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Changing Sink Strengths Influence Translocation of Phosphonate in Avocado (*Persea americana* Mill.) Trees

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Abstract

Translocation of phosphonic acid (H_3PO_3) in cv. Hass avocado trees was studied after trunk injection with 20% H_3PO_3, formulated as potassium phosphonate, at three stages of tree phenology during the growing season. Initially, translocation was solely acropetal in the xylem, and H_3PO_3 was detected in the leaves 24 h after treatment. Several days after injection, H_3PO_3 concentration in the bark of trunks and in roots increased, indicating basipetal phloem transport of H_3PO_3 from leaves. The rate of accumulation and the final concentration of H_3PO_3 in the roots were directly related to the sink strength of the shoot at the time of injection. For example, trunk injection at the beginning of spring growth flush, when renewal shoots were strong sinks, resulted in low H_3PO_3 root concentrations ($<9\ \mu g\ g_{fw}^{-1}$) which peaked about 45 days after treatment. When potassium phosphonate was injected after the transition of spring-grown shoots from sinks to sources, or at summer shoot maturity, root concentrations of H_3PO_3 increased to $\geq 25\ \mu g\ g_{fw}^{-1}$ by 30 days after treatment. These results suggest that strategic timing of injections according to phenological events may greatly improve fungicide efficacy when targeting specific organs for protection.

Keywords: avocado, phosphonates, phosphonic acid,*Phytophthora cinnamomi*, sink strength.

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). Owing 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 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 ionized 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).

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Phytophthora cinnamomi Rands is a devastating root disease of avocado (*Persea americana* Mill.) in most countries which grow this crop. The fungus invades the unsuberized roots, and less frequently attacks the suberized 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 colonization by the pathogen (Schutte *et al.* 1988; Guest and Grant 1991). The development of trunk injection of phosphonates during the 1980*s*, 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.

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. The asynchronous pattern of shoot and root growth in avocado is illustrated in phenology models developed for avocado (Whiley *et al.* 1988) which have since been confirmed by the observations of Ploetz *et al.* (1992) and Whiley and Schaffer (1994). If translocation of both photoassimilates and phosphonate is subject to similar controls, then natural variation (asynchrony) in root vs. shoot growth may well influence distribution patterns of trunk-injected H_3PO_3. Accordingly, we set out to establish whether H_3PO_3 movement to roots was influenced by sink-strength dynamics within trees at the time of application.

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° S., 650 m altitude), which has a cool, high rainfall subtropical climate.

Tree phenology was monitored by collecting fruiting shoots from each of the nine experimental trees immediately prior to treatment, then subsequently at *c.* 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 weights of the stems, leaves and fruit were determined separately after 72 h at 90°C 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. 4). 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. 4). Injections were carried out using Chemjet® tree injectors (Chemjet Trading Pty Ltd, Caboolture, Qld) 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 wood to a depth of 40 mm, thereby placing the injected H_3PO_3 into the xylem tissue and directly into 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; about nine injection sites per tree. Fresh samples of organs were collected from these trees before treatment and at 2–30 day intervals following injection, and analysed for H_3PO_3 content. Four subsamples 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. Subsamples of unsuberized roots were collected from each quadrant of the tree and 20 subsamples of leaves, stems and shoots were taken from positions representative of the entire canopy at each sampling time. Subsamples 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 8 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 weights 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. 4). Following injection, fresh unsuberized 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 Gas Chromatography

Concentrations of H_3PO_3 were measured in avocado tissues using an acid extraction and gas chromatography. Samples were extracted with dilute aqueous sulfuric acid and derivatized 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 phosphoric acid in a variety of tissues (leaf, root, fruit [including seed, skin and flesh], bark and wood) with low detection limits ($\leq 0{\cdot}1\ \mu g\ g_{fw}^{-1}$) and recoveries at $\geq$ 80%. (P. A. Hargreaves, unpubl. 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.

Results and Discussion

Distribution and Loss of Phosphonic Acid from the Tree (Pre-spring Shoot Growth Injection)

Prior to trunk injection, low levels of H_3PO_3 ($<3{\cdot}0\ \mu g\ g_{fw}^{-1}$) were detected in all parts of the tree (Fig. 1), 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 2 days of treatment, substantial increases in shoot and leaf concentrations were measured: $2{\cdot}2{\pm}0{\cdot}5$ to $77{\cdot}4{\pm}6{\cdot}9$ and $1{\cdot}1{\pm}0{\cdot}3$ to $52{\cdot}1{\pm}7{\cdot}20\ \mu g\ g_{fw}^{-1}$, 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 1 and 4). The H_3PO_3 concentration in these tissues peaked 8 days after injection and then rapidly declined in leaves (Figs 1*a* and 1*b*). 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 8 days of treatment, after which there was a rapid decline in concentration (Fig. 1*b*). 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 $2{\cdot}5\%$ 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. 1*a*). 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. 1*b*).

Concentrations of H_3PO_3 in the bark and wood of trunks were low compared to other organs of the tree (Fig. 1*c*). In the bark an increase in H_3PO_3 concentration

was measured 4 days after trunk injection (from 1·3±0·2 to 4·7±0·7 $\mu g\ g_{fw}^{-1}$), and peaked after 32 days (7·6±1·2 $\mu g\ g_{fw}^{-1}$), while in the wood the maximum concentration (5·4±0·5 $\mu g\ g_{fw}^{-1}$) was reached 8 days after treatment.

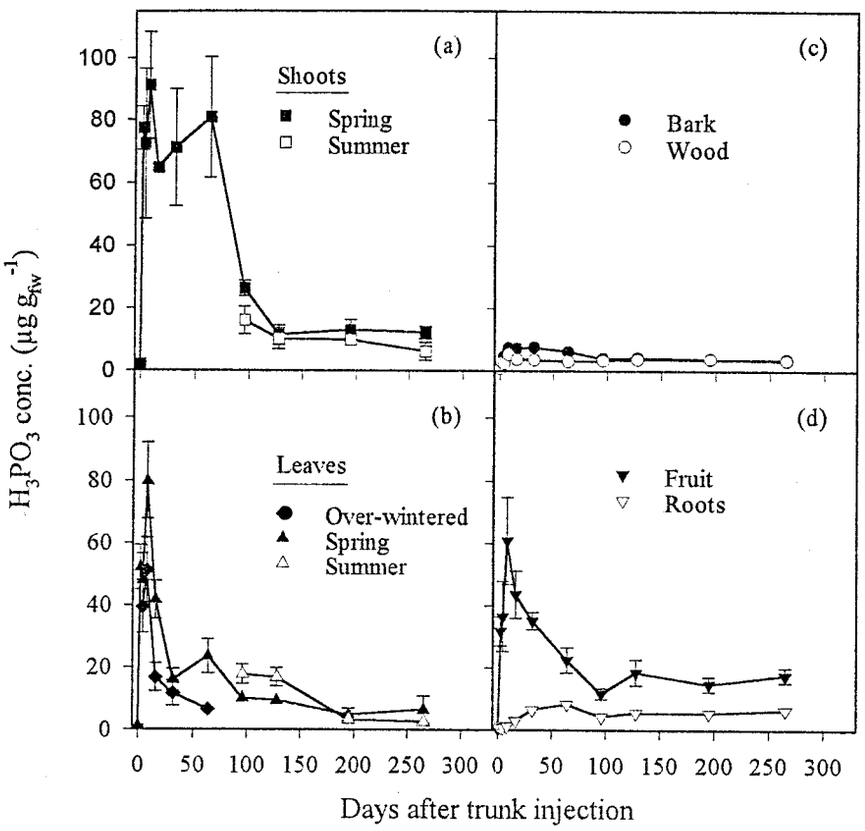

Fig. 1. Concentration flux of phosphonic acid (H_3PO_3) in avocado (*a*) shoots, (*b*) leaves, (*c*) trunk bark and wood, and (*d*) fruit and roots following trunk injection with 20% solution of potassium phosphonate at the beginning of spring shoot growth. Data points are mean values from three trees±s.e. bars (s.e. bars are obscured by symbols at some points).

Concentrations of H_3PO_3 in young fruit increased sharply following trunk injection, reaching 60·8±14·0 $\mu g\ g_{fw}^{-1}$ eight days after treatment. Thereafter, concentrations declined until stabilizing (at ≈17 $\mu g\ g_{fw}^{-1}$) 64 days after injection (Fig. 1*d*). The considerable H_3PO_3 concentration in fruit at an early stage of their ontogeny was in contrast to <1 $\mu g\ g_{fw}^{-1}$ H_3PO_3 detected following trunk injection when fruit were mature (Pegg and 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 $\mu g\ g_{fw}^{-1}$ for fruit compared with 12·3±2·0 $\mu g\ g_{fw}^{-1}$ 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 $\mu g\ g_{fw}^{-1}$ 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 $\mu g\ g_{fw}^{-1}$. The highest root concentration of H_3PO_3 was only 8·4±1·9 $\mu g\ g_{fw}^{-1}$, measured 64 days after injection (Fig. 1*d*). 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 $\mu g\ g_{fw}^{-1}$ 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 (50–80 $\mu g\ g_{fw}^{-1}$) with much lower concentrations in leaves (mature over-wintered) and twigs (10–20 $\mu g\ g_{fw}^{-1}$), thereby providing further evidence of the effect of sink strength on distribution (Cannell 1985). It was estimated that $6{\cdot}85 \pm 0{\cdot}98$ g (≈30%) of H_3PO_3 were lost from each tree from the time of injection until fruit harvest (296 days later). Approximately $3{\cdot}51 \pm 0{\cdot}28$ g (≈15%) of the total amount lost was attributed to loss through the litter cycle, while $3{\cdot}34 \pm 0{\cdot}57$ 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

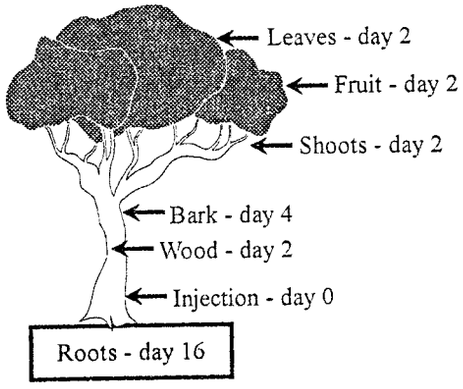

Fig. 2. Number of days after pre-spring shoot growth injection when significant increases in phosphonic acid concentration were measured in the different organs of the tree.

profiles of sucrose and phosphonate are almost identical (Martin and Edgington 1981; Dewey and Appleby 1983; Chamberlain *et al.* 1984). In our study the pattern of distribution of H_3PO_3 within avocado trees provides further evidence of the ambimobility of phosphonate. Following injection directly into the transpiration stream of trees, increased concentrations of H_3PO_3 were measured in stems and leaves of shoots and in fruit 2 days, and in bark of trunks, 4 days after treatment; however, there was no increase in root concentration until 16 days after trunk injection (Fig. 2). This time sequence suggests an apoplastic translocation pattern via the xylem to the leaves following trunk injection, whereafter symplastic entry into phloem resulted in active basipetal movement to the roots. The speed of the initial distribution within each canopy will thus vary with time of day that the injection is made owing to differences in transpirational flux.

Lateral Distribution of Phosphonic Acid in the Tree

Our studies showed that H_3PO_3 moved rapidly in an acropetal and basipetal direction, but had a 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. 3). However, the increase in concentration of H_3PO_3 in leaves and roots on the untreated side of the tree occurred more slowly, and reached only 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 cacao (*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.

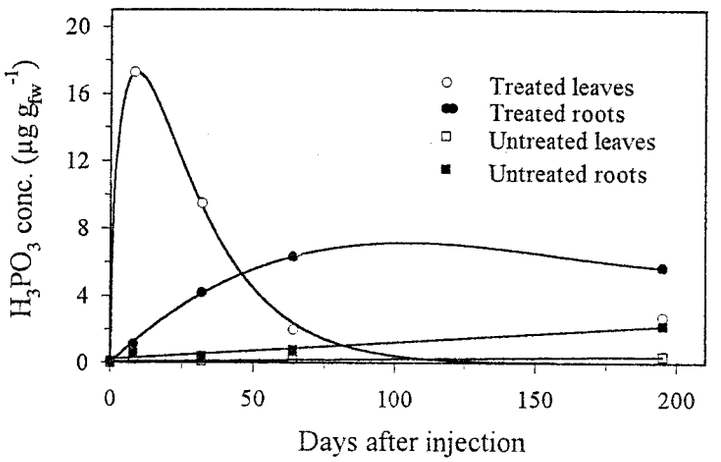

Fig. 3. Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following injection into one branch of a forked tree. The model for leaves on the treated branch is represented by $y = 9{\cdot}7x^{0{\cdot}48}e^{-0{\cdot}053x}$, $r^2 = 0{\cdot}88$ ($P < 0{\cdot}17$); for leaves on the untreated branch by $y = 0{\cdot}783{+}0{\cdot}002x$, $r = 0{\cdot}88$ ($P < 0{\cdot}05$); for roots on the side of the treated branch by $y = 0{\cdot}0023{+}0{\cdot}164x{-}1{\cdot}17e^{-4}x^2{+}2{\cdot}44327e^{-6}x^3$, $r^2 = 0{\cdot}99$ ($P < 0{\cdot}05$) and for roots on the side of the untreated branch by $y = 0{\cdot}22{+}0{\cdot}01x$, $r = 0{\cdot}97$ ($P < 0{\cdot}05$). Data points are mean values of three trees.

Effect of Strategic Timing of Injections on 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 our study, shoot phenology measured by dry matter accumulation was similar to that previously reported for avocado (Whiley *et al.* 1988; 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. 4). 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 quiescent (Fig. 4). Dry matter accumulation in the summer shoots was not as rapid as in the spring shoots, taking *c.* 100 days to accumulate 66% of the dry matter and 190 days to maximum dry matter. There was a linear increase in fruit dry weight from fruit set to maturity, a period of *c.* 300 days (Fig. 4).

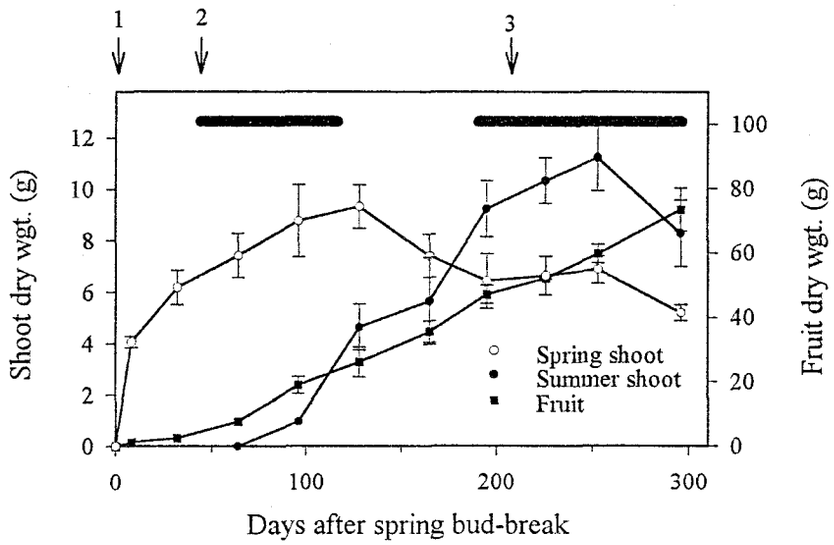

Fig. 4. Dry matter accumulation in spring and summer shoots and fruit, in cv. Hass trees during the period following trunk injection when phosphonic acid concentration fluxes in the tree were monitored. Trunk injection times are indicated by arrows where 1 = pre-spring shoot growth, 2 = spring shoot maturity, and 3 = summer shoot maturity. Horizontal bars indicate the major periods of root growth defined by Whiley *et al.* (1988), Ploetz *et al.* (1992) and Whiley and Schaffer (1994). Data points are mean values from nine trees±s.e. bars (s.e. bars 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.* 1988; 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 *c.* 60 days following spring bud-break and for a longer period *c.* 200 days after the beginning of spring growth (Fig. 4). 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 4 and 5). 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.

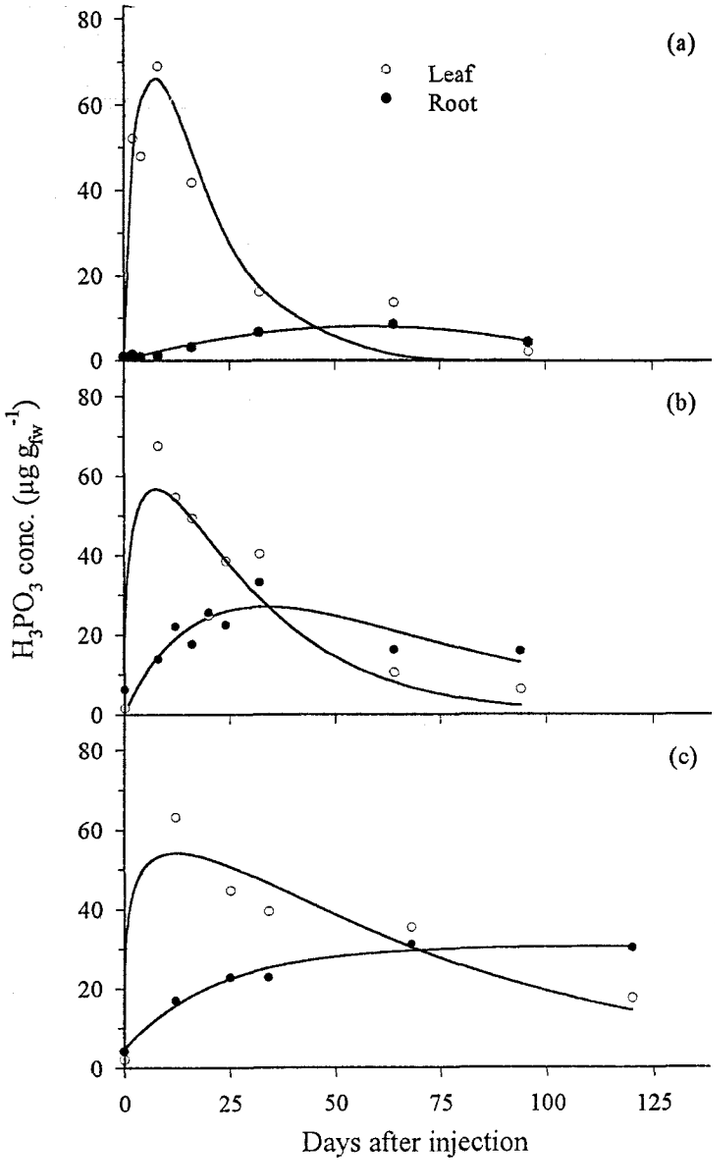

Fig. 5. Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection at (*a*) the beginning of spring shoot growth where the model for leaves is represented by $y = 36{\cdot}0x^{0{\cdot}649}e^{-0{\cdot}093x}$, $r^2 = 0{\cdot}69$ ($P < 0{\cdot}05$); and for roots by $y = 2{\cdot}94 + 0{\cdot}24x - 0{\cdot}0025x^2$, $r^2 = 0{\cdot}87$ ($P < 0{\cdot}01$); (*b*) the maturity of the spring shoot growth where the model for leaves is represented by $y = 39{\cdot}7x^{0{\cdot}356}e^{-0{\cdot}048x}$, $r^2 = 0{\cdot}73$ ($P < 0{\cdot}01$); and for roots by $y = 2{\cdot}59x^{0{\cdot}936}\, e^{-0{\cdot}0281x}$, $r^2 = 0{\cdot}58$ ($P < 0{\cdot}05$); (*c*) the maturity of summer shoot growth where the model for the leaves is represented by $y = 39{\cdot}7x^{0{\cdot}205}e^{-0{\cdot}0166x}$, $r^2 = 0{\cdot}98$ ($P < 0{\cdot}01$); and for roots $y = 30{\cdot}7 - 26{\cdot}02\ (0{\cdot}955^x)$, $r^2 = 0{\cdot}98$ ($P < 0{\cdot}01$). Data points are mean values of three trees.

The decrease in leaf concentration was faster when trunk injection was given prior to spring growth (Fig. 5*a*) 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. 5*c*). 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. 5). 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).

Conclusions

Our 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 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. Hence, for effective protection of roots, injection sites must be spaced around the full circumference of the trunk and with correct timing phosphonate translocation to roots may be increased by threefold. 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 colonization by *P. cinnamomi* during this critical period. However, H_3PO_3 concentrations in plant tissues decrease over time due to several factors, and reinjection of phosphonate fungicides will be necessary to prevent phytophthora root rot. Further research is needed to more closely define the optimum concentration of H_3PO_3 required for maximum root protection from fungal invasion.

Acknowledgments

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