Effects of Phosphorous Acid and Fosetyl-Al on the Life Cycle of *Phytophthora cinnamomi* and *P. citricola*

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ABSTRACT

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Mycelial growth of both *Phytophthora cinnamomi* and *P. citricola* was inhibited by low concentrations of phosphorous acid (H_3PO_3). Concentrations required for 50% inhibition (EC_{50}) ranged from 1.3 to 1.7 μg/ml for *P. citricola*, and from 4.1 to 6.2 μg/ml for *P. cinnamomi*. Sporangium formation by *P. citricola* and *P. cinnamomi* also was sensitive; EC_{50} values for H_3PO_3 inhibition were 1.4 and 1.8 μg/ml, respectively. Aluminum, calcium, and sodium tris-*O*-ethyl phosphonates (fosetyl-Al, fosetyl-Ca, fosetyl-Na) were as inhibitory to sporangium development as H_3PO_3. With *P. citricola* and *P. cinnamomi* the EC_{50} for inhibition of zoospore release by H_3PO_3 was 6 μg/ml. Cyst germination was not affected. H_3PO_3 inhibited oospore formation by *P. citricola*; 1 μg/ml caused 89–97% inhibition among different isolates. Oospore production by *P. cinnamomi* was less sensitive; 1 μg/ml caused 60–78% inhibition. The EC_{50} for H_3PO_3 inhibition of chlamydospore production by isolates of *P. cinnamomi* was 15–44 μg/ml. Fosetyl-Al and H_3PO_3, used at equivalent rates of phosphite, were very similar in their effects on chlamydospore formation by *P. cinnamomi* and *P. parasitica*.

Fosetyl-Al (Aliette®; Rhône-Poulenc Sanitaire, Lyon, France) possesses excellent systemic activity against several diseases caused by *Phytophthora* spp. (2,12,16,17,21), including those on citrus and avocados (6–8,19). Because of its phloem mobility, fosetyl-Al can be applied either to foliage (6) or as a trunk injection (7) for control of many soilborne *Phytophthora* spp. that cause plant diseases. Phosphorous acid (H_3PO_3), an important toxophore of fosetyl-Al, is a product of hydrolysis (1,18,20,21). Results of a comparison of the concentrations of chemical necessary to give 50% inhibition of mycelial growth (EC_{50} values) of several *Phytophthora* spp. showed that H_3PO_3 was from six to 14 times more active in vitro than was fosetyl-Al (11).

Information has been obtained on the effect of fosetyl-Al on the life cycle of *Phytophthora citrophthora* Smith and Smith (Leonian) and *Phytophthora parasitica* Dastur (9) but, excepting one abstract (3), no equivalent data exist for *Phytophthora citricola* Sawada. There are no data available on the effects of fosetyl-Al on *Phytophthora cinnamomi* Rands, an important pathogen of avocados in California. In particular, the effect of H_3PO_3 on the different spore stages in the life cycle of *Phytophthora* has not been studied. It has been demonstrated that the mycelial growth of *Phytophthora* spp. is sensitive to H_3PO_3 with EC_{50} values in vitro for inhibition ranging from 5 to 90 μg/ml (4). Since sporulation and spore germination are important in the epidemiology and pathogenicity of such pathogens, knowledge of the effects of these toxophores on these aspects of their life cycles is integral to a proper understanding of their biological mode of action.

In this study we examined the effects of both H_3PO_3 and fosetyl-Al on different phases of the life cycle of *P. cinnamomi* and *P. citricola* that were pathogenic on avocados in California. An isolate of *P. parasitica* was also included to provide a comparison of its chlamydospore production with that of *P. cinnamomi*.

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MATERIALS AND METHODS

Cultures. Cultures of *P. cinnamomi* used in these studies were Pc138 (A1 compatibility type) plus Pc356, Pc402, and Pc407 (A2 types). Three cultures of *P. citricola* were used: P1273, P1277, and P1315. One culture of *P. parasitica* (P1155) from citrus also was used. All cultures except P1155 were originally isolated from avocado (*Persea americana* Mill.). Cultures were maintained in the collection of *Phytophthora* at the University of California, Riverside, and had never been exposed to fosetyl-Al.

Mycelial growth response. Five-millimeter-diameter disks were taken from the margins of 5-day-old colonies of *P. cinnamomi* and *P. citricola* grown on clarified V8-$CaCO_3$ (V8C) agar. The disks were placed centrally on Ribeiro's modified synthetic agar medium (RMS) (15) containing 0.084 mM KH_2PO_4 and no β-sitosterol (11) at pH 6.2. Concentrations of H_3PO_3 (technical grade, 99.69% pure) incorporated into the medium were: 0.1, 0.5, 1.0, 2.5, and 5.0 μg/ml, respectively. Cultures were grown in the dark for 4 days at 24 C. There were three replicate plates of each concentration and the experiment was repeated once. Linear regressions were plotted of the log concentration of H_3PO_3 against percentage inhibition of radial growth compared to the unamended control to interpolate the EC_{50} values for mycelial inhibition of each culture.

Oospore production. Twelve-millimeter-diameter disks were cut with a sterile cork borer from the margins of 4-day-old cultures grown on V8C agar. Sixteen disks of each culture of *P. citricola*, and eight disks each of an A2 culture and A1 (Pc138) culture of *P. cinnamomi*, were blended in 65 ml of sterile distilled and deionized water at high speed for 5 sec (Waring blender). One milliliter of the blended inoculum was pipetted onto the surface of RMS containing 30 mg of β-sitosterol per liter and either 0, 1, 10, 50, or 100 μg/ml H_3PO_3 in 9-cm-diameter plastic petri dishes. There were three replicates of each H_3PO_3 treatment and cultures were incubated at 24 C in the dark for 33 days. The number of oospores produced per plate was determined with a Hawksley eelworm counting chamber. The experiment was repeated once.

Sporangium production. Five-millimeter-diameter disks of *P. cinnamomi* (Pc402) and *P. citricola* (P1273) were cut from the colony margins of 5-day-old cultures grown on V8C agar. Three disks were transferred to each 9-cm-diameter plate containing 20 ml of nonsterile filtered 1% soil extract and H_3PO_3 at 0.5, 2, 3.5, or 5

$\mu g/ml$. Following addition of H_3PO_3, the pH of the soil solution was adjusted to 6.8 using KOH. There were three replicate plates of each treatment. The cultures were incubated under constant illumination at 24 C for 2 days. Nine mycelial mats of each treatment were blended in 10 ml of sterile distilled and deionized water at high speed for 10 sec and sporangium production per milliliter determined for five samples using an eelworm chamber.

In two repeat experiments, three disks were incubated for 2 days in 10 ml of 1/5 diluted V8C broth in 9-cm-diameter plates. The mycelial mats that developed were rinsed twice with sterile distilled and deionized water and overlaid with 20 ml of soil extract and H_3PO_3 at 0.5, 3.5, or 5 $\mu g/ml$ at pH 6.8. Following incubation for 3 days at 24 C, sporangium production was determined.

Sporangium production in different fosetyl salt solutions. Five-millimeter-diameter disks were cut from 5-day-old cultures of *P. cinnamomi* (Pc402) and *P. citricola* (P1273) on V8C agar. The disks were transferred, three per 9-cm-diameter plate, and grown in 15 ml of 1/5 diluted V8C broth for 2 days at 24 C. The mycelial mats formed were rinsed twice with sterile distilled and deionized water and overlaid with soil extract amended with either fosetyl-Al (technical grade, 98% pure), fosetyl-Ca (80% wettable powder), fosetyl-Na (50% aqueous solution), or H_3PO_3 at 2.88, 3.14, 3.23, and 2.00 μg a.i./ml, respectively, all adjusted to pH 6.8 with KOH. The mats were incubated at 24 C for 3 days and sporangium production was determined by using an eelworm chamber as described previously. The experiment was repeated once.

Zoospore release (indirect sporangial germination) in the presence of H_3PO_3. Five-millimeter-diameter disks were cut from the margins of 5-day-old V8C agar cultures of *P. cinnamomi* (Pc402) and *P. citricola* (P1273). For each culture, five disks were placed in each of 15 9-cm-diameter plates containing 15 ml of 1/5 diluted V8C broth. Mycelial mats, produced overnight at 24 C, were washed twice with sterile distilled and deionized water and overlaid with 15 ml of 1% soil extract. After incubation for 5 days, the soil extract was removed and each culture was treated with 10 ml of either 0, 2, or 6 $\mu g/ml$ of H_3PO_3 at pH 6.2. There were five plates each containing five mycelial mats for each treatment. The mycelial mats were incubated overnight at 24 C in the dark. Zoospore discharge from sporangia (indirect germination) was initiated by placing the plates at 4 C for 30 min. The zoospores released were filtered through fluted paper (grade 515; Eaton-Dikeman, Mount Holly Springs, PA) and counted in a hemacytometer. Five samples of each treatment were counted and data calculated as zoospores released per milliliter. The experiment was repeated twice.

Chlamydospore production. Chlamydospore production was initiated by growing 5-mm-diameter disks of *P. cinnamomi* (isolates Pc356, Pc402, and Pc407), three per 9-cm-diameter plate, in 15 ml of half-strength carrot broth (5). Following incubation at 24 C for 48 hr, the mycelial mats produced were rinsed once with sterile distilled and deionized water and overlaid with 15 ml of aqueous solutions containing 0, 1, 5, 10, 50, or 100 $\mu g/ml$ of H_3PO_3 adjusted to pH 6.2 with KOH. There were three replicate plates of each culture at each H_3PO_3 concentration. The mycelial mats were incubated in the dark at 24 C for 35 days to allow chlamydospore production to occur. Chlamydospores were counted by blending the mycelial mats from each H_3PO_3 treatment in 10 ml of sterile distilled and deionized water at high speed for 30 sec. A 1:10 dilution of the suspension was made with sterile distilled and deionized water and the chlamydospores produced were counted in an eelworm chamber.

A comparison also was made of the effects of both fosetyl-Al (technical grade) and H_3PO_3 on chlamydospore production by *P. cinnamomi* (Pc402) and *P. parasitica* (P1155). For each treatment nine 5-mm-diameter disks of either Pc402 or P1155 were placed three per 9-cm-diameter plate, each containing half-strength carrot broth. Following incubation for 48 hr at 24 C, the mycelial mats were rinsed in sterile distilled and deionized water and overlaid with 15 ml of H_3PO_3 solution at either 0, 50, 100, 500, or 1,000 $\mu g/ml$ or alternatively with a solution of fosetyl-Al at 0, 72, 144, 720, or 1,440 $\mu g/ml$. All solutions were made up with sterile distilled and deionized water and adjusted to pH 6.8 with KOH. The mats were incubated at 18 C (the optimum temperature for chlamydospore production by *P. parasitica* [14]) for 26 days. The mycelial mats from each treatment were blended in 50 ml of sterile distilled and deionized water and chlamydospores were counted by using an eelworm chamber. The experiment was repeated once.

Chlamydospore germination. Chlamydospores were produced by growing mycelial mats of *P. cinnamomi* (Pc356, Pc402, and Pc407) on carrot broth for 48 hr at 24 C. These mats were rinsed with sterile distilled and deionized water and incubated, three per 9-cm-diameter plate, in 15 ml of sterile distilled and deionized water in the dark at 24 C for 20 days. Mycelial mats were rinsed with sterile distilled and deionized water, minced for 2 min (1 min at low speed and 1 min at high speed) in a Waring blender, and the chlamydospores were filtered through a nylon mesh with openings of 100 μm. The chlamydospore suspension was centrifuged for 90 sec at 6,000 g, the supernatant was discarded, the spores were resuspended in sterile distilled and deionized water, and the process was repeated. Chlamydospore suspensions, approximately 2,000 spores per milliliter, were streaked, 1 ml per 9-cm-diameter plate, onto RMS amended with H_3PO_3 at either 0, 1, 10, or 50 $\mu g/ml$ at pH 6.2. Three replicate plates per treatment were incubated overnight at 24 C and germination was determined by observing 100 chlamydospores per plate under a dissecting microscope. This experiment was repeated once.

Chlamydospore survival after treatment with either H_3PO_3 or metalaxyl. Three 5-millimeter-diameter disks of *P. cinnamomi*, either Pc402 or Pc407, were placed in each 9-cm-diameter plate, overlaid with 15 ml of half-strength carrot broth and grown for 48 hr at 24 C. The mycelial mats that formed were rinsed with sterile distilled and deionized water and overlaid with 15 ml of sterile distilled and deionized water for 3 wk to allow formation of chlamydospores.

Fungicide treatments consisted of H_3PO_3 at 100 or 1,000 $\mu g/ml$ and metalaxyl at 0.025 or 0.25 $\mu g/ml$, at pH 6.2. The control treatment was sterile distilled and deionized water at pH 6.2. Each plate contained 10 ml of test solution. There were four plates per treatment. The mycelial mats were incubated 24 hr at 24 C, minced in a Waring blender, and filtered through a nylon mesh with openings of 100 μm in a Büchner funnel. The filtrate was centrifuged for 3 min, the pellet was resuspended in sterile distilled and deionized water, and the chlamydospore suspension was streaked across plates of PARP medium (13), modified by substituting 125 $\mu g/ml$ ampicillin trihydrate for ampicillin sodium. Following incubation overnight at 24 C, chlamydospores were observed for germination by using a dissecting microscope. For each treatment, 500 spores were counted; the experiment was repeated once.

Mycelial survival. Five-millimeter-diameter disks of *P. cinnamomi* (Pc402) and *P. citricola* (P1273) were allowed mycelial growth for 24 hr in V8C broth, rinsed with sterile distilled and deionized water, and transferred (three per 9-cm-diameter plate) to solutions of fungicides. The fungicide concentrations for isolate Pc402 were: metalaxyl at 0.06 or 0.6 $\mu g/ml$, and H_3PO_3 at 5 or 50 $\mu g/ml$. For isolate P1273, the concentrations were: metalaxyl at 0.3 or 3.0 $\mu g/ml$ and H_3PO_3 at 1.3 or 13 $\mu g/ml$. All solutions were adjusted to pH 6.2 including the sterile distilled and deionized water control. At intervals of 3, 24, and 48 hr, three mycelial mats were removed from each treatment. The mats were placed on V8C agar and incubated at 24 C for 48 hr. Radial growth of the colonies was measured; the experiment was repeated once.

RESULTS

Linear growth response. The fungicide concentration required for 50% inhibition of mycelial growth by H_3PO_3 for three cultures of *P. citricola* ranged from 1.3 to 1.7 $\mu g/ml$ when mycelium was grown on a low-phosphate medium (Table 1). Corresponding EC_{50} values for *P. cinnamomi* ranged from 4.1 to 6.2 $\mu g/ml$. There were larger differences in EC_{90} values between the two species. The less-sensitive species of *P. cinnamomi* exhibited EC_{90} values from 27.9 to 71.7 $\mu g/ml$ (Table 1). Slope values for isolates of *P.*

cinnamomi ranged from 0.96 to 1.27 compared to 1.63 to 1.95 for *P. citricola*.

Oospore production. H_3PO_3 was highly inhibitory to oospore formation of *P. citricola*. For three cultures, inhibition ranged from 89 to 97% with only 1 μg/ml (Table 2). Inhibition of oospore production by *P. cinnamomi* was less severe, but still ranged from 60 to 78% at 1 μg/ml.

Sporangium production. Based on log-probit transformation, the EC_{50} values for inhibition of sporangium formation by *P. citricola* and *P. cinnamomi* were 1.4 and 1.8 μg/ml, respectively. Corresponding EC_{90} values for *P. citricola* and *P. cinnamomi* were 5.5 and 3.8 μg/ml, respectively (Fig. 1). The slope values for *P. citricola* and *P. cinnamomi* were 1.9 and 2.86, respectively. A

TABLE 1. Slope, EC_{50}, and EC_{90}[w] values[x] for inhibition of radial growth by phosphorous acid for individual cultures of *Phytophthora citricola* and *P. cinnamomi* grown on a modified Ribeiro's medium[y]

Species	Culture	Slope[z]	Inhibition of radial growth EC_{50}	EC_{90}
P. citricola	P1273	1.78	1.3 ± 1.1	4.7 ± 1.2
	P1315	1.63	1.3 ± 1.1	5.9 ± 4.1
	P1277	1.95	1.7 ± 1.1	6.1 ± 1.2
P. cinnamomi	Pc356	1.27	4.1 ± 1.2	27.9 ± 1.4
	Pc402	0.96	4.6 ± 1.2	64.2 ± 1.4
	Pc407	1.02	6.2 ± 1.2	71.7 ± 1.4

[w] EC_{50} and EC_{90} are the concentrations (micrograms per milliliter) required to inhibit radial growth 50 and 90%, respectively.
[x] Mean ± standard deviation of the mean based on a linear regression of the response (percent mycelial inhibition) plotted against the dosage (log concentration) H_3PO_3. The values are based on data from two experiments.
[y] Cultures grown on V8-$CaCO_3$ (V8C in the text) agar were transferred onto a modified Ribeiro's medium (11) at pH 6.2 containing 0.084 mM KH_2PO_4 and no β-sitosterol. Radial growth was measured after 4 days of incubation at 24 C.
[z] Correlation coefficients for all slope values were significant at $P = 0.01$.

TABLE 2. Influence of phosphorous acid (H_3PO_3) on the percent inhibition of production of oospores by *Phytophthora citricola* and *P. cinnamomi*[x]

Concentration of H_3PO_3 (μg/ml)	Percent inhibition of oospores: *P. citricola* P1277	P1273	P1315	*P. cinnamomi*[y] Pc407	Pc402	Pc356
1	97[z]	94	89	78	63	60
10	98	94	96	94	97	85
50	99	98	98	95	97	89
100	99	99	99	97	99	93

[x] Cultures were grown on a Ribeiro's modified synthetic medium (11) containing 0.084 mM KH_2PO_4 and 30 mg of β-sitosterol per liter for 33 days at 24 C. Inoculum consisted of minced mycelium.
[y] Mycelium of the A2 cultures of *P. cinnamomi* (Pc407, Pc402, and Pc356) was blended with an equal amount of the A1 culture (Pc138) for use as inoculum.
[z] Each value is the average of three replicates.

TABLE 3. Influence of phosphorous acid (H_3PO_3) and fosetyl salts (Al, Ca, and Na) on the number of sporangia produced by *Phytophthora cinnamomi* and *P. citricola*

Fungicide	Concentration of fungicide[x] (μg/ml)	Number of sporangia (× 10^2) produced per mycelial mat[y]: *P. cinnamomi* Pc402	*P. citricola* P1273
Control	–	23 a[z]	212 a
H_3PO_3	2.00	12 b	71 b
Fosetyl-Al	2.88	10 b	52 b
Fosetyl-Ca	3.14	8 b	34 b
Fosetyl-Na	3.23	7 b	37 b

[x] Fungicides were used at concentrations calculated to contain equal amounts of phosphite.
[y] Mycelial mats were grown in dilute V8C broth for 2 days at 24 C. Following rinsing, the mats were overlaid with 1% soil extract for 3 days to induce chlamydospore production.
[z] Nine mycelial mats were blended in 10 ml distilled water and sporangium production was determined by using a Hawksley eelworm counting chamber. Numbers in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Influence of phosphorous acid on the number of chlamydospores produced by three cultures of *Phytophthora cinnamomi* incubated at 24 C[x]

Concentration of H_3PO_3 (μg/ml)	Chlamydospores (10^3) produced by *P. cinnamomi*[y] Pc402	Pc407	Pc356	Chlamydospore production inhibition (%) Pc402	Pc407	Pc356
0.0	15.3 a[z]	10.7 a	9.5 a	–	–	–
1.0	14.0 a	8.2 b	10.3 a	–	–	–
5.0	12.4 a	7.2 b	10.0 a	–	–	–
10.0	14.5 a	6.9 b	8.6 a	5	36	9
50.0	10.0 b	4.9 b	6.0 b	35	54	37
100.0	4.4 c	3.7 b	4.0 b	71	65	58

[x] Cultures were grown in half-strength carrot broth for 48 hr and then overlaid with solutions of H_3PO_3 and incubated in the dark at 24 C for 35 days.
[y] Chlamydospore suspensions were prepared from nine mycelial mats which were blended in 10 ml of distilled water. Chlamydospores produced per 1.7 ml of a 1:10 dilution of this suspension were counted in a Hawksley eelworm counting chamber.
[z] Fungicide means followed by a common letter are not significantly different (LSD = 2.2, $P = 0.05$).

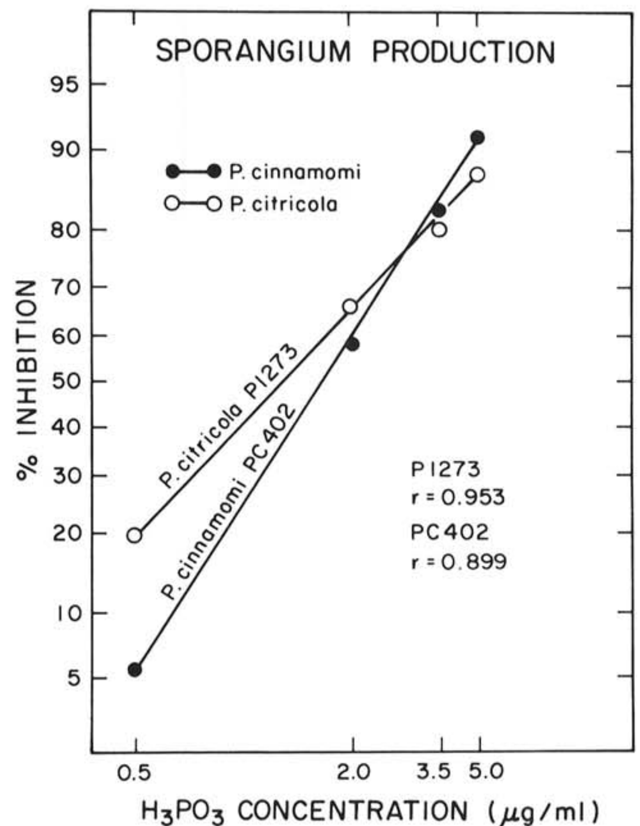

Fig. 1. Comparison of the inhibitory effects of phosphorous acid (H_3PO_3) upon sporangium production by *Phytophthora citricola* (O——O) and *P. cinnamomi* (●——●). Correlation coefficients (r) for the linear regression of combined data from two experiments were highly significant ($P = 0.01$). The slope values for *P. citricola* and *P. cinnamomi* were 1.91 and 2.86, respectively.

TABLE 5. Influence of phosphorous acid and fosetyl-Al on the percentage inhibition of production of chlamydospores by *Phytophthora cinnamomi* and *P. parasitica* at 18 C[x]

Fungicide	Concentration[y] (μg/ml)	Percent inhibition of chlamydospores: *P. cinnamomi* Pc402	*P. parasitica* P1155
H_3PO_3	50	−34.2[z]	96.5
	100	−14.6	100.0
	500	53.7	100.0
	1,000	100.0	100.0
Fosetyl-Al	72	−78.1	95.3
	144	−22.0	99.8
	720	100.0	100.0
	1,440	100.0	100.0

[x] Mycelial mats were grown in half-strength carrot broth for 48 hr at 24 C. Following a rinse in distilled water, different concentrations of H_3PO_3 or fosetyl-Al were added. The pH of the solutions was adjusted to 6.8 with KOH. The mats were incubated for 26 days at 18 C in these solutions before the number of chlamydospores produced was counted.

[y] Concentration series of H_3PO_3 and fosetyl-Al are equivalent in terms of their phosphite content.

[z] Each value is the average of three replicates.

TABLE 6. Influence of phosphorous acid and metalaxyl on percent inhibition of chlamydospore germination of *Phytophthora cinnamomi*[y]

Fungicide	Concentration (μg/ml)	Chlamydospore germination inhibition (%): Pc402	Pc407
Metalaxyl	0.025	11.3 a[z]	10.3 a
	0.25	11.6 a	8.1 a
H_3PO_3	100.0	11.1 a	7.1 a
	1,000.0	17.5 b	16.5 b

[y] Mycelial mats were grown for 48 hr in half-strength carrot broth at 24 C. The broth was replaced with distilled water and the mats incubated for 3 wk to allow for chlamydospore formation. The cultures were then incubated for 24 hr in fungicide solutions and then the chlamydospores were rinsed and germinated in a water suspension on V8C agar.

[z] Values are the means of five replicates. In each column different letters signify statistical differences ($P = 0.05$) according to Duncan's multiple range test.

comparison of the effect of H_3PO_3 and three fosetyl salts revealed that similar levels of inhibition were caused by all four compounds when used at equivalent phosphite concentrations (Table 3).

Zoospore release (indirect sporangium germination). In the presence of H_3PO_3 at 2 μg/ml, zoospore release with *P. cinnamomi* and *P. citricola* was inhibited by 39 and 30%, respectively. Based on two separate experiments, the EC_{50} values for inhibition by H_3PO_3 were ~6 μg/ml; this concentration caused a 58 and 51% reduction in zoospore release by *P. cinnamomi* and *P. citricola*, respectively. However, there was no effect on the subsequent germination of encysted zoospores at concentrations of H_3PO_3 up to 100 μg/ml.

Chlamydospore production. The formation of chlamydospores by *P. cinnamomi* at 24 C was sensitive to H_3PO_3 at concentrations greater than 10 μg/ml (Table 4). EC_{50} values for Pc356, Pc402, and Pc407 were 44±13, 37±10, and 15±4 μg/ml, and slope values were 33.8, 27.5, and 21.0, respectively, based on regression analysis of percent inhibition versus log concentration of H_3PO_3. In contrast, at 18 C chlamydospore production by *P. cinnamomi* was slightly stimulated by H_3PO_3 at 100 μg/ml (Table 5). The EC_{50} value of Pc402 for inhibition by H_3PO_3 at 18 C was near 400 μg/ml, based on a linear regression plot of the data. At 18 C, the inhibition of chlamydospore production by isolate P1155 of *P. parasitica* was 96.5% with 50 μg/ml of H_3PO_3 (Table 5). Fosetyl-Al at equivalent phosphite concentrations caused a similar effect on chlamydospore production; *P. parasitica* was almost completely inhibited by 72 μg/ml, whereas *P. cinnamomi* required 720 μg/ml for a similar effect (Table 5).

Chlamydospore germination and survival. With three isolates of *P. cinnamomi* (Pc356, Pc402, and Pc407) chlamydospore germination was unaffected by concentrations of H_3PO_3 up to 50 μg/ml compared to the control.

Chlamydospore germination in sterile distilled and deionized water on V8C agar following incubation for 24 hr in 100 or 1,000 μg/ml H_3PO_3 was only slightly inhibited. Low concentrations (0.025–0.25 μg/ml) of metalaxyl caused equivalent levels of inhibition (Table 6).

Mycelial survival. Following treatment of mycelial disks with either H_3PO_3 or metalaxyl at rates of 13–50 and 3–6 μg/ml, respectively, for periods of up to 48 hr, subsequent radial growth of the developing colonies was unaffected when compared to the untreated water control.

DISCUSSION

Mycelial growth of both *P. cinnamomi* and *P. citricola* was inhibited by low concentrations of H_3PO_3. In fact the EC_{50} values of 1.3–1.7 μg/ml recorded for three isolates of *P. citricola* were slightly lower than previously obtained (4). The range of EC_{50} and EC_{90} values for *P. cinnamomi*, 5–6 and 28–72 μg/ml, respectively, were very similar to those obtained previously (4,11).

Oospore production by *P. citricola* was extremely sensitive to H_3PO_3. As little as 1 μg/ml caused almost complete inhibition. In contrast, it took 50 μg/ml to achieve similar levels of inhibition of *P. cinnamomi*. The high sensitivity of oospore formation of *P. citricola* to H_3PO_3 parallels its effect on mycelial growth. In a study with *P. parasitica* it took 500 μg/ml of fosetyl-Al to completely inhibit oospore production (9), and the mycelial growth of this species also was relatively insensitive to the fungicide.

Sporangium production was extremely sensitive to all phosphite compounds tested. With both *P. citricola* and *P. cinnamomi*, EC_{50} values for inhibition were less than 3 μg/ml. With *P. citricola*, 3 μg/ml fosetyl-Al caused a 75% reduction in sporangium production. At the same concentration, production by *P. cinnamomi* was reduced by 50%. Similar effects of fosetyl-Al on sporangium production were observed with *P. citrophthora* and *P. parasitica* (9).

Zoospore release also was sensitive to H_3PO_3; EC_{50} values of 6 μg/ml were obtained with both *P. citricola* and *P. cinnamomi*. With *P. parasitica*, fosetyl-Al was also highly inhibitory to zoospore release; 10 μg/ml caused 90% inhibition (9). Zoospore release by *P. citrophthora* was much less sensitive, requiring 100 μg/ml to cause 21% inhibition (9). In contrast, mycelial growth of *P. citrophthora* was highly sensitive to both H_3PO_3 (4) and fosetyl-Al (9). Encysted zoospore germination of *P. cinnamomi* and *P. citricola* was unaffected by concentrations of H_3PO_3 up to 100 μg/ml. Similarly 100 μg/ml of fosetyl-Al had no effect on cyst germination of either *P. citrophthora* or *P. parasitica* (9).

At 24 C chlamydospore production by *P. cinnamomi* was inhibited by 50% with 15–44 μg/ml H_3PO_3. With *P. parasitica* at 18 C, H_3PO_3 was even more inhibitory to chlamydospore formation. The increased sensitivity of chlamydospore production of *P. parasitica* compared to *P. cinnamomi* was in contrast to the relatively low sensitivity of its mycelial growth to phosphite compounds (4,9). Even after incubation for 24 hr in 1,000 μg/ml H_3PO_3, chlamydospore germination of *P. cinnamomi* was inhibited by less than 20%. Equivalent results were obtained with *P. parasitica* by using fosetyl-Al (9).

In summary, sporangium formation and zoospore release were particularly sensitive to H_3PO_3 and fosetyl salts. Interestingly, zoospore release was inhibited by both fosetyl-Al (9) and H_3PO_3, but was unaffected by metalaxyl (10). Otherwise, the effects of these phosphite compounds paralleled those of metalaxyl (5,10), in that spore formation (especially sporangium production), rather than germination, was affected.

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