Project Number: AV10001

Horticulture Innovation Australia

# Final Report

## Improving yield and quality in avocado through disease management, Phase 2

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# Contents

# Summary

The overall project objective was to enhance avocado fruit standards by optimizing fruit yields, fruit quality and tree health, by improving management of insidious diseases affecting fruit, roots or whole trees.

The target audience is primarily the Australian avocado Industry, including growers in all production regions and other industry stakeholders such as consultants and agrichemical companies. New knowledge obtained in this project is of interest to the plant pathology community locally, and internationally.

Field and greenhouse project activities included:

- assessment of rootstocks under high Phytophthora disease pressure
- evaluation of improved applications of phosphorous acid
- evaluation of improved integrated approaches for managing postharvest diseases
- investigation of management options for brown root rot

Non-experimental activities undertaken by project staff included participation in avocado grower field days and scientific meetings, and other services to Industry.

Key outputs arising from the project were:

- Identification of SHSR-04 and Dusa rootstocks as highly tolerant of Phytophthora root rot (PRR). Rootstocks most capable of tolerating PRR had the highest yields.
- Improved concentration of phosphorous acid in roots achieved by adding a surfactant to low volume spray applications of phosphonate
- Industry standard fungicides effectively reduce postharvest disease, and strobilurin fungicides alone applied pre-harvest contribute significantly to higher fruit quality
- Some treatments are showing promise for reducing the viability of *Phellinus noxius* in woody root tissue (which can survive buried in soil >4 years). Alternative horticultural tree crops are less susceptible to Phellinus and could be grown in orchards currently infested with the fungus
- 3 research articles in peer-reviewed literature, 3 *Talking Avocados* articles, 1 co-authored book chapter, and contribution to several other Industry publications
- Key extension and Industry support via conference and field day presentations, and other activities

Key outcomes were identified:

- Significant increases in productivity can be achieved by PRR tolerant rootstocks
- Efficiencies in phosphonate application can be achieved by careful use of spreaders (low volume spray application) and monitoring to ensure critical levels of phosphonate are present in roots prior to peak infection periods
- Strategies to improve efficiency of treatments for anthracnose and stem end rot management have been identified, and these can be communicated to Industry
- Options for returning brown root rot-infested orchards to productivity have been identified, and require testing under field conditions
- Adoption of optimal disease management practices via a range of effective communication and extension activities
- Capacity for avocado disease R, D and E, and associated Industry support has been maintained in the project

Several recommendations at grower and R&D level were identified, and only a few are presented here:

- Growers who use low-volume spray technology/machinery (800L/Ha or less), could consider spraying phosphonate with DuWett at lower label rates (150-300mL/Ha), to increase the efficiency of uptake and translocation to roots
- Growers should monitor phosphorous acid root concentrations!  Levels of at least 80mg/kg are recommended, and preferably higher in growing climates where phosphorous acid is diluted by vigorousroot growth.
- Adoption of optimal disease management practices via a range of effective communication and extension activities
- Growers should review their spray technology and ensure that pesticides and other treatments are delivered as efficiently as possible. Pre-harvest strobulurin fungicide application can significantly improve fruit quality, even if protectants have not been adequately applied
- Proceed without further delay with the commercialisation of SHSR-04. Industry should do everything they can to support the avocado nurseries, and implement a new and improved ANVAS program
- Further work is necessary investigating Phellinus management, particularly evaluating Trichoderma and brassica biofumigation under field conditions
- It will be important to effectively communicate the research outputs, and ensure that capacity and continuity for avocado disease R&D and associated Industry support activities is maintained and enhanced into the future

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## Keywords

Avocado; integrated disease management; Phytophthora root rot; *Phellinus noxius*; anthracnose; stem end rot; *Calonectria ilicicola*;

# Introduction

The loss in productivity due to poor tree health and sub-standard quality fruit is a continuing concern for individual avocado growers and the whole Avocado Industry alike. While diseases such as Phytophthora root rot (PRR) and fruit diseases anthracnose and stem end rot, have been problematic since the early days of the industry in Australia, there have been other diseases, for example black and brown root rots, caused by *Calonectria ilicicola* and *Phellinus noxius*, respectively, which have been more recently identified as economic constraints to productivity. In addition to the specific activities outlined below, the project maintained capacity to investigate new diseases and provide support to Industry as they arose, and project team members participated in several grower field days and conferences to disseminate updates on integrated disease management practices.

The overall objective of this project was to enhance avocado fruit standards by optimising fruit yields, fruit quality and tree health, by improving management of insidious diseases affecting fruit, roots or whole trees. Project activities were directed to investigate improved management options for:

1) Phytophthora root rot. These studies included assessment of survival, health and yield of 'Hass' grafted to several different rootstocks under high disease pressure, with highly tolerant rootstocks identified. Another aspect was optimization of phosphorous acid applications, and determination of the critical minimum phosphorous acid concentration in roots required for acceptable PRR management. Our data supported the Minor Use permits and registration of an alternative formulation of phosphonate.

2) Postharvest fruit diseases anthracnose and stem end rot. The project evaluated alternative products applied as field sprays, including fungicides and non-pesticides. These were compared with Industry Standard copper and azoxystrobin applications, for an integrated approach to managing these fruit diseases and reducing reliance on copper fungicides.

3) Phellinus brown root rot. The project evaluated susceptibility of other horticultural tree crops which could be grown in infested sites, and efficacy of treatments to eliminate the long-living pathogen from woody debris in soil.

The research activities undertaken aligned to Objective 1 of the Strategic Plan for the Australian Avocado Industry (2011-2016), "To build a sustainable and competitive supply of Australian avocados to meet consumer needs".
(http://industry.avocado.org.au/AboutUs/documents/AvocadosStrategicPlan2011-16.pdf)
AV10001 followed from AV07000, "Improving yield and quality in avocado through disease management", and two previous projects AV04001 and AV01004. Rootstock assessment under high Phytophthora disease pressure was undertaken in conjunction with AV08000 "Rootstock improvement for the Australian avocado industry, Phase 3". A student project, AV09024, investigating mechanisms of tolerance to Phytophthora root rot, was undertaken with resource and personnel support from AV10001. AV10001 complements two recently-funded projects AV13021 "Exploring alternatives for managing Phytophthora root rot in avocados" and AV14012 "Investigating tree mortality during early field establishment".

# Methodology

The project focused on three key disease problems of avocado, Phytophthora root rot (PRR), *Phellinus noxius* brown root rot, and fruit diseases anthracnose and stem end rot. There were several experiments undertaken for each area, as outlined below. Further details are provided in Appendix 1. In addition to the experimental activities, significant efforts by the project leader and team were made throughout the project in extension activities and industry support services.

1) Phytophthora root rot

a) Rootstock evaluation

Assessment of survival and health of 'Hass' grafted to a range of different rootstocks continued from the previous project (AV07000). Yield data (kg) was collected for each tree in the Childers trial from 2009-2013. New field trials were planted in 2010 and 2013, in conjunction with Dr Tony Whiley (project AV08000), at Duranbah, NSW, a site with known high PRR disease pressure. Rootstocks planted in April 2010 included, but were not limited to, SHSR-04, SHSR-05, Dusa, Kidd and Reed. Rootstocks included in the 2013 trial were SHSR-04, Dusa, SHSR-08, SHSR-07, Reed and Velvick. SHSR rootstocks were selected and cloned by T. Whiley from individual mature trees which had survived high PRR disease pressure (eg. SHSR-04) or showed a high level of PRR tolerance in previous trials (eg. SHSR-07 and -08). Dusa is a rootstock developed by Westfalia Technological Services, and is highly tolerant to PRR (Smith et al 2011). Reed was included as a susceptible control.

All trees were assisted through the 12+ month establishment phase to prevent them from succumbing to PRR and dying before meaningful evaluation of rootstock effects could be recorded. Trees were treated with 0.1% solution of potassium phosphonate the day prior to planting, and 20g Ridomil (metalaxyl) immediately after planting. Prophylactic phosphonate (20% solution as a trunk paint), Ridomil, gypsum and mulch were applied throughout the establishment phase (12 months for the trial planted in 2010, and nearly 2 years for the 2013 trial). Periodic assessments of tree health were made.

b) Optimising phosphonate applications

Four trials were conducted to assess the efficacy of adding commercial surfactants to phosphonate spray applications to improve uptake and translocation of phosphorous acid to the roots. The Glasshouse Mountains trial (2011) with cv Hass, evaluated phosphonate at 0.25 and 0.5% with Pulse or DuWett. Subsequent trials in 2012 and 2013 were conducted in collaboration with commercial growers at Busselton, WA and Hampton, QLD, to evaluate phosphonate 0.5% applied with DuWett and copper (cuprous oxide). The aims were to determine if cost efficiencies could be achieved by combining phosphonate and copper spray applications with DuWett for improved PRR and fruit disease management. In 2013, 'Phostrol 500®' was provided for testing by strategic co-investment contributors Nufarm Australia, and trial data supported its registration in 2014.

c) Determination of critical phosphorous acid concentration in roots

Glasshouse assays were undertaken to determine the effective root concentration of phosphorous acid for effective PRR management. The trials also investigated the effect of rootstock, Reed, Velvick or Zutano, and graft type (ungrafted, self-graft or grafted with Hass), on phosphorous acid concentration in roots and root necrosis after phosphonate foliar spray and subsequent inoculation with *P. cinnamomi*. One sample of roots were harvested and analysed by SGS, while replicate samples were inoculated with Pc isolates with high or low sensitivity to phosphonate (as determined by in vitro EC50 studies). The *in vitro* and detached root assays were necessary due to the extreme difficulty with undertaking these studies in mature trees in the field.

2) Integrated approach to control fruit diseases

Pressure by consumers and international markets to reduce pesticide applications or shift towards "softer" options, was the impetus for trialing less conventional products and approaches to disease management. Several "non-traditional" chemical products were evaluated for their efficacy compared with industry standard copper and Amistar applications, to reduce postharvest diseases anthracnose and stem end rot. These included fungicides which are not currently registered in avocado, including a strobilurin, Cabrio; two protectants mancozeb and metiram (Rainshield and Polyram, respectively) as well as a formulation of calcium carbonate (NaturalGreen), an experimental compound from Nufarm Australia and saccharin, a known activator of defence responses in some plants.

Replicated trials were conducted in commercial orchards with 'Hass' at Glasshouse Mountains and Childers in the 2010/11, 2011/12 fruiting seasons and at Hampton in the 2012/13 season. Applications at Childers were undertaken by Dr John Leonardi, in conjunction with is project (AV08020). Treatments were sprayed at approximately 4-5 week intervals, and strobilurin fungicides (Amistar, Cabrio) applied 3 and 1 week prior to harvest, as per industry practice. Fruit were ripened at 23$^{o}$C and 65% relative humidity to encourage disease development, and assessed for anthracnose and stem end rot disease severity (% surface area or % fruit volume affected by disease, respectively) and incidence (% of fruit with symptoms) at the eating ripe stage. To determine whether NaturalGreen applications increased calcium in fruit, peel samples were collected from the untreated control and NaturalGreen treated fruit and analysed for N, Ca, Mg and K nutrient concentrations.

3) *Phellinus noxius*, brown root rot

a) *In vitro* evaluation of fungicide efficacy – Several isolates of Pn were collected in AV07000. Laboratory assays were undertaken to determine sensitivity of Phellinus to a range of available fungicides to identify those which may be part of an effective management option.

b) Field trials – There were 2 trials initiated in an orchard at Childers, known to be heavily infested with *Phellinus noxius* (Pn). One trial investigated treatments to existing trees to determine efficacy on reducing spread and deaths, and the second trial treated replant sites following tree death and removal to determine efficacy on eliminating Pn inoculum and establishment of new plantings. However, changes in farm management resulted in bulldozing of all trees in the trial block. Prior to this, soil was collected from each of the treated replant sites for glasshouse experiments.

c) Glasshouse trials – The soil treatment component of the field project was re-developed into a glasshouse based approach to evaluate soil treatment options to reduce the viability of Phellinus inoculum. The trial was conducted as closely as possible to field conditions using inoculum and soil obtained from commercial avocado orchards. A total of 9 treatments including soil fumigants, chemical soil drenches, cyclical waterlogging and a biological organism with previously observed *Phellinus* antagonistic properties were evaluated for their ability to reduce Pn inoculum viability.

4) Extension activities and industry support

Throughout the project Liz and other team members contributed significantly to field days, conferences, industry meetings and publications, and provided other pathology advice and support, as outlined in the "Outputs" section below.

# Outputs

This is a very brief account of project outputs. For further detailed information and results of individual experiments, please refer to Appendix 1.

1. *Identified SHSR-04 as highly Phytophthora tolerant.* Three independent trials undertaken in collaboration with Dr Tony Whiley (AV08000), at a site with very high Phytophthora root rot disease pressure, confirmed that this clonal selection had enhanced survival rates, and were healthier than other selections and commercial rootstocks tested. SHSR-04 is under a commercialisation plan.
2. *Rootstocks most capable of tolerating high PRR conditions had the highest yields.* Dusa™ and Velvick best tolerated high PRR in a rootstock trial at Childers (PRR was exacerbated by very high summer rainfall in 2011 and 2013), and consequently had the highest yields. Healthy trees had greater yields and larger fruit than trees declining from PRR. Differences among source of Velvick seed highlight the need for closed-pollinated Velvick blocks for nursery seed stocks.
3. *Graft union effects.* There were indications of graft type affecting translocation of major elements and phosphorous acid. Velvick and Zutano seedlings grafted with Hass had a reduction in phosphorous acid translocation from leaves to roots of approximately 50% compared with ungrafted plants. Trends in nutrient translocation from roots to leaves were inconsistent.

4. *Improved translocation of phosphonate to the roots.* Adding DuWett to low-volume (500L/Ha)

phosphonate sprays to Hass or Reed increased translocation to the roots and developing fruit by up to 2x compared with phosphonate sprays without DuWett, or standard injection. Phytotoxicity (leaf burn and drop) can result if using DuWett at high-volume rates. Although phosphorous acid in fruit peel was also increased, the temporary Maximum Residue Limit (MRL) established by the APVMA of 500mg/kg for avocados marketed and consumed in Australia was not exceeded.

5. *Critical concentration of phosphorous acid in roots must be above 80 mg/kg (80ppm).* Greenhouse assays with seedlings has demonstrated a negative relationship between phosphorous acid concentration in roots and % of necrosis after inoculation with *Phytophthora cinnamomi*. Velvick rootstock accumulated greater concentrations of phosphorous acid than Zutano, and had less severe root necrosis.

6. *Emergency use permits for phosphonate spray application.* Project data supported the application to APVMA for emergency use permits (eg. Permits 11828 and 12827) allowing 0.5% concentration of phosphonate for foliar spray, where the current registered label rate is ineffective at 0.1%. In addition, our 2013 field data supported registration of 'Phostrol 500®' by Nufarm Australia in 2014.

7. *Industry standard copper + strobilurin most effective at minimizing postharvest anthracnose disease*. Regular sprays of Norshield copper (cuprous oxide) through the season with two pre-harvest applications of strobilurin fungicides (either Amistar or Cabrio), were the most consistently effective treatments at reducing anthracnose and increasing fruit marketability. Stem end rot is much more difficult to manage with field fungicide applications, and industry standard treatment was not consistently effective. Mancozeb (Dithane Rainshield) consistently reduced disease compared with untreated controls, but at the rates tested, was not as efficacious as Industry Standard. NaturalGreen did not consistently improve fruit quality, and did not increase calcium content in fruit peel. Other "non-traditional" treatments were ineffective at the rates applied. Late season Amistar applications may account for significant improvement in fruit quality, irrespective of treatments through the season.

8. Phellinus noxius *can survive in woody debris buried in soil for >4 years.* The brown root rot fungus was successfully isolated onto media in 40% of woody root pieces, 2-4cm in diameter, encrusted with the characteristic "stocking", collected from a depth of 1m underneath where a tree had died from brown root rot and been removed.

9. *Greenhouse method for* Phellinus noxius *pathogenicity testing developed.* Several methods were tested to establish a reliable assay for evaluating brown root rot in controlled environment conditions. Inoculations with colonized grain (approximately a teaspoonful placed approximately 3cm below the potting mix surface, and close to the stem/crown of the plant) were the most successful when introduced in the warmer months of summer, November to May.

10. *Avocado seedlings are extremely susceptible to* Phellinus noxius. Greenhouse trials demonstrated near complete mortality of avocado seedlings inoculated with an isolate (originally isolated from avocado). Approximately 40% of macadamia seedlings also died. Passionfruit, citrus, and mango appear to be weak or non-hosts.

11. *Treatments to reduce viability of* Phellinus noxius *in woody debris identified.* The most effective treatments to "knock out" Pn in root debris in greenhouse pot trials were *Trichoderma* sp. and chloropicrin, with propiconazole and mustard biofumigation also somewhat effective. Urea, heavy irrigation and paclobutrazol treatments were ineffective. The *Trichoderma* sp. was isolated from a

previous greenhouse trial, where it detrimentally colonized Pn grain inoculum. It has been stored for future evaluation.

12. *Research publications.* Three research articles in peer-reviewed scientific journals and one online fact sheet have been published. Three technical articles have been published in *Talking Avocados.*

13. *Other publications.* Co-authored disease chapter in "The Avocado: Botany, production and uses, 2nd Ed" book, contributed to the "Industry Biosecurity plan for the Australian Avocado Industry, Version 2.0", and the "Orchard Biosecurity Manual", published in September 2011), and contributed photos and text to the avocado "Problem Solver" and poster for integrated management of PRR (both produced by Simon Newett, DAF).

14. *Extension activities and Industry support*. Throughout the project Liz and other team members were key participants in more than 10 avocado grower field days held throughout Australia. Project results were also presented at approximately 8 scientific meetings with broad international attendance, for example, the 7th World Avocado Congress (2011), International Horticulture Congress (2014), Aust/NZ Avocado Growers Conference (2013), Australasian Soilborne Disease Symposia (2012, 2014), Australasian Plant Pathology Society meeting (2013). Liz was also an invited participant (with others from USA, Chile and Spain) of the Westfalia Technological Services research forum, held in South Africa in 2013. Project members participated in Industry R&D meetings, the Strategic Agrichemical Review Process (SARP), the ANVAS review, the Orchard Productivity Review and the development of the Strategic Investment Plan. They also addressed numerous grower (and other stakeholder) queries.

# Outcomes

Key outcomes were identified:

1) *Significant increases in productivity can be achieved by adoption of Phytophthora root rot tolerant rootstocks, Dusa and SHSR-04.* Several field trials over a number of years have confirmed the superior tolerance of clonally propagated SHSR-04 and Dusa to Phytophthora root rot. The trials were conducted under extremely high PRR disease pressure, with prophylactic treatment only applied during the initial establishment phase. Dusa is currently available commercially from one nursery, although has now been licensed to another 3. Constraints to greater adoption of Dusa include higher cost compared with seedlings and limited availability. SHSR-04 is under a commercialization pathway. HIA is currently preparing a Request for Proposal document, so that potential commercial entities interested in progressing with commercialization of SHSR-04 can submit a proposal for consideration by HIA, the two other IP owners and industry.

2) *Greater productivity from healthier trees can be achieved by more efficient use of phosphonate.* Efficiencies in phosphonate application can be achieved by careful use of spreaders as low volume spray application to healthy trees. Root phosphonate levels can be greatly increased while reducing absolute phosphonate applied. We maintain that sick trees should be injected with phosphonate twice a year, when roots are actively growing. Monitoring is required to ensure that the critical concentration of phosphorous acid in roots is above 80 mg/kg (80ppm) throughout the infection period, otherwise

applications will be ineffective, particularly in wet years. In orchards where phosphonate has been used for many years, and in regions where root growth is vigorous, even higher levels may be required. Rootstocks with a degree of tolerance to PRR may require fewer phosphonate sprays, as they accumulate more phosphonate than less tolerant rootstocks.

3) *Industry standard fungicide sprays still provide the best quality fruit.* Our research did not identify a superior and more environmentally acceptable in-field treatment for reducing severity of fruit diseases, such as anthracnose and stem end rot. Industry standard copper + late season strobilurin sprays most consistently reduced damage caused by anthracnose, but stem end rot remains intractable. Late season strobilurin applications may account for significant improvement in fruit quality, irrespective of treatments through the season.

4) *Reduced economic impact of* Phellinus noxius*, causing brown root rot.* Options for returning brown root rot-infested orchards to productivity have been identified from greenhouse experiments, and require further testing under field conditions. An unexpected outcome was the identification of a Trichoderma sp. fungal isolate which was able to colonise and eliminate viable Phellinus in woody root debris. Trichoderma spp. are known biocontrol agents, but efficacy for reducing root diseases are often inconsistent under field conditions. Biofumigation with a brassica green manure was also effective. Chloropicrin fumigation was also effective, although may not be a commercially or environmentally feasible option. Replanting avocado or macadamia into Pn infested soil, where woody debris remains, would be economically irresponsible, as avocado is particularly susceptible, and macadamia moderately susceptible. Passionfruit, citrus, mango may be suitable (horticultural) alternatives, if infested sites cannot be fallowed.

5) *Adoption of optimal disease management practices via a range of effective extension and communication activities*. Science-based solutions to disease problems in avocado have been delivered throughout the project, via participation in field days and contributions to printed material (eg. peer-reviewed papers in scientific journals, Chapter in "The Avocado", articles in Talking Avocados, Phytophthora poster, Problem Solver Field Guide, etc.), and electronic media (eg. Best Practice Resource). The adoption rates and impact of these activities are difficult to measure, and are perhaps captured in specific extension projects. As the full extent of project outputs are now realised, further recommendations on improved disease management can be communicated in coming years. New recommendations may challenge some growers, but others will be quick to test and adopt improved management practices.

6) *Enhanced capacity for industry and the R&D team.* The project has allowed for the maintenance of a strong team working to help the industry with productivity constraints. Team continuity is extremely important to industry, and in this case has allowed for the development and funding of smaller projects (for example, AV13918, AV13021 and AV14012 arose from work done by the team in AV10001 and other HAL-funded projects), training of PhD students, and support for industry in advisory roles (eg. input into the biosecurity plans, SARP and ANVAS review). Not only has the project delivered specific outputs directly relevant to Industry, but the team's knowledge of the diseases and capacity to conduct high-quality research has increased tremendously.

As seen from the above, the majority of outcomes identified in the original proposal, developed in 2007, have been realised. Significant losses due to Phytophthora root rot can be checked by adoption of tolerant varieties and improved application of phosphonate, with monitoring to ensure critical root levels of phosphorous acid are achieved. An unexpected outcome was the identification of a Trichoderma isolate with activity against *Phellinus noxius*. The isolate was an unwanted contaminant in greenhouse trials. There are several commercial Trichoderma biocontrol products on the market, including one developed in Australia specifically for Phytophthora root rot in avocado (Phytoguard®), however, they have not been tested for efficacy against Pn. Another example of a useful outcome was being able to quantify the adverse effects of zero site preparation when replanting into sites heavily infested with Pc; 75% of the plant deaths occurred in sites where a sick tree had been removed in the 2 months prior to replanting. Additional benefits not captured under the outcome headings above, include data support for Emergency Use Permits for phosphonate sprays, registration of Phostrol by Nufarm Australia, identification of an emerging disease threat which is a significant problem in California and Israel (branch dieback associated with *Fusarium* sp. and vectored by a small beetle) and providing insect and disease diagnosis for growers. These outcomes, and potentially new ones arising from associated projects, will be realised in the medium to long term (5-20 years).

# Evaluation and Discussion

The project has been extremely successful, and will have significant impacts in the medium to long term (5-20+ years). The longer project timeframe (nearly 5 years) enabled continuity and a depth of study which is not usually possible in 3 year projects, particularly those with such a high proportion of field-based activities. All outputs identified are highly relevant to Industry at very practical levels. The project leader is committed to ongoing delivery of they key messages identified which will improve efficiency of disease management and thus enhance overall productivity, although such adoption and improvement is difficult to quantify.

Phytophthora root rot (PRR) remains the most important constraint to growers. The project identified the superior PRR tolerance of an Australian selection, SHSR-04. The delay in its commercialisation has been frustrating, but is outside the control of the current project. HIA is currently preparing a Request for Proposal document, so that potential commercial entities interested in progressing with commercialization of SHSR-04 can submit a proposal for consideration by HIA, and other IP owners. Dusa is also highly tolerant and is commercially available. Although Velvick does not have the level of tolerance as SHSR-04 or Dusa, it is moderately tolerant and tree health and productivity can be maintained with sound integrated PRR management. This is the first time that Zutano has been included in a replicated trial assessing tolerance to PRR. Zutano is frequently planted in the Tristate area, and now we have preliminary information suggesting it is not as tolerant as other rootstocks, so that growers planting this rootstock will have to be vigilant with their integrated PRR management. New information realised in the project includes the superior translocation if DuWett is added to phosphonate and sprayed at low volumes, and that levels of phosphorous acid in roots of at least 80mg/kg are required prior to peak infection and disease development periods. This new information can be communicated to Industry for immediate adoption. Care will have to be taken with the DuWett recommendation as trees could be negatively impacted if applications are not made very carefully and

according to label directions, ie. low volume, check nozzles and droplet size, don't spray when wet or misty etc.

There are several things we have learnt from project activities. Field and greenhouse studies with Phellinus are extremely difficult. Greenhouse inoculations were successful only during the warmer months, and the project was varied (extended) to accommodate the final trials. Field studies are also difficult due to the highly variable nature of Phellinus incidence (difficult to achieve satisfactory experimental replication) in the orchard situation. In retrospect, it would have been better to start with greenhouse evaluation of management candidates, rather than attempting two very large and time-consuming field trials. However, we have identified some promising treatments which should be included in future field-based evaluations. Passive injection of triazole fungicides into Phellinus-affected trees should be considered for high-value trees at risk of brown root rot development, and otherwise, our current recommendation of tree removal and installation of root barriers is working where implemented correctly.

While the project did not identify a fungicide-free alternative for reducing the incidence and severity of fruit diseases, we showed that the application of final strobilurin fungicides can contribute significantly to improving fruit quality, in the absence (or reduction) of the usual copper program. This is important for all who are concerned about the environmental effects of copper. Calcium carbonate applied as a foliar treatment in this project did not result in enhanced levels of Ca in fruit peel or reduced anthracnose development in fruit. It is likely that Ca has to be taken up by roots and translocated to fruit in the developing stages to have a positive effect on the balance of N:Ca and fruit quality.

Outside of direct interactions with colleagues and collaborators (listed in Acknowledgements), there were opportunities for Industry feedback on project activities. Perhaps the most useful were the annual R&D meetings, where short project updates were presented to the Board of Avocados Australia Ltd., followed by questions and discussion. Interaction with growers more widely occurred at the many field days attended (more than 10 during the project). Presentation of project results at conferences also provided valuable feedback from researchers and others within the Industry in Australia and overseas.

The Industry support and capacity components of this project cannot be understated. As well as delivering outputs and recommendations for improved disease management, the project allowed for the maintenance of a strong team to provide direct support for Industry and individual growers with pathology/disease issues as they arose. It is extremely important that this capacity is maintained and enhanced into the future, so that Australian horticultural R, D and E contributes to ongoing productivity and efficiency gains.

# Recommendations

Immediate practical recommendations to growers:

1. Take extreme care when planning and planting your orchards, particularly if replanting an old avocado block. Sites prepared in haste are likely not to be conducive to optimal orchard establishment. Pay particular attention to landform and drainage, and other Phytophthora management strategies. Our work has shown that trees are more likely to die if planted into a site soon after removal of declining trees, than if planted into a "fallow" site.

2. Growers who use low-volume spray technology/machinery (800L/Ha or less), could consider spraying phosphonate with DuWett at lower label rates (150-300mL/Ha). This will increase the efficiency of uptake and translocation to roots. Growers using spreaders need to be very careful with their rates, weather conditions etc. so that phytotoxicity and leaf drop is not severe. Our current recommendation regarding whether to inject or spray with phosphonate remains clear – growers who have declining trees should inject their trees (also consider major pruning), rather than spraying. Spraying achieves good results in healthy trees which have a good canopy to uptake and translocate phosphonate to the roots.

3. Growers should monitor phosphorous acid root concentrations! We keep emphasising this at field days, and would like to see greater adoption of this practice. We recommend levels of at least 80mg/kg, and preferably higher in growing climates where phosphorous acid is diluted by vigorous root growth. Fewer foliar applications may be required if trees are on more resistant rootstocks than susceptible rootstocks.

4. Industry standard copper + strobilurin fungicide sprays most consistently reduce postharvest anthracnose (and less consistently) stem end rot. Several other products were trialled. If growers operate a reduced or nil copper program, and have disease-conducive conditions throughout the season (high rainfall, older orchards), then pre-harvest applications of strobilurin (eg. Amistar), can improve marketability of fruit. These fungicides should be used according to label directions so that resistance does not develop, rendering them ineffective. Growers should review their spray technology and ensure that fungicide is delivered as efficiently as possible, without waste or negative off-target effects.

Recommendations to Industry and R&D investment decision makers:

1. It will be important to effectively communicate the research outputs, which will fulfill outcomes for many years to come. Many of the outputs have only been achieved in the last 1-2 years, so practical recommendations around improved disease management strategies, have not yet been communicated widely to Industry. These communication activities were conducted under the current project and should be included for funding in future projects.

2. Proceed without further delay with the commercialisation of SHSR-04. I understand that HIA is

currently preparing a Request for Proposal document, so that potential commercial entities interested in progressing with commercialisation of SHSR-04 can submit a proposal for consideration by HIA, the two other IP owners and industry.

3. Industry should do everything they can to support the avocado nurseries. More operations producing high quality trees should be encouraged to join the voluntary accreditation scheme (ANVAS). ANVAS has been reviewed and the key recommendations should be implemented as soon as possible. Industry should endorse the widespread adoption of clonally propagated trees, for superior PRR tolerance and orchard uniformity. Quality of such trees has improved in recent years, and time to generate them has decreased.

4. Evaluation of the economic impact of PRR in Australia. This has not been done, but requires collaboration with economists. Remote sensing may be a useful tool to rapidly determine extent of PRR decline over large areas, once the methodology has been optimised. Growers and Industry will then comprehend the cost of PRR to their operations, and the benefit of integrated management. This methodology will also enable quantification of outcomes.

5. Further work is necessary on phosphonate. Our work has shown a wide range in sensitivity to phosphonate exists among Pc isolates. We need to confirm what we suspect is happening, that is, continued use of phosphonate selects for populations of Pc which are less sensitive to the chemical, thus higher and higher concentrations in roots are required. There is no evidence for true resistance to phosphonate (a genetic mutation allowing the pathogen to overcome the chemical). Further work is required to confirm whether fewer foliar applications may be required if trees are on more resistant rootstocks than susceptible rootstocks.

6. There are many "new" products and application methods for delivering phosphonate to trees. Preliminary research has shown that some of the less expensive formulations are not as effective as known high quality products (eg. Agrifos). Phosphonate levels in roots were lower, despite equivalent application rates. Industry needs to decide if they want further work in this area. Perhaps such studies could be conducted in collaboration with growers who are already using the new chemical or application method (eg. Phoscap), or those who are interested in trialing them.

7. Continue to assess methods and/or new chemistries for PRR management, eg. Trichoderma, brassica biofumigation and mandipropamid. Mandipropamid is an anti-oomycete which has shown promise to reduce post-infection effects of Pc in greenhouse trials (AV13021). Syngenta have indicated interest in further trial work. There may be some value in evaluating a selection of biological amendments that are currently on the market. Investigations of soil health including suppressive soils is warranted, firstly through a revision of relevant literature, which will then guide a targeted experimental program. Further trials comparing Phytoguard®, a commercially available product containing Trichoderma, with the isolate of Trichoderma identified in this project, for their efficacy against *Phytophthora cinnamomi*, *Phellinus noxius* and *Calonectria ilicicola* (black root rot) are warranted.

8. Further testing of fungicides (eg. new chemistries) which may be used as alternatives to copper is not a high priority, unless a very promising new chemistry became available and testing in avocado was conducted in collaboration with the agrichemical industry. It would be preferable for industry to emphasise the importance of correct application for maximum efficacy, through a series of extension/communication channels, eg. spray nozzles and droplet size, coverage etc. Application with crop oils or organosilicone surfactants, for example Designer and DuWett for use with low

volume spraying, will improve the efficacy of the product and reduce costs and potential adverse environmental impacts.

9. Further work is necessary investigating Phellinus management, particularly evaluating Trichoderma (commercial formulation Phytoguard and the isolate identified in the current project) and brassica biofumigation under field conditions. Assess whether promising chloropicrin result deserves follow-up field trials. Infiltration of fungicides into trees is unlikely to be a viable option, unless there are single or a few trees which are highly valuable (eg. rootstock mother trees) and under threat from Phellinus. This could complement activities in AV14012 on the nectriaceous soilborne pathogens.

10. Further work is required on Fusarium dieback (vectored by the polyphagous shot hole borer) in avocado. Some orchards in NQ are having significant issues with this, and it is limiting productivity in some areas of California and Israel, but we know relatively little about it in Australia.

11. Ensure that capacity and continuity for avocado disease R&D and associated Industry support activities is maintained and enhanced into the future. There needs to be adequate funding for capacity included in future projects, as these activities are time-consuming but crucial for Industry.

# Scientific Refereed Publications


Journal articles

Kasson, M. T., O'Donnell, K., Rooney, A. P., Sink, S., Ploetz, R. C., Ploetz, J. N., Konkol, J. L., Carrillo, D., Freeman, S., Mendel, Z., Smith, J. A., Black, A., Hulcr, J., Bateman, C., Black, A. W., Campbell, P. R., Geering, A. D. W., Dann, E. K., Eskalen, A., Mohotti, K., Short, D. P.G., Aoki, T., Fenstermacher, K. A., Davis, D. D., Geiser, D. M., 2013. An inordinate fondness for *Fusarium*: Phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. *Fungal Genetics and Biology* **56**: 147-157.

Dann, E., Pegg, G., Shuey, L. 2012. *Phellinus noxius* (Corner) G.H. Cunningham et al. *Pathogen of the Month* http://www.appsnet.org/Publications/potm/pdf/Aug12.pdf

Dann, E. K., Cooke, A. W., Forsberg, L. I., Pegg, K. G., Tan, Y-P. Shivas, R. G., 2012. Pathogenicity studies in avocado with three nectriaceous fungi, *Calonectria ilicicola, Gliocladiopsis* sp. and *Ilyonectria liriodendri*. *Plant Pathology* **61**, 896-902.

Smith, L. A., Dann, E. K., Pegg, K. G., Whiley, A. W., Giblin, F. R., Doogan, V., Koppitke, R., 2011. Field assessment of avocado rootstock selections for resistance to Phytophthora root rot. *Australasian Plant Pathology* **40**: 39-47.

Chapter in a book or Paper in conference proceedings

Dann, E. K., Ploetz, R. C., Coates, L. M., Pegg, K. G., 2013. Foliar, fruit and soilborne diseases. In: Schaffer, B., Wolstenholme, N., Whiley, A. (Eds.), The Avocado: Botany, Production and Uses, 2nd Ed. ABI Publishing, Wallingford, UK, pp. 380-422.

# Intellectual Property/Commercialisation

The isolate of Trichoderma which deactivated *Phellinus noxius* in greenhouse tests has commercialisation potential. The IP is shared between Queensland Department of Agriculture and Fisheries and HIA. Further testing of this isolate is required. It is noted that Trichoderma is frequently isolated when root tissue from greenhouse or field trees are plated onto media. There are a large number of Trichoderma-containing biological amendments currently available, and nursery operators (and possibly also growers) are already using these. Phytoguard® is a product containing *Trichoderma hamatum* which was isolated from avocado roots at Mt Tamborine, and is being recommended by the manufacturers and distributors for reducing Phytophthora root rot in avocado. Given that this product is already formulated and available, further trials comparing Phytoguard with the isolate of Trichoderma identified in this project, for their efficacy against *Phytophthora cinnamomi*, *Phellinus noxius* and *Calonectria ilicicola* (black root rot) are warranted. If the current isolate is no more effective at reducing impacts of these pathogens than Phytoguard, there seems little point progressing with commercialisation.

Note that while DAF currently shares IP in the Trichoderma isolate, a new project is likely to be contracted through the University of Queensland, so that a background IP arrangement will be required at project commencement.

# References

Where required, references are provided in Appendix 1.

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# Appendices

Appendix 1. Summary of results for AV10001, prepared by Liz Dann, August 2015.

**Appendix 1:** Summary of results for AV10001, prepared by Liz Dann, August 2015

1) Phytophthora root rot

a) Rootstock evaluation

Original trials, planted at Duranbah and Childers May 2006.

Assessment of survival and health of 'Hass' grafted to a range of different rootstocks continued from the previous project (AV07000). The tree health data up to 2011 for these trials have been presented in Smith et al., (2011). Tree survival data 2011-2013 is presented in Table 1 and Table 2. At Duranbah in 2013 there were 60% of surviving trees on Dusa, and 70% on SHSR-04 and Hass (ungrafted), compared with only 10% of surviving trees on Duke 7, Reed and none on A10 rootstocks (Table 1). While the trees are clearly compromised, ie. have not achieved a desirable height for trees 8 years old, and are not yielding commercially acceptable volumes of fruit, the survival of Dusa and SHSR-04 demonstrates the strength and commercial desirability of these rootstocks under high PRR disease pressure. It is interesting that once the initial population of susceptible seedlings had died (ie. within the first 5 years), the rate of death amongst survivors was reduced, indicating their inherent tolerance to PRR.

At Childers, the Pc disease pressure was much less than at Duranbah, and the trees were able to establish well. In 2009, three years after planting trees, every rootstock but Reed and Velvick (clone) had minimal or no evidence of decline due to PRR (Figure 1). However, by 2010 there were large declines in tree health, with Hass on Reed rootstock showing the greatest decline, while Dusa™, Latas™, A8 and Velvick Lynwood seed (Velvick$^{L}$) rootstocks were the healthiest trees. In the absence of phosphonate applications and above average annual rainfall patterns, tree health continued to decline over 2010-2013. Hass on Dusa™ trees remained the healthiest over this time, and Velvick$^{L}$ and Velvick (clonal) were also significantly healthier than most other rootstocks in the trial. The wet summers of 2011 and 2013 saw rapid decline in tree health, and only 20% of the susceptible Reed survived. Dusa, Latas, Velvick clonal and Velvick$^{L}$ rootstocks all survived well under such high PRR pressure (Table 2).

Three years after planting (2009) the highest yields were from A8, Velvick (clonal), Velvick$^{L}$ and A10 rootstocks (Figure 2). The yields from these rootstocks were significanty higher than from Reed or Latas™. In 2010, highest yield per tree were from Velvick$^{L}$ rootstock, which was more than 3 times higher than the lowest yielding rootstock, Reed, but also significantly higher than from Velvick Andreson seed (Velvick$^{A}$) and Velvick (clonal) rootstocks.

The very severe weather events of late 2010 and early 2011, which saw major flooding in the Brisbane region, affected crop yields in south east Queensland significantly, and caused widespread tree deaths due to waterlogging. Thus, yields in 2011 were lower than in 2010. Yields were highest for A10, with Velvick$^{L}$ and Latas™ also yielding >30 kg

per tree (Figure 2). Continued above average rainfall in 2012 and the lack of phosphonate further impacted yields in 2012 and 2013. There were no significant differences among treatments, however the highest yielding trees were on Dusa™ and Velvick$^{L}$ rootstocks. Cumulative yield per tree (total yield across all years) was highest for Velvick$^{L}$, but significantly higher only than Velvick$^{A}$ and Reed.

Tree health had a significant effect on the proportion of trees yielding fruit, with a definite downward trend as the health of the trees decreased (data not shown). Trees not suffering decline due to PRR produce significantly larger fruit. The tree with a health rating of 3 had significantly larger fruit than trees rating 4 or greater (where 0=healthy and 10=dead), in 2013.

The superior performance of Velvick$^{L}$ compared with Velvick$^{A}$ seedling rootstocks with respect to tree health and yield is interesting, and demonstrates the potential for out-crossing to change seedling performance. The significance of this result should be checked with molecular studies to determine the extent of genetic variance among seedling lines of the same variety from different sources. It highlights the significant commercial benefits to be gained from producing seed for nursery use in isolation from out-crossing opportunities during flowering.

**Table 1.** Survival of trees (% alive compared with total number planted) grafted to different rootstocks in Duranbah trial planted in May 2006 (original trial)

| Rootstock | Source | Tree survival Oct 2011 | Tree survival June 2012 | Tree survival March 2013 |
|---|---|---|---|---|
| Latas ™ (clonal) | Birdwood | 30 | 30 | 30 |
| Dusa ™ (clonal) | Birdwood | 70 | 70 | 60 |
| Velvick (clonal) | Whiley | 20 | 20 | 20 |
| Velvick (seedling) | Andersons | 70 | 50 | 50 |
| Duke 7 (clonal) | Whiley | 10 | 10 | 10 |
| Barr Duke (clonal) | Whiley | 30 | 30 | 20 |
| Thomas (clonal) | Whiley | 30 | 30 | 30 |
| A10 (seedling) | Andersons | 30 | 0 | 0 |
| Reed (seedling) | Andersons | 10 | 10 | 10 |
| SHSR-04 (clonal) | Whiley | 70 | 70 | 70 |
| Hass (clonal) | Whiley | 70 | 70 | 70 |

**Figure 1.** Health of 'Hass' trees grafted to different rootstocks and planted in 2006 at Childers, QLD, with high PRR pressure.

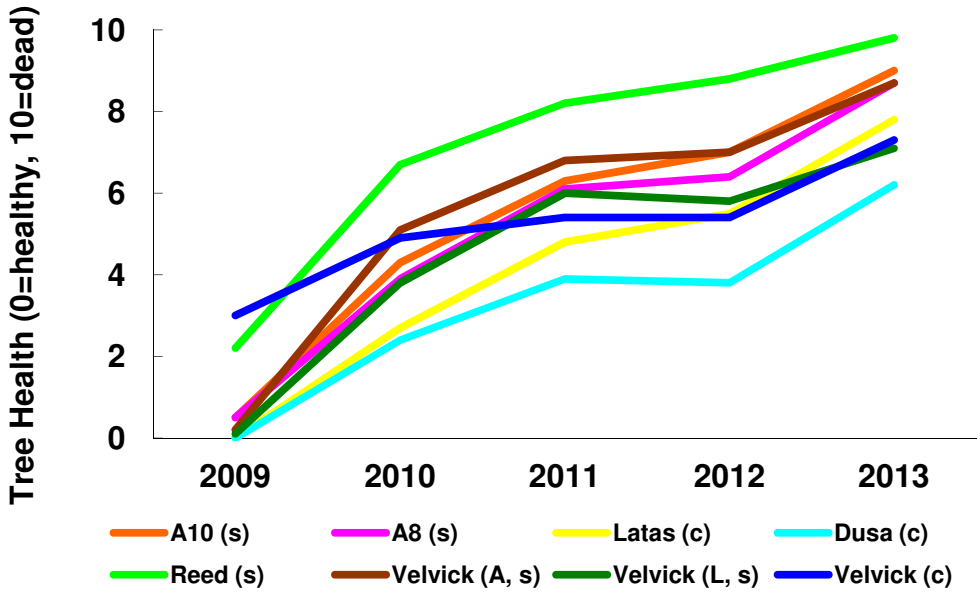
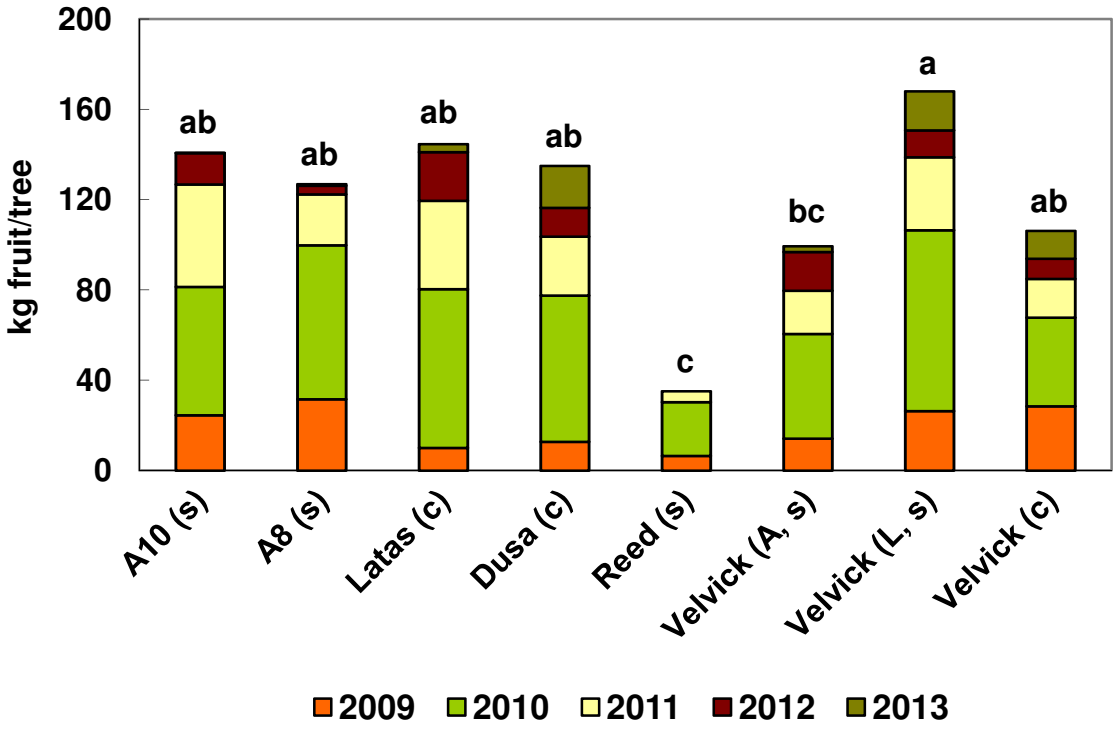



**Table 2**. Survival of trees (% alive compared with total number planted) grafted to different rootstocks in Childers trial planted in May 2006

| Rootstock | Tree survival Aug 2011 | Tree survival May 2012 | Tree survival April 2013 |
|---|---|---|---|
| Latas ™ (clonal) | 100 | 100 | 80 |
| Dusa ™ (clonal) | 100 | 100 | 100 |
| Velvick (clonal) | 100 | 100 | 78 |
| Velvick (Anderson seed) | 100 | 100 | 60 |
| Velvick (Lynwood seed) | 90 | 90 | 80 |
| A8 (seedling) | 90 | 90 | 60 |
| A10 (seedling) | 90 | 90 | 50 |
| Reed (seedling) | 90 | 70 | 20 |

**Figure 2.** Average yield (kg) per 'Hass' tree grafted to different rootstocks and planted in 2006 at Childers, QLD, with high PRR pressure.




Trials planted in 2010 and 2013

New field trials were planted in 2010 and 2013, in conjunction with Dr Tony Whiley's rootstock evaluation project, (AV08000), at Duranbah, NSW, a site with known high PRR disease pressure. Rootstocks planted in April 2010 included, but were not limited to, SHSR-04, SHSR-05, Dusa, Kidd and Reed. Rootstocks included in the 2013 trial were clonal SHSR-04, Dusa, SHSR-08 (the A10xVelvick seedling survivor), SS3-1, SHSR-07 (the surviving seedling of SHSR-02), and seedling Zutano, Reed and Velvick. SHSR-07 and SHSR-08 were sourced from healthy surviving trees from the rootstock trial planted at Duranbah in 2007. These trees (originally on seedling rootstock) were cut back to encourage rootstock to shoot, then new growth recovered and cloned. All trees were assisted through the 12+ month establishment phase to protect the young trees from succumbing to PRR and dying before meaningful evaluation of rootstock effects could be recorded. Trees were treated with 0.1% solution of potassium phosphonate the day prior to planting, and 20g Ridomil (metalaxyl) immediately after planting. Prophylactic phosphonate (20% solution as a trunk paint), Ridomil, gypsum and mulch were applied throughout the establishment phase (12 months for the trial planted in 2010, and nearly 2 years for the 2013 trial). Periodic assessments of tree health were made.

An assessment of tree health was made of trees planted to different rootstocks at Duranbah, NSW, in April 2010. Limited numbers of trees remain in this trial, as several trees which were dead or almost dead (health ratings of 7-10), were removed to make way for the trial planted in 2013. In June 2014, the highest percentages of surviving trees were those grafted to SHSR-04 at 53%, and the fewest survivors 5% for Reed rootstock (Table 4). SHSR-04 was significantly healthier than all rootstocks other than Reed/11, which is Reed seedling with a Reed scion although there was only 1 tree of the original 10 remaining.

Trees planted in May 2013 were assessed for health following the spring and summer leaf flushes in December 2013, May 2014, and March 2015. Differences in tree health among rootstocks were significant 12 months after planting (Table 3). Trees grafted to SHSR-04, SHSR-08 and Dusa were significantly healthier than Zutano, SS3-1 and the susceptible check Reed rootstocks. This is the first time that Zutano has been included in a replicated trial assessing tolerance to PRR. Zutano is frequently planted in the Tristate area, and now we have preliminary information suggesting it is not as tolerant as other rootstocks. The rapid decline in tree health, and high mortality rate across the trial, despite frequent metalaxyl and phosphonate applications, highlights the effect of high *P. cinnamomi* (Pc) disease pressure following tree decline and death/removal in orchards. The planting site history had a significant effect on plant mortality; 75% of the plant deaths occurred in sites where a sick tree had been removed in the 2 months prior to replanting. This information will be communicated to growers, and provides support for careful site preparation, including incorporation of chicken manure, and a period of fallow, prior to replanting.

Graft compatibility/incompatibility

The effect of the graft union on nutrient and phosphonate translocation, and reaction to Pc, was assessed in young plants grown in a glasshouse. Three graft treatments in two seedling rootstocks, Velvick and Zutano were used, vis, ungrafted, grafted with self, and grafted with Hass.

The pre-fertiliser leaf analyses revealed that the graft union was already affecting nutrient movement from the roots into the scion (Table 5). The presence of a graft union resulted in significantly ($P<0.05$) different leaf levels for all nutrients except zinc, relative to the ungrafted plants. There was no significant effect of rootstock (Velvick or Zutano) on the leaf nutrient levels in any scion combination. Ca, Mg and Mn was significantly lower in grafted plants of both varieties, compared with ungrafted, whereas K was significantly higher in grafted compared with ungrafted. The N:Ca ratio, known to be associated with fruit quality, was significantly increased in grafted plants compared with ungrafted for both varieties (Table 5).

There were some interesting and significant trends in the data when the effects of graft type on foliar nutrients after fertiliser treatments were analysed (simplified in Table 6). In Velvick, the type of graft had no effect on translocation of most nutrients to leaves (exceptions were C, K, N:Ca and Ca+Mg:N). However, there were significant differences

in Zutano, where levels of B, Ca, Mg and Mn reduced significantly with type of graft, from ungrafted, to self-graft, then lowest levels in Hass graft (Table 6). K in Hass leaves of Zutano and Velvick seedlings was higher than in their respective self-grafted and ungrafted plants. There were also differences among graft type for the ratios N:Ca and Ca+Mg:N. It is possible that the more vigorous Velvick rootstock was able to translocate essential cations through the graft union more effectively than Zutano.

The presence of a graft union affected the translocation of phosphonate from leaves into growing roots, although differences among rootstocks and graft type were not significant at the 5% level (P=0.089, data not shown). Velvick seedlings grafted back onto themselves or grafted with Hass had a 26% and 50% reduction in phosphonate root concentrations, respectively. Zutano seedlings grafted back onto themselves had a slightly higher concentration of phosphonate in roots compared with ungrafted Zutano, however, concentrations in roots of Zutano grafted with Hass were approximately 60% lower than those in ungrafted Zutano (data not shown). The trends in nutrient and phosphonate translocation were inconsistent among variety and graft type, and ideally, this experiment should be repeated.

**Table 3**. Health and % tree survival of trees grafted to different rootstocks 6 months, 1 and 2 years after planting at Duranbah in 2013

| Rootstock | Tree health 6 month | Tree health 1 year | Tree health 2 years | Tree survival 2 years |
|---|---|---|---|---|
| SHSR-08 (cl, best of AV10xVelvick) | 3.4 | 5.0 c | 4.5 | 91 |
| SHSR-04 (clonal) | 2.8 | 5.3 c | 4.8 | 85 |
| Dusa (clonal) | 2.8 | 5.5 bc | 5.7 | 70 |
| Velvick | 2.4 | 5.9 abc | 4.9 | 100 |
| SHSR-07 (cl, best Kidd 5RW) | 3.0 | 6.0 abc | 6.6 | 60 |
| Zutano | 2.8 | 7.5 ab | 7.3 | 90 |
| Reed | 3.9 | 7.6 ab | 8.6 | 70 |
| SS3-1 | 3.5 | 8.0 a | 8.6 | 30 |

Tree health is rated on a scale where 0=healthy and 10=dead, Tree survival is the % of living trees compared with total numbers planted.
Means followed by the same letter are not significantly different ($P$<0.05)

**Table 4.** Average health of trees grafted to different rootstocks in Duranbah trial planted in April 2010

| Rootstock | n | Source | Tree health Nov 2010 | | Tree health Apr 2011 | | Tree health Oct 2011 | | Tree health Jun 2012 | | Tree health Mar 2013 | | Tree health Jun 2014 | | Tree survival Jun 2014 (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SHSR-08/Dusa (clonal) | 20 | Whiley | 2.33 | bc | 2.15 | b | 2.65 | b | 6.8 | b | 8.1 | b | 5.5 | bc | 30 |
| SHSR-05 (seedling) | 19 | Whiley | 2.72 | ab | 2.58 | b | 2.58 | b | 5.7 | bc | 6.6 | cd | 5.2 | c | 42 |
| SHSR-04 | 17 | Whiley | 2.00 | cd | 1.58 | b | 2.12 | b | 4.1 | c | 5.5 | d | 2.9 | d | 53 |
| Reed | 20 | Andersons | 2.90 | a | 3.35 | a | 3.70 | a | 9.8 | a | 9.8 | a | 9.5 | a | 5 |
| 09 | 10 | Andersons | 2.11 | bcd | 2.30 | b | 2.50 | b | 6.8 | bc | 8.1 | abc | 7.4 | ab | 30 |
| HAW | 10 | Andersons | 2.70 | ab | 2.50 | ab | 3.10 | ab | 7.5 | ab | 9.0 | ab | 8.0 | a | 10 |
| Kidd (seedling Kidd) | 10 | Andersons | 1.90 | cd | 1.90 | b | 2.60 | b | 7.5 | ab | 8.8 | ab | 8.8 | a | 10 |
| Reed/11 | 10 | Andersons | 1.60 | d | 2.30 | b | 2.60 | b | 7.9 | ab | 8.9 | ab | 3.0 | d | 10 |

[a] Material was obtained from Tony Whiley's (Sunshine Horticultural Services, Nambour) selection program and also from Graham Anderson and Harold Taylor, Anderson's Nursery, Duranbah

Assessed using a rating scale of 1–10, where 1 = healthy and 10 = dead, at 7, 12 and 18 months after planting. Mean values within columns followed by the same letter are not significantly different at P=0.05

Tree survival is the % of living trees compared with total numbers planted.

**Table 5**. Effect of rootstock and graft compatibility on leaf nutrient analyses prior to fertilising

| Rootstock & graft type | | C | N | B | Ca | K | Mg | Mn | Zn | N:Ca | Ca+K:N | Mg+K:Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Velvick | Ungrafted | 44.8 cd | 2.5 a | 45 b | 2.2 a | 0.69 b | 0.68 a | 699 a | 19.1 | 1.2 c | 1.14 b | 0.6 c |
| | Self grafted | 44.5 d | 1.9 c | 32 c | 0.9 b | 0.97 a | 0.34 b | 264 b | 21.7 | 2.2 b | 0.98 cd | 1.5 b |
| | Hass graft | 46.3 a | 2.3 b | 61 a | 0.8 b | 1.05 a | 0.36 b | 298 b | 22.6 | 2.9 a | 0.81 e | 1.8 a |
| Zutano | Ungrafted | 44.7 cd | 2.2 b | 63 a | 2.2 a | 0.64 b | 0.75 a | 604 a | 22.3 | 1.0 c | 1.3 a | 0.6 c |
| | Self grafted | 45.3 bc | 1.9 c | 42 bc | 1.0 b | 1.02 a | 0.42 b | 281 b | 18.3 | 2.0 b | 1.03 bc | 1.5 b |
| | Hass graft | 45.8 ab | 2.1 bc | 47 b | 0.7 b | 1.06 a | 0.36 b | 251 b | 20.0 | 2.9 a | 0.85 de | 2.0 a |

**Table 6**. Effect of rootstock and graft type on leaf nutrient analyses after fertilising

| Rootstock & graft type | | C | N | B | Ca | K | Mg | Mn | N:Ca | Ca+Mg:N |
|---|---|---|---|---|---|---|---|---|---|---|
| Velvick | Ungrafted | 43 bc | 2.3 | 33.5 c | 1.2 b | 0.65 b | 0.44 b | 395 b | 1.9 b | 0.82 c |
| | Self grafted | 42 c | 2.2 | 31 c | 1.4 b | 0.63 c | 0.47 b | 406 b | 1.6 c | 0.93 b |
| | Hass graft | 44 a | 2.4 | 28.5 c | 1.6 b | 0.75 b | 0.45 b | 440 b | 1.9 b | 0.86 c |
| Zutano | Ungrafted | 43 b | 2.3 | 70.5 a | 1.6 a | 0.71 b | 0.58 a | 570 a | 1.5 cd | 1.0 a |
| | Self grafted | 42 c | 2.3 | 42.5 b | 1.7 a | 0.71 b | 0.58 a | 538 a | 1.4 d | 1.04 a |
| | Hass graft | 43 a | 2.4 | 33 c | 1.1 c | 0.81 a | 0.45 b | 383 b | 2.2 a | 0.82 c |

b) Optimising phosphonate applications

Four trials were conducted to assess the efficacy of adding commercial surfactants to phosphonate spray applications to improve uptake and translocation of phosphorous acid to the roots. The Glasshouse Mountains trial (2011) with cv Hass, evaluated phosphonate at 0.25 and 0.5% with Pulse or DuWett. Subsequent trials in 2012 and 2013 were conducted in collaboration with commercial growers at Busselton, WA and Hampton, QLD, to evaluate phosphonate 0.5% applied with DuWett and copper (cuprous oxide). The aims were to determine if cost efficiencies could be achieved by combining phosphonate and copper spray applications with DuWett for improved PRR and fruit disease management. In 2013, 'Phostrol 500®' was provided for testing by VC contributors Nufarm Australia, and trial data supported its registration in 2014.

2011 Glasshouse Mountains, QLD

A trial was conducted at Glasshouse Mountains to assess the effects on root and leaf concentrations by adding surfactants when applying phosphorous acid as a foliar spray. As expected, there were no significant differences in concentrations of phosphorous acid among treatments prior to the initial application in mid-June (Table 7). Due to some leaf burn and defoliation in treatments applied with surfactant, Phos alone was re-applied at the second application. Roots and leaves were sampled 6 weeks after the initial treatment. Trees which had received 2 applications of Phos alone, at either 0.25 or 0.5%, and those which had received 1 spray of Phos 0.5% + 0.2% Du-wett, had significantly higher levels of phosphorous acid in roots compared with unsprayed trees (Table 7). The other treatments resulted in root phosphorous acid levels which were more than 3 times greater than those from unsprayed trees, although the differences were not statistically significant. All treatments resulted in significantly higher phosphorous acid levels in leaves compared with unsprayed controls, except the 0.25% Phos + 0.045% Pulse treatment. Four months after treatment, levels of phosphorous acid in roots had declined across all treatments, however, were significantly greater than unsprayed control for the two rates of Phos without surfactant applied twice, as well as one application of Phos 0.5% + 0.2% Du-wett (Table 7). The levels in these roots may be sufficient to help protect against Phytophthora infection and damage in the critical spring and early summer months.

**Table 7.** Effect of foliar applications of phosphorous acid, with or without surfactants, on root and leaf concentrations (Glasshouse Mountains, 2011)

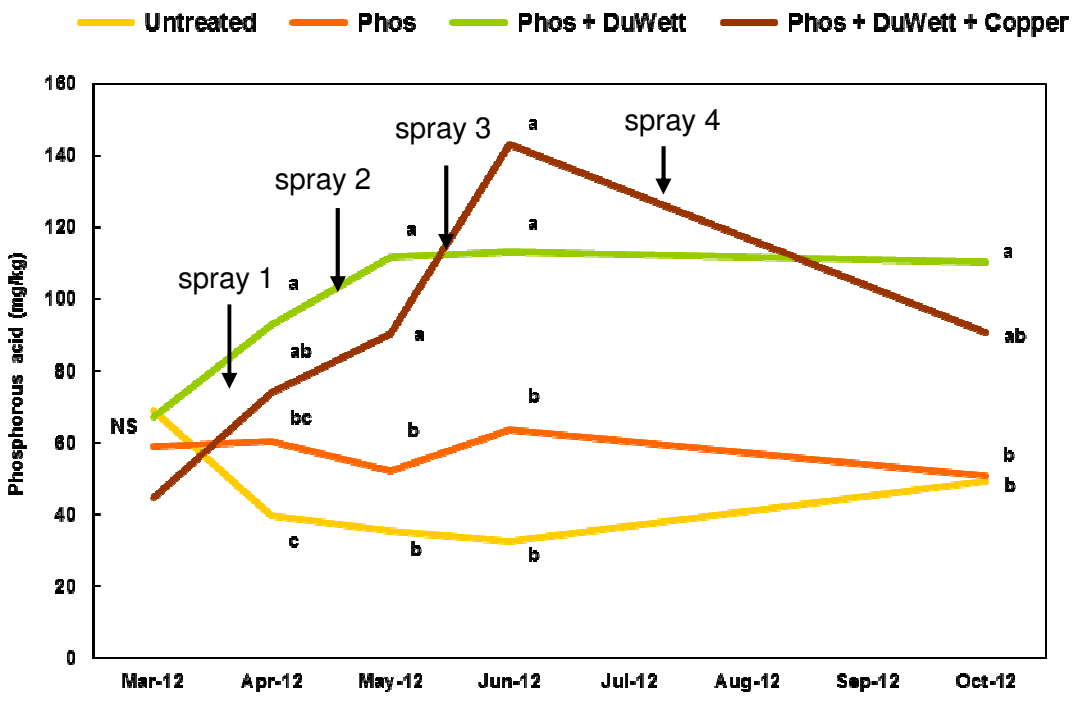
| Foliar spray treatment | No. app | Roots Baseline (June) | Roots July | Leaves July | Roots October |
|---|---|---|---|---|---|
| Control | 0 | 19.6 | 22.2 d | 3.6 e | 23.4 d |
| 0.5% Phos | 2 | 21.0 | 188.6 a | 99.0 a | 71.4 ab |
| 0.5% Phos + 0.045% Pulse | 1 | 23.8 | 68.2 d | 41.0 bc | 41.6 cd |
| 0.5% Phos + 0.2% Du-wett | 1 | 22.4 | 127.2 bc | 50.6 b | 49.0 bc |
| 0.25% Phos | 2 | 21.6 | 155.8 ab | 60.0 b | 73.6 a |
| 0.25% Phos + 0.045% Pulse | 1 | 28.8 | 79.8 cd | 18.2 de | 31.0 cd |
| 0.25% Phos + 0.2% Du-wett | 1 | 20.0 | 74.0 cd | 25.4 cd | 28.6 cd |

Within column, means followed by the same letter are not significantly different $P<0.05$

2012 Busselton, WA and Hampton, QLD

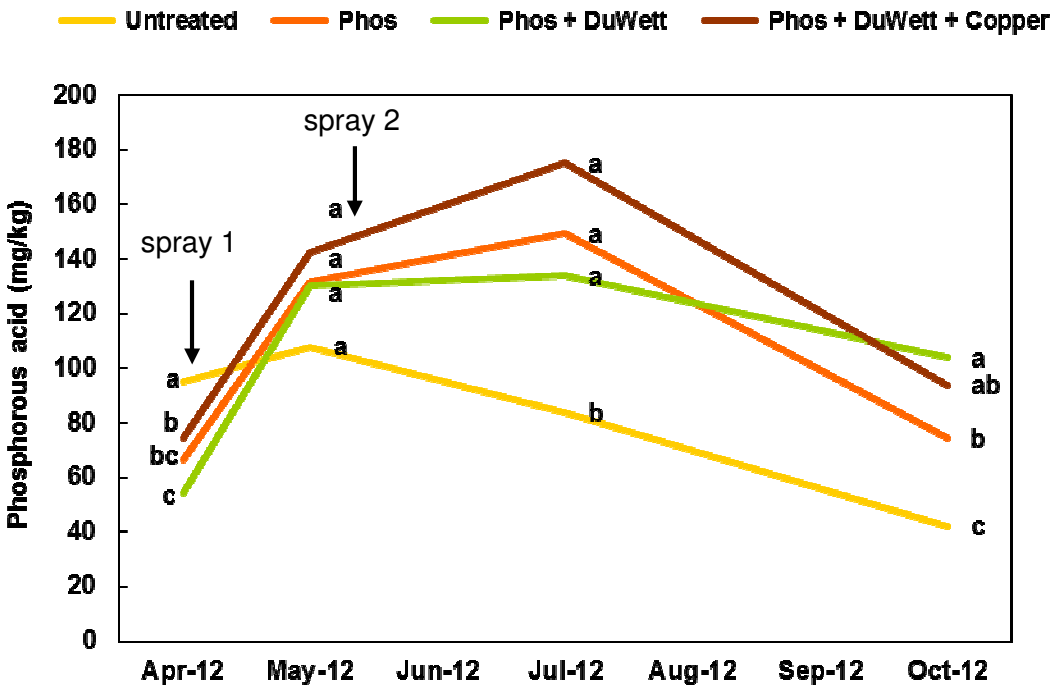
Two trials were conducted in collaboration with growers in SE Queensland and SW Western Australia to assess aspects of phosphonate foliar spray efficiency, specifically, to determine efficacy of applying phosphonate (Phos) with a spray adjuvant, and whether copper fungicides for fruit disease management could be applied with phosphonate + adjuvant treatment, ie. tank-mixed. At Busselton, WA, root phosphorous acid levels were significantly higher in trees sprayed with Phos + DuWett and Phos + DuWett + copper treatments, than in those untreated or sprayed with Phos alone, after a single spray application in March, and after two further applications in April and May (Figure 3). Concentrations in roots of unsprayed trees declined slightly through the year, and those in roots of trees treated with phosphonate alone were stable. At Hampton, QLD, baseline levels in April 2012 (ie. prior to treatment) of phosphorous acid in roots were different, with significantly higher levels in roots of trees assigned as untreated controls than those assigned to other treatments (Figure 4). After two spray applications, however, levels of phosphorous acid in roots were significantly higher for all phosphonate treatments than untreated controls. Figure 4 shows a dramatic decline in root levels across all treatments after the July sampling and during early spring, however, levels in roots from Phos + DuWett treatments were the highest at the October 2012 sampling. Phosphorous acid residues were determined in fruit flesh at commercial maturity. Figure 5 shows that across all treatments, levels of phosphorous acid were higher in fruit from Hampton, than in fruit from Busselton, which also reflects the general trend observed for roots (Figure 3 and Figure 4). At both sites, however, fruit from Phos + DuWett treatments had significantly greater residues of phosphorous acid than from Phos or Untreated fruit. Residues were the lowest for fruit not treated with phosphonate.

**Figure 3.** Effect of foliar applications with phosphonate alone or with DuWett and copper, on phosphorous acid levels in roots of avocado, cv Hass, (Busselton, WA) sampled 5 times from March to October, 2012.



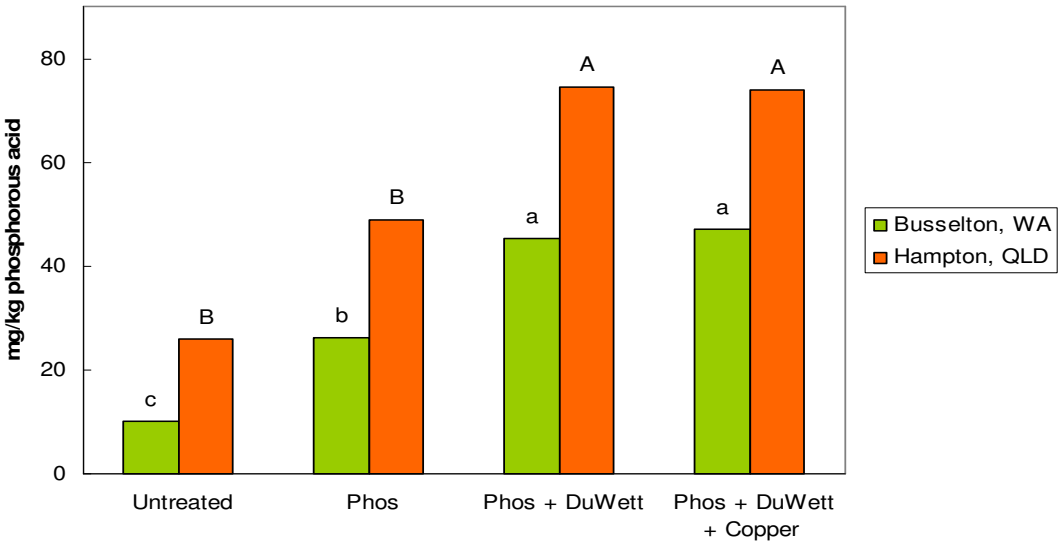

Approximate time of spray application is indicated by arrows. (within each sampling time, points on the figure followed by the same letter indicate mean concentrations of phosphorous acid in roots from those treatments are not significantly different, P>0.05).

**Figure 4.** Effect of foliar applications with phosphonate alone or with DuWett and copper, on phosphorous acid levels in roots of avocado, cv Reed, (Hampton, QLD) sampled 4 times from April to October, 2012.




Approximate time of spray application is indicated by arrows. (within each sampling time, points on the figure followed by the same letter indicate mean concentrations of phosphorous acid in roots from those treatments are not significantly different, P>0.05).

**Figure 5**. Effect of foliar applications with phosphonate alone or with DuWett and copper, on phosphorous acid levels in fruit of avocado, cv Reed, (Hampton, QLD) and cv Hass (Busselton, WA) sampled in November 2012.




At each site columns on the figure surmounted by the same letter indicate mean concentrations of phosphorous acid in fruit from those treatments are not significantly different, P>0.05).

2013 Hampton, QLD (with 'Phostrol 500®', Nufarm Australia)

The trial commenced in March 2013, with the collection and analyses of roots for baseline (pre-treatment) concentrations of phosphorous acid. Four foliar applications of Phostrol were made in April, May, July and September, 2013. Two treatments included DuWett with Phostrol, as our trial last year indicated higher root phosphorous acid levels when DuWett was included with Agrifos in the spray tank. Phostrol was applied as 0.5%, as per industry standard. Trees were injected with Phostrol (20%) in April and July 2013. Further root samples were collected in June, September and November 2013, and analysed by SGS Food and Agriculture Laboratory, for concentration of phosphorous acid. Mature fruit was harvested in November 2013 and samples of flesh analysed for phosphorous acid concentration. Root samples were also analysed in July and September from Reed trees outside of the trial area, which had received injections of Agrifos (20%).

Some leaf burn and drop was observed on trees where DuWett was included, after the April and May applications. The larger, healthier trees (southern end of trial) showed less leaf burn relative to those further north along each row (generally the less healthy trees). The label rate for DuWett is 150-600mL/Ha, and the first two applications were made with DuWett rates equivalent to 900mL/Ha (re-calculated after the foliar burn). The final sprays in July and September were applied with DuWett at 300mL/Ha and there was no further leaf burn after these treatments.

Table 8 summarises the phosphorous acid concentrations in roots throughout the experiment. Baseline levels of phosphorous acid in roots were similar among treatments and at a good level prior to autumn applications. Two months after the first injection there was no significant increase in phosphorous acid level in roots among treatments, however the Phostrol spray treatments with DuWett had higher root concentrations than all other treatments. Trees had received a total of three Phostrol sprays or two injections by September 2013, when roots were again sampled. There were no significant differences among treatments, however Phostrol sprays marginally increased root phosphorous acid levels compared with untreated controls. The final root sampling was in late November, after a total of four Phostrol spray applications. There were differences among treatments, where the addition of DuWett to Phostrol spray resulted in significantly higher concentrations of phosphorous acid in roots (Table 8). Concentrations of phosphorous acid in fruit flesh (at 75% moisture) were significantly different among treatments, with the lowest concentration in fruit harvested from untreated control trees. Levels in Phostrol + DuWett spray treatments were similar, and were significantly higher than in fruit from Phostrol alone spray, or Phostrol inject treatments (Table 8).

Similar results were obtained in the "commercial" block (outside of the trial) of 'Reed'. In July 2013, root phosphorous acid levels were 43 mg/kg and 57 mg/kg for "healthy" and "declining" trees, respectively. Trees were injected with Agrifos at 20% in mid-August 2013, and root phosphorous acid levels in September 2013 were 76 mg/kg and 67 mg/kg for "healthy" and "declining" trees, respectively. Thus, our results with Phostrol applied as an injection are at least consistent with a currently registered product, Agrifos.

It is likely that the apparent lack of response to phosphonate treatment this year was due to a very high crop load, which may have drawn photosynthate (and thus phosphonate) away from the roots and towards the fruit. Fruit flesh residue analyses supports this theory. Addition of DuWett with Phostrol improved translocation of phosphonate to the roots, but also to the fruit. The APVMA has established a temporary MRL at 500mg/kg for phosphorous acid, which applies only to avocados marketed and consumed in Australia. MRLs of importing countries are likely to be much lower, so that care must be taken if growers intend to use a surfactant when spraying phosponate on trees where fruit may be exported.

**Table 8.** Effect of Phostrol spray or injection of avocado cv. Reed on phosphorous acid levels in roots and fruit

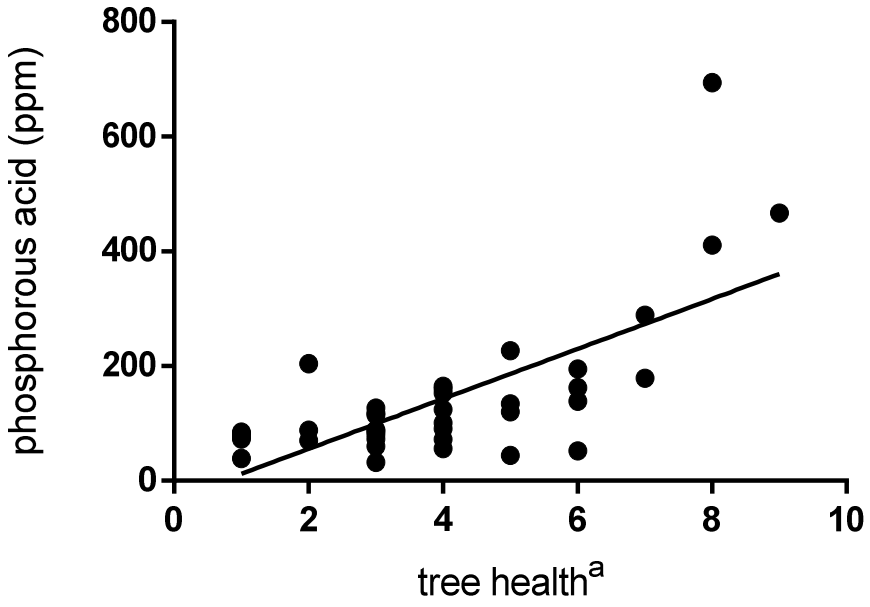
| Treatment | Phosphorous acid level in roots (mg/kg) | | | | Phosphorous acid level in flesh (mg/kg) |
|---|---|---|---|---|---|
| | 26 Mar 2013 | 24 Jun 2013 | 5 Sep 2013 | 28 Nov 2013 | 28 Nov 2013 |
| Untreated control | 75.3 | 78.8 | 75.5 | 74.5 b | 41.1 c |
| Phostrol spray | 74.2 | 76.8 | 90.8 | 73.5 b | 113.1 b |
| Phostrol + Duwett | 72.0 | 100.8 | 105.2 | 118.5 a | 196.3 a |
| Phostrol + Duwett + copper | 67.2 | 123.3 | 97.8 | 111.7 a | 176.1 a |
| Phostrol inject | 75.3 | 84.2 | 84.3 | 75.5 b | 124.9 b |

Within each column, means followed by the same letter are not significantly different at P=0.05

c) Determination of critical phosphorous acid concentration in roots

Glasshouse assays were undertaken to determine the effective root concentration of phosphorous acid for effective PRR management. The *in vitro* and detached root assays were necessary due to the extreme difficulty with undertaking these studies in mature trees in the field. Tree health and root phosphonate levels from over 40 'Reed' trees in the field were analysed, representing a range of canopy health from 1 (very healthy, green leaves, dense canopy) to 9 (near-death, tree almost completely defoliated). The relationship between tree health and root phosphonate in this declining block was highly significant ($P<0.001$). Figure 6 shows that as the health of the tree declines, the phosphonate concentrated into fewer roots, thus giving high readings for trees which were obviously sick. This shows that careful experimentation under glasshouse conditions was required for determination of "critical" phosphorous acid levels with respect to *Phytophthora cinnamomi* (Pc) infection and disease development.

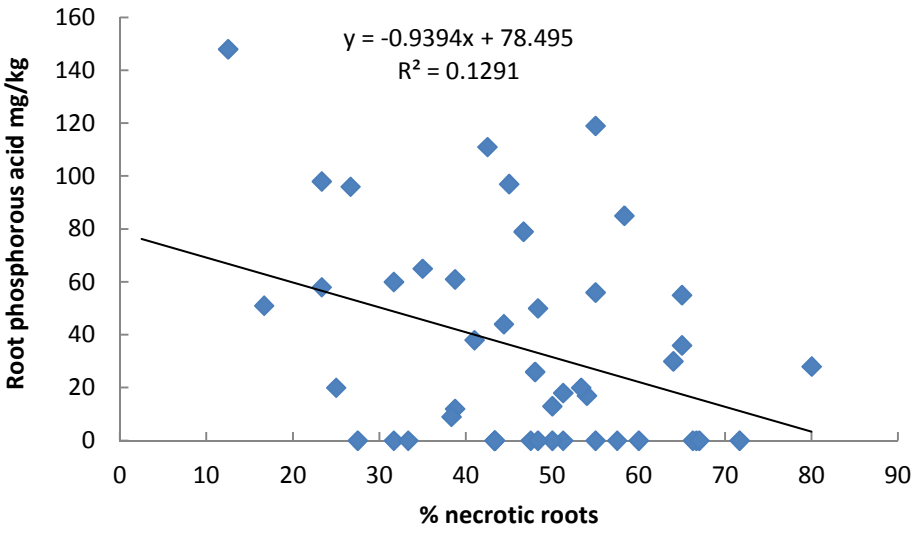
**Figure 6**. Relationship between tree health and phosphorous acid levels in roots of 'Reed' trees at Hampton




[a] Tree health is rated on a scale where 0=healthy and 10=dead

Glasshouse trials with a) intact seedlings or b) detached roots of seedlings were undertaken to investigate effects of phosphorous acid concentration in roots after phosphonate foliar spray and root necrosis following subsequent inoculation with *P. cinnamomi*. Intact seedlings were inoculated by repotting the plants with 5% v/v grain media colonised with Pc, subjected to flooding for 3 days then drained, and root necrosis assessed approximately 6 weeks after inoculation. Although there is large variation, the linear regression (significant at P=0.014), shows that there is minimal disease when concentrations of phosphorous acid approach 80 mg/kg and up to 80% root necrosis when there is no detectable phosphorous acid in roots (Figure 7).

**Figure 7**. Effect of phosphorous acid concentration in roots on root necrosis after inoculation with *P. cinnamomi,* intact seedling assay




Detached root experiments were undertaken with Reed, Velvick or Zutano plants sprayed with phosphonate. One sample of roots was harvested and analysed by SGS, while replicate samples were inoculated with Pc isolates with high or low sensitivity to phosphonate (as determined by in vitro EC50 studies). Detached roots were suspended over a beaker of water and a plug of Pc on agar media was introduced into the water (Figure 8).

**Figure 8**. Detached roots of avocado with necrotic areas evident after inoculation with *P. cinnamomi*

The phosphonate sensitive isolate had EC50 value (the concentration of phosphonate which inhibited growth of Pc by 50%) in the range 55 mg/kg, and the less sensitive isolate had EC50 104 mg/kg. In glasshouse Experiment 1, the higher level of phosphonate in roots of Velvick compared to Reed corresponded to less severe root disease in Assay 1, and similar levels of root disease in Assay 2 (Table 9). In glasshouse Experiment 2, phosphonate-treated Velvick trees had higher concentrations of phosphonate in roots than Zutano, and less root disease after inoculation with either isolate of Pc (Table 10). Thus, the magnitude of disease reduction corresponded to concentrations of phosphonate in roots and host resistance levels. Velvick is known to be more tolerant of Pc than Zutano and Reed, and these data show that the ability of Velvick to accumulate more phosphonate may contribute to this field observation. The data also demonstrate that necrosis is more severe in roots inoculated with the isolate of Pc less sensitive to phosphonate.

When data for rootstocks and Pc isolate were combined, there was a highly significant ($P<0.001$) negative relationship, with less disease with increasing levels of phosphorous acid in roots (Figure 9). In detached roots, an average of about 50 mg/kg prevented root necrosis, however there was a large spread of the data from 5 to 90 mg/kg.

Although variable, it would seem that root levels of at least 80 mg/kg phosphorous acid are required throughout the infection periods, and even higher levels required for orchards with long history of phosphonate use, which may have selected for isolates of Pc less sensitive to

phosphonate. Several years ago the minimum root level for protection from Pc was suggested to be 20 mg/kg, determined by survey rather than structured experiments (Whiley and Pegg, pers comm. 2012), ie. an arbitrary figure selected from the data. A South African study injected 6 month old seedlings with phosphonate and inoculated detached roots with Pc at various times thereafter. Root colonisation was reduced compared with controls by approximately 85% at root phosphonate concentrations of 9.8 to 53.2 mg/kg, and infection was never completely prevented (van der Merwe and Kotze, 1994). The current practical recommendation in Australia is that it is desirable to have root concentrations in commercial orchard trees of around 100 mg/kg prior to the onset of the "danger" periods for Pc infection and PRR development (Graeme Thomas, pers. comm.). Such a high level will ensure protection throughout the season, as concentrations decline with root growth.

Our study highlights differences between *in vitro* EC50 analyses, detached roots and *in planta* assays, and suggests that inherent plant defence mechanisms may be induced by phosphonate applications which contribute to retarding infection and development of Pc in the roots, ie. phosphonate has a dual mode of action. There is evidence for this in other systems (Daniel and Guest, 2006, Eshraghi, et al., 2011), but has not been studied in avocado.

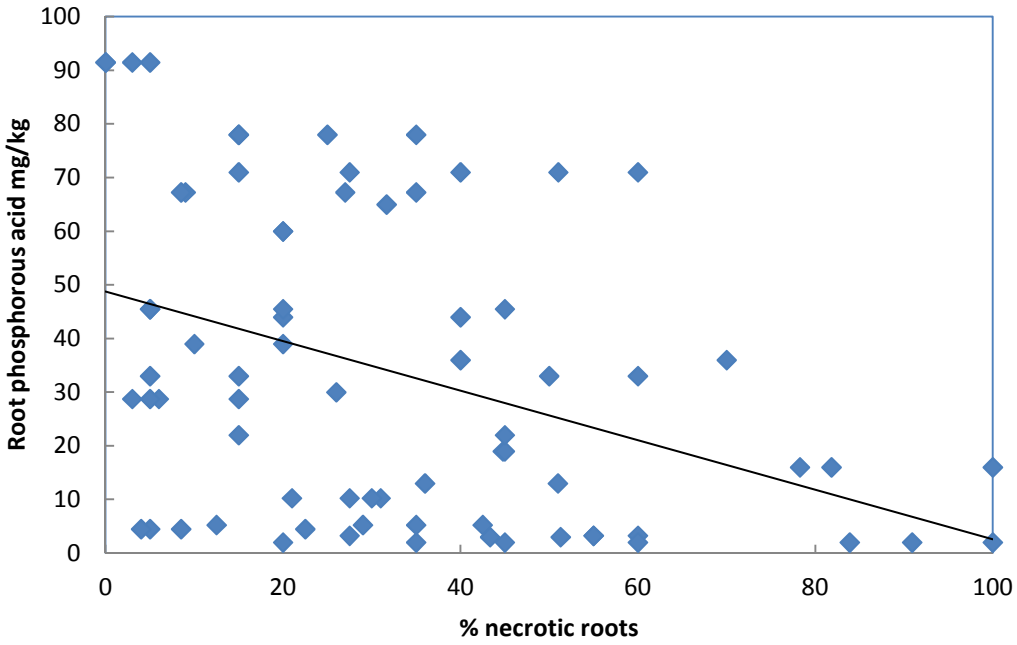
**Table 9** Concentration of phosphorous acid in roots, and root necrosis after inoculation of detached roots with isolates of Pc which had different sensitivities to Pc determined by *in vitro* tests. Glasshouse Experiment 1.

| | | Root necrosis (%) | | Root phos. acid conc. (mg/kg) |
|---|---|---|---|---|
| | | Phos sensitive Pc | Phos less sensitive Pc | |
| Assay 1 | | | | |
| | Reed | - | 74 | 9 |
| | Velvick | - | 40 | 42 |
| Assay 2 | | | | |
| | Reed | 32 | 48 | 25 |
| | Velvick | 37 | 44 | 47 |

**Table 10.** Concentration of phosphorous acid in roots, and root necrosis after inoculation of detached roots with isolates of Pc which had different sensitivities to Pc determined by *in vitro* tests. Glasshouse Experiment 2.

| | | Root necrosis (%) | | Root phos. acid conc. (mg/kg) |
|---|---|---|---|---|
| | | Phos sensitive Pc | Phos less sensitive Pc | |
| Velvick | Untreated | 24 | 26 | 7 |
| Velvick | Phos treated | 13 | 20 | 68 |
| Zutano | Untreated | 28 | 36 | 1 |
| Zutano | Phos treated | 14 | 21 | 59 |

**Figure 9**. Relationship between severity of Phytophthora root necrosis and concentration of phosphorous acid in roots, detached root assay. The relationship is significant (P<0.001).




2) Integrated approach to reduce fruit diseases

Pressure by consumers and international markets to reduce pesticide applications or shift towards "softer" options, was the impetus for trialling less conventional products and approaches for postharvest disease management. Several 'non-traditional' chemical products were evaluated for their efficacy compared with industry standard copper and strobilurin (Amistar) applications, to reduce postharvest diseases anthracnose and stem end rot. These included fungicides which are not currently registered in avocado, including a strobilurin (Cabrio), two protectants mancozeb and metiram (Rainshield and Polyram, respectively) as well as a formulation of calcium carbonate (NaturalGreen), an experimental compound from Nufarm Australia and saccharin, a known activator of defence responses in some plants (eg. Srivastava et al., 2011).

Replicated trials were conducted in commercial orchards with 'Hass' at Glasshouse Mountains and Childers in the 2010/11, 2011/12 fruiting seasons and at Hampton in the 2012/13 season. Treatments were sprayed at approximately 4-5 week intervals, and strobilurin fungicides (Amistar, Cabrio) applied 3 and 1 week prior to harvest, as per label recommendations. Fruit were ripened at 23$^{o}$C and 65% relative humidity to encourage disease development, and assessed for anthracnose and stem end rot disease severity (% surface area or % fruit volume affected by disease, respectively) and incidence (% of fruit with symptoms) at the eating ripe stage. To determine whether NaturalGreen applications increased calcium in fruit, peel samples were collected from the untreated control and NaturalGreen treated fruit and analysed for N, Ca, Mg and K nutrient concentrations.

In 2011, fruit were harvested in June. When fruit from the Glasshouse Mountains trial were ripened, there were no significant differences in the severity or incidence of anthracnose lesions among the treatments, despite five treatments (industry standard, Rainshield, Polyram, NaturalGreen + Amistar and saccharin + Amistar), having less than half the disease of untreated controls (Table 11). Stem end rot severity and incidence was significantly reduced by the industry standard treatment, and NaturalGreen + Amistar. Marketability, defined as fruit with less than 5% severity of anthracnose and no stem end rot, was greatest for fruit from the NaturalGreen + Amistar treatment though this was not significantly greater than for fruit from the Industry Standard, Polyram, Rainshield or saccharin + Amistar treatments.

Although anthracnose was less severe at the Childers site, higher severity of stem end rot reduced the overall quality and marketability of fruit, compared with the Glasshouse Mountains trial in 2011 (Table 12). There were no significant differences among treatments for any of the disease parameters measured, although anthracnose severity and incidence was lowest in fruit receiving Rainshield, and stem end rot was less severe in fruit sprayed with the industry standard coppers + Amistar (Table 12). Though not significant, marketability for fruit receiving the industry standard treatment was greatest and fruit from the saccharin treatment the least marketable.

**Table 11.** Postharvest disease severity and incidence in fruit treated with fungicides and "non-traditional" products (Glasshouse Mountains, 2010/11)

| Treatment | Severity side anthr.(%) | Total Severity stem end rot (%) | Incidence side anthr. (%) | Total Incidence stem end rot (%) | Marketability (%) |
|---|---|---|---|---|---|
| Untreated control | 32.1 | 6.84 abc | 69.0 | 25.0 abc | 43.0 bc |
| Industry standard | 15.0 | 1.87 d | 55.0 | 12.0 de | 64.0 ab |
| Rainshield | 12.6 | 5.33 bcd | 57.0 | 19.0 bcde | 57.0 abc |
| NaturalGreen | 28.4 | 7.14 abc | 74.0 | 28.0 ab | 35.0 c |
| NaturalGreen/Amistar | 4.8 | 2.35 d | 37.0 | 9.0 e | 79.0 a |
| Saccharin | 24.0 | 9.97 a | 73.0 | 33.0 a | 39.0 bc |
| Saccharin/Amistar | 13.4 | 8.38 ab | 47.0 | 24.0 abcd | 52.0 abc |
| Polyram | 12.1 | 3.65 cd | 55.0 | 14.0 cde | 62.0 abc |

Means followed by the same letter are not significantly ($P<0.05$) different
Fruit marketability = less than 5% severity of anthracnose and no stem end rot

**Table 12.** Postharvest disease severity and incidence in fruit treated with fungicides and "non-traditional" products (Childers, 2010/11)

| Treatment | Severity side anthr.(%) | Total Severity stem end rot (%) | Incidence side anthr. (%) | Total Incidence stem end rot (%) | Marketability (%) |
|---|---|---|---|---|---|
| Untreated control | 17.9 | 17.3 | 53.0 | 54.0 | 35.0 |
| Industry standard | 19.7 | 6.8 | 51.0 | 31.0 | 45.0 |
| Rainshield | 5.3 | 19.2 | 33.0 | 53.0 | 42.0 |
| NaturalGreen | 18.3 | 19.2 | 48.0 | 60.0 | 24.0 |
| NaturalGreen/Amistar | 13.7 | 15.3 | 42.0 | 52.0 | 34.0 |
| Saccharin | 19.8 | 20.5 | 60.0 | 67.0 | 19.0 |
| Saccharin/Amistar | 11.4 | 16.8 | 37.0 | 58.0 | 35.0 |
| Polyram | 19.9 | 14.8 | 49.0 | 44.0 | 34.0 |

Means followed by the same letter are not significantly ($P<0.05$) different
Fruit marketability = less than 5% severity of anthracnose and no stem end rot

In 2012 there were some significant differences among treatments in the numbers (incidence) of fruit from the Glasshouse Mountains site with side anthracnose or stem end rot, but no differences in severity among treatments (Table 13). Copper treatments with either Amistar or Cabrio as final sprays significantly reduced the incidence of fruit with stem end rot compared with untreated controls, (and halved the incidence of anthracnose, but the effect wasn't significant) and thus increased the percentage of marketable fruit. The NaturalGreen + Amistar treatment also had a significantly higher proportion of good quality fruit compared with controls (fruit in this treatment had an exceptionally low severity of side anthracnose, which contributed to the high marketability). NaturalGreen is a high calcium product. The plant extract NUL2580, provided for testing by NuFarm Australia alone did not significantly affect disease or fruit marketability compared with untreated controls.

There was a high level of postharvest disease pressure at Childers in 2012 and there were no significant differences in severity or incidence of anthracnose or stem end rot among treatments (Table 14). Interestingly, fruit treated four times from January to May 2012 with NUL2580 (100mL/L) had the least severe anthracnose disease, which was less than half the severity of the untreated controls. This translated to a percentage of marketable fruit similar to the industry standard fungicide treatments of regular copper applications plus two strobilurin applications close to harvest. (It must be noted that in both trials, Polyram was applied 4 times through the season, and copper applied 4 times in the Norshield + Cabrio treatment, compared with 6 applications of copper or mancozeb in the Norshield + Amistar and Rainshield + Amistar treatments, respectively. Similarly, NUL 2580 was applied 4 times from late January 2012).

**Table 13.** Effects of fungicide and non-traditional products as field spray treatments on severity and incidence of postharvest disease in 'Hass' fruit (Glass House Mountains, harvested June 2012)

| Treatment | Tot Sev Side (%) | Tot Sev Stem (%) | Tot Inc Side (%) | Tot Inc Stem (%) | Marketability (%) |
|---|---|---|---|---|---|
| Untreated control | 11.69 | 6.73 | 36.0 ab | 26.0 a | 58.0 d |
| Norshield/Amistar | 3.07 | 0.35 | 16.0 b | 7.0 b | 90.0 a |
| Norshield/Cabrio | 2.18 | 1.12 | 15.0 b | 7.0 b | 89.0 ab |
| Rainshield/Amistar | 4.29 | 2.79 | 30.0 ab | 17.0 ab | 73.0 bcd |
| Polyram/Amistar | 6.35 | 3.03 | 43.0 a | 20.0 a | 67.0 cd |
| NaturalGreen | 9.78 | 3.65 | 34.0 ab | 19.0 a | 72.0 abcd |
| NatGreen/Amistar | 0.71 | 4.23 | 14.0 b | 15.0 ab | 84.0 abc |
| Saccharin | 17.23 | 4.09 | 43.0 a | 19.0 ab | 63.0 d |
| Saccharin /Amistar | 9.46 | 3.84 | 29.0 ab | 17.0 ab | 72.0 bcd |
| NUL2580 50mL/L | 6.87 | 6.22 | 45.0 a | 22.0 a | 68.0 cd |
| NUL2580 100mL/L | 14.3 | 8.48 | 52.5 a | 31.2 a | 51.0 d |

**Table 14.** Effects of fungicide and non-traditional products as field spray treatments on severity and incidence of postharvest disease in 'Hass' fruit (Childers, harvested June 2012)

| Treatment | Tot Sev Side (%) | Tot Sev Stem (%) | Tot Inc Side (%) | Tot Inc Stem (%) | Marketability (%) |
|---|---|---|---|---|---|
| Untreated control | 34.4 | 3.39 | 78.0 | 16.0 | 38.0 |
| Norshield/Amistar | 22.9 | 4.04 | 61.0 | 24.0 | 52.0 |
| Norshield/Cabrio | 20.1 | 4.85 | 60.0 | 17.0 | 50.0 |
| Rainshield/Amistar | 24.2 | 3.81 | 59.0 | 20.0 | 45.0 |
| Polyram/Amistar | 20.0 | 6.64 | 61.6 | 28.3 | 40.4 |
| NaturalGreen | 26.6 | 10.1 | 72.0 | 38.0 | 35.0 |
| NatGreen/Amistar | 26.9 | 4.13 | 57.0 | 20.0 | 47.0 |
| Saccharin | 22.5 | 5.61 | 64.0 | 26.0 | 52.0 |
| Saccharin/Amistar | 23.1 | 9.18 | 67.0 | 35.0 | 31.0 |
| NUL2580 50mL/L | 31.7 | 5.43 | 69.9 | 22.0 | 35.4 |
| NUL2580 100mL/L | 14.2 | 4.03 | 48.0 | 22.0 | 52.0 |

The final trial assessing effects of alternative fungicides and "non-traditional" compounds on fruit disease in Hass was conducted in the 2012/2013 season at Hampton, Queensland. Severity and incidence of anthracnose disease was significantly less in fruit which had been sprayed throughout the season with the industry standard (Norshield copper + Amistar) or NUL2580 + Amistar, compared with untreated (control), or which had NaturalGreen or NUL2580 sprays only (Table 15). Stem end rot severity and incidence was the lowest in fruit from Norshield copper + Amistar, and severity was statistically lower than in fruit from all other treatments except NUL2580 + Amistar. Overall marketability was greatest in fruit from Norshield + Amistar and NUL2580 + Amistar treatments.

There were no significant effects of NaturalGreen applications on N, Ca, Mg and K nutrient concentrations in fruit peel, for any year or location (data not shown). Despite being a high calcium product, spray application of this product had no effect on increasing Ca in fruit. High Ca is desirable for improved fruit quality.

These results, and those from the previous years demonstrate that the current industry standard practice of monthly copper + 2 applications of Amistar 3 weeks and 1 week prior to harvest, give the fruit with the least postharvest disease. The results highlight the contribution of the final Amistar treatments to fruit quality, as NaturalGreen and NUL2580 alone were ineffective, however, when combined with Amistar, postharvest disease was reduced leading to increased outturn quality. Our trials have not unearthed a "silver bullet" ie. a new chemical or treatment combination which is considerably better than the current program. It must be noted that while we used Amistar in our trials, there are now several products on the market which have the same active ingredient (azoxystrobin), and another strobilurin, pyraclostrobin (active ingredient in Cabrio), was as effective as Amistar. Cabrio is not registered for use in avocado.

**Table 15.** Effects of fungicide and non-traditional products as field spray treatments on severity and incidence of postharvest disease in 'Hass' fruit (Hampton, harvested July 2013)

| Treatment | Severity anthracnose (%) | Severity Stem end rot (%) | Incidence anthracnose (%) | Incidence Stem end rot (%) | Marketability (%) |
|---|---|---|---|---|---|
| Untreated control | 20.2 a | 9.57 a | 79.0 a | 30.5 | 23.0 c |
| Norshield/Amistar | 13.7 b | 3.34 c | 58.3 bc | 16.1 | 45.7 ab |
| NaturalGreen | 27.7 a | 9.61 a | 82.0 a | 32.5 | 18.5 c |
| NG/Amistar | 16.0 ab | 7.38 ab | 73.5 ab | 28.5 | 31.0 bc |
| NUL2580 | 28.4 a | 7.09 ab | 82.0 a | 30.0 | 22.0 c |
| NUL2580/Amistar | 11.0 b | 5.50 bc | 56.4 c | 21.1 | 49.4 a |

3) Phellinus noxius brown root rot

a) *In-vitro* evaluation of fungicide efficacy

Laboratory assays were undertaken to determine sensitivity of *Phellinus noxius* (Pn) to a range of available fungicides to identify those which may be part of an effective management option. Paclobutrazol, propiconazole, azoxystrobin, prochloraz, metiram and mancozeb were highly inhibitory to growth of Pn in Petri dish assays, with EC50 (concentration at which mycelial growth is inhibited by 50%) ranging between 0.5 and 1.0 mg active ingredient/mL. The thiabendazole and cuprous oxide fungicide formulations were not inhibitory, but this was probably because the active ingredient was not effectively solubilised and/or distributed in the molten agar. Serenade (a *Bacillis subtilis* biocontrol agent) and Ecocarb were not inhibitory to growth of Pn. Field trials proceeded with two triazole group fungicides, paclobutrazol and propiconazole, based on their *in vitro* inhibition of Pn, and known efficacy against other basidiomycete fungi and their widespread use under field conditions eg. against *Armillaria* sp. which has a similar mode of infection and spread to Pn in other tree species (eg. Amiri and Schnabel, 2012).

b) Field trials

There were 2 trials initiated in 2011 in an orchard at Childers, known to be heavily infested with Pn. One trial investigated treatments to existing trees to determine efficacy on reducing spread and deaths, and the second trial treated replant sites following tree death and removal to determine efficacy on eliminating Pn inoculum and establishment of new plantings. However, changes in farm management in 2013 resulted in bulldozing of all trees in the trial block. Prior to this, soil was collected from each of the treated replant sites for glasshouse experiments, described below. Woody root debris from sites known to have had a tree die from Phellinus, was also collected and plated out to determine viability of Pn.

A large trial was designed utilising 75 trees in a site with high *Phellinus noxius* disease pressure. The trees in the trial were blocked using the number of trees adjacent still alive. Prior to treatment in 2011, there were no significant differences in tree health, number of fruit per tree, and total yield (kg) per tree among assigned treatments (data not shown). Approximately a year after treatment applications commenced, there were no significant differences among treatments in tree health (Table 16). However, trees drenched around the collar with paclobutrazol had a significantly ($P=0.01$) heavier crop load than all other treatments except those infiltrated with propiconazole (Table 16), based on visual rating of crop load. There were no significant differences among treatments in fruit quality, assessed as incidence and severity of postharvest anthracnose and stem end rot of fruit (Table 16). When the same trees were assessed in 2013, just prior to being bulldozed, overall tree health had declined considerably during the year, and crop loads prior to harvest were poor. There were no significant differences among treatments in tree health or crop yields (data not shown). The infiltration of trees with propiconazole was extremely difficult, and not likely to be an efficient practical treatment for several avocado trees in an orchard.

The remnant woody debris in the soil is particularly important for the long term survival of the fungus. Root debris buried up to 1 m deep was collected from sites where trees had died and been removed at least 3-4 years earlier. We confirmed presence of viable Phellinus by isolation onto media, in 40% of roots which were typically 2-4cm in diameter. These roots were encrusted with the characteristic "stocking", which most likely protected the internal root matrix with Pn colonisation, from decay.

**Table 16.** Tree health, crop yield and fruit quality assessments from the *Phellinus noxius* experiment at Childers, QLD, 2012

| Treatment | Tree health | Crop yield | % sev. anth. | % sev. stem end rot | % inc. anth. | % inc. stem end rot | % marketability |
|---|---|---|---|---|---|---|---|
| Kasil | 2.13 | 1.07 c | 10.5 | 12.9 | 48.2 | 44.9 | 41.3 |
| Propiconazole | 1.33 | 1.93 ab | 9.77 | 12.1 | 51.6 | 42.3 | 37.5 |
| Paclobutrazol | 2.20 | 2.20 a | 12.3 | 12.1 | 50.5 | 44.2 | 37.9 |
| Untreated | 2.53 | 1.50 bc | 10.7 | 14.0 | 42.5 | 44.8 | 38.9 |
| Untreated[a] | 3.80 | 1.29 bc | 6.09 | 7.83 | 47.1 | 32.5 | 55.4 |

Crop yield was a visual assessment where 0=no fruit, 1=low, 2=medium and 3=high crop load, tree health was assessed on a scale of 0 to 10, where 0=healthy and 10=dead, as per Darvas et al (1984)

[a] One of the intended treatments (thiabendazole fungicide) was not applied, as the product was not taken up by the tree upon attempting to inject and infiltrate

c) Greenhouse trials

There were two greenhouse-based areas of investigation with Pn.
The alternate host trial determined the relative susceptibility of alternate hosts to an isolate of *Phellinus* originating from infected avocado. The alternate hosts evaluated include Passionfruit rootstock #172, Kensington Pride mango, macadamia rootstock Beaumont, citrus rootstocks Troyer, Flying Dragon and Cleopatra, hoop pine, with Reed avocado seedlings included as a susceptible check. Ten percent of passionfruit seedlings died within one month after inoculation, but there were no further deaths. Within three months of inoculation all avocados had succumbed to *Phellinus*, and at the termination of the trial 6 months post-inoculation, almost 40% of macadamia seedlings had died (Figure 10). No deaths occurred in mango, citrus, or hoop pine. Interestingly, despite *Phellinus* being a significant cause of tree death in commercial hoop pine plantations, inoculation with the avocado isolate used in this experiment failed to result in hoop pine seedling death, although it significantly ($P<0.05$) reduced seedling height compared to non-inoculated plants.

Surviving plants from the trial were destructively harvested and assessed for further indications of Pn damage to root systems and/or reductions in plant vigour. In otherwise healthy looking

plants, the frequency of the presence of the characteristic Phellinus stocking (mycelia and soil encrusted areas on plant root systems) varied considerably among hosts. Stocking was absent on all passionfruit, present in 19-35% of citrus (excluding Citrus – Flying Dragon) and mango, and 75% of surviving macadamia plants (Table 17). In a small number of hoop pine and macadamia plants, tissue discolouration was evident underneath the stocking, potentially indicating early stages of pathogen growth into the plant. However, no discolouration was observed in internal tissues of mango or citrus roots underneath the stocking.

The alternate host trial data indicate that macadamia, at least rootstock Beaumont, would not be a suitable alternate tree crop to replace avocado orchards affected by *Phellinus*. Although further evaluation is required, it is likely that other macadamia rootstocks would show similar susceptibility. Although no stocking was observed on the surviving passionfruit seedlings, the deaths of two plants is consistent with a report from Thailand indicating that *Passiflora edulis* is susceptible to Pn. The presence of stockings on mango and citrus, despite the absence of plant death in the glasshouse trial would warrant further investigation under field conditions before providing recommendations to industry on replant options, particularly due to the limited numbers of confirmed tree deaths due to Phellinus in commercial mango orchards. In particular, the citrus rootstock Flying Dragon warrants further investigation based on the absence of both Phellinus infection stocking and vascular discolouration.

A second alternate host trial was established, and all Reed and Hass avocados had wilted and died within 2 months of inoculating with Pn colonised grain (Figure 11). The Pn inoculum was still viable and it appeared that the seed acted as a woody substrate prior to colonising the stem (Figure 11). There were only 2 citrus deaths (Flying Dragon) 2 months after inoculation, and Pn could not be isolated from crowns, however Trichoderma grew prolifically from stem and crown pieces with a high frequency of isolation. There were no macadamia deaths. The Pn inoculum in citrus and macadamia pots was black and dead, and most likely no longer viable. It is possible that Trichoderma contamination inactivated the Pn inoculum in citrus and macadamia before the Pn could colonise the woody stem tissue. Citrus was sourced from a commercial nursery which may have used a Trichoderma-containing microbial amendment. The macadamia seedlings were well established and in a non-pasteurised soil/mix, and could have already had natural Trichoderma colonisation. Avocados are extremely susceptible to Phellinus in our greenhouse tests.

**Figure 10.** Mortality of avocado and two alternate host crops following challenge inoculation with *Phellinus noxious* isolated from avocado

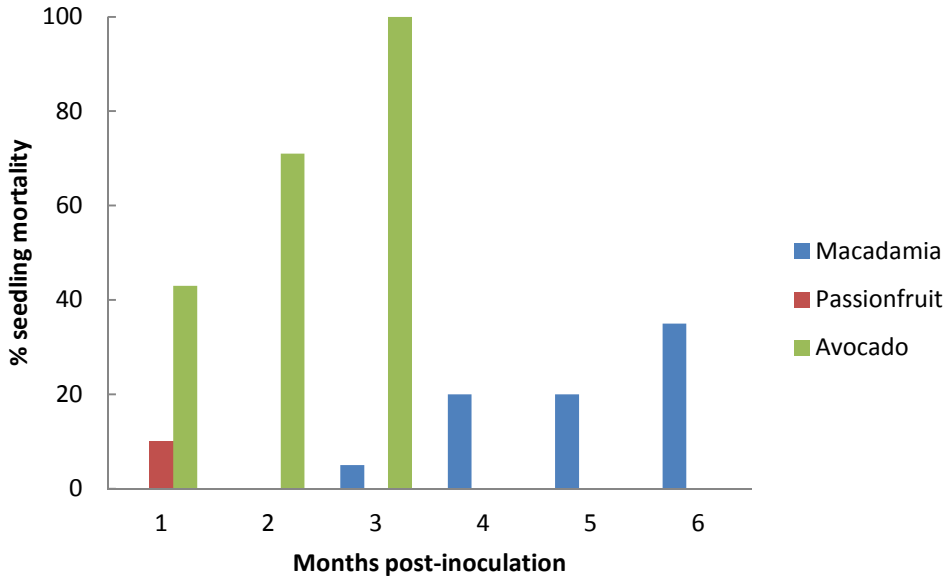



**Table 17.** Frequency of Phellinus stocking on alternate hosts 6 months post inoculation

| Host | Frequency of stocking observed in surviving plants (%) | Frequency of vascular discolouration (%) |
|---|---|---|
| Hass avocado | None surviving | - |
| Passionfruit | 0 | 0 |
| Citrus Flying Dragon | 0 | 0 |
| Citrus Troyer | 19 | 0 |
| Mango | 30 | 0 |
| Hoop Pine | 36 | 7.1 |
| Citrus Cleopatra | 38 | 0 |
| Macadamia | 75 | 8.3 |

**Figure 11.** Avocado seedling death 2 months after inoculation with Phellinus noxius (left) and colonisation of seed and crown (right)

Generating viable *Phellinus noxius* (Pn) inoculum, and identifying optimal environmental conditions conducive to *Phellinus* growth and infection were significant challenges associated with the glasshouse component of the project. Preliminary trials identified the best source of Pn inoculum, optimum temperatures required and the experiment duration to ensure pathogen growth and infection of plants. Several inoculations with Pn were necessary. During one inoculation, a *Trichoderma* sp. isolate (DNA sequence data could not distinguish between *Trichoderma* species *harzianum* and *tawa*) contaminant actively colonised, and eradicated, Phellinus inoculum. A culture of this isolate was stored and included as a treatment in the *Phellinus* management trial described below.

The soil treatment component of the field project was re-developed into a glasshouse based approach to evaluate soil treatment options to reduce the viability of Phellinus inoculum. The trial was conducted as closely as possible to field conditions using inoculum and soil obtained from commercial avocado orchards. A total of 9 treatments including soil fumigants, chemical soil drenches, cyclical waterlogging and a biological organism with previously observed *Phellinus* antagonistic properties were evaluated for their ability to reduce Pn inoculum viability. The *Phellinus*-infested material consisted of avocado root pieces, approx. 2-3cm diameter with characteristic infection "stocking". These were collected from trees which had recently died in northern NSW and placed into planter bags with a red krasnozem soil sourced from a Childers avocado orchard. The fumigant treatments include chloropicrin and a biofumigant mustard crop (cultivar Caliente 199). This crop was grown in pots and subsequently incorporated into the potting soil in an equivalent manner to the recommended field management practice. Three

separate chemical soil drench treatments included the fungicides paclobutrazol, propiconazole and a high dose of urea (ammonia fumigation). Additional treatments modifying soil moisture in the trial included periodic waterlogging (approximately every 3 weeks), maintenance of dry soil, and maintenance of an 'average' soil moisture (control treatment) equivalent to that required to grow the mustard crop. The biological treatment was a *Trichoderma* sp. fungal isolate obtained as a contaminant on *Phellinus* inoculum in a previous glasshouse experiment. Pieces of root were recovered and plated onto selective media at 3 and 6 months after initiation of the treatments, and observed for growth of Pn.

Viability of *Phellinus noxius* in root pieces was reduced most effectively by chloropicrin fumigation or with *Trichoderma* sp. three or two months after treatment, respectively (Figure 12). Heavy irrigation did not reduce viability of Pn, which is contrary to reports in the literature. Other treatments such as mustard biofumigation and propiconazole also reduced the percentage of viable Pn in root pieces. Interestingly, when the root pieces were recovered and dissected for sampling, there were differences in the internal structure and colour (Figure 13). Root pieces recovered from untreated pots were bright with brown Phellinus sclerotial plates clearly evident. However, root pieces recovered from chloropicrin and Trichoderma treated pots had a very dark grey internal colour (Figure 13). At 6 months there was a reduction in frequency of Pn isolation from all treatments except heavy irrigation (Figure 12), which was unexpected based on a previous study which showed that extended periods of wet soil reduced Pn viability (Chang, 1996). It was surprising that no Pn was recovered at 6 months from untreated and dry soil treatments. Ideally, this experiment should be repeated to at least verify the promising effects of Trichoderma, chloropicrin and biofumigation with a mustard green manure.

**Figure 12.** Frequency of isolation of *Phellinus noxius* from infested root pieces 3 and 6 months after treatment

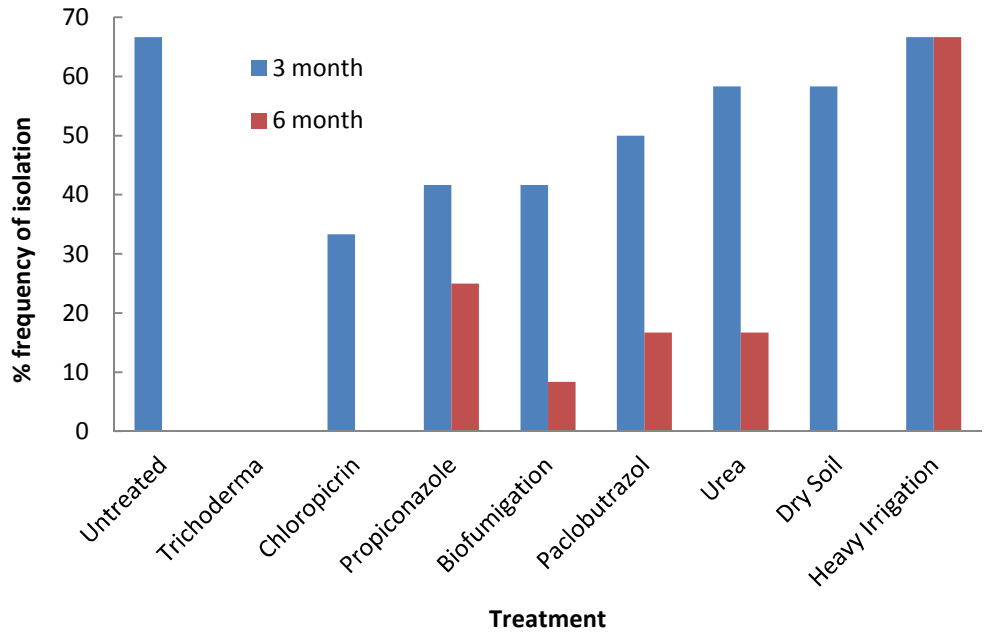


**Figure 13.** Root pieces initially colonised by *Phellinus noxius* buried in soil and recovered 3 months after nil treatment (top picture), chloropicrin fumigation (middle picture) or 2 months after *Trichoderma* treatment (bottom picture)

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