

Variation in Bentgrass Susceptibility to *Typhula incarnata* and in Isolate Aggressiveness Under Controlled Environment Conditions

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ABSTRACT

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Typhula incarnata, the causal agent of gray snow mold, is an important winter pathogen of turfgrasses in the northern United States. The relative susceptibility of cultivars of three bentgrass species (creeping, colonial, and velvet bentgrass) to *Typhula incarnata* and the aggressiveness of 15 *T. incarnata* isolates obtained from infected turfgrasses on golf courses in Michigan, Minnesota, and Wisconsin were evaluated under controlled conditions. A hypersensitive type of resistance response to *T. incarnata* was not observed in any cultivar. Disease severity increased with higher inoculum concentration of *T. incarnata*. Colonization by gray snow mold gradually decreased with increasing plant age from 11 weeks after seeding in most cultivars tested, suggesting that age-related resistance was expressed over time. There were significant differences in disease severity among the three bentgrass species, particularly between tetraploid (creeping and colonial) and diploid (velvet) species, and among cultivars within each species, indicating varying levels of susceptibility to *T. incarnata*. All 15 isolates were pathogenic on bentgrass and were significantly different in aggressiveness, but aggressiveness was not related to geographic origin. Therefore, turfgrass breeders should be able to use one or a few virulent representative isolates of the pathogen to screen for resistance.

Additional keywords: *Agrostis*, snow mold, *Typhula* blight

Bentgrass is the most widely used turfgrass species on golf course putting greens and fairways throughout the United States. Five bentgrass species are commonly used for turfgrass in the United States: creeping (*Agrostis stolonifera* L.), colonial (*A. capillaries* L.), velvet (*A. canina* L.), redbot (*A. gigantea* L.), and dryland (*A. castellana* L.). Among those species, creeping, colonial, and velvet bentgrasses are the most popular species used for high-quality tees and greens of golf courses (2,27,35).

Typhula incarnata Lasch ex Fr., the causal agent of gray snow mold, is an important winter pathogen of turfgrass in the northern United States (9,16,30). Symptoms of gray snow mold on turfgrass usually appear as circular, water-soaked, or straw-colored patches after snow melt in late winter or early spring (32). Plants in diseased patches may be matted, slimy, and covered with gray mycelium of the

pathogen (18). During the spring thaw, gray snow mold produces numerous yellow or reddish-brown sclerotia which may persist through the summer and early fall as resting structures. Later, they become the primary inoculum source when weather conditions during winter become more favorable (16,24).

At most golf courses, disease control generally relies on fungicide applications supplemented by cultural approaches, such as fertilization and snow removal (32). However, fungicides are expensive, time consuming to apply, and may have potential negative environmental impacts (6,16). In addition, fungicide applications may have limited efficacy due to long snow cover during winter. For these reasons, the planting of resistant cultivars would be an economical and effective method of controlling gray snow mold (6,36).

In the United States, however, there is no report of commercial bentgrass cultivars that are highly resistant to the gray snow mold pathogen (1,5). Gould et al. (15) reported that four common bentgrass species (creeping, colonial, velvet, and redbot) were susceptible to gray snow mold. Considerable differences in susceptibility to *T. incarnata* among cultivars within species also were reported by Årsvoll (1) and Vargas (32).

T. incarnata possesses wide genetic and ecological diversity (5,9,24,33). Matsu-moto and Tajimi (24) reported that *T. incarnata* populations had many different vegetative compatibility groups. Bruehl and Machtmes (5) suggested that there may be at least 39 alleles at the two incompatibility loci, A and B, in a sample of 32 field dikaryons. Chang et al. (9) also reported that *T. incarnata* isolates collected from Wisconsin, Michigan, Minnesota, and Utah have adaptability to diverse environmental conditions compared with *T. ishi-kariensis* and *T. phacorriza* because they were collected from every site sampled in those states. Vergara et al. (33), using molecular data obtained from random amplified polymorphic DNA (RAPD) analysis, suggested that high outcrossing and sexual recombination of *T. incarnata* may be key factors explaining a high level of genetic variation among isolates of *T. incarnata*.

Genetic diversity might explain the variation in aggressiveness of *T. incarnata* (24,28). Variation in aggressiveness of *T. incarnata* can play a critical role in the success of disease management strategies, including resistance screening of cultivars to gray snow mold (6,34). Previously completed resistance screenings of turfgrass to snow mold pathogens were accomplished mostly in field trials (29). However, snow mold development under field conditions can be erratic due to the inconsistency of weather conditions from year to year, because disease development requires unique weather conditions (4,9,17,29,30). Therefore, cultivar evaluations under controlled conditions, such as in growth chambers, can help to ensure adequate infection levels, to improve efficiency in terms of time, and to predict a field result (7,8,25,34), although the method has a limitation, in that field evaluation under various environmental factors is needed. Nonetheless, there is no information available on relative susceptibility of commercial bentgrass cultivars and on variation in aggressiveness of various isolates of *T. incarnata* under controlled conditions.

In the present study, therefore, we examined the effect of inoculum concentration and plant age on infection of bentgrass plants in controlled conditions. The relative susceptibility of nine cultivars repre-

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senting three bentgrass species (creeping, colonial, and velvet) to *T. incarnata* also was evaluated under these conditions. The variability in aggressiveness of 15 isolates of *T. incarnata* collected from different geographic areas (Wisconsin, Minnesota, and Michigan) on creeping bentgrass cultivars was examined.

MATERIALS AND METHODS

Plant materials. Four creeping bentgrass cultivars (L-93, Penncross, Pennlinks, and Providence), three colonial bentgrass cultivars (Bardot, SR 7100, and Tiger), and three velvet bentgrass cultivars (Barvaria, Greenwich, and Vesper) were examined in this study. Cultivars of creeping and colonial bentgrass (0.056 g of seed), and velvet cultivars (0.035 g of seed) were sown evenly in plastic pots (5.3 by 5.3 by 5.1 cm) filled with potting soil (Metro Mix 366-P; Scott's Company, Marysville, OH). The plants were grown in the greenhouse at 18 to 28°C with 16 h of light per day. The plants were mowed weekly with a scissors at a height of 0.6 cm from 2 weeks after germination until they were transferred to a controlled environment chamber after randomization. The chamber was set at 10°C and 10-h day length for 7 days, 5°C and 8-h day length for 7 days, and 2°C and 6-h day length for 7 days to simulate fall weather conditions before snow cover in nature. This process, called hardening, is very important before inoculation because low temperature and short day length are required for development of snow mold resistance within the plants (1,12). Water-soluble fertilizer (0.02-0.005-0.02 g of N-P-K per pot; SunGrow Company, Austin, TX) was applied biweekly from 4 weeks after germination until 2 weeks before hardening. Plants to be inoculated were placed into plastic trays containing distilled water to assure constant moisture.

Fungal isolates and inoculum preparation. Twenty isolates of *T. incarnata* were selected randomly from previously identified collections which were maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit) at 4 ± 1°C in the dark (9) (Table 1, Fig. 1). Inoculum preparation followed the same procedures described by Chang et al. (7). In brief, mycelium suspensions were produced by taking five culture plugs (5 mm in diameter) from the actively growing colony edge and transferring them to 20 ml of potato dextrose broth (PDB; Difco Laboratories) in 250-ml flasks. Broth cultures were grown at 10 ± 1°C in the dark. After 20 days, mycelium was harvested from four to six flasks, mixed together, and air dried for 30 min under a laminar flow hood. Residual water in air-dried mycelia finally was removed by vacuum filtration for 3 min under a pressure of 21 psi (1.5 kg/cm²) through cheesecloth, and mycelium then

was homogenized in a blender with 20 ml of sterile, distilled water for 30 s. These suspensions were adjusted with sterile, distilled water to desired concentrations according to experiments described below and used immediately for inoculation.

Inoculation procedure. Sterile pipettes were used to deliver 1 ml of inoculum solution directly on the soil line in the center of each pot. Following inoculation, the plants were arranged in a randomized complete block design and kept in a plastic container (70 by 40 by 15 cm; Rubbermaid, Wooster, OH) in which approximately 30% of the volume was filled with wetted potting soil (1:1 soil:distilled water in volume). Moisture was applied by evenly spraying the plants with distilled water with a hand sprayer until runoff from leaves was observed, and the plastic box was covered with a lid to maintain humidity required for disease development. The box was transferred to a controlled envi-

ronment chamber set at 2°C in darkness for 21 days, 5°C and 6-h day length for 7 days, and 10°C and 8-h day length for 10 days. Immediately after inoculation, the pots in the plastic container were returned to plastic trays containing water to assure constant moisture.

Effect of inoculum concentration and plant age on disease development. To evaluate the effect of inoculum concentration on disease development on 11-week-old plants, mycelial suspensions of *T. incarnata* isolates 11 and 19 were adjusted to 0.1, 0.2, 0.3, and 0.4 g/ml mycelium fresh weight (mfw) with sterile, distilled water. Six bentgrass cultivars—L-93, Penncross, Pennlinks, and Providence (creeping); Tiger (colonial); and Greenwich (velvet)—were inoculated with each inoculum concentration. Additionally, in order to evaluate the effect of plant age on disease development, 9-, 11-, 13-, and 15-week-old plants of the same cultivars were

Table 1. List of *Typhula incarnata* isolates used in this study

No.	Code	Source	Geographic origin (city, state)	Year collected
1	GLAD4.1	Gladstone Golf Course	Gladstone, MI	2002
2	IR 9. 2	Indian River Golf Course	Indian river, MI	2002
3	IR 15. 3	Indian River Golf Course	Indian river, MI	2002
4	LONG 9. 4	Lakewood Blackshire Golf Course	Oscoda, MI	2002
5	NEW 17. 3	Newberry Country Club	Newberry, MI	2002
6	RFR 5. 3	Red Fox Run Golf Course	Gwinn, MI	2002
7	CW 18. 3	Crosswoods Golf Course	Crosslake, MN	2002
8	LP 2. 1	Long Prairie Golf Course	Long Prairie, MN	2002
9	OC 11. 1	Oak Crest Golf Course	Roseau, MN	2002
10	SN 9	Superior National Golf Course	Lutsen, MN	2002
11	NE 108. 8. 3	Vernon Hills Golf Course	Peshigo, WI	2001
12	NE 110. 1. 5	Wander Springs Golf Course	Greenleaf, WI	2001
13	NW 4. 2. 1	Ashland Elks Golf Course	Ashland, WI	2001
14	NW 43. 2. 5	Madeline Island Golf Course	La Pointe, WI	2001
15	NW 88. 9. 2	Whitetail Golf Course	Colfax, WI	2001
16	SE 57. 3. 5	Lauderdale Lakes Country Club	Elkhorn, WI	2001
17	SE 90. 6. 3	Shoop Park Golf Course	Racine, WI	2001
18	SW 2. 6. 5	Baraboo Golf Course	Baraboo, WI	2001
19	SW 5. 4. 5	Blackhawk Golf Course	Janesville, WI	2001
20	SW 74. 10. 4	Sun Prairie Golf Course	Sun Prairie, WI	2001

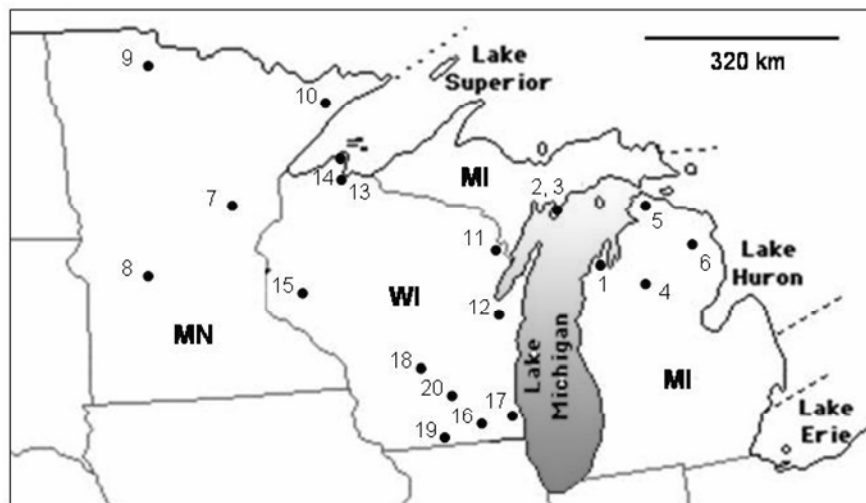


Fig. 1. Sampling sites of Michigan (MI), Minnesota (MN), and Wisconsin (WI). Solid circles with the numbers on the map indicate sampling locations, golf courses listed in Table 1.

inoculated with a mycelial suspension (0.3 g/ml mfw) of *T. incarnata* isolates 11, 13, and 19.

Evaluation of the susceptibility of bentgrass cultivars. To evaluate the susceptibility of different bentgrass cultivars to gray snow mold, inoculum (0.3 g/ml mfw) of each of five isolates (nos. 11, 12, 13, 16, and 19) was prepared separately and applied independently on 15-week-old plants of nine cultivars (L-93, Penncross, Providence, Bardot, SR 7100, Tiger, Barvaria, Greenwich, and Vesper).

Evaluation of the aggressiveness of *T. incarnata* isolates. To evaluate the aggressiveness of isolates of *T. incarnata* from different geographic regions, inoculum (0.3 g/ml mfw) from 15 isolates collected from three different states (Michigan: nos.

1, 2, 3, 4, 5, and 6; Minnesota: nos. 7, 8, 9, and 10; and Wisconsin: nos. 14, 15, 17, 18, and 20) was applied on 15-week-old plants of two creeping bentgrass cultivars (L-93 and Providence).

Disease evaluation and statistical analysis. Disease severity was assessed by visual determination of percentage of area colonized and infected by mycelia at 21, 28, and 38 days after inoculation. All three experiments described above were conducted twice in a factorial design with three replicates. Data were analyzed using mean values of two runs in each experiment because no significance was detected between the runs.

All statistical analyses were conducted using the general linear models procedure (PROC GLM) in SAS (version 6.12; SAS

Institute Inc., Cary, NC). Differences among plant ages, cultivars, and isolates were compared using Fisher's protected least significant differences (LSD) test at $P = 0.05$. Analysis of variance (ANOVA) was used to evaluate the effects of plant age on disease development and the disease severity of two creeping bentgrass cultivars by 15 isolates of *T. incarnata* with different geographic origin. Disease severity on the inoculum concentration of *T. incarnata* isolates 11 and 19 was determined using linear regression of the Sigmaplot program (version 5.0; SPSS Inc., San Rafael, CA).

For disease severity for nine cultivars of three bentgrass species to five isolates of *T. incarnata*, the source of variation for bentgrass species was partitioned into two

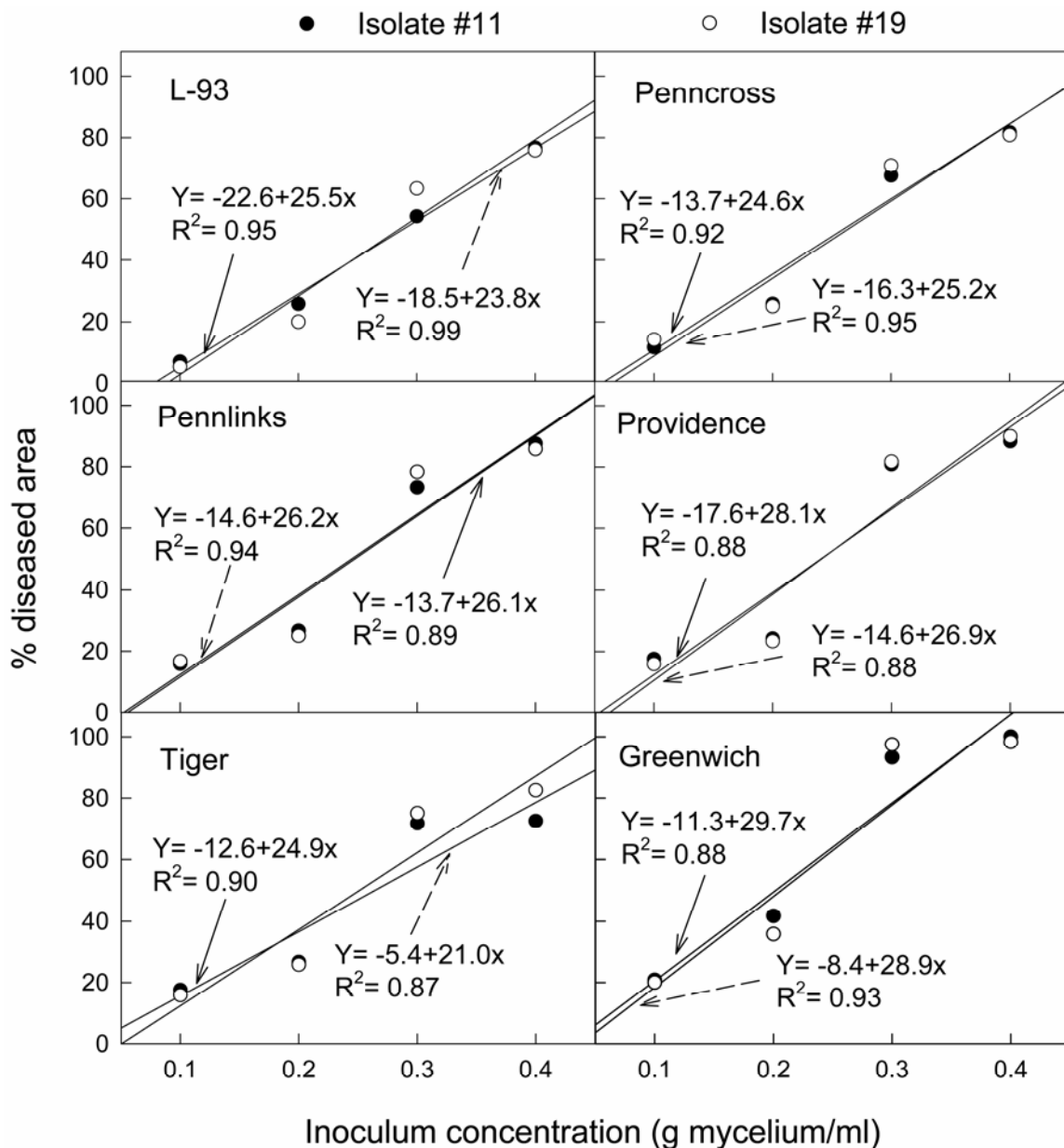


Fig. 2. Effect of inoculum concentration on disease development in six cultivars of three bentgrass species (creeping: L-93, Penncross, Pennlinks, and Providence; colonial: Tiger; and velvet: Greenwich) inoculated by one of two isolates of *Typhula incarnata* with four inoculum concentrations in the growth chamber. Short dash and solid arrows within each box indicate regression lines and equations of isolates 11 and 19, respectively.

orthogonal contrasts: velvet (A_1A_1) bentgrass versus creeping ($A_2A_2A_3A_3$), and colonial ($A_1A_1A_2A_2$) bentgrasses, and creeping versus colonial bentgrass. The

velvet bentgrass versus creeping and colonial bentgrass contrast was performed in order to test for differences between bentgrass species having diploid or tetraploid

genomes. Similarly, the creeping versus colonial bentgrass contrast served to test for differences between bentgrass species having different tetraploid genomes. This method also was used to partition the interactions of the bentgrass species with *T. incarnata* isolates. All treatments were assumed to be fixed and blocks were assumed to be random in all ANOVAs (SAS 6.12: SAS Institute Inc.). Contrasts were computed according to the procedures of Steel et al. (31).

Table 2. Analysis of variance for gray snow mold severity on four cultivars (L-93, Penncross, Pennlinks, and Providence) of creeping bentgrass inoculated with three isolates of *Typhula incarnata* at four plant ages (9-, 11-, 13-, and 15-week-old plants from germination to inoculation) in the growth chamber

Source of variation	df	Mean square	F value	P
Replication	4	75.2	0.94	0.4403
Cultivar	3	1,785.0	22.36	<0.0001
Plant age (cultivar)	12	6,391.8	80.05	<0.0001
Isolate	2	238.8	2.99	0.0522
Isolate × cultivar	6	139.1	1.74	0.1120
Isolate × plant age (cultivar)	24	470.1	5.89	<0.0001
Error	235	79.8

RESULTS

Disease severity significantly increased within cultivars as inoculum concentration increased from 0.1 to 0.4 g/ml mfw (Fig.

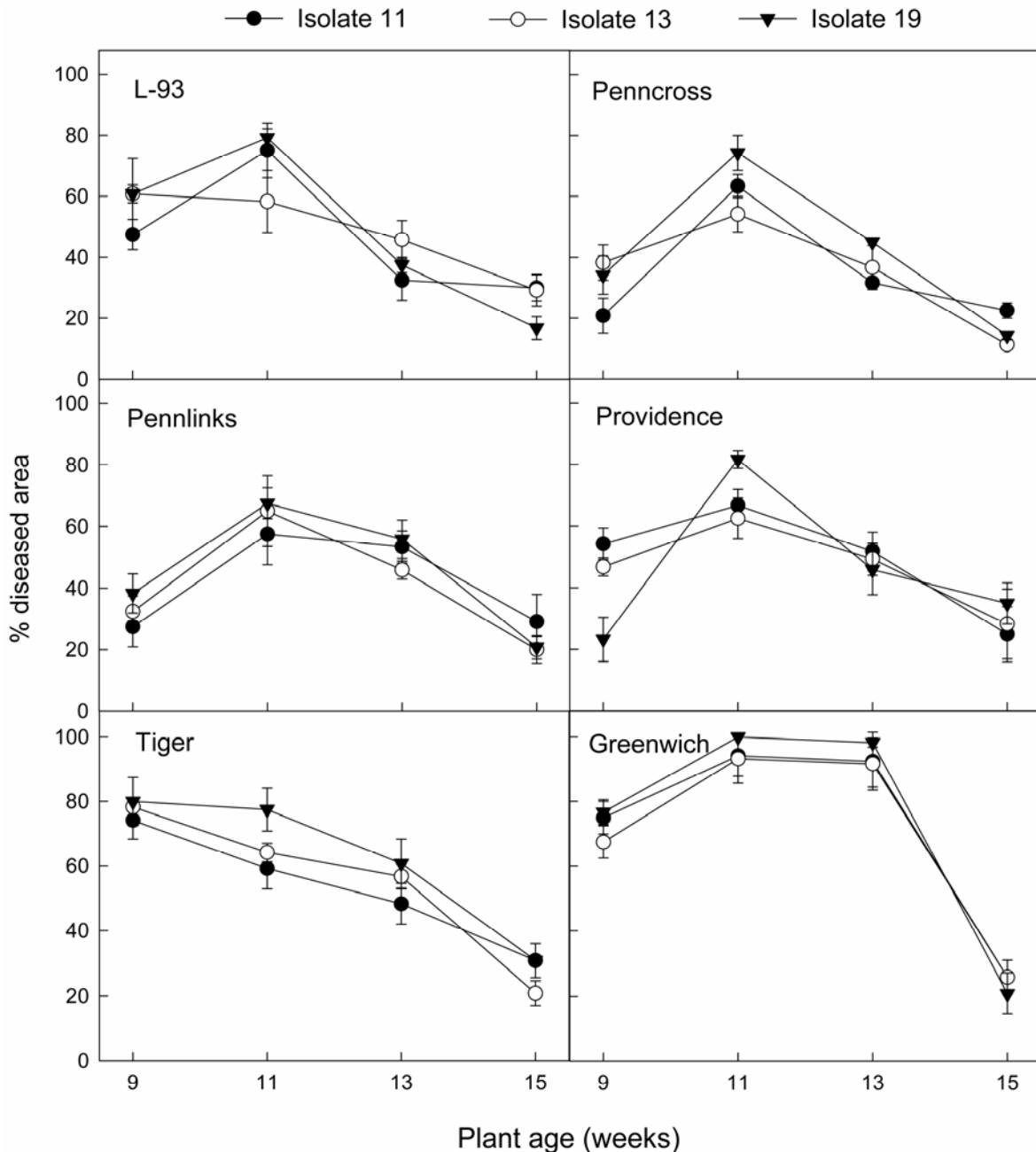


Fig. 3. Disease severity of six cultivars of three bentgrass species (creeping: L-93, Penncross, Pennlinks, and Providence; colonial: Tiger; and velvet: Greenwich) inoculated with each of three isolates of *Typhula incarnata* at four plant ages (9-, 11-, 13-, and 15-week-old plants from germination to inoculation) in the growth chamber. Disease severity was based on percent area diseased. Bars represent standard error of the mean.

2). A linear relationship was found between inoculum concentration and disease severity in all six bentgrass cultivars. Mycelial concentration used for the inoculation of the cultivars explained a minimum of 88% of the variation in disease severity. Typical gray snow mold symptoms with circular and water-soaked patches were induced on the plants inoculated with 0.1 g/ml mfw. At 0.4 g/ml mfw, the four creeping bentgrass cultivars were severely infected (more than 76% diseased areas), as were cultivars of velvet and colonial bentgrass (Fig. 2). Significant effects ($P < 0.0001$) on disease severity for cultivar and inoculum concentration within cultivar were detected, but no interactions between isolate and cultivar. Generally, disease severity was significantly less on L-93 at most inoculum concentrations than on other cultivars of creeping bentgrass. However, velvet bentgrass cv. Greenwich had much greater disease severity than cultivars of creeping and colonial bentgrasses (Fig. 2).

The ANOVA analysis showed highly significant effects of cultivar, plant age (cultivar), and isolate \times plant age (cultivar) on disease severity of four creeping bentgrass cultivars (Table 2). Disease severity

of all creeping bentgrass cultivars, as well as the velvet bentgrass cv. Greenwich and the colonial bentgrass cv. Tiger, was lowest for 15-week-old plants compared with 9-, 11-, and 13-week-old plants (Fig. 3). Bentgrass cultivars, with the exception of 9-week-old plants, became more resistant to *T. incarnata* as they aged. In 13- and 15-week-old plants, disease severity of colonial bentgrass cv. Tiger was similar to that of creeping bentgrass cultivars, but was lower than that of velvet bentgrass cv. Greenwich. Interestingly, 9-week-old plants of all creeping bentgrass cultivars tested, as well as the velvet bentgrass cv. Greenwich, showed much less disease severity in all three isolates than 11-week-old plants, but a similar trend was not observed in the colonial bentgrass cv. Tiger.

Disease severity among the three bentgrass species showed highly significant effects of ploidy contrast between tetraploid creeping and colonial bentgrasses and diploid velvet bentgrass ($P < 0.0001$) (Tables 3 and 4). Disease severity in creeping and colonial bentgrass cultivars was significantly less than velvet bentgrass cultivars for all isolates tested, whereas creeping and colonial bentgrass cultivars were not statistically different. The cvs. L-

93 (creeping bentgrass), Bardot (colonial bentgrass), and Vesper (velvet bentgrass) were less susceptible than the other two cultivars of each species tested in this study. A significant isolate effect ($P < 0.0001$) was observed (Tables 3 and 4). *T. incarnata* isolates 11 and 19 were found to be much more aggressive to most cultivars tested than the other isolates (Table 4).

No significant differences ($P < 0.3027$) were observed between the two runs of the comparison of 15 isolates for aggressiveness on two creeping bentgrass cultivars (*data not presented*); therefore, mean disease values of the runs were used for statistical analysis. All 15 isolates were pathogenic on the cultivars, ranging from 10.8 to 33.3% diseased area (Table 5; Fig. 4). Significant differences ($P < 0.0001$) in aggressiveness among isolates within state were detected. Isolates 3 (Michigan), 7 (Minnesota), 15, and 17 (Wisconsin) were capable of causing severe disease. On the other hand, isolates 5 (Michigan), 9 (Minnesota), and 14 (Wisconsin) were less aggressive. However, there were no significant differences among the three states in isolate aggressiveness.

DISCUSSION

The response of the cultivars to *T. incarnata* isolates was quantitatively controlled as observed in a previous study (34). Disease symptoms developed more slowly on less susceptible cultivars than susceptible ones tested. This same phenomenon also has been recognized in a wheat-snow mold pathosystem (3,13) and in a bentgrass-*T. ishikariensis* pathosystem (7).

Inoculum concentration significantly affected gray snow mold development on bentgrass cultivars. Generally, symptom development increased significantly as inoculum concentration increased. Disease severity was lower on L-93 at most inocu-

Table 3. Analysis of variance for gray snow mold severity on nine cultivars of three bentgrass species inoculated with five isolates of *Typhula incarnata* in the growth chamber

Source of variation	df	Mean square	F value	P
Replication	4	34.4	0.54	0.7098
Ploidy ^a	1	2,343.8	36.54	<0.0001
Genome (ploidy)	1	23.5	0.37	0.5459
Cultivar (ploidy \times genome)	6	353.0	5.50	<0.0001
Isolate	4	10,231.3	159.51	<0.0001
Isolate \times ploidy	4	67.5	1.05	0.3813
Isolate \times genome (ploidy)	4	204.4	3.19	0.0143
Isolate \times cultivar (ploidy \times genome)	24	96.9	1.51	0.0653
Error	220	64.1

^a Creeping and colonial bentgrass are allotetraploid with $A_2A_2A_3A_3$ and $A_1A_1A_2A_2$ genomes, respectively. Velvet bentgrass, with A_1A_1 genome, is diploid (20).

Table 4. Disease severity of nine bentgrass cultivars of three bentgrass species inoculated with five isolates of *Typhula incarnata* in the growth chamber

Species, cultivar	Isolate no.					Mean	LSD _{0.05} ^a
	11	12	13	16	19		
Creeping bentgrass							
L-93	53.3	15.8	15.0	22.5	30.8	27.5	8.9
Penncross	60.0	25.0	20.8	22.5	39.2	33.5	4.6
Providence	56.7	15.8	15.8	27.5	38.3	30.8	6.4
Mean	56.7	18.9	17.2	24.2	36.1	30.6	...
LSD _{0.05}	17.6	3.8	4.4	8.0	3.8
Colonial bentgrass							
Bardot	39.2	23.3	15.8	20.8	35.0	26.8	9.5
SR 7100	59.2	26.7	29.2	23.3	35.8	34.8	8.9
Tiger	52.5	18.3	25.0	29.2	36.7	32.3	10.8
Mean	50.3	22.8	23.3	24.4	35.8	31.3	...
LSD _{0.05}	16.5	9.2	8.3	3.8	4.6
Mean (creeping + colonial)	53.5	20.9	20.3	24.3	36.0	31.0	...
Velvet bentgrass							
Barvaria	61.7	31.7	29.2	36.7	42.5	40.4	9.5
Greenwich	62.5	25.8	30.8	32.5	34.2	37.2	5.2
Vesper	57.5	27.5	16.7	30.0	39.2	34.2	10.9
Mean	60.6	28.3	25.6	33.1	38.6	37.2	...
LSD _{0.05}	15.6	11.8	4.4	5.0	1.9

^a LSD_{0.05} = least significant difference.

lum concentrations than on other cultivars. However, difference in the disease severity among cultivars was not significant in the highest concentration (0.4 g/ml mfw). This suggests that, under the higher disease pressure, differences in cultivar susceptibility may be masked by overwhelming inoculum concentration. Therefore, for screening methods of bentgrass cultivars or germplasm for resistance to gray snow mold under controlled conditions, an inoculum concentration of 0.3 g/ml mfw is suggested for measurable separation among cultivars to be evaluated. However, further studies under field conditions should be required to verify the response of bentgrass plants to various inoculum concentrations.

As plants grew older, the three bentgrass species became less susceptible to *T. incarnata* (Table 2; Fig. 3). This result implies that expression of age-related resistance to *T. incarnata* may occur in bentgrass plants, as recognized in the interaction of other snow mold pathogens with grasses (1,7,11,12,26). Nakajima and Abe (26) reported that older, hardened winter wheat plants express higher resistance to snow mold than young plants similarly hardened under controlled conditions. This has been reported to be associated with the rapid accumulation of high levels of carbohydrates (3) during hardening and slower metabolism of the carbohydrates (21,37) in older plants. In the present study, however, disease severity of 9-week-old plants of bentgrass cultivars, with the exception of Tiger, was less than that of 11-week-old ones. A possible interpretation for this result may be the slower infection caused by fewer tillers in pots of 9-week-old plants than in pots of older plants.

The fact that disease severity of L-93, a less susceptible cultivar, indicated dramatically decreased susceptibility at 13 weeks, whereas Greenwich showed a significant decrease in susceptibility at 15 weeks, may be associated with earlier and higher accumulation of physiologically active substances involved with snow mold resistance in the former cultivar (14). At 15 weeks, susceptibility to gray snow mold was approximately equivalent in all bentgrass cultivars tested, suggesting that cultivars had acquired similar maximum levels of resistance to *T. incarnata*. Therefore, testing plants at more than 15 weeks might express a different level of resistance to gray snow mold under controlled conditions. Although significant interactions between isolate and plant age within cultivar were observed in this experiment, we could not determine the cause of the interactions. The fact that variation of disease severity among isolates within each cultivar, which was much higher on younger plants (9 and 11 weeks old) compared with older plants (13 and 15 weeks old) may indicate that infection and disease severity

was critically affected by infection time and disease progress, because plants in early ages have fewer tillers and are more susceptible responses to the pathogen (7).

The creeping and colonial bentgrass cultivars tested in this study were significantly less susceptible to *T. incarnata* than velvet bentgrass cultivars (Tables 3 and 4). No significant difference was found between creeping and colonial bentgrasses. These results differ from the report by Gould et al. (15), where field resistance of the three species were in the order of creeping > velvet > colonial bentgrass. This discrepancy may be caused by significant genotypic variations, such as those reported by Wang et al. (34), where differences in the relative susceptibility to *T. incarnata* were observed among 360 creeping bentgrass clones collected from old golf courses in Wisconsin. In addition, greenhouse tests may not be reliable predictors of field performance because field conditions such as host nutrient status, aggressiveness of isolate, and environmental conditions can affect bentgrass susceptibility (1,4,21). According to National Turfgrass Evalua-

tion Program data, under field conditions, disease severity within velvet bentgrass cultivars was more variable than severity within both creeping and colonial bentgrass cultivars.

Nevertheless, our result suggests that ploidy level contrast (tetraploid versus diploid) explained most of the variation among the species. It is possible that the resistance genes to gray snow mold may be conferred in the common subgenome (A_2A_2) of creeping ($A_2A_2A_3A_3$) and colonial ($A_1A_1A_2A_2$) bentgrasses. Velvet bentgrass, which is diploid, lacks the A_2A_2 subgenome (20). Further research would determine whether the effect is due to polyploidy itself or whether the A_2A_2 subgenome is responsible for increased resistance.

Significant differences in aggressiveness among isolates within each state were observed (Table 5; Fig. 4). Interestingly, there were no significant differences in aggressiveness between isolates collected from the three states. The significant variation among the isolates might be caused by high rates of outcrossing and sexual re-

Table 5. Analysis of variance for gray snow mold severity on two cultivars of creeping bentgrass inoculated with fifteen isolates of *Typhula incarnata* from Michigan, Minnesota, and Wisconsin in the growth chamber

Source of variation	df	Mean square	F value	P
Replication	4	24.7	0.66	0.6215
Isolate (location)	12	289.4	7.71	<0.0001
Location	2	34.2	0.91	0.4040
Cultivar	1	911.3	24.29	<0.0001
Cultivar × isolate (location)	14	236.0	6.29	<0.0001
Error	145	37.5

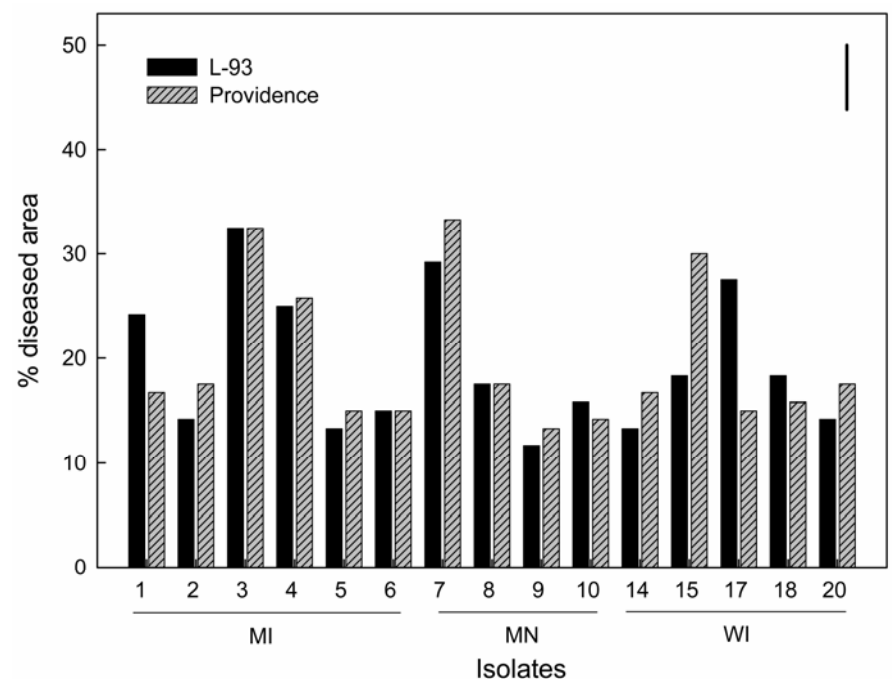


Fig. 4. Disease severity of two creeping bentgrass cultivars inoculated with 15 isolates of *Typhula incarnata* obtained from Michigan (MI), Minnesota (MN), and Wisconsin (WI) in the growth chamber. Disease severity was based on percent area diseased. Vertical lines represent the least significant differences ($P < 0.05$).

combination (5,33). In particular, weather conditions around the northern Great Lakes during fall such as moisture, temperature, and wind may give the fungus a favorable environment for production and dispersal of sexual structures called basidiocarps bearing basidiospores. Basidiocarps of *T. incarnata* and *T. ishkariensis* were observed at a driving range of Gateway Golf Club, Land O' Lakes, WI in the first week of November 2004 and last week of October 2005 (19).

Further, Vergara et al. (33) reported that no correlation was found between geographic distance and DNA marker-derived genetic distance of isolates collected from the three states. Even if sampling sites within each state or among three states show broad geographical distribution and long distance (Fig. 1), the low levels of variation in aggressiveness among the three states may be due to human interference by way of transport, equipment contaminated by their long survival period (10), and similar selection pressures from fungicide applications, the same source of infected sod (30), and similarity of host species or cultivars of turfgrass around the northern Great Lakes regions (33). In addition, an advantage of *T. incarnata* as a good saprophyte may allow the fungus to occupy a broader ecological niche (22,23).

Significant interactions detected between cultivar and isolate in our studies can be interpreted carefully as differential or race-specific, because the numbers of fungal isolates and bentgrass cultivars were not sufficient for such a conclusion. We suggest that further investigation using more samples and incorporating several methods of disease assessment are warranted in order to differentiate bentgrass and *T. incarnata* interactions. A cultivar effect ($P < 0.0001$) observed in this experiment might be explained by the genetic variation of isolates as well as cultivar interactions.

Overall, under controlled conditions, appropriate inoculum concentration of *T. incarnata* and the use of bentgrass plants at the proper age are very important for evaluation of the resistance capabilities of bentgrass cultivars or clones to *T. incarnata*. The information on quantitative variation in susceptibility of three bentgrass species to *T. incarnata* and in isolate aggressiveness also may be beneficial to bentgrass breeders so that a few virulent isolates can be utilized to screen bentgrass cultivars resistant to *T. incarnata*, saving both valuable space and time. However, further field evaluation is required to confirm the current results on variation in susceptibility of cultivars of bentgrasses and in aggressiveness of *T. incarnata* isolates with different geographic origins.

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