

# RUTGERS

New Jersey Agricultural  
Experiment Station

**PROCEEDINGS OF THE  
TWENTIETH ANNUAL  
RUTGERS TURFGRASS SYMPOSIUM**

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**January 13-14, 2011**

**Bruce B. Clarke, Director**

**William A. Meyer, Associate Director**

**The Center for Turfgrass Science**

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## **Proceedings of the Twentieth Anniversary Rutgers Turfgrass Symposium**

William A. Meyer and Barbara Fitzgerald, Editors

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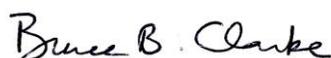
## Director's Opening Remarks:

Welcome to the Twentieth Anniversary of the Rutgers Turfgrass Symposium and the Center for Turfgrass Science at the School of Environmental and Biological Sciences/NJAES. The Symposium was established in 1991 to provide Rutgers faculty, students, and staff with an annual forum for the exchange of ideas on a wide range of topics in turfgrass science. Over the years, this format was expanded to include presentations by colleagues at other institutions. We are extremely fortunate this year to have many of our former graduate students from the Turfgrass Program present their research at the Twentieth Annual Turfgrass Symposium. I would like to thank Drs. Joseph Bischoff (USDA-APHIS), Michelle DaCosta (University of Massachusetts), Yuanhong Han (Noble Foundation), Gerald Henry (Texas Tech University), Josh Honig (Rutgers University), Patrick McCullough (University of Georgia), Lane Tredway (North Carolina State University), Eric Watkins (University of Minnesota), and Mr. Matt Koch (Rutgers University) for speaking at this year's symposium, as well as Drs. Stacy Bonos (Rutgers University), Karen Plumley (Mitchell Products, Inc), and John Inguagiato (University of Connecticut) for serving as session moderators. I would also like to recognize the Symposium Planning Committee comprised of Drs. Ning Zhang (Chair), Jim Murphy, Bruce Clarke, and Bill Meyer and Ms. Barbara Fitzgerald (co-editors of the Symposium Proceedings) for their hard work in the preparation of this year's program. Without their efforts, this year's Symposium would not have been possible.

The Rutgers Turfgrass program has a long and distinguished history dating back to the 1920s. In particular, we owe a debt of gratitude to Drs. C. Reed Funk, Ralph Engel and Henry Indyk for their pioneering efforts in turfgrass science that laid the foundation for the present-day turfgrass program. Since its inception in 1991, Center faculty have continued their efforts to develop outstanding research, undergraduate and graduate teaching, and continuing education programs in support of the turfgrass industry. With the tremendous support of the Rutgers Administration, the Center has hired some of the top academic talent in the country including Drs. Jim Murphy (1991), James White (1995), Bill Meyer (1996), Albrecht Koppenhofer (1998), Steve Hart (1999), Bingru Huang (2001), Stacy Bonos (2004), and Ning Zhang (2009). These scientists combined with the other 15 faculty in the Center form one of the most productive and well respected turfgrass programs in the world. To support our efforts, the Turfgrass Industry has donated over 4 million dollars over the last 20 years in the form of research grants, student scholarships (> \$100,000/yr), buildings (the Ralph Geiger Education Complex and the C. Reed Funk Equipment Facility at Hort Farm II), equipment, and gifts. We are indeed fortunate to have such a close partnership with the Turfgrass Industry in the state, region, and nation.

It is with deep pride and a sense of anticipation for the future of the Turf Center and its stakeholders that I welcome you to this year's Turf Research Symposium. I hope that you will find it an enjoyable and a worthwhile experience.

Sincerely,



Bruce B. Clarke, Director  
Rutgers Center for Turfgrass Science

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## TWENTIETH ANNIVERSARY RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 13-14, 2011

Foran Hall, Room 138A

### Thursday, January 13, 2011

- 5:00 – 5:45 PM Registration and Poster Set Up
- 5:45 - 6:00 PM Welcome and Introduction: **Dr. Bruce Clarke, Director - Center for Turfgrass Science**
- Opening Remarks: **Dr. Robert Goodman – Executive Dean of Agriculture and Natural Resources, NJAES**
- 6:00 – 7:30 PM **Turf Program Alumni Panel Discussion: *How Graduate School at Rutgers Prepared Me, or Did Not Prepare Me, For a Career in the Biological Sciences***  
(Moderator: Dr. William A. Meyer)
- 7:30 PM **Wine and Cheese Reception and Poster Session**

### Friday, January 14, 2011

- 8:15 – 8:50 AM **Registration, Coffee and Donuts**
- 8:50 – 9:00 AM Opening Remarks: **Dr. Larry Katz – Associate Director, Rutgers Cooperative Extension, NJAES**
- 9:00 - 10:45 AM **SESSION I: MANAGING ENVIRONMENTAL STRESS**  
Moderator: Dr. Karen Plumley, Mitchell Products
- 9:00 – 9:30 **Dr. Michelle DaCosta** (Department of Plant, Soil, and Insect Sciences, University of Massachusetts - Amherst): *Low-Temperature Physiology of Cool-Season Turfgrasses*
- 9:30 – 10:00 **Dr. Yuanhong Han** (Forage Improvement Division, Samuel Roberts Noble Foundation): *Development of Genetic Tools to Improve Drought Tolerance in Alfalfa*
- 10:00 – 10:30 **Dr. Gerald Henry** (Department of Plant and Soil Science, Texas Tech University): *Managing Turfgrass in a Semi-arid Environment*
- 10:30 – 10:45 **Discussion and Coffee Break**

- 10:45 – 12:30 PM     **SESSION II: PEST MANAGEMENT**  
 Moderator: Dr. John Inguagiato, Department of Plant Science and Landscape Architecture, University of Connecticut
- 10:45 – 11:15 **Dr. Lane Tredway** (Department of Plant Pathology, North Carolina State University): *Using Genomics to Unravel the Mysteries of Sclerotinia homoeocarpa*
- 11:15 – 11:45 **Dr. Joseph Bischoff** (USDA – APHIS): *Maintaining a Balance: The Role of APHIS in Working to Prevent the Introduction of Potential Invasive Plant Pathogens While Facilitating Global Trade and Commerce*
- 11:45 – 12:15 **Dr. Patrick McCullough** (Department of Crop and Soil Sciences, University of Georgia): *Enhancing Turfgrass Education with Mobile Application Technology*
- 12:15 – 12:30 **Discussion**
- 12:30 - 1:30 PM     **Lunch and Poster Session**
- 1:30 – 3:00 PM     **SESSION III: ADVANCES IN TURFGRASS BREEDING**  
 Moderator: Dr. Stacy Bonos, Department of Plant Biology and Pathology, Rutgers University
- 1:30 – 2:00     **Dr. Eric Watkins** (Department of Horticultural Science, University of Minnesota): *Developing Low-Input Turfgrass Cultivars for Cold Climates*
- 2:00 – 2:30     **Josh Honig** (Department of Plant Biology and Pathology, Rutgers University): *Characterization of Kentucky bluegrass (*Poa Pratensis* L.) Cultivars, Experimental Selections, Collections, and Hybrids Using Microsatellite Markers*
- 2:30 – 3:00     **Matthew Koch** (Department of Plant Biology and Pathology, Rutgers University): *Breeding Cool-Season Turfgrasses for Increased Salinity Tolerance*
- 3:00 - 3:30 PM     **Discussion/Closing Remarks**
- 3:30 PM             **Wine and Cheese Reception and Posters**

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## **PLENARY PRESENTATIONS**

## Low Temperature Physiology of Cool-Season Turfgrasses

Michelle DaCosta

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Low temperature injury and winterkill are major limitations in the management of some cool-season turfgrasses in northern climatic regions, including perennial ryegrass (*Lolium perenne* L.) and annual bluegrass (*Poa annua* L.). For example, greens and fairways comprised of considerable populations of annual bluegrass may experience as much as 70-90% turf loss from winterkill in severe winters, which can lead to costly re-establishment and significant decline in playability and aesthetic quality of golf course turf. As a result, research is needed to identify the underlying factors that contribute to differences in winter injury potential among cool-season grasses.

The primary mechanism by which turfgrasses develop freezing tolerance is through the process of cold acclimation, or cold hardening. The cold acclimation process is induced by decreases in temperature and daylength during fall months, which then trigger many physiological and structural changes inside the turfgrass cells. For example, plants accumulate numerous 'cryoprotective' compounds such as carbohydrates and proteins that help to lower the freezing point and stabilize cells at low temperatures. Climatic conditions and management practices (light levels, mowing height, nutrient availability, etc.) during the cold acclimation period can be important factors in determining freezing tolerance levels going into winter months. In addition, turfgrass species and cultivars vary in the maximal level of freezing tolerance that is achieved during cold hardening, which can be related to how well the plants can alter their cellular structure and accumulate protective compounds prior to winter. Based on these factors, we have conducted research to evaluate differences in cold acclimation capacity among several cool-season grasses, including perennial ryegrass, velvet bentgrass (*Agrostis canina* L.), and creeping bentgrass (*A. stolonifera* L.).

Freezing tolerance levels can be reversed (deacclimation) during freeze-thaw events throughout winter and into spring, thus leaving the plant susceptible to freezing injury. It has been reported that annual bluegrass may be more sensitive to deacclimation in response to elevated winter temperatures compared to other turf species; however, the causes for differences in deacclimation potential between these species is not understood. We are currently conducting research to examine early physiological and biochemical changes that are triggered by elevated temperatures. This research will ultimately help to identify predisposing factors responsible for differences in turfgrass winter injury.

## Development of Genetic Tools to Improve Drought Tolerance in Alfalfa

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Alfalfa (*Medicago sativa* L.) is one of the most important cultivated forage legumes worldwide. Drought is one of the biggest factors limiting alfalfa production. The objectives of our research were to identify quantitative trait loci (QTL) associated with enhanced biomass production under drought conditions and to develop genome-wide SNP marker resources in alfalfa. Two backcross alfalfa populations originating from a cross between two parents with contrasting phenotypes (*M. sativa* var. 'Chilean', drought-susceptible and *M. sativa* subsp. *falcata* var. 'Wisfal', drought-tolerant), were evaluated for biomass production under both irrigated and drought conditions at two locations (Las Cruces, NM and Burneyville, OK). Monthly aboveground biomass was measured in both populations at both locations for two years. Five QTLs associated with aboveground biomass production under drought conditions and two under irrigated conditions were identified. 454 sequencing of cDNA identified 40,661 candidate genome-wide SNPs both among and between the two parents. High-resolution melting (HRM) is a suitable SNP genotyping platform for polyploid species such as alfalfa that require sensitivity for allelic dosage detection and the ability to distinguish haplotypes in heterozygous genotypes. Confirmed SNPs represent valuable molecular marker resources that can be used to enhance marker density near QTL regions associated with biomass yield and develop alfalfa cultivars productive under limited water availability, as well as useful tools for association mapping and genome-wide selection in alfalfa.

## **Managing Turfgrass in a Semi-arid Environment**

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Managing turfgrass in West TX can be very difficult due to the extreme nature of the environment and severe weather conditions present on the TX High Plains. Located within the transition zone, Lubbock often experiences below freezing temperatures in the winter and several weeks above 100 °F during the summer. High soil pH (7.8 to 8.4), insufficient water quantity and quality (high salinity), and gusting winds make it challenging for most turfgrass species to survive year round.

Currently, only a few turfgrass species (tall fescue, bermudagrass, zoysiagrass, and buffalograss) are utilized in West TX. Therefore, a significant portion of our research program is dedicated to evaluating new turfgrass species and improving current species through selection and breeding. A collaborative project with the University of Georgia is aimed at increasing the tolerance of centipedegrass to high soil pH, cold temperatures, and salinity. A recent trip to the Island of Guana in the British Virgin Islands yielded 15 seashore paspalum accessions that were brought back to the United States. A second collaborative project with Rutgers University is geared toward the identification of drought, heat, and salt tolerant tall fescue progeny. Evaluation and breeding of buffalograss (excellent drought, cold, and salinity tolerance) continues to be a focus as well. The discovery and creation of more adaptive turfgrass species may help reduce costly inputs (fertilizers, irrigation water, and pesticides) that are often used in overabundance to maintain less adaptive grasses.

Water conservation efforts have recently intensified in the arid Southwest due to increases in urbanization and local drought conditions, which resulted in reduced amounts of water available for irrigating turfgrass. The fact that urban expansion is occurring simultaneously with exhaustion of historic water supplies creates a challenge for water conservation in this region. Therefore, we examined the effect of reduced irrigation inputs on the growth and survival of several cool-season turfgrass blends and mixtures. Decreasing irrigation from 2 to 1 inch/wk caused reductions in % turfgrass cover and % green leaf tissue regardless of turfgrass blend/mixture. Unfortunately, these reductions were below acceptable levels.

Turfgrass seedlings are often more susceptible to environmental stresses during establishment since they don't have extensive root systems and can't easily obtain water and nutrients. This is even more problematic in semi-arid regions where soil moisture is often limited and temperatures are high throughout the growing season. Establishment in these areas is often unsuccessful or poor. Amending the soil with conditioning materials may enhance seed germination, improve nutrient absorption, and increase root penetration. Humic/fulvic acid and calcium lignosulfonate exhibited significant increases in root and shoot biomass of bermudagrass compared to a traditional fertility program. The use of mulching material may also enhance turfgrass establishment. Gin trash mulch and hydro-mulch exhibited a 2 and 4-fold increase in buffalograss establishment 3 months after seeding, respectively, compared to no mulch.

The adaptability of several turfgrass pests to this region makes management even more difficult. Spring Dead Spot (SDS) is one of the most severe and difficult to control diseases of bermudagrass. High soil pH enhances the prevalence of this disease and renders certain fungicides less effective. Khakiweed is one of the most problematic weeds in West TX. Adaptation to soil compaction and salinity has led to its spread, while a thick cuticle and long taproot has limited its control. Investigation into the biology and ecology of SDS and khakiweed has provided clues to enhance the efficacy of pesticide applications and cultural management.

## Using Genomics to Unravel the Mysteries of *Sclerotinia homoeocarpa*

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Dollar spot, caused by the fungus *Sclerotinia homoeocarpa*, is the most important turf disease worldwide. This disease attacks every grass species that is grown for turf and can develop under a wide range of environmental conditions. Despite its significance, however, little is known about dollar spot or the fungus that causes it. Very little information is available regarding the mechanisms of infection, spread, and survival employed by this pathogen. The specific environmental conditions that trigger dollar spot epidemics have also remained elusive. Perhaps most remarkably, the correct taxonomic placement for the dollar spot pathogen is yet to be determined. It has been widely recognized since the 1970's that this fungus does not belong to the genus *Sclerotinia*, as originally described by F.T. Bennett in 1937, but efforts to determine the correct classification have failed.

Due to these gaps in our knowledge, and a historical lack of cultivars with resistance to dollar spot, turf managers rely heavily on fungicides for dollar spot management. It has been estimated that 40% of fungicide applications made to turf in the United States are for control of this single disease. Unfortunately, this dollar spot pathogen develops resistance to fungicides very quickly, which is severely limiting the number of tools that superintendents have at their disposal to control dollar spot. To fill these gaps in our knowledge, my research program has begun to focus almost exclusively on dollar spot, and in particular, the genetic structure of populations and how they change in response to fungicide applications. Toward this end, we have collected over 3000 isolates from the US, Dominican Republic, Argentina, Chile, United Kingdom, and Japan; collection trips to South Africa and Southeast Asia are also planned for 2011.

We elected to sequence the *S. homoeocarpa* genome to assist in the development of molecular markers for population analysis and identification of candidate genes involved in fungicide resistance. Sequencing runs were conducted by the J. Craig Venter Institute, using combination of Sanger and Illumina sequencing technologies to yield 70x coverage of the genome, which is estimated to be 39 Mb in size. The assembled genome data was loaded into the Mobyle SNAP Workbench to facilitate analysis and sharing with collaborators at NC State University and Ohio State University. The results of genome analyses and examples of the research facilitated by the availability of this genome data will be presented.

## **Maintaining a Balance: The Role of APHIS in Working to Prevent the Introduction of Potential Invasive Plant Pathogens While Facilitating Global Trade and Commerce**

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The role of the Plant Pathogen Identifiers and National Mycologists within the Plant Protection Quarantine (PPQ) program of APHIS-USDA is to safeguard US agricultural and natural resources from non-endemic pathogens that may enter, establish, and have deleterious effects on these resources. When a potential pathogen is found on a plant or plant product offered for import it is identified and determined if the potential pathogen in question could pose a threat to the resources of the United States. Identifiers try to provide determinations of the potential pests to the species level so the most detailed information regarding natural history and level of risk can be established. However, without a precise identification, to the level of species, it is often impossible to determine if the potential pathogen is endemic to the US or if it could be a threat to plants found within US borders. Frequently, potential pathogens can be identified only to genus-level or higher (e.g., family and order level) and are thus considered “reportable” by National Identification Services (NIS-USDA). “Action” is taken on most imported products with “reportable” pest determinations. In the case of plant pathogens “Action” requires that the product offered for import be either destroyed or re-exported. The economic impact of these imprecise determinations is significant and could be well over \$100 million per year. Imprecise determinations are often due to the fact that the diversity of fungal species is poorly characterized. It’s estimated that only 5%-10% of all fungal species have been described. In addition, the host ranges of many described pest species are not fully recognized and associating a pathogen found on an unreported host is difficult. This is especially true for fungal groups that are diverse and morphologically simplistic (e.g., *Colletotrichum*, *Pestalotiopsis*, *Phoma*, *Phomopsis*).

The rate of genus-level determinations for products entering the US is high. Since 2008 nearly half of all consumption based (e.g., fruits and vegetables) products that were offered for import and were found to be associated with a “suspect disease” were later considered “reportable” because an identification could only be made to the genus-level. For propagative material (i.e., plants for planting) the percentage was closer to 90%. Similar values are reflected in domestic identifications made by state diagnostic labs working within the National Plant Diagnostics Network (NPDN). While it is unlikely we will ever be able to identify all pathogens on plant and plant products coming through US ports of entry there are steps that can be taken to considerably improve the rate of precise and correctly identified samples.

One approach we are employing to eventually reduce the number of genus-level identifications is to isolate and sequence the pathogens encountered and deposit the collected material into the US National Fungus Collection (BPI). With these data we are creating a searchable database (i.e., BLAST) that will allow us to compare our collections from the many different pathways and hosts we encounter and utilize the publically available information in databases, such as GenBank. Furthermore, by maintaining the cultures and depositing herbarium samples these collections, representing material from our global trading partners and countless hosts, will be

made available to interested plant pathologist and mycologist. In just the last 6 months, since this initiative began, we have built a database of over 300 sequences from over 100 hosts and deposited over 100 new herbarium samples, all associated with cultures and rDNA ITS data. In one example of how this effort impacts trade and commerce; we were able to clarify the biogeography of a *Phomopsis* sp. that was not known to occur in the US but has been regularly found associated with tree peonies (*Paeonia suffruticosa*) from China and Japan. Previously, any shipment of peonies found associated with this particular *Phomopsis* (*Phomopsis* cf. *fukushii*) had been rejected from the ports at an estimated wholesale cost of \$3.6 million (since 2008). We were able to establish that this *Phomopsis* sp. appears to have a cosmopolitan distribution, including the continental US, and occurs on many different host genera. Therefore, “action” is not necessary and any future shipment of peonies found associated with this pathogen would be allowed into the US marketplace. This isolation and sequencing initiative was created to take advantage of the wealth of material entering the US on a daily basis. Through careful study and collaboration significant improvements in the precision of identifications is expected, helping to facilitate commerce and trade while better protecting US agriculture and natural resources.

## **Enhancing Turfgrass Education with Mobile Application Technology**

Patrick McCullough

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Traditional turfgrass extension through state universities support practitioners through publications, seminars, field days, and site visits. Conventional methods for turfgrass teaching and extension have limitations for delivering educational material such as resources, time, and technology. Recent advancements in mobile application technology allow users to access programs and real-time information from anywhere with potential to enhance university education in turfgrass science. Mobile applications are relatively new features to smart phones and iPods that have grown in popularity over the last two years. In 2009, an application called “Turfgrass Management” was developed by faculty at the University of Georgia. This application is a comprehensive program that contains pictures, information, and recommendations for managing turfgrass, weeds, diseases, and insects for use exclusively on smart phones and iPods. By December 2010, approximately 800 premium (subscription) applications have been sold and approximately 4,000 lite (free) applications have been downloaded on mobile devices in over 40 countries. Currently, a new mobile application is under development at the University of Georgia called “Turf Management Calculator”. The application calculates numerous measurements needed in turfgrass management including equipment calibration, fertilizer rate, irrigation, product price, sprayer calculations, and many others. The potential impact of mobile application technology on the future of turfgrass education will be presented with other applications currently under development at the University of Georgia.

## Developing Low-Input Turfgrass Cultivars for Cold Climates

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Turfgrass managers and other stakeholders in Minnesota and the surrounding region have two primary concerns. First, they need turfgrasses that can survive the harsh winter conditions that are common to the region. Second, they require turfgrasses that can provide acceptable turf with fewer inputs of water, pesticides, and fertilizer. In response to these concerns, the turfgrass breeding program at the University of Minnesota conducts research addressing three research areas: (i) making turfgrasses more sustainable through plant breeding; (ii) developing native grasses for use as turf; and (iii) evaluating and identifying alternative species for sustainable turf systems. Several ongoing projects demonstrate advancement in these research areas. Perennial ryegrass (*Lolium perenne*) is widely used in Minnesota for turf and the species is also grown for seed in northern Minnesota. Unfortunately, the species exhibits poor winterhardiness and high levels of susceptibility to rust disease; therefore, we have worked on improving winterhardiness through improved screening techniques while investigating the potential of using metabolomics-assisted breeding to improve crown and stem rust resistance in the species. We have also initiated an extensive effort to develop low-input cultivars of prairie junegrass (*Koeleria macrantha*), a species native to the short grass prairies found in much of the western United States, and we have found significant variation for important turfgrass quality traits in native germplasm collections. Major breeding challenges of prairie junegrass include susceptibility to rust disease, reduced mowing quality, and poor turf establishment. In addition to improving commonly used turfgrasses, such as perennial ryegrass, and developing new turfgrass species, such as prairie junegrass, we have conducted research projects to identify new uses for currently used low-input turfgrass species. We have found that several of the fine fescue species can provide acceptable low-input golf course fairway turf; therefore, we are beginning to improve germplasm specifically for this purpose.

## **Characterization of Kentucky bluegrass (*Poa Pratensis* L.) Cultivars, Experimental Selections, Collections, and Hybrids Using Microsatellite Markers**

Josh A. Honig, Stacy A. Bonos, and William A. Meyer

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Kentucky bluegrass (*Poa pratensis* L.) is an important facultative apomictic temperate perennial grass species utilized for both forage and cultivated turf. Through apomixis, this species is able to propagate diverse and odd ploidy levels, resulting in many genetically distinct phenotypes. A wide range of diverse cultivars and accessions of Kentucky bluegrass have been previously characterized based on common turf performance or morphological characteristics, as well as by random amplified polymorphic DNA (RAPD) markers. The objectives of the current study were to genotype 265 Kentucky bluegrass cultivars, experimental selections, collections, and hybrids using microsatellite (SSR) markers, and compare the results with the original Kentucky bluegrass classification system. Results based on SSR markers were compared with the original Kentucky bluegrass classification system based on pedigree, common turf performance and morphological characteristics. All cultivars, experimental selections, collections, and hybrids were uniquely identified with the current set of SSR markers. Genetic relationships of individuals as assessed by SSR markers closely matched known pedigrees. Furthermore, genetic relationships based on SSR markers more accurately reflected pedigree than genetic relationships based on morphological characteristics. The current set of SSR markers can be used to rapidly genotype and assign new cultivars/accessions to Kentucky bluegrass classification types and assess genetic relatedness among individuals, and should be considered for use in a Kentucky bluegrass Plant Variety Protection program.

## Breeding Cool-Season Turfgrasses for Increased Salinity Tolerance

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Turfgrass areas are potential sites for utilizing non-potable water sources; however, these water sources can be high in dissolved salts which can cause salt stress injury and poor turf quality. Before we can use saline irrigation water on cool-season turf, there is a need to develop salt tolerant cultivars. The objectives of this research were to: 1) develop germplasm screening techniques for identifying plants with increased salinity tolerance in the field and the greenhouse, 2) correlate salinity tolerance from different established screening techniques 3) determine the level of salinity tolerance of currently available cool-season turfgrass cultivars, and 4) determine the broad-sense and narrow-sense heritability of salinity tolerance in perennial ryegrass (*Lolium perenne* L.).

We developed a novel greenhouse screening technique that involved a recirculating spray chamber system that accurately mimics field conditions that a turf manager would experience with overhead irrigation (Koch and Bonos, 2010). A field screening technique was also developed that evenly applied saltwater irrigation overhead. Both of these new techniques proved extremely efficient at differentiating germplasm for different levels of salinity tolerance. These new methods were used to screen germplasm in different cool-season turfgrass species including perennial ryegrass, Kentucky bluegrass (*Poa pratensis* L.), and creeping bentgrass (*Agrostis stolonifera* L.).

Objective two was accomplished by screening perennial ryegrass genotypes using three different salinity-screening techniques. The first method was the novel screening technique that was developed in the greenhouse using the overhead spray chamber system (Koch and Bonos, 2010). The second technique was a hydroponic screening technique that was prepared similarly to how most salinity screening has been done in the literature. The third and final technique studied was the field screening technique that was developed where plants received equal amounts of saltwater applied overhead. Results from various measurements between the techniques indicate that each of the methods are significantly correlated to each other and it can be assumed based on this research that all three screening techniques can identify the same salt tolerance plants. The overhead irrigated methods, however, did cause a larger decrease in measurements when compared to the technique that did not include foliar irrigation applications. This difference in the methods could be due to the fact that the overhead irrigated methods included foliar stress that the hydroponic method did not include.

The third objective was accomplished using the field screening technique (described above). Twenty-two Kentucky bluegrass cultivars, 23 perennial ryegrass cultivars, and 17 bentgrass cultivars were screened with this method over a period of three years. Plants were irrigated with water with a concentration equal to  $10 \text{ dS m}^{-1}$  made from equal parts of NaCl and  $\text{CaCl}_2$ . Results from this study indicate this technique was efficient at differentiating cool-season turfgrass cultivars with varying levels of salinity tolerance. Kentucky bluegrass cultivars Liberator, Eagleton, and Diva had the highest percent green values after three years of data while

the most salt sensitive varieties included Julia and A03-84. Perennial ryegrass cultivars that ranked highly, with respect to salinity tolerance, included RKS, Gator 3, and MSH Comp. Fiesta III was the most susceptible stress after two years of visual ratings (2008 and 2010). Creeping bentgrasses Declaration, Kingpin, and 007 all performed well in this field experiment. Colonial bentgrass Tiger II and velvet bentgrass SR 7200 were the most susceptible after two years of data (2008 and 2010).

The final objective again utilized the same field screening technique as the previous objective. Heritability estimates are an important first step in the breeding process because it is an indication to how effective breeding will be at improving that trait. Broad-sense heritability is the ratio of the genotypic variance compared to the total phenotypic variance within a population of plants. To obtain this estimate, six replicated blocks of perennial ryegrass clones were planted in the field and irrigated overhead with water with increased salt concentrations. Visual percent green ratings were taken and compared to control plants receiving fresh water. Data from two years were analyzed using Proc Mixed in SAS and a broad-sense heritability estimate of 0.78 was obtained. This estimate indicates that the largest amount of variation, with respect to the saltwater applications, is due to genetic factors. Narrow-sense heritability is defined as the ratio of the additive gene effects compared to the total phenotypic variance. Since additive gene effects are the genetic factor that can be accurately predicted and passed down to progeny, narrow-sense estimates very important for breeding. To estimate narrow-sense heritability, 15 diallel crosses were made using parent plants with high salinity tolerance and others with low tolerance based upon previous salinity screening. Four replications of 96 progeny plants and the parents were planted in the field and overhead irrigated with saltwater. A mid-parent progeny regression was run on two repetitions of the study. The narrow-sense heritability estimate was 0.67. This estimate indicated that the majority of the genetic variance within the population was due to additive gene effects and also indicates that advances in salinity tolerance should be possible through recurrent selection methods.

### **Literature Cited**

Koch, M.J. and S.A. Bonos. 2010. An overhead irrigation screening technique for salinity tolerance in cool-season turfgrasses. *Crop Science*. 50:2613-2619.

## **POSTER PRESENTATIONS**

## Deep Transcriptome Analysis to Aid in Understanding the *Epichloë*-grass Symbiosis

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It is well established that the *Neotyphodium* and *Epichloë* fungal endophytes of grasses confer numerous benefits to their hosts. However, the details of the interaction are largely unknown. One of the outstanding questions regarding the plant-endophyte relationship is what are the factors contributing to maintenance of a compatible interaction. Previous studies have established that gene expression in the plant is altered in response to endophyte infection. Our hypothesis is that these changes are important for the maintenance of the symbiotic interaction. Our approach is to use SOLiD-SAGE to obtain a global quantitative comparison of the transcriptomes of endophyte-free and endophyte-infected plants. We have three samples in the comparison, all with the identical strong creeping red fescue plant genotype: 1) endophyte-free, 2) infected with an endophyte originally from strong creeping red fescue 3) infected with an endophyte originally from Chewings fescue. The SAGE libraries were prepared in triplicate. We have obtained a total of over 54 million SAGE tags, with between 4 and 10 million tags per replicate. We are supplementing the SOLiD-SAGE data with 454 transcriptome sequencing from the *Epichloë festucae* strain isolated from strong creeping red fescue and the plant infected with that strain. We have obtained over 200,000 454 sequences. We expect to obtain quantitative data on most of the expressed plant and fungal genes. Analysis of the data will reveal 1) general plant transcriptional changes in response to fungal endophyte infection, 2) plant transcriptional changes that are specific to the infecting fungal endophyte genotype, and 3) relative transcript levels for fungal endophyte genes for two endophyte genotypes each infecting the same plant genotype.

## Development of Molecular Methods for Identification of Turfgrass Rust Fungi Reveals Kentucky bluegrass as a Host for *P. coronata*

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Rust (*Puccinia* sp.) is a common fungal disease of cultivated turfgrasses. Over the past ten years, increased susceptibility to rust has been observed in several Kentucky bluegrass (*Poa pratensis* L.) cultivars, most notably the once highly resistant ‘Midnight’ types. This pattern suggests that new races or even new species of rust fungi may have emerged, however, the data required to test this hypothesis is currently lacking. In the present study, we developed and utilized molecular markers to identify turfgrass rust fungi. Sixty-six rust samples from 11 graminicolous hosts were collected in North America, the United Kingdom, Australia and Chile. The complete rDNA internal transcribed spacer (ITS) region and 5.8S ribosomal DNA portion of the samples were PCR amplified and sequenced. Phylogenetic analysis of the ITS sequences identified *Puccinia coronata*, *P. graminis*, and *P. striiformis* from the diseased tissue samples. *P. coronata* was the most prevalent species (68% of the samples) followed by *P. graminis* (27%) and *P. striiformis* (5%).

Not only was *P. coronata* the predominant rust species identified in this study, but, contrary to previous reports in the literature, this species was also frequently found in association with Kentucky bluegrass (21 samples). Based on the frequencies of rust species identified by sequence analysis and the unique host/pathogen relationship consistently observed between Kentucky bluegrass and *P. coronata*, 58% of the diseased Kentucky bluegrass samples collected in this study would have been incorrectly diagnosed as being caused by *P. striiformis* or *P. graminis* using traditional field identification techniques. Whether the *P. coronata*/Kentucky bluegrass association is new or has gone unnoticed due to misidentification is unknown. However, our findings suggest that the breakdown of resistance to rust in Kentucky bluegrass during the past decade may not have been due to the emergence of a new fungal race(s) as originally hypothesized. Rather, the upsurge of rust disease may have occurred because of an increased association of *P. coronata* with Kentucky bluegrass, or a recent transitioning of the pathogen from another grass population to this host.

To improve the accuracy of turfgrass rust fungus identification, we developed a real-time PCR molecular diagnostic protocol for *P. coronata*, *P. graminis*, and *P. striiformis* using ITS sequence data. Fluorescence labeled hydrolysis probes and primer pairs for *P. graminis* and *P. striiformis* were designed from the ITS-1 region, while the ITS-2 region was used for the *P. coronata* assay. The *P. graminis* and *P. striiformis* probes were designed within the same ITS-1 region and relied upon the same primer set to allow for multiplexing of the two probes for simultaneous detection of these pathogens. The resulting assays were 97% accurate for the identification of *P. graminis*, *P. coronata*, and *P. striiformis* from diseased turfgrass tissue and could detect the presence of multiple species in mixed infections. Species-specific

identifications were made using as few as 50-150 urediniospores (1-9 pg DNA), and were completed from DNA samples in approximately 1 hour.

This study represents the first DNA-based evaluation of turfgrass rust fungi and provides a quick and reliable molecular identification protocol that can be implemented by turfgrass breeding programs and disease diagnostic clinics as an alternative to field-based identification techniques. The real-time PCR assay developed in this study is currently being used by the Plant Diagnostic Laboratory at Rutgers University to identify rust species from diseased turfgrass samples.

## Single Nucleotide Polymorphisms and Real-Time PCR as a Method for Evaluating Unique Isolates of the Turfgrass Anthracnose Fungus

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Anthracnose, caused by the fungus *Colletotrichum cereale*, is a destructive disease of golf course putting greens. Molecular analysis of *C. cereale* has shown that there is considerable diversity within the species, with the fungus now subdivided into two major lineages and several host-specific populations. Isolates of *C. cereale* from prairie and cereal crops are members of populations that are distinct from those that attack turfgrass hosts. Turf pathogens from *Poa annua* and *Agrostis* sp. have been further subdivided into host specific populations. The ecological significance of this subdivision is currently unknown; although it is possible that membership in a given population may play a role in anthracnose disease development. Although previous DNA fingerprinting methods were able to identify wide-scale differences between *C. cereale* groups, numerous fine-scale, multilocus markers sampled at multiple points within the genome will be required to fully understand the diversity of *C. cereale*, and what if any role different populations of the fungus play in the disease cycle.

In the present work, we are developing new genomic resources and culture-independent molecular tools to better understand how populations and individuals of *C. cereale* have evolved in response to biotic and abiotic changes in their environment. The presence of unique single nucleotide polymorphisms (SNPs) in the genome are being used to generate high-resolution molecular markers to detect sub-specific differences in fungal populations, however, few SNPs are currently known in *C. cereale*. To identify SNPs that might serve as the basis for developing high-resolution molecular markers for this pathogen, restriction associated DNA (RAD) tagged Illumina sequencing was used to generate short-read sequence data in *C. cereale* and other *Colletotrichum* species. 28,699 RAD tags were sequenced from a sample of 59 *Colletotrichum* isolates obtained from graminicolous hosts (*Digitaria ciliaris*, *Echinochloa esculenta*, *Miscanthus sinensis*, *Panicum virgatum*, *Paspalum* spp., *P. annua*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays*) and cranberry (*Vaccinium macrocarpon*), including *C. cereale*, *C. graminicola*, *C. acutatum* and *C. gloeosporioides*. 39% of these sequences were mapped to the *C. graminicola* strain M1.001 reference genome, from which 4537 unique loci were identified. 68% of the RAD-tagged sequences were located within coding regions of this genome. Approximately 50% of the mapped loci possessed two or more alleles, with 1-6 SNPs present in each 49-bp sequence. For marker development, the greatest number of polymorphic loci were identified from grass-derived isolates of *C. graminicola* and its closest relatives (*C. navitas*, *C. nicholsonii*), while far fewer alleles were observed from the wider comparisons with isolates from turfgrass (*C. cereale*) and cranberry (*C. acutatum* and *C. gloeosporioides*). These data are currently being used to develop SNP markers for *C. cereale*.

Concurrently, we are conducting expanded studies of *C. cereale* using real-time PCR to evaluate populations of the fungus in a culture-independent manner that could facilitate high-throughput research in the system. Over 650 cultured *C. cereale* isolates previously obtained from turfgrass (*A. stolonifera*, *P. annua*), prairie grasses (*Dactylis glomerata*, *Elymus virginicanus*), and wheat (*Triticum aestivum*) hosts have been evaluated to date, along with new field collections of the pathogen from diseased turfgrasses made during 2010. Real-time PCR fluorescence-labeled hydrolysis probes were developed using the single-copy *Apn2* (DNA lyase) gene as a template to determine if *C. cereale* could be detected in plant tissue, and to broadly sample two major subspecific groups of the fungus, clades A and B. Two probes were designed; one for *C. cereale* clade A, the other for clade B. Both probes were specific for the pathogen, but differed from one another by 8-bp within the 33-bp probe target site. Application of the real-time assay in vitro showed that the probes were able to discriminate between known clade A and B isolates of *C. cereale* with 100% accuracy (avg.  $C_T$  clade A=28.15; avg.  $C_T$  clade B=26.82, where  $C_T$  = cycle threshold). Reactions performed using DNA extracted directly from diseased turf samples also detected the presence of *C. cereale* from 100% of the samples, with  $C_T$  values for uncultured samples at levels comparable to those achieved from cultured strains of the fungus. From our *Colletotrichum* collection, 93% of all samples were members of clade A and 89.5% of the samples obtained from turfgrasses were members of clade A.

The RAD-tagged database and the culture-independent real-time PCR assay developed here mark significant advances for genomic and molecular studies in *C. cereale*. Previous molecular research was limited by the lack of genomic data available and time consuming identification techniques; however, with the implementation of these new technologies, unique *C. cereale* isolates can now be identified rapidly through real-time PCR, and genome-wide variation can begin to be assessed using the RAD-tagged database. This represents an essential step to further our understanding of *C. cereale* population dynamics, as well as to determine the underlying ecological significance that population structure may play in the disease cycle of this pathogen. The genomic resources generated here are not only useful for *C. cereale* population-based analyses, but can also be used to expand our understanding of the entire *Colletotrichum* genus in areas such as epidemiology, pathogenicity, or fungicide sensitivity.

## **Using Plant Hormones and Osmoregulants to Promote Drought Tolerance and Post-drought Recovery in Creeping Bentgrass (*Agrostis stolonifera*)**

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Drought is one of the most common environmental stresses limiting plant growth. Previous research has shown that exogenous applications of plant hormones, applied prior to and during periods of limited irrigation, can reduce water consumption and improve stress tolerance of certain agronomic crops. Though even with such promising results, little work has been devoted towards utilizing plant growth regulators (PGRs) in turfgrass (i.e. golf courses, sports fields, home lawns). From a physiological perspective, there are various mechanisms involved in drought survival of plants, which can be categorized in three groups: low water usage prior to and during drought, tolerance to desiccation during water withholding, and rapid recovery of plants after drought conditions subside. Some plant growth regulators may regulated these mechanisms and incorporating plant growth regulators into current management protocols can be greatly beneficial for maintaining high stand quality with limited water resources. This study was designed to investigate effects of several PGRs on water use, drought survival and post-drought recovery in creeping bentgrass, including 1) Trinexapac-ethyl (TE): growth inhibitor for gibberellic acid synthesis and vertical growth suppressant; 2) Glycine betaine (GB): osmotic regulator to facilitate water retention in leaves; 3) Cytokinins (CK: Kinetin): tiller and root growth promoter for stand re-establishment after drought stress; 4) Gibberellic acid (GA): shoot growth promoter to help break dormancy for re-growth after drought stress has subsided. Field plots were subjected to drought conditions and post-drought rewatering between June and July in 2010, and physiological parameters, including leaf relative water content (RWC), osmotic adjustment (OA), evapotranspiration (ET), membrane stability, green leaf biomass (MSR), and visual rating of turf quality (TQ) were evaluated. During the drought period, plants treated with TE+GB or GB-only displayed significantly better TQ compared to controls. Enhanced turf performance was attributed to higher OA, lower ET rates, increased green leaf biomass, and higher TQ ratings. During the re-watering period, plants treated with a combination of GA, CK, and nitrogen (1 lb/1000 ft<sup>2</sup>) displayed somewhat better recovery compared to controls due to increased tiller density as well as an increased rate of tiller production and leaf elongation. The study will be repeated in 2011 to confirm the findings.

## **Evaluation of Bioenergy Characteristics of Fifty Switchgrass Clones on Prime and Marginal Soils in New Jersey**

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Switchgrass (*Panicum virgatum* L.) has been promoted as a model bioenergy species by the US Department of Energy (USDOE) and is currently being grown as a bioenergy crop in the US. Currently, the national strategy is to produce bioenergy crops on marginal cropland where there will be no competition with food production. Although perennial grasses such as switchgrasses are expected to be used as a biofuel crop on marginal land there has been little to no extensive research to evaluate their performance on marginal land. Biomass yield is considered a complex trait that is controlled by many genes and influenced by the environment. Extensive studies have been conducted to demonstrate that switchgrass cultivars vary widely in performance across different adaptation zones. Based on these data, it is likely that marginal land will affect the performance and biomass yields of switchgrass. Initial studies comparing switchgrass yields on marginal land vs. prime farmland found that performance across environments was not consistent and that the top performing entry is not the same on both soil types. These results indicate that breeding for biomass on marginal lands will require evaluation of breeding materials in those environments in order to successfully develop productive cultivars for marginal land use. This knowledge is critical both regionally and nationally for the successful development and use of biofuels nationwide to offset foreign oil dependency. The objectives of this study were to evaluate fifty clones of switchgrass for several traits including: lodging, anthracnose and rust resistance, tiller number, plant height, and biomass yield on three different soil types in NJ.

A total of three sites were utilized for this experiment, including two marginal sites and one prime site. The first marginal site (Somerset) was located in Somerset County, NJ and is a class IV Kleinsville shale. The second marginal site (Jackson) was located in Jackson, NJ and is a class V Evesboro sand. The prime farmland site (Freehold) was located in Freehold, NJ and is a class II Freehold sandy loam.

Fifty clones of switchgrass were identified from germplasm sources being evaluated in the Rutgers switchgrass breeding program. Five clones each from ten different populations representing both upland and lowland cultivars were vegetatively propagated and planted in the field to grow through the summer of 2008. This was done in order to produce enough vegetative clones to plant six replications in each of three locations (18 plants per clone, 900 plants total). These clones were planted in the three locations described above in June of 2009. All switchgrass clones received 60 kg N ha<sup>-1</sup> at establishment and in the spring of 2010. In 2010, data was collected on lodging, anthracnose disease (caused by the fungus *Colletotrichum navitas*), rust disease (caused by *Puccinia* spp.), tiller number, and plant height. Biomass yields will be determined in January 2011.

A significant location by clone interaction was observed for all traits evaluated. Significant differences were observed between switchgrass clones for lodging, anthracnose and rust resistance, tiller number, and plant height. Overall, clones exhibited less lodging at the two marginal sites. This could be due to the fact that the plants were shorter at the marginal sites than the prime site. For example at Somerset the tallest clone had a mean height of 174.2 cm (NSL 5), at Jackson 225.2 cm (Kanlow 3), and at Freehold the tallest clone had a mean height of 280.2 cm (NSL 5). Clones showed similar relative levels of anthracnose resistance across all three locations. Timber clones 1 and 4 were among the top 10 performing clones at each location, while Brooklyn clones 1 and 5 showed poor resistance to anthracnose at all locations. Rust resistance data was similar to anthracnose data in that several clones showed similar relative levels of resistance across all locations. Clones Timber 1 and NSL 4 were among the top 10 performing clones for rust resistance at all locations, while Brooklyn 2 and Cimarron 5 were among the worst performing clones at all locations. The Somerset location had the lowest overall incidence of rust, with mean ratings ranging from 9.3 to 6.8. Overall, clones at the prime site (Freehold) showed the highest number of tillers while clones at Jackson had the lowest tiller numbers. Again, several clones showed similar relative performance across all locations. Clones NSU 4, Alamo 1 and 4, Timber 1, and NSL 2 were all among the top 10 clones for tiller number while NSU 1 had the least number of tillers at all three locations. Data for several traits evaluated seems to indicate that while soil types may affect plant performance, relative levels of performance tend to be similar across all three soil types included in this study. This information will be important for determining breeding strategies for switchgrass improvement on marginal land. Data will continue to be taken in 2011 and heritability estimates will be calculated in order to determine the full effects of genotype by environment interactions on the traits evaluated in this study.

**Evaluation of Fine Fescues and Kentucky Bluegrass Response to Wear  
During the Summer of 2010 in Freehold, New Jersey**

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Fine fescue (*Festuca* spp.) and Kentucky bluegrass (*Poa pratensis* L.) are two common turfgrasses in the northern United States. While Kentucky bluegrass is much more widely used, fine fescue is a niche grass that can provide a strong turf stand in areas which, due to environmental conditions, are unable to support Kentucky bluegrass turfs. While these two grasses often are used in different environments, the goal of the use of both is to provide a quality turf. In order to meet this goal, both of these grasses must withstand numerous stresses. In many instances one of these stresses is wear.

In the summer of 2010 at the Adelpia Turfgrass Research Center, in Freehold, New Jersey, well established fields of experimental and commercial varieties of both fine fescue and Kentucky bluegrass were subjected to wear. To simulate this wear the Rutgers wear simulator was used. Over approximately 1 month the fine fescues were subjected to 10 passes with the Rutgers wear simulator while the Kentucky bluegrass was subjected to 30 passes over approximately 2 months. Evaluations of their responses to wear and their recovery from wear were recorded. In both the fine fescue and Kentucky bluegrass trials significant differences were found and in both cases there were experimental varieties that were found to perform better while being worn than numerous commercial cultivars. The top species of fine fescue were hard fescue (*F. Brevipila* R. Tracey), followed by Chewings fescue (*F. rubra* L. subsp. *Fallax* (Thuill.) Nyman). Creeping red fescues (*F. rubra* L. subsp. *rubra*) and (*F. rubra* L. var. *litoralis* Vasey ex Beal) were rated to have the lowest wear tolerance. Of the more than fifty commercial Kentucky bluegrass varieties evaluated in this study, Bewitched, Yankee, Mystique, and Argos were given the highest ratings after being worn.

## Biological Control of Black Cutworm on Golf Course Turf

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The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is one of the pests that damage turfgrass especially those established with bentgrass (*Agrostis* spp.) The black cutworm has multiple generations at the latitude of New Jersey and larvae occurring in these generations damage turfgrass in mid-May to late summer. Foraging birds attracted to black cutworm infested areas further disrupt the turf. Broad-spectrum insecticide (pyrethroids, trichlorfon, and carbaryl) application for the control of the black cutworm may adversely affect the natural enemies of the black cutworm and other pests. In the current project we studied biological control of black cutworm in turfgrass using the entomopathogenic nematodes.

Entomopathogenic nematodes are soil-dwelling insect parasitic nematodes that kill their hosts mainly due to their symbiotic bacteria that produce toxins against insects. These nematodes can be mass-reproduced and hence are available commercially. In a series of laboratory trials we first evaluated the virulence of several commercial nematode products against different black cutworm instars. Then efficacy of the highly virulent nematode products was tested under field conditions.

Results from laboratory experiments show that up to 100% control of fourth and fifth instars can be attained using *Steinernema carpocapsae* (Product name: Millenium®), *S. riobrave* (BioVector®), *Heterorhabditis bacteriophora* (Nemasys G®), or *H. megidis* (Nemasys H®). At 9/22 °C (night/day) or 19 °C (constant), temperatures similar to mid-May or late September, *S. feltiae* (Nemasys) was one of the most virulent products. Five field efficacy trials between June and September in 2009 and 2010 show that *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* can control up to 90% field-released black cutworm larvae. Compared to non-treated plots, the products prevented up to 85% of grass damage despite the extremely high density released in our experiments (10 fourth instars per 0.09 m<sup>2</sup>). *Steinernema carpocapsae* was the most consistent in its efficacy while efficacy of *H. bacteriophora* and *S. feltiae* varied in different field trials. In summary, nematode products can control black cutworm even at extremely high densities under average summer conditions. Under extremely warm conditions (highs consistently above 30 °C), nematodes product will not be effective. Unfortunately, the very heat-tolerant species *S. riobrave* was generally not very effective. However, even 90% control may not be good enough for black cutworm control on the greens of many golf courses and often not even on tees. Combination of two EPN species or sequential applications of lower rates may further improve nematode control but are unlikely to consistently reach the control levels of some of the presently available chemical insecticides. This study suggests that entomopathogenic nematode based products may be used for black cutworm control in turf with lower quality standards than exist on most golf course greens and offer a viable option for areas where use of chemical insecticides is restricted.

## **Anthracnose Severity on Annual Bluegrass Turf as Affected by Spring and Summer Topdressing**

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Anthracnose is a destructive disease of annual bluegrass [ABG; *Poa annua* L. f. *reptans* (Hauskn) T. Koyama] turf caused by the fungus *Colletotrichum cereale* Manns. Increased frequency and severity of this disease on putting greens over the last fifteen years has been attributed, in part, to stress-inducing management practices. Previous research has indicated that sand topdressing of ABG turf during the summer can reduce anthracnose severity but the effect of spring topdressing on this disease has not been examined. A field study was initiated in North Brunswick, NJ to evaluate the effect of spring topdressing (0, 1.2 and 2.4 L m<sup>-2</sup> applied as two split-applications on 20 April and 4 May 2009, and 14 and 28 April 2010) as well as the potential for this factor to interact with the effects of summer topdressing (0, 0.075, 0.15, 0.30 and 0.6 L m<sup>-2</sup> every 14-d from 1 June to 24 August 2009, and 24 May to mid-August 2010) on anthracnose severity. The trial used a 3 x 5 factorial arranged as a randomized complete block design with four replications and was conducted on ABG maintained at 3.2 mm on a Nixon sandy loam. Disease severity reached 10% on all treatments by early-August 2009 and mid-May 2010. Both spring and summer topdressing significantly reduced disease severity compared to no topdressing. Spring topdressing at 2.4 L m<sup>-2</sup> was more effective at suppressing disease than 1.2 L m<sup>-2</sup>. Generally, a summer topdressing rate of 0.30 L m<sup>-2</sup> every 14-d was required to reduce disease severity. The summer topdressing effect was more consistent at reducing disease than the spring topdressing effect in 2009, whereas spring topdressing was more consistent in 2010. Interaction data from both years suggest that an inverse relationship exists between total sand accumulation and anthracnose severity. Spring and summer topdressing can be used by turf managers as a tool to reduce anthracnose on ABG putting greens.

## **Genotypic Variations and Physiological Traits for Drought and Heat Tolerance in Creeping Bentgrass**

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Drought and heat are both major abiotic stresses, which often lead to decline in turf growth and quality in various grass species. Creeping bentgrass (*Agrostis stolonifera*), one of the widely-used species on golf courses, is sensitive to drought and heat stress. Improving tolerance to these abiotic stresses in creeping bentgrass is critically important for conserving resources and improving turf quality in water limiting and 'warm' climatic regions. The genetic variation is an invaluable source for selecting tolerant germplasm that can persist during stressful periods. The objectives of the study were to compare genetic variations in drought or heat tolerance among commercially available cultivars and to determine major physiological traits associated with the variations in either drought or heat tolerance. Eight cultivars of creeping bentgrass, 'Kingpin,' 'Tyee,' 'Shark,' '007,' 'Pro-a7,' 'Penncross,' 'Declaration,' and 'L-93,' were subjected to drought by withholding irrigation or heat stress in a growth chamber with elevated temperature (35 C). Several physiological measurements were made, including turf quality, leaf relative water content (RWC), leaf photochemical efficiency (Fv/Fm), chlorophyll content, leaf electrolyte leakage (EL), and content of malondialdehyde, a product of lipid peroxidation. Using these parameters cultivars were grouped into three groups of varying drought or heat tolerance of high, medium or low levels, using cluster analysis. 'Kingpin,' 'Tyee,' and 'Shark' consistently performed poorly under drought stress; 'Pro-a7,' 'Declaration' and '007' were the most tolerant. For heat tolerance, 'Declaration,' 'L93' and '007' performed the best while 'Kingpin' and 'Penncross' performed poorly under heat with the other cultivars being of intermediate tolerance. Drought tolerance in creeping bentgrass was mainly associated with the ability of leaves to maintain cellular hydration or water retention and membrane stability whereas heat tolerance was closely related to the leaf senescence or stay-green trait. Those physiological parameters closely correlated to overall turf performance under drought or heat stress could be used as selection criteria for breeding stress tolerant germplasms.

## Germinating Perennial Ryegrass Under Saline Conditions

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As water restrictions for golf courses continue to tighten, there is a need for identifying alternative water sources for irrigation. Though there are different solutions, effluent (waste) water is a likely choice for use on turfgrass sites. Many golf courses have made the switch to irrigate with effluent water in the past decade, either voluntarily or through water regulations, in order to keep their turf green and playable. Effluent water comes with an array of challenges including higher than normal salt concentrations. These salts can build up in the soil and become detrimental to plant growth causing drought-like conditions. This poses a problem for superintendents and introduces the need for salt tolerant turfgrass. This study reports on a screening technique for evaluating germination of turfgrass plants under saline conditions.

Perennial ryegrass (*Lolium perenne* L.) is an important turfgrass species due to its ability to germinate quickly. Because of this quality, this species of turfgrass is used extensively for overseeding dormant warm-season turfgrass on golf courses. Interestingly, many of the golf courses that do overseed are also those using alternative water sources including golf courses in Nevada and Arizona. Irrigating perennial ryegrass with saline water has been shown to reduce both establishment and germination. As more golf courses switch to effluent water, the need for perennial ryegrass with improved germination under saline conditions increases.

Eight cultivars or selections (Apple GL, KSA, Palmer III, Zoom, Linn, Paragon GLR, ESP, RKS) of perennial ryegrass were evaluated under seven salinity treatments. The treatments were as follows: Treatment 1 (control):  $0.25 \text{ dS m}^{-1}$ , Treatment 2:  $1.0 \text{ dS m}^{-1}$ , Treatment 3:  $2.0 \text{ dS m}^{-1}$ , Treatment 4:  $3.0 \text{ dS m}^{-1}$ , Treatment 5:  $4.0 \text{ dS m}^{-1}$ , Treatment 6:  $5.0 \text{ dS m}^{-1}$ , Treatment 7:  $6.0 \text{ dS m}^{-1}$ . Salinity concentrations were made using equal quantities of NaCl and CaCl<sub>2</sub> mixed with tap water. Three replicates of each cultivar within each salinity treatment were arranged in a completely random design. Four hundred Watt high pressure sodium supplemental lighting was used in the greenhouse under 14-hour day lengths throughout the 6-week study. Temperatures were maintained between 17 and 24°C. Sterilized soil from the Plant Biology Research and Extension Farm in Freehold, NJ was weighed and equal quantities were put into 4x4 inch plastic horticulture pots obtained from Griffin Greenhouse & Nursery Supplies, Ewing, NJ. Turfgrass plants were planted at a seeding rate of  $7.44 \text{ g/m}^2$ . Fertilizer was applied at a rate of  $1.49 \text{ g}$  of nitrogen/ $\text{m}^2$ . Four hundred ml/ $\text{m}^2$  of each saline water treatment was applied to each pot daily in the beginning of the experiment and reduced to two or three times per week once seeds germinated. Soil in pots were maintained at saturation. Saline water treatments were applied using a trigger sprayer (Spray Master, New Brunswick, New Jersey). The trigger sprayer simulates overhead irrigation that would be applied on a golf course. To quantify differences between cultivars and treatments, two visual ratings and digital images analyzed with SigmaScan were used. Visual ratings of seedling emergence were evaluated on a one to nine scale: one being extremely poor seedling emergence, nine being the highest seedling emergence observed). Visual ratings of percent green were also collected. Digital image analysis was also used to calculate percent green of perennial ryegrass in the 4x4 pots. Ratings were taken weekly for the

six week study. Visual ratings of seedling emergence and percent green were reported as a percentage of the control to eliminate genetic differences and seedlot effects on germination.

Seedling emergence and percent green ratings are reported for the first three weeks. At the present time, only three weeks of data have been collected and further data collection is currently underway. Preliminary results indicated that there was a significant difference between treatments (across all cultivars) and between cultivars (across all treatments) for percent green and seedling emergence. However in the initial three weeks of the experiment there were no differences between cultivars within salinity treatments. Increased salinity treatments caused a reduction in seedling emergence and visual percent green of all perennial ryegrass cultivars and selections with treatment seven (6 ds/M) exhibiting approximately 65% reduction in seedling emergence and visual percent green compared the control. This study will continue for three more weeks and final results will be reported. This study will also be repeated again in January to confirm results of the first experiment.

## **Progress Identifying New Hazelnut Germplasm Expressing Resistance to Eastern Filbert Blight**

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Eastern Filbert Blight (EFB) is a destructive fungal disease of hazelnuts caused by *Anisogramma anomala* (Peck) E. Müller. It is harbored by the wild North American hazel, *Corylus americana*, which is generally tolerant of the disease although not grown commercially due to its small-sized nuts. Most hazelnuts (filberts) grown for nut production and as ornamentals are the European species, *Corylus avellana*, which are typically very susceptible to EFB. In the past, only a small number of EFB-resistant *C. avellana* genotypes have been identified, with a large germplasm pool yet to be tested. At Rutgers, we have been collecting hazelnuts from new and diverse populations in Eastern and Northern Europe, Russia, and Central Asia since the inception of the project in 1996 by turfgrass breeder Dr. C. Reed Funk. As such, we have amassed a large body of germplasm (over 6,000 foreign accessions), while also developing improved methodologies to screen plants for genetic resistance to EFB. Searching for resistance in *C. avellana* for use in breeding shows great promise, as this species has the best nut production traits within the genus. Identifying EFB-resistant plants that produce acceptable nuts will greatly expedite the breeding process by reducing the number of generations needed to develop commercially acceptable, EFB-resistant cultivars adapted to the northeastern U.S., especially when compared to utilizing wild species in breeding. As a result of our work, we have identified over 45 individual *C. avellana* plants spanning more than fifteen distinct populations in Russia, Ukraine, Turkey, and Poland that express a high level of tolerance or complete resistance to EFB. Several of the plants from the earliest collections were found to produce high yields of medium to large size nuts with relatively high quality kernels. As such, they have been used as parents in our breeding efforts and genetic studies. Preliminary disease segregation results from our most promising selection, H3R13P40 of Russian origin, shows resistance is transmitted to offspring in a one resistant to one susceptible ratio, which is indicative of control by single dominant gene in a heterozygous state. These and other results bolster our desire to evaluate additional collections from new regions. To this end, we obtained a diverse collection of *C. avellana* seeds from the Republic of Georgia in 2009 and from the Napoli region of Italy in 2010 (both previously untested in NJ), and have begun to apply our protocol for rapid assessment of these valuable collections. In addition to evaluating discovered resistant plants for their nut characteristics (size, kernel percent, kernel yield, etc.), we are also very interested in understanding the genetic diversity present within and between collection locations and known sources of resistance, with particular interest in the relationships between possible EFB-resistance genes. In addition to our ongoing breeding evaluations, our goals for 2011 include characterization of our new resistant selections using microsatellite (SSR) markers to explore their relatedness and genetic diversity. This work will allow us to better understand the host-pathogen relationship and will provide support for our targeted use of a wide spectrum of resistance genes in our hazelnut genetic improvement program.

## **Dimeric Oligonucleotide Probes Enhance Diagnostic Macroarray Performance**

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Disease management demands fast and early pathogen detection and identification techniques. Here we describe the development of a macroarray diagnostic technique with enhanced sensitivity of detection and small sacrifice on specificity. With probes designed based on the internal transcribed spacer sequences of the rRNA genes of fungal and oomycete strains, we produced a macroarray, which included five types of probes: monomeric oligonucleotide probes (20-24 nt), dimers (40-48 nt), dimers with a poly-A spacer between the two repeats (50-58 nt), monomers with a poly-A tail of 10 bases (30-34 nt) and another of 20 bases (40-44 nt). The use of repeat sequence probes (dimers) remarkably improved the sensitivity of the macroarray. The dimeric probes could reliably detect 0.01 fg target genomic DNA, which is lower than the detection limits of most currently available molecular diagnostic methods, such as the conventional PCR and real-time PCR. Dimer probes also had lower signal variability, thereby increased the macroarray signal uniformity. However, in a few cases, specificity was compromised in dimer probes. Cross-hybridization occurred in highly similar sequences where the mismatch base was located near the end or in a chain of same base, which can be prevented in future array probe design.

## Comparing the Rutgers Wear Simulator and Cady Traffic Simulator

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Researchers have developed various simulators to impart wear or the combined stresses of wear and compaction on turfgrass. Wear simulators, including the Rutgers Wear Simulator (RWS) (Bonos et al., 2001), are designed to affect aboveground plant parts such as leaves, stems, and shoots and cause minimal soil compaction. Traffic simulators are typically designed to impart both wear and soil compaction similar to those stresses resulting from sports field play with cleated shoes. The Cady Traffic Simulator (CTS) was developed by Henderson et al. (2005) and is a recent example of a machine that imparts traffic on turf trials. The objective of this study was to compare the injury to Kentucky bluegrass (*Poa pratensis* L.) caused by the RWS and CTS.

A modified CTS was developed using a Toro Greens Aerifier (The Toro Co., Bloomington, MN). “Feet” were constructed according to specifications authored by Henderson et al. (2005). The RWS was an upgraded version of the machine described by Bonos et al. (2001). Rubber paddles were mounted on a Toro landscape mower that provided control of forward operating velocity. This modified version of the RWS was also equipped with a hydraulic control of the paddle rpm.

The trial area consisted of a 5-yr-old stand of ‘Midnight II’ Kentucky bluegrass established on a well-drained loam in North Brunswick, NJ. Thirty-six passes of the RWS and CTS were operated across two separate lanes consisting of four 1.5 x 1.2-m plots (reps) per lane on 16 July 2010. Every other passes was made in the opposite direction. The swaths of the RWS and CTS were 0.84- and 0.67-m, respectively. The RWS was operated at 4.0 km hr<sup>-1</sup> and the paddles rotated at 250 rpm. The CTS was operated in the forward direction at 1.6 km hr<sup>-1</sup>.

Visual assessments of turfgrass quality (1-9 scale; 9=most dense, non-bruised turf canopy) were made prior to simulator operation and after every four passes. Turfgrass quality was also assessed at 13, 24, and 33 days after simulation (DAS).

Digital images of plots were obtained with a Nikon CoolPix5000 (Nikon Inc., Melville, NY) camera in a box equipped with artificial lighting. The camera was mounted 0.53-m above the bottom of the box (length: 0.61-m; height: 0.56-m; width: 0.51-m). Camera settings included a shutter speed of 1/60 s, an aperture of F3.2, and ISO of 100 and an automatic focal length. Digital images captured 0.15 m<sup>2</sup> of each plot area and individual image size was 2560 x 1920 pixels. Images were taken from the same position in the plot for each image. Digital images were taken before simulator operation, after every fourth pass, and then 3, 13, 21, and 33 DAS. Images were imported into SigmaScan Pro (v. 5.0, SPSS, Inc., Chicago, IL) for digital image analysis (DIA). Percent green cover was determined according to methods described by Richardson et al. (2000) using batch analysis programming developed by Karcher and Richardson (2005). A hue range of 50

to 107 and a saturation range of 0 to 100 were used in the software to identify green leaves in the images.

Data were subjected to analysis of variance and an F test ( $p \leq 0.05$ ) was used to determine differences between CTS and RWS means at individual rating dates.

No differences in percent green cover (as measured by DIA) were apparent between the CTS and RWS prior to simulation or after 4 passes. However, greater turf damage from the RWS compared to the CTS was evident after 8, 12, 16, 20, 24, 28, 32, and 36 passes. The percentage decline in green cover was only <1% after 36 passes with the CTS; whereas the RWS reduced the percentage of green cover by 35% after 36 passes.

No differences in turfgrass quality were detected between the RWS and CTS prior to simulation or after 4, 8, 12, 16, 20, 24, 28, 32, and 36 passes. However, turfgrass quality was reduced from 9.0 to 6.8 after 36 passes of the CTS. Similarly, the RWS reduced turfgrass quality from 9.0 to 6.5 after 36 passes.

The discrepancy between DIA-measured percent green cover and turfgrass quality data from 0 and 36 passes of the CTS was attributed to the type of damage caused by each simulator. The rotating paddle action of the RWS resulted in the physical removal of leaf tissue from plots, while the CTS flattened the leaf canopy. Hence, visual ratings of turfgrass quality were able to detect the gradual thinning of the canopy (fewer upright leaves) caused by the CTS; whereas, DIA was not able to distinguish between the upright and decumbent green leaf tissue during wear and traffic simulation.

During recovery, the DIA-measured percentage green leaf cover was lower in RWS plots compared to the CTS plots at 3, 13, 21, and 33 DAS. Similarly, turfgrass quality was lower in RWS plots at 13 and 24 DAS; however there was no difference in turfgrass quality at 33 DAS between the two simulators.

Thus, our initial assessment of these two machines indicates that the RWS resulted in more rapid and greater initial injury to the turfgrass canopy than the CTS. Additionally, recovery from RWS injury was slower compared to injury caused by the CTS. This recovery difference was probably due to, at least in part, the greater initial level of damage caused by the RWS. Research in 2011 will study changes in soil physical properties and surface hardness caused by the two machines as well as effects on the turf characteristics of perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.).

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**Characterization of Type IV Pilus Function in the Bacterial Biocontrol Agent  
*Lysobacter enzymogenes* Strain C3**

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The microbial antagonist and plant-associated bacterium, *Lysobacter enzymogenes*, is capable of controlling a number of turfgrass diseases, including summer patch caused by *Magnaporthe poae*, grey leaf spot caused by *M. oryzae*, and leaf spot caused by *Bipolaris sorokiniana*. *L. enzymogenes* is also known to establish pathogenic interactions with a broad range of microbial eukaryotic hosts, including many fungal plant pathogens. We have been investigating the interaction between *L. enzymogenes* and fungal hosts to better understand the molecular basis of pathogenesis and improve biological approaches for turfgrass disease control. Type IV pili (T4P) are known to contribute to a variety of roles in bacteria, including gliding motility, extracellular polysaccharide (EPS) production, polar attachment to hosts and pathogenesis. Genes encoding for the biogenesis T4P have been identified in six unlinked *pil* loci within the genome of *L. enzymogenes* strain C3. We have taken a mutagenesis approach to evaluate the role of T4P in various functions in *L. enzymogenes* C3. Strains containing mutations within the major pilin subunit *pilA* gene or the secretin *pilQ* gene differed in gliding motility and EPS production compared with the wildtype strain. These mutant strains also differed from the wildtype strain during interactions with fungal hosts, in which they were no longer capable of polar attachment to host cell hyphae. These results provide evidence that the T4P contributes a key functional role during initial stages of *Lysobacter*/fungal host interactions and biocontrol of turfgrass diseases.

### **Immunoblot Screening for Presence of *Neotyphodium* spp. in *Festuca* spp.**

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We have screened tillers and seeds from *Festuca* spp. plants for the presence of endophyte (*Neotyphodium* spp.) using an immunoblot kit from Agrinostics, Ltd. Co. (Watkinsville, GA, USA). This kit is a solid phase stacked immunoblot assay in which monoclonal antibodies generated to *Neotyphodium* spp. cell wall proteins will react to *Neotyphodium* spp. proteins present in *Festuca* spp. tillers and seeds. The limit of detection of *Neotyphodium* spp. in seed is 50 ng/seed and in tiller it is 50 ng/ 1.6 mm tiller cross section. Immunoblot screening is a more rapid and accurate technique for *Neotyphodium* identification compared to microscopy.

We have screened over 1100 plants from a collection of *Festuca* spp. from the Atlas Mountain region of Morocco and from crossing blocks located at the Rutgers Plant Science Research and Extension Farm in Adelphia, NJ. Eighty % of seeds obtained from the Morocco collection were found to be endophyte positive (E+) and 20% were endophyte negative (E-), whereas only 6.4% of seeds from the forage tall fescue crossing blocks in Adelphia, NJ were E+. Seeds from the strong creeping red fescue crossing blocks in Adelphia, NJ were 51% E+, whereas 88% of seeds from plants from the tall fescue crossing blocks located in Adelphia, NJ were E+.

Plants from the tall fescue breeding program that were selected for rhizomatous growth habit were tested for the presence of endophyte. Tillers from 64 plants were all found to be E- when sampled during the summer 2010 under severe drought and heat stress. When some of these plants were retested after transplanting to the greenhouse in the fall, they were found to be E+, indicating that under adverse abiotic conditions, *Neotyphodium* spp. proteins are present at levels less than 50 ng/ 1.6 mm tiller cross section and therefore not detectable.

Endophytes are well-known to improve environmental and biological stress resistance in grasses, but some produce alkaloids that are detrimental to the health of forage animals. A rapid and accurate method for endophyte screening is critical for selecting plant material that is E+ for turfgrass breeding programs but E- for pasture breeding programs.

## **Performance of Tall Fescue Cultivars in Turfgrass Trials for Brown Patch Disease Resistance in 2010**

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Tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh. is a prominent cool season grass, grown widely in United States and used in athletic fields, golf course fairways, roughs and home lawns. During warm and humid climate, tall fescues become hosts of fungus *Rhizoctonia solani* Kühn, a causal agent of brown patch disease. Breeding methods for developing resistant cultivars is an effective way to control the disease. At Adelphia Turfgrass Research Station, Rutgers University, the breeding program has continued to develop brown patch resistant and improved tall fescue cultivars. Presently, thousands of germplasm have been gone through the cycles of selection and hybridization for the improvement. There is no completely brown patch disease resistant cultivar released but attempts are going on to develop cultivars which have improved resistance and high turf quality (viz. canopy density, uniformity, dark green color, growth habit and fine leaf texture).

Four Tall Fescue tests were established between 2006 and 2009 at Adelphia Research Station, Rutgers University. There are 300, 170, 176 and 169 entries respectively in each trial. In the 2006 National Tall Fescue test, the entries such as LS 1200, Colchise IV, Jamboree, Turbo, Bonanza II, Wolfpack II, Mustang 4, Titanium LS, Faith, Cannavaro, RK4, RK5, Hemi, Traverse SRP and Braveheart performed consistently well for Brown Patch disease resistance. In 2007 National Tall Fescue Test: BC3 Comp, BIZM Comp, Falcon V, IS-TF 177, PST-Syn-5A47, Apache III, FCE3 BS, IS-TF 155 and Wolfpack were exhibiting least disease among entries. In 2008 National Tall Fescue Test: IS-TF-177, IS-TF-178, Side Winder, PO1 Comp, Falcon V, PSG 8SP1, RK4, Speedway, Rocket and Essential displayed higher disease resistance. For 2009 National Tall Fescue Test: PST-5AWT-08, TF- 196, TF-225, F9E Comp, PST-5AWT-08, B31 Comp, PST-5MCD, LW-BS and PPG-TF-104 had high brown patch resistance. These varieties also displayed improved turf quality.

## Effect of High N Rate Fertilization on Anthracnose Severity of Annual Bluegrass Turf

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Anthracnose, caused by *Colletotrichum cereale* Manns, is a destructive disease of annual bluegrass [ABG; *Poa annua* L. f. *reptans* (Hauskn) T. Koyama] putting green turf that is exacerbated by inadequate or excessive fertilization. Previous work has indicated that N applied at 4.9 kg ha<sup>-1</sup> every 7-d or 9.8 kg ha<sup>-1</sup> every 14-d reduces anthracnose severity compared to lower N rates, but less is understood about the impact of higher N rates on this disease. A field study initiated in North Brunswick, NJ assessed the effect of high rates of soluble-N fertilization during the summer on anthracnose severity of ABG turf maintained at 3.2 mm. N-fertilizer (NH<sub>4</sub>NO<sub>3</sub>) treatments, arranged as a randomized complete block design with 4 replications, were applied at 4.9, 9.8, 14.7, 19.6 and 24.5 kg ha<sup>-1</sup> as a spray solution and irrigated. Each N treatment was applied every 7-d from 3 to 30 June and 23 July to 13 August 2009; the period of non-application (30 June and 23 July) was due to a desire to avoid excessive growth when disease development was delayed. In 2010, weekly treatments were initiated on 24 May and continued through 11 August. Initially, N applied at 4.9 kg ha<sup>-1</sup> every 7-d had the greatest disease severity within the range of treatments studied. As the season progressed each year, increased disease severity was observed in plots receiving the two highest N rates. In both years, the greatest anthracnose severity was observed in plots receiving N at 19.6 and 29.4 kg ha<sup>-1</sup> every 7-d while the least amount of disease was observed in plots receiving N at 9.8 kg ha<sup>-1</sup> every 7-d. The disease response (area under the disease progress curve) to total N applied during the summer was quadratic, with the greatest reduction in anthracnose severity observed at 125 and 175 kg ha<sup>-1</sup> of total N in 2009 and 2010, respectively. Results indicate that inadequate and excessive N fertilization intensifies anthracnose severity on ABG putting green turf.

## **Comparison of Switchgrass Stand Establishment in Marginal vs. Prime Farmland in Seven States**

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Switchgrass (*Panicum virgatum*) is a warm-season perennial grass native to North America. This species has several desirable attributes for biomass production: it has the potential for high biomass yield with low agrochemical input compared to other herbaceous crops; it has great adaptability to different environments; it has a positive influence on soil and water conservation, and it has high carbon sequestering characteristics. These characteristics make switchgrass a major candidate for sustainable biomass production on marginal land. However switchgrass establishment is difficult in adverse environmental and soil conditions due to seed dormancy, seedling morphology and weed competition. The majority of the breeding research to improve establishment and other important agronomic traits of switchgrass has been conducted on research facilities in high performing environments (i.e. prime farmland). Few studies have investigated the effects of marginal soil on stand establishment of switchgrass.

The objectives of this research study were to 1) compare the performance and establishment rates of switchgrass cultivars on marginal vs. prime farmland in seven states and 2) to identify switchgrass cultivars with a broad adaptation across regions and superior performance on specific marginal sites. Fourteen switchgrass cultivars and selections, representing both upland and lowland types, were evaluated. The cultivars were seeded in paired field trials (marginal vs. prime farmland) in NY, WI, and SD in 2008 and in NJ, PA, OH, and MD in 2009.

The results of this study indicate that there were significant differences in switchgrass establishment among locations. Establishment rate was better in prime sites compared to marginal sites in NJ and SD, but not in NY, MD, PA, and WI. There was no significant difference in establishment between prime and marginal sites in MD, PA and WI. Switchgrass cultivars established better in the marginal site compared to the prime site in NY. Central upland cultivars Blackwell and Pathfinder and the northern upland cultivar Summer had the best establishment across states. High Tide had the poorest establishment among the central upland cultivars (47.8%) across states. Southern lowland cultivars exhibited the poorest establishment (43-55%) across locations. This may be due to the northern locations of the trial sites.

We concluded that establishment between prime vs. marginal farmland varied by location. In general, upland cultivars performed better than lowland cultivars across locations. Biomass and agronomic data will be collected in upcoming years to determine the effects of marginal soil on biomass yield switchgrass performance. We plan to use this information to identify switchgrass cultivars with improved performance on marginal land.

## Horizontal Transmission of *Neotyphodium* Endophytes of Grasses

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For over 100 years it has eluded researchers how *Epichloë* and *Neotyphodium* endophytes are horizontally transmitted. It is commonly believed that *Neotyphodium* endophytes are limited to internal tissues of grass plants, including culms, leaves, ovules, and seeds – and only spread through seed of the host. However, several studies have shown that some *Epichloë* and *Neotyphodium* species produce epiphyllous structures, *i.e.*, mycelial networks with conidiogenous cells and conidia, growing mostly on leaf blades of host plants. The production of epiphyllous conidia suggests the possibility that some of these endophytes may have the capacity for plant-to-plant spread using surface produced conidia. These conidia are water transmitted and germinate to produce mycelium. Therefore, vertical transmission of these endophytes may not be the exclusive mode of transmission. The objective of this research was to demonstrate that germinating conidia of *Neotyphodium* can colonize and infect endophyte-free seedlings of grass. Using conidia of *Neotyphodium* and endophyte-free seeds of *Poa ampla* we show that when germinating seeds and developing seedlings of the plant were exposed to germinating conidia, 28-56% of seedlings became infected by the endophyte. Our results demonstrate that the assumption that endophytes are “trapped” within plant hosts is incorrect as endophytes may spread to uninfected grass hosts by conidia formed on the surfaces of plants.

## **Length of Residual *Poa annua* L. Control of Mesotrione Relative to Other Preemergent Weed Control Products**

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Field studies were conducted in the fall of 2009 and 2010 to evaluate the length of residual weed control of *Poa annua* on bare soil. Herbicides were applied to bare soil (Freehold sandy-loam with pH 6.4 and 2% organic matter). The compounds under evaluation are as follows: mesotrione at 0.25 and 0.38 lb ai/acre, dithiopyr at 0.25 and 0.38 lb ai/acre, prodiamine at 0.5 and 0.65 lb ai/acre, and bensulide at 8.3 and 10.5 lb ai/a. All applications were made using a single 9504E nozzle CO<sub>2</sub> pressured sprayer calibrated to deliver 40 gallons/acre at 30 psi. The bare ground trials were visually evaluated at 4 and 8 weeks after treatment (WAT) and the following spring for *Poa annua* control. The plots were evaluated for percent control and percent cover of *Poa annua*, both on a scale of 0 (no control or cover) to 100 (complete control or cover). Annual broadleaf weeds were evaluated also at 4 WAT. Control of *Poa annua* ranged from 70% to 90% between the four compounds examined. Mesotrione was the only compound which showed significant difference between rates applied, 70% and 86%, respectively. All other compounds provided between 79% and 90% control. In springtime, mesotrione was found to only provide 31% to 44% control of *Poa annua* as opposed to the 81% to 85% provided by dithiopyr, 81% to 89% provided by prodiamine and 82% to 85% provided by bensulide. Annual broadleaf weeds, *Stellaria media* (Common Chickweed) and *Lamium amplexicaule* (Henbit), were evaluated at 4 WAE. Mesotrione exhibited 99% control at both rates. The result of one year of research suggests that the broad spectrum herbicides (dithiopyr, prodiamine, and bensulide) control *Poa annua* better than mesotrione as demonstrated by decreased control of mesotrione over time.

## **Molecular Characterization of ER-stress Response Pathway as a Novel Target for Development of Compounds for Stress Tolerance Induction in Grasses**

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Drought and heat stresses are important environmental factors that can impact on turf quality and measures that seek to minimize them can be significant cost centers for turf maintenance. Abiotic stresses such as drought and heat have been shown to trigger programmed cell death (PCD) in plant cells to help plants sense and respond to these environmental challenges. Recent genetic evidence from studies of PCD mechanisms suggests the involvement of the Endoplasmic Reticulum (ER)-stress response pathway as a highly conserved integrator of diverse types of cellular stress. When cellular homeostasis is out of balance, accumulation of unfolded proteins results in activation of the unfolded protein response (UPR) which induces the expression of proteins such as molecular chaperones that can help maintain proper protein folding. However, when the threshold of misfolded proteins reached a certain level, PCD is induced. Thus, the level of misfolded proteins in the ER may be a conserved signal that can activate PCD. The observation that chemical chaperones such as Tauroursodeoxycholic acid (TUDCA) and Sodium 4-phenylbutyrate (PBA) can alleviate ER stress suggests application of these agents may increase plant tolerance to certain stresses by suppressing the accumulation of unfolded proteins. In our project, we evaluated the effects of TUDCA and PBA on turfgrass (*Agrostis stolonifera* L.) growth behavior under drought and heat conditions. The drought tolerance of turfgrass is found to be significantly improved with TUDCA while the chemical chaperones' effects on heat tolerance are relatively minor. Moreover, our molecular analyses showed that the expression of a grass bZIP60 gene is up-regulated under ER stress induced by Tunicamycin (TM) treatment and this up-regulation can be alleviated with application of TUDCA. Our results thus indicate the stress tolerance in plants can be modulated by chemical control through modulation of the ER stress pathway, which provides an approach to improve turf quality under most environmental stress conditions.

**Identification of Proteins Regulated by a Plant Growth Regulator  
(Trinexapac-ethyl) and Responsive to Drought Stress in Kentucky Bluegrass**

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Drought is one of the major limiting factors of plant production worldwide. Growth inhibitors may influence plant responses to stresses. The objective of this study was to investigate effects of pretreatment with trinexapac-ethyl (TE) on protein profiles in Kentucky bluegrass (*Poa pratensis* L.) under drought stress. Plants of 'Baron' were treated with TE twice with 14 d interval, then subjected to drought stress by withholding water for 15 days in growth chambers. Application of TE increased relative water content, photochemical efficiency and photosynthetic rate, and reduced electrolyte leakage during drought stress. Leaf proteins were extracted and separated two-dimensional electrophoresis. Approximately seventy protein spots were differentially accumulated in response to drought stress. These protein spots were analyzed using mass spectrometry and most spots were identified. TE foliar application increased the abundance of ferritin, catalase, and glutathione-S-transferase, suggesting that preconditioned plants with TE were able to reduce the production of active oxygen species and maintain active antioxidant defense system against the oxidative stress, which may contribute to better drought tolerance. The improved drought tolerance in TE-treated plants may also be related to the up-regulation of HSP70 and chaperonin that are involved in the repair and refolding of drought-damaged proteins.

## **Membrane Fatty Acid Composition and Saturation Levels Associated with Leaf Dehydration Tolerance and Post-drought Rehydration in Kentucky Bluegrass**

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Changes in fatty acid composition and saturation levels may be involved in leaf tolerance to dehydration during drought stress and recovery upon re-watering. The objective of this study was to compare changes in compositional and saturation levels of leaf fatty acids between two cultivars of kentucky bluegrass (*Poa pratensis*) contrasting in drought tolerance in response to drought stress and re-watering. Drought-tolerant 'Midnight' and sensitive 'Brilliant' were maintained well-watered (control) or subjected to drought for 15 d by withholding irrigation and then re-watered in a growth chamber. Compared to 'Brilliant', 'Midnight' maintained higher turf quality, leaf photochemical efficiency, relative water content, and membrane stability expressed as electrolyte leakage during drought stress. Following re-watering, 'Midnight' recovered more rapidly in each parameter than 'Brilliant'. The degree of fatty acid unsaturation decreased in both cultivars during drought stress, and the decrease was less pronounced and occurred later in 'Midnight'. Fatty acid unsaturation level resumed to the control level in 'Midnight' leaves, but did not fully recover in 'Brilliant' after re-watering. The alteration in fatty acid unsaturation level induced by drought and re-watering were mainly due to the changes in the composition of linolenic acids (18:3), linoleic acids (18:2), palmitic acids (16:0) and stearic acids (18:0). Our results suggest that leaf dehydration tolerance and post-drought recovery in kentucky bluegrass was associated with their ability to maintain relative higher proportion and level of unsaturated fatty acids, particularly linolenic acids and linoleic acids.

## Assessing Fungicide Effects on Turfgrass Soil Fungal Communities

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Quinone outside inhibitors (QoI) and sterol demethylation inhibitors (DMI) are two widely used fungicides in plant disease management. In this study, their impact on soil fungal communities following application to *Poa pratensis* L. was investigated. Starting from May 26<sup>th</sup> 2009, azoxystrobin (0.046 g a.i./sq meter; a QoI) and fenarimol (0.15 g a.i./sq meter; a DMI) were applied to *Magnaporthe poae* infested *P. pratensis* plots in New Brunswick, New Jersey, four times every 21 days for azoxystrobin and three times every 28 days for fenarimol treatment. Soil and root samples were taken on September 15, 2009. Molecular cloning and PCR denaturing gradient gel electrophoresis (DGGE) were used to assess the fungal community structure. For samples without fungicide treatment, ascomycetes were dominant in both rhizosphere and bulk soil. The closest internal transcribed spacer sequence match of the dominant taxon was *Phoma medicaginis* Malbr. & Roum. Chytridiomycota formed the second largest group in the rhizosphere fungal community, while the Basidiomycota was the second largest group in the bulk soil. After fungicide treatment, the frequency of Basidiomycota increased in rhizosphere soil but decreased in bulk soil. Fenarimol affected turf soil fungal communities more than azoxystrobin.

## **Development and Application of a TaqMan Real-time PCR Assay for Rapid Detection of *Magnaporthe poae*, the Summer Patch Pathogen of Turfgrass**

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Turfgrasses are ubiquitous in the urban landscape, athletic fields, golf courses, and residential lawns and city parks. Approximately 1.9% (16.3 million hectares) of the total continental U. S. area is covered by turfgrass. One of the most important diseases of turfgrass in North America is summer patch that is caused by the root-infecting fungus *Magnaporthe poae* Landsch. & Jacks. The pathogen affects the roots, crowns and rhizomes of several cool-season grasses under favorable environmental conditions. Detection and identification of *M. poae* are notoriously difficult and time-consuming using the conventional culture-based method that usually takes three weeks or longer. In this study, a culture independent TaqMan real-time PCR assay has been developed for *M. poae* that enables pathogen detection from the field samples within a few hours. The assay was validated with the target pathogen, its closely related fungal species and a number of other microorganisms that inhabit the same host and soil with the target pathogen. This assay will facilitate effective and sustainable turfgrass management by early and accurate diagnosis of this important disease and will reduce pesticide inputs.

## **Proteomic Analysis of Kentucky Bluegrass Leaves During Drought Stress and Recovery**

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Protein metabolism plays critical roles in plant adaptation to drought stress. The objective of this study was to identify proteins that are involved in drought survival and rehydration and re-growth upon rewatering in Kentucky bluegrass (*Poa pratensis* L.). Two cultivars differing in drought resistance, 'Midnight' (tolerant) and 'Brilliant' (sensitive) were exposed to well-watered conditions, drought stress 15 d by withholding irrigation, and then re-watered for 6 d in growth chambers. Physiological analysis demonstrated that 'Midnight' was better at maintaining leaf hydration and photochemical activities under drought stress and regrow and was rehydrated faster upon re-watering, compared to 'Brilliant'. Out of about 850 protein spots were reproducibly detected by two-dimensional gel electrophoresis, with 83 protein spots exhibiting a significant change in the abundance level during drought stress and recovery. Under drought stress, more protein spots were up-regulated in 'Midnight' than in 'Brilliant'; or less protein spots were down-regulated in 'Midnight' than in 'Brilliant'. By 6 d after rewatering, most proteins had restored largely or completely to the expression level of well-watered control in 'Midnight', but fewer proteins exhibited recovery in 'Brilliant'. Functions of the differentially-expressed proteins in response to drought stress and recovery between the two cultivars will be discussed.



*Cooperating Agencies:* Rutgers, The State University of New Jersey, U.S. Department of Agriculture, and County Boards of Chosen Freeholders. Rutgers Cooperative Extension, a unit of the Rutgers New Jersey Agricultural Experiment Station, is an equal opportunity program provider and employer.