Improved Biocontrol Efficacy of *Trichoderma harzianum* 1295-22 for Foliar Phases of Turf Diseases by Use of Spray Applications

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ABSTRACT

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Trichoderma harzianum strain 1295-22 is an effective biocontrol agent for several fungal diseases. The efficacy of granule and spray applications of strain 1295-22 for control of Pythium root rot, brown patch, and dollar spot of creeping bentgrass was investigated. Spray applications of conidial suspensions (SA) of strain 1295-22 significantly reduced all three diseases of creeping bentgrass turf in both greenhouse and field experiments. Control was greatest when Triton X-100 at 0.1% was added to aqueous spray suspensions. When SA were applied weekly, the biocontrol treatments were equivalent to standard fungicides. Broadcast granule applications (GA) also significantly reduced foliar symptoms of Pythium root rot, dollar spot, and brown patch; turf quality also was enhanced. The populations of *Trichoderma* spp. in the root zone of a bentgrass putting green treated with SA or GA of strain 1295-22 increased 10- to 100-fold after treatment compared with untreated plots. However, strain 1295-22 was present at high levels on bentgrass leaves only following SA. Collectively, the results suggest that strain 1295-22 possesses both rhizosphere and phylloplane competence. The combination of broadcast applications of granules followed by spray applications of conidia reduced damage from both root and foliar diseases.

Additional keywords: delivery system, soilborne pathogens

Biological disease control is a promising strategy for managing soilborne or foliar diseases in a wide range of crops (2,6,15,30). To be effective, biological disease control depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining population levels and activities of these organisms in associations with crops and plants (13,26). Regardless of the qualities of the biocontrol agents, the methods used to produce, formulate, and deliver these organisms may profoundly influence their efficacy (7,13).

Successful biocontrol requires an understanding of many factors: the cropping system; the epidemiology of the diseases to be controlled; the biology, ecology, and population dynamics of the biocontrol organisms; and the interactions among these variables (1,8,30). In previous stud-

ies, topdressing applications of a granular formulation of *Trichoderma harzianum* Rifai strain 1295-22 onto creeping bentgrass turf reduced incidence and severity of several important diseases (14,15). However, while this treatment reduced initial disease incidence, it did not control foliar phases of diseases such as dollar spot (15).

Improving the efficacy of biocontrol with strain 1295-22 may be possible when one considers the nature of the turfgrass ecosystem. This ecosystem contains a dense foliar canopy in which the foliage is continuously wounded by frequent mowing and can remain wet for extended periods of time. This creates a favorable environment for pathogens to spread from plant to plant (32). Many turfgrass pathogens can attack blades of fine-leaved grasses and spread aurally from blade to blade (22,24). Since granular applications of strain 1295-22 were applied to thatch and canopy, it is likely that the organism, when applied in this manner, cannot cover the entire plant or have the sufficient quality of inoculum to control aerial phases of the diseases caused by these pathogens.

Spraying conidial suspensions of *T. harzianum* has been used to control gray mold on fruits and foliage of various crops either alone or in combination with reduced levels or application frequencies of chemical fungicides (10,11). However, little information is available on the utility

of foliar sprays of *T. harzianum* for controlling soilborne pathogens that also infect foliage. Migheli et al. (17) reported that *T. harzianum* survived on the phylloplane of tomatoes for 2 weeks, which suggests that this biocontrol fungus may be potentially effective in controlling aboveground diseases of various crops.

The objectives of this study were to determine whether spray applications of *T. harzianum* strain 1295-22 enhance control of turf diseases, especially their aerial phases; to determine whether additives to spray applications enhance biocontrol efficacy; to determine whether this strain can establish and maintain effective population levels on turf leaves, i.e., whether it possesses phylloplane competence; and to use the information obtained to develop an effective system of biological control for turf diseases.

MATERIALS AND METHODS

Fungal isolates. The isolates of *Pythium graminicola* Subramanian (PRR-8), *Rhizoctonia solani* Kühn (RS-2), and *Sclerotinia homoeocarpa* F.T. Bennett (2L-1) used in this study were originally isolated and maintained as described previously (15). Strain 1295-22 (ATCC 20847) of *T. harzianum* Rifai was used in all experiments. The strain was obtained by protoplast fusion between *T. harzianum* strains T12 and T95 (28).

Preparation of pathogen inoculum for greenhouse tests. *P. graminicola* was grown either on 5% V8 agar plates for 3 days or on autoclaved wheat seeds (20 g of wheat seeds in 30 ml of water) for 10 to 14 days before use. *R. solani* and *S. homoeocarpa* were each grown on the wheat seed mixture in petri dishes. When this substrate was completely colonized, the petri dish lids were removed and the cultures were dried in a sterile airstream provided by a laminar flow transfer hood. The dried mixture was ground in a Waring blender and kept at 4°C until use (12).

Colonization of roots or leaves of creeping bentgrass following granule or spray application. A PVC pipe (70 mm long, 20 mm diameter) was sliced longitudinally into halves. The halves were held together and secured with rubber bands for the duration of the experiment. These cylinders were sealed at the bottom with a cotton plug and treated with either granules or spore suspensions, or left untreated.

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Each cylinder was filled with 30 g of an Arkport sandy loam soil (pH 6.3), and the resulting soil columns were each seeded with five seeds of creeping bentgrass (*Agrostis palustris* Huds.). Before the turfgrass seeds were sown, the soil moisture content was adjusted to approximately -72 mbars (5). For treatment with granules, 0.5% (wt/wt) granules was mixed with the soil prior to placement in tubes. For spray applications, 10-day-old seedlings of creeping bentgrass were sprayed with 3.3 ml of a suspension of conidia of strain 1295-22 containing 10^7 conidia per ml. The cylinders of each group were placed in a container (30 × 30 × 12 cm), and soil at the same moisture content was added to a depth of 5 cm to maintain a constant moisture level (23). These containers were sealed and placed in a growth chamber at 25°C under a 12-h diurnal light cycle. After 7 days, the cylinders were separated. Each seedling was carefully removed from the soil, washed twice with sterile distilled water, and transferred to *Trichoderma* selective medium (TSM) (25). The distribution of *Trichoderma* spp. over roots and leaves of each turfgrass seedling was recorded after incubation for 2 days on TSM at room temperature. This experiment was performed twice.

Quantitative determinations of root and leaf colonization were also performed. In separate tests, soil (200 g) was treated as previously described and placed in 10 × 10 × 5 cm plastic boxes. Each soil box was moistened with 36 ml of distilled water at the beginning of the experiment and incubated for 24 h. The creeping bentgrass seeds were then sown by sprinkling seed over the surface of the soil. Three boxes were seeded for each treatment, and each box was considered a replicate. Experiments were conducted at 23 to 25°C with a 12-h photoperiod using cool-white fluorescent lights, and additional water was added as required. After 10 days, seedlings were sprayed until runoff with conidial suspensions of *T. harzianum* strain 1295-22. The conidial suspensions were obtained by filtering a granular formulation suspended in water through four layers of cheesecloth. Suspensions were adjusted to a final concentration of 10^7 CFU/ml before use. The conidial suspensions of strain 1295-22 were mixed with 0.1% (vol/vol) Triton X-100 before spraying (20 ml/box). Seven days after spraying, leaves of all treatments were harvested with a sterile knife. Fresh leaf tissue (50 mg) was placed in 10 ml of sterile distilled water and shaken vigorously for 3 min on a vortex mixer. Samples were then diluted serially in sterile distilled water and plated on TSM. After incubation for 5 to 7 days at room temperature, colony counts from quadruplicate plates were converted to CFU per 50 mg fresh weight of turf leaves. Similarly, soil samples also were collected from each box and diluted

serially to obtain CFU counts per g dry weight of rhizosphere soil.

Evaluation of spray applications of 1295-22 in the greenhouse. Cornell mix (0.5 m³ of peat, 0.95 m³ of vermiculite, 2.6 kg of ground limestone, and 1.2 kg of Unimix [N-P-K, 10-20-5] pH 6.8 to 7.0) was placed in a shallow flat (50 × 30 × 5 cm), and the surface was smoothed. Seeds of creeping bentgrass were sown over the surface as described above. The flat was maintained in a greenhouse at 20 to 25°C and watered daily. The grass was cut with scissors to 1.5 cm every week, and the clippings were removed. After 10 days, seedlings were inoculated with the various pathogens. A small uniform quantity of *Pythium*, *Rhizoctonia*, or *Sclerotinia* was placed on two opposite corners of the flat. The *Pythium* inoculum consisted of four agar plugs (1 × 1 cm) or five infested wheat seeds, and inoculum of *Rhizoctonia* or *Sclerotinia* consisted of 0.2 g of wheat seed powder placed on opposite corners. When the symptoms of each disease appeared, 50 ml of the *Trichoderma* conidial suspension (10^7 CFU/ml) was sprayed weekly onto seedlings in each flat. Conidia were harvested by mixing a colonized peat moss-wheat bran mixture (11) with distilled water (1:50 wt/vol) and filtering through four layers of cheesecloth. The number of conidia was determined by counting in a Petroff-Hausser counting chamber. Efficacy of control measures was determined over time by estimating the percentage of the flat occupied by diseased plants.

The effects of additives on the efficacy of the biocontrol agent also were determined. Additives included surfactants (Triton X-100; Eastman Kodak Co., Rochester, NY, and Tween 20; Fisher Biotech, Fair Lawn, NJ) and a nutrient adhesive containing carboxymethyl cellulose and gum arabic (Pelgel; Liphatech, Inc., Milwaukee, WI). Triton X-100 or Tween 20 was added to conidial suspensions at the rate of 0.1% (vol/vol), and Pelgel was added at 1% (wt/vol).

Field evaluations. Experiments were conducted in 1994 on creeping bentgrass putting greens at the Cornell University Turfgrass Field Research Laboratory, Ithaca, New York. Conidial suspensions of strain 1295-22 from various sources were evaluated by spray applications to suppress the diseases of *Pythium* root rot, brown patch, and dollar spot of turfgrasses. The putting green was mowed to 5 mm, and a total of 1.3 kg of nitrogen per 100 m² was added prior to the establishment of treatments. The soil pH value was 6.4. Plots of 0.81 m² were established in locations where dollar spot and brown patch had been prevalent in previous years. These plots were then inoculated with *P. graminicola*. A 15-cm-diameter piece of sod was removed from the center of each plot to a depth of approximately 5 cm. As much of the loose soil as possible was removed from the bottom of the sod and returned to the green. Eighty cm³ of pathogen inoculum, grown on autoclaved wheat grain for 14 days as described earlier, was placed in the hole, and the sod was then placed back over the inoculum layer. Cores were tamped flush with the rest of the green surface to avoid damage during mowing (20).

Immediately after inoculation on 1 June and again on 1 July 1994, granules of 1295-22 were applied to plots at the rate of 6.4 g/m² (60 lb/A). Various conidial preparations were applied at different times. Conidial suspensions were prepared from (I) a peat moss-wheat bran mixture (11), (II) 2% wheat bran liquid culture (100 ml of 2% wheat bran [wt/vol] in a 250-ml flask was inoculated with 1 ml of a conidial suspension [10^6 spores/ml] and incubated at 30°C on a shaker [50 rpm] for 5 days), or (III) dry conidia. Conidial suspensions (I) and (II) were harvested by filtration through four layers of cheesecloth, while the dry powder preparation (III) was prepared by a prototype commercial process from BioWorks Inc. (Geneva, NY), and contained about 2×10^9 CFU/g.

Table 1. Populations of *Trichoderma* spp. in the rhizosphere or phylloplane of creeping bentgrass treated with strain 1295-22 of *Trichoderma harzianum*^w

Treatments	Rhizosphere populations (CFU/g soil)		Phylloplane populations (CFU/0.05 g leaves)	
	Autoclaved soil ^x	Natural soil	Autoclaved soil ^x	Natural soil
Untreated	2.9×10^3 c	1.8×10^4 c	3.6×10^3 b	6×10^4 b
Granular ^y application	2.4×10^5 a	1.0×10^5 b	6.7×10^3 b	7×10^4 b
Spray ^z application	1.0×10^5 b	1.8×10^5 a	4.0×10^5 a	3×10^5 a

^w Data were recorded 7 days after spraying spore suspensions on turfgrass seedlings. The *Trichoderma* levels in soil prior to treatments were 2 to 5×10^3 CFU/g for nontreated soils and 0.5 to 1×10^2 after autoclaving at the start of the experiment. After spray applications, CFU levels (3 h after application) were 5×10^5 CFU/g in soil and about 1×10^5 CFU/0.05 g of leaves.

^x The soil was autoclaved twice for 20 min (121°C, 15 lb). Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

^y Granular application of *T. harzianum* 1295-22 was mixed with autoclaved or unautoclaved soil before sowing turfgrass seeds.

^z Ten-day-old seedlings were treated with spray applications of *T. harzianum* 1295-22 that were derived from a granular formulation suspended in water and filtered through four layers of cheesecloth.

Fig. 1. Suppression of *Pythium* root rot (A), brown patch (B), and dollar spot (C) diseases on creeping bentgrass using spray applications of *Trichoderma harzianum* 1295-22 in greenhouse experiments. Disease severity represents the percentage of the total area of the test planting that contained diseased turf. Bars with differing letters are significantly different at $P = 0.05$.

Spray applications of strain 1295-22 (10^7 conidia/ml) were applied at a rate of 200 ml per plot. Conidial suspensions were mixed with 0.1% Triton X-100 before use. The granular formulation (15) was mixed with 200 cm³ of fine quarry sand (pH 7.5) before being applied as a topdressing. Granules were distributed by hand as uniformly as possible over the plot area on 1 June and on 1 July. Spray applications of strain 1295-22 conidial suspensions were applied at monthly intervals for conidial suspensions (I) and (II) beginning on 1 June. However, after 26 July, some of the spray applications were applied at weekly intervals when disease severity became high. The dry conidial preparation (III) was not available until September 1994, so this treatment was not applied before this time. This preparation also was applied monthly and weekly in separate trials. To test the effect of combined treatments, a monthly conidial spray (I) was applied over a granular treatment after 26 August when dollar spot became severe.

The efficacy of treatments for control of brown patch and dollar spot disease was compared with propiconazole {1-[(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl]-1H-1,2,4-triazole} applied at monthly intervals at the rate of 174 mg a.i./m². For *Pythium* root rot, metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxy-acetyl) alanine methyl ester] was applied on 10 June 1994 at a rate of 0.75 mg a.i./m². Disease severity of all plots was evaluated by estimating the percentage of plot area occupied by diseased plants.

Population dynamics of *Trichoderma harzianum* 1295-22. Population levels of *T. harzianum* in the soil were quantitated by serial dilutions onto TSM (25) for both greenhouse and field experiments. Soil samples were taken monthly immediately before treating plots and processed as described by Lo et al. (15). Briefly, 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 cores for each replicate was comminuted in 100 ml of water in a Waring blender for 1 min. Serial dilutions were 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 CFU/g of dry weight.

Experimental design and data analysis. All greenhouse and field experiments were established as a randomized complete block design with three and five replicates, respectively. All data were submitted to analyses of variance, and Duncan's multiple range tests were used for mean separations with the Statistical Analysis System program (SAS Institute, Cary, NC).

RESULTS

In greenhouse trials, granule and spray applications had varying effects on the distribution of 1295-22. Strain 1295-22 effectively colonized the roots of creeping bentgrass when applied either as granules or as sprays, but only the spray application of 1295-22 resulted in colonization on the creeping bentgrass foliage. Population levels of *Trichoderma* spp. in cores containing roots and soil increased relative to untreated rhizosphere soils of creeping bentgrass by about 100-fold and about 10-fold in autoclaved and nonautoclaved soils, respectively, following granular or spray applications of strain 1295-22 (Table 1). Some background levels of *Trichoderma* were present even in autoclaved soils. However, only spray applications significantly increased the population levels of strain 1295-22 on leaves when compared with untreated or granular-treated soils. Most of these increases in soil and on leaves were evident immediately after application and persisted for the duration of the experiment (7 days) (Table 1).

Application of conidial suspensions of strain 1295-22 in water reduced the severity of all three diseases (Fig. 1); however, in all tests, greater control was obtained when Triton X-100 was included with the conidial suspension. This treatment resulted in a 2.5-, 4-, and nearly 7-fold reduction in disease for Pythium root rot, brown patch, and dollar spot, respectively, relative to the water control by the end of the experiment. In addition, control of dollar spot in the absence of Triton X-100 was effective only for about 4 weeks; conversely, in the presence of this detergent, high levels of control were obtained with strain 1295-22 throughout the experiment (Fig. 1C). The addition of Tween 20 (data not shown) or Pelgel (Fig. 1A to C) did not provide increased levels of disease control relative to spore suspensions alone.

Triton X-100 alone affected the severity of foliar symptoms of Pythium root rot, brown patch, and dollar spot and was evaluated in separate but similar experiments. With all three diseases, symptoms were reduced by about 48% for dollar spot and brown patch and by about 54% for Pythium root rot following treatment with Triton X-100. With dollar spot and brown patch, disease severity 1 month after inoculation was significantly less with the combination of strain 1295-22 and the surfactant than with either material singly. However, Pythium root rot severity following treatment with Triton X-100 alone was only 1.5 times that of the combination of the surfactant plus 1295-22. This difference was not significant at $P = 0.05$ but was significant at $P = 0.1$.

In field evaluations, spray applications with spore suspensions of strain 1295-22 amended with 0.1% Triton X-100 reduced the severity of Pythium root rot, brown patch, and dollar spot on a creeping bent-

Fig. 2. Suppression of Pythium root rot (A), brown patch (B), and dollar spot (C) diseases using spray applications of *Trichoderma harzianum* 1295-22 on a creeping bentgrass putting green. Spore suspensions I, II, and III are conidia that were obtained from peat-bran, conidia from 2% wheat bran cultures in water and used directly from the liquid fermentation, or dry conidia, respectively. (W) and (M) represent spore suspensions sprayed weekly and monthly, respectively. Spore suspension I was applied 26 August 1994 to plots previously treated by broadcast application of the granular formulation. Spore suspension III was not applied until 1 September 1994. Disease severity represents the percentage of the total area of the plots that contained diseased turf. Bars with differing letters are significantly different at $P = 0.05$.

grass green when compared with untreated plots. Weekly applications of conidial suspensions were as effective as fungicide applications. Monthly spray applications of conidial suspensions significantly reduced disease severity but were less effective than weekly spray applications under severe disease pressure (Fig. 2C). Conidia obtained from different sources were equally effective in controlling diseases (Fig. 2A to C). Granular applications also significantly inhibited *Pythium* root rot and brown patch disease development, although disease se-

verity did not differ from untreated plots during the initial stage of disease development ($P = 0.05$) (Fig. 2A and B). Dollar spot severity was also reduced (data not shown) when compared with untreated plots during initial stages of dollar spot development ($P = 0.1$), although no differences were discerned at $P = 0.05$ (Fig. 2C). Plots treated with the granular formulation, but not the spray application, were noticeably greener and more vigorous when observed in November, 4 months after the last application (data not shown).

Introduction of 1295-22 to a creeping bentgrass putting green with either spray or granular applications was effective in establishing high population levels of *Trichoderma* spp. on roots. Strain 1295-22 survived and persisted at high populations (about 5×10^5 CFU/g) and remained stable or increased during the entire season regardless of the method of application. However, weekly spray applications of spore suspensions resulted in even higher populations (10^6 CFU/g) when compared with the other treatments (Fig. 3). In these experiments, it was not possible to reliably separate strain 1295-22 from all other *Trichoderma* strains, although colonies that appeared to be *T. virens* and therefore distinct in the appearance of conidiophores were excluded from counts. However, wild *Trichoderma* strains were present, and so only increases in total *Trichoderma* levels in plots could be determined.

DISCUSSION

Suppressing initial plant infection by soilborne pathogens is a logical strategy for controlling monocyclic diseases. The secondary infection of polycyclic diseases is an important factor for disease progress when primary inoculum is low (9,16). *Rhizoctonia* and *Sclerotinia* are monocyclic pathogens in some crops, but they may become polycyclic on plants maintained at high density (9). Turfgrasses contain a high-density canopy that is mowed daily on putting greens. Since many turfgrass pathogens can spread readily in turfgrass foliage, control of these diseases requires both suppression of initial plant infection and reduction of the infection rate. Granular applications of strain 1295-22 significantly inhibited disease severity during the initial stage of disease development, most likely by reducing levels of the pathogen inoculum in soil and thatch (15). However, dollar spot severity increased over the course of the season. It is apparent, therefore, that soil applications alone cannot effectively control the foliar phases of this disease. Conversely, in greenhouse and field experiments, we found that strain 1295-22 significantly reduced the foliar phases of *Pythium* root rot, brown patch, and dollar spot when spray applications of conidial suspensions containing Triton X-100 were used. Weekly spray applications were as effective as the standard (monthly) fungicide applications. These results indicate that the efficacy of strain 1295-22 against turf diseases, especially those involving secondary infections, is very strongly affected by the method of application.

Additives have been commonly used with fungicides to improve efficacy (18), and they also may enhance the ability of biocontrol agents to reduce plant disease. For example, Harman et al. (12) reported that seed treatment using 10% Pelgel with solid matrix priming markedly enhanced

Fig. 3. Population dynamics of *Trichoderma* spp. in a creeping bentgrass putting green treated with spray applications of *T. harzianum* 1295-22 at monthly (M) or weekly (W) intervals. Spore suspensions I, II, and III are conidia obtained from peat-bran, 2% aqueous wheat bran, or a commercial conidia source, respectively. In (A), spore suspension III was applied on 1 September 1994. All samples were collected just prior to each monthly application of granular or spray formulation.

the efficacy of *Trichoderma* strains to control *Pythium* spp. on various crops. Our results indicated that, for control of the three diseases of creeping bentgrass in this study, greater control was obtained when Triton X-100 was included than when no additives, Pelgel, or Tween 20 were used. The use of specific surfactants with strain 1295-22 seems essential to obtain levels of control equivalent to those achieved with chemical fungicides.

Detergents such as Triton X-100 may have several functions in biocontrol systems. They may slow the growth of pathogens (21,27,29) more than that of the biocontrol agents, or they may enhance wetting and adhesion of spores to infection courts. In preliminary experiments, both Tween 20 and Triton X-100 slowed the growth of both *T. harzianum* and the pathogens, but the ratio of the growth rates of *T. harzianum* and pathogens was greater with Triton X-100 than with Tween 20; i.e., Triton X-100 was more inhibitory to the pathogens than to *T. harzianum*. Tween 20 slowed growth of the biocontrol fungus less than did Triton X-100 (data not shown). This may be a factor in the reduced efficacy of Tween 20 as an adjuvant relative to Triton X-100.

A biocontrol preparation, because it contains a microorganism as the active ingredient, differs fundamentally from a chemical pesticide. Living organisms, in addition to yielding a large quantity of biomass of the bioprotectant fungus, must function effectively and reliably in each application (13). To test the efficacy of conidia harvested from various sources, we compared conidia harvested from moist peat-bran cultures, cultures produced in 2% aqueous wheat bran, or dry conidia suspended in water. All formulations provided equivalent levels of control, indicating that the method of spore production may not be a key factor in the efficacy of strain 1295-22 for controlling these diseases of creeping bentgrass.

To predictably and successfully use biological control agents for turfgrass disease control, it is critical that their biology and ecology be more completely understood. Biological control agents must grow and proliferate to be effective (19). Therefore, effective antagonists must become established in turfgrass ecosystems and remain active against target pathogens during periods favorable for plant infection. *T. harzianum* 1295-22 is a highly rhizosphere competent microbe (12,15,23). Broadcast applications of granules of strain 1295-22 resulted in establishment of stable and effective populations in turfgrass soils (15). Similarly, our data show that the populations of strain 1295-22 in soils or thatch treated with spray applications were as high as those in soils or thatch treated with granular formulations. Population levels of strain 1295-22 of 5×10^5 CFU/g of soil significantly reduced dollar spot,

Pythium blight and root rot, and brown patch diseases of turf (14,15).

Top-dressing putting greens with a granular formulation of strain 1295-22 apparently enhances the overall quality of creeping bentgrass relative to untreated plots or those sprayed either with fungicides or with the biocontrol agent, as evidenced by enhanced greenness (data not shown). However, spray applications, even though they resulted in numerically similar levels of root colonization, did not provide the same benefit. This may reflect the differences in inoculum potential of granules versus spray applications. Granules are applied as a several-millimeter-diameter particle that is fully colonized by the fungus. Conidial inocula, on the other hand, are much smaller and would therefore be expected to possess lower inoculum potentials than the granular formulation.

Enhanced root growth and plant vigor have been observed following application of *T. harzianum* to other crops. For example, Björkman et al. (4) reported that *T. harzianum* 1295-22 increased both root and shoot growth of corn. Enhanced turf quality may result from a similar enhancement of root development. Such increases in plant growth and development may result from (i) control of deleterious root microflora, including those not causing obvious disease (3), (ii) direct production of growth-stimulating factors (i.e., hormones or growth factors) (31), or (iii) increased nutrient uptake through enhanced root growth or promoted availability of necessary nutrients. Activity of biocontrol agents could also lessen concentrations of substances in soil that are inhibitory to plant growth (3).

The ability to survive on the phylloplane is also a desirable trait for strains of *Trichoderma* used as biocontrol agents against foliar diseases. Spray applications of strain 1295-22 resulted in disease-suppressive population levels on leaves, especially in the presence of Triton X-100. These populations were sufficient to suppress *Pythium* root rot, brown patch, and dollar spot over the entire season. Strain 1295-22 survived on turf leaves at least 4 weeks in growth chamber trials (data not shown). Thus, *T. harzianum* 1295-22 may possess a measure of phylloplane competence on creeping bentgrass turf.

The ideal biocontrol strategy attempts to introduce or promote the activity of biocontrol agents only when and where they are needed or are most effective and minimizes wasteful application of inoculum to nontarget habitats. Thus, for effective delivery, we need to consider plant-pathogen-antagonist interactions in terms of time and space. Based on our collective results, a possible strategy for effective control of turf diseases begins with a granule application in the spring to create disease-suppressive soils and possibly enhance plant vigor. *Pythium*, *Rhizoctonia*,

and *Sclerotinia* are important soilborne pathogens, and their survival structures in soil serve as primary inoculum. Consequently, suppression of the initial inoculum will be the first step in managing these diseases of creeping bentgrass. This granular application should be followed by monthly spray applications to suppress foliar phases of these diseases. Inhibition of the secondary infection and dissemination of these pathogens is also important for disease management. Because of the nature of turfgrass ecosystems, these pathogens may escape the granule treatment, infect foliage, and spread from blade to blade. Monthly spray applications of 1295-22 could provide a second step in protection of turfgrass foliage from attack by preventing these pathogens from initially infecting leaf blades and by reducing the spread of disease through mowing or other methods of inoculum dissemination. Finally, our results indicate that it will be necessary to apply weekly sprays for highly effective control of these diseases under severe disease situations.

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