

# Decrease in Incidence of Rhizoctonia Preemergence Damping-Off by Use of Integrated Chemical and Biological Controls

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## ABSTRACT

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The ED<sub>50</sub> value for control of Rhizoctonia preemergence damping-off of radish by benodanil was 1.3 µg a.i./g of soil. Seed treatment with conidial suspensions of *Trichoderma harzianum* was effective in reducing incidence of damping-off. Mixing conidia of the antagonist in soil did not control the disease. Incidence of damping-off decreased in the latter treatment in subsequent weekly replantings of radish until emergence was not significantly different from that in the uninoculated control. Control of damping-off by both seed treatment with *T. harzianum* and soil mix of benodanil was additive but not interactive. Integration of chemical and biological controls provides opportunities for enhancement and greater efficiencies in suppressing damping-off induced by *Rhizoctonia solani*.

Damping-off of seeds and seedlings induced by *Rhizoctonia solani* Kühn is responsible for considerable losses in many crops. Whereas control is achieved by treatment with relatively broad-spectrum fungicides (3), such measures may establish imbalances in the microbiological community unfavorable for activity of beneficial organisms. If fumigants or heat treatments are applied, increases in available substrates may allow an increase in inoculum potential of a reintroduced pathogen—the so-called “boomerang effect” elaborated by Kreutzer (21).

Many reports (1,5-7,9-18,22,24,25) propose use of *Trichoderma* spp. for control of damping-off; however, extensive commercial application of the agents to control such diseases has not occurred. The predominant reason is that biocontrol agents have not obtained

efficiencies matching those of currently available fungicides under all environmental conditions (26). For example, Harman et al (17) reported control of pathogens inducing damping-off by seed treatment with *T. harzianum* at temperatures favorable for activity of the biocontrol agent. At lower temperatures, control was not achieved. Again, control with this antagonist has proven difficult in alkaline soils (5,22).

An integrated approach could provide a solution to these problems. Pathogen-specific fungicides could be used to decrease detrimental effects on applied biocontrol agents and/or beneficial microorganisms already present in the soil (21). Furthermore, should environmental conditions be temporarily unfavorable for activity of the biocontrol agent, an associated fungicide could provide an effective backup system. Finally, there is opportunity for additive or synergistic control by a combination of chemical-biological control strategies (27).

In this study, we investigated the efficacy of a new pathogen-specific fungicide to control Rhizoctonia damping-off and strategies for integration of this fungicide and *T. harzianum* to control the disease. The experiments were designed to determine whether such integrated control would be possible under some of the conditions unfavorable for activity of the antagonist, namely, conducive soil of alkaline pH (5,22) and use of inoculum containing large propagules of *R. solani* (28) similar to those found in the field (19).

## MATERIALS AND METHODS

Nunn sandy loam soil (27) was used in all greenhouse experiments. Soil characteristics were pH 7.3, <1% lime, conductivity 0.4 mmhos/1 cm, organic matter 1.1%, 1 mg/g N (as NO<sub>3</sub>), 9 µg/g extractable Zn, and 3.2 µg/g extractable Fe. Soil was air-dried, sieved through 4-mm-mesh screen, and stored in covered containers before use.

Inoculum of *R. solani* isolate R3, AG4 (4), was grown in a chopped potato-soil (CPS) mixture prepared according to Ko and Hora (20). Large individual particles (589-1,000 µm) were plated on 2% water agar containing chloramphenicol (250 µg/ml). Seventy-six percent of the particles germinated within 17 hr of incubation at 26 ± 1 C. For the purpose of this study, the inoculum density (ID) of *R. solani* in the CPS culture was estimated as the number of particles (589-1,000 µm) per 0.764/g of air-dried soil.

After *R. solani* inoculum was introduced, soil was mixed thoroughly, moistened to 15% (w/v) at about -0.3 bar, and placed in plastic pots. Seeding and incubation procedures were done as described by Chet and Baker (5,6), using radish (*Raphanus sativus* L. ‘Early Scarlet Globe’) seeds with 98% germinability.

The fungicide benodanil was mixed thoroughly into soil in all experiments. The antagonist, *T. harzianum* Rifai, was derived from an isolate effective in inducing soil suppressiveness to *R. solani* (6). Conidia were grown for 10 days on malt-extract agar (2 g of yeast extract, 20 g of malt extract, 20 g of agar, and 1,000 ml of distilled water), washed from the surface of the cultures in sterile distilled water, and sieved through four layers of cheesecloth. The conidial suspension was centrifuged at 2,500 g for 15 min and resuspended in sterile distilled water three times to remove residual nutrients. For seed coating, conidia were suspended in a 10% (w/v) Pelgel (The Nitragin Co., Milwaukee, WI) solution containing 3.4 × 10<sup>8</sup> conidia per milliliter; 95% or more germinated on water agar. Aliquots (1 ml) of conidial suspensions

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were applied twice to 20 g of seeds 24 hr before sowing. For direct soil treatments, conidial suspensions (in sterile water) were mixed in soil that had been air-dried, ground, and mixed thoroughly. Evaluation of the propagule density of *T. harzianum* in soil was done by plate dilution on selective medium (8) in terms of colony-forming units.

Multiple comparisons of data were performed using Fisher's least significant difference (FLSD), and regression analysis was applied (when appropriate) at  $P = 0.05$  (23). Five replicates of each treatment were used in all experiments. Experiments were repeated at least twice with similar results; however, only the results of one trial are presented.

## RESULTS

The relationship between the ID of *R. solani* (large propagules) and disease incidence (DI) of preemergence damping-off of radish (Fig. 1) was transformed by the log-probit analysis (2). Regression analysis indicated a significant linear relationship between DI and ID. The ID inducing 50% DI was 18 propagules per 100 g of soil. Thus, in subsequent experiments, the ID was usually adjusted to one propagule per gram of soil.

In Figure 2, a dosage response curve is plotted relating the concentration of benodanil in soil to DI at an ID of one propagule per gram of soil. When dosage

response was transformed by log-probit analysis, there was a significant linear relationship between DI and concentration of benodanil. The  $ED_{50}$  was  $1.3 \mu\text{g a.i./g}$  of soil.

The  $ED_{50}$  for benodanil inhibition of radial growth of *R. solani* on potato-dextrose agar was  $0.28 \mu\text{g a.i./g}$  of medium (Fig. 3A); however, for *T. harzianum*, the  $ED_{50}$  value was  $38 \mu\text{g a.i./g}$  of medium (Fig. 3B). Analysis of log-probit transformations for dosage response indicated a significant linear relationship between concentration of benodanil and radial growth.

Applications of conidial suspensions of *T. harzianum* to soil infested with one propagule of *R. solani* per gram of soil did not reduce DI (Table 1). However, seed treatment significantly reduced incidence of damping-off compared with the inoculated control. Seed treatment together with  $0.5 \mu\text{g a.i.}$  of benodanil per gram of soil provided emergence values not significantly different from uninoculated controls, whereas the same level of application of benodanil alone gave only partial control.

To test the relatively long-term effect of infestation by *T. harzianum*, the antagonist was mixed into soil infested with *R. solani* (one propagule per gram of soil), and radishes were planted repeatedly at weekly intervals as described previously

(5,6,18,22,28). In the first crop, there was no reduction in disease by such treatment (Fig. 4A), which confirmed results of the previous experiment (Fig. 3). In successive plantings, however, disease incidence decreased to 22% in soil infested with *T. harzianum* in contrast with 100% damping-off in the inoculated control after four weekly plantings. Population densities of *Trichoderma* spp. remained stable during the experiment (Fig. 4B).

To test whether benodanil influenced the activity of *T. harzianum* and/or disease incidence during monoculture, the same experimental design was used, except benodanil ( $0.5 \mu\text{g a.i./g}$  of soil) was used alone and in combination with *T. harzianum*. Again, the soil was conducive to damping-off in the first crop of radishes when *T. harzianum* alone was mixed into soil, but suppressiveness developed later during the 5 wk of monoculture comparable to control with benodanil alone or in combination with *T. harzianum* (Fig. 4C). Benodanil consistently controlled damping-off during monoculture and did not affect the population density of *Trichoderma* spp. during the experiment (Fig. 4D).

Radish seeds were treated with preparations containing  $0, 10^7, 10^8,$  or  $10^9$  conidia per milliliter of *T. harzianum*. Seeds were planted in *R. solani*-infested soil (one propagule per gram) amended with benodanil at 0, 0.4, 1.6, or  $3.2 \mu\text{g a.i./g}$ , which resulted in a factorial treatment combination. Mean damping-

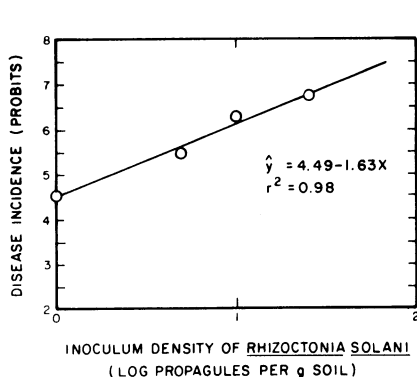


Fig. 1. Inoculum density-disease relationships for preemergence damping-off of radish induced by *Rhizoctonia solani*.

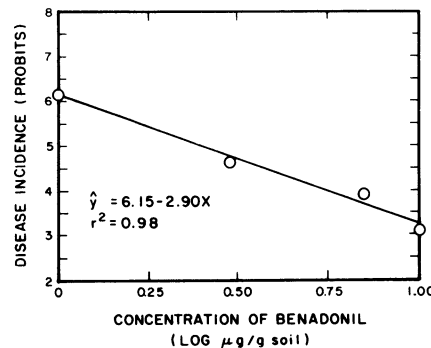


Fig. 2. Dosage response relating the concentration of benodanil in soil to incidence of preemergence damping-off of radish induced by *Rhizoctonia solani*.

Table 1. Mean percent emergence of radishes in soil infested with one propagule of *Rhizoctonia solani* per gram of soil and treated with benodanil and/or *Trichoderma harzianum*<sup>a</sup> mixed in the soil

Treatment	Benodanil ( $\mu\text{g a.i./g}$ of soil) <sup>b</sup>				
	0 (%)	0.5 (%)	1.5 (%)	3.5 (%)	5 (%)
<i>T. harzianum</i>					
Mixed in soil <sup>c</sup>	20 y	52 y	92 z	98 z	100 z
On seed <sup>d</sup>	74 z	90 z	92 z	94 z	100 z
No treatment with antagonist	16 y	44 y	88 z	98 z	98 z

<sup>a</sup> Figures are mean emergence of 10 seeds in each of five replicates per treatment. Emergence in uninoculated control was 90–94%.

<sup>b</sup> Numbers of each column followed by the same letter are not significantly different ( $P = 0.05$ ) by use of FLSD analysis.

<sup>c</sup> *T. harzianum* applied at  $2.5 \times 10^6$  conidia per gram of soil.

<sup>d</sup> Seeds were dipped in a conidial suspension with  $3.4 \times 10^8$  conidia per milliliter of Pelgel solution.

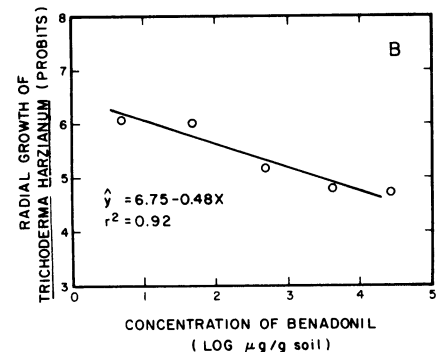
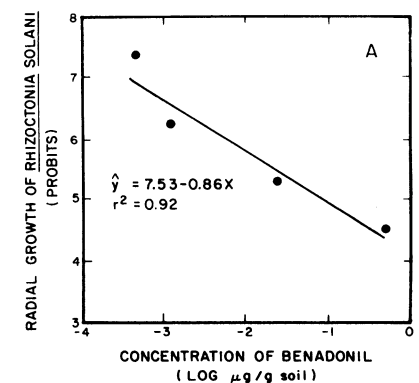


Fig. 3. Radial growth of (A) *Rhizoctonia solani* and (B) *Trichoderma harzianum* on potato-dextrose agar containing various concentrations of benodanil.

off incidence after 1 wk is given in Table 2. An analysis of variance was performed on the data and the treatment sum of squares partitioned into that for *T. harzianum*, benodanil, and their interactions. Significant *F* ratios for *P* = 0.05 were obtained for the *T. harzianum* and benodanil treatments but not for the benodanil × *T. harzianum* interaction.

## DISCUSSION

For integration of chemical and biological control of plant pathogens, the systems must be compatible. In this study, benodanil was not fungitoxic to *T. harzianum* (Fig. 2B) *in vitro* at rates far below those inhibitory to *R. solani* (Fig. 2A). The relatively low rate of benodanil inhibitory to radial growth of *R. solani* *in vitro* ( $ED_{50}$  = 0.28  $\mu\text{g a.i./g}$  of medium) was reflected in soil as measured by DI ( $ED_{50}$  = 1.3  $\mu\text{g a.i./g}$  of soil). Thus the two systems are potentially compatible.

In past research, it was difficult to induce suppressiveness to *R. solani* in alkaline soils by amendments of conidia of *T. harzianum* (5,22). This was especially true if large propagules of the pathogen were used as inoculum. These propagules are more typical of those found in field soil (19). Two strategies were used to solve these difficulties. The first was to apply the biological control agent to seed coats. This provided a barrier against the pathogen and resulted in significant control of damping-off compared with inoculated controls (Table 1). The second strategy involved implementation of monoculture in which repeated weekly plantings of radish induced suppressiveness in soil containing high population densities of *T. harzianum* (Fig. 4A,C). During these experiments, there was little change in the population density of *Trichoderma* spp. in the alkaline soil (Fig. 4B,D). This reflected previous results (22) and suggested that propagules of *R. solani* were inactivated during monoculture.

There was no evidence that benodanil enhanced the activity of *Trichoderma* spp. during monoculture (Fig. 4C,D); however, a possible interaction between seed treatment with *T. harzianum* and benodanil in soil was tested statistically by factorial design (Table 2). Analysis of variance indicated a nonsignificant *T. harzianum* × benodanil interaction, which demonstrated that disease control by *T. harzianum* and benodanil was additive but not synergistic.

Integrations of biological with compatible chemical controls, as reported here, can lead to enhanced efficiency in integrated pest management. These studies emphasize, however, the importance of strategic application of biocontrol agents (seed treatment in this case) to compensate for environmental conditions (e.g., high soil pH) and/or inoculum status (e.g., large propagules) unfavorable for efficient activity of such agents.

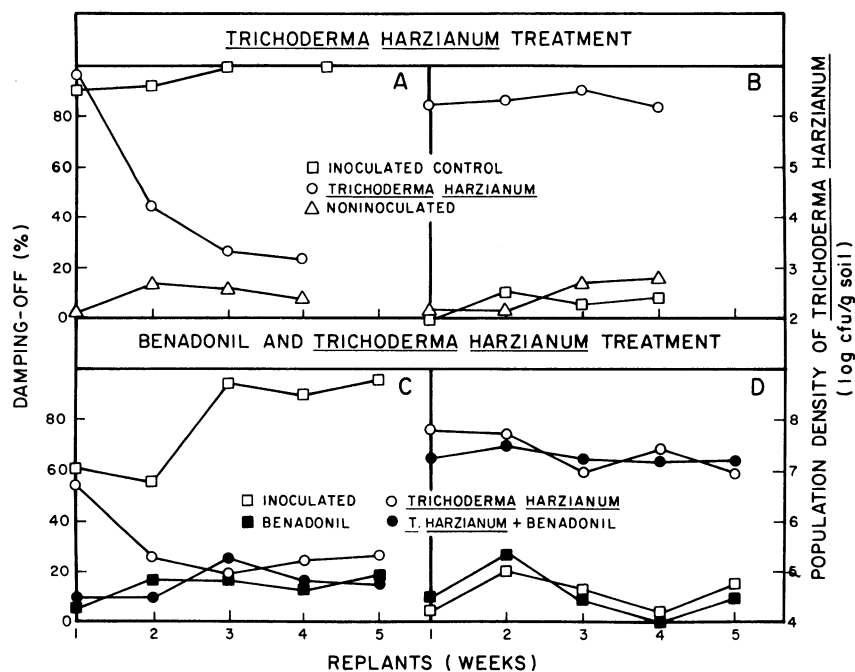


Fig. 4. Effect of *Trichoderma harzianum* and/or benodanil in soil infested with *Rhizoctonia solani* (one propagule per gram of soil) on (A,C) emergence of radish planted at weekly intervals and (B,D) the population density of *Trichoderma* spp. during this monoculture.

Table 2. Mean percent emergence of radishes in soil infested with one propagule of *Rhizoctonia solani* per gram of soil when benodanil was mixed into soil and *Trichoderma harzianum* seed treatment was used at various levels<sup>a</sup>

Benodanil ( $\mu\text{g a.i./g}$ of soil)	<i>T. harzianum</i> (conidia per milliliter of seed treatment suspension)			
	0 (%)	$10^7$ (%)	$10^8$ (%)	$10^9$ (%)
0	34	68	52	66
0.4	62	68	66	72
1.6	70	84	84	90
3.2	88	84	96	90

<sup>a</sup> Figures are mean emergence of 10 seeds in each of five replicates per treatment. Significant *F* ratios for *P* = 0.05 were obtained for the *T. harzianum* and benodanil treatments but not for the benodanil × *T. harzianum* interaction.

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