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In vitro Identification of *Trichoderma harzianum* as a Potential Antagonist of Plant Pathogens

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Abstract. When compared with a range of colonists of straw and other potential antagonists of glasshouse and cereal pathogens, *Trichoderma harzianum* IMI 275950 exhibited the greatest biocontrol activity in vitro. This activity was also observed in a gnotobiotic lettuce bioassay against the pathogen *Sclerotinia sclerotiorum*. Activity was dependent on antagonist/pathogen inoculum ratio and temperature.

Many studies have demonstrated the potential for microorganisms to control plant disease in vivo [3]. Commonly the antagonistic action has been identified by casual observation, and there may have been no attempt to identify the microbe–microbe interaction. In vitro studies are useful for screening for potential biocontrol systems and for identifying the mode(s) of action, although ultimately the activity must be established in vivo.

Of the range of biocontrol agents studied, *Trichoderma* spp. and particularly *T. harzianum* have shown much promise [7, 10, 14]. *T. harzianum* can act by mycoparasitism, together with the production of volatile pyrone antibiotics [2] and cell-wall-degrading enzymes [15]. It produces a powerful cellulase [4] and is a dominant colonist of straw [5], which is a major substrate for microorganisms in soils used for arable cultivation [9].

In the present report the potential use of *T. harzianum* against the pathogens, *Fusarium culmorum* and *Pythium ultimum*, has been studied in vitro. Its activity has been compared with that of other fungi which colonize straw, because this is one of the most abundant substrates in soil for which pathogens and their antagonists are likely to compete. *T. harzianum* has also been tested against some pathogens of glasshouse crops and compared with some other potential antagonists that had been suggested from casual observations in vivo or in vitro.

Materials and Methods

Organisms. Antagonists from the GCRI culture collection were *Acremonium* (*Cephalosporium*) sp. 637, *Cladosporium* sp. 638, and *Paecilomyces lilacinum* 580 (isolated from glasshouse soil with a tomato crop), *Gliocladium roseum* 588 (isolated from a carnation stem infested with *Fusarium oxysporum*), *P. lilacinum* 642 (isolated by Dr. B. Kerry from soil in Peru), and *Verticillium lecanii* (an insect pathogen). *Trichoderma harzianum* IMI 275950 [5], *T. harzianum* IMI 284726 [11], *Acremonium persicinum* IMI 284720, *Botryotrichum piluliferum* IMI 284721, *Penicillium corylophilum* IMI 284722, *P. echinulatum*, *P. viridicatum*, *Sordaria alcina* IMI 267236, *Trichoderma longibrachiatum* IMI 284728 [6], *Epicoccum nigrum*, and *Penicillium hirsutum* had all been isolated as colonists of decomposing straw. *Coniothyrium minitans* IMI 134523 [16] had been isolated from fungal sclerotia. The pathogens studied were *Botrytis cinerea* (from lettuce), *Fusarium avenaceum* (from wheat), *F. culmorum* IMI 239950 (from ryegrass), *F. oxysporum* (from wheat), *F. oxysporum* f. sp. *dianthi* (from carnation), *F. oxysporum*. f. sp. *narcissi* (from narcissus), *F. solani* (from wheat), *Phomopsis sclerotiodes* (from cucumber roots), *Pyrenochaeta lycopersici* (from tomato), and *Sclerotinia sclerotiorum* (from cucumber fruit).

Growth of organisms on agar. The antagonists and pathogens were inoculated onto 9-cm-diameter malt agar plates, each 3 cm from the edge of the plate with a common diameter. Controls were also set up with the antagonists or pathogens alone, so that a growth without interactions could be precisely measured. Growth of the fungus at 25°C was measured as the average diameter of the colony size in the four directions at right angles between 24 and 72 h after inoculation. However, assessments of interactions were made for a further 6 days.

Effects of spore concentration and temperature. *F. culmorum* was grown on potato sucrose agar, and *T. harzianum* IMI 275950 on malt agar, for 2 weeks. Spores were harvested and suspended in sterile distilled water at concentrations of 6.25×10^6 spores ml^{-1}. Dilutions of the spore suspensions were prepared by introducing 1 ml of stock solution into successive aliquots of 9 ml of sterile distilled water. Aliquots (1 ml) of antagonist and pathogen suspensions were mixed, and each concentration pair was streaked onto malt agar in Petri dishes (5 replicates) (9 cm diameter) as well as the monocultures. Plates were incubated at 5, 10, 15, 20, and 25°C, and colony growth of each fungus was assessed between 7 and 16 days after inoculation.

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Table 1. Mean radial growth rates of fungal colonies (assessed between 24 and 72 h) at 20°C

Potential antagonists	Daily growth rate (mm d^{-1})	Log daily growth rate	Pathogens	Daily growth rate (mm d^{-1})	Log daily growth rate
Cephalosporium sp. 637	2.6	1.0	*Botrytis cinerea*	21.3	3.1
Cladosporium sp. 638	2.3	0.8	*Fusarium avenaceum*	10.1	2.3
Coniothyrium minitans	5.6	1.7	*F. culmorum*	12.8	2.5
Gliocladium roseum	4.3	1.4	*F. oxysporum*	11.8	2.4
Paecilomyces lilacinum	4.0	1.4	*F. oxysporum* sp. *dianthi*	9.6	2.3
Paecilomyces sp. 580	4.0	1.4	*F. oxysporum* sp. *narcissi*	9.3	2.2
Trichoderma harzianum IMI 275950	26.1	3.3	*F. solani*	8.6	2.2
T. harzianum IMI 284726	20.1	3.0	*Phompsis sclerotiodes*	12.3	2.5
Verticillium lecanii	3.0	1.1	*Pyrenochaeta lycopersici*	2.0	0.7
			Sclerotinia sclerotiorum	24.1	3.2
LSD ($P = 0.05$)		0.1	LSD ($P = 0.05$)		0.1

Table 2. Screening of some potential microbial antagonist against plant pathogens[a]

Antagonist	*Botrytis cinerea*	*Fusarium avenaceum*	*F. culmorum*	*F. oxysporum*	*F. oxysporum* f. sp. *dianthi*	*F. oxysporum* f. sp. *narcissi*	*F. solani*	*Phomopsis* sp.	*Pyrenochaeta lycopersici*	*Sclerotinia sclerotiorum*
	Pathogen									
Cephalosporium sp.	0^+	1	X	0	0	0	0	0	0^+	0^+
Cladosporium sp.	X^+	0	1	0	0	0	0	1	0^+	0^+
Coniothyrium minitans	0^+	2	0	0	0	0	0	2	0^+	0^+
Gliocladium roseum	1^+	1	2	2	0	0	1	2	0	2^+
Paecilomyces lilacinum	0^+	3	0	0	0	0	0	3	0	2^+
Paecilomyces sp 580	2^+	3	3	1	2	1	2	0	0	2^+
Trichoderma harzianum IMI 275950	4^-	1,4	4	4	4	4	4	4	4	4^-
T. harzianum IMI 284726	4^-	4	4	4	0	4	4	4	4	4^-
Verticillium lecanii	1^+	0	0	0	0	0	0	0	0	0^+

[a] Key: X, pathogen overgrows antagonist after initial contact; 0, mutual inhibition where antagonist and pathogen meet; 1, inhibition zone around antagonist ca 1 mm; 2, inhibition zone around antagonist ca 2 mm; 3, inhibition zone around antagonist > 2 mm; 4, antagonist overgrows pathogen; $^+$, sclerotia; $^-$, sclerotia absent.

Effect of antagonists against *Sclerotinia sclerotiorum* in gnotobiotic culture of lettuce. Lettuce seeds were sterilized and germinated in sand moistened with plant nutrient solution and contained in boiling tubes [12]. The antagonists were buried in the sand at planting on discs (5-mm diameter) of malt agar with 7-day-old growth of the antagonist, and the pathogen (*Sclerotinia sclerotiorum*) of the same age was added on discs 5 days later. Three tubes, each containing three plants, were used for each treatment and for the control with no pathogen added. The plants were grown in a chamber at 18°C with a 16-h period of daylight of 24.5 Wm^{-2}. Plant survival was assessed after 18 days.

Results

Both strains of *Trichoderma harzianum* grew far more rapidly on agar than any other potential antagonists, but the pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum* had similar growth rates (Table 1). With the exception of *Pyrenochaeta lycopersici*, the remaining pathogens all grew faster than the other antagonists.

When both species of *T. harzianum* were grown with the pathogens, they completely overgrew the pathogens and inhibited sclerotia formation by those fungi with the ability to produce them (Table 2). Whereas some of the potential antagonists tested, especially *Paecilomyces* sp 580, showed antibiosis in varying degrees to the pathogens, none had the intensity or spectrum of activity exhibited by the *Trichoderma* spp., which is evident in Figs. 1 and 2.

A comparison of the activity of the *Trichoderma* spp. with other straw-degrading fungi against the pathogens *Fusarium culmorum* and *Pythium ultimum* indicated varying degrees of antagonism, but only *T. harzianum* IMI 275950 was totally effective in suppressing the growth of both pathogens (Fig. 3). Whereas growth on agar may not correlate well with growth on natural substrates, it is interesting that *T. harzianum* can grow rapidly on straw as well as on agar [6].

The activity of *T. harzianum* IMI 275950 was dependent of the relative inoculum size of the an-

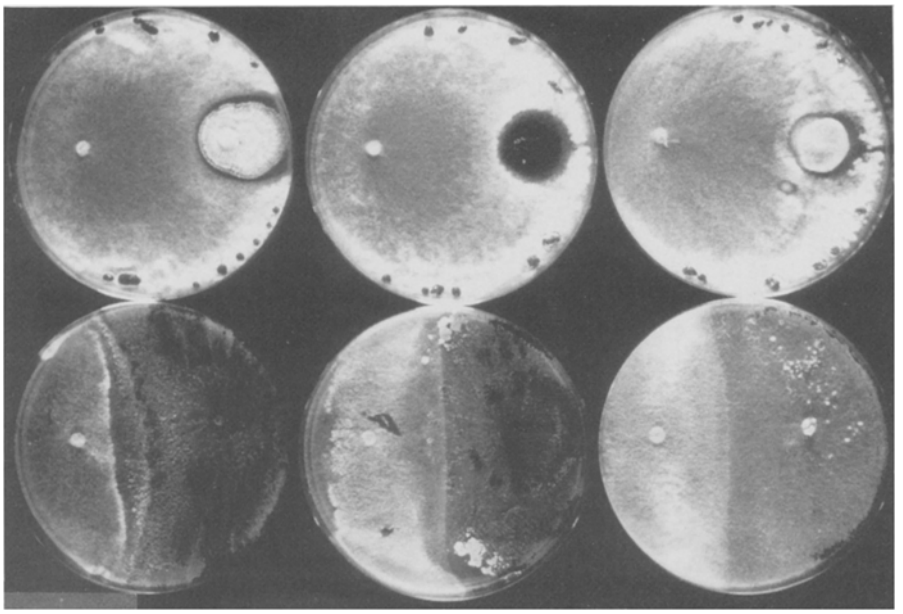

Fig. 1. Interactions between *Sclerotinia sclerotiorum* and potential antagonists. Top row (left to right): *Gliocladium roseum*, *Gladosporium* sp. 638, and *Paecilomyces* sp. 580. Bottom row: *Trichoderma harzianum* IMI 275950, *T. harzianum* IMI 284726, and *Trichoderma* sp. 647. The antagonist is inoculated on the right of each plate.

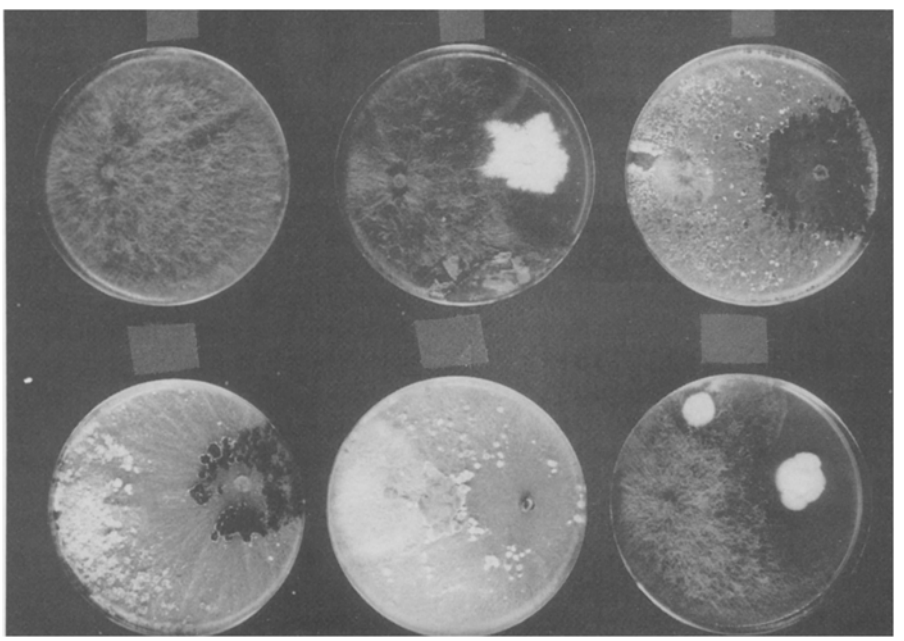

Fig. 2. Interactions between *Fusarium culmorum* and potential antagonists. Top row (left to right): pathogen only, *Gliocladium roseum*, and *T. harzianum* IMI 275950. Bottom row: *T. harzianum* IMI 284726, *Trichoderma* sp. 647, and *Paecilomyces* sp. 580.

tagonist to pathogen and on temperature when applied to the agar plate as a mixed spore inoculum (Fig. 4). Inoculum size was the most critical determinant of the interaction except at 5°C, when no growth of *Trichoderma* and only poor growth of *Fusarium* were observed in the time period.

Trichoderma harzianum IMI 275950 was the most effective of the antagonists tested in preventing death of lettuce seedlings caused by *S. sclerotiorum*, but there was an inhibitory effect on the root extension of survivors (Table 3).

Discussion

Field relevance of in vitro analyses of the biocontrol potential of antagonists of plant pathogens will always be equivocal. However, they can indicate the activity of organisms under ideal conditions prior to

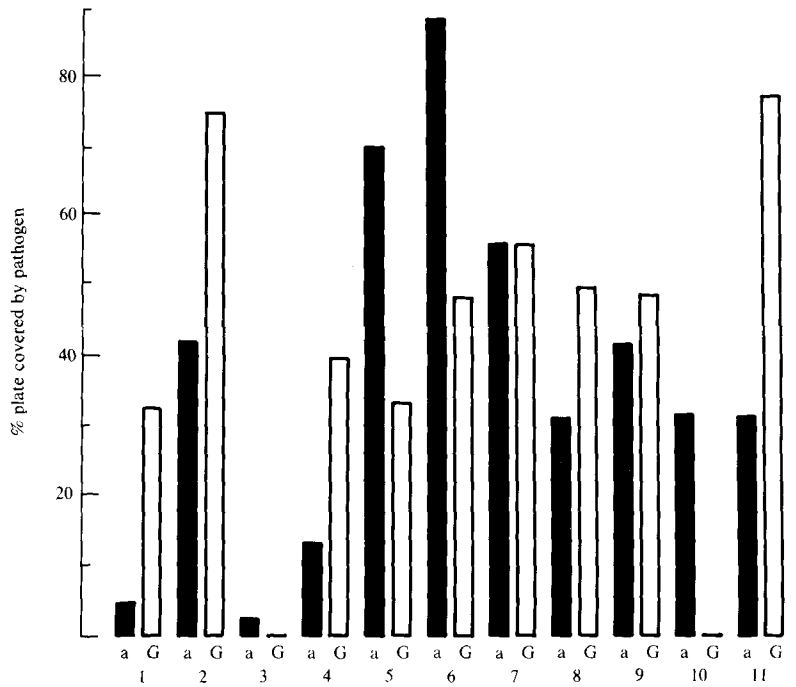

Fig. 3. Interactions between fungi isolated as colonists of straw and the pathogens *Fusarium culmorum* (*a*) and *Pythium ultimum* (*b*). *Trichoderma harzianum* IMI 284726; (2) *T. longibrachiatum*; (3) *T. harzianum* IMI 275950; (4) *Botrytrotrichum pilulferum*; (5) *Penicillium viridicatum*; (6) *P. corylophilum*; (7) *Epicoccum nigrum*; (8) *Sordaria alcina*; (9) *P. echinulatum*; (10) *P. hirsutum*; and (11) *Acremonium persicinum*. The pathogens fully covered the plate when no antagonist was present.

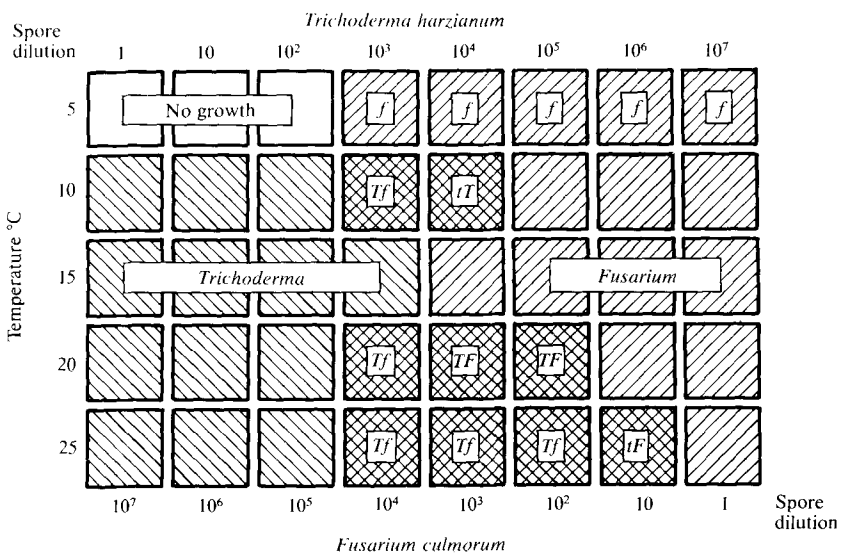

Fig. 4. Influence of inoculum (spores) levels and temperature upon the competitive abilities of *Fusarium culmorum* and *Trichoderma harzianum* on malt agar. The stippled areas indicate domination of the fungus indicated (left slope, *T. harzianum*; right slope, *F. culmorum*). Cross-hatching indicates co-existence of antagonist and pathogen. *T* and *F* refer to good growth, *t* and *f* to poor growth of *T. harzianum* and *F. culmorum*, respectively. The growth was assessed after 16 days.

the stresses created by the natural (soil) environmental constraints on the physical environment of the organisms, and from the present study it seems that *Trichoderma harzianum* may be less effective at low temperature. An earlier study [13] indicated that *T. harzianum* IMI 275950 is less tolerant of low water potential than is *Fusarium culmorum*. Such information will be useful in compiling a list of conditions for which the useful antagonistic properties of *T. harzianum* might be achieved in vivo. We have found that *T. harzianum* IMI 275950 sprayed as a suspension (spore ml^{-1}) onto straw in the field has completely suppressed the colonization of straw by *F. culmorum* (N. Magan, P. Hand, and J.M. Lynch,

Table 3. Control of *Sclerotinia sclerotiorum* by microbial antagonists in lettuce seedling tests

Antagonist	Plants surviving[a] (%)	Root length (mm)	Significance of difference for control
Trichoderma harzianum IMI 284726	11	21	$P < 0.05$
T. harzianum IMI 275950	66	15	$P < 0.001$
Paecilomyces sp. 580	22	36	n.s.[b]
None	0	0	
Control (no pathogen)	100	48	

[a] All treatments are significantly different from each other ($P < 0.05$) by an analysis of deviance with a binomial model.
[b] n.s., not significant.

unpublished). In gnotobiotic and field studies we and others have found *Trichoderma* spp. to be useful antagonists, particularly of sclerotia-forming fungi, but continued studies in vitro may lead to an understanding of why inoculants can sometimes fail.

A critical consideration in vivo is the relative size of pathogen to inoculant spore or biomass concentrations. It is clear for the present study and from an earlier study [8] with *Trichoderma* spp. against *Acremonium* spp. (*Cephalosporium*) that high pathogen concentrations may be overcome only by high concentrations of antagonist inocula. In a recent series of mature plant trials with protected lettuce grown in soil, we found that a range of biotypes of *T. harzianum* provided protection against *Rhizoctonia solani* when added in a ratio of 10 : 1, but not when the ratio of antagonist to pathogen was 1 : 1 (C.J. Ridout, J.R. Coley-Smith, and J.M. Lynch, unpublished).

A further consideration in the in vivo exploitation of antagonists selected in in vitro screens is their direct effect on plants. Recently we have shown in a mature plant trial with lettuce that *T. harzianum* IMI 275950 increased root and shoot growth, and this is consistent with other observations that *T. harzianum* can increase plant growth [1, 17].

In conclusion, this in vitro screening study led to the identification of a new biocontrol agent, *T. harzianum* IMI 275950, which is showing potential as a biocontrol agent in the field (N. Magan, P. Hand, and J.M. Lynch, unpublished); this activity is probably due at least in part to the production of some broad-spectrum antibiotics [2].

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Literature Cited

1. Chang Y., Chang Y, Baker R, Kleifeld O, Chet I (1986) Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis 70:145–148
2. Claydon N, Allan M, Hanson JR, Avent AG (1987) Antifungal alkyl pyrones of *Trichoderma harzianum*. Trans Brit Mycol Soc 88:503–513
3. Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. St Paul: American Phytopathological Society
4. Eveleigh DE (1985) *Trichoderma*. In: Demain AL, Solomon NA (eds) Biology of industrial micro-organisms. Menlo Park: Benjamin/Cummings Publishing Co, pp 487–509
5. Harper SHT, Lynch JM (1984) Nitrogen fixation by cellulolytic communities at aerobic–anaerobic interfaces in straw. J Appl Bact 57:131–137
6. Harper SHT, Lynch JM (1985) Colonization and decomposition of straw by fungi. Trans Brit Mycol Soc 85:655–661
7. Henis Y (1984) Ecological principles of biocontrol of soilborne plant pathogens: *Trichoderma* model. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. Washington DC: American Society for Microbiology, pp 353–361
8. Lai P, Bruehl GW (1968) Antagonism among *Cephalosporium gramineum*, *Trichoderma* spp and *Fusarium culmorum*. Phytopathology 58:562–566
9. Lynch JM (1983) Soil biotechnology: Microbiological factors in crop productivity. Oxford: Blackwell Scientific Publications
10. Lynch JM (1987) Biological control within microbial communities of the rhizosphere. In: Fletcher M, Gray TRG, Jones JG (eds) Ecology of microbial communities. Cambridge: Cambridge University Press, pp 55–82
11. Lynch JM, Slater JH, Bennett JA, Harper SHT (1981) The cellulase activities of aerobic micro-organisms isolated from soil. J Gen Microbiol 127:231–236
12. Lynch JM, White N (1977) Effects of some non-pathogenic microorganisms on the growth of gnotobiotic barley plants. Plant and Soil 47:161–170
13. Magan N, Lynch JM (1986) Water potential growth and cellulolysis of fungi involved in the decomposition of cereal residues. J Gen Microbiol 132:1181–1187
14. Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Annu Rev Phytopathology 23:23–54
15. Ridout CJ, Coley-Smith JR, Lynch JM (1986) Enzyme activity and electrophoretic profile of extracellular protein induced in *Trichoderma* spp by cell walls of *Rhizoctonia solani*. J Gen Microbiol 132:1181–1187
16. Tribe HT (1957) On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. Trans Brit Mycol Soc 40:489–499
17. Windham MT, Elad Y, Baker R (1986) A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518–521