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SUPPRESSIVE SOILS AND BIOLOGICAL CONTROL OF *PHYTOPHTHORA* ROOT ROT

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ABSTRACT

In South Africa avocado orchards were found in which some trees appeared healthy, while the rest of the orchard was severely affected by root rot. Some of these soils had the ability to significantly suppress the development of root rot of blue lupins and avocado when inoculated with the pathogen. These soils generally supported higher numbers of bacteria, fungi and actinomycetes than soil of diseased trees and contained higher percentages of micro-organisms antagonistic to the pathogen on agar plates. A number of bacteria, fungi and actinomycetes were able to suppress root rot of blue lupins significantly when inoculated simultaneously with the pathogen.

UITTREKSEL

In Suid-Afrika is boorde gevind met borne sonder wortelvrotsimptome, terwyl die res van die boord erg deur wortelvrot geaffekteer was. Sommige van die gronde het die vermoe gehad om wortelvrot van blou lupiene en avokadosaailinge betekenisvol te onderdruk wanneer ge'fnokuleer met die patogeen. Onderdrukkende gronde het oor die algemeen hoér getalle bakterieé, fungi en aktinomisete bevat as grond van siek borne, sowel as hoér persentasies mikro-organismes antagonisties teenoor die patogeen op agarplate. 'n Aantal bakterieë, fungi en aktinomisete het die vermoë gehad om wortelvrot van blou lupiene betekenisvol te onderdruk wanneer dit gelyktydig met die patogeen ge'inokuleer is.

INTRODUCTION

Avocado root rot, caused by *Phytophthora cinnamomi* Rands, is considered the most important root disease of avocado (Broad-bent & Baker, 1974; Pegg, Forsberg & Whiley, 1982; Kotzé, Moll & Darvas, 1987) and results in large losses in avocado producing areas throughout the world (Zentmyer, 1980). In 1982 *Phytophthora cinnamomi* was identified as the main cause of avocado root rot in South Africa (Milne, Brodrick & Hughes, 1975).

Control measures include treating trees with fungicides and, to a certain extent, application of fertilisers (Kotzé, Moll & Darvas, 1987) and cover cropping (Wolstenholme, 1979). Soils in Queensland, Australia were found to suppress disease, even though the pathogen was present (Broadbent, Baker & Waterworth, 1971). The suppressive nature of the soils was affirmed by greenhouse experiments (Broadbent *et al,* 1971) and was in part ascribed to higher numbers of micro-organisms as well as higher nitrogen and calcium content (Broadbent *et al,* 1974). The occurrence of suppressive soils in South Africa has not been investigated. However, certain trees have been identified (escape trees) that show exceptional vigour in orchards affected by root rot.

The purpose of this study was firstly to determine whether escape tree soils suppress *Phytophthora cinnamomi (Pc)* and secondly, to isolate possible antagonistic microorganisms for use as biocontrol agents.

MATERIALS AND METHODS Soil samples

Forty eight avocado trees (escape trees) in eastern and northern Transvaal without above ground symptoms of root rot, and which exhibited exceptional vigour in root rot infected orchards, were used for this study. The location and codes allocated to the various trees sampled is shown in Table 1. Four soil samples, collected to a depth of 15 cm from each tree, were pooled and stored in plastic bags at 20 — 25°C and processed within three to four days of sampling.

Test for suppressiveness of soils using *Lupinus angustifolius*

The ability of the pathogen to cause disease in escape tree soils was based on the bioassay method described by Broadbent *et al* (1971) in which New Zealand blue lupin seedlings *(Lupinus angustifolius)* were used as host indicator plants (Chee & Newhook, 1965) after infecting the test soil mix with inoculum of *Pc.*

In the present study inoculum of *Pc* was prepared by inoculating 1 ℓ Erlen-Meyer flasks containing 400 *mℓ* nutrient broth (1% glucose, 0,1% yeast extract) with actively growing discs of *Pc* cut from the margin of potato dextrose agar (PDA) plates. Flasks were incubated on a reciprocal shaker at 25°C for seven days after which the mycelium was harvested by filtration using Whatman no 1 filter paper, and washed twice with sterile distilled water to remove nutrients. Ten gram mycelium was added to 100 ml 0,1% water agar solution and mascerated for 15 s using an Ultra Turrax.

The different soil samples were dried at room temperature for 24 hours, ground with a mortar and pestle and mixed thoroughly with vermiculite at 5% (W/V) by shaking in a plastic bag (Malajczuk, McComb & Parker, 1977). As control soil from each locality was pooled, dried at room temperature for 24 h, and sterilised by gamma-irradiation [dosage 25 kGy, Isoster (Pty) Ltd, Kempton Park, South Africa] (Malajczuk *et al,* 1977).

Mascerated *Pc*-inoculum was added to each soil-vermiculite mixture (soil mix) at a rate of 0,05% (W/V) and mixed thoroughly by shaking, still in the same plastic bags. Each soil mix was divided between five cups and each cup planted with five blue lupin seeds. Seeds were surface sterilised by soaking in 70% ethanol for ten minutes and first germinated on water agar before planting. Seedlings were placed in a growth chamber at 25°C under constant fluorescent lighting and each cup received 30 ml of distilled water three times a week. Treatments containing sterile soil mix not inoculated with *Pc* were also included.

TABLE 1 Codes, location and description of soils sampled

Soil code	Location	Description
KM, CR	Nelspruit	Healthy
D1 — D9, D11 — D16	Nelspruit	Healthy
Q1, Q2, Q6 — Q9	Nelspruit	Healthy
L1 — L4, L6	Nelspruit	Healthy
2B1 — 2B3, AV	Louis Trichardt	Healthy
KMK	Nelspruit	Diseased
DK	Nelspruit	Diseased
QK	Nelspruit	Diseased
Z1 — Z4, C19	Tzaneen	Healthy
B1, B4, B12 — B14	Tzaneen	Healthy
A1 — A3, A5 — A7	Tzaneen	Healthy
BK	Tzaneen	Diseased
AK	Tzaneen	Diseased

Lupin seedlings were harvested after 7 — 10 days and the seedlings from each cup weighed. The

fresh mass of the seedlings obtained in this way was used as an indication of root rot severity (damping-off). Soils which reduced root rot of lupin seedlings were resampled and again bioassayed together with soil from five trees with visible root rot symptoms (diseased trees). Soils which proved suppressive (selected soils) were also tested for suppressiveness using avocado *(Persea americana)* and used for microbial analyses.

Test for suppressiveness of selected soils using *Persea Americana* (cv Edranol) seedlings

Edranol seedlings were planted in the selected soils and in diseased tree soils, after soils were inoculated with *Pc* at a concentration of 0,1% (W/V), as described above. Seedlings were planted in 2 ℓ plastic pots using ten replicates per treatment. Pots were then placed in a greenhouse with minimum and maximum temperatures of 18°C and 26°C and watered three times a week.

Seedlings were removed from pots after three months and the roots cleaned by washing gently in tap water. Disease severity was rated 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

The infection percentage was also determined by cutting feeder roots into small pieces, dipping the latter in 70% ethanol for 5 s, blotting dry and plating ten randomly selected pieces on corn meal agar plates (Zentmyer, 1963). The number of root pieces yielding growth of *Pc* was counted and expressed as the infection percentage.

Quantification of *PC*

Ten soil samples from each tree were collected to a depth of 15 cm, transported and stored in plastic bags at 20 — 25°C, and processed within 24 h of sampling. *Pc* was quantified in selected soils and diseased tree soils using the avocado leaf bait technique as described by Pegg (1977). Ten leaf discs were suspended on watery slurry of each soil sample (100 discs per tree) and incubated for 3 — 5 days at room temperature. After this period discs were plated on selective PARPH medium (Solel & Pinkas, 1984). The number of discs yielding "growth of *Pc* were counted and expressed as a percentage.

Isolation of micro-organisms for *in vitro* evaluation of antagonism to *PC*

Isolation of micro-organisms from selected soils and diseased tree soils was done within 48 h of sampling, by placing 10 g soil in 90 ml 0,1% water agar solution and Vales not followed by the same letter are significantly different according to Duncan's multiple range test (P = 0,05).

TABLE 2 Effect of soil from Nelspruit and Louis Trichardt areas on root rot of lupin seedlings in *PC*-inoculated vermiculite

Soil code	*Fresh mass (g)
Uninoculated control K5 (gamma-irradiated)	5,53 a
Uninoculated control K6 (gamma-irradiated)	5,50 a
KM	4,62 ab
D4	4,61 ab
D5	4,33 abc
Q2	4,21 bcd
Q8	4,17 bcd
Q7	4,11 bcd
D6	4,02 bcde
D3	4,01 bcde
AV	4,01 bcde
Q6	3,98 bcde
D12	3,94 bcde
D16	3,89 bcdef
CR	3,89 bcdef
D2	3,88 bcdef
D8	3,79 bcdef
D9	3,66 bcdef
D15	3,65 bcdef
Q9	3,52 bcdef
L1	3,47 bcdef
L4	3,45 bcdef
D11	3,45 bcdef
L6	3,44 bcdef
Q1	3,44 bcdef
D14	3,40 bcdefg
L2	3,38 bcdefg
D1	3,37 bcdefg
D13	3,19 bcdefgh
2B3	3,06 cdefgh
L3	3,04 cdefgh
D7	2,88 cdefgh
2B2	2,66 defgh
Inoculated control K5 (gamma-irradiated)	2,59 efgh
2B1	2,41 fgh
Inoculated control K6 (gamma-irradiated)	1,99 gh
Inoculated control K8 (gamma-irradiated)	1,86 h
Inoculated control K7 (gamma-irradiated)	1,78 h

Fresh mass is the mean of lupins of five replicate cups containing five seedlings each.

shaking on a reciprocal shaker for 20 min. Suspensions were then serially diluted and plated on four replicate plates of dried PDA (Butler & Hiñe, 1958) (novobiocin substituted with rifampicin at 50 ppm) plates for fungi; Chitin agar (Lingappa & Lockwood, 1962) for actinomycetes; Kings B for fluorescent pseudomonads; nutrient agar supplemented with 0,5% glucose (Bezuidenhout, 1978) for bacteria. Soil suspensions used for isolation of aerobic spore forming bacteria were first treated for 10 min at 80°C (Weste & Vithanage, 1977; Broadbent *et al,* 1971). Plates were then incubated at 25°C for three days, five days and 7— 14 days for isolation of fungi, bacteria or actinomycetes respectively.

Two to four isolates of fungi, bacteria or actinomycetes were placed on a PDA plate around a central, actively growing *Pc* culture (Broadbent, *et al,* 1971). Microorganisms inhibiting growth of *Pc* after five

days' incubation were isolated and evaluated for the control of root rot using blue lupin seedlings.

TABLE 3 Effect of soil from the Tzaneen area on root rot of lupin seedlings in *PC*-inoculated vermiculite

Soil code	*Fresh mass (g)
Uninoculated control K2 (gamma-irradiated)	5,30 a
Uninoculated control K1 (gamma-irradiated)	5,28 a
Z1	5,25 a
Z3	3,28 b
B13	3,21 b
A2	3,16 bc
B14	3,11 bc
A7	3,02 bc
B12	2,90 bcd
A1	2,89 bcd
A3	2,87 bcd
Z2	2,83 bcd
C19	2,55 bcd
A5	2,38 bcd
B4	2,33 bcd
A6	2,26 bcd
B1	1,71 bcde
Z4	1,38 cde
Inoculated control K3 (gamma-irradiated)	1,20 de
Inoculated control K1 (gamma-irradiated)	1,18 de
Inoculated control K2 (gamma-irradiated)	0,55 e

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

*Fresh mass is the mean of lupins of five replicate cups containing five seedlings each.

Evaluation of antagonistic microorganisms for control of root rot using *Lupinus angustifolius*

Micro-organisms attained as described above were individually cultured as described, using 250 ml Erlen-Meyer flasks containing 100 mf of broth. Fungi were harvested after seven days and mascerated as described for *Pc.* Gamma irradiated soil mix was inoculated with mascerated mycelium of *Pc* and an antagonistic fungus [at 0,05% (W/V) of each] as before. Cultures of bacteria (two days old) and actinomycetes (four days old) were harvested by centrifugation, resuspended in sterile distilled water, and 100 ml of a 10^7 suspension added to *Pc*-inoculated [0,05% (W/V)] sterile soil mix as above. Each soil mix was then divided between five cups, planted with pregerminated blue lupin seedlings, and evaluated after 14 days as previously described.

TABLE 4 Effect of soil from selected trees and diseased trees on root rot of lupin seedlings in *PC*-inoculated vermiculite

Nelspruit area		Tzaneen area	
Soil code	*Fresh mass (g)	Soil sample	*Fresh mass (g)
D4	5,91 a	Uninoculated control K1 (gamma-irradiated)	4,82 a
		Uninoculated control K3 (gamma-irradiated)	4,80 a
Uninoculated control K6 (gamma-irradiated)	5,52 ab	Uninoculated control K2 (gamma-irradiated)	4,77 a
Uninoculated control K7 (gamma-irradiated)	5,50 ab		
Uninoculated control K5 (gamma-irradiated)	5,49 ab	Z3	4,74 a
D5	5,36 abc		
KM	5,11 abc	Z1	4,50 ab
Q2	4,86 abcd		
Q7	4,73 abcd	A2	4,00 abc
Q8	4,69 abcd	B13	3,58 abcd
DK (diseased)	4,49 abcd	B14	3,38 abcde
QK (diseased)	4,15 bcde	BK (diseased)	3,14 abcde
KMK (diseased)	3,92 cdef	A7	3,02 bcde
Inoculated control K6 (gamma-irradiated)	2,95 ef		
Inoculated control K5 (gamma-irradiated)	2,92 ef		
Inoculated control K7 (gamma-irradiated)	2,70 f	Inoculated control K1 (gamma-irradiated)	2,62 cde
		Inoculated control K2 (gamma-irradiated)	2,34 de
		AK (diseased)	2,26 de
		Inoculated K3 (gamma-irradiated)	1,90 e

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

*Fresh mass is the mean of lupins of five replicate cups containing five seedlings each.

TABLE 5 Effect of selected soils on root rot of Edranol seedlings planted in *PC*-inoculated vermiculite

Selected soil code	[a]Root rot rating	[b]Infection percentage of roots
Inoculated control K1 (gamma-irradiated)	4,50 a	88 a
Inoculated control K3 (gamma-irradiated)	4,50 a	84 ab
Inoculated control K4 (gamma-irradiated)	4,33 ab	82 ab
Inoculated control K2 (gamma-irradiated)	4,17 abc	82 ab
KMK (diseased)	4,00 abc	80 ab
QK (diseased)	4,00 abc	80 ab
DK (diseased)	3,67 bcd	72 bc
Q8	3,17 de	54 ef
Q7	3,17 de	64 cde
Q2	3,00 de	56 def
D5	3,00 de	60 cdef
D4	2,50 e	48 fg
KM	2,50 e	48 fg
Z3	1,67 f	36 gh
Z1	1,17 f	24 h
Uninoculated control K1 (gamma-irradiated)	0,00 g	0 i
Uninoculated control K2 (gamma-irradiated)	0,00 g	0 i

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

[a]Each value is the mean rating of ten replicate avocado seedlings.

[b]Each value is the percentage infected root pieces.

TABLE 6 Semi-quantitative estimation of the *PC* populations of selected soils

Soil code	*Percentage of leaf pieces infected by *PC*
B14	30 a
A7	18 ab
A2	16 ab
AK (diseased)	15 ab
Z1	13 ab
KM	12 ab
Q2	9 b
B13	8 b
KMK (diseased)	8 b
Z3	6 b
QK (diseased)	3 b
BK (diseased)	2 b
DK (diseased)	1 b
Q7	1 b
Q8	1 b
D4	1 b
D5	1 b

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

*Percentage is the mean of ten leaf pieces of ten replicate plates each, infected with *PC*.

TABLE 7 Number of micro-organisms in soil from selected trees and diseased trees (per gram soil)

Soil code	*Bacteria ($X10^5$)	*Fungi ($X10^5$)	*Actinomycetes ($X10^4$)	*Aerobic spore forming bacteria ($X10^4$)	*Fluorescent pseudomonads ($X10^4$)
Z3	30,00 a	59,75 a	40,75 a	29,00 a	0,75 a
Z1	19,25 b	27,00 cd	39,00 ab	22,25 abc	0,50 a
KM	11,75 bcd	41,25 b	24,25 cde	25,25 ab	0,00 a
KMK (diseased)	10,75 cd	21,50 def	25,75 cde	23,25 ab	0,00 a
D5	9,75 cd	34,75 bc	23,25 cde	14,25 cde	0,25 a
DK (diseased)	9,00 cd	8,50 f	26,00 cde	9,50 e	0,75 a
Q2	8,25 cd	29,00 bcd	21,50 de	13,25 de	0,00 a
Q7	8,00 cd	20,00 def	32,75 abcd	12,75 de	0,25 a
D4	8,00 cd	12,50 ef	16,25 e	6,75 e	1,00 a
QK (diseased)	6,50 d	11,00 ef	22,25 de	14,25 cde	0,50 a
Q8	5,75 d	23,50 cde	27,00 bcde	23,75 ab	0,25 a

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

*Each value is the mean of five dilution plates.

TABLE 8 Number of micro-organisms tested as well as the percentage antagonistic to *PC in vitro*

Soil code	Bacteria		Fungi		Actinomycetes		Aerobic spore forming bacteria		Fluorescent pseudomonads	
	Number	% Antago-nistic	Number	% Antago-nistic	Number	% Antago-nistic	Number	% Antago-nistic	Number	% Antago-nistic
KM	16	50	38	42	19	95	20	85	0	0
KMK (diseased)	12	25	13	39	25	64	13	31	0	0
Z1	18	56	18	33	26	77	24	79	2	0
Z3	30	40	37	41	33	91	20	50	2	50
DK (diseased)	13	46	9	11	30	30	5	40	3	0
D4	14	57	10	40	18	94	5	60	4	75
D5	13	39	28	25	25	40	10	70	1	0
Q8	10	20	16	63	33	82	20	85	1	0
Q7	10	60	16	81	29	90	7	70	1	0
Q2	12	50	14	72	16	50	14	29	0	0
QK (diseased	10	60	11	36	23	17	6	50	2	0

TABLE 9 Effect of antagonistic bacteria on root rot of lupin seedlings planted in *PC*-inoculated vermiculite

Isolate code	Selected soil code	*Fresh mass (g)
Uninoculated control		5,33 a
B3	Z3	4,81 ab
B43	D5	4,27 bcd
B41	DK (diseased)	4,15 bcde
B5	Z1	4,15 bcde
B42	DK (diseased)	4,02 bcde
B9	KM	3,70 cdef
B4	Z1	3,70 cdef
B6	KMK (diseased)	3,56 cdef
B7	KM	3,51 cdef
B11	Q7	3,48 cdef
B12	Q8	3,41 def
B8	KM	3,30 ef
B10	Q7	3,30 ef
Inoculated control (without antagonist)		3,10 f

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

*Fresh mass is the mean of five replicate cups containing five seedlings each.

TABLE 10 Effect of antogonistic fungi on root rot of lupin seedlings planted in *PC*-inoculated vermiculite

Isolate code	Selected soil code	*Fresh mass (g)
Uninoculated control		5,45 a
F42	Q8	4,63 ab
F44	Q8	4,35 bc
F56	D5	4,01 bcd
F46	D5	4,04 bcd
F59	Z1	3,90 bcde
F51	Q7	3,87 bcde
F1	Z3	3,86 bcde
F57	Z3	3,85 bcde
F54	Z1	3,77 bcde
F43	DK (diseased)	3,65 bcde
F55	Q8	3,50 cde
F38	Q7	3,49 cde
F16	QK (diseased)	3,47 cde
F39	Q8	3,45 cde
F50	KM	3,43 cde
F48	KM	3,42 cde
F41	Q7	3,39 cde
F15	QK (diseased)	3,21 de
F53	KM	3,08 de
F47	KM	2,96 e
Inoculated control (without antagonist)		2,91 e

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

*Fresh mass is the mean of five replicate cups containing five seedlings each.

RESULTS

Test for suppressiveness of soils using *Lupinus angustifolius*

Of the 48 soils evaluated the first time, 12 significantly reduced root rot of blue lupin seedlings in comparison to the control treatments (Tables 2 and 3). When sampled again, together with soil from diseased trees from each orchard, evaluation of the 12 soils showed eight to consistently and significantly reduce root rot (Table 4).

However, one diseased tree soil also significantly reduced root rot when compared with the control treatments (Table 4).

Soils which proved to reduce root rot consistently were further selected and investigated, together with the diseased tree soils.

Test for suppressiveness of selected soils using Edranol seedlings

All selected escape tree soils significantly reduced the root rot severity of avocado seedlings, as well as the percentage of infected root pieces in comparison with the control (Table 5). No diseased tree soils had the ability to significantly reduce root rot of avocado seedlings or the percentage of infected root pieces (Table 5).

Quantification of *PC*

The number of leaf pieces yielding growth of *Pc* did not differ significantly for any of the soils, except that more *Pc* occurred in soil B14 than in seven escape tree soils and four diseased tree soils (Table 6). Soil B14 did not suppress root rot of avocado seedlings significantly (Table 4).

Isolation of micro-organisms for *in vitro* evaluation of antagonism to *PC*

Significantly more bacteria and fungi occurred in soil Z3 (Table 7) (which was significantly more effective in reducing disease severity than all other soils) (Tables 3, 5 and 6). Soil Z3 also contained significantly more actinomycetes than other soils, except for soils Z1 and Q7, as well as the highest number of aerobic spore forming bacteria (Table 7).

Overall, actinomycetes and spore forming bacteria were more antagonistic, followed closely by fungi and bacteria, while escape tree soils generally contained more microorganisms and higher percentages of antagonists than diseased tree soils from the same orchards (Table 8). Microorganisms strongly antagonistic to *Pc in vitro* were selected for further study.

Evaluations of antagonistic microorganisms for control of root rot using *Lupinus angustifolius*

When evaluating 50 micro-organisms for control of root rot of blue lupin seedlings, five isolates of bacteria and aerobic spore forming bacteria, four fungi and six actinomycetes significantly reduced root rot (Tables 9, 10 and 11). Disease severity of one bacterial, one fungal and two actinomycete treatments did not differ significantly from the uninoculated control (Tables 9, 10 and 11).

TABLE 11 Effect of antogonistic actinomycetes on root rot of lupin seedlings planted in *PC*-inoculated vermiculite

Isolate code	Selected soil code	*Fresh mass (g)
Uninoculated control		5,09 a
A1	Z1	4,55 ab
A5	Q7	4,28 abc
A17	KM (diseased)	4,07 bc
A46	Q8	4,02 bc
A47	Q7	4,01 bc
A11	KM	3,96 bc
A2	Z3	3,92 bcd
A4	Q7	3,90 bcd
A16	KM	3,90 bcd
A15	KM	3,85 bcd
A14	KM	3,85 bcd
A18	KM	3,84 bcd
A7	Q8	3,82 bcd
A6	Q8	3,75 bcd
A13	KM	3,61 bcd
A12	KM	3,48 cd
A10	KM	3,41 cd
Inoculated control (without antagonist)		2,90 d

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

*Fresh mass is the mean of five replicate cups containing five seedlings each.

DISCUSSION

The fact that trees without any visible root rot symptoms occurred in orchards affected by *Pc* was proof that some factor or factors were promoting the condition of the (escape) trees. As the *Pc* populations of escape tree soils were not lower than diseased tree soil from the same orchards, inoculum levels of different soils do not play a role in the health of the trees. This is the first report in which the criteria applicable to suppressive soils (Baker & Cook, 1974) were evaluated in South Africa.

Bioassays determining suppressiveness of soils have been used widely for various diseases and a variety of approaches and indicator plants (Chen, Hoitink & Madden, 1988; Shipton, Cook & Sitton, 1973; Wildermuth & Rovira, 1977; Broadbent *et al,* 1971; Baker *et al,* 1974). Such techniques may be used to determine the relative potential of the antagonistic population of a soil (Baker *et al,* 1974). Soils in which *Pc* occurred were thus found capable of significantly reducing disease severity in bio-assays, using blue lupin seedlings as hosts. However, of the 48 soils evaluated only ten consistently reduced disease severity of lupins and avocado seedlings.

This phenomenon has previously been described by Broadbent *et al* (1971, 1974) for soils in the Queensland area of Australia. The latter soils were reported to contain larger numbers of micro-organisms (especially *Bacillus* spp, actinomycetes and bacteria) than soils conducive to root rot (Broadbent *et al,* 1974; Halsall, 1982). The present study generally found suppressive soils to contain higher percentages of antagonistic micro-organisms than conducive soils, especially actinomycetes, aerobic spore forming bacteria, and fungi. The two soils most suppressive to root rot contained the highest numbers of actinomycetes and bacteria, while the most suppressive one also contained the highest number of fungi and the aerobic spore forming bacteria. Results obtained thus implicate the microflora of the soils for influencing the suppressiveness. The biological nature of suppression is confirmed by the fact that all suppressiveness is lost when the soils were first sterilised (Shipton, Cook & Sitton, 1973; Chen, Hoitink & Schmitthenner, 1987; Menzies, 1959).

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