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Final Report

Mechanisms of cultivar- and race-based disease resistance in avocado

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Summary

Phytophthora root rot caused by *Phytophthora cinnamomi* is a serious problem in avocado trees (*Persea americana*) resulting in low productivity and the production of small, poor quality fruit. If left untreated Phytophthora root rot will eventually kill affected trees. Current management practices involve an integrated approach, including; planting on well-draining soil with a high total cation exchange capacity, application of organic mulch, carefully monitored organic and inorganic nutrition, phosphonate applications and the use of tolerant rootstock varieties. Rootstocks with high levels of resistance to Phytophthora root rot are not available in avocado. However, differences in tolerance to the disease have been observed among commonly used rootstock varieties. Selection of rootstocks in

breeding programs is hampered by the fact that little is understood about the mechanisms underlying tolerance and resistance to this pathogen. The overall objective of this project was therefore to identify phenotypic differences across a range of avocado rootstock varieties with varying degrees of resistance/susceptibility that may be associated with known field susceptibility.

The first hypothesis being tested concerned determining if field resistant rootstock varieties have a greater root regenerative ability than susceptible varieties. This was tested using a glasshouse study to measure the inherent root growth of seedlings with and without inoculation to investigate root system health and wilting in response to infection by *P. cinnamomi.* To investigate whether inherent root growth was correlated with known field susceptibility of seedling rootstock varieties, a 2-pot root regeneration system was developed which allowed us to determine varietal differences in root: shoot ratio across seedling avocado varieties. Although 'Hass' produced the greatest amount of feeder roots relative to the surface area of mature leaves, the overall differences among nine different seedling varieties were not significant. The main finding of the glasshouse root growth studies was that there were no strong correlations between inherent root growth and root system necrosis correlated with Phytophthora root rot susceptibility in the field.

The second hypothesis tested was whether increased surface feeder root growth of mature trees and other tree physiology attributes such as stored non-structural starch, yield, tree health and growth measurements such as tree height and canopy volume are correlated to Phytophthora resistance in the field. At both experimental sites no significant differences or associations were found among stored starch, tree health, yield or tree growth parameters. Overall, results of field studies at both sites indicated that there was no evidence that increased feeder root growth or tree physiology attributes were associated with known field resistance.

The third hypothesis to be tested involved determining if there were differences in lesion length and extension of *P. cinnamomi* hyphae within tip-inoculated feeder roots of the field susceptible 'Reed' and moderately resistant 'Velvick' rootstock varieties. The growth of the pathogen in 'Velvick' was more rapid than in 'Reed' as lesion lengths of inoculated 'Velvick' roots were significantly longer than 'Reed' 4 days after inoculation. The visible margin between necrotic and healthy tissue was significantly more distinct in inoculated 'Reed' roots compared to 'Velvick'. Results from DNA testing for the presence of *P. cinnamomi* found no significant differences between the two cultivars, suggesting that restriction of *P. cinnamomi* growth through root tissue does not appear to be an effective resistance response for the field-resistant 'Velvick'.

Based on this work, root regenerative ability does not appear to be an important mechanism of tolerance / resistance to Phytophthora root rot. Rootstock differences in physiology parameters of mature trees are not associated with known field resistance. The impact of these findings is that there are as yet no shortcuts in breeding and selection for

Phytophthora resistant rootstocks to get around time consuming glasshouse and field screening studies.

Keywords

Avocado, Phytophthora root rot, resistance mechanisms, *Persea americana*, *Phytophthora cinnamomi*, Oomycete, plant disease, root development.

Introduction

The Australian avocado industry has rapidly expanded in size over recent decades, mainly due to successful implementation of integrated Phytophthora root rot management. The components of this strategy have been developed over the past 60 years, with the aim of allowing successful avocado production on sites where *P. cinnamomi* is present. Integrated management involves planting on soils that have a high total cation exchange capacity, selection of sites with good drainage, usage of root rot tolerant rootstocks, maintenance of soil calcium levels with gypsum, application of well aerated organic woody mulches, and the use of phosphonate trunk injections or foliar sprays as a means of systemic control. Planting on mounds or ridges is another measure to reduce temporary soil saturation and ensuring that soil is friable and well-aerated. The success of these approaches very much depends on the availability of rootstocks with good levels of resistance.

Project Objective

Due to the widespread presence of *P. cinnamomi*, a multi-faceted approach to understanding and treating Phytophthora root rot of avocado is important. Identification of rootstocks with high levels of resistance to the disease will help achieve effective and sustainable control of this significant disease. Therefore the overall objective of this PhD scholarship project was to gain an understanding of the mechanism behind resistance to Phytophthora root rot in avocado.

Resistance

Resistance of avocado to Phytophthora root rot is poorly understood. Evidence from various studies suggests that resistant clonal rootstocks of different heritage do not share the same resistance strategies to the same degree. ‘Resistance’ of high-performing rootstock selections is not complete and certain components of resistance, for example root regenerative ability, are technically mechanisms of tolerance.

Different mechanisms of resistance to Phytophthora root rot of avocado have been investigated using screening techniques to determine their potential application in avocado rootstock breeding programs. These aspects of resistance include the following:

1. root regenerative ability
2. attractiveness to zoospores in terms of either encystment rates or the concentration and composition of exudates produced by the roots
3. deposition of structural barriers such as callose and tyloses
4. the induction of various biochemical defence pathways within avocado roots in response to *P. cinnamomi* infection

1. Root Regeneration

The ability to grow more feeder roots than *P. cinnamomi* is able to destroy is one of the mechanisms by which avocado plants can tolerate root infection. Root regenerative ability as a component of resistance has been observed between *P. cinnamomi* and many other plant species. While the ability of avocado to combat root destruction by compensatory root growth has been identified as an important component of resistance, other pre- and post-penetration defence mechanisms may also play a role.

2. Zoospore attraction and encystment

Fundamental research into the epidemiology of *P. cinnamomi* infection of avocado conducted by George Zentmyer revealed that that zoospores are attracted to an area behind the root tip called the zone of elongation, and that zoospores and germ tubes are attracted to root exudates. Zentmyer first proposed the notion that zoospores were more strongly attracted to susceptible versus resistant avocado roots. Follow up studies indicate that the relationship between root susceptibility and *P. cinnamomi* zoospore attraction is highly dependent upon the host species.

3. Structural Barriers

Proliferation of *P. cinnamomi* within avocado root tissue may be effectively inhibited by physical barriers such as callose, tyloses, and fortification of cells that act as a physical barrier. Plants can reinforce their cell walls with lignin and callose, to obstruct pathogen growth. Production of tyloses (outgrowths of parenchyma cells into xylem cells) has been observed in the interaction of avocado roots and *P. cinnamomi* inoculation.

4. Biochemical Responses

Experimental work has demonstrated that biochemical factors, either induced or constitutive, may also effectively reduce the extent of disease symptoms. Plants produce many secondary metabolites that have antimicrobial activity, such as phenolic compounds, saponins, isoflavonoids and sesquiterpenes. These inhibitory compounds may be produced constitutively during normal growth and development (phytoanticipins), or induced in response to pathogen attack. Some studies suggest that phytoanticipins may constitute a substantial amount of resistance of avocado rootstocks to *P. cinnamomi.* Enzymatic activity may also be associated with resistance to *P. cinnamomi.* It has been observed that avocado root resistance to *P. cinnamomi* was associated with rapidly increased activity of β-1,3-glucanase in response to infection with *P. cinnamomi* and there were higher levels of superoxide dismutase. Enzymatic activity may also be associated with resistance to *P. cinnamomi.* It has been observed that avocado root resistance to *P. cinnamomi* was associated with rapidly increased activity of β-1,3-glucanase in response to infection with *P. cinnamomi* and there were higher levels of superoxide dismutase.

5. Genetic approaches

Phytophthora cinnamomi has a broad host range and host resistance is quantitative and polygenic and the *P. cinnamomi* / avocado interaction may involve complex genetic interactions, making it a difficult system for study and manipulation. Breeding and selection programs in perennial tree crops such as avocado progress slowly, and the time taken for plant growth from seed to bearing fruit may be several years. Therefore, effective methods are needed which allow accurate assessment of resistance or tolerance to Phytophthora root rot at an early stage in order to improve levels of resistance in avocado rootstock.

In order to obtain a better insight in Phytophthora resistance in avocado rootstocks the project focused on four key areas:

1) Development of an experimental root evaluation system.
2) Determining root regeneration of avocado seedling varieties
3) Investigations into feeder root growth among rootstock varieties in the field.
4) Determining disease lesion extension and *Phytophthora cinnamomi* growth in root tips.

Insight into mechanisms of resistance to Phytophthora root rot such as; root regenerative ability, differences in root growth of mature trees and restriction and containment of *P. cinnamomi* lesions on infected roots are important first steps to better understand resistance to this disease and may open up new avenues for more effective selection for resistance in breeding programs.

Methodology

1) Development of an experimental root evaluation system.

The ability of certain rootstock varieties to compensate for feeder root destruction has been identified as a possible mechanism of resistance to Phytophthora root rot of avocado. To assess root growth in the presence of *P. cinnamomi* and root regenerative ability relative to photosynthetic area of seedlings and to have easy access to root systems during subsequent experiments, methods such as hydroponics and training of root systems to grow two-dimensionally were trialed. Ultimately, a 2-pot root regeneration was developed to investigate possible differences between varieties in the output of feeder roots growing into a bottom pot in relation to total surface area of mature leaves and in response to mechanical root removal.

In order to determine whether root regenerative ability is a significant component of resistance to Phytophthora root rot, glasshouse experimentation using the 2-pot root regeneration system was used to determine root growth in the presence and absence of the pathogen. Seedlings of nine different seedling avocado varieties including rootstock and scion varieties spanning the three ecological races were tested for their root regenerative ability.

2) Determining root regeneration of avocado seedling varieties.

To augment glasshouse root growth studies and investigate whether root regenerative ability is a component of the resistance of mature trees to Phytophthora root rot, studies in the field were carried out to investigate possible differences between rootstock varieties in feeder root growth of mature trees during root flush events across two field sites. Data relating to yield, tree growth, health and vegetative parameters were measured as well as stored non-structural starch. Potential rootstock differences among rootstock varieties in root growth and these parameters were evaluated. To test whether the anti-oomycete fungicide Ridomil Gold® resulted in increased localised feeder root growth, the compound was applied on half the root window sites at both field locations. To discuss whether root growth measured in the current study is related to tolerance to Phytophthora root rot, the data were interpreted within the context of known field performance of rootstock varieties.

3) Investigations into feeder root growth among rootstock varieties in the field.

To gain insight into the root regenerative ability of different avocado rootstocks in relation to resistance to Phytophthora root rot in the field we sought to address the following research questions: i) Do some rootstock varieties in the field have greater surface feeder root growth than others? ii) Are there differences between varieties in root growth attributes of average root diameter or orientation around trees where most root growth occurs? iii) Does application of the anti-oomycete compound Ridomil Gold® result in increased localised feeder root growth? iv) Are there significant correlations between root growth and other tree physiology attributes?

A preliminary field study of root growth commenced in May 2013, at a trial site with high *P. cinnamomi* pressure at Duranbah, northern New South Wales. The mature trees selected were part of a rootstock trial planted in May 2006. A second field site was selected at Hampton on the Darling Downs to obtain field data across a broader variety of rootstocks. These trees were planted in 2005, and are clonal selections grafted with 'Hass' scions.

4) Determining disease lesion extension and *Phytophthora cinnamomi* growth in root tips.

The mechanisms by which 'Velvick' is better able to tolerate low levels of root rot disease than 'Reed' in the field are unknown. No detailed studies have been undertaken on histological differences between the rootstock varieties or rates of extension of *P. cinnamomi* within inoculated roots of 'Velvick' or 'Reed' plants. The current study sought to identify possible differences between 'Velvick' and 'Reed' seedling varieties in growth of *P. cinnamomi* hyphae within tip-inoculated feeder roots in relation to the length of the disease lesion. Specifically, the research questions were: i) Do growth rates of *P. cinnamomi* through inoculated feeder root tissue of two avocado rootstock varieties reflect known field resistance? ii) Are there differences between two varieties in the length of the *P. cinnamomi* lesion after inoculation?

The hypothesis tested in the first research question is that detection of *P. cinnamomi* in inoculated root tissue will reflect known field resistance i.e. growth rates of the pathogen were faster in the susceptible variety 'Reed' than the moderately resistant variety 'Velvick'. The hypothesis tested in the second research question is that a longer lesion will be visible for the susceptible variety 'Reed', in response to faster growth of the pathogen.

Disease lesions were defined as the continuous area of brown discoloration from the root tip. The lesion front was the boundary between brown and white/yellow root tissue. Diameter of roots was determined using a pixel measurement technique by taking the average of four measured diameter points along each inoculated root. For inoculated roots displaying a visible disease lesion, the junction between necrotic and healthy tissue was recorded as 'discrete' or 'gradual'. For roots displaying a gradual margin, the lesion front was half-way between 'healthy' white/yellow root tissue and the distal end of tissue that was dark brown in colour. The presence of *P. cinnamomi* in asymptomatic tissue ahead of the lesion front was assessed through a PCR test on root pieces at set distances from the infection point.

Outputs

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

1. Development of an experimental root evaluation system.

A two pot regeneration system was developed which allows the growth and manipulation of avocado seedlings in order to compare results of seedling root growth with performance of mature trees in the field.

The two pot system which was developed allows investigation of feeder root growth of avocado plants in response to different root trimming treatments in the glasshouse in relation to photosynthetic area of 'source' leaves.

Using the two pot system the root regenerative ability of 6 different avocado rootstocks was investigated (A8, Velvick, Esther, Edranol, Whitsell and Hass). The study of root: shoot ratio across six seedling varieties found that 'A8' had the greatest mass of excised roots relative to leaf surface area, followed by 'Velvick', 'Esther', 'Edranol', 'Whitsell' and 'Hass' produced the least roots compared to leaf surface area.

2) Determining root regeneration of avocado seedling varieties.

The root: shoot ratios (total projected area of excised roots/total surface area of mature leaves at 8 weeks) were not significantly different among ten varieties (G1, Velvick, Kidd, Hass, A10, Reed, Edranol, A10xV, Fuerte and Skhirate)

There were no significant varietal differences within trimming treatment groups. All rootstocks showed increased root: shoot ratios between 4 trims and 1 trim. The trend of increasing root: shoot ratio in the order of '4 trims' < '2 trims' < '1 trim' was observed for all varieties except 'Reed', 'Velvick' and 'G1', for which root: shoot ratios were greatest for '4 trims', followed by '1 trim' and '2 trims'. For 'Kidd' and 'Edranol', plants subjected to '4 trims' had significantly lower root: shoot ratios than '2 trims' and '1 trim' plants. For 'Fuerte' seedlings, plants whose roots were trimmed once had significantly higher root: shoot ratios than those trimmed twice or four times.

Measurements of root diameter showed that 'Reed' and 'Velvick' had significantly thicker roots than all other rootstock varieties except 'Kidd'. The rootstock with the narrowest roots was 'Hass', but this difference was only significant in comparison with 'Kidd', 'Reed' and 'Velvick'. Root diameters of 'A10', 'A10 x Velvick', 'Edranol', 'Fuerte', 'G1' and 'Skhirate' were all intermediate. By contrast, 'Skhirate' excised roots were the thickest in the second experiment, but the difference was only significant in comparison with 'Edranol', 'Hass' and 'A10'.

Plants inoculated with *P. cinnamomi* had significantly greater observable wilting than un-inoculated plants. In the first experiment, 'Fuerte' plants had the highest average wilting rating, 'G1' plants had the smallest average wilting rating. In the second experiment, 'Skhirate' plants had the largest average wilting rating and 'Edranol' plants had the least observable wilting.

3) Investigations into feeder root growth among rootstock varieties in the field.

At the Duranbah field site, there were no significant differences in root growth (projected area of roots) between 'Dusa' and 'SHSR-04' across all assessment times. .

Overall, Ridomil application increased feeder root growth, but when averaged over six assessments, the effect was not significant.

Root growth of different rootstock varieties at the Hampton site varied according to assessment time. For 'A10', 'Hass', 'Reed' and 'Velvick', the greatest amount of root growth was measured in November 2014, followed by December 2014 and the least amount of root growth was measured in May 2015. For 'SHSR-04' however, the greatest amount of root growth was measured in December 2014, with slightly less in November 2014, and less again in May 2015.

There were no significant differences between rootstock varieties in flowering intensity measured in October 2014. Significant differences were observed between varieties in the degree of determinate growth measured in October 2014.

Trees on 'Velvick' rootstocks had the greatest degree of observed determinate growth, followed by 'SHSR-04', 'Reed', 'Hass', and 'A10'. Determinate growth of 'Velvick' was significantly greater than all other rootstocks except 'SHSR-04'. 'SHSR-04' determinate growth was significantly greater than 'A10' and 'Hass' determinate growth.

The amount of stored non-structural starch measured in January 2015 was significantly different between rootstocks. 'Velvick' rootstocks had the greatest amount of starch, followed by 'Reed', 'SHSR-04', 'A10', and 'Hass' had the least amount of stored non-structural starch.

4) Determining disease lesion extension and *Phytophthora cinnamomi* growth in root tips.

Results showed that the disease lesion of tip-inoculated 'Velvick' roots was significantly longer than 'Reed', and that there was no evidence that 'Velvick' tissue was able to restrict pathogen growth.

Disease lesions of 'Reed' roots were significantly more likely to have a visually distinct margin with healthy root tissue than 'Velvick' disease lesions.

There was no significant association between lesion length and root diameter. Average root diameter had no significant association with margin type.

Visible lesions advance more quickly in 'Velvick' compared to 'Reed' but PCR analysis showed that *P. cinnamomi* can be detected about the same distance (7-9mm) from the lesion front in both varieties.

5) Non-refereed scientific publications

<u>Thesis</u>

Neilsen, M.J. 2016. Evaluation of Phytophthora Root Rot Resistance in Avocado. University of Queensland PhD Thesis 2016. Pp 185.

Conference Proceedings

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2013) Phytophthora root rot of avocado – why are some rootstocks more resistant? Proceedings of the 19th Australasian Plant Pathology Conference, 25-28 November, Auckland, New Zealand, p90.

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2013) Mechanisms of cultivar and race-based disease resistance to Phytophthora root rot in avocado. Presentation at the 5th New Zealand and Australian Avocado Growers' Conference – "Nutritional Values", 9-12 September, 2013, Tauranga, New Zealand.

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2014) Glasshouse studies of inherent root growth of seedling avocado rootstock varieties, International Horticultural Congress, 17-22 August, Brisbane, Australia.

Outcomes

Key outcomes were identified.

1. Development of an experimental root evaluation system.

A non-destructive method of measuring feeder root growth in response to mechanical root trimming was developed. Across six seedling avocado varieties, the 2-pot system was tested and found to be useful for assessing production of avocado feeder roots in relation to the total surface area of mature leaves. The technique is a novel way to measure seedling root growth without destroying plants.

2) Determining root regeneration of avocado seedling varieties.

The current study investigated differences in inherent root growth of young avocado seedlings and their ability to withstand infection by *Phytophthora cinnamomi*. Inherent root regenerative ability was assessed using a 2-pot root harvesting system with three root trimming events at different intervals. Root growth in the presence of the pathogen was studied following amendment of potting media with *P. cinnamomi*-colonised millet seed.
Although varietal differences were not significant, results of glasshouse investigations showed that root regenerative ability may be a component of resistance for moderately

resistant ‘Hass’ and resistant ‘Velvick’, but the susceptible variety ‘Reed’ also showed high root regenerative ability.

3) Investigations into feeder root growth among rootstock varieties in the field.

The ability to tolerate a certain level of disease through compensatory root growth has been identified as a possible mechanism of resistance of avocado to Phytophthora root rot caused by *Phytophthora cinnamomi*.

In the current study of avocado trees across six varieties at two field sites, no strong evidence was found that feeder root growth is a substantial component of the overall tolerance of varieties with known field resistance.

4) Determining disease lesion extension and *Phytophthora cinnamomi* growth in root tips.

Results suggest that there are physiological differences between ‘Reed’ and ‘Velvick’ in response to *P. cinnamomi* infection but restriction of pathogen proliferation through root tissue was not found to be a significant component of the field resistance of ‘Velvick’.

5) Training of a student

The study was undertaken by a research higher degree student at the University of Queensland. At the time of writing this report, the thesis had been submitted for external examination.

Evaluation and Discussion

The work described in this report was undertaken to identify possible mechanisms of resistance of avocado rootstock varieties to Phytophthora root rot of avocado caused by *Phytophthora cinnamomi.* Root regenerative ability in the absence and presence of the pathogen was investigated across ten seedling varieties, and feeder root growth of mature trees was investigated across six rootstock varieties and two field sites. Pathogen colonisation through tip-inoculated roots of ‘Reed’ and ‘Velvick’ was also assessed.

Results of the glasshouse root growth study showed that ‘Hass’ produced the most feeder roots relative to total mature leaf surface area after repeated root trimming, but ‘Hass’ root system necrosis was average and wilting was slightly more severe than the other varieties. Feeder root growth of ‘Hass’ trees in the field study under conditions of low disease pressure at Hampton was not significantly different to the other rootstock varieties: - susceptible ‘Reed’, moderately resistant ‘A10’, and resistant ‘Velvick’. Field studies have found that ‘Hass’ performs well under high Phytophthora root rot disease pressure. Results of the glasshouse root growth assay indicated that the ability to grow more feeder roots may be a component of the overall tolerance of ‘Hass’ to Phytophthora root rot, although results of the root system necrosis study suggested that disease still occurred and seedlings experienced hydraulic stress.

Variety ‘G1’was only available for the first round of glasshouse experiments, but had the least roots per leaf area of any variety. The variety had less root system necrosis than most other varieties and the least visible wilting after inoculation with *P. cinnamomi*. Although varietal differences were not significant, these results indicated that ‘G1’ had low inherent root growth ability, but root systems showed relatively low levels of necrosis after exposure to the pathogen and plants were showing a low level of hydraulic stress. These results indicate that root regenerative ability is not a component of the resistance of ‘G1’, but it is likely that other mechanisms contribute to the resistance of ‘G1’ to Phytophthora root rot.

‘SHSR-04’ is derived from a tree from an orchard near Bundaberg in central Queensland that displayed remarkable tolerance to the disease. No significant differences were found between ‘SHSR-04’ and ‘Dusa’ in tree health measured between eight and nine-and-a-half years after planting in a site infested with *Phytophthora cinnamomi* and where management practices to reduce Phytophthora root rot are not implemented. There was also no significant difference in yield between rootstocks, although a previous study on the same trees found that ‘SHSR-04’ rootstocks were significantly healthier assessed 22 months after planting. Although ‘Dusa’ has been shown to be more tolerant to Phytophthora root rot than ‘Duke 7’ in our field studies at Duranbah, no significant differences were observed between ‘Dusa’ and ‘SHSR-04’ in root growth characteristics.

The performance of rootstock variety ‘A10’ has been variable in previously published rootstock selection trials. It has been reported that that seedling ‘A10’ rootstocks performed poorly under high disease pressure, while clonal ‘A10’ performed comparatively well. In the current study, overall inherent root growth of seedling ‘A10’ was average but huge variability in response to trimming treatment was observed. Root system necrosis results were variable, with low levels of necrosis in the first experiment, but high levels in the second and average wilting in both. In the field study, measured feeder root growth at three time points over the 2014-15 season of ‘A10’ trees at Hampton was not significantly different to moderately resistant ‘Hass’, susceptible ‘Reed’ or resistant ‘Velvick’. Results of the current study support previous findings that ‘A10’ has variable health in the presence of disease caused by *P. cinnamomi,* and results on root regenerative ability are inconclusive.

In the current study ‘Edranol’ had consistently low inherent root growth, but performance in the root system necrosis assay was fair. Wilting of inoculated ‘Edranol’ seedlings was highly variable. A previous field study used seedling ‘Edranol’ rootstocks as susceptible controls found that the rootstock performed poorly under conditions of Phytophthora root rot pressure. Results of the current study indicate that ‘Edranol’ has average tolerance to the disease and low root regenerative ability.

'A10' x Velvick' had low levels of inherent root growth in the glasshouse study. Interestingly, these values were lower than both 'Velvick' and 'A10'. Performance of 'A10 x Velvick' in the root necrosis assays was poor to average. In both assays, root system necrosis of 'A10 x Velvick' was more severe in than 'Reed'. In the root system necrosis assays, the degree of visible wilting of 'A10 x Velvick' plants was variable. The parental identities of the 'A10 x Velvick' used in the current study are unknown, but results indicate that overall, 'A10 x Velvick' has low tolerance to Phytophthora root rot and low root regenerative ability.

'Fuerte' seedlings had low root: shoot ratios but root system health of 'Fuerte' was relatively good after *P. cinnamomi* inoculation, although corresponding above-ground symptoms were relatively more severe. Results of the current study indicate that 'Fuerte' has low root regenerative ability, fair root system health in the presence of *P. cinnamomi,* but the disease caused high levels of hydraulic stress.

The seedling rootstock variety 'Kidd' performed intermediately in the inherent root growth assay, however 'Kidd' root system necrosis after *P. cinnamomi* inoculation was consistently low and corresponding data showed that 'Kidd' also had relatively low levels of hydraulic stress. Overall, results of the current study suggest that 'Kidd' is a resistant rootstock variety, but root regenerative ability does not appear to be a substantial component of its resistance.

The field-susceptible variety 'Reed' showed high levels of root: shoot growth in the inherent root growth assays. Performance in the root necrosis assay was variable, with root systems of 'Reed' plants having average to low levels of root necrosis, while corresponding wilting ratings were average. Glasshouse results indicated that 'Reed' plants were not consistently susceptible, and root regenerative ability may be high. Possible reasons for the discrepancy in root necrosis results could be differences in temperature or seedling maturity.

In our glasshouse studies, 'Velvick' had high inherent root growth, average root system necrosis but correspondingly low levels of hydraulic stress. In the field study, while feeder root growth of 'Velvick' was not significantly different to less resistant rootstock varieties, 'Velvick' had the highest level of stored non-structural starch. The Australian avocado industry is based mostly on seedling 'Velvick' rootstocks, which usually perform well in soils where Phytophthora root rot is present Glasshouse results in the current study indicated that root regenerative ability may be a component of the resistance of 'Velvick', and in the presence of Phytophthora root rot, despite average levels of root necrosis, the degree of visible wilting was low.

In the inoculation study that compared lesion lengths and pathogen growth through tip-inoculated 'Reed' and 'Velvick' roots, lesions were significantly longer for inoculated 'Velvick' roots than for ‘Reed’. This finding supports the results obtained in the root system inoculation experiments, where infected ‘Velvick’ root systems were more necrotic than ‘Reed’, but visible wilting of inoculated plants was less severe for ‘Velvick’ than for ‘Reed. This indicates that while inoculated root systems of ‘Velvick’ appeared visually to be more diseased than ‘Reed’, ‘Velvick’ hydraulic function was less affected. Isolation data indicated that *P. cinnamomi* could be detected slightly further ahead of the lesion front in ‘Reed’ than ‘Velvick’, although the difference was only significant in the initial isolation experiment. Yet, PCR detection results combined with lesion length data suggest that relative to the root tip, *P. cinnamomi* also travelled further along inoculated ‘Velvick’ roots than ‘Reed’. These results are interesting because resistance to Phytophthora root rot in avocado has been associated with restriction of lesion extension and containment of the pathogen. In contrast, the disease lesion of field-resistant ‘Velvick’ was significantly longer than susceptible ‘Reed’ and there was no evidence of restriction of pathogen growth through ‘Velvick’ tissue. The mechanisms by which ‘Velvick’ is able to tolerate low levels of disease are yet to be elucidated.

Results of field studies indicated that overall, there was no observed association between known field resistance to Phytophthora root rot and surface root growth. While significant rootstock differences were observed in certain tree physiology parameters, these differences were also not associated with root growth. Results of glasshouse studies indicated that root regenerative ability may be a component of resistance of ‘Hass’ and ‘Velvick’ but the susceptible variety ‘Reed’ also showed high root regenerative ability.

Results of the necrosis study supported the high known field resistance of varieties ‘G1’ and ‘Kidd’, but both were observed to have low root regenerative ability. Restriction of pathogen growth through inoculated feeder roots was not observed to be a component of the resistance of the resistant variety ‘Velvick’ compared to the susceptible variety ‘Reed’, but necrotic lesions formed more rapidly in ‘Velvick’ than ‘Reed’.

Recommendations

Recommendations to Industry and R&D investment decision makers

The study did not provide clear advances in our understanding of mechanisms underlying field resistance in avocado rootstocks to Phytophthora root rot. Further investigation in this area is warranted, and could be included as a component in a larger disease management project. Abundant clonally-propagated plants of some new resistant varieties are required for further glasshouse work, and this material may be easier to obtain in future with new propagation techniques. A detailed microscopic study to observe infection processes and cellular defence responses combined with biochemical and molecular analyses, would assist with dissecting this question in greater detail. Responses of rootstock varieties to current root rot management strategies, eg. phosphonate and mulching, should be investigated as results from AV10001 suggest that phosphonate accumulation is higher in roots of Velvick compared to Reed and Zutano. Synergies between rootstock selection and management strategies should be identified.

If the glasshouse component were to be repeated, it is recommended that clonal material be used, and for experiments to be performed under standard climatic conditions.

> A major limitation of the glasshouse investigations into root regenerative ability in the presence and absence of *P. cinnamomi* was that seedling plant material was used instead of clones. Plants that have been clonally propagated from the same maternal plant are genetically identical whereas seedlings are the result of sexual recombination and are genetically variable. The large degree of statistical error encountered in glasshouse experiments may be partly explained by the use of seedling plant material. Also, due to time constraints, repeated glasshouse experiments took place at a different time of year to the first experiments, therefore plants experienced different environmental conditions. If the glasshouse component were to be repeated, it is recommended that clonal material be used, and for experiments to be performed under standard climatic conditions.

The 2-pot system may be applicable to studies of root growth for plant species other than avocado. If used again in future experiments, to ensure plants in replicate experiments are at the same stage of development and climatic conditions are identical. The same recommendations can be made for the study of root and shoot health due to *P. cinnamomi* infection.

Future studies should include the same number of replicates for each treatment, and larger pots to reduce possible effects of root restriction.

> Limitations of the fieldwork component of the study were that the same rootstock varieties were not available at each field site, and that at Hampton, trees that were not subject to yearly injections with phosphorous acid were not available, to determine if a possible interaction was present between the conjugate base phosphonate and Metalaxyl (Ridomil).

Future studies should further investigate the likely causes of the inhibitory effect of Ridomil on avocado feeder root growth.

> Our initial assumption that application of 'Ridomil Gold' granules would effectively act as a negative control for presence of *P. cinnamomi*, as the active ingredient Metalaxyl would inhibit pathogen growth, thereby resulting in a localised area of

increased feeder root growth relative to sites that did not receive the same treatment. Instead, application of the compound had complex and site-specific effects on feeder root growth.

Future field studies of avocado feeder root growth should also measure root growth at depth, to acquire more accurate data.

In this study only surface feeder root growth was measured. It is likely that particularly at Hampton, substantial sub-surface feeder root growth was present, as (surface) root growth levels from this site were low in comparison to Duranbah. Scraping away soil also showed that feeder roots were present several centimetres below the surface. Therefore, future field studies of avocado feeder root growth should also measure root growth at depth, to acquire more accurate data.

A non-destructive method to study root development is needed.

In the inoculation study much data had to be excluded due to breakages in roots coinciding with lesion margins. Also, suberisation of whole root systems occurred occasionally. This indicates that root systems experienced considerable stress during the inoculation procedure. For future inoculation studies, development of a less destructive method would be desirable. Also, in conjunction with studies of lesion extension and pathogen growth, associated histological analysis would provide some insights into possible biochemical and/or structural differences between 'Reed' and 'Velvick' tissue in response to infection with *P. cinnamomi.*

Future directions may need to include histological analysis to observe if the longer disease lesions observed in inoculated 'Velvick' tissue are associated with effective defence responses, for example, the formation of a protective cell layer between infected and healthy root tissue.

Investigate whether lesion extension and pathogen growth through root tissues cease at a certain time after inoculation, and whether this is associated with known field resistance.

Scientific Refereed Publications

None to report

<u>Thesis</u>

Neilsen, M.J. 2016. Evaluation of Phytophthora Root Rot Resistance in Avocado. University of Queensland PhD Thesis 2016. Pp 185.

Conference Proceedings

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2013) Phytophthora root rot of avocado – why are some rootstocks more resistant? Proceedings of the 19th Australasian Plant Pathology Conference, 25-28 November, Auckland, New Zealand, p90.

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2013) Mechanisms of cultivar and race-based disease resistance to Phytophthora root rot in avocado. Presentation at the 5th New Zealand and Australian Avocado Growers' Conference – "Nutritional Values", 9-12 September, 2013, Tauranga, New Zealand.

Neilsen, M., Drenth, A., Dann, E. and Campbell, P. (2014) Glasshouse studies of inherent root growth of seedling avocado rootstock varieties, International Horticultural Congress, 17-22 August, Brisbane, Australia.

Intellectual Property/Commercialisation

No commercial IP generated.

References

Where required, references are provided in Appendix 1.

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Appendices

Appendix 1: University of Queensland Thesis; "Evaluation of Phytophthora Root Rot Resistance in Avocado" by: Merran Jane Neilsen.