Comparative Antifungal Activity of Four Phosphonate Compounds Against Isolates of Nine *Phytophthora* Species

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ABSTRACT

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The systemic fungicide potassium phosphonate, three alkyl-substituted phosphonate compounds (monoethyl, dimethyl, and diethyl phosphonate), and potassium hypophosphonate were compared for their activity in vitro against 34 isolates of nine species of *Phytophthora*. Potassium phosphonate proved to be the most inhibitory compound against most isolates. In contrast, potassium hypophosphonate was the least inhibitory, in some cases over 100 times less than potassium phosphonate. Among the alkyl phosphonates, monoethyl phosphonate was generally more inhibitory than the two dialkyl phosphonates. Compared to diethyl phosphonate, dimethyl phosphonate was either equally or more inhibitory toward the majority of isolates tested. In vivo, the four phosphonate compounds were equally effective in controlling stem rots of *Persea indica* L. and pepper (*Capsicum annuum* L.) caused by *P. citricola* and *P. capsici*, respectively. Potassium hypophosphonate provided no control of either disease. In contrast, no phosphonate compound was effective in controlling stem rot of pepper caused by a laboratory-produced, phosphonate-resistant mutant of *P. capsici*. The rapid hydrolysis in plant tissues of the organic alkyl-substituted phosphonates to the more antifungal inorganic phosphonate anion probably explains the similar level of disease control achieved by all four phosphonate compounds.

Fosetyl aluminum (aluminum tris-*O*-ethyl phosphonate), known under the trade name Aliette (Rhone-Poulenc Ag Company, Research Triangle Park, NC) is a monoethyl phosphonate fungicide used for the systemic control of diseases caused by both foliar and soilborne members of the Peronosporales (2,3,5,8-10,14-16,21,22,24,25). Monoethyl phosphonate is ambimobile (13) and, because of its exceptional

phloem mobility, can be applied as either a foliar spray or, in the case of some tree crops, as a trunk injection for effective control of root rots caused by certain *Phytophthora* spp. (5,8–10,24).

Upon application of fosetyl Al to plants and soil, the monoethyl phosphonate moiety enters the plant and is metabolized to phosphonic acid, H_2PHO_3 (9), which in aqueous systems at physiological pH is ionized primarily to the dianion phosphonate, HPO_3^{-2} (23). Although the mode of action of monoethyl phosphonate is still unknown, the rapid production of relatively high amounts of phosphonate in plant tissues could be the primary determinant involved in disease control. Direct antifungal activity of potassium phosphonate toward sensitive *Phytophthora* spp. has been demonstrated (4,6,7,9,12,16–18). Once inside the plant, phosphonate is quite stable (24) and apparently is not readily oxidized to phosphate (HPO_4^{-2}) for utilization by the plant (20). This probably explains the exceptional duration of control encountered with some *Phytophthora* diseases in the field (5,8,10,21,25).

Other alkyl phosphonate compounds as well as different salts of phosphonic acid have been reported to control some diseases caused by Oomycetes (1,9), but specific data is lacking on their comparative antifungal activity, especially toward *Phytophthora*. Modification of the number and length of the alkyl side chains on the phosphonate molecule could influence antifungal activity. Effects might include altered toxicity toward the pathogen, different uptake rates into the plant, and differences in metabolism to phosphonate in the plant.

The purpose of this study was to evaluate the direct antifungal activity of four phosphonate compounds (monoethyl, diethyl, dimethyl, and potassium phosphonate) in inhibiting mycelial growth of 34 isolates of nine *Phytophthora* spp. Potassium hypophosphonate was also included because it is one oxidation state removed from phosphonate. In addition, the ability of these compounds to control stem rots of *Persea indica* L., caused by *P. citricola* Saw. and stem rot of green pepper (*Capsicum annuum* L.), caused by wild-type and phosphonate-resistant isolates of *P. capsici* Leonian, was also investigated.

MATERIALS AND METHODS

Chemicals. The chemical structures of the four phosphonate compounds and hypophosphonate are shown in Figure 1. Phosphorous acid (99.6%) and hypophosphorous acid (50, v/v) were obtained from Fisher Scientific Company. Their pH was adjusted to 6.2 with KOH to obtain potassium phosphonate and hypophosphonate, respectively. Dimethyl and diethyl phosphonate (99 and 98%, respectively) were obtained from Aldrich Chemical Company, Inc., Milwaukee, WI. Monoethyl phosphonate (fosetyl Al, technical grade, 98%) was obtained from Rhone-Poulenc Ag. Company. The formula weights of monoethyl, diethyl, and dimethyl phosphonate, and of phosphonic and hypophosphonic acid are 109, 138, 110, 82, and 66, respectively. Stock solutions of the fungicides, prepared directly before use, were buffered with MES hydrate (4-morpholine ethanesulfonic acid) at concentrations equivalent to those of the fungicides, and the pH was adjusted to 6.2 with KOH. The chemicals were added to autoclaved 0.5% Difco cornmeal agar after it had cooled to 50 C. Plugs of inoculum, 5 mm in diameter, were taken from the edge of actively growing cultures of different *Phytophthora* spp. grown on V8C agar (10% V-8 juice, 1% $CaCO_3$, clarified by centrifugation) and placed centrally onto the fungicide-amended agar. The fungi were placed in the dark for 96 hr at 24 C; then the colony diameter was measured and the plug diameter was subtracted. Percent inhibition of mycelial growth was obtained by comparison with unamended controls, transformed to probits, and plotted against the log of the fungicide concentration. Linear regression analysis was performed on pooled data from two separate experiments, each consisting of three replicate plates, to determine the EC_{50} values for each compound for the various *Phytophthora* isolates. The *Phytophthora* isolates examined were all obtained from the *Phytophthora* collection at the University of California, Riverside, and are shown in Table 1.

Production of zoospore inoculum. Zoospores of *P. capsici* isolates P1319 or P1361 were produced by first growing them on V8C agar for 4 days in the dark at 24 C, and then placing the plates under Sylvania Gro-Lux fluorescent lights at 24 C for 3 days to induce sporangium production. To initiate zoospore release, the plates were flooded with deionized water, placed at 4 C for 30 min, and then returned to 24 C. Maximum zoospore release occurred about 45 min later. Zoospores of *P. citricola* were obtained by placing 8-mm diameter disks, taken from the edge of an actively growing colony on V8C agar, into 100- × 25-mm petri dishes and adding 20 ml of 1/5 diluted V8C broth (200 ml of V-8 juice and 2 g of $CaCO_3$ in 800 ml of distilled water, autoclaved 20 min) to each dish. The petri dishes were incubated in the dark for 3 days; the 1/5 strength V8C broth was removed; and the mycelial mats were rinsed three times with deionized water. Twenty milliliters of nonsterile soil extract (10 g of soil per liter of water, filtered twice through a Whatman No. 1 filter) was added to each dish and the dishes placed in the dark at 24 C for 3 days. To release zoospores, the nonsterile soil extract was replaced with deionized water and the mycelial mats containing sporangia were incubated at 4 C for 30 min, then returned to 24 C. Maximum zoospore release occurred within 45 min.

In vivo efficacy against stem rot of pepper. Pepper plants, cultivar Yolo Wonder, were grown in the greenhouse for 5 wk in sterilized U.C. mix no. 5 (50% peat moss, 50% fine sand, 2.2 kg of dolomite, 1.5 kg of superphosphate, 148 g of KNO_3, and 148 kg of K_2SO_4 per cubic meter). They were then removed from the mix, and the soil was gently washed away from the roots with running water. The plants were used to evaluate both the protective and curative properties of the phosphonate compounds.

For the curative treatment, five pepper plants were placed into 300-ml Styrofoam cups containing enough deionized water to bring the level to the root-stem interface. To each cup was added inoculum consisting of 1×10^6 motile zoospores of either a wild-type isolate of *P. capsici* (P1319) or a phosphonate-resistant mutant (P1361), obtained by chemical mutagenesis of P1319 (4). After 12 hr, the plants were removed, rinsed well with running

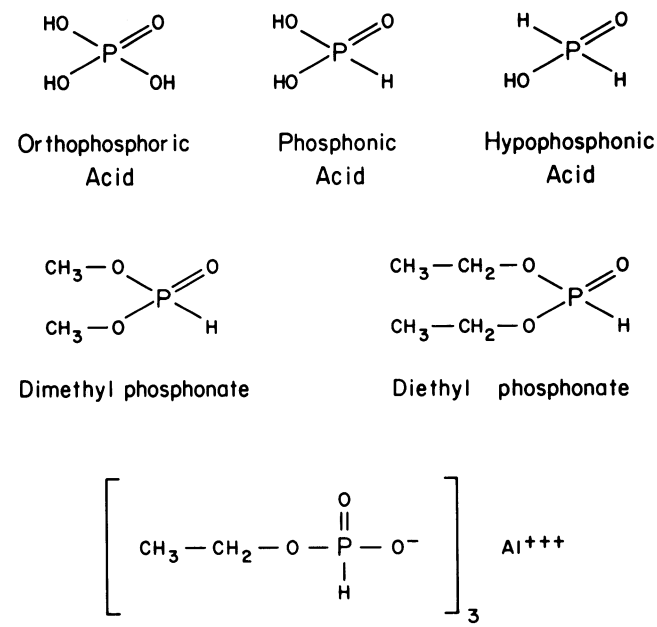

Fig. 1. Chemical structures of orthophosphoric, phosphonic, and hypophosphonic acid and dimethyl, diethyl, and monoethyl (fosetyl Al) phosphonates.

tap water, and then returned to the cups. The phosphonate compounds (in 250-ml volumes) were added at concentrations of either 1, 5, or 7 μeq of HPO_3^{-2} per milliliter, buffered with MES at a concentration equivalent to that of the test compound, and adjusted to pH 6.2 with KOH. Potassium hypophosphonate was added at 1, 5, or 7 mM. The plants were placed in a growth chamber for 6 days at 24 C with a daily 12-hr photoperiod providing 170 μE m^2 sec^{-1} at the plant surface. The plants were then removed from the solutions, rinsed with running tap water, and blotted dry on a paper towel. The resulting stem lesions were measured and 10 1-cm stem segments were excised from the crown upward, placed onto PARP medium (19), modified by the substitution of 125 μg of ampicillin trihydrate per milliliter (80%, Bristol Laboratories, Syracuse, NY) for 250 μg of sodium ampicillin per milliliter, and incubated at 24 C. The number of segments from which *Phytophthora* grew out for the next 4–6 days was counted to determine the percent stem infection.

To evaluate the protective efficacy of the phosphonate compounds, a similar procedure was followed except that the plants were placed for 24 hr in 250 ml of solutions of the phosphonate compounds with either 1, 5, or 10 μeq of HPO_3^{-2} per milliliter. They were then removed, rinsed thoroughly with water, and placed in 300-ml styrofoam cups with deionized water maintained at the level of the root-stem interface. The cups were then inoculated with 1×10^6 motile zoospores of *P. capsici* and allowed to incubate in the growth chamber for 6 days. For both the protective and the curative treatments, there were 10 plants per treatment, and the experiments were repeated twice.

In vivo efficacy against stem rot of *P. indica*. Seedlings of *P. indica* L. were grown from seed in the greenhouse for 6–7 wk in wooden flats containing sterile sand to which dilute Hoagland's solution was supplied daily. The plants were used for experiments similar to those described for pepper. For the curative experiments, five plants of *P. indica* were placed in 300-ml styrofoam cups and deionized water was added to the cup to bring the water level to the root-stem interface. Then inoculum consisting of 1×10^6 motile zoospores was added to each cup. The plants were incubated for 12 hr at 24 C, then removed and rinsed well with water. The infected plants were placed back into the cups, and 250 ml of the various phosphonate compounds were added at concentrations of either 1, 3, or 5 μeq of HPO_3^{-2} per milliliter, and potassium hypophosphonate was added at 1, 3, or 5 mM. The plants were allowed to incubate for 6 days in a growth chamber under a 12-hr photoperiod at 24 C. For the protective treatment, plants of *P. indica* were placed into 300-ml styrofoam cups containing 250 ml of each of the five compounds with HPO_3^{-2} at 1, 5, or 10 μeq/ml and allowed to incubate for 24 hr at 24 C. The plants were removed from the cups, rinsed well under running tap water, and returned to the cups. Deionized water was added to bring the level up to the root-stem interface, where it was maintained for the duration of the experiment. Next, 1×10^6 motile zoospores were added into each cup and the plants placed into a growth chamber with a 12-hr light regime at 24 C for 6 days. The plants were then

TABLE 1. In vitro antifungal activity of monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), and potassium phosphonate (PP) and of potassium hypophosphonate (PHP) toward isolates representing nine *Phytophthora* species[a]

Species	Isolate	Host	Inhibition (EC_{50})[b] by				
			PP	MEP	DMP	DEP	PHP
cactorum	P3083	Gypsophila	0.29	0.81	11.54	7.77	12.34
	P1686	Unknown[c]	0.25	0.59	6.47	16.04	24.05
	P3074	Cabbage tree	0.28	1.09	57.47	41.50	85.89
	P770	Apple	0.30	3.90	34.60	2.08	5.89
capsici	P890	Green pepper	0.17	1.18	1.18	0.99	4.98
	P891	Green pepper	0.23	1.17	1.33	8.22	6.25
	P1590	Cucumber	0.15	NT	1.12	1.11	4.82
cinnamomi	P411	Walnut	0.05	1.09	2.42	3.43	2.30
	P428	Avocado	0.08	0.14	3.32	5.70	3.02
	P440	Avocado	0.02	0.06	1.03	2.87	1.22
	P444	Avocado	0.05	0.34	4.87	2.18	1.27
citricola	P1315	Avocado	0.03	1.07	1.19	1.30	1.25
	P1356	Walnut	0.02	0.54	1.63	4.88	1.59
citrophthora	P1163	Citrus	0.08	0.33	0.09	0.97	8.20
	P1324	Citrus	0.11	0.28	0.13	1.63	5.61
	P3077	Citrus	0.08	0.23	0.47	0.87	6.67
	P1200	Cacao	0.09	0.22	5.87	7.06	15.96
cryptogea	P3147	Endive	0.34	0.47	9.55	13.92	19.47
megasperma	P509	Soybean	0.09	0.34	0.11	0.75	0.80
	P1724	Soybean	0.09	0.57	0.33	1.47	1.19
	P3153	Soybean	0.25	1.26	3.75	39.54	15.44
	P1331	Douglas fir	0.25	1.66	6.03	3.00	3.83
	P3160	Douglas fir	0.33	0.41	3.98	7.79	8.40
	P3161	Douglas fir	0.75	0.97	9.91	42.96	>100
palmivora	P436	Coconut	0.05	0.24	0.70	1.45	6.94
	P881	Cacao	0.11	0.19	1.15	3.96	10.54
	P376	Cacao	0.09	1.00	0.73	1.15	3.22
	P1399[d]	Cacao	0.94	>100	5.79	13.78	54.09
parasitica	P991	Citrus	0.55	0.62	5.64	5.77	24.14
	P1352	Tobacco	0.33	2.64	13.40	2.87	6.48
	P1962	Tomato	0.09	0.58	2.35	9.73	20.98

[a]Stock solutions of fungicides were prepared directly before use and buffered with 4-mopholine ethanesulfonic acid hydrate. The pH was adjusted to 6.2 with KOH. The chemicals were added to 0.5% cornmeal agar after autoclaving.

[b]Inhibition is based on comparison to unamended medium. Growth was measured after 4 days in the dark at 24 C. Values are expressed as the concentration in μeq of HPO_3^{-2} per milliliter (for PP, MEP, DMP, DEP) or mM (for PHP) required to cause 50% inhibition of mycelial growth. NT = not tested.

[c]Isolate P1686 is an American Type Culture Collection isolate (ATCC 16693).

[d]Isolate P1399 is a phosphonate-resistant strain of P376 produced by chemical mutagenesis using MNNG.

removed from the cups, rinsed under running tap water, and blotted dry on paper towels, and the percent stem infection was determined as already described for the pepper experiments. There were 10 plants per chemical treatment, and each experiment was repeated at least once.

Ion chromatographic analysis of phosphonate. Five seedlings of *P. indica* were placed in 250 ml of the four phosphonate compounds (HPO_3^{-2} at 5 μeq/ml) and potassium hypophosphonate (5 mM), as described earlier. After 24 hr, the seedlings were either removed from the cups, rinsed well, and placed into 250 ml of deionized water, or were allowed to remain in the fungicide solutions. At 24, 48, 96, or 168 hr after the initial fungicide treatment, three plants were selected at random from the cup and a 10-cm segment of the stem was excised from the crown upward, weighed, and rinsed well with running tap water. The tissue was chopped coarsely with a razor blade and ground to a fine powder under liquid nitrogen. The powder was transferred to a 15-ml vial and extracted with 5 ml of deionized water per gram of fresh weight tissue for 1 hr on a reciprocal shaker at 180 strokes/min. Next, 1 ml of the extract was transferred to an Eppendorf microfuge tube and centrifuged for 10 min in an Eppendorf microfuge, model 5414. The supernatant was diluted with deionized water and passed through a Sep-Pak C_{18} cartridge and a 0.22-μm filter before injection into the Dionex 2000i/P ion chromatography apparatus (Dionex Corp, Sunnyvale, CA). Phosphonate was analyzed as described previously, using conductivity detection and eluent suppression (23).

RESULTS

In vitro antifungal activity. Of the five compounds evaluated, with few exceptions, potassium phosphonate was the most inhibitory to *Phytophthora* (Table 1). Generally, sensitivity of isolates to potassium phosphonate within species was similar. Isolates of *P. citricola, P. cinnamomi, P. citrophthora,* and *P. palmivora* were the most sensitive, with EC_{50} values ranging from 0.02 μeq of HPO_3^{-2} per milliliter for *P. citricola* (P1356) and *P. cinnamomi* (P440) to 0.11 μeq/ml for *P. palmivora* (P1200) (Table 1). Isolates of *P. megasperma, P. cactorum,* and *P. capsici* were the least sensitive of those tested, with EC_{50} values ranging from 0.09 μeq of HPO_3^{-2} per milliliter for *P. megasperma* f. sp. *glycinea* from soybean to 0.75 μeq/ml for an isolate of *P. megasperma* (P3161) from Douglas fir (Table 1).

Potassium hypophosphonate was markedly less inhibitory than potassium phosphonate, sometimes by more than two orders of magnitude (Table 1). Variability in sensitivity of isolates was high, with EC_{50} values toward *P. megasperma* differing by 100 and EC_{50} values toward isolates of *P. cinnamomi* differing by a factor of less than three (Table 1).

Among the alkyl phosphonate compounds, monoethyl phosphonate was more inhibitory than dimethyl phosphonate against approximately two thirds of the isolates. With few exceptions, monoethyl phosphonate was more inhibitory than diethyl phosphonate, in some cases up to 40 times more so (Table 1). Dimethyl phosphonate was more inhibitory than diethyl phosphonate toward the majority of the *P. citricola, P. palmivora, P. megasperma,* and *P. citrophthora* isolates, respectively (Table 1).

Variability in the range of EC_{50} values for diethyl and dimethyl phosphonate toward isolates of *Phytophthora* was higher than for monoethyl phosphonate (Table 1). Excluding the two phosphonate-resistant mutants, *P. capsici* (P1361) and *P. palmivora* (P1399), EC_{50} values for monoethyl phosphonate ranged from 0.06 μeq of HPO_3^{-2} per milliliter for *P. cinnamomi* (P440) to 3.9 μeq/ml for the P770 isolate of *P. cactorum* (Table 1). By comparison, EC_{50} values for diethyl phosphonate ranged from 0.75 μeq of HPO_3^{-2} per milliliter for *P. megasperma* (P509) to 42.96 μeq/ml for *P. megasperma* (P3161), while EC_{50} values for dimethyl phosphonate ranged from 0.09 μeq/ml for *P. citrophthora* (P1163) to 34.6 μeq/ml for the P770 isolate of *P. cactorum* (Table 1).

Interestingly, monoethyl and dimethyl phosphonate were both more inhibitory than potassium phosphonate toward *P. capsici* isolate P1319, the isolate used to inoculate pepper plants for the in vivo efficacy evaluations (Table 2). The EC_{50} values for monoethyl and dimethyl phosphonate were 0.32 and 0.26 μeq of HPO_3^{-2} per milliliter, respectively, while the EC_{50} for potassium phosphonate toward this isolate was 0.51 μeq/ml (Table 2). The EC_{50} values for monoethyl, diethyl, dimethyl, and potassium phosphonate and for potassium hypophosphonate toward the phosphonate-resistant mutant P1361 were 23.33, 12.72, 4.62, and 4.09 μeq of HPO_3^{-2} per milliliter and 38.02 mM, respectively. The EC_{50} values for the phosphonate compounds against *P. citricola* P1287, the isolate used to inoculate *P. indica*, were 0.44, 3.42, 2.14, and 0.18 μeq of HPO_3^{-2} per milliliter for monoethyl, diethyl, dimethyl, and potassium phosphonate, respectively, and 3.64 mM for potassium hypophosphonate (Table 2).

In vivo efficacy of phosphonate compounds against stem rot of pepper. No compound was effective in controlling stem rot of pepper caused by the phosphonate-resistant mutant P1361, with 100% plant mortality resulting after 6 days. Monoethyl, diethyl, dimethyl, and potassium phosphonate provided partial control of stem rot caused by the wild-type isolate *P. capsici* P1319 when used either as a protective or a curative treatment, although no compound gave complete control (Figs. 2 and 3). When used as a protective treatment, all four phosphonate compounds demonstrated similar efficacy, with diethyl and dimethyl phosphonate more active in suppressing lesion development than monoethyl or potassium phosphonate at 1 μeq of HPO_3^{-2} per milliliter, while percent stem infection values were comparable at both 5 and 10 μeq/ml (Fig. 2A). Percent stem infection values were higher than lesion length values, ranging from 36 to 65% (Fig. 2B). Potassium hypophosphonate provided no control.

When used as a curative treatment, all four phosphonate compounds provided similar levels of control, with increased

TABLE 2. In vitro antifungal activity of monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), and potassium phosphonate (PP), and of potassium hypophosphonate (PHP) toward mycelial growth of the two *Phytophthora* species used in in vivo efficacy evaluations with *Persea indica* and *Capsicum annuum*

Fungicide[a]	Isolate[b]	EC_{50}[c]	Regression equation[d]	Correlation coefficient
MEP	P1361	23.33	$Y = 3.695 + 0.954X$	0.991
	P1319	0.32	$Y = -5.836 + 1.683X$	0.988
	P1287	0.44	$Y = -5.285 + 0.806X$	0.983
DEP	P1361	12.72	$Y = 2.388 + 2.365X$	0.993
	P1319	3.01	$Y = 4.293 + 0.147X$	0.902
	P1287	3.42	$Y = 4.699 + 0.563X$	0.910
DMP	P1361	4.62	$Y = 3.124 + 0.282X$	0.925
	P1319	0.26	$Y = -5.415 + 0.718X$	0.954
	P1287	2.14	$Y = 4.500 + 1.510X$	0.913
PP	P1361	4.09	$Y = 1.556 + 1.364X$	0.985
	P1319	0.51	$Y = -5.894 + 3.033X$	0.990
	P1287	0.18	$Y = -5.844 + 1.143X$	0.985
PHP	P1361	38.02	$Y = 2.971 + 1.284X$	0.988
	P1319	9.20	$Y = 3.448 + 1.611X$	0.958
	P1287	3.64	$Y = 4.400 + 1.068X$	0.950

[a] Fungicide stock solutions were prepared directly before use and buffered with 4-morpholine ethanesulfonic acid hydrate. The pH was adjusted to 6.2 with KOH. The compounds were added to 0.5% cornmeal agar after autoclaving.

[b] P1287 is an isolate of *P. citricola* from avocado. P1319 is an isolate of *P. capsici* from green pepper and P1361 is a phosphonate-resistant mutant of P1319 derived by chemical mutagenesis using MNNG.

[c] EC_{50} values are expressed as either microequivalents of HPO_3^{-2} per milliliter for the phosphonate compounds or mM for potassium hypophosphonate. Agar plugs, 5 mm in diameter, of the fungi were placed centrally on 0.5% cornmeal agar containing varying amounts of the phosphonate fungicides and grown in the dark at 24 C for 96 hr. The inhibition of mycelial radial growth was divided by the unamended control and transformed into probits, and regression analysis was performed to determine the log-dosage response.

[d] Y = probit of inhibition, X = log of fungicide concentration.

efficacy being achieved at the higher fungicide concentrations (Fig. 3A and B). At 1 μeq of HPO_3^{-2} per milliliter, dimethyl phosphonate was the most effective, while the 5 and 7 μeq/ml treatments resulted in similar lesion length values for all compounds (Fig. 3A). As with the protective treatment, percent stem infection values were greater than lesion length values for plants treated with all four compounds; values ranged from 20 to 70% (Fig. 3B). Again, potassium hypophosphonate provided no control.

In vivo efficacy of phosphonate compounds against stem canker of *P. indica*. All four phosphonate compounds provided effective control when applied as protective treatments. There were no differences in percent stem infection among plants treated in a protective manner with monoethyl, diethyl, dimethyl, or potassium phosphonate at concentrations of either 1, 5, or 10 μeq of HPO_3^{-2} per milliliter. *P. citricola* was recovered from 35–50% of the stem tissue, but no disease symptoms were observed. In contrast, both control and potassium hypophosphonate-treated plants had extensive stem necrosis, although plants treated with potassium hypophosphonate at 10 mM had about 45% less stem infection than the control.

When used as a curative treatment, plants treated with the four phosphonate compounds showed no stem necrosis and looked healthy. Again, *P. citricola* could be recovered from plants treated with these four phosphonate compounds (Fig. 4). The reduction in percent stem infection by potassium phosphonate was concentration-dependent (Fig. 4). Plants treated with 3 or 5 mM potassium hypophosphonate had extensive stem necrosis and showed little or no control of *P. citricola*, although stem infection was about 25% less than on the control plants.

Phosphonate analysis. After treatment of *P. indica* plants for 24 hr with monoethyl, diethyl, dimethyl, or potassium phosphonate at concentrations of 5 μeq of HPO_3^{-2} per milliliter, stem tissue contained 1.35, 0.96, 1.52, and 2.71 μeq of HPO_3^{-2} per gram fresh weight, respectively. The levels of phosphonate in the stems at 24 hr were not significantly different from the levels found at 168 hr in plants treated with monoethyl and potassium phosphonate and then transferred into water (Table 3). In contrast, stem tissue from plants treated with dimethyl and diethyl phosphonate for 24 hr and transferred to water contained significantly more phosphonate at 168 hr than at 24 hr. Higher levels of phosphonate were present in stems of plants that remained in the fungicide solutions for 168 hr compared to those that were transferred to water (Table 3). Only low levels of phosphonate (0.37 μeq of HPO_3^{-2} per gram fresh weight) were present in stem tissue of plants treated with potassium

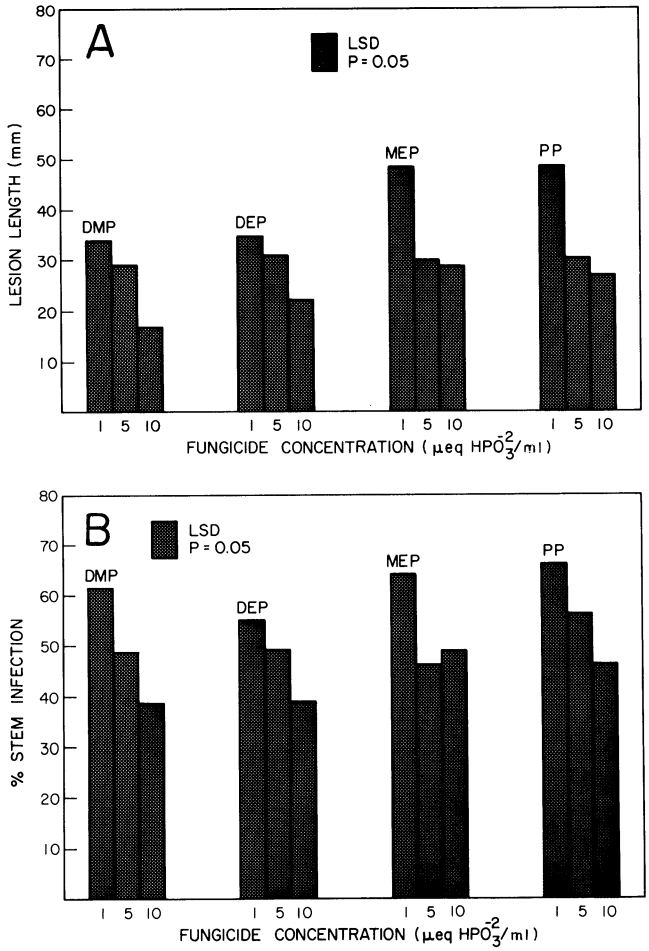

Fig. 2. Protective efficacy of monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), and potassium phosphonate (PP) in reducing stem rot and percent stem infection of 5-wk-old pepper plants caused by a wild-type isolate of *Phytophthora capsici* (P1319). **A,** lesion length. **B,** percent stem infection. Pepper plants were pretreated for 24 hr with the various phosphonate compounds at concentrations of 1, 5, or 10 μeq of HPO_3^{-2} per milliliter, then placed into cups containing water and inoculated with 1×10^6 zoospores. Six days later, stem rot severity and percent stem infection were determined by measurement of lesions and by plating out pieces of stem tissue onto PARP medium and observing them for the next 4–6 days for the presence of *Phytophthora*.

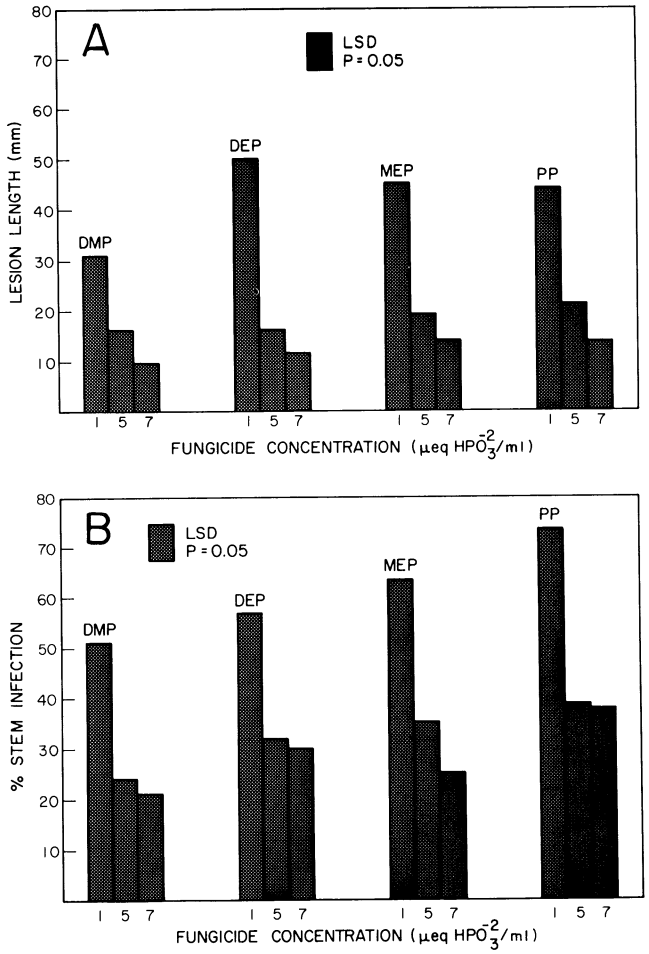

Fig. 3. Curative efficacy of monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), and potassium phosphonate (PP) in reducing stem rot and percent stem infection of 5-wk-old pepper plants caused by a wild-type isolate of *Phytophthora capsici* (P1319). **A,** lesion length. **B,** percent stem infection. Pepper plants were inoculated with 1×10^6 zoospores and 12 hr later were treated with the various phosphonate compounds at concentrations of 1, 5, and 7 μeq of HPO_3^{-2} per milliliter. Six days later, stem rot severity and percent stem infection were determined by measurement of lesions and by plating out stem tissue onto PARP medium and observed it for 4 6 days for the presence of *Phytophthora*.

hypophosphonate, even after 168 hr of continuous treatment (Table 3).

DISCUSSION

A comparative analysis of four phosphonate compounds and potassium hypophosphonate revealed that, in vitro, potassium phosphonate was generally the most inhibitory to mycelial growth of the majority of the isolates of nine *Phytophthora* spp. tested. Among the species evaluated, isolates of *P. citricola, P. cinnamomi, P. palmivora*, and *P. citrophthora* were generally very sensitive to potassium phosphonate, while those of *P. capsici, P. megasperma*, and *P. cactorum* were much less sensitive, which agrees with earlier reports (6,16). In most cases, substitution of one or more alkyl groups on the phosphonate molecule decreased activity toward *Phytophthora*, although there were several examples of increased inhibition. These biological differences might be explained by alterations in the site specificity of a metabolic process being selectively inhibited. Occasionally, one or more alkyl-substituted compounds can exert a stronger effect.

Although alkyl substitutions of the phosphonate molecule affected in vitro antifungal activity against *Phytophthora*, there was little difference in the phosphonate compound's efficacy in controlling stem rots of pepper and *P. indica* caused by *P. capsici* and *P. citricola*, respectively. For example, in vitro, potassium phosphonate was approximately 2.4, 12, and 19 times as fungitoxic as monoethyl, dimethyl, and diethyl phosphonate, respectively, toward an isolate of *P. citricola* (P1287). Yet in vivo, all four compounds exhibited similar efficacy in controlling stem rot of *P. indica* caused by this isolate. In addition, the in vitro antifungal activity of monoethyl and dimethyl phosphonate against an isolate of *P. capsici* (P1319) was about 1.6 and 2 times as great, respectively, as those of potassium phosphonate and 9.4 and 11.6 times as great, respectively, as those of diethyl phosphonate. However, all four phosphonate compounds demonstrated remarkably similar activity in reducing both stem rot and fungal development of this isolate on pepper plants.

Although it has been known for some time that ethyl phosphonate is degraded to phosphonate upon application to plants, only recently has research shown that treatment of plants with diethyl and dimethyl phosphonate can also result in high levels of phosphonate being present in plant tissues (23).

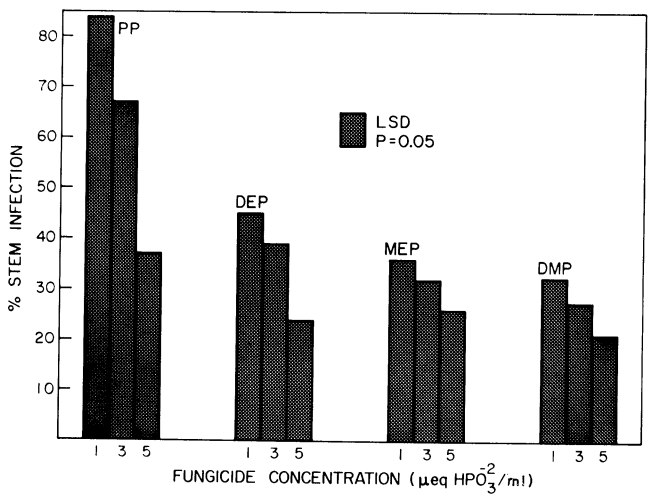

Fig. 4. Curative efficacy of monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), and potassium phosphonate (PP) in reducing percent stem infection of *Persea indica* by an isolate of *Phytophthora citricola* (P1287). *P. indica* plants were inoculated with 1×10^6 zoospores of *P. citricola* and 12 hr later were transferred to solutions of the various phosphonate compounds at concentrations of 1, 3, or 5 μeq of HPO_3^{-2} per milliliter. Six days later, percent stem infection was determined by plating out stem pieces onto PARP medium and observing them for the next 4–6 days for the presence of *Phytophthora*.

Additional phosphonate residue data obtained in this study support the concept that, for the alkyl-substituted phosphonates, in vivo metabolism to phosphonate is probably a primary factor in disease control. For example, phosphonate (HPO_3^{-2}) levels found in *P. indica* stem tissue treated with monoethyl, diethyl, and dimethyl phosphonate for 24 hr were 2.11, 2.83, and 3.66 μeq/g fresh weight, respectively. These in vivo phosphonate levels are 12–20 times higher than the EC_{50} values for potassium phosphonate for inhibition of mycelial growth of the pathogen *P. citricola* in vitro.

The lack of in vivo efficacy of potassium hypophosphonate in controlling stem rots of both pepper and *P. indica* is probably the result of two factors: the low in vitro antifungal activity of potassium hypophosphonate relative to that of potassium phosphonate and the lack of oxidation of hypophosphonate to phosphonate to any appreciable degree in the plant. Ion chromatographic analysis of stem tissue of *P. indica* treated with potassium hypophosphonate showed that only very low levels of phosphonate were present even 168 hr after continuous treatment.

While the mode of action of phosphonate remains unknown, phosphonate inhibition of *Phytophthora* might be explained by selective interference with a specific biochemical process, as is true with some other systemic fungicides such as metalaxyl (11). The lack of control afforded by all four phosphonate compounds in vivo against a phosphonate-resistant mutant of *P. capsici* on pepper stems in this study suggests a central role of the phosphonate anion in direct antifungal activity. These results are similar to those of Fenn and Coffey (17,18), who found that phosphonate-resistant isolates of *P. capsici* and *P. parasitica* var. *nicotianae* were not controlled on tomato leaves and tobacco seedlings, respectively, even though the seedlings contained high amounts of phosphonate which inhibited the wild-type parental isolates. In addition, Dolan and Coffey (12) found that phosphonate-resistant mutants of *P. palmivora* possessing moderate to high levels of phosphonate resistance in vitro were

TABLE 3. Phosphonate levels in *Persea indica* stem tissue after treatment with monoethyl phosphonate (MEP), diethyl phosphonate (DEP), dimethyl phosphonate (DMP), or potassium phosphonate (PP), at concentrations of 5 μeq of HPO_3^{-2} per milliliter, or of 5 mM potassium hypophosphonate (PHP), as determined by ion chromatography[x]

Time (hr)	Treatment[y]	Phosphonate levels[z] MEP	DEP	DMP	PP	PHP
24	Fungicide	1.35 d	0.96 d	1.52 c	2.71 b	nd
48	Fungicide	3.20 bc	2.32 cd	3.38 bc	2.13 b	0.15 bc
	Water	1.85 cd	1.56 cd	1.85 c	1.79 b	0.04 d
96	Fungicide	4.62 ab	4.46 b	5.66 b	2.50 b	0.15 bc
	Water	1.90 cd	2.20 cd	2.19 c	2.62 b	0.22 b
168	Fungicide	5.85 a	6.83 a	7.91 a	4.80 a	0.37 a
	Water	2.11 cd	2.83 c	3.66 b	1.91 b	0.09 cd

[x]Five 6-wk-old *P. indica* plants were placed in styrofoam cups containing 250 ml of the various phosphonate compounds and were either left in the chemical for 168 hr or were removed after 24 hr, rinsed well, and placed into the cups containing 250 ml of deionized water. The phosphonate compounds were adjusted to pH 6.2 with KOH and buffered with 5 mM 4-morpholine ethanesulfonic acid hydrate. The plants were kept in a growth chamber maintained at 24 C with a 12-hr photoperiod providing 170 $\mu E/cm^2/sec$ at the plant surface.

[y]Fungicide = plants were left in fungicide for this length of time; water = plants were left in fungicide for 24 hr and then transferred to water until this time.

[z]Stem segments, 10-cm long, were chopped coarsely with a razor blade, ground to fine powder under liquid nitrogen, placed in a 15-ml vial, and extracted with 5 ml of water per gram of fresh weight tissue for 1 hr on a reciprocal shaker at 180 strokes per minute. The extract was centrifuged, diluted with water if neccessary, and passed through a Sep-Pak C_{18} cartridge and a 0.22-μm filter before analysis by ion chromatography. Values are the mean of three replicates, and values with the same letter within columns are not significantly different according to Duncan's multiple range test. ND = none detected.

not controlled on tomato seedlings by phosphonate levels that controlled the parental wild-type.

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