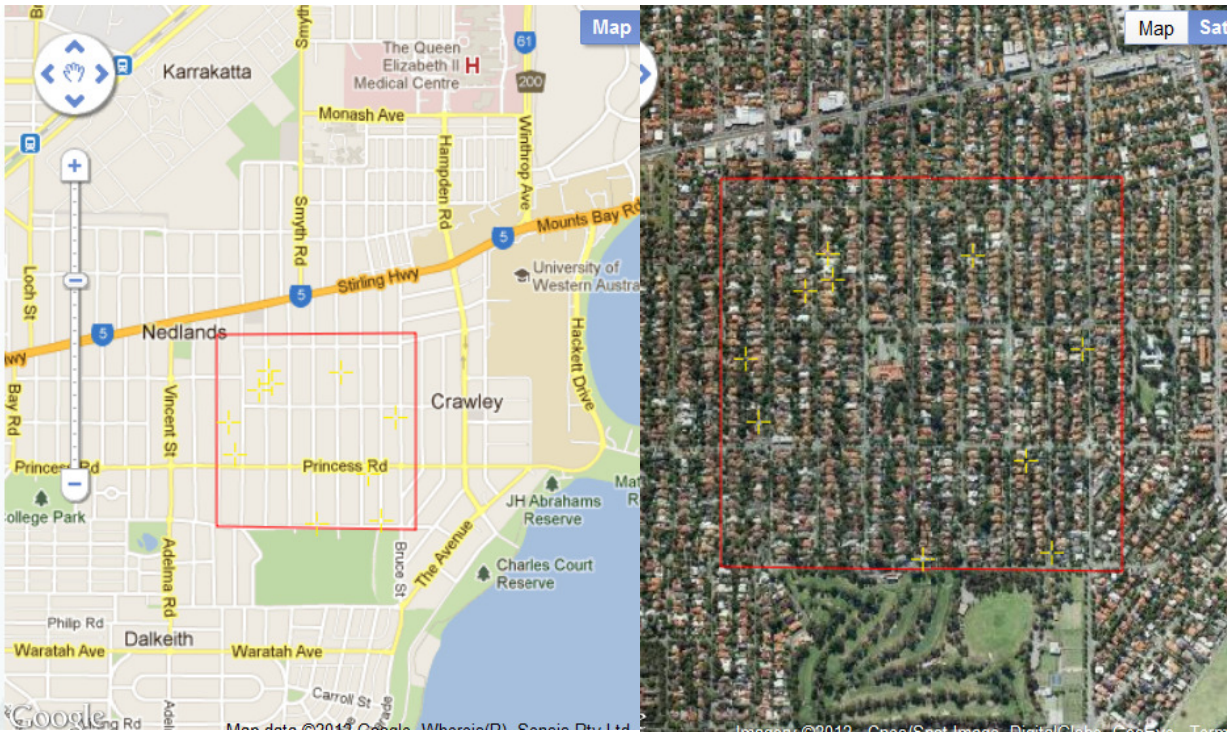
Perth – Inner-city

The three suburbs selected to represent the inner-city residential areas of Perth were Nedlands (inner west), Mount Lawley (inner north) and Kensington (inner south).

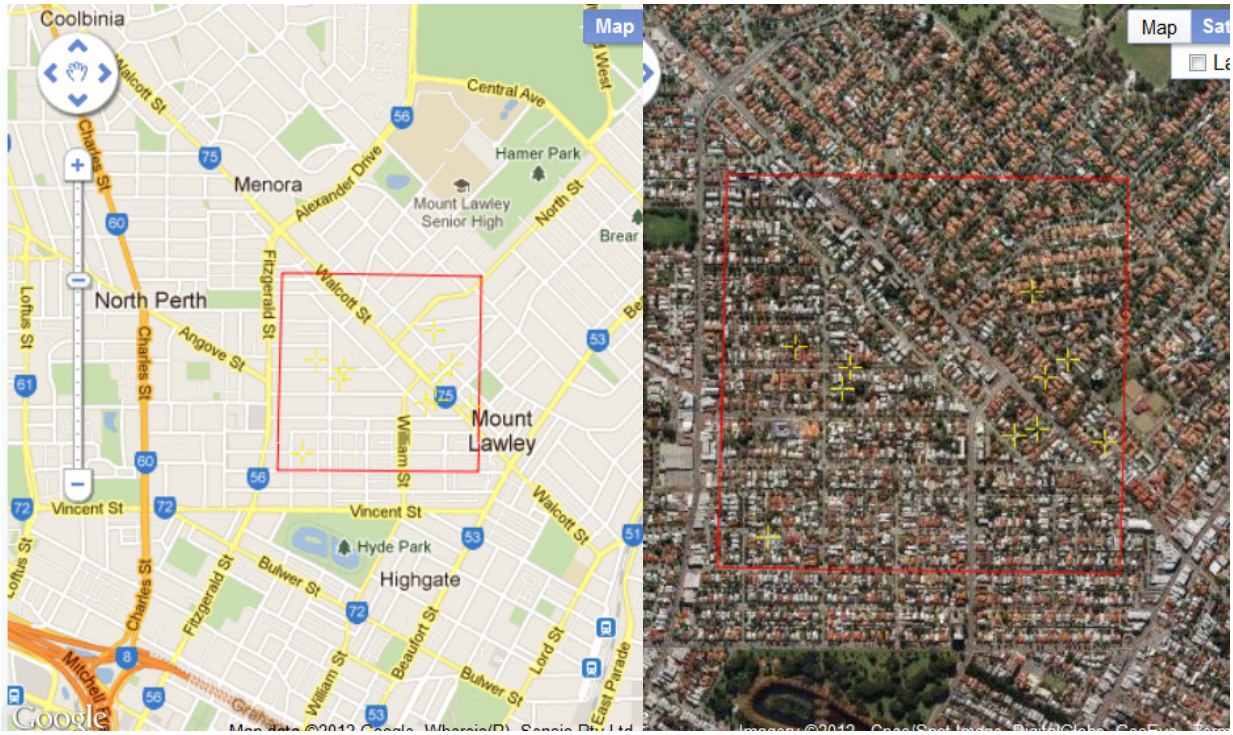
Table 3. Land surface cover types in Perth's inner-city residential areas

	Nedlands	Mount Lawley	Kensington
Tree/shrub	36.4 ±2.15	21.2 ±1.83	21.6 ±1.84
Grass	13.6 ±1.53	15.2 ±1.61	19.4 ±1.77
Imp. Ground	14.4 ±1.57	25.8 ±1.96	26.2 ±1.97
Imp. Roof	29.0 ±2.03	31.4 ±2.08	27.4 ±1.99
Soil/bare	4.8 ±0.96	6.2 ±1.08	5.2 ±0.99
Water	1.80 ±0.60	0.2 ±0.20	0.2 ±0.20

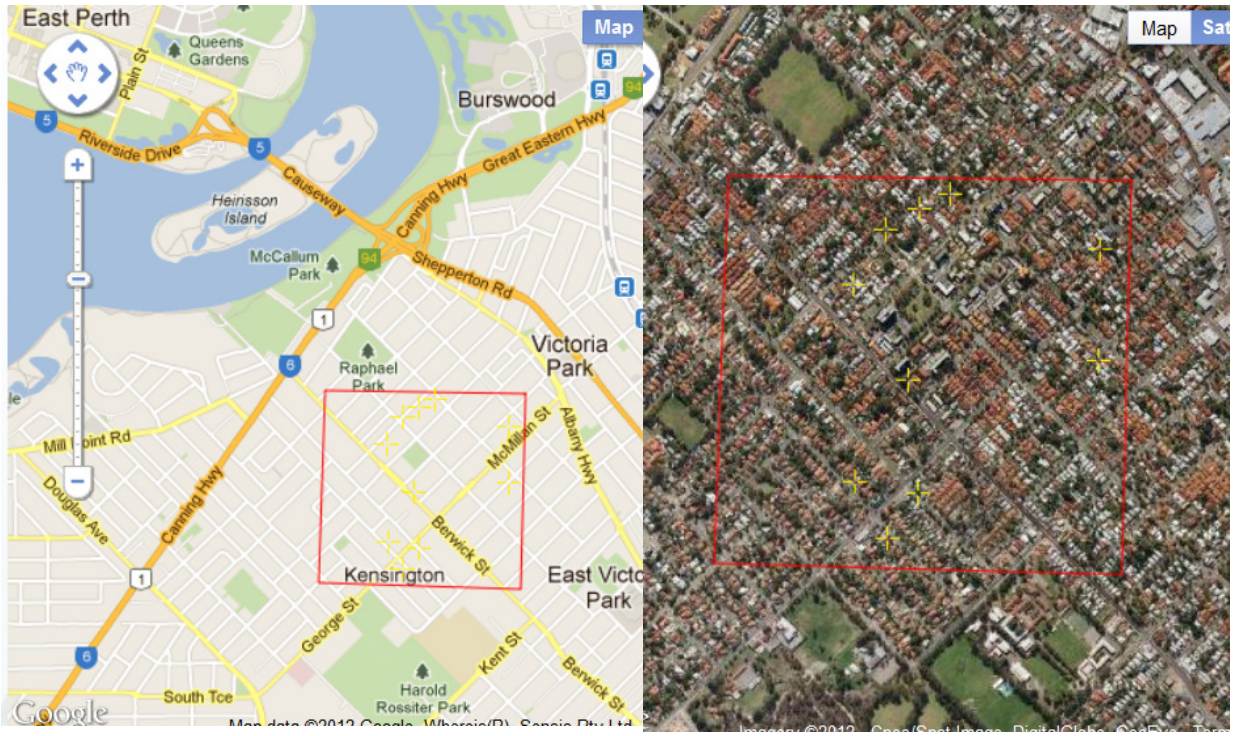
Nedlands



Mount Lawley



Kensington



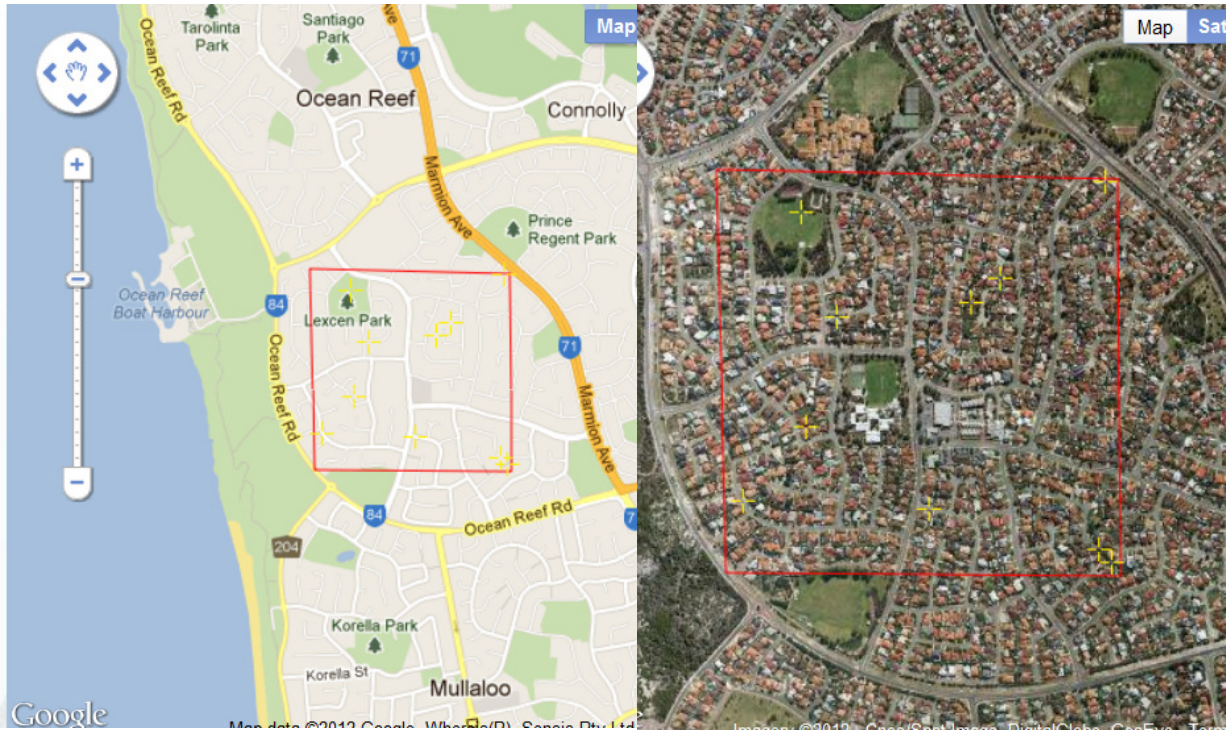
Perth – Outer suburbs

The three suburbs selected to represent the outer residential areas of Perth were Ocean Reef (outer north), Ballajura (outer north-east) and Armadale (outer south-east).

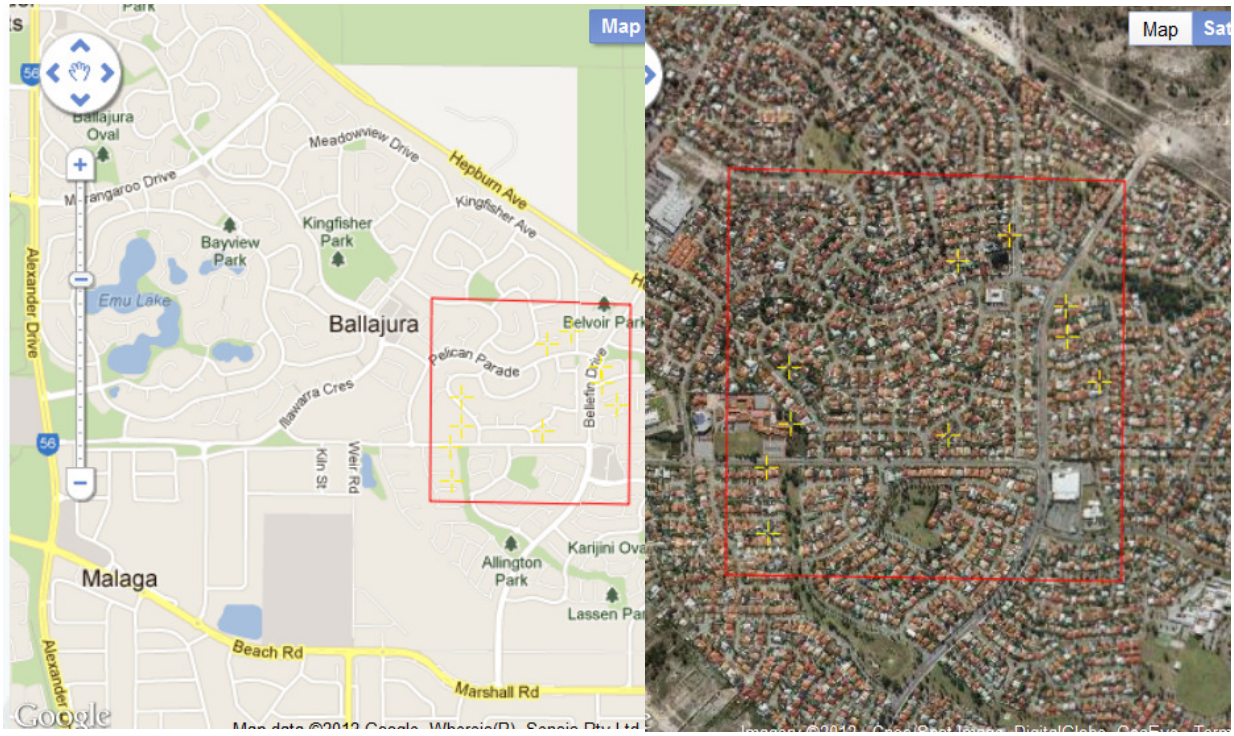
Table 4. Land surface cover types in Perth's outer suburb residential areas

	Ocean Reef	Ballajura	Armadale
Tree/shrub	16.0 ±1.64	13.8 ±1.54	21.4 ±1.83
Grass	20.0 ±1.79	22.4 ±1.86	27.2 ±1.99
Imp. Ground	29.8 ±2.05	23.6 ±1.90	18.2 ±1.73
Imp. Roof	25.6 ±1.95	31.6 ±2.08	23.2 ±1.89
Soil/bare	7.2 ±1.16	7.80 ±1.20	9.8 ±1.33
Water	1.4 ±0.53	0.8 ±0.40	0.2 ±0.20

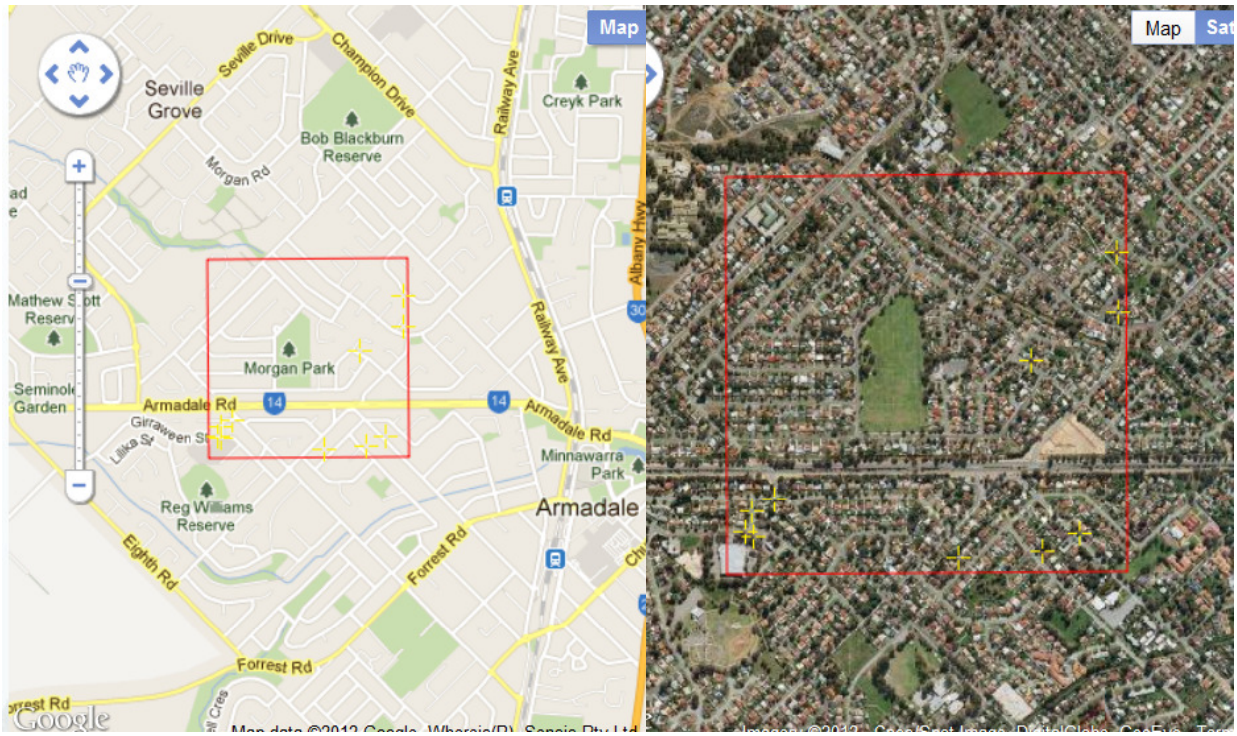
Ocean Reef



Ballajura



Armadale



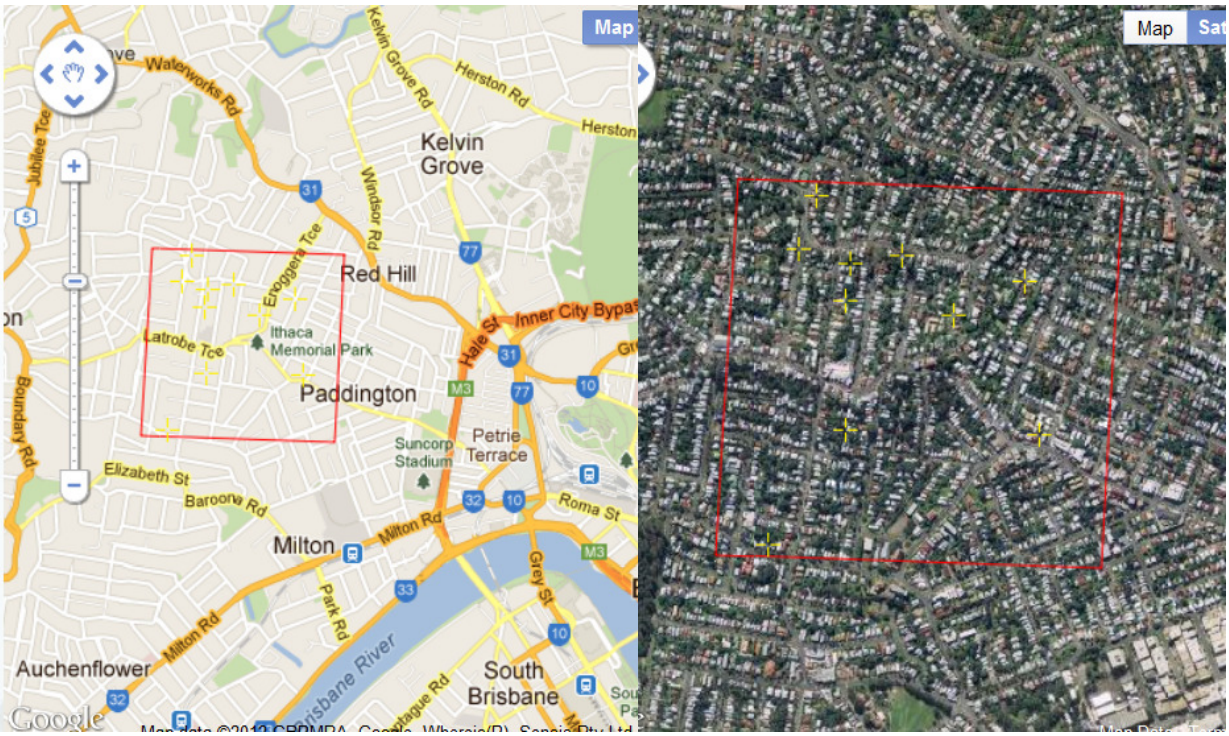
Brisbane – Inner-city

The three suburbs selected to represent the inner-city residential areas of Brisbane were Paddington (inner west), Clayfield (inner north) and Greenslopes (inner south).

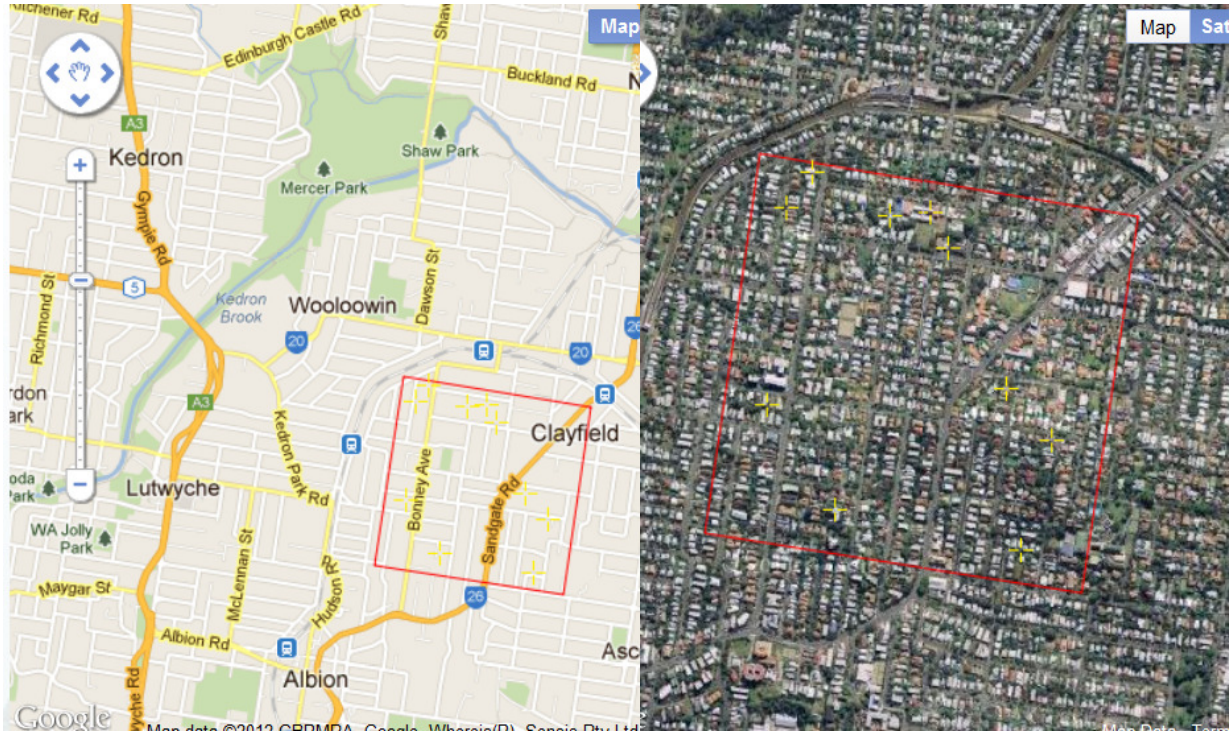
Table 5. Land surface cover types in Brisbane's inner-city residential areas

	Paddington	Clayfield	Greenslopes
Tree/shrub	30.6 ±2.06	27.6 ±2.00	33.0 ±2.10
Grass	14.8 ±1.59	17.0 ±1.68	18.6 ±1.74
Imp. Ground	24.4 ±1.92	23.6 ±1.90	22.4 ±1.86
Imp. Roof	27.8 ±2.00	28.6 ±2.02	23.8 ±1.90
Soil/bare	2.2 ±0.66	3.0 ±0.76	1.8 ±0.60
Water	0.2 ±0.20	0.2 ±0.20	0.4 ±0.28

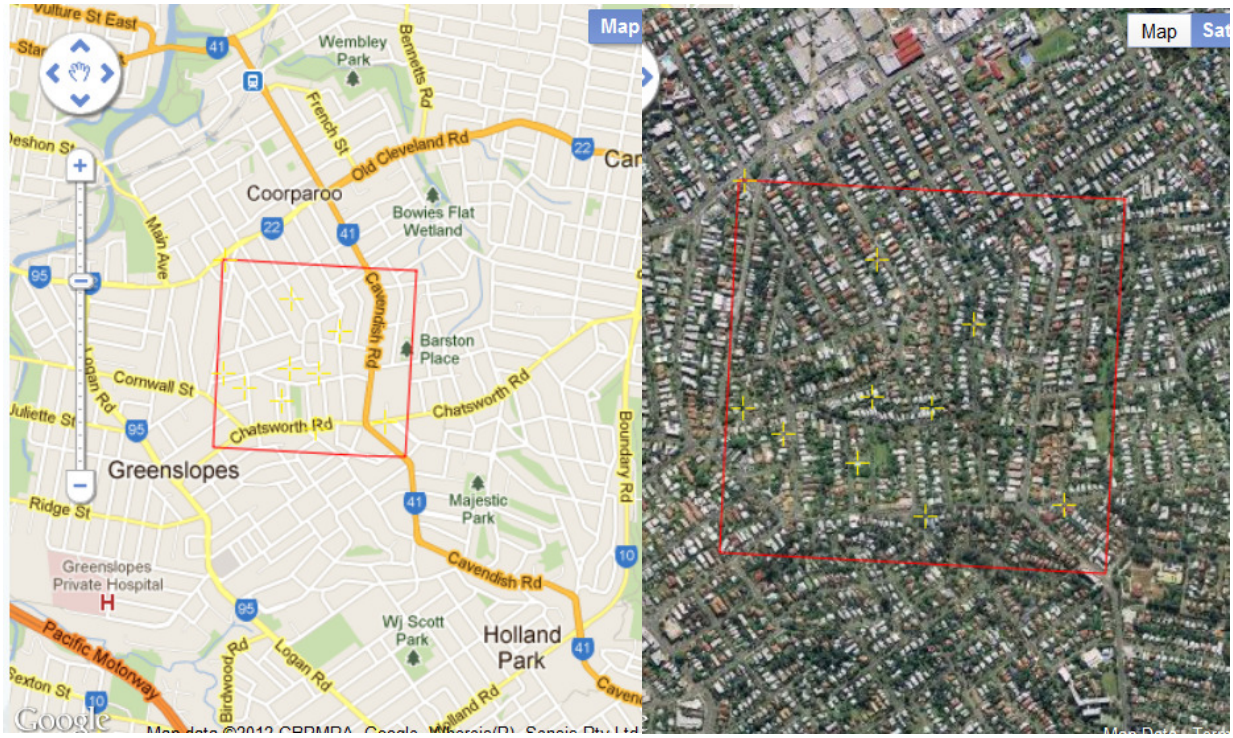
Paddington



Clayfield



Greenslopes



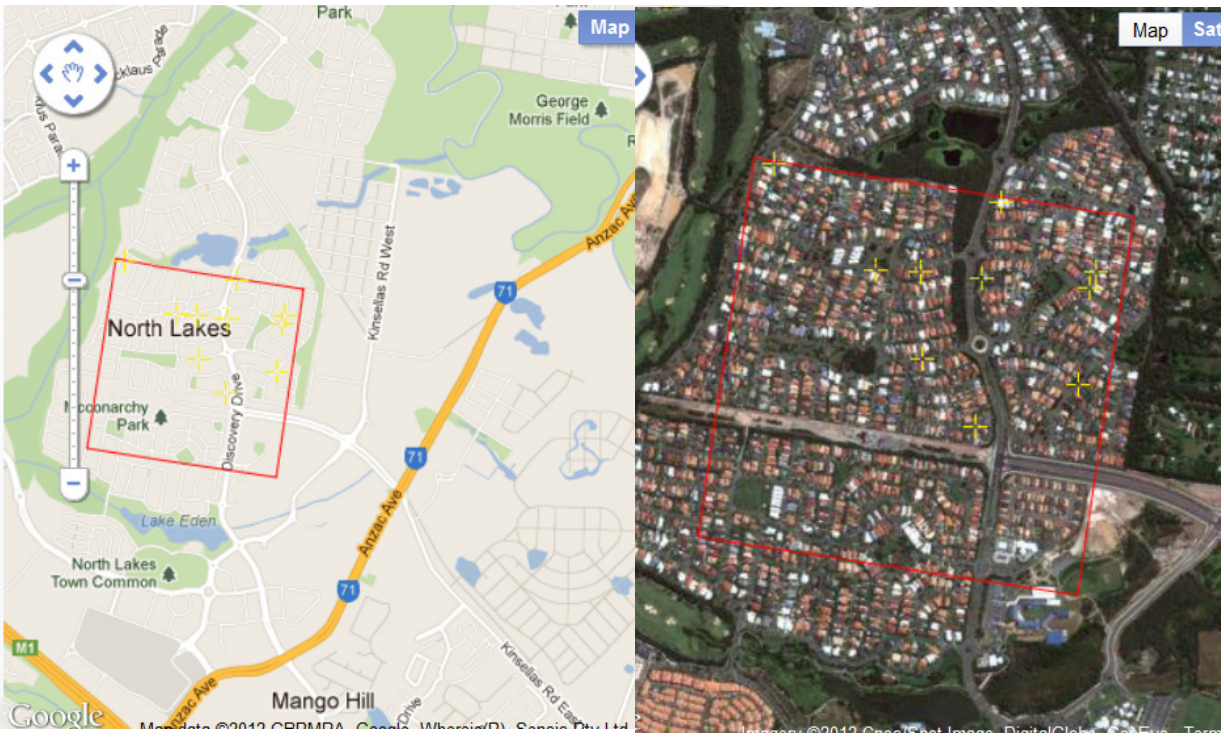
Brisbane – Outer suburbs

The three suburbs selected to represent the outer residential areas of Brisbane were North Lakes (outer north), Inala (outer south west) and Crestmead (outer south).

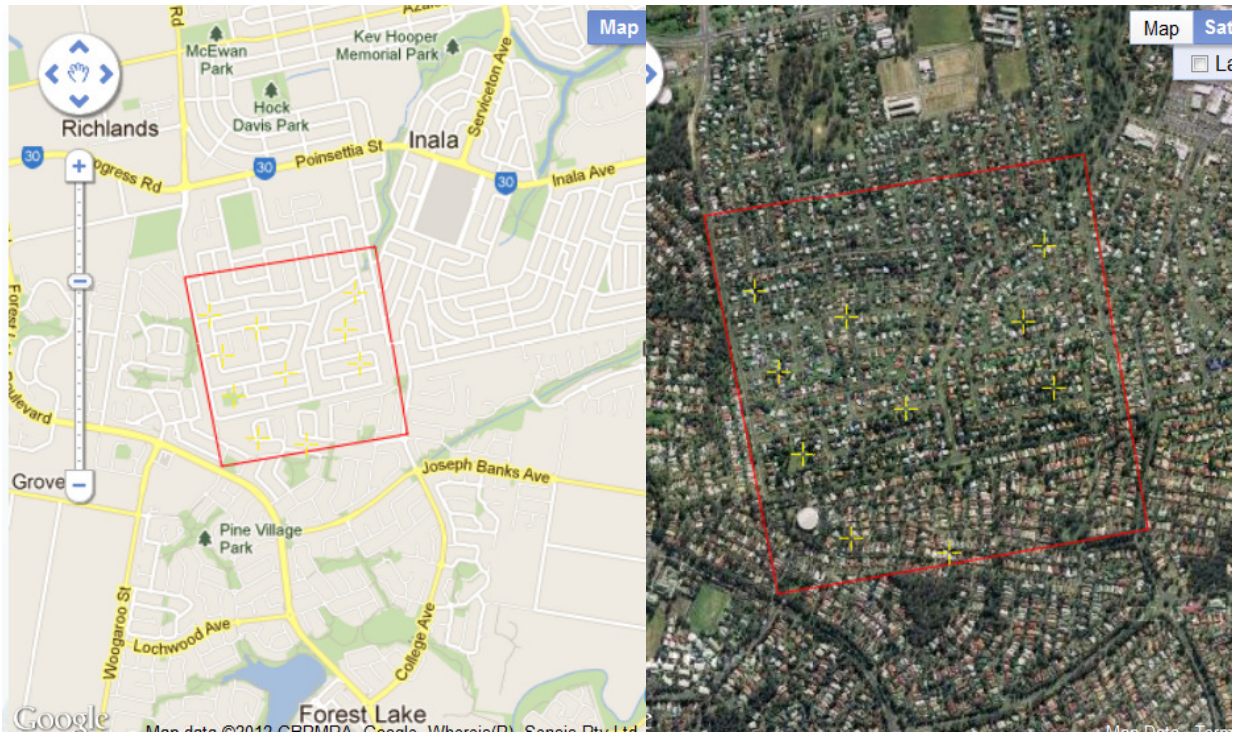
Table 6. Land surface cover types in Brisbane's outer suburb residential areas

	North Lakes	Inala	Crestmead
Tree/shrub	15.8 ±1.63	25.6 ±1.95	23.2 ±1.89
Grass	23.6 ±1.90	33.2 ±2.11	37.8 ±2.17
Imp. Ground	20.4 ±1.80	17.0 ±1.68	16.6 ±1.66
Imp. Roof	32.4 ±2.09	21.4 ±1.83	21.2 ±1.83
Soil/bare	7.4 ±1.17	2.0 ±0.63	1.0 ±0.45
Water	0.4 ±0.28	0.8 ±0.40	0.2 ±0.20

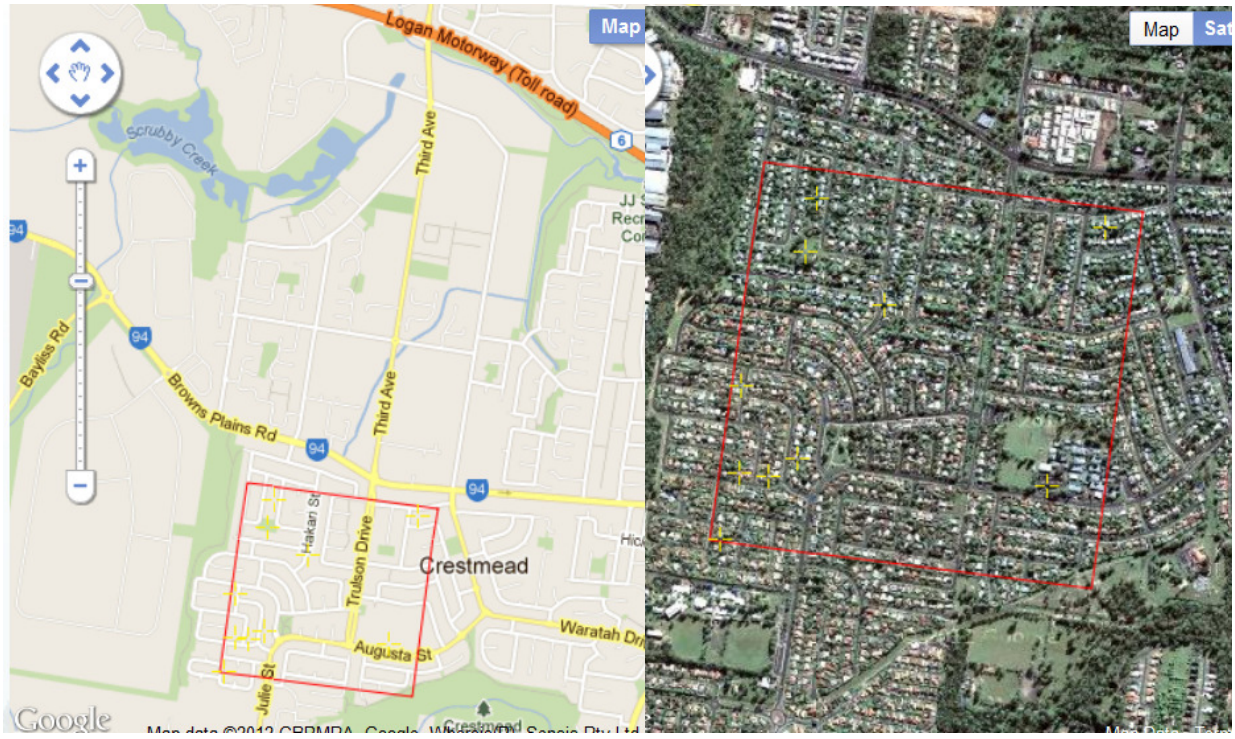
North Lakes



Inala



Crestmead



Canopy cover of residential areas within inner and outer suburbs

The tree/shrub canopy cover of residential areas within inner-city suburbs ranged between 19.2 and 30.4% and was significantly greater ($p \leq 0.01$) than that in outer suburbs where canopy cover ranged between 11.6 and 21.5% (Figure 3). This may simply be due to the younger age of these outer suburbs and consequently the younger age of the trees and lesser development and spread of the tree canopies. This would be supported by the inverse observation that there was a significantly smaller percentage of grass cover (without above tree canopy) within inner-city suburbs as compared to newer outer suburbs (Figure 3). However, this may simply indicate a changing preference from tall, perennial vegetation to amenity grass covered areas.

There was no significant difference in the cover of both impervious ground surfaces and impervious building roofs between inner and outer suburbs (Figure 3).

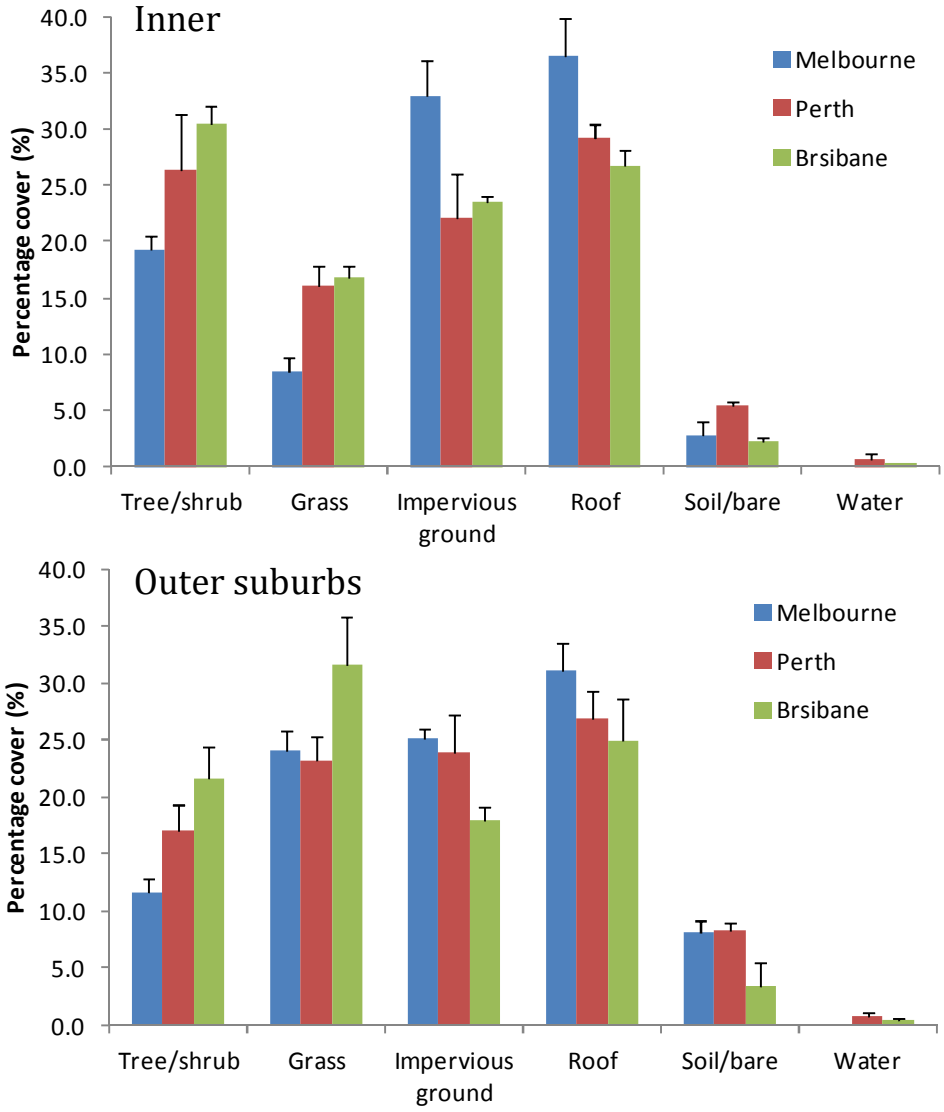


Figure 3. The percentage covers of different land surface types within residential areas of old inner (top) and new outer (bottom) suburbs in Melbourne, Perth and Brisbane

Canopy cover differences in Melbourne, Perth and Brisbane

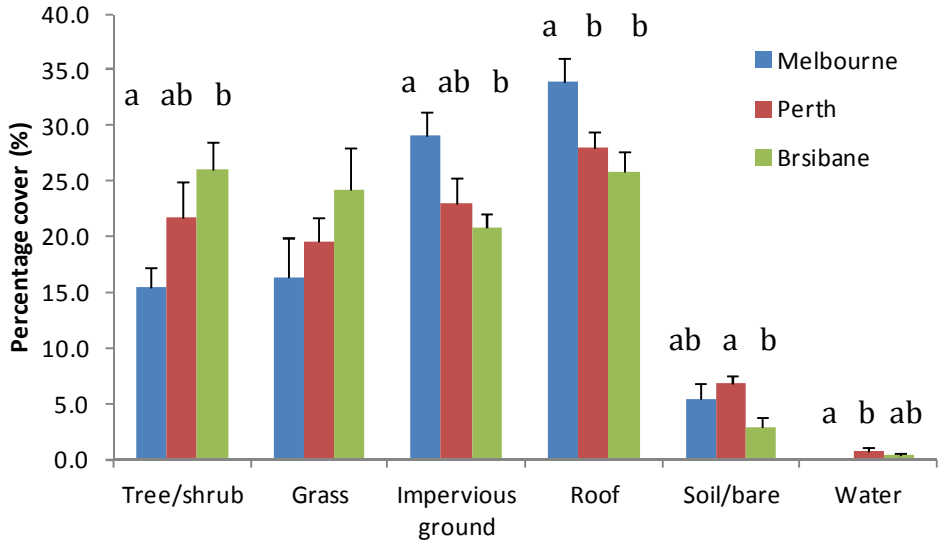


Figure 4. The percentage cover of different land surface types within Melbourne, Perth and Brisbane. Significant differences indicated at LSD $p \leq 0.05$.

Residential areas in Melbourne (15.4%) have significantly less tree and shrub canopy cover than in Brisbane (26.0%)and concurrently have a significantly greater area covered by impervious ground or roof surfaces, 62.8% versus 46.6% (Figure 4 and Table 7). This would suggest that the residential areas of Melbourne, both inner and outer, are more dense with a greater proportion of the land surface area covered by buildings or roadways with less space available for vegetation (tree, shrub or grass).

Table 7. Mean percentage cover of land surface types in three Australian cities. Significant differences presented according to LSD as determined from ANOVA (GENSTAT 14.0)

City	Tree/shrub	Grass	Imp. ground	Imp. roof	Soil/bare	Water
Melbourne	15.4 a	16.3	29.0 a	33.8 a	5.5 ab	0.0 a
Perth	21.7 ab	19.6	23.0 ab	28.0 b	6.8 a	0.8 b
Brisbane	26.0 b	24.2	20.7 b	25.9 b	2.9 b	0.4 ab
p value	0.035 *	0.261	0.031 *	0.020*	0.052	0.023*
LSD	7.81	9.81	6.14	5.49	3.17	0.52

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Appendix 8

THE EFFICACY OF IAA-PRODUCING PGPR IN DIFFERENT FORMULATIONS AS PLANT GROWTH REGULATOR

Apriwi Zulfitri

A thesis submitted for fulfillment of the requirements for the degree of

Master of Science in Agriculture

Faculty of Agriculture and Environment

The University of Sydney

New South Wales

Australia

March 2012

STATEMENT OF ORIGINALITY

I hereby certify that this thesis has not been submitted for any degree or diploma at any university and that to my best knowledge any help received in preparing this thesis and all reference materials used, have been acknowledged.

…….

Apriwi Zulfitri

March 2012

ABSTRACT

Intensive containerized-plant production systems in the ornamental nursery industry require high levels of water and chemical inputs to meet market demands and produce high quality plants over short periods of time. Inoculant based on plant growth promoting rhizobacteria (PGPR) are applied extensively to agricultural crops to improve plant growth and at the same time reduce chemical inputs including fertilizers and pesticides which can cause environmental degradation. However, PGPR application in the ornamental industry has not been widely studied.

Ornamental plant propagation is a critical part of the nursery industry to ensure aesthetic values which may be altered as a result of seed propagation. For that reason, many ornamental growers propagate ornamental plants vegetatively by cuttings. Inoculation of plant cuttings with IAA-producing PGPR may be a cost-effective alternative to using synthetic auxins in order to promote adventitious root growth.

The overall aim of this project was to evaluate the effectiveness of PGPR on the growth and development of ornamental plants of high value to the nursery industry by selecting the most responsive PGPR and ornamental host plant combination. This project also investigated the efficacy of several different formulations of PGPR to improve root growth parameters of cuttings and investigated the development of suitable formulations for IAA-producing PGPR.

The use of water as a growth medium and cuttings as a plant material was shown as an effective method to measure PGP effects of PGPR in ornamental plants and for evaluating the most responsive combination of PGPR-ornamental plant. However this method was limited by the lack of rhizosphere available for PGPR colonization and was also prone to pathogen contamination. The highest IAA producer, *Azospirillum brasilense Sp245* and the plant *Lavandula stoechas* (lavender) were selected to further evaluate the potential of PGPR in cutting propagation methods. When grown in sand and water media, Sp245 culture grown with tryptophan showed comparable effects to commercial rooting hormone in stimulating root growth parameters of *L. stoechas* cuttings. This formulation was better than other Sp245 treatments and commercial biofertilizer product. There was a positive relationship between

increased IAA concentration contained in the immersion solution and improved root growth parameters and this especially apparent in sand media. Less observable PGP effects in water medium may have been a result of an IAA dilution effect. Although there was only a weak positive relationship observed between numbers of viable cells recovered from cuttings with root growth parameters (only 0.6-19% variability in root growth parameters could be explained by number of viable cells recovered from cuttings), the positive correlation between IAA and root growth parameters showed that the PGP effect of IAA concentration contained in the supernatant of Sp245 with tryptophan did not improve root growth as effectively as Sp245 culture containing Sp245 cells together with IAA. This indicates that improved root growth parameters were not only due to the IAA concentration alone but may be partly due to other substance released by Sp245 cells. Formulations of Sp245 in peat, proven successful inoculant carrier, showed good survival rate but low IAA production and may be therefore ineffective for *L. stoechas* propagation. A better inoculants formulation for Sp245 that provides not only a sufficient number of inoculums but also be able to produce IAA to induce adventitious root growth in ornamental propagation.

ACKNOWLEDGMENT

My deepest gratitude goes to my both supervisors Dr. Meredith Wilkes and Dr. Rosalind Deaker for their invaluable guidance, knowledge and indefinite patience during the period of research work and in the completion of this thesis. I highly appreciate the considerable time and effort they have devoted in reviewing this thesis.

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I wholeheartedly am grateful to my dearest parents, Mahsun Gani and Sri Yatmini, for their unfailing support, for always believe in me and their continuous prayers for me.

The financial support for this master degree was provided by Australian Development Scholarship from AusAID

Above all, I praise“Almighty Allah” the most merciful for providing me with this invaluable experience.

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CHAPTER 1

ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylate
AMF	Arbuscular mycorrhiza fungi
BNF	Biological nitrogen fixation
cfu	Colony forming unit
CRF	Controlled-release fertilizer
DAPG	2,4-diacetylphloroglucinol
FYM	Farm yard manure
GFP	Green flourecent protein
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISR	Induced systemic resistance
MPN	Most probable number
PCA	Phenazine-1-carboxylic acid
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
PRHC	Pea and rice hull compost
RDF	Recommended dose fertilizer
RDN	Recommended dose Notrogen
SAM	S-adenosylmethionine
SAR	Systemic acquired resistance
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
VAM	Vesicular arbuscular mycorrhiza

CHAPTER 1 LITERATURE REVIEW

1.1 Introduction

Intensive containerized-plant production systems in the ornamental nursery industry require high levels of water and chemical inputs to meet market demands and produce high quality plants over short periods of time. Potting media used in containerized-plant production retains limited nutrients. In addition, the confined space and the small volume of the media limit potted plant roots to explore and obtain sufficient nutrients, therefore frequent nutrient amendments are required to support plant growth. The long term use of excessive chemicals, including fertilizer, may lead to run-off and degradation of the surrounding environment at levels similar to agricultural sites.

Inoculant-based on plant growth promoting rhizobacteria (PGPR) are applied extensively to agricultural crops to improve plant growth and at the same time reduce chemical inputs including fertilizer and pesticide which can cause environmental degradation. However, PGPR application in the ornamental industry has not been widely studied. Ornamental plant propagation is a critical part of the industry to ensure aesthetic values which may be altered as a result of seed propagation. For that reason, many ornamental growers propagate ornamental plants vegetatively by cuttings.

Research on PGPR has covered many aspects from the isolation of potential PGPR strains from plant roots, *in vitro* and field application of PGPR, to developing reliable inoculant formulations as biocontrols or biofertilizers. However, results are inconsistent and difficult to reproduce, due to the complexity of plant-PGPR interactions in nature. Positive results obtained from *in vitro*-based studies do not necessarily ensure a success in field trials. The promotional effects of PGPR may be more consistently achieved if plant growth promoting (PGP) mechanisms during the interaction are properly clarified using molecular approaches.

This chapter aims to review practices for ornamental production in the nursery industry and explore PGPR application as an environmentally friendly alternative to chemicals for improved plant growth. A summary of PGPR studies conducted in ornamental plants

including inoculation methods and positive responses observed from interaction of PGPR-ornamentals is also included.

1.2 Plant growth promoting rhizobacteria (PGPR)

The rhizosphere is the thin layer of soil adjacent to plant roots that is influenced by root activities. This term was first introduced by Lorenz Hiltner, a soil microbiologist, in the early 1900's after years of study on the role of different plant (legumes and non-legumes) root exudates in attracting different bacterial communities surrounding the root zone. He also studied how the bacteria colonizing the root surface and epidermis influence plant nutrient availability (reviewed in Hartmann et al., 2008). Hiltner's original definition of the rhizosphere has now been extended to cover the larger proportion of the soil around plant roots that is also affected by root growth and activities in terms of the soil physical, chemical and biological properties (McCully, 2005). The rhizosphere is an intense interactive zone as the root releases sugars, amino acids and other organic compounds that can be utilised by soil microorganisms, including bacteria, for their viability (Dobbelaere et al., 2003; Singh et al., 2004; Lambers et al., 2009). This nutritious environment results in a much higher population of bacteria in the rhizosphere but a lower diversity/species richness than in the bulk soil (van Loon and Bakker, 2003; Lugtenberg and Kamilova, 2009). The bacteria that occupy the rhizosphere are collectively termed rhizobacteria. Rhizobacteria can have profound effects on plant health and nutrition.

The interaction or communication between plants and rhizobacteria occurs through chemical signals released by both partners. The structure of the rhizobacterial community is affected by several factors including plant genotype and is determined by the amount and composition of root exudates (Marschner et al., 2004). In addition, the soil type and fertility are contributing factors that also shape the community (Innes et al., 2004). The rhizobacterial community may influence this interaction by exuding compounds as a means of communication that is recognisable by neighbouring bacteria and root cells of host plants (Bais et al., 2004; Gray and Smith, 2005). This form of communication can affect plant growth, nutrient status and also susceptibility to stress and pathogens in the host plant (Morgan et al., 2005).

Antoun and Prévost (2006) classified rhizobacteria as being neutral, deleterious or beneficial. The presence of the neutral group might be insignificant to the host plant, while deleterious rhizobacteria produce metabolites adverse to plant health. The concept of deleterious rhizobacteria is debatable because previous studies on this topic were mostly done in gnotobiotic and soil-less conditions without any challenge from native soil bacteria (cited in Antoun and Prevost, 2006) and these conditions are unlikely to exist naturally. In addition, Glick et al. (1999) stated that more destructive effects on agronomically important crops are mostly caused by phytopathogenic fungi, such as *Fusarium* and *Phytium* genera, thus the negative effects of deleterious rhizobacteria on plant growth are rarely discussed in relation to this topic. The beneficial category of rhizobacteria are able to promote plant growth and development, and are generally further grouped according to their physical interaction with the host plant (Glick et al., 1999). Beneficial rhizobacteria may form symbiotic interactions which involve modification of the host plant root morphology through nodule formation. Other beneficial rhizobacteria are free-living in the soil and employ associative relationships with the host plant. These free-living rhizobacteria are defined as plant growth promoting rhizobacteria (PGPR) and form associations with many different plant species (Kloepper et al., 1989).

PGPR are indigenous to soil and are able to competitively colonize plant roots. An effective root colonist is a fundamental trait for PGPR in order to survive in the rhizosphere and root surface, and to establish and effectively support host plant growth (Lugtenberg and Dekkers, 1999; Kamilova et al., 2005). Originally, the definition of PGPR only referred to free-living beneficial rhizobacteria (Kloepper et al., 1989), but over the years the definition has been extended to any root colonizing bacteria including symbiotic rhizobacteria (Antoun and Prevost, 2006). However, symbiotic rhizobacteria, especially rhizobia which are capable of fixing nitrogen in leguminous crops, are not usually considered as PGPR (Spaepen et al., 2009) and will not be discussed in this review. Recognition that root-associated bacteria can stimulate plant growth began in the mid 1920's and more recently win renewed interest in the 1970's. This field of research provides a potentially useful method for sustainable production of important staple food crops such as wheat, rice and maize (Dobbelaere et al., 2003).

PGPR comprise a broad range of soil bacterial taxa (Vessey, 2003; Lucy et al., 2004). Some common and well identified genera are *Azospirillum*, *Pseudomonas*, *Azotobacter*, and *Bacillus. Azospirillum* is a Gram negative, motile vibrio or spirillum, 1 µm in diameter, and is one of the most well studied genera since being identified as a free-living beneficial root associated bacteria (Saikia et al., 2010). The Bashan foundation, a non-profit scientific organization in Oregon, USA, has extensively studied and dedicated one of its major research programs to PGPR especially *Azospirillum*. The foundation provides a number of comprehensive papers on this particular genus, from the effective isolation and quantification methods from wheat roots, root colonization characteristics in different plant species, detailed plant growth promoting (PGP) mechanisms, ecology, agricultural applications, physical and molecular studies and also the future challenges and potential use of *Azospirillum* as a commercial PGP inoculant (Bashan and Levanony, 1985; Bashan et al., 1991; Bashan et al., 2004; Bashan and de-Bashan, 2010).

Another example of a well-identified PGPR is *Pseudomonas. Pseudomonas* is an aerobic Gram negative, fast growing, competitive root colonist, and is commonly found in the rhizosphere (Weller, 2007). Lugtenberg and Dekkers (1999) reviewed molecular based studies on identifying traits responsible for effective colonization of *Pseudomonas* by screening impaired mutants on different plants, then comparing their colonization ability with the wild type. The authors noted that slow growth and an inability to biosynthesize essential amino acids are among factors affecting the rhizosphere competence of PGPR. Kumar (2011) found that effective root colonization and survival in the presence of indigenous soil inhabitants, determine the rhizospheric competency of a PGPR.

Some *Pseudomona*s strains have been shown to improve plant growth by releasing a wide range of antifungal metabolites that suppress the growth of pathogens of agronomically important crops in both laboratory and field trials (Haas and Keel, 2003). Amein (2008) reported that a strain of *P. flourescens* provided consistent protection to field grown winter wheat seedlings from blight disease over two growing seasons. A considerable increase in plant survival rate and yield were also reported.

Bacillus is a Gram positive aerobic organism that can resist environmental stress by forming endospores (Kumar et al., 2011) and many strains of *Bacillus* and *Paenibacillus* are known to

stimulate plant growth. Emmert and Handelsman (1999) highlighted the endospore forming character of *Bacillus* as an important characteristic for a potential biocontrol inoculant as the spore can endure heat and desiccation ensuring the formulation will be stable over time. This genus is considered non-rhizosphere competent, unlike *Pseudomonas*, but given that rhizospheric competency is strain-dependent, some strains of *Bacilllus* may be rhizosphere competent (Kumar et al., 2011).

PGPR have attracted increasing attention over the years as more significant results in plant health and yield are reported. PGPR offer an environmentally friendly alternative for maintaining crop productivity in intensive agricultural practices. They may reduce excessive use of chemical inputs in agriculture and therefore decrease environmental degradation. Nutrient leaching and run-off can increase the nutrient content of environmental water, promote algal growth and decrease dissolved oxygen levels creating harmful conditions in water ecosystems. An example of a severe nutrient run-off occurred in the Gulf of Mexico in midsummer 2001, where 20,700 km^2 became permanently hypoxic (oxygen deficient) due to fertilizer run-off that was carried by the Mississippi river from agricultural sites (Rabalais et al., 2002). Other effects of chemical nitrogen (N) fertilizer include leaching of nitrate (NO_3^-) to ground water, high nitrous oxide (N_2O) emissions to the atmosphere and greenhouse gas emissions during N fertilizer production and transport (reviewed in Biswas et al., 2000).

PGPR may provide essential nutrients for plant growth or enhance nutrient availability, play a role in pathogen suppression, offer environmental sustainability and improvement of soil health in the long term (Vessey, 2003; Lucy et al., 2004; Lugtenberg and Kamilova, 2009) thereby potentially reducing the use of chemical fertilizers and pesticides.

1.3 Ornamental industry

Increasing attraction and demand for ornamental plants have made the floricultural industry one of the fastest growing agribusiness sectors worldwide. The main purpose of the ornamental industry is to provide high quality plant products, however this utilizes great amounts of water and chemical inputs such as fertilizer and synthetic growth hormones to obtain optimal plant growth and meet consumer demand. Even though the total land use by the ornamental industry may not be as large as that of crop plants, intensive containerized plant production potentially affects the surrounding environment, due to run-off from

excessive fertilizer use, at levels similar to intensive agricultural sites (Colangelo and Brand, 2001). The use of PGPR has been proposed as a way to reduce the negative effects of fertilizer use in this industry.

1.3.1 Ornamental production in the nursery industry

Over the years there has been a changing trend in the techniques of ornamental plant production. Traditionally, growers focused on extensive field systems to produce their plants, but since the early 1970's, they have shifted to high density production per unit area using containerized plants due to increasing resource costs (Majsztrik, 2010). Ornamental plants in nursery systems are usually produced in soil-less substrates or potting media. Soil substitutes were suggested as an alternative to soil, because confined soil in containers may cause impeded water drainage, poor aeration and disease development during production (Majsztrik, 2010). Peatmoss, pine bark or compost are common elements of potting media for containerized plants (Chen et al., 2002), however increasing peat prices due to mining and shipping costs has led to the evaluation of alternative materials (Wright et al., 2008; Moral et al., 2009). Pine bark and coconut coir are some examples of frequently-used peatmoss substitutes.

Containerized plant production represents an intensive horticultural practice, because in order to achieve high quality products, container-grown plants require large amounts of water and fertilizer as a result of nutrient limitation in the small potting media volume. Along with the shifting mode of production, these intensive practices are having negative impacts on the local environment. Since most potting media are porous in nature and have lower exchange capacities, they will stimulate nutrient leaching from the container (Bilderback et al., 2007).

Even though the use of chemical fertilizers is highly important to enhance plant growth and ornamental quality, their long-term overuse may cause environmental degradation (Gyaneshwar et al., 2002). In the nursery industry, there have been many efforts to discover better management practices related to the use of chemical fertilizer in efforts to minimize environmental damage. Several researchers have highlighted the over-application of water and fertilizer as a major issue in nursery production (Green et al., 1998; Lea-Cox et al., 2001; Chen et al., 2002; Ristvey et al., 2007) and Green et al. (1998) found that plants may capture

and utilize less than 10% of the water and fertilizer applied resulting in low nutrient uptake and nutrient loss through leaching (Ristvey et al., 2007). Low nutrient uptake in high-value ornamental plants may be a result of the use of artificial nutrients and water-rich confined systems continuously driving plants to focus on maximizing shoot growth. This results in ineffective root systems with less need to explore for water and nutrients in the growth substrate (Lea-Cox and Ristvey, 2003).

1.3.2 Application of chemical fertilizer

To date, container grown plant research has focused on efforts to increase efficiency of nutrient uptake in ornamental production while reducing nutrient. Water-soluble fertilizer (WSF) and controlled-released fertilizer (CRF) are types of efficient fertilizers which have been regularly used in the nursery industry (Chen et al., 2002). WSF is usually applied through irrigation systems such as overhead, drip, sub-irrigation or combinations between drip and subirrigation; this system is usually called fertigation. In this system, fertilizer can be simply adjusted to growth requirements of the plant (Majsztrik, 2010), however, each system has its own disadvantages.

The application of overhead fertigation has the possibility of fertilizer run-off and salt build up on the ground. Using drip irrigation or a combination between drip and subirrigation avoids leaf absorption of chemicals, employs less labour and allows better timing of fertilization of plants in line with their growth stage. Nevertheless, the holes in the irrigation tube are usually small and can be easily blocked by root growth, media particles, chemical precipitates or biological blockages such as bacteria and algae, so unequal fertilization may occur (reviewed in Carrasco and Urrestarazu, 2010).

CRF technology applies hydrophobic polymer coated elements on granules of fertilizer, ensuring the gradual release of nutrients suitable to each plant growth stage thereby improving fertilizer effectiveness and reducing contamination of the environment (Du et al., 2006). The first stage of constant fertilizer release mechanism of a single granule was described by Shaviv et al. (2003). As water vapour condenses through to the membrane of the granule coating, it dissolves a part of the dry nutrients thus causing an internal pressure change and the formation of a saturated nutrient solution. Then, the nutrient is released through a diffusion mechanism, due to resistance of the oating membrane to internal

pressure, by a 'concentration gradient across the coating'. The fertilizer is diffused at a gradual rate as long as the pressure gradient between the undissolved granule and saturated solutions remains constant. The rate and timing of fertilizer release are controlled by the type and thickness of the coating material (Lubkowski and Grzmil, 2007). CRF application in containerized ornamentals or woody plants has shown positive results in improving commercial plant quality and reducing environmental contamination at the same time (Wilson and Struve, 2006; Segura et al., 2007; Andiru, 2010).

Although the nutrient release rate is controlled by a coating diffusion mechanism, temperature appears to significantly affect the process and impacts on the CRF release characteristics since the rates will increase with a rise in temperature and vice versa (Huett and Gogel, 2000; Du et al., 2006). Husby et al. (2003) reported nutrient release rates of CRF were positively correlated with increase in temperature during 20 hours of observation. Since release rate is temperature dependent, the highest release rates would be during summer when plants require high amounts of water, potentially leading to high nutrient run-off because of intensive irrigation (Majsztrik, 2010). The author also suggested temperature-based fertilizer, such as CRF does not support plant nutrient uptake requirements because ornamentals require high nutrients mainly during relatively low temperature months. However, Merhaut et al. (2006) reported that CRF release rates performed under controlled temperature conditions did not seem to be affected by temperature. Management practices in the nursery industry including supervision and regularly checking on growing conditions along with appropriate irrigation when CRF is applied are required to avoid excess fertilizer run-off in the environment. Furthermore, selecting effective coating components which synchronize plant biological requirements with stable release rates under fluctuating field conditions need to be better assessed.

1.3.3 Application of organic fertilizer

Sustainable agricultural systems, including floriculture, that use organic materials such as farmyard manure, agricultural waste and vermicompost (plant and animal waste composted through the worm activity) are considered to be environmentally friendly alternatives for improving ornamental growth compared with chemical fertilizers. Biodegradable waste, municipal solid waste or household waste have also been shown to enhance organic N and C availability to plants, maintain soil pH and repair physical soil quality without changing the

soil bacterial community (Crecchio et al., 2001). The application of organic material has been reported to improve high-value ornamental plant quality significantly compared to those treated with inorganic fertilizer.

In a study on the effect of organic fertilizer in marigold (*Tagetes erecta* L.), vermicompost resulted in better plant performance compared with other soil amendments tested (including a commercial product originating from a mixture of animal manure and *Thiobacillus*). The vermicompost addition resulted in increased flower number, flower diameter, and root fresh and dry weight. Plant vermicompost could be applied as an alternative fertilizer in the ornamental industry considering vermicompost production is more cost effective compared to commercial products, and facilitates waste management due to the composting process which consumes urban waste as a raw material (Nazari et al., 2008). In a different study, Chang et al. (2010), used pea and rice hull compost (PRHC) as the only N source to grow anthurium in a soil-less substrate. The results showed similar growth, yield and cut flower quality to the plants receiving CRF or chemical nutrient solution, confirming sufficient nutrition from PRHC for anthurium cut flower production. In contrast, Cantaragiu and Toma (2008) found that poinsettia fertilized with cattle manure solution displayed growth and bract colouring quality below acceptable standards compared with inorganically fertilized and control treatment plants. These findings imply inconsistent responses of plants when organic material is used as the N source, especially in relation to maintaining ornamental quality in nursery production. Additionally, some organic fertilizers may release strong odours.

Increasing intensive livestock farming has resulted in a rise in the amount of organic waste. Thus, the application of composted manure as an ornamental substrate component could be a sustainable and effective waste treatment method; compost amended with manure enriches its N and P content (Moral et al., 2009). Although manure is able to significantly increase soil fertility and maintain pH, the high concentration of N combined with long term use may potentially lead to environmental pollution. Furthermore, manure application may also result in greenhouse gas emission (GHG) in the atmosphere due to the high content of N and C (Jarecki et al., 2008).

Studies on the effects of organic fertilizer have shown that the amendments not only improve nutrient availability to plants and physical soil quality, but also affect the soil microbial

community (Marschner et al., 2003; Pérez-Piqueres et al., 2006). Nutrient release of composted organic material that results in increasing soil quality is considered to be due to soil microorganism activity. Arancon et al. (2008) suggested that the influence of vermicompost on increasing germination rates, vegetative growth and number of flowers in petunias was a result of better potting media structure, increased population of beneficial microorganisms and hormones produced by these microbes. Given that both inorganic and organic fertilizers are at risk of stimulating environmental pollution, soil microbes including rhizobacteria have a promising future as environmentally friendly fertilizer in the ornamental industry.

1.4 Potential applications of PGPR

Potential applications of PGPR are mainly classified by the mechanisms used to stimulate plant growth and health during interactions with the host plant. PGPR may perform as a plant disease suppressor and/or plant growth promoter via several different mechanisms (Fig. 1) which is why PGPR can be applied as a biocontrol and/or a biofertilizer.

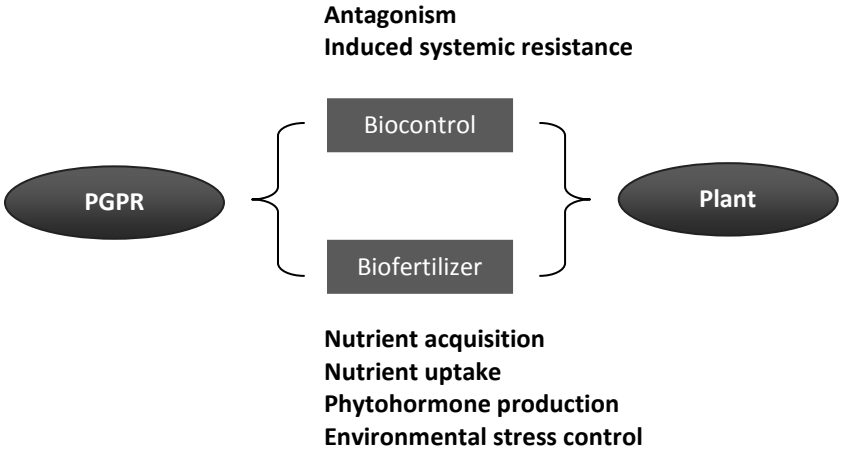

Fig. 1.1 Plant growth promoting mechanisms of PGPR. Adapted from Kumar et al. (2011). PGPR may increase plant growth by acting as a biocontrol and biofertilizer. Biofertlizer mechanisms involve nutrient acquisition and uptake, production of phytohormone and environmental stress control. PGPR may be act as a biocontrol through antagonism and induced systemic resistance against plant pathogens

PGPR control growth of plant pathogens by antagonistic activities and activating plant resistance to pathogenic microorganisms. The antagonistic mechanisms include antibiotic production, competition and parasitism, whereas biofertilizer mechanisms involve nutrient

acquisition and uptake, plant growth hormone production and reducing negative effects of environmental stress (Fig 1.1).

1.4.1 Biofertilizer

Providing nutrients to the host plant by synthesizing nutrients or increasing their availability is the main direct mechanism of PGPR in stimulating plant growth. PGPR which possess these mechanisms are referred to as biofertilizers and they act as an alternative to chemical fertilizer. Biofertilizers have attracted much attention due to their potential to reduce the use of chemical fertilizers which are hazardous to humans and animals and also pollute the environment in the long term or after excessive use. Several specific mechanisms have been reported and proposed to describe how PGPR directly improve the nutrient status of plants. Some bacteria may possess and utilize different mechanisms in the host plant depending on the life cycle or requirements of the host plant (Glick et al., 1999).

1.4.1.1 Associative biological nitrogen fixation

Despite its abundance in the atmosphere, N is known as a plant growth limiting factor. Consequently, the availability of N for plants is crucial. The N has to be reduced to ammonia so it can be utilized by plants to produce nucleic acids and proteins. Free-living diazotrophs, a group of bacteria which are able to convert atmospheric nitrogen (N_2) into readily usable ammonia, may be considered as PGPR. This is a high energy-requiring process which may be biologically catalysed by the nitrogenase enzyme and regulated by nitrogen fixation genes (*nif*) and is called biological nitrogen fixation (BNF) (Glick et al., 1999).

Although the inoculation with diazotrophic bacteria such as *Azospirillum, Azoarcus* and *Pseudomonas* spp. has shown improvement in growth, nitrogen content and yield in cereal grains (including wheat and rice), evidence of BNF being the main mechanism employed causing plant improvement was not significant (reviewed in Lucy et al., 2004). This result is in agreement with experiments using non-nitrogen fixing (Nif^-) mutants of *Azoarcus* done by Hurek et al. (1994) in rice seedlings. The authors observed that the bacteria were still capable of enhancing plant growth and development despite losing their nitrogen fixation ability, indicating that the enhanced plant growth may involve other mechanisms than direct transfer of nitrogen from the diazotrophic bacteria to the host plant.

Additionally, nitrogenase activity is inhibited by the presence of ammonia and is not likely to support plant growth by fixing nitrogen in highly nitrogen-fertilized environments, such as intensively-fertilized agriculture (Dobbelaere et al., 2003). Sevilla et al. (2001) investigated sterile sugarcane growth in two different growth media, N-sufficient and deficient, in combination with inoculation of non-nitrogen fixing (*nifD*$^-$) mutants of *Acetobacter diazotrophicus* which lack the ability to fix N_2. In this study, they found that in N-deficient conditions, plants inoculated with *nifD*$^-$ mutants had lower total nitrogen contents and also less growth than the plants treated with the wild type suggesting that the transfer of nitrogen fixed by the wild-type inoculants to the host plant might be involved in supporting sugarcane growth. When N was sufficient for plant growth, significantly better growth was observed in sugarcane inoculated with the mutant or wild type than uninoculated plants. However, there was no significant difference in the plant growth between the bacterial inoculations indicating involvement of other mechanism in *Acetobacter diazotrophicus* in enhancing plant growth other than BNF as the mutant was not capable of fixing N_2.

1.4.1.2 Increasing nutrient uptake

PGPR inoculation potentially reduces chemical fertilizer application and in the long term, may decrease chemical build up in agricultural soils. Chemical build up is caused by low plant nutrient uptake efficiency combined with years of overuse of chemical fertilizer. Chemicals, especially phosphorus (P), easily precipitates, sometimes up to 90%, after being applied to soil thus making the mineral less available to plants and remaining in the soil (Gyaneshwar et al., 2002). In addition, application of organic fertilizers such as manure and compost, which increase soil nutrient levels, may also cause nutrient build up and are possible run-off in the environment similar to chemical fertilizers (Mitchell and Tu, 2006).

Several studies by Adesemoye and colleagues have described enhanced plant nutrient uptake efficiency as a proposed PGP mechanism. They found that some PGPR strains were able to enhance plant nutrient uptake efficiency by substituting for chemical fertilizer. Adesemoye et al. (2008) reported that corn inoculated with commercial PGPR and arbuscular mycorrhiza fungi (AMF), either singly or combined in combination with two fertilisation amendments (ammonium nitrate and poultry litter), had better growth and significantly better yield than uninoculated corn. The significantly increased N, P and potassium (K) content in the corn grain and silage from plot with inoculants reflected enhanced plant nutrient uptake and the

level of those nutrients removed from the field plot. As this study was conducted for three years on a long-term corn field, the removal of N, P and K from the field plot (in the form of yield or plant removal) that had been planted with microbial-inoculated corn may lead to fertilizer build up reduction thus potentially reduce loss of the nutrients from the field to the surrounding environment.

Adesemoye et al. (2009) then investigated the relationship between inoculants and reduced rates of recommended N fertilizer doses in tomato plants. In this study, tomato plants were inoculated with PGPR, AMF or a mix of both, in combination with 0, 75 and 100% of the recommended N fertilizer rate. The results showed that mixed inoculation with 75% N demonstrated comparable plant growth and nutrient uptake (N and P) to the full rate fertilizer treatment without inoculants. A combination of inoculants and lower fertilizer rates (<75%) showed inconsistent results indicating that the inoculants were not able to fully replace the use of chemical fertilizer but potentially reduced its rate.

Further to these results, Adesemoye and colleagues (2010) analysed tomato plants fertilized with ^{15}N-depleted isotope that has lower ^{15}N concentration (0.01 atom%) than the natural abundance of ^{15}N (0.336%) to monitor N movement and to demonstrate that PGPR inoculation improved plant uptake of N from applied fertilizer and not only from residual N contained in the soil. PGPR used in this experiment was a mixture of *Bacillus amyloliquefaciens* strain IN937a and *B. pumilus* strain T4 that not capable of fixing N_2. Generally the authors found that the concentration of ^{15}N contained in per gram of plant tissue decreased as applied ^{15}N fertilizer level increased. The decrease was a result of applying fertilizer containing^{15}N-depleted isotope to plants. When using this depleted isotope, atom% of ^{15}N per gram of plant tissue decreased as ^{15}N uptake by plant tissue increased. Therefore further decrease in percentage of ^{15}N per gram of plant tissue indicating increased ^{15}N uptake from fertilizer applied as shown by tomato plants inoculated with PGPR mixture inoculation together with 80% fertilizer. These plants showed significantly lower ^{15}N% contain per gram of tomato tissue than those found in plants treated with 80% fertilizer suggesting that PGPR inoculation increased N uptake by the plants. From these results, they concluded that mechanisms, other than N_2, fixation must be involved in increasing N uptake since the bacterial strains used in the experiment were not capable of N_2 fixation.

1.4.1.3 Auxin production

Roots are the primary way a plant absorbs water and essential nutrients, consequently better root structure and development are required to enhance nutrient uptake efficiency and promote plant growth. Nutrient enhancement has been proposed as a mechanism of PGP due to specific alterations in root structure related to phytohormone production by PGPR during interactions with host plants. Some results in studies that examined N_2 fixation as a major PGPR mechanism in non-legume plants showed that bacterial fixed-N is an insignificant supply for host plant requirements (Spaepen et al., 2009), so recent PGPR research has given more attention to exploring other PGP mechanisms such as phytohormone production, especially auxin. PGPR may produce gibberellins and cytokinins but most attention has focused on the production of the auxin, indole-3-acetic acid (IAA). Barbieri et al. (1986) was first to report that plant responses to PGPR inoculations were more likely due to the auxin production mechanism than N fixation. They inoculated PGPR mutants impaired in N_2 fixation ability and auxin biosynthesis in wheat seedlings and found that root growth of the wheat seedlings was reduced when inoculated with a Nif-mutant that synthesized less IAA, while inoculation of a Nif-mutant that was a high IAA producer resulted in insignificantly different growth compared to the control.

Khalid et al. (2004b) isolated bacterial strains from the rhizosphere of several different cereal plants and found that up to 80% of the bacteria isolated were able to synthesize auxin without the presence of a precursor. The authors then compared auxin production in rhizosphere and non-rhizosphere soils by inoculating the highest bacterial auxin producers in rhizosphere and non-rhizosphere soil separately. The results showed that the hormone concentration was greater when the bacterial strains were inoculated in rhizosphere soil compared to non-rhizosphere soil, in the presence of the IAA precursor L-tryptophan, suggesting some inoculated bacterial strains may enhance auxin biosynthesis in the rhizosphere soil, despite the presence of the rhizosphere indigenous microorganisms, and subsequently induce plant growth.

IAA production has been widely observed in many rhizosphere microorganisms such as *Azospirillum, Enterobacter* and *Pseudomonas* (Patten and Glick, 2002; Dobbelaere et al., 2003; Baca and Elmerich, 2007). IAA is also produced naturally in plants and affects many important physiological processes including root proliferation and elongation (Salisbury and

Ross, 1992) The level of IAA produced by PGPR may affect host plants either positively or negatively (Glick et al., 1999). IAA may enhance root length and promote better-developed plant root systems, however, when the IAA concentration or inoculum are too high or too low, plant root growth may be inhibited. The effects of PGPR-produced IAA on root morphology and development in relation to IAA and inoculum concentrations was provided by Dobbelaere et al. (1999). Their experiments analysed wheat seedlings inoculated with increasing concentrations of wild type and mutants of *Azospirillum brasilense* Sp. 7 and Sp. 245 (10^6-10^9 cfu/mL). Inoculation with the wild type significantly inhibited root length and enhanced root hair formation compared to inoculation with dead cells, or the control. Conversely, wheat plants inoculated with mutant strains, impaired in the *ipdC* gene, which is involved in IAA synthesis, had very few root hairs, but no root length inhibition was observed. Furthermore, the authors also observed that the root length inhibition and root hair formation were more pronounced at high inoculant concentrations (10^8-10^9). Application of synthetic exogenous IAA to the roots, at increasing concentration (10^{-9}-10^{-4} M), showed identical effects to the PGPR inoculation treatment. It was concluded that the changes in root morphology might be a result of IAA produced by PGPR.

Even though some strains of rhizobacteria synthesize IAA in the absence of the precursor tryptophan in bacterial growth mediums, studies have shown that the concentration of IAA will increase significantly with the addition of tryptophan in the growth medium (Patten and Glick, 2002; Farah Ahmad et al., 2005; Khalid et al., 2004a). Khalid et al. (2004b) reported that plant roots also excrete tryptophan which may be used as an IAA precursor source for PGPR.

1.4.1.4 Ethylene regulation

Ethylene is the only gaseous plant hormone and has been considered as a plant growth inhibitor and also a growth promoter (Pierik et al., 2006). Ethylene also plays an important role in activating plant defence response mechanisms in abiotic and biotic stress conditions, such as drought, flooding, nutrient stress and the presence of pathogens (Glick, 2005; Hogan et al., 2006; Glick et al., 2007). Ethylene production is accelerated under stress conditions and stimulates flower senescence, leaf and petal abscission, and also premature ripening (Pierik et al., 2006). The effects of endogenous ethylene on plant growth and development are also affected by the hormone concentration and plant growth stage (Shaharoona et al., 2006b).

PGPR that possess the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase are capable of degrading plant-produced ACC. ACC is an immediate precursor for plant stress-induced ethylene production, thus by reducing ethylene levels, PGPR may protect host plants from the negative effects of ethylene and stimulate root growth (Glick, 2005; Tsavkelova et al., 2006a).

In 1998, Glick and colleagues proposed a model to clarify the growth promoting mechanisms caused by lowering plant ethylene levels (Fig 1.2). In this model, they postulated that IAA produced by PGPR, as a response to tryptophan contained in root exudates, may be taken up by the plant and enhance cell elongation and proliferation. Also, IAA produced by PGPR, together with endogenous IAA, converts S-adenosylmethionine (SAM) to ACC by activating ACC synthase. The stimulation of ACC synthase can also be enhanced by environmental stress, including physical stress such as floods, and biological stress such as plant pathogens (reviewed in Glick et al., 1999). The conversion of ACC to ethylene is catalysed by ACC oxidase. Nevertheless, some ACC might be secreted in root exudates and be taken up by ACC deaminase-containing PGPR and used as a nitrogen source. These bacteria then cleave the cyclopropane ring of the plant ACC to ammonia and α-ketobutyrate, thus decreasing the level of ACC outside of the plant. In order to maintain equilibrium between internal and external ACC levels, the plant secretes more ACC, subsequently lowering the internal ACC concentration and thereby reducing the amount of ethylene produced (Fig. 1.2).

Some PGPR have been tested for their ability in promoting plant growth via production of ACC deaminase under stress conditions such as in the presence of cadmium, a toxic heavy metal, (Safronova et al., 2006), inhibitory levels of environmental salinity (Mayak et al., 2004a; Cheng et al., 2007) and drought (Mayak et al., 2004b). The results showed that inoculation of PGPR possesing ACC deaminase was able to improve plant growth and stress tolerance. Co-inoculation of mung beans with *Bradyrhizobium* and PGPR with ACC deaminase activity enhanced plant growth and nodule production compared to mung beans inoculated with *Bradyrhizobium* only (Shaharoona et al., 2006a).

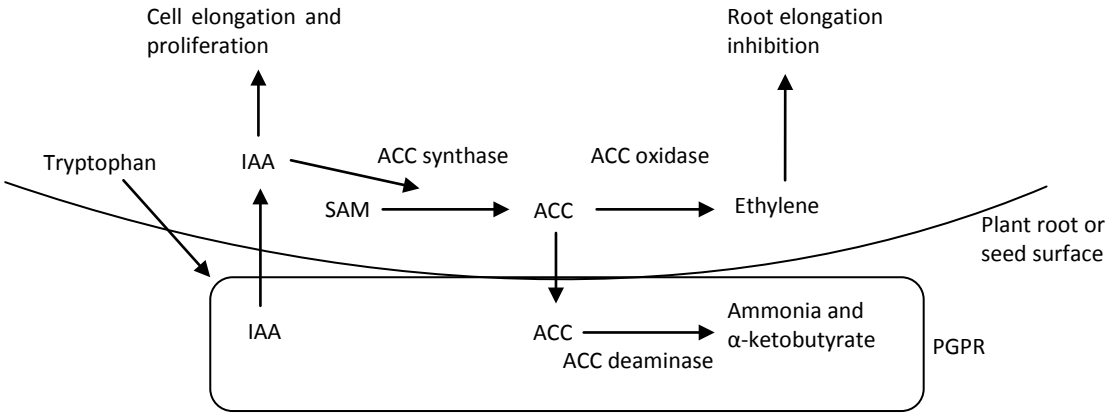

Fig. 1.2 Diagram of proposed PGPR mechanism to reduce plant ethylene levels (Glick et al., 1998). PGPR that possess ACC deaminase bound on plant or root surfaces synthesize IAA and together with plant endogenous IAA, stimulate cell growth. Synthesized IAA can also induce ACC synthase to convert SAM to ACC that will be metabolized to ethylene by ACC oxidase. Some of the formed ACC might be released from the plant and taken up by the PGPR. Hydrolysis of ACC by the PGPR lowers the level of outside ACC. The PGPR cause the plant to produce and exude more ACC thus subsequently reducing ACC levels inside the plant and then reduce ethylene formation. IAA : Indole-3-acetic acid; ACC: 1-aminocyclopropane-1-carboxylate; SAM: S-adenosylmethionine.

1.4.2 Biocontrol

The use of living organisms to limit the growth of pathogenic agents, thus reducing disease damage, is called biocontrol (Chinnasamy, 2006; Siddiqui, 2006). The application of PGPR as a biocontrol agent could be a more environmentally friendly alternative for supporting plant health as controlling plant pathogens using chemical agents is costly and destructive to the non target environment and, in the long term, may potentially induce pathogen resistance.

1.4.2.1 Antagonism

PGPR may act against pests, weeds or pathogenic microorganisms through antagonistic activities resulting from one or more specific actions such as competition for limited nutrients and niches on the root site, deleterious metabolite excretion or excretion of pathogen cell wall degrading enzymes (Barea et al., 2005). The antagonistic mechanisms mainly involve competition between PGPR and pathogens in the root environment (van Loon and Bakker, 2003).

Antagonism towards plant pathogens leads to their displacement and a subsequent stimulation of host plant growth (Whipps, 2001). Pliego et al. (2008) compared two efficient avocado root colonizing *Pseudomonas* strains in suppressing the fungal agent of white root

rot disease growth. The results showed that the two green fluorescent protein (GFP)-tagged *Pseudomonas* colonized different root sites when observed using confocal laser scanning microscopy. *P. pseudoalcaligenes* AVO110 was found abundantly in the intercellular space between epidermal root tip cells and root wounds while *P. alcaligenes* AVO73 inhabited root surface dispersedly and the proximity of lateral root tips. Suppression of fungal growth varied depending on the site colonised. *P. pseudoalcaligenes* AVO110 occupying the space between epidermal cells and root wounds was more likely to encounter the fungal hyphae because those particular sites are the preferential fungal penetration sites. In addition, the intercellular space is a nutrient rich site, attracting both PGPR and pathogens (Spaepen et al., 2009). From this study, Pliego et al. (2008) suggested that besides colonization ability, the root site occupied also contributes to effective pathogen biocontrol mechanisms. Studies on tomato roots observing competition between microorganisms using confocal laser scanning microscopy was conducted by Bolwerk et al. (2003). In this study, GFP-labelled PGPR (*P. fluorescens* WCS365) aggressively invaded the hyphae of GFP-labelled tomato foot and root rot (TFRR) pathogen, *Fusarium oxysporum* (forl), after competing for root exudates at the same site on the root which may decrease percentage of infected tomato plants.

Limited nutrients from plant root exudates or soil minerals, such as iron and phosphate, are some of the growth limiting factors for soil microorganisms (Kamilova et al., 2005). A well studied biocontrol mechanism using competition for limited nutrients is competition for iron, which occurs more intensively in the rhizosphere than in bulk soil (Elmerich and Newton, 2007). Despite its abundance in the soil, iron is mainly present as Fe^{3+} oxides which have low solubility (Spaepen et al., 2009). PGPR synthesize chelating mediators, such as high affinity siderophores, to bind and uptake the iron molecules. After siderophore-Fe^{3+} complexes are formed and bound to specific cell membrane receptors at the bacterial surface, the iron will be available as Fe^{2+} in the cytoplasm for bacterial metabolism (Couillerot et al., 2009; Lugtenberg and Kamilova, 2009). Insufficient or low affinity of plant pathogen produced-siderophores are incapable of competing with siderophores produced by PGPR on binding iron thus resulting in inadequate iron for pathogen growth (Dobbelaere et al., 2003). *Pseudomonas putida* WCS358 produces pyoverdines or pseudobactin, a yellow/green fluorescent iron-bound siderophore as a mechanism to control wilt disease caused by *Fusarium oxysporum* in radish (Devescovi et al., 2001; de Boer et al., 2003). In addition, Devescovi et al. (2001) found that the pseudobactin-impaired mutant demonstrated less

ability to suppress pathogens confirming siderophore involvement in the biocontrol mechanism.

PGPR biocontrol mechanisms also involve antibiotic and inhibitory metabolite production. Examples of well-characterized exuded metabolites include hydrogen cyanide (HCN), phenazines and 2,4-diacetylphloroglucinol (DAPG) (Raaijmakers et al., 2002; Haas and Defago, 2005; Spaepen et al., 2009). In their review of antibiotic production in *Pseudomonas* spp., Haas and Keel (2003) defined antibiotics as exuded metabolites by specific microorganisms which, at a certain concentration, have inhibitory or deleterious effects on other microorganisms . In a study to evaluate the antagonistic effect of three *Pseudomonas* strains on plant pathogens of chickpea, Akhtar and Siddiqui (2009) reported that secondary metabolites produced by the bacterial strains tested, especially *P. putida,* reduced the severity of damage by the pathogens. Hydrogen cyanide and antifungal metabolites produced by *P. putida* accounted for up to 59% of inhibition against hatching of the root-knot nematode *Meloidogyne incognita* and reduced fungal (*Macrophomina phaseolina*) root colonization by 64% *in vitro*. Subsequently the inoculated chickpeas had a better growth compared to uninoculated plants. In a similar study in coffee, *Pseudomonas* spp. and *Bacillus* spp. could potentially perform as biological control agents against coffee wilt disease caused by *Fusarium* spp. HCN and the production of antifungal lytic enzymes, including lipase and β-1,3 glucanase were considered as the major biocontrol mechanisms (Muleta et al., 2007). The toxicity of HCN to plant roots also allowed some host specific HCN-producing PGPR to be used as weed biocontrol agents as the host plants were likely to be cyanide-tolerant when inoculated at the seedling stage (Zeller et al., 2007).

In order to achieve effective plant protection from pathogens via biocontrol, PGPR not only have to inhabit the correct roots site, they also have to be present in sufficient number (Haas et al., 2000). Biocontrol of take-all disease of wheat in suppressive soils is one case that is related to the bacterial threshold density in suppressing soil-borne pathogens effectively. Suppressive soils have been described as soils, where despite containing soil-borne pathogens, the level of plant disease is low on crop roots due to the occurrence of indigenous soil bacteria (Haas et al., 2000; Mazzola, 2002; Weller et al., 2002). In 1998, Raaijmakers and Weller found DAPG-producing fluorescent *Pseudomonas* from wheat roots grown in suppressive soils in Quincy, Washington at sufficient numbers to provide significant disease

suppression. Further to this, the study demonstrated that the soil lost its suppressive properties after wet pasteurisation at 60°C for 30 min, confirming the role of the PGPR and antibiotic production in controlling disease. A high number of fluorescent *Pseudomonas* spp., which exuded HCN and DAPG, were also present in the rhizosphere of tobacco grown in soils suppressive to black root rot caused by *Thielaviopsis basicola* in Morens, Switzerland (Ramette et al., 2006). However, the authors suggested that the Morens soil suppressiveness may involve other unidentified soil microbial populations or environmental factors than only *Pseudomonas* or antibiotics produced, as they isolated antibiotic producing-*Pseudomonas* at similar abundance and diversity at the molecular level from a nearby conducive soil (the opposite of suppressive soil).

Another biocontrol mechanism is related to the synthesis of cell wall-degrading enzymes, mostly mycolytic enzymes, which lyse fungal cell walls. These enzymes include glucanases, proteases and chitinase and this mechanism is known as parasitism. For example, Fridlender et al. (1993) isolated a *P. cepacia* strain that produced β-glucanase that was able to damage hyphae of *Rhizoctonia solani*, *Sclerotium rolfsii* and *Phytium ultimum* and reduced the severity of disease caused by the fungal pathogens by up to 85% in greenhouse trials. *Enterobacter agglomerans* and *Bacillus* spp. also possess cell wall-degrading enzymes that are active against the fungal agent that causes rot root cotton disease, whereas another strain of *Bacillus* demonstrated parasitism towards *Curvularia lunata*, one of the main sorghum pathogens (Chernin et al., 1995; Pleban et al., 1997; Basha and Ulaganathan, 2002). A more recent study to explain the mechanism used by *P. fluorescens* to decrease the incidence of rot root disease in black pepper detected mycolytic enzyme excretion by PGPR which resulted in cytoplasmic coagulation in the mycelia of *Phytophthora capsici* when the microorganisms were cultured together (Diby et al., 2005).

1.4.2.2 Changing the plant susceptibility via induced systemic resistance (ISR)

Induced systemic resistance (ISR) is a plant-mediated defence system against a broad range of pathogens that may be induced by PGPR. The PGPR activate the system via chemical signals through pathways separate from systemic acquired resistance (SAR) which is induced by pathogen infection of the plant (Pieterse et al., 2003; Loon and Bakker, 2006a). *Pseudomonas flourescens* and *Bacillus* sp. are examples of widely studied strains that activate ISR in suppressing plant diseases (Bakker et al., 2007; Kloepper et al., 2004).

An ISR study in cucumber (Wei, 1991) used PGPR as a seed treatment to challenge *Colletotrichum orbiculare*, a causal agent of foliar anthracnose disease. In this study, the PGPR were used to inoculate seeds of a cucumber strain highly prone to anthracnose. After 21 days, the seedlings were inoculated with a conidial suspension of *C. orbiculare* on the second true leaf, and it was found that the inoculated plants had less severe damage compared to untreated plants in terms of number and diameter of lesions. As there was not any contact between the PGPR and the pathogen (because they were both localised in different parts of the plant), it enabled the conclusion to be made that the resistance is triggered by ISR instead of antagonistic mechanisms. Loon and Bakker (2006b) suggested that the PGPR may trigger plant roots to activate certain defence signals against pathogenic infections that systematically spread to aboveground tissue. The authors also proposed a priming mechanism pathway of PGPR ISR which involves increased jasmonic acid and ethylene sensitivities to activate defensive action against subsequent pathogenic attacks.

1.4.3 Studies of PGPR in ornamental plants

The application and mechanism of PGPR has been widely studied in agricultural crops, but there is little information on their effects in ornamental production systems. Even though some research has been conducted on ornamental seedlings using various strains of PGPR, generally the results have been inconsistent. This may be due to different growing media, environment conditions, sampling time, number of cells used for inoculation and different mechanisms possessed by each PGPR strain. The ornamental research in this review is focused on PGPR effects on field and greenhouse trials. *In vitro* or tissue culture experiments are not included because of differences in growth media and conditions. *In vitro* experiments involve controlled growth conditions that may affect plant growth as a result of high humidity, low light and poor gas exchange, and may result in changes to the structure and physiology of the plant compared to soil or potting mix-grown plants (Rout et al., 2006). Some studies on PGPR effects in ornamentals are summarized in Table 1.1. PGPR have been applied in a single inoculation or dual inoculation with mycorrhiza (Flores et al., 2007), cyanobacteria (Shanan and Higazy, 2009), in combination with organic fertilizers (Seema et al., 2006), in combination with graded levels of chemical N (Gadagi et al., 2002; Gadagi et al., 2004; Singh et al., 2008; Eid et al., 2009) or without any additional treatments (Gore and Altin, 2006; Srivastava and Govil, 2007). Generally, when inoculants were combined with increasing rates of N, the results indicated that reduced N rates achieved similar outcomes to

100% N without PGPR inoculation. Responses of ornamental plants to PGPR inoculation include increases in plant height, number of branches, number of leaves, leaf area, shoot and root weight, better root structure, number of flowers, improved nutrient uptake, accelerated flowering, and marketable flower quality characteristics.

The propagation method should be taken into consideration when producing high quality ornamental plants. Propagation from seed risks the possibility of genetic alterations which would affect the phenotype or the visual appearances of the plant. Therefore, many growers prefer vegetative propagation methods such as cuttings as maintaining the aesthetic value is an economically important aspect of ornamental production. Despite cuttings being one of the most common ornamental propagation techniques, there has been little research on the use of PGPR in ornamental cuttings (Li et al., 2005).

Table 1.1 Overview of PGPR tested in ornamental plants. The PGPRs were applied in singly or in combination with additional treatments using various inoculation methods and growth conditions.

PGPR	Plant and propagation technique	Inoculation method	Growth Conditions	Additional treatments	Plant response to PGPR inoculation	Reference
Azospirillum spp.	Chrysanthemum (40 day old seedlings)		Field	Graded levels of RDN	*Azospirillum* combined with 75% RDN plants produced higher flowers yield than 100% RDN amended plants	Gadagi et al., 2002
Azospirillum spp.	Blanket flower (40 day old seedlings)	Seedling dip for 1 hour (using aqueous slurry lignite-based inoculums)	Field	Different rates of N fertilizer (112 and 150 kg/ha)	• *Azospirillum* strain OAD-2+150 N kg/ha showed highest height, number of leaves, number of branches and dry matter • stimulated earlier flower emergence	Gadagi et al., 2004
• *A. brasilense* Cd1843 • *A. brasilense* Cd1843/pRKLACC (containing ACC-deaminase gene)	Carnation (cuttings)	Immersion in the bacterial solution, for 24 hours	Greenhouse	0.1% Indole butyric acid (IBA)	Inoculation with *A. brasilense* Cd184/pRKLACC: • induced larger number of adventitious roots • produced the longest roots • potentially saved commercial production time	Li et al., 2005
A. brasilense	Celosia (seed)	Direct inoculation with liquid inoculum	Greenhouse	Separate and combinations of: • different rates of N fertilizer (75 and 100%) • FYM	Inoculated plants + 75%N+FYM increased plant height, shoot and root fresh and dry weight, and number of inflorescence	Eid et al., 2006
P. fluorescence strain 51	Pelargonium, Chryasanthemum, and Dahlia (3 week old seedlings)	Drenching	Greenhouse	Nil	• Increased leaf surface, number of flower in Pelargonium • increased plant height and number of flowers in Chrysanthemum • decreased number of days required for flowering in Chrysanthemum • increased root length, number of flowers, fresh and dry weight and also accelerated flowering emergence in Dahlia	Gore and Altin, 2006
• *Azospirillum* spp. • Phosphate solubilising bacteria (PSB)	Marigold (30 day old seedlings)	Carrier-based inoculums	Field	Separate or combinations of • poultry manure • vermicompost • 75% RDN • 75% RDP P	Combination of vermicompost, poultry manure, *Azospirillum* and 75% RDN resulted in the best plant growth parameters, highest N and P uptake, and flower yield. This treatment also gained the maximum profit:cost ratio	Shubha, 2006

PGPR	Plant and propagation technique	Inoculation method	Condition	Additional treatments	Plant responses to PGPR inoculation	Source
• *Azotobacter chroococcum* • PSB	Gladiolus cv. American beauty (corm)	Corm dip for 30 minutes in the bacterial solution, respectively	Field	Nil	• *Azotobacter* inoculation increased plant height, number of leaves and number of spikes • PSB treatment showed better quality spike product • *Azotobacter* treatment hastened wilting of basal floret	Srivastava and Govil, 2007
Bacillus subtilis	Marigold (seed)	Direct inoculation with the bacterial suspensions	Greenhouse	With or without *Glomus facsiculatum* (VAM)	• *Bacillus* treatment showed higher total inflorescence production, flower fresh weight, accelerated flower maturity • Single inoculation of *Bacillus* or dual inoculated plants increased flower quality in terms of yellow colour properties	Flores et al., 2007
Mixture of *Azospirillum*, *Pseudomonas striata* and *Pseudomonas fluorescens*	Jasmine (three year old plantation)	Lignite based-culture	Field	• Combined with fungus *Trichoderma viridae*. • 50, 75 and 100% RDF of NPK • FYM (9 t/ha)	• Inoculated jasmine had better growth compared to NPK only treatment • 50% RDF of NPK + inoculation was as effective as 100% NPK in improving flower quality characteristics and chlorophyll content	Jayamma, N., 2008
• *Azotobacter* • PSB	Calendula (seedlings)	Seedling dip for 15 minutes (5% sugar slurry-based inoculums)	Field	• With or without PSB • FYM • Graded dose of N fertilizer (25, 50, 75 and 100%)	*Azotobacter*+PSB+75%N inoculated plants showed best plant height, diameter of main stem, number of leaves, number of branches and flower yield	Singh et al., 2008
• *Azotobacter chroococcum* • *Bacillus megaterium*	Stock (seed)	• *A. chroococcum* : Seed pre-sowing covering agent • *B. Megaterium* : Growth medium inoculant	Greenhouse	• Ammonium nitrate (2 or 4 g/pot) • Calcium superphospate • Adenosine triphosphate (ATP)	• Dual inoculation improved plant nutrient uptake (except N) among all treatments • ATP treated plants showed the best growth characteristics and highest total unsaturated fatty acid	Eid et al., 2009
Mixture of N fixing bacteria: *Azotobacter chroococcum*, *Azospirillum brasilense* and *Rhizobium* sp	Stock (3 week old seedlings)	Direct inoculation to growth medium with bacterial suspensions	Pot experiments	• Commercial mineral fertilizer • Cyanobacterial filtrate • Full rate of NH_4NO_3	Bacterial inoculation in combination with cyanobacterial culture increased plant height, number of leaves, leaf area and flower quality (florets number and diameter, fresh and dry weights of inflorescences)	Shanan and Higazy, 2009

RDN: recommended dose N, RDF: recommended dose fertilizer, RDP: recommended dose P, FYM: Farm yard manure

1.5 Aims of the project

The overall aim is to evaluate the effectiveness of PGPR on the growth and development of ornamental plants of high value to the nursery industry and determine if PGPR can substitute synthetic root growth hormone applied to ornamental plants thereby reducing input costs.

The specific aims of the project are to:

1. Determine the most effective way of growing ornamental plants to observe a PGP effect.
2. Determine the most effective ornamental plant host-PGPR interaction by screening a range of organisms and plants.
3. Measure changes in plant growth and development and nutrient composition after application of PGPR.
4. Investigate the most effective inoculation formulation to promote plant growth.

1.5.1 Hypotheses

1. PGP effects on ornamental plants will be dependent on specific plant-microbe interactions.
2. Where PGP effects are observed, PGPR will increase root development and nutrient use efficiency.

1.6 Project overview

The overall aim of this project was to evaluate the effectiveness of PGPR on growth and development of ornamental plants. The outcomes are presented in this thesis in three parts as described below.

Part one describes the development of methods used to observe PGP effects of in ornamental plant growth. A preliminary study was carried out using pansy seedlings grown in potting mix which were inoculated with PGPR after transfer to sterile sand. PGP effects were not observed in this system. PGPR selection was refined theoretically by reviewing the literature and experimentally by evaluating the ability of potential strains to produce IAA in a liquid growth medium. The highest IAA producer, *Azospirillum brasilense* Sp245, was chosen as the PGPR inoculant for further studies. The plant selection to determine the most responsive ornamental plant was done by immersing cuttings in solutions prepared from bacterial cultures and

observing root development. Cuttings were transferred to defined growth media to reduce the complexity of root system and minimise the risk of competition from other organisms present in potting mix. Lavender (*L. stoechas*) was the most responsive plant in this system. Further studies were carried out using *A. brasilense* Sp245 and *L. stoechas* as a model system.

In part two, improved root development in cuttings after immersion with different solutions was evaluated. Increased N use efficiency of propagated cuttings was also investigated.

In part three, different commercially available inoculant forms were evaluated for their potential to stimulate IAA production by PGPR and subsequent adventitious root formation in cuttings.

CHAPTER 2 SCREENING OF THE MOST EFFECTIVE PGPR-ORNAMENTAL PLANT INTERACTIONS

2.1 Introduction

The success of PGPR inoculation depends on many factors including specificity of the PGPR-host plant interaction (Nelson, 2004). A PGPR strain effective on one plant may not necessarily promote growth of another plant although there is likely to be a broad range of PGPR-plant specificities. Therefore, selection of an effective combination of PGPR and a responsive host plant is essential so that the PGPR inoculation provides the most beneficial effects on plant growth.

In selecting bacteria capable of a specific PGP mechanism, screening at the strain level within a bacterial species is important as a small difference in genotype may affect the PGP properties (Kloepper, 1996). As it is possible that a single strain is capable of PGP by several different modes of action (Vessey, 2003; López-Bucio et al., 2007), the selection should occur in conditions relevant to the growth promoting effects being sought. For example, in order to improve plant growth in an N_2-deficient environment, N_2-fixing PGPR inoculants may be applied to facilitate N_2-fixation, whereas if the purpose is to protect the host plant against fungal pathogen, a PGPR involved in controlling disease through ISR or the production of antifungal compounds may be applied.

The aims of the research presented in this chapter were to:

1. Develop a method to measure the effect of PGPR on ornamental plant growth.
2. Use the method to select bacterial strains capable of promoting growth of ornamental plants.
3. Determine the most responsive host plant.

The experiments were designed according to the following approaches:

- Selection of PGPR strains was done initially after reviewing the published literature and later experimentally, by measuring the rate of indole acetic acid (IAA) production.

- Selected strains were then evaluated for their effect on the growth of ornamental plants.
- The most responsive ornamental plant was selected by measuring growth parameters after inoculation with selected PGPR.

2.2 Materials and methods

The experiments described in this chapter include a preliminary study applying PGPR to pansy seedlings, screening of a range of PGPR for IAA production and determining the most responsive ornamental plant by inoculating plant cuttings with PGPR selected for high production of IAA.

2.2.1 Preliminary study of pansy seedling response to inoculation with PGPR

In the preliminary study, pansy seedlings were inoculated with inoculum prepared as described below. Pansy seedlings were obtained from Anthony Kachenko at Oasis Horticulture Pty Ltd. They were 14 weeks old and grown from seed in commercial potting mix. The seedlings were inoculated after transferring to pots containing sand. Plants were harvested and plant growth parameters were measured overtime.

2.2.1.1 Inoculum preparation and plant material

A. brasilense Sp7 was obtained from the SUNFix culture collection (Sydney University Centre for Nitrogen Fixation, Faculty of Agriculture, Food and Natural Resources). The selected strain fixes nitrogen in association with wheat roots and has been studied extensively. *A. brasilense* Sp7 was grown on nutrient agar (NA, Appendix A) and bacterial inoculant was prepared by suspending bacterial colonies in sterile water. The bacterial number was determined at the time of inoculation using viable plate counting. The bacterial suspension was serially diluted using sterilized distilled water. Aliquots (100 µL) of 10^{-5}-10^{-7} dilutions were spread on NA and incubated at 32°C until colonies were visible at which point they were counted.

2.2.1.2 Growth media preparation

Approximately 300 g of a mixture of fine and coarse sand was used to fill 125 mm diameter plastic pots that had been washed with 4% sodium hypochlorite and rinsed with water. A sheet of paper towel was placed on the bottom of each pot before being filled with the sand. The sand

was then moistened with 100 mL of water before a single seedling was transferred to each pot. A small container was placed under each pot to collect excess water.

2.2.1.3 Inoculation and cultural practices

Each of the seedlings was inoculated by pipetting 1 mL of *A. brasilense* Sp. 7 liquid inoculant into the sand at the base of the shoot then carefully covering with sand. Uninoculated pansies served as a control. All plants were watered daily and fertilised with 25 mL of quarter strength Hoagland's solution (Hershey, 1994, Appendix A) once a week. The pots were arranged in a randomized complete block design (RCBD) under a light bank, with 20 replicates per treatment.

2.2.1.4 Plant growth measurements

Sampling was carried out fortnightly by harvesting four plants from each treatment (total= 8) for measurement and analysis. Shoot height, chlorophyll content and photosynthetic rate were measured before the plant was uprooted from the sand.

2.2.1.4.1 Shoot height

The shoot height was measured from the cotyledonae node to the last node on the main stem of plants at each sampling time and expressed in cm.

2.2.1.4.2 Chlorophyll content

At 56 and 70 days after inoculation (DAI), a non-destructive method using a portable leaf greenness meter (SPAD-502Plus, Konica Minolta) was applied to determine chlorophyll content. For each treatment, five leaves were chosen randomly from different positions of each replicate. Four readings were averaged for each leaf to represent one observation. The measurement was recorded at each sampling time.

2.2.1.4.3 Photosynthetic rate

The photosynthetic rate was determined using a portable pulse amplitude fluorometer (PAM). Before measurement, the plants were stored in the dark for 20 minutes to stop any photosynthesis (Bulgarea and Boukadoum, 2001). Four leaves were chosen randomly from different positions on each replicate. For each leaf, readings were taken from five locations, and

then averaged to represent one observation. The photosynthetic rate was recorded at each sampling time.

2.2.1.4.4 Shoot and root fresh weight

Plants were removed from the pots and carefully shaken to remove excess soil from around the root system. The plant was then thoroughly washed under running tap water to remove attached soil and analysed for shoot and root characteristics.

Shoots and roots (free of sand) were separated and immediately weighed to obtain fresh weight. The shoots were then dried at 60-70°C and weighed to obtain shoot dry weight. Fresh root systems were transferred to tubes containing 50% ethanol and stored at 4°C for root length measurement.

2.2.1.4.5 Root length measurement

Stored fresh roots were washed free from ethanol and floated in deionised water in a clear perspex tray and scanned using a flatbed scanner at 600 dots per inch (dpi). Root analysis was performed using image analysis software WINRhizo Pro (Regent Instruments, Quebec City, Canada; Arsenault et al., 1995). The root mass was separated into parts, scanned separately in order to get a more accurate measurement and the data were combined as one observation per plant. The root length was measured in 0.04 mm diameter classes. The root mass was then dried overnight at 60-70°C and weighed to obtain root dry weight.

2.2.2 Selection of potential PGPR strains

The following experiment was carried out to screen bacterial strains for potential PGP characteristics by measuring IAA production in liquid growth medium. The IAA measurement was done each day for three days using colorimetric analysis. The experiment also assessed effects of IAA precursor, tryptophan, on IAA production and bacterial cell number during the three day incubation.

2.2.2.1 PGPR strains

PGPR strains were selected on the basis of their demonstrated ability to promote plant growth or to possess potential plant-growth promoting traits (Katupitiya et al., 1995; Dobbelaere et al.,

1999; Nguyen et al., 2003). PGPR strains were obtained from the SUNFix culture collection (Sydney University Centre for Nitrogen Fixation, Faculty of Agriculture, Food and Natural Resources).

Table 2.1 PGPR strains used in the study

PGPR isolates	Abbreviations used in this thesis	Reference
Citrobacter freundii 3C	3C	(Nguyen et al., 2003)
Pseudomonas fluorescens 1N	1N	(Nguyen et al., 2003)
Azospirillum brasilense Sp7	Sp7	(Katupitiya et al., 1995)
Azospirillum brasilense Sp7-S	Sp7-S	(Katupitiya et al., 1995)
Azospirillum brasilense Sp245	Sp245	(Dobbelaere et al., 1999)

2.2.2.2 Preparation of PGPR strains

2.2.2.2.1 Storage of PGPR strains

Fresh cultures of isolates of all the PGPR listed in Table 2.1 were frozen in glycerol broth (Appendix A) and stored at -80°C until required.

2.2.2.2.2 Subculture of stock cultures

Stock cultures stored at -80°C were revived by streaking on to NA and incubated at 28°C until colonies appeared (approximately 72 hours). In order to increase cell mass, the colonies were then re-streaked on fresh NA and incubated at 28°C for 72 hours. The plates were then stored at 4°C. When required, cultures were grown in liquid nutrient broth (NB, Appendix A) with shaking on an orbital shaker (*B braun*, Certomat R, UK) at 125 rpm for 72 hours.

2.2.2.3 Identification of PGPR strains

Preliminary identification of PGPR was done by observing their growth characteristics on selective and/or differential media and then by molecular analysis (16S rDNA sequence analysis).

2.2.2.3.1 Identification of PGPR strains using growth characteristics

Colonies of each strain were suspended in 1 mL of 0.85% sterile saline solution and mixed using a vortex mixer. An aliquot of 100 µL of each suspension was used to inoculate several selective media and incubated at 28°C.

Fermentation of L-sorbose in phenol red fermentation medium (Appendix A) was used to confirm typical physiology of 3C. Carbohydrate fermentation was indicated by a colour change in the media from red to yellow due to acid production by fermentation of the carbohydrate (Deaker et al., 2008). King's B growth medium (KB, Appendix A) was used for the preliminary identification of 1N by observing fluorescence under ultra violet (UV) light.

The presence of N_2-fixing bacteria, such as *Azospirillum* were detected by growing the bacteria in nitrogen-free bromothymol blue supplemented with malate (Nfb, Baldani and Döbereiner, 1980, Appendix A). These bacteria are capable of utilising atmospheric N_2 to support their growth to meet their N requirements. Formation of a rising white pellicle in semi-solid media is typical growth of *Azospirillum* sp. (Xie et al., 2003; Soares et al., 2006; Jolly et al., 2010).

2.2.2.3.2 Molecular confirmation of PGPR strains

Colonies of each isolate were suspended in 50 µL of sterile water and vortexed. The homogenized suspensions were then extracted by heating for 5 minutes at 95°C followed by centrifugation at 13,000 *g* for 5 minutes. The resulting supernatant was transferred to a new microcentrifuge tube and 16S rDNA wsa amplified by polymerase chain reaction (PCR). Three replicate extractions were carried out for each strain.

PCR was carried out with Mango Taq DNA polymerase (Bioline) and performed in a S1000 thermal cycler (Bio-Rad). An aliquot of supernatant containing DNA template (1 µL) was added to 24 µL Mango Taq mastermix (Appendix B). Sterile water instead of DNA template served as a negative control. Cycling conditions used were initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 45 seconds; then final extension at 72°C for 5 minutes.

In order to confirm the accuracy of DNA extraction and PCR conditions used, PCR products including the negative control were electrophoresed in a Bio-Rad sub-cell GT electrophoresis tank containing 1x TAE buffer using a 1% agarose gel at 100 V for 30 minutes.

PCR products from three replicates of each strain were pooled and purified using isolate PCR and gel kit (Bioline). The purified DNA fragments were sent to the Australian Genome Research Facility (AGRF) to be sequenced and the resulting sequence was compared with homologues in the Gene Bank database using BLASTn 2.2.26+ software (Zhang et al., 2000).

2.2.2.4 PGPR number of cell quantification

The number of PGPR cells per mL of liquid medium was calculated using viable plate counting. The culture was first serially diluted by transferring 100 µL of liquid bacterial culture to 900 µL of sterilized 0.85% saline solution and repeating until a dilution of 10^{-7} was reached. Finally, 100 µL of dilutions 10^{-5} to 10^{-7} were spread onto the surface of NA using a sterilized glass spreader. Plates were incubated at 28°C for 72 hours and resulting colonies were counted.

2.2.2.5 IAA production of PGPR strains in liquid culture

A colorimetric analysis based on the method of Gordon and Weber (1951) was used to measure bacterial IAA production. Starter cultures were prepared by inoculating 2 mL of Dworkin & Foster minimal medium (DF, Dworkin and Foster, 1958, Appendix A) with bacterial colonies and homogenising the suspension using a vortex mixer. The starter cultures (200 µL) were then dispersed in 10 mL of fresh DF minimal medium supplemented with filter sterilized (0.22 µm) L-tryptophan (Sigma-Aldrich) to a final concentration of 0.1%. Minimal medium without L-tryptophan was used as a control. The cultures were incubated for 3 days at 28°C on a shaker at 125 rpm. The amount of IAA produced was assayed daily for 3 days as described below.

The cultures were centrifuged at 15,000 *g* for 10 minutes and 2 mL of bacterial supernatant were transferred into test tubes followed by 4 mL of Salkowski reagent (Appendix A). The mixture was then vortexed immediately and incubated in the dark for 45 minutes at room temperature and absorbance was recorded at 525 nm. The presence of IAA was shown by the development of a pink colour (Figure 2.1). Absorbance values were then converted to IAA

concentration using an IAA standard curve in the range of 0-20 µg/mL. Uninoculated medium with and without tryptophan were used to zero the spectrophotometer before sample reading.

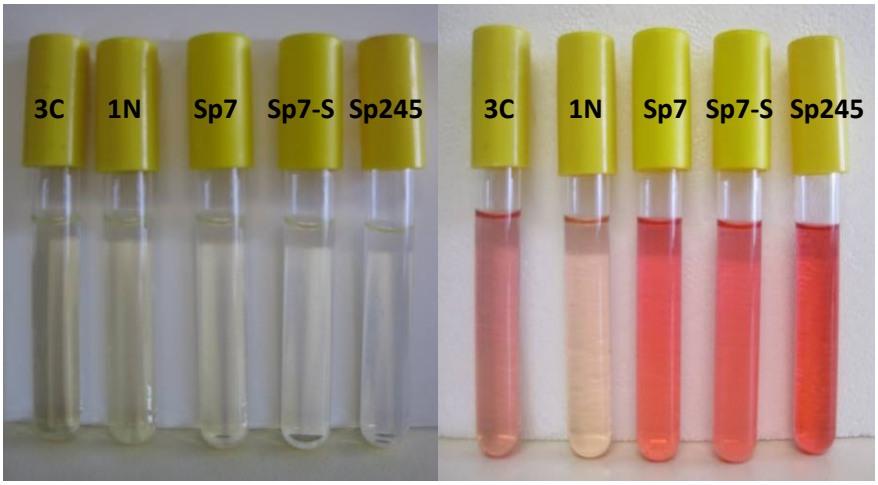

Fig. 2.1 Development of pink colour in supernatant of PGPR cultures indicating IAA production.

2.2.3 Selection of the most responsive ornamental plant to *A. brasilense* 245 inoculation

The aim of this experiment was to screen the most responsive ornamental plant to inoculation with the selected PGPR*, A. brasilense* Sp245. The plant selection was carried out using cuttings from a range of ornamental plants and performed in water medium to minimise contamination from other organisms.

2.2.3.1 Plant material preparation

Ornamental plants were purchased from local plant nurseries and are listed in Table 2.2.

Table 2.2 List of ornamental plants

Scientific name	Commercial name
Argyranthemum sp.	Marguerite daisy
Lavandula stoechas	Lavender avonview
Osteospermum sp.	The African daisy

Ornamental cuttings were prepared by selecting stems which were firm and approximately 4 cm in length. A third, to two thirds of the leaves at the lower part of the stem were removed and the cuttings were placed in water to maintain moisture while collecting more cuttings (Fig 2.2).

Fig. 2.2 Ornamental cutting preparation showing removal of leaves from the lower stems.

2.2.3.2 Growth media preparation

Water (25 mL) was added to 17 mm diameter test tubes which were covered with aluminium foil and autoclaved at 121°C for 15 minutes. To reduce cross-contamination, each treatment was placed in a separate rack and covered with plastic wrap that was supported by wooden sticks attached to each side of the rack (Fig. 2.3). The plastic cover was also intended to prevent evaporation during incubation. The racks were placed randomly in a growth chamber (NK System Biotron, Nippon Medical and Chemical Instrument, Tokyo, Japan) and the position of the racks was randomized each week. Temperature of the growth chamber was maintained at 23-24°C.

Fig. 2.3 Incubation of cuttings in water medium showing plastic covering to reduce cross-contamination and evaporation.

2.2.3.3 Preparation of treatments

The treatments applied to the plant cuttings were Sp245 culture grown with tryptophan and supernatant, synthetic IAA, heat killed Sp245 cells and sterile water as immersion solutions to stimulate adventitious root growth of the plant cuttings. All solutions were prepared under sterile conditions, except IAA.

Sp245 colonies were used to inoculate DF minimal medium and mixed using a vortex mixer. The thick homogenized culture was then aseptically dispersed in 1 mL aliquots to 50 mL of liquid DF minimal medium in a 250 mL conical flask. Filter sterilized L-tryptophan was added to a final concentration of 0.1% (w/v). The cultures were then incubated at 28°C for three days with shaking (125 rpm) and used as immersion solutions.

The supernatant of centrifuged Sp245 suspension was also included as a treatment and prepared by centrifuging a culture of Sp245 at 3,000 *g* for 30 minutes. The supernatant was then collected and used as immersion solution to treat the cuttings. Heat-killed Sp245 cell culture and water served as negative controls. To prepare the heat-killed Sp245 cell suspension, Sp245 culture was dispensed (1.5 mL aliquots) in microcentrifuge tubes and then incubated in a water bath at 90°C for 1 hour (Kamnev et al., 2004). The suspensions were then kept at room temperature to cool prior to inoculation.

Prior to inoculation, the IAA produced by Sp245 was measured (section 2.2.2.5). Analytical grade crystalline IAA (Sigma-Aldrich) was used as a positive control to compare the relative effectiveness of bacterial and IAA. The synthetic IAA was dissolved in sterile water to obtain a closest concentration of bacterial IAA and used to treat the cuttings.

2.2.3.4 Inoculation and general plant treatment

Plant experiments using cuttings were inoculated by immersing the cuttings individually for 6 hours (Tsavkelova et al., 2007a) in separate sterile microcentrifuge tubes containing 1 mL of immersion solution. After transfer to the growth tubes, water level was checked every three days and refilled if necessary to ensure the stem remained immersed below the water.

2.2.3.5 Recovery of A. brasilense Sp245 from cuttings

In order to confirm the presence of Sp245 in cuttings after 6 hours immersion, a differential medium was used to distinguish the presence of Sp245 from other microorganisms that may have grown during immersion. The number of Sp245 was estimated using the most probable number (MPN) technique. The MPN consists of sample dilution to extinction and multiple inoculations of media from each dilution. The number of bacteria of interest was estimated by comparing the number of positive reactions with published MPN tables (Woomer, 1990).

2.2.3.5.1 Cutting extraction and MPN

The PGPR were extracted from the cuttings by homogenizing the cutting using a mortar and pestle with 1 mL of sterilized 0.85% saline solution. The suspension was then transferred to microcentrifuge tube and vortexed. Each treatment was ground using a different pair of mortar and pestle to ensure there was no cross contamination.

The plant material suspensions were diluted 10 fold in series by diluting 100 µL of the suspension to 900 µL of sterilized 0.85% saline solution. Each dilution (100 µL) was then used to inoculate 3 mL of semi solid Nfb with malate as the sole carbon source. The growth of Sp245 was detected by a rising white pellicle in the semi-solid media after 72 hours incubation at 26-28°C. The number of Sp245 was calculated using computer software, MPN enumeration system (MPNes) from three replicates.

2.2.3.6 Adventitious root growth measurement in cuttings experiment

The following adventitious root growth measurements were conducted before the cuttings were removed from the medium.

2.2.3.6.1 Root formation

Root formation percentage was recorded at harvesting time by dividing the number of rooted cuttings by the total number of cuttings prepared for each treatment and multiplying the value by 100.

2.2.3.6.2 Number of main roots

The number of main roots was recorded each week on water-grown cuttings by visual inspection and at harvesting time for sand- and potting mix-grown cuttings.

2.2.3.6.3 Plant harvesting

The plants were harvested by pulling the cuttings out from media and removing excess moisture on a paper towel. The roots were separated from the stem and transferred to 50% ethanol in a screw cap conical tube and kept at 4°C for root length measurement.

2.2.3.6.4 Root length measurement

Adventitious root length was measured using WinRhizo as described in section 2.2.1.4.5 without root mass separation.

2.2.4 Data analysis

All data were subjected to statistical analysis of variance (ANOVA) using IBM SPSS statistics 20 and the differences between the means obtained were separated using Tukey's test (IBM SPSS statistics 20).

2.3 Results

2.3.1 Preliminary studies with nursery seedlings of pansies

In preliminary experiments, 14 week old pansy seedlings were inoculated with 1 mL of Sp7 culture before transplanting to pots containing sand. Uninoculated pansies served as a control. The bacterial strain selected had previously been reported as having plant growth promoting activity. The number of Sp7 applied was 3.4 x 10^5 cfu/mL. The destructive analysis was carried out on plants sampled fortnightly for 70 days.

In general, most of growth parameters increased significantly over the sampling time in the control and inoculated pansies and no significant PGP effect was observed in plants inoculated with Sp7 nor was there a interaction between inoculation and sampling time.

The pansies exhibited a considerable increase in plant height during the experiment in both control and inoculated pansies (Fig. 2.4). Inoculated pansies showed a better response in height

than the control, except at 14 and 42 days after inoculation (DAI). The maximum increase in plant height was noted on 56 DAI, followed by 70 DAI. The increase was 44.2% and 25.5% over the control respectively (Fig 2.4). Nevertheless, there was no statistically significant effect of inoculation on plant height (P=0.40) at any sampling time.

Analysis of roots using WinRhizo indicated that the control demonstrated a better response in total root length than the inoculated pansy at any sampling time, except on 70 DAI (Fig. 2.5) which showed a slight increase (4.88%) over the control. Although the control generally outperformed the inoculated plants, the root length increase was not significant (P=0.40.).

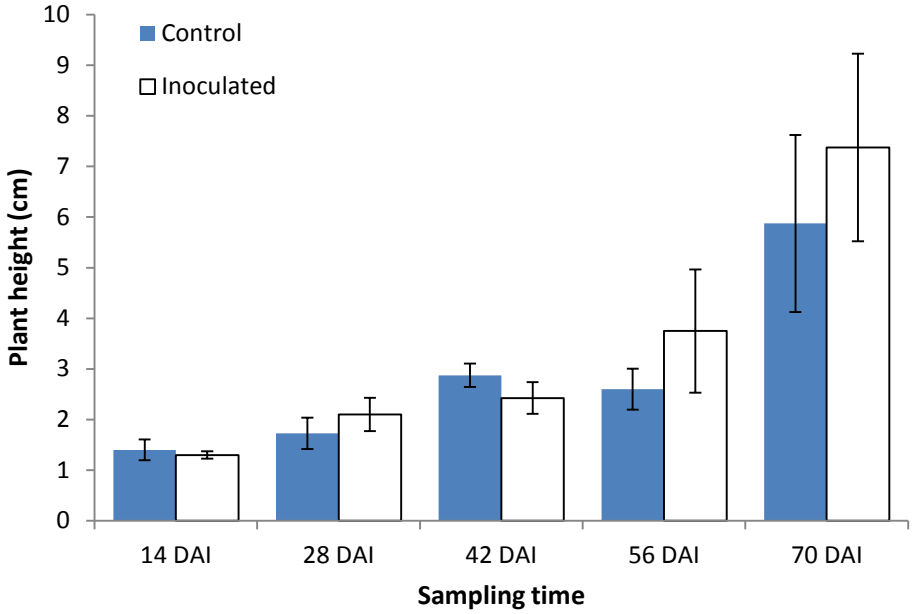

Fig. 2.4 Plant height of pansy seedlings at different sampling times. Error bars represent standard errors of the mean of four replicates. DAI: days after inoculation.

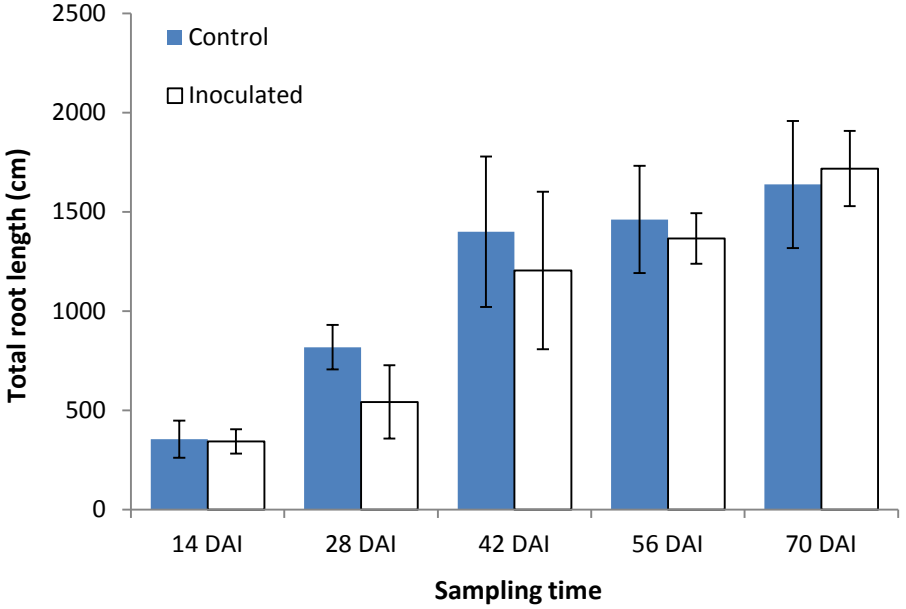

Fig. 2.5 Total root length of pansy seedlings at different sampling times. Error bars represent standard error of the mean of four replicates. DAI: days after inoculation.

The shoot and root fresh weights also increased with the age of the plant (Figs. 2.6 and 2.7). In both parameters, the control showed a better response than the inoculated pansies until 42 DAI. At the last two sampling times, the inoculated plants had slightly increased shoot and root fresh weight than the control. The maximum shoot and root fresh weight increase over the control was on 56 DAI with an average of 2.95 g (11%) and on 70 DAI with 1.82 g (16.7%), respectively. However, the increase in fresh weight parameters were not statistically significant (P=0.98 and P=0.68).

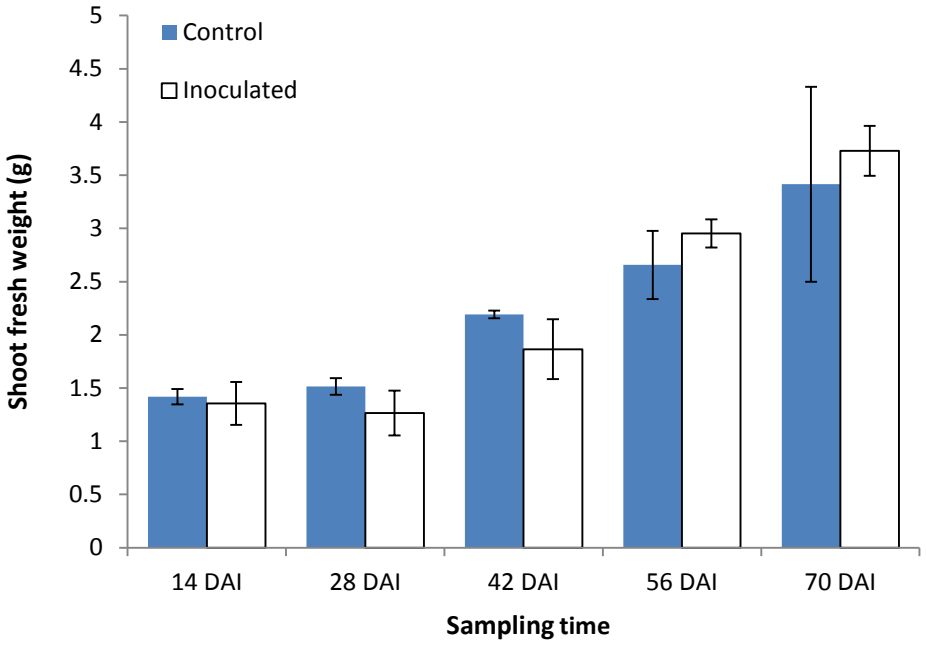

Fig. 2.6 Shoot fresh weight of pansy seedlings at different sampling times. Error bars represent standard error of the mean of four replicates. DAI: days after inoculation.

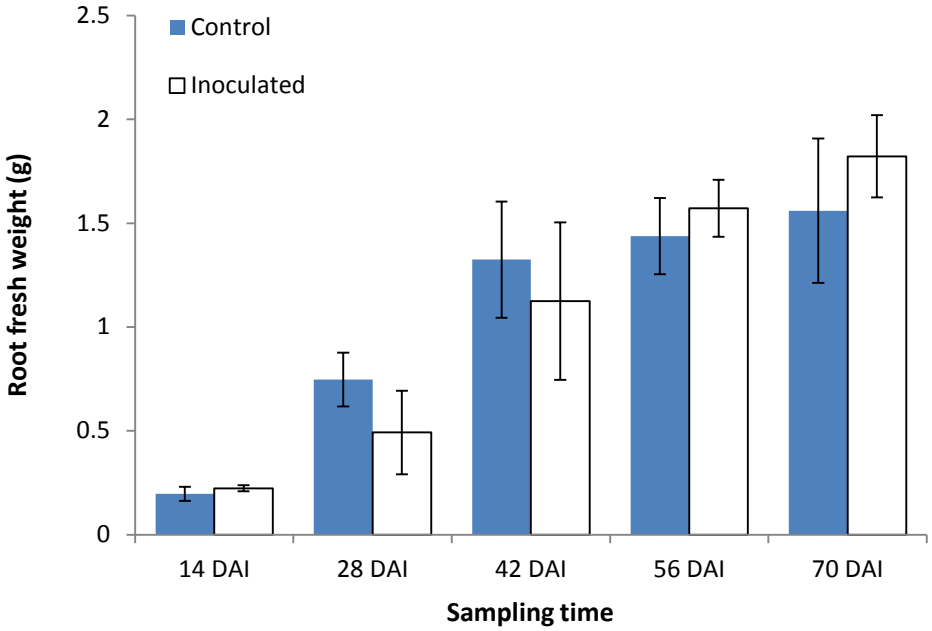

Fig. 2.7 Root fresh weight of pansy seedlings at different sampling times. Error bars represent standard error of the mean of four replicates. DAI: days after inoculation.

For shoot and root dry weight measurements in pansy seedlings, there was no significant effect of inoculation at P<0.05. The dry weights increased with the age of the plant for both control and inoculated plants (Fig 2.8 and 2.9), however the control pansies demonstrated higher dry weight compared to pansy treated with Sp7 at most sampling times. Inoculated pansies increased 25% shoot dry weight at 70 DAI and 5% root dry weight at 56 DAI over the control.

Chlorophyll content measurements demonstrated that at both sampling times, the inoculated pansies had slightly higher chlorophyll contents than the control by 2.25 and 9.7% on 56 and 70 DAI, respectively (Fig 2.10). There was a significant effect (P=0.005) of sampling time on chlorophyll content in control and inoculated plants but there was no significant effect of inoculation or the interaction between sampling time and inoculation on this parameter (P=0.23).

On the other hand, inoculated pansies showed a noticeable increase in maximum photosynthetic rate (Pmax) on 70 DAI by up to 120% (Fig 2.11), even though the control showed a better response on 56 DAI. The Pmax increase by inoculation was significant at P<0.001.

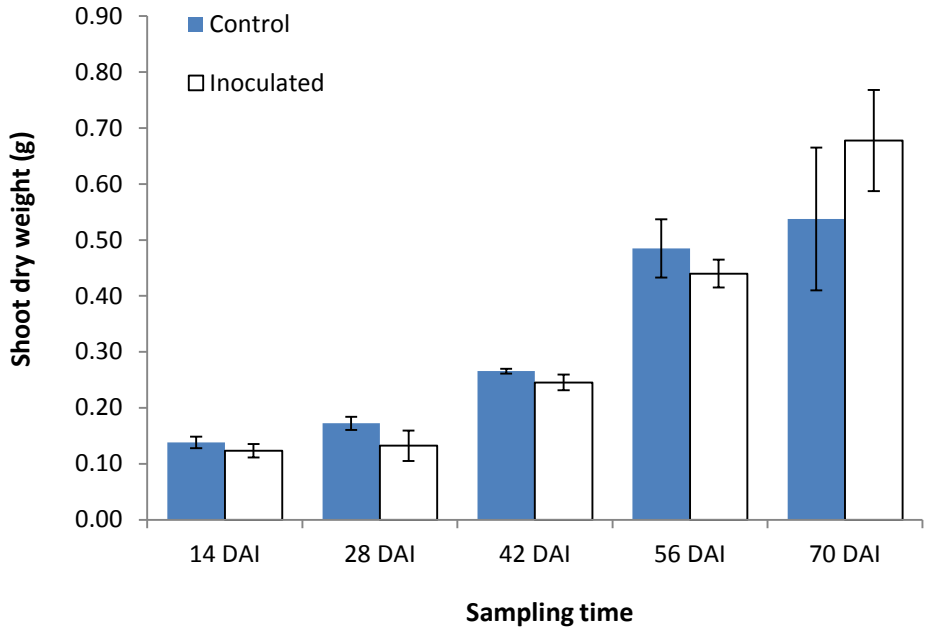

Fig. 2.8 Shoot dry weight of pansy seedling at different sampling times. Error bars represent standard errors of the mean of four replicates. DAI: days after inoculation.

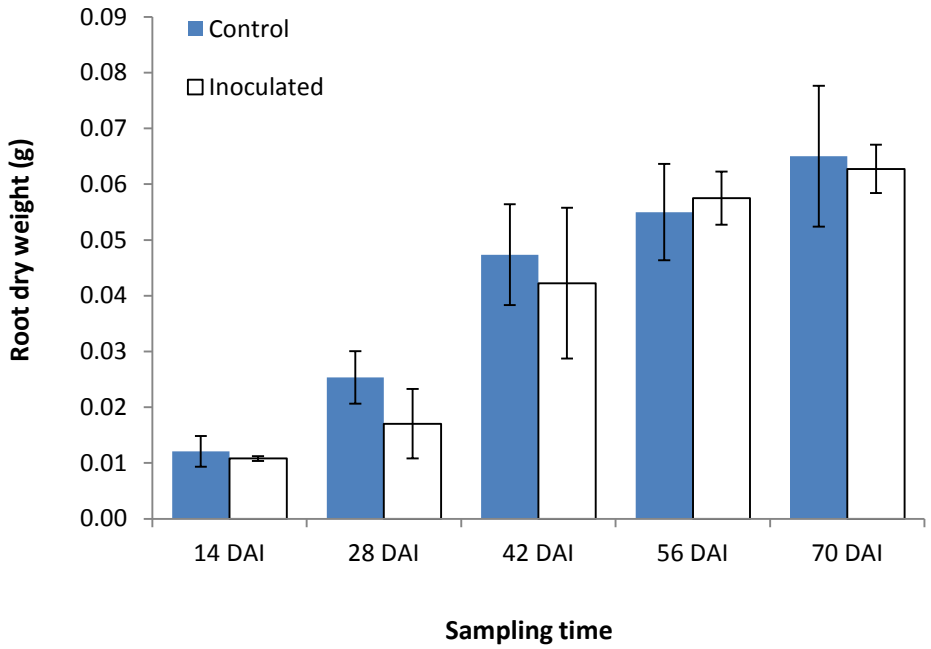

Fig. 2.9 Root dry weight of pansy seedling at different sampling times. Error bars represent standard errors of the mean of four replicates. DAI: days after inoculation.

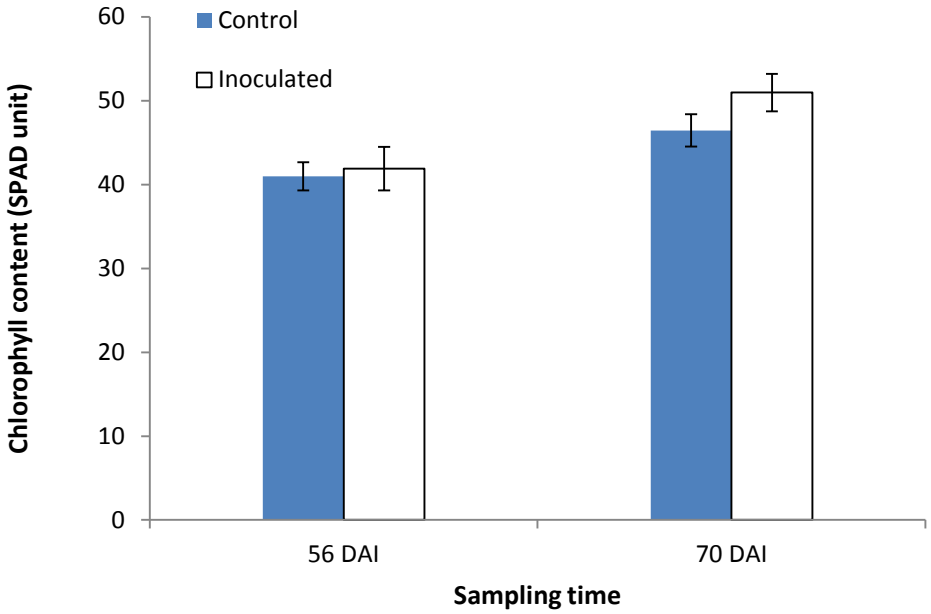

Fig. 2.10 Chlorophyll content in pansy seedling at different sampling times. Error bars represent standard errors of the mean of four replicates. DAI: days after inoculation.

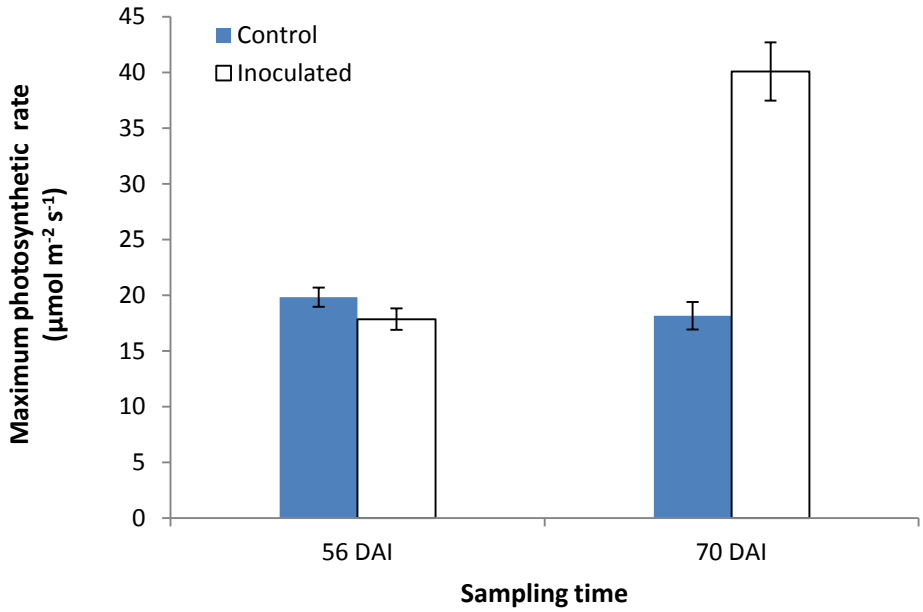

Fig. 2.11 Maximum photosynthetic rate in pansy seedlings at different sampling times. Error bars represent standard errors of the mean of four replicates. DAI: days after inoculation.

In this experiment, differences in response to inoculation on growth parameters were undetectable possibly because the pansy roots were already well colonised and Sp7 could not compete with the microorganisms present in the potting mix. To minimise competition, the next experiments were designed to apply inoculum to plant cuttings during propagation to reduce competition and to determine the effect on the development of adventitious roots. Plant propagation from cuttings was selected over seed propagation to reduce genetic variation. PGPR strains were also further selected for their ability to produce the phytohormone IAA in liquid culture. Selection of ornamental plants was performed by immersing the plant cuttings in the selected strain solution and measuring the adventitious roots produced to obtain the most responsive plant to the selected PGPR. It is a common commercial practice to immerse plant cuttings in chemical preparations of the phytohormone indole butyric acid (IBA) during propagation. By doing this experiment, the most effective combination of PGPR-ornamental plant may be established to further investigate the effects of PGPR inoculation on ornamental plants and the potential for PGPR application in the ornamental plant propagation industry.

2.3.2 Selection of potential PGPR strains

The PGPR strains used in the project were selected based on their ability to produce IAA in liquid medium (Table 2.1). Initially, the strains were identified by growing them on differential media to identify typical physiological characteristics and then on the basis of their 16S rDNA sequence. IAA production of PGPR strains was determined according to Gordon and Weber (1951) by growing the strains on liquid DF medium. Production of IAA was measured was taken daily during the 3 day incubation.

2.3.2.1 Growth characteristics of PGPR strains

L sorbose fermentation was found to be unique to *C. freundii* 3C in an API 50 CH test when compred with other enterobacteriaceae *Klebsiella pneumonia* 4P and *Enterobacter* Spp. 5P (Deaker et al., 2008). Phenol red fermentation medium supplemented with carbohydrate L-sorbose was used to distinguish *Citrobacter freundii* from the other four strains (*Pseudomonas flourescens* 1N, *Azospirillum brasilense* Sp7, *A. brasilense* Sp-7S and *A. brasilense* Sp245). Fermentation of the carbohydrate was detected by colour change of the medium from red to yellow, indicating acid production by *C. freundii* 3C. The color change in medium inoculated with 3C in Fig. 2.12 is clearly visualized while inoculation with other strains did not result in colour change of the medium.

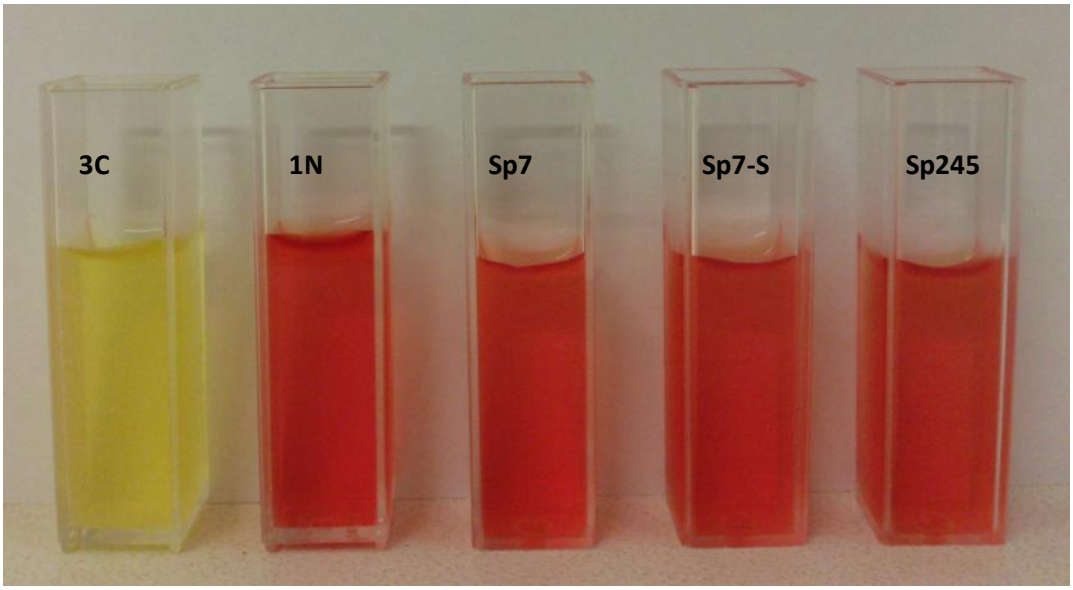

Fig. 2.12 Comparison of the PGPR strains growth in phenol red medium to distinguish *C. freundii* 3C from other PGPR. Colour change of phenol red media from red to yellow indicates fermentation of carbohydrate L-sorbose by 3C.

Production of fluorescent metabolites by 1N was performed using King's B medium then visualization of fluorescence under ultraviolet light (Fig. 2.13) exhibited response of *Pseudomonas fluorescence* strain 1N when grown on King's B medium. Fluorescence was not observed with any of the other selected strains

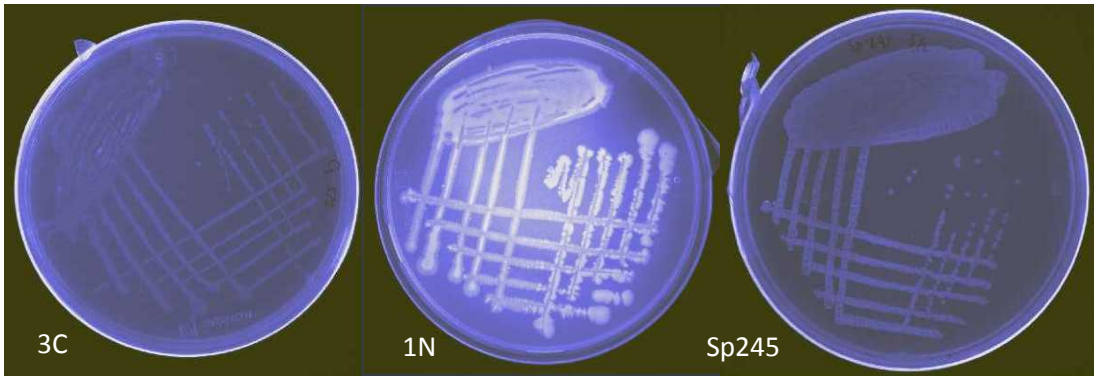

Fig. 2.13 Visualization of the PGPR strains growth on KB plate under UV light. 1: Fluorescence was only observed with 1N culture. Sp245 on KB plate was included to represent *A. brasilense* strains.

Typical growth of A. brasilense was measured using semisolid Nfb medium and detected by formation of a rising white pellicle on the subsurface of the medium. *A. brasilense* strains formed white pellicle after 72 hours incubation and no pellicle was observed in 3C and 1N (Fig. 2.14).

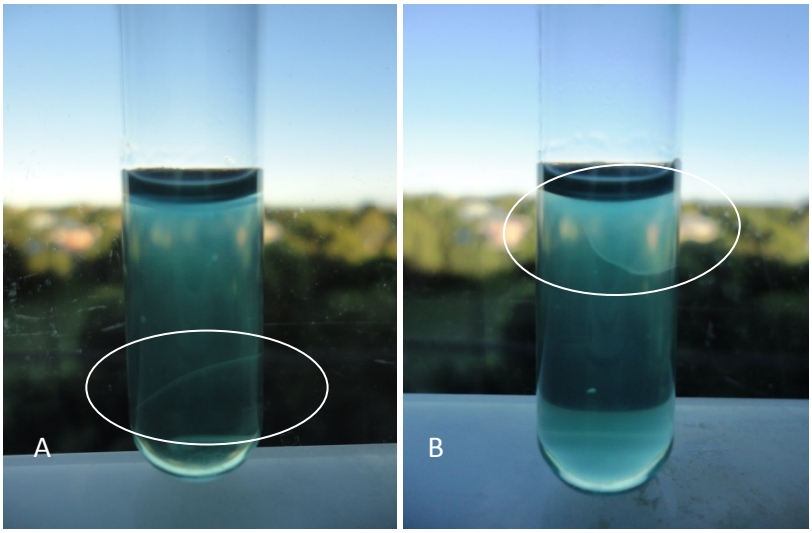

Fig. 2.14 Typical growth characteristics of *A. brasilense* in semi solid Nfb. Circles indicate rising pellicles in the media indicating the growth of the *Azospirillum* strains. A: the pellicle starts deepen in the medium and B: the pellicle rises as it consumes O_2.

2.3.2.2 Molecular analysis of PGPR strains

Sequencing of 16S rDNA followed by BLASTn analysis was used to confirm identification of PGPR strains. The identities of the PGPR strains according to Genebank database are liosted in Table 2.3. Strain 3C was identified as *Citrobacter freundii* and 1N as *Pseudomonas fluorescens*. BLASTn analysis confirmed the identity of Sp7 and Sp245 as *Azospirillum brasilense* Sp7 and Sp245, Sp7-S was confirmed as *A. brasilense*. Differentiation of strains for 1N, 3C and Sp7-S were not possible as they have not yet been included in the Genebank database.

Table 2.3 BLASTn report obtained from NCBI Genebank database on identification of PGPR strains

Strain	Confirmation of 16S rDNA on Genebank database	Coverage
3C	*Citrobacter freundii*	100%
1N	*Pseudomonas fluorescens*	100%
Sp7	*Azospirillum brasilense* Sp. 7	100%
Sp7-S	*A. brasilense*	97%
Sp245	*A. brasilense* Sp. 245	100%

2.3.2.3 IAA production of PGPR strains

Measurement of IAA was designed to determine the most effective IAA producing PGPR strain in a defined liquid medium (Dworkin and Foster, 1958) with and without the addition of tryptophan. Tryptophan is the biochemical precursor of auxin (including IAA) production in bacteria. Colorimetric analysis based on the colour change of Salkowski reagent was used to measure the phytohormone production. The effect of tryptophan on the number of viable bacteria cells was also determined over three day incubation (Table 2.4).

In the absence of tryptophan, the viable number of 3C cells reached its maximum on day 1 with $\log_{10}$ of 8. 7 cfu/mL (Table 2.4). Cell number of 3C decreased during the incubation period. A similar result was obtained when 3C was grown in the presence of tryptophan, the cell number decreased during three day incubation. The lowest viable cell number was observed at day 3 with $\log_{10}$ 6.6 cfu/mL of. Statistically, there was no effect of tryptophan addition (P=0.46) on the growth of 3C over the growing period.

Conversely, viable cell number of 1N grown without tryptophan increased over the four days and the maximum growth was noted on day 3 with $\log_{10}$ 9.1 cfu/mL. The number of viable cells of 1N increased on day 1 in the presence of tryptophan, then declined on the following day. The number then increased and reached the maximum number with $\log_{10}$ 8.9 cfu/mL . However, addition of tryptophan in the growth medium did not significantly affect the viable cell number (P=0.52).

Table 2.4 The number of viable bacterial cells/mL ($\log_{10}$) in the presence and absence of tryptophan

	Log_{10} cfu/mL (Non tryptophan medium)				Log_{10} cfu/mL (Tryptophan medium)			
Strain	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3
3C	8.6 ± 0.06	8.7 ± 0.13	8.1 ±0.03	6.6 ± 0.29	8.8 ± 0.30	8.2 ± 0.14	8 ± 0.06	7.7 ± 0.52
1N	7.5 ± 0.16	7.8 ± 0.13	8.4 ± 0.32	9.1 ± 0.38	7.8 ± 0.05	8.2 ± 0.04	5.8 ± 2.91	8.9 ± 0.54
Sp7	7.7 ± 0.14	7.7 ± 0.09	7.6 ± 0.06	8.2 ± 0.04	7.8 ± 0.04	7.8 ± 0.07	7.8 ± 0.10	8 ± 0.07
Sp7-S	7.4 ± 0.05	7.4 ± 0.06	7.6 ± 0.10	8.2 ± 0.36	7.9 ± 0.42	7.3 ± 0.17	8 ± 0.11	7.7 ± 0.14
Sp245	7.5 ± 0.09	6.8 ± 0.31	7.6 ± 0.14	8.1 ± 0.06	7.5 ± 0.09	7 ± 0.37	7.7 ± 0.33	8 ±0.03

Values are averages of three independent samples ± standard error.

Generally, *Azospirillum* strains grew more slowly than 3C and 1N during incubation. The number of Sp7 only changed slightly during three days of growth. Viable cell number reached a maximum at day 3 with $\log_{10}$ 8.2 cfu/mL without tryptophan and $\log_{10}$ 8 cfu/mL with tryptophan. The increase of viable cells on day 3 was significant ($P<0.05$), however, cell growth was not significantly affected by the presence of tryptophan (P=0.55). Similarly, the number of viable cells of Sp7-S culture reached a maximum on day 3 without tryptophan and day 2 with tryptophan. Cell growth of Sp245 showed same trend which the number of viable cell decreased on day 1 then increase on following days. There was no significant effect of tryptophan on the number of viable cells during growth period ($P>0.05$).

In the presence of tryptophan, all strains significantly increased their production of IAA compared with strains grown in the absence of tryptophan at $P<0.05$ (Table 2.5).The IAA content was also significantly affected by sampling time ($P<0.05$). The range of IAA production by PGPR strains was 0.04-0.41 μg/mL without tryptophan and up to ten times higher in the presence of tryptophan at 0.29-43 μg/mL (Table 2.5).

A significant increase in the production of IAA by all strains was recorded in the presence of 0.1% tryptophan. Sp245 showed a maximum increase on day 3 by more than 100% of the initial concentration on day 0, followed by Sp7 and Sp7-S. Meanwhile, the IAA production of 3C and 1N reached a maximum on day 1 and then slowly declined over day 2 and 3.

Initial IAA production at day 0 was higher when the medium was supplemented with tryptophan over the non tryptophan medium indicating that tryptophan was important for IAA production of all PGPR strains. The results on day 0 also indicated that tryptophan may induce the synthesis of bacterial IAA rapidly especially in *A. brasilense* strains, because the same starter culture was used to inoculate the both media, with and without tryptophan, the IAA measurement was performed immediately after all media were inoculated.

Table 2.5 Production of IAA (µg/mL) in the presence and absence of tryptophan

Strain	Non Tryptophan medium				Tryptophan medium			
	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3
3C	0.04 ± 0.01	0.05 ± 0.00	0.09 ± 0.00	0.22 ± 0.11	0.29 ± 0.06	7.64 ± 0.54	5.91 ± 0.41	4.72 ± 1.24
1N	0.05 ± 0.01	0.05 ± 0.00	0.37 ± 0.2	0.31 ± 0.09	0.79 ± 0.03	3.50 ± 0.35	2.36 ± 0.77	0.99 ± 0.14
Sp7	0.04 ± 0.00	0.05 ± 0.00	0.41 ± 0.18	0.05 ± 0.02	2.13 ± 0.21	7.64 ± 0.21	10.08 ± 0.89	20.56 ± 2.41
Sp7-S	0.04 ± 0.00	0.05 ± 0.00	0.12 ± 0.06	0.04 ± 0.00	1.95 ± 0.09	7.12 ± 0.24	8.87 ± 0.12	18.36 ± 1.01
Sp245	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.04 ± 0.00	1.99 ± 0.09	9.52 ± 0.90	29.5 ± 2.24	43.0 5± 1.71

Values are the mean of three independent samples ± standard error

In order to calibrate IAA measurement on the basis of cell density, the IAA concentration was calculated as µg/10^7 bacterial cells (Fig. 2.15). In general, production of IAA in the presence of tryptophan was up to 10 times higher than it was without tryptophan. When related to the number of viable bacterial cells, the highest amount of IAA production was observed in 3C at day 3 in non-tryptophan medium. However the quantities produced were significantly less when tryptophan present in the medium. In the presence of tryptophan, IAA production increased in all *Azospirillum* strains after 24 hours incubation, with Sp245 producing the highest amount of IAA. While the production of IAA by Sp7 increase consistently during the three day observation, both Sp7-S and Sp245 produced lower concentrations at day 2. The decrease was more noticeable in Sp7-S by 68%. At day 3, all *Azospirillum* reached their maximum, with the highest obtained by Sp245 of 4.92 µg/10^7 bacterial cells. 3C and 1N had maximum production on day 2 and day 1, respectively.

There were significant effects of sampling time and addition of tryptophan on IAA production on the basis of cell density by 1N, Sp7 and Sp7-S ($P<0.05$). However, the effect of time and tryptophan addition were not significant ($P=0.16$ and $P=0.10$) on IAA production by 3C. When Sp245 cells grown with tryptophan, the production of IAA significantly increased ($P=0.03$) however the production was not significantly different during three days of incubation ($P=0.39$)

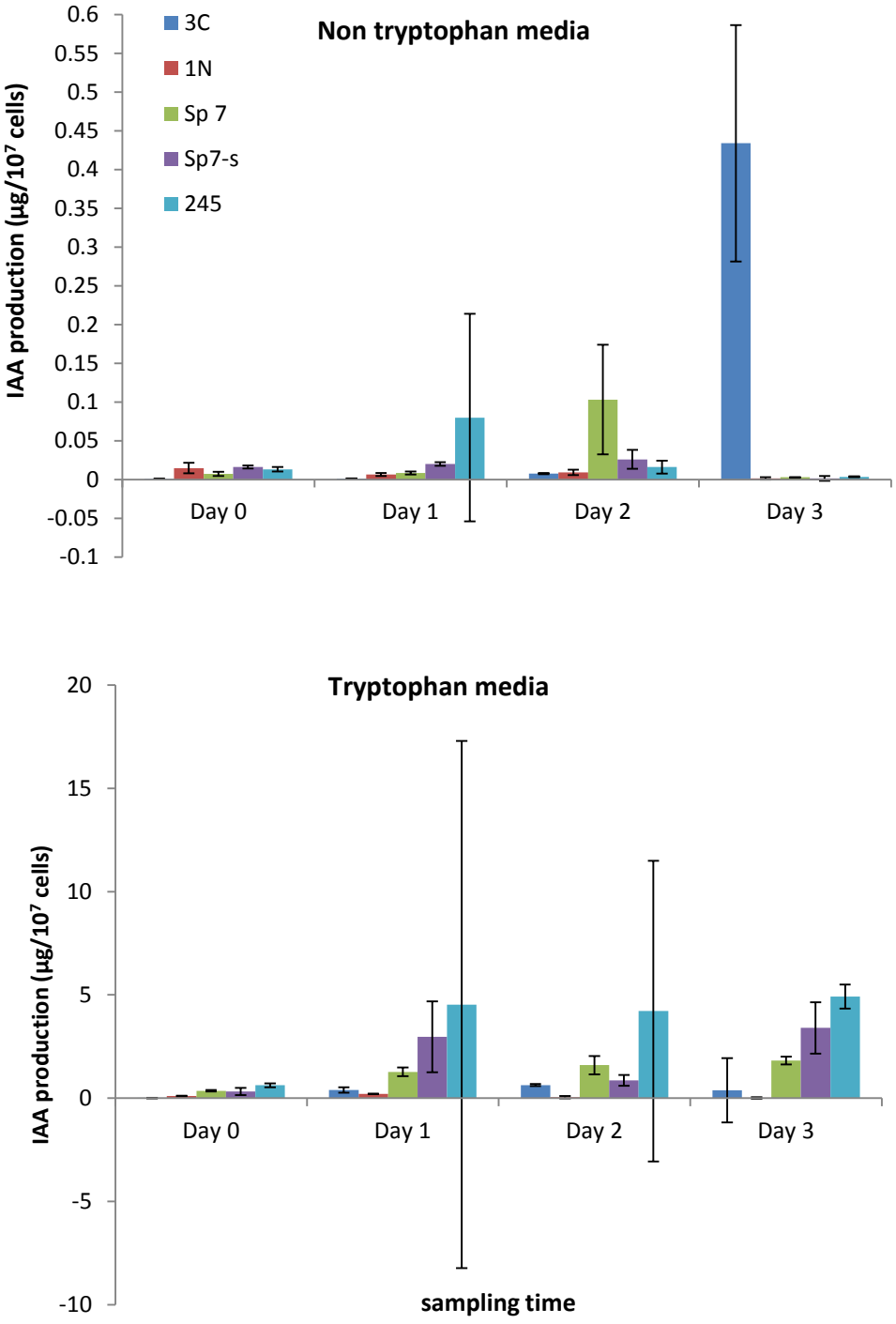

Fig. 2.15 IAA production expressed per number $10^7$of viable bacterial cells in the presence and absence of tryptophan. The values are means of three independent samples. Bars above values are standard errors.

2.3.3 The effect of various immersion solutions on the adventitious root growth parameters of ornamental cuttings in water medium

Ornamental plants used in the propagation experiment were obtained from a local plant nursery. The cuttings were immersed in different solutions including cultures of selected PGPR Sp245 to evaluate adventitious root formation. To confirm the presence of Sp245 in the cuttings after immersion, the bacterial cells were recovered using a semi solid Nfb medium.

The initial number of Sp245 cells in the Sp245 culture before inoculation of cuttings was $1.4x10^8$ cfu/mL and IAA concentration was 27 µg/mL. After 30 days, the three ornamentals that were tested had responded differently to each immersion solution (Fig 2.16). All immersion solutions induced adventitious root growth of *Argyranthemum* sp. cuttings. Root formation by cuttings was calculated as percentage of cuttings that formed roots per treatment. Cuttings treated with Sp245 supernatant grown with tryptophan and water had the highest percentage of root formation of 83.3%. The other treatments resulted in 66.7% root formation. The bacterial and IAA treatments stimulated root formation in *L. stoechas* cuttings, with the highest root growth observed in cuttings treated with 0.003% IAA. In the third week very small roots were detected in *Osteospermum sp.* cuttings immersed in Sp245 cultures with tryptophan and heat-killed cell solutions. The Sp245 cultures with tryptophan stimulated 33.3% root formation, whereas heat-killed cells caused 16.7% of the cuttings to produce roots (Fig 2.16).

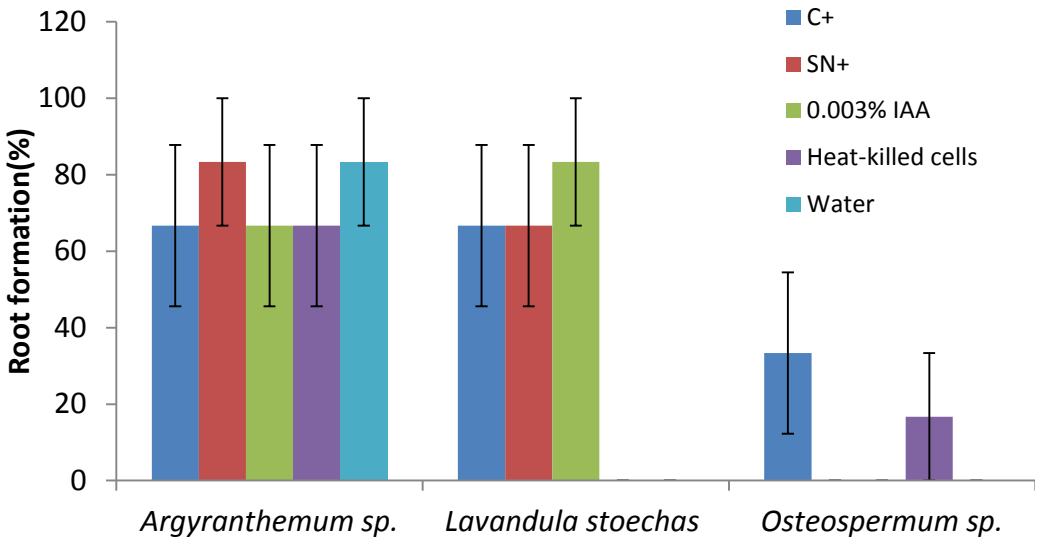

Fig. 2.16 The effect of various immersion solutions on adventitious root growth in ornamental cuttings tested. Results are presented as percentage of cuttings producing roots over the period of incubation. The values are means of six replicates. Error bars represent standard error of the means. C+: culture of Sp245 grown with tryptophan, SN+: supernatant of Sp245 grown with tryptophan.

Adventitious roots from ornamental plant cuttings had a similar visible appearance regardless of the various immersion solutions (Figure 2.17).

Argyranthemum sp. *L. stoechas* *Osteospermum* sp.

Fig. 2.17 Visual appearance of ornamental cutting root formation tested in various immersion solutions. Each ornamental showed similar adventitious appearances despite the various immersion solution used. A: Sp245 cultures, B: Sp245 supernatant, C: 0.003% IAA, D: Sp245 heat-killed cells and E: water

Very few roots were observed in treatments during the first week after immersion. However, all plants developed some roots by the third week of growth (Fig. 2.18). Of the various immersion solutions used to inoculate *Argyranthemum* sp. cuttings, the Sp245 supernatant grown with tryptophan treatment resulted in the highest number of roots over the observation time. Cuttings treated with Sp245 supernatant with tryptophan produced 26% more roots per cutting than the water treatment although they had the same rooting percentage

Sp245 cultures grown with tryptophan and supernatant stimulated the production of more roots in *L. stoechas* cuttings compared to 0.003% IAA (Figure 2.18). During the first 7 days, cuttings treated with 0.003% IAA produced the largest number of roots and there were no roots detected on the cuttings treated with the Sp245 supernatant with tryptophan. From day 7 to 14, the Sp245 supernatant with tryptophan treatment resulted in an average of 2.3 roots per cutting which was the highest of all treatments. However, at week 3 and 4 the highest number of roots per cutting was observed in the Sp245 culture treatments with an average of 4, 2 and 5 roots per cutting, respectively.

Osteospermum sp. cuttings had slower root growth compared to the other ornamentals. During the first two weeks of observation, there was no root growth detected in any treatment. During

week 3 and 4 very small roots appeared on cuttings treated with Sp245 cultures and heat-killed cells. No root growth was detected in other treatments (Fig 2.18).

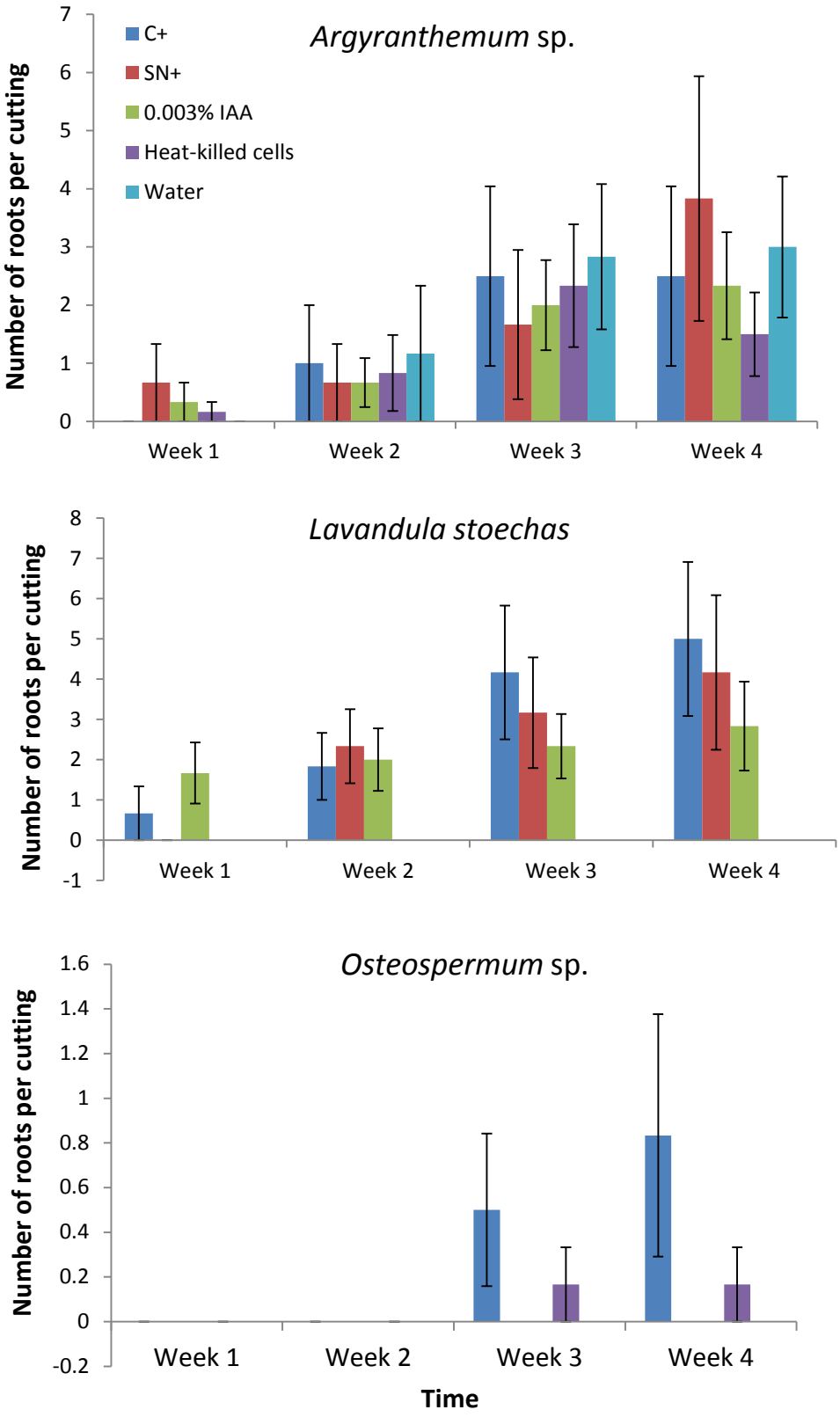

Fig. 2.18 The effect of various immersion solutions on the number of roots per ornamental cutting. The values are means of six replicates. Error bars represent standard error of the values. C+: culture of Sp245 grown with tryptophan, SN+: supernatant of Sp245 grown with tryptophan.

The total length and surface area of roots in ornamental cuttings are shown in Table 2.6. Although the *Argyranthemum* sp. cuttings immersed in water for 6 hours had the highest root length and the largest root surface area of all the treatments (3.95 cm and 0.60 cm^2), Fig 2.18 shows cuttings treated with Sp245 supernatant with tryptophan produced more roots than the water treatment did, illustrating that the Sp245 supernatant produced more roots of shorter lengths than the water treatment whereas the 0.003% IAA immersed cuttings had the shortest total root length of 1.85 cm on average. However, total root length or root surface between the cuttings did not vary significantly (P=0.68)

The experiment also demonstrated that *L. stoechas* and *Osteospermum* sp. inoculated with Sp245 cultures with tryptophan had the highest total root length and the largest total surface area than other treatments. The treatments developed total root length and surface area with averages of 3.04 cm and 0.31 cm^2 in *L. stoechas* and 0.05 cm and 0.01 cm^2 in *Osteospermum* sp. (Table 2.6).

Table 2.6 The effects of various immersion solutions on total root length and total root surface area in ornamental cuttings.

Treatment	*Argyranthemum sp.*		*L. stoechas*		*Osteospermum sp.*	
	Total length (cm)	Total surface area (cm^2)	Total length (cm)	Total surface area (cm^2)	Total length (cm)	Total surface area (cm^2)
C+	2.29±1.03	0.35±0.16	3.04 ± 1.88	0.31 ± 0.20	0.05 ± 0.03	0.01 ± 0.006
SN+	2.49±1.14	0.39±0.17	1.33 ± 0.48	0.14 ± 0.05	0	0
0.003% IAA	1.85±0.78	0.28±0.12	2.44 ± 0.94	0.25 ± 0.09	0	0
Heat-killed cells	2.20±0.78	0.33±0.0.12	0	0	0.02 ± 0.02	0.002 ± 0.002
Water	3.95±1.45	0.60±0.22	0	0	0	0

The values are means of six replicates ± standard error. C+: culture of Sp245 grown with tryptophan, SN+: supernatant of Sp245 grown with tryptophan.

2.3.4 Recovery of *A. brasilense* Sp245 from ornamental cuttings

The initial number of cells in the inoculum was 1.4 x 10^8 cfu/mL of medium. To investigate if the bacteria were absorbed by the cutting during inoculation, the cuttings were separated into two segments (upper and lower) immediately after 6 hours inoculation. Generally, the lower segment of all the ornamental cuttings had higher numbers of pellicle forming isolate compared

to the upper segment and contributed more than 50% of the total number of bacteria recovered (Table 2.7).

Table 2.7 Pellicle forming isolates in semi-solid Nfb from segments of inoculated cuttings

Cuttings	Segments of cutting	MPN of pellicle formed isolates /g of segment fresh weight
Argyranthemum sp.	Top	1.3×10^6 (6.12 ± 0.40)
	Lower	6.2×10^7 (7.79 ± 0.34)
Lavandula stoechas	Top	1.2×10^4 (4.09 ± 1.39)
	Lower	1.2×10^8 (8.09 ± 0.92)
Osteospermum sp.	Top	3.1×10^4 (4.98 ± 0.05)
	Lower	3.1×10^6 (6.50 ± 0.20)

The values are means of three replicates. Values in brackets are $\log_{10}$ transformation ± standard errors.

The total number of recovered Sp245 is presented in Table 2.8 and shows the highest recovery of bacteria was achieved in *L. stoechas* cuttings, where up to 86% of the initial inoculum was recovered, followed by *Argyranthemum* sp. (45.7%) and *Osteospermum* sp (2.3%). In the control (water) treatment, a very low number pellicle form isolates typical *Azospirillum* were detected in *Argyranthemum* sp. and *Osteospermum* sp. while *L. stoechas* cuttings had an undetectable levels of pellicle forming isolate.

Table 2.8 Total MPN of isolate forming pellicles in semi solid Nfb

Cuttings	MPN of pellicle forming isolates /g of cutting fresh weight	
	Sp245	Water
Argyranthemum sp.	6.4×10^7 (7.80 ± 0.33)	2.1×10^3 (3.33 ± 0.16)
Lavandula stoechas	1.2×10^8 (8.09 ± 0.90)	undetected
Osteospermum sp.	3.3×10^6 (6.51 ± 0.19)	2.1×10^4 (4.33 ± 0.45)

The values are means of three replicates. Values in brackets are $\log_{10}$ transformation ± standard errors.

2.4 Discussion

2.4.1 Selection of PGPR inoculant based on IAA production in liquid medium

The ability to synthesize IAA was used to select suitable PGPR as commercial ornamental plant growers often use auxins such as IBA to induce root formation in plant propagation techniques. IAA is an analog of IBA and is widely produced by rhizobacteria. Accordingly, IAA production may be a good marker for selecting an effective PGPR to support root formation in cuttings. Similar approaches have been reported by Khalid et al. (2004a) in selecting potential inoculants for improving wheat growth. The authors found that the highest auxin-producing-strains showed the most promising effects on wheat seedling growth parameters under contaminant-free (gnotobiotic) conditions. The strains also improved growth and yield of wheat in pot and field experiments, proposing that PGPR selection based on auxin production, including IAA, and evaluation the PGPR effects on plant growth *in vitro*, may be a reliable method to assess an effective PGPR.

This experiment demonstrated that in a liquid medium, all strains showed similar trends in their IAA production. The phytohormone production was significantly higher in the presence of tryptophan. This result is in agreement with other studies on bacterial IAA biosynthesis in liquid medium with and without the addition tryptophan (Khalid et al., 2004b; Ahmad et al., 2005; Tsavkelova et al., 2007b). The results indicated that the addition of tryptophan is essential to increase IAA production of PGPR inoculants.

The bacterial growth trend in the presence and absence of tryptophan in the culture was similar during the observation, with the highest rate of growth by strain 1N. Although 1N showed better growth in the tryptophan medium, Sp245 produced higher amounts of IAA in the same medium. This indicates that in the presence of tryptophan, the difference in IAA production between *Azospirilum* and the other strains was not related to the number of viable bacterial cells.

Azospirillum has been known to produce phytohormones, especially IAA, as one of the major mechanisms to promote plant growth (Tsavkelova et al., 2006b). After 24 hours incubation in the presence of tryptophan, *Azospirillum* strains showed high IAA production compared to two other strains with Sp245 producing the highest concentration. This result is supported by

Zakharova et al. (1999) who used HPLC measurements to demonstrate that *A. brasilense* Sp245 utilizes tryptophan as the main precursor for IAA production. Other compounds, that have been proposed as IAA precursors (anthranilic acid and indole), did not result in IAA production.

In both media (with and without tryptophan), *Azospirillum* strains showed the highest viable bacterial number at day 3 which coincided with a maximum IAA production, indicating that IAA synthesis increases with bacterial growth. Tsavkelova (2007b) reported that IAA may also been implicated in growth stimulation. The addition of increasing levels of exogenous IAA to bacterial cultures stimulated bacterial cell growth and biomass accumulation and the effect was strain dependent. The stimulating effects were shown actively at the microbial exponential phase (measured using optical density) and shortened stationary growth phase.

Patten and Glick (2002) demonstrated IAA production by *Pseusodomonas putida* with tryptophan levels lower (0-500 µg/mL) than those used in this experiment. The authors found that IAA production increased as tryptophan levels increased but the authors did not report the effects of tryptophan on the bacterial growth. IAA production in bacterial growth unit was expressed per bacterial growth based on observation at 600 nm (OD_{600}). Optical density may not be as a reliable method as spread plating in measuring bacterial growth because conventional spectrophotometer observations cannot differentiate between living and dead cells while spread plating allows only viable cells to be counted.

In another study (Ahmad et al., 2005) using higher levels of tryptophan (0-5000 µg/mL) similar results were reported. Increasing tryptophan concentration resulted in an increase in IAA production by *Azotobacter* spp. and *Pseudomonas* spp. after 7 and 15 days incubation. There was no report on the amount of bacterial growth.

A relationship between tryptophan concentration and *A. brasilense* Sp7 cell numbers was reported by Bar and Okon (1992). They found that when a high concentration of exogenous tryptophan was added to *A. brasilense* Sp7 growth medium, cell growth inhibition occurred and there was a particular change in transcription and protein synthesis. It was hypothesized that the increase in IAA production by *Azospirillum* may be related to bacterial survival against the plant-produced tryptophan toxicity in the rhizosphere. According to Glick (1999), rhizobacteria

may synthesize IAA to enhance plant growth, so in turn, they will be able to obtain plant metabolites to support their growth. In this experiment only a single level of tryptophan (0.1% or 1000 µg/mL) was used, so further effects of exogenous tryptophan on bacterial growth cannot be confirmed.

Given that Sp245 produced the highest amount of IAA during the three days of observation in this experiment, this strain was chosen to further evaluate PGPR effects on the growth of ornamental cuttings.

2.4.2 Selection of the most responsive ornamental cuttings to Sp245 inoculation

The use of water as a growth medium in this experiment was employed to obtain better observation of adventitious root growth over time. In addition, root observations could be carried out without removing the cuttings from the growth media which may destroy newly grown fragile roots. The synthetic IAA concentration used in this experiment (0.003%) was selected as the closest IAA concentration synthesized by Sp245 culture (27 µg/mL). The supernatant was included in this experiment to determine if the presence of Sp245 cells in immersion solution, that may subsequently be absorbed in the cuttings or attached on the cuttings surface, had a better effect than IAA containing supernatant only in stimulating root growth. Since there was no difference found between adventitious root growth parameters and appearances resulted from ornamental cuttings immersed in Sp245 culture or Sp245 supernatants, the adventitious root growth stimulation may have resulted mainly due to the effect of IAA synthesized by the PGPR.

Sp245 was capable of stimulating root formation in all of the ornamental cuttings tested. However, there were different responses between species to the various immersion solutions. *Argyranthemum* sp. did not appear to require specific stimulators to develop roots since all treatments were able to stimulate root formation. In fact, Sp245 and 0.003% IAA treatments stimulated less root formation than the water control. Both Sp245 cell culture and supernatant grown with tryptophan promoted root formation in *L. stoechas* at a similar rate which was less than the 0.003% IAA treatment. No roots were detected in cuttings treated with Sp245 heat-killed cells or water, suggesting that these cuttings required additional growth factors to form roots. The IAA contained in the culture and supernatant solution was effective as a root growth

promoter for the plant. While roots only formed on *Osteospermum* sp. cuttings treated with Sp245 cell culture and heat-killed cells solutions. It can be speculated that the roots grew randomly and were not a result of any treatment.

Even though there was no significant effects found, improvement over the IAA control, *L. stoechas* was the most responsive plant cutting to the Sp245 inoculant. This is also supported by the Sp245 cell recovery results from plant cuttings using Nfb medium and MPN counts that show that this plant had the highest Sp245 cell recovery. There were no *Azospirillum* detected from the water-treated *L. stoechas* cuttings, confirming the plant did not have an endemic *Azospirillu*m population which may affect Sp245 inoculations. Nfb medium has been used to isolate diazotrophic bacteria including *Azospirillum* from environments, such as the rhizosphere of wheat (Bashan and Levanony, 1985; Gosal et al., 2011), maize (Ilyas et al., 2008), rice (Jha et al., 2009), sugarcane (Moutia et al., 2010) and taro (Jolly et al., 2010).

The importance of host plant selection for PGPR inoculation was reported by Moutia et al. (2010). Their study showed that PGP effects of an *Azospirillum* mixed inoculant on sugarcane growth parameters under drought stress were dependent on the plant variety. Thus, host plant selection should be performed to ensure the effectiveness of PGPR inoculation. Host plant selection in this experiment was not carried out to a variety level, nevertheless, the different species clearly demostrate different responses to inoculation with Sp245. Different growth and yield responses between inoculated varieties of wheat in field trials was also reported by Khalid et al.(2004a), indicating that selection of responsive plants to selected PGPR inoculation will improve PGP effects.

2.5 Conclusion

In summary, since the use of ornamental seedlings did not result in detectable effects of PGPR inoculation, ornamental cuttings will be used as plant material to minimise competition between inoculated PGPR and microorganisms already present in potting mix. The most effective PGPR-ornamental plant interaction was shown to be by Sp245-*L.stoechas*. Sp245 was chosen as the potential inoculant due to its ability to produce the highest amount of IAA in the liquid medium. Furthermore, Sp245 inoculation of *L.stoechas* cuttings resulted in positive responses such as adventitious root formation, high number of Sp245 cell recovery from the

cuttings and no *Azospirillum* endemic populations. Accordingly, this pair will then be used in further experiments to evaluate PGPR effectiveness in ornamental plant cutting propagation techniques to compare their effectiveness to commercial root growth regulators.

CHAPTER 3 PLANT GROWTH PROMOTION OF *LAVANDULA STOECHAS* CUTTINGS AFTER INOCULATION WITH *AZOSPIRILLUM BRASILENSE* Sp245

3.1 Introduction

The nursery industry utilizes vegetative propagation to preserve essential ornamental properties such as flower colour, productivity, disease resistance and also to prevent plant variations that may result from seed propagation. Propagation by cuttings is considered an effective and rapid technique to maintain specific characteristics, especially in herbaceous plants (Cameron and Emmett, 2003). Successful ornamental cutting production relies on adventitious root growth of the plant. Synthetic auxin is one of the most widely used growth regulator in the industry and is used by ornamental growers to accelerate root growth (Miller, 2003). Auxin is naturally synthesised by plants, mainly as IAA, and is distributed throughout plant parts (Napier, 2003). However exogenous auxins, such as synthetic IBA and 1-naphthaleneacetic acid (NAA), are used to enhance cutting root growth.

Inoculation of plant cutting with IAA-producing PGPR may be cost-effective alternative to using synthetic auxins in order to promote adventitious root growth. *Azospirillum* is one of the most well-studied IAA-producing PGPR and has been shown to improve water and mineral uptake by developing root systems following inoculation to agricultural crop (Bashan and Levanony, 1990; Dobbelaere et al., 2001; Saubidet et al., 2002).

Plants belonging to genus *Lavandula* (lavender) are widely distributed throughout the Mediterranean area and are commercially cultivated as ornamental plants or for essential oil production (Angioni et al., 2006). Lavender oil is mainly used for food, aromatherapy and cosmetic purposes. In addition, studies have also shown that lavender oil is effective for its therapeutic and antimicrobial benefits (Cavanagh and Wilkinson, 2005; Angioni et al., 2006; Hanamanthagouda et al., 2010; Zuzarte et al., 2011) as well as antioxidant activity (Hui et al., 2010).

Inoculation of carnation cuttings with *Azospirillum* strains produced longer adventitious roots compared with 0.1% commercial rooting hormone (IBA) or water 24 days after inoculation (Li et al., 2005). Inoculation of chrysanthemum seedlings with *Azospirillum* combined with 75% recommended dose N (RDN) significantly increased growth parameters and flower yield compared to the uninoculated and 100% RDN plants indicating the bacterial inoculation substituted 25% of the fertilizer requirement (Gadagi et al., 2002). These studies indicate that *Azospirillum* may be used in the ornamental industry to reduce the use of both growth hormone and fertilizer.

Observation that PGPR application can enhance growth of various crops in both controlled conditions and field has stimulated interest in the mass production and commercialisation of microbial inoculants based on PGPR. Microbes formulated as inoculants must maintain viability and retain their functional PGP characteristics throughout product shelf life (Bashan, 1998). The most widely used commercial bacterial inoculants are based on the N_2-fixing rhizobia used to inoculate legumes (Deaker et al., 2004).

Bashan (1998) defined a bacterial inoculants as :

> "a formulation containing one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material, either organic, inorganic, or synthesized from defined molecules".

The properties of a carrier should support the bacterial growth and survival during production and storage and also allow adequate distribution of the inoculants bacteria to the target host (Smith, 1992; Deaker et al., 2004; Bashan, 1998). In addition, high quality inoculants should retain desirable biological, chemical and physical properties during production, be easy to manufacture, easy to handle, nontoxic, and environmentally friendly (Bashan, 1998)

Rhizobial inoculants are available as peat, liquid or broth, freeze-dried and granular products (Deaker et al., 2004). Although peat is the most successful carrier, it is not available worldwide (Bashan, 1998; Lucy et al., 2004). Rhizobial cells grown and stored in peat undergo physiological and morphological changes and generally survive better during delivery to the host plant, particularly when applied to seed (Feng et al., 2002; Deaker et al., 2004).

The aims of the experiments described in this chapter were to investigate the growth promoting effects of Sp245 on adventitious root growth of *L. stoechas* cuttings in different plant growth media and to determine if inoculation can stimulate nutrient uptake efficiency. Furthermore, the efficacy of different inoculant formulations containing Sp245 was tested and compared with a commercially available biofertilizer TwinN.

3.2 Materials and methods

This chapter describes four separate plant experiments carried out to evaluate the effects of *A. brasilense* Sp245 inoculation on adventitious root growth of *L.stoechas* cuttings. Sp245 was selected for this study because of its ability to produce the highest concentration of IAA when compared to other strains tested (as described in Chapter 2). The first three experiments investigated adventitious root growth responses of treated cuttings in sand and water. The final experiment investigated the effect of Sp245 on growth and nutrient uptake of treated *L. stoechas* shoots after transferring from sand to commercial potting mix.

3.2.1 Plant species and bacterial strains used in these experiments

Ornamental cuttings used in these experiments were harvested from *L. stoechas*, purchased from local nursery. The cuttings were prepared as described in section 2.2.3.1.

The source of strain *A. brasilense* Sp245 is described in section 2.2.2.1.

3.2.2 Plant growth media preparation

The effect of Sp245 on adventitious root growth of *L. stoechas* was performed in two different media, sand and water. Whereas investigation on the effect of Sp245 on nutrient uptake of *L. stoechas* cuttings was initially carried out in sand medium to grow adventitious roots after which the rooted cuttings were transferred to potting mix medium.

3.2.2.1 Sand medium

Propagating sand (Brunnings garden product Pty Ltd., Australia) was moistened with distilled water and autoclaved at 121°C for 1 hour. The bottom of each cell of seedling trays was covered with two sheets of paper towel of adequate size. The cells were then filled with the sand and were moistened with water. After cuttings were inserted in the sand, the tray was covered with

a transparent lid (Fig 3.1) to maintain humidity and reduce water loss. In order to prevent contamination between treatments, each tray contained a single treatment. The trays were placed under a light bank, randomly arranged and moved around each week during the propagating period to eliminate bias.

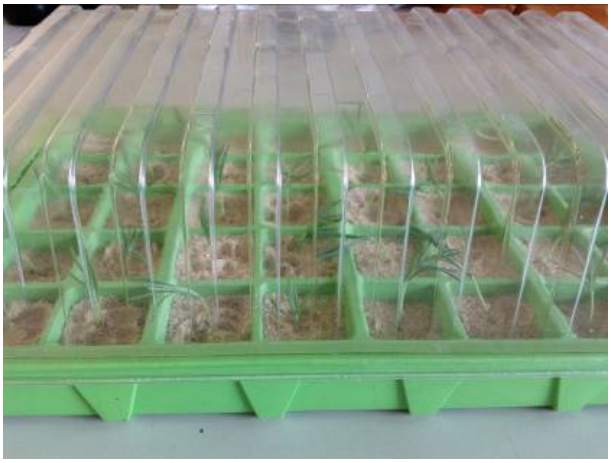

Fig. 3.1 Layout of sand grown cuttings experiment. The lid was used to reduce evaporation and maintain humidity.

3.2.2.2 Water medium

Water as a growth medium was prepared using sterile MilliQ water and plant growth assemblies were set up as described in section 2.2.3.2.

3.2.2.3 Potting mix medium

Commercial potting mix (Greenlife premium potting mix, Australian Native Landscapes Pty Ltd.) was used as the growth medium for cuttings that had produced adventitious roots in sand. Plastic pots (9.5 diameter x 9.5 cm deep) were sterilized by soaking in 4% sodium hypochlorite for 1 hour and rinsing with tap water. A sheet of paper towel was placed at the bottom of the pot and approximately 150 g of potting mix was added and moistened with 100 mL of water. A plastic container was placed under each pot to collect excess water. The plants were kept under a light bank for 30 days before harvesting.

3.2.3 Preparation and treatments of plant cuttings

3.2.3.1 Preparation of solutions used to treat cuttings of L. stoechas

Various solutions were prepared for application to plant cuttings before transfer to plant growth medium. All water was MilliQ water, unless mentioned otherwise and a detailed description of solutions used to treat cuttings and their preparation is provided below. For each experiment, inoculum properties including the viable number of Sp245, bacterial strains contained in commercial biofertlizer and IAA concentration were measured as described in section 2.2.2.4 and 2.2.2.5.

Table 3.1 Desription and coding of treatments

Description	Code of treatment used in table and figures
Sterile water	water
0.003% IAA	0.003% IAA
Commercial plant rooting hormone (IBA)	0.4% IBA
Supernatant of Sp245 grown without tryptophan	SN-
Supernatant of Sp245 grown with tryptophan	SN+
Culture of Sp245 grown without tryptophan	C-
Culture of Sp245 grown with tryptophan	C+
Peat culture of Sp245	PC
Commercial biofertilizer (TwinN)	CB

Treatment of synthetic IAA was prepared as described in section 2.2.3.3. The commercial plant rooting hormone Rootex-L (Bass laboratories, Australia) contains 0.4% indole-3-butyric acid (IBA) and was used as a positive control. The solution was prepared according to the manufacturer's instructions by diluting the hormone with water in a 1:1 ratio.

Colonies of Sp245 (prepared as in section 2.2.2.2.2) growing on NA were used to inoculate 2 mL of DF minimal medium and mixed using a vortex mixer. The thick homogenized culture was then aseptically dispersed in 1 mL aliquots to 50 mL of liquid DF minimal medium supplemented with filter sterilized L-tryptophan to a final concentration of 0.1%. The cultures were then incubated at 28°C for three days with shaking (125 rpm) and used as an immersion solution. Sp245 cultures without addition of tryptophan also included as treatment.

Cultures of Sp245 grown in DF minimal medium with and without tryptophan were centrifuged at 3,000 *g* for 30 minutes. The supernatant was then collected and used to treat plant cuttings. The supernatant in the first experiment was not filtered but in subsequent experiments the supernatant was filter-sterilized using a syringe filter (0.22 µm) to remove cells that may be still present in the supernatant after centrifugation. The supernatant of culture Sp245 grown with and without tryptophan were included in the project to investigate the effect of bacterial IAA in the immersion solution to stimulate adventitious root formation.

Peat culture of Sp245 was produced by inoculating a pack of sterilized peat (150 g) with 90 mL of Sp245 liquid culture without tryptophan using a sterile 25G syringe and incubated for 7 days at 28°C. In the first experiment, the Sp245 peat culture (1 g) was added into 99 mL of DF medium without tryptophan and vortexed thoroughly. The mixture was then centrifuged and the resulting supernatant was used as an immersion solution after filter sterilisation (0.22 µm). This was done to determine if there was any effect of IAA produced by peat-grown cells of Sp245. In order to increase inoculum size in subsequent experiment, 10 g of peat was suspended in 90 mL of DF medium and homogenized. The peat culture solution was not filter sterilized so that viable cell numbers of Sp245 were maintained in peat culture solution.

To prepare the washed Sp245 cell treatment, the Sp245 culture grown in the presence of tryptophan was prepared as above and the cells were harvested by centrifugation at 3,000 *g* for 30 minutes. The supernatant was then discarded and the cells were washed once with 0.03 M $MgSO_4$. The harvested cells were diluted with equal volume of 0.03M $MgSO_4$ (50 mL) and this bacterial suspension was then used as an immersion solution.

Biofertilizer immersion solution was made by dissolving 1 g of freeze-dried biofertilizer TwinN (Mapleton Agribiotec) into 99 mL of DF medium and vortexing. The manufacturer claims that the TwinN biofertilizer contains a mixture of propriety strains of N_2-fixing microbes.

Inoculum properties including bacterial number and IAA production in immersion solutions were determined before use (section 2.2.2.4 and 2.2.2.5). IAA measurement in peat culture was carried out using filtered supernatant to remove peat particles. For Sp245 in peat

culture and commercial biofertilizer IAA measurements, sterile water inoculated peat and DF medium were used as the respective blanks.

3.2.3.2 Treatment of cuttings

Cuttings were obtained and prepared as described in section 2.2.3.1. Cuttings were inoculated by immersing the cuttings individually for 6 hours (Tsavkelova et al., 2007a) in separate sterile microcentrifuge tubes containing 1 mL of immersion solution except for the commercial rooting hormone IBA treatment which was carried out based on the manufacturer's instructions. The lower end of the cuttings was immersed in IBA solution for 2 seconds and air dried on a paper towel before insertion in the growth medium.

3.2.3.3 Plant growth conditions

All plant experiments except water-grown cuttings were conducted under a light bank with 12 hours light. Water-grown cuttings experiment was conducted in temperature-controlled growth chamber at a constant temperature between 24-25°C.

The sand-or potting mix-grown cuttings were watered using a hand sprayer to maintain moisture. The water level in water grown cuttings was checked every three days and refilled if necessary to ensure the stem remained immersed below the medium. At the third week, 2 mL of Hoagland's solution was added to the media to support plant nutrition. Nutrient solution was not added in the potting mix-grown cuttings and efficacy of different formulations experiments.

3.2.4 Measurement of inoculation efficacy and plant growth

3.2.4.1 Recovery of Sp245 from L. stoechas cuttings after inoculation

Sp245 and N_2-fixing strains in commercial biofertilizer were recovered from cuttings after immersion for 6 hours in solutions and MPN was determined using the multiple tube fermentation method in semi solid Nfb medium (as described in section 2.2.3.5).

3.2.4.2 Harvesting and adventitious root growth measurement

Cuttings were harvested for analysis 30 days after planting. The cuttings were gently uprooted and shaken to remove excess sand around the roots. All the root growth parameters were measured as in section 2.2.3.6.

3.2.4.3 Measurement of N content in plant tissue

After 30 day of growth in sand medium, cuttings that had developed roots were transferred to potting mix medium. The plants were then grown for 30 days and harvested. The cuttings were harvested by pulling them from the potting mix carefully and shoots were excised. The shoots were then dried at 60-70°C overnight and prepared for Kjeldahl N content determination as described below.

3.2.4.3.1 Sample preparation

For each treatment, dried shoot materials were ground separately using a mortar and pestle. The resulting powder was stored in microcentrifuge tubes.

3.2.4.3.2 Kjeldahl methods

The digestion process was performed using a block digester system with a scrubber (Auto digest system K-437, BUCHI). Each sample (100 mg) was transferred to a Kjeldahl sample tube and a selenium catalyst (0.05 g), anti foaming agent stearic acid (0.5 g) and 15 mL of 98% sulphuric acid were added. A sample tube containing catalyst, anti foaming agent and sulphuric acid without any plant material served as a blank. All the tubes were then placed into the block digester in a fume hood which had been warmed prior to analysis. The digestion was conducted at 380°C until the solution became clear.

The cooled digested solution was placed in a flexible distillation unit (Auto Kjelflex K-437, Bio-rad) and distilled. The distilled solution was then titrated with 0.1 M HCl using a burette. The HCl was added to the distilled solution slowly until the solution colour changed to light purple. The blank and sample colours were compared to the colour of standard solution containing Kjeldahl indicator and boric acid. The volume of HCl required to produce the same colour as the standard was recorded.

3.2.4.3.3 N content calculation

N content in the powdered leaf sample was calculated according to formula as follows:

mg of nitrogen present in processed sample (mg N) = (T – B) x 14.0067 g/mol x conc. acid (mol/L).

mg of nitrogen present in plant sample = mg N x V_D/V_U

$$\text{Percent nitrogen (\% N)} = \frac{\text{mg N} \times V_D/V_U}{S} \times 100$$

Where: T = mL acid for sample titration

B = mL acid for blank titration

S = sample weight in milligrams

V_D = volume of plant digest

V_U = volume of plant digest used in distillation process

3.2.5 Data analysis

Difference between the means was analysed using analysis of variance (ANOVA, IBM SPSS statistics 20) and significantly different means were identified using Tukey's test (IBM SPSS statistics 20). Relationship between treatments and measurement parameters were examined using linear regression in Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, USA).

3.3 Result

3.3.1 Inoculum properties of immersion solutions

The initial number of viable cells and IAA concentration in immersion solutions of bacterial origin were measured before application to cuttings.

Viable number of Sp245 and bacterial strains in commercial biofertilizer was determined using a spread plate method on triplicate NA plates and IAA production was determined colorimetrically using supernatant of culture. The viable cell numbers and IAA concentrations contained in immersion solutions used in each experiment are presented in Tables 3.2, 3.3 and 3.4.

Within each experiment, there was no significant difference in the number of viable cells grown with and without tryptophan ($P>0.05$, Table 3.2). Inoculums characteristics of Sp245 and bacterial strains contained in commercial biofertilizer were similar between experiments. In experiment 3, the number of Sp245 applied to cuttings were 5.6×10^8 cfu/mL in Sp245 culture solution and 9.7×10^7 cfu/mL in Sp245 after washing and resuspending in 0.03M of $MgSO_4$ solution. The numbers indicated some cells were loss during the washing process, but the difference on cell numbers was not statistically significant ($P>0.05$).

In experiment 1, commercial biofertilizer contained significantly higher number of cells per mL with 1.7 x 10^9 cfu when compared to other solutions ($P<0.05$). This may have been because of the greater concentration of cells in the freeze-dried preparation.

Similarly to previous experiment in sand growth medium, in experiment 2, commercial biofertilizer had the highest number of bacteria per mL of immersion solution (2.1 x 10^9). However, in this case there was no significant difference ($P=0.058$) between the number of viable cells in commercial biofertilizer (TwinN) and Sp245 culture solutions.

As expected, there were no Sp245 colonies detected from peat culture extracts as a result of filter sterilization. Cells were removed in order to evaluate the effect of IAA production by peat-grown cells on *L. stoechas* propagation. In experiment 2, the peat solution was not filter sterilized to investigate the effect of both cells and IAA concentration on root growth stimulation. Statistically, the number of viable Sp245 cells in peat culture did not vary significantly to culture of Sp245 grown with or without tryptophan although the number of viable Sp245 cells in peat culture was two orders of magnitude lower than the culture of Sp245 in DF medium and three orders lower of magnitude compared to commercial biofertilizer. However, P value for the difference was low ($P=0.058$) and high variation in number of recovered cells from peat culture, which was shown by high standard error value (2.19, almost half of the data value). These might be the case of the insignificant difference in number of viable cells.

The presence of tryptophan significantly increased IAA production compared to Sp245 grown without tryptophan (Table 3.3). Cultures of Sp245 grown with tryptophan produced more IAA than peat cultures or commercial biofertilizer cultures

In experiment 1, IAA production in immersion solutions ranged from 0.1 ± 0.33 to 68.3 ± 0.30 µg/mL. The highest IAA production was recorded solutions were Sp245 had been grown with tryptophan (68.3 µg/mL) followed by commercial biofertilizer, peat culture of Sp245 and Sp245 without tryptophan. When expressed per 10^7 cfu, the highest amount of IAA production was still observed in Sp245 grown with tryptophan (5.34 µg/10^7 cfu) which was significantly higher than other treatments (Table 3.4). However, IAA concentration per 10^7 cells of Sp245 grown without

tryptophan and commercial biofertilizer was not significantly different (P=0.99). This indicates that the production of IAA was related to both the presence of tryptophan number of viable cells.

Similarly, in experiment 2, Sp245 cells grown with tryptophan produced the highest level of IAA (68.4 µg/mL). When number of viable cells was taken into account, IAA production by Sp245 grown with tryptophan was 3.13 µg/10^7 cfu which was significantly higher than IAA produced by Sp245 grown without tryptophan or commercial biofertilizer (0.03 and 0.04 µg/10^7 cfu, respectively). When values were adjusted to the same bacterial number, the production of IAA by Sp245 with tryptophan did not vary significantly from IAA produced by peat culture of Sp245.

In experiment 4, Sp245 grown in the presence of tryptophan showed significantly higher IAA production (P<0.01) when grown with tryptophan which was also observed when the production expressed per 10^7 cfu (P=0.02).

Table 3.2 The initial number of viable bacterial cells contained in immersion solutions for each experiment using spread plate method

Bacterial immersion solutions	Inoculum size (cfu/mL of immersion solution)			
	Exp. 1	Exp. 2	Exp.3	Exp 4
Sp245 grown without tryptophan	7.7×10^7 (7.9 ± 0.13)[b]	2.7×10^8 (8.4± 0.03)[a]		4.4×10^8 (8.64 ± 0.05)[a]
Sp245 grown with tryptophan	1.3×10^8 (8.1 ± 0.15)[b]	2.2×10^8 (8.3±0.07)[a]	5.6×10^8 (8.75 ± 0.33)[a]	3.8×10^8 (8.58 ± 0.11)[a]
Peat culture of Sp245	below the limit of detection	2.8×10^6 (4.4±2.19)[a]		
Commercial biofertilizer	1.7×10^9 (9.2 ± 0.35)[a]	2.1×10^9 (9.3 ±0.06)[a]		
Sp245 in $MgSO_4$			9.7×10^7 (7.9 ± 0.41)[a]	

The values are means of three replicates. Values in brackets are $\log_{10}$ transformations ± standard errors. The values in the same column with different lower case letters are significantly different at $p<0.05$. Exp 1: sand-grown cutting, Exp 2: water-grown cutting, Exp 3: washed Sp245 cells and Exp 4: growth and nutrient uptake in potting mix. Below the limit of detection means there was no growth of the bacteria. Diagonal streaked block indicates the solution(s) was not included in the relevant experiment. cfu: colony forming unit.

Table 3.3 The IAA concentration contained in immersion solutions for each experiment

Immersion solutions	IAA concentration (µg/mL)			
	Exp. 1	Exp. 2	Exp.3	Exp 4
Sp245 grown without tryptophan	0.3 ± 0.09[c]	0.9 ± 0.22[c]		0.89 ± 0.09[a]
Sp245 grown with tryptophan	68.3 ± 0.30[a]	68.4 ± 0.49[a]	11.6	93.9 ± 1.77[b]
Peat cultures of Sp245	0.1 ± 0.33[c]	1.2 ± 0.21[c]		
Commercial biofertilizer	5.2 ± 0.2[b]	8.1 ± 0.12[b]		
Sp245 with tryptophan in $MgSO_4$			*	

The values are means of three replicates ± standard error. The values in the same column with different lower case letters are significantly different at $p<0.05$. Exp 1: sand-grown cutting, Exp 2: water-grown cutting, Exp 3: washed Sp245 cells and Exp 4: growth and nutrient uptake in potting mix. Diagonal streaked block indicates the solution(s) was not included in the relevant experiment. The lack of standard error in Exp 3 column indicates the measurement was not replicated. *The IAA concentration was measured only in Sp245 culture because starter culture for both Sp245 in culture and Sp245 resuspended in $MgSO_4$ solutions was taken from the same source.

Table 3.4 IAA concentration contained in immersion solutions expressed per 10^7 cfu for each experiment

Immersion solutions	IAA concentration (µg/10^7 cfu)			
	Exp 1	Exp 2	Exp 3	Exp 4
Sp245 grown without tryptophan	0.05 ± 0.01[b]	0.03 ± 0.01[b]		0.02 ± 0.00[b]
Sp245 grown with tryptophan	5.34 ± 0.48[a]	3.13 ± 0.36[a]	0.21	2.5 ± 0.34[a]
Peat cultures of Sp245	*	3.04 ± 2.45[a]		
Commercial biofertilizer	0.09 ± 0.05[b]	0.04 ± 0.00[b]		
Sp245 with tryptophan in $MgSO_4$			#	

The values are means of three replicates ± standard error. The values in the same column with different lower case letters are significantly different at $p<0.05$. Exp 1: sand-grown cutting, Exp 2: water-grown cutting, Exp 3: Exp 3: washed Sp245 cells and Exp 4: growth and nutrient uptake in potting mix. Diagonal streaked block indicates the solution(s) was not included in the relevant experiment. The lack of standard error in Exp 3 column indicates the measurement was not replicated. * IAA concentration of peat culture can not be expressed per 10^7 cells because the lack of viable cell number. # The IAA determination was measured only in Sp245 culture because starter culture for both Sp245 in culture and Sp245 resuspended in $MgSO_4$ solutions was taken from the same source.

3.3.2 Relationship between initial number of viable bacterial cells and IAA production

There was a positive relationship between initial number of viable cells (Sp245 or N_2-fixing bacteria contained in commercial biofertilizer) and IAA production where 47% and 37%of the variability in IAA production was attributable to number of viable cells in the presence and absence of tryptophan, respectively (Fig. 3.2). This indicated that as number of viable cells increased, the IAA production increased. However, there was no effect of tryptophan on the number of viable cells.

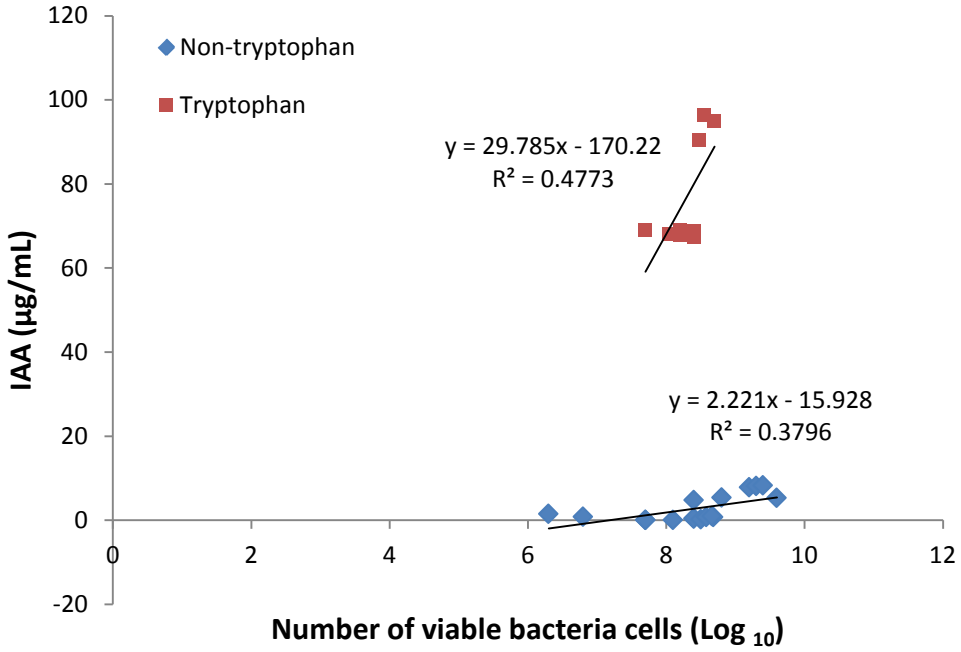

Fig. 3.2 Relationship between initial number of viable cells contained in immersion solutions and IAA production in the presence and absence of tryptophan. The graph shows that IAA production increased in the presence of tryptophan despite the fact that the number of viable cells in both media was within similar range indicating that tryptophan addition did not affect the number of bacterial cells. The data were pooled from all experiments, except experiment 3 where cells of Sp245 were washed and IAA was lower.

3.3.3 Recovery of Sp245 and other N_2-fixing bacteria from *L. stoechas* cuttings after 6 hours treated with different immersion solutions

After 6 hours immersion in various solutions, cuttings were ground in a mortar and pestle and the extracts were then used to inoculate semisolid Nfb media to estimate the number of N_2-fixing bacteria (including Sp245) attached to the cutting surface or absorbed by the cuttings through plant tissues. Nfb medium was used to distinguish N_2-fixing bacteria from resident

bacteria present on the cuttings . N_2-fixing bacteria were detected by formation of rising pellicles in semisolid Nfb.

The numbers of viable cells recovered from cuttings are presented in Table 3.5. In general, there were no viable Sp245 detected on cuttings that had been treated with bacteria-free immersion solutions (water, 0.003% IAA, culture supernatants with the exception of experiment 1 and commercial rooting hormone) in each experiment.

In experiment 1, the highest average recovery of cells from cuttings was observed after treatment with commercial biofertilizer with $8.45x10^6$ MPN/g cutting fresh weight, followed by the Sp245 culture with tryptophan treatment with $1.47x10^6$ MPN/g cutting fresh weight (Table 3.5). Viable Sp245 cells were also recovered from Sp245 supernatant with and without tryptophan treatments although they had the lowest cell numbers, $5.09x10^3$ and $2.16x10^3$ MPN/g cutting fresh weight respectively. The presence of Sp245 in cuttings treated with supernatant solutions was due to cells remaining suspended after centrifugation. Supernatants were passed through a 0.22 µm filter to remove cells in subsequent experiments.

In experiment 2, the number of cells recovered from cuttings ranged between $7.1x10^3$ to $2.5x10^4$ MPN/g cutting fresh weight (Table 3.5). The cell numbers of bacteria recovered from cuttings did not differ significantly (P=0.111) between any of the bacterial treatments after 6 hours immersion. As expected, Sp245 cells were not detected in cuttings immersed in filter sterilized supernatants of Sp245 (with or without tryptophan).

Table 3.5 The number of recovered Sp245 or N2-fixing bacteria viable cells from cuttings using MPN in Nfb media after 6 hours immersion in various solutions

Treatment	Viable cells recovery (MPN/g fresh weight of cuttings)			
	Exp 1	Exp 2	Exp 3	Exp 4
Water	Not detected	Not detected	Not detected	Not detected
0.003% IAA	Not detected	Not detected		
Commercial rooting hormone	Not detected	Not detected	Not detected	Not detected
Supernatant of Sp245 grown without tryptophan	$5.09x10^3$ $(3.71 \pm 1.24)^{bc}$	Not detected		Not detected
Supernatant of Sp245 grown with tryptophan	$2.16x10^3$ $(3.34 \pm 0.13)^{cd}$	Not detected		Not detected
Culture of Sp245 grown without tryptophan	$1.21x10^5$ $(5.08 \pm 0.12)^{ab}$	$6.9x10^3$ $(3.84 \pm 0.46)^{a}$		1.2×10^7 $(7.08 \pm 0.35)^{a}$
Culture of Sp245 grown with tryptophan	$1.47x10^6$ $(6.17 \pm 0.08)^{ab}$	$4.7x10^3$ $(3.67 \pm 0.37)^{a}$	1.2×10^5 $(5.11 \pm 0.34)^{a}$	5.4×10^7 $(7.73 \pm 0.30)^{a}$
Peat culture of Sp245	Not detected	$7.1x10^3$ $(3.85 \pm 1.43)^{a}$		
Commercial biofertilizer	$8.45x10^6$ $(6.93 \pm 0.47)^{a}$	$2.5x10^4$ $(4.40 \pm 0.13)^{a}$		
Sp245 with tryptophan resuspended in $MgSO_4$			1.87×10^5 $(5.27 \pm 0.26)^{a}$	

The values are means of three replicates. Values in brackets are $\log_{10}$ transformations ± standard errors. The values in the same column with different lower case letters are significantly different at $p<0.05$. Exp 1: sand-grown cutting, Exp 2: water-grown cutting, Exp 3: washed cells of Sp245 and Exp 4: growth and nutrient uptake in potting mix. Diagonal streaked block indicates the treatment(s) was not included in the relevant experiment. Not detected means there were no bacterial growth observed in any dilution tubes of Nfb media. MPN= most probable number.

3.3.4 Adventitious root growth responses of *L. stoechas* cuttings to immersion of various solutions in sand (Experiment 1)

The cuttings were harvested to evaluate root growth responses to various immersion solutions after 30 days growth in propagating sand. The adventitious root growth parameters included the percentage of cuttings that developed roots (root formation percentage), number of main roots per cutting and root length. Measurements were done using ten cuttings per treatment.

The effect of the various immersion solutions on root formation of *L. stoechas* cuttings in sand media are presented in Fig 3.3. In general, there was a trend observed in adventitious root growth of *L. stoechas* cuttings grown in sand. Cuttings treated with commercial rooting hormone or Sp245 culture grown with tryptophan showed significantly better responses in the root growth parameters over other treatments. Although adventitious roots on cuttings dipped in commercial rooting hormone exhibited higher number of main roots and longer total root length than those in Sp245 culture with tryptophan, the differences were not significant at $P<0.05$. Comparison of treatments containing Sp245 indicated that the presence of both tryptophan and cells resulted in better root growth responses in *L. stoechas* cuttings.

Root formation ranged from 30% to 100%. Cuttings treated with commercial rooting hormone (0.4% IBA) and Sp245 culture with tryptophan resulted in 100% formation (Fig 3.3A). The next most successful root formation was observed in treatment 0.003% IAA, followed by supernatant of Sp245 with tryptophan, water, and commercial biofertilizer. The lowest rooting percentage was observed in Sp245 formulations without tryptophan (culture, supernatant and peat).

The number of main roots ranged between 0.6 to 14 roots/cutting (Fig 3.3B). Similar to root formation percentage, commercial rooting hormone and Sp245 grown with tryptophan stimulated a significantly higher number of main roots compared to Sp245 without tryptophan ($P<0.05$). Although water treatment induced a higher percentage root formation than Sp245 formulations without tryptophan, the number of main roots between those treatments did not vary significantly (<0.05).

L. stoechas cuttings treated with commercial rooting hormone exhibited the highest total root length per cutting with an average of 105.5 cm, followed by cuttings treated with Sp245 culture with tryptophan with 77.7 cm (Fig 3.3C), but the difference between those two treatments was not significant (P=0.88). There was no significant difference in total root length observed in Sp245 formulations without tryptophan (culture, supernatant and peat), water, 0.003% IAA and commercial biofertilizer, even though the three latter treatments had higher percentage of root formation and number of main roots.

When only cuttings replicates that had formed root were taken into account for total root length measurements, the average total length values of all treatments increase except for commercial plant rooting hormone and culture of Sp245 with tryptophan as those treatments stimulated root growth in all replicates. Root length of cuttings treated with water, supernatant (with and without tryptophan) and biofertilizer was higher than that of peat culture treated cuttings. The average root length of cuttings treated with Sp245 supernatant with tryptophan was similar to that of Sp245 and commercial plant rooting hormone indicating that while the supernatant did not consistently stimulate root formation, development of roots once formed was similar for all three treatments.

Generally, all treatments resulted in similar visible appearances in adventitious roots. Differences in the abundance of *L. stoechas* adventitious roots in response to immersion solutions are illustrated in Fig 3.4. Cuttings treated with commercial plant rooting hormone and culture of Sp245 grown with tryptophan developed the most roots of all treatments, followed by supernatant of Sp245 with tryptophan and commercial biofertilizer. The lowest root mass was observed in cuttings treated with culture of Sp245 without tryptophan and peat culture of Sp245 at 30 days after planting.

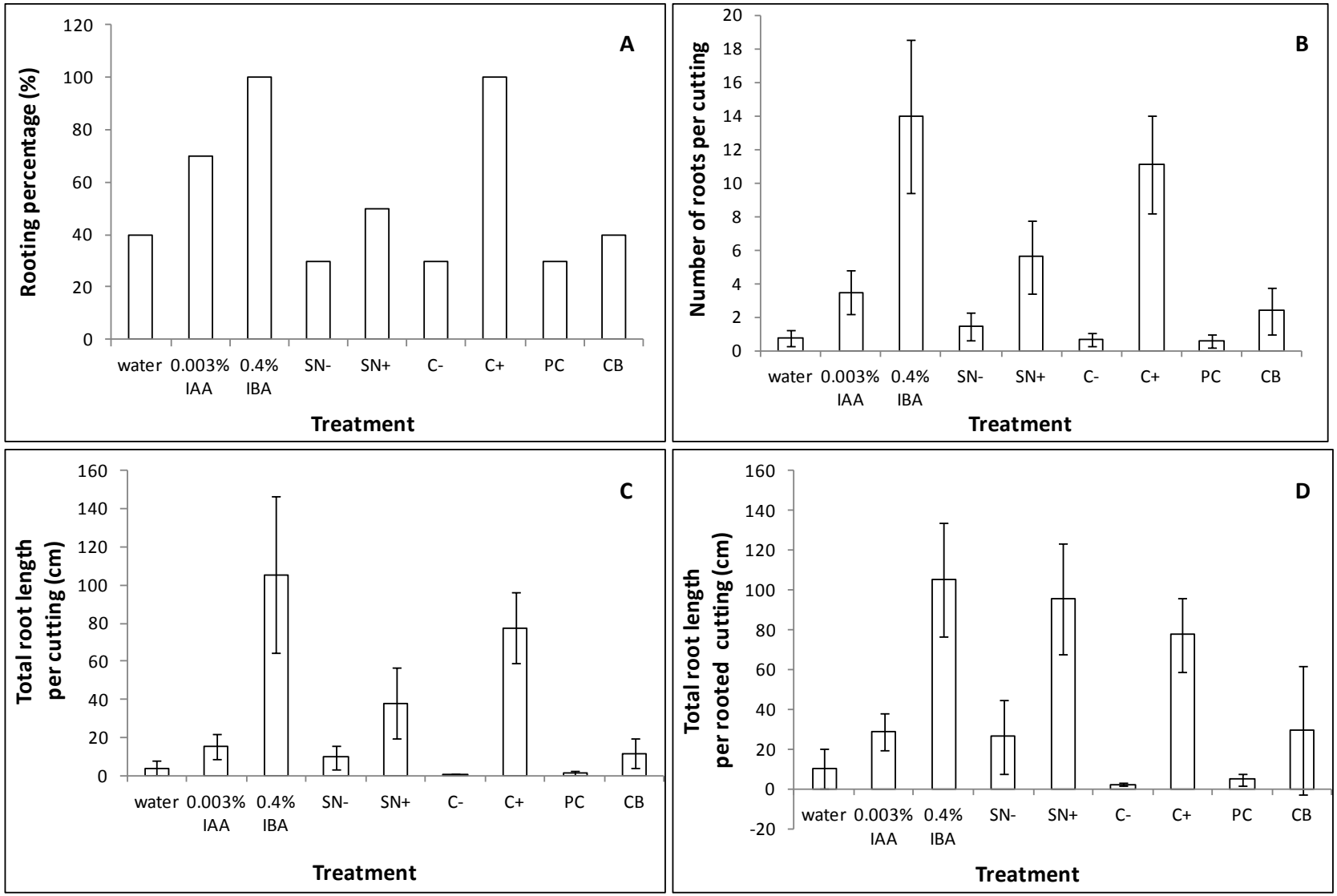

Fig. 3.3 The effect of various immersion solutions cutting of sand-grown *L. stoechas* cuttings 30 days after planting. A: Rooting percentage, B: Number of main root, C: Total root length, and D: Total root length per rooted cutting. The values are means of 10 replicates, except graph A which is total percentage of cutting that formed roots/10 cuttings. Error bars represent standard error of the mean. The lack of error bar in graph A because the values were not means, but total percentage. SN: supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertilizer. With/without tryptophan (-/+).

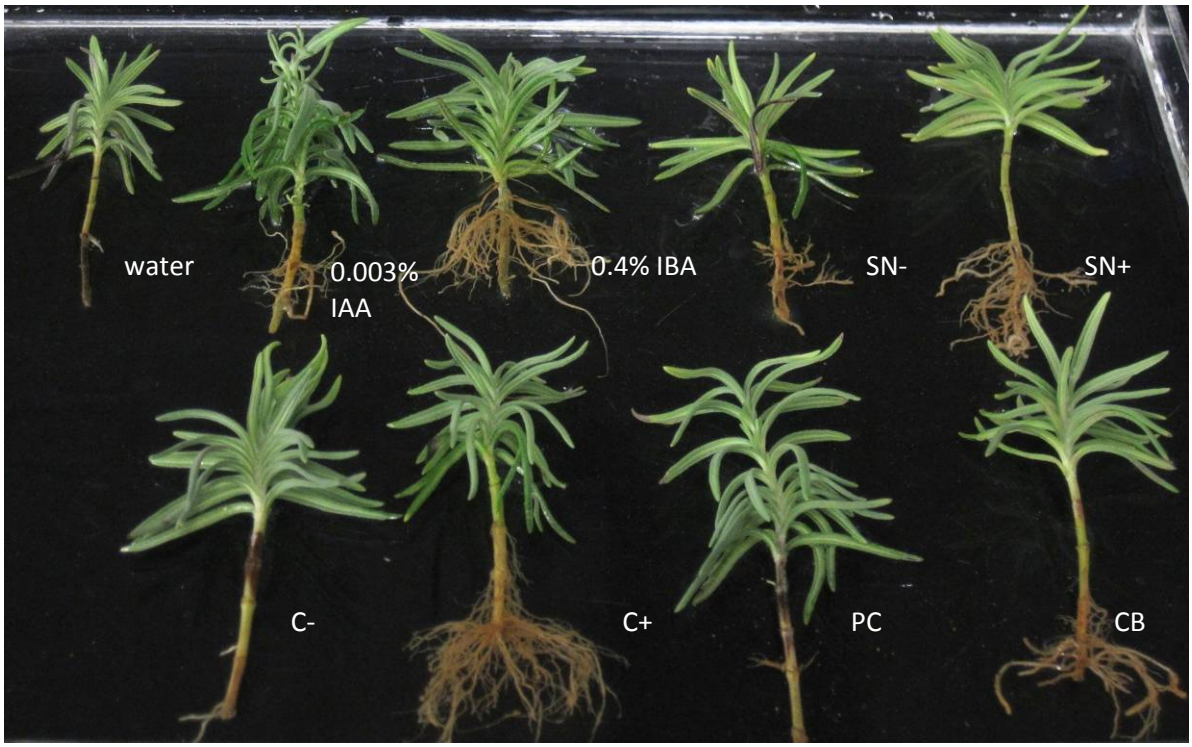

Fig. 3.4 The difference of root abundance and appearance of *L. stoechas* cuttings at 30 days after immersed with various solutions. SN: supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertilizer. Notion -/+ means without/with tryptophan.

3.3.5 Root growth responses of *L. stoechas* cuttings to various immersion solutions in water medium (Experiment 2)

In order to evaluate the responses of *L. stoechas* to various immersion solutions on adventitious root development, the following plant experiment was carried out in a water growth medium. By using this medium, the number of main roots was able to be observed over time without removing the cutting from the medium. Cuttings were treated with nine solutions listed in Table 3.1. Adventitious root development measurements included root formation percentage and numbers of main roots each week for 30 days and total root length at 30 days after planting using WinRhizo. There were 30 replicate cuttings per treatment and tubes for each treatment were placed in separate racks to reduce contamination. Racks were regularly rotated in the plant growth cabinet,

There was a variation in the number of main roots per cutting observed over 30 days. One week after immersion, only a few roots were detected in the cuttings treated with supernatant and culture of Sp245 with tryptophan with an average of 0.57 and 0.63 roots per cutting, respectively (Fig. 3.5). Cuttings treated with other treatments did not produce any adventitious root after one week. In the second week, *L. stoechas* cuttings treated with commercial rooting hormone and Sp245 culture without tryptophan started to develop roots. In the final week of growth (harvesting time), adventitious roots were found in all treatments except in cuttings treated with peat culture of Sp245. The highest number of adventitious roots was recorded on cuttings treated with commercial rooting hormone, producing 100 times more adventitious roots than those resulted from water treated cuttings. There was a significant effect of various immersion solutions in number of main roots at $P<0.05$.

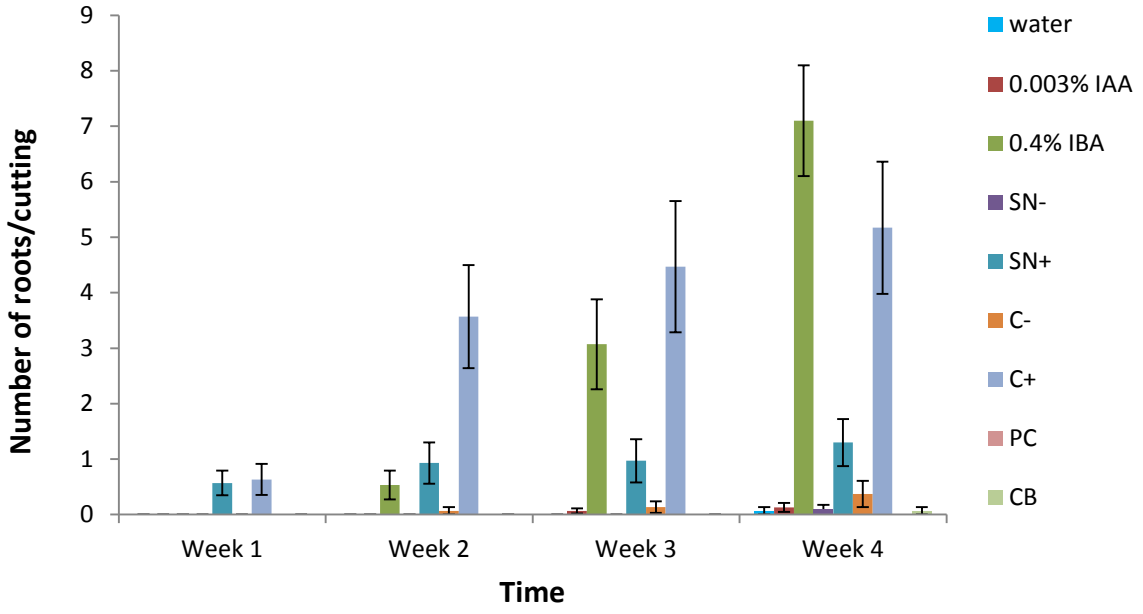

Fig. 3.5 The effects of various immersion solutions on number of the main roots of *L. stoechas* cuttings. Data are presented as number of main roots per cutting. Each treatment included 30 cuttings. SN: supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertilizer. Notion -/+ means without/with tryptophan.

The results in this experiment were similar to the result from experiment 1 where cuttings were grown in sand. Cuttings treated with commercial rooting hormone and Sp245 culture with tryptophan exhibited significant better root growth responses than other treatments. The effect of bacterial immersion solution on root growth of *L. stoechas* was more apparent compared to non-bacterial treatment, with the exception of Sp245 supernatant without

tryptophan. However, cuttings treated with peat culture of Sp245 did not grow any adventitious root.

Commercial rooting hormone and culture of Sp245 with tryptophan stimulated the highest percentage root formation with and total number of roots compared with other immersion solutions (Fig 3.6A and B). Cuttings treated with supernatant of Sp245 with tryptophan and Sp245 culture without tryptophan had a higher percentage of root development than cuttings treated in water and commercial biofertilizer treatment.

Culture of Sp245 grown with tryptophan significantly increased total length of adventitious roots per cutting in water medium (Fig 3.6C and D). After 30 days, the longest roots were observed in *L. stoechas* cuttings treated with Sp245 culture with tryptophan with 26.1 cm which was an average of 10 cm longer than commercial rooting hormone (16.6 cm) and three times longer than supernatant of Sp245 with tryptophan (8.23). Roots lengths were slightly higher when calculated per rooted cutting, however, the same general relationship between treatments was observed. There was a significant difference in total root length of cuttings treated with commercial rooting hormone and Sp245 culture with tryptophan compared to other treatments ($P<0.05$). The significant difference in total root length between cuttings treated with Sp245 culture grown with tryptophan and supernatant ($P<0.05$) indicates the important role of Sp245 cells in stimulating root growth than IAA alone.

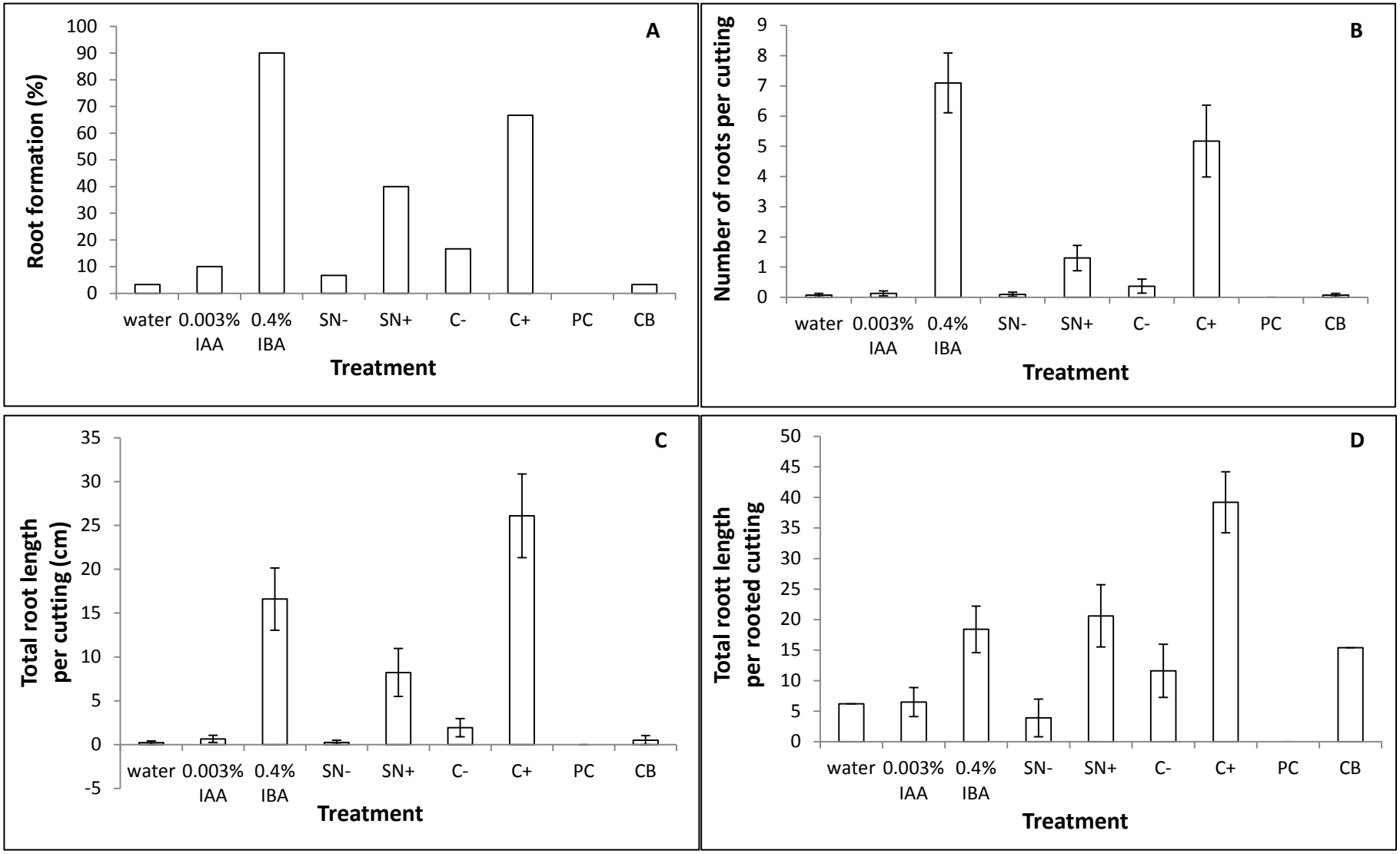

Fig. 3.6 The effect of various immersion solutions cutting of water-grown *L. stoechas* cuttings 30 days after planting. A: Root formation, B: Number of main root, C: Total root length, and D: Total root length per rooted cutting. The values are means of 10 replicates, except graph A which is total percentage of cutting that formed roots/10 cuttings. Error bars represent standard error of the mean. The lack of error bar in graph A because the values were not means, but total percentage. Column without error bars in graph D indicates number of rooted cutting was <2. SN: Supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertilizer. With/without tryptophan (-/+).

The most pronounced visible effect on adventitious root development in this experiment was observed after comparison of cuttings treated with Sp245 cultures grown with tryptophan and commercial rooting hormone (Fig. 3.7). Commercial rooting hormone treated cuttings produced more roots than the cuttings treated with Sp245 culture. However, roots developed from Sp245 culture with tryptophan were longer and more branched than roots from commercial rooting hormone treatment.

The difference in appearance of adventitious roots of *L. stoechas* between the different immersion solutions treatment were likely more pronounced due to difference in the levels of auxin contained in the immersion solutions. High levels of auxin, such as those in commercial rooting hormone (0.4% IBA), promoted more roots while lower auxin concentrations resulted in fewer but longer adventitious roots. In addition, hormone levels would be further diluted in the water-based plant-growth system. There was no difference in the shoots of cuttings during the 30 day growth incubation.

Fig. 3.7 The differences of adventitious root morphology and appearance of *L. stoechas* cuttings at 30 days were most obvious when observed between commercial rooting hormone and Sp245 culture with tryptophan treatments. Commercial rooting hormone treatment stimulated high number of but short main roots whereas Sp245 culture with tryptophan cuttings developed less number of but long and more branched roots.

3.3.6 Relationship between IAA concentration in immersion solutions and root growth parameters in different media

There was a positive relationship between IAA concentration in immersion solution and root growth parameters of *L. stoechas* cuttings (Figs 3.8, 3.9 and 3.10). In general, root development was better when cuttings were grown in sand than in water. Growth parameters increased with increased IAA concentration in both growth media. In sand medium, IAA concentration could explain 62% of the variation in root formation, 81% of the variation in the number of main roots and 79% of variation in root length. In comparison, IAA concentration accounted for 79% of variation in root formation, 58% of variation in number of roots and 64% of root length in water. This indicates that treatment with IAA consistently affects root formation and subsequent growth of roots (number and length) is affected by plant growth medium. Propagation of cuttings in water may reduce root growth responses to IAA compared with sand because of dilution.

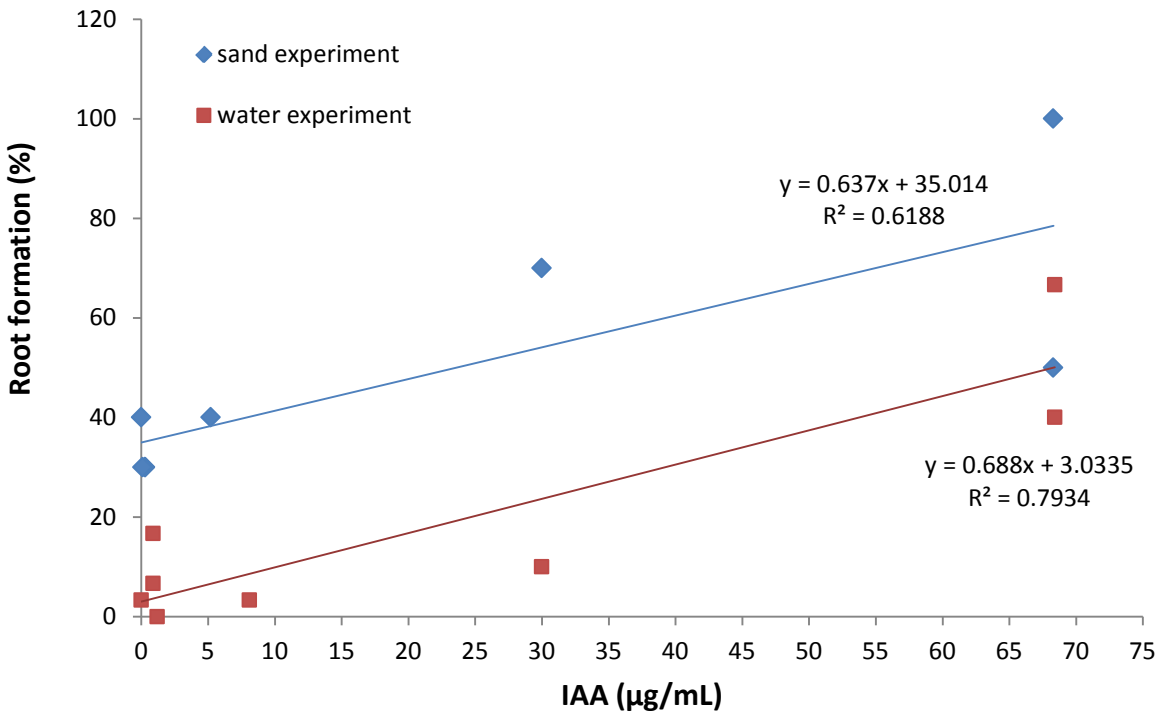

Fig. 3.8 The relationship of IAA concentration contained in immersion solutions and root formation of *L. stoechas* cuttings in different growth media. IBA treatment was not included because the high difference between IBA and other IAA concentrations.

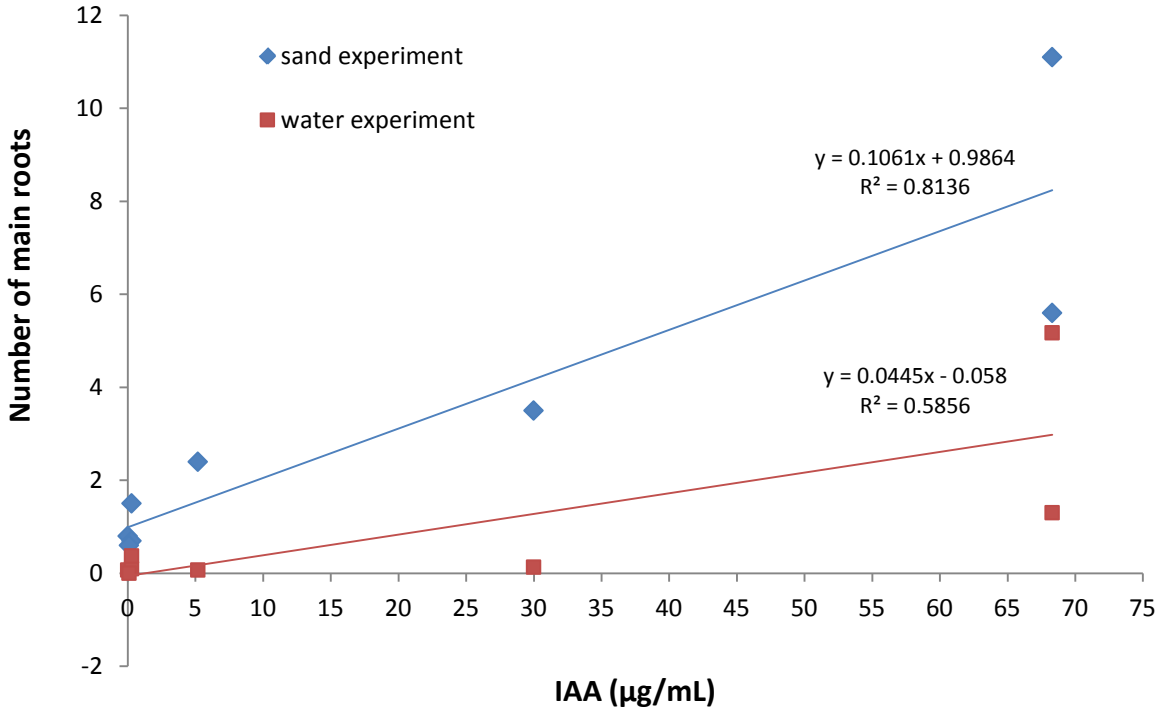

Fig. 3.9 The relationship of IAA concentration contained in immersion solutions and number of main roots of *L. stoechas* cuttings in different growth media. IBA treatment was not included because the high difference between IBA and other IAA concentrations.

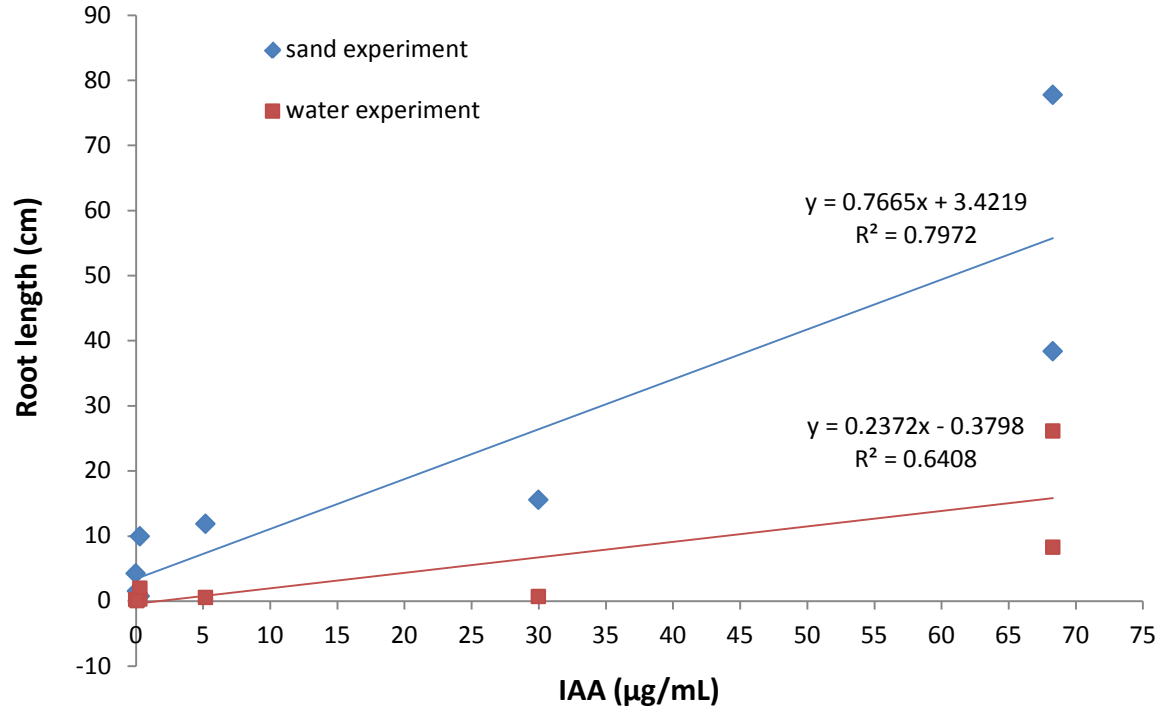

Fig. 3.10 The relationship of IAA concentration contained in immersion solutions and root length of *L. stoechas* cuttings in different growth media. IBA treatment was not included because the high difference between IBA and other IAA concentrations.

3.3.7 Relationship between number of recovered cells from cuttings and root growth responses of L. stoechas cuttings in different media

The relationship between number of viable cells recovered from cuttings after 6 hours immersion and root growth parameters are presented in Figs 3.11, 3.12 and 3.12. Generally, root responses in sand-grown cutting was more related to cell recovery number than water-grown cutting. However the relationship was weak indicated with low values of R^2. Recovered cells accounted for only 19, 17 and 15% of the variability in root formation, number of main roots and total root length of *L. stoechas* cuttings, respectively. These results indicate that the extent of initial colonisation of cuttings had little effect on subsequent root growth responses. These analyses include cells grown with and without tryptophan and as IAA production has a strong effect on root growth, the relationship between colonisation and root growth responses may be confounded by low IAA production in some treatments.

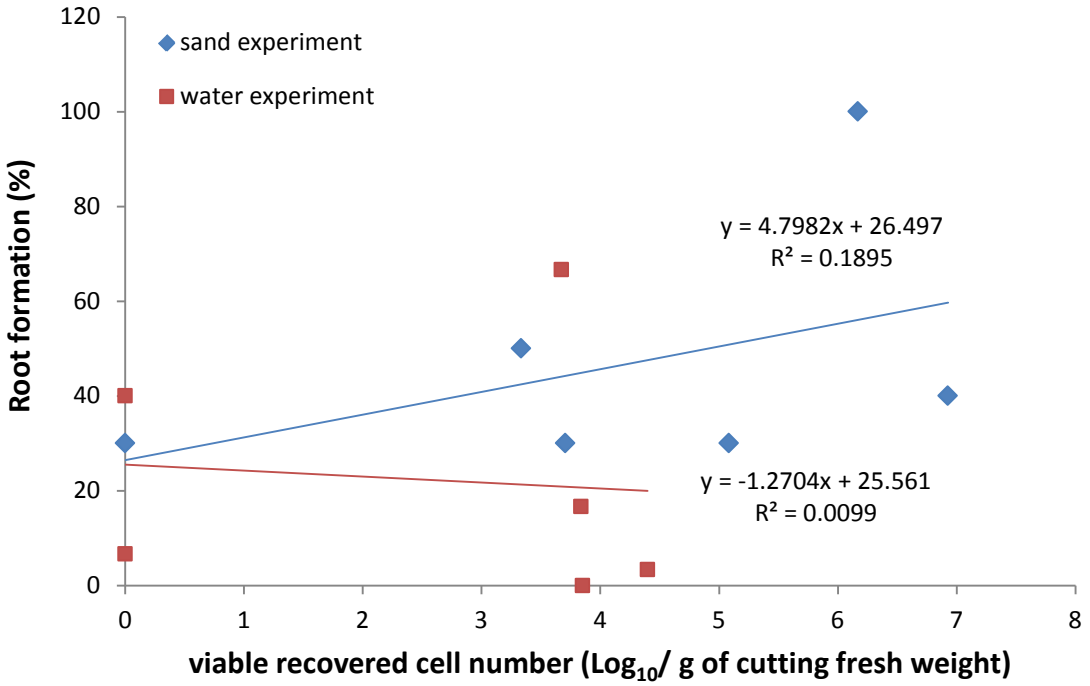

Fig. 3.11 The relationship of number of viable cells (Sp245 or N_2-fixing bacteria) recovered from cuttings and root formation in different media. The data were pooled from bacterial immersion treatments including supernatant of Sp245 with or without tryptophan

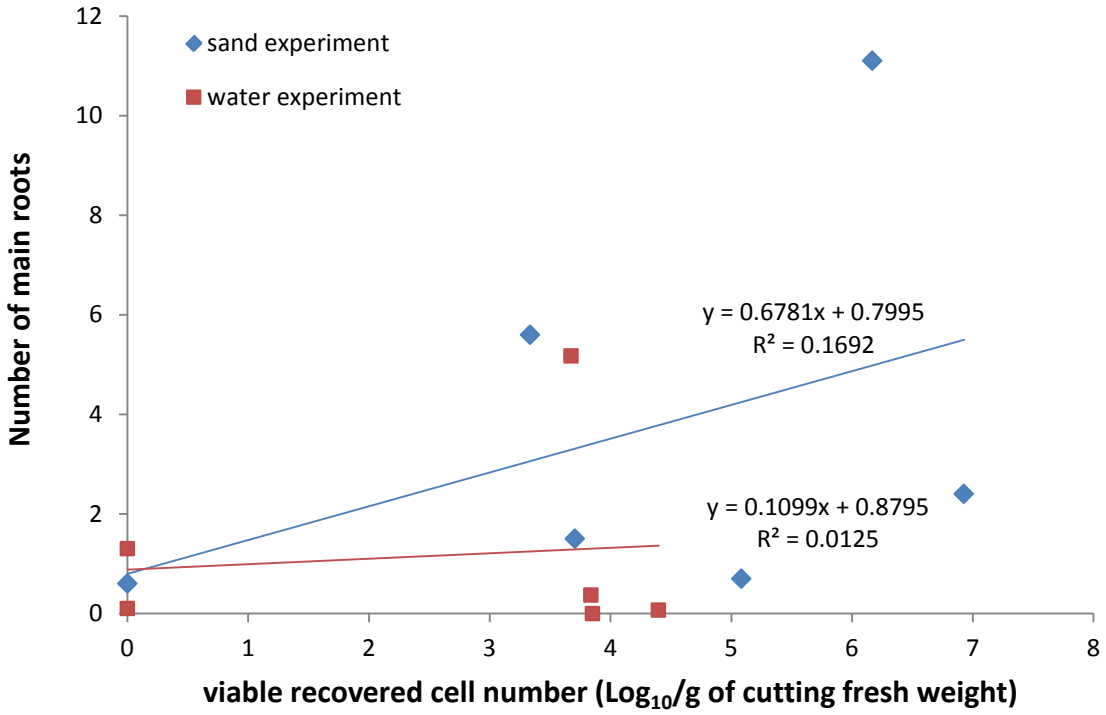

Fig. 3.12 The relationship of number of viable cells (Sp245 or N_2-fixing bacteria) recovered from cuttings and number of main roots in different media. The data were pooled from bacterial immersion treatments including supernatant of Sp245 with or without tryptophan

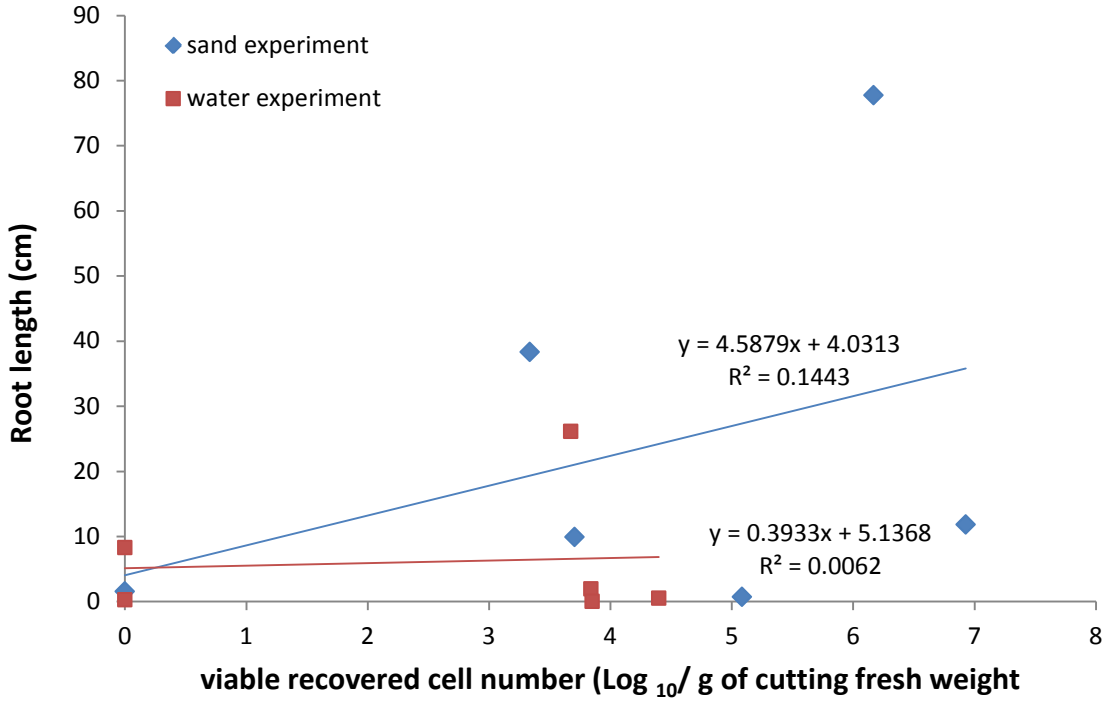

Fig. 3.13 The relationship of number of viable cells (Sp245 or N_2-fixing bacteria) recovered from cuttings and root length in different media. The data were pooled from bacterial immersion treatments including supernatant of Sp245 with or without tryptophan

3.3.8 The effects of Sp245 cells on root growth parameters of *L. stoechas* cuttings (Experiment 3)

In previous experiments, treatment of cuttings with Sp245 cells grown with tryptophan stimulated root development to commercial rooting hormone. It was also established that the presence of viable cells as well as IAA was important to promote maximum adventitious root growth of *L. stoechas* cuttings. Therefore, to further investigate the effect of Sp245 cells in *L. stoechas* propagation, cuttings were treated with a solution prepared by centrifuging cultures of Sp245 grown with tryptophan, washing and resuspending in MgSO4. After 30 days of growth, cuttings were gently removed from sand and adventitious root growth including root formation percentage, no of main roots and root length were measured.

In all the immersion solutions used, only cuttings treated with commercial rooting hormone formed adventitious roots after 30 days of growth (Fig 3.14).

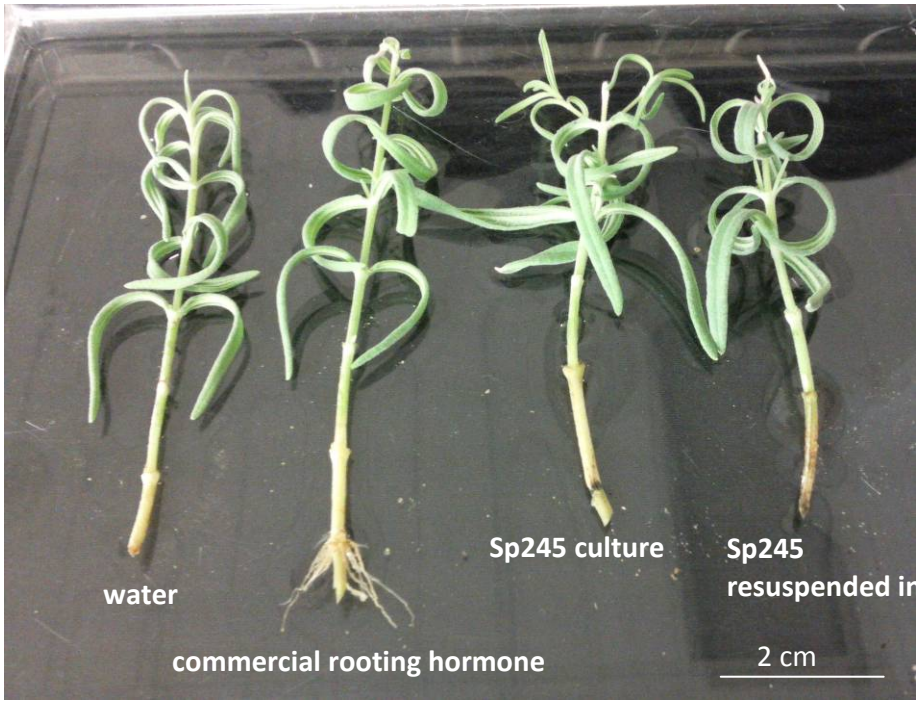

Fig. 3.14 *L. stoechas* cuttings after 30 days of growth. Only commercial rooting hormone treated cuttings formed adventitious roots, while the other treatments did not grow any roots. Cuttings treated with DF medium are not shown because none of the replicates survived.

Although cuttings immersed in water and $MgSO_4$ did not grow any adventitious roots, the shoots showed a similar appearance to shoots of commercial rooting hormone-treated cuttings. All cuttings treated with DF medium were colonized by fungi as early as first week of

growth (7 days after inoculation) and did not survive. Similar colonization by fungi conditions were also observed in some cuttings immersed in the Sp245 treatment at the third week of growth. The lack of adventitious root from cuttings treated with Sp245 culture or resuspended in $MgSO_4$ may have been because the very low IAA concentration in cultures produced for this experiment. Despite the lack of response in this experiment, the data are presented to highlight the importance of IAA production in cultures and that cell number and colonisation by IAA producing strains are not in themselves adequate to produce a plant growth promoting effect.

Adventitious root growth parameters measured in the commercial rooting hormone-treated cuttings were 49% lower (average of 7.1) than sand-grown cuttings that received the same treatment in the previous experiment. The total length of cuttings was an average of 6.37 cm, 93% shorter than root length developed after the same treatment in cuttings previously grown in sand (see Fig 3.3). This variation in plant-growth response may have been due to several factors including changes to growth conditions (e.g., temperature, light or a different source of sand) as well as genetic variation in the plant material from which cuttings were obtained.

3.3.9 The effects of various immersion solutions on the N status of *L. stoechas* shoots

The final experiment was conducted to determine the growth and N uptake of *L. stoechas* shoots after transfer of treated cuttings from sand to potting mix. Cuttings were treated with a selection the previously described immersion solutions including were water, commercial rooting hormone, supernatant of Sp245 with and without tryptophan and culture of Sp245 with and without tryptophan. Peat culture of Sp245 and commercial biofertilizer were not included in this experiment as their effects on root development were less significant than broth cultures of Sp245 grown with tryptophan. Sand-grown cuttings were transferred to potting mix after 30 days to investigate the survival of cuttings that had developed roots and N uptake. The cuttings were harvested after 30 days of growth in potting mix, the shoots were dried and ground for determination of the N content.

Fig 3.15 shows differences in the root abundance and appearance of the adventitious roots due to treatments before transfer to potting mix. Similar root morphology previous sand-grown cutting was observed (see Fig 3.4). Commercial rooting hormone-treated cuttings had

the most abundant roots, followed by supernatant and culture of Sp245 grown with tryptophan. The lowest root growth resulted from the water treatment.

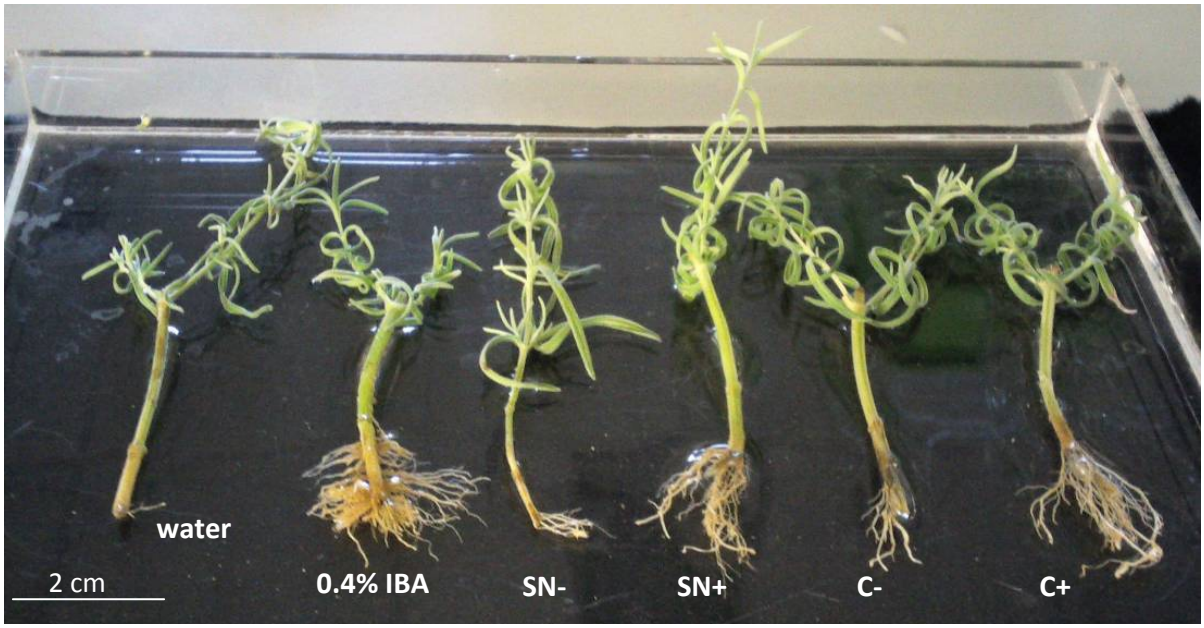

Fig. 3.15 Adventitious root growth morphology of 30 day old sand grown *L. stoechas* cuttings before transfer to potting mix medium. SN: Supernatant of Sp245, C: culture of Sp245. With/without tryptophan (-/+).

After 30 days further growth in potting mix, the shoots of water treated cuttings were dry whereas cuttings from other treatments displayed better vigour and survival after 30 days of growth in potting mix (Fig 3.16). Cuttings treated with commercial rooting hormone had the healthiest overall appearance. Cuttings treated with Sp245 cultures and supernatant containing tryptophan was similar in appearance and cuttings treated with supernatant from cultures without tryptophan were the least healthy.

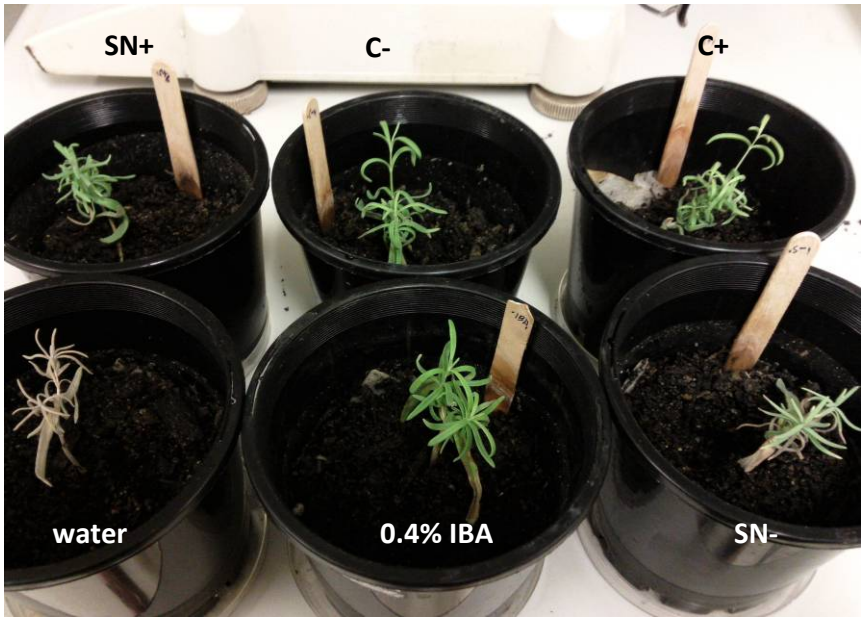

Fig. 3.16 *L.stoechas* cuttings at 30 days after transfer to potting mix medium. SN: Supernatant of Sp245, C: culture of Sp245. With/without tryptophan (-/+).

Of all the treatments, cuttings dipped in commercial rooting hormone had the highest N, however, there was no significant effect due to the of various immersion solutions on the N content of the shoot of *L stoechas* cuttings (P=0.924). The quantity of dried shoot material was too low to obtain detectable N values (Table 3.14).

Table 3.6 Dry weight and N content of shoot tissue of *L.stoechas* cutting

Treatments	Dry weight (g)	N content in shoot tissue of cutting (%)
water	0.06 ± 0.01	0.19 ± 0.05
0.4% IBA	0.11 ± 0.01	0.28 ± 0.002
SN-	0.06 ± 0.01	0.020 ± 0.19
SN+	0.07 ± 0.02	Below limit of detection
C-	0.06 ± 0.02	Below limit of detection
C+	0.06 ± 0.00	0.01 ± 0.2

The values are means of five replicates ± standard errors for dry weight and three replicates for N content measurement. Due to the low quantity of dried root, all five replicates were pooled and divided to three samples for subsequent N content measurement. The differences on dry weight and N content of shoot tissue were not significant at P<0.05. SN: Supernatant of Sp245, C: culture of Sp245. With/without tryptophan (-/+).

3.4 Discussion

3.4.1 Immersion solution properties and viable bacterial recovered from cuttings

As was observed in the initial experiments reported in Chapter 2, there was no significant effect of the presence of tryptophan on the number of viable Sp245 cells contained in the immersion solutions applied to *L. stoechas* cuttings in different growth media. Tryptophan addition to Sp245 culture significantly increased IAA production up to more than 100 times compared to Sp245 grown without tryptophan. Although N_2-fixing bacteria contained in the commercial biofertilizer (Twin N) produced more IAA than Sp245 grown without tryptophan, the production was still far lower than Sp245 cells grown with tryptophan. There was an indication of positive relationship between number of initial cells (Sp245 or N_2-fixing bacteria) and the production of IAA. However, the correlation was less than 50%. Increased number of viable cells will likely to stimulate higher the IAA production both in the presence and absence of tryptophan. A study on the relationship between Sp245 cell number and IAA production also showed that increasing cell numbers resulted in higher levels of IAA production without tryptophan addition (Dobbelaere et al., 1999).

Similar results were obtained in the MPN analysis of bacteria recovered from cuttings grown in different media. Bacterial cells were only recovered from cuttings treated with immersion solutions that contained bacterial cells. The only exception was observed in experiment 1 where low numbers of viable cells were recovered from the unfiltered supernatant of Sp245 cultures. This may have been due to incomplete cell pelleting during centrifugation of the culture and therefore some cells were still present in the supernatant. Additional filtering of Sp245 supernatants proved to be effective in removing Sp245 cells as there was no Sp245 cells recovered from cuttings in experiment 2. Inclusion of the Sp245 supernatant treatment was intended to differentiate the plant growth promoting effect of cells and cells extract. Furthermore, the presence and absence of tryptophan treatments was included to investigate the importance of bacterially produced IAA in the immersion solution or whether root growth can be promoted by IAA produced by the PGPR *in situ*. These results indicate that it is more important to have both IAA and IAA-producing cells in the immersion solution than to rely on IAA production by cells colonising the plant tissue.

3.4.2 Effects of *A. brasilense* 245 on adventitious root stimulation of *L. stoechas* cuttings.

Both active cultures and cell-free extracts of Sp245 grown with tryptophan consistently increased the percentage of root formation of *L. stoechas* cuttings grown in sand and water when compared with the water control. This indicates that root development was highly dependent on the level of IAA in the treatment solution. However, the effect was not significant when compared to the commercial rooting hormone 0.4% IBA. Bona et al. (2010) reported that *Lavandula* cuttings have poor rooting capability, but high levels of IBA will increase the rate of French lavender (*Lavandula dentate*) cuttings to stimulate adventitious root growth. The authors applied a range of commercial IBA solution concentration (0-0.3%) and found that increased levels of IBA enhanced the success of *Lavandula* rooting. In this study root development was strongly related to IAA concentration.

As well as increasing the percentage of cuttings that developed roots, treatment with the PGPR also significantly stimulated the number and total length of adventitious roots. Although the effects were not significant compared to the IBA treatment, there was a trend in increasing the number and total length of *L. stoechas* adventitious roots in response to the PGPR inoculation over the water control. Treatment of cuttings with commercial rooting hormone stimulated highest number of main roots per cutting than other treatments. The commercial rooting hormone concentration used in this study (0.4% or 4000 ppm IBA) was 58-fold higher and more than 1000-fold higher than the amount of IAA produced by Sp245 grown with and without tryptophan, respectively. The number of main roots is likely to increase when high levels of auxin are applied to *L.stoechas* cuttings. This is supported by the result of a study on promoting effects of *A. brasilense* Cd1843 in carnation rooting (Li et al., 2005). The authors reported an increased number of adventitious roots when cuttings were dipped or immersed in high levels of IBA or IAA.

In contrast, *Lavandula dentata* cuttings treated with IBA levels higher than 0.2% showed a decrease in the number of adventitious roots, indicating a root growth inhibition effect (Bona et al., 2010). This suggests that *Lavandula* species and other ornamental species may differ in their sensitivity to IBA or IAA concentration.

Sp245 grown with tryptophan induced rooting and increased the number of roots per cutting as early as 7 days after planting in water grown cuttings. The Sp245 culture developed more roots than the other treatments throughout the following weeks although cuttings treated with commercial rooting hormone produced the largest number of adventitious roots after four weeks.

Generally, growing cuttings in water after treatment with IAA-producing Sp245 cultures affected root length with longer total roots per cutting than the commercial rooting hormone treatment. Li et al. (2005) reported that carnation cuttings treated with *A. brasilense* Cd1843 stimulated total root length 2.5 times longer than 0.1% commercial IBA treatment after 24 days, even though the synthetic hormone treatment produced 20% more roots than the bacterial inoculation.

While increasing IAA concentration increased root growth parameters in experiments reported here, high concentration of auxin may also inhibit root length. A study on bacterial IAA effects on wheat seed roots by Dobbelaere et al.(1999) observed root length inhibition by high levels of auxin. Wheat seeds treated with increasing levels of synthetic IAA showed strong root length inhibition and the effect was similar to the effect of increasing cfu/mL of *A. brasilense* Sp245.

Although the recovery number of viable bacterial cells number in commercial biofertilizer was higher than in Sp245 cultures, better responses were found after treating cuttings Sp245 culture grown with tryptophan. Clearly, it is not only the number of viable cells applied to plants but the stimulation of IAA by cells that is important for improved adventitious root growth.

3.4.3 The effects of different formulations of Sp245 compared to commercially available inoculant formulation

In both cuttings grown in sand and water, treatment using Sp245 with tryptophan resulted in better root growth responses compared to cell-free extract of the same culture. This indicates that IAA concentration alone was not as effective as a combination of IAA and IAA-producing cells.

The potential of Sp245 cells to promote root development was measured by treating cuttings with cells that had been resuspended in $MgSO_4$, after growth with tryptophan. However, this formulation was ineffective due to the low level of IAA in the culture which 83% lower than the concentration obtained in the previous two experiments. Root development after treatment with commercial rooting hormone (0.4% IBA) in was also reduced in this experiment compared with the previous two experiments. Therefore responses to application of hormone may also have been affected by growth conditions or variation in plant material.

Peat culture of Sp245 was included in the study as it is currently the most common commercially available inoculant formulation. Peat-based formulations are a common technology used in the microbial inoculant industry. Legume seed inoculation with peat culture of rhizobia to stimulate nodulation and thus enhance N input to plants has been extensively exploited and rhizobial inoculums (Wakelin and Ryder, 2004). However, treatment of cuttings with Sp245 grown in peat culture resulted in poor root development. This might be a result of the low levels of IAA contained in the immersion solutions as also observed in commercial biofertilizer TwinN, 0.003% IAA and Sp245 grown without tryptophan that had poor root growth parameters.

3.4.4 Effects of different medium on adventitious root morphology of Sp245 treated cuttings

Cuttings treated with commercial rooting hormone had similar root morphology regardless where they were grown in sand or water. In contrast, the root morphology of *L. stoechas* cuttings treated with IAA-producing Sp245 cultures varied according to plant growth conditions. More abundant adventitious roots were observed in sand-grown cuttings than water-grown cuttings. Water grown cuttings had a lower number of roots but with more branching than sand-grown cuttings. It is possible that these differences were because of differences in colonisation pattern of cuttings in water and sand. In water, colonisation would be restricted to the cutting root surface whereas in sand, Sp245 would colonize both the cutting and surrounding sand. This is supported by the better relationship between IAA production by Sp245 and root growth of *L. stoechas* cuttings grown in sand compared with water

Propagating sand medium was used in this study to imitate real conditions in plant nursery practices because most growers use this medium to grow ornamental cuttings. The major difference in this study was that the sand was autoclaved prior to use in order to remove any microorganisms that may present. The use of a water medium was intended to improve root growth observations during the 30 day growth period and was assumed it would have been representative of what was occurring in to the sand grown cuttings. The use of the water medium enabled adventitious root growth observations without damaging newly grown adventitious roots, however the limitation of using this medium was removal of the rhizosphere.

3.4.5 The effects of Sp245 on N uptake of *L. stoechas* cuttings

When cuttings were grown for 30 days in potting mix, cuttings treated with water were dry to lack of adventitious root formation during propagation in sand and thus limited water uptake. Other treatments, especially commercial rooting hormone, resulted in green shoots and strong vigour.

However, the N content of *L. stoechas* cuttings grown in potting mix was not affected by the different immersion solutions including Sp245. The N content in leaves of all treatments was not significantly different to the control even though there were apparent differences in cutting growth between control and other treatments.

3.5 Conclusion

The effects of Sp245 on adventitious root growth of *L. stoechas* cuttings were more pronounced in water medium when total root length was compared to commercial rooting hormone. However, in general, root development of cuttings treated with Sp245 cultures was not significantly different compared to cuttings treated with commercial rooting hormone. There was a general increase in root formation, number of main roots and total length of adventitious root over the water control in both sand and water medium in response to Sp245 cultures grown with tryptophan treatment. The increase of root growth parameters positively correlated to increasing IAA concentration in immersion solutions. The plant growth media contributed to differences in *L. stoechas* adventitious root morphology particularly when cuttings were treated with Sp245 culture. There was no effect of Sp245 treatment on the N uptake by propagated cuttings. The presence of high levels of IAA,

together with Sp245 cells in the immersion solution is essential to obtain maximum effects of PGPR inoculation on adventitious root growth of *L. stoechas* cuttings. Current commercially available inoculants technology including peat and freeze-dried microorganisms are not likely to be effective as stimulators of root growth of *L. stoechas* cuttings because of low IAA production in these formulations, therefore new formulations of Sp245 need to be developed that stimulate IAA production as well as satisfy other criteria essential for efficacy of microbial inoculants.

CHAPTER 4 GENERAL DISCUSSIONS AND CONCLUSION

This project has experimentally evaluated the effectiveness of PGPR as plant growth regulators in ornamental plant propagation and to determine if PGPR can substitute synthetic root growth hormone applied to ornamental plants in the nursery industry thereby reducing production input costs. The efficacy of different formulations containing PGPR was also investigated.

4.1 Evaluation of the most effective PGPR-plant combination, inoculation method and growth medium

In the preliminary study, any effects of PGPR inoculation in pansy seedlings grown in potting mix were not observable when compared to the control treatment. The inoculated PGPR might have been competed by other microorganisms contained in the potting mix and colonized roots of the pansy before inoculation with the PGPR. In this regard, the use of cuttings as a plant material allowed reduction of contamination as well as complexity of the root systems which was already well-developed in the ornamental seedlings.

In general, the selection of the most compatible PGPR-host plant combination was done to ensure optimum delivery of the growth promoting effects by the PGPR to host plant. Determination of IAA production by the PGPR strains allowed the most effective IAA producer, *A. brasilense* Sp245, to be selected as a potential inoculant. This approach was useful for determinating the best inoculant because most ornamental cutting methods use propagation methods use rooting hormones (e.g., IBA) to induce adventitious root formation and produce improved root systems. Established root systems support young seedlings absorb water and nutrients from growth medium when transfer to a bigger media.

Bacterial IAA production in this project was determined during three days incubation and the maximum production was by Sp245 at day 3 in the presence of tryptophan. At the same day the maximum number of the viable cells was also observed, suggesting the concentration of IAA production, to some extent, has positive correlation to number of viable cells. This was supported by relationship between initial number and IAA concentration linear regression that indicated as number of viable cells (grown with or without tryptophan) increased, the

IAA production increased. Similar results were found in a study on the effect of IAA produced by Sp245 on altering wheat root morphology (Dobbelaere et al., 1999). The authors reported comparable effects between the inoculation of increasing number of Sp245 cells grown without tryptophan and increasing concentration of synthetic IAA were in wheat seeds, indicating that high numbers of viable Sp245 cells will produce high levels of IAA. mathematical model of IAA production by Sp245 based on controlled bioreactor trials has been proposed by Smets et al. (2004) to provide basic knowledge on future studies in elucidating IAA PGPR mechanism. The prototype model demonstrated that there was no effect of pH on the growth or IAA production by Sp245 but what is not clear in this relationship is whether IAA production by Sp245 was affected by the decreasing amount of malate (Sp245 growth substrate) as it is consumed by the Sp245, or the production stopped when tryptophan was depleted during the incubation time. In the experiments described in this project, the only correlation evident was when Sp245 reached maximum IAA production at day 3. The maximum number of the viable cells was also observed at this time suggesting the concentration of IAA production, to some extent, is positively correlated with the number of viable cells. For future studies, the relation of IAA production and the number of viable cells may be more apparent by extending the growth incubation period.

The need to select the most responsive ornamental host plant for PGPR inoculation to optimize beneficial effects of PGPR was based on the different growth requirements of ornamental cuttings to develop adventitious roots. *L. stoechas* cuttings demonstrated the best response to Sp245 inoculation and synthetic IAA but showed no root growth response to control treatments, indicating that the concentration of IAA produced by Sp245 has met the plant requirement to grow roots. Whereas the other ornamentals, *Argyranthemum* sp. seemed to not require an additional stimulator to grow roots and *Osteospermum* sp. showed a poor response to Sp245 inoculation. These results showed there was an indication of plant specificity in PGPR-plant interaction and different sensitivity of ornamental species to IAA. Furthermore, by selecting the best combination of PGPR and plant host, better interaction between both parties to improve plant growth may occur. However, a wider range of different ornamental plant types may be needed to obtain a better evaluation of the specificity of Sp245 promoting effects related to its IAA production.

Inoculation methods by immersing the cuttings in different solutions for six hours proved quite effective in introducing Sp245 to cuttings. This was concluded by the number of viable Sp245 cells recovered from the cuttings after six hours incubation and the effects observed after 30 days growth. The effect of different incubation times on the recovered cell number and root formation in cuttings would be required to investigate the relation between the lengths of incubation and the PGPR effectiveness. Studies have been conducted on the length of inoculations using PGPR in liquid inoculums to inoculate plant cuttings using immersion method and showed improved root growth of the host plant. For example, 30 minutes immersion to inoculate kiwi fruit (Erturk et al., 2010), 45 minutes for mint (Kaymak et al., 2008), 6 hours in kidney bean (Tsavkelova et al., 2007a) and 24 hours (Li et al., 2005) in carnation. Determining a minimum length of inoculation time will potentially save operation time in a commercial propagation industry as dipping cuttings in commercial rooting hormone only needs 2-5 seconds. Alternatively, the inoculation may also be done by pippeting the inoculants onto base of the cutting, however this method may not be effective if using water as a growth medium.

The use of water as a growth medium in PGPR-ornamental plant pair selection method was useful to observe the root stimulating effect of Sp245 in the early development of adventitious roots. The limitation of this method was that water does not contain any nutrients thus additional nutrition is required when cuttings are grown for more than three weeks in water. In addition, as the cuttings grown in water require light to grow, the light source and nutrient presence will attract the growth of plant pathogens (e.g., algae) which may affect or even colonize the newly grown roots of cuttings and may widely spread on the cutting stem. The method may be improved by adding different concentrations of very diluted nutrients and limiting the light exposure to the medium during propagation.

4.2 The effect of Sp245 on adventitious root growth of *L. stoechas* cuttings

Immersion of *L. stoechas* cuttings with Sp245 cultures grown with tryptophan induced adventitious root growth during 30 days of growth. The root growth stimulation effects were better than other treatments and comparable to commercial rooting hormone in water and propagating sand growth media. However, different growth media has induced root morphology variations in cuttings treated with cultures of IAA-producing Sp245. These differences were possibly due restriction of Sp245 colonization in water. When grown in

water medium, Sp245 could only colonize cutting surface while in sand Sp245 would colonize both cuttings and surrounding sand, therefore Sp245 might have had more opportunities to survive, proliferate and produce IAA. If this was the case, then it can be expected that IAA production in sand medium was higher than in water medium. Information on the concentration of IAA produced by Sp245 in both media during or at the end of the growth period may be needed to validate this hypothesis. Recommended methods to measure IAA content in sand medium is to dilute the sand in phosphate buffer after which tryptophan solution is added. IAA content in filtered sand solution is determined colorimetrically using Salkowski's reagent and further confirmed by HPLC-UV analysis (Sarwar et al., 1992; Khalid et al., 2004b).

A low IAA concentration or number of Sp245 have been reported to induce longer roots in wheat seeds, while more root numbers were observed in the presence of high IAA concentrations or cell number treatments (Dobbelaere et al., 1999). These contradicting effects have been considered as a result of ethylene production from IAA metabolism. Ethylene can inhibit root length triggered by environmental stress conditions or the presence of high concentrations of IAA. PGPR that produce ACC deaminase have been shown to hydrolyse ACC, the precursor of ethylene, and consequently reduce root inhibition. Further work to investigate threshold concentrations in IAA inhibiting root length and also the use of PGPR strains that are able to produce IAA as well as ACC deaminase may improve root growth stimulation in cutting propagation (Patten and Glick, 2002; Li et al., 2005). The effect of Sp245 on N uptake could not be detected in this experiment. Longer growth period of cuttings in potting mix may result in more detectable effects.

Molecular analysis such as proteomics may be applied to further investigate the mechanism used by Sp245 to promote root growth in *L. stoechas* cuttings. Some preliminary investigations on protein analysis of adventitious roots from Sp245 treatments were attempted in this project, however limited adventitious root material harvested from cuttings were not sufficient optimize protein analysis. The effectiveness of PGP by rhizobacteria will be optimally achieved if mechanisms underlying promotion mechanism of PGPR during plant microbe interactions under the influence of biotic and abiotic factors are properly understood. Thus future research is better aimed to understand these influences to provide more consistent result as a step further towards developing PGPR inoculants.

4.3 Effects of different formulations of Sp245 on root growth stimulation

Formulation of inoculants is crucial to ensure the effectiveness of PGPR, their affordability, and practicality for growers. The significant difference in total root length between cuttings treated with Sp245 culture grown with tryptophan and supernatant indicates the important role of Sp245 cells in stimulating root growth than IAA alone. However, formulation using Sp245 cells washed and resuspended in $MgSO_4$, to further investigate the effect of Sp245 cells in *L. stoechas* propagation, was ineffective to stimulate *L. stoechas* cuttings. In contrast to this result, the same formulation of inoculum using PGPR cells resuspended in $MgSO_4$ buffer to inoculate ornamental carnation cutting was reported to stimulate higher root growth over control treatment and was comparable to commercial rooting hormone treatment(Li et al., 2005). In other report, mung bean cuttings treated with dilution of IAA-deficient PGPR mutant in water contributed in the development of mung bean root systems even after removal of supernatant containing IAA (Patten and Glick, 2002). Sp245 resuspended in $MgSO_4$ formulation may be improved by first determining the minimum number of viable cells that stimulate root growth in cutting propagation. A different composition of medium may be used to grow Sp245 as DF medium is too costly. A range of tryptophan level may also be applied to Sp245 growth medium in order to determine the most efficient concentration in relation to IAA production and subsequent root growth promoting effect. A study reported that tryptophan levels less than 0.1% already showed effectiveness for bacterial IAA production and subsequent plant growth (Patten and Glick, 2002). Reducing tryptophan levels may decrease input costs for mass production.

If Sp245 cells diluted in $MgSO_4$ formulation showed potential by stimulating root growth of cuttings over control treatments and comparable to commercial rooting hormone, further development using freeze-dried Sp245 cells could be applied. Freeze dried cell formulations are more stable and easier to handle than liquid inoculants (Sp245 culture grown with tryptophan). Other studies showed effectiveness of Peat cultures of Sp245 showed moderate of bacterial survival rate, however, the Sp245 cells produced much lower IAA than Sp245 grown with tryptophan in broth cultures and consequently demonstrated less root growth response of cuttings. A better formulation that improves IAA production as well as sustains sufficient viable cells is required to ensure inoculant effectiveness. Further work on peat formulation of Sp245 grown with tryptophan may be needed to be developed because peat formulation can also be an alternative to liquid inoculants.

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Appendix A

Composition of media, buffers and solutions.

Media/buffer/solution	Composition/L
Bacterial cultures	
Glycerol nutrient broth	
Peptone	0.5 g
Yeast extract	1 g
NaCl	0.5 g
Lab lemco powder	1 g
Glycerol	12.5 g
water	100 mL
Nutrient agar	
Dehydrated nutrient broth (Difco ™)	8 g
Agar	15 g
water to	1L
Nutrient broth	
Dehydrated nutrient broth (Difco ™)	8 g
Water to	1 L
Semi solid nitrogen free broth medium (Nfb)	
L-malic acid	
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
$CaCl_2$	0.02 g
Agar	2 g
Fe-EDTA (1.645 solution)	4 mL
Trace elements solution *	2 mL
Bromothymol blue (0.5%, w/v in 0.2.M KOH solution)	2 mL
Vitamin #	1 mL
water to	1 L

(pH was adjusted to 6.8 with KOH, the solution should be green in colour and autoclaved at 121°C for 15 minutes)

*Trace elements stock solution

$Na_2MoO_4.2H_2O$	0.2 g
$MnSO_4.H_2O$	0.235 g
H_3BO_3	0.28 g
$CuSO_4.5H_2O$	0.008 g
$ZnSO_4.7H_2O$	0.024 g
water to	1 L

#Vitamin stock solutions

Biotin	0.01 g
Pyridoxin	0.02 g
water to	20 mL

Diluted from stock, 50 times and added 1 mL/L medium

Dworkin & Foster minimal medium

$(NH_4)_2SO_4$	2 g
KH_2PO_4	4 g
Na_2HPO_4	6 g
$MgSO_4.7H_20$	0.2 g
$FeSO_4.7H_20$ *	0.1 mL
Micro element	0.1 mL

Micro elements :

B (as H_3BO_3)	10 µg
Mn (as MnSO4)	10 µg
Cu (as $CuSO_4$)	50 µg
Zn (as $ZnSO_4$)	70 µg
Mo (as MoO_3)	10 ug
water to	1 L

Each chemical was added one at a time until completely dissolved, then the solution pH was adjusted to 7.2. The solution was then autoclaved at 121°C for 20 minutes. Once cooled, glucose was added aseptically through Milipore filtration (0.2 µ) to a final concentration of 0.5% (w/v)

Stock solution:

$FeSO_4.7H_20$	100 mg
water to	10 mL

Micro element stock:

H_3BO_3	10 mg
$MnSO_4$	10 mg
$ZnSO_4$	70 mg

$CuSO_4$	50 mg
MoO_3	10 mg
water to	100 mL

King's B

Protease peptone	20 g
Glycerol	10 g
K_2HPO_4	1.5 g
$MgSO_4.\ 7H_2O$	1.5 g
Agar	15 g

pH was adjusted to 7.2 after which glycerol and agar were added. Medium was autoclaved at 121°C for 15 minutes

Phenol red carbohydrate fermentation medium

$(NH_4)_2SO_4$	2 g
Yeast extract	0.5 g
Tryptone	1 g
Phenol red	0.18 g
Trace elements	10 mL

The solution was made to 1 L with phosphate buffer and autoclaved at 121°C for 20 minutes allow the solution to room temperature. The carbohydrate stock solution was added aseptically through milipore filtration (0.2 μm) to a final concentration of 0.5%

Carbohydrate stock solution

L-sorbose or D-glucose	100 g
Water to	1 L

Trace elements

$Na_2\ MoO_4.\ H_2O$	0.2 g
$MnSO_4.H_2O$	0.235 g
H_3BO_3	0.28
$CuSO_4.\ 5H_2O$	0.008 g
$ZnSO_4.\ 7H_2O$	0.024 g

Phosphate buffer

K_2HPO_4	1.21 g
KH_2PO_4	0.34 g

IAA determination

Salkowski reagent

water	50 mL
12M H_2SO_4 (sp. gr. 1.84)	25 mL
0.5M FeCl3	5 mL

The acid was slowly added to the water and the solution was let cool in room temperature. $FeCl_3$ was added and mixed well

IAA standard solution (500 µg/mL)

IAA standard	0.05 g
Ethanol : water (50:50)	100 mL

IAA working solution (10 µg/mL)

IAA standard solution	1 mL
water	50 mL

Buffer

Saline solution (0.85%)

NaCl	8.5 g
Water to	1 L

Plant nutrient solution

Hoagland's nutrient solution

1 M $NH_4H_2PO_4$	1 mL
1 M KNO_3	6 mL
1 M $Ca(NO3)_2.4H_2O$	4 mL
1 M $MgSO_4.7H_2O$	2 mL
1 M Fe-EDTA (0.5% aqueos)	2 mL
Micronutrient	1 mL
Water to	1 L

Micronutrient stock	g/L of H_2O
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.22
$CuSO_4.5H_2O$	0.08
$H_2MoO_4.H_2O$	0.02

Appendix B

Molecular reagents

All molecular reagents, enzyme, buffer and primers were purchased from Bioline

MangoTaq mastermix

	Final concentration	25µL reaction
MilliQ water		16.85 µL
5x MangoTaq buffer colored	1x	5 µL
MgCl2 (50 mM)	2 mM	1 µL
dNTPs (10 mM)	0.2mM	0.5 µL
Primer 27F (50 µM)	0.4 µM	0.2 µL
Primer 1492R (50 µM)	0.4 µM	0.2 µL
MangoTaq DNA polimerase	0.5 U	0.25 µL

	Sequence (5'-3')
Primer 27F	AGAGTTTGATCMTGGCTCAG
Primer 1492R	GCYTACCTTGTTACGACTT

Appendix 9

Australian Government
Australian Pesticides and Veterinary Medicines Authority

PERMIT TO ALLOW MINOR USE OF AN AGVET CHEMICAL PRODUCT

FOR THE CONTROL OF SPECIFIED FUNGAL DISEASES IN NURSERY STOCK

PERMIT NUMBER - PER13328

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows any person to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 28 MAY 2012 TO 31 MAY 2015.

Permit Holder:
NURSERY & GARDEN INDUSTRY AUSTRALIA LTD
Level 1,
16-18 Cambridge St
EPPING NSW 2121

Persons who can use the product under this permit:
Persons generally.

CONDITIONS OF USE

Products to be used:
CUNG FU 350 SC FUNGICIDE
PLUS OTHER REGISTERED PRODUCTS
Containing: 350 g/L COPPER PRESENT AS COPPER HYDROXIDE
as their only active constituent.

YATES FUNGUS FIGHTER COPPER FUNGUS SPRAY
PLUS OTHER REGISTERED PRODUCTS
Containing: 375 g/kg COPPER PRESENT AS COPPER HYDROXIDE
as their only active constituent.

BLUE SHIELD DF COPPER FUNGICIDE
PLUS OTHER REGISTERED PRODUCTS
Containing: 500 g/kg COPPER PRESENT AS COPPER HYDROXIDE
as their only active constituent.

Directions for Use:

Crop	Diseases	Product & Rate
Nursery stock (non-food only) - seedlings and plugs, potted trees and shrubs, foliage plants, palms, grasses and fruit trees (non-bearing only).	Fungal leaf spots *Alternaria* spp, *Colletrotrichum* spp. Downy mildew *Peronospora* spp. Myrtle rust *Uredo rangelii*	**350 g/L COPPER HYDROXIDE** 150mL/100L of water **375 g/kg COPPER HYDROXIDE** 140g/100L of water **500 g/kg COPPER HYDROXIDE** 100g/100L of water

Critical Use Comments:

- Begin application at first sign of disease.
- Ensure complete and thorough coverage of foliage and/or crop. Use a minimum spray volume of 250 L/ha using air-blast spray or boomspray.
- DO NOT apply more than 6 applications per crop with a re-treatment interval of 7-14 days between consecutive application.

TO AVOID CROP DAMAGE:
The products included in this permit have NOT been fully evaluated for crop safety on all species or in all situations where treatment may be undertaken. It is therefore recommended to treat a sample area and assess appropriately prior to whole crop treatment to help minimise potential for any phytotoxic damage. This action cannot guarantee crop safety as application, environmental and crop conditions may vary from test treatment to whole of crop treatment. Any instances of phytotoxic damage should be reported to the permit holder or the APVMA.

Therefore crops to be treated under this permit must be fully evaluated by the user for phytotoxicity prior to broad scale application.

Jurisdiction:
ACT, NSW, QLD, SA, TAS, NT & WA
(Note: Victoria is not included in this permit, as their 'control-of-use' legislation means a permit is not required to legalise this off-label use in that state)

Additional Conditions:
This Permit provides for the use of a product in a manner other than specified on the approved label of the product. Unless otherwise stated in this permit, the use of the product must be in accordance with instructions on its label.

Persons who wish to prepare for use and/or use products for the purposes specified in this permit must read, or have read to them, the details and conditions of this permit.

Issued by

Delegated Officer

Appendix 10

BioSecure *HACCP*
Development Progress (July 2012)

John McDonald
Industry Development Manager
NGIQ

The Nursery & Garden Industry Queensland (NGIQ), on behalf of and with the national peak industry body NGIA, have been continuing to progress the development of BioSecure *HACCP* as a third market access instrument. This has been through the ongoing development of governance and administration documents plus the web based Audit Management System (AMS). The concept of BioSecure *HACCP* is closely aligned to the ICA system with growers self certifying via Plant Health Assurance Certificates (PHAC) based on the application of agreed movement control procedures (pest specific) of the importing jurisdiction.

Note: Governments will still provide plant health inspectors issuing Plant Health Certificates and manage the Interstate Certification Assurance (ICA) arrangements allowing industry to self certify under Plant Health Assurance Certificates.

BioSecure *HACCP* has a range of potential benefits for industry and government including:

Government benefits	Industry benefits
Shared responsibility/co-regulation	Shared responsibility/co-regulation
Red tape reduction	Recognition of good horticultural practice
Resource utilisation improvements	Enhanced trading flexibility
Efficiency through electronic system	Improved productivity
Enhanced traceability	Cost reductions
Building a robust biosecurity system	Prompt return to trade after EPP detection

The Domestic Quarantine & Market Access Working Group (DQMAWG) has given an "in principle" support for the concept through a letter dated April 2010 (**see Attachment 1**). A progress report was presented, by Biosecurity Queensland, to the national Plant Health Committee on 7th June 2012 with positive responses from all jurisdictions.

BioSecure *HACCP* is an industry biosecurity systems approach program that requires growers to maintain a high health management system at all times for all pests (not just a specific quarantinable pest) and document all procedures and systems. Auditing of the system on-farm (twice per annum) will be through technical officers (appropriately trained under ICA auditor certification) of peak industry bodies (Qld, NSW, Vic, TAS, SA, WA & NT) through partnerships with state/territory biosecurity agencies.

Overall document management/certification/compliance/etc is managed by industry (NGI's), via the national Audit Management System, and audited on-farm for program compliance. The

BioSecure *HACCP* system validation audits of NGI's (desktop & on-farm audits) are completed by state/territory biosecurity agencies (**see Attachment 2 Flow Charts**).

NGIQ have been liaising extensively with Biosecurity Queensland as the development of the program has progressed including:

- Review of ICA processes and templates
- Consideration of draft BioSecure *HACCP* process development
- ICA data management (Plant Health Inspection System)
- Review of BioSecure *HACCP* system design

BioSecure *HACCP* Project (NGIA/HAL) is progressing with the contracting for key deliverables completed including:

- Development of Governance and Administration Guidelines:
    - Heads of Agreement documents for NGIA – NGI's MOU
    - Heads of Agreement documents for NGI's – Government MOU
    - Terms & Conditions documents for NGI's - Grower
- Increasing the capacity and security of the Audit Management System:
  (see Attachment 3 "Story Boards")

The nursery industry is preparing documents to go to the (DQMAWG) for consideration as a working model to be viewed at the final face to face meeting in September 2012.

BioSecure HACCP components:

With standard high health practices in place and supported by **mandatory** Nursery Industry Accreditation Scheme Australia (NIASA) accreditation BioSecure *HACCP* certified businesses will comply with jurisdictional pest specific entry requirements/entry conditions/movement controls/etc via Entry Condition Compliance Procedures or ECCP's. A demonstration draft is attached as **Attachment 4**). Documentation will be electronic and generated within the AMS with appropriate security and traceability provided. The Plant Health Assurance Certificate (PHAC) is the document of choice for certifying consignments meet entry conditions with consignment details attached and "locked" at the time of saving/printing/emailing (**see Attachment 5**)

BioSecure HACCP certified growers will automatically comply with the following activities as standard business practice and will require documented procedures and records to support compliance. Auditing will verify procedures and records.

Approved Supplier Register	Biological Organism Release Record
Materials Import Inspection Record	Disinfestation/Sanitation Record
Materials Despatch Inspection Record	Site Pathogen Testing Record
Register of Authorised Inspector Record	Soil Pathogen Testing & Disinfestation Record
Register of Authorised Person Record	Site Inspection Record

Visitor Record	Growing Media Disinfestation Record
Vehicle Inspection Record	Growing media Specification Record
Corrective Action Report	Growing Media Quality Record
Calibration Schedule Record	Pesticide Application Record
Crop Monitoring Record	Water Disinfestation Record
Sticky Trap Monitoring Record	Irrigation Water Quality Record
Weed Monitoring Record	

ATTACHMENT 1

Plant Health AUSTRALIA

Tel **02 6260 4322** Fax 02 6260 4321
Suite 5, 4 Phipps Close Deakin ACT 2600
E-mail admin@phau.com.au
www.planthealthaustralia.com.au

Plant Health Australia Ltd
ACN 092 607 997
ABN 97 092 607 997

Dear John

Thank you for the work you have been undertaking with the Domestic Quarantine and Market Access Working Group (DQMAWG) relating to the proposed BioSecure HACCP system for the nursery industry.

Currently where interstate movement controls exist businesses have two possible certification options, a Plant Health Certificate issued by a government inspector or a Plant Health Assurance Certificate issued by businesses that are accredited under the Interstate Certification Scheme (ICA) for a relevant ICA protocol. The DQMAWG sees the BioSecure HACCP system as an innovative approach to certification. The DQAMWG suggests that after further development it could be considered as a third option for certification where appropriately qualified organisations could be accredited to undertake various regulatory activities. The BioSecure HACCP system could be the first step in a system that eventually involves a wider range of industries.

While the DQMAWG accepts the principles of the BioSecure HACCP system, additional development is needed before further consideration and any formal approval of the system. Issues that have been raised include:

- The need to meet jurisdictions' entry conditions. What elements of the manual meet each specific jurisdiction's entry requirements.
- What type of auditing system is proposed eg independent, self auditing, and where would verification audits by regulators fit in.
- The system should be discussed with the Australian Quarantine and Inspection Service as it could possibly link with export certification, particularly where crop monitoring during the growing season is a requirement.
- Acceptance of such a system may require jurisdictions to make legislative changes.

Initial consideration by the DQMAWG could be on a similar basis as the ICA Scheme, which has been adopted nationally following signing of a Memorandum of Understanding (MOU) between all jurisdictions, with an agreement to principles of the scheme and a description of how the system is managed.

I have been advised that you have held further discussions with Mr Mike Ashton and Mr Matt Rogers from Biosecurity Queensland, Department of Employment, Economic Development and Innovation regarding further development of the system. I suggest that the draft MOU, principles and description of an enhanced scheme be sent to Cameron Tree, Queensland member of DQMAWG in the first instance.

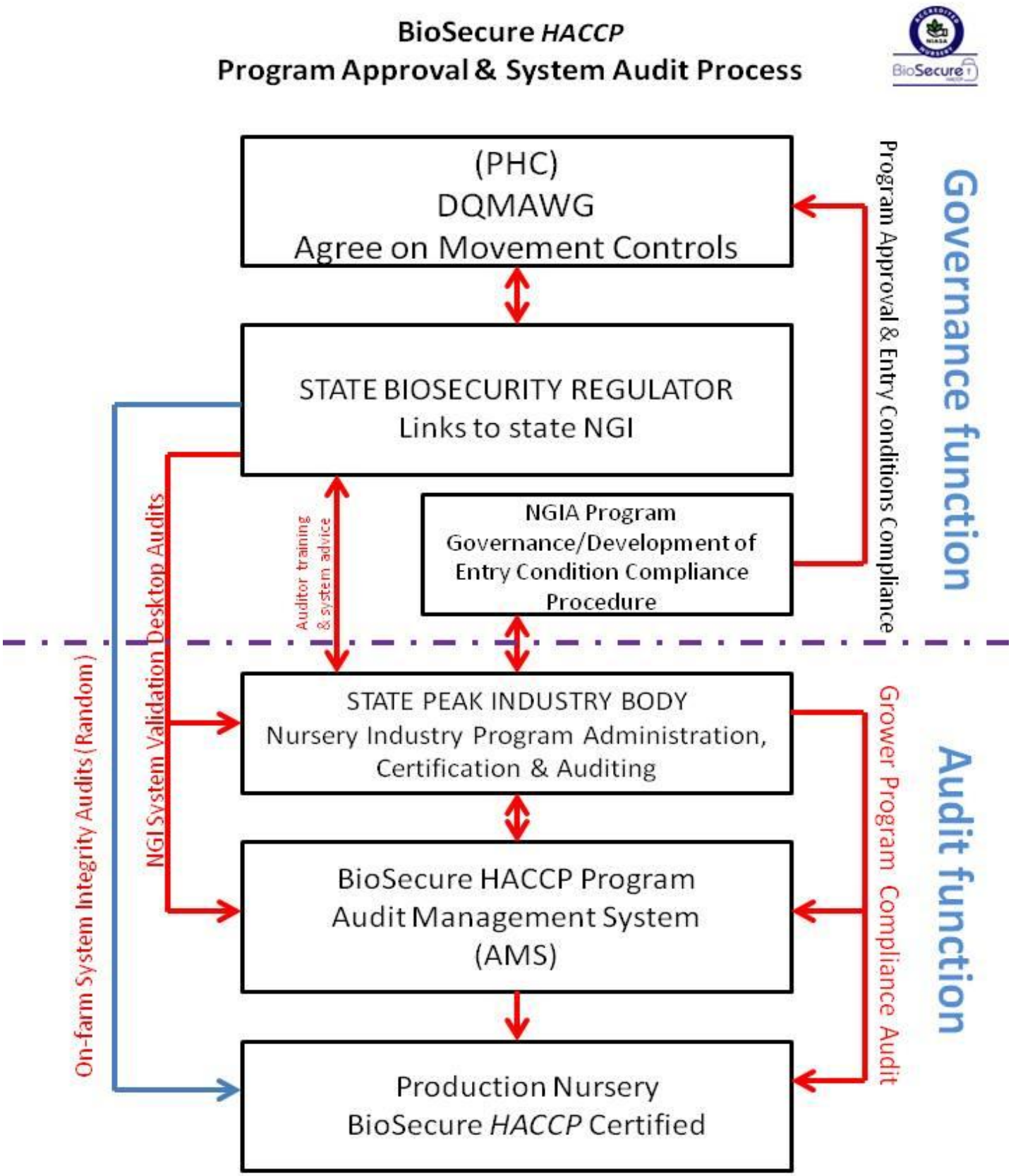
Once again thank you for this innovative and forward thinking work.

Yours sincerely,

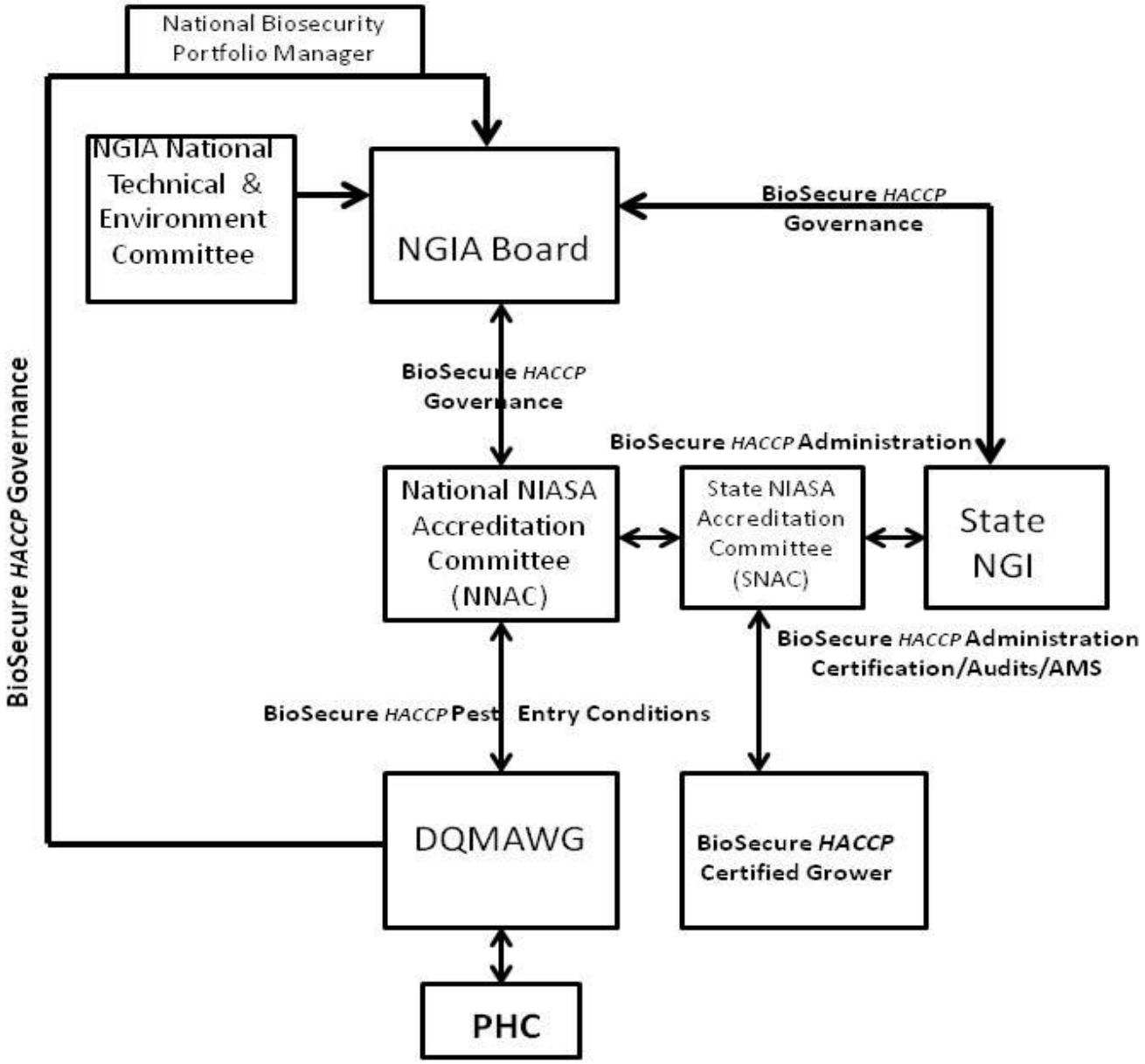
Rod Turner
Chair
Domestic Quarantine and Market Access Working Group

7/4/10

ATTACHMENT 2 **FLOW CHARTS**



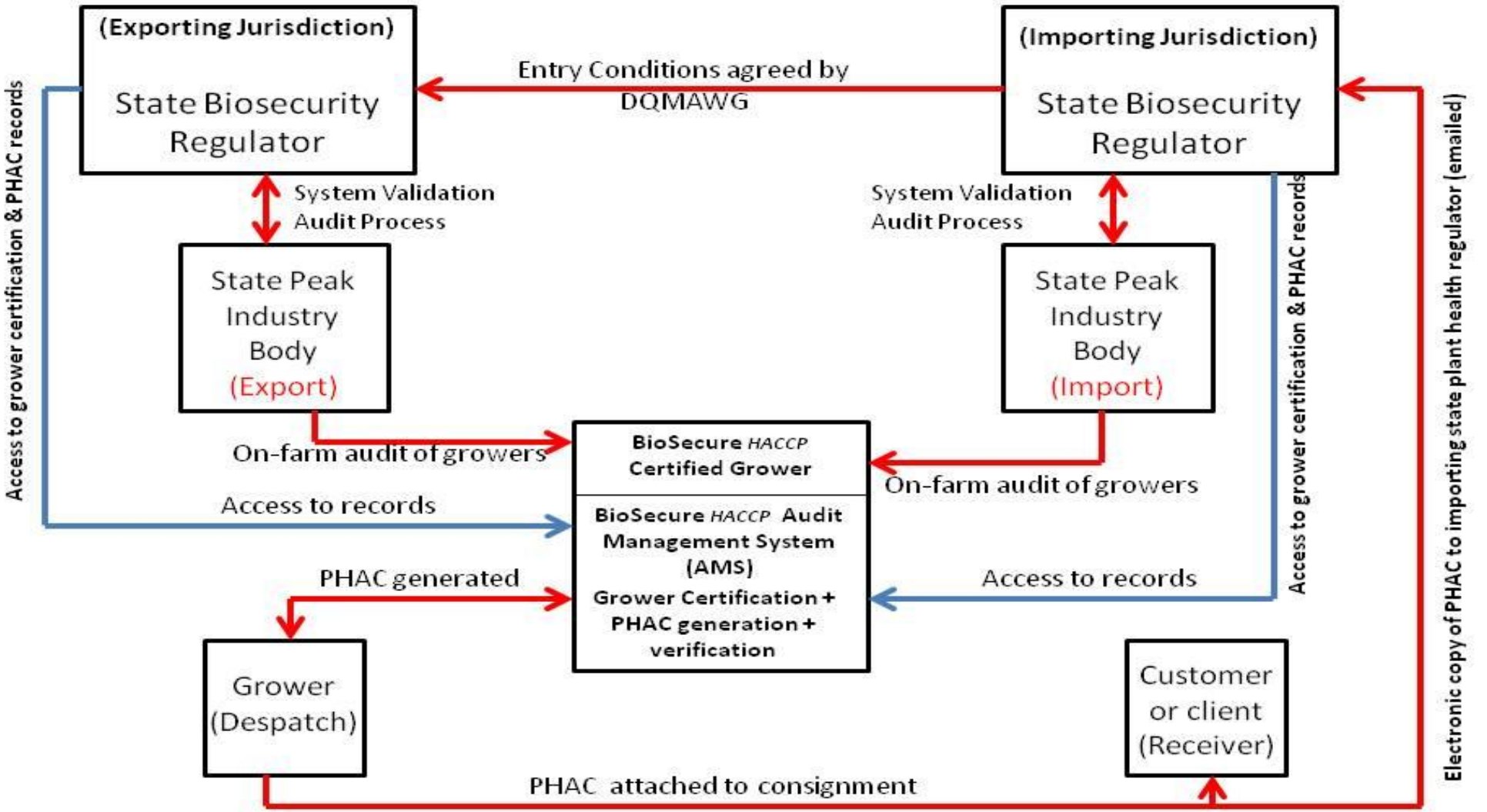
BioSecure *HACCP* Flow Chart
Industry Governance/Administration



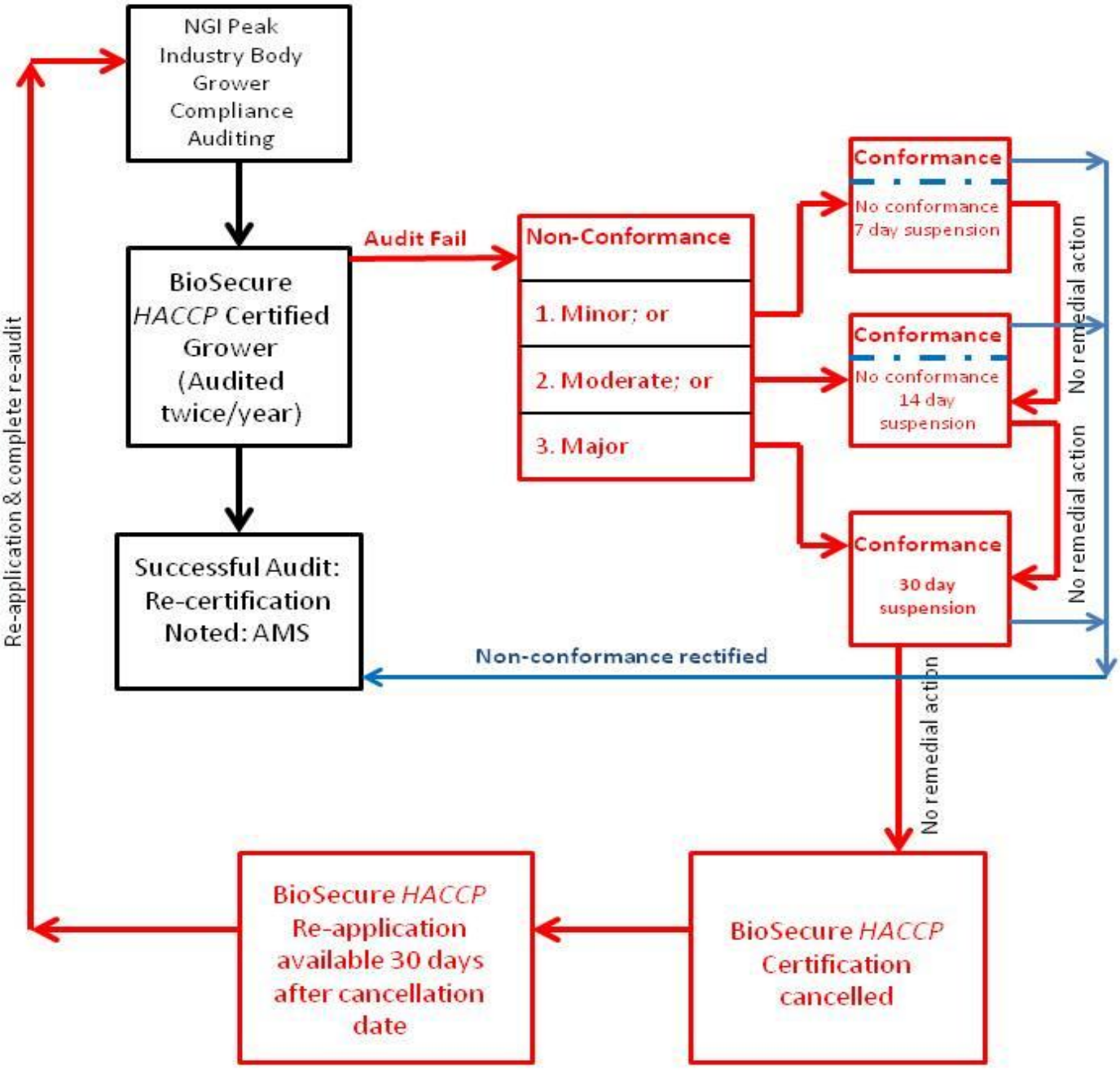
Abbreviations

- **DQMAWG -** Domestic Quarantine & Market Access Working Group
- **NGI -** Nursery & Garden Industry
- **NGIA -** Nursery & Garden Industry Australia
- **NIASA -** Nursery Industry Accreditation Scheme Australia
- **PHC -** Plant Health Committee

BioSecure *HACCP* Consignment Certification Process (Plant Health Assurance Certificate (PHAC)



BioSecure *HACCP*
On-farm Audit and Non-Compliance Process



BioSecure *HACCP* Non-conformance Reinstatement Parameters

- **Minor non-conformance – Certification reinstated on compliance**
- **Moderate non-conformance – Certification reinstated upon compliance**
- **Major non-conformance – Certification reinstated after 30 day suspension period is completed**
- **denotes a return to certified status after non-conformance is rectified**

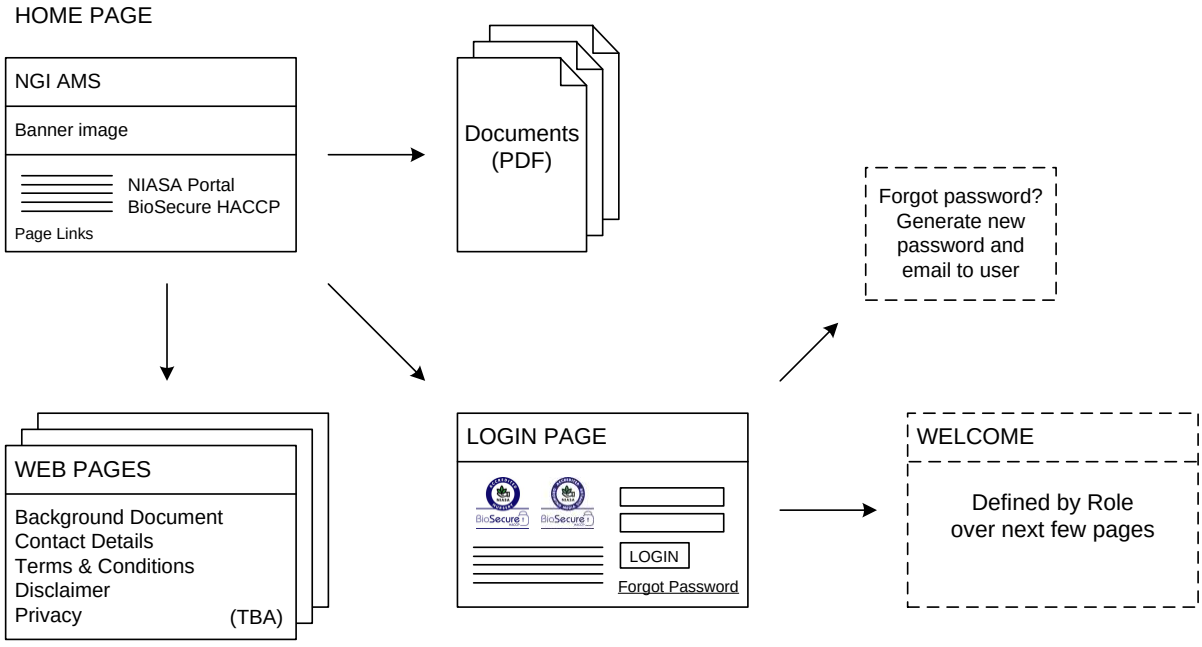
ATTACHMENT 3

BIOSECURE *HACCP* AUDIT MANAGEMENT SYSTEM (AMS)

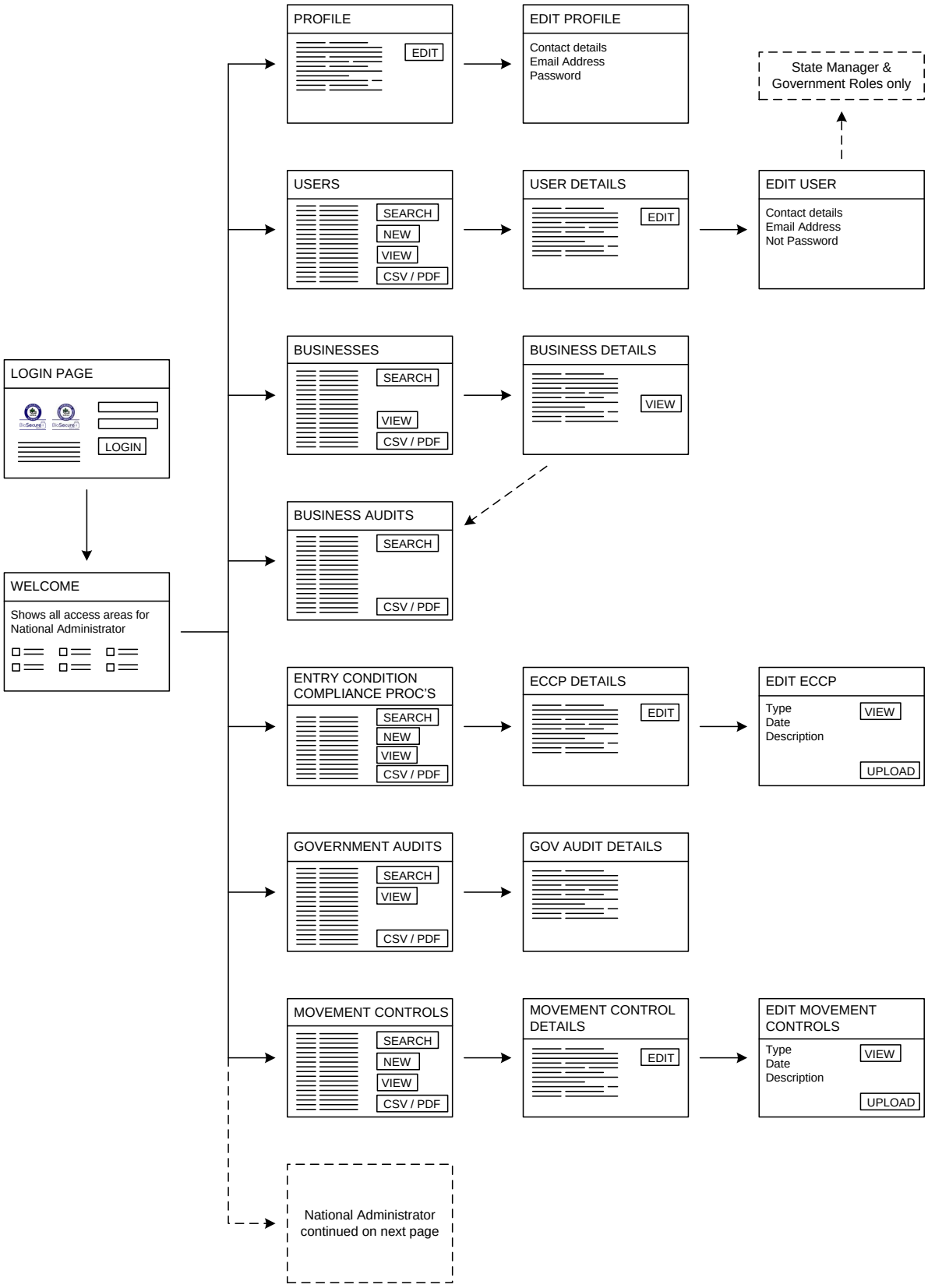
DRAFT HIGH-LEVEL OVERVIEW (STORY BOARDS)

A draft high-level overview of the AMS and its processes has been proposed below. This has been included to assist all parties in conceptualising the requirements, and is subject to change.

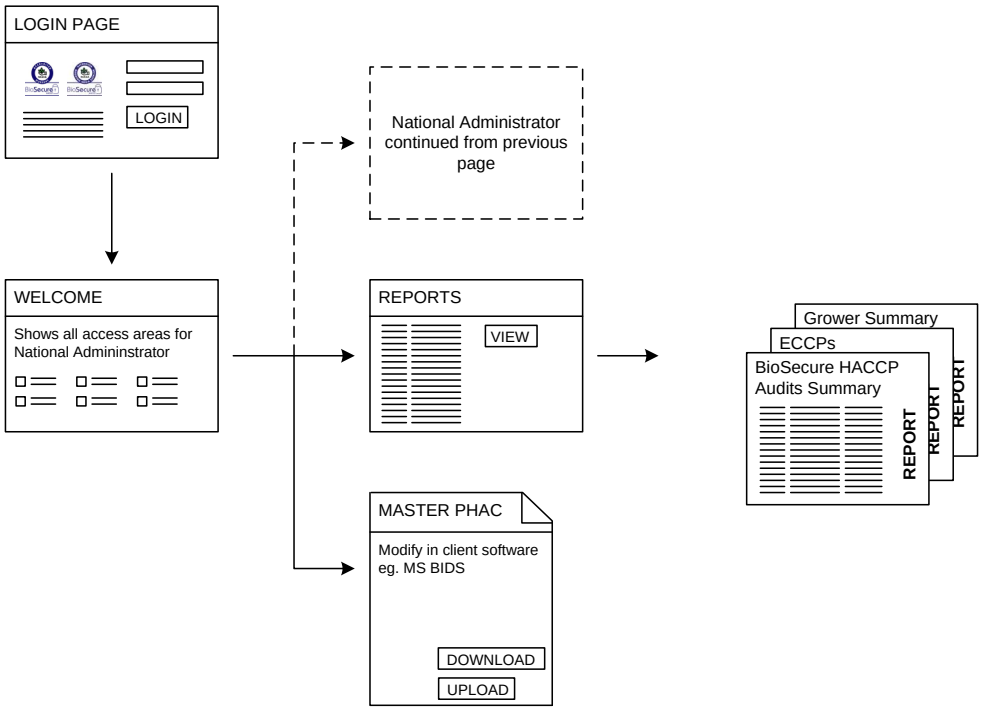
Common Landing Pages



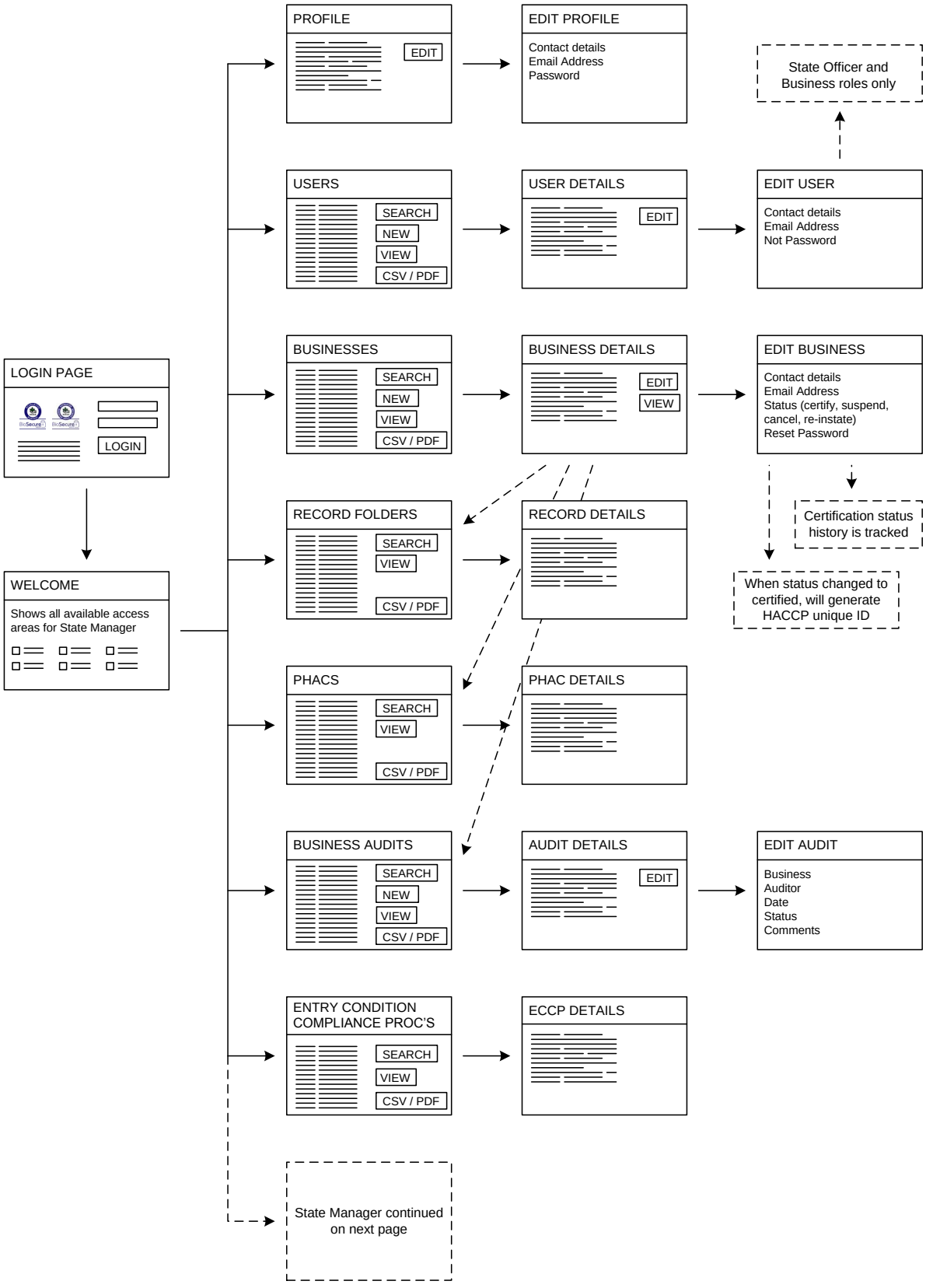
National Manager (Administrator) Role



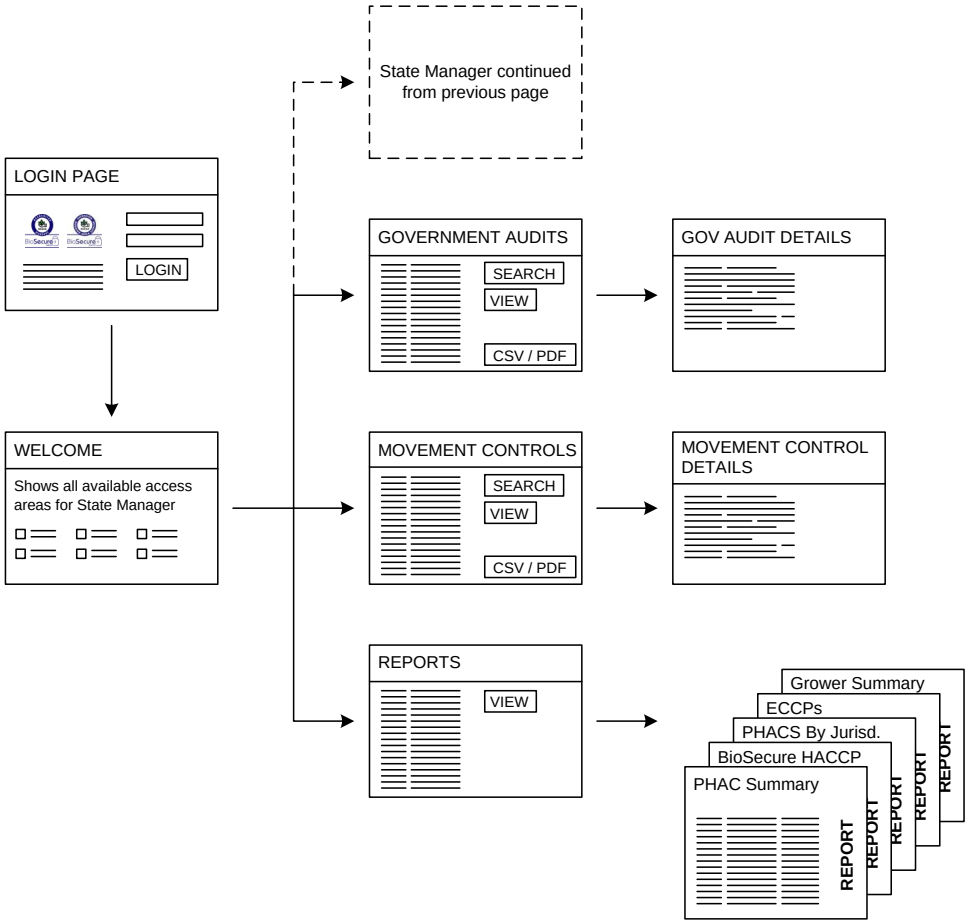
National Manager (Administrator) Role, Continued...



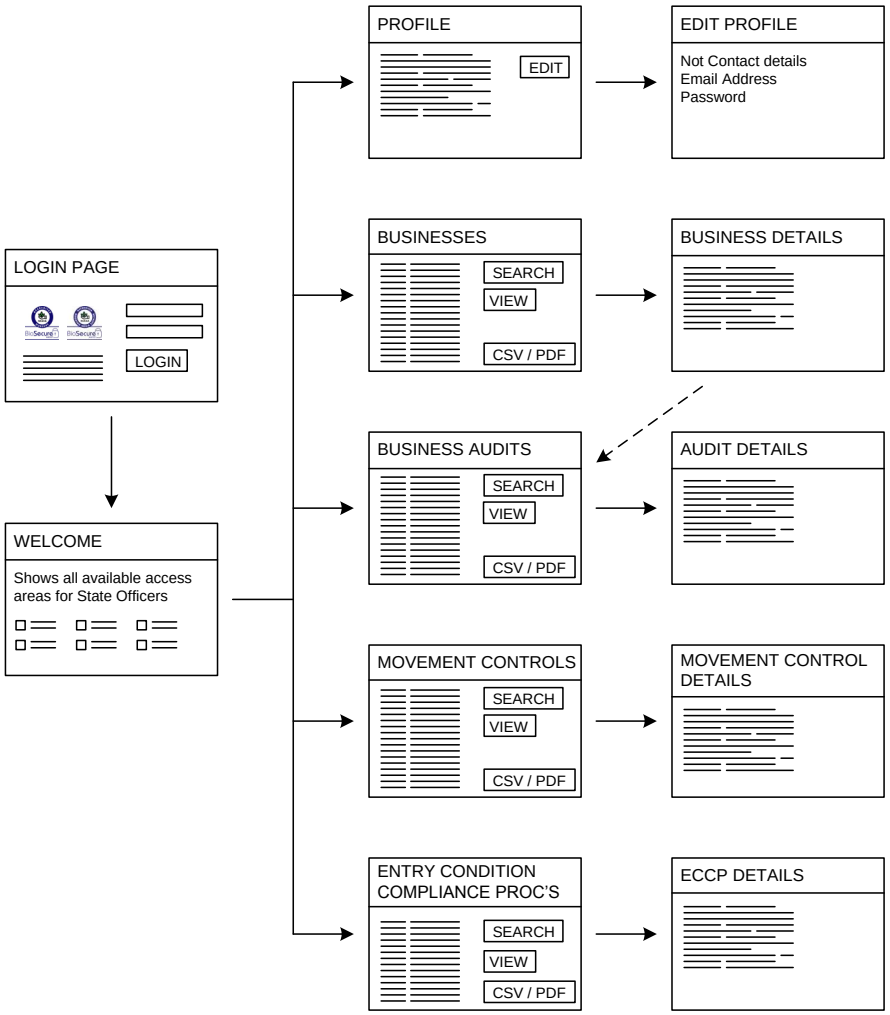
State Manager Role



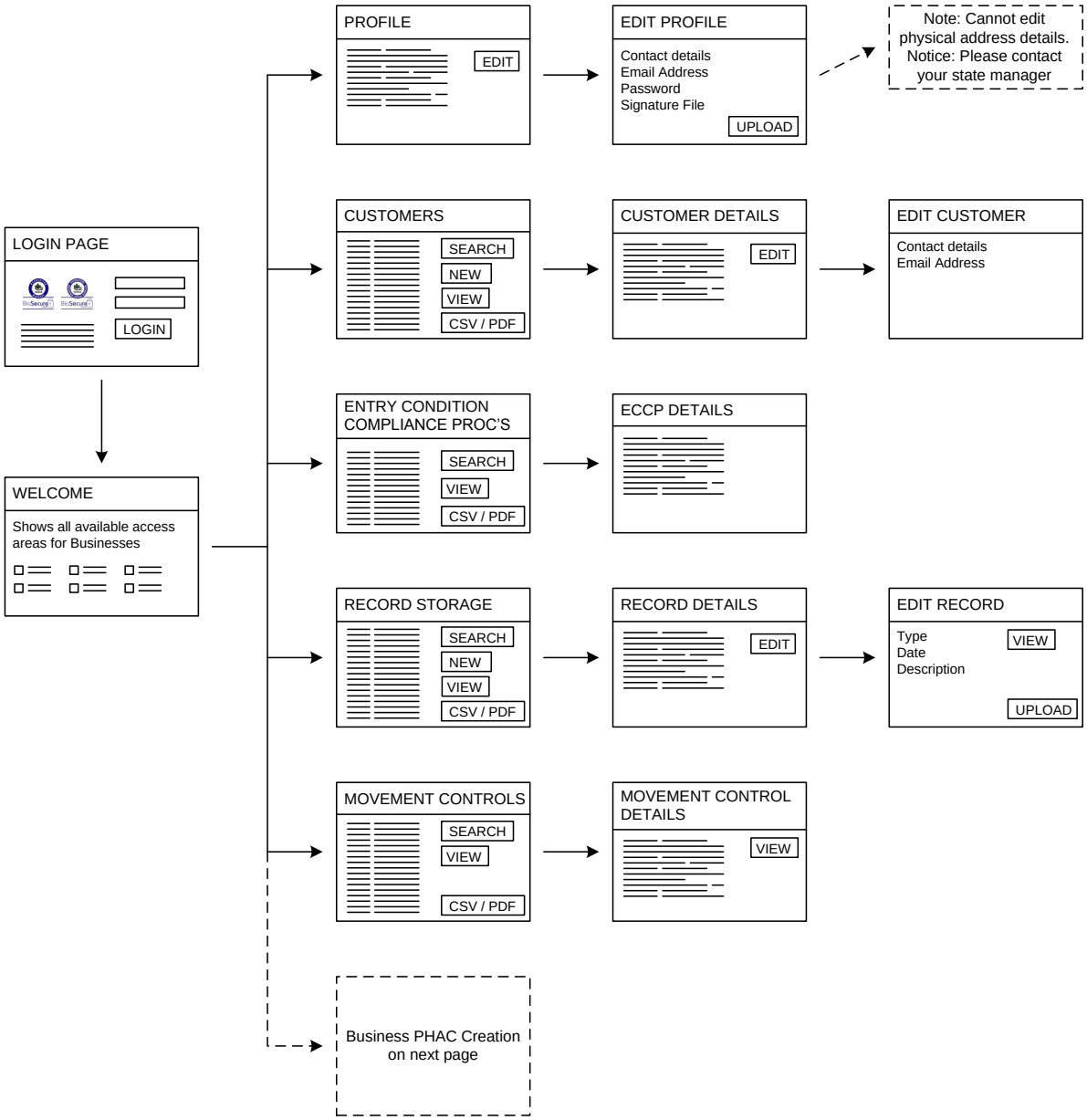
State Manager Role, Continued...



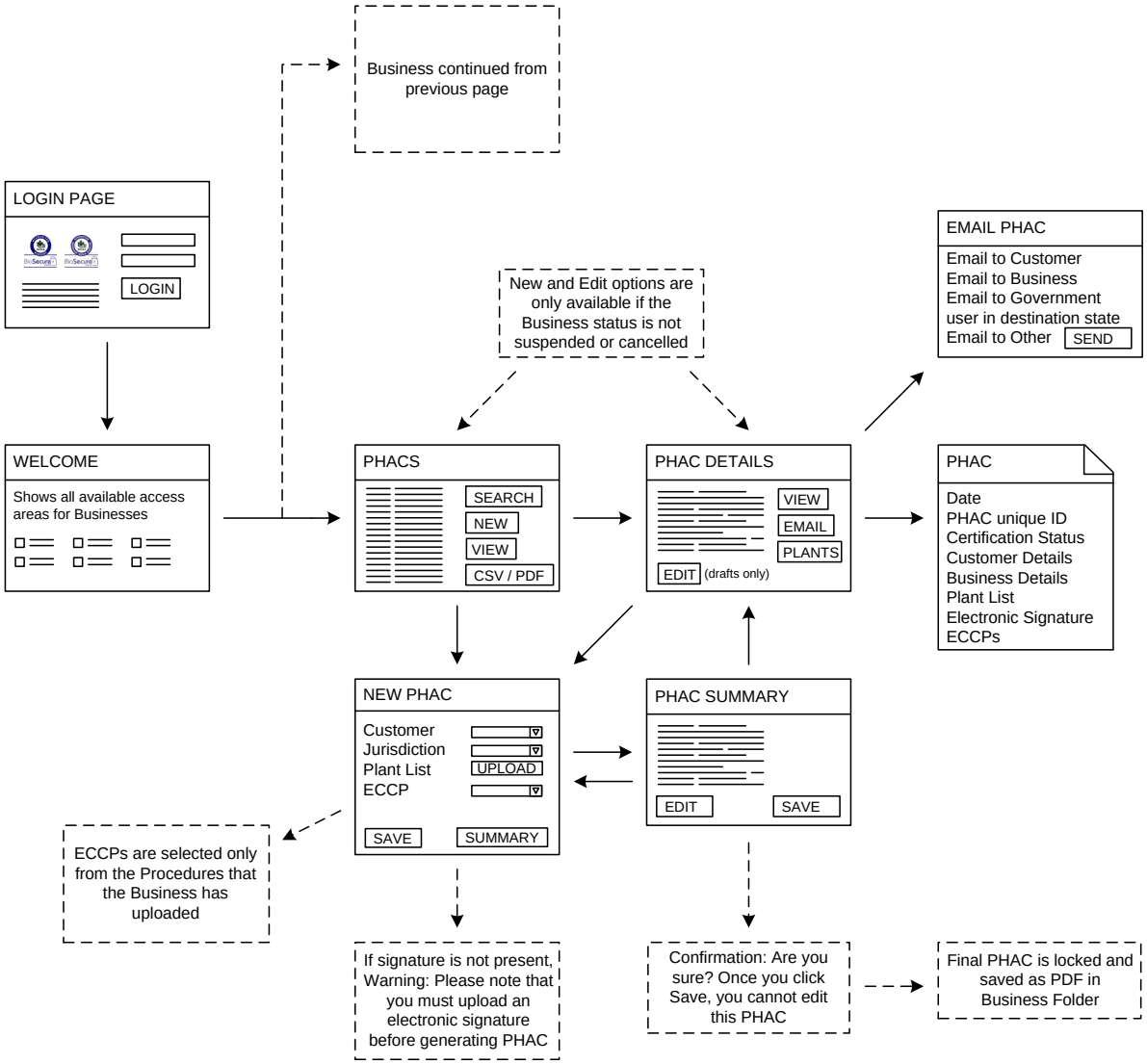
State Officer Role



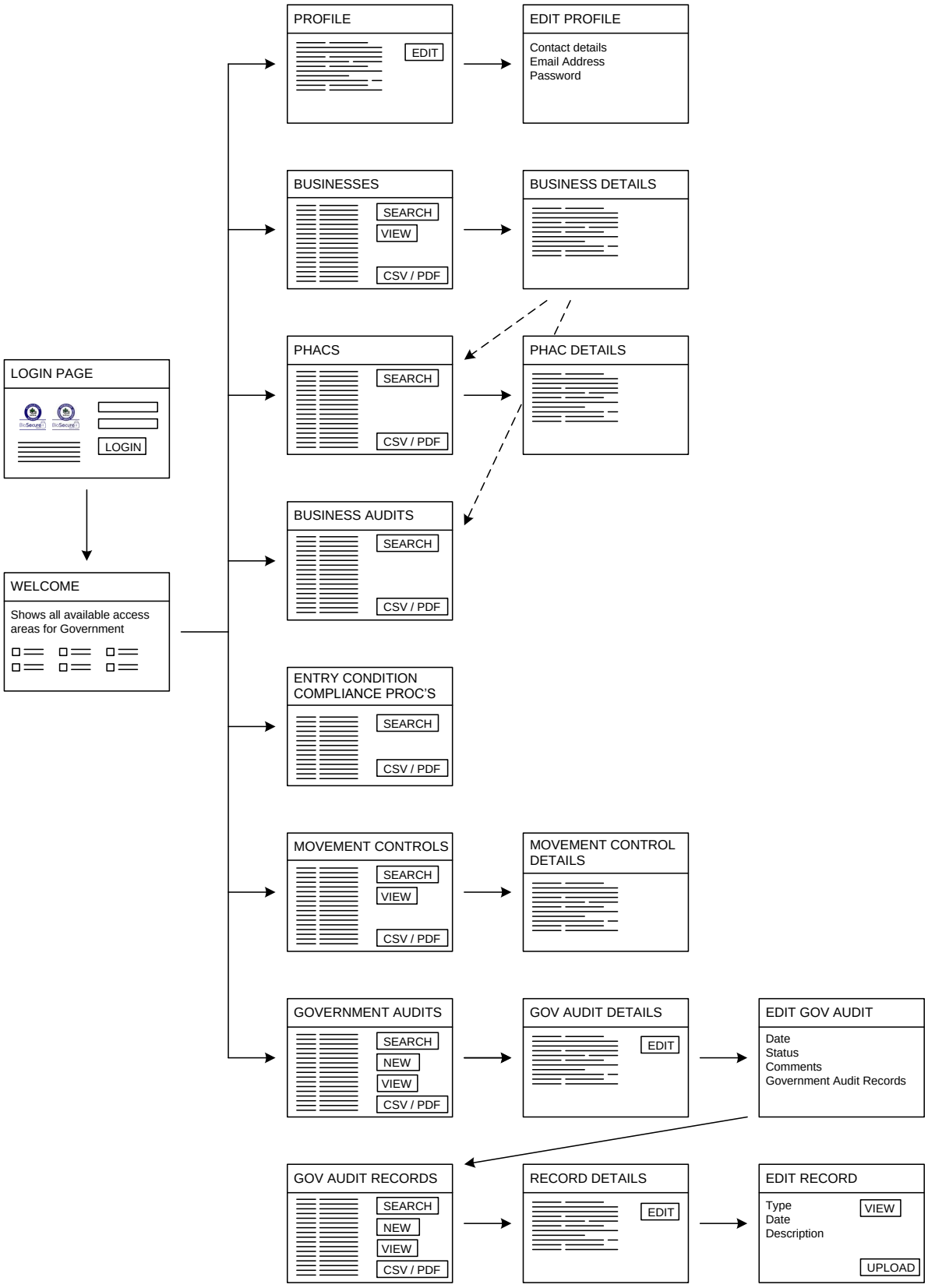
Business (Grower) Role



Business (Grower) Role, Continued...



Government Role



Attachment 4

ECCP Procedure # ECCP01

BioSecure *HACCP*

Entry Conditions Compliance Procedure

(ECCP)

Pest name: Tomato Yellow Leaf Curl Virus (TYLCV)

Market Entry Compliance Procedure for the following jurisdiction(s):

QLD	NSW	VIC	TAS	SA	WA	NT	ACT
☐	☒	☐	☐	☐	☐	☐	☐

***Note:** **Growers denied access to a new BioSecure *HACCP* ECCP until training is completed.**

Pest Details

1. **Common name: Tomato Yellow Leaf Curl Virus (TYLCV)** _____
2. **Biological name:** ___As above_____
3. **Pest/Disease Biology:**

Tomato leaf curl disease is caused by viruses in the geminivirus family of plant viruses. These viruses are spread by whiteflies.

Tomato leaf curl disease is not transmitted in seed, soil or from plant to plant by handling. It is harboured in infected plants, some of which may be hosts that do not show symptoms. The virus causing tomato leaf curl disease is spread from plant to plant by **silverleaf whitefly** (SLW) the biotype B of *Bemisia tabaci.* SLW is a serious pest in tomatoes and other vegetable crops in the coastal areas of Queensland and New South Wales; it is an established pest in cotton production systems in Queensland; and an established pest in Western Australia.

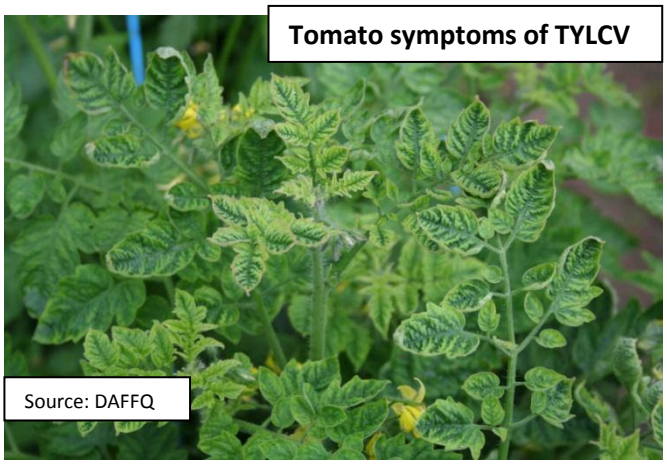
Although the immature nymphal stages of the whitefly can acquire virus from infected plants, it is the active adult insects that are responsible for almost all virus spread into and within crops.

The whitefly acquires the virus while feeding using the piercing-sucking mouthpart to pierce plant cells and suck sap through a stylet. The virus persists in the insect which can then transmit the virus throughout its life. Whiteflies need to feed on infected plants for at least 15 minutes to acquire the virus, and then they need to feed for 15 to 30 minutes to transmit the virus to another host plant. Transmission efficiency increases as the duration of the feeding times increases.

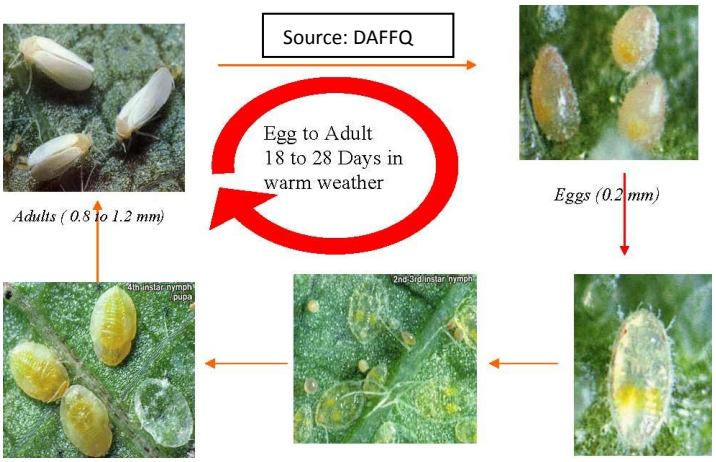
Although the transmission efficiency of individual insects may be low, the enormous populations of SLW moving within and between crops can result in rapid spread and high disease levels. TYLCV is not carried from generation to generation through the SLW egg.

This virus is not spread by other sap-sucking insects such as aphids or leafhoppers nor by leaf-eating pests such as grasshoppers, Heliothis or beetles.

Pest/symptoms images



Vector images (Silverleaf whitefly)



4. Host Material (Plants/plant Parts/Growing Media/etc.)

Tomato is the major host of TYLCV. However, many other species are also hosts of TYLCV, including:

Host species	Host species	Host species	Host species
Boerhavia erecta	*Cyanchum acutum*	*Macroptilium* spp.	*Polygonum* spp.
Capsicum annuum	*Datura stramonium*	*Malva parviflora*	*Sida* spp.
Capsicum chinense	*Euphorbia* spp.	*Mercurialis ambigua*	*Solanum nigrum*
Cleome viscose	*Eustoma grandiflora*	*Phaseolus vulgaris*	*Solanum luteum*
Croton lobatus	*Lycopersicon esculentum*	*Physalis* spp.	*Wissadula* spp

NB: this list is not exhaustive

5. Host symptoms/damage

Tomato plants affected by TYLCV grow slowly and become stunted or dwarfed. Leaflets are rolled upwards and inwards. Leaves are often bent downwards and are stiff rather than limp as with wilted plants. Leaves are thicker than normal, of leathery texture, show interveinal chlorosis and are somewhat wrinkled. Young leaves are slightly chlorotic (yellowish). The flowers appear normal. Fruit, if produced at all, are small, dry and unsaleable.

TYLCV can be confused with several other tomato conditions such as tomato big bud, tomato yellow top, physiological leaf roll and phosphate and magnesium deficiency. Tomato big bud can be distinguished because it produces green flowers. Tomato yellow top virus causes leaflets to be reduced in size and become rounded, with yellowish, down-curled or up-curled margins. Physiological leaf roll due to water stress does not stunt plants and the young expanding leaf tissue is soft rather than rigid.

Phosphate deficiency causes stiff, stunted plants with a purplish tinge and all parts of the plant are reduced in size. Magnesium deficiency causes yellowing of the interveinal areas of the middle and lower leaves. With TYLCV, only new growth produced after infection is reduced in size. As well, phosphate and magnesium deficient plants tend to be more or less evenly distributed throughout a planting, whereas virus-affected plants tend to be distributed randomly or in patches.

Use seedling plants produced in an area free from virus and whiteflies. Destroy old crops as soon as possible after harvesting ceases. Control SLW adults before destroying crops to reduce the migration of SLW to other crops.

Plant new crops as far away as practicable from existing crops which may harbour the virus and whitefly carriers.

Control whiteflies using appropriate chemicals and application strategies.
Maintain a high standard of weed control within and around crops to reduce hosts of both the virus and whitely hosts.
(Source: DAFFQ)

5. Pest Specific Movement Controls:

The importation, introduction or bringing into New South Wales of any host plant that originates from or has moved through a property situated in Queensland or a State or Territory with a known outbreak of Tomato Yellow Leaf Curl Virus, but only where that **property is within 20 kilometres** of the area of the known outbreak.

Plants regulated under Proclamation P169 are only to be imported, introduced or brought into New South Wales if they are accompanied by a Plant Health Certificate or Plant Health Assurance Certificate certifying that the plants meet the conditions of entry into New South Wales.

Entry Conditions

- Plants originating from within 20km of a known infestation
- ***host plant*** means any tomato (Lycopersicon esculentum), bean (Phaseolus vulgaris), lisianthus (Eustoma grandiflora), lobed croton (Croton lobatus), Capsicum spp., Euphorbia spp. or Physalis spp. plant, but specifically excludes the seed, fruit or flower of any of these plants

1. The plants have been grown under a pest management program which prevents the introduction of Silverleaf whitefly and Tomato Yellow Leaf Curl Virus (TYLCV) which includes:

   (a) grown in a Silverleaf whitefly proof production facility such as a screen house or glass house; **or**

   (b) grown using a treatment and weed control program involving:-

      (i) a program of chemicals registered for the control of Silverleaf whitefly; and
      (ii) the removal of weeds from inside the production areas and areas surrounding production areas; **and**

   (c) the production facility and surrounding area is monitored for the presence of Silverleaf whitefly; **and**

   (d) in the case of material propagated vegetatively from cuttings, the mother plants from which the material is sourced are tested every six months and found free of TYLCV; **and**

2. All host plants to be introduced to NSW are inspected and found free of Silverleaf whitefly and symptoms of TYLCV – for commercial consignments the rate of inspection is to be 600 plants per consignment (if the consignment contains less than 600 plants, then all plants are to be inspected); **and**

3. The host plants are packed so as to exclude the entry and prevent infestation with Silverleaf whitefly; **and**

4. The package containing the host plants must be clearly labelled with the name of the grower and address of the property on which the host plants were grown and the name and address of the business that packed the host plants into the package (where the packer can provide trace back information then 'grower' details are not required').

Chemicals registered for Silverleaf whitefly are:

Mode of Action Group	Active	Trade Name	Schedule
Group 1B Insecticide	dimethoate(400g/L)	4FARMERS DIMETHOATE 400 SYSTEMIC INSECTICIDE	6
Group 1B Insecticide	dimethoate(400g/L)	APVMA OPEN USE PERMIT 9583- DIMETHOATE	U
Group 1B Insecticide	dimethoate(400g/L)	SABOTEUR SYSTEMIC INSECTICIDE	6
Group 3A Insecticide	bifenthrin(80g/L)	SCOTTS PROCIDE 80SC INSECTICIDE/MITICIDE	6
Group 3A Insecticide	pyrethrins(0.3g/L)+pip.but.(1.2g/L)	GARD & GROW NATURAL PYRETHRUM INSECT KILLER	0
Group 4A Insecticide	acetamiprid(225g/L)	SCOTTS CROWN 225SL SYSTEMIC INSECTICIDE	6
Group 4A Insecticide	imidacloprid(50g/Kg)	APVMA PER11560 SuSCon MAXI SOIL INSECTICIDE	5
Group 4A Insecticide	imidacloprid(200g/L)	CONFIDOR 200 SC INSECTICIDE	5
Group 7C Insecticide	pyriproxyfen (100g/L)	APVMA PER12659 ADMIRAL	5
Group 9B Insecticide	pymetrozine (500g/Kg	APVMA PER11973 CHESS	5
Group 12A Insecticide	diafenthiuron (500g/L)	APVMA PER11971 PEGASUS	5
Group 16 Insecticide	buprofezin(440g/L)	APVMA PER11553 APPLAUD INSECTICIDE	U
Group 22A Insecticide	azadirachtin A & B (29.55g/L)	AZAMAX INSECTICIDE	5
Insecticide	fatty acids - K salt(285g/L)	NATRASOAP INSECTICIDAL SOAP SPRAY	0
Insecticide	paraffinic oil(815g/L)	BIOCLEAR PARAFFINIC OIL	5
Insecticide	petroleum oil(840g/L)	BIOCOVER HORTICULTURAL OIL	5

NOTE: Select at least three (3) products from the table above from three (3) different **Mode of Action Groups** and rotate based on treatments as specified on each product label.

Tomato Yellow Leaf Curl Virus Audit Checklist

The production nursery complies with the following:

A) Grown in a silverleaf whitefly proof production facility

☐ **Auditor to verify facility is silverleaf whitefly proof**

B) Grown using a treatment and weed control program involving:-

1. A program using chemicals registered for silverleaf whitefly control

☐ **Auditor to verify chemical program is documented and in place**
☐ **Auditor to verify chemical program is/has been applied (records)**

2. Removal of weeds from production areas and areas surrounding production areas

☐ **Auditor to verify weed monitoring program is documented and in place (records)**
☐ **Auditor to verify weed monitoring program is effective**
☐ **Auditor to verify chemical weed program is/has been applied (records)**

3. The production facility and surrounds are monitored for silverleaf whitefly

☐ **Auditor to verify silverleaf whitefly monitoring program is in place (records)**

4. Mother plants are tested and found free of TYLCV at six monthly intervals

☐ **Auditor to verify TYLCV monitoring/testing program is undertaken (records)**

5. All host plants are inspected and found free of silverleaf whitefly and symptoms of TYLCV at the rate of 600 plants per consignment (all plants inspected if total is less than 600)

☐ **Auditor to verify silverleaf whitefly/TYLCV inspection program is undertaken (records)**

6. Host plants are packed to exclude entry and prevent infestation with silverleaf whitefly

☐ **Auditor to verify packaging excludes and prevents infestation of silverleaf whitefly**

7. Host plant packaging must be marked with the name and address of the grower where the host plants were grown and the name and address of the packer

☐ **Auditor to verify host plant packaging is marked with grower and packer address details for traceback**

Attachment 5 Plant Health Assurance Certificate

Consignment Details

Certificate number: e.g. PHAC1001Q

Consignor

Name
Address
Telephone

Consignee

Name
Address
Telephone

Reconsigned To (Splitting consignments or reconsigning whole consignments)

Name
Address
Telephone

Method of Transport (provide details where known)

☐ Road	Truck/Trailer Registration
☐ Rail	Consignment number
☐ Sea	Airline/Flight no.
☐ Air	Vessel Name & Voyage no.

Certification Details

Certified Business that Prepared the Product

Name
Address
Telephone

Grower or packer

Name
Address
Telephone

BH No. of Certifying Business. **Brand Name** or Identifying Marks (as marked on Packages) **Date Code** (as marked on packages)

e.g. BH123Q		

ECCP Procedure Code*	Pest	ECCP Procedure Code*	Pest

*** Note:** Entry Condition Compliance Procedure (ECCP)

Number of packages	Type of Packages (eg. Trays, cartons)	Type of Produce	Authorisation for Split Consignment

Additional Certification

Declaration

I, an Authorised Signatory of the accredited business that prepared the plants or plant produce described above, hereby declare that the plants or plant produce have been prepared in the business's approved facilicies in accordance with the certification(s) granted to the business under BioSecure HACCP and the details shown above are true and correct in every particular.

Authorised Signature Name	**Signature**	**Date**
		/ /

Appendix 11

Nursery Production Farm Management System – Benefit Cost Analysis

Report- August 2012

ABN 41 107 715 364
Michael Clarke
Cathy Moore
P: (02) 9817 5888
E: clarke@AgEconPlus.com.au
W: www.AgEconPlus.com.au

Contents

Abbreviations

DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
HACCP	Hazard Analysis and Critical Control Point
HAL	Horticulture Australia Limited
IDO	Industry Development Officer
IPM	Integrated Pest Management
KPI	Key Performance Indicator
NGIA	Nursery and Garden Industry Australia
NIASA	Nursery Industry Accreditation Scheme Australia
NIDO	Nursery Industry Development Officer
NPFMA	Nursery Production Farm Management System
OHS	Occupational Health and Safety
PC	Productivity Commission
R&D	Research and Development
RD&E	Research, Development and Extension
RDCs	Research and Development Corporations

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- Grant Dalwood, South Australia Nursery Industry Development Officer
- Karen Brock, Brocklands Tasmania

Without whose assistance the cooperation of nursery industry businesses would not have been secured.

Executive Summary

This document reports a series of cost benefit analyses on Nursery and Garden Industry Australia's (NGIA) Nursery Production Farm Management System (FMS). It was prepared to provide an evidence base for communication to industry and Horticulture Australia Limited.

Three benefit cost analyses were completed. The first addressed the value of the FMS to individual businesses. The second analysis quantified the FMS's value to the whole nursery industry while the third identified benefits to the broader Australian community.

Not all nursery businesses that invest in a FMS receive a financial return and many adopt the FMS for reasons that are not purely financial. Amongst those who did receive a financial gain from adoption, the return is substantial and reflected in new markets accessed, reduced stock wastage, management efficiencies, labour and chemical savings. Less easily quantified benefits include improved access to technology, risk reduction, brand building, staff culture, continuous improvement and ease of compliance with environmental regulations. Business costs include both capital expenses (up to $150,000 to retrofit an older nursery) and annual operating outlays of as much as $50,000 per annum. The formal benefit cost analysis showed a positive return on business investment with a five year payback period.

To deliver these benefits to individual businesses, NGIA and Horticulture Australia Limited (HAL) have supported twenty two levy funded projects totalling almost $1.3 million. Contributions have also been made by various state governments. Ongoing costs include annual administration and the Industry Development Officer (IDO) network.

Quantification of industry benefits from total investment is dependent on the number of adopting businesses and the number of these businesses that receive a financial benefit. The analysis has been completed using the assumption that around half of those who adopt the FMS receive a financial benefit. On this basis the FMS has delivered a strong return for industry – net present value of $71.22 million with a benefit cost ratio of 8.01 and a return on investment of 40.5%.

Sensitivity analysis completed on industry returns demonstrated that even with only 25% of adopters receiving a financial benefit from FMS implementation, additional industry revenue more than covered industry investment costs.

Benefits to the Australian community from the nursery industry's investment in the Nursery Production FMS were identified and analysed across the environmental, social and economic 'triple bottom line'. The most important environmental benefits realised by the Australian community were improved biosecurity (less chance of invasive weeds, pests and diseases) and improved chemical management. Community social benefits included increased demand for gardening with associated positive spin offs for health, social and visual amenity. Community economic benefits included employment and regional development.

1 Introduction

This document is a benefit cost analysis of the Nursery Production Farm Management System (FMS). It was prepared for Nursery and Garden Industry Australia (NGIA) by AgEconPlus between December 2011 and September 2012.

1.1 Analysis Purpose

The purpose of the benefit cost analysis was to provide an objective and independent evidence base for communication to industry. Completion of benefit cost analysis is also consistent with Horticulture Australia Limited (HAL) requirements.

1.2 Background

Farm Management Systems are a framework endorsed by industry and government to ensure a sustainable future for primary producers. The Nursery Production FMS is aimed at guiding change and technology adoption and includes three key on farm programs:

1. Nursery Industry Accreditation Scheme Australia (NIASA) – a Best Management Practice program to improve business efficiency whilst being mindful of the environment.
2. EcoHort – an Environmental Management System which offers risk assessment, a continuous improvement pathway and opportunity to demonstrate sound environmental stewardship.
3. BioSecure *HACCP* – a biosecurity program which helps business assess their pest, disease and weed risks for both imported and exported material.

Businesses must be NIASA accredited in order to be eligible for EcoHort and BioSecure *HACCP* certification. The Nursery Production FMS is relevant to production nurseries, growing media manufacturers and greenlife markets. The Nursery Production FMS has been adopted by 274 mainly production nursery businesses (Table 1.1). There are approximately 3,500 nursery production businesses in Australia (AgEconPlus and Agtrans Research 2009).

The Nursery Production FMS is supported by a formal recognition process, on farm technical and pathology support. An annual accreditation / certification charge is levied by State or Territory Associations based on NGIA membership for these support services (Table 1.1).

Table 1.1 Number of Accredited/Certified Nursery Industry Businesses

Program	Number of Businesses	Cost per annum NGIA Member ($)	Cost per annum NGIA Non Member ($)
NIASA	274	400 - 530	730 – 880
EcoHort	100	0 - 195	0 - 390
BioSecure *HACCP*	2	0 - 195	0 - 390

Source: NGIA September 2011

1.3 Study Approach

The benefit cost analysis was completed at three levels:

1. The first analysis addressed the value of Nursery Production FMS to an individual business that had implemented the system.
2. The second analysis quantified the farm management system's value to the whole nursery industry since inception.
3. The third analysis assessed benefits to the broader Australian community across the economic, social and environmental 'triple bottom line'.

The Nursery Production FMS was analysed as a 'whole' inclusive program rather than attempting separate evaluations for each of the NIASA, EcoHort and BioSecure *HACCP* programs. At this point in time it was deemed too difficult to separate out benefits associated with adoption of each Nursery Production FMS component.

The project was delivered using benefit cost analysis techniques described in the Council of Rural Research and Development Corporation (CRRDC) Evaluation Guidelines (updated 2009).

Data to inform the analysis was sourced from a survey of participating nursery industry businesses. A copy of the survey questionnaire is included as Appendix 1. Twenty seven complete data sets were collected from Nursery Production FMS accredited/certified businesses and these were aggregated into appropriate business types (see Table 1.2).

Table 1.2 Nursery Industry Businesses Analysed

Business Type	Description	Data sets collected
Production nurseries	• Includes the seedling and potted colour sector; tree and shrub growers; propagation specialists; and indoor plant growers. • Businesses identified with the assistance of NGIA Nursery IDOs.	21
Growing media manufacturers	• Includes manufacturers of growing media. • Media manufacturers were identified through Compost Australia's media manufacturers list and with the assistance of NGIA.	4
Greenlife Markets	• Greenlife markets provide a plant wholesaling service to the industry. • Only two greenlife markets have adopted NPFMS and this has only occurred since 2010. It was therefore necessary to complete the survey on the basis of actual costs and expected benefits.	2
	Total	27

Surveys were completed as both face-to-face and telephone interviews. Telephone interviews were used to ensure the study was delivered cost effectively. More than fifty nursery businesses were contacted and those contacted were mostly enthusiastic about their participation in NPFMS. Time pressures associated with operating a nursery business prevented many of those contacted from participating in what was a comprehensive, and therefore time consuming, survey.

NGIA were keen to secure a mix of data sets across businesses operating in both tropical and temperate production environments. From the twenty seven data sets secured, seventeen were from businesses in southern Australia (NSW, Victoria, Tasmania and South Australia) and ten were located in Queensland.

A survey sample of twenty seven, ten per cent of those who have adopted an NPFMS is a reasonable sample size and provides confidence in the resulting analysis.

1.4 Review of Literature

Survey design was informed by the relevant NIASA literature. FreshLogic (2007) reviewed NIASA nursery growth, market share and perceived advantages and found:

- NIASA accredited businesses were more likely to be larger operations with substantial market share.
- Buyers of plants were aware of the NIASA program. Government sector buyers had a policy of purchasing from NIASA accredited nurseries, retail buyers were less committed. Buyers of propagation stock were reassured that NIASA reflected minimum quality standards.
- NIASA member feedback acknowledged that the scheme had provided valuable assistance in managing their nursery operations. The program provided 'another pair of eyes', but marketing upside was presently less apparent.
- NIASA member nurseries were performing ahead of the market.

Kachenko *et al* (2010) surveyed NIASA participants and concluded:

- Nursery production businesses became accredited to enhance their business reputation; to create a marketing advantage; to manage business risk; to access the Industry Development Officer (IDO) network; and to deliver on their environmental ethos.
- Most businesses recognised that NIASA accreditation satisfied their inter-state quarantine requirements.
- NIASA accreditation entitled businesses to a further discount on insurance with OAMPS.
- Business risk management and environmental responsibility were important drivers for the industry and are key components of the NIASA.
- Accredited businesses use NIASA within their marketing material which supports the ranking of business reputation as a reason for becoming accredited.

- More than 75% of businesses who are NIASA accredited would recommend accreditation to other businesses.

2 Value of NPFMS to an Individual Business

Aggregations of survey data for each of production nurseries, growing media manufacturers and greenlife markets are presented in this chapter. Costs and benefits are analysed and return on investment reported.

2.1 Production Nurseries

Surveyed production nurseries included tree / shrub producers, propagation specialists, tube stock growers, seedlings and potted colour nurseries. Enterprises tended to grow a mix of these product types.

Production Nursery Costs

In most instances Nursery Production FMS participation resulted in at least some additional capital costs and always resulted in additional annual operating expenses. The average of these capital and operating costs across twenty one data sets is reported in Table 2.1.

Table 2.1 NPFMS Capital and Operating Costs – Production Nursery Average

Cost Item	Average Cost per Business ($)	Comments
Capital Costs		
Water treatment plant	17,310	Purchased by some nurseries regardless of Nursery Production FMS requirements
Steam steriliser for pots and tubes	3,810	Some purchased regardless of Nursery Production FMS
Drainage – pipes, gravel and bunds	15,500	Nil runoff required for EcoHort accreditation
Growing surface upgrades	4,762	Required by only three surveyed businesses
Storage facilities, conveyors, etc.,	4,510	Typically storage for chemicals or soil
Systems development (e.g. Quality Assurance, Occupational Health and Safety)	2,214	Often to support Nursery Production FMS reporting
Integrated Pest Management development	1,052	Identified by only two survey respondent
Total	**49,158**	**(maximum of $150,000, minimum of zero)**
Annual Operating Costs		
Accreditation costs	510	Program subscription to state association
Labour – administration	1,502	Incurred by most accredited/certified businesses
Labour – staff training	736	Some considered his a 'base case' cost
Labour – quality / safety checking	887	Required infrequently
Laboratory testing costs	360	Some nurseries have in-house testing
Nursery maintenance	1,425	Extra maintenance required post Nursery Production FMS
Sterilisation and water treatment	1,600	Incurred by most nurseries
Research & Development (R&D), continuous improvement	2,880	Major annual cost item for some nurseries
Total	**9,900**	**(maximum of $50,000, minimum of $465)**

There was a wide variation in the cost of capital investment attributed to Nursery Production FMS by production nurseries. Some nurseries identify major capital items including water treatment plants, steam sterilisation facilities, nursery drainage, new growing surfaces and systems development while other surveyed nurseries claimed that these costs would have occurred regardless of accreditation. New production nurseries were less likely to incur capital costs as compared to established facilities attempting to 'retro-fit' to meet Nursery Production FMS requirements. Capital costs therefore vary from between $100,000 and $150,000 for those attributing major capital upgrades to no cost at all for those businesses that felt that Nursery Production FMS requirements were part of 'base case' good business practice.

Nursery Production FMS operating costs were similarly affected by the business manager's attitude to what constitutes 'base case' business practice. Annual operating costs attributable to the Nursery Production FMS ranged from as little as $465, the cost of nursery accreditation, to over $50,000 per annum for large operations which attributed significant R&D and continuous improvement investments (e.g. conveyors, OHS railings, concreted work surfaces and propagation equipment)to their FMS.

Production Nursery Benefits

Production nursery benefits from Nursery Production FMS were found to be of two types – those that are readily quantified, making a positive contribution to the financial performance of the business and those that are important but less tangible. The average of quantifiable Nursery Production FMS benefits along with a relevant explanation is summarised in Table 2.2.

Table 2.2 Nursery Production FMS Financial Benefits – Production Nursery Average

Benefit Item	Average Benefit per Business ($)	Comments
Reduced insurance premiums	182	Insurance broker OAMPS had until recently offered a 10% premium reduction. This was discontinued following floods in 2011.
Reduced throw out rate - extra plant sales	25,238	Less poor quality plants produced and there is a ready market for additional saleable plants (e.g. throw out rates reduced from 5% to 3% with Nursery Production FMS). NB: throw out rate reductions not observed for tube stock.
New markets accessed - extra sales	59,690	Certification has facilitated increased sales via marketing advantage or enhanced reputation. New markets accessed have included interstate sales[#] and access to markets requiring plants that meet food safety standards e.g. fruit tree sales to commercial growers.
Management efficiencies	12,429	Includes access to innovation and business information provided by IDOs. Nursery Production FMS accreditation is also reported to be more cost effective than alternative systems.
Input savings - labour	5,857	Nursery Production FMS has led to the adoption of labour saving technologies e.g. pot cleaning equipment.
Input savings - chemicals	1,571	Includes savings on chemicals (e.g. fungicides) and fertilisers.
Input savings – electricity	476	Benefit only quantified by a few nurseries.
Input savings - water	225	Volumes saved can be significant one nursery saved up to a mega litre per annum of potable water.
Total	**105,668**	**(maximum of $702,000, minimum of $0[^])**

While NPFMS has in some instances facilitated interstate plant sales, the majority of Plant Health Certification is still completed by State or Territory Departments of Primary Industries
^ 7 of 21 interviews completed stated that there were no financial benefits associated with Nursery Production FMS accreditation

It is worthy of note that, on average, input labour savings, a benefit, were greater than additional labour costs, an expense. As with all Australian horticulture, reduction in expenditure on high cost labour is essential for long term industry profitability. Labour saving is an important 'selling point' for Nursery Production FMS adoption.

As with capital and operating costs there was considerable variation in quantified benefits. Large production nurseries that were supportive of Nursery Production FMS identified financial benefits to their business through additional sales and new markets. Other production nurseries were strongly of the opinion that the whole supply chain is driven by price alone and that there was no financial benefit from Nursery Production FMS participation. These owners and managers were Nursery Production FMS accredited/certified in order to realise a series of non-financial benefits.

Less easily quantified benefits of Nursery Production FMS participation identified by nursery production businesses included:

- Access to IDO which bring 'fresh eyes' and new knowledge to the production nursery business – financial, environmental, human resource and community management benefits were associated with IDO visits.
- Time savings associated with keeping up to date on innovation and changing legal requirements (e.g. OHS, human resources, insurance, chemical management, myrtle rust, etc.). 'Someone keeps across the issue for you then sends you information about what to do'. This non-financial benefit was also linked to the IDO network.
- Risk reduction – there is a lower probability of say a catastrophic production failure with the Nursery Production FMS in place (e.g. major pest or disease incursion such as myrtle rust within the business). While this benefit is certainly financial in nature, its quantum was difficult to estimate by production nursery businesses.
- Enhanced business reputation – additional confidence provided to existing customers and a point of differentiation when securing tendered contracts. Nursery Production FMS is all part of building the adopting business's brand. It demonstrates consistency, market positioning and credibility. It builds goodwill which will bring long term financial benefit to the business.
- Creation of a beneficial staff culture – Nursery Production FMS accreditation/certification helps bring about a professional and positive business outlook. Staff take pride in keeping up to the standard and management implement what they may have otherwise delay. Having an externally prescribed and audited system provides a discipline that ensures the team take training and required standards seriously.
- Continuous improvement within the business – the Nursery Production FMS encourages the questioning of the standard and current business practices, how can this be improved, what R&D is required and how can this proceed at a commercially appropriate rate. Improvements are prioritised over many years and implemented when they are affordable.
- A body of evidence demonstrating environmental best practice. This body of evidence assists with the management of some business's environmental ethos and is also useful in the event of disputes or to support business expansion plans. Production nurseries have found local government authorities to be much more sympathetic to businesses that can demonstrate sound environmental stewardship. EcoHort is often used by local government as a standard that must be met prior to building approval.

Production Nursery Return on Investment

A financial analysis of nursery production business Nursery Production FMS accreditation/certification was completed and is reported in Table 2.3. The impact on the business was analysed over ten years, to allow amortisation of capital, and a 5% interest rate was assumed.

Other data used to drive the analysis included:

- Capital costs associated with 'retro-fitting' a large established nursery business. Capital costs of $100,000 were assumed so as not to overestimate the financial benefit of Nursery Production FMS accreditation/certification. Capital costs modelled included a water treatment system ($20,000), a steam steriliser for pots and tubes ($20,000), improved growing surfaces ($10,000), a drainage upgrade ($40,000), miscellaneous capital ($5,000) and improved operating systems ($5,000). The cost of these capital items was amortised over a ten year period i.e. the annual additional cost of capital was $10,000 per annum.

- Additional annual operating costs were modelled on the average of survey responses and totalled $9,900 per annum. Major additional operating costs included labour ($3,125), investment in R&D and continuous improvement ($2,880), additional maintenance costs ($1,425), and sterilisation / water treatment expenses ($1,600). Program costs of $510 per annum were assumed and were a relatively minor expense item.

- No benefit was allocated for reduced insurance premiums as the OAMPS discount has been discontinued. Reduced plant stock throw out rates were assumed to start two years after commencement at $5,000 per annum and increase to $25,000 per annum within ten years of commencement.

- New markets and additional sales were the single largest benefit modelled. When fully realised, ten years after Nursery Production FMS implementation, the value of new sales achieved either interstate or in new local markets was assumed to be worth $45,000 per annum. Larger production nurseries that were Nursery Production FMS accredited/certified were able to demonstrate additional sales of more than $500,000 per annum. As previously noted, other businesses realised no additional sales as a result of the program.

- Other financial benefits associated with Nursery Production FMS accreditation/certification were assumed to include management efficiencies along with labour and other input savings.

Table 2.3 Return on Investment – Production Nursery (over ten years, 5% interest rate)

Financial Indicator	Result
Gross revenue - average annual increase	$105,669
Operating costs - average annual increase	$9,900
Capital costs – average annual cost over ten years	$10,000
Net revenue increase	$85,769
Return on investment	31%
Break-even on investment	5 years

Definitions:
Gross revenue: additional business receipts before allowance for associated costs
Operating cost: annual costs directly relevant to the generation of business revenue
Capital costs: business investments required to meet Nursery Production FMS requirements and secure additional revenue
Net revenue: gross revenue after allowance for operating costs and annualised capital costs
Return on investment: the yield generated from Nursery Production FMS
Break-even on investment: number of years required to recoup Nursery Production FMS related outlays

Return on investment and time to break even are, in AgEconPlus's experience commercially acceptable for Australian small to medium business enterprises.

2.2 Growing Media Manufacturers

Growing Media Costs

Surveyed growing media manufacturers who adopted Nursery Production FMS incurred additional capital and operating costs – Table 2.4.

Table 2.4 Nursery Production FMS Capital and Operating Costs – Growing Media Manufacturer Average

Cost Item	Average Cost per Business ($)	Comments
Capital Costs		
Water treatment plant	1,700	Some form of upgrade required by three of four businesses surveyed
Steriliser equipment	6,800	Most considered this essential equipment
Drainage – pipes, gravel and bunds	8,125	To ensure no offsite impacts
Production surface upgrades	14,825	To ensure no offsite impacts
Storage facilities, conveyors, etc.,	375	Minor cost for all businesses surveyed
Systems development (e.g. QA, OHS)	5,950	Includes electronic and paper systems
Total	**37,775**	**(maximum of $101,000, minimum of $2,000)**
Annual Operating Costs		
Accreditation costs	490	Cost is similar to production nurseries
Labour – administration	1,250	Additional cost for only two businesses
Labour – staff training	750	Additional cost for only two businesses
Labour – quality / safety checking	3,125	Important to all surveyed
Laboratory testing costs	1,625	Important to all surveyed
Business maintenance	0	Not identified as being linked to Nursery Production FMS
Sterilisation and water treatment	4,100	Most important cost item
R&D, continuous improvement	0	Not identified as being linked to NPFMS
Total	**11,340**	**(maximum of $25,865, minimum of $465)**

On average, additional capital costs associated with farm management system adoption were less for growing media manufacturers than they were for production nurseries. Growing media manufacturers spent more on annual operating expenses.

Growing Media Benefits

The average of financial benefits for growing media manufacturers is shown in Table 2.5.

Table 2.5 NPFMS Financial Benefits – Growing Media Manufacturer Average

Benefit Item	Average Benefit per Business ($)	Comments
Reduction in substandard product - extra growing media sales	60,000	Manifest as fewer disputes with production nurseries and subsequent interruption to sales.
New markets accessed - extra sales	30,000	Linked to enhanced business reputation and reluctance of customers to purchase if the business was not accredited/certified under the Nursery Production FMS. Estimated by some at around 10% of turnover.
Input savings - labour	3,750	Nursery Production FMS has led to the adoption of labour saving technologies e.g. a systems based approach resulting in leaner and more efficient operations.
Input savings - water	1,000	Water was saved during production.
Total	**94,750**	**(maximum of $202,000, minimum of $0^)**

^ 1 of 4 interviews completed stated that there were no financial benefits associated with Nursery Production FMS accreditation

Financial benefits of adoption, exceeded annual cash costs.

Other benefits of Nursery Production FMS participation identified by growing media manufacturers included:

- Staff meetings required as part of the NPFMS allow for additional input and planning and a more efficient business operation.
- Laboratory test results on growing media products are available to share with customers i.e. production nurseries.
- Improved product quality, consistency and safety meeting both customer and regulatory requirements.
- NPFMS participation safeguards media manufacturing businesses against claims by customers that growing media are to blame for greenlife losses.

Growing media manufacturers concluded that they must be accredited/certified or their customers will not buy from them. This situation is different from production nurseries whose customers are yet to insist on this requirement.

Growing Media Return on Investment

Growing media manufacturing business return on Nursery Production FMS investment was estimated using an average of capital cost, operating cost and financial benefit data presented in the above tables. Results are shown in Table 2.6.

Table 2.6 Return on Investment – Growing Media Manufacturer

Financial Indicator	Result
Gross revenue - average annual increase	94,750
Operating costs - average annual increase	11,340
Capital costs – average annual cost over ten years	8,000
Net revenue increase	75,410
Return on investment	29%
Break-even on investment	7 years

Definitions:
Gross revenue: additional business receipts before allowance for associated costs
Operating cost: annual costs directly relevant to the generation of business revenue
Capital costs: business investments required to meet Nursery Production FMS requirements and secure additional revenue
Net revenue: gross revenue after allowance for operating costs and annualised capital costs
Return on investment: the yield generated from Nursery Production FMS
Break-even on investment: number of years required to recoup Nursery Production FMS related outlays

As with production nurseries, the financial evaluation of growing media manufacturer investment in Nursery Production FMS shows that return on investment and time to break even are commercially acceptable for Australian small to medium business enterprises.

2.3 Greenlife Markets

The small size of this sector and the risk of individual firm identification prevented reporting of survey results by individual cost and benefit item. Only two businesses were surveyed.

In aggregate, additional capital costs associated with NPFMS adoption were modest (<$1,000) as were additional operating costs included labour (approximately $8,000) and maintenance (approximately $850). One respondent reported no financial benefits associated with Nursery Production FMS accreditation while the other identified modest labour savings. It is noted that at the time of survey Greenlife Markets had been Nursery Production FMS accredited for less than two years.

3 Value of NPFMS to the Australian Nursery Industry

This chapter analyses the financial return to the Australian nursery industry from investment in the Nursery Production FMS. Data was collated from HAL levy funded projects, NGIA secured grants and returns to individual nursery businesses to create an industry wide analysis. As with the individual nursery business analyses, non-financial benefits from investment in the Nursery Production FMS were also considered.

3.1 Cost of Nursery Production FMS to the Nursery Industry

Between 1992 and 2011 NGIA and HAL supported twenty two levy funded Nursery Production FMS development, implementation and administration projects, a total investment of almost $1.3 million – see Table 3.1.

Table 3.1 Industry Investment in NPFMS 1992 to 2011

Code	Project Title	Cost ($)
NY138	Compilation of guidelines for a proposed NIASA	8,200
NY417	NIASA Technical Officers Workshop No. 1	13,116
NY541	NIASA Management Committee and Technical Officers Group Conference	13,500
NY504	Implementation of NIASA in South Australia	56,391
NY95004	Implementation of NIASA in South Australia	22,640
NY602	NIASA Annual Conferences (cont'd NY9602)	15,560
NY96002	NIASA Annual Conferences (cont'd NY602)	66,472
NY99006	Strategic planning, ongoing development and evaluation of NIASA	75,168
NY02013	Ongoing development of NIASA	30,000
NY03005	Planning and development of the NIASA	157,000
NY03014	Development, Environmental Management System framework for NIASA	39,900
NY04030	Adoption of *HACCP* by NIASA	10,000
NY04029	Adoption of EMS by NIASA and AGCAS	50,000
NY04014	Ongoing development of NIASA	15,000
NY06018	Manage and Administration – Nursery Accreditation and Awards - NIASA	90,000
NY07009	Manage and Administration – Nursery Accreditation and Awards - NIASA	103,000
NY09013	Nursery Industry Accreditation and Awards - Manage and Administration	110,000
NY06015	NY06015 Industry & Stakeholder Marketing	40000
NY07501	NY07501 Nursery Industry and Stakeholder Marketing	54400
NY08009	NY08009 Industry & Stakeholder Marketing	95000
NY09017	NY09017 Industry & Stakeholder Marketing	100000
NY10502	NY10502 Industry & Stakeholder Marketing	130000
	Total	**1,287,967**

Source: NGIA June 2012

Contributions to Nursery Production FMS development were also made by the Queensland Department of Employment, Economic Development and Innovation (DEEDI) and the South Australian Research and Development Institute (SARDI).

In addition to levy funded Nursery Production FMS development projects, NGIA also invested in the ongoing operation of the program. Annual ongoing investments were associated with annual administration costs and IDO network costs not covered by accreditation fees. An annual allowance of $300,000 was made in the benefit cost analysis for these industry operating expenses after consideration of NGIA data.

3.2 Benefit of Nursery Production FMS to the Nursery Industry

Nursery Production FMS financial benefit to the Nursery industry was estimated as the sum of individual business returns and was quantified using the following data:

- At the time of writing this report there were 274 Nursery Production FMS accredited/certified businesses. These businesses include production nurseries, growing media manufacturers and greenlife markets.
- Production nursery benefit was estimated as the net increase in revenue after operating and capital costs i.e. $85,769 per annum (see Table 2.3 above). Growing media manufacturer net benefit was estimated at $75,410 (Table 2.6) Greenlife market net benefit was not estimated due to both the small Australian population of greenlife markets and survey sample size consulted.
- An attribution factor of 50% was applied to this net benefit in recognition of survey results which indicated that a large share of those businesses adopting Nursery Production FMS received no financial benefit. This attribution factor is tested with sensitivity analysis.
- Benefits begin to accrue to adopting businesses who receive a financial benefit five years after the Nursery Production FMS was completed, reach a maximum impact for early adopters in 2010 and stay at this level for ten years. By 2020 it is assumed that any increase in business revenue attributable to Nursery Production FMS efficiencies is no longer relevant.

3.3 Financial Return to the Nursery Industry

Industry benefit cost analysis results using the above data are summarised in Table 3.2.

Table 3.2 Benefit Cost Analysis Results - Industry Impact

Criterion	Core Assumptions ($' million, 30 year analysis period, 5% discount rate)
Present value of industry benefits ($'m)	81.37
Present value of industry costs ($'m)	10.15
Net present value ($'m)	71.22
Benefit cost ratio	8.01
Internal rate of return (%)	40.5

Definitions:
Present value of benefits and costs: current lump sum value of future industry benefits or costs after allowing for the time value of money.
Time value of money estimated using a real, i.e. inflation adjusted, discount rate of 5%.
Net present value: is the present value of benefits less the present value of costs
Benefit Cost Ratio: is the present value of benefits divided by the present value of costs
Internal rate of return: is equivalent to yield achieved on the Nursery Production FMS investment

From an industry perspective and using the assumption that 50% of those who adopt the Nursery Production FMS receive a financial benefit, the Nursery Production FMS has delivered a strong industry benefit – net present value of $71.22 million with a benefit cost ratio of 8.01 and a return on investment of 40.5%.

Sensitivity analysis was used to test the assumption that 50% of those who adopt Nursery Production FMS receive a financial benefit. A pessimistic scenario assumed 25% of adopters receive a financial benefit while an optimistic scenario assumed 75% of adopters receive a financial benefit. The optimistic scenario takes account of industry comment that Nursery Production FMS has created a 'trickle down' impact for non-accredited/certified nurseries.

Table 3.3 Sensitivity Analysis Results - Industry Impact

Criterion	Pessimistic Scenario (25%)	Core Assumptions	Optimistic Scenario (75%)
Present value of industry benefits ($'m)	40.69	81.37	122.06
Present value of industry costs ($'m)	10.15	10.15	10.15
Net present value ($'m)	30.54	71.22	111.91
Benefit cost ratio	4.01	8.01	12.02
Internal rate of return (%)	28.1	40.5	48.3

The sensitivity test shows that even with only 25% of adopters receiving a financial benefit from Nursery Production FMS adoption (i.e. 69 nursery businesses), additional industry revenue more than covers investment costs.

3.4 Non-Financial Return to the Nursery Industry

In addition to increasing industry revenue, the Nursery Production FMS has generated a range of less easily quantified benefits for the Australian nursery industry. Other benefits identified by surveyed nursery production businesses included:

- Increased industry professionalism – implementation of the Nursery Production FMS has taken what can be a 'backyard' industry and provided best management practices along with systems for continuous improvement. In the case of nursery production businesses adopting the Nursery Production FMS, they are thought to account for a large share of Australian greenlife production.
- Trickle down benefits – even though only 274 businesses have adopted and become accredited/certified under the program, there has been a general lift in industry standards. Non accredited businesses are aware of Nursery Production FMS requirements and have adopted many low cost/high impact practices. Examples provided by industry include simple low cost initiatives such as routine sterilisation of secateurs and work benches?.
- Improved industry biosecurity – having the Nursery Production FMS in place has provided systems and knowledge to assist industry with the control of endemic

and exotic pests and diseases. Examples provided include phytophthora, western flower thrip and most recently myrtle rust.

- Protection of the industry's social licence to operate – local and state government planning departments are familiar with EcoHort and have been reassured of the industry's environmental credentials. This has resulted in an ongoing willingness to accommodate the industry within local communities.

No non-financial costs were identified by the study.

4 Value of NPFMS to the Australian Community

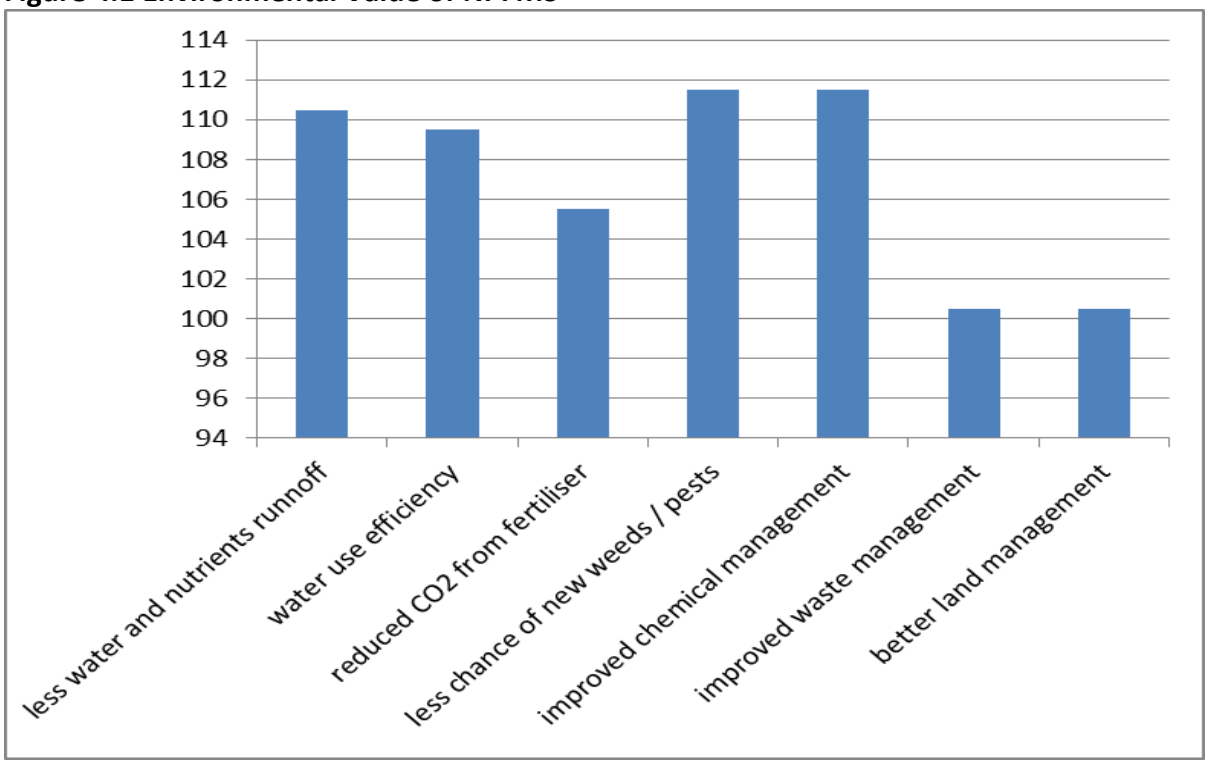
Chapter four addresses 'spillover' benefits to the Australian community from the nursery industry's investment in the Nursery Production FMS. The analysis is informed by survey data.

The survey of participating nursery industry businesses included questions on NPFMS benefits to the Australian community. Nursery industry businesses were asked to rank community benefits based on their observations, on a scale of one to five with 'five' being most important. Histograms presented in this chapter show the sum of these rankings.

4.1 Community Environmental Benefits

Seven major groups of community environmental benefits were identified and ranked using survey data.

Figure 4.1 Environmental Value of NPFMS

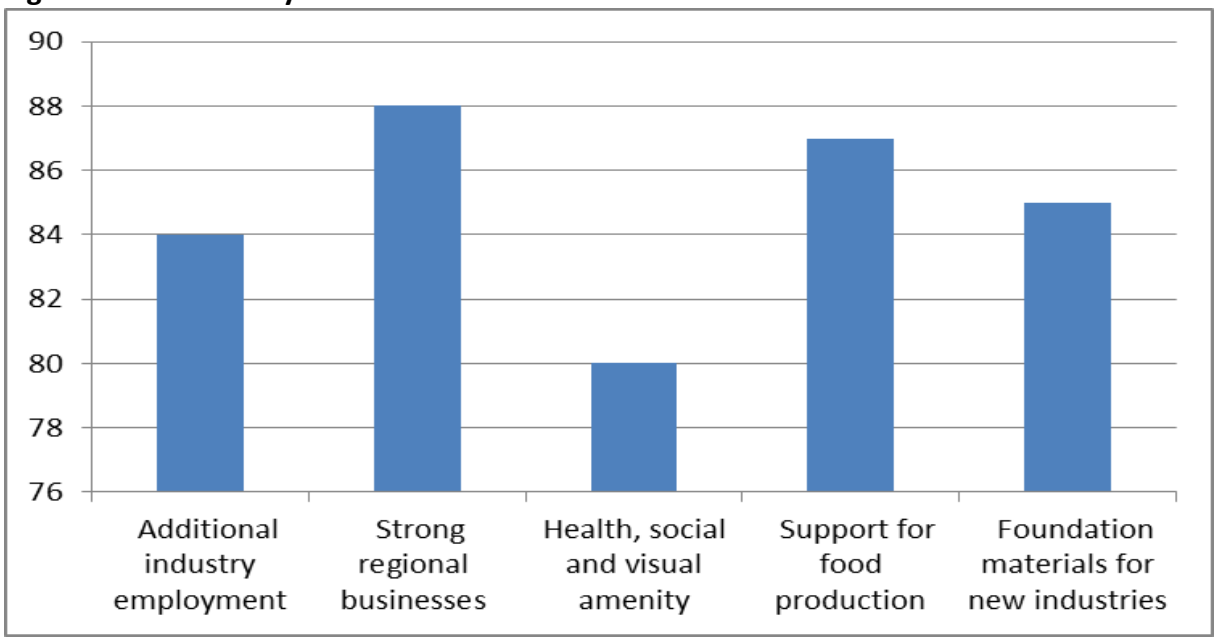


According to this data the most important spillover environmental benefits were improved biosecurity (less chance of invasive weeds and pests) and improved chemical management.

4.2 Community Social Benefits

Industry identified five groups of social benefits arising from Nursery Production FMS adoption. Strong regional businesses was ranked most highly. Foundation materials for new industries included provision of starter plants for the fibre (timber) industry – see Figure 4.2.

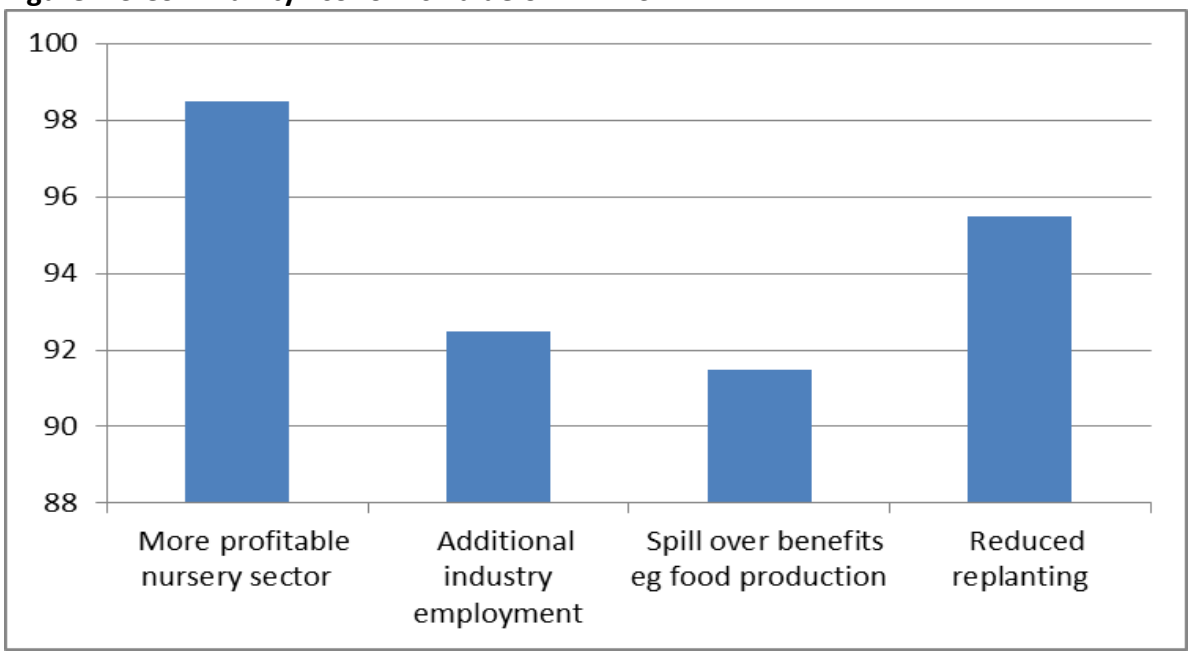
Figure 4.2 Community Social Value of NPFMS



4.3 Community Economic Benefits

Four major groups of broader community economic benefits were identified and ranked using survey data (Figure 4.3). A more profitable nursery sector was the most significant.

Figure 4.3 Community Economic Value of NPFMS



4.4 Community Economic Costs

No costs in addition to industry, NGIA and HAL investments described in Chapter 3 were identified.

5 Study Conclusions

Three types of benefit cost analysis have been completed. Results from each of the individual business, whole of industry and community spillover analysis show that benefits exceed Nursery Production FMS investment costs.

Not all adopting businesses have received a financial return. This study has shown that even if only one quarter of those who adopt receive a financial benefit, then HAL and NGIA's investment in the Nursery Production FMS has been worthwhile.

References

Chudleigh, Simpson and Clarke (2009) An Economic Analysis of HAL Investment in Projects in the Industry Development Cluster

Compost Australia (2010) Growing Media Volume Data 2010

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FreshLogic (2009) Market Monitor, access at: http://www.ngia.com.au/Category?Action=View&Category_id=105&Highlight1=market%20monitor&Highlight2=market%20monitor

Kachenko, A, Gibbs J and Walker, N (September 2010) NIASA Stakeholder Survey Summary of Findings

NGIA Investment in NIASA spreadsheets provided in December 2011 and updated June 2012.

NGIA Explanation of NIASA, EcoHort and BioSecure HACCP available at: http://www.ngia.com.au/Category?Action=View&Category_id=119

NGIA Nursery Trade Register and location of nursery businesses is available at http://www.ngia.com.au/Category?Action=View&Category_id=598

Appendix 1 – Survey Questionnaire, NPFMS Benefit Cost Analysis

Survey Purpose

To understand the 'bottom line benefits' of the industry's Nursery Production Farm Management System (NPFMS), Nursery and Garden Industry Australia (NGIA) requires an objective and independent analysis of the benefits and costs to individual businesses, the industry as a whole and the Australian community. By taking the time to complete this confidential questionnaire you will be assisting with the development of an evidence-base to support, refine and increase the adoption of the Nursery Production Farm Management System. Individual survey responses will not be reported.

Questions

1. In which program(s) of the Nursery Program Farm Management System (NPFMS) are you accredited/certified:
    a. Only the Nursery Industry Accreditation Scheme Australia (NIASA)
    b. NIASA plus EcoHort
    c. NIASA plus BioSecure
    d. All three programs i.e. NIASA, EcoHort and BioSecure HACCP?
2. In what year did you receive accreditation/certification in:
    a. NIASA _____
    b. EcoHort _____
    c. BioSecure _____
3. What enterprise type best describes your business:
    a. Production Nursery: indoor, tree/shrub, propagation, seedling / bloomers, in ground
    b. Growing media manufacturer
    c. Greenlife market
4. In what state/territory is most of your operation based?

    _____

Costs and Benefits for Individual Businesses

5. Are you an NGIA member? Are the following NPFMS accreditation costs correct for your business?

Cost of accreditation/certification programs (circle relevant cost)

Program	$ per annum NGIA Member	$ per annum NGIA Non-Member
NIASA	400 – 530	730-880
EcoHort	0-195	0-390
BioSecure HACCP	0-195	0-390

Source: NNAC meeting Dec 2011

As well as accreditation and auditing costs, we are interested in additional *operating* costs and *capital* costs incurred by your business. Question 6 deals with operating costs and Question 7 with capital costs.

6. Besides accreditation and auditing, what additional operating costs are incurred by your business– type and amount per year? Please complete the table below. The table contains some examples which may or may not be relevant to your business. There is also room for you to add other costs.

Operating Costs to individual business to meet the requirements of NPFMS

Cost Type	Annual cost to your business (Examples only)
Administration costs	• Paperwork associated with NPFMS takes our office administrator one day per week i.e. 0.2 FTE a total cost of $8,000
Staff training	• Annually we invest in NPFMS training for all our staff. Including lost work time this costs about $5,000
Labour for internal quality checking	• We employ the equivalent of an internal auditor for 0.25 FTE at a cost of $10,000
Analytical testing	• Labour costs for pathogens costs us an extra $500 per year and we do this to comply with BioSecure HACCP
Facilities: may be operational costs but are also relevant in capital costs below	• Beds • Water treatment • Hygiene
Continuous Improvement	• Required by NIASA: annual cost to your business?

7. What additional capital costs were incurred by your business in adopting the NPFMS? Please complete the table below by inserting capital items and cost when incurred. The table contains some examples which may or may not be relevant to your business. There is also room for you to add other capital costs.

Capital Costs to individual business to meet the requirements of NPFMS

Cost Type	**Annual cost to your business (examples only)**
Drainage	• Before we could get certified we had to invest $25,000 in internal site drainage and we spent this money in 2005.
Record keeping software and training	• Software cost $500 and one off onsite training was a further $1000
Beds	• Gravel maintenance cost us $*x* per annum
Hygiene	• Disinfecting
Continuous improvement	• 3 items to be listed here as per NIASA

Benefits

8. What benefits are there from adopting the NPFMS for your business – qualitative and quantitative? (Benefits that we can quantify are important.) Please complete the table below.

Benefit of NPFMS to your business

Benefit Type	Value to your business (examples only)
OAMS insurance discount	• 10% off a premium of $5,000 per year.
Market access – NIASA assists us in meeting my interstate trade requirements	• Alternative certification arrangements would have been more expensive for my business and interstate sales are now worth $500,000 per year
Improved product quality	• We attribute the quality 'dividend' to additional sales of 1% or 400 six inch pots valued at $0.50 each
Access to the Industry Development Officer (IDO) network	• Working with the IDO we have identified and adopted new technology that improves efficiency. We wouldn't have identified this opportunity outside the NPFMS. As a result we expect to save $2,000 per year on our energy bill
Enhanced business reputation	• See quality 'dividend' above.
Reduced stock throw out rate	• Our throw out rate was 17% now 4% • This translates into sales of 4,000 extra six inch pots with a wholesale value of $1.50 each
Input savings E.g. labour, energy, water (Smart Approved WaterMark), fertiliser, chemicals, etc.	•
Meeting customer requirements (NPFMS may become a precondition of purchase)	•
Other: Management efficiencies Risk reduction Marketing advantages	•
NPFMS sets a standard for staff to meet - preventative	• Proactive rather than reactive – forces cultural shift in attitude
	•

Costs and Benefits for Nursery Industry as a Whole

9. What benefits are there from the NPFMS for the industry as a whole?

   On a scale of 1 to 5 where 5 is the most important and 1 is the least important, what is the importance of the following potential nursery industry benefits?

   Please complete the table below.

Benefit of NPFMS to the nursery industry

Benefit Type	Value to the nursery industry on a scale of 1 - 5
Access to additional markets	
Community support for nursery industry	
More favourable regulation	
Improved profitability through efficiency	
Savings in disputes and litigation	
Major Biosecurity breaches avoided	
Quality assurance for customers	
Other (please specify)	

10. Besides the cost of development, are there any costs to the nursery industry as whole of having a NPFMS in place? If yes – list them

_____

_____

_____

Costs and Benefits for the Australian Community from NPFMS

11. What benefits are there for the Australian community from having the NPFMS?

    On a scale of 1 to 5 where 5 is the most important and 1 is the least important, what is the importance of the following potential benefits for the Australian community? Please complete the table below.

Benefit of NPFMS to the Australian community

Benefit Type	Value to Australian community on a scale of 1 - 5
Better environmental and natural resource outcomes For example: • Less water and nutrients leaving the nursery • Less demand for water for production (water use efficiency) • Reduced fertiliser use resulting in carbon emission savings • Less chance of new plant weeds/pests • Improved chemical management (best practices) • Improved waste management • Improved land management (erosion, etc)	
Economic benefits For example: • More profitable nursery sector • Additional industry employment • Spill over benefits to other industries eg landscape industry, food and fibre production • Reduced replanting – inappropriate plants and failure of unhealthy stock	
Social benefits For example: • Additional industry employment • Strong nursery businesses in regional Australia • Increased demand for gardening with associated positive spin offs for health, social amenity, visual amenity • Support food production in Australia underpinning food security and clean and green produce • Provide starter plants for the fibre industry (timber) across Australia	
Other benefits?	

1. Are there other comments you would like to make in relation to the operation of the NPFMS?

_____

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_____

Thank you for your time, the cost benefit analysis will be posted on the NGIA website