Published online April 25, 2006

Response of Bentgrass Cultivars to *Sclerotinia homoeocarpa* Isolates Representing 10 Vegetative Compatibility Groups

Nanda Chakraborty, Taehyun Chang, Michael D. Casler, and Geunhwa Jung*

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

ABSTRACT

Dollar spot caused by *Sclerotinia homoeocarpa* F.T. Bennett is a major disease of turfgrass throughout the world. Current control strategies depend heavily on fungicide application. Host resistance is an alternate management strategy, but a thorough understanding of the pathogen and host interaction is required to utilize it successfully. Seventy-nine clones of 10 cultivars of creeping (*A. stolonifera* L.), colonial (*A. capillaris* L.), dryland (*A. castellana* Boiss. & Reut.), and velvet (*A. canina* L.) bentgrass species were inoculated with an isolate of *S. homoeocarpa* in the greenhouse. A large degree of genetic variation in response to *S. homoeocarpa* at the species, cultivar, and clone levels was detected. In addition, 18 isolates of *S. homoeocarpa* representing 10 vegetative compatibility groups (VCGs) were used to inoculate 12 bentgrass cultivars. Disease severity evaluations showed significant difference among bentgrass cultivars and species in their response to dollar spot. The colonial and velvet bentgrass cultivars were significantly less susceptible to all the isolates of *S. homoeocarpa* compared to the creeping bentgrass cultivars. The isolates of *S. homoeocarpa* showed significant differences in levels of aggressiveness. However, data from pathogenicity tests indicated a lack of race-specific interactions. Therefore, turfgrass breeders should be able to select for resistance to one or a few highly virulent isolates of the pathogen, and obtain resistance to a wide array of isolates.

THE GENUS *Agrostis* (bentgrass) comprises over 200 species (Hitchcock, 1951). Only five species are commonly used for turfgrass in the USA: colonial, velvet, dryland, redtop (*A. gigantea* Roth.), and creeping. These species are perennial, outcrossing, cool-season grasses used for lawns, athletic fields, and golf courses. Currently, the stoloniferous, allotetraploid creeping bentgrass ($2n = 4x = 28$) is the most adapted species for use on golf course fairways and greens (Wipff and Fricker, 2001). Most bentgrass cultivars are susceptible to dollar spot, caused by *S. homoeocarpa*, but there are significant differences in their response to dollar spot (Baldwin and Newell, 1992; www.ntep.org/data/bt03g/bto3g_05-2/bt03g05t19.txt; verified 19 Jan. 2006).

Dollar spot is a major disease of turfgrass throughout the world. This disease is the most prevalent and economically important turf disease in North America, particularly on intensively managed golf course putting greens and closely mown fairways (Couch, 1995; Vargas, 1994). Symptoms occur from spring through fall, but are most prevalent during warm and humid days with cool nights in spring, early summer, and fall. The optimum temperature for disease development is 21 to 27°C, though the fungus will grow over a wider range of temperature, 21 to 32°C. On bentgrass turf maintained at low mowing heights such as golf greens or fairways, symptoms of dollar spot appear as round or irregularly shaped sunken, straw colored patches approximately 3 cm in diameter or about the same size as a silver dollar (Walsh et al., 1999). When disease pressure is high, isolated spots coalesce to form large, irregular patches. Since this fungus is not known to produce conidia or a sexual stage in North America, the organism most likely spreads via mycelia or transport of colonized leaf tissue by wind, water, machinery such as mowers, or by human traffic (Baldwin and Newell, 1992).

Dollar spot severity can be reduced by management practices such as blending seeds of resistant and susceptible cultivars (Abernathy et al., 2001). Cultural practices include removal of foliar dew by mowing or poling (Williams et al., 1996), and applications of late spring N fertilizer or multiple applications of composts (Boulter et al., 2002). Even though cultural practices reduce disease severity, dollar spot management is still highly dependent on fungicide applications. However, isolates of *S. homoeocarpa* have developed resistance to benzimidazole, dicarboximide, and demethylation inhibitor fungicides (Cole et al., 1968; Warren et al., 1974; Detweiler et al., 1983; Golembiewski et al., 1995). Moreover, some fungicides have not been reregistered due to environmental concerns. These factors have further stimulated research into alternative disease management strategies such as host resistance.

Development of creeping bentgrass cultivars that exhibit resistance to *S. homoeocarpa* would greatly reduce the costs and environmental impacts of fungicide applications. Although host resistance to *S. homoeocarpa* is reported (Bonos et al., 2003), the genetic mechanisms of dollar spot resistance are poorly understood. A thorough understanding of both pathogen and host biology is required to develop resistant cultivars for deployment. Knowledge of the genetic variability of the pathogen is needed to predict genetic shifts affecting pathogenicity, which affects the durability of host resistance.

Information about the genetic composition of *S. homoeocarpa* populations can be derived from the study of vegetative compatibility groups (VCGs). Sonoda (1988) identified 54 VCGs among 119 dollar spot isolates from *Paspalum notatum* Flueggé in Florida, while Bernick (2002) detected seven different VCGs using 1297 isolates collected from different locations in the midwestern U.S. and the Netherlands. A high degree of genetic similarity (≥90%) was found among the isolates

Nanda Chakraborty, Taehyun Chang, and Geunhwa Jung, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; Michael D. Casler, USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI 53706. Received 25 Apr. 2005. *Corresponding author (jung@plantpath.wisc.edu).

Published in Crop Sci. 46:1237–1244 (2006).
Turfgrass Science
doi:10.2135/cropsci2005.04-0031
© Crop Science Society of America
677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: VCG, vegetative compatibility group.

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

(Bernick, 2002), similar to previous studies by Raina et al. (1997) and Hsiang and Mahaku (1999). The authors hypothesized that due to a lack of sexual reproduction, isolates of *S. homoeocarpa* were genetically similar and did not form isolated populations representing particular geographical locations. Previous studies have shown that there is more variation among the VCGs compared to variation within a VCG (Bernick, 2002). But there has not been any study on the aggressiveness of isolates representing different VCGs and susceptibility of bentgrass cultivars and species.

Bentgrass cultivar field evaluations by the National Turfgrass Evaluation Program (NTEP) (www.ntep.org/data/bt03g/bt03g_05-2/bt03g05t19.txt; verified 19 Jan. 2005) have demonstrated significant differences among cultivars in their susceptibility to *S. homoeocarpa*. Moreover, field studies (Bonos et al., 2003) using multiple dollar spot isolates have shown that resistance to *S. homoeocarpa* is a quantitatively inherited trait. It is unknown if these results were due to major resistance genes specific to each isolate, or due to a number of genes indicating that resistance to dollar spot is a quantitative trait.

Therefore, studying host resistance and race specificity interactions using isolates representing different VCGs will provide valuable information about the nature of resistance. This information will help the breeders to make decisions about sources of dollar spot resistance through germplasm screening and developing breeding lines for improved dollar spot resistance.

The objectives of this study were to define the genetic relatedness of 23 isolates of *S. homoeocarpa* representing 11 VCGs, evaluate susceptibility of 79 clones representing 10 bentgrass cultivars by greenhouse inoculations with one isolate of *S. homoeocarpa*, followed by greenhouse inoculations of 12 bentgrass cultivars with 18 isolates of *S. homoeocarpa* representing 10 VCGs, and examine the aggressiveness of the isolates and the presence of race specific interactions.

MATERIALS AND METHODS

Genetic Relatedness of *S. homoeocarpa* Isolates Using RAPD-PCR

Twenty-three isolates of *S. homoeocarpa* representing 11 VCGs were provided by Dr. Jon Powell, University of Minnesota (Table 1). These isolates were collected from sites in northern Illinois, Michigan, Arkansas, Florida, and Minnesota from 1995 to 2001 (Bernick, 2002; Powell and Vargas, 2001). The VCGs were determined by Dr. Powell's group based on the ability of the hyphae of two fungal isolates to fuse and form a stable heterokaryon (Powell and Vargas, 2001).

Each isolate was grown by placing a 4-mm-diam plug of actively growing hyphae on a Difco potato dextrose agar (PDA) plate (BD Diagnostics, Franklin Lakes, NJ) for 5 d at room temperature. Mycelia were scraped from the colony and 0.2 g was transferred to a 2-mL Eppendorf tube for DNA extraction. A modified Dellaporta extraction method (Dellaporta et al., 1983) was used to extract fungal DNA. Fungal mycelia were frozen overnight at −80°C. After the samples were thawed for 15 min in a 65°C water bath, 600 μL of Dellaporta buffer was added (100 m*M* Tris pH 8.0, 50 m*M* ethylenediamine-tetraacetate, 500 m*M* NaCl, 10 m*M* β mercaptoethanol; Fisher Scientific Co. LLC, Pittsburgh, PA). The mycelia were ground in a FastPrep FP120 machine (BIO 101 Inc., Carlsbad, CA) using a ceramic bead for 40 to 45 sec. Then 70 μL of 20% sodium dodecyl sulfate (SDS, Fisher Chemicals) was added and the mixture was incubated for 1 h at 65°C, and spun for 10 min at 12 000 rpm (5415C Microcentrifuge, Eppendorf Inc., Westbury, NY). Four hundred microliters of 5 *M* potassium acetate (Sigma-Aldrich Corp., St. Louis, MO) was added to the supernatant. The tubes were incubated for 20 min at room temperature and spun for 10 min at 12 000 rpm. To 450 μL of supernatant, an equal volume of isopropanol was added, mixed slowly, incubated for 30 min at room temperature, and spun for 10 min at 13 000 rpm to precipitate the pellet. The supernatant was discarded and 70% (v/v) ethanol was added to wash the pellet. The pellet was air dried and 0.1× Tris EDTA (60 μL) was added to dissolve it. The DNA was quantified with a DNA Fluorometer Model TKO-100 (Hoefer Scientific Instruments, San Francisco), and diluted to 4 ng μL^{-1} in 0.1× Tris EDTA with tartrazine for use in PCR.

Table 1. The 23 isolates of *Sclerotinia homoeocarpa,* their vegetative compatibility groups (VCGs), and the site of collection.

VCG	Isolates	Site of collection
A	L36	Unknown golf course, IL
	MN1	Unknown golf course, MN
	FL17	Unknown golf course, FL
B	Southbrook	Southbrook, MN
	ARK	Unknown golf course, AR
	33A-24	Hancock Turf Research Center, East Lansing, MI
C	30B-13	Lakewood Shores, MI
	30B-24	Lakewood Shores, MI
	33A-9	Hancock Turf Research Center, East Lansing, MI
D	48–54	North Shore County Club, Chicago, IL
	30B-48	Lakewood shores, MI
E	64–14	Evergreen Golf, Hudson, MI
	64–41	Evergreen Golf, Hudson, MI
	64–49	Evergreen Golf, Hudson, MI
F	46–3	Forest Akers Golf, East Lansing, MI
	32–47	Evergreen Golf, Hudson, MI
	32F(SH8)	Evergreen Golf, Hudson, MI
G	I16	Les Bolstead, St. Paul, MN
	I18	Les Bolstead, St. Paul, MN
H	A7	Southbrook, MN
J	Les Bolstead	Les Bolstead, St. Paul, MN
K	TB64D	Unknown
L	BRS	Unknown

Thirty Operon RAPD primers were arbitrarily chosen (B11, 17, C11, 19, D2, 3, 16, 20, E3, 6, 14, F13, 14, G7, 10, 11, H4, 13, 14, K2, 8, 9, 12, P8, T14, Y5, 9, 13, 14, 15; Operon Technologies Inc., Alameda, CA) and were utilized for the RAPD reactions. The reactions were performed in 96 well plates with a total volume of 10 μL using an MJ PTC-100 thermocycler (MJ Research, Watertown, MA) programmed to the same conditions as described by Scheef et al. (2003). Thermal cycling conditions of 91°C for denaturation, 42°C for annealing, and 72°C for elongation were used for the first cycle followed by 39 cycles with first cycling time of 60 s for denaturation, 15 s for annealing, and 70 s for elongation. Subsequent cycles were the same except for a 15-s denaturation period. PCR products were separated by a 1.5% (w/v) agarose gel for 2 h at 300 V, stained in ethidium bromide (1.5 μg mL^{-1}) for 30 min and subsequently destained in distilled water for 20 to 30 min. Only bright and reproducible bands were scored manually for the presence or absence of bands as 1 and 0, respectively. One data matrix was generated by pooling categorical data from thirty primers and used for genetic relatedness analysis.

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

S. homoeocarpa Inoculum Preparation and Greenhouse Inoculations

Of the 23 isolates in Table 1, only 18 isolates representing 10 VCGs were selected by considering several factors such as VCGs, genetic relatedness, and the growth ability of mycelium. These isolates were grown on PDA for 1 wk at 21°C under constant fluorescent light. Inoculum was prepared by twice autoclaving 15 g of oat (*Avena sativa* L.) seeds with 20 mL Difco potato dextrose broth (PDB) in 125 mL Pyrex flasks. Four 4-mm-diameter culture plugs were excised from the growing edge of each fungal colony and transferred to the oat seed medium and allowed to grow for 3 wk at room temperature, with 12 h of light and daily shaking to prevent clumping of the seeds.

Seventy-nine clones of 10 cultivars of the creeping, colonial, velvet, and dryland bentgrass species were grown from individual seeds for the 2001 inoculation experiments in May and September. Three replications were used for each experiment, and different vegetative replicates of the same clones were used in each experiment, maintained in the greenhouse and established in 6.5-cm-diameter pots using identical protocols for each experiment. Two inoculation experiments were conducted in 2003 using 12 seeded bentgrass cultivars in factorial combinations with 18 isolates of *S. homoeocarpa* under similar conditions of temperature, humidity, and inoculum dose in September and December. The plants were started from 0.05 g of seeds evenly spread on soilless potting media (Metro Mix 366-P, the Scotts Company, Marysville, OH) on each 6.5-cm-diameter pot. Turf was trimmed twice every month to a height of 1 to 1.5 cm for 4 mo. There were four replications of each of the cultivars, and plants were inoculated when they completely filled the pots and mimicked a short-trimmed green or tee.

For all four experiments in 2001 and 2003, plants were inoculated by placing four infested oat seeds on the surface of the turf canopy in the center of each pot. This number of seeds was chosen based on previous inoculation data because the number gave most reliable disease under infection and colonization conditions used in the study. In these previous inoculations (N. Chakraborty, unpublished data, 2003), it was noted that changing the number of seeds by one, such as from two to three seeds or three to four seeds, did not lead to a significant difference in dollar spot severity. Therefore the amount of mycelia on each seed was estimated visually and seeds with equal amount of mycelia were used as inoculum.

After inoculation, the pots were arranged in a randomized complete block design and then placed in a mist chamber for 3 d with humidity >90% and temperature ranging from 22 to 27°C which is optimal for maximum pathogenicity (Walsh et al., 1999). The duration of daylight varied according to the season during the four experiments due to lack of artificial lights in the mist chamber. For the inoculation experiment in May, the average daylength was 14 h, for the September experiment it was 12 h, whereas for the December inoculation, it was approximately 9 to 10 h. After 3 d, the pots were moved from the mist chamber to the greenhouse. Two days later, the infested seeds were removed and disease symptom severity was scored as a percentage of area of symptomatic, whitish colored turf in each pot. To increase accuracy and precision of estimating disease severity, transparencies with grids of 1 cm^2 (Apollo, Ronkonkoma, NY) were used to trace the area of symptomatic bleached tissue area in each pot and then converted into percentage.

Statistical Analysis

RAPD data pooled from 30 primers were analyzed using the NTSYS software package (Rohlf, 1993). The genetic similarity matrix was constructed based on the simple matching coefficient method (Sneath and Sokal, 1973). The unweighted pair group method with arithmetic averages (UPGMA) was then used to analyze the matrix for hierarchical clustering. Analysis of Molecular Variance (AMOVA, Arlequin version 2.000) was used to estimate the variance among the isolates within a VCG and the variance between VCGs (Schneider et al., 2000).

Each pair of pathogenicity experiments (2001 and 2003) was analyzed by ANOVA, combined over experiments and analyzed separately for each experiment. In both cases, the combined ANOVA detected significant clone or cultivar × experiment interaction, so only the separate analyses were used in reporting results. For the 2001 experiments, the source of variation for bentgrass species was partitioned into three orthogonal contrasts: velvet bentgrass vs. others (diploid genome A_1A_1 vs. tetraploid genomes A_2A_2 A_3A_3 and A_1A_1 A_2A_2), creeping bentgrass vs. colonial and dryland bentgrasses (different tetraploid genomes A_2A_2 A_3A_3 vs. A_1A_1 A_2A_2), and colonial vs. dryland bentgrass (similar tetraploid genomes A_1A_1 A_2A_2 vs. A_1A_1 A_2A_2). Cultivars within species, and clones within cultivars comprised the remainder of the variability among the 79 clones.

For the 2003 experiments, the source of variation for bentgrass species was partitioned into two orthogonal contrasts: velvet bentgrass vs. creeping and colonial bentgrasses, and creeping vs. colonial bentgrass. The method of contrasts was also used to partition the interactions of the two bentgrass species contrasts with *S. homoeocarpa* VCG and isolates within VCG. All effects were assumed to be fixed, except for replicates, clones (in the 2001 experiments), and experiments (for the combined analyses), which were assumed to be random (SAS Institute, 1999). Contrasts were computed according to the procedures of Steel et al. (1996). Mixed models analysis was used to compute standard errors of least squares treatment means.

Normality of the residuals was tested by the Shapiro–Wilk test and heterogeneity of residual variances was determined by visually inspecting plots of residuals vs. predicted values (SAS Institute, 1999). For both the 2001 and 2003 experiments, there was some evidence for non-normality, largely by a slightly elevated frequency of extreme observations. However, the linearity of normal probability plots and the general absence of variance heterogeneity suggested that data transformation was not necessary.

Field data on dollar spot disease ratings of seven bentgrass cultivars were obtained from the NTEP web site (NTEP, 2001, 2003). The data consist of means over 10 locations, representing a wide range of climatic regions, and were based on 1 to 3 yr of data at each location under natural inoculum loads. Ratings were made on a 1-to-9 scale, where 1 = completely diseased and 9 = no disease. Rank correlation coefficients and Kendall's coefficient of concordance were used to compare average disease rankings for the greenhouse studies with the NTEP field data (Conover, 1971; Steel et al., 1996).

RESULTS

Genetic Variability of *S. homoeocarpa* Isolates

A total of 64 polymorphic bands were scored from 23 isolates using 30 RAPD primers. Each primer produced one to four polymorphic amplified bands ranging from 300 to 2000 bp in size. We did not find any distinct banding pattern for a specific isolate or particular VCG. A genetic similarity of 48% was detected among all the isolates collected from golf courses in Arkansas, Florida,

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

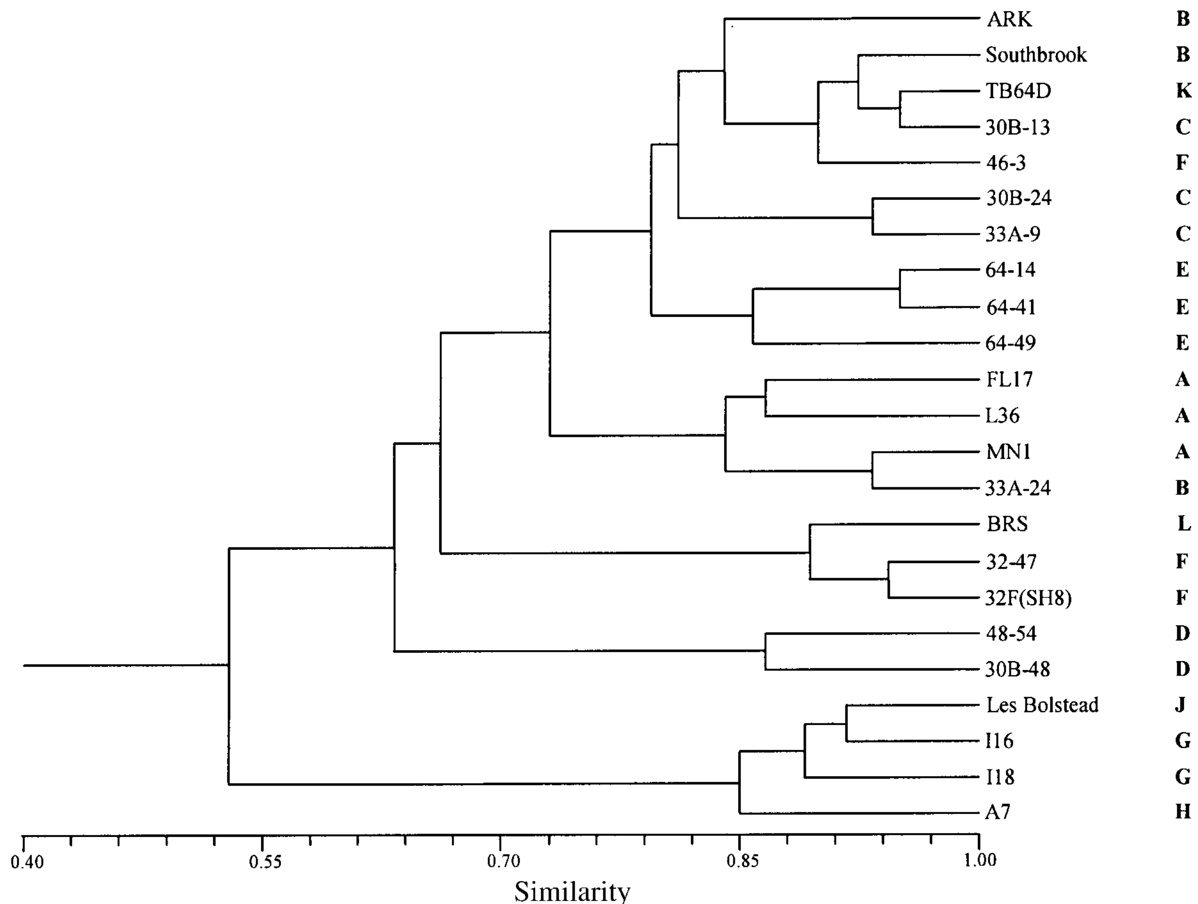

Fig. 1. Dendrogram showing the relationship among the 23 isolates of *Sclerotinia homoeocarpa* based on 64 RAPD markers. Bold letters after each name represent vegetative compatibility groups. The dendrogram was constructed by the NTSYS program using UPGMA (unweighted pair-group method with arithmetic averaging).

Illinois, Michigan, and Minnesota (Fig. 1). Three isolates from a golf course in Les Bolstead, MN, were ≥84% similar to each other but they digressed along with isolate A7 from the rest and shared only 48% similarity with all the other isolates. The other 19 isolates shared ≥62% similarity between them.

Most of the isolates within a particular VCG are more closely related (>85%) than between VCGs but there were some exceptions. AMOVA showed that percentages of variation were 54 and 46% among and within the VCGs, respectively. Specifically isolates in VCG F which came from the same golf course were >90% similar to each other. Similarly, isolates in VCG E were from the same golf course and formed their own group. In contrast, some isolates collected from diverse areas fell in the same VCGs, for example, FL17 was from Florida but it was genetically closest to L36, which was from Illinois.

Susceptibility of Bentgrass Cultivars, Aggressiveness, and Race Specificity of *S. homoeocarpa* Isolates

In the first pathogenicity experiment of 2001, a significant cultivar effect ($P < 0.0062$) was detected (Table 2). In addition, a significant clone effect ($P < 0.0002$) was found within cultivars. These clone and cultivar effects were noted in the first experiment, but both sources of

Table 2. Analysis of variance of two greenhouse experiments conducted in 2001 using 79 bentgrass clones and isolate MN1 of *Sclerotinia homoeocarpa*.

	Experiment 1, May 2001				Experiment 2, Sept. 2001			
Source of variation	df	MS	*F* value	*P* value	df	MS	*F* value	*P* value
Replicates	2	111.3	2.74	0.0676	2	406.0	2.54	0.0818
Velvet vs. creeping, colonial, and dryland	1	27.5	0.68	0.5624	1	224.6	1.41	0.3115
Creeping vs. colonial and dryland	1	4731.9	116.60	<0.0001	1	9701.8	60.81	<0.0001
Colonial vs. dryland	1	0.0	0.00	0.9847	1	0.1	0.00	0.9829
Cultivar (species)	6	269.7	6.65	0.0062	6	266.2	1.67	0.3009
Clone (cultivar species)	69	81.2	2.00	0.0002	69	216.1	1.35	0.0635
Error	155	40.6			152	159.5		

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

Table 3. Mean disease (%) caused by isolate MN1 of *Sclerotinia homoeocarpa* on 10 bentgrass cultivars in two greenhouse experiments, 2001.

			Mean disease (%), SD	
Species	Cultivar	Number of clones	Experiment 1, May 2001	Experiment 2, Sept. 2001
Velvet	Greenwich	6	15.7	34.5
Colonial	Astoria	10	11.7	30.3
	Bardot	10	11.4	23.5
	Alister	5	9.7	27.3
	Tiger	10	11.0	26.5
	SR7100	10	13.0	23.7
Species mean			11.4 ± 1.0	26.3 ± 1.7
Dryland	Highland	10	11.5	26.2
Creeping	PennG1	5	23.6	37.8
	Penncross	6	29.2	46.7
	Providence	7	16.3	41.4
Species mean			23.0 ± 1.3	42.0 ± 2.3
LSD (0.05)			5.4	8.9

variation were of marginal significance ($P = 0.06$–0.07) in the second experiment. Nevertheless, the rank order of cultivars remained similar with a rank correlation coefficient of $r = 0.73$ ($P < 0.05$). More importantly, differences among species were also consistent between the two experiments. In both 2001 experiments, the colonial bentgrass cultivars (mean percentage of disease 11.4 ± 1.0 in Experiment 1; 26.3 ± 1.7 in Experiment 2) were significantly less susceptible to *S. homoeocarpa* isolate MN1 than the creeping bentgrass cultivars (23.0 ± 1.3 in Experiment 1; 42.0 ± 2.3 in Experiment 2) (Table 3).

A significant difference in percent disease occurred between creeping vs. colonial and dryland bentgrass cultivars in both experiments. Disease severity was not significantly different between colonial vs. dryland bentgrass cultivars (Table 2). The difference between the creeping bentgrass vs. colonial and dryland bentgrasses explained ≥98% of the variance among species in both 2001 experiments (Table 2). The velvet bentgrass cultivar, which is diploid with the A_1 genome, was not significantly different in susceptibility from the other tetraploid bentgrass cultivars with the A_1 and A_2 genomes.

In both experiments of 2003 involving susceptibility of 12 commercial cultivars from three species of bentgrass to 18 isolates of *S. homoeocarpa* from 10 different VCGs (Table 4), cultivar and isolate main effects explained most of the variability in disease reaction. A significant cultivar effect ($P < 0.0001$) was detected (Table 4) and it explained 49 and 76% of the variance among bentgrass cultivars in the first and second experiments, respectively. The remainder of the variance among cultivars was explained by the difference between the creeping and colonial bentgrass cultivars (52 and 18% in the first and second experiments, respectively, Table 4). Disease severity did not differ between creeping and colonial bentgrass cultivars compared to the velvet bentgrass cultivars (Table 4). Colonial and velvet bentgrass cultivars were generally less susceptible than creeping bentgrass cultivars (Table 5). Cultivar × isolate interactions were generally not significant (Table 4).

Table 5. Mean disease (%) of 12 bentgrass cultivars for 18 isolates of *Sclerotinia homoeocarpa* in two greenhouse experiments, 2003.

		Mean disease (%), SD	
Species	Cultivars	Experiment 1, Sept. 2003	Experiment 2, Dec. 2003
Colonial	Tiger	17.4	30.3
	SR7100	16.5	27.8
	Glory	14.9	29.5
Species mean		16.2 ± 1.3	29.2 ± 1.3
Velvet	SR7200	19.5	31.8
	Bavaria	19.0	34.9
	Vesper	20.9	30.6
Species mean		19.8 ± 1.0	32.4 ± 2.2
Creeping	Crenshaw	27.5	35.3
	L-93	20.2	29.2
	PennA4	20.6	35.8
	Penncross	22.1	33.0
	Pennlinks	19.6	29.1
	Providence	22.0	28.5
Species mean		22.0 ± 2.9	31.8 ± 3.3
LSD (0.05)		2.6	3.1

A significant effect of isolates ($P < 0.0001$), or significant differences in aggressiveness among isolates was detected in both 2003 experiments (Tables 4 and 6). Isolates A7, BRS, 64-41, and 33A-9 from geographically different locations caused less disease in all the cultivars compared to isolates MN1, 30B-24, and 46-3. Due to under representation of several VCGs, comparison of disease severity among VCGs was beyond the scope of this study despite a significant effect of VCG ($P < 0.0001$) detected in both 2003 experiments.

Table 4. Analysis of variance of two greenhouse experiments conducted in 2003 using 12 bentgrass cultivars and 18 isolates (10 vegetative compatibility groups [VCGs]) of *Sclerotinia homoeocarpa*.

	Experiment 1, Sept. 2003				Experiment 2, Dec. 2003			
Source of variation	df	MS	*F* value	*P* value	df	MS	*F* value	*P* value
Replicate	3	3407.30	54.22	<0.0001	3	3264.78	36.09	<0.0001
Creeping vs. colonial cultivars	1	3494.69	55.61	<0.0001	1	848.28	9.38	0.0023
Creeping and colonial vs. velvet cultivars	1	16.87	0.27	0.6046	1	318.39	3.52	0.0612
Cultivar (species)	9	355.91	5.66	<0.0001	9	402.31	4.45	<0.0001
VCG	9	756.12	12.03	<0.0001	9	1778.55	19.66	<0.0001
Isolate (VCG)	8	711.79	11.33	<0.0001	8	880.19	9.73	<0.0001
Creeping vs. colonial × VCG	9	38.04	0.61	0.7929	9	127.77	1.41	0.1794
Creeping and colonial vs. velvet × VCG	9	47.67	0.76	0.6551	9	157.84	1.74	0.0763
Cultivar (species) × VCG	81	50.44	0.80	0.8893	79	74.18	0.82	0.8622
Creeping vs. colonial × isolates (VCG)	8	48.45	0.77	0.6287	8	96.88	1.07	0.3819
Creeping and colonial vs. velvet × isolates (VCG)	8	141.45	2.25	0.0228	8	70.68	0.78	0.6192
Cultivar (species) × isolates (VCG)	72	54.33	0.86	0.7756	71	77.40	0.86	0.7904
Error	508	62.85			523	90.45		

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

Table 6. Mean disease caused by 18 *Sclerotinia homoeocarpa* isolates from 10 vegetative compatibility groups (VCGs) on 12 bentgrass cultivars in two greenhouse experiments, 2003.

		Mean disease (%)	
VCG	Isolates	Experiment 1, Sept. 2003	Experiment 2, Dec. 2003
A	L36	21.3	39.1
	MN1	23.9	37.9
	FL17	21.5	31.5
B	Southbrook	18.8	25.4
	ARK	22.6	33.7
C	30B-13	21.4	33.1
	30B-24	26.6	34.9
	33A-9	13.0	22.3
D	48–54	23.1	37.6
	30B-48	22.8	42.3
E	64–14	16.5	27.9
	64–41	12.5	30.3
F	46–3	25.5	33.4
G	I16	25.4	28.5
	I18	20.4	24.2
H	A7	13.4	28.6
K	TB64D	18.7	23.0
L	BRS	13.3	30.0
LSD (0.05)		3.5	4.2

The ranking of dollar spot resistance of bentgrass cultivars from greenhouse tests was generally positively correlated with the ranking of means across 10 locations in the NTEP field trials (Table 7). The second experiment in 2003 was the only exception out of four experiments in 2 yr. Kendall's coefficient of concordance was $\tau = 0.62$ ($P = 0.19$) across all four experiments.

DISCUSSION

A distinct RAPD banding pattern for a specific isolate or particular VCG was not detected in this study as previously reported by Bernick (2002). Our study was based on a much smaller sample size compared to previous studies (Bernick, 2002; Raina et al., 1997; Sonoda, 1988) but we included six isolates belonging to three additional VCGs (F, J, L) that were not included in those studies. Due to a small number of the isolates, our goal was not to understand the genetic relatedness between the VCGs but to understand the general relatedness between the isolates irrespective of their individual VCGs.

We found a similarity of 48% between the 23 isolates collected from golf courses in Arkansas, Illinois, Florida, Michigan, and Minnesota. This result is similar to Hsiang and Mahaku (1999) in which they found 66% similarity among isolates from Canada, but unlike those of previous studies (Raina et al., 1997) which have shown ≥80% genetic similarity among isolates from the USA. The lower level of genetic similarity in our study might be due to the small sample size of 23 isolates in comparison to the other studies. The genetic relatedness study was the first step to understand the similarity among the different isolates but additional isolates representing each VCG are required to further clarify our findings. The outcome of this relatedness study was sufficient to show the genetic dissimilarity among the isolates, so that we can choose different isolates representing multiple VCGs and significant genetic diversity for studying host resistance in bentgrass cultivars.

Previous studies (Hsiang and Cook, 1992), including NTEP, have performed field disease evaluation experiments with different bentgrass species relying on natural inoculum. Results from NTEP have shown significant difference among the commercial bentgrass cultivars in their response to dollar spot, but so far there have been no published data on the performance of the commercial cultivars based on individual isolates of the pathogen under greenhouse conditions.

An observed cultivar effect in the first pathogenicity experiment of 2001 indicated that the bentgrass cultivars were significantly different from each other in susceptibility. A significant cultivar effect in the pathogenicity experiments of 2003 using 18 isolates of *S. homoeocarpa* was similar to the findings of the 2001 experiments (Table 2). The significant clone effect within each cultivar in these experiments (Table 2) indicated that clones within individual cultivars showed significantly different disease responses. This was a reasonable outcome since commercial cultivars are all synthetic varieties and individual clones are genetically different from each other.

A possible explanation for finding significant differences in susceptibility among clones within cultivar as well as cultivars within species in only the first experiment and not in the second of 2001 was due to a higher MS error despite the similar inoculation procedures used (Table 2). Possible causes are differences in the age of the clones inoculated and in temperature during an incubation period between the two experiments. The second experiment included older plants which were maintained for a longer period of time (approximately 8 mo) and in general, had more disease compared to the

Table 7. Rankings of seven bentgrass cultivars evaluated in the 2001 and 2003 tests and at 10 locations in the National Turfgrass Evaluation Program (NTEP).

		2003		2001		
Species	Cultivar	Experiment 1	Experiment 2	Experiment 1	Experiment 2	NTEP†
Colonial	Glory	1‡	5	–	–	1
Colonial	Tiger	3	6	1	2	2
Colonial	SR7100	2	1	2	1	3
Creeping	L-93	5	4	–	–	4
Creeping	Pennlinks	4	3	–	–	5
Creeping	Penncross	7	7	4	4	6
Creeping	Providence	6	2	3	3	7
Correlation with NTEP		0.89*	−0.18	0.80*	0.60*	

* Correlation coefficient significant at $P < 0.05$.
† Ranking of means from 10 locations, ranging from 1 to 3 yr of data per location. Data obtained from www.ntep.org.
‡ 1 = most resistant for all tests.

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

first one (4 mo). This might be due to more senescent tissue, thatch built up and nutrient stress in the plants used in the second experiment. The temperature inside the growth chamber was influenced by outside temperature. Nonetheless, both the 2001 experiments were still similar in respect to the rank order ($r = 0.73$) of the cultivars and the species. These results also support the idea that dollar spot resistance is affected by different environmental conditions as reported by other researchers (Vincelli et al., 1997).

We detected a significant difference in percentage of disease between creeping vs. colonial and dryland bentgrass cultivars in both 2001 experiments, and this difference explained ≥98% of the variance among species. Creeping bentgrass is allotetraploid with A_2 and A_3 genomes whereas colonial and dryland bentgrass are also allotetraploid with common genomes A_1 and A_2 (Jones, 1956). Thus, colonial and dryland bentgrasses are more closely related to each other than to creeping bentgrass. The fact that differences in disease response were not significant between colonial vs. dryland bentgrass cultivars provides further support of this. Velvet bentgrass with A_1 genome, which is diploid, was not significantly different in susceptibility from all the other tetraploid bentgrass species. This might be because velvet bentgrass has a genomic affinity to the colonial bentgrass in being less susceptible to dollar spot than the creeping bentgrass. It is possible that the A_1 genome common in the velvet and colonial bentgrass species is actually conferring the dollar spot resistance but further experiments are needed to validate this hypothesis. Similarly, in the two 2003 experiments, the colonial and velvet bentgrass cultivars were more resistant to all the isolates of *S. homoeocarpa* compared to the creeping bentgrass cultivars.

One of the criteria used for selecting the 18 fungal isolates (Table 6) for the pathogenicity tests in the 2003 experiments was their genetic difference compared to the other isolates based on RAPD derived genetic similarity (Fig. 1). Isolates with significant genetic diversity will help to mimic the array of natural inocula, thereby providing a better understanding of the pathogenicity and host resistance which will facilitate bentgrass breeding for dollar spot resistance.

There was a significant isolate effect in both 2003 experiments, which shows that the isolates differ in their aggressiveness irrespective of cultivars and species. But the interactions between bentgrass cultivars and species with isolates were not significant; therefore differences in susceptibility of bentgrass cultivars to *S. homoeocarpa* appear to be race nonspecific although significant difference in aggressiveness was observed among isolates. This is very important information for turfgrass breeders, because it suggests that turfgrass breeders should be able to select for resistance to one or a few virulent isolates of the pathogen, and obtain resistance to a wide array of isolates irrespective of different VCGs and genetic diversity.

Moreover, the only exception in the ranking of dollar spot resistance of bentgrass cultivars from greenhouse tests was in the second experiment in 2003 (Table 7). This was due to compression of cultivar differences in the second experiment, which had higher disease pressure. Excluding Experiment 2 of 2003, increased the estimate of Kendall's coefficient of concordance to $\tau = 0.81$ ($P = 0.10$). These results suggest that there is, generally, a positive rank correlation between field and greenhouse rankings for resistance to this pathogen.

Greenhouse assays used in our current study were different than field assays in several respects, particularly in the nature and amount of inoculum, plant age, and environmental factors. In our greenhouse study, we only noted the disease severity, whereas field ratings were based on both disease severity and the number of lesions. This limitation to greenhouse studies reduces the potential range of disease severity that can be observed among cultivars. Modification of certain variables, including plant age, temperature, and light duration will improve the reproducibility and the sensitivity of our greenhouse experiments. As with field studies, greenhouse evaluations must be repeated in time, allowing assessment of host symptoms across potentially different environmental conditions and disease severity levels.

Even with these limitations, data from pathogenicity tests have demonstrated significant differences among bentgrass clones, cultivars, and species in their response to the dollar spot pathogen, significant differences in levels of aggressiveness among *S. homoeocarpa* isolates and finally, a lack of race-specific interactions. Race nonspecific resistance is much more likely to be durable across a wide array of environments in which bentgrass is grown. Therefore, our findings assure turfgrass breeders that new bentgrass cultivars with improved dollar spot resistance will have broad utility for the golf course industry, irrespective of geographic variation in *S. homoeocarpa* isolates. The genetic transfer of resistance from colonial bentgrass to creeping bentgrass should be a relatively simple process, because these species readily hybridize with each other (Belanger et al., 2004). However, because hybrids between velvet bentgrass and creeping bentgrass are sterile, it will be more difficult to transfer resistance from the diploid velvet bentgrass to the tetraploid creeping bentgrass (Brilman, 2003).

ACKNOWLEDGMENTS

We thank Jon Powell for providing us the dollar spot isolates and Leon Burpee, Zahi Atallah, and Craig Grau for valuable discussion and critical reviews for the manuscript. We thank Joe Curley and Steve Abler for their excellent technical assistance. Funding for this project was supported by Hatch Formula Fund (WIS04777) and by the Wisconsin Turfgrass Association.

REFERENCES

Abernathy, S.D., R.H. White, P.F. Colbaugh, M.C. Engelke, G.R. Taylor, II, and T.C. Hale. 2001. Dollar spot resistance among blends of creeping bentgrass cultivars. Crop Sci. 41:806–809.

Baldwin, N.A., and A.J. Newell. 1992. Field production of fertile apothecia by *Sclerotinia homoeocarpa* in *Festuca* turf. J. Sports Turf Res. Inst. 68:73–76.

Belanger, F.C., S. Bonos, and W.A. Meyer. 2004. Dollar spot resistant hybrids between creeping bentgrass and colonial bentgrass. Crop Sci. 44:581–586.

Bernick, S.C. 2002. The assessment of diversity within Minnesota

Reproduced from Crop Science. Published by Crop Science Society of America. All copyrights reserved.

populations of *Sclerotinia homoeocarpa* and the development of species specific PCR primers for the detection of North American *Sclerotinia homoeocarpa* isolates. M.S. thesis. Univ. of Minnesota, St. Paul.

Bonos, S.A., M.D. Casler, and W.A. Meyer. 2003. Inheritance of dollar spot resistance in creeping bentgrass. Crop Sci. 43:2189–2196.

Boulter, J.I., G.J. Boland, and J.T. Trevors. 2002. Evaluation of compost for suppression of dollar spot (*Sclerotinia homoeocarpa*) of turfgrass. Plant Dis. 86:405–410.

Brilman, L.A. 2003. Velvet bentgrass (*Agrostis canina* L.). p. 201–205. *In* M.D. Casler, and R.R. Duncan (ed.) Turfgrass biology, genetics, and breeding. John Wiley & Sons, Hoboken, NJ.

Cole, H., B. Taylor, and J. Duich. 1968. Evidence of differing tolerances to fungicides among isolates of *Sclerotinia homoeocarpa*. Phytopathology 58:683–686.

Conover, W.J. 1971. Practical nonparametric statistics. John Wiley & Sons, NY.

Couch, H.B. 1995. Disease of turfgrasses. 3rd ed. Krieger Publ., Malabar, FL.

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19–21.

Detweiler, A.R., J.M. Vargas, Jr., and T.K. Danneberger. 1983. Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. Plant Dis. 67:627–630.

Golembiewski, R.C., J.M. Vargas, Jr., A.L. Jones, and D.R. Detweiler. 1995. Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homoeocarpa* populations. Plant Dis. 79:491–493.

Hitchcock, A.S. 1951. Manual of the grasses of the United States. USDA Misc. Publ. 200. U.S. Gov. Print. Office, Washington, DC.

Hsiang, T., and S. Cook. 1992. Resistance of bentgrass cultivars to dollar spot disease. Biol. Cult. Tests 8:110.

Hsiang, T., and G.S. Mahaku. 1999. Genetic variation within and between southern Ontario populations of *Sclerotinia homoeocarpa*. Plant Pathol. 48:83–94.

Jones, K. 1956. Species differentiation in *Agrostis* II. Cytological relationships in *Agrostis canina* L. J. Genet. 54:370–376.

NTEP. 2001. 1998 National Bentgrass (Putting Green) Test. 2001 Data [Online]. Prog. rep. NTEP no. 02-3. Available at www.ntep.org/reports/bt98g/bt_02-3/bt98g_02-3.htm (verified 19 Jan. 2006). NTEP, Beltsville, MD.

NTEP. 2003. 2003 National Bentgrass (Putting Green) Test. 2004 Data [Online]. Prog. rep. NTEP no. 05-2. Available at www.ntep.org/reports/bt03g/bt03g_05-2/bt03g_05-2.htm (verified 19 Jan. 2006). NTEP, Beltsville, MD.

Powell, J.F., and J.M. Vargas, Jr. 2001. Vegetative compatibility and seasonal variation among isolates of *Sclerotinia homoeocarpa*. Plant Dis. 85:377–381.

Raina, K., N. Jackson, and J.M. Chandlee. 1997. Detection of genetic variation in *Sclerotinia homoeocarpa* isolates using RAPD analysis. Mycol. Res. 101:585–590.

Rohlf, F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, NY.

SAS Institute Inc. 1999. SAS/STAT User's Guide, Version 7–1, SAS Inst., Cary, NC.

Scheef, E., M. Casler, and G. Jung. 2003. Development of species-specific SCAR markers in bentgrass. Crop Sci. 43:345–349.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dep. of Anthropology, Univ. of Geneva, Switzerland.

Sneath, P.H.A., and R. Sokal. 1973. Numerical taxonomy. The principles and practice of numerical classification. Freeman, San Francisco.

Sonoda, R.M. 1988. Vegetative compatibility groups among *Sclerotinia homoeocarpa* from leaves of *Paspalum notatum*. Proc. Soil Crop Sci. Soc. Fla. 48:35–36.

Steel, R.G.D., J.H. Torrie, and D.A. Dickey. 1996. Principles and procedures of statistics: A biometrical approach. 3rd ed. McGraw-Hill, New York.

Vargas, J.M., Jr. 1994. Management of turfgrass diseases. 2nd ed. Lewis Publ., Boca Raton, FL.

Vincelli, P., J.C. Dooney, and A.J. Powell. 1997. Variation among creeping bentgrass cultivars in recovery from epidemics of dollar spot. Plant Dis. 81:99–102.

Walsh, B., S.S. Ikeda, and G.J. Boland. 1999. Biology and management of dollar spot (*Sclerotinia homoeocarpa*); an important disease of turfgrass. HortScience 34:13–21.

Warren, C.G., P.L. Sanders, and H. Cole, Jr. 1974. *Sclerotinia homoeocarpa* tolerance to benzimidazole configuration fungicides. Phytopathology 64:1139–1142.

Williams, D.W., D.W. Powell, Jr., P. Vincelli, and C.T. Dougherty. 1996. Dollar spot on bentgrass influenced by displacement of leaf surface moisture, nitrogen, and clipping removal. Crop Sci. 36:1304–1309.

Wipff, J.K., and C. Fricker. 2001. Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera* L.) in the Willamette valley, Oregon. Int. Turfgrass Soc. Res. J. 9:224–242.