Reducing reliance on phosphonates for managing Phytophthora root rot

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INTRODUCTION

The success of the South African and Australian avocado industries today is attributed to the targeted research, development and widespread adoption of the phosphonate group of pesticides for management of Phytophthora root rot (PRR). However, an integrated approach, as recommended by Pegg and Wolstenholme and their colleagues since the 1970s, remains the current best practice strategy. Growers in our respective industries have been constantly reminded of this, and the "Pegg Wheel" has been a key feature of presentations, posters, publications and discussion for many years (e.g. Wolstenholme & Sheard, 2010). A recent study has demonstrated that a multi-faceted approach to PRR management reduced disease severity and improved fruit quality more effectively than individual control methods (Ramírez-Gil *et al.,* 2016). No apology is offered for the continual reiteration of the basic dogma of integrated disease management, which is applicable to any pest or disease problem, not just Phytophthora root rot.

The key elements of integrated Phytophthora management include:

1) careful site selection and preparation, including adequate drainage to reduce the build-up of free soil water,
2) selection of rootstocks which are tolerant to *Phytophthora cinnamomi* (Pc), such as Dusa, Bounty, Velvick, and new selections,
3) planting Phytophthora-free trees sourced from accredited nurseries,
4) application of mulches and/or composts to improve soil structure, encourage root regeneration and stimulate microbial activity to reduce the survival of Pc propagules,
5) optimal irrigation management and tree nutrition, including calcium amendment to suppress Pc, and
6) Judicious use of chemicals, such as phosphonates and metalaxyl, applied correctly and supported by root and fruit residue analyses where available.

The South African industry exports much of the avocado crop to European markets, where the maximum residue limit (MRL) is 50 mg/kg, with <20 mg/kg imposed by some importing retailers (Nortjé, 2016, and pers. comm., 2017). Hence, there is considerable pressure on avocado producers to minimise (and optimise) the use of these compounds. This is in contrast to the situation in Australia, where only 3% of the crop is currently exported to mostly Asian countries (Avocados Australia, 2016), and our regulator, the Australian Pesticides and Veterinary Medicines Authority, have set the temporary MRL at 500 mg/kg for fruit marketed domestically. Despite the more relaxed state of affairs for Australian fruit, it is pertinent to maintain research efforts to reduce and/or optimise applications of any pesticide, and explore alternative or complementary management strategies.

This article will present our research group's recent efforts to identify Phytophthora tolerant rootstocks and investigate the interaction between phosphonate and *Phytophthora cinnamomi*.

RESULTS AND DISCUSSION

Rootstock evaluation

Selection and breeding of material to resist or tolerate diseases has been a crucial management strategy for, arguably, all agriculturally-important crops. A series of field trials since 2006 evaluated several rootstocks grafted with Hass for survival and yield performance under high PRR conditions at Duranbah, northern NSW and one trial at Childers, central Queensland.

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

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

Tree health is rated on a scale where 0=healthy and 10=dead (Darvas *et al.,* 1984)
Tree survival is the % of living trees compared with total numbers planted
Means followed by the same letter are not significantly different (P<0.05)

The trials were established in conjunction with Dr Tony Whiley's rootstock evaluation projects. The results from these trials have been reported (Smith *et al.,* 2011; Dann *et al.,* 2013), but briefly, Dusa, Latas and SHSR-04 were highly tolerant, Velvick was moderately tolerant and Reed was consistently highly susceptible to PRR. Yields were evaluated in the Childers trial 2009-2013 (Dann *et al.,* 2013). Cumulative yield per tree (total yield across all years) was highest for Velvick^L, but significantly higher only than Velvick^A and Reed. Above average rainfall in 2011-2013 impacted yields and tree health, and the highest yielding trees were on Dusa and Velvick^L rootstocks. SHSR-04 is an Australian selection, and is currently being considered for commercialisation. It was not included for evaluation in the Childers trial where yields were obtained.

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

A subsequent field trial was planted in 2013, at Duranbah, NSW, and included SHSR-07 and SHSR-08, which were selections from trees surviving under high PRR pressure in a rootstock trial planted in 2007. Differences in tree health among rootstocks were significant 12 months after planting (Table 1). Trees grafted to SHSR-04, SHSR-08 and Dusa were significantly healthier than Zutano, SS3-1 and the susceptible Reed rootstocks. This is the first time that Zutano has been included in a replicated trial assessing tolerance to PRR. Trees on Zutano are being increasingly planted, mostly due to the strong demand for planting material and availability of seed from New Zealand. We now have preliminary information suggesting it is not as tolerant as other rootstocks, and certainly a poor choice for replant sites. The rapid decline in tree health and high mortality rate across the trial (despite frequent metalaxyl and phosphonate applications in the first two years) highlights the effect of high *P. cinnamomi* disease pressure. Seventy five percent of tree deaths occurred in sites where a sick tree had been removed in the 2 months prior to replanting, where Pc inoculum would have been high. This stresses the importance of careful site preparation prior to replanting which should include a period of fallow to allow Pc inoculum to naturally decline.

Bounty is available for Australian growers but has not been widely tested. Several of the promising Westfalia Fruit Ltd-SAAGA co-owned rootstocks have cleared Australian quarantine and are awaiting testing under Australian conditions.

Phosphonate studies

The Australian industry has continually revised recommendations on phosphonate use to growers, as new information from research has become available. Current "best practice" revolves around testing feeder rootlets for phosphonate concentration to allow informed decision on applications and other management strategies. The South African industry (SA Avocado Growers' Association) has recently supported the development and optimisation of such methodology, and growers are encouraged to contact Dr Adele McLeod at the University of Stellenbosch, for more details.

The timing, mode of delivery and frequency of phosphonate applications depend on location (and rainfall, temperature), tree phenology and health of trees. Injections are mandatory for sick (declining) trees and multiple foliar sprays as a preventative measure for healthy trees. The main application window is in autumn/winter, when the root flush is active. Hass fruit should have sized-up by then, and are unlikely to be a resource sink for phosphonate. The other application window is late spring/early summer, after the spring flush has hardened off.

However, this application window coincides with early fruit development and may result in fruit residues of phosphonate which are above acceptable limits for the European markets. SAAGA is actively funding further research in this area.

What is the "critical" concentration of phosphonate required in roots? This question can be interpreted in two ways, and there is not a simple answer. Firstly, "critical" could mean the concentration which is required at any point in time to reduce the infection of roots by Pc such that only low (acceptable) levels of disease occur. Secondly, "critical" could be interpreted, for example by growers, to mean the concentration of phosphonate required at the end of the application window to protect roots for several months until next treatments can be applied, with the knowledge that concentrations are diluted in actively growing roots. Petri dish tests where several isolates of Pc were grown on agar media augmented with different concentrations of phosphonate, has demonstrated there is a wide range of sensitivity to phosphonate among isolates of Pc (Fig. 1). The inhibitory concentration at which Pc growth was inhibited by 50% (IC50) ranged from less than 1 ppm for an isolate from an orchard at Robinvale, Victoria, where phosphonate is not used, to 120 ppm for an isolate from Beechmont, Queensland, which is a high rainfall area and phosphonate is trunk-injected twice annually. Weinert *et al.* (1997) also demonstrated a wide range of sensitivity amongst isolates collected from trees which had been continuously treated with phosphonate for 10 years, where Pc growth could be completely arrested at concentrations of 50-1000 ppm. Growth of isolates collected from untreated trees was completely inhibited at 50 or 100 ppm. Thus, it is likely that repeated use of phosphonate has selected for populations of Pc which are less sensitive thus pushing up the "critical" phosphonate recommendation. This data does not suggest that Phytophthora is mutating to become resistant to phosphonate. When considering "critical" phosphonate concentration, it is important to remember that phosphonate has a dual mode of action (Guest and Bompeix, 1990), and the direct inhibition combined with the activation of plant defences in the roots are likely to provide effective management at root concentrations less than those inhibiting Pc growth in Petri dish assays.

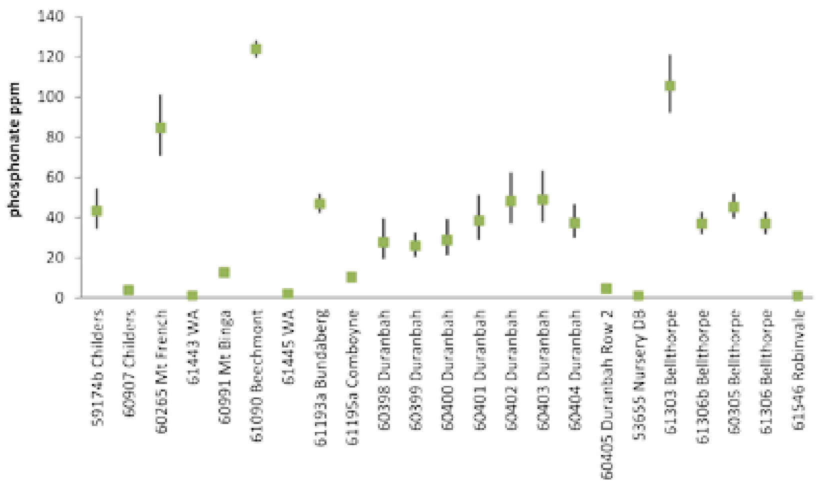

Figure 1. Inhibitory concentration of phosphonate at which growth of *Phytophthora cinnamomi* is reduced by 50% (IC50), *in vitro* assay.

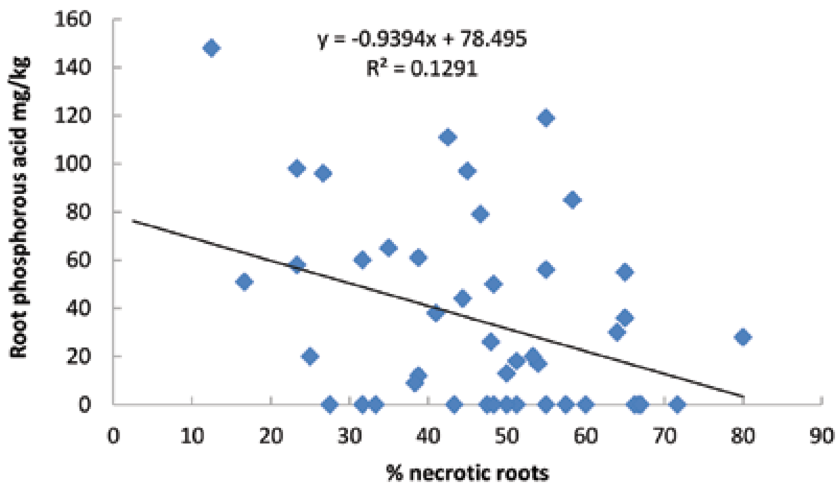

Figure 2. Effect of phosphorous acid concentration in roots on necrosis after inoculation with *P. cinnamomi*, intact seedling assay.

Glasshouse trials with intact seedlings were undertaken to investigate phosphonate accumulation in roots and disease development following subsequent inoculation with *P. cinnamomi*. Intact seedlings were inoculated by repotting the plants with 5% v/v grain media colonised with Pc, subjected to flooding for 3 days then drained, and root necrosis assessed approximately 6 weeks after inoculation. Although there is large variation, the linear regression (significant at P=0.014), shows that there is minimal disease when root concentrations of phosphonate approach 80 mg/kg and up to 80% root necrosis when there is no detectable phosphonate in roots (Fig. 2). A comparable study with detached roots showed a similar highly variable but significant (P<0.001) negative relationship, with less disease occurring with increasing levels of phosphonate in roots (results not shown). In detached roots, an average of about 50 mg/kg prevented root necrosis, however there was a large spread of the data from 5 to 90 mg/kg (not shown).

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Detached root experiments were undertaken with Reed, Velvick and Zutano seedlings sprayed with phosphonate, to investigate relative accumulation of phosphonate, and PRR in susceptible or resistant rootstocks. For each treatment, one sample of roots was harvested and analysed for phosphonate concentration, while replicate samples were inoculated with Pc isolates determined from the *in vitro* IC50 study to have high or low sensitivity to phosphonate. In glasshouse Experiment 1, the higher level of phosphonate in roots of Velvick compared to Reed corresponded to less severe root disease in Assay 1, and similar levels of root disease in Assay 2 (Table 3). In glasshouse Experiment 2, phosphonate-treated Velvick plants had higher concentrations of phosphonate in roots than Zutano, and less root disease after inoculation with either isolate of Pc (Table 4). Thus, the magnitude of disease reduction corresponded to concentrations of phosphonate in roots and host resistance levels. Velvick is known to be more tolerant of Pc than Zutano and Reed (as described above and in Smith *et al*., 2011), and these data show that the ability of Velvick to accumulate more phosphonate may contribute to this field observation. The data also demonstrate that necrosis is more severe in roots inoculated with the isolate of Pc less sensitive to phosphonate.

Although variable, it would seem that root levels of at least 80 mg/kg phosphorous acid are required throughout the infection periods, and even higher levels may be required for orchards with long history of phosphonate use, which may have selected for isolates of Pc less sensitive to phosphonate. Several years ago the minimum root level for protection from Pc was suggested to be 20 mg/kg, determined by survey rather than structured experiments (Whiley and Pegg, pers comm. 2012), i.e. an arbitrary figure selected from the data. A South African study injected 6 month old seedlings with phosphonate and inoculated detached roots with Pc at various times thereafter. Root colonisation was reduced compared with controls by approximately 85% at root phosphonate concentrations of 9.8 to 53.2 mg/kg, and infection was never completely prevented (Van der Merwe and Kotze, 1994). Several factors must be considered when interpreting results of root analyses, and determining optimum root phosphonate concentrations. These include pre-application phosphonate concentration in roots, tree health and other management practices, (e.g. mulching, calcium applications, irrigation), tree age and size, rootstock, location (rainfall, cyclones, flood risk etc.), tree and root vigour and crop load. A "critical" root concentration recommendation for well-managed trees on Dusa rootstock in a low PRR prone region will be less than that for an orchard on Reed or Zutano rootstock, in poorly drained soils in a high rainfall location.

Table 3. Concentration of phosphorous acid in roots, and root necrosis after inoculation of detached roots with isolates of Pc which had different sensitivities to phosphonate determined by *in vitro* tests. Glasshouse Experiment 1.

		Root necrosis (%)		Root phos acid conc. (mg/kg)
		Phos sensitive Pc	Phos less sensitive Pc	
Assay 1				
	Reed	Not tested	74	9
	Velvick	Not tested	40	42
Assay 2				
	Reed	32	48	25
	Velvick	37	44	47

Table 4. Concentration of phosphorous acid in roots, and root necrosis after inoculation of detached roots with isolates of Pc which had different sensitivities to phosphonate determined by *in vitro* tests. Glasshouse Experiment 2.

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

CONCLUSION

In addition to the tolerant rootstock evaluations and phosphonate optimisation research, our group continue to evaluate other PRR management strategies, such as new anti-oomycete chemistries, microbial amendments, brassica biofumigants and soluble silicon. Of these, soluble silicon shows promise for improving fruit quality and marketability although its effects on PRR and recovery of declining trees have been inconsistent when assessed in short-term glasshouse and field trials. Efficacy of any management strategy will vary according to local conditions, and there is no guarantee that new products tested in the laboratory or in a limited number of commercial orchards will suit every situation. Growers are in an enviable position of having sufficient trees and equipment to conduct their own mini-research trials. The most important thing to remember is to have adequate "control" treatments, so that you can compare new treatments or products with current management practices. Growers in both Australia and South Africa will need to remain vigilant and eat, sleep, breathe the Pegg Wheel to be triumphant against Phytophthora and maintain productive orchards.

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