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DISEASES

Genetic resistance to snow mold fungi in bentgrass

By Michael Casler, Jeff Gregos, Zhichun Wang and John Stier

Winter diseases of turfgrass, collectively referred to as snow molds, are a major problem on golf courses and other turf areas in Wisconsin and similar regions. Golf course greens, fairways and tees are of primary concern because of their high dollar value. Nearly all golf course superintendents spray putting greens with fungicides to inhibit snow mold fungi. Most superintendents also spray tee boxes, while many also spray their fairways.

This control method is highly expensive, it has limited effectiveness and it may adversely affect the environment. In addition, some fungal pathogens have developed resistance to fungicides after years of repeated applications.

Our objective was to determine if existing cultivars of creeping bentgrass (Agrostis palustris L.); colonial bentgrass (Agrostis tenuis Sibth.); creeping red fescue (Festuca rubra L.); and Chewings fescue (Festuca rubra L. commutata Gaud.) differ in snow mold reaction. In addition, our goal was to determine if snow mold resistance is genetically inherited in creeping bentgrass.

Nearly all golf course superintendents spray putting greens with fungicides to inhibit snow mold fungi. This is expensive, has limited effectiveness and may adversely affect the environment.

Creeping bentgrass is a highly desirable species for golf courses, but most cultivars are generally considered to be highly susceptible to various snow mold pathogens.

Snow mold fungi

Snow mold fungi are facultative parasites, capable of surviving and growing on necrotic tissue, becoming particularly serious when susceptible hosts are compromised either through injury or stress. These pathogens are most active at temperatures ranging from 32 to 55°F and are favored by extended snow cover. Disease symptoms begin as small, round patches (2 to 4 in. in diameter) with a water-soaked appearance. As the pathogen grows, the turf foliage dies, leaving brown patches that coalesce into extensive areas of severely damaged turf. In Wisconsin, areas of golf courses that routinely receive severe snow mold damage will have a low population of perennial turf grasses and a high population of annual-type Poa annua that regenerates in late spring from the soil seed banks.

There are four common snow mold fungi in Wisconsin. Pink snow mold is caused by Microdochium nivale and occurs throughout the state. Gray snow mold is caused by Typhula

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incarnata and occurs throughout Wisconsin. Speckled snow mold is caused by Typhula ishikariensis and occurs largely in the northern half of Wisconsin. Another fungus of unknown pathogenicity, Typhula phacorrhiza, occurs largely in the far northern part of Wisconsin.

In the spring of 1998, we initiated a series of experiments to compare several cultivars of creeping bentgrass (Cato, Penncross, Penneagle, Seaside II, and SR 1119); colonial bentgrass (Astoria, Highland, SR7100, Tendez, and Tiger); creeping red fescue (Dawson, Jasper, and Pennlawn); and Chewings fescue (SR5100, Tiffany, and Victory). Plots were seeded at three locations in Wisconsin: O.J. Noer Turfgrass Research and Education Facility near Verona (southern WI), Sentryworld Golf Course near Stevens Point (central WI), and Gateway Golf Course near Land O' Lakes (northern WI). All plots were managed as golf course fairways with a mowing height of 1/2 in. at Verona and Stevens Point and 3/4 in. at Land O' Lakes.

Each experiment was planted in spring 1998 and late summer 1999. The experimental design was a split-split-split-plot with host genus as whole plots, host cultivar as subplots, pathogen as sub-sub-plots, and inoculated (cultured inoculum) vs. non-inoculated (natural inoculum) as sub-sub-sub-plots.

Isolates of each pathogen were collected from each experimental site and used to inoculate plots only at their respective site. Isolates were cultured and multiplied in the laboratory on millet seed. Because T. phacorrhiza was not found at the two southern sites and T. ishikariensis was not found at O.J. Noer, these treatments were not included at these two sites. Plots were inoculated in October of the establishment year and visually rated for snow mold damage the following spring.

Cultivar variation

The fine fescue cultivars were consistently more resistant to all snow mold fungi than the bentgrass cultivars (Table 1). The differences were greatest at the central and northern locations where there was greater average snow mold damage. T. ishikariensis was the most pathogenic to bentgrass at the central and northern locations and to fescue at the northern location. T. incarnata was most pathogenic to fescue at the central location and to both species at the southern location.

There was a considerable amount of natural inoculum at each site, as indicated by the damage to non-inoculated plots. Inoculation with T. ishikariensis or T. incarnata significantly increased damage to bentgrass at all locations. Inoculation with T. ishikariensis at the northern site or T. incarnata at the central site significantly increased damage to fescue. M. nivale and T. phacorrhiza did not cause increased damage to either bentgrass or fine fescues.

Compared to creeping bentgrass, colonial bentgrass was damaged less by all snow mold fungi at all locations (Table 2). This difference was significant ($P<0.05$) in nearly all cases, the only three exceptions being the most pathogenic isolate (T. ishikariensis at the northern site) and the two treatments with the least damage (non-inoculated and M. nivale at the southern site).

In some cases, creeping bentgrass cultivars had more than double the damage to colonial bentgrass cultivars. Increases in damage to creeping and colonial bentgrass cultivars, due

RELEVANT LITERATURE

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Posselt, U.K. and F. Altpeter. 1994. Improvement of snow mould resistance by conventional and in vitro techniques. Euphytica 77:251-255.

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to inoculation, were generally similar, with the exception of T. incarnata at the central and southern sites.

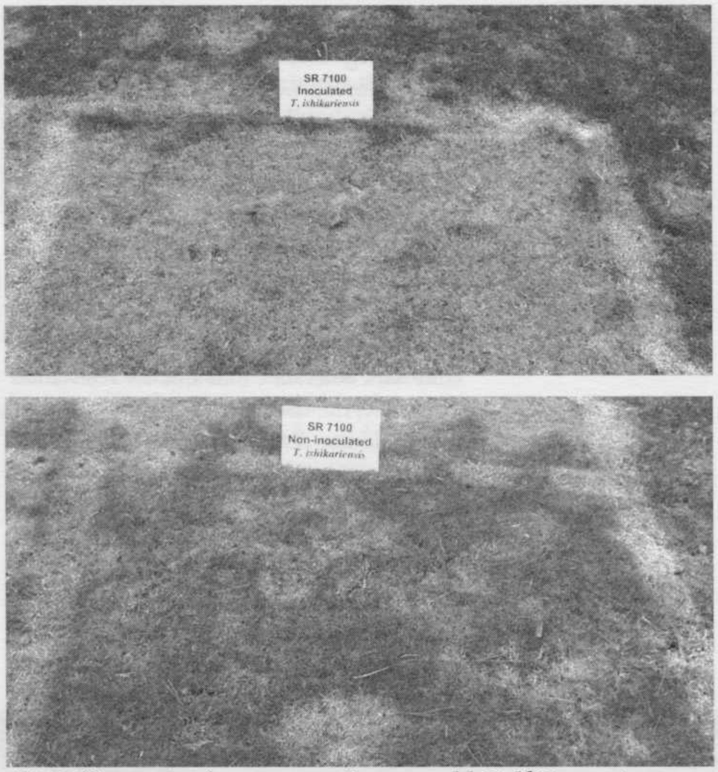
For creeping bentgrass, cultivar x environment interactions were frequent. Creeping bentgrass and fine fescue cultivar rankings were highly inconsistent across locations, years, and fungal species. Colonial bentgrass cultivar rankings were somewhat consistent across these environmental factors. Across all locations, years, and fungal species, SR7100 and Tiger ranked lowest in damage (30 and 34%, respectively) compared to Astoria and Highland (42 and 44%, respectively).

Creeping bentgrass clonal collections

In 1997, we began a creeping bentgrass breeding program with the objective of developing genetic resistance to snow mold. Seeded cultivars of creeping bentgrass consist of heterogeneous seed mixtures. Each seed potentially contains a different combination of genes so that its appearance, or phenotype, will differ from that of its neighbors. On golf courses, this leads to natural selection between neighboring plants for adaptation to various stresses and management factors. One can easily see this phenomenon on golf courses by observing patchiness of a single species on putting greens or fairways.

In our search for genetic resistance to snow mold, we focused on fairways of Wisconsin golf courses with three criteria: north of U.S. Hwy 10; infrequent or no treatment with fungicides to control snow mold fungi; and frequent snow mold damage following a typical winter. We sampled plants that had a large diameter, green color and absence of snow mold patches within two weeks of the final snow melt. We also collected plants from golf course greens in both northern and southern Wisconsin, with selection based on large diameter, bright green color, fine leaf texture and absence of Poa annua within the bentgrass patch.

We screened 326 of these clones for reaction to an isolate of T. ishikariensis during the summer of 1999. The clones were split into six pieces, grown in 1.25 x 1.25 x 2-inch containers, and managed to simulate a fairway with a half-inch mowing height. The clones were arranged in a randomized complete block design with six replicates. Flats were placed in a growth chamber to simulate a fall hardening period, with a gradual temperature reduction to 41°F and a gradual reduction in day length.



SR 7100 creeping bentgrass at Sentryworld Golf Course inoculated with T. ishikariensis (top) not inoculated (bottom).

Four of the six replicates were inoculated with T. ishikariensis and all plants were kept in the dark for 8 weeks. Plant chlorosis/necrosis was scored weekly using a 0-to-10 scale, where 0 = completely green plant and 10 = completely dead plant. Plants were then placed in a greenhouse where they were scored two more times, using the same rating scale.

We screened a subset of 72 clones twice in a second experiment. The second experiment was similar to the first, but included an isolate of T. incarnata and a second isolate of T. ishikariensis. There were three replicates each of the control and the three fungal isolates and two separate runs of the second experiment.

Five creeping bentgrass clones will be intercrossed to make an experimental synthetic population in summer 2001.

Clonal variation in creeping bentgrass

Plant chlorosis/necrosis scores were highly repeatable and significant differences (P<0.01) were found among clones for the control (non-inoculated) treatment, the inoculated treatment, and the difference. Clonal variation within the control was due to variable tolerance to extended cold/dark conditions. Clonal variation within the inoculated treatment was due to a combination of cold/dark tolerance and disease resistance. Therefore, the difference was used as a measure of disease reaction.

Chlorosis and necrosis symptoms developed gradually during the dark period. Scores for inoculated and non-inoculated treatments were highly correlated during the first three ratings, because disease symptoms were not highly expressed. As disease symptoms became highly expressed during the last three weeks of cold/dark conditions, the correlation between inoculated and non-inoculated approached zero. All data in this paper are taken from means over the last three weeks of cold/dark conditions and the two weeks of recovery in the greenhouse.

Clones selected from fairways had a mean difference between inoculated and non-inoculated of 1.9 compared to clones selected from greens with a mean of 3.0 (P<0.01; Fig. 1). If we define snow mold resistant clones as those with mean differences <1, fairway collections had 22% resistant plants and green collections had 3% resistant plants. As expected, based on snow mold management, there appears to be more natural selection for snow mold resistance in low-maintenance golf course fairways than on putting greens.

There were also large differences among golf courses in the frequency of resistant plants, with a range of 0 to 42% resistant

TABLE 1

BENTGRASS VS. FINE FESCUE: PERCENTAGE OF PLOT SHOWING SNOW MOLD DAMAGE IN SPRING OF THE 2ND YEAR.

Location/	Inoculum [a]				
Host genus	None	Mniv	Tinc	Tish	Tpha
NORTHERN WI					
Bentgrass	47.0	44.0	71.3*	80.0*	52.5
Fine fescue	15.3	16.8	14.0	54.8*	17.1
P-value [b]	<0.01	<0.01	<0.01	<0.01	<0.01
CENTRAL WI					
Bentgrass	39.4	38.6	59.5*	69.7*	—
Fine fescue	34.6	27.3	49.1*	39.2*	—
P-value [b]	0.01	0.03	<0.01	<0.01	
SOUTHERN WI					
Bentgrass	10.8	12.5	18.5*	—	—
Fine fescue	3.6	1.9	5.3	—	—
P-value [b]	<0.01	<0.01	<0.01		

* SIGNIFICANTLY DIFFERENT FROM THE RESPECTIVE NON-INOCULATED VALUE AT P<0.05.
A MNIV = MICRODOCHIUM NIVALE, **TINC** = TYPHULA INCARNATA, **TISH** = TYPHULA ISHIKARIENSIS, AND **TPHA** = TYPHULA PHACORRHIZA.
b = PROBABILITY THAT DECLARING A DIFFERENCE BETWEEN BENTGRASS AND FINE FESCUE MEANS WILL RESULT IN AN INCORRECT STATEMENT.

plants on the northern-WI golf courses. Thus, local conditions and/or the source of the original bentgrass germplasm has a lot to do with the likelihood of finding snow mold resistance.

The proof of the above snow mold challenge would come with repetition, which could only be done on a smaller set of clones. The 72 clones chosen for the second experiment were based on the full range of reactions in the first experiment. The repeatability of these ratings was similarly low to that observed for creeping bentgrass cultivars in the field studies reported above. Correlations between experiments, runs of experiment #2, and between fungal isolates of experiment #2 were all positive, but low, ranging from 0.1 to 0.4.

Despite this low apparent repeatability, each experiment was internally highly repeatable, with significant differences observed among clone means for reaction to all isolates and to the control in each run and averaged over runs of experiment #2.

After sifting through all this data on these 72 clones, were able to find five clones which appeared to have higher-than-average resistance to each snow mold isolate (Table 3). These clones had mean disease reactions consistently lower than the other clones for all four inoculations. They did not differ from the other clones in chlorosis/necrosis as control plants, indicating that they appeared to be unique in having repeatable snow mold resistance but were average for cold/dark tolerance.

Because the comparisons made in Table 3 are data-based (i.e., they were suggested by the results), the P-values are not correct, but they nevertheless suggest the type of consistency that is necessary in order to make successful selections.

TABLE 2

CREEPING VS. COLONIAL BENTGRASS: PERCENTAGE OF PLOT SHOWING SNOW MOLD DAMAGE IN SPRING OF THE 2ND YEAR

Location / Inoculum [a]					
Host genus	**None**	**Mniv**	**Tinc**	**Tish**	**Tpha**
NORTHERN WI					
Creeping bentgrass	54.5	55.5	78.5*	81.8*	63.7
Colonial bentgrass	38.7	31.4	63.8*	77.6*	40.0
P-value [b]	<0.01	<0.01	0.01	0.43	<0.01
CENTRAL WI					
Creeping bentgrass	52.2	48.2	85.7*	83.5*	—
Colonial bentgrass	26.2	29.0	29.9	54.7*	—
P-value [b]	<0.01	0.05	<0.01	<0.01	
SOUTHERN WI					
Creeping bentgrass	11.8	14.3	24.0*	—	—
Colonial bentgrass	9.8	10.7	13.0	—	—
P-value [b]	0.22	0.06	<0.01		

* SIGNIFICANTLY DIFFERENT FROM THE RESPECTIVE NON-INOCULATED VALUE AT P<0.05.

A MNIV = MICRODOCHIUM NIVALE, **TINC** = TYPHULA INCARNATA, **TISH** = TYPHULA ISHIKARIENSIS, AND **TPHA** = TYPHULA PHACORRHIZA.

b = PROBABILITY THAT DECLARING A DIFFERENCE BETWEEN CREEPING AND COLONIAL BENTGRASS MEANS WILL RESULT IN AN INCORRECT STATEMENT.

These five creeping bentgrass clones will be intercrossed to make an experimental synthetic population in summer 2001. This population will be tested on golf course fairways to determine if this selection protocol was successful in increasing resistance to snow mold fungi. Our future plans are to continue selecting for increasing levels of snow mold resistance, in new germplasm and in the experimental synthetic, using field-based screening techniques. We have also made crosses to study the inheritance of resistance and to map resistance genes in collaboration with Dr. Geunhwa Jung of the Department of Plant Pathology.

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TABLE 3

MEAN CHLOROSIS/NECROSIS SCORES FOR 72 CREEPING BENTGRASS CLONES CHALLENGED WITH THREE ISOLATES OF FUNGAL SNOW MOLD PATHOGENS OR GROWN UNDER COLD/DARK CONDITIONS WITHOUT INOCULATION

Experiment/Inoculum [a]						
Measurement/Group	Expt. 1 Control	Expt. 1 Tish 3	Expt. 2 Control	Expt. 2 Tish 3	Expt. 2 Tish 1	Expt. 2 Tinc 1
COLD/DARK REACTION [b]						
Best five clones	2.93	3.55	3.85	5.63	5.53	5.17
Other 67 clones	3.19	6.00	3.75	4.05	6.23	5.64
P-value [c]	0.36	<0.01	0.46	<0.01	0.01	0.02
RECOVERY REACTION [b]						
Best five clones	1.50	1.30	2.69	6.79	5.21	4.63
Other 67 clones	1.58	3.68	2.75	8.03	6.31	5.60
P-value [c]	0.71	<0.01	0.84	<0.01	0.01	0.01

A TISH = TYPHULA ISHIKARIENSIS AND TINC = TYPHULA INCARNATA.
B COLD/DARK = MEAN OF RATINGS TAKEN AT 6, 7, AND 8 WEEKS AFTER INOCULATION. ALL PLANTS WERE MAINTAINED IN THE DARK AT 41°F. RECOVERY = MEAN OF RATINGS TAKEN AT 1 AND 2 WEEKS AFTER REMOVAL FROM COLD/DARK CONDITIONS TO A GREENHOUSE WITH A 16-HR DAYLENGTH AND 65-72°F.
C PROBABILITY THAT DECLARING A DIFFERENCE BETWEEN CREEPING AND COLONIAL BENTGRASS MEANS WILL RESULT IN AN INCORRECT STATEMENT.