Dissecting the Disease - Dollar Spot

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When I applied to UW-Madison from India, I wanted to get trained as a plant pathologist with specialization in disease resistance mechanisms. I was specifically interested in studying a disease pathosystem, emphasizing on how some hosts fight back against the pathogen attack with an array of resistance genes, thereby having insignificant damage, whereas others succumb to the pathogen and cause us economic loss in our commercially important plants. I wanted to apply my knowledge of the resistance genes to the development of better plants by classical breeding and molecular biology techniques. These genetically improved plants with introgressed resistance gene/genes will have a better ability to withstand the disease impact and allow us to reduce the application of costly management practices and chemicals.

So when I learned about Dr. Jung's project of studying the genetic variability of bentgrass resistance to dollar spot using different Vegetative Compatibility Group (VCG) isolates, I was glad to work on it and contribute my understanding of the host and dollar spot pathogen interaction to deciphering the nature of resistance and find the resistance genes. The ultimate goal of my PhD project is to estimate the number and effects of dollar spot resistance genes and to develop resistant cultivars/lines using molecular techniques in creeping bentgrass.

Why are we interested in this disease?

Bentgrass or *Agrostis* is a large genus of over 100 species. Only about four species are used for turfgrass in the US; these are colonial, velvet, highland, and creeping. These species are perennial, outcrossing cool-season grasses and are used for lawns, athletic fields, and golf courses. Currently, the stoloniferous creeping bentgrass is the most adapted species for use on golf course fairways and greens. All modern bentgrass cultivars are susceptible to dollar spot but there are significant differences in their

susceptibility.

Dollar spot caused by *Sclerotinia homeocarpa* F. T. Bennett is a major disease of turfgrass throughout the world, and is the most prevalent and economically important turf disease in North America, particularly on intensively managed golf course putting greens and closely mown fairways. The disease occurs from spring through fall, but is most active during humid periods of warm days with cool nights in spring, early summer, and fall. The optimum temperature for disease development is 70°-80°F, though the fungus will grow over a wider range, 50°F to 90°F. On low height turf such as greens or fairways, the symptoms appear as round or irregularly shaped, sunken, straw to brown colored patches approximately 3 cm in diameter or about the same size as a silver dollar. When disease pressure is high, isolated spots grow together to form large, irregular patches. Since this fungus is not known to produce conidia or a sexual stage in the U.S., the organism most likely spreads via mycelia or transport of infected leaf tissue by wind, water, machinery such as mowers, or by human traffic.

One of the recent Grass Root articles, written by Kevin L. Hensler in the July/August 2002 issue, discusses the different current research that can be utilized in the management strategies to combat this disease.

Dollar spot management, like most other turf diseases, is highly dependent on chemical fungicide application. The causal fungus has, however, developed resistance to several important classes of fungicides such as benzimidazoles, dicarboximides, and demethylation inhibitors (DMIs). Some fungicides have not been reregistered due to environmental concerns. This has stimulated research into alternative disease management strategies such as host resistance, one of three major components of "Disease Triangle".

How is our work different?

Previous reports indicate variability among bentgrass cultivars in their susceptibility to dollar spot. It has been well known that the colonial bentgrass species is naturally more resistant to dollar spot than creeping bentgrass. Two greenhouse inoculation experiments were performed in our lab using eighty-one clones of ten cultivars of the creeping, colonial, dryland, and velvet bentgrass species. The clones were inoculated with the dollar spot isolate MNI obtained from Dr. Jon Powell, University of Minnesota. Large genetic variation was detected at the species, cultivar, and clone level in bentgrass.

When differential response was noted between the clones using only one isolate, we were interested to determine the disease response which means the ability of causing disease and the amount of disease, caused by different isolates belonging to different VCGs on the creeping bentgrass clones. Information about the genetic similarity and the virulence of the different isolates will further strengthen the program to breed for disease resistant cultivars. The breeder needs to be absolutely certain that the cultivar that he breeds for resistance is able to resist a number of different isolates of the pathogen.

So, seven new fungal isolates were provided to us by Dr. Jon Powell. We studied the genetic relatedness or how the isolates are genetically related to each other to find if there is a correlation between their genetic similarity and their grouping under the different VCGs. We did not find any significant correlation but we found that the isolates under VCG A were genetically closely related to each other than to any other isolates belonging to the other VCGs.

The next step was to find the correlation between VCGs and virulence. We used eight isolates belonging to five VCGs, along with the two creeping bentgrass clones (372 and 549). The clones were identified from more than 300 clones collected by Dr. Mike Casler, Univ. of Wisconsin-Madison, from fairways and greens in golf courses throughout Wisconsin. There was an overall clone and isolate effect. The clone 372 is highly resistant while 549 is susceptible to dollar spot. Our previous results indicated that the level of resistance detected in the 372 clone was approximately the same as that of cultivars of the colonial species. Some of the isolates did not show significant difference in disease response, that is, they caused almost similar damage to the two clones but two isolates, MN1-VCG A and Les Bolstead -VCG J, did show a significant clone effect. These two isolates caused less damage to 372 but caused more damage to 549. Our results indicated that there was no race specific interaction based on the clones tested. Absence of race specificity means that the clones are either more susceptible to all the isolates studied so far, or they are more resistant to all of them than the other clone. The clone 372 was more resistant than the clone 549 for all the eight isolates. This information can help in judicious management of fungicide application by understanding the amount of fungicides to be applied to the cultivars, depending on their disease response to the isolates. We will further study the race specific interaction and virulence by including sixteen more isolates belonging to eleven VCGs which were grouped recently.

Since we have noticed a significant difference in disease response or disease resistance between the two creeping bentgrass clones 372 and 549, it suggests that progeny individuals created from the cross will segregate or have different levels of resistance for dollar spot disease. So a mapping population was created by crossing clone 372 by 549 this spring of 2002. We will use the

progeny comprising the mapping population to estimate locations on the chromosomes, numbers, and effect of dollar spot resistance genes using molecular markers.

Currently more than two hundred barley, oat and rice Restriction Fragment Length Polymorphism (RFLP) anchor probes are being screened using the two parental DNAs. These probes which are segments of DNA obtained from barley, oat and rice, are used to find homology or similarity with the DNA of the bentgrass clones. The molecular markers or probes serve as landmarks on the chromosomes to help us find the location of the disease resistance genes and a large number of them are arranged on the chromosome to form a linkage map.

In our lab, the probes or markers tested so far hybridized or combined with and detected abundant polymorphisms or difference in both bentgrass DNAs, indicating their potential transferability between the species. Thus all the necessary tools and expertise are already in place to successfully create the linkage map in bentgrass. The linkage map, the data obtained from field and greenhouse inoculations of the progeny plants, and a statistical software program will be used to help researchers create durably resistant cultivars using multiple resistance gene markers obtained through QTL (Quantitative Trait Loci) analysis. QTL means a statistical correlation between the disease resistance genes and the molecular markers. These molecular markers linked to QTL will help the breeder to select the plants from the population which have disease resistance genes for the dollar spot pathogen. This is called marker-assisted selection and it will help to speed up the breeding process of developing resistant cultivars/lines.

No other research groups have yet studied race-specific interactions in clones, cultivars, and species using multiple isolates of the dollar spot pathogen. If any race-specific interactions are noted, results from the experiment using multiple isolates from eleven VCGs can be used to analyze whether the QTLs for disease resistance are similar or different for different isolates.

What is the potential benefit of our work?

Our research program incorporates molecular and genetic methods, plant pathology, plant breeding, and quantitative genetics. This is important because every year, public health concerns due to synthetic chemicals applied on golf courses and other grassy areas are significantly increasing worldwide. Development of dollar spot resistant bentgrass cultivars would considerably decrease the need for fungicide application on bentgrass. In many cases, molecular markers are available for marker-assisted selection of novel genes such as disease resistance. However, markers for resistance to dollar spot and other turfgrass diseases are currently unavailable. By pairing markers with novel resistance genes, producers and private industry will be able to access the germplasm diversity held within the public sector in improved forms well into the 21st century. This marker-assisted selection can result in higher gain from selection than phenotypic selection, or trying to select plants through visual comparison of the disease. The linkage map will also be useful to other researchers on this topic in the future, as our map will be becoming the reference map, and they will be able to compare dollar spot and other turfgrass disease resistance QTLs with our QTLs.

Who will directly gain from our work?

These research results will help turfgrass breeders to speed the selection process by using molecular markers, with the ultimate goal of developing disease resistant breeding lines and cultivars. Ultimately, golf course superintendents, private seed companies and anyone using bentgrass will receive nearly immediate benefit from the development of disease resistant cultivars. In the end, the amount of fungicides used for the control of dollar spot will be dramatically reduced.