Studies on the In Vitro and In Vivo Antifungal Activity of Fosetyl-Al and Phosphorous Acid

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ABSTRACT

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In a low-phosphate medium fosetyl-Al showed a much higher activity in vitro against *Phytophthora* than previously reported in the literature. Both fosetyl-Al, and more particularly phosphorous acid (H_3PO_3), were highly inhibitory in vitro against several species of *Phytophthora*. Phosphorous acid was much less inhibitory in vitro against *Pythium* and had only low activity against a selection of non-oomycetous fungi. The EC_{50} values for an isolate of *Phytophthora cinnamomi*, cultured on a low phosphate medium, were 0.05 PO_3 meq of H_3PO_3 (4 μg/ml) and 0.45 PO_3 meq of fosetyl-Al per liter (54 μg/ml). An increase in the level of phosphate reduced the inhibition of mycelial growth due to fosetyl-Na, but there was little or no effect of phosphate on the inhibition caused by H_3PO_3. In vivo, either 12.7 PO_3 meq of H_3PO_3 (1.0 g/L) or 12.7 PO_3 meq of fosetyl-Al per liter (1.5 g a.i./L), applied as a foliar spray or soil drench, gave equivalent control of root rot caused in seedlings of *Persea indica* by *Phytophthora cinnamomi*. Compared to fosetyl-Al, H_3PO_3 had a similar, though generally higher efficacy, in reducing stem infection of *Persea indica* seedlings by *Phytophthora citricola*. The EC_{50} values for inhibition of stem infection were 0.09 PO_3 meq of H_3PO_3 (8 μg/ml) and 0.22 PO_3 meq of fosetyl-Al per liter (26 μg a.i./ml).

Additional key words: aluminum tris-*O*-ethyl phosphonate, sodium ethyl phosphonate.

Fosetyl-Al (aluminum tris-*O*-ethyl phosphonate) is the active ingredient of a formulated systemic fungicide, known as Aliette®, developed by Rhône-Poulenc Phytosanitaire in France (28,29,38,40). Fosetyl-Al has high efficacy against some diseases caused by members of the Peronosporales, particularly the downy mildew fungi and several *Phytophthora* species (2,3,6–9,11,14,15, 18,20–24,26–30,32,33,36,38–41). Previous reports have indicated that fosetyl-Al prevented in vitro fungal growth only at concentrations of 1,000 μg/ml or greater (9,12,29–31,34,36,37,39). Since fosetyl-Al was found to have low activity against mycelial growth in vitro, it has been proposed that rather than exerting a direct effect on the pathogen it may act indirectly by triggering a host resistance response (4,10,13,19,24,29,35,37,39,40). Fosetyl-Al has been reported to cause a stimulation of host defense responses in detached tomato leaves infected with *Phytophthora capsici* (4,10,13,19,35,37) and in detached grape leaves infected with *Plasmopara viticola* (19,24).

Fosetyl-Al is degraded to H_3PO_3 in plant tissue (4,35,37,38). In this paper we report on the in vitro and in vivo activity of fosetyl-Al and H_3PO_3. The influence of the phosphate level of the culture medium on the in vitro activity of fosetyl-Al, fosetyl-Na, and H_3PO_3 is also investigated.

MATERIALS AND METHODS

Phytophthora root rot control with fosetyl-Al and H_3PO_3. Nine-week-old *Persea indica* seedlings growing in UC mix (50% blow sand, 50% peat moss, plus 2.2 kg dolomite, 1.5 kg superphosphate, 148 g KNO_3, and 148 g K_2SO_4 per cubic meter (1) in 6-cm-diameter peat pots were transplanted into 3.8-L metal containers with soil naturally infested with *P. cinnamomi* from an avocado grove on the campus at the University of California, Riverside (UCR). In the control treatment the soil was treated with aerated steam for 1 hr at 60 C to destroy propagules of *Phytophthora*. On the day following transplanting, one set of the plants was given a foliar spray until run-off, with either distilled water or aqueous solutions of fosetyl-Al (1.5 g a.i./L) or H_3PO_3 (1.0 g/L). At these concentrations fosetyl-Al and H_3PO_3 contained 12.7 PO_3 meq/L. The concentration of fosetyl-Al needed to give consistent control of root rot was determined in preliminary greenhouse experiments. Paper towels were used to cover the soil to prevent any run-off solution from contacting the soil. The same solutions were applied as 25-ml soil drenches to each pot. The foliar spray and soil drench treatments were repeated at 7 days, and at 8 days a third foliar spray was applied. Treatments were replicated five times. All solutions contained 0.027 M MES buffer (2-[*N*-morpholino]ethane sulfonic acid) adjusted to a pH of 6.2. Plants were grown in the greenhouse and watered with tap water and dilute Hoagland's solution.

Six weeks after transplanting, the increase in plant height was measured, the soil was washed from the roots, and the extent of root rot assessed visually. Roots and shoots were placed in separate paper bags, and dried at 65 C for 2 days to obtain their dry weights.

Control of stem infection by *P. indica* by *Phytophthora citricola* with fosetyl-Al and H_3PO_3. Nine-week-old *P. indica* seedlings were placed in 946-ml styrofoam cups containing 650 ml of distilled deionized water, to which were added 2×10^4 motile zoospores per plant of *P. citricola*, isolate P1273 from the *Phytophthora* collection at UCR. Twenty-seven hours after inoculation, seedlings of *P. indica* were transferred to solutions containing different concentrations of H_3PO_3 or fosetyl-Al in 0.03 M MES buffer at pH 6.2. Five days later, the stems were cut into 12–14 pieces ~ 0.7 cm in length, the number of pieces depending upon stem length, dipped in 70% ethanol, blotted, and plated onto PARP medium (17) modified by the substitution of 125 μg/ml ampicillin trihydrate (85%, Bristol Laboratories, Syracuse, NY 13201) for 250 μg sodium ampicillin per milliliter. On the first, second, and third day after plating, the stem pieces from which *Phytophthora* was recovered were marked and the percentage of stem pieces infected was calculated. There were six replicates of each treatment.

Expression of PO_3 concentrations. To avoid confusion, the concentration of all PO_3-containing compounds (H_3PO_3, fosetyl-Na, and fosetyl-Al) are expressed as either PO_3 meq/L or μg/ml. The former expression allows for a comparison of compounds on the basis of their PO_3 content. Fosetyl-Na and H_3PO_3 have one PO_3 group per molecule, while fosetyl-Al has three PO_3 groups due to the trivalency of aluminum. Values expressed as PO_3 meq/L can be converted to micrograms per milliliter by multiplication with the

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following conversion factors: 82 (for H_3PO_3), 118 (for fosetyl-Al), or 132 (for fosetyl-Na).

Growth in liquid culture. *Phytophthora cinnamomi* (isolate Pc356) and *P. citricola* (isolate P1273), both from the UCR *Phytophthora* collection, were cultured in Ribeiro's synthetic liquid medium (25), modified as outlined below. The glucose concentration was 9.0 g/L, β-sitosterol was omitted, and KH_2PO_4 was added at concentrations of either 0.084, 0.84, or 8.4 mM. At each of the three phosphate levels the effect of either 0.57 PO_3 meq of H_3PO_3 (47 μg/ml) or 1.0 PO_3 meq of fosetyl-Na per liter (132 μg/ml) was tested. MES buffer was used at a final concentration of 0.03 M and the pH adjusted to 6.2. Each treatment was in triplicate and the experiment was repeated once. Fifty milliliters of liquid medium was added to 250-ml Erlenmeyer flasks, stoppered with cotton plugs, and autoclaved for 20 min at 1.05 kg cm^2 (15 psi) and 121 C. After cooling to room temperature, 0.05 ml of a 1 mg of thiamine HCl solution per milliliter, sterilized by filtration through a 0.22-μm Millipore filter, was added aseptically to each flask containing 50 ml of medium. Inoculum was prepared by culturing the fungus on a solid medium containing 0.5% Difco cornmeal agar (CMA) and 1.3% Difco Noble agar. Two 0.5-cm-diameter agar disks from the margins of the fungal colony were added to 50 ml of Ribeiro's modified liquid medium containing 0.084 mM KH_2PO_4. After 6 days of stationary culture in the dark at 24 C the culture was minced in a Sorvall Omni-Mixer for 20 sec at medium speed. One milliliter of this minced mycelium was added to each 250-ml flask containing 50 ml of medium. The cultures were incubated in the dark without shaking at 24 C for 6 days. The cultures were then filtered through 2.5-cm-diameter Whatman glass fiber filter disks, oven dried, and weighed. Mycelial dry weights were calculated by subtracting the filter disk dry weight from the dry weight of the filter plus mycelium.

Fosetyl-Na was used instead of fosetyl-Al in liquid culture experiments, since use of the latter caused the formation of a gelatinous precipitate, presumably aluminum hydroxide (16).

Growth on solid media. Fungi from different taxonomic groups were grown in the presence of either H_3PO_3 or fosetyl-Al on Ribeiro's modified synthetic agar medium (RMSM) or on CMA. MES buffer, adjusted to pH 6.2, was added to the CMA. In some experiments with H_3PO_3, a comparison was made of the effects of 0.084, 0.84, or 8.4 mM KH_2PO_4 on fungal inhibition. RMSM was prepared with 15 g/L of Difco purified agar and the pH was adjusted to 6.1. After autoclaving, thiamine HCl was added (1 mg/L), and 15 ml of medium was dispensed into 8.5-cm-diameter plastic petri plates. A 0.5-cm-diameter agar disk, taken from an actively growing colony on 0.5% CMA plus 1.3% Noble agar was placed with the fungal side downward in the center of each plate. Plates were incubated in the dark at 24 C. The pH of the RMSM after 8 days of fungal growth ranged from 5.9 to 6.6 in the various treatments. Radial growth was determined by measuring colony diameters at two points on each petri plate and taking the average value, having subtracted the diameter of the fungal plug.

In addition, three isolates of *Phytophthora infestans* (P1293, P1294, and P1297) (from the UCR *Phytophthora* collection) and one isolate of *P. cinnamomi* (Pc356), were tested for sensitivity to H_3PO_3 using rye seed agar medium (RSM) (5). MES buffer was added to give a final concentration of 0.02 M and the pH was adjusted to 6.2. Fifteen milliliters of RSM was dispensed into each 8.5-cm petri plate. Agar disks, 0.5 cm in diameter, from colonies on RSM were placed inverted onto the solidified medium. The isolates of *P. infestans* were incubated at 21 C, while the isolate of *P. cinnamomi* was at 24 C. Radial growth was measured, and treatments were replicated six times.

Calculation of EC_{50} and EC_{90} values. EC_{50} and EC_{90} values for radial growth inhibition due to H_3PO_3 and fosetyl-Al were obtained from regression lines plotting percentage inhibition on a probit scale versus log concentration.

RESULTS

Phytophthora root rot control with fosetyl-Al and H_3PO_3. No significant differences were found in shoot or root dry weights between the noninfested steamed soil treatment and the H_3PO_3 and fosetyl-Al foliar and soil treatments of plants grown in infested soil (Table 1). Foliar or soil drench treatments, with either H_3PO_3 or fosetyl-Al, gave values for shoot growth and percentage healthy roots that were not significantly different from those of the noninfested control treatment. In contrast, plants grown in infested soil, and not treated with either H_3PO_3 or fosetyl-Al, showed very little shoot growth and the roots were completely rotted (Table 1).

Control of stem infection of *P. indica* by *P. citricola* with fosetyl-Al and H_3PO_3. Seedlings of *Persea indica* previously inoculated with zoospores of *P. citricola*, were immersed in buffered solutions of fosetyl-Al and H_3PO_3. In untreated plants, *P. citricola* was often isolated from stem tissue extending from the original soil line to 8–10 cm above the soil line and stem necrosis was extensive. At effective H_3PO_3 and fosetyl-Al levels the pathogen was usually not isolated from stem tissue more than 2 cm above the original soil line and stems did not become necrotic. Compared on a PO_3 meq basis, H_3PO_3 was significantly more effective than fosetyl-Al in preventing stem infection ($P = 0.05$) at three of six concentrations (Fig. 1). However, at 0.25, 0.38, and 0.51 PO_3 meq/L, fosetyl-Al and H_3PO_3 did not significantly differ in the level of control

TABLE 1. Control of *Phytophthora cinnamomi* root rot of seedlings of *Persea indica* in the greenhouse with fosetyl-Al and H_3PO_3[x]

Treatment[y]	Root dry weight (g)	Shoot dry weight (g)	Shoot growth (cm)	Percent healthy roots[z]
Noninfested steamed soil + H_2O	2.81 a	7.81 a	16.7 ab	92.4 a
Infested soil + H_2O	0.17 b	3.02 b	1.4 c	0.0 b
Infested soil + fosetyl-Al-foliar	2.09 a	6.13 a	16.5 ab	81.8 a
Infested soil + fosetyl-Al-soil	1.53 ab	6.31 a	16.1 ab	84.2 a
Infested soil + H_3PO_3-foliar	2.31 a	6.92 a	19.9 a	84.6 a
Infested soil + H_3PO_3-soil	2.31 a	7.42 a	10.9 b	84.4 a

[x]Values 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, $P = 0.05$.

[y]Soil 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 H_3PO_3 per liter, which are 12.7 PO_3 meq/L concentrations.

[z]Based on visual observation of roots rinsed free of soil.

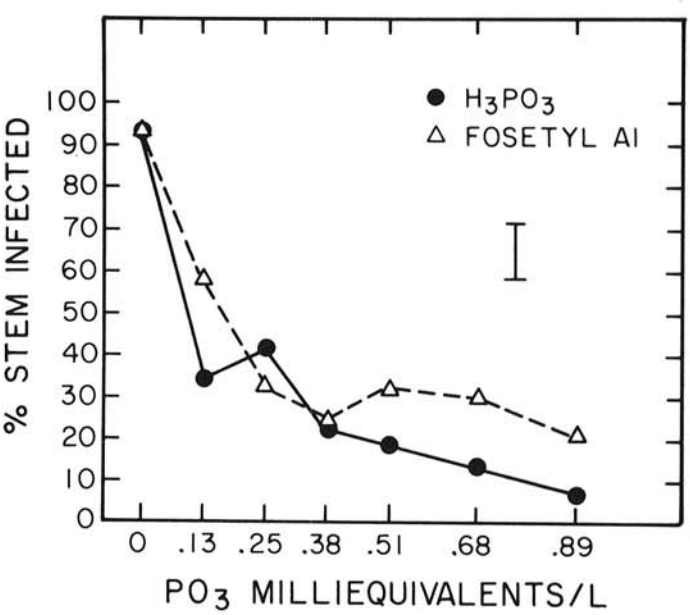

Fig. 1. Percentage of stems of *Persea indica* infected by *Phytophthora citricola*, isolate P1273, as a function of the PO_3 meq concentration of H_3PO_3 or fosetyl-Al. Seedlings were immersed in either H_3PO_3 or fosetyl-Al 27 hr after inoculation with 2×10^4 zoospores per plant. Vertical bar represents LSD ($P = 0.05$).

achieved. The EC_{50} values for inhibition of stem infection were 0.09 PO_3 meq/L (8 μg/ml) for H_3PO_3 and 0.22 PO_3 meq/L (26 μg/ml) for fosetyl-Al.

Growth in liquid culture. In liquid culture, 47 μg/ml H_3PO_3 (0.57 PO_3 meq/L) caused 82–84% growth inhibition of *P. citricola*, isolate P1273, grown in media containing either 0.084, 0.84, or 8.4 mM KH_2PO_4 (Fig. 2). *P. cinnamomi* was less inhibited by H_3PO_3 than was *P. citricola* (Fig. 2). With *P. cinnamomi*, increasing the KH_2PO_4 concentration from 0.084 mM to 8.4 mM, in the presence of 0.57 mM H_3PO_3, caused a small, but significant, decrease in percent growth inhibition from 67 to 52%.

Fosetyl-Na, at 1.0 PO_3 meq/L, was less inhibitory to the growth of both *P. cinnamomi* and *P. citricola* than was H_3PO_3 at 0.57 PO_3 meq/L (Figs. 2 and 3). Fosetyl-Na caused a 16–54% growth inhibition of *P. citricola*, and a 12–58% growth inhibition of *P. cinnamomi*, the extent of the inhibition depending on the phosphate concentration of the medium (Fig. 3). Increasing the KH_2PO_4 concentration tenfold from 0.084 mM to 0.84 mM resulted in a 38 or 46% reduction in inhibition due to fosetyl-Na, with *P. citricola* or *P. cinnamomi*, respectively. Of the three KH_2PO_4 concentrations used, 0.84 mM was optimal for growth of *P. cinnamomi* and *P. citricola* in liquid culture. Increasing the KH_2PO_4 concentration to 8.4 mM resulted in a 5% decrease in growth with *P. citricola* and an 11% decrease with *P. cinnamomi*.

Growth on solid media. Table 2 compares the in vitro antifungal activity of H_3PO_3 and fosetyl-Al towards mycelial growth of *P. capsici*, isolates P1091 and P1314, and *P. cinnamomi*, isolate Pc356, cultured on RMSM containing 0.084 mM KH_2PO_4. The EC_{50} values for growth inhibition ranged from 2.5 μg/ml to 5.4 μg/ml with H_3PO_3 and 44.5 μg/ml to 53.7 μg/ml with fosetyl-Al. The EC_{90} values for H_3PO_3 ranged from 39.4 μg/ml to 110.7 μg/ml, and with fosetyl-Al the EC_{90} values were between 170.1 μg/ml and 196.3 μg/ml. Comparing the EC_{50} values for H_3PO_3 and fosetyl-Al on a PO_3 meq basis (one molecule of fosetyl-Al contains three PO_3 groups), H_3PO_3 was from 5.8 to 14.2 times more inhibitory towards mycelial growth of *Phytophthora* in vitro than was fosetyl-Al. The same comparison for the EC_{90} values showed H_3PO_3 to be from 1.2 to 3.0 times more inhibitory to mycelial growth than fosetyl-Al. Figure 4 compares the growth of *P. cinnamomi* isolate Pc356, at a range of H_3PO_3 and fosetyl-Al concentrations after 5 days of culturing on RMSM containing 0.084 mM KH_2PO_4.

Fungi from a broad range of taxonomic groups were tested on

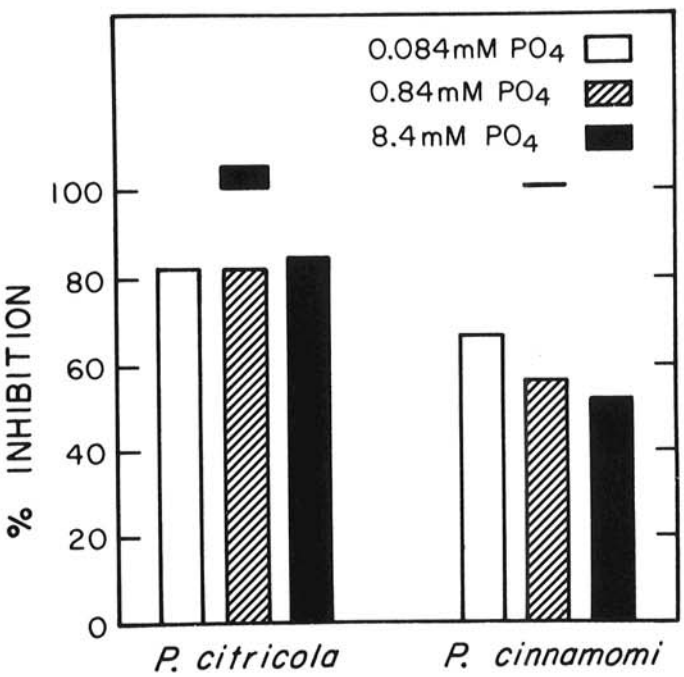

Fig. 2. Percentage growth inhibition of *Phytophthora citricola*, isolate P1273, and *Phytophthora cinnamomi*, isolate Pc356, grown in Ribeiro's modified synthetic liquid medium at three KH_2PO_4 levels in the presence of 0.57 PO_3 meq of H_3PO_3 per liter (47 μg/ml). LSD values ($P = 0.05$) for each fungus are represented by bars located above the histograms.

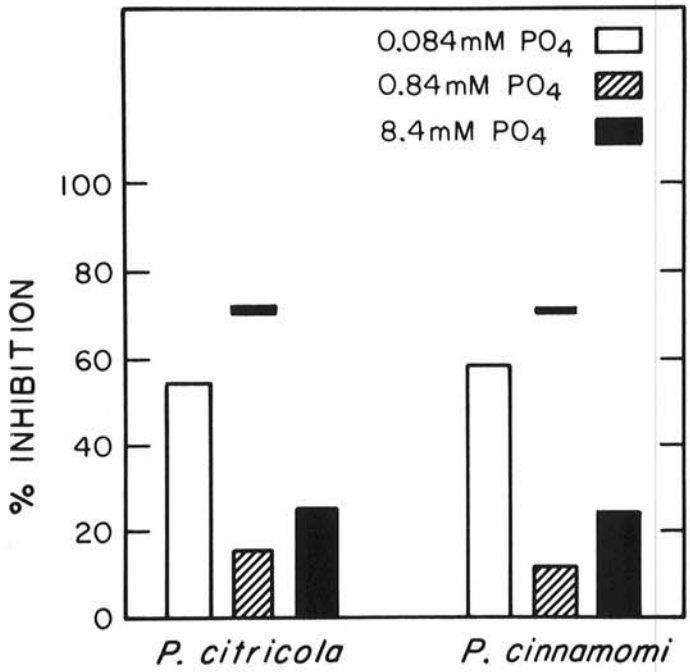

Fig. 3. Percentage growth inhibition of *Phytophthora citricola*, isolate P1273, and *Phytophthora cinnamomi*, isolate Pc356, grown in Ribeiro's modified synthetic liquid medium at three KH_2PO_4 levels in the presence of 1.0 PO_3 meq of fosetyl-Na per liter (132 μg/ml). LSD values ($P = 0.05$) for each fungus are represented by bars located above the histograms.

TABLE 2. Comparison of the in vitro inhibitory activity of fosetyl-Al and H_3PO_3 towards mycelial growth of three fungi[w]

Fungus	H_3PO_3		Fosetyl-Al		EC ratio fosetyl-Al/H_3PO_3[y] (PO_3 meq basis)
	μg/ml	PO_3 meq/L[x]	μg/ml	PO_3 meq/L[x]	
EC_{50}[z]					
P. capsici (P1091)	5.4	0.07	44.5	0.38	5.8
P. capsici (P1314)	2.5	0.03	50.3	0.43	14.2
P. cinnamomi (Pc356)	4.2	0.05	53.7	0.45	8.9
EC_{90}[z]					
P. capsici (P1091)	110.7	1.35	193.1	1.64	1.2
P.capsici (P1314)	55.3	0.67	196.3	1.66	2.5
P. cinnamomi (Pc356)	39.4	0.48	170.1	1.44	3.0

[w] Fungi were grown for five days on Ribeiro's modified synthetic medium containing 0.084 mM KH_2PO_4 and 0.03 M MES buffer at pH 6.2.
[x] One millimole of H_3PO_3 contains one PO_3 meq whereas one millimole of fosetyl-Al contains three PO_3 meq.
[y] The numbers in this column are ratios of EC_{50} or EC_{90} values for fosetyl-Al compared to H_3PO_3 on a PO_3 meq basis.
[z] EC_{50} and EC_{90} values for inhibition of radial growth were obtained from regression line equations for a plot of percent probit inhibition versus log concentration.

RMSM or CMA for inhibition of mycelial growth in the presence of H_3PO_3. On CMA only mycelial growth of the *Phytophthora* species was strongly inhibited by H_3PO_3 (Table 3). Fungi from other taxonomic groups were much less sensitive to H_3PO_3.

At a low KH_2PO_4 concentration (0.084 mM), 0.84 mM H_3PO_3 caused significant inhibition of mycelial growth of most fungi grown on RMSM (Table 4). At the higher KH_2PO_4 levels strong inhibition was only apparent with *P. cinnamomi*, and to a lesser extent with *Pythium aphanidermatum* (Table 4).

Of four *Pythium* species tested in another experiment, *P. myriotylum* was stimulated by both 69 and 138 μg of H_3PO_3 per milliliter, and *P. ultimum* was stimulated by 69 μg of H_3PO_3 per milliliter; while *P. polymorphon* was not affected by 69 μg of H_3PO_3 per milliliter, *P. aphanidermatum* was inhibited 31% by this concentration of H_3PO_3 (Table 5). The two isolates of *Phytophthora capsici* tested were inhibited by 56–66% at 69 μg of H_3PO_3 per milliliter.

P. infestans, grown on RSM, was insensitive to H_3PO_3 compared to *P. cinnamomi* (Fig. 5). The EC_{50} values for the isolates of *P. infestans* ranged from 173 to 221 μg of H_3PO_3 per milliliter, while that for *P. cinnamomi*, isolate Pc356, was 7.3 μg of H_3PO_3 per milliliter.

DISCUSSION

It has been reported that in vitro >1,000 μg of fosetyl-Al per milliliter was required to completely inhibit mycelial growth of *Phytophthora* species (9,12,29–31,34,36,37,39). However, the results of our studies have demonstrated that with a low phosphate medium <200 μg/ml was required for inhibition of mycelial growth. The EC_{50} and EC_{90} values for an isolate of *P. cinnamomi* on RMSM containing 0.084 mM KH_2PO_4 were 54 and 170 μg of fosetyl-Al, respectively, per milliliter.

Previous investigators have considered H_3PO_3 to be essentially nonfungitoxic (4,37). Our experiments have revealed that both in vitro and in vivo, H_3PO_3 had high efficacy against *Phytophthora*. With isolates of *P. capsici* and *P. cinnamomi* the EC_{50} values for inhibition of mycelial growth in vitro were 0.03 to 0.07 PO_3 meq/L (2.5 to 5.4 μg/ml) for H_3PO_3, compared to 0.38 to 0.45 PO_3 meq/L (45 to 54 μg a.i./ml) for fosetyl-Al. In vivo, H_3PO_3 was found to be at least as effective as fosetyl-Al in controlling both root rot caused by *P. cinnamomi* and stem canker caused by *P. citricola* in *Persea indica*. The EC_{50} values for H_3PO_3 and fosetyl-Al for inhibition of

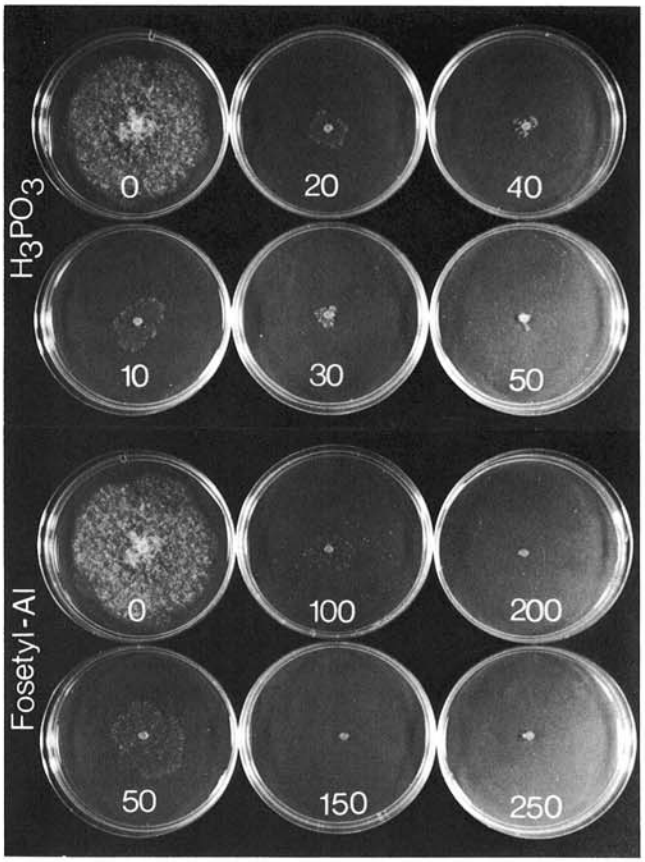

Fig. 4. Colony size of *Phytophthora cinnamomi*, isolate Pc356, after 5 days of growth at 24 C on Ribeiro's synthetic agar medium (RMSM) containing 0.084 mM KH_2PO_4. Numbers are μg of H_3PO_3 or fosetyl-Al per milliliter (×0.29).

TABLE 3. Percentage growth inhibition of various fungi on cornmeal agar at four concentrations of phosphorous acid

Fungus	Percentage inhibition of radial growth[z] at H_3PO_3 concentrations (μg/ml) of: 69	276	414	552
Phytophthora citricola (P1273)	100 a	100 a	100 a	100 a
Phytophthora cinnamomi (Pc356)	100 a	100 a	100 a	100 a
Phytophthora megasperma f. sp. *medicaginis* (P1057)	45 c	96 ab	98 a	100 a
Phytophthora megasperma f. sp. *medicaginis* (P1253)	49 c	92 b	99 a	100 a
Phytophthora cactorum (P1235)	60 b	80 c	89 b	99 a
Alternaria alternata	14 d	24 d	40 c	59 b
Rhizoctonia solani	8 e	18 e	29 d	38 c
Thielaviopsis basicola	6 ef	9 f	10 e	10 e
Phomopsis viticola	2 fg	11 f	12 e	14 e
Neurospora tetrasperma	1 fg	8 fg	27 d	27 d
Colletotrichum phonioides	0 g	4 g	7 f	10 e

[z]Percentage based on colony growth on cornmeal agar without H_3PO_3. Values are means of five replications. Letters compare means of the various fungi at each H_3PO_3 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 4. Percentage growth inhibition of various fungi on Ribeiro's synthetic agar medium (RMSM) containing 0.84 mM H_3PO_3 (69 μg/ml) at three phosphate concentrations

Fungus	Percentage inhibition of radial growth[z] at KH_2PO_4 concentrations (mM) of: 0.084	0.84	8.4
Phytophthora cinnamomi (Pc356)	100 a	93 a	90 a
Pythium aphanidermatum	53 b	56 b	31 b
Rhizopus stolonifer	52 b	30 c	0 c
Fusarium oxysporum f. sp. *apii*	42 b	5 d	1 c
Verticillium dahliae	49 b	0 d	1 c
Schizophyllum commune	38 b	0 e	2 c
Rhizoctonia solani	3 c	0 e	0 c

[z]Percentage based on colony growth on identical medium without H_3PO_3. Values are means of four or five replications. At a particular KH_2PO_4 concentration, values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 5. Percentage growth response of *Pythium* species and *Phytophthora capsici* on cornmeal agar containing phosphorous acid

Fungus	Colony diameter (percentage of control)[z] at H_3PO_3 concentrations (μg/ml) of: 69	138	276	414	552
Pythium myriotylum	191 a	174 a	58 a	47 a	30 a
Pythium polymorphon	100 c	93 b	6 b	10 c	0 c
Pythium aphanidermatum	69 d	51 c	30 a	24 b	17 b
Pythium ultimum	128 b	57 c	0 b	0 c	0 c
Phytophthora capsici (P1314)	34 e	14 d	2 b	0 c	0 c
Phytophthora capsici (P1091)	44 e	8 d	0 b	0 c	0 c

[z]Control was colony diameter on cornmeal agar without H_3PO_3. At a particular H_3PO_3 concentration, values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

infection of *P. indica* by *P. citricola* were 0.09 PO_3 meq/L (8 μg/ml) and 0.22 PO_3 meq/L (26 μg/ml), respectively.

The in vitro antifungal activity of H_3PO_3 was hardly affected by the phosphate concentration in the medium, contrasting with the activity of fosetyl-Na which was greatly reduced by an increase in the phosphate level. Increasing the phosphate concentration 100-fold did not reduce the efficacy of H_3PO_3 (0.57 PO_3 meq/L) against *P. citricola* in liquid culture and only resulted in a 15% reduction of inhibition with *P. cinnamomi*. However, a 10-fold increase in phosphate resulted in a 38 and 46% reduction in inhibition due to fosetyl-Na (1.0 PO_3 meq/L) with *P. citricola* and *P. cinnamomi*, respectively. Similarly, Bompeix et al (4) found phosphate to interfere with the in vivo activity of fosetyl-Al when detached tomato leaves, inoculated with *P. capsici*, were floated on a fosetyl-Al solution containing phosphate buffer. Possibly, the loss of efficacy was due to competition for uptake with phosphate.

In vitro, *P. infestans* was the only *Phytophthora* species tested which was not very sensitive to H_3PO_3. Fosetyl-Al has been reported to be less effective against late blight than some other diseases caused by Peronosporales (3,29,38). The four *Pythium* species were also much less sensitive to H_3PO_3 than the *Phytophthora* species tested, though they were more inhibited than any of the non-oomycetous fungi. The high activity in vitro of H_3PO_3 and fosetyl-Al specifically against *Phytophthora* closely parallels their in vivo behavior, and provides good circumstantial evidence of a direct mode of action against the pathogen.

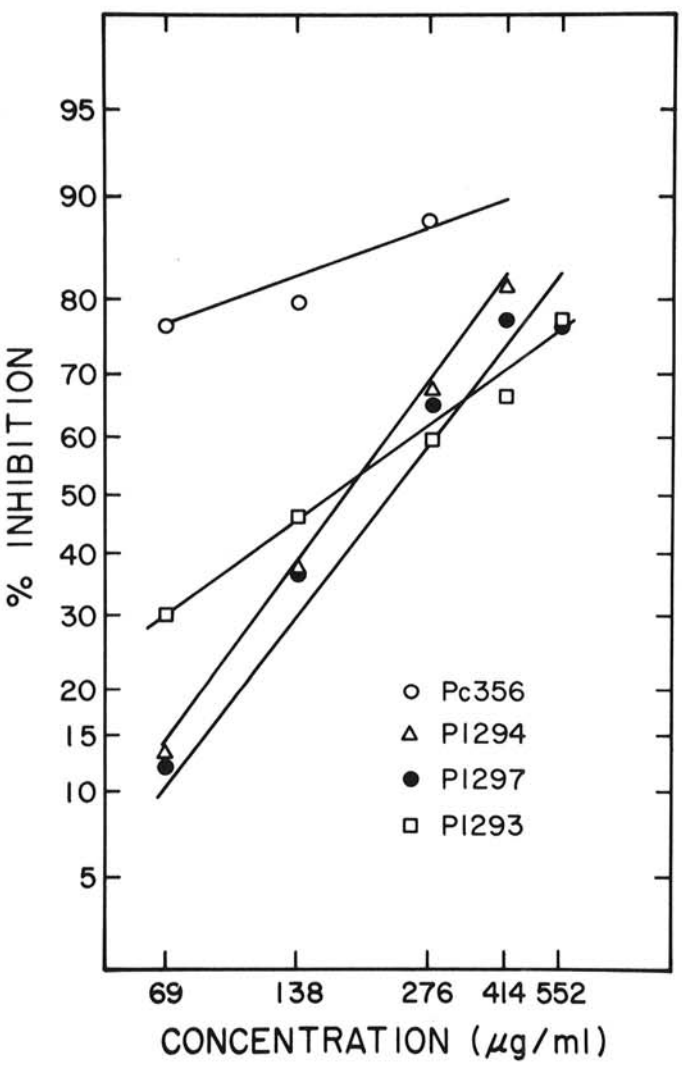

Fig. 5. Dosage response curve for *Phytophthora cinnamomi*, isolate Pc356, and three isolates of *P. infestans* on Rye seed agar medium containing H_3PO_3. Pc356 was cultured at 24 C while the isolates of *P. infestans* were grown at 21 C. Correlation coefficients (r) ranged from 0.91 to 0.98 and were all significantly positive ($P<0.01$).

There was a close correspondence between the in vitro and in vivo EC_{50} values for inhibition by H_3PO_3. In vitro the values for several isolates of *Phytophthora* ranged from 0.03 to 0.07 PO_3 meq/L, while in vivo the value for *P. citricola* was 0.09 PO_3 meq/L. However, with fosetyl-Al, the equivalent in vivo EC_{50} value of 0.22 PO_3 meq/L was lower than the range of in vitro EC_{50} values (0.38 to 0.45 PO_3 meq/L) for several isolates of *Phytophthora*. Phosphorous acid appeared to have very similar activity in vitro and in vivo, while fosetyl-Al, even on a low phosphate medium, had lower efficacy in vitro than in vivo. The higher activity of fosetyl-Al in vivo might be explained in terms of its rapid conversion to phosphorous acid, presumably by plant enzymes. Fosetyl-Al has been reported to degrade to H_3PO_3 in plant tissue (4,35,37,38) but no data has been published concerning the rate of degradation. Williams et al (38) reported that H_3PO_3 was the major residue in plant tissue following foliar or soil application of fosetyl-Al.

It has been reported that detached tomato leaves, inoculated with *P. capsici*, produced more phenolic compounds and formed "necrotic blocking zones" when floated on fosetyl-Al solutions (4,10,13,35,37). In addition, detached grape leaves, inoculated with *Plasmopara viticola*, produced more antifungal stilbenes and flavanoids and had fewer symptoms when treated with fosetyl-Al (19,24). These results were interpreted as being due to stimulation of a host defense response (4,10,24,37). Our results would appear to allow at least a partial reinterpretation of these data based on a directly inhibitory effect of fosetyl-Al or H_3PO_3 on the pathogen. The increase in antifungal compounds observed in grape and tomato could be a secondary host response, following the primary effect of these compounds on the pathogen.

In summary, the spectrum of in vitro biological activity for H_3PO_3 and fosetyl-Al closely parallels the known in vivo behavior of fosetyl-Al. The high efficacy of H_3PO_3 in vitro against *Phytophthora* was an unsuspected finding of this study. The potentiality of using H_3PO_3, rather than fosetyl-Al, as a fungicide for control of plant diseases caused by *Phytophthora* deserves further investigation.

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