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THE INFLUENCE OF CALCIUM ON SAPROPHYTIC GROWTH AND PATHOGENICITY OF *PHYTOPHTHORA CINNAMOMI* AND ON RESISTANCE OF AVOCADO TO ROOT ROT

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ABSTRACT

The effect of calcium on saprophytic growth and pathogenicity of Phytophthora cinnamomi *Rands was studied as well as the influence of calcium on the resistance of avocado to root rot caused by the pathogen.*

Saprophytic growth of the pathogen was increased significantly by calcium, although the pathogenicity of the fungus was not influenced significantly. Ungrafted Edranol, Duke 7 and Martin Grande (G755) trees showed a significant decrease in susceptibility to Phytophthora cinnamomi *when treated with calcium sulphate (as well as with calcium carbonate for Edranol). The same tendency (although not significant) was observed when these rootstocks were grafted with Hass.*

UITTREKSEL

Die uitwerking van kalsium op saprofitiese groei en patogenisiteit van Phytophthora cinnamomi Rands *is bestudeer, sowel as die invloed van kalsium op die weerstandbiedendheid van avokadoboompies teen wortelvrot, veroorsaak deur die patogeen.*

Saprofitiese groei van die patogeen is betekenisvol verhoog deur kalsium, alhoewel die patogenisiteit van die swam nie betekenisvoi bel'nvloed is me. Ongeénte Edranol, Duke 7 en Martin Grande (G755) onderstamme net 'n betekenisvolle verlaging in vatbaarheid vir Phytophthora cinnamomi *getoon na behandeling met kalsiumsulfaat (sowel as Edranol behandel met kalsiumkarbonaat). Dieselfde tendens, alhoewel onbetekenisvol, is gevind by hierdie onderstamme wat geént is met Hass*

INTRODUCTION

High levels of calcium, nitrogen, microbial activity and organic matter in soils suppressive to *Phytophthora cinnamomi* Rands *(PC),* the causal organism of root rot of avocado *(Persea americana)* (Milne, Brodrick & Hughes, 1975), have been implicated as the main reasons for disease reduction in these soils (Broadbent & Baker 1974). Subsequently, cover cropping and applications of dolomite and nitrogen-rich fertilisers have been recommended (Chalker, 1979).

Reports on the effect of calcium on growth of *PC* (Chee & Newhook, 1965; Erwin, 1968) and on root rot severity (Lee, 1979; Snyman, 1984; Snyman & Darvas, 1982; Boughton, Malajczuk & Robson, 1978; Broadbent, Trochoulias, Biagent, Abbot & Dettmann, 1989; Halsall, 1980) are often contradictory and little is known about the effect of calcium on the pathogenicity of the fungus. However, high concentrations of calcium or leachates from calcium amended soil were shown to decrease sporangium production, while optimum levels of calcium maximised production of sporangia (Halsall & Forrester, 1977; Lee, 1979).

The aim of this study was to establish the effect of calcium on saprophytic growth and pathogenicity of *PC* and to evaluate the effect of calcium on the resistance of avocado trees (grafted and ungrafted) to root rot caused by the fungus.

MATERIALS AND METHODS

To measure the effect of calcium on mycelial growth of *PC,* the fungus was cultured in 250 *mℓ* Erlen Meyer flasks which were cleaned, using a method described by Lopateci and Newton (1956). Each of the six replicate flasks contained 150 *mℓ* liquid basal medium consisting of 1% glucose and 0,1 % yeast extract which were amended with calcium at 200 ppm, or unamended for the control treatment. Calcium chloride, calcium sulphate or calcium carbonates were used as sources of calcium. Each flask was inoculated with ten discs taken from the margin of an actively growing culture of *Phytophthora cinnamomi* on potato dextrose agar (PDA) plates. Cultures were incubated at 25°C in a shaker and harvested after 14 days, using Whatman no 1 filter paper. Mycelium was then washed three times with sterile de-ionised water to remove nutrients, and the dry mass measured by weighing after drying in an oven at 65°C for 24 h.

To determine the effect of calcium on the pathogenicity of the fungus, *PC* was cultured in 1 ℓ Erlen-Meyer flasks containing 400 mℓ of liquid basal medium amended with calcium (or unamended for the control) as described above. Mycelium was harvested and washed after seven days of incubation as described. Excess water was removed by blotting on filter paper and the mycelium mascerated with an Ultra Turrax for 15 s in 0,1% water agar [0,5% (W,V)].

These mycelial suspensions were used as inoculum.

Edranol seedlings were removed from nursery bags, rinsed under running tap water, and dipped in the various mycelial suspensions (treatments) for 10s. Trees were subsequently planted in vermiculite in 2 ℓ plastic pots and placed on a bench in the greenhouse of which the temperature fluctuated between 18 and 26°C. Ten replicates of each treatment were used and plants were watered three times a week.

After one month plants were removed from pots and evaluated for root rot severity by rating on a percentage scale as follows:

0 = No visible sign of disease
1 = Root rot symptoms on less than 20% of root area
2 = Root rot symptoms on 21 — 40% of root area
3 = Root rot symptoms on 41 — 60% of root area
4 = Root rot symptoms on 61 — 80% of root area
5 = Root rot symptoms on more than 80% of root area

To determine the influence of calcium on avocado trees, Edranol, Duke 7 and G755 root stocks, ungrafted and grafted with Hass, were used. Trees were planted in vermiculite in 2 ℓ plastic pots and grown in a greenhouse with minimum and maximum temperatures of 18°C and 26°C. Plants received 250 mℓ of water three times a week to which nutrition (without Ca) was added once a week. Calcium carbonate and calcium sulphate were used as sources of calcium and added to the water at 200 ppm calcium once a week, while nothing was added to the water the rest of the time. Trees not receiving any calcium served as controls.

After three months plants were removed from pots and infected with *PC* by dipping each root system in a mycelial suspension [0,5% (W/V)] of the fungus which was obtained as described previously. Trees were then replanted in clean vermiculite. One month later trees were removed from pots and the roots washed under running tap water. Root rot severity was rated on a percentage scale as described previously.

RESULTS

All calcium sources increased dry mass significantly (Table 1), although pathogenicity of *PC* was not reduced significantly when compared to the control (Table 2). Susceptibility of ungrafted Edranol seedlings was reduced significantly by both calcium carbonate and calcium sulphate (Table 3). Susceptibility of ungrafted Duke 7 and G755 trees was only reduced significantly by calcium sulphate, while the effect of calcium carbonate was not significant (Table 3). Although differences were not significant, calcium carbonate and calcium sulphate also reduced susceptibility of trees when grafted with Hass (Table 4).

TABLE 1 Effect of calcium sources on mycelial growth of *Phytophthora cinnamomi*

Treatment	Dry mass of mycelium (g)
Calcium chloride	0,320 a
Calcium carbonate	0,314 a
Calcium sulphate	0,304 a
Control	0,250 b

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P = 0,05).

TABLE 2 Effect of calcium sources on the pathogenicity of *Phytophthora cinnamomi*

Treatment	Root rot rating
Control	4,500 a
Calcium carbonate	4,500 a
Calcium chloride	4,500 a
Calcium sulphate	4,250 a

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P = 0,05).

TABLE 3 Effect of calcium on the susceptibility of three avocado root stocks (ungrafted) to root rot

Treatment	Root stock	Root rot rating
Control	Edranol	5,000 a
Control	Duke	4,200 ab
Control	G755	4,000 bc
Calcium carbonate	Edranol	4,000 bc
Calcium sulphate	Edranol	4,000 bc
Calcium carbonate	Duke 7	3,800 bcd
Calcium carbonate	G755	3,600 bcd
Calcium sulphate	Duke 7	3,200 cd
Calcium sulphate	G755	3,000 d

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P = 0,05).

TABLE 4 Effect of calcium on the susceptibility of three avocado root stocks (grafted with Hass) to root rot

Treatment	Root stock	Root rot rating
Control	Edranol	5,000 a
Control	G755	4,400 ab
Control	Duke 7	4,200 ab
Calcium carbonate	Edranol	4,200 ab
Calcium carbonate	G755	4,000 ab
Calcium sulphate	Edranol	3,800 ab
Calcium carbonate	Duke 7	3,800 ab
Calcium sulphate	Duke 7	3,600 b
Calcium sulphate	G755	3,200 b

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P = 0,05).

DISCUSSION

While no growth-promoting effect of calcium on *Phytophthora cinnamomi was* found to occur by Ghee & Newhook (1965), Erwin (1968) reported that calcium increased the mycelial weight of *PC* consistently. Results of the present experiments are in agreement with the latter finding in that calcium significantly increased mycelial dry mass. However, the ability of *PC* to cause disease does not depend on saprophytic growth or the nutrients evaluated, as amendments which increased saprophytic growth did not necessarily increase disease incidence.

Calcium was found to reduce root rot of *Persea indica* (Lee, 1979; Zentmyer & Lewis, 1975), avocado (Snyman, 1984; Snyman & Darvas, 1982) and Jarrah (Boughton, Malajczuk & Robson, 1978). However, according to Broadbent, Trochoulias, Baigent, Abbot & Dettmann (1989), calcium had no effect on the behaviour of the pathogen in soil leachates of calcium amended soil or on root rot of avocado, nor did calcium nutrition of Eucalypt seedling prevent seedling infection or reduce mortality (Halsall, 1980). Results of this study show that calcium increased resistance of avocado trees to root rot. The fact that the effect of calcium was more pronounced on ungrafted trees than on trees grafted with Hass, is in agreement with an increase in susceptibility of root stocks when grafted, especially with Hass, as found by Botha & Kotzé (1989). The effect of calcium sulphate was more beneficial than calcium carbonate and may be explained by reports by Trochoulias, Broadbent & Baigent (1986) that leaves of trees treated with calcium sulphate contained higher levels of calcium than trees treated with calcium carbonate. Calcium had a greater effect on increasing resistance of the avocado plants than on lowering the pathogenicity of the pathogen and may be due to calcium making the host more resistant to colonisation (Lee, 1979) or to reducing the spread of *PC* in the plant (Bellamy, Heather & Pratt, 1971).

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