

Symposium Organizing Committee

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Proceedings of the Thirty-Second Annual Rutgers Turfgrass Symposium

Bradley Park and Barbara Fitzgerald, Editors

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Director's Remarks

Welcome to the 32nd Annual Rutgers Turfgrass Symposium. Established in 1991, the Symposium provides Rutgers faculty, students, staff, and guests the opportunity to exchange ideas and encourage collaboration on research topics in turfgrass science. One of the foci of this year's symposium is the discussion of a multi-omics approach to plant improvement, which hopefully will encourage ideas for a future meeting and research on genetic sequencing of turfgrasses.

I thank our invited speakers, Dr. Gary Muehlbauer (Department of Agronomy and Plant Genetics, University of Minnesota) who will present a keynote on "Genomics Enabled Gene Discovery and Crop Improvement in Barley", as well as Dr. Renaud Rincent (French National Institute for Agricultural Research), Dr. J. Mitch Elmore (USDA – ARS, Cereal Disease Lab), Dr. Glen Groben (USDA – ARS, Foreign Disease-Weed Science Research Unit), and Dr. Ming Yi Chou (Department of Plant Pathology, University of Wisconsin - Madison), and all the Center faculty and students who have agreed to present at this year's symposium.

I also thank Drs. Thomas Gianfagna, Ning Zhang, Rong Di, and Mr. Brad Park for serving as session moderators and the Planning Committee comprised of Drs. Faith Belanger (Symposium Chair), Stacy Bonos, Rong Di, Mr. Brad Park, and Ms. Barbara Fitzgerald for their contributions to the Symposium. Co-editors of the Symposium Proceedings were Mr. Brad Park and Ms. Barbara Fitzgerald. We appreciate the technical support of Mr. Bernard Ward who made it possible to live stream this year's Symposium.

Our graduate students continue to be recognized for their excellence. Three graduate students were recognized during oral and poster paper competitions at the annual meeting of the Crop Science Society of America in Baltimore, MD. Mr. William Erickson received first place for his oral presentation in the Molecular Techniques, Genetics, Microbiome, and Turfgrass Breeding session and first place for his poster presentation in the Turfgrass Physiology, Molecular Biology, Microbiome, and Genetics session. Mr. Pingyuan Zhang was awarded first place for his poster presentation in Turfgrass Pest Management: Disease, Insects, Weeds – Golf session and third place for his oral presentation in the Turfgrass Pest Management: Disease, Insects, Weeds session. Ms. Stephanie Rossi received second place for her oral presentation in the Crop Physiology and Metabolism session.

Ms. Stephanie Rossi (Ph.D. candidate) and Dr. Cathryn Chapman (now at the University of Connecticut) were awarded a Watson Fellowship from the Golf Course Superintendents Association of America, which recognizes graduate students who have been identified as scientists that will go on to be leaders in turfgrass management. I am also pleased to announce that, due to the generosity of Mr. Sean Pattwell, a new award for graduate students studying turfgrass science was developed during 2022. Ms. Katherine Diehl was the first recipient of the Sean S. Pattwell Graduate Student Internship, which allowed her to expand her educational experience at Bandon Dunes Golf Resort in Bandon, Oregon.

Finally, we are indebted to the outstanding industry partnerships from throughout the state, region, and nation, which provides invaluable intellectual, material, and financial support. The Center for Turfgrass Science is better for it.

We are glad that you chose to spend time with us and hope that you enjoy the many opportunities that the Rutgers Turfgrass Symposium has to offer.

Sincerely,

A handwritten signature in cursive script that reads "James A. Murphy". The signature is written in black ink and is positioned below the word "Sincerely,".

James A. Murphy, Director

Table of Contents

Symposium Organizing Committee	1
Director's Remarks.....	2
Table of Contents	4
Schedule	7
PLENARY PRESENTATIONS	9
Alleviation of Heat-induced Leaf Senescence in Creeping Bentgrass by Application of Protease Inhibitors Associated with Suppression of Protein Degradation	10
<i>Stephanie Rossi and Bingru Huang</i>	
Mobile Remote Sensing-based and Artificial Intelligence-guided Turfgrass Water Conservation Programs	11
<i>Bingru Huang</i>	
Optimizing the Use of Annual Bluegrass Weevil to Control Annual Bluegrass in Creeping Bentgrass Fairways	12
<i>Albrecht M. Koppenhöfer, Anna Luiza Sousa, Matthew Elmore, and Katherine Diehl</i>	
Phenomic Selection: a Low-cost and Efficient Alternative to Genomic Selection	14
<i>Renaud Rincent</i>	
Using Genomics to Help Guide Breeding for Summer Patch Tolerance in Kentucky Bluegrass.....	15
<i>Stacy A. Bonos, Christine Kubik, Jennifer Vaiciunas, Olivia Wright, and Josh Honig</i>	
Genomics Enabled Gene Discovery and Crop Improvement in Barley	16
<i>Gary J. Muehlbauer</i>	
Golf, Agronomy and Research at Bandon Dunes (the 2022 Sean M. Pattwell Scholarship).....	17
<i>Katherine Diehl</i>	

Topdressing Sand Size Effects on Mat Layer Development of of Creeping Bentgrass.....	18
<i>James A. Murphy, Hui Chen, James Hempfling, Chas Schmid, Zhongqi Xu, Susan Butterworth and Bradley S. Park</i>	
Investigating Turfgrass Disease Suppressive Soil Microbiome - Metagenomics, Culturomics, and Metabolomics.....	22
<i>Ming Yi Chou</i>	
Integrative Multi-omics Approaches to Dissect Plant Stress Responses.....	23
<i>J. Mitch Elmore</i>	
Oxford Nanopore Technologies Sequencing Approaches for Mycobiome Analysis of Tall Fescue Under Drought Stress and Diagnostics of Cool-Season Turfgrass Diseases	24
<i>Glen Groben, Bruce B. Clarke, Lee J. Kerkhof, Stacy A. Bonos, William A. Meyer, Yuanshuo Qu, Jing Luo, Emily Walsh, Ning Zhang</i>	
The <i>Epichloë festucae</i> Antifungal Protein Can Reduce Dollar Spot Symptoms on Creeping Bentgrass.....	26
<i>Faith C. Belanger, Patrick Fardella and Bruce B. Clarke</i>	
POSTER PRESENTATIONS.....	27
Dithiopyr Resistance and Alpha-Tubulin Mutations Vary by Ecotype for Goosegrass (<i>Eleusine indica</i>) in New Jersey	28
<i>Katherine Diehl and Matthew Elmore</i>	
Gene Editing of Creeping Bentgrass to Improve Dollar spot Disease Resistance.....	29
<i>Alison Dineen, Stacy Bonos and Rong Di</i>	
Colonization Stability and Efficacy of Plant-Growth-promoting Rhizobacteria in Creeping Bentgrass.....	30
<i>William Errickson, Khasif Jaleel, Bingru Huang</i>	
Investigating White Grub Resistance in Turf-Type Tall Fescue	31
<i>Jennifer Halterman and Stacy Bonos</i>	
Characterizing Major Gene Resistance Expression to Eastern Filbert Blight in New Jersey.....	32
<i>D. Jacobs, S.A. Mehlenbacher, J. M. Capik, and T. J. Molnar</i>	
Establishment of Zoysiagrass and Bermudagrass in New Jersey	34
<i>Mark LaBarge, Stacy A. Bonos, Daniel L. Ward, James A. Murphy, and Kevin Sullivan</i>	

Evaluation of Creeping Bentgrass (<i>Agrostis stolonifera</i>) Shade Tolerance Under Simulated Shade.....	36
<i>Eric MacPherson, Daniel Wu, Domonic Petrella, and Stacy A. Bonos</i>	
Physiological Regulation and Mitigation of Summer Decline of Annual Bluegrass Using Plant Heath Products	37
<i>Sean McBride, James Murphy, Bingru Huang</i>	
Precision Breeding of Turfgrass to Enhanced Resistance to Fungal Pathogens	38
<i>John E. McLaughlin and Rong Di</i>	
Response of Kentucky Bluegrass to Traffic During 2021	41
<i>Bradley S. Park and James A. Murphy</i>	
Morphactin-mediated Amelioration of Heat-induced Leaf Senescence Associated with Alterations in Chlorophyll Metabolism in Creeping Bentgrass	42
<i>Stephanie Rossi and Bingru Huang</i>	
QTL Mapping of Anthracnose Disease Severity in a Switchgrass F1 Mapping Population	43
<i>Christopher Tkach, Christine Kubik, Jennifer Vaiciunas, Joshua Honig, John E. Carlson, Stacy Bonos</i>	
Efficacy of Preemergence Herbicides for False-Green Kyllinga (<i>Kyllinga gracillima</i>) Control from Seed	44
<i>Trevor S. Watson, Matthew T. Elmore, Aaron J. Patton, Brandon C. McNally, and Daniel P. Tuck</i>	
Genetic Mapping of Summer Patch Resistance in Hard Fescue	45
<i>Daniel (Shidi) Wu, Austin Grimshaw, Shaun Bushman, Matthew Robbins, Jennifer N. Vaiciunas, Josh A. Honig, Phillip L. Vines, William A. Meyer and Stacy Bonos</i>	
Bentgrass Cultivar and Autumn-applied Fungicide Timing Effects on Suppression of Dollar Spot	46
<i>Pingyuan Zhang, Glen Groben, Patrick Fardella, Bruce Clarke, James Murphy</i>	
Lipid Metabolism Associated with Heat Tolerance in Hard Fescue	48
<i>Krishna Katuwal</i>	
PRE-REGISTERED PARTICIPANTS	49

THIRTY-SECOND ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

March 16, 2023

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

8:30 - 9:00 AM	Registration
9:00 AM	Welcome – Laura Lawson (<i>Executive Dean of Agriculture and Natural Resources</i>)
9:10 - 10:40 AM	SESSION I: Stress Tolerance (Moderator: Thomas Gianfagna)
9:10 - 9:30	Stephanie Rossi (<i>Department of Plant Biology, Rutgers University</i>) – Alleviation of Heat-induced Leaf Senescence in Creeping Bentgrass by Application of Protease Inhibitors Associated with Suppression of Protein Degradation
9:30 - 9:50	Bingru Huang (<i>Department of Plant Biology, Rutgers University</i>) – Mobile Remote Sensing-based and Artificial Intelligence-guided Turfgrass Water Conservation Programs
9:50 - 10:10	Albrecht Koppenhöfer (<i>Department of Entomology, Rutgers University</i>) – Optimizing the Use of Annual Bluegrass Weevil to Control Annual Bluegrass in Creeping Bentgrass Fairways
10:10 - 10:40 AM	Discussion and Break
10:40 - 12:00 PM	SESSION II: Breeding / Genomics (Moderator: Ning Zhang)
10:40 – 11:00	Renaud Rincent (<i>French National Institute for Agricultural Research</i>) – Phenomic Selection: a Low-cost and Efficient Alternative to Genomic Selection
11:00 – 11:20	Stacy Bonos (<i>Department of Plant Biology, Rutgers University</i>) – Using Genomics to Help Guide Breeding for Summer Patch Tolerance in Kentucky Bluegrass
11:20 - 12:00	KEYNOTE: Gary Muehlbauer (<i>Department of Agronomy and Plant Genetics, University of Minnesota</i>) – Genomics Enabled Gene Discovery and Crop Improvement in Barley
12:00 – 1:00 PM	Lunch Break and Poster Session

1:00 – 1:40 PM	SESSION III: Turf Management (Moderator: Bradley Park)
1:00 – 1:20	Katherine Diehl (<i>Department of Plant Biology, Rutgers University</i>) Golf, Agronomy and Research at Bandon Dunes (the 2022 Sean M. Pattwell Scholarship)
1:20 – 1:40	James Murphy (<i>Department of Plant Biology, Rutgers University</i>) Topdressing Sand Size Effects on Mat Layer Development of Creeping Bentgrass
1:40 – 2:00 PM	Discussion Session
2:00 – 3:20 PM	SESSION IV: Pathology (Moderator: Rong Di)
2:00 – 2:20	Ming Yi Chou (<i>Department of Plant Pathology, University of Wisconsin - Madison</i>) Investigating Turfgrass Disease Suppressive Soil Microbiome – Metagenomics, Culturomics, and Metabolomics
2:20 – 2:40	J. Mitch Elmore (<i>USDA – ARS, Cereal Disease Lab</i>) Integrative Multi-omics Approaches to Dissect Plant Stress Responses
2:40 – 3:00	Glen Groben (<i>USDA</i>) Oxford Nanopore Technologies Sequencing Approaches for Mycobiome Analysis of Tall Fescue under Drought Stress and Diagnostics of Cool-Season Turfgrass Diseases
3:00 – 3:20	Faith Belanger (<i>Department of Plant Biology, Rutgers University</i>) – The <i>Epichloë festucae</i> antifungal Protein Can Reduce Dollar Spot Symptoms on Creeping Bentgrass
3:20 – 3:45 PM	Discussion Session and Closing Remarks
3:45	Social Hour and Poster Session

PLENARY PRESENTATIONS

Alleviation of Heat-induced Leaf Senescence in Creeping Bentgrass by Application of Protease Inhibitors Associated with Suppression of Protein Degradation

Stephanie Rossi and Bingru Huang

Department of Plant Biology, Rutgers University

Creeping bentgrass (*Agrostis stolonifera*) is a cool-season turfgrass that is sensitive to high temperatures, which accelerate leaf senescence and reduce turf performance. Protein hydrolysis is a process occurring during leaf senescence in which protease enzymes catalyze the degradation of pigments, such as chlorophyll, and is responsible for the symptomatic yellowing of leaves. The objectives of this study included examining whether exogenously applied protease inhibitors may suppress heat-induced leaf senescence in creeping bentgrass and to determine whether the effects of protease inhibitors on heat tolerance are associated with an alteration in protein metabolism. Creeping bentgrass plants were exposed to heat stress (35/30 °C, day/night) or non-stress control (22/18 °C, day/night) temperatures for 35 d using environment-controlled growth chambers and leaves were exogenously treated with 1 µM leupeptin, 10 µM aprotinin, or 10 µM pepstatin A weekly. Plants treated with leupeptin or pepstatin A had significantly higher turf quality from 14 through 35 d of heat stress, while turf quality was significantly higher in aprotinin-treated plants from 14 through 28 d of heat stress. Photochemical efficiency was significantly higher in plants treated with any of the protease inhibitors at 0 d and from 21 through 35 d of heat stress. Leupeptin- and aprotinin-treated plants had significantly higher chlorophyll content from 7 through 35 d of heat stress, while pepstatin A-treated plants maintained significantly higher chlorophyll content at 7 d and from 21 through 35 d of heat stress. Under heat stress, endogenous protein content was significantly higher in all protease inhibitor-treated plants while general protease activity and the content of a majority of proteinogenic amino acids were significantly lower, suggesting that proteins were hydrolyzed at a lower rate. These findings indicate that exogenous application of protease inhibitors can suppress heat-induced leaf senescence and enhance turf performance by delaying proteolysis.

Mobile Remote Sensing-based and Artificial Intelligence-guided Turfgrass Water Conservation Programs

Bingru Huang

Department of Plant Biology, Rutgers University

This presentation presents a USDA-SCRI funded project on mobile remote sensing-based and artificial intelligence-guided turfgrass irrigation program for how to use remote sensing technology to develop precision irrigation management programs for water conservation and high through-put phenotyping to select drought-tolerant turfgrass germplasms. Remote sensing technology can be used to monitor plant health and soil moisture content in turfgrass areas, allowing for better irrigation management and water conservation. The presentation will outline the potential benefits of remote sensing-based phenotyping and precision irrigation systems for turfgrass management, such as improved water conservation, reduced costs, and improved turfgrass health. The presentation concludes by discussing the challenges associated with implementing remote sensing-based precision irrigation systems and potential solutions to these challenges.

Optimizing the Use of Annual Bluegrass Weevil to Control Annual Bluegrass in Creeping Bentgrass Fairways

Albrecht M. Koppenhöfer¹, Anna Luiza Sousa¹, Matthew Elmore² and Katherine Diehl²

¹*Department of Entomology, Rutgers University*

²*Department of Plant Biology, Rutgers University*

The annual bluegrass weevil (ABW) is a serious and expanding pest with demonstrated ability to develop resistance to a range of insecticides under the commonly used management regimes. In previous research, we found that threshold-based ABW control (treat only if damage starts to appear) can reduce annual bluegrass (ABG) cover compared to industry-standard control programs while allowing only minimal and temporary loss in turf quality. ABW offered similar selective ABG control as 1 year's worth of monthly paclobutrazol applications. ABW controlled ABG more effectively at lower ABG percentages, while at the lower ABG populations damage was less obvious. Hence, in areas with high ABG percentages, paclobutrazol alone could be used to reduce ABG cover to the point where ABW activity can be tolerated without compromising quality. At that point, paclobutrazol applications may be reduced or even eliminated and ABG management provided by ABW alone, thereby reducing the use of both PGRs and insecticides. However, one key component in the implementation of this approach still needed to be explored in more detail: the effect of CBG cover on ABG control and turfgrass quality (i.e., the point when paclobutrazol applications could be reduced or stopped).

In the ongoing study, we continued these studies in fields maintained as fairways with a mix of ABG and CBG with plots at the beginning of the study having approximately 0%, 10%, 25% and 50% ABG cover, the rest CBG. In the 1st experiment, ABG percentage was combined with three different ABW management programs: no control, threshold-based control, and preventive control. In the 2nd experiment, the same ABG percentages were all managed with threshold-based control. Both experiments were conducted in 2 runs. Here we report primarily about the 1st runs of both experiments that were concluded in October 2022; the 2nd runs will be concluded in October 2023.

In both experiments, ABG cover tended to decline throughout the season especially in plots with higher ABG cover at the beginning of the year. The decline was the strongest between May and June when spring generation ABW larvae were active. The greater the decline in ABG, the greater was the loss in turf quality. However, in all treatments turf quality loss was never below 5 (1-9 scale) and was never below 6 for more than about 2 weeks, except for 3 weeks in 2021 in the non-treated 50% plots.

During 2021, there was no clear and significant effect of ABW management program on ABG cover. Low ABW population densities and well above average precipitation rates throughout much of the growing season limited ABW damage to ABG and allowed ABG to recover better from it. During 2022, ABG declined more in the threshold and untreated treatments, but there was a less clear effect on turf quality than in 2021. Above average rainfall during the time when ABW spring larvae were active may have limited the negative effects of their feeding activity on the turf quality. And when rainfall dropped dramatically thereafter, the hot and dry conditions may have limited the recovery of ABG in all treatments.

Overall, the threshold approach for ABW management seems to be a viable alternative to the preventive approach for ABW management that will only result in very limited and transient reduction in turfgrass quality while allowing ABW to help reduce the ABG cover. Based on our observations to date, this approach should be viable with initial ABG cover of at least up to 30%. Whether a higher initial ABG cover might be feasible will depend on the tolerance to transient turf quality decline of the specific golf course. If the goal is to more dramatically reduce ABG cover, either in speed or percent reduction, the approach will likely have to be combined with other methods to reduce ABG such as periodical applications of the PGR paclobutrazol as done in our previous study.

Phenomic Selection: A Low-Cost and Efficient Alternative to Genomic Selection

Renaud Rincent

French National Institute for Agricultural Research

Genomic selection - the prediction of breeding values using DNA polymorphisms - is a disruptive method that has widely been adopted by animal and plant breeders to increase genetic gain. It was recently shown that other sources of molecular variations such as those resulting from transcripts or metabolites could be used to accurately predict complex traits. These endophenotypes have the advantage of capturing the expressed genotypes and consequently the complex regulatory networks that occur in the different layers between the genome and the phenotype. However, obtaining such omics data at very large scales, such as those typically experienced in breeding, remains challenging. As an alternative, we proposed using near-infrared spectroscopy (NIRS) as a high-throughput, low cost and non-destructive tool to indirectly capture endophenotypic variants and compute relationship matrices for predicting complex traits, and coined this new approach "phenomic selection" (PS). We tested PS on bread wheat (*Triticum aestivum* L.) diversity panels and breeding material using NIRS measured on various tissues (grains, leaves). We showed that one could reach predictions as accurate as with molecular markers, for developmental and productivity traits, even in environments radically different from the one in which NIRS were collected. Our work constitutes a proof of concept and provides new perspectives for the breeding community, as PS is theoretically applicable to any organism at low cost and does not require any molecular information.

References:

- Rincent R, Charpentier J-P, Faivre-Rampant P, et al (2018) Phenomic Selection Is a Low-Cost and High-Throughput Method Based on Indirect Predictions: Proof of Concept on Wheat and Poplar. *G3: Genes|Genomes|Genetics* g3.200760.2018. <https://doi.org/10.1534/g3.118.200760>
- Robert P, Auzanneau J, Goudemand E, Oury F-X, Rolland B, Heumez E, Bouchet S, Le Gouis J, Rincent R. (2022) Phenomic Selection in Wheat Breeding: Identification and Optimisation of Factors Influencing Prediction Accuracy and Comparison to Genomic Selection. *Theor. Appl. Genet.* **2022**. <https://doi.org/10.1007/s00122-021-04005-8>.

Using Genomics to Help Guide Breeding for Summer Patch Tolerance in Kentucky Bluegrass

Stacy A. Bonos, Christine Kubik, Jennifer Vaiciunas, Olivia Wright, and Josh Honig

Department of Plant Biology, Rutgers University

Summer patch disease caused by *Magnaporthiopsis poae* and *Magnaporthiopsis meyeri-festuceae* is one of the most important diseases affecting Kentucky bluegrass (*Poa pratensis* L.) in the mid-Atlantic and transition zone regions of the United States and other regions of the world. Selection for summer patch tolerance requires optimal growing conditions that include warm temperatures and significant rainfall during the spring and early summer. Inoculations are possible but it requires growing the fungus on sterilized oats and placing the inoculum next to Kentucky bluegrass roots up to 18 months before symptoms are visible.

Certain cultivars including Midnight, Bolt and Cabernet are tolerant of summer patch disease but due to the apomictic breeding behavior of Kentucky bluegrass, it is not always possible to recover or combine summer patch tolerance with other important agronomic traits such as seed yield into new hybrids that are developed. Additionally, the breeding cycle for Kentucky bluegrass takes between 5-10 years before a new cultivar is commercially produced. This means if one of the parents in a cross turns out to be susceptible to summer patch after crosses are made it will take several years to resolve.

The DNA Genotyping Lab at Rutgers University conducted a SNP marker genetic diversity study in Kentucky bluegrass and categorized more than 200 Kentucky bluegrass cultivars and selections including all the entries in the 2017 National Kentucky Bluegrass Test sponsored by the National Turfgrass Evaluation Program (NTEP). The results of this genetic diversity study were compared to the phenotyping data for summer patch tolerance collected from the 2017 NTEP Kentucky bluegrass test at the Adelphia Research and Extension Farm in 2020 and 2021.

The results indicate that certain groups are more susceptible (Shamrock) to summer patch disease than other groups (Midnight). The Shamrock types have been used extensively in crosses because they have improved seed yield, however this new finding indicates that it may be beneficial to use different parents with improved summer patch tolerance or screen Shamrock crosses more aggressively for summer patch tolerance.

These results will help breeders select parents for future Kentucky bluegrass hybrid crosses. Ultimately, the goal is to identify the genetic region and/or identify SNP markers that are linked to summer patch susceptibility so that we can select against that trait while retaining high seed yield potential and other useful agronomic traits.

Genomics-Enabled Gene Discovery and Crop Improvement in Barley

Gary J. Muehlbauer

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN

The international barley genetics community has generated rich resources of genomics tools that have been used for both gene discovery and barley improvement. Two examples of the utility of these resources are discussed here. 1) *Fusarium* head blight, caused by the fungal pathogen *Fusarium graminearum*, is a major problem in barley production worldwide primarily due to the accumulation of trichothecene mycotoxins in the grain during infection. We used transcriptomics combined with selection in yeast to identify a barley gene, *HvUGT13248*, encoding a UDP-glucosyltransferase that detoxifies trichothecene mycotoxins. Subsequently, we developed transgenic barley plants overexpressing *HvUGT13248* and showed that these plants were highly resistant to FHB and reduced the amount of toxin in the grain. We next used a barley TILLING population to identify two *HvUGT13248* mutant alleles and showed that these plants were highly susceptible to FHB, demonstrating that this gene is important for barley resistance to FHB. Sequencing of *HvUGT13248* sequence from 496 accessions (elite varieties, landraces and wild barleys) resulted in identifying only 11 mutations, of which none of them conferred a susceptible or resistant phenotype. Our results indicate that *HvUGT13248* is a highly conserved gene and functional across a large set of barley germplasm. 2) The wild barley diversity collection (WBDC) is a key resource for identifying beneficial alleles for barley improvement. To exploit the WBDC, we developed an advanced backcross nested association mapping (AB-NAM) population by backcrossing twenty-five diverse wild barley accessions to the spring six row cultivar Rasmusson and derived 796 BC₂F_{4,6} lines. The population was genotyped with over 265,000 markers (384 SNP markers, and imputation of exome capture sequence data and 9K SNP markers from the parents) and phenotyped for numerous agronomic traits (e.g., yield, heading date, height, productive tiller number, wax production). We conducted genome-wide association mapping to identify QTL in the AB-NAM population and showed that there are key QTL that can be employed in barley breeding programs. This population can be used for both gene discovery and barley improvement.

Golf, Agronomy and Research at Bandon Dunes (the 2022 Sean M. Pattwell Scholarship)

Katherine Diehl

Department of Plant Biology, Rutgers University

“Links golf” is thought to be the oldest and original design and playing style of the game. Characterized by coastal terrain where grass is the natural vegetation, links golf courses are usually designed using the natural landscape and highlight its natural beauty. Many players prefer the firmer surfaces and distinct style of play required for links golf. Links style courses have become widely popular in the US, notably since 1999, following the opening of Bandon Dunes in Oregon, the first public links course in the US. The links courses at Bandon Dunes are among the most popular and highly-ranked courses in the country, making the result a top global golf destination.

The Pattwell Scholarship was initiated in 2022 to fund a summer internship for a turfgrass graduate student to learn and be inspired by links-style golf course management. This talk will discuss the agronomic practices and climate-based challenges learned by working as a greenskeeper for one month at Bandon Dunes resort. Personal insight on the daily practices and BMPs, and resource management constraints will be summarized to illustrate the capabilities and challenges faced by the Bandon Dunes agronomy department. Subject to eccentric architectural designs to non-stop golf traffic, they product high-quality turf and excellent course conditions for year-round links golf. As researchers we may learn a lot from their agronomic experience and philosophy; better understanding their agronomic needs is a valuable path to identifying problems warranting future research.

Topdressing Sand Size Effects on Mat Layer Development of Creeping Bentgrass

¹James A. Murphy, ²Hui Chen, ³James Hempfling, ⁴Chas Schmid, ¹Zhongqi Xu,
¹Susan Butterworth and ¹Bradley S. Park

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The application of topdressing sand to putting greens during the season is often avoided or applied at very low application rates (dusting) due to the potential of coarse sand particles interfering with play and dulling mower blades. Topdressing incorporates into the turf canopy better if the sand lacks very coarse and coarse particles. However, there is concern that finer topdressing sand may negatively alter the physical characteristics of putting surfaces. The objectives of this research were to determine the combined effects of eliminating coarse particles from topdressing sand and core cultivation on physical properties of a creeping bentgrass (*Agrostis stolonifera*) turf.

A field trial was initiated in May 2016 on a 19-month-old ‘Shark’ creeping bentgrass maintained at 2.8-mm height of cut on a sand-based root zone. The experimental design was a 3 x 2 x 2 factorially arranged randomized complete block with four blocks. The factors were sand size (medium-coarse, medium-fine, fine-medium), quantity of mid-season topdressing (244- or 488-g m² every 10 to 14 days totaling ten applications from June through early October), and cultivation (non-cultivated or core cultivated in April/May and October). Controls (no mid-season topdressing) at each level of cultivation were also included for comparisons resulting in 14 total treatments (Table 1). The medium-coarse (1.0-mm) sand used in this trial meets the USGA particle size recommendation for construction, whereas that of the medium-fine and fine-medium sands do not. The quantity of fine and very fine particles in the medium-fine (0.5-mm) and fine-medium (0.25-mm) sands exceed the USGA recommendations, and these sands contain little to no coarse particles (Table 2).

Topdressing creeping bentgrass turf with medium-fine sand (particles ≤ 0.5 -mm) has diluted and modified thatch accumulation similar to that of the coarse-medium sand (particles ≤ 1.0 -mm), despite the medium-fine sand not conforming to USGA sand size guidelines. Over time, surface wetness data indicated that differences among treatment factors in this trial have intensified over time. Most notable is that topdressing with fine-medium (0.25-mm) sand has increased the fine sand content of the mat layer, which has increased the capillary porosity and water retention of the mat layer of those plots. Additionally, the surface wetness of plots has been significantly drier on plots that were core cultivated.

Core cultivation has increased water infiltration of creeping bentgrass regardless of the topdressing sand size. Thus, the negative effects of topdressing with finer sands on water infiltration can be reduced by core cultivation.

Surface hardness has been strongly affected by treatment, but the effect was dependent on the sand size as well as the level of cultivation. Under relatively dry conditions, topdressing with finer sands reduced surface hardness; there were no differences among sand size on plots that were core-

cultivated twice per year. Volumetric water content (VWC) was inversely related to surface hardness; thus, treatments that had a drying effect on the surface increased surface hardness. Topdressing has reduced surface VWC compared to the controls and this effect was most pronounced for plots that were not core-cultivated. Among the two controls, the core-cultivated control frequently had dramatically lower surface water content than the non-cultivated control.

Surface water retention differences among plots eventually required hand-watering of individual plots to avoid over- or under-watering. During 2022, the effect of sand size on the number of hand-watering events depended on the level of cultivation. Under non-cultivated conditions, the number of hand-watering events decreased as the topdressing sand size became finer. There were nearly 44 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand. The impact of sand size on the number of hand-watering events was much smaller under core-cultivated conditions; there were only 12 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand.

We have received a grant to continue this trial with an alteration of the cultivation factor; the level of hollow tine cultivation twice per year will be changed to solid tine cultivation twice per year. This will determine whether solid tine cultivation can provide benefits like those observed with core cultivation.

Table 1. Description of treatment combinations of sand size, topdressing rate, and cultivation factors as well as two controls (no mid-season topdressing) evaluated on a ‘Shark’ creeping bentgrass turf seeded in 2014 and grown on a sand-based rootzone. Treatments initiated in May 2016.

Treatment no.	Sand size ^a	Topdressing rate during mid-season ^b	Cultivation ^c	Annual quantity of sand applied
		g m ⁻²		kg m ⁻²
1	medium-coarse	244	none	6.3
2	medium-coarse	244	core + backfill	8.3
3	medium-coarse	488	none	8.8
4	medium-coarse	488	core + backfill	10.8
5	medium-fine	244	none	6.3
6	medium-fine	244	core + backfill	8.3
7	medium-fine	488	none	8.8
8	medium-fine	488	core + backfill	10.8
9	fine-medium	244	none	6.3
10	fine-medium	244	core + backfill	8.3
11	fine-medium	488	none	8.8
12	fine-medium	488	core + backfill	10.8
13	none	0	none	0
14	none	0	core + backfill	5.9

^a First-mentioned size class represent the predominant size fraction in the sand.

^b Ten applications of topdressing applied every two weeks from June through early October. Topdressing at 2.44g m⁻² represented a ‘dusting’ quantity (O’Brien and Hartwiger, 2003); whereas topdressing at 488 g m⁻² filled the surface thatch and lower verdure layers.

^c Core cultivation to the 38-mm depth was performed twice a year (April/May and October) using 13-mm diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with medium-coarse sand at 2.9 kg m⁻². At the time of core cultivation, non-cultivated plots were topdressed with the respective sand at 2.0 kg m⁻² to fill the verdure and surface thatch layers to the same extent as the cored and backfilled plots.

Table 2. Sand size distributions of the three topdressing sizes, mat layer and the underlying rootzone at the initiation of the trial; USGA construction specification provided for references. Weighted averages based on distributions of each sand delivery through Oct. 2018.

Topdressing Sand Size	Particle diameter (mm) / Size class ^a				
	2.0-1.0 very coarse	1.0-0.5 coarse	0.5-0.25 medium	0.25-0.15 fine	0.15-0.05 very fine
	----- % retained (by weight) -----				
Medium-coarse	0	34.8	57.7	8.4	0.1
Medium-fine	0	0.1	76.7	22.7	0.5
Fine-medium	0	5.7	25.8	66.8	1.7
Mat Layer ^b	0.1	25.3	56.4	15.4	2.7
Rootzone	6.9	25.3	44.6	17.2	4.1
USGA construction specification	≤ 10	----- ≥ 60 -----		≤ 20	≤ 5

^a Sieve opening and mesh: 2-mm = no. 10; 1-mm = no. 18; 0.5-mm = no. 35; 0.25-mm = no. 60; 0.15-mm = no. 100; 0.05-mm = no. 270

^b Sand size distribution of 45 core samples of the mat layer collected before treatment initiation in May 2016.

Investigating Turfgrass Disease Suppressive Soil Microbiome – Metagenomics, Culturomics, and Metabolomics

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Specific disease suppressive soil is soil that provides disease suppression through antagonistic microbial activities. Despite being well-documented in many economically important crop-pathosystems, empirical evidence of specific disease suppressive soils in turfgrass is currently lacking. To address this gap, we collected field soil from 14 sites across two geological regions and five states, which exhibited varying degrees of disease pressure and management intensities. We transplanted the soil microbiome to sterile potting mix, established 'Penncross' creeping bentgrass, and inoculated it with *Clariireedia jacksonii* to examine the dollar spot suppressiveness of different turfgrass-associated microbial communities. Our results indicate that disease severity, as measured by turfgrass greenness, varied significantly depending on the soil source, suggesting different levels of microbial disease suppression. Correlation analyses also revealed a significant association between reduced fungicide use and increased dollar spot suppressiveness. Building on this preliminary study, my program plans to study turfgrass disease suppressive soil targeting the diseases that are important in New Jersey and the Northeast. We will identify candidate microbial strains, genes, and metabolites responsible for disease suppression using culturomics, metagenomics, and metabolomics techniques. This project will ultimately contribute to the development of improved turfgrass disease management, such as fungicide programs that facilitate the formation of disease suppressive soil, and biological control strategies.

Integrative Multi-omics Approaches to Dissect Plant Stress Responses

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Plants respond to abiotic and biotic stresses through complex, multi-level signaling networks. By reconstructing the topology of these networks, we can begin to understand how signals are propagated and predict the genes that have the largest influence on plant phenotypes. We used integrative multi-omics to dissect the interplay between brassinosteroid hormone and drought responses in *Arabidopsis*. Wild-type and brassinosteroid signaling mutants were subjected to a 30-day drought experiment using a robotic platform to precisely control water levels and collect detailed images for accurate phenotyping. Tissue was collected and multi-omics profiling revealed differentially regulated transcripts, proteins, and phosphosites in the mutants under well-watered and drought conditions. A systems-biology approach was used to integrate these orthogonal datasets into multi-level models of cellular signaling. Gene regulatory networks predict the transcription factors that regulate modules of differentially expressed transcripts. Phospho-signaling networks identify activated kinases and their phosphorylated targets. The reconstructed networks have high predictive power, and genes with high centrality are being functionally validated in drought and hormone assays. This strategy represents a powerful tool to identify and test the genes that regulate important phenotypes.

Oxford Nanopore Technologies Sequencing Approaches for Mycobiome Analysis of Tall Fescue Under Drought Stress and Diagnostics of Cool-Season Turfgrass Diseases

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Sequencing technologies have enabled scientists to better understand the microbial communities associated with plants. Sanger sequencing was the first sequencing technology which could sequence up to 1 kb of DNA from one sample. Next generation sequencers have since been developed with the capabilities to sequence many different fragments of DNA at once from multiple unique samples. The most common of them are the Illumina sequencers which can sequence millions of copies of DNA from samples but is limited to a sequence size of 300 bp in both the forward and reverse direction (600 bp total if overlapping). The latest sequencing platforms from PacBio and Oxford Nanopore Technologies (ONT) are not limited to the 600 bp totals and can consistently generate millions of reads over 10 kb in length. These Next Generation Sequencers are commonly used for genomics, transcriptomics, and microbiome research. Currently, microbiome studies are conducted using Illumina sequencers targeting a ~600 bp barcoding region like the fungal internal transcribed spacer (ITS). These sequences are identified using a reference database to determine taxonomic identification, but most fungi can not be identified to species using only the ITS region. The latest sequencers can generate reads that span multiple gene regions which will help improve taxonomic identification in microbiome studies and microbial identification.

In a two-year study, we compared the short-read Illumina MiSeq and long-read ONT MinION sequencers capabilities to evaluate the mycobiome associated with tall fescue exposed to prolonged periods of drought stress in a rainout shelter at the Rutgers Plant Science Research and Extension Farm in Freehold, NJ. Twelve plants, comprised of six sets of half-sib progeny, one exhibiting a drought tolerant phenotype and the other a susceptible phenotype were selected for analysis each year. Each half-sib was separated into a shoot, root, and rhizosphere soil component. Both platforms sequenced portions of the fungal nuclear ribosomal RNA genes. The Illumina MiSeq sequenced the ITS (600 bp) region, while the ONT MinION covered the small subunit, ITS, and the partial large subunit (4,600 bp). Comparative analysis of the mycobiome associated with the roots, shoots, and rhizosphere soil showed significant differences between the two platforms. The ONT MinION identified more diverse fungal lineages and had higher taxonomic resolution compared to the Illumina MiSeq. Furthermore, the MinION sequencer was able to identify a phenotype effect between the tall fescue plants that the MiSeq could not detect. Our results also indicated that root pathogens may play a role in tall fescue drought stress tolerance.

The results from the mycobiome analysis showed that the ONT sequencer was able to consistently identify an extremely diverse number of fungi with higher taxonomic resolution (genus/species).

This makes it appealing as a one size fits all approach for pathogen diagnostics. To test if the ONT sequencer could be used for pathogen diagnostics, diseased turfgrass core samples were collected on 26 August 2022 from Rutgers Horticultural Research Farm 2 in New Brunswick from fields displaying active infections of anthracnose (*Colletotrichum cereale*), brown patch (*Rhizoctonia solani*), dollar spot (*Clarireedia jacksonii*), gray leaf spot (*Pyricularia oryzae*), and summer patch (*Magnaporthiopsis poae*). Samples were processed with the same molecular approach used in the microbiome study to see if species level identification of the causal agents of each disease could be obtained. The ONT sequencing approach was able to generate reads of each causal agent that were confirmed with microscopic and laboratory culturing methods. Although a more thorough evaluation of the ONT sequencer's ability to identify pathogens from environmental samples is still needed, this preliminary study highlights an exciting new opportunity for plant disease diagnostics.

The *Epichloë festucae* Antifungal Protein Can Reduce Dollar Spot Symptoms on Creeping Bentgrass

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When strong creeping red fescue (*Festuca rubra* subsp. *rubra*) is infected by the fungal endophyte *Epichloë festucae* it exhibits good resistance to dollar spot disease caused by the fungal pathogen *Clarireedia jacksonii* (Clarke et al., 2006). This endophyte-mediated disease resistance is unique to the fine fescues and has not been observed in other commercial grass species infected with other *Epichloë* spp. Our research program has been focused on understanding the mechanism of the observed disease resistance in endophyte-infected strong creeping red fescue since this may lead to new approaches to manage dollar spot in creeping bentgrass (*Agrostis stolonifera*). Dollar spot disease on creeping bentgrass is a major problem on golf courses and superintendents currently rely heavily on fungicides for control.

We have been pursuing the possibility that the disease resistance in endophyte infected strong creeping red fescue may be due to the presence of an abundant *E. festucae* antifungal protein, designated *Efe-AfpA*. The transcript for the antifungal protein was highly expressed in the infected plants (Ambrose and Belanger, 2012) and the purified antifungal protein suppressed the growth of *C. jacksonii* in culture (Tian et al., 2017). In greenhouse assays, topical application of purified antifungal protein to endophyte-free strong creeping red fescue plants and creeping bentgrass plants protected them from developing severe symptoms caused by *C. jacksonii* (Fardella et al., 2022). These results support the hypothesis that the endophyte antifungal protein is a major contributor the dollar spot resistance seen in endophyte-infected strong creeping red fescue. It may be possible to develop the antifungal protein as an alternative or complement to fungicides for the management of dollar spot disease on turfgrasses.

Acknowledgments

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POSTER PRESENTATIONS

Dithiopyr Resistance and Alpha-Tubulin Mutations Vary by Ecotype for Goosegrass (*Eleusine indica*) in New Jersey

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The objective of this research was to confirm herbicide resistance of multiple putative resistant goosegrass biotypes in New Jersey and elucidate mechanisms of resistance within each biotype. We hypothesized that resistance to mitotic herbicides (dithiopyr and prodiamine) is underreported in cool-season turfgrass and widespread in New Jersey, following decades on reliance on these preemergence herbicides for goosegrass and crabgrass control on golf courses.

Plants from three putative-resistant biotypes (JB, CV, WL; from New Jersey golf courses), and two putative-susceptible biotypes (SK and DD; a home lawn and tree farm) were harvested and propagated in a greenhouse for seed. In 2021, dose-response experiments were conducted in 10 by 10 cm pots of 80:20 sand/peat (v/v), biotype were also included. Prodiamine and dithiopyr (0, 50, 100, 500 and 5000 g/ha) treatments were applied to all biotypes within 24 hr of seeding. A known dithiopyr-resistant (PB-R) and known-susceptible (HF-S) biotype were included for control. Goosegrass seedling counts and aboveground biomass were measured at 28 DAT. Four plants from each biotype were randomly selected from >100 plants grown from seed for genetic sequencing of the α -tubulin gene. Within biotype, sequences were not different between replicates and were subjected to multiple sequence alignment across biotypes in comparison to a reference genome (GenBank accession AJ005598-9). Seedling count data from dose response experiments were subjected to nonlinear regression in GraphPad Prism, to generate IC_{50} values used to determine levels of resistance for each biotype.

Herbicide resistance was confirmed for suspected resistant biotypes. Two α -tubulin mutations were identified and varied by biotype. Dithiopyr resistant PB-R and JB (IC_{50} = 34 and 121, respectively) and susceptible (SK, DD, IC_{50} = <10 g/ha) biotypes shared Leu-136-Phe mutations. Prodiamine-resistant WL and CV biotypes (IC_{50} = 80 and 175, respectively) differed significantly in their tolerance to dithiopyr, but shared Thr-239-Ile mutations.

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. Many cultivars of creeping bentgrass are susceptible to dollar spot disease caused by *Clarireedia jacksonii*. Genetic engineering technologies allow us to knock-out (KO) dollar spot susceptibility genes in creeping bentgrass, resulting in plants that confer dollar spot disease resistance. We used the CRISPR-Cas9 gene editing platform to KO *AsCPK12*, a calcium-dependent protein kinase (CDPK) that has been identified as a negative regulator for rice blast disease resistance. We have constructed the CRISPR-gene editing vector (pRD302) with the expression of the guide RNA (gRNA) targeting *AsCPK12* under the control of the wheat U6 promoter and the monocot codon-optimized Cas9 nuclease gene under the control of maize ubiquitin promoter. We have also constructed another transient vector pRD577 to KO *AsNPR3*, a potential gene involved in negative immunity regulation. We utilized both biolistic bombardment and *Agrobacterium*-mediated transformation methods to deliver the CRISPR vector into embryogenic calli of the creeping bentgrass cultivar “Crenshaw”. We produced many transgenic plants in tissue culture and transferred plants to soil. The region of the *CPK12* target site in transgenic plants was amplified and sequenced, and we identified multiple gene-edited plants. We selected two plants with loss of function mutations, 302-30 and 302-28, and conducted further analysis to evaluate dollar spot disease resistance. Detached leaves from aforementioned plants were inoculated with mycelium of *C. jacksonii* on water agar. Lesion length was measured 3 and 5 days post inoculation (dpi). The average lesion length on detached leaves from both edited plants was smaller than the non-edited wild type plants. Additionally, eight clones of 302-30 were inoculated with *C. jacksonii* in a growth chamber. Two weeks post inoculation, total DNA was isolated from these plants and the *C. jacksonii* fungal level was quantified with qPCR. Our results showed that the *C. jacksonii* fungal levels in 302-30 plants were significantly lower compared to the non-edited wild type plants. These results indicate that loss of function mutations in *CPK12* can improve dollar spot disease resistance in creeping bentgrass. pRD577 has been delivered into Crenshaw calli and the plantlets are being regenerated.

Colonization Stability and Efficacy of Plant Growth-promoting Rhizobacteria in Creeping Bentgrass

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Inoculation with plant growth promoting rhizobacteria (PGPR) is a novel approach to improve growth and abiotic stress tolerance of cool season turfgrasses. Several endophytic PGPR colonize plant roots and produce ACC deaminase, which reduces the production of stress-induced ethylene, effectively reducing leaf senescence. However, for this symbiosis to have the intended effects on improving stress tolerance, the roots must first be colonized by the PGPR using successful and confirmed inoculation methods. Additionally, these methods must be confirmed under fields conditions, which can be challenging due to fluctuating temperatures and moisture, as well as the presence of native soil organisms. In this study, field plots of creeping bentgrass (cv. Pencross) were inoculated with two novel strains of *Paraburkholderia aspalathi* bacteria that have demonstrated growth promoting properties using a foliar spray and soil drench inoculation method. The inoculation treatments were applied to well-watered plots and plots that were subjected to 28 days of deficit irrigation (60% ET) followed by 14 days of rewatering (100% ET). To evaluate the colonization efficiency of bacteria, roots were sampled from the plots to examine the presence and quantity or density of the bacteria strains that were applied. The presence of bacterial inoculants in plant tissues was determined by bacterial streaming and PCR analysis that was designed based on signature sequences of the 16S rDNA in *P. aspalathi*. Both analytical techniques were able to confirm that the soil drench method was the most effective inoculation method. Plots inoculated with *P. aspalathi* using the soil drench method also demonstrated the greatest improvements in drought stress tolerance and post-drought recovery, suggesting that inoculation with these novel strains of *P. aspalathi* using the soil drench method is an effective approach to improving drought stress tolerance and reducing water use in creeping bentgrass.

Investigating White Grub Resistance in Turf-Type Tall Fescue

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The complex of annual white grubs, including the larvae of Asiatic garden beetles, oriental beetles, Japanese beetles, and northern masked chafers, are the most destructive and widespread insect pest affecting turfgrass in the northeastern U.S. Increased tolerance to white grub feeding has been observed in tall fescue populations at the Rutgers Plant Science Research Farm in Freehold, NJ. The preliminary observations warrant more detailed investigations into the mechanisms for white grub tolerance. The research objectives include determining if there is a preference for white grubs to lay eggs on certain tall fescue populations and assess the ability of white grubs to survive among different populations, evaluate white grub feeding patterns for potential feeding preference on roots of different populations, compare populations for their ability to compensate for white grub feeding and assess populations for fungal endophytes to determine if there is an association with white grub feeding. A field trial was established at the Rutgers Adelphia Plant Science Research and Extension Farm in Freehold, NJ with sixteen cultivars and inoculated with oriental beetle and northern masked chafer in October 2022 to assess egg-laying preferences and if the eggs and larvae are able to survive on certain cultivars more than others. Sixty-four rhizotron boxes will be used to determine grub feeding patterns and preferences and for drought studies to investigate root architecture differences among the sixteen cultivars used in the field study using WinRhizo root scanning software. These cultivars will also be evaluated for the presence of endophytes to identify potential associations between endophyte presence and white grub tolerance in tall fescue.

Characterizing Major Gene Resistance Expression to Eastern Filbert Blight in New Jersey

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Eastern filbert blight, caused by the fungus *Anisogramma anomala*, is the historical limiting factor of European hazelnut (*Corylus avellana*) production in eastern North America. The pathogen is native to a wide area of the eastern U.S. and was inadvertently spread to the Pacific Northwest in the 1960s where it devastated commercial hazelnut production in the Willamette Valley of Oregon. Fortunately, resistance to EFB was found in the late blooming pollinizer ‘Gasaway’, which was subsequently used in breeding at Oregon State University (OSU) to develop new cultivars, such as Jefferson and Yamhill, that are now being widely planted. A significant effort was more recently placed on identifying additional sources of resistance in Oregon to support breeding efforts. From this work, dozens of additional cultivars and selections were eventually found to be resistant and are now being used in breeding (Mehlenbacher, 2018; Mehlenbacher and Molnar, 2021). Of concern, however, is that hazelnuts identified as resistant in Oregon may not hold up to the pathogen in the eastern U.S. The *A. anomala* in Oregon is believed to stem from a single point introduction (Gottwald and Cameron, 1980) and genetic diversity of *A. anomala* in the region appears to be limited compared to populations found naturally occurring in the East (Muehlbauer et al., 2019). A targeted study by Tobia et al. (2017) strongly supports this premise as samples collected across orchards spanning a ~245 km transect of Washington and Oregon had nearly identical simple sequence repeat profiles and were placed in a single genetic group in contrast to five different genetic groups resolved from cankers collected in plots at Rutgers University alone. Thus, breeding for resistance in Oregon likely relies on selection under a reduced diversity of *A. anomala* isolates which presents a scenario that may have implications regarding durability of identified resistance genes. In other words, plants found resistant under Oregon conditions may not maintain a similar level of resistance when challenged with a wider diversity of *A. anomala*. Previous work at Rutgers supports this concern as greenhouse inoculation and field studies in New Jersey strongly suggested the existence of pathogenic variation but plant materials available for study at the time were limited (Capik and Molnar, 2012; Molnar et al., 2010); thus, the many new sources of resistance identified in Oregon warrant testing under eastern conditions.

In this study, 50 EFB-resistant cultivars and breeding selections (10 trees each) originating at OSU were planted in 2019 at Rutgers University Hort Farm 3 (East Brunswick, NJ) and exposed to naturally occurring high EFB pressure. The genotypes comprise a wide diversity of backgrounds and are known to be protected by major *R*-genes mapped to respective linkage groups (LGs): 6 contain *R*-genes mapped to LG 2, 27 to LG 6 (the region of ‘Gasaway’ resistance), 10 to LG7, and 7 are currently unmapped. Trees were evaluated for disease incidence and severity in 2022. Results showed plants protected by *R*-genes mapped to LG2 and LG7 are maintaining resistance, with only one genotype per LG and few total trees expressing EFB (8.6 % of trees for LG2, 1.1% for LG7). In contrast, 25 of 27 (93%) of genotypes protected by *R*-genes mapped to LG6 expressed EFB; 87.6% of total trees have cankers and 17 genotypes show 100% incidence of infection across replications. Importantly, disease in LG6 trees was severe, indicating a lack of usefulness for most *R*-genes mapped to this region of the genome. Unlike our findings in New Jersey, the trees with

resistance mapped to LG6 remain resistant or highly tolerant to EFB under Oregon conditions, which strongly supports the presence of pathogenic variation. These results also inform breeding efforts regarding the available sources of resistance being used in the OSU breeding program.

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Establishment of Zoysiagrass and Bermudagrass in New Jersey

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Climate change is causing more extreme temperature fluctuations with variable conditions in the transition zone. 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). 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).

High temperatures negatively affect the performance of creeping bentgrass (*Agrostis stolonifera* L.) and result in increased inputs for adequate playability. Zoysiagrass (*Zoysia* spp.) and bermudagrass (*Cynodon* spp.) are warm-season turfgrasses used in the transition zone and southern portion of the US. Evaluating the performance of cold-hardy zoysiagrass and bermudagrass germplasm and their potential for fairway use in the northern transition zone can provide an option for superintendents to potentially save money, resources, and have less of an impact on the environment.

Zoysiagrass selections (13) and bermudagrass selections (16) were established on June 30, 2022 from sprigs at a rate of approximately 500 bushels/acre in 98 sq ft plots in a Randomized Complete Block Design with three replications. Data was collected on irrigation, fertilizer usage, pesticide usage, and mowing frequency. Visual ratings and image data were also collected. From July 7 to October 6, 2022, visual establishment ratings were taken weekly on a 0-10 rating scale representing percent green cover. Turfgrass quality ratings, on a 1-9 scale, were taken monthly starting on August 26, 2022 until the plots entered winter dormancy. In addition, fall color retention ratings were recorded on a weekly basis from September 30 to December 1, 2022 on a 1-9 scale. Digital image data was collected using a Canon PowerShot G15 (PC1815) with a 20 x 24 inch lightbox with 4 florescent bulbs. Images were taken on August 5, 2022 and then weekly from September 23 to December 1, 2022. In addition to lightbox images, a full plot image was taken using the same camera to provide an accurate representation of the entire 98 sq ft plot. It was observed that all plots were dormant by December 1, 2022 with the largest decrease in color retention from November 17 to December 1, 2022. Visual ratings were statistically analyzed using SAS 9.4 to perform an ANOVA test. Overall, bermudagrasses established quicker than zoysiagrasses. Most plots filled in eventually, but 5 zoysiagrass selections did not establish well even by October 20, 2022. Within the bermudagrasses, Rio, Monaco, and Hollywood had the quickest establishment. It is interesting to note that the plots with quickest establishment are (typically) seeded bermudagrass cultivars. Empire, FZ 1727 and DALZ 1701 had the quickest establishment among the zoysiagrasses. The bermudagrass and zoysiagrass with the highest turfgrass quality were FB 1628 and Empire, respectively. We will continue to evaluate percent cover, turf quality, spring green up, winter kill (if present) and disease resistance (if present). This

trial will also be used to identify some of the best performing bermudagrass and zoysiagrass for NJ climactic conditions. The best performing selections will be utilized for the additional research projects in the coming years.

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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 identify shade tolerant cultivars? Additionally, this study also aimed to evaluate 41 common commercial cultivars and experimental selections of creeping bentgrass. The study was carried out firstly in a greenhouse under both full sun and simulated foliar shade, followed by a second experiment in a growth chamber again under both full sun and simulated foliar shade. Foliar shade for both studies was accomplished using a photosensitive 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 in both studies, which is indicative of shade avoidance characteristics. Of the cultivars studied, L-93XD and Oakley demonstrated a small percent change in height indicating a strong shade tolerance for this trait, in both the greenhouse and the growth chamber. A positive shade tolerant chlorophyll concentration was seen in the cultivars, Matchplay and MacDonald, in both the greenhouse and growth chamber studies. 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 and Oakley 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.

Physiological Regulation and Mitigation of Summer Decline of Annual Bluegrass Using Plant Health Products

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Annual bluegrass (*Poa annua*) is a cool season turfgrass species commonly found on golf courses all over the world. It's low tolerance to heat stress and prolific seed production makes it a poor performing turfgrass that is difficult to manage. Attempts to control *Poa* are not always successful, and it has the potential to develop resistance to currently utilized herbicides. The goal of this study is to identify effective plant-health products for improving *Poa* summer performance and heat tolerance on putting green conditions. Fourteen different treatments were applied, including biostimulant seaweed extracts, amino acids, plant growth regulators, fungicides and an untreated control. Treatments were foliar sprayed to 3x4' plots, with four replicates per treatment, every 14 days throughout the summer. Plots were arranged using a Randomized Complete Block Design. The following measurements were taken weekly to quantify plant health and performance: visual turf quality (TQ), canopy temperature, dark green color index (DGCI), percent green canopy cover, normalized difference vegetative index (NDVI), stress index (SI), and leaf area index (LAI). Of the treatments, the seaweed extracts and fungicides were effective in promoting *Poa* summer performance.

Precision Breeding of Turfgrass to Enhanced Resistance to Fungal Pathogens

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Plant non-specific lipid transfer proteins (nsLTPs) are classified as pathogenesis-related (PR) proteins which function as part of the innate immune system (Gao et al. 2022; Kader 1996; Stotz et al. 2013; Liu et al. 2015). nsLTPs are small cysteine-rich proteins with the consensus sequence C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C which forms four conserved disulfide bridges (Kader 1996). This conserved sequence generates a hydrophobic pocket which accepts and binds lipids (Lerche et al. 1997; Salminen et al. 2016). nsLTPs have been classified into gene families and subfamilies based on gene expression patterns and other characteristics such as motif structure. A majority of nsLTPs contain a signal peptide that targets the protein to the apoplast. The best characterized nsLTP is DEFECTIVE IN INDUCED RESISTANCE1 (DIR1) in *Arabidopsis* which plays a role in the systemic acquired resistance (SAR) response (Champigny et al. 2013; Carella et al. 2017). nsLTPs have been recognized as possessing potent antifungal properties and have been linked with both biotic and abiotic stress reduction (Hairat et al. 2018; Molina et al. 1993; Salminen et al. 2016; Kader 1996; Xu et al. 2018; Zhu et al. 2012; Gonorazky et al. 2005). nsLTPs have been found to bind lipids using *in vitro* assays (McLaughlin et al. 2021; Bogdanov et al. 2016; Lerche et al. 1997; Heinemann et al. 1996) and this lipid binding functionality is central to the antifungal nature of these proteins (Regente and De La Canal 2000; Molina et al. 1993; Wang et al. 2004). In *Arabidopsis*, wheat, and rice where either cDNA libraries have been generated or when genomic data permits genome-wide analysis of gene families, classification has permitted a better understanding of nsLTPs but functional data on individual nsLTPs is lacking (Boutrot et al. 2008; Xue et al. 2022; Wang et al. 2010).

Previous research in our laboratory has identified antifungal nsLTPs in both *Arabidopsis* and wheat which have been shown to provide significant protection against *Fusarium graminearum* both *in vitro* and when overexpressed in wheat (McLaughlin et al. 2015; McLaughlin et al. 2021). The proteins AtLTP4.4 and TaLTP9, are effective *in vitro* against four major turf fungal pathogens, Summer Patch (*Magnaporthe poae*), Snow Mold (*Monographella nivalis*), Brown Patch (*Rhizoctonia solani*), and Dollar Spot (*Clarireedia jacksonii*). This suggests that heterologous overexpression of nsLTPs in turfgrasses may provide broad spectrum resistance to fungal pathogens. To this end, we are transforming the creeping bentgrass (*Agrostis stolonifera*) with several different *Arabidopsis* and wheat nsLTPs which have shown promise as antifungal proteins. We also propose to identify creeping bentgrass nsLTPs that may function as antifungals. Information about nsLTPs in response to biotic and abiotic stresses in turfgrasses is very limited due to the lack of genomic resources. Searching for nsLTPs using nested PCR with degenerate primers together with the use of 5' and 3' RACE-PCR methods will be used to discover endogenous nsLTP genes in bentgrass. These specific nsLTPs can then be compared using expression analysis to determine response to different stresses, including fungal pathogen exposure. These novel nsLTPs would then serve as targets for CRISPR activation (CRISPRa) to generate novel disease resistance in bentgrass. CRISPR-Cas9 and CRISPRa, respectively, are ideal for creating modifications in susceptibility loci and to overexpress key resistance genes such as nsLTPs which show strong antifungal responses. Because nsLTPs have also been shown to

enhance abiotic resistance to cold, drought, and heat conditions, the edited bentgrass lines can be tested for these traits as well.

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Response of Kentucky Bluegrass to Traffic During 2021

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Traffic tolerance is an important attribute in Kentucky bluegrass (*Poa pratensis* L.) needed to provide quality surfaces at recreational sites. The objective of this field trial was to determine the response of Kentucky bluegrasses in the 2017 NTEP test to traffic during 2021. Three replications of 89 entries were seeded in September 2017 on a loam in North Brunswick, NJ. Traffic was applied in a strip across half of each plot using a combination of the Rutgers Wear Simulator and the Cady Traffic Simulator; two passes wk^{-1} with each machine were applied May through August 2021; the other half of each plot did not receive traffic. Plots received traffic applied to the same strip using a combination of the two machines during 2018 to 2020. Uniformity of turf cover (UTC) was visually assessed on traffic and no traffic plots before traffic was initiated (3 May 2021) and after 28, 56, and 72 traffic passes on 14 June, 3 August, and 30 August 2021, respectively. Area under the UTC progress curve (AU_{UTCPC}) was calculated for each plot to integrate the cumulative effects of traffic. Data were analyzed as a 2 (no traffic and traffic) \times 89 (entries) factorial strip-plot design. Kentucky bluegrass had lower AU_{UTCPC} values in trafficked plots compared to non-trafficked plots. A significant traffic \times entry interaction was detected for AU_{UTCPC} ; few differences in AU_{UTCPC} were detected among entries that did not receive traffic, while there were many differences among entries within the trafficked strip. Entries with the greatest AU_{UTCPC} under the trafficked condition were Barvette HGT, PST-K15-172, and BAR PP 7K426; whereas, Orion (PST-K13-143), Starr (GO-2628), Blue Knight, A06-8, DLFPS-340/3364, DLFPS-340/3444, PPG-KB 1320, Skye, Amaze (NAI-14-133), NAI-14-132, RAD-1776, Pivot, Aviator II (NAI-15-84), Heartland (NAI-14-187), Dublin (PST-K15-157), A16-1, NK-1, DLFPS-340/3553, NAI-15-80 had the lowest AU_{UTCPC} under traffic.

Morphactin-mediated Amelioration of Heat-induced Leaf Senescence Associated with Alterations in Chlorophyll Metabolism in Creeping Bentgrass

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Heat stress is a major abiotic stress that hinders the growth and performance of cool-season turfgrasses, such as creeping bentgrass (*Agrostis stolonifera*), which experiences premature leaf senescence when exposure to high temperatures is prolonged. Morphactins are plant growth regulators that have been utilized as foliar treatments for the control of leaf senescence, but their effects under heat stress and in cool-season turfgrasses are not yet understood. The objectives of this study were to determine whether application of chlorflurenol-methyl (CM), a morphactin with senescence-inhibiting properties, can control leaf senescence in creeping bentgrass under heat stress and to examine the regulatory effects that CM may have on chlorophyll metabolism. Mature creeping bentgrass plants were subjected to heat stress (35/30 °C, day/night) or non-stress control (22/18 °C, day/night) temperatures for a duration of 25 d in climate-controlled growth chambers and were foliar-treated with 10 µM CM or water only every 7 d. Under heat stress, plants treated with CM had enhanced turf quality at 25 d, while chlorophyll content was significantly enhanced from 14 through 25 d of heat stress. Activity of the chlorophyll synthesis enzyme, porphobilinogen deaminase, was significantly higher in CM-treated plants from 21 through 25 d of heat stress, while activities of the chlorophyll degradation enzymes, chlorophyllase and chlorophyll-degrading peroxidase, were significantly lower from 14 through 25 d of heat stress. The activity of pheophytinase, another chlorophyll-degrading enzyme, was significantly lower in CM-treated plants at 7, 21, and 25 d of heat stress. The results of this study suggest that foliar application of CM inhibits heat-induced leaf senescence by suppressing chlorophyll degradation and enhancing chlorophyll synthesis.

QTL Mapping of Anthracnose Disease Severity in a Switchgrass F1 Mapping Population

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Switchgrass (*Panicum virgatum*) has been identified as a model bioenergy crop by the US Department of Energy due to its potential for high biomass yields and its wide range of adaptability throughout the United States. A major roadblock to the widespread use of switchgrass in the Northeastern US is the prevalence of anthracnose disease caused by the fungal pathogen, *Colletotrichum navitas*. To overcome this challenge quantitative trait loci (QTL) map-based approach was carried out using a switchgrass mapping population consisting of 202 full-sib F1 progeny that segregate for anthracnose infection severity. This population was developed previously as part of the Northeast Woody/Warm-season Biomass (NEWBio) Consortium project. This population has been screened for severity of anthracnose disease ratings and growth parameters during six separate growing seasons (2014, 2015, 2019, 2020, 2021 and 2022). Preliminary data collected during these years indicate that the 202 full-sib F1 mapping population approaches a normal frequency distribution in disease response, as would be expected. Using a genotyping by sequencing approach, small nucleotide polymorphisms (SNPs) were identified using Stacks and allele dosage for each SNP was then subsequently estimated using polyRAD to obtain polyploid genotype calls. A total of 4,129 SNP markers were mapped to 19 linkage groups, with a cumulative length of 1907.6 cM and an average of 2.08 SNP markers per cM. QTL mapping identified one significant QTL for anthracnose disease severity on chromosome 8K which accounts for ~20% of the phenotypic variation. A second putative QTL was also identified on chromosome 1K that lies just below the threshold for significance. The identification of these QTLs serves to improve our understanding of anthracnose disease resistance and can be used as an aid in the generation of improved, anthracnose disease-resistant cultivars that will be well suited for biomass production in the northeastern United States.

Efficacy of Preemergence Herbicides for False-Green Kyllinga (*Kyllinga gracillima*) Control from Seed

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Greenhouse experiments were conducted to determine the efficacy of herbicides used in cool season turfgrass against false-green kyllinga (*Kyllinga gracillima*) emergence from seed in soil. A replicated preliminary experiment evaluated various herbicides at 100 and 10% typical use rates for cool season turfgrass. Herbicides and 100% use rates included bensulide (11,200 g ha⁻¹), corn gluten meal (880,000 g ha⁻¹), dimethenamid (1,680 g ha⁻¹), dithiopyr (560 g ha⁻¹), isoxaben (1,120 g ha⁻¹), mesotrione (280 g ha⁻¹), methiozolin (516 g ha⁻¹), oxadiazon (4,500 g ha⁻¹), pendimethalin (3,360 g ha⁻¹), proflam (840 g ha⁻¹), siduron (13,500 g ha⁻¹), and sulfentrazone (280 g ha⁻¹). The 100 and 10% use rate applications of corn gluten meal, pendimethalin, methiozolin, and siduron were less effective than sulfentrazone, dithiopyr, isoxaben, oxadiazon, proflam, dimethenamid, and bensulide against false-green kyllinga from seed. Herbicides deemed effective in the first experiment were evaluated in a greenhouse dose-response experiment.

Treatments were a complete factorial of weed species (false-green kyllinga, and smooth crabgrass), herbicide, and herbicide rate. Herbicides were applied at 100, 50, 25, 12.5, 6.3, and 3.1% of typical use rates for cool season turfgrass. Herbicides evaluated were bensulide, dimethenamid, dithiopyr, and sulfentrazone. Local collections of false-green kyllinga seed were sowed to 10 by 10 cm pots filled with local field soil. Smooth crabgrass (*Digitaria ischaemum*) was seeded to separate pots as a standard for comparison. Treatments were replicated four times. Seedlings were counted 42 days after application and expressed as a percent of the non-treated control. Data were analyzed as a three-way complete factorial using the GLIMMIX procedure in SAS (P=0.05). Fisher's protected LSD test was used to separate means.

Main effect interactions were detected (P < 0.05) for seedlings count expressed as a percent of the non-treated control 6 weeks after treatment. Bensulide, dimethenamid, and oxadiazon provided >94% false-green kyllinga control at all rates. Dithiopyr, proflam, sulfentrazone, and isoxaben provided less false-green kyllinga control than bensulide and oxadiazon at the 3.1 and 6.3% rates. Proflam, isoxaben, and oxadiazon provided more smooth crabgrass control than false-green kyllinga control at 6.3 and 3.1% rates. Future research should evaluate the efficacy of bensulide, dimethenamid, and oxadiazon in the field for false-green kyllinga control from seed.

Genetic Mapping of Summer Patch Resistance in Hard Fescue

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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 create a mapping population, construct a genetic linkage map, and identify Quantitative Trait Loci (QTL) for summer patch resistance in hard fescue. The parental clones R10 (resistant) and S5 (susceptible) were selected in a preliminary study based on the extreme phenotypic performance toward summer patch resistance. Full-sib progeny populations were constructed by crossing R10 (♀) x S5 (♂) and S5 (♀) x R10 (♂). Ninety-one progeny for population R10 (♀) x S5 (♂) and 87 progeny for population S5 (♀) x R10 (♂) (178 progeny total) were established in three identical mowed spaced-plant trials. 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 were assessed by visual rating on a scale of 1 to 10 during the summers of 2018 through 2021. Next Generation Sequencing was performed, and the sequence data were demultiplexed by Stacks with a reference genome to find SNPs. We obtained 6577 SNPs and 7230 SNPs shared by 90% of samples for R10 x S5 and S5 x R10 populations, respectively. A linkage map with 21 linkage groups was constructed using 1273 SNPs, covering 1593 cM in total. Eight QTLs, with significant LOD scores above the genome-wide LOD thresholds, were identified in four regions on three linkage groups. These QTLs explained 6.5% to 13.5% phenotypic variation for summer patch stress. This is the first report of QTL mapping of summer patch resistance in hard fescue and will help the development of hard fescue cultivars with improved resistance to summer patch in a more efficient method.

Bentgrass Cultivar and Autumn-applied Fungicide Timing Effects on Suppression of Dollar Spot

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Anecdotal observations and preliminary experimental evidence suggest that autumn-applied fungicide has the potential to reduce dollar spot, caused by *Clarireedia jacksonii*, on fairway turf during the subsequent growing season. However, the optimal timing of autumn-applied fungicides and potential interactions with cultivar susceptibility are unclear.

Twelve autumn fungicide timings, including a non-treated control, and three creeping bentgrass (*Agrostis stolonifera*) cultivars were evaluated in factorially arranged randomized complete block design with four replications for the suppression of dollar spot the subsequent growing season. Seven calendar-based timings were applied as a tank mixture of fluazinam and propiconazole either once (three timings), twice (three timings), or thrice (one timing) on 24 September, 15 October and/or 5 November during 2021. An eighth calendar-based timing received chlorothalonil applied thrice. Two additional fungicide timings were based on the Smith-Kerns Logistic Regression Model where fluazinam plus propiconazole was applied when the risk index output reached 20 or 40%. Finally, a twelfth timing consisted of fluazinam and propiconazole applied curatively on a threshold basis when 314 mm² per 3 m² of diseased foliage was observed.

All fungicide timings were applied to three creeping bentgrass cultivars: ‘Coho’ (least susceptible), ‘007’ (moderately susceptible), and ‘Independence’ (most susceptible to dollar spot) managed as a fairway turf at 9.5 mm. Plots were inoculated with *C. jacksonii* in March 2021. After all plots expressed dollar spot symptoms, chlorothalonil was applied on 8 and 31 July, 14 August, and 10 September 2021 to arrest the disease and ensure turf recovery. No disease symptoms were evident at the initiation of fungicide timing treatments on 24 September 2021. Both model-based timings were applied on 24 September and 15 October. The curative threshold timing resulted in one application for each cultivar on 9 or 11 October 2021.

Leaf tissue was collected on 18 Nov. 2021 for qPCR analysis to quantify pathogen load. The area exhibiting dollar spot symptoms on each plot was measured every 1 to 7 days from May through mid-August in 2022. Disease severity data was log₁₀ transformed to correct for heteroscedasticity and used to calculate the area under the disease progress curve (AUDPC). AUDPC was analyzed using the mixed model in GLIMMIX, SAS with fungicide treatment and cultivar as main effects.

While both factors affected dollar spot development in the subsequent season, cultivar had a greater impact compared to fungicide timing. Disease onset was delayed by 1- and 3-weeks on 007 and Coho, respectively, compared to Independence. All fungicide timings that included a September application of fluazinam plus propiconazole provided the best suppression of dollar spot measured as AUDPC the next growing season; whereas a single application of fluazinam plus propiconazole applied in October or November, and chlorothalonil applied thrice, had no effect on disease severity compared to the non-treated control. The qPCR analysis indicated that fluazinam

plus propiconazole applied in September (six timings) or once in early-October (curative threshold timing) provided the greatest suppression of the *C. jacksonii* population. The tank mix of fluazinam plus propiconazole applied on 15 October and/or 5 November did not suppress the *C. jacksonii* population compared to the non-treated check. This confirms that the suppression of the *C. jacksonii* population (inoculum load) in leaf tissue with autumn applications of fluazinam plus propiconazole can delay the onset of dollar spot symptoms and reduce disease severity the subsequent growing season.

Interestingly, the application of chlorothalonil applied thrice was among treatments with the greatest suppression of the *C. jacksonii* population in autumn; however, this treatment had no effect on disease severity during the subsequent growing season compared to the non-treated control. Thus, the timing effect of different fungicide chemistries applied in autumn on dollar spot during the subsequent growing season needs further evaluation.

Lipid Metabolism Associated with Heat Tolerance in Hard Fescue

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A detailed understanding of molecular mechanisms is required for improving heat tolerance in cool-season turfgrasses. The objective of the current study was to identify major lipids and oxygenated fatty acids (oxylipins) and their associated metabolic roles in driving heat tolerance in two hard fescue genotypes with distinct levels of heat tolerance. Harf fescue genotypes '219' and '141' were exposed to control (22/18 °C; day/night) and heat stress (35/30 °C; day/night) treatments in growth chambers for 21 days. No differences in turf quality were observed between the two genotypes throughout the control treatment. However, genotype '141' maintained greater turf quality, percent green cover, photochemical efficiency, and non-photochemical quenching compared to '219' at 21 days of heat stress, indicating its superior heat tolerance. The lipidomic analysis identified several differentially regulated lipids and oxylipins in '141' and '219' genotypes in response to 21 days of heat stress. Identified lipids and oxylipins are crucial underlying metabolites associated with heat tolerance in hard fescue and can potentially serve as biomarkers for improving heat tolerance in cool-season turfgrasses.

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