Proceedings of the International Society of Citriculture

Physiological Interactions of Phosphorous Acid and Control of Root Pathogens

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Abstract. **Phosphorous acid (H_3PO_3) is neutralized with either potassium carbonate or potassium hydroxide to form potassium phosphonate. This compound is used worldwide as an anti-oomycete fungicide. Potassium phosphonate can be applied either as a root drench, a stem paint, stem injection or foliar application. In the plant, it breaks down rapidly into phosphonate anions, which reduce the mycelial growth of several species of *Phytophthora*, thereby inhibiting fungal growth. In citrus, this inhibition allows time for the plant's natural defence mechanisms to further inhibit fungal growth through accelerated production of the phytoalexin scoparone. Scoparone levels in citrus bark inoculated with *Phytophthora citrophthora* can be increased two to four fold by treatments of H_3PO_3 at 125 g/m . Many questions still exist about the relationship between the direct and the indirect action of the phosphonates. It is known that there is a phosphonate/phosphate antagonism within the fungal cell and that phosphate can in certain instances reverse the antifungal action of phosphonate. Potassium phosphonate applied as a foliar spray is broken down to phosphonate ions which peak in the roots after 28 days. Phosphorous acid results in higher phosphonate levels in citrus than fosetyl-Al, which is also broken down into phosphonate ions. Foliar applications of potassium phosphonate control brown rot on the fruit in addition to**

controlling *Phytophthora* root and foot rot, whereas stem applications and drenches control the latter only. Although most of the phosphonate usage to date is against the oomycetes there is evidence that the spectrum is considerably wider and its effectiveness has also been shown against other root pathogens, e.g. *Rhizoctonia, Fusarium* and *Armillaria.*

Until the 1970's there were no fungicides available for the treatment of soil-borne diseases caused by oomycetes. At that stage, two new groups of systemic fungicides active against these fungi were introduced, viz. the acylalanines and the phosphonates (Schwinn & Staub, 1987). The acylalanines are used widely for agricultural purposes but are exposed to the development of resistance (Davidse, 1981; Le Roux, *et al.,* 1993).

Unlike the acylalanines the phosphonates have a remarkable ability of translocation via both the phloem and the xylem. There has thus far also been no confirmed report of resistance to phosphonates in the field, despite the relative ease with which mutants with reduced sensitivity can be induced in the laboratory (Bower & Coffey, 1985; Dolan & Coffey, 1988; Duvenhage, 1994; Neema, *et al.*, 1998, Sanders & Coffey, 1990).

The term phosphonate is used to describe the salts or esters of phosphonic acid, $HPO(OH)_2$. The term phosphorous acid has been widely applied to phosphonic acid and phosphates to its alkali metal salts (Boenig, *et al.*, 1982). Phosphonic acid and its metal salts are commodity chemicals which are used widely in manufacturing and in chemical industries. The best known novel phosphonate compound used in agriculture is the herbicide, glyphosate [N-(phosphomethyl) glycine] (Toy & Walsh, 1987).

In 1977, Aliette WP (fosetyl-Al), the aluminium salt of phosphonic acid was released. Nina, the sodium salt of ethyl phosphonate and the calcium salts were tested but never commercially released (Anding *et al.*, 1987; Muchovej, *et* al., 1980). Darvas (1984) injected avocado trees with both phosphorous acid and fosetyl-Al in the early 1980's and found that phosphorous acid was more effective against *Phytophthora cinnamomi* than fosetyl-Al. It also became clear that the alkyl phosphonates were degraded in the plant to phosphonate anions (Bompeix *et al*., 1980; Bompeix & Saindrenan, 1984; Bower & Coffey, 1985; Coffey & Joseph, 1985; Dolan & Coffey, 1988; Fenn & Coffey, 1984; 1985).

A successful challenge of the patent protection obtained by Rhône-Poulenc in Australia led to the registration of potassium phosphonate and stimulated research into its efficacy as a treatment for a number of plant diseases (De Boer, *et al*., 1990; Pegg *et al*., 1985; Piccony *et al*., 1987). Schutte *et al*., (1991), determined the phosphite concentrations and distribution in mature citrus trees after trunk injections and foliar sprays either with phosphorous acid or fosetyl-Al and a trunk paint with fosetyl-Al. It was found that phosphorous acid (H_3PO_3) used as a trunk injection resulted in the highest phosphite levels over the longest period of time. Application intervals therefore depend upon the application method and formulation used and should not exceed 42 days. Fosetyl-Al, potassium phosphonate and phosphorous acid cannot be treated as identical in terms of efficacy. These differences are as a result of differing rates of assimilation and transport within the plant, and in the case of the alkyl phosphonates, different rates of breakdown to phosphonate in different plant species. The cation associated with alkyl phosphonate could in certain cases have a supplementary protective antifungal effect (Guest *et al*., 1996).

Physiological interactions of phosphorous acid

Phosphorous (phosphonic) acid shows very low mammalian toxicity. The toxicity is largely dependant on the cation. Sodium phosphonate has comparable toxicity to table salt whereas that of potassium phosphonate is even lower. Neither mammals nor plants have the capacity to oxidize phosphonate to phosphate. Non-enzymic oxidation is dependant on pH but is very slow at biological temperatures (Smillie *et al*., 1988).

Results by Bower & Coffey (1985) and Dolan & Coffey (1988), suggest that the phosphonates first act within the fungus and then within the plant's defence system. The concentrations required to inhibit mycelial growth of strains with reduced sensitivity in the laboratory were never more than 20-fold that of the parental strains. This falls within the range of natural occurring isolate sensitivities and are not the same as, e.g. insensitivity to the benzimidazoles where a 1000-fold concentration increase could not give satisfactory control of the diseases.

The direct effect of phosphonate ions on the fungus is but part of the mode of action of this compound. Ouimette & Coffey (1989b) discovered that crops were protected after the residues could no longer be detected. It was also found that phosphonates could control *Phytophthora in vitro* at concentrations (<28μM) which were ineffective against the mycelial growth on medium (Farih *et al*., 1981). De Boer *et al*. (1990) showed that phosphonates could provide effective control of *P. clandestine* which causes root rot of subterranean clover with as little as 0.3kg potassium phosphonate/ha. Black shank of tobacco caused by *P. nicotianae* was also controlled by application of 0.1mM of phosphonates whereas the ED_{50} for the pathogen was 1mM (Smillie *et al*., 1989).

Bompeix *et al*., 1980) observed enhanced activities of the plant's defence system after phosphonate applications. This was confirmed by Afek & Sztejnberg (1989) and Guest (1984b; 1986). The response stimulated by phosphonates were rapid cytological changes, nuclear migration, papilla deposition and hypersensitive cell death (Durand & Sallé, 1981; Guest, 1982) ethylene biosynthesis, phenylalanine ammonia lyase activation (Nemestothy & Guest, 1990, Saindrenan, *et al*., 1988) lignification (Nemestothy & Guest, 1990), respiration and pentose phosphate metabolism and phytoalexin accumulation in infected plants (Afek & Sztejnberg, 1989; Guest, 1984b).

Effect of phosphonates on scoparone in citrus

The phytoalexin scoparone (6,7-dimethoxycoumarin) is involved in defence mechanisms of citrus against several pathogens such as *Phytophthora citrophthora* (Smith & Smith), Leonian (Afek & Sztejnberg, 1988; 1993; Afek *et al.*, 1986; Sulistyowati *et al.*, 1990). Scoparone exists in low concentrations in healthy citrus bark and fruit peels (Tatum & Berry, 1977) but increases rapidly following the inoculation with pathogens (Afek & Sztejnberg, 1988; De Lange *et al*., 1976). Treatments with chemicals such as fosetyl-Al and phosphorous acid increased scoparone in citrus bark inoculated with *P. citrophthora* much more than untreated citrus (Afek & Sztejnberg, 1989). In doing so, the phosphonates induce resistance in citrus. Afek and Sztejnberg (1989) also showed that to obtain the same scoparone accumulation, the concentration of H_3PO_3 required for the same effect as fosetyl-Al was 41%.

The work done by Afek & Sztejnberg was conducted on four citrus spp., viz. macrophylla and sour orange (*Phytophthora* resistant) and rough lemon and niva (*Phytophthora* susceptible). The first three had the potential to produce scoparone and therefore the potential for resistance. As a result of this, only 300µg of fosetyl-Al or 125µg of H_3PO_3 per millilitre was required to stimulate scoparone in the first three species. In niva, 500µg of fosetyl-Al and 200µg of H_3PO_3 per millilitre decreased lesion size. This was without influencing the scoparone, indicating a more direct mode of action against the pathogen. Their research also led to the suggestion that the phosphonates affect *Phytophthora* in citrus in two ways, (i) it increases the host defence mechanisms at low level treatments; and (ii) it acts directly as a fungistat at higher levels.

Phosphonates used on citrus in South Africa: Fosetyl-Al, formulated as Aliette WP was the first phosphonate used on citrus in South Africa. It was used as a foliar application at 250g/100ℓ of water. It was later registered as a stem paint, initially at 500g/ℓ which was reduced to 300g/ℓ. During the 1980's Aliette Ca was formulated for stem injections not only on avocado trees but also for citrus. Le Roux, *et al.* (1991) reported a 66% increase in income 44 months after citrus trees were injected with Aliette CA at 0.4g a.i./m^2 canopy volume.

When the patent protection held by Rhône-Poulenc expired during the early 1990's, research was conducted to compare stem applications of potassium phosphonate with those of Aliette. The results showed that a 20% formulation of potassium phosphonate was superior to Aliette stem applications. This formulation was registered as Phytex by Horticura CC. It provided excellent control of *Phytophthora* foot and root rot when applied as a stem application, by painting 30-40cm of the trunk from just above the soil surface. However, the stem applications did not give any brown rot control. Le Roux, *et al* . (1999) tested it as a foliar spray and obtained control of brown rot for more than 8 weeks. Growers could now apply Phytex one month prior to harvesting in order to ensure protection against the development of brown rot as a post-harvest disease. The Phytex is applied at 1ℓ/100ℓ of water as a light cover spray (2000ℓ/ha). It is important not to spray trees which are under drought stress or when the day temperatures exceed 30°C. The foliar applications control feeder root rot, foot rot and brown rot. Orchards infected with *Phytophthora* receive three Phytex applications commencing with the rainy season. This would mean during October in the summer rainfall areas and during April in the winter rainfall areas. The applications are two months apart and co-incide with the increase of inoculum/disease severity rather than with the root growth flushes as is the case with avocado root rot (Whiley, *et al*., 1995). If necessary, due to excessive rains, a fourth foliar application is applied one month prior to harvesting to prevent the development of brown rot. When stem applications are used, a dye is added to the Phytex by the manufacturer. This does not only show which trees have been painted but also increases the sticking capabilities of the product.

De Lange *et al.* (1976) showed that scoparone was induced in citrus peel by black spot, *Guignardia citricarpa* Kiely. Rhône-Poulenc combined fosetyl-Al with mancozeb to produce a formulation known as Mikal-M to control black spot and *Phytophthora* with the same spray. It was decided to test potassium phosphonate with mancozeb as well. The results, however, indicated that the potassium phosphonate was less effective against *Phytophthora* when used in a mixture with mancozeb than when used on its own. Mason (Pers. comm., 1998) experienced the same when applying Phytex with and without mancozeb against powdery mildew (*Plasmopara viticola*) on grapes.

Phytex was also tested as an aerial spray. The same amount of product (15ℓ Phytex/ha) was applied using a crop sprayer which could apply 90ℓ mixture/ha instead of the 1800-2000ℓ which is applied by normal tractor driven spray machines. The aerial applications failed to deliver brown rot control and the effect on feeder root rot control was also not convincing. This concept was therefore abandoned.

It is known that the phosphonates are stable in plants but that they are oxidised in the soil by soil microbes (Adams & Conrad, 1953; Malacinski & Konetzka, 1967). The use of phosphonates were therefore never recommended in orchards. Aliette was, however, registered as a drench in nurseries. With the change to open hydroponic irrigation systems, where the water and nutrients applied through the system are absorbed by the roots within minutes after application, it was decided to put Phytex through these systems. The results to date are promising and sufficient data will be available during the coming season for registration.

Other root diseases

Phosphonate is effective against *Rhizoctonia solani* Kühn when applied as soil treatments to wheat (Wehner *et al*., 1987) and Medicago pastures (Pankhurst *et* al., 1998) and against *Fusarium oxysporum* on a number of different plants (Pankhurst, *et al*., 1998; Yamada *et al*., 1986). However, the current registrations held by the phosphonates are mainly for the systemic control of diseases caused by foliar and soilborne members of the Peronosporales and even within that group the efficacy differ from specie to specie. Voegele *et al*. (1997) showed that in the case of *Rhizobium* (*Sinorhizobium) meliloti* phosphate uptake by the plants were severely inhibited by phosphonates under phosphate limiting conditions.

Conclusion

Low concentrations of the citrus phytoalexin scoparone exist naturally in healthy citrus bark and fruit peel. This phytoalexin, which is associated with resistance against *Phytophthora*, increases rapidly following inoculation with the pathogen. In trees treated with phosphonates, the scoparone concentration in citrus bark inoculated with *Phytophthora* increased more than in untreated citrus. Potassium phosphonate proved to be the most inhibitory of the phosphonate formulations tested against most *Phytophthora* isolates.

Phytex (potassium phosphonate) controls feeder root rot and foot rot effectively for 8 weeks after a stem paint application. A foliar application will also control brown rot in the fruit effectively. Farmers have an inexpensive and long-lasting control of most of the *Phytophthora* root rots in the form of phosphonates.

Improvements in the effectiveness of phosphonates in disease control may be expected once there is an improved understanding of the factors that control phosphonate distribution in plants and by timing applications to co-incide with particular growth stages or disease pressure.

Literature cited

Adams, F. & J.P. Conrad. 1953. Transition of phosphite to phosphate in soils. Soil Science 75: 361-371.

Afek, U. & A. Sztejnberg & S. Carmely. 1986. 6,7-Dimethoxy-coumarin, a citrus phytoalexin conferring resistance against *Phytophthora* gummosis. Phytochemistry 25: 1855-1856.

Afek, U. & A. Sztejnberg. 1988. Accumulation of scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora.* Phytopathology 78:1678-1682.

Afek, U. & A. Sztejnberg. 1989. Effect of fosetyl-Al and phosphorous acid on scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora.* Phytopathology 79:736-739.

Afek, U. & A. Sztejnberg. 1989. Effects of fosetyl-Al and phosphorous acid on scoparone, a phytoalexin associated with resistance of *Citrus* to *Phytophthora citrophthora.* Phytopathology 79:736-739.

Afek, U., & A. Sztejnberg. 1993. Temperature and gamma irradiation effects on scoparone, citrus phytoalexin conferring resistance to *Phytophthora citrophthora.* Phytopathology 83: 753-758.

Anding, C.A., B. Merindol & J-M. Gouot. 1987. Mode d'action du phoséthyl-Al. Symposium Internationale Aliette, Lyon/Paris, 24-28 June, p.3.

Boenig, I.A., M.M. Crutchfield & C.W. Heitsch. 1982. Phosphorous compounds. In Encyclopaedia of Chemical Technology 17:490-539.

Bompeix, G., A. Ravisé, G. Raynal, F. Fettouche & M.C. Durand. 1980. Modalités de l'obtention des necroses bloquantes sur feuilles detaches de tomate par l'action du tris-*o*-éthyl phosphonate d'aluminium (phoséthyl d'aluminium), hypotheses sur son mode d'action in vivo. Annales de Phytopathologie 12:337-351.

Bompeix, G. & P. Saindrenan. 1984. In vitro antifungal activity of fosetyl-Al and phosphorous acid on *Phytophthora* species. *Fruit* 39:777-786.

Bower, L.A. & M.D. Coffey. 1985. Development of laboratory tolerance to phosphorous acid, fosetyl Al and metalaxyl in *Phytophthora capsici.* Canadian Journal of Plant Pathology 7:1-6.

Coffey, M.D. & M.C. Joseph. 1985. Effects of phosphorous acid and fosetyl-Al on the life cycle of *Phytophthora cinnamomi* and *P. citricola.* Phytopathology 75:1042-1046.

Darvas, J.M., J.C. Toerien & D.L. Milne. 1984. Control of avocado root rot by trunk injection with phosethyl-Al. Plant Disease 68:691-693.

Davidse, L.C. 1981. Resistance to acylalanine fungicides in *Phytophthora megasperma* f. sp. *medicaginis.* Netherlands Journal of Plant Pathology 87:11-24.

De Lange, J.H., A.P. Vincent, L.M. Du Plessis, P.J. Van Wyk & L.G.J. Ackerman. 1976. Scoparone (6,7-dimethoxycoumarin) induced in citrus peel by black spot, *Guignardia citricarpa* Kiely. Phytophylactica 8:83-84.

De Boer, R.F., F.C. Greenhalgh & K.G. Pegg. 1990. Phosphorous acid treatments control *Phytophthora* diseases in Australia. Bulletin OEPP/EPPO 20:193-197.

Dolan, T.E. & M.D. Coffey. 1988. Correlative *in vitro* behaviour of mutant strains of *Phytophthora palmivora* expressing different resistances to phosphorous acid and fosetyl-Na. Phytopathology 78:974-978.

Durand, M.C. & G. Sallé. 1981. Effet du tris-*o*-éthyl phosphonate d'aluminium sur le couple *L. esculenium – P. capsici.* Etude cytologique et cytochemique. Agronomie I:723-732.

Duvenhage, J.A. 1994. Monitoring the resistance of *Phytophthora cinnamomi* to fosetyl-Al and H_3PO_3. Yearbook of the S.A. Avocado Growers Association 17:35-37.

Farih, A., P.H. Tsao & J.A. Menge. 1981. Fungitoxic activity of Efosite-Al on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora,* Phytopathology 71:934-936.

Fenn, M.E. & M.D. Coffey. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74, 606-611.

Fenn, M.E. & M.D. Coffey. 1985. Further evidence for the direct mode of action of fosetyl-Al and phosphorous acid. Phytopathology 75:1064-1068.

Guest, D.I. 1982. Chemically induced host resistance – The mode of action of some systemic anti-oomycetes disease control chemicals. PhD. thesis. University of Sydney, NSW, Australia.

Guest, D.I., 1984a. The influence of cultural factors on the direct anti-fungal activities of fosetyl-Al, propamocarb, metalaxyl, SN 75196 and Dowco 444, Phytopathology 111:155-164.

Guest, D.I. 1984b. Modification of defence responses in tobacco and capsicum following treatment with fosetyl-Al [Aluminium tris (O-ethyl phosphonate)]. Physiological Plant Pathology. 25: 123-134.

Guest, D.I., K.G. Pegg & A.W. Whiley. 1996. *Phytophthora* diseases. Part I. Their control. Australian Plants 18:148, 366-369.

Le Roux, H.F., J.J. Bester & M.C. Pretorius. 1993. Effect of systemic and non-systemic fungicides on *Phytophthora nicotianae* resistant to furalaxyl in sand-bark medium. Proc. IV World Congress of the Int. Soc. of Citrus Nurserymen. South Africa. p. 216-222.

Le Roux, H.F., M.H. Mason & M.C. Pretorius. 1999. Efficacy of Phytex (potassium phosphonate) in controlling *Phytophthora* brown rot in citrus fruit. XIVth International Plant Protection Congress, Jerusalem, Israel.

Le Roux, H.F. , F.C. Wehner, J.M. Kotzé & N.M. Grech. 1991. Combining fosetyl-Al trunk injection or metalaxyl soil drenching with soil application of aldicarb for control of citrus decline, Plant Disease 75: 1233-1236.

Malacinski, G.M. & W.A. Konetzka. 1967. Orthophosphite-nicotinamide adenine dinucleotide oxido-reductase from *Pseudomonas fluorescens,* Journal of Bacteriology 93:1900-1910.

Muchovej, J.J., L.A. Maffia & R.M.C. Muchovey. 1980. Effects of exchangeable soil aluminium and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper, Phytopathology 70:1212-1214.

Neema, C., G. Bompeix & X. Mourichon. 1988. Induction of *Phytophthora citrophthora* mutants resistant to fosetyl-Al and phosphorous acid and study of their pathogenicity towards citrus. Fruits 43:499-505.

Ouimette, D.G. & M.D. Coffey. 1989a. Phosphonate levels in avocado seedlings and soil following treatment with fosetyl-Al or potassium phosphonate. Plant Disease 73: 212-215.

Ouimette, D.G. & M.D. Coffey. 1989b. Comparative antifungal activity of four phosphonate compounds against isolates of nine *Phytophthora* species. Phytopathology 79: 761-767.

Pegg, K., A.W. Whiley, J.B. Saranah & R.J. Glass. 1985. Control of *Phytophthora* root rot of avocado with phosphorous acid. Australasian Plant Pathology 14:25-29.

Piccone, M.F., A.E. Whiley & K. Pegg. 1987. Curing root rot in avocados. Australian Horticulture (May), p. 5-15.

Pankhurst, C.E., B.G. Hawke, H.J. McDonald & R. Johnson. 1998. Role of root disease in the poor establishment of Medicago pastures after cereal cropping in South Australia. Plant Pathology 47:749-758.

Sanders, F.L. & M.D. Coffey. 1990. Laboratory-induced resistance to fosetyl-Al in a metalaxyl-resistant field isolate of *Phythium aphanidermatum.* Plant Disease 74:690-692.

Schutte, G.C., J.J. Bezuidenhout & J.M. Kotzé. 1991. Timing of application of phosphonate, fungicides using different application methods as determined by means of gas-liquid-chromatography for *Phytophthora* root rot control of citrus. Phytophylactica 23:69-71.

Schwinn, F.J. & T. Staub. 1987. Phenylamides and other fungicides against oomycetes. In Modern Selective Fungicides : Properties, Application and Mechanisms of Action (ed. H. Lyr), pp. 259-274. Longman Scientific & Technical, Harlow, UK.

Smillie, R.H., B.R. Grant & R.L. Cribbes. 1988. Determination of phosphate and phosphite in plant material by gas chromatography-mass spectrometry and ion chromatography. Journal of Chromatography 455:253-261.

Smillie, R.H., B.R. Grant & D.I. Guest. 1989. The mode of action of the phosphite: evidence for both direct and indirect modes of action on three species of *Phytophthora* in plants. Phytopathology 79:921-926.

Tatum, J.H. & R.E. Berry. 1977. 6,7-Dimethoxycoumarin in the peels of citrus. Phytochemistry 16: 1091-1092.

Toy, A.D.F. & D.N. Walsh. 1987. Organic phosphorous plant growth control agents, herbicides and fungicides. Phosphorous Chemistry in Everyday Living, pp. 331-342. American Chemical Society, Washington, DC.

Voegele, R.T., S. Bardin & T.M. Finan. 1997. Characterization of the *Rhizobium* (*Sinorhizobium*) *meliloti* high and low-affinity phosphate uptake systems. Journal of Bacteriology. 179:23, 7226-7232.

Wehner, F.C., E.M. Smith, R.O. Barnard.& J.M. Kotzé. 1987. Control of crater disease of wheat, caused by *Rhizoctonia solani*, using metalaxyl and phosphorous acid. Phytophylactica 19: 495-498.

Whiley, A.W., P.A. Hargreaves, K.G. Pegg, V.J. Doogan, L.J. Ruddle, J.B. Saranah & P.W. Langdon. 1995. Changing sink strengths influence translocation of phosphonate in avocado *(Persea Americana* Mill.) trees. Australian Journal of Agricultural Research 46:5, 1079-1090.

Yamada, Y., T. Oishi, K. Mukai & T. Kato. 1986. Protective activities against *Fusarium* diseases of phenylphosphonic acids and related compounds. Journal of Pesticide Science 11: 627-629.