Chapter 5

An aggressiveness assay for *Typhula incarnata*, *T. ishikariensis* and *T. phacorrhiza* on creeping bentgrass

ABSTRACT

A compact assay was developed to determine the relative aggressiveness of the *Typhula ishikariensis* complex, *T. incarnata* and *T. phacorrhiza*. Incubation of non-hardened creeping bentgrass at the optimum temperatures for growth of the fungi (5° and 10° C) reduced the time traditionally required for an estimation of aggressiveness of the fungi. Significant differences among the aggressiveness of the *Typhula* species were evident 21 days after inoculation. *Typhula ishikariensis* and *T. incarnata* were very aggressive at both temperatures but not significantly different from each other. There were no differences between the two biological species of the *T. ishikariensis* complex. *Typhula phacorrhiza* did cause symptoms in this assay but was significantly less aggressive than *T. ishikariensis* and *T. incarnata*. The *T. phacorrhiza* isolates failed to completely kill the host at both temperatures, which indicates it can be weakly aggressive to non-hardened creeping bentgrass. This new technique allowed for the rapid and efficient assessment of aggressiveness of the *Typhula* species associated with turfgrasses.

INTRODUCTION

Typhula blights are a major turfgrass disease problem of Wisconsin golf courses and control measures sometimes fail (Worf, 1988). A survey of Wisconsin golf courses (Chapter 2), dikaryon-monokaryon mating experiments (Chapter 3) and sequencing of the compleate internal transcribed spacer region of nrDNA (Chapter 4) have revealed *T. incarnata* Lasch ex Fr., *T. phacorrhiza* Reichard ex. Fr. and two groups of *T. ishikariensis* Imai were present in Wisconsin golf courses. Typhula blights are caused by *T. incarnata*, the causal agent of gray snow mold, and *T. ishikariensis*, the causal agent of speckled snow mold.

Speckled snow mold is believed to be more difficult to control than gray snow mold (Worf, 1988; Tani and Beard, 1997; Stienstra, 1980). On orchardgrass, it has been demonstrated that *T. ishikariensis* is more aggressive than *T. incarnata*, while *T. ishikariensis* biological species II is more aggressive than *T. ishikariensis* biological species I (Matsumoto, 1989). However, Couch states, "There is no evidence that *Typhula ishikariensis* is the more pathogenic [to turfgrasses] of the two species (Wernham and Chelton, 1943). Rather, since it [*T. ishikariensis*] is found primarily in regions where snow cover persists for a longer period of time, the greater disease severity is probably due to the fact that the energy reserves of the turfgrasses become more depleted, thus increasing their vulnerability to colonization by the fungus (Smith, 1987)," (Couch, 1995).

The third species, *Typhula phacorrhiza*, is a closely related fungus, reported as a common saprophyte (Remsburg, 1940), a pathogen of wheat (Schneider and Seaman, 1986), a biological control agent (Burpee et al., 1987; Wu and Hsiang, 1997a, Wu and Hsiang, 1997b) and an associated snow mold collected from Wisconsin golf courses (Millett and Maxwell, 1997). Successful biological control efforts involving *T. phacorrhiza* have been ongoing for more than a decade, but it is yet unknown as to whether the Wisconsin *T. phacorrhiza* isolates are turfgrass pathogens or saprophytes that colonize necrotic tissues.

Fungicide evaluations, turfgrass breeding efforts and other Typhula blight management

research at the University of Wisconsin require accurate identification and subsequent characterization of *Typhula* species. Unfortunately, the relative aggressiveness of the *Typhula* species found in Wisconsin golf courses has not yet been determined and the conventional methods for aggressiveness testing in the field or under controlled environmental conditions require extended incubation periods (Smith, 1981).

Various methods have been designed to determine the aggressiveness of the pathogenic *Typhula* species (Nakajima and Abe, 1989). However, they all require extended periods of incubation at or near 0° C (Wernham and Chelton, 1943; Cormak and Lebeau, 1959; Sunderman, 1964; Bruehl et al., 1966; Årsvoll 1975; Abe and Matsumoto, 1981; Amano and Ozeki, 1981; Smith, 1981; Gaudet and Chen, 1987) even though optimum temperatures *in vitro* for growth of pathogenic *Typhula* species range from 5° to 15° C (Remsburg, 1940; Matsumoto, 1989).

This chapter reports the development of a rapid and efficient aggressiveness assay that indicates *T. incarnata*, the *T. ishikariensis* complex and *T. phacorrhiza* vary in their ability to cause Typhula blights of creeping bentgrass.

This study integrates the screening method of Nakajima and Abe (1989), the bioassay of Nelson and Craft (1992), and the Wisconsin Fast Plants (1994; 1996) growing systems. Nakajima and Abe (1989) developed a method for assessing resistance to the snow molds *T. incarnata* and *Microdochium nivale* in winter wheat incubated at the optimum growth temperature ranges of the fungi. Their method decreased the time and expenses required to assess Typhula blight resistance in wheat. Nelson and Craft (1992) developed a miniaturized and rapid bioassay for the selection of soil bacteria suppressive to Pythium blight of turfgrasses. The Nelson and Craft assay reduced the time, expenses and space required for estimation of disease suppressiveness. Finally, the Wisconsin Fast Plants program (1994; 1996) created small and inexpensive growing systems using trash and everyday items, such as 35 mm film cans and Rubbermaid™ containers.

The objective of this study was to develop a rapid and efficient laboratory screening technique to determine the relative level of aggressiveness of the *Typhula* species on creeping bentgrass. The hypotheses tested were i) an efficient assay conducted at the optimum temperatures of the fungi can be developed to estimate the relative level of aggressiveness of the three *Typhula* species and ii) *T. incarnata* and *T. ishikariensis* are more aggressive that *T. phacorrhiza*.

The *Typhula* species aggressiveness assay has demonstrated that the aggressiveness of the *Typhula* species can be ascertained using less space, time and money when incubation temperatures are at the optimum growth temperatures for the fungi. The results indicate that the isolates of *T. incarnata* and *T. ishikariensis* were equal and strongly aggressive at 5° and 10° C while *T. phacorrhiza* was weakly aggressive at both temperatures.

MATERIALS AND METHODS

Turfgrass growing system

A turfgrass/pathogen growing system was developed by integrating the Wisconsin Fast Plants growing system (Wisconsin Fast Plants, 1994; Wisconsin Fast Plants, 1996), the miniature bioassay of Nelson and Craft (Nelson and Craft, 1992) and the resistance screening method of Nakajima and Abe (1989). This system allowed for rapid growth of both the creeping bentgrass and the *Typhula* species.

Thirty-five mm film cans (3 cm diameter x 5 cm deep), styrofoam board (36 cm x 21 cm x 3 cm) and plastic storage containers (36 cm x 21 cm x 9 cm) with lids were surface disinfested for at least 24 hours with a 50% bleach solution. A sharp metal pipe was used to cut holes into the styrofoam board to house the film cans, (five rows by ten columns for a total of 50 cans per plastic container). Three holes were burned into the bottom of the film cans with a metal rod (3 mm in diameter) to allow for bottom watering of the turf cans. The surface disinfested turf cans were filled with vermiculite, which had been autoclaved for at least 12 hours. 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) seed was surface disinfested with a 2.5% sodium hypochlorite solution (50% Clorox bleach; Chun et al., 1997) for one hour. The disinfested seed (0.8 g wet weight) was sown on top of the vermiculite-filled turf cans. The plastic containers were then filled with Type I reagent grade water (< 10.0 colony forming units/Ml; E-pure™ water purifier, Barnstead, Dubuque, IA) to allow bottom watering of the turf cans. The containers were then covered with clear plastic film to prevent air contamination during seed germination. The turfgrass was allowed to grow for 7 to 10 days at 24° C with a 12 hr photoperiod. The plastic film was removed immediately before the turfgrass was inoculated.

Inoculation method

Fungal isolates (Table 5.1) were grown in potato dextrose broth at either 10° C (*T. incarnata*, *T. ishikariensis* and the unidentified *Typhula* species) or 15° C (*T. phacorrhiza*) for

Table 5.1. Descriptions of *Typhula* isolates used in the aggressiveness assay.

Fungus	Isolate	Origin
T. incarnata	1.35	Meadow Springs Golf Club Jefferson, WI
T. incarnata	2.100	Sentry World Golf Course Stevens Point, WI
T. incarnata	3.114	Trout Lake Golf & Country Club Woodruff, WI
T. incarnata	3.279	Eagle Bluff Golf Course Hurley, WI
T. ishikariensis Wisconsin Group 1	1.93	Christmas Mountain Village Wisconsin Dells, WI
T. ishikariensis Wisconsin Group 1	2.183	Glenn Cairn Golf Course Ogdensburg, WI
T. ishikariensis Wisconsin Group 1	3.120	Trout Lake Golf & Country Club Woodruff, WI
T. ishikariensis Wisconsin Group 2	1.31	Meadow Springs Golf Club Jefferson, WI
T. ishikariensis Wisconsin Group 2	2.105	Sentry World Golf Course Stevens Point, WI
T. ishikariensis Wisconsin Group 2	3.122	Trout Lake Golf & Country Club Woodruff, WI
T. phacorrhiza	2.230A	Marshfield Country Club Marshfield, WI
T. phacorrhiza	3.117	Trout Lake Golf & Country Club Woodruff, WI
T. phacorrhiza	3.120B	Trout Lake Golf & Country Club Woodruff, WI
T. phacorrhiza	3.253	Timber Ridge Golf Course Minocqua, WI
Unidentified *Typhula* species	3.129	Trout Lake Golf & Country Club Woodruff, WI

21 to 35 days. The dry weight of the mycelia was determined by drying a small piece of the mycelial mat in a microwave oven. The mycelial mats were vacuum filtered and briefly macerated in a Warring™ blendor with sterile, distilled water. Sterile plastic pipettes with 0.5 cm of the tip cut off were used to deliver 0.09 g dry weight mycelia in 2 ml of sterile distilled water to each turf can. The containers were then covered with their lids, placed in black plastic garbage bags to ensure high humidity and uninterrupted darkness, and then placed in Percival™ growth chambers without light at 5° or 10° C.

Aggressiveness scale

The disease severity was visually rated by estimating the area of diseased turfgrass. The turfgrass plants were assigned a rating from a modified scale of Stanosz et al. (1990): 0 = 0 %, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75% and 4 = 76 to 100%. The turfgrass plants were rated 21 and 28 days after inoculation (DAI). After the 28 DAI rating, the containers were transferred to a 24° C growth chamber with a 12 hour photoperiod, removed from the black plastic bags, lids were taken off to allow the turfgrass to recover and then the turf cans were rated again at 35 DAI.

Statistical methods

Data were analyzed with the one-way Anova on means (fit continuous Y by nominal or ordinal X) platform of the JMP® statistical software (JMP®, SAS® Institute Inc., Cary, N.C.). Means and standard errors of the means were calculated along with multiple comparison with the control. Results of both experiments were combined and comparison of group means were visually represented by comparison circles. Dunnett's test (Dunnett, 1955) was employed to compare the set of means against the mean of the control group. Group means were compared by visually examining how the comparison circles intersect. The outside angle of intersection indicates whether group means are significantly different. Circles for means that are significantly different either do not intersect or intersect slightly so that the outside angle of intersection is less than 90 degrees. If the circles intersect by an angle of more

than 90 degrees or if they are nested, the means are not significantly different. In the Dunnett's report, |d| quantile is shown and can be used in a manner similar to a Student's *t* statistic. If the value is positive, its mean is more than the LSD apart from the control group mean and is thus significantly different.

RESULTS

The experiments were performed twice with similar results (Tables 5.2, 5.3, 5.4). There were differences between the three species at 35 DAI (Figs. 5.1, 5.2 and 5.3) and individual turf can comparisons illustrate this point (Fig. 5.4). Twenty-one, 28 and 35 days after inoculation differences between the species were observed at both temperatures (Figs. 5.5, 5.6, 5.7, 5.8, 5.9 and 5.10). There were no significant rating differences between 5° and 10° C (data not shown). However, the turfgrass controls that were incubated at 10° C had greater chlorosis than those at 5° C (Fig. 5.4).

The ratings from 21, 28 and 35 DAI at both 5° and 10° C were pooled and the analysis of this data set is presented in Fig. 5.11. Comparison with the control using both Dunnett's Method and comparison circles indicates that *T. ishikariensis* and *T. incarnata* are not significantly different from each other but are significantly different from the control (Fig. 5.11). Also, *T. phacorrhiza* is significantly different than the control, *T. ishikariensis* and *T. incarnata* (Fig. 5.11).

Table 5.2. Mean disease incidence rating for creeping bentgrass inoculated with *Typhula ishikariensis* isolates at incubation temperatures of 5° and 10° C.

Experiments with *Typhula ishikariensis* isolates	5° C						10° C					
	21 DAI[a]		28 DAI		35 DAI		21 DAI		28 DAI		35 DAI	
	mean	SE[b]	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
1.31.1[c]	3.1	± 0.1	4.0	± 0.0	4.0	± 0.0	2.9	± 0.2	4.0	± 0.0	4.0	± 0.0
1.31.2	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
1.93.1	3.6	± 0.2	4.0	± 0.0	4.0	± 0.0	3.5	± 0.2	4.0	± 0.0	4.0	± 0.0
1.93.2	2.6	± 0.2	3.4	± 0.1	3.9	± 0.1	3.4	± 0.1	4.0	± 0.0	4.0	± 0.0
2.105.1	3.0	± 0.0	4.0	± 0.0	4.0	± 0.0	2.1	± 0.2	3.5	± 0.1	4.0	± 0.0
2.105.2	3.3	± 0.1	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
2.183.1	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
2.183.2	3.8	± 0.1	4.0	± 0.0	4.0	± 0.0	3.9	± 0.1	4.0	± 0.0	4.0	± 0.0
3.120.1	3.8	± 0.1	3.8	± 0.1	3.9	± 0.1	3.2	± 0.1	4.0	± 0.0	4.0	± 0.0
3.120.2	3.7	± 0.1	4.0	± 0.0	4.0	± 0.0	3.9	± 0.1	3.9	± 0.1	4.0	± 0.0
3.122.1	3.6	± 0.4	3.8	± 0.2	4.0	± 0.0	3.9	± 0.1	4.0	± 0.0	4.0	± 0.0
3.122.2	3.0	± 0.2	3.7	± 0.1	4.0	± 0.0	2.9	± 0.4	3.5	± 0.4	4.0	± 0.0

[a] DAI = days after inoculation.

[b] SE = standard error of the mean.

[c] Experiment 1 = 1.31.1, experiment 2 = 1.31.2, etc.

Table 5.3. Mean disease incidence rating for creeping bentgrass inoculated with *Typhula incarnata* isolates at incubation temperatures of 5° and 10° C.

Experiments with *Typhula incarnata* isolates	5° C						10° C					
	21 DAI[a]		28 DAI		35 DAI		21 DAI		28 DAI		35 DAI	
	mean	SE[b]	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
1.35.1[c]	3.1	± 0.1	4.0	± 0.0	4.0	± 0.0	2.7	± 0.2	3.6	± 0.1	4.0	± 0.0
1.35.2	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	3.8	± 0.1	4.0	± 0.0	4.0	± 0.0
2.100.1	3.0	± 0.0	4.0	± 0.0	4.0	± 0.0	3.4	± 0.2	3.9	± 0.1	4.0	± 0.0
2.100.2	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
3.114.1	3.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
3.114.2	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
3.279.1	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0
3.279.2	4.0	± 0.0	4.0	± 0.0	4.0	± 0.0	3.8	± 0.2	3.9	± 0.1	4.0	± 0.0

[a] DAI = days after inoculation.
[b] SE = standard error of the mean.
[c] Experiment 1 = 1.35.1, experiment 2 = 1.35.2, etc.

Table 5.4. Mean disease incidence rating for creeping bentgrass inoculated with *Typhula phacorrhiza* isolates and one unidentified *Typhula* isolate at incubation temperatures of 5° and 10° C.

Experiments with *Typhula phacorrhiza* and an unidentified isolate	5° C						10° C					
	21 DAI[a]		28 DAI		35 DAI		21 DAI		28 DAI		35 DAI	
	mean	SE[b]	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
2.230.1[c]	0.9	± 0.2	0.9	± 0.2	1.6	± 0.3	1.1	± 0.2	1.2	± 0.2	1.5	± 0.3
2.230.2	1.0	± 0.0	3.4	± 0.2	3.4	± 0.2	1.3	± 0.3	2.6	± 0.2	2.6	± 0.2
3.117.1	1.0	± 0.0	1.0	± 0.0	1.8	± 0.1	1.1	± 0.1	2.6	± 0.3	2.7	± 0.3
3.117.2	1.1	± 0.3	1.3	± 0.3	1.3	± 0.3	1.1	± 0.1	1.4	± 0.2	1.6	± 0.2
3.120.1	0.6	± 0.1	1.0	± 0.0	2.1	± 0.1	0.8	± 0.2	1.8	± 0.2	1.8	± 0.3
3.120.2	2.5	± 0.2	2.6	± 0.2	3.9	± 0.1	2.2	± 0.1	2.3	± 0.2	3.6	± 0.1
3.253.1	1.0	± 0.0	1.3	± 0.3	2.1	± 0.1	1.1	± 0.1	2.5	± 0.2	2.7	± 0.3
3.253.2	0.5	± 0.1	0.5	± 0.1	0.7	± 0.2	1.1	± 0.1	1.5	± 0.2	1.9	± 0.2
3.129.1[d]	2.2	± 0.1	2.3	± 0.1	1.3	± 0.1	2.3	± 0.1	2.3	± 0.1	2.9	± 0.2
3.129.2	2.5	± 0.1	2.5	± 0.1	1.4	± 0.1	2.0	± 0.1	1.9	± 0.1	2.5	± 0.3

[a] DAI = days after inoculation.
[b] SE = standard error of the mean.
[c] Experiment 1 = 1.35.1, experiment 2 = 1.35.2, etc.
[d] 3.129 = unidentified *Typhula* species.

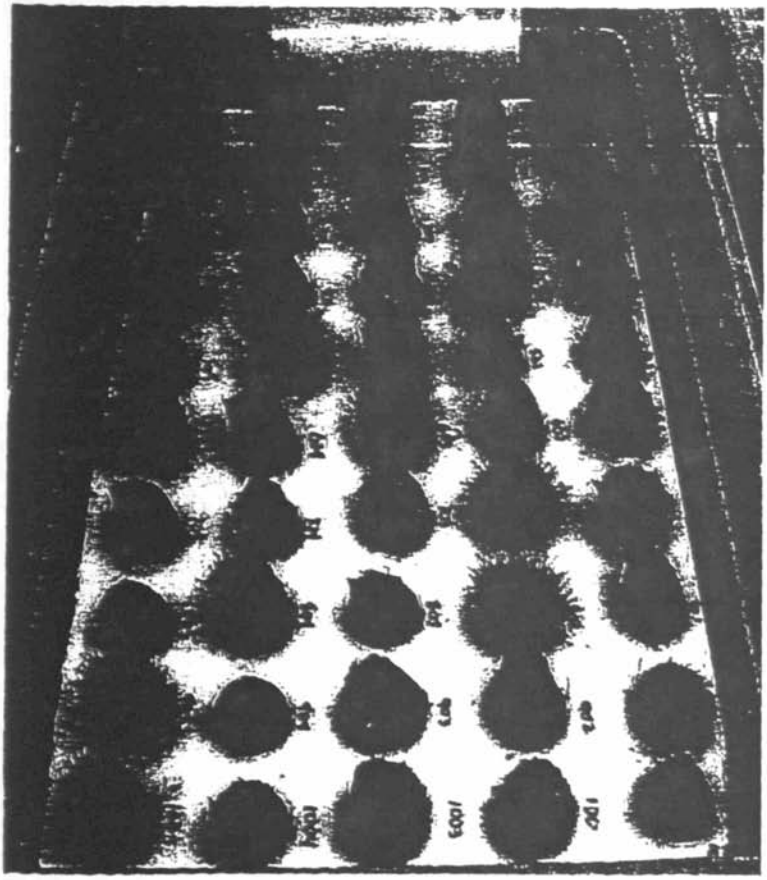

Figure 5.1. Aggressiveness assay of *Typhula incarnata* incubated at 5°C. Picture was taken 35 days after inoculation.

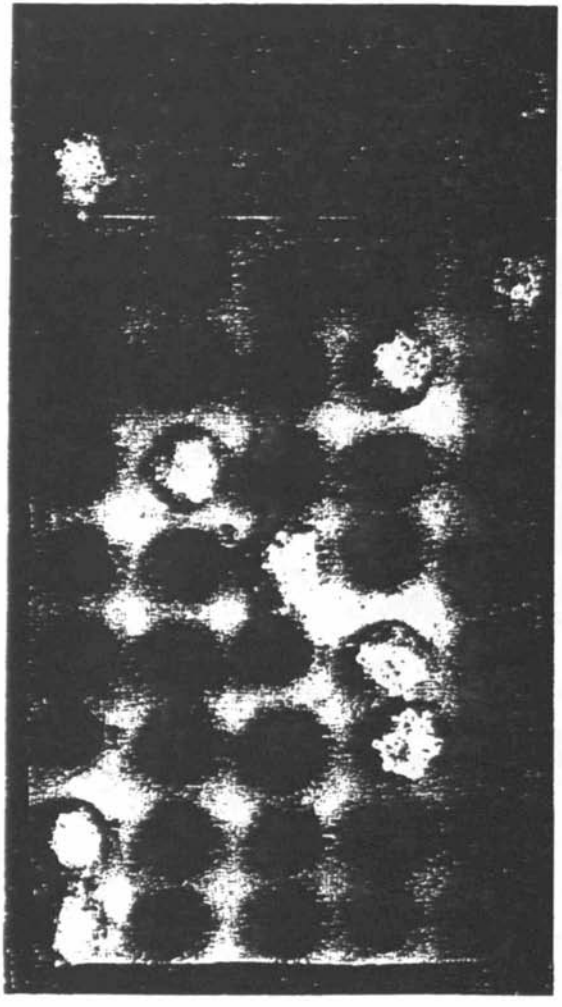

Figure 5.2. Aggressiveness assay of *Typhula ishikariensis* incubated at 5° C. Picture was taken 35 days after inoculation.

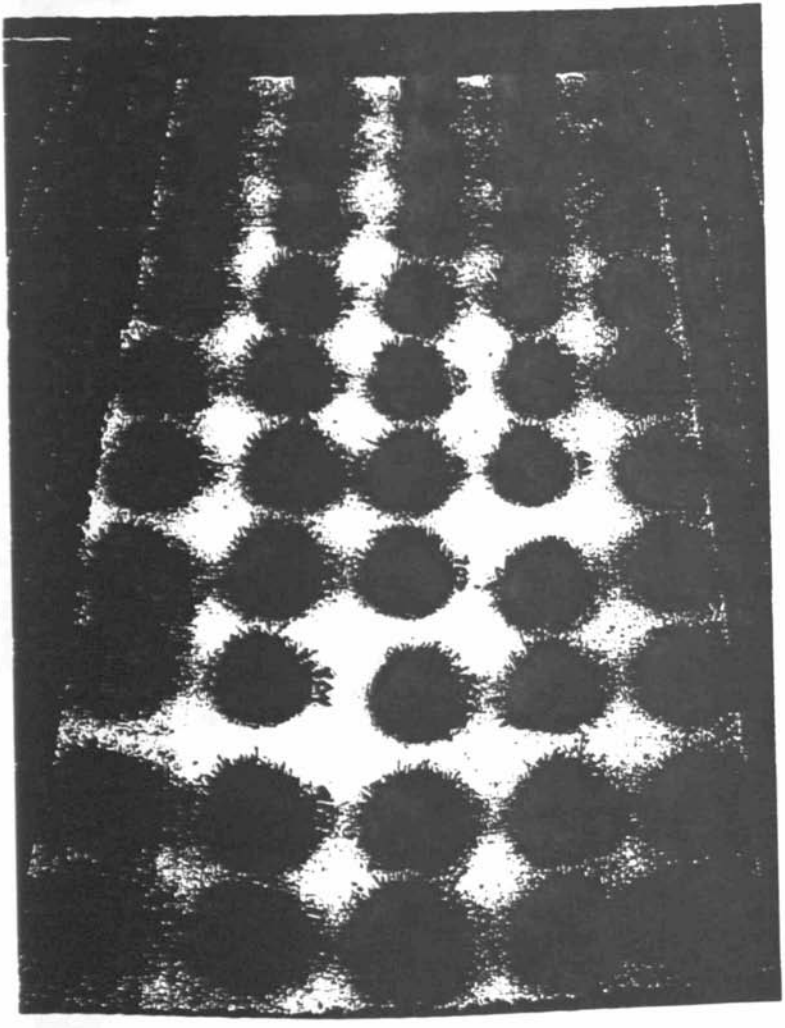

Figure 5.3. Aggressiveness assay of *Typhula phacorrhiza* incubated at 5° C. Picture was taken 35 days after inoculation.

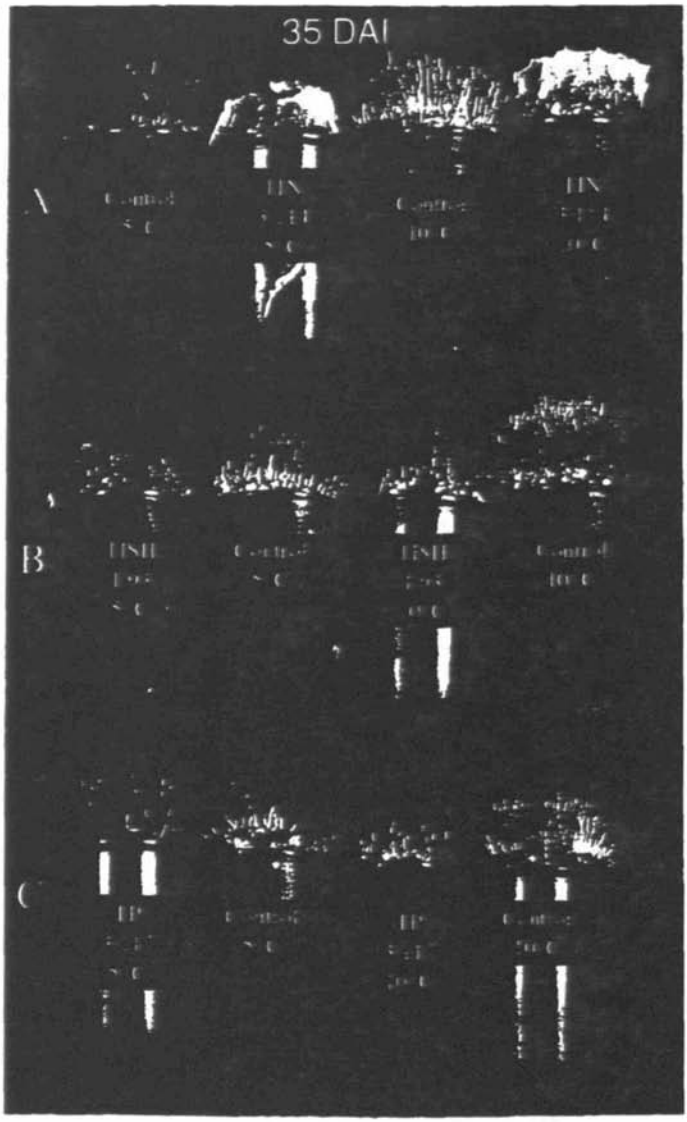

Figure 5.4. Turf cans 35 days after inoculation (DAI) with A) *Typhula incarnata* isolate 3.114, B) *T. ishikariensis* isolate 1.93 and C) *T. phacorrhiza* isolate 3.117.

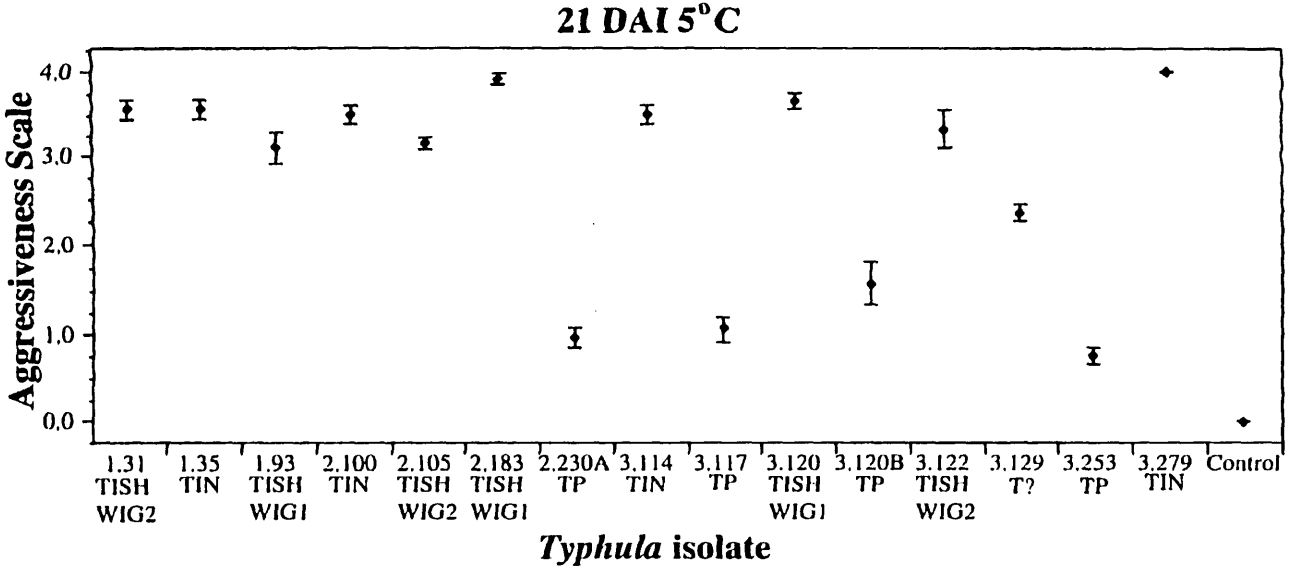

Figure 5.5. Aggressiveness of the *Typhula* species 21 days after inoculation (DAI) at 5°C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

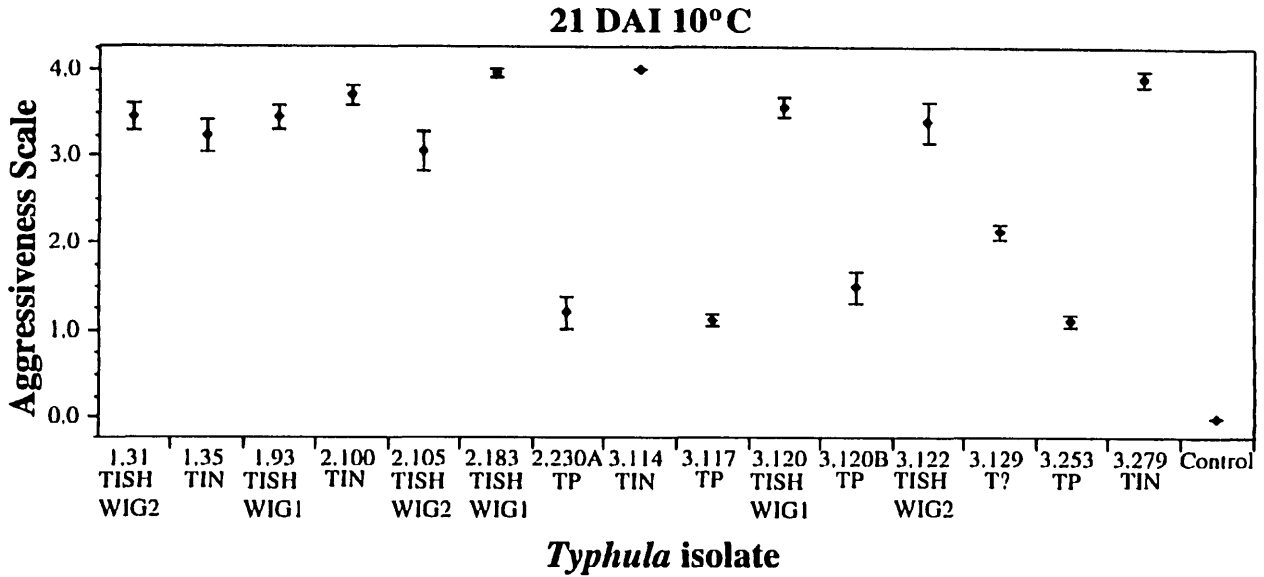

Figure 5.6. Aggressiveness of the *Typhula* species 21 days after inoculation (DAI) at 10° C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

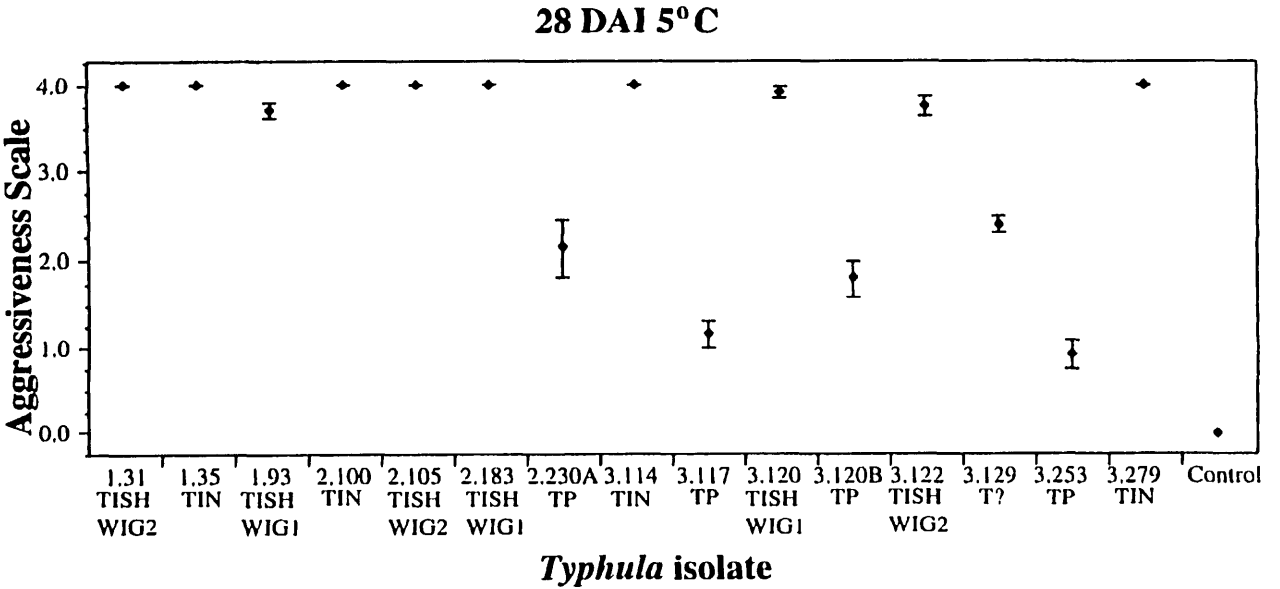

Figure 5.7. Aggressiveness of the *Typhula* species 28 days after inoculation (DAI) at 5° C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

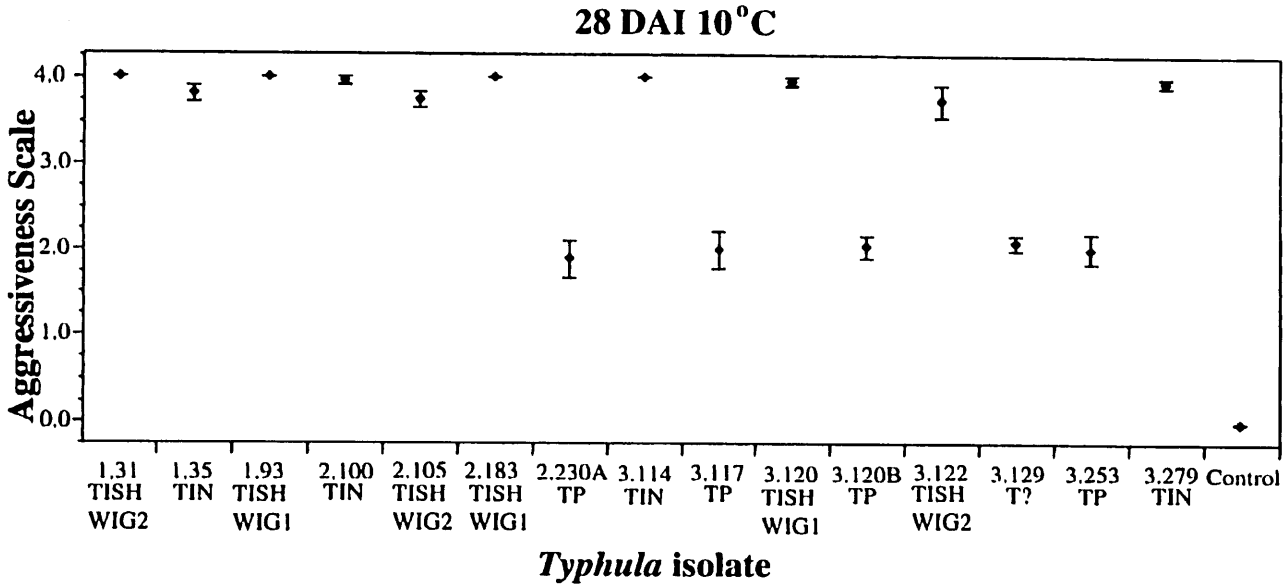

Figure 5.8. Aggressiveness of the *Typhula* species 28 days after inoculation (DAI) at 10°C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

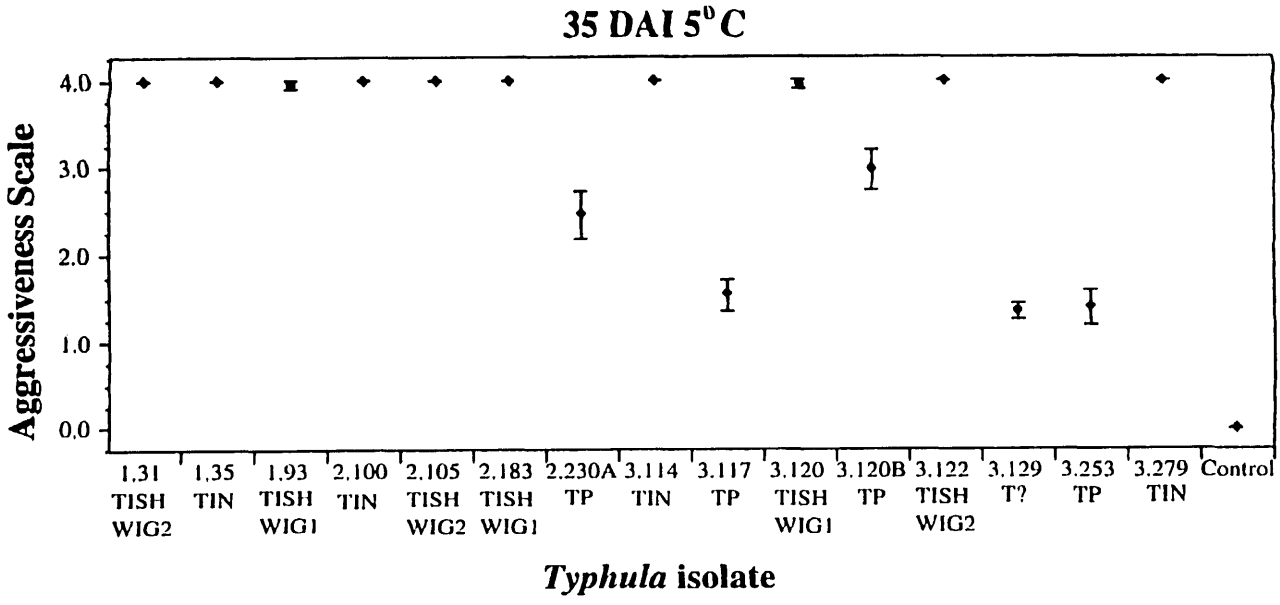

Figure 5.9. Aggressiveness of the *Typhula* species 35 days after inoculation (DAI) at 5°C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

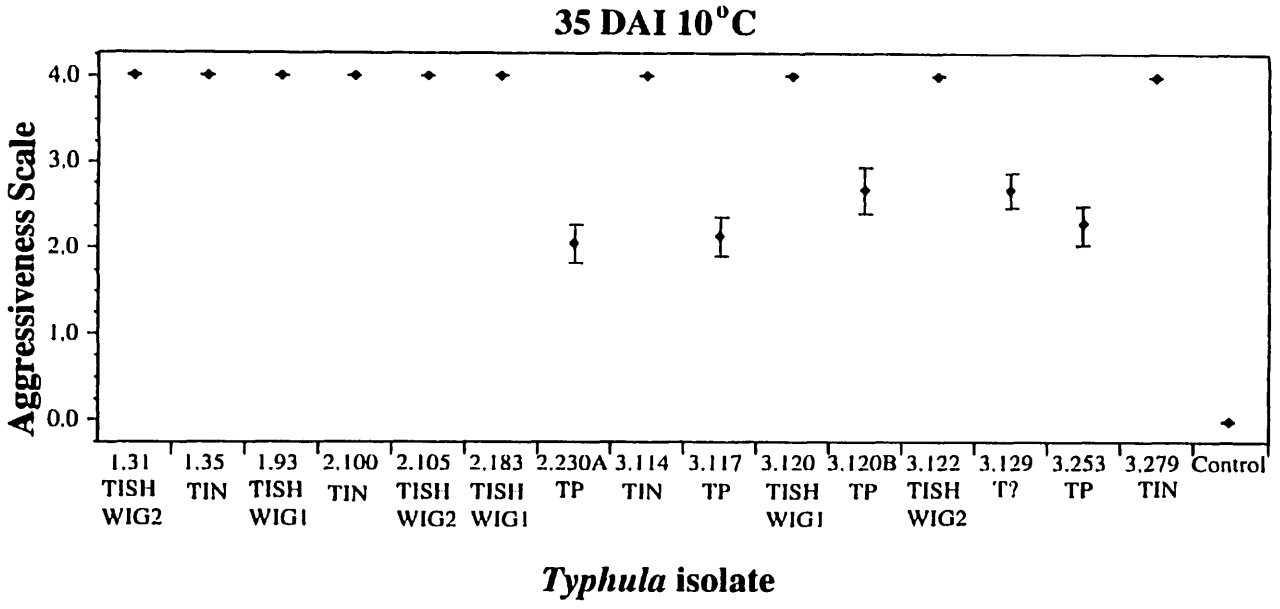

Figure 5.9. Aggressiveness of the *Typhula* species 35 days after inoculation (DAI) at 10°C. Results from both experiments were combined. TISH = *Typhula ishikariensis*, WIG1 = Wisconsin group 1, WIG2 = Wisconsin group 2, TIN = *T. incarnata*, TP = *T. phacorrhiza* and T? = unidentified *Typhula* species. Bars represent the standard error of the mean. The aggressiveness scale is 0 = 0% disease severity, 1 = 1 to 25% disease severity, 2 = 26 to 50% disease severity, 3 = 51 to 75% disease severity and 4 = 76 to 100% disease severity.

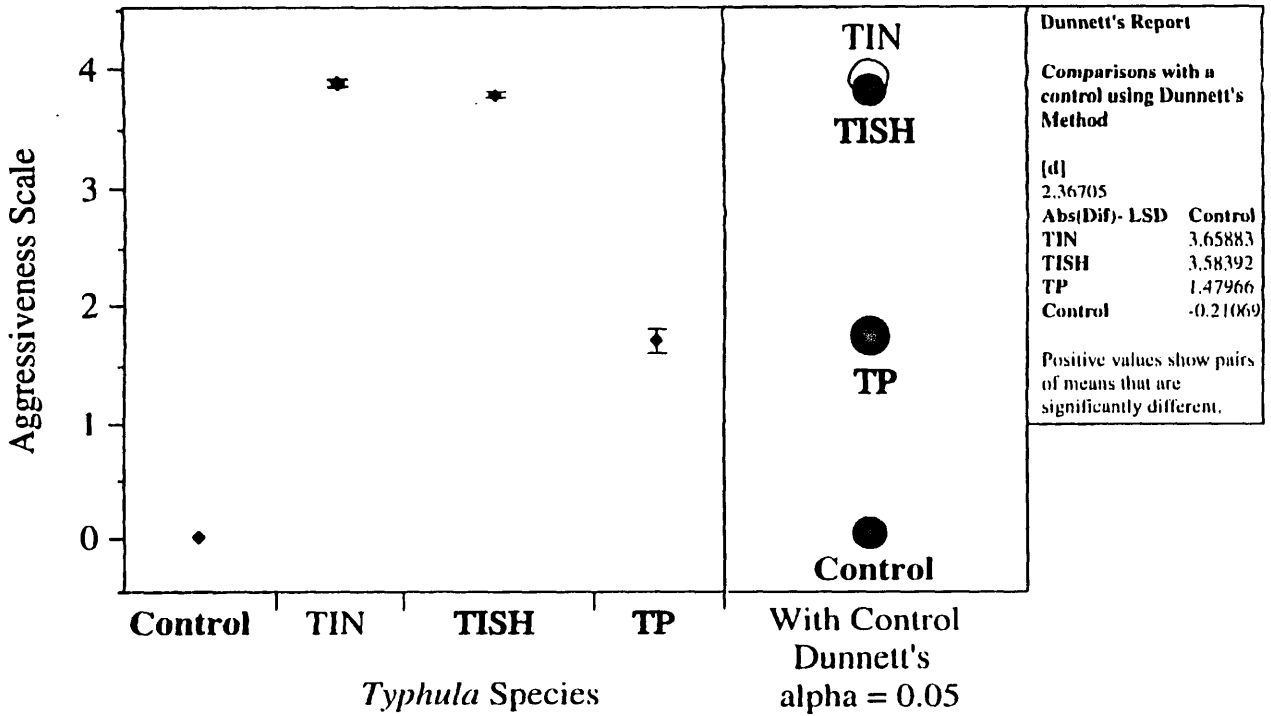

Figure 5. 11. Aggressiveness of *Typhula* species as determined by the aggressiveness assay. TIN = *T. incarnata* (mean = 3.9, SE = 0.04), TISH = *T. ishikariensis* (mean = 3.8, SE = 0.05) and TP = *T. phacorrhiza* (mean = 1.7, SE = 0.12). Comparison circles of the means using Dunnett's method (Dunnett, 1955).

DISCUSSION

Typhula ishikariensis and *T. incarnata* have equally aggressive at 5° and 10° C as determined by the aggressiveness assay reported here. These results agree with those of Wernham and Chelton (1943) that indicate *T. incarnata* and *T. ishikariensis* do not differ in their aggressiveness towards orchardgrass. Speckled snow mold is believed to be more difficult to control than gray snow mold (Worf, 1988; Tani and Beard, 1997; Stienstra, 1980). However, controlling a disease should not be confused with the aggressiveness of a fungal pathogen. It could be that *T. incarnata* and *T. ishikariensis* are equal in their aggressiveness to bentgrass, but that they vary in their ability to be managed. One explanation could be that the more soil-borne *T. ishikariensis* is escaping the foliar applied fungicide treatments, whereas *T. incarnata* is not. Furthermore, Couch states, "There is no evidence that *Typhula ishikariensis* is the more pathogenic [to turfgrasses] of the two species (Wernham and Chelton, 1943). Rather, since it [*T. ishikariensis*] is found primarily in regions where snow cover persists for a longer period of time, the greater disease severity is probably due to the fact that the energy reserves of the turfgrasses become more depleted, thus increasing their vulnerability to colonization by the fungus," (Couch, 1995).

On the other hand, these results do conflict with studies by Matsumoto (1989) involving orchardgrass. Lack of hardening could be responsible for this. On hardened orchardgrass under field conditions, *T. incarnata* was the least aggressive, followed by the moderately aggressive *T. ishikariensis* biological species I and the most aggressive *T. ishikariensis* biological species II, (Matsumoto, 1989). In our experiments, there were no differences between biological species I (WIG1) and II (WIG2) of the *T. ishikariensis* complex (data not shown). Additionally, Årsvoll (1977) reported that *T. incarnata* and *T. ishikariensis* caused significantly more damage to non-hardened grasses as compared to hardened grasses. In this assay, the one-week-old turfgrass hosts were non-hardened and it has been reported that host plants must be hardened to fully display their genetic abilities (Årsvoll, 1977; Gaudet and

Chen, 1987). However, this assay was developed to assess the aggressiveness of the *Typhula* species on non-hardened turfgrass and not to assess the level of host resistance.

Figure 3.10 of Matsumoto and Sato (1983) illustrates the importance of hardening on aggressiveness estimation. As this figure illustrates, host plants that are not hardened provide a favorable opportunity for *T. incarnata* and *T. ishikariensis* to prevail. Our results support this theory.

With this in mind, it is clear that the Wisconsin isolates of *T. phacorrhiza* are not aggressive, since the isolates assessed in our study killed a lower percentage of plants. This is supported by Burpee who stated, "Initially, the fungus (*T. phacorrhiza*) was assumed to be an unreported pathogen of turfgrass, but inoculations of various species in controlled environments and the field failed to produce symptoms (unpublished)," (Burpee, 1994).

A more accurate description of *T. phacorrhiza* in Wisconsin golf courses could be that it is mainly saprophytic but can act as a senectopathic disorder (Couch, 1995). This hypothesis is supported by the fact that *T. phacorrhiza* was found mainly associated with patches on top of patches caused by *T. ishikariensis* and *T. incarnata* (Chapter 2). One explanation is that during the end of winter (near 15° C), *T. phacorrhiza* colonized the already diseased turfgrass leaves. Yet another explanation is that it acted only as a saprophyte, which colonized the partially consumed turfgrass leaves at a period when *T. ishikariensis* and *T. incarnata* were becoming less active.

Future research on the aggressiveness of the *Typhula* species associated with turfgrasses could center on improving and expanding the aggressiveness assay. The assay reported here could be repeated at higher temperatures, especially at the optimum growth temperature of *T. phacorrhiza* (15° C).

This assay could be used to screen for Typhula blight resistance in turfgrasses. However, turfgrass seed is needed for this assay since field collected turfgrass sprigs may produce varying results. Fungal or bacterial contaminants from non-sterile soil and/or debris

of a "dirty system" would antagonize the *Typhula* isolates and therefore reduce the amount of disease and produce inaccurate results. Commercially available turfgrass varieties could be used because seed is readily available. A hardening period would be required at the beginning of the assay in order to let the host fully display their genetic abilities.

Finally, the next logical step for assessing the aggressiveness of the *Typhula* species is conducting field experiments. Unfortunately, field experiments, conducted at the O. J. Noer Turfgrass Research and Education Facility in Verona, WI, with the isolates used in this study did not produce symptoms during the winter of 1998 to 1999 (data not shown).

In conclusion, assessing the aggressiveness of *Typhula* species using temperatures higher than traditionally used for producing Typhula blight appears feasible. This technique could reduce the space, time and cost normally required to assess fungal aggressiveness. The order of magnitude based on the aggressiveness assay finds *T. ishikariensis* and *T. incarnata* to be strongly aggressive to creeping bentgrass and *T. phacorrhiza* being weakly aggressive.

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