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Biological control of Phytophthora root rot *in vitro* and *in vivo*

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ABSTRACT

Bacterial isolates were tested in *vitro for antagonism against the causal fungus of avocado root rot. Blue lupins were used in a bio-assay to evaluate bacterial antagonists. The use of alginate pellets imbedded with skim milk powder and antagonistic bacteria proved much more effective than a seed dip of Edranol seed into the same alginate, skim milk powder and bacteria suspension.*

INTRODUCTION

Studies on biological control of the avocado root rot *(Phytophthora cinnamomi* Rands) *(Pc)* are becoming more frequent [Baker, 1978; Gees & Coffey, 1989; Maas & Kotzé, 1990 and Duvenhage *et al,* (1991)] as pressure increases for banning the use of chemicals. The fear of resistance by *Pc* to Fosetyl-Al became a reality as an isolate of *Pc* in a French nursery proved to be highly resistant to H_3PO_3 and to Fosetyl-Al in culture (Vegh *etal,* 1985). Fosetyl-Al completely failed to control the disease on *Chamaccyparis lawsoniana .*

The aim of this study is to evaluate bacteria as bio-control agents of *Pc in vitro* and *in vivo,* and to test new methods of applying these antagonists to the planting mixture or seeds.

MATERIALS AND METHODS

Soil collected from nine avocado nurseries in the Transvaal was processed according to the method described by Maas and Kotzé (1989) to obtain bacteria. Forty-five bacterial isolates were obtained and tested *in vitro* for antagonism against *Pc* on PDA plates (Broadbent *et al,* 1971). Bacterial isolates showing moderate to vigorous antagonism against *Pc* were leophylized.

Pc inoculum was grown in a broth consisting of 1% glucose plus 0,1% yeast extract for 14 days at 25°C in a shaker. .The fungal mass was harvested by filtration through Whatman no 1 filter paper and was blotted dry with paper cloth. Mycelium was added to 0,1% agar solution at a rate of 0,5% (W/V) and mascerated for 30 s with an Ultra Turax.

A planting medium Hygrotec-seedling mix was added to vermiculite at 5% (W/V). The mascerated inoculum suspension was then added to the planting mixture at a ratio of 10% ("V/V) and added to polystyrene cups.

Lupin bio-assay

Due to the sensitivity of lupins to *Pc* (Darvas, 1979), the blue lupin *(Lupinus angustifolius)* was used to evaluate *in vivo* antagonism of the amended bacteria. Five lupin seeds were each planted in one of five cups for each isolate

The bacteria were grown in a broth consisting of 1% glucose and 0,1% yeast extract for 48 h on a shaker at 25°C. Fifty mℓ bacterial suspension was added to 300 mℓ planting mixture before lupins were planted. Controls were grown in the absence of antagonists, with or without *Pc.*

The avocado leaf disc technique described by Pegg (1977) was used to determine the incidence of *Phytophthora* 14 days after adding the different bacteria to the soil mixture. Ten leaf discs were floated for four days on each of the five cups in each treatment. The discs were then plated on PARPH selective medium. The percentages of discs yielding growth of *Pc* were recorded.

TABLE 1 Effect of bacterial antagonists on disease incidence in lupins inoculated with *Phytophthora cinnamomi*

Treatment	Number of dead lupins	Number of standing lupins	% *Pc* recovered from plant mixture
Control	0	23	0
Control + *Pc*	21	0	100
HH4	3	19	100
AH3	0	21	100
AH4	0	21	84
HH4 X	7	9	98
HH2	8	13	86
HH3	6	18	100
AH1	13	2	100
AW1	13	7	98

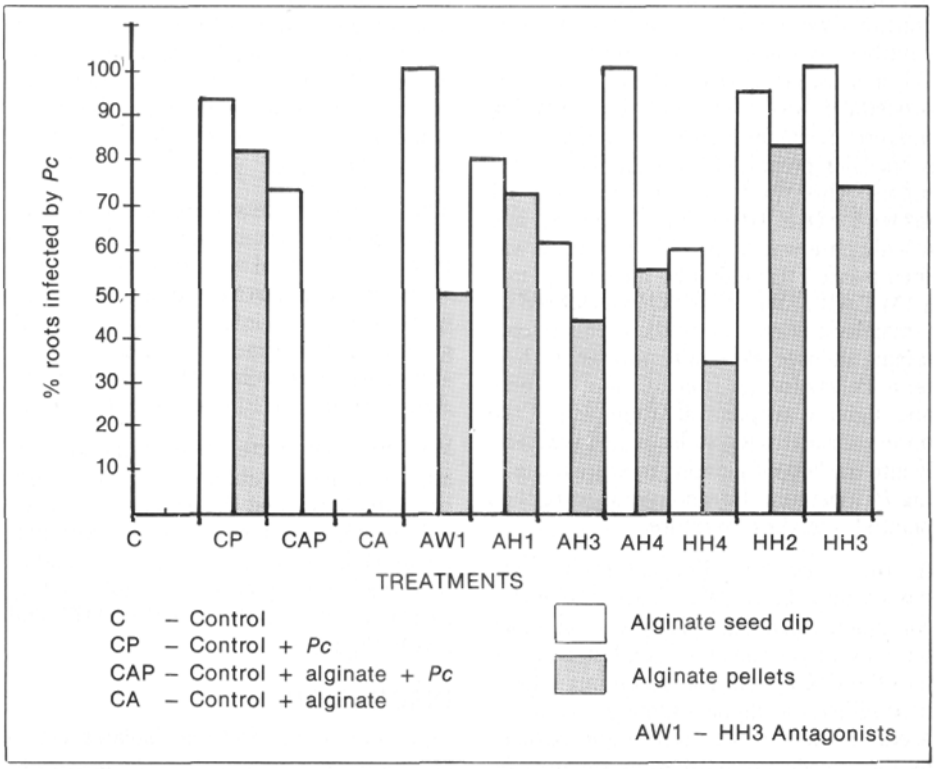

Fig 1 Percentage of Edranol seedling roots infected with *Phytophthora cinnamomi* six months after planting and treatment with alginate seed dip and alginate pellets containing antagonists.

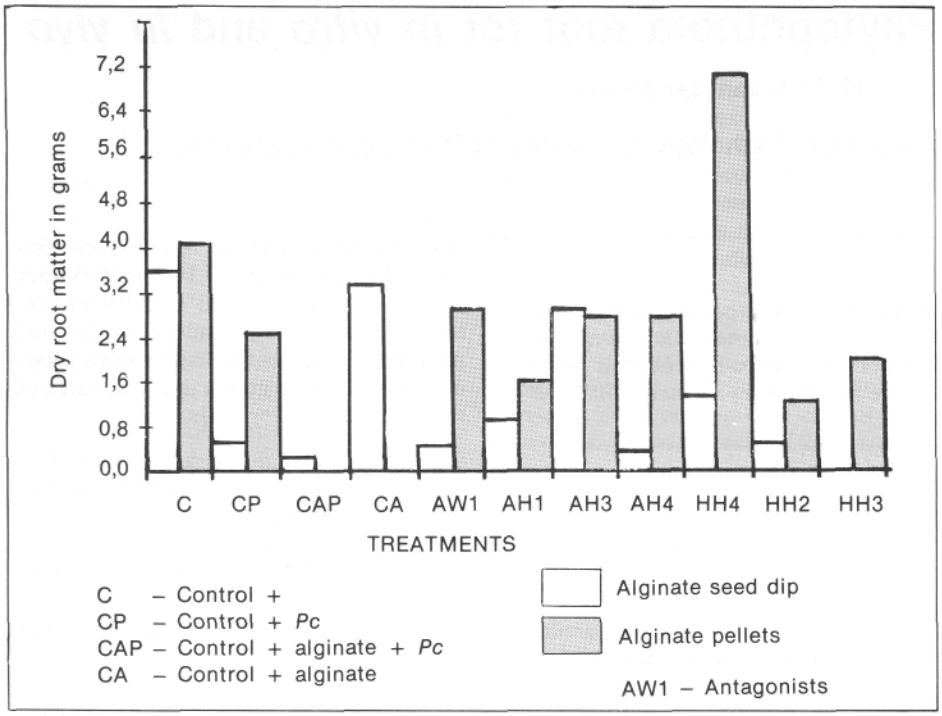

Fig 2 Dry root matter of Edranol seedlings six months after planting in artificially inoculated *Phytophthora* mixture and treatment with alginate seed dip and alginate pellets containing antagonists.

Edranol seed bio-assay

The bacterial antagonist was evaluated for control of *Pc* on seedlings of the highly susceptible avocado *Persea Americana* cv Edranol (Snyman *et al,* 1984). The same procedure was used regarding planting mixture, inoculum preparation and bacterial suspension preparation as described for the lupins. After the bacterial suspension has grown for 48 h, 10% skim milk powder and 4% alginate was added. This mixture was then pushed through a 20 mℓ syringe into a 1M $CaCl_2$ solution, which was stirred on a magnetic stirrer to produce pellets containing alginate, skim milk powder and bacteria. Pellets formed from 50 mℓ of bacterial and alginate suspension from each of the different isolates were also mixed separately into the 300 mℓ planting mixture containing *Pc* inoculum. Edranol seeds were then planted into these mixtures.

At the same time Edranol seeds were dipped into the same alginate suspension containing skimmed milk powder and bacteria, as mentioned above, and then placed into the $CaCl_2$ solution which immediately solidified the alginate around each of the seeds. Each seed was repeatedly covered by the suspension until approximately 50 mℓ of the suspension adhered to the seed. The seeds were then planted into the planting mixture containing *Pc.*

RESULTS

Of the 45 bacterial isolates tested *in vitro* against *Pc,* only eight isolates showed antagonism. Of these eight isolates tested for control of *Pc* by means of blue lupins, two isolates gave 100% disease control. Four isolates had less than 70% lupins still standing after 14 days (Table 1), five isolates did not reduce the amount of *Pc* recovered, while one did reduce it with 16% and one to 14% (Table 1).

The alginate pellets were superior to alginate seed dip when comparing the percentage roots infected with *Pc* in the Edranol seed trial. The control and control plus alginate showed no infected roots. In the alginate pellet treatment, the most effective antagonist was isolate HH4 followed by AH3, AW and AH1 in that order (Figure 1).

With dry root matter taken as parameter, alginate pellets again proved to be superior to alginate seed dip in all but one instance. With antagonist HH4 dry root matter of avocado seedlings was even higher than the control plants and is followed by treatment with isolates AH4, AH3 and AW1 (Figure 2).

DISCUSSION

Eight out of 45 bacterial isolates (18%) proved to be antagonistic to *Pc in vitro* to some degree, inhibiting mycelial growth of the fungus on PDA. This suggests that metabolites are produced by the bacteria, inhibiting the growth of *Pc.*

Testing these bacteria as biological control agents against lupins indicated that some of the isolates are effective against *Pc in vivo,* but none of these succeeded in dramatically reducing the incidence of *Phytophthora* in the soil. The lupins gave a good indication of which antagonists have potential for further testing, except in the case of isolate AW1.

During this study four bacterial isolates were found, with potential as biological control agents. It was shown that the method by which these organisms were applied to the plant or soil, has a great influence on the degree of disease control acquired.

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