The role of rootstocks and nutrition in the quality of 'Hass' avocado

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AV00013

Final report of the project

"The role of rootstocks and nutrition in the quality of 'Hass' avocado"

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1 Media summary

The Australian avocado industry has a strong commitment to continually improve the quality of avocado fruit offered to the consumer. The industry, in partnership with Horticulture Australia Ltd and the Department of Primary Industries and Fisheries, Queensland, has funded numerous projects to improve fruit quality. In the past these have largely concentrated on chemicals to control fruit rots, which is one of the major factors affecting quality. However, there is growing evidence that there are a number of non-chemical means of improving quality. These are associated with improving the "vitality" of the fruit so that it is better able to prevent rots and other disorders. In particular, fruit with more calcium (Ca) and less potassium (K), often have less rots and disorders. By finding ways of improving fruit nutrition, we may be able to improve fruit quality offered to the consumer and at the same time reduce the need for chemicals.

Fruit trees are often grafted onto different rootstocks which allow the tree to produce more and better fruit. We tested whether some to these avocado rootstocks can take up more Ca, which could then improve Ca concentrations in the fruit. We found that the Velvick rootstock can have more Ca, and less K in the leaves than other avocado cultivars. This may explain why Hass avocado fruit has less rots when it is grown on Velvick rootstocks. We also noticed that the graft (the point where the Velvick rootstock joins to the Hass scion) in Hass on Velvick trees does not seem to restrict Ca from moving from the roots to the leaves, but there is a restriction in movement with some other rootstocks. Therefore, careful selection of the rootstock could improve fruit health and quality without the need for additional chemicals. The avocado industry is funding another project to find rootstocks that will produce better fruit quality, and the above results will help identify these better rootstocks more quickly.

We know from other research that the balance of nutrients in the soil could affect the uptake of Ca into the plant. We confirmed that high applications of K can reduce Ca uptake into the branch sap and the leaves. Therefore, re-evaluating the amount of K required by the tree, and how this is applied, may increase fruit Ca uptake.

We also know that fruit from higher yielding trees often have better fruit quality. This project showed that about 60 leaves per fruit gave good fruit size and fruit retention on the tree. The fruit Ca concentration was higher with less leaves per fruit, but the reduction in fruit size and the larger fruit loss with less leaves per fruit could reduce overall profitability.

We also looked at the accumulation of minerals into the different fruit tissues during fruit growth. It was thought that this may indicate ways of increasing the Ca concentration in the flesh and the skin, where rots develop. However, there was little evidence that accumulation in the different tissues could be manipulated to increase concentrations in the skin and flesh.

In summary, we now have a better understanding of some of mechanisms by which rootstocks and soil treatments may improve fruit nutrition and quality, and this information can be used to develop better rootstocks. We also have a better idea of the number of leaves per fruit that gives a good balance between fruit yield and quality. These results will help the avocado industry produce better quality fruit and reduce its dependence on chemicals to improve fruit quality.

2 Technical summary

The Australian avocado industry has a strong commitment to continually improve the quality of avocado fruit offered to the consumer. Consumers are concerned about the quality of the avocados they buy, since fruit on the retail shelf can contain rots and discoloured flesh. Previous studies have shown that high fruit Ca and Mg, and low K concentrations are related to less internal disorders and rots. Several factors such as rootstocks and pruning systems can reduce rots and disorders, and it is thought that these have their effect partly through improving fruit minerals concentrations. Thus, this project studied the rootstock/scion characteristics that might affect the movement of cations from the soil to the roots, and from the rootstock to the scion, as well as the effect of leaf to fruit ratio on distribution to the fruit, and the partitioning of cations between the fruit tissues.

To determine the potential of the rootstock to affect cation uptake, four seedling rootstock cultivars were grown under adequate and marginal Ca nutrition in an inert growing medium in the glasshouse. There were no significant cultivar effects on total root and suberised root Ca concentrations. However, there was a strong trend for higher Ca concentrations in Velvick leaves compared with Hass, Fuerte and Duke 7. In contrast, Velvick had significantly lower leaf K and higher root K concentrations, compared with the other cultivars. As a result Velvick had the lowest ratios of K/Ca, Mg/Ca and (K+Mg)/Ca in the leaves. This suggests that Velvick may be better able to increase Ca and decrease K concentrations in the leaves, which could be related to the higher Ca concentrations in fruit from Hass on Velvick trees.

Growing several avocado cultivars in soil from a commercial avocado orchard with differing K and Ca soil concentrations, indicated that excess soil K can reduce Ca concentrations in the branch sap and leaves. This again confirms the significance of K in Ca nutrition, and justifies a re-examination of K nutrition with a view to reducing application rates and improving timing and application systems. Further work is required in this regard.

To determine whether the graft union could affect the translocation of cations from the rootstock to the scion, several graft combinations were injected with strontium (Sr) just below the graft of young trees, and the Sr concentration determined in stem sections below and above the graft 15 minutes after injection. Strontium was used because of its similarities to Ca and because of its low natural concentrations in plants. The graft union in Velvick on Velvick and Hass on Velvick did not appear to retard translocation of Sr across the union. However, movement across the Hass on Duke 7 union was slower, possibly due to some form of incompatibility. All treatments with Hass as the rootstock appeared to have lower rates of Sr movement up the stem, but the graft union did not appear to restrict Sr movement.

Previous results have indicated that there are often less defects in fruit from higher yielding trees, but there have been no specific studies to determine appropriate leaf to fruit ratios. To test this, fruit were removed from selected branches and a girdle placed on the branch to produce 30-120 leaves per fruit above the girdle. The branches were girdled to prevent carbohydrates from the leaves above the girdle being translocated away from the fruit to other parts of the tree. In general, more leaves per fruit increased fruit size and the percentage of fruit retained. However, the fruit Ca concentration decreased with more leaves per fruit. There was no effect on fruit quality because of low rots severity and the fact that the fruit were not stored. The results suggest that no more than 60 leaves per fruit

would be a good compromise between fruit size, retention and fruit mineral concentrations.

Finally, cation concentrations in fruit tissues during fruit growth were measured over time. Calcium concentrations in the seed, flesh and skin were highest at 4 weeks after flowering and declined thereafter. The Ca concentration was higher in the skin compared with the flesh during early fruit growth only. In contrast, the K concentration increased until 4-15 weeks after flowering then declined. The results did not show unexpected patterns of cation accumulation in the fruit tissue. For example, there is no evidence of accumulation in the seed at the expense of the flesh, which might be manipulated to improve the cation ratio in the flesh or skin.

In summary, Velvick, which is associated with better fruit quality when grafted under Hass, accumulates less K and possibly more Ca in the leaves, thereby resulting in more favourable ratios with Ca, K and Mg. This could provide a good early screening test in rootstock selection programs. In addition, the results suggest that there is less restriction in Ca movement across the graft in Hass on Velvick compared with Hass on Duke 7, and that Hass may have an inherently slower Ca translocation rate than Velvick and Duke 7. Further studies are required to determine the causal mechanisms, but there is potential to also use this as a screening test for potential rootstock/scion combinations. Potassium nutrition should be re-visited with a view to reducing soil K concentrations and improving Ca uptake. In relation to distribution of Ca between leaves and fruit, a leaf to fruit ratio of about 60 appeared to give a good balance between potential yield and fruit mineral concentration. This target ratio needs to be considered in canopy management studies.

3 General introduction

The Australian avocado industry has a strong commitment to continually improve the quality of fruit offered to the consumer. Surveys have indicated that consumers are concerned about the quality of the avocados they buy, and that the fruit on the retail shelf often contain rots and discoloured flesh (Hofman and Ledger 1999).

Research in the early 1990s indicated large variation in tree yield (Thomas 1997) and fruit quality between adjacent trees in the same orchard (Hofman *et al.* 2002). Given the fact that most of these trees were Hass on random seedling rootstocks, it was suggested that this variation may be largely due to the performance of the rootstock (Hofman *et al.* 2002). Since then additional work has confirmed the potential benefits that rootstock selection can provide (Willingham *et al.* 2001; Marques *et al.* 2003).

There is strong evidence that adequate fruit nutrition can reduce fruit rots and internal disorders in fruit after harvest. A number of studies have shown that high fruit Ca and Mg, and low K concentrations are related to reduced internal disorders and rots in avocados, and in other fruits (Hofman and Smith 1994). Rootstocks can also affect scion fruit quality in a number of fruit, including 'Hass' avocado (Willingham *et al.* 2000; Marques *et al.* 2003). These and other studies (Vuthapanich 2001) have suggested that fruit minerals are likely to be involved in this rootstock/scion interaction.

The Australian avocado industry relies on grafted rootstock/scion combinations. In the past, rootstock selection was based on the availability of rootstock material or the propagationist's preference (Whiley and Schaffer 1994). In contrast, other horticultural industries such as citrus, have developed specific rootstock/scion combinations to maximise performance under specific environmental conditions. Ben-Ya'acov *et al.* (1992) recognises the most efficient means of improving tree performance in each environment is by selecting suitable rootstock/scion combinations. Rootstocks differ markedly in their ability to obtain nutrients from the soil (Reuther *et al.* 1958), and may be an effective means of optimising fruit minerals for improved quality.

Unfortunately, the lack of understanding of the benefits of rootstock selection has reduced the ability of growers to produce consistent yields and fruit quality. Specifically, Whiley *et al*. (1997) identify the use of seedling rootstocks as a major constraint to producing predictable and consistent fruit quality in the Australian avocado industry. However, recent evidence now confirms the potential benefits of rootstock selection for yield and quality.

It is also likely that varying degrees of physiological incompatibility at the rootstock/scion interface may inhibit the transport of nutrients to the fruit. For example, Coetzer *et al.* (1994) observed an initial accumulation of boron (B) in the roots of Duke 7 rootstocks grafted to Hass, while Whiley *et al.* (1996) reported higher concentrations of B in Hass leaves grafted to Velvick compared to those grafted to Duke 7.

It is generally observed that a high leaf to fruit ratio results in larger fruit of lower quality. In avocado, fruit from higher yielding trees can have less disease and internal disorders, and be smaller but with higher fruit Ca concentrations (Hofman *et al.* 2002). Some recent studies confirming rootstock effects on fruit quality (Willingham *et al.* 2001; Hofman *et al.* 2002; Marques *et al.* 2003) may have been mediated through higher tree yields in those trees, thus producing better quality fruit. Therefore, leaf to fruit ratios may be important in fruit quality, but there is little understanding on the optimum leaf to fruit ratio for fruit size and quality.

Several studies have shown that the Ca concentration in avocado fruit flesh increases up to about 6-10 weeks after flowering, but decreases thereafter (Bower 1988; Witney *et al.* 1990a). However, there is little understanding of the partitioning of Ca between the flesh, seed and skin, which may have some bearing on Ca accumulation in the flesh and skin.

The above shows that fruit mineral nutrition can have a large effect on fruit quality, and that there is considerable commercial potential in investigating the mechanisms whereby rootstocks influence fruit quality. This would help develop commercial recommendations for suitable rootstock/scion combinations, and identify those rootstock/scion characteristics that are associated with improved fruit quality.

This project investigated the nature of minerals (especially Ca) uptake and distribution by several avocado rootstocks and rootstock/scion combinations. The concept used was to try to understand the movement of Ca across the various barriers; from the soil to the roots, across the graft union into the scion, into the fruit, and finally distribution within the fruit to the skin and flesh.

4 Uptake of minerals into the roots and leaves of seedling trees

4.1 Introduction

The most active soil zone for nutrient uptake in avocado is in the top 30 cm (Durand 1987; Whiley *et al.* 1988). Characteristics of avocado roots include low hydraulic conductivity, high oxygen requirement (Stolzy *et al.* 1971) and sparse root hairs (Zilberstine *et al.* 1991). The absence of root hairs and low water conductivity. features which are typical of shade-adapted plants, indicate that avocado leaves have limited transpiration efficiency (Wolstenholme 1987).. Since there is generally a high correlation between transpiration and Ca uptake (Kirkby 1979), this characteristic can be counter to the requirements for fruit quality, especially when water is limiting. In addition, nutrient uptake is primarily through the non-suberised secondary "feeder" root system (Whiley and Schaffer 1994). However, since most of the avocado root system is suberized (Zilberstine *et al.* 1991), they generally scavenge water and nutrients inefficiently.

There are numerous reports of rootstock effects on tree/leaf nutrition, but there is relatively little data on what rootstock factors can contribute to these effects, especially in avocado. In general terms, the place and number of root laterals along a primary root, together with their diameter and angle to the primary root, influence the soil exploration capacity of the root system. Consequently, this will affect the availability of mineral nutrients and water for plant growth. Borys et al. (1985) studied the architecture and growth of seedling rootstock populations of the West Indian, Guatemalan and Mexican races and found significant differences between seedling populations within races as well as between races. They reported that West Indian race avocados had a larger root system than Mexican and Guatemalan races, with significantly more first, second and third order laterals. There was a direct correlation between the root system and the size of the above-ground growth, with West Indian race seeds producing larger trees. This can be partly attributed to a root system more capable of exploiting the available soil environment. However, rootstock/scion interactions also occur, since the scion can alter the size of the root system and its horizontal and vertical extension (Wallace *et al.* 1955; Whiley 1994).

This chapter investigated the impact of rootstock on Ca uptake from the soil into the roots and leaves of seedling trees. The experiments were carried out in inert potting medium to reduce unknown soil interactions. A pilot trial using two rootstocks identified the nutrient concentrations required for adequate and marginal Ca nutrition based on leaf norms. This was required since Ca translocation can be affected by the other minerals (Himelrick *et al.* 1983). The main trail used 4 rootstocks grown under adequate and marginal Ca.

Above ground vegetative growth was monitored. Also, changes in leaf stomatal conductance (which is closely related to transpiration) were determined because Ca uptake is influenced by transpiration and water movement in plants.

4.2 Materials and methods

4.2.1 Pilot trial

4.2.1.1 Treatments

Hass (Guatemalan) and Sharwil (Mexican) seedlings were grown in inert 50:50 vermiculite/perlite (coarse grade 3) sterile mix under 5 concentrations of Ca (100µM or 4.1g Ca/L, 200µM or 8.2 g Ca/L, 300µM or 12.3 g Ca/L, 400µM or 16.4 g Ca/L and 2000µM or 82 g Ca/L). The trees were watered every second day with 500mL of the relevant Ca solution. Six replicate trees were used for each treatment.

The highest Ca concentration was based on half strength Hoaglands solution (Hoagland *et al.* 1950). All other minerals were half strength Hoaglands solution and kept at the same concentration in all the treatments. See Appendix 1 for details.

4.2.1.2 Plant material and growing conditions

Hass and Sharwil seed was collected from mature avocado trees at the Centre for Subtropical Fruits, Maroochy Research Station (MRS) (latitude 260S, altitude 30 m). The trees had been previously tested and certified free from Sunblotch viroid, a potentially serious disease of avocado (Da-Graca 1985). Care was taken to minimise the risk of Phytophthora contamination by making sure the fruit and seeds did not come in contact with the ground, treating the seed with copper, and using sterile media.

The seedlings were initially grown in Mary River sand:peat moss (1:1) with a standard nutrient mix. At the start of the nutrient treatments the sand/peat moss was washed from the roots, the seed removed (to eliminate any mineral reserves left in the seed), and the trees re-potted into 5 L containers with an inert 50:50 vermiculite/perlite (coarse grade 3) sterile mix. The trees were left to stabilise for 10 days and watered using distilled water every second day.

The trial was conducted in a glasshouse at MRS from March to June 2002. Weekly average, maximum and minimum glasshouse air temperatures were 24, 35 and 14°C respectively, as recorded with a Tiny Talk Plus data logger (Gemini Data Loggers, UK). The photon flux density of photosynthetically active radiation between 10:00 and 12:00 on full sunlight days ranged between 1000 and 1400 µmol m^{-2} s^{-1} as determined by a line quantum sensor (LI-COR Inc., USA).

4.2.2 Full trial

4.2.2.1 Treatments and plant material

One Guatemalan (Hass), one predominantly West Indian (Velvick) and 2 Mexican cultivars (Fuerte and Duke 7) were used. Velvick and Duke 7 represent rootstocks commonly used in Australia, and Hass and Fuerte are commercial scion cultivars.

The trial consisted of the 4 cultivars and 2 Ca concentrations of 400µM and 2000µM, with the other essential nutrients at the same concentration in both solutions. Each tree was watered daily with 500ml of the relevant Ca solution. Ten replicate trees were used for each cultivar per treatment.

The plant material was propagated, prepared and grown as described in section 4.2.1.2.

4.2.2.2 Vegetative growth and performance

To stimulate new stem and leaf growth each tree was cut back to a height of approximately 67 cm at the beginning of the experiment (day 0). New shoot growth was measured at 0,

31, 62, and 82 days. New cumulative shoot growth was expressed as the combined length of primary shoot (from the main stem to shoot tip) plus secondary and tertiary axillary shoots.

Stomatal conductance was determined between 8.00am and 10.00am on 21-day-old leaves, at roughly weekly intervals. The day of the week was determined by the need for cloudless conditions to ensure similar conditions between sampling times. The sample leaves were located 2 nodes below the shoot apex. The readings were taken from 3 leaves per tree and 3 trees per cultivar (for each treatment) using a Licor 1600 porometer (LI-COR Inc., USA).

4.2.2.3 Leaf and root samples

The trial was harvested when the first leaf flush had hardened (approximately 86 days after the nutrient regime commenced). Five fully expanded, mature leaves were taken from around the canopy of each plant for mineral analyses. The leaves were cleaned from dust and chemical residues with a wet cloth, then rinsed with distilled water (Kadman *et al.* 1972).

The potting mix was carefully removed from the roots and the roots rinsed with distilled water. The white non-suberised roots were separated from the brown suberised roots for each tree and weighed. The total root weight was determined by addition. The roots were again rinsed in distilled water before drying and mineral analysis.

4.2.3 Dry weight and minerals analyses

The % dry weight was determined by drying the leaves and roots at 60^0C in a dehydrating oven (Thermoline L+M, Australia) for 3 to 5 days until constant weight. Twenty grams of each tissue for each tree was then ground to 100 mesh size in a Udy Mill (Udy Corp., USA). The sample for total roots was derived by adding non-suberised and suberised root samples together in the same ratio as non-suberised to suberised root weights. All samples were re-dried at 60^0C for at least 3 hours immediately before analysis.

The ground leaf and roots tissue (0.5 gm per tree) were digested using a wet digestion method (Baker and Smith 1974). Each sample was digested in a 50 mL erlenmeyer flask with 15 ml of a digestion solution containing 50 mL nitric acid, 250 mL perchloric acid and 60 mg of ammonium metavanadate dissolved in 10 mL of hot deionised water. The samples were pre-digested at ambient temperature for at least 2 hours, then heated on a hot plate to approximately 80°C for about 30 minutes. The heat was then gradually increased over 1 hour to 190°C and continued for another 30-60 minutes. The cooled, digested sample was transferred to a volumetric flask and made up to 25 ml with distilled water. The concentration of B, Ca, K and Mg were determined with an inductively coupled plasma atomic emission spectrophotometer (ICPAES) Spectroflame P (Spectro Analytical Instruments, Germany). Two certified samples of known nutrient concentrations (avocado leaf and oat herbage) were included as references. The results were checked for contaminants against blanks which only had the digestion solution added to the flasks (3 blanks for each digestion batch of 50 samples).

4.2.4 Statistical analysis

At least 2 trees from each cultivar were blocked together on 4-8 different tables in the glasshouse. The Ca treatments were allocated 4 tables each with 10 trees per table.

Data were analysed with Genstat 5® (Release 4.21) for Windows (Lawes Agricultural Trust, The United Kingdom). The protected least significant difference (lsd) procedure at

P=0.05 was used to test for differences between treatment means (Steel and Torrie 1980). The results were analysed by the residual maximum likelihood method. The initial model considered treatments, cultivars and their interaction as fixed effects and blocks (pairs of tables) tables within blocks, and trees within tables as random effects. Where estimates of the variance components for random effects were negative the relevant effect was removed from the model and the analysis re-run.

The full model was used for analyses of mineral concentrations, mineral ratios and dry weights. Since there was no effect of blocks or tables the model was reduced to include only trees as a random effect.

Analysis of variance was used for stomatal conductance. The effects of Ca concentration, cultivar, day, and all their interactions were considered. Estimates of variability between leaf measurements within trees, trees within tables, tables within blocks, and blocks were made. As the variance component for tables had a negative estimate the variation was ignored so that the final analysis considered variability between leaf measurements within trees, trees within blocks and blocks.

In the full trial there was no effect of Ca concentration on leaf or root mineral concentrations, stomatal conductance etc, so the results were averaged across the two concentrations and the means presented.

4.3 Results

4.3.1 Pilot trial

The leaf Ca concentrations in the 2000μM Ca treatment were within the adequate concentration range for both cultivars (Table 4.1). The leaf Ca concentration in the 400μM treatment was within the marginal range, and below the marginal range for 100μM, 200μM and 300μM. The concentrations for all other elements were within the adequate range or the lower end of the high range. Ca deficiency symptoms were present in the leaves of the 100-300μM treatments. On this basis, 2000 and 400 was used in the full trial.

Table 4.1. Pilot Trial. Mineral concentrations in the leaves of Hass and Sharwil avocado seedlings supplied with modified Hoaglands solution containing 100μM, 200μM, 300μM, 400μM and 2000μM Ca. The recommended marginal, adequate, high and toxic concentrations in mature avocado leaves are presented (Reuter and Robinson 1997).

Cultivar	Ca conc. (μM)	Ca %	K %	Mg %	Mn (mg kg^{-1})	Na (mg kg^{-1})	P %	S %	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Hass	2000	2.81	1.86	0.73	475.24	130.37	0.25	0.24	89.88	14.71	53.81	53.23
Hass	400	0.81	1.63	0.74	372.00	101.80	0.21	0.16	98.62	14.59	59.41	55.32
Hass	300	0.25	1.57	0.60	374.82	112.21	0.21	0.16	95.61	13.49	52.46	55.74
Hass	200	0.22	1.85	0.53	236.70	150.66	0.26	0.24	86.86	12.65	55.51	57.13
Hass	100	0.08	1.11	0.47	263.71	194.51	0.25	0.20	83.91	14.70	57.39	53.24
Sharwil	2000	2.91	1.75	0.55	317.58	66.90	0.24	0.23	84.18	20.54	55.43	53.81
Sharwil	400	0.63	1.88	0.65	323.54	66.87	0.28	0.18	79.36	15.21	53.29	52.13
Sharwil	300	0.23	1.82	0.56	368.38	62.46	0.29	0.17	85.37	20.34	54.25	55.48
Sharwil	200	0.27	1.10	0.48	210.82	71.55	0.25	0.27	89.17	20.80	50.75	55.74
Sharwil	100	0.09	1.82	0.45	253.08	70.77	0.23	0.19	82.09	20.30	52.61	52.39
	Marginal	0.5 - 0.9	0.35 - 0.74	0.15 - 0.24	15 - 29		0.05 - 0.08	0.05 - 0.15	10 - 39	3 – 4	40 - 49	12 - 29
	Adequate	1.0 - 3.0	0.75 - 2.0	0.25 - 0.80	30 - 500	<0.25	0.08 - .25	0.15 - 0.6	40 - 100	5 - 15	50 - 200	30 - 50
	High	3.1 - 4.0	2.1 - 3.0	0.81 - 1.0	501 - 1000		0.26 - 0.3			16 - 25		51 - 300
	Toxic	>4.0	> 0.3	>1.0	> 1000		> 0.3		>100	> 25		> 300

Values are means of 6 plants per cultivar.

In most instances there was little effect of cultivar on leaf mineral concentrations, except for higher Na in Hass and higher Cu in Sharwil (Table 4.1). There was a trend for decreasing Mg and Mn with reducing Ca treatment.

4.3.2 Full trial

4.3.2.1 Mineral concentrations

There were no significant cultivar effects on total root and suberised root Ca concentrations (Table 4.2). However, there was a trend for higher Ca concentrations in Velvick leaves compared with the other cultivars (significant at P=0.1).

In contrast, Velvick had significantly lower K concentrations in the leaves and higher concentrations in all parts of the roots, compared with the other cultivars. In addition, Velvick had lower leaf Mg concentrations than Fuerte and Hass, but Velvick root concentrations were either intermediate with the other cultivars, or there was no cultivar effect. Boron concentrations were higher in the Velvick leaves compared with Fuerte and Hass.

Velvick had the lowest ratios of K/Ca, Mg/Ca and (K+Mg)/Ca in the leaves compared with the other cultivars (Table 4.3), mainly as a result of its higher Ca and lower K and Mg concentrations. In addition, there were strong trends of higher ratios in the Velvick roots because of higher K concentrations.

Cultivar differences were also noted for the other cations in the leaves (Table 4.4). In particular, Zn and Fe was higher, and Na was lower in Velvick, compared with the other cultivars.

Table 4.2 Main trial. Mineral concentrations in the mature leaves, and total, suberised and non-suberised roots of Duke 7, Fuerte, Hass and Velvick avocado seedlings supplied with modified Hoaglands solution containing 400 and 2000μM Ca. The results are averaged across the two Ca concentrations. Means within each column and tissue followed by the same letter are not significantly different at lsd of P<0.05.

Cultivar	Mineral concentrations (mg/kg dry weight)			
	Ca	K	Mg	B
Leaf				
Duke 7	3838	5556[b]	5296[ab]	39.17[b]
Fuerte	3757	5821[bc]	5566[b]	31.37[a]
Hass	3807	6101[c]	5735[b]	31.48[a]
Velvick	4374	4719[a]	4843[a]	40.72[b]
P value	0.093	0.001	0.005	0.005
lsd	283	496.8	533.2	6.8
Total root				
Duke 7	2085	4614[a]	4851[a]	67.36[a]
Fuerte	2168	5572[b]	5729[b]	76.05[b]
Hass	1822	5021[ab]	5766[b]	68.01[a]
Velvick	2046	6892[c]	5122[ab]	72.64[bb]
P value	ns	0.001	0.022	0.002
lsd		875.4	737.8	5.2
Suberised root				
Duke 7	2464	4511[a]	4618	
Fuerte	2101	4290[a]	4679	
Hass	2005	4167[a]	4610	
Velvick	2275	5606[b]	4609	
P value	ns	0.001	ns	
lsd		721		
Non-suberised root				
Duke 7	2217	9689[a]	5104	
Fuerte	1711	10182[a]	4559	
Hass	1540	9976[a]	5280	
Velvick	1842	13655[b]	5204	
P value	0.093	0.001	ns	
lsd		1482		

P value = probability of significant difference
lsd = least significant difference at P=0.05.
ns = no significant difference (P=0.05)

Table 4.3 Main trial. K/Ca, Mg/Ca and (K+Mg)/Ca ratios in the mature leaves of Duke 7, Fuerte, Hass and Velvick avocado seedlings supplied with modified Hoaglands solution containing 400 and 2000μM Ca. The results are averaged across the two Ca concentrations. Means within each column and tissue followed by the same letter are not significantly different at lsd of P<0.05.

Cultivar	Mineral ratios		
	K/Ca	Mg/Ca	(K+Mg)/Ca
Leaf			
Duke 7	1.60[b]	1.49[b]	3.10[b]
Fuerte	1.62[b]	1.54[b]	3.16[b]
Hass	1.72[b]	1.59[b]	3.30[b]
Velvick	1.12[a]	1.15[a]	2.26[a]
P value	< 0.001	< 0.001	< 0.001
lsd	0.20	0.17	0.35
Total root			
Duke 7	2.62	2.84	5.45
Fuerte	2.73	2.86	5.59
Hass	2.94	3.31	6.26
Velvick	3.87	2.77	6.64
P value	0.051	ns	ns
lsd	0.84		
Suberised root			
Duke 7	2.28	2.41	4.69
Fuerte	2.36	2.56	4.91
Hass	2.15	2.40	4.58
Velvick	2.67	2.24	4.95
P value	ns	ns	ns
Non-suberised root			
Duke 7	5.254[a]	2.89	8.22
Fuerte	6.696[ab]	2.99	9.74
Hass	6.795[ab]	3.63	10.59
Velvick	7.971[b]	3.09	11.25
P value	0.017	ns	0.065
lsd	1.42		1.99

P value = probability of significant difference
lsd = least significant difference at p=0.05.
ns = no significant difference (P=0.05).

Table 4.4 Main trial. K/Ca, Mg/Ca and (K+Mg)/Ca ratios in the mature leaves, and total, suberised and non-suberised roots of Duke 7, Fuerte, Hass and Velvick avocado seedlings supplied with modified Hoaglands solution containing 400 and 2000μM Ca. The results are averaged across the two Ca concentrations. Means within each column followed by the same letter are not significantly different at lsd of P<0.05.

Cultivar	Mineral concentrations (mg kg^{-1} DW)						
	Mn	Na	P	S	Cu	Fe	Zn
Duke 7	826.4^{b}	27.44^{b}	674.5^{b}	1147^{ab}	36.8^{b}	173.1^{b}	19.41^{b}
Fuerte	487.6^{a}	23.34^{b}	568.2^{a}	1043^{a}	14.8^{a}	133.6^{a}	15.50^{a}
Hass	671.6^{b}	23.3^{b}	692.4^{b}	1274^{b}	13.9^{a}	148.3^{ab}	16.99^{ab}
Velvick	770.3^{b}	18.82^{a}	688.6^{b}	1181^{b}	19.8^{b}	209.5^{c}	24.42^{c}
P value	<0.001	<0.001	<0.001	.011	<0.001	<0.001	<0.001

P value = probability of significant difference

4.3.2.2 Vegetative growth and dry weight

There were no significant Ca treatment effects on vegetative growth or dry weight for any of the cultivars at all measurement dates, so the results were averaged across the two Ca concentrations.

Fuerte had the highest cumulative new shoot length at days 31, 62 and 82 compared with Hass and Velvick (Figure 4.1). Hass had lower growth than Duke 7 and Fuerte at 62 and 82 days. There was no difference in the cumulative new shoot growth between Duke 7 and Velvick.

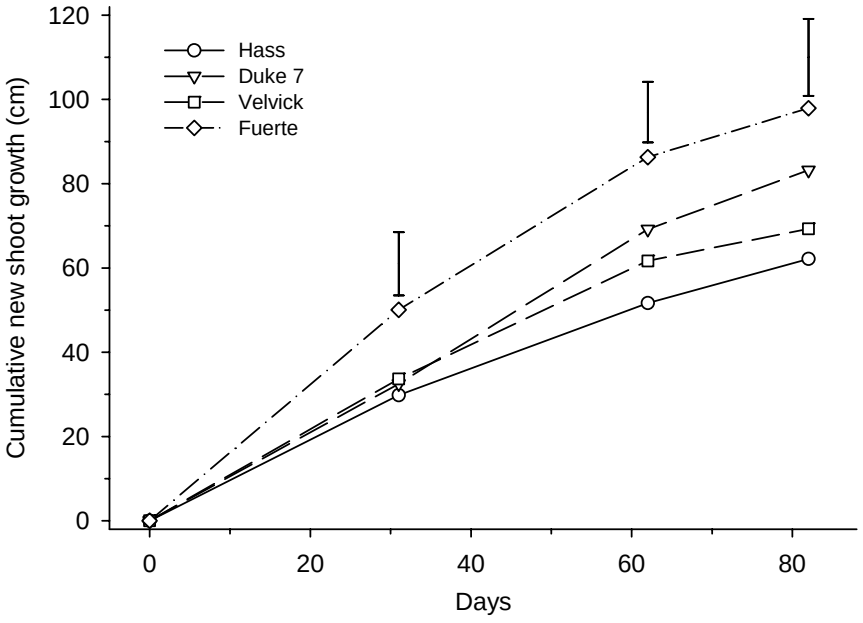

Figure 4.1 Cumulative new shoot growth (cm) of Hass, Duke 7, Velvick and Fuerte avocado seedlings from the start of the nutrient regime. Cumulative growth is expressed as new shoot length, including secondary and tertiary shoots. The results are averaged across the two Ca concentrations. The vertical bars indicate lsd (P<0.05) for each measurement time.

Hass had lower total and suberised root dry weights than Velvick and Fuerte at the end of the experiment (Figure 4.2). There was no difference between cultivars in the suberised root dry

weight. These results do not take into account weights at the start of the experiment, so the results may reflect lower root weight at the start rather than differences in growth rate during the experiment. However, the lower total root mass but similar non-suberised roots mass in Hass suggests a greater proportion of non-suberised roots in this cultivar.

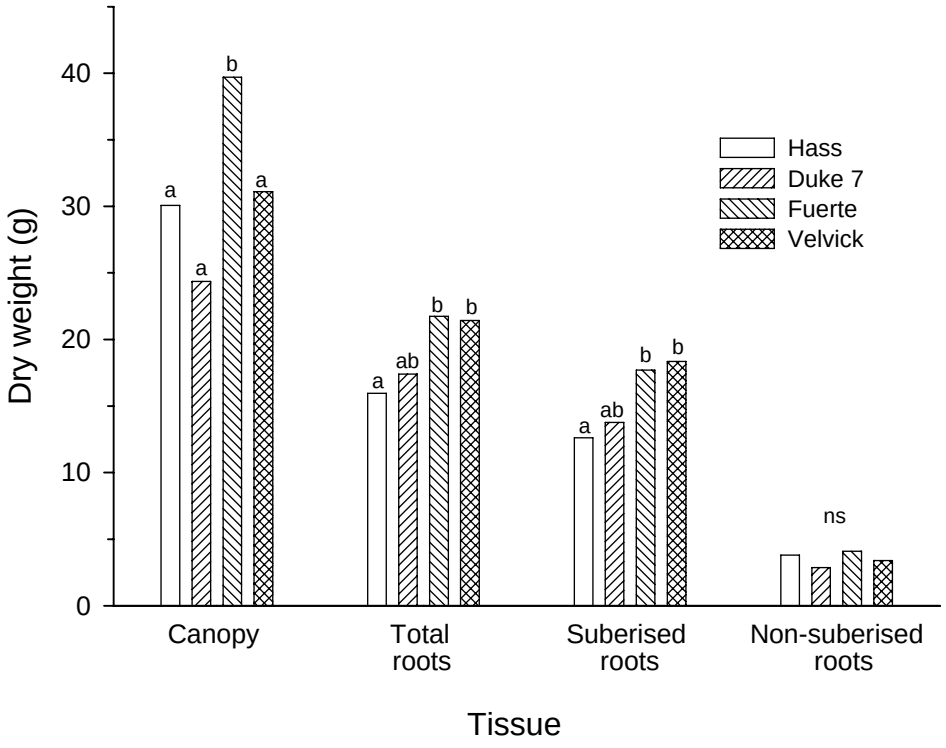

Figure 4.2 Main trial. The total root, suberised root and non-suberised root dry weights of Hass, Duke 7, Velvick and Fuerte avocado seedlings after 82 days. The results are averaged across the two Ca concentrations. Bars with the same letter within each tissue are not significantly different at p=0.05. ns=no significant difference.

4.3.2.3 Stomatal Conductance

Stomatal conductance increased in all cultivars from 42 days (Figure 4.3). At 52 days stomatal conductance was higher in Duke 7 compared with the other cultivars. There were no differences in stomatal conductance between Fuerte, Velvick and Hass, and no cultivar differences at all other measurement times.

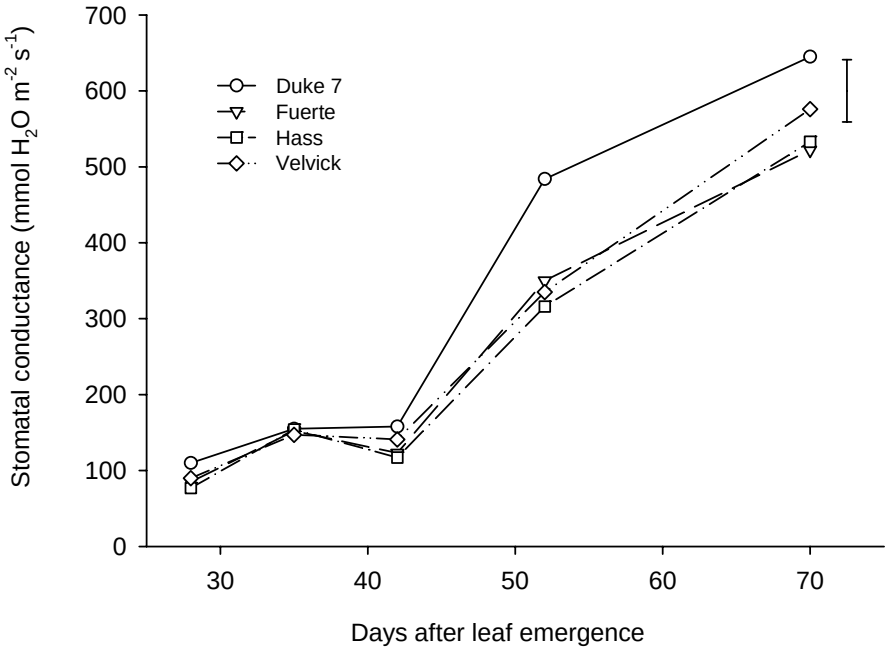

Figure 4.3 Stomatal conductance of the leaves from Duke 7, Fuerte, Hass and Velvick avocado seedling trees measured between 0800-1000 hours from 21 to 72 days after leaf emergence. The readings were taken from 3 leaves per tree and 3 trees per cultivar (for each treatment). The results are averaged across the two Ca concentrations. The vertical bars indicate lsd ($P < 0.05$) for all measurement times.

4.4 Discussion

These results indicate that cultivars have differing capacities for mineral accumulation in roots and leaves. Similar results have been obtained by other researchers. For example, 'Hass' grafted to Guatemalan rootstocks had greater leaf Ca concentrations than 'Hass' on Mexican rootstocks (Haas 1950; Embleton *et al.* 1962; Ben-Ya'acov *et al.* 1992; Willingham *et al.* 2000). Also, higher leaf B concentrations were found in Hass trees grafted to Velvick compared with Duke 7 (Whiley *et al.* 1996).

The higher leaf Ca in Velvick compared with the other cultivars suggests a greater capacity to either take up and/or translocate Ca to the leaves. The fact that the root Ca concentrations were similar, and that Velvick root Ca concentration was mid-range with the other cultivars, suggests that there is no accumulation of Ca in Velvick roots. Hence a potentially higher Velvick leaf Ca suggests good translocation from the roots to the leaves, as well as good uptake into the roots.

In contrast, the lower K in Velvick leaves and higher K in Velvick roots compared with the other cultivars suggests that translocation of K to the leaves is limiting in this cultivar. It is difficult to determine whether the increased K in the roots is due to the lower translocation to the leaves only, or due to greater uptake into the roots as well. The lower Mg concentration in Velvick leaves was likely due to reduced root uptake since the root concentrations were similar or intermediate with the other cultivars. Although Velvick leaf Mg concentration was lower than several (but not all) of the other cultivars, absence of differences in the roots suggests that the cultivar effect on Mg is not as strong as with K.

The present trial suggests some interaction between Ca, K and Mg, since Velvick had potentially higher leaf Ca concentration associated with lower K and Mg concentrations, which was reflected in lower ratios of K/Ca, Mg/Ca and (K+Mg)/Ca. Consequently, Ca may have reduced the uptake of Mg and K, which suggests a competitive inhibition of Mg and K uptake in Velvick trees. Such competition is plausible on the basis of competition between ions of the same electrical charge for exchange sites in plant tissue (Bartal *et al.* 1996). Cations such as Ca, Mg and K are transported in the xylem via negatively charged exchange sites on the xylem wall (Marschner 1986). If the Ca concentration is high or the concentration of other cations such as K and Mg is low, less competition for the exchange sites would occur and Ca uptake may increase.

Other studies have demonstrated the negative effects of K and Mg on Ca uptake. For instance, in sugar beet (*Beta vulgaris* L.) Mg interfered with Ca uptake and caused Ca deficiency symptoms in the leaves (Mostafa *et al.* 1976). Also, increasing Mg and K in the nutrient solution reduced the Ca concentration in the tissues of winter wheat forage (*Triticum aestivium* L. 'Centurk') (Ohno *et al.* 1985).

The cultivar differences in leaf B may be related to the suggested close association between B and Ca (Shear 1975). Boron can help maintain plant Ca in soluble form (Faust and Shear 1968) and has been linked with increased movement of Ca into apple leaves (Yamauchi *et al.* 1986). The higher leaf B in Velvick may also be a result of cation interaction since Velvick had higher leaf B and lower leaf Mg and K than Fuerte and Hass. For example, high levels of K in nutrient solution suppressed the uptake and distribution of B into avocado leaves (Jaime *et al.* 1992).

Ca uptake is restricted mainly to the non-suberised roots, since suberin in the endodermis restricts the delivery of Ca to the xylem (White 1998). Ca uptake can also occur at branching points with lateral roots or root hairs due to the disruption of the impermeable layer at these points (White 1998). In this study there was no difference in the total mass of non-suberised roots between the cultivars. However, the smaller mass of total roots in Hass would suggest that the ratio of non-suberised to total roots was greater in Hass compared with Velvick and Fuerte. This did not result in higher root or leaf Ca concentration in Hass. Other factors, such as a larger above-ground mass diluting the Ca may be involved, but does not appear to be supported by the lower shoot growth of Hass. Another factor may be the influence of root branching (root architecture) on Ca uptake. Certainly, differences in avocado cultivars with respect to root architecture have been reported (Whiley 1994), but it is unclear if there were architecture differences between the cultivars used in this study. These aspects require further investigation.

There was no indication of the influence of stomatal conductance in leaf Ca concentrations, since Duke 7 had greater stomatal conductance, but did not have higher leaf Ca concentrations.

In conclusion, the results confirm the potential for the avocado cultivar to influence leaf mineral concentrations. The fact that Velvick had lower (Mg+K)/Ca (generally associated with better fruit quality), and can produce better Hass fruit quality in several field trials compared with other rootstocks (Willingham *et al.* 2001; Marques *et al.* 2003), suggests that leaf mineral concentrations of the rootstock might be useful as an early selection criterion for fruit quality. Boron may also be involved in the ability of Velvick to improve fruit quality, but field studies have found little relationship between fruit quality and B across rootstocks (Marques *et al.* 2003).

5 Calcium uptake into the xylem sap and leaves under different Ca/K soil conditions

5.1 Introduction

Section 4 demonstrated that rootstocks accumulate cations in their roots and leaves to varying degrees. This characteristic may contribute to the observed effects of rootstocks on Hass avocado fruit quality (Willingham *et al.* 2001; Marques *et al.* 2003). However, it is unclear whether cation accumulation in the rootstock leaves indicates the capacity of the rootstock to contribute to higher Ca in the scion fruit.

Calcium uptake into the roots is influenced by water movement to the root surface, growth of the roots to the Ca in the soil solution, and the degree of branching and number of root hairs. Once absorbed into the roots, the Ca is transported to the leaves and fruit in the xylem sap. Hence, the xylem sap Ca concentration can indicate the concentration of Ca available to the leaves and fruit.

Cation uptake by the roots is affected by the cation ratios in the soil solution (Himelrick *et al.* 1983). It is possible that the rootstock capacity to take up Ca into the xylem sap will vary depending on the Ca activity ratio in the soil solution.

Project AV02009 (Optimising the post-harvest qualities of Hass avocado through improved calcium nutrition) includes a field trial based at Bundaberg aimed at increasing fruit Ca concentrations (and thereby fruit quality) by applying Ca to the soil. Results to date indicate that microfine gypsum applications just before flowering have little impact on fruit Ca and quality, probably because most of the Ca is leached from the top 0-30 cm of soil within 70 days of application. This suggests that Ca is more mobile in avocado soils than first expected, and that more information is required to reliably increase soil solution Ca during the critical first 70 days of fruit growth.

The following experiment tested the potential of several avocado rootstocks representing the 3 races to take up Ca from the soil in the above field trial, with the exchangeable cation suite in the soil adjusted to several different Ca/K ratios. Seedling trees were grown in soil at these different ratios in the glasshouse. The soil exchangeable cations, and concentrations in the soil solution xylem sap and leaf were determined.

5.2 Materials and methods

5.2.1 Plants and growing conditions

Seedlings of Velvick (Mexican), Reed (Guatamalan), Smerdon (GxM) and Toro Canyon (M) (30 seedlings of each cultivar) were germinated and grown under standard conditions (section 4.2.1.2). When about 50 cm tall, the plants were cut back to leave 3-5 buds on the stem. When the new shoot growth was about 10 cm long, the potting medium was washed from the roots, the remaining seed removed, and the plants placed in 5 L plastic pots with sandy loam soil from a typical avocado orchard in the Bundaberg district. The soil was taken from about 5-30 cm depth (top organic layer removed). The soil was sieved to about 10 mm, then steam sterilised at about 70°C for 45 min.

The plants were re-potted again after 3 months because of Mn toxicity due to water logging caused by soil compaction. Soil from the same Bundaberg site was used, but about 20% by volume of perlite (coarse grade) was added to increase aeration. To further reduce the risk of water logging, each plant was given 200 mL of water only when the top of the soil was very dry (every 3-4 days).

Any leachate was collected in trays under each pot and returned to the soil. The plants were grown in a glasshouse at 30°C day/20°C night.

Nitrogen was applied as a 1% urea spray weekly as required, and as 200 mL per pot of ammonium nitrate at 26.5 g/100 L fortnightly.

5.2.2 Treatments

The following soil treatments were applied to 10 seedlings from each cultivar:

- No additional soil treatment (Control)
- Double the current exchangeable Ca content of the soil (Ca treatment). 3.27 g $CaSO_4.2H_2O$ (gypsum)/kg dry soil was added, equivalent to 1.88 cmol Ca/kg, or 0.752 g Ca/kg, or roughly 3 tonnes gypsum /ha.
- Quadruple the current exchangeable K content of the soil (K treatment). 0.73 g K_2SO_4/kg dry soil was added, equivalent to 0.78 cmol K/kg or 0.305 g K/kg or roughly 300 kg K/ha.

The K rate was high in order to change the ratio of exchangeable K to exchangeable Ca on the soil's cation exchange surfaces. All treatments were applied on an oven dry soil basis by drying a sub-sample of soil at 105°C for 24 h.

5.2.3 Harvest and minerals analysis

All plants were watered with 200 mL the afternoon before the trial was harvested to bring the soil to field capacity.

The stem of each plant was removed at ground level. The bark was removed from the bottom 10 mm of stem and the stem inserted into a vacuum device to remove the sap from the wood. This sap represents the water and solutes transported from the roots to the leaves via the xylem. About 95 mm of vacuum was used and small sections were continually cut from the tip of the branch to expose new tissue and increase sap flow. About 0.1-0.5 mL sap was collected per plant. The sap samples were placed in the fridge until analysis.

About 10 mature leaves per plant were sampled, dried at 65°C for about 3 days, then ground to about 1 mm. Leaf samples were analysed as described in section 4.2.3. The sap samples were placed in a freezer overnight just before analysis, then thawed to remove any particulate matter. They were then diluted 2:1 with concentrated nitric acid and analysed by ICPAES. The results are expressed as mg/mL of sap.

All soil samples were re-wetted to near field capacity where necessary and stored at 3 °C prior to extraction. 250 g of wet soil was vacuum filtered through Whatman No 1 filter paper. The filtrate was then acidified with concentrated HCl and stored at 4 °C prior to analysis by ICPAES. A sub-sample of the wet soil was then air dried, ground to pass a 2mm screen and a 5 g sample weighed into a 50 mL centrifuge tube and extracted with 1M ammonium acetate on an end-over-end shaker for 30 minutes. The tubes were then centrifuged at 3000 rpm for 10 minutes and a 5 mL sample of the supernatant transferred to a plastic vial for analysis by ICPAES (Australian Standard Method 15D3). The results represent the cations held on the exchange complex plus those in the soil solution, and are expressed as cmol+/kg soil (oven dry weight).

5.2.4 Statistical analysis

The trees were randomly placed on 4-5 benches in the glasshouse. The trees were randomised several times during the experiment to minimise any position effects.

Data were analysed with Genstat 5® (Release 4.21) for Windows (Lawes Agricultural Trust, The United Kingdom) using a 2 way analysis of variance. The protected least significant difference

(lsd) procedure at P = 0.05 was used to test for differences between treatment means (Steel and Torrie 1980).

5.3 Results

5.3.1 Soil

5.3.1.1 Soil exchangeable cations

Added Ca doubled the exchangeable Ca in the soil compared with control and added K (Table 5.1). Added K increased exchangeable K by 4 times compared with control and Ca. Added Ca marginally reduced exchangeable Mg, but there was no effect of K on exchangeable Mg compared with the control. Added Ca had the highest CEC, while the CEC of the K treatment was slightly higher than the control.

Because a 'closed pot' system was used, these results were expected because all the cations added would be recovered as the sum of cations taken up by the plant and 'exchangeable' cations.

Table 5.1. The exchangeable cations, and the cation exchange capacity (CEC) of the soil when approximately 2 times the Ca (as $CaSO_4$) or 4 times the K (as K_2SO_4) was added compared to no added fertilizer (Control). Means within each column followed by the same letter are not significantly different at lsd of P<0.05.

Soil treatment	Soil exchangeable cations (cmol+/kg dw)				
	Ca	**K**	**Mg**	**Na**	**CEC**
Control	1.9 [a]	0.10 [a]	1.06 [b]	0.07 [a]	3.15 [a]
2 x Ca	4.2 [b]	0.12 [a]	0.94 [a]	0.07 [a]	5.36 [c]
4 x K	1.8 [a]	0.64 [b]	1.06 [b]	0.10 [b]	3.61 [b]
lsd	0.19	0.02	0.09	0.007	0.24
P value	<0.001	<0.001	0.009	0.001	<0.001

P value = probability of significant difference
lsd = least significant difference at P<0.05.
ns = no significant difference (P>0.05)

5.3.1.2 Soil solution cations

Added soil Ca increased soil solution Ca concentration by about 4 times compared with the control (Table 5.2). Additional soil K slightly increased solution Ca above the control. Added K increased solution K by about 12 times compared with the control. Both treatments increased solution Mg compared with the control, but Ca resulted in the highest concentration. Added Ca and K reduced soil solution P concentrations, while solution S concentrations increased because of the presence of sulphate in both fertilisers.

Table 5.2. The concentration of cations in the soil solution when approximately 2 times the Ca (as $CaSO_4$) and 4 times the K (as K_2SO_4) was added compared to no added fertilizer (Control). Means within each column and treatment type followed by the same letter are not significantly different at lsd of P<0.05.

Treatment	Soil solution minerals (mg/L)					
	Ca	**K**	**Mg**	**Mn**	**P**	**S**
Control	39.1 [a]	11.0 [a]	19.9 [a]	0.24 [a]	0.43 [b]	40.3 [a]
2 x Ca	182.5 [c]	25.0 [a]	61.0 [c]	2.09 [c]	0.28 [a]	185.0 [c]
4 x K	69.8 [b]	141.0 [b]	42.3 [b]	1.05 [b]	0.30 [a]	143.5 [b]
lsd	17.8	19.9	9.7	0.32	0.07	28.2
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

P value = probability of significant difference
lsd = least significant difference at P<0.05.
ns = no significant difference (P>0.05)

5.3.2 Sap

There was no rootstock effect on Ca concentration in the sap (Table 5.3). Velvick had the lowest K, B and S concentrations in the sap, and lower Mg concentrations than most of the other cultivars.

Adding extra Ca to the soil did not increase sap Ca, K or Mg concentrations compared with the control (Table 5.3). Both Mn and S sap concentrations were increased with added soil Ca. In contrast, added soil K increased sap K concentrations by over 2 times. Extra soil K also reduced sap Ca by almost half compared with the control and Ca treatments. Added K also resulted in the lowest sap Mg concentration, and lower Mn concentrations than the Ca treatment.

Table 5.3. The concentration of cations in the xylem sap of four avocado cultivars grown in soil where either no additional fertiliser was added (Control), or approximately 2 times the Ca (as $CaSO_4$) or 4 times the K (as K_2SO_4) was added compared with the Control. Means within each column and treatment type followed by the same letter are not significantly different at lsd of P<0.05.

Treatment	Sap concentration (mg/L)					
	Ca	**K**	**Mg**	**B**	**Mn**	**S**
Rootstock						
Reed	22.2	185.2 [b]	24.5 [ab]	0.55 [b]	2.36	7.68 [ab]
Smerdon	25.0	177.3 [b]	31.0 [c]	0.56 [b]	1.75	9.60 [bc]
Toro Canyon	21.5	192.9 [b]	28.9 [bc]	0.56 [b]	1.78	11.20 [c]
Velvick	19.0	143.8 [a]	21.2 [a]	0.42 [a]	2.04	6.64 [a]
lsd	ns	33.4	4.6	0.12	ns	2.25
P value	0.193	0.024	<0.001	0.046	0.125	<0.001
Soil						
Control	25.6 [b]	135.1 [a]	26.8 [b]	0.44 [a]	1.16 [a]	5.64 [a]
2 x Ca	26.0 [b]	158.4 [a]	30.2 [b]	0.59 [b]	2.80 [c]	10.80 [b]
4 x K	14.2 [a]	230.9 [b]	22.2 [a]	0.54 [b]	1.98 [b]	9.84 [b]
Lsd	4.8	28.9	4.0	0.10	0.49	1.94
P value	<0.001	<0.001	<0.001	0.01	<0.001	<0.001

P value = probability of significant difference
lsd = least significant difference at P<0.05.
ns = no significant difference (P>0.05)

Rootstocks responded differently to soil treatments in relation to sap Mg concentration (Table 5.4). In most rootstocks extra Ca did not affect sap Mg, expect in Toro Canyon where the sap Mg increased. Reed and Toro Canyon were the least affected by added K, while added K to Smerdon and Velvick decreased sap Mg compared with the control.

In relation to the other nutrients, Velvick had lower sap B than Smerdon and Toro Canyon (Table 5.3). The increase in sap B with added Ca and K was mainly due to the large treatment response of Toro Canyon, while the sap B concentrations in other 3 cultivars was not affected by soil treatment (Table 5.4). Toro Canyon had the highest sap Zn concentration compared with the other cultivars, and sap Mn concentration was higher with added Ca compared with the control or added K (Table 5.3).

Table 5.4. The concentration of magnesium and boron in the xylem sap of four avocado cultivars grown in soil where either no additional fertiliser was added (Control), or approximately 2 times the Ca (as $CaSO_4$) or 4 times the K (as K_2SO_4) was added compared with the Control. The results show the interaction between soil treatment and rootstock. Means within each cation followed by the same letter are not significantly different at lsd of P<0.05.

Rootstock	Sap concentration (g/kg)		
	Control	2 x Ca	4 x K
Magnesium			
Reed	24.1 ^{abc}	28.9 ^{cde}	20.4 ^{ab}
Smerdon	35.2 ^{ef}	32.5 ^{def}	25.3 ^{bcd}
Toro Canyon	22.3 ^{abc}	38.2 ^{f}	26.2 ^{bcd}
Velvick	25.5 ^{bcd}	21.1 ^{abc}	16.9 ^{a}
lsd (interactions)		7.96	
P value		0.015	
Boron			
Reed	0.45 ^{abcd}	0.62 ^{def}	0.59 ^{cdef}
Smerdon	0.64 ^{def}	0.50 ^{bcde}	0.53 ^{bcde}
Toro Canyon	0.26 ^{a}	0.74 ^{f}	0.68 ^{ef}
Velvick	0.40 ^{abc}	0.49 ^{bcde}	0.36 ^{ab}
lsd (interactions)		0.20	
P value		0.001	

P value = probability of significant difference
lsd = least significant difference at P<0.05.

5.3.3 Leaf

Smerdon and Velvick had higher leaf Ca concentrations than Toro Canyon (Table 5.5). Velvick had the lowest leaf K concentration of all cultivars tested. Smerdon had higher leaf Mg compared with the other cultivars, but concentrations were similar in Velvick, Reed and Toro Canyon. Of the other minerals, Reed had lower leaf B, Toro Canyon had lower leaf Mn, and Velvick had higher leaf P, compared with the other cultivars.

Additional soil Ca did not significantly increase leaf Ca, but increased leaf Mg and Mn compared with the control (Table 5.5). In contrast, added soil K significantly reduced leaf Ca by 43%, and increased leaf K by 136%, compared with the control. Added K also reduced leaf Mg and increased leaf Mn compared with the control.

There were significant interactions between rootstocks and soil treatments for K and Mg, but these interactions were small, and did not affect the general response of added K increasing leaf K and decreasing leaf Mg, and Ca increasing leaf Mg (except in Velvick) compared with the control (Table 5.6).

Table 5.5. The concentration of cations in the leaves of four avocado cultivars grown in soil where either no additional fertiliser was added (Control), or approximately 2 times the Ca (as $CaSO_4$) or 4 times the K (as K_2SO_4) was added compared with the Control. For the cultivar effects, the results are the average of all soil treatments, and for soil effects the results are the average of all cultivar treatments. Means within each column and treatment type followed by the same letter are not significantly different at lsd of P<0.05.

Treatment	Leaf concentration (mg/kg)				
	Ca	**K**	**Mg**	**B**	**Mn**
Rootstock					
Reed	10790 [ab]	13410 [c]	5917 [a]	50 [a]	1339 [b]
Smerdon	12570 [c]	12040 [bc]	7476 [b]	71 [b]	1279 [b]
Toro Canyon	9480 [a]	11880 [b]	5851 [a]	71 [b]	988 [a]
Velvick	12120 [bc]	10160 [a]	6147 [a]	74 [b]	1422 [b]
lsd	1446	1441	475	14	242
P value	<0.001	<0.001	<0.001	0.005	0.004
Soil					
Control	12830 [b]	8190 [a]	6578 [b]	67 [ab]	738 [a]
2 x Ca	13680 [b]	8000 [a]	7490 [c]	56 [a]	1837 [c]
4 x K	7200 [a]	19440 [b]	4975 [a]	77 [b]	1196 [b]
lsd	1252	1248	411	12	210
P value	<0.001	<0.001	<0.001	0.005	<0.001

P value = probability of significant difference
lsd = least significant difference at P<0.05.
ns = no significant difference (P>0.05)

Table 5.6. The concentration of potassium and magnesium in the leaf of four avocado cultivars grown in soil where either no additional fertiliser was added (Control), or approximately 2 times the Ca (as $CaSO_4$) or 4 times the K (as K_2SO_4) was added compared with the Control. The results show the interaction between soil treatment and rootstock. Means within each cation followed by the same letter are not significantly different at lsd of P<0.05.

Rootstock	Leaf concentration (mg/kg)		
	Control	2 x Ca	4 x K
Potassium			
Reed	9880 [b]	9750 [b]	20610 [d]
Smerdon	7740 [ab]	7000 [a]	21390 [d]
Toro Canyon	7660 [ab]	8040 [ab]	19950 [d]
Velvick	7470 [ab]	7190 [a]	15810 [c]
lsd (interactions)		2496	
P value		0.036	
Magnesium			
Reed	5920 [cd]	6830 [e]	5000 [ab]
Smerdon	8240 [f]	9087 [g]	5100 [abc]
Toro Canyon	5770 [bcd]	6922 [e]	4860 [a]
Velvick	6380 [de]	7120 [e]	4940 [a]
lsd (interactions)		820	
P value		<0.001	

P value = probability of significant difference
lsd = least significant difference at P=0.05.
ns = no significant difference (P=0.05)

5.4 Discussion

The soil Ca and K treatments achieved the desired effect of increasing both the exchangeable and soil solution Ca and K by 2-12 times. However, despite the large increase in available Ca to the

roots, there was no significant soil Ca effect on xylem sap or leaf Ca concentrations in any of the cultivars tested. This suggests that the maximum Ca uptake capacity had already been reached under the conditions of this trial. It is possible that this could represent Ca uptake saturation under typical field conditions, however the experimental conditions may have influenced Ca uptake capacity. For example, root growth was slow because of poor soil aeration (even though perlite was added and water frequency was minimised). These conditions affected root growth, and may have affected root architecture (degree of branching etc), which can potentially affect Ca uptake (White 1998). Therefore, even though soil solution Ca was increased with Ca treatment, the roots may not have been capable of absorbing this additional Ca effectively. Also, Ca uptake is heavily influenced by transpiration (Cline and Hanson, 1992). Higher humidity conditions in the glasshouse may have reduced water loss from the leaves, and therefore uptake from the roots. These factors may also have contributed to the nil effect of cultivar on leaf Ca.

However, the results again confirm the importance of added K in decreasing Ca uptake as shown in the sap and leaf analytical results. The negative effect of added K on leaf Ca concentration has been reported in other plants (Ohno *et al.* 1985). Therefore, there may be benefit to investigating K requirements of avocado with a view to minimising K fertilisation. Both reducing the annual application rates and targeting applications to growth cycles could be studied. In particular, frequent small fertigation applications, and reducing applications during early fruit growth should be considered.

While there were no cultivar interactions in response to K application, the results described in section 4.3.2.1 suggest that these may exist. Velvick had higher root K and lower leaf K than Duke 7, Fuerte and Hass (section 4.3.2.1) and lower sap and leaf K than Reed, Toro Canyon and Smerdon (this trial), which indicates a capacity for Velvick to accumulate excess K in the roots and prevent K translocation to the leaves. This mechanism could minimise the negative effects of excess K on Ca translocation to the leaves and possibly fruit, and result in the higher leaf Ca noted here, and higher leaf and fruit flesh Ca concentrations in Velvick compared with Duke 7 in the field (Marques *et al.* 2003).

In relation to responses of the soil used in this trial to cations, the increase in solution Ca with high K would be expected as a result of a 'mass action' effect (the addition of K in excess of the amount of Ca would displace Ca from the exchange surfaces), but a stronger affinity of the exchange complex for K than for Ca may also have occurred. This latter effect is confirmed by the very small increase (ns at $p<0.05$) of K concentration in the soil solution following Ca addition.

Finally, the soil treatments produced similar responses in cation concentrations in the sap and the leaf. Therefore, there is potential to use branch sap to monitor tree nutrition status. Leaf Ca concentration is not well related to fruit Ca concentration because leaves are a stronger sink for water flow and therefore Ca. However, branch sap may give better indications of the capacity of the fruit to accumulate Ca. Extracting sap samples is more difficult that leaf sampling, but the reduced analysis cost (at least 50% cheaper), and potentially more useful information, may outweigh this disadvantage.

6 Effect of the graft union on scion minerals

6.1 Introduction

Sections 4 and 5 demonstrated that the rootstock itself can affect mineral accumulation in the rootstock leaves. The next potential barrier to mineral translocation to the fruit is the graft union, however little is known about how the interaction between the rootstock/scion influences mineral translocation across the graft union. To date, most studies have investigated the transport of nutrients from the roots to the scion without considering the role of the graft union (Tromp 1975; Granger *et al.* 1983; Barnard 1991). Trunk injection of these elements below the graft union directly into the vascular system is a more direct way of determining the graft union effects since it eliminates soil/root interactions (Sanchez-Zamora *et al.* 2000). Trunk injections have been used for the treatment of diseases (Guest *et al.* 1994) or correction of nutrient deficiencies (Whiley *et al.* 1991; Fernandez-Escobar *et al.* 1993), but injections have not been used much to study the translocation of nutrients from the rootstock to the scion.

The availability of minerals and nutrients to the scion will be reduced if physiological differences exist between a rootstock and scion (Barnett and Weatherhead 1968). Such incompatibilities can result from graft union necrosis leading to the death of the scion. However, while some incompatibilities between a rootstock and scion are not morphologically apparent (Schoning *et al.* 1997), anatomical differences at the graft union can still affect the free exchange of water and nutrients between scion and rootstock (Errea *et al.* 1994).

The development of callus between the graft components represents one of the prerequisites for the formation of a successful graft union (Barnett and Weatherhead 1968). The callus acts as an effective pathway between the scion and rootstock, enabling water and nutrients to by-pass the vascular tissues damaged during graft assembly, until the graft components unite and produce new xylem and phloem. However, the rootstock and scion do not always constitute a successful graft, resulting in graft incompatibility. This can be classified into two categories. Translocated incompatibility is characterised by alterations in the translocation pattern, while localised incompatibility is characterised by anatomical alterations at the union area that cause mechanical weakness (Errea *et al.* 1994).

This section reports on four experiments designed to determine the influence of the graft union on Ca translocation in selected avocado rootstock/scion combinations. The first experiment aimed to determine the injected Ca concentration required for detection of the injected Ca above the background Ca, and to determine the time delay between injection and sampling of tissue in order to "capture" the injected Ca. Radioactively labelled Ca was the preferred approach but was not possible because of restrictions in use. Previous experiments had determined that only 0.5 mL of solution could be effectively injected in to the stems, but this was later reduced to 0.2 ml.

The first experiment indicated that using Ca was not feasible because sufficient Ca could not be injected to raise Ca concentrations above background concentrations. Higher Ca concentrations were considered impractical because of the risk of tissue damage. Therefore, strontium (Sr) was used in future experiments because of its similar behaviour to Ca in plants (Andersen 1963) and the very low background Sr concentrations in avocado wood tissue. Two experiments determined the most suitable concentration, and the durations between injection and sampling that would ensure that the injected Sr would be contained in the sampled wood, rather than in the rest of the canopy. These experiments identified the best conditions for the final experiment (Experiment 4) which tested the translocation of Sr in several graft combinations.

6.2 Materials and Methods

6.2.1 Plants and growing conditions

Each rootstock plant was propagated from seed from mature avocado trees located at MRS. All propagation material had been previously tested and certified free of Sunblotch viroid. Initially each tree was grown in Mary River sand and peat moss (1:1) containing slow release fertiliser (Osmocote). Once the Osmocote was depleted (about 3 months) a supplemental standard nutrient solution (Aquasol) was applied every fortnight. The plants were grown in a shadehouse until ready for use.

One week prior to each experiment the nutrient applications were discontinued. The plants were transferred to a controlled environment (CE) glasshouse maintained at 25°C and 50% relative humidity, and all treatments applied under these conditions. The four experiments were conducted from October 2002 to January 2003.

6.2.2 Grafting combinations

The following graft combinations were used:

- Non-grafted seedling trees. These provided the treatment controls for the homografts.
- Plants grafted with the scion from the same plant (self grafted or homograft) which provided controls for the heterografts, and
- Different rootstock/scion cultivars (heterografts) (Yeoman *et al.* 1976).

The scion bud wood required for the homograft combinations was collected from the tree it was to be grafted back to. To stimulate bud wood growth, each seedling was heavily pruned one month before grafting (early December 2001). The scion bud wood for the heterograft combinations was collected from a single mature Hass tree at MRS, which was known to produce superior wood and true-to-type fruit. The bud wood was collected early in the morning between growth cycles (just before the summer flush cycle, mid- January 2002; Saranah, personal communication 2001).

Each scion bud stick had at least two sound, healthy and full dormant buds. Grafting commenced immediately after cutting using the splice graft technique. Each scion was covered with a plastic bag to maintain higher humidity, then with a paper bag to reduce sunburn of the new growth. The bags were removed when the grafts had started to grow (approximately 3-4 weeks after grafting). The grafting tape was removed when sufficient callus had appeared and had hardened (approximately two months after grafting). Any new vegetative growth on the rootstock was removed. Once the scion was well established all but the most dominant branch was removed.

6.2.3 Experiment 1: Ca concentration and duration

To determine the appropriate Ca concentration and the required time between injection and sampling, 0.5 ml of 0.082, 0.82 and 1.64 g/L Ca (as $Ca(NO_3)_2.2H_2O$) was injected into the stems 43 cm above the soil surface (just below section 1 referred to below). This was equivalent to 0.041, 0.41 and 0.82 mg Ca per tree. The syringe (0.45 x 13 mm**)** was gently pushed through the bark into the xylem and Ca slowly injected over about 1 minute.

Two, 5, 10 and 20 minutes after injection, 3 cm stem sections were taken at 44-47, 47-50 and 53-56 cm above the soil. These are called sections 1, 2 and 3 respectively. The grafts in the other experiments were located at 50-53 cm above the soil, so that sections 1 and 2 were below the graft union and section 3 was above the graft union for those experiments where grafted seedlings were used.

All injections were done between 900 and 1100. All leaves were removed between sections 1 and 4 about 24 hours before injection to eliminate leaf uptake of Ca. Each tree was well watered with deionised water before injection to maximise transpiration. Three Velvick and 3 Thomas seedlings (replications) were used for each replication. A control treatment with no injection was used.

6.2.4 Experiment 2: Strontium concentration

The highest Ca amount (0.82 mg) in experiment 1 failed to increase the Ca concentration in the xylem tissue above the background. Increasing the volume and concentration increased the risk of tissue damage. Therefore Sr was used in subsequent experiments.

0.2 mL of 4.1, 8.2, 16.4 and 32.8 g Sr/L (in the form of $SrCl_2$) was injected into the stem. This was equivalent to 0.82, 1.64, 3.28 and 6.56 mg Sr, respectively. A 10 minute duration was used based on experience from the previous experiment. A 0.2 ml injection was used to reduce tissue damage. Stem sections were harvested after 10 minutes as outlined in section 6.2.3. All other procedures were as described in section 6.2.3.

Three Velvick seedlings (replications) were used for each concentration.

6.2.5 Experiment 3: Strontium sampling time

To determine best time between Sr injection and sampling, 5 harvest times (0, 5, 10, 15, and 20 minutes) were tested. 0.1 mL of 32.8 g/L Sr (3.28 mg) was injected into the stems of Hass on Reed and Hass on Barr Duke. 0.1 mL (equivalent to 3.28 mg Sr) was injected to further reduce the risk of tissue damage during injection. Three seedlings (replications) of each of the rootstock/scion combinations were used for each concentration and harvest time. A control treatment with no injection was used.

For each harvest time, 5 stem sections (3 cm long), were taken. These were 41-44, 44-47, 47-50, 53-56 and 56-59 cm from the soil surface. The graft union was located between 50-53 cm above the soil. Thus, sections 1, 2 and 3 were below the graft union and sections 4 and 5 were above the graft union. All other procedures were as described in section 6.2.3. The stem section containing the graft union was not measured because of cost considerations.

6.2.6 Experiment 4: Rootstock/scion effects on translocation across the graft union

To determine the effect of the graft union on Sr translocation, non-grafted seedling trees and grafted trees with combinations from Mexican, Guatemalan and West Indian races (see Table 6.1 for details) were injected with 0.1 ml of 32.8 g Sr/L (3.28 mg Sr, as $SrCl_2$) below the graft union, or at similar heights for the non-grafted treatments. The non-grafted trees were considered as trees with no graft or genetic differences to affect Sr movement. The homograft combinations would indicate the effect of the graft in the absence of genetic differences. Non-grafted trees with no Sr injection were also included to determine the background Sr concentration.

Table 6.1 Experiment 4. Non-grafted and grafted (heterograft and homograft combinations) trees used in experiment 4. The trees were injected with 0.1 ml of 32.8 g Sr/L below the graft union.

Heterograft combinations	Homograft combinations	Non-grafted
HC:H = Hass scion[1] onto Hass rootstock (G x M:G x M)	H:H = Hass grafted back to itself (G x M:G x M)	H = Hass seedling tree (G x M)
HC:V = Hass scion[1] onto Velvick rootstock (G x M:WI)	V:V = Velvick grafted back to itself (WI x WI)	V = Velvick seedling tree (WI)
HC:D7 = Hass scion[1] onto Duke 7 rootstock (G x M:M)	D7:D7 = Duke 7 grafted back to itself (M:M)	D7 = Duke 7 seedling tree (M)

[1]Hass budwood sourced from a commercial Hass tree of proven performance.
G = Guatemalan, M = Mexican, WI = West Indian race

Each treatment tree was injected with 0.1 ml of Sr solution 44 cm above soil level, and 3 cm below section 1. Eight single tree replicates were used for each graft combination. Three cm stem sections were sampled 15 minutes after injection from just below (47-50 cm above the soil) and just above the graft union (53-56 cm above the soil). All other procedures were as described in section 6.2.3.

Stomatal conductance was measured as described in section 4.2.2.2. The stomatal conductance was measured 3 times over several weeks before the experiment, when clear, non-cloudy conditions prevailed. More measurement times were attempted but clear weather was not available. The results are averaged over the 3 measurement times.

The diameter (mm) of the stem just above and just below the graft union was also recorded as an indicator of graft compatibility.

6.2.7 Minerals analysis

Each stem section was dried at 60°C for 3-4 days or until there was no further weight loss, then analysed for mineral concentrations as described in section 4.2.3.

6.2.8 Statistical analysis

The mineral concentrations and trunk diameter were analysed by analysis of variance using Genstat 5® (Release 4.21) for Windows (Lawes Agricultural Trust, The United Kingdom). Cultivars (graft combinations), treatments (injection volume and time, when included), sections and their interactions were considered as fixed effects and trees and pieces within trees as random effects. Since the comparison of non-injected controls with the injected treatment resulted in very large differences in Sr levels for the injected treatments, trees from the non-injected and injected treatments were analysed separately. The vegetative growth and conductance data were analysed by analysis of variance but with cultivars, treatments and their interactions as fixed effects and trees as random effects.

All measurements had equal sample size (balanced data) and the protected least significant difference (lsd) procedure at $P = 0.05$ was used to test for differences between treatment means (Steel and Torrie 1980).

6.3 Results

6.3.1 Experiment 1: Ca concentration and duration

The highest injection concentration of 0.81 mg/0.5 mL Ca resulted was equivalent to about 13% of the background, assuming that all of the injected Ca stayed in the first 3 cm section above the injection site. This was insufficient to raise the Ca concentration in the stems above the background Ca. As a result there was no significant Ca concentration, time from injection to sampling, or stem section effects on stem Ca concentrations in either cultivar (Table 6.2). In most cases the Ca concentration in the control (non-injected) trees was similar to, or not lower than the Ca concentration in the injected trees, and there was no consistent increase in Ca concentration with increased Ca injection concentration. On this basis there was little benefit in using Ca in future experiments.

6.3.2 Experiment 2: Strontium concentration

Injection with 6.56 mg Sr increased Sr concentration in the stem sections between the injection site and the graft union (sections 1 and 2) (Figure 6.1). The Sr concentration in the section above the graft union was no different to that in the non-injected control and in the trees injected with lower concentrations. This suggests that a longer time between injection and sampling is required to allow Sr to move across the graft interface.

The highest Sr injected resulted in a 60 mg/kg increase in concentration over the background in sections 1 and 2. Assuming little injected Sr reached section 3, this represented only 0.48 mg Sr, or 1.5 % of the injected Sr.

Table 6.2 Experiment 1. Calcium concentration (mg/kg dry weight), in sections 1, 2, and 3 of Thomas and Velvick avocado trees injected with 0.041, 0.41 and 0.82 mg Ca, then stem sections sampled 2, 5, 10 and 20 minutes after injection. Two, 3 cm sections were taken just below the graft union (sections 1 and 2) and one 3 cm section taken just above the graft union (section 3). The Ca was injected just below section 1.

mg Ca injected, and duration between injection and sampling	Ca concentration (mg/kg dry weight)							
	Thomas				Velvick			
	Section 1	Section 2	Section 3	Average	Section 1	Section 2	Section 3	Average
2 minutes								
Control (no Ca injected)	3186	3477	3682	3448	3710	3756	3984	3816
0.82	3208	3768	3322	3432	4911	3791	3630	4110
0.41	3559	3632	3381	3523	4238	4103	3836	4059
0.041	3094	3254	3251	3199	4266	4228	3724	4072
P value = 0.248	ns	ns	ns	ns	ns	ns	ns	ns
5 minutes								
Control (no Ca injected)	3186	3477	3682	3448	3710	3756	3984	3816
0.82	3209	2967	2940	3038	3644	3673	3376	3564
0.41	3330	3074	2664	3022	3636	3613	3105	3451
0.041	3346	3298	2626	3090	3671	3714	3309	3564
P value = 0.248	ns	ns	ns	ns	ns	ns	ns	ns
10 minutes								
Control (no Ca injected)	3186	3477	3682	3448	3710	3756	3984	3816
0.82	3121	3714	3252	3362	3966	3777	4532	4091
0.41	3004	3641	3259	3301	3624	3627	3953	3734
0.041	3570	3306	3529	3468	3896	3419	4469	3928
P value = 0.248	ns	ns	ns	ns	ns	ns	ns	ns
20 minutes								
Control (no Ca injected)	3186	3477	3682	3448	3710	3756	3984	3816
0.82	3100	3578	3419	3365	3536	3515	3140	3397
0.41	3151	3408	3618	3392	3688	3639	3151	3492
0.041	3209	3442	3531	3394	3943	3586	3463	3664
P value = 0.248	ns	ns	ns	ns	ns	ns	ns	ns

P value = probability of significant difference.
ns = no significant difference ($P<0.05$). Values are means of 3 plants per cultivar.

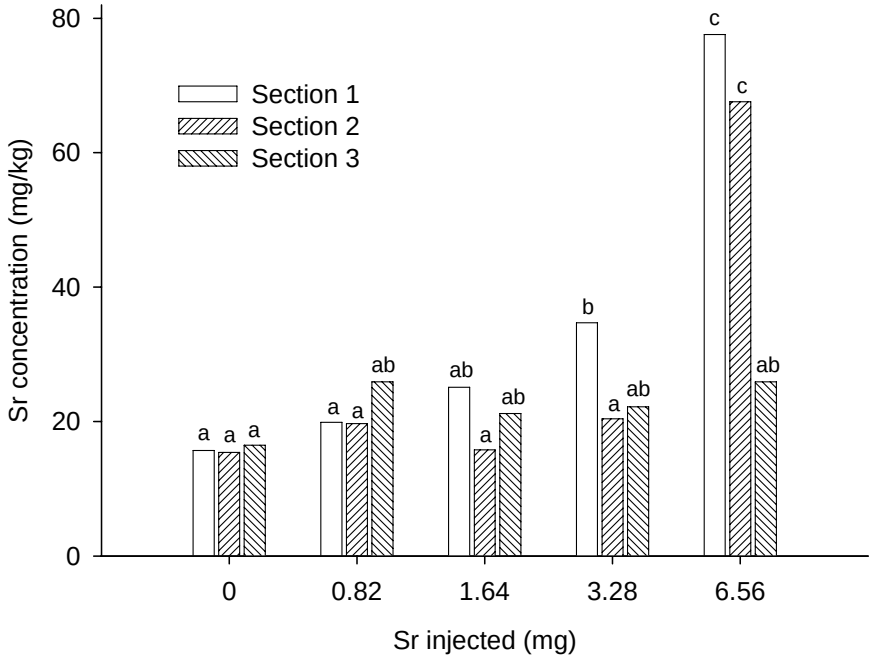

Figure 6.1 Experiment 2. Strontium (Sr) concentration (mg/kg dry weight), in sections 1-3 of Velvick avocado seedlings injected with 0, 0.82, 1.64, 3.28 and 6.56 mg Sr, then stem sections sampled 10 minutes after injection. Two, 3 cm sections were taken just below the graft union (sections 1 and 2) and one 3 cm section taken just above the graft union (section 3). Bars with the same letter are not significantly different at lsd of P<0.05.

6.3.3 Experiment 3: Strontium translocation rate and sampling duration

The injected Sr was detected in sections 1 and 2 directly above the injection site immediately after injection (Figure 6.2) (0 minutes). By 10 minutes after injection the concentration in sections 1 and 2 in Hass on Reed declined and the concentration in section 3 increased. By 15 minutes section 4 (above the graft union) in both combinations had Sr concentrations higher than the control, but by 20 minutes the concentrations in sections 3-5 were similar to the controls.

The concentrations in sections 1 and 2 did not decline, or declined slightly between 10 and 20 minutes, suggesting that this portion of the injected Sr was less mobile, and perhaps bound.

Similar results were obtained for both graft combinations, except that at 15 minutes the Sr concentration in sections 1-3 was higher than sections 4 and 5 (above the graft union) with Hass on Barr Duke, while there was no difference between sections 2-5 with Hass on Reed.

Based on these results, 15 minutes was selected as the duration time for experiment 4 since by this time the injected Sr was detected across the graft union.

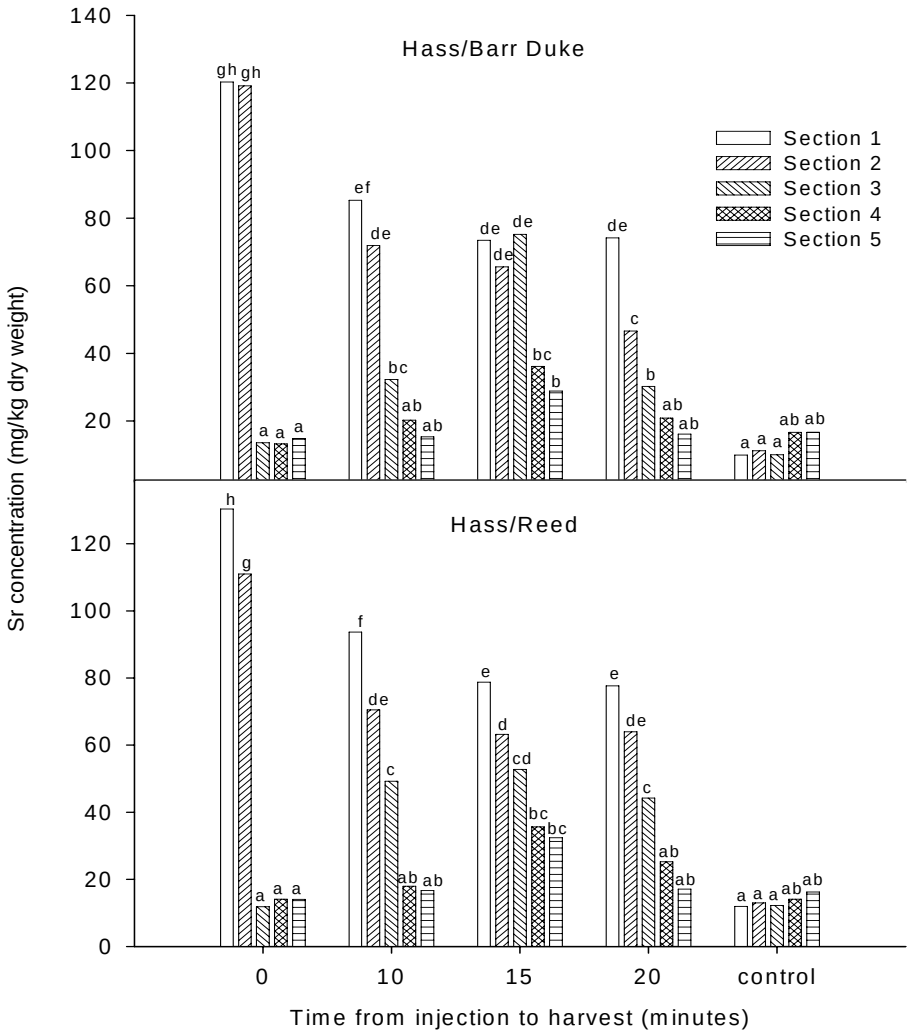

Figure 6.2 Experiment 3. Strontium (Sr) concentration (mg/kg dry weight), in sections 1-5 of Hass on Barr Duke and Hass on Reed avocado seedlings injected with 3.28 mg Sr, then stem sections sampled 0-20 minutes after injection. Three, 3 cm sections were taken just below the graft union (sections 1-3) and two 3 cm sections taken above the graft union (sections 4 and 5). The control trees were not injected. Bars within each graph with the same letter are not significantly different at lsd of P<0.05.

6.3.4 Experiment 4: Rootstock/scion effects on translocation across the graft union

Figure 6.3 shows no differences in Sr concentration between the rootstock and scion in all control treatments. In the injected treatments the Sr concentrations in the Velvick combinations were similar in the rootstock and in the scion. In Hass grafted to Duke 7, the rootstock Sr concentration was higher than the scion concentration, but the concentrations were similar for the non-grafted and self-grafted Duke 7. In Hass, all combinations resulted in higher Sr concentrations in the rootstock than in the scion. Also, the Sr concentration in the non-grafted Hass at the location equivalent to below the graft union in the grafted trees, had higher Sr concentration than at the height equivalent to above the union.

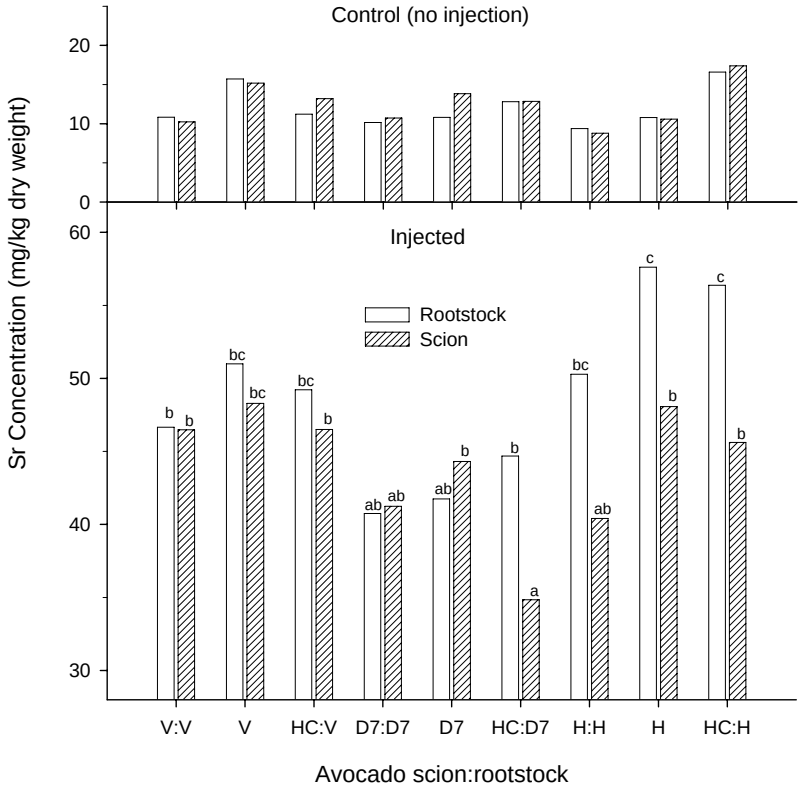

Figure 6.3. Experiment 4.The Sr concentrations (mg/kg dry weight) from below (rootstock) and above (scion) the graft union of 9 avocado graft combinations. The trees were either not injected (control) or injected with 3.28 mg Sr (Injected) 6 cm below the graft union. Stem sections (3 cm long) were taken between the injection site and the graft union (rootstock), and just above the graft union (scion). V:V=Velvick grafted to Velvick, V=non-grafted Velvick, HC:V=Hass grafted to Velvick, D7:D7= Duke 7 grafted to Duke 7, D7=Non-grafted Duke 7, HC:D7=Hass grafted to Duke 7, H:H=Hass grafted to Hass from the same plant, H=non-grafted Hass, and HC:H= commercial Hass grafted to Hass. Bars within each graph with the same letter are not significantly different at lsd of P<0.05. ns= no significant treatment effects for the controls (no Sr injection).

With Hass as the scion, Duke 7 rootstock resulted in lower stomatal conductance than with both Velvick and Hass rootstock (Figure 6.4). There was no difference in the stomatal conductance between different scions within each rootstock, except where Hass on Velvick had higher conductance compared with the other Hass rootstock combinations.

The Hass scion had a smaller stem diameter at harvest than its Velvick rootstock, suggesting scion undergrowth (Figure 6.5). There was no difference in the rootstock and scion diameters for all the other combinations. The diameter of each graft component was not measured at grafting, so it is possible that these differences resulted from differing diameters at grafting. However, that fact that the Hass scion diameter on Velvick is larger than all the other Hass scions suggests that this is not the case.

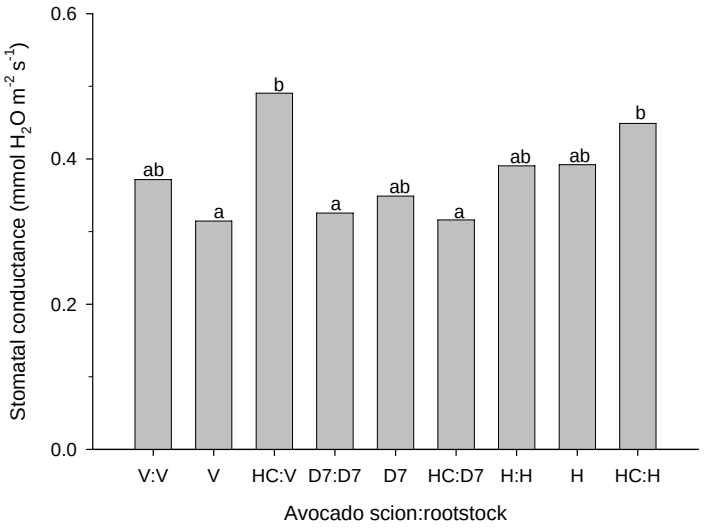

Figure 6.4. Experiment 4. Stomatal conductance of the leaves of 9 avocado graft combinations, measured between 0800-1000 hours at 3 dates before injection. The average of the 3 dates is presented. The readings were taken from 3 leaves per tree and 3 trees per cultivar (for each treatment). V:V=Velvick grafted to Velvick, V=non-grafted Velvick, HC:V=Hass grafted to Velvick, D7:D7= Duke 7 grafted to Duke 7, D7=Non-grafted Duke 7, HC:D7=Hass grafted to Duke 7, H:H=Hass grafted to Hass from the same plant, H=non-grafted Hass, and HC:H= commercial Hass grafted to Hass. Bars with the same letter are not significantly different at lsd of P<0.05.

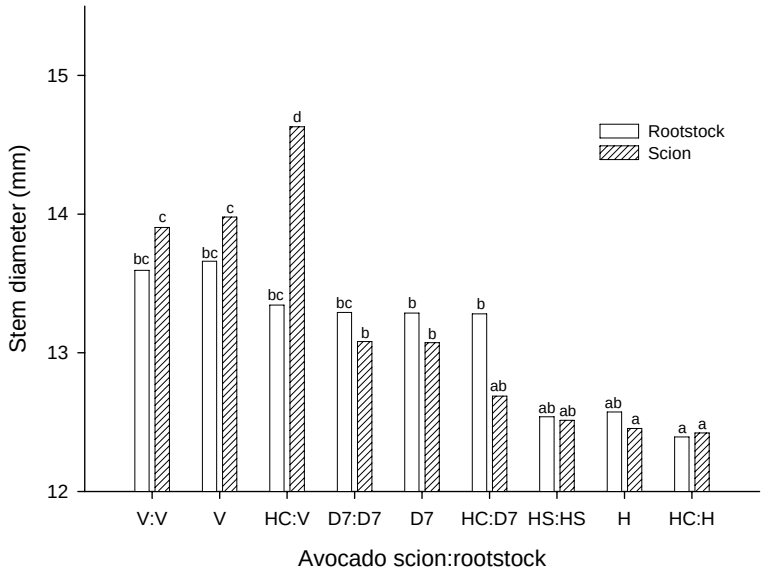

Figure 6.5. Experiment 4. Diameter (mm) of the stem above (scion) and below (rootstock) the graft union of nine avocado graft combinations. There were no treatment differences between Sr injected and control trees. Therefore the data has been pooled. V:V=Velvick grafted to Velvick, V=non-grafted Velvick, HC:V=Hass grafted to Velvick, D7:D7= Duke 7 grafted to Duke 7, D7=Non-grafted Duke 7, HC:D7=Hass grafted to Duke 7, H:H=Hass grafted to Hass from the same plant, H=non-grafted Hass, and HC:H= commercial Hass grafted to Hass. Bars with the same letter are not significantly different at lsd of P<0.05.

6.4 Discussion

This study suggests that 1) the graft union significantly affected Sr transport from rootstock to the scion, 2) the deposition of Sr in the stem at the point of injection was significant, 3) different cultivars exhibited different graft compatibilities and 4) the rootstock/scion stem diameter was not necessarily indicative of the capacity of the graft union to impede Sr, and presumably Ca.

The ability to examine the effects of the graft union and genetics on movement of Ca was based on the capacity to inject sufficient Ca into the stem to raise concentrations above natural or background concentrations. However, the high background concentration and the concern of excessively high Ca causing cell damage (Prof David Edwards, personal communication, 2001), suggested that Ca was not suitable. Strontium is very similar to Ca, so that Sr was expected to reliably indicate the pattern of Ca transport across the graft union (Lembrechts *et al.* 1990; Veresoglou *et al.* 1995).

The presence of Sr in the 6 cm of stem just above the injection site at time 0 (sections 1 and 2 in Experiment 3) suggests that 0.1 ml injection volume was large enough to "push" Sr up the stem without translocation. However, movement into other sections would presumably be due to translocation alone. In addition, only about 1.5% of the injected Sr was detected in the two sections above the injection point at time 0, suggesting that a large percentage moved down the stem during injection or remained near the injection site. A significant percentage of the injected Sr also appeared to be immobilised in sections 1 and 2, since concentrations did not change between 10 and 20 minutes (Experiment 3). Despite these observations, it was clear sufficient Sr movement had occurred to allow conclusions regarding movement and the effects of grafting.

Experiment 4 suggested cultivar and grafting effects on Sr movement. The higher Sr concentrations in the sections below the graft union, compared with those above the graft union in Hass on Duke 7 suggested that Sr translocation across the graft union was restricted in this combination, while the similar concentrations in sections below and above the graft union in Hass on Hass suggests that the graft union per se is not a restricting factor. Thus, because there were no concentration differences in Duke 7 non-grafted and homograft Duke 7, the restriction in Hass on Duke 7 is likely associated with some form of graft incompatibility between Hass and Duke 7. It could be argued that the Hass scion itself may have reduced the "pull" of Sr to the scion, which could have reduced the Sr concentration in the Hass scion rather than some graft union barrier. However, the fact that there were no scion differences in the Velvick combinations, and that the stomatal conductance was the same in all the Duke 7 combinations, suggests that this was most likely not the case.

Similar reductions in Ca concentrations reaching the scion between different scion rootstock combinations has been found in other species such as Bartlett pear (Woodbridge 1973) and apple (Jones 1974).

In contrast, all Hass rootstock combinations resulted in higher Sr concentrations below compared with above the graft union, and even in non-grafted Hass the Sr concentration was higher at the height equivalent to below the graft union in the grafted treatments, compared with the equivalent height above the graft union. This suggests inherently slower movement in Hass rootstock compared with Velvick and Duke 7. The charge density and the exchange mechanism in the xylem vessels may be a factor (Hanger 1979;

Atkinson *et al.* 1992), which can vary between cultivars (Kirkby and Pilbeam 1984). The fact that there were similar Sr gradients in all the Hass rootstock combinations suggests that there was no negative effect of the graft union on Sr translocation in these combinations.

The presence of overgrowth or undergrowth in grafts is often considered to indicate some level of incompatibility and possible restriction in flow between rootstock and scion. This is often associated with phloem degeneration and necrosis, resulting in higher starch concentrations where the overgrowth occurs (Whiley *et al.* 1997). In this study there was no evidence that the slight overgrowth in Hass on Velvick resulted in reduced translocation, since the Sr concentrations were similar below and above the graft union in this combination.

This study demonstrated that different rootstock scion combinations can potentially affect Sr (and Ca) movement across the graft union, presumably because of some level of incompatibility. The indication of little restriction with Hass on Velvick compared with Hass on Duke 7, is in line with better fruit quality observed with the former compared with the latter combination (Willingham *et al.* 2000; Marques *et al.* 2003). This study also demonstrated that the traditional measure of incompatibility does not necessarily indicate restrictions in xylem nutrient movement. Further investigations into different rootstock/scion combinations to identify optimal combinations for new plantings and cultivar selection are warranted in order to maximise the probability of increased fruit quality by selecting rootstocks that minimise Ca transport disruption.

7 Effect of leaf to fruit ratio on fruit growth, quality and minerals

7.1 Introduction

The avocado exhibits polyaxial growth, typified by a synchronous growth pattern with alternating shoot and root flushes (Thorp *et al.* 1993). Bearing avocado trees have two major vegetative flushes per year. The first begins in spring towards the end of flowering, while the second occurs during summer (Scholefield *et al.* 1985). Each flush is followed by a period of enhanced root growth, resulting in a cyclic pattern of shoot and root growth.

New leaves become net exporters of carbohydrates after about 60-80 days. Before that stage the developing leaves compete with developing fruits for available assimilates, water and nutrients during this critical stage of fruit development (Blanke and Notton 1991). While the spring and summer vegetative flushes compete with fruit growth and may increase fruit drop, they are essential for the long term productivity of the tree.

Avocado fruits are borne on two types of inflorescences. Determinate inflorescences terminate with a fruit, whereas indeterminate inflorescences terminate with a vegetative shoot (Blanke *et al.* 1998). Unlike most fruits, both cell division and cell growth in avocados contributes to fruit growth as long as a fruit is attached to the tree (Lee and Young 1983).

Many studies have demonstrated that most of the calcium moves into fruit during the first weeks of development (Wiersum 1966; Adams and Ho 1989). During this time the cell walls and membranes are developing and are sinks for calcium, and most of the water is supplied via the xylem which contains more Ca than phloem (Hanger 1979; Adams and Ho 1989). As the fruit matures water supply is mainly via the phloem, and the role of the xylem decreases. Mobile nutrients such as K and Mg are translocated in the phloem and are available to the fruit during the whole growing season. In contrast, less mobile nutrients such as Ca are in low concentrations in the phloem, so that Ca availability in the latter stages of fruit growth is restricted (Atwell *et al.* 1999).

The leaf to fruit ratio is significant in relation to Ca accumulation in fruit. Since Ca moves mainly in the xylem, plant tissues such as leaves that have the greatest water use will generally accumulate more Ca. In addition, the fruit Ca concentration is determined by a balance in the uptake of Ca, water and carbohydrates into the fruit. If carbohydrate supply is high, for example when the leaf to fruit ratio is high, the uptake of carbohydrates and water can be greater than Ca uptake. In this case the fruit may be large but have lower Ca concentrations (Beverly *et al.* 1993), with negative effects on fruit quality.

This study investigated the influence of leaf to fruit ratio on fruit growth, minerals, and quality. A preliminary trial in 2000/01 used ratios of 10 to 60 leaves per fruit, plus several controls. Ten leaves per fruit was found to be too low, so 30-120 leaves per fruit were investigated in 2002/03. Girdles were applied to restrict movement of carbohydrates away from the leaves and subtending fruit, and minimise the contributions from stored carbohydrates. Movement of minerals to these fruit via the xylem would have been unaffected (Davie and Stassen 1997).

7.2 Materials and methods

7.2.1 Experimental site

The 2 trials were conducted in a commercial avocado orchard at Beerwah, south east Queensland, Australia (latitude 26.90°S, longitude 152.80°E, altitude 30 m). The climate was subtropical with an average annual rainfall of 1,752 mm, with less rain in winter than in summer. The first trial was conducted from September 2000 to May 2001. A second trial was abandoned at the end of 2001 due to large fruit drop caused by unfavourable weather conditions, but was repeated between September 2002 to May 2003.

Four, visually healthy and uniform Hass trees were selected close together in each of 5 adjacent rows (total of 20 trees). The trees were 8 years old on seedling rootstocks of unknown origin. The site was on sandy loam soil, on a gentle slope with good drainage. The trees were irrigated with under-tree sprinklers. Peak flowering occurred in the first 2 weeks of September for both trials. The trees were managed under standard commercial practices (Newett *et al.* 2001).

7.2.2 Treatments

Suitable indeterminate panicles (about 5 per tree) were tagged at flowering. Within 4 weeks of flowering, excess fruit were removed and a girdle applied at a location on the branch that resulted in 10, 30, 60 or 120 leaves per fruit above the girdle. The girdles were applied when the spring flush was fully expanded at about 4 weeks after flowering. A 10 mm wide strip of bark was removed by cutting the bark with two razor blades mounted 10 mm apart on a wooden block. The girdles were maintained during fruit growth by removing any scar tissue. Vegetative growth occurring within the girdled branch was removed during fruit growth to maintain the correct leaf to fruit ratio.

The following treatments were applied in 2000/01:

- 10 leaves per fruit
- 30 leaves per fruit
- 60 leaves per fruit
- Control non-girdled (CNG; no leaf or fruit removal, and no girdling). This resulted in about 60 leaves per fruit on the branch.
- Control girdled (CG), with no leaf or fruit removal, but girdling at about 30 cm from the terminal of the branch. This was about the average distance for all girdled treatments. There were about 40 leaves per fruit above the girdle.

In 2002-03, 30, 60 and 120 leaves per fruit were used. The two control treatments were the same as 2000/01. The CNG treatment had about 80 leaves per fruit. The girdle in GC was placed about 80 cm from the terminal, with about 100 leaves above the girdle.

7.2.3 Fruit development and quality

Fruit size was determined by measuring length and breadth fortnightly using digital callipers. Percentage fruit retention was determined by counting the number of tagged panicles that retained fruit. The average leaf area for each treatment was determined by taking 10 leaves per treatment per tree from 5 representative trees, and determining leaf area using a leaf area meter.

The fruit were harvested at commercial maturity (late May). The fruit were individually labelled, measured for weight, length and breadth, then ripened at 20°C (no ethylene). In

the 2000/01 trial only, the fruit were treated with 0.55 ml L^{-1} Sportak® (a.i. 450g L^{-1} Prochloraz) for 30 s within 2 hours of harvest for disease control.

At the eating ripe stage, the fruit were weighed. Fruit firmness was determined by gently squeezing the fruit in the palm of the hand. Hand assessment was regularly calibrated using an 'Anderson Firmometer' (Anderson Manufacturing and Toolmaking, New Zealand). Calibration was based on the distance (mm x 10) a 17 mm diameter curved probe penetrated into the fruit (skin not removed) in 10 sec using a 200 g weight (White *et al.* 2001). The days to ripe (DTR) was recorded as the number of days after harvest for fruit to attain a reading of approximately 80 on the firmometer. This corresponded to a firmness of about 4-5 N when measured with an Instron Universal Testing Machine model 1122 (Instron Ltd, UK), fitted with an 8 mm-diameter hemispherical probe (probe penetration 2 mm).

The skin colour at ripe was rated using the Avocare Assessment Manual (White *et al.* 2001) skin colour rating scale (Table 7.1).

Table 7.1 Colour rating scale used for Hass avocado skin colour

Scale	Skin Colour
1	Emerald green
2	Forest green (fruit is not shiny)
3	Some black on green; fruit mainly green (>50% green)
4	Some green on black; fruit is mainly dark (>50% dark)
5	Purple; skin is completely dark
6	Black

The ripe fruit were then cut longitudinally into quarters and the seed and skin removed. The quarters were visually rated for rots and internal flesh disorders as the percentage of the flesh volume affected. The results are presented as severity (% of the flesh affected), and incidence (% of fruit with at least 1% of the flesh volume affected).

Rots were rated based on the location of the lesion on the fruit. Body rots, mainly caused by *Colletotrichum* spp. (Coates *et al.* 1995), were characterised as those developing from the skin into the body of the fruit. Stem end rots, mainly caused by *Dothiorella* spp. (Coates *et al.* 1995), were rated as those starting from the stem end of the fruit. Diffuse discolouration (generally associated with chilling damage) was characterised as areas of grey or grey/brown discolouration with poorly defined margins. Bruising was characterised as areas of distinct dark grey to black discolouration often with air pockets in the flesh. Vascular browning was present when parts of the vascular bundles were discoloured.

7.2.4 Percent dry matter and minerals

The % dry matter and mineral concentrations were determined for individual fruit as described in Section 4.2.3.

7.2.5 Statistical analysis

In both years each tree (replicate) had one replicate (branch) for all 5 treatments, evenly distributed around the tree. Thus, 5 branches were used per tree, each with one treatment. Twenty trees were used.

Data were analysed with Genstat 5® (Release 4.21) for Windows (Lawes Agricultural Trust, UK) using residual maximum likelihood because the data was unbalanced, with treatments having different numbers of observations on different trees.

Fruit weight and the weight of individual fruit components (seed, skin and flesh) used the initial model of treatments as fixed effects and trees, branches within trees, and fruit within branches as random effects. The estimate of the variance for branch indicated no branch effect, so the final model included only trees and fruit within trees as random effects.

Percent fruit retention was analysed with trees as random effects and treatments as fixed effects, but the tree effect was estimated as negligible (negative variance) and was removed from the model. Fruit size was analysed with treatments as fixed effects and trees, branches within trees, and fruit within branches as random effects.

The models for fruit quality parameters were:

Tree, branch and fruit for % dry matter
Tree and fruit for DTR, fruit colour, stem end rots and body rots
Branch and fruit for bruising and diffuse discolouration
Fruit for vascular browning

For minerals, the final models used were:

Tree, branch and fruit for Mg
Branch and fruit for B, Ca and K

7.3 Results

7.3.1 Fruit size

In both seasons avocado fruit showed sigmoidal fruit growth, with a gradual increase until 8 weeks, followed by a rapid increase until 20 weeks then reduced growth until 44 weeks after fruit set (Figure 7.1).

In both years there was no treatment difference for the first 10-12 weeks after flowering. In 2000/01 CNG fruit were significantly larger than 10 leaves per fruit from 12 weeks on, and from about 20 weeks on were also larger than the 30 leaves per fruit. During the latter stages of fruit growth 10 leaves per fruit had smaller fruit than 60 leaves per fruit.

Similar treatment responses were noted in 2002/03, with CNG fruit being larger than 30 leaves per fruit from about week 16 on, and 30 leaves per fruit being smaller than 120 leaves per fruit from about 24 weeks. There was little difference between 60 and 120 leaves per fruit and CG fruit throughout fruit development.

In both years the treatment effects on fruit growth became obvious during rapid fruit growth, with the differences increasing as the fruit matured.

Leaf to fruit ratio also affected the ratio of the length to breadth (l:b) (Figure 7.2). In both years CNG fruit had a higher l:b (narrower fruit) than 10 leaves (2000/01) and 30 leaves (2002/03) but not at all measurement times. There were no significant differences between the other treatments. A leaf to fruit ratio greater than 30 leaves was required to achieve l:b ratios similar to the control fruit (CG and CNG). More leaves per fruit resulted in a more elongated, less round fruit.

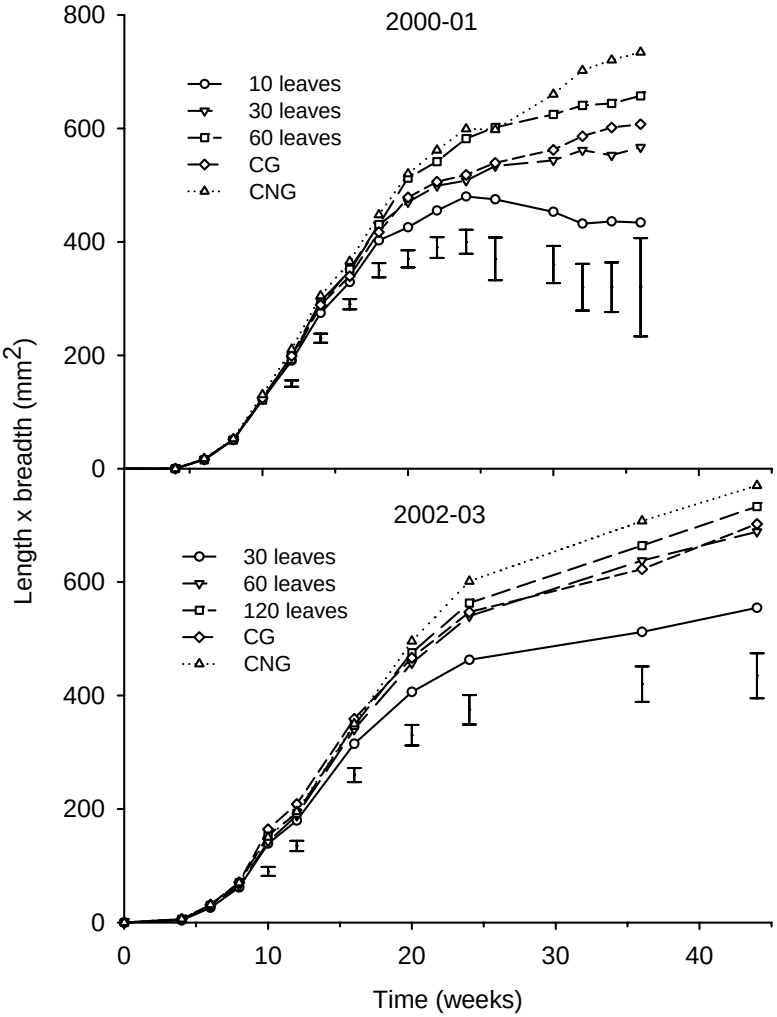

Figure 7.1 The length by breadth of Hass avocado fruit from branches with differing leaf to fruit ratios, and with either no girdling (CNG), or girdling but no leaf or fruit removal (CG). The results are from the 2000-01 and 2002-03 seasons from 4 weeks after flowering. The vertical bars indicate lsd ($P<0.05$) for comparison between treatments at each harvest. There were no significant treatment effects at harvest times with no lsd bar.

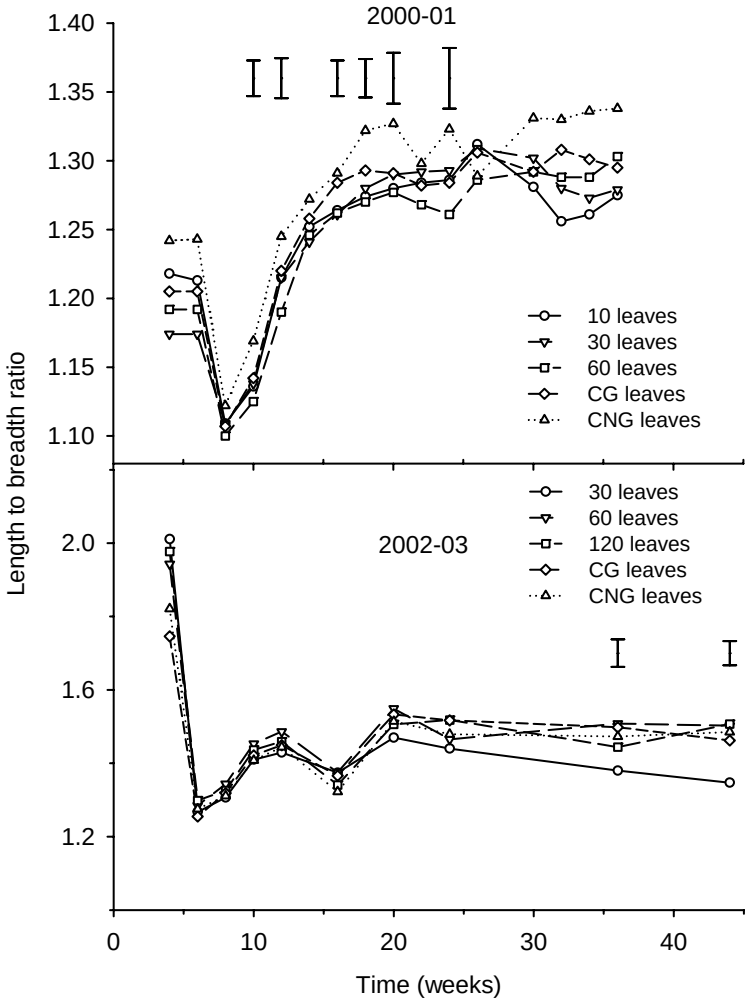

Figure 7.2 Fruit length to breadth ratio of Hass avocado fruit from branches with differing leaf to fruit ratios, and with either no girdling (CNG), or girdling but no leaf or fruit removal (CG). The results are from the 2000-01 and 2002-03 seasons, commencing 4 weeks after flowering. The vertical bars indicate lsd (P<0.05) for each harvest. There were no significant treatment effects at harvest times with no lsd bar.

7.3.2 Fruit retention

There were no significant treatment effects on % fruit retention between 0-12 weeks in 2000/01. From about 14-18 weeks, 30 and 60 leaves per fruit retained more fruit than the 10 leaves and CG treatments (Figure 7.3). However, from weeks 20 to 44 the 60 leaves and CNG treatments had higher fruit retention than the CG, 10 and 30 leaf treatments. In 2002/03 from 8 weeks on, CNG had higher % retention than 30 and 60 leaves. At most sampling times after 8 weeks, the 120 leaf treatment had higher fruit retention than the 30 leaf treatment. In both years CNG had higher fruit retention than CG in the latter stages of fruit growth.

In 2000/01, initial fruit drop occurred between 6-8 weeks, with a very large decrease in % retention occurring between 14-20 weeks. In 2002/03 an initial fruit drop occurred in weeks 6-8 for the lower leaf:fruit ratio treatments, with no large fruit drop period thereafter.

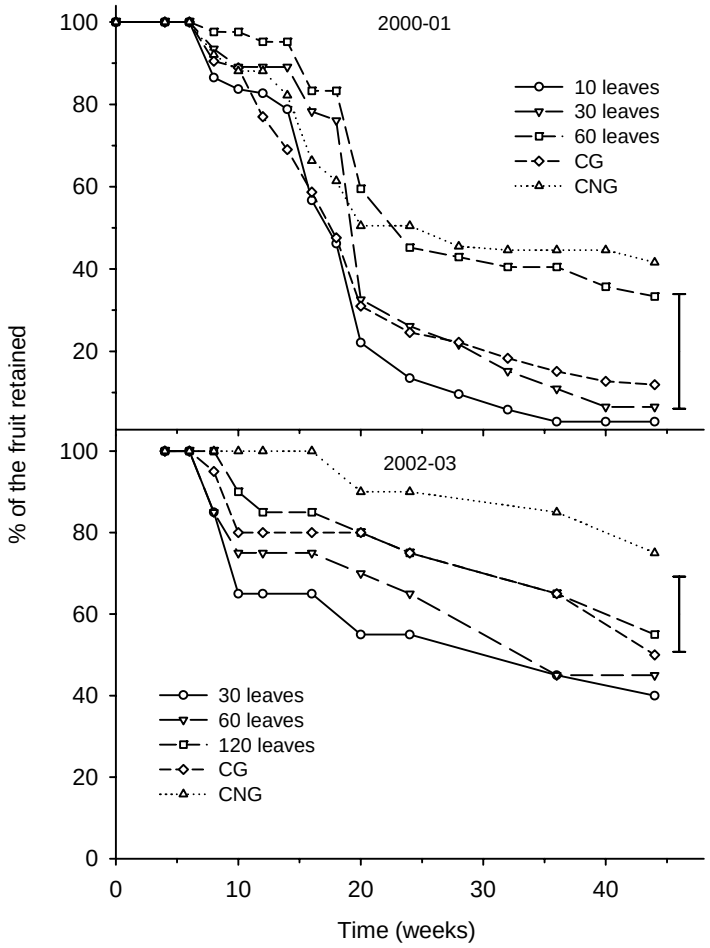

Figure 7.3 The percentage of fruit retained from branches with differing leaf to fruit ratios, and either no girdling (CNG), or girdling but no leaf or fruit removal (CG). The results are for the 2000-01 and 2002-03 seasons, from 4 weeks after flowering. The vertical bars indicate lsd (P<0.05) for comparison between harvest and treatments.

7.3.3 Fruit mineral concentrations

In 2000/01 the flesh Ca concentration was higher in the 10 leaf treatment compared with all other treatments except 30 leaves per fruit (Table 7.2). The non-girdled control had lower Ca concentrations compared with all the girdled treatments apart from the girdled control.

In 2002/03, the 30 leaf treatment had higher fruit Ca concentration than all the other treatments, and there were no significant differences between these remaining treatments (Table 7.3).

In 2000/01 the 10 leaf treatment had higher flesh Mg concentrations than the CNG treatment, but there was little consistent effect between the other treatments. There was no significant treatment effect on Mg in 2002/03, or on flesh K in both years.

In both years the lowest leaf to fruit ratio (10 leaves for 2000/01 and 30 leaves in 2002/03) had the lowest K/Ca, Mg/Ca and (K+Mg)/Ca ratios, mainly as a result of the higher Ca in these treatments (Table 7.2 and Table 7.3). In 2000/01, the 30 leaf treatment had lower

ratios compared with CNG, but there was no significant difference between 60 leaf, CG and CNG treatments. In 2002/03, there were no significant differences in the ratios between 60, 120, CG and CNG treatments.

Table 7.2 2000/01. Mature 'Hass' avocado fruit flesh B, Ca , Mg and K concentrations (mg/kg dry weight) from branches with differing leaf to fruit ratios, and either no girdling (CNG), or girdling (CG) but no leaf or fruit removal. The ratios of K/Ca, Mg/Ca, and K+Mg)/Ca are also presented. Means within each column followed by the same letter are not significantly different at lsd of P<0.05.

Treatment	Minerals concentration (mg/kg dry weight)						
	B	Ca	K	Mg	K/Ca	Mg/Ca	(K+Mg)/Ca
10 leaves per fruit	95.36	798.6 [c]	24143	1442 [b]	25.3 [a]	1.82 [a]	27.1 [a]
30 leaves per fruit	83.15	651.5 [bc]	26387	1324 [ab]	32.2 [a]	1.96 [a]	34.1 [a]
60 leaves per fruit	83.10	611.3 [b]	25293	1446 [b]	56.8 [ab]	2.80 [ab]	59.6 [ab]
CG	100.19	522.9 [ab]	20093	1358 [ab]	41.3 [ab]	2.43 [ab]	43.7 [ab]
CNG	86.83	398.9 [a]	20990	1172 [a]	72.2 [b]	3.20 [b]	75.4 [b]
P value	ns	<0.001	ns	<0.001	0.007	0.023	0.007
lsd value		163.76		215.2	39.83	1.217	40.99

P value = probability of significant difference
lsd value = least significant difference at P<0.05.
ns = no significant difference (P>0.05).

Table 7.3 2002/03. Mature 'Hass' avocado fruit flesh B, Ca , Mg and K concentrations (mg/kg dry weight) from branches with differing leaf to fruit ratios, and either no girdling (CNG), or girdling (CG) but no leaf or fruit removal. The ratios of K/Ca, Mg/Ca, and K+Mg)/Ca are also presented. Means within each column followed by the same letter are not significantly different at lsd of P<0.05.

Treatment	Minerals concentration (mg/kg dry weight)						
	B	Ca	K	Mg	K/Ca	Mg/Ca	(K+Mg)/Ca
30 leaves per fruit	58.6	320 [b]	14752	811	49.5	2.64 [a]	52.2 [a]
60 leaves per fruit	62.6	261 [a]	15165	807	61.1	3.21 [b]	64.3 [b]
120 leaves per fruit	60.7	247 [a]	14597	823	60.6	3.47 [b]	64.1 [b]
CG	63.9	257 [a]	14439	818	59.3	3.33 [b]	62.4 [b]
CNG	65.8	258 [a]	15310	867	64.7	3.55 [b]	68.2 [b]
P value	ns	0.01	ns	ns	0.09	0.02	0.01
lsd value		36.8			9.7	0.499	10.1

P value = probability of significant difference.
lsd value = least significant difference at P<0.05.
ns = no significant difference (P>0.05).

7.3.4 Fruit weight and quality

In 2000/01 there were no treatment effects on fruit weight at harvest or when ripe, the weight of the flesh, skin or seed or % dry matter when ripe (data not presented). There were also no treatment effects on rots or internal disorders, mainly because of little disease and no flesh internal disorders.

However, in 2002/03 the 30 leaves per fruit treatment had significantly lower unripe and ripe fruit weight, flesh weight and % flesh weight compared with the other treatments, and significantly greater % of the total fruit weight as seed and skin (% seed and % skin weight) (Table 7.4). CG fruit had significantly higher fruit and flesh weight than the other treatments except 120 leaves per fruit, and significantly greater % flesh weight than 30 and 60 leaves per fruit. There was little difference between the other treatments.

Table 7.4 2002/03. The weight of Hass avocado fruit at harvest and when ripe, the weight of the ripe fruit flesh, and the proportion of the skin, seed and flesh relative to whole fruit weight. The % of the ripe flesh volume with bruising is also presented. The fruit were obtained from branches with differing leaf to fruit ratios, and with either no girdling (CNG), or girdling but no leaf or fruit removal (CG). Means within each column followed by the same letter are not significantly different at lsd of P<0.05.

	Weight (g)			% of total fruit weight			Flesh
Treatment	**Unripe fruit**	**Ripe fruit**	**Flesh**	**Skin**	**Seed**	**Flesh**	**Bruising (%)**
30 leaves	184 [a]	169.9 [a]	113 [a]	17.53 [c]	15.29 [b]	67.21 [a]	0.13
60 leaves	229.6 [b]	214.7 [b]	155 [b]	15.51 [b]	12.49 [a]	71.99 [b]	1.10
120 leaves	247.6 [bc]	231.2 [bc]	169 [bc]	15.42 [b]	12.01 [a]	72.57 [bc]	1.97
CG	266.1 [c]	249.9 [c]	186 [c]	13.85 [a]	12.03 [a]	74.13 [c]	1.70
CNG	235.1 [b]	221.6 [b]	162 [b]	14.53 [a]	12.08 [a]	73.4 [bc]	1.43
P value	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.095
Lsd value	23.68	22.45	17.32	1.5	1.01	1.72	

P value = probability of significant difference.
lsd value = least significant difference at P<0.05.

There were no significant treatment effects on the DTR or the fruit dry matter. There were also no significant effects on stem end or body rots and no vascular browning or diffuse discolouration was observed. There was a trend of less bruising in the 30 leaves per fruit treatment, but the bruising severity was very low.

7.3.5 Leaf area

In 2002/03, leaves from the 30 and 60 leaves per fruit treatment were smaller compared with those from the other treatments (Table 7.5).

7.4 Discussion

In avocado, competition between young developing fruit and the spring vegetative flush can reduce fruit set and fruit size (Snijder *et al.* 2000). The developing leaves are not net exporters of photosynthate until about 60-80 days, during which time they compete with the developing fruits for carbohydrates. In many fruits, reducing competing vegetative vigour increases fruit size and retention, and similar results have been obtained in avocado (Penter and Stassen 1999). This has been achieved by managed N nutrition (Embleton *et al.* 1968) pruning the spring flush (Blumenfeld *et al.* 1983) and by foliar growth retardant sprays (Köhne and Kremer-Köhne 1987). Most of these treatments have their effect through controlling leaf to fruit ratio, but specific studies on the leaf to fruit ratio in avocado have not been studied.

The present results suggest that leaf to fruit ratios of about 60 resulted in similar fruit size and final fruit weight to at least the CNG control. The higher % fruit retention with 60 leaves compared with 30 leaves per fruit in 2002/03 suggests that 60 leaves per fruit is a good balance between fruit size and retention. There was little additional benefit of 120 leaves per fruit in relation to fruit size or retention.

Girdling removes the phloem connection to the rest of the tree, restricting the movement of carbohydrates from other parts of the tree to the girdled tissue, and *vice versa*. Thus, the higher % fruit retention in CNG compared with CG and the lower leaf ratio treatments confirms the role that carbohydrate reserves in other parts of the tree, or other phloem factors, play in fruit retention. This is supported by studies where girdling reduced fruit yield in other fruit crops (Ferguson and Watkins 1992; Volz *et al.* 1993). In addition,

removing all the leaves on non-girdled, fruiting branches did not affect apple fruit yield when compared with control branches (no leaf removal and no girdle) (Chalmers *et al.* 1975) because of supplies from other parts of the plant.

Table 7.5 The average leaf area (mm^2) from branches with differing leaf to fruit ratios, and either no girdling (CNG), or girdling but no leaf or fruit removal (CG). The results are for 2000-01 and 2002-03. Means followed by the same letter within each year are not significantly different at lsd of P<0.05.

Treatment	Leaf area (mm^2)
2000/01	
10 leaves	124.1
30 leaves	137.9
60 leaves	150.9
CG (approx 40 leaves)	139.9
CNG (approx 60 leaves)	142.2
P value	ns
2002/03	
30 leaves	139.0 [a]
60 leaves	150.47 [a]
120 leaves	178.88 [b]
CG (approx 80 leaves)	168.33 [b]
CNG (approx 100 leaves)	173.48 [b]
P value	0.042
Lsd value	16.63

P value = probability of significant difference.
Lsd value = least significant difference (P<0.05).
ns = no significant difference (P>0.05).

The smaller leaf area in the lower leaf:fruit ratio treatments would suggest that, under higher carbohydrate demand, leaf area could be reduced. However, the leaves from the lower leaf:fruit ratio treatments may have higher photosynthetic activity, as was observed in mango (Simmons *et al.* 1998). These leaves also had lower starch concentrations because the extra energy demands from the fruit reduced accumulation of starch in these leaves.

Avocado flesh Ca concentration, and ratios with K and Mg, have been related to physiological disorders such as chilling injury, pulp spot and vascular browning (Thorp *et al.* 1995; Davie and Stassen 1997), as well as fruit rots (Penter and Stassen 2000; Hofman *et al.* 2002). The fact that these relationships were not observed in these trials may be related to the low rots severity in 2000/01, and the fact that physiological disorders are uncommon in non-stored fruit.

The higher flesh Ca concentration in the 10 (2000/01) and 30 (2002/03) leaves per fruit treatment is similar to the higher Ca concentrations in fruit from trees with higher yield (Vuthapanich 2001). Vuthapanich (2001) did not determine leaf:fruit ratio directly, but the experimental trees were of the same visual size, so fruit yield would have been indicative of the leaf:fruit ratio. They found that higher tree yield was associated with smaller fruit, higher fruit Ca concentrations and reduced rots and diffuse discolouration. This association between crop load and fruit quality is confirmed by the fact that, on a whole of industry basis, on-years in South Africa (presumably due to higher yields per tree) are associated with better outturn quality in export markets due to less defects (Korsten, personal

communication, 2001). Similar trends have been observed where rootstocks that produced better quality scion fruit also often had higher yield (Willingham *et al.* 2003).

These results illustrate the significance of maintaining a correct balance between the accumulation of carbohydrates, water and minerals into the developing fruit (Beverly *et al.* 1993). Higher leaf:fruit ratios result in greater carbohydrate supply to the fruit, which also increases the movement of water to the fruit by osmotic pressure. However, if Ca accumulation cannot keep pace with the increase in carbohydrates and water, then sub-optimal flesh mineral concentrations can result.

In both years this higher Ca appeared to be the main factor in the lower ratios of K/Ca, Mg/Ca and (K+Mg)/Ca. Higher K and Mg concentrations in the girdled compared with the control treatments may also have contributed to the lower ratios in 2000/01, but this was not the case in 2002/03. Therefore, the leaf:fruit ratio and perhaps fruit size appears to have minimal influence on flesh Mg and K concentrations.

In summary, this trial has shown that fruit weight and retention was influenced by the leaf:fruit ratio. This could also potentially affect fruit quality by influencing fruit minerals concentrations, so that a balance is required between fruit size and quality. Although care is needed to extrapolate the data to whole trees, the results suggest that about 60 leaves per fruit is a good reference point for commercial production. There is little benefit to more leaves per fruit, and fewer leaves per fruit may result in higher fruit Ca concentrations, but less fruit retention and yield.

8 Accumulation and distribution of Ca in the developing fruit

8.1 Introduction

The processes just before flowering and during fruit development determine many quality attributes of fruits, such as texture, colour, taste, and nutritional value (Duester 2001). One of the processes that is known to be important to final fruit quality is the accumulation of fruit minerals, especially Ca (Thorp *et al.* 1995; Hofman *et al.* 2002).

Calcium, water and other essential nutrients are distributed within the fruit via the vascular network arising from the seed coat (Moore-Gordon *et al.* 1998). The uptake of Ca during the first 10 or so weeks is crucial because this is the period of rapid cell division when new cell walls and membranes are formed (Adams and Ho 1989). Calcium most likely accumulates in these developing fruits because of the rapid growth creating a sink (Hanger 1979), and also because of the relatively higher transpiration of young compared with older fruit because of their larger surface area to volume ratio. Unlike most fruit, cell division is a significant part of avocado fruit growth during the whole fruit growth period (Whiley and Schaffer 1994).

The seed, which regulates fruit growth, may also influence the distribution of Ca and other nutrients within the fruit (Wolstenholme *et al.* 1985). The seed itself is actively growing and attracting nutrients. In addition, the seed is generally considered as a source of plant growth substances, which are essential in promoting cell division in fruit (Bangerth 1979). Consequently, the seed may increase the flow of Ca to the seed itself and to the flesh and skin by influencing growth in the surrounding fruit tissue. In this respect a study of the distribution of Ca between the fruit tissues could suggest how to increase Ca concentrations in the flesh.

Competition between cations such as Mg and K can also modify Ca transport through non-vascular tissue and during xylem unloading (Harker *et al.* 1988). However, their partitioning between seed, flesh and skin during fruit development is unclear. It is also unclear whether the presence of fruit increases or decreases the sink strength of Ca to the branch, which would influence the source/sink relationship between the fruit and surrounding vegetative tissues.

This section reports on the changes in Ca, Mg and K in the seed, flesh and skin of Hass avocado fruit at several stages during fruit growth. In addition, cation concentrations were determined in leaves close to fruit and on non-fruit branches to determine if the fruit influences cation accumulation into the leaves.

8.2 Materials and methods

8.2.1 Plant material

The experiment was conducted in a commercial orchard at Flaxton in south east Queensland, from October 2001 (fruit set) to August 2002 (harvest). The climate was cool, mesic subtropical, with an average annual rainfall of 2000 mm, in a summer wet, winter dry pattern.

Twenty, ten year old 'Hass' trees located in 4 adjacent east-west oriented rows (blocks for statistical analysis) were used. The trees were on seedling rootstocks of unknown origin. The soil was a krasnozem (ca. 60% clay fraction) of basaltic origin. The soil was well drained and 10 to 16 m deep. The trees were irrigated with under-tree sprinklers, and managed under standard commercial practices (Newett *et al.* 2001).

8.2.2 Treatments

Approximately 150 indeterminate panicles per tree were tagged 2 weeks after flowering. The panicles were selected from all sides of the tree. Fruit were harvested from the tagged panicles at 2 weekly intervals from 4 weeks after flowering until week 12, and at weeks 16 and 34. Ten fruit per tree were sampled at 4 weeks, 5 per tree at 6 and 8 weeks, and 3 fruit per tree for the remaining harvests.

In addition, 5 leaves from branches containing no fruit (called non-fruit leaves; NFL) and from branches directly supported fruit (fruit leaves; FL) were harvested from around the canopy of each tree. The leaves were approximately 40 days old at the first sampling (4 weeks after flowering), or approximately 80% expanded.

The leaves and fruit were cleaned with a wet cloth, then rinsed with distilled water. Within 2 hours of harvest the fruit were separated into skin, flesh and seed. The skin was removed from the fruit with a potato peeler, and the flesh separated from the seed after cutting the fruit in half.

8.2.3 Mineral analysis

The samples were dried, and the dry weight and mineral concentrations determined as described in section 4.2.3.

8.2.4 Statistical analysis

Individual trees were treated as replications and arranged in 4 blocks (rows).

Data were analysed with Genstat 5® (Release 4.21) for Windows (Lawes Agricultural Trust, UK). Mineral concentrations was analysed using analysis of variance (randomised block design) with time as the treatment factor. Variability was assessed between blocks, between trees within blocks and between measurements within trees. The ratios of mineral concentrations (K/Ca, Mg/Ca and (Mg+K)/Ca) were analysed using residual maximum likelihood, with fruit components as fixed effects and variation between blocks, variation between trees within blocks, and variation between samples within trees as random effects.

Mineral ratios were analysed using residual maximum likelihood, with components as fixed effects and variation between blocks, variation between trees within blocks, and variation between samples within trees as random effects.

The dry weight of skin, seed and flesh were analysed using analysis of variance considering time as the treatment factor. Variability was assessed between blocks, between trees within blocks and between measurements within trees. The first analysis considered variation between blocks, between trees within blocks, between measurements on a sampling date within trees, and between units measured within a sampling date within trees. The estimate for the variance component for trees was negative so the variation between trees was excluded from the model in the second analysis. The data was log base e transformed, and the back transformed data presented.

The dry weight of leaf type was analysed as a tissue by harvest time factorial using transformed data, and the back-transformed data presented.

All measurements had equal sample size (balanced data) and the protected least significant difference (lsd) procedure at P = 0.05 was used to test for differences between treatment means (Steel and Torrie 1980).

8.3 Results

8.3.1 Fruit and leaf growth

The dry weight of seed, skin and flesh increased from week 4 to week 34 (Figure 8.1). The flesh dry weight was greater than seed and skin at all sampling times. The dry weight of the seed and flesh increased more rapidly from 12 weeks after flowering.

There was no significant difference in the dry weight of the different leaf types, and there was no interaction between harvest time and leaf type. Leaf weight reached a maximum by 12 weeks after flowering (Figure 8.2).

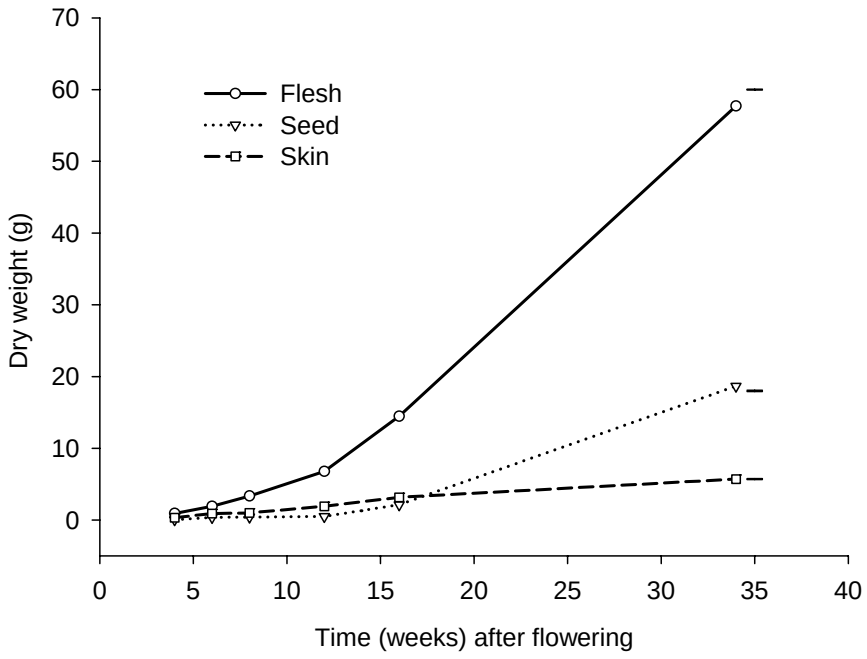

Figure 8.1 The dry weight (g) of Hass avocado fruit flesh, seed and skin from 4 weeks after flowering to maturity. The vertical bar represents lsd (P<0.05) for comparison between harvest times within each tissue.

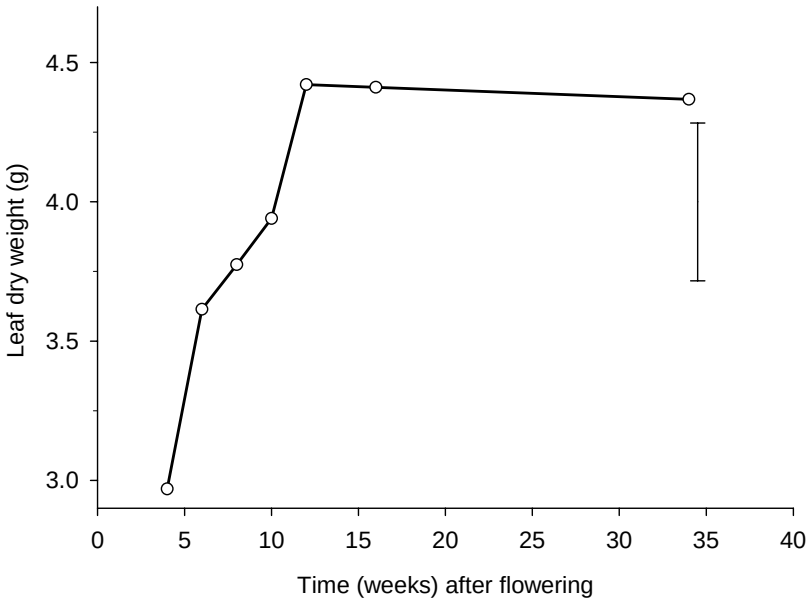

Figure 8.2 The average dry weight (g) of leaves from branches containing no fruit and from branches directly supporting fruit from Hass avocado trees from 4 weeks after flowering to fruit maturity. There were no significant differences (at P<0.05) between the leaf types so the averages are presented. The vertical bar represents lsd at P<0.05.

8.3.2 Mineral concentrations

Calcium concentrations in the seed, flesh and skin was highest at 4 weeks after flowering and generally declined thereafter (Figure 8.3). Concentrations in the skin were higher than in the flesh up to 6 weeks after flowering, but were similar thereafter. Similar changes were noted with Mg.

In contrast, the flesh K concentration increased to week 16 then declined. The seed K concentration increased to about 10 weeks after flowering and declined thereafter, while skin K concentration declined from week 4 onwards.

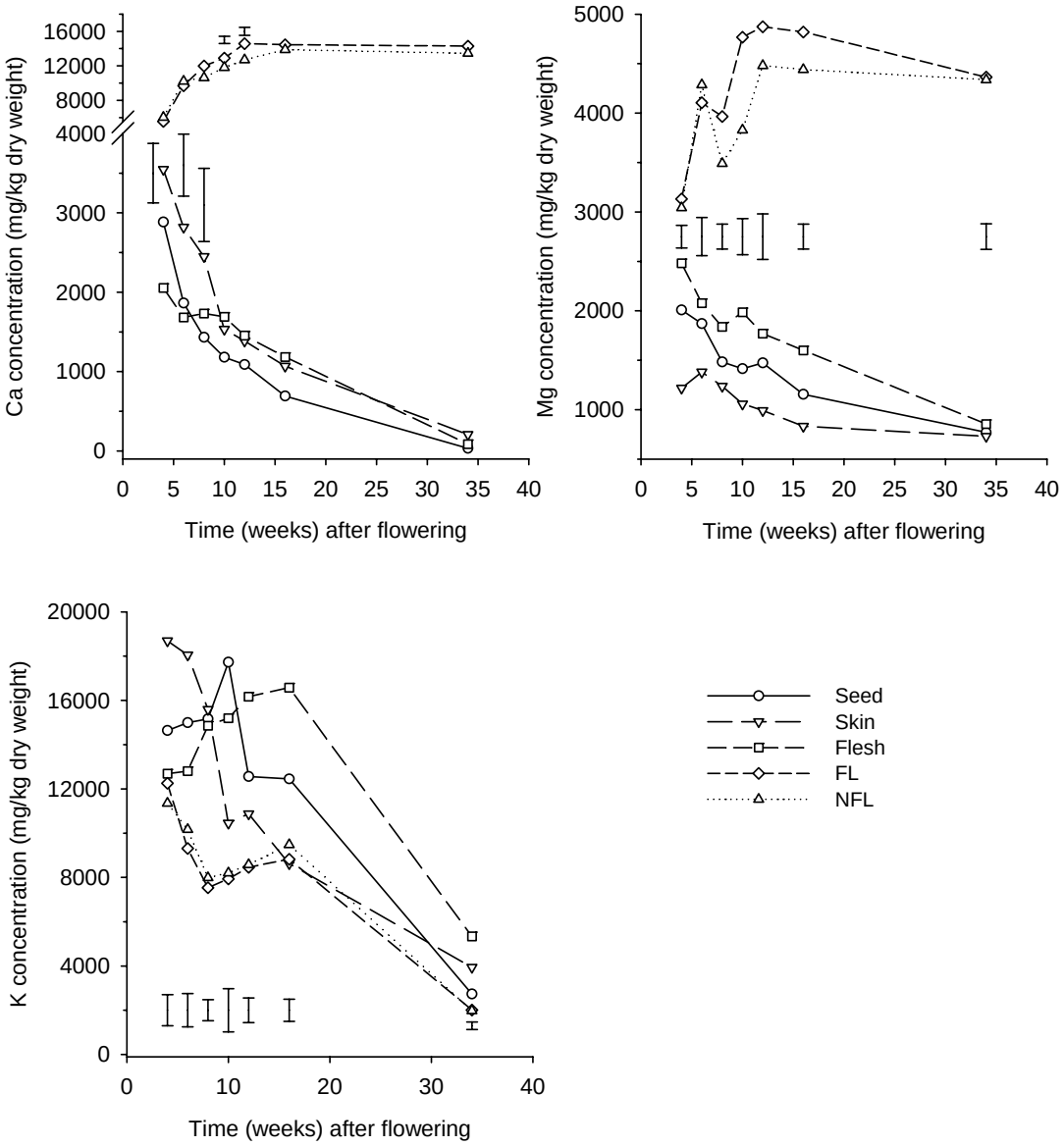

Figure 8.3 Ca, Mg and K concentrations (mg/kg dry weight) in the seed, skin and flesh tissue of Hass avocado fruit, and in the leaves from branches containing no fruit (non-fruit leaves; NFL) and from branches directly supporting fruit (fruit leaves; FL), during fruit development. The vertical bars represents lsd ($P<0.05$) for comparison between tissues at the same harvest. For Ca concentration, lsd bars are presented only for those harvest times with significant differences between flesh, seed and skin, and between the FL and NFL.

Calcium and Mg concentrations were higher in the leaves compared with the fruit tissues at all harvest times, while leaf K concentrations were often similar to the fruit tissues (Figure 8.3). Also, Ca and Mg concentrations increased in the leaves up to 10-12 weeks after flowering, which was opposite to the changes in the fruit tissues. The K concentrations in both leaf types decreased between 4 and 10 weeks and again from 16 to 35 weeks. Ca and Mg concentrations were higher in the FL than the NFL tissue. However, there was no difference in FL and NFL K concentrations.

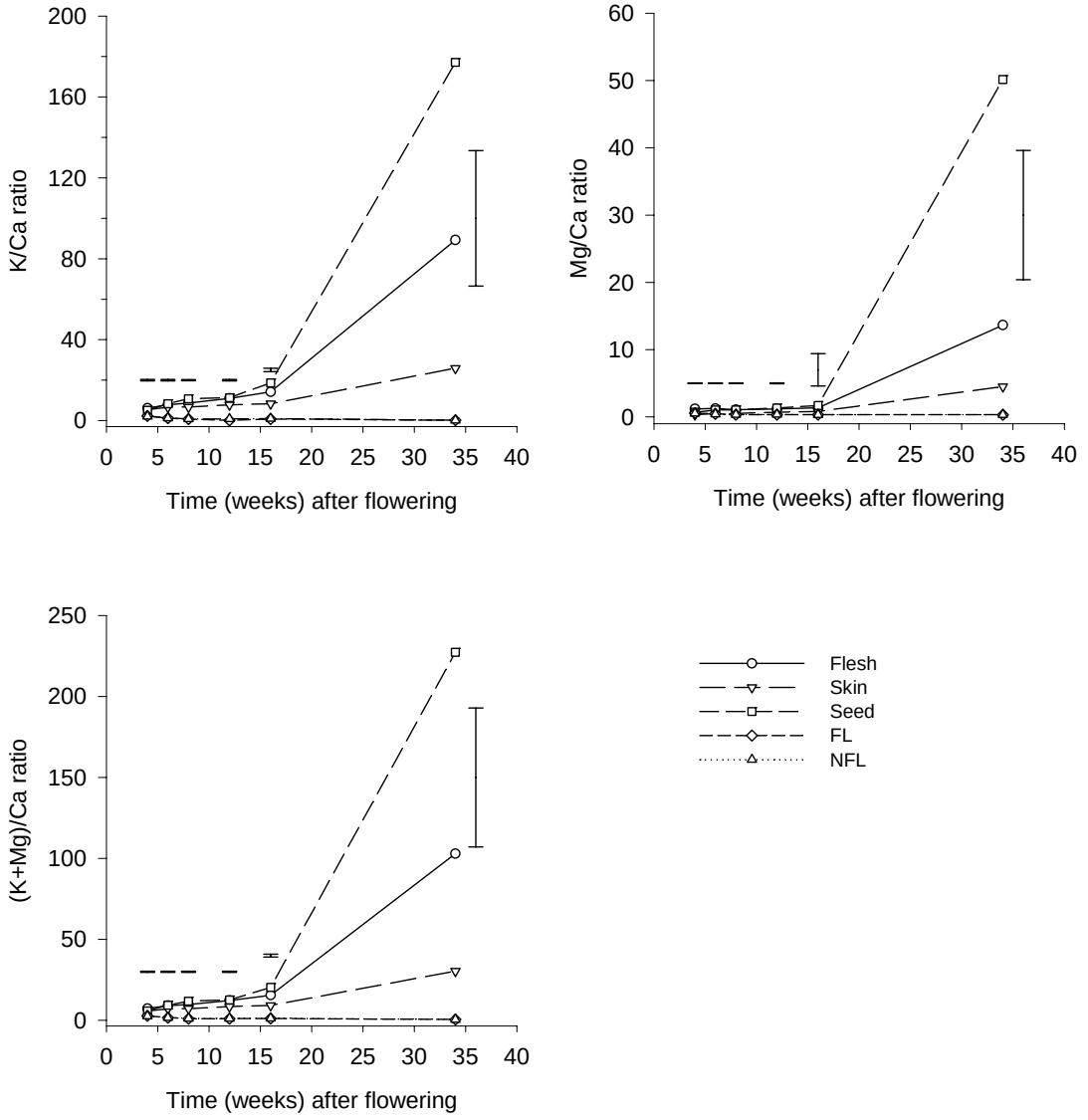

Figure 8.4 The K/Ca, Mg/Ca and (K+Mg)/Ca ratios in the seed, skin, and flesh of Hass avocado fruit, and leaves from branches containing no fruit (called non-fruit leaves; NFL) and from branches directly supporting fruit (fruit leaves; FL), at several sampling times after flowering. The vertical bars represent lsd ($P<0.05$) for comparison between tissues at each harvest.

The K/Ca, Mg/Ca and (K+Mg)/Ca ratios in the skin, seed and flesh increased significantly from 16 weeks after flowering (Figure 8.4), mainly due to the Ca concentration decreasing to a greater extent than Mg and K. In general, the skin had lower K/Ca, Mg/Ca and (K+Mg)/Ca ratios than the flesh at most assessment times, mainly because of the higher Ca concentration and the lower K concentration in the skin.

The ratios in the leaves were lower than in the fruit tissues at most sampling times, mainly because of the higher Ca concentration in the leaves than in the fruit tissue (Figure 8.4). There was little difference in the ratios between the leaf types.

8.4 Discussion

The changes in the minerals concentrations in the leaf and fruit tissues can be interpreted based on the relative sink strength of the tissues and the way in which the cations move in the plant.

Calcium moves mainly in the xylem, and is accumulated in plant tissue primarily based on its evapo-transpiration. Therefore, leaves lose more water than fruit flesh and typically accumulate more Ca. Most of the Ca is rendered insoluble once it enters the tissue, so there is very little re-distribution in the phloem (Ho *et al.* 1995). Potassium, on the other hand, remains soluble and can be re-distributed in the phloem. Hence, its accumulation is influenced more by metabolic activity of the tissue (Van Den Dool and Wolstenholme 1983). Magnesium is also phloem-mobile, but to a lesser extent than K.

As a result, leaf Ca concentrations increased with age and did not decline in the mature leaves. This reflected the import of Ca in the xylem as a result of water loss from the leaves and the Ca being bound in the leaf. The leaf K concentration generally decreased, while the Mg concentration increased then decreased. This reflected the greater mobility of K, and to a lesser extent of Mg.

In the fruit tissue the higher Ca concentration in the skin compared with the flesh during early fruit growth was likely due to the greater water loss from the skin. The continued decrease in Ca concentration in the flesh has been noted in other studies from about 6-10 weeks after flowering (Witney *et al.* 1990a). The decrease in concentration is likely due to a dilution effect from the rapid fruit growth, since the Ca content (mg Ca per fruit) per fruit continued to increase with fruit growth, but the concentration decreased (Witney *et al.* 1990a). Magnesium appears to be more similar to Ca than to K in relation to concentration changes and phloem mobility.

Given the above, the higher K concentration in the young skin may be a result of higher metabolic activity. It is possible that the skin was a source of K for the seed and flesh during the time when skin K concentration was decreasing and flesh and seed K concentration was increasing.

There is also a component of active Ca uptake. Indoleacetic acid (IAA) is a plant growth-regulating substance, and IAA export from tissues is positively correlated with high metabolic activity (Banuelos *et al.* 1988). In addition, there is a direct relation between the export of IAA and the import of Ca into tissues. The IAA concentrations in avocado seed peak at about 4 weeks after flowering, then decline until 20 weeks (Blumenfeld and Gazit 1970). This is a similar pattern as Ca concentrations in the flesh, suggesting that IAA export may be implicated with Ca import into avocado fruit during early fruit growth. Magnesium displayed the same pattern, which suggests that a similar mechanism may also be involved (Marschner 1986).

In most other studies, Ca concentrations increase to week 6-10 after flowering, then decrease (Bower 1985; Witney *et al.* 1990a). It is unclear why the fruit Ca concentrations did not show similar patterns as in these studies. Also, in other studies the Ca concentration in the skin was generally higher than in the flesh and seed. This was observed only in early fruit growth in this study.

The decline in Ca, K and Mg concentrations with fruit growth reflect less uptake of these minerals compared with carbohydrates and water, so that the fruit grew at a greater rate than the accumulation of minerals (Beverly *et al.* 1993). The xylem connections to the fruit decline with age, and because there is little Ca in the phloem, the Ca uptake into the fruit declines. With K, the demand for K from other tissues results in less K entering the fruit via the phloem, or mobilisation from the fruit to the other tissues.

The higher Ca and Mg concentrations in FL tissue may suggest that the fruit increases the flow of these minerals to the leaves. A similar argument has been given for the higher fruit Ca concentrations in indeterminate avocado fruit compared with determinate fruit (Woolf, personal communication, 2003). During early fruit growth the indeterminate fruit had lower Ca concentration than determinant fruit, possibly because the young developing leaves near the indeterminate fruit were stronger sinks for Ca. However, as these leaves matured the indeterminate fruit had higher Ca concentrations than the indeterminate fruit, presumably because the leaves now increased the water flow past the fruit. It is also possible though that the greater metabolic activity in leaves close the fruits (because of the greater demand for energy from the nearby fruits) could directly increase cation movement past these fruit.

The results again confirm that the leaf is a very efficient competitor for Ca, and once in the leaf there is little relocation to other plant tissues, including the fruit. These factors confirm the significance of maintaining transpiration flow to the fruit during growth. Factors that increase the competition for water will reduce water flow to the fruits during early fruit growth at least, and potentially reduce Ca uptake.

9 Conclusions

The following conclusions are based on the observation that Hass on Velvick produced better quality fruit than Hass on Duke 7 in several field studies.

Section 4 indicated that there were significant rootstock effects on leaf and root K and Mg, and possible effects in Ca. Velvick had significantly less K in the leaves and more in the roots compared with the other cultivars, but only some suggestion of higher Ca in the leaves. Therefore it is possible that the better fruit quality associated with Velvick may be related to ability to reduce K uptake into the leaves, as much as an ability to increase Ca. Marques (2003) also noted that Hass on clonal Velvick had lower leaf K concentrations and higher rootstock wood, scion wood, leaf, fruit skin and flesh Ca concentrations than Hass on clonal Duke 7. Therefore, these results suggest that analysis of root and leaf Ca, Mg and K may be good early screening test for rootstocks that could produce fruit with good mineral balance and quality.

Growing avocado cultivars under several K to Ca ratios also confirmed the importance of K in Ca nutrition. A re-examination of K nutrition with a view to reducing application rates and improving timing and application systems is warranted.

There were also strong indications that the rootstock/scion combination can affect movement of Ca across the graft, based on the assumption that Sr shows similar characteristics in the plant as Ca. The Hass/Velvick combination appeared to show little restriction in Sr movement across the graft union, while Hass/Duke 7 showed some restriction. This again agrees with improved fruit quality and higher fruit Ca concentrations than Hass/Duke 7 (Marques *et al.* 2003). This characteristic may be synergistic with the greater restriction in K uptake and enhanced Ca accumulation in

Velvick leaves. Using graft transmission as an early screening method is more difficult than leaf and root mineral analysis because of the need for more controlled conditions, but it may have a role as a later screening test following further technique development.

A leaf to fruit ratio of about 60 was considered an appropriate compromise between fruit size, fruit retention and the desire for high fruit Ca concentrations. Similar conclusions were obtained with mango (Simmons *et al.* 1998). This recommendation was developed using single branches and girdling, so extrapolating to the whole tree needs to be done with some caution. However, for a mature Hass tree, the recommendation relates to about 7 fruit per cubic metre of leaf canopy. For a typical mature orchard with well pruned trees a ratio of 60 equates to about 340 fruit per tree, assuming a total canopy volume of about 100 m^3, and about 50% of the canopy with leaves. The average fruit number per tree for a typical commercial orchard in 2004 was 365 fruit, indicating that a leaf to fruit ratio of 60 is commercially attainable. This equates to a yield of 20 t/ha with 300 trees/ha. Aiming for lower ratios would have further benefits for fruit quality, although the compromise with fruit size needs to be considered.

Other factors can also influence the competition between leaves and fruit and reduce fruit Ca concentrations. The main factor is the presence of competing vegetative flush during early fruit growth. Removing this flush can increase fruit Ca (Cutting and Bower 1990). Pruning systems that increase vegetative flushing during this period can reduce fruit Ca and fruit quality (Leonardi 2003). Therefore, the target of 60 or less leaves per fruit is a guide that needs to be considered with these factors in mind.

The changes in mineral concentrations in the fruit tissues and the leaves were in line with the known physiological behaviour of these nutrients. The results support the importance of water relations to ensure adequate water (and Ca) flow to the developing fruit, and the need to get the right balance between fruit growth and mineral uptake to ensure adequate Ca concentration. There were no indications of how the seed/skin/flesh characteristics can be altered to improve the minerals balance in the flesh.

10 Technology transfer

Minimal activities have been done because of the nature and challenges of the PhD program that conducted the research. Several articles will be published in Talking Avocados this year. The results will also be presented at the national conference in New Zealand in 2005.

Communication has been maintained with members of the AAL R&D committee, especially Dr A Whiley. The results of the project will be discussed in depth with Dr Whiley with a view to maximising the benefits of the results for his avocado rootstock project (AV01007).

11 Recommendations

When rootstocks are being selected for the potential to produce quality scion fruit, roots and leaf mineral concentrations could be measured at the seedling stage to identify the selections that have high Ca and low K in the leaves. This could be considered within the avocado rootstock project (AV01007).

Assessing for movement of Ca (using Sr as a "marker") across the graft union would require further refinement, but could be considered as an additional test.

The K nutritional requirements should be re-examined, since high soil K reduced sap and leaf Ca concentrations in all avocado cultivars tested. Both the annual requirements and the timing and method of application should be considered. Further work is warranted on using branch sap and soil solution for tree nutrition monitoring.

The suggested target of 60 leaves per fruit or less should be considered in the current Canopy management project (AV04008) to determine how pruning practices can help achieve this ratio. Obtaining consistent leaf to fruit ratios from year to year would improve the consistency and predictability of fruit quality.

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14 Appendices

14.1 Appendix 1

Nutrient solutions used in "Uptake of minerals into the roots and leaves of seedling trees", based on Hoagland's nutrient solution (Hoagland and Arnon 1950).

2000 μM Ca per L

The following macronutrient stock solutions were prepared from half strength Hoagland's solution and prepared to 1 L final volume of nutrient solution:

- Stock 1 = 1.00 M $Ca(NO_3)_2$ = 164 g/L ÷2 = 82 g/L (use 4 ml/L of nutrient solution).
- Stock 2 = 1.00 M $NH_4H_2PO_4$ = 115 g/L ÷2 = 57.5 g/L (use 1 ml/L of nutrient solution).
- Stock 3 = 1.00 M KNO_3 = 101 g/L ÷2 = 50.5 g/L (use 6 mL/L of nutrient solution).
- Stock 4 = 1.00 M $MgSO_4$ = 120 g/L ÷2 = 60g/L (use 2 ml/L of nutrient solution).

The following micronutrient stock solution (stock 5) was prepared from double strength Hoagland's solution and prepared to 1 L final volume of nutrient solution (using 1 ml/L of nutrient solution):

- 5.72 g of H_3BO_3
- 3.62 g of $MnCl_2.4H_2O$
- 0.44 g of $ZnSO_4.7H_2O$
- 0.16 g of $CuSO_4.5H_2O$
- 0.04 g of $H_2MoO_4.H_2O$

The following Iron (Fe) stock (stock 6) was prepared from full strength Hoagland's solution and 1 mL/L should be added to the above 5 stocks for 1 L of nutrient solution.

- First dissolve 26.1 g of EDTA in 286 mL of water that has 19 g of KOH. Next dissolve 24.9 g of $FeSO_4.7H_2O$ in 500 mL of water. Slowly add the iron sulfate solution to the potassium EDTA solution and aerate this solution overnight with stirring. Make to 1 L final volume and store in a dark container.

400 μM Ca per L

The same stock solutions will be used as in 2000 μM of Ca. However, the lower concentrations of Ca in this treatment will also result in lower N concentration as compared to treatment 1 in stock 1. As a consequence, stock 4 must be modified to balance the N factor and an additional stock solution of $MgSO_4$ (stock 7) must be made to balance the Mg factor. Therefore the following stocks solutions are required for treatment 2:

Stock 1 = 1.00 M $Ca(NO_3)_2$ = 16.4 g/L (use 4 ml/L of nutrient solution).

Stock 4 = 1.00 M $MgNO_4$ = 40 g/L (use 2 ml/L of nutrient solution).

Stock 7 = 1.00 M $MgSO_4$ = 12 g/L (use 2 ml/L of nutrient solution).

300 μM Ca per L

The same stock solutions will be used as in 2000 μM of Ca. However, the lower concentrations of Ca in this treatment will also result in lower N concentration as compared to treatment 1 in stock 1. As a consequence, stock 4 must be modified to balance the N factor and an additional stock solution of $MgSO_4$ (stock 7) must be made to balance the Mg factor. Therefore the following stocks solutions are required for treatment 3:

Stock 1 = 1.00 M $Ca(NO_3)_2$ = 12.3 g/L (use 4 ml/L of nutrient solution).

Stock 4 = 1.00 M $MgNO_4$ = 42.5 g/L (use 2 ml/L of nutrient solution).

Stock 7 = 1.00 M $MgSO_4$ = 9 g/L (use 2 ml/L of nutrient solution).

200 μM Ca per L

The same stock solutions will be used as in 2000 μM of Ca. However, the lower concentrations of Ca in this treatment will also result in lower N concentration as compared to treatment 1 in stock 1. As a consequence, stock 4 must be modified to balance the N factor and an additional stock solution of $MgSO_4$ (stock 7) must be made to balance the Mg factor. Therefore the following stocks solutions are required for treatment 2:

Stock 1 = 1.00 M $Ca(NO_3)_2$ = 8.2 g/L (use 4 ml/L of nutrient solution).

Stock 4 = 1.00 M $MgNO_4$ = 45 g/L (use 2 ml/L of nutrient solution).

Stock 7 = 1.00 M $MgSO_4$ = 6 g/L (use 2 ml/L of nutrient solution).

100 μM Ca per L

The same stock solutions will be used as in 2000 μM of Ca. However, the lower concentrations of Ca in this treatment will also result in lower N concentration as compared to treatment 1 in stock 1. As a consequence, stock 4 must be modified to balance the N factor and an additional stock solution of $MgSO_4$ (stock 7) must be made to balance the Mg factor. Therefore the following stocks solutions are required for treatment 3:

Stock 1 = 1.00 M $Ca(NO_3)_2$ = 4.1 g/L (use 4 ml/L of nutrient solution).

Stock 4 = 1.00 M $MgNO_4$ = 47.5 g/L (use 2 ml/L of nutrient solution).

Stock 7 = 1.00 M $MgSO_4$ = 3 g/L (use 2 ml/L of nutrient solution).

Final Solution

Each Ca treatment (2000μM, 400μM, 300μM, 200μM and 100μM) were made up to 200 L and the solutions were stored in separate tanks in the glass-house. Fresh nutrient solutions for the treatments were required every 60 days for the pilot study and every 7 days for the trial. The following volumes from the stock solutions were required to make up 200 L of nutrient solution for each individual treatment:

Stock 1 use 800 mL per Ca treatment.

Stock 2 use 200 mL per Ca treatment.

Stock 3 use 1200 mL per Ca treatment.

Stock 4 use 400 mL per Ca treatment.

Stock 5 use 200 mL per Ca treatment.

Stock 6 use 50 mL per Ca treatment.

Stock 7 use 400 mL for the 400μM, 300μM, 200μM and 100μM of Ca.