Biological Control of Turfgrass Diseases with a Rhizosphere Competent Strain of *Trichoderma harzianum*

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ABSTRACT

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Trichoderma harzianum strain 1295-22 is a commercially available biocontrol agent that is strongly rhizosphere competent and able to control several plant pathogenic fungi. Two formulations were tested for their ability to control brown patch caused by *Rhizoctonia solani*, dollar spot caused by *Sclerotinia homoeocarpa*, and Pythium root rot and blight caused by *Pythium graminicola*. In growth chamber trials, soils planted with creeping bentgrass were amended with the granular formulation to give 10^6 cfu/g. All three diseases were significantly reduced by this treatment. Populations of *Pythium* spp. were suppressed under laboratory conditions by strain 1295-22. In field trials conducted over 4 years, strain 1295-22 reduced dollar spot severity relative to untreated plots. Monthly applications of granular or peat-based formulations of *T. harzianum* 1295-22 reduced initial disease severity by as much as 71% and delayed disease development by up to 30 days. The persistence of strain 1295-22 in soil core samples from treated creeping bentgrass greens was also measured. After application of strain 1295-22, soil populations of *Trichoderma* spp. increased 100-fold relative to populations in untreated plots. Population levels remained at least an order of magnitude greater in treated than in untreated plots. Even after overwintering, population levels remained at or above 10^5 cfu/g of dry weight of the sample.

Additional keywords: IPM, soilborne pathogen, topdressing

Dollar spot caused by *Sclerotinia homoeocarpa* F.T. Bennett, Pythium root rot caused by *Pythium graminicola* Subramanian, and brown patch caused by *Rhizoctonia solani* Kühn are among the most common diseases on golf course turf in the United States (24). Creeping bentgrass (*Agrostis palustris* Huds.) is highly susceptible to these pathogens. Typically, control of these diseases on golf course turf is achieved by repeated applications of fungicides (27). However, repeated applications of broad spectrum or persistent chemicals may result in soil contamination, fungicide resistance, or harmful effects to nontarget organisms (23,29). Thus, the development of nontoxic alternatives to chemical fungicides for control of these diseases would be useful in reducing undesirable environmental effects and public exposure to pesticides.

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Biological control is an attractive alternative strategy for the control of turf diseases. Recent studies indicate that individual microbial antagonists are capable of reducing the severity of turfgrass diseases (24). For example, in field studies, *Typhula phacorrhiza* (Reichard:Fr.) Fr. reduced the severity of gray snow mold incited by *T. incarnata* Fr. and *T. ishikariensis* Imai (5). Binucleate *Rhizoctonia* spp. reduced brown patch (4,36), and strains of the bacterium *Enterobacter cloacae* (Jordan) Hormaeche & Edwards have been effective in suppressing dollar spot (26). Similarly, isolates of *Gliocladium virens* J.H. Miller, J.E. Giddens, & A.A. Foster and *Fusarium heterosporum* Nees:Fr. have been reported to suppress dollar spot on bermudagrass (15,21). To our knowledge, however, none of these agents are commercially available.

Although there are a limited number of microbial-based fungicides commercially available, only *Trichoderma harzianum* Rifai strain 1295-22 has been registered with the U.S. Environmental Protection Agency (EPA registration no. 68539-3) for turfgrass disease control. Strain 1295-22 is a highly rhizosphere-competent biocontrol microbe (30) produced by protoplast fusion between two strains of *T. harzianum* (20,33). Much of our knowledge of the disease control efficacy of this agent comes from studies of diseases on a wide range of crops including turf. However, the turfgrass ecosystem is quite different from crop agroecosystems. Therefore, the purpose of this study was to determine whether strain 1295-22 of *T. harzianum* used as a granular formulation would become established in turfgrass soils and effectively suppress disease development of dollar spot, Pythium root rot and blight, and brown patch of creeping bentgrass. A preliminary report from this study has been published (22).

MATERIALS AND METHODS

Fungal isolates and media. The isolate of *S. homoeocarpa* (DS-21) used in this study was isolated from creeping bentgrass lesions by plating affected foliage on water agar (WA). Pure cultures were then maintained on Bacto potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI). *P. graminicola* (strain PRR-8) used in this study was isolated originally from turfgrass roots (25) and maintained on corn meal agar (Difco). *R. solani* (strain RS-2) was obtained from soil (20) and maintained on PDA. Strain 1295-22 (ATCC 20847) of *T. harzianum* was used in all experiments and maintained on PDA.

In all laboratory and growth chamber experiments, soil was infested with inoculum of *S. homoeocarpa*, *P. graminicola*, or *R. solani* to achieve the desired disease incidence. Inoculum was prepared by growing *S. homoeocarpa* or *R. solani* on an autoclaved mixture consisting of 20 g of wheat grains and 30 ml of water in petri dishes. When this substrate was completely colonized (i.e., the entire contents of the plate fused into a single mass with hyphae), the petri dish lids were removed, and the cultures were dried in a sterile air stream in a laminar flow transfer hood. The dried mixture was ground in a Waring Blender and kept at 4°C until use (20). *P. graminicola* was grown in 10% V8 juice broth for 28 days, then comminuted with a Waring blender and mixed into sandy loam soil (pH 6.4). Preliminary experiments were conducted to determine minimum inoculum levels of each pathogen that permitted about 50% seedling survival after 7 days. These dosages were 0.5 g and 0.04 g, respectively, of autoclaved wheat preparations of *S. homoeocarpa* and *R. solani* inoculum per 100 g of soil. The LD_{50} level of *P. graminicola* was 2 cfu/g of soil as determined by dilution plating on *Pythium* selective medium (PSM). PSM

contained (per liter): cornmeal agar 17 g, Igepal 630 (Alltech Assoc. Inc., Deerfield, IL) 0.1 ml, pimaricin (2.5% aqueous suspension, Sigma Chemical Co., St. Louis, MO) 10 mg, rifampicin (Sigma) 10 mg, ampicillin (Sigma) 250 mg, benomyl (50% WP, DuPont de Nemours & Co., Wilmington, DE) 20 mg, and rose bengal (Eastman Kodak Co., Rochester, NY) 50 mg.

Preparation of 1295-22 formulations. In most experiments, a granular formulation was prepared on montmorillonite clay granules that contained *T. harzianum* at about 5×10^8 cfu/g. This material is manufactured by TGT Inc., Geneva, NY, and is available for sale by TGT as T-22G and by the Wilbur-Ellis Co. (Fresno, CA) as BioTrek T-22G. However, in experiments in 1992, *T. harzianum* was cultured on a 1:1 (wt/wt) wheat bran–peat mixture for 2 weeks (29). After drying, this peat preparation also contained about 5×10^8 cfu/g.

Controlled environment evaluations of *T. harzianum* 1295-22. In growth chamber tests, the granular formulation of *T. harzianum* 1295-22 was mixed with sandy loam soil (pH 6.3) infested previously with *S. homoeocarpa*, *P. graminicola*, or *R. solani* as described above. In most experiments, the populations of strain 1295-22 in the granular formulation were determined on *Trichoderma* selective media (TSM) (32) and then adjusted to about 10^5, 10^6, and 10^7 cfu/g of soil. In some experiments, the conidial numbers in granules were counted directly using a Petroff-Hausser and Helber counting chamber. Granules of strain 1295-22 were then diluted to give the expected populations of conidia in the infested soil. Two hundred grams of the infested soil mixture were placed in $10 \times 10 \times 5$ cm plastic boxes and incubated 24 h. Creeping bentgrass was then sown over the surface of the soil by sprinkling seeds with the aid of a salt shaker. Each treatment was distributed among three replicate boxes. Each soil box was moistened with 36 ml of distilled water at the beginning of each experiment, and additional water was added as needed. Experiments were conducted at 23 to 25°C with a 12-h photoperiod provided by cool-white fluorescent lights. Disease severity was assessed over time by determining the percentage of the turf area occupied by symptomatic plants. All of the experiments described above were repeated at least twice, and the data presented are from a representative test.

Population dynamics of *T. harzianum*. The total population of *T. harzianum* in soil (both introduced and native) from field plots was enumerated on TSM. Plate counts were obtained by pooling five soil cores (approximately 1×4 cm) from each replicate of each treatment. Sample cores contained leaves, roots, rhizomes, thatch, and soil. A 10-g subsample from the core sample from each replicate was comminuted in 100 ml of distilled water in a Waring blender for 1 min. A dilution series was prepared from each treatment replicate and plated on TSM. The diluted core samples were placed in an oven (105°C for 24 h) for dry weight determinations. After incubation of the dilution plates at room temperature for 5 to 7 days, the colonies were enumerated and population levels were expressed as colony forming units per gram of dry weight. Observation of colonies under a dissecting microscope was sometimes required to determine whether colonies were *Trichoderma* or *Gliocladium* spp.

Field studies. Three field tests were performed during 1990, 1992 to 1993, and 1994 to evaluate 1295-22 for the control of dollar spot. Field plots (0.91×0.91 m^2) were established on creeping bentgrass (*Agrostis palustris* Huds.) putting greens naturally infested with *S. homoeocarpa* at the Cornell University turfgrass field research facility in Ithaca, NY. Dollar spot severity of all plots was rated as the percent area of the plot that was diseased. Dollar spot incidence was rated on a scale of 0 to 10 for which 0 = no disease, 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70, 8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100 dollar spots per plot. Typical patch diameters were 4 cm. Granules were applied on 18 July and 16 August 1990 to all plots (soil pH 6.4) at 3.2 g (T-22a) or 6.4 g (T-22b) per m^2. *T. harzianum* granules were mixed with 200 cm^3 of sand, distributed as uniformly as possible over the plot area, and lightly rubbed by hand into the turf canopy. Because dollar spot symptoms did not appear until August, all plots were evaluated for dollar spot severity 2 months after the initial application. The fungicide propiconazole (173 mg a.i./m^2) was applied on 18 July and 16 August 1990 as a standard.

On 28 April 1992, biocontrol treatments were again applied as described above, except that the peat–wheat bran formulation was used. Plot areas (soil pH 8.0) for the 1992 study did not overlap those from the 1990 study. Treatments were applied at monthly intervals thereafter, and the fungicide propiconazole was applied only at the beginning of the season (28 April) in 1992.

To assess the survival of 1295-22, a separate study on a second putting green (soil pH 6.4) was treated as described above. Granules (6.73 g/m^2) of 1295-22 were applied on 26 August and 26 September 1992. The same plots were not treated again until 24 May 1993. Thereafter, treatments were applied at monthly intervals, and disease severity was rated monthly as described above. In 1994, a separate putting green (soil pH 6.4) was treated as described in 1993. However, the granular formulation of 1295-22 was applied only on 1 June and 1 July 1994, and the fungicide propiconazole was applied monthly.

Experimental design and data analysis. All field experiments were established as a randomized complete block design with four replicates in 1990 and five replicates in 1992 to 1993, and 1994. All data were analyzed using analysis of variance, and means were separated using LSD tests at alpha values of 0.05 and 0.1 (SAS Institute, Cary, NC). The data in 1993 and 1994 also were analyzed using the general linear model programs to compare time durations required for development of 50% of maximum disease level.

RESULTS

In growth chamber experiments, *T. harzianum* 1295-22 significantly reduced the severity of foliar symptoms of dollar spot, brown patch, and Pythium root rot disease at 14 days after planting only when the population level of this strain was greater than 10^5 cfu/g of soil. *T. harzianum* at 10^7 cfu/g provided a significantly greater level of dollar spot control at 14 days than did lower population levels (Table 1).

In a separate time-course experiment, populations of *T. harzianum* remained at approximately 5×10^5 cfu/g of soil throughout a 45-day experimental period (Fig. 1A). In the absence of the biocontrol agent, *Pythium* populations and disease increased rapidly. However, when *T. harzianum* 1295-22 was present, the increase, both in *Pythium* populations and in disease severity, was delayed significantly (Fig. 1B and C).

In 1992 field evaluations, strain 1295-22 of *T. harzianum* significantly reduced dollar spot severity during initial stages of

Table 1. Suppression of dollar spot, brown patch, and Pythium root rot on creeping bentgrass with *Trichoderma harzianum* 1295-22 in growth chamber experiments

Inoculum density of 1295-22 (cfu/g soil)	Disease severity[x] (%)		
	Dollar spot	Brown patch	Pythium root rot[y]
0	85 a[z]	86.7 a	83 a
10^5	86 a	90.0 a	80 a
10^6	42 b	53.3 b	13 b
10^7	27 c	43.3 b	11 b
LSD ($P = 0.05$)	11	20.6	6

[x] Data were recorded 14 days after planting.
[y] Soil was amended with soil containing *Pythium graminicola* at 2 cfu/g estimated by a modified Pythium selective media.
[z] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

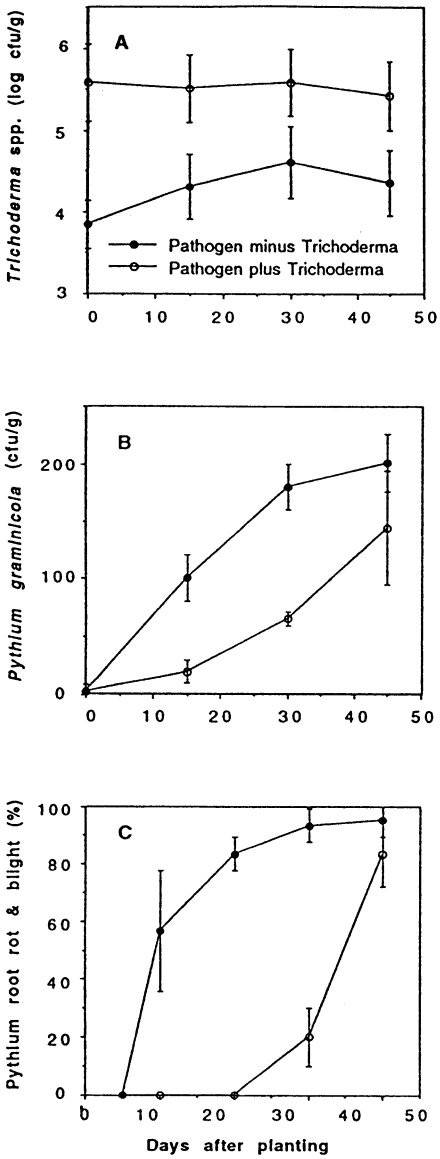

Fig. 1. Population dynamics of **(A)** *Trichoderma* spp., **(B)** *Pythium graminicola*, and **(C)** suppression of Pythium root rot and blight after treating with granules of *Trichoderma harzianum* strain 1295-22 in creeping bentgrass under controlled environments. Error bars represent standard deviations of means.

disease development. Similarly, dollar spot severity was also significantly (P = 0.1) reduced in 1990, 1993, and 1994. In 1990, this strain was not as effective as propiconazole in reducing dollar spot severity 20 days after application but was more effective than the fungicide 60 days after application (Table 2). At least 38% disease control was achieved in every field experiment. In 1993 and 1994, monthly applications of *T. harzianum* provided up to 71 and 64% disease control, respectively (Table 2).

When dollar spot disease progress in plots treated with *T. harzianum* 1295-22 was compared with that in untreated plots, there was a significant treatment effect (P < 0.01) and time effect (P < 0.001), particularly during August to October in 1993. However, the rate of disease progression in plots treated with strain 1295-22 did not differ from that in untreated plots. A 33-day delay in disease development remained constant in 1295-22 treated plots over the duration of this experiment with monthly topdressing applications (Fig. 2A). Similarly, in 1994, disease development was also delayed by about 17 days with granular applications, even though granules of strain 1295-22 were applied only in June and July. There was a significant treatment (P < 0.01) and time (P < 0.002) effect (Fig. 2B).

Introduction of strain 1295-22 as peat–bran or granular formulations was effective in establishing high populations in turfgrass soil despite a relatively high soil pH (8.0 and 6.4 in 1992 and 1993, respectively) (Fig. 3A and B). For example, in 1992, topdressing applications of 1295-22 established initial recoverable populations of 3 to 5 × 10^5 cfu/g dry weight sample. This population level increased significantly during a 3-month period in alkaline (pH 8.0) soil (Fig. 3A). Moreover, in 1993, 1295-22 survived and persisted at high levels (3 to 5 × 10^5) for at least 8 months, including the entire winter (from 26 September 1992 to 24 May 1993), in a soil of pH 6.4 (Fig. 3B). After 24 May 1993, populations stabilized at approximately 3 to 5 × 10^5 cfu/g dry weight sample even with continued monthly application (Fig. 3B).

DISCUSSION

Control of soilborne diseases (20) and increased plant yields by biocontrol agents are well-documented (1,8). Effective agents for the control of root-infecting pathogens may be introduced either on seeds or as a soil treatment if the agent in question is rhizosphere competent, i.e., able to effectively colonize roots (8,16). Either bacterial or fungal biocontrol agents may be rhizosphere competent (7), but it has often been considered that long-term efficacy of individual biocontrol strains is unlikely. For example, Deacon (11) states, "Both evidence and theory suggest that progress (in control of soil-borne pathogens) has been limited by the use of single clonal strains of biocontrol agents, which inevitably are ecologically constrained in competitive soil environments." However, Nelson and Craft (26) have reported that *E. cloacae* remained at levels greater than 10^4 cells per gram of soil for up to 13 weeks and was detectable in the spring of the next year. Although only a few rhizosphere competent strains of *T. harzianum* are known (1,17), our data demonstrate that *T. harzianum* strain 1295-22 can survive at least 8 months and remain at levels of 10^5 to 10^6 cfu/g dry weight sample following applications of the biocontrol agent to soil at either pH 8.0 or pH 6.4. These results confirm that strain 1295-22 is a strongly rhizosphere competent biocontrol agent and that it is able to survive at effective levels on creeping bentgrass roots even over cold winter months in New York.

Chet and Baker (6) reported that the minimal effective dosage of *Trichoderma* spp. was around 1 × 10^6 cfu/g of soil to control damping off of radish caused by *R. solani*. Our data indicate that population levels of 3 to 5 × 10^5 cfu/g of soil are suf-

Table 2. Control of dollar spot on a creeping bentgrass putting green with *Trichoderma harzianum* strain1295-22

	1990				1992		1993		1994	
Treatments	Rating1[v]	Control (%)	Rating2[v]	Control[w] (%)	Rating[v]	Control (%)	% area per plot[x]	Control (%)	% area per plot[x]	Control (%)
Untreated	1.0	...	2.3	...	1.6	...	21.2	...	0.22	...
Sand	1.0	0	2.0	11	NT[y]	...	NT	...	NT	...
T-22A[z]	0.5	50	2.7	0	1.0	38	NT	...	NT	...
T-22B[z]	0.5	50	1.3	44	1.0	38	6	71	0.08	64
Propiconazole	0.0	100	2.0	11	1.2	25	NT	...	0.00	100
LSD (P = 0.05)	0.5	1.2	0.5	16.2	0.14					
(P = 0.1)	0.4	0.9	0.4	13.3	0.12					

[v] Disease incidence rating scale: 1 = 1 to 10 infection centers per plot area. Ratings 1 and 2, 1990, were measured 20 and 60 days after applications respectively; ratings in 1992, 1993, and 1994 were measured at first dollar spot appearance. In 1992, strain 1295-22 was treated at monthly intervals that started from 28 April 1992; propiconazole was treated one time at the beginning in 1992. In 1993, these treatments were treated monthly with *T. harzianum* strain 1295-22. In 1994, the plots were treated by *Trichoderma* granules from 1 June 1994.

[w] Based on a percentage of the disease severity in plots not treated with *T. harzianum* strain 1295-22.

[x] Disease: rating at first month after dollar spot appearance.

[y] NT = not measured.

[z] T-22 represented *T. harzianum* strain 1295-22; A and B were dosages of granules that were applied at 3.2 and 6.4 g/m^2, respectively.

ficient to reduce dollar spot, brown patch, and Pythium root rot disease severity in growth chamber tests. Similarly, strain 1295-22 populations of about 5×10^5 cfu/g of soil were required to reduce Pythium root rot and blight. In field trials, populations of strain 1295-22 with granular applications remained at or above 3 to 5×10^5 cfu/g of soil, and these levels reduced the initial severity of dollar spot disease relative to untreated plots. The results demonstrated that strain 1295-22 can survive in association with creeping bentgrass plants and remained at the minimal effective level following granular applications.

The results reported here are consistent with the known rhizosphere competence of *T. harzianum* strain 1295-22. Harman et al. (20) applied this strain as a seed treatment on peas in a field trial. At bloom, pea roots were sampled and more than 95% of root segments were colonized by this strain. The ability of this strain to colonize either corn or cotton roots was compared to that of two other strains, and strain 1295-22 was most effective (30). Datnoff et al. (10) used 1295-22 as an amendment in greenhouse potting soil for production of tomato seedlings for transplanting. Plants produced in the presence of *T. harzianum* developed less Fusarium crown and root rot than control plants, suggesting that rhizosphere competence permitted it to persist on tomato roots at effective levels. Similarly, treatment of bean seeds with 1295-22 increased yields and decreased root rot of dry beans (34), which also indicates effective levels of root colonization.

The ability of strain 1295-22 to survive over the winter has not been reported previously. Earlier studies with annual crops demonstrated that the organism did not overwinter at effective levels in soil (T. E. Stasz and G. E. Harman, *unpublished*), but living roots were not present over the winter in those studies. More recently, studies on rye grain cover crops indicate that strain 1295-22 overwinters on the roots at populations of about 10^5 cfu/g but not in the surrounding soil. Levels of strain 1295-22 on overwintered, colonized rye roots were sufficient to colonize a subsequent corn crop and increase plant yields (3).

Populations of strain 1295-22 in soils of pH 8 were as high as those in soil of pH 6.4. However, the activity and growth of *Trichoderma* spp. are favored by acidic conditions (1,35). This apparent contradiction may arise from several factors. Possibly, higher soil pH may increase the period in which hyphae are present before sporulation, and thus more reproductive units may occur if hyphae form conidia and/or chlamydospores (1). Further, the activities of competitive microbes may be different in acidic and alkaline soils. Generally, lower fungal populations should be present in alkaline than in more acidic soils, and these fungi may be primary competitors of *T. harzianum*. Finally, the pH at the root surface may be lower than that in the surrounding soil because the roots release H^+ to form H_2CO_3 to overcome adverse physiochemical conditions (12,13).

Most biological control strategies are directed at suppressing initial disease induced by soilborne pathogens (14). Our results indicate that treatments with granular or peat-based formulations of *T. harzianum* strain 1295-22 significantly reduced dollar spot severity and delayed disease development. Similarly, strain 1295-22 inhibited the increase of *Pythium* populations and disease development for 3 to 4 weeks. These results suggest that granules of 1295-22 reduced pathogen inoculum in the soil. However, subsequent disease development may occur, and so other methods of application of this strain should be studied. Alternatively, compatible chemical fungicides may be required for complete disease control.

Mechanisms by which *T. harzianum* strain 1295-22 suppresses dollar spot disease development are not fully understood. Cell-wall-degrading enzymes and mycoparasitism play a role in the control of some diseases (8,19,23) and have been

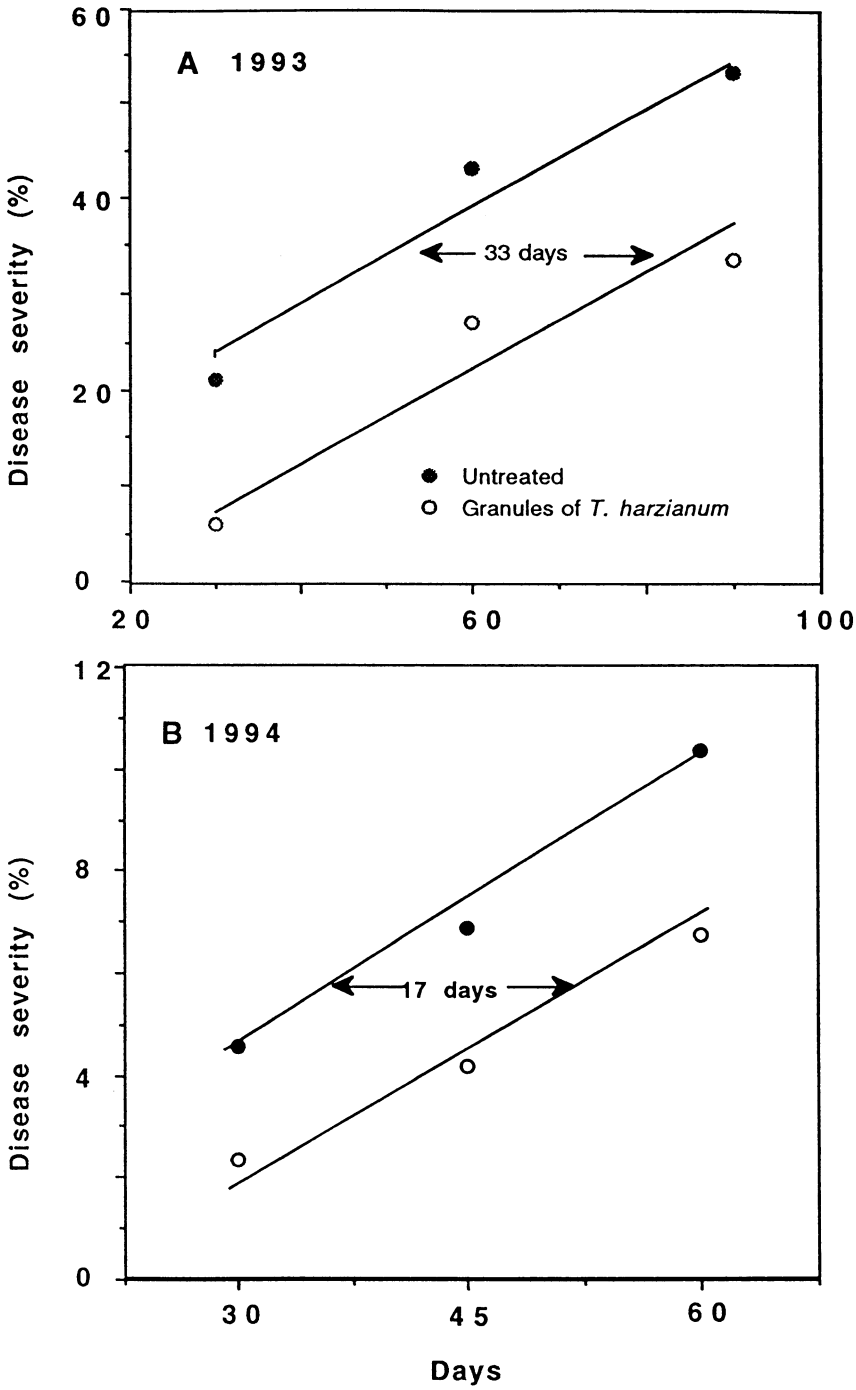

Fig. 2. Dollar spot disease progress in untreated and *Trichoderma harzianum* strain 1295-22-treated plots in **(A)** 1993 and **(B)** 1994. Lines were fitted using the general linear model procedure (SAS Institute, Cary, NC). Treatment and time effects were significant ($P < 0.01$) in all cases.

observed with this strain (G. E. Harman, *unpublished*). However, other indirect interactions with the pathogen, such as competition, may also occur (28). For example, seed treatment of corn with 1295-22 resulted in significant root colonization and a 25% reduction in electrolyte leakage from the root (T. Bjorkman, Cornell University, *unpublished*). Reduction in tissue leakage may be an important mechanism for limiting plant diseases by reducing ectotrophic pathogen growth (8), especially if the biocontrol agent is able to intercept metabolites critical for triggering germination of pathogen propagules in soil (19).

There has been a significant increase over the past few years in the amount of fungicides used in turfgrass management (9). Pesticide marketing figures for 1989 show that more fungicides were sold in the United States for use on turfgrass than on any other commodity, including the various food crops (2). More recent figures confirm this trend (L. D. Houseworth, 1992, *personal communication*). Although fungicides are convenient and available for the control of most turfgrass diseases, excessive use of broad spectrum or persistent chemicals may result in soil contamination, fungicide resistance, or other harmful effects (23,31). Thus, effective, nontoxic, biologically based fungicides will be a useful alternative if they are reliable and commercially available. Strain 1295-22 of *T. harzianum* has been registered with the U.S. Environmental Protection Agency for a range of crops including turfgrass and has been tested widely against crop disease caused by *Pythium* spp., *R. solani*, *Botrytis cinerea*, and *Sclerotium* spp. (18,20). Our data show that this strain provides biological control for dollar spot and suppresses other diseases in controlled environments on creeping bentgrass. Topdressing of putting greens is a common cultural practice used to maintain a smooth, firm surface, and granular fertilizers and other materials are regularly applied to golf courses (26,27). Therefore, application of *T. harzianum* as a granular topdressing formulation is compatible with existing practices of turf management.

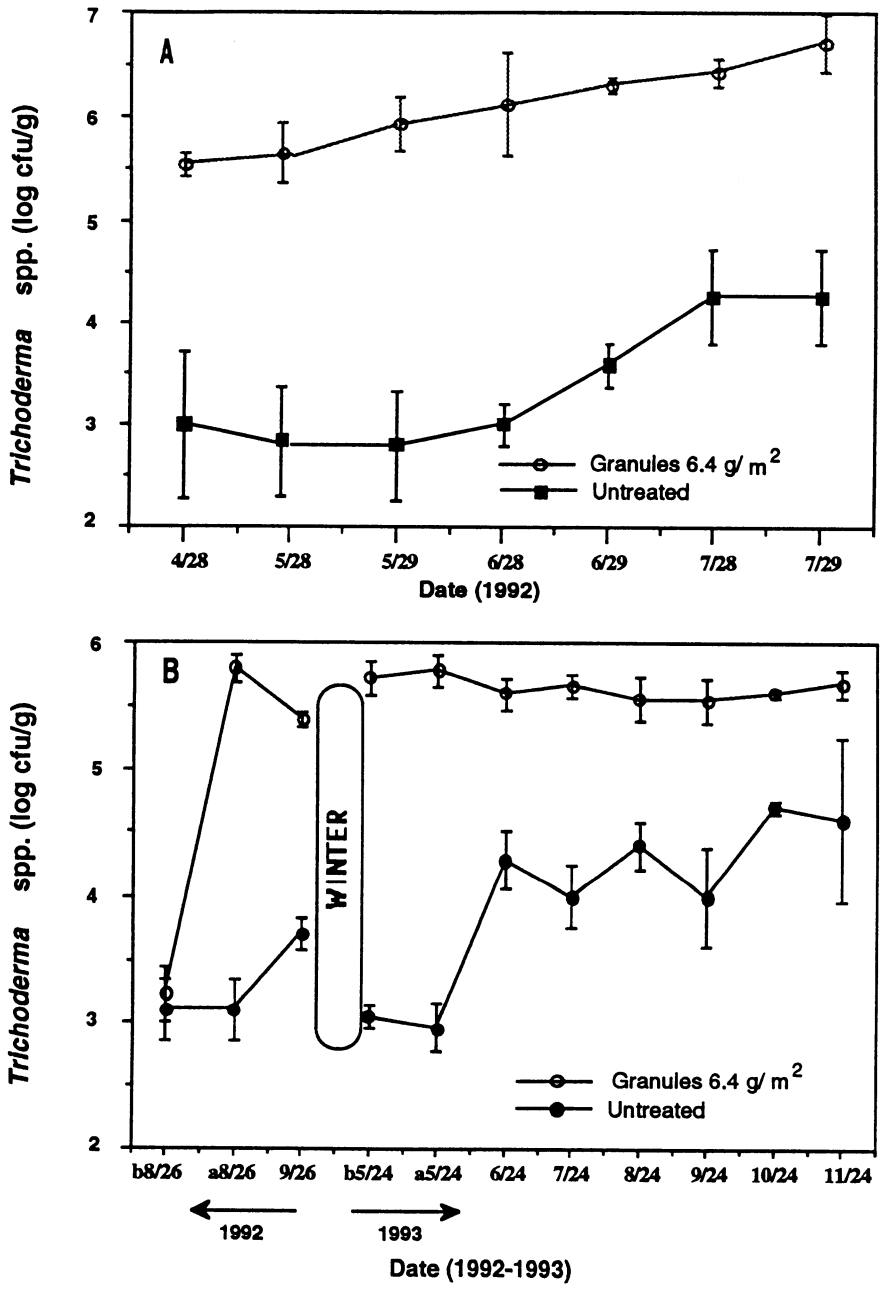

Fig. 3. Population dynamics of *Trichoderma* spp. in a creeping bentgrass putting green treated with *Trichoderma harzianum* strain 1295-22. **(A)** 1992 population in pH 8.0 soil; **(B)** 1992 to 1993 populations in pH 6.4 soil. Ratings were measured before (b) and after (a) topdressing applications on 26 August 1992 and 26 May 1993. Error bars represent standard deviations of means.

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