CSIRO Publishing

Australian Journal of Agricultural Research

VOLUME 52, 2001

A journal for the publication of original contributions
towards the understanding of an agricultural system

All enquiries and manuscripts should be directed to:

Australian Journal of Agricultural Research
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7628
Fax: +61 3 9662 7611
Email: ajar@publish.csiro.au

CSIRO PUBLISHING

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/ajar

Aust. J. Agric. Res., 2001, **52**, 1017–1022

Rootstock influences postharvest anthracnose development in ‘Hass’ avocado

S. L. Willingham[A], *K. G. Pegg, A. W. Cooke, L. M. Coates, P. W. B. Langdon, and J. R. Dean*

Queensland Horticulture Institute, QDPI, 80 Meiers Road, Indooroopilly, Qld 4068, Australia.
[A]Corresponding author; email: willins@dpi.qld.gov.au

Abstract. Rootstock studies conducted on ‘Hass’ avocado found that rootstock had a significant impact on postharvest anthracnose susceptibility. This is the first record of such an effect for avocado. The severity and incidence of anthracnose was significantly lower on ‘Hass’ grafted to ‘Velvick’ Guatemalan seedling rootstock compared with the ‘Duke 6’ Mexican seedling rootstock. Differences in anthracnose susceptibility were related to significant differences in concentrations of antifungal dienes in the leaves and mineral nutrients in the leaves and fruits from trees grafted to different rootstocks. Leaf diene concentrations were up to 1.5 times higher in ‘Hass’ trees on the ‘Velvick’ than the ‘Duke 6’ rootstock. In ungrafted nursery stock trees, diene concentrations were around 3 times higher in ‘Velvick’ than ‘Duke 6’ leaves. The ‘Velvick’/‘Hass’ combination also had a significantly lower leaf N concentration, a significantly higher fruit flesh Mn concentration, and significantly lower and higher leaf N/Ca and Ca+Mg/K ratios, respectively. A significant correlation (r = 0.82) between anthracnose severity and skin N/Ca ratio was also evident.

Additional keywords: Colletotrichum gloeosporioides, diene, nutrition.

Introduction

Anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the most serious postharvest disease of avocado (*Persea americana* Mill.). Stem-end rot, another important postharvest disease of avocado, can be caused by a number of different fungi including various *Dothiorella* spp., *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl., and *Phomopsis perseae* Zerova. *C. gloeosporioides* can also cause rots at the stem-end. Postharvest disease symptoms of anthracnose arise from quiescent field infections that do not become apparent until after the fruit is harvested and begins to ripen (Binyamini and Schiffmann-Nadel 1972; Verhoeff 1974). The quiescent infection stage has been shown to be the germinated appressoria and infection peg structure embedded in the wax and cuticle of the fruit skin (Prusky *et al.* 1991; Coates *et al.* 1993).

Much research has focussed on how immature or unripe fruits remain resistant to fungal infection and the mechanisms regulating pathogen quiescence, with the aim to manipulate natural resistance for new disease control strategies. In avocado, quiescence of the anthracnose pathogen is thought to be due to fungitoxic concentrations of preformed antifungal compounds present in the skin of the fruit (Prusky *et al.* 1982, 1983). Of the 5 major antifungal compounds identified, 1-acetoxy–2-hydroxy-4-oxo-heneicosa–12,15 diene (diene) was shown to be the most active against *C. gloeosporioides* (Prusky *et al.* 1982; Adikaram *et al.* 1992). During ripening, the concentration of diene in the fruit skin declines around 10-fold to subfungitoxic levels allowing the *C. gloeosporioides* infections to resume colonisation of the fruit (Prusky *et al.* 1988). Cultivars of avocado naturally more resistant to anthracnose have been shown to have higher concentrations of the diene as well as a slower rate of decline of diene concentration during ripening (Prusky *et al.* 1988; Ardi *et al.* 1998).

Three ecological races are identified within *P. americana* and given varietal status within the species: *P. americana* var. *drymifolia* (Mexican race), *P. americana* var. *guatemalensis* (Guatemalan race), and *P. americana* var. *americana* (West Indian race) (Ben-Ya’acov and Michelson 1995). At present, more than 95% of avocado production in Australia is based on Guatemalan cv. Hass. The main seedling rootstocks in use are selections of the Guatemalan (‘Velvick’) and Mexican (‘Duke 6’, ‘Duke 7’) races (Ben-Ya’acov and Michelson 1995). A key criterion for rootstock selection has been resistance to root rot caused by *Phytophthora cinnamomi* Rands. The Mexican Duke cultivars were introduced from California due to their moderate physiological resistance to *P. cinnamomi* (Phillips *et al.* 1987) and their ability to regenerate roots quickly (Erwin and Ribeiro 1996). ‘Velvick’ was a local selection and was also chosen for its ability to quickly regenerate roots. Root rot resistance has been the driving force behind rootstock selections, and other characteristics affected by rootstock, such as yield and

 10.1071/AR01015 0004-9409/01/101017

quality, have not received much attention until the last decade or so. Differences in fruit yield (Arpaia *et al.* 1992; Kremer-Kohne and Kohne 1992; Smith 1993), tree vigour, fruit physiological storage disorders (e.g. grey pulp, vascular browning) (Smith 1993), fruit shape (Kohne 1992), and fruit maturity (Young 1992) have been recorded for different avocado rootstocks. Rootstock has also been shown to affect leaf and fruit mineral nutrient concentrations (Ben-Ya'acov and Michelson 1995).

Fruit mineral nutrition, in particular calcium (Ca), is known to be very important for sound fruit quality (Chaplin and Scott 1980; Wills and Tirmazi 1982). High fruit Ca concentration has been correlated with low levels of postharvest disease in apple (Conway and Sams 1983, 1984, 1987; Sams *et al.* 1993; Conway *et al.* 1999), peach (Conway *et al.* 1987), and avocado (Hofman *et al.* 1999). The ability of Ca to retard ripening and senescence and reduce the postharvest development of diseases is thought to be due to its ability to strengthen cell walls and maintain membrane selective permeability and integrity (DeMarty *et al.* 1984). Calcium strengthens cell walls by cross-linking the pectic polymers in the cell wall and middle lamella (Carpita and Gibeaut 1993), thus making them less accessible to attack by fungal pectolytic enzymes (Bateman and Lumsden 1965; Stockwell and Hanchey 1982; McGuire and Kelman 1986; Conway *et al.* 1992). Restricting tissue maceration and the subsequent release of free Ca ions from the cell wall may also reduce decay by preventing host signalling and the activation of enzymes involved in pathogenesis (Pagel and Heitefuss 1990).

To date, the influence of rootstock on postharvest disease susceptibility has not been reported for avocado. In this paper, we make the first report of a rootstock effect on the postharvest development of anthracnose in 'Hass' avocado fruit, and relate this to differences in mineral nutrient and leaf diene concentrations.

Materials and methods

Field site and rootstocks

These experiments were conducted during the 1998–99 cropping season in a commercial orchard at Duranbah, northern New South Wales (28.19ºS). The climate of this region is termed warm subtropical and received an above average annual rainfall of 4064 mm during the 1998–99 season. All of the trees had received normal commercial management practice and had been trunk-injected with 20% di-potassium phosphonate when the spring and summer leaf flushes had matured, to limit infection by *P. cinnamomi*. The postharvest disease susceptibility of fruit from the Guatemalan cv. Hass was compared when grafted to seedling Guatemalan ('Velvick') or Mexican ('Duke 6') race rootstocks. The postharvest disease susceptibility of 'Hass' fruits from these two rootstock races was first studied in young (3½-year-old) trees and then again in older (8-year-old) trees. For both studies, trees of the same age grafted to different rootstock races were planted in adjacent rows of the same block of the orchard and, thus, were under the same cultural and environmental conditions. Twenty fruits were harvested from each of 6 young 'Hass' trees grafted to seedling 'Velvick' or 'Duke 6' rootstocks and 40 fruits were harvested from each of 5 or 6 older 'Hass' trees grafted to seedling 'Velvick' or 'Duke 6' rootstocks, respectively.

Postharvest disease assessments

Harvested fruits were placed at 22°C (65% RH) to ripen before being assessed for disease. Fruits were judged ripe when soft enough to yield to gentle hand pressure. The number of days each fruit took to ripen was recorded as a measure of fruit shelf life. Ripe fruits were sliced longitudinally into quarters, peeled, and examined for anthracnose and stem-end rot lesions. Anthracnose and stem-end rot severity were estimated as the percentage of fruit surface area affected by either disease. The incidence of anthracnose and stem-end rot was calculated as the percentage of fruit affected by either disease. The percent acceptable fruit was calculated as the percentage of fruits with ≤5% anthracnose severity and no stem-end rot. The cause of lesions at the stem-end was determined by isolation onto potato dextrose agar.

Diene analysis

Two leaves from each of 6 tree replicates were collected from a uniform height and aspect from young (3½-year-old) and older (8-year-old) 'Hass' trees on seedling 'Velvick' and 'Duke 6' rootstocks for diene analyses. Two leaves from each of 5 tree replicates were also collected from ungrafted seedling 'Velvick' and 'Duke 6' nursery stock trees for diene analyses. Leaf diene concentration was measured by a Varian 3300 gas chromatograph using the technique as described by Carman *et al.* 1998.

Mineral nutrient analyses

Fifteen leaves and 0.5 g wood tissue from both above and below the graft union from each of 6 tree replicates were collected from around the tree at a uniform height from young 'Hass' trees on each rootstock for mineral analyses. Five fruits from each of 5 or 6 tree replicates were also collected from around the tree at a uniform height from young 'Hass' trees grafted to 'Duke 6' or 'Velvick' rootstocks, respectively, to measure mineral concentrations in the flesh and skin tissues. Fruit flesh, skin, leaves, and wood tissue from above and below the graft union were oven dried at 60°C to a constant weight, ground to a fine powder using an electric coffee grinder (Philips, Groningen, The Netherlands), and digested in 5 : 1 (v/v) nitric-perchloric acid (Baker and Smith 1974). Total mineral nutrient concentrations were then measured by inductively coupled plasma atomic emission spectrophotometry (ICPAES). Total N concentration was measured by a LECO CNS 2000 analyser at 1100°C. As there were no differences in fruit flesh dry matter percentage of 'Hass' fruits from different rootstock races, flesh mineral concentrations were expressed on a DW basis (Fidler *et al.* 1973).

Statistical analyses

Statistical analyses were conducted using GENSTAT 5 release 4.1 data analysis software (Lawes Agricultural Trust, Rothamsted Experimental Station) for a completely randomised design analysis of variance. Arcsine angular transformations were made on percentage data; however, examination of residual plots indicated that transformation did not improve the distribution of residuals. Hence, untransformed data are presented. Pair-wise testing between means was done using the least significant difference (l.s.d.) procedure at $P = 0.05$.

Results

Postharvest diseases

Fruits from 3½-year-old 'Hass' trees grafted to 'Velvick' took slightly longer to ripen and had significantly less severe (82% lower) and a lower incidence (34% lower) of

Table 1. The effects of rootstocks on fruit shelf life (days), anthracnose, and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit acceptability (% of fruits with ≤5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruits harvested from young (3½-year-old) and older (8-year-old) trees and ripened at 22°C (65% RH)
Mean values within columns followed by the same letter are not significantly different at $P = 0.05$ ($n = 6$ for young trees and $n = 5$ or 6 for 'Velvick' or 'Duke 6', respectively)

Rootstock	Shelf life	% Anthracnose		% Stem-end rot		Acceptable fruit
		Severity	Incidence	Severity	Incidence	
			Young trees			
Velvick	7.0a	7.7b	61.9b	0.3a	1.7a	66.1a
Duke 6	6.7b	41.8a	93.2a	0.6a	4.3a	13.6b
			Older trees			
Velvick	9.1a	15.6b	50.0b	1.2a	10.1a	64.5a
Duke 6	8.9a	39.5a	77.0a	1.4a	11.7a	33.6b

anthracnose compared with fruits from 'Hass' trees grafted to 'Duke 6' (Table 1). As such, fruit acceptability was significantly increased by 79% for 'Hass' on the 'Velvick' rootstock compared with the 'Duke 6' rootstock (Table 1). Levels of stem-end rot were very low in this study, and no significant differences in disease severity or incidence between fruits from the different rootstocks were evident (Table 1). 'Hass' fruits from 8-year-old trees grafted to 'Velvick' also had significantly less severe (61% lower) and a lower incidence (35% lower) of anthracnose than fruits from trees grafted to 'Duke 6' (Table 1). Fruit acceptability on 'Velvick' was also significantly increased in older trees by 48% compared with 'Duke 6' (Table 1). Again, stem-end rot levels were low, and no significant differences in disease severity or incidence were evident between the two rootstocks (Table 1).

Dienes

In ungrafted nursery trees, 'Velvick' had leaf diene concentrations 3 times higher than 'Duke 6' (Table 2). Diene concentrations measured in young and older trees were up to 1.5 times higher in 'Hass' leaves from the 'Velvick' rootstock compared with the 'Duke 6' rootstock (Table 2).

Table 2. The effect of rootstocks on the diene concentration (mg/g_{FW} leaf) of 'Hass' avocado leaves harvested from young (3½-year-old) and older (8-year-old) trees and the diene concentration of avocado leaves harvested from ungrafted seedling nursery trees
Mean values followed by the same letter are not significantly different at $P = 0.05$ ($n = 6$ for young and older trees and $n = 5$ ungrafted nursery trees)

Rootstock	Diene
	Young trees
Velvick	2.5a
Duke 6	1.7b
	Older trees
Velvick	3.3a
Duke 6	2.6b
	Ungrafted nursery trees
Velvick	0.20a
Duke 6	0.06b

Mineral nutrient concentrations

Mineral nutrient concentrations of fruit flesh, skin, leaf, and scion wood and rootstock wood tissue were measured for the young trees only. Leaves from 'Hass' trees on 'Duke 6' had a significantly higher concentrations of N compared with leaves from 'Hass' on 'Velvick' (Table 3). The leaves from the 'Duke 6'/'Hass' combination also had a significantly higher N/Ca ratio and a lower Ca+Mg/K ratio than the 'Velvick'/ 'Hass' combination (Table 3).

'Hass' fruits from the 'Velvick' rootstock trees had a significantly lower K concentration, a significantly higher concentration of Mn, and a higher Ca+Mg/K ratio in the flesh tissue, compared with 'Hass' fruits from the 'Duke 6' rootstock trees (Table 3). No significant differences in mineral nutrient concentrations in the skin tissue were evident between rootstocks (Table 3).

'Hass' scion wood tissue, collected above the graft union, had significantly lower concentrations of Mg and K when grafted to 'Velvick' compared with 'Duke 6' (Table 3). 'Velvick' rootstock wood tissue, collected below the graft union, had significantly lower concentrations of N, Ca, Mg, and K, and a lower N/Ca ratio, than 'Duke 6' (Table 3).

Significant positive correlations were found between fruit skin and flesh N concentrations and anthracnose severity (Table 4). Non-significant negative correlations were found between fruit skin and flesh Ca concentrations and anthracnose severity (Table 4). In the skin tissue, a significant positive correlation was found between the N/Ca ratio and anthracnose severity. The same trend, although weaker and non-significant, was found in the flesh tissue. Non-significant negative correlations were found between fruit skin and flesh Ca+Mg/K ratios and anthracnose severity (Table 4).

Discussion

The differences in anthracnose susceptibility of 'Hass' avocado grafted to different seedling rootstocks found in these studies have not been previously reported. To date, recorded differences in postharvest performance of avocado

Table 3. The effects of rootstocks on 'Hass' avocado leaf, fruit flesh, fruit skin, scion wood, and rootstock wood nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K), and manganese (Mn) mineral nutrient concentrations harvested from 3½-year-old trees
Mean values within columns followed by the same letter are not significantly different at $P = 0.05$ ($n = 5$ for fruit flesh and skin tissue and $n = 6$ for leaf and wood tissue)

Rootstock	N (% $_{DW}$)	Ca (mg/kg $_{DW}$)	Mg ratio	K ratio	Mn	N/Ca	Ca+Mg/K
			Leaf				
Velvick	2.3b	2.7a	0.6a	0.9a	303a	0.9b	3.6a
Duke 6	2.5a	2.4a	0.6a	1.2a	212a	1.1a	2.5b
			Fruit flesh				
Velvick	1.2a	0.012a	0.1a	2.8b	7.0a	103.4a	0.05a
Duke 6	1.3a	0.008a	0.1a	3.2a	6.0b	233.2a	0.04b
			Fruit skin				
Velvick	1.1a	366a	0.1a	1.9a	8.5a	0.003a	194a
Duke 6	1.3a	306a	0.1a	2.2a	8.1a	0.004a	144a
			Scion wood				
Velvick	0.8a	0.6a	0.12b	1.0b	22.6a	1.3a	0.7a
Duke 6	0.9a	0.7a	0.14a	1.4a	19.9a	1.4a	0.6a
			Rootstock wood				
Velvick	0.6b	0.4b	0.1b	0.8b	16.1a	1.6b	0.7a
Duke 6	1.1a	0.6a	0.2a	1.5a	8.2a	2.0a	0.5a

fruits from trees grafted to different rootstocks have been limited to physiological storage disorders, fruit shape, and maturity (Ben-Ya'acov and Michelson 1995). The increased anthracnose susceptibility of 'Hass' fruits on the 'Duke 6' rootstock cannot be attributed to stress caused by stem-pitting, as no stem-pitting, which is thought to have a viral aetiology (Ploetz and Korsten 1992), was found in the trees at the Duranbah field site. There were also no visible signs of graft incompatibility between the scion and the rootstocks (e.g. overgrowth at graft union).

The greater disease resistance of 'Hass' fruits on the 'Velvick' rootstock is suspected to be related to the higher concentration of antifungal diene measured in the leaves of young, older, and ungrafted 'Velvick' nursery stock trees. Elevating avocado fruit diene concentration or slowing the decline of diene during ripening has been shown to reduce anthracnose levels after harvest (Prusky *et al.* 1988). However, only leaf diene concentrations were measured in our studies due to our inability to extract tissues with a high oil content such as the fruit skin. Although the relationship between leaf and fruit diene concentrations in avocado is unknown, it is that probable they would be positively correlated as the same specialised idioblast cells where dienes are produced (Leikin-Frenkel and Prusky 1998) are found in all parts of the avocado tree (Platt and Thomson 1992). However, final confirmation of our hypothesis that rootstock was influencing anthracnose development due to differences in diene concentrations awaits a comparison of diene concentrations in the fruit skin.

Table 4. Correlation coefficients (*r* values) for linear regressions between fruit skin or flesh nitrogen (N), calcium (Ca), magnesium (Mg), and potassium (K) mineral nutrient concentrations and anthracnose severity for 3½-year-old 'Hass' avocado trees on 'Velvick' and 'Duke 6' rootstocks

Mineral concentration (% $_{DW}$)	Pooled r value ($n = 11$)
Skin tissue	
N	0.72*
Ca	–0.46n.s.
N/Ca ratio	0.82**
Ca+Mg/K ratio	–0.54n.s.
Flesh tissue	
N	0.73**
Ca	–0.44n.s.
N/Ca ratio	0.47n.s.
Ca+Mg/K ratio	–0.58n.s.

* $P < 0.05$; ** $P < 0.01$; n.s., not significant.

The inherently higher concentration of antifungal diene in the 'Velvick' rootstock persisted following grafting of nursery stock trees. The magnitude of the recorded differences between grafted rootstock trees, however, was not as great as ungrafted nursery stock trees. The mechanisms by which the rootstocks are able to influence the concentration of antifungal diene in the leaves of the scion are not known. Avocado rootstocks have been reported to affect the final expression of biochemical activities of enzymes in the scion (Bower and Nel 1982), and thus, it is possible that enzymes involved in either the synthesis or degradation (e.g. lipoxygenase, epicatechin) of diene were affected. It is also interesting to note that there appeared to be much higher concentrations of diene in grafted trees than in ungrafted nursery trees. These differences may have been due to a graft incompatibility effect or perhaps an aging effect since dienes showed an increasing trend with tree age.

Leaves of trees grafted to Guatemalan race rootstocks have been reported to have lower concentrations of N and K and higher concentrations of Ca and Mg than Mexican race rootstocks (Haas 1950; Embleton *et al.* 1962; Ben-Ya'acov *et al.* 1992). This study found similar effects for N but not for the other mineral nutrients. However, there were similar trends of higher Ca and lower K concentrations in the leaves of trees grafted to Guatemalan 'Velvick' than the Mexican 'Duke 6' rootstock.

The increased severity and incidence of anthracnose in 'Hass' fruits from trees on the 'Duke 6' rootstock may be related to the higher leaf N concentration, as elevated N concentration and a low N/Ca ratio have previously been associated with poor keeping quality and increased postharvest decay of other crops such as pear (Sugar *et al.* 1992), chicory (Schober and Vermeulen 1999), and onion (Wright 1993). Increasing the N concentration may result in weaker cell walls and thus increase their susceptibility to fungal pectolytic enzymes (Bateman and Basham 1976). The effect of N on the cell wall is indirect and is mediated by this nutrient's negative effect on the sink : source relationships in the tree. By increasing the N concentration and thus the photosynthetic ability of the tree, the leaves (source) will transpire more actively and thus outcompete the developing fruit (sink) for water and Ca (Kirkby and Pilbeam 1984). This causes an imbalance of Ca in the fruit, which is essential to strengthen cell walls (DeMarty *et al.* 1984). Therefore, often the balance of mineral nutrients is found to be more closely related to differences in quality and disease as found in this study. Although a significant correlation was found between anthracnose severity and N/Ca, only a weak non-significant trend was evident for Ca. This is contrary to what has been previously recorded in the literature for avocado (Hofman *et al.* 1999) and other fruits such as apple (Conway and Sams 1983, 1984, 1987; Sams *et al.* 1993; Conway *et al.* 1999) and peach (Conway *et al.* 1987).

The higher concentration of Mn present in the flesh of 'Hass' fruits from the 'Velvick' rootstock compared with the 'Duke 6' rootstock may have also contributed to its greater disease resistance. Manganese is an important cofactor for a number of key reactions involved in the synthesis of secondary metabolites (e.g. phenols, lignin) via the shikimic acid pathway, which are necessary for plant defence (Burnell 1988). Manganese is also important for plant disease resistance as it not only affects plant enzymes (e.g. phenylalanine ammonia-lyase) but also pathogen enzymes. For example, elevated concentrations of Mn have been found to inhibit fungal pectin methylesterase, an exoenzyme (Sadasivan 1965).

Tissue pH has also been reported to affect decay by affecting the enzymes involved in pathogenesis (Pagel and Heitefuss 1990). Recently, the importance of skin pH in avocado for the development of quiescent anthracnose infections was confirmed (Yakoby *et al.* 2000). The possibility that rootstocks may also be influencing skin pH levels and thus anthracnose development is currently being investigated.

This study has revealed that rootstocks can have a significant effect on postharvest anthracnose susceptibility of avocado, most likely by influencing the accumulation of antifungal diene and mineral nutrients in the scion. The mechanisms by which rootstocks can influence these scion characteristics are not understood and warrant further investigation. This finding provides a valuable opportunity for the development of new long-term disease control strategies for avocado that are less reliant on chemical control, by matching rootstock/scion combinations.

Acknowledgments

We thank the Australian Avocado Growers Federation, the Horticultural Research and Development Corporation, and the Queensland Horticulture Institute for funding this project. We also thank Dr Ray Carman and Mr Paul Handley for diene analyses, Mr Graham Anderson for allowing access to his orchard, and Mr Harold Taylor for help in identifying different rootstock races and supplying nursery trees.

References

Adikaram NKB, Ewing DF, Karunaratne AM, Wijeratne EMK (1992) Antifungal compounds from immature avocado fruit peel. *Phytochemistry* **31**, 93–96.

Ardi R, Kobiler I, Jacoby B, Keen NT, Prusky D (1998) Involvement of epicatechin biosynthesis in the activation of the mechanisms of resistance of avocado fruits to *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology* **53**, 269–285.

Arpaia ML, Bender GS, Witney GW (1992) Avocado clonal rootstock production trial. *Proceedings of the Second World Avocado Congress* (*Riverside*, *California*) **1**, 305–310.

Baker AS, Smith RL (1974) Preparation of solutions for atomic absorption analyses of Fe, Mn, Zn, and Cu in plant tissue. *Journal of Agricultural and Food Chemistry* **22**, 103–107.

Bateman DF, Basham HG (1976) Degradation of plant cell walls and membranes by microbial enzymes. *Encyclopedia of Plant Physiology New Series* **4**, 316–355.

Bateman DF, Lumsden RD (1965) Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhizoctonia solani*. *Phytopathology* **55**, 734–738.

Ben-Ya'acov A, Michelson E (1995) Avocado rootstocks. *Horticultural Reviews* **17**, 381–429.

Ben-Ya'acov A, Michelson E, Sela I (1992) Field study of avocado (*Persea americana* Mill.) seeded rootstocks and scion sources: final report of experiments planted as commercial orchards. 1. Investigation of rootstock, scion and manure at Bene-Deror Fuerte orchard planted in 1969 (in Hebrew with English summary). *Alon HaNotea* **46**, 769–782.

Binyamini N, Schiffmann-Nadel M (1972) Latent infection in avocado fruit due to *Colletotrichum gloeosporioides*. *Phytopathology* **62**, 592–594.

Bower JP, Nel ME (1982) Avocado stock-scion interactions as evidenced by the electrofocusing technique of electrophoresis and enzyme analysis. *Crop Product* **11**, 92–94.

Burnell JN (1988) The biochemistry of manganese in plants. In 'Manganese in soils and plants'. (Eds RD Graham, RJ Hannam, NC Uren) pp. 125–137. (Kluwer Academic Publishers: London)

Carman RM, Duffield AR, Handley PN, Karoli T (1998) Chemistry around the avocado antifungal diene. *ACIAR Proceedings* **80**, 186–189.

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal* **3**, 1–30.

Chaplin GR, Scott KJ (1980) Association of calcium in chilling injury susceptibility of stored avocados. *HortScience* **15**, 514–515.

Coates LM, Muirhead IF, Irwin JAG, Gowanlock DH (1993) Initial infection processes by *Colletotrichum gloeosporioides* on avocado fruit. *Mycological Research* **97**, 1363–1370.

Conway WS, Greene GM, Hickey KD (1987) Effects of preharvest and postharvest calcium treatments of peaches on decay caused by *Monilinia fructicola*. *Plant Disease* **71**, 1084–1086.

Conway WS, Jansiiewicz WJ, Klein JD, Sams CE (1999) Strategy for combining heat treatment, calcium infiltration, and biological control to reduce postharvest decay of 'Gala' apples. *HortScience* **34**, 700–704.

Conway WS Sams CE (1983) Calcium infiltration of Golden Delicious apples and its effect on decay. *Phytopathology* **73**, 1068–1071.

Conway WS, Sams CE (1984) Possible mechanisms by which postharvest calcium treatment reduces decay in apples. *Phytopathology* **74**, 208–210.

Conway WS, Sams CE (1987) The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. *Journal of the American Society for Horticultural Science* **112**, 300–303.

Conway WS, Sams CE, McGuire RG, Kelman A (1992) Calcium treatment of apples and potatoes to reduce postharvest decay. *Plant Disease* **76**, 329–334.

DeMarty M, Morvan C, Thellier M (1984) Calcium and the cell wall. *Plant Cell and Environment* **7**, 441–448.

Embleton TW, Matsumura M, Storey WB, Garber MJ (1962) Chloride and other elements in avocado leaves, as influenced by rootstock. *Proceedings of the American Society of Horticultural Science* **80**, 230–236.

Erwin DC, Ribeiro OK (1996) 'Phytophthora diseases worldwide.' (The American Phytopathological Society Press: St Paul, MN, USA)

Fidler JC, Wilkinson BG, Edney KL, Sharples RO (1973) 'The biology of apple and pear storage.' (Commonwealth Agricultural Bureaux: Slough, England)

Haas ARC (1950) Rootstock influence on the composition of scion avocado leaves. *California Avocado Society Yearbook* **34**, 149–152.

Hofman P, Whiley T, Marques R (1999) Fruit quality and nutrition—the role of rootstocks. *Talking Avocados* **10**, 26–27.

Kirkby EA, Pilbeam DJ (1984) Calcium as a plant nutrient. *Plant Cell and Environment* **7**, 397–405.

Kohne JS (1992) Field evaluation of 'Hass' avocado grown on Duke 7, G6 and G755C rootstocks. *Proceedings of the Second World Avocado Congress* (*Riverside*, *California*) **1**, 301–303.

Kremer-Kohne S, Kohne JS (1992) Yield and fruit quality of Fuerte and Hass on clonal rootstocks. *South African Avocado Growers' Association Yearbook* **15**, 69.

Leikin-Frenkel A, Prusky D (1998) Ethylene enhances the antifungal lipid content in idioblasts from avocado mesocarp. *Phytochemistry* **49**, 2291–2298.

McGuire RG, Kelman A (1986) Calcium in potato tuber cell walls in relation to tissue maceration by *Erwinia carotovora* pv. *atroseptica*. *Phytopathology* **76**, 401–406.

Pagel W, Heitefuss R (1990) Enzyme activities in soft rot pathogenesis of potato tubers: effects of calcium, pH and degree of pectin esterification on the activities of polygalacturonase and pectate lyase. *Physiological and Molecular Plant Pathology* **37**, 9–25.

Phillips D, Grant BR, Weste G (1987) Histological changes in the roots of an avocado cultivar, Duke 7, infected with *Phytophthora cinnamomi*. *Phytopathology* **77**, 691–698.

Platt KA, Thomson WW (1992) Idioblast oil cells of avocado: distribution, isolation, ultrastructure, histochemistry and biochemistry. *International Journal of Plant Science* **153**, 301–310.

Ploetz RC, Korsten L (1992) So you have root rot under control, is black streak, bacterial canker, or some other disease reducing yield? How are these diseases managed in the various avocado-growing countries? *Proceedings of the Second World Avocado Congress* (*Riverside*, *California*) **2**, 681–684.

Prusky D, Keen NT, Eaks I (1983) Further evidence for the involvement of a preformed antifungal compound in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. *Physiological Plant Pathology* **22**, 189–198.

Prusky D, Keen NT, Sims JJ Midland SL (1982) Possible involvement of an antifungal diene in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. *Phytopathology* **72**, 1578–1582.

Prusky D, Kobiler I, Fishman Y, Sims JJ, Midland SL, Keen NT (1991) Identification of an antifungal compound in unripe avocado fruits and its possible involvement in the quiescent infections of *Colletotrichum gloeosporioides*. *Journal of Phytopathology* **132**, 319–327.

Prusky D, Kobiler I, Jacoby B (1988) Involvement of epicatechin in cultivar susceptibility of avocado fruits to *Colletotrichum gloeosporioides* after harvest. *Journal of Phytopathology* **123**, 140–146.

Sadasivan TS (1965) Effect of mineral nutrients on soil microorganisms and plant disease. In 'Ecology of soil-borne plant pathogens'. pp. 460–469. (University of California Press: California)

Sams CE, Conway WS, Abbott JA, Lewis RJ, Ben-Shalom N (1993) Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatments. *Journal of the American Society of Horticultural Science* **118**, 623–627.

Schober BM, Vermeulen T (1999) Enzymatic maceration of witloof chicory by the soft rot bacteria *Erwinia carotovora* subsp. *carotovora*: the effect of nitrogen and calcium treatments of the plant on pectic enzyme production and disease development. *European Journal of Plant Pathology* **105**, 341–349.

Smith DG (1993) Horticultural performance of imported cultivars and rootstocks in the north-eastern Transvaal. *South African Avocado Growers' Association Yearbook* **16**, 28–30.

Stockwell V, Hanchey P (1982) Cytohistochemical techniques for calcium localization and their application to diseased plants. *Plant Physiology* **70**, 244–251.

Sugar D, Righetti TL, Sanchez EE, Khemira H (1992) Management of nitrogen and calcium in pear trees for enhancement of fruit resistance to postharvest decay. *HortTechnology* **2**, 382–387.

Verhoeff K (1974) Latent infections by fungi. *Annual Review of Plant Physiology* **12**, 99–110.

Wills RBH, Tirmazi SIH (1982) Inhibition of ripening of avocados with calcium. *Scientia Horticulturae* **16**, 323–330.

Wright PJ (1993) Effects of nitrogen fertiliser, plant maturity at lifting and water during field-curing on the incidence of bacterial soft rot of onions in store. *New Zealand Journal of Horticultural Science* **21**, 377–381.

Yakoby N, Kobiler I, Dinoor A, Prusky D (2000) pH regulation of pectate lyase secretion modulates the attack of *Colletotrichum gloeosporioides* on avocado fruits. *Applied Environmental Microbiology* **66**, 1026–1030.

Young PJ (1992) Inherent influence of rootstock race on avocado fruit maturity. *Proceedings of the Second World Avocado Congress* (*Riverside*, *California*) **1**, 149–154.

Manuscript received 17 January 2001, accepted 21 May 2001