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Proceedings of the Twenty-Sixth Annual Rutgers Turfgrass Symposium

Albrecht Koppenhöfer and Barbara Fitzgerald, Editors

Rutgers Cooperative Extension educational programs are offered to all without regard to race, religion, color, age, national origin, gender, sexual orientation or disability.

Director's Opening Remarks:

Welcome to the Twenty-Sixth Annual Rutgers Turfgrass Symposium 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 has expanded to include presentations by colleagues at other institutions. I would like to thank Dr. Kristen Nelson (Department of Forest Resources, University of Minnesota) for presenting this year's keynote address entitled, "*Public and Private Land Managers Examining Fine Fescues as a Vegetation Choice for the Future,*" as well as Mr. Mark Kuhns (Director of Grounds at Baltusrol Golf Club, Springfield, NJ) and all of the Turf Center faculty and students who have agreed to present their research at this year's symposium. I would also like to thank Drs. Stacy Bonos, Bill Meyer, Jim Murphy and Jim White for serving as session moderators, and the Symposium Planning Committee comprised of Drs. Josh Honig (Chair), Bill Meyer and Bruce Clarke, as well as Dr. Albrecht Koppenhöfer 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 faculty, students and staff in the Turfgrass Center continue to be recognized for excellence in research, teaching and outreach. In 2016, Dr. Bill Meyer was named the first C. Reed Funk Endowed Faculty Scholar in Plant Biology and Genetics, Dr. Stacy Bonos became a fellow in the Crop Science Society of America (CSSA), and Dr. Bruce Clarke was the recipient of the CSSA Fred V. Grau Turfgrass Science Award and the Green Section Award from the United States Golf Association. Also in 2016, the New Jersey Turfgrass Association (NJTA) presented Dr. Bingru Huang with the NJTA Hall of Fame Award and Mr. Brad Park with the Recognition Award. Our graduate students also received several major awards for their research accomplishments. Dr. Lisa Beirn received the Musser International Turfgrass Foundation's Research Scholarship Award and Ms. Zipeng Tian received 2nd place honors in the Graduate Student Oral Paper Competition at the annual CSSA meeting held in Phoenix, AZ

Over the past 26 years, Turf Center faculty have continued to conduct outstanding research, undergraduate and graduate teaching, and continuing professional education and service programs in support of the Turfgrass Industry. In return, the Turfgrass Industry have donated their time and over $5 million in the form of research grants, student scholarships (> $80,000/yr.), fellowships (the Henry Indyk Endowed Graduate Fellowship), buildings (the Ralph Geiger Education Complex and the C. Reed Funk Maintenance Facility at Hort Farm II), equipment, and gifts to the Rutgers Turfgrass Program. We are indeed fortunate to have such a close partnership with our Turfgrass Industry colleagues in the state, region and nation.

It is with deep pride and a sense of anticipation for the bright 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

Table of Contents

TWENTY-SIXTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 13, 2017

Foran Hall, Room 138A

Friday, January 13, 2017

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

9:00 - 10:00 AM **SESSION I: PATHOLOGY AND SYMBIOSIS**
(Moderator: Dr. Stacy Bonos)

9:00 – 9:20 **Dr. Thomas Gianfagna** (Department of Plant Biology, Rutgers University) *A New Antibody for Grass Endophyte Evaluation and the Identification of Acremonium strictum in Tall Fescue*

9:20 – 9:40 **Dr. Faith Belanger** (Department of Plant Biology, Rutgers University) *The Epichloë festucae Antifungal Protein Has Activity Against the Dollar Spot Pathogen*

9:40 – 10:00 **Richard Buckley** (Plant Diagnostic Lab, Rutgers University) *25 Years of the Plant Diagnostic Laboratory: Trends in Turf Disease Diagnosis*

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

10:30 – 12:00 PM **SESSION II: TAXONOMY AND ECOLOGY**
(Moderator: Dr. William Meyer)

10:30 – 10:50 **Dr. Ning Zhang** (Department of Plant Biology, Rutgers University) *Phylogenomic and Comparative Genomic Analysis of the Magnaporthales Fungi*

10:50 – 11:10 **Dr. Joshua Honig and Dr. Thomas Molnar** (Department of Plant Biology, Rutgers University) *Combining Field and Lab Data to Reach New Heights in the Rutgers Hazelnut Breeding Program*

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

11:20 – 12:00 **Keynote: Dr. Kristen Nelson** (Department of Forest Resources, University of Minnesota) *Public and Private Land Managers Examining Fine Fescues as a Vegetation Choice for the Future*

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

1:00 – 2:10 PM **SESSION III: TURF MANAGEMENT**
(Moderator: Dr. James Murphy)

1:00 – 1:30 **Mark Kuhns** (Director of Grounds, Baltusrol Golf Club) *The Role of the Modern Golf Course Superintendent: It's More Than Just Growing Grass*

1:30 – 1:50 **Hui Chen** (Department of Plant Biology, Rutgers University) *Traffic Tolerance of Fine Fescues: Techniques for Screening Germplasm*

1:50 – 2:10 **Dr. Patrick Burgess** (Bayer Crop Science) *Turfgrass Stress Tolerance and Management in Response to Elevated Atmospheric Carbon Dioxide*

2:10 – 2:30 PM **Discussion and Coffee Break**

2:30 – 3:30 PM **SESSION IV: PEST MANAGEMENT**
(Moderator: Dr. James White)

2:30 – 2:50 **Dr. Albrecht Koppenhöfer** (Department of Entomology, Rutgers University) *Understanding and Managing Insecticide Resistance in the Annual Bluegrass Weevil*

2:50 – 3:10 **Dr. Matthew Elmore** (Department of Plant Biology, Rutgers University) *The Future of Turfgrass Weed Science at Rutgers*

3:10 – 3:30 **James Hempfling** (Department of Plant Biology, Rutgers University) *Bentgrass Tolerance and Fungicide Timing Effects on Dollar Spot*

3:30 - 3:45 PM **Discussion and Closing Remarks**

3:45 PM **Social Hour and Poster Session**

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PLENARY PRESENTATIONS

A New Antibody for Grass Endophyte Evaluation and the Identification of *Acremonium strictum* in Tall Fescue

Thomas Gianfagna, Jeanne Peters, Marshall Bergen, James White and William Meyer

Department of Plant Biology, Rutgers University

Endophytes are well-known to improve environmental and biological stress tolerance in grasses where they occur naturally, but the alkaloids they produce may also be toxic to grazing animals. Screening grasses for endophytes is an important part of a grass breeding strategy for turf and pasture. Identification of endophytes in grass tillers can be done by microscopy, PCR, or using an immunoblot assay. For determining endophyte status of the large number of samples generated by breeding programs, the only practical option is the immunoblot assay. There is one commercially available immunoblot kit (Agrinostics, GA), which we have used satisfactorily, but it is expensive for large sample screening and cross-reacts with *Claviceps* and some other fungal species, creating the potential for false positives. To create a more specific test kit, we made antibodies to a fungal chitinase that is secreted by the grass endophyte *Epichloë coenophiala* into the apoplast space of infected grasses. The antibody was made in rabbits and the antigen designed so that it would not cross react with *Claviceps* or other fungal and plant chitinases. To test the efficacy of the antibody, culture filtrates of *Epichloë coenophiala*, *Claviceps purpurea*, and *Acremonium strictum*, an endophyte that we have identified in tall fescue, were extracted and the chitinase was partially purified and tested against the antibody by Western blotting. In addition, apoplastic fluid from leaves of endophyte-infected and non-infected grasses was extracted by vacuum infiltration and assayed for reactivity to the antibody.

The *Epichloë festucae* Antifungal Protein Has Activity Against the Dollar Spot Pathogen

Zipeng Tian, Ruying Wang, Bruce B. Clarke, Faith C. Belanger

Department of Plant Biology, Rutgers University

Many grasses in their natural environment are associated with fungal endophytes from the genus *Epichloë* (Clay, 1988). Some *Epichloë* spp. have been confirmed to confer enhanced resistance to abiotic and biotic stresses to the plant hosts. Endophyte-mediated resistance to the fungal diseases red thread, caused by *Laetisaria fuciformis* (McAlp.) Burdsall, and dollar spot, caused by *Sclerotinia homoeocarpa* Bennett, has been well documented in fine fescues infected with *E. festucae* (Bonos et al., 2005; Clarke et al., 2006). Such disease resistance is not a general effect of endophyte infection in other grass species/*Epichloë* spp. interactions. The underlying mechanism of the endophyte-mediated disease resistance has not yet been established.

Festuca rubra L. subsp. *rubra* (strong creeping red fescue) exhibits endophyte-mediated fungal disease resistance. *Epichloë festucae* Leuchtm., Schardl & M.R. Siegel, the fungal endophyte of *F. rubra* subsp. *rubra*, produces an abundant antifungal protein that is secreted into the apoplast of the infected plant (Ambrose and Belanger, 2012). We have purified the antifungal protein from the apoplastic fluid of endophyte-infected plants and found that it does have activity against the dollar spot pathogen. We expressed the antifungal protein in the yeast *Pichia pastoris* in order to obtain larger quantities of the protein for additional testing. The *E. festucae* antifungal protein purified from the yeast culture filtrate had activity against the dollar spot pathogen in several different assays. These results support the hypothesis that the antifungal protein is a factor in the disease resistance observed in endophyte-infected strong creeping red fescue. We are currently working on approaches to easily produce enough of the antifungal protein to test on plants.

We are also working on generating a knock-out of the antifungal protein gene to determine the effect on disease susceptibility in strong creeping red fescue. The standard approach to generating knock-outs in *E. festucae* relies on homologous recombination of long DNA fragments, generally greater than 1000 base pairs, flanking the gene of interest. This approach was not successful for the *E. festucae* antifungal protein, likely because it resides in an unusual genomic region where the gene is flanked by repeated sequences. We are now working on developing a CRISPR approach to knocking out the gene to overcome this problem.

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Clarke, B.B., J.F. White Jr., R.H. Hurley, M.S. Torres, S. Sun, and D.R. Huff. 2006. Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Dis. 90:994–998.

25 Years of the Plant Diagnostic Laboratory: Trends in Turf Disease Diagnosis

Richard Buckley and Sabrina Tirpak

Rutgers Plant Diagnostic Laboratory

The Rutgers Plant Diagnostic Laboratory (PDL) opened for business in July 1991. Our mission is to provide New Jersey citizens with the diagnosis of plant health problems. These services are to be provided in an accurate and timely manner to meet the increasing agricultural and environmental needs of the State. The laboratory performs a number of pay-for-diagnosis services that include: disease and insect pest diagnosis, plant and weed identification, insect identification, fungus and mold identification, and nematode assays. There are 2 full-time diagnosticians. To date, the PDL has evaluated more than 50,600 samples with revenues that exceed 3.6 million dollars. 92% of our sample submissions consist of turfgrass and ornamental plants, which reflect New Jersey's status as an urban agriculture state and Rutgers as a turfgrass research powerhouse. Turfgrass alone accounts for 40% of our totals. Golf courses from 43 states use the Rutgers Plant Diagnostic Laboratory. Each year the outcome of all the turfgrass samples are tallied. In any given year, the PDL diagnoses one or more samples of most turf diseases and turf insect pests. The top five most common problems, however, remain consistent from year to year. These maladies include: temperature and moisture stress, *Pythium* root diseases, the root-infecting patch diseases summer patch and take all, anthracnose, and high populations of plant parasitic nematodes. All turf samples are subject to a standard protocol of observation to eliminate bias. As such, our diagnostic output reflects our sample inputs. Each of the most common turf problems we diagnose is characterized by non-descript symptom expression in the field, are root related, or has fungal signs that are not easily identified without laboratory microscopes. These are the problems that are the most difficult for practitioners to solve in the field, so they are the most common outcomes from samples submitted to the PDL.

Phylogenomic and Comparative Genomic Analysis of the Magnaporthales Fungi

Ning Zhang[1], Jing Luo[1], Huan Qiu, Guohong Cai[2], Debashish Bhattacharya[3]

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The order Magnaporthales (Ascomycota, Fungi) includes devastating pathogens of cereals and grasses, such as the rice blast fungus *Pyricularia* (*Magnaporthe*) *oryzae* and the summer patch pathogen *Magnaporthiopsis* (*Magnaporthe*) *poae*. Magnaporthales also includes saprotrophic species associated with grass roots and submerged wood. Despite its scientific and economic importance, the phylogenetic position of Magnaporthales within Sordariomycetes and the interrelationships of its constituent taxa, remain controversial. In this study, we generated novel transcriptome data from 21 taxa that represent key Magnaporthales lineages of different infection and nutrition modes and phenotypes. Phylogenomic analysis of >200 conserved genes allowed the reconstruction of a robust Sordariomycetes tree of life that placed the monophyletic group of Magnaporthales sister to Ophiostomatales. Among Magnaporthales, three major clades were recognized: 1) an early diverging "wood" clade comprised of saprotrophs associated with submerged woods; 2) a "blast" clade that includes the rice blast fungus and other pathogens that cause blast diseases of monocot plants. These species infect the above-ground tissues of host plants using the penetration structure, appressorium; and 3) a "root" clade comprised primarily of root-associated species that penetrate the root tissue with hyphopodia. The well-supported phylogenies provide a robust framework for elucidating evolution of pathogenesis, nutrition modes, and phenotypic characters in Magnaporthales.

Combining Field and Lab Data to Reach New Heights in the Rutgers Hazelnut Breeding Program

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At Rutgers University, we have been researching hazelnuts and many other nut trees including walnuts, pecans, hickories, and almonds, since the program was started in 1996 by Dr. C. Reed Funk. Hazelnuts were identified early on as one of the best-adapted species and the one with the lowest input requirements and highest potential demand both locally and worldwide for their tasty, yet healthy kernels. However, the fungal disease eastern filbert blight (EFB), caused by *Anisogramma anomala* (Peck) E. Müller, makes it almost impossible to grow the crop in the eastern United States, and host genetic resistance would be needed to have success in this region. Fortunately, in the 1970s a resistant cultivar named 'Gasaway' was found in Washington. It was shown to carry a dominant R-gene and was subsequently used in breeding at Oregon State University with great success. Today, a majority of hazelnut orchards in Oregon are protected by this R-gene and the industry is thriving and expanding yearly. Unfortunately, work at Rutgers has shown that this R-gene does not provide sufficient protection in the East (Capik and Molnar, 2012; Molnar et al, 2010a,b). Most 'Gasaway'-related plants develop EFB over time and succumb to the disease, with pathogenic variation of the fungus being one of the most likely contributing factors to the R-gene's ineffectiveness (Molnar et al., 2010a; Muehlbauer et al., unpublished). However, despite its lack of utility in New Jersey, the existence of 'Gasaway' spurred us to make wide germplasm collections to search for additional sources of resistance to use in the hazelnut breeding program. Starting in 2002, we made a number of seed-based collections of hazelnut across its native range in Europe and the Caucuses, grew many thousands of trees, and exposed them to the fungus. While most trees died, surprisingly about 2% of the plants were found to be resistant and subsequent field tests are showing this resistance to be highly heritable. When combining these new plants with work ongoing in Oregon, we collectively have access to resistant parent plants from Spain, Turkey, Italy, Russia, Crimea, Georgia, Estonia, Latvia, Moldova, Chile, Poland, and Serbia (Capik et al., 2013; Colburn et al., 2015; Muehlbauer et al., 2014; Leadbetter et al., 2015, 2016).

Despite a substantial pool of sources of resistance to EFB, the strong evidence of pathogenic variation present within the pathosystem challenges us with the need to develop durable forms of resistance, especially in hazelnuts where traditional orchard life expectancies are greater than 35 years. Thus, considerable efforts are underway to characterize these new sources of resistance to target more effective breeding strategies. A first step was to use simple sequence repeat (SSR) markers to study the genetic diversity of our new resistant accessions in comparison to 'Gasaway' and other known resistance sources that were representative of much of the world's germplasm of European hazelnut. This work showed tremendous diversity exists within our collection (Muehlbauer et al., 2014), which is vital to support breeding efforts in a highly heterozygous, clonally propagated crop. However, collection origin and genetic relationships can be independent of R-gene allelic variation. These data do not provide sufficient evidence that our new sources of resistance actually differ from one another in respect to their R-genes (they may carry the same R-genes), and more specifically, they may carry the same R-gene as 'Gasaway'. Thus, further work is needed to better understand the genetic resources available for breeding including mapping and comparing quantitative trait loci (QTLs) associated with EFB resistance.

In this study, a tree originating from seed collected in Holmskij, Russia (H3R07P25) in 2002, was selected based on its complete resistance to EFB and disparate origin (SSR-based genetic relationship) from 'Gasaway'. It was crossed in 2011 with an improved EFB-susceptible selection (OSU 1155.009) from Oregon State University to grow out a pseudo F_2 mapping population of 281 individuals. Trees were planted at the Rutgers Horticultural Farm 3 in 2012 and exposed to EFB through natural field infection. In 2015 and 2016 the trees were evaluated using a scale of 0 = no cankers to 5 = all stems containing canker.

In conjunction with the above field evaluations, DNA was extracted from 119 H3R07P25 x OSU 1155.009 mapping population progeny for the purpose of creating a genetic linkage map. Genotyping by Sequencing (GBS) was performed on all 119 mapping population progeny and parents using a *Pst*I-*Msp*I ddRAD-seq procedure similar to the methods described by Elshire et al. (2011) and Poland et al. (2012). The GBS analysis resulted in 2133 single nucleotide polymorphism (SNP) markers being placed on 11 linkage groups using JoinMap at a LOD score of 20. Eighty-four SSR markers, from previous OSU genetic linkage maps, were also placed on the current H3R07P25 x OSU 1155.009 linkage map. These SSR markers were used as "anchor markers" for linkage group (LG) assignment/comparison to previously developed OSU genetic linkage maps. A putative major QTL (LOD > 50) for EFB resistance was identified on hazelnut linkage group 2 using MapQTL. The 'Gasaway' resistance QTL was previously identified on hazelnut linkage group 6 (Mehlenbacher et al., 2006; Sathuvalli et al., 2012). The current results help to confirm the existence of EFB resistant germplasm in the Rutgers hazelnut breeding program that is different than the previously identified 'Gasaway' resistance. This finding is particularly important when considering that the 'Gasaway' resistance source has been shown to break down under high disease pressure in the eastern United States.

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Public and Private Land Managers Examining Fine Fescues as a Vegetation Choice for the Future

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Public and private land managers are interested in turfgrass for a diverse range of landscape management alternatives. While turfgrass is a dominant vegetation choice for urban public and private land cover, cool season fine fescues (*Festuca* spp.), which provide an opportunity to reduce maintenance and input requirements, are not widely utilized. Public land managers balance many factors in turf selection such as cost, maintenance requirements, the amount of use, and tolerance of environmental stress. So what are public land managers' perceptions about low-input turfgrasses in relation to their fine fescue trait preferences? For example, we found that while all traits are important to public land managers, tolerance traits are most important and these traits are perceived most negatively in low-input turfgrasses such as fine fescues (Meyer et al.). Among private residential landowners, what are consumers' preferences and willingness to pay for turfgrass attributes? For example, we identified three consumer segments based on participants' heterogeneity in their preferences for turfgrass attributes: *Balanced Consumers*, *Low-input Conscious Consumers* and *Appearance Conscious Consumers* (Yue et al.). To select and manage cool season fine fescues, consumers need adequate information about turfgrass attributes and lawn maintenance practices when they choose what types of turfgrasses to grow in their home lawns. We found turfgrass consumers trusted information from families, university extension, and garden centers the most (Yue et al.). Finally, public land managers are responsible for making complex decisions about vegetation choices and the subsequent management of those spaces. Despite their potential role in promoting environmentally sustainable practices, their perceptions and beliefs are largely unknown as it relates to vegetation management, specifically low-input turfgrasses. Overall, we found that managers demonstrated an interest in sustainability on the lands they managed through favorable views towards low-input turfgrasses, and support of municipal programs to convert both public and private lands to low-input turfgrasses (Barnes et al.).

The Role of the Modern Golf Course Superintendent: It's More Than Just Growing Grass

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The modern day superintendent has to have more than just an Agronomy or Turfgrass Science degree. There was a time when the superintendents were called greenkeepers and many had no agronomic skills or formal training to prepare them for a career in Turfgrass Management. At one time, golf professionals were in charge of the course maintenance. If you could play the game well then you should be able to maintain the turf. One greenkeeper that I am aware of was selected primarily because he was a good player and he was Scottish.

I will briefly discuss the expectations of the facilities, members and players of today. A Turfgrass degree or certificate is now a standard prerequisite and a requirement in most job descriptions. Many courses today will prefer to hire a superintendent with a bachelors or master's degree. Many of the Associate degree and certificate programs will provide an individual with the basic skills and scientific knowledge to fulfill an individual's need in an entry level position. Most students today will complete several comprehensive internships at various facilities and in different climatic zones of warm and cool season grasses.

The educational needs of turf managers today vary widely. Turf Managers today must possess a wide array of scientific, business and technical skills to be successful. Aside from a thorough knowledge of Turfgrass, managers must have a firm grasp of Plant Pathology, Personnel Management, Accounting, Horticulture, Arboriculture, Environmental science, Irrigation systems management, drainage, carpentry, basic electricity, plumbing, and a host of other technical skills. Successful superintendents must demonstrate a mastery of both verbal and written communication skills.

Today's superintendents must also possess a strong work ethic and understanding that the they are dealing with a living entity that in some cases requires many hours of observation and nurturing. Golf turf managers should be able to play and understand the game of golf to better deal with the player issues at various facilities. Preparing for major events is also something that may have to be addressed during their career path. Hosting Major events and dealing with entities such as the USGA, PGA of America and the PGA Tour may also require a different set of skills when dealing with events of that magnitude.

Most of a superintendent's career will involve building strong relationships and working with consultants, universities and associations to develop a successful strategy. Superintendents must constantly take advantage learning opportunities such as field days and local and national conferences and shows. Continuing education should always be on the path to a successful career.

Traffic Tolerance of Fine Fescues: Techniques for Screening Germplasm

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Fine fescues (*Festuca* spp.) are low maintenance grasses with better shade and drought tolerances compared to other cool-season grasses, and are often used as low-input components in mixtures with other grasses. However, these grasses are not utilized to the same extent as other species partially due to poor traffic tolerance and recuperative ability. Enhancing traffic tolerance and recuperative ability of fine fescues requires the development of germplasm screening methods to assess the response of fine fescues to traffic. It is not clear whether the performance of fine fescues under traffic stress is dependent on the form of traffic and/or the time of year that traffic response is assessed. It is also not clear whether plant characteristics such tissue fiber and protein content are associated with traffic tolerance. Therefore, the objectives of our research are to evaluate the aspects of traffic form and season of traffic as potential factors related to traffic tolerance of fine fescue and determine the relationship of tissue fiber and protein content with traffic tolerance of fine fescues.

Two studies were initiated in 2012 to evaluate the effect of traffic form (abrasive wear vs. trampling) and season of wear (spring, summer or autumn) on ten fine fescues using split-plot designs with four replications. The traffic factors were arranged as main plots. The ten fine fescues entries were arranged as the subplot factor and consisted of: 'Aurora Gold' and 'Beacon' hard fescue (*Festuca brevipila* R. Tracey), 'Culumbra II' and 'Radar' Chewings fescue [*F. rubra* L. subsp. *fallax* (Tuill.) Nyman], 'Marvel' and 'Garnet' strong creeping red fescue (*F. rubra* L. *rubra*), 'Shoreline' and 'Seabreeze GT' slender creeping red fescue (*Festuca rubra* L. var. *littoralis* Vasey ex Beal), 'Quatro' sheep fescue (*F. ovina* L.) and 'Blueray' blue x hard fescue (*F. glauca* Vill. x *F. brevipila* R. Tracey). Traffic treatments were initiated in autumn 2013, and concluded after autumn in 2016. Uniformity of turf cover (1 to 9 scale; 9 being most uniform visual appearance), fullness of turf canopy (100% representing a full canopy), and leaf bruising (1 to 9 scale; 9 representing no bruising) were visually rated after each wear period. Green cover was measured using digital image analysis at the end of each wear period. Surface bulk density and volumetric water content were measured by gamma-ray scattering and detection of thermalized neutrons (Troxler, Model 3440), respectively, in each year.

Traffic Form Study

Over the ten seasons of assessment (autumn 2013 to autumn 2016), analysis of variance (ANOVA) indicated little to no difference in the loss of turf uniformity between the two traffic forms until spring 2015. From spring 2015 to autumn 2016, abrasive wear caused greater reduction in uniformity of turf cover compared to trampling. The amount of variation in the loss of turf uniformity was attributable to the entry factor was greater than the traffic form factor during the first half of the study (autumn 2103 to spring 2015); whereas, the traffic form factor was responsible for a greater amount of the variation than the entry factor from summer 2015 to autumn 2016. The uniformity response of fine fescues was independent of traffic form except during the seasons of autumn 2014 through autumn 2015, when more differences among fine fescue entries were evident under abrasive wear than trampling.

Similarly, the ANOVA of the reduction in the fullness of turf canopy (FTC) indicated little to no difference between two forms of traffic until summer of 2015. From autumn 2015 to autumn 2016, however, abrasive wear caused a greater reduction in FTC compared to trampling. The amount of variation in FTC explained by the fine fescue entry factor was greater than the traffic form factor from autumn 2013 to spring 2015; whereas, the traffic form factor accounted more for the total variation than the fine fescue factor from autumn 2015 to autumn 2016. The FTC response of fine fescues was independent of traffic form through most of the study except during spring and summer 2015. Greater differences in FTC among fine fescues were observed under abrasive wear than trampling during spring and summer 2015.

Leaf tissue bruising was more evident under abrasive wear than trampling throughout most of the study. Fine fescues were more prone to leaf bruising during autumn and summer compared to spring. The leaf bruising response of fine fescues depended on the traffic form during five out of the ten assessment seasons; greater differences in leaf bruising among fine fescues occurred under abrasive wear than trampling. Quarto exhibited the least bruising during autumn but was more prune to leaf bruising during spring and summer. In contrast, leaf bruising of Radar was more severe during autumn than spring and summer.

The ANOVA of green cover indicated that abrasive wear caused a greater reduction in green cover compared to trampling during all assessment seasons. The amount of variation attributable to the traffic form factor was greater than entry factor during nine out of the ten seasons of assessment. The green cover response of fine fescues was always independent of traffic form throughout the study.

Surface bulk density, measured after the third, sixth, and ninth seasons of traffic indicated that trampling compacted the turf and soil more than the untreated control. Compaction resulting from abrasive wear was intermediate to the untreated control and trampling treatment. Surface bulk density decreased over time due the development of shoot and thatch biomass as the grass matured.

Season of Wear Study

Over the three years of assessment (autumn 2013 to summer 2016), the ANOVA indicated no difference in the seasonal loss of turf uniformity until the third year (autumn 2015 to summer 2016). Summer wear caused a greater reduction in turf uniformity than autumn wear; the uniformity of turf cover during spring wear was intermediate to summer and autumn wear. The fine fescue factor explained more of the variation in uniformity response than the season factor throughout the study, and interacted with the season factor during the second and third year of the study. This interaction indicated that greater differences in uniformity among fine fescue were observed during summer wear; fine fescue differences during spring wear tended to be greater than during autumn wear when there were few to no differences.

Similarly, the loss in FTC was not affected by the season of wear until the third year, when summer wear caused a greater reduction in FTC than spring and autumn. The amount of variation in FTC attributable to the entry factor was greater than the season factor in all three years; however, the fine fescue by season interaction explained a large amount of the variation in FTC during the third year. During the first two years, the performance of Blueray, Quarto and Beacon were consistently better than the strong creeping red fescues (Marvel and Garnet) and slender creeping red fescues (Shoreline and Seabreeze GT). During the third year of the study, these relative differences among cultivars were only evident during summer wear; fewer differences among fine fescues were observed during spring and autumn wear.

Leaf bruising of fine fescues was more severe during autumn and summer wear than spring wear in all three years. Leaf bruising was minimal during spring of year 1 and 3, and mild during spring of year 2. Additionally, the differences in leaf bruising among fine fescues was dependent on the season of wear in all three years. Quatro, Shoreline and Seabreeze GT were more vulnerable to leaf bruising during summer than autumn while Radar, Garnet and Marvel were bruised more during autumn than summer.

The season of wear had a significant effect on the loss of green cover during all years and interacted with the fine fescue factor during the third year of the study. Autumn wear reduced more green cover than spring and summer wear during the first year, whereas spring wear and summer wear caused the greatest reduction in green cover in years two and three, respectively. Garnet, Shoreline and Seabreeze GT were always among the cultivars exhibiting the greatest loss of green cover during the first two years. During the third year, the greatest loss of green cover occurred during summer; Chewings fescue (Radar and Culumbra II), strong creeping red fescue (Marvel and Garnet) and Shoreline slender creeping red fescue lost 30 to 36% of green cover during summer wear.

Summary

During the first half of the study Initially, the ability of fine fescue turf to maintain uniformity and FTC under traffic stress was influenced more by the entry than the form of traffic. As the study progressed, however, the form of traffic (abrasive vs. trampling) became the more dominant factor suggesting that that fine fescue germplasm has a finite influence on persistence under long term traffic.

The ability of fine fescue turf to maintain a uniform and full canopy in different seasons was largely effected by genetic difference (entry factor) rather than season of wear. However, the persistence of fine fescue during the third of study indicated that summer wear resulted in a greater damage among fine fescues than spring and summer.

The leaf bruising response of fine fescue to traffic is complicated by the fact that responses varied with season, and some cultivars maintained a relatively uniform, dense canopy but were prone to severe leaf bruising. Evaluation of this characteristic needs to be evaluated in both the summer and autumn.

Potential mechanisms associated with traffic tolerance among fine fescues have not been well investigated. Studies have been initiated to evaluate the effects of species, age of turf, and N fertilization on the tissue fiber and protein contents of fine fescues. Data analysis to identify potential correlations of these factors with traffic tolerance among fine fescues is ongoing. Factors strongly associated with traffic tolerance could be useful as germplasm screening parameters within a breeding program.

Turfgrass Stress Tolerance and Management in Response to Elevated Atmospheric Carbon Dioxide

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Drought and heat stress are primary abiotic stresses of cool-season plant species and impose significant limitations to turfgrass performance and aesthetics throughout the world. Within the context of global climate change, mean air temperatures are steadily rising and precipitation due to rainfall events is becoming less frequent in many areas. One of the primary contributors to altered weather patterns is the gradual increase of atmospheric carbon dioxide (CO_2), which has nearly doubled since the beginning of industrial era. The physiological effects of elevated CO_2 on various aspects of plant growth and development are generally considered to be positive, though the majority of previous research has been conducted on agronomic crop species with minimal focus on turfgrass species. Healthy turfgrass stands serve key functions throughout the environment including erosion control, surface water detoxification, and control of allergens and disease. Given the wide distribution and utilization of turfgrass in North America (≥35,000 square kilometers), a greater understanding of potential changes in turfgrass growth and stress response with increasing atmospheric CO_2 concentration is critically important and may have far-reaching implications across many aspects of environmental stewardship and turfgrass management. Our lab has recently investigated how the major turfgrass species used for golf or lawn and landscape settings respond to heat and/or drought stress under elevated CO_2 environments. The results, which detail a wide array of turfgrass physiological and biochemical responses under these conditions, are beginning to suggest potential implications for future generations of turfgrass managers and will be discussed.

Understanding and Managing Insecticide Resistance in the Annual Bluegrass Weevil

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Insecticide resistance is caused by overuse and misuse of insecticides which commonly occurs in commodities with low tolerance to pest damage and a dearth of effective alternatives to the insecticides. The annual bluegrass weevil (ABW), *Listronotus maculicollis* (Coleoptera: Curculionidae), is an excellent example for this resistance development scenario. ABW larvae can cause severe damage to tees, fairways, collars, and greens on golf courses. Due to the presence of several generations per year and concurrent presence of multiple life stages with increasing asynchrony during the growing season, ABW is one of the most difficult-to-manage turfgrass insect pests in North America. Alternatives to synthetic insecticides are only more recently being explored.

Golf course superintendents have heavily relied on synthetic insecticides for ABW management, primarily using broad-spectrum adulticides (pyrethroids, chlorpyrifos) to control overwintered adults in spring before egg-laying starts. Resistance to pyrethroids was first reported from New England and seems to be on the rise. A recent survey (McGraw and Koppenhöfer, 2017) indicated that throughout the area affected by the pest (all states of Mid-Atlantic and Northeast regions of the United States and southeastern Canada), 19% of golf courses experience problems in controlling the pest, regionally up to 55% (Long Island). To make matters worse, the only other effective adulticide, chlorpyrifos, and many, in extreme cases most, available larvicides seem to be less effective against pyrethroid-resistant ABW populations. This broad resistance is likely related to the fact that enhanced enzymatic detoxification, a rather non-specific resistance mechanism, is at least in part responsible for the resistance.

There is a dire need to develop a better understanding of the extent and scope of insecticide resistance as a base for optimizing the use and longevity of existing insecticides for ABW management.

Laboratory and greenhouse studies

Previously we had determined through topical applications in the laboratory the resistance levels of adult ABW from nine populations to two pyrethroids and also found limited resistance to several other insecticides from different insecticide classes. The populations collected at Rutgers Horticultural Farm 2 (HF), North Brunswick, NJ and at Pine Brook GC (PB), Manalapan, NJ were determined to be pyrethroid-susceptible. The other populations had various levels of pyrethroids resistance/tolerance, with resistance ratios (RR_{50} = LD_{50} of resistant / LD_{50} of susceptible population) ranging from 14 to 343 for bifenthrin and 8 to 324 for λ-cyhalothrin. Pyrethroid resistant populations also demonstrated elevated tolerance to chlorpyrifos (RR_{50} 3.3–15.5), clothianidin (RR_{50} 2.9–9.7), and spinosad (RR_{50} 3.0–5.1). Topical assays with indoxacarb and chlorantraniliprole did not yield meaningful dose-response curves due to low mortality for the resistant populations. Different types of assays may need to be employed to study these compounds. We have since then repeatedly observed moderate to high levels of resistance ($RR_{50} > 20$) among these ABW populations which have not change significantly over the years of the study.

Resistance levels in adult ABW of the tested populations were significantly reduced in the presence of the enzyme inhibitors PBO and DEF, confirming that enzymatic detoxification plays important role in ABW resistance to pyrethroids. Combinations of synergists (oxidase inhibitor PBO, glutathione transferase inhibitor DEM, esterase inhibitor DEF) and bifenthrin or chlorpyrifos were tested in laboratory bioassays against adults from seven ABW populations. Bifenthrin toxicity was significantly increased in presence of PBO (8–20 fold) and DEF (9–39 fold), which indicates involvement of oxidase and esterase systems as possible resistance mechanisms. DEM had a weak effect on bifenthrin toxicity for most populations. Synergists did not significantly affect chlorpyrifos toxicity in our study.

To determine and compare the level of adult and larval resistance to major insecticide modes of action, selected insecticides of different chemical classes (see above) were tested against susceptible and resistant ABW populations in greenhouse experiments. For adult assays, 10 adults were caged in *Poa annua* pots 2 h before treatments were applied using a Generation III Research sprayer. For larval assays, larval populations were created by caging adults (3 pairs) in containers with established *P. annua* for 1 week to lay eggs. Treatments were applied 10 days after adult removal (average larval stage ~3-3.5 instar), and mortality was evaluated 10 days after application. Results of our greenhouse adult bioassays were consistent with results obtained in other assay types including the topical laboratory test on resistance levels (RR_{50}) of various populations to different insecticides. Larvae of the resistant populations were less susceptible to chlorantraniliprole, bifenthrin and chlorpyrifos compared to susceptible populations. These insecticides provided higher percent reduction in susceptible populations (80–90%) compared to resistant populations (up to 57% reduction). Percent reduction provided by spinosad and indoxacarb differed only between the most resistant and susceptible populations.

Field Experiments

Any to-date field observations on resistance originate from product efficacy testing trials that are generally poorly designed to truly understand how resistance affects product efficacy and that have, if at all, characterized resistance in simple yes/no Petri dish assays. Only by studying the individual tools separately (different products applied only once at specific times) will it be possible to understand how to put together optimal management programs for different resistance levels. Our ongoing research is specifically designed to test the efficacy of individual applications of the commonly used adulticides and larvicides on fairways at four golf courses representing the full spectrum of pyrethroid-resistance as clearly characterized in our lab studies. Resistance ratios (RR_{50}s) to the pyrethroid bifenthrin at the four courses were 2, 30, 95, and 343.

To keep the size of experiments manageable, insecticide applications targeting adults were tested in separate experiments from those targeting larvae. Adulticides (Table 1) were applied at the optimal timing to control overwintered adults, i.e., when most adults have moved onto the short mown areas in spring but before females start laying eggs. Timing was determined by weekly vacuum sampling of adults, degree day accumulation (base 50 °F starting March 1) (120 GDD_{50}), and indicator plant phenology (*Forsythia* sp. half gold : half green). Adulticides applied were bifenthrin (Talstar; 0.1 lb ai/ac = 112 g ai/ha), chlorpyrifos (Dursban; 1.0 lb ai/ac = 1121 g ai/ha), indoxacarb (Provaunt; 0.225 lb ai/ac = 252 g ai/ha), and spinosad (Conserve; 0.4 lb ai/ac = 448 g/ha). Adulticides were watered in with 0.04" (1 mm) of overhead irrigation.

Larvicides were applied to target young larvae around late bloom of flowering dogwood (200 GDD_{50}) and mid-size larvae around full bloom of hybrid Catwba rhododendron, 400 GDD_{50}).

Treatments were evaluated at around 700 GDD_{50} when most developmental stages were around the fifth instar. Early larvicides applied were chlorantraniliprole (Acelepryn; 0.156 lb ai/ac = 175 g ai/ha), cyantraniliprole (Ference; 0.156 lb ai/ac = 175 g ai/ha), and clothianidin (Arena; 0.247 lb ai/ac = 277 g ai/ha). Early larvicides were watered in with 0.1" (2.5 mm) of overhead irrigation. Late larvicide applied were chlorantraniliprole (Acelepryn; 0.156 lb ai/ac), cyantraniliprole (Ference; 0.156 lb ai/ac), and clothianidin (Arena; 0.247 lb ai/ac), indoxacarb (Provaunt; 0.225 lb ai/ac), spinosad (Conserve; 0.4 lb ai/ac), and trichlorfon (Dylox; 6.0 lb ai/ac = 6725 g ai/ha). Adulticides were watered in with 0.2" (5 mm) of overhead irrigation.

For adulticides, we observed no interaction between resistance level and insecticides. Control of the 2x and 30x resistant populations was higher than at 95x, and control was the lowest at 342×. All insecticides caused significant control but there were no differences among insecticides. At 2× and 30×, bifenthrin (62–66%), indoxacarb (55–61%), and spinosad (50–58%) but not chlorpyrifos (39–42%) significantly reduced ABW populations; at 100× Talstar (44%) but not spinosad (32%), chlorpyrifos (23%), and indoxacarb (18%) caused significant reduction; and at 343x none of the insecticides caused significant reduction (0–22%).

For larvicides (Fig. 1, right), timing of application did not affect efficacy of Acelepryn, Ference, and Arena. Resistance level and insecticide interacted significantly. Ference was not affected by resistance level (69–94%); Conserve only showed a difference between 30× (90%) and 343× (52%), but not between any other timings (72–84%); and all other insecticides were significantly affected by resistance, although, due to high variability in the data, not always consistently. Provaunt was the only remaining insecticide that was effective up to the 95× resistance level (68–84%) but was ineffective (8%) at 343×. Acelepryn efficacy (dates combined) declined from 54–59% at 2× and 30× to 25% at 95× (not significant) and 6% at 343× (not significant). Arena efficacy (dates combined) declined from around 50% at 2× and 30× to 6–18% at 95× and 343× (not significant). And trichlorfon efficacy declined from 68–70% at 2× and 30× to 24–28% at 95× and 343× (not significant).

Repeating these experiments should further clarify the effects of resistance on the efficacy of various insecticides. However, the findings already indicate that Ference might be the most effective insecticide against highly resistant ABW population followed by Conserve. Provaunt seems to be the only other insecticide that does not seem to be affected up to the 95× level, but it is completely ineffective at the 343× level.

The Future of Turfgrass Weed Science at Rutgers

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The turfgrass weed science program will see a new research focus under the direction of a new faculty member. The new program will focus on three main objectives. The first objective is to develop best management practices for perpetually problematic weeds such as annual bluegrass (*Poa annua*) as well as weeds that have become more problematic in recent years such as kyllinga (*Kyllinga* spp.) and goosegrass (*Eleusine indica*). Exploring new cultural and chemical management strategies in combination with new turfgrass varieties will guide the development of more economical and effective weed control strategies.

The program will also investigate biological and natural products for weed control. For example, a field research effort will investigate annual bluegrass weevil (*Listronotus maculicollis*) as a biocontrol agent for annual bluegrass in creeping bentgrass (*Agrostis stolonifera*) fairways. A laboratory and greenhouse project will investigate previously isolated bacterial endophytes on a large spectrum of economically relevant weeds and desirable turfgrass through laboratory and greenhouse trials.

The third objective will be to understand what influences weed competitiveness in turfgrass systems. The influence of certain abiotic factors will be investigated, but more effort will be devoted to elucidate microbial associations with various weeds and their influence on weed competitiveness. Another facet of the project will try to elucidate how weeds or weed control practices influence the microbiome as part of a much larger effort to better understand the turfgrass microbiome.

Bentgrass Tolerance and Fungicide Timing Effects on Dollar Spot

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Dollar spot disease, caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett, is a common and persistent disease of turf throughout the world. More money is spent to control this disease on golf courses than on any other in the United States. Thus, practices that reduce fungicide inputs needed to control dollar spot on golf courses could provide substantial economic and environmental benefits.

The timing of fungicide applications for dollar spot control could be more effective if outbreaks were reliably predicted, potentially reducing annual fungicide inputs. Two weather-based models have been recently developed for forecasting dollar spot activity on bentgrass (*Agrostis* spp.) turf. The first model uses growing degree days (GDD) to predict the first occurrence of dollar spot symptoms during the spring. This GDD model was developed by Ryan et al. (2012) and uses a base air temperature of 15°C (59°F) and a start date of April 1st. The second model uses air temperature and relative humidity as variables in a logistic regression approach to predict the development of dollar spot epidemics throughout the entire growing season (Smith 2013). These models have not been validated on bentgrass cultivars that are tolerant (e.g., Declaration) to this disease and have not been field tested in the metropolitan region around New Jersey.

The objectives of this field study were to evaluate the effect of initial (pre-symptomatic) and subsequent timings of fungicide application on dollar spot incidence and development of susceptible and tolerant bentgrass cultivars maintained at 12.7 mm (0.5 in). The goal was to determine the extent that pre-symptomatic fungicide application(s) affect total fungicide usage over a growing season when subsequent fungicide applications are based on either a disease-threshold or predictive-model. Treatments in this trial were arranged as factorial combinations of bentgrass tolerance to dollar spot, initial fungicide application timing, and subsequent fungicide timing. The cultivars Declaration (more tolerant) and Independence (susceptible) were used for the bentgrass tolerance factor. Initial fungicide application timings included threshold-based (< 2 infection centers / 0.74 m^2 [8 ft^2]); calendar-based (20 May); logistic regression model-based (20% risk index); and five GDD ranges (20-30, 30-40, 40-50, 50-60, or 60-70). Three subsequent fungicide timings were based on the logistic regression model, disease threshold, or were withheld completely to assess the long-term effects of the initial fungicide timings. A calendar-based application program (three week intervals from 20 May to 21 and 23 November during 2015 and 2016, respectively) was also included for comparison. All possible combinations of initial and subsequent fungicide timings were applied to both cultivars. Boscalid (Emerald 70WG, BASF) at 0.384 kg a.i. / ha (0.18 ounces product / 1,000 ft^2) was used for all fungicide applications. Threshold-based plots were monitored as often as daily for dollar spot incidence. The number of applications to each plot was recorded.

The factors of subsequent fungicide timing and bentgrass cultivar had a much greater impact on long-term (May through November) disease control than the initial fungicide application factor during 2015 and 2016. Excellent [<2 infection centers / 0.74 m^2 (8 ft^2)] long-term control of dollar spot was achieved for both cultivars each year when subsequent fungicide timing was based on either the logistic regression model or the calendar-based program. The logistic regression model either had no effect on or reduced fungicide inputs by one or two applications (depending on the initial fungicide timing) compared to the calendar-based program (nine applications). Good to excellent, long-term disease control was also achieved when subsequent fungicide timing was based on a threshold program. The total fungicide usage and the level of disease control achieved by the threshold timing depended on the cultivar and, to a lesser extent, the initial fungicide timing. Subsequent, threshold-based fungicide applications on Declaration plots produced excellent disease control; a total of three and one fungicide applications were made during 2015 and 2016, respectively, regardless of the initial fungicide application timing. In contrast, Independence plots received six or seven threshold-based subsequent applications during 2015 and four or five applications during 2016, depending on the initial fungicide timing. Moreover, disease incidence on Independence plots occasionally surpassed the threshold value during the growing season (up to 6 and 9 infection centers / 0.74 m^2 (8 ft^2) during 2015 and 2016, respectively), which may not be considered an acceptable incidence by some golfers. This research will be continued during 2017.

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POSTER PRESENTATIONS

Fiber and Protein Content of Fine Fescues as Affected by N Fertilization

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Fine fescues (*Festuca* spp.) are low maintenance grasses that could be more widely used if the traffic tolerance of these species were improved. Fiber and protein affect shoot tensile strength, rigidity and elasticity and thus may have effects on wear tolerance. The objective of this study was to assess the effect of nitrogen fertilization on fiber and protein content of fine fescues. The trial was arranged in a 3 × 4 factorial split-plot design with 4 replications. The main plot factor consisted of three fine fescue species (*Festuca brevipila* R. Tracey 'Beacon', *F. rubra* L. *rubra* 'Garnet', *F. rubra* L. subsp. *fallax* (Thuill.) Nyman 'Rushmore') seeded in September 2012 on a loam in North Brunswick, NJ. The subplot factor was four levels of nitrogen fertilization: 0, 49, 98, and 146 kg ha^{-1} yr^{-1}. Nitrogen fertilizer treatments were applied in four split applications in May, June, August and September. The trial was mowed at 6.4 cm and irrigated to avoid drought stress. Pests were controlled as needed. Turf quality, turf color, chlorophyll contents and normalized difference vegetation index (NDVI) were assessed monthly. Verdure samples were collected before the first N application (May) and after the second (July) and fourth (October) N application in 2015. The same sampling sequence will be repeated in 2016. Verdure samples were used to determine leaf water content, biomass, fiber analysis (total cell wall, hemicellulose, lignocellulose, lignin and cellulose content), and protein content. As expected, better turf quality, darker turf color and higher chlorophyll content were observed for fine fescues fertilized with N during the first year of the trial. Nitrogen fertilization also increased leaf water content but had no significant on fiber content of the three fine fescue species. Results from the second year will be reported.

Turfgrass Disease Protection Using Bacterial Endophytes *Bacillus amyloliquefaciens, Bacillus pumilus* and *Pantoea agglomerans*

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Increasing evidence suggests that many plants in nature host endosymbiotic microbes. Although the interaction mechanisms between endosymbiotic microbes and plants are not well understood so far, several studies suggest that in some cases, endophytes may serve to increase fitness of plants. For example, fungal endophytes have been shown to enhance the resistance of host plants to abiotic and biotic stresses. In grasses, fungal endophytes of the genus *Epichloë* are able to provide resistance to insects and diseases. Research on plant root-associated endophytic bacteria demonstrates that they also promote plant growth in a variety of ways, such as enhancing seed germination rates and increasing seedling growth. In our study, we isolated several endophytic bacterial strains from cool-season turfgrasses, identified these as *Bacillus amyloliquefaciens*, *Bacillus pumilus* and *Pantoea agglomerans* using DNA sequence analysis. Most *B. amyloliquefaciens* strains showed antifungal effects on PDA, creating a 3–8 mm inhibition zones between fungal pathogens and bacterial endophytes. All three bacterial endophytes promoted growth and increased host fitness of tall fescue and perennial ryegrass under conditions of pathogen exposure. In presence of pathogens *Sclerotinia homoeocarpa* and *Rhizoctonia solani*, grasses with endophytic bacteria grew better than the control without bacterial endophytes. Grasses inoculated with endophytes showed larger root systems with a greater dry weight than plants without endophytes. MALDI-TOF MS analysis and PCR assays suggest that the effective antifungal compounds from *B. amyloliquenfaciens* were from three groups of lipopeptides, iturin, fangycin and surfactin.

CRISPR - Gene Editing of Creeping Bentgrass to Improve Stress Tolerance and Disease Resistance

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Creeping bentgrass is one of the most widely used cool-season grass species on golf courses. However, there is not one cultivar of creeping bentgrass that is considered completely resistant to dollar spot disease caused by *Sclerotinia homoeocarpa* F.T. Bennet. Creeping bentgrass is also stressed by heat and drought during summer months. In this project, we use the CRISPR/Cas (clustered regularly interspaced short palindromic repeats-associated endonuclease)-gene editing technology to knock-out genes involved in disease susceptibility and negative stress regulation in creeping bentgrass to improve disease resistance and stress tolerance. Using bioinformatics tools, we have identified EST (expressed sequence tag) sequences for the following three genes that are related to disease and stress susceptibility in creeping bentgrass Crenshaw cultivar: *BONL* (*BONZAI1-like*), *CPKL* (*calcium-dependent protein kinase 12-like*) and *DREBL* (*dehydration responsive element binding 1c-like*). We have cloned the partial cDNAs of these three genes from Crenshaw creeping bentgrass by RT-PCR (reverse transcription-polymerase chain reaction). We have also cloned and sequenced partial genomic DNA (gDNA) sequences of these three genes from Crenshaw. The gene target sites have been identified in these genes' gDNA sequences, and the CRISPR-editing vectors containing the gRNA (guide RNA) sequences and the Cas9 nuclease cassettes have been constructed. We have developed the tissue culture system for Crenshaw creeping bentgrass transformation, and the CRISPR-gene editing vectors have been transformed into callus tissues by gene gun bombardment. The CRISPR-gene editing platform is being extended into other turfgrass species.

Rolling and Dew Removal Effects on Dollar Spot Disease of Creeping Bentgrass

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Dollar spot (caused by *Sclerotinia homoeocarpa*) is a prevalent disease on golf course turfs throughout the United States. The effect of lightweight rolling on dollar spot incidence of creeping bentgrass (*Agrostis stolonifera* L. 'Independence') was assessed in a field study maintained at 12.7 mm on a sandy loam in North Brunswick, NJ. A 2 x 2 factorial arranged in a randomized complete block design with four replications was used to evaluate the time of day [morning (AM) or afternoon (PM)] and frequency (3 or 6 d wk^{-1}) of lightweight rolling during the fall of 2015, spring of 2016 and fall of 2016. Two additional treatments were included in the design: morning removal of dew using an absorbent fabric and an untreated control. Plots rolled in the morning when dew was present had the greatest impact on disease incidence, reported as the area under disease progress curve (AUDPC), reducing AUDPC by 52, 33 and 39% compared to PM rolling during the fall 2015, spring 2016 and fall 2016, respectively. The frequency of rolling interacted with time of day to affect dollar spot incidence on a few observation dates in fall 2015. The interactions suggested that more frequent AM rolling (6 d wk^{-1}) may be more effective at reducing disease incidence compared to less frequent AM rolling (3 d wk^{-1}), whereas rolling frequency did not affect disease with PM rolling. There was no effect of rolling frequency on disease during the spring regardless of the time of day. A time of day by frequency interaction during fall 2016 again suggested that more frequent AM rolling (6 d wk^{-1}) reduced disease incidence compared to less frequent AM rolling (3 d wk^{-1}); whereas, more frequent PM rolling (6 d wk^{-1}) increased disease incidence compared to less frequent PM rolling (3 d wk^{-1}). Removal of dew using an absorbent fabric reduced AUDPC by 74, 53 and 60% during the fall 2015, spring 2016 and fall 2016, respectively, compared to the untreated control. This reduction from dew removal with an absorbent fabric was similar to the disease reduction from AM rolling 6 d wk^{-1} during fall 2015, spring 2016 and fall 2016. This research will be continued during the spring of 2017.

Heritability of Simulated Wear Tolerance in Three Fine Fescue Species

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Communities and local governments around the country are currently focusing on reducing environmental effects and reducing the costs of recreational and homeowner landscapes. One of the main areas of concern is maintaining turfgrass areas. Currently research is being conducted in all facets of turfgrass breeding to develop lower maintenance turfgrass cultivars. Fine fescues (*Festuca* spp.*)* have been identified as potential candidate species for immediate application. Fine fescues have been shown to be drought resistant and require less mowing and fertilization compared to traditional species used in lawn and recreational sites. However, one of their drawbacks is that they are regarded to be less tolerant to wear and traffic. Information is lacking regarding the heritability and potential for improvement of many performance characteristics in the fine fescues including wear tolerance. Two individual replicated field studies, which included 159 chewings fescue (*F. rubra* L. subsp. *fallax* (Thuill.) Nyman), 158 hard fescue (*F. brevilipa* R. Tracey), and 148 strong creeping red fescue (*F. rubra* L. subsp. *rubra*) entries, were conducted in New Brunswick, NJ and Minneapolis, MN during 2015 and 2016 to determine the response and broad-sense heritability estimates for wear tolerance. Heritability estimates for the three species on a clonal basis fell between ($0.68 \leq H^2 \leq 0.81$) in the New Jersey location and ($0.44 \leq H^2 \leq 0.66$) in the Minnesota location. On a single plant basis heritability estimates for the three species fell between ($0.30 \leq H^2 \leq 0.45$) in the New Jersey location and ($0.08 \leq H^2 \leq 0.17$). These estimates indicate that genotypic variance led to response and show the possibility for improvement of wear tolerance in fine fescues through recurrent breeding methods based on selection of clonal plants versus single plants.

Screening Kentucky Bluegrass Cultivars and Selections Under Reduced Irrigation and Fertility

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Kentucky bluegrass (*Poa pratensis* L.) is a cool-season turfgrass species that is frequently used in the United States and Canada for golf courses, sports fields, and home lawns. The utility of Kentucky bluegrass as a turfgrass is attributed to characteristics such as good traffic tolerance and winter hardiness. In addition, the highly rhizomatous growth habit of Kentucky bluegrass provides a dense turf canopy and makes it the primary species grown for sod production.

Over the past decade, the Rutgers breeding program has developed over 60 new Kentucky bluegrass cultivars. Experimental selections are screened for traits such as improved turfgrass quality, disease resistance and better abiotic and biotic stress tolerance compared to older cultivars. After approximately five years of screening, the top performing selections are advanced as new, commercially available cultivars.

One area of research/breeding that has been lacking, is the evaluation and development of cultivars under reduced inputs such as fertilizer and irrigation. The sustainability of the turfgrass industry, as a whole, is centered around the ability to conserve finite resources while maintaining superior turfgrass conditions. Traditionally, Kentucky bluegrass is maintained using frequent applications of fertilizer and irrigation; however, it is not well known how Kentucky bluegrass performs when these resources are limited. Consequently, the main objective of this research was to evaluate cultivars and experimental selections of Kentucky bluegrass managed with reduced mowing, irrigation, pesticide, and fertilizer applications in order to make cultivar recommendations for turfgrass managers and to develop new commercially available cultivars.

In September 2013, 317 cultivars and selections were seeded at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ. Turf quality (1 – 9, 9 = best overall quality) was visually rated during the growing seasons of 2015 and 2016 while percent green (1 – 100%) was visually measured as a means of estimating drought tolerance. Differences in turf quality and percent green were detected among the cultivars and selections for each rating date/year combination. The experimental selection A00-1400 had better turf quality compared to many of the other cultivars and selections evaluated when averaged over 2015-2016 and for several other rating dates during the summer of 2015-2016. In addition, this selection had high percent green ratings in July 2015 (66.7%), July 2016 (83.3%), and August 2016 (63.3%). In some cases, certain cultivars (such as ‘Green Star’) had high percent green ratings (July 2015; 80%), but low turf quality (July 2015, 5.3). Overall, this information will aid in the breeding of cultivars that will have both good turf quality and drought tolerance when managed under reduced inputs.

Kentucky Bluegrass Response to Wear and Traffic During Autumn

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Tolerance to wear and trampling stresses is important on recreational and sports turf. The objective of this field trial was to evaluate the effects of the Rutgers Wear Simulator (RWS) and the Cady Traffic Simulator (CTS, trampling stress) on entries in the 2011 NTEP Kentucky bluegrass (*Poa pratensis* L.) trial seeded October 2011 on a loam in North Brunswick, NJ. Three traffic strips (RWS, CTS, and non-treated) were applied using a strip-plot design with three replications across 92 Kentucky bluegrass entries. Twenty-four passes of the RWS and CTS were made from 30 Sep. to 4 Nov. 2015 (4 passes wk^{-1} for 6 wk). Uniformity of turf cover was visually assessed and green turf cover was determined by digital image analysis. After 24 passes, green turf cover averaged 93%, 92%, and 58% for the non-treated, CTS and RWS strips, respectively; the uniformity and green turf cover of entries depended on the traffic level. After trampling with the CTS, 79 and 83 entries were in the top statistical group for uniformity and green turf cover, respectively. After RWS treatment, only 12 and 7 entries ranked within the top statistical group for uniformity and green turf cover, respectively. 'Barvette HGT', 'Rubix', A06-46, SRX 2758, and 'Nu Chicago' ranked among entries with both the best uniformity and greatest green cover after RWS traffic. 'Arrowhead', SRX 5321, 'Bewitched', 'Midnight', 'Bluebank', J-1853, PST-T10-18, H99-1653 and 'Aramintha' were among entries with either the best uniformity or greatest green turf cover after RWS treatment. Thus, Kentucky bluegrass was more tolerant of trampling stress than wear, and wear (RWS) was more effective at distinguishing entries.

Phytotoxicity Evaluation of Poacure (Methiozolin) on Different Cultivars of Fine Fescue

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Poacure (Methiozolin) is a relatively new herbicide that has been reported to selectively control annual bluegrass (Poa annua L.) in various cool and warm season turfgrasses. The objective of this study was to evaluate phytotoxicity of Poacure on different fine fescue cultivars. The experiment was conducted in a randomized completely block design with three replications, and repeated. Nine different cultivars of fines fescue were seeded in 10*10 cm2 square pots. Methiozolin (Poacure 250EC) was then applied with five different rates, 0.2, 0.4, 0.6, 0.8, 1 gal/acre at four different application timings, four weeks before seeding (4 WBS), two weeks before seeding(2 WBS), at seeding(AtS) and two weeks after germination(2 WAG). Non-treated controls were included for each cultivar. Pictures of each pots were taken with a light box biweekly after germination. Green percent coverage data were collected using SigmaScan. Above ground tissue was harvested 8 weeks after germination, fresh and dry weight were measured. Significant reduction in green percent coverage was observed when Poacure was applied before germination. It is relatively safer to apply Poacure two weeks after germination, however, higher rate also lead to reduction in green percentage cover. ANOVA analysis showed cultivar, application rate, application timing all have significant effect on the green percentage cover. Interaction between rate and application time was also significant, suggesting that changes in application rate will affect the green percent coverage of fine fescue differently depending on the application timing.

Brown patch in Tall Fescue: Evaluation of Turf Trials Inoculated with Rhizoctonia soloni and Breeding for Improved Resistance to the Disease Here at Rutgers

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Tall fescue [*Lolium arundinacea* (Schreb.) Darbyshire], a cool season grass native to Europe and some parts of Africa, was introduced into the United States in the 1800s as a forage grass. Tall fescue has become one of the major cool season turf species in United States because of its winter hardiness, persistence, adaptability to wider range of soils, and tolerance to shade and drought. Tall fescue has a deep root system that enhances drought tolerance and allows the plant to stay green longer in dry conditions. Tall fescue also has among the best heat tolerance of the cool-season grasses. These qualities have increased the use of tall fescue in home lawns, sports fields, golf course roughs, recreational fields, sod farms, and roadsides. One of the major limitations of tall fescue is susceptibility to brown patch, caused by the fungus *Rhizoctonia solani*, in warm and humid regions. Brown patch is a soil borne disease of both cool season and warm season turfgrasses which causes blighted, circular to irregularly-shaped patches to form in the turf which quickly fade to light brown. Breeding for disease resistance is one of the main objectives of the Rutgers breeding program. Here at Rutgers mowed space plant nurseries of thousands of individual plants are inoculated with the brown patch fungus, and individuals that exhibit no symptoms of infection are used in the breeding material for cultivar development. Many of the newer cultivars and selections in the turf trails have been developed using these methods. Five tall fescue trials were established at the Rutgers Plant Biology and Pathology Research and Extension Farm at Adelphia, NJ between 2012 and 2015. All tests were established in September by hand sowing a 5.9 lb. per 1000 ft^2 rate in a 3ft x 5 ft turf plot. All tests were arranged in randomized complete block design with three replications, and each plot had a 6-inch unseeded border to limit contamination. All turf trials were inoculated with *Rhizoctonia solani* infected sterilized Kentucky bluegrass seeds at a rate of 1 gram per square meter. Trails were managed to promote disease development by frequent irrigation and fertilization. Once symptoms of brown patch developed the trials were rated on a 1-9 scale with 1 being brown patch infection throughout the whole plot and 9 being no symptoms present. All data were summarized and subjected to an analysis of variance. Means were separated using Fisher's protected least significant difference (LSD) means separation test. The means for all trials ranged from 1.5 to 8.7. The cultivars and selections that were most resistant were Amity, Avenger II, GTO, and 3B3 composite. Even though these cultivars performed very well there is still room for improvement to brown patch in tall fescue and this will remain a top priority of the Rutgers breeding program.

Development of the ‘I × A89’ Perennial Ryegrass Mapping Population

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An overhead irrigation technique was used from June to August 2015 to screen 403 perennial ryegrasses (*Lolium perenne* L.) genotypes (3 replicates per genotype) for salinity tolerance. The tolerance of a given genotype was visually assessed according to green color retention when exposed to increased levels of soil salinity. Salinity tolerant (n = 20) and sensitive (n = 9) genotypes were identified as candidate parental lines and transplanted into a field nursery on 24 September 2015. Parental candidates were visually evaluated for leaf color and growth habit morphology during May 2016. Simultaneously, candidate genotypes were maintained in a separate spaced plant nursery where they were screened for resistance to dollar spot, a foliar disease caused by the fungal pathogen *Sclerotinia homoeocarpa* F.T. Bennett. In addition to phenotypic evaluations, variability of genetic backgrounds in candidate parental lines was also assessed by genotyping with 24 microsatellite DNA markers. Parental lines were isolated, in pairs, based on flowering compatibility and contrasting phenotypic traits. Fourteen biparental crosses were conducted from late May to early July 2016. Seed was harvested from both parents in all biparental crosses and threshed on 22 August 2016. The biparental cross ‘I × A89’, was chosen for the mapping population as determined by variation in phenotypes and polymorphism of genetic backgrounds. Seed from the ‘I × A89’ cross was planted on 22 August 2016 and plantlets were transplanted on 14 September 2016 to establish the mapping population. The ‘I × A89’ mapping population consists of the two parents (15-8325-‘I’ and 15-8343-‘A89’) and 368 progeny (184 progeny from each parent) and will be useful for genetic studies of important traits in perennial ryegrass breeding.

Entomopathogenic Nematodes for the Control of Annual Bluegrass Weevil, *Listronotus maculicollis*, larvae: Optimizing Efficacy Through Split Applications and Combinations with Imidacloprid

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The annual bluegrass weevil (ABW), *Listronotus maculicollis* (Coleoptera: Curculionidae), is a major pest of short-mown golf course turfgrass (fairways, tees, collars, greens) in the northeastern USA and eastern Canada. It has 2–3 generations per year. Damage potential is the greatest from late May to mid-June, caused by the spring-generation larvae. Eggs are laid in the grass stems. Young larvae (L1–3) tunnel stems, killing individual grass tillers. Severe damage is caused by older larvae (L4–5) that feed externally at ground level on the grass crowns and may kill large areas of high profile turf.

ABW is particularly problematic due to the development of resistance to commonly used insecticides. Entomopathogenic nematodes (EPN) offer a safer, more sustainable alternative. Adult ABWs are a poor target for EPN. But several EPN species have provided acceptable control (50–80%) of ABW larvae in golf course fairways. The most consistent control was provided by the species *Steinernema carpocapsae*, but *S. feltiae* and *Heterorhabditis bacteriophora* often gave the same level of control (McGraw & Koppenhöfer 2009, McGraw et al. 2010). However, control rates are too variable and low to make wide adoption by golf courses likely. In the present study we examined whether split EPN applications and combinations of EPN with the neonicotinoid imidacloprid could be used to improve control of ABW larvae and thereby increases the likelihood of their adoption by golf courses.

Greenhouse studies

Imidacloprid is widely used for white grub control and can be applied for that purpose at the same time when ABW larvicides need to be applied. Annual bluegrass, *Poa annua*, was grown from plugs taken from established fields with no ABW history. Plugs were washed free of soil and grown for 1 month in the greenhouse in 540-ml clear plastic cups. To obtain turf infested with ABW larvae, plugs were transferred into taller 850-ml cups and ABW adults caged in the cups for 1 week for egg-laying. After adult removal, cups were left in the greenhouse. Treatments were applied in tap water when larvae were primarily early 4th instars. Treatments were a low and high rate ($0.3 / 0.6 \times 10^9$ IJs/ha) of the EPN spp. *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*; a low and high rate of the insecticide Merit (ai: imidacloprid) (118 / 336 g ai/ha); and combinations of the EPNs and imidacloprid. At 14 DAT, the soil and grass were manually examined for ABW stages followed by submersion in water saturated with table salt which irritates small larvae out of the plant material and floats all stages to the water surface.

The three EPN species provided similar levels of control and control levels tended to increase with EPN rate (36–50% at 0.3×10^9 IJs/ha; 63–77% at 0.6×10^9 IJs/ha). Merit alone provided 45% at the lower rate and 71% at the higher rate. Control levels in all combinations were additive as determined with a χ^2 test with 89–92% in the combination of the high EPN and imidacloprid rate.

Field efficacy

Experiments were conducted on golf course fairways with a history of ABW infestations but without resistance to pyrethroid insecticides. Experiments were laid out along the fairway edge no more than 5 m deep into the fairway. Grass was a mix of creeping bentgrass and *P. annua*. Individual replicates consisted of 91.5× 91.5 cm turf areas with 30.5 cm buffer around each replicate. Larval populations were monitored weekly; applications were made when late 3rd instar and 4th instar larvae peaked. Treatments were applied using watering cans in the equivalent of 2 mm water followed by about 4 mm overhead irrigation (total about 6 mm). Treatments were imidacloprid (336 g ai/ha), two rates (1.25 / 2.5 × 10^9 IJs/ha) of *S. carpocapsae* and *H. bacteriophora*, and their combinations. Treatments were evaluated after 10–14 days by taking 8 cores (57 mm diam × 38 mm deep) from each plot at least 20 cm from the border of the plot. Cores were extracted in the laboratory by submersion in salt-saturated water.

The two EPN species provided similar levels of control with 45–57% at the lower rate and 68–70% at the higher rate. Imidacloprid provided 59% control. Mortality in the combination treatments was additive and was also similar for the two EPN in the various combinations with imidacloprid with 74–77% for *H. bacteriophora* and 81–83% for *S. carpocapsae*.

Split Applications

Since ABW larvae emerge from the plants over a period of time and EPN densities tend to decline rapidly after application, we hypothesized that splitting treatments and applying them in two halves several days apart would increase efficacy. This was tested in two field experiments against a pyrethroid-susceptible and one against a pyrethroid-resistant ABW population using the same methodology as for the previous field test. Treatments were Merit (336 g ai/ha); *S. carpocapsae* (1.25 / 2.5 × 10^9 IJs/ha) applied at 0 DAT or split evenly between 0 and 4–6 DAT; and the combinations of *S. carpocapsae* with Merit.

Imidacloprid provided 21–39% control. *Steinernema carpocapsae* control was not affected by rate or pyrethroid-resistance and ranged 65–81%. Control in the combinations was additive but statistically only higher than Merit alone and was not affected by *S. carpocapsae* rate and pyrethroid-resistance, ranging 77–84%. Splitting *S. carpocapsae* did not significantly increase control but, averaged across all *S. carpocapsae* alone and combination treatments, provided 10% more control than the non-split treatments. However, combinations of imidacloprid with split *S. carpocapsae* had numerically the highest control in all three experiments and were the only treatments that provided significantly higher control (87–95%) than both imidacloprid and non-split *S. carpocapsae*. Treatments were similarly effective against pyrethroid-susceptible and -resistant ABW.

Conclusions

EPNs, particularly *S. carpocapsae*, can provide good ABW control. Splitting *S. carpocapsae* applications in two halves applied about 4–7 days apart tends to increase the efficacy and may be particularly useful when emergence of larvae from the plants is spread out over a longer period of time due to weather conditions. Combinations of imidacloprid and *S. carpocapsae*, particularly split *S. carpocapsae*, can provide excellent control of ABW larvae in pyrethroid-susceptible and -resistant populations, while at the same time also providing white grub control due to the long residual activity of imidacloprid in the soil.

Kentucky Bluegrass Collection Incorporation

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Kentucky Bluegrass is a more popular cool season turf grass, second only to tall fescue. There is an abundance of variability in Kentucky bluegrasses and the ability to retain that variability through naturally occurring apomixis, which is asexual reproduction via seed. Seed yield is a critical trait that Kentucky bluegrass has been lacking, resulting in higher prices and less production. The goal is to incorporate Kentucky bluegrass collections from around the world to improve key characteristics such as seed production, disease resistance, drought tolerance, heat tolerance and uniformity in our existing germplasm.