Symposium Organizing Committee

James F. White, Jr., Chair
Bruce B. Clarke
Rong Di
Barbara Fitzgerald
Phillip Vines

Proceedings of the Twenty-Ninth Annual Rutgers Turfgrass Symposium

Rong Di and Barbara Fitzgerald, Editors

ASSOCIATE DIRECTOR'S REMARKS

Welcome to the Twenty-Ninth 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. Recently, we have included scientists outside of Rutgers to broaden the scope of the Symposium. I would like to thank Dr. Carolyn Young of the Noble Research Institute, Ardmore, OK, for providing this year's keynote address. I would also like to thank Dr. James White (Chair), Dr. Rong Di, Dr. Bruce Clarke, Dr. Phillip Vines, and Barbara Fitzgerald for serving on the Symposium Planning Committee. Dr. Rong Di and Barbara Fitzgerald served as co-editors of the Proceedings.

The faculty, students and staff in the Rutgers Turfgrass Program continue to be recognized for excellence in research, teaching and extension. At the Crop Science Meetings for the C-5 Turf division in San Antonio, TX in November 2019, the following graduate students received awards: Cathryn Chapman received first place for her oral presentation and second place for her poster; Ms. Stephanie Rossi won first place for her oral presentation; William Errickson got first place for his oral presentation and second place for a poster; Yuanshuo (Henry) Qu received a third place award for his oral presentation; and Hui (Eric) Chen got second place recognition for his oral presentation. There were many students in this competition from universities throughout the U.S. and our students were outstanding. Eric Chen also received a Chris Stiegler travel grant. In 2019, Dr. Phillip Vines won the Musser International Turfgrass Foundation's Fellowship, which is the highest award for graduate students in Turf Science. I am very pleased to report that Dr. Vines has now been hired as an Assistant Professor at Rutgers in the Center for Turfgrass Science to be part of the Turfgrass Breeding Team. We also congratulate Drs. James Murphy for receiving the Fred V. Grau Turfgrass Science Award from the C-5 division of the Crop Science Society of America in 2019.

Over the past 29 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. The turfgrass breeding team at Rutgers has developed over 400 new improved cultivars in cooperation with over 30 seed organizations. The royalties from this program support the turf farms and research projects in the Center. The Turfgrass Industry in New Jersey has also been a great supporter of the Center for Turfgrass Science over the past 29 years.

We are pleased that you have chosen to attend this year's Symposium.

Sincerely,

W. A. Meyer

William A. Meyer, Associate Director

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TWENTY-NINTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 10, 2020

Foran Hall, Room 138A

Friday, January 10, 2020

8:30 - 9:00 AM	**Registration, Posters, Coffee and Donuts**
9:00 - 10:00 AM	**SESSION I: Stress Tolerance / Bioinformatics** (Moderator: Dr. Donald Kobayashi)
9:00 – 9:20	**Dr. Bingru Huang** (*Department of Plant Biology, Rutgers University*) Regulatory Mechanisms for Heat-Induced Leaf Senescence in Cool-Season Turfgrass
9:20 – 9:40	**Dr. Dana Price** (*Department of Entomology, Rutgers University*) Turfgrass Science in the Age of Informatics
9:40 – 10:00	**Dr. Dale Bremer** (*Horticulture and Natural Resources, Kansas State University*) Evaluating Drone and Ground-Based Remote Sensing Applications to Measure Turfgrass Properties, Including Drought Stress
10:00 - 10:30 AM	**Discussion and Coffee Break**
10:30 – 11:10 AM	**SESSION II: The Microbiome** (Moderator: Dr. James White)
10:30 – 10:50	**Dr. Thomas Gianfagna** (*Department of Plant Biology, Rutgers University*) A New Antibody for Grass Endophyte Evaluation
10:50 – 11:10	**Dr. Ning Zhang** (*Department of Plant Biology, Rutgers University*) Microbiome Studies: Drought Resistance of Tall Fescue and Dollar Spot Resistance of Creeping Bentgrss
11:10 – 11:20 AM	**Discussion Session**
11:20 – 12:00 PM	**KEYNOTE: Dr. Carolyn Young** (*Noble Research Institute*) The Value of Endophytes: Hidden Treasures of Cool-Season Grasses
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1:00 – 2:00 PM	**SESSION III: Breeding and Data Management** (Moderator: Dr. Rong Di)
1:00 – 1:20	**Dr. Phillip Vines** (*Department of Plant Biology, Rutgers University*) Breeding for Gray Leaf Spot Disease Resistance in Cool-Season Turfgrasses
1:20 – 1:40	**Dr. Stacy Bonos** (*Department of Plant Biology, Rutgers University*) Breeding Progress Toward the Perfect Putting Green
1:40 – 2:00	**Dr. Galen Collier** (*Office of Advanced Research Computing, Rutgers University*) Research Computing at Rutgers OARC and the Amarel Cluster
2:00 – 2:30 PM	**Discussion Session**
2:30 – 3:30 PM	**SESSION IV: Stress Tolerance and Pest Management** (Moderator: Dr. James Murphy)
2:30 – 2:50	**Katherine Diehl** (*Department of Plant Biology, Rutgers University*) Annual Bluegrass Weevil (*Listronotus maculicollis*) for Annual Bluegrass (*Poa annua*) Control in Fairways
2:50 – 3:10	**Bradley Park** *(Department of Plant Biology, Rutgers University*) Kentucky Bluegrasses Differ in Tolerance to Traffic
3:10 – 3:30	**Henry Yuanshuo Qu** (*Department of Plant Biology, Rutgers University*) Genetic Analysis of Tall Fescue Populations Under Rainout Shelter Selection
3:30 - 3:45 PM	**Discussion and Closing Remarks**
3:45 PM	**Social Hour and Poster Session**

Pre-registered Participants

Dr. Michael Agnew
Syngenta
302 Rose Glen Lane
Kennett Square, PA 19348

Mr. Shaun Barry
GCSANJ Foundation
1133 Somerset Street
Somerset, NJ 08873

Dr. Faith Belanger
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Stacy Bonos
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Dale Bremer
Department of Horticulture and
Natural Resources
Kansas State University
3608 Throckmorton
Manhattan, KS 66506

Mr. Richard Buckley
Rutgers Plant Diagnostic Lab
20 Indyk Engel Way
North Brunswick, NJ 08902

Mr. John Capik
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Chris Carson
Echo Lake Country Club
515 Springfield Avenue
Westfield, NJ 07090

Ms. Cathryn Chapman
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Hui (Eric) Chen
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Qiang (Richard) Chen
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Joseph Clark
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Bruce B. Clarke
Director, Center for Turfgrass Science
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Galen Collier
Director, Rutgers Office of Advanced
Research Computing
96 Frelinghuysen Road
Piscataway, NJ 08854

Mr. Ryan Daddio
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Dennis DeSanctis, Jr.
Double ‘D’ Turf
385 Morrison Avenue
Hightstown, NJ 08520

Dr. Rong Di
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Katherine Diehl
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Pre-registered Participants

Mr. Jonathan Drake
Turf Farm, Inc.
44 Sandy Pond Road
Hope Valley, RI 02832

Ms. Limei Du
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Matthew Elmore
Department of Plant Biology
Foran Hall, 59 Dudley Rd.
New Brunswick, NJ 08901

Dr. William Errickson
Rutgers Cooperative Extension of
Monmouth County
4000 Kozloski Road
Freehold, NJ 07728

Mr. Patrick Fardella
Department of Plant Biology
Foran Hall, 59 Dudley Rd.
New Brunswick, NJ 08901

Mr. Kyle Genova
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Thomas Gianfagna
Department of Plant Biology
Foran Hall
59 Dudley Road
New Brunswick, NJ 08901

Dr. Robert Goodman
Executive Dean of Agriculture
and Natural Resources
88 Lipman Drive
New Brunswick, NJ 08901

Mr. Glen Groben
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Jennifer Halterman
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Joseph Heckman
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Bradley Hillman
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Joshua Honig
Department of Plant Biology
Foran Hall
59 Dudley Road
New Brunswick NJ 08901

Dr. Bingru Huang
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Donald Kobayashi
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Albrecht Koppenhőfer
Department of Entomology
Blake Hall, 93 Lipman Drive
New Brunswick, NJ 08901

Ms. Olga Kostromytska
Department of Entomology
Blake Hall, 93 Lipman Drive
New Brunswick, NJ 08901

Mr. Keith Kubik
New Jersey Turfgrass Association
c/o Grass Roots Inc.
P. O. Box 336
Mt. Freedom, NJ 07970

Pre-registered Participants

Mr. T. J. Lawson
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Jing Luo
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Melinda Martin
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. William Meyer
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Thomas Molnar
Department of Plant Biology
Foran Hall
59 Dudley Road
New Brunswick, NJ 08901

Dr. Megan Muehlbauer
Agriculture and Natural Resources
88 Lipman Drive
New Brunswick, NJ 08901

Dr. James Murphy
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Stephanie Murphy
Rutgers Cooperative Extension
Soil Testing Lab
ASB II - 57 US Highway 1
New Brunswick, NJ 08901

Mr. Bradley Park
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Jeanne Peters
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901-8520

Dr. Karen Plumley
60 Blackbone Hill Road
Millstone, NJ 08510

Ms. Amanda Carolina Prado de Moraes
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Dana Price
Department of Entomology
180 Jones Avenue
Floor 1, Room 218
New Brunswick, NJ 08901

Mr. Yuanshuo (Henry) Qu
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. James A Quinn
Dept. Ecology, Evol. & Natural
Resources
1050 George Street, Apt. # 8K
New Brunswick, NJ 08901-1050

Ms. Stephanie Rossi
Department of Plant Biology
Foran Hall
59 Dudley Road
New Brunswick, NJ 08901

Mr. Marcus Rountree
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Hiranthi Samaranayake
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Pre-registered Participants

Yu Shi
Department Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Dirk Smith
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Ms. Sabrina Tirpak
Rutgers Plant Diagnostic Lab
20 Indyk-Engel Way
North Brunswick, NJ 08902

Mr. Daniel Tuck
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mrs. Jennifer Vaiciunas
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. Phillip Vines
Department of Plant Biology
Foran Hall
59 Dudley Road
New Brunswick, NJ 08901

Ms. Emily Walsh
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Eric Weibel
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Dr. James White
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Daniel Shidi Wu
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Shaohui Wu
Department of Entomology
96 Lipman Drive
Thompson Hall
New Brunswick, NJ 08901

Dr. Carolyn Young
Noble Research Institute
2510 Sam Noble Parkway
Ardmore, OK 73401

Mr. John Zajac
Z Seeds LLC
PO Box 8
Berlin, MD 21811

Dr. Ning Zhang
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

Mr. Pingyuan Zhang
Department of Plant Biology
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901

PLENARY PRESENTATIONS

Regulatory Mechanisms for Heat-Induced Leaf Senescence in Cool-Season Turfgrass

Stephanie Rossi, Jing Zhang, Guohui Yu, and Bingru Huang

Department of Plant Biology, Rutgers University

Leaf senescence is a typical characteristic of heat stress, which attributes to the reduction of photosynthesis and plant growth. However, metabolic and molecular factors regulating heat-stress induced leaf senescence are still unclear. Our studies found that heat-induced leaf senescence in cool-season turfgrass was mainly due to accelerated chlorophyll degradation. Heat-activated activity of chlorophyll degrading enzymes and genes contributed to breakdown of chlorophyll degradation and accelerated leaf senescence. Several molecules, including salicylic acid, cytokinins (CK), ethylene, abscisic acid (ABA), and Ca were identified as key signaling molecules of several senescence-related genes, including *PPH* as a direct downstream target gene of transcription factors in the ABA and CK signaling pathways. The molecular factors for senescence-related signaling and transcription factors controlling heat-induced leaf senescence will be further discussed.

Turfgrass Science in the Age of Informatics

Dana Price

Department of Entomology, Rutgers University

In only the last few years, the genome research field - fueled largely by next-generation sequencing techniques - has seen an unprecedented growth in size and applied scope. Multi-dimensional omics datasets and analyses are driving research into plant biological processes with significantly reduced costs and compressed timelines. In this talk, I will discuss recent developments in genome sequencing technologies, platforms, techniques and applications that further the next-generation of molecular turf research.

Evaluating Drone and Ground-Based Remote Sensing Applications to Measure Turfgrass Properties, Including Drought Stress

Dale Bremer[1], Mu Hong[1], and Deon van der Merwe[2]

[1]*Department of Horticulture and Natural Resources, Kansas State University*
[2]*GD Animal Health, Deventer, the Netherlands.*

In turfgrass systems, remote sensing is increasingly used to measure a number of properties of the turf surface, including but not limited to plant stress (abiotic and biotic) and visual quality. This report includes results from several studies at Kansas State University that utilized ground-based (hand held) proximal spectral reflectance sensors (e.g., to measure normalized difference vegetation index [NDVI]), and drone-mounted spectral reflectance sensors and thermal cameras (e.g., to measure canopy temperature) to evaluate early detection of drought stress in turfgrass, visual quality, and other aspects of turfgrass systems.

Small unmanned aircraft systems (sUAS) have emerged onto the scene as potential tools for turfgrass management. Despite much rhetoric, the use of sUAS for this purpose is not well understood. The use of sUAS and sensors may improve accuracy and efficiency in turfgrass research and management compared with conventional methods. We evaluated the ability of sUAS combined with ultra-high spatial resolution remote sensing to detect early drought stress in turfgrass. Results were compared with ground-based techniques in fairway-height (15.9 mm) creeping bentgrass (*Agrostis stolonifera* L.) irrigated from well-watered to severe water deficit (100 to 15% evapotranspiration [ET] replacement). Small UAS-based measurements with a modified digital camera included three reflectance bands (near infrared [NIR, 680–780 nm] and overlapping green [G] and blue [B] bands [400–580 nm]) and eight derived vegetation indices (VIs). Ground-based measurements included soil volumetric water content (VWC), turfgrass visual quality (VQ), percentage green cover (PGC), soil temperature, and reflectance with handheld optical sensors. Declines in VWC in deficit-irrigation treatments were detected with NIR and six of eight VIs from sUAS, and the NDVI and red band reflectance from a handheld sensor, before symptoms appeared in VQ and PGC. The most consistently sensitive parameters of sUAS throughout the 3-yr study were NIR and GreenBlue VI [$(G - B)/(G + B)$], which detected drought stress >5 d before decreases in VQ (Hong et al., 2019a).

In a related study in the same plots as above, we used a sUAS-mounted thermal camera to: (1) evaluate the ability of canopy temperature (Tc) imaging to detect drought stress early in turfgrass; (2) compare early drought-stress detection ability of Tc measurements with that of the sUAS-mounted spectral reflectance sensors described above, as well as with handheld proximal sensors; and (3) evaluate thermal data's relationship to spectral reflectance from sUAS-mounted and handheld sensors, VWC, soil temperature, VQ, and PGC. Results indicated Tc measurements via the sUAS detected rises of Tc in 15 and 30% compared with 100% ET plots, corresponding with declines in VWC, before drought stress became visible. This was comparable to the best spectral parameters on companion flights, and Tc was closely correlated with spectral data from sUAS-mounted ($|r| = 0.52–0.69$) and handheld sensors ($|r| = 0.75–0.82$). Thermal data were more strongly correlated with VQ (r = -0.60 to -0.77) and PGC (r = -0.58 to -0.78) than with VWC (r = -0.43 to -0.63) and soil temperature ($|r| = 0.27–0.41$) (Hong *et al.*, 2019b). Overall, results from these studies indicated that ultra-high spatial resolution remote sensing and thermal imaging *via* sUAS detected drought stress before it was visible to a human observer, and could be valuable for improving irrigation management in turfgrass.

In addition to early detection of drought stress, canopy spectral reflectance (e.g., NDVI) is used to provide objective evaluations of visual quality in turfgrass. However, evaluations of quality may be confounded by differences among cultivars and species and by cultural practices such as mowing height that affect reflectance. In a study near Manhattan, KS, we examined effects of different cultivars and species on relationships between NDVI and visual quality ratings in Kentucky bluegrass (*Poa pratensis* L., 'Apollo'), two Kentucky bluegrass × Texas bluegrass (*Poa arachnifera* Torr.) hybrids ('Thermal Blue' and 'Reveille'), and tall fescue (*Festuca arundinacea* Schreb., 'Dynasty'). A broad range of visual quality was imposed on all four grasses through deficit irrigation, and NDVI was measured using broadband spectral radiometry across this range for each grass. Distinct linear regression models of visual quality were found for each grass, and models were also distinct among years in each grass. Relationships between NDVI and visual quality were stronger in the bluegrasses (r^2 = 0.41 to 0.83) because they had a greater range in quality under deficit irrigation than tall fescue. Results indicated that the requirement to develop separate models for each grass and in each year, combined with relatively wide confidence intervals, represents a confounding factor when estimating VQ with NDVI (Bremer *et al*., 2011).

In a related study we evaluated the effects of mowing height on relationships between NDVI and VQ in KBG and in a hybrid bluegrass. Mowing heights were 7.62 cm and 3.81 cm. The NDVI averaged 4.5 to 7% higher in high- than in low-mown plots. Distinct regression models of VQ were found at each mowing height and in each species (r^2 from 0.40 to 0.81); separate relationships between NDVI and VQ were also found between years in the same plots. Correlations between NDVI and VQ were stronger at high than at low mowing heights, possibly because of more green biomass at high mowing heights. Results indicate that when using NDVI to evaluate turfgrass VQ, evaluations should be limited to plots maintained at the same mowing height and with the same species to reduce variability in NDVI (Lee *et al*., 2011).

Other applications of remote sensing in turfgrass have been investigated at Kansas State University including: 1) using hyperspectral radiometry as a non-destructive method for estimating green leaf area index (LAI) in turfgrass; green LAI is an important indicator of the photosynthetic capacity of turfgrass canopies; 2) estimating transpiration in turfgrass from thermal measurements of a turfgrass canopy with infrared thermometers (IRTs); and 3) evaluating how factors of the turfgrass canopy (e.g., percentage green cover, canopy density, visual quality) affect NDVI and its component reflectances (i.e., red and NIR bands).

References

Hong, M., D.J. Bremer, and D. van der Merwe. 2019a. Using small unmanned aircraft systems for early detection of drought stress in turfgrass. Crop Sci. 59:2829-2844. doi:10.2135/cropsci2019.04.0212

Hong, M., D.J. Bremer, and D. van der Merwe. 2019b. Thermal imaging detects early drought stress in turfgrass utilizing small unmanned aircraft systems. Agrosystems, Geosciences & Environment 2:190028. doi:10.2134/age2019.04.0028

Bremer, D.J., Lee, H., K. Su, and S.J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: I. Variation among species and cultivars. Crop Sci. 51:2212–2218.

Lee, H., D.J. Bremer, K. Su, and Steven J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in turfgrasses: Effects of mowing height. Crop Sci. 51:323-332.

A New Antibody for Grass Endophyte Evaluation

Thomas J. Gianfagna, Jeanne S. Peters, and William A. Meyer

Department of Plant Biology, Rutgers University

Endophytes improve environmental and biological stress tolerance in grasses where they occur naturally, but the alkaloids they produce may also be toxic to grazing animals. Screening grasses for endophytes is an important part of a grass breeding strategy for turf and pasture. Identification of endophytes in grass tillers can be done by microscopy, PCR, or using an immunoblot assay. For determining endophyte status of the large number of samples generated by breeding programs, the only practical option is the immunoblot assay. There are now two commercially available immunoblot kits from Agrinostics (Watkinsville, GA) and CropMark Seeds (Christchurch, New Zealand). Both are expensive for large sample screening, and cross-react with *Claviceps* and some other fungal species, creating the potential for false positives. We have made some improvements to the existing test kits. We discovered that the natural-occurring plant and fungal phosphatases were the source of the false positive results in the existing commercial test kits that use an alkaline phosphatase linked enzyme conjugated to the secondary antibody. To solve this problem we screened phosphatase inhibitors and now use the compound levamisole.

To create a more specific test kit, we made antibodies to a fungal chitinase that is secreted by the grass endophyte, *Epichloë coenophiala* into the apoplast space of infected grasses. The antibody was made in rabbits and the antigen designed so that it would not cross react with *Claviceps* or other fungal and plant chitinases. To test the efficacy of the antibody, culture filtrates of *Epichloë coenophiala*, *Claviceps purpurea*, and *Acremonium strictum*, an endophyte that we have identified in tall fescue, were extracted and the chitinase was partially purified and tested against the antibody by Western blotting. In addition, apoplastic fluid from leaves of endophyte-infected and non-infected grasses was extracted by vacuum infiltration and assayed for reactivity to the antibody will be discussed.

GigA is an abundant secreted protein that is exclusively found in *Epichloë* spp. It has been identified in tall fescue, fine fescue, and perennial ryegrass infected with *Epichloë* spp. The *gigA* gene produces a small protein (GigA), which is cleaved to produce epichloëcyclins, cyclic peptides whose function is not known. GigA and the epichloëcyclins have been sequenced. We now have an antibody to the GigA protein and will report on the specificity of GigA to *Epichloë* and other fungi.

Microbiome Studies: Drought Resistance of Tall Fescue and Dollar Spot Resistance of Creeping Bentgrass

Ning Zhang, Glen Groben, Henry Qu, Stacy Bonos, William Meyer,
James Murphy, and Bruce B. Clarke

Department of Plant Biology, Rutgers University

Microbiomes refer to the microbial community and their genomes in a system. Preliminary research indicates that turfgrass associated microbiomes have the potential to help solve some of the important problems in turfgrass management. Our long-term goal of the project is to describe the microbiomes associated with turfgrasses, understand the causality of microbiomes and the plant health, and to translate this knowledge to develop new sustainable management strategies and other applications.

One of our ongoing projects is evaluating the microbiome associated with tall fescue genotypes grown in a rainout shelter at the Rutgers Plant Science Research and Extension Farm in Adelphia, New Jersey after prolonged periods of drought stress. This is a three-year project. Each year, 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 different tissue types showed clear differences. The two phenotypes showed no significant differences in microbiome composition but certain microbial species differed in abundance. For example, the Magnaporthales species were higher in the susceptible grass populations for the root samples and soil samples. The culture results also support this finding since we isolated seven *Magnaporthiopsis* culture from the susceptible 2017 root samples and only four from the resistant roots. Magnaporthales fungi are known root pathogens of grasses. To see if there are any significant differences in microbial functions, the metatranscriptome of the same set of siblings is currently being evaluated.

Another ongoing project is looking at changes in the microbiome before and after dollar spot symptoms develop for a susceptible and tolerant cultivar maintained at fairway height. Dollar spot is caused by the fungal species in the genus *Clarireedia* and is a very economically important disease. Understanding what constitutes a healthy microbiome versus a diseased microbiome for this disease may lead to the development of better biocontrols and cultural practices. A TaqMan qPCR assay has been developed to fast and accurately detect and quantify the dollar spot pathogens.

The Value of Endophytes: Hidden Treasures of Cool-Season Grasses

Carolyn A. Young

Noble Research Institute LLC, Ardmore, Oklahoma

Grasses from the subfamily Pooideae are often infected with endophytic *Epichloë* species that represent dominant and stable members of the plant's microbiome. These endophytic fungi are known to enhance host tolerance to drought, improve nutrient acquisition and provide protection from pests and pathogens. *Epichloë* species represent a dynamic group of endophytes that systemically infect above ground host tissue and vary in transmission strategies (horizontal, vertical or both), reproduction (sexual or asexual), genome content (varying ploidy levels) and production of bioactive alkaloids (e.g. ergot alkaloids, indole-diterpenes, lolines and pyrrolopyrazine). Protection against herbivory has been associated with the production of bioactive alkaloids, but some alkaloids have also been shown to reduce livestock performance. Despite this, grasses with endophytes are valued because of their persistence. Understanding the diversity of endophytic *Epichloë* species and identifying the genetic and biochemical differences between strains has advanced cultivar development for forage purposes.

Endophytes improve forage systems

Tall fescue and perennial ryegrass are valuable forage grasses with improved persistence due to the association with *Epichloë* species. Initially, the endophytes in these two hosts were recognized as the causative agents of fescue toxicosis and ryegrass staggers. To overcome the poor livestock performance but maintain persistence of the grass, elite cultivars were developed with improved persistence along with inclusion of *Epichloë* strains that are considered safe for livestock. These endophyte strains differ in their ability to produce specific alkaloids and have been selected as natural variants from host species found at the center of origin. Synthetic associations of endophytes with grass breeding lines can be established through a seedling inoculation protocol. Nevertheless, breeding with endophytes presents an extra layer of complexity to maintain the integrity of both the symbiont and host. We developed a PCR-based quality assurance pipeline to assess all grass lines under evaluation for cultivar development. Individual seeds or tillers are tested by PCR for endophyte presence and strain identification to ensure consistency of seed stocks, plots and pastures. As part of our grass breeding efforts, we routinely track endophyte infection rate and strain identity, testing both seed and tillers from the greenhouse to the field. This process helps us more rapidly identify problems with endophyte transmission, which enables improved selection and development of endophyte-infected cultivars. Searching for the hidden treasure of novel endophytes to employ in breeding lines can also come from characterizing crop wild relatives.

Endophytes in native grasses

Grasslands provide sources of many crop wild relatives, and are among the largest and most widely distributed ecosystem representing 26% of the world land area. Crop wild relatives have potential to provide improved genetic traits (biotic and abiotic stress resistance) through breeding with closely related domesticated crop species. But could crop wild relatives such as native grasses also represent a source of beneficial symbionts?

Plant material sourced either through the USDA National Plant Germplasm System or from ecological collections were evaluated for presence of *Epichloë* species. Molecular analyses of symbiont genetic traits among and between host populations allowed us to explore resident endophyte incidence and diversity in host species across multiple grass tribes. *Epichloë* species within a host and between host populations were found to exhibit considerable genotypic and chemotypic diversity associated with the biosynthetic pathways of the bioactive alkaloids. Phylogenetic analyses of intron-rich housekeeping genes (*tefA* and *tubB*) and mating-type genes were used to infer hybrid and nonhybrid origins, and designate species. Inheritance patterns of the mating-type idiomorphs signified hybrid species that have arisen from independent hybridizations of the same parental species. Multiple *Epichloë* taxa or single taxa with multiple chemotypes were found to associate independently with a single host species. This approach has resulted in identification of many new *Epichloë* species and new hosts for known endophytes. However, the selective role these endophytes provide to the host are yet to be elucidated.

Genetic diversity results in chemotypic diversity

Considerable genotypic and chemotypic diversity has been identified within the symbiont alkaloid biosynthetic pathways. Genome sequencing and genotyping indicate symbiont variation can occur within a host and between host populations. An excellent example of how genetic diversity can impact the breadth of possible endpoint products exists with evaluation of the indole-diterpene biosynthetic pathway.

Indole-diterpenes represent a structurally diverse class of bioactive alkaloids with core moieties of indole and cyclic diterpene. The indole-diterpene lolitrem B, a potent tremogen, was first recognized as a secondary metabolite produced during the symbiotic association of *Epichloë festucae* var. *lolii* with perennial ryegrass that negatively impacted livestock productivity. Discontinuous distribution of indole-diterpene (*IDT)* biosynthesis genes was observed for *Epichloë* species*,* even within a single species. Common features of the *IDT* loci are the sub-telomeric location and integration of large regions of AT-rich repeats. Functionality of the *IDT* gene clusters were confirmed by examining the indole-diterpene composition of *Epichloë*-infected plant material using high-performance liquid chromatography−high resolution mass spectrometry (HPLC−HRMS) with the samples compared to known indole-diterpenes produced by endophyte-infected *Ipomoea* species. Much of the indole-diterpene diversity seen in *Epichloë* can be explained by the likely erosion of the *IDT* cluster from the chromosome end. Many of the same chemotypes have been reinvented in different species, which suggests that the different indole-diterpenes may provide a selective advantage.

Endophytes in outreach

Like any hidden treasure, novel endophyte technology can only become useful and valuable when unearthed and shared. To this end, we are developing outreach units tailored to reach diverse learners from school students and teachers to agricultural producers to introduce them to the inconspicuous symbiotic relationship of endophytes in grasses. We have established a Noble Education Fellowship with the focus of providing science teachers with research experience and resources they can take back to the classroom. One lab exercise reveals endophytes in common grasses using microscopes and cell phones to record findings. Because of the diversity of groups and individuals we desire to reach, the technical aspects of this outreach unit can be adjusted depending on the materials and time frame available for a given group. For example, an activity from this unit has been implemented to demonstrate to agricultural producers the presence of endophytes inside tall fescue and to heighten awareness of factors to consider when planting and maintaining fields intended for grazing.

Putting our discoveries to work in cool season grass pastures demonstrates the value of endophytes. In any quest to find hidden treasure it first has to be found and understood before it is actually of tangible worth. We provide this value through continued research toward understanding *Epichloë* species, developing novel endophyte cultivars and educating producers and students.

Breeding for Gray Leaf Spot Disease Resistance in Cool-Season Turfgrasses

Phillip L. Vines, Ryan M. Daddio, Ronald F. Bara, Dirk A. Smith,
Stacy A. Bonos, and William A. Meyer

Department of Plant Biology, Rutgers University

Gray leaf spot, caused by the fungal pathogen *Pyricularia oryzae*, has been recognized as a common and destructive disease of perennial ryegrass (*Lolium perenne*) turf for greater than 20 years. More recently, gray leaf spot disease symptoms have been observed on tall fescue (*Schedonorus arundinaceus*) and hard fescue (*Festuca brevipila*) cool-season turf, with more widespread observations of the disease on tall fescue. Initial gray leaf spot disease symptoms include small, water-soaked lesions on leaf blades that develop into dark, necrotic spots. The infections expand into circular to oblong lesions and become gray to grayish brown with dark brown to purple borders. As the disease progresses, lesions coalesce and cause tip blighting, which often leads to twisting or flagging of leaf blades.

Breeding for gray leaf spot disease resistance has been a major focus of the perennial ryegrass breeding program at Rutgers University since the first major outbreak, which occurred in 2000. During that year, most cultivars and selections were devastated by the disease; however, two experimental populations exhibited very good gray leaf spot disease resistance. There were 89 single-plant progeny turf plots in the 2000 variety trial that had excellent gray leaf spot resistance. The plants that were used to produce the seed of those 89 plots were clonally replicated in a nursery and used to make crossing blocks during the spring of 2001. This was the basis for the gray leaf spot disease resistance breeding program for perennial ryegrass at Rutgers University. To date, over 50 gray leaf spot resistant perennial ryegrass cultivars have been developed through collaborations between the Rutgers turfgrass breeding program and industry cooperators.

In 2018, a significant gray leaf spot disease outbreak was observed in two tall fescue variety trials at the Plant Biology research farm in Adelphia, NJ. Although gray leaf spot had been previously documented on tall fescue turf in states farther to the south such as North Carolina, this was the first occurrence of this level of gray leaf spot disease pressure at the research farm in NJ. Similar to the first gray leaf spot epidemic in perennial ryegrass at the Plant Biology research farm, some experimental populations appeared to have good gray leaf spot disease resistance. There were 39 single-plant progeny turf plots in the 2018 variety trial that had excellent gray leaf spot disease resistance. The plants that were used to produce the seed of those 39 plots were clonally propagated, established in a nursery, and used to make crossing blocks in the spring of 2019. There were six populations derived from these crosses; this was the basis for the gray leaf spot disease resistance breeding program for tall fescue at Rutgers University.

Also in 2018, the characteristic gray leaf spot symptoms were observed in a hard fescue variety trial at the Rutgers turfgrass research farm in Adelphia, NJ and in a seeded fine fescue trial at the Horticultural Research Farm number 2 in North Brunswick, NJ. Six isolates of *P. oryzae* were obtained from the diseased hard fescue leaf tissue. Pathogenicity trials were conducted, and Koch's postulates were fulfilled to confirm that *P. oryzae* can incite gray leaf disease symptoms on hard fescue. Considering the historical significance of gray leaf spot in other turfgrass species, this disease could become a concern for managing hard fescue turf.

There is much work to be done in breeding for gray leaf spot disease resistance in cool-season turf. A great deal of progress has been made in perennial ryegrass, with a large number of improved cultivars available on the turfgrass seed market today. However, breeding efforts for gray leaf spot resistance in tall fescue have just begun. The Rutgers turfgrass breeding program will continue to focus on studying this disease on tall fescue and strive to develop improved tall fescue cultivars with gray leaf spot disease resistance. Additionally, future work will include monitoring both tall fescue and hard fescue turf for gray leaf spot disease symptoms across a larger geographic area and collecting isolates of *P. oryzae* from symptomatic turf, conducting additional studies to better understand differences such as virulence and host preference among *P. oryzae* isolates, and evaluating breeding lines to select for improved resistance to gray leaf spot in perennial ryegrass, tall fescue, and hard fescue.

Breeding Progress Toward the Perfect Putting Green

Stacy A. Bonos[1], Eric N. Weibel[1], P.L. Vines[1], Tracy J. Lawson[1], Lindsey Hoffman[2], and William A. Meyer[1]

[1]*Department of Plant Biology, Rutgers University*
[2]*University of Massachusetts – Amherst*

Bentgrass breeding at Rutgers has long history, even predating the earliest turf-type perennial ryegrass (*Lolium perenne* L.) and tall fescue cultivars developed by Dr. C. Reed Funk. Howard B. Sprague, the resident agronomist at the New Jersey Agricultural Experiment Station during the 1930's, released 'Raritan' velvet bentgrass (*Agrostis canina* L.) in 1940. After several decades of 'rebuilding' the turfgrass program following World War II, Dr. Ralph Engel released 'Cobra' (Engel *et al*., 1994) and Viper creeping bentgrass (*Agrostis stolonifera* L.) in cooperation with International Seeds (now DLF International Seeds) (Funk and Meyer, 2001). Drs. C. Reed Funk and Richard Hurley reinitiated the bentgrass breeding program in the late '80s and early '90s and released 'Southshore' (Hurley *et al*., 1990) in 1992 and Loft's L93 (Funk and Meyer, 2001). Loft's L93 quickly became world renowned as one of the first cultivars developed with improved tolerance to dollar spot disease (caused by *Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch sp. nov.). The bentgrass breeding program at Rutgers was advanced further with the addition of Dr. William Meyer in 1996 and Dr. Stacy Bonos in 2001 and became one of the major focuses of Dr. Bonos' breeding efforts.

Screening for disease has been a major focus of the turfgrass breeding program as a whole. Initially, tolerance to dollar spot disease was the main focus of the bentgrass breeding program since this disease accounts for the majority of pesticide applications applied to golf course turf in the temperate U.S. Major breakthroughs in tolerance to dollar spot were achieved with the release of 'Declaration', '13M' 'Memorial' and 'Kingpin' cultivars. Although improvements in dollar spot tolerance were made, tolerance to additional diseases including brown patch (caused by *Rhizoctonia solani* Kuhn), anthracnose (*Colletotrichum cereale*), copper spot (*Gleosocospora sorghi*) and root Pythium (*Pythium* spp.) were still needed. Breeding work continued to 'stack' multiple disease tolerance into single cultivars. This led to the development of 'Piranha', 'Chinook', 'L93-XD', 'Coho', and 'Match Play' which have improved tolerance to anthracnose, copper spot and brown patch diseases.

In addition to disease tolerance, an important breeding objective is improved quality and performance for fairway and putting green management. To this end, several screening techniques have been implemented to accomplish this goal. Firstly, new cultivars need to have good wear tolerance. Wear tolerance was evaluated by applying the Rutgers Wear Simulator (RWS) (Bonos *et al*., 2001) to half of each plot and evaluating percent damage and recovery. 'L93 XD' was specifically developed for improved wear tolerance under putting green height. Seventy-nine creeping bentgrass entries in a putting green trial and 60 entries in a fairway trial were replicated three times in a randomized complete block design in a turf trial seeded in 2016. In 2019, 66 and 84 passes with the RWS were applied between July 11 and August 21, 2019 to the putting green and fairway trials, respectively. In the putting green trial, MGH Comp, EFB

Comp, DSF Comp, Coho, 777, AP 23, LFC Comp, Macdonald, MGS Comp, Match Play, Piranha and Chinook had excellent wear tolerance while Proclamation, Penn A-1, V8, 13M, Alpha, Crystal BlueLinks, Putter and Penncross had poor wear tolerance. In the fairway trial, TourPro, Piranha, LFC Comp, RH 93, DSF Comp, Chinook, Declaration, LSG Comp, Coho, 777 and Shark exhibited good wear tolerance while Mackenzie, Alpha, Penncross, SR 1119, L-93XD, Cobra 2, Century, Mariner, PC2.0, Putter, and Focus exhibited poor wear tolerance.

Another important trait for fairway performance is recovery from divot injury. Divots were made on the 2014 NTEP fairway trial using a divot simulator similar to Fry *et al*. (2008) during the growing seasons of 2016, 2017 and 2018. Cultivars with the quickest recovery to divots were Penncross, Crystal Blue Links, Chinook, Luminary, Nightlife, Armor, and Piranha. Cultivars with the slowest recovery from divot injury were L93XD and Barracuda. 007 had variable performance among years. Interestingly, consistently in all three years, colonial bentgrasses (*Agrostis capillaris* L.) had lower initial injury and recovered more quickly than all creeping bentgrass cultivars except Penncross.

The goal will be to combine disease tolerance with wear tolerance, divot recovery and other traits including drought and heat tolerance to develop bentgrass cultivars with improved turf quality under low (less than 0.50 inch) cutting heights for golf courses around the world.

References

Bonos, S.A., E. Watkins, J. A. Honig, M. Sosa, T. Molnar, J.A. Murphy and W.A. Meyer. 2001. Breeding cool-season turfgrasses for wear tolerance using a wear simulator. International Turfgrass Society Research Journal Vol. 9:137-145.

Engel, R. E., G. W Pepin, and C. W. Edminster. 1994. Registration of 'Cobra' creeping bentgrass. Crop Sci. 34:306.

Funk, C.R. and W.A. Meyer. 2001. 70 years of turfgrass improvement at the New Jersey Agricultural Experiment Station: The Garden State’s Rutgers University has long been in the forefront of turfgrass development. USGA Green Section Record 39:19-23.

Hurley, R. H., V G. Lehman, J. A. Murphy, and C. R. Funk. 1994. Registration of 'Southshore' creeping bentgrass. Crop Sci. 34: 1124-1125.

Fry, L.R., M. D. Richardson and D. E. Karcher. 2008. An improved method for simulating divot injury on turfgrass. Crop Sci. 48:2459–2462. doi: 10.2135/cropsci2008.01.0038

Research Computing at Rutgers OARC and the Amarel Cluster

Galen Collier
Director, Research Support

Rutgers Office of Advanced Research Computing

The Office of Advanced Research Computing (OARC) manages advanced computing, networking, and storage systems freely available to all Rutgers students, staff, and faculty. Our office, reporting to both ORED and OIT, was created approximately three years ago with the goal of positioning Rutgers as a nationally and internationally recognized leader in research computing and data cyberinfrastructure to advance the frontiers of research, pedagogy, and innovation. We provide research computing expertise and support to all Rutgers campuses through next generation computing, data science, networking, cloud services, and creative learning environments. We are also a well-known, active partner for local, regional, and national programs and for individuals within focused academic, research, science, engineering, and medical communities. Some areas of special emphasis for us include innovation in campus cyberinfrastructure, partnering on research proposal development, and supporting regional or national partnerships.

Our team includes experienced computational scientists available for consulting, training, or partnering on grant activities (e.g., proposal development and embedded project support). The discipline-specific expertise represented among our team members includes biochemistry, bioengineering, business administration, chemistry, genomics, mathematics, mechanical engineering, microbiology, and physics. We have a dedicated business operation, workforce development, and commercial partnership team, as well as an infrastructure administration team that, like our hardware, is distributed across all major Rutgers sites.

Each semester, we host a variety of support and outreach events such as small custom workshops, in-class guest instructor engagements, large departmental-scale training events, and a range of introductory workshops for students, staff, faculty from all disciplines. An overarching goal of each workshop is to help researchers at all computing skill levels get off to a good start with the basics of using research computing resources and with managing their vital research data or software workflows.

Our extensive computing, networking, and storage infrastructure is constantly growing. We operate using a hybrid University- and community-supported model where hardware is expanded each year and there are opportunities for researchers or departments to purchase dedicated compute and storage resources within our centrally-managed infrastructure. Our primary computing system, the Amarel cluster (named after Saul Amarel, Rutgers computer science pioneer), is a multi-campus system available for use by all current students, staff, and faculty at no cost. Beyond its extensive compute capabilities, the Amarel cluster offers a variety of key features such as many types of GPU accelerators and a large parallel-I/O storage system designed for handling very large datasets. The Amarel cluster spans multiple Rutgers campuses using a dedicated high-performance internal network and it offers low-latency connectivity to regional service providers and other institutions.

Annual Bluegrass Weevil (*Listronotus maculicollis*) for Annual Bluegrass (*Poa annua*) Control in Fairways

Katherine H. Diehl, Matthew T. Elmore, James A. Murphy, and Albrecht M. Koppenhöfer

Department of Plant Biology, Rutgers University

Annual bluegrass (*Poa annua*) is one of the most problematic weeds of cool-season turfgrass. The annual bluegrass weevil (*Listronotus maculicollis*; ABW) is a turfgrass pest that showcases ovipositional preference for annual bluegrass over creeping bentgrass (*Agrostis stolonifera*). Turfgrass managers control ABW preventatively, to avoid any turfgrass damage. We hypothesized that withholding ABW control until a damage threshold was met would reduce annual bluegrass cover in creeping bentgrass fairways. The effect of ABW damage was tested alone and in combination with various rates of paclobutrazol.

Research was conducted in 2018 and 2019 on adjacent sites located on a simulated creeping bentgrass fairway infested with annual bluegrass at the Rutgers Horticulture Farm No. 2 in North Brunswick, NJ. Three insecticide programs were combined with four rates of paclobutrazol in a three by four factorial. Treatments were replicated four times and arranged in a randomized block design.

The four paclobutrazol rates were 210, 105, 70, and 0 g ha^{-1} (referred to as high, medium, low, and none hereafter). Paclobutrazol was applied monthly from May through October. The first insecticide program (preventative) was an industry-standard that controlled early larval stages to prevent any turfgrass injury. In the second program (threshold), no insecticides were applied until visual ratings determined that ABW damage resulted in unacceptable turfgrass quality. Once this damage threshold was met, an insecticide was applied to prevent future turfgrass injury. No insecticides were applied to the third program (no-insecticide) for ABW control.

To evaluate the efficacy of the insecticide programs, cores (6 cm in diameter) were taken from each plot and larvae were extracted in salt solution. Extracted larvae were counted and used to determine the average larval density per plot. Turfgrass quality, annual bluegrass quality and creeping bentgrass quality were evaluated monthly on a 1-9 scale from poor to excellent (where 6=acceptable). Lightbox photos were taken monthly and subjected to digital analysis (Turf Analyzer software). Percent annual bluegrass cover was evaluated visually each month and grid counts were taken in November. Data were subjected to ANOVA in SAS (v9.4) as a 4 by 3 factorial RCBD ($\alpha=0.05$) and Fisher's Protected LSD ($\alpha=0.05$) was used to separate means.

Data were analyzed separately by year, as treatment-by-year interactions occurred on multiple dates. Although larval densities were higher in 2019, the preventative program provided excellent ABW larval control both years (87-98% control, relative to the non-treated). Insecticide program affected turfgrass quality from June to August in 2018 and in June 2019, but did not have a season-long effect either year. While quality was lower in plots receiving high rates paclobutrazol in May, paclobutrazol did not have a lasting effect on turfgrass quality.

All paclobutrazol treatments reduced annual bluegrass cover from May to November in 2018 and from July to October in 2019. In 2018, there were no cover differences between the middle and high rates, and there were no cover differences between any rates in 2019. The non-paclobutrazol plots had the most annual bluegrass cover both years. Insecticide program also affected annual bluegrass cover both years. Annual bluegrass cover was highest in the preventative program on all rating dates in 2018 and 2019.

An interaction between main effects of paclobutrazol and insecticide program occurred at the end of each season. There were no cover differences between insecticide programs treated with high rate of paclobutrazol either year. On 30 November 2018, the effect of insecticide program was apparent for the middle rate of paclobutrazol, and in the non-paclobutrazol treated plots. In both no and middle rates, cover was highest in the preventative program (83% and 33%, respectively) and similar between the threshold and no-insecticide programs (56-68% and 8-13%, for no and middle rates, respectively). In October 2019, within the no, low, and middle rates, cover was higher in the preventative program (56%) than the threshold and no-insecticide programs (36% and 26%, respectively).

Kentucky Bluegrasses Differ in Tolerance to Traffic

Bradley S. Park and James A. Murphy

Department of Plant Biology, Rutgers University

Kentucky bluegrass (*Poa pratensis* L.) is a cool-season turfgrass that is frequently established on sport fields and other highly trafficked recreational surfaces in New Jersey and throughout temperate climates of the world. Machines are often used to impart wear or the combined stresses of wear and compaction to evaluate the tolerance of Kentucky bluegrass to traffic.

The Rutgers Wear Simulator (RWS) was designed to primarily abrade and wear aboveground plant parts such as leaves and surface stems with minimal compaction of the soil (Bonos *et al*., 2001). Other machines such as the Cady Traffic Simulator (CTS) have been designed to impart both wear and compaction of the soil similar to trampling caused by athletes with cleated shoes (Henderson *et al*., 2005).

The RWS and CTS were used independently to separately assess wear and trampling tolerance of Kentucky bluegrass in the 2011 National Turfgrass Evaluation Program (NTEP) Test (Park *et al*., 2016). Additionally, the RWS and CTS have been used in combination to assess the traffic stress of tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort.) cultivars and experimental selections (Park and Murphy, 2017) and Kentucky bluegrass cultivars and experimental selections (Park *et al*., 2019). The objective of this research was to evaluate the traffic stress tolerance of Kentucky bluegrasses in the 2017 NTEP test during summer and autumn 2019.

The 89 Kentucky bluegrass entries comprising the 2017 National Turfgrass Evaluation Program [NTEP] Test were seeded into 2.4 x 1.8-m plots on a loam in North Brunswick, NJ in October 2017. Each entry was replicated three times. Plots were mowed two or three times per week at a height of 3.8-cm and irrigation was applied at no less than 80% ET in the absence of rain and once the loam was well-below field capacity.

Two levels of traffic (Traffic and No Traffic) were evaluated using a strip-plot design across each entry during summer and autumn evaluation periods in 2019. The RWS (16 passes) and CTS (16 passes) were both operated on the trafficked-strip for a total of 32 passes during 1 to 24 July 2019.

Mechanical problems disabled the CTS during autumn 2019; thus, a vibratory pavement roller (1173 kg) was used to impart compaction during the autumn evaluation period. The RWS (14 passes) and vibratory roller (14 passes) were both operated on the trafficked strip for a total of 28 passes during 11 Sept. to 7 Oct. 2019. Every other pass with each machine was made in the opposite direction. The RWS was operated at 4.0 km hr^{-1}; the paddles rotated at 250 rpm. The CTS was operated in the forward direction at 1.6 km hr^{-1}. The pavement roller was operated at 3.8km hr^{1} with vibration enabled.

At the conclusion of summer and autumn 2019 traffic, the non-trafficked and trafficked portions of Kentucky bluegrass entries were visually assessed for uniformity of turf cover (1 to 9 scale; 9=most uniform cover). A digital image of the center 21.5 x 16.2-inch of each plot was obtained with a Canon PowerShot G16 (Canon USA, Inc., Lake Success, NY) camera mounted in a box equipped with artificial lighting. The camera was set at a shutter speed of 1/40 s, aperture of F2.8, ISO of 100 and focal length of 8-mm. Digital image size was 3000 x 4000 pixels.

Images were digitally analyzed for the percentage of green cover using a hue range of 50 to 107 and a saturation range of 0 to 100 in the Turf Analyzer image analysis software (Green Research Services, Fayetteville, AR).

Data were subjected to analysis of variance using a 2 x 89 factorially arranged strip-plot design. Means were separated using a Fisher's least significant difference (LSD) test at $p < 0.05$.

Traffic reduced the uniformity of turf cover and green cover of Kentucky bluegrass (KBG) during summer and autumn 2019. Moreover, the interaction of the traffic and KBG factors was significant during both traffic periods, which indicated that there were few to no differences in the uniformity of turf cover and green cover among KBG entries under the conditions of no traffic. Whereas, under the trafficked conditions, there were substantial differences among KBG entries.

Summer 2019

Six KBG entries were very tolerant of traffic in summer 2019 based on visual assessment of the uniformity of turf cover; A-16-17, BAR PP 7K426, BAR PP 71213, BAR PP 7309V, 'Barvette HGT', and DLFPS-340/3549 were not affected by traffic. Similarly, four entries were very tolerant of summer traffic based on the percentage of green cover in digital images; BAR PP 7K426, PPG-KB 1131, PST-11-7, BAR PP 7309V and 'Prosperity' were not affected by traffic.

Entries with the best uniformity of turf cover were BAR PP 7K426, A-16-17, BAR PP 71213, BAR PP 7309V, 'Barvette HGT', DLFPS-340/3549, KH3492, 'Yellowstone' (A12-7), NAI-A16-3, NAI-14-178, PST-K15-172, DLFPS-340/3552, PPG-KB 1131, and PST-K15-167. Entries with the greatest green cover after summer 2019 traffic were PST-11-7, 'Prosperity', A-16-17, PPG-KB 1131, DLFPS-340/3552, BAR PP 7K426, BAR PP 7309V, DLFPS-340/3494, A11-26, DLFPS-340/3550, NAI-13-14, J-3510, PST-K15-177, PST-K15-167, BAR PP 71213, 'Selway', 'Midnight', NAI-13-132, 'Blue Devil', DLFPS-340/3549, BAR PP 79494, DLFPS-340/3556, PST-K15-172, NAI-A16-3, DLFPS-340/3500, 'After Midnight', KH3492, DLFPS-340/3455, and GO-22B23.

Entries with the poorest uniformity of turf cover after summer 2019 traffic were NAI-14-132, NAI-15-84, J-1319, 'Pivot', DLFPS-340/3364, RAD-1776, DLFPS-340/3553, NK-1, and NAI-15-80. One entry had the lowest green cover after summer 2019 traffic, NAI-15-80.

Autumn 2019

Eight KBG entries were not affected by autumn traffic based on the uniformity of turf cover; Barvette HGT, BAR PP 71213, BAR PP 7K426, PST-K15-167, PST-K15-172, A-16-17, DLFPS-340/3549, and Yellowstone (A12-7) had the same uniformity of turf cover as the non-trafficked strip-plot. Autumn traffic did not affect the green cover of 41 KBG entries.

Entries with the best uniformity of turf cover after autumn 2019 traffic were Barvette HGT, BAR PP 71213, PST-K15-167, PST-K15-172, BAR PP 7K426, A-16-17, DLFPS-340/3549, Yellowstone (A12-7), and RAD 553. Entries with the greatest green cover after autumn traffic were NAI-14-178, Barvette HGT, A-16-17, Prosperity, BAR PP 71213, PST-11-7, A11-26, DLFPS-340/3494, RAD 553, PST-K11-118, and KH3492.

Entries with the poorest uniformity of turf cover after autumn 2019 traffic were A16-7, A12-34, NAI-14-176, NAI-14-122, PPG-KB 1320, NAI-14-187, A99-2897, DLFPS-340/3446, A06-8, RAD-1776, Pivot, NAI-14-132, NAI-15-84, DLFPS-340/3364, J-1319, DLFPS-340/3553, NK-1, NAI-15-80, and PST-K15-157. Entries with the least green cover after autumn traffic were NK-1, NAI-15-80, and PST-K15-157.

Thus, eleven KBG entries exhibited a high tolerance of traffic during both traffic periods in 2019. The KBG entries that had the best uniformity of turf cover during both periods were A-16-17, BAR PP 7K426, BAR PP 71213, Barvette HGT, DLFPS-340/3549, PST-K15-172, and A12-7. Entries with the greatest green cover during both periods were PST-11-7, Prosperity, A-16-17, DLFPS-340/3494, A11-26, BAR PP 71213, and KH3492.

National Turfgrass Evaluation Program tests continue to be an excellent source of non-biased performance data of commercially available turfgrass cultivars as well as experimental selections. Traffic tolerance is an important selection criterion for those managing high traffic sports fields and grounds as well as sod producers servicing this market.

These data are also important for the turfgrass seed industry. Currently, only 15 of the 89 entries comprising the 2017 NTEP Kentucky bluegrass test are commercially available. Seed company decision makers can use these data on traffic tolerance as part of the determination to commercialize experimental selections.

References

Bonos, S.A., E. Watkins, J.A. Honig, M. Sosa, T. Molnar, J.A. Murphy and W.A. Meyer. 2001. Breeding cool-season turfgrasses for wear tolerance using a wear simulator. Int. Turfgrass Soc. Res. J. 9:137-145.

Henderson, J.J., J.L. Lanovaz, J.N. Rogers III, J.C. Sorochan, and J.T. Vanini. 2005. A new apparatus to simulate athletic field traffic: The Cady Traffic Simulator. Agron. J. 97:1153-1157.

Park, B.S. and J.A. Murphy. 2019. Response of Kentucky bluegrass to traffic in autumn, 2018. Rutgers Turf. Proc. 50:235-249.

Park, B.S. and J.A. Murphy. 2017. Using digital image analysis to assess tall fescue traffic tolerance during spring, summer, and autumn. ASA, CSSA, and SSSA Int. Annu. Meet. p. 107151.

Park, B.S., H. Chen, and J.A. Murphy. 2016. Kentucky bluegrass response to wear and traffic during autumn. ASA, CSSA, and SSSA Int. Annu. Meet. p. 100269.

Genetic Analysis of Tall Fescue Populations Under Rainout Shelter Selection

Henry Yuanshuo Qu[1], Edwin J. Green[2], Stacy A. Bonos[1], and William A. Meyer[1]

[1]*Department of Plant Biology, Rutgers University*
[2]*Department of Ecology, Evolution & Natural Resources, Rutgers University*

Rain-out shelters have been widely used in the breeding and selection of tall fescue [*Festuca arundinacea* (Schreb.)] for improved drought tolerance. Green persistence during drought stress is an important trait with obvious phenotypic variations upon which to base selection. In this study, we closely observed and documented two consecutive generations of tall fescue populations as well selected genotypes evaluated in rain-out shelter trials and seek to optimize this selection procedure by a thorough genetic analysis combining field phenotyping data, historic breeding records, and laboratory sequencing data. Low heritability estimates (~ 0.10) of green persistence measured by normalized difference vegetation index were obtained. Given the experimental design of 20 maternal half-sibs and 10 blocks of maternal clones in this study, the prediction accuracy of commonly used selection methods ranked as follows: parental selection > stratified mass selection > family selection > mass selection. In stratified mass selection, the incorporation of relationship matrices obtained from maternal pedigree and single nucleotide polymorphisms (SNPs) in the prediction of breeding values for selection genotypes improved mean prediction accuracy by 20%, compared with the naïve model wherein independence of selection genotypes was assumed.

POSTER PRESENTATIONS

Effects of Plant Growth Regulators and Nitrogen on Post-Drought Recovery in Creeping Bentgrass

Cathryn Chapman and Bingru Huang

Department of Plant Biology, Rutgers University

Drought is a major abiotic stress that limits the growth and development of plants, and can cause severe damages in stoloniferous creeping bentgrass (*Agrostis stolonifera*). Drought stress damages can be improved upon re-watering through the use of plant growth regulators (PGR). PGRs, including hormones, as well as nitrogen can promote rapid recovery from drought, which is critical for regrowth and reestablishment of important growing regions, including daughter plants from stolon nodes as well as new tillers. The objective of this study was to determine the effectiveness of gibberellic acid (GA) and cytokinin (CK) alone or combined, as well as ammonium nitrate alone or combined with GA and CK for overall recovery from a prolonged period of drought stress, and to determine the morphological effects that these compounds have on tiller formation and regeneration of daughter plants from stolons upon re-watering from drought stress. This study found that exogenous application of these compounds stimulated overall leaf elongation upon re-watering following 35 days of drought stress, and that there were differential PGR treatment effects on stolon and tiller re-growth. More specifically, the study concluded that GA mainly contributed to increased stolon elongation and production, while CK was responsible mainly for increased tiller elongation and production. Ammonium nitrate did not significantly contribute to either stolon or tiller regrowth and was mainly responsible for promoting leaf elongation. Therefore, using GA and CK could be the key for further enhancing post-drought recovery through increased growth of tillers and daughter plants.

Microbiome Analysis of Annual Bluegrass Seeds

Qiang Chen, Bushra Aleem, James F. White, and Matthew Elmore

Department of Plant Biology, Rutgers University

Plants vector numerous microbes that contribute to host nutrition, development, stress responses and phenotypes. Plant-associated microbes are generally acquired from the surrounding environment or vertically transmitted with seeds. Seed-vectored bacteria can be mutualistically associated with the host seedlings. Without seed-vectored bacteria, seedlings can lose root gravitropism, fail to develop root hairs, and be susceptible to soil-borne pathogens. *Poa annua* seedlings are very competitive in turfgrass systems and this could be due in part to mutualistic associations with microbes. However, the microbiome associated with *P. annua* has not been explored. We hypothesize that part of the *P. annua* seed microbiome is conserved across locations and management inputs (e.g., fertilizer) within these locations. In this study, we conducted metagenomic analysis of microbial community associated with *P. annua* seeds from three locations with sandy soils. The Dormie Club in West End, North Carolina, Tuckahoe Sod Farm in Hammonton, New Jersey, and Hollytree Golf Course in Tyler, Texas. *P. annua* plants were collected from adjacent areas with high (i.e., fairways and sod) and low management inputs at each location. Seeds were collected from *P. annua* plants and subjected to surface sterilization or left non-sterilized. The bacterial and fungal communities were characterized by Illumina MiSeq high-throughput sequencing of 16S rRNA V3-V4 regions and internal transcribed spacer 2 (ITS2) region, respectively. Regardless of locations and seed treatments, the bacterial community associated with *P. annua* seeds was composed of 13 phyla, 18 classes, 38 orders, 61 families and 100 genera. At genus level, *Pantoea* (49%) and *Pseudomonas* (35%) were the most abundant. Sterilization procedure reduced the diversity of bacterial community associated with NC seeds but not NJ or TX seeds. ITS2 analysis revealed that the fungal community associated with *P. annua* seeds was composed of 10 phyla, 29 classes, 40 orders, 63 families and 257 genera. At the class level, it mainly consisted of Dothideomycetes (48%), Tremellomycetes (30%), Sordariomycetes (9%), and Microbotryomycetes (9%). The abundant genera included *Neoascochyta* (16%), *Cladosporium* (10%), an unknown Pleosporales genus (10%), *Filobasidium* (8%), *Cystofilobasidium* (5%), *Vishniacozyma* (5%), *Alternaria* (4%), *Holtermanniella* (4%), *Microdochium* (4%), *Cuivibasidium* (3%), and *Aureobasidium* (3%). Sterilization did not affect the diversity of fungal communities on TX samples but reduced the diversity of fungal communities on NJ samples. Fungal communities associated with *P. annua* seeds from all three locations shared 9 fungal genera. Among the genera we found on/in *P. annua* seeds, some are known to contain plant-beneficial microbes, e.g. *Pantoea*, *Pseudomonas*, *Cladosporium*, and *Aureobasidium*.

Effect of Ethephon Applications on Levels of Apomixis in Rockstar Kentucky Bluegrass

Ryan M. Daddio, Joshua A. Honig, Thomas J. Gianfagna, B. Shaun Bushman,
Stacy A. Bonos, and William A. Meyer

Department of Plant Biology, Rutgers University

Apomixis is asexual reproduction via seed. This mode of reproduction can be exploited if you identify an improved genotype, as it can be fixed and reproduced with ease. The problem becomes identifying novel, improved genotypes. Temporarily disabling apomixis would be an ideal approach by providing a higher proportion of hybrids to select from. Kentucky bluegrass is a facultative apomict which has led to difficulty in selecting improved cultivars that retain a high level of apomictic reproduction, a desired trait in modern varieties. Through review of literature, it has been hypothesized that ethylene may play a role in the development of the asexual embryo. To test this hypothesis, 'Rockstar' Kentucky bluegrass was pollinized by 'Avalanche' Kentucky bluegrass after a series of treatments with ethephon in a growth chamber. Four timing levels (Pre-culm emergence from the boot, Post-culm emergence from the boot, Elongation from the boot and all three timings in combination) and two treatment levels (water control and 1000 ppm ethephon) were tested among three replications for a total of 24 experimental units. A sample of 48 progeny from each experimental unit were subjected to analysis with 10 SSR markers to evaluate the effect of ethephon on reproductive outcomes.

Growth and Physiological Effects of *Burkholderia* on Drought Stress Tolerance and Post-Drought Recovery in Creeping Bentgrass

William Errickson[1,2] and Bingru Huang[2]

[1]Rutgers Cooperative Extension
[2]Department of Plant Biology, Rutgers University

Improving drought tolerance and turf recovery upon re-watering are valuable traits. This study was designed to investigate effects of a new strain of *Burkholderia* (plant growth promoting rhizobacteria, PGPR) on creeping bentgrass during drought stress and re-watering. Bacteria were used to inoculate the roots of creeping bentgrass (cv. 'Penncross'). The plants were subjected to 35 days of drought stress treatments, followed by a 15 day recovery period in growth chamber trials. Turf quality, canopy density and tiller production were measured throughout the study. Root biomass, length, surface area and volume were also quantified at the conclusion of the drought stress and recovery periods. Inoculated plants produced more tillers and maintained higher percent green coverage in the canopy during drought stress and exhibited a more rapid response to re-watering during the recovery period. Root growth was also increased in inoculated plants under drought stress compared to non-inoculated plants. These results suggest that inoculation with this novel strain of *Burkholderia* was able to confer enhanced drought tolerance and recovery to creeping bentgrass by maintaining greater canopy density through increased tiller production and improving root growth.

Epichloë festucae Antifungal Protein Purification and Gene Knock-Out

Patrick Fardella III, Ruying Wang, Simin Luo, Bruce B. Clarke, and Faith C. Belanger

Department of Plant Biology, Rutgers University

Control of dollar spot disease on creeping bentgrass, caused by *Clarireedia jacksonii*, is a major problem for golf course managers and currently relies heavily on fungicide applications. Ongoing efforts to address this problem have focused on breeding tolerant cultivars and on improving management protocols. We are pursuing a different and complementary approach, which is to understand the mechanism of dollar spot resistance in a fungal endophyte (*Epichloë festucae*) infected strong creeping red fescue. Endophyte-mediated disease resistance is well established in fine fescues (Clarke *et al*., 2006), but is not a general feature of other endophyte-infected grasses such as perennial ryegrass or tall fescue. If we can uncover the mechanism of the endophyte-mediated disease resistance in fine fescues, it may be possible to adapt it for use in other turfgrasses such as creeping bentgrass, which are not infected with *Epichloë* endophytes.

Previously we identified an abundant endophyte transcript for an antifungal protein. The antifungal protein gene found in *E. festucae* infecting strong creeping red fescue is not present in most *Epichloë* genomes for which whole genome sequences are available (Ambrose and Belanger, 2012). The transcript abundance and the limited existence of the antifungal protein gene among *Epichloë* spp. suggested the *E. festucae* antifungal protein may be a component of the unique endophyte-mediated disease resistance observed in strong creeping red fescue.

We are taking two approaches to address the importance of the *E. festucae* antifungal protein in the disease resistance of strong creeping red fescue. In one approach we are optimizing purification methods for producing large amounts of the protein for testing in direct application to plants. In the other approach we have knocked out the antifungal protein gene with the objective of determining the effect on the dollar spot resistance in plants carrying the knock-out isolate. Our results to date are described below.

E. festucae antifungal protein purification

The *E. festucae* antifungal protein is a highly expressed fungal protein in the infected host grass (Ambrose and Belanger, 2012), but purification from plant tissue is not practical since it is overall a minor protein in the mixed fungal/plant tissue. Moreover, it is not expressed when the fungus is grown in culture. We, therefore, have explored generating the antifungal protein in several established protein expression systems. The objective is to identify a protein expression system that can generate a large amount of active antifungal protein in the simplest way. The antifungal protein has been successfully expressed in the yeast *Pichia pastoris* (Tian *et al*., 2017) and in the bacterium *Escherichia coli.* The *E. festucae* antifungal protein is similar to a protein from another fungus, *Penicillium chrysogenum,* which is designated PAF (*Penicillium* antifungal protein) and which also has antifungal activity (Marx, 2004). We have obtained an engineered PAF overexpression strain of *P. chrysogenum* from Dr. F. Marx (Medical University of Innsbruck, Innsbruck, Austria) so that we could directly compare the activities of PAF and the *E. festucae* antifungal protein. The antifungal activity of PAF and the *E. festucae* antifungal protein produced in yeast and in bacteria against *Neurospora crassa* conidia, a model fungus used in such systems, was compared. At the lowest concentrations, PAF had the best activity, but at greater than 5 ug/mL the activity of the *E. festucae* antifungal protein produced in yeast was similar to PAF. At concentrations greater than 20 ug/mL the activities of all three samples were similar.

Since large amounts of PAF can be easily purified from *P. chyrsogenum*, we are now working on expressing the *E. festucae* antifungal protein in a PAF knock-out strain of *P. chrysogenum* (obtained from Dr. F. Marx) using the PAF promoter to drive expression. Several *P. chrysogenum* transformants with the *E. festucae* antifungal protein gene have been identified and will soon be tested for production of the *E. festucae* antifungal protein. If successful, the *P. chrysogenum* expression of the *E. festucae* antifungal protein should provide us with sufficient quantities of the protein for assessing efficacy against the dollar spot pathogen in turfgrass.

***E. festucae* antifungal protein gene knock-out**

Two independent gene knock-out isolates of *E. festucae* were generated by using the CRISPR-Cas9 technology. The objective was to inoculate the knock-out isolates back into endophyte-free strong creeping red fescue and assess the level of dollar spot resistance of the plants. If the antifungal protein is indeed a factor in the dollar spot resistance, as we suspect it is, then the plants harboring the knock-out isolates would be expected to exhibit less or no dollar spot resistance. However, extensive attempts over a 12-month period to inoculate plants with the knock-out isolates were unsuccessful. Interestingly, the wild type isolate was successfully inoculated into endophyte-free plants. These results were unexpected and have raised new questions about the possible importance of the *E. festucae* antifungal protein to the symbiotic association with strong creeping red fescue. Is the antifungal protein an important factor in the symbiotic interaction or is the inability to inoculate plants with the knock-out isolates unrelated to the lack of the antifungal protein gene but rather due to some other change in these isolates as the result of removing this gene?

The interaction of fungal plant pathogens and symbionts with their hosts involves effector proteins, characterized as small-secreted proteins, that can be important for colonization or for evasion of host defenses (Plett and Martin 2015; Uhse and Djamei 2018). The *E. festucae* antifungal protein is a small secreted protein and is considered a candidate effector protein (Hassing et al., 2019). We are addressing the possibility that the *E. festucae* antifungal protein may have a role in the symbiotic interaction, in addition to being an antifungal protein, using two approaches. First, we are working on generating new knock-out isolates to determine if they also have the same phenotype as the original isolates. Second, we are also rescuing the two knock-out isolates that we previously generated by reintroducing the antifungal protein to see if the presence of the antifungal protein will then allow inoculation into plants.

Acknowledgements
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References

Ambrose, K.V., Belanger, F.C. (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PLoS ONE 7(12):e53214

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

Hassing, B., Winter, D., Becker, Y., Mesarich, C.H., Eaton, C.J., and Scott, B. (2019) Analysis of *Epichloë festucae* small secreted proteins in the interaction with *Lolium perenne*. PLoS ONE 14:e0209463

Marx, F. (2004) Small, basic antifungal proteins secreted from filamentous ascomycetes: a comparative study regarding expression, structure, function and potential application. Applied Microbiology and Biotechnology 65, 1330142

Plett, J.M., and Martin, F. (2015) Reconsidering mutualistic plant–fungal interactions through the lens of effector biology. Current Opinion in Plant Biol. 26:45-50

Tian, Z., Wang, R., Ambrose, K.V., Clarke, B.B., Belanger, F.C. (2017) The *Epichloë festucae* antifungal protein has activity against the plant pathogen *Sclerotinia homoeocarpa*, the causal agent of dollar spot disease. Scientific Reports 7:5643

Uhse, S., and Djamei, A. (2018) Effectors of plant-colonizing fungi and beyond. PLoS Pathogens 14:e1006992

TaqMan Quantitative PCR Detection of *Clarireedia* spp., the Causal Agent of Dollar Spot in Turfgrasses

Glen Groben[1], Ning Zhang[1], Bruce Clarke[1], James Murphy[1], Sangkook Lee[1],
Joanne Crouch[2], and Paul Koch[3]

[1] *Department of Plant Biology, Rutgers University*
[2] *Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA-ARS*
[3] *Department of Plant Pathology, University of Wisconsin-Madison*

Dollar spot is one of the most economically important and destructive diseases of both warm- and cool-season turfgrasses. A recent taxonomic revision has placed the causal agents in a new genus *Clarireedia* with four species. All species of *Clarireedia* are capable of causing dollar spot disease in turfgrass. The main goal of the current study was to develop a TaqMan quantitative polymerase chain reaction (qPCR) molecular detection system to rapidly quantify the abundance of *Clarireedia* from environmental (field) samples. TaqMan primers and probes were developed based on the sequences of the internal transcribed spacer 1 (ITS1) region of the RNA genes. The qPCR assay was able to detect all four *Clarireedia* species but not fungi phylogenetically related to *Clarireedia*, other turfgrass pathogens, or fungal species commonly isolated from turfgrass. Genomic DNA concentrations of *Clarireedia* ranging from 38.0 ng (3.8×10^{-8} g) to 38.0 fg (3.8×10^{-14} g) were consistently detected using this assay, with results obtained in as little as three hours including DNA isolation and qPCR amplification. The qPCR assay was also able to consistently detect *Clarireedia* from symptomatic and asymptomatic creeping bentgrass (*Agrostis stolonifera* L.) samples when using foliar tissue and surface thatch layer (0-5 mm depth). The pathogen was not consistently detected in the lower thatch (~ 5-10 mm depth) and soil indicating that the pathogen is not widely distributed in these layers and primarily found in the foliar tissue and surface thatch layer. Interestingly, lower levels of the pathogen population were detected in asymptomatic samples taken in June compared to asymptomatic samples from August. This suggests that creeping bentgrass can tolerate a certain quantity of the pathogen in leaves or thatch before disease symptoms appear. However, further research is needed to verify this hypothesis.

A Microsclerotial Granular Formulation of the Entomopathogenic Fungus *Metarhizium brunneum* F52 Strain for Annual Bluegrass Weevil Management: Efficacy and Compatibility with Fungicides

Albrecht M. Koppenhöfer[1], Olga S. Kostromytska[1,2], and Shaohui Wu[1,3],

[1]*Department of Entomology, Rutgers University*
[2]*Currently: Stockbridge School of Agriculture, University of Massachusetts*
[3]*Currently: Department of Entomology, University of Georgia, Tifton*

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a serious and expanding pest of golf course turf in the Northeast and Mid-Atlantic regions of the United States and southeastern Canada. Its larvae can cause severe damage to tees, fairways, collars, and greens. This pest is one of the most difficult-to-manage turfgrass insect pests in North America due to the presence of several generations per year and concurrent presence of multiple life stages with increasing asynchrony during the growing season. Its ability to develop resistance to a wide range of insecticides warrants the development of alternative control methods.

Products based on the conidial spores of entomopathogenic fungi have thus far given unreliable control of ABW adults and larvae in the field, but the use of fungal microsclerotia may improve economy and efficacy of fungus-based products. Microsclerotia are survival structures naturally formed in soil by fungi. Applied microsclerotia granules produce infective conidial spores over several weeks, thus prolonging the residual effect of the fungus application. Improvements in conidial production by microsclerotia in soil can be made by the addition of hydrogels. Hydrogels have a capacity to hold large volumes of water when moistened and can slowly release this retained water over time, making it available to plants, fungi and other organisms.

The granular formulation of *M. brunneum* F52 microsclerotia used in this study was prepared at the United States Department of Agriculture, Agricultural Research Service, Crop Bioprotection Research Unit in Peoria, IL using diatomaceous earth as the carrier material. The majority of granules (~85%) were 0.25-1.0 mm in size giving about 2,500 granules per gram of formulation.

Compatibility with common turfgrass fungicides

Fungicides are frequently applied to golf course turf to combat the multitude of fungal disease than can afflict it. As some of these fungicides may have detrimental effects on entomopathogenic fungi, we tested the compatibility of commonly used turf fungicides with *Metarhizium brunneum* F52 microsclerotia. The tested fungicides were from different classes and included propiconazole (Banner Maxx II: 14.3% A.I.), iprodione (Chipco 26 GT: 23.3% A.I.), chlorothalonil (Daconil WeatherStick: 54% A.I.), and the combination products TwinLine (12% pyraclostrobin and 7.4% metconazole) and Stratego (11.4% propiconazole and 11.4% trifloxystrobin). In the laboratory, the fungicides were incorporated into 1.2% water agar at rates that included and exceeded typical field rates. Clay-based microsclerotial granules were added to each Petri dish and incubated at 26 °C for 9 days before the number of viable spores produced was determined. In vitro, chlorothalonil was not detrimental to conidial production and viability at all concentrations (1-1000 mg a.i./l). Iprodione reduced conidial production at $\geq$ 100 mg a.i./l but had no effect on viability. Propiconazole reduced conidial production at $\geq$ 10 mg a.i./l and reduced viability at 1000 mg a.i./l. A pyraclostrobin + metconazole combination reduced conidial production at ≥ 10 mg a.i./l and viability at ≥ 100 mg a.i./l. A propiconazole + trifloxystrobin combination reduced conidial production at all concentrations and viability at ≥ 100 mg a.i./l.

Based on our laboratory findings, three fungicides were applied at two rates (propiconazole: 0.5 and 1.8 kg ai/ha; iprodione: 1.5 and 6 kg ai/ha; chlorothalonil: 2.2 and 8.6 kg ai/ha) to pots with creeping bentgrass in the greenhouse. Microsclerotia granules had been applied to the pots 1 day earlier. After 10, 20 and 30 days, the number of fungal colony forming units (CFUs) in the top 2.5 cm of soil and the grass was determined. Propiconazole suppressed colony forming unit (CFU) counts at both low and high rates; chlorothalonil and iprodione had no inhibitory effect on fungal growth. The number of CFUs recovered did not differ significantly 10, 20 or 30 days after treatment.

In conclusion, M. brunneum F52 microsclerotia sensitivity varied with fungicides and concentration, and the suppressive effects were less profound in soil than in vitro. Chlorothalonil and iprodione should be fully compatible with microsclerotia-based formulations of M. brunneum F52 in turfgrass. Propiconazole, on the other hand, may need to be applied some time before or after the application of the entomopathogenic fungus to minimize any suppressive effects. However, the benefit of separating the applications in time needs to be investigated in future studies. Our study provides baseline reference for the potential commercial use of the microsclerotia in pest control with regard to fungicide compatibility especially in turfgrass, which may require frequent fungicide applications. Future research may be directed to testing the compatibility of the microsclerotia granules with other fungicide groups, e.g., the succinate dehydrogenase inhibitors (SDHI), comparison between microsclerotial and conidial formulations in susceptibility to fungicides, and time intervals between fungus and fungicide applications to maximize the fungal activities in pest control.

Efficacy against ABW adults and larvae

In laboratory experiments, adult ABW were susceptible to *M. brunneum* F52 applied as microsclerotia. However, no significant effect on survival of ABW adults and larval population densities was observed in greenhouse experiments with grass when treated with microsclerotia.

In field experiments in 2017, 2018, and 2019, microsclerotia applied to target the mid-sized larvae of ABW in spring, provided no significant control (0 – 18% suppression) while the insecticide imidacloprid alone provided 27 – 45% control. In combinations of the fungus with imidacloprid, mortality was additive, resulting in 34 – 64% control. The addition of hydrogel in 2017 and 2018 did not significantly increase mortality rates. In 2019, a conidia-based liquid formulation of *M. brunneum* F52 was included in the field experiment at two rates and also combined with imidacloprid. The 2019 experiment was conducted at four golf courses simultaneously and the data combined for analysis. Imidacloprid alone gave 40% control. The low concentration of the conidial formulation resulted in 29% control alone and 55% control in combination with imidacloprid. The high concentration provided 51% control by itself and 70% in combination with imidacloprid. Mortality in all combination treatments was additive. It should be noted that imidacloprid applied in these combination treatments in spring would also control white grubs for the season.

The effect of all fungal treatments was likely somewhat limited by the relatively low temperatures during the experiments that were conducted in spring. Higher efficacy may be achieved when targeting ABW larvae in summer. However, the need for frequent fungicide applications on golf course turf during summer to suppress fungal turf diseases would make coordination of *M. brunneum* treatments with fungicides treatments to avoid negative interactions challenging.

Introducing the New Rutgers Hazelnut Cultivars Raritan, Monmouth, Somerset, and Hunterdon

Thomas J. Molnar, Emil Milan, and John Capik

Department of Plant Biology, Rutgers University

The Rutgers University nut tree breeding program was initiated by Dr. C. Reed Funk in 1996. The title of the project at the time was the "Underutilized Perennial Food Crops Genetic Improvement Program". Its early goal was to systematically explore the potential of a number of temperate nut tree species and then develop targeted breeding programs for those that held the most promise for New Jersey and the northeastern United States. Several years into the program, hazelnuts (*Corylus* spp.) clearly stood out above the other species, which included at the time black and Persian walnuts (*Juglans nigra* and *J. regia*, respectively), heartnuts (*Juglans ailantifolia*), pecans and hickories (*Carya* spp.), almonds (*Prunis dulcis*), and gingkoes. Beyond having a shorter generation time for breeding, hazelnuts proved well adapted to our climate, produced good early yields, had few pest or disease problems, and were already widely known as a low input, high value crop in regions of the world where they have been grown previously. Based on these points, it was our assumption that if farmers could grow local hazelnuts successfully, there would be a very strong market for them.

The stem canker disease eastern filbert blight (EFB) caused by *Anisogramma anomala* was identified as the primary reason hazelnuts had not been previously commercialized in the northeastern United States. Thus, identifying resistance to EFB became the early target of the breeding program, in addition to selecting for high yields of good quality kernels. By 2002, a significant focus was placed on hazelnuts in comparison to the other nut tree species and a sizable breeding program was initiated that included extensive germplasm collections from around the world. From these efforts, a wide germplasm base was assembled and many sources of EFB resistance were identified over the next decade (Capik and Molnar, 2012; Muehlbauer *et al*., 2014; Leadbetter *et al*., 2015, 2016; Molnar *et al*., 2018, 2019; Honig *et al*., 2019). This foundational material was used in systematic breeding, bolstered by a close relationship with the hazelnut breeding and genetics program at Oregon State University. On top of this, substantial support from the Rutgers Turfgrass Center and the New Jersey Agricultural Experiment Station, as well as a number of competitive grant opportunities including the USDA NIFA Specialty Crops Research Initiative, allowed for a sizable hazelnut breeding effort to be developed at Rutgers.

Currently, the program has shown significant progress in meeting the goals of developing well adapted, EFB-resistant hazelnut plants that produce high yields of excellent quality nuts. Presented here are the very first cultivars to be released from the program, named 'Raritan', 'Monmouth', 'Hunterdon', and 'Somerset'. All of these plants are pure European hazelnuts (*Corylus avellana*) that express a high level of tolerance or resistance to EFB. The source of disease resistance was carefully considered in these first releases, based on the knowledge that the pathogen is highly genetically diverse in the region (Muehlbauer *et al*., 2019). The first three are protected by horizontal resistance, also known as quantitative or multi-genic resistance, meaning that they are not immune to the pathogen, but highly tolerant. They are protected by multiple genes, which we hope will reduce selection pressure of the fungus to overcome the genetic resistance in these plants over time. This is a serious concern since hazelnut trees are expected to be planted in long-lived orchards of 35-50 years or more. The fourth cultivar (Somerset) is protected by a single *R*-gene from the Spanish cultivar 'Ratoli' (Sathuvalli *et al*., 2001). This gene has held up under high disease pressure in the field in New Jersey and Oregon for more than two decades, as well as in controlled inoculations with the pathogen collected from additional locations around the United States (Molnar *et al*., 2010, 2019; Capik and Molnar, 2012). To help manage the pathogen over time, it is suggested that the four new cultivars are planted together in equal proportions in the orchard. This strategy will present multiple resistance sources in one location, which should reduce selection pressure of the fungus for any single source. The cultivars were also selected to have similar nut and kernel characteristics, bloom phenology, and maturity dates to work well together in combination.

The first of these new trees, currently being multiplied through micropropagation/tissue culture, will be planted on test farms across the region in 2020 and become more widely available from commercial propagators in 2021. They represent the very first releases from the tree breeding program initiated by Dr. Funk and will be marketed under the Rutgers Landmark™ hazelnut series.

Capik, J. M., and Molnar, T. J. 2012. Assessment of host (*Corylus* sp.) resistance to eastern filbert blight in New Jersey. Journal of the American Society for Horticultural Science 137:157-172.

Honig, J. A., M.F. Muehlbauer, J.M. Capik, C. Kubik, J. N. Vaiciunas, S. A. Mehlenbacher, and T. J. Molnar. 2019. Identification and mapping of eastern filbert blight resistance quantitative trait loci in European hazelnut using double digestion restriction site associated DNA sequencing. Journal of the American Society for Horticultural Science 144: 295-304

Leadbetter, C.W., J.M. Capik, M. Pisetta, and T.J. Molnar. 2015. Sources of resistance to eastern filbert blight in hazelnuts from the Republic of Georgia. Scientia Horticulturae 193:269–275.

Leadbetter, C.W., J.M. Capik, S.A. Mehlenbacher, and T.J. Molnar. 2016. Hazelnut accessions from Russia and Crimea transmit resistance to eastern filbert blight. Journal of the American Pomological Society 70:92-109

Muehlbauer, M. F., J.A. Honig, J.M. Capik, J.N. Vaiciunas, and T.J. Molnar, T. J. 2014. Characterization of eastern filbert blight-resistant hazelnut germplasm using microsatellite markers. Journal of the American Society for Horticultural Science 139:399-432

Molnar, T.J., J.C. Goffreda, and C.R. Funk. 2010. Survey of *Corylus* resistance to *Anisogramma anomala* from different geographic locations. HortScience 45:832–836.

Molnar, T.J., J.J. Lombardoni, M.F. Muehlbauer, J.A. Honig, S.A. Mehlenbacher, and J.M. Capik. 2018. Progress breeding for resistance to eastern filbert blight in the eastern United States. Acta Hort 1226:79-85

Molnar, T.J., P. Engel, S.A. Mehlenbacher, and J. Capik. 2019. Multiple sources of eastern filbert blight resistance provide breeding utility in New Jersey. Journal of the American Pomological Society 73: 178-192 2019

Sathuvalli, V.R., H.L. Chen, S.A. Mehlenbacher, and D.C. Smith. 2011. DNA markers linked to eastern filbert blight resistance in 'Ratoli' hazelnut. Tree Genetic and Genomes 7:337–345.

Physiological Effects of Aminoethoxyvinylglycine on Improving the Heat Tolerance of Creeping Bentgrass

Stephanie Rossi and Bingru Huang

Department of Plant Biology, Rutgers University

Heat stress is a major factor contributing to summer bentgrass decline in creeping bentgrass (*Agrostis stolonifera* L.), a cool-season turfgrass susceptible to premature leaf senescence when exposed to elevated temperatures for prolonged periods. The objective of this study was to determine the physiological effects of the ethylene inhibitor, aminoethoxyvinylglycine (AVG), on leaf senescence in creeping bentgrass exposed to heat stress. Plants were maintained in environmentally-controlled growth chambers under non-stress (22/18 ^{o}C, day/night) or heat stress (35/30 ^{o}C, day/night) temperature conditions for 25 d, and turf quality, electrolyte leakage, and chlorophyll content were measured. Plants were foliar sprayed with AVG prior to and during heat stress. At 25 d of heat stress, all treated plants had significantly higher levels of chlorophyll *a* and *b* as well as total chlorophyll content in comparison to the untreated controls. Electrolyte leakage was affected differentially by AVG under heat stress. The current results suggest that application of AVG may have suppressed leaf senescence and improved membrane stability.

Koch's Postulates for Gray Leaf Spot Disease on Hard Fescue (*Festuca brevipila*)

Phillip L. Vines, Ryan M. Daddio, Ruying Wang, Jing Luo, Ning Zhang, Bruce B. Clarke,
Ronald F. Bara, Dirk A. Smith, William A. Meyer, and Stacy A. Bonos

Department of Plant Biology, Rutgers University

Gray leaf spot, caused by the fungal pathogen *Pyricularia oryzae*, is a serious disease of perennial ryegrass (*Lolium perenne*) and tall fescue (*Schedonorus arundinaceus*) cool-season turf in the United States. Initial disease symptoms include small, water-soaked lesions on leaf blades that develop into dark, necrotic spots. The infections expand into circular to oblong lesions and become gray to grayish-brown with dark brown to purple borders. As the disease progresses, lesions coalesce and cause tip blighting, which often leads to twisting or flagging of leaf blades. These characteristic gray leaf spot symptoms were observed in a hard fescue (*Festuca brevipila*) variety trial at the Rutgers turfgrass research farm in Adelphia, NJ and in a seeded fine fescue trial at the Horticultural Research Farm number 2 in North Brunswick, NJ in October 2018. Thus, the objective of this study was to conduct Koch's postulates to determine if *P. oryzae* was the causal organism of gray leaf spot symptoms in hard fescue.

Samples of fine fescue turf exhibiting symptoms of gray leaf spot disease were collected from field trials at Adelphia, NJ and North Brunswick, NJ during October 2018. Leaves with symptomatic lesions were surface disinfested, dried in a laminar flow hood, placed on diluted-strength potato dextrose agar (PDA), and incubated at 25 C. Apical tips of hyphal strands were subcultured on standard PDA to purify fungal cultures. Fungal isolates were identified based on morphological assessments and phylogenetic analyses of genomic DNA.

Two single-conidium isolates of *Pyricularia oryzae*, one from Adelphia, NJ (D300452) and one from North Brunswick, NJ (181001P12) were used for pathogenicity studies. Inoculum was prepared for each isolate with the final conidial concentration adjusted to 4×10^4 conidia mL^{-1}. Two hard fescue cultivars ('Beacon' and 'Reliant IV') were seeded into 3.8 cm diameter conetainers and maintained with daily irrigation and biweekly fertility to encourage growth. The plants were established in light- and temperature-controlled growth chambers for 3 weeks prior to inoculation with daytime conditions at 29 C and 90% relative humidity and nighttime conditions at 22 C and 90% relative humidity. For inoculation, 1 mL of Tween 20 was added to the conidial suspension and the solution was sprayed onto the plants until runoff; control plants were sprayed with sterile-distilled water and Tween 20 in a similar manner. Inoculated and control plants were placed into clear bins within the growth chambers for physical separation. After inoculation, the plants were maintained for 3 weeks with daytime conditions at 29 C and 95% relative humidity and nighttime conditions at 22 C and 95% relative humidity.

Treatment factors in this study were fungal isolate and hard fescue cultivar; there were three levels of fungal isolate (D300452, 181001P12, and untreated control) and two levels of hard fescue cultivar (Beacon and Reliant IV). The treatments were arranged as a 3 × 2 factorial in a completely randomized design with four replications for each treatment combination. Conetainers were repositioned three times per week within an isolation bin to increase homogeneity of environmental conditions within each growth chamber. The experiment was repeated in a second growth chamber. Disease evaluations included visual assessments of gray leaf spot disease incidence and severity. Incidence was scored as a count of the number of gray leaf spot lesions on all leaves within a given conetainer. Severity was scored using a 0 to 10 rating scale where 0 represented no gray leaf spot disease symptoms present and 10 represented 100% gray leaf spot diseased plant tissue. Data were subjected to analysis of variance using the GLIMMIX procedure of SAS, Version 9.3. After plants were evaluated for disease symptoms, leaf samples were collected for reisolation of the pathogen.

Six fungal isolates were obtained from gray leaf spot disease symptoms on hard fescue turf during Oct. 2018; four isolates were from North Brunswick, NJ and two isolates were from Adelphia, NJ. All isolates were identified as *P. oryzae*, based on morphological and phylogenetic investigations. Inoculation with *P. oryzae* resulted in gray leaf spot disease symptom development at 7 days after inoculation. Mean disease incidence was significantly higher for plants inoculated with *P. oryzae* compared to the non-inoculated control plants. Furthermore, disease incidence for isolate D300452 was significantly higher than isolate 18100P12 in one replication of the study (Figure 3A). These findings suggest there may be a difference in virulence among isolates of *P. oryzae*. Similarly, mean disease severity was significantly higher for inoculated plants compared to non-inoculated controls. Likewise, in one replication of this study, isolate D300452 incited greater disease severity than isolate 18100P12. Again, this evidence indicates a difference in virulence for the isolates of *P. oryzae* evaluated in this study. At 3 weeks after inoculation, hard fescue seedlings inoculated with either *P. oryzae* isolate were devastated with gray leaf spot disease symptoms while the non-inoculated control seedlings were healthy in appearance.

Koch's postulates were fulfilled by morphological evaluations and DNA sequencing of the reisolated fungi. This study confirms that *P. oryzae* can incite gray leaf spot disease on hard fescue. In addition, given the historical significance of gray leaf spot disease in other turf species, this study suggests that gray leaf spot could be a major concern for managing hard fescue turf in the future. Future work will include continuing to monitor hard fescue turf for gray leaf spot disease symptoms across a larger geographic area and collect isolates of *P. oryzae* from symptomatic turf, conducting additional studies to better understand the apparent difference in virulence between isolates D300452 and 18100P12 as well as other isolates of *P. oryzae*, and evaluating breeding lines to select for improved resistance to gray leaf spot in hard fescue.

Inheritance of Summer Patch Disease Resistance in Hard Fescue

Daniel S. Wu, Austin L. Grimshaw, Henry Y. Qu, Phillip L. Vines, Eric N. Weibel,
William A. Meyer, and Stacy A. Bonos

Department of Plant Biology, Rutgers University

Hard fescue (*Festuca brevipila* Tracey) is a cool-season turfgrass known for exceptional performance under low-maintenance conditions. The widespread application of fine fescue is hindered by summer patch disease. Summer patch is a root disease and has been long known caused by *Magnaporthiopsis poae*. Recently, *Magnaporthiopsis meyeri-festucae* has been identified as another causal pathogen of summer patch. The objective of this study was to investigate the inheritance of summer patch resistance in fine fescue populations. The experimental populations were created by crossing three summer patch resistant parents and three susceptible parents in a diallel crossing design. In the spring of 2018, 100 progenies from each of the 15 crosses and reciprocals were established in a mowed spaced-plant trial. All populations, as well as selected parental genotypes, were arranged in a randomized complete block design with four replications. Three cubic centimeters of summer patch inoculum was placed under all plants prior to planting with a mixture of both an *M. meyeri-festucae* isolate (SCR9) and an *M. poae* isolate (C11). This procedure was repeated in the spring of 2019. Visual rating of disease severity and disease incidence, as well as corresponding normalized difference vegetation index (NDVI) were assessed during summer 2018 and 2019. According to the data collected in 2018, the narrow-sense heritability estimates based on mid-parent progeny regression analysis were (0.56 ± 0.03) by visual rating data and (0.37 ± 0.03) by NDVI data.

Dollar Spot Control as Affected by Fungicide Programming and Bentgrass Susceptibility

Pingyuan Zhang, Kyle Genova, Bruce B. Clarke, and James A. Murphy

Department of Plant Biology, Rutgers University

The severity of dollar spot (caused by *Clarireedia jacksonii*) differs among bentgrass (*Agrostis* spp.) cultivars. A 3 × 6 factorial arranged as a randomized complete block design was used to assess the effectiveness of threshold-based fungicide applications to control dollar spot on six cultivars: 'Capri' colonial bentgrass (*A. capillaris*), and 'Declaration', '007', 'Shark', 'Penncross' and 'Independence' creeping bentgrass (*A. stolonifera*). A calendar-based program (21-d interval, nine applications) and two threshold programs that applied fungicide at a damage threshold (314-mm^2 across four replications in 2018 and 471-mm^2 across six replications in 2019) within either 24-h or the next spray-day (Monday) were evaluated. Disease severity was measured every 1 to 3 days as the active dollar spot infection area within each plot and summarized as the area under disease progress curve for disease outbreaks that exceeded the disease threshold. Both threshold programs reduced the number of fungicide applications compared to the calendar-based program. The number of fungicide applications were further reduced on cultivars that were less susceptible to dollar spot. Declaration received 2 to 3 threshold fungicide applications while 5 to 6 applications were applied on Independence. Fungicide program was the dominant factor that influenced the dollar spot outbreaks in both years; 21 disease outbreaks occurred on threshold treatments while no outbreaks occurred on calendar-based treatments. When differences between threshold programs were apparent, outbreaks were more severe for the next spray-day program. Disease outbreaks on plots treated with threshold fungicide programs depended on the cultivar factor during 13 of the 21 outbreaks and indicated that disease outbreaks occurred when the environment conditions were conducive to disease and a given cultivar was no longer protected by fungicide (usually more than 21-d after previous application). Declaration, 007, Capri, Shark, Penncross and Independence ranked among the cultivars (either as a main effect or an interaction with fungicide program) with the most severe disease during 1, 1, 5, 6, 7, 10 of the 21 outbreak periods, respectively. Thus, a cultivar with higher susceptibility was more likely to sustain the most severe disease during outbreaks.