

Rootstock Improvement for the Australian Avocado Industry – a Preliminary Report

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SUMMARY

The “Rootstock Improvement for the Australian Avocado Industry” project has been developed to address a number of key issues important to the long-term sustainability of avocado production in Australia. Australian avocado orchards are currently planted on seedling rootstocks, which are genetically diverse. 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 postharvest rots which negatively impacts on consumers. These differences have been attributed to different rootstocks exerting changes on scion physiology. This project reports on the first 3 years of a planned 10-year program to identify superior rootstocks and develop efficient clonal propagation systems so that genetic uniformity of orchards and the benefits arising can be introduced to the avocado industry.

Results from this project have clearly demonstrated improved efficiency of avocado seed germination in nurseries through seed scarification techniques. Cutting the seed and removing the seedcoat before planting reduced the time and increased the germination by 30-50%. In relation to clonal propagation the study of wounding techniques to encourage root development discovered that a 360° scrape of the etiolated shoot prior to the application of KIBA (a root promoter) consistently gave a “collar of roots”. This is particularly important for those rootstocks that are difficult to clonally propagate.

As plant improvement research has a long timeframe in the interim period leading up to the development of recommendations growers are encouraged to identify and record rootstocks used when planting new orchards.

Key words: anthracnose, clonal propagation, KIBA, seed germination

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-Khöne 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. It is unlikely that significant *Phytophthora* resistance will be achieved without the intervention of biotechnology to develop inter-specific hybrids through protoplast fusion techniques as is currently being researched in Florida (Pliego-Alfaro, 2001). However, this research is in its infancy and it is likely to be many years before commercial benefits will be achieved. In the interim period 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 race) compared to fruit from trees grafted to 'Duke 6' (Mexican race). 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 3 years activities of a proposed 10-year rootstock improvement program covering aspects of propagation and rootstock diversity to anthracnose susceptibility.

MATERIALS AND METHODS

Seed Germination Studies

Seed germination studies were carried out on 'Kidd' which is a seedling of Guatemalan origin. Pre-germination treatments included: (1) undamaged seed with the seedcoat intact (control); (2) a 5 mm slice cut from the top of the seed; (3) a pole to pole 5 mm deep longitudinal cut made in each quarter of the seed; (4) a pole to pole 5 mm deep longitudinal cut made once in each seed; (5) hot water treatment of the seed to 50°C for 30 min; and (6) the seedcoat removed without damaging the seed. Treatments were replicated 10 times with 10 seeds in each replication. Seed were planted in a composted pine bark potting mixture, which was irrigated with overhead misters to maintain moisture. A TinyTalk[®] logger was used to monitor the temperature in the germination medium for the duration of the experiment. Germination was judged to have occurred when a shoot from each seed emerged above the soil level.

Clonal Propagation Studies – Stem Wounding

'Velvick' was used across all treatments as the "nurse seed" in the cloning process which basically followed the method of Ernst (1999). The advantages of this seed are the vigour it produces and the large seed mass containing ample carbohydrates for growth in a dark chamber. The cloning research was carried out using the following rootstocks: 'Barr Duke', 'Duke 7', 'Franceschi', 'Thomas' (Mexican race); 'Zutano' (Mexican/Guatemalan hybrid); 'A10', 'Edranol', 'Hass' (Guatemalan/Mexican hybrids);

'A8', 'Nabal', 'Reed' (Guatemalan); 'Velvick' (West Indian). Post-etiolation wounding was investigated using the following treatments: 1) a 10 mm long sliver of bark cut from the etiolated tissue; 2) two vertical cuts 10 mm long on either side of the etiolated stem; 3) four vertical cuts 10 mm long equidistant around the etiolated stem; and 4) a light scraping of the bark for about 10 mm in a vertical direction completely around the etiolated stem. In each case the wounded area was painted with a 0.8% KIBA gel in the form of Clonex[®] (Growth Corp. Pty Ltd, WA). Following post-etiolation treatment the rootstocks were held at ambient temperatures under 60% shade for 4 weeks after which they were transferred to 30% shade. The experiments were carried out from October to December, 2002. About 2-3 months after applying the wound and KIBA treatments the rootstocks were grafted to 'Hass' while still attached to the "nurse seed". Once the grafted 'Hass' scions had produced 3-5 leaves the rootstocks were cut from the "nurse seed" and the rooting of the micro-cloned rootstocks evaluated.

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', 'SHS 1', 'Thomas', 'Toro Canyon', 'Zutano', 'A10', 'Edranol', 'Hass', 'SHS 2', 'A8', 'SHS 3', 'Nabal', 'Reed', 'Plowman', 'SHS 4', '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.

RESULTS AND DISCUSSION

Seed Germination Studies

The temperature in the germination bed ranged from 14-36°C over the duration of the experiment with a mean temperature of 24.4°C. The germination results are presented in Fig. 1. Ten weeks after planting there were significant differences between all pre-germination treatments and untreated seed. Approximately 32% of the untreated seed had germinated compared with about 64% of seed that had either been hot-water treated or had a single pole to pole side cut. Germination improved to 70% where the seed coat had been removed and to about 88% where seed had either been cut 4 times from pole to pole or had 5 mm sliced off the top of the seed. The rate of germination was also highest for seed that had either received four pole to pole cuts or had 5 mm sliced off the top of the seed with about 18% of seed germinating after 6 weeks. At this time no seed from other treatments had germinated. Eight weeks after planting about 82% of seed from the four side cuts and top cut treatments had germinated while for the seedcoat removed and one side cut treatments germination was 47 and 49%, respectively (Fig. 1).

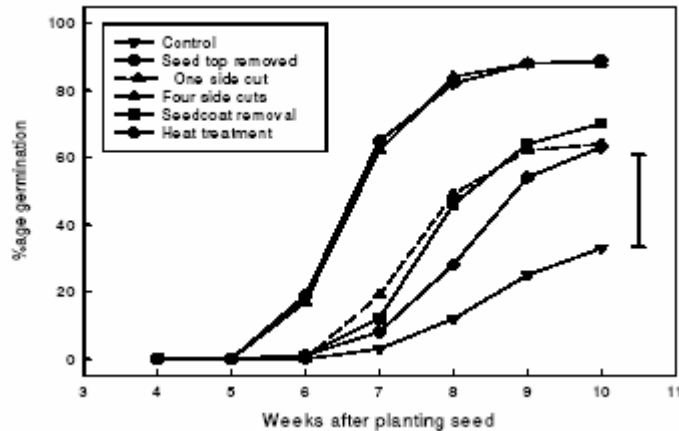


Fig. 1. Percentage germination of 'Kidd' seed following various scarification treatments. The experiment was conducted at Anderson's Nursery, Duranbah, NSW from October to December, 2002. The vertical bar represents the LSD ($P \leq 0.05$) at 10 weeks.

The results from this germination study conclusively demonstrate the benefits of scarification of avocado seed that has been freshly extracted from fruit and confirm the findings of earlier research (Kadman, 1963; Sauls and Campbell, 1980 and Bergh, 1988). These authors reported that the removal of the seedcoat reduced the time for seed germination however, the mechanism involved is not understood. There is some evidence of a biochemical inhibitor mechanism operating in the seedcoat (Perumal, 1961; Leal *et al.*, 1976) but alternatively germination may be inhibited due to a physical barrier being imposed by the seed coat (Bergh, 1988).

Results reported herein differ from previously reported research in that top or side cutting (four cuts from pole to pole) consistently gave superior results to seedcoat removal. Benefits from the more severe form of scarification made have its origins in seed dispersal mechanisms developed during the evolution of *Persea* sp. Barlow (2000) suggests that the avocado evolved with now extinct mega-fauna (e.g. glyptodonts, gomphotheres and mastodons) with the fruit being swallowed whole and passed through the digestive system before being deposited in a pile of dung far from the parent tree. The avocado seed has high mammalian toxicity so the consuming animal would need to carefully strip the flesh away with its teeth but in the process is likely to damage both the seedcoat and cotyledons. Hence seed finding its passage through the gut would emerge scarified and ready for germination.

Clonal Propagation Studies – Stem Wounding

There was a difference in rooting observed between the different groups of rootstocks. The Mexican race group responded to all methods of wounding, producing root systems of commercial quality in all cases. However, the scraped-wound treatment in general produced the most robust root systems with a "collar" of roots around the etiolated stem above the wound. Rooting of the Mexican/Guatemalan and Guatemalan/Mexican hybrid rootstocks and the Guatemalan race rootstocks was in general similar to the Mexican race group. However, there was a more marked difference between the wounds with the 4 vertical cuts and the scraped bark treatments generally superior to the other

wound treatments. 'Velvick' (West Indian) was the most difficult rootstock to root. In most instances a large mass of callus tissue grew at the wound site (particularly with the three cutting wounds) with either very sparse or zero root growth (Fig. 2). The 360° scrape wound gave the most consistent result with 'Velvick' (Fig. 3) although not all plants treated this way produced good root systems. From these results it is obvious that further research is required with 'Velvick' to develop cloning methodology sufficiently robust for commercial application. Aspects that can be further investigated are KIBA concentrations and temperature regimes during rooting.



Fig. 2. Excessive wound callusing on 'Velvick'.



Fig. 3 Successful growth of "collar of roots" on 'Velvick' following 360°wound.

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 1.

It is evident from the data (Table 1) 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°/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 1. Variance in anthracnose (*Colletotrichum gloeosporioides*) susceptibility in a population of avocado rootstocks. 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	5b	Hass	G x M	2a
Duke 7	M	5b	SHS 2	G x M	2a
Parida	M	5b	A8	G	1a
SHS 1	M	5b	SHS 3	G	0a
Thomas	M	5b	Nabal	G	0a
Toro	M	5b	Reed	G	1a
Canyon Zutano	M x G	4b	Plowman	G x WI?	0a
A10	G x M	2a	SHS 4	WI x M?	1a
Edranol	G x M	3ab	Velvick	WI	0a

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

** 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.

CONCLUSIONS

The commercial implication of the research results are that scarification, be it either seedcoat removal or damage to the cotyledons can provide an effective tool to reduce the time from planting to germination and in some instances increase the total percentage of seeds germinating. Some nurseries have waited for fruit to ripen before extracting seed and under these conditions the seedcoat firmly adheres to the seed. However, the extraction of seed from unripened fruit requires a knife to cut through the flesh. By applying sufficient force to cut through into the cotyledons the seed comes away cleanly from the seedcoat and the cotyledons are cut hence scarification on two fronts is applied during the seed extraction process.

During clonal propagation the type of wound applied to the etiolated stem prior to KIBA treatment can determine the success and quality of rooting. Easy-to-root rootstocks require minimum wounding while a full 360° scrape is usually required for those that root with greater difficulty.

At this point in time it is not clear if and how much effect diene levels in rootstocks might have on the postharvest control of anthracnose in fruit of the scion variety. Past research has determined there are a number of factors involved growing fruit with low

disease risk. These include maintaining consistent high yields on trees; producing fruit with high flesh calcium concentrations or lower N:Ca ratios; and harvesting fruit at the optimum eating quality. In addition, Willingham *et al.* (2001) found that rootstocks also influence disease susceptibility of the scion variety fruit. In studies with 'Hass' grown on 'Velvick' (West Indian race) and 'Hass' grown on 'Duke 6' (Mexican race) rootstocks there was significantly less postharvest anthracnose in ripened fruit from the 'Hass'/'Velvick' trees. The 'Hass'/'Velvick' trees also had significantly higher levels of leaf diene than the 'Hass'/'Duke 6' trees suggesting that diene is implicated in lower disease levels of fruit from 'Hass'/'Velvick' trees. However, fruit nutrient profiles were also different between fruit from the different rootstocks so the results may not be solely attributable to diene levels.

The results reported in this article and by Willingham *et al.* (2001) are complementary and suggest that with current knowledge Mexican race rootstocks and their hybrids should generally be avoided when planting new orchards in summer-wet districts of eastern Australia. However, field testing is still required to substantiate the arguments presented above and obviously it will be some time before conclusive data is available.

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