Hort Innovation

Final Report

Understanding the mode of action of phosphite in avocado for enhanced management of Phytophthora root rot

Project leader:

Assoc. Prof. Elizabeth Dann

Delivery partner:

University of Queensland

Project code:

AV19005

Project:

Understanding the mode of action of phosphite in avocado for enhanced management of Phytophthora root rot (AV19005)

Disclaimer:

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Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

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Public summary

Phytophthora root rot has plagued the Australian avocado industry since the early days of commercial production, and growers have been encouraged to adopt an integrated disease management strategy, based upon principles of the "Pegg wheel", which includes the use of anti-oomycete chemicals metalaxyl and phosphonic acid, and both are still used in industry today.

Since their introduction in 1986-87, there has been much research in Australia and internationally on phosetyl-Al and potassium phosphonate in several crop and model plant species, and multiple modes of action are accepted to explain efficacy. In AV16007, we have demonstrated the highly stable and mobile nature of phosphite (the breakdown anion of phosphonate) in avocado, and shown that considerable concentrations of phosphite end up in fruit pulp and seed at commercial maturity. In addition, phosphite can be measured for several months in seedlings grown from seed with high phosphite concentrations.

The current project, AV19005, was undertaken to investigate mode of action of phosphite in avocado. Effects on growth and inherent plant defence responses were studied in glasshouse and laboratory trials, while links with the carbohydrates starch and soluble sugars, were investigated by comparing various tissues at different times from phosphonate sprayed versus unsprayed trees. Our results show there are no growth stimulation effects of phosphite, in the absence of the pathogen. That is, phosphite is not acting as a fertiliser. Quantitative PCR analysis demonstrated that prior to and during infection by *Phytophthora cinnamomi*, phosphite primes and activates genes associated with the host defence response in roots, which has previously been shown in model plant species, but not in avocado. Phosphite clearly impacts carbohydrate availability and/or metabolism in avocado. At the autumn sampling, there was a trend for starch (and phosphite) to be increased in expanding flush leaves, corresponding to reductions in soluble sugars in those tissues. The reverse occurred in mesocarp (flesh) of maturing fruit. This trend was not observed so clearly at other sampling times. Overall, a positive correlation between phosphite and the soluble sugars glucose, fructose and sucrose was demonstrated. The mode of action studies highlights that phosphite affects multiple pathways within the plant, and we are just beginning to understand the impacts.

Extensive field trials have helped to understand translocation patterns of phosphite, and the differences amongst climatic regions and tree age. Phosphite accumulates in fruit when applied in the window preceding commercial harvest, whether it be the summer application window to Shepard trees in north Queensland, or autumn/winter applications to Hass in southeast Queensland. Shifted timing of applications in southeast Queensland shows promise for fewer applications at more favourable times of the year for more sustained translocation of phosphite to roots, where it is required.

Industry support, education and extension activities have been key components of the project. Project outputs will facilitate more targeted and effective applications of phosphite, contributing to healthier and more productive orchards.

Keywords

Phytophthora cinnamomi, phosphonate, phosphorous acid, carbohydrate, defence genes

Introduction

Phytophthora root rot remains one of the largest constraints to orchard productivity in Australia and globally, costing the Australian industry an estimated $17 million annually in lost production, treatment and tree replacement (Hall and Dann, unpublished). This disease is one of the major contributors to the average 50% yield gap relative to that achieved by the most successful growers. Returns to growers have, predictably, fallen over the last couple of years, given the increase in production (>122,000 T in 2021-2022, compared with 78,000T in 2020-2021, AAL Facts at a Glance), along with Covid-19-related increased costs of inputs, including fertilisers, pesticides, transport, labour etc. It is imperative that our industry lift productivity and quality to remain globally competitive. Optimising tree health and yields through improved management of Phytophthora root rot will form part of the solution.

Applications of phosphite, (primarily as potassium phosphonate formulations), have been a critical component in the integrated management of Phytophthora root rot since the 1980s. This early research included studies on the optimum mode, concentration and timing of application, and potassium phosphonate became widely used as a trunk injection and foliar sprays. Growers in Australia have access to a laboratory testing service, to ensure that application, by either registered method, results in increased concentrations in roots, where it is required. Since then, on-farm usage patterns have increased, frequently without expected increases in root accumulation or disease control. This prompted the question from growers “Where is the phosphite going?”, particularly when the cost of potassium phosphonate has increased two-fold in the last couple of years. While growers frequently send rootlet samples for analyses of phosphite, however, there has been no regular testing for residues in fruit pulp. Activities in related project AV16007, demonstrated that phosphite (the breakdown product of phosphonate), is translocated to the fruit, even when this organ was thought not to be a “sink” for photosynthate and phosphite. In addition, limited testing of other tissues showed that phosphite is stored, and/or continuously translocated within the tree. These interesting results show that our understanding of metabolism of phosphite within the tree, and its interactions with the pathogen is limited, warranting more detailed study.

The studies in AV19005 addressed the mode of action of phosphite within the plant, specifically indirect inhibition of *P. cinnamomi* via the activation of inherent plant defence responses; translocation and accumulation of phosphite and carbohydrates in different tissues and optimal timing of phosphite application for maximum efficacy as a crop protectant. Project outputs will facilitate more targeted and effective applications of phosphite, contributing to healthier and more productive orchards.

At commencement in June 2020, the project was aligned with the avocado industry Strategic Investment Plan (SIP) (2017-2021) Outcome 4:

> “By 2021, productivity (marketable yield per hectare) has improved by 15 per cent on average, without increased production costs per kilogram. The strategic intent here is to accelerate the application of proven good practices in production as a means of improving on-farm profitability, business resilience and ability to compete in domestic and international markets.”

A new SIP (2022-2026) has recently been developed, and the project specifically aligns with Outcome 2: “Industry supply, productivity and sustainability”, and Strategy 4. “Develop and optimise fit for purpose pest and disease management strategies.”

Methodology

<u>Mode of action studies</u>

a) Plant defence responses

Seedlings (40) of ‘Reed’ were transferred to the “two pot system” and sprayed with potassium phosphonate. One week later, 2-3 g roots sampled for phosphite concentration. 20 plants were inoculated with zoospores of *Phytophthora cinnamomi*, 20 left uninoculated. Roots were sampled at 0, 3, 6, 12, 24 and 48 h after inoculation and stored -80°C. Total RNA extracted, quantitative PCR used to measure the relative abundance of defence genes, phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), pathogenesis-related protein 5 (PR5), metallothionein (METAL), glutathione–S–transferase (GSH), endochitinase, and putative MAPK4 signalling gene. Abundance of genes was calculated relative to endogenous avocado control gene (actin). *P. cinnamomi* in roots was quantified by measuring RNA transcripts of cytochrome c oxidase II gene (cox II). The experiment undertaken twice, and generalised additive model (GAM) analysis was used to predict the relationships between the three variables including gene transcript levels, root phosphite concentration (mg/kg) and time (hours post inoculation; hpi).

b) Growth stimulation effects

Glasshouse studies have been undertaken as part of a PhD student project to assess whether there is a “biostimulant” effect, for example, whether applications of phosphonate (or metalaxyl) improve plant growth (including the strength and volume of the root flush) in the absence of Pc, and the relative curative (post-infection) and preventative (pre-infection) efficacy of phosphite applications. Glasshouse trials were repeated.

<u>Storage, metabolism and translocation of phosphite in avocado trees</u>

Carbohydrates were measured in several different tissues at different time points over two years. Samples were collected from the field trial at Ravensbourne, QLD, (described below), from the unsprayed plot and trees sprayed at both application times (summer and autumn/winter, as industry standard practice). Samples were extracted for starch and soluble sugar analyses. Total starch analyses were done by project staff in Brisbane, utilising the colorimetric assay (Megazyme), while quantification of soluble sugars, sucrose, fructose, glucose, perseitol and mannoheptulose, was completed by HPLC by project collaborators at CSIRO, Adelaide.

Maximising uptake and efficacy of phosphite

Field trials at Capel (WA), Walkamin (north QLD) and Ravensbourne were undertaken.

The trial at Capel was in collaboration with Jasper Farms, and staff managed all aspects of spray treatments and root, fruit collection, in consultation with the project leader. The Hass trees were young, planted November 2018, and had not received any phosphonate sprays prior to the commencement of the trial treatments in December 2020. Treatments were control (no sprays), summer sprays, autumn/winter sprays and sprays at both summer and autumn/winter. A single row of trees was assigned to each treatment, with two buffer rows between treatment rows. Samples were collected from several trees in each row, and pooled to give one sample per treatment.

The Walkamin (north Queensland) trial with Shepard avocados was undertaken in collaboration with Dept Agriculture and Fisheries staff, at Walkamin Research station. Treatments were as for Capel trial, described above, except that the trial was designed as a randomised complete block, with 4 replicate plots per treatment. Each plot comprised 2 or 3 trees, and root and fruit samples from each plot were pooled. Appendix 3 describes the treatments and results from the Walkamin trial.

Project staff completed all activities from September 2020 to July 2022, with Hass trees at the Sunnyspot Farms orchard at Ravensbourne, southeast QLD. As well as unsprayed control, summer and autumn/winter applications, additional treatments included sprays at the leaf flush, at slightly different times to the currently recommended strategy, and application via soil drench. Treatments were applied to plots of 9-12 consecutive trees in a row, with some treatments having two plots. The samples of various tissues for phosphorous acid and carbohydrate analyses were collected from the nil (unsprayed) and phosphonate sprayed at both application windows.

Roots, vegetative tissue and fruits have been collected at key times and analysed for phosphorous acid (phosphite) by MA Analytical Services.

Additional field trials aligned with a PhD student project are in progress, to assess efficacy of pre- and post-planting and methods of phosphonate application (and other oomycete fungicides), on establishment of trees in sites with high *Phytophthora cinnamomi* pressure.

Industry support, steering committee, communications, training and extension

Three steering (PRG) committee meetings were held during the project, and the minutes of each meeting have been provided with previous milestone reports. The project leader and team staff and students actively participated in many extension, education, workshop events across Australia during the project, and these are summarised in Appendix 5. These were in collaboration with industry or extension projects and personnel (e.g. AAL, AV17005), or other regional consultants or resellers.

Training of a PhD student, post-docs and early career researchers and other technical staff has been a key component of the project.

Results and discussion

Mode of action studies

a) Plant defence responses

In the model plant *Arabidopsis thaliana*, phosphite primes defences by suppressing the activation of mitogen activated protein kinase 4 (MAPK4), a negative regulator of biotic stress signaling and salicylic acid accumulation. In AV19005, we identified the expression of defence-related genes in avocado associated with suppression of *P. cinnamomi* root infection at a range of phosphite concentrations. MAPK4 was suppressed by phosphite in uninoculated roots, with greater suppression at higher concentrations, suggesting roots are “primed” prior to infection. After inoculation and infection by *P. cinnamomi*, MAPK4 expression was higher from 6h compared with uninoculated roots, and reached a peak at 24h after inoculation in roots with low phosphite concentrations. This could mean that during the early biotrophic phase of infection, the jasmonic acid pathway is activated and salicylic acid pathway is downregulated.

Pathogenesis-related 5 gene (PR5) gene expression increased in uninoculated roots with increasing phosphite concentrations, reaching a peak at 12h in roots with high phosphite concentrations. PR5 expression was even higher after inoculation with *P. cinnamomi*, increasing with time and phosphite concentration. Relative quantities of defence genes lipoxygenase and endochitinase were enhanced in roots at low-medium phosphite concentrations in inoculated seedling roots (see Appendix 4). There were inconsistent and inconclusive relationships for the other defence genes studied.

b) Growth effects

Repeated glasshouse trials conducted by the PhD student have shown that foliar sprays with phosphonate or metalaxyl applied as granules to potting media, do not affect growth of avocado seedlings in the absence of Phytophthora. This shows there is no "growth stimulating" effects of phosphonate, as reported in some studies. Phosphite is not the form of phosphorus that is readily available for plant growth, and it is not converted to PO_4, the fertiliser form, in plants. There is some evidence that it may be converted slowly by microbial action in soil. Growth effects previously reported may be attributable to reducing soilborne disease and/or effects of potassium, in the potassium phosphonate formulations. This is supported by work in the current project, where phosphite concentration was significantly and positively correlated with accumulation of potassium in fruit pulp harvested from the Ravensbourne field trial ($p=0.042$, Figure 1). Industry needs to be mindful of phosphite formulations being sold as fertilisers, and not crop protectants (and thus not registered with APVMA), for example Phoscare® (https://www.zadco.com.au/product/phoscare-0-28-25/). Also to note, there are separate (independent) trials progressing for registration of ammonium phosphonate in Australia. It will be important to monitor nitrogen accumulation (as well as phosphite), in fruit pulp, as high nitrogen is frequently associated with more severe fruit anthracnose disease and pulp breakdown.

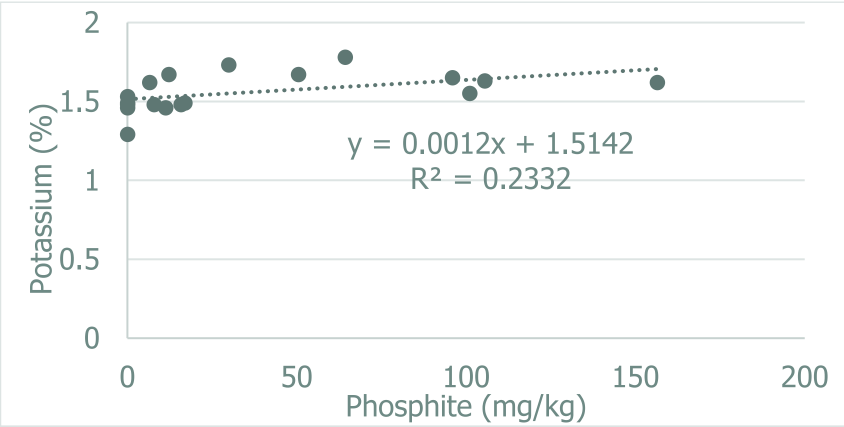

Figure 1. Relationship between phosphite concentration and potassium in pulp of commercially mature fruit

Metalaxyl treatments were consistently more effective at protecting roots from severe root rot after inoculation of glasshouse seedlings with *P. cinnamomi*, and this was reflected in enhanced growth parameters of metalaxyl + Pc seedlings compared with untreated or phosphonate + Pc.

Field trials are currently in progress at Maroochy Research Station, and Ravensbourne, QLD. Results to date from the Maroochy trial are showing that phosphonate drench prior to planting in October 2022, or metalaxyl post-planting with or without sprays of phosphonate, are aiding establishment of avocado nursery trees planted into a site with high Phytophthora root rot pressure (Appendix 1). This trial has now been terminated and data undergoing analysis. The Ravensbourne trial includes oomycete chemistry not previously evaluated for root rot in avocado in Australia, but which have shown promise in trials in California (Belisle et al 2019). In the early stages of the trial, one product is having a significant positive effect on health of replants compared with untreated controls and another oomycete product. This trial continues to be monitored. These trials provide useful information to industry on best practice for tree establishment under high PRR disease pressure. Congratulations, and thank you if you are actually reading this report.

<u>Storage, metabolism and translocation of phosphite in avocado trees</u>

Extensive fruit residue monitoring activities in AV16007 demonstrated that phosphonate sprays in the weeks prior to commercial harvest resulted in phosphite translocating to mature fruit pulp and seed, an application time previously shown not to result in high fruit residues (Whiley et al., 1995, 2001), although trunk injection in spring resulted in higher residues in fruit than roots and shoots at harvest more than 250 days later (Whiley et al., 1995). Our more recent result was supported by a study from project collaborators in South Africa (McLeod et al., 2020). Further testing showed accumulation of phosphite in many tissues not directly sprayed with phosphonate, suggesting that this substance may be stored and translocated with carbohydrates.

In AV19005, root, stem, leaf, fruit and flower tissues were collected from trees across two years from two treatment plots (phosphonate sprayed during the spring/summer and autumn application windows, or unsprayed control), in a field trial at Ravensbourne. For the leaves and stems, mature (hardened) and expanding leaves (flush) were sampled. Maturing fruits (approx. 18% dry matter) were harvested in April and mesocarp (flesh) and seed were separated. The Ravensbourne trial was established as a demonstration to investigate effects of spray timing and application method on phosphorous acid accumulation in roots, fruit and other tissues (described in more detail in the "Maximising uptake and efficacy of phosphite" section. For the carbohydrate study, tissues were collected from 5 pseudo-replicates within each treatment block (phos acid sprayed or untreated), and each replicate consisted of 2 tree plots. These tissues were analysed for phosphorous acid concentrations (MA Analytical), starch and soluble sugar measurement, by UQ project staff and CSIRO collaborators (Drs Harley Smith and Marc Goetz), respectively.

At the first sampling in April 2021, phosphonate had been sprayed twice, in November and December 2020, and there were significantly higher phosphite concentrations in flush roots, stems (both soft flush stems and hardened mature stems), and in seed of maturing fruit, compared with the same tissues from unsprayed (nil) trees (Figure 2). Starch concentration was highest in the stem samples and expanding flush leaf tissue, but there were no significant differences between treatments except that starch concentration in mesocarp of maturing fruit from phos sprayed trees was lower than in fruit from unsprayed (nil) trees (Figure 2). Both glucose and fructose were lower in expanding flush leaves of phos sprayed trees compared with unsprayed, but there were no other significant treatment effects in other tissues in those sugars or sucrose. Perseitol and mannoheptulose were also lower in expanding flush leaves of phos sprayed trees compared with unsprayed. Mannoheptulose was lower in flush stems and perseitol was lower in immature fruit flesh of sprayed trees compared with respective tissues in unsprayed trees. There were no other treatment effects. Similar results of starch accumulation were observed in tissue samples collected in April 2022.

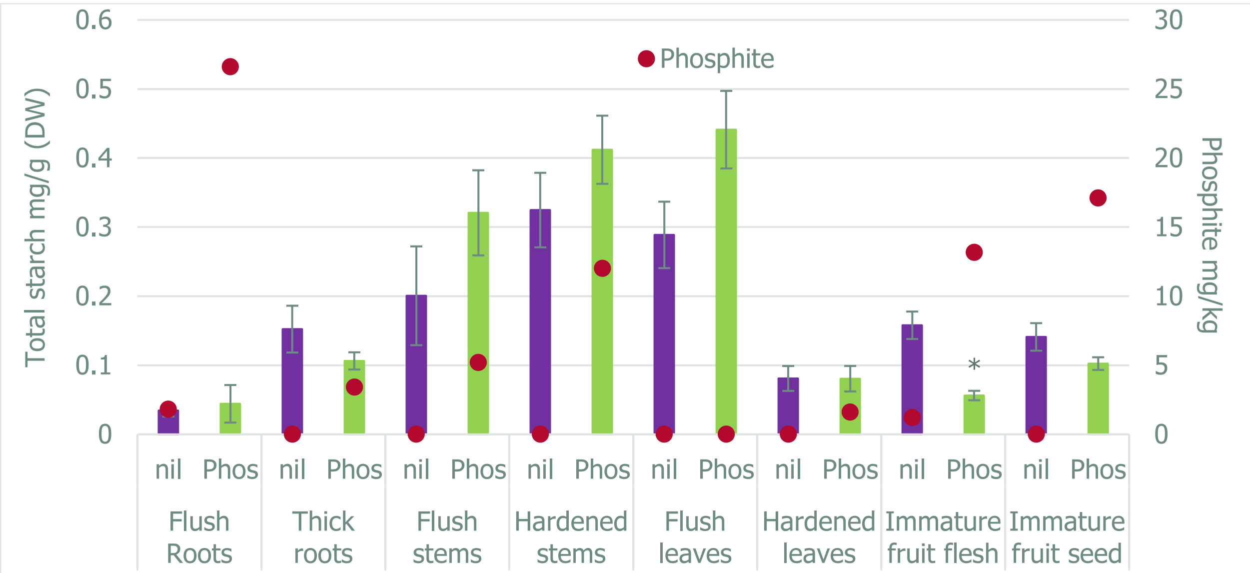

Figure 2. Total starch and phosphite in tissue samples collected from phosphonate sprayed (Phos) or unsprayed (nil) trees in April 2021. "Phos" trees were sprayed on 10 Dec 2020 and 14 Jan 2021 (* indicates significantly different at p=0.05 to nil control for the same tissue)

Unfortunately, farm staff inadvertently sprayed the entire experimental block (including the unsprayed (nil) control plots) with phosphonate in early July 2021, prior to the second tissue collection time point, which has compromised comparisons between sprayed and nil treatments after the first tissue collection in April 2021. However, the accidental spray of nil plots and subsequent tissue analyses provides further information about the longevity and translocation of phosphite over time. One day after spraying, phosphite was detected in flesh (10mg/kg) and seed (<5 mg/kg) of mature fruit. Phosphite in fruit flesh and seed from sprayed trees was higher at 68 and 33 mg/kg, respectively, as expected, following 5 scheduled sprays applied to phos treatment between December 2020 and June 2021. Two weeks later, it was detected in flower buds (146 mg/kg in nil and 674 mg/kg in phos), and four months later in flowers (47mg/kg), flush stems (17 mg/kg), leaves (5 mg/kg), and roots (19 mg/kg). Nine months after the accidental spray, phosphite was no longer detected in flush stems, but was present in flush roots and immature fruit, albeit at very low concentrations (average <5 mg/kg). Phosphite could not be detected in tissues sampled at 12 or 15 months after the initial single accidental spray.

Analyses to demonstrate correlations between traits (phosphite, starch and each of the sugars) were completed. Data points for undetectable (zero) phosphite concentration were removed, as we wanted to look for relationships between phosphite concentrations and different carbohydrates. Appendix 2 shows the matrix of Pearson correlations amongst the measured traits, both combined across tissue types and for individual tissue types, where the direction and significance of the correlation are represented by "+" or "-". Across all tissue types, phosphite concentration is significantly positively correlated with the soluble sugars glucose, fructose and sucrose (these sugars increase in concentration with increasing phosphite), and negatively correlated with the C7 complex sugar mannoheptulose (decreases with increasing phosphite). Looking at specific tissue types, there are highly significant correlations between phosphite and sucrose in stems, flowers and mesocarp (flesh) of immature fruit. Phosphite is also positively correlated with starch in seed of mature fruit, and perseitol in flower buds. There are negative correlations of phosphite with sucrose and glucose in flush leaves, with glucose in immature fruit flesh, starch in flush stems and perseitol in immature fruit seed.

It is interesting to look at correlations amongst the carbohydrates, independent from phosphite. As expected, glucose, fructose and sucrose are highly positively correlated with each other across all tissue types, and many individual tissues, e.g. flowers, leaves and stems. Glucose and fructose are also significantly positively correlated with both mannoheptulose and perseitol (overall, and in many tissues), and sucrose is positively correlated with perseitol. Mannoheptulose and perseitol are positively correlated across all tissues and other tissues individually (Appendix 2).

Results in this section suggest that phosphite reduces starch accumulation in the mesocarp of fruit undergoing maturation. In growing and maturing tissues, starch functions as a carbohydrate reserve to buffer tissues when sugar availability is reduced, or metabolism is increased. Reduced starch levels in response to phosphite treatment during fruit maturation suggests that phosphite may reduce carbohydrate availability to developing fruits. Alternatively, phosphite may act to increase carbohydrate metabolism resulting in an augmentation of starch degradation in the mesocarp of fruit undergoing maturation. As sucrose metabolism generates glucose and fructose, decreased levels of these two hexoses in expanding flush leaves suggests that sucrose metabolism may be reduced by treatment with phosphite. However, while the levels of starch in expanding flush leaves derived from phosphite and control treated were not significantly different, the apparent trend for higher starch levels in phosphite treated leaves is intriguing. It corresponds to significant reductions in all soluble sugars except sucrose in expanding flush leaves after phosphite treatment compared with those from control (nil) treatment. For example, an increase in starch biosynthesis could also reduce glucose and fructose levels in expanding flush leaves. Therefore, whether phosphite decreases sucrose metabolism and/or increases starch biosynthesis could explain the lower levels of glucose and fructose in expanding flush leaves. Overall, results in this project support a hypothesis that phosphite impacts carbohydrate availability and/or metabolism. If phosphite acts to increase plant defense responses, which consumes carbohydrates, then applications of phosphite may change carbohydrate flux and metabolism in developing organs of the shoot system. Effects of phosphite on defence responses was only studied in flush roots in the current project, and independently from the carbohydrate work.

<u>Maximising uptake and efficacy of phosphite</u>

Capel

The trial with Hass trees in WA confirmed anecdotal reports of difficulty in increasing phosphite levels in roots of young trees, despite repeated sprays. Four sprays between December 2020 and May 2021 failed to increase root levels above 20 mg/kg, with residues of <20 mg/mg (fresh weight) also detected in young stems fruit pulp at commercial harvest in July 2021. Two sprays in February 2022 resulted in root concentrations in March 2022 of 27 and 51 mg/kg, however 4 further sprays in March to June did not give increased root levels when sampled in July 2022, and as in the previous year, phosphite was measured in young stems (up to 25 mg/kg) and fruit pulp concentrations at harvest were less than 20 mg/kg. It is clear that considerable amounts of phosphite are present in young stems, presumably to then be translocated to other tissues over time. It is possible that spray concentrations or volumes were not optimal, or that vigorous growth of healthy young trees and roots diluted the available pool of phosphite after each application. Highest root levels were achieved after February sprays, when vegetative growth is still very active (leaf and root flushes generally occur concurrently over spring and summer), and presumably driving phosphite down to the roots.

Walkamin

Results are presented in detail in Appendix 3. Briefly, phosphite levels never exceeded >75ppm at any time over the 2 years, despite 4 sprays about 2 weeks apart at each of the application windows. Root phosphite levels never exceeded >25ppm at the 2021 or 2022 pre-summer sample times, indicating that the autumn/winter applications did not sustain high levels through vigorous spring growing months. But there were very low fruit residues in mature fruit from trees with autumn/winter only sprays. This is likely because fruit were harvested prior to that window of sprays, so are not a sink for phosphite. Phosphite applications (in autumn or summer) always resulted in significantly higher root phosphite levels in the post-spray root sample. It is important to note however that fruit phosphite levels (see next section) were much higher after summer applications than in the roots, indicating that the foliar-applied phosphite in summer was being translocated to the fruit, not the roots, and thus may pose a risk for exceeding MRL. It is feasible that, with the vigorous growing conditions in north Queensland, root growth may exceed *Phytophthora* infection and loss of roots to root rot, so that the summer sprays could be dropped, and alternative strategies utilised. Applications could resume after harvest, with first applications commencing in March, prior to pruning, to ensure sufficient canopy to drive phosphite to the roots. Suggest that subsequent applications could be spread monthly rather than fortnightly.

Ravensbourne

Sprays were applied at the summer root flush, autumn/winter root flush, and at leaf flushes as well as via soil drench. Phosphite residue testing showed that applications to spring/summer leaf flush (i.e. before leaves had fully hardened off), resulted in higher root concentrations in April 2021 and 2022, than applications at the root flushes, and lower residues in fruit (July 2021). Sprays during the traditional autumn/winter application window increased root levels but also resulted in very high levels in early flowers in July, consistent with a previous report (Nartvaranant et al., 2004). While *in vitro* studies showed that high concentrations of phosphite were phytotoxic to pollen germination and pollen tube growth, there was a small effect of reduced pollen germination and growth when phosphonate was applied at early flowering in the field (although the impact on yield was not assessed), leading to the recommendation that applications cease at least 6 weeks prior to anthesis (Nartvaranant et al., 2004).

October, November sprays to leaf flushes increased levels in roots in December, prior to the onset of the normal wet season. Further flush sprays in February, March, April maintained high root levels in April 2022, and also through July and December 2022 without further application, although residues in fruit were also elevated. Spray applications only at the spring/summer root flush (Dec, Jan) in this growing region are likely not sufficient to protect roots from Pc infection, and concentrations in roots did not exceed 30mg/g at any sampling time.

Despite transient high concentrations of phosphite in roots after soil drench treatments, levels could not be sustained over a long period of time, and this application method is not effective.

The results from the trial at Ravensbourne show that if conditions are favourable, sprays when trees are vegetatively flushing may be advantageous, as trees can transiently store phosphite in young stems and leaves, and it is translocated to roots and other sink strengths as those leaves mature and become net exporters of photoassimilates. The earlier suggested timing of application prior to autumn root flush is also supported by a recent study in Tzaneen, South Africa, published by project collaborators (Jolliffe et al., 2023), showing that Pc quantities in roots in late autumn are significantly higher than in other seasons, and correlated with the number of hours of higher soil temperatures 2 months prior, i.e. late February-March (late summer). Further on-farm trials could assess sprays in October, November, late February and March alongside sprays in traditional application windows (particularly the autumn/winter), for efficacy and fruit residues.

<u>Industry support, steering committee, communications, training and extension</u>

The project leader and team staff and students actively participated in many extension, education, workshop events, academic conferences across Australia during the project, and at the World Avocado Congress in New Zealand, April 2023, and these are summarised in Appendix 5. These were in collaboration with industry or extension projects and personnel (e.g. AAL, AV17005), or other regional consultants or agronomists. One article has been published in *Talking Avocados* so far, and in the scientific peer-reviewed literature, and further articles are in preparation, pending finalisation of experiments and data analysis.

Training of a PhD student, post-doc and early career researchers and other technical staff has been a key component of the project. Liz has co-supervised a MSc student (with collaborator Prof Adele McLeod), at Stellenbosch University, South Africa, and this has resulted in a paper published in the peer reviewed scientific literature (see Jolliffe et al., 2023). Other manuscripts are being drafted for publication in due course.

Outputs

Table 1. Output summary

Output	Description	Detail
A guide for optimal phosphonate concentrations to effectively prime avocado plant defences against *P. cinnamomi* infection	Role of phosphite in activating plant defences vs direct (fungistatic) inhibition of Pc	Defence priming was shown to occur at relatively low root phosphite concentrations, <60 mg/kg, a manuscript is currently in preparation. The link between activated defences and reduction of Pc infection of roots was not demonstrated
Develop a guide detailing the optimal timing of phosphite application required to achieve maximum efficacy as a crop protectant with acceptable fruit residues	Information from field trials in north QLD and southern QLD measuring root and fruit phosphite after different application regimes	Results have been discussed amongst project team, and presented at workshops in Bundaberg, Manjimup and Walkamin in 2022. Reports provided as attachment.
Prepare a report detailing optimal application regimes specific to different growing regions	Key messages from field trials to inform and support decisions on timing of phosphite applications	These are included as appendices to this report. Manuscripts for publication, where appropriate will be prepared in coming months. Factors to consider include timing in relation to leaf flush, harvest and pruning
Updates and recommendations to be published in the Avocado Industry BPR and Talking Avocados	Several	See Appendix 1. Outputs will be communicated as appropriate
Posters and presentations delivered at meetings to increase training, extension and communication capacity Collaborative interactions with domestic and international avocado pathology network Scientific publications in peer-reviewed journals	Several outputs at industry meetings, field days, Best Practice Resource, Talking Avocados, World Avocado Congress, scientific (plant pathology) conferences	See Appendix 1. Ongoing discussion and interaction with project collaborators, Prof David Guest and Prof Adele McLeod Scientific manuscripts in preparation: Scarlett, Guest, Van Ogtrop, Dann (?)Phosphite primes defences against Phytophthora in avocado roots Jose, Drenth, Dann (?) Effects of phosphonate and metalaxyl fungicides on growth of avocado seedlings
Register of technical services, advice and assistance provided to growers	See detail	Email correspondence regarding activities and grower enquiries for AV19005 and AV16007 has been saved.

Documents associated with milestone reports, M&E, communications plan and risk register	Yes, all available upon request.	All Milestones submitted to Hort Innovation, M&E, Comms, risk register etc. available as attachments to MS #102

Outcomes

Table 2. Outcome summary

Outcome	**Alignment to fund outcome, strategy and KPI**	**Description**	**Evidence**
< List the outcome (e.g. knowledge, awareness, practice change, commercialisation, availability of new knowledge for next phase project) >	< Align to the relevant Fund outcome, strategy and KPI >	< Describe and define the outcome in terms of how it was realised by the target stakeholder group(s). Explain how the outcome is relevant at the Fund level >	< What forms of evidence were collected to identify and understand the outcome (e.g. survey, observation, feedback) >
End of project outcomes			
Provide growers with scientific evidence-based best practices strategies in the use of phosphonates to minimise the impact of Phytophthora root rot	**SIP 2017-2021** SIP Outcome 4 "By 2021, productivity (marketable yield per hectare) has improved by 15 per cent on average, without increased production costs per kilogram" 15% increase in productivity (yield per hectare) from healthier trees improved via disease management practices (where adopted) 15% improvement in fruit quality measured by packout data and postharvest quality assessments (where adopted) Enhanced capacity in orchard productivity, RD&E through mentoring and training and strengthening linkages within Australia and internationally **SIP 2022-2026** Outcome 2: "Industry	Current best practices in tree establishment, maintaining tree health, integrated management of Phytopthora root rot communicated to industry by various methods.	Survey results of participants from several workshops available (from AV16007 and AV17005 activities). Participants rated the workshops as very useful or extremely useful, and indicated that they intended to make changes as a result of attending the workshop Growers and agronomists revising phosphonate sprays
Increased grower awareness of best practices and strategies to improve fruit quality and maximise orchard productivity			
Adoption of recommended strategies by growers			
Continue to strengthen industry relationships and networks to encourage and support high level awareness of best practices			

	supply, productivity and sustainability", and Strategy 4. "Develop and optimise fit for purpose pest and disease management strategies."		
Short term (immediate) outcomes			
Identify optimal phosphonate application rates and times to limit the impact of root rot disease and maximise orchard productivity	As above	As above. Further dissemination and implementation of project-related outputs in coming months.	
Communicate to the avocado industry the benefits and importance of adopting recommended activities and strategies		As above and ongoing.	As above, and ongoing.
Maintain strong, collaborative relationships with industry stakeholders who will benefit from the project		Ongoing linkages with other projects and disciplines as well as all industry stakeholders in Australia and internationally are expected.	A above, and ongoing.
Enhanced capacity to support pathology activities required for the avocado industry		Student project linked to phosphonate and Phytophthora root rot work is continuing, outputs to be communicated as appropriate.	Ongoing training and involvement of a student in industry activities. Strong industry engagement through project team's on-farm research trials and collaboration with colleagues in QLD DAF (north QLD) and CSIRO. Adoption of enhanced techniques for identification of oomycetes causing root rot in avocado.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project investigated the mode of action, translocation and storage of phosphite, and timing of application of phosphonates to	The project has delved into research areas not previously tackled in avocado. This includes defence gene activation work and analyses of key carbohydrates. The results suggest	Review methodology and what worked, what didn't work. Extracting good results showing significant treatment differences from field trials and variable seedling material

manage tree health and fruit quality?	that phosphite moves with the simple carbohydrates (sugars) and may not be "stored" with complex carbohydrates (starch). Phosphite induces defence responses in roots but does not stimulate growth of avocado in the absence of Phytophthora. The project has demonstrated that applications to avocado should be late summer/early autumn, and not into late autumn and winter.	in the glasshouse is difficult. Could perhaps use clonal material.
To what extent has the project addressed the needs of avocado industry levy payers for optimising phosphonate application for the management of PRR?	Consistent messaging regarding practices to manage the key diseases has been delivered throughout this project.	Further targeted extension of new results/outputs still to be delivered to industry.
To what extent did extension activities produce response and engagement by industry levy payers? Have regular project updates been provided through linkage with the industry communication providers? To what extent have the updates addressed industry interest and need?	Dedicated disease management workshops were extremely interactive, with a very high level of engagement Several articles in Talking Avocados, Guacamole, industry forums, etc.	Working with small study groups (5-6 growers/agronomists) in each region might also help to extend best practice and new strategies to industry. As indicated above, further scientific and extension outputs to come from the project. Will work with extension and comms teams to provide this material in the most effective way possible
What efforts did the project make to improve efficiency? In what ways has project improved its efficiency and effectiveness?	Huge efficiencies were made through utilising students and visiting international research staff (not paid by the project) to assist paid project staff with experiments. This has increased our project output considerably, and assisted in training new generation of horticulturalists. Field work undertaken as efficiently as possible, and attempts made to coordinate travel with other tasks, e.g. combining workshop or conference with field trial activity. Sharing staff across other projects has largely been successful, and ensured continuity of employment of key staff.	Good students and staff are hard to secure. Perhaps industry could provide some awards/small scholarships as incentives for Hons students to work with researchers on their projects. Greater use of third party providers to assist with field work conducted away from primary location. Greater awareness and linkages across projects and industries

Recommendations

- The on-farm trials and phosphite analyses of multiple tissues, including leaves and stems, has provided very useful information relevant to management of timing of phosphonate application. Further coordinated on-farm trials in each of the major growing regions to demonstrate efficacy of earlier applications of phosphonate, applied during leaf flush, e.g. Late February to early April, compared with May-July. Sample and test root, fruit and perhaps other tissues for phosphite. It is clear that timing of application relative to fruit harvest and pruning requires more thought and field-based research.
- Analyses of the effects of additional potassium (when potassium phosphonate is applied) may be useful. Potassium was positively correlated with phosphite in fruit flesh, and, depending on timing of application, may have impacts on fruit size (potassium in early fruit development increases fruit size), or quality (potassium may compete with calcium in critical stages of fruit development).
- Subcontracting an independent research provider or spray operator in each location may be useful. Quarantine rows within commercial orchards with excellent signage to ensure growers don't accidentally spray with phosphonate. This worked well in AV16007, and is highly recommended for future on-farm trials.
- Progress research/evaluation of untested alternatives and approaches for managing Phytophthora root rot. While there are some new anti-oomycete chemistries available, they must be used as part of an integrated strategy. The PhD student aligned with this project is evaluating a selection of these new products. Efficacy of claimed biological or "soft" crop protectants should also be evaluated. Products to test must be carefully chosen based on reliable data from other crops, and not on "data-free observations" (DFOs), or pressure from third parties with commercial interests. Consider further research on evaluating treatments to stimulate root growth, for example soil conditioners, and whether these can improve natural soil microbial communities and activity and contribute to managing (outgrowing) Phytophthora root rot.
- After several decades of delivering presentations on best practice integrated disease management, (where very little has changed), most growers within industry are still not adopting the basics, and rather seeking "the silver bullet", fungicide or other magical treatment. Perhaps a different extension model/s could be trialed?? For example, utilising small groups of grower volunteers, who are prepared to contribute to discussions and run demo trials to showcase trial results to the group and broader industry, if appropriate.

Refereed scientific publications

Journal articles

1. Dann, EK and McLeod, A (2020) Phosphonic acid: a long-standing and versatile crop protectant, *Pest Management Science*, October 2020, DOI 10.1002/ps.6156.
2. Jolliffe, J. B., Dann, E. K., van der Rijst, M., Masikane, S. L., Novela, P., Mohale, P. and McLeod, A. (2023) Seasonal colonisation of avocado roots by *Phytophthora cinnamomi* in South African orchards, *Plant Disease*, https://doi.org/10.1094/PDIS-07-23-1457-RE

Chapter in a book or paper in conference proceedings

Conference presentations (abstracts)

1. Jose, J et al (2022), Effect of phosphonate and metalaxyl on avocado growth in the presence and absence of *P. cinnamomi,* Australasian Soilborne Disease Symposium, Cairns, August 2022 (oral presentation)
2. Scarlett, K. et al (2022) Plant defence activation against Phytophthora root rot in phosphite primed avocado, Australasian Soilborne Disease Symposium, Cairns, August 2022 (oral presentation)
3. Dann, E. et al (2023) Plant defence activation against Phytophthora root rot in phosphite-primed avocado, 10th World Avocado Congress, Auckland, New Zealand, April 2023 (poster presentation, Appendix 4)
4. Dann, E. et al (2023) Understanding the mode of action of phosphite in avocado, 10th World Avocado Congress, Auckland, New Zealand, April 2023 (oral presentation, slides available at https://industry.nzavocado.co.nz/wp-

content/uploads/2023/04/0905-Elizabeth-Dann.pdf)

5. Jose, J et al (2023) Evaluating the efficacy of phosphite and metalaxyl in Phytophthora root rot control in avocado, International Congress of Plant Pathology, Lyon, France, August 2023 (J. Jose et al, poster presentation, Appendix 1.)

References

Belisle RJ, Hao W, McKee B, Arpaia ML, Manosalva P, Adaskaveg JE (2019) New oomycota fungicides with activity against Phytophthora cinnamomi and their potential use for managing avocado root rot in California. Plant Disease, 103 (8):2024-2032. doi:10.1094/PDIS-09-18-1698-RE

McLeod, A, Novela, P, Mohale, P and Masikane, S (2020) Investigating phosphonic acid residues in late hanging fruit and in tree roots from trees that received one versus two annual trunk injections, South African Avocado Growers' Association Yearbook, 43: 58-63.

Nartvaranant, P, Hamill, S, Leonardi, J, Whiley, AW and Subhadrabandhu, S (2004) Seasonal effects of foliar application of phosphonate on phosphonate translocation, *in vitro* pollen viability and pollen germination in `Hass' avocado (*Persea americana* Mill.), *Journal of Horticultural Science and Biotechnology*, 79:1, 91-96.

Whiley, AW, Hargreaves, PA, Pegg, KG, Doogan, VJ, Ruddle, LJ, Saranah, JB, Langdon, PW, 1995. Changing sink strengths influence translocation of phosphonate in avocado. *Australian Journal of Agricultural Research*. 46: 1079–1090.

Whiley, A.W., 2001. Avocado Canopy Health and Management, Final report. HRDC project AV96004.

Intellectual property

No project IP or commercialisation to report.

Appendices

Appendix 1. Jacob Jose_ICPP poster

Appendix 2. Carbohydrate correlation analyses

Appendix 3. AV19005 Walkamin DAF report 31012023

Appendix 4. Scarlett Poster WAC 29032023

Appendix 5. Extension outputs DANN AV19005 15062023

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The owners and staff of several businesses have been extremely generous with their products, orchards, useful discussions and time over several years. Without their support and collaboration, this project would not have delivered the practical outputs identified. Thank you to Daryl Boardman and Tyson Cross (Sunnyspot Farms), Jacinta Foley and Sam Nixon (Jasper Farms), and Adam Fitzgerald and Matt Goodwin (MA Analytical).

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We acknowledge the help and support of the project PRG team, Clayton Lynch, Doug Pow and Garth Sanewski (QLD DAF).

Project team and collaborators

Project team and collaborators: From top left, Hydro Harley, Kelly Scarlett, David Guest (University of Sydney), Harley Smith, Marc Goertz (CSIRO, Adelaide), Jacinta Foley, Sam Nixon (Jasper Farms), Dale Bennett, Geoff Dickinson (Queensland DAF, Walkamin, QLD), Lara-Simone Pretorius, Imsu Nokdy, Liz Dann, Jacob Jose (University of Queensland), Adele McLeod (Stellenbosch University), Daryl Boardman, Sally Boardman, Tyson Cross (Sunnyspot Farms).

Evaluating the efficacy of phosphite and metalaxyl in Phytophthora root rot control in avocado

Jacob Jose, Elizabeth Dann and Andre Drenth

Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Australia

Introduction

- Avocado root rot, caused by ***Phytophthora cinnamomi***, is the most economically important disease in avocado worldwide, necrotising fine feeder roots, which results in the expression of several above-ground symptoms ranging from leaf chlorosis to tree death, in trees of all ages and sizes (Figure 1A,B).
- As *P. cinnamomi* inoculum can stay viable in infested soils for up to 8 years, orchard soils with a history of pathogen infestation remain contaminated for years, **affecting establishment** of avocado nursery trees (Figure 1B). .
- Anti oomycete chemistries **phosphite and metalaxyl** are valuable tools in the integrated management of avocado root rot, which includes chemical control, tolerant rootstock, cultural, biological and legislative control.
- Efficacy of phosphite and metalaxyl in **improving field establishment** of avocado nursery trees in infested sites have not been evaluated.
- Effect of **application timing** and **mode of application** of phosphite and metalaxyl in improving field establishment of avocado nursery trees have not been assessed.

Figure 1: A) Adult avocado tree showing Phytophthora root rot decline symptoms, **B)** Declining avocado nursery tree 10 months after planting

Research Question

Do phosphite and metalaxyl improve field establishment of avocado nursery trees in soils with high *P. cinnamomi* disease pressure?

Methods

- **Chemical treatments** (*n* = 10): Applied phosphite as root drench or foliar spray 2 days before planting (Figure 2A), metalaxyl as soil mix at planting, and/or at 3 and 10 weeks after planting, or phosphite foliar spray programme once every two months since planting.
- **Avocado nursery trees**: 90 ten-month-old 'Hass' on 'Reed' planted (Figure 2B) in a *P. cinnamomi*-infested orchard with declining adult avocado trees (Figure 1A)
- **Establishment parameters**: Tree health scores (on a scale of 1-5, with 1 = healthy, and 5 = dead) were recorded once every month, tree height and tree diameter were measured quarterly. The experiment ran for 10 months.

Figure 2: **A)** Nursery avocado trees ready to be pre-treated before planting, **B)** Nursery trees immediately after planting.

Results

- All phosphite and metalaxyl treated plants **except pre-planting phosphite spray** treated plants showed significantly lower tree health score ($p < 0.001$) (i.e. **trees were healthier**) compared to that of control (Figure 3D).
- **All metalaxyl treated** plants and **phosphite pre-plant drench treated** plants **grew significantly better** (height and/or diameter) compared to that of control ($p < 0.001$) (Figure 3E).

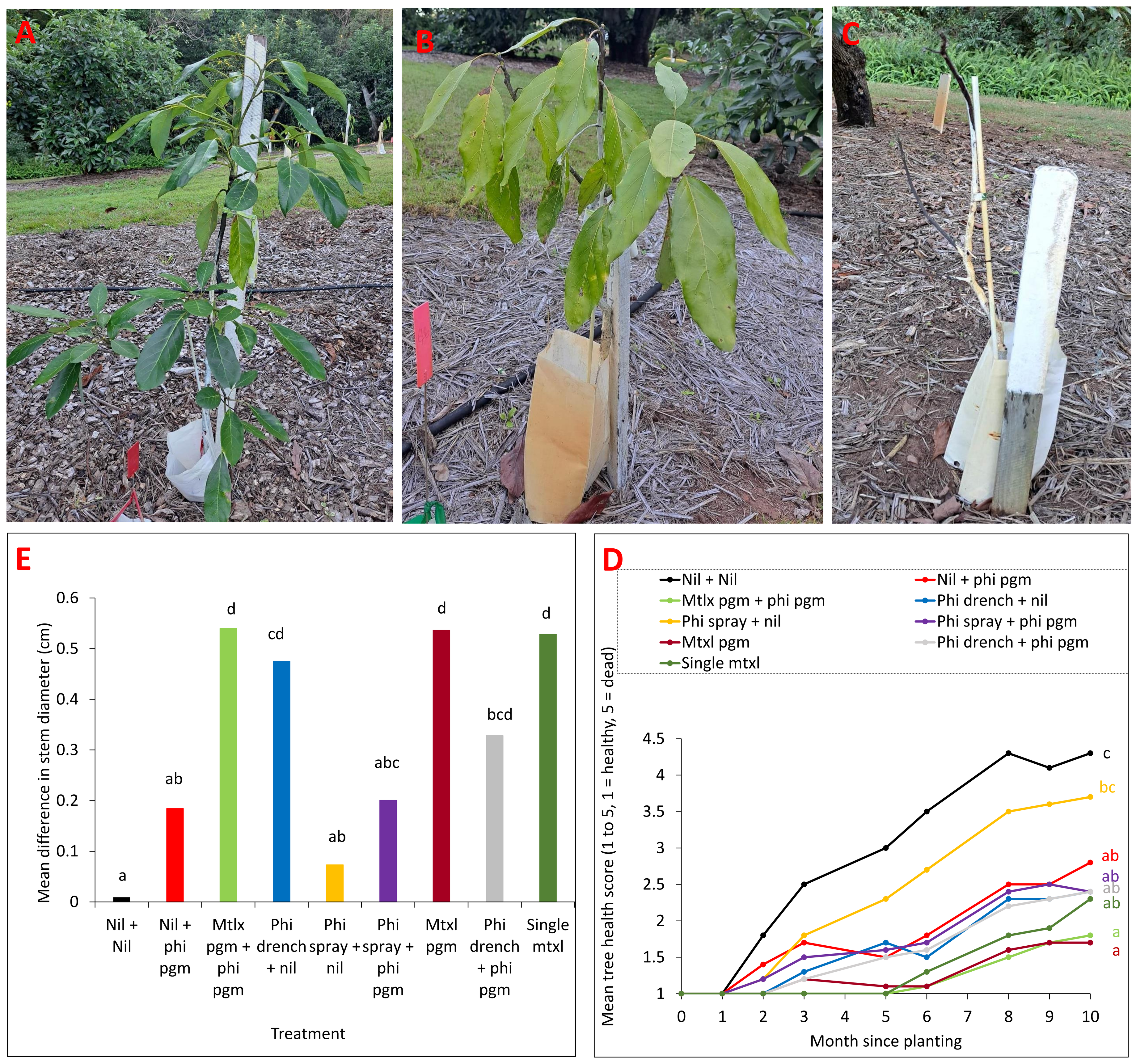

Figure 3: Tree health and growth 10 months after planting **A)** A healthy tree (health score = 1), **B)** Tree in moderate decline (health score = 3), **C)** Dead tree (health score = 5) from control treated group, **D)** Tree health scores across 10 months, where trees were scored on a scale of 1 to 5, with 1 = healthy, and 5 = dead, **E)** Change in plant stem diameter between month 10 and month 0.

Conclusion

1. **Metalaxyl treatments improve field establishment** of avocado nursery trees in soils with high *P. cinnamomi* disease pressure.
2. **Phosphite applied as root drench before planting improves establishment** of avocado nursery trees in *P. cinnamomi* infested soils.
3. **Phosphite applied as foliar spray after planting improves tree health** of avocado nursery trees in *P. cinnamomi* infested soils.

Hort Innovation
Strategic levy investment
AVOCADO FUND

- jacob.jose@uq.net.au
- Jacob Jose

Queensland Alliance for Agriculture and Food Innovation

The University of Queensland Australia
QAAFI Queensland Alliance for Agriculture and Food Innovation
Queensland Government

Appendix 2. AV19005 carbohydrate and phosphite correlation analyses, across tissue types

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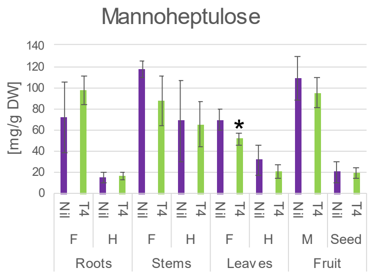
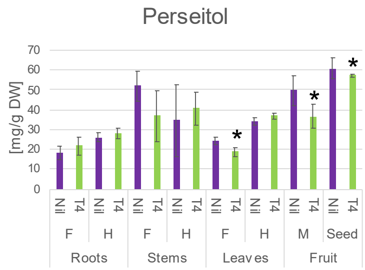
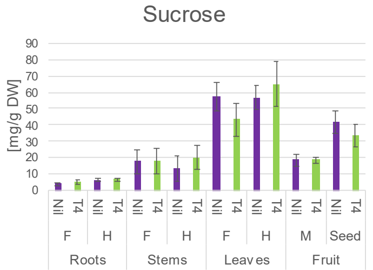
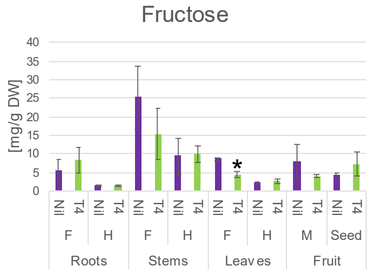
Soluble sugars – samples collected 16 April 2021

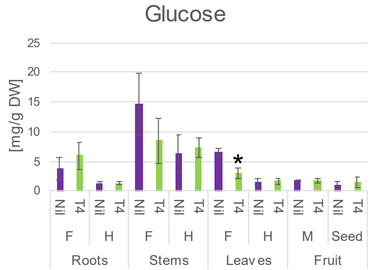
F=Flush
H=Hardened
M=Mesocarp











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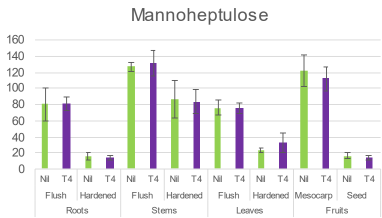
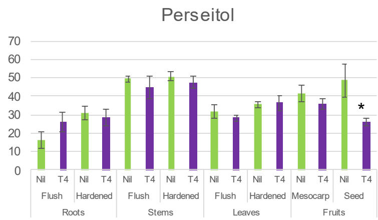
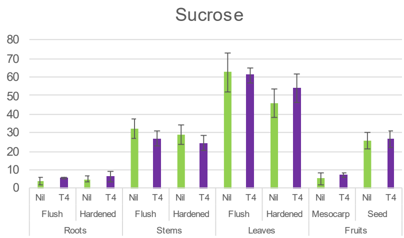
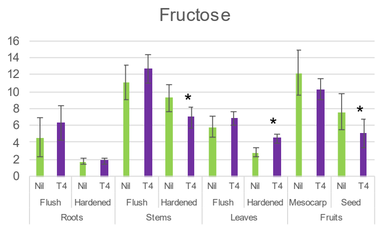
Soluble sugars – samples collected 13 April 2022

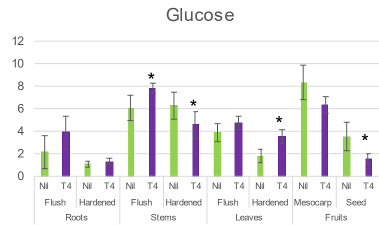
F=Flush
H=Hardened
M=Mesocarp











AV19005 – Understanding the mode of action of phosphite in avocado for enhanced management of Phytophthora root rot.

MS106 – DAF Activities (July 2022 – January 2023)

Prepared by Geoff Dickinson, Dale Bennett and Carole Wright, DAF, Mareeba (Due 31/01/2023)

ACTIVITY ONE – Participation in Extension and Communication activities

1. AV19005. Industry workshop and field trial visit – DAF Walkamin Research Facility, 02/08/2022, 8AM – 1PM.

The AV19005 project team; Dr Elizabeth Dann, Mr Clayton Lynch, Dr Geoff Dickinson and Ms Dale Bennett organised and facilitate a successful project event with 26-invited industry stakeholders in attendance. The goal of the event was to provide AV19005 updates and information to those stakeholders with a higher-level of understanding of phytophthora root rot management and phosphite application methodologies. The format of the morning involved a series of presentations by Dr Liz Dann on significant project activities and outcomes, interspaced with a field walk and data presentation for the AV19005 NQ Field trial located at the DAF Walkamin Research Facility, presented by Dr Geoff Dickinson.

Dr Liz Dann presenting at the AV19005 Industry workshop, 02/08/2022.

2. AV19005. Project Reference Group meeting – Hilton Hotel, Cairns/Zoom, 02/08/2022, 4PM-6PM.

The PRG meeting included the PRG members and invited guests; Liz Dann, Kelly Scarlet, Lara-Simone Pretorius, Imsu Nokdy, Harley Smith, Marc Goetz, Geoff Dickinson and Dale Bennett. Chaired by Liz Dann, the meeting format included updates on Lab studies, Field Trials (Capel, WA; Walkamin, NQ, Ravensbourne, SQ) and Training, extension and communications activities.

ACTIVITY TWO – NQ field trial treatments and phosphite monitoring completed

NQ Trial – Agronomy operations

Management and monitoring of the AV19005 replicated trial on 9-year-old, Shepard avocado trees at the DAF Walkamin Research Station continued throughout the MS106 period (July 2022 – January 2023). This included regular trial agronomy including nutrient/herbicide application every 2-3 months and irrigation 2-3 times per week.

NQ Trial – Phosphite treatment applications

The four experimental treatments are as follows:

Treatment	Phosphite treatments	Autumn program	Summer program
1	No sprays		
2	Summer foliar sprays only		X
3	Autumn foliar sprays only	X	
4	Summer and Autumn foliar sprays	X	X

The 7-month extension of project AV21005, has allowed another application of the Autumn phosphite foliar sprays (conducted on the 19/05/22, 02/06/22, 16/06/22 and 30/06/22). These operations was conducted as per previous applications using a hand-held, pressurised Silvan sprayer (200l tank capacity), with phosphite applied as Agri-Fos 600 (Agri-Chem) at a rate of 825ml product/100L water at high volume to the point of run-off, ensuring all leaves (surface and underside) and branches are covered (approx. 5-10l/tree).

Metalaxyl was applied to control treatment trees on the 19/05/22 and 30/06/22. These operations were conducted as per previous applications using granular Zee-Ml 50 g (Metalaxyl 50g/kg) applied at a rate of 50g/m^2 of product to bare soil, at an area around the trunk of 3mx3m = 9m^2. This is a product application rate of 450g/tree. Mulch was raked away from this area prior to application and then returned after one both applications were conducted.

Application of phosphite treatments at Walkamin with Silvan sprayer

NQ Trial – Autumn 2022 phosphite monitoring activities

Root sampling was conducted on the 10/05/2022 prior to phosphite treatment application, then post-application on the 27/07/22 and 27/10/22. All 16 root samples x 3 collection dates were oven dried at 50^{0}C and then sent to MA Analytical Services for phosphite analysis.

ACTIVITY THREE – Final analysis and summary of NQ trial results

By Carole Wright (Senior Biometrician), Geoff Dickinson and Dale Bennett, DAF Mareeba.

Methodology

A trial was conducted at DAF Walkamin Research Facility to compare the effect of phosphite applied at different times on the residual levels in roots, fruit and seeds. Four treatments were trialled:

Treatment	Phosphite treatments
1	No sprays
2	Spring sprays only (November)
3	Autumn sprays only (May)
4	Spring and Autumn sprays (November and May)

Each treatment was replicated four times in a randomised complete block design. Phosphite levels in the roots, fruit and seeds were assessed. Root samples were taken on 8 occasions, fruit tissue was assessed on 3 occasions, and seed samples on 2 occasions.

The phosphite limit of detection is 5 ppm. The March 2022 fruit samples were returned with a limit of detection of 10 ppm. This set of samples has been analysed twice, with the limit of detection set at 5 ppm and at 10 ppm. Due to the phos acid level of some samples being below the limit of detection, the data were initially analysed using the censored approach of Taylor (1973)[1], but the algorithm was not able to consistently converge. The inability to converge is most likely due to the high proportion of data below the limit of detection. An alternative approach is to set the censored values at half the limit of detection.

There is no interest in comparing the sampling occasions and therefore each individual sampling occasion has been analysed separately using analysis of variance (ANOVA). In all analyses, replicate was fitted as a random term and treatment fitted as the fixed effect. All significance testing was performed at the 0.05 level. If a significant effect was found, the 95% least significant difference (lsd) was used to make pairwise comparisons.

Root samples – Results

A summary of the results from the ANOVA on root sample data are presented in the Table 1. For each individual sampling occasion the treatment means, F-statistic, p-value, standard error (se) and 95% lsd are presented. Only 2 plot samples gave phosphite levels above the limit of detection in November 2021. These were 2 of the Autumn & Summer treatment samples. This sampling occasion has not been analysed.

For all sampling occasions analysed, a significant treatment effect was detected ($p < 0.05$).

- In February 2021 and 2022, April 2021, and May 2022, the mean phosphite levels for the control and Autumn-only treatment were significantly lower than the Autumn & Summer and Summer-only treatments.
- In July 2021 and 2022, the mean phosphite levels for the control and Summer-only treatment were significantly lower than the Autumn-only and Autumn & Summer treatments.
- In Oct 2022 the mean phosphite level was significantly higher for the Autumn & Summer treatment compared to all other treatments.

All samples for the control were less than the limit of detection. The only change in pairwise comparisons when the control is excluded from the dataset, is for the significant difference at 27 October 2022 between Autumn-only and Summer-only is now not significant. This is likely an effect of the smaller dataset resulting in a decrease in the degrees and freedom and an increase in the lsd.

	12 October 2020* Pre-summer - Baseline	2 Feb 2021 Post-summer	13 April 2021 Pre-autumn	21 July 2021 Post-autumn	8 Nov 2021 Pre-summer	4 Feb 2022 Post-summer	10 May 2022 Pre-autumn	27 July 2022 Post-autumn	27 Oct 2022 Pre-summer - Final
Treatment									
Control	20.0	2.5 b	2.5 b	2.5 b	2.5	2.5 b	2.5 b	2.5 b	2.5 c
Autumn only	-	2.5 b	2.5 b	19.8 a	2.5	4.6 b	2.5 b	55.5 a	14.0 b
Autumn & Summer	-	33.5 a	44.2 a	24.5 a	4.8	74.2 a	27.5 a	73.0 a	25.0 a
Summer only	-	28.2 a	50.5 a	2.5 b	2.5	75.0 a	22.8 a	8.0 b	4.8 c
$F_{(3,9)}$	-	66.82	28.65	12.15	-	94.69	21.42	18.17	17.37
p-value	-	<0.001	<0.001	0.002	-	<0.001	<0.001	<0.001	<0.001
se	-	2.02	4.86	3.30	-	4.22	2.85	8.18	2.46
95% lsd	-	6.47	15.56	10.55	-	13.49	9.13	26.17	7.87

Figure 1: Root phosphite levels (ppm) across the 4 treatments at 8 sampling times + the initial baseline time. (*1 sample analysed only - Bulk of 4 control plots)

Root samples - Discussion

- It is accepted that the minimum desirable root phosphite level is ≥25ppm (BPR, 2020) and optimum level is ≥80ppm (BPR, 2023).
- Root phosphite levels in all 3 foliar phosphite treatments were only recorded at below optimum levels (<80ppm).
    - Root phosphite levels never exceeded >75ppm at any time over the 2 years.
    - Root phosphite levels never exceeded >25ppm at the 2021 or 2022 pre-summer sample times.
    - Root phosphite levels were extremely low <5ppm at the 2021 pre-summer sample time
- It is important to note however that fruit phosphite levels (see next section) were much higher than the roots (up to 327.5ppm), indicating that the foliar-applied phosphite in Summer was entering the tree, it was however being translocated to the fruit, not the roots.
- Phosphite applications (in Autumn or Summer) always resulted in significantly higher root phosphite levels in the post-spray root sample
    - However: Autumn applications never exceeded > 56ppm
    - However: Summer applications never exceeded > 75ppm
- Phosphite applications in one season only (Autumn or Summer), only ever gave minimum protection (≥ 25 ppm) for 6 months or less.
- Phosphite application in two seasons (Autumn <u>and</u> Summer), gave minimum protection (≥ 25ppm) for the majority of the 2-year trial period (except at the time of the Nov 21 pre-summer sample).
- The method of applying Phosphite to an orchard using foliar-sprays:
    - May not be a suitable application method where phosphite levels > 80ppm are required over the whole year.
    - Will need to be conducted in both Autumn and Summer to achieve the minimum (≥ 25 ppm) root phosphite levels over the whole year.
    - May pose MRL risks when applied in Summer, due to the phosphite being translocated to the fruits rather than just the roots.

Fruit Samples – Results

A summary of the results from the ANOVA on fruit sample data are presented in Table 2. For all sampling occasions analysed, a significant treatment effect was detected ($p < 0.05$).

- At all sampling occasions the mean phosphite levels for the control and Autumn-only treatment were significantly lower than the Autumn & Summer and Summer-only treatments.
- At February 2022 the mean phosphite level for the Summer-only treatment was significantly higher than all other treatments.
- The conclusions remain consistent for the March 2022 samples with two different limits of detection.

When the control samples are excluded from the analysis, the only change in pairwise comparisons is no significant difference is detected between the Autumn & Summer and Summer-only means in February 2022. This is likely an effect of reduced degrees of freedom and a higher 95% lsd.

	2 February 2021* Pre-harvest	**7 April 2021** Harvest time	**7 February 2022** Pre-harvest	**30 March 2022** Harvest time 5ppm detection	**30 March 2022** Harvest time 10ppm detection
Treatment					
Control	< 5.0	2.5 b	2.5 c	2.5 b	5.0 b
Autumn only	-	2.5 b	2.5 c	13.1 b	15.0 b
Autumn & Summer	110.0	13.0 a	265.0 b	157.5 a	157.5 a
Summer only	-	12.2 a	327.5 a	151.8 a	151.8 a
$F_{(3,9)}$	-	22.82	79.58	40.64	39.68
p-value	-	0.001	<0.001	<0.001	<0.001
se	-	1.22	19.23	13.92	13.28
95% lsd	-	3.92	61.51	42.61	42.47

Figure 2: Fruit flesh phosphite levels (ppm) across the 4 treatments and 3 sampling times + 1 baseline sample in February 2021. (*2 samples analysed only - Bulk of 4 control plots and Bulk of 4 Autumn & Summer plots)

Fruit samples – Discussion

- Phosphite application in Autumn-only, resulted in minimal fruit flesh phosphite levels (2.5-13.1ppm) at both the pre-harvest (8 weeks before harvest) and harvest sample times.
- Phosphite application in both the Autumn & Summer and Summer-only treatments resulted in high fruit flesh phosphite levels (265.0-327.5ppm) at pre-harvest which then declined (151.8-157.5ppm) by harvest time.
- The high fruit flesh phosphite levels in orchards which have received Summer foliar phosphite applications, may be a concern if MRLs for export markets are introduced or reviewed.

Seed Samples – Results

A summary of the results from the ANOVA on the seed sample data are presented in Table 3. For both sampling occasions analysed, a significant treatment effect was detected ($p < 0.05$).

- At both sampling occasions the mean phosphite levels for the control and Autumn-only treatment were significantly lower than the Autumn & Summer only treatments.
- At February 2022 the mean phosphite level for the Summer-only treatment was significantly higher than all other treatments.
- Excluding the control samples results in no changes to the conclusions.

	7 Feb 2022 Pre-Harvest	**30 March 2022** Harvest time
Treatment		
Control	2.5 c	2.5 b
Autumn only	4.9 c	4.4 b
Autumn & Summer	99.5 b	83.8 a
Summer only	120.0 a	88.0 a
$F_{(3,9)}$	124.18	147.50
p-value	<0.001	<0.001
se	5.55	3.92
95% lsd	17.75	12.55

Figure 3: Fruit seed phosphite levels (ppm) across the 4 treatments and 2 sampling times.

Seed samples – Discussion

- Phosphite application in Autumn-only, resulted in minimal fruit seed phosphite levels at pre-harvest (2.5-4.9ppm) and harvest (2.5-4.4ppm) sample times.
- Phosphite application in both the Summer-only and Autumn and Summer treatments resulted in high fruit seed phosphite levels at pre-harvest (99.5-120.0ppm) which declined slightly at harvest time (83.8-88.0ppm).
- Seed phosphite levels were higher in the Summer-only treatment than the Autumn & Summer treatment at pre-harvest time.
- The high fruit seed phosphite levels within the two Summer foliar phosphite treatments may have implications (and benefits) when using this seed for nursery propagation purposes.

REFERENCES

[1] J. Taylor (1973). The analysis of designed experiments with censored observations. Biometrics, 29, 35-43.

Plant defence activation against Phytophthora root rot in phosphite-primed avocado

Kelly Scarlett[1,2], Floris van Ogtrop[2], David Guest[2] and Elizabeth Dann[1]

1 Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, Australia
2 Sydney Institute of Agriculture, School of Life and Environmental Sciences, University of Sydney, Australia

e.dann@uq.edu.au

Introduction

One component of the integrated management of Phytophthora root rot is the application of phosphite, a phloem-mobile anion of phosphorous acid. While a dual mode of action of phosphite is generally accepted, the relationship between *in planta* phosphite concentration and plant defence activation has only been investigated in model plants, such as *Arabidopsis*, where low concentrations of phosphite "prime" plants to respond more rapidly when they are subsequently infected by a pathogen. The activation of inherent defence responses in avocado rootstocks with resistance to *P. cinnamomi* is, however, well established (van den Berg *et al.* 2021).

The mitogen-activated protein kinase (MAPK) pathway is important in many physiological processes, including plant defence signalling. MAPK4 negatively regulates biotic stress signalling and salicylic acid accumulation (required for defence). At low concentrations, phosphite interrupts the MAPK pathway, MAPK4 is downregulated, leading to accumulation of salicylic acid and activation of defence genes after pathogen challenge (Massoud *et al.* 2012).

Research questions

- **Is phosphite a negative regulator of MAPK4 in avocado?**
- **At what concentration/s does phosphite prime plant defence in avocado roots?**
- **Does *in planta* suppression of *P. cinnamomi* correlate with root phosphite concentration?**

Methods

- 40 'Reed' seedlings transferred to "two pot system" and sprayed with potassium phosphonate
- 1 week later, 2-3 g roots sampled for phosphite concentration
- 20 plants inoculated with zoospores of *Phytophthora cinnamomi*, 20 left non-inoculated
- Roots sampled at 0, 3, 6, 12, 24 and 48 h after inoculation, stored -80°C
- Total RNA extracted, quantitative PCR used to measure the relative abundance of defence genes, phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), pathogenesis-related protein 5 (PR5), metallothionein (METAL), glutathione–S–transferase (GSH), endochitinase, and putative MAPK4 signalling gene
- *P. cinnamomi* measured by relative abundance of cytochrome c oxidase II gene (cox II)
- Abundance of genes was calculated relative to endogenous avocado control gene (actin)
- Experiment undertaken twice, data analysed by generalised additive models (GAM)

Figure 1. Seedling growth, root inoculation and root sampling

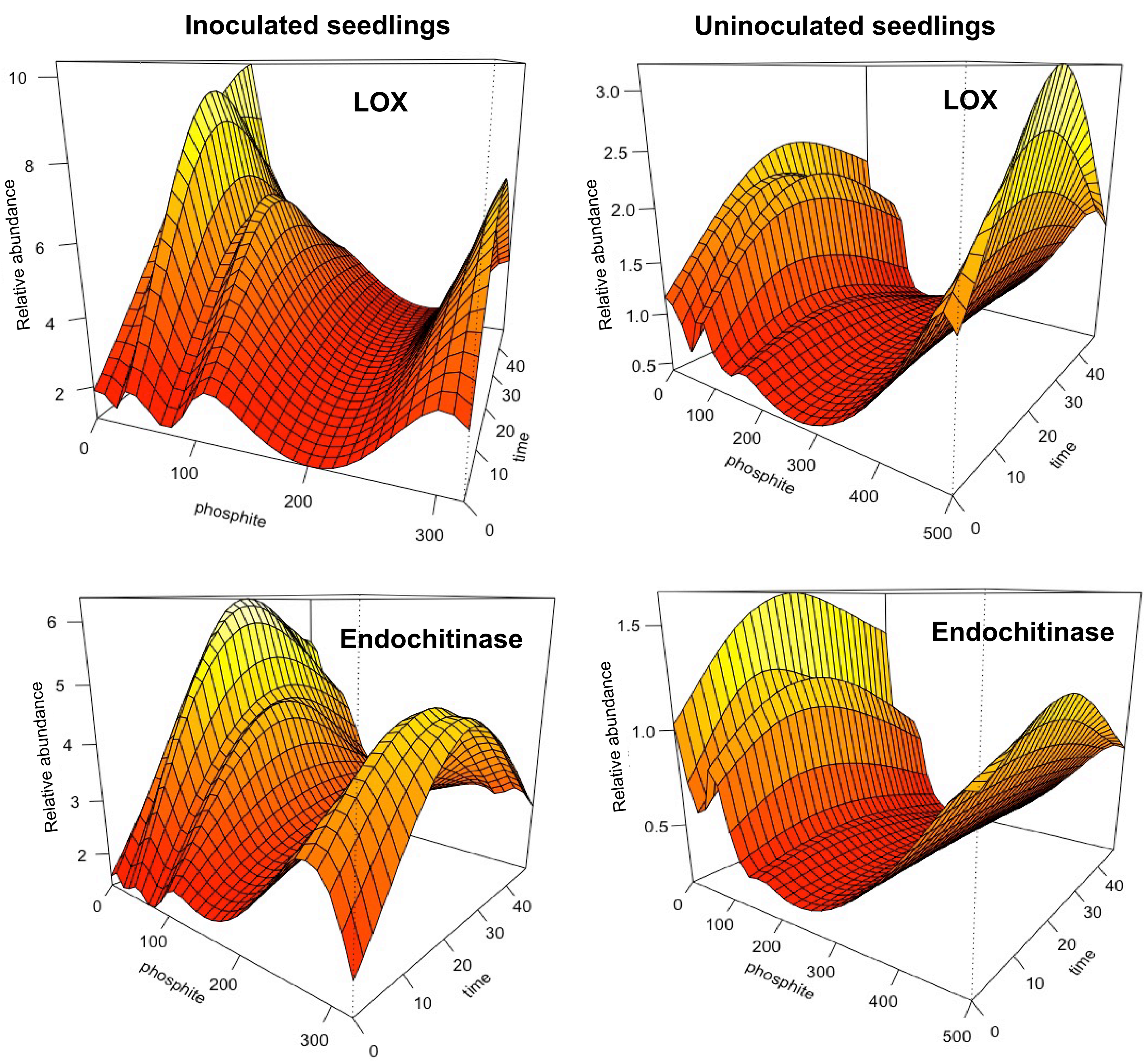


Figure 2. Defence gene activation in roots over time and phosphite concentrations

Results and conclusions

- Large variation in root phosphite concentrations, <5 mg/kg – 500 mg/kg, from spraying with phosphonate or translocated from seed
- Phosphite concentration and sampling time have significant effects on MAPK and defence gene activation
- Between 30-60 mg/kg, phosphite appears to be a negative regulator of MAPK4 in avocado following infection
- At phosphite concentration of 30-50 mg/kg, LOX and endochitinase genes are rapidly upregulated in roots, compared with other phosphite concentrations and the non-inoculated seedlings (Fig. 2)
- Response of GSH is not as strong
- No apparent effects of phosphite on PAL, PR5 or METAL defence genes
- No defence gene activation in roots with phosphite concentrations greater than 120-250 mg/kg
- Cox II transcripts (*P. cinnamomi*) first detected at 3h after inoculation, and decreases after 6h in roots with phosphite concentrations of 30-50 mg/kg.
- Further data analyses is currently underway

In healthy trees, phosphite at relatively low concentrations (30-60 mg/kg) can activate defence responses and reduce infection of roots by *P. cinnamomi*.

Phosphite is only one part of an integrated strategy to manage Phytophthora root rot. Other management factors include good drainage and water management, tolerant rootstocks, disease-free nursery trees, mulching and optimal tree nutrition.

Massoud et al (2012) Plant Physiology, www.jstor.org/stable/41496263
Van den Berg et al (2021) Frontiers in Plant Science, doi.org/10.3389/fpls.2021.636339

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Appendix 1. AV19005 extension outputs, 15/06/2023

Type of extension activity	Details
Dedicated Phos acid workshop (AV19005 and AV17005) **Dedicated advanced disease management workshops (AV16007 and AV19005)**	Liz Dann was keynote presenter at the avocado phos acid (phosphonate) workshop, held at Mareeba on 21 April 2021. The workshop was organised by Dr Geoff Dickinson and team (collaborators in AV19005, and members of extension project AV17005) 4 face-to-face workshops were held in 2022 to cover the basics of disease management (Phytophthora and fruit diseases), and present results from current research projects. Each workshops was split into two parts Part 1 "Striving for the best quality fruit", Part 2 "The battle against Phytophthora root rot", with extended informal presentations by Liz. 1) Bundaberg, 27 April 2022. Held by Liz in conjunction with Syngenta and EE Muir, with Kath Adams (Syngenta) presenting on maximum residue limits (MRLs), and Scott Matthews (Campbells) discussing global pressures on pesticides and fertilisers. Attended by approx. 58 people, evaluation available. 2) Manjimup, 5 May 2022. Held by Liz in conjunction with Syngenta and Farmlink, with Shell Xiao (Syngenta) presenting on maximum residue limits (MRLs), and Zac Starkie (Farmlink) discussing global pressures on pesticides and fertilisers. Attended by approx. 45 people, evaluation available. 3) Port Macquarie, 15 June 2022. In conjunction with AAL Regional Export Forum. Evaluation available. 4) Walkamin, 2 August 2022. Invite only for advanced growers and agronomists/consultants. Organised in conjunction with Clayton Lynch (Australian Produce Partners) and Geoff Dickinson (DAF).
AV17005	Phytophthora poster & video - Significant contribution to poster and video, produced by the industry extension projects (AV10002 and AV17005), and added to the Best Practice Resource website https://avocado.org.au/wp-content/uploads/2019/09/Manage-Phytophthora-Root-Rot-Poster-1.pdf https://www.youtube.com/watch?v=0T2Kz5tNfX0
Avoskills workshops (AV17005)	Two presentations by Liz at each of the two day "Avoskills" workshops "Diseases (other than Phytophthora) and their management in avocado" "Phytophthora root rot of avocado: The disease and how to manage it"

	- Manjimup, WA, 10-11 March, 2020 - Bundaberg, QLD, 21-22 September 2021 - Mildura, VIC, 12-13 May 2022 - Port Macquarie, NSW, 14-15 June 2022 All the Avoskills presentations (slides) are available on the AAL Best Practice Resource
Australian industry hosted events e.g. Qualicado, Regional Forum Field days Workshops AV17005	"Phosphonate, field trials and flower blight (AV 16007 Project update)", Manjimup, WA, 12 March 2020 "Fruit diseases of avocado and how to manage them" "Phytophthora root rot of avocado and how to manage it" - Tamborine Northern Rivers Regional Forum, Alstonville, 1 June 2022. These presentations are available on the Best Practice Resource, https://avocado.org.au/wp-content/uploads/2022/06/3.-Fruit-diseases-Liz-Dannv2.pdf; https://avocado.org.au/wp-content/uploads/2022/06/6.-Phytophthora-root-rot-Liz-Dannv2.pdf
***Talking Avocados* articles**	"New projects to improve productivity through disease management" Autumn 2018 "Improving avocado orchard productivity through disease management" L. Dann, A. Prabhakaran, E. Lancaster, K. Bransgrove, M. Hickey, E. Singh, Winter 2019, Talking Avocados 30 (2): 56-59 "Phosphorous acid (phosphonate): research update and new project activities" Talking Avocados, Summer 2021 Volume 31 (4):61-67 Talking Avocados (TA) is published quarterly and distributed widely within the avocado industry. Past editions of TA are available on the AAL website https://avocado.org.au/news-publications/talking-avocados/past-editions/
Refereed paper	Dann, EK and McLeod, A (2020) Phosphonic acid: a long-lived and versatile crop protectant, *Pest Management Science*, 77:2197-2208 (review) https://doi.org/10.1002/ps.6156
Grower/industry presentations	"Avocado diseases and their management" to Nutrien agronomists from Queensland and northern NSW, as part of their North Eastern Coastal Agronomy Conference, held in Brisbane 20 April 2021

Academic presentations	Australasian Soilborne Disease Symposium, Cairns, August 2022 • Effect of phosphonate and metalaxyl on avocado growth in the presence and absence of *P. cinnamomi* (J. Jose presentation) • Plant defence activation against Phytophthora root rot in phosphite primed avocado (K. Scarlett presentation) International Congress of Plant Pathology, Lyon, France, August 2023 • Evaluating the efficacy of phosphite and metalaxyl in Phytophthora root rot control in avocado (J. Jose et al, abstract accepted for poster presentation)
WAC, New Zealand, April 2023	• Plant defence activation against Phytophthora root rot in phosphite-primed avocado (E. Dann poster presentation) • Understanding the mode of action of phosphite in avocado (E. Dann oral presentation, slides available at https://industry.nzavocado.co.nz/wp-content/uploads/2023/04/0905-Elizabeth-Dann.pdf)