

Phosphonate Fungicides for Control of Diseases Caused by *Phytophthora*

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ABSTRACT

In greenhouse experiments, seedlings of *Persea indica*, an avocado relative, planted into soil naturally infested with *Phytophthora cinnamomi* did not develop root rot when treated with a foliar spray or soil drench of potassium phosphonate or fosetyl-aluminum (trade name = Aliette®). Potassium phosphonate was prepared by adding potassium hydroxide to phosphorous acid in water. Potassium phosphonate and fosetyl-aluminum also controlled stem canker of bare-rooted seedlings of *P. indica* caused by *P. citricola*. Phosphonate incorporated into culture media was more inhibitory toward growth of *Phytophthora* species than was fosetyl-aluminum. Two days after treating plant tissue with fosetyl-aluminum, more than 95% of the parent compound was degraded to phosphonate. Concentrations of phosphonate in plants treated with fosetyl-aluminum or with potassium phosphonate were sufficiently high to directly inhibit growth of *Phytophthora*. In the laboratory, use of chemical mutagenesis procedures led to the development of pathogenic isolates of *Phytophthora* which were resistant to high concentrations of phosphonate. In the field, a breakdown in control by fosetyl-aluminum of root rot, caused by *P. cinnamomi*, has been recorded in France. The risk of this occurring with avocados under California conditions needs to be assessed if these valuable fungicides are to be used intensively and over extended periods of time.

INTRODUCTION

In the late 1970s, Rhone-Poulenc Agrochimie in France introduced the newly discovered phosphonate fungicides for control of downy mildews and diseases caused by species of *Phytophthora* (2). The commercial product, which is marketed under the trade name Aliette®, is an aluminum salt of tris-O-ethyl phosphonate. The common chemical name for Aliette® is fosetyl-aluminum (fosetyl-Al). The discovery of the unique downward systemic transport properties of these phosphonate fungicides was of great significance for the control of soil-borne diseases caused by *Phytophthora*. Fosetyl-Al is the only commercially available fungicide known to be systemically transported in a downward direction in plants. This property permits the fungicide to be applied to foliage or tree trunks in order to control root diseases caused by *Phytophthora*.

The fosetyl-Al story became even more intriguing when beginning in 1979, published reports stated that fosetyl-Al did not significantly inhibit mycelial growth of *Phytophthora* in culture, even though diseases caused by *Phytophthora* were controlled with fosetyl-Al (3, 8). Researchers then focused their efforts on demonstrating that the mode of action of fosetyl-Al involved a stimulation of natural disease resistance mechanisms in plants (3, 6, 8). It was reported that the principal breakdown product of fosetyl-Al was phosphonate, which was thought to be inactive against growth of *Phytophthora*, although no specific data on its antifungal activity had been published (8).

It should be noted that at physiological pH values, such as those that occur in plant tissue, phosphorous acid is actually a phosphonate salt and is therefore most accurately referred to as phosphonate. The negatively-charged phosphonate ion is counter-balanced by a positively charged ion such as potassium, sodium, calcium, or magnesium. In this study phosphonate refers to the potassium salt derivative of phosphorous acid.

This report describes experiments investigating the in vitro and in vivo efficacy of phosphonate and fosetyl-Al. Evidence is presented that phosphonate, which is the major breakdown product of fosetyl-Al in plants and soil, is the primary active ingredient inhibiting growth of *Phytophthora* in plants treated with either fosetyl-Al or potassium phosphonate.

MATERIALS AND METHODS

Terminology of phosphonate compounds

Aliette ® is the trade name for the commercial formulation of the generic compound fosetyl-aluminum, which in chemical terms is the aluminum salt of tris-O-ethyl phosphonate. In plants and in soil, the ethyl group of fosetyl-Al is cleaved leaving phosphonate as the primary degradation product. In this study, aqueous solutions of fosetyl-Al or of phosphorous acid (phosphonate) were adjusted to a pH of 6.2-6.5 by addition of potassium hydroxide (KOH). Adding KOH to a solution of phosphorous acid leads to formation of a mixture of monobasic and dibasic potassium phosphonate.

Control of Diseases Caused by *Phytophthora cinnamomi* and *P. citricola*

The efficacy of fosetyl-Al and potassium phosphonate for control of root rot of *Persea indica*, an avocado relative extremely susceptible to the disease, was tested by planting nine-week-old seedlings in soil obtained from an avocado grove infested with *P. cinnamomi*. In the control treatment, the soil was treated with aerated steam for 1 hour at 60 C to destroy propagules of *P. cinnamomi*. One day after transplanting, the plants were given a foliar spray until runoff or a 25-ml soil drench of 1.5 g/L of fosetyl-Al or of 1.0 g/L of potassium phosphonate. Treatments were repeated 7 days later, and a third foliar spray was applied 8 days after transplanting. Six weeks after transplanting, the increases in plant height and dry weight of shoots and roots were measured, and the extent of root rot assessed visually.

Bare root seedlings of *P. indica* were placed in styrofoam cups containing 650 ml of distilled deionized water and 2 x 1Q4 motile zoospores of *P. citricola*. One day later, the seedlings were transferred to solutions of potassium phosphonate or fosetyl-Al. Five days later, the stems were cut into 0.7-cm-long sections, surface sterilized, and plated

onto an agar medium containing a mixture of antibiotics selective for *Phytophthora*. On the three following days, stem pieces from which *Phytophthora* was recovered were marked, and the percentage of stem pieces infected was calculated.

Antifungal Activity of Phosphonate and Ethyl Phosphonate

Potassium phosphonate was added to cornmeal agar at various concentrations in order to determine the effect of phosphonate on mycelial growth of species of *Phytophthora* and fungi from other taxonomic groups. A 0.5-cm-diameter agar disk, taken from an actively growing fungal colony on agar without fungicide, was placed fungal side downward in the center of each plate. Plates were incubated in the dark at 24 C for 5-6 days. Radial growth was then determined by measuring colony diameters at two points on each petri plate.

Potassium phosphonate was added to Ribeiro's synthetic liquid medium. Three different concentrations of phosphate were used in preparing the liquid medium in order to determine the effect of phosphate on the efficacy of phosphonate.

Quantification of Phosphate, Phosphonate, and Ethyl Phosphonate in Plant Tissue

Concentrations of phosphate, phosphonate, and ethyl phosphonate in extracts of tomato leaflets floating on solutions of fosetyl-Al were determined by high performance ion chromatography (HPIC) using a Dionex ion chromatograph according to the method of Ouimette and Coffey (5).

Development of Phosphonate-Resistant Isolates of *Phytophthora*

Isolates of *P. capsici* and *P. parasitica* resistant to high concentrations of phosphonate were obtained by treating zoospores with 30 µg/ml of the chemical mutagen N-methyl-N'-nitro-N-nitrosoguanidine and overlaying with media containing potassium phosphonate (4). Isolates which grew through the agar overlay containing potassium phosphonate were sub-cultured and further selected for normal growth characteristics and pathogenicity.

RESULTS AND DISCUSSION

Potassium phosphonate was as effective as fosetyl-Al for the control of root rot of the avocado relative *P. indica* (Table 1). Both compounds were also equally effective in the control of stem canker of *P. indica* caused by *Phytophthora citricola* (Fig. 1). Sixty-nine µg/ml of potassium phosphonate incorporated into cornmeal agar completely inhibited mycelial growth of *P. citricola* and *P. cinnamomi*, but even higher concentrations had little effect on growth of unrelated fungi (Table 2). These results and other reports on the spectrum of activity of fosetyl-Al demonstrate that phosphonate fungicides are selectively active against *Phytophthora* and related fungi (2, 4).

Tomato leaflets floated on solutions of fosetyl-Al for 2 days contained very little ethyl phosphonate. More than 95% of the parent compound had been degraded to phosphonate (Table 3). These results indicate that phosphonate is the predominant antifungal compound responsible for control of *Phytophthora* in plants treated with fosetyl-Al.

In our experiments, increasing the phosphate concentration of a defined synthetic liquid

medium 100-fold from 0.084 mM to 8.4 mM phosphate did not affect the efficacy of potassium phosphonate against *P. citricola* and only caused a 15% reduction in inhibition with *P. cinnamomi*. These results suggest that differing phosphate levels present in avocado tissues are unlikely to affect the disease control efficacy of phosphonate fungicides.

Media commonly used for culturing *Phytophthora* contain phosphate levels inhibitory to the antifungal activity of fosetyl-Al. For example, V8 juice agar contained 1.38 mM phosphate, a level which severely reduces the antifungal activity of fosetyl-Al. In earlier studies, investigators found that fosetyl-Al was inactive against *Phytophthora* in culture because the phosphate levels of the culture media employed interfered with the activity of fosetyl-Al. Of more significance is the fact that in plants and soil, fosetyl-Al degrades to phosphonate which is much less inhibited by phosphate.

Isolates of *P. capsici* and *P. parasitica* resistant to high concentrations of phosphonate were readily obtained by chemical mutagenesis. Sensitive isolates had EC50 values ranging from less than 50 to 58 µg/ml of phosphonate. The EC50 values for the corresponding resistant mutants ranged from 277-835 µg/ml phosphonate. The phosphonate-sensitive isolate of *P. capsici* was effectively controlled by treatment with fosetyl-Al, whereas the phosphonate-resistant isolate was not controlled even in leaflets containing 367 µg phosphonate per g fresh weight of leaf tissue.

TABLE 1. Control of *Phytophthora cinnamomi* root rot of seedlings of *Persea indica* in the greenhouse with fosetyl-Al and potassium phosphonate^x.

Treatment ^y	Root Dry Weight (g)	Shoot Dry Weight (g)	Shoot Growth (cm)	Percent Healthy Roots ^z
Noninfested steamed soil+H ₂ O	2.81a	7.81a	16.7ab	92.4a
Infested soil + H ₂ O	0.17b	3.02b	1.4c	0.0b
Infested soil + fosetyl-Al-foliar	2.09a	6.13a	16.5ab	81.8a
Infested soil + fosetyl-Al-soil	1.53ab	6.31a	16.1ab	84.2a
Infested soil + HPO ₃ ²⁻ -foliar	2.31a	6.92a	19.9a	84.6a
Infested soil + HPO ₃ ²⁻ -soil	2.31a	7.42a	10.9b	84.4a

^xValues at 6 wk after planting are the means of five replicates. Values with the same letter are not significantly different according to Duncan's multiple range test, P=0.05.

^ySoil drenches were applied 1 and 7 days after transplanting 9-wk-old seedlings into soil

naturally infested with *P. cinnamomi*. Foliar sprays were applied 1, 7, and 8 days after transplanting. Concentrations of the chemicals used were 1.5 g.a.i. of fosetyl-Al and 1.0 g HPO₃ per liter, which are 12.7 meq/L concentrations.

²Based on visual observation of roots rinsed free of soil.

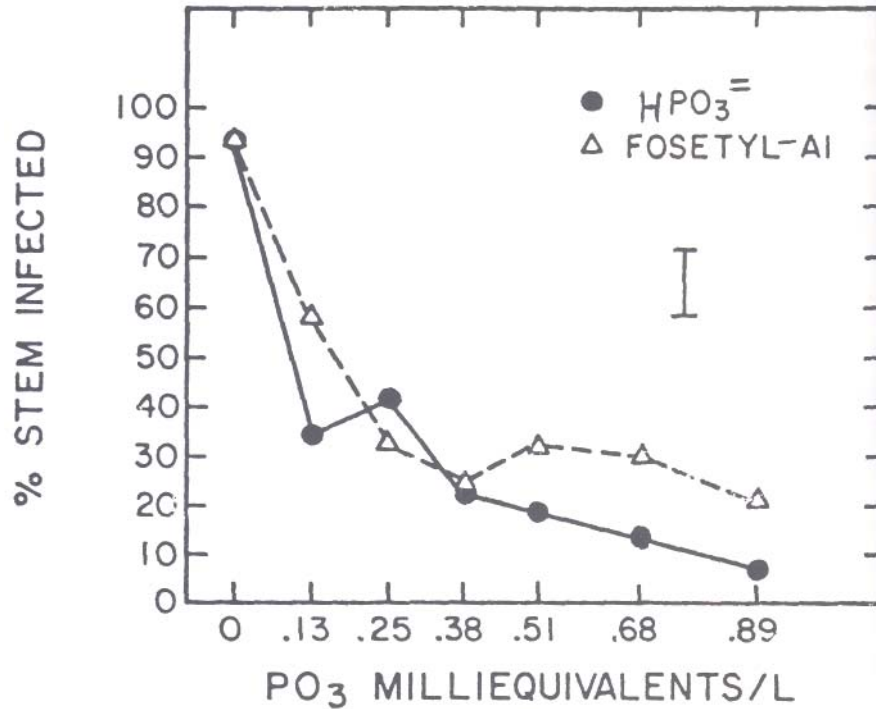


Fig. 1. Percentage of stems of *Persea indica* infected by *Phytophthora citricola*, isolate P1273, as a function of the PO₃ meq concentration of HPO₃ or fosetyl-Al. Seedlings were immersed in either HPO₃ or fosetyl-Al 27 hr after inoculation with 2×10^4 zoospores per plant. Vertical bar represents LSD (P=0.05).

In 1985, there was a report from a nursery near Paris, France, of a naturally-occurring isolate of *P. cinnamomi* which was no longer controlled effectively by treatment with fosetyl-Al (7). The development of phosphonate-resistant and pathogenic isolates of *Phytophthora* in the laboratory, and the loss of control that occurred with *P. cinnamomi* in a nursery, indicate that there is a potential for appearance of resistant isolates of *Phytophthora* in the field if sufficient selection pressure is applied via heavy use of phosphonate fungicides. This risk needs investigating in depth to avoid failure of control once these fungicides become widely used in the avocado industry.

In summary, potassium phosphonate was as effective as fosetyl-Al in controlling both root rot and stem canker of *P. indica*, an avocado relative, in greenhouse and laboratory experiments. Phosphonate is the predominant active compound which inhibits *Phytophthora* in plants treated with either fosetyl-Al or potassium phosphonate. The development of phosphonate-resistant isolates of *Phytophthora* in the laboratory, and the discovery of a naturally-occurring tolerant isolate of *P. cinnamomi* (7) in a French nursery, suggest the potential for phosphonate resistance occurring in situations where these fungicides are used excessively. Research into the risk of this occurring in avocado groves should be an important priority. Reducing the risk by using resistant rootstocks and developing alternative control methods (e.g., biological control)

represents the best approach for maintaining the long-term efficacy of these unique fungicides (1).

TABLE 2. Percentage growth inhibition of various fungi on cornmeal agar at four concentrations of phosphonate.

Fungus	Percentage inhibition of radial growth ^z at HPO ₃ ⁻ concentrations (µg/ml) ³ of:			
	69	276	414	552
<i>Phytophthora citricola</i> (P1273)	100a	100a	100a	100a
<i>Phytophthora cinnamomi</i> (Pc356)	100a	100a	100a	100a
<i>Phytophthora megasperma</i> f. sp. <i>medicaginis</i> (P1057)	45c	96ab	98a	100a
<i>Phytophthora megasperma</i> f. sp. <i>medicaginis</i> (P1253)	49c	92b	99a	100a
<i>Phytophthora cactorum</i> (P1235)	60b	80c	89b	99a
<i>Alternaria alternata</i>	14d	24d	40c	59b
<i>Rhizoctonia solani</i>	8e	18e	29d	38c
<i>Thielaviopsis basicola</i>	6ef	9f	10e	10e
<i>Phomopsis viticola</i>	2fg	11f	12e	14e
<i>Neurospora tetrasperma</i>	1fg	8fg	27d	27d
<i>Colletotrichum phonioides</i>	0g	4g	7f	10e

^zPercentage based on colony growth on cornmeal agar without HPO₃. Values are means of five replications. Letters compare means of the various fungi at each HPO₃ concentration separately (within a column). Means with the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

TABLE 3. Phosphonate (HPO_3^-) and ethyl phosphonate concentration of detached tomato leaflets floating on solutions containing fosetyl-Al as determined by high performance ion chromatography (HPIC)^x.

Fosetyl-Al concentration of floating solution ($\mu\text{g/ml}$)	HPO_3^- concentration in leaflets ($\mu\text{g/g fr. wt.}$)	ethyl phosphonate concentration in leaflets ($\mu\text{g/g fr. wt.}$)	Lesion length (cm) ^z	
			P1319 sensitive isolate	P1361 resistant isolate
0	0	0.0	4.1	4.6
80	49	0.5	1.4	4.2
160	88	3.0	0.4	4.4
240	181	4.0	0.4	3.5
320	222	10.0	0.5	3.6
400	358	14.0	0.4	3.6

^xPhosphate content of leaflets was 8.9 mM as determined by HPIC.

^yUninoculated leaflets were floated on water or a solution containing 80-400 $\mu\text{g/ml}$ fosetyl-Al for 48 hr prior to extraction of HPO_3^- and ethyl phosphonate.

^zLesion length data are from the same experiment in which, following 24 hr of uptake, leaflets were inoculated with *Phytophthora capsici* and floated on the respective solutions for 4 additional days.

A final word of caution is necessary regarding the use of phosphonate as a fungicide. At present, phosphonate is not registered in the state of California for fungicide usage. Aliette® (fosetyl-Al) is registered for use on non-bearing avocado trees. Aliette® is currently undergoing the registration process for use as a trunk injection and foliar spray on bearing avocado trees.

REFERENCES

1. Coffey, M.D. 1987. *Phytophthora* root rot of avocado—an integrated approach to control in California. Plant Disease 71:1046-1052. Also, this Yearbook.
2. Cohen, Y., and Coffey, M.D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.
3. Durand, M.C., and Salle, G. 1981. Effet du tris-0-ethyl phosphonate d'aluminium sur le couple *Lycopersicum esculentum* Mill.-*Phytophthora capsici* Leon. Etude cytologique et cytochimique. Agronomie 1:723-731.
4. Fenn, M. E. 1986. Evidence for the direct mode of action of fosetyl-Al and

- phosphorous acid. Dissertation. University of California, Riverside, California.
5. Ouimette, D. G., and Coffey, M. D. 1988. Quantitative analysis of organic phosphonates, phosphonate and other inorganic anions in plants and soil using high performance ion chromatography. *Phytopathology* 78: in press.
 6. Raynal, G., Ravise, A. and Bompeix, G. 1980. Action du tris-O-ethyl phosphonate d'aluminium (phosethyl d'aluminium) sur la pathogenic de *Plasmopara viticola* et sur la stimulation des reactions de defense de la vigne. *Ann. Phytopathol.* 12:163-175.
 7. Vegh, I., Leroux, P., LeBerre, A., and Lanen, C. 1985. Detection sur *Chamaecyparis lawsoniana* 'Elwoodii' d'une souche de *Phytophthora cinnamomi*. Rands resistente au phosethyl-Al. *P.H.M.-Rev. Hortic.* 262:19-21.
 8. Vo-Thi, H., Bompeix, G., and Ravise, A. 1979. Role du tris-O-ethyl phosphonate d'aluminium dans la stimulation des reactions de defense des tissus de Tomate contre le *Phytophthora capsici*. *C.R. Acad. Sci. Ser. D288*:1171-1174.