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Fungicide Levels in Avocado Roots and Soil Following Treatment with Phosphonate Fungicides

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Aliette® is a systemic fungicide effective against important *Phytophthora* diseases of citrus and avocado, such as avocado root rot and root rot and gummosis of citrus. Aliette® is unique among systemic fungicides in that it is translocated in both the xylem and phloem conducting tissues, and hence can move in both an upward and downward direction in the plant. This property permits its use as a foliar spray, or in the case of avocado, as a trunk injection for control of root rot.

The active ingredient of Aliette® is ethyl phosphonate, which breaks down to phosphonate (HPO_3^{-2}) in plants and soil (8). Although the mode of action of ethyl phosphonate still remains unknown, it is likely that it exerts a direct effect on disease control mediated by its breakdown product, phosphonate, which is capable of inhibiting growth and sporulation of *Phytophthora* (5, 6, 8, 12, 13, 14). Phosphonate itself has been used successfully in controlling root and heart rot of pineapple caused by *P. cinnamomi* and *P. parasitica* (20), root rot of avocado caused by *P. cinnamomi* (9, 19), and *Phytophthora* gummosis caused by *P. parasitica* and *P. citrophthora* (17). Unbuffered phosphonic (phosphorous) acid is phytotoxic because of its low pH, and so is commonly used as a salt, such as sodium or potassium phosphonate in the range of 6.2 to 6.7 (8, 13).

Registration of Aliette® on nonbearing avocados to control avocado root rot has been recently granted in California, with registration for bearing avocados anticipated in 1990. Currently, potassium phosphonate is not registered for use as a fungicide for any crop in the U.S.; but it is registered for use in Queensland, Australia, as a trunk injection for the control of avocado root rot.

At present, little is known concerning the fate of ethyl phosphonate or the more fungitoxic metabolite phosphonate in avocado trees and soil. To further evaluate the disease potential of Aliette® and potassium phosphonate against avocado root rot, it is desirable to determine the persistence of fungicide residues and their distribution within the plant, especially the roots. In addition, some knowledge of the persistence of ethyl phosphonate and phosphonate in soil would be useful, as Aliette® is used as a soil drench in avocado replant situations and in nurseries for the control of ornamental diseases caused by various *Phytophthora* species.

At present, there is much interest among California avocado growers and researchers in the use of trunk injections of Aliette® and potassium phosphonate to control root rot, stimulated by the encouraging results obtained in South Africa and Australia with this

method. We have been experimenting with trunk injections of phosphonate fungicides for over five years and have had only limited success in returning severely diseased trees back into good production. The reasons for this are not clear, but one possibility may be that the levels of phosphonate in avocado roots after trunk injections are not sufficiently high to actively suppress the pathogen, *P. cinnamomi,* under soil and environmental conditions prevalent in California.

In this present study, we used high-performance ion chromatography to determine both the persistence and distribution of ethyl phosphonate and phosphonate in young avocado trees grown in the greenhouse following either a foliar or soil application of Aliette® or potassium phosphonate. In addition, we monitored the phosphonate levels in feeder roots of mature avocado trees in a field plot after trunk injection with three different phosphonate fungicides: Aliette® potassium phosphonate, and a related compound, dimethyl phosphonate.

Materials and Methods

Greenhouse studies. Avocado seedlings (Topa Topa) were grown in 4 liter pots containing potting soil for 12 weeks. The plants then were treated with either 2,100 ppm potassium phosphonate or 3,000 ppm Aliette® as either a foliar or soil application. The soil application consisted of 500 ml per pot applied approximately four hours after irrigation. The foliar application involved immersion of the foliage in the fungicide solution and then placement of the pot contaminating the soil. The same foliar application was repeated 24 hours later. There were six plants per fungicide treatment per experiment, and the plants were arranged randomly on a greenhouse bench. Additionally, 0.1% Triton B-1956 was used as a surfactant with the foliar applications of both Aliette® and potassium phosphonate.

At 1, 2, 4, 6, and 8 weeks after fungicide application, a soil sample was removed from six pots of each treatment, to a depth of 10 centimeters from four points approximately 10 centimeters from the base of the plants, using a 15 millimeter-diameter cork borer. The plants then were removed from the pots and washed thoroughly with running water to remove soil from the roots plus any removable fungicide residue remaining on the foliage surface. After the excess water on the plants had dried, they were separated into roots, stems, leaves and, beginning 2 weeks after initial application, newly emerged leaves. The tissue was chopped finely with a razor blade, mixed well, and a 2 gram fresh weight sample removed for fungicide analysis. Six replicates were made of each tissue analysis for each treatment.

Field injection study. An existing fungicide injection plot located in Rancho California, treated twice yearly since December 1985, was used for the purpose of monitoring phosphonate levels in roots after trunk injection of three different phosphonate fungicides: Aliette® potassium phosphonate, and dimethyl phosphonate. Dimethyl phosphonate is a compound with a similar chemical structure to that of Aliette® and has been shown to also break down to phosphonate in plants. In July, 1988, 15 year-old trees (Hass scion on seedling rootstocks) were injected with 80 milliliters (4 x 20 ml) of either 9% dimethyl phosphonate, 10% Aliette® or 7 % potassium phosphonate. These trees had received previous fungicide injections for the past 21/2 years as part of an

ongoing fungicide evaluation trial. One, 4, and 8 weeks after injection, samples of feeder roots from five of the healthiest trees of each treatment were obtained and brought back to lab for fungicide residue determination as described previously (18).

Results

Greenhouse Studies

Ethyl phosphonate and phosphonate residues in avocado tissues following soil application of fungicides. One week after soil application of Aliette® avocado roots and stems contained 2 and 3 micrograms ethyl phosphonate/gram fresh weight (ppm), respectively, while none was detected in leaves. Ethyl phosphonate was not detectable in any tissue after 2 weeks.

With Aliette® treatment, the phosphonate levels in the roots after 1, 4, and 8 weeks were 208, 522, and 488 ppm, respectively (Table 1). After potassium phosphonate soil treatment, the phosphonate levels in the roots were 356 ppm after 1 week, increasing to 1399 ppm after 4 weeks, and then decreasing steadily to 213 ppm by 8 weeks (Table 1). The phosphonate levels in roots during the 8 week experiment were not different with the two fungicide treatments, with the exception at week 4, in which the potassium phosphonate treatment yielded higher levels. The levels of phosphonate in avocado stems were not different after treatment with either compound (Table 1). After 1 week, plants treated with potassium phosphonate had almost four times as much phosphonate in the leaves compared to the Aliette® treatments (Table 1). Thereafter, the levels of phosphonate in the leaves were not different with the two fungicide treatments (Table 1). With the two soil fungicide treatments, the levels of phosphonate in the new leaves were not significantly different, with the exception of the Aliette® treatment at week 6, in which there was a several-fold increase (Table 1).

Levels of ethyl phosphonate and phosphonate in avocado tissues following foliar treatment of fungicides. One week after foliar applications of Aliette® no ethyl phosphonate was detected in any tissue. During the 8 week duration of the experiment, there was no difference in the phosphonate levels in the roots treated with either fungicide (Table 2). The levels of phosphonate in the stems were not different between the two compounds, with the exception of the fourth week in which plants treated with potassium phosphonate had higher levels. After 1 week, Aliette® treatment resulted in a three-fold difference in phosphonate levels of leaves compared to the potassium phosphonate treatment, with no difference in the levels thereafter (Table 2). There were no differences in the phosphonate levels in the new leaves with either treatment (Table 2). Overall, the patterns of distribution of phosphonate in avocado tissues were almost identical after treatment with either compound for the duration of the experiment (Table 2).

Levels of ethyl phosphonate and phosphonate in soil following soil application of fungicides. One week after soil application of Aliette® the soil contained 7 ppm ethyl phosphonate; none was detected at 2 weeks and thereafter. At 1, 2, and 4 weeks after Aliette® soil treatment, the soil contained 88, 58, and 13 ppm phosphonate, respectively. One and 2 weeks after treatment with potassium phosphonate, the soil

contained 146 and 44 ppm, respectively; none was detected after 4 weeks.

Table 1. Levels of phosphonate in avocado seedlings (Topa Topa) up to 8 weeks after soil treatment with 500 milliliters of 3,000 pm Aliette® or 2,100 ppm potassium phosphonate.[x]

		ppm phosphonate[z]			
Fungicide[y]	Week	Roots	Stems	Leaves	New leaves
potassium phosphonate	1	356 c[z]	221 d	221 a	
	2	510 bc	534 cd	105 bcd	175 c
	4	1399 a	1561 a	132 bc	298 ab
	6	512 bc	784 bc	72 bcd	116 c
	8	213 c	382 d	47 d	131 c
Aliette	1	208 c	191 d	57 cd	
	2	751 b	566 cd	136 b	211 bc
	4	522 bc	1140 b	131 bc	337 a
	6	706 b	1084 b	86 bcd	317 a
	8	488 bc	543 cd	105 bcd	116 c

[x]Soil application consisted of a 500 milliliter drench to each 4-liter pot approximately 4 hours after irrigation.

[y]Fungicides were adjusted to pH 6.2 with KOH.

[z]Values within columns for each tissue sample followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

Table 2. Levels of phosphonate in avocado seedlings (Topa Topa) up to 8 weeks after foliar treatment with either 3,000 ppm Aliette® or 2,100 ppm potassium phosphonate.[x]

		ppm phosphonate[y]			
Fungicide[z]	Week	Roots	Stems	Leaves	New leaves
	1	15 a[z]	21 c	42 de	
potassium	2	13 a	70 bc	118 abc	128 ab
phosphonate	4	18 a	209 a	114 abc	103 bc
	6	11 a	130 ab	74 cde	37 d
	8	14 a	75 bc	19 e	24 4 d
Aliette	1	16 a	43 bc	140 ab	-
	2	8 a	126 ab	166 a	157 a
	4	14 a	59 bc	85 bcd	73 c
	6	15 a	121 ab	73 cde	30 d
	8	12 a	95 bc	49 de	13 d

[x]Foliar treatment consisted of completely immersing the foliage in fungicide solution then laying the pot on its side while the foliage dried, preventing any fungicide from reaching the soil. The same treatment was repeated 24 hours later.

[y]Fungicides were adjusted to pH 6.2 with KOH and buffered with 25.5 mM MES hydrate. Triton B-1956 was added at 0.1% to act as a surfactant.

[z]Values within columns for each tissue sample followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

Field Studies

Phosphonate residues in roots of trees injected with phosphonate fungicides. One week after trunk injection of mature trees with either of the three different phosphonate fungicides, only very low levels (1-4 ppm) of phosphonate were present in feeder roots (Table 3). After 4 weeks, the phosphonate levels in trees injected with dimethyl phosphonate and Aliette® increased to 25 and 17 ppm, respectively while phosphonate levels in trees injected with potassium phosphonate remained the same at 4 ppm. After 8 weeks, the phosphonate levels in roots were almost identical with the three fungicide treatments, ranging from 5-8 ppm (Table 3).

Table 3. Phosphonate (HPO_3^{-2}) residues in feeder roots of avocado trees up 8 weeks after trunk injection with either 10% Aliette®, 7% potassium phosphonate or 9% dimethyl phosphonate.[z]

Fungicide	1 week	4 weeks	8 weeks
Aliette	1	17	5
Potassium phosphonate	4	4	7
Dimethyl phosphonate	3	25	8

Residues are expressed as micrograms phosphonate per gram fresh weight tissue (ppm) and each value is the mean of five feeder root samples taken from the healtiest trees in each treatment.

Discussion

In greenhouse studies with avocado seedlings, it was determined that ethyl phosphonate (Aliette®) was short-lived in both soil and avocado tissues, with no detectable residues found in avocado or soil 2 weeks after soil treatment, or 1 week after foliar application. In contrast, high levels of phosphonate were detected in both soil and for at least 8 weeks in avocado tissue. This strongly suggests that phosphonate, rather than ethyl phosphonate, is the primary molecule involved in control of avocado root rot. The levels of phosphonate in all tissues were almost identical after soil or foliar treatments with either Aliette® or potassium phosphonate, which probably explains the similar efficacy in controlling Phytophthora diseases with either Aliette® or potassium phosphonate, as reported by others (8, 13, 19, 20).

Compared to the soil drench treatment, considerably lower levels of phosphonate were detected in avocado tissue after foliar treatment with either Aliette® or potassium phosphonate, and these levels also persisted for at least 8 weeks. Most likely, this is due to the much higher amounts of chemical applied with the soil treatment compared to the foliar treatment. It is likely that the avocado roots can continue to take up phosphonate available in soil solution, and this could explain the good control of root rot achieved with young trees in replant situations when Aliette® is applied as soil drench to the sleeve one or two days prior to planting in the field (7).

The phloem-mobility of phosphonate was confirmed by the detection of phosphonate in the roots after careful foliar application of either phosphonate or Aliette® In addition, the persistence of phosphonate in avocado tissue 8 weeks after treatment with either Aliette® or potassium phosphonate indicates that it probably is not readily oxidized by the plant to phosphate for use as a fertilizer. This is in agreement with previous findings, where no growth response was detected in plants growing in soil where phosphonate was used as the sole source of phosphorus fertilizer (15). Such properties may account

for the persistence of phosphonate in the avocado plant and explain the relatively long-term control achieved with some *Phytophthora* diseases (8,9,17,20).

Persistence of phosphonate in soil was less than in plants. After 4-6 weeks, no phosphonate was detectable in soil following either potassium phosphonate or Aliette® treatment, respectively. It is known that soil microorganism (fungi, bacteria) can oxidize phosphonate (HPO_3^{-2}) to phosphate (HPO_4^{-2}) as part of the natural phosphorus cycle in the environment (1,3,16). A similar mechanism may have occurred in these studies, resulting in the disappearance of phosphonate from the soil over a relatively short time period. In addition, the high water solubility of phosphonate (>50%), may have facilitated some leeching from the soil container.

Once inside the plant, phosphonate appears to be quite stable, although the ultimate fate of the molecule is unknown. While the exact mechanisms involved in disease control by Aliette® are still not fully understood, critical determinants must be the concentration and persistence of the active metabolite phosphonate in the plant tissues targeted by the pathogen. By comparison with previous in vitro studies on the inhibitory effects of potassium phosphonate towards *P. cinnamomi,* the levels of phosphonate in roots of greenhouse-grown avocados in the absence of *P. cinnamomi* after applications of potassium phosphonate or Aliette® are sufficient to account for a direct inhibition of *P. cinnamomi,* especially with the soil treatment. Coffey and Joseph (6) found that potassium phosphonate was highly inhibitory to critical stages in the life cycle of *P. cinnamomi;* the EC_{50} values for inhibition of sporangium production and zoospore release were 2ppm and 6ppm, respectively. Because the rapid increase in zoospores by *P. cinnamomi* under conducive environmental conditions is primarily responsible for the highly destructive nature of avocado root rot, interference of zoospore production by persistent, inhibitory levels of phosphonate in the roots undoubtedly is a major factor in successful disease control. In addition, recent research in our lab has shown that phosphonate is exuded from the roots of *Persea indica* (an avocado relative) after application of potassium phosphonate. This opens up the possibility that low levels of phosphonate exuding from avocado roots after treatment with phosphonate fungicides may contribute to disease control by providing a chemical barrier. The phosphonate levels in feeder roots of avocado trees growing in the field which had been injected with various phosphonate fungicides were low, with the amounts at one and eight weeks after injection ranging from 1-8 ppm. The reasons for these low amounts are not known, but the physiology of the tree appears to play a large role. It is possible that the fungicide is being translocated to and being retained by the foliage rather than being translocated down to the roots, where it must be present to suppress *P. cinnamomi.* The marginal soil conditions of the experimental plot used for this study are not conducive to good root growth even in the absence of *P. cinnamomi.* Generally, the only place that feeder roots could be found during sampling was in the vicinity of the lone minisprinkler. Recently, growers in some areas of the state where water is extremely expensive have been reducing their irrigation frequency, which increases the stress on the tree and reduces the potential for root growth even more. Aliette® has been most successful used as trunk injections in areas of the world such as Australia and South Africa with high natural rainfall and deep soil profiles with abundant organic matter, all of which encourage good root growth. Because an active and healthy root system is necessary to provide a "sink" for phosphonate, we believe that Aliette® will be maximally effective

in situations favorable for good root growth. This can be encouraged by paying attention to proper irrigation, choosing well-drained soils, and perhaps by the addition of organic mulches to the soil in order to conserve moisture and encourage root growth.

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