Methods of Fosetyl-Al Application and Phosphonate Levels in Avocado Tissue Needed to Control Stem Canker Caused by *Phytophthora citricola*

Z. A. El-Hamalawi and **J. A. Menge,** Department of Plant Pathology, and **C. J. Adams,** Department of Statistics, University of California, Riverside 92521

ABSTRACT

El-Hamalawi, Z. A., Menge, J. A., and Adams, C. J. 1995. Methods of fosetyl-Al application and phosphonate levels in avocado tissue needed to control stem canker caused by *Phytophthora citricola.* Plant Dis. 79:770-778.

The efficacy of several methods of fosetyl-Al application to control avocado stem canker disease, caused by *Phytophthora citricola,* was evaluated under greenhouse conditions. Fosetyl-Al was applied to the canker area as paint alone (0.4 g a.i. fosetyl-Al + 1.0 ml water), combined with Tree Seal (0.4 g a.i. fosetyl-Al + 0.5 g Tree Seal + 0.5 ml water), or applied alone followed by Tree Seal on either scraped bark or bark cut in a fish-scale-like pattern (30 cm along the stem). The use of fosetyl-Al as a soil drench (3.2 g a.i. fosetyl-Al per liter) was also evaluated. The most effective method was either using the fosetyl-Al:Tree Seal:water formulation on heavily scraped areas of the stem or applying fosetyl-Al alone on the bark cut in a fish-scale-like pattern. Applying fosetyl-Al as a soil drench was also effective in controlling stem canker disease, but to a lesser degree than the paint application method. Phosphonate, the anionic metabolite of fosetyl-Al in plants, was quantified in the bark, leaves, and roots of treated avocado plants by high-performance liquid chromatography following the different application methods of fosetyl-Al. Application of the fosetyl-Al:Tree Seal:water formulation on heavily scraped stem areas resulted in the highest level of phosphonate residue in the canker area and was the most efficacious in controlling the stem canker pathogen. Phosphonate residue in the plant inhibited infection by *P. citricola* for about 6 months after its application. There was a strong negative correlation ($r = -0.978$) between the phosphonate level in the stem bark and the size of the stem canker caused by *P. citricola.*

Avocado trunk canker disease, commonly known as citricola canker, is caused by *Phytophthora citricola* Sawada. This disease was first described by Fawcett (17) and Barrett (1), but it was not until 1973 that Zentmyer et al. (40) identified the pathogen as *P. citricola.* In recent years, *P. citricola* has caused increasing losses in avocado (*Persea americana* Miller) groves throughout California (5,6). *P. citricola* affects the crown, lower trunk, and sometimes the main structural roots (6,41). Typical symptoms of the disease include bark cracking and exudation of a white, sugary material, usually near the base of the trunk. Scraping the bark reveals a blackened, necrotic lesion in the inner bark and phloem. In advanced stages, defoliation and twig dieback occur, and if the canker encircles the trunk the tree will die. *P. citricola* can be isolated from feeder roots, cankers, and soil from beneath infected avocado trees.

Fosetyl-Al is a systemic anti-Oomycete fungicide and is used worldwide to control diseases caused by members of the *Peronosporales*, especially root and crown rots caused by various soilborne *Phytophthora* species and foliar diseases caused by some downy mildews (4,8,11,14,26). Phosphonate has been used successfully in controlling root and heart rot of pineapple caused by *Phytophthora cinnamomi* and *P. parasitica* (33), root rot of avocado caused by *P. cinnamomi* (2,10,18,31,32), and Phytophthora gummosis caused by *P. parasitica* and *P. citrophthora* (24). Phosphonate activity can be explained by a combination of both fungitoxicity to *Phytophthora* and the elicitation of a defense reaction in the host (19). The importance of each component depends on the sensitivity of the pathogen to direct inhibition of growth and sporulation by phosphonate in the specific environment and on the strength of the host plant's defense response in the tissue under attack (19). Several methods of application of fosetyl-Al have been employed, such as soil drenching (15), trunk injection (2,10,32), trunk paint or spray (24), and foliar sprays (22,34,36).

Fosetyl-Al is more effective than metalaxyl and copper fungicides for curing existing avocado stem canker caused by *P. citricola* (12). Fosetyl-Al (aluminum tris-O-ethyl phosphonate, trade name Aliette) is the first commercially available ambimobile phosphonate fungicide (15,16). Fosetyl-Al is unique among fungicides in that it is translocated in both xylem and phloem. The phloem mobility of the highly water-soluble phosphonate was confirmed by its detection in root tissues following careful foliar application of fosetyl-Al (9). Fosetyl-Al is short-lived in both soil and plant tissues, with no detectable residues found in plant or soil 2 weeks after soil treatment (28). Once in aqueous systems in the soil or inside the plant, fosetyl-Al is rapidly hydrolyzed to phosphonic acid (syn. phosphorus acid) (39), which is the active component in fosetyl-Al. Plants cannot readily oxidize phosphonate to phosphate (23). The phosphonate anion is extremely inhibitory to critical stages in the life cycles of certain *Phytophthora* spp., especially to sporulation (30).

The objective of this study was to evaluate several methods of fosetyl-Al application for their effects on the level and persistence of phosphonate in avocado plant tissues and on the efficacy of fosetyl-Al for controlling stem canker disease of avocado.

MATERIALS AND METHODS

Materials. Avocado seedlings of the cultivar Topa Topa were grown from seeds planted in UC No. 4 soil mix (25) in plastic liners (5.5 × 11.0 cm) with perforated bases for drainage at 24 ± 2°C in the greenhouse. After 6 weeks, seedlings were transplanted into 4- or 20-liter pots containing the same soil mix, watered with dilute (14%) Hoagland's solution (38) as needed. Plants of *Persea indica* L., a noncommercial close relative of avocado that is extremely susceptible to *Phytophthora* infection, were grown from seed in the same greenhouse in flats containing sand. Forty-five days after sowing, *Persea indica* seedlings were transplanted individually into 4-liter pots containing UC No. 4 soil mix. All plants were maintained in the greenhouse before being used in the study.

Fosetyl-Al (Aliette, 80% a.i.) was obtained from Rhône-Poulenc Agric. (Monmouth, NJ), and Tree Seal (Asphaltum 45%, siliceous material 15%, water 40%) was purchased from Morrison's Orchard Supplies (Yuba City, CA). Fosetyl-Al paint (0.5 g Aliette; 0.4 g a.i. fosetyl-Al + 1 ml water), fosetyl-Al:Tree seal formulation paint (0.5 g Aliette; 0.4 g a.i. fosetyl-Al + 0.5 ml Tree Seal + 0.5 ml water), and the fungicide preparation applied as a soil drench (4 g Aliette; 3.2 g a.i. fosetyl-Al per liter) were prepared immediately before use.

Corresponding author: Z. A. El-Hamalawi
E-mail: plpath@ucrvms

Accepted for publication 4 April 1995.

Preparation of inoculum and inoculation method. *P. citricola* isolate (cc-6) was originally isolated from cankers on avocado trees. Stock cultures were maintained on slants of V8C-agar medium (12) and stored at 18°C in the dark. Fresh cultures were grown on V8C-agar plates incubated at 24°C in the dark. To maintain the pathogen's virulence, avocado seedlings were inoculated and the pathogen was reisolated monthly from colonized bark tissue by plating on a selective PARPH (27) agar medium. Cultures were examined microscopically to confirm the identity of *P. citricola* using the taxonomic key of Stamps et al. (37). Inoculation of the stems of avocado plants was made by removing a disk from the bark using a cork borer (4 mm diameter) to expose the cambium on which a culture agar plug (4 mm) of *P. citricola* was placed. The wound was moistened with a drop of water after inoculation and wrapped with a strip of Parafilm. Disease incidence and canker development were assessed 2 weeks after inoculation by measuring lesion areas in square centimeters (12). Each canker size is the mean of two experiments, each with 10 plants and four different inoculation sites per plant.

Phosphonate determination. High-performance liquid chromatography (HPLC) was used to determine phosphonate in the avocado tissue samples. All chromatographic equipment, column and reagents (21) were obtained from Alltech (Deerfield, IL). Bark strips (5 cm long) were collected at distances of 30, 60, 90, 120, and 150 cm above the fungicide-treated sections of the avocado stem. The bark samples were chopped finely with a razor blade and combined, and a 1-g sample was taken representing the overall plant for the fungicide analysis. Washed roots or leaves were chopped finely, and a 1-g sample was taken from the roots or leaves to represent the plant tissue. Plant samples were placed in a round-bottomed centrifuge tube containing 10 ml of 10 mM succinic acid, pH 3.5. Samples were macerated using a tissue homogenizer at medium speed for 30 s, centrifuged at 15,000 rpm in a Sorvall RC-5B centrifuge (Ivan Sorvall, Inc., Norwalk, CT) for 20 min, and filtered through a 0.45-µm millipore membrane with a syringe. An aliquot of the sample prepared for phosphonate residual analysis (100 µl) was injected into the LC module with 10 mM succinic acid, pH 3.5, as mobile phase, at a flow rate of 1.0 ml/min. Phosphonate standard was prepared to cover a range of 5 to 50 ppm. Each phosphonate determination is the mean of two experiments with five plants each.

Methods of fungicide application. In all fungicide application experiments, 10 avocado plants were used for each data point and each experiment was performed twice. In order to facilitate the diffusion of the fungicide into plant tissue, stem sections (30 cm long) located 20 cm above the soil line were prepared for fungicide paint application by using one of the following methods: (i) light scraping: removing the cuticle and part of the epidermis around the circumference of the stem with a razor blade; (ii) heavy scraping: removing the cuticle, epidermis, and outer layer of bark around the circumference of the stem; (iii) scraping in a fish-scale-like pattern: making incomplete cuts through the bark (30-cm-long sections) around the circumference of the stem to form small grooves, with the unattached ends of the scales oriented upward (Fig. 1). When using the fungicide as a paint on a preexisting stem canker, the canker and a surrounding margin of healthy tissue (5 cm wide) were scraped either lightly or heavily, as described.

The previously scraped areas of healthy or cankered stems of avocado plants were painted with either fosetyl-Al, fosetyl-Al followed by Tree Seal, or fosetyl-Al:Tree Seal formulation in thin layers, using a brush. The fungicide paint was applied to cover the area of scraped stem or cankers and surroundings approximately 10 to 20 cm above and below the margins. The amounts of fungicide paint used were 3 and 6 g for 8- to 12-month-old and 18-month-old avocado plants, respectively.

Soil drench experiments were performed on 8-month-old *Persea indica* and *Persea americana* and 18-month-old *Persea americana* avocado plants grown in 4- and 20-liter pots, respectively. Each avocado plant was drenched with either 500 ml per pot (8-month-old plants) or 1 liter per pot (18-month-old plants) of the fungicide preparation. Each plant received half the amount of the fungicide preparation initially, followed by the other half 2 h later. Saucers were placed under the pots for 1 day to prevent the fungicide loss. Two days after fungicide application, the plants were irrigated regularly. All the experiments in the study were performed twice and are described as follows:

Experiment 1. The objective of the experiment was to evaluate the effect of fungicide paint on scraped active stem cankers and the development of stem canker caused by new *P. citricola* inoculations made after the fungicide application. The experimental design was a three-factor factorial: (i) fungicide treatment as a stem paint with three levels (control, fosetyl-Al, and fosetyl-Al:Tree Seal formulation), (ii) stem preparation with two levels of scraping (light and heavy), and (iii) time of inoculation with seven levels (1, 2, 3, 4, and 5 weeks and 5 and 6 months after fungicide application). *Persea americana* plants, 10 months old, were stem inoculated with *P. citricola* as described above. The plants were maintained under greenhouse conditions for a 2-month period during which the cankers expanded (12 to 18 cm long) and developed on the stems. The canker and a surrounding margin of healthy tissue (5 cm wide) were scraped either lightly or heavily and painted with either fosetyl-Al paint or fosetyl-Al:Tree Seal formulation as previously described. Because scraping the canker obliterated the margins of the canker, measurements of the sizes of cankers were not made. Instead, isolations were made onto the PARPH agar from the region of the canker at 0, 1, 2, 3, 4, and 5 weeks and 5 and 6 months after fungicide application. The stems of the plants received new inoculations with *P. citricola* at 0, 1, 2, 3, 4, and 5 weeks and 5 and 6 months after the fungicide had been applied as described. Two weeks after inoculation, disease was assessed. The phosphonate concentrations in the stem bark were determined 5 and 6 months after fungicide treatment by using HPLC, as described above. The data for inoculations at 1 to 5 weeks were statistically analyzed separately from those inoculations at 5 and 6 months.

Experiment 2. To evaluate the effect of fungicide applied as paint on heavily scraped avocado stems prior to infection on disease development and phosphonate concentrations in the plant tissues, an ex-

Fig. 1. Stem of 8-month-old *Persea americana* cv. Topa Topa plant cut in a fish-scale-like pattern by making incomplete cuts through the bark (30-cm-long section) around the circumference of the stem to form small grooves with the unattached ends of the scales oriented upward.

periment was designed as a two-factor factorial: (i) fungicide treatment as a stem paint with four levels (control, fosetyl-Al, fosetyl-Al followed by Tree Seal, and fosetyl-Al:Tree Seal formulation), and (ii) time of inoculation with three levels (3, 6, and 9 weeks after fungicide application). The stems of 8-month-old *Persea americana* plants were heavily scraped and then painted either with 3 g of fosetyl-Al paint per plant or fosetyl-Al paint followed by Tree Seal or fosetyl-Al:Tree Seal formulation as described above. At 3-week intervals after fungicide application, plants were stem inoculated with *P. citricola*. Phosphonate was determined in the stem, leaf, and root tissues of inoculated plants by using HPLC. Disease development was assessed 2 weeks after inoculation, and a sample of the inoculation site was cultured on PARPH agar.

Experiment 3. The objective of the experiment was to evaluate the effect of fungicides applied as paint to stem scraped in a fish-scale-like pattern on stem canker development and tissue phosphonate levels. The treatment, designed as two-factor factorial, included: (i) fungicide treatment as a stem paint with three levels (control, fosetyl-Al, and fosetyl-Al:Tree Seal formulation), and (ii) time of inoculation with six levels (1, 2, 3, 4, 12, and 20 weeks after fungicide application). The stem bark of a group of 12-month-old Topa Topa plants was cut in a fish-scale-like pattern, then painted with the fungicide. Stem inoculation with *P. citricola* and the determination of the residual phosphonate in the stem bark were performed 0, 1, 2, 3, 4, 12, and 20 weeks after the fungicide treatment. At each inoculation time, bark samples were taken to determine phosphonate levels. Two weeks after each inoculation, the canker size was assessed and a sample of the inoculation site was cultured on PARPH agar to detect *P. citricola*.

Table 1. Effects of application of fosetyl-Al or fosetyl-Al:Tree Seal formulation as a paint over scraped stem cankers of *Phytophthora citricola* on 12-month-old *Persea americana* cv. Topa Topa plants on the development of canker caused by new inoculations made after fungicide application

Inoculation (weeks after treatment)	Canker size (cm^2)[a] on scraped[b] and fungicide-treated[c] stems					
	Light scraping			Heavy scraping		
	Control	Fosetyl-Al	Fosetyl-Al: Tree Seal	Control	Fosetyl-Al	Fosetyl-Al: Tree Seal
1	19.00	1.13	0.48	30.84	0.10	0.00
2	16.38	0.98	0.40	28.31	0.08	0.00
3	17.33	0.80	0.28	24.18	0.05	0.00
4	19.84	0.70	0.08	26.39	0.00	0.00
5	15.67	0.30	0.19	23.25	0.00	0.00
Mean	17.65	0.78	0.29	26.59	0.05	0.00

[a] Canker size is the mean of two experiments, each with 10 plants and four inoculation sites per plant; canker areas were measured 2 weeks after inoculation and averaged for all sites. When canker was zero, *P. citricola* was not recovered on *Phytophthora* selective PARPH media.

[b] The stem canker was scraped along the circumference of the stem by removing the cuticle and part of the epidermis (light scraping) and the outer layer of the bark (heavy scraping).

[c] Each avocado plant received 3 g of the fosetyl-Al paint (0.4 g a.i. fosetyl-Al + 1 ml water) or fosetyl-Al:Tree Seal formulation (0.4 g a.i. fosetyl-Al + 0.5 ml water + 0.5 g Tree Seal) to cover the scraped lesion.

Table 2. Effects of fosetyl-Al or fosetyl-Al:Tree-Seal formulation as a paint on scraped cankers of *Phytophthora citricola* on 12-month-old *Persea americana* cv. Topa Topa on phosphonate (PHO_3^{-2}) concentration in stem bark and on cankers caused by new inoculations made 5 and 6 months after fungicide treatment

Stem scraping[a]	Inoculation (mo after treatment)	Canker size (cm^2)[b]			PHO_3^{-2} (μg/g fresh wt)[c]	
		Control	Fosetyl-Al[d]	Fosetyl-Al: Tree Seal[d]	Fosetyl-Al[d]	Fosetyl-Al: Tree Seal[d]
Light	5	18.58	2.80	2.62	17.70	17.83
	6	20.50	12.10	11.24	5.24	6.94
	Mean	19.54	7.45	6.93	11.47	12.39
Heavy	5	25.70	2.17	0.00	23.10	246.53
	6	27.38	6.56	0.00	10.21	129.00
	Mean	26.54	4.36	0.00	16.66	187.77

[a] The stem was scraped with a razor blade to remove the cuticle and part of the epidermis (light scraping) and the outer layer of the bark (heavy scraping).

[b] Canker size is the mean of two experiments, each with 10 plants and four inoculation sites per plant. When canker size was zero, *P. citricola* was not recovered on *Phytophthora* PARPH agar.

[c] Phosphonate level is the average of two experiments, each with five replicates. Moisture content of stem bark was 73%.

[d] Each avocado plant received 3 g of the fosetyl-Al paint (0.4 g a.i. fosetyl-Al + 1 ml water) or fosetyl-Al:Tree Seal formulation (0.4 g a.i. fosetyl-Al + 0.5 ml water + 0.5 g Tree Seal) to cover the scraped lesion.

Experiment 4. The objective of the experiment was to evaluate the effect of fungicide application as a soil drench on stem canker development and phosphonate concentrations in the tissues of *Persea indica* and *Persea americana*. The treatment design was a three-factor factorial and included: (i) avocado species with two levels (*Persea indica* and *Persea americana*), (ii) fungicide treatment with two levels (control and fosetyl-Al), and (iii) time of inoculation with five levels (3, 6, 9, 12, and 15 weeks after fungicide application). At 3-week intervals after fungicide application, plants were stem inoculated with *P. citricola*, and the phosphonate was determined in the stem, leaf, and root tissues. Two weeks after inoculation, the disease development was assessed and samples of the inoculation sites were cultured on PARPH agar.

Experiment 5. The objective of the experiment was to compare stem paint and soil drench applications of fosetyl-Al on tissue phosphonate levels and stem canker development. The treatment design was a two-factor factorial: (i) fungicide treatment with three levels (control, stem paint, and soil drench), and (ii) time of inoculation with five levels (3, 6, 9, 12, and 15 weeks after fungicide application). *Persea americana* plants (18 months old) grown in 20-liter pots were divided into two groups: the first group of plants was drenched with 1 liter of fosetyl-Al preparation as previously described. The stems of the second group of plants were cut in a fish-scale-like pattern and then painted with 6 g of fosetyl-Al paint per plant. At 3-week intervals after fungicide application, plants were stem inoculated with *P. citricola* and the phosphonate was determined in stem, leaf, and root tissues. The disease development was assessed 2 weeks after inoculation, and samples of the inoculation sites were cultured on PARPH agar.

Statistical analysis. The experimental design used throughout the study was completely randomized. The design of the treatments was either a two-factor or a three-factor factorial. There were 10 replicate plants for each treatment combination. Canker size and phosphonate concentration were transformed to logarithms to homogenize the variance. Any treatment combination for which no cankers developed for any of the replicate plants was excluded from the calculation of the error term. Analysis of variance was done to test all main effects and interactions. Single degree of freedom contrasts were done to test for mean separation, linear or quadratic trends over time, and specific interaction effects. Data were analyzed with the general linear models procedure (GLM) using the SAS computer program (35).

RESULTS

Fosetyl-Al as a paint on stem cankers (experiments 1, 2, and 3). Fungicide

paint application on either lightly or heavily scraped active stem cankers (experiment 1) was effective in controlling treated cankers. One week after application of either fosetyl-Al or fosetyl-Al:Tree Seal formulation on scraped active stem cankers on 12-month-old *Persea americana* plants, cankers ceased to expand and *P. citricola* was not recovered from canker sites. This curative effect was confirmed by the absence of *P. citricola* over a 6-month period (the duration of the experiment) following the fungicide treatment.

Fungicide treatments were also effective in protecting the avocado plants against new stem infections, since either very small or no cankers developed after inoculations with *P. citricola* at sites distant from the fungicide-treated cankers (Tables 1 and 2). Analysis of variance of canker size indicated no significant interaction with experiment and no significant difference between experiments. All other main effects and interactions were significant (Tables 3 and 4). Cankers were significantly larger for untreated stems than for the stems treated with fungicide. The fungicide application as a stem paint to heavily scraped stems of 12-month-old *Persea americana* was more effective in controlling *P. citricola* stem cankers than fungicide applied to lightly-scraped stems (Tables 1 and 2). The interaction of scraping by fungicide treatment was significant, since heavy scraping resulted in the development of larger cankers than light scraping for the untreated stems but showed the reverse effect for the two fungicide treatments of fosetyl-Al. The fungicide by scraping by week interaction was also significant. One component of this significant interaction was the fact that heavy scraping combined with fosetyl-Al:Tree Seal formulation treatment showed no trend over the course of the experiment, since cankers were not formed (Table 1). The other five combinations of scraping by fungicide treatments showed a general trend toward decreasing canker size over the length of the experiment (Table 3). Canker size changed from 19 to 15.7 cm^2 for the untreated control with light scraping but from 30.8 to 23.3 cm^2 for heavy scraping for inoculations done 1 to 5 weeks after fungicide treatment (Table 1). The linear trend over weeks for heavy scraping was not significant compared to light scraping for the two treatments with fosetyl-Al but was the reverse for the control, which showed a greater decrease in canker size over time with heavy scraping than with light scraping (Tables 1 and 3).

Treating heavily scraped stems with fosetyl-Al:Tree Seal formulation (experiment 1) completely protected avocado plants against the stem canker for a 6-month period (the duration of the experiment), and *P. citricola* was not recovered from the inoculation sites. Using fosetyl-Al alone resulted in significantly less protection (76% protection) after the same period of time (Tables 2 and 4). On lightly scraped stems, the decline in the protective effect of both fosetyl-Al and fosetyl-Al:Tree Seal formulation was evidenced by the significantly larger canker size at 6 than at 5 months (Table 2). When fosetyl-Al alone, fosetyl-Al followed by Tree Seal, or fosetyl-Al:Tree Seal formulation was applied as a paint on heavily scraped stem sections of 8-month-old *Persea americana* (experiment 2), no stem cankers developed

Table 3. Summary of analysis of variance and contrasts[a] for the effect of application of fosetyl-Al or fosetyl-Al:Tree Seal formulation as a paint over scraped stem cankers of *Phytophthora citricola* on 12-month-old *Persea americana* cv. Topa Topa plants on the development of canker caused by new inoculations made after fungicide application

Source of variation	df[b]	*F* value[c]	Prob > *F*
Experiment[d]	1	0.01	0.913
Week	4	49.59	0.000
Scraping treatment	1	156.09	0.000
Fungicide treatment	2	>999.99	0.000
Control vs. fungicide treatment	1	>999.99	0.000
Fosetyl-Al vs. fosetyl-Al:Tree Seal	1	219.72	0.000
Scraping × week	4	9.07	0.000
Scraping × fungicide	2	744.76	0.000
Scraping for control	1	524.94	0.000
Scraping for fosetyl-Al	1	916.64	0.000
Scraping for fosetyl-Al:Tree Seal	1	204.27	0.000
Fungicide × week	8	8.90	0.000
Scraping × fungicide × week	8	12.44	0.000
Linear regression with time for			
Light scraping control	1	5.11	0.024
Light scraping fosetyl-Al	1	86.16	0.000
Light scraping fosetyl-Al:Tree Seal	1	66.77	0.000
Heavy scraping control	1	50.26	0.000
Heavy scraping fosetyl-Al	1	9.10	0.003
Heavy scraping fosetyl-Al:Tree Seal	1	0.00	1.000

[a] Statistical analysis of data given in Table 1.
[b] Error df = 414.
[c] Canker size + 1 transformed to logs for analysis.
[d] All interactions with experiment were not significant and they were not included in the table.

Table 4. Summary of analysis of variance and contrasts[a] for the effect of fosetyl-Al or fosetyl-Al:Tree-Seal formulation as a paint on scraped cankers on 12-month-old *Persea americana* cv. Topa Topa on phosphonate (PHO_3^{-2}) concentration in stem bark and on canker caused by *Phytophthora citricola* made 5 and 6 months after fungicide treatment

	Canker size[b]			Residual phosphonate[b]		
Source of variation	df[c]	*F* value	Prob > *F*	df[c]	*F* value	Prob > *F*
Experiment[d]	1	0.04	0.845	1	0.45	0.506
Month	1	610.28	0.000	1	277.97	0.000
Scraping treatment	1	776.10	0.000	1	938.75	0.000
Fungicide treatment	2	>999.99	0.000	1	612.98	0.000
Control vs. fungicide	1	>999.99	0.000	...	...	...
Fosetyl-Al vs. fosetyl-Al:Tree Seal	1	698.34	0.000	...	...	...
Scraping × month	1	135.02	0.000	1	7.61	0.008
Fungicide × month	2	144.50	0.000	1	2.91	0.093
Scraping × fungicide	2	752.75	0.000	1	494.04	0.000
Light scraping						
Control vs. fungicide	1	874.67	0.000	...	...	...
Fosetyl-Al vs. fosetyl-Al:Tree Seal	1	1.52	0.219	1	3.20	0.078
Heavy scraping						
Control vs. fungicide	1	>999.99	0.000	...	...	...
Fosetyl-Al vs. fosetyl-Al:Tree Seal	1	>999.99	0.000	1	>999.99	0.000
Scraping × fungicide × month	2	51.45	0.000	1	80.44	0.510
5-mo vs. 6-mo						
Light scraping						
Control	1	2.21	0.139	...	...	...
Fosetyl-Al	1	409.50	0.000	1	118.82	0.000
Fosetyl-Al:Tree Seal	1	405.27	0.000	1	72.78	0.000
Heavy scraping						
Control	1	1.15	0.285	...	...	...
Fosetyl-Al	1	198.83	0.000	1	55.92	0.000
Fosetyl-Al:Tree Seal	1	0.00	1.000	1	41.41	0.000

[a] Statistical analysis of data given in Table 2.
[b] Canker size + 1 and residual phosphonate + 1 transformed to logs for analysis.
[c] Error df = 190 and 64 for canker size and residual phosphonate, respectively.
[d] All interactions with experiment were not significant and they were not included in the table.

and *P. citricola* was not recovered on PARPH agar during a 9-week duration of the experiment.

The application of fosetyl-Al alone as a paint on stem bark cut in a fish-scale-like pattern (experiment 3) was slightly more effective in protecting 12-month-old *Persea americana* plants against *P. citricola* than fosetyl-Al:Tree Seal formulation. The effect of fosetyl-Al alone lasted for a 20-week period (the duration of the experiment, Table 5), while fosetyl-Al:Tree Seal formulation did not prevent canker development after 12 weeks of fungicide treatment. The analysis of variance showed that applying fosetyl-Al alone was significantly more effective in controlling stem canker development compared with fosetyl-Al:Tree Seal formulation (Table 6). The treatments resulted in 100% inhibition after 4 weeks with fosetyl-Al and after 12 weeks with fosetyl-Al:Tree Seal formulation (Table 5). The linear and quadratic regressions of canker size with time were not significant for the control, but they were significant for either fungicide treatment (Table 6).

Soil drench of fosetyl-Al on stem cankers (experiments 4 and 5). The application of fosetyl-Al as a soil drench (experiment 4) was also effective in protecting 8-month-old avocado plants against stem canker disease caused by *P. citricola*. Fifteen weeks after fungicide application, *Persea americana* plants were still resistant to *P. citricola*, while *Persea indica* developed small cankers after 9 weeks of the fungicide treatment (Table 7). The fungicide applied as a soil drench (experiment 5) prevented stem infection of 18-month-old *Persea americana* for 9 weeks and showed a protective effect that lasted for the 15-week duration of the experiment (83.35% canker inhibition after 15 weeks, Table 8). The fungicide application as a stem paint prevented canker development throughout the 15-week duration of the experiment. In all cases where no lesions developed, *P. citricola* was not recovered. The fungicide applied as a stem paint was more efficacious than soil drench in controlling stem canker (Table 8).

Phosphonate levels in fungicide-treated avocado tissues (experiments 1 to 5). The phosphonate levels in the plant tissues varied according to the method of fungicide application and the time following the fungicide treatment. Analysis of variance of the phosphonate concentrations showed no significant interaction with experiment and no significant difference between experiments. There was no significant difference in the phosphonate level of the stem bark (experiment 1) after the application of either fosetyl-Al alone or fosetyl-Al:Tree Seal formulation as a paint on lightly scraped stem bark (Tables 2 and 4). The fosetyl-Al:Tree Seal formulation resulted in significantly higher residual phosphonate in the bark than fosetyl-Al alone when the fungicide was applied on heavily scraped stems (Table 4). Six months after fungicide treatment of a heavily scraped stem, the phosphonate concentration in the bark with fosetyl-Al paint was only 8% of that with fosetyl-Al:Tree Seal formulation (Table 2). Also, the concentration of phosphonate in the bark of lightly scraped stems treated with fosetyl-Al:Tree Seal formulation was only 5% of that on heavily scraped stems (Table 2). The residual phosphonate level in the bark was the highest with fosetyl-Al:Tree Seal formulation on heavily scraped stems and the lowest with fosetyl-Al alone on a lightly scraped stem.

Applying the fungicide as a paint on heavily scraped stems of 8-month-old *Persea americana* by using either fosetyl-Al alone, fosetyl-Al followed by Tree Seal, or fosetyl-Al:Tree Seal formulation (experiment 2) did not result in significant differences in the phosphonate levels in the leaves, but did result in significantly different phosphonate levels in the stem and the roots (Fig. 2 and Table 9). Fosetyl-Al:Tree Seal formulation resulted in significantly higher phosphonate levels than Tree Seal applied separately following fosetyl-Al application in roots and stems of avocado (Table 9). The analysis of variance for residual phosphonate levels showed significant interactions between time and fungicide treatment in the roots and stems but not in the leaves. The pattern of change in phosphonate levels over time, for either roots or stems, was not significantly differ-

Table 5. Levels of phosphonate in stem bark of 12-month-old *Persea americana* cv. Topa Topa following application of fosetyl-Al or fosetyl-Al:Tree Seal formulation as a paint on stem bark cut in a fish-scale-like pattern[a] and the effect of residual phosphonate on disease incidence and stem canker development caused by *Phytophthora citricola*

	Canker size[c] (cm^2)			Phosphonate (μg/g fresh wt)[d]	
Time[b] (wk)	Control	Fosetyl-Al[e]	Fosetyl-Al: Tree Seal[f]	Fosetyl-Al[e]	Fosetyl-Al: Tree Seal[f]
1	26.59	5.92	9.05	9.85	8.32
2	28.34	2.50	4.00	16.53	13.54
3	22.60	0.13	1.45	23.55	17.35
4	25.27	0.00	0.30	32.20	19.80
12	27.42	0.00	0.00	54.80	26.90
20	24.95	0.00	4.91	32.65	11.84
Mean	25.86	1.43	3.28	28.76	16.29

[a] Incomplete cuts were introduced to the bark around the circumference of the stem to form small grooves in a fish-scale-like pattern.
[b] Time of phosphonate determination and inoculation by *P. citricola* after fungicide application.
[c] Canker size is the mean of two experiments, each with 10 plants. When canker size was zero, *P. citricola* was not recovered on *Phytophthora* PARPH agar.
[d] Phosphonate level is the mean of two experiments, each with five replicates. The moisture content of the stem bark was 73%.
[e] Each plant received 3 g of fosetyl-Al paint (0.4 g a.i. fosetyl-Al + 1 ml water).
[f] Each plant received 3 g of fosetyl-Al:Tree Seal formulation (0.4 a.i. fosetyl-Al + 0.5 g Tree Seal + 0.5 ml water).

Table 6. Summary of analysis of variance and contrasts[a] for the levels of phosphonate in stem bark of 12-month-old *Persea americana* cv. Topa Topa following application of fosetyl-Al or fosetyl-Al:Tree Seal formulation as a paint on stem bark cut in a fish-scale-like pattern and the effect of residual phosphonate on disease incidence and stem canker development caused by *Phytophthora citricola*

	Canker size[b]			Residual phosphonate[b]		
Source of variation	df[c]	*F* value	Prob > *F*	df	*F* value	Prob > *F*
Experiment[d]	1	0.27	0.602	1	0.09	0.768
Week	5	491.24	0.000	5	127.48	0.000
Fungicide treatment	2	>999.99	0.000	1	200.48	0.000
Control vs. fungicide	1	>999.99	0.000	...	...	...
Fosetyl-Al vs. fosetyl-Al: Tree Seal	1	556.50	0.000	1	25.00	0.000
Fungicide × week	10	166.97	0.000	5	17.48	0.000
Regression with time						
Control, linear	1	0.03	0.869	...	...	...
Quadratic	1	0.33	0.564	...	...	...
Fosetyl-Al, linear	1	644.21	0.000	1	237.80	0.000
Quadratic	1	500.75	0.000	1	223.86	0.000
Fosetyl-Al vs. fosetyl-Al: Tree Seal, linear	1	19.17	0.000	1	5.99	0.016
Quadratic	1	>999.99	0.000	1	200.38	0.000

[a] Statistical analysis of data given in Table 5.
[b] Canker size + 1 and residual phosphonate + 1 transformed to logs for analysis.
[c] Error df = 252.
[d] All interactions with experiment were not significant and they were not included in the table.

ent between the two treatments with Tree Seal, but fosetyl-Al alone showed a significantly different pattern from the Tree Seal treatments (Fig. 2). The phosphonate levels declined over time in stems, with a slightly steeper rate of decline for the Tree Seal treatments than for the treatment without Tree Seal (Fig. 2).

Stem bark cut in a fish-scale-like pattern (experiment 3) and then treated with fosetyl-Al alone showed significantly higher levels of phosphonate in the bark tissue after 20 weeks than did those treated with fosetyl-Al:Tree Seal formulation (Table 5). Analysis of variance of phosphonate concentration indicated no significant interaction with experiment and no significant differences between experiments (Table 6). The phosphonate level in the stem bark after the fungicide application as a paint on stems cut in a fish-scale-like pattern increased with time and reached its maximum 12 weeks after the treatment (Table 5). After applying fosetyl-Al as a soil drench to 8-month-old *Persea indica* and *Persea americana* (experiment 4), the phosphonate levels were significantly higher in the stem bark of *Persea americana* after 3 weeks compared with *Persea indica* (Tables 7 and 10). The residual phosphonate (experiment 4) was significantly higher in the stems and leaves of *Persea americana* compared to *Persea indica* (Tables 7 and 10). There was no significant difference in residual phosphonate in the roots of *Persea americana* and of *Persea indica* (Table 10). Six weeks after the fungicide treatment there was no significant difference in phosphonate levels in the stem bark of both avocado species. Fifteen weeks after treatment, *Persea americana* plants were still resistant to *P. citricola*, while *Persea indica* developed small cankers 9 weeks after the fungicide treatments (Table 7).

The phosphonate levels of all plant tissues tested after fungicide application as a paint on bark cut in a fish-scale-like pattern (experiment 5) were higher compared to that of the tissues of plants treated with fungicide as a soil drench (Tables 8 and 11). Six to 15 weeks after fungicide application, the phosphonate concentration was lowest in the roots and highest in the leaves in both methods of fungicide application (Table 8). The time required for the residual phosphonate to reach its maximum level was 6 weeks when applying the fungicide as a paint on stem bark cut in a fish-scale-like pattern and 3 to 6 weeks for soil drench treatment (Tables 7 and 8). The correlation analysis showed a strong negative correlation ($r = -0.99$) between phosphonate concentration and time. The percent inhibition of canker development increased as the level of phosphonate increased in the bark. The correlation analysis showed a strong negative correlation ($r = -0.978$) between phosphonate level and canker size (Fig. 3). Stem bark phosphonate concentration of 21 µg/g fresh tissue bark is required to completely inhibit cankers caused by *P. citricola*.

DISCUSSION

This study showed that the application of fosetyl-Al as a stem paint on heavily scraped active stem cankers was effective in curing the existing infections and protecting avocado plants against new infections. Scraping cankerous tissues is one of the cultural practices growers use to remove active cankers with the intention of curing diseased avocado trees. However, canker scraping alone greatly increased *P. citricola* canker disease since it resulted in rapid movement of the pathogen through the phloem, spreading the disease to the rest of the tree (13). It is recommended to use the scraping technique only when followed by fosetyl-Al application as a stem paint for curing diseased plants. While

Table 7. Levels of phosphonate in the tissues of 8-month-old plants of *Persea indica* and *Persea americana* cv. Topa Topa following application of fosetyl-Al as a soil drench[a] and the effect of residual fungicide on disease incidence and stem canker development caused by *Phytophthora citricola*

		Phosphonate (µg/g fresh wt)[c]			Canker size (cm²)[d]	
Plant species	Time (wk)[b]	Root	Stem	Leaves	Control	Treated
Persea indica	3	283.0	211.6	130.1	140.1	0.0
	6	237.5	139.5	213.5	150.3	0.0
	9	200.1	111.6	146.7	98.2	0.2
	12	140.0	75.0	98.3	75.5	0.5
	15	79.4	51.4	77.3	67.9	1.0
	Mean	187.97	117.65	133.27	106.4	0.3
Persea americana	3	308.8	571.6	621.7	24.3	0.0
	6	193.9	148.6	428.5	21.9	0.0
	9	180.7	112.5	297.0	22.7	0.0
	12	163.0	101.8	227.8	18.0	0.0
	15	90.2	88.2	140.1	15.0	0.0
	Mean	187.35	204.52	343.13	20.4	0.0

[a] The fungicide treatment consisted of a 500-ml (3.2 g a.i. fosetyl-Al/liter water) per 4-liter pot applied as soil drench.
[b] Time of phosphonate determination and inoculation by *P. citricola* after fungicide application.
[c] Phosphonate level is the mean of two experiments with five replicates each. The moisture contents of the stem bark, roots, and leaves were 74, 86, and 86%, respectively.
[d] Canker size is the mean of two experiments, each with 10 plant replicates and four inoculation sites. When canker was zero, *P. citricola* was not recovered on *Phytophthora* PARPH agar.

Table 8. Levels of phosphonate in tissues of 18-month-old *Persea americana* cv. Topa Topa plants following application of fosetyl-Al as a soil drench or as a stem paint after cutting the bark in fish scale-like pattern and effects of residual fungicide on disease incidence and stem canker development caused by *Phytophthora citricola*

		Phosphonate (µg/g fresh wt)[b]			Canker size
Fungicide treatment	Time (wk)[a]	Root	Stem	Leaves	(cm²)[c]
Stem paint[d]	3	54.9	60.6	256.1	0.0
	6	80.0	202.8	320.0	0.0
	9	67.8	190.0	401.9	0.0
	12	43.0	151.2	260.8	0.0
	15	16.0	82.4	119.3	0.0
	Mean	52.3	137.4	271.7	0.0
Soil drench[e]	3	36.4	30.2	126.5	0.0
	6	48.9	92.5	191.7	0.0
	9	22.0	50.2	108.2	0.0
	12	9.5	13.8	42.5	2.0
	15	6.0	9.7	26.3	4.3
	Mean	24.55	39.27	99.0	1.3
Control	3	...	...	...	30.0
	6	...	...	...	27.1
	9	...	...	...	41.2
	12	...	...	...	30.7
	15	...	...	...	25.2
	Mean	...	...	...	30.8

[a] Time of phosphonate determination and inoculation by *P. citricola* after fungicide treatment.
[b] Phosphonate level is the mean of two experiments, each with five replicates. The moisture contents of root, stem bark, and leaves were 88, 73, and 85 %, respectively.
[c] Canker size is the mean of two experiments, each with 10 plants and four inoculation sites per plant. When canker size was zero, *P. citricola* was not recovered on *Phytophthora* selective PARPH agar.
[d] The stem bark of each plant was cut in a fish-scale-like pattern, then painted with 6 g of fungicide paint (0.4 g a.i. fosetyl-Al + 1 ml water) using a brush.
[e] Plants grown in 20-liter pots received 1-liter of fungicide preparation (3.2 g a.i. fosetyl-Al/liter).

scraping young plants with a razor blade may not completely reflect field treatments, the intent was to duplicate treatments carried out by growers with an ax or a machete on large trees in the field. Results of this study indicate that fosetyl-Al treatments to control *P. citricola* canker can be greatly facilitated by scraping or cutting the bark in a fish-scale-like pattern before applying the fungicide.

The phosphonate levels in avocado plant tissues and the protective effects of phosphonate against infection by *P. citricola* seemed to be affected by: (i) the method of fungicide application, (ii) fungicide concentration and formulation, (iii) method of stem preparation before stem paint application, and (iv) time following fungicide application. Applying the fungicide as a stem paint was about twofold more effective in delivering the phosphonate into the plant tissues than the soil drench method (Table 8). Heavy scraping followed by fosetyl-Al application as a stem paint gave a higher residual phosphonate than applying the fungicide either after light scraping or after cutting the stem in a fish-scale-like pattern. The phosphonate reached its maximum level in the stem bark in about 3

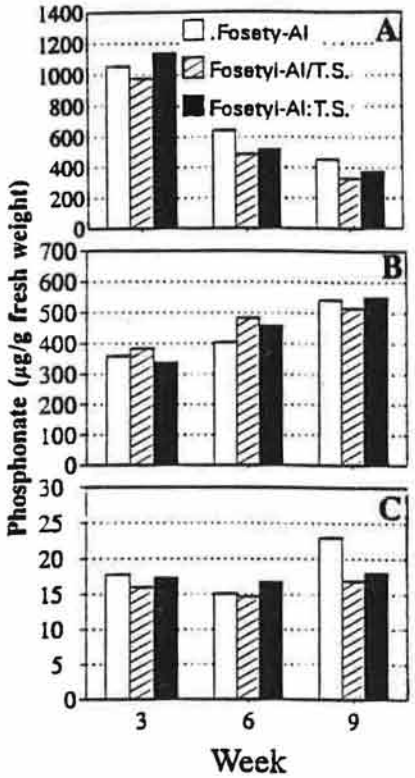

Fig. 2. Levels of residual phosphonate in the tissues of 8-month-old *Persea americana* cv. Topa Topa plants after 3, 6, and 9 weeks of fosetyl-Al, fosetyl-Al followed by Tree Seal (fosetyl-Al/T.S.), or fosetyl-Al:Tree Seal formulation (fosetyl-Al:T.S.) application as a paint on heavily scraped stems. Bars of **(A)** stems, **(B)** leaves, and **(C)** roots are the average of two experiments, each with 10 plants. No stem cankers developed in all cases after *Phytophthora citricola* inoculation.

Table 9. Summary of analysis of variance and contrasts[a] for the levels of residual phosphonate in the tissues of 8-month-old *Persea americana* cv. Topa Topa plants following the application of fosetyl-Al or fosetyl-Al:Tree Seal formulation as a paint on heavily scraped stems

		Root[c]		Stem[c]		Leaves[c]	
Source of variation	df[b]	*F* value	Pr > *F*	*F* value	Pr > *F*	*F* value	Pr > *F*
Experiment[d]	1	0.00	0.959	0.12	0.732	0.00	0.981
Week	2	11.75	0.000	887.85	0.000	40.87	0.000
Linear	1	6.60	0.012	>999.99	0.000	81.55	0.000
Quadratic	1	16.89	0.000	45.74	0.000	0.20	0.657
Fungicide treatment	2	5.77	0.005	42.98	0.000	0.73	0.483
Fosetyl-Al vs. fosetyl-Al with Tree Seal	1	6.92	0.010	7.65	0.000	1.73	0.153
Fosetyl-Al followed by Tree Seal vs. fosetyl-Al: Tree Seal	1	4.61	0.030	57.43	0.000	1.07	0.305
Fungicide × week	4	2.52	0.048	28.54	0.000	0.40	0.529
Fosetyl-Al vs. fosetyl-Al with Tree Seal × week							
Linear	1	3.81	0.055	23.03	0.000	0.04	0.837
Quadratic	1	5.82	0.018	6.16	0.015	3.95	0.051
Fosetyl-Al followed by Tree Seal vs. fosetyl-Al: Tree Seal × week							
Linear	1	0.01	0.933	0.08	0.779	2.82	0.098
Quadratic	1	0.45	0.504	1.34	0.251	0.10	0.753

[a] Statistical analysis of data presented in Figure 2.
[b] Error df = 72.
[c] Residual phosphonate + 1 transformed to logs for analysis.
[d] All interactions with experiment were not significant and they were not included in the table.

Table 10. Summary of analysis of variance and contrasts[a] for the levels of phosphonate in the tissues of 8-month-old plants of *Persea indica* and *Persea americana* cv. Topa Topa following application of fosetyl-Al as a soil drench and the effect of residual fungicide on disease incidence and stem canker development caused by *Phytophthora citricola*

		Residual phosphonate[c]					
		Root		Stem		Leaves	
Source of variation	df[b]	*F* value	Prob > *F*	*F* value	Prob > *F*	*F* value	Prob > *F*
Experiment[d]	1	0.00	0.977	0.04	0.840	0.04	0.836
Week	4	328.64	0.000	482.58	0.000	279.70	0.000
Linear	1	>999.99	0.000	>999.99	0.000	>999.99	0.000
Quadratic	1	42.33	0.000	139.54	0.000	64.56	0.000
Plant species	1	0.41	0.523	226.58	0.000	>999.99	0.000
Plant species × week	4	9.38	0.000	48.80	0.000	54.23	0.000
Linear	1	7.24	0.009	13.34	0.000	114.43	0.000
Quadratic	1	12.49	0.000	153.88	0.000	47.06	0.000

[a] Statistical analysis of data given in Table 7.
[b] Error df = 80.
[c] Residual phosphonate + 1 transformed to logs for analysis.
[d] All interactions with experiment were not significant and they were not included in the table.

Table 11. Summary of analysis of variance and contrasts[a] for the levels of phosphonate in the tissues of 18-month-old *Persea americana* cv. Topa Topa plants following application of fosetyl-Al as a soil drench or as a stem paint after cutting the bark in fish-scale-like pattern and effects of residual fungicide on disease incidence and stem canker development caused by *Phytophthora citricola*

		Residual phosphonate[c]					
		Root		Stem		Leaves	
Source of variation	df[b]	*F* value	Prob > *F*	*F* value	Prob > *F*	*F* value	Prob > *F*
Experiment[d]	1	0.04	0.918	0.00	0.973	0.22	0.643
Week	4	327.02	0.000	327.60	0.000	394.24	0.000
Linear	1	970.70	0.000	203.48	0.000	>999.99	0.000
Quadratic	1	226.80	0.000	773.30	0.000	438.44	0.000
Fungicide treatment	1	615.78	0.000	>999.99	0.000	>999.99	0.000
Fungicide × week	4	31.00	0.000	113.86	0.000	82.95	0.000
Linear	1	48.47	0.000	331.81	0.000	180.68	0.000
Quadratic	1	28.10	0.000	1.24	0.269	8.28	0.005

[a] Statistical analysis of data given in Table 8.
[b] Error df = 80.
[c] Residual phosphonate + 1 transformed to logs for analysis.
[d] All interactions with experiment were not significant and they were not included in the table.

.o 6 weeks in all methods of fungicide application. The high phosphonate absorption rate and the effectiveness in controlling cankers associated with heavy scraping are probably due to the direct exposure of the phloem to the fungicide, which facilitates fungicide uptake. Although cutting the bark in a fish-scale-like pattern resulted in phloem exposure, the extent of surface area exposed to the fungicide was probably less than in the case of heavy scraping. The level of residual phosphonate in the tissue was higher when fosetyl-Al:Tree Seal formulation was applied as a paint on the scraped stems, probably because Tree Seal allowed the fungicide to adhere tightly to the phloem, resulting in longer contact time for continuous fungicide uptake. Fosetyl-Al alone was more effective when applied on fish-scale-like cuttings, since it apparently was trapped in pockets, allowing continued contact of the fungicide with the bark.

Canker size decreased as the level of phosphonate in the bark increased (R^2 = 0.957). The results indicated that the residual phosphonate level required for complete disease prevention is 21 µg or higher per g of fresh bark tissue. Fosetyl-Al and its breakdown product, phosphonate, have been experimentally proved to give protection against diseases caused by *Phytophthora* spp. by inducing a number of physiological changes in the plant (3). Coffey and Joseph (7) found that phosphonate was highly inhibitory to critical stages in the life cycle of *P. cinnamomi* such as oospore production, sporangium production, and zoospore release. Phosphonates also inhibited the mycelial growth of *P. citricola* (29).

Ethyl phosphonate is hydrolyzed to ethanol and phosphonic acid in vivo, and ethyl phosphonate is short-lived in both soil and avocado tissues, with no detectable residues 1 week after foliar application (20,39). In contrast, Ouimette and Coffey (29) found high levels of phosphonate in avocado plant tissues. Our data support those of Ouimette and Coffey, in that phosphonate concentration and its persistence in plant tissues are factors that would be expected to play a major role in the efficacy of fosetyl-Al in controlling avocado stem canker disease. Six months after treatment with fosetyl-Al:Tree Seal formulation as a paint on heavily scraped stem (Table 2), the residual phosphonate in the avocado bark was high enough (129.0 µg/g fresh bark) to completely control stem canker disease. Sandler et al. (34) also reported a reduction in the lesion size of Phytophthora gummosis in citrus 30 but not 60 or 100 days after phosphonate application. Once inside avocado plants, phosphonate appears to be stable. This phenomenon agrees with the conclusion presented by MacIntire et al. (23) that higher plants cannot readily oxidize phosphorus compounds to phosphates and that no growth response was detected in plants growing in soil where phosphonate was used as the sole source of phosphorus.

Although there was no significant difference between the phosphonate levels of the stem bark of *Persea americana* and *Persea indica* 9 weeks after the fosetyl-Al application as a soil drench, the fungicide was more effective in controlling the disease in *Persea americana* than in *Persea indica*. Since *Persea americana* is known to have better resistance to *P. citricola* than does *Persea indica,* their differences in response to treatment with fosetyl-Al favor the complex mode of action that included components of direct antifungal activity and a stimulated host defense response from the infected plant.

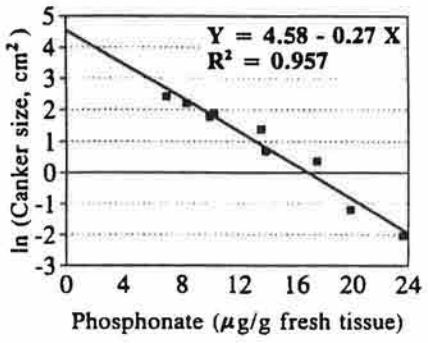

Fig. 3. Correlation between phosphonate concentrations in the bark (moisture content = 73%) and the size of cankers developed on stems of avocado plants after fungicide application on scraped stem areas and inoculation with *Phytophthora citricola* (see text for details on fungicide treatment and inoculation procedure). Level of residual phosphonate was determined at the time of each inoculation, and canker size was measured 2 weeks after inoculation.

ACKNOWLEDGMENTS

We thank C. J. Lovatt for using the HPLC in her laboratory, G. A. Zentmyer for reviewing the manuscript, and California Avocado Commission for financial assistance in this project.

LITERATURE CITED

1. Barrett, J. T. 1917. *Pythiacystis* related to *Phytophthora*. Phytopathology (Abstr.) 7:150.
2. Bezuidenhout, J. J., Darvas, J. M. and Toerien, J. C. 1987. Chemical control of *Phytophthora cinnamomi*. S. Afr. Avocado Growers' Assoc. Yearbook 10:106-108.
3. Bompeix, G., Ravise, A., Raynal, G., Fettouche, F., and Durand, M. C. 1980. Modalites de l'obtention des necroses bloquantes sur feuilles detachees de Tomate par l'action du tris-O-ethyl phosphonate d'aluminum (phosetyl d'aluminum), hypothyses sur son mode d'action in vivo. Ann. Phytopathol. 12:337-351.
4. Bruin, G. C. A., and Edgington, L. V. 1983. The chemical control of diseases caused by zoosporic fungi a many-sided problem. Pages 193-232 in: Zoosporic Plant Pathogens. S. T. Buczacki, ed. Academic Press, New York.
5. Coffey, M., Oudemans, P., and Ouimette, D. 1988. *Phytophthora citricola*: Another cause of avocado decline. Calif. Avocado Soc. Yearbook. 72:127-131.
6. Coffey, M. D. 1987. Phytophthora root rot of avocado: An integrated approach to control in California. Plant Dis. 71:1046-1052.
7. Coffey, M. D., and Joseph, M. C.1985. Effects of phosphorous acid and fosetyl-Al on the life cycle of *Phytophthora cinnamomi* and *P. citricola*. Phytopathology 75:1042-1046.
8. Coffey, M. D., and Ouimette, D. G. 1989. Phosphonates: Antifungal compounds against Oomycetes. Pages 107-129 in: Nitrogen, Phosphorus and Sulphur Utilization by Fungi. L. Boddy, R. Marchant, and D. J. Read, eds. Symposium of the British Mycological Society, April 1988. Cambridge University Press, Cambridge.
9. Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. 24:311-338.
10. Darvas, J. M., Toerien, J. C., and Milne, D. L. 1984. Control of avocado root rot by trunk injection with Phosethyl-Al. Plant Dis. 68:691-693.
11. Dercks, W., and Buchenauer, H. 1987. Comparative studies on the mode of action of aluminum ethyl phosphite in four *Phytophthora* species. Crop Prot. 6:82-89.
12. El-Hamalawi, Z. A., and Menge, J. A. 1994. Effect of wound age and fungicide treatment of wounds on susceptibility of avocado stems to infection by *Phytophthora citricola*. Plant Dis. 78:700-704.
13. El-Hamalawi, Z. A., Menge, J. A., and Guillemet, F. B. 1995. Infection court and factors affecting the expansion of stem canker of avocado caused by *Phytophthora citricola*. Plant Dis. 79:384-388.
14. Erwin, D. C. 1983. Viability within and among species of Phytophthora. Pages 149-165 in: Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
15. Farih, A., Menge, J. A., Tsao, P. H., and Ohr, H. D. 1981. Metalaxyl and efosite aluminum for control of *Phytophthora gummosis* and root rot on citrus. Plant Dis. 65:654-657.
16. Farih, A., Tsao, P. H., and Menge, J. A. 1981. Fungitoxic activity of efosite aluminum on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71:934-936.
17. Fawcett, H. S. 1916. A bark disease of avocado trees. Calif. Avocado Assoc. Annu. Rep. 1916:152-154.
18. Fenn, M. E., and Coffey, M. D. 1984. Studies on the in vitro and in vivo antifungal activity of fosetyl-Al and phosphorus acid. Phytopathology 74:606-611.
19. Griffith, J. M., Davis, A. J., and Grant, B. R. 1992. Target sites of fungicides to control Oomycetes. Pages 69-100 in: Target Sites of Fungicide Action. W. Koller, ed. Chem. Rubber Co. Press, Boca Raton, FL.
20. Guest, D., and Grant, B. 1991. The complex action of phosphonates as antifungal agents. Biol. Rev. 66:159-187.
21. Henderson, I. K., Saari-Nordhaus, R., and Anderson, J. M. 1991. Sample preparation for ion chromatography by solid-phase extraction. J. Chromatogr. 546:61-71.
22. Laville, E. Y., and Chalandon, A. J. 1981. Control of *Phytophthora gummosis* in citrus with foliar sprays of fosetyl-Al, a new systemic fungicide. Proc. Intl. Soc. Citricult. 1:346-349.
23. MacIntire, W. H., Winterberg, S. H., Hardin, L. J., Sterges, A. J., and Clements, L. B. 1950. Fertilizer evaluation of certain phosphorus, phosphorous, and phosphoric materials by means of pot cultures. Agron. J. 42:543-549.
24. Matheron, M. E., and Matejka, J. C. 1988. Persistence of systemic activity for fungicides applied to citrus trunks to control *Phytophthora gummosis*. Plant Dis. 72:170-174.
25. Matkin, O. A., and Chandler, P. A. 1957. The

U.C.-type soil mixes. Pages 68-85 in: The U.C. System for Producing Healthy Container-Grown Plants. K. F. Baker, ed. Calif. Agric. Exp. Stn., Extension Service, Berkeley, CA.
26. Menge, J. A. 1986. Use of new systemic fungicides on citrus. Citrograph 65:245-252.
27. Mitchell, D. J., Kannwischer-Mitchell, M. E., and Zentmyer, G. A. 1986. Isolating, identifying, and producing inoculum of *Phytophthora* spp. Pages 63-66 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
28. Ouimette, D. G., and Coffey, M. D. 1988. Quantitative analysis of organic phosphonates, phosphonate, and other inorganic anions in plants and soil by using high-performance ion chromatography. Phytopathology 78:1150-1155.
29. Ouimette, D. G., and Coffey, M. D. 1989. Comparative antifungal activity of four phosphonate compounds against isolates of nine *Phytophthora* species. Phytopathology 79:761-767.
30. Ouimette, D. G., and Coffey, M. D. 1990. Symplastic entry and phloem translocation of phosphonate. Pestic. Biochem. Physiol. 38:18-25.
31. Pegg, K., Whiley, A. W., Saranah, J. B., and Glass, R. J. 1985. Control of Phytophthora root rot of avocado with phosphorous acid. Australas. Plant Pathol. 14:25-29.
32. Pegg, K. W., Langdon, P. W., and Saranah, J. B. 1987. Comparison of phosetyl-Al, phosphorous acid and metalaxyl for the long-term control of *Phytophthora* root rot of avocado. Aust. J. Exp. Agric. 27:471-474.
33. Rohrbach, K. G., and Schenck, S. 1985. Control of pineapple heart rot, caused by *Phytophthora parasitica* and *P. cinnamomi*, with metalaxyl, fosetyl Al and phosphorous acid. Plant Dis. 69:320-323.
34. Sandler, H. A., Timmer, L. W., and Graham, J. H. 1986. Timing of application of metalaxyl and fosetyl-aluminum for control of Phytophthora foot rot. Proc. Fla. State Hortic. Soc. 99:57-59.
35. SAS Institute. 1989. SAS/STAT User's Guide. Version 6.0, 4th ed. SAS Institute, Cary, NC.
36. Schutte, G. C., Bezuidenhout, J. J., and Kotze, J. M. 1991. Timing of application methods as determined by means of gas-liquid-chromatography for Phytophthora root rot control of citrus. Phytophylactica 23:69-71.
37. Stamps, D. J., Waterhouse, G. M., Newhook, F. J., and Hall, G. S. 1990. Revised tabular key to the species of *Phytophthora*. Mycological Papers 162. C.M.I., Kew, England.
38. Tuite, J., ed. 1969. Plant Physiological Methods, Fungi and Bacteria. Burgess Publishing., Minneapolis, MN.
39. Williams, D. J., Beach, B. G. W., Horriere, D., and Marechal, G. 1977. LS74-783; a new systemic fungicide with activity against Phycomycete diseases. Pages 565-573 in: Proc. Br. Crop Prot. Conf., Brighton, England.
40. Zentmyer, G. A., Jefferson, L., and Hickman, C. J. 1973. Another species of *Phytophthora* on avocado in California. Calif. Avocado Soc. Yearbook 56:125-129.
41. Zentmyer, G. A., Jefferson, L., Hickman, C. J., and Chang-Ho, Y. 1974. Studies of *Phytophthora citricola*, isolated from *Persea americana*. Mycologia 66:830-845.