Phosphonate Levels in Avocado (*Persea americana*) Seedlings and Soil Following Treatment with Fosetyl-Al or Potassium Phosphonate

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ABSTRACT

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The levels of ethyl phosphonate and phosphonate in avocado seedlings and soil were determined using high-performance ion chromatography at 1, 2, 4, 6, and 8 wk following foliar or soil applications of either 3 mg/ml of fosetyl-Al or 2.1 mg/ml of potassium phosphonate. After soil treatment with either potassium phosphonate or fosetyl-Al, phosphonate persisted in soil for 2 and 4 wk, respectively. With fosetyl-Al, low levels of ethyl phosphonate were present in soil, roots, and stems 1 wk after application, but none was detected thereafter. In contrast, no ethyl phosphonate residues were detected in either soil or avocado tissue 1 wk following foliar application of fosetyl-Al. Soil treatment with both potassium phosphonate and fosetyl-Al resulted in much higher phosphonate levels being present in all tissues compared with foliar treatment (up to 78 and 94 times more in the root samples following potassium phosphonate and fosetyl-Al treatment, respectively). Following both soil and foliar applications of the two fungicides, high phosphonate levels were maintained in avocado tissues for the 8-wk period of the experiments, suggesting that phosphonate is stable in plants. The phosphonate levels found in roots after either soil or foliar applications were sufficiently high to account for a direct antifungal effect in controlling avocado root rot caused by *Phytophthora cinnamomi*.

Fosetyl-Al is an ethyl phosphonate fungicide shown to be efficacious against some important diseases caused by soilborne *Phytophthora* spp. as well as some downy mildew diseases (3,6–13, 19,21). Fosetyl-Al is unique among fungicides in that it is translocated in both the xylem and phloem (9). This property permits its use as a foliar spray or, as in the case of some tree crops, a trunk injection for control of root rots caused by *Phytophthora* sp. (5,10,21).

Fosetyl-Al is hydrolyzed to phosphorous acid in plants and soil (9,20). In aqueous systems, phosphorous acid (H_3PO_3) is in equilibrium with phosphonic acid (H_2PHO_3) such that essentially all of the molecules are in the phosphonic acid state. Two phosphonate anions ($HPHO_3^-$ and PHO_3^{-2}) can result from the ionization of phosphonic acid, which has pK_a values of 1.3 and 6.7 (9). Most previous literature has used the terms phosphorous acid and phosphite (1,4–7,9,14,15,17–19,21–24) to denote the phosphonate moiety. According to the International Union of Pure and Applied Chemistry (16), the correct term for the anionic form of phosphonic acid is phosphonate. This term will be used here. Unbuffered phosphonic acid is phytotoxic because of its low pH, and so is commonly used as a salt, such as sodium or potassium phosphonate, in the pH range of 6.2–6.7 (9,20).

Although the mode of action of fosetyl-Al still remains controversial, it is likely that it exerts a direct antifungal effect on disease control mediated by its breakdown product, the phosphonate anion, which is capable of inhibiting growth and sporulation of a pathogen such as *Phytophthora* (6,7,9,14,15). Phosphonate itself has been used successfully in controlling root and heart rot of pineapple caused by *P. cinnamomi* Rands and *P. parasitica* Dastur (24), root rot of avocado caused by *P. cinnamomi* (14,21), and Phytophthora gummosis caused by *P. parasitica* and *P. citrophthora* (R. & E. Sm.) Leonian (19).

Registration of fosetyl-Al on nonbearing avocados to control avocado root rot has been granted recently in California, with full registration anticipated once toxicological studies have been completed. At present, little is known concerning the fate of ethyl phosphonate or the more fungitoxic metabolite phosphonate in avocado tissues and soil (9). To fully evaluate the efficacy of fosetyl-Al and potassium phosphonate against avocado root rot, it is desirable to determine the nature and levels 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 both plants and soil would be useful, because this has a direct bearing on the number of applications of fungicide that will be required.

In this paper we employ high-performance ion chromatography (HPIC) to determine both the persistence and distribution of ethyl phosphonate and phosphonate in avocado seedlings following either a foliar or soil application of fosetyl-Al or potassium phosphonate.

MATERIALS AND METHODS

Fungicide treatments. Avocado seedlings (*Persea americana* Mill. 'Topa Topa') were grown in 4-L pots containing U.C. mix no. 5 (50% peat moss, 50% fine sand, plus 2.2 kg of dolomite, 1.5 kg of superphosphate, 148 g of KNO_3, and 148 g of K_2SO_4 per cubic meter) for 12 wk. The plants then were treated with equivalent rates (25.5 meq phosphonate)

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of either 2.1 mg/ml of potassium phosphonate or 3 mg/ml of fosetyl-Al as either a foliar or soil application. Phosphorous acid (99.6%) was obtained from Fisher Scientific, Tustin, CA. Fosetyl-Al (96.4% technical grade) was kindly supplied by Rhône-Poulenc Ag. Co., Research Triangle Park, NC. Titration of phosphorous acid with 10 M KOH to 6.2 yielded a mixture of monobasic and dibasic potassium phosphonate. Both chemicals were buffered with 25.5 mM MES-hydrate (4-morpholine ethane sulphonic acid).

The soil application method consisted of 500 ml per pot applied as a drench approximately 4 hr after irrigation. The method for foliar application involved immersion of the foliage in the fungicide solution and then placement of the pot on its side while the foliage dried, so as to prevent any fungicide runoff from contaminating the soil. The same foliar application was repeated 24 hr later. There were six plants per fungicide treatment per experiment and the plants were arranged randomly on a greenhouse bench. Additionally, 0.1% (v/v) Triton B-1956 (Rohm and Haas, Philadelphia, PA) was used as a surfactant with the foliar applications of both fosetyl-Al and potassium phosphonate.

At 1, 2, 4, 6, and 8 wk after fungicide application a soil sample was removed from six pots of each treatment, to a depth of 10 cm from four equidistant points around the base of the plants, using a 15-mm-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 wk after initial application, newly emerged leaves. The tissue was chopped finely with a razor blade, mixed well, and a 2-g fresh weight sample was removed for fungicide analysis. Five additional 2-g samples of each tissue were dried thoroughly in a forced-air oven at 65 C for 3 days to determine their dry weight. Six replicates were made of each tissue analysis for each treatment.

Plant and soil analysis. Plant tissue samples were ground to a fine powder in liquid nitrogen using a mortar and pestle. The powder was transferred to a 15-ml plastic vial and 10 ml of deionized water was added. For soil analysis, moist samples weighing 5 g were placed in 15-ml plastic vials and 10 ml of water was added. The vials were shaken for 1 hr on a reciprocal shaker at 180 strokes/min and then allowed to stand for 30 min to allow the debris to settle. Next, 1.5 ml of the supernatant was removed, placed in an Eppendorf microfuge tube, and centrifuged for 10 min. Finally, the supernatant was transferred to a clean microfuge tube and either analyzed immediately or stored at −20 C. Before injection into the chromatography apparatus, the extract was diluted if necessary, passed through a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) on the end of a 3-ml disposable syringe, and into another 3-ml syringe with a Swinney holder (Fisher Scientific) containing a 13-mm GS-type filter, pore size 0.22 μm. Five duplicate soil samples were allowed to dry in a forced-air oven at 65 C for 3 days to determine soil dry weight.

Ion chromatography system. The HPIC apparatus was a Dionex 2000i/P with a model AMMS-1 anion micromembrane suppressor coupled with conductivity detection (Dionex Corp., Sunnyvale, CA). For phosphonate analysis, an AS4A separator column was used along with two types of guard columns, an MPIC-NG1 and an HPIC AG4A. The eluent for the AS4A column consisted of 0.53 mM $NaCO_3$ and 1.54 mM Na_2HCO_3 used at a flow rate of 2.2 ml/min. For ethyl phosphonate analysis, an AS6 separator column was used along with an MPIC-NG1 and an HPIC AG6 guard column. The eluent consisted of 20 mM NaOH run at a flow rate of 2.2 ml/min. The suppressor regenerants for phosphonate and ethyl phosphonate analysis were 15 mM H_2SO_4 run at 2.0 ml/min and 30 mM H_2SO_4 run at 3.5 ml/min, respectively. The detector sensitivity was set at 3 μS and the chromatograms were compared with standards of ethyl phosphonate or phosphonate as well as spiked and untreated samples. The data were recorded on a Spectra-Physics 4270 integrator, and fungicide levels were determined by comparing peak height with a standard curve (20).

RESULTS

Fungicide residues in avocado tissues following soil application. One week after soil application of fosetyl-Al, avocado roots and stems contained 2 and 3 μg of ethyl phosphonate per gram fresh weight, respectively, while none was detected in leaves. Ethyl phosphonate was not detectable in any tissue after 2 wk.

Phosphonate levels in avocado roots during the 8-wk experiment were not different after treatment with either fungicide, with the exception of week 4, in which the potassium phosphonate treatment yielded higher levels. With the fosetyl-Al treatment, the phosphonate levels in the roots ranged from 208 to 751 μg/g fresh weight, respectively (Table 1). After potassium phosphonate treatment, the phosphonate levels were 356 μg/g fresh weight at 1 wk, increasing to 1,399 μg/g fresh weight after 4 wk and then decreasing steadily to 213 μg/g fresh weight by 8 wk.

The phosphonate levels in stems during the 8-wk experiment were similar after treatment with either fungicide, with the exception of week 4, in which the potassium phosphonate treatment yielded higher levels (Table 1). The phosphonate levels at 8 wk after treatment with either fungicide were not significantly different from those found after 1 wk.

After 1 wk, plants treated with potassium phosphonate contained almost four times as much phosphonate in their leaves compared with the fosetyl-Al treatment (Table 1). Thereafter, the levels of phosphonate in the leaves were not different with the two fungicide treatments.

Fungicide residue in avocado tissues following foliar treatment. One week after foliar applications of fosetyl-Al, no ethyl phosphonate was detected in any

Table 1. Levels of phosphonate in seedlings of *Persea americana* 'Topa Topa' up to 8 wk after soil treatment with 500 ml of either 3 mg/ml of fosetyl-Al or 2.1 mg/ml of potassium phosphonate[w]

Fungicide[x]	Week	Phosphonate (μg per gram fresh weight)[y]			
		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	1,399 a	1,561 a	132 bc	298 ab
	6	512 bc	784 bc	72 bcd	116 c
	8	213 c	382 d	47 d	131 c
Fosetyl-Al	1	208 c	191 d	57 cd	···
	2	751 b	566 cd	136 b	211 bc
	4	522 bc	1,140 b	131 bc	337 a
	6	706 b	1,084 b	86 bcd	317 a
	8	488 bc	543 cd	105 bcd	116 c

[w] Soil application consisted of a 500-ml drench to each 4-L pot approximately 4 hr after irrigation.
[x] Fungicides were adjusted to pH 6.2 with KOH and buffered with 25.5 mM MES-hydrate.
[y] Samples of avocado tissue (2 g) were ground to a fine powder in liquid nitrogen and extracted with 10 ml of water for 1 hr in a 15-ml plastic vial on a reciprocal shaker at 180 strokes/min. The extract was passed through a Sep-Pak C_{18} cartridge and a 0.22-μm filter before injection into the chromatography apparatus. Data is the mean of six replicates. To obtain μg phosphonate per gram dry weight, multiply the fresh weight values for roots, stems, and leaves by 5.4, 4.2, and 3.2, respectively. The limit of detection using ion chromatography is 0.5 μg phosphonate/g fresh weight.
[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.

tissue. During the 8-wk duration of the experiment, there was no difference in the phosphonate levels in the roots treated with either fungicide (Table 2). After 1 wk, fosetyl-Al treatment resulted in a threefold difference in phosphonate levels of leaves compared with the potassium phosphonate treatment, with no difference in the levels thereafter (Table 2). The patterns of distribution of phosphonate in avocado tissues were almost identical after treatment with either compound for the 8-wk 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 fosetyl-Al, the soil contained 7 μg of ethyl phosphonate per gram of soil dry weight; none was detected at 2 wk and thereafter. At 1, 2, and 4 wk after fosetyl-Al soil treatment, the soil contained 88, 58, and 13 μg of phosphonate per gram dry weight, respectively. One and two weeks after treatment with potassium phosphonate, the soil contained 146 and 44 μg of phosphonate per gram dry weight, respectively; none was detected after 4 wk.

DISCUSSION

In these studies it was determined that ethyl phosphonate was short-lived in both soil and avocado tissues, with no detectable residues found in avocado or soil 2 wk after soil treatment, or in avocado 1 wk after foliar application. In contrast, high levels of phosphonate were detected in both soil and avocado tissues and these residues persisted for 4 wk in soil and for the 8-wk duration of the experiments in avocado tissue.

Compared with the soil treatment, considerably lower levels of phosphonate were detected in avocado tissue after foliar treatment with either fosetyl-Al or potassium phosphonate, and these levels also persisted for the duration of the 8-wk experiment. The phloem mobility of phosphonate was confirmed by its detection in the roots following careful foliar application of either phosphonate or fosetyl-Al. No ethyl phosphonate was detected in root tissues following foliar application, even at 1 wk after foliar application of fosetyl-Al.

The persistence of phosphonate in avocado tissue 8 wk after chemical treatment provides circumstantial evidence that it is not readily oxidized to phosphate by the plant. This is in agreement with previous findings that no growth response was detected in plants growing in soil where phosphonate was used as the sole source of phosphorus fertilizer (17). Robertson and Boyer (23) concluded that phosphonate and phosphate are separate biological entities, apparently due to a difference in distribution of electrical charges on the phosphonate molecule relative to that of the phosphate molecule, despite the fact that the molecules have a very similar atomic spatial arrangement (23). They also determined that phosphonate solutions were resistant to oxidation by molecular oxygen at temperatures up to 60 C and over a pH range of 1.5–7.6, and suggested that phosphonate might be a useful buffer for biological studies because of its chemical stability and relative biological inactivity (22). Such physiochemical and biological properties may account for the persistence of phosphonate in the avocado plant and explain the long-term disease control achieved with some target pathogens (9,10,24).

Persistence of phosphonate in soil was less than that found in plants. After 4–6 wk, no phosphonate was detectable in soil following either potassium phosphonate or fosetyl-Al treatment. However, the behavior of phosphonate in an artificial potting mix, such as the one used in this study, may differ from the behavior in field soils. Adams and Conrad (1) investigated the transition of phosphonate to phosphate in soil and found that its oxidation proceeded only when microbial activity was not restricted by the presence of toluene. It has also been found that soil microorganisms can take up phosphonate from a simple synthetic media, oxidize it to phosphate in the cell, and release it upon autolysis of the cells (1,4,18). 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 leaching from the soil container.

With few exceptions, there were no sustained differences found in the level of phosphonate in avocado tissue following foliar application of potassium phosphonate or fosetyl-Al. Once inside the plant, phosphonate appears to be quite stable, although the ultimate fate of the molecule is unknown. Whereas the exact mechanisms involved in disease control by fosetyl-Al 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 lowest levels of phosphonate (8–18 μg/g fresh weight) found in the roots after foliar application of potassium phosphonate or fosetyl-Al in the present study are sufficient to account for a direct inhibition of *P. cinnamomi*. For example, Fenn and Coffey (14) and Coffey and Bower (6) reported that EC_{50} values of potassium phosphonate for inhibition of radial growth of mycelium of *P. cinnamomi* on solid media ranged from 4.2 μg/ml to 9.0 μg/ml. In addition, Coffey and Joseph (7) found that potassium phosphonate was highly inhibitory to critical stages in the life cycle of *P. cinnamomi*; 1 μg/ml of potassium phosphonate inhibited oospore production by 60–78%, while the EC_{50} values for inhibition of sporangium production and zoospore release were 1.8 and 6 μg/ml, respectively. Because the rapid increase in secondary inoculum by *P. cinnamomi* under conducive environmental conditions is primarily responsible for the highly destructive nature of

Table 2. Levels of phophonate in seedlings of *Persea americana* 'Topa Topa' up to 8 wk after foliar treatment with either 3 mg/ml of fosetyl-Al or 2.1 mg/ml of potassium phosphonate[w]

Fungicide[x]	Week	Phosphonate (μg per gram fresh weight)[y]			
		Roots	Stems	Leaves	New leaves
Potassium phosphonate	1	15 a[z]	21 c	42 de	···
	2	13 a	70 bc	118 abc	128 ab
	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 d
Fosetyl-Al	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

[w] 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 hr later.

[x] 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.

[y] Samples of avocado tissue (2 g) were ground to a fine powder in liquid nitrogen and extracted with 10 ml of water for 1 hr in a 15-ml plastic vial on a reciprocal shaker at 180 strokes/min. The extract was passed through a Sep-Pak C_{18} cartridge and a 0.22-μm filter before injection into the chromatography apparatus. Data is the mean of six replicates. To obtain μg phosphonate per gram dry weight, multiply the fresh weight values for roots, stems, and leaves by 5.4, 4.2, and 3.2, respectively. The limit of detection using ion chromatography is 0.5 μg phosphonate/g fresh weight.

[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.

avocado root rot, interference with these processes by persistent, inhibitory levels of phosphonate in the roots must be a major factor in the successful disease control that is achieved. In addition, it has recently been shown that *Phytophthora* can accumulate phosphonate actively against a concentration gradient (2), raising the possibility that the levels of phosphonate in the roots necessary to inhibit *P. cinnamomi* may be actually lower than those required had the molecule entered the fungus by passive diffusion alone.

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