Horticulture Innovation Australia

Final Report

Exploring alternatives for managing Phytophthora root rot in avocado

Dr Elizabeth Dann
The University of Queensland

Project Number: AV13021

AV13021

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

The University of Queensland
PQ Australia Pty Ltd
Syngenta Australia Pty Ltd
Avocado Ridge
Coastal Avocados
Syngenta Australia Pty Ltd

ISBN 978 0 7341 3974 0

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

Contents

Summary

Protection of the avocado trees and developing fruit from pests and diseases will always be of prime concern to growers. Industry must remain vigilant and prepared to evaluate new products, such as mandipropamid and potassium silicate, to ensure delivery of high quality fruit from healthy trees. This will enhance productivity and maintain competitive advantage at the grower and whole of Industry level.

The question that this project addressed was ***"Can mandipropamid and/or potassium silicate improve avocado tree health and productivity by reducing Phytophthora root rot?"***

The aims of the project were to evaluate:

1) The efficacy of mandipropamid to control Phytophthora root rot (PRR) under glasshouse conditions, as a "proof of concept" prior to extensive field trials.

2) The effect of soil drench or foliar applications of potassium silicate on tree health, fruit yield and quality and root regeneration under high PRR field conditions.

Specifically, the activities included:

1) Petri dish assays testing 13 isolates of *P. cinnamomi* (Pc) for sensitivity to mandipropamid

2) Glasshouse tests with avocado seedlings to evaluate efficacy of mandipropamid applied pre or post inoculation with Pc on severity of root necrosis, comparing with metalaxyl

3) Collaborating with growers and packing shed operators at Childers and Comboyne to assess effects of silicon (Agsil) drench applications on tree health, yield and packout

4) Conducting our own field trial on a grower's orchard at Beechmont, to test effects of Agsil drench, spray or injection on tree health, root growth and fruit quality

The key outputs are:

1) All isolates of *Phytophthora cinnamomi* (Pc) tested were highly sensitive to mandipropamid *in vitro*.
2) Label rates of metalaxyl applied **before** or mandipropamid applied **after** Pc inoculation resulted in significant reduction of disease caused by Pc in glasshouse trials.
3) Indication that Agsil applied as a drench treatment to declining trees improves tree health, yields and quality of fruit.

The key outcomes are

1) Mandipropamid shows promise as an effective tool in the management of Phytophthora root rot

2) Improved use pattern for metalaxyl, resulting in increased productivity

3) Demonstration of beneficial effects of Agsil drench application on yields and fruit quality, potentially boosting net return by 20% or more.

Recommendations to Industry

1) Industry should support field trials to evaluate efficacy of mandipropamid (and other new anti-oomycete chemistries) for improving health and productivity of trees declining from severe PRR. Assessments of fruit quality and packouts should be included in this activity.

2) Liaise with the SARP coordinator and agrichemical companies, to ascertain intentions on further evaluation and registration for anti-oomycete products.

3) Evaluate Agsil other available silicon products against existing grower standard practice for effects on tree health, productivity (yield), packout rates and fruit quality. These trials would best be undertaken in collaboration with orchards at sites across the major production areas, and with support from local packing sheds.

Keywords

Phytophthora cinnamomi, anti-oomycete, silicon

Introduction

This project was initiated under the HAL "Voluntary Contribution" (VC) scheme, when the project leader was approached by PQ Australia to conduct independent trials to evaluate efficacy of potassium silicate (Agsil) in avocado orchards. Many growers were already using the product, applied as a soil drench or through fertigation, however, rigorous testing producing data to support its use in Australia were lacking. Around the same time, Syngenta Australia indicated they had a new anti-oomycete active which warranted small scale glasshouse testing in seedlings for efficacy against Phytophthora. The project commenced in March 2014 and concluded in April 2017, and was funded by VCs from PQ Australia and Syngenta Australia, matched by the Australian Government. There was no avocado levy support.

Phytophthora root rot (PRR) remains the key orchard challenge for growers in Australia, particularly in the eastern tropical and sub-tropical regions which have encountered high rainfall in recent years, favouring this root disease. PRR has significantly impacted productivity at orchard and whole-industry level due to tree decline and death, an abundance of small fruit which can be difficult to market, and poorer quality fruit from PRR affected trees. *Phytophthora cinnamomi* (Pc) is an oomycete pathogen with thick-walled survival spores and motile zoospores which favour its persistence in the soil and dispersal in free water, and make management of this disease difficult. It is wise for the industry to remain open-minded about alternative management options, which currently rely heavily on the use of phosphonates. The successful management of this disease will rely on an integrated approach, which utilises as many cultural, chemical and agronomic options as possible.

This project assessed soluble potassium silicate and mandipropamid for their efficacy in the integrated management of PRR. The effects of silicon applications on reducing plant disease are well known, and some studies demonstrating reduced severity of PRR and fruit anthracnose, support this. Mandipropamid is currently registered in Australia as Revus® (Syngenta) for control of downy mildew oomycete pathogens in grapes and poppies. Mandipropamid belongs to the carboxylic acid amides (FRAC Group 40), and preliminary results indicate that it inhibits steps in the biosynthesis of phospholipids. It offers protection and control through contact and translaminar activity. It does not stop the release of zoospores from sporangia, but prevents germination of zoospores, and has some effect on mycelial growth and formation of infection structures. While most applications so far are for foliar diseases, it has efficacy against stem and root *Phytophthora* species (including *P. cinnamomi*) in ornamental crops when applied as a soil drench. Efficacy of mandipropamid to reduce Phytophthora root rot in avocado has not been determined.

Methodology

Part A Laboratory and glasshouse trials with mandipropamid

In vitro assays were undertaken to determine EC50 for mandipropamid with several isolates of *Phytophthora cinnamomi* isolated from avocado roots at different locations across Queensland, NSW and Western Australia. The assays involved amending fungal growth media with several dilutions of mandipropamid, inoculating plates with plugs of mycelium of Pc, and measuring colony diameters. Data were analysed in GraphPad Prism, comparing against growth on non-amended media.

Two glasshouse experiments were conducted to investigate efficacy of mandipropamid (applied either before Pc inoculation, or after infection has already taken place), in Hass seedlings. Metalaxyl applied pre or post-inoculation was included as an industry standard control treatment. Mandipropamid drench treatments were applied to the soil at label (0.5mL Revus/L) and 1/100 label rates, and metalaxyl (granular) was applied at the label rate (100g/m^2). Pre-inoculation treatments were applied 12 days prior to inoculation, and post-inoculation treatments applied 12 days after inoculation with Pc.

The *P. cinnamomi* inoculum was prepared using a colonised wheat:sand substrate to infest potting soil. A mixture of wheat, sand and water (1:1:1) was prepared and autoclaved on two consecutive days prior to colonisation with *P. cinnamomi* (BRIP 60404) for a period of 10 days at room temperature, and frequent shaking to disperse inoculum evenly. Challenge inoculation with Pc was performed by potting seedlings from 50mm tubes into 150mm pots containing Searles potting mix infested with the colonised wheat:sand mix at a rate of 2.5% by volume inoculum. Plants were watered to the point of soil saturation on the day of inoculation, and thereafter carefully watered. Seedlings were watered heavily once per week until trial harvest to ensure sufficient soil moisture for disease expression, but allowed to reach a surface dry point before further watering to avoid excessive disease pressure.

Seedlings were monitored and at 3-4 weeks after inoculation, plant heights were measured, roots washed and assessed for % healthy roots and plant/root biomass determined. There were approximately 10 seedlings per treatment in each experiment.

Part B Field trials with potassium silicate

Field trial sites were conducted at 4 locations between Central Queensland and the mid New South Wales coast to assess the effect of potassium silicate on tree health and productivity by reducing Phytophthora root rot (PRR). Each site was visited between 14 April and 10th June 2014 to establish trial sites, establish baseline tree health and establish communication links with growers for potassium silicate application and ongoing management. Grower collaborators were responsible for Agsil applications at three sites (all drench applications), and the pathology team independently conducted the 4th trial on an orchard at Beechmont, QLD. The selected trial sites enabled evaluation of the potential for potassium silicate application to manage Phytophthora root rot across 3 growing regions, with unique orchard management activities at each site.

Details are provided in the Appendix, but briefly, at each site Agsil was applied as a drench at 300mL/tree delivered in 20L to the active root zone. Application was planned to coincide with periods of active root flush (autumn and summer), however frequency and timing varied across sites for a number of reasons. Harvest data (estimated yields per tree) was collected from Sites 1 and 2 in 2015, and Site 4 in 2016. Additionally, commercial packout data was obtained for fruit from Site 1 in 2015 and Site 4 in 2016. Fruit was harvested from trees in the fully replicated trial at Site 4 in 2015 and 2016, ripened and assessed for postharvest anthracnose and stem end rot.

Part C Glasshouse trial with potassium silicate and humic acid

A single glasshouse trial is currently being conducted with Reed avocado seedlings evaluating treatments of potassium silicate and humic acid applied in combination and alone, for efficacy in reducing severity of PRR. This is in response to anecdotal evidence by some growers that tree health is improved by silicon applied with humic acid than when applied alone. Agsil and humic acid (Seasol Humate Plus) were each applied alone at 10%v/v, and in a combined treatment. Two applications were made, 10 days apart, and all plants were inoculated with Pc colonized grain a week after the final treatment. Plant growth and root necrosis will be evaluated in mid-June 2017.

Outputs

1) All isolates of *Phytophthora cinnamomi* (Pc) tested were highly sensitive to mandipropamid *in vitro*.

The mycelial growth of thirteen isolates of Pc from Bundaberg, Childers, Mt Binga, Beechmont, Mt French, and Bellthorpe (Queensland), Comboyne, Duranbah (NSW) and Manjimpu (WA) was strongly inhibited by mandipropamid in Petri dish assays. The IC50, that is, the concentration at which mycelial growth is inhibited by 50%, ranged between 2.2×10^{-7} to 1.5×10^{-6} g active ingredient (a.i.)/mL, which represents concentrations 100-1000x lower than the label (registered) rate for foliar application of Revus® to control downy mildew in grapes and oilseed poppies.

2) Label rates of metalaxyl applied **before** or mandipropamid applied **after** Pc inoculation resulted in significant reduction of disease caused by Pc in glasshouse trials.

The two glasshouse pot trials have provided an initial indication of the efficacy of mandipropamid in controlling *P. cinnamomi* in avocado. The efficacy of mandipropamid soil drench was compared to the industry standard treatment of soil with granular metalaxyl.

The application of 0.5mL Revus (mandipropamid) per L water (the label rate) as a soil drench 12 days after Pc inoculation, or 100g/m^2 Ridomil (metalaxyl) as granules to soil surface 12 days before inoculation, significantly reduced the severity of avocado root necrosis when assessed 4 weeks after inoculation, compared with untreated inoculated controls. There were no significant differences in plant height or root dry weights among treatments in the first experiment, however in the second experiment the pre-inoculation treatment with metalaxyl resulted in significantly greater root biomass than untreated inoculated controls. Application of 1/100 label rate of Revus was not effective, even though *in vitro* analyses indicated inhibitory effects at much lower concentrations.

This is extremely useful information for industry, as it identifies that Revus® (mandipropamid) has post-infection efficacy against *Phytophthora cinnamomi*, under very high disease pressure glasshouse inoculation trials. Revus® is currently registered in Australia for controlling downy mildew in grapevine and oilseed poppy. The data also confirms that metalaxyl (already registered in avocado) has greatest efficacy when applied before Pc inoculation. This improves the existing management strategy for trees declining due to Phytophthora root rot.

3) Indication that Agsil applied as a drench treatment to declining trees improves tree health, yields and quality of fruit.

Trials were conducted on-farm at Childers, Goodwood, Beechmont (QLD) and Comboyne (NSW). At Site 1, Childers, there was a slight but statistically insignificant improvement in tree health after applications of Agsil (300mL Agsil per tree delivered in 20L water on 3 occasions annually) 8 months-1 year after the initiation of the trial, however, by the final assessment two years after the start of treatments, all trees were improving in health rating, and there were no differences between trees treated with Si and controls. Yields were recorded mid-way through the trial in July 2015, and were nearly 15% lower for Agsil drenched trees compared with non-drenched trees, however the difference was not significant. Single bins of fruit from each treatment were run through Superpak commercial packing shed, and while here was only a slight increase in the percentage of fruit in premium grade and total % packouts from the Agsil drench treated trees, there was a 40% increase in 2nd grade fruit (and decrease in 3rd grade fruit) from Agsil-drenched trees compared with non-drenched trees.

The trial at Site 2, Goodwood, consisted of two rows treated with Agsil drench in two separate blocks, with untreated rows either side assessed as the untreated controls. Agsil did not consistently improve tree health compared with untreated trees at any time point, however tree yields from Agsil drenched trees were approximately 13% and 39% higher than trees in the untreated rows for the two blocks, but the increase was not statistically significant.

Treatments to trees at Site 3 were undertaken by project staff, (ie. not the grower), and included AgSil soil drenches as per trial sites 1 & 2 (300mL Agsil per tree, delivered in 20L of water), foliar spray (50mL Agsil per tree delivered in 5L water) and trunk injection (approx. 1mL Agsil per tree in 100mL water). Tree health was not significantly affected by Agsil treatment, although trees sprayed with Agsil had improved health over 2015 and the first half of 2016, however, by the end of the trial in August 2016 trees had similar scores to those in all other treatments. A tray of fruit from each trial tree was harvested at commercial maturity in 2015 and 2016 and assessed for postharvest disease. There were no significant differences in severity or incidence of anthracnose among treatments in either 2015 or 2016, however fruit from trees sprayed or drenched with Agsil had significantly less severe stem end rot than those that were injected in 2015, and all Agsil treatments reduced stem end rot severity, (although not significantly) in 2016 compared with untreated trees. The reduction in SER resulted in greater proportions of marketable fruit.

Agsil was applied 6 times to selected trees in the trial at Site 4, Comboyne, between August 2014 and June 2016. Health of Agsil drenched trees had improved by the final assessment compared with untreated controls, but the effect was not statistically significant, however estimated yield was 14% higher for the Agsil drenched trees. One bin of fruit from each treatment was transported to Coastal Avocados to obtain packout data. While the percentage of fruit in the Premium grade was similar for fruit from the two treatments, there were more fruit in A Grade from Agsil treated trees than from untreated controls. Library trays held for defect analyses showed that there was less pepper spot (4%) in fruit from Agsil treated trees and no anthracnose compared with 8% pepper spot and 3% anthracnose in fruit from untreated control trees. The net return estimated by the packing shed, based on prices received at the time of packing, was $2.63/kg and $3.20 for fruit from untreated trees and Agsil treated trees, respectively.

4) Rates of Agsil at 10% v/v are phytotoxic to seedlings in the glasshouse

The final activity for the project is a glasshouse experiment with Reed avocado to assess Agsil and humic acid applied alone or in combination, for their efficacy on PRR after inoculation with Pc. However, the selected rate of 10%v/v Agsil applied per pot, has been phytotoxic to seedlings and many have died. The humic acid (10% v/v) has not been phytotoxic, and results of root rot severity will be available soon.

Outcomes

1) Mandipropamid shows promise as an effective agent against Phytophthora

2) Improved use pattern for metalaxyl, resulting in increased productivity

3) Demonstration of beneficial effects of Agsil drench application on yields and fruit quality, potentially boosting net return by 20% or more.

Evaluation and Discussion

The project has delivered significant outputs, likely to have a large impact on industry, with a very small budget (less than $54,000 over 3 years). Firstly, the project identified that Revus® (mandipropamid) has post-infection efficacy against *Phytophthora cinnamomi*, under very high disease pressure in glasshouse inoculation trials. Revus® is currently registered in Australia for controlling downy mildew in grapevine and oilseed poppy. Results have been communicated to Syngenta, Australia, who were interested and surprised at the post-infection efficacy. Syngenta are keen to pursue further field-based trials with this active. The data from the glasshouse experiments also confirms that metalaxyl (already registered in avocado) has greatest efficacy when applied before *P. cinnamomi* inoculation. This aligns with current recommended use pattern of application when planting nursery stock into Pc infested soil. Metalaxyl is rarely used in mature trees, due to the cost, and enhanced biodegradation (hence reduced efficacy) when used frequently. The study also demonstrates that the agrichemical companies have anti-oomycete chemicals available for testing, and that perhaps further efforts to interact with these companies and test their pipeline actives, will result in identification of industry-useful products.

While certainly not a "silver bullet" the 4 trials conducted on growers' orchards at Childers, Beechmont and Comboyne demonstrates that Agsil may have a role in orchard management. Tree health was generally improved in Agsil treated trees, but not significantly compared with untreated trees. In 2 of 3 trials where yields were measured, average yields per tree were 13-39% greater from Agsil-drenched trees compared with untreated trees. Fruit quality and packout data demonstrates that fruit from Agsil treated trees had: higher proportions of fruit in 2nd grades (but not Premium grade), superior quality with reduced pepper spot and stem end rot, resulting in higher marketability and an estimated 20% increase in net return to the grower. Fruit postharvest evaluations from one trial showed that stem end rot was significantly reduced in both years of assessment, resulting in a greater proportion of marketable fruit. It is possible that more frequent applications of Agsil targeted more closely to autumn and spring root flushes, may have improved efficacy. A limitation of this trial has been the number of replicate trees receiving Agsil at each site. At 3 sites there were approximately 10 trees per treatment, which is clearly not sufficient due to high tree-to-tree variability. Since the commencement of this trial, we have become aware of other high silicon products which should also be tested for their effects on tree health, fruit quality and packout rates. Future trials should evaluate treatments applied to whole rows (eg. 30 trees), compared with grower standards, at different orchards across Australia (for replication), and across at least 3 years. Whole-row treatments can be assessed for productivity (yield) as well as commercial packout and quality. This approach would give more meaningful and realistic outputs, and be simpler to manage for the project team and collaborating orchards.

Recommendations

1) Industry should support field trials to evaluate efficacy of mandipropamid (and other new anti-oomycete chemistries) for improving health of trees declining from severe PRR

2) Liaise with the SARP coordinator and agrichemical companies, to ascertain intentions on further evaluation and registration for anti-oomycete products.

3) Evaluate Agsil other available silicon products against existing grower standard practice for effects on tree health, productivity (yield), packout rates and fruit quality. These trials would best be undertaken in collaboration with orchards at sites across the major production areas, and with support from local packing sheds.

Scientific Refereed Publications

There have been no refereed scientific publications arising from this project to date.

Intellectual Property/Commercialisation

No commercial IP generated.

Acknowledgements

Mr Louie Lim (PQ Australia) and Dr Leanne Forsyth (Syngenta Australia) are acknowledged for their collaboration and technical input in this project. Project collaborators Chris Nelson and Jim Carney helped to identify orchards and growers or managers to assist with field trials. Coastal Avocados and Superpak packing sheds provided data on bin weights and packouts. Kevin Debreceny and staff (Comboyne Avocados), Craig Perkins (Beechmont), Laurie McCloskey (Peirson Homes Trust, Goodwood) and the team at Avocado Ridge (Childers) were all fantastic collaborators and their assistance with conducting the field trials is greatly appreciated.

Appendices

Appendix 1: AV13021 results including data tables.

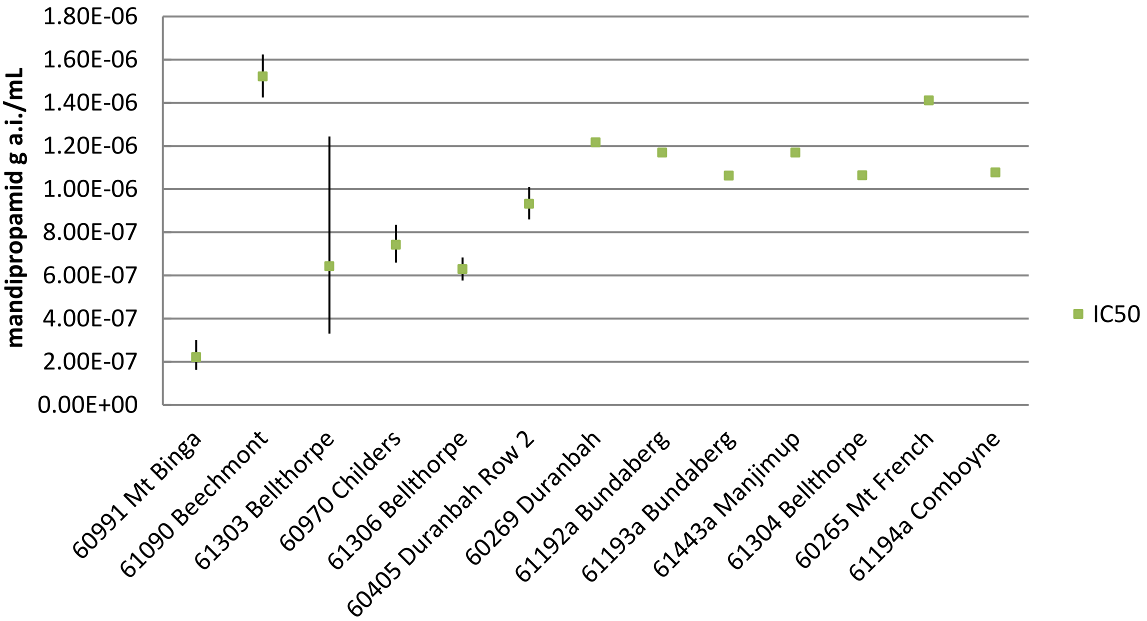
Appendix 1: AV13021 Milestone report #190 results including data tables

Elizabeth Dann, QAAFI, 14th June 2017

Part A Laboratory and glasshouse trials with mandipropamid

In vitro assays have been completed to assess inhibitory effects of mandipropamid several isolates of *Phytophthora cinnamomi* from a range of sites across Queensland, NSW and Western Australia. The IC50, that is the concentration at which mycelial growth is inhibited by 50%, ranged between 2.2 x 10^{-7} to 1.5 x 10^{-6} g active ingredient (a.i.)/mL (Figure 1), indicating that all isolates tested were extremely sensitive to mandipropamid. Note that the label rate of Revus® (50mL Revus® per 100L for spray application of downy mildew on grapes and oilseed poppies) contains 1.25 x 10^{-4} g a.i./mL. This shows that mandipropamid inhibits growth of Phytophthora at concentrations 100-1000x lower than the label (registered) rate for foliar application.

Figure 1. Sensitivity of several Pc isolates to mandipropamid determined by in vitro plate assays



The two glasshouse based pot trials have provided an initial indication of the efficacy of mandipropamid in controlling *P. cinnamomi* in avocado. The efficacy of mandipropamid was compared to the industry standard treatment of soil with the systemic fungicide Metalaxyl. Mandipropamid drench treatments were applied to the soil at label and 1/100 label rates, both prior to and after challenge inoculation of avocado seedlings with *Phytophthora cinnamomi*. Each of the two experiments thus comprised a total of 8 treatment groups: Mandipropamid (4 soil treatment types), metalaxyl at the label rate both pre and post-challenge inoculation (2 treatment types) as well as challenge inoculated and uninoculated controls.

The application of mandipropamid as a soil drench after Pc inoculation significantly reduced the severity of avocado root necrosis. In Trial 1, mandipropamid at label rates applied post-inoculation and metalaxyl applied pre-inoculation were the most effective treatments, with significantly ($P< 0.05$) healthier roots than all other treatments except for the plants receiving no treatment or Pc (Table 1). Application of 1/100 label rate of mandipropamid was not effective, even though in vitro analyses indicated inhibitory effects at much lower concentrations. There were no significant differences in plant height or root dry weights among treatments. In Trial 2, mandipropamid at label rates applied post-inoculation and metalaxyl applied pre-inoculation were again the most effective treatments, with significantly ($P< 0.05$) healthier roots than all other treatments except for the plants receiving no treatment or Pc, which had significantly healthier roots (Table 2). Mandipropamid pre- and metalaxyl post-treatments had similar levels of root necrosis, significantly less than for 1/100 mandipropamid applications or untreated inoculated plants. Plant height was reduced significantly by 1/100 mandipropamid treatments compared with untreated (and uninoculated), label rates of mandipropamid or metalaxyl applied post- or pre-inoculation respectively. There were significant reductions in dry root biomass of 1/100 rates mandipropamid or untreated controls (inoculated with Pc) compared with untreated and uninoculated controls.

Table 1. Avocado seedling height and root health following chemical and *P. cinnamomi* challenge inoculation treatments (Hass), Trial 1

Treatment	Timing relative to Pc inoc.[a]	% healthy roots	Plant height (cm)	Root dry weight (g)
UTC	na	87.0 a	42.1	1.31
Mandipropamid	Post	85.0 a	47.0	1.82
Metalaxyl	Pre	81.0 a	47.7	1.52
Mandipropamid	Pre	66.0 b	51.0	1.54
1/100 Mandipropamid	Pre	63.0 b	54.5	1.42
1/100 Mandipropamid	Post	56.0 b	49.8	1.15
UTC Pc	na	52.0 b	54.8	1.53
Metalaxyl	Post	52.0 b	54.6	1.37
LSD ($P\leq0.05$)		14.6	ns	ns

[a] Plants were inoculated with Pc 12 days after pre treatment, and post treatments applied a further 11 days later. Assessments were made 3 weeks after post-treatments (approx. 5 weeks after inoculation).

Table 2. Avocado seedling height and root health following chemical and *P. cinnamomi* challenge inoculation treatments (Hass), Trial 2

Treatment	Timing relative to Pc inoc. [a]	% healthy roots	Plant height (cm)	Root dry weight (g)
UTC	na	96.0 a	43.0 a	1.50 ab
Mandipropamid	Post	70.0 b	40.2 a	1.17 bc
Metalaxyl	Pre	67.0 b	41.5 a	1.68 a
Mandipropamid	Pre	45.0 c	37.8 ab	1.49 ab
1/100 Mandipropamid	Pre	20.0 d	31.0 c	1.07 c
1/100 Mandipropamid	Post	6.0 d	33.5 bc	1.09 c
UTC Pc	na	12.0 d	39.8 ab	0.91 c
Metalaxyl	Post	40.0 c	39.0 ab	1.11 bc
LSD (P≤0.05)		11.3	6.6	0.39

Means followed by the same letter are not significantly different at P<0.05

[a] Plants were inoculated with Pc 17 days after pre-treatment, and post treatments applied a further 7 days later. Assessments were made 3 weeks after post-treatments (approx. 4 weeks after inoculation).

Part B Field trials with potassium silicate

Four field trials were undertaken on grower orchards.

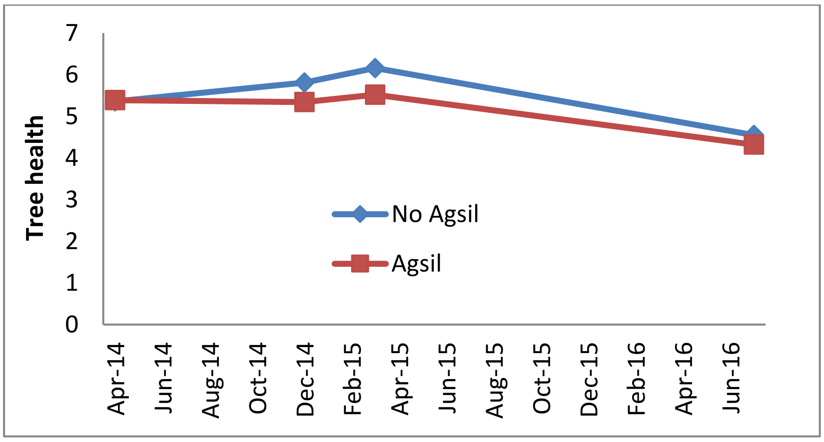
Trial site 1: Project collaborator trial site within an orchard where potassium silicate is already an established orchard treatment, Childers Qld.

The orchard owner is a re-seller of the potassium silicate product Agsil. The entire orchard is currently treated with a low dose of potassium silicate on a monthly basis by fertigation. There is no phosphorous acid used in this orchard. Sick/declining trees are treated at a high 'treatment' rate of 300mL Agsil per tree on 3 occasions annually. Given the entire orchard currently receives Agsil, this trial was set up to examine the effects of withdrawing Agsil treatment from selected trees with obvious signs of decline due to PRR. The trial site was selected in an area of the orchard where declining trees were receiving the treatment dose of Agsil. A total of 22 trees were selected for monitoring over the duration of the trial, with 11 of these clearly identified for Agsil treatment withdrawal. At the trial initiation, the average health ratings of trees subject to Agsil withdrawal and continuing treatment was 5.36 and 5.67 respectively. The tree health rating system used across all trial sites is based on the established system, where 1 = totally healthy and 10 = dead tree. Root windows to facilitate measurements of root growth were installed under a selection of Agsil drench and non-drenched trees. Trees received 300mL Agsil per tree, delivered in 20L of water at each treatment date throughout the trial (February, May July and October 2014; February, May August and December 2015 and May 2016).

At each assessment time from 2014 to 2016 there was a slight improvement in canopy health of the Agsil-drenched treated trees although the difference was not significant (Table 3 and Figure 2). In April 2015 images of root growth under the windows were captured, and analysed with WinRhizo software. There were no significant differences in root lengths, area or diameter between the trees treated with Agsil drench vs non-drenched trees (Table 4), although root length, area and volume were slightly higher for Agsil drenched trees. Yields per tree were nearly 15% lower for Agsil drenched trees compared with non-drenched trees, however the difference was not significant (Table 3). Fruit from the different treatments were deposited into different bins at harvest in May 2015, and analysed separately by Superpak commercial packingshed (no statistical analysis of data possible on single bins from each treatment). There was only a slight increase in the percentage of fruit in premium grade and total % packouts from the Agsil drench treated trees, however there was a 40% increase in 2nd grade fruit (and decrease in 3rd grade fruit) from Agsil-drenched trees compared with non-drenched trees (Table 5).

Table 3 and Figure 2. Tree health and yield data for 2014-2016 from Agsil amendment trial - Site 1, Childers

		Tree health				Yield (kg/tree)
	n	April 2014	Dec 2014	March 2015	July 2016	May 2015
No Agsil Drench	11	5.36	5.81	6.18	4.55	79.9
Agsil Drench	23	5.39	5.34	5.52	4.74	68.1
p		0.958	0.457	0.300	0.732	0.497



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

Table 4. Root analyses data collected April 2015 from Agsil amendment trial - Site 1, Childers

	n	Root length (mm)	Surface area	Projected area	Volume cm^3	Diameter mm
No Agsil Drench	11	484	159	50.5	4.20	1.30
Agsil Drench	9	667	202	64.2	4.92	1.26
p		0.377	0.469	0.469	0.602	0.785

Table 5. Packout data collected May 2015 from Agsil amendment trial - Site 1, Childers

	% packout by fruit grade			
	Premium	AustAvo	Generic	Total
No Agsil Drench	50.3	21.1	26.2	97.6
Agsil Drench	51.3	36.5	10.8	98.6

AustAvo=2nd grade, and Generic=3rd grade fruit

No Agsil drench – downgrades due to limb rub (70%), sunburn (20%), insect chew (5%) and pepper spot (5%)

Agsil drench – downgrades due to limb rub (45%), hail damage (35%), sunburn (20%) and insect chew (5%)

Trial site 2: Independent grower trial in Childers, QLD.

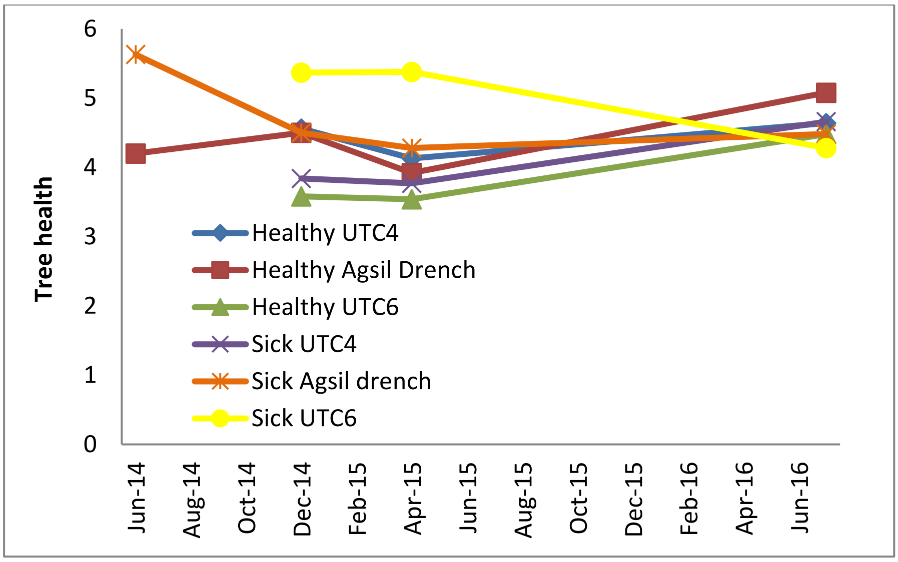
This trial site was established on a well-managed orchard with no history of potassium silicate treatment. The trial consisted of two treatment rows in which potassium silicate soil drenches were applied, one in a block of relatively "healthy" trees, and the other row within a block of "sick" declining trees. Rows either side of the Agsil treated rows were assessed for canopy health as the untreated controls. Trees received 300mL Agsil per tree, delivered in 20L of water at each treatment date throughout the trial (July, October and December 2014; October and November 2015 and March 2016).

There were significant differences in tree health among treated or untreated rows in both "healthy" and "sick" blocks in December 2014 and July 2016, however, the Agsil treated rows were not the healthiest (Table 6 and Figure 3). There may have been a better chance of seeing effects if Agsil had been applied more frequently, eg. 2 applications in Autumn 2015 and 2016 to coincide with autumn and spring/summer root flushes.

Yield data was collected in May 2015. While there was no significant difference, tree yields from Agsil drenched trees were approximately 13% and 39% higher than trees in the untreated rows for the "healthy" and "sick" blocks respectively (Table 6). Packout data was not obtained for this fruit due to a mix-up at the packingshed.

Table 6 and Figure 3. Tree health and yield data for 2014-2016 from Agsil amendment trial - Site 2, Childers

		Tree health				Yield Kg/tree
	n	Jun 2014[a]	Dec 2014	Apr 2015	Jul 2016	May 2015
"Healthy" block						
Row 4 untreated	25	ND	4.56 a	4.13	4.64 ab	ND
Row 5 Agsil drench	25	4.2	4.50 a	3.92	5.08 a	73.7
Row 6 untreated	25	ND	3.58 b	3.54	4.48 b	65.4
p			0.001	0.057	0.027	0.362
"Sick" block						
Row 4 untreated	32	ND	3.84 c	3.77 b	4.66 a	ND
Row 5 Agsil drench	31	5.63	4.50 b	4.28 b	4.48 ab	47.2
Row 6 untreated	32	ND	5.37 a	5.38 a	4.28 b	33.9
p			<0.001	<0.001	0.05	0.161



[a] pre-treatment assessment of tree health, Tree health is rated on a scale where 0=healthy and 10=dead, ND Not determined.

Trial site 3: Independent grower in Beechmont, QLD.

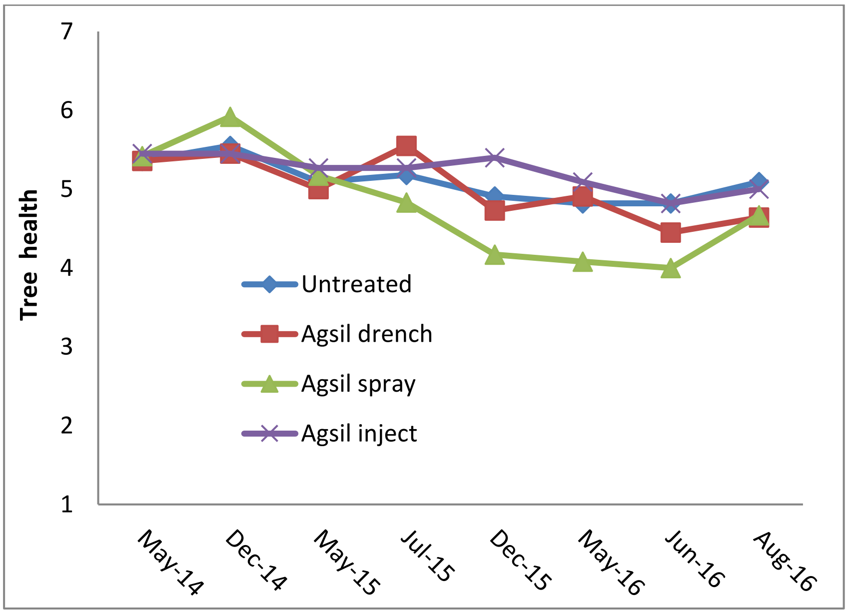
This trial site was established on a small orchard with no history of potassium silicate treatment. Trees currently receive a phosphorous acid injection annually, however an area of the orchard in mid-decline was selected to evaluate a range of potassium silicate application methods. Treatments were undertaken by project staff, (ie. not the grower), and included AgSil soil drenches as per trial sites 1 & 2 (300mL Agsil per tree, delivered in 20L of water), foliar spray (50mL Agsil per tree delivered in 5L water) and trunk injection (approx.

1mL Agsil per tree in 100mL water). In 2014 trees were treated in June (all treatments) and December (spray and drench), 2015 trees treated in May (all treatments) and December (spray and drench), 2016 treated in May (spray and drench) and June (all treatments).

Tree health assessments have been obtained at least twice each year, and fruit were harvested for quality assessments in 2015 and 2016. Canopy health was not significantly affected by Agsil treatment, although trees sprayed with Agsil had improved health over 2015 and the first half of 2016, however, by the end of the trial in August 2016 trees had similar scores to those in all other treatments (Table 7 and Figure 4). There were no sig diffs in severity or incidence of anthracnose among treatments in either 2015 or 2016 (Table 8 and Table 9). Fruit from trees sprayed or drenched with Agsil had significantly less severe stem end rot than those that were injected in 2015 (Table 8), and all Agsil treatments reduced stem end rot severity, although not significantly) in 2016 compared with controls in 2016. The reduction in SER resulted in greater proportion of marketable fruit from Agsil sprayed and drenched trees in 2015, and from all Agsil treated trees in 2016.

Table 7 and Figure 4. Tree health data for 2014-2016 from Agsil amendment trial - Site 3, Beechmont

Treatment	n	Tree health May 2014	Dec 2014	May 2015	Jul 2015	Dec 2015	May 2016	Aug 2016
Untreated	11	5.36	5.55	5.09	5.18	4.91	4.82	5.09
Agsil drench	11	5.36	5.45	5.00	5.55	4.73	4.91	4.64
Agsil spray	12	5.42	5.92	5.17	4.83	4.17	4.08	4.67
Agsil inject	11	5.45	5.45	5.27	5.27	5.4	5.09	5.00
p		0.979	0.903	0.958	0.702	0.253	0.290	0.652



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

Table 8. Fruit quality data collected from fruit harvested in August 2015 from Agsil amendment trial - Site 3, Beechmont

	n	Severity side	Severity stem		n	Incidence side	Incidence stem	Marketability
Untreated	220	9.60	6.97	abc	11	55.9	19.1	51.4
Agsil drench	220	9.04	3.65	c	11	45.5	11.4	58.6
Agsil spray	240	9.03	5.09	bc	12	41.7	16.3	58.8
Agsil drench no phos	220	12.77	8.26	ab	11	52.7	20.0	45.9
Agsil inject	219	9.79	9.16	a	11	48.6	20.9	50.5
p		0.091	0.018			0.417	0.411	0.455

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 9. Fruit quality data collected from fruit harvested in August 2016 from Agsil amendment trial - Site 3, Beechmont

	n	Severity side	Severity stem	n	Incidence side	Incidence stem	Marketability
Untreated	209	4.77	12.8	11	25.4	33.0	56.5
Agsil drench	209	5.34	9.48	11	27.8	26.8	61.7
Agsil spray	228	5.72	9.61	12	27.6	26.3	64.0
Agsil inject	209	3.79	8.61	11	20.6	26.3	63.2
p		ns	ns		ns	ns	ns

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

Trial site 4: Independent grower in Comboyne, NSW.

This trial site was established on an orchard with no history of potassium silicate treatment. Trees have been receiving recent spray applications of phosphorous acid to restore tree health, however a row of trees in mid-decline was identified as a treatment site. Nine and ten trees, respectively, were selected for untreated controls or Agsil drench applications (300mL Agsil per tree, delivered in 20L of water). Agsil was applied by farm staff in August 2014, February, June, November and December 2015 and May and June 2016. Trees were either pruned heavily or staghorned in March 2015. This trial was visited twice only, prior to treatment application and at the end of the trial in 2016.

All trees in the trial were harvested at the time of final tree health assessment (August 2016) and one bin of fruit from each treatment were transported to Coastal Avocados to obtain packout data. There was a full bin of fruit (430kg) from trees in each treatment, and an estimated additional 120kg fruit from the final Agsil treated tree to be picked. This fruit would not fit into the bin for transporting to the packingshed. The estimated yield per tree was 14% higher for the Agsil drenched trees (Table 10). While the percentage of fruit in the Premium grade was similar for fruit from the two treatments, there were more fruit in A Grade from Agsil treated trees than from untreated controls. Library trays held for defect analyses showed that there was less pepper spot (4%) in fruit from Agsil treated trees and no anthracnose compared with 8% pepper spot and 3% anthracnose in fruit from untreated control trees (Table 11). The net return estimated by the packingshed, based on prices received at the time of packing, was $2.63/kg and $3.20 for fruit from untreated trees and Agsil treated trees, respectively.

Table 10. Tree health data for 2014 and 2016 and estimated yield 2016 from Agsil amendment trial - Site 4, Comboyne

Treatment	n	Tree health		Est. Yield kg/tree
		Jun 2014	Aug 2016	Aug 2016
Untreated	9	5.3	5.6	47.8
Agsil drench	10	5.2	4.3	55.0
p		0.903	0.128	

Table 11. Packout data collected August 2016 from Agsil amendment trial - Site 4, Comboyne

	% packout by fruit grade			
	Premium	A Grade	Class 1	Processing
Untreated	56.5	27.8	13.2	2.5
Agsil Drench	55.6	32.6	9.4	2.3

A Grade=2nd grade, Class 1=3rd grade fruit, Processing=defect
Untreated – downgrades due to caterpillar damage (16%), hail (13%), wind rub (19%), sunburn (22%), pepper spot (8%) and anthracnose (3%)
Agsil drench – downgrades due to caterpillar damage (18%), hail (21%), wind rub (22%), sunburn (24%), and pepper spot (4%). No anthracnose damage.