

Dollar Spot Resistant Hybrids between Creeping Bentgrass and Colonial Bentgrass

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ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is an important turfgrass species used extensively on golf courses. It is highly susceptible to the fungal pathogen *Sclerotinia homoeocarpa* F.T. Bennett, the causal agent of dollar spot disease. There is a wide range in degree of susceptibility among current cultivars, with a limited number that have improved resistance. Colonial bentgrass (*A. capillaris* L.), a related species, has good resistance to dollar spot. We have produced interspecific hybrids between creeping bentgrass and colonial bentgrass and field-tested them against dollar spot. Some of the hybrids had excellent dollar spot resistance, exhibiting essentially no disease symptoms. This is the first formal report of evaluation of interspecific hybrids between creeping bentgrass and colonial bentgrass for an agronomically important trait. The results presented here suggest that the use of interspecific hybrids may be a useful new approach in *Agrostis* breeding programs.

CREeping BENTGRASS is the predominant turfgrass species used on golf course greens and fairways in temperate climates of North America (Warnke, 2003). It is highly susceptible to the fungal pathogen *Sclerotinia homoeocarpa*, the causal agent of dollar spot disease. Dollar spot is a foliar disease favored by conditions of high humidity, warm days, and cool nights. Symptoms include necrotic areas of turf the size of a silver dollar that may coalesce to form large areas of dead turf (Vargas, 1994). Each year a hundred million dollars worth of fungicides is applied to golf courses, much of it to control dollar spot (Watson et al., 1992). There is a wide range in degree of dollar spot susceptibility among creeping bentgrass cultivars, with only a limited number of cultivars that have improved resistance (Plumley et al., 2000). Improvement in dollar spot resistance is therefore one of the main objectives in current creeping bentgrass breeding programs (Meyer and Belanger, 1997).

We have previously investigated the potential for interspecific hybridization between creeping bentgrass and related *Agrostis* species under both open-pollinated field conditions (Belanger et al., 2003a) and controlled crosses performed under greenhouse conditions (Belanger et al., 2003b). Interspecific hybrids with colonial bentgrass (*A. capillaris* L.), velvet bentgrass (*A. canina* L.), redtop bentgrass (*A. gigantea* Roth), and dryland bentgrass (*A. castellana* Boiss. and Reut.) were recovered at low frequencies and were fertile. These results suggested that interspecific hybridization may be a useful new approach in creeping bentgrass breeding since these other *Agrostis* spp. have some traits, such as resis-

tance to certain diseases and better heat tolerance, which would be beneficial in creeping bentgrass. Interspecific hybridization has been widely used in cultivar improvement of other crops (Stalker, 1980; Kalloo, 1992) but has not yet been used in breeding of *Agrostis* spp. (Brilman, 2001).

Colonial bentgrass is a turfgrass species that is used on golf courses in Europe and occasionally in the USA (Ruemmele, 2003), although generally creeping bentgrass is preferred. Colonial bentgrass is recognized by breeders as having good resistance to dollar spot, although it can be infected under high disease pressure (Plumley et al., 2000). Since creeping bentgrass can hybridize with colonial bentgrass and they both have the same chromosome number (Jones, 1956), it may be possible to introgress dollar spot resistance from colonial bentgrass into creeping bentgrass. Before beginning a breeding program utilizing interspecific hybridization, however, it will be important to determine if the primary hybrids have the trait of interest. The objective of this study was to determine if hybrids between creeping bentgrass and colonial bentgrass had enhanced resistance to dollar spot, relative to the creeping bentgrass parents.

MATERIALS AND METHODS

Plant Materials

Colonial bentgrass × creeping bentgrass interspecific hybrids were recovered from crosses between transgenic creeping bentgrass and colonial bentgrass plants under both field and greenhouse conditions (Belanger et al., 2003a, 2003b). Two lines (4475 and 5061) of transgenic creeping bentgrass expressing the *bar* gene, which confers resistance to the herbicide glufosinate {4-[hydroxy(methyl)phosphino]DL-homocysteine}, were used as the pollen parents. Hybrid progeny from the crosses were identified by screening progeny from the colonial bentgrass plants for herbicide resistance. Twenty-three hybrids were obtained from the creeping bentgrass pollen parent 4475 and 12 hybrids were obtained from the creeping bentgrass pollen parent 5061. Plants of 'Cobra' creeping bentgrass (International Seeds, Inc., Halsey, OR) and 'SR7100' colonial bentgrass (Seed Research, Corvallis, OR) were included in the study as controls to compare with the hybrids. Individual seedlings of the hybrids and the creeping and colonial plants were established in the greenhouse and maintained in 100-cm² pots.

Field Tests

Field evaluation of the interspecific hybrids was done as part of larger field trials of many individual creeping bentgrass plants. A field trial was established at the Rutgers Turfgrass Research Facility at North Brunswick, NJ, on 16 May 2001. The trial was established as a randomized complete block design with six vegetatively propagated replications of each of the interspecific hybrids as well as six replicates of the parental creeping bentgrass lines 4475 and 5061. Also included

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Table 1. Analysis of variance of percent dollar spot disease of the creeping bentgrass pollen parents and their interspecific hybrid progeny in the 2001 (i) and 2002 (ii) field tests.

(i)	Creeping parent	Days after inoculation							
		18	32	40	46	53	61	69	76
2001									
	4475	***	***	NS	***	**	***	NS	NS
	5061	NS	NS	***	***	***	***	***	***
(ii)	Creeping parent	Days after inoculation							
		20	26	41	48	53	61	70	81
2002									
	5061	NS	**	NS	***	NS	NS	*	**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS is not significant.

in the trial were 24 creeping bentgrass progeny from both lines 4475 and 5061, 48 plants of Cobra, and 48 plants of SR7100. The plants were placed 45 cm apart and maintained as mowed spaced plants at a height of approximately 2.54 cm with a rotary mower. Weeds were removed manually as needed.

A mixture of three isolates of *S. homoeocarpa* was used to inoculate the field trial. These isolates were used in previous bentgrass field tests (Dai et al., 2003) and were known to result in dollar spot disease. One isolate was collected from creeping bentgrass turf. One isolate was from a perennial ryegrass (*Lolium perenne* L.) host and another was from a collection of *S. homoeocarpa* isolates present at Rutgers University. Single hyphal tips of each isolate were transferred from water agar to plates of potato dextrose agar. In preparation for inoculation, isolates were grown separately on sterilized Kentucky bluegrass seed. Two hundred grams of Kentucky bluegrass seed were autoclaved for 15 min at 151°C. Seventy-five milliliters of sterile H₂O were added to the autoclaved bluegrass seed and incubated overnight. For each isolate, fungal mycelium from one half of a Petri dish was cut into 1- by 1-cm pieces and transferred to a flask of sterilized bluegrass seed. After approximately 3 wk of growth on the bluegrass

seed, the inoculum was dried on newspaper for 3 d. Each batch of inoculum was forced through a seed sieve and equal weights of the three batches mixed together. The inoculum was applied to the field with a drop spreader (Model #28-07175, Gandy Co., Owatonna, MN) at a rate of 1.75 g m⁻² on 24 June 2001. Light irrigation was applied to enhance fungal growth from the inoculum onto the turfgrass leaves. Dollar spot symptoms appeared 2 wk following inoculation. The inoculated bentgrass plants were rated weekly for 8 wk for percentage diseased turf for each replicate. This visual rating method is the standard used in the Rutgers Turfgrass Breeding Program for cultivar evaluation (Plumley et al., 2000). The data at each rating date were subjected to analysis of variance (ANOVA) followed by Dunnett's test to compare the interspecific hybrids against their creeping bentgrass parental line. Significance was evaluated at $P < 0.05$.

A second field trial was established 20 May 2002, that included five replicates of interspecific hybrids 8, 14, and 15, and the parental creeping bentgrass line 5061. The plants were prepared as described above. The field was inoculated on 21 June 2002, and rated for dollar spot at weekly intervals as described above.

RESULTS

Field Tests

In 2001, 35 colonial bentgrass × creeping bentgrass hybrids were evaluated for dollar spot resistance. The creeping bentgrass controls showed a high incidence of dollar spot, averaging 67 to 90% disease at the latest rating date. The colonial bentgrass controls showed good resistance, averaging only 4 to 22% disease throughout the rating period. Analysis of variance of the data from the creeping bentgrass pollen parents and their interspecific hybrid progeny revealed significant differences at most rating dates (Table 1i). Some of the interspecific hybrids from the creeping bentgrass parental line 4475 exhibited delays in disease development through 61 d

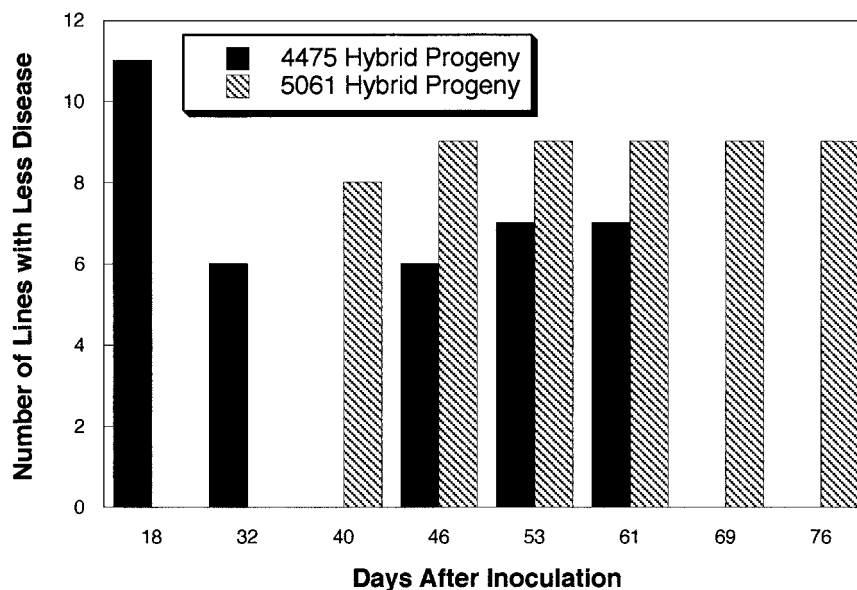


Fig. 1. Number of colonial bentgrass × creeping bentgrass interspecific hybrid lines exhibiting significantly ($P < 0.05$) less disease than their creeping bentgrass parent at each rating date in 2001. At 18 and 32 d after inoculation there were no hybrid progeny with significantly less disease than the creeping bentgrass parent 5061. At 69 and 76 d after inoculation there were no hybrid progeny with significantly less disease than the creeping bentgrass parent 4475.

after inoculation. The number of resistant hybrid lines at the different rating dates are shown in Fig. 1. Ultimately, however, all of these hybrids did develop disease to levels that were not significantly different from their creeping bentgrass parent 4475. Similarly, some of the interspecific hybrids from the creeping bentgrass parental line 5061 also had significantly less disease throughout the season (Fig. 1). Three of the interspecific hybrids from pollen parent 5061, numbers 8, 14, and 15, stood out as having essentially no disease throughout the entire rating period. The disease levels of these three interspecific hybrids throughout the season are compared with those of the creeping bentgrass and colonial bentgrass controls in Fig. 2. The response of these three

interspecific hybrids to dollar spot was more similar to that of colonial bentgrass than creeping bentgrass.

The three resistant interspecific hybrids were again subjected to a field test in 2002, along with the creeping bentgrass pollen parent 5061. In 2002, the creeping bentgrass parent was not as severely affected as in 2001, but did exhibit significant disease symptoms. Analysis of variance of the data again revealed significant differences (Table 1ii). In 2002, the three interspecific hybrids again showed essentially no disease throughout the season (Fig. 3). Because of the large variation in the ratings of the 5061 parental line, the ratings of the hybrids were not statistically significantly different from the parent at all the rating dates, even though they

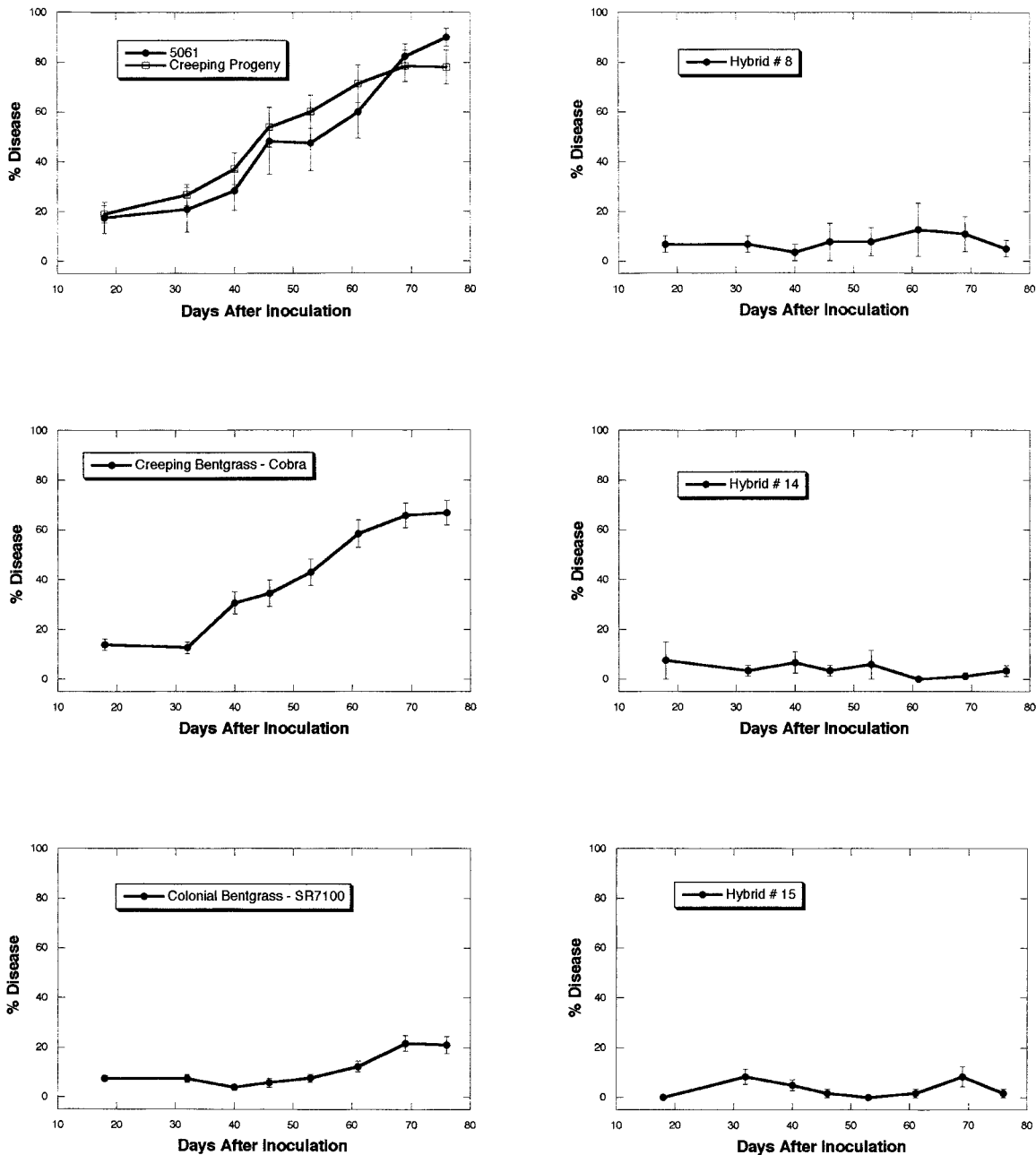


Fig. 2. Disease ratings of plants from the 2001 field test after inoculation with the dollar spot fungus. Data shown are the means and standard errors at each rating date.

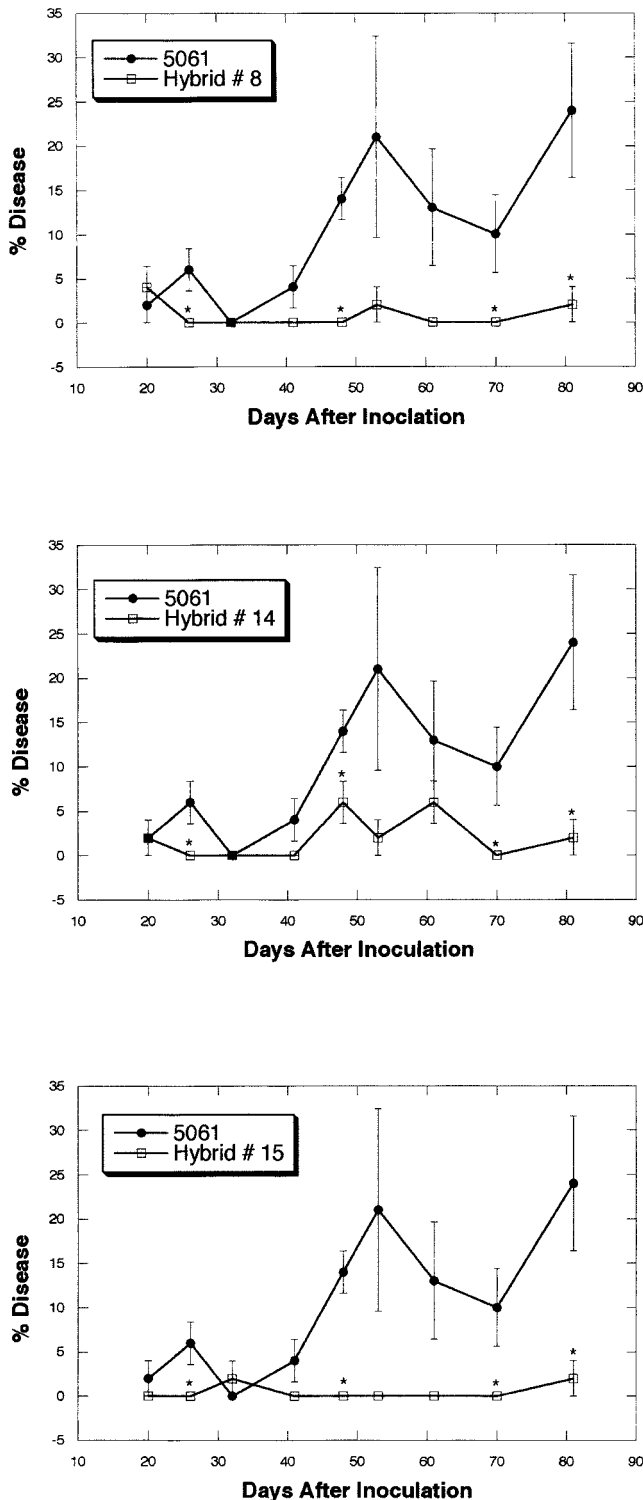


Fig. 3. Disease ratings of creeping bentgrass parental line 5061 and three interspecific hybrids from the 2002 field test after inoculation with the dollar spot fungus. Data shown are the means and standard errors at each rating date. Asterisks indicate statistical significance at $P < 0.05$.

exhibited very little disease symptoms. In field testing for disease resistance there is often a large standard error observed with susceptible genotypes. With truly resistant genotypes, the standard error is small. At the

last two rating dates when line 5061 was exhibiting disease symptoms, the hybrids were significantly different and had almost no disease. Figure 4 illustrates the extent of damage suffered by creeping bentgrass parent 5061 compared with interspecific hybrid number 15 in both 2001 and 2002. The photos were taken at the end of each rating period. Interspecific hybrids 8 and 14 were similarly unaffected by dollar spot in both years.

DISCUSSION

Dollar spot is one of the major management problems encountered with creeping bentgrass (Walsh et al., 1999). The current method of disease control relies heavily on the use of fungicides. Development of improved creeping bentgrass cultivars that require less fungicide use would clearly be of major environmental benefit. In this study we found that some colonial bentgrass \times creeping bentgrass interspecific hybrids had excellent dollar spot resistance, whereas the creeping bentgrass pollen parents were susceptible, as were creeping bentgrass progeny from the pollen parents. The dollar spot resistant phenotype of the interspecific hybrids, therefore, was derived from the colonial bentgrass parents. The growth habit of the resistant hybrids was similar to that of colonial bentgrass, being more upright and having fewer stolons than creeping bentgrass. Whether morphological traits may contribute to the observed dollar spot resistance is not yet known.

Colonial bentgrass as a species is recognized by breeders as having good dollar spot resistance (Plumley et al., 2000). Our field test results indicate the gene(s) conferring dollar spot resistance to colonial bentgrass, whether for morphological traits or for particular resistance genes, are being expressed in the resistant interspecific hybrids. Since we know the hybrids can be fertile (Belanger et al., 2003b), these results are encouraging regarding the possibility of introgressing dollar spot resistance into creeping bentgrass. Interspecific and intergeneric hybridization have been successful in transferring disease resistance traits to numerous crop species (Goodman et al., 1987; Stalker, 1980).

Not all of the colonial bentgrass \times creeping bentgrass hybrids, however, exhibited dollar spot resistance, which emphasizes the importance of screening before beginning a backcrossing program. The two creeping bentgrass genotypes used as the pollen parents of the interspecific hybrids were not selected based on prior evaluation of their response to dollar spot. Rather, they were selected based on the 1:1 segregation of the *bar* gene in their progeny, which was used to identify the hybrids (Belanger et al., 2003a, 2003b). The colonial bentgrass parents were also random selections. In future use of interspecific hybridization between creeping bentgrass and colonial bentgrass, screening the parents for the trait of interest may enhance the proportion of useful primary hybrids.

Colonial bentgrass has good resistance to dollar spot but it is very susceptible to another fungal disease, brown patch (caused by *Rhizoctonia solani* Kühn), whereas creeping bentgrass has good resistance to brown patch (Plumley et al., 2000). Interspecific hybridization be-

2001

5061



Hybrid #15



2002

5061



Hybrid #15



Fig. 4. Photographs comparing the extent of disease in creeping bentgrass parental line 5061 with the resistant interspecific hybrid #15. Photos were taken on 1 Sep. 2001 and 7 Sep. 2002.

tween colonial bentgrass and creeping bentgrass may also be useful in improving the brown patch resistance of colonial bentgrass.

Currently nothing is known regarding the mechanism of dollar spot resistance in colonial bentgrass and the interspecific hybrids. From the original interspecific crosses only one to five hybrid individuals were recovered from any single cross. Estimation of the number of genes involved in conferring the dollar spot resistant phenotype from our current data is therefore problem-

atic. This type of analysis will be performed in the future from a larger backcross population. Stability of the dollar spot resistance trait will also need to be evaluated throughout the backcrossing process.

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