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The objective of this project was to develop elite clones of creeping bentgrass with multiple pest resistances and stress tolerances that can be delivered to the seed industry for use in synthesizing new creeping bentgrass varieties broadly adapted to a range of ecological and environmental conditions, including reduced pesticide application. The findings reported here are the result of the first three years of the Bentgrass Breeding Consortium between the USDA-ARS, the University of Wisconsin, University of Illinois, and Michigan State University. Shown above is snow mold damage to the Gateway Golf Club driving range immediately after snow melt in April 2004.

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## PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of \$25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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# Development of Creeping Bentgrass with Resistance to Snow Mold and Dollar Spot

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## SUMMARY

Creeping bentgrass (*Agrostis stolonifera*) is the premier grass for golf course putting greens and is one of the most desirable grasses for fairways and tee boxes for much of the USA. Recent breeding advances have demonstrated that genetic variation exists within creeping bentgrass for a range of pest resistances and stress tolerances. For many golf courses, maintenance of a high quality turf requires frequent, varied, and intensive pesticide applications. Our goal was to identify creeping bentgrass clones with multiple pest resistances for use in breeding new bentgrass varieties.

- Field evaluations of dollar spot and snow mold resistance of creeping bentgrass clones were highly successful, resulting in wide variation among clones.
- Multiple field sites and collaborators at different locations were essential, because of relatively low correspondence among locations, particularly for snow mold.
- The lack of correspondence among locations suggests the possibility of some fungal species-specific resistance for snow molds, and possibly some isolate-specific resistance for dollar spot.
- The small number of creeping bentgrass clones selected for superior resistance to dollar spot and snow molds represent a valuable genetic resource that should be useful in beginning to breed new varieties of creeping bentgrass and creeping x colonial bentgrass hybrids for multiple disease resistance.

Creeping bentgrass (*Agrostis stolonifera*) is the premier grass for golf course putting greens and is one of the most desirable grasses for fairways and tee boxes for much of the USA. Recent breeding advances have demonstrated that genetic

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variation exists within creeping bentgrass for a range of pest resistances and stress tolerances. Many of these traits allow bentgrass to be grown in environments and under conditions that were impossible just a few years ago. For example, increased heat tolerance has extended the range of bentgrass for use on putting greens in the southern USA.

For many golf courses, maintenance of a high quality turf requires frequent, varied, and intensive pesticide applications. Pesticide costs can consume up to 10% of the total budget for a highly managed golf course. Intensive management, including frequent and low mowing, irrigation, and heavy play, serves to enhance and/or spread the development of pest problems, particularly fungal diseases. In addition to their expense, pesticides represent a potential health and environmental hazard, both to golfers and to the surrounding environment, and they have limited efficacy (3, 8) and lead to fungal resistance (2, 7).

Genetic resistance to disease pests is a



The above image shows the "chamber of death" and damage on creeping bentgrass clones due to inoculation with Pythium disease in August 2005.





Four creeping bentgrass clones in a ryegrass fairway at Gateway Golf Club, showing differences in genetic resistance to dollar spot in August 2005

widespread phenomenon in agricultural and horticultural plants. Disease resistance, identified and developed by plant breeders and plant pathologists, has been used to protect economically important plants for more than 90 years. There are many examples in which genetic resistance has been durable for over 30 years without any need for fungicidal protection or increase in disease incidence.

While there has been much research on genetics and breeding of creeping bentgrass for individual pest resistances, there has not been a concerted effort to develop multiple-pest-resistant germplasm. Plants with multiple pest resistance will be required to have a significant impact, nationwide and locally, on pesticide use. There is strong evidence for genetic resistance to snow mold and dollar spot in some clones of creeping bentgrass (1, 9, 10).

There are currently several bentgrass breeding programs scattered around the USA, including New Jersey, Pennsylvania, Texas, Rhode Island, Michigan, Illinois, Wisconsin, and Oregon. Many of these programs operate somewhat independently of each other. While there is some collaboration between public and private programs, particularly in the seed production and commercialization of publicly developed cultivars, both public and private programs compete in the development of cultivars to support the industry. As such, individual programs have difficulty

in identifying and developing germplasm with multiple pest resistances. Each program has expertise, local knowledge, and environmental conditions to support identification of resistances/tolerances to a small number of pest problems. Only with collaboration among several diverse locations/programs, can we hope to identify germplasm with the multiple pest/stress resistances that will be necessary to solve most of the stress problems limiting adaptation of creeping bentgrass.

The objective of this project was to develop elite clones of creeping bentgrass with multiple pest resistances and stress tolerances that can be delivered to the seed industry for use in synthesizing new creeping bentgrass varieties broadly adapted to a range of ecological and environmental conditions, including reduced pesticide application. The findings reported here are the result of the first three years of the Bentgrass Breeding Consortium between the USDA-ARS, the University of Wisconsin, University of Illinois, and Michigan State University.

## Project Design

Three populations of creeping bentgrass clones were developed for this study. The Wisconsin population consists of a cross between



One of the final creeping bentgrass plants selected for resistance to snow mold and dollar spot, combined with excellent turf quality, compared to neighboring plants highly susceptible to dollar spot at Verona, WI in August 2005

Statistic	Wisconsin <sup>†</sup>			Michigan <sup>‡</sup>				
	TW4	TWR4	TW5	TM4	TMR4	TM5	TMR5	TYPH <sup>§</sup>
Mean	3.6	4.7	4.4	4.0	4.0	3.8	5.4	4.4
Standard deviation	1.3	1.4	1.2	1.5	1.4	1.1	1.1	0.6
Minimum	0.5	0.0	0.5	0.0	0.0	1.1	1.1	1.5
Maximum	7.5	9.0	9.0	6.2	6.2	6.8	7.6	5.9
LSD <sub>(0.05)</sub>	3.1	3.2	2.2	2.1	2.0	2.3	2.3	1.6
Repeatability	0.23	0.31	0.56	0.73	0.74	0.43	0.31	0.37

<sup>†</sup> TW4 = speckled snow mold rating immediately after snow melt in 2004, TWR4 = speckled snow mold rating after 4 weeks of recovery in 2004, and TW5 = speckled snow mold rating immediately after snow melt in 2005.  
<sup>‡</sup> TM4 = gray snow mold rating in spring 2004, TMR4 = recovery after grey snow mold infection in 2004, TM5 = gray snow mold rating in spring 2004, and TMR5 = recovery after grey snow mold infection in 2004.  
<sup>§</sup> TYPH = mean overall snow mold ratings.

**Table 1.** Statistics for 600 creeping bentgrass clones evaluated for snow mold reaction in Wisconsin and Michigan (0 = no reaction; 9 = highly susceptible)

two clones that differed in resistance to speckled and gray snow mold pathogens. This cross, consisting of 200 clones, is also being utilized in genetic linkage mapping (4) and disease-resistance mapping (6). The Michigan population consists of 200 clones collected from old golf courses in Michigan, largely for high turf quality and large patch size. The Illinois population consists of 200 clones that represent two generations of random mating of a population of clones collected from old golf courses in Illinois.

Each plant was vegetatively propagated in the greenhouse and clonal material was exchanged among the three locations near Chicago in February 2003. Clones were evaluated for disease and insect reactions as described below. Unless described otherwise, all ratings were made on a scale of 0 to 9, where 0 = plant completely brown from disease and 9 = no symptoms (plants with high values were most resistant and desirable). The rating scales were approximately linear with respect to the percentage of diseased tissue, so that a mean rating of 4.5 would represent approximately 50% diseased leaf tissue.

## University of Wisconsin and USDA-ARS

### Speckled snow mold (*Typhula ishikariensis*)

The 600 clones were transplanted to the practice fairway at Gateway Golf Course, Land 'O Lakes, WI in July 2003. The experimental design was a randomized complete block with four replicates and a split-plot randomization with populations as whole plots and clones as sub-plots. Plants were transplanted from 2-inch x 2-inch pots onto 18-inch centers into a perennial ryegrass fairway. Each plant was transplanted into a 9-inch circle in which the perennial ryegrass had been killed with glyphosate. Mowing height was maintained at approximately 1/2 inch.

Natural infection by *T. ishikariensis* was sufficiently severe and uniform to allow ratings to be made in spring 2004. Ratings were made in mid-April immediately after snow melt and again in early May following recovery. In October 2004, plots were inoculated with a mixture of isolates representing all three biological varieties of this fungus and all known to be particularly virulent on creeping bentgrass. Plots were rated again in early spring 2005, shortly after snow melt.

Statistic	Wisconsin <sup>†</sup>	Michigan <sup>‡</sup>				Illinois <sup>§</sup>		
	DW4	DM4	DMR4	DM5	DMR5	DI6	DI7	DS
Mean	4.3	2.2	4.0	4.3	4.4	7.4	6.5	4.8
Standard deviation	1.3	1.2	1.4	0.9	1.2	0.6	0.7	0.6
Minimum	0.0	0.0	0.0	1.4	0.0	5.3	2.7	3.0
Maximum	9.0	5.6	6.2	6.2	6.5	8.8	8.4	7.1
LSD <sub>(0.05)</sub>	1.9	2.3	2.0	2.0	2.6	1.0	1.1	1.3
Repeatability	0.65	0.56	0.74	0.35	0.27	0.41	0.54	0.47

<sup>†</sup> DW4 = dollar spot rating in 2004.  
<sup>‡</sup> DM4 = dollar spot rating in 2004, DMR4 = dollar spot rating after recovery in 2004, DM5 = dollar spot rating in 2004, and DMR5 = dollar spot rating after recovery in 2005.  
<sup>§</sup> DI6 = dollar spot rating in August and DI7 = dollar spot rating in September.  
DS = mean over all dollar spot ratings.

**Table 2.** Statistics for 600 creeping bentgrass clones evaluated for dollar spot reaction in Wisconsin, Michigan, and Illinois (0 = no reaction; 9 = highly susceptible)

### Pythium blight (*Pythium spp.*)

The 600 clones were transplanted into a perennial ryegrass fairway at the O.J. Noer Turfgrass Research and Education Facility near Verona, WI in June 2003. The experimental design and planting arrangement were as described above. The fairway was covered with a metal-framed hoop house with the plastic cover-

ing normally removed. Plots were inoculated with a mixture of isolates of *Pythium spp.* in early August 2004, and covered with plastic to increase the temperature and humidity of the local environment. Plants were rated following two weeks of exposure to the pathogen under these conditions.

State	Wisconsin <sup>†</sup>			Michigan <sup>‡</sup>				
	TW4	TWR4	TW5	TM4	TMR4	TM5	TMR5	TYPH <sup>§</sup>
Illinois	3.4	4.4	4.1	3.7	4.0	4.2	5.6	4.3
Michigan	3.7	4.6	4.5	4.5	4.4	3.5	5.5	4.5
Wisconsin	3.9	5.1	4.5	3.9	3.5	3.9	5.2	4.5
LSD <sub>(0.05)</sub>	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1

<sup>†</sup> TW4 = speckled snow mold rating immediately after snow melt in 2004, TWR4 = speckled snow mold rating after 4 weeks of recovery in 2004, and TW5 = speckled snow mold rating immediately after snow melt in 2005.  
<sup>‡</sup> TM4 = gray snow mold rating in spring 2004, TMR4 = recovery after grey snow mold infection in 2004, TM5 = gray snow mold rating in spring 2004, and TMR5 = recovery after grey snow mold infection in 2004.  
<sup>§</sup> TYPH = mean over all snow mold ratings.

**Table 3.** Means for three groups of creeping bentgrass clones deriving from Illinois, Michigan, or Wisconsin and evaluated for snow mold reaction in Wisconsin and Michigan (0 = no reaction; 9 = highly susceptible)

State	Wisconsin <sup>†</sup>	Michigan <sup>‡</sup>				Illinois <sup>§</sup>		
	DW4	DM4	DMR4	DM5	DMR5	DI6	DI7	DS
Illinois	4.6	2.2	4.0	4.5	4.4	7.5	6.6	5.0
Michigan	3.7	2.3	4.4	4.6	4.9	7.2	6.3	4.8
Wisconsin	4.5	2.0	3.5	3.9	4.0	7.4	6.6	4.8
LSD <sub>(0.05)</sub>	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1

† DW4 = dollar spot rating in 2004.  
‡ DM4 = dollar spot rating in 2004, DMR4 = dollar spot rating after recovery in 2004, DM5 = dollar spot rating in 2004, and DMR5 = dollar spot rating after recovery in 2005.  
§ DI6 = dollar spot rating in August and DI7 = dollar spot rating in September.  
DS = mean over all dollar spot ratings.

**Table 4.** Means for three groups of creeping bentgrass clones deriving from Illinois, Michigan, or Wisconsin and evaluated for dollar spot reaction in Wisconsin, Michigan, and Illinois (0 = no reaction; 9 = highly susceptible)

**Pink snow mold (*Microdochium nivale*)**

The 600 clones were transplanted into another perennial ryegrass fairway at O.J. Noer in August 2003. The experimental design and planting arrangement were as described above. Plots were mowed at 1/2 inch, allowing the clones to grow laterally for the 2004 growing season. In October 2004, plots were inoculated with an isolate of this fungus that had previously shown vir-

ulence against creeping bentgrass. Due to lack of snow cover and mild winter conditions, there were no pink snow mold symptoms in spring 2005 and 2006.

**Dollar spot (*Sclerotinia homoeocarpa*)**

In August 2004, the speckled snow mold experiment at Land 'O Lakes, WI was inoculated with an isolate of the dollar spot pathogen that has

Variable <sup>†</sup>	TW4	TWR4	TW5	TM4	TMR4	TM5	TMR5
TWR4	0.92 <sup>‡</sup>						
TW5	-0.20	-0.25					
TM4	0.10	0.11	-0.04				
TMR4	0.03	0.04	-0.06	0.75			
TM5	-0.01	0.02	-0.10	-0.13	0.19		
TMR5	0.07	0.07	-0.15	0.09	0.14	0.11	
TYPH	0.63	0.63	0.05	0.37	0.39	0.28	0.37

† Wisconsin: TW4 = speckled snow mold rating immediately after snow melt in 2004, TWR4 = speckled snow mold rating after 4 weeks of recovery in 2004, and TW5 = speckled snow mold rating immediately after snow melt in 2005; Michigan: TM4 = gray snow mold rating in spring 2004, TMR4 = recovery after grey snow mold infection in 2004, TM5 = gray snow mold rating in spring 2004, and TMR5 = recovery after grey snow mold infection in 2004; TYPH = mean over all snow mold ratings.  
<sup>‡</sup> r = 0.09, P < 0.05; r = 0.11, P < 0.01.

**Table 5.** Phenotypic correlation coefficients among snow mold ratings for 600 creeping bentgrass clones evaluated in Wisconsin and Michigan (0 = no reaction; 9 = highly susceptible)



Variable†	DW4	DM4	DMR4	DM5	DMR5	DI6	DI7
DM4	0.22‡						
DMR4	0.09	0.59					
DM5	0.16	0.39	0.93				
DMR5	-0.06	0.17	0.24	0.27			
DI6	0.34	0.20	0.07	0.07	-0.12		
DI7	0.45	0.21	0.07	0.11	-0.06	0.52	
DS	0.51	0.63	0.59	0.62	0.35	0.42	0.46

† Wisconsin: DW4 = dollar spot rating in 2004; Michigan: DM4 = dollar spot rating in 2004, DMR4 = dollar spot rating after recovery in 2004, DM5 = dollar spot rating in 2004, and DMR5 = dollar spot rating after recovery in 2005; Illinois: DI6 = dollar spot rating in August and DI7 = dollar spot rating in September; DS = mean over all snow mold ratings.

‡  $r = 0.09$ ,  $P < 0.05$ ;  $r = 0.11$ ,  $P < 0.01$

**Table 6.** Phenotypic correlation coefficients among dollar spot ratings for 600 creeping bentgrass clones evaluated in Wisconsin, Michigan, and Illinois (0 = no reaction; 9 = highly susceptible)

been shown to be highly virulent against creeping bentgrass (5). Ratings were made approximately four weeks after inoculation.

#### Black cut worm (*Agrotis ipsilon*)

Two replicates of the pink snow mold experiment were inoculated with second-instar black cutworm larvae. In the middle of each bentgrass plant, a PVC pipe, 10 cm in diameter and 10 cm long, was driven approximately 1 cm into the ground. Five larvae were placed in the pipe and the pipe was covered with nylon mesh to prevent birds from eating the larvae. After 10 days, pipes were removed and the number of surviving larvae were counted. The plots were mowed and two days later damage was scored on a 0 to 9 scale, where 0 = no feeding damage and 9 = plant completely brown (no regrowth). One replicate was inoculated and scored in mid-August and one replicate was inoculated and scored in mid-September.

#### **University of Illinois**

##### Dollar spot (*Sclerotinia homoeocarpa*)

The 600 clones were transplanted into a perennial ryegrass fairway at Champaign, IL in

June 2003. The experimental design and planting arrangement were as described above for each of the University of Wisconsin field trials. Plots were mowed at 1/2 inch allowing the clones to grow laterally for the 2004 growing season. Plots were inoculated with the dollar spot pathogen in early June and ratings were made in late June and mid-July.

#### **Michigan State University**

##### Gray snow mold (*Typhula incarnata*)

The 600 clones were transplanted into a perennial ryegrass fairway at East Lansing, MI in June 2003. The experimental design and planting arrangement were as described above for each of the University of Wisconsin field trials. Plots were mowed at 12 mm (approximately 1/2 inch) allowing the clones to grow laterally for the 2004 growing season. Each plant was rated for reaction to gray snow mold in April 2004 and 2005, based on infections from natural inoculum.

##### Dollar spot (*Sclerotinia homoeocarpa*)

Three isolates of the dollar spot pathogen were mixed and used to inoculate the entire trial at East Lansing in early July 2004. Plots were rated



for dollar spot reaction two weeks later using the same rating scale as for snow mold, which was also converted as described. Recovery from dollar spot infection was rated five weeks after inoculation. This disease was rated again in 2005.

## Results

### Pink Snow Mold, Pythium Blight, and Black Cutworm

Inoculations with pink snow mold, Pythium blight, and black cutworm failed to provide meaningful differences among the creeping bentgrass clones. In the case of Pythium blight, the disease pressure was so severe and uniform that all plants were heavily or completely damaged. All creeping bentgrass clones in the study were highly susceptible under the extreme conditions of this inoculation. For pink snow mold, the relatively few minor symptoms were due to mild winters with little significant snow cover. For black cutworm, the lack of variation among creeping bentgrass clones could only be attributed to extreme variation in the inoculation technique and/or the measurement of symptoms. Apparent differences among clones were not repeatable, indicating that there are environmental and/or cultural factors that obscure genetic differences among clones.



A creeping bentgrass clone highly susceptible to speckled snow mold in April 2004



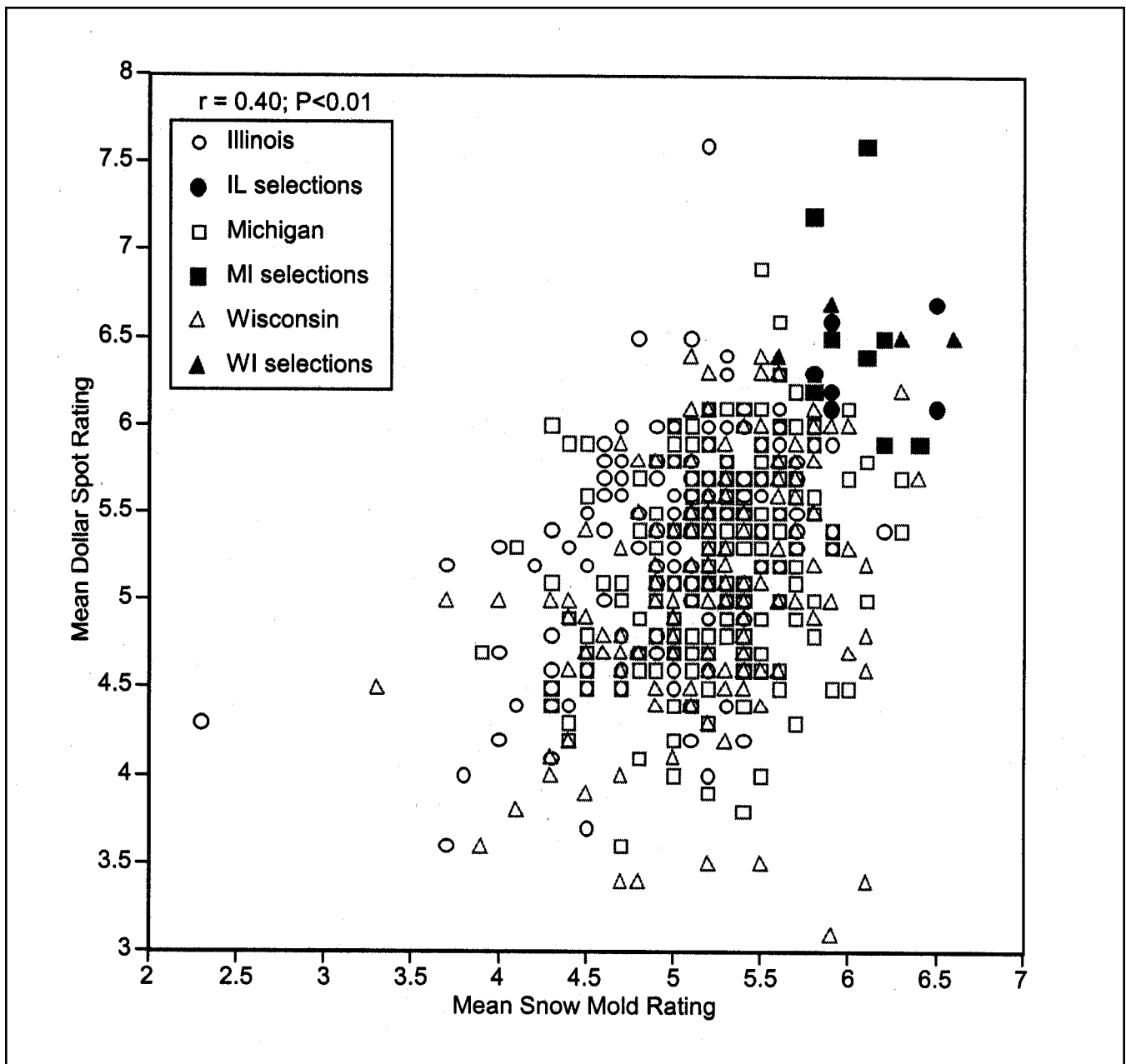
A creeping bentgrass clone highly resistant to speckled snow mold in April 2004

Variation among clones within populations was significant for all measures of snow molds (Table 1) and dollar spot reaction (Table 2). There was a large range among clones for all variables, generally consisting of at least 75% of the scale of measurement. LSD values were all small relative to the range among clone means. Repeatability was moderate to high for all dollar spot and snow mold ratings. These results demonstrated that there are large and consistent differences among clones for both dollar spot and snow mold reaction.

### Grey and Speckled Snow Mold and Dollar Spot

The three populations of creeping bentgrass clones differed for most measures of snow mold and dollar spot reaction (Tables 3 and 4). For snow mold, the Wisconsin population had the highest ratings for *T. ishikariensis*, while the Michigan population had the highest ratings for *T. incarnata*. Similarly for dollar spot, the Wisconsin population had nearly the highest mean rating in Wisconsin, the Michigan population generally had mean ratings in Michigan, and the Illinois population had the highest mean ratings in Illinois.

Most snow mold ratings were uncorrelated with each other (Table 5). The only exceptions were ratings of snow mold reaction and recovery that were taken within a few weeks of each other in either Wisconsin or Michigan. Despite these results, the moderate repeatability of the average



**Figure 1.** Scatterplot of mean dollar spot rating (seven ratings in Wisconsin, Michigan, and Illinois) against mean snow mold rating (seven ratings in Wisconsin and Michigan) for 600 creeping bentgrass clones

snow mold reaction, across all ratings, indicated the presence of some clones with fairly consistent results across all ratings. This is remarkable, particularly given that most snow mold symptoms at the East Lansing, MI and Land 'O Lakes, WI locations were caused by two different snow mold pathogens. Dollar spot ratings were considerably more consistent across ratings made at different locations or years (Table 6). This was probably

due to the use of a constant source of inoculum at all locations and the fact that dollar spot is caused by only one organism. The pooled correlation coefficient was  $r = 0.23 (\pm 0.05)$ .

#### **Value and Future Use of Disease Resistant Clones**

These results suggest that there may be some race specificity for host resistance to these

two diseases. Both results are surprising, because factorial studies of host genotypes and pathogen isolates have shown, in both cases, a general lack of host genotype x pathogen isolate interaction (5, 10). These results may be an indication of more long-term evolution of race-specific disease resistance on golf courses, a phenomenon that may not have been detected from evaluation of collections within a limited region. Particularly for snow molds, plants resistant to snow mold from one golf course may not be resistant to snow molds from all other courses. These results underscore the importance of collaboration between researchers at different locations allowing evaluation of each disease across a wide range of environmental conditions and potential pathogen isolates.

Based on these results, an overall disease index was created as the sum of all 12 disease ratings. Index values of the 600 creeping bentgrass clones ranged from 14 to 72, with a mean of 54 and standard deviation of 10. Twenty clones with the highest disease indices and superior turf quality were selected for potential release to private companies (Figure 1) for use in breeding new varieties of creeping bentgrass. These 20 clones had a mean index value of 68, a mean over all snow mold ratings of 5.9, and a mean over all dollar spot ratings of 6.3. These clones are also being crossed with additional clones with superior dollar spot, snow mold, and brown patch resistance, to generate a new set of genetic materials for evaluation and selection.

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