CHAPTER 7

TRANSLATION OF PHENO/PHYSIOLOGY RESEARCH INTO MANAGEMENT STRATEGIES

The phenological and physiological aspects of avocado presented earlier in this thesis have been important in providing mechanisms for structured research to advance understanding of how the avocado tree grows, and more specifically defining its responses to the environment. However, ultimately the basic principles of tree mechanisms uncovered by research must be taken through to applied management strategies for their full potential to be realised. This chapter discusses two aspects of applied research which have utilised knowledge gained through the more basic areas of investigation. More specifically, the research methodology outlined in section 7. 1 was developed from phenology and carbon partitioning principles reported in Chapters 2 and 5, while the current investigations reported in section 7. 2 have been developed from the pheno/physiological studies detailed in Chapters 2 and 3.

7. 1 CONTROL OF PHYTOPHTHORA ROOT ROT WITH TRUNK-INJECTED PHOSPHONATE[‡]

7. 1. 1 Introduction

Phosphonates (viz. salts or esters of phosphonic acid) were the first commercially used ambimobile fungicides in plants (Zentmyer 1979; Lüttringer and De Cormis 1985). They are particularly effective in controlling diseases caused by Oomycetes such as the Phytophthora and Pythium species and downy mildews which cause severe economic losses in agricultural crops worldwide (Cohen and Coffey 1986). Due to effective translocation of phosphonates within plants several methods of application have been employed to control diseases. These include the traditional methods of foliar sprays and soil drenches (Pegg *et al*. 1985; Rohrbach and Schenck 1985) and painting or sponge banding the trunks of trees with phosphonate formulations (Snyman and Kotzé

[‡] **Whiley, A.W., Hargreaves, P.A., Pegg, K.G., Doogan, V.J., Ruddle, L.J., Saranah, J.B. and Langdon, P.W., 1995.** Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Aust. J. Agric. Res.* Manuscript accepted 17 Oct 1994. **APPENDIX 4**

and Kotzé 1983). The term phosphonate is widely used for the salts and esters of phosphonic acid (H_3PO_3). Once phosphonates are introduced into plant tissues they are rapidly hydrolysed to H_3PO_3 and subsequently ionised to the phosphonate anion, HPO_3^{-2} (Ouimette and Coffey 1990). This anionic form of H_3PO_3 is more correctly known as phosphonate (Ouimette and Coffey 1989).

Phytophthora cinnamomi Rands is a devastating root disease of avocado (*Persea americana* Mill.) in most countries which grow this crop. The fungus invades the unsuberised roots, and less frequently attacks the suberised woody tissue of major roots or the collar of the tree (Pegg *et al*. 1982). When injected into the xylem tissues of the trunk or major limbs, the phosphonate anion is ultimately translocated to the roots limiting colonisation by the pathogen (Schutte *et al*. 1988; Guest and Grant 1991). The development of trunk injection of phosphonates during the 1980s, was an unconventional application technique which has subsequently been shown to control phytophthora root rot in avocados (Darvas *et al*. 1984; Pegg *et al*. 1985). Initially, research with trunk-injected phosphonates for the control of avocado root rot, focused on curing diseased trees (Darvas *et al*. 1984; Pegg *et al*. 1985, 1987, 1990). It was demonstrated that trees rating 9 on the health scale of 0, healthy to 10, dead (Darvas *et al*. 1984) could be restored to full health within two years by a trunk injection program with phosphonate fungicides (Pegg *et al*. 1987). However, within a few years of the commercial development of trunk injection, the focus on tree health shifted from curative to preventative management procedures, which required more strategic and efficient use of the technology.

The symplastic distribution within plants of ambimobile herbicides such as 2, 4-D and glyphosate, and the nematicide oxamyl, has been shown to be source/sink related, with the respective compounds accumulating in organs with greatest sink strength at the time of application (Leonard and Crafts 1956; Crafts and Yamaguchi 1958; Tyree *et al*. 1979; Dewey and Appleby 1983). It is likely that the ambimobile phosphonate exhibits similar behaviour following entry into the symplast.

In studies with young, container-grown avocado trees, Whiley and Schaffer (1993) reported that shoot and root sink activity were temporally separated; leaves were the strongest sinks for ^{14}C-

photosynthates during early shoot growth, and the sink strength of roots increased once shoots became quiescent (see also Chapter 5). The asynchronous pattern of shoot and root growth in avocado is illustrated in phenology models developed for avocado (Whiley *et al*. 1988a) which have since been confirmed by the observations of Ploetz *et al*. (1992) and Whiley and Schaffer (1994). The purpose of the research described in this Chapter was to relate the translocation of H_3PO_3 to roots, following trunk injection with a formulation containing mono- and di-potassium phosphonate (potassium phosphonate), to sink-strength dynamics within the tree at the time of application.

7. 1. 2 Materials and Methods

Trees selected for the experiment were 12-year-old, healthy 'Hass', approximately 12 m in canopy diameter, grafted to 'Velvick' Guatemalan seedling rootstock and growing in a site where *Phytophthora cinnamomi* was not present so that phosphonate fungicides had not been previously used. The trees were growing in a commercial orchard at Maleny in coastal S.E. Queensland (latitude 27^o S, 650 m altitude), which has a cool, high rainfall subtropical climate.

Tree phenology was monitored by collecting fruiting shoots from each of the 9 experimental trees immediately prior to treatment, then subsequently at ≈ 30 day intervals following the first injection through to fruit maturity and harvest 296 days later. Three current seasons fruiting shoots were collected from each tree and oven dry mass of the stems, leaves and fruit were determined separately after 72 hrs at 90^oC in a forced-draught oven. Trees were trunk-injected with potassium phosphonate at three different phases of tree phenology during spring and summer, thereby spanning the fluctuating relationships between shoot and root growth (Fig. 38). During each phenology phase, three trees were injected with the fungicide.

The first group of trees was trunk-injected with a 20% solution of potassium phosphonate towards the end of anthesis just as spring shoot growth commenced (4 October) (Fig. 38). Injections were carried out using Chemjet$^{(R)}$ tree injectors (Chemjet Trading Pty. Ltd., Caboolture, Australia) at the rate of 15 mL m^{-1} of canopy diameter (Pegg *et al*. 1987). Each injection site was prepared by drilling 6 mm diameter holes into the trunk, penetrating the xylem tissue to a depth of 40 mm, thereby giving the injected H_3PO_3 direct access to the xylem tissue and the transpiration stream.

Each injector was filled with 20 mL of the fungicide and injection sites were equally spaced around the circumference of the trunk; ca. nine injection sites per tree. Fresh samples of organs were collected from these trees before treatment and at intervals following injection, and analysed for H_3PO_3 content. Four sub-samples of bark and wood tissues (from tree trunks) were collected at each sampling time and care was taken to ensure that they were not selected from positions in close proximity to injection sites. Sub-samples of unsuberised roots were collected from each quadrant of the tree and 20 sub-samples of leaves, stems and shoots were taken from positions representative of the entire canopy at each sampling time. Sub-samples were bulked for H_3PO_3 analysis. For each tree, the amount of H_3PO_3 lost via fruit harvest and organ senescence was estimated over an eight month period. The senescence of flowers, leaves, twigs and fruit was monitored by placing 9 l containers in each quadrant of the canopy to collect a representative sample of material shed during the experiment. The containers were emptied at 14-day intervals, the material sorted into the various organs, dry mass determined and each component analysed for H_3PO_3 content. Fruit yield was recorded at harvest and H_3PO_3 content determined from samples from each of the three trees.

A second group of trees was trunk-injected when the spring shoots had completed extension growth and leaves were fully expanded (9 December), and a third group of trees was injected once the summer vegetative growth had matured (3 May) (Fig. 38). Following injection, fresh unsuberised root and leaf samples were collected at intervals from these two groups of trees and the H_3PO_3 content determined separately for each organ. The trunk injection treatments were repeated at the beginning of spring shoot growth and at the end of spring shoot maturity the following season and leaf and root samples collected and analysed over 96 days (data not presented).

To test lateral distribution of H_3PO_3, another group of three trees, each with trunks which formed two main vertical branches within 1 m of soil level was selected. At the beginning of spring shoot growth, only one of the main branches on each tree was injected with a 20% solution of potassium phosphonate at a rate calculated to treat the whole tree based on 15 mL m^{-1} diameter of canopy. Leaf and root samples were collected at intervals from the treated and untreated sides of the tree and the H_3PO_3 concentration was determined in each organ.

Phosphonic acid analysis by GC

Concentrations of H_3PO_3 were measured in avocado tissues using an acid extraction and gas chromatography. Samples were extracted with dilute aqueous sulphuric acid and derivatised using diazomethane to form the dimethyl ester. This extract was injected into a gas chromatograph equipped with a glass chromatographic column (Carbowax 20M) and a flame photometric detector. Dimethyl phosphonate was detected as a peak on the chromatogram and the concentration determined by comparison with a known standard. Residue concentrations of H_3PO_3 were calculated and expressed as $\mu g\ g_{fw}^{-1}$ for each sample. This method allowed a rapid and quantitative analysis of phosphonic acid in a variety of tissues (leaf, root, fruit [including seed, skin and flesh], bark and wood) with low detection limits ($\leq 0.1\ \mu g\ g_{fw}^{-1}$) and recoveries of $\geq 80\%$ ([‡] P.A. Hargreaves, unpublished data).

Data analysis

The H_3PO_3 content in leaves was monitored for each injection time and the concentration flux fitted to the non-linear regression model derived by Wood (1967) where $y = ax^b e^{-cx}$. Linear and non-linear regression analyses were used to relate the concentration flux of H_3PO_3 in roots to the time elapsed after trunk injection.

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7. 1. 3 Results and Discussion

Distribution and loss of phosphonic acid from the tree

Prior to trunk injection, low levels of H_3PO_3 (< 3.0 μg g_{fw}^{-1}) were detected in all parts of the tree (Fig. 35), although there was no previous history of the use of phosphonate fungicides on the experimental trees or in the orchard. It is unlikely that these pre-treatment concentrations of H_3PO_3 were derived from natural sources (Hilderbrand 1983). Weeds were regularly controlled in the orchard by using glyphosate [N-(phosphono-methyl) glycine], an ambimobile phosphonate based herbicide (Dewey and Appleby 1983). Glyphosate is completely degraded to CO_2 by microorganisms in the soil with the main intermediary metabolite being aminomethylphosphonic acid (Carlisle and Trevors 1988; Pipke and Amrhein 1988). It is likely that H_3PO_3 is a metabolite from the degradation of aminomethylphosphonic acid, in which case it may have been taken up by the tree thereby accounting for its presence in tissues before treatments were applied.

Following trunk injection at the beginning of spring shoot growth, H_3PO_3 concentrations increased in all tissues. Within two days of treatment, substantial increases in shoot and leaf concentrations were measured: 2.2 ± 0.5 to 77.4 ± 6.9 and 1.1 ± 0.3 to 52.1 ± 7.20 μg g_{fw}^{-1}, respectively. The highest H_3PO_3 concentrations were measured in the spring shoots (stem and leaves) which were actively growing at the time of trunk injection (Figs. 35 & 38). The H_3PO_3 concentration in these tissues peaked eight days after injection and then rapidly declined in leaves (Figs. 35a & 35b). However, in the stems of spring shoots there was a high H_3PO_3 level until 96 days after injection when concentration in those tissues fell rapidly. This coincided with the beginning of summer shoot growth and the development of a new leaf sink (Whiley and Schaffer 1993).

Phosphonic acid levels in mature, over-wintered leaves peaked within eight days of treatment after which there was a rapid decline in concentration (Fig. 35b). The difference in maximum H_3PO_3 concentration between spring and over-wintered leaves was probably due to different sink strengths at the time of injection. Whiley and Schaffer (1993) showed that following exposure of a mature leaf to $^{14}CO_2$, 27% of the ^{14}C-photosynthate was recovered from actively growing leaves at the terminal of the shoot while only 2.5% was found in mature leaves adjacent to the treated leaf. These mature leaves are a strong source of photoassimilates during spring shoot growth (Whiley

1990) and probably account for the more rapid loss of H_3PO_3 compared with spring leaves which remain strong sinks during development.

Ninety-six days after injection, sufficient summer shoot growth was present to allow sample collection. The concentration of H_3PO_3 in the stems of these new shoots was initially lower than in the stems of spring shoots at the same sampling time, but thereafter was similar despite temporal separation of their development in relation to the time of the trunk injection (Fig. 35a). During early growth, leaves on summer shoots had higher concentrations of H_3PO_3 than the adjacent spring leaves but by 195 days after trunk injection there was no significant difference between spring and summer leaves (Fig. 35b).

Concentrations of H_3PO_3 in the bark and wood of trunks were low compared to other organs of the tree (Fig. 35c). In the bark an increase in H_3PO_3 concentration (from 1.3 ± 0.2 to 4.7 ± 0.7 μg g_{fw}^{-1})

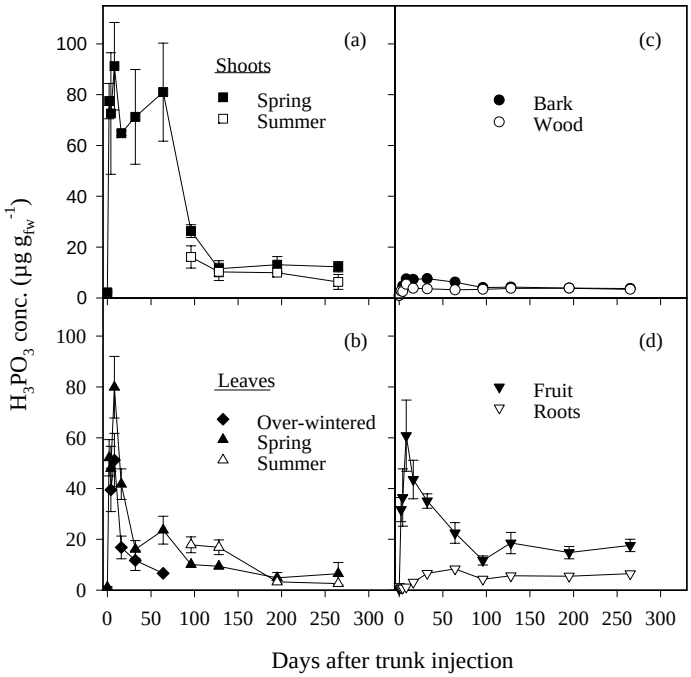

Fig. 35 Concentration flux of phosphonic acid (H_3PO_3) in avocado (a) shoots; (b) leaves; (c) trunk bark and wood; and (d) fruit and roots following trunk injection with 20% solution of di-potassium phosphonate at the beginning of spring shoot growth. Data points are mean values from three trees ± vertical SE bars which are obscured by symbols at some points.

was measured four days after trunk injection and peaked after 32 days (7.6 ± 1.2 μg g_{fw}^{-1}) while in the wood the maximum concentration (5.4 ± 0.5 μg g_{fw}^{-1}) was reached eight days after treatment.

Concentrations of H_3PO_3 in young fruit increased sharply following trunk injection, reaching 60.8 ± 14.0 μg g_{fw}^{-1} eight days after treatment. Thereafter, concentrations declined until stabilising (at ≈ 17 μg g_{fw}^{-1}) 64 days after injection (Fig. 35d). The considerable H_3PO_3 concentration in fruit at an early stage of their ontogeny was in contrast to < 1 μg g_{fw}^{-1} H_3PO_3 detected following trunk injection when fruit were mature (K.G. Pegg and A.W. Whiley, unpublished data). At the time of harvest, fruit (in this study) had maintained the highest H_3PO_3 concentration compared with other tissues, viz. 17.6 ± 2.4 μg g_{fw}^{-1} for fruit compared with 12.3 ± 2.0 μg g_{fw}^{-1} for stems of spring shoots. This was probably due to the comparatively strong sink status of the fruit throughout ontogeny (Cannell 1985), but is well below the maximum residue level of 100 μg g_{fw}^{-1} set for avocado fruit in Australia.

The accumulation of H_3PO_3 in roots was slower than in spring shoots and fruit, with no detectable increase until 16 days after treatment: from 1.4 ± 0.4 to 3.1 ± 0.5 μg g_{fw}^{-1}. The highest root concentration of H_3PO_3 was only 8.4 ± 1.9 μg g_{fw}^{-1}, measured 64 days after injection (Fig. 35d). Following this peak there was a slight decline in root concentration which remained relatively constant for the balance of the monitoring period. The pattern of both leaf and root accumulation of H_3PO_3 immediately following treatment was similar to that reported by Schutte *et al.* (1988). However, in their study, following a gradual increase in H_3PO_3 concentration in roots from 2 to 20 μg g_{fw}^{-1} during the first 35 days after injection, there was a 300% increase in H_3PO_3 between 35 and 42 days after treatment followed by a sharp decline in concentration.

At the time of treatment, 23 g of H_3PO_3 were injected into the trunks of each of the three trees. The residual H_3PO_3 concentration in senesced tree organs was highest in the inflorescence and fruitlets (50 to 80 μg g_{fw}^{-1}) with much lower concentrations in leaves (mature over-wintered) and twigs (10 to 20 μg g_{fw}^{-1}), thereby providing further evidence of the effect of sink strength on distribution (Cannell 1985). It was estimated that 6.85 ± 0.98 g (≈ 30%) of H_3PO_3 were lost from each tree from the time of injection until fruit harvest (296 days later). Approximately 3.51 ± 0.28 g (≈ 15%) of the total amount lost was attributed to loss through the litter cycle while 3.34 ± 0.57 g (≈ 15%)

was removed in harvested fruit. These estimates did not take into account other losses through root senescence and leakage (Ouimette and Coffey 1990) or possible oxidation in plant tissues to PO_4^{--} by bacteria as suggested by Bezuidenhout *et al*. (1987). The other significant factor responsible for declining tissue concentrations of H_3PO_3 was dilution by growth and its impact will largely depend on tree vigour.

Ouimette and Coffey (1990) concluded that symplastic entry of phosphonate across the plasmalemma occurs by active transport (Epstein 1973) which is dependent on metabolic energy. Several researchers using [^{14}C]sucrose as a standard for phloem-transported material, have demonstrated that translocation profiles of sucrose and phosphonate are almost identical (Martin and Edgington 1981; Dewey and Appleby 1983; Chamberlain *et al*. 1984). Following injection directly into the transpiration stream, the pattern of distribution of H_3PO_3 within avocado trees in this study provides further evidence of the ambimobility of phosphonate. Increased concentrations of H_3PO_3 were measured in stems and leaves of shoots and in fruit two days, and in bark of trunks, four days after treatment; however, there was no increase in root concentration until 16 days after trunk injection (Fig. 36). This time sequence suggests an apoplastic translocation pattern via the xylem to the leaves following trunk injection, whereafter symplastic entry into phloem resulted in

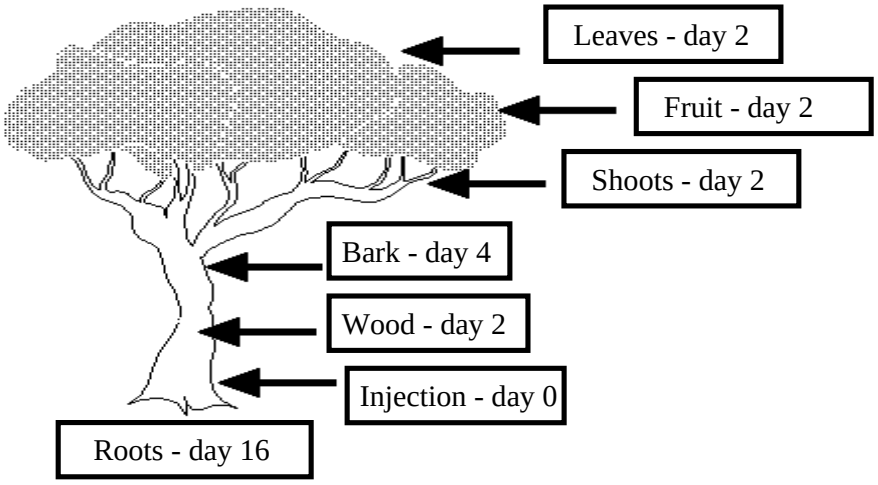

Fig. 36 Days after pre-spring shoot growth injection when significant increases in phosphonic acid concentration were measured in the different organs of the tree.

basipetal movement to the roots.

Lateral distribution of phosphonic acid in the tree

This investigation showed that H_3PO_3 moved rapidly in an acropetal and basipetal direction, but had much less effective lateral translocation. Leaves and roots on the treated side of the tree showed a substantial increase in H_3PO_3 concentration within 8 (leaves) to 32 (roots) days after trunk injection (Fig. 37). However, the increase in concentration of H_3PO_3 in leaves and roots on the untreated side of the tree occurred more slowly, and only reached 2 and 35% of the peak concentrations of leaves and roots from the treated side of the tree, respectively. In contrast, studies with translocation of phosphonate fungicides in cocoa (*Theobroma cacao* L.) have shown that trunk injection into one site in the tree is sufficient to disseminate adequate levels of H_3PO_3 throughout the tree, thereby providing protection from pod rot (*Phytophthora palmivora* Butler) (Guest *et al*. 1994). This may be due to the less complex structure of cacao plants which have a central trunk (chupon) producing lateral plagiotropic branches at given intervals (the jorquette) (Purseglove 1968). In studies with trunk-injected phosphonate in monocotyledons, Darakis *et al*. (1985) found that there was an excellent distribution throughout the plant from a single injection site. This is likely due to the presence of many short xylem vessels with numerous cross-connections which facilitate both vertical and lateral translocation within these plants.

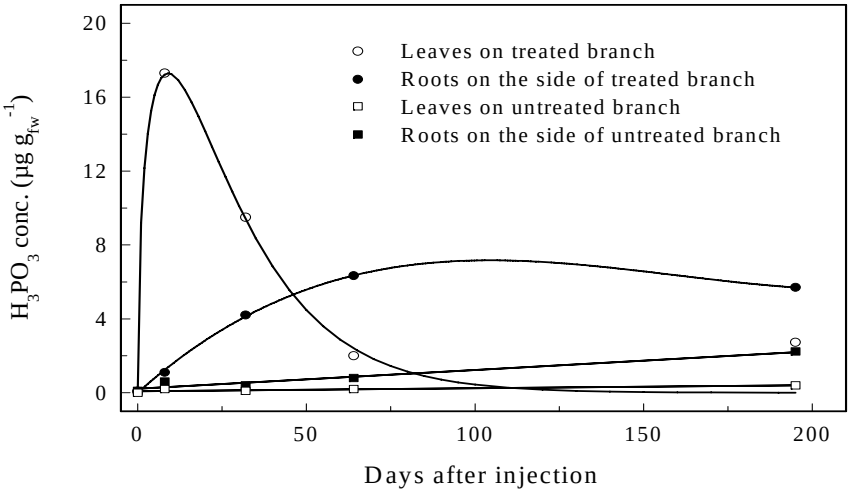

Fig. 37 Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection into one side of a dual-branched tree. The model for leaves on the treated branch is represented by $y = 9.7x^{0.48} e^{-0.053x}$, $r^2 = 0.88$ ($P < 0.17$); for leaves on the untreated branch by $y = 0.783 + 0.002x$, $r^2 = 0.88$ ($P < 0.05$); for roots on the side of the treated branch by $y = 0.0023 + 0.164x - 1.17e^{-4}x^2 + 2.44327e^{-6}x^3$, $r^2 = 0.99$ ($P < 0.05$) and for roots on the side of the untreated branch by $y = 0.22 + 0.01x$, $r = 0.97$ ($P < 0.05$). Data points are mean values of three trees.

Effect of sink strength and phosphonic acid root concentrations

The efficiency of translocation of H_3PO_3 to the roots appears directly related to the sink/source status of the leaves at the time of injection. In this study, shoot phenology measured by dry matter accumulation was similar to that previously reported for avocado (Whiley *et al*. 1988a; Ploetz *et al*. 1992; Whiley and Schaffer 1994). There were two major periods of shoot growth corresponding to spring and summer. Spring shoots grew vigorously for the first 32 days following bud-break, during which time they accumulated 66% of their final dry matter (Fig. 38). Thereafter, the growth rate declined with the maximum shoot dry matter attained 128 days after bud-break. Summer shoot growth began 96 days after spring shoot bud-break, at a time when spring growth was relatively

was relatively quiescent (Fig. 38). Dry matter accumulation in the summer shoots was not as rapid as in the spring shoots, taking ≈ 100 days to accumulate 66% of the dry matter and 190 days to maximum dry matter. There was a linear increase in fruit dry mass from fruit set to maturity, a period of ≈ 300 days (Fig. 38).

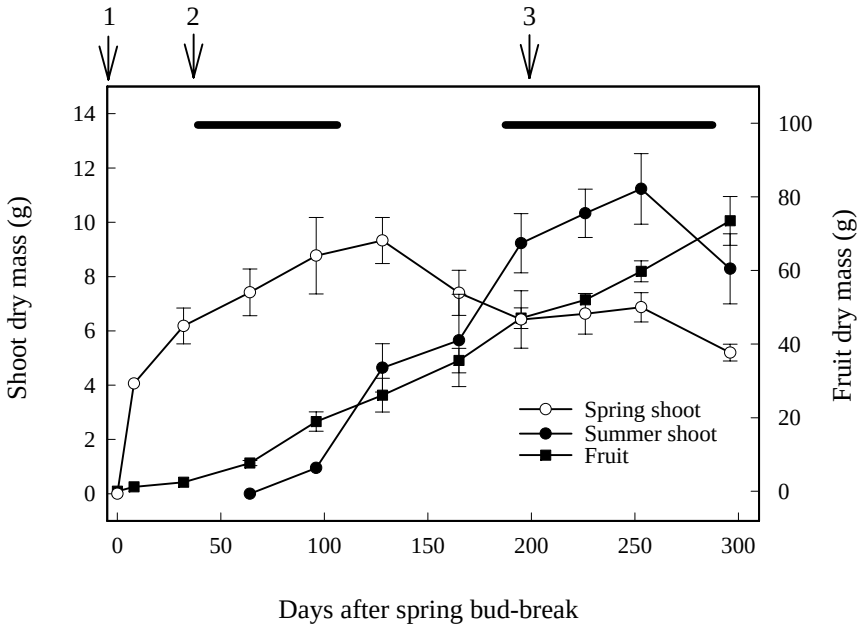

Fig. 38 Dry matter accumulation in spring and summer shoots and fruit, in cv. Hass trees during the period following trunk injection when phosphonic acid concentration fluxes in the tree were monitored. Trunk injection times are indicated by arrows where 1 = pre-spring shoot growth; 2 = spring shoot maturity; and 3 = summer shoot maturity. Horizontal bars indicate the major periods of root growth defined by Whiley *et al.* (1988a), Ploetz *et al*. (1992) and Whiley and Schaffer (1994). Data points are mean values from nine trees ± vertical SE bars which are obscured by symbols at some points.

While root growth data were not collected in this experiment, corroborating evidence from other studies (Whiley *et al*. 1988a; Ploetz *et al*. 1992; Whiley and Schaffer 1994) suggests that root growth (hence sink strength) was greatest when shoots were relatively quiescent, i.e. for a short

time ≈ 60 days following spring bud-break and for a longer period ≈ 200 days after the beginning of spring growth (Fig. 38). This is further substantiated by Whiley and Schaffer (1993) who reported that ^{14}C-photosynthate was largely retained in new, actively growing shoots (38% in shoots compared with 14.5% in roots) when trees were exposed to $^{14}CO_2$ shortly after new shoot growth had commenced. However, once all leaves on shoots were fully expanded, exposure to $^{14}CO_2$ resulted in a larger proportion of ^{14}C-photosynthate being translocated to the roots (32% in roots compared with 13% in the new shoots).

Concentration fluxes of H_3PO_3 in leaves and roots of trees trunk-injected at different stages of phenological development, mirrored the dynamics of temporal sink separation (Figs. 38 & 39). At each time following trunk injection of potassium phosphonate, there was a rapid increase in the leaf concentration of H_3PO_3 reaching between 50 and 70 $\mu g\ g_{fw}^{-1}$ within 8 to 12 days after treatment. The subsequent decline in leaf H_3PO_3 was also quite rapid, reflecting the exporting capacity of the leaves as H_3PO_3 crossed the symplast and became phloem-mobile. The decrease in leaf concentration was faster when trunk injection was given prior to spring growth (Fig. 39a) than at the other selected stages of phenology, and could be attributed to a combination of dilution by growth and translocation. However, prior to spring growth, the root sink was weak (Whiley and Schaffer 1994) which was reflected by the low concentration of H_3PO_3 that accumulated in the roots when trees were injected at that time. Conversely, leaf H_3PO_3 concentrations following treatment at summer shoot maturity, when vegetative and fruit sinks had weakened, took longer to decline (Fig. 39c). This was at a time when the root sink was strong, and resulted in the highest root concentration of H_3PO_3, which was sustained for a longer period than root concentrations resulting from injections at either the beginning or end of spring shoot growth (Fig. 39). Similar relationships with respect to leaf and root H_3PO_3 concentrations were obtained the following spring from different groups of trees treated in the same manner (data not presented).

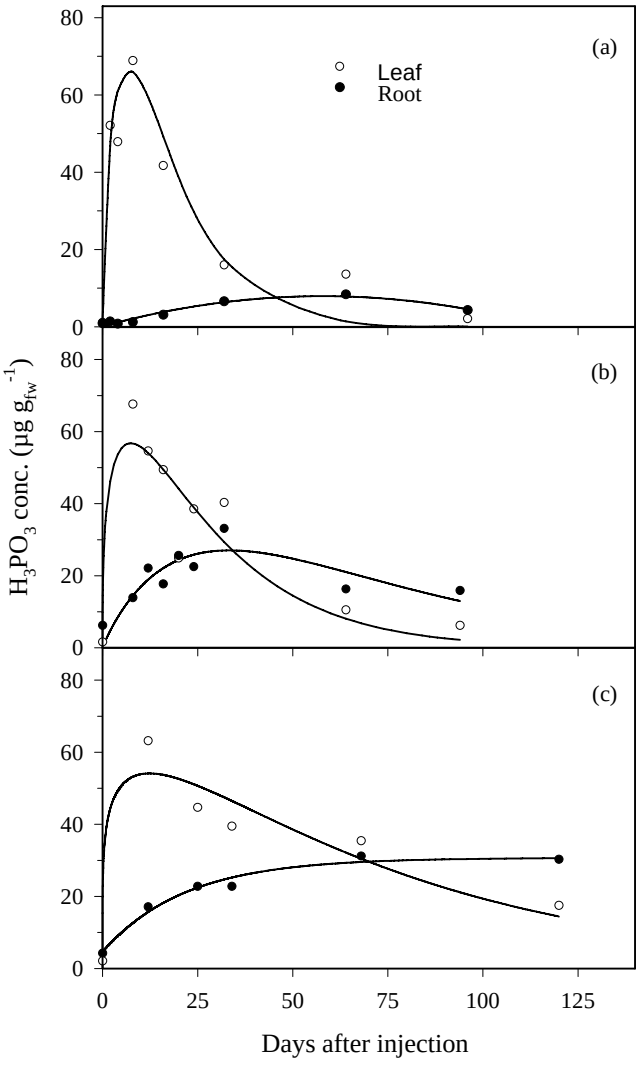

Fig. 39 Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection at (a) the beginning of spring shoot growth where the model for leaves is represented by $y = 36.0x^{0.649}e^{-0.093x}$, $r^2 = 0.69$ ($P < 0.05$); and for roots by $y = 2.94 + 0.24x - 0.0025x^2$, $r^2 = 0.87$ ($P < 0.01$); (b) maturity of the spring shoot growth where the model for leaves is represented by $y = 39.7x^{0.356}e^{-0.048x}$, $r^2 = 0.73$ ($P < 0.01$); and for roots by $y = 2.59x^{0.936}\ e^{-0.0281x}$, $r^2 = 0.58$ ($P < 0.05$); (c) during summer shoot growth where the model for the leaves is represented by $y = 39.7x^{0.205}e^{-0.0166x}$, $r^2 = 0.98$ ($P < 0.01$); and for roots $y = 30.7 - 26.02\ (0.955^x)$, $r^2 = 0.98$ ($P < 0.01$). Data points are mean values of three trees.

7. 1. 4 Conclusions

This study confirms previous reports that phosphonate is ambimobile in plants. Following trunk injection there is rapid acropetal movement in the xylem from the treatment site to the leaves. The dynamics of subsequent phloem translocation is determined by the strength of competing sinks when the H_3PO_3 enters the symplast. However, there was little redistribution of phosphonate to suggest lateral movement across the tree, demonstrating it to be slow and relatively inefficient compared with vertical movement. Phosphonate translocation to roots following trunk injection can thus increase three fold with correct timing. In subtropical Australia, disease pressure is greatest during the summer and autumn months when soil temperatures and moisture are optimum for growth and development of the pathogen, and when rapid fruit development imposes further stress on roots of heavily cropping trees. Strategically timed injections of phosphonate fungicides at either spring shoot growth maturity and/or during the mid to late summer months will protect the roots of the tree from colonisation by *P. cinnamomi* during this critical period. However, H_3PO_3 concentrations in plant tissues decrease over time due to several factors, and re-injection of phosphonate fungicides will be necessary to prevent phytophthora root rot. Further research is required to more closely define the optimum concentration of H_3PO_3 required for maximum root protection from fungal invasion, and to pre-empt a possible lowering of the MRL as a consequence of the “anti-pesticide” lobby.

7. 2 EFFECT OF SPRING APPLIED NITROGEN AND PACLOBUTRAZOL ON 'HASS' YIELD AND FRUIT SIZE

7. 2. 1 Introduction

In the last 10 years 'Hass' has become the major cultivar of the Australia avocado industry and is grown in all production regions from the highland tropics of the Atherton Tableland (latitude 17^{o}S) to the cool Mediterranean climate of south Western Australia (latitude 32^{o}S). Environmentally the cultivar is most suited to a cool subtropical climate such as that found at Maleny in S.E. Queensland (latitude 26.5^{o}S, altitude 520 m) where sustainable average yields are > 24 t ha^{-1} (Whiley and Winston 1987). Production of 'Hass' in the warm subtropics of Australia results in reduced yield and smaller fruit compared with the cooler mesic conditions of Maleny (Whiley and Winston 1987; Chapter 6). A similar phenomenon occurs in South Africa when yield and fruit size is compared between the cooler production areas of Natal and warmer areas of the Eastern and Northern Transvaal (B. N. Wolstenholme, pers. comm.[‡]).

Chemical manipulation at critical phenological stages has resulted in a commercially significant increase in 'Hass' fruit size. In a warm, subtropical climate in South Africa, preliminary results showed an increase in fruit size of cv. Hass after dipping small fruitlets in forchlorfenuron (Köhne 1991), a chemical with cytokinin-like activity in some crops which potentially increases cell division. Wolstenholme *et al*. (1990) and Whiley *et al*. (1991) reported increased fruit size and yield of 'Hass' avocado following mid-anthesis foliar sprays of the growth retardant paclobutrazol (PBZ) which they attributed to reduced competition in spring from vegetative sinks. Paclobutrazol at 2.5 g a. i. l^{-1} resulted in larger fruit by the time spring shoot growth had fully matured and this advantage was maintained through to harvest (Wolstenholme *et al.* 1990) provided that the spring vegetative flush was not too severely retarded.

Blanke and Whiley (1995) report that cv. Hass fruit have comparatively higher respiration (R_d and R_l) rates than cv. Fuerte, which is not affected by lower yields and smaller fruit in warm

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subtropical climates (Whiley and Winston 1987). They suggest that the higher energy requirement of 'Hass' may be responsible for smaller fruit size when grown in warmer climates (respiration is a direct function of temperature). Increased assimilate supply to 'Hass' fruitlets during early ontogeny may improve fruiting performance of this cultivar as this is the most rapid stage of cell division (Valmayor 1967) and fruit size of 'Hass' is directly related to cell numbers (Moore-Gordon *et al*. 1995). During this period assimilates are available from storage (starch), and current photoassimilates from over-wintered leaves which have lost photosynthetic efficiency due to photoinhibition and depleted nitrogen content (Chapter 2). Net CO_2 assimilation (*A*) is directly related to leaf nitrogen concentration (Lugg and Sinclair 1981; DeJong 1982; Syvertsen 1984). However, nitrogen fertilisation of avocados in spring reduced yield due to stimulation of competitive vegetative sinks during a critical stage of fruit development (A.W. Whiley, unpublished data).

The objective of this experiment was to improve both yield and fruit size of 'Hass', by increasing *A* of the over-wintered "source" leaves of the canopy during the early stages of fruit ontogeny with pre-bloom soil applications of nitrogen, while concurrently and temporarily suppressing vigour of the indeterminate spring shoots with mid-anthesis foliar sprays of PBZ. Forchlorfenuron (CPPU) was also included to evaluate its potential to increase fruit size. Data collected over a two year period are presented. However, the experiment has not been completed and will be continued for at least another year. Data presented in this thesis cover the period from July 1992 until October 1994.

7. 2. 2 Materials and Methods

Eight-year-old cv. Hass trees grafted to Guatemalan race seedlings and growing in a commercial orchard at Childers, S.E. Queensland (latitude 25^{o}S, altitude 40 m) with a warm, subtropical climate were selected in 1992 for this study. Temperature and rainfall data are summarised in Table 14.

For the duration of the experiment, trees were injected with 20% di-potassium phosphonate as recommended (Pegg *et al*. 1985, Whiley *et al*. 1995) to limit infection by Phytophthora root rot. Rainfall was supplemented with irrigation (micro-sprinklers) and scheduled with tensiometers to ameliorate the development of water stress (Whiley *et al*. 1988a; Banks 1992). All trees were

Table 14 Temperature and rainfall data for Childers, S.E. Queensland. Data are mean values for 1992 and 1993 and were collected with an automatic weather station (Monitor Sensors, Caboolture, AUST.) sited in the orchard.

Months	Rainfall (mm)	Temperature ^{o}C	
		Min.	Max.
Jan	51.6	20.5	32.5
Feb	272.8	20.6	29.6
Mar	19.8	18.3	27.4
Apr	11.0	16.5	25.0
May	9.4	13.5	23.9
Jun	12.5	10.3	21.9
Jul	15.6	9.3	20.4
Aug	4.7	10.0	23.1
Sep	68.2	12.5	24.7
Oct	66.4	16.1	29.6
Nov	56.2	18.1	30.1
Dec	200.0	19.3	31.3

fertilised with nitrogen, potassium and boron following recommendations from leaf analyses which were carried out on mature summer-grown leaves collected in May each year (Table 15). Leaf analyses were provided by a commercial laboratory (INCITEC, Brisbane, AUST.).

There were seven experimental treatments (Table 16) which were replicated five times in a randomised block design. The additional nitrogen was soil-applied as urea within the dripline of trees when inflorescence growth had begun on ≥ 80% of shoot terminals but before anthesis had commenced; 04/08/92 and 05/08/93, respectively. Immediately following application of urea, trees were irrigated for 5 hrs to move the fertiliser into the soil to reduce volatilisation of the nitrogen.

Table 15 Base fertiliser schedule for cv. Hass trees during 1993 and 1994.

Application Time	Nitrogen[1] (g tree^{-1})	Potassium[2] (g tree^{-1})	Boron[3] (g tree^{-1})
Feb 93	384	320	
Apr 93	96	160	22
May 93	96		
Sep 93			11
Jan 94	240	250	
Apr 94	144	200	11

[1] Nitrogen was applied as Urea (48% N)
[2] Potassium was applied as Muriate of Potash (50% K)
[3] Boron was applied as Solubor (22% B)

Paclobutrazol (as Cultar$^{(R)}$, ICI Australian Operations (Pty) Ltd, Melbourne, AUS.) was formulated with a non-ionic surfactant (Agral$^{(R)}$ at 0.05%) and applied at mid-anthesis (14/9/92 and 16/9/93, respectively) with a Stihl SG-17 motorised knapsack sprayer. Trees were sprayed to the point of run-off using between 5 and 7 l tree^{-1} (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). CPPU (supplied by INCITEC, Brisbane, AUST.) was also formulated with 0.05% Agral$^{(R)}$ and foliar applied with the aforementioned sprayer unit (21/10/92 and 24/10/93, 21/11/93) to the point of run-off.

Table 16 Experimental treatments used on cv. Hass trees in 1992, 1993 and 1994.

1992	Treatments
1	Control (commercial practice - Table 15)
2	480 g tree^{-1} of N at inflorescence emergence
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis
6	CPPU foliar spray (10 mg a. i. l^{-1}.) 3 weeks after fruit set
7	CPPU foliar spray (20 mg a. i. l^{-1}.) 3 weeks after fruit set
1993‡	
1 to 5	As above
6	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis
7	CPPU foliar spray (10 mg a. i. l^{-1}.) 3 and 7 weeks after fruit set

‡ In 1994 the CPPU treatment was discontinued.

Immediately prior to the emergence of the inflorescence (pre-anthesis), samples of the most recently matured leaves were collected for nitrogen analysis, and net CO_2 assimilation of three mature leaves from three trees of treatments 1 to 3 and 5 were measured with either a LI-COR 6200 portable photosynthesis meter in 1992 (LI-COR, Nebraska, USA) or a CIRAS-1 portable photosynthesis meter in 1994 (PP Systems, Herts, UK). Leaves for nitrogen analyses were dried and analysed following the procedures detailed in Chapter 2. Nitrogen leaf analyses and measurements of *A* were repeated on similar leaves from treatments 1 to 3 and 5 on fruiting shoots two weeks after the completion of anthesis (post-anthesis) when young fruitlets were growing at a rapid rate. Trees were harvested on 26/07/93 and 08/06/94 and yield and fruit size recorded. As nitrogen and foliar PBZ have the potential to affect fruit quality by regulation of tree vigour (Faust 1989; Witney *et al.* 1990), fruit from treatments 1 to 3, 5 and 7 were evaluated for post-harvest storage performance from the 1992/93 crop. Twenty fruit from each tree of the five

treatments were packed into standard industry fibre-board cartons immediately after harvest and stored in a cool room at 7^{o}C. After 10 days of storage fruit were examined each day to detect softening. Samples were judged as being "sprung" when 50% of the fruit in each carton had softened (judged by hand), and the number of days post-storage was recorded.

Data analysis

Statistical analysis of yield data was by ANOVA and fruit size data were first analysed by covariance to account for the effect of crop load on each of the trees. Mean values ± SEs are presented for other data.

7. 2. 3 Results and Discussion

Although chlorophyll fluorescence was not determined, mean minimum night temperatures during July were below 10^{o}C (Table 14) and photoinhibition of PS II could be expected, reducing photosynthetic efficiency of over-wintered leaves during the late winter and early spring (Chapters 2 & 3). Due to restricted research funding and the servicing requirements of equipment, nitrogen content and *A* of leaves were only measured at pre- and post-anthesis stages of growth in 1992 and 1994. In these years there were no significant differences among treatments in pre-anthesis leaf N concentrations and *A*. Nitrogen concentrations were between 2.0 and 2.2% and *A* determined during the first week of August across all treatments was ca. 12 μmol CO_2 m^{-2} s^{-1} (Table 17). Post-anthesis measurements taken 9 to 10 weeks later showed a substantial reduction in leaf N concentrations and *A* across all treatments. However, there were differences among treatments with leaf N concentrations higher than control where additional nitrogen was applied pre-anthesis. There were also differences between the two nitrogen treatments, with higher leaf N concentration on trees sprayed at mid-anthesis with PBZ. Paclobutrazol also maintained higher post-anthesis leaf N concentrations compared to control trees suggesting that where PBZ was applied, suppression of spring shoot growth reduced translocation of nitrogen from over-wintered leaves to the new growth. There are numerous other reports documenting increased leaf N concentrations in fruit crops following applications of PBZ (Atkinson and Crisp 1982; Raese and Burts 1983; Wang *et al*. 1985). Post-anthesis *A* closely followed leaf N concentrations and was significantly higher in those treatments where additional nitrogen and PBZ were applied to trees. Net CO_2 assimilation was also

also higher in trees where only PBZ was applied compared with *A* of leaves on control trees (Table 17).

In studies with soil-applied PBZ on pecan seedlings, Wood (1984) was unable to show significant differences in *A* of treated trees compared with controls, although there was a trend in the data which showed increasing *A* as the concentration of applied PBZ increased. The lack of a significant response may be due to the study being carried out with container-grown trees which is known to restrict *A* (Arp 1991; Thomas and Strain 1991). Wood (1984) reported A_{max} of 11.4 μmol CO_2 m^{-2} s^{-1} which is considerably lower than the A_{max} measured by Anderson and Brodbeck (1988b) for pecan (22 μmol CO_2 m^{-2} s^{-1}).

Due to the high coefficients of variation typical of orchard trees (27.4% and 45.5%) there were no significant yield differences among treatments in either the 1992/93 or 1993/94 crops (Tables 18 and 20). However, in 1992/93 trees receiving additional nitrogen and PBZ produced on average ca. 33% more fruit than control trees suggesting improved performance of these trees (Table 18). The trend in yield of the PBZ treatments and their relationship to control trees and to each other is in line with results described by Whiley *et al.* (1991). From three consecutive years of data they reported that 'Hass' yield was significantly increased by mid-anthesis foliar sprays of PBZ and that 1.25 g a. i. l^{-1} gave higher yields than 2.5 g a. i. l^{-1}. However, the latter increased fruit size in relation to the control trees. The higher concentration of CPPU (treatment 7) gave mild phytotoxic symptoms in new shoot growth with chlorotic areas developing in some of the leaves and though not significantly, average yield was lower than controls. Following a covariance analysis adjusting for yield, there were no significant differences in mean fruit size among treatments (Table 18).

Table 18 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on yield and fruit size of cv. Hass at Childers in 1992/93. Data are mean values from five trees for each treatment and fruit size data have been subjected to covariance analysis adjusting for yield. Fruit size values in parenthesis are unadjusted means. There were no significant differences between values in columns as tested by ANOVA.

	Treatments	Yield (kg tree^{-1})	Fruit size(g)
1	Control	114.0	244.5 (242.5)
2	480 g tree^{-1} of N at inflorescence emergence	102.5	275.7 (265.0)
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	112.9	245.5 (242.4)
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	123.9	231.7 (236.6)
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	151.6	216.6 (241.8)
6	CPPU foliar spray (10 mg a. i. l^{-1}) 3 weeks after fruit set	112.1	231.9 (228.2)
7	CPPU foliar spray (20 mg a. i. l^{-1}) 3 weeks after fruit set	103.1	273.7 (263.4)
Regression coefficient			0.730**

With exception of treatment 2, there was no treatment effect on fruit shape. Additional nitrogen increased the fruit length/diameter ratio resulting in “neckier” fruit when compared with other treatments (Table 19). As fruit from control trees had similar length/diameter ratios as those from PBZ treatments it would appear that the latter had no effect on fruit shape. These results are in contrast to earlier reports where foliar applications of PBZ at mid-anthesis reduced the fruit length/diameter ratio of cvs. Hass and Fuerte avocados, effectively producing rounder fruit (Wolstenholme *et al*. 1990). The only major difference between these experiments were the environmental conditions, the latter being carried out in cooler, higher rainfall areas at more southerly latitudes than Childers.

The application of additional nitrogen and PBZ to trees significantly increased the post-harvest storage life of fruit when held at 7^{o}C compared with all other treatments. Although not statistically

Table 19 Fruit shape (length/diameter ratio) and storage life at 7°C until 50% of the fruit had detectable softening. Data for fruit shape and number of storage days are treatment means ± SEs of 20 fruit from each of five trees.

	Treatments	Fruit shape (l/d ratio)	‡N°. of days stored at 7°C
1	Control	1.36 ± 0.02	33.6 ± 1.1
2	480 g tree^{-1} of N at inflorescence emergence	1.42 ± 0.03	31.8 ± 1.3
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	1.35 ± 0.01	34.0± 1.3
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	1.36 ± 0.02	37.4 ± 1.1
7	CPPU foliar spray (20 mg a. i. l^{-1}) 3 weeks after fruit set	1.35 ± 0.02	34.5 ± 1.7

‡ The author acknowledges the assistance of Dr P. J. Hofman and Mr L. Smith, Queensland Department of Primary Industries in the collection of these data.

significant, there was a trend for fruit from trees receiving only additional nitrogen (treatment 2) to soften earlier than other treatments. Witney *et al.* (1990) reported that suppression of vigour in avocado trees increased fruit calcium concentrations thereby improving the storage potential of fruit. In their studies an increase in fruit Ca concentration of 1000 mg kg^{-1} extended shelf life by six days. Whiley (unpublished data) found that mid-anthesis foliar applications of PBZ (1.25 g a. i. l^{-1}) to 'Hass' increased Ca concentrations of young fruitlets by up to ca. 20% during the first 12 weeks of ontogeny, a critical period for the development of cell wall and membrane integrity (Bower 1985; Cutting and Bower 1989). Cutting and Bower (1990) also report increased Ca concentrations in 'Hass' fruit after trunk injection of PBZ at mid-anthesis; a treatment which also reduced spring shoot vigour.

Unavoidable circumstances relating to grading and packing fruit from the experiment delayed the harvest in 1993 which occurred some time after floral induction and development had begun. Treatment 5 trees were carrying very large crops and flowering was greatly reduced in this treatment which was reflected in the 1994 yield (Table 20). Post-harvest evaluations were not carried out in 1994 because some trees had too few fruit at maturity.

Table 20 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on yield and fruit size of cv. Hass in 1993/94. Data are mean values from five trees for each treatment and fruit size data have been subjected to covariance analysis adjusting for yield. Fruit size values in parenthesis are unadjusted means. There were no significant differences between values in columns as tested by ANOVA.

	Treatments	Yield (kg tree^{-1})	Fruit size(g)
1	Control	108.2	194.9 (195.9)
2	480 g tree^{-1} of N at inflorescence emergence	93.2	200.7 (205.5)
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	131.0	216.2 (211.8)
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	144.0	206.4 (198.8)
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	94.7	211.8 (216.2)
6	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	111.0	215.6 (214.0)
7	CPPU foliar spray (10 mg a. i. l^{-1}) 3. and 7 weeks after fruit set	105.2	186.2 (187.7)
Regression coefficient			-0.243**

The 1993/94 yield data suggested a trend for mid-anthesis PBZ at 2.5 and 1.25 g a. i. l^{-1} to carry heavier crops than other treatments but differences were not significant. Once covariance adjustment had been made for crop load there were no significant differences among treatments in mean fruit size (Table 20).

With respect to the cumulative yield for the two years of the study there were significant differences among treatments (Table 21). Mid-anthesis PBZ at 1.25 g a. i. l^{-1} produced the most fruit on average, although not significantly more than PBZ at 2.5 g a. i. l^{-1} or nitrogen plus PBZ at 2.5 g a. i. l^{-1}. The cumulative yields of each of these three treatments were greater than those of the

controls and trees where additional nitrogen alone was given. There were no significant differences in mean fruit size among treatments.

There was no significant effect of CPPU on fruit yield or mean fruit size (Table 21). This is in contrast to the earlier report of Köhne (1991) where mean fruit size of 'Hass' was significantly increased after dipping fruitlets in a 10 mg a. i. l^{-1} a. i. solution when about 3 to 5 mm in diameter.

Table 21 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on cumulative yield and mean fruit size of cv. Hass for 1992/93 and 1993/94. Data are mean values from five trees for each of the treatments. Values not having a common letter are significantly different ($P \leq 0.05$) as tested by ANOVA.

	Treatments	Yield (kg $tree^{-1}$)	Fruit size(g)
1	Control	194.7c	219.7a
2	480 g $tree^{-1}$ of N at inflorescence emergence	189.3c	238.2a
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	243.9ab	230.8a
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	267.9a	219.1a
5	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	246.3ab	214.2a
6	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis‡	223.1bc	233.8a
7	CPPU foliar spray (10 mg a. i. l^{-1}.) 3. and 7 weeks after fruit set	208.2bc	229.9a

‡ In the first year of the study these trees received CPPU at 10 mg a. i. l^{-1}.

However, a more recent study where trees were sprayed with CPPU shortly after fruit set reported a significant decrease in yield and no increase in fruit size from the treatment (Köhne *et al.* 1993). Wolstenholme (pers. comm.‡‡‡) was also unable to increase 'Hass' fruit size with forchlorfenuron sprays in an environment in Natal which is conducive to relatively large fruit size.

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7. 2. 4 Conclusions

These preliminary results indicate that higher yield (averaged over two seasons) and improved storage life can be achieved for 'Hass' growing in warm, subtropical climates. Nitrogen applied before flowering in combination with mid-anthesis foliar sprays of PBZ gave increased net photosynthesis, yield and storage life, while PBZ alone also increased fruit yield compared with trees receiving standard commercial recommendations. There was no improvement in fruit size from any of the chemical treatments. However, despite higher yields these treatments were able to maintain fruit size in relation to lower producing treatments. The pre-anthesis nitrogen treatment plus mid-anthesis PBZ in 1993 produced extremely heavy crops which led to failure of flowering the following season. The latter may be due to insufficient root growth during the late summer and early winter at the time of floral induction as discussed in earlier chapters. These trees have flowered and set heavily again in 1994 and large crops are expected in 1995. The combination of pre-flowering nitrogen and mid-anthesis PBZ on fruit set and retention is probably causing over-cropping in years when trees flower normally. While no direct evidence has been produced from this study, it is likely that the increased *A* at a critical stage of development together with restricted shoot growth has improved the opportunity for fruit retention. Further research is required to see if reliable management strategies can be developed to effectively utilise the concept being tested. It is clear that a high level of management expertise would be necessary to reliably assess tree condition and the likely response to treatment. Finally, in view of the practical impossibility of applying CPPU exclusively to young fruits, further research with this growth regulator would appear to be contra-indicated