CHAPTER IV

Disease Resistance Screening of Bentgrass to *Typhula incarnata* Lasch

ABSTRACT

Bentgrass (*Agrostis* spp.) is susceptible to a wide range of diseases. One economically devastating disease is gray snow mold blight, caused by *Typhula incarnata* Lasch. There are no known resistant cultivars of creeping bentgrass (*A. palustris*) or any other turfgrass species to *T. incarnata*. Research into snow mold disease resistance is hampered by the absence of rapid screening techniques, in addition to poor identification of pathogenic strains of the fungus. Using a controlled screening procedure described herein, we have selected 20 creeping bentgrass genotypes from 890 samples from old Northern Michigan golf courses and identified 3 accessions of colonial bentgrasses (*A. capillaris*) with strong resistance to the gray snow mold. Six commercial creeping bentgrass cultivars 'L-93', 'Penn A4', 'Penn G2', 'Penncross', 'Providence' and 'Emerald' were all found susceptible. The resistant genotypes identified will be useful to the development of creeping bentgrass cultivars.

Key words: Snow mold resistance, controlled screening, creeping bentgrass, colonial bentgrass

INTRODUCTION

Snow mold diseases are important causes of winter injury in grasses and cereals in North America, Canada, Russia, Japan, and the Nordic countries. *Typhula* snow molds are known by different names such as gray snow mold (*T. incarnata),* snow scald, speckled snow mold (*T. ishikariensis)* and *Typhula* blight (collectively caused by *T. incarnata* and *T. ishikariensis*) (Vargas, 1994). Gray snow mold (*T. incarnata*) causes serious damage in turfgrass and is common on golf courses in climates with less than 90 days of snow cover (Millet, 2000). The pathogen is very slow growing and symptoms caused by the different species are difficult to distinguish (Hsiang et al, 1999). Symptoms usually appear as circular, water-soaked or straw-colored patches measuring 5 to 15 cm across. Plants may be matted and appear slimy with mycelium. Usually only the leaves appear diseased and dead and the crown may survive to produce new leaves in the spring. Mycelium, basidiospores or sclerotia that are produced may be sources of inoculum that lead to new infection. Colonized dead plant tissues decompose and disintegrate, and then the sclerotia fall to the thatch and soil where they oversummer, and remain as resting bodies during the summer months. Susceptible plants generally show the water-soaked lesions and a yellowish decaying appearance. The thin crust of mycelium is white and may be covered with dust giving it a gray-white appearance (hence the name "gray snow mold") (Jackson and Fenstermacher, 1969).

Gray snow mold is a cold-loving or 'psychrophilic' organism. Snow molds have the ability to modify their intracellular conditions favorable to survival (Snider et al., 2000). The lack of high ice nucleation activity combined with the presence of antifreeze activity in all fungal fractions indicates that snow molds can moderate their environment

to inhibit or modify intra- and extracellular ice formation, which helps explain their ability to grow at subzero temperatures under snow cover. Pathogenicity of the fungi is not dependent on ice nucleation activity to cause freeze wounding of host plants. Another factor contributing to snow mold survival may be the pathogen's capacity to utilize a broad range of substrates, from live tissues to dead organisms. Jacobs and Bruehl (1986) theorized that *T. incarnata* is unable to establish itself sufficiently in its host to cause disease problems, while Matsumoto and Tajimi (1985) described the fungus more as an 'opportunistic parasite' attacking senescent or moribund plant tissues beneath the snow, where the low temperature reduces the activity of other antagonistic microflora.

There is little information regarding the pathogenicity and variability of the gray snow mold. Snow mold resistance evaluation among bentgrass cultivars is currently done in the field with the National Turfgrass Evaluation Procedure (NTEP) trials using naturally infected plots. In the 1995 NTEP trial, twenty seven *A. palustris* varieties from twenty four sites were observed to show a wide range of resistance to the *Typhula* species, but no variety was found to be strongly resistant. Results from NTEP scored in 2000 at the Michigan State University Hancock Research Center showed snow mold disease infection ranged from 20% to 90% of the plot area. Although NTEP provides information on the performance of bentgrass cultivars, compounding biotic or abiotic factors, e.g. the presence of multiple pathogens, competition, and the moisture or dryness in some areas, limit the applicability of the findings and do not give an accurate description of a cultivar's resistance. Field studies are also dependent on the duration of snow cover and melt.

Under controlled conditions, Millet (2000) inoculated the creeping bentgrass (*A. stolonifera sp. palustris*) cultivar 'Penncross' with isolates of the three snow molds (*T. incarnata, T. ishikariensis* and *T. phacorrhiza*) from Wisconsin area using the Fast Turf screening system. Creeping bentgrass was grown in 35-mm film cans and incubated in cold chambers for three weeks at temperatures of 41 ^{o}F and 50 ^{o}F. The disease was rated at 21, 28 and 35 days. The results indicated that gray and speckled snow molds were not significantly different from each other in their ability to cause disease. *T. phacorrhiza* was not as strongly aggressive and the author postulated its role as a decomposer, termed 'senectophatic disorder', which colonized dying grass as the other snow molds were becoming less active. The study by Millet (2000) is conceptually important because it highlighted developing a controlled screening procedure against snow mold. Resistance in the host plants however was not precisely determined because all three fungal species were used as inoculants concurrently, disregarding the probability of competition between *Typhula* species. Under field conditions, a thin line separates the circular damaged spots created by different species and these disease patches never overlapped.

Studies measuring resistance in turfgrass and other crops to gray snow mold or snow molds are generally few in number. In grasses, *Lolium perenne* is considered the most susceptible to gray snow mold, followed by *Festuca arundinaceae, A. palustris, Poa annua, P. pratensis* and *F. rubra.* (Hsiang, 1999). Within each species, there can be a broad range of susceptibility among cultivars. Wu and Hsiang (1998) found a strong positive correlation between susceptibility of 12 turfgrass species to *T. incarnata* and their susceptibility to *T. ishikariensis.* There are no known resistant cultivars or species of bentgrass and the severity of damage warrants research to find a resistant genotype

under controlled conditions. Despite the low resistance found in creeping bentgrasses, selection of varieties for durable resistance can be done because heritability estimates for snow mold resistance in grasses are high (Gaudet et al., 1999).

Bentgrass, *Agrostis* sp. a derivation from Greek: grass, forage, has about 220 species distributed throughout the world (Watson and Dallwitz, 1992). It is a perennial or annual outcrossing polyploid (x=7, 2n=14, 21, 28, 42, etc.). The wide genetic variability implies potential for finding a resistant plant among the bentgrass species. Other possible sources for snow mold resistance may be in naturally selected clones of creeping bentgrass. To identify such sources, creeping bentgrass (*A. stolonifera sp. palustris*) samples were collected from old Northern Michigan golf courses that have not been sprayed with fungicide or overseeded for the last 10 years. Natural selection and recombination events could have played a significant role in creating resistant creeping bentgrass. Identifying resistant clones through controlled screening experiments would contribute to turfgrass improvement programs. Controlled screening would also enable rapid testing of several materials independent of the presence of snow and with high repeatability.

The objectives of this study were to develop a controlled physiological screening system for resistance to *T. incarnata* and to search for plants resistant to the pathogen from several creeping bentgrass cultivars, naturally selected clones obtained from Michigan and plant introduction lines of *Agrostis* spp.

MATERIAL AND METHODS

Plant materials

The materials were divided into three groups:

a. Creeping bentgrass collected in April 2000 from golf courses in Northern Michigan - Plugs were collected from areas bordering snow mold circular patches. A total of 890 genotypes were used as follows: 220 samples (Population A) for developing the procedure and another 670 samples (Population B) for additional screening.

b. Commercial creeping bentgrasses, 8 to 10 plants each of cultivars, 'L-93', 'Penn A4', 'Penn G2', 'Penncross', 'Providence' and 'Emerald' were used.

c. Forty accessions of *Agrostis* sp. plant introductions, representing 14 species obtained from USDA Washington Pullman Station were used. Seeds of 25 plants per accession were germinated on filter paper and transferred into pots (1 seedling /pot) (Table 4.1).

The plants were grown in No. 2 pots (4x4x4 inches) containing peat soil mixture and supplemented with Peter's fertilizer. Plant height was maintained by clipping at 1.0 inch. Several 'Penncross' plants were also grown in pots and used as a susceptible plant control.

Pathogen

Sclerotia of gray snow mold (*T. incarnata*) were collected from Hancock Turfgrass Research Center in April 2001 and grown in potato dextrose agar (PDA, 39g/L with streptomycin and penicillin antibiotics) at 5 °C for 2 months. Each isolate was then transferred to sterilized cornmeal mixture (1 part cornmeal to 2 parts silica sand with 5% PDA broth, autoclaved for 40 minutes) for multiplication. Growth and incubation in cornmeal was made at 5 °C for another two months. A phase contrast microscope was used to verify the presence of *T. incarnata* in the cornmeal mixture by checking for the

Table 4.1. List of *Agrostis* spp. screened for snow mold resistance and their geographic origins.

Species	No. of accessions	Geographic origins
A. canina	2	Netherlands, Iran
A. capillaris	7	Europe
A. castellana	10	USA, Spain, Portugal
A. palustris	5	USA, Turkey, Europe
A. stolonifera	6	USA, Europe, Russia
A. mongolica	1	Mongolia
A. lachnantha	2	Africa
A. munroana	1	Iran
A. hygrometrica	1	Uruguay
A. scabra	1	Canada
A. trinii	1	Russia
A. vinealis	1	Russia
A. transcaspica	1	Russia
A. gigantea	2	USA, Turkey

presence of mycelia and visually checking for the presence of orange to brown sclerotia. The fastest growing and virulent isolate identified from pre-screening was chosen and used for the controlled screening procedures.

Controlled screening and rating procedure

Initial screening was done in controlled plant growth chambers using day temperature of 7 °C and night temperature of 4 °C for 4 weeks. However the disease did

not progress well. After the optimum temperature of 5 °C was determined, all succeeding tests were performed in the cold room available at the Crop & Soil Sciences farm at Michigan State University. In the first screening, 220 creeping bentgrasses (Population A) from the N. Michigan collection were randomly put in trays at 10 plants per tray. The plants were brought into the cold room (5 °C) for 3 days for acclimation prior to disease inoculation. Equal amounts of inoculum (1 g) were put into the center of each pot and covered with moistened cheesecloth. To compare treatments of inoculated versus uninoculated plants, only 9 plants/tray were inoculated and 1 pot was left untreated. The trays were filled with water and put inside plastic bags to maintain high humidity and optimize disease severity. Visual inspection and scoring for resistant and susceptible plants was done at 4 and 6 weeks using Horsfall-Barratt system with a scale of 0 = no disease to 10 = completely dead plant. Susceptible plants were classified by the growth of the infection area as characterized by increased area of soft, watery, yellow or brown lesions and widespread development of mycelia. Two people rated and the means of two ratings were used for analysis. Plants were returned to the greenhouse (GH) after 6 weeks and scores were taken again after 3 days. The previously yellowing leaves would appear brown and dried at this time. Recovery was scored after another 10 days (1, 3, 5, 7, 9, 10 scale, with 10 as 100% recovered). Statistical analysis using Proc GLM option of SAS was done to test significance between uninfected and infected treatments. The plants were ranked based on the disease scores and recovery.

Candidate resistant plants were divided into three clones and screened for a second time using 'Penncross' as the susceptible check, and all plants in the tray were inoculated. Screening for the resistant lines from Population B (670 creeping bentgrass),

the 6 commercial cultivars and 40 plant introductions followed the procedure described above using completely randomized design (CRD). Statistical Analysis Software (SAS version 8.0) was used for data analyses. Arcsin transformation on percentage means for each score was calculated and F-Test was generated using the PROC GLM with LSD options of SAS.

RESULTS AND DISCUSSION

Screening creeping bentgrass populations

The growth conditions in the cold room of 5 ^{o}C temperature and high humidity were found favorable for *T. incarnata*. At 4 weeks, fungal mycelia were visible on creeping bentgrass and leaves appeared water-soaked and slightly yellowed (Figure 4.1.A). At 6 weeks, lesions were turning brown on whole leaves of some of the inoculated plants with a wider spread of infected area (Figure 4.1.B). Some plants appeared to be entirely damaged and this was more apparent when plants were brought to the greenhouse and scored after 3 days (Figure 4.1.C). The results of the controlled screening system using two populations of creeping bentgrass from old Michigan golf courses: population A (220 plants) and population B (670 plants), are shown in Table 4.2. In both populations, significant differences between the two treatments, inoculated and uninoculated (as control) were observed at the 4th week, 6th week and after 3 days in the greenhouse. Control plants were mostly rated as 0 (no disease) but a few plants were rated as 1 or 2 (for slightly yellowed). This is largely in contrast to the inoculated plants where the mean disease scores in six weeks ranged from 5.51 (50-60% infected) to 7.53

Figure 4.1. **A.** Creeping bentgrass at the 4th week of snow mold infection, left bottom pot in the tray is uninoculated. **B.** Disease after the 6th week, left top pot in the tray is uninoculated. **C.** Trays on benches at 3 days in the greenhouse **D.** PI lines showing susceptible plants and accessions with good recovery. *"Images in this dissertation are presented in color."*

Table 4.2. Analysis of variance of snow mold disease inoculation in creeping bentgrass using inoculated and uninoculated (control) as treatments in two populations from N. Michigan.

Population	Treatments and F-values[3]	N	Snow mold Rating[1]			Recovery Means[2]
			4th week	6th week	GH 3d	
A. 220 plants	Control	23	0.95 ± 1.5	1.24 ± 2.0	1.87 ± 2.7	8.2 ± 2.8
	Inoculated	197	5.51 ± 1.2	6.46 ± 1.2	7.53 ± 1.1	3.2 ± 2.1
	F-value		228.15**	241.97**	266.07**	190.04**
B. 670 plants	Control	63	0.84 ± 1.0	1.40 ± 1.6	2.40 ± 1.9	8.07 ± 2.2
	Inoculated	607	5.67 ± 1.1	6.83 ± 1.1	8.02 ± 1.0	2.73 ± 2.2
	F-value		898.78**	993.72**	787.1**	480.28**

[1] Disease severity was rated on as Horsfall-Baratt scale of 0 (no infection) to 10 (completely dead). Scores are the means of N (number of samples).

2 Recovery rated on a scale of 0 (no recovery) to 10 (complete recovery).

[3] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation on done on the percentage values and run using Proc GLM option of the SAS system.

* *Treatment means were found significant different at $P<0.05$.

(70-80%, highly infected). There were significant differences between the two treatments, uninoculated and inoculated, suggesting the inoculation treatment was effective to cause disease. The frequency pattern of snow mold disease scores in population B was graphed and generally follows a bell distribution curve (Figure 4.2). The continuous distribution pattern is suggestive of a horizontal type of resistance controlled by a few minor genes. In contrast, if resistance trait was a vertical resistance type controlled by a major gene, frequency data distribution will be in 2 discrete columns.

Recovery was measured after another 10 days in the greenhouse. Uninoculated creeping bentgrasses recovered significantly better than the inoculated plants. Recovery for infected plants was generally low at 27% to 32%.

From population A, 28 plants were chosen as candidate resistant plants after ranking. Another 59 plants were chosen as candidate resistant plants from population B. The candidate resistant plants were divided into three clones and subjected to a second screening. A second replicated screening was necessary to determine accuracy of the first rating for the resistant genotypes as disease escapes could be factor for erroneous rating. Replication and testing against a highly susceptible check, 'Penncross' ensured better selection for the resistant genotype.

The results of replicated screening of selected genotypes from population A and B are shown in Table 4.3 and Table 4.4. Analysis of variance (F-test) and t-tests (LSD) showed significant differences in the 6^{th} week, GH 3 days rating and the means of the three ratings (Table 4.3.1). Ratings were not found significant across the 28 creeping bentgrass genotypes at the 4^{th} week ($P<0.10$) and but were highly significant at the 6^{th} week and GH at $P<.05$. Nine genotypes (Nos. 15, 4, 21, 6, 8, 26, 22, 24 and 28)

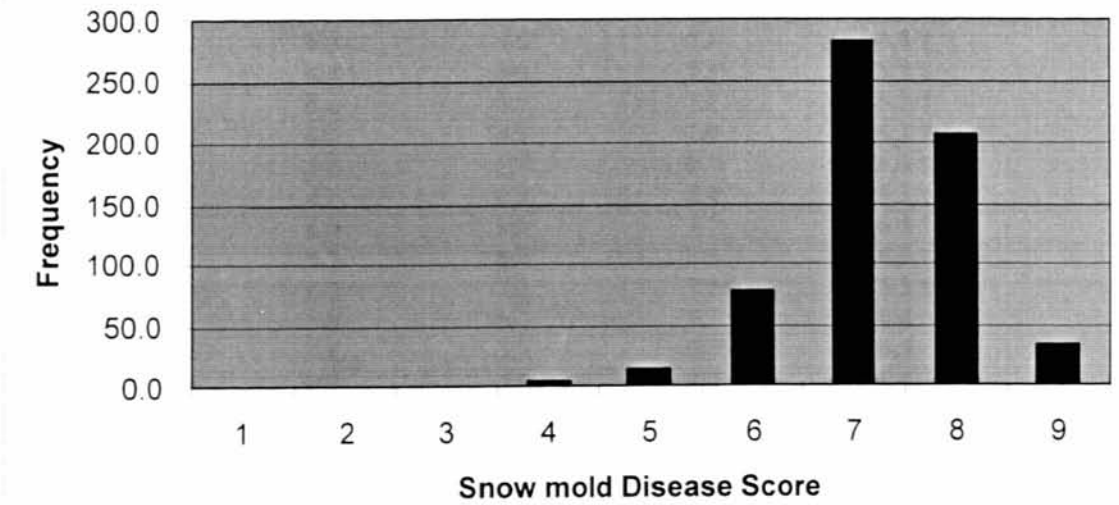

Figure 4.2. Distribution of disease rating scores in 670 creeping bentgrass genotypes.

Table 4.3.1. Disease ratings and analysis of variance of candidate resistant lines of creeping bentgrass from Population A to snow mold (*Typhula incarnata*) using a completely randomized design with 3 replicates.

Candidate Resistant line [3]	Snow mold Rating[1, 2]			
	4th Week	6th Week	GH 3 days	Means
15	3.5	4.5	5.5	4.8 †
4	4.5	5.3	7.8	5.9 †
21	6.0	5.5	7.2	6.2 †
6	5.7	6.3	7.0	6.3 †
8	4.5	7.7	6.7	6.3 †
26	4.7	6.3	8.0	6.3 †
22	5.0	6.8	7.7	6.5 †
24	5.7	6.0	7.8	6.5 †
28	5.2	7.0	7.3	6.5 †
2	5.8	6.3	7.7	6.6
9	5.8	6.7	7.3	6.6
20	6.0	5.8	8.0	6.6
27	5.8	6.8	7.2	6.6
17	6.2	6.7	7.3	6.7
19	5.8	7.2	7.3	6.8
25	5.7	5.8	7.3	6.8
3	6.0	7.5	7.3	6.9
11	6.3	7.1	7.3	6.9
12	5.8	6.8	8.2	6.9
23	6.5	6.7	7.5	6.9
10	5.3	7.5	8.2	7.0
7	6.2	7.5	7.5	7.1
13	6.8	7.2	7.5	7.1
16	6.5	7.3	7.3	7.1
1	6.7	7.5	7.5	7.2
5	6.5	7.2	8.0	7.2
18	6.5	7.8	7.3	7.2
14	7.3	7.8	7.5	7.5
Penncross	7.0	7.8	8.3	7.7
F-test (P<.0001)[4]	1.62	1.69**	2.07**	1.84**
LSD (P=0.05)	1.8	1.8	1.0	1.1

[1] Disease severity rated on a Horsfall-Barratt scale of 0 (no infection) to 10 (completely dead).

[2] Each score was taken independently by 2 raters and the mean was calculated for 3 replicates. '4th' and '6th' week ratings were taken in the cold room at 5°C. 'GH 3 days' ratings were taken 3 days in the greenhouse after pots were taken out of the cold room.

[3] A total of 28 candidate resistant lines were selected from 220 individual creeping bentgrass genotypes and divided into three replicates.' Penncross' was used as the susceptible plant control.

[4] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation was done on the percentage values and run using Proc GLM option of the SAS system for calculation of F values.

* Scores were found significantly different at P<0.10.
** Scores were found significantly different at P<0.05.

† Disease rating means were found to be significantly different from 'Penncross'.

Table 4.3.2. Recovery ratings of 28 selected creeping bentgrass lines (Population A) from snow mold infection using CRD with 3 replicates (ranked from the best genotype).

	Recovery Rating[1, 2]
Candidate Resistant Line	Means
2	8.7 †
8	8.0 †
15	8.0 †
22	7.7 †
24	7.7 †
9	7.0 †
17	7.0 †
25	7.0 †
26	7.0 †
7	6.7 †
23	6.7 †
6	6.3 †
18	6.3 †
21	6.3 †
3	6.0 †
5	6.0 †
27	6.0 †
28	6.0 †
14	5.3 †
19	5.3 †
20	5.3 †
10	5.0
13	4.7
11	4.0
16	4.0
Penncross	4.0
1	3.7
4	3.3
12	2.0
F test (P<0.0001)[3]	2.33**
LSD(P=0.05)	2.90

[1] Recovery rated on a scale of 0 (no recovery) to 10 (complete recovery).

[2] Each score was taken independently by 2 raters and the mean for 3 replicates.

[3] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation on done on the percentage values and run using the Proc GLM option of the SAS system.

** Scores were found significantly different at P<0.05.

† Recovery ratings were found to be significantly better than 'Penncross'.

Table 4.4.1. Disease ratings and analysis of variance of candidate resistant lines of creeping bentgrass from Population B to snow mold using a completely randomized design with 3 replicates.

Candidate Resistant line [3]	Snow mold Rating[1, 2]			
	4th Week	6th Week	GH 3 days	Means
336	3.0	4.3	5.0	4.1 †
226	3.0	3.5	6.7	4.4 †
223	3.2	5.0	5.2	4.4 †
560	3.2	4.8	5.3	4.4 †
367	3.2	3.5	6.7	4.4 †
191	3.3	3.8	6.3	4.5 †
585	3.3	3.3	7.0	4.6 †
79	3.5	4.8	5.8	4.7 †
249	3.5	4.3	6.3	4.7 †
645	3.2	4.0	7.0	4.7 †
247a	4.0	4.5	6.0	4.8 †
247b	3.7	5.2	6.0	4.9 †
484	4.3	4.5	6.0	4.9 †
261	3.3	4.7	7.2	5.1 †
587	4.0	4.2	7.2	5.1 †
595	3.5	4.7	7.2	5.1 †
148	4.5	4.8	6.2	5.2
152	3.8	5.2	6.7	5.2
242	4.5	5.2	6.0	5.2
110	4.7	5.2	6.0	5.3
194	4.3	5.3	6.2	5.3
121	4.3	5.2	6.5	5.3
122	3.7	4.7	7.7	5.3
404	4.5	5.3	6.3	5.4
156	4.5	5.2	6.7	5.4
368	4.5	4.2	7.7	5.4
640	4.5	4.2	7.7	5.4
117	4.0	5.0	7.7	5.6
175	4.7	5.0	7.0	5.6
239	4.3	5.8	6.5	5.6
648	4.2	4.7	7.8	5.6
544	4.3	5.3	7.2	5.6
639	4.7	4.3	8.2	5.7
313	4.2	4.8	8.2	5.7
656	4.3	4.7	8.2	5.7
406	5.0	4.8	7.5	5.8
392	5.0	5.5	7.0	5.8
227	5.2	5.7	6.8	5.9
647	4.8	5.8	7.0	5.9
193	4.7	5.8	7.5	6.0
370	4.5	5.8	7.7	6.0
435	4.5	5.3	8.2	6.0
527	4.5	5.7	7.8	6.0
39	4.5	6.0	7.7	6.1
636	5.0	6.2	7.0	6.1
49	5.2	5.5	7.5	6.1
289	5.3	5.8	7.0	6.1
635	5.2	5.3	7.7	6.1
6	5.2	5.3	7.8	6.1
528	5.5	4.7	8.3	6.2

Penncross	3.5	6.8	8.8	6.2
146	4.8	6.3	7.7	6.3
604	5.8	5.5	7.7	6.3
644	5.7	5.5	8.2	6.4
145	5.5	5.7	8.3	6.5
570	5.7	6.0	8.2	6.6
291	5.2	6.7	8.2	6.7
649	5.5	6.7	8.0	6.7
522	5.8	6.7	7.8	6.8
F-test (P<.0001)[4]	2.28**	2.37**	2.92**	3.20**
LSD (P=0.05)	1.4	1.4	1.4	1.0

[1] Disease severity rated on a Horsfall-Barratt scale of 0 (no infection) to 10 (completely dead).

[2] Each score was taken independently by 2 raters and the mean for 3 replicates. '4th' and '6th' week ratings were taken in the cold room at 5°C. 'GH 3 days' was ratings taken 3 days in the greenhouse after pots were taken out of the cold room.

[3] A total of 59 breeding lines were selected from 670 individual creeping bentgrass genotypes and divided into three replicates. 'Penncross' was used as the susceptible plant control.

[4] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation was done on the percentage values and run using Proc GLM option of the SAS system.

** Scores were found significantly different at P<0.05.

† Disease rating means were found to be significantly less than 'Penncross'.

Table 4.4.2. Recovery ratings of selected creeping bentgrass lines to snow mold (*Typhula incarnata*) using CRD with 3 replicates.

	Recovery Rating[1, 2]
Breeding Line	Means
194	7.9 †
336	7.4 †
79	7.3 †
152	7.2 †
6	7.1 †
242	7.1 †
554	6.9 †
175	6.7 †
404	6.7 †
247b	6.6 †
640	6.5 †
484	6.3 †
570	6.3 †
223	6.2 †
560	6.2 †
585	6.2 †
122	6.1 †
522	6.1 †
247a	6.0 †
587	6.0 †
645	6.0 †
110	5.9 †
249	5.8
227	5.8
239	5.8
39	5.7
370	5.7
649	5.7
636	5.5
639	5.5
604	5.4
49	5.3
648	5.3
289	5.3
121	5.2
145	5.2
226	5.2
656	5.2
117	5.0
291	5.0
644	5.0
595	4.9
191	4.8
313	4.8
146	4.8
261	4.7
367	4.4
647	4.3
368	4.2
406	4.2

193	4.1
148	4.0
528	3.8
635	3.7
435	3.5
527	3.0
Penncross	2.9
F test (P<0.0001)[3]	2.77**
LSD(P=0.05)	2.08

[1] Recovery rated on a scale of 0 (no recovery) to 10 (complete recovery).

[2] Each score was taken independently by 2 raters and the mean for 3 replicates.

[3] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation on done on the percentage values and run using the Proc GLM option of the SAS system.

† Recovery means were found to be significantly better than 'Penncross'.

** Scores were found to be significantly different at P<0.05.

performed significantly better than susceptible check 'Penncross' from the replicated trials and candidate resistant line No. 15 was found to be the most resistant genotype. Recovery from disease was also found to be significantly different among genotypes (Table 4.3.2). Genotype No. 15 was ranked third in recovery. Twenty-one lines recovered better than 'Penncross'. Genotype No. 4 which had the second best resistant score or low disease rating had poor recovery indicating that resistance and recovery are independent traits. Eight genotypes could be considered as potential breeding materials with good snow mold resistance and significant recovery from cold and disease treatments.

Significant differences among disease rating means of 59 candidate resistant plants were found from Population B at the 4^{th} week, 6^{th} week and GH 3 days rating stages (Table 4.4.1). Sixteen genotypes were found with significantly better disease resistance than 'Penncross'. Genotype No. 336 was found to be the most resistant and with the second best recovery (Table 4.4.2). From these 16 best resistant genotypes, only 12 plants were selected as breeding lines because 4 genotypes did not recover well. The line with the second least disease rating, No. 226, did not perform better than 'Penncross' supporting previous findings that resistance and recovery are independent traits. A total of 20 resistant genotypes were found from 890 clones taken from Northern Michigan old golf courses.

Screening commercial creeping bentgrass cultivars

Six of the most popular commercial creeping bentgrass cultivars 'L-93', 'Penn A4', 'Penn G2', 'Penncross', 'Providence' and 'Emerald', were subjected to the

developed snow mold screening procedure using complete randomized design with 8 to 10 replicates per cultivar. Significant differences were found among them for disease rating means and percentage recovery (Table 4.5). 'L-93', 'Penn A4', 'Penn G2', 'Penncross' and 'Providence' were grouped together by t-tests (LSD) and were not significantly different in mean disease ratings. Cultivar 'Emerald' was grouped separately and hence appeared as more susceptible than the first group. No commercial cultivar was found resistant.

The six commercial cultivars significantly varied in recovery performance with 'L-93' having the best rating. The results support the ratings from NTEP that 'L-93' is the superior creeping bentgrass cultivar. 'Penncross' showed 39% mean recovery and this is close to the previous recovery ratings of 4.0 (40% recovery) in the trials using Populations A and B in the first two tests. 'Penn G2', 'Penn A4' and 'Providence' had low recovery ratings and did not differ as a group based on t-tests.

Screening of PI Lines

Forty plant introductions were subjected to the snow mold screening procedure, with 24 to 25 representative genotypes per accession. Disease rating means and analysis of variance showed that ten accessions significantly performed better than 'Penncross' (Table 4.6.1). The ten accessions were species of *A. canina*, *A. capillaris*, *A. vinealis* and *A. mongolica*. The best performing colonial bentgrasses (*A. capillaris*) came from Europe. *T. incarnata* is widely distributed in Europe, Russia and parts of Northern Hemisphere and bentgrasses originating from these areas may have potential resistance to the pathogen. None of the creeping bentgrass species (*A. stolonifera* or *A. palustris*) was

Table 4.5 . Performance and analysis of variance of commercial creeping bentgrass cultivars using CRD in controlled snow mold screening experiments, their disease rating means[1] and percentage recovery [2].

Genotype	No. of Samples	Disease Rating Means[3]	Percentage Recovery[3]
L-93	10	5.9 ± 0.54^{a}	$61.00\ \% \pm 10.22^{a}$
Penn A4	8	5.6 ± 0.48^{a}	$19.37\ \% \pm 13.74^{c}$
Penn G2	10	5.4 ± 0.41^{a}	$27.00\ \% \pm 13.78^{c}$
Penncross	10	5.8 ± 0.76^{a}	$39.00\ \% \pm 7.74^{b}$
Providence	10	5.5 ± 0.20^{a}	$16.50\ \% \pm 7.09^{c}$
Emerald	10	6.5 ± 0.71^{b}	$29.50\ \% \pm 3.29^{bc}$
F-test		5.28**	31.54**
LSD		0.9	16.08

[1]Disease severity rated on Horsfall-Baratt scale of 0 (no infection) to 10 (completely dead). Disease rating means is the average of mean ratings at the 4th week, 6th week and at 3 days in the greenhouse taken independently by 2 raters and mean for given number of samples.

[2] Recovery was taken at 17 days in the greenhouse and means of the percentage recovery of the number of samples.

[3] Means followed by the same letter are not significantly different.

** Scores were found significantly different at $P<0.05$

Table 4.6.1. Disease ratings and analysis of variance of resistance to gray snow mold of different bentgrass species with 'Penncross' (as susceptible control) using CRD with 24 to 25 genotypes per accession.

		Snow mold Rating[1, 2]			
Species (MSU No.)	N	4th Week	6th Week	GH 3 days	Means
A. capillaris MSU-4	24	4.1	6.4	6.6	5.7 †
A. capillaris MSU-6	25	4.5	6.3	6.4	5.7 †
A. canina MSU-2	25	4.4	6.3	6.9	5.9 †
A. canina MSU-1	25	4.0	5.7	8.8	6.2 †
A. capillaris MSU-3	25	4.2	6.6	7.7	6.2 †
A. capillaris MSU-8	25	5.9	7.2	5.4	6.2 †
A. vinealis MSU-37	25	4.5	6.4	7.8	6.2 †
A. capillaris MSU-9	25	5.8	7.3	5.7	6.3 †
A. mongolica MSU-32	25	4.3	6.7	7.9	6.3 †
A. capillaris MSU-5	25	5.9	7.2	6.0	6.4 †
A. gigantea MSU-40	25	4.9	6.6	8.1	6.5
A. capillaris MSU-7	25	5.5	7.1	7.1	6.6
A. lachnantha MSU-30	25	4.6	6.5	8.6	6.6
A. hygrometrica MSU-33	25	4.3	6.7	8.7	6.6
A. scabra MSU-35	25	4.5	6.0	9.2	6.6
A. trinii MSU-36	25	5.5	6.9	7.5	6.6
A. stolonifera MSU-29	25	4.7	6.9	8.4	6.7
A. castellana MSU-16	25	4.9	7.0	8.2	6.7
A. castellana MSU-18	25	5.0	6.7	8.5	6.7
A. castellana MSU-13	25	5.2	6.9	8.2	6.8
A. castellana MSU-15	24	5.5	7.2	7.9	6.9
A. castellana MSU-17	25	5.5	7.4	7.9	6.9
A. palustris 'Penncross'	25	5.5	7.4	7.9	6.9
A. castellana MSU-14	25	5.3	7.3	8.2	6.9
A. castellana MSU-12	25	5.6	7.2	8.1	7.0
A. castellana MSU-11	25	5.0	7.1	8.8	7.0
A. stolonifera MSU-27	25	5.2	6.9	8.9	7.0
A. transcaspica MSU-38	25	5.6	7.3	8.2	7.0
A. palustris MSU-20	25	5.3	7.2	8.6	7.0
A. gigantea MSU-39	25	5.5	7.2	8.4	7.0
A. stolonifera MSU-25	25	5.0	7.5	8.8	7.1
A. stolonifera MSU-26	25	5.9	7.4	8.0	7.1
A. palustris MSU-21	25	5.8	7.3	8.4	7.2
A. palustris MSU-23	25	5.6	7.3	8.6	7.2
A. munroana MSU-34	25	5.4	6.9	9.3	7.2
A. castellana MSU-10	25	5.6	7.1	9.0	7.2
A. stolonifera MSU-24	25	5.8	7.7	8.3	7.3
A. castellana MSU-19	25	5.6	7.2	9.0	7.3
A. palustris MSU-22	25	5.9	7.7	8.3	7.3
A. stolonifera MSU-28	25	5.8	7.3	9.2	7.4
A. lachnantha MSU-31	25	5.4	7.7	9.3	7.5
F test (P<0.0001)[3]		6.5**	5.8**	11.2**	8.38**
LSD(P=0.05)		0.6	0.5	0.7	0.4

[1] Disease severity rated on a Horsfall-Barratt scale of 0 (no infection) to 10 (completely dead).

2 Each score was taken independently by 2 raters and the mean for 25 replicates. '4th' and '6th' week

[3] Ratings were taken in the cold room at 40°F. 'GH 3 days' were ratings taken 3 days in the greenhouse after pots were taken out of the cold room.

[4] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation on done on the percentage values and run using the Proc GLM option of the SAS system.

† Disease rating means found to be significantly different from 'Penncross'.

** Scores were found significantly different at $P<0.05$.

Table 4.6.2. Recovery ratings of accesssions of *Agrostis* species to gray snow mold (*Typhula incarnata*) using CRD with 24 to 25 genotypes per accession.

		Recovery Rating[1, 2]
MSU No.	Species	Means
MSU-8	*A. capillaris*	6.1 †
MSU-9	*A. capillaris*	6.0 †
MSU-5	*A. capillaris*	5.3 †
MSU-7	*A. capillaris*	4.1
MSU-26	*A. stolonifera*	3.9
MSU-6	*A. capillaris*	3.7
Penncross	*A. palustris*	3.5
MSU-2	*A. canina*	3.4
MSU-4	*A. capillaris*	2.9
MSU-14	*A. castellana*	2.9
MSU-36	*A. trinii*	2.9
MSU-12	*A. castellana*	2.8
MSU-13	*A. castellana*	2.8
MSU-17	*A. castellana*	2.8
MSU-32	*A. mongolica*	2.8
MSU-15	*A. castellana*	2.6
MSU-16	*A. castellana*	2.6
MSU-22	*A. palustris*	2.5
MSU-20	*A. palustris*	2.4
MSU-3	*A. capillaris*	2.1
MSU-21	*A. palustris*	2.1
MSU-30	*A. lachnantha*	2.1
MSU-18	*A. castellana*	2.0
MSU-37	*A. vinealis*	2.0
MSU-40	*A. gigantea*	1.9
MSU-23	*A. palustris*	1.7
MSU-38	*A. transcaspica*	1.7
MSU-24	*A. stolonifera*	1.6
MSU-39	*A. gigantea*	1.6
MSU-19	*A. castellana*	1.5
MSU-25	*A. stolonifera*	1.5
MSU-29	*A. stolonifera*	1.5
MSU-1	*A. canina*	1.4
MSU-11	*A. castellana*	1.2
MSU-27	*A. stolonifera*	1.1
MSU-33	*A. hygrometrica*	0.9
MSU-10	*A. castellana*	0.8
MSU-28	*A. stolonifera*	0.3
MSU-34	*A. munroana*	0.3
MSU-35	*A. scabra*	0.3
MSU-31	*A. lachnantha*	0.2
F test (P<0.0001)[3]		14.96**
LSD(P=0.05)		1.0

1 Recovery rated on a scale of 0 (no recovery) to 10 (complete recovery).
2 Each score was taken independently by 2 raters and the mean for 25 replicates.
[3] Ratings were converted into percentages as in 1=10% and 10=100%. Arcsin transformation on done on the percentage values and run using the Proc GLM option of the SAS system.
† Recovery means were found to be significantly different from 'Pencross'.
** Scores were found significantly different at α =0.05.

found to be significantly different from the susceptible check 'Penncross' (*A. palustris).* Colonial bentgrasses and *A. mongolica* have the same ploidy level (2n=4x, tetraploid) as creeping bentgrasses and may potentially be donors of snow mold resistance. However *A. mongolica* had very poor recovery. Only three accessions showed significant recovery after snow mold infection (Table 4.6.2). These three accessions were all colonial bentgrasses.

In summary, a suitable physiological screening technique was developed against gray snow mold (*T. incarnata*) that would enable rapid, reproducible and controlled determination of resistance. We selected 20 resistant creeping bentgrass genotypes from 890 samples from old Northern Michigan golfcourses. Original populations of bentgrasses in temperate North America were derived from highly heterogenous populations of South German bentgrass mixtures (Casler et al., 2003). Decades of natural selection must have eliminated many unadapted plants in favor of plants with pest resistance and stress tolerances needed to survive management and edaphic factors that define their local environment (Casler et al., 1996 and 2003). Natural variation existing in old golf courses has been useful as a foundation for several creeping bentgrass programs (Engelke et al., 1995; Hurley et al., 1994).

Current top commercial creeping bentgrasses, 'L-93', 'Penn A4', 'Penn G2', 'Penncross', 'Providence' and 'Emerald' were all found susceptible to gray snow mold. Among the PI accessions or 14 *Agrostis* species, some accessions of colonial bentgrasses (*A. capillaris*) appears to have the best resistance to snow mold. The 2001 National Bentgrass test sponsored by the USDA and National Turfgrass Federation findings showed that from the snow mold complex ratings of 26 bentgrass cultivars grown on a

fairway or tee, only one cultivar of colonial bentgrass, SR 7100 was found to be resistant to snow mold. Future experiments need to compare the colonial bentgrass selections and re-check SR7100 resistance to gray snow mold, and or screen for resistance against other isolates of gray, speckled or pink snow molds.

REFERENCES

Anonymous. 1996. 1993 National Bentgrass (Green) Test - 1995. Data, Table 21. Percent *Typhula* ratings of Bentgrass Cultivars on a Green. NTEP 97-6, Beltsville, MD.

Anonymous. 2001. National Bentgrass Test -1998 . Data, Table 26. Snow mold complex ratings of bentgrass cultivars grown on a fairway or tee. Rating of Bentgrass Cultivars on a Green. 2001 data, Beltsville, MD.

Casler, M.D., J.F. Pedersen, G.C. Eizenga and S.D. Startton. 1996. Germplasm and cultivar development. In LE Moser et al (eds.) Cool-season forage grasses. American Society of Agronomy, Madison, WI. Pp. 413-469.

Casler, M.D., Y. Rangel, J. Stier and G. Jung. 2003. RAPD Marker Diversity among creeping bentgrass clones. Crop Sci. 43:688-693.

Engelke, M.C., V.G. Lehman, W.R. Kneebone, P.F. Colbaugh, J.A. Reinert and W.E. Knoop. 1995. Registration of 'Crenshaw' creeping bentgrass. Crop Sci. 35:590.

Gaudet, D.A. and A. Larouche A. 1999. Towards an understanding of snow mould resistance. *In* Intl Workshop on Plant-Microbe Interactions at Low Temperatures under Snow and Intl Comm. On Global Climate and Plant Environmental Stress, Akureyri, Iceland.

Hurley, R.H., V.G. Lehman, J.A. Murphy and C.R. Funk. 1994. Registration of 'Southshore' creeping bentgrass. Crop Sci. 34:1124-1125.

Hsiang T., N. Matsumoto and S. Millet. 1999. Biology and management of *Typhula* snow molds of turfgrass. Plant Disease. 83(9):788-798.

Jackson, N and Fenstermacher J.M. 1969. *Typhula* blight: its cause, epidemiology and control. J. Sports Turf Res. Inst. 45: 67-73.

Jacobs, D.L. and G.W. Bruehl. 1986. Saprophytic ability of *Typhula incarnata, Typhula idahoensis*, and *Typhula ishikariensis*. Phytopathology 76(7): 695-698.

Matsumoto, N. and A. Tajimi. 1985. Field survival of sclerotia of *Typhula incarnata* and *T. ishikariensis* biotype A. Can. J. Bot. 63: 1126-1128.

Millet, S. 2000. The distribution, molecular characterization and management of snow molds in Wisconsin Golf Courses. Ph.D. thesis. University of Wisconsin-Madison, under DP Maxwell adviser.

Snider, C.S., T. Hsiang, G. Zhao and M. Griffith. 2000. Role of ice nucleation and anti-freeze activities in pathogenesis and growth of snow molds. Phytopathology 90(4):354-361.

Vargas, J.M., Jr. 1994. Management of turfgrass diseases. CRC Press, Boca Raton, Fl.

Watson, L. and M.J. Dallwitz. 1992. Grass Genera of the World. Wallingford Oxon, UK CAB Intl. 1038p.

Wu, C. and T. Hsiang. 1998. Pathogenicity and formulation of *T. phacorrhiza*, a biocontrol agent of gray snow mold. Plant Disease. 82: 1003-1006.