Effect of Rate and Frequency of Application of *Typhula phacorrhiza* on Biological Control of Typhula Blight of Creeping Bentgrass

M. B. Lawton and L. L. Burpee

Graduate student and associate professor, Department of Environmental Biology and the Guelph Turfgrass Institute, University of Guelph, Guelph, Ontario N1G 2W1.

Research was supported by a graduate scholarship to M. B. Lawton from the Natural Sciences and Engineering Research Council of Canada and by a grant from Philom Bios Inc. of Saskatoon, Saskatchewan.

We thank L. G. Goulty for excellent technical assistance.

Accepted for publication 7 August 1989 (submitted for electronic processing).

ABSTRACT

Lawton, M. B., and Burpee, L. L. 1990. Effect of rate and frequency of application of *Typhula phacorrhiza* on biological control of Typhula blight of creeping bentgrass. Phytopathology 80:70-73.

Field studies were conducted from 1985 to 1988 to determine the effect of rate and frequency of application of *Typhula phacorrhiza* (isolate T016) on control of Typhula blight of creeping bentgrass. In 1985 and 1986, plots of creeping bentgrass were treated with 0, 50, 100, 200, and 400 g/m^2 of autoclaved grain infested with isolate T016. The increase in application rate resulted in: 1) a significant decrease in intensity of Typhula blight, 2) a reduction in time required for turfgrass to recover from injury by pathogenic species of *Typhula*, 3) an increase in number of sclerotia of *T. phacorrhiza* recovered from turfgrass thatch, and 4) a decrease in number of sclerotia of *T. ishikariensis* var. *ishikariensis* plus *T. incarnata* recovered from thatch. The intensity of Typhula blight, the time required for turfgrass recovery, and the number of sclerotia of *T. i. ishikariensis* plus *T. incarnata* recovered from thatch were significantly reduced in plots of creeping bentgrass that received applications of grain colonized by isolate T016 in 1985 and 1986 compared with that of plots that received applications in 1985 only. After applications of colonized grain in the fall of 1985 and 1986, in 1988 residual activity of isolate T016 was detected via a reduction in intensity of Typhula blight and an increased number of sclerotia of *T. phacorrhiza* recovered from thatch.

Additional keywords: Agrostis palustris, snow mold.

Typhula blight (gray or speckled snow mold) caused by *Typhula ishikariensis* Imai and *T. incarnata* Fr. is a common disease of turfgrasses in areas where sufficient inoculum exists and snow cover exceeds 90 continuous days. Fungicides containing mercury or pentachloronitrobenzene (PCNB) provide excellent control of Typhula blight of turfgrass in Canada (2,12). However, environmental and health concerns about the use of mercury (6) and the toxicity of PCNB to certain species of grasses (3,5) limits use of these fungicides for control of Typhula blight. Studies by Burpee et al (4) revealed that plots of creeping bentgrass (*Agrostis palustris* Huds.) treated with grain infested with isolate T011 of *T. phacorrhiza* (Reichard:Fr.) Fr. exhibited significantly less Typhula blight than untreated plots. When grain infested with isolate T011 was applied at 100 g/m^2 in 1983 and at 200 g/m^2 in 1984, the intensity of Typhula blight was suppressed by 63 and 74%, respectively (4). However, residual control of Typhula blight was not observed in the plots the following year (10).

In the present study, the efficacy of isolate T016 of *T. phacorrhiza* applied at different rates to creeping bentgrass was examined over a 3-yr period. Applications of grain infested with the biological control agent were made in the first 2 yr, with residual activity monitored in the third year. Disease intensity, population densities of *Typhula* spp., and time required for recovery (i.e., regrowth) of turfgrass after Typhula blight damage were measured each year.

MATERIALS AND METHODS

Suppression of Typhula blight by isolate T016 of *T. phacorrhiza* was evaluated over a period of 3 yr on a 4- × 9-m sward of 8-yr-old creeping bentgrass (cultivar Penncross) with a history of severe Typhula blight incited by *T. ishikariensis* var. *ishikariensis* Arsvoll & Smith and *T. incarnata*. The turfgrass was mowed, fertilized, and irrigated as prescribed for bentgrass golf putting greens (1). Experiments were conducted at the Ontario Ministry of Agriculture and Food Horticulture Research Station located in Cambridge, Ontario.

Isolate T016 of *T. phacorrhiza* was collected as a sclerotium from the foliage of winter wheat near Cambridge, Ontario, in 1982. Cultures of the fungus were produced by inducing myceliogenic germination of the sclerotium at 10 C in the dark on Basidiomycete agar for systematics, modified by Smith (11). Inoculum was prepared by soaking a mixture of equal weights of four grains (wheat, oats, barley, and corn) in water for 4 hr. One-liter mason jars were half-filled with the grain and autoclaved twice for 20 min at 121 C and 137 kPa. Three to five mycelial plugs of isolate T016 from actively growing colonies on Basidiomycete agar for systematics were placed in each jar. After incubating the cultures for 10–12 wk at 10 C, infested grain was air dried in a laminar-flow microbial transfer hood at 23 C for 24 hr.

Applications of 50, 100, 200, or 400 g/m^2 of colonized grain (1.75×10^3, 3.5×10^3, 7.0×10^3, and 1.4×10^4 colony-forming units [cfu], respectively) and 100, 200, and 400 g/m^2 of heat-killed colonized grain were made by hand to the surface of plots (1 m^2) of creeping bentgrass on 4 December 1985. Remaining plots were left untreated, or they received an application of PCNB 17G at 30 kg ai/ha. The fungicide was applied with a drop-type fertilizer spreader (O.M. Scott, Marysville, OH). The nine treatments were arranged in a randomized complete block design with four replicates.

Snow covered the plots on 4 December 1985 and melted on 25 March 1986, resulting in 111 days of continuous snow cover. The Horsfall-Barratt rating scale (7) was used to estimate disease intensity (percent necrotic foliage per plot) on 30 March 1986. Recovery of the creeping bentgrass from snow mold damage (i.e., time required for >97% of the turfgrass canopy to redevelop from stolons) was determined from Horsfall-Barratt ratings recorded at weekly intervals from 30 March to 26 May 1986. Five soil cores (10 cm deep and 5 cm in diameter) containing turfgrass stolons and thatch were removed from each plot on 31 March 1986 to estimate the density of sclerotia of *Typhula* spp. per plot. Cores were teased apart with forceps and examined

with a dissecting microscope (10×) for the enumeration of sclerotia.

On 28 November 1986, identical treatments were applied to the same plots of creeping bentgrass. Snow covered the plots on 2 December 1986 and melted on 28 March 1987, resulting in 116 days of continuous snow cover. Disease intensity was recorded on 30 March 1987. Recovery of turfgrass was monitored at weekly intervals until 25 May 1987. Sclerotia were counted as described previously from five soil cores removed from each plot on 6 April 1987.

In the fall of 1987, all plots were left untreated to monitor residual disease suppression resulting from colonized grain of isolate T016 applied in the fall of 1985 and 1986. Snow covered the plots from 15 December 1987 to 19 January 1988 and from 2 February to 27 March 1988, resulting in 89 days of discontinuous snow cover. Disease intensity was recorded on 31 March 1988, recovery of turfgrass was monitored at weekly intervals until 12 May 1988, and sclerotia from five soil cores per plot were counted from samples taken on 7 April 1988.

A $\log(x+1)$ transformation of application rate (x) of colonized grain of isolate T016 was used in the derivation of regressions to detect significant changes in disease intensity, recovery of turfgrass, and number of sclerotia formed per plot in 1986, 1987, and 1988 in response to increasing concentrations of isolate T016. Analysis of covariance (13) was used to compare slopes of regressions from different years.

RESULTS

Mean levels of intensity of Typhula blight in untreated plots after snow melt were 84, 96, and 56% in the spring of 1986, 1987, and 1988, respectively. Recovery of sclerotia of *T. i. ishikariensis* from necrotic foliage of turfgrass in all 3 yr and recovery of sclerotia of *T. incarnata* in 1987 and 1988 indicated that these species were the incitants of Typhula blight in the experimental plots.

A highly significant decline in disease intensity occurred in 1986 ($P = 0.001$), 1987 ($P = 0.001$), and 1988 ($P = 0.01$) in response to increases in concentration of isolate T016 of *T. phacorrhiza* applied in the fall of 1985 and 1986 (Fig. 1). The slope of the line generated when disease intensity was plotted against the log of application rate of isolate T016 was significantly ($P = 0.01$) greater in 1987 than in 1986 (Table 1). The slope of the regression of disease intensity versus the log of application rate of isolate T016 was not significantly different between 1986 and 1988, but it was significantly ($P = 0.01$) less in 1988 than in 1987.

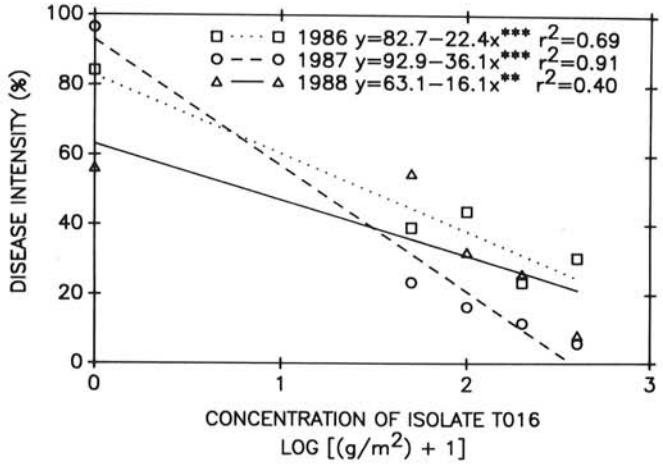

Fig. 1. Regressions of concentration of isolate T016 of *Typhula phacorrhiza* applied to creeping bentgrass versus intensity of Typhula blight in 1986, 1987, and 1988. Slopes are significantly different from zero at $P = 0.01$ (**) and $P = 0.001$ (***). Equations and coefficients of determination (r^2) are based on values from four replicate plots. Mean values are represented as points on the regression line.

As the concentration of isolate T016 increased, the time required for turfgrass to recover to <3% necrotic tissue decreased significantly in 1986 ($P = 0.05$) and 1987 ($P = 0.001$), but not in 1988 (Fig. 2). The slope of the line generated when recovery time was plotted against the log of application rate of isolate T016 was significantly ($P = 0.01$) greater in 1987 than in 1986 (Table 1). The slope of the regression of recovery time versus log of application rate of isolate T016 in 1988 was not significantly different from the 1986 regression, but it was significantly ($P = 0.01$) less than the 1987 regression.

A significant ($P = 0.001$) increase in the number of sclerotia of *T. phacorrhiza* was detected in plots in the spring of 1986, 1987, and 1988 in response to increases in concentration of grain colonized by isolate T016 of *T. phacorrhiza* applied in the fall of 1985 and 1986 (Fig. 3). The slopes of the regressions between number of sclerotia of *T. phacorrhiza* recovered and the log of application rate of isolate T016 were not significantly different in 1986 and 1987 (Table 1). The slope of the regression between number of sclerotia of *T. phacorrhiza* recovered and the log of application rate of isolate T016 in 1988 was not significantly different from the slope for 1986, but it was significantly ($P = 0.01$) less than the slope for the 1987 regression.

In response to increases in concentrations of grain colonized by isolate T016 of *T. phacorrhiza* applied in the fall of 1985 and 1986 (Fig. 4), a significant decrease in number of sclerotia of *T. i. ishikariensis* plus *T. incarnata* was detected in plots in the spring of 1986 ($P = 0.01$) and 1987 ($P = 0.001$), but not in 1988. The slope of the regression between number of sclerotia of *T. i. ishikariensis* plus *T. incarnata* recovered and the log of application rate of isolate T016 was significantly ($P = 0.01$) greater in 1987 than in 1986 (Table 1). The slopes of the 1986 and 1988 regressions for number of sclerotia of *T. i. ishikariensis* plus *T. incarnata* recovered and log of application rate of isolate T016 were not significantly different; however, the slope of the regression in 1988 was significantly ($P = 0.01$) less than that of the 1987 regression.

Disease intensities, recovery times, and numbers of sclerotia of pathogenic *Typhula* spp. were similar in plots treated with heat-killed colonized grain and in plots left untreated over all 3 yr. Plots treated with PCNB had <3% necrotic tissue, recovered

TABLE 1. Comparison of slopes of regressions of intensity of Typhula blight, time required for >97% of the turfgrass canopy to redevelop after Typhula blight, number of sclerotia of *Typhula phacorrhiza* recovered from turfgrass thatch, and number of sclerotia of *T. ishikariensis* var. *ishikariensis* plus *T. incarnata* recovered from thatch versus log (concentration +1) of isolate T016 of *T. phacorrhiza* applied to plots of creeping bentgrass[a]

Parameter	Year	Slope	*F*-Value[b]
Disease intensity	1986 vs. 1987	−22.4 vs. −36.1	9.730**
	1986 vs. 1988	−22.4 vs. −16.1	1.162
	1987 vs. 1988	−36.1 vs. −16.1	13.985**
Recovery time	1986 vs. 1987	−0.94 vs. −2.19	7.863**
	1986 vs. 1988	−0.94 vs. −0.53	0.666
	1987 vs. 1988	−2.19 vs. −0.53	13.935**
No. of sclerotia of *T. phacorrhiza*	1986 vs. 1987	16.7 vs. 22.1	0.842
	1986 vs. 1988	16.7 vs. 8.82	3.278
	1987 vs. 1988	22.1 vs. 8.82	8.435**
T. ishikariensis and *T. incarnata*	1986 vs. 1987	−10.6 vs. −28.5	18.501**
	1986 vs. 1988	−10.6 vs. −5.05	2.122
	1987 vs. 1988	−28.5 vs. −5.05	33.946**

[a]The incitant of Typhula blight was *T. i. ishikariensis* in 1986 and a combination of *T. i. ishikariensis* and *T. incarnata* in 1987 and 1988. Plots were treated on 4 December 1985 and 28 November 1986 with 0, 50, 100, 200, or 400 g/m² of grain colonized by isolate T016 of *T. phacorrhiza*.

[b]Slopes of regressions are significantly different from each other at $P = 0.01$ (**).

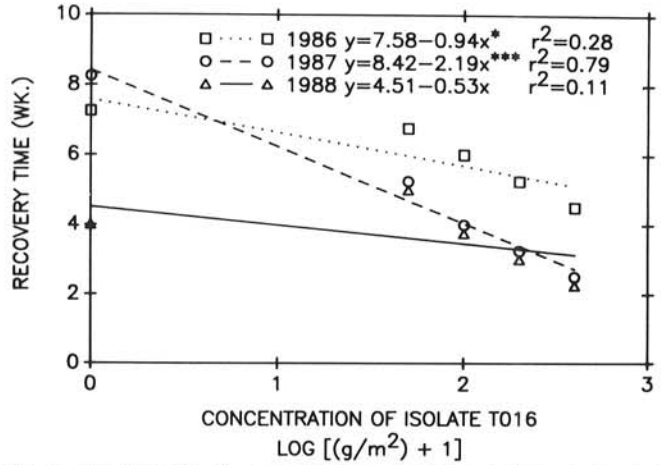

Fig. 2. Regressions of concentration of isolate T016 of *Typhula phacorrhiza* applied to creeping bentgrass versus time required for recovery of turfgrass in 1986, 1987, and 1988. Slopes are significantly different from zero at $P = 0.05$ (*) and $P = 0.001$ (***). Equations and coefficients of determination (r^2) are based on values from four replicate plots. Mean values are represented as points on the regression line.

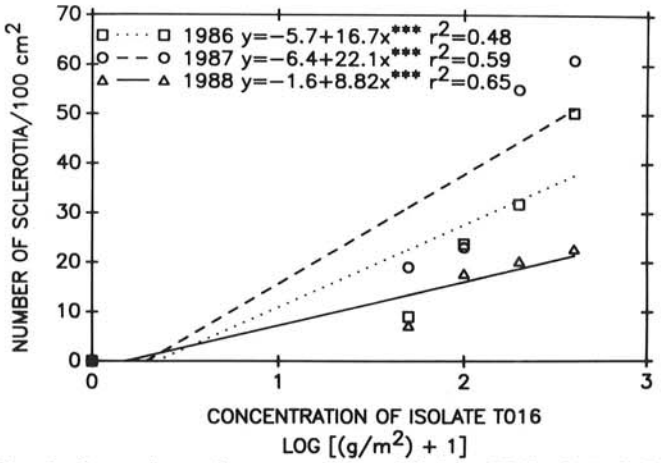

Fig. 3. Regressions of concentration of isolate T016 of *Typhula phacorrhiza* applied to creeping bentgrass versus number of sclerotia of *T. phacorrhiza* recovered from turfgrass in 1986, 1987, and 1988. Slopes are significantly different from zero at $P = 0.001$ (***). Equations and coefficients of determination (r^2) are based on values from four replicate plots. Mean values are represented as points on the regression line.

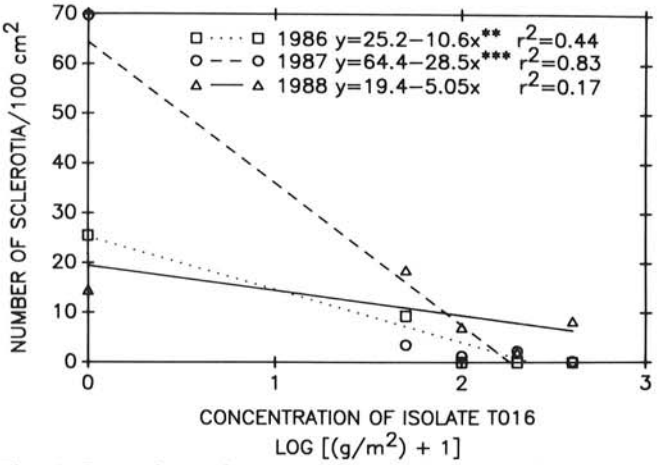

Fig. 4. Regressions of concentration of isolate T016 of *Typhula phacorrhiza* applied to creeping bentgrass versus number of sclerotia of *T. ishikariensis* var. *ishikariensis* plus *T. incarnata* recovered from turfgrass in 1986, 1987, and 1988. Slopes are significantly different from zero at $P = 0.01$ (**) and $P = 0.001$ (***). Equations and coefficients of determination (r^2) are based on values from four replicate plots. Mean values are represented as points on the regression line.

in <1 wk, and had <1 sclerotia of pathogenic *Typhula* spp. per 100 cm^2 after snow melt in 1986 and 1987. No residual activity was observed in these plots in 1988. Sclerotia of *T. phacorrhiza* were not recovered in 1986, 1987, or 1988 from untreated plots, from plots treated with PCNB, or from plots treated with heat-killed colonized grain.

DISCUSSION

Success of *T. phacorrhiza* as a biological control agent of Typhula blight of creeping bentgrass depends on the concentration of the antagonist applied. High rates of the agent (>100 g of colonized grain per m^2) were effective because a greater number of particles of colonized grain were applied per unit area from which *T. phacorrhiza* could initiate mycelial growth.

Although it is important to determine application rates of biological control agents that maximize disease control, relying on excessive rates may be unacceptable because of high costs of production and dissemination. At present, a rate of 200 g/m^2 of grain colonized by *T. phacorrhiza* (7.0×10^3 cfu) is required to provide suppression of Typhula blight that is equivalent to suppression achieved with PCNB (4). However, selection of isolates with potential for enhanced disease suppression and use of alternative delivery systems may lead to a reduction in application rates. Recently, we reported (9) that isolates of *T. phacorrhiza* vary significantly in their potential to suppress Typhula blight. In addition, *T. phacorrhiza* has been incorporated into alginate pellets (14) for dissemination in the field (8). Incorporation of highly disease-suppressive isolates of *T. phacorrhiza* into alginate pellets may improve the efficacy of biological control.

The presence of sclerotia of isolate T016 of *T. phacorrhiza* in turfgrass thatch suggests that, in a perennial crop such as creeping bentgrass, a resident population of the antagonist may develop over time, resulting in residual suppression of Typhula blight. Lawton et al (10) reported that a single application of isolate T011 of *T. phacorrhiza* failed to control Typhula blight 16 mo after application. However, in our study, significant differences in responses to the concentration of *T. phacorrhiza* (isolate T016) in 1987 compared with 1986 suggests that sclerotia produced after the first application germinated and suppressed disease the following year. Reductions in disease intensity recorded 16 mo after two annual applications of isolate T016 (Fig. 1) indicate that residual activity was operative. This suggests that survival of the biocontrol agent in turfgrass thatch and/or soil, coupled with a reduction in the population density of pathogenic *Typhula* spp., can result in suppression of Typhula blight after annual applications of *T. phacorrhiza* have ceased.

The importance of a balance between population densities of the antagonist and the pathogen has been demonstrated (4). Isolate T011 of *T. phacorrhiza* suppressed Typhula blight in plots of creeping bentgrass infested naturally with sclerotia of *T. i. ishikariensis* but not in plots treated with additional inoculum of the pathogen. Our results indicate that at least 2 yr of application of isolate T016 is required to establish a balance between antagonist and pathogen before residual suppression of Typhula blight is detected.

The mechanism of antagonism of *T. phacorrhiza* remains unclear. Dual pairings of the antagonist and the pathogen showed no evidence of antibiosis or parasitism (4,8). Preliminary studies (8) suggest that competitive colonization of turfgrass thatch may be a factor in the suppression of Typhula blight. Colonization and possession of thatch by *T. phacorrhiza* may limit mycelial growth of the pathogen by competition for nutrients and/or space or by production of inhibitory compounds. Unidentified staling products or water-diffusable metabolites are thought to be involved in mutual antagonism between pathogenic species of *Typhula* (12). Selection of isolates of *T. phacorrhiza* with an increased potential to colonize and establish a population in turfgrass thatch may result in increased efficacy of biological control of Typhula blight of creeping bentgrass.

LITERATURE CITED

1. Beard, J. B. 1982. Turf Management for Golf Courses. Burgess Publishing, Minneapolis, MN. 642 pp.
2. Burpee, L. L. 1988. Preventative control of cold-weather diseases. Golf Course Management 56(8):62-68.
3. Burpee, L. L., and Goulty, L. G. 1984. Evaluation of fungicides for control of pink and grey snow mold on creeping bentgrass. Pages 6–7 in: Turfgrass Research Annual Report. R. W. Sheard, ed. University of Guelph, Guelph, Ont. 38 pp.
4. Burpee, L. L., Kaye, L. M., Goulty, L. G., and Lawton, M. B. 1987. Suppression of gray snow mold on creeping bentgrass by an isolate of *Typhula phacorrhiza*. Plant Dis. 71:97-100.
5. Fushtey, S. G. 1980. Chemical control of snow mold in bentgrass turf in southern Ontario. Can. Plant Dis. Surv. 60:25-31.
6. Granhall, I. 1971. Environmental aspects of using persistent pesticides. Proc. Br. Insect. Fungic. Conf. 3:735-740.
7. Horsfall, J. G., and Cowling, E. G. 1978. Phytopathometry: The measurement of plant disease. Pages 120–136 in: Plant Disease. An Advanced Treatise. Vol. 1. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York. 465 pp.
8. Lawton, M. B. 1989. Studies on the control of snow molds on turfgrass and winter wheat in Ontario. Ph.D. thesis. University of Guelph, Guelph, Ont. 189 pp.
9. Lawton, M. B., and Burpee, L. L. 1988. Variation in suppression of Typhula blight (gray snow mold) of turfgrass by isolates of *Typhula phacorrhiza*. (Abstr.) Can. J. Plant Pathol. 10:368.
10. Lawton, M. B., Burpee, L. L., and Goulty, L. G. 1986. Factors influencing the efficacy of a biofungicide for control of grey snow mould on turfgrass. Proc. Br. Crop Prot. Conf. 1:393-398.
11. Smith, J. D. 1981. Snow molds of winter cereals: Guide for diagnosis, culture, and pathogenicity. Can. J. Plant Pathol. 3:15-25.
12. Smith, J. D. 1987. Winter-hardiness and overwintering diseases of amenity turfgrasses with special reference to the Canadian Prairies. Tech. Bull. 1987-12E Agric. Canada. Saskatoon, Sask. 193 pp.
13. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods, 7th ed. Iowa State University Press, Ames. 507 pp.
14. Walker, H. L., and Connick, W. J., Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Sci. 31:333-338.