Effect of *Trichoderma harzianum* Seed Treatment and *Rhizoctonia solani* Inoculum Concentration on Damping-Off of Snap Bean in Acidic Soils

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ABSTRACT

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The efficiency of biocontrol on damping-off of bean (*Phaseolus vulgaris*) induced by *Rhizoctonia solani* depends on soil reaction and inoculum concentration of the pathogen. Soil was acidified to pH 3.5 or 5.6 and infested with *R. solani* inoculum at concentrations of zero, 0.1, 1.0, 5.0, or 10.0 g/kg of soil. Seeds were coated with conidia of *Trichoderma harzianum* and planted in the acidified, infested soils. As inoculum concentration of *R. solani* increased from 0.1 g/kg of soil, there was a corresponding increase in disease incidence (proportion of plants damped off). Disease incidence was reduced by 32% in soil of pH 3.5 compared with soil of pH 5.6 and by 65% when seeds were treated with conidia of *T. harzianum* as opposed to untreated seed. Piecewise linear regression indicated that the rate of increase of damping-off was greater when the range of *R. solani* inoculum was from 0 to 0.1 than from 0.1 to 10.0 g/kg of soil. The results suggested that *T. harzianum* seed treatment of snap bean reduces incidence of *R. solani* damping-off in acidic soils.

Of notable importance among methods of biological control is the utilization of antagonistic microorganisms. Among these antagonists, members of the genus *Trichoderma* have been widely employed (4,9,10). In particular, *T. harzianum* Rifai has been demonstrated to attack many fungal hosts, especially in an acidic environment (2,6). A promising strategy of deploying this inimical microbe is to treat seed with it prior to planting. This is a direct approach that alters the microbiological environment of the developing seed, presumably to the detriment of the would-be pathogen.

This study was designed to determine the efficacy of *T. harzianum* as a seed treatment in controlling damping-off of snap beans in two acidic soils containing several inoculum concentrations of *Rhizoctonia solani.*

MATERIALS AND METHODS

Greenhouse soil (peat moss, sand, and top soil, 1:1:1 [v/v]) was sieved through a 4-mm screen into preweighed seed flats (26.5 × 54.0 × 7.0 cm) and dried at 37 C for 30 min with periodic mixing. This predried soil was pasteurized by being heated for 30 min with aerated steam (60–65 C). After cooling, 0.1 *N* hydrochloric acid (HCl) was added to the soil and allowed to equilibrate. The soil was then adjusted to pH 3.5 or 5.6 and measured by adding soil (1:5, w/v) to distilled water, pH 6.9. Soil pH was measured periodically and adjusted as necessary with Hoagland's nutrient solution of pH 3.5 or 5.6.

A virulent isolate of *R. solani* from naturally infested soil was grown on autoclaved oat seed:oat hull mixture (1:1, v/v) for 2 wk at 25 C for inoculum. Soil was artificially infested by thoroughly mixing the appropriate amount of inoculum with acidified soil. Final *R. solani* inoculum concentrations were 0, 0.1, 1.0, 5.0, and 10.0 g/kg of soil.

T. harzianum isolate MD 177 was cultured on Difco potato-dextrose agar (PDA) for 5 days at 25 C. Conidia were collected in sterile distilled water with a sterile rubber policeman and stored at 5 C. The concentration of conidia was approximately 1.0×10^6/ml. Snap bean (*Phaseolus vulgaris* L. cv. Blue Lake) seeds were immersed for 5 min in the conidial suspension (100 seeds per 500 ml) and then dried for 1–2 hr. Coated seeds were planted at 100 per flat. Flats were placed in a 24.5 ± 2 C greenhouse with a 16-hr light period of a quantum flux of 500 μE/m^2 per second. Damping-off was evaluated by stand counts 18 days after planting.

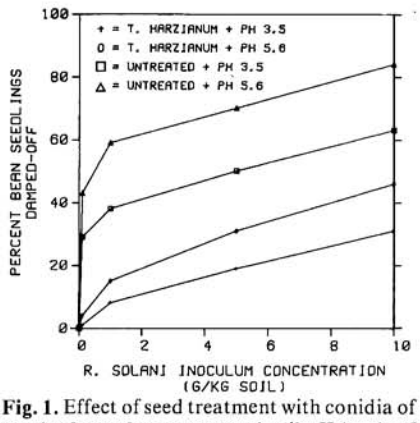

Fig. 1. Effect of seed treatment with conidia of *Trichoderma harzianum* and soil pH levels of 3.5 and 5.6 on damping-off of bean seedlings at increasing inoculum concentrations of *Rhizoctonia solani.* Each point represents the mean of four replicates in each of two experiments.

Mycelial agar plugs (4 mm diameter) from the edge of 1-wk-old cultures of *R. solani* or *T. harzianum* were each transferred to the center of five petri plates (9 cm diameter) containing PDA acidified to pH 3.5 or 5.6 with 1*N* HCl. Daily measurements of colony diameter were recorded for determination of growth rates.

The greenhouse experiment was a complete multifactorial with a randomized complete block design. Each treatment combination was replicated four times, and the entire experiment was repeated twice for a total of 160 observations.

A multifactor analysis of variance was performed on the transformed ($\log_e$ 1/1-Y) proportion of diseased plants. Aptness of the analysis of variance was tested by the Burr-Foster Q-test for homogeneity of variances and the Shapiro and Wilk W-test for normality (1). Means were separated by the Student-Newman-Keuls test (1). Piecewise linear regression was also performed on the data. Residual analysis was used to test appropriateness of the regression (8).

RESULTS

Both pH and seed treatment with *T. harzianum* conidia had significant (P = 0.05) effects on disease incidence as measured by the proportion of plants damped off (Fig. 1). There was an overall decrease in disease incidence of 32% compared with plants grown in soil of pH 5.6. When seed was treated with *T. harzianum* conidia, there was an overall decrease of 65% in disease incidence compared with untreated seed. Best control resulted when seed treated with *T. harzianum* conidia were grown in soil of pH 3.5. Neither pH nor seed treatment were phytotoxic to bean seedlings.

The disease incidence/inoculum curve for the four treatment combinations indicated that as the inoculum concentration increased, the ratio of disease to inoculum decreased (Fig. 1). Piecewise linear regression of the four curves indicated a rapid increase in the rate of disease incidence from 0 to 0.1 g of *R. solani* per kilogram of soil and a slower

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Table 1. Rates of increase of bean seedling damping-off as affected by *Rhizoctonia solani* inoculum concentration, soil pH, and seed treatment with *Trichoderma harzianum*

Treatment	*R. solani* concentration (g/kg of soil)	
	0–0.1	0.1–10.0
T. harzianum + soil, pH 3.5	0.318[z]	0.035
T. harzianum + soil, pH 5.6	0.803	0.056
Untreated + soil, pH 3.5	3.800	0.064
Untreated + soil, pH 5.6	6.594	0.124

[z]Rates are the slopes of the regression of disease incidence on inoculum concentration of *R. solani*. All regressions had coefficients of determination (R^2) greater than 0.90.

increase in the rate from 0.1 to 10.0 g of *R. solani* per kilogram of soil (Table 1). Thus, for *T. harzianum*-treated seed planted in soil of pH 3.5, the expected disease incidence is estimated to increase by 0.318 for each unit increase in inoculum up to 0.1 g of *R. solani* per kilogram of soil and by 0.035 thereafter up to 10.0 g of *R. solani* per kilogram of soil.

The logarithm of increased colony diameter regressed on time shows that *T. harzianum* grew at a faster rate on acidified PDA than did *R. solani* (Table 2). However, for each organism, the rates were not significantly different at the two pH levels.

DISCUSSION

The results indicated that soil acidity and seed treatment with conidia of *T. harzianum* each reduce incidence of bean damping-off. Moreover, by integrating treated seed with low soil pH, significant control can be achieved. The literature on this enhancement effect of pH on biocontrol by *T. harzianum* has been somewhat equivocal. Elad et al (5) reported increased growth rate and antagonistic activity of *T. harzianum* at pH levels of 7.0–8.5. Liu and Baker (7) and Chet and Baker (2) contended that *T. harzianum* induced suppressiveness to *R. solani* as soil pH was lowered. Danielson and Davey (3) stated that the optimum pH for growth in the genus *Trichoderma* ranged from pH 3.7 to 4.7. It generally seems that acidic soil conditions favor growth and antagonistic properties of *T. harzianum*.

Table 2. Comparison of growth rates of *Rhizoctonia solani* and *Trichoderma harzianum* on potato-dextrose agar (PDA) having pH of 3.5 and 5.6

Treatment	Growth rate[y] on PDA having pH	
	3.5	5.6
T. harzianum	3.16 a[z]	2.89 a
R. solani	1.33 b	1.73 b

[y]Growth rates represent the slope of regression line of colony diameter (centimeters) regressed on time (days).
[z]Values followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls test.

The proportion of infectible sites to the number of infectious propagules is low at high inoculum levels. This is evident from the shape of the curves in Figure 1, which indicate competition for susceptible infection sites. Moreover, the rate of disease increase for all four treatment combinations from 0 to 0.1 g of *R. solani* per kilogram of soil was on the order of 90–100% greater than the rate from 0.1 to 10.0 g of *R. solani*. Therefore, little competition occurred at inoculum concentrations of *R. solani* less than 0.1 g/kg of soil.

Although the shape of the disease incidence/inoculum curves was similar for all treatment combinations, the rate of disease increase was significantly reduced as soil pH was decreased or as seed were treated with conidia of *T. harzianum*. The apparent mode of action of low soil pH was to reduce the growth rate of *R. solani* while simultaneously increasing the growth rate of *T. harzianum*.

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