Biological Control of *Rhizoctonia solani* on Tall Fescue Using Fungal Antagonists

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ABSTRACT

Yuen, G. Y., Craig, M. L., and Giesler, L. J. 1994. Biological control of *Rhizoctonia solani* on tall fescue using fungal antagonists. Plant Dis. 78:118-123.

A binucleate *Rhizoctonia* sp. (isolate GM 460) from soil and *Gliocladium virens* (isolate TRBG) from creeping bentgrass consistently inhibited Rhizoctonia blight on tall fescue in laboratory bioassays. When *R. solani* was inoculated onto tall fescue seedlings treated with either antagonist, 20–60% of the foliage was blighted after 10 days, whereas 80–100% of the foliage was affected in the controls. Suppression of blight was associated with reduced growth of *R. solani* on grass blades. GM 460 and TRBG differed in their tolerance of decreasing relative humidity. At 100% relative humidity, TRBG grew on grass blades to a greater extent than GM 460; at 95% relative humidity, GM 460 grew on grass blades but TRBG did not. When tall fescue seedlings treated with the fungi were subjected to 35–45% relative humidity for 3 days before being inoculated with the pathogen at greater than 95% relative humidity, TRBG did not inhibit the disease, whereas GM 460 was suppressive. A combination of GM 460 and TRBG was as or more effective than the individual isolates, but combinations of either fungus with other fungal isolates often resulted in decreased efficacy. When GM 460 was applied to tall fescue in the field, it persisted on treated turf for a 1-mo period and reduced brown patch development from 35% blighted turf in the control to less than 20%. Applications of GM 460 or TRBG in a subsequent field experiment, however, were ineffective. The only effective treatment in this experiment was a combination of GM 460 and TRBG, which decreased the percent blighted turf from the 30–36% in the controls to 14–26%.

Additional keywords: *Festuca arundinacea*

Rhizoctonia solani Kühn, which causes Rhizoctonia blight or brown patch disease, is one of the most destructive pathogens of turfgrass. The disease is widespread geographically and affects both warm- and cool-season turfgrasses. Several anastomosis groups (AG) are involved, and these vary among geographic regions and species of grass (2). Cultural procedures affect disease occurrence and severity (20), but fungicidal control currently is the primary means of reducing damage due to *R. solani.*

Tall fescue (*Festuca arundinacea* Schreb.), which is susceptible to *R. solani,* is becoming an increasingly popular low-maintenance turfgrass because of low water and fertilizer requirements and tolerance to shade, insects, and diseases. The aesthetic value of tall fescue has been greatly improved through the development of cultivars with fine blades and dense canopies. These improved cultivars, however, are associated with increased problems due to brown patch (20), perhaps because the dense canopies provide more favorable microenvironments for the pathogen. Differences in tolerance to brown patch among cultivars of tall fescue have been reported (3), but in the absence of high levels of resistance, greater fungicidal inputs may be necessary to protect dense-canopied cultivars. This, in part, may negate the advantages of tall fescue as a low-maintenance turfgrass. Therefore, methods are needed that can provide long-term control of brown patch and that are compatible with tall fescue.

Microorganism-based biological control may be one alternative to fungicides for controlling brown patch. Effective biocontrol of *R. solani* diseases on crop plants has been found in numerous studies (1,6,8,14,15). Bacterial and fungal biocontrol agents effective against other turfgrass diseases, such as dollar spot (7,17), Typhula snow mold (5), and Pythium blight (18), also have been reported. Few biocontrol agents, however, have been reported to be effective against *R. solani* on turfgrass. Brown patch on creeping bentgrass was inhibited by avirulent binucleate *Rhizoctonia* spp., albeit for short (8-day) periods (4). *Laetisaria arvalis* was more effective than low-virulence isolates of *Rhizoctonia zeae* and binucleate *Rhizoctonia* spp. in reducing brown patch infection levels on live tall fescue leaf clippings (22). Little information is available on the effectiveness of antagonistic fungi from other plant systems and on the potential of using combinations of fungi to control brown patch.

Our objectives were 1) to identify biocontrol agents effective against brown patch on tall fescue from among fungi isolated from turfgrass and other habitats; 2) to determine the effects of relative humidity on their growth on grass blades and on disease suppression; and 3) to evaluate the antagonists alone and in combination for efficacy in suppressing brown patch on tall fescue turf. Preliminary results have been reported (24).

MATERIALS AND METHODS

Fungal isolates and inoculum production. Isolate R212 of *R. solani* AG 1-IA, from Kentucky bluegrass, was used as the pathogen in all experiments. The fungus was stored and propagated on potato-dextrose agar (PDA). Mycelial plugs (4-mm-diameter) from 5- to 7-day-old PDA cultures were used as laboratory inoculum. For field inoculations, the fungus was cultured on autoclaved oat seed (21).

Two isolates of biocontrol agents were studied most extensively. *Gliocladium virens* J.H. Miller, J.E. Giddens, & A.A. Foster (isolate TRBG) was isolated from *R. solani*-infected creeping bentgrass. GM 460, a binucleate *Rhizoctonia* sp. AG-B(o), was isolated from soil and was antagonistic to *R. solani* causing root rot of sugarbeet (8,11). Other fungi tested in this study included isolates of binucleate *Rhizoctonia* sp., *Fusarium* spp., *Gliocladium* spp., *Penicillium* spp., and sterile fungi from tall fescue, Kentucky bluegrass, perennial ryegrass, and creeping bentgrass turfs. Mycelial plugs from PDA cultures were used to apply all fungi to tall fescue in the laboratory. Sporulating fungi also were applied as conidia collected from 5- to 10-day-old PDA cultures and suspended in sterile water at 10^5 to 10^6 conidia per milliliter. For field application of TRBG, the fungus was cultured in a molasses-yeast medium and formulated into alginate-bran pellets (13). GM 460 was prepared for field application by culturing the fungus on sterilized millet seed (21).

Journal Series Paper 10400 of the Agricultural Research Division, University of Nebraska.

Accepted for publication 14 October 1993.

Laboratory inoculation of tall fescue. A plant bioassay was used in evaluating fungal isolates for inhibition of brown patch. Tall fescue cv. Fawn was seeded into 150-ml plastic drinking cups at approximately 100 seeds per cup. The growth medium was a pasteurized mixture of equal volumes vermiculite, sand, and Sharpsburg silty clay loam. Following germination, seedlings were maintained at 23 C with 16 hr of fluorescent light (30 W/m^2) and clipped regularly to 5-cm height. When the seedlings were 2–3 wk old, test fungi were applied as two mycelial plugs placed in the center of the cup on the growth-medium surface. Alternatively, 5 ml of spore suspension were sprayed onto seedlings using a hand-pumped sprayer. Each cup was then enclosed in a plastic bag and incubated for 2–4 days in a growth chamber with 12 hr of light (30 W/m^2) at 28 C and 12 hr of darkness at 23 C. Relative humidity (RH) in the bags exceeded 95%, as determined by a relative humidity and temperature probe (Model HMP 35A, Vaisala, Inc., Woburn, MA). Following incubation with biocontrol agents, the seedlings were inoculated with two mycelial plugs of *R. solani* isolate R212 and incubated for 10 more days. Every other day, the percentage of the foliage with blight was visually assessed. Five to eight replicate cups were used, and each biocontrol treatment was tested in at least two experiments. Controls in each experiment were cups of seedlings inoculated with R212 only. Noninoculated seedlings to which no biocontrol agents were applied also were included in each experiment, but because disease did not develop on any of these units, they were not included in the statistical analysis. Analysis of variance and Duncan's multiple range test were performed on the data after arcsine transformation.

Measurements of fungal occurrence and growth. Biocontrol fungi and R212 applied to cups of tall fescue seedlings were detected by culturing segments of grass blades on agar media. At various intervals following the application of fungi, all of the grass blades in each cup were cut into 2-cm-long segments using sterile scissors or razor blades. Fifty segments were selected at random and placed on each type of agar media. V8 juice agar (13) amended with streptomycin sulfate and penicillin G, both at 100 mg/L, was used to isolate TRBG. For isolating GM 460 and R212, water agar was amended with 50 mg/L pencycuron (technical grade, 96% a.i.) or 50 mg/L prochloraz (technical grade, 95.2% a.i.), respectively, in addition to streptomycin and penicillin. Pencycuron is inhibitory to *R. solani,* and prochloraz is inhibitory to binucleate *Rhizoctonia* spp. (12). Cultures were examined after 2 days of incubation for *Rhizoctonia* isolates and after 3 days for TRBG. Hyphal-tip transfers were made onto PDA to confirm identity of the isolates. When one of the fungi was found to grow from one-half of a leaf-blade segment, the fungus was considered to occur on or to occupy 1 cm of blade. The relative occurrence of a fungus on grass blades was expressed as centimeters of blade with positive isolation per 100 cm.

The growth of GM 460, TRBG, and R212 on grass blades at 95, 98, and 100% RH was compared in one experiment in which mycelial plugs from PDA cultures were placed on one end of 10-cm-long detached tall fescue leaf blades. The blades were taped onto stiff plastic mesh and sealed in large Mason jars over saturated solutions of KNO_3, K_2SO_4, and distilled water. The solutions provided 95, 98, and 100% RH, respectively (23). The temperature was maintained at 25 $\pm$ 1 C by placing the jars in an incubator. Conditions within the jars were measured with a relative humidity and temperature probe. Each jar contained three grass blades for each fungal isolate, and there were two jars for each humidity level. After incubation for 3 days, each blade was dissected aseptically into 0.5-cm-long segments starting from the end opposite that inoculated with a fungus. The segments were placed on agar media. The blade segment most distant from the point of inoculation from which a given fungus was isolated determined the distance that organism grew on a grass blade. The experiment was designed as a split plot with relative humidity levels being the main treatments and fungal isolates the subtreatments. The experiment was performed twice; because heterogeneity of error variance was not indicated, data from the two experiments were pooled for analysis of variance with the experiments being treated as subsubtreatments.

Field experiments. Experiments were conducted in 1991 and 1992 on established turf of cv. Rebel at the University of Nebraska John Seaton Anderson Turfgrass Research Facility located near Mead. The turf was fertilized monthly from May through September with 23 kg N/ha in the form of urea (46N-0P-0K) and maintained at 6.5 cm height. During the experiments, the plots were irrigated at night with approximately 4 cm of water per week to increase leaf moisture. No fungicides were applied. There were four 1.5 $\times$ 1.5 m replicate plots per treatment arranged in a randomized block design.

On 1 August 1991, R212-colonized oat seed was used to infest turfgrass at 94 g/m^2. Only GM 460 was evaluated as the biocontrol agent; there were two treatments, with GM 460 being applied once (31 July) and twice (31 July and 7 August). In both treatments, GM 460-colonized millet seed was applied at 115 g/m^2. Plots not treated with the biocontrol agent and plots not infested with R212 were the controls.

In 1992, R212 inoculum was applied on 1 July at 38 g/m^2 and repeated on 10 August at 33 g/m^2. GM 460 and TRBG were tested as separate treatments, applied once and three times, and as a combined treatment applied three times. For each application, GM 460 was delivered as 83 g/m^2 of colonized millet seed, and TRBG was applied as 47 g/m^2 of alginate-bran pellets. Single-application treatments were made on 25 June, 1 wk before infestation with R212. Triple-application treatments were made on 25 June, 27 July, and 21 August. Three applications of a systemic fungicide, thiophanate-methyl, also were included as a treatment in this experiment. A granular formulation containing 2.3% a.i. was applied on the above three dates at the rate of 5.5 g material/m^2. Only R212 inoculum was applied to control plots.

Percent turf showing blight symptoms was estimated visually every other week through September. During periods in 1992 when blight was absent or in low levels, i.e., less than 20% blight, foliage within 96-cm^2 areas was examined for lesion development on leaf blades and sheaths, and rated on a 0 to 10 scale (0 = no lesions, 10 = most severe lesion development). A rating of 10 approximated 20% blighted turf. Three measurements were made per plot and averaged prior to statistical analysis. The arcsine transformation was performed on percent blight data when variance heterogeneity was indicated. Lesion ratings and percent blight data were subjected to analysis of variance and the LSD test.

In 1991, relative occurrences of binucleate *Rhizoctonia* spp. and *R. solani* in the turf were determined. Thirty days after the last application of GM 460 (9 September), three subsamples of at least 10 grass blades each were collected from each plot and bulked in sterile plastic bags. The samples were assayed for the fungi by isolation according to laboratory procedures described above.

RESULTS

Efficacy of biocontrol agents in the laboratory. Out of more than 150 isolates of fungi from turfgrass and other sources, TRBG and GM 460 were the most effective and consistent in inhibiting blight development on tall fescue in the laboratory. In one experiment (Fig. 1), GM 460 and TRBG delayed blight development and reduced the amount of blight after 10 days to less than 60% of the control. Similar levels of blight suppression were provided by GM 460 and TRBG in more than 10 other experiments (partial data presented in Tables 1–4). Most of the other fungi, including other isolates of *Gliocladium* and binucleate *Rhizoctonia* spp., had no effect on brown patch. Some isolates, such as binucleate *Rhizoctonia* sp.

WRT1, only delayed disease development (Fig. 1), and levels of disease suppression varied among experiments (Fig. 1, Table 1).

GM 460 and TRBG were considered compatible when applied in combination because disease levels resulting from treatment with the combination were equal to or lower than individual isolate treatments. The biocontrol agents often were incompatible with other fungal isolates. For example, the combination of TRBG and GM 460 in one experiment (Table 1) suppressed disease on day 10 to the same level as GM 460 alone (22%), whereas the control had 80% blight. When GM 460 or TRBG was applied in combination with binucleate *Rhizoctonia* sp. WRT1, disease levels were higher than with the individual isolates. Similar results occurred when these treatments were tested in other experiments.

TRBG was effective in inhibiting disease when applied as mycelial plugs or as alginate-bran pellets, but not as a conidial suspension (Table 2). In one experiment, treatment with mycelial plugs of TRBG or with alginate-bran pellets at 17 mg per cup, which was comparable to the concentration used in field applications, limited blight severity by day 10 to less than 50% blighted foliage. An application of TRBG as a spore suspension had no effect on blight development. In the same experiment, GM 460 applied in colonized millet seed at 30 mg per cup, approximately field application rate, was as effective as as mycelial plugs (Table 2). All of these treatments were repeated in other experiments with similar results.

Suppression of blight by GM 460 and TRBG was related to decreased occurrence of R212 on grass blades. When R212 was inoculated onto tall fescue seedlings treated 2–4 days previously with GM 460 or TRBG, the frequency of R212 isolation from nonsymptomatic grass blades 4 days later was reduced by 60–82% (Table 3).

Influence of humidity on fungal growth and biocontrol efficacy. When GM 460, TRBG, and R212 were applied to detached leaf blades placed in 95, 98, and 100% RH, maximum growth for all three isolates occurred at 100% RH (Fig. 2). There was a significant ($P = 0.03$) relative humidity × isolate interaction. At 100% RH, GM 460 grew 2.6 cm in 3 days on grass blades, which was significantly less than R212 (4.5 cm in 3 days). TRBG, on the other hand, grew the same distance as R212. The three isolates were similar in growth at 98% RH. At 95% RH, minimal growth of GM 460 and R212 occurred, while TRBG failed to grow. Infection of grass blades by R212 and development of lesions occurred at 100% RH but not at 95 or 98% RH (*data not shown*).

GM 460 and TRBG differed in their capacity to inhibit blight following exposure to low humidity (Table 4). When seedlings treated with GM 460 or TRBG were incubated in plastic bags at

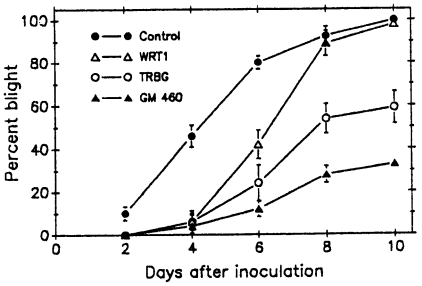

Fig. 1. Brown patch disease severity (percent blighted foliage) on tall fescue seedlings as affected by antagonistic fungi applied 4 days prior to inoculation with *Rhizoctonia solani* R212. GM 460 and WRT1 are isolates of binucleate *Rhizoctonia* spp. TRBG is an isolate of *Gliocladium virens*. All fungi were applied to cups of seedling as two agar plugs. Vertical bars denote standard error.

Table 1. Effects of binucleate *Rhizoctonia* sp. isolates GM 460 and WRT1 and *Gliocladium virens* TRBG applied alone and in combination on the severity of brown patch caused by *Rhizoctonia solani* R212 on tall fescue seedlings

Treatment[y]	Blighted foliage[z] (%)
GM 460	21 d
TRBG	48 c
WRT1	35 cd
GM 460 + TRBG	22 d
GM 460 + WRT1	50 bc
TRBG + WRT1	69 a
GM 460 + TRBG + WRT1	22 d
Nontreated control	80 a

[y]Treatments consisting of two agar plugs per isolate were applied 2 days prior to inoculation of seedlings with two agar plugs of R212.
[z]Disease severity was determined 10 days after inoculation with R212. Data presented are nontransformed means of six replications. Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test applied to arcsine-transformed data.

Table 2. Effects of binucleate *Rhizoctonia* sp. GM 460 and *Gliocladium virens* TRBG applied by different delivery methods on the severity of brown patch caused by *Rhizoctonia solani* R212 on tall fescue seedlings

Fungus	Delivery method[y]	Blighted foliage (%)[z] Day 4	Day 10
GM 460	Agar plugs	0 b	25 c
GM 460	Colonized millet seed	0 b	23 c
TRBG	Agar plugs	0 b	48 b
TRBG	Alginate-bran pellets	0 b	33 bc
TRBG	Conidial suspension	73 a	100 a
None	...	85 a	95 a

[y]Biocontrol treatments were applied 2 days prior to inoculation of seedlings with R212. Two agar plugs per cup were used in the applications of GM 460, TRBG, and R212. GM 460-colonized millet seed and TRBG alginate-bran pellets were applied at 30 and 17 mg/cup, respectively.
[z]Values presented are nontransformed means of six replications. Numbers within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test applied to arcsine-transformed data.

Table 3. Effects of binucleate *Rhizoctonia* sp. GM 460 and *Gliocladium virens* TRBG on the occurrence of *Rhizoctonia solani* R212 on nonsymptomatic foliage of tall fescue seedlings and the severity of brown patch measured 4 days after inoculation of seedlings with R212

Treatment[w]	R212 isolation frequency (cm blades/100 cm)[x]	Blighted foliage[y] (%)
Experiment 1		
GM 460	7*[z]	12*
Nontreated control	38	44
Experiment 2		
TRBG	21*	22*
Nontreated control	52	66
Experiment 3		
GM 460	11*	15*
TRBG	18*	28*
Nontreated control	47	62

[w]GM 460 and TRBG were applied 2 days prior to inoculation of seedlings with R212, except in experiment 1, in which GM 460 was applied 4 days prior to R212.
[x]Isolation frequency was determined by culturing 50 2-cm-long segments of nonsymptomatic blades on prochloraz-amended water agar. Values represent means of eight replications in experiments 1 and 2, and of six replications in experiment 3.
[y]Values are nontransformed means of eight replications in experiments 1 and 2, and of six replications in experiment 3.
[z]* = Treatment was significantly different from the control at $P \leq 0.05$ according to F test (experiments 1 and 2) and LSD test (experiment 3). Analysis of percent blighted foliage data was performed after arcsine transformation.

greater than 95% RH for 3 days prior to inoculation with *R. solani,* blight severity measured 10 days after inoculation was less than 40%, compared to 100% in the control. When GM 460-treated seedlings were incubated uncovered in the growth chamber at 35–45% RH prior to pathogen inoculation and then enclosed in plastic bags following inoculation to maintain high humidity, blight severity was 68% but still significantly lower ($P = 0.05$) than in the control. In contrast, TRBG incubated on seedlings for 3 days under low humidity had no effect on disease.

Biocontrol efficacy on field turf. In 1991, one and two applications of GM 460 reduced the severity of brown patch on tall fescue turf 27 days after the last application (Table 5). Development of disease during the first month following infestation with R212 was low: less than 15% blight was measured in the infested control, which was not significantly different from that occurring in the noninfested plots. Higher levels of brown patch were not found until 28 August, when 35% of the turf in the infested control was blighted. In contrast, there was less than 20% blight in both GM 460 treatments, and they did not differ significantly. Subsequently, subnormal temperatures during September arrested further disease development, and less than 10% disease was found in all plots.

GM 460 persisted in the turf for 30 days after application (Table 5). Binucleate *Rhizoctonia* spp. indistinguishable from GM 460 were detected at low levels in treated plots, but not in the noninfested control. The highest frequency (3 cm of blades per 100 cm) was found in plots with two applications of GM 460. These plots had a frequency significantly ($P = 0.10$) greater than plots treated only once with GM 460 or the controls. *R. solani* was isolated from plots treated with GM 460 at reduced frequencies compared to the infested control, but the differences were not statistically significant.

In the 1992 experiment comparing TRBG, GM 460, and a combination of the two, the most consistently effective treatment was the combination applied three times (Fig. 3). During the period of greatest disease severity (5 to 21 August), there was 30–36% blight in the control; percent blight in the combination treatment was reduced ($P = 0.05$) at least 10%. Treatments involving TRBG alone reduced percent blight only on 21 August. During other periods, there were no significant differences in percent blighted turf among treatments, with all having less than 10% (*data not shown*). When disease severity was assessed as lesion ratings, the combination treatment was found to reduce lesion development ($P = 0.05$) compared to the control through the end of August, or 7 days after the last application of the biocontrol agents (Fig. 3). TRBG and GM 460 alone reduced lesion ratings only on the first reading on 9 July. There was no difference between one and three applications of the individual isolates. None of the biocontrol treatments differed significantly from the control by 3 September. Treatment with thiophanate-methyl was ineffective in reducing disease and resulted in disease levels that were generally as high or higher than those of the control. Disease levels in the fungicide-treated plots were significantly higher than levels in the GM 460 + TRBG combination at nearly every reading.

Table 4. Effects of incubating tall fescue seedlings treated with binucleate *Rhizoctonia* sp. GM 460 and *Gliocladium virens* TRBG at high and low humidity levels on the severity of brown patch caused by *Rhizoctonia solani* R212

Fungus[x]	Humidity[y]	Blighted foliage[z] (%)
GM 460	High	25 c
GM 460	Low	68 b
TRBG	High	37 c
TRBG	Low	100 a
None	High	95 a
None	Low	100 a

[x]All fungi were applied to cups of seedlings as two agar plugs per isolate. GM 460 and TRBG were applied 2 days prior to inoculation with R212.

[y]Humidity treatments were applied to biocontrol agent-treated seedlings during the 2-day incubation period prior to inoculation with R212. High relative humidity levels (>95%) were maintained by enclosing individual cups of seedlings in plastic bags. Low levels (35–45%) were maintained by incubating cups of seedlings open in a growth chamber. Following R212 inoculation, all of the cups of seedlings were placed in >95%. Relative humidity was measured using a relative humidity/temperature probe.

[z]Percent blighted foliage was determined 10 days after inoculation with the pathogen. Values represent nontransformed means from two experiments, each with five replications. Data from the two experiments were tested for error variance heterogeneity and transformed using arcsine transformation prior to pooled analysis of variance. No significant experiment effect or treatment × experiment interaction was found. Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test on arcsine-transformed data.

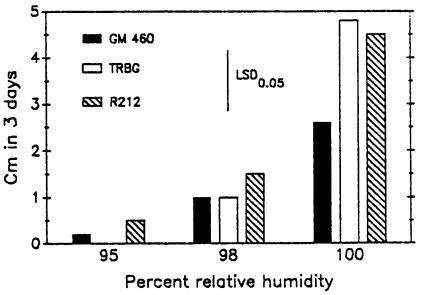

Fig. 2. Growth of binucleate *Rhizoctonia* GM 460, *Gliocladium virens* TRBG, and *R. solani* R212 on detached leaf blades of tall fescue as affected by relative humidity. Values represent means of two experiments. The vertical bar denotes LSD for comparing isolates within a relative humidity level.

DISCUSSION

We found biological control agents that can consistently inhibit the development of brown patch in the laboratory and provide reductions in disease severity in the field. The plant bioassay used to evaluate these organisms was effective in identifying competent antagonists that would be eliminated by commonly used in vitro screening systems. Antibiosis and mycoparasitism against *R. solani* by TRBG was observed in vitro, but GM 460 exhibited neither of these attributes (9; G. Y. Yuen, *unpublished*). Our findings support the contention that plant assays provide the best assessments of microbial biocontrol potential (18).

Disease severity in the field was measured by rating lesion severity on foliage through close inspection and by the more common method of estimating the blight severity from a distance. Brown patch disease in high-cut grasses such as tall fescue is manifested initially as discrete

Table 5. Inhibition of brown patch on tall fescue turf in 1991 by binucleate *Rhizoctonia* sp. GM 460 and detection of GM 460 and *Rhizoctonia solani* (Rs) on grass blades

Treatment[x]	Blight severity (% of turf)[y]	Isolation frequency (cm blades/100 cm)[z]	
		GM 460	Rs
Infested control	35 a	0.5 b	6.5 a
GM 460 one application	19 b	1.0 b	3.5 a
GM 460 two applications	13 b	3.0 a	3.0 a
Noninfested control	12 b	0.0 b	2.5 a

[x]The first application of GM 460, as colonized millet seed at 115 g/m^2, was made 1 wk prior to infestation of the turf with the pathogen. The second was made at the time of infestation.

[y]Blight severity was determined 27 days after infestation. Data presented are nontransformed means of four replications. Values within the column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$) on arcsine-transformed data.

[z]Isolation frequency was determined 30 days after infestation by culturing 100 cm of grass blades per sample on water agar amended with selective fungicides. Values within each column followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.10$.

lesions on blades and sheaths. Blighting of blades can occur subsequently as lesions expand in size and in number, but factors such as insects, environmental stress, and other diseases also can contribute to blight symptoms. Because biological control agents would more likely affect brown patch development in the early stages of an epidemic, we consider measurement of lesion development to be the more appropriate method for assessing biocontrol efficacy in the field. Although we observed biocontrol effects in 1992 using both methods, Sutker and Lucas (22) found brown patch biocontrol to occur only when they measured disease on live leaf clippings.

The capacity of GM 460 to persist and inhibit *R. solani* on tall fescue turfgrass was surprising, as it was originally isolated from soil (8), although other AG-B(o) isolates have been obtained from sugarbeet and various weed hosts (11). This finding suggests that the most effective antagonists against pathogens on turfgrass are not necessarily limited to organisms isolated from turf, but can also include organisms from other sources. The capacity of GM 460 to grow on turfgrass conceivably may be due to its inherent flexibility as a plant surface colonizer (10), but also may be related to the unique environment of the turfgrass canopy with its prolonged periods of leaf wetness and high humidity.

Our finding that TRBG can grow on green grass blades also is unique because *Gliocladium* and closely related *Trichoderma* are more commonly known as secondary colonizers of soil organic matter and the rhizosphere (19). Although growth of TRBG within grass blade tissues was not examined in this study, the fact that the spread of the fungus on grass blades was greatly limited by relative humidity suggests that it existed on the phylloplane. Unlike GM 460, TRBG could not inhibit blight after being subjected to low humidity, perhaps because of an inability of TRBG to produce survival structures while on the leaf surface. As evidence, TRBG produced no chlamydospores in molasses-yeast medium (G. Y. Yuen, *unpublished*), in contrast to *G. virens* GL-21, which produces great numbers of chlamydospores in the same substrate (13).

The two biocontrol agents in this study have very different attributes, and this diversity may be advantageous in the field. Although the combination of the two fungi in laboratory tests was not more effective than GM 460 alone, the combination was more consistent in suppressing disease in the field. Complex mixtures of microorganisms may be responsible for the effectiveness of particular composts and organic fertilizers in controlling brown patch (16). Our findings show that not all combinations of antagonists are beneficial and that compatibility between organisms must be considered. Population monitoring may have provided some indications as to why the combination of GM 460 and TRBG was effective in 1992, whereas the individual isolates were largely ineffective. Such studies, however, are logistically limited until markers become available to distinguish the biocontrol agents from similar resident fungi.

Using current delivery technology, disease suppression conferred by the biocontrol agents is not at levels that would economically justify their application. Increased efficacy may be possible through improvements in formulation. Biological control also will be more applicable when integrated with fungicides, resistance, and cultural management.

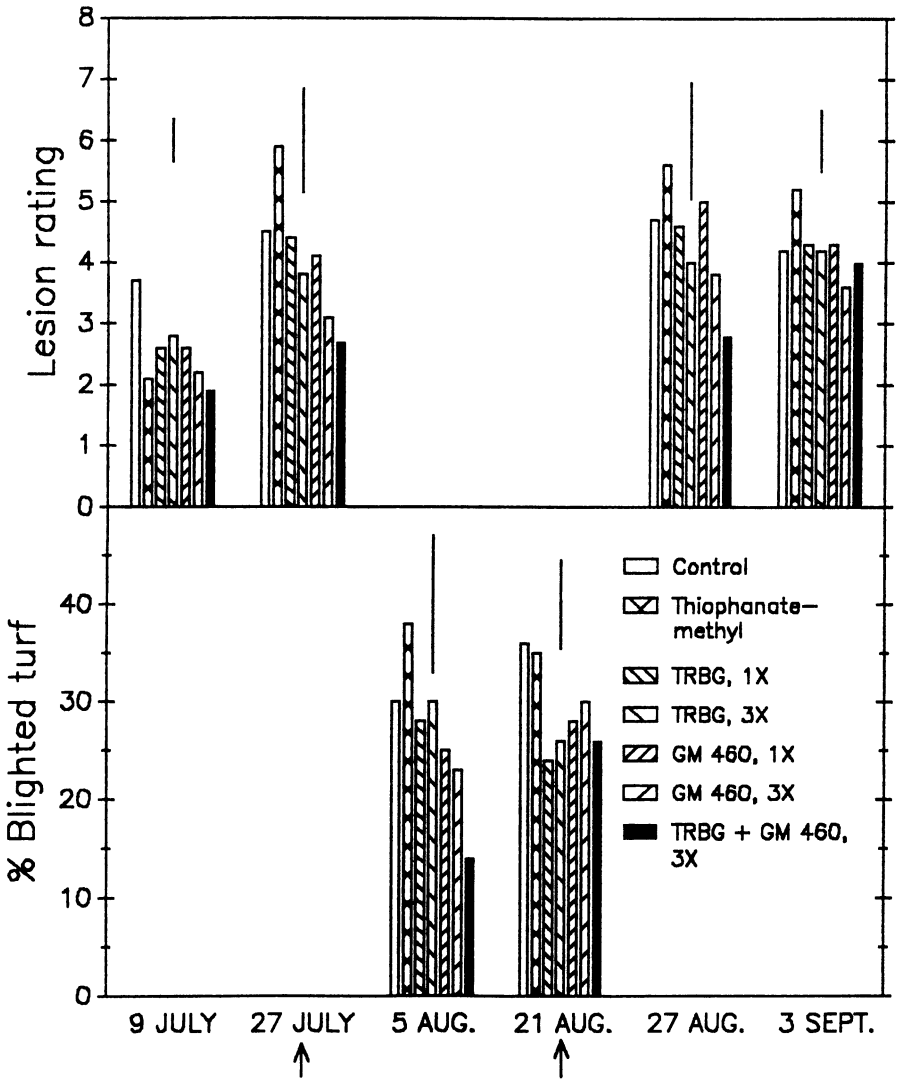

Fig. 3. Effects of binucleate *Rhizoctonia* GM 460, *Gliocladium virens* TRBG, a combination of the two isolates, and thiophanate-methyl on the severity of brown patch on tall fescue turf in 1992. Disease severity was measured as percent turf showing blight symptoms and as lesion ratings on a 0 to 10 scale (0 = no disease, 10 = most severe lesion development). Height of vertical bars indicates LSD values ($P = 0.05$) for each date. The arcsine transformation was not performed on percent blight data because variance heterogeneity was not found. Percent blight data for 9 and 27 July, 27 August, and 3 September are not presented because all treatment means were less than 10% and there were no significant treatment effects. Lesion ratings were not obtained on 5 and 21 August. GM 460 and TRBG were tested as single- and triple-application treatments. The combination treatment and the fungicide were applied three times. The first application of all treatments was made on 25 June, and subsequent applications are indicated by arrows. GM 460 and TRBG were applied as colonized millet seed and as alginate-bran pellets, respectively.

ACKNOWLEDGMENTS

We thank Leonard Herr, fellow committee member of NC-125 (Biocontrol of Soilborne Plant Pathogens), for providing isolate GM 460. This research was funded in part by grants from the National Turfgrass Evaluation Program and the University of Nebraska Research Council.

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