RUTGERS

New Jersey Agricultural
Experiment Station

Proceedings of the Twenty-First Annual Rutgers Turfgrass Symposium

January 6, 2012

Bruce B. Clarke, Director
William A. Meyer, Associate Director

The Center for Turfgrass Science

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Donald Kobayashi
Ning Zhang

Proceedings of the Twenty-First Annual Rutgers Turfgrass Symposium

Donald Kobayashi and Barbara Fitzgerald, Editors

Associate Director's Remarks:

Welcome to the twenty-first Annual Rutgers Turfgrass Symposium at the School of Environmental and Biological Sciences/NJAES, Rutgers University. This symposium was started 21 years ago as an annual meeting to update Center for Turfgrass Science members and stakeholders on current issues in turfgrass science. This year we are especially pleased to have Dr. Mark Sorrells, a professor of Plant Breeding and Genetics at Cornell University, as our keynote speaker. It is important to recognize the Organizing Committee of Drs. Ning Zhang, Bruce Clarke, Donald Kobayashi, Ms. Barbara Fitzgerald and myself. I would also like to thank Dr. Donald Kobayashi and Barbara Fitzgerald for doing an excellent job editing this year's Proceedings.

The faculty of the Turf Center continue to be recognized for excellence in research, teaching and extension. Dr. Bingru Huang was named a fellow of the prestigious American Association for the Advancement of Science (AAAS) and the Ralph Geiger Endowed Chair in Turfgrass Science. Dr. Stacy Bonos was promoted to Associate Professor with tenure and received the Rutgers University Board of Trustees Fellowship Award for Scholarly Excellence. Drs. Bonos, Clarke, Hillman, Koppenhöfer, Murphy and Zhang were the Rutgers faculty who received the Award for Excellence for Multi-State Research from the USDA. Dr. Bruce Clarke received the Award of Merit from the American Phytopathological Society – Northeast Division and the Visionary Leadership Award from the Epsilon Sigma Phi National Extension Society. I was fortunate to receive the Breeder's Cup Award for developing Mallard Kentucky bluegrass from the Turfgrass Breeders Association of the Crop Science Society of America. In addition our graduate students won several research awards at the annual meeting of the Crop Science Society of America in San Antonio, Texas in October. Lisa Beirn and Davis Jespersen received awards for their oral presentations and Emily Merewitz, Katelyn Venner, and James Cross for their poster presentations. In addition, James Hempfling was awarded 2nd place in the Graduate Student Oral Presentation Competition at the Northeast Division American Phytopathological Society meeting in New Brunswick.

The faculty and staff of the Turf Center continue to have major national and international impact in turfgrass science. Dr. James Murphy did an outstanding job this year leading the C-5 division of the Crop Science Society of America, and he represents the United States as a board member of the International Turfgrass Society (ITS). Dr. Clarke also serves on the board of ITS and will become President when the ITS meeting is held at Rutgers in 2017. Finally, over $90,000 in scholarships were awarded to our graduate and undergraduate students this past year by more than a dozen turf industry groups. We greatly appreciate our relationship with the turfgrass industry and look forward to working with them in 2012.

Thanks for participating in this year's symposium.

Sincerely,

W. A. Meyer

William A. Meyer
Associate Director
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Table of Contents

TWENTY-FIRST ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 6, 2012

Foran Hall, Room 138A

Friday, January 6, 2012

8:30 - 9:00 AM **Registration, Coffee and Donuts**

9:00 - 10:00 AM **SESSION I: PEST MANAGEMENT**
(Moderator: Dr. Albrecht Koppenhöfer)

9:00 – 9:20 **Dr. Olga Kostromytska** (Dept. of Entomology, Rutgers University) *Annual Bluegrass Weevil IPM: Plant Resistance/Tolerance and Semiochemicals for Monitoring and Management*

9:20 – 9:40 **Dr. Bruce B. Clarke** (Dept. of Plant Biology and Pathology, Rutgers University) *Improving Disease Control with Products that Induce Resistance in Turf*

9:40 – 10:00 **Dr. Donald Kobayashi** (Dept. of Plant Biology and Pathology, Rutgers University) *Novel Strategies for Biorational Approaches to Turfgrass Disease Control – A Genomics Perspective*

10:00 - 10:30 AM **Discussion and Coffee Break**

10:30 – 12:00 PM **SESSION II: TURF IMPROVEMENT AND BREEDING**
(Moderator: Dr. William A. Meyer)

10:30 – 10:50 **Dr. Faith Belanger** (Dept. of Plant Biology and Pathology, Rutgers University) *Effects of Fungal Endophyte on Host Gene Expression*

10:50 – 11:10 **Dr. Stacy Bonos** (Dept. of Plant Biology and Pathology, Rutgers University) *Improvement in Breeding for Disease Resistance in Bentgrass Using Classical and Molecular Approaches*

11:10 – 11:20 AM **Discussion session**

11:20 – 12:00 **Keynote: Dr. Mark Sorrells** (Department of Plant Breeding and Genetics, Cornell University) *Molecular Breeding Strategies for Plant Improvement*

12:00 - 1:00 PM **Lunch and Poster Session**

1:00 – 2:00 PM **SESSION III: PHYSIOLOGY AND MANAGEMENT**
(Moderator: Dr. Bingru Huang)

1:00 – 1:20 **Bradley Park** (Dept. of Plant Biology and Pathology, Rutgers University) *Traffic Tolerance of Tall Fescue From 2007 Through 2011*

1:20 – 1:40 **Emily Merewitz** (Dept. of Plant Biology and Pathology, Rutgers University) *Elevated Cytokinin Content in ipt-Transgenic Creeping Bentgrass Promotes Drought Tolerance Through Regulation of the Metabolite Profile*

1:40 – 2:00 **Thomas Gianfagna** (Dept. of Plant Biology and Pathology, Rutgers University) *Immunoblot Screening for the Presence of Neotyphodium spp. in Festuca spp.*

2:00 – 2:30 PM **Discussion and Coffee Break**

2:30 – 3:30 PM **SESSION IV: BIOLOGY AND SYSTEMATICS**
(Moderator: Dr. Barbara Zilinskas)

2:30 – 2:50 **Dr. Thomas Molnar** (Dept. of Plant Biology and Pathology, Rutgers University) *From Turf to Nuts: Where Have We Gone in 16 Years of Plant Breeding?*

2:50 – 3:10 **Lisa A. Beirn** (Dept. of Plant Biology and Pathology, Rutgers University) *What's in a Name? Determining the True Identity of the Dollar Spot Pathogen of Turfgrass*

3:10 – 3:30 **Dr. Ning Zhang** (Dept. of Plant Biology and Pathology, Rutgers University) *A Six-Gene Phylogeny Reveals the Evolution of Mode of Infection in the Rice Blast Fungus and Allied Species*

3:30 - 4:00 PM **Discussion, Closing Remarks and Poster Session**

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PLENARY PRESENTATIONS

Annual Bluegrass Weevil IPM: Plant Resistance/Tolerance and Semiochemicals for Monitoring and Management

Olga S. Kostromytska[1], Albrecht M. Koppenhöfer[1], Cesar Rodriguez-Saona[1], and Stacy A. Bonos[2]

[1] Department of Entomology and [2] Department of Plant Biology & Pathology, Rutgers University

The annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), is a severe pest of golf courses throughout the northeastern United States. Because of insecticide overuse and the resulting development of resistance, ABW management can no longer rely solely on chemical control. Practical and reliable monitoring tools and host plant resistance/tolerance are cornerstone strategies for the development of sustainable ABW management program. The objectives of this study are: (1) to investigate use of ABW semiochemicals for improved monitoring tools and as potential new management tools; and (2) to investigate plant host resistance and tolerance as a new sustainable ABW management option which will suppress ABW populations.

Plant Resistance/Tolerance

ABW damage is commonly reported from the areas with high *P. annua* percent. But whether *P. annua* is less tolerant to ABW feeding than bentgrasses or/and more preferred by weevils is not clear. Suppressing *P. annua* in favor of more tolerant/resistant grasses seems to be the best way to reduce problems with ABW. However, *P. annua* is extremely difficult to suppress in operating golf courses. Thus, it will be important to select bentgrasses that are not only more tolerant of ABW feeding but also poor hosts of ABW (i.e., resistant). Otherwise, ABW population could build up on the tolerant but not resistant turf and then damage adjacent *P. annua* patches or even the bentgrasses themselves.

Host plant resistance has two major components: plant tolerance to insect feeding and true resistance. Tolerant plants can support insect populations, but can compensate the feeding and not show significant damage. Resistant plants are detrimental to insect growth, development and reproduction through antixenosis (non preference) or antibiosis (adverse effect on insect biology).

The susceptibility of three bentgrass species (seven cultivars) to ABW in comparison with annual bluegrass was investigated in choice and no-choice laboratory oviposition experiments and greenhouse larval survival experiments. Behavioral bioassays clearly showed that ABW females prefer *P. annua* for oviposition in choice and no choice situation. Even though, physiological state of females seemed to be an important factor in host preference. The percentage of eggs laid in bentgrasses in choice tests increased from 12% with adults collected before spring dispersal to 34% with adults collected in spring from short-mown turf. Although no distinct preferences for oviposition were observed among bentgrass species/cultivars, females

tended to lay more eggs in the creeping bentgrass cvs. ‘Penncross’ and ‘L-93’ cvs. and the colonial bentgrass cv. ‘Capri’. Adult ABWs fed equally on all grass species tested. ABW developed from eggs to pupae on all bentgrass cultivars tested. Most cultivars (with the exception of ‘Villa’ and ‘Capri’) had lower larval density than *P. annua,* however the lowest larval density in this experiment (cvs. ‘L-93’, ‘Penncross’, ‘TigerII’) was higher than proposed damage threshold for ABW. The data suggested that all tested bentgrasses appeared to be more tolerant than *P. annua.*

Semiochemicals

Presently available ABW monitoring methods are ineffective or impractical and/or too labor intensive and hence rarely used. Plant and/or insect derived attractants can simplify and improve monitoring and already widely used for monitoring of many insect pests. Semiochemicals may also be used in combination with insecticides in the ‘attract-and-kill’ approach optimizing use of insecticides.

In choice experiments in which ABW antennae were blocked, no host preferences were observed, indicating that chemical cues play an important role in ABW host recognition. Females were attracted to *P. annua* plugs in Y-tube olfactometer assays and repelled by some creeping bentgrass cultivars. Thus, recent laboratory studies confirm that host plant volatiles are significant factors in ABW female behavior and potentially may be used as attractants for monitoring and/or management strategies as main lure compounds or at least as pheromone synergists.

Host plant volatiles and weevil pheromones were investigated as possible attractants for monitoring and management. Head space volatiles of males or females ABW with or without *P. annua* as a food source and volatiles were collected and processed in GC-MS analysis. No sex specific pheromones were detected. In the volatiles collected from males and females feeding on *P. annua* two compounds were tentatively identified as E-β-Ocimene and β Muurolene. Volatiles of six bentgrass cultivars and *P. annua* were collected and analyzed, compounds unique to *P. annua* identified. More studies are needed to determine effects of these compounds on ABW behavior.

Improving Disease Control with Products that Induce Resistance in Turf

Bruce B. Clarke

Department of Plant Biology and Pathology, Rutgers University

Induced resistance in plants is a physiological state in which environmental, chemical, or biological activators initiate a signal transduction pathway causing specific genes to be expressed and chemicals to be produced that protect plants from disease. Two of the most widely studied types of induced disease resistance in plants are systemic acquired resistance (SAR) and induced systemic resistance (ISR). Although they both induce disease resistance, each has a different signaling pathway; SAR stimulates the production of salicylic acid, while ISR causes an enhancement of jasmonic acid and ethylene.

SAR is induced when a pathogen, stress, or activator triggers the salicylic acid pathway releasing systemic signals in the plant that induce defense response genes and the production of antimicrobial compounds called pathogenesis-related proteins (PR proteins). Recent research has shown that a new turfgrass fungicide called Daconil Action (a premixed product sold by Syngenta Crop Protection containing the fungicide chlorothalonil and an SAR activator acibenzolar-S-methyl) can stimulate the production of PR proteins leading to enhanced suppression of diseases such as dollar spot (*Sclerotinia homoeocarpa*), anthracnose (*Colletotrichum cereale*), and Pythium blight (*Pythium* spp.) compared to chlorothalonil alone.

ISR responses are typically activated in advance of infection when non-pathogenic, root-associated microbes (e.g., rhizobacteria or fungi) or a chemical stimulus triggers the plant to release ethylene and jasmonic acid as internal signals. This results in the systemic priming of the plant so that resistance genes are quickly activated in response to subsequent invasion by a pathogen. Most examples of ISR activated by plant growth promoting rhizobacteria have been observed in dicots, but bacterial activated ISR has also been documented for several monocots including rice (*Oryza sativa*), pearl millet (*Pennisetum glaucum*), and creeping bentgrass (*Agrostis stolonifera*). Recently, ISR was found to be activated by a new fungicide called Civitas (a mixture of a food-grade synthetic isoparaffin and an emulsifier pigment marketed by Petro-Canada) resulting in the suppression to varying degrees of anthracnose, dollar spot, and pink and gray snow mold (*Microdochium nivale* and *Typhula* spp., respectively) compared to untreated turf.

This presentation will review the concept of enhancing a plant's natural defense against disease with the application of commercially available products that either have SAR or ISR activity. Non-target effects, such as phytotoxicity during high temperatures, and potential strategies for successfully utilizing SAR and ISR activators in the field will also be discussed.

Novel Strategies for Biorational Approaches to Turfgrass Disease Control – A Genomics Perspective

D. Kobayashi[1], N. Patel[1], N. Donofrio[2] and B. Hillman[1]

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The genome sequence of the bacterial biocontrol agent *Lysobacter enzymogenes* indicates the presence of several genes encoding pathogenicity mechanisms used by bacterial pathogens of animals and plants. There is strong supportive evidence that *L. enzymogenes* uses these mechanisms to establish unique pathogenic interactions with fungal plant pathogens. Many of these pathogenicity mechanisms, such as type III, type IV and type VI secretion systems, function to deliver virulence effector molecules into host cells. While it has yet to be established that *L. enzymogenes* delivers virulence effectors into fungal cells, it is well known that bacterial pathogens of plants and animals use such molecules to disrupt host defense responses to reduce host fitness and facilitate infection. Furthermore, although host defenses of animals and plants represent well studied systems, fungal host defense responses to pathogen attack are poorly understood.

To gain a better understanding of fungal host defenses in response to bacterial infection, the transcriptome of *Magnaporthe oryzae*, causal agent of rice blast and gray leaf spot of turf, during interactions with *L. enzymogenes* was determined using an RNA-Seq approach. Evaluation of *M. oryzae* whole genome expression patterns identified several genes altered in expression when the fungus was challenged with either a wildtype strain or one of several different pathogenicity mutant strains of *L. enzymogenes* compared with untreated fungus. Many of these genes are homologues of those involved in basal defense responses used by plants and animals to combat pathogen invasion. These results provide strong supportive evidence that *L. enzymogenes* uses effector molecules to alter fungal host defense responses. The implication of bacterial effectors and identification of fungal host defense genes open a new area of research with potential for new approaches to fungal disease control.

Effects of Fungal Endophyte on Host Gene Expression

Faith C. Belanger and Karen V. Ambrose

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It is well established that the *Neotyphodium* and *Epichloe* 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 factors contribute 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 are using a strong creeping red fescue (*Festuca rubra*) genotype that is endophyte-free and the same genotype that is infected with an endophyte originally from strong creeping red 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. Our analysis of quantitative differences between the endophyte-free and endophyte-infected samples has revealed hundreds of genes showing statistically significant moderate changes in gene expression. We have also found that a few fungal genes constitute an extremely high percentage of the overall fungal gene expression.

Improvements in Breeding for Disease Resistance in Bentgrass Using Classical and Molecular Approaches

Stacy A. Bonos, Eric N. Weibel, Tracy J. Lawson, Josh Honig, Martin Majewski, Eric Koch, Matthew Koch, and Laura Cortese

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Bentgrass species are particularly susceptible to a number of important diseases including dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Bennett), copper spot (caused by *Gleosercospora sorghi* D.C. Bain & Edgerton); anthracnose (caused by *Colletotrichum cereale* Manns sensu lato Crouch, Clarke and Hillman) and brown patch (caused by *Rhizoctonia solani* Kühn). One of the main goals of the Rutgers turfgrass breeding program is to identify genetic resistance to diseases and incorporate resistant sources of germplasm into new cultivars. The utilization of genetic resistance is a promising component of sound integrated disease management programs and can help reduce fungicide use.

Dollar spot resistance in creeping bentgrass (*Agrostis stolonifera* L.) has been the most researched host/pathogen system in the bentgrasses. Dollar spot resistance seems to be quantitatively inherited because: 1) a continuous distribution of phenotypes and transgressive segregation was observed in crosses between resistant and susceptible clones (Bonos et al., 2003); 2) gene number calculations ranged from 2-5; 3) it was affected by the environment (Bonos et al., 2003; Bonos, 2006); and 4) inheritance was most likely due to additive gene action (Bonos, 2011).

We recently initiated molecular marker studies to identify loci associated with dollar spot resistance in an effort to improve the efficiency of breeding efforts. In order to find molecular markers that could be incorporated into a traditional breeding program we first developed a PCR-based linkage map of creeping bentgrass using several types of molecular markers. Secondly, we identified four quantitative trait loci associated with dollar spot resistance. Most recently we conducted a confirmation study to see if the QTL markers identified in previous studies remain associated with dollar spot resistance.

For the QTL confirmation study, we evaluated 100 progeny from a cross between L93-10 (dollar spot resistant) and 7418-3 (dollar spot susceptible) in a randomized complete block design with three replications. The progeny and parents were inoculated with a virulent dollar spot isolate obtained from the University of Massachusetts (courtesy of Dr. Geunhwa Jung) and evaluated for dollar spot disease in 2009, 2010 and 2011. Approximately twelve molecular markers flanking each of the four putative QTL (a total of 50) were selected and tested for polymorphism by genotyping the 100 progeny using an ABI 3130 genetic analyzer and sized using Genemapper 3.7 software (Applied Biosystems). A one-way analysis of variance was conducted to determine the association of the marker genotype to the dollar spot phenotype.

Three markers were significantly associated with dollar spot resistance. Two of the markers were SSR markers (GT423(120) located on linkage group 4.1[7418-3] and GA1681F(203) located on linkage group 5.2[L93-10]) located in close proximity to two of the QTLs previously identified. The third marker, BRSC4_008N240, found in close proximity to a QTL on linkage group 1.1(L93-10), encodes for an Auxin Response Factor 8 (ARF 8) (Tian et al., 2004) which has been shown to activate defense related genes (Domingo et al., 2009). These three markers confirm the potential for using molecular markers for screening for dollar spot resistance in a plant breeding program to enhance selection of resistant germplasm.

Breeding bentgrasses for resistance to other diseases including anthracnose, copper spot and brown patch are in their infancy compared to breeding for dollar spot resistance. However, progress has been made in identifying germplasm sources not only with resistance to each disease but also with improved resistance to multiple diseases. Research will continue to identify additional sources of germplasm with improved resistance to important diseases and to incorporate molecular tools to improve the selection efficiency of the breeding program to maximize cultivar development.

References

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Molecular Breeding Strategies for Plant Improvement

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New selection methods and technologies are the basis for more efficient plant breeding strategies. Advancements in genotyping technologies are rapidly reducing marker costs and increasing genome coverage allowing the routine use of molecular markers for plant breeding. Knowledge of the level of genetic diversity and historical relationships among cultivated wheat germplasm can be effectively exploited for the assessment of genetic variation, association breeding, marker-assisted recurrent selection (MARS) and genomic selection (GS) for plant improvement.

Association breeding and MARS are based on the selection of statistically significant, marker-trait associations such as quantitative trait loci (QTL). Association breeding facilitates the discovery of novel alleles whose relative allelic value can be assessed as often as necessary. Complementary to MAS strategies that are best suited for simply inherited traits, is a new method called genomic selection (GS). GS can efficiently improve agronomically important, complex traits controlled by many genes. Genomic selection incorporates genome-wide marker information in a breeding value prediction model, thereby avoiding biased marker effect estimates and capturing more of the variation due to small effect QTL.

To use GS, a training population representative of the breeding germplasm is genotyped with genome-wide markers and extensively phenotyped in a target set of environments. That data is used to train a prediction model that is used to estimate the breeding values of lines in a population using only the marker scores of those individuals. Prediction models can incorporate performance over multiple environments, G x E effects, specific screening techniques, and novel traits. Because of reduced generation time, annual genetic gain for GS is predicted to be two to three fold greater than for a conventional phenotypic selection program, even with only a modest prediction accuracy of 0.50. This new approach to crop improvement will facilitate a better understanding of the dynamic genome processes that generate and maintain new genetic variation

Traffic Tolerance of Tall Fescue From 2007 Through 2011

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Tall fescue (*Festuca arundinacea* Schreb.) is well adapted to the transition zone and breeding efforts have produced newer cultivars with better turf quality that can be used for parks and sports fields. An assessment of the seasonal performance of these newer cultivars subjected to traffic stress would be useful. The objective of this study was to assess the traffic tolerance of tall fescue in spring, summer, and fall. The 113 entries comprising the 2006 National Turfgrass Evaluation Program Tall Fescue Test were established in September 2006 on a Nixon loam in North Brunswick, NJ. The test was mowed at 7.6-cm and irrigated to avoid severe drought stress during 2007 through 2010. No irrigation was applied in 2011. Twenty-four wear passes were applied over two to three days in April 2009 and May 2011 (Spring), October 2007 and 2009 (Fall), and July 2010 (summer). Sixteen wear passes were applied during July 2008 (Summer). Ten passes of a vibratory roller (1362 kg) were applied 6 to 14 days after wear. Traffic tolerance was evaluated by assessing the fullness of the turfgrass canopy as well as the canopy loss. The fullness of the turfgrass canopy (0-100% scale; 100%=full canopy) for each entry was rated before wear (C) and 8 to 9 days after rolling (C_T). Canopy loss after traffic was calculated as: C_T-C. Analysis of variance was performed on data using a randomized complete block design with three replications in each season. Replications were nested within season-year (Fall 2007 and Spring 2009; Fall 2009, Summer 2010, and Spring 2011). Means were separated using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$. Entries had lower C_T after traffic applied in Fall 2007 compared to Spring 2009. Similarly, entries had lower C_T after traffic applied in Fall 2009 compared to Summer 2010 and Spring 2011. Canopy loss after traffic was also more severe (lower C_T- C) for Fall 2007 compared to Spring 2009 and more severe after Fall 2009 compared to Summer 2010 and Spring 2011. Analysis of variance of Fall 2007 and Spring 2009 data, and Fall 2009, Summer 2010, and Spring 2009 data determined a significant entry effect and season-year by entry interaction for C_T. Cultivar differences for C_T were more pronounced after traffic in spring and fall compared to summer. Eleven and ten entries were in the top statistical grouping for C_T after Spring 2009 and 2011 traffic, respectively. Seventeen entries were the highest ranked for C_T after Fall 2009 traffic. However, fifty-four and forty entries were in the top statistical category for C_T after Summer 2008 and 2010 traffic, respectively. Despite the season-year by entry interaction, there were entries that had consistently better traffic tolerance across all season-years. Entries that were ranked with the group of entries with the greatest C_T after all six traffic periods were LS 1200, Falcon V, Traverse SPR, and Bullseye. These cultivars should be given strong consideration for establishment on high traffic sports fields and recreational sites receiving multi-seasonal use. In addition to traffic tolerance data, turfgrass quality and brown patch susceptibility are useful selection criteria for tall fescue grown on sports fields and recreational sites.

Elevated Cytokinin Content in *ipt*-transgenic Creeping Bentgrass Promotes Drought Tolerance Through Regulation of the Metabolite Profile

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Increased endogenous plant cytokinin (CK) content through transformation with an isopentyl transferase (*ipt*) gene has been found to be an effective method to promote plant drought tolerance. This study aimed to analyze the effects of differential CK content on the accumulation of major leaf metabolites in creeping bentgrass (*Agrostis stolonifera* L.) exposed to drought stress. Null transformant (NT) and transgenic plants transformed with *ipt* controlled by a senescence activated promoter (*SAG12-ipt*) were exposed to drought stress in an environmental growth chamber until the moisture content of the 1:1 sand:soil growing mix reached approximately 5%. A metabolite profile consisting of 45 distinguishable metabolites were identified and categorized as amino acids, carbohydrates, organic acids, and organic alcohols. The enhanced drought tolerance of *SAG12-ipt* plants may be related to the maintenance of several important metabolites, particularly the amino acids: proline, γ-aminobutyric acid, alanine, and glycine and carbohydrates: sucrose, fructose, maltose, and ribose. *SAG12-ipt* plants also exhibited differential regulation of several important organic acids, particularly those involved in the citric acid cycle. Specific metabolite changes identified over the course of drought stress and at the same level of leaf relative water content (47% RWC) in NT compared to *SAG12-ipt* plants are discussed, which may be particularly important to understanding the involvement of CK in the drought response.

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

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We 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.

In 2010, we 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 percent 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+. In 2011, we isolated the endophytes from the E+ tall fescue collection. These fungi can be used for alkaloid screening to obtain endophytes that produce low levels of the alkaloids that are toxic to mammals. Perennial ryegrass selections from the NTEP were also evaluated for endophyte in 2011.

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. This finding suggests that a high temperature screen could be used to select for heat-stable endophytes that could provide environmental stress tolerance as well as insect resistance at the upper temperature for tall fescue.

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.

From Turf to Nuts: Where Have We Gone in 16 Years of Plant Breeding?

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A program for the genetic improvement of temperate nut trees was initiated at Rutgers University by Dr. C. Reed Funk in 1996. Dr. Funk, being a very long-sighted and ambitious plant breeder, began the nut tree program after already having a successful career in turfgrass breeding, which he started shortly after receiving his Ph.D. from Rutgers in 1962. The principles followed in the establishment of the nut tree program were based on the methodology developed by Dr. Funk during his long career as a turfgrass breeder (Funk et al., 2008). The effectiveness of his methods can be observed first hand by walking across most home lawns, parks, and sports fields in the U.S.A where improved cool season grasses are grown, species including Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*), and others. It was Dr. Funk's great success with turfgrass and the continued success of the program after his retirement, under the direction of Dr. William Meyer, which allowed for the initiation and subsequent proliferation of the nut tree program at Rutgers.

Dr. Funk began the nut tree breeding program for several reasons. Besides his general love of trees and interest in walnuts that began as a child (his father grew Persian and eastern black walnuts in his hometown of Richmond, Utah), during his career he was able to directly observe the impact a dedicated breeder could have on substantially improving an underutilized perennial species. One good example is perennial ryegrass. Before Dr. Funk worked on the species, it was used primarily as a forage grass in Europe and the U.S.A. Now, largely through breeding at Rutgers, perennial ryegrass is a very widely used component of high quality turf, including that of golf courses, stadiums, parks, and home lawns. Similar improvements were made with Kentucky bluegrass, tall fescue (*Festuca arundinace*a), fine fescues (*Festuca* spp.) and several other species. Based on the striking improvements made with these once underutilized plants, Dr. Funk strongly believed similar gains could be made with nut trees and other underutilized perennial crops for food, bioenergy, and environmental enhancement. This idea, combined with Dr. Funk's love of trees, is where the Rutgers nut tree program got its beginnings.

Due to their different growth habits, reproductive behaviors, and propagation requirements, working with tree crops is very different than working with perennial grasses. However, many of the breeding fundamentals remain the same. Similar to the early turf program, a major focus of the early nut tree program was, and still is, obtaining and rigorously evaluating a very large collection of diverse germplasm. Access to superior, diverse genetic resources is essential when working with long generation-cycle species. As such, it is necessary

to collect, evaluate, and identify the best available genetic resources for use as parents in controlled hybridization programs to ultimately produce superior offspring in as short a period of time as possible. Our first step was to assemble and evaluate a diverse collection of walnuts (*Juglans* spp.), hazelnuts (*Corylus* spp.), pecans and hickories (*Carya* spp.), and several other species. We hoped to identify species best adapted to our region that also showed the most potential for rapid genetic improvement. From there, we would initiate more intensive germplasm collection efforts, systematic evaluations, and a focused improvement program using the best genetic material to greatly enhance the species' usefulness for New Jersey and the eastern U.S.A.

Since 1996, our nut tree program has expanded in size yearly. Our research nurseries are planted across five Rutgers research farms located in Cream Ridge, Adelphia, and North Brunswick, New Jersey. Currently, our collection holds almost 34,000 trees, all of which are undergoing evaluations in our nurseries and greenhouses. These include approximately 30,000 hazelnuts; 2,000 eastern black walnuts; 1,500 Persian walnuts; 500 pecans and hickories; 100 heartnuts (*Juglans ailantifolia* var. *cordiformis*); 50 chestnuts (*Castanea* spp.); and 25 miscellaneous accessions including almonds (*Prunus dulcis*), edible oaks (*Quercus* spp.), and paw paws (*Asimina triloba*). Our collection increases by about 5,000 trees each year. However, we have been culling the same or an even greater number of trees from our nurseries yearly, as individuals prove poorly adapted or are inferior and not useful for breeding purposes. Therefore, in recent years the total number of trees in our holdings has remained fairly consistent.

Our most advanced work to date is with hazelnuts. Part of the reason for this advanced state is hazelnuts' relatively short generation time (4-5 years from seed to seed and only 2-3 years for production when planting a grafted tree), their small plant size relative to the other tree nut species, the wide genetic diversity available and high inter-fertility of the genus *Corylus*, and the relative ease of obtaining large numbers of hybrid seed. Hazelnuts also consistently produce abundant crops in our trials with very little inputs (no pesticides or fungicides)—this fact alone drew our attention to the species from the onset of the program. Currently, 99% of the U.S. production of hazelnuts occurs in the Willamette Valley of Oregon. Our primary goal is to develop productive commercial quality hazelnut cultivars for New Jersey and the northeastern U.S.A. that are highly resistant to the fungal disease eastern filbert blight (EFB), caused by *Anisogramma anomala*. This disease is the number one limiting factor of hazelnut culture in this area and is the reason hazelnuts are not grown here commercially (Thompson et al., 1996). As such, much of our research efforts center on identifying and developing resistance to this disease. One strategy we have used is to make hybridizations between *Corylus avellana*, the European hazelnut of commerce, and our native species *C. americana*. While our native species generally produces small, thick-shelled nuts, it is naturally adapted to our climate and most plants are highly resistant to EFB. By making hybridizations between select *C. americana* plants and improved *C. avellana* cultivars, it is possible to recover offspring that are resistant to EFB and sufficiently cold hardy, while also retaining the superior nut quality of the European parent. We

have also had success in identifying novel sources of resistance to EFB in our new collections of European hazelnuts. In 2002, 2004, and 2006, collection trips were made to southern Russia, the Crimean peninsula of Ukraine, and Poland. Hazelnut seeds were brought back to Rutgers from a diversity of research institutes, roadside markets, and bazaars. As expected, a very high percentage of the seedlings succumbed to EFB. However, a small percentage remained healthy, showing no signs or symptoms of infection. We have used a number of these new resistant plants in breeding and now have thousands of improved, EFB-resistant *C. avellana* seedlings that are undergoing evaluations from which we hope to identify new potential cultivars. Further, from our first generation of hybrids planted in 2000, working closely with Oregon State University, we selected 14 EFB-resistant plants that produced high yields of excellent quality nuts. These plants were propagated through grafting and layering and in 2010 were planted in replicated yield trials in NJ, PA, NY, NE, and Ontario, Canada. It will be from these trials where we should select our first Rutgers hazelnuts for release, which we anticipate in three or four years. Similar work is underway with the other nuts species, especially black walnuts; however, due to their relatively large plant size, long-generation time, and the greater amount of land needed to evaluate germplasm collections and progeny, work is progressing at a much slower rate.

It should be noted that Dr. Funk also developed a sister program to ours at Rutgers. Improving Perennial Plants for Food and Bioenergy (www.IPPFBE.org), a non-profit research and breeding organization, was established near Dr. Funk's family home in Richmond, Utah just a few years after he began the Rutgers nut program. The focus of IPPFBE includes temperate nut trees as well as other perennial crops, and allows us to test plant material in two distinct climatic areas, the Mid Atlantic and Inner Mountain West regions of the U.S. We feel the development and widespread use of productive, underutilized perennial food and bioenergy species will be of great help in diversifying agriculture, eliminating hunger, and improving health, prosperity, self-reliance, and productivity in many regions of the world. Perennial crops can be grown in an environmentally sustainable manner on steep, rocky slopes and other lands not suitable for cultivated annuals, as discussed by J. Russell Smith (1950) in “Tree Crops: a Permanent Agriculture”. Ultimately, we feel the development of perennial crops for lands not suitable for annual crop production will greatly increase the world’s food and bioenergy production capacity. This increased production will be needed as the world’s population reaches a projected nine billion in 2050, while clean water and petroleum resources dwindle and increasing amounts of level, fertile farmland is used to produce annual energy crops. Through our nut tree genetic improvement program at Rutgers, IPPFBE in Utah, and other similar programs we may help inspire, it is our goal to make a significant contribution towards enhancing the sustainability and longevity of agricultural systems in the U.S. and around the world.

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What's in a Name? Determining the True Identity of the Dollar Spot Pathogen of Turfgrass

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Dollar spot (*Sclerotinia homoeocarpa*) is one of the most important fungal diseases of warm- and cool-season turfgrasses. The destructive and persistent nature of this fungus has resulted in the extensive use of fungicide-based control measures that often require repeat applications and can be extremely costly. Despite the economic importance of this disease, the taxonomy of the causal agent has been known to be inaccurate for a number of years. In this seminar, we will provide an overview of the taxonomic inconsistencies that have surrounded the dollar spot pathogen for the past 75 years, beginning with its description in 1937. We will introduce the morphological characteristics lacking in *S. homoeocarpa* that demonstrate that the fungus is not a true *Sclerotinia* species, and will discuss why other taxonomic treatments suggested for this pathogen are not appropriate. Special emphasis will be placed on ongoing molecular phylogenetic research at Rutgers and collaborating institutions that are investigating the 'true' identity of the dollar spot fungus. At present, eight molecular markers (the rDNA internal transcribed spacer region, calmodulin, translation elongation factor 1 alpha, DNA replication factor Mcm7, actin, beta tubulin, and portions of rDNA large and small subunits) have been PCR amplified and sequenced from 58 isolates of the dollar spot pathogen and 6 closely-related Sclerotiniaceae and Rutstroemiaceae members- *Ciboria, Monilinia, Lambertella, Lanzia, Poculum*, and *Rutstroemia*. An additional 258 herbarium specimens were obtained from the U.S. National Fungus Collections in Beltsville, MD are being used to compare modern and historical collections of these fungi. Preliminary phylogenetic results will be presented, as well as expected taxonomic outcomes and implications of this research for those studying the dollar spot pathogen and related fungi.

A Six-Gene Phylogeny Reveals the Evolution of Mode of Infection in the Rice Blast Fungus and Allied Species

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The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as *Magnaporthe oryzae* (rice blast and gray leaf spot fungus, formerly known as *M. grisea*), *M. poae* (summer patch pathogen of turf grasses), and *Gaeumannomyces graminis* (take-all fungus of various cereals and grasses), which are popular model organisms in fungal biology and host-pathogen interaction studies. Despite their ecological and economic importance, the phylogenetic relationships among the constituent species remain ambiguous due to the lack of convincing morphological characters and paucity of molecular data for the majority of the non-model species in the family. In this study, our multilocus phylogeny suggests that both *Magnaporthe* and *Gaeumannomyces* are polyphyletic genera. The phylogeny also provides insights into fungal biology and pathogenesis. *Magnaporthe oryzae* formed a basal clade, while *M. poae* and *M. rhizophila* formed another well-supported clade with *G. incrustans* and *G. graminis*. The basal species infect both root and aerial parts of the plant host, while the aerial infection capacity seems to be lost in the taxa of the latter clade. The phylogeny is corroborated by evolution of the anamorphs and a cAMP-dependent protein kinase (*CPKA*) gene. *Magnaporthe oryzae* produces *Pyricularia*, while taxa in the latter clade all produce *Phialophora*-like anamorphs. *CPKA* is present in animals and many fungal lineages with various functions. In *M. oryzae*, *CPKA* is essential for the formation of functional appressoria for leaf penetration. In root-infecting *G. graminis* var. *tritici* and *M. poae*, however, only non-functional *CPKA* homologous pseudogenes were found in their genomes. The study indicates that anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among these taxa.

POSTER PRESENTATIONS

The Application of Next-Generation Sequencing Technology Is Helping Us Elucidate the Genetics of Turfgrass-Endophyte Symbiotic Interaction

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Our research aims to gain insight into the biological process of plant-microbe symbiotic interactions using high-throughput DNA sequencing. Primarily, we are interested in how the plant's gene expression profile is impacted by the presence of a naturally occurring fungal endophyte. Symbiosis between fungi and plants in nature is well documented. Yet, much research is still needed to fully comprehend the workings of this important interaction in regards to plant evolution, breeding and physiological performance. One such symbiotic association of interest takes place between *Festuca rubra*, a commercially important grass species known as fine fescue, and *Epichloë festucae*, a fungal endophyte that lives within its grass host. Studies have revealed that *Epichloë* endophytes live entirely within the plant, and provide a wide range of benefits to their grass hosts, principally resistance to insect and mammalian herbivores. *E. festucae* infection of *F. rubra* also confers fungal disease resistance to the host. These observations are important because resistance to diseases has not been documented in other cool-season grass-endophyte interactions.

We have utilized transcriptome analysis to address fundamental aspects of plant-endophyte symbiosis in order to facilitate large-scale comparative analysis. Based on our hypothesis that differential gene expression is imperative for the maintenance of the symbiotic interaction, we have used SAGE (Serial Analysis of Gene Expression) to obtain a global quantitative comparison of the transcriptome of the endophyte-free (E-) and endophyte-infected (E+) plants. The SAGE libraries were prepared in triplicates, and sequenced using ABI's SOLiD platform. The >54 million SAGE tags we obtained were supplemented with >200 000 454 transcriptome sequences of the *E. festucae* endophyte strain isolated from its grass host plus the plant infected with that strain. The SAGE tags were mapped to a reference database consisting of our 454 transcriptome, and *Festuca* and *Epichloë* sequences downloaded from NCBI.

On-going analysis of the data has revealed plant transcriptional changes in response to fungal endophyte infection. Ultimately, the findings will enrich our overall understanding of how symbiotic relationships in nature, a complex interplay between host plants and their microbe symbionts, are maintained. Specifically, the research will contribute to the knowledge of the symbiosis between a grass host plant and its fungal endophyte by identifying the important genes responsible for maintaining the equilibrium, where both plant and endophyte thrive.

Effects of Plant Growth Regulators and Osmoregulants on 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. From a physiological perspective, there are various mechanisms involved in drought survival of plants, which can be categorized into 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 regulate 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 and osmoregulants 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) Gibbrellic 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-July in 2010 and 2011. 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 displayed better recovery compared to controls due to increased tiller density as well as an increased rate of tiller production and leaf elongation.

Differentiating Heat and Drought as Components of Summer Stress Among Experimental Tall Fescue Genotypes

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Heat and drought are two abiotic stressors of turfgrass which are responsible for the decline of cool season grasses during the summer months. These two stresses are often grouped together and referred to as summer stress. While these two stresses often occur simultaneously, quantification and comparison of the stress induced decline caused by each stress individually will help both turfgrass managers and breeders combat this issue.

Breeding of tall fescue (*Lolium arundinacea*) has recently produced cultivars which have made this grass, which was once suitable solely for low maintenance utility applications, a viable option for areas of moderate cultural intensity such as home lawns and athletic fields. This is attributed to these new cultivars being finer textured, more laterally growing, and darker in color. While tall fescue has been bred to be functionally more like turfgrass species such as perennial ryegrass (*Lolium perenne* L.) and Kentucky bluegrass (*Poa pratensis* L.), it continues to exhibit relatively high levels of tolerance to summer stress. Though this rather high summer stress tolerance is well documented, little research has been done to characterize it. This study has been set up to determine whether heat stress or drought stress is the bigger factor contributing to the decline of tall fescue during summer months.

Twenty-four tall fescue clones were selected based on field performance as spaced plants; twelve that were perceived to be tolerant and twelve that were perceived to be sensitive to summer stress. These twenty-four clones were subjected to heat and drought stress in combination and alone by placing them in growth chambers and controlling their temperature and water supply. A number of physiological measurements were taken, including turf quality, relative water content, electrolyte leakage, and photochemical efficiency. Overall, the effects of heat+drought resulted in the most stressful conditions followed by drought alone and clones subjected to heat stress alone appeared to maintain their overall quality for the longest period. However, this is confounded by the performance of the plants selected as summer stress tolerant compared to the plants selected as summer stress sensitive. In the control, drought, and heat+drought treatments there were generally no significant differences between these two groups in any of

the measurement parameters. Conversely, in the heat stressed plants, the selected summer stress tolerant group performed significantly better, in all measurement parameters, than the summer stress sensitive group. In addition, certain clones were found that performed well in only certain conditions, others were found that performed well under all conditions, while still others were found which performed poorly under all conditions.

Genomic Assessment of *Colletotrichum* Causing Anthracnose Disease in Grasses Used For Turfgrass, Food, Fuel and the Environment

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The fungal genus *Colletotrichum* is comprised of at least 68 species that attack >3200 monocot and dicot plants. The genus includes 14 closely related species pathogenic to grasses, collectively causing billions of dollars of losses in turfgrass, bioenergy feedstocks, and cereal crops each year. In turfgrass systems, anthracnose caused by *C. cereale* is a destructive disease of cool-season golf course putting greens, with a typical U.S. golf course spending ~$20k annually on preventative fungicide treatments to provide acceptable levels of control.

Elucidating the connection between genotype, phenotype, and adaptation in fungal populations is fundamental to the study of plant pathogens. To overcome the systematic bias that may occur in these studies through the use of nucleotide characters sampled from only one or a few molecular markers, researchers are increasingly developing datasets from larger numbers of orthologous genes or even whole genomes. Yet species and population history in the genus *Colletotrichum*, like that of most non-model organisms, has not been investigated at the whole-genome level. In this study, we set out to determine whether Illumina-sequenced restriction-associated DNA tagging (RAD-Seq) technology could be used to efficiently study genome-scale variation in *Colletotrichum*, and whether the relationships generated through analysis of this data set was equivalent to data from other molecular analyses. Illumina sequencing was performed from 43 *Colletotrichum* pathogens isolated from cereals and grasses and 9 *Colletotrichum* pathogens isolated from cranberry and blueberry (~56 Mb/genome, 12% repetitive DNA, 64.8X coverage). The sample included 10 *Colletotrichum* species, representing different levels of divergence, including 4 *C. cereale* isolates from annual bluegrass (*Poa annua*) turf, 20 isolates of *C. graminicola* pathogens of corn, and a sampling of isolates from switchgrass, miscanthus, sorghum, sugarcane and other grasses. To target the non-repetitive portion of the genome, a RAD-Seq approach was employed, utilizing the methylation sensitive restriction enzyme *Sbf*I and short, unique multiplex barcode tags. Between 34.8−99.8% of the data from grass pathogens mapped to the *C. graminicola* M1.001 reference genome, but only 5.1−8.5% of the cranberry and blueberry pathogen data matched a *C. graminicola* sequence (threshold 88% identity). After removing monomorphic and non-orthologous sequences, 1257 unique loci were represented in the dataset, with 67% located in coding regions, spanning 84% of the 10 major chromosomes, providing 1-6 SNPs per sequence marker, and, 1 marker/30 kb.

Overall, 16.7% of the aligned 72,263 nucleotides were variable in two or more *Colletotrichum* samples. Results from three different analytic methods showed that the species level distinctions between the sampled taxa agreed with previously hypothesized patterns of diversification. Population subdivision was also observed. However, shared ancestral polymorphisms were detected throughout the phylogenetic tree, resulting in poor resolution of older, basal relationships. The results of this unbiased, whole-genome approach shows that markers currently used for species delineation and diagnosis of grass pathogenic *Colletotrichum* accurately reflect species relationships, and provides a valuable resource for SNP marker development for ongoing population scale studies.

Effect of Scarification Depth on Anthracnose Severity of Annual Bluegrass Putting Green Turf

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Anthracnose is a destructive disease of annual bluegrass [ABG; *Poa annua* L. f. *reptans* (Hausskn) T. Koyama] putting green turf caused by the fungus *Colletotrichum cereale* Manns. Although not required for host penetration, wounding has been reputed to enhance infection of host plant tissue by *C. cereale*. There is limited research concerning the effect of wounding on anthracnose severity of ABG putting green turf. In particular, the impact of mechanical injury from the cultural practice of scarification (vertical cutting) on disease severity remains unclear. A field study was initiated on 23 July 2010 in North Brunswick, NJ to evaluate the effect of scarification depth (0, 1.3- and 7.6-mm) on anthracnose severity. The trial used a completely random design with ten replications and plot size of 0.5- by 1.5-m. The study was conducted in 2010 and repeated twice in different locations in 2011. Scarification treatments were applied once in each study (23 July 2010, 6 July 2011 and 3 Aug 2011). Disease development was monitored at 10 positions along each of three 25-cm transects within each plot (30 observations per plot). Transects overlaid the scarification lines of treated plots and were randomly positioned over turf in non-scarified plots. All transects were marked so that ratings over time were performed at the same locations within each plot. Anthracnose severity was evaluated immediately after scarification and every 1- to 5-d thereafter. Disease severity was 5% at the initiation of the first and second runs of the study, and 27% for the third run. Scarified plots had slightly greater disease (3%) than the control on only one of 32 observation dates in 2010 and 2011. These findings do not support the hypothesis that wounding from scarification increases anthracnose severity.

Molecular Markers Linked to Heat Tolerance in Bentgrass Species

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Heat stress is a major abiotic stress which affects cool-season plants. Plants exposed to high temperatures often experience decreases quality and performance and stress related injury. Previous studies using suppression subtractive hybridization and gel electrophoresis have identified several genes predicted to encode proteins which may be of key importance for heat tolerance. A creeping x colonial bentgrass hybrid mapping population was tested and found to have differences in heat tolerance. Markers for genes associated with possible heat tolerance related proteins were developed. Markers for these candidate genes were then tested in the mapping population and added to an existing linkage map. Six markers were successfully added to the map which correspond with expansin, catalase, cysteine protease and several heat shock proteins (HSP26, HSP 70 and HSP101 kDa).

Screening Perennial Ryegrass Cultivars For Germination Under Salinity Stress

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Water restrictions are a current issue that many golf courses are facing. As these restrictions tighten there will be a need for identifying turfgrasses that can withstand irrigation from alternative water sources with higher than normal salt concentrations such as effluent water. 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 and provide a turf stand in a short period of time. Therefore, this species is used for overseeding dormant warm-season turfgrass on golf courses (a practice used by many golf courses worldwide). However, irrigating perennial ryegrass with saline water has been shown to reduce both establishment and germination.

Eight cultivars or selections (Apple GL, KSA, Palmer III, Zoom, Linn, Paragon GLR, ESP, RKS) of perennial ryegrass were evaluated under seven salinity treatments: Control; 0.25 dS/m; 1.0 dS/m; 2.0 dS/m; 3.0 dS/m; 4.0 dS/m; 5.0 dS/m; 6.0 dS/m. Saline water was made using equal quantities of NaCl and $CaCl_2$ mixed with tap water. Three replicates of each cultivar within each salinity treatment were arranged in a completely random design. 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. Turfgrass plants were planted at a seeding rate of 7.44g/m^2. Fertilizer was applied at a rate of 1.49 g of nitrogen/m^2. Four hundred ml/m^2 of each saline water treatment were applied to each pot three times/week to maintain soil moisture for the first two weeks. After two weeks, application of treatments were reduced to two times/week Saline water treatments were applied using a trigger sprayer (Spray Master, New Brunswick, New Jersey) to simulate overhead irrigation on a golf course. Two visual ratings and digital images were analyzed with SigmaScan. Visual ratings of seedling emergence were evaluated on a one to nine scale: one being poor seedling emergence, nine being the highest seedling emergence. Visual ratings of percent green were also collected. Digital image analysis was also used to calculate percent green. 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. Run 1 of the experiment occurred during winter of 2010 and run 2 occurred during spring of 2011.

Significant differences were observed between treatments (across all cultivars) and between cultivars (across all treatments) for percent green and seedling emergence. However, in both runs 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 ryegrasses. Perennial ryegrass seedlings exposed to the 6 ds/m salinity treatment exhibited a 60%-77% reduction in seedling emergence and a 63%-77% reduction in visual percent green compared to the control. In most instances Linn, Zoom, and Paragon GLR had the highest percent green values (visual ratings and digital image analysis) across both runs when averaged across all treatments. Linn has very course leaves and an upright growth habit, so both the digital image analysis and percent green ratings therefore are slightly skewed due to this cultivar's larger leaf blades. Interestingly, RKS, a cultivar selected for mature plant salinity tolerance, was among the least tolerant in this study. This supports previous research that mature plant salinity tolerance is unrelated to salinity tolerance at seedling stage (Hughes et al, 1975; Qian and Suplick, 2001). More research is needed to develop plants that are tolerant to salinity levels at every stage of growth.

Hughes, T.D., J.D. Butler, and G.D. Sanks. 1975. Salt tolerance and suitability of various grasses for saline roadsides. Journal of Environmental Quality. 4:65-68.

Qian, Y.L., and M.R. Suplick. 2001. Interactive effects of salintity and temperature on Kentucky bluegrass and tall fescue seed germination. International Turfgrass Society. 9:206-2

Identification of Genes Associated with Enhanced Cytokinin Content and Drought Tolerance in *ipt*-Transgenic Creeping Bentgrass by Suppression Subtractive Hybridization (SSH)

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Increased endogenous plant cytokinin (CK) content through transformation with an isopentyl transferase (*ipt*) gene has been found to be an effective method to promote plant drought tolerance. This study aimed to identify differentially-expressed genes associated with CK synthesis and drought tolerance of creeping bentgrass (*Agrostis stolonifera* L.). Null transformant (NT) and transgenic plants transformed with *ipt* controlled by a senescence activated promoter (*SAG12-ipt*) were exposed to drought stress in an environmental growth chamber until the moisture content of the 1:1 sand:soil growing mix reached approximately 5%. The subtraction of cDNA libraries from well-watered and drought stressed NT and *SAG12-ipt* leaves was performed. The results show that enhanced drought tolerance of *SAG12-ipt* plants may be related to the maintenance of several important differentially expressed genes. Specific gene changes will be presented.

Microsatellite Markers Reveal a Microgeographic Population Expansion and a Stochastic Long Distance Dispersal of *Waitea circinata* var. *circinata* **Infecting Turfgrass**

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Waitea circinata var*. circinata* is an emerging pathogen of turfgrass in North America. It causes brown ring patch of turfgrass in golf courses and amenity areas. To understand and characterize its population biology, we isolated eight promising microsatellite markers from an enriched genomic library. Seven of these were used to study the genetic diversity of eastern and western of USA populations of *W. c.* var. *circinata* and the association with phenotypic characteristic and geography. In general, the microsatellite markers so developed were highly polymorphic, with eastern population showing a mean observed heterozygosity 0.54 compared to 0.50 for the western population. Thirty six genotypes were revealed among the 39 isolates assayed and one was shared among the two populations. No host associated UPGMA tree clustering was observed among the genotypes suggesting a lack of host specialization. Analysis of population structure based on isolation by distance model revealed a significant population expansion, r^2= 0.11, *P* = 0.01, of *W. c.* var. *circinata* within a radius of about 150 km but it became more stochastic with increase in distance. Despite so, the two populations were weakly but significantly differentiated from each other with presence of immigrant individuals across boundaries. At micro-geographic level, the sclerotia phenotype also showed $Q_{ST} > F_{ST}$ suggesting that natural selection forces were causing divergent evolution of the sclerotia phenotype as an adaptation to local eco-geographical conditions. However the low Q_{ST} values indicates that the 2 populations have not been isolated for long periods of time.

Determination of the Gene Effects Controlling Rapid Lateral Tillering Rate and Rhizome Formation in Tall Fescue [*Lolium arundinaceum* (Schreb.) Darbysh.]

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Tall fescue [*Lolium arundinaceum* (Schreb.) Darbys.] is a cool season, perennial, self-incompatible, bunch type turfgrass, which spreads primarily by erect tillers. However, some types have been found that have a rapid lateral tillering rate and rhizome formation. The rapid tillering rate and rhizome formation are beneficial in tall fescue as they help plants to spread faster and recover from damage. Tall fescue clones with three types of growth habit classified as: 'Bunch type' which spreads by erect tillers; 'broad base type' which spreads by lateral tillers; and 'rhizome type' which produce rhizomes along with tillers were used in controlled diallel crosses to study the inheritance of rapid lateral tillering rate and rhizome formation in tall fescue. Two genotypes from each growth type were selected and allowed to cross in isolation in all possible combinations. Progenies were planted in the field in randomized complete block design with four replications. Turf density (to represent rapid lateral tillering) and number of rhizomes were collected on the progeny of the controlled crosses. Narrow sense heritability estimates, maternal effects and heterosis were calculated.

Significant heterosis and maternal effects were found for turf density in the broadbase type parent crosses and rhizome type parent crosses, including reciprocals. Significant heterosis was found in the bunch type parent crosses and when bunch type parents were crossed with broad base type parents. Significant maternal effects were found in the crosses between rhizome type and bunch type parents and their reciprocals. The narrow sense heritability estimates was very high for turf density. Broadbase type parents and bunch type parents had the highest mid parent-progeny mean followed by the broadbase type parents. Rhizome type parents have the lowest mid parent-progeny mean.

For rhizome formation, significant heterosis and maternal gene effects were found in the rhizome type parent crosses including reciprocals, the broad base type crossed with bunch type parents and the broad based type crossed with the rhizome type parents, respectively. Based on the narrow sense heritability estimates, rhizome formation is heritable, but less than turf density. Rhizome type parents have the highest mid parent-progeny mean.

Significant heterosis and maternal effects for turf density and rhizome formation in tall fescue might be due to dominant gene effects, which allow the expression of contributed genes from one parent than other parent in a cross, resulting in desired trait

formation. High narrow sense heritability suggests that additive gene effects may be strongly influencing turf density and rhizome formation. However, further analysis of combining ability will be helpful to confirm the type of gene actions influencing these traits. Diallel analysis has proven to be useful to evaluate the performance of parents based on progeny tests, selecting superior parents, and improving breeding efficiency for the rapid tillering rate and rhizome formation in tall fescue.

An Oxidative Mechanism For Nutrient Acquisition From Epiphytic/Endophytic Microbes in Tall Fescue

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Tall fescue (*Festuca arundinacea* Screb.) is a cool-season perennial grass that harbors nitrogen-fixing bacteria on glumes and paleas adherent to seeds. When seeds germinate, these bacteria colonize seedling roots. These roots, in turn, secrete peroxide, which results in the oxidation/digestion of bacteria on root surfaces. We refer to this mechanism as 'Oxidative Nutrient/Nitrogen Scavenging' (ONS) and we hypothesize that it provides nutrients, especially nitrogen, to the growing seedling.

The generalized process of ONS is outlined as follows:

1. Plant seeds carry nitrogen-fixing bacteria on the seed coat or within seeds.
2. Microbes grow on and within seedling roots and fix nitrogen.
3. Roots secrete reactive oxygen species (ROS), primarily H_2O_2, onto microbes.
4. ROS causes partial breakdown of microbe membranes, proteins, and nucleic acids.
5. Roots absorb smaller compounds (e.g., peptide and nucleic acid fragments) from oxidizing microbes.

The overall objectives of this research are to:

1. Evaluate the ONS mechanism through stable isotope studies.
2. Identify ONS symbioses and the nitrogen-fixing bacteria on a range of grasses.
3. Demonstrate that ONS results in enhanced production of antioxidants and stress-adaptive factors.
4. Conduct field tests to evaluate performance of ONS plants.
5. Patent specific microbes present in plants that show superior growth using ONS.

The ONS mechanism may be particularly critical to plants in soils deficient in nitrogen. Plants that undergo ONS may be more resistant to oxidative stress than plants nourished by inorganic fertilizers because they must adapt to high levels of ROS used to oxidize symbiotic microbes. Stresses from factors such as drought, high heat, fungal diseases, and heavy metals in soils generally result in tissue damage through the resulting internal production of ROS. Plants adapt to ROS through enhanced production of antioxidants (e.g., phenolics like anthocyanins and tannins; enzymes like peroxidases,

superoxide dismutases) and stress-adaptive factors (e.g. heat shock proteins, dehydrins). Plants that have enhanced levels of antioxidants and stress-adaptive factors have reduced sensitivity to a host of oxidative stresses.

Significance: This work will lay a foundation for understanding the biology and importance of the Oxidative Nutrient/Nitrogen Scavenging mechanism. ONS may enable plants to grow and flourish despite of the absence of adequate soil nutrients, infrequent soil moisture, and suppressed soil microbial activity. An understanding of the relationship of ONS to stress tolerance in plants may provide a new approach to the creation of hardier plants for extreme environments.

Anthracnose Severity of Annual Bluegrass Turf as Influenced by Nitrogen Fertilization Programming

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Anthracnose, caused by *Colletotrichum cereale* Manns, is a destructive fungal disease of annual bluegrass [ABG; *Poa annua* L. f. reptans (Hausskn) T. Koyama] putting green turf. Proper nitrogen (N) management can reduce disease severity and enhance turf recovery. Previous work has shown that both granular- and liquid-N fertilization play an important role in anthracnose suppression; however, the potential interactive effect of both factors on disease severity has not been reported. The objectives of this study were: i) to evaluate the impact of late- or early-season granular-N fertilization rate on anthracnose severity; and ii) to determine whether late- or early-season granular-N fertilization alters the effect of frequent low rate soluble-N fertilization on anthracnose. The field study was initiated in 2008 in North Brunswick, NJ on annual bluegrass turf maintained at 3.2 mm on a Nixon sandy loam. The experiment used a 2 x 3 x 4 factorial arranged as a randomized complete block design with three replications. The primary season of granular-N fertilization (2/3 of total N applied in the fall or spring), annual granular-N rate (73, 146 and 219 kg ha^{-1}), and frequency of summer soluble-N (4.6 kg ha^{-1} of soluble-N applied every 0, 1, 2 or 4 weeks from mid-May through August 2009 and 2010) were the main factors. The main effects explained most of the variation in anthracnose severity. Summer soluble-N applied every week (18.3 kg N ha^{-1} $month^{-1}$) reduced disease severity 36 and 27 % by the end of 2009 and 2010, respectively, compared to turf receiving no soluble-N. Granular-N fertilization applied primarily in the spring reduced disease severity compared to autumn based granular-N fertilization on all but two rating dates in the study. The rate of granular-N fertilization also affected disease severity; N applied at an annual rate of 219 kg N ha^{-1} decreased disease severity by 21 and 17 % compared to 73 kg ha^{-1} by the end 2009 and 2010, respectively. The interaction between season and granular-N rate indicated that autumn based fertilization at 219 kg N ha^{-1} yr^{-1} was needed to reduce anthracnose; whereas, the spring granular program reduced disease severity at the annual rate of 146 kg N ha^{-1}. Turf managers should place an emphasis on applying N fertilization during the summer as well as spring as a component of best management practices to suppress anthracnose disease.

Use of Mesotrione for Annual Bluegrass (*Poa annua* L.) Control at Kentucky Bluegrass Establishment

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Field studies were conducted in the fall of 2007 to the spring of 2009 to evaluate the response of newly seeded Kentucky bluegrass cultivars to mesotrione applied at planting (PRE), and PRE followed by (fb) sequential treatments four weeks after turfgrass emergence (WAE) at rates ranging from 0.28 to 2.24 kg ai/ha. In separate studies annual bluegrass control in newly seeded 'Midnight II' Kentucky bluegrass was evaluated with mesotrione applied PRE fb sequential treatments 4 and 8 WAE at 0.14 to 0.56 kg/ha. All applications were made with a single 9504E nozzle CO_2 pressured sprayer calibrated to deliver a total 375 L/ha at 220 kPa. Experimental designs were a strip-plot with four replications for the Kentucky bluegrass cultivar study and a randomized complete block with four replications for the annual bluegrass control study. Kentucky bluegrass cultivars 'America', 'P-105', 'Midnight II', 'Avalanche', Kingfisher', 'Washington', 'Bedazzled', 'Thermal', and 'Award' were seeded on 8-28-07, and 9-16-08 at 1.7 kg/ha in 1.8 m rows with a drop spreader. Annual bluegrass control studies were initiated on 9-13-07 and 09-22-08. Kentucky bluegrass cover and annual bluegrass control were visually evaluated in December and the following spring on a scale of 0 (no cover or control) to 100 (complete cover or control). In the Kentucky bluegrass cultivar study significant cover reductions were not evident across all Kentucky bluegrass cultivars at rates of 0.28 and 0.56 kg/ha. Cover reductions were evident on some cultivars at rates of 1.12 and 2.24 kg/ha and sequential applications further reduced cover. A high degree of intraspecific variability was evident with cultivars 'Thermal' and 'Washington' showing the most tolerance while 'Kingfisher' and 'Avalanche' were the most sensitive. In the annual bluegrass control studies, nearly complete control of winter annual broadleaf weeds such as chickweed, henbit, oxalis and veronica were observed at all application rates. 'Midnight II' cover was not significantly reduced by mesotrione. Annual bluegrass control ranged from 61 to 94% and increased with increasing mesotrione rate. Annual bluegrass control was 83% at 0.28 kg/ha averaged across the three application regimes. Applying a sequential application of mesotrione at 4 WAE increase annual bluegrass control to 80% from 74% compared with a single PRE application. Applying a third application of mesotrione at 8 WAE did not further increase annual bluegrass control compared with two applications. The results of these studies suggest that the overall tolerance of Kentucky bluegrass is excellent and mesotrione can be safely used at establishment for high levels but not complete annual bluegrass control.

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