

Symposium Organizing Committee

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Proceedings of the Thirty-First Annual Rutgers Turfgrass Symposium

Faith Belanger and Barbara Fitzgerald, Editors

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Associate Director's Remarks:

Welcome to the Thirty-First Annual Rutgers Turfgrass Symposium. The Symposium was established in 1991 to provide an opportunity for Rutgers faculty, staff and students to discuss current research and share ideas on a broad range of topics in turfgrass science. This year, I would like to extend a special welcome to our keynote speaker, Mark Farman (Department of Plant Pathology, University of Kentucky) who will present a seminar on 'Evolutionary genetics of *Pyricularia oryzae*'. I would also like to extend a warm welcome and thank you to our invited speakers this year, Dana Sullivan (Turf Scout), Peter Oudemans (Department of Plant Biology, Rutgers University), Thierry Besancon (Department of Plant Biology, Rutgers University), and Eric Patterson (Department of Plant and Soil Sciences, Michigan State University) and all of the turf center faculty and students who have agreed to present their research at this year's symposium. I would also like to thank our session moderators Dr. Phillip Vines, Dr. Rong Di, Mr. Brad Park, and Dr. Faith Belanger and the Symposium Planning Committee, Dr. Phillip Vines (Symposium Committee Chair), Rong Di, Jim Murphy, Stacy Bonos, Stephanie Rossi (Graduate Student Member), and Dr. Faith Belanger and Ms. Barbara Fitzgerald (Co-Editors of the Symposium Proceedings) for their hard work in planning this year's symposium. We also appreciate the technical support of Mr. Bernard Ward, Deborah Andriano, Phil Wisneski and Becky Rathmill for their help with live streaming and website postings. Without their efforts, the symposium would not be possible.

Faculty, staff and students in the turfgrass science program have been recognized nationally for their excellent research. This year, five graduate students were recognized at the National Crop Science Society Annual Meeting in Salt Lake City, UT. William Errickson won first place in the Genetics Oral presentation category in the Division of Turfgrass Science. He also won first place in the Oral presentation category in the Division of Crop Physiology and Metabolism. Cathryn Chapman won second place in the Genetics Oral presentation category in the Division of Turfgrass Science. Brandon McNally won second place in the Turf Oral presentation category in the Division of Turfgrass Science. Stephanie Rossi won third place in the Genetics Poster presentation category and Zhongqi (Mercer) Xu won third place in the Golf Poster presentation category in the Division of Turfgrass Science. Brandon McNally also was selected for the Outstanding Graduate Student Award (MS) from the Northeastern Weed Science Society.

This year, Dr. Bingru Huang was appointed Editor-in-Chief for the Crop Science Society of America. Bingru will have editorial oversight of 9 journals that are published by the society and its affiliates. This year also marks the retirement of Dr. Bruce Clarke, who provided excellent leadership to the Center for Turfgrass Science at Rutgers University for thirty years. In recognition for his leadership and commitment of service to the turfgrass industry, the New Jersey Turfgrass Foundation announced the initiation of the Bruce B. Clarke Endowed Graduate Fellowship, which will support a graduate student in turfgrass science. This endowment requires significant monetary support which the turfgrass industry is committed to support. This is a testament to the amazing

relationship that Rutgers turfgrass faculty have with the turfgrass industry in the state and beyond. We are fortunate for the unique partnership we have with the turfgrass industry and are grateful for the support they provide to the turfgrass program at Rutgers University. We are glad that you have decided to spend the day with us to discuss the exciting topics in turfgrass science presented at today's Symposium!

Sincerely,

Stacy A. Bonos, Associate Director

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THIRTY-FIRST ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

March 17, 2022

Institute for Food, Nutrition, and Health Building, Room 101

- 8:30 - 9:00 AM** **Registration**
- 9:00 AM** **Welcome – Laura Lawson** (*Interim Executive Dean of Agriculture and Natural Resources*)
- 9:10 - 10:40 AM** **SESSION I: Remote Sensing Research and Perspectives of Turfgrass Breeding** (Moderator: Phillip Vines)
- 9:10 - 9:30 **William Meyer** (*Department of Plant Biology, Rutgers University*)
A Perspective on Breeding Cool-Season Turfgrasses at Rutgers University for the Past 60 Years
- 9:30 - 9:50 **Dana Sullivan** (*Turf Scout*)
Remote Sensing for Turfgrass Research
- 9:50 - 10:10 **Peter Oudemans** (*Department of Plant Biology, Rutgers University*)
Applications for Remote Sensing in Cranberry Crop Management
- 10:10 - 10:40 AM** **Discussion and Break**
- 10:40 - 12:00 PM** **SESSION II: Molecular Genetics Approaches to Study Turfgrass Pathogens** (Moderator: Rong Di)
- 10:40 – 11:00 **Glen Groben** (*Department of Plant Biology, Rutgers University*)
Quantifying the Concentration of *Clariireedia* in the Field for Dollar Spot Susceptible and Tolerant Creeping Bentgrass Cultivars
- 11:00 – 11:20 **Ning Zhang** (*Department of Plant Biology, Rutgers University*)
Pyricularia oryzae and Related Fungi
- 11:20 - 12:00 **KEYNOTE: Mark Farman** (*Department of Plant Pathology, University of Kentucky*) Gray Leaf Spot of Perennial Ryegrass is a Recently-Emerged Disease with a Complex Evolutionary Origin that was Intimately Intertwined with that of Another Recently Emerged Disease – Wheat Blast
- 12:00 – 1:00 PM** **Lunch Break**

- 1:00 – 2:00 PM** **SESSION III: Weed Genomics and Management**
(Moderator: Brad Park)
- 1:00 – 1:20 **Matthew Elmore** (*Department of Plant Biology, Rutgers University*)
Influence of Phosphorus on Annual Bluegrass Competitiveness in
Creeping Bentgrass
- 1:20 – 1:40 **Thierry Besancon** (*Department of Plant Biology, Rutgers University*)
Ecology and Approaches to Weed Control on Carolina Redroot
- 1:40 – 2:00 **Eric Patterson** (*Department of Plant, Soil and Microbial Sciences, Michigan
State University*) Developing Molecular Tools for Resistance Trait Discovery
and Diagnostics in Turfgrass Systems
- 2:00 – 2:30 PM** **Discussion and Break**
- 2:30 – 3:50 PM** **SESSION IV: New Approaches for Turfgrass Stress Management**
(Moderator: Faith Belanger)
- 2:30 – 2:50 **Patrick Fardella** (*Department of Plant Biology, Rutgers University*)
Purification and Activity of the *Epichloë festucae* Antifungal Protein
- 2:50 – 3:10 **James White** (*Department of Plant Biology, Rutgers University*)
Endosymbiotic Bacteria in Cells of Grasses Enhance Nutrient Status,
Growth, Abiotic Stress Tolerance and Pest Resistance of Hosts
- 3:10 – 3:30 **Albrecht Koppenhöfer** (*Department of Entomology, Rutgers University*)
Native Persistent Entomopathogenic Nematodes for Long-Term Insect
Pest Suppression
- 3:30 – 3:50 **Cathryn Chapman** (*Department of Plant Biology, Rutgers University*)
Interaction of Elevated Carbon Dioxide and Drought Stress on the Recovery of
Three Cool-Season Turfgrass Species
- 3:50 – 4:30 PM** **Discussion Session and Closing Remarks**

PLENARY PRESENTATIONS

A Perspective of Breeding Cool-Season Turfgrasses at Rutgers University for the Past 60 Years

William A. Meyer, C. Reed Funk Professor of Turfgrass Breeding

Department of Plant Biology, Rutgers University

Researchers have been involved in breeding cool-season turfgrass species at Rutgers University (School of Environmental and Biological Science) and the New Jersey Agricultural Experiment Station (NJAES) for the past 60 years (Meyer and Funk, 1989). In 1961 Dr. C. Reed Funk initiated the program with a broad breeding goal to develop improved, widely adapted pest and stress-tolerant cultivars to be planted throughout the world. Dr. Funk collected 1000's of turfgrass germplasm sources from throughout the USA. These collections of turfgrasses from old turfs have created the genetic base for improved cultivars that are available today.

In 1996, the leadership of this program was transferred to Dr. William A. Meyer. A new emphasis was initiated to continue the program with an additional push for expansion of the germplasm base by collecting from old turf areas in the centers of origin (mostly throughout Europe) for nine cool-season species. In the past 25 years, over 13,293 collections out of 42,813 total collections from 52 countries have been screened at Rutgers to be used for integration of the germplasm pool of the different turfgrass species. The Rutgers breeding program has collaborated and provided leadership in releasing over 500 improved turfgrass cultivars that exhibit superior quality characteristics. Collaboration with seed companies and universities has helped to develop cultivars that are superior to older standard cultivars with a new emphasis placed on new cultivars that have higher seed yields and floret fertility that will benefit seed farmers.

At Rutgers, the breeders were fortunate to have two research farms in the upper US transition zone with different soil types. In Freehold, NJ the 200-acre Adelphia farm has sandy loam soil and at HortFarm II in North Brunswick, NJ it is in the piedmont and has Nixon sandy loam. Both of these farms have supplemental irrigation and are ideal sites to evaluate turfgrass germplasm in turf plots and mowed and unmowed spaced plants. It is possible to screen for heat, drought, disease, and insect tolerance with the warm and humid summers. The process of cultivar development involves extensive exchange and testing each of generations of genetic resources between our collaborators and Rutgers breeders. The objective is to have a continual supply of advanced turfgrass germplasm to be developed cooperatively and released by the collaborating turfgrass seed companies.

At Rutgers, we have over 40,000 turf plots seeded and being evaluated each year. The species being developed include Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), and tall fescue [*Schedonorus arundinaceus* (Shreb) Dumort]. A program has also been carried on for fine fescues including strong creeping red fescue (*Festuca rubra* L. subsp. *arenaria* (Osbeck), Chewings fescue (*Festuca rubra* L. subsp. *commutata* Markgr. Dann.), hard

fescue (*Festuca brevipilia* R. Tracey), and slender creeping red fescue [*Festuca rubra* subsp. *litoralis* (G. Meyer) Auguier]. Since 1996, a new emphasis has been put on bentgrass species for the golf industry. The three species developed were creeping bentgrass (*Agrostis stolonifera* L.), colonial bentgrass (*Agrostis capillaris* L.), and velvet bentgrass (*Agrostis canina* L.).

KENTUCKY BLUEGRASS

Until the late 1970's Kentucky bluegrass was the leading turf species with over 150 -180 million pounds of seed produced in North America each year. Today the production is around 60-70 million pounds (Paul Hedgepeth, personal communication). The main reason this production is reduced results from competition from other species. Dr. Funk always referred to Kentucky bluegrass as the Cadillac of the turfgrasses. This is a unique turfgrass species in that it reproduces through an asexual process called apomixis. With this process, clones are produced through seed generations. The breeders at Rutgers were able to make sexual crosses through a greenhouse approach by pollinating different strains between midnight and 4:00 AM when the flowers were found to open up (Pepin and Funk, 1971).

At Rutgers, many Kentucky bluegrasses seedlings are grown out in the greenhouse and each spring approximately 40,000 are planted in spaced plant nurseries to grow about 13 months. Selections showing a combination of improved density, freedom from disease, and high seed head number are identified. Grow out tests are conducted with a pinch of seed from each selection. Those lines that demonstrate a uniform progeny are used to start a 48-plant uniformity trial as spaced plants. It is desirable to have an 85% or higher apomixis level with weak off-types for a commercially acceptable cultivar. Lines are not sent out to collaborators until 2-3 years of testing in turf. The most promising lines are sent out to Northwestern US cooperators for seed production trials. Approximately 10-15 lines are selected each year for advanced trials. This whole process can take over 9 years. In Table 1 (Morris, 2020), the turf quality ranking of the cultivars from Rutgers is compared to the standard cultivars in the 2017 national turfgrass evaluation program (NTEP). Rutgers has a proud history in bluegrass breeding from their release of Midnight in the 1980s (Meyer et al., 1984). It is still ranked in the top 25% of most Kentucky bluegrass turf trials today.

PERENNIAL RYEGRASS

There were no true turf-type perennial ryegrasses available until the late 1960s when Dr. Funk released Manhattan perennial ryegrass in 1967 (Funk et al., 1969). This cultivar resulted from collections made from old turf in Central Park in New York City. They were 16 surviving clones that had survived many years in mowed turf. This cultivar started a turfgrass seed industry on perennial ryegrass that reached a high of 200 million pounds per year in the early 2000s and is still somewhere between 150-180 million pounds (Paul Hedgepeth, personal communication) per year (Meyer and Funk, 1989). One of my first cooperative projects with Dr. Funk was when I was a private breeder in Oregon. It was a project to improve genetic stem rust resistance

(*Puccinia graminis* Pers.) in seed production fields of perennial ryegrass. The first successful collaborative project resulted in Manhattan II perennial ryegrass with improved stem rust resistance (Funk et al. 1984).

Perennial ryegrass has become a widely used species in northern turf mixtures and used to overseed dormant bermudagrass in southern areas of the US. In the 1990s gray leaf spot (*Pyricularia oryzae* Cooke, Sacc.) became a major disease of perennial ryegrass. One of the great achievements of the Rutgers breeding program was the discovery of gray leaf spot resistance in 17 lines of perennial ryegrass from Rutgers sources and 17 from recent European collected sources (Bonos et al., 2004). These sources have been incorporated into the Rutgers adapted germplasm and resulted in many gray leaf spot resistant cultivars that are being marketed. In Table 2 are the top 13 cultivars across 17 locations (Morris, 2020) in the 2016 NTEP perennial ryegrass trial (114 entries) developed with our collaborators in the Rutgers breeding program. We are pleased that we have been able to maintain a continued effort in supplying our collaborators with improved perennial ryegrass germplasm for cultivars.

At Rutgers, there have been efforts to select perennial ryegrass cultivars with improved salinity tolerance. Because of water quality issues, especially in arid regions, and the availability of water, there is a demand for salinity tolerance. When Dr. Matt Koch was a graduate student of Dr. Stacy Bonos, he developed a greenhouse salinity technique using overhead watering with salt water. He confirmed these results with field studies using salt water applied in the field of mowed spaced plants (Koch and Bonos, 2010, 2011). Dr Bonos' students have developed other species to be more tolerant to salinity. A number of tall fescues and slender creeping red fescues have resulted from this work.

TALL FESCUE

Until 1980, the only tall fescue used widely was Kentucky 31(Ky-31), which was found growing on a hillside in Kentucky that was seeded originally from European sources. It was released in the early 1940s for mostly forage and some for turf usage. There have been quantities of Ky-31 produced (mostly in Missouri) in the 1970s-1990s from 70-120 million pounds per year. At this time, there are still 20-40 million pounds (Paul Hedgepeth personal communication) of Ky-31 produced each year (Funk and Meyer, 1989).

Rebel tall fescue was released in the 1980s (Funk et al., 1981), which started the new turf-type seed industry that has grown today to 160-190 million pounds per year (Paul Hedgepeth, personal communication). The project that resulted in Rebel took 18 years of improvement work. Rebel is considered a landmark cultivar producing a finer, denser turf with a slower rate of vertical growth, brighter darker green color, and tolerance to shade and wear (Funk and Meyer, 2001). Since Rebel was developed, there has been a continual release of new improved tall fescues with Rutgers cooperators. In Table 3 are the top 15 cultivars in the 2018 NTEP tall fescue trial out of 132 that were all developed with participation of the Rutgers breeding program and various seed companies.

The level of leaf spot (*Drechslera dictyoides*) resistance has been improved in the last 15-20 years in the new tall fescue cultivars. The major diseases in tall fescue have been brown patch

(*Rhizoctonia solani* Kuhn) for over 40 years and more recently gray leaf spot, especially in new seedlings (Watkins et al., 2009). There has been a continual improvement in brown patch of tall fescue but it still needs further improvement. There have also been improvements in gray leaf spot resistance. Stem rust is a very serious disease of tall fescue in seed fields in the northwest US. At Rutgers, there has been an effort to improve the resistance by screening mature clones and maintaining them vegetatively to use for further breeding work in the next season (Meyer et al. 2017).

Another advancement in tall fescue research at Rutgers has been the construction of a rain-out shelter. By selecting a few percent of the most tolerant lines each generation of testing, it has been possible to maintain green turf in tall fescue with very limited water in the New Jersey summer. The recent drought test coordinated by NTEP has shown that selections like RS4 tall fescue have had the top performance in drought compared to other entries (Morris, 2020).

FINE FESCUES

The fine fescue species are an underutilized group of cool-season species. The three primary species being improved at Rutgers are Chewings, strong creeping red fescue, and hard fescue. There has been one cultivar of slender creeping fescue developed at Rutgers. One important trait of fine fescues is that many sources contain the *Epichloë festucae* fungal endophyte (Funk and White 1997). The presence of the maternally borne endophyte can control above ground feeding insects and dollar spot disease in fine fescues.

The most popular fine fescue species in the USA is strong creeping fescue, which has an annual production of 7-10 million pounds in the United States and 45-60 million in Canada; this is mostly the cultivar Boreal (Paul Hedgepeth, personal communication). The strong creepers are popular because they have thick rhizomes like Kentucky bluegrass and can be maintained for many years in seed production fields and help the turf recover after hot summers. Chewings is a bunch type fescue that can be mowed closely and used for golf course fairways and bowling greens. Each year there are 7-10 million pounds of seed produced in North America (Paul Hedgepeth, personal communication). Hard fescue usually has top turf performance and disease resistance. The results of the 2014 NTEP fine fescue turf trial in Table 4 show that the hard fescues were the top turf performers from 2015 to 2019 (Morris, 2019). Unfortunately, only 450,000 pounds of hard fescue are produced each year (Paul Hedgepeth, personal communication). Because of the low volumes of fine fescues, there is less interest in breeding these species. At Rutgers, we continue to breed these species because of their low maintenance potential and the need to increase seed production (Bonos and Huff, 2013).

BENTGRASSES

The golf course industry relies heavily on creeping bentgrasses for greens, tees, and fairways in the cool-season growing areas. The cultivar Pennncross is a seeded creeping bentgrass that was developed at Penn State University; since the 1950s it has been planted on many golf courses. At Rutgers, the cultivars Cobra (Engel et al., 1994) and Lofts L-93 were developed later and are available as seed. In the 2014 NTEP trial (Tables 4 and 5) 5 creeping bentgrasses were the top 5

entries (Morris, 2020) in the putting grass trial and Penncross was number 20 out of 20. In the fairway trial, the top 3 entries were creeping bentgrass and were significantly better than Penncross. All of these cultivars were developed by Dr. Stacy Bonos from Rutgers and her cooperators in the Northwestern US. The greatest impact has been from developing dollar spot (*Clarireedia* spp.) resistance in cultivars (Bonos et al., 2003). Dollar spot is the major disease in creeping bentgrass. The pounds of creeping bentgrass produced each year is less than most other species but the price per pound is higher.

Colonial and velvet bentgrass are both valuable species with lower volumes than creeping bentgrass but better dollar spot resistance. The major limitation of colonial bentgrass is brown patch disease with high demand for improved cultivars. Velvet bentgrass is a high-quality putting green grass but requires very careful maintenance. This species is resistant to both dollar spot and brown patch but not widely accepted by the golf industry.

SUMMARY

At Rutgers University there has been a lot of progress over the last 60 years to improve cool-season turfgrass species (over 500 cultivars released with our collaborators). The wide germplasm collection from the centers of origin of the cool-season species for the last 26 years is a major asset for Rutgers. The key to the success of this program has been the excellent collaboration with corporations and other organizations. One important aspect of being at Rutgers was the progress and growth of the 11 graduate students under my direction at Rutgers. I have really enjoyed my 26 years in New Jersey at Rutgers as the Director of the 2 research turf farms and the Turfgrass Breeding Project. Dr. Stacy Bonos was named as the Director of the Adelphia research farm and Turfgrass Breeding Project January 1, 2021.

**TABLE 1 2017 NTEP Kentucky Bluegrass 2020 Data in 12 Locations with 89 Entries.
Maintained at Medium Maintenance.**

<u>Rank</u>	<u>Entry*</u>	<u>TQ</u>
1	Starr	6.3
2	Bombay	6.3
3	Cloud	6.2
4	PPG-K6-1304	6.1
5	Skye	6.1
9	PPG-KB-1131	6.0
13	Syrah	5.9
14	Midnight	5.9
17	All-26	5.8
20	DLF-340/3550	5.8
21	A99-2897	5.8
22	DLF-340/3500	5.8
25	All-40	5.8
26	A10-280	5.8
88	KenBlue	4.8
89	Blue Knight	4.8
LSD		0.2

***Numbers of top 26 refer to rank in Turf Quality. All varieties listed in top 26 were developed with participation of the Rutgers Turfgrass Breeding Program**

TABLE 2 2016 NTEP Perennial Ryegrass Trial in 17 Locations with 114 Entries.

<u>Rank</u>	<u>Entry*</u>	<u>Mean Quality</u>	<u>Max % in top 25</u>
1	PPG-PR-421	6.3	88.2
2	NP-2	6.1	64.7
3	NP-3	6.3	64.7
4	Mystique	6.2	64.7
5	Slugger 3GL	6.1	58.8
6	LDLFPS 236/3546	6.2	58.8
7	PPG PR-372	6.2	58.8
8	Zoom II	6.2	58.2
9	Apple 3GL	6.1	58.0
10	Furlong	6.3	52.9
11	JR 97	6.1	52.9
12	Alloy	6.2	52.9
13	Stellar 3GL	6.2	52.9
114	Linn Standard	3.1	0.0
LSD Value		0.3	

***Top 13 varieties developed with participation of the Rutgers Turfgrass Breeding Program**

TABLE 3 2018 Tall Fescue NTEP Trial in 28 Locations with 32 Entries. Percentage of Time Entry in Top 25% out of 132 Entries.

<u>Rank</u>	<u>Entry*</u>	<u>TQ</u>	<u>Max % in top 25</u>
1	Titanium GLS	6.4	68.2
2	RH3	6.3	63.6
3	Spyder 2LS	6.4	59.1
4	K18-RS6	6.4	69.1
5	AH2	6.4	59.1
6	Bonfire	6.3	59.1
7	AH1	6.3	54.5
8	PPG-TF323	6.4	54.5
9	DLFPS 321/3705	6.4	54.5
10	PPG-TF 267	6.3	54.5
11	Raptor LS	6.2	54.5
12	PPG-TF 338	6.3	50.0
13	Stealth	6.3	50.0
14	DLFPS 321/3705	6.4	50.0
15	PPG TF 262	6.3	50.0
132	Ky 31 Standard	4.2	0.0
LSD Value		0.2	

*Top 15 varieties developed with participation of the Rutgers Turfgrass Breeding Program

TABLE 4 2014 NTEP Fine Fescue Trials Turfgrass Quality Data 2015-2019 in 25 Locations with 42 Entries.

Rank

	<u>Species</u>	<u>Entry*</u>	<u>TQ</u>	<u>Max % in top 25</u>
1	Hard	Gladiator	5.7	66.7
2	Hard	DLFPS TL/3066	5.6	66.7
3	Hard	Resolute	5.6	58.3
4	Hard	MNHF-14	5.5	58.3
5	Hard	Beacon	5.4	58.3
6	Chewings	DLF-PS Trc/3338	5.6	50.0
7	Hard	Jetty	5.6	50.0
8	Hard	DLFPS FL/3066	5.5	50.0
36	Chewings Cascade Standard		4.8	0
44	Strong Creeper Boreal Standard		4.2	0

***All varieties developed with participation of the Rutgers Turfgrass Breeding Program**

TABLE 5 2014 Creeping Bentgrass NTEP Putting Greens Trials in 19 Locations with 20 Entries

<u>Rank</u>	<u>Entry*</u>	<u>TQ</u>	<u>Max % in top 25</u>
1	Tour Pro (GDE)	6.4	64.3
2	Piranha (DC-I)	6.5	64.3
3	L93-XD	6.5	57.1
4	DLFPS AP/3058	6.5	50.0
5	McDonald	6.4	50.0
20	Penncross	4.4	0
LSD		0.3	

***Top 5 varieties developed with participation of the Rutgers Turfgrass Breeding Program**

TABLE 6 2014 Fairway NTEP Bentgrass Trial Results from 2015-2019 in 17 Locations with 17 Entries.

<u>Rank</u>	<u>Entry*</u>	<u>TQ</u>	<u>Max % in top 25</u>
1	Piranha	6.6	70.0
2	Chinook	6.5	60.0
3	007	6.4	60.0
17	Penncross STD	4.4	
LSD		0.3	

***All varieties developed with participation of the Rutgers Turfgrass Breeding Program**

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Remote Sensing for Turfgrass Research

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Remote sensing in its simplest form is defined as the acquisition of information about an object without coming into contact with that object. More specifically, as applied to living vegetation, remote sensing has been used to measure the amount of energy reflected from a plant canopy. This measurement provides some information about how well the plant is utilizing and dissipating irradiant energy, most commonly for photosynthesis. Remote sensing of turfgrass encompasses a wide range of tools ranging from ground based (handheld or vehicle mounted), drones, manned aircraft and satellites. This presentation will explore the foundational concepts that drive the interpretation of remotely sensed data and the technological advancements that have buoyed remote sensing and digital image analyses in turfgrass research.

Remote sensing of turfgrass canopies is a measurement of reflected or emitted light energy from the surface of the turfgrass canopy. From a plant perspective, irradiant energy from the sun can be reflected, absorbed or transmitted. Absorbed energy may be utilized for photosynthesis, emitted as long-wave energy (sensible heat flux from an energy balance perspective) or released via transpiration (latent heat flux). Thus, measurements of reflected (short-wave or emitted energy (long-wave) are indicative of how efficiently the turfgrass canopy is utilizing energy from the sun. Typically, living vegetation will absorb visible light energy and is highly reflective in the near-infrared region (NIR). Near-infrared (NIR) reflectance is most affected by biomass production, canopy geometry, and consequent light scattering (Hatfield et al. 2008, Sullivan et al. 2007). Alternatively, visible spectra are correlated with energy absorption for photosynthesis and generally exhibit a much smaller range in reflectance compared to the NIR.

Capturing reflected or emitted (long-wave) energy from a turfgrass canopy has evolved significantly over the past 20 years. While many of the earliest ground sensors are still in use today, practitioners now have access to drones, multispectral sensors, and more affordable high resolution satellite imagery. Drones in particular, bring accessibility and efficiency to many research applications. However, understanding the foundational science, the importance of platform choice and defining best operational procedures are keystones to deriving accurate and repeatable remotely sensed measurements.

Applications for Remote Sensing in Cranberry Crop Management

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Cranberries are produced in low-lying wetland fields (beds) with low pH and typically sandy soils. The crop yield varies from 10,000 to over 50,000 lb/acre and is determined by genetics, soil type, proper nutrition, water availability, pathogen effects, sun exposure, insect feeding, weed competition as well as a variety of other physical disturbances. Scouting on these beds can be destructive since the vines are easily broken or damaged. Use of remote sensing and drones is a very useful approach to scouting cranberry beds. In this presentation I will highlight two research programs where research on drone applications is ongoing. Following this I will also present ideas for current and future applications.

Quantifying the Concentration of *Clariireedia* in the Field for Dollar Spot Susceptible and Tolerant Creeping Bentgrass Cultivars

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Dollar spot is one of the most economically important turfgrass diseases. The causal agents of dollar spot disease are fungi in the *Clariireedia* genus. They infect both warm- and cool-season turfgrasses. More money is spent on fungicides to control dollar spot disease than any other turfgrass disease. One strategy for controlling dollar spot is the use of tolerant cultivars. We previously developed a qPCR assay that can quantify the *Clariireedia* concentration in asymptomatic and symptomatic turfgrass. The goal of this three-year study was to quantify the *Clariireedia* concentration in a tolerant and susceptible creeping bentgrass (*Agrostis stolonifera*) cultivar throughout the growing season. The tolerant cultivar was 'Declaration' and the susceptible cultivar was 'Independence'.

Two types of turf samples were collected weekly from 0.91 m by 1.52 m replicated plots for each cultivar in 2019, 2020, and 2021 from the first week of May and through the second week of August, for a total of fifteen weeks. The first type of turf sample was created by collecting ten cores, 1 cm in diameter x 2.5 cm deep, from a randomly chosen 0.09 m² area within each plot during 2019 and 2020. In 2021, the ten cores were randomly collected from within the entire 1.4-m² of each plot. Clippings were also collected in 2021 using a Toro Greensmaster Flex 21 (The Toro Company, MN) equipped with a twelve blade verticut reel set at a 2.3-mm depth. Clippings were collected every other week starting the first week of May 2021 and ending the second week of August. An additional sample was collected on the fourth week which was the last week of May for a total of nine clipping collections. The ten cores and clippings were ground in liquid nitrogen of which 0.25 g of ground tissue was used for DNA isolation. DNA was analyzed in triplicate with the qPCR assay. On each sampling date, the number of dollar spot lesion centers were counted for the entire plot and within the 0.09 m² area.

The 2019 composite samples included foliage and the entire thatch layer, and only 13.6% of the composite samples had detectable *Clariireedia* with the qPCR assay. Samples taken the last week of August 2019 only contained the foliar and the top 5 mm of the thatch layer. These composite samples all detected *Clariireedia* concentrations. The average *Clariireedia* concentration for Independence was 3.16E-11 while the average *Clariireedia* concentration for Declaration was 8.24E-13. The *Clariireedia* concentration on Independence was 38.4-fold higher than Declaration and statistically different. Thus, these cultivars with different resistance to dollar spot had correspondingly different *Clariireedia* concentrations.

Clarireedia was detected in all of the 2020 composite samples. The composite samples used only foliar and the top 5 mm of the thatch from each core of the ten cores. Independence had a statistically higher *Clarireedia* concentration than Declaration in week 5, 9, and 14 of the study. During week 5 there were no visual disease symptoms in either cultivar. Linear model analysis showed that the two cultivars started with the same *Clarireedia* concentration but increased at different rates throughout the growing season. The *Clarireedia* concentration in Independence increased by 67.7% per week while Declaration increased by 32.1%. The difference from initial and final concentration was 2,326-fold increase for Independence and 65.3-fold increase for Declaration. At the end of the experiment the *Clarireedia* concentration in Independence was 35.6-fold more than Declaration.

The 2021 composited cores of foliar and surface thatch showed that Independence had a statistically higher *Clarireedia* concentration than Declaration in week 1, 2, 3, 8, 10, 13, 14, and 15 of the study. Linear model analysis indicated that the initial concentration of *Clarireedia* in Independence was 3.52-fold more than Declaration at the beginning of the year in asymptomatic tissue and the concentration increased at different rates for each throughout the growing season. *Clarireedia* increased by 30.9% each week in Independence, while the concentration in Declaration increased by 9.3%. The difference between initial and final concentration was 56.8-fold for Independence and 3.8-fold for Declaration. At the end of the experiment Independence had a 14.9-fold greater *Clarireedia* concentration than Declaration.

The 2021 verticutting clippings were collected from the same plots as the 2021 composite samples. The clippings obtained from verticutting showed that Independence had a statistically higher *Clarireedia* concentration than Declaration in week 3, 5, 7, 9, 13, and 15. Thus, the qPCR assay was able to identify differences in *Clarireedia* concentrations for the two cultivars from verticutting samples of asymptomatic turf, as well as symptomatic turf during, and at the end of the growing season. Linear model analysis showed that the two cultivars had different initial concentrations but similar rates of change. Independence had a 10.4-fold higher *Clarireedia* concentration than Declaration at the beginning of the experiment. The *Clarireedia* concentration increased at similar rates in both cultivars; 40.2% per week for Independence and 37.5% per week for Declaration. The difference in the initial and final *Clarireedia* concentration was 160.0-fold for Independence and 118.3-fold for Declaration. At the end of the experiment Independence *Clarireedia* concentration was 14.0-fold more than Declaration.

Overall, results from this study indicated that the qPCR assay can identify significant differences in *Clarireedia* concentration in both asymptomatic and symptomatic turf of two cultivars that vary in susceptibility to dollar spot. In each linear model analysis, the more susceptible cultivar Independence always had the higher *Clarireedia* concentration compared to the more tolerant cultivar, Declaration. The ability of the qPCR assay to quantify the concentration of *Clarireedia* in the field during the growing season opens up new opportunities to study the epidemiology of dollar spot disease.

***Pyricularia oryzae* and Related Fungi**

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The fungal order *Magnaporthales* contains economically and scientifically important cereal and grass pathogens, such as the rice blast fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), the take-all root rot pathogen of cereals *Gaeumannomyces graminis*, and the summer patch pathogen of turfgrasses *Magnaporthiopsis poae* (syn. *Magnaporthe poae*), which are model organisms in host-pathogen interaction studies. To date, over 200 species have been described in *Magnaporthales*, about 50% of which are pathogens of domesticated and wild monocotyledon plants. The best-studied species in this order is the rice blast fungus, which is one of the most devastating threats to food sources in the world. Each year this pathogen destroys enough rice to feed 60 million people as a conservative estimate. It also infects turfgrasses causing the gray leaf spot of perennial ryegrass and tall fescue. The rice blast fungus is fast evolving. The wheat isolates arose in Brazil in 1985 and cause wheat blast, a new disease of wheat that has dispersed to other countries in South America and South Asia, and has become a potential threat to wheat production in the world.

Despite their scientific and economic importance, the taxonomy and phylogenetic relationships of this group of fungi had been unsettled for a long time due to a paucity of study and sequence data for the non-model species in *Magnaporthales*. The rice blast fungus and many other species in *Magnaporthales* have had various scientific names applied to them due to their complex life cycle and difficulty in resolving the taxonomic and nomenclatural issues. For example, *Magnaporthe grisea*, *M. oryzae*, *Pyricularia grisea*, and *P. oryzae* have been used for the rice blast fungus in scientific journal articles, books or databases, which has caused inconsistency and confusion among scientists, and other user communities around the world. Recent advancement in gene, transcriptome, and genome sequencing of these fungi has resulted in robust phylogenies, which correspond well with their pathogenicity, ecology, and biology. The taxonomy has thereby been revised in recent studies.

The knowledge of taxonomy, phylogeny, biogeography and pathology of the *Magnaporthales* is scattered through an increasingly wide range of periodicals and books, which extend back for over 140 years. A monograph of *Magnaporthales* fungi is going to be published in order to provide an overview of these fungi, which includes both historic records in literature and recent advancements. Morphological descriptions, diagnostic illustrations, type designation, geographical distribution, host range, and references are provided for representative taxa, especially the type or economically important species. Dichotomous keys to the three families and 38 genera in *Magnaporthales* are generated, and all accepted species names are included. This comprehensive monograph will facilitate future work on systematics, biodiversity, genetics, plant protection and quarantine.

Gray Leaf Spot of Perennial Ryegrass is a Recently-Emerged Disease with a Complex Evolutionary Origin that was Intimately Intertwined with that of Another Recently Emerged Disease - Wheat Blast

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Gray Leaf Spot (GLS) is a devastating disease of perennial ryegrass (*Lolium perenne*) turf caused by the blast pathogen *Pyricularia oryzae* (syn. *Magnaporthe oryzae*). In the mid- to late-1990s, GLS caused serious and widespread damage to golf course fairways and athletic fields from Nebraska to Rhode Island, and it is now emerging in Europe. GLS was first reported by Landschoot and Hoyland in 1992 but a "blast" disease of annual ryegrass (*Lolium multiflorum*) forage was recorded 20 years earlier in Louisiana and Mississippi. Early studies that were started mid-GLS epidemic showed that the disease was caused by a new *P. oryzae* population most closely related to the one causing wheat blast - another new disease that had emerged just a few years earlier (1985). Using whole genome sequence data for a large collection of GLS and wheat blast isolates, we now show that they co-evolved in Brazil, with all isolates having descended from a single founder individual belonging to the population responsible for the 1971 outbreak on annual ryegrass in LA and MS. This individual - a hybrid between strains specialized on goosegrass (*Eleusine* sp.) and signalgrass (*Urochloa* sp.) - initiated a series of matings involving at least five more strains from three additional host-adapted lineages, which resulted in the formation of a hybrid swarm. Mating within the swarm generated strains with exceptionally high genetic diversity and infection capability not only on wheat and *Lolium*, but at least 10 additional host genera. Finally, with the level of detail achievable through whole genome analyses, we were able to determine that the clonal lineage responsible for GLS was already established in Brazil by 1987, because a member isolate was recovered that year from a wheat plant in Palotina, Paraná. In addition to providing unprecedented insights into the emergence of two new plant diseases, our findings have broad implications in evolutionary biology.

Influence of Phosphorus on Annual Bluegrass Competitiveness in Creeping Bentgrass

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Annual bluegrass (ABG; *Poa annua* L.) is a problematic weed of turfgrass. Phosphorus (P) influences ABG as growth, establishment, and infestation is favored in high P soils. Years of research show ABG is more sensitive to P deficiencies than creeping bentgrass (CBG; *Agrostis stolonifera* L.). However, until knowledge gaps are addressed to better understand this difference in P sensitivity, a clear set of guidelines for turfgrass managers to exploit this sensitivity and suppress ABG will not be available. The objective of this research was to evaluate the effect of P application rate on sward composition when ABG and CBG were grown in competition. Two experiments, each repeated in time were conducted at the New Jersey Agricultural Experiment Station in New Brunswick, NJ in a glasshouse.

In the first experiment, treatments consisted of five phosphorus rates (from one application of triple superphosphate at 0, 12.5, 25, 50, 100 kg P ha⁻¹), which were slightly adjusted in run 2 (0, 6.25, 12.5, 25, 50 kg P ha⁻¹). The growing medium was a silica sand and peat moss mix (9:1 v/v) with a Mehlich-3 P concentration of 3 mg kg⁻¹. Annual bluegrass and CBG were sown in 25-cm diameter pots at a seeding rate of 55 and 165 seeds pot⁻¹, respectively. Line-intersect grid counts were collected monthly to determine coverage of ABG, CBG, and bare soil in each pot. Digital image analysis was used to determine percent green cover. Phosphorus applied at 12.5 to 100 kg ha⁻¹ did not influence ABG cover 90 days after seeding; however, ABG cover was slightly reduced at the 6.25 kg ha⁻¹ P treatment. The 0 kg ha⁻¹ P treatments failed to reach 7% green cover. This indicates that P rates between 0 and 12.5 kg P ha⁻¹ should be examined in more detail.

In the second experiment, treatments consisted of five P rates (0, 3, 6, 9, and 12 kg ha⁻¹ P) in a factorial with two soil pH levels (5.6 and 7.1). Eight tillers of ABG and eight tillers of CBG were planted in an alternating sequence to each 15-cm pot with a sand and peat growing medium containing 4 mg kg⁻¹ Mehlich-3 P. Treatment responses were evaluated in the same manner as described above. Additionally, visual assessments of turfgrass quality were conducted using a 1 to 9 scale where 1 equaled low-quality turf and 9 equaled high-quality turf. Phosphorus applied at 3 kg P ha⁻¹ in the low pH soil provided the greatest CBG cover (72%), which was 5-fold greater than ABG cover. The highest P rate in the high pH soil resulted in the most ABG cover (61%). All P-receiving treatments (3 to 12 kg ha⁻¹) in the low pH soil had similar green cover, but the greatest competitive advantage for CBG was observed in the 3 kg ha⁻¹ P treatment. Turfgrass quality was greatest when ≥ 6 kg P ha⁻¹ was applied to low pH soil, but only slightly reduced in the 3 kg ha⁻¹ treatment. Regardless of pH, 0 kg ha⁻¹ P treatments resulted in lowest ABG cover (< 4%) and poor overall green cover (< 30%).

Phosphorus applications that resulted in 4 to 6 mg kg⁻¹ Mehlich-3 P reduced ABG competitiveness in CBG, especially in the low pH soil. This competitive advantage for CBG was not found at > 6 mg kg⁻¹ in either soil. Field research is needed to evaluate this concept in sand rootzones with low soil P.

Ecology and Approaches to Weed Control on Carolina Redroot

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Because of the cranberry requirement for sandy acidic soil (pH 4 to 5), good drainage, and abundant rainfall, New Jersey commercial production is located in the Pine Barrens area, which offers optimal cranberry growth conditions. Limited herbicide options and the lack of soil cultivation associated with cranberry cropping foster the development of perennial weed species. Carolina redroot [*Lachnanthes caroliniana* (Lam.) Dandy] is a frequent perennial weed of New Jersey cranberry bogs that competes with the crop for nutritional resources. This species often forms monoculture patches in New Jersey cranberry beds where its development is associated with cranberry vine death caused by fairy ring (*Thanatophytum* sp.) disease as well as other “stand opening” conditions of natural and anthropic origin that damage the cranberry canopy. Carolina redroot can rapidly colonize open patches because of its rhizome sprouting capacity and abundant seed production with on average 2,500 seeds produced per inflorescence. This presentation will highlight the research efforts that have been conducted over the last 5 years to evaluate the quantitative and qualitative impacts of Carolina redroot on cranberry production, explore cultural practices that could potentially suppress its development, and successfully develop management strategies based on overlapping of residual herbicide applications.

Developing Molecular Tools for Resistance Trait Discovery and Diagnostics in Turfgrass Systems

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Turfgrass is a tightly controlled plant ecosystem: acre-for-acre, more is spent on turfgrass pest management than in any other system. Furthermore, turf systems are perennial and therefore many of the cultural, mechanical, and chemical control methods available in agronomic settings do not apply. For weed control, managers rely on a somewhat restricted number of chemistries, which are often applied in succession without rotating among other, diverse, control techniques. This puts strong evolutionary pressure on turf weeds and ultimately selects for biotypes resistant to the commonly used chemistries. Resistance then further limits weed control options and increases the cost of pest management. Understanding the genetic changes causing resistance helps researchers measure the extent of the problem and make recommendations to stop the spread of resistance; furthermore, once the genetic changes are known, molecular assays can be developed to diagnose these changes in field weeds. These assays are cheap, rapid, and accurate. Resistance is assumed if these changes are present, and with the rapid turnaround the results can be used to guide subsequent management strategies. Although resistance mechanisms are known for many of the most common turf weeds and chemistries, assays have yet to be developed. In this talk I will briefly introduce how researchers find novel mechanisms of resistance and describe molecular diagnostic assays in current use to help in the battle against herbicide resistance evolution.

Purification and Activity of the *Epichloë festucae* Antifungal Protein

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Epichloë festucae is a Class I Clavicipitaceous endophyte found in turfgrasses, supporting its hosts with antiherbivory compounds and other physiological benefits (Kuldau and Bacon, 2008). In strong creeping red fescue, (*Festuca rubra* subsp. *rubra*), the fungal endophyte provides resistance to dollar spot disease caused by the ascomycete pathogen *Clarireedia jacksonii* (Clarke et al., 2006). This endophyte-mediated resistance can also be seen against red thread disease caused by the basidiomycete *Laetisaria fuciformis* (Bonos et al., 2005). While endophyte infected fine fescues can be utilized to reduce disease, some turfgrasses such as creeping bentgrass (*Agrostis stolonifera*) do not have naturally occurring endophytes that provide such resistance. Understanding this endophyte-mediated resistance could lead to utilizing the underlying mechanism to protect other grasses from these diseases.

Previously we identified an *E. festucae* antifungal protein, *Efe-AfpA*, that was highly expressed *in symbio* and localized to the plant apoplast, but not produced in *E. festucae* pure culture (Ambrose and Belanger, 2012). This protein has amino acid sequence similarity to two well-established antifungal proteins: PAF produced by *Penicillium chrysogenum* and AFP produced by *Aspergillus giganteus* (Lacadena et al., 1995; Kaiserer et al., 2003). *Efe-AfpA* was previously produced heterologously in a yeast *Pichia pastoris* expression system and determined to have activity against *C. jacksonii* (Tian et al., 2017). Since attaining completely pure *Efe-AfpA* was an issue in the *P. pastoris* system, we tested a bacterial (*Escherichia coli*) and a fungal (*Penicillium chrysogenum*) system to determine which could produce the most *Efe-AfpA* with the easiest purification method. After comparing all three systems, the fungal *P. chrysogenum* system was chosen as it produced the highest yield with a single step purification on CM-cellulose. This expression system was provided by Dr. F. Marx along with a PAF overexpression strain (Sonderegger et al., 2016), which allows us to compare the activity of PAF with that of *Efe-AfpA*. The *P. chrysogenum* expression system has allowed us to further test the efficacy of *Efe-AfpA* against plant pathogenic fungi and to evaluate its potential as a treatment for fungal disease.

Both *C. jacksonii* and *L. fuciformis* were susceptible to *Efe-AfpA* on PDA plates amended with increasing concentrations of the protein, with the former showing a higher degree of inhibition. *C. jacksonii* was inhibited greatly by concentrations ranging from 10 to 100 $\mu\text{g mL}^{-1}$, while *L. fuciformis* was inhibited to a lesser extent mostly by the 50 and 100 $\mu\text{g mL}^{-1}$ concentrations. This is in stark contrast with *C. jacksonii* and *L. fuciformis* plated on PAF amended PDA plates, where no to minimal levels of inhibition were seen. Although these two proteins are similar in sequence, we can hypothesize that there are key differences between them that result in this difference in activity.

Two greenhouse experiments were conducted to test the ability of *Efe-AfpA* to control dollar spot disease on creeping bentgrass ‘Crenshaw’. Applications of 1mL of *Efe-AfpA* at 20 and 100 $\mu\text{g mL}^{-1}$ were applied as a spray either daily or only twice in a week-long experiment. In both

cases, 100 $\mu\text{g mL}^{-1}$ showed highly reduced disease symptoms as compared to the control while 20 $\mu\text{g mL}^{-1}$ showed slight differences. These results support the hypothesis that the *Efe-AfpA* protein is a major factor in the endophyte-mediated disease resistance seen in the *F. rubra* – *E. festucae* – *C. jacksonii* system. It may be possible to develop *Efe-AfpA* as a potential chemical fungicide alternative for disease control on grasses, such as creeping bentgrass, that do not have an endophyte providing resistance.

With a reliable greenhouse inoculation methodology for dollar spot infection, more extensive greenhouse trials will be conducted with *Efe-AfpA* on both dollar spot and red thread disease on creeping bentgrass. This inoculation method has also proven successful on strong creeping red fescue, providing us with another way to support the hypothesis that *Efe-AfpA* is a major component for the endophyte-mediated disease resistance. Endophyte infected (E+) and endophyte non-infected (E-) strong creeping red fescue will be challenged with dollar spot and treated using *Efe-AfpA*.

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Endosymbiotic Bacteria in Cells of Grasses Enhance Nutrient Status, Growth, Abiotic Stress Tolerance and Pest Resistance of Hosts

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Plants naturally carry microbes (bacteria and fungi) on, and within, seeds to facilitate development and early survival of seedlings. Other microbes may be acquired by plants from healthy soils. In this presentation, the ways that plants (especially grasses) cultivate soil bacteria and internalize them into cells of roots and leaves will be discussed. In addition, the mechanisms that grasses and plants in general use to extract nutrients from bacteria will be described. These mechanisms include the 'rhizophagy cycle' for extraction of soil-acquired nutrients from bacteria, and the 'nutrient trap mechanism' for extraction of nitrogen from nitrogen-fixing bacteria in leaves and leaf sheath cells. White et al. (2018) described the rhizophagy cycle and showed how it is used by plants to acquire soil nutrients (potassium, nitrogen, iron, calcium, etc.). Chang, Kingsley and White (2021) outlined evidence for the 'nutrient trap mechanism' for atmospheric nitrogen fixation by intracellular endophytes and transfer to plant cells (see graphic below). This mechanism is a nutrient trap, because once microbes are engaged in nutrient exchange with a plant cell, the microbes cannot escape from the process without being degraded by superoxide. In this mechanism, intracellular bacteria secrete ethylene, which triggers plant cells to grow and supply intracellular bacteria with carbohydrates that fuel nitrogenase activity; simultaneously, the root cell produces superoxide, triggering bacteria to secrete antioxidant forms of nitrogen (nitric oxide or ammonia) that combine with superoxide to produce nitrate that is absorbed directly into plant cells. Nitric oxide-secretion is thought to result in the chemical reaction $\text{NO (nitric oxide)} + 2\text{O (superoxide)} \rightarrow \text{NO}_3 \text{ (nitrate)}$; while secretion of ammonia results in the chemical reaction $2\text{NH}_3 \text{ (ammonia)} + 9\text{O (superoxide)} \rightarrow 2\text{NO}_3 \text{ (nitrate)} + 3\text{H}_2\text{O (water)}$. It is notable that secretion of ammonia by bacterial cells results in a nearly five-fold antioxidant capacity and twice the nitrate yield. Through exposure to superoxide produced by the plant cell, diazotrophic bacteria are forced to secrete antioxidant forms of nitrogen to prevent their own degradation. The function of nitrogen fixation in the nutrient trap mechanism in plant cells is closely tied to cell growth where microbial cells are provided with nutrients to support replication and nitrogen fixation. The extensive fibrous root systems of grasses with numerous root tips engaged in the rhizophagy cycle, and prolific tiller and leaf production in grasses may be adaptations to maximize nutrients extracted from endosymbiotic bacteria.

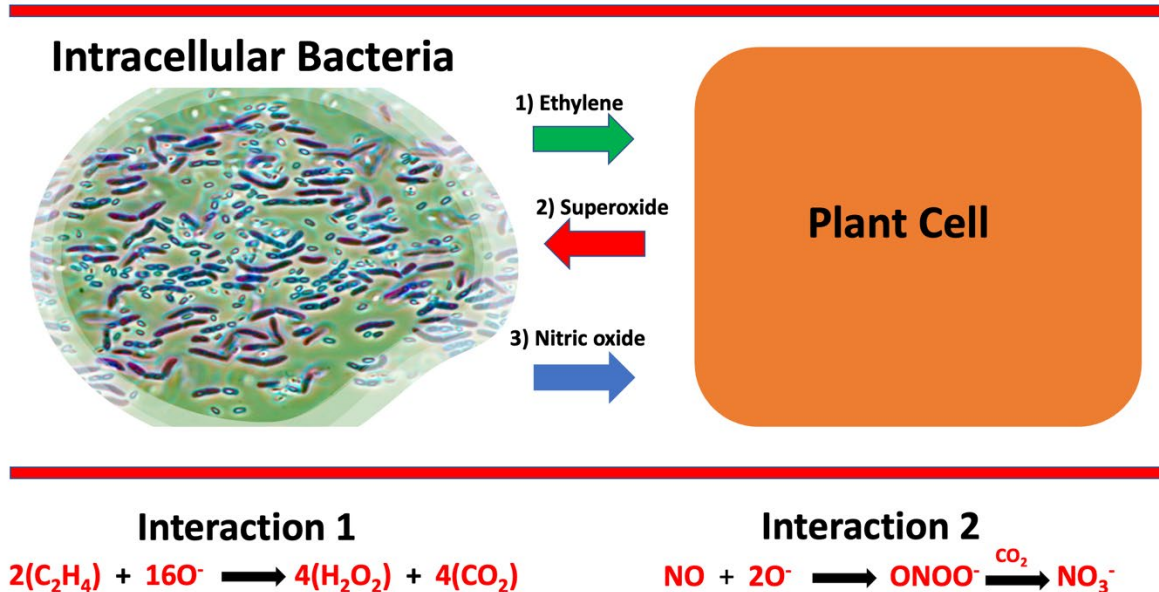


Figure 1. Graphic that shows the chemical exchange between intracellular bacteria and the host grass cells (nutrient trap mechanism). These chemical interactions are expressed as equations in interactions 1 and 2. As a result of the nutrient trap mechanism plant cells acquire nitrate to support growth and development.

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Native Persistent Entomopathogenic Nematodes for Long-Term Insect Pest Suppression

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Turfgrass is afflicted by many insect pests which are primarily controlled with synthetic insecticides, commonly in a preventive mode over large areas, which can suppress many natural enemies of turf insect pests. Insecticide overuse leads to reduced insecticide efficacy through insecticide resistance and increased microbial insecticide degradation. There is a need for the development of alternative insect control methods, that, ideally, can provide long-lasting pest suppression.

Entomopathogenic nematodes (EPNs) have shown potential for the control of various turf insect pests including white grubs, caterpillars, weevils, and crane flies. EPN research has focused on using them as biopesticides applied inundatively with little concern for long-term effects. However, several studies in field crops have shown that inoculative applications of native EPN strains adapted to the local conditions and maintained to preserve their ability to persist in the environment, can effectively suppress pest populations for several years. In turfgrass, oriental beetle larvae were effectively suppressed for up to 4 years after a single application of the white grub-adapted species *Steinernema scarabaei*.

Field study methodology. During 2019 we extensively surveyed one fairway each at Pine Brook Golf Course (PB, Manalapan, NJ) and Howell Park Golf Course (HP, Farmingdale, NJ), both in Monmouth County, central New Jersey for native EPNs. These fairways had received only limited insecticide applications for many years. Soil samples were taken from the fairway and the adjacent rough. The majority of EPNs collected were either *Heterorhabditis bacteriophora* or *Steinernema carpocapsae*. Isolates of each species were mixed together to increase the genetic diversity of the populations to be used in inoculative applications. The EPN populations were mass reared in wax moth, *Galleria mellonella*, larvae and used to inoculate the field plots in early June 2020.

Field plots (20 m × 10 m), located at PB and HP, were arranged along the fairway edge, with one half in the fairway, the other in the rough. Plots were separated by ≥ 10 m. EPNs were applied at a total of 1.25 × 10⁹ infective juvenile nematodes/ha. Treatments were *H. bacteriophora*, *S. carpocapsae*, a 1:1 mixture of both species, and an untreated control. There were two replicates per treatment at each golf course. To minimize border effects when sampling for EPN and insect populations, samples were taken in each plot from a 4 m × 4 m area in the rough and one in the fairway that was 2 m distance from the fairway edge, and 3 m distance from the sides of the plot. For more uniform coverage, the sampling area was further divided into four 2 m × 2 m subplots. Subplots were sampled separately, but for analysis the data for the four subplots were combined so that there was one data point for each variable for each side (fairway vs. rough) of each plot. Data were analyzed with repeated measures ANOVA with treatment and golf course as between-subject factors and grass type (fairway vs. rough) and sampling date as within-subject factors.

EPN populations. EPN populations in the plots were determined 1 week before application to gauge the scale of resident populations and again 1 (July 2020), 4 (October 2020), 6 (December 2020), 13 (July 2021), and 15 (September 2021) months after application. Ten soil cores (7.5 cm depth × 2.5 cm diameter) were taken per subplot, mixed thoroughly, and a subsample of 150 grams

was placed into a plastic cup and baited with five waxworms for three consecutive 3-day rounds. Dead waxworms were replaced with live ones after 3 and 6 days (max. number of infections 15 per subplot). EPN-infected waxworms were collected and incubated to determine EPN species by the color of the cadavers (indicates the bacterial symbiont species associated with the EPN species) and the size and behavior of the infective juveniles emerging from the cadavers (measured and observed under a dissection microscope).

EPN detection (i.e., number of infected waxworms) was highly variable. Generally, higher EPN numbers were detected in the rough vs. the fairway. However, treatment effects were similar in rough and fairway. Thus, EPN detection tended to be higher in the treated plots than the untreated plots for the species with which the plots were treated. For *S. carpocapsae*, this effect was very strong and persistent in the plots treated with this species alone but only weak in the plots treated with both species. For *H. bacteriophora*, the effect was weaker, except for a high peak in October 2020, but was observed in both the *H. bacteriophora* alone treatment and in the combination treatment.

A third EPN species, *Steinernema cubanum* or a closely related, possibly new species, was also found regularly in many plots at both golf courses in both the fairway and the rough side. It was found generally in higher numbers late in the season. Isolates of this species recently found by us elsewhere in New Jersey have shown high virulence to white grubs. It is possible that this species, that has very large IJs, uses primarily larger white grub instars as hosts in late summer to early fall but not in spring when soil temperature might be too cool. These native isolates of *S. cubanum* hold promise for inoculative releases in white grub populations in NJ and surrounding areas.

Annual bluegrass weevil (ABW) populations in June. In mid-June 2020 and 2021, plots were surveyed for ABW populations including all life stages from first larval instar through adult. Eight turf/soil cores (3.8 cm depth × 5.4 cm diameter) were taken per subplot and extracted in the laboratory by submersion in warm tap water saturated with table salt. The number of ABW life stages were recorded for each sample along with any other insects found in the samples.

ABW densities were generally very low in the plots in both years (10-20 larvae and pupae per 0.1 m²) which is several times lower than in previous years in untreated areas in those sites (reasons unknown). Numbers were significantly higher in the untreated fairway than in the untreated rough. In both years, numbers in the fairway were significantly lower in the plots treated with both EPN species (47% lower in 2020, 89% lower in 2021) than in the untreated plots and the ones treated with *S. carpocapsae* only. No differences among treatments were detected in the rough. The only other insects found in significant numbers, similar to ABW, during the ABW sampling were larvae of the black turfgrass atenioides (BTA), numbers of which were not significantly affected by EPN treatments. The low numbers of ABW and BTA recovered likely made detection of significant treatment effects difficult.

Surface insect populations. Surface-active insect populations were determined in July and early September of 2020 and 2021 via soap flushes. In each 4 m × 4 m sampling area, two 30 cm × 30 cm areas were treated with 500 ml of 0.8% soap solution at the start of the sampling and again after 5 minutes. Any insects found within 20 minutes were collected and identified in the laboratory. Soap extraction revealed many insects including billbug adults, wireworm adults, sod webworm larvae, cutworm larvae, crane fly larvae, ground beetle adults. But only adults of ABW and especially BTA were found in numbers high enough for meaningful analysis. ABW numbers

were higher in the fairway than the rough. In the fairway, they were significantly lower in the plots treated with *S. carpocapsae* and the species combination than in the untreated plots. BTA numbers were higher in the fairway than the rough. In the fairway, they were significantly lower in all EPN treatments than in the untreated plots.

White grub/soil insect populations. Populations of white grub and other soil insects were determined in September 2020 and 2021. Four turf/soil cores (7.5 cm depth × 10.5 cm diam) were taken per subplot and destructively sampled. Any soil insects found were collected and identified in the laboratory. Sampling showed a mix of annual white grub species consisting of oriental beetle followed by Japanese beetle and a few northern masked chafers. Due to the low densities, the three species were pooled for analysis. White grubs were more common in the rough than in the fairway, albeit also at low densities. In the rough, densities were significantly lower in the plots treated with *H. bacteriophora* than in the untreated plots; due to the low densities, no treatment effect could be detected in the fairway. BTA larvae were more common in the fairway than in the rough but were not affected by EPN treatment in either rough or fairway. Numbers of other insects (i.e., ABW larvae and pupae, crane fly larvae, wireworm larvae) detected were too low and variable for meaningful analysis.

Future plans. We are planning to continue sampling in the field plots through 2022 to better determine the long-term establishment of the EPNs and their effects on ABW, white grubs, and other insect pests. We believe that conducting a similar experiment in grub infested rough areas on golf courses using the field isolates of *S. cubanum* found in this study, provided it reproduces well in white grubs, holds some promise and may be initiated in late summer 2022.

Interaction of Elevated Carbon Dioxide and Drought Stress on the Recovery of Three Cool-season Turfgrass Species

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Prolonged drought stress limits the growth and productivity of cool-season turfgrass species and may negatively impact the potential for regrowth once rewatering occurs by inducing dormancy or mortality of critical growth organs (rhizomes, stolons, or crowns) that can result in permanent loss of turf stands. Thus, the ability to maintain active growth during prolonged stress periods or to quickly recover once the stress has subsided is highly dependent upon the viability or level of damage to the meristematic tissues in these growing organs.

The concentration of atmospheric carbon dioxide is projected to double from its current approximate concentration ($400 \mu\text{L L}^{-1}$) to between 800 and $900 \mu\text{L L}^{-1}$ by the next century. Elevated atmospheric carbon dioxide has been found to enhance drought tolerance in many crop species and some turfgrass species, but more information is still needed on the mechanisms behind the level of drought tolerance and postdrought recovery within different turfgrass species. Additionally, more information is needed regarding whether improved drought tolerance by elevated CO_2 is associated with the protection of rhizomes, stolons, or crowns and if this differs across turfgrass species exhibiting these different growth habits.

Two studies were conducted with the following objectives: 1) to determine whether different species of turfgrass differing in growth habit (rhizomatous, stoloniferous, or bunch-type) exhibited differential responses to elevated CO_2 and drought stress, and 2) to determine whether elevated CO_2 concentration could alleviate drought damage in the critical growth organs to enhance drought tolerance and postdrought recuperative potential. In both studies, plants were grown at ambient CO_2 ($400 \mu\text{L L}^{-1}$) or elevated CO_2 concentration ($800 \mu\text{L L}^{-1}$) for 28 d, subsequently subjected to either irrigation (control) or drought stress (irrigation completely withheld) for 28 d, and then rewatered for 18 d.

In the first study, plants of rhizomatous Kentucky bluegrass (*Poa pratensis* L.) that were grown at elevated CO_2 concentration under drought stress maintained higher membrane stability, leaf water content, and visual turf quality, and Kentucky bluegrass rhizome nodes exhibited lesser damages, as manifested by higher viability and decreased levels of endogenous abscisic acid and auxin compared with plants at ambient CO_2 . Upon rewatering, plants that were exposed to drought stress under elevated CO_2 exhibited increased growth of total shoot and daughter plant biomasses and an increased number of rhizomes, as well as higher percent turfgrass cover compared to plants at ambient CO_2 concentration. The results suggest that the enhanced drought tolerance and subsequent regrowth at elevated CO_2 could have protective effects on rhizomes, facilitating drought survival of Kentucky bluegrass.

In the second study, plants of stoloniferous creeping bentgrass (*Agrostis stolonifera* L.) and bunch-type tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort] that were grown at elevated CO_2 concentration under drought stress maintained greater leaf relative water content,

turf quality, and less damages to membrane stability, compared with plants at ambient CO₂. After prolonged drought stress, creeping bentgrass stolon nodes exhibited lesser damages, as manifested by significantly higher tissue viability due to elevated CO₂. Rewatering of drought stressed creeping bentgrass plants exposed to elevated CO₂ showed increases in total shoot biomass and percent turfgrass cover compared to plants exposed to ambient CO₂ concentration. However, elevated CO₂ had no significant effects on the viability of crowns with meristematic tissues in tall fescue during drought, or on shoot biomass or percent turfgrass cover during rewatering. These results indicate that the stoloniferous turfgrass species was more responsive to elevated CO₂ compared to the bunch-type species for improving regrowth during postdrought rewatering, and that elevated CO₂ concentration may have protective effects on stolons to mitigate drought damage. Such knowledge can be advantageous when making appropriate turfgrass selections under the interaction of drought stress and the anticipated rising concentration of CO₂.

POSTER PRESENTATIONS

Environmental and Economic Comparison of Zoysiagrass, Bermudagrass, and Creeping Bentgrass Fairways in the Northern Transition Zone

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Zoysiagrass (*Zoysia* spp.) and bermudagrass (*Cynodon* spp.) are warm-season turfgrasses used in the lower transition zone and southern portion of the US. As of 2015, golf courses in the transition zone account for approximately 78% of the 10,375 hectares of zoysiagrass in the US (Patton et al., 2017) and bermudagrass is the most widely used warm-season turfgrass in the southern and transition zone (Wu et al., 2020). Increasing temperatures from climate change are a concern for the feasibility of cool-season grasses in these regions. The southern portion of the northeast will experience significantly more days per year above 90°F between 2041 and 2070 under both low or high emission scenarios (Horton et al., 2014). Seasonal shifts have already been seen in the northeast with a warmer late-winter, higher spring temperatures, and an earlier emergence of plants from winter dormancy (Dupigny-Giroux et al., 2018). Additionally, the warming trend has shifted the plant hardiness zone with a migration of warm-season grasses northward (Hatfield, 2017; USDA, 2012). Management tactics to mitigate the impact of climate change on the turfgrass industry are limited (Hatfield, 2017). Cool-season grasses, like creeping bentgrass (*Agrostis stolonifera* L.), that are reaching their adaptation limits in this warming environment bring about complications in sustainable golf course management. Given the development of cold-hardy zoysiagrass and bermudagrass cultivars, these warm-season grasses offer potential options for superintendents to save money, resources, and have less of an impact on the environment.

The proposed series of studies (1-4) will commence in the summer of 2022. The aim is to determine and quantify the agronomic performance, economic benefits, environmental impact, golfer perceptions, and potential shortcomings of zoysiagrass and bermudagrass used for fairways in the northern transition zone. Study 1 will include 12 zoysiagrass selections and 14 bermudagrass selections. For consistency in establishment, all zoysiagrass and bermudagrass selections will be sprigged at a 10:1 expansion ratio. Plots will each be 100 sq ft in a completely randomized design with 3 replications. This warm-season turfgrass trial will be grown in a sod farm environment. Data will be collected on establishment, turf quality, spring green-up, winter kill (if it occurs), and disease resistance (if present). This will provide superintendents and sod growers with performance data on species that have not been evaluated in the region for 30 years.

Study 2 will compare the standard fairway grass in the northeast (creeping bentgrass) with zoysiagrass and bermudagrass. Selections of each species will be made by selecting the 2 best zoysiagrass and bermudagrass cultivars identified in study 1. These will be compared to the 2 best creeping bentgrass cultivars and a standard (according to previous trials in NJ). The trial will be established as sod for all species and kept under fairway maintenance practices. Plots will be 4x6 ft in a completely randomized design with 3 replications. Data will be collected on how much labor, chemicals and natural resources are required to establish and maintain each species

at an acceptable turfgrass quality. If disease, weeds, or insects require chemical treatment, we will document fungicide, herbicide, and insecticide usage. This information will be used to calculate the Environmental Impact Quotient (Kovach et al., 1992) required to obtain adequate quality in each species. We will use this information to write an Environmental Assessment to determine the environmental impact of each species and make comparisons. Our economic component entails evaluating the costs associated with producing zoysiagrass, bermudagrass, and creeping bentgrass to demonstrate cost effectiveness of using warm-season grasses on golf courses, and to highlight cost areas where savings take place. An economic cost model will be constructed in MS Excel under conventional management systems for each species. Cost models will be based on the following: experiment records from PI's research project regarding turfgrass maintenance, including frequency and amount of irrigation, fertilizer, chemical treatments, mowing, and labor hours; interviews with golf course superintendents (approximately three to five) to determine current golf course practices regarding fairway maintenance; and current prices for chemicals, equipment, and labor.

The 3rd and 4th study will determine irrigation requirements and golfer preference in grass species, respectively. For study 3, irrigation data will be collected according to the NTEP warm-season drought trial protocol (https://ntep.org/data/ws18w/ws18w_21-4/ws18w21tproto.pdf). The single top performer of each species will be used with the same layout as study 2. Study 4 will consist of a survey sent to public and private courses in NJ with several short questions. Questions will assess whether golfers prefer a certain species of turfgrass and if they are more likely to consider playing a course that is more environmentally friendly. Data will be collected on preference of zoysiagrass, bermudagrass, creeping bentgrass, or perennial ryegrass. Perennial ryegrass will be included on the survey to prevent bias that may arise. Golfer responses will serve as the dependent variable to determine if there is a preferred turfgrass species.

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Glyphosate and Fluazifop Application Timing Affects Deertongue Grass (*Dichanthelium clandestinum*) Control in Naturalized Fine Fescue

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Deertongue grass (*Dichanthelium clandestinum*) is a broad-bladed perennial with deep roots and thick rhizomes that is difficult to control in no-mow, or “naturalized” golf course areas. In 2020, research was conducted at the Mendham Golf and Tennis Club (Mendham, NJ) to evaluate the efficacy of single applications of fluazifop (280 g ha⁻¹ with NIS 0.25% v/v) and glyphosate (560 g ha⁻¹) in a complete factorial with five different application timings for deertongue grass control. Applications were made at 75 and 175 growing degree-days (GDD; April 28 and May 26, respectively) during full-bloom of first spring flowering (June 18), in mid-July (July 22), and at 25 cooling degree-days (CDD; September 22). Treatments were applied to 2 by 3 m plots, replicated five times and arranged in a RCBD. Plots were mowed once in October, two weeks after the CDD-timed treatments were applied. Deertongue grass injury was visually assessed on a 0 (healthy) to 100 (necrotic) scale from May through October. Deertongue grass green cover was visually evaluated on a 0 (none) to 100 (complete cover) percent scale. Grid intersect counts were conducted in October 2020 and May 2021 at the conclusion of the Mendham experiment. All data were subject to ANOVA using the GLIMMIX procedure in SAS (v. 9.4).

An interaction between effects of herbicide and application timing on deertongue control occurred 14 to 21 weeks after treatment (WAT); trends were similar on each date. Glyphosate applied at 175 GDD and full-bloom provided greater deertongue grass control (89 to 97%.) than glyphosate applied at 75 GDD and 25 CDD (36 and 62%) and all fluazifop treatments (11 to 59%) by the final rating on 22 October 2020. Glyphosate applied at or after 175 GDD was the most effective treatment, providing 81 to 94% control at spring greenup in May 2021.

Based on the result of the 2020 trial, an experiment was initiated at Hort Farm 2 (North Brunswick, NJ) to evaluate integrated weed control programs in 2021. The site was fine fescue artificially infested with deertongue grass. This experiment investigated glyphosate and fluazifop efficacy at two different application timings in a factorial with monthly mowing. Herbicides were applied at 175 GDD (May 13) and 25 CDD (September 27). These timings are also practical as the canopy height allowed for equipment to be driven through fine fescue without concern for tire tracks disrupting aesthetics. The 175 GDD fluazifop treatment was also treated with fluazifop at 25 CDD, as it became apparent that a single application of fluazifop, even combined with mowing, was ineffective. Mowing was initiated three weeks after the 175 GDD application on June 9 and concluded on October 5. A walk-behind push mower bench set to 6” was used.

In the 2021 trial, glyphosate provided more deertongue grass control than fluazifop. Glyphosate injury to fine fescue was not observed at any time. Mowing improved deertongue grass control on one rating date in August. Effects of mowing alone and in combination with herbicides will be best evaluated in early 2022 at spring greenup. This experiment will be repeated at a similar location in 2022.

Gene Editing of Creeping Bentgrass to Improve Dollar Spot Disease Resistance

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Creeping bentgrass (*Agrostis stolonifera* L., *As*) is one of the most widely used cool-season grass species on golf courses. Besides being stressed by heat and drought during summer months, many commonly used cultivars of creeping bentgrass are highly susceptible to dollar spot disease caused by *Clariireedia jacksonii*. We have utilized the CRISPR (clustered regularly interspaced short palindromic repeats-associated endonuclease)-gene editing technology to precisely knock-out stress negative regulators to enhance creeping bentgrass stress tolerance and disease susceptible genes to improve plant disease resistance. We have constructed our own CRISPR-gene editing vector with the wheat U6 promoter driving the expression of guide RNA (gRNA) targeting the chosen gene and the monocot codon-optimized Cas9 nuclease gene under the control of maize ubiquitin promoter. We have developed an efficient plant transformation protocol for 'Crenshaw' creeping bentgrass by both gene gun bombardment and *Agrobacterium*-mediated gene delivery. The Crenshaw complete genomic DNA (gDNA) sequence of *AsCPK12* encoding calcium-dependent protein kinase (CDPK), a proven negative regulator for rice blast disease resistance, was identified by bioinformatics analysis and cloned by PCR (polymerase chain reaction). The 26-nucleotide *AsCPK12-SacI* target sequence was chosen and the CRISPR-gene editing vectors pRD302 was constructed to transform the embryogenic calli initiated from Crenshaw creeping bentgrass seeds. Many transgenic Crenshaw plants have been produced by both gene gun and *Agrobacterium* transformation methods. The gDNA fragments spanning the *AsCPK12* target site from each transgenic plant were PCR-amplified, analyzed by DNA sequencing and ICE (inference for CRISPR editing) analysis. Several *AsCPK12*-edited Crenshaw plants have been identified. One of the *AsCPK12*-edited plant, RD302-A30, was clonally propagated. Eight RD302-A30 clones and eight non-edited Crenshaw, wild type plants were inoculated by sprinkling *C. jacksonii*-infected Kentucky bluegrass seeds. Total DNAs were isolated from the infected plants two weeks post inoculation. The *C. jacksonii* fungal level in each plant was quantitated by qPCR analysis using the fungal ITS (internal transcribed spacer)-specific primers and the host actin gene as the endogenous control. Our results showed that the *C. jacksonii* fungal levels in RD302-A30 plants were much lower compared to those in the non-edited wild type plants, indicating that editing *AsCPK12* can improve creeping bentgrass resistance to dollar spot disease.

Use of the *Epichloë festucae* Antifungal Protein as a Treatment for Fungal Diseases

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Epichloë festucae is a Class I Clavicipitaceous endophyte found in turfgrasses, supporting its hosts with antiherbivory compounds and other physiological benefits (Kuldau and Bacon, 2008). In strong creeping red fescue (*Festuca rubra* subsp. *rubra*), the fungal endophyte provides resistance to dollar spot disease caused by the ascomycete pathogen *Clarireedia jacksonii* (Clarke et al., 2006). This endophyte mediated resistance can also be seen against red thread disease caused by the basidiomycete *Laetisaria fuciformis* (Bonos et al., 2005). While endophyte infected grasses can be utilized to avoid disease, some turfgrasses such as creeping bentgrass (*Agrostis stolonifera*) do not have naturally occurring endophytes that provide such resistance. Understanding this endophyte-mediated resistance could lead to utilizing the underlying mechanism to protect other grasses from these diseases.

Previously we identified an *E. festucae* antifungal protein, *Efe-AfpA*, that was highly expressed *in symbio*. It was produced heterologously in a yeast *Pichia pastoris* expression system and was found to have activity against *C. jacksonii* (Ambrose and Belanger; 2012, Tian et al., 2017). We currently use a more efficient *Penicillium chrysogenum* expression system, previously used to express similar antifungal proteins, to produce large quantities of easily purifiable *Efe-AfpA* (Sonderegger et al., 2016). This has allowed us to further test its efficacy as a treatment for dollar spot and red thread disease. Purified *Efe-AfpA* was active against both *Clarireedia jacksonii* and *Laetisaria fuciformis* in plate assays. In greenhouse assays *Efe-AfpA* was effective in preventing dollar spot disease symptoms on creeping bentgrass.

Efe-AfpA may also be applicable to other plant-pathogen systems. The structurally related AFP protein from *Aspergillus giganteus* was effective against several fungal pathogens in both direct application and transgenic approaches (Vila et al., 2001; Girgi et al., 2006). Given its wide host range and well characterized infection methods, we are also determining *Efe-AfpA*'s efficacy against grey mold caused by *Botrytis cinerea* on apples (Zhang et al., 2014).

With a reliable greenhouse inoculation methodology for dollar spot infection, more extensive greenhouse trials will be conducted with *Efe-AfpA* on both dollar spot and red thread disease on creeping bentgrass. *B. cinerea* biocontrol experiments will also be conducted on apples to determine if *Efe-AfpA* is effective at controlling grey mold.

Acknowledgments

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Optimal Conidial Concentration for Gray Leaf Spot Disease Evaluation on Tall Fescue Turfgrass in Controlled Environment Studies

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Gray leaf spot is a major foliar disease of turfgrasses in the United States. This disease is caused by *Pyricularia oryzae*, which is also recognized as a pathogen of other economically important crops, causing diseases such as blast in rice (*Oryza sativa*) and wheat (*Triticum aestivum*). On turfgrass, gray leaf spot disease symptoms begin as pinpoint, water-soaked leaf lesions, which progress into dark, necrotic spots. As the disease progresses, lesions expand and coalesce to cause blighting of leaf tips and twisting of leaf blades. Severe infections can kill entire turfgrass plants and decimate turf stands. Breeding for gray leaf spot disease resistance has been an effective means of disease control in species such as perennial ryegrass (*Lolium perenne*). Traditional screening methods for gray leaf spot disease resistance are based on field evaluations during natural epiphytotic, which are often sporadic in occurrence and not certain for any given year. The objective of this study was to develop a growth chamber-based screening method to provide a reliable and controlled selection strategy for gray leaf spot resistant germplasm. Nine tall fescue (*Festuca arundinacea*) populations were selected based on field performance to gray leaf spot disease; the selected populations ranged from very susceptible to very tolerant to gray leaf spot disease in field evaluations. Three virulent *Pyricularia oryzae* strains were used for inoculations, which were conducted by spraying conidial concentrations of 0, 50, 500, 5,000, and 50,000 conidia mL⁻¹ onto the plant leaves. Evaluations of growth chamber experiments included visual assessments of plant health and percent disease. Plant health ratings were scored on a 1 to 9 rating scale, where 1 represented heavily diseased, dead turfgrass plants and 9 represented non-diseased, green, healthy turfgrass plants. Percent disease ratings were scored on a 0 to 100 scale, where 0 represented no visible disease symptoms and 100 represented complete disease devastation. Tall fescue populations exhibiting high levels of field tolerance were identified as the most tolerant populations using conidial concentrations of 50,000 conidia mL⁻¹. This is a promising observation for developing controlled screening techniques to identify gray leaf spot resistant tall fescue germplasm. However, further testing on additional populations should be conducted to ensure the reproducibility of this method.

Evaluation of Creeping Bentgrass (*Agrostis stolonifera*) Shade Tolerance Under Simulated Shade

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Shade stress and shade avoidance responses (SAR) are a major problem for plants grown under foliar shade. Creeping bentgrass (*Agrostis stolonifera*) is a popular turfgrass grown on golf courses under a wide variety of growing conditions including foliar shade. However, shade tolerance and shade avoidance are still understudied in creeping bentgrass, and which traits of interest are important in shade tolerance are still ambiguous. The objective of this study was to distinguish what morphological traits are important to identify shade tolerance in creeping bentgrass. If certain morphological traits could be identified that reflected shade tolerance, these could be used to develop shade tolerant cultivars. Additionally, this study also aimed to evaluate 31 common commercial cultivars and experimental selections of creeping bentgrass. The study was carried out in a greenhouse under both full sun and simulated foliar shade. Foliar shade was accomplished using a photoselective filter, which decreased light intensity by ~50% and reduced the red to far-red ratio (R/FR) by ~33%. Several traits were monitored including height, tiller count, biomass, and total chlorophyll concentrations. Of the traits evaluated, height and chlorophyll were significantly affected by shade, which is indicative of shade avoidance characteristics. Of the cultivars studied, L-93XD demonstrated a negative percent change in height indicating a strong shade tolerance for this trait. A positive shade tolerant chlorophyll concentration was seen in the cultivars, Penncross, Matchplay, MacDonald, and Luminary. Cultivars that exhibited both smaller heights and increased chlorophyll concentrations when grown in the shade are cultivars that are most adapted for shade, and both traits should be the focus moving forward. L-93XD showed promise in both categories. Future creeping bentgrass shade studies should prioritize height and chlorophyll concentration measurements. These morphological traits are also good phenotypic selectors in improving creeping bentgrass to become more shade tolerant through directed breeding programs.

Effects of Hormones and Plant Growth Promoting Bacteria on Annual Bluegrass (*Poa annua*) Tolerance to Heat Stress

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Annual bluegrass (*Poa annua*) is known to have relatively shallow roots and is more susceptible to heat stress compared to other turfgrass species. Enhancing root growth may be key to improving heat stress tolerance in *Poa annua* and maintaining quality putting greens. The objective of this study is to determine the effects of selected growth-promoting hormones and ethylene-inhibiting bacteria on rooting characteristics in *Poa annua* grown under heat stress conditions. *Poa annua* sods were grown at control (22/17 °C day/night) and heat stress (35/30 °C day/night) conditions in growth chambers. Plants were treated weekly for 21 days with exogenous applications of the hormone strigolactone (SL); ethylene inhibitor aminoethoxyvinylglycine (AVG); ethylene suppressing plant growth promoting bacteria (PGPR) with 1-aminocyclopropane-1-carboxylic acid deaminase (ACCd) activity; and water as untreated control. Plant heat responses were evaluated as visual turf quality (TQ), canopy temperature change, and leaf water content (LWC). Total root length was analyzed using WinRHIZO software, and total root biomass was measured at the end of the experiment. Application of SL, AVG, and PGPR resulted in significantly higher TQ on days 14 and 21 of heat stress, higher LWC on day 21, and lower canopy temperature change on day 21 compared to the control. Of the treatments, the ethylene inhibitors (AVG and ACCd PGPR) had a more significant effect on TQ and LWC than SL. Root length and biomass was significantly greater for SL, AVG, and PGPR treatments under heat stress compared to the control. These results indicate that the application of growth-promoting hormones and ethylene inhibitors on *Poa annua* can enhance root growth and improve *Poa annua* heat tolerance.

Non-Specific Lipid Transfer Proteins (nsLTPs) Have Antifungal Activity Against Select Turfgrass Fungal Pathogens

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We have identified antifungal proteins in *Arabidopsis* and wheat that have been shown to provide significant protection against *Fusarium graminearum* both *in vitro* and when overexpressed in wheat. The proteins AtLTP4.4 and TaLTP9, both non-specific lipid transfer proteins (nsLTPs), have been shown to be effective antifungal molecules and, in addition to *F. graminearum*, are effective *in vitro* against four major turf fungal pathogens, *Magnaporthe poae* (summer patch), *Monographella nivalis* (snow mold), *Rhizoctonia solani* (brown patch) and *Clarireedia jacksonii* (dollar spot). nsLTPs have been recognized as possessing potent antifungal properties and have been linked with both biotic and abiotic stress reduction. Using an activation tagging approach, we identified that overexpression of a non-specific lipid transfer protein (nsLTP) enhanced resistance of *Arabidopsis* to trichothecenes, small molecule mycotoxins produced by *Fusarium graminearum* that function as potent virulence factors during infection. Plant nsLTPs are small cysteine-rich proteins with the consensus sequence of C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C which forms four conserved disulfide bridges. When broken, those disulfide bridges generate free thiols, which may function to scavenge ROS molecules. We showed that exogenous trichothecene exposure to *Arabidopsis* and wheat leaf tissue leads to substantial ROS accumulation and overexpression of AtLTP4.4 and TaLTP3 significantly reduced ROS accumulation compared to the vector control. Hence, a potential mechanism by which AtLTP4.4 may confer resistance is by scavenging ROS and reducing or suppressing oxidative stress that modulates disease progression in addition to the inherent antifungal properties of this protein. Information about the role of nsLTPs in response to biotic and abiotic stresses in turfgrasses is very limited. A recent publication reported that an nsLTP (c120612_g1) in Kentucky bluegrass (*Poa pratensis* L.) is significantly induced by a combination of drought and ethephon treatment. This is consistent with previous reports where nsLTPs are upregulated by both abiotic and biotic stresses. In addition, the finding that nsLTPs can both protect against the accumulation of ROS during stress and serve as antifungal proteins suggests that overexpressing nsLTPs may be useful in a breeding scheme to enhance fungal pathogen resistance in turfgrasses. Creeping bentgrass (*Agrostis stolonifera*) nsLTPs may serve as targets for CRISPR activation (CRISPRa) to generate novel disease resistant bentgrass. As gene editing advances and may not involve the introduction of foreign DNA, CRISPR editing may be an ideal tool for creating modifications in susceptibility loci and to overexpress key resistance genes.

Investigating Unexpected Disease Symptoms of Bacterial Blight (*Xanthomonas arboricola*) on Hazelnut in New Jersey

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Xanthomonas arboricola pv. *corylina* is the causal agent of bacterial blight on hazelnut, a disease first described in Oregon (Barss, 1913) and currently found worldwide. Symptoms are expressed in the spring and include bud necrosis, spots on husks (involucres) and nuts, and water-soaked lesions on stems that lead to girdling and eventual dieback of young branches (Lamichhane and Varvaro, 2014). The disease has greatest impact on young trees and can cause mortality in severe cases. Outbreaks result in substantial losses in young orchards and nursery systems. In New Jersey, symptoms similar to bacterial blight have been observed on hazelnut, including bacterial spots on leaves, necrotic lesions on stems, and high incidence of husk spots. However, there are discrepancies between these symptoms and what is currently observed in Oregon. The most noticeable dissimilarity is the lack of characteristic branch dieback. Instead, trees seem to grow through infections with dramatic twisting of the leaves and branches where necrotic lesions are present. Also, there is considerable proliferation of coalescing husk spots unlike that observed in Oregon. Further, these symptoms persist and develop through the entire growing season, rather than solely in the spring. Multilocus sequence analysis using partial sequences of seven housekeeping genes was conducted to confirm the pathogen's species identity, however phylogenetic analysis according to Fischer-Le Saux et al. (2015), revealed the New Jersey isolates were genetically different from *X. arboricola* pv. *corylina* found in Oregon. Interestingly, New Jersey isolates cluster more closely with *X. arboricola* pv. *pruni*, which infects stone fruits and almond, and was not previously reported to infect hazelnut.

Investigations of the disease are underway, and a new disease response rating scale has been developed. This rating scale was used to evaluate a wide germplasm collection in the field in 2021 and results revealed considerable variation across hazelnut genotypes but consistency among clonal material. These findings have implications for breeding programs, as breeding for resistance is an important component of disease management. Currently, the only strategy to manage bacterial blight in hazelnut orchards is copper application (Lamichhane and Varvaro, 2014); however, there is risk of bacterial resistance to copper, especially with repeated applications. This is of particular concern because the prolonged disease period in New Jersey allows ample opportunity for bacteria to proliferate and spread, therefore requiring multiple applications in a season for effective management. To protect future yields, by proxy of maintaining the trees' photosynthetic ability and overall health, improved management is necessary.

The differences described here suggest there may be a new (genetically distinct) strain of *X. arboricola* infecting hazelnuts in New Jersey. This discovery has implications for orchard

management and hazelnut breeding targets. The disease response rating scale will be used in future years to evaluate resistance among germplasm in the field and to lay the framework for understanding mechanisms of host-pathogen interactions in future research.

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Dollar Spot and Spring Green-up Responses of Kentucky Bluegrass Subjected to Traffic

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We are researching traffic applications to Kentucky bluegrass (*Poa pratensis* L.) cultivars and experimental selections to improve the playing surface quality of recreational sites. We observed several unexpected Kentucky bluegrass responses to traffic during 2020 and 2021; the objective of this abstract is to report on dollar spot disease (caused by *Clarireedia jacksonii*) and spring green-up responses of Kentucky bluegrass subjected to traffic. The 2017 NTEP Kentucky bluegrass test (three replications of 89 entries) was seeded in September 2017 on a loam in North Brunswick, NJ. Traffic was applied in a strip across $\frac{1}{2}$ of each plot using a combination of the Rutgers Wear Simulator and the Cady Traffic Simulator during autumn 2018, summer and autumn 2019, and summer and autumn 2020; the other approximate $\frac{1}{2}$ of each plot did not receive traffic (no traffic). Dollar spot and spring green-up were visually evaluated using a 1 to 9 scale where 9 equaled the least dollar spot and best spring green-up, respectively. Data were analyzed as a 2 (no traffic and traffic) x 89 (entries) factorial strip-plot design. Greater dollar spot incidence was observed in no traffic plots compared to traffic plots on 25 June and 20 July 2020. A significant traffic x entry interaction was detected on 25 June 2020; entries that exhibited the most severe dollar spot incidence in no traffic plots were DLFPS-340/3549, DLFPS-340/3548 Blue Knight, RAD 553, and A11-38. Trafficked Kentucky bluegrass plots exhibited better spring green-up compared to no traffic plots on 9 April 2021; a significant traffic x entry interaction was detected on this rating date. Fifty-one entries had better spring green-up under the level of traffic compared to no traffic. Entries with the best spring green-up under both levels of traffic were Barvette HGT, Barserati, A11-40 and A99-2897.

Improvement of Heat Tolerance in Creeping Bentgrass By Sitosterol Involving Regulation of Antioxidant Metabolism

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Cool-season turfgrasses, such as creeping bentgrass (*Agrostis stolonifera* L.) are adversely affected by prolonged heat stress and experience loss of yield and performance resulting from premature leaf senescence. The objectives of this study included determining whether exogenous application of sitosterol, an organic membrane-stabilizing compound, could enhance heat tolerance in creeping bentgrass and to understand the manners in which sitosterol may affect antioxidant metabolism. Mature creeping bentgrass plants were placed under heat stress (35/30 °C, day/night) or optimal (22/18 °C, day/night) temperature conditions for a duration of 28 d within climate-controlled growth chambers and were foliar-treated on a weekly basis with 400 µM sitosterol. Plants treated with sitosterol had significantly higher turf quality and chlorophyll content than untreated controls at 21 and 28 d of heat stress while electrolyte leakage was significantly lower at those dates. Additionally, malondialdehyde content was significantly lower in treated plants at 21 and 28 d of heat stress. The activities of four antioxidants, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were significantly increased in response to sitosterol treatment from 14-28 d of heat stress. These findings indicate that application of sitosterol may improve heat tolerance in plants under heat stress mainly by decreasing lipid peroxidation of membranes and promoting antioxidant metabolism.

Biomass Yield Trial of Experimental Switchgrass Breeding Selections

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Switchgrass (*Panicum virgatum*) has been classically used as a forage crop. More recently switchgrass has gained a lot of attention as a potential bioenergy crop due to its potential for high biomass yields and wide range of adaptability across the continental United States. A major challenge preventing the widespread use of switchgrass in the warm and moist climate of the Northeastern US is the fungal pathogen *Colletotrichum navitas*, which is the causal agent of anthracnose disease of switchgrass. To overcome this challenge, breeding efforts have been undertaken to select for new switchgrass cultivars that are anthracnose resistant, high biomass yielding and suitable for growth on marginal lands. This study screened 8 different experimental cultivars that were selected by teams at Rutgers and Cornell Universities and compared them to four current commercial cultivars of switchgrass. Each cultivar was seeded in an 8 ft x 3 ft plot in quadruplicate in the spring of 2019 and was subsequently screened for anthracnose resistance and biomass yield in 2020 and 2021. Two years of biomass data collection found that the experimental upland cultivar mineland-upland-biomass (MUB) produced significantly more biomass when compared to the commercial cultivar Carthage at an alpha level of 0.05. Going forward, additional selections from this MUB population can be made from breeding nurseries to further increase biomass yield potential and anthracnose resistance.

Genetic Mapping of Summer Patch Resistance in the Hard Fescue R10 X S5 Mapping Population

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Hard fescue (*Festuca brevipila* Tracey) is a cool-season turfgrass known for exceptional performance under low-maintenance conditions but is damaged by summer patch disease. Summer patch is a root disease caused by *Magnaporthiopsis poae* and *Magnaporthiopsis meyeri-festuciae*. The objectives of this study were to develop the mapping population, construct a genetic linkage map, and identify quantitative trait loci (QTL) for summer patch resistance in hard fescue. The parental resistant clone R10 and parental susceptible clone S5 were selected in a preliminary study based on the phenotypic performance toward summer patch resistance. Full-sib progeny populations were constructed by R10 (♀) x S5 (♂) and S5 (♀) x R10 (♂). One hundred progeny for each population (200 progeny total) were established in three identical mowed spaced-plant trials in 2017 (Trial 1), 2019 (Trial 2), and 2020 (Trial 3). The populations were arranged in a randomized complete block design with four replications. A mixture of an *M. meyeri-festuciae* isolate (SCR9) and an *M. poae* isolate (C11) served as inoculum for the trials. The disease severity of hard fescue clones was assessed by visual rating on a scale of 1 to 10 during the summers of 2018, 2019, 2020, and 2021. Next Generation Sequencing was performed, and the sequence data were analyzed by Stacks to find the SNPs. We obtained 7914 SNPs shared by 90% of samples by SNPs detection with the reference genome involved. A linkage map was constructed using 1090 SNP markers spanning 990.03 cM, and interval mapping analysis identified four QTL that explained 8.6-16% of phenotypic variance associated with the summer patch resistance. This is the first report for QTL mapping of summer patch resistance in hard fescue and will help in the development of hard fescue cultivars with improved resistance to summer patch in a more efficient method.

Tall Fescue Seeding Improves False-Green Kyllinga (*Kyllinga gracillima*) Control with Herbicides

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False-green kyllinga (*Kyllinga gracillima* Miq.) is a problematic C₄ perennial sedge (Cyperaceae) species in turfgrass systems. Previous research found that multiple spring herbicide applications are required for postemergence false-green kyllinga control, but non-chemical strategies or late summer applications have not been examined. The objective of this research was to evaluate the effect of tall fescue (*Festuca arundinacea*) interseeding in combination with late summer postemergence herbicide applications for false-green kyllinga control.

Replicate field experiments were conducted adjacently from 2019 to 2020 (Year 1) and 2020 (Year 2) to 2021. The site was a mature stand of false-green kyllinga (>95% kyllinga cover in mid-summer) established by sodding turf from an infested golf course fairway at Woodlake Country Club in Lakewood, NJ. Seven herbicide treatments and a non-treated control were arranged in a complete factorial with tall fescue interseeding or no interseeding designed in a strip-plot randomized complete block design with three replications. Herbicide treatments consisted of single applications of halosulfuron-methyl (70 g ha⁻¹), imazosulfuron (420 g ha⁻¹), and sulfentrazone + carfentrazone (280 + 30 g ha⁻¹) applied 4 weeks before tall fescue seeding (4 WBS) and on the day of seeding. Glyphosate (220 g ae ha⁻¹) applied on the day of seeding was also included. Turf-type tall fescue was seeded at 360 kg PLS ha⁻¹ in September. False-green kyllinga control was evaluated visually on a 0 (no control) to 100 (complete control) percent scale in June, July, and August. A grid intersect count was conducted in July and August. Turfgrass quality was assessed only in the seeded strips on a 1 to 9 scale. Data were subjected to ANOVA in SAS (P = 0.05) with means separated using Fisher's protected LSD test. The combination of tall fescue interseeding with imazosulfuron or halosulfuron applied 4 WBS was the most effective program in both years. Imazosulfuron 4 WBS combined with interseeding resulted in ≤ 1% false-green kyllinga cover in both years and 15 and 42% cover in Year 1 and 2, respectively, without interseeding. Similarly, halosulfuron 4 WBS and glyphosate at seeding resulted in ≤ 12% cover with interseeding and 49 to 71% cover without interseeding in both years. Interseeding without herbicides resulted in 46 and 67% false-green kyllinga cover compared to 100 and 91% cover in the non-seeded control, in Year 1 and 2, respectively. Tall fescue interseeding improved herbicide efficacy of all herbicides in Year 2 and all herbicides except imazosulfuron in Year 1. Turfgrass quality data were pooled across years. Imazosulfuron, halosulfuron, and sulfentrazone + carfentrazone applied the day of interseeding reduced turfgrass quality to <6.0 in May. Among treatments applied 4 WBS, only imazosulfuron reduced turfgrass quality in May, but these quality reductions were no longer apparent by June.

Applying imazosulfuron (420 g ha⁻¹) or halosulfuron (70 g ha⁻¹) in late summer followed by interseeding turf-type tall fescue as soon as four weeks later is an effective strategy for selective POST false-green kyllinga control in cool-season turfgrass; glyphosate applied the day of interseeding is an effective non-selective option. Herbicides or interseeding alone provided poor false-green kyllinga control.

Soil pH Effect on Anthracnose of Annual Bluegrass

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Anthracnose (*Colletotrichum cereale* Manns *sensu lato* Crouch, Clarke, and Hillman) is a devastating disease of annual bluegrass [*Poa annua* L. f. *reptans* (Hauskn) T. Koyama] putting green turf. Previous research has indicated that anthracnose on annual bluegrass was more severe when the mat layer pH was lower than 5.5. Applications of lime increase soil pH and soil Ca, but it is not known whether both affect anthracnose severity. Therefore, the objective of this research was to determine whether anthracnose severity on annual bluegrass putting green turf responds to soil pH and/or soil Ca. A trial was conducted on an annual bluegrass mowed at 2.8-mm in North Brunswick NJ from 2019 to 2021. Five rates of lime and two rates of gypsum and sulfur were applied in 2020 and 2021. All materials were applied on 17 Feb in 2020 and 10 Mar and 5 Apr in 2021. Three additional gypsum applications were applied at a 4- to 6-wk interval to maintain increased calcium availability during the growing season. Anthracnose was rated every week after symptoms appeared and the area under the disease progress curve (AUDPC) was calculated to evaluate disease severity. Soil amendments had a significant effect on AUDPC, mat layer pH, mat layer Ca, and shoot tissue Ca in all years. However, only the mat layer pH response to soil amendment was independent of year; the response of AUDPC, mat layer Ca, and shoot tissue Ca to soil amendment were not independent of year. There was a linear decrease in anthracnose severity as lime rate increased in all years. Gypsum treatments decreased disease in 2019 and 2020 compared to the control but had no effect in 2021. Thus, under acidic soil conditions, both lime and gypsum reduced anthracnose severity compared to the control, although the effect of gypsum was less consistent. Inconsistency may be related to differences between gypsum and lime effects on mat layer pH and Ca, shoot tissue Ca, or other nutrients. Further data analysis to clarify the extent to which anthracnose severity is affected by mat layer pH and Ca and shoot tissue Ca is being conducted.

Autumn-applied Fungicide Timing Effects on the Development of Dollar Spot

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Anecdotal evidence suggests that autumn-applied fungicide has the potential to reduce dollar spot caused by *Claviceps jacksonii* during the following growing season; however, the appropriate timing is not well understood since multiple autumn applications are typically made on golf courses. Eight fungicide treatments and a non-treated control were arranged in a randomized complete block design with four (2019) and six (2020) replications to evaluate the suppression of dollar spot during the subsequent growing season. The trial was conducted on a 3- and 4-year-old '007' creeping bentgrass (*Agrostis stolonifera*) turf mowed at 9.5 mm in North Brunswick, NJ. Dollar spot developed naturally in the trial area before the initiation of the study in September 2019. Fungicide treatments (fluazinam at 0.7 kg a.i. per ha) were initiated after pre-trial suppression of dollar spot on the entire study area; seven treatments received a tank mix of fluazinam (0.7 kg a.i. per ha) and propiconazole (1.5 kg a.i. per ha) once (three timings), twice (three timings), or thrice (one timing) in September, October and/or November in 2019 and 2020. An eighth treatment received chlorothalonil (15.3 kg a.i. per ha) thrice. The turf area with dollar spot infection centers was assessed every 1 to 7 days from September to November in 2019 and 2020, and May to the termination of study in July 2020 and August 2021. Except for the single November application in 2019, treatments that used the tank mix of fluazinam and propiconazole suppressed dollar spot severity (25 to 42% in 2020 and 7 to 41% in 2021) during the subsequent growing season in both years, compared to the non-treated check. However, the chlorothalonil treatment resulted in slight (15% in 2020) or no (2021) reduction in disease severity, compared to the non-treated check even though chlorothalonil suppressed disease symptoms the previous autumn in both years. The tank mix application of fluazinam and propiconazole in September-October or September-October-November provided the best suppression of dollar spot the following spring and early-summer in both years. The potential of the November timing of fluazinam and propiconazole to reduce disease was probably dependent on environment conditions at the time of application. Dollar spot was not active during the colder weather in November 2019, whereas warmer weather resulted in active dollar spot in November 2020. Research is in progress to determine the impact of host susceptibility, inoculum load, fungicide chemistry, and autumn-applied fungicide timing based on calendar date, disease threshold, and disease predictive model output on the development of dollar spot during the subsequent growing season.

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