

MODE OF RESISTANCE IN AVOCADOS AFTER TREATMENT WITH PHOSPHOROUS ACID

T BOTHA, J H LONSDALE and G C SCHUTTE

Dept of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002

ABSTRACT

*Antifungal compounds occur in avocado roots, but the concentration of these compounds are not affected after treatment with H_3PO_3 . Phosphite concentrations in avocado trees did not reach levels that are toxic to *Phytophthora cinnamomi*, but H_3PO_3 concentration in the roots increased to levels inhibitory to sporangium production and zoospore release. An H_3PO_3 concentration of 57 ppm was measured 42 days after treatment. The inhibitory effect on *P. cinnamomi* was observed two weeks after an H_3PO_3 treatment.*

OORSIG

*Antifungiese stowwe kom voor in avokadowortels, maar die konsentrasie bly onveranderd na 'n H_3PO_3 -behandeling. Fosfietkonsentrasies in avokadobome het nooit letale vlakke vir *Phytophthora cinnamomi* bereik nie, maar die H_3PO_3 -konsentrasie in die wortels het gestyg tot 'n vlak wat inhiberend was teenoor sporongiaproduksie en soospoorvrystelling. Die H_3PO_3 -konsentrasie in wortels was 57 dpm 42 dae na behandeling. Geïnduseerde weerstand is twee weke na behandeling waargeneem.*

INTRODUCTION

Phosetyl-Al is degraded to phosphorous acid (H_3PO_3) in plants (Saindrenan, Darakis and Bompeix, 1985). Avocado root rot control is more effective with H_3PO_3 than with phosetyl-Al (Darvas and Bezuidenhout, 1987). H_3PO_3 is translocated both acropitally and basipitally in the plant (Zentmyer, 1979); initially to the leaves via the xylem and then with the photosynthate to the roots via the phloem (Piccone, Whiley and Pegg, 1987).

There is uncertainty concerning the disease control mechanism of H_3PO_3 . Fenn and Coffey (1985) ascribed the controlling effect to the direct action of H_3PO_3 on *P. cinnamomi*, while Bompeix and Saindrenan (1984) and Kotzé, Moll and Darvas (1987) suggested that H_3PO_3 acts indirectly by activating the defence mechanism of the plant against the pathogen.

To date the relationship between H_3PO_3 concentration, accumulation of antifungal compounds and induction of resistance in avocado roots, has not been investigated. This paper therefore reports the results of a study in which the theoretical basis for the mode of action of H_3PO_3 was compared under similar conditions.

MATERIALS AND METHODS

A 10 per cent H_3PO_3 solution (pH adjusted to 5,8 with KOH) was used in all experiments. Glasshouse Experiment Ten-month-old clonal Duke 7 seedlings (Mass scion) were used in all experiments. Each tree was injected with 0,5 ml 10 per cent H_3PO_3 . Control trees were each injected with 0,5 ml sterile distilled water.

Phosphite analysis

H_3PO_3 concentration was determined with gas liquid chromatography (GLC) according to the method described by Bezuidenhout, Darvas and Kotzé (1987) seven, 14 and 21 days after injection (three seedlings / treatment).

Detection of antifungal compounds Extraction and purification

Stem segments (15 g) were cut from the seedlings seven, 14 and 21 days after injection (three replicates / treatment) and extracted by means of the method described by Dubery and Schabort (1987). The extract was concentrated to a volume of 10 ml and 30 μl (of each concentrate were spotted on silica gel 60 thin-layer chromatography (TLC) plates with fluorescent indicator. TLC plates were chromatographed with benzene:ethyl acetate (2:1 v/v).

Detection

Chromatograms were subjected to two bioautography methods. In the first, TLC plates were sprayed with a spore suspension of *Cladosporium cladosporioides* (Fresen) de Vries in a glucosemineral salts solution (Homans and Fuchs, 1970). After spraying, the TLC plates were incubated in a moist chamber for five days at 25°C. Inhibition zones indicated the presence of fungitoxic compounds. In the second method, zoospore germination of *P. cinnamomi* was used as criterium. Potato dextrose agar (PDA) was poured to a thickness of 3 mm on a glass plate and transferred to the TLC plates after solidification. Two millilitre zoospore suspension of *P. cinnamomi* (10^5 spores/ml) were evenly spread over the PDA surface. Clear zones indicating inhibition of zoospore germination were recorded after two days.

FIELD EXPERIMENT

Three-year-old clonal Duke 7 trees were used for determining antifungal compounds, phosphite concentration and resistance to *P. cinnamomi*. Experimental trees were injected with H_3PO_3 (0,4 g ai/m² leaf canopy), while control trees were injected with the same volume of sterile distilled water.

Phosphite analysis

H₃PO₃ concentration was determined at various intervals (three, seven, 14, 21 and 35 days after treatment) with the method of Bezuidenhout *et al* (1987).

Detection of antifungal compounds Extraction and purification Young feeder roots (30 g) were collected from each tree (three, seven, 14, 21 and 35 days after treatment) and three methods were used to extract fungitoxic compounds. The first method was described by Dubery *et al* (1987). The second extraction was done with 150 ml chloroform and the extract concentrated to a volume of 10 ml . With the third method, roots were homogenised in 100 ml HCl (0,1 M) and phenolic compounds were extracted according to the technique of Ribereau -Gayon (1972).

Detection

The two methods described under glasshouse experiment were used, and TLC plates were examined under UV light (366 nm) for fluorescent properties.

Determination of resistance

Ten freshly cut root tips (15 mm long), collected 0, 14, and 28 days after injection from each tree, were suspended in sterile distilled water in test tubes (five replicates / treatment). The method of Zilberstein and Pinkas (1987) was used to determine resistance to infection by *P. cinnamomi*. Electrical conductivity of bathing solutions containing the root tips, was measured 48 hours after inoculation with zoospores.

RESULTS

Glasshouse Experiment Phosphite analysis

A significant increase in H₃PO₃ concentration was noted 14 and 21 days after treatment (Figure 1).

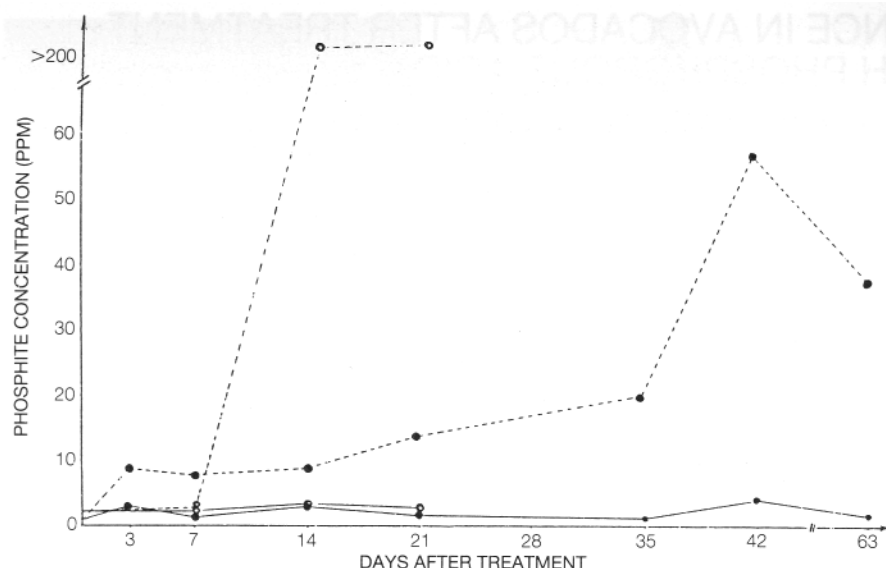


Fig 1 Phosphite concentration in avocado roots of : treated glasshouse plants (○-----○), control glasshouse plants (○—○), treated field trees (●-----●) and control field trees (●—●).

Detection of antifungal compounds

Two antifungal substances were detected (seven, 14 and 21 days after treatment) in both treated and nontreated seedlings. These substances had R_f values of 0,11 and 0,88 respectively. The concentration of the two substances did not differ significantly between treatments.

Field Experiment

Phosphite analysis

The H_3PO_3 concentration increased gradually in the roots after treatment. Significant differences between treatments occurred 21 to 63 days after treatment (Figure 1).

Detection of antifungal compounds

Method 1

Two antifungal substances with R_f values 0,10 and 0,88 were detected in treated as well as untreated trees at all the intervals.

Method 2

A single inhibition zone (R_f 0,98) occurred on the TLC plates from treated as well as untreated trees at all the intervals.

Method 3

A single inhibition zone (R_f 0,89) was observed on the TLC plates from treated as well as

untreated trees at all the time intervals (Figure 2). The antifungal compounds had no fluorescent properties under UV light (366 nm).

Determination of resistance

The electrical conductivity (EC) did not differ significantly between treatments at commencement of the experiment. However, two weeks after treatment, the EC of the roots from treated trees was significantly lower than that of roots from untreated trees. The results obtained four weeks after treatment could not be interpreted, due to bacterial contamination of the bathing solutions (Table 1). application of H_3PO_3 . Although concentration of H_3PO_3 in the tissues never reached levels fungicidal to *P cinnamomi*, partial inhibition of growth of the pathogen probably enabled the plants to effectively activate other host defence mechanisms. Such mechanisms might have included morphological changes like formation of suberine and tylose (Phillips, Grant and Weste, 1987). A correlation exists between resistance according to Zilberstein *et al* (1987) and H_3PO_3 accumulation in roots.

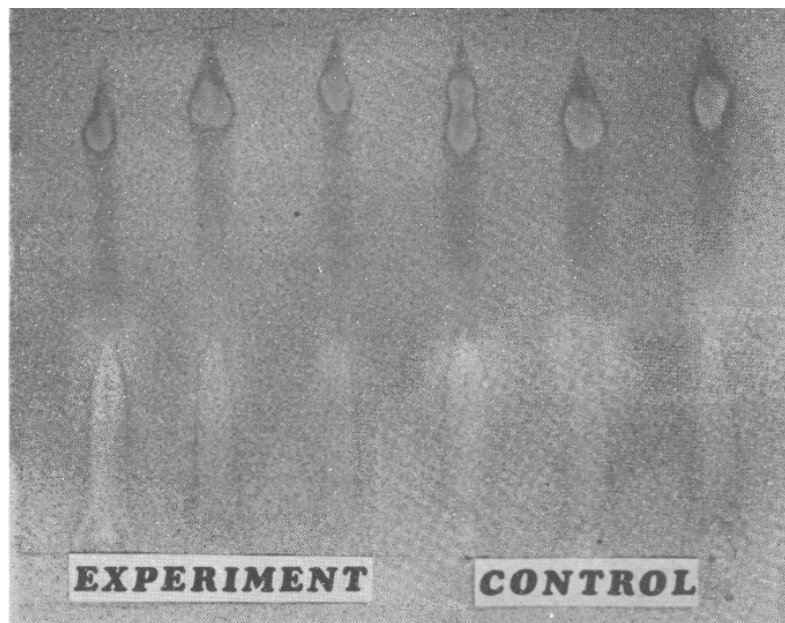


Fig 2 Inhibition of *Cladosporium cladosporioides* on a TLC plate chromatographed with extracts from avocado roots, treated (experiment) and not treated (control), with H_3PO_3 .

TABLE 1 Relative electrical conductivity of bathing solutions incubated with avocado roots inoculated with *P cinnamomi*

Days after treatment	Treatment	
	Control	Experiment
0	0,13b	0,11b
14	0,23a	0,09b

Each value is the mean of five replicates : Values on non-inoculated root segments were subtracted from the inoculated ones. Values not followed by the same letter are significantly different ($P=0,05$), according to Duncan's multiple range test.

REFERENCES

- BOMPEIX G & SAINDRENAN P, 1984, *In vitro* antifungal activity of fosetyl-AI and phosphorous acid on Phytophthora species. *Fruits* 39, 777 - 786.
- COFFEY M D & BOWER L A, 1984, *In vitro* variability among isolates of eight Phytophthora species in response to phosphorous acid. *Phytopathology* 74, 738 - 742.
- DARVAS J M & BEZUIDENHOUT J J, 1987. Control of Phytophthora root rot of avocados by trunk injection. *S A Avocado Growers' Assoc Yrb* 10, 91 - 93.
- DUBERY I A & SCHABORT J C, 1987. 6, 7, Dimethoxycoumarin: a stress metabolite with antifungal activity in irradiated citrus peel. *S A Journal of Science* 83, 440 - 441.
- FENN M E & COFFEY M D, 1985. Further evidence for the direct mode of action of fosetyl-AI and phosphorous acid. *Phytopathology* 75, 1064 - 1068.
- HOMANS A L & FUCHS A, 1970. Direct bioautography on thin layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography* 51, 327 - 329.
- KOTZE J M, MOLL J N & DARVAS J M, 1987. Root rot control in South Africa : Past, present and future. *S A Avocado Growers' Assoc Yrb* 10, 89 - 91.
- PHILLIPS D, GRANT B R & WESTE G, 1987. Histological changes in the roots of an avocado cultivar, Duke 7, infected with *Phytophthora cinnamomi*. *Phytopathology* 77, 61 - 69.
- PICCONE M F, WHILEY A W & PEGG K G, 1987. Curing root rot in avocados. *Australian Horticulture* 5, 5 - 115.
- RIBEREAU-GAYON P, 1972. Plant phenolics, Oliver & Boyd Edinburgh.
- SAINDRENAN P, DARAKIS G & BOMPEIX G, 1985, Determination of ethyl phosphite, phosphite and phosphate in plant tissues by anion-exchange high performance liquid chromatography and gas chromatography. *Journal of Chromatography* 347, 267 - 273.
- ZENTMYER G A, 1979. Effect of physical factors, host resistance and fungicides on root infection at the soil root interface in the soil interface, ed J L Harley, R Scott-Russell, pp 315 - 328. London: Academic 4478 pp.
- ZENTMYER G A, 1984. Avocado diseases. *Tropical Pest Management* 30, 388 - 400.
- ZILBERSTEIN M & PINKAS Y, 1987. Detached root Inoculation a new method to evaluate resistance to Phytophthora root rot in avocado trees. *Phytopathology* 77, 841 - 844.