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DISTRIBUTION OF PHOSPHITE IN AVOCADO TREES AFTER TRUNK INJECTION WITH PHOSPHOROUS ACID AND ITS POSSIBLE RESPONSE TO *PHYTOPHTHORA CINNAMOMI*

G C SCHUTTE, T BOTHA, J J BEZUIDENHOUT[1] and J M KOTZÉ
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002
[1] Westfalia Estate, P O Box 14, Duivelskloof 0835

ABSTRACT

A 10 per cent phosphorous acid (H_3PO_3) solution with pH adjusted to 5,8 with KOH, was injected into avocado trees at a rate of 15 mℓ /m^{-2} canopy diameter. Gas-liquid-chromatograph (glc) analysis showed that the phosphite (PO_3^{3-}) concentration increased to 133 ppm in the leaves, three days after trunk injection, and steadily decreased after seven days. During the same period the PO_3^{3-} concentration in the roots increased after 21 days to a maximum of 57 ppm over a six-week-period. This concentration inhibits mycelial growth of Phytophthora cinnamomi *in vitro by 92 per cent.*

OORSIG

'n Tien persent fosforige suur (HsPOs) oplossing waarvan die pH na 5,8 aangepas is met KOH, is in avokadobome ingespuit teen 'n dosis van 15 mℓ /m^{-2} druparea. Gas-vloeistof-chromatograaf (gvc) analise wys dat die fosfiet (PO_3^{3-})-konsentrasie in die blare gestyg het tot 133 dpm drie dae na inspuiting, waarna dit geleidelik afgeneem het na sewe dae. Gedurende dieselfde tydperk het die PO_3^{3-}-konsentrasie in die wortels na 21 dae toegeneem tot 'n maksimum van 57 dpm oor 'n perlode van ses weke. In vitro, inhibeer hierdie konsentrasie PO_3^{3-} 92 persent van die miseliumgroei van P cinnamomi*.*

INTRODUCTION

Phytophthora root rot causes extensive damage to feeder roots of avocados, which results in a progressive decline in tree health and death (Sterne, Kaufmann and Zentmyer, 1978: Whiley, Pegg, Saranah and Forsberg, 1986; Whiley, Pegg, Saranah and Langdon, 1987).

Phosetyl-AI (aluminium tris-o-ethyl phosphanate) is the first commercially produced fungicide that moves in a basipetal direction after foliar application (Bertrand, Ducret, Debourge and Horriere, 1977). Darvas, Toerien and Milne (1984) demonstrated that trunk injections with phosetyl-AI was an effective cure for Phytophthora root rot control in avocados.

In vitro phosphorous acid (H_3PO_3) was found to be more active than phosetyl-AI against mycelial growth of several *Phytophthora* spp and the medium had little or no effect on the efficacy. H_3P0_3 was six to 14 times more active *in vitro* than phosetyl-AI (Fenn and Coffey,

1984). H_3PO_3 appears to be the toxophore responsible for inhibition of *Phytophthora* spp when host plants are treated with phosetyl-Al (Coffey and Bower, 1984).

Phosetyl-Al is degraded to H_3PO_3 and ethanol in plant tissue (Luttringer and De Cormis, 1985; Saindrenan, Dorakis and Bompeix, 1985) and in soils (Piedallu and Jannet, 1985). Research on disease control with phosphorous acid is taking place in many crops and in many countries (Wehner, Smith, Barnard and Kotzé, 1987). At present Australia is the only country where patent rights have been overcome and has been registered (Fos-ject 200) as a trunk injectable fungicide for root rot control in avocados.

The trunk injection method for treating diseased avocado trees is used extensively, but there is no reliable method to monitor phosphite in the roots. This paper describes the distribution and concentrations of phosphite in avocado trees over a period of time, after trunk injection with H_3PO_3 and the correlation thereof with *in vitro* inhibition of *P cinnamomi.*

MATERIALS AND METHODS

Field experiment

A 10 per cent H_3PO_3 solution (pH adjusted to 5,8 with 10 per cent KOH) at a rate of 15 mℓ /m^{-2} canopy area was injected into trunks of three-year-old avocado trees at the University of Pretoria's experimental farm. Three replications were used per treatment and three trees, injected with sterile distilled water, were used as control. The phosphite concentrations were measured 0, 3, 7, 14, 21, 35, 42 and 63 days after initial injection, by means of a gas-liquid-chromatograph (glc).

Assay for phosphite by means of glc Extraction

Ten g of each root and leave sample was homogenised by mortar and pestle in liquid nitrogen. One g of the fine sample was added to 10 mℓ distilled water and further homogenised with an ultraturrax.

The sample was centrifuged for five min at 8 000 rpm. Two mℓ methoxyethanol and 9,1 mi of the sample was transferred to a vial and 10 mℓ formic acid was added to the solution. A standard H_3PO_3 series with ooncentrations of 1, 5, 10, 15 and 20 ppm was processed the same way as above.

Derivation

Two solutions, 10 per cent KOH and 20 per cent N-methyl-N nitroso-p-toluolsulfonamide, were separately dissolved in 50 mℓ 2-methoxy-ethanol. Nitrogen gas was bubbled through a mixture of the two solutions that turned the clear sample slightly yellow, whereupon another 10 mℓ formic acid was added to clear the solution again.

Glc analysis

The samples were analysed by glc under conditions presented in Table 1. One mℓ of both the standard and sample solutions were injected into the glc. Series of phosphite concentrations were graphically analysed and used as standard to determine the phosphite concentrations in the various samples.

TABLE 1 Gas liquid – chromatograph conditions for the determination of phosphite concentrations

Detector	Type	NPSD (Carlo Erba)
	Temperature	190°C
Colomn	Type	Carbowax
	Temperature	140°C
	Length	2 metres
Injector	Temperature	150°C
"Carrier" Gas	Type	N_2
	Flow rate	0,80 kg/cm^{-2}
Air	Flow rate	0,80 kg/cm^{-2}
Hydrogen Gas	Flow rate	0,80 kg/cm^{-2}
Registrator	Sensitivity	1 mV
	Paper speed	30 cm/h
Sample size		1 µℓ

RESULTS

Glc analysis

Four minutes after injecting the samples into the glc, the phosphite peaks were drawn by the recorder on graphic paper (Figure 1). A series of concentrations were used to graphically determine the phosphite concentrations. Glc analysis showed that the phosphite concentration in the leaves increased to 133 ppm, three days after injection of phosphorous acid into the trees. After seven days the phosphite concentration steadily decreased to 18,0 ppm over a period of nine weeks, while the phosphite content in the control stayed more or less the same, with means of 8 ppm (Figure 2; Table 2). During the same period, the phosphite concentration in roots increased from one to 57 ppm While the phosphite content in the control was slightly less with means of 2,38 ppm (Figure 3; Table 3).

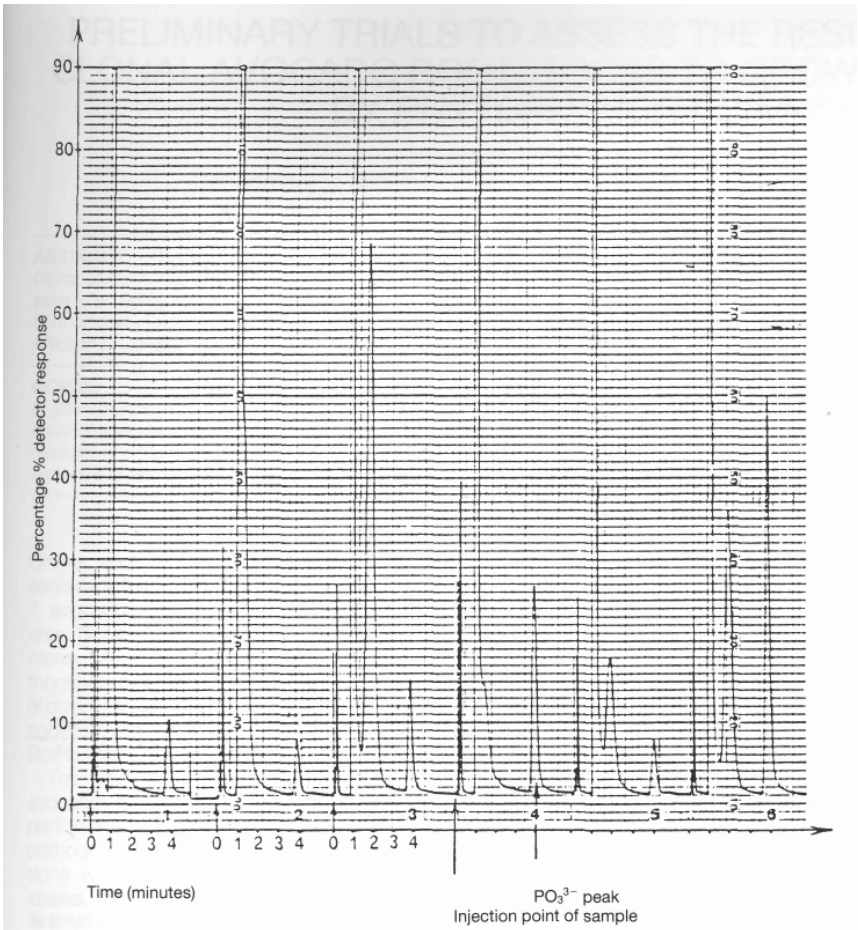

Fig 1 Determination of PO_3^{3-} concentrations in plant tissue after injection into a gas-liquid-chromatograph.

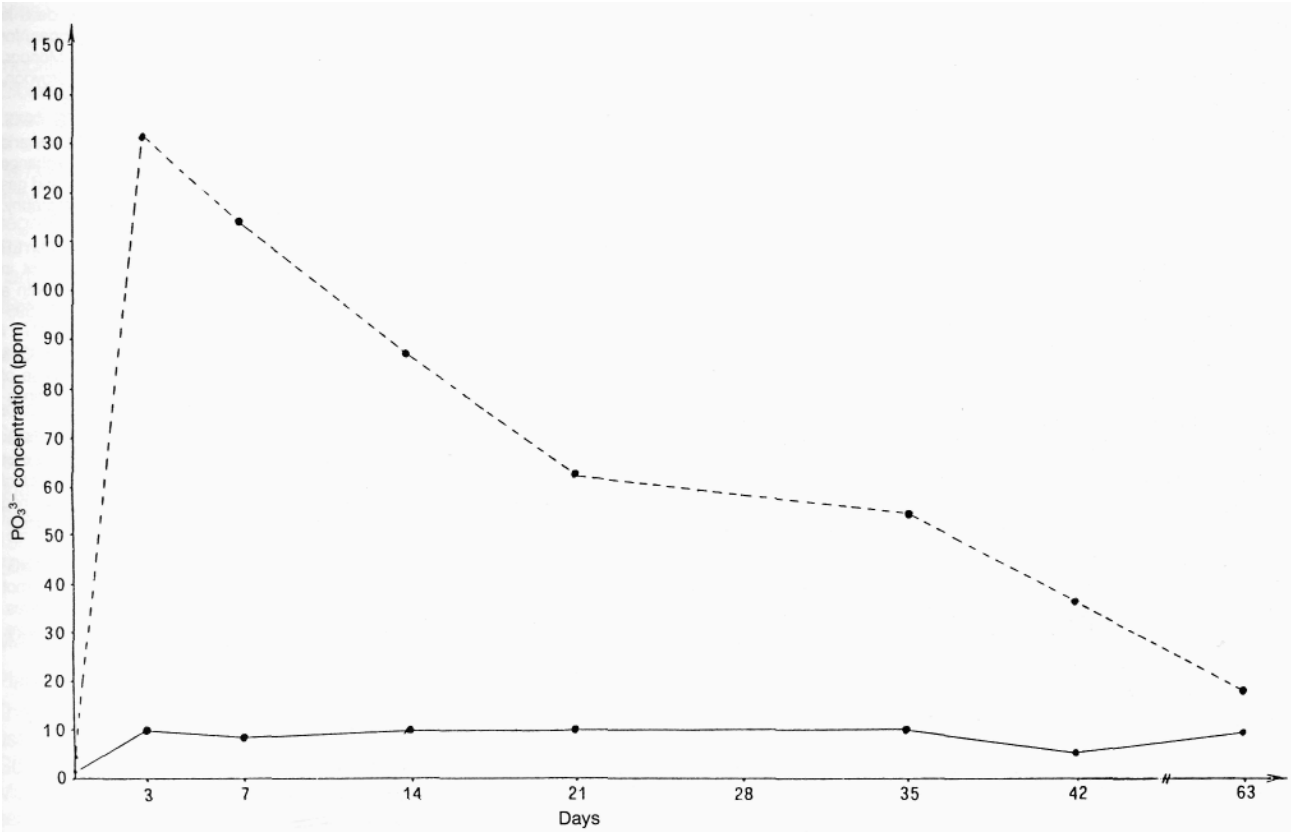

Fig 2 Distribution of phosphite in avocado leaves over a period of nine weeks in experimental trees (●---●) and in trees receiving no treatment (●——●).

TABLE 2 Mean phosphite concentrations in avocado leaves over a nine-week-period

Day	Phosphite concentrations (ppm) in leaves	
	Control	Experiment
0	1,67d	5,00d
3	10,00cd	133,33a
7	9,00cd	115,00a
14	10,00cd	88,33ab
21	9,67cd	63,33bc
35	7,67cd	55,00bcd
42	5,00d	35,50cd
63	7,67d	18,00cd

Mean values are based on three replicates; values with the same letter are not significantly different (P=0,05) according to Duncan's multiple range test.

TABLE 3 Mean phosphite concentrations in avocado roots over a nine-week-period

Day	Phosphite concentrations (ppm) in roots	
	Control	Experiment
0	1,00c	1,00c
3	3,33c	9,00bc
7	1,67c	8,00bc
14	3,00c	9,33bc
21	2,33c	14,00ab
35	1,33c	20,33a
42	4,00c	57,00a
63	2,33c	38,00a

Mean values are based on three replicates; values with the same letter are not significantly different (P=0,05) according to Duncan's multiple range test.

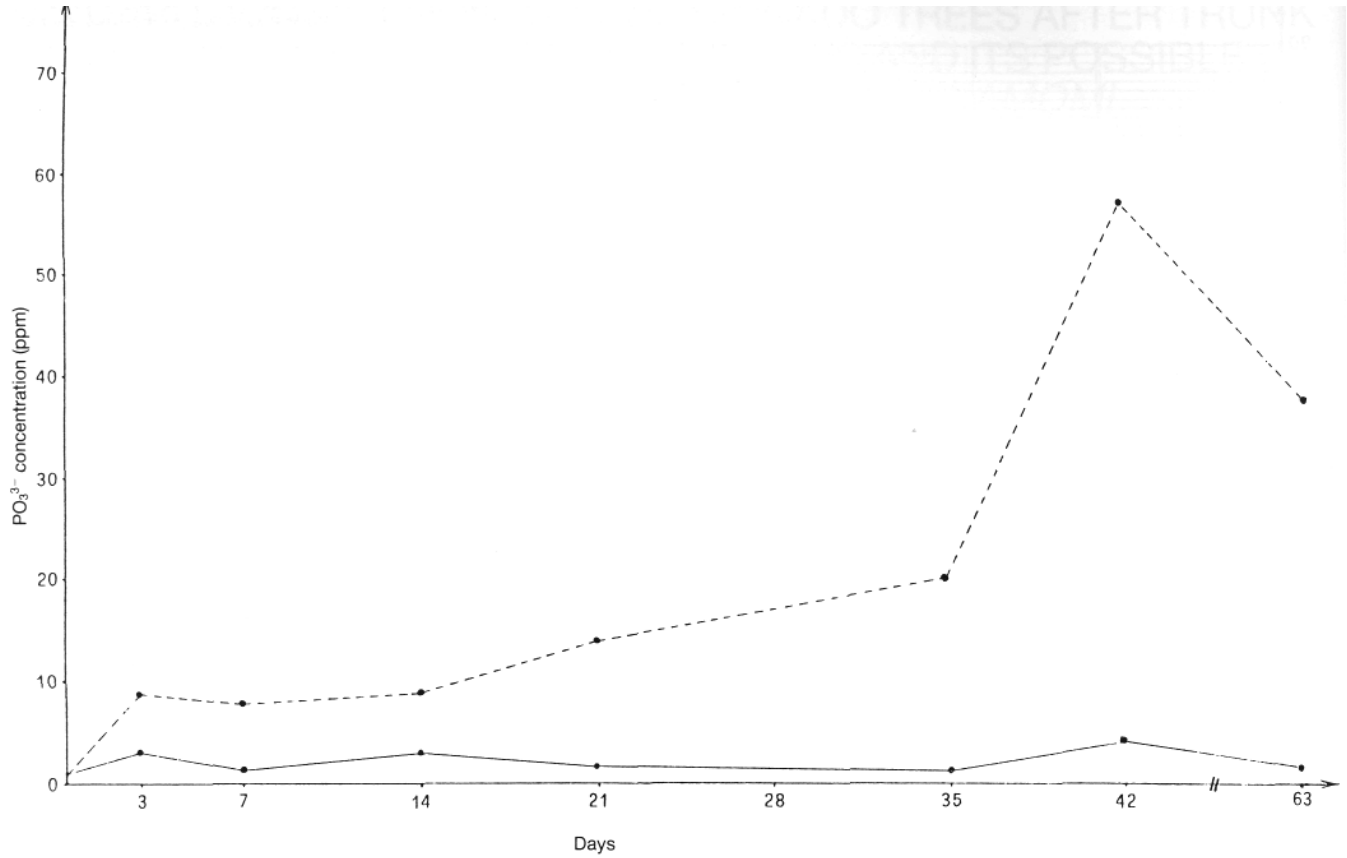

Fig 3 Distribution of phosphite in avocado roots over a period of nine weeks in experimental trees (●---●) and in trees receiving no treatment (●——●).

DISCUSSION

Bezuidenhout, Darvas and Kotzé (1987) determined the distribution of PO_3^{3-} in avocado trees treated with phosetyl-Al and found that the PO_3^{3-} content in the aerial parts of the tree, viz mature leaves, branches and fruit, steadily increased while the PO_3^{3-} content in

the roots increased two weeks after injection. A maximum of about 17 ppm was reached after four weeks. This PO_3^{3-} concentration will, according to Coffey and Bower (1984), only control 60 per cent of the mycelial growth of *P cinnamomi.* Although the PO_3^{3-} concentration decreased in the roots, the aerial parts had a higher PO_3^{3-} content than the roots after 10 weeks.

The glc analysis showed that after three weeks the roots had a statistically significant increase of PO_3^{3-}, which increased to a maximum of 57 ppm after seven weeks. According to Coffey and Bower (1984) this concentration will inhibit *in vitro* 92 per cent of the mycelial growth of *P cinnamomi.*

The high biological activity of H_3PO_3 against *P cinnamomi* indicates a specific effect of this toxophore against this pathogen. Although the movement of phosphite ions is initially upwards towards the leaves, the way down to the roots is quickly found and given protection from about 21 days after injection.

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