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Evaluation of drench treatments with phosphonate derivatives against *Pseudomonas syringae* pv. *syringae* on pear under controlled environment conditions

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Abstract

Several phosphonate derivatives including the oomycetic antifungal agents phosphonate and tris-o-ethylphosphonate (fosetyl), the ethylene-releasing compound 2-chloroethylphosphonate (ethephon), and the antibiotic 2-epoxypropylphosphonate (phosphomycin) were evaluated for *in vitro* and *in planta* activity against *Pseudomonas syringae* pv. *syringae*. Inhibition of colony growth in CYE agar by phosphonate, fosetyl and etephon was very slight (minimal inhibitory concentrations MIC= 0.31–0.62 g HPO_3^{2-}/l). Also, survival of *P. syringae* pv. *syringae* in aqueous solutions of phosphonate or fosetyl was high. Only phosphomycin showed significant antibacterial activity *in vitro* (MIC=10-20 μg HPO_3^{2-}/ml) compared to streptomycin (1-2 μg a.i./ml). Potted pear plants irrigated with these chemicals and inoculated with *Pseudomonas syringae* pv. *syringae* had significantly less disease than non-treated controls ($P<0.001$). Phosphomycin was the most active compound with a median effective dose (ED_{50}) of less than 0.62 g HPO_3^{2-}/l. Activities of the other phosphonates were weak but consistent between experiments.The ED_{50}s on whole plants were 2.1, 3.3, and 6.9 g HPO_3^{2-}/l for ethephon, phosphonate and fosetyl, respectively. The $ED_{50}$of *P. syringae* pv. *syringae* increased from 6.5 in non-treated controls to 7.7-8.8 $\log_{10}$ cfu/ml on plants treated with phosphonates at 1.86 g HPO_3^{2-}/l. It was concluded that drench treatment with fosetyl is not a practical option for control of *P. syringae* pv. *syringae* on pear.

Introduction

Blast of pear caused by *Pseudomonas syringae* pv. *syringae* is one of the bacterial diseases that limit pear production throughout the world. Symptoms are characterized by blast of buds, blossoms, leaves or fruits which occur in periods of cool wet weather during bloom and post-bloom stages (Jones and Aldwinckle, 1990). Large resident epiphytic populations of *P. syringae* pv. *syringae* with ice-nucleation activity commonly develop on apparently healthy pear surfaces and may cause disease under suitable environmental conditions (Gross et al., 1983; Lindow et al., 1982; Mansvelt and Hattingh, 1988; Montesinos and Vilardell, 1991; Whitesides and Spotts, 1991).

Control of bacterial blast of pear with chemicals is difficult and is based on copper compounds and antibiotics which are recommended according to the specific legal restrictions of each country (De Waard et al., 1993; Jones and Aldwinckle, 1990). However, their use is limited by factors such as low efficacy, phytotoxicity to the plant or development of resistance of the pathogen (Andersen et al., 1991).

Phosphonates are a class of chemical compounds derived from phosphorous acid based on O-P (e.g. fosetyl) or C-P bonds (e.g. ethephon) some of which have applications in crop protection (Hilderbrand, 1983). Fosetyl-Al and potassium phosphonate are used to control diseases caused by Oomycete fungi (Bompeix et al., 1990; Coffey and Joseph, 1985; Darvas et al., 1984;

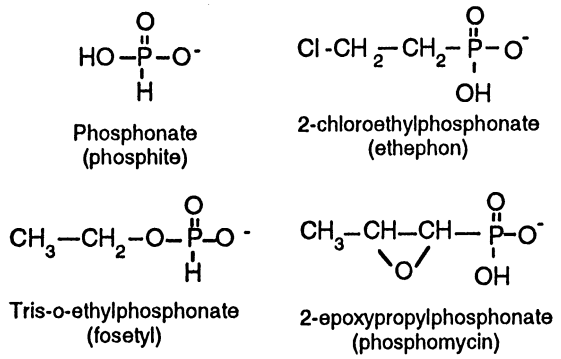

Figure 1. Chemical structures of phosphonate derivatives used in this study.

Dolan and Coffey, 1988; El-Hamalawi et al., 1995). Fosetyl-Al has been evaluated in greenhouse and field trials for the control of several bacterial diseases caused by *Erwinia chrysanthemi*, *Pseudomonas cichorii* and several *Xanthomonas campestris* pathovars on ornamental plants (Chase, 1993), *X. arboricola* pv. *citri* on citrus (McGuire, 1988), and *Erwinia amylovora* on pear and apple (Paulin et al., 1990). However, the main problems reported in these trials were inconsistency of results, moderate efficacy and the high dose and number of applications needed to obtain reliable disease control. Ethephon was reported to give some degree of protection on plants against viral infections (Bellés et al., 1990, Van Loon, 1977). Phosphomycin is an antibiotic used in the control of certain human and animal diseases (Shoji et al., 1986) but has not been tested on plants.

The purpose of this work was to determine the efficacy of fosetyl, phosphonate, phosphomycin, and ethephon in controlling *Pseudomonas syringae* pv. *syringae* on pear under conditions conducive to disease development. To decrease sources of variability clonal plant material was employed, phosphonates were applied by irrigation; the pathogen was inoculated using a detached leaf assay or by local infiltration of leaves on whole plants, and disease development was performed under controlled environment conditions.

Materials and methods

Phosphonate derivatives

Phosphonates used were potassium phosphonate (Alexin95 PS^R, Comercial Química Massó S.A., Barcelona, Spain), aluminium tris-o-ethylphosphonate (fosetyl-Al, Aliette^R, Rhône Poulenc Agro, France), 2-chloroethylphosphonate (ethephon, Ethrel48^R, Compagnie Francaise de Produits Industrielles, France) and 2-epoxypropylphosphonate (phosphomycin, kindly given by Fermentaciones y Síntesis Españolas, S.A., Spain). The products were selected because they have a common phosphonate group, but there are differences in chemical structure (Figure 1). Streptomycin (Sigma Chemical Co., USA) was used as a reference antibiotic. The concentrations of all phosphonate derivatives are expressed as $[HPO_3]^{2-}$ which permits a comparison on the basis of their $[HPO_3]^{2-}$ content. Values given can be converted to the corresponding weight of active ingredient by multiplication using the following conversion factors: 1.38 (for fosetyl), 1.02 (for phosphonate), 1.81 (for ethephon), and 1.73 (for phosphomycin).

Table 1. List and origin of bacterial strains

Bacteria	Host plant	Source[a]
Erwinia amylovora 6076	pear	PMV
P. syringae pv. *syringae* EPS17a	pear	TAG-EPS
P. syringae pv. *syringae* EPS94	pear	TAG-EPS
P. syringae pv. *syringae* MV4	pear	TAG-EPS
P. syringae pv. *syringae* LL3Y	pear	TAG-EPS
P. syringae pv. *syringae* 2027-37	pear	CFBP
P. syringae pv. *syringae* 1392	pear	CFBP
P. syringae pv. *syringae* S*	pear	OSU
Xanthomonas campestris pv. *campestris* 1119	cabbage	CFBP
X. arboricola pv. *corylina* 2565	hazelnut	CFBP
X. arboricola pv. *juglandis* 1317.3	walnut	IVIA
Pantoea herbicola EPS494	pear	TAG-EPS
Pseudomonas fluorescens EPS288	corn	TAG-EPS
P. viridiflava 2107	bean	CFBP

[a] CFBP – Collection Francaise de Bactéries Phytopathogènes, INRA Angers, France; IVIA – M. López, Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain; OSU – D. Gross, Oregon Stat University, USA; PMV – Laboratoire de Pathologie Moleculaire et Vegetale- INRA/INA-PG, Paris, France; TAG-EPS – Unitat de Tecnologia Agroalimentària-University of Girona, Spain.

Assessment of in vitro *activity*

The effect of fosetyl, phosphonate, ethephon and phosphomycin on the growth of 14-bacterial strains of plant pathogenic and non-pathogenic bacteria (Table 1) was determined by a growth inhibition agar incorporation test. Bacteria from either lyophilized or −80°C stored

cultures, were streaked onto King's B agar (King et al., 1954) or Yeast extract-Dextrose-Calcium carbonate (YDC) agar (Schaad, 1988) depending on the species, and grown at 25 °C for 2-to-3 days. For growth inhibition assays, the low-complexing non-amended phosphate medium casitone-yeast extract-glycerol (CYE) was used (Andersen et al., 1991). The medium contained per liter 1.7 g casitone, 0.35 g yeast extract, 2.0 g glycerol, and 15.0 g of purified agar. Total phosphorus content was assessed in CYE agar transformed to ash, extracted by acidic digestion and determined as phosphate by the phosphovanadomolybdate method (Jeffrery et al., 1989). Mean concentration of total phosphorus was 25-30 μg $[HPO_4]^{2-}$/g f.w.

Stock solutions of the chemical compounds made up in distilled water were added to molten CYE agar at 50 °C on Petri plates to achieve concentrations of 634, 317, 158, 79, 39, 19, 10, 5, 2.5, 1.25, 0.62, 0.31, and 0 μg $[HPO_3]^{2-}$/ml. For streptomycin the concentrations assayed were in μg a.i./ml. Bacterial suspensions (ca. 10^9 cfu/ml) were inoculated onto the agar surface with a sterile toothpick and the plates incubated at 25 °C. Colony growth after 5 days was taken as tolerance to the corresponding dose of the chemical. The experimental design consisted of combinations of five products by thirteen concentrations, and three replicates per treatment. Non-amended Petri plates were used as non-treated controls. Each Petri plate contained the fourteen strains tested. The experiment was conducted twice.

Survival of *P. syringae* pv. *syringae* in solutions of fosetyl and phosphonate was studied with strains EPS17a and EPS94. Suspensions of 10^8 cfu/ml of the bacteria were made up in either 0, 0.16, 0.31, 0.62, or 1.24 g $[HPO_3]^{2-}$/l of each phosphonate derivative. Samples were taken at 0 and 60 min, 10-fold diluted in series and plated onto King's B agar. Inoculated Petri plates were incubated at 25 °C and viable colonies counted after 3 days. The experimental design consisted of two strains, two phosphonate derivatives at five different concentrations, and three replicates per treatment.

Plant growth and treatment with phosphonates

Self-rooted pear plants of cultivar Conference (CAV clone) obtained by micropropagation (Agromillora Catalana, S.A., Barcelona, Spain) were used. Plants were 2-to-3 years old, about 50 cm high and were grown in 1 liter capacity plastic containers filled with a commercial peatmoss/vermiculite/perlite potting mix (type BVU, Prodeasa, Girona, Spain). Plants were chilled during winter, pruned and forced to bud in the greenhouse 2-to-3 months before experiments began. Actively growing plants were maintained in the greenhouse and fertilized once a week with a solution of 200 ppm N-P-K (20-10-20). One month before starting experiments plants were fertilized with only a N-K solution (20-20), to prevent interference of phosphate phosphorus on the phosphonate trials. The phosphonate derivatives were applied by irrigation with 100 ml of either 0.62, 1.86, 3.72 or 6.20 g $[HPO_3]^{2-}$/l solutions depending on the experiment. This volume of solution was retained by the potting mix in the container. Treatments were applied once a day for the five days preceeding inoculation of the bacterial pathogens. Therefore, final amounts of phosphonate applied to the potting mix were 0.31, 0.93, 1.82 and 3.1 g $[HPO_3]^{2-}$, respectively. Plants irrigated with water alone were used as non-treated controls. The pH of the phosphonate derivative solutions and of the potting mix after treatment with phosphonate derivatives was determined. The pH of the potting mix was assessed by the slurry method which consists of mixing 10 g of material with 25 ml of either distilled water or 0.1M KCl (Wild, 1973).

Preparation of bacterial inoculum

Strains EPS17a and EPS94 of *P. syringae* pv. *syringae*, isolated from buds of pear trees from commercial orchards located near Girona (Catalunya, Spain) were used. Bacterial suspensions were prepared in sterile distilled water from King's B agar cultures grown at 25 °C for 24 h. The concentration of bacteria in the inoculum was adjusted by dilution of a concentrated suspension to 10^8cfu/ml. Concentration was determined using absorbance at 600 nm and interpolation in a previously obtained calibration curve.

Detached leaf assays

Thirty plants were used for each treatment and treatments consisted of irrigation with each phosphonate derivative at a dose of 1.86 g $[HPO_3]^{2-}$/l as described above. For inoculation of *P. syringae* pv. *syringae* using the detached leaf assay (Yessad et al., 1992), two young apical leaves of each plant were collected, disinfected by immersion for 5 min in a 1% solution of commercial sodium hypochlorite, and rinsed three times with sterile distilled water. Leaves were placed in square 12-cm sterile Petri dishes containing water agar (10 g/l) with

a sterile filter paper on the surface. For inoculation, the midvein of each leaf was cut with a scalpel and a 20 μl drop of the bacterial suspension was deposited onto the wound. Leaves inoculated with sterile distilled water were used as controls. The Petri dishes were sealed with plastic film and incubated at 25 °C using a 16 h light- 8 h dark photoperiod (150 μE.m^{-2}.s^{-1}) for 48 h. The experimental design consisted of five treatments (four phosphonate derivatives and a non-treated control), two bacterial strains (EPS17a and EPS94) and three replicates of six leaves for each treatment-strain combination. The experiment was conducted twice.

Whole plants trials

P. syringae pv. *syringae* was inoculated into whole pear plants by local infiltration of leaves (Ercolani, 1973). Infiltration was performed using a modified Hagborg's device (Hagborg, 1970). The apparatus consisted of forceps with the inner side tips provided with two soft rubber discs to avoid leaf damage. A hypodermic needle with a bevel edge tip was introduced through a hole made in one side of the forceps tip until the needle tip contacts the surface of the opposite rubber disc. The hypodermic needle was connected by means of a plastic tubing to a 500 μl gastight syringe (Hamilton-Bonaduz, Switzerland). The syringe was operated by an automatic precission microburette unit (Crison model Microbu 2031, Crison Instruments, S.A.). The microburette was controlled by a computer program operated by a PC compatible computer which permitted selection of injection volume and elapsed time between injections. For infiltration each leaf was held between the forceps by gentle pressure at the selected area and 20 μl of the bacterial inoculum were injected by the microburette through the hypodermic needle into the mesophyll of the leaf. Six local infiltrations of about 5 mm diameter per leaf were distributed on both sides of the midrib and all the leaves of each plant were inoculated (20-25 leaves) to make a total of 120–150 inoculations per plant. Inoculated plants were incubated at 25 °C and 85% relative humidity using a 16 h light- 8 h dark photoperiod (150 μE.m^{-2}.s^{-1}) for 5 days in a controlled environment chamber (Conviron, model PGR15, Winnipeg, Manitoba, Canada).

To study the effect of phosphonate derivatives on pathogen virulence, three doses (10^6, 10^7 and 10^8 cfu/ml) of *P. syringae* pv. *syringae* EPS94 and a water control were inoculated into plants treated with 1.86 g $[HPO_3]^{2-}$/l of each phosphonate derivative as described above. The experimental design consisted of three replicate plants per phosphonate type-pathogen-dose combinations. The experiment was conducted twice.

For the effect of dose of phosphonate derivative on disease, four doses of each phosphonate were tested (0.62, 1.86, 3.72 and 6.20 g $[HPO_3]^{2-}$/l). Plants irrigated with water were used as non-treated controls. Inoculations were performed using a dose of 1x10^8 cfu/ml of *P. syringae* pv. *syringae* EPS94. The experimental design consisted of three replicate plants for each phosphonate type-dose combination. Six inoculations were performed per leaf and all leaves on each plant were inoculated. The experiment was conducted twice.

Disease assessment and data analysis

A five level disease severity rating was used for the detached leaf assay according to the following scale: 0, no infection; 1, necrosis located at the inoculation point; 2, necrosis affecting the inoculation point and the midvein; 3, necrosis expanding from the inoculation point through the midvein and additional veins; 4, necrotic area affecting veins and leaf tissues over more than 50% of the leaf surface. A four level scale was used for local infiltration of leaves on whole plants according to the following scale: 0, no infection; 1, necrosis located around the needle puncture; 2, necrosis affecting only the infiltrated area; 3, necrosis expanding from the infiltrated area to neighbouring tissues. Disease severity (*S*) was calculated for each of the six replicate leaves in the case of the detached leaf assay and for each plant in the whole plant assay. The following formula was used:

$$S = \frac{\sum_{n=1}^{N} I_n}{N \cdot I_{max}}$$

where, I_n is the corresponding rating of infection for each inoculation, *N* is the number of leaves inoculated per replicate box in the detached leaf assay or the number of infiltrations per plant in the whole plant assay. I_{max} is the maximum rating for either the detached leaf assay or local infiltration of leaves on whole plants. Severity was transformed to probit before analysis of data by ANOVA using the PC-SAS (SAS Institute, Cary, NC). Testing the hypothesis for the effect of product type, dose and pH of phosphonate derivative solution was carried out with an analysis of covariance using pH and dose as covariates. Half effective dose (ED_{50}) for *P. syringae* pv. *syringae* EPS94 and for each

Table 2. Minimum inhibitory concentrations (μg/ml)[a] for streptomycin (Str), phosphomycin (Phm), fosetyl (Fos), phosphonate (Pho) and ethephon (Etp) for several bacterial strains on amended CYE agar

Bacteria	Product				
	Str	Phm	Fos	Pho	Etp
Erwina amylovora (1 strain)	1–2	20–40	317–634	>634[b]	317–634
Pantoea herbicola (1 strain)	8–16	>80	317–634	>634	80–158
Pseudomonas fluorescens (1 strain)	16–32	20–40	158–317	>634	40–80
P. syringae pv. *syringae* (7 strains)	1–2	10–20	317–634	>634	317–634
P. viridiflava (1 strain)	0.5–1	20–40	158–317	>634	317–634
Xanthomonas spp. (3 strains)	2–16	2.5–20	317–634	>634	317–634

[a] For phosphonates the concentration is in equivalents of phosphonate.
[b] the highest dose tested.

chemical compound was calculated from disease-dose relationships according to Finney (1971).

Results

In vitro *activity*

Inhibition of colony development on low complexing CYE agar amended with phosphonate derivatives is shown in Table 2. The antibacterial activity of phosphonate, fosetyl and ethephon was very low for all bacterial strains tested. Phosphonate was the least active compound since the MIC was higher than 0.63 g $[HPO_3]^{2-}$/l. Twelve out of fourteen strains had a MIC for fosetyl higher than 0.32 g $[HPO_3]^{2-}$/l. Eleven out of fourteen strains had a MIC for etephon higher than 0.32 g $[HPO_3]^{2-}$/l. Phosphomycin showed higher *in vitro* activity than the other phosphonates and the MIC values ranged from 2.5 to 80 μg $[HPO_3]^{2-}$/ml. All phosphonates showed in general lower antibacterial activity than streptomycin. At the corresponding MIC, the pH of the CYE medium was 3.7 for fosetyl, 6.5 for phosphonate, 2.8 for ethephon and 7.5 for phosphomycin. *P. syringae* pv. *syringae* survived relatively well in solutions of fosetyl or phosphonate (Figure 2). There were no significant differences in viable bacteria depending on the strain (P=0.414) or the type of phosphonate derivative (P=0.474). A slight effect of the concentration of phosphonate derivative on bacterial viability was observed (P=0.012), but differences were not significant at concentrations up to 0.6 g $[HPO_3]^{2-}$/l.

Effect of phosphonates on disease levels

Disease severity in plants irrigated with the four phosphonate derivatives at 1.86 g $[HPO_3]^{2-}$/l in comparison with non-treated plants are shown in Table 3.

Plants irrigated with phosphonate derivatives and tested with the detached leaf assay showed significantly lower severity levels than non-treated controls ($P<0.001$). There were no significant differences neither between the two strains of *P. syringae* pv. *syringae* tested (P=0.276) nor between the two independent experiments (P=0.187) performed. The most effective treatments were ethephon (76% disease reduction) and phosphomycin (68%). Fosetyl and phosphonate were equally effective (49%) (P=0.236). Phytotoxicity was not observed at the dose applied for phosphonate, fosetyl or phosphomycin. However, ethephon produced some defoliation and inhibition of internodal elongation on plants.

Plants treated with phosphonate derivatives and inoculated with *P. syringae* pv. *syringae* by local infiltration of leaves, also showed a significant decrease in disease levels ($P<0.001$) in comparison with non-treated controls. No significant differences were observed between the two experiments (P=0.195). The most active compound was phosphomycin (85% dis-

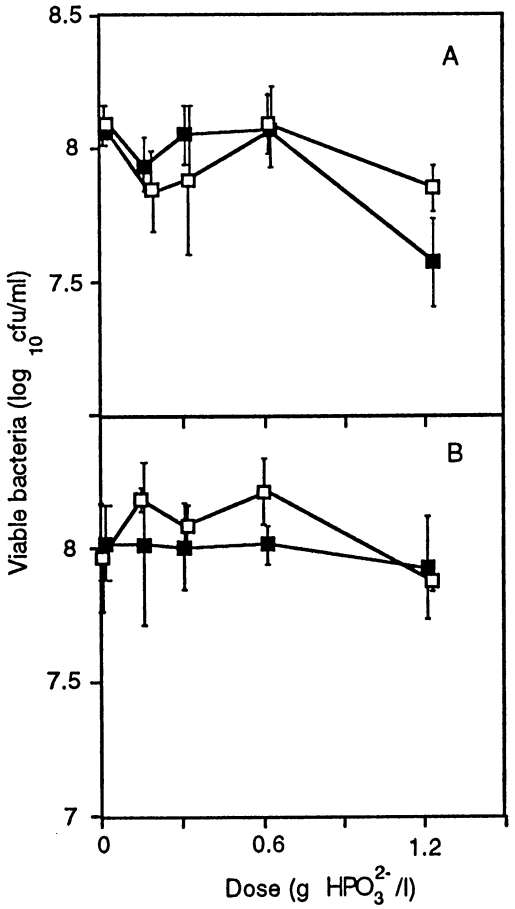

Figure 2. Survival of strains EPS17a (□) and EPS94 (■) of *P. syringae* pv. *syringae* exposed for 60 min to solutions of A), fosetyl, or B), phosphonate at different concentrations. Values are the means of three replicates. Error bars indicate confidence intervals (*P*=0.01).

ease reduction), followed by phosphonate and ethephon (43% disease reduction), and by fosetyl which was the least active chemical compound (16%).

The pH of aqueous solutions of phosphonate derivatives used for plant treatment were different. In the range of concentrations studied, pH was 3.2-4.6 for fosetyl, 5.7-5.8 for phosphonate, 2.0-3.3 for ethephon, and 7.3-9.1 for phosphomycin. At a rate of 1.86 g $[HPO_3]^{2-}$/l the pH of the potting mix was not significantly different between non-treated and phosphomycin treated plants (P=0.146) with mean values of 6.5. Differences were also not significant between ethephon, fosetyl and phosphonate (P=0.196) with mean values of 5.5. However, differences were significant between phosphomycin or water and fosetyl, ethephon or phosphonate (P<0.001).

Table 3. Disease severity[x] on pear plants cv. Conference inoculated with *Pseudomonas syringae* pv. *syringae* after treatment[y] with fosetyl, phosphonate, ethephon or phosphomycin as a soil drench

Treatment	Detached leaf assay	Whole plant assay
Non-treated	0.81 a[z]	0.93 a
Fosetyl	0.40 b	0.78 a
Phosphonate	0.42 b	0.53 c
Etephon	0.19 c	0.50 c
Phosphomycin	0.26 c	0.14 d

[x] Disease severity data were transformed to probit (*S*) before ANOVA and means separation tests were performed. Numbers are backtransformed mean probit values from two experiments with three replicates per experiment. Only strain EPS94 of *Pseudomonas syringae* pv. *syringae* was used for local infiltrations on whole plant trials whereas strains EPS17a and EPS94 were used for the detached leaf assay.

[y] Plants were irrigated five times with a solution of 1.86 g $[HPO_3]^{2-}$/l of phosphonate derivative before pathogen inoculation.

[z] Means within the same column and followed by the same letter do not differ significantly (P=0.05) according to Tukey's mean separation test.

Table 4. Virulence of *Pseudomonas syringae* p.v. *syringae* EPS94, and ED_{50} values of phosphonate derivatives for control of leaf necrosis on pear plants cv. Conference

Treatment[x]	ED_{50} pathogen[y] ($\log_{10}$(cfu/ml))	ED_{50} phosphonate[z] derivative (g $[HPO_3]^{2-}$/l)
Non-treated	6.54 ± 0.19 b[w]	–
Fosetyl	7.73 ± 0.47 ab	6.9 ± 0.8 a
Phosphonate	7.72 ± 0.36 ab	3.3 ± 0.6 b
Ethephon	8.02 ± 0.59 ab	2.1 ± 0.3 b
Phosphomycin	8.76 ± 1.01 a	<0.6

[x] Plants were irrigated once a day for the five days preceeding inoculation with the pathogen.

[y] Leaves were locally infiltrated with 20 μl of bacterial suspensions of either 0, 10^6, 10^7, or 10^8 cfu/ml. The concentration of each phosphonate derivative used was 1.86 g $[HPO_3]^{2-}$/l. ED_{50} was determined from Probit (*S*)-$\log_{10}$ dose plots of Figure 3. Numbers represent the mean and confidence interval (P=0.05) of pooled data from two independent experiments.

[z] Plants were treated with either 0.62, 1.86, 3.72, or 6.20 g $[HPO_3]^{2-}$/l of each phosphonate derivative before inoculation by local leaf infiltration with 20 μl of a bacterial suspension of 1×10^8 cfu/ml. ED_{50} for each chemical was determined from Probit (*S*)-dose plots of Figure 4. Numbers represent the mean and confidence interval (P=0.05) of pooled data from two independent experiments.

[w] Means within the same column and followed by the same letter do not differ significantly (P=0.05) according to Tukey's mean separation test.

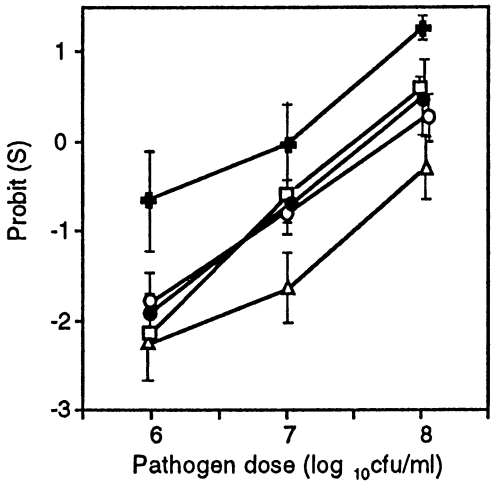

Figure 3. Dose-response relationships between disease levels and pathogen dose on potted pear plants cv. Conference irrigated with water (+), fosetyl (□), phosphonate (●), ethephon (○), or phosphomycin (△). Plants were inoculated by local infiltration of leaves with 20μl suspensions of 1x10^6, 1x10^7, and 1x10^8 cfu/ml of *P. syringae* pv. *syringae* EPS94. The dose of phosphonate was 1.86 g $[HPO_3]^{2-}$/l and a total amount of 0.83 g HPO_3^{2-} per plant was applied by irrigation. Values are the mean of two independent experiments and three replicate plants per experiment. Error bars indicate confidence intervals (P=0.01).

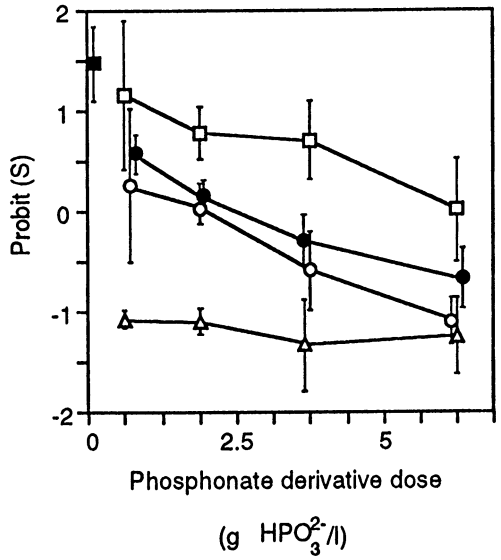

Figure 4. Effect of concentration of fosetyl (□), phosphonate (●), ethephon (○), or phosphomycin (△) on disease levels on potted pear plants cv. Conference inoculated by local infiltration of leaves with 20μl of suspensions of 1x10^8 cfu/ml of *P. syringae* pv. *syringae* EPS94. Phosphonates were applied by irrigation and plants were treated five times before inoculation with the pathogen. Values are the mean of two independent experiments and three replicates per experiment. Error bars indicate confidence intervals (P=0.01).

Effect of phosphonates on pathogen virulence

There were significant differences in the ED_{50} of the pathogen between phosphonate treated plants at a dose of 1.86 g $[HPO_3]^{2-}$/l and non-treated controls ($P<0.01$) (Table 4). The effect of phosphomycin was significantly different from non-treated control and increased by 130 times the ED_{50} of the pathogen (from 6.54 to 8.76 $\log_{10}$ cfu/ml). However, the effect of fosetyl-Al, K-phosphonate and ethephon was not significantly different from the non-treated control or from phosphomycin. The slope of the probit disease-pathogen dose response curve was not significantly different between treatments (P=0.065) (Figure 3).

Phosphonate derivative dose-disease relationships

There were significant differences in disease levels on whole plants depending on the phosphonate type ($P<0.001$) and dose of application ($P<0.001$) (Figure 4). When pH of treatment-dose combinations was used as a covariate, its effect on disease was not significant (P=0.300). Differences were not significant between the independent experiments performed (P=0.215). Therefore, data from both experiments were pooled to calculate the ED_{50} of phosphonates. Differences in ED_{50} among phosphonate derivatives were also significant ($P<0.001$) (Table 4) and ED_{50} ranged from less than 0.6 to 6.9 g $[HPO_3]^{2-}$/l. Fosetyl was the least active compound and showed the highest ED_{50}. Phosphonate was not significantly different from ethephon (P=0.232) and both were significantly more active than fosetyl. Phosphomycin was the most active phosphonate derivative. The slope of the probit disease-phosphonate dose was not significantly different between phosphonate and etephon (P=0.074) but was different between these phosphonates and fosetyl ($P<0.001$). The slope of phosphomycin was extremelly shallow and significantly different from the other phosphonates ($P<0.001$).

Discussion

Drenching the root system of pear plants with either phosphonate, fosetyl, ethephon or phosphomycin, decreased significantly and consistently disease levels after inoculation with *P. syringae* pv. *syringae*. Control levels obtained with a dose of 1.86 g $[HPO_3]^{2-}$/l ranged

from 45 to 80% in the detached leaf assay and from 16 to 84% in whole plants. The efficacy was comparable to that previously reported in other bacterial disease trials based on foliar application of fosetyl-Al for the control of *Erwinia amylovora* on pears (Paulin et al., 1990), and of several pathovars of *Xanthomonas* spp., *Erwinia chrysanthemi*, and *Pseudomonas cichorii* on ornamental plants (Chase, 1993) when successful control was achieved. However, these authors reported a high degree of inconsistency between trials.

The control levels obtained with bacterial diseases contrasted with the higher efficacies which were observed when fosetyl-Al or phosphonate were used for the control of fungal diseases caused by *Phytophthora* spp. on tomato, tobacco, capsicum, pepper and avocado (Bompeix et al., 1980; Coffey and Joseph, 1985; Darvas JM et al., 1984; Dolan and Coffey, 1988; El-Hamalawi et al., 1995) and by *Plasmopara viticola* on grape (Dercks and Creasy, 1989). These studies revealed that fosetyl-Al is absorbed and translocated in the plant, when applied either as a foliar spray or by irrigation (El-Hamalawi et al., 1995; Ouimette and Coffey, 1988) and is partially degraded into phosphonate (Coffey and Joseph, 1985; Fenn and Cofey, 1984; Williams et al., 1977). Plant tissue concentrations of phosphonate of 0.22 g $[HPO_3]^{2-}$/Kg f.w. have been reported in young avocado plants (El-Hamalawi et al., 1995) and of 0.54 g $[HPO_3]^{2-}$/Kg f.w. in tobacco seedlings which were irrigated three times with rates of fosetyl-Al of 1.0 to 2.1 g $[HPO_3]^{2-}$/l (Fenn and Cofey, 1984). These levels disappeared very slowly with time since plants cannot readily oxidize phosphonate to phosphate (El-Hamalawi et al., 1995; McIntire et al., 1950).

Control levels of bacterial blast of pear increased with the phosphonate derivative dose applied by irrigation and this observation allowed a calculation of ED_{50}values. The high ED_{50} obtained for fosetyl (6.9 g $[HPO_3]^{2-}$/l) indicates a weak *in vivo* activity of this chemical against *P. syringae* pv. *syringae* on pear by root application. However, the other phosphonates were more active. Our finding of a significant dose-response relationship for fosetyl contrasted with data reported by Chase (Chase, 1993) indicating that disease control on several ornamental plants was more or less independent of the dose.

The virulence of *P. syringae* pv. *syringae* was also affected by phosphonate derivative treatment of pear plants. A slightly significant increase was observed in the ED_{50} of *P. syringae* pv. *syringae* in pear plants treated with fosetyl, phosphonate and ethephon at 1.86 g $[HPO_3]^{2-}$/l in comparison with non-treated controls. These findings contrast with other reports showing that *Xanthomonas campestris* introduced into host plants by leaf infiltration reached similar numbers whether or not plants were treated by foliar application of fosetyl-Al at 3.47 g $HPO_3{}^{2-}$/l (Chase, 1993).

The high antibacterial activity of phosphomycin in pear plants agreed with inhibition of bacterial growth on amended CYE agar and with the antibiotic nature of the compound which is produced by some strains of *Pseudomonas syringae* (Shoji et al., 1986). The low activity of fosetyl in pear plants against *P. syringae* pv.*syringae* agreed also with a weak *in vitro* activity on CYE agar. However, the moderate efficacy of phosphonate and ethephon in plant trials contrasted with the weak *in vitro* activity. The MICs observed were much higher than those considered effective for currently used antibacterial or antifungal compounds (De Waard et al., 1993; Norelli and Gilpatrick, 1982) and were 20-100 times higher than those observed for phosphonate and fosetyl-Al in Oomycete fungi (Fenn and Cofey, 1984).

The weak activity *in vitro* of fosetyl, phosphonate and ethephon could be due to complexing with or interference by, the media components. For example, the activity of fosetyl-Al was reported to decrease in growth media containing high concentrations of phosphate (0.5-4.5 g $[HPO_4]^{2-}$/l) (Fenn and Cofey, 1984). However, this is not likely to occur in our case since the phosphate contents of CYE agar (25-30 μg $[HPO_3]^{2-}$/g f.w.) was 20-40 times lower than the MICs observed for phosphonate, fosetyl or ethephon. Also, the low antibacterial activity of fosetyl and phosphonate in CYE agar was consistent with the finding of a high survival of *P. syringae* pv. *syringae* in concentrated aqueous solutions of these chemical compounds. This high survival agreed with similar observations in some phytopathogenic bacteria and with the fact that recovery levels of *X. arboricola* pathovars introduced into the surface of plants previously sprayed with fosetyl-Al or water were similar (Chase, 1993). Furthermore, these results agreed also with the known ability of some *Pseudomonas* spp. to utilize a range of structurally diverse organophosphonates as sources of phosphorus, nitrogen or carbon (Zboinska et al., 1992).

The activity of phosphonate, fosetyl and ethephon may be due to the pH of the solutions used rather than to its chemical nature. Chase (Chase, 1993) reported that although pH of unbuffered solutions of fosetyl-Al may be one factor affecting some plant bacteria, pH sensitivity cannot explain the relative recovery of others. However, independently of the phosphonate derivative

amended, the pH of aqueous solutions used for irrigation of plants was not related to disease control levels achieved, probably because of the buffering capacity of the potting mix that restricted pH values to 5.2-6.2.

The study of the mechanisms by which phosphonate derivatives affect disease development upon infection of pear by *P. syringae* pv. *syringae* are beyond the scope of this paper. Several reports claim that fosetyl-Al stimulates natural defense mechanisms in tomato, bean, pepper, tobacco and grapevine plants innoculated with some Oomycete fungi by inducing blocking necrosis, phytoalexin accumulation and other biochemical and structural defence processes (Bompeix et al., 1980, 1981; Dercks and Buchenauer, 1987; Dercks and Creasy, 1989; Guest, 1984, 1986). However, the role of fosetyl-Al as a resistance inducing compound *in planta* is controversial because there are not enough data to support this hypothesis (Fenn and Coffey, 1984; Kessmann et al., 1994). Ethephon has been reported to delaying symptom expression in tobacco plants inoculated with tobacco mosaic virus (Van Loon, 1977) and in *Cynara aurantiaca* inoculated with citrus exocortis viroid (Bellés et al., 1990), but the effect has been attributed mainly to the release of ethylene as a breakdown product.

Except for phosphomycin, the ED_{50} for the other phosphonate derivatives applied as a root drench may be too high for practical use for the control of bacterial blast of pear in the field. These ED_{50}s were 100 to 700 times higher than those observed in control of stem rot of avocado seedlings (10-20 mg HPO_3^{2-}/l), a fungal disease caused by *Phytophthora* spp. (Fenn and Coffey, 1984). The weak activity of fosetyl against bacterial blast of pear agreed with the finding of inconsistent efficacy reported in other studies of control of bacterial diseases of ornamental plants (Chase AR, 1993) and of fire blight of pear (Paulin et al., 1990). However, the activity found by us was consistent from trial to trial because we designed our experiments to reduce variability.

More research should be performed to define the potential applicability of the phosphonate derivatives studied in this work for the control of bacterial blast of pear. Although the ED_{50} of fosetyl, phosphonate and ethephon applied by irrigation to pear plants is too high for a practical use in the field, it is expected that improvement of application methods may increase the efficiency of these compounds. Further greenhouse and field trials are being performed to evaluate different modes of application of phosphonate derivatives to pear plants to increase their effective concentrations in plant tissues.

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References

Andersen GL, Menkissoglou O and Lindow SE (1991) Occurrence and properties of copper-tolerant strains of *Pseudomonas syringae* isolated from fruit trees in California. Phytopathology 81: 648–656

Bellés JM, Granell A and Conejero V (1990) Impairment of viroid infection in *Gynaura auranticaca* plants by treatment with 2-chloroethylphosphonic acid (ethephon). Can J Plant Pathol 12: 175–179

Bompeix G, Fettouche F and Saindrenan P (1981) Mode d'action du Phosethyl-Al. Phytiatrie-Phytopharmacie 30: 257–272

Bompeix G, Ravisé A, Raynal G, Fettouche F and Durand MC (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

Chase AR (1993) Efficacy of fosetyl-Al for control of some bacterial diseases on ornamentals. Plant Dis 77: 771–776

Coffey MD and Joseph MC (1985) Effects of phosphorous acid and Fosetyl-Al on the life cycle of *Phytophtora cinnamoni* and *P. citricola*. Phytopathology 75: 1042–1046

Darvas JM, Toerien JC and Milne DL (1984) Control of avocado root rot by trunk injection with phosethyl-Al. Plant Dis. 68: 691–693

Dercks W and Buchenauer H (1987) Comparative studies on the mode of action of aluminium ethyl phosphite in four *Phytophtora* species. Crop Protection 6: 82–89

Dercks W and Creasy LL (1989) Influence of fosetyl-Al on phytoalexin accumulation in *Plasmopara viticola*- grapevine interaction. Physiol Mol Plant Pathol 34: 203–213

De Waard MA, Georgopoulos SG, Hollomon DW, Ishii H, Leroux P, Ragsdale NN and Schwinn B (1993) Chemical Control of Plant Diseases: Problems and Prospects. Annu Rev Phytopathol 31: 403–421

Dolan TE and Coffey MD (1988) Correlative *in vitro* and *in vivo* behavior of mutant strains of *Phytophtora palmivora* expressing different resistances to phosphorous acid and fosetyl-Al. Phytopathology 78: 974–978

El-Hamalawi ZA, Menge JA and Adams CJ (1995) Methods of fosethyl-Al application and phosphonate levels in avocado tissue needed to control stem canker caused by *Phytophtora citricola*. Plant Dis 79: 770–778

Ercolani GL (1973) Two hypothesis on the aetiology of response of plants to phytopathogenic bacteria. J Gen Microbiol 74: 83–95

Fenn ME and Coffey MD (1984) Studies on the *in vitro* and *in vivo* antifungal activity of Fosetyl-Al and phosphorous acid. Phytopathology 74: 606–611

Finney JC (1971) Probit Analysis. 3rd. Edition. Cambridge Univ. Press. Cambridge, England

Gross DC, Cody YS, Proebsting EJJr, Radamaker GK and Spotts RA (1983) Distribution, population dynamics, and characteristics of ice nucleation-active bacteria in decidious fruit tree orchards. Appl Environ Microbiol 46: 1370–1379

Guest DI (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

Guest DI (1986) Evidence from light microscopy of living tissues that fosetyl-Al modifies the defence response in tobacco seedlings following inoculation by *Phytophtora nicotianae* var. *nicotianae*. Physiol Mol Plant Pathol 29: 251–261

Hagborg WAF (1970) A device for injecting solutions and suspensions into thin leaves of plants. Can J Bot 48: 1135–1136

Hilderbrand RL (1983) The role of phosphonates in living systems. CRC Press, Boca Raton, Fla

Jeffrery GH, Bassett J, Mendham J and Denney RC (1989) Vogel's Quantitative Chemical Analysis. Longman, Harlow, England

Jones AL and Aldwinckle HS (eds.) (1990) Compendium of apple and pear diseases. American Phytopathological Society Press, MN

Kessmann H, Staub T, Hofmann C, Maetzke T and Herzog J (1994) Induction of systemic acquired disease resistance in plants by chemicals. Annu Rev Phytopathol 32: 439–459

King EO, Ward MK and Raney DE (1954) Two simple media for demonstration of pyocianin and fluorescin. J Lab Clin Med 44: 301–307

Lindow SE, Hirano SS, Barchet WR, Arny DC and Upper CD (1982) Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiol 70: 1090–1093

Mansvelt EL and Hattingh MJ (1988) Resident populations of *Pseudomonas syringae* pv. *syringae* on leaves, blossoms, and fruits of apple and pear trees. J Phytopathol 121: 135–142

McGuire RG (1988) Evaluation of bactericidal chemicals for control of *Xanthomonas* on citrus. Plant Dis 72: 1016–1020

McIntire WH, Winterberg SH, Hardin LJ, Sterges AJ and Clements LB (1950) Fertilizer evaluation of certain phosphorus, phosphorous, and phosphoric materials by means of pot cultures. Agron J 42: 543–549

Montesinos E and Vilardell P (1991) Relationships among population levels of *Pseudomonas syringae*, amount of ice nuclei, and incidence of blast of dormant flower buds in commercial pear orchards in Catalunya, Spain. Phytopathology 81: 113–119

Norelli JL and Gilpatrick JD (1982) Techniques for screening chemicals for fire blight control. Plant Dis 66: 1162–1165

Ouimette DG and Coffey MD (1988) Quantitative analysis of organic phosphonates, phosphonate, and other inorganic anions in plants and soil by using high-performance ion chromatography. Phytopathology 78: 1150–1155

Paulin JP, Chartier R, Lecomte P, Brisset MN, Lachaud G and Larue P (1990) Experiments with Aliette (Phosetyl-Aluminium) in fire blight control. Acta Horticul 273: 383–390

Schaad NW (1988) Laboratory Guide for Identification of Plant Pathogenic Bacteria. 158 pp. American Phytopathological Society Press, St. Paul, MN

Shoji J, Kato T, Hinoo H, Hattori T, Hirooka K, Matsumoto K, Tanimoto T and Kondo E (1986) Production of fosfomycin (phosphonomycin) by *Pseudomonas syringae*. J Antibiotics 39: 1011–1012

Van Loon L C (1977) Induction by 2-chloroethylphosphonic acid of viral like lesions, associated proteins and systemic resistance in tobacco. Virology 80: 417–420

Whitesides SK and Spotts RA (1991) Induction of pear blossom blast caused by *Pseudomonas syringae* pv. *syringae*. Plant Pathol 40: 118–127

Wild A (ed) (1973) Russell's Soil Conditions and Plant Growth. Longman Scientific and Technical, New York 991 pp.

Williams DJ, Beach BGW, Horrière D and Marechal G (1977) LS 74–783, a new systemic fungicide with activity against phycomycete diseases. Pages 565–573. In: Proc Br Crop Prot Conf Pests and Diseases. 21–24 Nov 1977, Brighton, England. 1045 pp

Yessad S, Manceau C and Luisetti J (1992) A detached leaf assay to evaluate virulence and pathogenicity of strains of *Pseudomonas syringae* pv. *syringae* on pear. Plant Dis 76: 370–373

Zboinska E, Lejczak B and Kafarski P (1992) Organophosphonate utilization by the wild-type strain of *Pseudomonas fluorescens*. Appl Environ Microbiol 58: 2993–2999