

Suppression of Gray Snow Mold on Creeping Bentgrass by an Isolate of *Typhula phacorrhiza*

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ABSTRACT

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Field studies were conducted in 1983 and 1984 to determine the effects of *Typhula phacorrhiza* (isolate T011) and *T. ishikariensis* var. *ishikariensis* (*T. i.* var. *ishikariensis*) (isolate T004) alone and in combination on creeping bentgrass. Isolate T011 was nonpathogenic; isolate T004 caused foliar blight and crown decay. Significantly less foliar necrosis was observed on bentgrass inoculated with a combination of isolates T011 and T004 than on bentgrass inoculated with isolate T004 alone. Sections of a creeping bentgrass golf green with a history of infection by *T. ishikariensis* showed 44 and 70% less gray snow mold when infested with 100 and 200 g/m² of wheat-grain inoculum of *T. phacorrhiza* in 1983 and 1984, respectively. Laboratory tests failed to reveal parasitism or hyphal interference among isolates of *T. phacorrhiza*, *T. incarnata*, and *T. i.* var. *ishikariensis* in culture. However, results of additional experiments indicated that growth of *T. incarnata* and *T. i.* var. *ishikariensis* was suppressed on BASM agar that had been exposed to *T. phacorrhiza*. Application of BASM broth to the exposed agar reduced the suppressive effects.

Additional key words: biological control, low-temperature-tolerant fungi, turfgrass

At least six species of low-temperature-tolerant plant-pathogenic fungi incite snow mold diseases of turfgrasses in cool humid and cool subhumid climates (17,18,22). Isolates of three snow mold fungi, *Microdochium nivale* (Fries) Samuels & Hallet (= *Fusarium nivale* Ces. ex Sacc.), *Typhula incarnata* Lasch. ex Fr., and *T. ishikariensis* Imai var. *ishikariensis* (*T. i.* var. *ishikariensis*), have been collected frequently from turfgrasses in southern Ontario (L. L. Burpee, unpublished).

Applications of fungicides in late autumn are recommended to protect turfgrass from infection by snow mold fungi (17,22); however, few chemicals provide acceptable control (<3% disease) when inoculum levels are high and turfgrass is covered with snow for 3 mo or longer (3,4). Fungicides containing mercury and PCNB are used extensively for winter disease management on turfgrasses in eastern Canada (L. L. Burpee, unpublished). The cost of applying these fungicides, coupled with environmental and health concerns about the use of mercury (12) and problems associated with the toxicity of PCNB to certain species of grasses (3,7), have prompted us to seek alternative approaches to snow mold management.

Few studies have been conducted to determine the feasibility of using microbial antagonism as a management tool for snow mold diseases. In laboratory studies, Harder and Troll (13) found that the viability of sclerotia of *Typhula incarnata* was significantly reduced in soil infested with *Trichoderma* spp. These researchers concluded that cultural practices designed to enhance growth of *Trichoderma* in soil may lead to a reduction in the incidence of gray snow mold. However, results of field experiments were not reported. Smith and Davidson (20) observed that isolates of *Acremonium boreale* Smith & Dav., a weakly virulent low-temperature-tolerant pathogen of grasses, were antagonistic in culture to five species of snow mold fungi including *Typhula incarnata* and *T. ishikariensis*. Antagonism was observed at temperatures ranging from -3 to 10 C. To our knowledge, *A. boreale* has not been evaluated as a biocontrol agent under field conditions.

Smith (18) has reviewed reports of interspecific and intraspecific antagonism among snow mold fungi. Antagonism appears to be quite common, particularly among isolates of *Typhula* spp., and it deserves further evaluation as a possible means of managing snow mold diseases.

In 1983, we collected sclerotia of *T. phacorrhiza* Fr. from the thatch layer of Kentucky bluegrass (*Poa pratensis* L.) near Cambridge, Ontario. *T. phacorrhiza*, assumed to be a low-temperature-tolerant saprophyte (15), has been observed frequently in southern Ontario on corn stover and other organic debris after at least 80 days of snow cover (L. L.

Burpee, unpublished). Recently, Seaman and Schneider (16) reported that isolates of *T. phacorrhiza* ranged from avirulent to highly virulent on winter wheat (*Triticum aestivum* L.). The induction of pathogenesis or disease suppression by *T. phacorrhiza* has not been reported on other cereals or grasses.

In the present study, the influence of an isolate of *T. phacorrhiza* alone or in combination with *T. ishikariensis* was examined on creeping bentgrass (*Agrostis palustris* Huds.) maintained as a golf putting green.

MATERIALS AND METHODS

Three experiments were conducted during 1983 and 1984 on a 7-yr-old stand of creeping bentgrass (cultivar Pennncross) maintained at the Ontario Ministry of Agriculture and Food Horticultural Research Station, Cambridge. Mowing, fertilization, and irrigation schemes were similar to those prescribed for bentgrass golf putting greens (2). Isolates of *Typhula* spp. that were used in the experiments included isolate T011 of *T. phacorrhiza* collected from the thatch layer of Kentucky bluegrass near Cambridge, Ontario, in 1982 and isolate T004 of *T. i.* var. *ishikariensis* collected from foliage of creeping bentgrass near Galt, Ontario, in 1982. Initial cultures of both isolates were derived from single sclerotia.

Pathogenicity and isolate interactions.

A field study was conducted from November 1983 through April 1984 to evaluate virulence of isolates T011 and T004 alone and in combination on creeping bentgrass. Inoculum was prepared by transferring mycelial plugs from colonies on BASM agar (19) to 1-L mason jars containing moist, autoclaved wheat grain (100 cm³ grain, 20 ml H₂O). Cultures were incubated for 12 wk at 10 C.

A sward (1 × 6 m) of creeping bentgrass was selected as an inoculation site. Snow mold was not observed on the site the previous spring. Treatments included inoculations of turf with autoclaved (heat-killed) and nonautoclaved inocula of isolate T004, isolate T011, and a mixture of T004 and T011. Twenty- or 40-cm³ aggregates of grain inoculum were placed 25 cm apart on the turf on 29 November 1983. Treatments were arranged in a randomized complete block design with four replicates.

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Immediately after inoculation, the turfgrass foliage was sprayed with water until runoff and the turf was covered with wooden frames (300 × 100 × 15 cm) overlain with 4-mil transparent plastic sheeting (6). The frames were used to maintain an extended period of leaf wetness for disease development.

The frames were removed 127 days after inoculation. A quantitative estimate of disease was made by measuring the diameter of the necrotic area of turf

surrounding each aggregate of inoculum. Values were subjected to analysis of variance, and means were statistically separated using Duncan's modified (Bayesian) least significant difference test (21). Samples of leaf tissue and fragments of grain inoculum were collected after the measurements were made. The material was washed for 1 hr in running tap water, blotted dry, and placed on acidified potato-dextrose agar (APDA), in petri dishes stored at 10 C. Fungi growing from the samples were identified after 2–4 wk of incubation.

Disease suppression studies. The snow mold suppression potential of *T. phacorrhiza* (isolate T011) was evaluated on creeping bentgrass turf with a history of severe infection by *T. ishikariensis*. Grain inoculum of isolates T011 and T004 was prepared as described before. Plots (1 m²) of turf were infested with 100 g of inoculum of one or both isolates on 18 November 1983 or with 200 g of inoculum on 21 November 1984. Inoculum was dispersed by hand onto the turf surface. Additional treatments included uninfested plots and, in 1984, plots infested with autoclaved, uncolonized grain. Treatments were arranged in a randomized complete block design with four replicates.

The Horsfall-Barratt rating system (14) was used to estimate disease intensity (percent necrotic foliage per plot)

beginning 140 and 147 days after inoculation in 1983 and 1984, respectively. Statistical procedures and postevaluation sampling and isolation techniques were the same as those described before.

Laboratory studies. *T. phacorrhiza* (isolate T011), *T. incarnata* (isolate T003), and *T. i. var. ishikariensis* (isolate T004) were paired on culture media to observe possible antagonism among colonies. Mycelial plugs from actively growing cultures of the test organisms were placed 4 cm apart on BASM agar in plastic petri dishes. Cultures were incubated at 1 or 10 C and observed for hyphal antagonism 1, 2, and 4 wk after pairing.

The isolates were also paired on strips of cellophane for microscopic observations of hyphal interactions. Mycelial plugs from colonies growing on BASM were placed 2 cm apart on strips 3.5 × 3 cm cut from a single layer of dialysis tubing. The strips had been soaked for 1 hr in sterile distilled water, separated into single layers, then autoclaved in distilled water before placement on the medium. After 5–7 days of incubation at 10 C, the strips were removed from the media and placed on microscope slides. The area of hyphal contact between isolates was treated with lactophenol and examined at 100 and 400×.

An additional method, based on the cellophane membrane technique of Dennis and Webster (8), was used to assess antibiotic and/or nutrient deprivation effects induced by *T. phacorrhiza* (isolate T011) on *T. incarnata* (isolate T003) and *T. i. var. ishikariensis* (isolate T004). A mycelial plug (4 mm in diameter) of isolate T011 was placed in the center of a sterile, 47-mm-diameter cellulose acetate filter (0.22-μm pore size) that had been placed on 6 ml of BASM agar in a plastic petri dish. Five replicate cultures were incubated for 10 days at 10 C in the dark. Dishes containing uninfested filters served as controls. After incubation, the diameter of each colony was about 32 mm. The filters were removed with sterile forceps, and a mycelial plug of isolate T003 or isolate T004 was placed on the agar surface in the center of each dish. Cultures were incubated at 10 C in the dark. The diameter of each colony was measured and oven-dry weights of sclerotia were determined at 7 and 30 days, respectively. Antagonism was assessed by comparing values obtained from untreated media with values obtained from media that had been pretreated with isolate T011.

To determine if the addition of nutrients could reduce or prevent antagonism induced by *T. phacorrhiza* (isolate T011), the cellulose acetate filter technique was modified to include applications of BASM broth to the media after the membranes were removed. Concentrations equivalent to 0.01×, 0.1×, and 1× BASM were

Table 1. Interaction between *Typhula phacorrhiza* (isolate T011) and *T. ishikariensis* var. *ishikariensis* (isolate T004) on creeping bentgrass

Treatment	Size of inoculum aggregate ^a (cm ³)	Diameter of necrotic turf ^b (cm)
Isolate T004	20	5.6 a ^c
Isolate T011	20	0.0 b
T004 + T011	20 + 20	2.9 c
T004 + autoclaved T011	20 + 20	4.1 ac
Autoclaved T011	20	0.0 b
Untreated	...	0.0 b

^aPlaced on turf surfaces in November 1983.

^bMean of four replicates (127 days after inoculation).

^cValues followed by the same letter are not significantly different according to Duncan's LSD at *P* = 0.05.

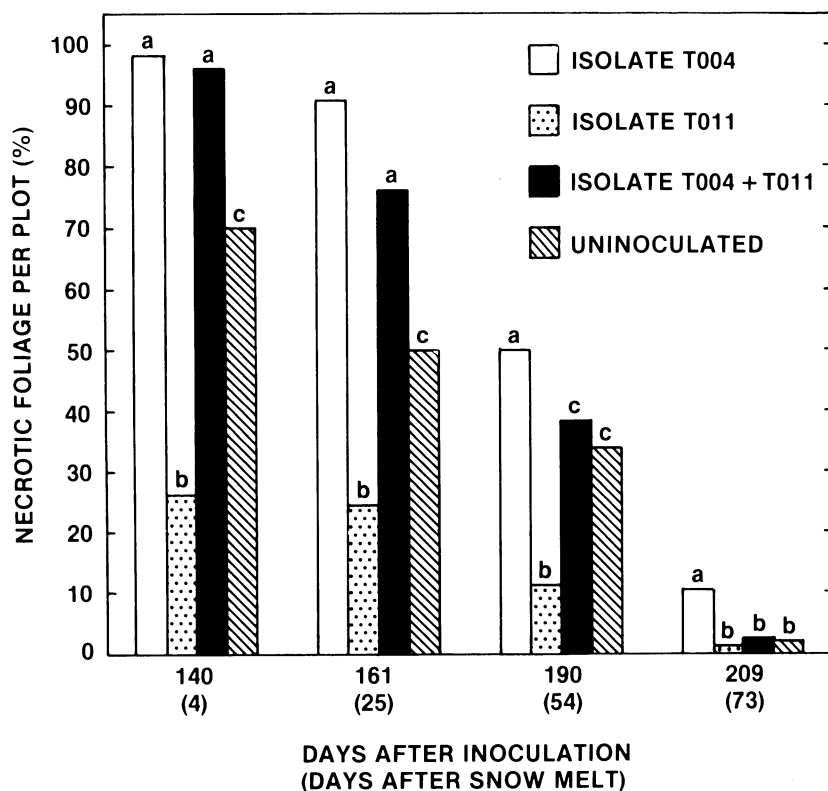


Fig. 1. Intensity of necrotic foliage in plots of creeping bentgrass in 1984 after 140–209 days of exposure to autoclaved wheat grain infested with *Typhula ishikariensis* var. *ishikariensis* (isolate T004) and/or *T. phacorrhiza* (isolate T011). All plots were infested with sclerotia of *T. ishikariensis* from previous epidemics of gray snow mold. Bars with the same letter do not represent significantly different values (*P* = 0.05) according to Duncan's least significant difference test.

obtained by adding 0.6 ml of BASM broth, from dilutions of a 10 \times -strength stock solution, to each petri dish of BASM agar that had been exposed to isolate T011. Where no nutrient was added, 0.6 ml of sterile distilled water was applied. Volumes were allowed to absorb into the medium overnight before placing mycelial plugs of isolates T003 or T004 on the agar surface. Five replicate dishes of each concentration were prepared for each isolate. Diameter of colonies and dry weights of sclerotia were determined as described before. Simple linear regressions were used to detect a significant change in colony diameter and/or sclerotial dry weight with increasing concentration of nutrients.

RESULTS

Pathogenicity and isolate interactions. *T. i. var. ishihikariensis* (isolate T004) was pathogenic on creeping bentgrass, and *T. phacorrhiza* (isolate T011) was nonpathogenic (Table 1). Significantly less necrotic foliage was observed in plots infested with a mixture of isolates T004 and T011 than in plots infested with isolate T004 alone. The intensity of necrotic foliage was not limited significantly in plots infested with a mixture of isolate T004 and autoclaved inoculum of isolate T011. *T. phacorrhiza* was isolated from symptomless foliage and wheat grain collected from plots infested with isolate T011. *T. ishihikariensis* was isolated from necrotic foliage and wheat grain collected from plots infested with isolate T004.

Disease suppression studies. All plots were infested naturally with sclerotia of *T. ishihikariensis* from previous epidemics of gray snow mold. Gray snow mold was the only disease observed in the experimental plots after snow melt in 1984 and 1985. A 70% intensity of necrotic foliage was observed initially in uninoculated (naturally infested) plots in 1984 (Fig. 1). This was significantly less than the intensity of necrosis in plots infested with grain inoculum of *T. i. var. ishihikariensis* (isolate T004) or a mixture of inoculum of isolate T004 and *T. phacorrhiza* (isolate T011). For at least

190 days after inoculation (54 days after snow melt), turfgrass inoculated with isolate T011 alone showed significantly less necrosis than turf subjected to the other treatments (Figs. 1 and 2). A similar effect was noted in 1985 (Figs. 3 and 4). In both years, intensity of necrosis declined in all plots after snow melt because of the growth of symptomless foliage. *T. phacorrhiza* was isolated from symptomless and senescent foliage collected from plots infested with isolate T011. Sclerotia of *T. phacorrhiza* were observed on senescent leaves and stolons. *T. ishihikariensis* was isolated from necrotic foliage collected from all plots.

Laboratory studies. With the exception of a weak barrage observed between colonies of *T. incarnata* (isolate T003) and *T. phacorrhiza* (isolate T011), antagonism was not evident among any isolates paired on BASM agar. No evidence of cellular lysis or hyperparasitism was found on cellophane in areas of intrasolate and interisolate mycelial contact.

Exposure of BASM agar to *T. phacorrhiza* (isolate T011) for 10 days at 10 C (Table 2) resulted in significant ($P = 0.05$) reduction in mycelial growth and sclerotial dry weight of *T. incarnata* (isolate T003) and *T. i. var. ishihikariensis* (isolate T004). Applications of various concentrations of BASM broth to BASM agar, after the agar was exposed to isolate T011, resulted in a significant ($P = 0.001$) linear increase in dry weight of sclerotia in isolates T003 and T004 and

a significant ($P = 0.001$) linear increase in colony diameter in isolate T004 (Table 3).

DISCUSSION

Isolate T011 of *T. phacorrhiza* is a low-temperature-tolerant saprotroph that can grow under snow, colonize senescent and nonsenescent foliage of creeping bentgrass, and suppress the development of gray snow mold caused by *T. i. var. ishihikariensis*. Observations of interactions among hyphae of isolate T011 and isolates of *T. i. var. ishihikariensis* and *T. incarnata* suggest that the mechanism of disease suppression is not hyperparasitism or cellular lysis induced by an antibiotic or by hyphal contact. A nutrient-mediated reduction in the suppression of growth of *T. i. var. ishihikariensis* and *T. incarnata* on agar that was exposed to isolate T011 (Table 3) suggests that *T. phacorrhiza* may inhibit other species of *Typhula* via nutrient competition. However, use of the cellulose acetate filter technique does not preclude the possibility that secondary metabolites and/or staling products are involved in antagonism. This problem must be addressed in further studies using cellfree extracts of liquid media exposed to isolate T011.

Other possible mechanisms of antagonism induced by *T. phacorrhiza* include substrate possession (i.e., competition for space) and induced resistance. Preliminary observations have revealed that pathogenic species of *Typhula* penetrate turfgrass leaves via stomata and/or wounds (L. L. Burpee, unpublished). Direct penetration of epidermal cells has not been observed. If hyphae of *T. phacorrhiza* colonize leaves in a similar fashion, then antagonism may result from competition for penetration sites and/or from the induction of antimicrobial substances in wounds (1). It is possible that these types of antagonism may function in inhibiting infection by other snow mold fungi (e.g., *Coprinus psychromorbidus* Redh. & Trag. and *Microdochium nivale* (Fr.) Samu. & Hall) that penetrate via stomata and wounds (9,11).



Fig. 2. Suppression of gray snow mold by *Typhula phacorrhiza* (isolate T011) in 1-m² plots of creeping bentgrass in 1984. Turfgrass in the plots on the left and right was exposed for 140 days to autoclaved wheat grain (100 g/m²) infested with isolate T011. Turfgrass in the central plot was not exposed to isolate T011.

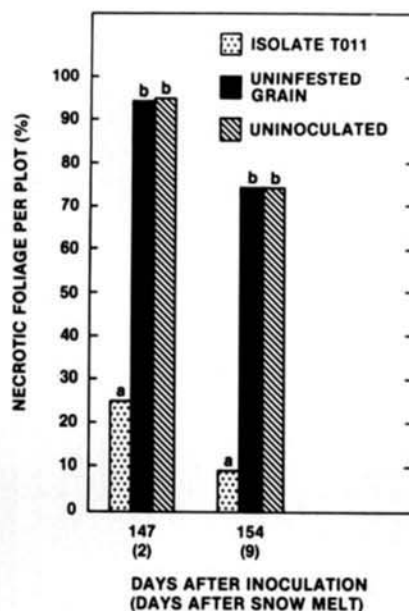


Fig. 3. Intensity of necrotic foliage in plots of creeping bentgrass in 1985 after 147 and 154 days of exposure to autoclaved wheat grain infested with *Typhula phacorrhiza* (isolate T011). All plots were infested with sclerotia of *T. ishihikariensis* from previous epidemics of gray snow mold. Bars with the same letter do not represent significantly different values ($P = 0.05$) according to Duncan's least significant difference test.

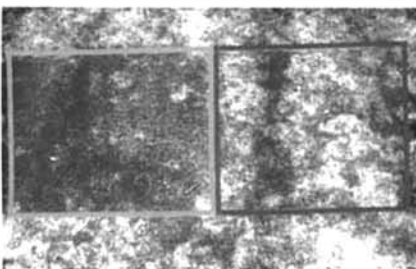


Fig. 4. Suppression of gray snow mold by *Typhula phacorrhiza* (isolate T011) in 1-m² plots of creeping bentgrass in 1985. Turfgrass in the plot on the left was exposed for 147 days to autoclaved wheat grain (200 g/m²) infested with isolate T011. Turfgrass in the plot on the right was exposed to uninfested wheat grain.

Table 2. Influence of the exposure of BASM agar to *Typhula phacorrhiza* (isolate T011) on mycelial growth and sclerotial dry weight of *T. incarnata* (isolate T003) and *T. ishihariensis* var. *ishikariensis* (isolate T004)

Treatment ^a	Isolate T003 ^b		Isolate T004 ^b	
	Colony diameter (mm)	Sclerotial weight (mg)	Colony diameter (mm)	Sclerotial weight (mg)
Isolate T011	26.2 a ^c	10.7 a	26.9 a	4.2 a
Untreated	31.2 b	30.6 b	28.9 b	16.2 b

^aA culture of isolate T011 was incubated at 10 C for 10 days on a cellulose acetate membrane (0.22-μm pore size) placed on BASM agar. The membrane was then removed and mycelial plugs of isolates T003 or T004 were placed on the agar.

^bDiameters of colonies were measured and oven-dry weight of sclerotia was determined after incubation at 10 C for 7 and 30 days, respectively. Values represent means of five replicates.

^cWithin a column, values followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's least significant difference test.

Table 3. Linear regression of colony diameter and sclerotial dry weight of *Typhula incarnata* (isolate T003) or *T. ishihariensis* var. *ishikariensis* (isolate T004) versus concentration of BASM broth applied to BASM agar after exposure of the agar to *T. phacorrhiza* (isolate T011)^a

Isolate	Parameter ^b	Slope coefficient	r ²	t value ^c
T003	Colony diameter	-0.64	0.05	0.94
	Sclerotial dry wt	200.51	0.97	25.84***
T004	Colony diameter	3.29	0.69	6.29***
	Sclerotial dry wt	107.83	0.96	20.41***

^aA culture of isolate T011 was incubated at 10 C for 10 days on a cellulose acetate membrane (0.22-μm pore size) placed on BASM agar. The membrane was then removed, BASM broth was applied to the agar at concentrations of 0.01 X, 0.1 X, and 1 X, and mycelial plugs of isolates T003 or T004 were placed on the agar.

^bDiameters of colonies were measured and oven-dry weight of sclerotia was determined after incubation at 10 C for 7 and 30 days, respectively.

^cValues of t marked with asterisks are significant at $P = 0.001$.

Results of field experiments indicate that isolate T011 suppressed development of gray snow mold in plots of creeping bentgrass infested with natural inoculum (i.e., sclerotia from previous epidemics) of *T. ishihariensis* but not in plots infested with a combination of natural inoculum and unnatural inoculum (i.e., infested grain). This suggests that disease suppression by *T. phacorrhiza* is influenced by the quantity and/or quality of inoculum of *T. ishihariensis* in a turfgrass sward. Further studies are required to determine how the concentration of sclerotia of *T. ishihariensis* in soil and the nutrient status of the soil (e.g., content of organic matter) influence the disease suppression potential of *T. phacorrhiza*.

We believe that isolate T011 can be formulated into a mycofungicide for control of gray snow mold of creeping bentgrass and possibly other turfgrasses, forage grasses, and winter cereals. Isolate T011 can be cultured easily on wheat or rye grain with a technique similar to that used in the mushroom spawn industry (5). Furthermore, preliminary studies indicate that mycelial and/or sclerotial fragments of isolate T011 can be

formulated with sodium alginate (10,23) into pellets of a uniform size that can be applied to turfgrass with standard commercial fertilizer spreaders (M. B. Lawton, unpublished). Application of these "artificial sclerotia" in late autumn, before snowfall, may lead to a high incidence of foliar colonization by *T. phacorrhiza* under conditions (>90 days of snow cover) that are conducive for infection by *T. ishihariensis*. In addition, the production of sclerotia of *T. phacorrhiza* on senescent leaves of creeping bentgrass, after infestation with isolate T011, suggests that disease suppression may be observed for more than a single season.

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