Correlative in Vitro and in Vivo Behavior of Mutant Strains of *Phytophthora palmivora* Expressing Different Resistances to Phosphorous Acid and Fosetyl-Na

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ABSTRACT

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Mutant strains of an isolate (PO376) of *Phytophthora palmivora* were selected in the laboratory from populations of germinating cysts treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Five mutants were selected for their low, moderate, or high resistance in vitro to phosphorous acid (H_3PO_3) buffered to pH 6.2. The EC_{50} values for inhibition of radial growth of mycelium by H_3PO_3 on 0.5% cornmeal agar for these mutants ranged from 36.6 to 130.3 μg/ml compared to 6.6 μg/ml for the parental isolate. The effect of H_3PO_3 on inhibition of zoosporangium production also was investigated. The parental isolate had an EC_{50} of 0.1 μg/ml of H_3PO_3, whereas values for the mutant strains ranged from 4.5 to 14.8 μg/ml. Similarly with fosetyl-Na, values for zoosporangium inhibition ranged from 10.6 to 45.6 μg/ml for the mutants, compared to only 0.3 μg/ml for the parental isolate. Using tomato seedlings as a susceptible host for in vivo tests, EC_{50} values for inhibition of infection ranged from 38 μg/ml for the parental type and 47 to 437 μg/ml for the five mutants. For these mutants, the order of their in vivo sensitivity to both H_3PO_3 and fosetyl-Na paralleled that observed in vitro for inhibition of their mycelial growth by H_3PO_3. The results of these experiments support the hypothesis that H_3PO_3 and fosetyl-Na have a direct mode of action against *P. palmivora*.

Fosetyl salts (Na^+, Ca^{++}, Al^{+++}) and their breakdown product phosphorus acid (H_3PO_3) are active both in vitro and in vivo against various *Phytophthora* species (3–5,7–9,11–18,22). Different hypotheses exist concerning the mode of action of these phosphonate fungicides (3,8,12,13,20). Several workers have provided evidence that there may be a significant role for an indirect mode of action of fosetyl-Al involving a stimulation of a host defense mechanism (3,15,17,22). Other chemically unrelated fungicides, such as metalaxyl, which are active directly against *Phytophthora*, also are capable of affecting host defense mechanisms (2,23). Metalaxyl, however, is known to have a primary mode of action involving interference with RNA synthesis of the pathogen (10), and the indirect effects on host metabolism are believed to be of secondary importance.

Recently, evidence has accumulated that H_3PO_3 is highly active in vitro against some *Phytophthora* species (4,5,7–9,12–14). In addition, it was shown that a laboratory-derived mutant of *Phytophthora capsici* Leonian, highly resistant to H_3PO_3 in vitro, also was resistant in vivo to both H_3PO_3 and fosetyl salts (5,14). However, although substantial evidence now exists to suggest that phosphonate fungicides have a direct mode of action against *Phytophthora* (9,11), the importance of this direct fungistatic activity in host-parasite relationships in vivo has yet to be evaluated.

A close correspondence between in vitro and in vivo performance of fungal isolates varying in resistance to both H_3PO_3 and fosetyl salts would strengthen the concept that the primary mode of action of these phosphonates is directly against the pathogen. In this study we selected an isolate (PO376) of *Phytophthora palmivora* Butler because of its extreme sensitivity to H_3PO_3. Sensitivity to H_3PO_3 and fosetyl-Na was compared both in vitro and in vivo using a series of laboratory-derived mutant strains of PO376, selected because they possessed a range of resistances to H_3PO_3 in vitro. Tomato was chosen as a suitable host because seedlings were highly susceptible to this isolate of *P. palmivora* (6,20).

MATERIALS AND METHODS

Phytophthora. The isolate of *P. palmivora* used in this study is maintained as PO376 in the collection of *Phytophthora* at the University of California, Riverside. It was originally isolated from cacao in Malaysia, and tomato seedlings were determined to be a susceptible host (6,20). The fungus was grown on cleared V8C agar (10% V-8 juice, 1% $CaCO_3$, clarified by centrifugation) at 24 C. Zoosporangia were produced on V8C incubated 3 days in the dark at 28 C followed by 4 days at 24 C under fluorescent lights. Zoosporangium release was initiated by overlaying the cultures with distilled water and chilling them for 30 min at 4 C.

Fungicides. Stock solutions of H_3PO_3 (99.69% pure solid) and fosetyl-Na (technical grade, 500 mg/ml in H_2O) were prepared either with distilled water for H_3PO_3 or with 0.03 M 2-morpholinoethanesulfonic acid (MES) buffer for fosetyl-Na (13). Solutions were adjusted to pH 6.2 with 10 N KOH and stored in amber glass bottles at 4 C.

Mutagenesis. Encysted zoospores (3.5×10^7/experiment) of PO376 were exposed to the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) at 30 μg/ml for 15 min as previously described (5,10,18). Resistant colonies were selected by overlaying the encysted zoospores with a low phosphate, modified Ribeiro's

synthetic agar medium (13,21) amended with 30 μg/ml of H_3PO_3 and adjusted to pH 6.2 before autoclaving. Rifampicin (5 μg/ml) and vancomycin (100 μg/ml) were added to the medium after autoclaving to prevent bacterial contamination. Selection of resistant colonies involved overlaying the original plates with a thin layer of fresh Ribeiro's medium containing 20 μg/ml of H_3PO_3 at pH 6.2. Colonies that grew through this layer were then transferred to 0.5% cornmeal agar (CMA) amended with 30 μg/ml of H_3PO_3 at pH 6.2, and radial growth rates of the resistant mutants were compared with those of the parental isolate on 0.5% CMA. Five mutants with different H_3PO_3-resistance levels, but with radial growth rates and sporulation capacities similar to the parental isolate, were selected.

Mycelial growth. In vitro resistance of the mutants to H_3PO_3 was determined by placing 4-mm-diameter disks, taken from the margins of 4-day-old colonies, onto 0.5% CMA amended with 0, 30, 50, 100, or 200 μg/ml of H_3PO_3 at pH 6.2. Radial growth was measured after 7 days' incubation in the dark at 24 C. The EC_{50} values were computed using linear regression analysis of the percent inhibition and log concentration of H_3PO_3. Plates were prepared in triplicate for each treatment, and the experiments were repeated twice.

Zoosporangium production. Five-millimeter-diameter disks of the parental isolate and H_3PO_3-resistant strains were cut from the colony margins of 4-day-old cultures grown on V8C agar in the dark at 28 C. Five disks were transferred to each 9-cm-diameter petri plate, and 20 ml of one-fifth strength V8C broth was added. The plates were incubated 24 hr in the dark at 28 C. After incubation, disks were washed twice with sterile deionized distilled water and treated with H_3PO_3 (0, 5, 10, 20, 50, 100 μg/ml in distilled water at pH 6.2) or fosetyl-Na (0, 80, 160, 320, 480, 800 μg/ml in 0.03 M MES buffer at pH 6.2). The cultures were incubated under constant illumination at 24 C for 24 hr. Each culture was blended at high speed 10 sec in 30 ml of sterile deionized distilled water, and sporangium production per milliliter was determined using a Hawksley eelworm counter. For each strain there were three replicate plates for each treatment, and the experiment was repeated three times. The results were presented as a probit percent inhibition of zoosporangium production against log concentration of H_3PO_3 or fosetyl-Na.

In vivo assessment. The tomato cultivar Bonnie Best was used in all experiments. Seed was sown in the greenhouse in UC mix (1) consisting of 50% blow sand, 50% peat moss, plus 2.2 kg of dolomite, 1.5 kg of superphosphate, 148 g of KNO_3, and 148 g of K_2SO_4, per cubic meter, overlaid with a thin layer of vermiculite in 15-cm-diameter clay pots. A concentration series of H_3PO_3 (0, 10, 20, 50, 70, 100, 200, 500 μg/ml at pH 6.2) or fosetyl-Na (0, 112, 240, 320, 480, 800, 1,100, 1,600, 3,000 μg/ml at pH 6.2) was prepared, each in 100 ml of distilled water in 180-ml Styrofoam cups. Two-week-old tomato seedlings were placed six to a cup, and 1 ml of 1×10^5 zoospores of a strain of *P. palmivora* was added immediately. The plants were incubated in the light at 24 C for 5 days. After 5 days, the seedling stem was cut into 0.5-cm sections, dipped briefly in 70% alcohol, rinsed twice in distilled water, blotted on paper towels, and plated on PARP medium (19) modified by substitution of 125 μg/ml ampicillin trihydrate for 250 μg/ml of sodium ampicillin. The plates were incubated at 24 C. The degree of infection was expressed as the percent root pieces yielding colonies. The data were plotted as the probit percent inhibition of infection against log concentration of H_3PO_3 or fosetyl-Na. The experiment was repeated six times, and the data for each isolate were pooled.

To compare the performance of H_3PO_3 and fosetyl-Na, concentrations also were expressed as micrograms per milliliter were converted to PO_3 meq/L by dividing by either 82 for H_3PO_3 or 132 for fosetyl-Na.

Phosphate influence on in vivo efficacy. The parent PO376 and a resistant strain with high resistance to H_3PO_3, L3, were chosen. Concentration series were prepared as previously described except that each solution was amended with either 0, 1, or 10 mM of potassium phosphate buffer at pH 6.2. Tomato seedlings were inoculated with zoospore suspensions as before. The tomato stems were plated on PARP medium (19) after 5 days, and percent infection was determined as previously described. The results were plotted as probit percent inhibition against log concentration of H_3PO_3 or fosetyl-Na. The experiment was repeated five times.

RESULTS

Mycelial growth of resistant mutants in vitro. Using the chemical mutagen MNNG, a total of 68 single-zoospore strains of *P. palmivora* displaying resistance to H_3PO_3 were recovered. An average of six resistant mutants were recovered per 1×10^7 population of encysted zoospores exposed to MNNG. Mutant strains were selected for their similar radial growth rates on CMA and similar sporulation capacities on V8C as compared to the parental strain PO376. The parental strain was very sensitive to H_3PO_3, exhibiting an EC_{50} value of 6.6 μg/ml. Ultimately five mutant strains designated 18, X3, N3, L3, and V1 were selected for further experimental analysis (Table 1). These mutant strains possessed a range of EC_{50} values from 36.6 to 130.3 μg/ml H_3PO_3 for inhibition of radial mycelial growth on 0.5% CMA. Two strains (X3, 18) exhibited low resistance, one strain (N3) moderate resistance, and two strains (L3, V1) high resistance to H_3PO_3 (Table 1, Fig. 1).

Zoosporangium production. Table 2 is a comparison of the EC_{50} values for inhibition of zoosporangium production for H_3PO_3 and fosetyl-Na. For H_3PO_3, the EC_{50} value for PO376 was 0.1 μg/ml of H_3PO_3, whereas that of the resistant mutants ranged from 4.5 to 14.8 μg/ml. The EC_{50} values for fosetyl-Na ranged from 0.3 μg/ml

TABLE 1. EC_{50} values for phosphorous acid (H_3PO_3)-resistant mutants of *Phytophthora palmivora* grown on 0.5% cornmeal agar with H_3PO_3

Strain[a]	EC_{50} (μg/ml)[b]	Regression equations	Correlation coefficient[c]
PO376	6.6	$Y = 3.78 + 56.36\ X$	0.99
18	36.6	$Y = -7.80 + 36.96\ X$	0.91
X3	39.2	$Y = -3.46 + 33.92\ X$	0.95
N3	83.6	$Y = -43.23 + 48.50\ X$	0.99
L3	117.7	$Y = -71.53 + 59.12\ X$	0.99
V1	130.3	$Y = -101.55 + 71.60\ X$	0.99

[a] PO376 is an H_3PO_3-sensitive parental isolate. The remaining strains are H_3PO_3-resistant mutants produced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment of PO376.
[b] EC_{50} values are based on linear regression of percent mycelial growth inhibition plotted against log concentration of H_3PO_3 for each isolate.
[c] Correlation coefficients are significant ($P = 0.01$).

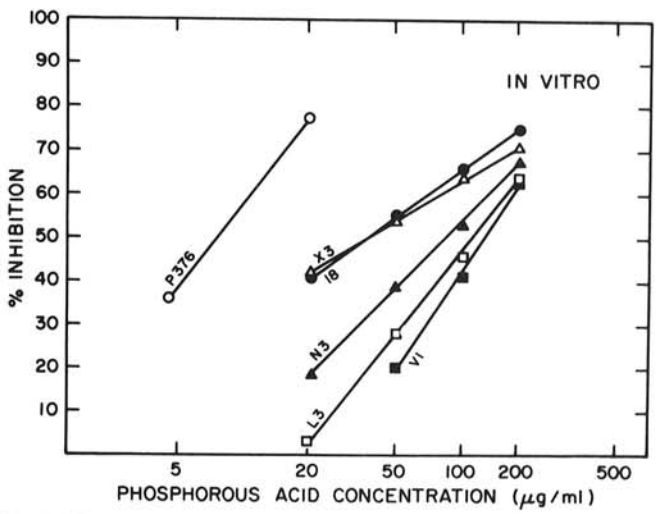

Fig. 1. Linear regression analysis of the in vitro relationship between percent inhibition of mycelial growth and concentration of phosphorous acid. Isolates were grown 4 days at 24 C on 0.5% Difco cornmeal agar containing different levels of buffered H_3PO_3 adjusted to pH 6.2 with 10 N KOH.

for PO376 to 45.6 μg/ml for V1. In general, the degree of resistance observed paralleled that obtained for mycelial growth. However, strain 18, which in terms of mycelial growth inhibition (Table 1) had low resistance to H_3PO_3, was the one exception (Table 2).

In vivo assessment. The regression lines for each strain for probit percent inhibition of infection of tomato stem pieces versus log concentration are presented for H_3PO_3 (Fig. 2) and fosetyl-Na (Fig. 3). The data in Table 3 represent a comparison of EC_{50} values obtained by interpolation from regression lines. These data indicate that isolates PO376, X3, and 18 show the greatest sensitivity to H_3PO_3 and fosetyl-Na. An analysis of covariance between the slopes and adjusted means of the regression lines (Fig. 2) determined that isolates PO376, X3, and 18 were not significantly different in their response to H_3PO_3. The slopes and adjusted means of the remaining lines were significantly different ($P > 0.01$). Isolates V1 and L3 were the most resistant to both compounds in vivo, with EC_{50} values of 263 and 437 μg/ml, respectively, for H_3PO_3. Strain N3 demonstrated intermediate sensitivity to H_3PO_3 and fosetyl-Na with EC_{50} values of 113.4 and 6735.3 μg/ml, respectively (Table 3). The EC_{50} ratios of fosetyl-Na to H_3PO_3 based on equivalent phosphite content also are listed in Table 3. They indicate that H_3PO_3 is always more effective than fosetyl-Na under the conditions of these experiments. In addition, this EC_{50} ratio increased markedly with increasing resistance of the strain to H_3PO_3.

Phosphate influence on in vivo efficacy. The effect of three levels of potassium phosphate buffer on efficacy of both compounds was determined by repeating the in vivo experiments with the parental isolate PO376 and strain L3, which exhibited a high in vitro resistance to H_3PO_3 (Table 4). With PO376, percent inhibition of infection was significantly increased by both the 1 and 10 mM phosphate treatments at all concentrations of H_3PO_3 and fosetyl-Na. The data obtained for strain L3 generally paralleled that of PO376 but were more complex (Table 4). At 0.85 phosphonate meq/L with both H_3PO_3 and fosetyl-Na, percent inhibition of infection was increased with increasing phosphate concentration. At 2.43 meq/L, only the highest level of phosphate enhanced the inhibitory effects of H_3PO_3 or fosetyl-Na. Again 10 mM potassium phosphate levels with either 6.10 meq/L of H_3PO_3 or fosetyl-Al were the most inhibitory to the resistant strain L3 (Table 4).

TABLE 2. Comparison of the in vitro EC_{50} values of phosphorous acid (H_3PO_3) and fosetyl-Na for percent inhibition of zoosporangium formation with strains of *Phytophthora palmivora* that exhibit a range of resistances to H_3PO_3[a]

Isolate	EC_{50} values of H_3PO_3		EC_{50} values of fosetyl-Na		EC_{50} ratio of fosetyl-Na to H_3PO_3 (PO_3 meq)
	μg/ml[b]	meq/L[c]	μg/ml[b]	meq/L[c]	
PO376	0.1	0.001	0.3	0.002	1.7
X3	4.5	0.06	10.6	0.08	1.3
18	7.7	0.10	45.1	0.34	3.4
N3	8.9	0.09	13.2	0.10	1.1
V1	11.6	0.15	45.6	0.34	2.3
L3	14.8	0.19	40.4	0.31	1.6

[a] Mycelial mats were grown in dilute V8C broth 24 hr at 28 C. After rinsing, the mats were overlaid with solutions of H_3PO_3 or fosetyl-Na and incubated under lights 24 hr at 24 C. Mycelial mats were blended and zoosporangium production was determined using a Hawksley eelworm counting chamber.

[b] EC_{50} values are based on linear regression analysis. The correlation coefficients ranged from 0.87 to 0.96 for H_3PO_3 and 0.88 to 0.97 for fosetyl-Na.

[c] Values for PO_3 meq/L were determined by dividing micrograms per milliliter by the conversion factors: 82 (H_3PO_3) or 132 (fosetyl-Na).

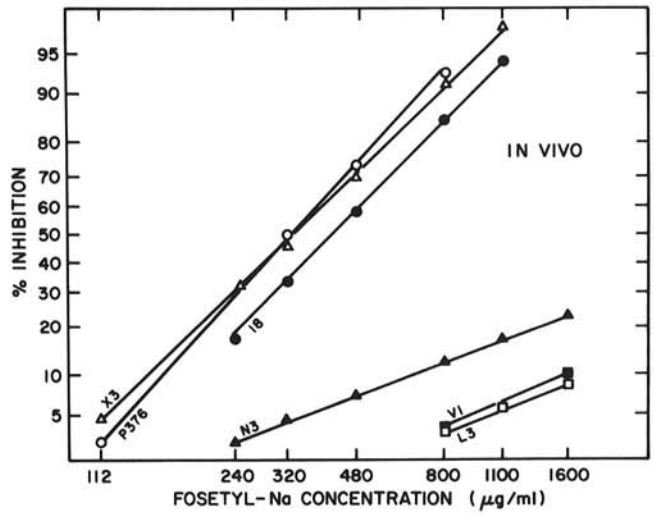

Fig. 3. Linear regression analysis of the in vivo relationship between percent inhibition of infection and concentration of fosetyl-Na (pH 6.2 with MES buffer). Tomato seedlings were exposed to a range of concentrations of fosetyl-Na and inoculated with zoospores of a strain of *Phytophthora palmivora*.

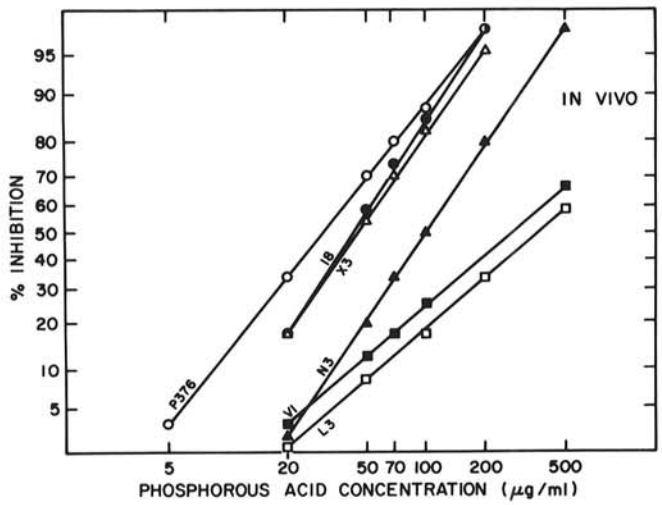

Fig. 2. Linear regression analysis of the in vivo relationship between percent inhibition of infection and concentration of phosphorous acid. Tomato seedlings were exposed to a range of concentrations of buffered H_3PO_3 (pH 6.2 with 10 N KOH) and inoculated with zoospores of a strain of *Phytophthora palmivora*.

TABLE 3. Comparison of the in vivo EC_{50} values of phosphorous acid (H_3PO_3) and fosetyl-Na for percent inhibition of tomato seedling stem infection with strains of *Phytophthora palmivora* that exhibit a range of resistances to H_3PO_3 in vitro[a]

Isolate	EC_{50} values of H_3PO_3		EC_{50} values of fosetyl-Na		EC_{50} ratio of fosetyl-Na to H_3PO_3 (PO_3 meq)
	μg/ml[b]	meq/L[c]	μg/ml[b]	meq/L[c]	
PO376	38	0.5	356	2.7	5.6
X3	47	0.6	437	3.3	5.6
18	50	0.6	341	2.6	4.1
N3	113	1.4	6,735[d]	51.0	3.60
V1	263	3.3	157,752[d]	1,195.1	364.3
L3	437	5.5	218,234[d]	1,653.3	302.2

[a] Bare-rooted seedlings were placed in solutions of H_3PO_3 and of fosetyl-Na and immediately inoculated with zoospores. Four days after inoculation, the stem of each seedling was plated in 0.5-cm segments on PARP medium to determine percent infection.

[b] EC_{50} values are based on linear regression analysis. The correlation coefficients ranged from 0.87 to 0.93 for H_3PO_3 and 0.73 to 0.95 for fosetyl-Na.

[c] Values for PO_3 meq/L were determined by dividing micrograms per milliliter by the conversion factors: 82 (H_3PO_3) or 132 (fosetyl-Na).

[d] Extrapolated values for EC_{50} are based on the linear regression analysis.

TABLE 4. Effect of 1 or 10 mM potassium phosphate levels on the percent inhibition of infection of tomato seedlings treated with phosphorous acid (H_3PO_3) or fosetyl-Na and inoculated with either the parental isolate of *Phytophthora palmivora* (PO376) or a mutant strain (L3) exhibiting high resistance to H_3PO_3

Treatment (PO_3 meq/L)[z]	Phosphate level (mM)	Percent inhibition of infection[y]			
		PO376		L3	
		H_3PO_3	Fosetyl-Na	H_3PO_3	Fosetyl-Na
0.85	0	57 d	6 g	17 f	0 c
2.43		100 a	36 e	59 c	0 c
6.10		100 a	100 a	71 b	2 c
0.85	1	72 c	19 f	39 e	3 c
2.43		100 a	42 d	48 d	4 c
6.10		100 a	99 a	73 b	3 c
0.85	10	87 b	69 c	45 de	39 b
2.43		100 a	91 b	75 b	45 a
6.10		100 a	100 a	82 a	45 a

[y] Bare-rooted seedlings were placed in solutions of H_3PO_3 and of fosetyl-Na with 0, 1, or 10 mM potassium phosphate buffer and inoculated immediately with zoospores. Four days after inoculation, the stem of each seedling was plated in 0.5-cm segments on PARP medium to determine percent infection. Values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

[z] Values for PO_3 meq/L were determined by dividing micrograms per milliliter by the conversion factors: 82 (H_3PO_3) or 132 (fosetyl-Na).

DISCUSSION

Current ideas regarding the mode of action of phosphonate fungicides are controversial (3,9,11,13,15,22). It has been demonstrated with the use of metabolic inhibitors, selected for their ability to interfere with biosynthetic pathways involved in phytoalexin production, that the effects of the phosphonate fosetyl-Al can be nullified (3,22). Consequently, a hypothesis has been proposed that explains the mode of action of fosetyl-Al as due to the stimulation of metabolic changes in the host that result in an enhanced defense response (3,15,17,22). However, a significant body of evidence has been accumulated that demonstrates high in vitro sensitivity of *Phytophthora* isolates to the principal metabolite of fosetyl-Al in plants, H_3PO_3 (4,5,7–9,11,12,14). In addition, strains of *P. capsici* exhibiting resistance both in vitro and in vivo to different phosphonate compounds (H_3PO_3, fosetyl-Na, and fosetyl-Al) have been generated through chemical mutagenesis procedures (5,12,14). This fact supplies strong circumstantial evidence for the direct mode of action of these fungicides (9).

In the current study, mutant strains of *P. palmivora*, with a range of in vitro resistances to H_3PO_3, exhibited the same degree of resistance in vivo to both H_3PO_3 and fosetyl-Na. This provides compelling evidence for a direct mode of action for these fungicides.

Zoosporangium formation in the parental isolate PO376 of *P. palmivora* was very sensitive to inhibition by both H_3PO_3 and fosetyl-Na. This was consistent with the findings for *P. cinnamomi* and *P. citricola*, where there was equivalent efficacy of these compounds against formation of zoosporangia, chlamydospores, and oospores (8). One additional reason for the success of these compounds in controlling some *Phytophthora* diseases undoubtedly relates to their inhibitory effects on spore formation.

In this study, the in vivo efficacy of the two phosphonate compounds tested was not equivalent; H_3PO_3 was five times as active on a phosphonate milliequivalent basis as fosetyl-Na in controlling the parental strain of *P. palmivora* on tomato seedlings. Further, as the level of resistance of the mutant strains increased, the observed EC_{50} ratios of fosetyl-Na to H_3PO_3 became greater. This may relate to the fact that fosetyl-Na must be first metabolized to H_3PO_3 before a strong direct effect on mycelium growth can occur (9). Because the experiments were of short duration and small seedlings were used, this metabolism was probably still incomplete.

The enhanced level of control of *P. palmivora* in vivo in the presence of increasing levels of phosphate was unexpected. It contradicted findings obtained with the interaction between *P. cinnamomi* and *Persea indica*, where phosphate was shown to reduce the efficacy of both compounds (12,13). This indicates that phosphate influence on host and fungal metabolism may be an important factor affecting the efficacy of phosphonate fungicides (4). The relationship between phosphate concentration in tissues, host-parasite metabolism, and the mode of action of phosphonate fungicides could be complex. There is a need for expanded research in this area to clarify the role of the host in these interactions.

However, we believe one clear conclusion emerges from this study: Parallel orders of resistance to H_3PO_3 were achieved in vitro and in vivo. We believe this provides the strongest evidence to date for a direct mode of action in vivo of the phosphonate fungicides H_3PO_3 and fosetyl-Na against *Phytophthora*.

LITERATURE CITED

1. Baker, K. F., ed. 1957. The U. C. System for producing healthy container-grown plants. Univ. Calif., Div. Agric. Sci., Agric. Exp. Stn. 322 pp.
2. Barak, E., Edgington, L. V., and Ripley, B. D. 1984. Bioactivity of the fungicide metalaxyl in potato tubers against some species of *Phytophthora*, *Fusarium*, and *Alternaria*, related to polyphenoloxidase activity. Can. J. Plant Pathol. 6:304-308.
3. Bompeix, G., Ravisé, A., Raynal, R., Fettouche, F., and Durand, M. C. 1980. Modalités de l'obtention des nécroses bloquantes sur feuilles détachées de Tomate par l'action du tris-*O*-éthyl phosphonate d'aluminium (phoséthyl d'aluminium), hypothèses sur son mode d'action in vivo. Ann. Phytopathol. 12:337-351.
4. Bompeix, G., and Saindrenan, P. 1984. In vitro antifungal activity of fosetyl-Al and phosphorous acid on *Phytophthora* species. Fruits 39:777-786.
5. Bower, L. A., and Coffey, M. D. 1985. Development of laboratory tolerance to phosphorous acid, fosetyl-Al, and metalaxyl in *Phytophthora capsici*. Can. J. Plant Pathol. 7(1):1-6.
6. Chee, K. H. 1969. Hosts of *Phytophthora palmivora*. Rev. Appl. Mycol. 48:337-344.
7. Coffey, M. D., and Bower, L. A. 1984. In vitro variability among isolates of eight *Phytophthora* species in response to phosphorous acid. Phytopathology 74:738-742.
8. 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.
9. Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.
10. Davidse, L. C. 1981. Resistance to acylalanine fungicides in *Phytophthora megasperma* f. sp. *medicaginis*. Neth. J. Plant Pathol. 87:11-24.
11. Dercks, W., and Buchenaner, H. 1987. Comparative studies on the mode of action of aluminium ethyl phosphite in four *Phytophthora* species. Crop Prot. 6:82-89.
12. Fenn, M. E. 1986. Evidence for direct mode of action of fosetyl-Al and phosphorous acid. Ph.D. thesis. University of California, Riverside.
13. Fenn, M. E., and Coffey, M. D. 1984. Studies on the in vitro and in vivo antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74:606-611.
14. Fenn, M. E., and Coffey, M. D. 1985. Further evidence for the direct mode of action of fosetyl-Al and phosphorous acid. Phytopathology 75:1064-1068.
15. Guest, D. I. 1984. Modification of defence responses in tobacco and capsicum following treatment with fosetyl-Al [Aluminium tris (*O*-ethyl phosphonate)]. Physiol. Plant Pathol. 25:125-134.
16. Guest, D. I. 1984. The influence of cultural factors on the direct antifungal activities of fosetyl-Al, propamocarb, metalaxyl, SN 75196 and Dowco 444. Phytopathol. Z. 111:155-164.
17. Guest, D. I. 1986. Evidence from light microscopy of living tissues that fosetyl-Al modifies the defence response in tobacco seedlings following inoculation by *Phytophthora nicotianae* var. *nicotianae*. Physiol. Plant Pathol. 29:251-261.
18. Joseph, M. C., and Coffey, M. D. 1984. Development of laboratory resistance to metalaxyl in *Phytophthora citricola*. Phytopathology 74:1411-1414.
19. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
20. Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, Vaduz, FL. 417 pp.
21. Ribeiro, O. K., Erwin, D. C., and Zentmyer, G. A. 1975. An improved

synthetic medium for oospore production and germination of several *Phytophthora* species. Mycologia 67:1012-1019.
22. Vo-Thi, H., Bompeix, G., and Ravisé, A. 1979. Rôle du tris-*O*-éthyl phosphonate d'aluminium dans la stimulation des réactions de défense des tissues de Tomate contre le *Phytophthora capsici*. C. R. Acad. Sci. Ser. D. 228:1171-1174.
23. Ward, E. W. B. 1984. Suppression of metalaxyl activity by glyphosate: Evidence that host defence mechanisms contribute to metalaxyl inhibition of *Phytophthora megasperma* f. sp. *glycinea* in soybeans. Physiol. Plant Pathol. 25:381-386.