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Evaluating micro-organisms from avocado soils for antagonism to *Phytophthora cinnamomi*

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ABSTRACT

Bacteria (including actinomycetes) and fungi isolated from different avocado soils were tested for in vitro *antagonism to* Phytophthora cinnamomi (Pc). *Significantly more bacteria (13 per cent) inhibitory to* Pc *were recovered from pathogen-free soil than from soil in which* Pc *occurred (2 per cent). Fungi in general were more antagonistic to* Pc *than bacteria when compared after 21 d.* Penicillium *spp were most frequently isolated from healthy soil and large numbers of pseudomonads occurred in soils harbouring* Trichoderma spp.

UITTREKSEL

Bakterieé (insluitende akitinomisete) en swamme geisoleer uit verskillende avokadogronde is in vitro *getoets vir antagonismo teen* Phytophthora cinnamomi (Pc). *Betekenisvol meer bakterieé (13 persent) wat antagonisties is teenoor* Pc*, is verkry uit patogeenvry gronde*, as *uit grond waarin* Pc *voorgekom het (2 persent). Na 21 d was swamme in die algemeen meer antagonisties teenoor* Pc as *bakterieé.* Penicillium spp *kon geredelik geisoleer word uit gesonde gronde en groot getalle pseudomonade het in gronde voorgekom waarin* Trichoderma *spp volop was.*

INTRODUCTION

Yield losses due to root rot of avocado *(Persea Americana* Mill) caused by *Phytophthora cinnamomi (Pc)* Rands are effectively reduced by disease-free nurseries, resistant rootstocks, judicious use of fungicides and sound cultural practices. (Kotzé, Moll, & Darvas, 1987).

Other measures such as biological control are being investigated (Coffey, 1986; Darvas & Bezuidenhout, 1987), especially since the discovery of fungicide resistant *Pc* strains in soils after continuous treatment with metalaxyl (Darvas & Becker, 1984).

The purpose of this study was to obtain potential antagonists to *Pc* while simultaneously investigating soil ecology.

MATERIALS AND METHODS

Soils

To initiate this study one soil sample (approx 2 kg) from each of five avocado trees on the University of Pretoria Experimental Farm, was collected to a depth of 10 cm, air-dried and stored at room temperature. The particulars of each soil are tabulated in Table 1.

TABLE 1 Description of trees from which soil was collected

A: }	Healthy avocado tree, approximately 10 years old. *P c* absent.
B: }	Healthy avocado tree, approximately three years old. *P c* absent.
C: }	Healthy avocado tree, approximately six years old. *P c* absent.
D: }	Stunted tree, approximately six years old and five m from tree C. *P c* could not be isolated.
E:	Diseased tree, approximately 10 years old. *P c* isolated from lupin bait plants

Brackets indicate trees in close proximity to each other.

Microbial activity

The number of fungi, bacteria, pseudomonads and actinomycetes was determined according to the method described by Maas & Kotzé (1989).

Microbial antagonism

Potato-dextrose agar (PDA) plates centrally inoculated with *Pc* were simultaneously inoculated with five microbes from the enumeration plates. Thirty bacteria, 20 fungi and 15 actinomycetes from each soil type were evaluated for antagonism to *Pc*. The number of bacteria and actinomycetes inhibiting growth after 18 d incubation at 25°C was counted and expressed as the percentage of the total number of isolates tested. The effect of fungi was determined by measuring colony diameter after 10 d.

RESULTS

The number of microbes isolated from the various soils is shown in Table 2. Significantly more bacteria and pseudomonads occurred in the two diseased soils, compared with the healthy soils from which significantly more actinomycetes were isolated. A significantly greater number of fungi occurred in the *Pc* diseased soil.

TABLE 2 Effect of avocado soils on the recovery of fungi, bacteria, actinomycetes and pseudomonads

Soil type[y]	Microbe[x]			
	Bacteria^{-5}	Fungi^{-4}	Pseudomonads^{-4}	Actinomycetes^{-4}
A	75,7a	18,3a	0,3a	52a
B	137,7b	19,6a	7,0b	42a
C	71,0a	16,6a	1,0a	29b
D	223,0c	21,0a	11,3c	23b
E	195,0c	35,3b	14,3c	13c

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P=0,05).
x: mean of three replicate plates expressed per gram dry soil mass.
y: see Table 1 for descriptions.

The number of bacteria and actinomycetes inhibiting growth of *Pc* after 21 d is shown in Table 3. A significantly higher number of inhibitory isolates occurred in the three healthy soils. No isolates from the *Pc* diseased soil inhibited growth after 18 d.

TABLE 3 Percentage bacteria and actinomycetes inhibiting growth of *P c* after 21 days

Soil Type[x]				
A	B	C	D	E
16a	10a	15a	1b	0b

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P=0,05).
x: 45 isolates per soil type were tested.

The effect of fungi obtained from the various soils on growth of *Pc* is shown in Table 4. Although no significant difference in inhibition between fungi from various soils occurred, they significantly reduced the growth of *Pc* in comparison to the control. The identity and mean number of fungi occurring in the various soils is shown in Table 5.

TABLE 4 Effect of fungi obtained from five avocado soils on colony diameter (mm) of *P c* determined after 10 days

	Soil Type[x]					
	Control	A	B	C	D	E
Colony Diameter	42a	28b	31b	29b	28b	34b

Values not followed by the same letter are significantly different according to Duncan's multiple range test (P=0,05).
x: 20 isolates per soil type were tested.

TABLE 5 Identity and mean number of fungi occurring in five avocado soils obtained from the University of Pretoria Experimental Farm

Genus	Soil Type[x]				
	A	B	C	D	E
Alternaria spp	0a	0a	0a	2a	0a
Aspergillus spp	2a	3a	0a	0a	2a
Cladosporium spp	0a	1a	0a	1a	8a
Fusarium spp	8a	12a	10a	13a	10a
Gliocladium spp	1a	2a	0a	0a	23b
Microdochium spp	1a	1a	3a	0a	3a
Myrothecium spp	0a	0a	1a	0a	2a
Mucorales	3a	0a	1a	0a	0a
Penicillia	18a	12a	15a	13a	4b
Trichoderma spp	3a	0a	2a	20b	0a
Unidentified	26a	30a	21a	24a	28a

Values in horizontal lines not followed by the same letter, differ significantly according to Duncans' multiple range test (P=0,05).

Gliocladium roseum occurred very frequently in the *Pc* diseased soil in comparison to the other soils. In addition, very few penicillia occurred in the latter soil. Significantly more *Trichoderma* spp were encountered in soil obtained from the stunted tree.

DISCUSSION

The discovery of soils that suppress disease even in the presence of the pathogen led to an increased interest in soil ecology. (Malajczuk, 1983). Malajczuk & McComb (1979) and Weste & Vithange (1977) found microbial populations to differ qualitatively and quantitatively, with greater numbers occurring in suppressive soil. Contradictory to this, greater number of microbes were found to occur in the diseased soils in the present study. However, each soil harboured specific dominant genera. *Trichoderma* spp and *Gliocladium roseum* occurred in greater numbers in each of the diseased soils whereas penicillia were predominantly associated with the three healthy soils.

Greater numbers of pseudomonads were also found to occur in the *Pc* diseased soil and the soil collected from the stunted tree. Pseudomonads have been reported to stimulate sporangium formation of *Pc* (Marx & Haasis, 1965).

Malajczuk (1983) showed that there was a correlation between the number of antagonistic bacteria and actinomycetes and the suppressive potential of soils. The results of the present study corroborate this tendency, as a significantly greater number of antagonistic bacteria and actinomycetes were found to occur in the healthy soils.

The extent of interactions between *Phytophthora* and other fungi under *in vitro* and *in vivo* conditions and their possible importance in the mechanisms of *Phytophthora* propagule reduction remains largely unexplored (Malajczuk, 1983). In the present study fungi in general were more antagonistic to *Pc* than bacteria and actinomycetes, as all the fungi inhibited colony increase to some extent, whereas only some bacteria and actinomycetes inhibited growth for periods longer than 21 d.

The fact that micro-organisms are involved in the suppression of diseases caused by *Phytophthora* spp strongly suggests that the manipulation of these microbes by various

management techniques may result in the biological control of these diseases in both agriculture and forestry. However, more work is necessary to develop a better understanding of the interaction of soil and rhizosphere micro-organisms with *Pc*.

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