South African Avocado Growers' Association Yearbook 1992. 15:12-14

The influence of nitrogen and calcium on mycelial growth and disease severity of *Phytophthora cinnamomi* and the effect of calcium on resistance of avocado to root rot

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ABSTRACT

The effect of nitrogen and calcium on mycelial growth and disease severity of Phytophthora cinnamomi *was studied, as well as the influence of calcium on the resistance of avocado to root rot caused by the pathogen.*

Mycelial growth was increased significantly by calcium and nitrate nitrogen, whereas ammonium nitrogen and urea had no significant effect compared to the (unamended) control. The disease severity of the fungus to avocado seedlings receiving ammonium sulphate as nitrogen source, was significantly lower than when urea or nitrate nitrogen was used. No calcium or nitrogen source significantly influenced disease severity of the fungus, compared to the (unamended) control. Ungrafted Edranol, Duke 7 and Martin Grande (G755) trees showed a significant decrease in susceptibility to P cinnamomi *when treated with calcium sulphate (as well as with calcium carbonate for Edranol).*

INTRODUCTION

High levels of calcium, nitrogen, microbial activity and organic matter have been implicated as the main reasons for disease reduction (Broadbent & Baker, 1974) in soils suppressive to *Phytophthora cinnamomi* Rands *(Pc),* the causal organism of root rot of avocado (*Persea americana* Mill) (Milne *et al,* 1975). Consequently, cover cropping and application of dolomite and nitrogen-rich fertilizers have been recommended to accomplish this purpose (Chalker, 1979).

Reports on the effect of calcium on growth of *Pc* (Chee & Newhook, 1965; Erwin, 1968) and on root rot severity (Boughton *et al,* 1978; Broadbent *et al,* 1989; Hassall, 1980; Lee, 1979; Snyman, 1984; Snyman & Darvas, 1982; Zentmyer & Lewis, 1974/75) are often contradictory. Little is known about the effect of calcium and nitrogen on the disease severity of the fungus. It is unclear whether the reported root rot reducing effect of calcium (Boughton *et al,* 1978; Lee, 1979; Snyman, 1984; Snyman & Darvas, 1982; Zentmyer & Lewis, 1974/75) is the result of an effect on the disease severity of *Pc,* or on resistance by the host. However, nitrogen was found to be toxic to *Pc* (Gilpatric, 1969; Tsao & Oster, 1981; Zentmyer & Bingham, 1956) and high concentrations of calcium or leachates from calcium-amended soils were shown to decrease sporangium production, whereas optimum levels of calcium maximized production of sporangia (Halsall &

Forrester, 1977; Lee, 1979).

The aim of this study was to establish the effect of calcium and nitrogen on mycelial growth and disease severity of *Pc* and to evaluate the effect of calcium on the resistance of avocado trees (grafted and ungrafted) to root rot caused by *Pc.*

MATERIALS AND METHODS

Effect of calcium and nitrogen on mycelial growth of *Pc*

A freshly isolated culture of *Pc* (PREM 50801) was cultured in 250 ml Erlenmeyer flasks, which were cleaned by soaking overnight in 10% nitric acid, rinsed and filled with de-ionised water, autoclaved and rinsed again before use (Lopatecki & Newton, 1956). Each of the 10 replicate flasks contained 150 ml basal liquid medium consisting of 1% glucose and 0,1% yeast extract. The medium was amended with calcium or nitrogen at 200 mg/ml, or unamended for the control treatment. Calcium chloride, calcium sulphate or calcium carbonate were used as sources of calcium, and magnesium nitrate, calcium nitrate, urea, ammonium sulphate and ammonium phosphate were used as nitrate or ammonium nitrogen sources. Each flask was inoculated with 10 discs (2 mm diameter) taken from the margin of an actively growing culture of *Pc* on water agar. Cultures were incubated at 25°C in a reciprocal 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 determined by weighing after drying at 65°C for

24 h.

Effect of calcium and nitrogen on disease severity of *Pc*

Pc was cultured in 1 L Erlenmeyer flasks containing 400 ml basal liquid medium, amended with calcium or nitrogen (or unamended for the control). Mycelium was harvested and washed after seven days of incubation, as described previously. Excess water was removed by blotting on filter paper and the mycelium macerated with an Ultra Turrax (Janke & Kunkel, Staufen) for 15 s in 0,1% water agar. These mycelial suspensions (0,5% [W/V]) were used as inoculum.

Ungrafted seedlings of the avocado cv Edranol-(three months old) were removed from nursery bags, rinsed under running tap water, and dipped in the various mycelial suspensions (treatments) for 10 s. Seedlings were then planted in vermiculite in 2 L plastic pots and placed in a greenhouse, where 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 as follows:

0 = No visible sign of disease

1 = Root rot symptoms on 20% or less 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

Effect of calcium on the susceptibility of three avocado rootstocks to root rot

Edranol, Duke 7 and Martin Grande (G755) rootstocks, ungrafted and grafted with cv Hass, were used. Trees (eight months old) 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 respectively. Plants received 250 ml of water three times a week, to which nutrients (without Ca) were added once a week. Calcium carbonate and calcium sulphate were used as sources of calcium and added to the water at 200 mg/ml calcium once a week. Trees not receiving any calcium served as controls.

After three months, plants were removed from pots and inoculated with *Pc* by dipping each root system in a mycelial suspension of the fungus, 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

Effect of calcium and nitrogen on mycelial growth of *Pc*

Addition of calcium resulted in the greatest effect on vegetative growth of the fungus. Significantly more mycelial growth took place in media amended with calcium chloride, calcium carbonate, calcium sulphate, calcium nitrate and magnesium nitrate, than in media supplemented with ammonium phosphate, urea, ammonium sulphate and the unamended control treatment (Table 1).

TABLE 1 Effect of calcium and nitrogen 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
Calcium nitrate	0,294 a
Magnesium nitrate	0,290 a
Ammonium phosphate	0,252 b
Control	0,250 b
Urea	0,230 b
Ammonium sulphate	0,226 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 and nitrogen sources on the disease severity of *Phytophthora cinnamomi*

Treatment	Root rot rating
Urea	5,0 a
Magnesium nitrate	5,0 a
Calcium nitrate	4,9 ab
Control	4,5 abc
Calcium carbonate	4,5 abc
Calcium chloride	4,5 abc
Ammonium phosphate	4,4 abc
Calcium sulphate	4,2 bc
Ammonium sulphate	4,1 c

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

Effect of calcium and nitrogen on disease severity of *Pc*

Although none of the treatments significantly affected disease severity of the fungus in comparison to the control, ammonium sulphate significantly decreased disease severity in comparison to calcium nitrate, magnesium nitrate, and urea (Table 2). Calcium sulphate only reduced disease severity in comparison with magnesium nitrate and urea (Table 2).

Effect of calcium on the susceptibility of three avocado rootstocks to root rot

Calcium carbonate and calcium sulphate significantly reduced root rot of ungrafted Edranol seedlings, while only the calcium sulphate treatment resulted in significantly less root rot in ungrafted Duke 7 and G755 rootstocks (Table 3). When the three rootstocks were grafted with Hass, neither calcium carbonate nor calcium sulphate were effective in reducing root rot (Table 4).

DISCUSSION

Although the increase in mycelial growth of *Pc* as a result of calcium amendment of a complete medium has been reported previously (Erwin, 1968), the singular growth promoting effect of calcium on *Pc* obtained in this study is a new finding. Also, no previous reports on the growth promoting effect on *Pc* by the nitrate nitrogen sources used in this study could be found.

None of the amendments, when supplied to the mycelium only, significantly influenced disease severity, compared to that of the (unamended) control. However, certain amendments such as ammonium sulphate and calcium sulphate did result in a decrease in disease severity when compared with supplements of urea, magnesium nitrate or calcium nitrate. The latter finding corroborates previous results, showing high levels of ammonium to occur in soils suppressive to *Pc* (Broadbent & Baker, 1974). The disease-enhancing effects of nitrate and urea have also been reported for other diseases such as take-all on wheat (Garrett, 1948).

The ability of *Pc* to cause disease does not depend on its mycelial growth potential or on the nutrients evaluated in this study, as amendments which increased mycelial growth were not necessarily responsible for increased disease incidence. Previous reports for this phenomenon could not be found for *Pc.*

Calcium sulphate has been indicated to reduce root rot of ungrafted avocado seedlings (Snyman, 1984). Results of the present study are in agreement with this work, as calcium sulphate and calcium carbonate effectively reduced root rot of ungrafted Edranol seedlings, while only calcium sulphate was effective on ungrafted Duke 7 and G755 rootstocks. The fact that the effect of calcium was insignificant when trees were grafted with Hass, conforms to a general reduction in resistance of grafted trees (Botha & Kotzé, 1989). These results show that the major effect of calcium in reducing root rot of avocado is in decreasing the susceptibility of avocado plants, while the reduction in disease severity of *Pc* is insignificant.

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

Treatment	Rootstock	Root rot rating
Control	Edranol	5,0 a
Control	Duke 7	4,2 ab
Control	G755	4,0 bc
Calcium carbonate	Edranol	4,0 bc
Calcium sulphate	Edranol	4,0 bc
Calcium carbonate	Duke 7	3,8 bcd
Calcium carbonate	G755	3,6 bcd
Calcium sulphate	Duke 7	3,2 cd
Calcium sulphate	G755	3,0 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 rootstocks (grafted with Hass) to root rot

Treatment	Rootstock	Root rot rating
Control	Edranol	5,0 a
Control	G755	4,4 ab
Control	Duke 7	4,2 ab
Calcium carbonate	Edranol	4,2 ab
Calcium carbonate	G755	4,0 ab
Calcium sulphate	Edranol	3,8 ab
Calcium carbonate	Duke 7	3,8 ab
Calcium sulphate	Duke 7	3,6 b
Calcium sulphate	G755	3,2 b

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

ACKNOWLEDGEMENTS

The authors wish to thank Westfalia Estate for kindly supplying trees for trials, and Professors R O Barnard and F C Wehner for useful discussion.

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