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# PRELIMINARY RESULTS FROM AVOCADO ROOTSTOCK RESEARCH IN AUSTRALIA

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Australian avocado orchards are currently planted on seedling rootstocks, which are genetically diverse encompassing genotypes from the three botanical races of *Persea americana*. This diversity increases the difficulty of getting a uniform outcome from standard management practices. For example, over a 6-year period a 400% difference in yield was measured between 'Hass' trees in the same orchard under identical management. Additionally, large differences have been recorded between trees in the susceptibility of fruit developing post harvest rots which negatively impacts on consumers. These differences have been attributed to different rootstocks exerting changes on scion physiology/chemistry. *Phytophthora cinnamomi* was present in eastern Australia before avocados were introduced in the late 19th century. Thus, avocado seedling rootstocks have been subjected to selection pressure by *P. cinnamomi* for a long time. In current research rootstocks have been recovered from old grafted trees still growing well in areas where most surrounding trees have died from root rot. These cloned rootstocks grafted to Hass are being compared with resistant rootstocks developed overseas.

Results discussed in this paper include the implications of botanical race on cloned rootstock propagation and *Colletotrichum gloeosporioides* tolerance together with preliminary yield results from genotypic x environment experiments and rootstock responses to *Phytophthora cinnamomi*.

**Key words** *Colletotrichum gloeosporioides*, rootstocks, *Phytophthora cinnamomi*, yield efficiency

# RESULTADOS PRELIMINARES DE LA INVESTIGACION SOBRE PORTAINJERTOS DE PALTOS EN AUSTRALIA

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Los huertos de palto australianos actualmente se plantan sobre portainjertos de semilla, los cuales son genéticamente diversos, en los que se incluyen genotipos de las tres razas botánicas de Persea americana. Esta diversidad incrementa la dificultad de obtener un resultado uniforme de las prácticas de cultivo estándar. Por ejemplo, durante un período de 6 años, se determinó un 400% de diferencia en producción entre paltos 'Hass' en el mismo huerto, bajo cultivos idénticos. Además, se han registrado grandes diferencias entre árboles respecto a la susceptibilidad de la fruta que se ve afectada por pudriciones en post cosecha, con un impacto negativo en el consumidor. Estas diferencias han sido atribuidas a diferentes portainjertos que ejercen cambios fisiológicos / químicos sobre la variedad. Phytophthora cinnamomi ha estado presente en el este de Australia desde antes que los paltos fueran introducidos a fines del siglo 19. De esta manera, los portainjertos de semilla de paltos han estado sujetos a presiones de selección por P. cinnamomi durante un largo tiempo. En estudios actuales, se han recuperado portainjertos desde árboles viejos injertados, aún creciendo bien en áreas donde la mayoría de los árboles de los alrededores han muerto por pudrición de raíces. Estos portainjertos clonados injertados a 'Hass' están siendo comparados con portainjertos resistentes desarrollados en otros países.

Los resultados discutidos en este texto incluyen las implicancias de la raza botánica en la propagación de portainjertos clonales y tolerancia a Colletotrichum gloeosporioides junto con resultados preliminares productivos de experimentos por genotipo x ambiente y repuesta de portainjertos a Phytophthora cinnamomi.

## 1. Introduction

In California, Webber (1926) observed "no factor of the avocado industry is more important than rootstocks, and there is no problem that we know less about, or which requires a longer time to solve." Since then a considerable body of knowledge has been accumulated on the effect of rootstocks on salinity and alkalinity tolerance, mineral nutrient uptake and *Phytophthora* root rot tolerance (Gabor *et al.*, 1990; Kremer-Köhne and Duvenhage, 2000; Lahav and Whiley, 2001; Whiley *et al.*, 1996). However, despite the documented differences in environmental and edaphic responses between the botanical races of *P. americana*, with the exception of the Israeli rootstock program there has been little progress made on the selection and development of avocado rootstocks to improve productivity and fruit quality. Considerable effort has been expended on the search for *Phytophthora*-resistant rootstocks. This area of investigation has been largely unsuccessful based on the investment/outcomes ratio as after 40 years of research we appear no closer to having rootstocks with commercial resistance to *Phytophthora*, i.e. rootstocks that will stand up to root rot without the application of fungicides. There are good reasons for this lack of progress when the genesis of crop and disease are considered, viz. the evolutionary centres of the tree and the pathogen are in completely different geographic regions. As Phytophthora root rot in avocados is recognised as a "new encounter" disease it is highly unlikely that any rootstock will be immune or highly resistant to the pathogen. Thus identification and use of rootstock lines that either show some tolerance to *Phytophthora* root rot or respond favourably to phosphonate applications should be encouraged provided they also impart productivity.

There is some evidence to suggest that physiological incompatibility at the inter-racial level may be affecting crop performance, particularly with respect to fruit quality. For instance, it is known that trees of the same variety grafted to Mexican or Guatemalan race rootstocks will have different mineral nutrient profiles (Haas, 1950; Whiley *et al*., 1996; Bard, 1997; Lahav and Whiley, 2001). Similarly, different race rootstocks change the carbohydrate accumulation profile in trees of the same variety, which is known to drive productivity (Whiley, 1994). Furthermore, the research of Willingham *et al*. (2001) into postharvest anthracnose control of Hass (predominantly Guatemalan) has shown that less disease developed in fruit from trees grafted to Velvick (West Indian) compared to fruit from trees grafted to Duke 6 (Mexican). In each of these studies there has been a relatively narrow genetic base of material evaluated and this requires expansion before conclusive results can be obtained. Knowledge from such studies is important in the context of avocado production in Australia, which straddles a diverse range of soil types and environments. These range from the deep, red clay loams of the summer rainfall, subtropics through to the sands of the semi-arid, winter rainfall regions of Western Australia. With such diverse climate and soil conditions it is unlikely that one rootstock line will perform well in all situations. For example, Velvick is a vigorous rootstock when used in subtropical Australia but when grown in California there are difficulties with establishment and growth of trees is slow (J. Menge, Riverside, 1996, personal communication). In the latter environment, Mexican race types are favoured as rootstocks. Additionally, if inter-racial incompatibility proves to be a problem then it is likely that scion lines will require different rootstocks, e.g. Hass is predominantly Guatemalan while Shepard is predominantly Mexican race.

The efficiency of commercial fruit growing is generally increased by selecting the best performing varieties for an area and reducing or eliminating the genetic variability between production units. For a chosen avocado variety this is relatively simple as scions are grafted onto rootstocks however, in Australia the latter are mostly of seedling origin with wide genetic diversity. The rootstock cloning technique of Frolich and Platt (1972) and the various modifications that have since developed (Bender and Whiley, 2001) have provided the technology to produce genetic uniformity in avocado orchards. This has mostly been exploited to retain "*Phytophthora* root rot tolerance" with trees grafted to cloned Duke 7 and more recently other elite rootstocks that have been identified with some tolerance to this disease. Such trees have been widely planted in California and South Africa. A copy tree program has also been carried out in Israel where the rootstock and scion of high performance trees have been cloned and replanted in orchards. It is claimed that this program has been responsible for marked increases in avocado production in this country (Ben-Ya'acov and

Michelson, 1995). Such a program has also been proposed for Australia by Thomas (1997) based on the identification of superior performing trees through perusal of orchard records.

There is no published data available from any country comparing the production from trees grown on cloned rootstocks to those on seedling rootstocks from the same maternal source. Due to the high cost of cloning trees under Australia conditions, reliable comparative data on the performance of cloned and seedling rootstocks is required to validate which material is best used by industry.

This paper reports on the first 5 years activities of a proposed 10-year rootstock improvement program covering preliminary aspects rootstock yield efficiency, anthracnose susceptibility and Phytophthora tolerance.

## 2. Materials and Methods

### 2.1 Study of rootstock yield efficiency when grafted to Hass

Hass trees with both seedling and cloned rootstocks of Mexican, Guatemalan and West Indian race were planted in different climatic zones of Australia where avocados are commercially produced. At each site best commercial practice for establishing trees was followed. The experimental sites were planted between January and April 2005. Trees flowered and set fruit in spring 2006 and were measured (canopy height and diameter) and harvested when fruit were mature in 2007. The cubic volume of trees was calculated and the yield efficiency ($kg.m^{-2}$) estimated from the data.

### 2.2 Study of anthracnose tolerance of rootstocks

Rooted cuttings of 18 potential rootstock lines were planted in a composted pine-bark media in 90 mm square pots and placed in a controlled temperature chamber at 20/30°C (day/night) with 90-100% RH. There were 6-11 plants of each of the rootstock lines which were: Barr Duke, Duke 7, Parida, SHSR-01, Thomas, Toro Canyon, Zutano, A10, Edranol, Hass, SHSR-02, A8, SHSR-03, Nabal, Reed, Plowman, SHSR-04, Velvick. At the beginning of the experiment all of the plants had a minimum of six mature leaves. After growing plants for four weeks in the experimental chamber the health of plants was rated using a 0-5 scale where 0 = nil leaf lesions and 5 = lesions covering greater than 90% of the leaf surface and defoliation. During the course of the experiment leaf samples with active lesions were submitted to DPIF Plant Protection Group, Indooroopilly for identification of the pathogen invading leaf tissues. Lesion rating data was analysed by ANOVA.

2.3. Study of Phytophthora root rot tolerance of rootstocks

To select for greater tolerance to root rot, field experiments have been established in replant sites heavily infested with *P. cinnamomi.* Ten trees of each rootstock grafted to Hass were planted in each experiment. As rootstocks are extremely sensitive to *Phytophthora cinnamomi* for 12-18 months after planting, remedial treatments were applied so that tolerance could be expressed. As broad spectrum fumigants which drastically reduce the population of the pathogen can no longer be used as preplant treatments, fungicides (metalaxyl, potassium phosphonate) were used to protect transplants. Metalaxyl was applied to the soil surface at planting and again three months later. Potassium phosphonate was used to drench nursery trees and after planting was applied to the foliage and stems at regular intervals. The foliage of individual trees was visually rated at monthly intervals on a scale of 0 -10 (Darvas *et al.,* 1984) where 0 = healthy and 10 = totally defoliated.

3. Results and Discussion

3.1 Study of rootstock yield efficiency when grafted to Hass

Yield efficiencies of Hass on the respective rootstocks grown at Goodwood, central Queensland are presented in Table 1. Of the seedling rootstocks SHSR-02 (G x M), Peasley (G) and Toro Canyon (M) had the highest yield efficiencies while for cloned rootstocks Duke 7 (M), SHSR-02 (G x M), Zutano (M x G), Thomas (M) and Velvick (WI) had the highest yield efficiency of this group.

**Table 1** Yield efficiency (kg.m$^{-3}$) of 10 rootstocks propagated as either seedlings or clones and grafted to Hass and grown at Goodwood, central Queensland. Data are from the first year crop and values in columns are means of 10 trees.

| Seedling rootstock | Yield efficiency | Cloned rootstock | Yield efficiency |
|---|---|---|---|
| A8 | 0.45 | A8 | 0.08 |
| A10 | 0.51 | A10 | 0.29 |
| SHSR-01 | 0.64 | Duke 7 | 0.51 |
| Nabal | 0.49 | Nabal | 0.23 |
| Peasley | 0.81 | SHSR-02 | 0.44 |
| Reed | 0.36 | Thomas | 0.34 |
| SHSR-02 | 1.00 | Velvick | 0.33 |
| Toro Canyon | 0.79 | SHSR-04 | 0.27 |
| Velvick | 0.63 | Zutano | 0.35 |
| Velvick/interstock | 0.24 | | |
| SHSR-04 | 0.44 | | |

Yield efficiencies of Hass on the respective rootstocks grown at Hampton, southern Queensland are presented in Table 2. Of the seedling rootstocks

SHSR-02 (G x M), A10 (G x M) SHSR-04 (WI x G) had the highest yield efficiencies while for the cloned rootstocks Duke 7 (M), A8 (G) A10 (G x M) and Zutano (M x G) had the highest yield efficiency of this group. It is stressed that the results from both experiments are from the first year of cropping and should be treated with caution until data from future years cropping is available.

**Table 2** Yield efficiency (kg.m$^{-3}$) of 10 rootstocks propagated as either seedlings or clones and grafted to Hass and grown at Hampton, southern Queensland. Data are from the first year crop and values in columns are means of 10 trees.

| Seedling rootstock | Yield efficiency | Cloned rootstock | Yield efficiency |
|---|---|---|---|
| A8 | 0.17 | A8 | 0.52 |
| A10 | 0.76 | A10 | 0.52 |
| Duke 7 | 0.06 | Duke 7 | 0.60 |
| Kidd | 0.31 | Hass | 0.26 |
| Nabal | 0.31 | Nabal | 0.30 |
| Plowman | 0.09 | Plowman | 0.88 |
| Reed | 0.18 | Reed | 0.18 |
| SHSR-02 | 0.90 | SHSR-02 | 0.48 |
| Velvick | 0.36 | Velvick | 0.42 |
| Velvick/interstock | 0.31 | Zutano | 0.50 |
| SHSR-04 | 0.53 | | |

3.2. Study on anthracnose tolerance of rootstocks

Within two weeks after placing the plants in the experimental chamber leaf lesions appeared on mature leaves of some of the rootstock lines. After a month at high temperature and RH conditions complete defoliation of plants in some lines occurred which, was due to the severity of lesion development. Pathology isolations from leaf tissues confirmed that the causal organism was *Colletotrichum gloeosporioides*, commonly known as anthracnose. Lesion severity ratings made four weeks after placing plants in the experimental chamber are presented in Table 3.

It is evident from the data (Table 3) that a pattern of susceptibility based on racial origin of rootstock lines is present. For example, those lines of Mexican race origin were the most susceptible to anthracnose with plants being completely defoliated by the end of the treatment period; those of Guatemalan race origin had a higher level of resistance with only the odd lesion developing on leaves while those of West Indian race origin also had high resistance to disease.

It is likely that there is an eco-evolutionary reason for the divergence in botanical variety response to anthracnose based on the disease pressure present during the evolution of the species. For example, the Mexican race population developed under relatively cool temperatures and low rainfall (ca. 16.0°C/786 mm) compared with the Guatemalan race (ca. 19.6°C/1394 mm) while the West Indian race was exposed to conditions of highest disease pressure (ca. 28.0°C/1137 mm) (Wolstenholme, 2002). Based on the data of Prusky *et al.* (1988) it is likely that disease resistance in leaves is related to the diene concentration in trees with the Mexican race having the lowest and the West Indian race the highest concentrations. However, this requires confirmation through the analysis of leaves from the experimental population of rootstocks.

**Table 3** Variance in anthracnose (*Colletotrichum gloeosporioides*) susceptibility in a population of avocado rootstocks. Susceptibility is defined by leaf lesion ratings on a scale of 0-5 where 0 = 0 lesions and 5 = lesions covering 90% of the leaf surface with defoliation. All values in either of the "leaf rating" columns with different superscript letters are significantly different ($P \leq 0.05$).

| Rootstock | Race* | Leaf rating** | Rootstock | Race | Leaf rating |
|---|---|---|---|---|---|
| Barr Duke | M | $5^{b}$ | Hass | G x M | $2^{a}$ |
| Duke 7 | M | $5^{b}$ | SHS 2 | G x M | $2^{a}$ |
| Parida | M | $5^{b}$ | A8 | G | $1^{a}$ |
| SHSR-01 | M | $5^{b}$ | SHS 3 | G | $0^{a}$ |
| Thomas | M | $5^{b}$ | Nabal | G | $0^{a}$ |
| Toro Canyon | M | $5^{b}$ | Reed | G | $1^{a}$ |
| Zutano | M x G | $4^{b}$ | Plowman | G x WI? | $0^{a}$ |
| A10 | G x M | $2^{a}$ | SHS 4 | WI x M? | $1^{a}$ |
| Edranol | G x M | $3^{ab}$ | Velvick | WI | $0^{a}$ |

* M = Mexican; G = Guatemalan; WI = West Indian

3.3. Study of Phytophthora root rot tolerance of rootstocks

At the Walkamin site (Table 4) there was minimal commercial damage to trees that had mean health ratings less than 1. In the population of Hass trees on cloned rootstocks the healthiest trees were either grafted to Velvick, A10 or Thomas or planted on their own roots (Table 4). Reed, Barr Duke and Nabal showed the greatest decline in health of the cloned rootstocks. Hass trees grafted to SHSR-01, Velvick, Barr Duke and SHSR-03 were the healthiest trees within the seedling rootstock population (Table 4) while A10, Nabal, Duke 7 and Reed had the greatest decline in tree health.

In a separate experiment at Hampton, Velvick seedlings performed well under low disease pressure when compared with the susceptible rootstocks A8 and A10 (Table 5). Mineral analyses of roots have shown that rootstocks vary in their nutrient absorption (data not presented) and this may correlate to Phytophthora tolerance in rootstocks. In this study Velvick had significantly higher Ca and Fe in roots and lower N, K and Mg when compared with A8 and A10.

**Table 4** Performance of 10 rootstocks propagated as either seedlings of clones and grafted to Hass during the establishment phase in a replant orchard site at Walkamin, North Queensland. Rootstock rating: 0 = healthy, 10 = dead. Values in columns are means of 10 trees and with different superscript letters are significantly different ($P \leq 0.05$) as tested by ANOVA.

| **Seedling rootstock** | **Health rating (0-10)** | **Cloned rootstock** | **Health rating (0-10)** |
|---|---|---|---|
| A8 | $2.6^{b}$ | A8 | $1.8^{bc}$ |
| A10 | $1.9^{b}$ | A10 | $0.6^{ab}$ |
| Barr Duke | $0.8^{a}$ | Barr Duke | $2.6^{c}$ |
| Duke 7 | $2.3^{b}$ | Duke 7 | $0.9^{b}$ |
| SHSR-01 | $0.4^{a}$ | Hass | $0.3^{a}$ |
| Nabal | $2.2^{b}$ | Nabal | $4.7^{c}$ |
| Reed | $2.9^{b}$ | Reed | $2.3^{c}$ |
| SHSR-03 | $1.0^{a}$ | Thomas | $0.6^{ab}$ |
| Velvick | $0.4^{a}$ | Velvick | $0.1^{a}$ |
| Zutano | $1.5^{ab}$ | Zutano | $0.9^{b}$ |

**Table 5** Performance of three seedling rootstocks in Phytophthora infested replant land at Hampton, Queensland. (Rootstock rating, 0 = healthy and 10 = dead).

| **Seedling rootstock** | **Mean tree health (0-10 rating)** | |
|---|---|---|
| | Low disease pressure | High disease pressure |
| Velvick (WI) | 1.3 | 6.6 |
| A10 (G x M) | 5.8 | 7.2 |
| A8 (G) | 5.9 | 7.3 |

In the replant site at Hampton, Hass on cloned Dusa were the healthiest trees together with Hass grafted to cloned Barr Duke, seedling SHSR-01, cloned Toro Canyon and seedling Velvick (Table 6). The health rating of Dusa at this site verifies the results from South Africa (Kremer-Köhne and Duvenhage, 2000 and Kremer-Köhne *et al*., 2001, 2002) with this rootstock and provides a standard with which to compare other Phytophthora tolerant rootstocks. At this site SHSR-01 is also demonstrating promise as a rootstock tolerant to Phytophthora root rot (Table 6) and confirms the results reported from the replant site at Walkamin.

At the high pressure Phytophthora site at Duranbah, Hass grafted to cloned GE showed a very high level of tolerance to *P. cinnamomi* (Table 7) thereby demonstrating the merit in recovering and testing rootstocks from isolated survivors growing in orchards where trees have been subjected to long term selection pressure by *P. cinnamomi*. Additionally Hass clones also showed a high level of root rot tolerance at this site. Graft unions have a physiological effect on trees (Whiley, 1994) and can reduce the flow of carbohydrates to roots (carbohydrates drive root growth and defence mechanisms). In this case the absence of a graft union may allow Hass clones to establish under high disease pressure through increased ability to regenerate roots.

**Table 6** Performance of 18 month old seedling and clonal rootstocks in Phytophthora infested replant land at Hampton, Queensland. Rootstock rating: 0 = healthy, 10 = dead. Values in columns with different superscript letters are significantly different ($P \leq 0.05$) as tested by ANOVA.

| Rootstock | Mean tree health (0-10 rating) |
|---|---|
| Velvick1-seedling | $4.79^{a}$ |
| A10 seedling | $4.44^{ab}$ |
| Velvick seedling | $2.33^{abc}$ |
| Toro Canyon clone | $2.11^{abc}$ |
| SHSR-01 seedling | $1.88^{abc}$ |
| Barr Duke clone | $1.67^{bc}$ |
| Dusa clone | $0.11^{c}$ |

The performance of seedling rootstocks has been variable but this is not surprising as Zentmyer originally showed that less than 1% of seedlings from Duke 7 inherited the tolerance factor. The performance of the Merensky clones (Latas and Dusa) at Duranbah (Table 7) may have been compromised by the presence of *Cylindrocladium, Cylindrocladiella* and *Cylindrocarpon* sp. which were isolated from the roots of nursery supplied trees.

**Table 7** Performance of 12 month old seedling and clonal rootstocks in heavily infested replant land at Duranbah, New South Wales. Rootstock rating: 0 = healthy, 10 = dead. Values in columns with different superscript letters are significantly different ($P \leq 0.05$) as tested by ANOVA.

| Rootstock | Mean tree health (0-10 rating) |
|---|---|
| Latas clone | $5.0^{a}$ |
| Dusa clone | $3.5^{bcd}$ |
| Velvick clone | $4.0^{bcd}$ |
| Velvick seedling | $4.3^{abcd}$ |
| Duke 7 clone | $4.4^{abcd}$ |
| Barr Duke clone | $4.8^{abc}$ |
| Thomas clone | $3.7^{bcd}$ |
| A10 seedling | $4.9^{ab}$ |
| Reed seedling | $6.8^{a}$ |
| GE clone | $2.0^{d}$ |
| Hass clone | $2.1^{cd}$ |

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