Techniques

Quantitative Analysis of Organic Phosphonates, Phosphonate, and Other Inorganic Anions in Plants and Soil by Using High-Performance Ion Chromatography

D. G. Ouimette and M. D. Coffey

Graduate research assistant and professor, respectively, Department of Plant Pathology, University of California, Riverside 92521-0122.
We acknowledge the financial assistance of the California Avocado Commission (Production Research Committee) and Rhône-Poulenc Inc. in this project and thank BettyAnn Merrill for preparation of the manuscript.
Accepted for publication 21 March 1988 (submitted for electronic processing).

ABSTRACT

Ouimette, D. G., and Coffey, M. D. 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.

The current analytical method for quantitation of the systemic fungicide aluminum tris-O-ethyl phosphonate (Aliette) and its anionic metabolite phosphonate (HPO_3^{-2}) in plants is extremely tedious and requires the use of both high-pressure liquid chromatography and gas-liquid chromatography. We report here a simple and improved method that employs high-performance ion chromatography, using eluent suppression and conductivity detection, to determine residues of ethyl, diethyl, and dimethyl phosphonate, as well as the inorganic anions HPO_3^{-2}, HPO_4^{-2}, NO_3^-, and SO_4^{-2} in aqueous extracts of plants and soil. The method has proved sensitive, reproducible, and requires only simple sample preparation. Two types of column and eluent are employed, one for inorganic anions and the other for organic phosphonate compounds. The efficiency of aqueous extraction of ethyl phosphonate and HPO_3^{-2} from plants is about 70%. Aqueous extraction efficiency of organic phosphonates from soil is 90%, whereas that of HPO_3^{-2} using 0.5 M $NaHCO_3$ is 85%. Under the conditions employed, the limits of detection of the organic phosphonates from plants and soil are 2.0 μg/g and 5.0 μg/g, respectively; those of HPO_3^{-2} and other inorganic anions in plants and soil are 0.5 μg/g and 0.2 μg/g, respectively.

The systemic fungicide fosetyl-Al (aluminum tris-O-ethyl phosphonate), known under the trade name Aliette (Rhône-Poulenc Agrochimie, Lyon, France), is used worldwide to control diseases caused by members of the Peronosporales, especially root and crown rots caused by various *Phytophthora* species and foliar diseases caused by some downy mildews (4,22,23). Concepts concerning the mode of action of ethyl phosphonate are contradictory, with some evidence suggesting that it operates by stimulation of the host's natural defense mechanisms (1,2,13,14, 15,19) and other data indicating a direct effect on the pathogen (5,6,7,8,10,11,12). Ethyl phosphonate is hydrolyzed to ethanol and phosphonic acid (H_3PO_3) in vivo (23). At physiological pH, phosphonic acid is ionized primarily to HPO_3^{-2}, due to pKa values of 1.3 and 6.7 for the hydroxyl groups (7). The HPO_3^{-2} anion has been shown to be up to 15 times as active as ethyl phosphonate against various *Phytophthora* species (7,11). There is little data available on the fate of HPO_3^{-2} in the plant and especially on its concentration and persistence in plant tissues. Such factors would be expected to play a major role in the efficacy of disease control, as is true with other systemic fungicides (4,7), so they are deserving of critical investigation. Because HPO_3^{-2} is only one oxidation state removed from HPO_4^{-2}, it is possible that HPO_3^{-2} may behave like HPO_4^{-2} in plants, being capable of active uptake by the roots, or loading into the phloem and subsequent translocation throughout the plant, ultimately accumulating in the meristematic regions of roots and shoots.

MacIntire et al (17) evaluated certain phosphorous compounds as fertilizers in pot cultures using red clover, ryegrass, alfalfa, brown-top millet, and soybeans as test plants and found no effect in the first year with phosphonic acid (H_3PO_3) but did find beneficial effects in the second year. It would appear that plants cannot readily oxidize HPO_3^{-2} to HPO_4^{-2} and use it as a phosphorous source. They attributed the second-year response to oxidation of HPO_3^{-2} to HPO_4^{-2} in the soil by microorganisms.

Other chemically-related organic phosphonate compounds such

© 1988 The American Phytopathological Society

as dimethyl phosphonate also possess both in vivo and in vitro efficacy against *Phytophthora* sp. and in some cases are more efficacious than mono ethyl phosphonate (18). To study the behavior of ethyl phosphonate, phosphonate, and other organic phosphonate compounds in plants and soil, a reproducible method for quantitative residue determination is required. Ryder (20) used single column ion chromatography with conductivity detection to separate and quantify orthophosphoric, phosphonic acid, and hypophosphorous acid by using succinic acid as the mobile phase, but the separation was done in the absence of plant or soil extracts and no organic phosphonate compounds were analyzed. Bradfield and Cooke (3) used ion chromatography to determine inorganic ions in aqueous extracts of plants and soils and achieved good separation of SO_4^{-2}, NO_3^-, HPO_4^{-2}, and CL^-. However, it is doubtful if this method would separate HPO_3^{-2} from the other inorganic anions, nor could it be used for analysis of organic phosphonate compounds. A technique to determine ethyl phosphonate, HPO_3^{-2}, and HPO_4^{-2} in plant tissue has been described (21). The protocol, however, involving both high-pressure liquid chromatography (HPLC) and gas-liquid chromatography (GLC), is extremely tedious and time-consuming. Ethyl phosphonate, HPO_3^{-2}, and HPO_4^{-2} must be separated from the majority of the extract components by HPLC. Following HPLC separation the samples must be further purified and then derivatized before final quantitative GLC analysis.

We report here an improved method for residue determination of ethyl, diethyl, and dimethyl phosphonate and the inorganic anions HPO_3^{-2}, HPO_4^{-2}, SO_4^{-2}, and NO_3^- in aqueous extracts of plants and soil. This method involves high-performance ion chromatography (HPIC) using both eluent suppression and conductivity detection.

MATERIALS AND METHODS

Ion chromatography system. The HPIC apparatus used was a Dionex 2000i/P with a model AMMS-1 anion micromembrane suppressor and a conductivity detector (Dionex Corp., Sunnyvale, CA). For anion analysis, an AS4A separator column was used with two types of guard columns, an MPIC-NG1 and an HPIC-AG4A. The regenerate for the AMMS-1 suppressor was .015 N H_2SO_4 used at a flow rate of 2.0 ml/min. The eluent for the AS4A column consisted of 0.53 mM $NaHCO_3$ and 1.45 mM Na_2CO_3 used at a flow rate of 2.2 ml/min. For organic phosphonate analysis, an AS-6 separator column was used with a MPIC-NG1 and an HPIC-AG6 guard column. The eluent consisted of 20 mM NaOH at 2.2 ml/min, and the suppressor regenerate was .030 N H_2SO_4 at 3.5 ml/min. The sensitivity of the conductivity meter was set at 3 μS for generation of standard curves and for determination of residues in plants and soil and 1 μS for determination of detection limits of HPO_3^{-2} and other inorganic anions in plant and soil extracts. The sample was delivered via a 50-μl injection loop and the data recorded on a Spectra-Physics 4270 integrator (Spectra-Physics, San Jose, CA).

Generation of standard curves. Standards of analytical grade fosetyl-Al (99.3%, Rhône-Poulenc), H_3PO_3 (99.6%, Fisher Scientific Company, Pittsburgh), dimethyl and diethyl phosphonates (99% and 98%, respectively, Aldrich Chemical Company, Inc., Milwaukee), potassium phosphate, potassium sulfate, and sodium nitrate were prepared in glass-distilled water. Standard curves for the compounds were generated by plotting their concentration (μeq/ml or μg/ml) against the peak height. Linear regression analysis was performed to determine the linearity of the response. The standard concentrations ranged from 0 to 7.5 μg/ml for the inorganic anions and 0 to 100 μeq phosphonate per milliliter for the organic phosphonates. To investigate the possibility that on-column hydrolysis of ethyl phosphonate might be occurring due to the basicity of the eluent (20 mM NaOH), 5 μg/ml of ethyl phosphonate was exposed to 20 mM NaOH for 1 hr before injection into the HPIC apparatus and compared with an equal amount added to water. The chemical structures of the phosphonate compounds used in this study are shown in Figure 1.

Analysis of plant material. Pepper plants (*Capsicum annuum* L.) were grown in the greenhouse in U.C. mix #1 for 5 wk, then removed from the soil and the roots rinsed gently with running tap water. Five plants were transferred to 250-ml styrofoam cups containing 125 ml of 5.0 mM solutions of the various phosphonate compounds buffered with 5.0 mM MES hydrate (4-morpholine ethane-sulfonic acid) and adjusted to pH 6.2 with KOH. Titration of phosphonic acid with KOH yielded a mixture of mono- and dipotassium phosphonate. The plants were placed in a growth chamber for 24 hr at 24 C with an initial 12-hr photoperiod followed by dark, then removed from the solutions, rinsed well under running tap water, and blotted dry on paper towels. The five plants were combined, and samples weighing 2 g (fresh weight) of either roots, stem, or leaves were chopped coarsely with a razor blade and transferred individually to a ceramic mortar. Liquid nitrogen was then added and the tissue ground into a fine powder. The powder was transferred to a 15-ml vial, 10 ml of glass-distilled water added (5 ml/g fresh-weight tissue), and the vial shaken for 1 hr at room temperature on a reciprocal shaker operating at approximately 180 strokes per min. The vials were allowed to stand for 20 min to settle debris from the extract, and a 1.5-ml aliquot was transferred to an Eppendorf microfuge tube and centrifuged for 10 min by using an Eppendorf Microfuge (model 5414). The supernatant was then transferred to a clean microfuge tube and either analyzed immediately or stored at −20 C for future analysis. Before injection into the HPIC, 100 μl of the extract was diluted with 900 μl of water in an Eppendorf microfuge tube. The diluted sample was then passed through a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) on the end of a 3-ml disposable syringe and into another syringe with a Swinney holder (Fisher Scientific) containing a 13-mm GS-type filter, pore size 0.22 μm (Millipore Corp., Bedford, MA), to remove particulate matter. The C_{18} cartridge removes pigments, phenolics, and other high molecular weight organic substances. Percent extraction of HPO_3^{-2} and ethyl phosphonate was determined by spiking a 0.5-g segment of pepper stem with 20 μl of a 10 mg/ml solution (200 μg per segment) and extracted as described already. There were five replications per treatment in each experiment.

Soil analysis. Five grams of a sandy loam soil taken from an avocado grove in southern California was added to an aluminum weighing boat, and 500 μl of a 1 mg/ml solution of either HPO_3^{-2} or the organic phosphonates was dispensed evenly throughout the soil in approximately 50 μl droplets. The soil was mixed well with a metal spatula and allowed to dry. Before adding the chemical, the soil was oven-dried at 100 C for 2 hr and passed through a #5 sieve to remove large clods and rocks. The soil was then added to a 15-ml vial and, in the case of organic phosphonates, was extracted with 10 ml of glass-distilled water, while HPO_3^{-2} was extracted from soil with either glass-distilled water or 0.5 M sodium bicarbonate ($NaHCO_3$). $NaHCO_3$ was compared to water extraction because it

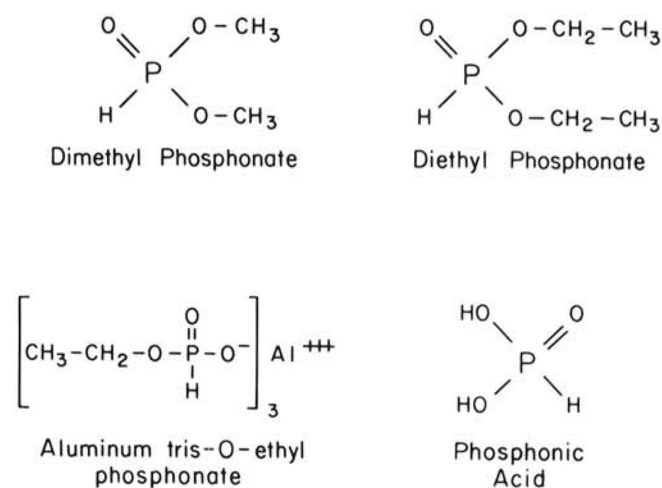

Fig. 1. Chemical structures of phosphonic acid and organic phosphonate compounds (monoethyl, diethyl, and dimethyl phosphonate).

is the standard extractant for phosphate from calcareous soils common to the western U.S.A. (9). The experiment consisted of five replications, and percent extraction from soil was determined by comparing the amount extracted from soil with an equal amount added to water.

Limits of detection of organic phosphonates, HPO_3^{-2}, and other inorganic anions from plants and soil. Limits of detection were determined by spiking undiluted plant or soil extract with known amounts of HPO_3^{-2} and organic phosphonate compounds before passage through a C_{18} cartridge and 0.22 μm filter and injection into the HPIC. Because plant and soil extracts contain NO_3^-, SO_4^{-2}, and HPO_4^{-2} naturally, these ions were not added to the extracts. Instead, the limits of detection of these anions in water was used to extrapolate the values in plant and soil extracts. This was possible because of negligible interference of the extract components with peak height or anion separation.

RESULTS

Standard curves. The standard curves for the organic phosphonate compounds are shown in Figure 2. In the concentration range tested (0–100 μeq/ml), the response was linear for all three compounds with correlation coefficients of 0.996 for diethyl phosphonate and 0.999 for ethyl and dimethyl phosphonate. Detector responses for the compounds differed, with diethyl phosphonate showing less response than ethyl and dimethyl phosphonate at the higher concentrations and to a lesser degree at the lower levels (Fig. 2). The standard curves for the inorganic anions are shown in Figure 3. The correlation coefficient for HPO_4^{-2} and SO_4^{-2} was 0.999, for HPO_3^{-2} was 1.0, and for NO_3^- was 0.951. No hydrolysis of ethyl phosphonate occurred after incubation of 5.0 μg/ml in 20 mM NaOH for 1 hr before analysis, indicating that on-column hydrolysis was unlikely, because during HPIC analysis the chemical was in contact with NaOH for less than 10 min.

Analysis of plant material. Aqueous plant extracts from roots, stems, and leaves treated with organic phosphonate compounds contained both the parent compound and HPO_3^{-2}, indicating that partial metabolism to HPO_3^{-2} had taken place with all three organic phosphonate compounds (Table 1). Dimethyl phosphonate yielded the most HPO_3^{-2} in both the roots and leaves compared with other compounds tested (Table 1). Treatment with ethyl phosphonate yielded the least amount of HPO_3^{-2} in the roots and stems compared with the other treatments, but there were higher levels in the leaves relative to the diethyl phosphonate and HPO_3^{-2} treatments. In all cases there was more of the parent organic phosphonate compounds in the plant than HPO_3^{-2}, indicating that only partial metabolism to HPO_3^{-2} had occurred during the 24-hr duration of the experiment (Table 1). Separation of all the major anions HPO_4^{-2}, NO_3^-, SO_4^{-2}, as well as HPO_3^{-2}, was accomplished with sharp, distinct peaks obtained free from interference (Fig. 4c). The order of elution off the column was NO_3^-, HPO_3^{-2}, HPO_4^{-2}, and SO_4^{-2}, with approximate retention times of 4.3, 6.8, 8.5, and 9.8 min, respectively (Fig. 4a).

The organic phosphonates eluted off the column at about 7.2 min. Under the conditions employed, the three organic phosphonate compounds had almost identical retention times, but because in any instance the plant extract only contained one such compound, this did not present a problem. The identity of a compound was verified by injection of anion standards.

Extraction efficiencies. Ethyl phosphonate and HPO_3^{-2} were extracted from pepper stem tissue with water with an extraction efficiency of approximately 70%. Determination of the extraction efficiency of ethyl phosphonate was complicated by the fact that the molecule was being hydrolyzed during either the spiking and/or the extraction phase. In this case, meq of HPO_3^{-2} was converted to meq ethyl phosphonate and the amount recovered compared to an equal amount added to water. An attempt was also made to compare the extraction efficiency of water to that of 5% trichloroacetic acid. However, even after extensive washings of the sample with diethyl ether to remove the trichloroacetic acid, enough remained to coelute with the HPO_3^{-2} and HPO_4^{-2} peaks, and it was therefore considered unsuitable as an extractant.

Soil analysis. Determination of organic phosphonate compounds and inorganic anions in soil was readily achieved, with the chromatogram for the inorganic anions looking similar to those derived from plant material (Fig. 4b). The $NaHCO_3$ extractant did not interfere with the anion analysis because the eluent itself was composed in part of $NaHCO_3$ (Fig. 4b). The chromatogram for the organic phosphonate compounds from soil was slightly cleaner than that derived from plant extract (Fig. 5b). Percent recovery for HPO_3^{-2} from soil was 68% with water and 85% with $NaHCO_3$ (Table 2). Recovery from soil of the organic phosphonate compounds using water as the extractant was 87%,

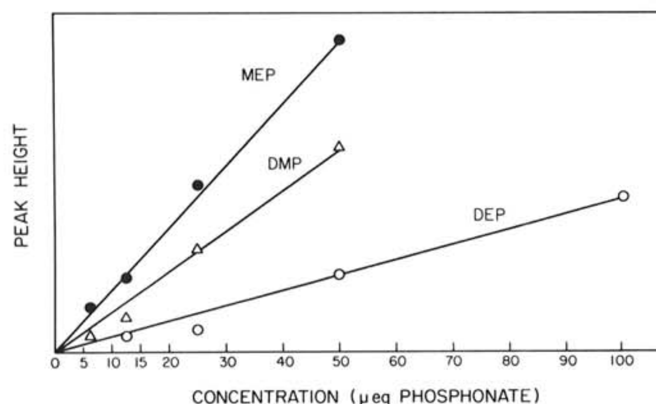

Fig. 2. Standard curves of monoethyl, diethyl, and dimethyl phosphonate (MEP, DEP, and DMP, respectively). Standards were prepared in glass-distilled water, and detector response was 3 μS.

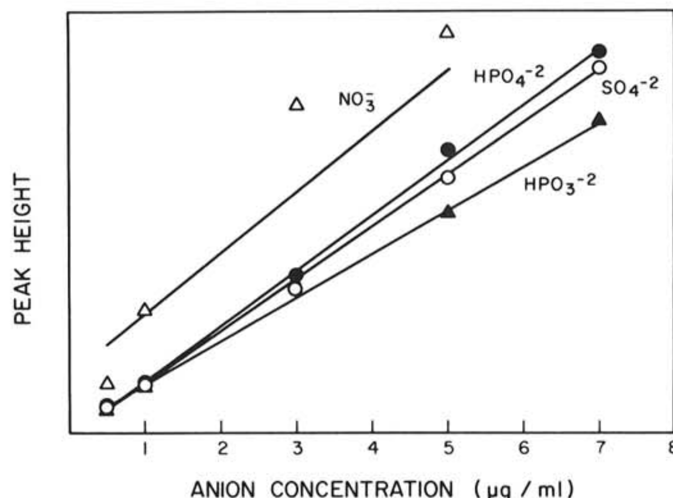

Fig. 3. Standard curves of phosphonate (HPO_3^{-2}) and the major plant anions nitrate (NO_3^-), phosphate (HPO_4^{-2}), and sulfate (SO_4^{-2}). Standards were prepared in glass-distilled water, and the detector response was 3 μS.

TABLE 1. Concentration of organic phosphonates and inorganic phosphonate in pepper tissue after application of 5.0 mM solutions of various phosphonate compounds to pepper plants for 24 hr[a]

	Concentration (μg/g fresh weight)[b]					
	Parent compound			Phosphonate		
Compound	Roots	Stems	Leaves	Roots	Stems	Leaves
Monoethyl phosphonate	1,024	450	174	81	82	95
Diethyl phosphonate	614	571	372	166	133	65
Dimethyl phosphonate	784	590	189	397	176	138
Potassium phosphonate	···	···	···	342	186	44

[a] Solutions were buffered with 5.0 mM 4-morpholine ethane sulfonic acid and the pH adjusted to 6.2. Pepper plants were maintained at 24 C and supplied with 12 hr of artificial light during the 24-hr experiment.
[b] Concentration values have been corrected for 70% extraction efficiency.

91%, and 92% for diethyl, dimethyl, and ethyl phosphonate, respectively (Table 2). $NaHCO_3$ was not used for the organic phosphonate compounds because of the high extraction efficiency of water.

Limits of detection. Because of the higher efficiency of the AS4A column, the conductivity detector sensitivity could be increased from 3 μS to 1 μS, allowing detection of 10 times the amount of inorganic anions compared with the organic phosphonates. The limits of detection for the inorganic anions was 0.2 and 0.5 μg/g and for the organic phosphonate compounds was 2.0 and 5.0 μg/g in soil and plant extracts, respectively.

DISCUSSION

In the past, anion analysis of plant and soil extracts using HPIC presented a potentially formidable task. Because many laboratories had access to conventional HPLC equipment with the standard type bulk property detectors such as refractive index and conductivity, as well as solute-specific type detectors such as UV/VIS, there was a tendency to try to adapt this equipment for anion analysis. Unfortunately, bulk property detectors tend to have poor selectivity and sensitivity. In addition, such detectors are temperature dependent and very sensitive to changes in the flow rate of the eluent and have a limited linear dynamic range (16). Whereas solute-specific detectors such as UV are the optimum type of detector because they possess none of the drawbacks described for the bulk property detectors, their advantage may be lost when analyzing anions, especially inorganic ions, because the ion of interest may not absorb UV light. In these cases, as with the technique described by Saindrenan et al (21), a UV-absorbing eluent is used to separate the anions. When the nonabsorbing anions pass through the detector, a void is created, and if the leads on the recorder are reversed, a peak will appear. However, in this case, UV detection apparently could not be used to quantitate the phosphonate residues because of the lack of linear response of anion concentration to detection; hence, extensive sample clean-up, derivatization, and subsequent GLC analysis was needed (21). This is an example of a solute-specific type of detector (UV) being used as a bulk detector with all the disadvantages previously described.

In contrast, detection of anions using HPIC allows the opposite to occur; that is, a bulk property conductivity detector can be used as a solute-specific detector by eliminating the conductivity of the eluent after it has separated the anions by using an eluent suppression device. The process is described in more detail by Johnson and Haak (16). By neutralizing the eluent, a standard conductivity detector can become a sensitive, reliable, and easy way to detect anions because only those anions of interest remain conductive. In addition, the sample clean-up procedure necessary for anion analysis with this method is extremely simple and rapid. Also, because of the high water solubility of the phosphonate compounds, water can be used as the sole extractant, eliminating the need for partitioning into other solvents and eliminating additional sources of error. The sensitivity of detection also allows for high dilutions of the samples, resulting in cleaner chromatograms as well as minimizing the potential for column contamination by plant extract components.

Finally, although it was known that ethyl phosphonate was hydrolyzed to HPO_3^{-2} in plants (23), the fate of other organic phosphonate compounds in soil and plants had not been determined previously. Table 1 shows that all three organic phosphonate compounds tested in this study are hydrolyzed to HPO_3^{-2} to varying degrees in plants and that the parent compound can also enter the plant in relatively high amounts. It is yet to be determined whether these molecules, especially phosphonate, are taken up by diffusion alone or if active uptake phenomena are involved. The metabolism to HPO_3^{-2} of organic phosphonates should make them potentially useful for control of plant pathogens, especially *Phytophthora* species and downy mildew organisms.

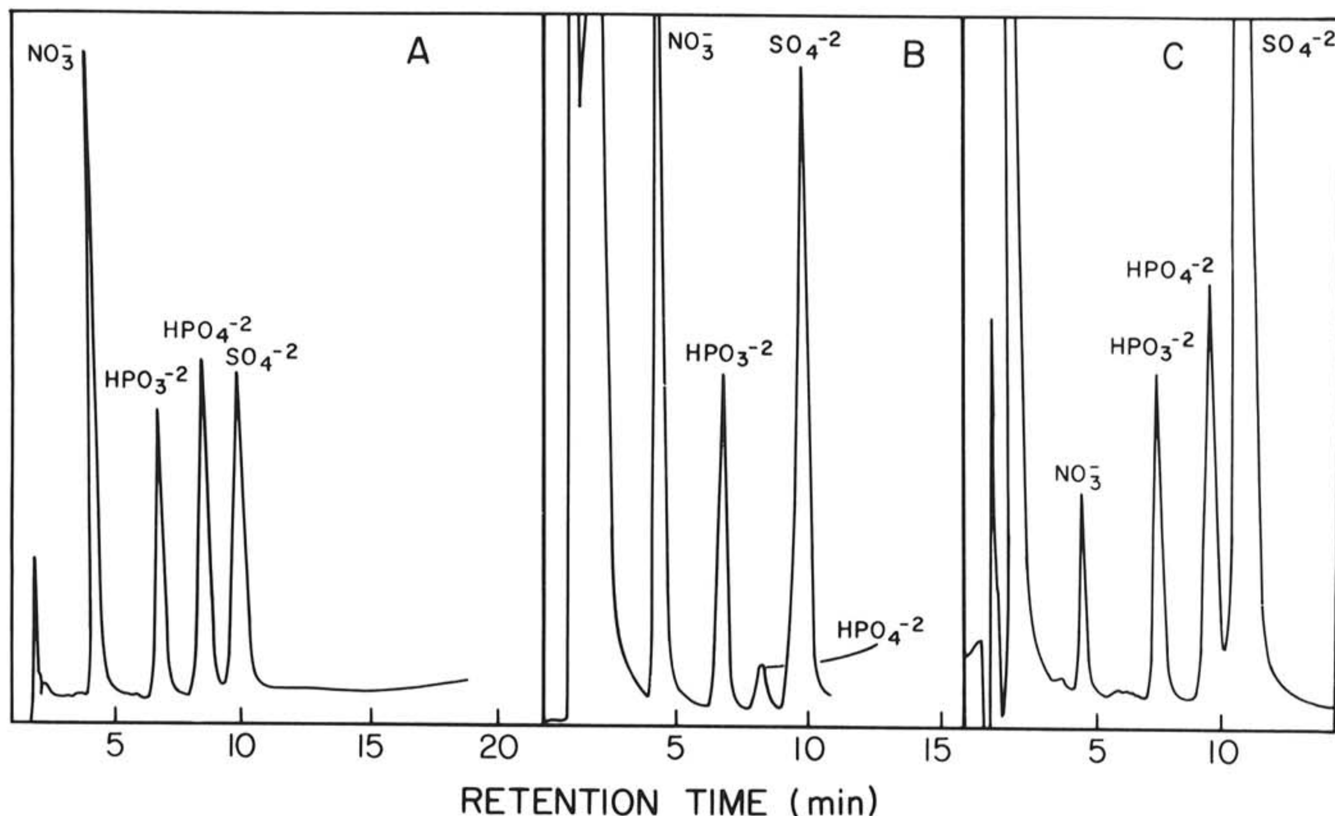

Fig. 4. Chromatograms of (**A**) 5 μg/ml of ethyl phosphonate standard prepared in glass-distilled water; (**B**) aqueous extracts of a sandy loam soil to which ethyl phosphonate was added and extracted with water, 2 ml/g of soil; and (**C**) aqueous extracts of pepper stems (*Capsicum annuum* L.) after treatment with 5.0 mM fosetyl-Al for 24 hr. Pepper tissue was extracted with water, 5 ml/g fresh weight. Both soil and plant tissue was extracted for 1 hr, the extracts diluted 10:1 with water and passed through a 0.22 μm filter and a Sep-Pak C_{18} cartridge before injection into the HPIC. The conductivity detector was set at 3 μS.

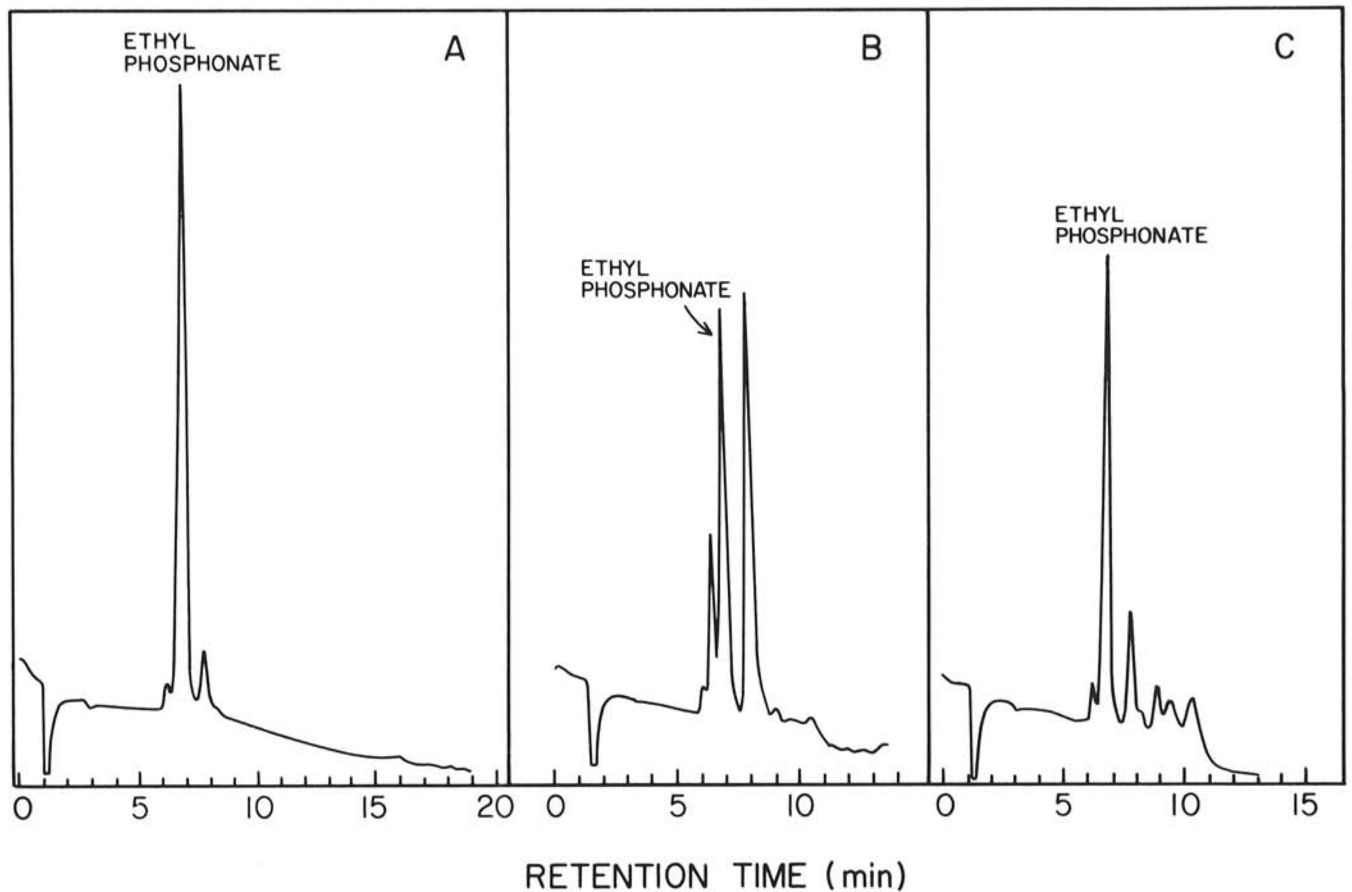

Fig. 5. Chromatograms of (**A**) 2.5 μg/ml of standards of nitrate (NO_3^-), phosphonate (HPO_3^{-2}), phosphate (HPO_4^{-2}), and sulfate (SO_4^{-2}) prepared in glass-distilled water; (**B**) sandy loam soil to which phosphonate was added and extracted with 0.5 M $NaHCO_3$ at 2 ml/g of soil; and (**C**) aqueous extracts of pepper stems (*Capsicum annuum* L.) previously treated with 5.0 mM K_2HPO_3 for 24 hr. Pepper tissue was ground to a powder in liquid nitrogen and extracted with water, 5 ml/g fresh weight. Both soil and plant tissue was extracted for 1 hr, the extracts diluted 10:1 with water and passed through a 0.22 μm filter and a Sep-Pak C_{18} cartridge before injection into the HPIC. Conductivity detector was set at 3 μS.

TABLE 2. Extraction efficiency from soil of organic phosphonates and inorganic phosphonate using either water or sodium bicarbonate[a]

Compound	Extractant	% recovery[b]
Monoethyl phosphonate	Water	91.2 ± 8.7
Diethyl phosphonate	Water	86.6 ± 13.4
Dimethyl phosphonate	Water	90.0 ± 5.9
Phosphonate	Water	67.6 ± 7.6
Phosphonate	0.5 M $NaHCO_3$	84.4 ± 0.5

[a] Soil was a sandy loam and the percent recovery values are the mean of five replicates.

[b] Soil was extracted by placing 5 g of soil into a 15-ml vial to which 10 ml of extractant was added. The samples were shaken for 1 hr on a reciprocal shaker at 180 strokes per min.

LITERATURE CITED

1. Bompeix, G., Fettouche, F., and Saindrenan, P. 1981. Mode d'action du phoséthyl Al. Phytiatr. Phytopharm. 30:257-272.
2. Bompeix, G., Ravisé, A., Raynal, G., Fettouche, F., and Durand, M. C. 1980. Modalités de l'obtention des necroses 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.
3. Bradfield, E. G., and Cooke, D. T. 1985. Determination of inorganic ions in water extracts of plants and soils by ion chromatography. Analyst 110:1409-1410.
4. Bruin, G. C. A., and Edgington, L. V. 1983. The chemical control of diseases caused by zoosporic fungi. Pages 193-232 in: Zoosporic Plant Pathogens. S. T. Buczaki, ed. Academic Press, New York. 252 pp.
5. 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.
6. 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.
7. Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.
8. Dercks, W., and Buchenauer, H. 1987. Comparative studies on the mode of action of aluminium ethyl phosphite in four *Phytophthora* species. Crop Protect. 6:82-89.
9. Donahue, R. C., Miller, R., and Shickluna, J. C. 1977. Soils: An introduction to soils and plant growth. 4th Ed. Prentice-Hall, Inc., New Jersey. 626 pp.
10. Farih, A., Tsao, P. H., and Menge, J. A. 1981. Fungitoxic activity of efosite aluminum on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71:934-936.
11. 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.
12. 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.
13. Guest, D. I. 1983. Induced resistance as the mode of action of the systemic anti-oomycete compound, Aliette. (Abstr.) Page 225 in: Abstracts of Papers, Fourth International Congress of Plant Pathology, 17-24 August 1983, Melbourne, Australia. 273 pp.
14. Guest, D. I. 1984. Modification of defense responses in tobacco and capsicum following treatments with fosetyl-Al [aluminium tris (O-ethyl phosphonate)]. Physiol. Plant Pathol. 25:125-134.
15. Guest, D. I. 1986. Evidence from light microscopy that fosetyl-Al modifies the defense response in living tobacco seedlings following inoculation by *Phytophthora nicotianae* var. *nicotianae*. Physiol. Plant Pathol. 29:251-256.
16. Johnson, E. L., and Haak, K. K. 1984. Anion analysis by ion chromatography. Pages 263-300 in: Liquid Chromatography in Environmental Analysis. J. F. Lawrence, ed. Humana Press, Clifton,

New Jersey. 374 pp.

17. McIntire, W. H., Winterberg, S. H., Hardin, L. J., Serges, A. J., and Clements, L. B. 1950. Fertilizer evaluation of certain phosphorus, phosphorous, and phosphoric materials by means of pot cultures. Agron. J. 42:543-549.
18. Ouimette, D., and Coffey, M. D. 1985. In vivo efficacy of five phosphite compounds against *Phytophthora capsici* on pepper plants. (Abstr.) Phytopathology 75:1330.
19. Raynal, G., Ravisé, A., and Bompeix, G. 1980. Action du tris-O-éthyl-phosphonate d'aluminium (phoséthyl d'aluminium) sur la pathogénie de *Plasmopora viticola* et sur la stimulation des réactions de défense de la vigne. Ann. Phytopathol. 12:163-175.
20. Ryder, D. S. 1986. Separation of orthophosphoric acid, phosphorous acid, and hypophosphorous acid using single column ion chromatography with conductivity detection. J. Chromatogr. 354:438-441.
21. Saindrenan, P., Darakis, G., and Bompeix, G. 1985. Determination of ethylphosphite, phosphite and phosphate in plant tissues by anion-exchange high performance liquid and gas chromatography. J. Chromatogr. 347:267-273.
22. Stevens, C. C., and Mercer, R. T. 1981. The control of certain phycomycete diseases of strawberries, apples, vines and hops with applications of fosetyl aluminium. Pages 579-587 in: Proc. Brit. Crop Protect. Conf., Pests Dis. 16-19 Nov. 1981, Brighton, England. 930 pp.
23. Williams, D. J., Beach, B. G. W., Horřiere, D., and Marechal, G. 1977. LS74-783, a new systemic fungicide with activity against phycomycete diseases. Pages 565-573 in: Proc. Brit. Crop Protect. Conf., Pests Dis. 21-24 Nov. 1977, Brighton, England. 1045 pp.