Symposium Organizing Committee

Albrecht Koppenhöfer, Chair
Bruce B. Clarke
Matthew Elmore
Barbara Fitzgerald
James Murphy

Proceedings of the Twenty-Seventh Annual Rutgers Turfgrass Symposium

Matthew Elmore and Barbara Fitzgerald, Editors

*Rutgers Cooperative Extension educational programs are offered to all without regard to race, religion, color, age, national origin, gender, sexual orientation or disability.*
ASSOCIATE DIRECTOR’S REMARKS:

Welcome to the Twenty-Seventh Annual Rutgers Turfgrass Symposium at the School of Environmental and Biological Sciences/NJAES. The Symposium was established in 1991 to provide Rutgers faculty and staff with an annual forum for the exchange of ideas on a wide range of topics in turfgrass science. This was later expanded to include scientists from outside of Rutgers to present their research and serve as keynote speakers. I would like to thank Dr. Jeffrey Steiner (Division Director for Plant Production in the Institute of Food Production and Sustainability, USDA-NIFA) for giving this year’s keynote address. I would also like to thank Dr. Ning Zhang, Dr. Stacy Bonos, Dr. James Murphy, and Dr. Albrecht Koppenhöfer for serving as moderators and the Symposium Planning Committee, comprised of Drs. Albrecht Koppenhöfer (Chairperson), Bruce Clarke, Matt Elmore (Editor), James Murphy, and Ms. Barbara Fitzgerald (Co-Editor).

The faculty, students and staff in the Rutgers Turfgrass Program continue to be recognized for excellence in research, teaching and extension. A great accomplishment in 2017 was having Rutgers host the 13th International Turfgrass Research Conference (ITRC). This was the first time that the ITRC was held in the United States since 1993 and was a very successful and well-attended conference. Almost 500 people from 24 countries attended the ITRC, and Ms. Stephanie Rossi took 2nd place honors in the Graduate Student Oral Paper Competition. At the Annual Crop Science Meetings in Tampa, FL, graduate students Hui Chen, Cathryn Chapman and James Hempfling received awards in the graduate student oral and poster competition. Moreover, two of our students, James Hempfling and Yuanshuo Qu, were recognized by the Golf Course Superintendents Association of America with Watson Fellowships. We also congratulate Drs. James Murphy and Bruce Clarke who received the Recognition Award in 2017 from the New Jersey Turfgrass Association.

Over the past 27 years, Turf Center faculty have continued to conduct outstanding research, undergraduate and graduate teaching and continuing professional education and service programs in support of the Turfgrass Industry. In return, the Turfgrass Industry has donated their time and over $5 million in the form of research grants, student scholarships and building funds to the Rutgers Turfgrass Program. In addition, the Cultivated Sod Association of New Jersey, in collaboration with the New Jersey Turfgrass Association, the New Jersey Landscape Contractors Association and the Golf Course Superintendents Association of New Jersey, raised funds for a permanent pavilion at the Adelphia Research Farm in honor of Dr. Steve Hart, former Extension Specialist in Turfgrass Weed Science at Rutgers University. The Hart Pavilion is a beautiful addition to the Adelphia Farm and will be used for a wide range of meetings and Field Days for many years to come.

It is with deep pride and a sense of anticipation for the bright future of the Turf Center that we welcome you to this year’s Symposium. We are pleased that you have chosen to attend this year’s Turfgrass Symposium.

Sincerely,

William A. Meyer, Associate Director
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TWENTY-SEVENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University
January 12, 2018
Foran Hall, Room 138A

Friday, January 12, 2018

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

9:00 - 10:00 AM SESSION I: Microbes Associated with Turfgrasses
(Moderator: Dr. Ning Zhang)

9:00 – 9:20 Dr. James F. White (Rutgers University, Department of Plant Biology) Seed-Vectored Bacterial Endophytes of Grasses: What Are They and How Do They Work?

9:20 – 9:40 Glen Groben (Rutgers University, Department of Plant Biology) The Microbiome Associated with Tall Fescue Under Drought Stress

9:40 – 10:00 Dr. Joseph Roberts (University of Maryland, Department of Plant Science and Landscape Architecture) Identification and Management of Bacteria Associated with Etiolation on Cool-Season Golf Course Fairways

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

10:30 – 11:10 AM SESSION II: Weed Science
(Moderator: Dr. Stacy Bonos)

10:30 – 10:50 Trent M. Tate (Rutgers University, Department of Plant Biology) Breeding Fine Fescues for Increased Tolerance to Mesotrione

10:50 – 11:10 Dr. Matthew Elmore (Rutgers University, Department of Plant Biology) Early Efforts to Understand and Control False-Green Kyllinga (Kyllinga gracillima) and Goosegrass (Eleusine indica)

11:10 – 11:20 AM Discussion session

11:20 – 12:00 PM KEYNOTE: Federal Funding in Turfgrass Science
Dr. Jeffrey Steiner (USDA – NIFA, Institute of Food Production and Sustainability) Resourcing the Next Generations of Turfgrass Science

12:00 - 1:00 PM Lunch and Poster Session
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<td>1:00 – 2:10 PM</td>
<td><strong>SESSION III: Turfgrass Management and Stress Physiology</strong>&lt;br&gt;(Moderator: Dr. James Murphy)</td>
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<td>1:00 – 1:30</td>
<td><strong>Mark Kuhns</strong> (Director of Grounds, Baltusrol Golf Club) <em>Building a Successful Career in Golf Course Management: It’s More than Just Growing Grass</em></td>
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<td>1:30 – 1:50</td>
<td><strong>Brad Park</strong> (Rutgers University, Department of Plant Biology) <em>Assessing Grasses for Establishment on Two New Jersey Roadsides</em></td>
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<td>1:50 – 2:10</td>
<td><strong>Dr. Bingru Huang</strong> (Rutgers University, Department of Plant Biology) <em>Molecular and Metabolic Regulation of Stress-Induced Leaf Senescence in Turfgrass</em></td>
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<td>2:10 – 2:30 PM</td>
<td><strong>Discussion and Coffee Break</strong></td>
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<td>2:30 – 3:30 PM</td>
<td><strong>SESSION IV: Pest Biology and Management</strong>&lt;br&gt;(Moderator: Dr. Albrecht Koppenhöfer)</td>
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<td>2:30 – 2:50</td>
<td><strong>Dr. Shaohui Wu</strong> (Rutgers University, Department of Entomology) <em>Reproductive Diapause in the Annual Bluegrass Weevil</em></td>
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<td>2:50 – 3:10</td>
<td><strong>Kyle Genova</strong> (Rutgers University, Department of Plant Biology) <em>Mat Layer pH Affects Anthracnose of Annual Bluegrass Turf</em></td>
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<td>3:10 – 3:30</td>
<td><strong>Austin Grimshaw</strong> (Rutgers University, Department of Plant Biology) <em>Evaluation of Pathogenicity for a Newly Discovered Summer Patch Causal Pathogen</em></td>
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<td>3:30 - 3:45 PM</td>
<td><strong>Discussion and Closing Remarks</strong></td>
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<td>3:45 PM</td>
<td><strong>Social Hour and Poster Session</strong></td>
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PLENARY PRESENTATIONS
Seed-Vectored Bacterial Endophytes of Grasses: What Are They and How Do They Work?

James F. White, Jr.¹, Qiang Chen¹, Kathryn L. Kingsley¹, Matthew T. Elmore¹, Satish K. Verma²

¹ Department of Plant Biology, Rutgers University,
² Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, UP, India

Turfgrass seeds carry a small community of symbiotic bacteria on paleas and lemmas, on the surfaces of seeds—and sometimes within the seed itself. These bacteria include species of Bacillus, Pseudomonas, and Pantoea, among others. When the seed germinates the symbiotic bacteria colonize seedling root and shoot meristems and become distributed throughout the plant; simultaneously bacteria colonize the rhizosphere where they reduce growth of soil pathogens and suppress colonization of seedlings by pathogenic fungi. Bacteria also internally colonize cells of the plant—in roots colonizing root cells to establish the ‘rhizophagy symbiosis’. In the rhizophagy symbiosis plants obtain nutrients from bacteria that alternate between a root intracellular endophytic phase and a free-living soil phase. Bacteria acquire soil nutrients in the free-living soil phase; nutrients are extracted from bacteria oxidatively in the intracellular endophytic phase [1]. We conducted experiments on seed-vectored pseudomonad endophytes from Phragmites australis using Poa annua as surrogate host. We found that initially the symbiotic pseudomonads grow on the rhizoplane in the exudate zone behind the root meristem. Bacteria enter root tip meristem cells—locating within the periplasmic space between cell wall and plasma membrane. In the periplasmic spaces of root cells bacteria convert to wall-less L-forms. As root cells mature, bacteria are exposed to reactive oxygen (superoxide) produced by NADPH oxidases on the root cell plasma membranes. Reactive oxygen degrades some of the intracellular bacteria—effectively extracting nutrients from them. Surviving bacteria in root epidermal cells trigger root hair elongation [1-3], and as hairs elongate bacteria exit at the hair tips, reforming cell walls and rod shapes as they emerge into the rhizosphere where they may obtain additional nutrients. Later attraction of bacteria to the root exudate zone behind the root tip meristem again places bacteria in position to enter root meristem cells. Experiments involving grass seedlings with and without endophytic bacteria grown on 15N-labeled proteins suggest that the rhizophagy cycle could account for 30% of the nutrients absorbed by grass roots [4]. Other experiments using seedlings of Poa annua and bacteria that differed in resistance to oxidative degradation suggest that bacteria that participate in the rhizophagy symbiosis with plant roots must be susceptible to oxidative degradation within plant cells, but resistant enough for some cells to survive to trigger root hair elongation to exit into the rhizosphere. Bacteria resistant to oxidative degradation may enter cells but nutrients cannot be extracted from them and their intracellular numbers cannot be regulated, resulting in parasitisms where plant root growth is suppressed. The extent to which plants rely on symbiotic bacteria to obtain soil nutrients is not yet clear. It is also not known whether families of plants may differ in the extent to which they employ the rhizophagy cycle to obtain nutrients. Due to the central role that root-tip meristems play in the rhizophagy symbiosis, it seems probable that grasses where roots...
ramify prolifically, producing many root tips, may rely heavily on the rhizophagy symbiosis to obtain soil nutrients. Because the rhizophagy symbiosis involves oxidative interactions with intracellular bacteria one outcome of this nutritive symbiosis is increased resistance to oxidative stresses, both of biotic and abiotic origins. This work suggests that bacterial endophytes play a significant role in how plants acquire nutrients through root systems and highlights a possible target for new control measures for weeds and invasive non-native plants through inhibition of the rhizophagy symbiosis. Additional research is needed to determine how bacterial endophytes alter fitness and increase performance of turfgrass species and other crop plants.

References

The Microbiome Associated with Tall Fescue Under Drought Stress

Glen Groben, Jing Luo, Emily Walsh, Henry Qu, William Meyer, Stacy Bonos, Bruce Clarke, Ning Zhang

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The effects microbiomes have on physiological traits in turfgrasses are poorly understood. Drought tolerance is an economically important trait, which can be influenced by certain microbes. One example is the mycorrhizae association, which has been shown to improve drought tolerance of many plant species. In this study, we evaluated the microbiome associated with tall fescue genotypes grown in a rainout shelter after prolonged periods of drought stress. Twelve plants were selected for analysis, comprised of six sets of siblings, one exhibiting a drought tolerant phenotype and the other a susceptible phenotype. The microbiome associated with the shoot, root, and rhizosphere soil was evaluated for each tall fescue half-sib pair. Microbiome analysis was preformed utilizing an Illumina NGS metabarcoding approach that sequenced the 16S and ITS barcoding region to determine the composition of the bacterial and fungal communities, respectively. Comparative analysis of the microbiomes associated with the two phenotypes will be used to determine which microbes are associated with improved drought tolerance and drought susceptibility.
Identification and Management of Bacteria Associated with Etiolation on Cool-Season Golf Course Fairways

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Bacterial etiolation and subsequent decline has emerged as a significant issue on multiple cool-season turfgrasses. Recent reports of etiolation have been predominately on golf course putting greens; although the same symptoms are often observed on higher cut turf, particularly in areas comprised of perennial ryegrass (*Lolium perenne* L.) and annual bluegrass (*Poa annua* L.). In addition to identifying bacteria infecting susceptible turfgrasses, previous reports have implicated fertilization source to play a role in symptom expression. In 2016, symptoms of etiolation were observed on a mixed stand of perennial ryegrass and annual bluegrass managed as a golf course fairway in Phoenix, MD. Turf exhibiting etiolation was surface sterilized in 0.6% NaOCl for 2 min, rinsed and placed in sterile H2O for examination of bacterial streaming at 400X magnification. Positive bacterial streaming was then plated onto nutrient agar + 1% sucrose using an Autoplate Spiral Plater. The most frequently isolated bacteria were identified as *Pantoea* sp. through sequencing of the 16S subunit. In addition to completing Koch’s postulates for confirmation of pathogenicity, a 2-year field trial was established at the initial isolation site to examine the impact of both fertility and antimicrobials on reducing bacterial etiolation symptoms. The study design was a 2 x 9 factorial with treatments arranged in a randomized complete block design. The main plot consisted of fertilizer source (urea vs. ammonium sulfate) applied as 10 kg N ha⁻¹ (0.2 lb. N 1000 ft⁻²) every 2 weeks. The subplot factor included antimicrobial or plant growth regulator treatments [propiconazole (Banner Maxx), prohexadione calcium (Aneuw), trinexapac-ethyl (Primo Maxx), chlorothalonil + acibenzolar-S-methyl (Daconil Action), pigmented fosetyl-Al (Signature Xtra Stressgard), mineral oil (Civitas), tebuconazole (Mirage), paclobutrazol (Trimmit), or none] applied at label rates every 2 weeks. Fertilizer and antimicrobial treatments were applied as a tank mix to the foliage in 820 L H2O ha⁻¹ (2 gallons H2O per 1000 ft²). Turf was rated visually every 2 weeks for percent etiolation development. Data was summarized across each season through calculation of area under etiolation progress curve (AUEPC) values. All data was square root transformed and subjected to analysis of variance in SAS 9.4 using the mixed procedure. Means were separated using Tukey’s HSD (*Pr<0.05*). Symptoms of etiolation developed naturally throughout the experimental site during 2016 and 2017, which were different based on statistical analysis. Fertilization source was not significant when comparing AUEPC values in either year of study. Plant growth regulator treatments significantly impacted AUEPC in both growing seasons whereas additional antimicrobials showed no effect. Prohexadione calcium and trinexapac-ethyl applications resulted in the lowest AUEPC across both seasons regardless of fertilization source, although trinexapac-ethyl was also statistically similar to the none check in 2017. Contrary to previous reports, these results illustrate some plant growth regulator chemistries as a viable option to limit etiolation caused by *P. ananatis*. 
Breeding Fine Fescues for Increased Tolerance to Mesotrione

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The fine fescues (Festuca spp.) are a group of cool-season grasses that are better adapted to cool, dry, shaded environments, infertile, acidic soils and drought conditions. They also exhibit the best performance under lower fertility levels than other cool-season turfgrasses. These qualities give them the reputation of being low maintenance grasses.

mesotrione (Tenacity) is an HPPD inhibiting herbicide that provides pre- and post-emergent control of many problematic grassy weeds including Poa annua. Currently the Tenacity label does not recommend use in fine fescues at seeding. The Rutgers Turfgrass Breeding program has been working to breed mesotrione tolerant fine fescues by using a recurrent selection breeding strategy so the herbicide can be utilized safely at seeding. In addition to the breeding work, two studies were conducted to better understand the tolerance mechanism of fine fescue to mesotrione. Three experimental selections of each fine fescue species (hard (F. brevipila), Chewings (F. rubra ssp. commutata), strong creeping red (F rubra ssp. rubra)) were used to quantify tolerance levels using a rate titration study and quantify the absorption and translocation levels using ¹⁴C radiolabeled mesotrione. In both studies, plants were established from vegetative plugs and kept in a growth chamber maintained at 25/15 °C day/night temperature and 50% humidity and ten hour photoperiod. Herbicide treatments were applied in a spray chamber set to deliver 280 L ha⁻¹ at rates of 0, 17.5, 35, 70, 140, 280, 560, 1121, 2242, 4483, and 8966 grams a.i. ha⁻¹ + 0.25% non ionic surfactant. Visual percent injury was assessed and means separated using Fisher’s Protected LSD. Hard fescues were the most tolerant species and strong creeping red fescue the least tolerant species. In addition to the rate titration, the same nine vegetative lines were used in a study to evaluate foliar absorption and translocation of ¹⁴C labeled mesotrione herbicide at 24 and 96 hours after treatment (HAT) and the root absorption and translocation of ¹⁴C labeled mesotrione herbicide. Foliar absorption and translocation of radioactivity was not associated with differential tolerance levels to mesotrione. Interestingly, the most susceptible lines of Chewings and strong creeping fescue exhibited greater root uptake of ¹⁴C-mesotrione more tolerant lines. Our results demonstrate that differences in root absorption contribute to differences tolerance, but there are likely other factors that contribute and further studies are needed to better understand the mechanism of increased tolerance. Overall the breeding of mesotrione tolerance in fine fescues has shown promising improvement for hard and Chewings fescue and with further breeding and testing we should have commercially acceptable resistance levels.
**Early Efforts to Understand and Control False-Green Kyllinga (Kyllinga gracillima) and Goosegrass (Eleusine indica)**

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False-green kyllinga and goosegrass are two of the most problematic turfgrass weeds in the Northeastern US. Two greenhouse experiments and four field experiments were conducted to evaluate the efficacy of various post-emergence herbicides against false-green kyllinga in New Jersey and Indiana during 2017. Other field research was conducted to determine seasonal goosegrass emergence patterns in New Jersey.

Previous field research demonstrated that halosulfuron-methyl, imazosulfuron, and sulfentrazone have efficacy against false-green kyllinga. The objective of this research was to evaluate the efficacy of single and sequential applications of these herbicides at various rates for post-emergence false-green kyllinga control.

Two experiments were conducted at Stone Harbor Golf Club in Cape May Courthouse, NJ. Site 1 was maintained as a rough at 6.35 cm and Site 2 was maintained as a creeping bentgrass (Agrostis stolonifera) fairway. Initial and sequential applications were made on 13 June and 13 July, respectively. Two additional experiments were conducted in IN, one at Bloomington Country Club (Bloomington, IN) and Victoria National Golf Club (Newburgh, IN) on creeping bentgrass fairways. Applications were made on May 30th and June 30th. Treatments were arranged in a randomized block design and applied using standard small-plot spray equipment at 410 L ha⁻¹ in New Jersey and 810 L ha⁻¹ in IN.

Treatments included both single and sequential applications of imazosulfuron (420 g/ha), halosulfuron-methyl (70 g/ha), sulfentrazone (110 g/ha), a single application of sulfentrazone (110 g/ha) + imazosulfuron (420 g/ha), and a non-treated control. False-green kyllinga injury was evaluated visually from 0-100% (with 0% being no injury and 100% completely necrotic). Control was determined by assessing the percent cover of false-green kyllinga in each plot compared to the non-treated control at 4, 8, and 12 weeks after initial treatment (WAIT). Grid intersect counts were also conducted at 12 WAIT. Data were analyzed in SAS (v9.4) and Fisher’s Protected LSD (α=0.05) was used to separate means.

Only the NJ data are presented for brevity. In NJ field experiments, all treatments containing imazosulfuron provided >97% false-green kyllinga control at 12 WAIT. Two sequential applications of halosulfuron-methyl provided >95% control but the single application only provided 64% control at the rough height site. Sulfentrazone provided 50% at the fairway trial (Site 2) and no control in the rough trial (Site 1) at 12 WAIT.

A greenhouse experiment was conducted to further explore efficacy of single applications of imazosulfuron, halosulfuron-methyl, imazosulfuron and imazosulfuron + carfentrazone-ethyl on false-green kyllinga. Herbicides were applied at 0.1, 0.25, 0.5, 0.75, 1, 2, 4 and 10 times registered use rates. Treatments included: a non-treated control, imazosulfuron (42, 105, 210,
315, 420, 840, 1680 and 4200 g/ha), halosulfuron-methyl (6.8, 17.3 34, 52.5, 70, 140, 280 and 700 g/ha), sulfentrazone (28, 70, 140, 210, 280, 560, 1120 and 2800 g/ha), and sulfentrazone + carfentrazone-ethyl (77, 153, 230, 306, 640, 1230, and 3060 g/ha). Treatments were replicated four times and arranged in a completely randomized design; the experiment was repeated in time. Treatments were applied using a track sprayer with water carrier at 410 L ha\(^{-1}\) to pots of false-green kyllinga in a peat-based growing medium. Percent visual injury (0-100\%) was measured weekly and clipping yield was collected biweekly. Regression analyses were conducted in SAS to characterize kyllinga responses and will be presented.

Economical and effective management programs for goosegrass depend on predicting seedling emergence. The objective of this study was to determine goosegrass seedling emergence patterns in bare ground and cool season turfgrass. Experiments were initiated in April 2017 at the Rutgers Horticultural Research Farm No. 2 in North Brunswick, NJ and the Tamarac Golf Course in East Brunswick, NJ. Plots at the Rutgers site were subjected to three different ground cover treatments; bare ground, perennial ryegrass (PRG; *Lolium perenne*) mowed at 1.25 cm, and PRG mowed at 6.4 cm. Plots measured 1.0 by 1.0 m and goosegrass seedlings were counted and removed on a weekly basis from fixed circles measuring 1000 cm\(^2\) within each plot. Soil temperature and soil volumetric water content were monitored at a depth of 5.0 cm. At the Tamarack location, the experiment was performed on two sites approx. 100 m apart on the same fairway. Plots were maintained as a golf course fairway at a 1.25 cm mowing height. Plots measured 1.0 by 1.0 m and goosegrass seedlings were counted and removed on a weekly basis from fixed circles measuring 500 cm\(^2\) within each plot. Soil temperature was monitored at a depth of 5.0 cm. Data were subjected to ANOVA in SAS and regression analyses were conducted using Prism software.

At the Hort. Farm site, seedling emergence was first observed on May 26 and last observed on October 20. Ground cover treatment affected seedling emergence. More seedlings emerged in bare soil (8518 seedlings m\(^{-2}\)), than at the 1.25 cm mowing height (2468 seedlings m\(^{-2}\)), and 6.4 cm mowing height (1563 seedlings m\(^{-2}\)). Patterns of emergence were affected by ground cover treatment greater proportion seedlings emerged earlier in the season in bare soil plots as compared to plots with PRG at 1.25 and 6.4 cm mowing height. At both Tamarack Golf Course sites, goosegrass seedling emergence was first observed on May 10 and last observed on September 14. Emergence patterns and the total number of seedlings emerged were different between the two Tamarack sites.
Resourcing the Next Generation of Turfgrass Science

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The USDA National Institute of Food and Agriculture (NIFA) provides leadership and funding for research, education, and extension programs that advance agriculture-related sciences. We invest in and support initiatives that ensure the long-term viability of agriculture, including turfgrass. NIFA applies an integrated approach to ensure that groundbreaking discoveries in agriculture-related sciences and technologies reach the people who can put them into practice. NIFA has multiple funding mechanisms that can be used to help advance the turfgrass industry and bring new scientific discoveries and technologies to practice, as well as prepare the future workforce of turfgrass practitioners. In this presentation, the range of NIFA programs that are applicable to turfgrass science are described, with examples given for how these have been used to-date. A value-chain approach is given to envision how multiple applicable programs could be utilized in a coordinated fashion to systematically address industry priorities and solve the challenges they face today, and in the future.
Building a Successful Career in Golf Course Management:
It’s More Than Just Growing Grass

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

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

The educational needs of turf managers today vary widely. Turf managers today must possess a wide array of scientific, business and technical skills to be successful. Aside from a thorough knowledge of turfgrass, managers must have a firm grasp of plant pathology, personnel management, accounting, horticulture, arboriculture, environmental science, irrigation systems management, drainage, carpentry, basic electricity, plumbing, and a host of other technical skills. Successful superintendents must demonstrate a mastery of both verbal and written communication skills.

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

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

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The highway roadside environment presents challenges to establishing and maintaining vegetative cover due to an inconsistent supply of plant available water, low soil fertility and minimal fertilization inputs, and salted-soil conditions resulting from deicing materials applied during winter months. Identifying grasses that are better adapted to these conditions could result in more persistent vegetative cover in locations that would otherwise be subject to soil erosion and ultimately deterioration of roadway edges. University of Minnesota, University of Wisconsin, Michigan State University, Rutgers University and their respective State Departments of Transportation (DOT) recently collaborated to develop a multi-state research program with the objective of assessing the establishment and persistence of numerous grass species and mixtures seeded on roadsides during 2016.

Two roadsides were selected in New Jersey during summer 2016, which represented ‘urban’ and ‘rural’ locations based on the presence of a curb (US Highway 1, North Brunswick, NJ) or no curb (Interstate 287, South Plainfield, NJ), respectively. Each roadside location had relatively few interruptions from signs and other structures to accommodate the end-to-end arrangement of 1.8-m long plots along a 1.2-m strip immediately adjacent to the roadway or curb.

The existing vegetation in the 1.2-m wide strip adjacent to the roadways was treated with glyphosate in September 2016. Following nonselective control, a seedbed was prepared at each location by rototilling to a 5-cm depth and raking to remove coarse debris and smooth the soil surface. Three replications of sixty entries were seeded into 1.2 x 1.8-m plots at each location and included individual varieties of the following species: perennial ryegrass (including tetraploid) \textit{(Lolium perenne} L.); Kentucky bluegrass \textit{(Poa pratensis} L.); hard fescue \textit{(Festuca brevilipa} R. Tracey); strong creeping red fescue \textit{(F. rubra} L. subsp. \textit{rubra}); Chewings fescue \textit{(F. rubra} L. subsp. \textit{fallax} [Thuill.] Nyman); slender creeping red fescue \textit{(F. rubra} L. var. \textit{littoralis} Vasey ex Beal); tall fescue \textit{(Schedonorus arundinaceus} [Schreb.] Dumort.); alkaligrass \textit{(Puccinellia} spp.); and smooth brome \textit{(Bromus inermis} Leyss). Various DOT-specified (Michigan, Minnesota, Nebraska, New Jersey, and Wisconsin) seed mixtures for roadides were also seeded. The New Jersey rural and urban test locations were seeded on 4 and 5 October 2016, respectively. Immediately following seeding, a complete starter fertilizer (10-10-10) was applied at 49 kg N ha\textsuperscript{-1} and trials were covered with a biodegradable erosion control blanket to aid in the establishment of vegetative cover.

Soil samples were collected from both locations during late summer 2016 and tested for physical and chemical properties including soil salinity (measured as electrical
conductivity [EC] of 1:1 soil:water suspension). Soil samples were also collected in spring 2017 to evaluate soil fertility and salinity. Plots were assessed for bare soil, weed and desirable grass cover during December 2016 and May 2017 using a 60-cell soil. Data were subjected to ANOVA and means were separated using Duncan’s Multiple Range Test (α = 0.05).

Single variety Kentucky bluegrass entries exhibited poor establishment in December 2016 at both the rural (< 44%) and urban (< 34%) locations in New Jersey. In contrast, single-variety perennial ryegrass entries (non-tetraploid) established greater than 70 and 88% cover at the rural and urban sites, respectively. The Nebraska Urban Roadsides and Lawn DOT-specified mixture (‘Titanium’ tall fescue [88%]; ‘Park’ Kentucky bluegrass [7%]; and ‘Evening Shade’ perennial ryegrass [5%], by weight) established greater than 90% grass cover at the rural and urban locations in December 2016.

By May 2017, forty-nine entries had less than 10% grass cover at the rural location; only seven entries had greater than 50% grass cover and all of these entries were alkaligrass (‘Fults’, ‘SeaSalt’, BAR PD06N17, ‘Ocean Maritima’, and ‘Salton Sea’) or a DOT-specified mixture that contained alkaligrass: MI DOT THV Mixture (‘Pennlawn’ creeping red fescue [44.3%]; ‘Pennant’ perennial ryegrass [29.1%]; ‘Baron’ Kentucky bluegrass [14.7%]; and ‘Fults’ alkaligrass [9.8%]) and MI DOT TUF Mixture (‘Dawson’ creeping red fescue [39.5%]; ‘Pennant III’ perennial ryegrass [19.4%]; ‘Reliant IV’ hard fescue [19.5%]; ‘Baron’ Kentucky bluegrass [9.8%]; and ‘Fults’ alkaligrass [9.8%]).

Of the nine entries that established less than 10% grass cover at the urban location by May 2017, eight of these entries were Kentucky bluegrass (single variety or experimental selection). Single variety and experimental selection entries with the greatest (top statistical ranking) grass cover (47 to 83%) at the urban site in May 2017 consisted of the following species: perennial ryegrass (‘Gray Fox’, ‘Premium’, ‘Stellar 3 GL’, 16-14-Lp 145, BAP Lp 10966, and Replicator [tetraploid]); tall fescue (‘Saltillo’, ‘Thunderstruck’, ‘Black Tail’, ‘Birmingham’, ‘Avenger II’, and ‘Fayette’); alkaligrass (Oceana Maritima, BAR PD06N17, and Fults); smooth brome (BAR BIF 1GRL); and slender creeping red fescue (‘Seabreeze GT’ and ‘Shoreline’). DOT-specified mixtures with the greatest (top statistical ranking) grass cover at the urban location in May 2017 were NE DOT Urban Roadsides and Lawns (84% grass cover); MI DOT THV Mixture (71% grass cover); MI DOT TUF Mixture (67% grass cover); and MN DOT 25-131 Low Maintenance Turf (67% grass cover) (‘Park’ Kentucky bluegrass [16.3%]; Sheep fescue [11.3%]; ‘Boreali’ creeping red fescue [28.6%]; ‘Radar’ Chewings fescue [19.8%]; ‘Chariot’ hard fescue [13.6%]; perennial ryegrass [9.3%]).

The poor establishment of many entries at the rural location was attributed to elevated soil salinity levels resulting from the use of deicing products on the road surface and the lack of a curb to minimize salt accumulation on the establishing grass. Soil EC values ranged from 6.0 to 7.7 mmhos cm⁻¹ (excessive [i.e., crop failure] at >3.0 mmhos cm⁻¹) at the rural site in early spring 2017; whereas, EC values of 0.6 to 0.8 (medium [i.e., satisfactory]: 0.4 – 1.0 mmhos cm⁻¹) were measured at the curb-protected urban site in spring 2017. Thus, the presence of a curb may be beneficial for successful establishment
of grass cover on roadsides that receive frequent application of deicing materials during winter. Additionally, the lack of a curb at the rural location allowed for much greater wheel damage from vehicle traffic on the establishing grasses.

Kentucky bluegrass exhibited very poor establishment during autumn and subsequently very low spring grass cover along both roadsides. Kentucky bluegrass is well-known for it slow establishment rate from seed; thus, these species may be more effective when used as a component of mixtures and contributing to roadside cover over the long-term. The use of alkaligrass to establish grass cover on salt-affected soil adjacent to roadways was generally the most successful species choice over the first 8 months of the rural test. The persistence of this species in New Jersey, however, is uncertain. Evaluation of both trial locations through spring 2018 will provide insight into alkaligrass persistence under non-irrigated conditions during summer months and ultimately improve state DOT seed (species/mixture) recommendations.
Molecular and Metabolic Regulation of Stress-Induced Leaf Senescence in Turfgrass

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Leaf senescence is a typical characteristic of heat stress, which reduces photosynthesis and plant growth. However, molecular factors regulating heat-stress induced leaf senescence are still unclear. Through genetic analysis, RNAi transformation, and transcriptomic profiling of perennial ryegrass, several genes (SGR, PPH, NYC1, PAO, and NOL) related to chlorophyll degradation were found to regulate heat-induced leaf senescence. Several molecules, including salicylic acid, cytokinins (CK), ethylene, abscisic acid (ABA), and Ca were identified as key signaling molecules of those senescence-related genes. LpPPH was a direct downstream target gene of transcription factors in the ABA and CK signaling pathways. The molecular factors for PPH signaling and transcription factors that may activate or deactivate PPH expression and control heat-induced leaf senescence will be discussed.
Reproductive Diapause in the Annual Bluegrass Weevil

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Diapause is the arrestment of development to increase an organism’s survival potential in response to unfavorable environmental conditions. Varying with insect species, it may occur at different stages of the life cycle from egg to adult. In adults, diapause is characterized by halted reproductive maturation and is thus often defined as reproductive diapause. During reproductive diapause, the reproductive organs of males and females are kept at minimal size to conserve metabolic reserves and do not resume development until diapause termination. The annual bluegrass weevil (ABW), *Listronotus maculicollis* Kirby, undergoes reproductive diapause during hibernation. The ABW is a major pest of short-mown turfgrass on golf courses and grass tennis courts, and is considered one of the most difficult-to-manage turfgrass insect pests in eastern North America. Understanding the role of environmental cues, especially temperature, may be critical to monitoring the spring emergence of the overwintered ABW adults. Effective monitoring of ABW populations may largely reduce unnecessary applications and optimize timing and, with that, efficacy of warranted applications.

Diapausing ABW adults were collected from their hibernating sites at different times in fall and winter, and subjected to different conditions to observe diapause termination by dissecting and measuring the reproductive organs. When diapausing weevils were maintained under laboratory cold conditions (6 °C / 4 °C, LD 10:14) from early December to late March, the sizes of reproductive organs of both sexes increased or fluctuated slightly, and very few females had developing oocytes, suggesting that most adults did not resume development during the chilling period. When diapausing weevils (chilled for 40 to 83 days) were transferred to warm conditions (21 °C, LD 14:10) for different lengths of time, reproductive organ sizes in both sexes increased as chilling period prolonged, implying that chilling played an important role in diapause termination. Diapausing weevils collected in September without chilling did not develop successfully despite exposure to warm conditions. In contrast, 87% of males and 93% of females collected from the field on January 21 had initiated reproductive development after 5 days of exposure to warm conditions, indicating the necessity of chilling for diapause termination. Male and female reproductive organ sizes increased faster and to a greater final size as the preceding chilling period increased. The prolonged chilling period in the field resulted in more synchronized and advanced development in ABW when exposed to warm conditions.

Growing degree day (GDD) model (base temperature 10 °C, starting March 1) is often used for timing of adulticide applications. However, whether ABW adults terminate reproductive diapause at 10 °C has never been investigated physiologically. To explore the threshold temperature and length of time required for diapause termination, diapausing ABW adults collected from the overwintering sites were stored under laboratory cold conditions (6 °C / 4 °C, LD 10:14) and then transferred to different warm-up temperatures at LD 12:12. When weevils were transferred to 7, 14 and 21 °C in December and late January, the sizes of male and female reproductive organs were significantly smaller at 7 °C than at 14 and 21 °C. When ABW were transferred to 7, 9, 11, 13 and 15 °C in late January, higher temperatures facilitated the diapause termination process. The sizes of male and female reproductive organs were larger and diapause
termination rate was higher at higher temperatures. At 7 °C in males and at 7 and 9 °C in females, organs did not grow significantly; percentage of diapause termination was very low and did not increase significantly. At 11, 13 and 15 °C, the time required for 50% individuals to terminate diapause was 18, 13 and 8 days in males, and 19, 14 and 8 days in females, respectively. The threshold temperature causing 50% diapause termination was around 9 °C in males and was 10 – 11 °C in females, indicating potentially earlier emergence of males than females in spring.

In the observation of reproductive status for ABW collected bi-monthly from the field from late October to late March, an apparent peak of reproductive development was observed on 7 January when 80% of males and 53% of females had resumed growth of reproductive organs. However, the percentage of male and female maturity and insemination rate were low until early March, but all adults reached 100% by late March.

In conclusion, temperature may serve as a major environmental cue in regulating diapause termination in ABW. Low temperature maintained the diapause status and post-diapause quiescence, and a period of chilling resulted in more rapid and synchronized development of reproductive organs under warm conditions. In late January after the required chilling period was met, higher temperatures favored the diapause termination process and the resumption of reproductive development. Male weevils had a lower threshold temperature than the females in triggering the development of reproductive systems, although on average the threshold temperature was very close to the base temperature of 10 °C in the currently used GDD model.
Mat Layer pH Affects Anthracnose of Annual Bluegrass Turf

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Anthracnose (caused by Colletotrichum cereale Manns sensu lato Crouch, Clarke and Hillman) is a common disease on golf course putting greens in the United States. The influence of mat layer pH on anthracnose severity of annual bluegrass [Poa annua L. f. reptans (Hausskn) T. Koyama] was assessed in a 6-yr field study maintained at 2.8 mm on a sandy loam in North Brunswick, NJ. Lime (CaCO₃) treatments were applied at five rates (41, 199, 414, 608, 785 kg Ca ha⁻¹ in 2011; 42, 311, 569, 750, 914 kg Ca ha⁻¹ in 2014; 133, 481, 1056, 1570, 2535 kg Ca ha⁻¹ in 2015; and 0, 0, 0, 1067, 1794 kg Ca ha⁻¹ in 2016) to attain target pH levels of 5.8, 6.3, 6.8, 7.3 and 7.8, respectively, in the mat layer. Elemental sulfur treatments were applied at two rates as a wettable powder (24 and 49 kg ha⁻¹ in 2011, 2015 and 2016, and 12 and 24 kg ha⁻¹ in 2014) to decrease mat pH. Gypsum (CaSO₄*2H₂O) treatments were applied at two rates (195 and 377 kg Ca ha⁻¹ in 2011; 357 and 652 kg Ca ha⁻¹ in 2014; 303 and 665 kg Ca ha⁻¹ in 2015; and 434 and 750 kg Ca ha⁻¹ in 2016) to match rates of Ca applied in the second and third lowest limestone rate treatments with target pH levels of 6.3 and 6.8, respectively. Treatments were applied on 12 Dec. 2011, 1 Apr. 2014, 21 Dec. 2015 and 28 Dec. 2016 to allow for greater reaction time during the winter and early spring.

Data from September 2017 indicated that the pH in the mat layer ranged from 5.3 (greatest S rate) to 7.1 (greatest lime rate). Generally, lower pH (untreated and two sulfur rates) resulted in an increase of anthracnose severity as well as a decrease in turfgrass color and quality during the study. Lime treated plots had significantly less disease than sulfur treated and untreated plots. Under severe disease pressure (> 40% infested turf), only the four greatest lime rates decreased anthracnose. Better turfgrass quality and darker green color were observed on limed plots in 2012, 2013, 2014 and 2015. In 2016 and 2017, the four greatest lime rates produced the best turfgrass quality and darkest green color. During this period, plots treated with gypsum had greater disease severity compared to the corresponding lime treated plots, and were not different from untreated plots underscoring the impact that pH has on anthracnose disease of annual bluegrass turf. This research will be continued during in 2018.
Evaluation of Pathogenicity for a Newly Discovered Summer Patch Causal Pathogen

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A new Magnaportheopsis species, M. meyeri-festucae was isolated from the diseased roots of fine fescue (Festuca spp.) turfgrasses. It is described and illustrated based on phenotypic characteristics and DNA sequences of SSU, ITS, LSU, MCM7, RPB1 and TEF1 genes.

Inoculation experiments performed on “Beacon”, and “Predator” fine fescues, and “Midnight” and “Baron” Kentucky bluegrasses in temperature- and light-controlled growth chambers resulted in disease symptoms and fulfillment of Koch’s postulates. Assessments for pathogenicity confirmation included visual ratings of foliar and root tissue, measurements of root length, and shoot and root weights. The fungus was recovered from all inoculated samples, and a representative of each cultivar was used for identification via ITS sequencing. Morphological characteristics of recovered strains fit well with the type stain FF2 that was used for inoculation, and all ITS sequences matched that of the original strain with 100% identity.

Foliar health of the plants in this study ranged from 1 to 4 (1=diseased, 4=healthy) (mean = 2.6). Midnight (mean = 3.3), Predator (mean = 2.8), and Beacon (mean = 2.8) had significantly greater foliar health than Baron (mean = 1.8). With respect to the main effect of fungal inoculation, foliar health was significantly reduced in inoculated samples compared to non-inoculated samples. The shoot weight averaged 958 mg and ranged from 70 to 2450 mg for all samples. Shoot weight was not affected by the factors included in this study. Root health ranged from 2 to 6 (2=diseased, 6= healthy), and the overall average root health was 4.3. As with foliar health, mean root health was independently and significantly affected by cultivar (p = 0.0197) and fungal isolate (p < 0.0001). With respect to root health, Midnight (mean = 5.0) was the best, whereas Baron (mean = 4.1) and Predator (mean = 3.8) were the worst, and Beacon (mean = 4.3) was similar to all cultivars. Inoculated samples had significantly lower root health than non-inoculated samples. Mean root length was 168.2 mm for all inoculated and non-inoculated samples, and the values ranged from 45 to 205 mm. Root length was significantly affected by the main effect of fungus (p = 0.0007). The root length of inoculated samples was 14% shorter than that of non-inoculated samples. Root weight ranged from 60 to 1590 mg (mean for all samples = 681 mg). Root weight was also significantly affected by the fungus (p = 0.0386). The root weight of inoculated samples was 26% less than the root weight of non-inoculated samples. This work is a starting
point to increase our understanding of this new fungal species. Summer patch is a devastating turfgrass disease and continued research will be aimed at breeding resistance into new cultivars as well as developing better management strategies for the disease.
POSTER PRESENTATIONS
Removal of Coarse Sand from Topdressing Applied to Putting Green Turf

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Sand topdressing of putting greens during the season is often avoided due to the potential of coarse sand particles interfering with play and dulling of mower blades. This study evaluated the effects of eliminating coarse particles from topdressing sand on the surface wetness and turf quality of a creeping bentgrass (Agrostis stolonifera) turf. This trial was initiated in May 2016 on a 19-month-old ‘Shark’ creeping bentgrass maintained at 2.8-mm on a sand-based root zone. A 3 x 2 x 2 factorially arranged randomized complete block design with four replications included the factors of sand size (medium-coarse, medium-fine, fine-medium), quantity of mid-season (June to September) topdressing (2,440 and 4,880 kg ha\(^{-1}\) every 2 weeks), and cultivation (non-cultivated or core cultivation plus backfill in May and October). Two non-topdressed controls (at both levels of cultivation) were included for comparisons resulting in 14 total treatments. Turf quality was visually rated June through October. Mower clippings were collected from each plot to determine the quantity and particle size distribution of sand collected during mowing. Core samples were collected before and one-year after treatment initiation to characterize the thickness of the thatch-mat layer and content of sand and organic matter (OM). Volumetric water content (VWC) of the surface 0- to 38-mm depth zone was monitored routinely.

Better turf quality was observed on topdressed plots than non-topdressed plots, regardless of sand size or topdressing rate. Turf quality was acceptable on all plots but slightly poorer on core cultivated plots compared to non-cultivated plots throughout most of 2016. Topdressing with medium-coarse sand increased the quantity and portion of sand collected during mowing compared to medium-fine and fine-medium sands. The portion of topdressing sand collected by the mower increased positively with topdressing rate. Topdressing increased the depth of the mat layer and decreased the OM concentration compared to non-topdressed controls. Topdressing at 4,880 kg ha\(^{-1}\) increased the thickness of mat layer depth and reduced OM concentration compared to topdressing at 2,440 kg ha\(^{-1}\). Core cultivation did not influence mat layer depth but did reduce OM concentration. Sand size of topdressing affected the sand size distribution of the mat layer, and interacted with topdressing rate and cultivation. Fine-medium and medium-fine sand topdressing increased the fineness of sand in the mat layer compared to topdressing with medium-coarse sand. Topdressing at 4,880 kg ha\(^{-1}\) with fine-medium sand intensified this response; whereas the fineness of sand in the mat layer was not strongly affected by the topdressing rate of medium-fine sand. Core cultivation and backfilling with medium-coarse sand offset any increase in fineness of mat layers formed by topdressing with fine-medium and medium-fine sand. Core cultivation decreased VWC at the 0- to 38-mm depth zone throughout the year compared to non-cultivated plots. Medium-coarse and medium-fine sand produced a drier surface compared to plots topdressed with fine-medium sand; however, this sand size effect on surface wetness frequently was not observed in cultivated plots (sand size factor interacted with cultivation factor). Under core cultivation, the VWC of non-topdressed control plots was similar to topdressed plots.
Metagenomic Analysis of Bacterial Communities Associated with Seeds of Cool-Season Turfgrasses

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Endophytes of grasses have been shown to enhance field performance by improving germination rates, growth and increasing resistance of grasses to biotic and abiotic stresses. Similar benefits to grass hosts have been shown for both fungal and bacterial endophytes. It has been observed that seed germination tends to be better in grasses from climates where seed is exposed to moisture during the seed maturation process. We hypothesized that seed germination is better in moist climates due to a greater diversity of bacterial endophytes that successfully colonize seed surfaces. To test this hypothesis we obtained seed samples from a diversity of locations. Locations were classified as either high moisture or low moisture during the seed maturation period. Seed samples were then subjected to metagenomic analysis to examine the diversity and families of bacteria associated with seeds. We conducted a metagenomic study by sequencing the V3-V4 region of 16S rDNA sequences with Illumina MiSeq. By comparing the bacterial community in several grass genera, we found that Lolium (including Lolium arundinaceum; average of 20851 reads/sample) possessed more than twice the reads of fine fescues (average of 9211 reads/sample). This overall correlation could explain why Lolium seeds tend to germinate better than fine fescues in our experimental assays. The results also showed that seed from moist climates tended to show a higher diversity of bacteria associated with seeds. Further, it is suggested that there is a positive correlation between the content (proportion) of beta-Proteobacteria and visual merit ratings of turf cultivars based on data from the British Society of Plant Breeders (2017). We further found negative correlations between Actinobacteria abundance and live ground cover based on data from the British Society of Plant Breeders (2017). A negative association was also indicated between Sphingobacteria abundance and resistance to red thread disease based on data from the British Society of Plant Breeders (2017). In our seed germination tests, alpha-Proteobacteria and Sphingobacteria abundance were positively associated with germination time and negatively associated with germination rate. Gamma-Proteobacteria abundance showed a positive correlation with germination rate and a negative correlation with germination time. These correlations seem to suggest that particular bacterial endophytes in beta-Proteobacteria and gamma-Proteobacteria are beneficial in turf, while alpha-Proteobacteria, Actinobacteria and Sphingobacteria may be parasitic. At least two of these groups, alpha-Proteobacteria and Sphingobacteria, contain species that are known to be intracellular parasites of Eukaryotes. While, additional experiments are needed to confirm these correlations, confirmation that beta- and gamma-Proteobacteria are beneficial endophytes may enable improvement of turf cultivars using endophytes in these groups.
**Poa pratensis Cultivar Responses to Mesotrione in a Controlled Environment.**

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Mesotrione is a relatively new chemistry for pre-emergent and post-emergent control of broadleaf and grassy weeds, such as annual bluegrass (*Poa annua*), in managed turfgrass and seed production. Ninety cultivars of Kentucky bluegrass were evaluated for tolerance to a maximum label rate (280 g ha\(^{-1}\)) of mesotrione (Tenacity). Kentucky bluegrass cultivars were seeded to pots, filled with fine silica sand and sphagnum peat moss-based potting mix blend at a 1:1 ratio by volume and arranged in a randomized complete block design with four replications. Treatments consisted of a non-treated control (water + non-ionic surfactant) and mesotrione applied at 280 g ha\(^{-1}\) (water + non-ionic surfactant + mesotrione). Treatments were applied using a track sprayer with water carrier at 410 L ha\(^{-1}\) on the day of seeding. Percent bleaching was evaluated every six days after application for a total of three ratings. Digital image analysis was conducted to quantify percent bleaching, establishment, and ground cover. A cultivar dependent response was detected.
Bacteria Associated with Smooth Crabgrass (*Digitaria ischaemum*) Seed Inhibit Competitor Plant Species

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Smooth crabgrass (*Digitaria ischaemum* (Schreb) Schreb ex Muhl Schreb.) and annual bluegrass (*Poa annua* L.) are competitive early successional species that are usually considered weeds in agricultural and turfgrass systems. Bacteria and fungi associated with these weeds may contribute to their competitiveness. Smooth crabgrass and annual bluegrass seed may be a mechanism to vector important microbes. We hypothesized that bacteria associated with smooth crabgrass and annual bluegrass seeds would affect seedling growth and antagonize competitor forbs such as common dandelion (*Taraxacum officinale*), and white clover (*Trifolium repens*). Smooth crabgrass and annual bluegrass seeds were surfaced-sterilized through vigorous agitation in water and 4.125% NaOCl solution for 40 minutes. Seeds were placed on Petri plates of various media and outgrowing bacteria and fungi were isolated for study in axenic culture. After treatment with NaOCl solution, annual bluegrass seed did not produce any outgrowing bacteria or fungi, so a separate sample of seed was rinsed with sterile deionized water only to isolate outgrowing bacteria and fungi. Twenty-four bacterial strains and two fungal species were isolated and inoculated onto dandelion seeds to evaluate their effects on seedling growth. Ten strains were antagonistic to dandelion seedling growth and of these, four were antagonistic enough to increase seedling mortality. All four bacterial strains that increased dandelion mortality were isolated from smooth crabgrass seed while none of the 14 isolates from annual bluegrass seed increased mortality. Two of the four bacterial isolates (characterized as *Pantoea* spp. through 16S subunit sequencing) were inoculated on to surface-sterilized smooth crabgrass, clover and annual bluegrass seed alone and in combination with a *Curvularia* sp. fungus also isolated from smooth crabgrass seed. These bacteria caused >65% clover seedling mortality but did not affect annual bluegrass seedling mortality. Effects on smooth crabgrass seedling mortality were inconsistent. Whether alone or in combination with bacteria, *Curvularia* was highly pathogenic to smooth crabgrass and clover but not annual bluegrass. This *Curvularia* sp. was growth promotional in annual bluegrass, increasing the gravitropic response of annual bluegrass roots. These experiments demonstrate that bacteria associated with smooth crabgrass seeds may be antagonistic to competitor forbs. The weedy character of smooth crabgrass could at least in part stem from possession of bacteria that are antagonistic to competitor species. Future research should further explore the effect of these bacteria on smooth crabgrass competitiveness and elucidate the mechanism by which these bacteria cause mortality in competitor species.
Potassium Effects on Pink Snow Mold Incidence of Annual Bluegrass
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Pink snow mold (caused by Microdochium nivale) is a prevalent disease on golf course turfs in cool, humid regions of the United States. A 2-yr field study assessed the effect of late-season, K fertilization on pink snow mold incidence on annual bluegrass [Poa annua L. f. reptans (Hausskn) T. Koyama] (ABG) maintained at a 2.8-mm height on a sandy loam in North Brunswick, NJ. A randomized complete block design with five replications was used to evaluate three K rates: 0, 20, and 40 kg ha\(^{-1}\) applied as a potassium sulfate solution on 23 and 24 November in 2015 and 2016, respectively. Plots were inoculated with 8.6 and 4.3 g m\(^{-2}\) of oats infested with M. nivale isolates PPCC12012 and PP42013 on 11 December 2015 and 12 December 2016, respectively, and covered with two layers of permeable growth cover to enhance disease development. During the winter of 2015-2016, disease severity, measured as area under the disease progress curve (AUDPC), was not different among plots treated with or without K. During the winter of 2016-2017, plots treated with 40 kg K ha\(^{-1}\) reduced the AUDPC by 13% compared to the plots where no K was applied. This research will be continued during the winter of 2017-2018.
Dollar Spot Disease Forecasting on Bentgrass Fairway Turf in New Jersey

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The species and cultivar of bentgrass (Agrostis spp.) influences the incidence and severity of dollar spot disease (caused by Sclerotinia homoeocarpa F.T. Bennett) epidemics. A field trial was initiated in North Brunswick, NJ to assess the reliability of two weather-based models for predicting dollar spot epidemics on bentgrasses with a wide-range in susceptibility. ‘Independence’, ‘Penncross’, ‘Shark’, ‘007’, and ‘Declaration’ creeping bentgrass (A. stolonifera L.), and ‘Capri’ colonial bentgrass (A. capillaris L.) were seeded in a randomized complete block design with 25 blocks on 29 Sept. 2014. The trial area was inoculated with S. homoeocarpa isolates NJDS003 and NJDS007 on 7 Apr. 2015; epidemics occurred naturally during 2016 and 2017. The onset of disease symptoms was compared to forecasts from a growing degree-day (GDD, base 15°C and biofix 1 April) and a logistic regression model. Subsequent disease progress (incidence) was assessed every 2- to 18-d for each cultivar and was compared to disease forecasts from the logistic model. Unintended dollar spot suppression from fludioxonil applications made to control anthracnose disease precluded the assessment of disease progress during mid- to late-2016. Disease symptoms first appeared in highly susceptible cultivars at 73-, 27-, and 92-GDD during 2015, 2016, and 2017, respectively; whereas, symptoms appeared at 79-, 140-, and 112-GDD for moderate and low susceptibility cultivars. The logistic regression model forecasted a risk index of 20% for dollar spot at 7-, 7-, and 21-d before disease symptoms appeared in highly susceptible cultivars during 2015, 2016, and 2017, respectively; whereas, a 20% risk index occurred at 11-, 29- and 28-d before disease onset on tolerant cultivars. Subsequent outbreaks were accurately forecasted in susceptible cultivars using the logistic regression model throughout 2015, early-2016, and 2017. Disease progress in moderate and low susceptibility cultivars appeared to coincide with a model risk index greater than 20%. Interestingly, disease recovery occurred during periods when the risk index was sharply declining, albeit greater than 20%.
The wild American hazelnut, *Corylus americana*, is native to a wide area of land in North America, bounded by the Rocky Mountains in the west and the Atlantic Ocean in the east, and spanning from southern Canada to the southeastern US. The species is adapted to a variety of climates including very cold regions and is also resistant to eastern filbert blight (EFB), caused by the fungus *Anisogramma anomala*—a disease that is devastating to the commercial European hazelnut *C. avellana*. Unfortunately, *C. americana* has thick-shelled, tiny nuts and clasping husks that prevent harvesting from the orchard floor, which makes it largely unsuitable for commercial production. However, it is cross-compatible with the commercial European hazelnut and can serve as a genetic donor for disease resistance, early nut maturity, cold tolerance, etc. Programs to develop commercial-quality hybrid hazelnuts have been ongoing for several decades in the Upper Midwest, but more breeding and research work is required to develop and then clonally propagate and distribute widely adapted plants with nut quality and yields comparable to standard European hazelnuts. A wider germplasm base is also necessary to serve as a foundation to support future breeding efforts, especially when developing plants expressing durable resistance to EFB and adaptation to widely fluctuating climatic events that will likely be encountered in the future.

As part of the Hybrid Hazelnut Consortium, consisting of Rutgers University, Oregon State University, the University of Nebraska-Lincoln, and the Arbor Day Foundation, we have been collecting *C. americana* germplasm since 2009 with the help of partners, colleagues, and the interested public around the U.S. Today, we have a total field collection of 1,899 seedlings obtained from 128 seed lots that span 23 states and one Canadian province. The bushes are being evaluated for response to EFB, presence of big bud mites, flowering and vegetative bud break phenology, cold hardiness and frost tolerance of catkins, nut yield, and nut and kernel characteristics. The genetic diversity and population structure of the collection is also undergoing evaluation using several approaches including genotyping by sequencing and subsequent SNP analysis and chloroplast haplotype determination. Using traditional and molecular approaches, we hope to select superior, diverse genotypes for use in breeding efforts to develop new hybrid cultivars and pollenizers adapted to the eastern USA.
Endogenous Phosphatase Activity in Turfgrass and Fungi Generates False Positives in Commercial Endophyte Testing Kit

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One method to determine the presence of *Epichloë* spp. endophyte in turfgrasses is by immunoblot. Currently, a commercial kit sold by Agrinostics is available. It has been reported in the literature that the primary antibody to the *Epichloë* spp. protein in the kit lacks specificity and reacts to other fungal species commonly found in turfgrasses. The kit uses a secondary antibody with a conjugated color reacting enzyme. The conjugated enzyme is alkaline phosphatase. The alkaline phosphatase activity is detected using a substrate that produces the chromogen naphthol-fast red.

The method begins by placing a seed, tiller section, or plug from a fungal culture on a prewetted nitrocellulose membrane. After overnight incubation, the seed, tiller, or plug is removed from the membrane, non-specific protein binding is blocked, and then the membrane is probed with the primary antibody. After washing, a secondary antibody specific to the host species of the primary antibody is added. The color detection reagents for alkaline phosphatase are added and if a pink color develops this indicates that the tiller, seed, or fungus has tested positive for *Epichloë* spp.

We found that incubating the tillers, seeds or fungal plugs in the naphthol/fast red chromogen solution alone without prior incubation with the primary and secondary antibodies results in the tested materials turning pink, giving a false positive result. This was also found with apoplastic fractions isolated from tillers and with fungal extracts of *Acremonium* spp. and *Claviceps* spp. among others. Further investigation determined that the apoplastic fractions from the tillers and the fungal extracts had natural endogenous phosphatase activity that was responsible for the false positive result. In this report we show that the endogenous phosphatase activity could be inhibited by the phosphatase inhibitor levamisole prior to incubating the membrane with primary antibody, greatly improving the accuracy of the commercial test kit.
Application of Bayesian Statistics to Selection of Drought Tolerant Tall Fescue

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Mean and variance are important parameters in the population breeding. Despite popularity, the ANOVA method is not necessarily the best approach to analyze population breeding data. This paper briefly explained the assumptions and limitations of ANOVA in point estimation of mean and variance. More importantly, two Bayesian statistical models were developed using data collected from thirty tall fescue populations subject to drought treatment in a rain-out shelter, and the two models are compared using deviance information criterion (DIC).
A Perennial Ryegrass (Lolium perenne L.) Genetic Linkage Map Constructed with Single Nucleotide Polymorphism Markers

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Perennial ryegrass (Lolium perenne L.) is a cool-season grass native to temperate regions of Asia, North Africa, and Europe. This species is important for forage and turfgrass applications, and it is grown throughout much of the temperate world. In general, perennial ryegrass breeding efforts are directed toward selection for improved turfgrass quality and growth habit, increased disease resistance, higher seed yields, and enhanced tolerance to abiotic and environmental stresses. The efficiency of the selection process can be improved via complementation with molecular markers, which requires the development of a genetic linkage map. For this study, an F₁ generation mapping population was established by crossing the parents I06 and A89. The mapping population consisted of the 2 parents and 126 F₁ progeny lines. Double digest restriction-site associated DNA libraries were constructed and sequenced for each individual using the Illumina MiSeq and HiSeq 2500 sequencing platforms. The total sequencing output generated exceeded 1.9 × 10⁹ reads, and approximately 10× greater coverage was achieved for the parental lines, compared to progeny lines. Sequence data was used to identify single nucleotide polymorphism (SNP) markers and construct the genetic linkage map. The genetic linkage map included 1,698 SNP markers across 7 linkage bars with a total length of 4,260 cM. On average, there were 243 SNP markers per linkage bar. This genetic linkage map provides a solid foundation for our current and future work. We are currently working to improve the quality of this genetic linkage map by using microsatellite markers to anchor the linkage bars to recognized perennial ryegrass linkage groups. Future research includes using the I06 × A89 mapping population to identify and map quantitative trait loci for salinity tolerance, dollar spot resistance, leaf color, and growth habit.
Application of CRISPR-Cas9 Technology to Knock Out the *Epichloë festucae* Antifungal Protein Gene

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Strong creeping red fescue (*Festuca rubra* subsp. *rubra*) is a commercially important, low-maintenance turfgrass that is often naturally infected with the fungal endophyte *Epichloë festucae*. Endophyte-mediated enhanced tolerance to dollar spot disease, caused by the fungal pathogen *Sclerotinia homoeocarpa* F. T. Bennett, has been observed in the field (Clarke et al., 2006). In a previous quantitative transcriptome study of the *E. festucae*–strong creeping red fescue interaction, the second most abundant fungal transcript was found to encode a protein similar to antifungal proteins previously identified in *Penicillium* and *Aspergillus* species (Ambrose and Belanger, 2012). The purified protein has been shown to inhibit the growth of *S. homoeocarpa* in plate assays (Tian et al., 2017). To further study the role of this protein in the endophyte-mediated disease tolerance *in planta*, we carried out a gene knockout of the *E. festucae* antifungal protein gene. The conventional fungal gene knockout approach that relies on homologous recombination was unsuccessful, likely because this gene is flanked by repeated sequences. However, we successfully applied CRISPR-Cas9 technology to the non-model fungus *E. festucae*. The Cas9 endonuclease generated a double-strand break at the target site of the antifungal protein gene, and a mutation was introduced when repairing the break. To date, we have identified one mutant with a single base pair insertion at the Cas9 target site, which introduced an early stop codon, and two mutants with large insertions from the fragments of the transformation vector. Next, the knockout isolate will be reintroduced into strong creeping red fescue to evaluate disease susceptibility of the host plant to dollar spot disease using an established greenhouse inoculation assay.


