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PHYTOPHTHORA REVISITED

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Phytophthora root rot remains a damaging disease of avocado in many countries where it causes significant tree deaths and reduces yield. Some members of our research team witnessed and worked through the spectacular Phytophthora epidemics of the 60s and 70s in Australia when entire orchards were destroyed. The disease threatened the existence of the industry. The question of importance to researchers was how such losses could be reduced. In pursuit of answers they considered and evaluated many things. They made good progress in the cultural and chemical management of the disease. This led to a significant reduction in the effects of the disease and gave them a good appreciation of the disease biology. They demonstrated the importance of clean planting material, and found that soil organic matter influenced Phytophthora activity by increasing antagonistic microbial populations. There was direct lysis of hyphae and inhibition of chlamydospore germination in high organic matter soils. Others found that calcium (gypsum) was a weak fungicide against Phytophthora and that animal manures reduced Phytophthora populations through microbial suppression and the production of ammonia.

A major breakthrough occurred in the early 80s when cost effective injections of phosphorous acid (phosphonate), a practice unsuccessfully challenged in the Federal Court of Australia, were found to reduce the disease. During the 90s there was ongoing testing of phosphonate application strategies with particular emphasis on foliar spraying. During these early years there was also involvement in the pursuit of host resistance. The root rot tolerant rootstock 'Velvick' (West Indian race) was selected by Dr. Tony Whiley during this period.

We believe that good progress was made in understanding and reducing the impact of the pathogen but it has not been enough. There are still many important questions to be answered. We have been motivated to conduct further research in three main areas:

- further testing of phosphonate application strategies
- the pursuit of useful field tolerance in rootstocks
- the evaluation of plant activators.

(1) Phosphonate application strategies

Phosphonate (PO_3^{2-}) is systemic in the avocado tree and high concentrations can occur in developing fruit, shoot and root tips. Phosphonate is believed to work against

Phytophthora by

(a) directly inhibiting the growth of *Phytophthora cinnamomi.* This occurs at high phosphonate concentrations which do not kill the fungus but merely retard fungal growth. This prevents the fungus establishing a close relationship with the host and host defences remain unchanged.

(b) indirectly stimulating plant defence mechanisms (e.g. promoting salicylic acid production in infected plants). This occurs when phosphonate levels are low within the roots and release of stress metabolites from Phytophthora link the pathogen with host defence systems. These natural plant defence systems then bring the invasion under control. In addition, low levels of phosphonate significantly reduce sporulation of *P. cinnamomi*.

Phosphonate is a stable compound and there is no evidence that plants can oxidise phosphonate to phosphate. However, phosphonate can be oxidised by soil microorganisms into phosphate. Phosphonate rarely causes visible damage to vegetative growth in avocado (Guest *et al.* 1995).

Further evaluation of phosphonate application strategies was stimulated by results from research into the control of *Phytophthora ramorum* in USA forests. *P. ramorum* is thought to have been introduced from Europe into USA in 1994. Because of the damage it is causing in American forests, it has been classified as a bioterrorist. Working with the Australian company Agrichem, scientists in America have developed an organosilicate bark penetrating translocation aid (Pentrabark) to allow phosphonate to be absorbed through the bark of oak trees at an adequate concentration for disease control. It has previously been shown for avocado that the concentration of phosphonate required to protect or rejuvenate feeder roots could not be absorbed through the bark of older trees.

In our 1980 injection trials, we demonstrated rapid basipetal redistribution of phosphonate to actively growing feeder roots when trees showed strong and advanced vegetative flushes. If injections were made before the completion of the foliar flush, most of the phosphonate ended up in the canopy where it was not needed. This occurs because the translocation to root tissue is affected by source/sink relationships at the time of injection.

In our earlier studies we did not closely investigate the effect of phosphonate on the growth of feeder roots. We were primarily concerned with root health and tree recovery; we generated useful information which was made available to growers.

During the past year we have conducted more rigorous studies on the influence of phosphonate on the development of the feeder root system.

In a field trial at Hampton, where treatments were applied at early vegetative flushing, we compared trunk injections with basal trunk sprays containing Pentrabark and critically examined the root systems. We found that feeder root development was inhibited under injected trees (Table 1). The concentration of phosphonate in feeder roots was also significantly higher under the injected trees (Table 2). This suggests that high phosphonate levels in root tips in the early stage of the feeder root flush can have an adverse effect on root growth. As this reduction in root mass may be detrimental to yield, it reinforces the recommendation to delay injections until late April/early May in subtropical Queensland when most of the canopy is in a quiescent stage. It may be even more appropriate to delay injections until the root flush is complete, i.e. when the feeder root system is fully developed. This is a practice which has been advocated by Graeme Thomas, G.L.T. Horticultural Services Pty Ltd, Toowoomba, Queensland (personal communication) for some time. He has found that growers achieve a higher root concentration and this concentration persists longer by delaying injections until June/July in subtropical Queensland when the feeder root system is fully developed but before flower bud development is advanced.

Table 1: The effect of trunk injection or basal trunk spray at Hampton on feeder root mass four months after treatment

Application method	Mean root mass[1]
Trunk injection[2]	2.14 b
Basal bark spray[3]	2.86 a
p	0.004
lsd	0.44

1. 1 = roots sparse, few roots, 2 = roots present, network not developed, 3 = roots abundant, network developed.
2. Injection 20% phosphonate.
3. Sprays 50% phosphonate (20% soln) + 50% water + 2.5% by volume Pentrabark.

Table 2: The concentration of phosphonate in leaf and root samples from trunk injection/basal trunk sprays at Hampton; trees treated February 2005.

	24.2.05*		**15.3.05***		**14.4.05***		**16.5.05***	
	Roots (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)	Leaves (mg/kg)	Roots (mg/kg)	Leaves (mg/kg)
Trunk injection	30.4a	220 a	47.1 a	228 a	58.0 a	125.1 a	52 a	95 a
Basal trunk spray	9.1b	5 b	15.3 b	9 b	23.0 b	5.0 b	24.7 a	6 b
p	0.002	<0.001	0.019	0.002	0.034	<0.001	0.111	0.005
lsd	11.39	60.1	25.75	119.3	31.97	31.51	ns	57.2

* Some samples were at non-detectable levels, i.e. less than 5 mg/kg – for statistical purposes these values were changed to 5 mg/kg

It is not known why high levels of phosphonate in root tips inhibit feeder root growth. It is possible that there is:

- a specific reaction to PO3^{2-} ions and an interaction with root phosphate levels
- osmotic stress caused by a high concentration of PO_3^{2-} ions
- production of chromosome abnormalities in root tips which interfere with cell division (mitosis). Cytological research is required to determine whether phosphonate affects cell division in avocado root tips and causes a reduction of root growth.

Even though root levels of phosphonate may be less in trees receiving basal bark sprays containing Pentrabark, this treatment was as effective as trunk injection for the recovery of severely affected trees in a field trial at Duranbah (Table 3). Also results indicate (Table 2) that the phosphonate applied in this way is transported only in the phloem and remains in the roots (a strong metabolic risk) thus reducing unwanted fruit residues.

Table 3: Improvement in health in Hass trees severely affected by Phytophthora root rot at Duranbah.

Treatment	Improvement in tree health (%)
Untreated	0
Trunk injection	15.8
Basal trunk spray	12.2

In current experiments we are comparing the efficacy of Pentrabark and Pulse (a bark penetrant used with Round-up for woody plants) for use in basal trunk applications with phosphonate. Pulse has the advantage that it does not cause flocculation of the blue vegetable dye present in commercial preparations of phosphonate. This flocculation with Pentrabark tends to block spray nozzles.

(2) Tolerant rootstocks

The selection of rootstocks with superior root rot tolerance has become a vital component of the Australian rootstock improvement programme. High resistance is unlikely in *Persea americana* because *P. cinnamomi* is not a coevolved pathogen. The pathogen is thought to have originated in Asia, whereas avocados evolved in Central America where *P. cinnamomi* was not present. Thus the host has no natural resistance to the pathogen. Also *P. cinnamomi* has a very wide host range and generally it is much more difficult to find resistance to pathogens which have a wide rather than narrow host range.

When evaluating rootstocks for useful field tolerance, the Phytophthora pressure within a given field must be taken into account. The severity of root rot in a given field will depend upon the level of tolerance in the rootstock, the quantity and distribution of inoculum in the soil, and the extent to which soil moisture, soil temperature, soil type and soil organic matter levels influence *P. cinnamomi* activity. This is clearly demonstrated in Table 4.

Table 4: Tolerance to *P. cinnamomi* in 18-month-old Hass grafted to three rootstocks planted in infested soil at Hampton.

	Mean tree health (0-10)[A]	
Rootstock	**Low disease pressure**	**High disease pressure**
'Velvick' (West Indian race)	1.3	6.6
'Anderson 10' (Guatemalan race)	5.8	7.2
'Anderson 8' (Guatemalan race)	5.9	7.3

A = tree health on 0 (healthy) to 10 (dead) scale (Darvas *et al.* 1984)

'Velvick' has shown superior root rot tolerance under low disease pressure, but this level of tolerance is insufficient under conditions of high disease pressure. 'Anderson 10', a rootstock with an outstanding ability to rapidly replace damaged feeder roots, has failed in both situations. This illustrates that the capacity to replace roots, lost to disease or other factors, is alone insufficient to cope with the pathogen. It appears that 'Velvick', under reasonable disease pressure, has natural defence mechanisms to minimise infection. Thus any studies in the pursuit of greater tolerance to root rot will benefit from a greater understanding of the host/pathogen interaction. Too little is known about these defence systems which play a critical role in arresting pathogen invasion. The capacity of these natural defence responses will vary considerably with rootstock. It is assumed that root rot affected rootstocks will probably also vary in their ability to respond to phosphonate applications. Menge and Ploetz (2003) list 'D9', 'Duke 7', 'Thomas', 'Merensky 1' and 'Merensky 2' rootstocks as having Phytophthora tolerance.

3. Evaluation of plant activators

The Fruit Pathology Team (Horticulture and Forestry Science, DPI&F) is using new strategies to control plant diseases by using activators (these are chemicals but not fungicides) that stimulate the expression of natural defence responses in plants. It has been found that the salicylic acid analogue, Bion® (acibenzolar-S-methyl) activates systemic acquired resistance in plants and can increase resistance to Phytophthora (Ali *et al.* 2002). Dann and Muir (2002) have shown that potassium silicate, when incorporated in a growing medium, significantly increased the activity of plant resistance proteins (chitinase, β-1,3-glucanase) in peas and reduced disease caused by the foliar pathogen *Mycosphaerella pinoides.* Injections of potassium silicate were found to reduce postharvest anthracnose in ‘Hass’ avocado fruit (Anderson *et al.* 2004).

In a preliminary field experiment we injected potassium silicate into avocado trees severely affected by Phytophthora root rot; these trees had an average rating of 5.5 on the 0 (healthy) to 10 (dead) scale used in Phytophthora research (Darvas *et al.* 1984). These injections stimulated the rapid growth of dormant epicormic buds with an eventual significant increase in canopy density (Table 5). We did not believe that this rapid vegetative response was due to Phytophthora control.

Table 5: Effect of potassium silicate injections on mean tree health improvement in Phytophthora affected trees.

Treatment	Mean tree health improvement (%)
Control	-3.6 a
Potassium silicate injection	+31.1 b

In other experiments, where we drenched young trees affected by Phytophthora with potassium silicate, we also recorded a growth response. Once again the response was so rapid that we did not consider that potassium silicate was affecting Phytophthora activity.

We therefore evaluated potassium silicate on ‘Reed’ avocado seedlings growing in a growth cabinet in Phytophthora infested growing medium. Treatments were applied to plants prior to inoculation. Pots were subjected to cyclic waterlogging to promote Phytophthora infection. Results are given in Table 6.

Table 6: The effect of potassium silicate and phosphorous acid treatments on root tip health and tree health of ‘Reed’ avocado seedlings inoculated with *P. cinnamomi* in a growth cabinet experiment in May/June 2005.

Treatment	Healthy root tips (%)	Seedling health (1-5)
Control	0.33 a	4.17 b
Silicon drench	0.00 a	4.17 b
Phosphonate drench	67.50 b	1.67 a
Phosphonate + Silicon drench	67.50 b	1.83 a
p	<0.001	<0.001
lsd	10.53	0.5273

Seedling health on 1 (healthy) to 5 (dead) scale.

Silicon did not control root rot and, when mixed with phosphonate, was not detrimental to its ability to reduce root rot. We do not yet know if silicon, when combined with phosphonate, is able to give enhanced Phytophthora control as has been reported for Bion. Further research is needed to establish whether silicon will become a component of the integrated strategy to enable avocados to be produced economically in the presence of *P. cinnamomi*.

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