

RUTGERS

New Jersey Agricultural
Experiment Station

PROCEEDINGS OF THE TWENTY-THIRD ANNUAL RUTGERS TURFGRASS SYMPOSIUM

January 17, 2014

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The Center for Turfgrass Science

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Symposium Organizing Committee

Faith Belanger, Chair
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Donald Kobayashi
Thomas Molnar

Proceedings of the Twenty-Third Annual Rutgers Turfgrass Symposium

Thomas Molnar and Barbara Fitzgerald, Editors

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Associate Director's Opening Remarks:

Welcome to the twenty-third Annual Rutgers Turfgrass Symposium at the School of Environmental and Biological Sciences/NJAES, Rutgers University. This symposium was started 23 years ago as an annual meeting to update members of the Center for Turfgrass and stakeholders on current issues in turfgrass science. This year we are pleased to have Dr. Zeng Yu Wang from the Forage Improvement Division of the Noble Foundation in Ardmore, OK as our keynote speaker. He is an expert in the genetic manipulation of forage and bioenergy crops. We also are honored to have Dr. Karl Guillard, from the University of Connecticut, speak about *Nitrogen fate* and Dr. Megan Kennelly, from Kansas State University, presenting her research on disease control in Zoysiagrass. It is important to recognize this year's Symposium Organizing Committee: Drs. Faith Belanger (chairperson), Tom Molnar, Don Kobayashi, Bruce Clarke, Ms. Barbara Fitzgerald and myself. Also, I would like to thank Tom Molnar and Barbara Fitzgerald who did an excellent job editing the proceedings.

The faculty staff and students of the Turf Center continue to be recognized for their excellence in research, teaching, and outreach. Ms. Lisa Beirn received the Gerald Mott Graduate Student Award which is a very high honor presented each year to one of the top graduate students in plant science in the country. Chas Schmid was recognized at the Crop Science Meetings in Tampa as one of the top graduate student presenters by the C-5 division. Drs. Bruce Clarke and James Murphy were recognized as President and USA board member of the International Turfgrass Society, respectively. Rutgers was also selected to be the host for the International Turfgrass Science meetings in 2017. Dr. Bruce Clarke was recognized with the John Morely Distinguished Service Award from the Golf Course Superintendents Association of America, which is the highest award presented by this international stakeholder organization. Dr. Jim Murphy recently developed the fertilizer certification program for NJ as mandated by the legislature, and has done an excellent job overseeing its implementation. Finally, the Rutgers Turfgrass Breeding Program continues to develop many of the top turfgrass cultivars in the world as recognized by their dominance in the National Turfgrass Evaluation Trials for all of the major cool-season turfgrass species. The development of improved varieties with excellent disease resistance continues to be a major objective of the Rutgers Turfgrass Breeding Program.

The faculty in the Turf Center are especially proud of their graduate students who have finished advanced degrees and have moved on to become leaders at other universities and corporations throughout the US. This past year the Center awarded over \$86,000 in scholarships to graduate, undergraduates and 10 week turfgrass students. These scholarships were supported by more than a dozen turf industry groups. The Center appreciates the support we receive from the Turfgrass Industry. In this regard, special recognition is due Chris Carson for chairing the Turf Expo in Atlantic City and Shaun Barry for chairing the Rutgers Golf Classic each year.

Thanks for participating in this year's symposium.

Sincerely,



William A. Meyer

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TWENTY-THIRD ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 17, 2014

Foran Hall, Room 138A

Friday, January 17, 2014

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

9:00 - 10:00 AM **SESSION I: FUNGAL BIOLOGY AND PEST MANAGEMENT**
(Moderator: Dr. Bruce B. Clarke)

9:00 – 9:20 **Dr. Albrecht Koppenhöfer** (Department of Entomology, Rutgers University) *Advancing Integrated Management of Annual Bluegrass Weevil*

9:20 – 9:40 **Dr. James White** (Department of Plant Biology and Pathology, Rutgers, University) *Potential Benefits of the Use of Bacillus Endophytes in Grasses and Other Monocots*

9:40 – 10:10 **Dr. Megan Kennelly** (Department of Plant Pathology, Kansas State University) *Effects of Cultural Practices and Fungicide Programs on Large Patch of Zoysiagrass, and Screening Zoysiagrass Germplasm Lines for Resistance*

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

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

10:30 – 10:50 **Dr. Bingru Huang** (Department of Plant Biology and Pathology, Rutgers University) *Improving Creeping Bentgrass Performance Using PGR and Biostimulants*

10:50 – 11:10 **Eric Koch** (Department of Plant Biology and Pathology, Rutgers University) *Breeding and Evaluation of Perennial Ryegrass (*Lolium perenne* L.) for Improved Salinity Stress*

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

- 11:20 – 12:00 **Keynote: Dr. Zeng Yu Wang** (Forage Improvement Division, The Samuel Roberts Noble Foundation) *Genetic Manipulation of Forage and Bioenergy Crops*
- 12:00 - 1:00 PM Lunch and Poster Session**
- 1:00 – 2:00 PM SESSION III: TURFGRASS MANAGEMENT**
(Moderator: Dr. Thomas Molnar)
- 1:00 – 1:30 **Dr. Karl Guillard** (Department of Plant Science and Landscape Architecture, University of Connecticut) *Predicting Turfgrass Response to Nitrogen Fertilization by Measuring the Labile Nitrogen and Carbon Fractions of Soil Organic Matter*
- 1:30 – 1:50 **Charles Schmid** (Department of Plant Biology and Pathology, Rutgers University) *Annual Bluegrass Response to Potassium Fertilization*
- 1:50 – 2:10 **Dr. James Murphy** (Department of Plant Biology and Pathology, Rutgers University) *Evaluation of Traffic Tolerance Among Fine Fescues*
- 2:10 – 2:30 PM Discussion and Coffee Break**
- 2:30 – 3:30 PM SESSION IV: TURFGRASS GENOMICS**
(Moderator: Dr. Faith Belanger)
- 2:30 – 2:50 **Dr. Ning Zhang** (Department of Plant Biology and Pathology, Rutgers University) *Second Generation Diagnostics: Turf Pathogen Barcoding, Real-Time PCR, and Diagnostic Array*
- 2:50 – 3:10 **Karen Ambrose** (Department of Plant Biology and Pathology, Rutgers University) *Symbiosis and Sequencing: The Discovery of Horizontal Gene Transfer of a Bacterial Insect Toxin Gene Exclusively into the Epichloë Fungal Endophytes of Grasses*
- 3:10 – 3:30 **Dr. Joshua Honig** (Department of Plant Biology and Pathology, Rutgers University) *Genetic Diversity of Bentgrass (Agrostis) Cultivars and Accessions Using Microsatellite (SSR) Markers*
- 3:30 - 3:45 PM Discussion and Closing Remarks**
- 3:45 PM Social and Poster Session**

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PLENARY PRESENTATIONS

Advancing Integrated Management of Annual Bluegrass Weevil

Olga S. Kostromytska, Cesar Rodriguez-Saona, and Albrecht M. Koppenhöfer

Department of Entomology, Rutgers University

The annual bluegrass weevil (ABW) is a severe pest of golf courses throughout the Northeast and into surrounding areas in the Mid-Atlantic and eastern Canada. Due to insecticide overuse, insecticide-resistant ABW populations are a growing problem. Because of the involvement of enhanced metabolic detoxification by up to three enzyme families, resistant populations are resistant in varying degrees to most available insecticides. There is a 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. But there is also a dearth of effective and feasible monitoring techniques and alternative controls for ABW. The overall goal of this project is to optimize existing ABW monitoring methods, develop alternative management strategies, and integrate them in order to achieve significant ABW suppression with reduced chemical input.

Semiochemicals for Monitoring and Management

Available ABW monitoring methods are not effective and/or are too labor intensive and hence rarely used. One main focus of this project is to search for semiochemical attractants (pheromones, host-plant volatiles) and study the behavioral and physiological responses of male and female ABW to these compounds and blends thereof, with the long-term goal of developing attractant-based monitoring and/or management tools. 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, 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. The *P. annua* emitted volatiles (+)- β -pinene, (E)-2-hexenal, 6-methyl-5hepten-2-one, (Z)-3-hexenyl acetate, decanal, hexanal, nonanal, octanal, and phenyl ethyl alcohol were tested in the behavior Y-tube assays as potential ABW attractants. At the highest rate (20 μ g), all tested compounds were repellent to males and females. At the lower rates, only 3-hexenyl acetate and phenyl ethyl alcohol were preferred by ABW females. Combination of these compounds was field tested. During March to May, in the beginning of the experiment, more ABW adults were collected in the baited traps than in non-baited traps. During June-July, weevil numbers were too low and differences diminished. In the summer experiment, no differences between baited and control traps were observed, partially due to relatively low ABW numbers. This finding suggests that grass volatiles alone were not strong enough attractants to pull ABW from the fairways. To compare volatiles emitted by *P. annua* and bentgrasses, headspace volatiles of six bentgrass cultivars and *P. annua* were collected in the greenhouse and from established bentgrass and *P. annua* fields. Cultivars differed in the quality and quantity of compounds produced. Green leaf volatile 3-hexenyl acetate and terpenoid linalool were produced by *P. annua* in much greater quantities than by the bentgrasses. Bentgrass headspace had more terpenoid volatiles (ocimene, borneol, camphene, sabinene, and

terpenolene), which commonly serve roles in plant defense and might be involved in bentgrass resistance to ABW.

To determine whether specific compounds are present in the headspace of ABW males and females (\pm *P. annua* as a food source), we collected volatiles using a pull system. Samples were processed using gas chromatography-mass spectrometry analysis. No sex specific compounds were detected in the headspace of ABW males and females; however, in the headspace of ABW males and females feeding on *P. annua*, two specific compounds, α -muurolene and E- β -ocimene, were detected. In ongoing studies, we are examining the presence of sex or aggregation pheromones in different phenological stages of ABW.

Alternative control methods

Entomopathogenic nematodes (EPN) have already shown potential for ABW larval control, but their performance was variable and declined at high ABW densities. EPN efficacy may be synergized by combination with the neonicotinoid imidacloprid. Such synergistic interactions have been shown with nematode-neonicotinoid combinations for white grub control. These combinations seem quite feasible since they could be applied at the appropriate time in spring to control both ABW larvae while the neonicotinoid should still provide white grub control later in the season. Alternatively, the combinations could be applied in summer to control ABW larvae and white grubs at the same time. Nematodes-imidacloprid combinations for ABW larval control were studied in greenhouse and field experiments. In greenhouse tests, a low and a high rate of imidacloprid and the nematodes *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae*, were tested alone and in combination against ABW fourth instars. In the field experiment, two rates of *H. bacteriophora* and *S. carpocapsae* were tested alone or in combination with the labeled rate of imidacloprid. In the greenhouse experiment, ABW mortality was generally additive in the combinations with no significant synergistic effect observed. In the field experiment, no significant differences among treatments were observed after 7 days, but all treatments differed from control after 14 days. In addition, plots treated with combinations numerically tended to have fewer ABW larvae. The interactions were generally additive with no significant synergistic interactions observed. Future experiments will examine the effect of delayed and split application of nematodes on this interaction.

Potential Benefits of the Use of *Bacillus* Endophytes in Grasses and Other Monocots

James White and Qiang Chen

Department of Plant Biology and Pathology, Rutgers University

We are exploring the potential application of some novel seed-transmitted bacterial endophytes in turf grasses. Recently, we encountered the bacterial species *Bacillus amyloliquefaciens* as a common endophyte of tall fescue and perennial ryegrass. This is a bacterium that has found application as a commercial biological agent to control fungal diseases in many crops. The bacterium is effective for disease control because it produces antifungal lipopeptides that inhibit hyphal growth and pathogen colonization of plant tissues. The antifungal lipopeptides of *B. amyloliquefaciens* are also known to cause increased expression of plant defense genes and this further enhances resistance of plants treated with the bacterium. Studies that we have conducted on vanilla orchids, which also contain this endophyte, suggest that the bacterium may colonize plants externally around stomata and internally in vascular tissues. This distribution of the bacterium in plants may enable the bacterium to prevent entry of pathogens through stomatal openings and reduce transit through vascular tissues. In this research we intend to do the following:

1. Survey cultivars in several species of turf grasses to determine how widespread *Bacillus* spp. are in grasses.
2. Examine distribution of the bacterium in plants as they develop.
3. Examine production of antibiotic lipopeptides by endophytic bacteria.
4. Test effectiveness of the new endophytes in turf grasses against disease agents.
5. Develop methods for infecting grasses (seedlings) with the bacterial endophytes.
6. Patent the most potent endophytes for use in enhancing resistance of turfgrasses.

Effects of Cultural Practices and Fungicide Programs on Large Patch of Zoysiagrass and Screening Zoysiagrass Germplasm Lines for Resistance

Megan Kennelly¹, Kehinde Obasa¹, Jack Fry², Dale Bremer², Rodney St. John³

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Large patch, caused by the fungus *Rhizoctonia solani* anastomosis group (AG) 2-2 LP, is the most common and severe disease of zoysiagrass (*Zoysia* spp.) in the Transition Zone. Despite the importance of this disease, more information is needed on the effects of cultural practices, host resistance, and chemical controls in respect to its control. Kansas State University, in partnership with colleagues at other institutions, has recently conducted a number of studies on large patch management. Some of the key objectives of this work were to: (1) Determine the effects of cultivation (aerification, verticutting, and sand topdressing) and timing of fertilization on disease severity; (2) Evaluate new zoysiagrass germplasm lines from parental crosses including *Z. japonica*, *Z. matrella*, and *Z. pacifica*; and (3) Evaluate the effects of different fall and spring applications of various fungicides. The cultural practices/fertility study was conducted at three sites (Manhattan, Olathe, and Haysville, KS) over several years. Cultivation did not affect soil moisture, temperature, or patch sizes, or influence turf recovery rate from large patch. In several site-years, spring and fall N fertility was associated with lower percentages of non-green turf in both cultivated and non-cultivated plots. In the germplasm screening studies, fourteen new progeny from parental crosses including *Z. japonica*, *Z. matrella*, and *Z. pacifica* were evaluated in the field and growth chamber in comparison to 'Meyer' as a control. All progeny had similar disease levels compared to 'Meyer' in the growth chamber, but only six consistently had disease levels as low as 'Meyer' in the field. Growth chamber results did not correlate to field results and further work is needed to optimize screening methods. In the fungicide studies, various fungicide programs were evaluated over several years. In general, one fall application was as effective as two fall applications. Early spring applications reduced disease compared to later spring applications. Ongoing studies include further evaluations of fungicides, fertility practices, and new germplasm lines.

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Improving Creeping Bentgrass Performance Using PGR and Biostimulants

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Creeping bentgrass (*Agrostis stolonifera* L.) is a cool-season grass species that is widely used on putting greens. The grass exhibits excellent turf characteristics when grown under optimal conditions. However, during the summer months in many regions, it succumbs to summer bentgrass decline syndrome. Summer bentgrass decline syndrome is a major concern of golf course superintendents, especially in southern states and the transitional zone. Heat stress is a primary causal factor of this syndrome, which leads to a decline in turf quality and physiological activities. Summer bentgrass decline syndrome is characterized by a thinning of the turf canopy, leaf senescence, and root dieback. Root dieback inhibits water and nutrient uptake, as well as other metabolic pathways such as the synthesis of hormones, including cytokinins. A decline in cytokinin content may limit shoot growth and cause leaf senescence. Therefore, maintaining quality bentgrass putting greens during the summer months remains a major challenge in bentgrass management.

Some golf courses apply various compounds on bentgrass, hoping to improve turf growth during the summer months. Incorporation of natural products or plant growth regulators (PGRs) may promote shoot and root growth and would favor creeping bentgrass survival. Some natural products that can stimulate plant growth and development are classified as plant growth promoters, collectively named biostimulants. Biostimulants may include carbon sources, humates, microbial suspensions or powders, and hormone-containing products such as seaweed extracts. Their effectiveness of growth promotion varies greatly with plant species, physiological conditions of the plants, product formulation/composition, application rate, and timing. Currently, there is a lack of season-long field experiments and controlled-environment studies to confirm manufacturer claims. With the increasing use of PGRs and biostimulants on creeping bentgrass putting greens, understanding their efficacy and how they work would help turf managers develop more efficient summer stress management practices.

In the current project, we evaluated treatments combining different PGRs and biostimulants based on their biological functions for alleviating summer bentgrass decline and promoting summer bentgrass performance. We conducted the experiment on a 6-year-old 'Putter' bentgrass green built to USGA greens specifications located at Hort Farm II, Rutgers University. The experiment was conducted from May to October, 2012 and 2013. We tested several commercial products and experimental materials containing seaweed extracts, amino acids, hormones, hormone-inhibitor, and humic acids. The application of the experimental seaweed extracts from Ocean Organics promoted significantly higher turf quality, green leaf biomass, and plant density compared to the control during most of the experimental periods. The combined treatments of Primo and cytokinins, an ethylene inhibitor, or nitrogen had some beneficial effects in promoting turf density (turf quality and

plant density) and green color (chlorophyll content). The effects from the application of these products were more pronounced during the spring. During the summer months, the positive effects of treatments were still present, but less pronounced than that in the spring.

Breeding and Evaluation of Perennial Ryegrass (*Lolium perenne* L.) for Improved Salinity Stress

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Potable water is becoming more restricted for use on golf courses and home lawns around the world. An alternative water source known as effluent water is a viable source with which to irrigate. This water source is cost effective but comes with an array of challenges, including a high level of salinity. Even in low concentrations, consistent irrigation with salt-affected water can cause accumulation in soils. These salts can cause extreme reductions in turfgrass stands.

Perennial ryegrass (*Lolium perenne* L.) is an important cool-season turfgrass species due to its ability to germinate quickly and provide a turfgrass stand within a short period of time. Because of this quality, this species is used extensively to overseed dormant warm-season turfgrass on golf courses. Improved varieties of perennial ryegrass can contain symbiotic fungi known as endophytes. These fungi live intercellularly within leaf, sheath, and stem tissues. The presence of the *Neotyphodium* endophyte has the ability to convey abiotic and biotic stress tolerance in perennial ryegrass cultivars (Van Zijl de Jong et al., 2008).

The first study conducted was the evaluation of four perennial ryegrass cultivars in both the presence and absence of the fungal endophyte under salinity stress. Salinity treatments were applied using the methods described by Koch et al. (2011). Early results from have shown that the fungal endophyte does not convey salt tolerance, and in some instances causes a reduction in plant growth and overall plant health under salinity stress.

Two studies were conducted to evaluate the effects of salinity stress on germinating perennial ryegrass seedlings. The first germination study was conducted in a rain-out shelter located in Freehold, New Jersey. Three replicates of 145 entries consisting of both cultivars and experimental lines were tested for tolerance to salinity under two treatments: 0.5 dS m⁻¹ (control) and 10 dS m⁻¹. Irrigation was applied using a boom sprayer. The second germination study was carried out in a growth chamber to maintain constant temperature and humidity while evaluating multiple morphological characteristics of ten perennial ryegrass cultivars (which were also included in the first study). Irrigation was applied using an ebb and flow technique, where pots were submerged into one of the two treatments. Interestingly, both germination studies showed no cultivar by treatment interaction. Both studies also showed significant cultivar differences.

Future research will be conducted to understand the technique needed to efficiently breed for perennial ryegrass seedlings under salinity stress.

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Genetic Manipulation of Forage and Bioenergy Crops

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Forage and bioenergy crops play important roles in sustainable agriculture and the development of alternative sources of energy. Genetic engineering offers effective ways to improve grasses and legumes. We have established genetic transformation systems for several important forage, turf, and bioenergy species including tall fescue, switchgrass, ryegrasses, maize, alfalfa, white clover, and *Medicago truncatula*. In particular, we established a high throughput transformation system for switchgrass, a dedicated bioenergy grass as identified by the Department of Energy.

To better understand plant development in compound leaf species, we use *M. truncatula* as a model species. By screening a large population of *Tnt1* retrotransposon-tagged mutants of *M. truncatula*, we identified several types of mutants that exhibited defects in compound leaf development, leaf senescence, and seed development. Detailed characterization of the leaf mutants revealed that leaf margin formation is cooperatively regulated by the auxin/SLM1 (ortholog of *Arabidopsis PIN-FORMED1*) module, which influences the initiation of leaf margin teeth and the *trans*-acting short interfering RNA pathway (*TAS3* ta-siRNA), which determines the degree of margin indentation.

On the applied side, the target traits are forage quality, drought tolerance, phosphate uptake, and improved biofuel production. Forage digestibility is a limiting factor for animal productivity. We produced transgenic tall fescue plants that showed reduced lignin content, altered lignin composition, and increased dry matter digestibility. We also generated stay-green alfalfa that showed increased crude protein content. Drought tolerance is an important trait for improvement in perennial forages. We characterized novel ERF transcription factor genes from the model legume *M. truncatula*. Overexpression of the genes in alfalfa and white clover led to a significant increase in cuticular wax loading on leaves, decreased water loss, and enhanced drought tolerance. Phosphorus is immobile and often deficient in pasture soils. We cloned and characterized a constitutive promoter, two root-specific promoters, a novel phytase gene and a purple acid phosphatase gene from *M. truncatula*. Transgenic expression of the phytase gene or the purple acid phosphatase gene in alfalfa and white clover led to significant improvement in organic phosphorus uptake and plant growth. Switchgrass is an important bioenergy crop. Lignification of grass cell walls negatively affects enzymatic hydrolysis and utilization of structural polysaccharides. We have cloned major lignin genes from switchgrass and regenerated transgenic switchgrass plants with RNAi constructs of the lignin genes. Improved sugar release and increased ethanol production was obtained from the transgenics.

Predicting Turfgrass Response to Nitrogen Fertilization by Measuring the Labile Nitrogen and Carbon Fractions of Soil Organic Matter

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The responsiveness of turfgrass plants to N fertilizers depends greatly on the N-supplying capacity of the soil on which they are grown. If mineralization potential is high due to relatively large amounts of labile C and N fractions of the soil organic matter, it is unlikely that the turfgrass would benefit much from additional N supplied by fertilizers. In these cases, the inherent capacity of the soil to supply N would most likely be sufficient, or nearly sufficient, for turfgrass plant growth and quality demands. Thus, little if any supplemental N fertilizer would be required to meet expected performance goals (assuming conditions for mineralization are favorable during the growing season). Application of N in these situations is economically wasteful and increases concerns of environmental nutrient pollution and risks of certain insect and disease infestations. On the other hand, if soil mineralization potential is low, then it is likely that a marked turfgrass response would be observed following N fertilization. This is because the inherent N supply is less than what is needed to meet turfgrass plant demands under higher quality and growth expectations.

The ability to predict the mineralization potential of any site beforehand, and therefore its expected response to N, would be a valuable tool for turfgrass nutrient management. Measuring the mineralization potential of a soil can be accomplished, but it has traditionally been expensive, time-consuming (many weeks for some tests), or only available from a limited number of commercial or governmental laboratories. Therefore, the practice has not been used on a routine basis. Further, it becomes more complicated when organic fertilizers, amendments, and composts of varying decomposition and mineralization potentials are applied to turf.

My current research has shown that the mineralization potential of turfgrass soils, and subsequent turfgrass response to N fertilizers, can be estimated with two new soil tests that were developed for field crops (primarily corn [*Zea mays* L.]). One test is called the Illinois Soil N Test (ISNT), which measures the highly labile amino-sugar N fraction of soil organic matter along with ammonium and some amides. The higher the ISNT-N value, the greater the soil's potential for supplying available N to plants. This translates to a reduced need for supplemental N fertilizer to reach performance goals. My data suggests that once the ISNT-N concentrations reach 250 mg kg⁻¹ or greater, it is unlikely that any further greening of Kentucky bluegrass or tall fescue lawn color would occur in response to N fertilizer application (Geng et al., 2014). When ISNT-N concentrations are 200 to 250 mg kg⁻¹, there is a moderate chance that N fertilization would result in greening of the turf. When concentrations are < 200 mg kg⁻¹, there is a very high probability that turf would become greener in response to N.

The second new test is called the soil permanganate-oxidizable C (POXC) test, which measures the highly labile C fractions in the soil organic matter. This test is also referred to as the "Soil Active-Carbon Test". The higher the concentrations of POXC, or active C, the greater the potential for the soil to supply available N to plants. My data suggests that once the POXC

concentrations reach 1,300 mg kg⁻¹ or greater, there is a low probability of any further increase in Kentucky bluegrass or tall fescue lawn color in response to N fertilization (Geng et al., 2014). When POXC concentrations are 1,100 to 1,300 mg kg⁻¹, there is a moderate chance that some supplemental N fertilization would result in greening of the turf. When concentrations are < 1,100 mg kg⁻¹, there is a high probability that turf would become greener in response to supplemental N fertilization.

The capacity to categorize a specific turfgrass site in terms of its responsiveness to N fertilization in relation to inherent mineralization potentials would be a major improvement to the current state of cool-season lawn fertilization recommendations. Typically, in most turf situations, set rates of N (usually 25 to 50 kg N ha⁻¹) are applied two to four times or more across the growing season at a site without accounting for inherent N-supplying capacity of the soil. Having an estimate of the soil's capacity to supply N during the growing season would better guide the planning and management of N fertilization programs. This would result in positive economic benefits, with less N fertilizer being wasted, as well as environmental benefits from the more efficient use of N fertilizer, since it would be applied according to recommendations based on objective estimations of the inherent N-supplying capacity of the site.

The ISNT and POXC tests are applicable to turfgrass soils and can estimate Kentucky bluegrass or tall fescue lawn responsiveness to N fertilization. Although these tests are not widely available in commercial or governmental soil testing laboratories, they are amenable for inclusion into a routine testing protocol. Testing on other turf species for other purposes (e.g. golf, athletic fields) and in other climates will be needed to develop more robust N fertilizer recommendations based on these procedures.

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Annual Bluegrass Response to Potassium Fertilization

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Potassium (K) is the second most abundantly used element by plants. Previous reports indicate that K fertilization can increase stress tolerance (heat, cold, drought, and wear) in cool-season grasses. Preliminary research investigating anthracnose (caused by *Colletotrichum cereale* Manns) disease of annual bluegrass (*Poa annua* L. f. *reptans* (Hauskn) T. Koyama; ABG) turf has shown that fertilization with potassium nitrate can improve turf quality and reduce disease severity compared to other nitrogen (N) sources. However, it is unclear whether K fertilization affects anthracnose severity and, if so, how much K is required for optimum turf performance. The objective of this field study was to 1) determine the effect of K source and rate on anthracnose severity and turf performance of annual bluegrass and 2) establish K sufficiency ranges for ABG turf based on the relationship among soil test level, tissue concentration, and turf performance. Four K sources, potassium chloride (KCl), potassium sulfate (K_2SO_4), potassium nitrate (KNO_3), and potassium carbonate (K_2CO_3) were applied at a 1:1 N:K molar-adjusted ratio. KCl and K_2SO_4 were also applied at 2:1 and 4:1 N:K molar-adjusted ratios. A no N check (with KCl applied at a rate equal to the 1:1 treatments) and a no K check were also included. Treatments were applied biweekly immediately after a 4.9 kg N ha^{-1} application of urea. A total of 16 applications were made beginning in April and ending in November of 2012 and 2013.

Treatments that applied both N and K had significantly less anthracnose severity than either nutrient applied alone. Interestingly, the no N check (K only) treatment lowered anthracnose severity more than the no K check (N only) treatment by the end of 2012 and throughout 2013. Disease severity was similar among the four K sources in both years, although KCl had slightly greater disease severity than the other K sources on a few dates at the end of 2013. There were no differences in anthracnose severity among the three N:K ratios in 2012; however, by the end of 2013, K applied at 1:1 and 2:1 N:K had lower anthracnose severity compared to the 4:1 treatment. Thatch/mat K increased from 35 mg kg^{-1} in no K check plots to 100 mg kg^{-1} in the 1:1 N:K plots by September 2012. These results indicate that K nutrition is important for ABG; deficiencies in soil K can result in increased anthracnose severity.

Evaluation of Traffic Tolerance Among Fine Fescues

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The fine fescues include several *Festuca* species that form turf with a very fine leaf texture. These species have good tolerance of shade and low-input management compared to other cool-season grasses but are not utilized to the same extent due, in part, to poor traffic tolerance and recuperative ability. The performance of fine fescue species under traffic has not been extensively studied. Thus, the relative tolerance of fine fescues to wear and the combination of wear and compaction or the influence that time of year has on wear tolerance is not known. The objectives of this project were to evaluate five fine fescue species for (1) tolerance to wear or wear and compaction and (2) tolerance of wear applied during different seasons.

Separate field trials were seeded in September 2012 at Hort Farm No. 2, Rutgers University, to address each objective. Each trial contained ten fine fescues: ‘Aurora Gold’ and ‘Beacon’ hard fescue (*Festuca brevilipa* R. Tracey), ‘Culumbra II’ and ‘Radar’ Chewings fescue [*F. rubra* L. subsp. *fallax* (Thuill.) Nyman], ‘PPG-FRR-106’ and ‘Garnet’ strong creeping red fescue (*F. rubra* L. subsp. *rubra*), ‘Shoreline’ and ‘Seabreeze GT’ slender creeping red fescue (*F. rubra* L. var. *littoralis* Vasey ex Beal), ‘Quatro’ sheep fescue (*F. ovina* L.), and ‘Blueray’ blue × hard fescue (*F. glauca* Vill. × *Festuca brevilipa* R. Tracey). The ten fine fescue entries were seeded as subplots within traffic treatment main plots.

The traffic type trial (objective 1) was a 3 x 10 factorial arranged in a split-plot design with four replications. The three levels for the traffic type factor consisted of wear applied with the Rutgers Wear Simulator (RWS), trampling applied with the Cady Traffic Simulator (CTS), and an untreated control. Eight passes (one pass per week) of each simulator were applied to the respective main plots from 24 Sep. to 10 Nov. 2013. Traffic treatments (eight passes; one pass per week) will resume for the periods of April to May, July to August, and September to November 2014, and April to May and July to August 2015.

The seasonal wear trial (objective 2) was a 4 x 10 factorial arranged in a split-plot design with four replications. The four levels of the seasonal wear factor were none, autumn, spring and summer. Autumn wear was applied as one pass per week for eight weeks with the RWS from 24 Sep. to 10 Nov. 2013. The same intensity of wear will be applied for the periods of April to May, July to August, and September to November 2014, and April to May and July to August 2015.

Turf quality was visually evaluated once a month from April to October 2013. Leaf tissue bruising, uniformity, and density of cover (UDC), and fullness of turf canopy (FTC) were

visually assessed on 24 Sep. (before traffic application) and 11 Nov. 2013 (after traffic application). Green cover was determined by digital image analysis (DIA). Images were taken before (24 Sep.) and after (11 Nov.) application of traffic treatment in the same area of each plot in 2013.

As expected, all traffic treatments applied in the autumn of 2013 had detrimental effects on fine fescues in both trials. Wear (RWS) caused more bruising injury than trampling (CTS) in the traffic type trial; whereas, reductions in UDC and FTC were more dramatic with trampling than wear stress. Differences among fine fescues for bruising injury were more evident when wear (RWS) stress was predominant as opposed to trampling (CTS). 'Quatro' sheep fescue had the least bruising of all fine fescues while 'Radar', 'Blueray', 'Shoreline', and 'Garnet' had the greatest bruising damage. Bruising injury was not closely associated with loss of UDC or FTC. Differences among fine fescues were most evident for FTC under the trampling type of traffic (CTS). 'Seabreeze GT' and 'Aurora Gold' had the lowest FTC, which was probably due, in large part, to the poor FTC of these entries before traffic. 'Beacon' and 'Radar' had the greatest FTC under trampling (CTS).

Similar to the traffic type trial, wear (RWS) during autumn in the seasonal trial bruised leaf tissue and reduced UDC and FTC for all entries relative to the control in 2013. Bruising damage was greatest on 'Culumbra II' and 'Radar', while 'Quatro' had the least bruising injury. The UDC and FTC responses of fine fescues to autumn wear were similar. 'Radar', 'Beacon', and 'Blueray' (blue × hard fescue) had the greatest UDC after autumn wear while 'Aurora Gold' and 'Seabreeze GT' had the lowest UDC.

Continued evaluation of fine fescue's responses to wear and trampling and wear during different seasons will improve our understanding traffic tolerance among fine fescue species. Responses will also provide insight needed to efficiently screen fine fescue germplasm for improved persistence under traffic.

Second Generation Diagnostics: Turf Pathogen Barcoding, Real-Time PCR, and Diagnostic Array

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Turfgrasses are ubiquitous in the urban landscape, golf courses, athletic fields, residential lawns, and city parks. Approximately 1.9% (16.3 million hectares) of the total continental U.S. area is covered by turfgrass. There are about 200 fungal species that have been recognized as turfgrass pathogens. Disease management demands fast and early pathogen detection and identification techniques. In this presentation, DNA barcoding and modern diagnostic tools for turf pathogens will be introduced. One of the most important diseases of turfgrass in North America is summer patch, which is caused by the root-infecting fungus *Magnaporthiopsis (Magnaporthe) poae*. The pathogen affects the roots, crowns, and rhizomes of several cool-season grasses under favorable environmental conditions. Detection and identification of *M. poae* is notoriously difficult and time-consuming using conventional culture-based methods that usually take three weeks or longer to complete. A culture independent TaqMan real-time PCR assay has been developed for *M. poae* that enables pathogen detection from field samples within only a few hours. We have also been developing a diagnostic DNA macroarray technique, which offers fast, culture-independent, and simultaneous detection of multiple pathogens from field samples. A total of 246 probes for 55 fungal and oomycete pathogens, six grass hosts, and two bacterial species have been designed and printed on the multi-pathogen diagnostic array (PathoCHIP). To date, the array has been tested with various pathogens and field samples. The PathoCHIP is now ready for the next phase, which is the development of a portable tool for on-site detection of turf pathogens that can be easily used by plant diagnostic clinics and golf course superintendents.

**Symbiosis and Sequencing: The Discovery of Horizontal Gene Transfer
of a Bacterial Insect Toxin Gene Exclusively into the *Epichloë* Fungal
Endophytes of Grasses**

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The widespread presence of symbiotic plant-microbe relationships is well known. Such interactions span the continuum of mutual benefit to having detrimental effects on the plant host. In turf, an interaction of broad significance is that of turfgrass-fungal endophyte mutualism. Agriculturally and economically significant turfgrasses, such as *Festuca* spp. and *Lolium* spp., are known to harbor epiphytic *Epichloë* fungi in their aerial tissues. This infection is asymptomatic and often confers benefits, including resistance to herbivory and drought tolerance, to its plant host. The plant, in turn, makes nutrients accessible to its fungal symbiont. An intriguing aspect of this mutualism is manifested in the endophyte-mediated disease resistance unique to the *Festuca rubra* (red fescue)–*Epichloë festucae* interaction.

Previous field studies have shown that the devastating disease dollar spot, caused by the fungus *Sclerotinia homoeocarpa*, is effectively hindered in endophyte-infected *Festuca rubra* and not in endophyte-free plants (Clarke et al., 2006). Similarly, another study demonstrated that endophyte-infected *F. rubra* is highly toxic to chinch bugs (Yue et al., 2000). In order to understand the mechanisms driving these advantages, we generated SOLiDTM-SAGE quantitative transcriptome libraries from clonally propagated plants of endophyte-free and *Epichloë festucae*-infected *F. rubra* (Ambrose and Belanger, 2012). These samples were analyzed for differential plant gene expression. A myriad of physiological processes were found to be affected due to the presence of *Epichloë festucae*, with >200 differentially expressed plant transcripts. Analysis of the data revealed that the most abundant endophyte transcript constituted >10% of its transcriptome. Strikingly, the second most abundant endophyte transcript encoded a small secreted antifungal protein that could be a candidate for fungal disease resistance.

Additional *Epichloë* transcripts that may be involved in conferring biotic resistance to endophyte-infected plants were also discovered. One such transcript was identified as a horizontally transferred gene of bacterial origin that encodes the TcdA/TcdB pore forming domain and shares similarity with the highly toxic proteins *Makes caterpillar floppy 1* and *2* (*Mcf1* and *Mcf2*) in *Photorhabdus* and *Xenorhabdus* (Daborn et al., 2002; Waterfield et al., 2006) and *fitD* in *Pseudomonas* (Péchy-Tarr et al., 2008). *Mcf* and *fitD* proteins have been shown to possess anti-insecticidal properties. *Photorhabdus* and *Xenorhabdus* are bacterial symbionts of entomopathogenic nematodes. Remarkably, screening for a similar toxin gene in the fungal genome database in NCBI revealed that the gene is found only in *Epichloë* endophytes of

grasses and not in any other fully sequenced fungal genomes in the repository. In order to test for toxicity, the *Epichloë poae* Mcf gene was cloned into the vector pColdII for expression in *Escherichia coli*. Injection of the *Escherichia coli* cells expressing the *Epichloë poae* Mcf protein into black cutworms (*Agrotis ipsilon*) resulted in 100% mortality (N = 6) at 48 hours post-injection. As a control, black cutworms were injected with *Escherichia coli* cells containing the empty vector. These cutworms had 100% survival at 48 hours. These findings indicate that the *Epichloë poae* Mcf protein does have insecticidal activity.

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**Genetic Diversity of Bentgrass (*Agrostis*) Cultivars and Accessions
Using Microsatellite (SSR) Markers**

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The bentgrasses (*Agrostis* spp.) are important temperate perennial grass species utilized for forage and cultivated turf. Creeping bentgrass (*A. stolonifera* L.), colonial bentgrass (*A. capillaris* L.), and velvet bentgrass (*A. canina* L.) are adapted to low mowing heights, making these species highly suitable for fine turf applications on golf course putting greens, tees, and fairways. Numerous studies have shown that the genus *Agrostis* has been difficult to characterize taxonomically. The objectives of the current study were to genotype 74 bentgrass cultivars, experimental selections, and accessions using both genomic and chloroplast microsatellite (SSR) markers. Results based on genomic SSR markers showed that colonial and velvet bentgrasses were more similar to each other than either species was to creeping bentgrass, while chloroplast SSR markers indicated that velvet bentgrass was more closely related to creeping bentgrass. All cultivars, experimental selections, and collections, with the exception of the creeping bentgrass cultivars Shark and Authority, were uniquely identified with the current set of genomic SSR markers. Genetic relationships of individuals as assessed by genomic SSR markers generally matched known pedigrees. Bayesian clustering analysis indicated that many of the newer creeping bentgrass cultivars have been developed by recombination of similar breeding populations. The current set of genomic SSR markers can be used to genotype new creeping bentgrass cultivars/accessions to assess genetic relatedness among populations and should be considered for use in a creeping bentgrass Plant Variety Protection program.

POSTER PRESENTATIONS

Development of Molecular and Greenhouse-Based Resources to Facilitate Controlled *in Planta* Studies of *Colletotrichum cereale*

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Anthrachnose, caused by the ascomycete fungus *Colletotrichum cereale*, is a destructive disease of *Poa annua* putting green turf that has been increasing in frequency and severity in recent years. Considerable progress has been made towards the development of best management practices to control anthracnose in the field; however, many unanswered questions remain about the basic biology of this fungus. Advancements in this area have been hindered by the lack of available tools to conduct controlled, *in planta* studies. Our objective was to develop key tools required for experimental studies of this pathogen including: (a) a reproducible greenhouse inoculation protocol; and (b) a real-time PCR protocol to detect *C. cereale* directly from infected tissue. *P. annua* biotypes 98226 and 99112, shown in previous field studies to be susceptible to anthracnose, were inoculated with a conidial suspension (10^{-6}) of *C. cereale*, placed in a mist chamber inside a growth chamber, and maintained under conditions of 12 h daylight ($500 \mu\text{E m}^{-2}\text{s}^{-1}$), 100% relative humidity (RH), 30°C day, and 26°C night for 24 h. Two different fungal isolates were tested. Misting occurred for a duration of 1 h every 2 h. After 24 h, plants remained in the growth chamber under the conditions described above, except at 80% RH. The study was repeated twice and included negative controls. Plants exhibited chlorosis and thinning 10-14 d after inoculation. Morphological inspection revealed signs of the fungus (appressoria and occasionally acervuli). After stringent surface sterilization with 10% NaClO and 70% EtOH for 2 min., *C. cereale* was re-isolated from symptomatic tissue. To further confirm infection, we developed a real-time PCR assay based on the *Apn2* molecular marker. Dual-labeled hydrolysis probes and primer pairs were designed to discriminate between the two major *C. cereale* subgroups responsible for anthracnose disease, termed clades A and B. These assays were 100% effective at detecting the pathogen from 700 samples, including DNA extracted from cultured isolates (n=575), symptomatic plant tissue (n=38), and herbarium specimens (n=87) ranging from 70 to 105 years old. Using this protocol, *C. cereale* infection was verified in all inoculated plants (average cycle threshold value = 32.47). The development of a reliable, greenhouse-based inoculation protocol marks significant progress for advancing *in planta* studies of *C. cereale*. This tool, combined with the real-time PCR assays, will serve as a foundation for investigating the biology, infectivity, and lifestyle of this important pathogen.

Some Like it Basic: Graminicolous *Colletotrichum* Species Moderate Environmental pH by Secreting Ammonium

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The genus *Colletotrichum* represents a large group of filamentous fungi that are capable of causing anthracnose disease on a wide range of host plants. In fruit crops, *Colletotrichum* species are known for their ability to increase pH in localized areas via the secretion of ammonium in order to enhance pathogenicity and induce disease. This process is not well understood for graminicolous *Colletotrichum* species, but could provide important insight into their infection cycle in these hosts. The objectives of this study were: (1) to evaluate select graminicolous *Colletotrichum* species for their ability to raise the pH of their environment; and (2) to examine whether ammonium secretion plays a role in this process. Isolates of the following anthracnose pathogens were evaluated: *C. caudatum* (from *Sorghastrum nutans*), *C. cereale* (from *Poa annua*), *C. graminicola* (from *Zea mays*), *C. navitas* (from *Panicum virgatum*), and *C. sublineola* (from *Sorghum bicolor*). One representative isolate from each species was examined, except for *C. cereale* and *C. navitas*, from which four and two isolates were evaluated, respectively. An additional isolate, *C. gloeosporioides* (from *Cucumis pepo* fruit), a *Colletotrichum* species known to raise pH in plant tissue, was included for comparison purposes. All ten isolates were grown on solid, rich media (5g KNO₃, 4g K₂HPO₄, 2g MgSO₄·7H₂O, 0.01g FeCl₃, 10g yeast extract, 30g agar) at 25°C under constant fluorescent light at an initial pH of 4, 6, and 8. The pH of each culture was measured 1.5, 3.0 and 4.0 cm from the point of inoculation. To test for ammonium production, isolates were grown in liquid, rich media (5g KNO₃, 4g K₂HPO₄, 2g MgSO₄·7H₂O, 0.01g FeCl₃, 10g yeast extract) on a shaker at 25°C under constant light at an initial pH of 4. At 0, 24, 48, 72, and 96 h post-inoculation, pH and ammonium production were measured using colorpHast pH indicator strips (EMD Millipore, Billerica, MA) and a colorimetric ammonium test kit (EMD Millipore, Billerica, MA), respectively.

All five graminicolous species raised the pH of the solid media throughout the plates, but the effect was greatest closest to the point of inoculation (1.5 cm away from the center of the plate). When grown at an initial pH of 4, all isolates increased the pH to 7, 1.5 cm from the point of inoculation, whereas a pH of 8 or higher was achieved at the same distance when grown on media with an initial pH of 6 or 8. The isolate of *C. gloeosporioides* maintained a pH of 7 or higher at all distances from the point of inoculation. There was a positive relationship between increased pH and time; however, pH and ammonium production did not increase significantly until 48 hr post-inoculation. All isolates were capable of producing ammonium but to different degrees. For example, after 72 hr, *C. cereale* isolate 17DSouth produced 80 mg/L ammonium, whereas *C. cereale* isolate 11-100 only produced 35 mg/L. The *C. gloeosporioides* isolate consistently produced more ammonium than all other isolates.

Our results clearly show that several graminicolous *Colletotrichum* species are capable of increasing the pH of their environment, and that ammonium production plays a major role in this process. The importance of alkalization in the disease cycle remains unknown; however, it seems likely that increased pH around the point of inoculation may enhance the ability of graminicolous *Colletotrichum* species to colonize and infect their hosts.

Breeding Switchgrass for Anthracnose Resistance

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As switchgrass (*Panicum virgatum*) gets more widely planted for biofuel production, pests will become increasingly problematic. Anthracnose disease (caused by *Collitotrichum navitas*) in particular is becoming more prevalent on switchgrass cultivars in the northeast US. Fourteen switchgrass cultivars were planted in paired fields on both prime and marginal land in NJ. Significant differences were observed between cultivars in paired trials. Lowland cultivars, in general, had less anthracnose disease compared to upland cultivars selected from the mid-west. ‘Timber’ exhibited the most consistent resistance to this disease across locations, whereas ‘Pathfinder’ and KY 1625 were the most susceptible. There were also differences in cultivar rankings between locations. For example, ‘BoMaster’ had the best disease resistance in one location established on marginal land, but had only moderate tolerance at a different location where the trial was planted on prime land. ‘Sunburst’ had moderate tolerance at the marginal site, but was the most susceptible cultivar at the prime location. Additionally, we found a significant N effect on anthracnose disease at the different locations. Nitrogen application for most cultivars increased disease resistance, with the exception of ‘High Tide’. ‘High Tide’ exhibited more disease susceptibility with nitrogen compared to the no nitrogen treatment. We plan to use these data to identify superior cultivars and select genotypes to use as parents in the breeding program. Future research will include evaluation of a mapping population segregating for anthracnose resistance and the determination of the influence of anthracnose disease on biomass yield in switchgrass.

Phylogeography of the Boxwood Blight Fungus, *Calonectria pseudonaviculata*

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Boxwood (*Buxus* sp.) are long-lived evergreen ornamental shrubs popular in the landscape due to their shaping ability and deer-resistance. Boxwood is the most popular plant for topiaries; they are used in many U.S. historic sites, including the White House and Monticello, and are frequently planted on the grounds of golf courses and country clubs. A destructive new disease of boxwood, a blight caused by the ascomycete fungus, *Calonectria pseudonaviculata*, was first described in Europe in the 1990s and New Zealand in 1998. Boxwood blight was first reported in the U.S. states of Connecticut and North Carolina in October 2011. Within six months, the disease was reported in Maine, Maryland, New York, Ohio, Oregon, Pennsylvania, Rhode Island, and Virginia, as well as British Columbia and Ontario, Canada. More recently, the disease spread throughout the eastern seaboard into New Jersey, Maryland, and Delaware. Boxwood blight causes rapid defoliation of infected plants, followed by death. Boxwood production is worth over \$100 million a year in the U.S., with 43% of revenue derived from states reporting boxwood blight.

It is important to understand how much genetic diversity is present in different populations of this fungus to predict how that diversity could impact the evolution of important phenotypes, such as fungicide resistance. The objectives of this study were to develop genome-scale molecular markers in *C. pseudonaviculata* to estimate world-wide genetic diversity and to determine the source of the boxwood blight fungus in the U.S. One hundred and seventy-five isolates from Europe, Asia, U.S., and New Zealand were genotyped using AFLP markers. Data suggest at least two introductions of *C. pseudonaviculata* into the U.S., with subsequent movement of genotypes around the U.S. Estimates of genetic diversity were higher than expected for a recent introduction of what is considered a highly clonal species. In addition, multiple genotypes of the fungus were collected from individual plants, indicating that plants may host populations of fungi rather than individual clones. If the increased genetic diversity is associated with increased phenotypic diversity, these data suggest that mitigation of *C. pseudonaviculata* will require study of multiple isolates of the fungus to best encompass the evolutionary potential of the pathogen.

Nitrogen Fertility, Mowing Height, and Topdressing Effects on Anthracnose of Annual Bluegrass

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Anthracnose, caused by the fungus *Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman, is a destructive disease of annual bluegrass [ABG; *Poa annua* L. forma *reptans* (Hauskn.) T Koyama] putting green turf. Previous research has identified nitrogen (N) fertility, mowing height, and sand topdressing as best management practices (BMPs) that can be used to reduce anthracnose severity of ABG. However, questions remain about how combinations of these BMPs may interact to affect disease severity and playability [ball roll distance (BRD; Stimpmeter) and turf quality] of ABG turf. A field trial was initiated in 2012 in North Brunswick, NJ, to evaluate the effects of mowing height, N fertility, and sand topdressing on anthracnose severity and playability of ABG turf.

Treatments were arranged in a 2 x 2 x 2 factorial using a split-split-plot design with four replications. Main plots were mowing height (2.3 and 3.2 mm), subplots were N fertility (100 and 200 kg ha⁻¹ yr⁻¹), and sub-subplots were sand topdressing (46.4 and 97.6 Mg ha⁻¹ yr⁻¹ during 2012; 36.6 and 80.6 Mg ha⁻¹ yr⁻¹ during 2013).

Nitrogen fertility accounted for 82% and 35% of the variation in anthracnose severity during 2012 and 2013, respectively; as expected, greater N reduced disease severity up to 39% and 17% during 2012 and 2013, respectively, compared to the lower N rate. Sand topdressing and mowing height accounted for a similar amount of the variation in anthracnose severity. Higher mowing height reduced disease severity up to 13% compared to lower mowing height on three of seven rating dates during 2012, and five of nine rating dates during 2013. Greater sand topdressing reduced disease up to 10% and 11% compared to the lower sand topdressing treatment on five of seven rating dates during 2012 and seven of nine rating dates during 2013, respectively. To date, interaction effects on anthracnose severity among these management factors have not been consistent.

The relative importance of management factors for BRD differed from that observed with disease severity responses. Mowing height accounted for 71% and 62% of the BRD response during 2012 and 2013, respectively, and N fertility accounted for slightly more of the BRD response than the sand topdressing factor. Additionally, the changes in BRD caused by N fertility and sand topdressing were not likely to be detectable by golfers since BRD differences were typically 0.2 m or less. Across all treatments, the occurrence of an acceptable BRD (≥ 2.9 -3.2 m) in 2013 ranged from 19 to 94% of the evaluation dates, which differed from 2012 when all treatments had acceptable BRD on 69 to 97% of evaluation dates. Frequent rain during early summer 2013 was the most likely reason for the lower frequency of acceptable BRD during 2013, especially at the high mowing height.

In summary, increased N fertility produced the greatest reductions in disease severity compared to higher mowing height or greater sand topdressing. However, increased N fertility and sand topdressing had a minimal effect on BRD; whereas, higher mowing height had a substantial negative effect on BRD. Thus, priority should be given to lowering mowing height rather than

reducing N fertility or sand topdressing rates when adjusting BMPs to improve playability (green speed) without greatly increasing the risk for anthracnose.

Nitrogen Fertility and Mowing Height Effects on Fungicide Control of Anthracnose

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It has been suggested that fungicide control of anthracnose disease (*Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman) of annual bluegrass [ABG; *Poa annua* L. forma *reptans* (Hauskn.) T Koyama] may be less efficacious if cultural practices are not optimized. Greater nitrogen (N) fertility and increased mowing height are best management practices (BMPs) that have been shown to decrease anthracnose severity and thus may help reduce fungicide inputs needed to control this disease. Reductions in fungicide use can be quantified by either a decreased number of threshold-based fungicide applications (increased interval between sprays) or a reduced rate of fungicide that provides acceptable disease control. A field trial was initiated in 2012 in North Brunswick, NJ to evaluate the effects of mowing height, N fertility, and fungicide programming on anthracnose disease control of ABG turf grown on a Nixon sandy loam. Treatments were arranged in a 2 x 2 x 6 factorial using a split-split-plot experimental design with four replications. The three factors were mowing height (2.3 and 3.2 mm) applied as the main plot, N fertility (100 and 200 kg ha⁻¹ yr⁻¹) applied as the subplot, and fungicide program [no fungicide, threshold-based applications (> 5% turf area infected and > 7-d since a previous fungicide spray), and calendar-based applications at four fungicide rates (25%, 50%, 75%, and 100%)] applied as the sub-subplot. All fungicide treatments consisted of a tank mix of chlorothalonil and fosetyl-Al. All threshold applications were applied at the 100% rate of 8.1 and 9.8 kg a.i. ha⁻¹ of chlorothalonil and fosetyl-Al, respectively.

Nitrogen fertility and fungicide programming and the interaction between these factors accounted for 91 and 89% of the area under the disease progress curve (AUDPC) response during 2012 and 2013, respectively. As expected, greater N fertility reduced disease severity up to 25% and 15% compared to the lower N level across all rating dates during 2012 and 2013, respectively. Higher mowing height reduced disease severity up to 6% compared to lower mowing height on five of nine rating dates during 2012 and 8% on seven of eleven rating dates during 2013. Nitrogen fertility interacted with fungicide rate on most disease observations dates during both years. This interaction indicated that under higher N fertility acceptable disease control (<10% turf area infected) could be achieved at fungicide rates as low as 25% of label rate during 2012 and 50% of label rate during 2013; whereas, under lower N fertility, an acceptable level of disease control could not be consistently achieved at any fungicide rate during either year. The efficacy of fungicide treatments depended on mowing height as well as the N fertility rate (3-way interaction) on five of eleven dates during 2013. This interaction indicated that mowing height had no effect on AUDPC (Fig. 1) under either N level at the 50, 75 and 100% fungicide rates. And maximum disease control at these fungicide rates was achieved at the greater N level. Whereas, lower mowing height increased disease severity at the 0 and 25% fungicide rates regardless of the N level. At the 0% fungicide rate, increasing the mowing height and N fertility was the most effective management for suppressing disease severity.

The findings from this trial indicate that increasing N fertility can reduce anthracnose severity more than increasing mowing height; fungicides are more efficacious under greater N fertility; and lower mowing height can reduce fungicide efficacy at low fungicide rates. Moreover, a

higher mowing height can cause the greatest reduction in disease severity on plots that receive greater N fertility and no fungicides. These data indicate that fungicide rate could be reduced by up to 75% and still provide acceptable disease control when greater N fertility is applied. It also appears that threshold-based management of fungicide applications is feasible (total applications were reduced 80% in 2012) when BMPs are used. However, another year of evaluation is needed to confirm these observations.

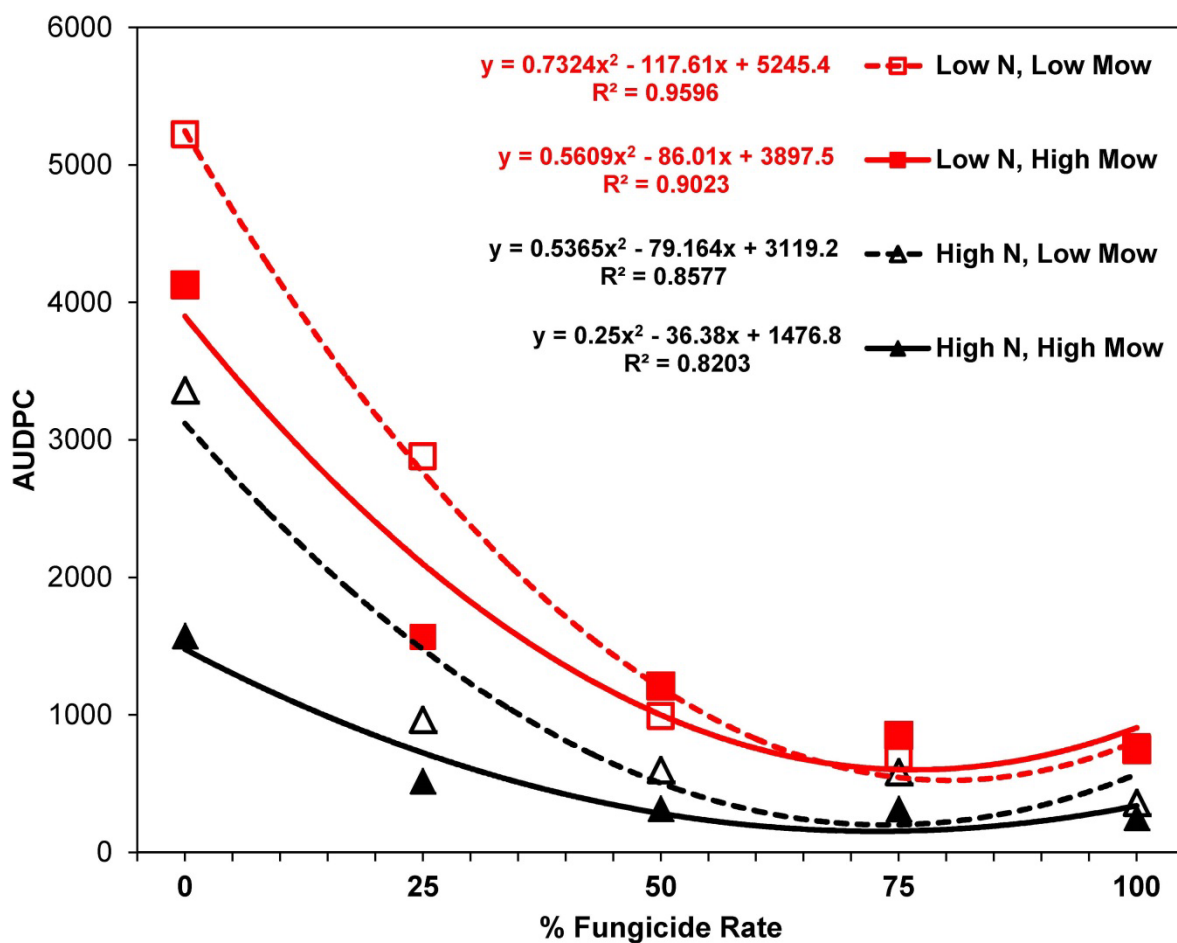


Figure 1. Area under disease progress curve (AUDPC) response to mowing height, N fertility, and fungicide rate on annual bluegrass turf in North Brunswick, NJ during 2013.

Host Plant Resistance and Tolerance to Annual Bluegrass Weevil

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The annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby, is a serious and expanding pest of short cut turfgrass. Presently, chemical control is the only effective and commonly used management strategy. Development of resistance and compromised efficacy of most available insecticides against resistant populations has emphasized the need for alternative strategies. Annual bluegrass, *Poa annua* L., is generally considered to be a preferred host of ABW and/or is particularly susceptible to it. However, there is an increasing number of field observations on ABW damage to creeping bentgrass (CBG), *Agrostis stolonifera* L. As for now, the only evidence of increased creeping bentgrass tolerance was obtained in limited experimental studies. 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, and currently, it is impossible to completely eliminate *P. annua* (re)establishment over time. Therefore, it will be important to select bentgrasses that are not only more tolerant of ABW feeding but also poor hosts for ABW (i.e. resistant). The main goal of this study is to investigate the potential of host plant resistance as an alternative strategy for ABW management.

ABW ovipositional and feeding preferences were determined in the laboratory and field in no-choice and choice experiments. Larval survival and growth on selected bentgrasses and grass tolerance to larval feeding were studied in greenhouse assays. Throughout our various experiments, older and newer cultivars of three bentgrass species were evaluated for resistance to ABW considering three major components: antixenosis, antibiosis, and tolerance. They were compared to wild type *P. annua* as the assumed most preferable and suitable ABW host. Because CBG is the most commonly used bentgrass species on golf courses, four cultivars were included: 'L93' and 'Penncross' (older, widely known cultivars), and '007' and 'Declaration' (newer, high-quality cultivars). Two cultivars of colonial bentgrass, *A. capillaris*, 'Tiger II' (better known) and 'Capri' (newer, better quality), and two cultivars of velvet bentgrass, *A. canina*, 'Greenwich' and 'Villa', were used in the experiments.

Compared to the assumed preferred host annual bluegrass, all tested bentgrass cultivar were less preferred for ABW oviposition in no-choice and choice laboratory and field experiments. Oviposition was observed in all tested bentgrasses. 'Villa', 'Greenwich' and 'Declaration' were the least preferred among bentgrasses. *Poa annua* was the most suitable host for ABW larval survival and development. It had significantly higher numbers of ABW life stages than all tested bentgrasses. Moreover, larvae weighed more and developed faster in *P. annua* than in most of the bentgrasses. Suitability as a host for ABW larvae varied among bentgrass species and cultivars. Larval densities were relatively high in 'Villa' and 'Capri'. Among the bentgrasses, creeping bentgrass cultivars were the least preferred and suitable for ABW survival and development. Nonetheless, ABW larvae survived and developed on all of the tested bentgrasses.

Two greenhouse studies in which grasses were exposed to larval feeding and cumulative effect of adult, young, and late larvae feeding demonstrated that bentgrasses were more tolerant to ABW feeding than *P. annua*. Damage in *P. annua* pots become apparent sooner and at lower ABW densities than in bentgrasses. Our data model predicts that bentgrasses generally can

tolerate 2-3 times higher densities of ABW larvae than *P. annua* before sustaining the same damage level. However, at higher population densities, damage was observed in some bentgrass cultivars, with the most severe damage occurring in velvet and colonial bentgrass. Thus, creeping bentgrass is preferable as an overseeding option in areas with high ABW populations.

To investigate possible mechanisms responsible for differences in resistance among bentgrasses and compared to *P. annua*, silicon and fiber content of leaf and stem tissue were determined for all of the above grass species and cultivars. However, results showed that neither silicon or fiber content was correlated to ABW larval densities observed in the tested grasses, and thus, they were not likely involved in bentgrass resistance to ABW.

**Comparative Genomics of the Newly Emergent Boxwood Blight Pathogen
Calonectria pseudonaviculata Reveals Global Diversity in the
Frequency of the Mating-Type Locus**

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Calonectria pseudonaviculata, a destructive ascomycete fungal pathogen responsible for boxwood blight disease, has spread in the past fifteen years throughout the United Kingdom, New Zealand, and most of Europe. Subsequently, the pathogen was reported in western Asia and most recently was found in North America in late 2011. Affected plants show dark brown to black spots or diffuse dark areas on leaves, followed by defoliation, severe dieback, and eventual plant death. In the U.S., boxwood are commercially important evergreen ornamental plants with an annual market value of over \$103 million. The factors driving the global dispersal of this destructive pathogen are currently undetermined. For plant pathogenic fungi, generation of genetic diversity stemming from sexual recombination is known to facilitate the rapid adaptation of pathogens to host resistance genes or fungicides or lead to increased virulence. As such, an understanding of the sexual reproductive potential of *C. pseudonaviculata* holds strong implications for boxwood blight disease epidemiology, risk assessment, and the development of effective control strategies.

In this study, we used comparative genomics to assess the primary determinant of mating, the *MATI* gene, from a global population of 300 *C. pseudonaviculata* isolates originating from four continents. Isolates were collected since the first outbreaks of boxwood blight in 1994 through to the present day. Genomic DNA libraries for paired-end and mate pair reads were constructed for 15 *C. pseudonaviculata* isolates using Illumina Nextera tagmentation chemistry and sequenced using an Illumina MiSeq instrument (2x250 and 2x300; 219 million assembled reads). Four isolates representing the two major subgroups of *C. pseudonaviculata* (G1 and G2) were sequenced to high depth of coverage (310x to 58x; maximum N50=60k); eleven isolates were sequenced to an average coverage of 23x to 34x. Genome assemblies (~53 Mb) were used to create local BLAST databases and then searched for homologues of the idiomorphic genes *MATI-1* and *MATI-2*. From each isolate, only one of the two idiomorphs was present in the genome at the *MATI* locus, indicating that *C. pseudonaviculata* is a heterothallic (outcrossing) fungus. Based on the genome assemblies, two sets of PCR primers were designed to amplify differently sized regions of the *MATI-1* and *MATI-2* genes in a duplex reaction, with scoring of the mating type performed using capillary gel electrophoresis visualization of amplicon size. PCR products of the *MATI-1* and *MATI-2* idiomorphs were sequenced, analyzed and compared against the reference genome assemblies of isolates CB45 (*MATI-1*) and cpsCT1 (*MATI-2*) using BLASTn. *MATI* nucleotide sequences of the 300 isolates showed 100% identity to the reference isolate sequences. PCR analysis showed that the *MATI-2* genotype is present worldwide, but exclusively in the G1 lineage. *MATI-1* is solely present in five European countries from isolates of the G2 lineage. Since the G2 lineage only first appeared in 2005, it

appears that the fungus lacked the ability to undergo sexual recombination until the introduction of G2.

It is currently unknown whether the G1 and G2 isolates are able to mate, as extensive divergence between the two lineages suggests that G1 and G2 may be distinct species. For example, although the *MAT1* genes themselves were invariable, comparative analysis of the genome region immediately surrounding *MAT1* showed that 26% of the 35kb contig exhibited fixed variation between the G1 and G2 isolates. Furthermore, within gene coding regions, 10% of the nucleotide variants produced nonsynonymous base changes, resulting in amino acids changes in G2 versus G1 isolates. 100% of the isolates from North America were solely of the *MAT1-2*/G1 genotype; therefore, our data suggest that the fungus is currently unable to reproduce sexually in the U.S.

Enhancing the Evaluation of New Hazelnut Germplasm from Turkey and the Baltic Region with the Use of Molecular Tools

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At Rutgers University, we have been collecting European hazelnut (*Corylus avellana*) germplasm from across its native range in Eastern and Northern Europe, Russia, and the Caucasus region since the project was started in 1996 by C. Reed Funk. A primary goal of these collections was to identify plants resistant to the fungal disease eastern filbert blight (EFB), caused by *Anisogramma anomala*, that also produce nuts of improved quality. Over the past decade, we have been successful in this effort. Through the evaluation of 2449 open-pollinated *C. avellana* seedlings from Russia, Ukraine, and Poland, we identified 84 new trees that were resistant to EFB and 14 others that only expressed a few small cankers (Molnar et al., 2007; Capik et al., 2013). A number of these trees also produced excellent quality kernels, and preliminary progeny tests are showing that resistance is being transmitted to their offspring in a manner indicative of a dominant gene. One limitation of this approach, however, is the uncertain origin and genetic relationships of the seedlings. Many of the seed lots were collected from roadside markets and bazaars in rural regions where the parent trees were unknown. To better understand the resistant accessions, 17 simple sequence repeat (SSR) markers were used to investigate their genetic diversity and population structure in comparison to a panel of known and well-characterized reference cultivars. The resulting co-allelic data was then used to construct a UPGMA dendrogram and STRUCTURE diagram to clarify relationships between trees. Results showed that our new EFB-resistant or tolerant accessions were found throughout most of the final 11 consensus groups resolved in the study, providing evidence that EFB-resistance is common across the *Corylus* genus. Further, seedlings collected from common geographic locations tended to group together with reference accessions of similar origins, providing additional insight into their genetic backgrounds (Muehlbauer et al., 2013). The information generated from the study greatly aids the selection of breeding parents when goals are to maintain a high level of genetic diversity in the offspring.

A more recent collection of hazelnut germplasm was made from Turkey and the Baltic region. Since 2005, nearly 1000 trees originating from Turkey, Latvia, and Lithuania have been evaluated in the field. In 2013, we identified 46 resistant and tolerant accessions from this collection, several of which also produced nuts of improved quality. Based on the success of our previous study, we again used SSR markers to characterize these new accessions in comparison to 26 reference accessions (including a number of our new resistant seedlings from Russia, Ukraine, and Poland) representing each of the 11 consensus groups derived from our original study. Allele summary statistics for each of the 17 markers showed the following results; average allele number=11.5, average H_e =.768, average H_o = .81, and average PIC value= .741. The summary statistics indicate that this group of hazelnut accessions (in particular the new Turkish, Latvian and Lithuanian seedlings) are very genetically diverse. The observed and expected heterozygosity values and PIC values are similar to those derived from our previous hazelnut diversity study, although the number of alleles found in this study was only about half of that found in our prior study, likely because this study only included a fraction of the number of

accessions in the original study. The UPGMA dendrogram showed three main clusters of accessions: a group of nearly all of the Turkish seedlings and cultivars, a group of nearly all of the Latvian and Lithuanian seedlings, and a final group holding most of the remaining reference cultivars.

In general, the SSR results show that these new accessions from Turkey and the Baltic region represent new sources of resistance from regions where few resistant accessions were previously available. Together with our earlier efforts, our new resistant plants at Rutgers University provide a very strong foundation to continue breeding efforts with the goal of developing EFB-resistant plants that produce excellent quality kernels. The combination of phenotypic characteristics and molecular tools allow us to identify and utilize the best plants from the different genetic groups in our breeding efforts to better maintain high genetic diversity while also selecting for rare, elite plants expressing EFB resistance with improved nut traits.

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Potassium and Iron Fertilization Effects on Tolerance of Velvet Bentgrass to Foot Traffic

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Interest in using velvet bentgrass (*Agrostis canina* L.; VBG) for golf course putting green turf has been renewed over the past decade. Improved VBG cultivars exhibit excellent turf performance under wear compared with many creeping bentgrass (*A. stolonifera* L.) cultivars; however, there is a lack of research on the effects of fertility on VBG performance. The objective of this trial was to determine what effect, if any, potassium (K) and iron (Fe) nutrition have on the performance of VBG subjected to foot traffic. A field trial was initiated in 2010 on a 6-yr-old 'Greenwich' VBG turf in North Brunswick, NJ. A 2x2x5 factorial study arranged in a split-split plot design with three replications was used for this trial. The factor of foot traffic (none or 200 rounds per day; manually applied using golf shoes equipped with Softspike Pulsar® cleats) was applied as main plots. Sub-plots were the iron sulfate factor applied at 0 or 0.12 g m⁻² of Fe every two weeks. Sub-sub-plots were the potassium sulfate factor applied at N:K of 1:0, 1:0.4, 1:0.8, 1:1.7, and 1:3.3 every two weeks. Nitrogen was applied biweekly at a rate of 4.9 kg N ha⁻¹. As expected, foot traffic reduced turf quality and color compared to no foot traffic from June through October in 2010 and 2011, especially during periods of high temperature stress. Biweekly iron sulfate applications increased turf color throughout the growing season, but improvement in turf quality was much more subtle. Biweekly applications of potassium sulfate at a N:K ratios of 1:1.7 and 1:3.3 lowered turf quality and color from July through October compared to the lower ratios or no application of potassium. High rate K fertilization created elevated K levels (>116 mg kg⁻¹) in the thatch/mat layer, which eventually had a negative impact on turf quality and color. Our results indicate that biweekly applications of iron sulfate can be used to mask discoloration of VBG caused by foot traffic. Biweekly applications of K were detrimental at greater rates of K to turf performance.

Effects of the Rutgers Wear Simulator and Cady Traffic Simulator on the 2011 NTEP Kentucky Bluegrass Trial

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Researchers have developed machines to impart wear and the combined stresses of wear and compaction on turfgrass. The objective of this trial was to evaluate the effects of the Rutgers Wear Simulator (RWS) and the Cady Traffic Simulator (CTS) on Kentucky bluegrass (*Poa pratensis* L.). The field trial included all entries of the 2011 NTEP Kentucky Bluegrass Trial seeded in October 2011 on a loam soil in North Brunswick, NJ. Three separate machine treatment strips (RWS, CTS, and non-treated control) were applied across 92 Kentucky bluegrass entries in a strip-plot design with three replications. Sixteen passes of the RWS and CTS were made (2 passes per week) during 26 Sept. to 14 Nov. 2012 (8 weeks). Fullness of turf canopy (FTC; 0 to 100% scale) and turf quality (1 to 9 scale) were visually assessed and percent green cover was determined by digital image analysis. After 16 passes, machine treatments ranked as control>CTS>RWS for FTC, turf quality, and percent green cover and the factors of machine and entry interacted for each of these parameters. The RWS reduced the FTC and percent green cover for all entries relative to the control. The CTS did not reduce the turf quality, percent green cover, nor FTC of three, twelve, and sixteen entries respectively, compared to the control. BAR VV 0709 and Pp 10847 had the best turf quality and greatest FTC and percent green cover after 16 passes of the RWS. Entries with the best turf quality and greatest FTC and percent green cover after operation of the CTS were AKB 2282, A03-1017, BAR Pp 110358, J-1770, Pick 4340, and BAR 8PP 504. Entries with the poorest turf quality and least FTC and percent green cover after 16 passes of both machines were PST-K4-3, A05-329, A06-47, PST-K9-97, and PST-K9-90.

Identification of Low Ergovaline Containing Forage Tall Fescue

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Endophyte safe tall fescue has been developed for the Central Plains and southern regions of the U.S. There has been no endophyte safe tall fescue cultivars bred for the Continental climate typically found in the northeastern U.S. Endophyte safe tall fescue is desirable in that it produces low levels of alkaloids toxic to mammals, but high levels of alkaloids toxic only to insects, and has stand persistence. We have screened 191 forage tall fescue plants from the Rutgers Turfgrass breeding program that are grown in a Continental climate for horse pasturage in New Jersey and have identified over 20 possible candidates.

Application of Droplet Digital PCR Technology for *In Situ* Genotypic Analysis of Plant Pathogen Populations

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Population studies of plant pathogens are essential to understanding the biology of current epidemics that threaten crop production and yield. These studies can provide information on the genetic changes of different populations over space and time and allow us to better manage plant protection programs. Challenges to genotyping individuals arise when the organisms of interest are obligate biotrophs, have multiples individuals per leaf, or have multiple ploidy levels.

In this work, we evaluate the ability of the Droplet Digital PCR (ddPCR™) system implemented on the BioRad QX100 instrument to genotype pathogen samples *in situ*. For our experiments, we evaluated populations of *Plasmopara obducens*, the diploid oomycete pathogen responsible for the recent downy mildew disease epidemics on cultivated impatiens (*Impatiens walleriana*). Population studies of this pathogen are difficult due to its biotrophic nature and diploid genome. Because *P. obducens* cannot be cultured and DNA is extracted directly from environmental samples, genotyping of samples can also be hindered by the presence of heterogeneous DNA. The high sensitivity provided by the ddPCR™ could potentially help in accurately genotyping samples in difficult systems like this pathosystem. ddPCR™ enables the absolute quantification of nucleic acids in a sample, using the same primers and probes as real-time PCR but with greater sensitivity and precision. The ddPCR™ system involves the emulsification of a DNA sample, probes, primers, and PCR master mix with oil to create 20,000 aqueous droplets containing target DNA. Each aqueous droplet functions as an independent amplification event within a single sample. The emulsified samples are then transferred to a standard PCR thermal cycler for amplification. After thermal cycling, the samples are transferred to the BioRad QX100 droplet reader for endpoint fluorescence analysis. Droplets that contain target DNA will exhibit a higher fluorescence relative to negative droplets. Furthermore, droplets containing multiple genotypes (i.e. due to aneuploidy or multiple nuclear populations) will also be detected by this system. The data is then analyzed using Poisson statistics to determine the concentration of target DNA in each sample.

In this study, we identified five single nucleotide polymorphisms (SNP) by performing Sanger sequencing of the nuclear ribosomal DNA of 134 *P. obducens* individuals. Taqman hydrolysis probes were designed for four SNPs and used to genotype 895 *P. obducens* samples, including 87 herbarium samples collected between 1881-2004, using standard real-time PCR (qRT-PCR) and ddPCR™. The ddPCR™ revealed allelic ratios departing from 1:1 for multiple heterozygote samples, with 100 of 449 samples analyzed to date exhibiting a 3:1 allelic skew. For two of the

SNPs analyzed, this 3:1 allelic ratio was observed in 30-38% of all the modern samples and only in 4-6% of herbarium samples. This observed 3:1 allelic ratio departs from the expected 1:1 ratio in diploid organisms. The observed skew could be a result of mixed genotypes in individual samples, or the presence of aneuploidic states in single isolates, or both. The ddPCRTM system proves to be a valuable tool for diagnosis for the presence of a single or multiple genotype within a sample. In addition, it has higher precision and sensitivity than qRT-PCR.

Soil pH Modification Effects on Annual Bluegrass Growth, Quality, and Anthracnose

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Soil pH can affect many soil chemical and biological properties, such as nutrient availability, ion toxicity, and microbial activity. Generally, annual bluegrass [*Poa annua* L. f. *reptans* (Hauskn) T. Koyama; ABG] is not considered to be tolerant of low soil pH; however, the soil pH level that inhibits annual bluegrass growth and decreases turfgrass quality is not clear. The objectives of this field study were: 1) to quantify the responses of ABG over a range of soil pH and identify a critical level; and 2) to determine the effect, if any, of soil pH on anthracnose severity of annual bluegrass turf. The trial was initiated in 2011 on ABG turf that had an initial pH value of 5.3 in the 60-mm deep mat layer and 6.0 in the underlying soil. Granular limestone (CaCO_3) was applied at five rates (118, 569, 1184, 1739, and 2247 kg $\text{CaCO}_3 \text{ ha}^{-1}$) based on target pH levels of 5.8, 6.3, 6.8, 7.3, and 7.8, respectively, in the mat-thatch layer. Elemental sulfur was sprayed as a wettable powder at two rates (24 and 49 kg S ha^{-1}) to decrease pH. All treatments were applied 12 Dec. 2011 to provide reaction time over the winter and early spring of 2012.

Soil test data from August 2012 indicated that the pH of the mat layer ranged from 4.3 (49 kg S ha^{-1} treatment) to 5.8 (2,247 kg $\text{CaCO}_3 \text{ ha}^{-1}$). Sulfur treatments decreased turfgrass quality and color throughout 2012 and 2013 compared to limestone treatments and the check. In 2012, anthracnose severity was variable across the research plots; however, sulfur treatments and the untreated check (low pH treatments) generally had greater disease severity compared to the highest limestone rate (2247 kg $\text{CaCO}_3 \text{ ha}^{-1}$). Disease severity was more consistent during 2013. Once again, sulfur treatments and the untreated check had the greatest anthracnose severity and the two highest limestone rates (1739, and 2247 kg $\text{CaCO}_3 \text{ ha}^{-1}$) decreased disease severity compared all other treatments except 1184 kg $\text{CaCO}_3 \text{ ha}^{-1}$.

Effects of Elevated Carbon Dioxide Concentration on Kentucky Bluegrass Responses to Increasing Temperatures and Drought Stress

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Global climate change is associated with increased CO₂ concentration in the atmosphere, elevated temperatures, and a decline in water availability. The objective of this study was to investigate whether elevated CO₂ may mitigate the adverse effects of increasing temperature and drought stress on a cool-season turfgrass species. Kentucky bluegrass ('Baron') plants were grown in growth chambers with ambient (400 ppm) or elevated (800 ppm) CO₂ and day/night temperatures of 15/12, 20/17, 25/22, 30/27, 35/32 °C under well-watered conditions or deficit irrigation to replace 50% of the evapotranspiration rate (drought stress). Turf quality declined with increasing temperature and during drought stress, and the highest turf quality was found in the combined treatment of 20/17 °C, elevated CO₂, and well-watered conditions. The lowest turf quality occurred at the combined treatment of 35/32 °C, drought stress, and ambient CO₂. Increasing temperatures had no effects on leaf wilting score for the well-watered control at both ambient and elevated CO₂, but drought stress caused severe leaf wilting, particularly at temperatures above 25/22 °C for both ambient and elevated CO₂. Elevated CO₂ mitigated drought-induced leaf wilting under high temperatures. Increasing temperatures and drought stress caused significant decline in shoot and root dry weight, but plants at elevated CO₂ maintained significantly higher shoot and root biomass at the different temperatures and under drought stress compared to plants in ambient CO₂. Single-leaf photosynthetic rate (P_n) was the highest at 20/17 °C and decreased with increasing temperatures. The decline was more severe under drought stress compared to well-watered conditions. Plants at elevated CO₂ had significantly higher P_n under all temperature regimens for both well-watered conditions and drought stress compared to plants in ambient CO₂. Respiration rate increased with increasing temperatures and elevated CO₂ suppressed the rate of increase. Results show that elevated CO₂ mitigated the adverse effects of high temperatures and drought stress on Kentucky bluegrass as demonstrated by the improvement in turf quality, leaf wilting, and biomass production, which could be related to the suppression of carbohydrate consumption through respiration and the promotion of carbohydrate production through photosynthesis.

Effects of Sand Size for Topdressing Velvet Bentgrass Putting Green Turf

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Topdressing sand is applied to smooth the surface of putting greens and maintain desirable rootzone characteristics. Sand size can impact the ease of incorporation and interference to mowing and play. A field trial was initiated in July 2010 on 'Greenwich' velvet bentgrass (*Agrostis canina* L.) to determine the effects of sand particle size distribution and application rate on the ease of incorporation, turf quality, surface hardness (Clegg Impact Soil Tester), and water retention. Treatments were arranged in a 2 x 2 factorial and included an untreated control in an RCBD with three replications. Topdressing was applied biweekly at 0.15 and 0.3 L m⁻² with medium-coarse and medium-fine size sands. Differences in hardness and turf quality were not apparent during 2010; however, all topdressing treatments had better turfgrass quality than the non-topdressed check during 2011, 2012, and 2013. Additionally, plots topdressed at 0.3 L m⁻² had better turfgrass quality than plots topdressed at 0.15 L m⁻². Topdressing with medium-fine sand produced equivalent or better turf quality than plots topdressed with medium-coarse sand. Surprisingly, surface hardness decreased for 17% and 70% of the 0.5- and 2.25-kg Clegg measurements, respectively, compared to the non-topdressed control during 2012 and 2013. Volumetric water content (VWC) at 0- to 3.8-cm depth zone was greater in non-topdressed plots than topdressed plots on 50% of observation dates. No substantial differences in VWC were found among sand sizes and topdressing rates. Topdressing at 0.3 L m⁻² or with medium-coarse sand increased the time for the sand to incorporate or be removed from the turf surface with mowing compared to 0.15 L m⁻² and the medium-fine sand, respectively. The amount of medium-coarse sand removed by mowing increased dramatically as the topdressing rate increased from 0.15 to 0.3 L m⁻²; whereas there was no increase in medium-fine sand removal between these topdressing rates during 2012 and 2013. In 2013, all sand treatments significantly increased water infiltration measured with mini disk infiltrometers at near saturation, indicating sands were improving the rootzone by increasing macropores. Water infiltration was not different among sand sizes and topdressing rates.



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