Trichoderma harzianum: A Biocontrol Agent Effective Against *Sclerotium rolfsii* and *Rhizoctonia solani*

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ABSTRACT

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An isolate of *Trichoderma harzianum* capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani* was isolated from a soil naturally infested with those pathogens. In culture, *T. harzianum* grew better than *S. rolfsii* and invaded its mycelium under growth conditions adverse to the pathogen; eg, high pentachloronitrobenzene concentrations, high pH levels, or low temperatures. Under greenhouse conditions, incorporation of the wheat-bran inoculum preparation of *T. harzianum* in pathogen-infested soil significantly reduced bean diseases caused by *S. rolfsii, R. solani,* or both, but its biocontrol capacity was inversely correlated with temperature. The wheat bran preparation of *T. harzianum* increased growth of bean plants in a noninfested soil and it controlled *S. rolfsii* more efficiently than a conidial suspension of the same antagonist. An uninoculated wheat bran preparation increased disease incidence. In naturally infested soils, wheat bran preparations of *T. harzianum* inoculum significantly decreased diseases caused by *S. rolfsii* or *R. solani* in three field experiments with beans, cotton, or tomatoes, and they significantly increased the yield of beans.

Additional key words: integrated control, PCNB.

Biological control of soilborne plant pathogens by the addition of antagonistic microorganisms to the soil is a potential nonchemical means for plant disease control. The species of *Trichoderma* capable of hyperparasitizing pathogenic fungi, are highly efficient antagonists (2,5). Weindling (12) reported the parasitism of *Trichoderma lignorum* (Tode) Harz on *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kühn. This effect was also shown, under field conditions, by Wells et al (13) using *T. harzianum* grown on ryegrass. Similarly, Backman and Rodriguez-Kabana (1) controlled *S. rolfsii* in peanuts by using molasses-enriched clay granules as a food base for *T. harzianum*. Recently, Hadar et al (6) found that *T. harzianum* directly attacked *R. solani* mycelium. Wheat-bran-grown cultures of this antagonist added to soil in greenhouse plantings reduced damping-off caused by *R. solani* in beans, tomatoes, and eggplants. The efficiency of *Trichoderma* was improved when integrated with pentachloronitrobenzene (PCNB) at sublethal doses (3).

In the present study, an antagonistic strain of *T. harzianum* capable of controlling both *S. rolfsii* and *R. solani* was isolated from pathogen-infested soil and its behavior was studied under laboratory, greenhouse, and field conditions.

MATERIALS AND METHODS

Fungi were isolated from the soil on Martin's agar medium as described (8). Their antagonistic activity towards pathogens was determined according to the method of Dennis and Webster (4), but using a solidified synthetic medium (SM) (10). Different pH levels were obtained by the addition of 0.1 N NaOH or 0.1 N HCl to the medium before sterilization. PCNB as Terraclor (75% a.i. Olin Chemicals, Little Rock, AR 72200) was added to SM agar before sterilization.

Greenhouse experiments. These were carried out in an alluvial vertisol soil (30% sand, 17.5% silt, 52% clay, and 0.5% organic matter; pH 7.95) either artificially or naturally infested with *S. rolfsii*.

Artificial infestation of soil was accomplished by adding *S. rolfsii* sclerotia from a 10-day-old dried SM-agar culture. Unless otherwise stated, final sclerotial concentration was 0.1 g (dry wt)/kg soil.

The antagonistic fungus was grown on a wheat bran: sawdust: tap water mixture (3:1:4, v/v) autoclaved for 1 hr at 121 C, on 2 successive days. Autoclavable plastic bags, containing this medium, were inoculated with *T. harzianum* and incubated in illuminated chambers for 14 days at 30 C. The *T. harzianum* preparation (65% moisture), at various concentrations, was mixed with the soil before planting. Experiments with beans (*Phaseolus vulgaris* L. 'Brittle Wax') were carried out in plastic boxes (9 × 9 × 10 cm) each containing 500 g of soil. Greenhouse temperatures were kept at 23–30 C and treatments in all experiments were replicated six times in randomized blocks.

Conidial suspension was prepared from an SM-agar-grown culture of *Trichoderma*. Numbers of conidia (haemocytometer count) were adjusted to those of the wheat-bran *T. harzianum* preparations.

Field experiments. The wheat-bran preparation of *T. harzianum* inoculum was mixed with the soil to a depth of 7–10 cm with a rotary hoe. Three experiments were conducted, all in a naturally infested alluvial soil and in a six-replicate, randomized block design. In the first experiment (*T. harzianum* preparation concentration 150 g [fr wt]/m^2) beans (cultivar Tender green) were sown on 21 September 1978, in three 12-m rows. In the second experiment (*T. harzianum* inoculum preparation concentration 30 g [fr wt]/m of row), cotton (*Gossypium hirsutum* L. 'SJ-1') was sown 2 June 1978 in a 5-m row in a commercial cotton field. The percentage of diseased plants was recorded on 1 July 1978. In the third experiment (*T harzianum* preparation concentration 50 g [fr wt]/m^2), tomatoes (*Lycopersicon esculentun* Mill 'VF 317') were sown 20 April 1978, in a 10 × 3.6-m plot and the percentage of diseased plants was recorded on 20 August 1978. Analysis of

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variance was used for statistical analysis. The significance level of $P = 0.05$ was used throughout.

RESULTS

Antagonistic activity. Fungi were isolated from a field soil naturally infested with both *S. rolfsii* and *R. solani*. Of the 150 isolates tested, it was mainly the *Trichoderma* spp. that were antagonistic to *S. rolfsii*, and usually this was expressed as a strong lysis of the mycelium.

One isolate, identified as *T. harzianum* Rifai aggr. (11) was used throughout this study. Its spectrum of antagonism, tested in culture, was found to include *R. solani* and *Pythium aphanidermatum* Edson (Fitz), but not *Fusarium oxysporum* f. sp. *vasinfesctum, Sclerotium bataticola* Taub, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Dematophora necatrix* Hartig, *Verticillium dahliae* Kleb., or a *Phytophthora* sp. This isolate of *T. harzianum*, therefore, differs from the one used by Hadar et al (8), its antagonistic ability extends in addition to *R. solani, P. aphanidermatum*, and *S. rolfsii*.

The growth rate of *T. harzianum* in culture was greater than that of *S. rolfsii* at high PCNB concentrations (10–30 μg/g culture medium) or pH levels (7.0–8.5), or at low temperatures.

T. harzianum invaded *S. rolfsii* mycelium in encounters on agar culture media. A disc of mycelium from the zone where *S. rolfsii* grew was therefore removed daily at various distances from the meeting line of the two fungi, and transferred to SM agar in order to determine the presence of *T. harzianum*. *T. harzianum* progressed faster through the *S. rolfsii* mycelium at lower temperatures (<27 C) and at high PCNB concentrations or high pH levels which were more favorable for growth of *T. harzianum*.

Greenhouse studies. A wheat bran inoculum preparation of *T. harzianum* was added to *S. rolfsii*-inoculated soil sown with beans. A more efficient biological control of the pathogen was achieved at higher concentrations of the *T. harzianum* preparation and lower concentrations of the *S. rolfsii* inoculum in the soil (Fig. 1).

In another experiment, *T. harzianum* (3 g/kg) was added to soil naturally infested with pathogens capable of inducing damping-off. Thereafter, beans were sown in this soil. The proportion of plants attacked by *S. rolfsii* in the control and the *Trichoderma*-treated soils were 47 and 8%, respectively; the corresponding data for plants attacked by *R. solani* were 42 and 18%, respectively. In both cases, the differences between the treatments were statistically significant. The efficiency of *S. rolfsii* control by *T. harzianum* declined with the increase in temperatures (Fig. 2).

Biological control by *T. harzianum* depended on the type of inoculum and the time of soil inoculation (Table 1). The wheat bran preparation was much more efficient than the conidial suspension, especially in a delayed sowing. In contrast, autoclaved wheat bran, with or without autoclaved *T. harzianum*, increased the incidence of disease, caused by *S. rolfsii* in beans by 21–46%, and wheat bran, inoculated with a mixture of microorganisms from a natural soil, did not affect disease incidence at all. When the *Trichoderma* preparation was mixed with autoclaved wheat bran (1:3, w/w) its efficiency remained unimpaired. When applied to noninfested soil, the wheat bran preparation of *T. harzianum* significantly increased both the size and weight of bean plants (Table 2). Uninoculated wheat bran, however, had no significant effect on the plants.

Field studies. Beans were sown in a soil naturally infested with *S. rolfsii* and *R. solani*. Throughout the growth period, dead seedlings with typical symptoms of damping-off caused by either *S. rolfsii* or *R. solani*, were recorded. *T. harzianum* significantly delayed the progress and incidence of both diseases for 9 wk (Fig. 3). Eleven

TABLE 1. Effect of type of *Trichoderma harzianum* inoculum and time of application on biological control of a bean disease caused by *Sclerotium rolfsii*

Time of sowing[y] (days)	Diseased plants (%)		
		Inoculum type	
	Not inoculated	Conidial suspension	Wheat bran preparation
0	64 b[z]	45 c	20 e
15	40 c	28 d	19 e
30	80 a	43 c	4 f

[y]Number of days between *T. harzianum* application to soil and sowing.
[z]Numbers followed by the same letter are not significantly different ($P = 0.05$). There were six replications.

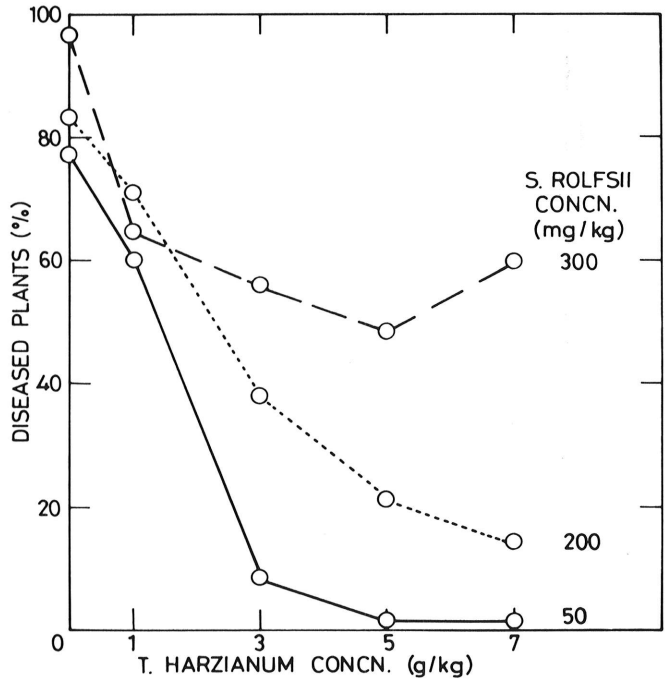

Fig. 1. Effect of inoculum concentrations of fungal antagonist *Trichoderma harzianum* and pathogen *Sclerotium rolfsii* on the incidence of bean disease caused by *S. rolfsii*, 14 days after inoculation. Each number represents the mean of at least six replications.

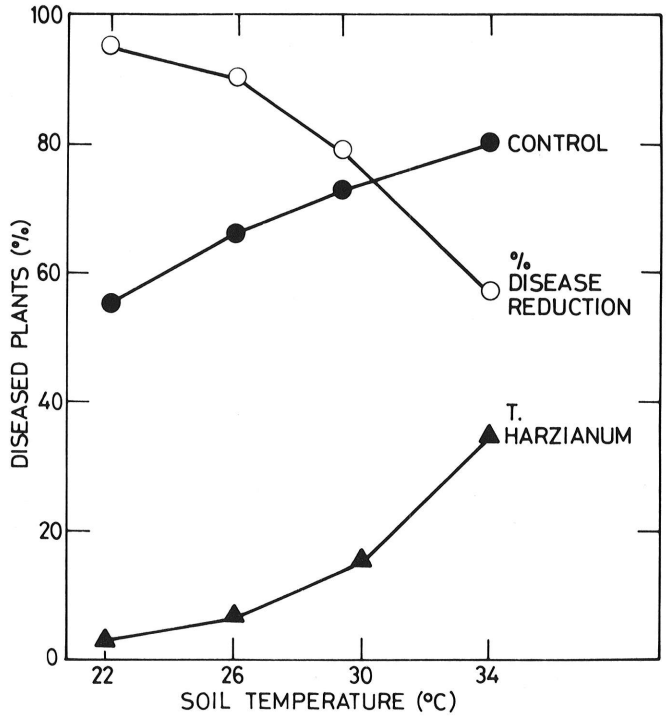

Fig. 2. Effect of soil temperature on the incidence of bean disease caused by *Sclerotium rolfsii* and on its biological control by fungal antagonist *Trichoderma harzianum*. Plants were grown in pots placed in Wisconsin-type controlled-temperature tanks, at six replications.

TABLE 2. Effect of a wheat bran preparation of *Trichoderma harzianum* (TH) or uninoculated wheat bran (WB) (both added to soil at 3 g [fr wt]/kg) on the growth of bean seedlings, 25 days after sowing

Parameter	Preparation		
	TH	WB	None
Plant height (cm)	28.1 A[a]	23.9 B	22.8 B
No. of leaves per plant	4.0 A	3.1 B	2.7 B
Wet weight (grams per plant)	2.8 A	2.1 AB	1.3 B
Dry weight (grams per plant)	0.33 A	0.28 AB	0.23 B

[a]Numbers followed by the same letter in each row are not significantly different ($P = 0.05$). There were six replications and 20 plants were sampled in each replication.

weeks after sowing, plants were removed and disease index (on a scale of 0–4, in which 0 = healthy plants and 4 = completely girdled ones) for *R. solani* was determined. Indices in the untreated and *T. harzianum*-treated plots were 1.12 and 0.87, respectively. The green pod yield of the *T. harzianum*-treated plots increased 20% (to 5,570 kg/ha) compared with the control.

A second field experiment was carried out with cotton in a soil naturally infested with *R. solani*. Twenty-nine days after planting, the proportions of diseased plants in the untreated and *T. harzianum*-treated plots were 44 and 23%, respectively.

A third field experiment was carried out with tomatoes sown in soil naturally infested with *S. rolfsii*. One hundred and twenty-three days after planting, *T. harzianum* had significantly ($P = 0.05$) reduced the percentage of diseased plants by 20%.

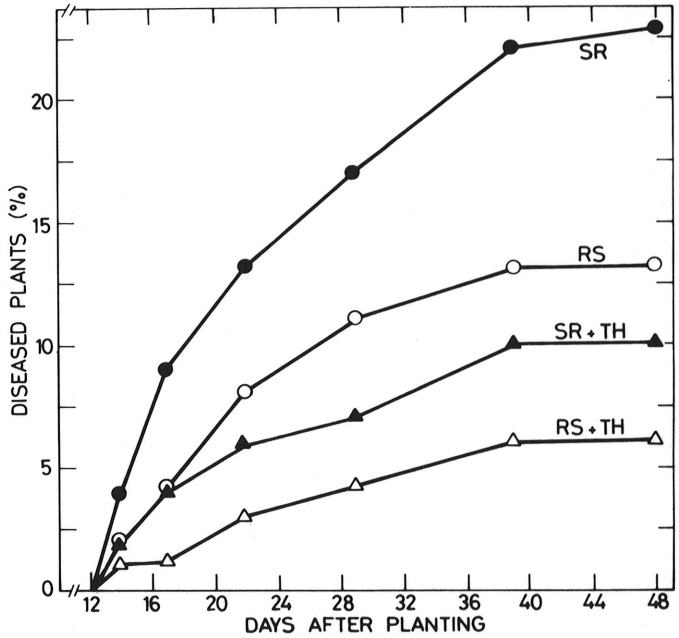

Fig. 3. Effect of fungal antagonist *Trichoderma harzianum* (TH) added in a wheat-bran inoculum preparation (1,500 kg[fr wt]/ha) on the percentage of bean plants diseased by *Sclerotium rolfsii* (SR) or *Rhizoctonia solani* (RS) in a field experiment. Data are the averages of six replications.

DISCUSSION

Trichoderma harzianum was found to be an effective biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* and *R. solani* under both greenhouse and field conditions. The *T. harzianum* isolate used in this study was obtained from a soil naturally infested with both *S. rolfsii* and *R. solani*. It was capable of directly attacking and lysing both pathogens in culture. The ability of *Trichoderma* to attack different fungi was shown by Durrell (5).

Growth rates of *T. harzianum* and *S. rolfsii* as well as antagonistic activity in culture were higher at high PCNB dosages and pH levels or low temperatures. Similarly, biological control in soil was more efficient at low temperatures (Fig. 2), which correlated with the influence of temperature on the comparative growth rates of *T. harzianum* and *S. rolfsii*. The improved antagonism under conditions adverse for the pathogen emphasizes the potential of integrating various means of control; eg, a combination of PCNB at sublethal doses and *T. harzianum* as recently shown (3,6). Similarly, Munnecke et al (9) found that *Trichoderma* is more tolerant than is *Armillaria mellea* to methyl bromide and carbon disulfide.

In greenhouse experiments, biological control of *S. rolfsii* (up to 97% reduction of disease incidence in bean seedlings) and *R. solani* (57% reduction of disease incidence in bean seedlings) was achieved in soil naturally and artifically infested with both pathogens. The efficiency of the biological control correlated positively with the rate of *T. harzianum* wheat bran preparation applied to the soil and negatively with the level of infestation by the pathogen.

As also shown elsewhere (1,6,13), efficient biological control depends, among other things, on the presence of living cells of *T. harzianum* and the presence of a food base (Table 1). Uninoculated wheat bran even increased disease incidence; this was reported by Kelley (7) who used molasses as a food base for *Phytophthora cinnamomi*.

Under field conditions, *T. harzianum* significantly increased the yield and decreased disease incidence, although less efficiently than under greenhouse conditions. Improved methods for applying *T. harzianum* in the field may produce better results. However, in this study the field results obtained from three different locations, with three plant species and two pathogens (which frequently coexist), point to the high potential and reproducibility of biological control. The *T. harzianum* preparation used actually improved plant growth (Table 2)—an additional advantage over pesticides which frequently cause some phytotoxicity.

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