

Effect of phosphite in avocado roots on the zoospores of *Phytophthora cinnamomi*

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ABSTRACT

Levels of phosphite inhibitory to *Phytophthora cinnamomi* were reached in roots of six-month-old *Persea americana* cv *Edranol* within seven days after injection with phosphorous acid. Scanning electron microscope (SEM) studies showed no significant differences in the cyst's ability to germinate and infect phosphorous acid treated or control roots. SEM showed severe deterioration of root surfaces in untreated control roots compared to phosphorous acid treated roots.

The detached root technique described by Botha et al (1989) proved to be an excellent in vitro method of testing the effectiveness of antifungal activity of systemic fungicides when a highly susceptible plant is used.

INTRODUCTION

Research on chemical control of avocado root rot caused by *Phytophthora cinnamomi* Rands dates back many years (Zentmyer, 1955). Direct injection of the chemical Fosetyl-Al into the stem of avocado trees, was invented much later (Darvas et al, 1984). Fosetyl-Al is degraded to H_3PO_3 and EtOH in plant tissue (Luttringer & De Cormis, 1985; Saindrenan et al, 1985). H_3PO_3 was found to be 14 times more inhibitory to *Phytophthora* spp *in vitro*, than Fosetyl-Al (Fenn & Coffey, 1984). It was found that the phosphite concentration in avocado roots reached its peak six weeks after injection with phosphorous acid.

The aim of this study is to determine the reaction of zoospores of *P cinnamomi* on H_3PO_3 treated roots compared to untreated roots, as well as to determine the antifungal activity occurring in the roots after treatment, by means of the detached root technique described by Botha et al (1989).

MATERIALS AND METHODS

Plant material

Susceptible *Persea americana* Edranol seedlings were used throughout all experiments.

Treatment

Plants were injected with a 10% phosphorous acid solution at a rate of 0,8 g aim⁻² canopy area. To reduce the effects of phytotoxicity to the trees, the H₃PO₃ solution was partially neutralized with potassium hydroxide to a pH of 6,1. Control plants were left untreated.

Pathogen isolate and zoospore production

P cinnamomi isolated from avocado roots in the Nelspruit area was used. The pathogen was grown on pea-agar (Chen & Zentmyer, 1970) and a method of Gisi *et al* (1980) was used for production of sporangia. Zoospore release was induced as described by Botha *et al* (1989). Concentrations of 10³ to 10⁴ ml⁻¹ zoospores were obtained in this manner.

Roots

The use of excised root tips for determining tolerance in avocado rootstocks was described by various researchers (Kellam & Coffey, 1985; Dolan & Coffey 1986 and Botha *et al*, 1989). To determine the antifungal activity induced by H₃PO₃ injections the method of Botha *et al* (1989) was used. Roots were excised from ten plants injected with H₃PO₃, and from ten untreated plants, seven and 14 days after treatment. Two root tips, ca 50 mm in length, from each plant were placed perpendicularly over two parallel glass rods imbedded in 15 ml water-agar in petri-dishes (10 root tips per plate). Each root tip was inoculated at the region of elongation, with 10 ml of a *P cinnamomi* zoospore suspension, containing 4 x 10³ ml⁻¹ motile zoosporas in distilled water and plates, were incubated in the dark at 25°C. After 48 hours the roots were surface disinfected for 5 S in 70% ethanol, cut aseptically into 4 mm segments and plated out sequentially on a selective medium (PARPH) and incubated at 25°C. After two to three days the number of segments from which *P cinnamomi* developed were recorded.

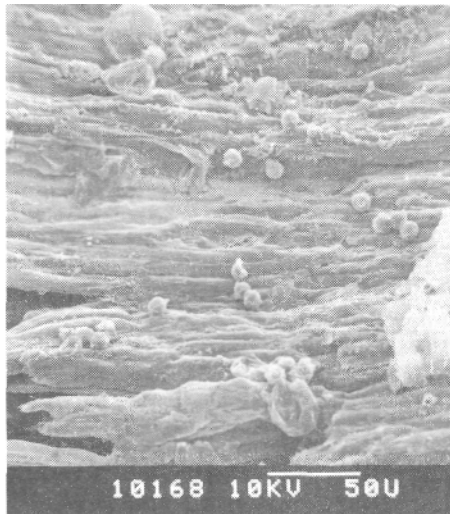


Fig 1 Encysted zoospores of *P. cinnamomi* on the surface of an untreated Edranol root 30 minutes after inoculation. Germ tubes are just emerging on some cysts (Mag: 400X).

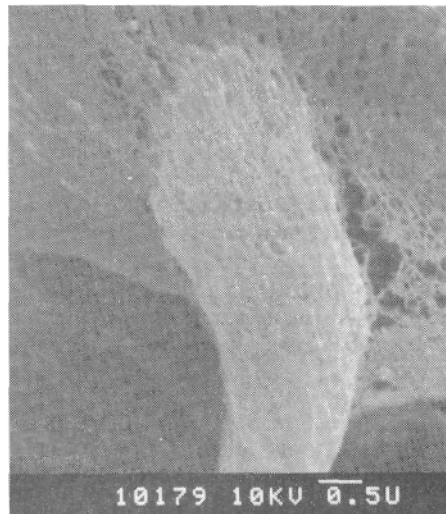


Fig 2 An appressorium of *P. cinnamomi* fixed to the surface of an untreated Edranol feeder root 2 h after inoculation with zoospores (Mag: 18 000X).



Fig 3 A cyst of *P. cinnamomi* with the germ tube clearly evident and fading away underneath an untreated root surface, 3 h after inoculation with zoospores. No appressorium present (Mag: 2 500X).



Fig 4 The germ tube from a cyst of *P. cinnamomi*, penetrating an untreated Edranol feeder root 6 h after inoculation with zoospores. No appressorium present (Mag: 22 000X).

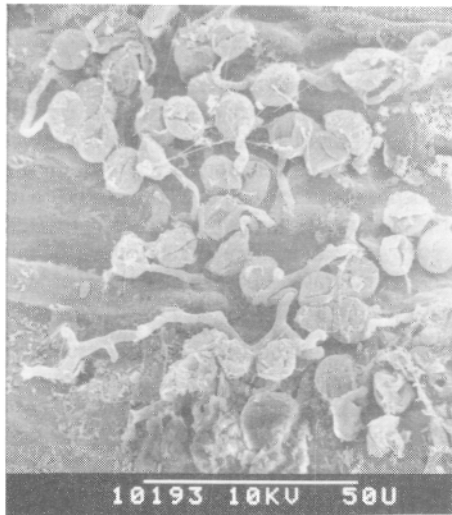


Fig 5 Cysts and mycelium of *P cinnamomi*, 5 h after inoculation with zoospores on an untreated root (Mag: 950X).

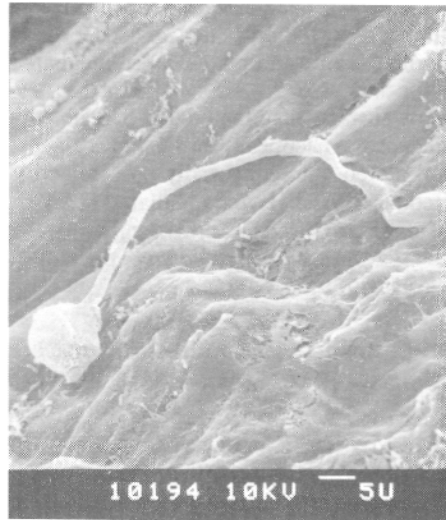


Fig 6 Cyst, germ tube and appressorium of *P cinnamomi* on the surface of an untreated Edranol root 6 h after inoculation with zoospores (Mag: 1 500X).

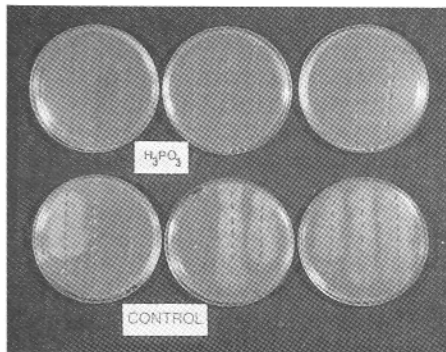


Fig 7 Linear colonisation of excised Edranol feeder roots 48 h after inoculation with motile zoospores of *P cinnamomi*, 14 days after H_3PO_3 injections (top), compared to the roots of the untreated control trees (bottom). Linear colonisation was measured when plated out, by measuring the root segments from which *P cinnamomi* was recovered.



Fig 8 Edranol root from untreated control tree 26 h after inoculation with zoospores of *P. cinnamomi*. Note the deterioration in structure of the root surface (Mag: 41X).

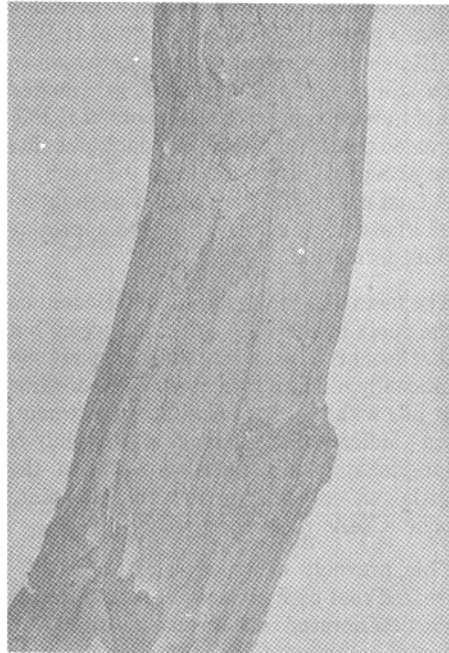


Fig 9 Edranol root from an H_3PO_3 treated tree, two weeks after H_3PO_3 injection and 26 h after inoculation with zoospores of *P. cinnamomi*. Cysts and germ tubes are clearly visible on root surface. Note the structure of root surface still intact (Mag: 41X).

TABLE 1 Linear colonisation of excised root tips of six-month-old Edranol seedlings injected with H_3PO_3 , 48 hours after inoculation with zoospores of *P. cinnamomi*

Days after treatment	Linear colonisation of roots (mm) after 48 h	
	Control (Tree untreated)	Phosphite (Trees injected with H_3PO_3)
7	24,00 a	1,82 b
14	20,27 a	0,36 b

Preparation for Scanning Electron Microscope (SEM)

After roots were inoculated and/or incubated root tips ca 10 mm were fixed overnight in a 2,5% Gluter aldehyde solution pH 7,3. The roots were then washed three times in a 0,075M phosphate buffer, followed by washing once for 15 minutes in each of a 20, 50, 70 and 90% ethanol solutions, followed by washing three times in a 100% ethanol solution for 15 minutes. Samples were critical point dried with CO_2 and mounted for gold

coating in a Eiko IB 2 ion coater. The Hitachi S450 scanning electron microscope was used throughout all experiments.

Roots from untreated plants were inoculated with zoospores and treated as described by Botha *et al* (1989), except that roots were taken out of incubation at different time intervals, 0 (not inoculated), 30 minutes, 1, 2, 3, 4, 5, 6, 9, 12, 24 and 48 hours after incubation.

RESULTS

Roots from untreated trees, taken out of incubation at different time intervals after inoculation with motile zoospores of *P cinnamomi*, revealed that at 30 minutes after inoculation the zoospores have already encysted and germ tubes were just visible (Figure 1). After 2 h, the appressoria were clearly visible (Figure 2). It also appeared that appressorium formation was not always necessary for root penetration (Figures 3 and 4). At 5 h the typical mycelium growth of *P cinnamomi* was evident on some of the root surfaces (Figure 5). Formations of appressoria were generally very common (Figure 6). a deterioration in structure of root surfaces were evident 26 h after inoculation (Figure 8).

A significant reduction in linear colonisation of excised Edranol root tips, inoculated with zoospores of *P cinnamomi*, were evident seven days after injecting with H_3PO_3 , when compared with untreated roots. Fourteen days after H_3PO_3 treatment linear colonisation almost stopped (Figure 7 and Table 1).

Comparing the excised root tips of untreated control trees, to excised root tips of H_3PO_3 treated trees, 14 days after injection and 26 h after inoculation with motile zoospores of *P cinnamomi*, the structure of the root surfaces from the H_3PO_3 treated trees were much more intact (Figure 9) than the deteriorated structure of the root surface from untreated control trees (Figure 8).

SEM also showed that zoospores had the ability to encyst, germinate and form appressoria on roots from both control trees as well as on roots from H_3PO_3 treated trees. Even though the linear colonisation of the Edranol roots by zoospores of *P cinnamomi* were almost halted within 14 days after injections with H_3PO_3 , the zoospores still had the ability to encyst, germinate and form appressoria on these treated roots.

Roots were inoculated with zoospores near the tip at the region of elongation. After 48 h roots were cut into segments and the segments plated on selective medium (PARPH). Each value is the mean of 10 replicates. Values in rows, not followed by the same letter, differ significantly according to Duncan's multiple range test ($P = 0,05$).

DISCUSSION

According to Coffey and Joseph (1985), H_3PO_3 is inhibitory to sporangium formation and chlamyospore production. Zoospore release oospores and cyst germination is generally highly sensitive to H_3PO_3 . SEM showed that zoospores of *P cinnamomi* still recognised, encysted, germinated and formed appressoria on roots of H_3PO_3 treated trees. This possibility indicates that no phosphite is exuded from the roots, or at least

not in quantities high enough for inhibition of cyst germination and appressorium formation.

The facts that the cysts still germinated on the roots of the H₃PO₃ treated trees, but that linear colonisation of the roots were significantly reduced to almost no colonisation at all, within two weeks after treatment, may indicate that the level of phosphite is fungitoxic inside the root, but that the pathogen only encounters the phosphite once it penetrates the root.

This prevents destruction of the H₃PO₃ treated root by *P cinnamomi*, compared to the deteriorated root surface of the control roots.

The detached root technique, used by Kellam and Coffey (1985); Dolan and Coffey (1986) and modified by Botha *et al* (1989), for determining tolerance of avocado rootstocks, can be used for *in vitro* testing of efficacy and distribution of systemic fungicides such as H₃PO₃ and Fosetyl-Al in avocado roots.

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