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Biological and chemical control of root rot

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ABSTRACT

Phytophthora cinnamomi **isolates from trees treated for a prolonged period with Aliette were significantly less sensitive to H_3PO_3 in vitro when compared to *Phytophthora cinnamomi* isolates from untreated trees. *Phytophthora cinnamomi* isolates from H_3PO_3 treated trees also tended to be less sensitive to H_3PO_3 in vitro (though not statistically significant) when compared to *Phytophthora cinnamomi* isolates from untreated trees.**

Application of antagonistic fungi (*Paecilomyces lilacinus*, *Aspergillus candidus* and *Trichoderma hamatum*) to nursery trees before and after planting in the orchard, as well as to already established orchard trees was tested for control of *Phytophthora cinnamomi* root rot since 1992. Results showed that out of the three antagonistic fungi evaluated, *A. candidus* and *T. hamatum* established and survived well in the soils. Suppressiveness of soils to *P. cinnamomi* was enhanced, and *P. cinnamomi* populations were decreased to some extent by treatments of *A. candidus* and *T. hamatum*. The condition of trees treated with phosphonates or *A. candidus* improved, while no yield differences occurred between trees from different treatments.

Efficacy of trunk sprays of Aliette WP and H_3PO_3 was compared to H_3PO_3 trunk injections by analysing the roofs of treated trees for phosphonic acid residues. Trunk spray of Aliette WP (0.8g a.i./m^2) or H_3PO_3 (0.8g a.i./m^2) applied every six weeks resulted in similar phosphonic add levels than the standard two rounds of trunk injections. Unlike injections, the trunk spray is not detrimental to the wood of the tree and the method holds much promise for future root rot control.

RESISTANCE OF *P. CINNAMOMI* TO H_3PO_3

The avocado industry is totally reliant on phosphonates for chemical control of root rot, as there is no other fungicide available for continuous use. Phosphonates have been used world wide for root rot control since the development of the trunk injection technique at Westfalia Estate in the early 1980's (Darvas *et al*, 1983). However, it was found for the first time in 1992 (Duvenhage, 1994) that *P cinnamomi* isolates which were exposed to phosphonates for a prolonged period (isolated from the original trial orchard at Westfalia Estate treated since 1980) were less sensitive to phosphonates *in vitro* than isolates from untreated trees. Subsequently, similar results were also found in Australia by Weinert ef al (1998).

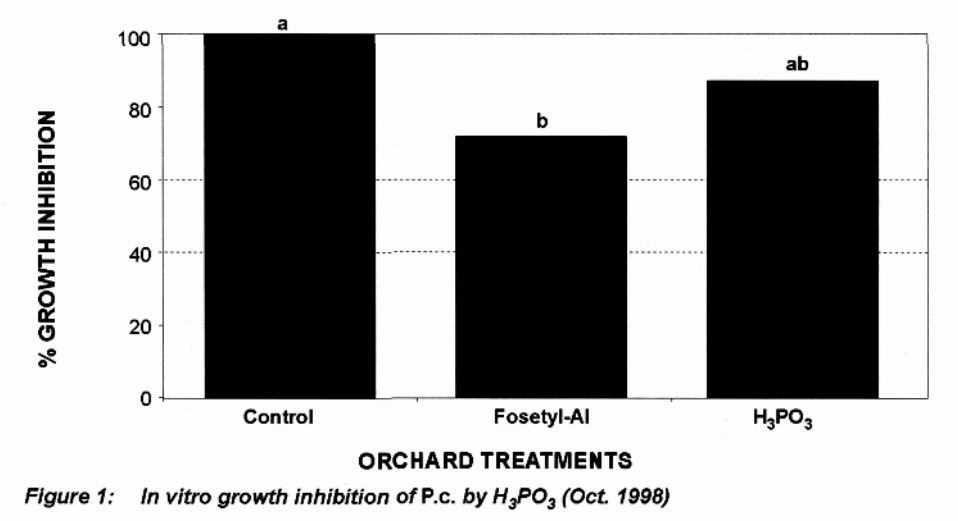

Figure 1: In vitro growth inhibition of P.c. *by* H_3PO_3 *(Oct. 1998)*

In the initial tests done in South Africa (1992 and 1993), five isolates each of Aliette treated, H_3PO_3 treated, or untreated trees were tested (Duvenhage, 1994), while in subsequent tests the number of isolates were increased to 10 each (Duvenhage & Köhne, 1995, 1996, 1997). The tests are repeated bi-annually to keep track of possible further decreases in the sensitivity of isolates to H_3PO_3. Although the *in vitro* testing of the sensitivity of isolates towards phosphonates only reflects on the direct mode of action of phosphonates and not on the indirect mode of action, it still gives an indication of the relative sensitivity of isolates from phosphonate treated or untreated trees.

Results from tests done in 1998 (Fig. 1) showed that *P cinnamomi* isolates from trees treated with Aliette or H_3PO_3 were respectively 21% or 10% less sensitive to H_3PO_3 than isolates from untreated trees (as indicated by *in vitro* inhibition of mycelium growth). The average decrease in the sensitivity (over the period 1992-1998) of isolates from phosphonate treated trees is about 13% when compared to isolates from untreated trees. Also, it was reported in Australia (Weinert *et al.*, 1998) that the EC50 values of phosphonate, for *P cinnamomi* isolates from untreated trees were 9 ppm ±1.41, while the EL50 values for isolates from phosphonate treated trees were 98 ppm ± 90.4. All these results indicate a shift in the sensitivity of *P cinnamom*i after long term treatment with phosphonates.

BIOCONTROL OF ROOT ROT - FINAL REPORT

Because the development of resistance to phosphonates remains a threat, it is necessary to implement other ways of root rot control, and to scale down the use of phosphonates. This will decrease the pressure for development of resistance to phosphonates in *Phytophthora cinnamomi* populations, thereby prolonging the effective lifetime of phosphonates. The development of an antagonist treatment for the biocontrol of root rot could make a meaningful contribution to the avocado industry becoming less reliant on phosphonates for root rot control.

The discovery of suppressive soils in South Africa (Duvenhage & Maas, 1990; Duvenhage *et al*., 1991) led to the isolation of antagonistic microorganisms which were able to control root rot of avocado under greenhouse conditions (Duvenhage & Kotzé, 1993). Three fungal antagonists (*Aspergillus candidas* [PREM. 50935], *Trichoderma hamatum* [PREM. 50938] and *Paecilomyces lilacinus* [PREM. 50933]) were evaluated since 1992 under orchard conditions for root rot control. Forty newly planted (reduced to 20 after orchard thinning in 1997) and twenty established trees were used for each treatment (Duvenhage & Köhne 1995, 1996, 1997, Duvenhage & Kremer-Köhne, 1998). Antagonists were applied as spore suspensions (individually or in combination) to the nursery medium or orchard soil during rain or irrigation. For comparison, untreated control and standard phosphonate treatments were included.

Antagonist and *P. cinnamomi* populations, suppressiveness of soils to *P cinnamomi*, root and tree health, and yield were monitored:

- During March of each year, soil samples were taken from the topi 5cm of soil (pooling three subsamples from each tree), and used for the following:
    - Antagonist populations were monitored by doing colony counts on dilution plates (using potato dextrose agar amended with chloramphenicol). *P cinnamom*i populations were monitored using the avocado leaf bait technique as described by Pegg (1977). Ten leaf discs were suspended on a watery slurry of each soil sample and incubated for 3-5 days at room temperature. The discs were then plated on PARPH selective medium (Solel & Pinkas, 1984), and the percentages of discs yielding growth of *P cinnamomi* determined. Suppressiveness of soils was based on a bioassay method described by Broadbent *et al.* (1971), and New Zealand blue lupin seedlings *(Lupinus angustifolius)* were used as indicator plants (Chee & Newhook, 1965).

Soil samples were dried at room temperature, ground, and mixed with vermiculite at 5 % (w/v). Mycelium of *P cinnamom*i (PREM 50801) was then incorporated at a rate of 0.05 % (W/V). Ten to fourteen days after planting, the seedlings were weighed, and the fresh mass used as an indication of root rot severity (relating to suppression of disease).

- Root health of trees was rated visually during May 1 998 on a 0 5 scale where:
    1. = No visible sign of disease
    2. = Less than 20 % of roots affected by root rot
    3. = 21-40 % of roots affected by root rot
    4. = 41-60 % of roots affected by root rot
    5. = 61-80 % of roots affected by root rot
    6. = More than 80 % of roots affected by root rot
- Tree condition was rated visually during July/August every year on a 0-10 scale, where 0 = completely healthy (no root rot symptoms above ground), and 1 0 = completely dead.
- Yield was rated visually during February every year according to fruit density, on a 1 5 scale where:
    1. = Poor fruit density

2. = Below average fruit density
3. = Average fruit density
4. = Above average fruit density
5. = Outstanding fruit density

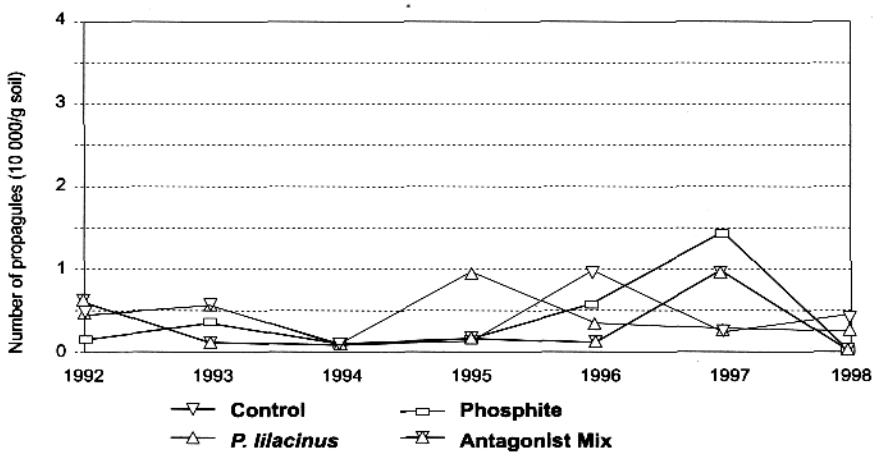

Figure 2: Propagation of P. lilacinus in soil of trees from different treatments

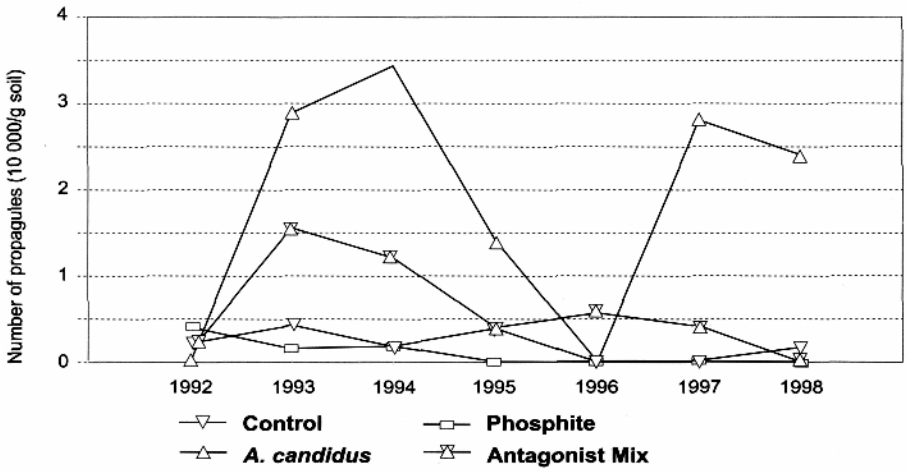

Figure 3: Propagation of A. candidus in soil of trees from different treatments

Of these three antagonists, only soil treatments of *Aspergillus candidus* or *Trichoderma hamatum* increased their populations over the trial period, when compared to soil from the control and phosphonate treated trees (Fig. 2, 3 & 4). Phosphonate, *Aspergillus Candidas* and *Trichoderma hamatum* treatments resulted in lower *P cinnamomi* populations in soils when compared to the control and *Paecilomyces lilacinus* treatments (Fig. 5). Soils from antagonist treated trees tended to be more suppressive to *P cinnamomi* than soil from the control or phosphonate treated trees (Fig. 6). At the end of the trial period, trees of all the antagonist treatments and the phosphonate treatment had better root health than the control trees - the trees from the *Aspergillus candidus* treatment had perfectly healthy roots (Fig. 7). Although tree health did not differ much over the period of the trial, trees from the phosphonate and *Aspergillus*

candidus treatments tended to have better tree health since 1994 (Fig 8). No differences in the yield of trees from different treatments occurred (Fig. 9).

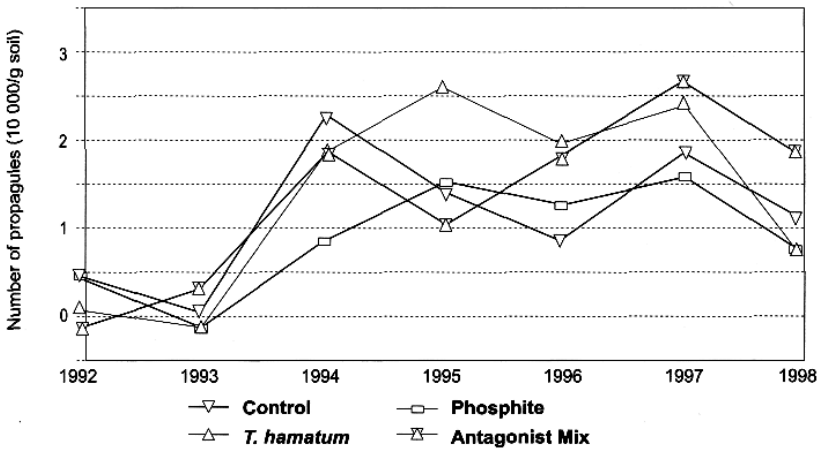

Figure 4: Propagation of* T. hamatum *in soil of trees from different treatments

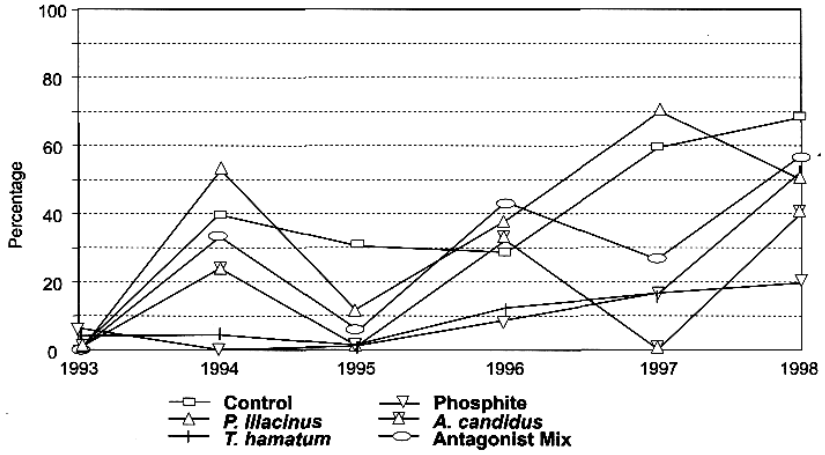

Figure 5: % P.c. infected leaf disks from soil of different treatments

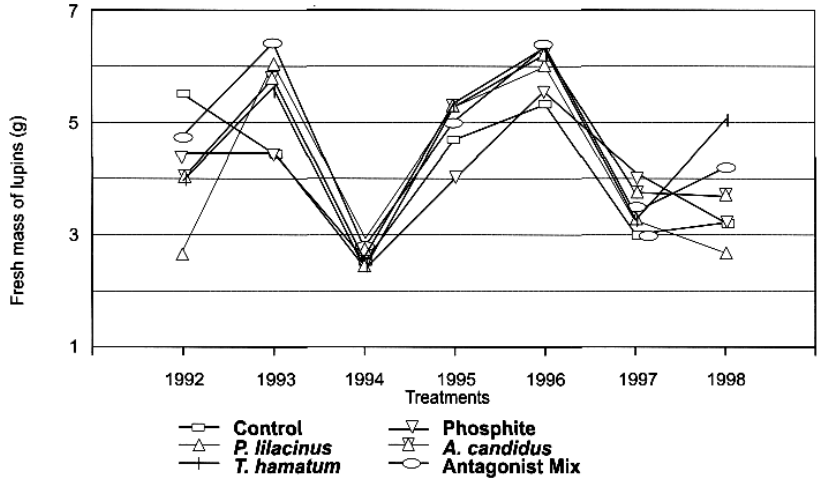

Figure 6: % Suppressiveness of soils from different treatments

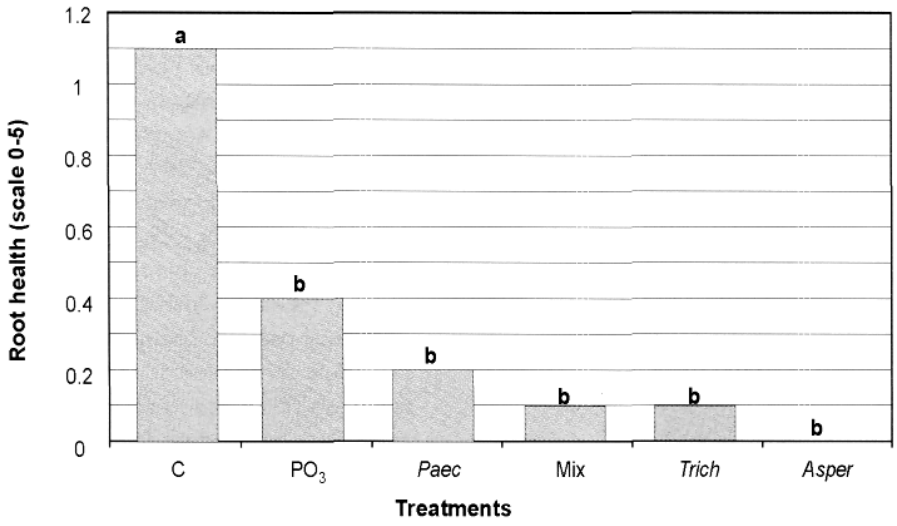

Figure 7: Root health as influenced by different treatments (1998)

In general, the results show *Aspergillus candidus* and *Trichoderma hamatum* to be the most beneficial biocontrol treatments evaluated. These biocontrol organisms should now be developed further by culturing on a larger scale, and commercial application to the planting medium of nursery trees. The fact that the untreated control trees in the trial did not decline rapidly over the six year period of the trial, indicated that healthy trees could be managed with fewer applications of H_3PO_3 than the standard two injection rounds which are normally used.

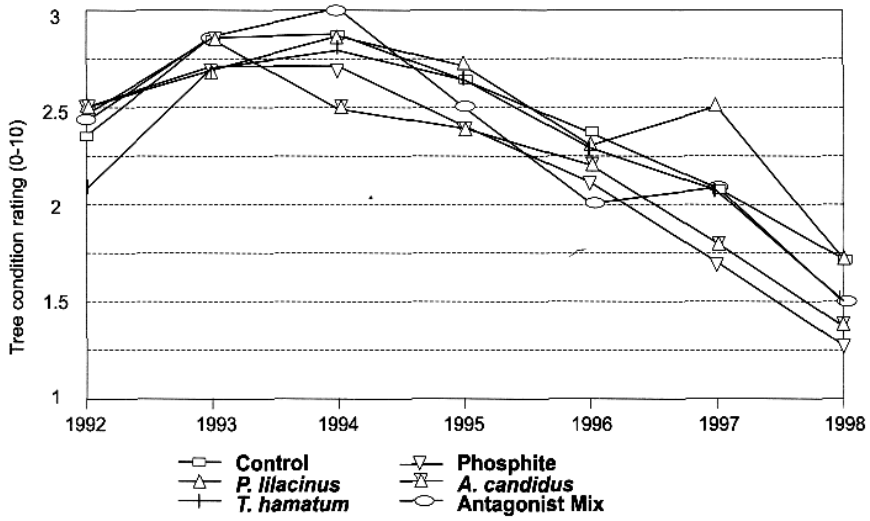

Figure 8: Tree health as influenced by different treatments

STEM PAINT APPLICATION OF H_3PO_3

Low pressure injection (by using syringes) of avocado trees with H_3PO_3 is still the most popular method of injecting, although high pressure injection equipment has been evaluated previously in South Africa (Duvenhage & Könne, 1995). However, injection of H_3PO_3 is labour intensive and time consuming, and causes injury to the wood tissue of the trunk with prolonged use (Robbertse & Duvenhage, 1999). In 1996 it was reported

that a 5% H_3PO_3 trunk paint (applied to the thin bark at a rate of 0.4 to 0.8 g a.i. /m^2 canopy area) after spring and summer growth flushes gave promising results for maintaining tree health (Duvenhage & Köhne).

As a satisfactory alternative for injection was still needed, the trunk paint technique was developed further during 1996 to 1998, aiming to develop an effective technique for application of H_3PO_3 to large avocado trees.

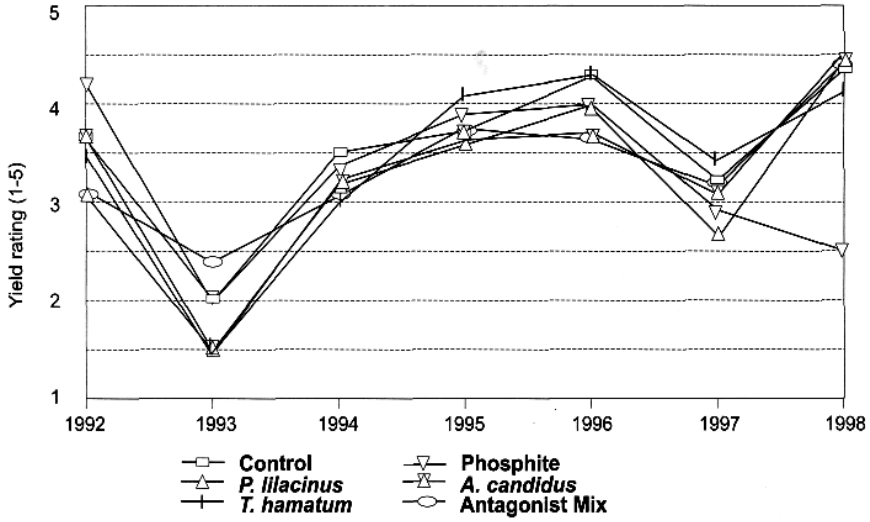

Figure. 9: Yield as influenced by different treatments

Table 1. Phosponic acid residues (mg/kg) in roots after phosphonate treatment

TREATMENT*	After 28 days	After 56 days
Control	0	0
H_3PO_4 inject (0.4g; 0.2g)	14	24.3
H_3PO_4 spray (0.8g; 0.4g)	3	not determined
Aliette WP spray (0.8g; 0.8g; 0.8g; 0.8g)	13.4	9.3
H_3PO_4 spray (0.8g; 0.8g; 0.8g; 0.8g)	14.7	13
H_3PO_4 spray (1.6g; 1.6g; 1.6g; 1.6g)	10	not determined
Aliette WP spray (rinsed) (0,8g; 0,8g; 0,8g; 0,8g)	19.8	not determined
H_3PO_4 spray (rinsed) (0,8g; 0,8g; 0,8g; 0.8g)	8.5	not determined

*Figures in brackets indicate the rate of active ingredient applied per m^2 canopy area for each application.

Fuerte avocado trees, planted in 1980 were used for the trial (six trees per treatment). For the standard injection treatment a 100g/litre H_3PO_3 solution was used in November (after the spring flush; at 0.4 g a.i./m^2 canopy area), as well as a 50 g/ℓ solution in January (after the summer flush; at 0.2 g a.i./m^2 canopy area), while control trees were untreated. For comparison, H_3PO_3 trunk spray (200g/ℓ was applied at the same dates as injection treatments (at 0.4 g a.i./m^2 or 0.8 g a.i./m^2 canopy area). Further more fosetyl-

Al trunk spray (Aliette WP at 500 g/ℓ) and H_3PO_3 trunk spray (200 g/ℓ) applied from mid September to mid January at six week intervals (at 0.8 g a.i./m^2 or l.6 g a.i./m^2) canopy area, to the thin bark these treatments were duplicated, and the trunks rinsed with water 2 days after application to simulate the effect of rain (Table 1). Samples of feeder roots were taken from each tree (near to the trunk, at the drip line, and in between) and pooled for two trees (resulting in three root samples per treatment). The root samples were taken 28 or 56 days after the last application of each treatment, and analysed for phosphonic acid residues by the South African Bureau of Standards.

Root samples taken 28 days after application of treatments, showed that two H_3PO_3 trunk sprays resulted in much lower phosphonic acid residues than two H_3PO_3 injection applications (Table 1). However, Aliette or H_3PO_3 trunk sprays (at 0.8 g a.i./m^2 canopy area) applied four times resulted in phosphonic acid residues comparable to two H_3PO_3 injection applications. Rinsing of the trunks two days after H_3PO_3 trunk spray applications resulted in reduced phosphonic acid residues when compared to non-rinsed H_3PO_3 trunk sprays, while rinsing of the trunks two days after Aliette WP trunk spray applications did not result in reduced phosphonic acid residues when compared to non-rinsed Aliette WP trunk sprays. At 56 days after application of treatments, Aliette WP or H_3PO_3 trunk sprays (at 0.8 g a.i./m^2 canopy area) applied four times resulted in phosphonic acid residues which were lower than that of two H_3PO_3 injection applications (Table 1).

Trunk sprays of H_3PO_3 or Aliette WP (at 0.8 g a.i./m^2 canopy area) applied every 6 weeks from mid September to mid January to the thin bark of the tree (and not to the thick old bark) were as effective as two standard injection rounds for the application of phosphonates to large trees.

Comparison of the phosphonic acid residues after 28 and 56 days showed a quicker decline in the trunk spray applications than in the injection treatments, and confirms that 6 - 7 week application intervals are needed for trunk sprays.

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