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Field assessment of avocado rootstock selections for resistance to Phytophthora root rot

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Abstract Phytophthora root rot (PRR), caused by *P. cinnamomi*, is a primary constraint on avocado productivity in Australia. Numerous field trials at sites in northern NSW and southern QLD have demonstrated significant variation in tree health amongst commercial rootstocks and recently selected material, grown under high PRR disease pressure. Selections 'SHSR-02', 'SHSR-04', ungrafted 'Hass' (rooted cuttings from clonal propagation) and the commercial rootstock 'Dusa™' were significantly healthier over time than other rootstocks, many of which died during the course of the trials. 'Reed' was consistently highly susceptible. In many cases superior tree health was associated with increased tree height and trunk girth. The trials also clearly demonstrate the negative impact of Phytophthora root rot on establishment of new avocado production blocks, and the importance of identifying and selecting avocado rootstock material that can withstand high *P. cinnamomi* disease pressure.

Keywords *Persea americana* · *Phytophthora cinnamomi* · PRR

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Introduction

Phytophthora root rot (PRR), caused by the soilborne pathogen *Phytophthora cinnamomi* (*Pc*), is the most destructive and important disease of avocado (*Persea americana*) (Pegg et al. 2002) and is a major factor limiting avocado fruit production in Australia (Ploetz et al. 2002). PRR affects feeder roots and disrupts the absorption of water and nutrients and their distribution within the plant; ultimately trees decline and may die. A definitive control for avocado root rot has not been found, but its impact is currently reduced using an integrated approach (Menge and Ploetz 2003), with component strategies including ensuring adequate drainage, promoting active and healthy root growth via optimum nutrition, mulching, the use of potassium phosphonate (Pegg et al. 1985), and the use of PRR-resistant rootstocks.

The search for PRR-resistant avocado rootstocks was initiated by Zentmyer in California in the 1940s and 1950s (Zentmyer et al. 1963), with the selection of the moderately resistant Duke 7 cultivar which became the first Phytophthora resistant rootstock to be commercially accepted. More

recently, several laboratory and glasshouse screening tests with *Persea* sp. have been conducted and compared for their close correlation to performance under field conditions (Gabor and Coffey 1990a, 1991a; Gabor et al. 1990a). These reports concur that the cultivar Topa Topa is highly susceptible to *P. cinnamomi* and 'Thomas', 'Martin Grande' (a hybrid of *P. americana* and *P. schiedeana*) and 'Barr Duke' seedlings are more resistant or tolerant. Differential resistance was also exhibited in callus tissue of three cultivars, concurring with whole-plant responses, with 'Topa Topa' classified as susceptible, 'Duke 7' as moderately resistant and 'Martin Grande' as resistant to *P. cinnamomi* (Phillips et al. 1991).

The selection and development of PRR-resistant rootstocks is continuing in many avocado-producing countries, including Australia, the U.S.A., South Africa, Spain (the Canary Islands) and the Philippines (Lahav and Lavi 2009). Important sources of resistance are so called 'escape trees' which have survived in fields despite high inoculum pressure (Kotze et al. 1987; Zentmyer and Schieber 1987). Australia is ideally suited to the natural selection of such trees as its industry is based on seedling rootstocks that represent the three ecological races of avocado and which have been exposed to over 100 years of PRR selection pressure (Tryon 1905; Whiley 1982). The recovery, cloning and testing of rootstocks from these 'escape' trees (or their seedling progeny) form one of the approaches adopted in our PRR resistance research program.

In Australia, scions are usually grafted onto genetically diverse seedling rootstocks. In contrast, superior rootstocks that have been identified in California and South Africa are now cloned to ensure genetic uniformity. When assessing the performance of rootstocks from 'escape' trees (or their seedling progeny) in the present PRR-resistance program, clones are often tested.

The aim of this study was to evaluate a range of seedling and clonal rootstocks with 'Hass' scions, for field resistance to root rot when young trees were grown in avocado replant sites heavily infested with *Pc.* The rootstocks included some of those currently utilised by the industry in Australia, and selections from Australian and overseas rootstock improvement programs. 'Reed' seedling rootstocks were included as susceptible controls.

Materials & methods

The experiments were carried out in commercial orchards at Duranbah, northern New South Wales, Hampton, near Toowoomba in south east Queensland, and at Childers, near Bundaberg, Queensland. Sites were chosen based on the high water retention characteristics of their soils, and the recent removal of avocado trees declining as a result of severe PRR.

Tree preparation

For seedling rootstocks mature seeds were collected from single maternal trees of each of the selected varieties to reduce the degree of genetic variability within each population. Seed were extracted while the fruit was still mature green and placed in composted pine bark in shallow trays for germination. Upon germination seeds were planted in 5 L nursery bags and grown until approximately 600 mm tall before they were grafted with 'Hass' scions.

Cloned rootstocks were produced using the Ernst micro-cloning technique (Bender and Whiley 2002). "Nurse" seeds were planted in composted pine bark in 90×90 mm pots and grafted 50 mm above the medium surface with scions of the chosen rootstocks. Once scions grew, plants were placed in complete darkness at 27±3°C for 12–15 days, during which time approximately 300 mm of etiolated growth occurred. They were then removed from these conditions and the base of the etiolated shoot treated with 0.8% potassium indole butyric acid. A 50×50×120 mm ribbed tube was slid down over the shoot and filled with composted pine bark and then placed in 30% shade until roots appeared at the bottom of the tube. Once rooted these shoots were cut from the parent plant, placed in a composted pine bark medium in 5 L poly nursery bags and grown until they were large enough to graft with 'Hass' scions. Cloned 'Hass' rootstocks were not grafted, (ie. they were planted as rooted cuttings). The trees on both types of rootstocks were grown to approximately 800 mm before planting in the experimental sites.

Tree treatment

Trees used in the trials were obtained from certified disease-free nurseries and were 9 (on seedling rootstocks) or 15 (on clonal rootstocks) months old at planting. The day before planting, each tree was drenched with 1 L of a 0.1% *v*/*v* potassium phosphonate solution (Agri-Fos 600®, Agrichem, Australia). At planting, 60 g of metalaxyl-M (Ridomil® Gold 25 G, Syngenta) and 60 g of a commercial general compound fertiliser (CK77, CK Life Sciences International (Holdings) Inc. and Rustica Plus, Campbells Fertiliser Australasia) were applied to the soil around each tree. Phytophthora protection measures were imposed during the establishment period so that new trees could begin to grow vigorously to produce a more favourable root: shoot ratio and have the opportunity to express resistance once measures were discontinued. Potassium phosphonate was applied monthly to young trees either as a foliar spray of 0.5% *v*/*v* Agri-Fos 600® adjusted to a pH of 7.2 applied to runoff using a backpack spray unit or as a bark application of 20% *v*/*v* potassium phosphonate in 2% *v*/*v* bark penetrant Pulse® (Nufarm Australia Ltd) applied to the trunk of trees using a paint brush or backpack spray unit to 1 m above

ground level. Trees were regularly fertilised throughout the trial and irrigated as required to promote growth.

Tree health assessments

For trials that had been established for >2 years, the effect of PRR on tree health was assessed regularly using a standard tree health scale used in *Phytophthora* research (Darvas et al. 1984), where 0 = vigorous and healthy, to 10 = dead. For small trees (<2 years old), a modified scale was used where 0 = vigorous and healthy, to 5 = completely defoliated (Gabor et al. 1990b). After the final tree health assessment, measurements were taken of tree height from ground level to the canopy apex and trunk girth 2 cm above the graft union.

Confirmation of *Phytophthora cinnamomi* in soil and roots

Soil samples were collected at each site at completion of the trials to confirm the presence of *P. cinnamomi.* Soil samples were taken randomly from beneath trial trees and were bulked to provide a composite sample. Replicate subsamples were baited for *P. cinnamomi* using germinated New Zealand blue lupins, *Lupinus angustifolius*, (Chee and Newhook 1965). Roots of lupin seedlings were assessed 5 days after baiting for necrosis and collapse, and isolation from a sub-sample of root rot affected lupins was made onto the selective cornmeal agar media P_{10}VP containing 10 ppm Pimaricin, 200 ppm Vancomycin, 100 ppm Terraclor (pentachloronitrobenzene (PCNB)) amended with 50 ppm Hymexazol (Tachigaren). Phytophthora infection was confirmed after characteristic corraloid hyphae were observed upon microscopic examination.

Root samples were also collected from 20% of surviving trees that were randomly selected at the completion of each trial to confirm infection by *P. cinnamomi*. Roots showing symptoms of *Pc* infection were sampled, washed with tap water to remove loose soil and then immersed in 50% *v/v* ethanol for one minute to surface sterilise. Roots were then rinsed with sterile distilled water and dried with sterile blotting paper. Isolations from infected tissue were made onto P_{10}VP media as described above. Petri dishes were incubated in the absence of light at room temperature for 72 h and corraloid hyphae visually identified.

Duranbah Trial 1

The first trial at Duranbah was established on a site that had previously been cleared of mature avocado trees that were declining due to PRR. Two planting rows were prepared on each bed at a distance of 3 m apart. Planting spaces were prepared at 3 m intervals along rows and irrigation installed with a single sprinkler per planting space.

The trial comprised 11 rootstock varieties of both seedling and clonal origin sourced from three nurseries (Table 1). Ten replicate trees of each rootstock were used in the trial except 'SHSR-04' for which only eight plants were available. Trees were planted in May 2006 in a randomized block design, and the PRR management regime described above was maintained until November 2007. Tree health ratings were obtained 11 times between November 2006 and March 2008 and tree height and trunk girth measurements were taken in April 2008.

Statistical analyses of tree health data were conducted separately for each assessment using GenStat 11 data analysis software (GenStat 2008) for a randomised block design analysis of variance. Since there was a substantial proportion of missing values due to the deaths of trees, the height and girth data were analysed with a mixed model using REML in GenStat 11 with rootstock as the fixed effect and replicate + residual as the random effects. Fisher's protected least significant difference (LSD) test (P=0.05) was used for pair-wise comparisons of means.

Duranbah Trial 2

A second trial at Duranbah was planted in the same location in May 2007. Three seedling rootstock lines were compared to a known susceptible 'Reed'. The three rootstocks of interest were SHSR-02, 'A10' × 'Velvick' cross where 'A10' was the maternal tree and 'Velvick' × 'A10' cross where 'Velvick' was the maternal tree. Seed for the 'A10' × 'Velvick' crosses were collected from 'A10' and 'Velvick' trees growing adjacent to each other whose limbs had been entwined to encourage cross pollination. Although the pollen parent of trees that were grown from these seeds was not known, it was assumed to be the neighbouring tree. Forty-seven replicate trees of 'Reed' and 'SHSR-02' were used in the trial along with 23 replicate trees of 'A10' ♀ × 'Velvick' and 24 replicate trees of 'Velvick' ♀ × 'A10'.

Trees were planted in a systematic layout. Management to control PRR ceased 3 months after planting. Trees were rated for health six times between November 2007 and April 2008 using a scale of 0–5 described above. Measurements of tree height and trunk girth were taken in May 2008.

The tree health data were analysed with a mixed model using REML in GenStat. The random effects model was Plot + Tree.Time where Tree.Time is the residual variance. To account for correlation over time within each tree, various covariance structures for the residual variance were tested with the best being an unstructured covariance model. The fixed effects model was Rootstock + Time + Rootstock × Time interaction. This enabled an assessment of whether patterns of mean ratings over time differed for the different rootstocks. Height and girth data were analysed with a mixed model using REML in GenStat 11, where the random effects

Table 1 Summary of rootstocks used in the field trials

Rootstock	Propagation type	Ecological race	Background	Source[a]	Duranbah Trial 1	Duranbah Trial 2	Duranbah Trial 3	Hampton	Childers
A8	Seedling	Guatemalan	Seedling selection, Duranbah, New South Wales, 1950s	2					✓
A10	Seedling	Guatemalan × Mexican	Seedling selection, Duranbah, New South Wales, 1950s	2	✓			✓	✓
Barr Duke	Clonal	Mexican	Duke 6 seedling, California	3	✓			✓	
Dusa™ (Merensky 2)	Clonal	Mexican × Guatemalan	escape tree, South Africa	1	✓			✓	✓
Latas™ (Merensky 1)	Clonal	Mexican	Fuerte escape tree, South Africa,	1	✓				✓
Duke 7	Clonal	Mexican	Duke seedling, California, 1950's	3	✓				
Hass	Clonal	Guat. with some Mexican	Unkown parentage, La Glabra Heights, California 1935	3	✓				
Reed	Seedling	Guatemalan	Anaheim × Nabal, Carlsbad, California, 1948	2	✓	✓	✓		✓
Rigato	Seedling	Guatemalan × Mexican	Seedling selection Walkamin, Queensland				✓		
SHSR-02	Seedling	Guatemalan	From Mt Tamborine escape tree	3		✓	✓	✓	
SHSR-04	seedling	Mexican	From Bundaberg escape tree	3					
SHSR-04	Clonal	Mexican		3	✓				
SHSR-05	Seedling	Guat. with some WI	Seedling selection Bundaberg, Queensland				✓		
Thomas	Clonal	Mexican	Fuerte escape tree, Escondido, California, 1979	3	✓				
Toro Canyon	Clonal	Mexican	Cloned from survivor tree, California	3				✓	
Velvick (Whiley)	Clonal	Guat. with some WI	Seedling selection, Queensland	3	✓				✓
Velvick (Anderson)	Seedling	Guat. with some WI	Seedling selection, Queensland	2	✓				✓
Velvick (Lynwood)	Seedling	Guat. with some WI	Seedling selection, Queensland	3				✓	✓
V1	Seedling	Guat. with some WI	Seedling selection Childers, Queensland	3				✓	
A10♀xVelvick	Seedling		cross pollinated	2		✓			
Velvick♀xA10	Seedling		cross pollinated	2		✓			

[a] 1 = Birdwood Nursery, Nambour, QLD; 2 = Anderson's Nursery, Duranbah, NSW; 3 = Sunshine Horticultural Services P/L, Nambour, QLD

model was Plot + Residual and fixed effects model was Rootstock (GenStat 2008). Fisher's protected LSD test (P=0.05) was used for pair-wise comparisons of means.

Duranbah Trial 3

A third trial at Duranbah was planted in the same location in July 2007 to compare the PRR responses of three seedling rootstocks, 'Rigato', 'SHSR-02' and 'SHSR-05', to a known susceptible, 'Reed'. Eighteen replicate trees of 'Rigato', eight reps of 'SHSR-02', three replicates of 'SHSR-05' and 17 replicates of 'Reed' were planted in the trial in a systematic layout. Management of Phytophthora control ceased 1 month after planting. Tree health was rated six times during the trial using a scale of 0–5 described above and measurements of tree height and trunk girth were taken in May 2008.

The data were analysed with a mixed model using REML in GenStat 11 (GenStat 2008). To account for the non-randomised design, the random effects model included terms for both row and position. Fisher's protected LSD test (P=0.05) was used for pair-wise comparisons of means.

Hampton trial

A trial was established in December 2005 on a commercial farm in Hampton, south east Queensland. The block used had previously been cleared of old avocado trees and recent plantings had begun to show signs of decline from PRR at the time of planting the current trial. Trees were interplanted with existing trees in a randomised block design across three rows, 8 m apart with 2.5 m between new and existing trees.

Seven rootstocks were replicated 10 times in the trial (Table 1). Based on visual examinations of existing trees, replicates 1–3 were in a "low" disease area, and replicates 4–10 were in a "high" disease pressure area. PRR management ceased after April 2007. Tree health was assessed seven times between December 2006 and June 2009, and tree heights and trunk girths were measured in September 2009.

Tree health ratings were analysed as a randomised complete block design using a repeated measures analysis in GenStat 11 (GenStat 2008). Comparisons between means were made using Fisher's protected LSD test. The variance ratios and LSDs for the time and interaction terms were adjusted for the degree of auto-correlation between times by the Greenhouse-Geisser epsilon test (Greenhouse and Geisser 1959). Health ratings and height and girth measurements of trees in the high disease area (Replicates 4–10) were analysed by ANOVA, and comparisons between means were made using Fisher's protected LSD test.

Childers trial

This trial was established in May 2006 in a commercial orchard in a block that had been fallow for less than a year, after old, declining trees had been removed. Trial trees were planted along a single row within the orchard at a spacing of 5 m with 5 m between rows. Irrigation was installed with a single sprinkler per planting space.

There were 10 replicates of each of 8 rootstocks of seedling and clonal origin (Table 1), except for 'Velvick' clonal (Whiley) where there were only 9 replicates. PRR management ceased after April 2007. Tree health was assessed in February 2007, December 2007 and March 2009 and trunk girth was measured in March 2009.

Data for each assessment time were analysed with a mixed model using REML in GenStat 11 (GenStat 2008) with rootstock as the fixed effect and replicate as the random effect. Comparisons between means were made using Fisher's protected LSD test.

Results

Duranbah Trial 1

Six months after the establishment of the first trial, differences in tree health were already evident, with 'Reed' being significantly less healthy (highest rating) than 'Velvick' clonal, 'Duke 7', 'SHSR-04' and 'Hass' rootstocks (Table 2). After 11 months, 'Reed' was significantly less healthy than 'Dusa™', 'Velvick' clonal, 'Thomas', 'SHSR-04' and 'Hass'. With time, the rootstocks with intermediate tree health ratings became progressively less healthy, and by 19 and 22 months after planting, when PRR management had been withdrawn, the healthiest trees were on 'SHSR-04' rootstock, which were significantly (P<0.05) healthier than those on all other rootstocks with the exception of 'Hass' (Table 2).

After 22 months less than 70% of trees in the trial had survived with rootstocks 'Latas™', clonal 'Velvick', 'Barr Duke' and 'Thomas' having 50% or more trees die (results not shown). The interaction of tree health and rootstock did not have a significant effect on tree height (results not shown, P=0.076) but was correlated with trunk girth (P= 0.002). Girths of trees on 'SHSR-04' and 'Thomas' were significantly greater than those on 'Velvick' seedling, 'Duke 7', 'A10' and 'Reed' (Table 3).

Duranbah Trial 2

For tree health, there was a significant interaction (P=0.004) between Rootstock and Time. At all assessment times, the healthiest trees were on 'SHSR-02' rootstock (data for the first and final assessment times are shown in Table 4). The superior

Table 2 Average health of trees grafted to different rootstocks in Duranbah Trial 1. Assessed using a rating scale of 0–10, where 0 = healthy and 10 = dead, at 6, 11, 19 and 22 months after planting. Mean values within columns followed by the same letter are not significantly different at $P=0.05$

Rootstock	Time of assessment after planting			
	6 months	11 months	19 months	22 months
Latas™	4.9 abcde	5.0 ab	7.9 a	8.5 a
Dusa™	5.1 abcd	3.5 bcd	5.5 bc	5.4 bc
Velvick clonal	4.4 bcde	4.0 bcd	7.9 a	7.7 a
Velvick seedling	4.8 abcde	4.3 abcd	6.6 ab	7.0 ab
Duke 7	3.6 de	4.4 abcd	7.8 ab	8.2 a
Barr Duke	5.6 abc	4.9 abc	7.0 ab	8.0 a
Thomas	4.8 abcde	3.7 bcd	7.0 ab	7.7 a
A10	5.3 abc	4.9 ab	8.1 a	7.9 a
Reed	6.2 a	6.8 a	8.5 a	8.3 a
SHSR-04	4.3 cde	2.2 d	1.8 d	2.7 d
Hass	3.3 e	2.1 cd	3.8 cd	4.8 cd

tree health on ‘SHSR-02’ was also demonstrated in increased height and girth measurements compared to the other rootstocks, with the exception that trees on the ‘Velvick’♀ × ‘A10’ rootstock had similar trunk girths (Table 4).

Duranbah Trial 3

Significant rootstock effects developed only 9 months after planting ($P=0.023$). ‘Reed’, was the least healthy rootstock with a rating of 2.9, and was significantly different from ‘SHSR-02’ and ‘SHSR-05’, with ratings of 1.5 and 1.4, respectively. ‘Rigato’ had a rating of 2.4, and was not significantly different from any of the other rootstocks. Trees on the ‘SHSR-05’ rootstock were significantly taller than those on all other rootstocks, while trees on ‘SHSR-05’ and ‘SHSR-02’ rootstocks had greater girth measurements than those from ‘Rigato’ and ‘Reed’ (data not shown).

Hampton trial

Since markedly different results were obtained in the high and low disease areas, results from the two areas were not combined (Table 5). Mean health ratings for the different areas were averaged over time (12–48 months after planting). In the low disease area (replicates 1–3 of each rootstock), the interaction between tree health ratings for rootstocks and time was not significant ($P=0.352$) nor was the effect of rootstock ($P=0.075$), however time was significant ($P<0.001$). Therefore no PRR effect was apparent on tree health.

In the high disease area (replicates 4–10 of each rootstock), the interaction between tree health ratings for rootstocks and time was not significant ($P=0.253$), but time ($P<0.001$) and rootstock were significant ($P=0.002$). Table 5 presents mean health ratings for rootstocks for the high and low disease areas averaged over time. ‘Dusa™’ trees were the healthiest overall, and were significantly healthier than ‘A10’, ‘Velvick’, ‘V1’ and ‘Toro Canyon’ (Table 5).

Table 6 presents mean health ratings for rootstocks for the high disease pressure area at three assessment times. At 15 months after planting, trees on ‘Dusa™’ and ‘Barr Duke’ rootstock were significantly healthier than those on all other rootstocks except ‘SHSR-02’, indicating that these three rootstocks are able to establish well after planting in areas heavily infested with *P. cinnamomi*. At 26 months after planting, the order of healthiest to least healthy rootstocks was ‘Dusa™’, ‘SHSR-02’, ‘Barr Duke’, ‘Toro Canyon’, ‘A10’, ‘Velvick’ and ‘V1’ (Table 6). At the final disease assessment 42 months after planting ‘Dusa™’, ‘Barr Duke’ and ‘SHSR-02’ were still the healthiest trees though not significantly healthier than the other rootstocks except for ‘V1’. ‘Velvick’ and ‘Dusa™’ trees were the tallest at over 2 m, and these rootstocks also had the greatest trunk girths (Table 6).

Table 3 Average trunk girth of trees grafted to different rootstocks in Duranbah Trial 1. Trunk girth measurements were taken immediately above the graft union 2 years after planting. Mean values followed by the same letter are not significantly different at $P=0.05$

Rootstock	Girth (mm)
Latas™	127.6 bcd
Dusa™	112.3 bcd
Velvick clonal	124.9 bcd
Velvick seedling	99.3 d
Duke 7	107.5 cd
Barr Duke	131.9 abcd
Thomas	179.4 ab
A10	83.7 d
Reed	78.4 d
SHSR-04	179.1 a
Hass	143.3 abc

Childers trial

Nearly 3 years after the Childers trial was planted, trees were thriving, in contrast to those in the Duranbah and Hampton

Table 4 Average health, height and girth of trees grafted to different rootstocks in Duranbah Trial 2. Average tree health ratings were assessed using a rating scale of 0–5, where 0 = healthy and 5 = completely defoliated, at 6 and 11 months after planting. Measurements of tree height and trunk girth were taken 12 months after planting. Mean values within columns followed by the same letter are not significantly different at P=0.05

Rootstock	Tree health		Height (cm)	Girth (mm)
	6 months	11 months		
Reed	2.52 a	2.96 a	87.8 b	76.4 b
SHSR-02	1.53 b	1.68 b	114.2 a	102.2 a
A10♀ × Velvick	2.75 a	2.83 a	90.8 b	82.0 b
Velvick♀ × A10	2.25 a	2.46 a	95.4 b	96.9 a

trials, and this is reflected in the lower tree health ratings and the larger trunk girths. (Tables 2, 3, 4, 5, 6, and 7). The least healthy trees at final assessment were from 'Reed' rootstock, which was significantly less healthy than all other rootstocks. Trunk girths were similar among most rootstocks, however 'Reed' had significantly smaller trunk girths than all other rootstocks except 'A10' (Table 7).

Confirmation of *Phytophthora cinnamomi* in soil and roots

At the completion of each trial, soil and root samples were collected from beneath trial trees to confirm the presence of *P. cinnamomi* in the soil and infection of roots.

Baiting of a composite soil sample collected from across the three trials at the Duranbah site resulted in 100% of lupin seedlings becoming necrotic and collapsed and *P. cinnamomi* was isolated on selective media from all of the sub-samples of infected seedlings. *P. cinnamomi* infection was confirmed in 94% of trees sampled for root isolations.

Baiting of soil from the Hampton site resulted in 98% seedling collapse, where 85% of those were confirmed as being infected with *P. cinnamomi*. *P. cinnamomi* infection was confirmed in 81% of trees sampled for root isolations.

Table 5 Average tree health of trees grafted to different rootstocks in 'low' and 'high' PRR pressure areas at Hampton. Average tree health was assessed using a rating scale of 0–10, where 0 = healthy and 10 = dead. Tree health ratings were averaged over time. Mean values within columns followed by the same letter are not significantly different at P=0.05

Rootstock	Low disease area	High disease area
A10	5.86	5.41 ab
V1	2.03	7.45 a
Velvick	1.00	4.98 ab
Toro Canyon	1.33	4.31 b
SHSR-02	1.62	3.53 bc
Dusa™	1.57	1.67 c
Barr Duke	2.33	2.84 bc

Baiting of soil sampled from the Childers site resulted in 74% seedling necrosis and collapse, where 95% of those were confirmed as being infected with *P. cinnamomi*. *P. cinnamomi* infection was confirmed in 90% of trees sampled for root isolations.

Discussion

The trials demonstrated significant variation in tree health amongst rootstocks grown under high PRR disease pressure. Root examination, isolation of roots on selective media and lupin baiting for Phytophthora confirmed an extremely high population of *P. cinnamomi* at all trial sites. These trials also demonstrated the high susceptibility of 'Reed' seedlings. However, they are a preferred rootstock in some regions, particularly Western Australia, as they are vigorous, highly productive and respond well to phosphonate sprays and injections.

The rootstocks which consistently remained the healthiest under high PRR disease pressure were 'SHSR-02', 'SHSR-04', 'Dusa™', and ungrafted 'Hass'. 'SHSR-02' is seedling material obtained from an 86 year old 'escape' tree planted at Mt Tamborine, Queensland, in 1923 (Hauser 2008). It is of Guatemalan origin and adjacent to 'Hass' trees, so out-crossing is possible. SHSR-04 was recovered and cloned from another 'escape' tree in the Bundaberg region of Queensland, and is of Guatemalan and Mexican origin. 'Dusa™' was developed from an 'escape' tree at Westfalia estates (South Africa) in the late 1970s, and is most likely a Mexican × Guatemalan hybrid (D. Roe, pers. comm.). In the current trials, 'Dusa™' was superior to 'Duke 7' at Duranbah, which is consistent with reports from severe PRR areas in South Africa and California (Newett et al. 2002). 'Dusa™' is available commercially in many avocado-producing countries (including in Australia) and is considered a standard against which other resistant rootstocks should be compared.

'Duke 7' and 'Barr Duke' are reported to be moderately resistant (Gabor and Coffey 1990b, 1991b; Gabor et al.

Table 6 Average health, height and girth of trees grafted to different rootstocks at Hampton, grown under high PRR disease pressure. Average tree health ratings were assessed using a rating scale of 0–10, where 0 = healthy and 10 = dead, at 15, 26 and 42 months after planting. Girths and heights were measured 46 months after planting. Mean values within columns followed by the same letter are not significantly different at P=0.05

Rootstock	Tree health			Height (cm)	Girth (mm)
	15 months	26 months	42 months		
A10	5.14 a	5.00 ab	5.86 b	131.5 d	162.1 d
V1	5.40 a	7.80 a	9.40 a	199.5 abc	224.5 cd
Velvick	4.57 a	5.43 ab	5.43 bc	245.5 a	341.7 a
Toro Canyon	3.57 a	4.57 abc	4.43 bc	176.1 cd	254.8 bc
SHSR-02	2.50 ab	3.50 bc	4.02 bc	164.0 cd	308.1 abc
Dusa™	0.71 b	1.71 c	1.14 c	230.7 ab	323.1 ab
Barr Duke	1.29 b	3.57 bc	3.14 bc	189.8 bc	280.4 abc

1990b; Phillips et al. 1991) but succumbed to PRR at the Duranbah site. At the Hampton site, 'Barr Duke' was one of the three most resistant cultivars. This discrepancy suggests that environmental or other biotic factors affected the establishment of 'Barr Duke' at these sites. Better understanding of G × E (genotype × environment) interactions are needed for these and other avocado rootstocks.

The performance of clonal 'Hass' trees was unexpected. These trees remained healthy in the single trial in which they were assessed. We suspect that the absence of a graft union and thus potential graft union incompatibility in these plants may result in a more vigorous root system that is able to overcome damage caused by PRR. Although 'Hass' was originally selected as a scion and may not possess the characteristics which confer specific rootstock advantages (eg. salinity tolerance, adaptation to calcareous soils, smaller growth habit, high sustainable yields), its performance at Duranbah is noteworthy. Further testing of the growth and yield performance of clonal 'Hass' growing on its own root systems is underway in separate trials conducted in major avocado production regions around Australia.

Table 7 Average health, height and girth of trees grafted to different rootstocks in Childers trial. Average tree health ratings were assessed using a rating scale of 0–10, where 0 = healthy and 10 = dead, at 18 and 34 months after planting. Measurements of trunk girth were taken 34 months after planting. Mean values within columns followed by the same letter are not significantly different at P=0.05

Rootstock	Tree health		Girth (cm)
	18 months	34 months	
A8 seedling	0.0	0.5 a	42.9
A10 seedling	0.8	0.5 a	38.69
Latas™ clonal	0.5	0.0 a	41.98
Dusa™ clonal	0.0	0.0 a	43.02
Reed seedling	1.3	2.2 b	33.6
Velvick seedling (Anderson seed)	0.7	0.2 a	42.46
Velvick seedling (Lynwood seed)	0.0	0.1 a	47.49
Velvick clonal (Whiley)	0.3	0.3 a	43.1
P	0.172	0.003	0.001
LSD (5%)	ns	1.08[a]	5.71[b]

[a] Except for comparisons withVelvick clonal (Whiley) where LSD = 1.11

[b] Except for comparisons with Velvick clonal (Whiley) where LSD = 5.87

New rootstock selections will have to undergo rigorous testing in the field under high PRR disease pressure. For example, the trial at Hampton demonstrated that differences in tree health were not significant when trees were planted in areas of low disease pressure. Due to the widespread nature of PRR, establishment of new blocks in avocado replant land will almost certainly have to include more resistant rootstocks together with PRR management practices to ensure successful return of land to production. These trials have identified individual trees with superior PRR resistance, and these will be cloned and included in continuing rootstock assessments. New rootstocks should also be evaluated for traits other than PRR resistance, such as fruit quality and susceptibility to postharvest disease (Marques et al. 2003; Willingham et al. 2001), yield (Arpaia et al. 1992) and tree vigour and fruit physiological disorders (Smith 1993). Ungrafted 'Hass', 'Reed' and 'SHSR-04' are among a range of rootstocks included in trials established in 2005 and replicated across four growing regions in Australia for assessment of these growth and quality parameters.

There is limited information on the anatomical and physiological traits that are associated with PRR tolerance. Root regeneration capacity and reduced development of necrosis in individual roots are involved in the tolerance of different rootstocks (Gabor and Coffey 1990a; Kellam and Coffey 1985). Similarly, there are only a few studies showing that scion variety or rootstock can influence the plants' physiology and/or biochemistry, which may then

have an effect on the resistance to *Pc*. One study investigated the interaction of *Pc* and avocado utilising undifferentiated callus masses (Phillips et al. 1991). The rate of fungal infection was lower and cells underwent a hypersensitive-like response (ie. rapid cell necrosis in the inoculated areas) when callus tissue of the more resistant 'Duke 7' and 'Martin Grande' was inoculated with *Pc*, than when cells of 'Topa Topa' were inoculated. There is also some indication that presence and activity of peroxidase isoenzymes may be influenced by rootstock-scion combinations (Bower and Nel 1982) and that peroxidases may be associated with resistance of avocado to *Pc* (van Lelyveld and Brodrick 1975). A limitation with most of the physiological and biochemical studies to date is that the majority of avocado varieties or rootstocks tested are from the Mexican ecological race of *Persea americana*, with only one representative from the Guatemalan race, and none from the West Indian race. Much more work is needed on these processes and on diverse genotypes of this crop.

Further studies in our laboratory will examine the biochemical and physiological processes that are associated with field resistance in diverse avocado germplasm. The questions of how root regeneration and graft compatibililty relate to PRR resistance should also be addressed.

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