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A PRELIMINARY REPORT

The influence of N and Casources on pathogenicity of *Phytophthora cinnamomi* and of Ca-sources on resistance of avocado roots to infection by the fungus

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ABSTRACT

Saprophytic growth of Phytophthora cinnamomi *indicated that the pathogenicity was not enhanced by addition of N- or Ca-sources to the basal growth medium. Mycelium obtained from the N and Ca-enriched sources and used to inoculate blue lupin seedlings indicated that the pathogenicity of the fungus was reduced by the addition of either N or Ca to the fungus cultures.*

The detached root technique was used to determine the effect of Ca treatments on the susceptibility of avocado seedling roots cv Edranol to Phytophthora cinnamomi. *After treating seedlings for two months with $CaCO_3$ or $CaCI_2$ no difference in resistance could be found.*

INTRODUCTION

Fertilisation of avocado *(Persea americana* Mill) with N and Ca-sources is common practice. N-sources were found to reduce root rot of avocado (Broadbent & Baker, 1974; Zentmyer & Bingham, 1956; Bingham, Zentmyer & Martin, 1958 and Gilpatric, 1969). Tsao & Oster (1981) showed that both NH_3 and NHO_3 reduced propagule germination of *Phytophthora cinnamomi* Rands *(P.c.).* In. addition $CaCO_3$ was found to reduce root rot of *Eucalyptus marginata* (Boughton, Malajczuk & Robson, 1978) and that suppressive soils contained high concentrations of calcium (Broadbent, Baker & Water worth, 1971). Lee (1979) and Snyman (1984) reported that addition of Ca to soil reduced root rot and Halsall & Forrester (1977) showed that Ca concentration influenced sporangium formation of *P.c.*

This study aims to determine the effect of N and Ca-sources on pathogenicity of P.c. and the effect of Ca-sources on the susceptibility of avocado seedlings to *P.c.* invasion.

MATERIALS AND METHODS

Effect of N and Ca-sources on saprophytic growth of *P.c.*

To determine the effect of nitrogen and calcium sources on saprophytic growth of *P.c.,* an isolate was grown in basal medium consisting of 0,1% yeast extract and 1% glucose.

This basal medium was supplemented with either $Ca(NO_3)_{2'}$, $Mg(N0_3)_{2'}$, $(NH_4)_2S0_{4'}$, $(NH^4)_{2'}$, HPO_4 or urea at a rate of 200 ppm N and 200 ppm Ca when using $CaCI_2$ or $CaCO_3$. The control consisted of unamended basal medium. Each treatment was replicated five times in 250 mℓ Erlen-meyer flasks containing 100 mℓ medium each. Flasks were incubated on a shaker for 14 days at 25°C, after which mycelium was harvested by filtration through Whatman no 1 filter paper, washed twice with sterile distilled water to remove nutrients, and dried at 65°C for 24 h before weighing.

TABLE 1 Effect of N- and Ca-sources on mycelial dry mass on *P.c.*

Amendment	Dry mass (g)*
$CaCO_3$	0,246, a
$CaCl_2$	0,236 ab
$Ca\ (NO_3)_2$	0,220 abc
$(NH_4)_2HPO_4$	0,220 abc
Urea	0,212 abc
Control	0,196 bcd
$Mg\ (NO_3)_2$	0,178 cd
$(NH_4)_2SO_4$	0,162 d

*Values followed by the same letter do not differ significantly according to Duncan's analysis of variance (P = 0,01).

TABLE 2 Effect of N- and Ca-sources on pathogenicity of *P.c.*

Amendment	Lupin dry mass (g)*
$(NH_4)_2SO_4$	0,238 a
$Mg\ (NO_3)_2$	0,158 b
$(NH_4)_2HPO_4$	0,148 bc
$CaCO_3$	0,146 bc
Urea	0,136 cd
$CaCl_2$	0,124 de
$Ca(NO_3)_2$	0,110 e
Control	0,040 f

*Values followed by the same letter do not differ significantly according to Duncan's analysis of variance (P = 0,01).

TABLE 3 Effect of Ca-sources on avocado root invasion by *P.c.*

Amendment	Root length infected by *P.c.* (mm)*
$CaCO_3$	25,17 a
Control	21,83 a
$CaCl_2$	19,00 a

*Values followed by the same letter do not differ significantly acccording to Duncan's analysis of variance (P = 0,01).

Effect of N and Ca-sources on pathogenicity of *P.c.*

To determine the effect of nitrogen and calcium sources on pathogenicity of *P.c.,* the same proceduree as described earlier was followed, except that the fungus was grown for seven days instead of 14 days before harvesting. The mycelium was blotted dry and 1,5 g of each treatment homogenised in 200 mℓ sterile 0,1% water agar. Of this homogenate 100 mℓ was used to inoculate 1ℓ vermiculite which was then dispensed into five polystyrene cups (250 mℓ capacity). Each cup was planted with five pre-germinated lupin seeds and placed in a growth chamber with constant fluorescent lighting and ambient temperature of 25°C. Plants were watered twice a week with equal volumes of water and removed after seven days. After drying at 65°C for 48 h plants from each cup were weighed to determine dry mass.

Effect of Ca-sources on resistance of Edranol seedling roots to infection by *P.c.*

Four-month-old Edranol seedlings planted in vermiculite were used for the purpose of this experiment. Treatments consisted of supplementing a complete nutrient solution with $CaCl_2$ or $CaCO_3$ at a rate of 200 ppm Ca. Control plants received only the nutrient solution. Plants were placed in a greenhouse of which the temperature fluctuated between 18°C and 27°C and received treatment three times a week. After two months feeder roots were removed and tested for resistance to invasion by P.c. using the detached root technique as described by Zilberstein & Pinkas (1987). To determine how far P.c. has invaded the roots, the method described by Botha, Wehner & Kotzé (1989) was followed.

RESULTS

Effect of N and Ca-sources on saprophytic growth of *P.c.*

$CaCO_3$ amended media significantly increased dry mycelial mass above that of the control and the $Mg(NO_3)_2$ and $(NH_4)_2SO_4$ amended media (Table 1). None of the other treatments differed significantly from the control (Table 1). $(NH_4)_2SO_4$ amended media did however significantly decrease mycelial mass in comparison with $CaCO_3$', $CaCl_2$', $Ca(NO_3)_2$', $(NH_4)_2HPO_4$ or urea amended media (Table 1).

Effect of N and Ca-sources on pathogenicity of *P.c.*

All amendments significantly decreased the ability of *P.c.* to cause disease with the greatest reduction caused by $(NH_4)_2SO_4$ (Table 2).

Effect of Ca-sources on resistance of Edranol seedlings to invasion by *P.c.*

No differences in the colonisation of avocado roots by *P.c.* were detectable after treatment with $CaCO_3$ or $CaCl_2$ (Table 3).

DISCUSSION

Although Chee & Newhook (1965) could not show increased growth of *P. cinnamomi* by Ca addition, Erwin (1968) did find $CaCO_3$ to increase growth of *P.c.* and confirms the results of this experiment. The tendency of Ca to increase saprophytic growth did however have no effect on the pathogenicity of the fungus. Although all N and Ca-sources reduced the pathogenicity of *P.c.* it might in some cases be related to a reduction in vigour of the fungus as can be seen from the fact that $(NH_4)_2SO_4$ and Mg $(NO_3)_2$ reduced aprophytic growth as well as pathogenicity. The effect of N and Ca-sources reducing pathogenicity of *P.c.* might play a role in suppressive soils, which are rich in Ca and N (Broadbent, *et al).*

Although Ca was found to reduce root rot of *Eucalyptus* (Boughton, ef *al)* and avocado (Lee, 1979 and Snyman, 1984), the mechanism of root rot reduction has not been investigated. Present results tend to indicate that Ca does not increase the resistance of treated plants but rather reduces the ability of the fungus to cause disease.

REFERENCES

BINGHAM, F T, & ZENTMYER, G A, 1954. Relation of hydrogen-ion concentration of nutrient solution to Phytophthora root rot of avocado seedlings. *Phytopathology* 44: 611 - 614.

BOTHA, T, WEHNER, F C, & KOTZÉ, J M, 1989. An evaluation of *in vitro* screening techniques for determining tolerance of avocado rootstocks to *Phytophthora cinnamomi. S A Avocado Growers' Assoc Yrb,* 11: 60 - 63.

BOUGHTON, T J, MALAJCZUK, N, & ROBSON, A D, 1978. Suppression of the infection of Jarrah roots by *Phytophthora cinnamomi* with application of calcium carbonate. *Aust J Bot* 26: 611 - 615.

BROADBENT, P, & BAKER, K F, 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Aust J Agric Res* 25: 121 - 137.

BROADBENT, P, BAKER, K F, & WATERWORTH, Y, 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Aust J Biol Sci* 24: 925 - 944.

CHEE, K H, & NEWHOOK, F J, 1965. Nutritional studies with *Phytophthora cinnamomi* Rands. *N Z J Agric Res* 8: 523 - 529.

ERWIN, D C, 1968. The effect of calcium on mycelial growth of *Phytophthora megasperma* and *P cinnamomi. Mycologia* 60: 1112 - 1116.

GILPATRIC, J D, 1969. Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. *Phytopathology* 59: 973 - 978.

HALSALL, D M, FORRESTER, F I, 1977. Effects of certain cations on the formation of *Phytophthora* zoospores. 1. Effects of calcium, magnesium, potassium and iron ions *Can J Microbiol.* 23: 994 - 1001.

LEE, B S, 1979. The influence of calcium and nitrogen on disease caused by *Phytophthora cinnamomi* and two morphological forms of *Phytophthora palmivora.* PhD Thesis, University of California, Riverside.

SNYMAN, C P, 1984. The effect of calcium on avocado root growth and avocado root rot caused by *Phytophthora cinnamomi. S A Avocado Growers' Assoc Yrb* 7: 91 -

92.
TSAO, P H & OSTER, J J, 1981. Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with organic substances. *Phytopathology* 71(1): 1981.
ZENTMYER, G A & BINGHAM, F T, 1956. The influence of nitrate on the development of *Phytophthora* root rot of avocado. *Phytopathology* 46: 121 - 124.
ZILBERSTEIN, M & PINKAS, Y, 1987. Detached root inoculation — A new method to evaluate resistance to *Phytophthora* root rot in avocado trees. *Phytopathology* 77(6): 841 - 844.