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Evaluation of *Trichoderma* for Biological Control of Avocado Root Rot in Bark Medium Artificially Infested with *Phytophthora cinnamomi*

A. McLeod, N. Labuschagne and J.M. Kotzé
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, RSA

ABSTRACT

Trichoderma spp. isolated from avocado roots and rhizosphere soil as well as composted pine bark, were previously evaluated in vitro for antagonism against Phytophthora cinnamomi (Pc). Eight Trichoderma isolates that overgrew and halted growth of Pc, were subsequently evaluated in the green house for biogical control of Phytophthora root rot of avocado seedlings grown in presterilised pine bark medium. Millet seed inoculum of Trichoderma harzianum (C4 & BB5) and T. hamatum (F56) significantly reduced root rot and stimulated root regeneration of seedlings. T. harzianum (BB5) isolated from pine bark, caused an increase in root mass of avocado seedlings grown in the absence of Pc. Pc populations were significantly reduced by T. harzianum (C4) and T. hamatum (F56), but not by T. harzianum (BB5).

UITTREKSEL

Trichoderma spp. geisoleer uit avokado-risosfeer grond en -wortels, sowel as gekomposteerde dennebas is in vitro geévalueer vir antagonisme teen Phytophthora cinnamomi (Pc). Agt Trichodermo-isolate wat groei van Pc geinhibeerhet, isgevolglikin 'n glashuis eksperiment geévalueer vir biologies beheer van Phytophthora-wortelvrot by avokadosaailinge in 'n gesteriliseerde dennebas-medium. Mannasaad-inokulum van T. harzianum (C4 & BB5) en T. hamatum (F56) het wortelvrot verlaag en wortelgenerasie van saailinge betekenisvol verhoog. T. harzianum (BB5), geisoleer uit dennebas, het wortelmassa van saailinge betekenisvol verhoog in die afwesigheid van die patogeen. Pc populasies is verlaag deur T. hamatum (F56) en T. harzianum (C4), maar nie deur T harzianum nie (BB5).

INTRODUCTION

Species of *Trichoderma* have provided varied levels of control of a number of important soilborne plant pathogens, including *Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium rolfsi* (Sivan *et al.,* 1984). However, research on *Trichoderma* spp. as biocontrol agents of root diseases of perennial plants caused by *Phytophthora* spp. is limited (Kelley, 1976; Roiger & Jeffers, 1991).

Trichoderma spp. have been exceptionally good models for studying biocontrol because they are ubiquitous, easy to isolate and culture, grow rapidly on many substrates, are rarely pathogenic on higher plants, act as mycoparasites, compete well for food and site, produces antibiotics, and have enzyme systems capable of attacking a wide range of plant pathogens (Wells, 1988).

Trichoderma spp. has been implicated as microorganisms involved in control of soilborne plant pathogens in suppressive soils and composted bark media (Nelson & Hoitink, 1983; Elad *et al,* 1980). This genus is frequently isolated from suppressive soil (Smith *et al,* 1990), composted bark media (Nelson & Hoitink, 1983) and plant roots infected with pathogens (Roiger & Jeffers, 1991).

To find effective antagonists for application to soil or planting material, the best approach is to seek them where the pathogen is suppose to cause disease but isn't (Cook, 1985). There can, however, be exceptions to this rule, and Cook (1985) stated that "antagonists are where you find them". Cook (1985) further states that where we look for antagonists should be determined by the job to be done: if the antagonist is to live in soil, we should seek our candidates from soil. Introduced antagonists should be able to function as residents (Cook, 1985).

The purpose of this study was to evaluate *Trichoderma* spp. isolated from *Phytophthora* suppressive soil, and avocado roots growing in suppressive soil as well as composted pine bark, for control of root rot of avocado seedlings incited by *Phytophthora cinnamomi* Rands. The *Trichoderma* isolates were previously screened *in vitro* and subsequently *in vivo* on lupine and avocado seedlings.

MATERIALS AND METHODS

ISOLATION OF *TRICHODERMA*

The origin of various *Trichoderma* isolates used in biocontrol experiments is summerised in Table 1.

Table 1
Trichoderma isolates used in biological control studies of *Phytophthora cinnamomi* and their origin

Isolate designation[a]	*Origin*[b]
D2	Soil Z5 (roots)
C5	Soil Z4 (roots)
A1	Soil Z3 (roots)
C4	Soil Z4 (roots)
A4	Soil Z3 (roots)
BB1	Pine bark
BB5	Pine bark
F56	Soil D2 (soil)

[a]Codes of *Trichoderma* isolates used in biocontrol studies
[b]*Trichoderma* was isolated from *Phytophthora*-suppressive avocado rhizosphere soil, avocado roots as well as composted pine bark.

Soil and roots were collected from the rhizosphere of avocado trees (Z3, Z4 and Z5) at Tzaneen. Soil Z3 has previously been shown to be suppressive to *Phytophthora* root rot of avocado by Duvenhage (1993). Soil surrounding Z4 was not found to be suppressive by Duvenhage (1993), although this tree and the tree in soil Z5 showed exceptional vigour. Soil Z5 was not tested for suppression of *Phytophthora.*

Trichoderma was isolated from avocado soils collected from the topsoil (10 cm deep) under each tree. Three soil samples were taken from each tree, placed in plastic bags, kept at 20°C, and assayed as soon as possible after collection. From each sample, two 10g subsamples were taken,

added to 90 ml sterile water, and shaken on a reciprocal shaker for 30 minutes. Serial ten-fold dilution series were made in quarter-strength Ringer's solution (Oxoid), and plated on *Trichoderma* selective medium (Chen *et al.,* 1988). After 8 days of incubation at 25°C, colonies were picked at random and transferred to potato-dextrose agar (PDA).

For isolation of *Trichoderma* from root surfaces, one gram avocado roots were added to 90 ml of sterile water and shaken for 20 min on a reciprocal shaker. Dilution series and further processing were carried out as described above.

Two 10 g composted pine bark samples were collected from bags containing composted pine bark (Bark Enterprises, PO Box 3622, Brits). These bark samples were processed in the same manner as the avocado soil samples.

The freshly-isolated *Trichoderma* spp. were compared in terms of root rot control, with a standard isolate of *T. hamatum* (F56; Prem. 50938) with known biocontrol ability of *P. cinnamomi* (Duvenhage & Kotzé, 1993).

Two *P. cinnamomi* isolates were used. One isolate was obtained from J.A. Duvenhage (Merensky Technological Services, Duivelskloof) and the second isolate (WB1) was freshly isolated from avocado soil with the lupine bait technique described by Darvas (1979). The identity of the latter isolate was confirmed by Dr. W.J. Botha, Plant Protection Research Institute, Private Bag X134, Pretoria.

Growth medium

Biological control studies of *P. cinnamomi* were carried out in a mixture of composted pine bark, sand, and peat (3:1:1 v/v) This medium is hereafter referred to as bark medium. The bark medium was steam sterilised at 80°C for one hour on three consecutive days.

Chemical analysis of the medium by Outspan laboratories (P.O. Box 14105, Verwoerdburg) revealed the following: 1 mg/ml phosphorous; 22 mg/ml potassium; 36 mg/L calcium; 5 mg/ml magnesium; 25 mg/ml sodium; pH 5,8; 32 ms/m conductivity, 12% porosity and 588 ml/L water-holding capacity.

Evaluation of antagonistic *Trichoderma* isolates for suppression of root rot of *Persea Americana*

Preparation of inoculum of Trichoderma isolates

120 g of millet seed was mixed with 65 ml of water in 250 ml Erlenmeyer flasks, and autoclaved for 15 min on two consecutive days. After cooling, ten discs (5 mm diameter) from the margin of *Trichoderma* isolates C4, Al, A4, C5, D2, F56, BB1 & BB5 growing on PDA, were transferred to the millet seed and incubated at room temperature for ten days.

Preparation of Phytophthora cinnamomi *inoculum*

Phytophthora cinnamomi was cultured in 500 ml Erlenmeyer flasks containing 200 ml broth (1% glucose and 0,1% yeast extract). Each flask was inoculated with five agar discs from the margin of a *P. cinnamomi* (WB1) culture growing on PDA and incubated on a reciprocal shaker for two weeks. Mycelium was harvested by using Whatman no. 1 filter paper and then washed three times with sterile water to remove nutrients. Excess water was removed by blotting on filter paper and 10g mycelium was macerated with a Ultra Turrax (Ira & Kunkel) for 15 seconds in 100 ml water agar (0,1%).

Inoculation of bark medium and planting of avocado seedlings

Millet seed inoculum of each *Trichoderma* isolate was added at 1,5% (w/v) to sterilised bark medium and mixed thoroughly by hand. The *Trichoderma* -inoculated medium was incubated for six days at greenhouse temperatures fluctuating between 18°C and 36°C, and shaken twice to promote even colonisation.

P. cinnamomi macerated mycelium was added to a) *Trichoderma-amended* bark medium, b) sterilised and c) unsterilised bark medium, at 0,1% (w/v).

Control treatments consisted of a) sterilised bark, b) unsterilised bark medium, c) sterilised bark medium amended with each of the *Trichoderma* isolates and d) sterilised bark amended with uncolonised millet seed (1,5% w/v).

Four-month-old Duke 7 seedlings were planted in the inoculated bark medium in 2,5L plastic bags, with six replicates per treatment. Seedlings were placed in the greenhouse with temperatures ranging between 18°C and 30°C, and watered three times a week.

Evaluation on avocado seedlings

Treatments were evaluated according to: incidence of root rot, regeneration of new roots, reduction in dry lateral root mass and shoot weight, and increase in plant growth.

Seedlings were removed and roots washed under running tap water after 7 weeks. Root rot severity was rated on a percentage scale, where 0% indicates no root rot and 100% indicates that all the roots showed root rot symptoms. The generation of new roots was also determined on a percentage scale, where 0% shows no generation of new roots and 100% indicates that all the roots were white newly generated roots.

The lateral roots were cut from the tap root (Snyman *et al.,* 1984), and roots of each seedling were placed separately in brown paper bags and dried at 50°C for three days. Shoot mass was similarly determined. The percentage reduction in dry lateral root and shoot mass was determined relative to root and shoot mass of seedlings in uninfested sterilised control. The length of seedlings was determined at planting and at the end of the experiment and expressed as percentage increase in plant height.

Quantification of antagonists

The populations of each *Trichoderma* isolate was determined six days after amending of the bark medium, and 7 weeks after Duke 7 seedlings were planted in *P*. *cinnamomi* infested bark medium. Two 10 g bark medium subsamples of each treatment was added to 90 ml sterile water, and shaken on a rotatory shaker for 20 minutes. Serial dilutions series were prepared in quarter strength Ringer's solution and spread on three replicate plates of *Trichoderma* selective medium (Chen *et al,* 1988). Plates were incubated at 24°C in the dark, and colonies counted after six days.

Quantification of P. cinnamomi

The population of *P. cinnamomi* in treatments inoculated with *P. cinnamomi* was estimated with the avocado leaf bait technique as described by Pegg (1977). The bark medium of each treatment was bulked together and four 250 ml polystyrene cups were filled with 60 g of bark medium and 200 ml sterile water. The slurry was mixed and ten avocado leaf discs were suspended on the surface of each sample. Cups were incubated for four days in the dark, and discs plated on PARPH medium (Solel & Pinkas, 1984). The number of discs yielding growth of *P. cinnamomi* was determined after 7 days.

Trichoderma isolates effective in controlling avocado root rot were identified by Professor F. C.

Wehner (Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002).

RESULTS

Evaluation of *Trichoderma* for control of *Phytophthora* root rot of Duke 7 seedlings

Five *Trichoderma* isolates (C5, Al, C4, BB5 & F56) significantly reduced root rot and stimulated root regeneration of avocado seedlings grown in *P. cinnamomi*-infested medium, compared to sterilised bark medium infested with *P. cinnamomi* (Figures 1 & 2). *Trichoderma* isolates C4, BBS and F56 were superior to other isolates in controlling disease, as measured by root rot development and root regeneration (Figures 1 & 2). The unsterilised bark medium was not able to suppress root rot and stimulate root regeneration of avocado seedlings in *P. cinnamomi*-infested medium (Figures 1 & 2)

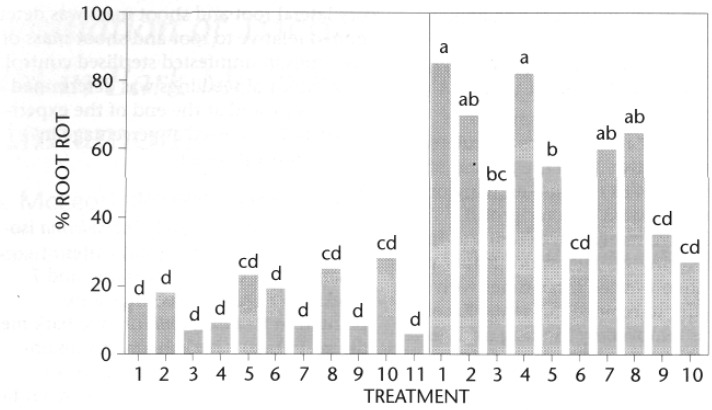

Figure 1

Effect of millet seed inoculum of *Trichoderma* isolates on mean percentage root rot of Duke 7 seedlings in pre-sterilised bark medium not infested (left) and infested (right) with *Phytophthora cinnamomi* (*Pc*). Bars followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).
1 =Sterilised control; 2 = Unsterilised control; 3 = *Trichoderma* isolate C5; 4 = *Trichoderma* isolate D2; 5 = *Trichoderma* isolate A1; 6 = *Trichoderma* isolate C4; 7 = *Trichoderma* isolate A4; 8 = *Trichoderma* isolate BB1; 9 = *Trichoderma* isolate BB5; 10 = *Trichoderma* isolate F56; 11 = Uncolonised millet seed

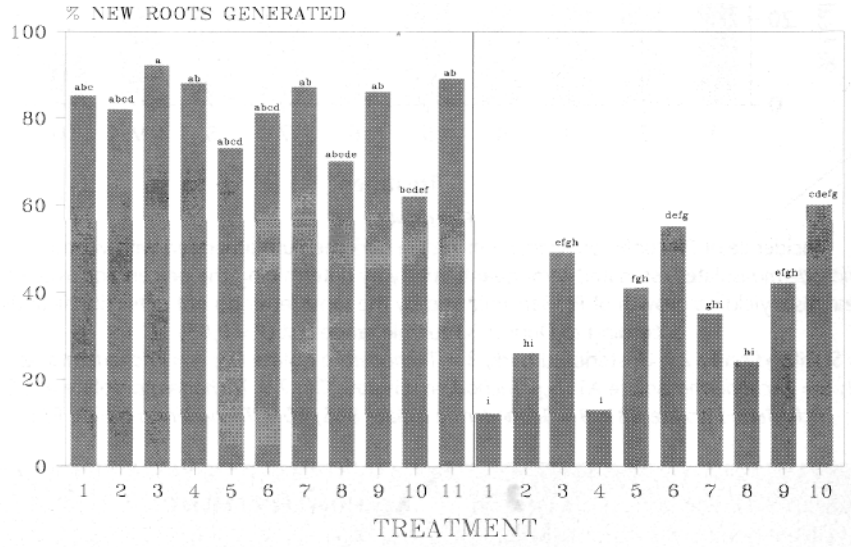

Figure 2

Effect of millet seed inoculum of *Trichoderma* isolates on mean percentage new roots generated by Duke 7 seedlings in pre-sterilised bark medium not infested (left) and infested (right) with *Phytphthora cinnamomi* (*Pc*). Bars followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).
1 = Sterilised control; 2 = Unsterilised control; 3 = *Trichoderma* isolate C5; 4 = *Trichoderma* isolate D2; 5 = *Trichoderma* isolate A1; 6 = *Trichoderma* isolate C4; 7 = *Trichoderma* isolate A4; 8 = *Trichoderma* isolate BB1; 9 = *Trichoderma* isolate BB5; 10 = *Trichoderma* isolate F56; 11 =Uncolonised millet seed

Trichoderma isolate BB5 and uncolonised millet seed caused a significant increase in dry lateral root mass of seedlings grown in the absence of the pathogen (Figure 3). The reduction in root mass is difficult to interpret due to variation in specific treatments. Although *Tríchoderma* isolate BB5 stimulated root growth, as was evident from thick white roots of seedlings, no measurements were made, except lateral dry root mass. There were no significant differences in dry shoot weight and increase in plant growth between different treatments (Table 2), probably due to the short period of growth of seedlings.

Table 2
Effect of *Trichoderma* isolates on percentage increase in plant growth and dry shoot weight of avocado seedlings in pre-sterilised bark medium infested or uninfested with *Phytophthora cinnamomi*

Treatment[a]	*% Increase in plant height*[b]	*% Reduction in dry shoot mass*[b]
Sterilised control	6.88 ab	
Unsterilised control	5.97 ab	–02.63 ab
Uncolonised millet seed	5.52 ab	–06.24 ab
Trichoderma isolate C5	7.17 a	–02.78 ab
Trichoderma isolate D2	3.84 ab	–00.95 ab
Trichoderma isolate A1	4.90 ab	·04.01 ab
Trichoderma isolate C4	3.02 ab	10.10 ab
Trichoderma isolate A4	3.46 ab	03.49 ab
Trichoderma isolate BB1	4.36 ab	07.31 ab
Trichoderma isolate BB5	5.45 ab	–08.00 ab
Trichoderma isolate F56	3.12 ab	–01.78 ab
Sterilised control + *Pc*	1.51 b	11.54 ab
Unsterilised control + *Pc*	2.92 ab	–04.45 ab
Trichoderma isolate C5 + *Pc*	2.84 ab	14.26 b
Trichoderma isolate D2 + *Pc*	2.41 ab	01.36 ab
Trichoderma isolate A1 + *Pc*	3.80 ab	17.80 b
Trichoderma isolate C4 + *Pc*	5.50 ab	05.30 ab
Trichoderma isolate A4 + *Pc*	5.50 ab	–00.08 ab
Trichoderma isolate BB1 + *Pc*	1.57 b	18.69 ab
Trichoderma isolate BB5 + *Pc*	3.27 ab	14.95 b
Trichoderma isolate F56 + *Pc*	5.03 ab	–17.38 a

[a]Uncolonised millet seed and millet seed inoculum of *Trichoderma* isolates were incorporated at 1,5 % (w/v) in container media and *P. cinnamomi* at 1 g homogenised mycelium per ℓ container media.
[b]Mean of six replicates; percentage increase in plant growth and reduction in dry shoot mass were determined 7 weeks after planting; reduction in dry shoot mass was determined relative to the sterilised control; values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)

Quantification of Trichoderma

After 6 days of incubation of *Trichoderma* amended bark media, *Trichoderma* isolate BB5 established significantly better than other isolates (Table 3). Propagule numbers of *Tríchoderma* isolate Al increased with a factor of ten in the period that seedlings were grown in pathogen infested medium (Table 3). Populations of isolates F56, D2, C5, BB1 and C4 remained stable after 7 weeks. In contrast to these isolates, the propagule number of isolate BB5 and A4 declined with a factor of 30 and 10 respectively after 7 weeks of seedling growth (Table 3).

Table 3
Growth and survival of *Trichoderma* isolates inoculated into presterilised bark medium

Trichoderma *isolate*[a]	Trichoderma *cfu* g^{-1} *bark medium* (20×10^6)	
	6 days[b]	*7 weeks*[b]
C5	10.0 c	42.7 c
D2	22.5 cb	67.0 ab
A1	6.0 c	57.0 bc
C4	14.3 c	18.1 d
A4	63.0 b	6.7 d
BB1	64.0 b	79.0 a
BB5	185.0 a	5.9 d
F56	14.0 c	11.0 d

[a]Millet seed inoculum of *Trichoderma* was incorporated at 15 g ℓ^{-1} container medium
[b]*Trichoderma* isolates enumerated on the medium of Chen *et al.* (1988), 6 days and 7 weeks after amendment of nursery medium with *Trichoderma*; values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)

Quantification of P. cinnamomi

The percentage avocado leaf discs yielding growth of *P. cinnamomi* was significantly less in bark medium infested with *Trichoderma* isolates, except *Trichoderma* isolate BB5 (Figure 4). The unsterilised bark medium did not suppress *P. cinnamomi* populations (Figure 4).

Trichoderma isolates BB5 and C4 were both positively identified as *T. harzianum.*

DISCUSSION

Some *Trichoderma* isolates (eg. D2) showed strong antagonism against *P. cinnamomi* in dual culture tests, but failed to control root rot of avocados and lupines. In previous tests (McLeod, unpublished) the same phenomenon was observed by Smith *et al.* (1990) when testing *Trichodertna* for control of *P. cactorum* crown and root rot of apple seedlings. It is recognised that the *in vitro* method has limitations, but can be effective in identifying some effective antagonists such as *T. hamatum* (F56) and *T. harzianum* (C4).

The lupine bio-assay method did not always correlate with the biocontrol potential of *Trichoderma* against root rot of avocados (McLeod, unpublished). This was apparent with isolate D2 which controlled damping-off of lupins but failed in the avocado bio-assay. Again this method has limitations, but can be effective in identifying some potential antagonistic *Trichoderma* isolates.

High densities of *Trichoderma* spp. are present in suppressive soil and pine bark (Kuter *et al*. 1983; Cásale, 1990). This indicates that these taxa are involved in disease suppression and are capable of establishing in bark medium. Results obtained from this study support this, as root rot was

controlled with *T. hamatum* (F56) and *T. harzianum* (C4 & BB5) which were isolated from suppressive bark (McLeod unpublished) and soil.

There is a great deal of controversy as to the isolation source of antagonists (Cook, 1985). Results from this study agree with that of Myatt *et al.* (1992), indicating that there is no correlation between biocontrol and the isolation source (pine bark or soil) and method of isolation (root surface and rhizosphere soil). In contrast, Smith *et al.* (1990) found no effective *Trichoderma* biocontrol agents from sites were the pathogen was not present.

Initial establishment of all *Trichoderma* isolates in the bark medium was high, with propagule numbers of 10^6/g or more. However, high populations of *Trichoderma* did not always result in control. Smith *et al.* (1990) also found a lack of dependence between qualitative root rot ratings of apple seedlings and presence of biocontrol agent.

T. harzianum (BB5) reached high populations after 6 days, but declined drastically after 7 weeks, yet it gave good control. The good initial establishment probably reflects a favourable growth environment for this isolate, which was originally isolated from pine bark. There is no apparent reason for the decline of populations, except that most energy is presumably spent on the production of secondary metabolites and not reproduction.

P. cinnamomi populations were reduced with more than 50% by all *Trichoderma* isolates, except BB5. Cásale (1990) also found that in disease suppressive soils rhizosphere populations of *P. cinnamomi* was very low in the presence of *Trichoderma,* but pathogen populations were high in soils where *Trichoderma* was absent.

According to Baker (1968) a decrease in pathogen populations reflects that biocontrol might be due to antibiosis or lysis. In other studies, hyperparasitism has been postulated as the mode of action, since coiling of *Trichoderma* around hyphae of *P. cinnamomi* has been observed (Cásale, 1990). *Trichoderma* can produce enzymes such as glucanase, chitinase and cellulase that can degrade fungal cell walls (Hadar *et al. 1979;* Chet & Baker, 1981). The latter is effective on cell walls of oomycetes (Chet & Baker, 1981). Since all *Trichoderma* isolates except BB5 decreased *P. cinnamomi* populations it is clear that hyperparasitism is not the only mode of action involved in suppression of root rot by isolates F56 and C4. Mycoparasitism is probably never exclusively responsible for biocontrol of the pathogen, but rather a combination of mycoparasitism, antibiosis and competition (Wells, 1988).

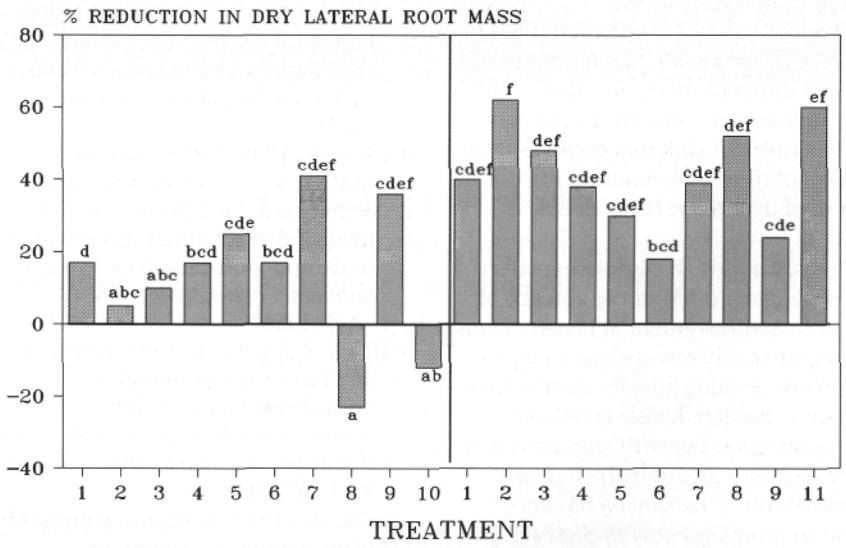

Figure 3

Effect of millet seed inoculum (1,5% w/v) of *Trichoderma* isolates on mean percentage reduction in dry lateral root mass of Duke 7 seedlings in pre-sterilised bark medium not infested (left) and infested (right) with *Phytophthora cinnamomi* (*Pc*). Bars followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)
1 = Unsterilised bark control; 2 = *Trichoderma* isolate C5; 3 = *Trichoderma* isolate D2; 4 = *Trichoderma* isolate A1; 5 = *Trichoderma* isolate C4; 6 = *Trichoderma* isolate A4; 7 = *Trichoderma* isolate BB1; 8 = *Trichoderma* isolate BB5; 9 = *Trichoderma* isolate F56; 10 = Uncolonised millet seed; 11 = Sterilised bark

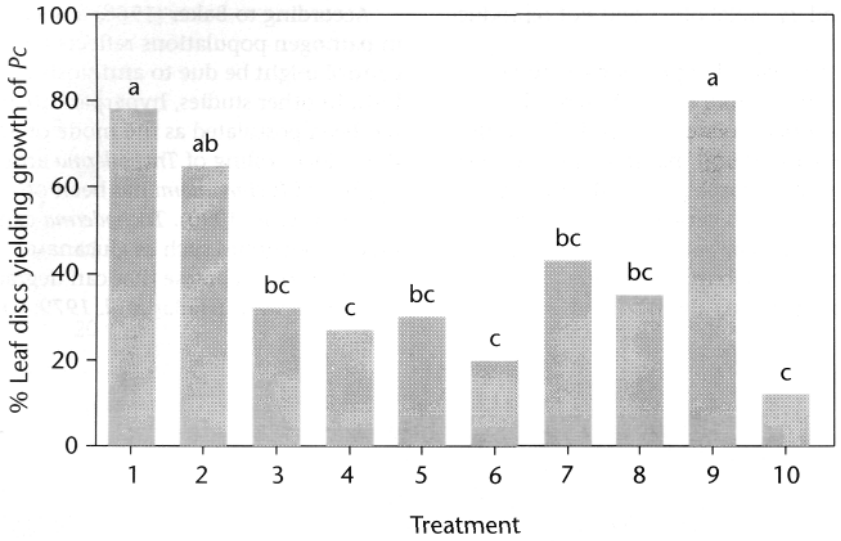

Figure 4

Incidence of *Phytophthora cinnamomi* (*Pc*) in bark medium amended with various *Trichoderma*-isolates estimated semi-quantitatively by determining the percentage avocado leaf discs yielding growth of *Pc*. Bars followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)
1 = Sterilised bark; 2 = Unsterilised bark; 3 = *Trichoderma* isolate C5; 4 = *Trichoderma* isolate D2; 5 = *Trichoderma* isolate A1; 6 = *Trichoderma* isolate C4; 7 = *Trichoderma* isolate A4; 8 = *Trichoderma* isolate BB1; 9 = *Trichoderma* isolate BB5; 10 = *Trichoderma* isolate F56

The inability of *T. harzianum* (BB5) to decrease *P. cinnamomi* populations might reflect a different mode of action of disease suppression. This antagonist may create a nutrient sink that deprives *P.*

cinnamomi of nutrients, resulting in poor infection of host tissue (Chet & Baker, 1981).

The plant growth stimulating effect of *T. harzianum* (BB5) in the absence of the pathogen is a potential benefit. Considering that this was evident after only 7 weeks of seedling growth, further investigation is needed. Roiger & Jeffers (1991) also found growth stimulation of apple seedlings grown in *Trichoderma* amended soil. *T. harzianum* has been found to produce a growth-regulating factor that increases dry weight of shoots and stems (Windham *et al,* 1986).

Results from the present study do not support previous data showing pine bark to be suppressive (McLeod, unpublished). Hoitink *et al.* (1991) also found no consistent suppression of *Pythium* damping-off among batches of composted pine bark.

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REFERENCES

BAKER, R. 1968. Mechanisms of biological control of soil-borne pathogens. *Annual Review of Phytopathology* 6: 263 - 294

CÁSALE, W L. 1990. Analysis of suppressive soils and development of biological control methods for *Phytophthora* root rot of avocado. *California Avocado Society Yearbook* 74: 53 - 56.

CHEN, W, HOITINK, H. A. J. & MADDEN, L. V. 1988. Microbial activity and biomass in container media for predicting suppressiveness to damping-off caused by *Pythium ultimum. Phytopathology* 78: 1447 - 1450.

CHET, I. & BAKER, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from suppressive soil naturally suppressive to *Rhizoctonia solani. Phytopathology* 71: 286 - 290.

COOK, R.J. 1985. Biological control of plant pathogens: Theory to application. *Phytopathology* 75: 25 - 29.

DARVAS, J. M. 1979. Lupine bait technique for the semi-quantitative analysis of *Phytophthora cinnamomi* and other root pathogens in avocado soils. *South African Avocado Grower's Association Yearbook* 3: 29 - 30.

DUVENHAGE, J. A. 1993. Microbial and nutritional factors involved in soils suppressive to *Phytophthora* root rot of avocado. M.Sc.(Agric) Thesis. University of Pretoria, Pretoria.

DUVENHAGE, J.A. & KOTZE, J.M. 1993. Biocontrol of root rot of avocado seedlings. *South African Avocado Growers' Association Yearbook* 16: 70 - 72.

ELAD. Y,I, CHET, I. & KATON, J. 1980. *Trichoderma harzianum:* A biocontrol agent effective against *Sclerotium rolfsi* and *Rhizoctonia solani. Phytopathology* 70: 119 - 121.

HADAR, Y., CHET, I. & HEÑÍS, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma hamatum. Phytopathology* 69: 64 - 68.

HOITINK, H.A.J., INBAR, Y. & BOEHM, M. J. 1991. Status of compostamended potting mixes naturally suppressive to soilborne diseases of floricultural crops. *Plant Disease* 75: 869 - 873.

KELLEY, W.D. 1976. Evaluation of *Trichoderma harzianum* impregnated clay granules as biocontrol for *Phytophthora cinnamomi* damping-off of pine seedlings. *Phytopathology* 66: 1023 - 1027.

KUTER, G.A., NELSON, E.B., HOITINK, H.A.J. & MADDEN, L.V. 1983. Fungal populations in container media amended with composted hard wood bark suppressive and conducive to *Rhizoctonia damping-off. Phytopathology* 73: 1450 - 1456.

MYATT, P.M., DART, P.J. & HAYWARD, A.C. 1992. Potential for biological control of *Phytophthora* root rot of chickpea by antagonistic root-associated bacteria. *Australian Journal*

of Agricultural Research 44: 773 - 784.
NELSON, E.B. & HOITINK, H.A.J. 1983. The role of microorganisms in the suppression of *Rhizoctonia solani* in container media amended with composted hardwood bark. *Phytopathology* 73: 274 - 278.
PEGG, K.G. 1977. Soil application of elemental sulphur as a control of *Phytophthora cinnamomi* root rot of pineapple. *Australian Journal of Experimental and Agricultural and Animal Husbandry* 17: 859 - 865.
ROIGER, DJ. & JEFFERS, S.N. 1991. Evaluation of *Trichoderma* spp. for biological control of Phytophthora crown and root rot of apple seedlings. *Phytopathology* 81: 910 - 917.
SIVAN, A. ELAD, Y. & CHET, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum. Phytopathology* 74: 498 - 501.
SMITH, V.L., WILCOX, W.F. & HARMAN, G.E. 1990. Potential for biological control of *Phytophthora* root and crown rots of apple by *Trichoderma* and *Gliodadium* spp. *Phytopathology* 80: 880 - 885.
SNYMAN, A.J., SNYMAN, C.P. & KOTZÉ, J.M. 1984. Pathogenicity of avocado root rot fungi to Edranol seedlings and Duke 7 rooted cuttings. *South African Avocado Growers' Association Yearbook 7:* 80 - 81.
SOLEL, Z. & PINKAS, Y. 1984. A modified selective medium for detecting *Phytophthora cinnamomi* on avocado roots. *Phytopathology* 84: 506 - 508.
WINDHAM, M.T., ELAD, Y. & BAKER, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopahtology* 76: 518 - 521.
WELLS, H.D. 1988. *Trichoderma* as biocontrol agent. In *Biocontrol of plant diseases,* Mukerji, K.G & Garg, K.L., (eds.). CRC Press. New York. I: 71 - 82.