Development of Creeping Bentgrass with Multiple Pest Resistance

University scientists adopt a team approach to improve this important turfgrass species.

BY MICHAEL CASLER, G. JUNG, S. BUGHRARA, A. HAMBLIN, C. WILLIAMSON, AND T. VOIGT

Creeping bentgrass (*Agrostis stolonifera*) is the premier grass for golf course putting greens and is one of the most desirable grasses for fairways and tees for much of the USA. Recent breeding advances demonstrate that genetic 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 many golf courses, maintenance of a high-quality turf requires frequent, varied, and intensive pesticide applications. Pesticide costs can consume up to 10% or more 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 widespread phenomenon in agricultural and horticultural plants. Disease resistance has been used to protect economically important plants for more than 90 years. There are many examples of genetic resistance that has been durable for more than 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 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 programs in 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 among 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 meet the challenges limiting adaptation of creeping bentgrass.

PROJECT DESIGN

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 that are 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, the University of Illinois, and Michigan State University, supported in part by the USGA.

Three populations of creeping bentgrass clones were developed for this study. The Wisconsin population consists of a cross between two clones that differed in resistance to speckled and gray snow mold pathogens. This cross, consisting of 200 clones, also is 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 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.

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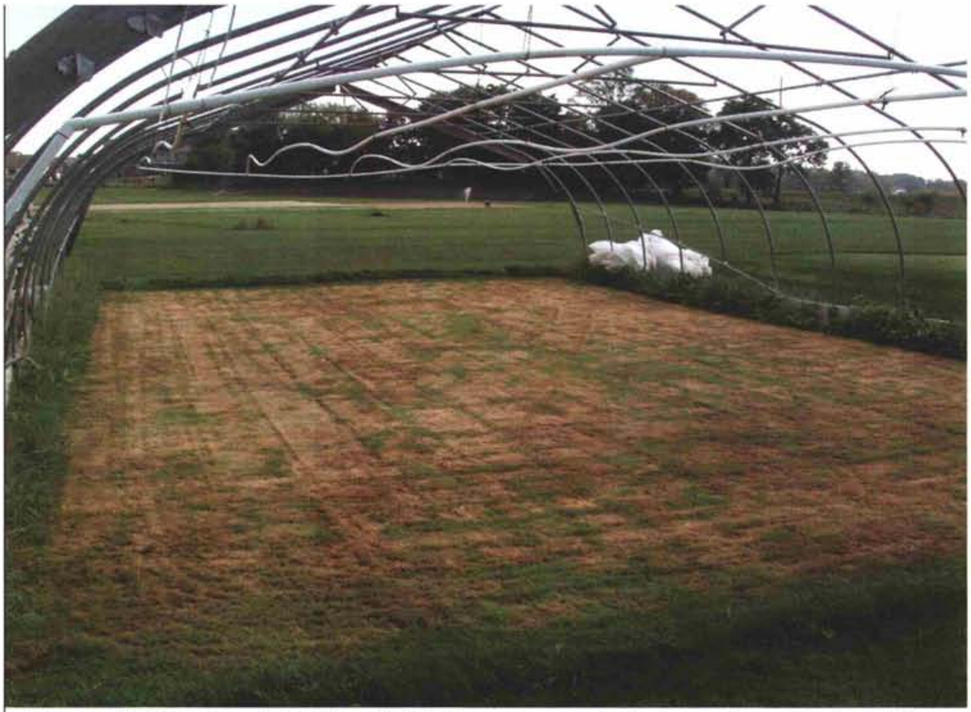
Speckled Snow Mold (*Typhula ishikariensis*)

The 600 clones were transplanted to the practice fairway at Gateway Golf Course, Land O' Lakes, Wis., in July 2003. 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 — all known to be particularly virulent on creeping bentgrass. Plots were rated again in early spring 2005, shortly after snow melt.

Pythium Blight (*Pythium spp.*)

The 600 clones were transplanted into a perennial ryegrass fairway at the O. J. Noer Turfgrass Research and Education Facility new Verona, Wis., in June 2003. The fairway was covered with a metal-framed hoop house with the plastic covering normally removed. Plots were inoculated with a mixture of isolates of *Pythium spp.* in early August 2004, and they were 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.



The so-called "chamber of death" resulted in damage to creeping bentgrass clones after inoculation with *Pythium* disease in August 2005.

Pink Snow Mold (*Microdochium nivale*)

The 600 clones were transplanted into another perennial ryegrass fairway at O. J. Noer in August 2003. In October 2004, plots were inoculated with an isolate of this fungus that had previously shown virulence 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, Wis., was inoculated with an isolate of the dollar spot pathogen that has been shown to be highly virulent against creeping bentgrass.[5] Ratings were made approximately four weeks after inoculation.

Black Cutworm (*Agrostis 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 was 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, Ill., 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 ½ 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, Mich., in June 2003. 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. 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.

Variation among clones within populations was significant for all measures of snow molds and dollar spot reaction. There was a large range among clones for all variables, and 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.

Gray 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. 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. 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 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, Mich., and Land O' Lakes, Wis., locations were caused by two different snow mold pathogens.

Dollar spot ratings were considerably more consistent across ratings made at different locations or years. 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.

In August 2005, four creeping bentgrass clones planted in a research trial on a ryegrass fairway at Gateway Golf Club (Land O' Lakes, Wis.) show differences in genetic resistance to dollar spot.

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 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, 20 clones with the highest disease indices and superior turf quality were selected for potential release to private companies for use in breeding new varieties of creeping bentgrass. 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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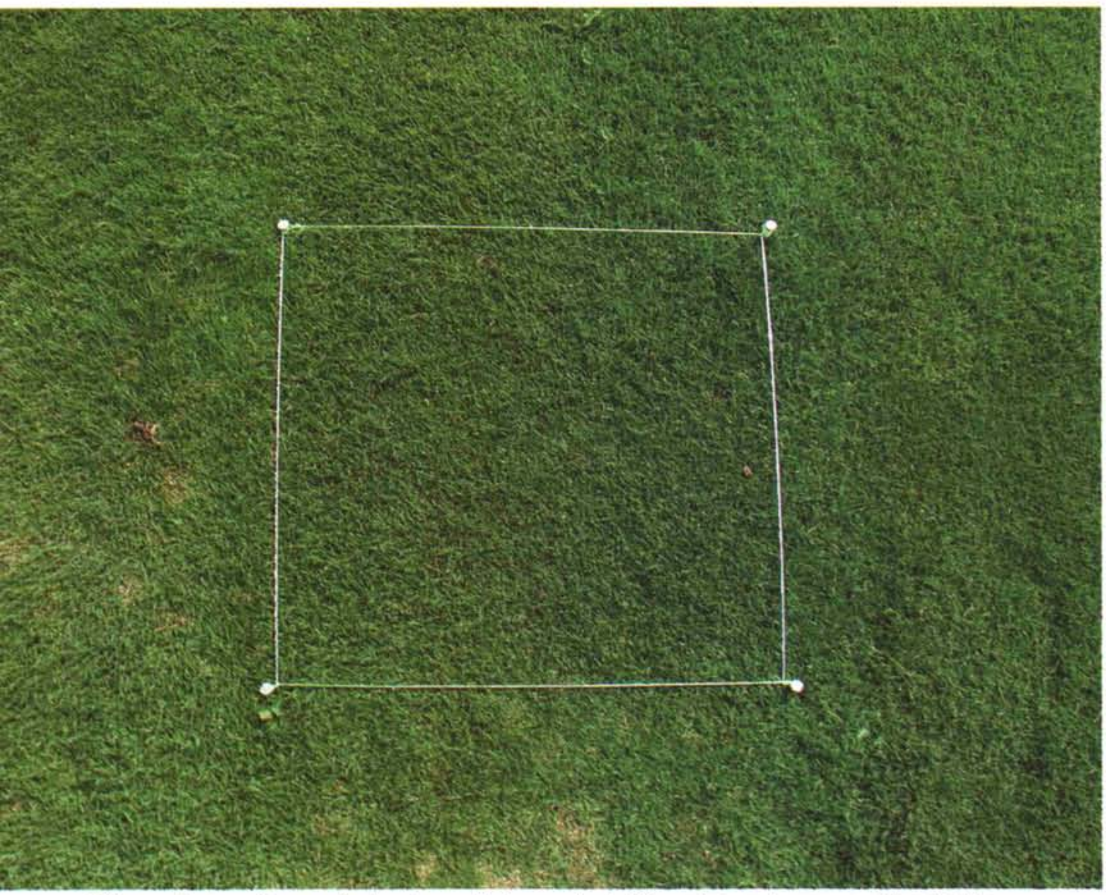

This excellent-quality creeping bentgrass plant was selected for resistance to snow mold and dollar spot at Verona, Wis., in August 2005.

Editor's Note: A more complete version of this research can be found at USGA Turfgrass and Environmental Research Online: http://usgatero.msu.edu/v05/n18.pdf.

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CONNECTING THE DOTS

A Q&A with* Dr. Michael Casler*, University of Wisconsin, regarding development of creeping bentgrass with multiple pest resistance.

Q: How common is it for universities located in different states to be cooperatively funded for long-term projects such as the Bentgrass Breeding Consortium? Do you think this is a trend that will increase as funding becomes more competitive?

A: Cooperative funding for long-term projects among universities is very rare. Short-term funding for cooperative projects is more common, usually for two or three years. I don't see this trend changing, as most opportunities for long-term funding have disappeared. In fact, as universities become more competitive, I see this trend continuing. Most of these decisions are made on a case-by-case basis — if the scientists make an excellent proposal, they can be funded, whether at one or more universities.

Q: Do you think grant administrators in most universities are open to interstate teamwork approaches such as yours, or do you feel that they would be reluctant to readily adopt this approach based on current grant funding and reporting procedures?

A: As long as the funding comes from outside the university, I don't think they care one way or the other. Administrators are interested in professors bringing funds into the university and in accomplishing research results. If a collaborative project accomplishes these two things, then it's not viewed any differently. The main impediment is intellectual property, such as improved germplasm — who owns it and who benefits from its development. In our case, this is all shared equally, but it required many months to work out wording that all three universities could accept.

Q: Spreading research dollars over a broad geographic area for a project such as the Bentgrass Breeding Consortium seems to make a lot of sense in that germplasm for multiple resistances can be identified and tested. Are there not-so-obvious drawbacks to this approach, as well?

A: The only drawback we observed was the distances among the collaborators and the difficulty in getting together to discuss the research. Most of these can be overcome by email and professional meetings.

Q: In your research, you focus on germplasm improvement for snow mold and dollar spot resistance in creeping bentgrass. What other resistance(s) do you feel would be priorities for creeping bentgrass, and would this require expanding the number of participating universities in the Bentgrass Breeding Consortium (i.e., a more southern location for bentgrass heat tolerance)?

A: Ideally, we would like to include several other locations, such as Rutgers, Texas A&M, and perhaps some others. This would allow us to cover a wider range of stresses and pests. Complicating this would be two things — introducing more traits to evaluate drastically reduces the probability of success, and adding more collaborators increases the political and administrative problems in creating the consortium and negotiating its rules and boundaries. In the long term, our vision is a nationwide bentgrass breeding consortium, but we feel that it's important to build slowly, rather than try to get everyone on board at once. Funding and organizational limitations are the main reasons for this.

Q: You stated that your objective for the Bentgrass Breeding Consortium is to develop multiple-resistance germplasm in creeping bentgrass. How much feedback have you gotten from seed producers for your germplasm that they could incorporate into new bentgrass cultivars?

A: There is quite a bit of interest among the private bentgrass breeders. However, because the clones have not yet been released to the breeders, they have not been able to provide any feedback at this time. They will have their first access to the clones in spring 2007.

Q: When more resistant germplasm is identified, how soon can superintendents expect it to show up in improved bentgrass cultivars?

A: This process will probably require a number of years to evaluate the clones, incorporate them into new varieties, test the varieties, and conduct seed increases of the new varieties. This process may require a minimum of six to eight years before the first materials reach commercialization.

Jeff Nus, Ph.D., *manager, Green Section Research.*