2019 Research Summaries

TURFGRASS AND ENVIRONMENTAL RESEARCH PROGRAM

CONFIDENTIAL — NOT FOR PUBLICATION

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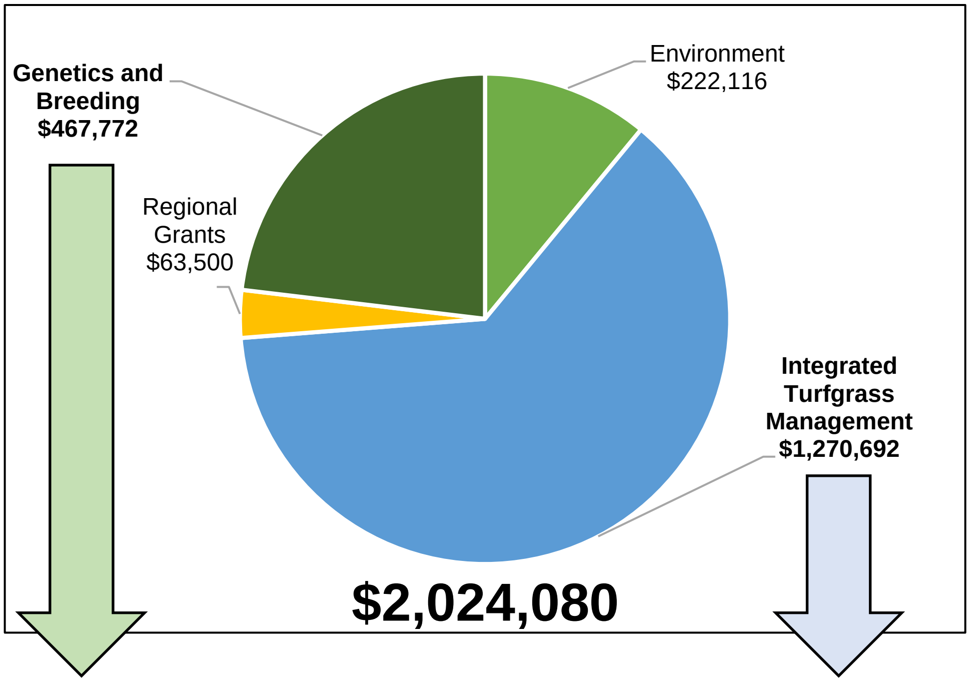
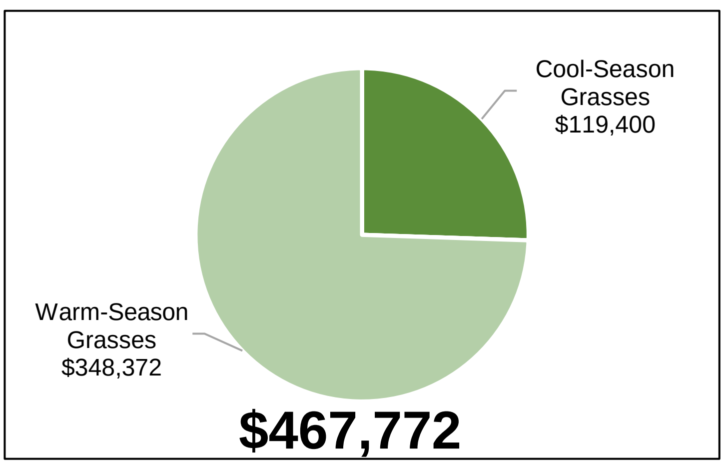
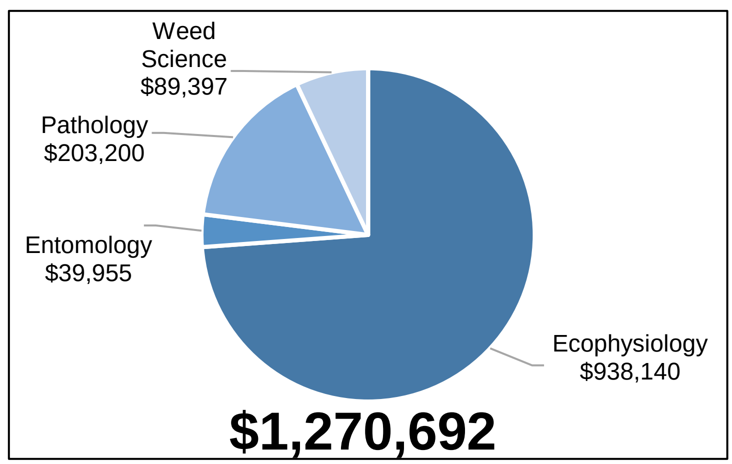
Turfgrass and Environmental Research Grants - 2019



Genetics and Breeding



Integrated Turfgrass Management



1. GENETICS AND BREEDING

The quality and stress tolerance of turf is a product of the environment, management practices, and genetic potential of the grass plant. In many cases, major limitations to turf quality are stress effects, many of which can be modified or controlled through plant improvement. Projects are directed toward the development of turf cultivars that conserve natural resources by requiring less water, and fewer pesticides and fertilizers. Among the characteristics most desirable in the new turfgrasses are:

- Reduced requirements for irrigation, mowing, and fertilization
- Tolerance of non-potable water
- Reduced need for pesticides by increasing resistance to disease, insects, nematodes, and weed encroachment
- Ability to survive high and low temperature extremes
- Increased shade tolerance
- Tolerance of intensive traffic and poor-quality soils.

USGA ID#: 1983-01-001

Title: Breeding and Evaluation of Kentucky Bluegrasses, Tall Fescues, Fine Fescues, Perennial Ryegrasses, and Bentgrasses for Turf

Project Leaders: William A. Meyer, Phillip L. Vines, and Stacy A. Bonos
Affiliation: Rutgers, The State University of New Jersey

Objectives:

1. Collect and evaluate useful turfgrass germplasm and associated endophytes.
2. Continue population improvement programs to develop improved cool-season cultivars and breeding synthetics.
3. Develop and utilize advanced technology to make current breeding programs more effective and efficient.

Start Date: 1982
Project Duration: Continuous
Total Funding: $10,000 per year

Summary Points:

- Continued progress was made in obtaining new sources of turfgrass germplasm. These sources are being used to enhance the Rutgers breeding program.
- Modified population backcrossing and continued cycles of phenotypic and genotypic selection combined with increasing sources of genetic diversity in turfgrass germplasm has resulted in the continued development and release of top performing varieties in the NTEP
- Five perennial ryegrasses, six new tall fescues, five fine fescues, two Chewings fescues, one hard fescue, two creeping red fescues, four Kentucky bluegrasses, and two creeping bentgrasses were named and released in 2019
- Published 2 referred journal articles and 12 non-referred journal articles in 2019.
- Fourteen Plant variety certificates issued and 6 PVP's applied for in 2019.

Summary Text:

As of October 30,2019 over 1,500 promising turfgrasses and associated endophytes were collected in Slovenia, Poland, Croatia, Italy, Hungary, Serbia, Austria, and Romania. These were evaluated in the spring of 2019 in the Netherlands and over 515 had seed produced in the summer of 2019 and will be evaluated in New Jersey starting in fall 2019. Over 9,675 new turf evaluation plots, 87,258 spaced-plant nurseries plants, and 11,000 mowed single-clone selections were established in 2019 in New Jersey.

Over 11,232 seedlings from intra- and inter-specific crosses of Kentucky bluegrass were screened for promising hybrids under winter greenhouse conditions, and the superior plants were put into spaced-plant nurseries in the spring. Over 12,276 tall fescues, 2,628 Chewings fescues, 2,000 hard fescues, 22,796 perennial ryegrasses, and 13,596 bentgrasses were also screened during the winter in greenhouses, and superior plants were put in spaced-plant nurseries. Over 118 new intra- and inter-specific Kentucky bluegrasses were harvested in 2019.

The following crossing blocks were moved in the spring of 20I9: five hard fescues (148 plants), seven Chewings fescues (166 plants), five strong creeping red fescues (131 plants), nineteen perennial ryegrasses (846 plants), ten tall fescues (370 plants), nine creeping bentgrasses (180 plants), four velvet bentgrasses (158 plants), and six colonial bentgrasses (203 plants).

The breeding program continues to make progress breeding for disease resistance and improved turf performance. New promising named and released perennial ryegrass varieties in 2019 were Umqua, Alloy, Vision, Lover, and Infusion. New tall fescues were Genius, Honeymoon, Annapolis, Corbett, Rebel 5, and Estrena. There were also two Chewings fescues named Momentum and Conductor, one hard fescue named Clarinet, and two creeping red fescues named Chorus and Ruddy. There were two new creeping bentgrasses named Mac Donald and S-1. The new Kentucky bluegrasses were Babe, Chloe, Tattoo, and Jersey.

Figure 1. A drone image (100-ft altitude) showing gray leaf spot activity in Adelphia, NJ. Note the less-susceptible varieties beginning in the fifth column from the right.

Figure 2. Tall fescue germplasm are screened for brown patch symptoms following inoculation.

USGA ID#: 2007-05-346

Title: Collection, Enhancement and Preservation of Turfgrass Germplasm

Project Leader: Kevin Morris
Affiliation: National Turfgrass Federation (NTF)

Start Date: 2017
Project Duration: 3 years
Total Funding: $30,000

Summary Points:

- Federal funding for priority research needs identified at a Fall 2017 workshop, including genome sequencing of turfgrass species, research to document the inherent value of turfgrass and drought turfgrass cultivars is now being requested.
- As a result of the workshop, USDA-ARS has not only allocated new funding for genome sequencing of turfgrass species, but also has developed a consortium of turfgrass researchers to initiate new studies.
- To determine the acreage, uses and economic value of the turfgrass industry in the U.S., NTF has collaborated with a group of universities and non-profit organizations to apply for a $1,000,000 USDA grant.
- The Foundation for Food and Agriculture Research (FFAR) has agreed to consider providing matching funds for priority turfgrass research projects identified in 2020.

Summary Text:

Background

Turfgrass is an estimated $60 billion, 60 million-acre industry in the U.S., making turfgrass the third largest agricultural crop in the U.S. by acreage. With tens of millions of home lawns, millions of miles of turf on roadsides, a million or more athletic fields, thousands of parks, golf courses, institutional grounds and other sites, turfgrass is an ubiquitous crop in the U.S., but often taken for granted as to its importance and value. To that end, federal government turfgrass research funding falls far below research funding for other comparably sized agricultural industries, averaging just over $1,000,000 annually. With the industry facing serious challenges such as water shortages, concerns about pesticide use, fertilizer restrictions and economic issues, research is needed to help the turfgrass industry overcome these challenges and thrive over the next 25-30 years.

To better address industry challenges, in September 2017 about 40 attendees participated in a professionally facilitated workshop, hosted by the National Turfgrass Federation and the U.S. National Arboretum, to discuss turfgrass research needs, priorities and funding strategies. Attendees included representatives from golf, parks, seed and sod, lawn and landscape, irrigation, equipment and the plant protection/enhancement industries; as well as university research, non-profits and the federal government.

The historical context and development of the 2004 National Turfgrass Research Initiative (NTRI) was presented, as well as presentations outlining federal government, non-profit organization and commercial turf industry research accomplishments. Two days of discussions within small groups, and one large group resulted in twenty-eight research needs that were consolidated considering overlap of ideas, resulting in eighteen broader research topics.

Voting by all participants resulted in multiple priorities on the research needs list. A few of the highest rated research needs included: 1) genomic studies to identify genes that infer drought, disease, cold and other tolerances in turfgrasses, 2) studies to quantity and maximize ecosystem services benefits of turfgrasses, and 3) new approaches to reduce water use and utilize more recycled water on turfgrass. Discussions ensued on potential research partners including, USDA-ARS, universities, non-profit organizations and private industry.

Results to Date

As a result of this workshop and the interest shown by participants in increasing research funding, USDA-ARS has committed new funding to genome sequencing of turfgrasses at several locations. Consequently, USDA-ARS turfgrass researchers have formed a research consortium to encourage collaboration among ARS units in Tifton, GA, Beltsville, MD, Corvallis, OR and Logan, UT. The researchers are now organizing, collaborating and developing their research plans. Much like the potential of genome sequencing to develop new treatment strategies for human health issues, this funding will contribute foundational information to aid the development of better heat, cold, drought, disease and insect resistant grasses.

The National Turfgrass Federation (NTF) worked to develop and include in the 2018 U.S. Farm Bill language that outlines not only the importance of turfgrass research (through the National Turfgrass Research Initiative), but also broad national turfgrass research needs. We have organized groups and have met with various members of Congress and federal government officials, including Secretary of Agriculture Sonny Perdue to discuss our needs. The next steps are to obtain funding for turfgrass research projects within the federal government, as well as competitive grants within and outside the federal system.

The first turfgrass funding request is $3,000,000 for FY2020 to be spread across six USDA-ARS labs. Priority research topics include additional funding for genome sequencing, drought tolerant grasses, and ecosystem service maximization from turfgrass areas. We are also continuing our efforts to obtain additional turfgrass competitive grant funding opportunities through USDA-NIFA.

In addition, we have initiated discussions with a major non-profit, the Foundation for Food and Agriculture Research (FFAR) to identify turfgrass projects for potential funding. Congress created FFAR in the 2014 Farm Bill to increase funding for agricultural research. In the 2018 Farm Bill, FFAR was reauthorized with $185 million in federal funding available. All FFAR projects require a 1:1 match with industry and/or non-profits. In 2020, FFAR will work with the turfgrass industry to develop mutually agreeable research projects and will then match funding committed by industry and non-profits.

Future Plans

Another priority need identified at the workshop is the gathering and publishing of acreage, scope, usage and economic value data on the U.S. turfgrass industry. This information is critical to documenting the economic and societal impact of the turfgrass industry within the U.S. NTF has selected a firm to conduct a national turfgrass survey and recently, in collaboration with several universities and non-profit organizations, has applied for a $1,000,000 USDA grant to fund the survey. If funded, this data should be available in 2022. Turfgrass acreage, use and economic value is another essential piece in demonstrating the need for turfgrass research nationally, and in our states and communities. Increased initial funding opportunities, a comprehensive national survey, as well as an updated National Turfgrass Research Initiative using needs identified at the 2017 workshop, are important steps to reducing inputs and improving turfgrass management practices.

Figure 1. National Turfgrass Federation meeting with the U.S. Secretary of Agriculture, Sonny Perdue.

USGA ID#: 2016-25-575

Title: Genetic Engineering of Turfgrass for Enhanced Multi-Stress Resistance

Project leader: Hong Luo
Affiliation: Department of Genetics and Biochemistry, Clemson University

Objectives:

The main objective of this project is to genetically engineer enhanced tolerance to multiple adverse environmental conditions, such as drought, salt, heat and nutrient deficiency in turfgrass using agricultural biotechnology approaches. We proposed to develop methodology to evaluate and demonstrate the feasibility of genetically engineering multi-stress tolerance in transgenic turfgrass through simultaneous overexpression of three genes encoding a vacuolar H+-pyrophosphatase, *AVP1* from Arabidopsis, a SUMOylation E3 ligase, *OsSIZ1* from rice, and a flavodoxin, *Fld* from cyanobacterium. Specifically,

1. We will prepare a chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/ p35S-*bar* containing CaMV35S promoter-driven *AVP1*, corn ubiquitin promoter- driven *OsSIZ1* and *Fld* genes together with a CaMV35S promoter-driven selectable marker gene, *bar*, for herbicide resistance.
2. We will conduct *Agrobacterium*-mediated turfgrass transformation to produce transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* construct and overexpressing *AVP1*, *OsSIZ1* and *Fld* genes.
3. We will analyze putative transgenic plants for transgene insertion and expression.
4. We will Examine plant growth and development in transgenics and evaluate plant performance under various abiotic stress conditions including drought, heat, salt, P and N starvation in comparison with wild type plants and transgenic lines expressing a single transgene (AVP1, OsSIZ1 or Fld).

Start Date: 2016
Project Duration: 3 years
Total Funding: $60,000

Summary Points:

- Three representative transgenic lines harboring the chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, and expressing high level of the three stress-related genes, *AVP1*, *OsSIZ1* and *Fld* were grown in greenhouse and asexually multiplied for performance evaluation. Another three transgenic lines expressing AVP1, OsSIZ1 and Fld, respectively, as well as non-transgenic wild type (WT) plants were also simultaneously grown in greenhouse and used as controls for comparison.
- Assessed plant growth under normal conditions comparing different transgenic lines and wild type plants and observed that the transgenic lines expressing three genes together (AVP1, OsSIZ1 and Fld) outperformed wild type and transgenic plants expressing only one of the three genes (AVP1, OsSIZ1 and Fld), exhibiting rapid plant growth with more biomass production (examples shown in Fig. 1).

- Conducted salinity and drought stress and nitrogen deficiency tolerance test with all the transgenic lines and wild type plants. The transgenic lines expressing three genes together (AVP1, OsSIZ1 and Fld) outperformed wild type and transgenic plants expressing only one of the three genes (AVP1, OsSIZ1 and Fld), exhibiting enhanced tolerance to the stress conditions tested (examples shown in **Fig. 2 & 3**).
- Our data showed that simultaneous introduction of three transgenes into turfgrass not only boosted plant growth, but also significantly enhanced plant performance under various adverse environmental conditions. Assessment of plant response to additional abiotic stresses including oxidative stress, P starvation and study of other characteristics are currently being completed. The results obtained demonstrate the great potential of extending the same strategy to other turf and forage species as well as vegetable and food crops, significantly enhancing agriculture production. The plant materials could be directly used for cultivation upon completion of the government deregulation process, or used as foundational breeding materials for products of wide environmental adaptation.

Summary Text:

In the face of a global scarcity of water resources, the increased salinization of soil and water as well as elevating environmental pollution caused by fertilization, enhancing crop plant tolerance to various abiotic stresses is the big challenge of modern agriculture practice. This project aimed to genetically engineer turfgrass with multiple genes involved in plant stress response for enhanced plant performance under adverse environmental conditions. In our previous summaries presenting our research data obtained in 2016 and 2017, we reported the construction of a chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* that contains expression cassettes for three genes, *AVP1*, *OsSIZ1* and *Fld* together with a selectable marker gene, *bar* for herbicide resistance, and its introduction into creeping bentgrass plants. Transgenic analysis confirmed foreign gene insertion and expression in the twenty primary T0 transgenic lines using PCR and Northern hybridization. Three representative transgenic lines with high level of foreign expression were selected and asexually propagated for further analysis. We have continued to conduct greenhouse experiments evaluating transgenic plants for their growth, development and performance under various abiotic stresses in comparison with wild type controls and transgenic lines expressing individual transgenes, AVP1, OsSIZ1 and Fld respectively. As shown in **Fig. 1**, when overexpressed individually in transgenic plants, both AVP1 and OsSIZ1 led to enhanced plant growth compared to wild type controls. Remarkably, transgenic plants overexpressing all three genes grew even faster than AVP1 and OsSIZ1 transgenic plants, producing the highest biomass among all plant lines tested, indicating that co-expression of three genes together in transgenic plants are able to significantly boost plant growth.

When assessing plant performance under various environmental adversities, we found that transgenic plants overexpressing the three genes, AVP1, OsSIZ1 and Fld exhibited enhanced tolerance to multiple abiotic stresses, such as salinity, drought and nitrogen deficiency (examples shown in **Fig. 2** and **Fig. 3**).

Publication resulting from the project:

1. Yuan, S., Zhao, J., Li, Z., Hu, Q., Yuan, N., Zhou, M., Xia, X., Noorai, R.E., Saski, C., Li, S., **Luo, H.** (2019) MicroRNA396-mediated alteration in plant development and salinity stress response in creeping bentgrass. Nature Horticulture Research 6:48.
2. Zhao, J., Yuan, S., Zhou, M., Yuan, N., Li, Z., Hu, Q., Bethea F.G. Jr., Liu, H., Li, S., **Luo, H.** (2019) Transgenic creeping bentgrass overexpressing Osa-miR393a exhibits altered plant development and improved multiple stress tolerance. Plant Biotechnology Journal 17:233-251.
3. Zhou, M., Zhao, J., Li, D., Yuan, S., Yuan, N., Li, Z., Jia, H., Gao, F., San, B., Hu Q., **Luo, H.** (2019) Biolistic DNA delivery in turfgrass embryonic callus initiated from mature seeds. In: Methods in Molecular Biology - Biolistic DNA Delivery in Plants, Rustgi, S & **Luo, H.** (eds.) Springer Science+Business Media, NY (in press).
4. Wu, P., Cogill, S., Qiu, Y., Li, Z., Zhou, M., Hu, Q., Chang, Z., Noorai, R., Xia, X., Saski, C., Raymer, P., **Luo., H.** (2019) Comparative transcriptome profiling provides insights into plant salt tolerance in Seashore paspalum (*Paspalum vaginatum*) (revision submitted).

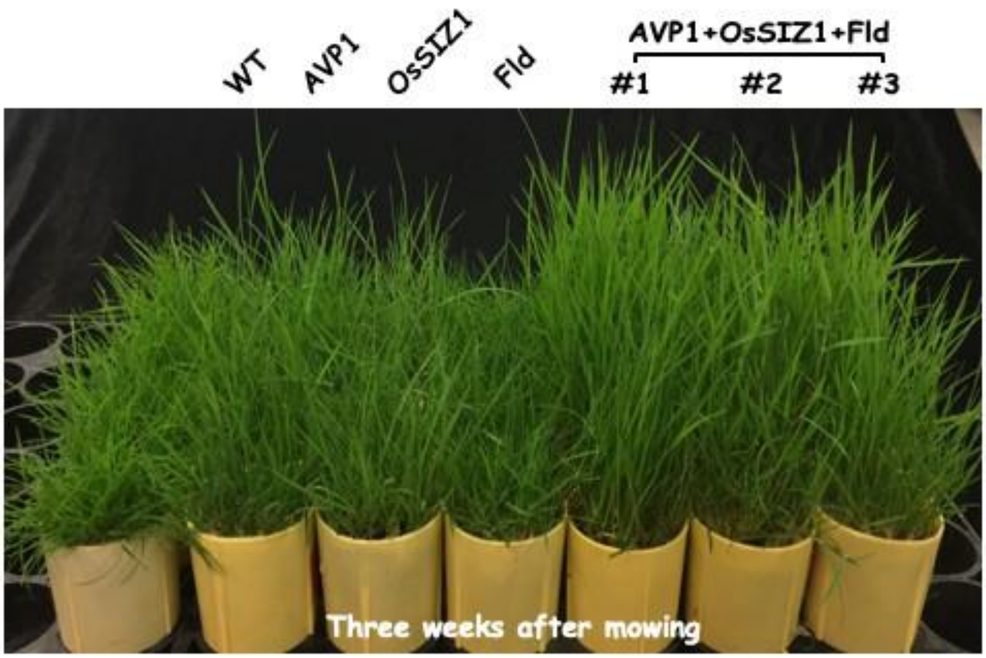


Figure 1. Transgenic creeping bentgrass plants harboring the chimeric gene construct p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* that overexpress the three genes, *AVP1, OsSIZ1* and *Fld* (AVP1+OsSIZ1+Fld, lines #1, 2 & 3) exhibited significantly enhanced growth. Wild-type (WT) control plants, the three transgenic lines expressing AVP1, OsSIZ1 and Fld, respectively, and the three representative transgenic lines expressing all three genes, *AVP1, OsSIZ1* and *Fld* were fully developed in cone-tainer for 8 weeks, then mowed and grown for three weeks in growth room under normal conditions before photographing.

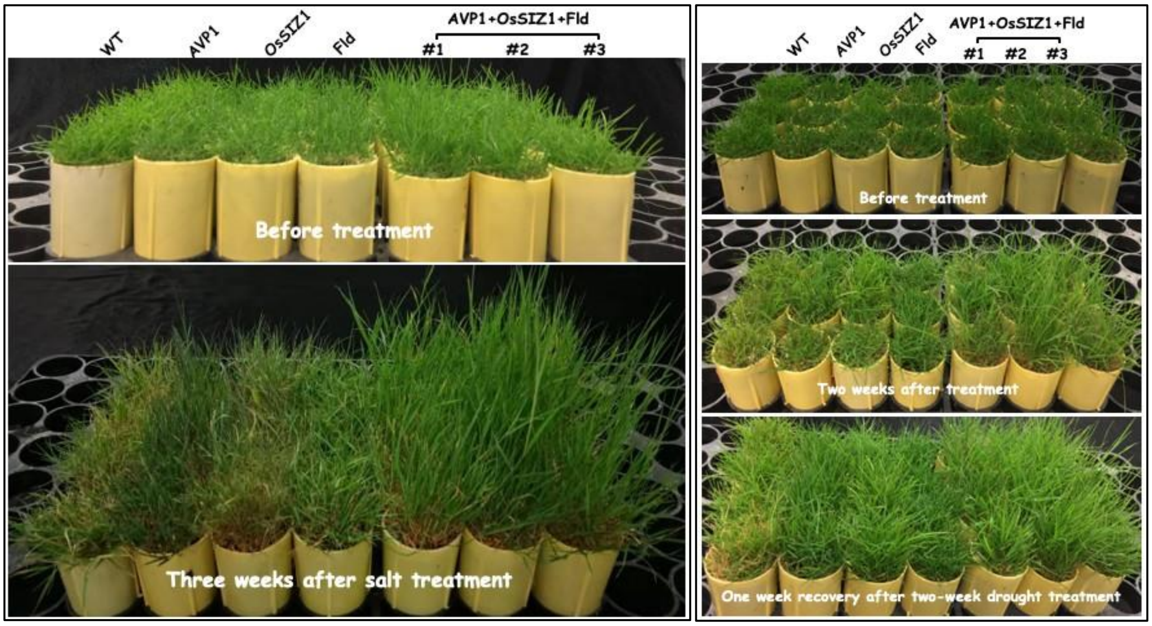


Figure 2. Transgenic creeping bentgrass plants harboring the chimeric gene construct p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* exhibited enhanced tolerance to multiple environmental stresses. Wild-type (WT) control plants, the three transgene line expressing *AVP1, OsSIZ1* and *Fld*, respectively, and additional three representative transgenic lines expressing all three genes, *AVP1, OsSIZ1* and *Fld* were fully developed in cone-tainer for 8 weeks under normal conditions in growth room **(left).** To evaluate plant performance under salt stress, both WT and transgenic plants expressing individual or a group of three genes were maintained in the growth room and subjected to a three-week treatment of 200 mM NaCl. As shown in the picture, the transgenic lines expressing *AVP1*, *Fld* or three genes (*AVP1*, *OsSIZ1* and *Fld*) together exhibited significantly higher tolerance salt stress than WT controls and the transgenic line expressing OsSIZ1. The former remained green with no apparent stressed symptom, whereas the latter became wilted and yellowish showing severely damaged symptom **(right).** To evaluate plant performance under drought stress, both WT and transgenic plants expressing individual or a group of three genes were maintained in the growth room and subjected to a two-week water withholding. Plants were photographed two weeks after water withholding and one week after recovery for documenting plant response to drought stress. As shown in the picture, the transgenic lines harboring either individual or three genes all performed better than WT controls, surviving drought treatment and continued their growth, whereas the WT controls became wilted and failed to completely recover upon resumed watering.

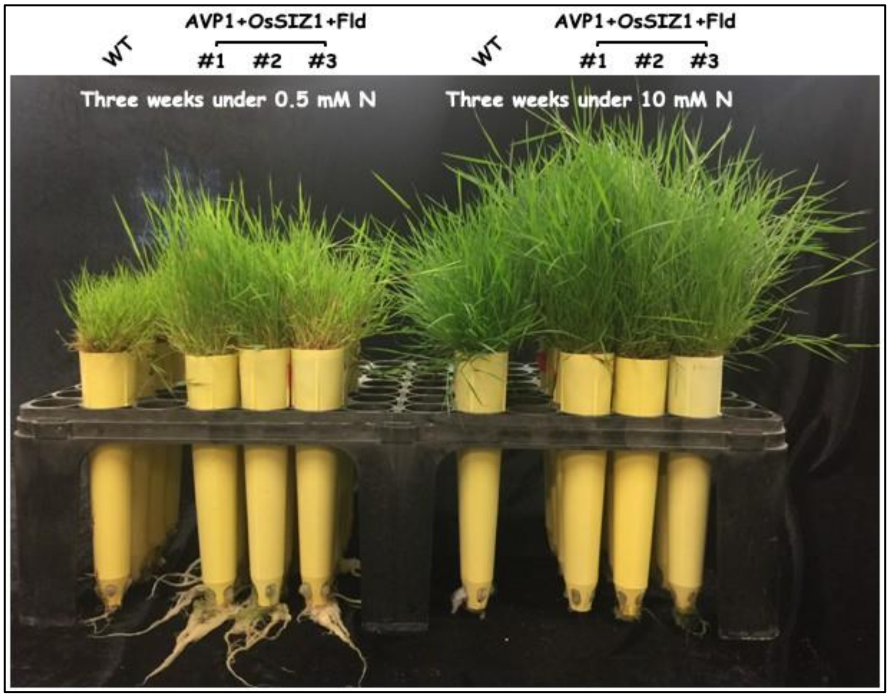


Figure 3. Transgenic creeping bentgrass plants harboring the chimeric gene construct p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi- *FNR:Fld*/p35S-*bar* exhibited enhanced tolerance to nitrogen (N) deficiency. Wild-type (WT) control plants, the three transgene lines expressing all three genes, *AVP1, OsSIZ1* and *Fld* were fully developed in cone-tainer for 8 weeks under normal conditions in growth room. All the plants were then grown on MS medium containing 0.5- or 10-mM N for three weeks. As shown in the picture, the transgenic lines expressing *AVP1*, *OsSIZ1* and *Fld* grew better and produced more shoot biomass under both N deficiency (0.5 mM) and normal N supply (10 mM). In addition, N deficiency also significantly boosted plant root growth in transgenic lines compared to WT controls.

USGA ID#: 2017-12-622

Title: Pre-Breeding for Bentgrass Germplasm Improvement

Project Leaders: Keenan Amundsen[1], Scott Warnke[2], Bill Kreuser[1]
Affiliation: [1]University of Nebraska-Lincoln; [2]USDA-ARS

Objectives:

The goal of this research is to develop genetically narrow but diverse bentgrass families with enhanced abiotic stress tolerance.

Start Date: 2017
Project Duration: 3 years
Total Funding: $51,040

Summary Points:

- Water-deficit stress tolerance between creeping and colonial bentgrass was assessed.
- Transcript expression differences between colonial and creeping bentgrass in response to water-deficit stress were identified.
- Genotype-specific genetic resources were developed to reduce cultivar development time and support variety discrimination.

Summary Text:

There are principally five bentgrasses used in turf systems including colonial bentgrass, creeping bentgrass, velvet bentgrass, redtop bentgrass, and highland bentgrass. Creeping bentgrass (*Agrostis stolonifera*) is the most prevalent, due to its adoption by the golf industry for its ability to form an exceptional golf course putting green playing surface. Creeping bentgrass is naturally adapted to ditch banks and areas prone to periodic flooding, and it is often considered a “water loving” species. Improved creeping bentgrass cultivars can maintain a relatively high visual and functional quality even with modern turfgrass management practices designed to keep the turf lean and fast with reduced management inputs.

There is demand for reduced water utilization in turf systems and improving drought tolerance of managed turf species is one way to address the need. If water-limiting stress tolerance of creeping bentgrass is considered, there is variability across cultivars and germplasm selections. However, there are limits to the level of drought tolerance in creeping bentgrass, which impedes the ability to make significant advancements for drought tolerance through conventional plant breeding. Colonial bentgrass, which is naturally adapted to dryer environments, is more drought tolerant than creeping bentgrass. It is possible to introduce traits from certain bentgrass species to others and as an example, dollar spot resistance from colonial bentgrass was introduced into creeping bentgrass [Belanger et al., 2004. Crop Science 44(2):581-86]. A similar approach could be used to introduce improved drought tolerance from colonial bentgrass into creeping bentgrass, or other desirable traits from other extant bentgrass species. When introducing desirable traits from unimproved accessions to elite breeding lines, traits that negatively impact quality are also introduced and require several rounds of breeding and selection to remove the undesirable traits, slowing the breeding process and time-to-release new cultivars.

Our project aims to use a pre-breeding approach to introduce desirable traits from wild or unimproved bentgrasses into high quality breeding lines, followed by additional plant breeding cycles to remove the undesirable traits that were introduced through the initial crosses. The resulting germplasm could more easily be used by bentgrass breeders to improve their elite lines. We evaluated wild bentgrass accessions from the National Plant Germplasm System in our previous project in preparation for this study. We identified wild material that performed as good as or better than standard entries for establishment rate, color, and density. From the previous project we also identified bentgrass accessions with potential for use in lawns. Buffalograss is commonly found in lawns in many coastal regions but that end use is not a priority for the bentgrass industry.

In 2018, we developed genotype-specific markers that could confirm hybrid formation between creeping and colonial bentgrasses. Due to the success of the genotype-specific markers for their ability to discriminate accessions, we focused our current efforts on expanding bentgrass genetic resources by identifying gene candidates that can be used as genetic markers to accelerate the breeding process. A greenhouse study was done where BCD colonial bentgrass, and Providence and Declaration creeping bentgrasses were grown under well-watered or water-limiting conditions. Total RNA was extracted and sequenced, generating 567 million 150 bp paired-end reads. Contaminants and poor-quality reads were removed and the cleaned sequences were separately assembled into reference transcriptomes (Table 1). Declaration had the most assembled transcripts and Providence the least, with 126,620 average number of assembled transcripts for the three cultivars. The final transcriptome assemblies ranged from 49.4 to 59.8 Mb, with an average transcript size of 443 bp.

The sequencing reads for each genotype treatment were mapped to each of the three reference transcriptomes. The number of sequencing reads mapped to a given transcript is an indication of the relative expression of that transcript when that sample was collected per genotype in response to the water treatment. Differences in read counts can then be used to infer changes in transcript expression between genotypes or treatments. The Bioconductor edgeR package was used to assess differential gene expression (Table 2). Differential transcript expression analysis was done in reference to each variety transcriptome in order to identify variety-specific transcripts responding to the water treatment in addition to those with different expression profiles. When basal transcript expression is compared between BCD colonial and Providence or Declaration creeping bentgrasses, the average number of transcripts differentially expressed was 1,237 and not much difference was observed between varieties. Similarly, when the water deficit treatments were compared, there was a slight increase in the number of transcripts differentially expressed between BCD colonial and Providence creeping bentgrass compared to BCD colonial and Declaration creeping bentgrass, but again the number of differentially expressed transcripts was consistent among treatments. However, when a comparison was made between the water deficit and control samples for each variety, BCD colonial had 2.5 to 5 fold increase in the number of differentially expressed genes compared to the creeping bentgrass samples suggesting that BCD colonial is actively responding to the stress. Research is continuing beyond the funded project to characterize the transcripts involved in the response. The data generated from this project will serve as a reservoir for genotype-specific markers that can be further used for variety discrimination or tracking water deficit stress tolerance in breeding programs.

Table 1. Summary of sequencing and transcriptome assembly for BCD colonial, and Providence and Declaration creeping bentgrasses.

Variety	Water treatment	Rep	Raw Reads	Cleaned Reads	Assembled Transcripts
Providence	Control	1	30,385,608	25,184,056	115,991
		2	39,615,872	33,955,862	
		3	31,862,466	26,724,082	
	Deficit	1	37,103,630	31,781,812	
		2	44,912,066	38,016,330	
		3	24,262,698	20,821,486	
BCD Colonial	Control	1	18,832,482	15,746,322	126,042
		2	22,592,358	19,153,928	
		3	22,404,136	18,136,700	
	Deficit	1	49,290,640	41,304,212	
		2	35,251,384	28,706,308	
		3	25,697,642	21,236,668	
Declaration	Control	1	32,886,360	28,506,068	137,829
		2	22,220,692	18,636,070	
		3	20,739,064	17,598,868	
	Deficit	1	20,106,548	16,825,144	
		2	26,058,582	21,452,114	
		3	62,953,348	52,284,296	

Table 2. Differentially expressed transcripts between BCD colonial, and Providence and Declaration creeping bentgrasses in response to deficit water stress.

Treatment A		Treatment B		Reference Transcriptome		
Variety	Condition	Variety	Condition	BCD Colonial	Declaration	Providence
BCD Colonial	Control	Declaration	Control	1,062	1,186	1,370
BCD Colonial		Providence		1,411	1,965	882
Declaration		Providence		1,673	2,676	552
BCD Colonial	Deficit	Declaration	Deficit	1,492	2,005	1,766
BCD Colonial		Providence		2,187	3,029	2,073
Declaration		Providence		752	878	347
BCD Colonial	Control	BCD Colonial	Deficit	5,223	4,294	4,243
Declaration		Declaration		2,080	2,117	1,940
Providence		Providence		1,071	1,042	1,427

USGA ID#: 2018-11-661

Title: Evaluation of activity of a fungal endophyte antifungal protein against dollar spot infected creeping bentgrass

Project Leaders: Faith C. Belanger and Bruce Clarke
Affiliation: Rutgers University

Objectives:

The goal of this project is to produce the *Epichloë festucae* antifungal protein in bacteria and test its activity in dollar spot infected creeping bentgrass and fine fescue. If the purified protein is effective, this could represent an additional method to control dollar spot and reduce fungicide inputs.

Start Date: 2018
Project Duration: 3 years
Total Funding: $120,000

Summary Points:

1. The fungal endophyte (*Epichloë festucae*) that infects strong creeping red fescue produces an abundant antifungal protein that is not found in most *Epichloë* species. It may be involved in the disease resistance observed in endophyte-infected strong creeping red fescue.

2. Gene knock-outs of the antifungal protein were produced, but extensive efforts to move the antifungal protein minus-isolates back into endophyte-free plants have been unsuccessful. These results raise the possibility that: 1) the antifungal protein may be important in the symbiosis between the turfgrass host and the endophyte and that without it the endophyte may be identified as a pathogen by the host and eliminated, or 2) the lack of ability to inoculate plants with the knock-out isolates is due to some other change in those isolates as the result of removing this gene.

3. The activities of the *E. festucae* antifungal protein produced in yeast and bacteria, and the activity of a similar protein, PAF, from *Penicillium chrysogenum*, were assessed against the model fungus *Neurospora crassa*.

Summary Text:

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

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

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

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

Two independent gene knock-out isolates of *E. festucae* were generated by using the CRISPR-Cas9 technology. The objective was to inoculate the knock-out isolates back into endophyte-free strong creeping red fescue and assess the level of dollar spot resistance of the plants. If the antifungal protein is indeed a factor in the dollar spot resistance, as we suspect it is, then the plants harboring the knock-out isolates would be expected to exhibit less or no dollar spot resistance. However, extensive attempts over a 12-month period to inoculate plants with the knock-out isolates were unsuccessful (Table 1). Interestingly, the wild type isolate was successfully inoculated into endophyte-free plants. These results were unexpected and have raised new questions about the possible importance of the *E. festucae* antifungal protein to the symbiotic association with strong creeping red fescue. Is the antifungal protein an important factor in the symbiotic interaction or is the lack of ability to inoculate plants with the knock-out isolates unrelated to the lack of the antifungal protein gene but rather due to some other change in those isolates as the result of removing this gene?

Table 1. Inocluation of strong creeping red fescue seedlings with *Epichloë festucae* wild-type Rose City (RC) and antifungal protein gene knock-out isolates 1a-7t8s3 and 1c-3s5.

	Not infected	Infected	Died	Total
Wild type RC isolate	18	5	17	40
Knock-out 1a-7t8s3	39	0	32	71
Knock-out 1c-3s5	51	0	17	68

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

E. festucae antifungal protein purification

The *E. festucae* antifungal protein is a highly expressed fungal protein in the infected host grass (Ambrose and Belanger, 2012), but purification from plant tissue is not practical since it is overall a minor protein in the mixed fungal/plant tissue. Moreover, it is not expressed when the fungus is grown in culture. We, therefore, have explored generating the antifungal protein in several established protein expression systems. The objective is to identify a protein expression system that can generate a large amount of active antifungal protein in the simplest way. The antifungal protein has been successfully expressed in the yeast *Pichia pastoris* (Tian et al., 2017) and in the bacterium *Escherichia coli.* The *E. festucae* antifungal protein is similar to a protein from another fungus, *Penicillium chrysogenum,* which is designated PAF (*Penicillium* antifungal protein) and which also has antifungal activity (Marx, 2004). We have obtained an engineered PAF overexpression strain of *P. chrysogenum* from Dr. Florentine Marx (Medical University of Innsbruck, Innsbruck, Austria) so that we could directly compare the activities of PAF and the *E. festucae* antifungal protein. A comparison of antifungal activities of PAF and the *E. festucae* antifungal protein produced in yeast and in bacteria against *Neurospora crassa* conidia, a model fungus used in such systems, is shown in Fig. 1. At the lowest concentrations, PAF had the best activity, but at greater than 5 ug/mL the activity of the *E. festucae* antifungal protein produced in yeast was similar to PAF. At concentrations greater than 20 ug/mL the activities of all three samples were similar.

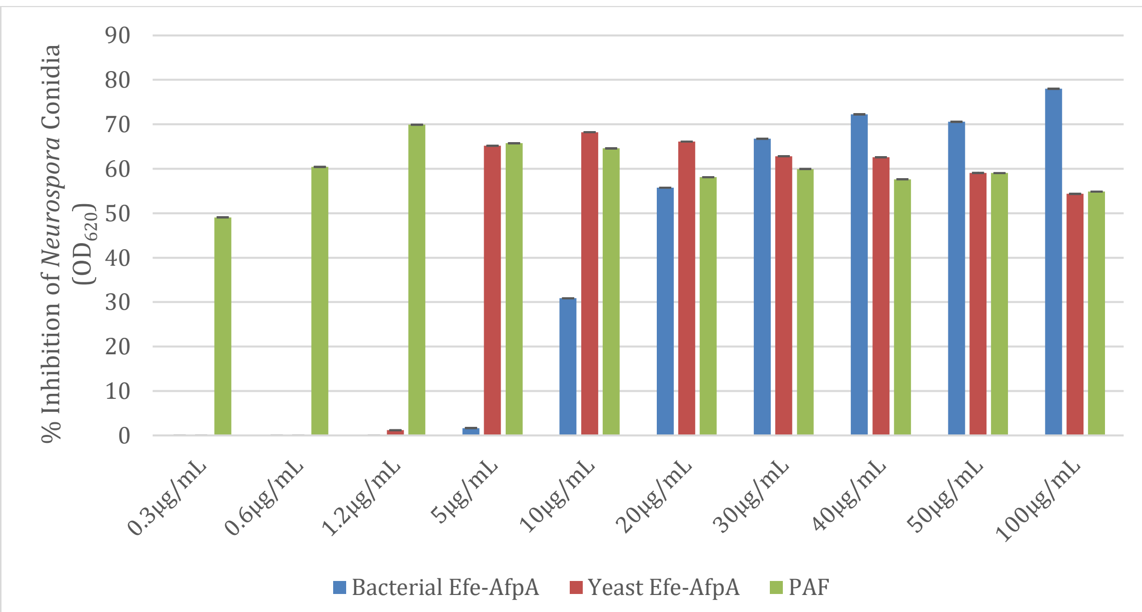


Figure 1. Comparative percent inhibition of growth of *Neurospora* conidia by the *E. festucae* antifungal protein and PAF. Increasing concentrations of both bacterially and yeast produced *E. festucae* antifungal protein (Efe-AfpA), and *Penicillium chrysogenum* produced PAF were assayed in triplicate against *Neurospora* conidia and growth was measured after 24 hours. Percent inhibition was measured by absorbance at OD_{620}. Standard deviation is represented by error bars.

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

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USGA ID#: 2019-15-685

Title: Deciphering the relationship between environmentally induced epigenetic modification and dwarfism in greens-type *Poa annua* L.

Project Leader: David R. Huff and Chris W. Benson
Affiliation: Pennsylvania State University

Objectives:

Objective 1 (Month 1-6): Elucidate the global methylation status of mowed and unmowed *Poa annua* using traditional ecological methods such as MSAP and enzyme-linked immunosorbent assays (ELISA).

Objective 2 (Month 18-30): Elucidate downstream transcriptional changes as a response to epigenetic modification during imposed environmental stress on clonal *Poa annua*.

Objective 3 (Month 1-18): Use methods in genomic sequencing to resolve the methylation status of candidate loci identified in the RNA-seq analysis.

Objective 4 (Month 12-24): Evaluate transgenerational retention of morphological characters and epigenetic signatures in subsequent generations of *Poa annua* mowed and unmowed.

Objective 5 (Month 24-36): Use methyl-sensitive restriction enzymes as a marker to guide the breeding of elite and stable cultivars of *Poa annua* for commercial release for golf-course putting greens.

Start Date: 2019
Project Duration: 3 years
Total Funding: $91,824

Summary Points:

- *Poa annua*'s range of phenotypes is echoed by its range of genome architectures.
- Under stress of mowing, *Poa annua* experiences increased global DNA methylation.
- Reduced shoot size in the offspring of mowed clones suggests that *Poa annua* can pass environmental cues to its offspring in a transgenerational fashion.
- Transgenerational inheritance of mowing stress is correlated with heritable patterns of DNA methylation, but the causal mechanism of transgenerational inheritance and adaptive plasticity remains unclear.

Summary Text:

Heritability of acquired traits caused by environmental cues is a possible driving factor in *Poa annua's* global proliferation and wide-ranging morphologies. To study transgenerational inheritance of acquired phenotypes, we grew and phenotyped progeny plants from mowed and unmowed *Poa annua.* Parental genotypes had either been mowed at 3 mm for eight months or left unmowed for eight months. Seed was grown from the mowed and unmowed clones and phenotyped periodically to test for transgenerational differences in shoot area of the progeny plants. Progeny from mowed clones were on average 37% smaller than progeny grown from unmowed clones (n=35, p=0.04, **Fig. 1**). Reduction in shoot area of the offspring from mowed parental plants were observed up to 95 days after germination when the experiment ended. Reduced shoot size in the offspring of mowed clones suggests that *Poa annua* can pass environmental cues to its offspring in a transgenerational fashion. Because epimutations occur more frequently than genetic mutations, it is likely that that observed transgenerational plasticity is conferred at least in part by epigenetic mechanisms (Kronholm and Collins 2016).

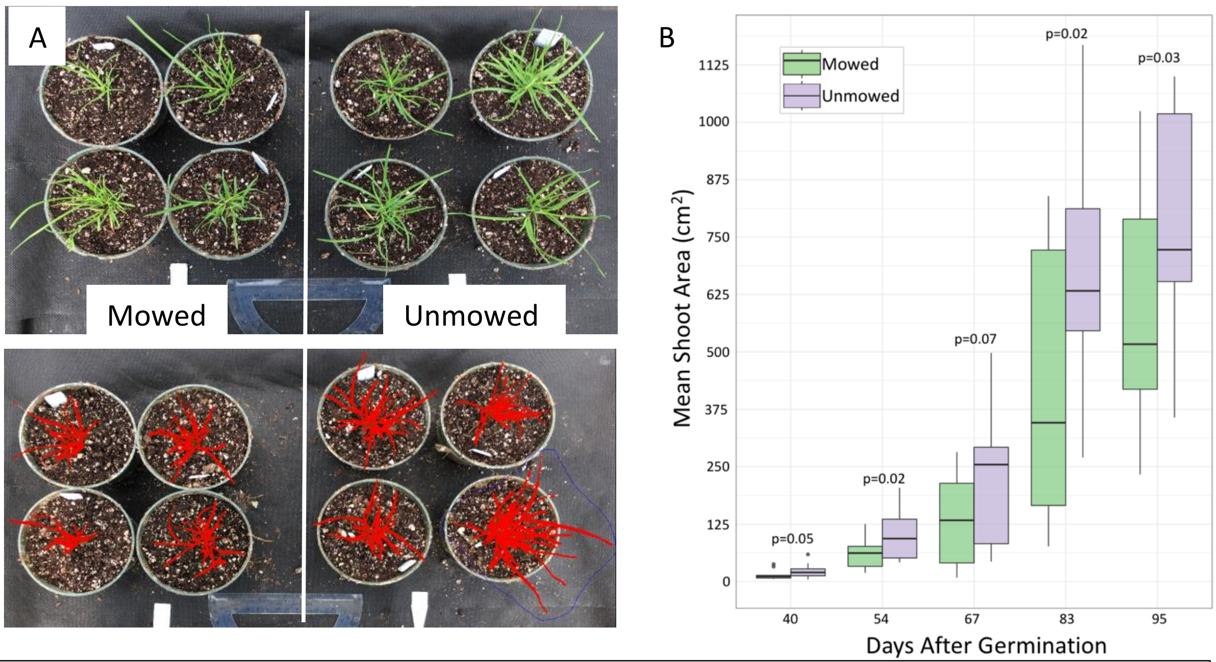

Figure 1: Transgenerational priming of *Poa annua* to mowing stress. (A) example images show the progeny of mowed and unowed clones during image analysis. (B) boxplots depict the shoot area of offspring *Poa annua* when the clonally propagated parent was either mowed at 3 mm for eight months or left unmowed for eight months. Plants were measured periodically between 40 and 95 days after germination (n=35). p-values above the boxes show the likelihood that the observed differences in shoot area are biologically significant at each timepoint. Shoot area was measured in imageJ.

To study epigenetic modification and phenotypic plasticity in *Poa annua* under mowing stress, we used MSAP to survey the global methylation profiles of clonally propagated plants under simulated mowing maintenance. Of the 448 identified MSAP loci, 154 were susceptible to methylation and 294 were identified as being not susceptible to methylation. Methyl-susceptible loci were used to assess epigenetic variation while non-methyl susceptible loci were used to assess genetic variation in a similar fashion as a traditional AFLP experiment. Of the methyl-susceptible loci, 84% (129) were polymorphic between mowing treatments while only 32% (93) of the non-methyl susceptible loci were polymorphic between treatments. A Shannon's diversity index corroborated that epigenetic loci are more diverse than genetic loci (methyl-susceptible loci, I=0.5789 and SD=0.115; non-methyl susceptible loci, I=0.1854 and SD=0.086), providing further confidence that variations in epigenetic methylation status were more prevalent than variation in genetic mutational status between mowed and unmowed clones, Wilcoxon rank sum test with continuity=11874 (P<0.0001).

Despite having higher epigenetic variation than genetic variation, neither global genetic (non-methyl susceptible) nor global epigenetic (methyl-susceptible loci) differences were detected between mowed and unmowed treatments across genotypes (AMOVA, ϕ_{ST}= 0.01376, P=0.1446 and ϕ_{ST}= -0.0234, P=0.93, respectively) suggesting that although methylated loci were more diverse than genetic loci, their diversity was irregular and did not associate with mowing treatments across all genotypes and all 5'-CCGG methylation contexts. Diversity of genetic loci was predictably low due to our experimental design that included identical genotypes present in both the mowed and unmowed treatments.

Methylation at the target 5'-CCGG restriction site was divided into subcategories where presence of both the *EcoRI-HpaII* and *EcoRI-MspI* bands indicates an unmethylated state, presence of one of the restriction sites represents a hemimethylated or internally methylated state, and absence of both *EcoRI-HpaII* and *EcoRI-MspI* is considered fully methylated. It is important to note that absence of both restriction sites is uninformative in experiments with genetic diversity because this banding pattern could also be caused by genetic mutation. Our experimental design circumvents the mutation problem by analyzing genotypes that had been clonally propagated thus, excluding genetic diversity and allowing us to call absence of both restriction sites as fully methylated. Interestingly, only fully methylated CCGG sites were significantly different during pairwise comparisons of mowed and unmowed genotypes (p=0.002, **Fig. 2**) with mowed genotypes being, on average, 6.8% more fully methylated (hyper-methylated) than their unmowed clone. Variation between the remaining contexts, hemi, internal, and unmethylated, were not significant between mowing treatments.

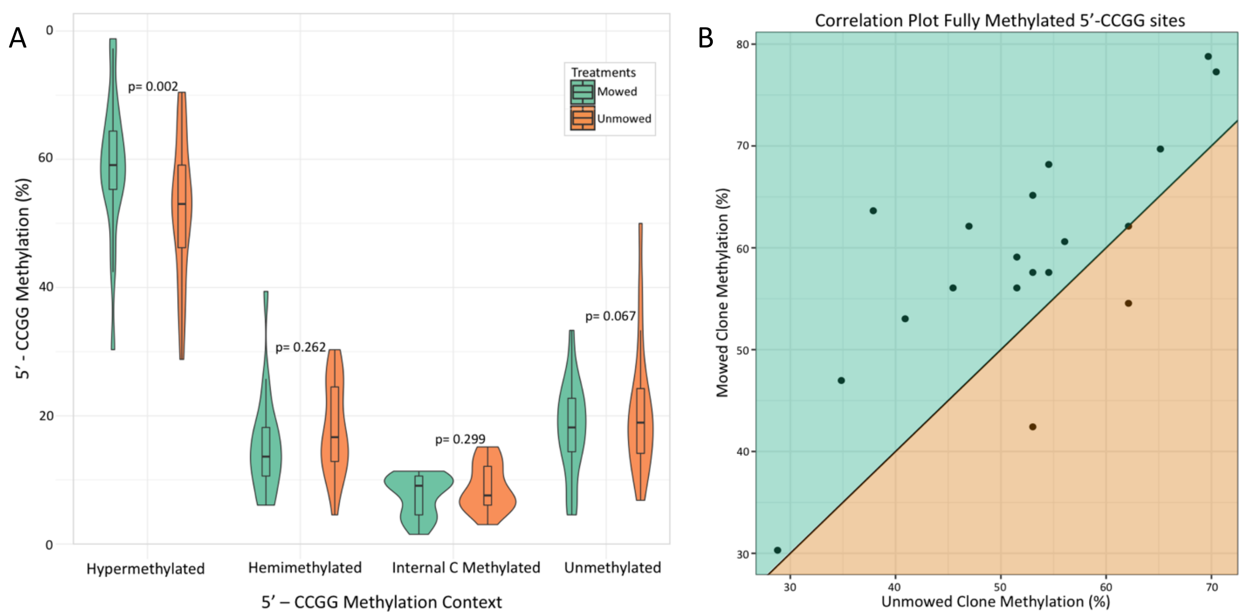

Figure 2: Mowing treatment induces full (hyper-) methylation of CCGG loci in *Poa annua*. Violin and correlation plots compare methylation states of clonal *Poa annua* after eight months of either being mowed or unmowed. (A) Violin plots compare hypermethylated, hemimethylated, internal cytosine methylation, and unmethylated CCGG sites under mowing stress. (B) Correlation plot shows the tendency of mowed clones to be more methylated than unmowed clones. Only two genotypes were more fully methylated in the unmowed clone than the mowed.

ELISA-based colorimetric assays have been developed to survey the fraction of 5-mC DNA over the entire genome with no preference toward sequence context (i.e., CG, CHG, and CHH). We used ELISA to compare genomic methylation between clonally propagated genotypes of *Poa annua* that were either subjected to mowing stress for eight months or left unmowed for eight months. Global cytosine methylation varied between 0.28% and 15.84% in the sixteen measured *Poa annua* genotypes. Pairwise comparison indicated that, on average, mowed clones had 3.04% higher global cytosine methylation than their unmowed counterpart (p=0.02). Our results indicate that mowing stress is a likely contributor to measurable differences in global cytosine methylation patterns in *Poa annua* (**Fig. 3**).

Epigenetic memory such as DNA methylation could play a vital role in transgenerational priming and stress-adaptation in *Poa annua*. To study transgenerational memory in *Poa annua,* we collected seed and surveyed the offspring from each of the mowed and unmowed clones. Offspring plants were allowed to grow unmowed for three months before conducting another ELISA assay to survey for transgenerational retention of global methylation patterns in the offspring of mowed and unmowed plants. Global cytosine methylation varied between 0.82% and 6.35%. Progeny of mowed clones averaged 1.34% greater cytosine methylation than progeny of unmowed clones, suggesting that there may be some transgenerational retention of the methylation landscape in progeny plants (**Fig. 3**, p=0.08).

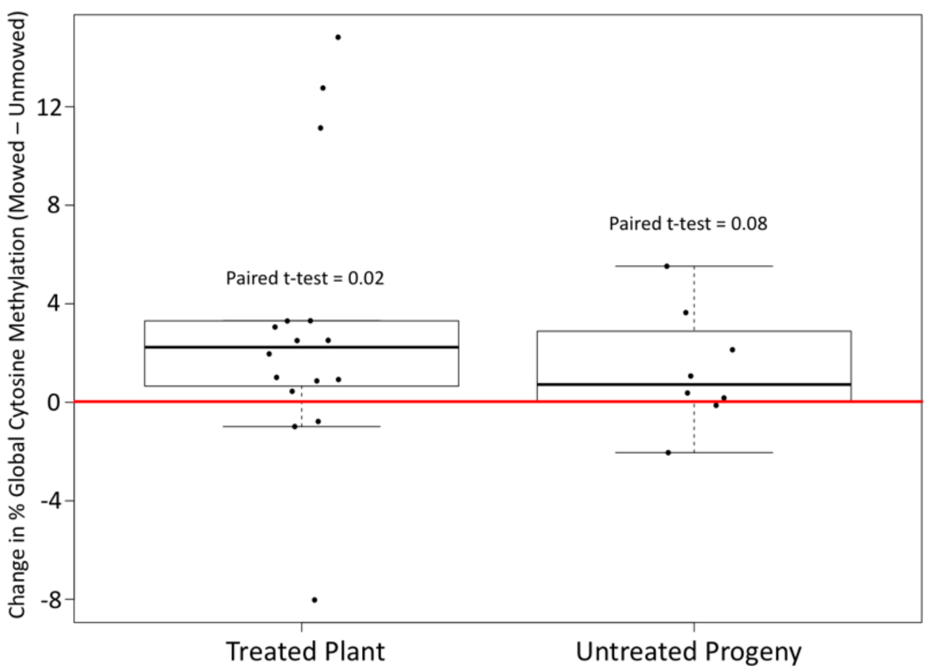

Figure 3: Global DNA methylation is elevated in mowed clones compared to unmowed clones and those methylation patterns are passed to offspring. Boxplots show the difference in global cytosine methylation in *Poa annua* between mowing treatments. Treated plants were clonally propagated and subjected to eight months of mowing treatment. Mowed plants were kept at 3mm mowing height while unmowed plants were allowed to grow without mowing. Y-axis depicts the difference in cytosine methylation between the mowed plant and its unmowed clone (i.e., mowed clone – unmowed clone). Progeny plants were grown from seed of the mowed and unmowed plants. Progeny plants were allowed to grow for three months without mowing treatment before measuring global cytosine methylation. For progeny plants, the y-axis depicts the difference in global cytosine methylation between plants grown from the mowed clone versus plants grown from the unmowed clone. Points above the red line were more methylated in the mowed clone.

Poa annua's observed epigenetic rewiring and patterns of non-Mendelian inheritance on golf course putting greens is largely driven by its status as a neo-allopolyploid between diploid species, *Poa infirma* and *Poa supina*. Allopolyploidy may result in 'genome dominance' and 'biased fractionation' where one of the ancestral diploid subgenomes is repressed while the other subgenome retains higher gene expression levels and fewer DNA mutations. Genome dominance and biased fractionation is regulated in part by epigenetic signals such as cytosine methylation, histone modification, and small RNA silencing. It is highly likely that our observed methylation differences in *Poa annua* is related to its biased expression of subgenomes under mowing stress.

From our research to date, and after consulting with numerous colleagues and collaborators, we have come to realize that improving our resolution of methylation patterns in a non-model species such as *Poa annua* is fraught with difficulties and is unlikely to be the most fruitful avenue of investigation. However, these same colleagues and collaborators have encouraged an alternative approach for unraveling the phenotypic instability of *Poa annua* by utilizing *Poa annua*'s known subgenome architecture. Therefore, rather than continuing with our originally proposed methylome analyses, we believe that the USGA financial resources would be better utilized to unravel the downstream effect of epigenetic reprogramming on genome dominance and bias fractionation of *Poa annua's* subgenomes. We believe that studying the genomic architecture and transcriptional profiles of *Poa annua, Poa supina,* and *Poa infirma* is the most likely avenue to elucidate *Poa annua*'s phenotypic instability and thereby enhancing our efforts to develop commercial cultivars of elite and stable *Poa annua* for use on golf course putting greens. With your approval, we would like to reallocate our current funding towards the study of *Poa annua's* allopolyploid genomic architecture. Budgetary details of our proposed changes will be submitted in a separate document.

USGA ID#: 2016-01-551

Title: Development of New Bermudagrass Varieties with Improved Turfgrass Quality and Increased Stress Resistance

Project Leaders: Yanqi Wu, Dennis Martin, Justin Quetone Moss, and Nathan Walker
Affiliation: Oklahoma State University

Objectives:

1. Improve bermudagrass germplasm for seed production potential, turf performance traits, and stress resistance.
2. Develop, evaluate and release seed- and vegetatively-propagated turf bermudagrass varieties for use on fairways, tee boxes and putting greens.
3. Assemble, evaluate and maintain *Cynodon* germplasm with potential for contributing to the genetic improvement of the species for turf.

Start Date: 2016
Project Duration: 6 years
Total Funding: $300,000

Summary Points:

- Three OSU clonally propagated, fine-textured experimental genotypes of turf bermudagrass were advanced into the 2019 NTEP Warm-season Greens Test.
- Five OSU putting greens-type bermudagrass genotypes including Tahoma 31™ exhibited significantly greater freeze tolerance than Champion Dwarf bermudagrass
- Five OSU clonally propagated and three seeded bermudagrass entries were included in the 2019 NTEP National Bermudagrass Test.

Summary Text:

Bermudagrass is the most widely used turfgrass in the southern USA and throughout tropical and warmer temperate regions of the world. Global warming arguably has increased use of turf-type bermudagrass in climates typically dominated by cool-season turfgrasses, however various challenges in these locations still exist. Turfgrass managers and consumers desire new bermudagrass varieties with greater cold tolerance, enhanced turf quality, improved drought tolerance, increased host plant disease resistance [*i.e*., spring dead spot (SDS), leaf spot disease, etc.], improved insect resistance (mites, armyworms, etc.), reduced requirements for mowing and fertilization, better shade tolerance, and faster divot recovery rate. The Oklahoma State University (OSU) grass breeding program released seed-propagated turf-type bermudagrass cultivars ‘Yukon’ in 1996 and ‘Riviera’ in 2000, and vegetatively-propagated cultivars ‘Patriot’ in 2002, ‘Latitude 36’ and ‘NorthBridge’ both in 2010, and ‘Tahoma 31’ in 2017 for commercial use by the turfgrass industry. The long-term goal of the OSU program is to continue the development of new cultivars with high turfgrass quality and improved resistance to abiotic and biotic stresses.

Developing putting green-type bermudagrass cultivars is an important component of the current research grants funded by the US Golf Association and the Oklahoma Center for the Advancement of Science and Technology. Since 2015, six field trials have been established to test performance under golf putting greens management conditions at the OSU Turf Research

Center (TRC). In 2019 we completed two trials, one established in 2016 and another in 2017. We selected three experimental entries, OKC0805, OKC0920, and OKC3920 to advance into the 2019 National Turfgrass Evaluation Program (NTEP) warm-season putting green test (Figure 1). In 2017, 2018, and 2019 replicated plots of several experimental selections and two standards (Champion Dwarf and TifEagle) were established for testing disease resistance. In 2018 the 2017 planting was infested with root-knot nematodes (*Meloidogyne marylandi*), leaf spot (*Bipolaris cynodontis*), and the cause of root-decline of warm-season grasses (*Gaeumannomyces graminis*) the three most common diseases in the region on ultradwarf bermudagrasses. The 2018 planting was infested in 2019 and the 2017 established plots were evaluated for nematode reproduction, leaf spot and root decline severities.

The 2018 established putting green mowing trial, including 19 OSU experimental selections and three commercial cultivars (‘Champion Dwarf’, ‘TifEagle’, and ‘Sunday’), was continued this year. Flash flooding in May 2019 washed away some sand base and caused damage to the trial. By August, the test green was restored but turf performance data were not collected to the level planned. We will continue this test in 2020.

We established a 2019 putting green mowing test encompassing 16 OSU experimental selections and four cultivars (Champion Dwarf, TifEagle, ‘Mini Verde’, and Sunday) in early May. Unfortunately this new test was also damaged by the flooding so we had to re-prepare plant materials in a greenhouse and replant the test in the summer of this year. In addition, 10 new experimental selections as local entries were included in the 2019 NTEP warm-season putting greens trial as field space was available at the OSU TRC. This group of experimental selections constitutes our 6th set of materials for putting green tests.

One of our objectives in developing new putting green cultivars is to improve cold hardiness. To quantify cold hardiness of the advanced selections from the first three putting green mowing trials, Ms. Lakshmy Gopinath under the direction of Dr. Justin Moss performed three freeze tolerance tests, one in 2018 and two in 2019 (Figure 2). The three tests consistently indicated that ‘Tahoma 31^{TM}’ bermudagrass was the most cold-hardy variety in the tests. The OSU experimental selections for putting greens, 63x18, OKC0805, 16x09, OKC0920, and OKC3920 were significantly better than Champion Dwarf, indicating good progress made in improving the targeted trait. The lethal temperatures to kill 50% (LT50) of OKC3920 was similar to that of Tahoma 31, indicating a high level of freeze tolerance.

A replicated trial established at the OSU TRC in the summer of 2017 was continued in 2019. The trial included 35 OSU vegetatively-propagated experimental selections, four vegetatively-propagated commercial cultivars (‘Astro,’ ‘Latitude 36^{TM},’ Tahoma 31^{TM} and ‘TifTuf’), 11 seed-propagated experimental synthetics and two seed-propagated commercial cultivars (‘Riviera’ and ‘Monaco’). We collected data for spring greenup, turf quality, disease response, and drought resistance. Another replicated trial established at the Oklahoma Panhandle Research and Extension Center, Goodwell, OK in the summer of 2017 was continued this year. As part of graduate student Mr. Shuhao Yu’s PhD dissertation research, this test included 78 OSU experimental selections and six commercial cultivars (Latitude 36^{TM}, ‘NorthBridge^{TM},’ TifTuf, ‘Tifway,’ Astro and ‘U3’). We collected data for spring green up, turf quality, seedhead prolificacy, drought resistance, and fall color retention. Based on the two field-based trials as well as on other trials, three seeded entries and five clonal genotypes were selected for inclusion in the 2019 NTEP National Bermudagrass Test.

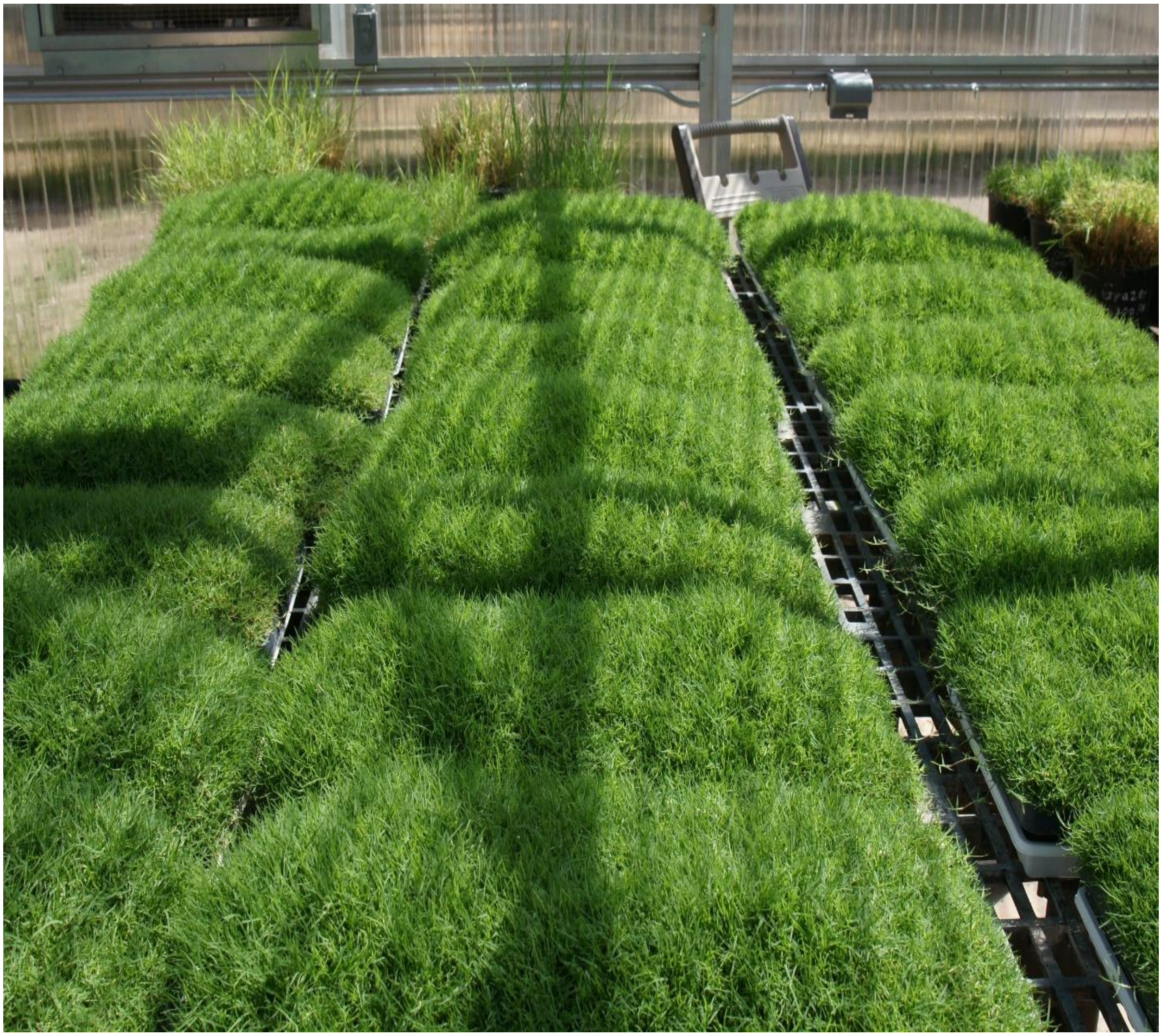

Figure 1. Planting materials of experimental bermudagrass selections prepared in a greenhouse on the Agronomy Farm of Oklahoma State University for the 2019 National Turfgrass Evaluation Program Warm-season Green Test.

Figure 2. Ms. Lakshmy Gopinath, Ph.D. student under Dr. Justin Moss, is evaluating freeze tolerance of experimental bermudagrass genotypes bred for putting green use in a chamber experiment at Oklahoma State University.

USGA ID#: 2017-14-624

Project Title: Development of a shade-tolerant bermudagrass cultivar(s) suitable for fine turf use

Project Leaders: Charles Fontanier and Yanqi Wu
Affiliation: Oklahoma State University

Objectives:

1. Screen for fine turf qualities and shade resistance in newly developed common and hybrid bermudagrass germplasm,
2. Further develop an existing bermudagrass breeding population for superior fine turf characteristics, shade resistance and seed yield, and
3. Develop and validate a high throughput method for screening plants for shade resistance.

Start Date: 2017
Number of Years: 3
Total Funding: $90,000

Summary Points:

- 75 bermudagrass genotypes have been screened for shade tolerance under greenhouse conditions with the top 20 genotypes and industry checks planted in the field for further evaluation.
- The majority of seeded entries and several clonal selections did not survive their first winter in the field trial.
- An additional 30 genotypes were screened for shade tolerance under greenhouse conditions.

Summary Text:

<u>Background and Rationale</u>
Bermudagrass is a desirable turfgrass for use in the transition zone due to its relatively good drought, heat, disease, and insect resistance, and reasonably good cold hardiness. The main factor that prevents more widespread use of bermudagrass is its poor shade tolerance. Beginning in 2007, 45 common bermudagrasses [*Cynodon dactylon* (L.) Pers. var. *dactylon*] collected from China, Africa, and Australia that exhibited good seed production were tested along with four bermudagrass varieties for shade tolerance and overall turf quality. Of those 45 bermudagrasses, the 10 best-performing selections were chosen for further development. Polycrossing combinations of those 10 selections in 2011 produced three synthetic populations. Two of these experimental cultivars, OKS 2011-1 and OKS 2011-4, were tested for shade tolerance and the third OKS 2011-3 was retained for further selection. OKS 2011-1 and OKS 2011-4 did not outperform existing seeded-type cultivars in severe shaded conditions. From the OKS 2011-3 breeding population, the best performing 90 plants were selected after two years of shade pressure. These plants were tested in the field for turf quality and major seed yield related traits. This project seeks to build on previous work to continue selecting for shade tolerance among common bermudagrasses and interspecific hybrids.

Methods

A rapid throughput screening method was developed to identify genotypes showing enhanced shade tolerance under greenhouse conditions. This was done to reduce cost and time associated with multi-year field trials. In June 2017, 75 bermudagrass genotypes were established from sprigs within 2.5-in diameter conetainers under three light environments (0, 51, and 63% shade) within a research greenhouse. Once uniformly established (~8-weeks), plants were subjected to shade treatments using neutral density black fabric for 4 months and clipped biweekly at 1.5-in to promote rapid stress. Fertilizer was applied using a commercial soluble fertilizer (Peter's 20-20-20) biweekly at carefully metered amounts (0.125 lb N M^{-1}) to ensure uniform application. Turf quality, leaf elongation rate, and above-ground biomass (verdure) were assessed at the conclusion of the 4-month shade treatment. The entire trial was repeated in Spring 2018 using only the heavy shade and non-shaded treatments as there was little value gained from the intermediate shade level. Data from greenhouse trials were subjected to a factor analysis to identify entries that performed well across all metrics.

In June 29, 2018, the top 20 performing genotypes were then planted as 2.5-inch plugs in a field study alongside industry standard cultivars (Patriot, Celebration, TifGrand, Latitude36) and five seeded populations from the OSU breeding program. Plots measured 3-ft by 3-ft and each treatment combination was replicated 3 times. The study site was split into two environments: heavily shaded versus non-shaded. The heavily shaded site was characterized by evergreen trees along the western edge and deciduous trees along the southern and eastern edge of the space. Plots were mowed weekly at 1.5-inches using a rotary mower, and fertilizer was applied monthly at 1lb N per 1000 square feet

An additional set of 30 plants from the OSU breeding program were selected and planted in conetainers using sprigs. The plants were evaluated in the greenhouse for a rapid screening of shade tolerance similar to the one conducted previously. Top performers from this second greenhouse trial will be planted in 2020 within the existing shade field trials.

Early Results

Data from the greenhouse trial were reported on in 2017. The industry standard 'TifGrand' demonstrated a mean turf quality score of 4.7, while 18 of OSU's experimental cultivars exceeded this value. Similar to a previous field trial, 'Patriot' was one of the worst performing cultivars under the greenhouse screening method. The top-performing cultivar ('2014-4x2') showed minimal shade avoidance response (etiolation), while the worst-performing cultivar ('2014-29x19') developed a 'stemmy' and etiolated growth habit under heavy shade. Results of the second experimental run were similar and thus the method was considered to be reproducible.

In the most recent greenhouse screening trial, variation in response to shade stress was again evident (Fig. 1). Data related to turf quality, leaf elongation rate, leaf angle, and biomass were collected in Oct 2019. Analysis of these results is ongoing.

Regarding the field study, data collection began in 2019 but due to substantial winter kill, these data were limited in their usefulness (Fig. 2). Furthermore, it was decided to delay application of supplemental shade until 2020. Evaluation of spring green-up under the severe shade treatment showed 7 grasses belonging to the top statistical category including OSU1439, OSU1403, and 'Celebration'. Seeded entries including the standards were all poor performers for this trait despite appearing to have established very well in the prior summer and fall.

Future Expectations

Tree shade in the heavily shaded treatment will be supplemented with artificial shade from neutral density fabric beginning in May 2020 unless the project undergoes further winter injury. Ratings of percent cover, normalized difference vegetation index, and turf quality will be collected monthly using digital images, a spectral reflectance meter, and visual ratings. The top 10 to 20 entries from the ongoing greenhouse screening trials will be planted in summer 2020 on an adjacent plot area and subjected to shade using a large polywoven fabric as early as fall 2020.

Findings from the greenhouse trials will be compared to those from the field trial in order to validate the screening method and further work towards development of a new cultivar.

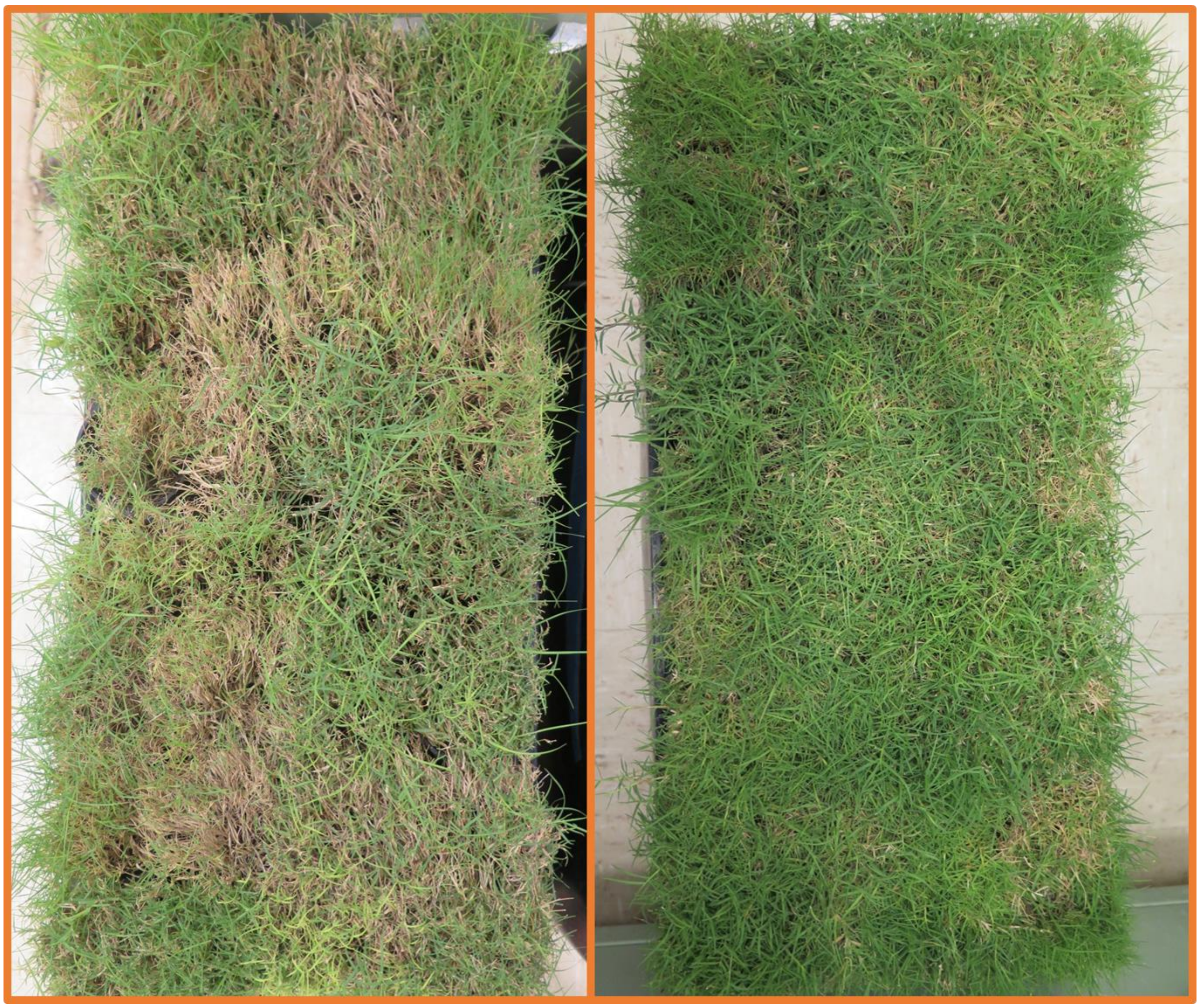

Figure 1. Overview of selected grasses after 12 weeks of shade treatment (left) under greenhouse conditions compared to those from ambient conditions (right).

Figure 2. Field plots in May 2019 showing poor coverage and loss of stand among many entries.

USGA ID#: 2016-34-604

Title: Identification of bermudagrass and zoysiagrass with green color retention at low temperature

Project Leaders: Joseph G. Robins and B. Shaun Bushman
Affiliation: USDA-ARS Forage and Range Research

Objectives:

1) Screen germplasm of Bermuda grass and zoysiagrass for green color retention when exposed to cool temperature growth.
2) Identify germplasm sources for ongoing selection for increased color retention under cool temperatures.

Start Date: 2017
Project Duration: 5 years
Total Funding: $225,000

Summary Points:

- The first run of the zoysia grass evaluation revealed significant differences among the genotypes.
- We will run the zoysiagrass evaluation twice more and bermudagrass evaluation three times.
- We will use the five high and low zoysiagrass and bermudagrass genotypes for candidate gene analysis cool temperature color retention.

Summary Text:

Bermudagrass and zoysiagrass are warm-season grasses that provide high quality turf production in hotter areas with lower water requirements than most cool-season grass alternatives. One weakness of these species is their tendency to lose green color and turf quality during cooler winter months. One response to this tendency is for turf managers to over-seed warm-season turf with either annual or perennial ryegrass during cooler winter months. The ryegrass establishes rapidly and provides green color and turf quality but requires substantial inputs of irrigation and fertilization during winter months. Another option is to paint winter dormant warm-season grasses. While not requiring irrigation and fertilization this option is still expensive. A potential alternative is to identify genetic variation within bermudagrass and zoysiagrass for green color and quality retention under cool temperatures. Germplasm sources with increased green color retention would provide plant breeders with the necessary starting material to develop cultivars of these species that possess high turf quality and performance during hot summer and cool winter months and result in lower inputs and cost. The objective of this research is to characterize a large collection of bermudagrass and zoysiagrass genotypes for green color retention under decreasing temperatures in a growth chamber experiment. The University of Florida (Gainesville), Oklahoma State University (Stillwater), and Texas A&M University (Dallas) provided the germplasm resources for the study. This research seeks to complement and supplement research being conducted at the University of California at Riverside for in field evaluation of this trait.

The USDA-ARS Forage and Range Research Unit received bermudagrass and zoysiagrass genotypes from the three universities during the summer and fall of 2017. Plants were placed in a greenhouse at Logan, UT and allowed to acclimate. Beginning in the winter of 2017/2018, plants were cloned to create replicates for the growth chamber cool temperature evaluations. Fifty cell (4.7 cm across × 6.4 cm deep) plant trays were used for the evaluation. Individual clonal ramets from each genotype were randomly assigned to individual cells in a tray. Each plant was replicated three times for the randomized complete block design. Each block was placed in a separate growth chamber. Once cloning was completed, the plants were allowed to grow in the cells for one month prior to being placed in the growth chambers.

The first bermudagrass evaluation was initiated in spring 2018. After acclimating in the growth chamber for one week, temperatures were decreased by 20 °C to 5 °C over the course of eight weeks. Each week digital images were taken of each flat using a digital camera and a customized light box. This first run was a pilot study to ensure proper functioning of the chambers and to fine tune the experimental approach. While the bermudagrass clones were in the growth chamber, the zoysiagrass genotypes were clones and prepared, in the same manner, for the cool temperature evaluation. The zoysiagrass evaluation began in December 2018 and continued through January 2019. Unfortunately, this also coincided the U.S. government shutdown. Fortunately, due to officially allowed contingencies, the data collection could proceed. However, the shutdown prevented the re-cloning of the bermudagrass plants and a substantial delay in our ability to begin another run of the study. In fact, following the zoysiagrass study, it was fall 2019 before the next run could begin. At the end of the December/January zoysiagrass evaluation all digital images were processed for analysis. This included using software to “cut” the image of each individual cell from the overall flat picture and then convert all images to ratings of dark green color index using Turf Analyzer software. The resulting ratings were then analyzed as a mixed model and overall mean dark green color index values were calculated for each plant.

The first run of the zoysiagrass evaluations resulted in significant differences among the included populations for green color retention under cool temperatures. We now hope to verify these results through two additional runs for the zoysiagrass and three runs for the bermudagrass. We are confident to complete these evaluations by the end of 2020. Following the growth chamber studies, we will identify five high and low color retention genotypes from each species and complete a modified bulk segregant analysis using candidate genes to characterize potential genetic controls for this trait in both species. We will also compare the results obtained in the field studies in California to determine the relationship between growth chamber and field results. We hope the result will be a high-throughput methodology to characterize turfgrass species for cool-season color retention.

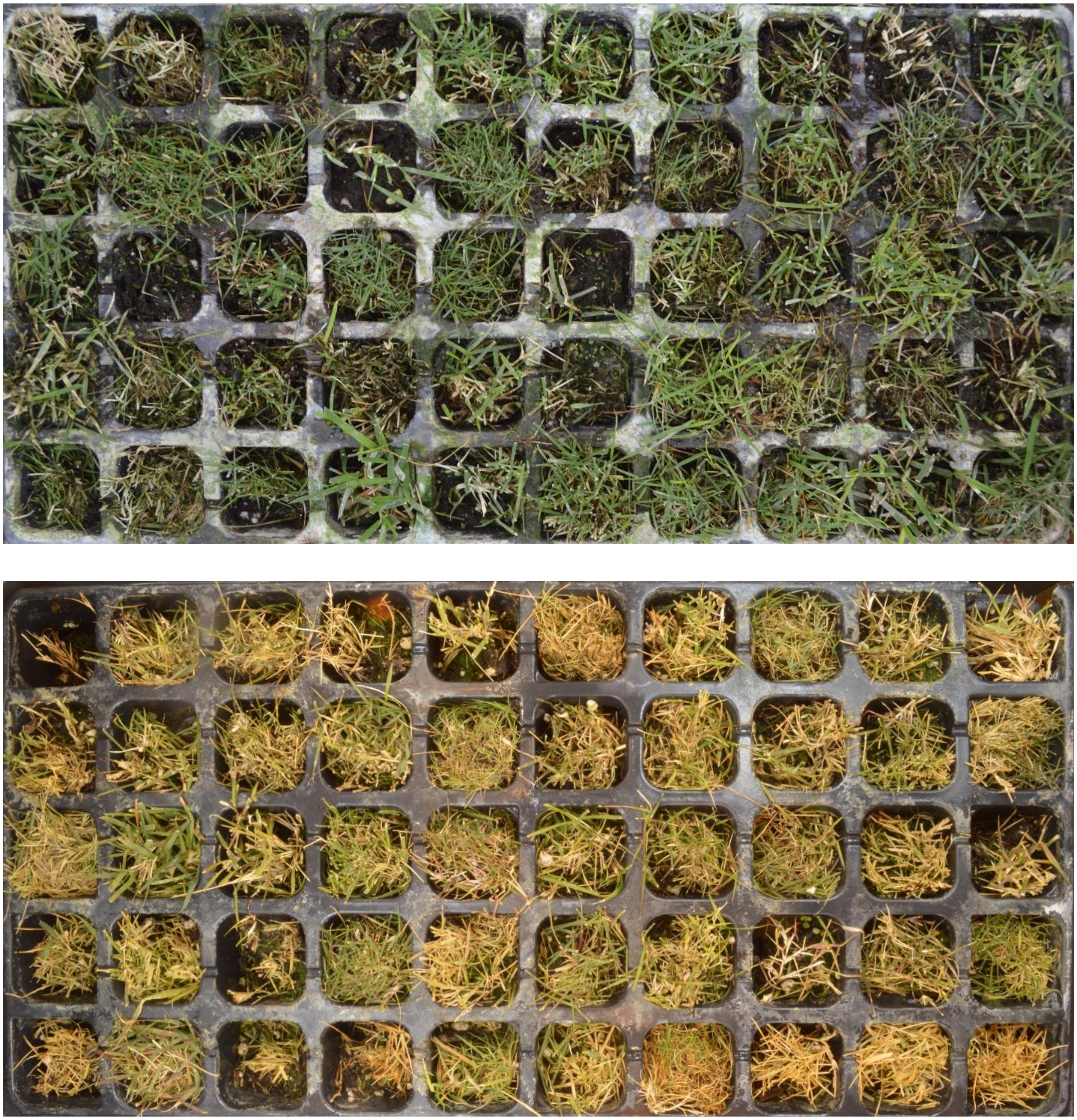

Figure 1. Digital images of trays containing 50 individual genotypes (clones) at different time points in the cool-season color retention evaluation.

USGA ID#: 2016-38-608

Title: Breeding for Resistance to Winter Dormancy in Bermudagrass and Zoysiagrass

Project Leaders: Kevin Kenworthy, John Erickson, Kenneth Quesenberry
Affiliation: University of Florida

Objectives:

1) Develop germplasm and cultivars of bermudagrass that are winter dormant resistant.
2) Develop germplasm and cultivars of zoysiagrass that are winter dormant resistant.

Start Date: 2016
Project Duration: 5 years
Total Funding: $150,000

Summary Points:

- New trials of bermudagrass and zoysiagrass were planted using entries based on improved winter performance.
- Selected entries were entered into the new bermudagrass and zoysiagrass NTEP studies.
- Wear was applied during the fall of 2019 to older zoysiagrass and bermudagrass trials. Wear provides good separation of entries and shows promise to identify lines most suitable for maximizing winter performance of fairways for use in Florida. Selections have been made in both species to plant new fairway and putting green (zoysiagrass) trials in 2019.
- New crossing blocks of bermudagrass were planted.

Summary Text:

The Florida turfgrass industry is among the largest and most dynamic turfgrass industries worldwide. Florida has more golf courses and acres in sod production than any other state in the U.S. To aid golf course superintendents and ensure the continued growth of golf in Florida, better turfgrass cultivars are needed. The majority of golf in Florida is played through the winter months when turfgrass growth, density and turf quality have declined. We propose to improve two warm-season genera of turfgrass with the major objective to screen and breed new cultivars that lack an ability to enter winter dormancy. Sub-objectives for improvement include sting nematodes, drought and large patch resistance.

Bermudagrass
In 2018, we completed evaluations of two advanced nurseries of bermudagrass. These two nurseries contained 340 experimental lines. Winter performance data from these nurseries and from evaluations at UC Riverside were used to retain approximately 70 lines which were replanted in a randomized complete block design with two replications. Fourteen lines were selected and planted in a new fairway trial with nine bermudagrass controls (Tifway, TifTuf, Celebration, Bimini, NorthBridge, Latitude 36, Tahoma 31, Landrun and Iron Cutter). This experiment is still establishing. Four lines were entered in the new bermudagrass NTEP trial. Entries for NTEP were selected with an emphasis on

winter performance data from UC Riverside. Additionaly, new bermudagrass crossing blocks were planted in Jay, FL and Citra, FL.

Recent 2019 data from a 2017 planted bermudagrass trial is shown in Table 1. Overall turf quality values are low because irrigation was withheld in May 2019 during a period of significant drought stress. The quality ratings reflect those entries that recovered faster during the summer rainy period.

Table 1. 2019 turfgrass quality ratings following severe drought stress of bermudagrass entries in Citra, FL.

Entry	**July 11**	**July 30**	**Aug**	**Sep**	**TPI***
FB1630	5.0	5.0	5.3	6.0	4
FB1634	5.7	5.0	5.7	6.0	4
TifTuf	4.0	4.7	5.3	5.3	3
FB1633	4.0	4.7	5.3	5.0	3
FB1636	4.3	5.3	5.0	5.3	3
FB1632	4.3	5.0	4.7	5.0	2
FB1631	4.3	4.7	5.0	4.7	2
Bimini	3.3	4.7	4.0	4.3	1
SW02	3.3	4.3	4.3	4.0	1
FoxFire03	3.8	4.7	4.3	4.0	1
FB1628	2.7	3.7	4.3	4.3	0
FB1629	3.3	3.0	3.3	3.3	0
Celebration	3.0	3.3	4.0	3.3	0
Tifway	2.0	2.3	4.0	3.3	0
LSD (0.05)#	1.1	1.2	1.0	1.2	

*TPI = Turfgrass Performance Index, the number of times an entry occurred in the top statistical group.

#ratings within a column that differ by more than the lsd value are significantly different ($p < 0.05$).

In October 2019, plots were split into wear and no wear treatments on the 2017 bermudagrass fairway trial using a Baldree Traffic Simulator. One pass from the similator was equivalent to wear from one football game. During the traffic period, one pass was applied twice per week. Wear was applied on the following dates: 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November. Wear data is found in Figures 1 and 2.

Figure 1 Shows that on average wear decreased percent green cover by approximately 20 percent during the rating period. Figure 2 shows individual genotype responses to wear for percent green cover compared to no wear applied. FB1628, FB1630 and ‘TifTuf’ had minimal loss of cover through the period of wear. Some entries lost cover, but quickly initiated regrowth when wear was terminated. These entries were Bimini, FB1630, FB1633, FB1634, FB1636 and FoxFire03. ‘Tifway’, ‘Celebration’, and FB1629 showed significant loss of cover and limited capacity for recovery. This data provides evidence of bermudagrass growth comparisons during periods of wear, decreasing temperatures and decreasing daylength. This type of information could be useful in the selection of bermduagrasses best adapted to tolerate traffic during the winter when Florida golf courses experience more rounds of play. Figure 3 is an image of the 2017 bermudagrass trial with wear patterns.

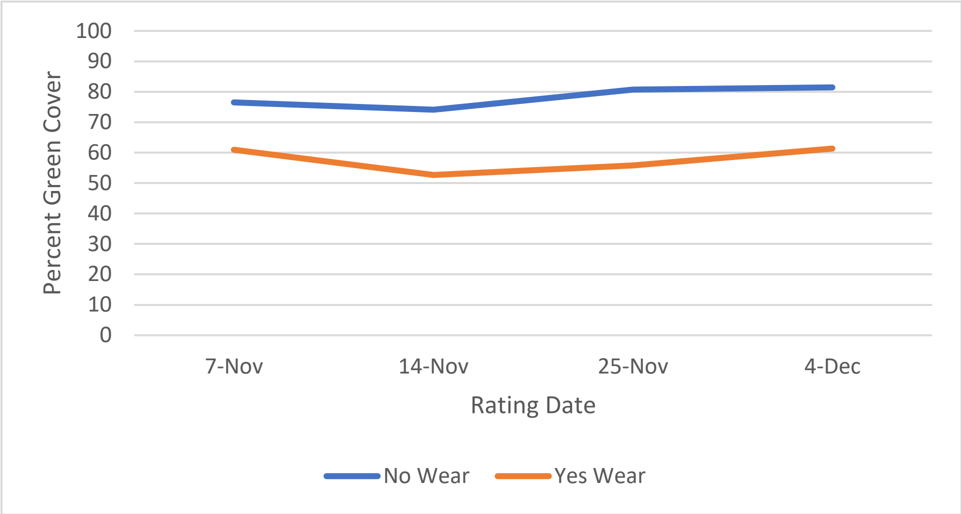

Figure 1. Average percent green cover ratings of 15 bermudagrass genotypes compared between wear and no wear treatments. A single pass of wear was applied using a Baldree Traffic Simulator on 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November.

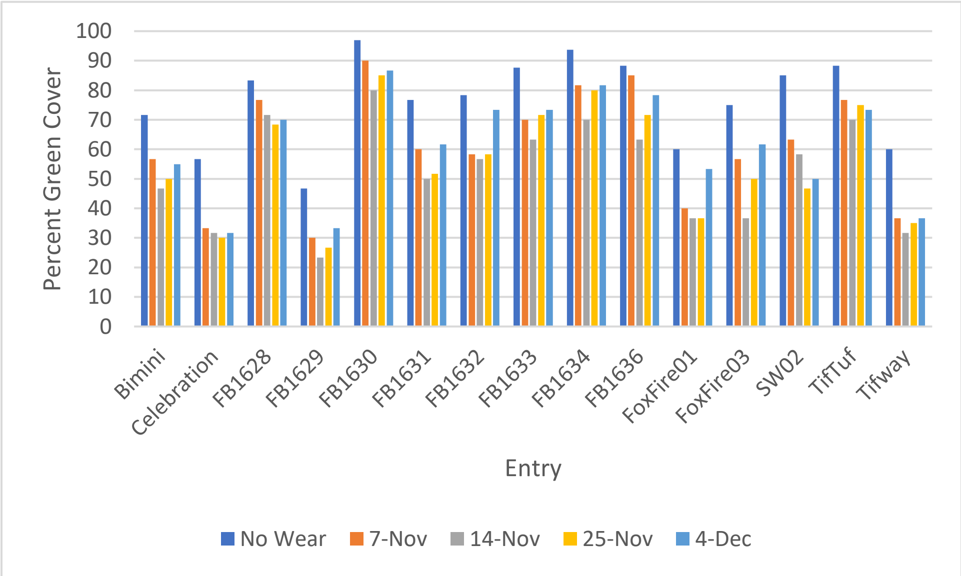

Figure 2. Percent green cover ratings of 15 bermudagrass genotypes mowed at 0.5” through the fall of 2019 with a single pass of traffic applied with a Baldree Traffic Simulator on 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November.

Zoysiagrass

In 2019, a new space plant nursery of zoysiagrass was planted in Hague, FL and two new zoysiagrass trials were planted at Citra, FL. A zoysiagrass putting green trial was planted with 12 entries comprised of 10 experimental lines and 'Diamond' and 'Lazer' as commercial standards. Lines included in the study were selected based on their persistence and disease resistance. Twenty-seven entries were planted in a new fairway trial with 'Zeon' utilized as the commercial standard. Experimental entries were selected based on their drought tolerance in Florida and winter performance in Florida and California. Both of these experiments are still establishing. In addition, 11 entries were included in the new zoysiagrass NTEP trial. Their inclusion was based on winter performance data from UC Riverside.

Recent 2019 data from a 2017 planted zoysiagrass trial is shown in Table 2. In 2018, the trial was mowed at 0.5"; however, it was left unmowed for most of 2019 to serve as a crossing block. Turf quality ratings reflect those plots that maintained quality through the summer without mowing.

Table 2. 2019 Turfgrass quality ratings of unmowed zoysiagrass plots in Citra, FL.

Entry	July 11	July 30	Aug	Sep	TPI*
1329	8.0	8.7	7.3	6.3	4
1335	7.7	8.3	7.3	6.3	4
1313	7.0	7.7	6.3	6.0	4
1305	7.3	7.0	5.3	4.3	2
1337	6.0	5.7	5.3	5.0	2
1306	6.0	5.0	4.3	5.0	1
Zeon	5.7	5.7	5.0	4.0	1
1309	5.7	6.0	4.7	5.0	1
LSD (0.05)#	1.1	1.7	2.5	1.9	

*TPI = Turfgrass Performance Index, the number of times an entry occurred in the top statistical group.

#ratings within a column that differ by more than the lsd value are significantly different ($p < 0.05$).

In October 2019, plots were split into wear and no wear treatments on the 2017 zoysiagrass trial using a Baldree Traffic Simulator. One pass from the similator was equivalent to wear from one football game. During the traffic period, one pass was applied twice per week. Wear was applied on the following dates: 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November. Wear data is found in Figures 4 and 5.

Figure 4 shows the average effect of wear across genotypes. On 7 November 2019 the difference in cover between no wear and wear treatments was 25%. This loss occurred after six wear events. Loss of green cover continued through November in the wear plots, but green cover remained consistent in the non-wear plots. Figure 5 provides genotypic percent green cover responses to wear through November 2019 compared to no wear at the onset of the wear applications. 'Zeon' and FAES1305 were the most susceptible to these twice per week wear events during the fall 2019. It is worth noting that these two lines had significant dollar spot (not rated) affecting their cover (reflected in the low cover ratings in the non-wear plots). FAES1313, FAES1329 and FAES1335 were the most tolerant of wear during the fall 2019 with decreasing temperatures and daylength. These lines show potential for use on golf courses to maximize playing conditions during the winter months in Florida during periods of increased play.

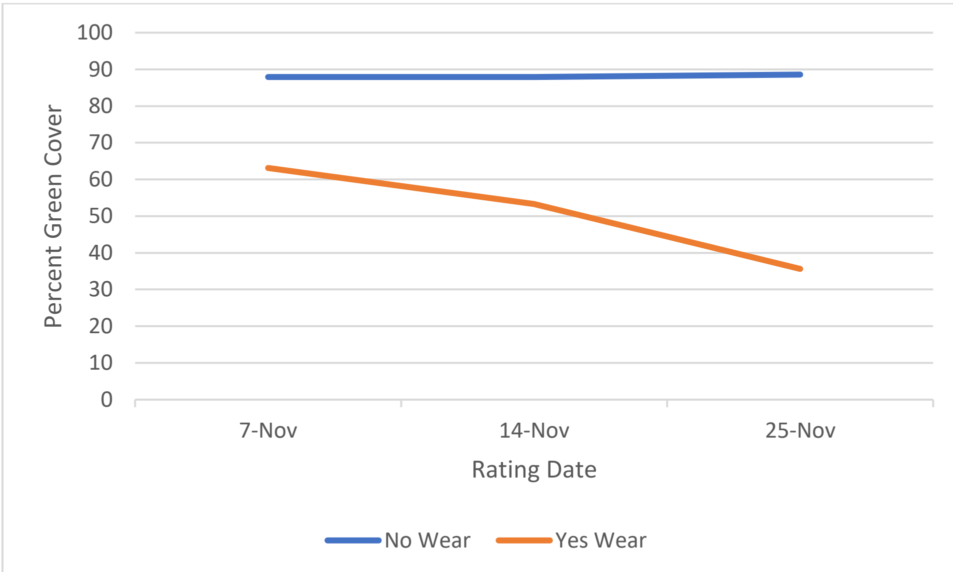

Figure 3. Average percent green cover ratings of 8 zoysiagrass genotypes compared between wear and no wear treatments. A single pass of wear was applied using a Baldree Traffic Simulator on 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November.

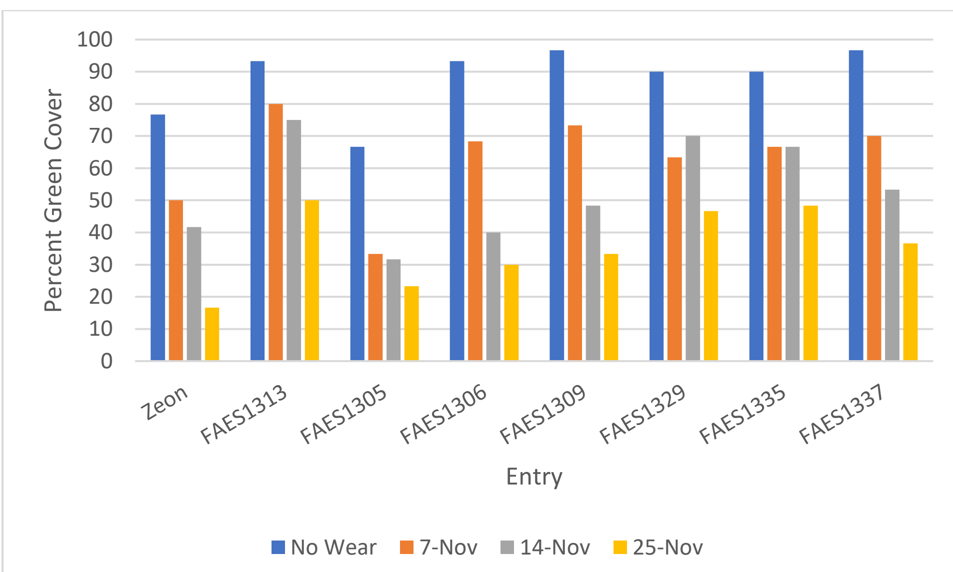

Figure 4. Percent green cover ratings of eight unmowed zoysiagrass genotypes through the fall of 2019 with a single pass of traffic applied with a Baldree Traffic Simulator on 21, 24, 28 and 30 of October; and 4, 7, 12, 14 and 18 of November.

USGA ID#: 2017-21-631

Title: Improvement of Bermudagrass, Zoysiagrass, and Kikuyugrass for Winter Color Retention and Drought Tolerance

Project Leaders: Adam J. Lukaszewski, Marta Pudzianowska, and James H. Baird
Affiliation: University of California, Riverside

Objectives:

1. Develop bermudagrass, kikuyugrass, and zoysiagrass turf-type genotypes with improved winter color retention and drought tolerance for Mediterranean and arid climates.
2. Utilize Diversity Arrays Technology (DArT) markers to aid in breeding efforts and marker-assisted selection.
3. Develop techniques to reduce kikuyugrass ploidy level to diploid by androgenesis in order to reduce aggressiveness and improve turf quality and playability characteristics.

Start Date: 2017
Project Duration: 5 years
Total Funding: $250,000

Summary Points:

- Hybridization of existing UCR bermudagrass accessions continued, with emphasis on genotypes with desirable winter color retention, early spring green-up, and drought tolerance. A large amount of hybrid seed was produced and germinated, 864 new hybrids were planted.
- Evaluation of 12 our most promising bermudagrass hybrids in replicated trials across several climatic zones in California revealed 2 hybrids (17-8 and TP6-3), with better or comparable quality and winter color retention relative to commercial cultivars (Bandera, Tifway and TifTuf).
- New studies including UCR best performers (17-8, TP6-3, BF2 and 10-9) and 7 commercial cultivars were started at two golf courses in northern California to test their performance under regular traffic and maintenance on fairways.
- Kikuyugrass accessions were intercrossed and selfed, 280 seedlings were obtained and planted.
- Selection among kikuyugrass continues for less aggressive growth and finer texture.
- Testing and selection of bermudagrass and kikuyugrass for drought tolerance was initiated.
- Evaluation of winter color retention continues among entries of bermudas, kikuyus and zoysias.

Summary Text:

Warm-season or C4 turfgrass species including bermudagrass, zoysiagrass, and kikuyugrass are much better adapted to heat, drought, and salinity compared to cool-season grasses, but they go dormant during winter months making them less desirable choices for lawns, athletic fields, and golf courses. Clear differences in winter color retention, drought tolerance, and water use efficiency exist among warm-season grasses, and within individual species, which indicates that genetic improvements are possible. Our objectives are to develop improved genotypes of these three species with emphasis on winter color retention and drought tolerance for Mediterranean and arid climates.

Starting in spring 2017 the person responsible for the general advancement and day-to-day operations of this project is Dr. Marta Pudzianowska. Christian Bowman started as a new Ph.D. student in Fall 2019. His focus will be on genetics and genomics in this project.

Bermudagrass
In addition to the existing collection of seven *Cynodon* species (over 100 accessions), a collection of bermudagrass genotypes from the University of Florida (195 accessions) and Oklahoma State University (350 accessions) was planted in 2016 and maintained in years 2017-2019. The collection is continuously supplemented with samples collected locally or donated to us by others. As in previous years, new hybrids are being produced. Seeds from 2018 crosses (detached tiller crosses and open pollination) were germinated and 864 new hybrids were planted in 2019. They are being screened for color retention and turf quality, together with collection accessions and 770 hybrids planted in 2018. Top performing collection accessions were again intercrossed in 2019 (detached tiller crosses). These seeds will be germinated and planted in 2020.

Based on overall quality, color retention and preliminary dry-down test performed in 2016, 71 best hybrids and collection accessions developed and collected in previous years were planted in dry-down area, together with 5 commercial cultivars (Bandera, Celebration, Santa Ana, TifTuf and Tifway II). Plots were established in May 2019, in completely randomized design with 3 replicates. Irrigation was turned off on August 1st and restored on October 1st. Preliminary results show considerable variation among tested genotypes in response to drought, with some UCR hybrids outperforming commercial cultivars. Tests will be continued in 2020. Accessions with the latest onset of dormancy and the earliest green-up will be intercrossed, on the assumption that the next generation hybrids may show reduced dormancy period. After re-screening hybrids for suitability for roughs or homeowners use (2” mowing height) 12 hybrids with the best quality and winter color retention were selected and planted in Coachella Valley (Thermal, low desert) and Carmel-by-the-Sea (Northern California, Monterey area) in June and July 2019, respectively and will be evaluated in 2020.

After 2 years of evaluation of 12 most promising UCR hybrids in Southern and Northern California the study was terminated in June 2019. Based on the quality and color retention assessment, both visually and with Normalized Difference Vegetation Index (NDVI) and Dark Green Color Index (DGCI), 2 UCR hybrids, TP6-3 and 17-8, were selected as the best of all tested accessions. They were comparable or better than commercial cultivars (Bandera, TifTuf and Tifway) in all 3 locations (University of California, Riverside (Riverside, Inland Southern California); Coachella Valley (Thermal, Low Desert) and Fairfax (Northern California)). Sod of these 2 hybrids, along with 2 other well performing entries: BF2 and 10-9, was produced in 2018 and 2019 at the West Coast Turf fields in the Coachella Valley, and planted on the fairways of two golf courses in Northern California: Napa Golf Course in May and Almaden Golf & Country Club in San Jose in August 2019. Study includes 7 commercial cultivars: Bandera, Celebration, Latitude 36, Santa Ana, Tifway II, TifTuf and Tahoma 31 (added later). Plots (20 x 12 ft) are placed on 2 fairways at each golf course and maintained like the rest of the fairway. Plots are being evaluated for their performance under regular golf course traffic and management and for winter color retention.

Kikuyugrass
After evaluation of 203 kikuyugrass accessions from the collection established in years 2016-2018, genotypes showing the best quality and the highest morphological and genetic diversity were selected for intercrossing and self-pollination. Crosses were performed in the greenhouse during winter and spring of 2019, based on observations of the pollination mode conducted in 2017-2018. Seeds were germinated, and 280 seedlings were planted in July 2019. These plants will be evaluated for quality, growth habit, texture, flowering and winter color retention. Intercrossing and selfing of kikuyugrass accessions will continue during winter and spring of 2020.

Preliminary drought tolerance assessment performed in 2017 on collection accessions showed considerable variation for the character. To confirm these preliminary results and further investigate drought tolerance of best performing genotypes 38 kikuyugrass accessions were planted in dry-down area. Plots were established in June 2019 in 3 replicates. Assessment of drought tolerance of these accessions will start in 2020.

Zoysiagrass
A large collection of zoysiagrass genotypes from the University of Florida (155 accessions) and Texas A&M (219 accessions) was planted in 2016 and maintained during 2017, 2018 and 2019. Collection was supplemented with 14 UCR hybrids obtained from the breeding program conducted by Dr. V. B. Youngner and V. A. Gibeault, which resulted in releasing cultivars ‘El Toro’, ‘De Anza’ and ‘Victoria’. During the years 2017-2019 color retention and the overall quality of these genotypes were evaluated visually. Considerable variation in color retention and quality was observed among zoysiagrass genotypes with the average color values being higher than those of bermudagrass.

Figure 1. Bermudagrass hybrids developed from crosses performed in 2018 at UCR Agricultural Operations field in Riverside, CA. Plots were established on 29 June 2019. Photo taken on 30 August 2019.

Figure 2. Bermudagrass hybrids selected for roughs and lawns at West Coast Turf farm in Thermal, Coachella Valley, CA. Plots were established on 17 June 2019. Photo taken on 7 November 2019.

Figure 3. Bermudagrass genotypes from UCR and commercial cultivars on fairway at Napa Golf Course in Napa, CA. Plots were established on 22 May 2019. Photo taken on 28 August 2019. ‘Santa Ana’ in foreground. UCR 17-8 bermudagrass in adjacent plot above 17-8.

Figure 4. Bermudagrass genotypes from UCR and commercial cultivars on fairway at Napa Golf Course in Napa, CA. Plots were established on 22 May 2019. Photo taken on 3 December 2019. UCR 17-8 in foreground. 'Celebration' bermudagrass in adjacent plot above 17-8.

USGA ID#: 2017-11-621

Title: Development of Seeded Zoysiagrass Cultivars with Improved Turf Quality and High Seed Yields

Project Leaders: A. Dennis Genovesi and Ambika Chandra
Affiliation: Texas A&M AgriLife Research - Dallas

Objectives:

1. Development of finer-textured germplasm/cultivar(s) of zoysiagrass with high seed yields that offer an economical alternative to vegetative types with the potential for rapid turf establishment.
2. Breed to improve characteristics such as turf quality, competitive ability and persistence under biotic and abiotic stresses.

Start Date: 2017
Project Duration: 3 years
Total Funding: $89,559

Summary Points:

- Seed was harvested from the 2017 Isolation Blocks for germination during the winter of 2019 - 2020 and planting in a Spaced Plant Nursery in 2020.
- A total of 24 new selections (12 red and 12 yellow) were made from the 2017 Isolation Blocks and the 2015 SPN and planted in 2019 Isolation Blocks.
- Johnston Seeds is in the process of screen our germplasm and seed parents for cold hardiness in Enid, OK.

Summary Text:

Introduction

Zoysiagrass (*Zoysia* spp.) is a warm season, perennial grass that is being used on golf courses and home lawns due to their lower level of maintenance as compared to other turfgrasses (Murray and Morris, 1988). Most cultivars are vegetatively propagated since they offer a uniform, high quality turf stand, but an alternative, relatively inexpensive, way is to propagate zoysiagrass by seed (Patton et al 2006). Availability of seeded varieties is limited to *Z. japonica* Steud. types such as ‘Zenith’ and ‘Compadre’. The focus of this research project is the development of multi-clone synthetic varieties that exhibit leaf textures finer than Zenith with seed yields that meet the production goals needed to make it profitable to produce. Since the inception of the project in 2010, our breeding strategy has been to utilize the classical plant breeding method known as phenotypic recurrent selection. Our approach has been to alternate between Spaced Plant Nurseries (SPN) for progeny selection, and isolation crossing blocks to promote outcrossing and recombination. This strategy should allow for the gradual accumulation, over multiple generations, of desirable alleles affecting both seed yields and finer leaf texture.

Progress Update

In 2017, we began our fourth cycle of recurrent selection with the planting of 23 advanced lines identified from 1,750 progeny planted in the 2015 SPN. These 23 lines were planted in isolation blocks, Red #1 (7 entries), Red #2 (9 entries) and Yellow (7 entries), based on their seedhead color, seedhead density, height of inflorescence exertion and texture. They were planted late in 2017 (9/26/17) so were allowed to grow in during 2018. Seedheads were harvested on 23 and 28 May 2019 and stored for processing. Seedheads will be processed during the winter of 2019-2020 to produce a clean seed product. Seed will be scarified with 30% NaOH (Yeam, et. al. 1985) and seed germinated in potting mix to produce seedlings for planting in the field in 2020. Our goal will be to produce 25 seedlings from each seed parent to be planted in a 2020 SPN resulting in a population size of nearly 600.

In addition, in 2019 two new Isolation Blocks were planted 15 Aug. 2019. Selections were made both from the 2017 Isolation Blocks (Fig. 1 A and B) and the 2015 SPN (Fig. 1C) resulting in 12 red seedhead types being planted in one isolation block and 12 yellow seedhead types in the other isolation block. Five of our best red seeded parents were chosen from the 2017 Isolation Blocks to be combined with six new selections from the 2015 SPN (Fig. 1A) and one of our Cold Hardy/Large Patch Tolerant advanced lines from another USGA sponsored project. Also, we chose two of our best yellow seeded parents from the 2017 Isolation Blocks to be combined with 10 new selections from the 2015 SPN. Thus in 2019 we planted two new isolation blocks consisting of 12 red and 12 yellow seeded types for recombination.

In 2018 were entered into a collaboration with Johnston Seed with the transfer of vegetative material from our most advanced synthetic parents. On 31 July 2018, one - 18 cell tray of each of 13 synthetic parents with intermediate texture were shipped to Johnston Seeds on MTA for evaluation for cold hardiness. Those parental lines were planted in Enid, OK on 2 Aug. 2018. In addition, 535 progeny from 16 medium coarse textured families were planted on 6 July 2018 for evaluation under their growing conditions. Data was collected by Johnston Seed Co. in May, June and July of 2019. Approximately 10 hybrid progeny had good establishment, turfgrass quality and flowering characteristics with no signs of mite damage and therefore, show promise as seeded zoysia parental lines. The 13 synthetic parents were evaluated for spring green-up, vigor, flowering suitable for seed production and the absence of mites. Potential seed parents that were noted to be the best: 6086-21, 6087-15, 6596-05, 6585-34 and 6598-38. The future plan is to evaluate the promising hybrid progeny and synthetic parents for spring green-up in OK in 2020, and then assemble them into isolation blocks for another cycle of recombination and/or synthetic variety blocks for varietal evaluation.

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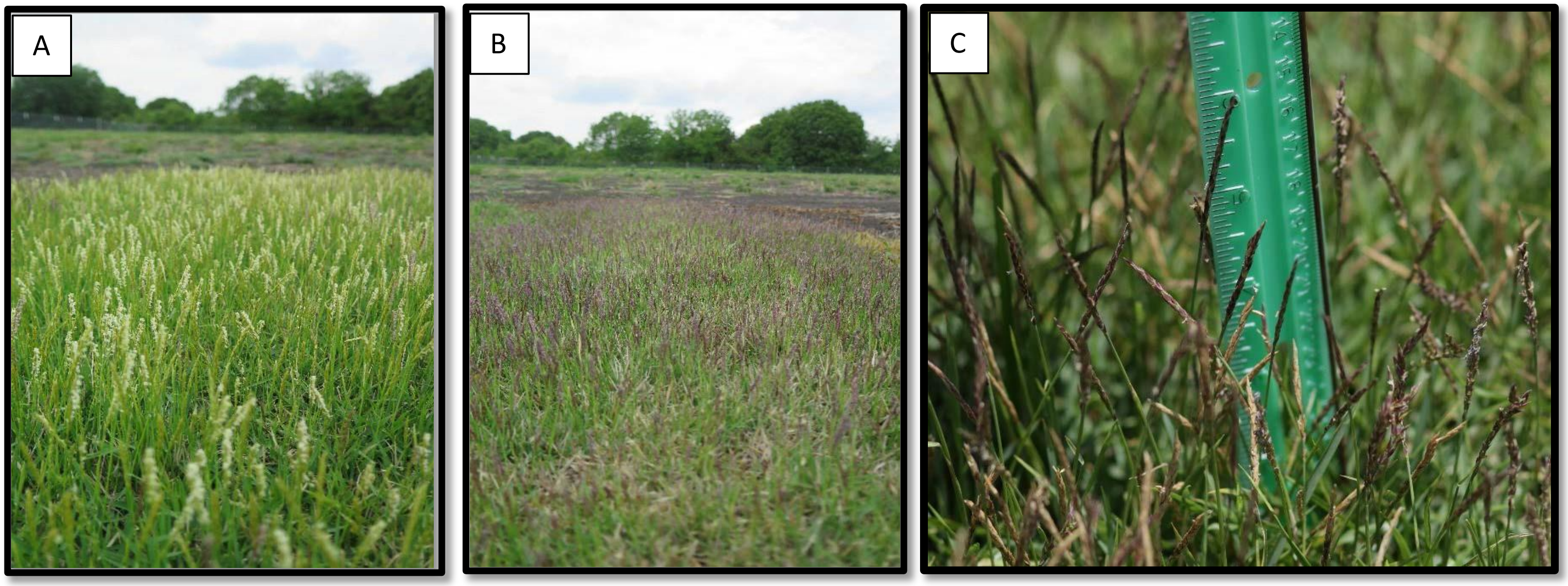

Figure 1. TAES 6598-38, a yellow seeded parent selected from the 2017 isolation block (A); TAES 6596-05, a red seeded parent selected from the 2017 isolation block (B); a red seeded selection from the 2015 spaced plant nursery advanced to the 2019 isolation block (C).

USGA ID#: 2018-01-651, 2018-02-652, 2018-03-653

Title: Development of Cold Hardy Zoysiagrass Cultivars for Golf Courses in the Transition Zone

Project Co-Leaders: Dennis Genovesi[1], Ambika Chandra[1], Jack Fry[2], Megan Kennelly[2], Aaron Patton[3], Meghyn Meeks[1], Manoj Chhetri[2] and Ross Braun[3]

Affiliation: Texas A&M AgriLife Research-Dallas[1], Kansas State University[2], Purdue University[3]

Objectives:

1. Phase I (year 1): Pairwise crossing of cold hardy zoysiagrasses adapted to the transition zone with under-utilized finer-textured zoysia accessions and large patch-tolerant zoysia germplasm was completed in 2017/2018 at Texas A&M AgriLife-Dallas. Progeny populations were distributed to three test locations, Manhattan, KS, West Lafayette, IN, and Dallas, TX, in 2018 for evaluations (Table 1). Phase I is complete.

2. Phase II (year 2 and 3): Field evaluation began during the winter of 2018/2019 in the form of non- replicated spaced plant nurseries (SPN) comprised of the newly generated progeny populations in Manhattan, West Lafayette, and Dallas. The objective of Phase II is to identify those experimental hybrids with superior cold tolerance as well as excellent turfgrass quality for different playing surfaces. Notes are taken for entries that exhibits no visible symptoms of large patch or billbug feeding as a result of the natural infestations, as well as any prevalent stress. Phase II is in progress.

3. Phase III (year 4-6): A set of 75 hybrids will be selected in 2020 based on their superior performance for field evaluation in the form of replicated field trials (RFT) at Dallas, Manhattan, and West Lafayette. RFT in Dallas will be conducted under full sun and shade (63% PAR reduction). Evaluation of these advanced hybrids to large patch disease tolerance will be conducted in Manhattan, KS and tolerance to hunting billbug will be conducted in West Lafayette, IN. RFTs will also be planted at five to eight additional locations in the transition zone in 2021, 2022 and 2023.

Summary Points:

- Phase II (year 1): Data were collected for progeny in spaced plant nurseries planted at three locations: 1,370 progeny at Olathe, KS where only 5.2% survived; 1,624 progeny at West Lafayette, IN where 20.7% survived; and 1,633 progeny at Dallas TX where 72.2% survived (Table 1).
- The objective of Phase II field testing is the selection of experimental lines that have comparable/superior cold tolerance to that of Meyer with tees to greens turfgrass quality for the golf industry in the transition zone. Emphasis will be on the advancement of entries that exhibit no visible symptoms of large patch disease or hunting billbug susceptibility.

Summary Text:

Zoysiagrass is a warm-season grass that provides an excellent playing surface for golf with the added benefits of low nutrient and pesticide requirements making it an ideal turfgrass for use in transition zone (Fry et al., 2008). In the transition zone, 'Meyer' (*Z. japonica*) has been the cultivar of choice since its release in 1951 (Grau and Radko, 1951), primarily because it has excellent freezing tolerance. However, Meyer is relatively slow to establish and recover from divots and is coarser textured and less dense than *Z. matrella* cultivars (Fry and Dernoeden, 1987; Patton, 2009).

Researchers at Texas A&M AgriLife Research-Dallas and Kansas State University have worked together since 2004 to develop and evaluate zoysiagrasses with better quality than Meyer but adapted to the transition zone. From this work, a number of advanced lines derived from paired crosses between *Z. matrella* and *Z. japonica*, were identified (e.g. – KSUZ 0802, KSUZ 0806 and KSUZ 1201) with a level of hardiness equivalent to Meyer (Okeyo et al., 2011), but with finer texture and better density than Meyer. Because of its superior performance, KSUZ 0802 ('Innovation') was recently co-released by TAM AgriLife and KSU as a new commercial variety (Chandra et al., 2017).

TAM AgriLife, KSU and Purdue University have been working on a USGA-funded project since 2012 where the main objective is to incorporate large patch (*Rhizoctonia solani* AG 2-2LP) tolerance, along with cold hardiness and improved quality, into new transition zone adapted zoysiagrasses. In 2018, the top ten hybrids with intermediate leaf texture (out of over 2,800) exhibiting large patch tolerance and cold hardiness were selected for advanced evaluations by the three collaborating institutions.

For the current project, we have initiated new crosses between these intermediate texture types with cold hardiness available in the pipeline and under-utilized and finer-textured *Zoysia* species (*Z. pacifica, Z. minima and Z. pauciflora*) available in our germplasm collection. The focus of this project is to develop cold hardy zoysiagrasses with quality suitable for golf course fairways, tees, and putting greens. In addition to cold hardiness and turfgrass quality, experimental hybrids that are advanced will also be evaluated for large patch, hunting billbug and shade tolerance.

We are partnering with Dr. Jack Fry, Turfgrass Scientist at Kansas State University (K-State), Dr.Aaron Patton, Extension Turfgrass Specialist at Purdue University and Dr. Megan Kennelly, Plant Pathologist at K-State. These professors have extensive experience with testing turfgrasses adapted to the transition zone for cold hardiness and disease susceptibility.

Progeny populations were produced in TAM AgriLife-Dallas and shared with our collaborators located at Olathe, KS and West Lafayette, IN (Phase I was completed in 2018) (see Table 1). Phase II was begun when three spaced plant nurseries were planted: (1) summer 2018 in West Lafayette, IN by Aaron Patton and Ross Braun, (2) in 2017 and 2018 in Olathe, KS by Jack Fry and Manoj Chhetri and (3) in 2017 and 2018 in Dallas, TX by Ambika Chandra, Dennis Genovesi, and Meghyn Meeks. Table 1 describes the numbers surviving by location. At West Lafayette, IN out of 1,624 progeny planted, 336 (20.7 %) survived. Of the 336, there were a range of differences in leaf texture (8.0 to 3.5), genetic color (8.0 to 4.3), summer quality (7.5 to 4.9), fall turf cover (75.5 to 10%) and fall color retention (8.0 to 3.0) (data not presented; Figure 1 and 2). In Olathe, KS, out of 1,370 progeny planted only 71 (5.2%) survived with a range of different leaf textures (7.5 to 3.5) and vigor (8.0 to 2.0). In Dallas, TX, we planted 1,633 with 1,188 (72.7%) surviving. Since climate is kinder to hybrids with less cold hardiness, we saw a range of ratings for survival (100 to 14.3%), spring greenup (4.3 to 2.0), establishment (5.2 to 2.3) and turf quality (8.0 to 2.0). It is interesting to note that preliminary data (2018-2019) shows survival of hybrids originating from crosses between cold hardy *Z. matrella/japonica* types and finer-textured *Z. minima* and *pacifica* accessions that are known to generally lack cold hardiness. A few of these surviving hybrids have leaf texture similar to 'Zorro' and 'Zeon'. This suggests that our breeding effort was successful in potentially combining these desirable traits. Data will be collected in the spring of 2020 to continue assessing the cold hardiness of the surviving hybrids. In summary, a total of 1,595 hybrids out of 4,627 survived the winter of 2018/2019 (34% survival), and 75 elite hybrids out of these surviving 1,595 hybrids (4%) will be selected and advanced to Phase III replicated field trials.

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Table 1. Progeny populations developed and distributed across locations with numbers surviving in 2019.

Lineage	Number of Progeny Tested					
	Purdue West Lafayette, IN		K-State Olathe, KS		TAM AgriLife Dallas, TX	
	# Planted	# Survived (%)	# Planted	# Survived (%)	# Planted	# Survived (%)
CH Intmd. x *Z. pacifica* or Recip.	333	10 (3)	492	1 (0.2)	895	677 (73)
CH Intmd. x *Z. pauciflora* hybrid or Recip.	62	14 (22.6)	30	1 (3.3)	57	41 (71.9)
CH Intmd. x *Z. minima* hybrid or Recip.	148	11 (7.4)	101	5 (5)	88	72 (83.7)
CH Intmd. x CH Intmd.	374	177 (47.3)	225	46 (20.4)	239	160 (67.5)
Z. matrella x CH Intmd. or Recip.	305	66 (21.6)	190	6 (3.2)	257	184 (71.6)
LPT x CH Intmd.	83	2 (2.4)	69	1 (1.4)	21	19 (90.5)
CH LPT x CH Intmd. or Recip.	51	15 (29.4)	43	3 (7)	11	4 (36.4)
Seeded Type Intmd. x CH Intmd. or Recip.	155	13 (8.4)	130	4 (3.1)	37	19 (51.4)
Z. minima hybrid x CH *Z. japonica* or Recip.	26	5 (8.2)	21	0 (0)	3	1 (33.3)
Z. minima hybrid x CH-LPT	3	0 (0)	3	0 (0)	0	0 (0)
Z. matrella x CH *Z. japonica*	50	21 (42)	43	3 (7)	15	6 (40)
CH *Z. japonica* x *Z. pacifica*	12	0 (0)	10	1 (10)	3	1 (33.3)
Z. sinica x CH Intmd.	5	1 (20)	4	0 (0)	3	2 (66.7)
Z. matrella x CH-LPT	10	1 (10)	8	0 (0)	4	2 (50)
Seeded Type Intmd. x CH LPT	1	0 (0)	1	0 (0)	0	0 (0)
Total by location	**1,624**	**336 (20.7)**	**1,370**	**71 (5.2)**	**1,633**	**1188 (72.7)**

CH – Cold Hardy
LPT – Large Patch Tolerant
CH Intmd. – Cold Hardy intermediate (*Z. japonica x Z. matrella*)
Z. pauciflora hybrid – *Z. pauciflora x Z. matrella*
Z. minima hybrid – *Z. minima x Z. matrella*
Seeded Type Intmd. – Seeded *Z. japonica x Z. matrella*
Recip. – Reciprocal cross

Figure 1. Visual differences for 2018-2019 winterkill of hybrid progeny taken 31 May 2019 in West Lafayette, IN.

Figure 2. Tees to Greens Spaced Plant Nursery located in West Lafayette, IN taken June, 2019 with 336 promising cold hardy zoysiagrasses.

USGA ID#: 2018-16-666

Title: Exploring the Use of Coarse Zoysiagrass Phenotypes as a Low-Input Turf for Golf Course Roughs

Project Leaders: Aaron Patton[1], Ross Braun[1], Susana Milla-Lewis[2], and Brian Schwartz[3]
Affiliation: [1]Purdue University, [2]North Carolina State University, [3]University of Georgia,

Collaborators: Evergreen Turf in both Escondido, CA and Chandler, AZ.

Objectives:

Evaluate coarse zoysiagrass phenotypes for their performance and playability in multiple climates (warm-arid, warm-humid, transition zone) as a potential grass for golf course roughs and other low-maintenance areas.

Start date: 2018
Project duration: 3 years (2018-2020)
Total funding: $61,846

Summary Points:

- In 2019 (year 2), zoysia plots were maintained at a golf course rough mowing height (3.0 inches) under low-maintenance regimes receiving minimal-to-no pest control, nitrogen fertilization, and supplemental irrigation.
- The 2018-2019 winter resulted differences in winterkill damage among entries in West Lafayette, Indiana, which 19 out of 87 zoysiagrass plots, including zoysia checks, had significant winter damage and these plots had either 0% or below 5% turfgrass cover by the end of the 2019 growing season.
- In 2019 (year 2), preliminary results based on turf quality and other collected data indicates several entries that are consistently in the top performing group across multiple sites.
- In 2020 (year 3), data collection and attention will continue to be paid to the selection of entries that exhibit minimal-to-no visible symptoms of drought stress, insect and disease susceptibility, and winterkill, and the best golf ball lie to help reach the goal in identifying entries that perform well under low-input conditions, which will assist in lowering golf course maintenance budgets.
- In 2020 (year 3), golf course superintendents visiting the experimental locations for university field days will provide feedback and ratings of plots via a survey instrument.

Summary Text:

Zoysiagrass roughs are amongst some of the most easily played (improving pace of play) and easily managed (few inputs required with excellent weed suppression) of all the species used in golf course roughs. Breeding programs have “coarse-textured” germplasm available that has excellent stress and pest tolerance and fast establishment when managed with no inputs. These coarse *Z. japonica* genotypes have the ability to offer a superior golf course rough surface with little to no inputs and fewer long-term maintenance costs. Our research team has existing collections of zoysiagrasses collected from unmanaged areas or as part of germplasm collections. These existing collections have not been explored for their potential use, but they offer great promise as a low-input zoysiagrass for golf course roughs.

In 2018 summer, propagated zoysiagrass germplasm was planted at five replicated sites: 1) West Lafayette, IN; 2) Raleigh, NC; 3) Tifton, GA; 4) Chandler, AZ; 5) Escondido, CA. Plot sizes are at least 1.5 x 1.5 m, with 0.5 m borders, with 3 replications arranged in a randomized, complete-block design at each site. In year 1 (2018), all sites were fertilized (1.0 lb N/1000 ft^2), watered, and pest control was applied to promote establishment. In year 2 (2019), plots were mowed as needed at a golf course rough height and maintained with minimal-to-no inputs (fertilization, irrigation, pest control) and will continue to be in year 3 (2020). This low-maintenance regime will help to identify entries that perform well under these conditions and assist in lowering golf course maintenance budgets.

Data collection, from 2018 to 2020, is similar to typical NTEP trials in order to identify those that are best suited to for golf course roughs. In addition, golf ball lie was measured in the summer and fall of 2019 for each entry using the method developed by Richardson et al. (2010) at three sites and will be measured again in 2020. Golf course superintendents visiting the experimental locations in 2020 for university field days will be asked to provide feedback and ratings of plots via a survey instrument. Superintendents will be asked for feedback on turf quality, ball lie (acceptable and optimal), and other potential turf characteristics.

Winterkill ratings (1 to 9 scale, 9=fully green, 1=no green tissue) were evaluated at all sites, except Georgia in the spring of 2019 (Fig. 1 and Table 1). The following entries typically exhibited the greatest winterkill damage in West Lafayette, Indiana: XZ14069, XZ14071, XZ14074, ZG09062, 09-TZ-54-9, 09-TZ-89-73,1 10-TZ-1254, 15-TZ-11766, 16-TZ-12783, 16-TZ-13463 (Table 1). In addition, 19 out of 87 zoysiagrass plots, including zoysia checks, had either 0% turfgrass cover or never recovered above 5% cover by the end of 2019 in Indiana (data not shown). Winterkill ratings from the other locations also reveal differences among entries, although damage appears to be not as severe as Indiana.

For each location, turf quality ratings, averaged across multiple ratings dates in 2019 within each location, display several entries that are consistently performing well at multiple sites (Table 2). Preliminary results based on turf quality ratings (Table 2) and other data collected (turf cover, digital image analysis, uniformity, density, spring and fall color, drought tolerance (Fig. 2), and summer color) (data not shown) indicate XZ14069, ZG09062, 09-TZ-54-9, 15-TZ-11766, 16-TZ-12783, and 16-TZ-13463 are entries that are in the top performing group at three or more sites. While other entries such as XZ14071, XZ14074, 10-TZ-1254, and 16-TZ-14114 are in the top performing group at two of the five sites. However, none of the previously mentioned 10 entries are in the top performing group at Indiana due to the majority of these entries resulted in severe winterkill from the 2018-2019 winter. Golf ball lie data from one or two data collection events across Indiana, North Carolina, and Georgia locations also revealed differences among entries (Fig. 3 and 4). Performance of entries, additional golf ball lie data, and superintendent feedback will continue to be evaluated in 2020. Further analysis of data may indicate the need for recommendations of specific entries based on regional climatic differences.

Results from this study in combination with feedback from golf course superintendents on species choices for golf course roughs pertinent to their maintenance, performance, and playability may potentially identify *Z. japonica* germplasm suitable for low-maintenance golf course roughs that can be used over a wide geographic region.

References

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Table 1. Winterkill ratings (1-to-9 scale, 9=fully green, 2=some green tissue, 1=no green tissue) for each testing location, except Georgia, in 2019 compared to five zoysiagrass checks, one bermudagrass check, and three cool-season grass checks (entries, not checks, sorted by Indiana location).

	2019 Winterkill Ratings			
Entries	IN	NC	AZ	CA
ZG09004	8.0 ab	6.7 b-e	6.3 c-f	6.0 c-e
PURZ 1606	7.3 bc	4.3 ij	7.3 a-e	6.0 c-e
10-TZ-994	7.3 bc	6.0 d-h	5.0 f	5.0 e
ZG09055	7.3 bc	5.0 g-i	--‡	5.0 e
PURZ 1701	7.0 bc	5.3 f-i	6.7 b-f	8.0 a-c
PURZ 1602	6.3 cd†	3.3 j	6.0 d-f	7.0 b-d
PURZ 1603	6.0 cd	4.3 ij	6.0 d-f	6.3 c-e
XZ14070	5.3 de	8.0 ab	6.7 b-f	4.7 e
PURZ 1702	5.0 d-f	4.3 ij	7.0 a-f	--‡
XZ14072	5.0 d-f	7.7 a-c	7.0 a-f	8.0 a-c
XZ14055	4.0 e-g	5.3 f-i	7.3 a-e	--
16-TZ-12036	3.7 f-h	6.0 d-h	7.7 a-d	8.3 ab
XZ14092	3.7 f-h	4.7 h-j	5.3 ef	5.3 de
16-TZ-14114	3.3 gh	6.0 d-h	7.7 a-d	7.0 b-d
XZ14069	2.7 g-j	8.7 a	8.0 a-d	8.7 ab
10-TZ-1254	2.7 g-j	7.3 a-d	9.0 a	8.0 a-c
XZ14071	2.7 g-j	6.7 b-f	6.3 c-f	6.3 c-e
ZG09062	1.7 i-k	5.3 f-i	8.0 a-d	8.3 ab
09-TZ-54-9	1.7 i-k	6.7 b-f	8.7 ab	8.0 a-c
09-TZ-89-73	1.7 i-k	6.7 b-f	8.0 a-d	8.3 ab
XZ14074	1.3 jk	7.0 b-e	7.7 a-d	9.0 a
15-TZ-11766	1.0 k	7.0 b-e	7.7 a-d	7.3 a-c
16-TZ-12783	1.0 k	5.7 e-i	8.0 a-d	7.0 b-d
16-TZ-13463	1.0 k	5.7 e-i	7.0 a-f	8.0 a-c
'Chisholm' zoysiagrass	4.0 e-g	6.7 b-f	8.3 a-c	7.7 a-c
'Meyer' zoysiagrass	7.3 bc	6.3 c-g	5.3 ef	5.0 e
'Empire' zoysiagrass	2.7 g-j	5.3 f-i	7.7 a-d	8.0 a-c
'Jamur' zoysiagrass	3.3 gh	6.0 d-h	7.7 a-d	7.7 a-c
'Zenith' zoysiagrass	3.0 g-i	6.0 d-h	8.7 ab	7.0 b-d
'Riviera' bermudagrass	2.3 h-k	3.3 j	--	--
Fine fescue mixture	9.0 a	--‡	--	--
'Bluenote' Kentucky Bluegrass	9.0 a	--	--	--
'Mustang 4' Tall fescue	9.0 a	--	--	--
P-value	<0.0001	<0.0001	0.0109	<0.0001

† Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher's protected least significant difference test.
‡ Entry or check either not planted at site or did not survive during establishment in 2018.

Table 2. Turf quality ratings (1-to-9 scale, 9=best quality, 6=minimum acceptability) of zoysiagrass entries averaged across multiple rating dates for each testing location in 2019 compared to five zoysiagrass checks, one bermudagrass check, and three cool-season grass checks (entries, not checks, sorted by overall mean).

	2019 Turf Quality				
Entries	IN	NC	GA	AZ	CA
XZ14069	3.1 g-i	7.2 a	7.3 ab	6.7 ab	7.0 a-c
16-TZ-14114	6.6 b-d	6.0 c-e	6.3 a-f	5.5 c-f	6.1 c-f
09-TZ-54-9	2.3 i-k	7.2 a	6.5 a-e	6.8 a	7.3 ab
XZ14070	5.7 d-f	6.0 c-e	6.0 b-g	5.7 c-e	6.2 c-f
XZ14072	6.7 b-d	6.5 a-c	4.7 g-l	4.8 e-h	6.2 c-f
ZG09004	7.5 a-c	5.5 c-e	5.8 b-g	4.5 g-j	5.6 f-h
16-TZ-12783	1.0 k	7.0 ab	7.8 a	5.8 b-d	6.7 b-e
16-TZ-12036	3.7 g-i	6.7 a-c	6.5 a-e	5.3 c-g	6.1 c-f
09-TZ-89-73	3.6 g-i	5.7 c-e	6.5 a-e	6.2 a-c	6.4 b-f
ZG09055	7.4 a-d	5.8 c-e	4.7 g-l	--‡	4.7 hi
ZG09062	2.9 h-j	6.7 a-c	6.8 a-c	5.8 b-d	6.0 c-f
15-TZ-11766	1.0 k	6.3 a-c	7.8 a	5.0 d-h	7.8 a
10-TZ-1254	5.7 d-f	6.3 a-c	4.7 g-l	5.8 b-d	6.2 c-f
16-TZ-13463	1.0 k	5.8 c-e	6.7 a-d	6.0 a-c	6.9 a-d
10-TZ-994	7.3 a-d	5.8 c-e	3.3 k-m	3.8 jk	3.9 ij
PURZ 1602	6.5 b-d†	6.0 c-e	4.2 h-m	4.7 f-j	4.1 ij
XZ14055	4.7 e-g	5.8 c-e	4.2 h-m	5.2 d-g	--‡
XZ14071	3.5 g-i	6.2 b-d	4.8 f-k	4.5 g-j	5.4 f-h
XZ14074	1.1 jk	6.8 a-c	4.0 h-m	6.0 a-c	5.8 e-g
PURZ 1701	7.1 a-d	5.2 e	4.0 h-m	4.2 h-k	3.2 jk
PURZ 1606	6.7 b-d	5.2 e	4.7 g-l	3.5 k	2.5 k
PURZ 1603	5.9 c-f	5.5 c-e	3.2 lm	3.8 jk	4.0 ij
XZ14092	3.0 g-i	5.1 e	4.0 h-m	5.5 c-f	3.7 j
PURZ 1702	4.7 e-g	5.2 e	3.7 i-m	4.0 i-k	1.0 l
‘Chisholm’ zoysiagrass	5.7 d-f	5.8 c-e	5.5 c-h	6.2 a-c	5.9 d-f
‘Meyer’ zoysiagrass	7.1 a-d	5.3 de	5.0 e-j	4.8 e-h	4.8 g-i
‘Empire’ zoysiagrass	4.6 f-h	6.2 b-d	6.3 a-f	5.5 c-f	6.2 c-f
‘Jamur’ zoysiagrass	6.3 b-f	6.0 c-e	6.2 b-g	5.5 c-f	6.0 c-f
‘Zenith’ zoysiagrass	3.1 g-i	5.7 c-e	3.5 j-m	5.0 d-h	3.9 ij
‘Riviera’ bermudagrass	3.9 g-i	--‡	5.0 e-j	--‡	--‡
Fine fescue mixture	8.0 ab	5.4 c-e	5.2 d-i	--	--
‘Bluenote’ Kentucky Bluegrass	6.4 b-e	--	2.7 m	--	--
‘Mustang 4’ Tall fescue	8.6 a	5.4 c-e	3.2 lm	--	--
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.
‡ Entry or check either not planted at site or did not survive during establishment in 2018.

Figure 1. Differences in spring green-up and winterkill among plots in West Lafayette, IN site on 17 June 2019.

Figure 2. Visible differences in response to drought stress among entries and standard zoysiagrass checks in West Lafayette, IN on 7 Aug. 2019.

Figure 3. Golf ball lie data collection at the West Lafayette, IN site on 12 Aug. 2019.

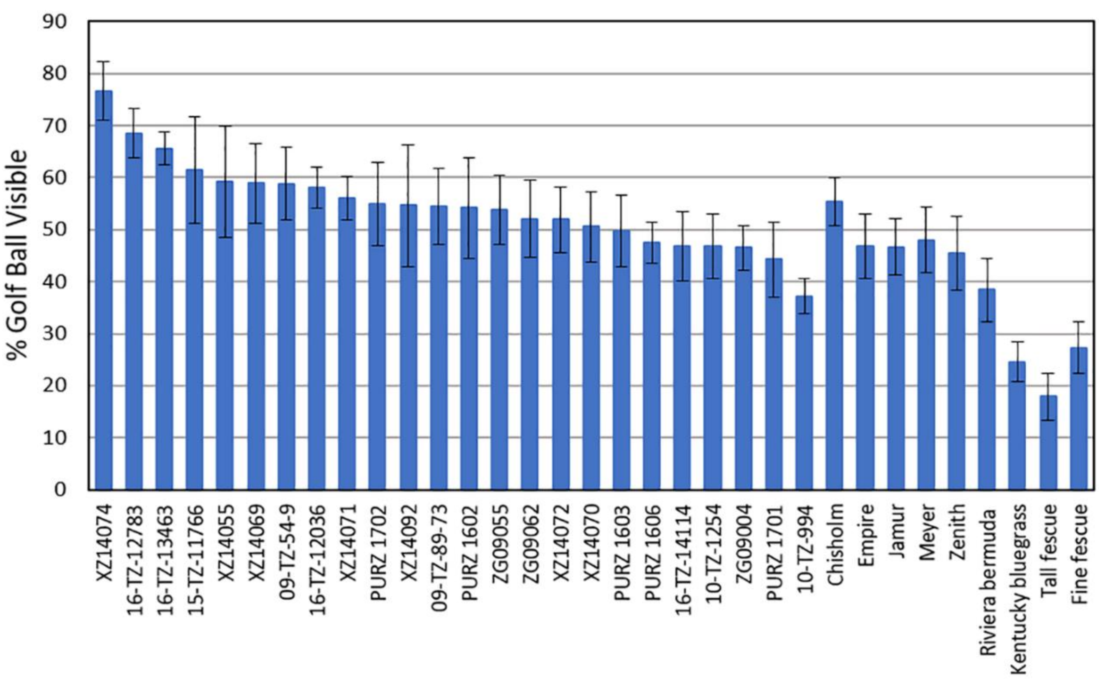

Figure 4. Golf ball lie data from one or two data collection events across Indiana, North Carolina, and Georgia locations. Error bars represent standard error of the mean.

USGA ID#: 2016-35-605

Title: Developing phenotypic and genomic tools to study salt-tolerance in seashore paspalum

Project Leaders: Elizabeth Kellogg, Kenneth Olsen, Ivan Baxter; grad student David Goad
Affiliation: Donald Danforth Plant Science Center

Objectives:

Increase diversity in available seashore paspalum germplasm, generate genome-wide SNP markers for seashore paspalum germplasm, identify phenotypic and genetic basis for variation in salt tolerance between lines.

Start Date: 2016
Project Duration: 2 years
Total Funding: $69,997

Summary Points:

- Generated genome-wide SNP markers and used these to genotype over 200 accessions
- Determined that the “coarse” ecotype of seashore paspalum is a hybrid between the “turf” ecotype and a so-far unidentified paspalum species.
- Extracted high-molecular weight DNA and generated long PacBio reads, which have substantially improved the quality of the reference genome assembly.
- Prepared manuscript on diversity and population genetics in paspalum species; target submission in February 2020
- Identified genotype specific ionomics responses to increased salinity
- Completed a high-throughput phenotyping experiment, growing paspalum accessions under varying salinity levels. 140,000 images were produced from which to calculate biomass.
- Completed RNA sequencing to determine which gene are involved in response to changing salinity.

As outlined below, all data collection is complete for this project, and we are currently analyzing data and writing up results for publication. The project has been led by Washington University graduate student David Goad, and forms the basis for his dissertation.

Summary Text:

Seashore paspalum (*Paspalum vaginatum*) is an extremely salt tolerant grass that has found an economic niche as a turfgrass in coastal regions, particularly on golf courses. Additionally, it has recently seen increased interest from the broader plant science community as a potential model system for the study of salt tolerance in plants. Both groups will undoubtedly benefit from any advancements made in understanding the biology of the species. With funding from the USGA, we have taken the first steps toward making seashore paspalum a scientific model system. Specifically we have improved the quality of the reference genome, increased the diversity of the available germplasm, and quantified genetic and phenotypic diversity in the species. These tools are now available to the turfgrass community to serve as a springboard for future work in the system by breeders and basic scientists alike.

1. Increase the pool of available germplasm. David collected 200 wild seashore paspalum and the closely related non-salt-tolerant species *Paspalum distichum* from a diverse range of freshwater and saline environments along the US Gulf and Atlantic Coasts. He intentionally included the “coarse” ecotype of seashore paspalum, which has previously been mostly ignored, in the collections because we believed it may contain useful physiological traits for breeding despite its undesirable morphology.
2. Generate genome-wide SNP markers. David genotyped all of our samples along with the USDA GRIN collection using a Genotyping-by-Sequencing approach to generate genome-wide SNP markers.
3. Population genetics analysis. The SNP data showed that the “coarse” ecotype of seashore paspalum is actually a hybrid of the “turf” ecotype and another still-unidentified *Paspalum* species. This conclusion is supported by the lack of genetic clustering of the coarse type with either *Paspalum distichum* or the turf ecotype of seashore paspalum (Fig. 1). The coarse type also shows high genome-wide heterozygosity, which appears to reflect its hybrid origin. These hybrids vary in ploidy with 2x, 3x and 4x individuals represented in our collection, while the “turf” ecotype is entirely diploid (2x). The hybrid should likely be considered its own species and we are pursuing documenting it as such. Despite the hybrid’s lack of favorable turf qualities, it has been used as a dune stabilizer, particularly the cultivar “Brazoria”. David also found that many wild plants are clonal and that identical genotypes can be found in distant locations which has implications for future germplasm collection. Finally, he has confirmed that *P. distichum* and *P. vaginatum* are genetically distinct species, which had been an open question. A publication detailing this work is nearing completion and expected to be submitted to Molecular Ecology in early 2020.
4. Artificial tide flow experiment. David designed and built automated flood trays in which pots were submerged in a nutrient solution with varying salinity levels twice daily (mimicking tidal inflows) (Fig. 2). We collected the total above-ground tissue from each genotype at each salinity level to measure biomass and the tissue concentration of twenty different ions using the ionomics pipeline in Ivan Baxter’s lab at the Danforth Center. We are currently analyzing these data with mixed models and preliminary results are promising. *P. distichum* is indeed less salt tolerant than both ecotypes of *Paspalum vaginatum*. We also identified differences in ionomic profile between coarse and turf ecotypes despite their similar salt tolerance levels. Within the “turf” ecotype of *P. vaginatum* we identified differences in ion concentration between genotypes and in some cases, such as potassium, these were correlated with growth rate (Fig. 3).
5. High-throughput phenotyping on the Lemnatec automated phenotyper. This project made use of the state-of-the-art phenotyper at the Danforth Center. Plants were grown in individually barcoded pots, which were placed on a conveyor belt. Plants travel through the growth room on the conveyor belt, passing automatic water stations where they are weighed and then watered according to a set percentage of water. Plants were subjected to one of five treatments: no salt, gradual daily salt application at a high or low concentration, and a large salt application applied at the beginning of the experiment at a high or low concentration. Each day, the conveyer belt moves the plants past cameras. Color and infrared images were taken daily of each plant from three different angles to measure growth rate, color, and other morphological changes induced by exposure to the salt solution (Fig. 4). Over 140,000 images were taken. Computational analysis of these images with the *PlantCV* pipeline is pending. We expect to identify subtle changes in morphology associated with increased resistance to salt that are invisible or difficult to measure with the naked eye. By comparing the ionomic results to the image analysis we should be able to develop a clear picture of both the morphological and physiological responses to salinity in seashore paspalum.

6. Gene expression analysis (RNA-seq). Using the plants grown in the Lemnatec, David also collected root and shoot tissue to measure gene expression (RNA-seq). We wished to identify genes that are more highly expressed under saline conditions and therefore likely to be involved in salt tolerance. The saline treatments include tissue collected shortly after first exposure to a high salt concentration to measure the salt "shock" response due to a sudden change in osmotic pressure as well as after two weeks of gradual salt build-up to investigate genes involved in long term stress tolerance due to the toxicity of sodium ions. We have generated just over 1 billion paired-end reads across 36 samples. Bioinformatics analysis of these reads to measure differential expression is in progress. Advancements made to the reference genome described below have been instrumental in this process.
7. Improved reference genome. In addition to the main goals described above, we also extracted high molecular weight DNA for PacBio sequencing in collaboration with the Joint Genome Institute of the Department of Energy. We generated 74.03x coverage of long reads and used them along with a linkage map produced by the Devos lab at the University of Georgia to produce a highly contiguous genome assembly. Seventy-five percent of the genome can now confidently be assigned to its location on a chromosome. This new reference assembly represents a dramatic improvement over the previous draft and will form the backbone of future genetic studies in the system.

In summary, the data collection portion of this project has been completed and has generated several large genetic and phenotypic datasets which are currently at various stages of analysis. By the end of 2020 we expect to have submitted or published no fewer than 3 peer reviewed journal articles from this work. Future work in the species will aim to focus on identifying candidate genes and breeding markers not only for salt tolerance, but other traits of importance as well.

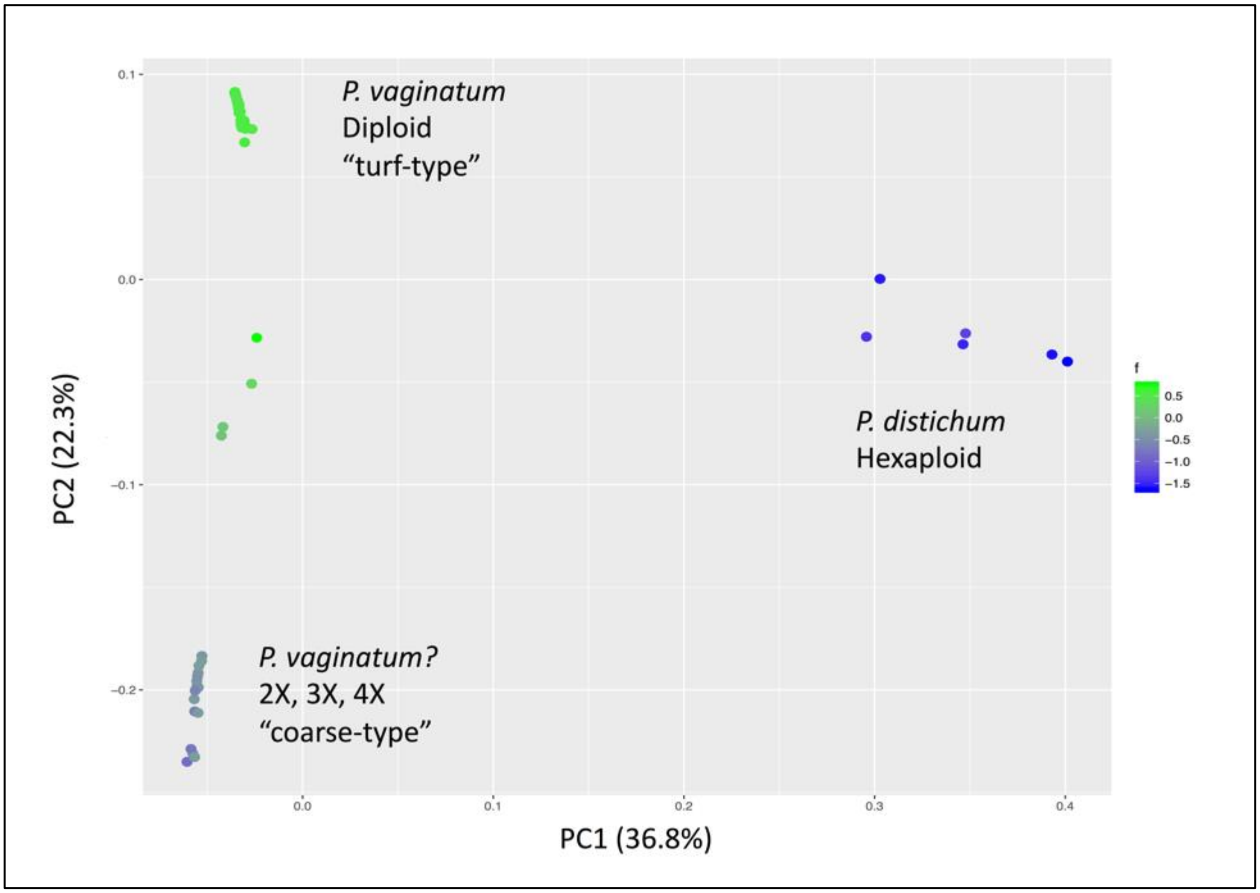

Figure 1. PCA of all *P. vaginatum* and *P. distichum* samples based on genome-wide SNP markers. Three clearly distinct clusters correspond to the "turf" ecotype, "coarse" ecotype, and *P. distichum.* Values next to group name represent inferred ploidy based on flow cytometry genome size estimates. Darker shades of blue represent higher genome-wide heterozygosity as measured by the individual inbreeding coefficient (f).

Figure 2. Paspalum accessions being watered by a pump system controlled by a Raspberry Pi. Three of the six trays were irrigated with a saline solution which increased in concentration every two weeks.

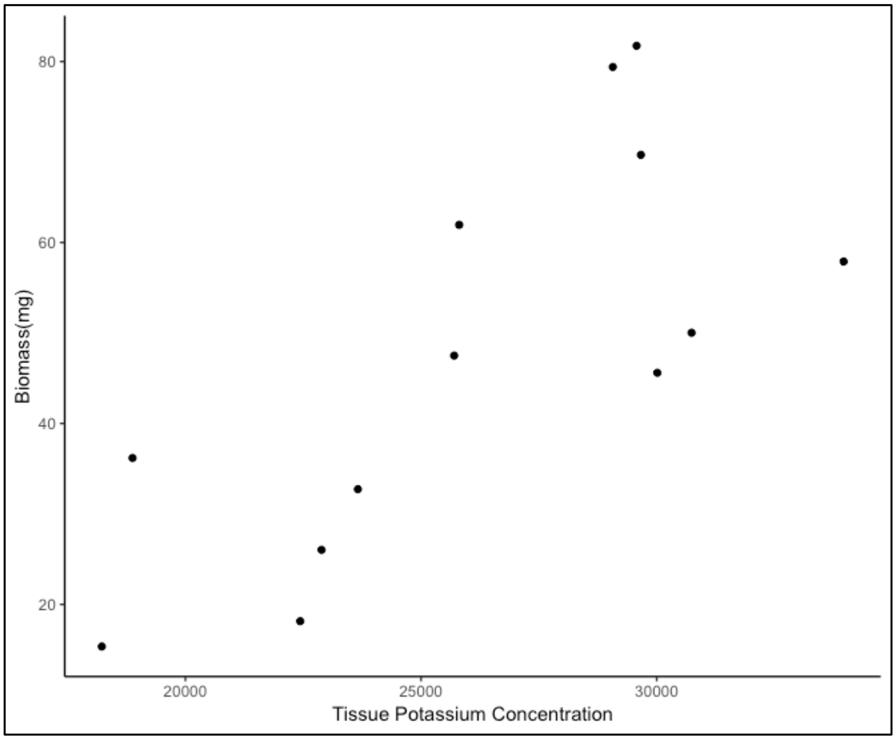

Figure 3. The relationship between tissue potassium concentration and growth rate as measured by total above ground biomass of different genotypes of the turf ecotype of seashore paspalum after being exposed to a 30ECw saline solution for one week.

Figure 4. Side-view image of seashore paspalum taken on the *Lemnatec* automated phenotyper.

USGA ID#: 2003-36-278

Title: Buffalograss Breeding and Genetics

Project Leader: Keenan Amundsen
Affiliation: University of Nebraska-Lincoln

Objectives:

The primary objective of this study is to develop, through selection and plant breeding, buffalograss suitable for golf course fairways, tees, and roughs.

Start Date: 2018
Project Duration: Continuous
Total Funding: $30,000

Summary Points:

- The ploidy level of new buffalograss accessions were characterized and ranged from diploid to hexaploid.
- Genetic diversity of the new buffalograss collection demonstrated uniqueness of the accessions.
- Concentrations of plant hormones important for buffalograss seed dormancy differed in response to seed soaking treatments.

Summary Text:

Buffalograss [*Buchloë dactyloides* (Nutt.) Engelm. syn *Bouteloua dactyloides* (Nutt.) Columbus] is considered a model for low-input turfgrass species. It has exceptional drought and heat tolerance and can be maintained at an acceptable quality level with minimal fertilizer applications, pesticide applications, and supplemental irrigation. Buffalograss is ideally suited for low-input golf course roughs or lawns. Throughout the Midwest, buffalograss can also be found on golf course fairways because of its ability to tolerate low mowing, but fairway use is limited. It is important to test and evaluate modern cultivars to revise use recommendations as appropriate. As a test case, Sundancer buffalograss was established in a fairway at Holmes Park Golf Course in Lincoln, NE. The city golf course maintenance coordinator indicated that to his surprise the only criticism of buffalograss from golfers was its winter dormancy response. Buffalograss is a warm-season species and goes dormant in the fall in response to the first hard frost and breaks dormancy late in the spring after soil temperatures warm. At Holmes Park, buffalograss was dormant and straw-colored for at least one month while the cool-season surrounding species were green and actively growing. From a reduced management perspective, the early dormancy response is beneficial because that is a month when no management is necessary, contributing to reduced labor and equipment costs. There are still questions about the ability of buffalograss to recover from traffic stress and damage (divots) occurred during dormancy and is an interest for future research.

The University of Nebraska-Lincoln (UNL) buffalograss breeding program is focused on improving the visual and functional quality of managed buffalograss, while increasing seed yield, sod strength, and establishment rate from seed or plugs for seed and vegetative buffalograss producers. Sundancer, released in 2014, was a significant improvement over existing buffalograss lines for seed yield and turfgrass quality. Sundancer is a synthetic variety, ultimately derived from two male and four female progenitors. A significant effort of most breeding programs is evaluation of germplasm, selections, and elite material. In 2017, the UNL buffalograss germplasm collection was increased by a collection of new buffalograsses collected from parts of Colorado, Kansas, Nebraska, New Mexico, Oklahoma, and Texas. Preliminary field performance of the collection was reported in 2018. Buffalograss represents a ploidy series including diploids, tetraploids, pentaploids, and hexaploids. When hybrids are formed from parents that differ in ploidy levels, the resulting progeny are often sterile due to mismatched chromosome pairing during meiosis. Therefore it is critically important for any buffalograss breeding program to characterize the ploidy level of breeding lines. Flow cytometry was used to measure nuclear DNA content and infer the genome size and ploidy level of 69 accessions from the collection (Figure 1a and 1b). In the collection, there were four diploids, nine tetraploids, 30 pentaploids, and 26 hexaploids. This information helps inform future breeding decisions.

In 2018, genetic resources were developed for studying buffalograss including genotype-specific simple sequence repeat markers (SSRs). Those genetic markers were used to assess the amount of genetic diversity present among the new collection and elite buffalograss cultivars. An unweighted pair group method with arithmetic mean method was used to cluster the accessions based on the molecular marker data (Figure 2). Most accessions were similar, with dissimilarity scores less than 0.22 except for Gg6_2 which was a blue grama accession that was included as an outgroup. A relatively large group of 12 accessions clustered with the cultivar 95-55, but otherwise most accessions were distinct based on the molecular markers tested. Results from this study demonstrate that the collection adds unique genetics to the UNL buffalograss breeding program, introducing more genetic diversity in support of future breeding efforts.

Seed dormancy is an important issue for buffalograss seed producers since post-harvest seed treatments add to production costs and relatively high seed costs for turfgrass managers. Preliminary research was conducted to determine if harvest timing influences maternal or seed development factors that impact seed dormancy. Seed was harvested from a five-year-old buffalograss production field on July 17th, August 2nd, August 16th, and September 5th. A significant difference was observed, and seed harvested later had reduced dormancy. Even with the late harvest date, seed dormancy was not reduced enough to overcome the need for post-harvest seed treatments.

Previously it was reported that buffalograss post-harvest seed treatments impact permeability of the bur allowing water imbibition [Kreuser et al. 2016. HortSci 51(12):1566-72]. As part of that previous study, hormone profiling was also done to identify key metabolites influencing seed dormancy mechanisms in buffalograss. The hormone profiling study was inconclusive, likely due to the timing of sample collection following post-harvest seed treatments. In a current study, a hormone profiling experiment was done to compare how water and potassium nitrate seed soaking treatments influence seed dormancy. Seed samples were collected at 1, 2, 4, 8, 24, and 48 hrs after soaking treatments and sent to the UNL Metabolomics Facility for hormone profiling. Abscisic acid and trans-zeatin concentrations were significantly different between soaking treatments. Both hormones are important for plant growth and development and have been implicated for playing a role in seed dormancy. An RNA-seq study was also done to complement the hormone profiling study but results are pending and expected in early 2020. This research on characterizing seed dormancy mechanisms in buffalograss along with expanding germplasm and genetic resources contribute to the success of UNL buffalograss breeding efforts to address buffalograss manager and producer needs.

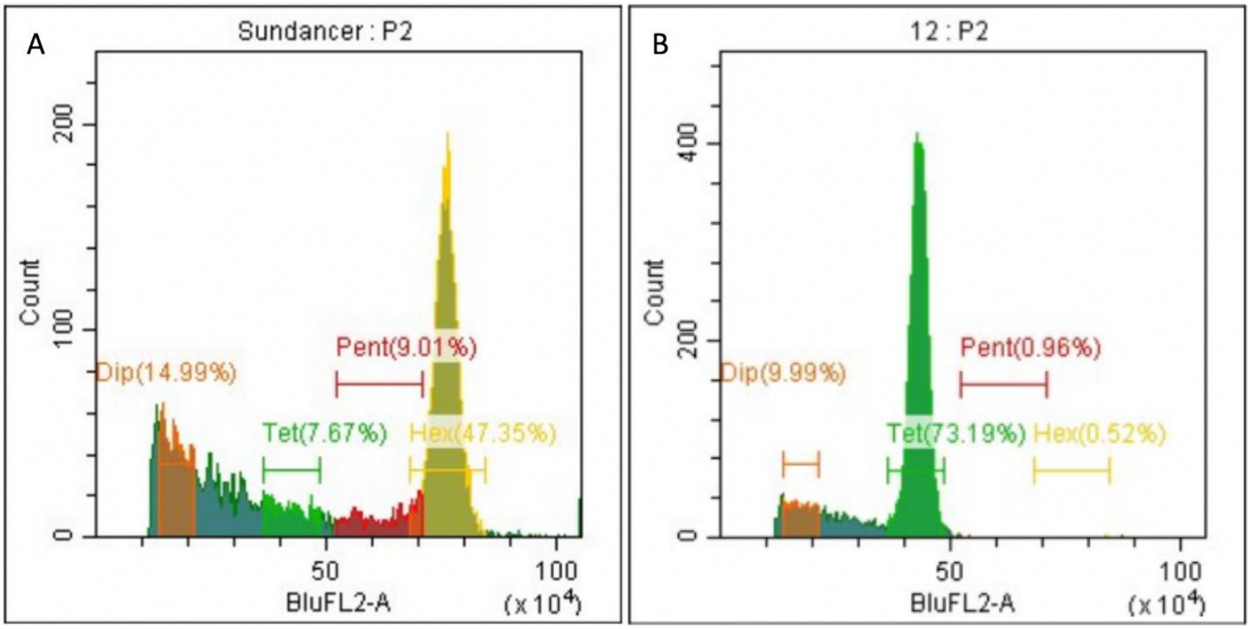

Figure 1. Ploidy analysis of Sundancer (panel A) and an unknown sample from a buffalograss collection (panel B). The ploidy analysis results are consistent for Sundancer, a known hexaploid. The unknown sample was classified as a tetraploid based on the flow cytometry results.

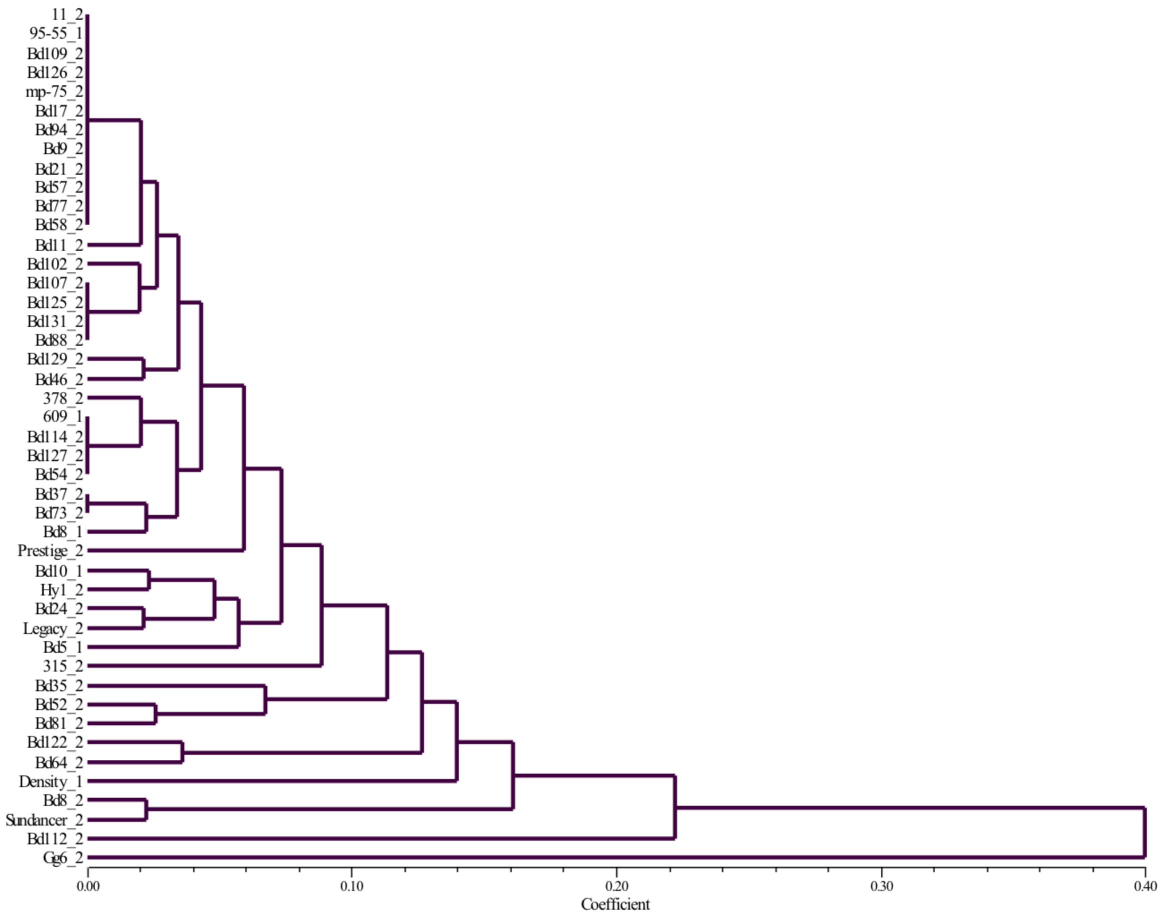

Figure 2. A phylogram depicting genetic diversity among a buffalograss collection. The study included the cultivars 95-55, 378, 609, Prestige, Legacy, 315, Density, and Sundancer. Samples 11_2, mp-75_2, and Hy1_2 are accessions from the University of Nebraska-Lincoln buffalograss breeding program. Gg6_2 is a blue grama sample used as an outgroup. All other samples (Bd*) are from a new and previously uncharacterized buffalograss collection. A coefficient of dissimilarity was used in creation of the phylogram, so a lower coefficient value (along the horizontal axis) indicates a closer genetic relationship based on the molecular markers used in the analysis.

USGA ID#: 2016-05-555

Title: Improved Wheatgrass Turf for Limited Irrigation Golf Course Roughs

Project Leaders: Joseph G. Robins and B. Shaun Bushman
Affiliation: USDA-ARS Forage and Range Research

Objectives:

1) Evaluate performance of elite wheatgrass turfgrass populations for turfgrass quality in monoculture and mixture conditions.
2) Characterize the effect of mowing height and irrigation replacement on wheatgrass turfgrass quality.

Start Date: 2015
Project Duration: 3 years
Total Funding: $40,440

Summary Points:

- Wheatgrass turf receives higher turf quality ratings when grown in mixture with Kentucky bluegrass and/or hard fescue rather than in monoculture.
- Partial irrigation (50% ET_0 replacement) results in greater turfgrass quality for wheatgrass monocultures and mixtures.
- The effect of mowing height on wheatgrass turf quality is inconsistent and smaller.
- Wheatgrass dark green color index correlates negatively with turfgrass ground cover, density, uniformity, and quality.

Summary Text:

The term wheatgrass is a catch-all for cool-season perennial grass species from several genera in the tribe Triticeae. Previous taxonomic treatments considered many wheatgrass species to be members of the *Agropyron* genus. However, more recent treatments separated many of the species into different genera, including *Agropyron*, *Elymus*, *Pascopyrum*, and *Thinopyrum*, among others (Dewey, 1983). The wheatgrasses are native to arid and semi-arid regions of the temperate word, including Eurasia and western North America. They possess great tolerance to various stresses, including harsh winter conditions and extended summer drought. For these reasons, the wheatgrasses are valued by livestock producers and land managers for forage production and revegetation of disturbed rangeland sites, and plant breeders have developed and released dozens of cultivars of the major wheatgrass species. Because of these same traits the wheatgrasses are also used in low-maintenance turf production, such as roadsides and recreational properties. In the past, wheatgrass turf seeding relied on forage cultivars or low-growing revegetation cultivars. However, over the past twenty years the USDA-ARS Forage and Range Research Unit in Logan, UT has had a dedicated wheatgrass breeding program. This program focuses on improving crested (*Agropyron cristatum*), intermediate (*Thinopyrum intermedium*), thickspike (*Elymus lanceolatus*), and western (*Pascopyrum smithii*) wheatgrasses for turfgrass production. The objectives of this research were to characterize wheatgrass turfgrass populations under two irrigation levels, two mowing levels, and monoculture and mixture production conditions with Kentucky bluegrass and fine fescue. This work may be of interest to the golf industry because of the potential of wheatgrass turf to save on irrigation costs and amounts, particularly in more distal parts of rough and out-of-bounds areas.

Plots were established at a Millville, UT research site in August 2016 by hand-sowing and raking. Following sowing, uniform irrigation was used through the fall of 2016 to ensure stand establishment (Figure 1). The plant materials were ten cultivars or experimental populations – one cultivar and one experimental population from each of the four wheatgrass species and one cultivar each of Kentucky bluegrass and fine fescue. Irrigation (no irrigation and 50% ET_0 replacement) and mowing (51 mm and 76 mm) were initiated in spring 2017 and continued during the 2017 and 2018 growing seasons. Plots were irrigated and mowed weekly. Beginning in late June of each year, once water stress began, and continuing into early fall digital images were taken of each plot biweekly using a digital camera and a custom light box (Bushman et al., 2012). Images were then converted to ratings of ground cover, dark green color index, density, uniformity, and quality using the Turf Analyzer software. These ratings were then statistically analyzed using the R 3.4.2 statistical software (R Core Team, 2017), the ASReml-*R* (Version 4, Butler et al., 2018) package based on a mixed model analysis of a split-split-plot over time experimental design.

The results of the mixed model analysis across irrigation levels, mowing heights, and mixtures were significant differences between the irrigation levels and turfgrass entries for ground cover, dark green color index, density, uniformity, and quality. But there were significant differences between the mowing heights only for ground cover, color, and density. The greatest turfgrass trait values corresponded to the 50% ET_o replacement irrigation treatment and the 51 mm mowing treatment, although the effect of mowing was less consistent. Despite these differences, the mixed model analysis also found strong evidence of interactions between the turfgrass entries and the irrigation and mowing treatments, including irrigation × turfgrass mixture, mowing height × turfgrass mixture, and irrigation × mowing height × turfgrass mixture interactions. Due to these interactions, the analysis was repeated based on the four individual irrigation level and mowing height combinations, for example the no irrigation and 51 mm mowing height combination.

Within each of these irrigation level-mowing height combinations there were significant differences among the turfgrass entries for ground cover, dark green color index, density, uniformity, and quality (Figure 3 contains the turfgrass quality results for each of the entries). None of the turfgrass entries possessed high ground cover in all four irrigation level-mowing height combinations. However, four turfgrass entries (Park KB, Experimental crested wheatgrass (CW)-Park KB, Tegmar intermediate wheatgrass (IW)-Durar HF, and Experimental WW-Park KB) contained high ground cover in three of the combinations. Four turfgrass entries (RoadCrest CW, Experimental CW-Durar HF, Sodar thickspike wheatgrass (TW)-Durar HF, and Tegmar IW) possessed high dark green color index values in all four combinations. Two turfgrass entries (Rosana WW-Durar HF-Park KB and Rosana WW-Park KB) possessed high density in three combinations. Tegmar IW was the only turfgrass entry to possess high uniformity in all four combinations. The Experimental CW-Park KB and Rosana WW-Durar HF-

Park KB entries possessed high turfgrass quality in all four combinations. Five other entries and Park KB possessed high turfgrass quality in three of the combinations.

The highest environmental correlations for the same trait were between the two mowing heights at the same irrigation level, in other words between MH51 and MH76 at the ET0 and ET50 irrigation levels. Environmental correlations for the same trait across irrigation levels, for example MH51 at ET0 correlated to MH51 at ET50, were generally lower. Between traits the highest correlation was between density and quality ($\rho = 0.87$ to 0.90). The relationship between dark green color index and the other four traits was always negative.

Overall, the higher irrigation level consistently resulted in higher turfgrass trait ratings. The effect of mowing height was less consistent, although the lower height often resulted in higher ratings. However, even the no irrigation plots approached a rating of 5 for turfgrass quality. To receive the highest ratings, the wheatgrasses had to be accompanied by either Park and/or Durar. However, the wheatgrasses also added to the trait ratings, because Park and Durar rarely received highest ratings when grown in monoculture. We have finished the data analysis and are now in the process of writing the forthcoming peer-reviewed journal article. These results will also prove useful for making recommendations to turfgrass managers, including golf course superintendents.

References

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Butler, D.G., B.R. Cullis, A.R. Gilmour, B.J. Gogel, and R. Thompson (2018) ASReml-R reference manual. Version 4. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Dewey, D.R. (1983) Historical and current taxonomic perspectives of *Agropyron*, *Elymus*, and related genera. Crop Science 23:637-642.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Figure 1. Wheatgrass turf monoculture and mixture plots following establishment at Millville, UT in October 2016.

Figure 2. Wheatgrass turf monoculture and mixture plots treated with two irri gation levels and two mowing heights at Millville, UT in August 2017. No irrigation plots are in the background and 50% ET_0 replacement irrigation plots are in the foreground.

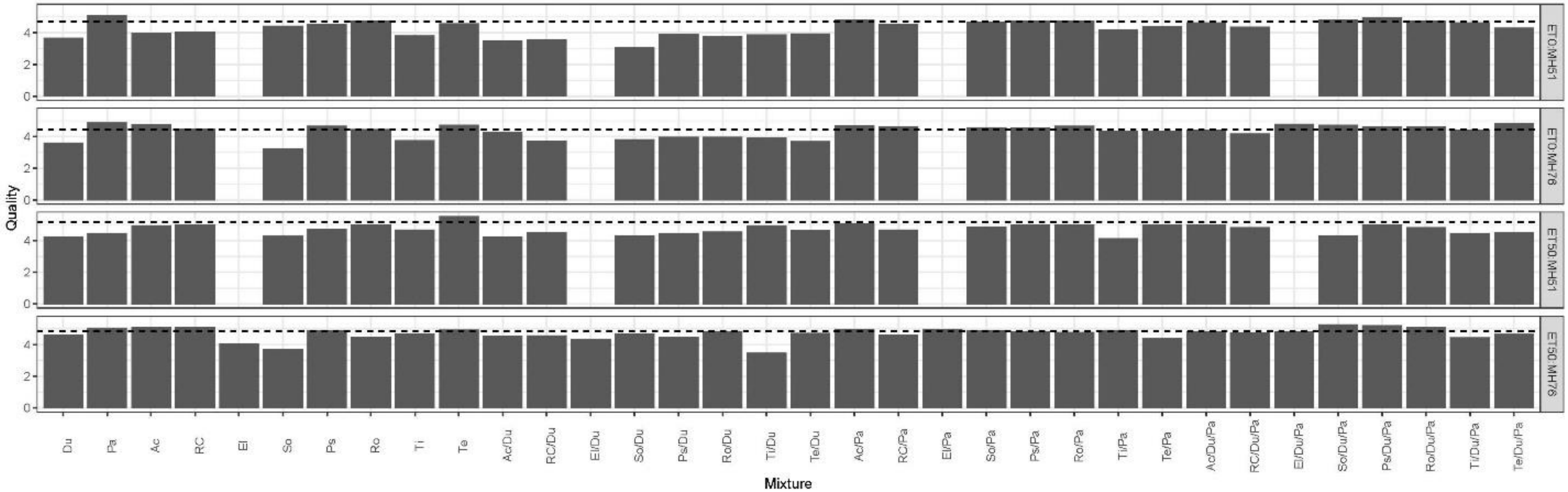


Figure 3. Turfgrass quality values of ten wheatgrass, Kentucky bluegrass, and hard fescue cultivars or experimental populations, eight Kentucky bluegrass-wheatgrass two-way mixtures, eight hard fescue-wheatgrass two-way mixtures, and eight Kentucky bluegrass-hard fescue-wheatgrass three-way mixtures evaluated during 2017 and 2018 at a Millville, UT field site under two irrigation levels (ET0 and ET50) and two mowing heights (MH51 and MH76). Values are summarized within each irrigation level and mowing height combination. The dashed lines correspond to the cutoffs for least significant difference between the numerically highest value and the remaining values. Cultivar and experimental population abbreviations are Du – 'Durar' hard fescue, Pa – 'Park' Kentucky bluegrass, Ac – experimental crested wheatgrass, RC – 'RoadCrest' crested wheatgrass, El – experimental thickspike wheatgrass, So – 'Sodar' thickspike wheatgrass, Ps – experimental western wheatgrass, Ro – 'Rosana' western wheatgrass, Ti – experimental intermediate wheatgrass, and Te – 'Tegmar' intermediate wheatgrass.

USGA®

2. INTEGRATED TURFGRASS MANAGEMENT

Turfgrasses developed for use on golf courses require management practices that provide quality playing surfaces while conserving natural resources and protecting the environment. Projects focus on reducing the use of water, pesticides, fertilizers, and energy. The objectives of these studies include:

- Developing cultural practices that allow efficient turfgrass management under unique conditions such as drought and deficit irrigation, irrigation with marginal quality water, poor quality soils, and shade
- Determining the range of adaptability and stress tolerances of turfgrasses
- Evaluating direct and interacting effects of two or three cultural practices such as irrigation, mowing, fertilization, cultivation, compost utilization
- Investigating pest management practices such as biological, cultural, and mechanical controls; application of turf management practices utilizing IPM and reduced inputs; and pest modeling and forecasting

USGA ID#: 2017-09-619

Title: Smart Tools to Improve and Accelerate the Turfgrass Evaluation Process

Project Leaders: Ning Wang[1], Yanqi Wu[1], Justin Moss[1], Charles Fontanier[1], Jack Fry[2], and Dale Bremer[2]

Affiliation: [1]Oklahoma State University; [2]Kansas State University

Objectives:

The ultimate goal of the proposed project is to develop a rapid, quantitative, multi-trait turfgrass quality rating platform to improve the efficiency of turfgrass management in golf courses, accelerate the selection process and improve the selection accuracy of turfgrass breeding.

Start Date: 2017
Project Duration: 3 Years
Total Funding: $89,305

Summary Points:

1. The developed ground-based turfgrass evaluation system was refined and optimized to accommodate field environment conditions. A few new hardware and software modules were added and tested.
2. A drone-based turfgrass evaluation system with an RGB and a thermal camera was also tested primarily. A new drone platform has been developed from fall 2019.
3. Turfgrass quality indices were modified based on the collected sensor data, data processing and interpretation results, and manual ground-truth measurements.
4. Field tests were conducted using the developed systems in a turfgrass nursery (40 rows x 20 columns) at the OSU Agronomy Farm.

Summary Text:

Rational

Bermudagrass (*Cynodon* spp.) is the most commonly used turfgrass for golf courses, lawns, parks, and sports fields in the southern USA and throughout tropical and warmer temperate regions in the world. At Oklahoma State University, the turf bermudagrass breeders have been conducting intensive research and field trials to develop new varieties with greater cold tolerance, enhanced turf quality, improved drought tolerance, increased host plant disease resistance, reduced requirements for mowing and fertilization, better shade tolerance, and faster divot recovery rate. However, quality evaluation of turfgrass has been one of the major and tedious work inputs in golf course management. Visual quality evaluation of turfgrass plots are widely used by turfgrass breeders and researchers, which is slow, subjective and laborious. The collected data are highly variable and difficult to repeat. *This research will develop a field evaluation tool with a goal of accelerating field data collection and data processing and analysis*.

Methodology and Project Progress

Figure 1 shows a project workflow map (presented in the 2017 and 2018 Annual Report) with percentages to show the up-to-date progress status.

Ground-based Platform

1. *Optimize the field data acquisition system and interface*

In the updated data acquisition system, we integrated an RTK GPS, an MS Kinect sensor, an ultrasonic sensor, and a GoPro camera into a complete system, simplified the user interface, and added a sensor calibration option (Figure 2).

As the data processing algorithms mainly depend on the location-stamped data. Hence, the accuracy of the GPS data is critical. The RTK GPS system used in this system is a newly commercialized, low-cost RTK GPS. We conducted a series field tests to establish a protocol to set up the GPS system and read the cm-accuracy location stamps. A GPS-to-local coordination system conversion software module was developed, tested, and finalized.

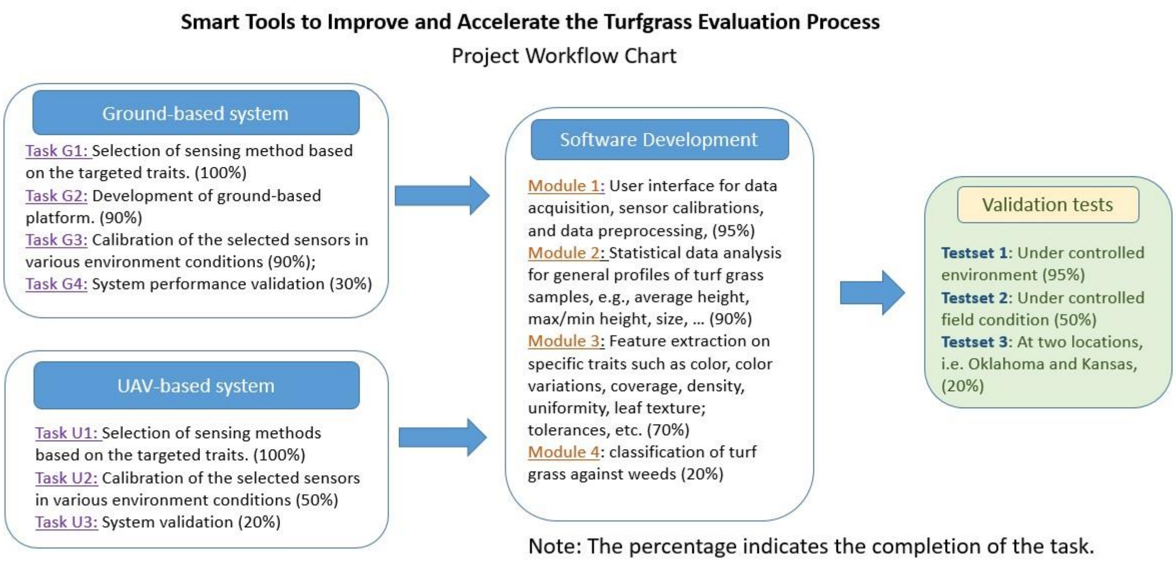


Figure 1. Updated project workflow map.

To improve the system "on-the-go" performance, the data acquisition system and preprocessing algorithms need to be run rapidly. The 2017-2018 version software limited the golf cart speed no more than 3 mph. In the updates software, we re-organized the software modules for the data acquisition and improve the data processing modules to accommodate variations of the driving speed of the golf cart.

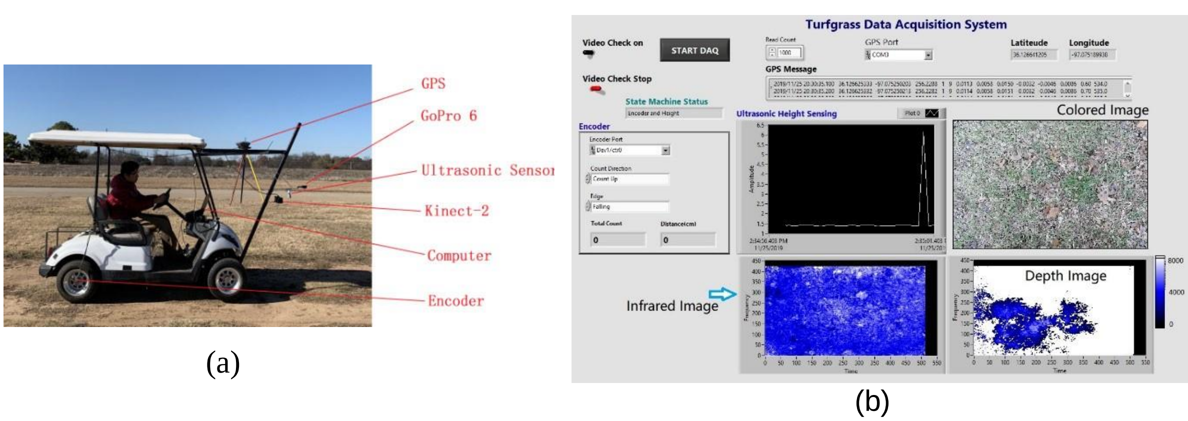


Figure 2. Golf cart with sensors: (a) System setup, (b) Front panel for system operations.

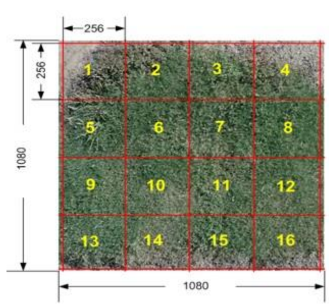
2. *Sensor data preprocessing software and algorithms*

We added a module to automate image preprocessing, i.e. collection, stitching, noise reduction, and quality improvements (Figure 3). In previous software, these were mainly done by manually extracting or selecting 'good' images from collected sample images and videos. This module not only speeded up the data preprocessing, but also provided consistent quality of the images for the following feature extraction and image analysis.

3. *Interpretation of the features extracted from collected images related to turfgrass rating*

In 2017-2018, we developed an algorithm to analyze the collected turfgrass images, extract features, and calculate turfgrass quality indices related to turfgrass quality rating based on NTEP evaluation criteria. At the beginning of this year, we presented the definition of every index in the developed algorithm to Dr. Yanqi Wu, verified the correctness of interpretation, and revised the software and algorithms according to his comments. Two color indices (average greenness and DGCI (the Dark Green Color Index)) and six texture indices (Figure 4 and 5) are used in our current algorithm.

Figure 3. Image preprocessing module for combining multiple images collected during the movement of the golf cart to form a sample image for further image processing and analysis.



6 texture characteristics:

- **Average Intensity**
- **Average Contrast**
- **Smoothness**
- **Third Moment**
- **Uniformity**
- **Entropy**

Block Index	Average Intensity	Average Contrast	Smooth-ness	Third Moment	Uniformity	Entropy
1	111.1683	35.2397	0.0187	0.0418	0.0081	7.1753
2	95.9156	34.4374	0.0179	-0.0907	0.0082	7.1211
3	97.0916	34.6498	0.0181	-0.1199	0.0081	7.1284
4	104.4021	36.7097	0.0203	-0.0893	0.0075	7.2153
5	102.3299	32.6406	0.0161	0.0195	0.0087	7.0683
6	103.9733	33.7211	0.0172	-0.0619	0.0083	7.0978
7	105.7711	31.5504	0.0151	-0.0511	0.0088	7.0002
8	110.0410	32.8456	0.0163	-0.0852	0.0085	7.0579
9	95.1776	33.1547	0.0166	0.1443	0.0088	7.0721
10	86.6907	33.4220	0.0169	0.0288	0.0085	7.0773
11	92.5176	30.6489	0.0142	-0.0261	0.0093	6.9496
12	103.0003	31.8535	0.0154	-0.0799	0.0088	7.0135
13	89.0741	36.2362	0.0198	0.4083	0.0086	7.1590
14	75.4252	33.1633	0.0166	-0.0221	0.0086	7.0406
15	87.7439	34.9712	0.0185	0.0813	0.0084	7.1285
16	90.9143	32.9366	0.0164	-0.0024	0.0085	7.0673
Max	111.1683	36.7097	0.0203	0.4083	0.0093	7.2153
Min	75.4252	30.6489	0.0142	-0.1199	0.0075	6.9496
Avg	96.9523	33.6363	0.0171	0.0060	0.0085	7.0858
STDDEV	9.6520	1.6592	0.0017	0.1287	0.0004	0.0683

Figure 4. Texture indices for turfgrass evaluation.

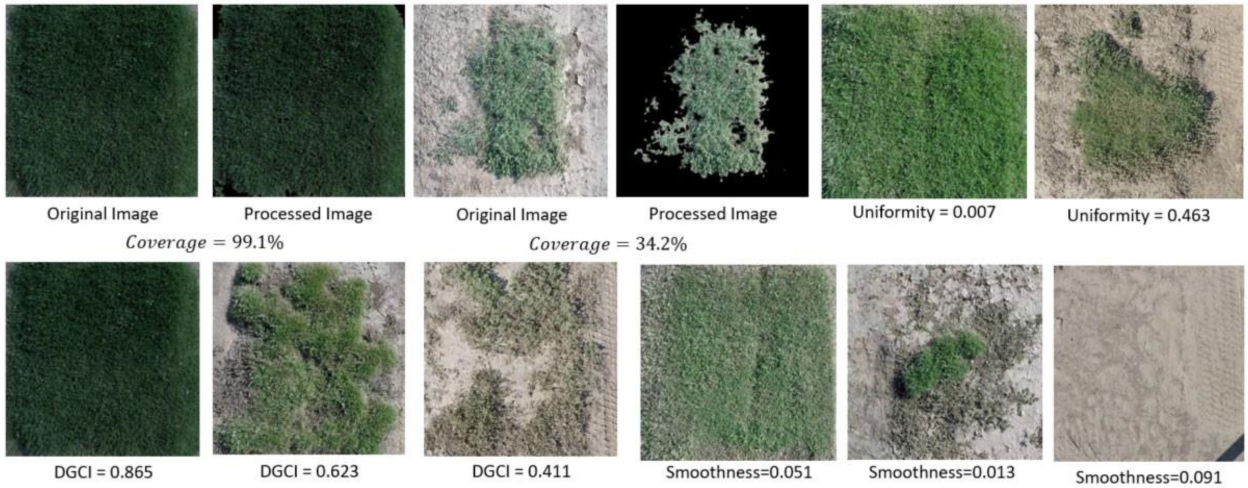


Figure 5. Examples of calculated color and texture indices.

<u>UAV-based Platform</u>

We continued collecting RGB color images of the testing field with a DJI P3 drone at the OSU experimental farm and develop a program to process the UAV images. We also designed mounting mechanisms for a FLIR Duo thermal/RGB camera, a gimbal and a remote controller for the FLIR camera (Figure 6). However, the DJI P3 could not provide consistent flight performance due to the added payloads. It crashed during an experiment in summer 2019 and destroyed the gimbal. In fall 2019, we started to design and fabricate a drone, which could hold much higher payload, better flight performance, and additional functions on path planning and control.

(a) The off-the-shelf DJI P3 drone with an RGB camera

(b) The drone with four extended legs

(c) The drone with added gimbal, FLIR Duo, and remote controller

Figure 6. The updated DJI drone platform.

Future Expectations

The major tasks for the coming year will be: 1) finalizing the hardware and software systems for data acquisition and processing; 2) finalizing the design and fabrication of new drone system; and 3) conducting field experiment from May 2010 in Oklahoma and Kansas. We expect to deliver the hardware and software systems by the end of 2020.

USGA ID#: 2018-14-664

Title: On-Site Golf Course Evaluation of New Turfgrasses for Putting Greens

Project Leader: Brian Schwartz
Affiliation: The University of Georgia

Objectives:

1. Evaluation of advanced experimental turfgrasses for putting greens under realistic management intensity and performance expectations.
2. Initiation of a USGA sponsored graduate student worker position in the UGA Turfgrass Breeding Program at Tifton, GA.

Start Date: 2018
Project Duration: 3 years (2018 – 2020)
Total Funding: $19,000 to date

Summary Text:

During 2012 we began testing new hybrid bermudagrasses and zoysiagrasses as a way for me to develop relationships with golf course superintendents who had collaborated with the Tifton program in the past. Since then, we have had research trials at the Atlanta Athletic Club, Atlanta Country Club, Big Canoe Golf Course, Country Club of Columbus, East Lake Golf Club, Ford Plantation, Landings Club, Sea Island Golf Club, Streamsong Golf Resort, TPC Sawgrass, University of Georgia Golf Course, and Valdosta Country Club. In addition to the 7 ongoing putting green trials planted during 2018 or before, we established new tests at East Lake Golf Club, Meadows Country Club, and Olde Florida Golf Club during 2019.

1) **Country Club of Columbus**

William Smith renovated the old research green prior to planting on July 15th, 2015. All three bermudagrasses (TifEagle, 12-TG-101, and 12-TG-143) established very quickly. A picture of the green during August 2019 is below, as well as a summary of the 14 stimp measurements taken to-date.

C.C. of Columbus (14 Stimp Measurements)			
(2015 – 2019)		Fastest	Overall Avg.
Bermuda	12-TG-101	**12.1’**	**9.9’**
	TifEagle	**12.0’**	**9.7’**
	12-TG-143	**11.7’**	**9.3’**
	TifEagle Green	**12.0’**	**10.0’**

2) Landings Club

Chris Steigelman renovated a portion of the old practice green prior to planting on July 21st, 2015. All bermudagrasses (12-TG-39, 12-TG-101, and 12-TG-143) established very quickly. A picture of the green during the fall of 2019 is below, as well as a summary of the 10 stimp measurements taken to-date.

The Landings Club (10 Stimp Measurements)			
(2015 – 2019)		Fastest	Overall Avg.
Bermuda	TifEagle	**11.4'**	**9.7'**
	12-TG-39	**11.4'**	**9.6'**
	12-TG-101	**12.2'**	**9.5'**
	12-TG-143	**10.5'**	**9.3'**
	TifGrand	**12.5'**	**8.6'**
Paspalum	SeaStar	**11.5'**	**8.7'**

3) Valdosta Country Club

Barry Bennett renovated the old practice green prior to planting on May 25th, 2016. Tom Howard and Randall Bice have been managing the green for the last two years. All five bermudagrasses (Tifdwarf, TifEagle, 12-TG-39, 12-TG-101, and 12-TG-143) established fairly quickly. A picture of the green during September of 2019 is below, as well as a summary of the 12 stimp measurements taken to-date.

Valdosta C.C. (12 Stimp Measurements)			
(2016 – 2019)		Fastest	Overall Avg.
Bermuda	12-TG-39	**10.7'**	**9.3'**
	12-TG-101	**10.4'**	**9.2'**
	12-TG-143	**10.8'**	**9.1'**
	TifEagle	**11.5'**	**9.0'**
	Tifdwarf	**10.4'**	**8.6'**

4) Atlanta Country Club

Mark Esoda constructed a new research green adjacent to his bentgrass research green prior to planting on June 16th, 2016. Scott Lambert has been managing the green for the last two years. All bermudagrasses (TifEagle, 12-TG-101, and 12-TG-143) established quickly, but six large trees surrounding the green were removed September 6th, 2016 because the green was only getting 2 hours of sunlight. A picture of the green during May of 2019 is below, as well as a summary of the 12 stimp measurements taken to-date.

Atlanta Country Club (12 Stimp Measurements)			
(2016 – 2019)		Fastest	Overall Avg.
Bermuda	12-TG-101	**11.4’**	**8.4’**
	12-TG-143	**10.9’**	**8.0’**
	TifEagle	**10.4’**	**7.9’**
Bent	A-1	**10.5’**	**9.5’**

5) Big Canoe Golf Course

Lydell Mack converted a bentgrass nursery green to a research plot during 2017. This test site is divided in two equal areas, one treated as a “no-till” soil profile and the other “cored-out” and refilled with a new green’s mix. Two bermudagrasses (TifEagle and 12-TG-101) and two zoysiagrasses (Diamond and Primo) were planted in long strip-plots that span across both soil profiles on May 25th, 2017. Pictures of the green during 2019 are below, as well as a summary of the 5 stimp measurements taken to-date. The most important information to be gleaned from this trial will be the long-term survival potential of each genotype over several winters when covered, and where left unprotected during the winters.

Big Canoe Golf Course (5 Stimp Measurements)			
(2017 – 2019)		Fastest	Overall Avg.
New Rootzone	12-TG-101	**11.3’**	**9.1’**
	TifEagle	**11.0’**	**8.8’**
	Primo	**10.6’**	**8.8’**
	Diamond	**9.5’**	**8.2’**
No-Till	12-TG-101	**10.4’**	**8.7’**
	TifEagle	**10.7’**	**8.7’**
	Primo	**9.7’**	**7.8’**
	Diamond	**8.9’**	**7.7’**

6) TPC Sawgrass

Jeff Plotts constructed a large research site during the summer of 2017. Our experimental bermudagrass (12-TG-101) is being compared to four bermudagrass (TifEagle, Sunday, Imperial, and G12) and three zoysiagrass (Primo, Prizm, and DALZ1308) cultivars. A picture of the plots during May 2019 is below, as well as a summary of the 2 stimp measurements taken to- date.

TPC Sawgrass (2 Stimp Measurements)			
(2017 – 2019)		Fastest	Overall Avg.
Bermuda	G12	**8.8'**	**8.8'**
	12-TG-101	**8.8'**	**8.6'**
	Imperial	**9.5'**	**8.6'**
	TifEagle	**8.7'**	**8.6'**
	Sunday	**8.4'**	**8.0'**
Zoysia	Prizm	**8.6'**	**8.1'**
	Primo	**8.2'**	**8.1'**
	DALZ1308	**8.3'**	**7.8'**

7) Streamsong Golf Resort

Rusty Mercer constructed a new research site during 2018 to compare MiniVerde, TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. The goals of this research site are to test adaptation to long-season growing environments and very intense topdressing and growth regulator management. A picture of 12-TG-101 during June of 2019 is below, as well as the first stimp measurements taken.

Streamsong (1 Stimp Measurement)			
(2018 – 2019)		Fastest	Overall Avg.
Bermuda	TifEagle	**10.2'**	-
	Mach 1	**10.1'**	-
	12-TG-101	**10.1'**	-
	MiniVerde	**9.0'**	-
	MiniVerde Green	**12.1'**	-

8) East Lake Golf Club

Ralph Kepple constructed a new research site to compare MiniVerde, TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. Sprigs were planted during May of 2019 and established very quickly. A goal of this research site was to test grow-in time with Lexicon Intrinsic fungicide applications. Four stimp measurements and pictures of the green during planting, 21, and 31 days later are below.

East Lake Golf Club (4 Stimp Measurements)			
(2019 – 2019)		Fastest	Overall Avg.
Bermuda	MiniVerde	**11.5’**	**10.5’**
	TifEagle	**12.0’**	**10.3’**
	12-TG-101	**12.0’**	**10.3’**
	Mach 1	**11.4’**	**10.2’**

9) Meadows Country Club

Pat Franklin constructed a new research site during 2019 to compare TifEagle and the UGA experimental variety 12-TG-101. Sprigs were planted during August of 2019 and have been establishing very quickly. The goals of this research site are to test adaptation to warm, long-season environments and a grow-in protocol that included Lexicon Intrinsic fungicide. Pictures of the green during planting and five weeks later are below.

10) Olde Florida Golf Club

Darren Davis renovated his research site during 2019 to compare TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. He will also test TifGrand and the UGA experimental variety 11-T-56. Sprigs were planted during September of 2019 and have been establishing very quickly. The goals of this research site are to test adaptation to the long-season growing environments, winter-time play and recovery, and very intense topdressing and growth regulator management. Pictures of the green during planting and four weeks later are below.

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11) <u>USGA Sponsored Students</u>

Mr. Jonathon Fox successfully defended his M.S. thesis “Methods for Analyzing Shade Tolerance in Warm Season Turfgrasses” in December of 2018 and was hired in a full time position with the UGA Tifton turfgrass breeding program this fall to concentrate on developing grasses for golf course use. Mr. Matthew Mathis is currently pursuing a B.S. in Environmental Horticulture on the Turfgrass & Golf Course Management track. Matthew has taken ownership of several on-campus putting green trials and has been instrumental in propagating plant materials for the USGA sponsored research trials, as well as for those planted at the UGA Tifton Campus and at Pike Creek Turf.




USGA ID#: 2017-04-614

Project Title: Bermudagrass rough conversion to no-mow, low-input grass area

Project Leader: Maggie Reiter
Affiliation: University of California Cooperative Extension

Objectives:

1. Evaluate the performance of low-input, alternatives grasses as an out-of-play area on a Central California golf course
2. Compare establishment rates of those alternative species
3. Test methods for bermudagrass termination
4. Develop best management practices for subsequent weed control

Start Date: 2017
Duration: 3 years
Total Funding: $50,000

Summary Points:

- Survival varied across species (Figure 2)
- California brome and spike bentgrass maintained acceptable cover without irrigation after an establishment period
- Organic herbicides were characterized by an immediate burndown and ultimately complete recovery after one application (Figure 4)
- Conventional herbicides like glyphosate and fluazifop were more effective at controlling bermudagrass

Summary Text:

To conserve natural resources, increase economic savings, and comply with legislative restrictions, golf course managers have to maintain their landscapes at healthy conditions with lower inputs of water, fertilizer, pesticides, and energy. A worthwhile option to reduce inputs is using native or naturalized grass species that perform well under reduced inputs. Among golf course turfgrass areas, the rough is the largest component of maintained turfgrass and the most reasonable area to integrate lower-input grasses on a large scope with effective outcomes. Currently, bermudagrass (*Cynodon dactylon*) is the most dominant species on golf course roughs throughout California and the Southwest United States. Golf course managers are looking for ways to convert bermudagrass areas, especially in rough areas that are seldom in play.

The goal of this project is to determine what alternative grass species will perform well and remain playable as an unmowed golf course rough, and to develop best management practices to terminate bermudagrass and establish a functional, low-input stand of vegetation.

Objectives 1 and 2
One of 3 initial field trials continued data collection in 2019: a cool-season variety trial at a UC Research and Extension Center in Parlier (Fresno), CA (Figure 1a). Ten cool-season grass species were established in fall 2017, and the majority of entries were California native grasses

(Table 1). Plots were arranged in a randomized complete block with 4 replications. This field trial was not irrigated after April 2018, and plots received season rainfall from October 2018 to April 2019. Additionally, no weed control was provided after spring 2018.

Data were collected to measure persistence with no irrigation inputs after a 6-month establishment period. Normalized Difference Vegetation Index (NDVI) was collected with a Trimble GreenSeeker handheld crop sensor, desirable plant cover was measured with a visual rating, and weed cover was measured with a visual rating. Data were collected in spring 2019, after seasonal rainfall concluded.

To demonstrate treatment effects and statistical significance, confidence intervals (95%) were constructed for the sample means in R.

NDVI measurements could not differentiate between weed species and desirable grasses, so visual ratings were a more useful measurement of vegetation. California brome (*Bromus carinatus*) plots had over 95% cover, spike bentgrass (*Agrostis exarata*) had over 50% cover, and dune bentgrass (*A. pallens*) and purple needlegrass (*Stipa pulchra*) plots had approximately 20% cover. Remaining species had less than 5% cover (Figure 2). Weed pressure was greater than 70% cover for all species except spike bentgrass (*A. exarata*) and California brome (*B. carinatus*) (Figure 3). Figure 1b shows the trial in spring 2019 after it degraded to weeds. California brome (*B. carinatus*), spike bentgrass (*A. exarata*), and dune bentgrass (*A. pallens*) persisted with reseeding and recruitment of new individuals. Purple needlegrass (*S. pulchra*) persisted with survival of tillers.

Objective 3
A healthy sward of 'TifSport' hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) was subjected to different termination methods in summer 2019. The trial area was managed as a golf course rough, and contained about 5% swinecress (*Coronopus* spp.) cover. Treatments for bermudagrass removal included conventional herbicides and non-selective organic herbicides (Table 2).

Herbicide treatments were applied on July 31. Plots were arranged in a randomized complete block with 4 replications. Herbicides were applied with a CO_2 backpack sprayer delivering 935 L/ha water. Applications were made around 12:00 PM with 0% cloud cover and air temperatures approximately 32C. Bermudagrass injury was measured with normalized difference vegetation index (NDVI). Data were collected before initial applications and every 1 to 7 days after application. Statistical analysis was conducted in R, using Dunnett's test to compare differences from the untreated control at each rating date.

The organic herbicides Avenger, Finalsan, Suppress, and WeedPharm showed significant injury 2 days after application, compared to untreated control plots. Suppress and Finalsan plots completely recovered by 19 days after initial applications, while Avenger and WeedPharm recovered by 28 days after application. Throughout the entire trail, Burnout induced no injury compared to controls (Figure 4). Glyphosate-containing treatments Ranger PRO and Ranger PRO + Fusilade II took longer to separate from the untreated control, and reached significant injury 5 days after the initial application. The glyphosate-containing treatments maintained significant injury throughout the duration of data collection, up to 42 days after initial application (Figure 4).

Objective 4

Weed management in these systems is long-term and complex. A publication of guidelines for weed management in naturalized areas of golf course roughs will be developed. The publication will include integrated concepts and will be framed in an Adaptive Management approach.

Table 1. Cool-season grass species seeded fall 2017 in Fresno.

Spike bentgrass	*Agrostis exarata**
Dune bentgrass	*Agrostis pallens* 'Camp Pendleton'*
California brome	*Bromus carinatus**
Tufted hairgrass	*Deschampsia cespitosa**
Sheep fescue	*Festuca ovina* 'Bighorn'
Hard fescue	*Festuca brevipila* 'Predator'
Molate fescue	*Festuca rubra* 'Molate'*
Chewings fescue	*Festuca rubra* ssp. *commutata* 'Heathland'
Prairie junegrass	*Koeleria macrantha**
Purple needlegrass	*Stipa pulchra**

*California native

Table 2. Herbicides trade names, active ingredients, and application rates for the bermudagrass termination experiment.

Trade name	Active ingredient	Application rate
Avenger	d-limonene	25% v/v solution
Burnout	Citric acid + clove oil	25% v/v solution
Finalsan	Ammoniated soap of fatty acids	16.7% v/v solution
Suppress[1]	Caprylic acid + capric acid	9% v/v solution
WeedPharm	Acetic acid	100% solution (no dilution)
Ranger PRO[2]	Glyphosate	5 qt/acre (1.25% v/v solution)
Ranger PRO + Fusilade II[2]	Glyphosate + fluazifop	5 qt/acre + 24 fl oz/acre

[1]An acidifier (BioLink) was added at a rate of 1% v/v solution

[2]A nonionic surfactant (Prefer 90) was added at a rate of 0.25% v/v solution

Figure 1a. Plots of alternative and native cool-season grass species during spring 2018 in Fresno.

Figure 1b. Plots of alternative and native cool-season grass species during spring 2019 in Fresno. The field has degraded to annual weeds and little desirable grass cover.

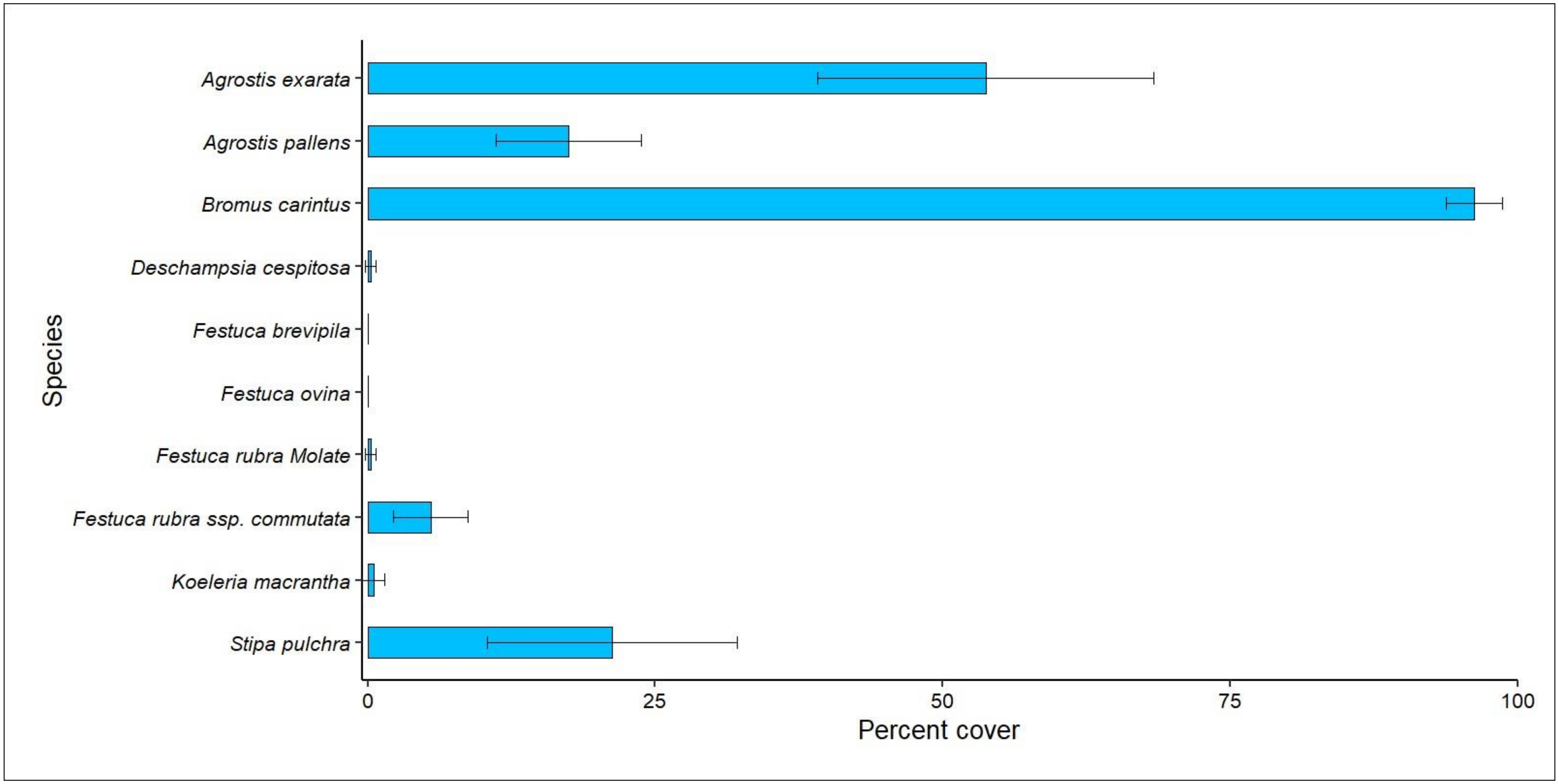

Figure 2. Percent desirable grass cover in spring 2019 in Fresno. Bars represent means, and error bars represent 95% confidence intervals.

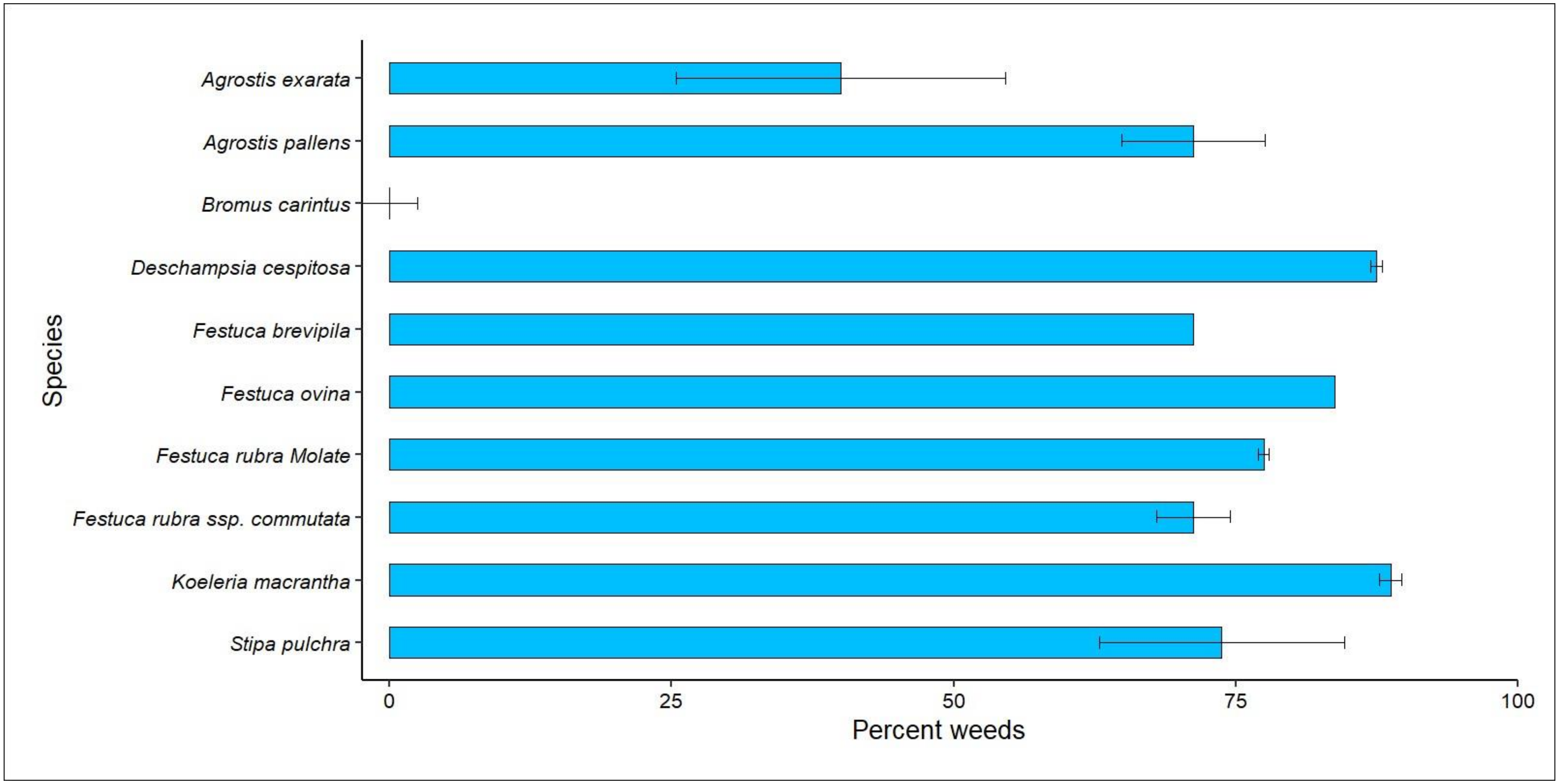

Figure 3. Percent weed cover in spring 2019 in Fresno. Bars represent means, and error bars represent 95% confidence intervals.

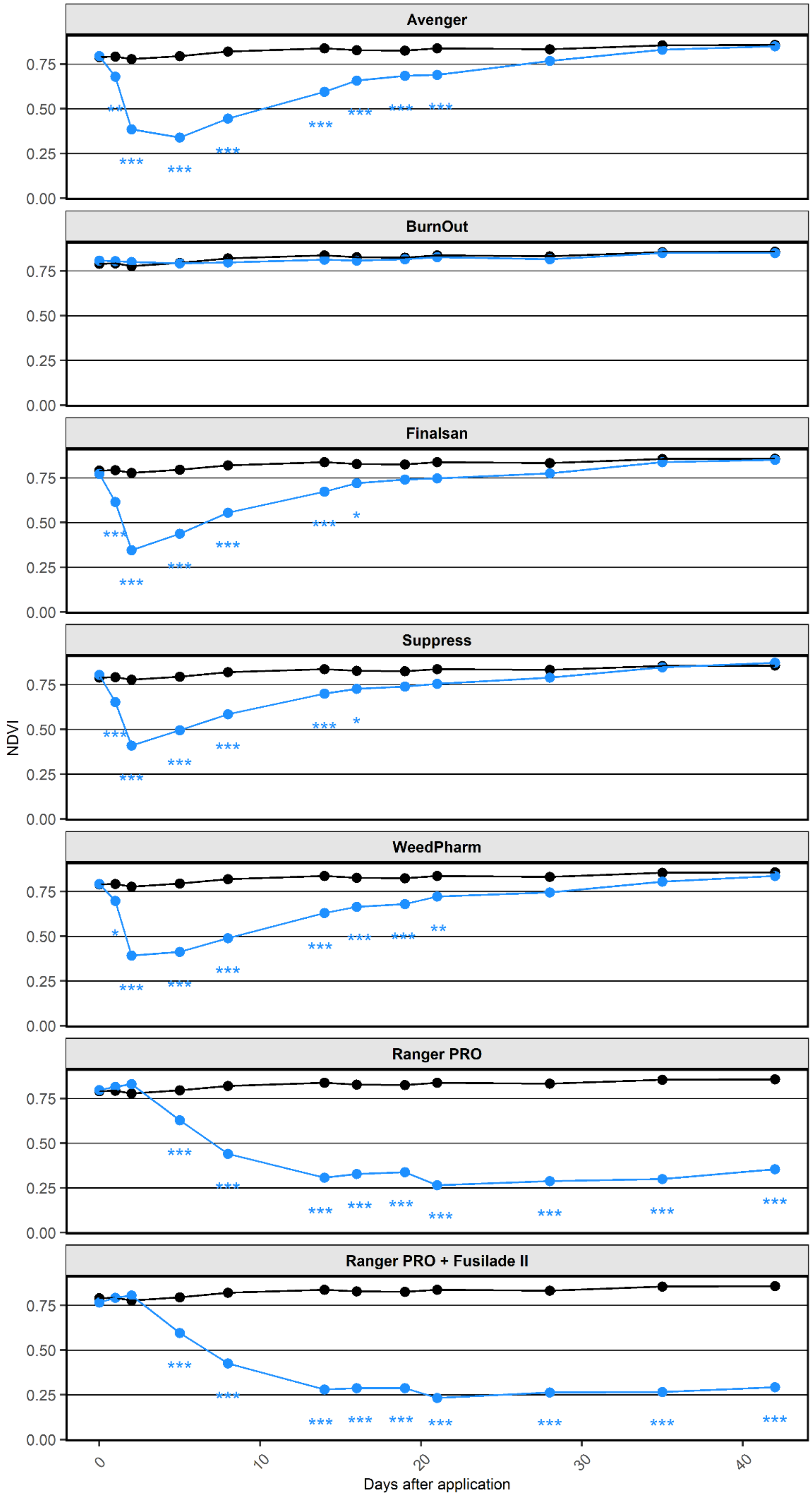

Figure 4. NDVI for herbicide treatments (blue) compared to untreated control (black) ***p<0.001, **p<0.01,*p<0.05 according to Dunnett's test.

USGA ID#: 2019-05-675

Title: Native Grasses and Alternative Groundcovers for the Southwest

Project Leaders: Kai Umeda and Worku Burayu
Affilation: University of Arizona

Objectives:

1. Evaluate and compare the adaptation and performance of nativegrasses and alternative groundcovers in the low desert southwest United States as a low input turfgrass replacement in non-play areas of golf courses.
2. Generate local research-based information about the feasibility of growing new groundcovers and the nativegrasses by assessing their interactions with insect pests and weeds, water use and fertility requirements.
3. Increase the awareness of stakeholders about the characteristics of nativegrasses and alternative groundcovers for low water use requirements and potential water saving capacity.

Start Date: 2019
Project Duration: 3 years
Total Funding: $45,000

Summary Points:

- Seeded nativegrasses emerged and established at varying rates.
- Nativegrasses exhibited characteristically differential height and growth habits.
- Both white- and pink- flowered cultivars of Kurapia established well when planted in the late spring.
- The white-flowered Kurapia spread more rapidly across the surface area and was shorter statured compared to the pink-flowered cultivar.

Summary Text:

In the low desert southwest United States, water quantity and quality issues have led to water use regulations and greater conservation efforts. There is a demand for appropriate alternative low water use plant materials for landscaping residential and commercial properties in the desert region. Seeking and investigating alternative plant materials to replace turf from non-play areas on golf courses is gaining more interest. The University of Arizona Cooperative Extension Turfgrass Science program in Maricopa County started evaluating the adaptation and performance of nativegrasses and alternative groundcovers and in the first three years of research identified prospective nativegrasses and a promising new groundcover for the irrigated low desert Arizona conditions. To develop recommendations for best management practices, we are continuing the existing research and expanding investigations of additional nativegrasses and two cultivars of the groundcover, Kurapia (Table 1). The focus of the current projects is to evaluate and determine the performance of nativegrasses and Kurapia at low irrigation levels. Two field experiments, one for nativegrasses and one for Kurapia were established at the Wigwam Golf Club in Litchfield Park, AZ. In the first experiment, nativegrass species were seeded into 8 ft by 8 ft plots arranged in a randomized complete block design (RCBD) with 3 replicates. The second experiment compares two cultivars of a promising new groundcover, Kurapia (pink- and white-flowered) with four replications in a RCBD. The irrigation requirements of the two cultivars will be evaluated and determined under varying regimes of 80, 50 and 20% ETo under drip irrigation.

This first year report of a three year investigation emphasizes the evaluation of establishing different species of nativegrasses with respect to their characteristics for height, greenness, surface ground cover, and growth uniformity. The overall visual quality rating was evaluated for greenness and ground cover following the procedures developed by National Turfgrass Evaluation Program, 1998d as (1 brown or yellow, 5 light green, and 9 dark green). Visual ground cover ratings from 1 to 9 were measured for plots with almost no vegetative cover (1) and complete coverage of the area with no visible soil (9). Data were analyzed using JMP ver. 14.3 statistical software and means compared using Student's t-test. The project's ultimate goal is to provide golf course superintendents and other professionals of the Arizona green industry specific recommendations for the best management practices of selected plant materials.

Results

In general, the seeded nativegrasses germinated, emerged, and established well over time (Figure 1). Nativegrasses exhibited variable performance and significant differences for growth in height (Figure 2), for surface area coverage (Figure 3), and greenness (Figure 4). *Hilaria jamesii*, *Bouteloua dactyloides*, *Bouteloua gracilis*, *Sporoborus aeroides*, *Bouteloua curtipendula,* and *Sporobolus cryptandrus* exhibited the best surface area coverage. *Sporoborus aeroides* was the tallest followed by *Bouteloua curtipendula*, and *Bouteloua gracilis*. The shortest statured grasses were *Bouteloua dactyloides*, and the bluestems at under 12 inches in height.

Both white- and pink-flowered cultivars of Kurapia established very well. The white-flowered cultivar spread more rapidly (Figure 5) across the surface area. It was also shorter in height compared to the pink-flowered cultivar.

Table 1. Nativegrasses and groundcover evaluated in the low desert Arizona (2019).

Common Name	**Scientific Name**
Alkali sacaton	*Sporobolus airoides*
Blue grama	*Bouteloua gracilis*
Bluestem, Little "Cimarron"	*Schizachyritm scoparium*
Bluestem, Sand "Chet"	*Andropogon halii*
Buffalograss	*Bouteloua dactyloides*
Galleta, "Viva"	*Hilaria jamesii*
Grama, Sideoats "Vaughn"	*Bouteloua eurtipendula*
Lovegrass Sand, "Bend"	*Eragrostis trichodes*
Purple threeawn	*Aristida purpurea*
Sand dropseed	*Sporobolus cryptandrus*
Kurapia	*Lippia nodiflora*

Figure 1. Establishment of nativegrasses, 8 weeks after planting (planted June 25, 2019) at Litchfield Park, AZ.

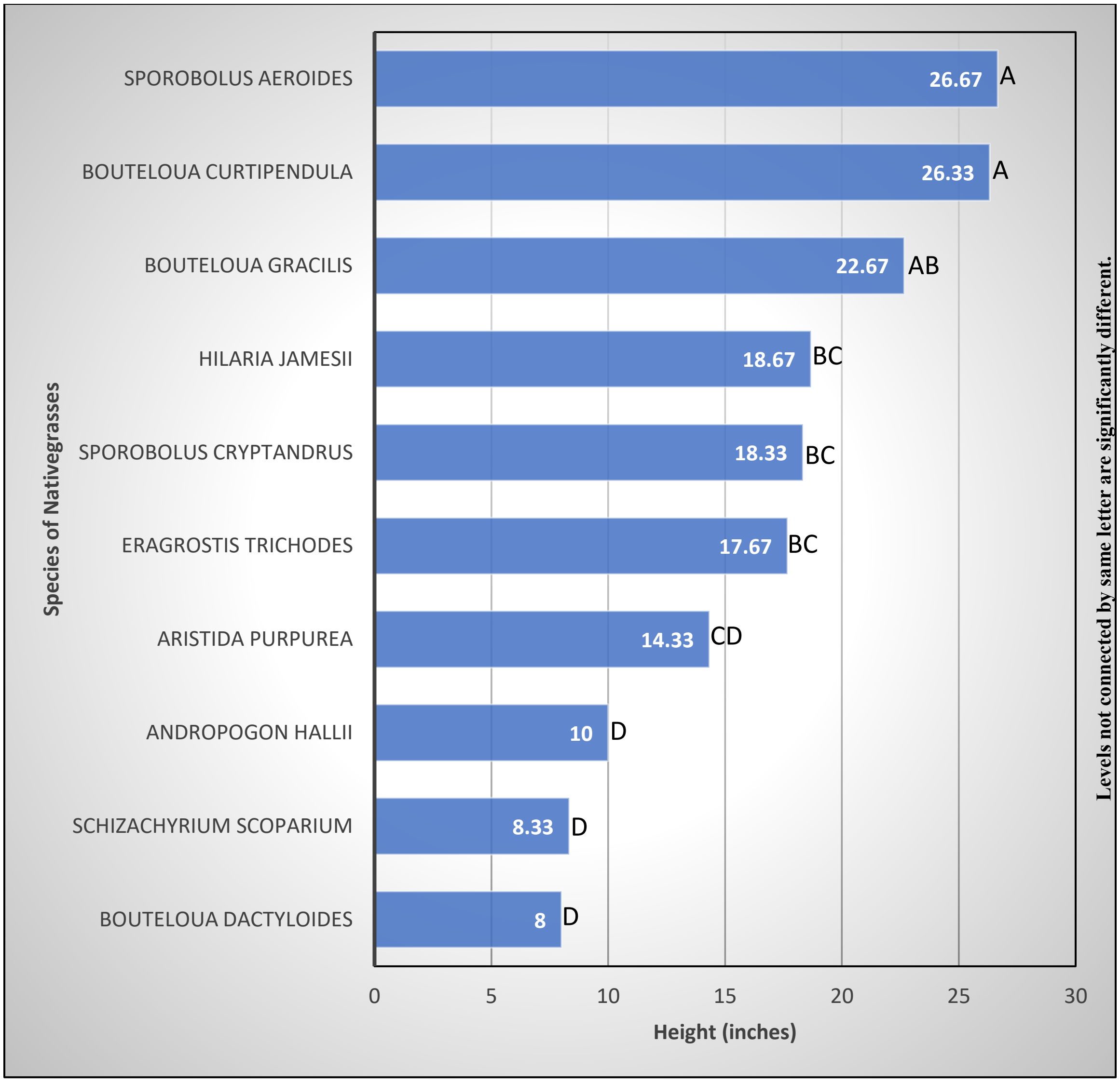

Figure 2. The height of nativegrasses at 12 weeks after planting at Litchfield Park, AZ in 2019.

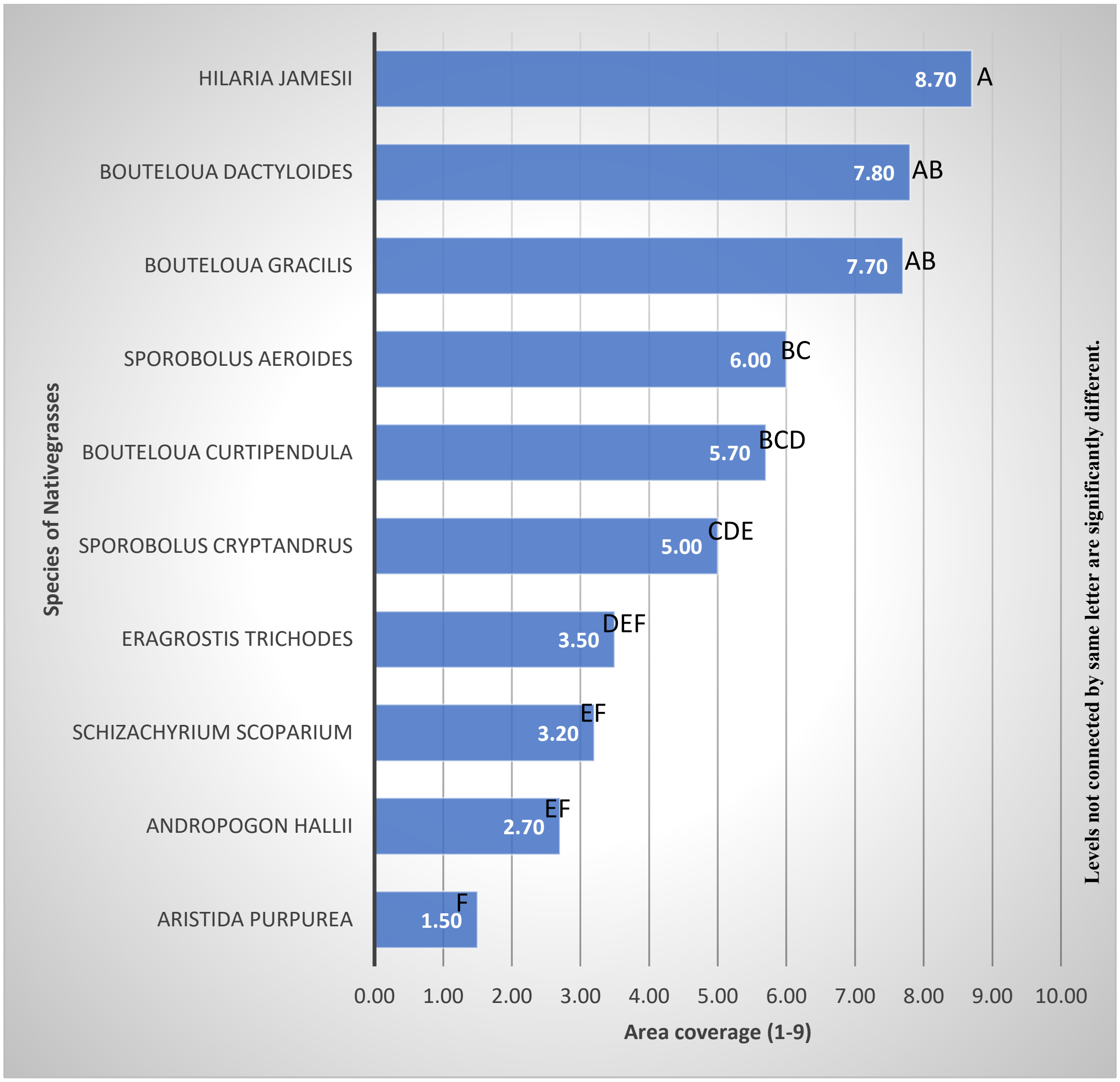

Figure 3. Surface area coverage by nativegrasses at 12 weeks after planting at Litchfield Park, AZ in 2019. (1 = bare ground visible, 9 = no bare gound visible)

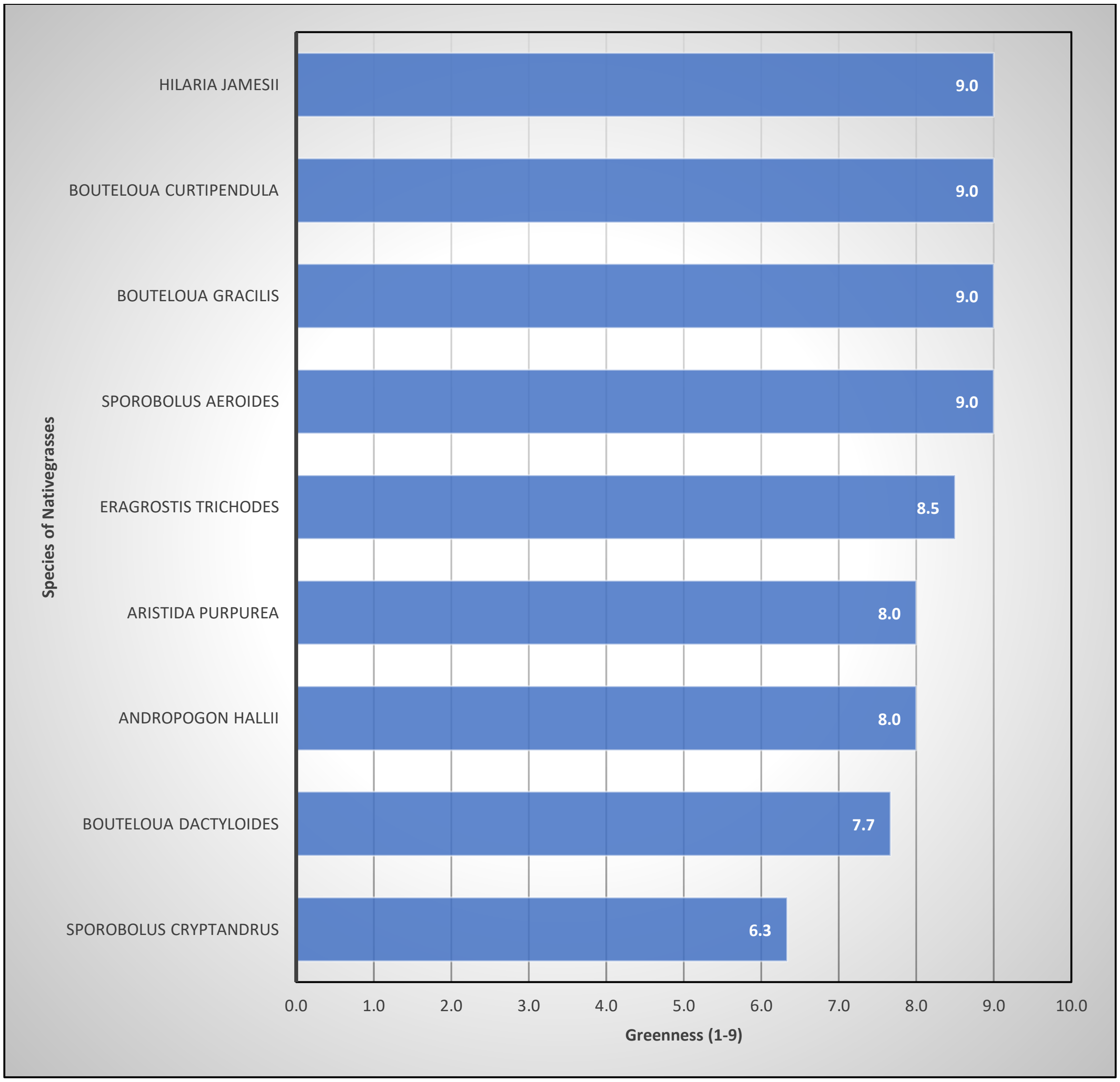

Figure 4. The greenness of nativegrasses at 12 weeks after planting at Litchfield Park, AZ in 2019. (1 = not green, 9 = dark green)

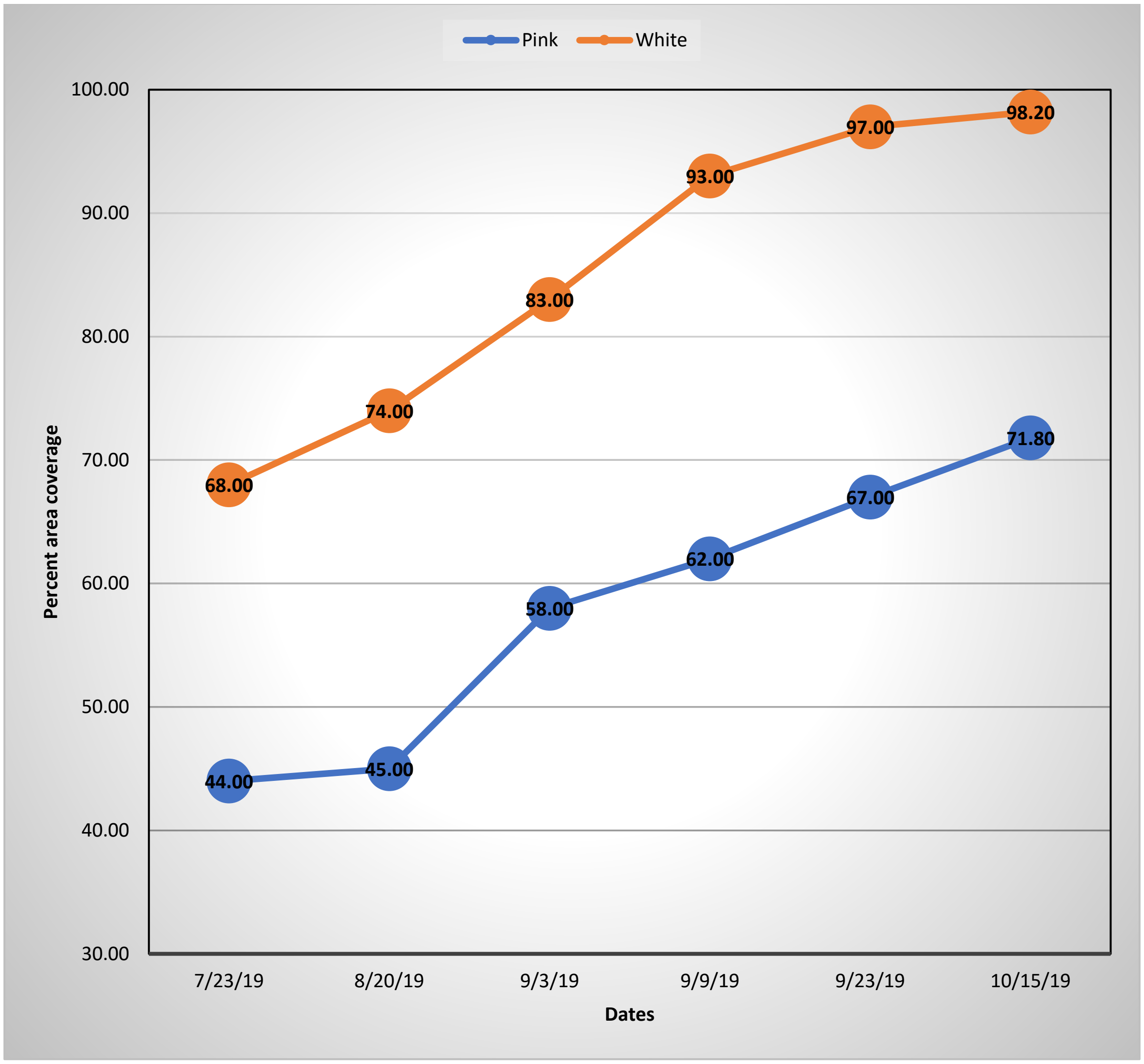

Figure 5. The performance of white and pink cultivars of Kurapia in percent area coverage in Litchfield Park, AZ in 2019 (Planted 5/13/2019).

USGA ID#: 2018-15-665

Title: Evaluation of Warm-Season Species, Blends and Mixtures to Reduce Golf Course Rough Inputs

Project Leader: Kevin Morris
Affiliation: National Turfgrass Evaluation Program (NTEP)

Objectives:

This project evaluates warm-season grasses, blends and mixtures that reduce inputs and maintenance costs of golf course roughs.

Start Date: 2018
Project Duration: Three years
Total Funding: $45,000

Summary Points:

- A national evaluation of warm-season grasses to reduce golf course rough inputs was established at eleven locations in the southeastern, southern and western U.S.
- Ten entries, consisting of bermudagrass, buffalograss, zoysiagrass and a mixture of buffalograss, curly mesquite and blue grama were mailed to trial locations and planted in summer 2018.
- Full establishment of all entries lingered in 2019 at some locations, with significant difference among species and entries.
- The trial is now maintained at a mowing height of two inches or greater, with minimal fertilization, irrigation and weed control to simulate a low input golf course rough.
- Data on establishment rate, turfgrass quality, winter survival, spring greenup, percent living cover, freedom from weeds, and rate of top growth has been collected, and will be analyzed and presented in spring 2020.

Summary Text:

Due to droughts in California, Oklahoma, Texas, the southeast U.S. and other locations, the golf course industry needs grasses that perform well with little, if any, supplemental irrigation. In addition, fertilizer and pesticide restrictions in various states or localities require golf courses to use less of these inputs. Finally, as a result of the recent recession and subsequent economic pressures, golf courses are investigating new cost saving strategies.

To address these issues within golf, and the turf industry in general, NTEP initiated a national low input trial in 2015, evaluating cool-season (C3) species, blends and mixtures. This trial of 32 entries, including several C3 grass species and even mixtures of various clover types, is planted at seventeen locations in mid and northern-tier U.S. states. With very minimal inputs of fertilizer, water and pesticides, and reduced mowing requirements, this trial has yielded very interesting results in its first two years.

Several interesting new native warm-season (C4) species, some resulting from USGA funding, are currently under development. Additionally, improvements in buffalograss, bermudagrass, zoysiagrass and other more traditional turf species may show that significant reductions in water, fertilizer, pesticides and mowing are possible. Therefore, we feel the time is now to evaluate C4 species, blends and potentially, even mixtures of species (and legumes) for their ability to reduce input in golf course roughs.

Information from this project will be valuable to the golfing industry because it will determine the adaptation of C4 grasses for golf course use. Information obtained from these evaluations will be of interest to plant breeders, researchers, extension educators, USGA agronomists, golf course architects, and superintendents who need to select the best adapted species, cultivars, blends and/or mixtures to reduce maintenance and inputs.

Location and Number of Trial Sites

The evaluation trials are jointly sponsored by the United States Golf Association (USGA) Green Section and the National Turfgrass Evaluation Program (NTEP). An advisory committee consisting of turfgrass researchers, breeders and NTEP personnel developed trial protocols, evaluation parameters and selected trial locations.

Trial sites are located at land grant university research sites, or in close proximity of a land grant university with a research component. Eleven (11) evaluation trial sites throughout the southern and western U.S. were selected, in accordance with the number of expected entries.

Trial Specifics and Protocols

NTEP is the coordinating agent for this five-year cultivar trial. Daily maintenance is conducted by the host universities. Trials are maintained according to the following procedures developed by the advisory committee and approved by the NTEP Policy Committee (to conform with management used in roughs):

Management protocol during establishment

- Standard irrigation and fertility to enhance establishment
- Weed control as needed, including pre-emergent applications

Management protocol after establishment period

- Mowing height of 2” or higher
- Mowing frequency: once per week during growing season
- Nitrogen rate: 0 – 2 lbs./1000 sq. ft/year
- Irrigation: 50% ETo or lower (depends on location) or irrigation only during severe drought tress
- Pest control: minimal weed control to avoid significant stand loss

Data Collection and Publication

The research cooperator is responsible for data collection. The following is representative of the data to be collected annually:

1. Percent establishment every 14 days until plots are fully established
2. Percent living ground cover of planted species in spring to assess winter survival

3. Spring greenup ratings in years two through five
4. Turfgrass quality ratings each month throughout the growing season
5. Percent living ground cover of planted species monthly throughout each growing season
6. Percent grassy and broadleaf weed encroachment two times per year (excluding planted species)
7. Canopy height measurements monthly just prior to mowing (average of three locations in each plot)

NTEP will request annual data by December 15th of each year, organize, review and statistically analyze submitted data, and will publish on the NTEP web site (www.ntep.org) in spring or summer of the following year.

Progress to Date

Ten (10) entries were received by NTEP in late May/early June 2018. The entries consist of eight vegetatively-established and two seed-established entries. Species included in the trial include multiple entries of bermudagrass, zoysiagrass, buffalograss, as well as one mixture entry consisting of buffalograss, curly mesquite and blue grama. Entries were shipped to cooperators in mid-June 2018 and subsequently planted at the eleven locations.

Data on establishment rate has been collected from the initial planting date into 2019 until all entries are fully established. Data on turfgrass quality, percent living ground cover and other measurements were also collected in 2019 with initial results showing significant differences among entries in establishment rate, color and density. The entirety of 2018-2019 data will be analyzed and published by NTEP in early 2020. We anticipate that over the five-year trial period, this trial will provide much meaningful data on grasses for reduced input roughs.

Figure 1. Establishment of trials in College Station, TX during July 2018.

USGA ID#: 2017-06-616

Project Title: Establishment and Maintenance Practices for No-Mow Fine Fescue Golf Course Roughs

Project Leaders: Eric Watkins, Sam Bauer, Andrew Hollman
Affiliation: University of Minnesota

Objective:

Determine optimum seeding rates and biomass removal strategies for no-mow fine fescue.

Start Date: 2017
Duration: 3 years
Total Funding: $44,272

Summary Points:

- No-mow fine fescues can serve as a low-input vegetation option in golf course roughs.
- Initial seeding rate influenced seed head density, grassy weed coverage, and biomass.
- Mowing treatment influenced broadleaf weed coverage, bare soil/dead vegetation coverage, and golf ball visibility.
- The results from this project will help to clarify fine fescue rough establishment and management strategies.

Summary Text:

With continued local restrictions and social pressures of input use on turf areas, golf course superintendents are increasingly using fine fescues in out-of-play rough areas. The use of unmown fine fescues can result in decreased maintenance costs while increasing aesthetic value due to seed head production during late spring and early summer. Since these stands are managed differently than in-play areas, superintendents may be faced with management of different weed species and different amounts of competition. The density and height of the stands may alter golf ball visibility and reduce the pace of play. Research that guides superintendents of proper establishment and management of unmown fine fescues is lacking. In this project, we are investigating seeding rates and mowing regimes for optimal weed suppression, golf ball visibility, and aesthetics in now-mow fine fescues.

Establishment of the project began in July of 2017 at the University of Minnesota Turfgrass Research Outreach and Education Center in St. Paul and at Rush Creek Golf Club in Maple Grove, MN. Each location was seeded with ‘Beacon’ hard fescue in a 3 x 4 factorial design with four replications. The two factors included seeding rate based on pure live seed (PLS) (3 levels: 1, 2, and 3 PLS cm^{-2}) and mowing regime (4 levels: spring mowing; fall mowing; spring and fall mowing, and no mowing). The height of cut for each mowing event was 10.2 cm and clippings were removed. All plots were mowed in the fall of 2017 to provide uniform stands prior to 2018 data collection.

The following data were collected in 2018-2019 in St. Paul, and 2019 in Maple Grove: living fine fescue coverage and weed pressure (grid counts); seed head density (culms in a 0.09 m^2 subsample per plot); overall quality; total biomass at each mowing (dry biomass weights of

0.09 m^2 subsample per plot); maturity (days after April 1 until seed head is fully emerged); lodging (visual assessment as needed); and golf ball lie (golf ball visibility image analysis).

Seed heads and uniform fine fescue stands of high turfgrass quality provide aesthetic value to low maintenance areas on golf courses. Full seed head emergence occurred by 1366 growing degree days in 2018 and 1094 growing degree days in 2019 at St. Paul. Density of the culms were influenced by the seeding rate in 2018 at St. Paul, and 2019 at Maple Grove. Culm density increased as seeding rate decreased. Additionally, culm density in 2019 at St. Paul was only 8-11% of what it was during the first reproductive year in 2018 (Fig. 1). Each location differed in broadleaf weed coverage as influenced by mowing treatment. In 2019 at St. Paul, broadleaf weed coverage was highest with fall only and spring + fall mowing treatments (Fig 2), while Maple Grove plots had more broadleaf weeds in spring mowing treatments than in fall mowing treatments. This is likely a function of what weeds were present: broadleaf weeds prominent at St. Paul included white clover and black medic, whereas Maple Grove had many thistles and milkweed. The highest percentage of grassy weeds in St. Paul was with the 1 PLS cm^{-2} treatment in the spring 2018; however, this decreased to less than 1% of the total plot coverage when averaged across mowing treatments after summer 2018. Grassy weeds were more problematic at Maple Grove: the 1 PLS cm^{-2} treatments had significantly more grassy weeds in the summer and fall of 2019 than the other seeding rates. Bare soil and dead matter were significantly influenced by mowing treatment in 2019 at St. Paul. The no mowing treatment did not recover from winter as well as the other mowing treatments which contributed to an estimated 10% dead vegetation coverage in those plots.

Unmown fine fescue stands are commonly mowed in the fall, and biomass removal can be laborious. In general, all seeding rate treatments decreased in biomass from fall 2018 to fall 2019, with the exception of the 3 PLS cm^{-2} treatment, which remained the same. Seeding rate did not influence biomass at Maple Grove. Collected biomass included both weed and fine fescue species, which likely reduced our ability to detect seeding rate influences on this variable.

Playability in unmown fine fescues is difficult as it serves as a penalty for unfortunate golf shots. To quantify this playability characteristic, we tossed a red golf ball at waist height into the plot, and then took a digital image of the golf ball from a vantage point directly over the plot. This allows for quantifying the pixels of the golf ball that are visible. Fewer red pixels would indicate a ball that is more difficult to locate and more difficult to play. Image analysis has been performed on images taken from St. Paul in fall 2019. Results indicate the no mowing treatment is less visible than the fall mowing and spring and fall mowing treatments. The difference between the two sets of treatments is about 3% of the total image (including surrounding vegetation), which may not be of practical importance.

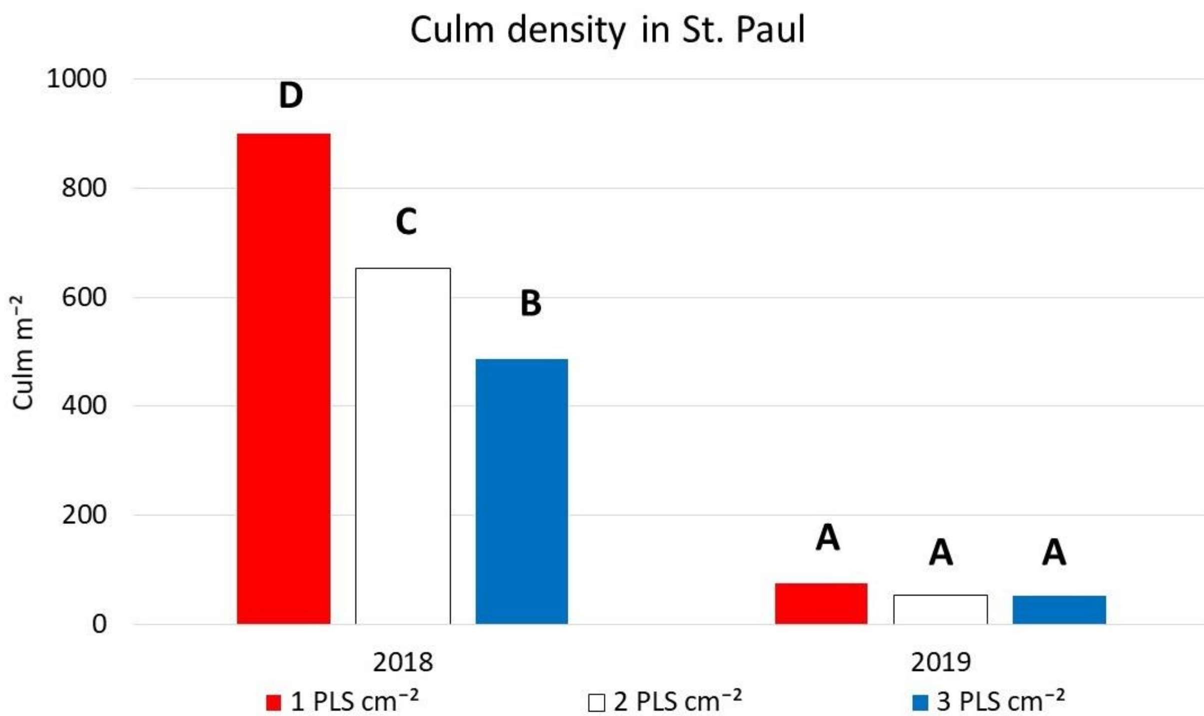

Figure 1. Estimated mean culm density averaged across mowing treatment at St. Paul each year by seeding rate. Mean separation was determined using Tukey's method at α = 0.05. Bars with the same letter are not significantly different.

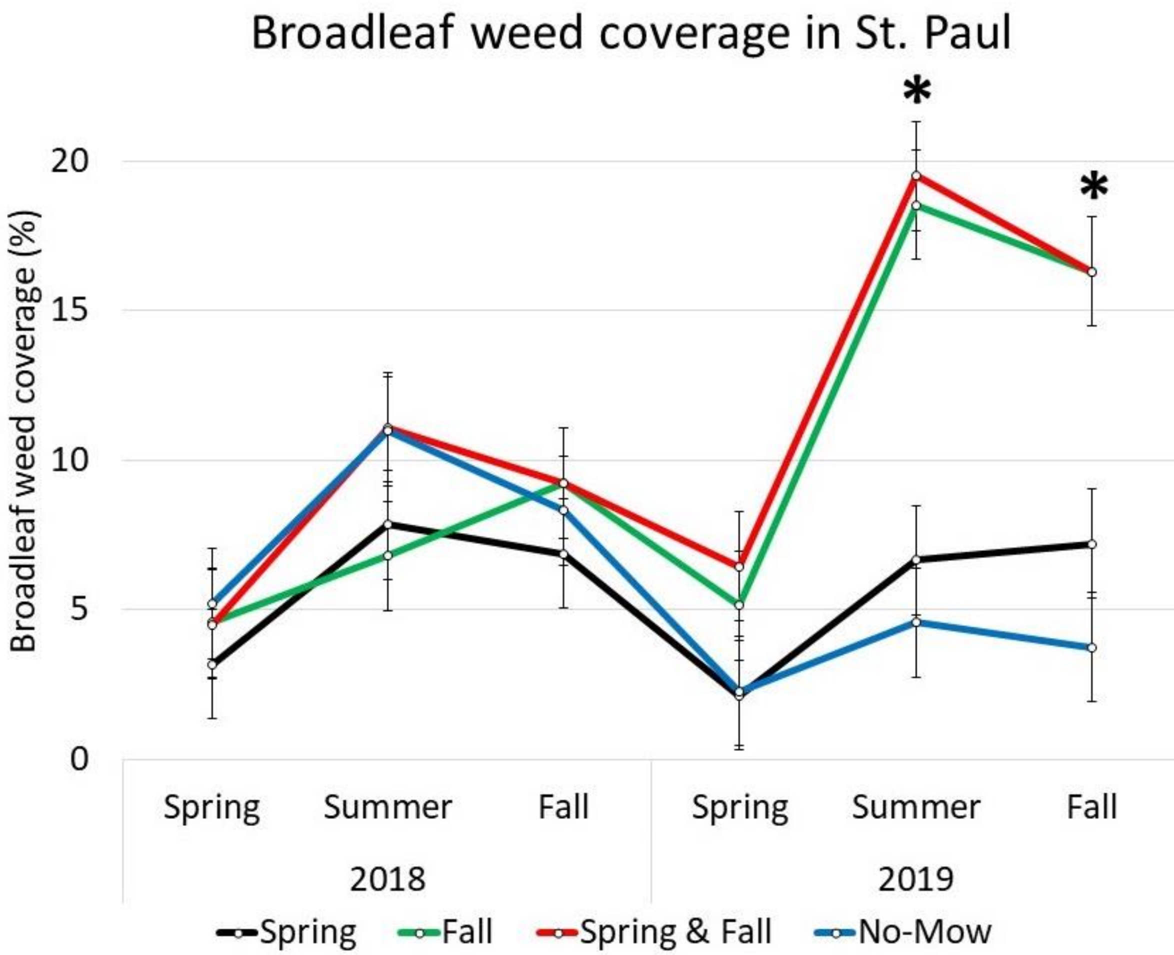

Figure 2. Estimated mean coverage of broadleaf weeds averaged across seeding rate at St. Paul each year by mowing treatment. Error bars are standard error = 1.4%. Dates with significant differences were determined using Tukey's method at α = 0.05, and are indicated by (*).

Figure 3. The original (A) and analyzed image (B) of a golf ball in a 2 PLS cm^{-2}, fall mowing treated plot in fall 2019 at St. Paul.

USGA ID#: 2017-13-623

Title: Golfer Perception of Input-Limited Fairway Management in the Northcentral U.S.

Project Leaders: Bill Kreuser, Michael Carlson, Keenan Amundsen
Affiliation: University of Nebraska-Lincoln

Objectives:

1. Document annual inputs for buffalograss, Kentucky bluegrass and creeping bentgrass fairways under traditional and input-limited management in the northcentral U.S.
2. Determine the fairway species preference, and expected quality level, for golf course superintendent and professional and amateur golfers when inputs are known and unknown.
3. Link golfer quality expectations to annual management inputs.
4. Determine the combined effects of irrigation regiment and nitrogen fertility on pest incidence, and corresponding total pesticide use in fairways of an improved buffalograss cultivar.

Start Date: 2017
Project Duration: 3 years
Total Funding: $69,020

Summary Points:

- Quality ratings were lower this year than prior years in the Kentucky bluegrass and buffalograss treatments because of the lack of control of invasive grass weeds in both the pest control and no pest control treatments.
- The species, pest control and fertilizer treatments had a larger impact on differences in visual quality than the irrigation treatments in 2019.
- Species was the biggest factor in differences of the NDRE values in 2019, while the interaction of species x irrigation x fertilizer was the highest order interaction. Pest control did not impact the NDRE values in 2019.
- The threshold fertilizer treatments had the highest NDRE values within buffalograss treatments, whereas threshold fertilizer treatments were lower than the standard and higher than the non-fertilized treatments in both cool-season grasses.
- Golfer perception data was collected this summer from a survey and data is currently being analyzed.

Summary Text:

Rational:
It's commonly assumed that buffalograss (*Buchloe dactyloides*) fairways require fewer management inputs in the northcentral U.S. compared to more commonly used species such as

Kentucky bluegrass (KBG; *Poa pratensis*) or creeping bentgrass (CBG; *Agrostis stolonifera*). However, negative opinions of buffalograss are common among golfers and superintendents, despite improved color and density characteristics of recently-released cultivars. Golfers may be more likely to accept buffalograss if savings compared to other species are quantified.

Methodology:

We established 'Prestige" buffalograss, 'Barvette' KBG, and 'Pure Select' CBG in three, 20 ft x 30 ft plots with three replications for a total of nine plots each during 2016 in Lincoln, NE. These plots are arranged in a randomized complete block design and serve as the whole plot treatment factor for the experiment. Sub-plots are arranged in a 2 irrigation x 3 fertilizer x 2 pest control factorial treatment structure. Irrigation levels are 1) no supplemental irrigation or 2) standard references evapotranspiration (ET_0) replacement (i.e. 80% ET_0 for CBG and BG or 60% ET_0 for buffalograss). Fertilizer levels are 1) unfertilized, 2) "standard" fertilizer (1 lb N/1000 ft^2 in May, Sept., Oct. and Nov. for CBG and KBG, 1 lb N/1000 ft^2 in June and July for buffalograss), or 3) a threshold program where 0.25 lbs N/1000 ft^2 is applied when NDRE values of the plots are beneath a threshold value. Pest control levels are 1) untreated or 2) "standard" strategies to control weeds, disease and insects. Experimental management for these plots began in May of 2019. The entire area was mowed when needed over the duration of the season, generally three times per week. Soil moisture was measured using a Spectrum Technologies Field Scout TDR 300 with 3 in probes in plots that received supplemental irrigation. Plots that received pest control were checked for broadleaf weed pressure and dollar spot pressure bi-weekly throughout the season. The entire plot was applied with a broadleaf herbicide when these weeds were spotted. Dollar spot was treated when the Smith-Kerns model indicated high pressure for the disease, this model was found on GreenKeeper and checked before application of the fungicide. Turfgrass visual quality and normalized difference red edge (NDRE) were rated weekly. Turfgrass visual quality was rated on a 1- to -9 scale where 1 represented exposed soil, 9 was perfect turf and 6 was minimally acceptable. NDRE was measured using a Holland Scientific RapidScan handheld crop sensor (active light source) over the length of the plot.

Preliminary Results:

Quality:

The combined effects of species x fertilizer x irrigation and species x irrigation x pest control produced the highest-order interaction affecting the quality of the plots. The species receiving pest control had the highest quality throughout the study except for the non-irrigate creeping bentgrass that still had relatively poor quality from the mismanagement of the turf two years prior. The KBG that received standard or threshold fertilizer that was either irrigated or not had the highest mean quality, whereas the creeping bentgrass treatments had the lowest on average throughout 2019. When quality measurements were averaged from 23 May to 6 Sept. 2019 the quality remained below acceptable for all treatment combinations in both the species x fertilizer x irrigation and species x irrigation x pest control interactions. Many of the no pest control plots had large influxes of grass weeds that were no controlled for in the winter and with the broadleaf pest control applications during the growing season, that reduced the quality for these plots.

NDRE:

The pest management effect was not significant, so the combined effect of species x irrigation x fertilizer produced the highest-order interaction affect the NDRE values of the plots. The biggest difference in NDRE values was driven by the species, and then fertilizer treatments. The highest NDRE values recorded from 23 May to 6 Sept. 2019 were the Kentucky bluegrass and creeping bentgrass with standard fertilizer treatments regardless of irrigation and averaged 0.3146. The threshold fertilizer treatments from the Kentucky bluegrass and creeping bentgrass had lower NDRE values than the standard fertilizer, whereas it was higher than the non-fertilizer treatments. The buffalograss treatments had the lowest NDRE values, with the threshold fertilizer treatments at the top of the buffalograss treatments.

Golfer Perception:

These data were collected in the form of a survey this past summer from a group of superintendents at the UNL Turfgrass Field Day, and from Professional Golf Management students, who golf regularly. The survey respondents were asked to rate the visual quality and playability as either acceptable or unacceptable as their expectations as a golfer with no prior knowledge of maintenance costs, and then again after learning the relative maintenance costs for each plot. These data are currently being analyzed.

Table 1. Average turfgrass visual quality ratings and NDRE values from 23 May to 6 September 2019. Letters denote significance at p = 0.05.

Species	Irrigation	Fertilizer	Quality	NDRE
Kentucky Bluegrass	Irrigated	Standard	5.36 ab	0.3146 ab
		Threshold	4.59 fgh	0.2933 c
		Non-fertilized	4.56 fgh	0.2833 cd
	Non-irrigated	Standard	5.48 a	0.3210 a
		Threshold	5.13 bc	0.2910 c
		Non-fertilized	4.58 fgh	0.2792 de
Creeping bentgrass	Irrigated	Standard	4.98 cde	0.3059 b
		Threshold	4.35 hi	0.2857 cd
		Non-fertilized	3.97 j	0.2793 de
	Non-irrigated	Standard	5.06 cd	0.3171 a
		Threshold	4.13 ij	0.2868 cd
		Non-fertilized	3.56 k	0.2669 f
Buffalograss	Irrigated	Standard	4.72 efg	0.2719 ef
		Threshold	4.52 gh	0.22617 fg
		Non-fertilized	4.48 gh	0.2347 i
	Non-irrigated	Standard	4.65 fgh	0.2543 gh
		Threshold	5.06 cd	0.2628 fg
		Non-fertilized	4.82 def	0.2511 h

USGA ID#: 2019-11-681

Project Title: Targeted assessment of bermudagrass growth in a shaded environment.

Principal Leaders: Charles Fontanier
Affiliation: Oklahoma State University

Objectives:

1) *Quantify the effect of simulated shade structure height, material, and density on the energy balance of a turfgrass surface.*
2) *Characterize the spectral properties of light transmitted through various shade fabrics and plastics.*
3) *Quantify bermudagrass growth and development under varying light quality.*

Start Date: 2019
Number of Years: 3
Total Funding: $112,233

Summary Points:

- Ten commercial lens filters have been tested for transmission of PPF for potential use in light quality study.
- A propagation technique has been selected for testing the effect of light quality on seedling growth and development.
- A graduate student has been hired to complete the remaining objectives in 2020-21.

Summary Text:

Background and Rationale
Management of shaded turfgrass systems can be complex. Understanding how turfgrasses respond to shaded environments is an ongoing research need for all turfgrass sites ranging from putting greens to home lawns. Commonly, neutral density shade fabric is used to screen for shade tolerance or provide a simulated shade treatment for management studies. Criticisms of these methods suggest they do not accurately simulate real-world shaded conditions that often reduce light quality (red:far red ratio), include tree root competition for water and nutrients, or otherwise influence the energy balance differently than a shade fabric might.

The currently accepted method for developing minimum light requirements for turfgrasses involves calculation of the daily light integral (DLI) for the accumulated photosynthetically active radiation (PAR). This is an improvement over historical recommendations based on 'hours of sunlight', but the results may be limited due to the variation in 'types of shade' that exist in the real world. Furthermore, the scientific community has not established a standard design for shade research which has resulted in variation in how investigators simulate shade.

Methods
The proposed research has three primary objectives: characterize the energy balance under real world and simulated shade, characterize the light spectrum under real world and simulated shade, and conduct a bioassay of the bermudagrass shade response to varying light quality.
To characterize the energy balance of simulated shade structures, a uniform block of bermudagrass has been reserved at the OSU Turfgrass Research Center. Shade structures will be built and tested using varying heights (e.g., 1, 2, 3, 10-ft tall), shade densities (e.g., 50%, 80%

shade), and shade materials (neutral or red selective). Sensors will be installed to measure net radiation, wind speed, ambient temperature, relative humidity, and surface temperature. Data will be collected under shaded and non-shaded conditions for each shade type to determine their effect on microclimatic conditions including long-wave radiation and evaporative demand. Similar measurements will be made under real-world shade conditions varying in tree species and shade severity.

To complete the second objective, a spectroradiometer (Flame S, Ocean Optics) will be used to measure light spectral properties under the real-world and simulated shade environments. Measurements will be taken up to five times per day to determine how sun angle influences the performance of various shade structure designs. Data will be analyzed to determine the red:far red ratio for each shade type and multivariate analyses will be used to identify which methods most accurately simulate real world shade.

Objective 3 of this project will test the effect of reduced red to far red light ratio on bermudagrass seedling growth. An 8-week study will be conducted at the OSU Horticulture Research Greenhouses. A seeded bermudagrass cultivar will be planted as a single seed within 4mm diameter cone-tainer filled with a soilless growing medium. Treatments will include shade provided by either 1) conventional neutral density shade fabric, 2) red-selective plastic sheets, 3) conventional neutral density shade fabric plus red-selective plastic sheets, and 4) ambient conditions in the greenhouse (non-shaded). Specific shade materials will be selected after analysis of field data from objectives 1 and 2. Plants will be maintained under non-mown conditions. At the conclusion of 8 weeks, measurements of vertical elongation, above ground and below-ground dry mass, specific leaf area, and number of tillers will be measured to determine if light quality is a critical factor when evaluating bermudagrass light requirements.

<u>Early Results</u>

Ten commercial lens filters from the Lee Filter company were tested for transmission of PPF under the ambient conditions within the OSU research greenhouses (Fig. 1). Results were very similar to the commercial specifications published for each filter suggesting future filter selection can be based largely on published transmission data. The R:FR ratio for the selected filters varied from near 0 for the Dark Blue to near 1 for the Pink (Fig. 2). Neutral colored filters such as the Gray and Black products reduced PPF uniformly but allowed increased Far Red light, thus performing differently than typical shade fabric which reduces both PPF and other wavelengths uniformly. Based on these data, the Light Blue 203 product appears most appropriate for the proposed study and will likely be used in conjunction with polywoven shade fabric to target specific light environments.

The light quality environments were proposed to be applied to seedling bermudagrasses. Preliminary testing with ‘Rio’ bermudagrass using a commercially available peat-based media resulted in consistent germination and establishment (Fig. 3). Concurrent tests using sand, fritted clay, and agarose gel did not result in consistent germination, likely due to moisture availability (sand and fritted clay) and disease pressure (agarose).

<u>Future Expectations</u>

Activities related to objective 1 will begin in Summer 2020 with measurement of wind, temperature, and light conditions under various man-made shade structures as well as various tree canopy types. Activities related to objective 3 will continue in Spring 2020 with anticipated completion by June.

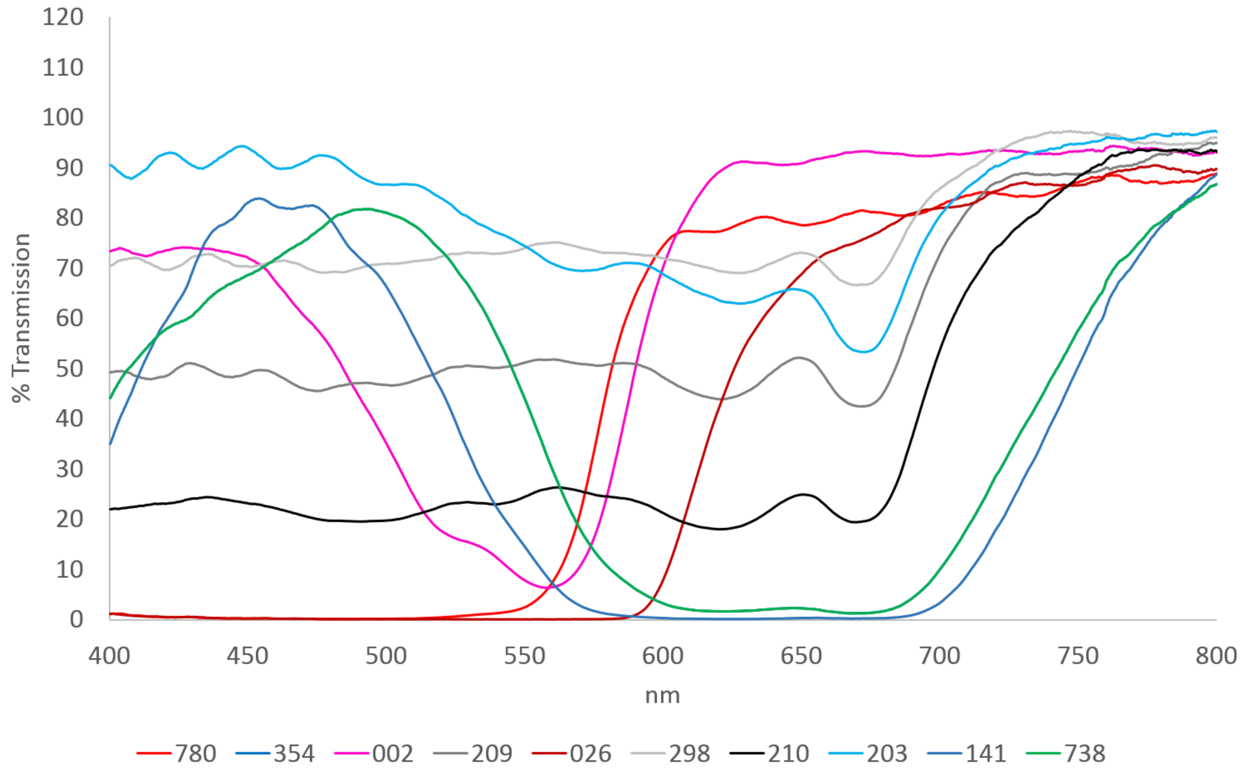

Figure 1. Relative transmission of PPF and Far Red light under ten commercially available lens filters.

	% Transmittance				
Filter #	Blue	Red	Far Red	R:FR	PPF
Red 780	0%	80%	85%	0.94	34%
Green 738	75%	3%	30%	0.10	37%
Blue 354	75%	3%	35%	0.09	37%
Pink 002	50%	90%	93%	0.97	59%
Gray 209	45%	50%	90%	0.56	49%
Dk Red 026	0%	80%	90%	0.89	22%
Lt Gray 298	70%	65%	95%	0.68	72%
Black 210	20%	23%	85%	0.27	24%
Lt Blue 203	90%	60%	85%	0.71	77%
Dk Blue 141	80%	0.20%	20%	0.01	30%

Figure 2. Relative transmission of blue, red, far red, and PPF light and corresponding R:FR ratio for ten commercially available lens filters.

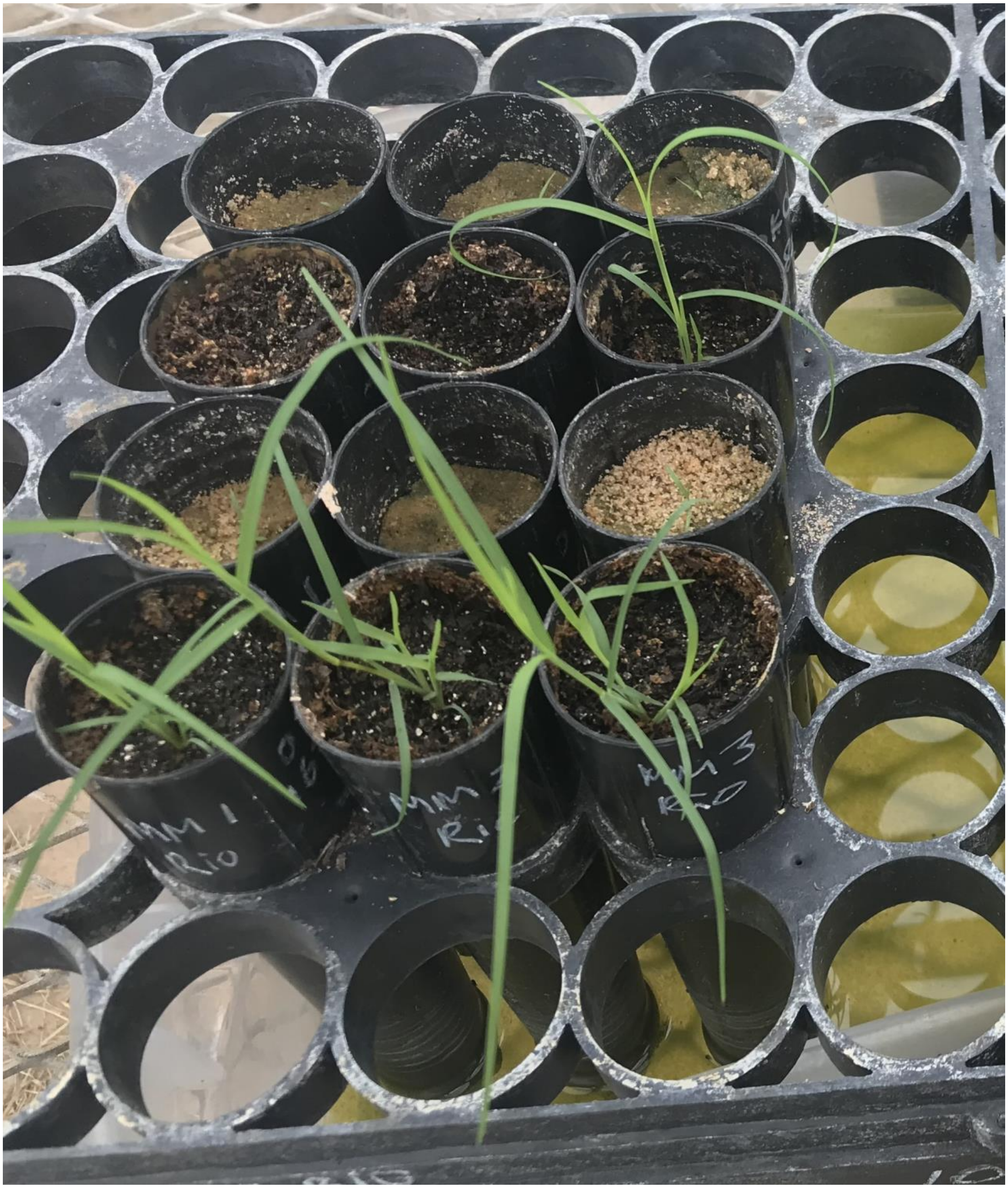

Figure 3. ‘Rio’ bermudagrass established under greenhouse conditions was best in the peat-based media.

USGA ID#: 2019-08-678

Title: Increasing Winter Soil Temperatures with Air Gaps on Ultradwarf Bermudagrass Putting Greens

Project Leaders: Mike Richardson, Eric DeBoer, Doug Karcher, Thomas Walton, and John McCalla

Affiliation: University of Arkansas, Department of Horticulture

Start Date: 2019
Project Duration: 2 years
Total Funding: $69,428

Summary Text:

As ultradwarf bermudagrass (*Cynodon dactylon* x *C. transvaalensis*) putting greens move further north in the transition zone, there is an increased risk of sustaining winter injury from low-temperature exposure, resulting in the need to cover greens periodically to prevent winter injury. The benefits of covers for winter protection of putting greens have been well-documented in both cool-season and warm-season turfgrasses (Goatley et al., 2005; Goatley et al., 2007; Roberts, 1986; Shashikumar and Nus, 1993). In a recent USGA-funded study (DeBoer et al., 2019), it was determined that reducing the predicted low-temperature to cover greens from -4.0 °C to as low as -9.4 °C did not significantly reduce winter survival. However, under extreme low temperatures, some winter injury was observed in all covered plots, suggesting that the use of industry standard covers alone may not always be enough to protect greens from winter injury.

Several cases have been recently observed where covers were being deployed in a conservative manner for ultradwarf greens in the transition zone and significant winterkill still occurred. On golf courses and in research trials, a consistent observation has been patterns of winterkill that suggest that cover thickness or the presence of air under the covers improves winter survival (Photo 1). Superintendents have tried various methods to raise covers off the turf canopy and create an air gap, such as placing pine straw, irrigation pipe or even Styrofoam "pool noodles" on the green before placing protective covers (Jared Nemitz, Peninsula Club, North Carolina, personal communication). Although the use of materials such as pine straw to create an air gap, in conjunction with covers, has been practiced by superintendents, the effect of an air gap under protective covers on soil temperature has not been tested experimentally on ultradwarf bermudagrass greens in the transition zone.

A preliminary trial was conducted during the 2018/2019 winter season at the University of Arkansas Research and Extension Center in Fayetteville AR. The trial was placed on a USGA-constructed green containing large, replicated plots (4 x 12 m) of Tifeagle ultradwarf bermudagrass. Cover treatments were applied to the green when low temperatures were predicted to fall below – 6.7 °C (20 °F). A permeable, black woven polypropelene cover (Xton, Inc. Florence, Alabama) was used to cover 4 of the 5 plots, with one plot treated as an uncovered control. Three batting treatments (Hendrix Batting, High Point, NC) were installed on plots (1.8 x 1.8 m) prior to placing the cover (Photo 2) and included batting weights of 229, 305 or 336 g m^{-2}. In addition, one additional plot was covered with the Xton cover, but had no batting (covered control). Data collected in the study included soil temperature at a depth of 2.5 cm. Soil temperatures were measured either early in the morning (8:00-9:00 am) or in the afternoon (3:00-4:00 pm). Although winter survival and greenup data were collected routinely in the spring, the only statistical significance was between the uncovered controls and all cover treatments (Photo 3).

On all sampling dates, the covered control and all batting treatments had significantly warmer soil temperatures than the uncovered controls (Figure 1). On average, the covered control increased soil temperature 1-2 °C compared to the uncovered control. On most dates, the inclusion of the batting increased the 2.5 cm depth soil temperature by 2-3 °C compared to the cover only control. There were no statistical differences in soil temperature under the various batting weight treatments.

This preliminary study suggests that the creation of an air gap under a protective cover can enhance soil temperature and may provide additional protection to warm-season putting greens under extreme low temperatures. Additional air gap treatments will be examined in a more comprehensive study during the 2019/2020 season.

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Photo 1. Winterkill of ultradwarf bermudagrass in Fayetteville AR, with patterns suggesting enhanced turfgrass survival under seams and air ripples in protective cover.

Photo 2. Example batting material treatments prior to placing the cover over the batting.

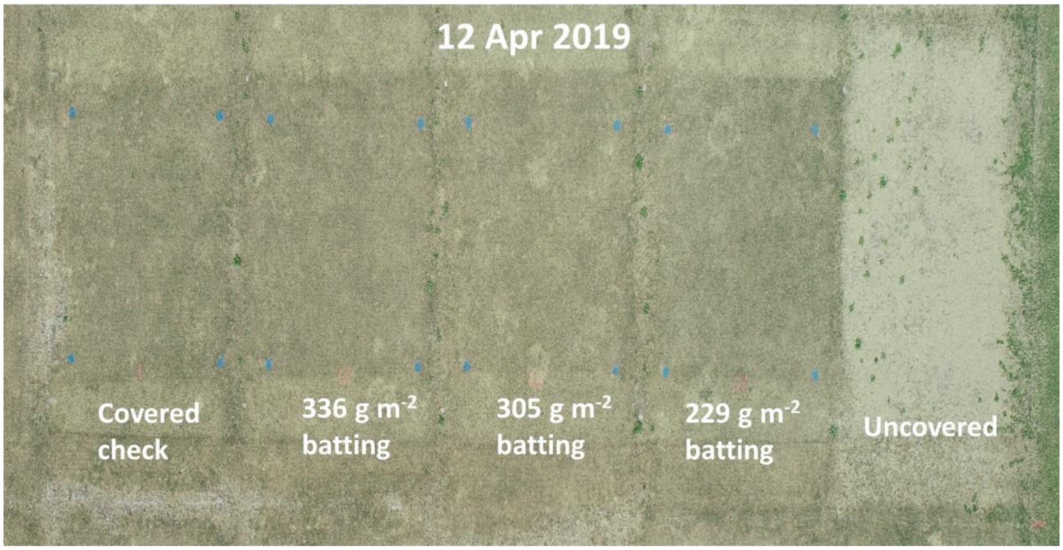


Photo 3. Effect of protective covers and batting material weight on spring greenup of Tifeagle bermudagrass.

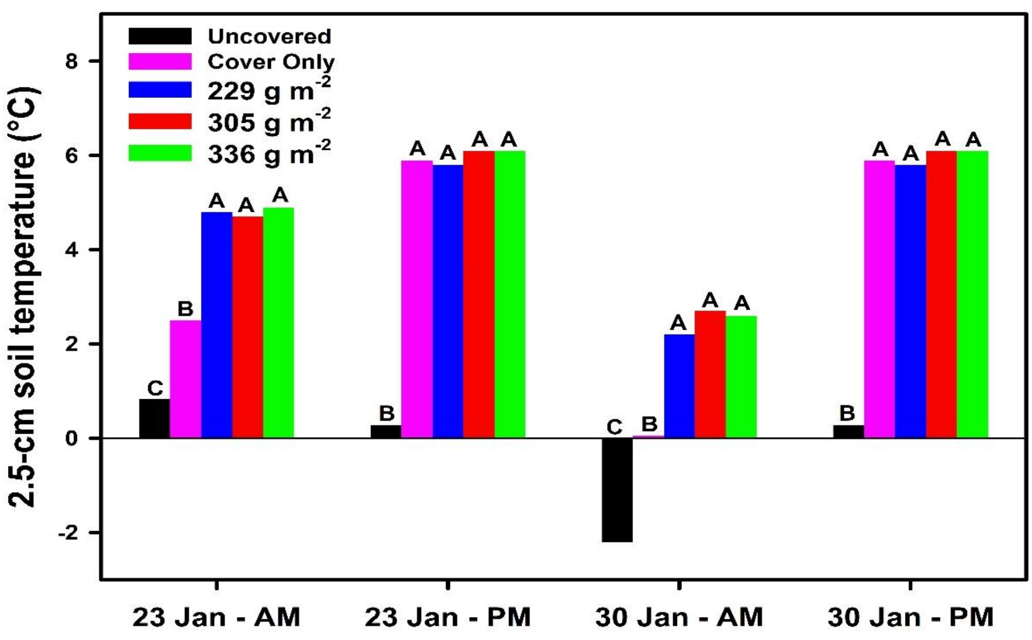


Figure 1. Effect of protective covers and various weights of batting fabric on the 2.5-cm morning (AM) and afternoon (PM) soil temperature of an ultradwarf bermudagrass green on two dates in 2019. Different letters within each bar grouping indicates a significant difference according to a least significant difference test (P=0.05)

USGA ID#: 2019-16-686

Title: Best Management Practices for Ultradwarf Bermudagrass Survival and Management in the Transition Zone

Project Leaders: David McCall, Mike Goatley, Xunzhong Zhang, Shawn Askew, and Jordan Booth

Affiliation: Virginia Tech

Objectives:

Objective 1a. Evaluate the impact that traditional cultivation practices have on ultradwarf bermudagrass putting greens cold tolerance. 1b. Examine the role that organic matter accumulation plays in ultradwarf bermudagrass putting greens cold tolerance.

Objective 2. Evaluate the effects on canopy/soil temperatures and spring recovery of various UDB cultivars when adding a second layer of cover of varying colors and composition to a standard black LPC applied according to the golf superintendent.

Objective 3. Assess the impact of fall and winter TE applications on winter survival of UDB.

Objective 4. Develop methodology to rapidly assay winter-related injury and spring recovery potential for UDB and other bermudagrasses.

Start Date: 2019
Project Duration: 3 years
Total Funding: $90,000

Summary Points:

- Aeration treatments were conducted in August of 2018 and July of 2019. Cold tolerance/spring green up data were collected in spring of 2019, aeration recovery data were collected following aeration in 2019 and TruFirm and Volumetric Water Data were collected monthly during the summers of 2018 and 2019.
- Cover treatments were installed in December of 2019 to evaluate impact of double cover techniques on soil temperature.
- Trinexapac-ethyl treatments were made during the fall/winter of 2018/19 and have been initiated for 2019/20 on four different ultradwarf putting greens (G12 and TifEagle) with four replications at each location.
- Methods have been evaluated for assessing winter-related ultradwarf bermudagrass injury and treatments have been developed. Plugs will be pulled in later winter 2020 and assessed for injury and spring recovery potential.

Summary Text:

Aeration Treatments (Obj. 1) were initiated in Summer of 2018 and Repeated in Summer of 2019. Initial firmness measurements in 2018 were correlated with greens construction methods (no-till greens were less firm than new construction). Most recent measurements correlated with % disruption). 5% Surface disruption and no aeration plots had the lowest TruFirm ratings and were statistically firmer than all other treatments. There were no treatment x location differences, indicating that aeration treatments may have a greater impact on surface firmness than construction methods. Aeration treatments had no impact on cold tolerance in the first year but did have a significant impact on turfgrass quality during summer ratings with aerated plots exhibiting higher turf quality than plots receiving less surface disruption.

Cover treatments will be installed as needed based on forecasted low temperatures. Soil temperatures under the various cover treatments will be recorded every 30 minutes using Onset ® HOBO ® data loggers and covers will be removed when air temperatures rise above threshold. This study began in December 2019 and will be repeated whenever forecast temperatures are below 25°F.

Fall and Winter applications of trinexapac-ethyl (Primo Maxx, Syngenta Crop Protection) improved (Obj. 3) turfgrass quality and prevented an early dormancy break in the winter of 2018/2019 when compared to the untreated. Rate had little impact, but the impact of fall and winter applications lasted longer than fall applications alone. Treatments were initiated in October of 2018 and made until March of 2019. Winter 2019/20 treatments began in October of 2019.

Methods will be evaluated and refined in February 2020 to rapidly assess winter-related injury and spring recovery of ultradwarf bermudagrasses. Other laboratory components include looking at impact of trinexapac-ethyl on cold tolerance of ultradwarf bermudagrass in the growth chamber on dormancy and spring green up following alternating periods of mild and cold temperatures.

Figure 1. Experimental plots after aeration treatments in July 2019.

Figure 2. Aeration treatments had no impact on cold tolerance in the first year but did have a significant impact on turfgrass quality during summer ratings.

Figure 3. Cover treatments will be installed as needed based on forecasted low temperatures. Soil temperatures under the various cover treatments will be recorded every 30 minutes using Onset ® HOBO ® data loggers and covers will be removed when air temperatures rise above threshold. This study began in December 2019 and will be repeated whenever forecast temperatures are below 25°F.

Figure 4. Fall and Winter applications of trinexapac-ethyl improved turfgrass quality and prevented an early dormancy break in the winter of 2018/2019 when compared to the untreated.

USGA ID#: 2019-17-687

Title: Understanding Factors Associated with Successful Re-Establishment of Golf Course Putting Greens Following Winterkill

Project Leaders: Michelle DaCosta[1] and Eric Watkins[2]

Additional Cooperators: Scott Ebdon[1], Lindsey Hoffman[1], Dominic Petrella[2], Trygve S. Aamlid[3], Tatsiana Espevig[3], Wendy Waalen[3], Sigridur Dalmannsdottir[3], and Carl-Johan Lönnberg[3]

Affiliation: University of Massachusetts[1], University of Minnesota[2], Norwegian Institute of Bioeconomy Research[3]

Objectives:

The objectives of the project are to examine the impacts of temperature, light intensity, and priming agents on seed germination and seedling vigor of genetically diverse creeping bentgrass cultivars.

Start Date: 2019
Project Duration: 3 years
Total Funding: $119,999

Summary Points:

- A set of 12 creeping bentgrass cultivars were evaluated for differences in germination traits at low (10°C) versus optimal (25°C) temperatures.
- Growth chambers were optimized for testing the effects of different light intensities and temperatures on seedling vigor and establishment.
- A novel method using chlorophyll fluorescence imaging was developed to improve photosynthetic efficiency screening of turfgrass seedlings.
- An international collaborative project on spring re-establishment was initiated with the Norwegian Institute of Bioeconomy Research (NIBIO) and the Scandinavian Turfgrass and Environment Research Foundation (STERF).

Summary Text:

Winter damage of golf turf is a persistent challenge in the northern U.S., particularly for species such as annual bluegrass (*Poa annua*) and creeping bentgrass (*Agrostis stolonifera*). In the last decade, widespread winter damage resulted in significant turf loss on putting green surfaces across the northern U.S., resulting in costly re-establishment, delays in course openings, and lost revenue. Reseeding is often a necessary and costly investment to promote recovery and to maintain adequate density and uniformity for play. However, adverse conditions such as cold soil and air temperatures, poor seedbed quality, and sub-optimal light intensity and spectral composition typical of early spring plantings can often delay seed germination, diminish establishment vigor, and increase competition to weeds and summer stress.

The overall goal of our research is to evaluate factors affecting spring re-establishment of creeping bentgrass, which is the most widely used turfgrass on golf course greens and fairways in the northern U.S. The specific objectives are to evaluate the genetic variability among creeping bentgrass cultivars for post-germination seedling vigor, particularly interactions with low temperatures and variable light intensities typical of spring plantings at northern latitudes. In addition, we will examine effectiveness of chemical priming agents to specifically enhance seedling vigor at low temperatures. These data will then help to adjust plant selection and management practices for golf course superintendents to utilize more effective strategies to enhance re-establishment success in spring months.

A unique aspect to our research is the establishment of an international research collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO) and the Scandinavian Turfgrass and Environment Research Foundation (STERF). Similar to the U.S., winterkill issues are also common on golf courses in the Nordic countries. Consequently, we have collaboratively defined our research objectives to more broadly explore potential barriers and identify solutions for successful spring re-establishment in northern climates.

General Methodology & Preliminary Results

Twelve creeping bentgrass cultivars were selected that represented a range of cold germination or spring green-up traits as identified in our previous research at UMass and UMN. Seed was shipped to UMass directly from breeders, and then the same seed lot from each cultivar was shipped to UMN so all research studies were conducted using the same seed. In the first year of experiments, the objectives were as follows: (i) establish baseline germination of creeping bentgrass at optimal (25°C, or 77°F) and low (10°C, or 50°F) temperatures, (ii) examine seedling cold tolerance and vigor following germination, and (iii) examine the interaction between low temperatures and high light intensities on seedling establishment following germination.

Differences in seed germination capacity at low temperatures were tested using petri dishes containing moistened filter paper that were placed into a growth chamber. The selected germination temperatures of 25°C (optimal) and 10°C (low) and were found to be discriminatory temperatures based on prior germination testing in our lab. Germination was recorded daily until maximum germination percentage was reached. Three replicate petri plates containing 50 seed for each cultivar were used per experiment, and then the experiment was repeated over time for a total of three experimental runs.

There were differences in germination rate and total percent germination among the 12 cultivars of creeping bentgrass both at optimal and low temperatures (Figure 1). However, we had significant concerns about the total percent germination, with most cultivars exhibiting less than 80% total germination even under optimal temperatures. Although these tests were conducted at UMass, the team at UMN also detected low germination among the seed. We subsequently found that there was an issue with seed storage cooler room, where all of the seed were located while awaiting shipments from breeders. Consequently, we requested new seed and experiments commenced again in October to establish germination traits for the current seed lots. During this time period, we also conducted tests to optimize measurements of seedling vigor at different temperatures, including an agar-based method to non-destructively monitor root and shoot growth and the use of chlorophyll fluorescence imaging as a tool to monitor differences in seedling photosynthetic capacity (Figure 2). We expect to use chlorophyll fluorescence imaging to assess genetic differences among creeping bentgrass cultivars for seedling photosynthetic efficiency and as a screening tool to detect leaf injury in response to low temperatures following germination.

To test the interactions of low temperatures and light intensity on seedling establishment, optimization of growth chamber conditions were first required to allow for seedling exposure to different light treatments within one chamber. We found that certain shade cloths could significantly alter the target temperature in the chamber, potentially confounding the temperature treatments. The maximum light intensity at plant height was determined to be approximately 800 µmol m^{-2} s^{-1} (without shade cloth), with a target to achieve 0, 50% and 90% reduction in the light intensity at different locations of the chamber. Different combinations and layers of white and black shade cloths were tested at both 10 and 22°C to identify the optimal shade cover regime while minimizing any differences in temperature underneath the shade cloths. For a 50% reduction in light intensity, this required a combination of 1 layer each of 90% white and 20% white shade cloths. For a 90% reduction in light intensity, this required 1 layer of 70% black and 3 layers of 20% white shade cloths.

To better understand the role of light intensity and photoinhibition as a potential factor affecting seedling establishment, a novel method was developed using chlorophyll fluorescence imaging. The photochemical efficiency (Fv/Fm) was measured at different time points following exposure from 22 to 10°C. The Fv/Fm of all pixels in the captured image were then used to calculate the percentage of pixels at given Fv/Fm values. Upon transfer from 22 to 10°C, there was an increase in the percentage of pixels at lower Fv/Fm values, indicating a decrease in photochemical efficiency upon exposure to low temperature (Figure 3). Based on quantification of the slope of the pixel distribution, a more negative slope was found to be associated with higher photochemical efficiency. In addition, based on regression analyses, we determined the slope of pixel distribution to be highly correlated ($r^2 = 0.95$) to the average Fv/Fm of individual leaves. Therefore, this method can provide us with a more representative response for a larger number of seedlings rather than measuring the average Fv/Fm of individual leaves in an image.

Next Steps

With new seed and optimized experimental conditions, we expect to complete the initial screening experiments for seed germination and seedling cold and light responses this winter. Results from growth chamber experiments will then be used to guide field trials at UMass, UMN, and NIBIO (Landvik, SE Norway) starting in spring 2020. Based on the results from the growth chamber and field studies, we then plan to select the top performing and bottom performing cultivars to test the effects of chemical priming compounds for enhancing seed germination and/or seedling establishment. In collaboration with NIBIO, we also established a cultivar evaluation trial (SCANGREEN) at UMass, UMN, and four sites in the Nordic countries in fall 2019. The objectives of this NTEP-style trial are to evaluate performance of various species and cultivars for putting greens in northern climates (including fine fescues and creeping bentgrass, colonial bentgrass, and velvet bentgrass).

Figure 1. Differences in seed germination among 12 creeping bentgrass cultivars. Seeds were germinated in petri dishes at either 25°C or 10°C in controlled environment chambers. Data are presented as the average over three different experimental runs at each temperature.

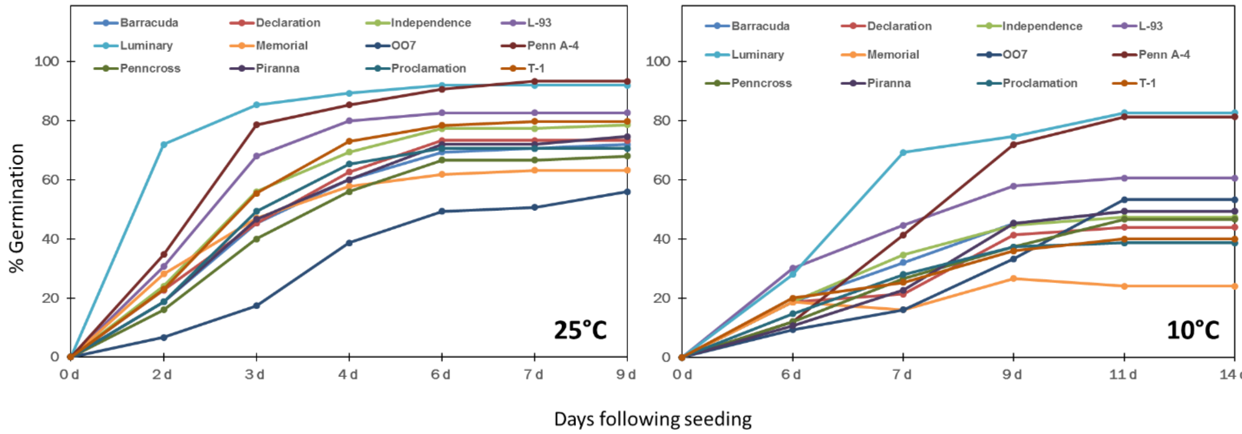


Figure 2. Seedling growth of creeping bentgrass cultivar, Luminary, at 25°C. Seed were germinated in flats containing USGA sand media. Middle and right panels show the same flat using chlorophyll fluorescence imaging to represent leaf photochemical efficiency (Fv/Fm) of seedlings at 7 and 14 d following seeding. Higher Fv/Fm values (as indicated by colors of yellow, orange and red) are a measure of a higher capacity for light harvesting and photosynthetic efficiency.

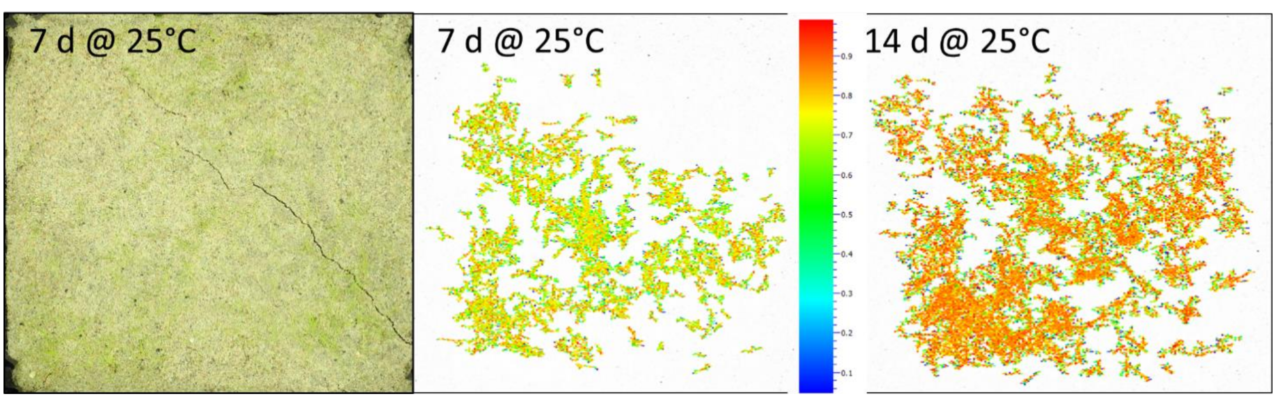
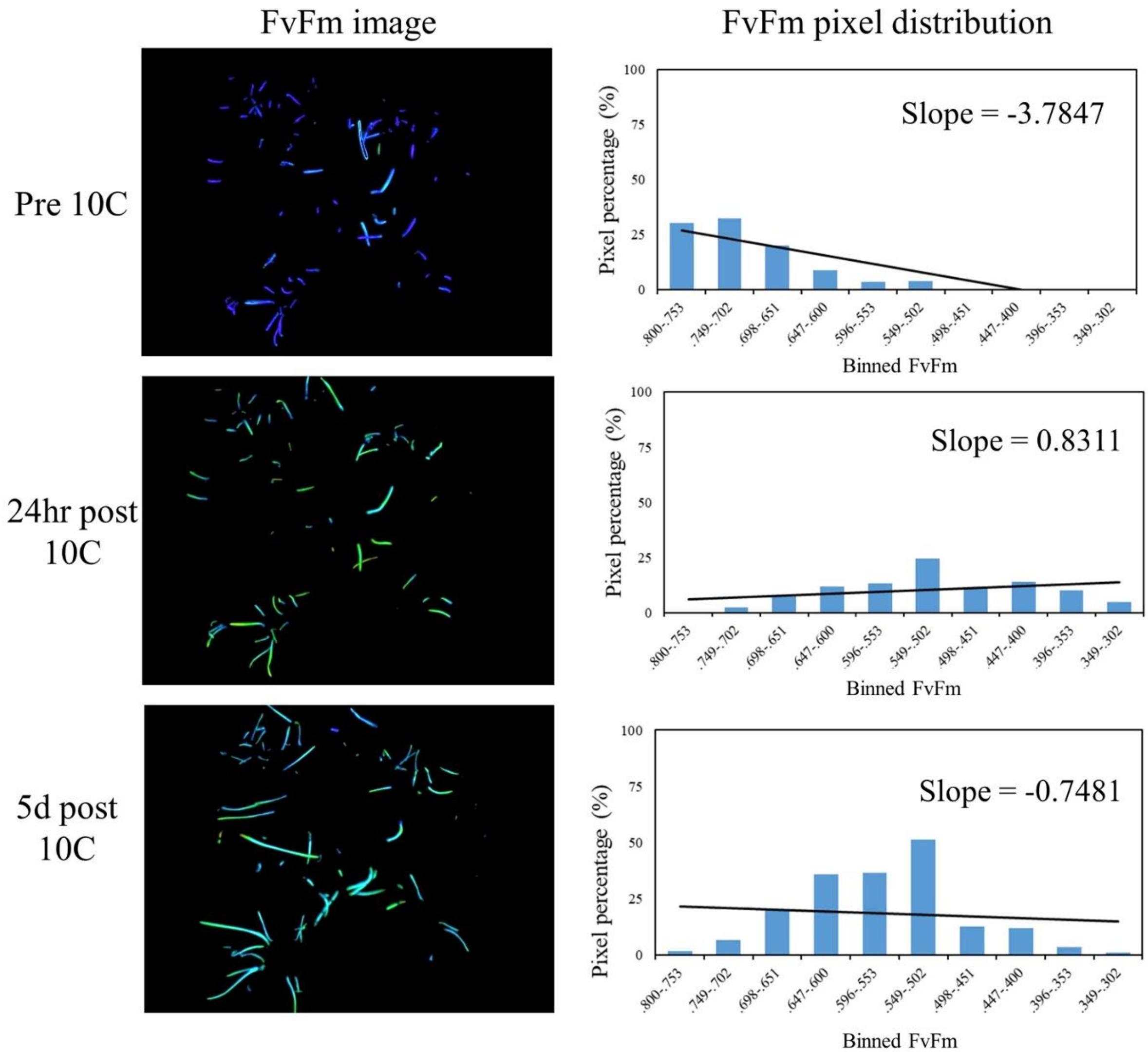


Figure 3. Pixel analysis based on chlorophyll fluorescence imaging of photochemical efficiency (Fv/Fm). The Fv/Fm of plants were analyzed at three time points, prior to being transferred to 10˚C, 24 hr after 10˚C, and 5 d after being at 10˚C. The slope of the line fit to the bar charts can be used to compare treatment effects.



USGA ID#: 2019-14-684

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

Project Leaders: Bingru Huang and James Murphy
Affiliation: Rutgers University

Objectives:

1. Determine physiological factors associated with *Poa* responses to heat stress and summer decline.
2. Identify effective plant-health products and application rates for controlling *Poa* summer decline or improving heat tolerance.
3. Test the effectiveness of plant-health products for promoting summer performance of annual bluegrass on putting green conditions.

Start Date: 2019
Project Duration: 3 years
Total Funding: $118,552

Summary Points:

- Poa ecotypes with greater canopy density maintained lower canopy temperature.
- Several products, including amino acid- or seaweed-based biostimulants, PGRs, and fungicides could effectively enhance Poa performance under heat stress (35/30 ^{o}C, day/night).
- Improved Poa performance under heat stress with different plant-health products was associated with the maintenance of better turf quality and more active shoot growth, as well as greater leaf cellular membrane stability.
- The combination of Primo and Proxy applied at the rates for seedhead control suppressed Poa growth.

Summary Text:

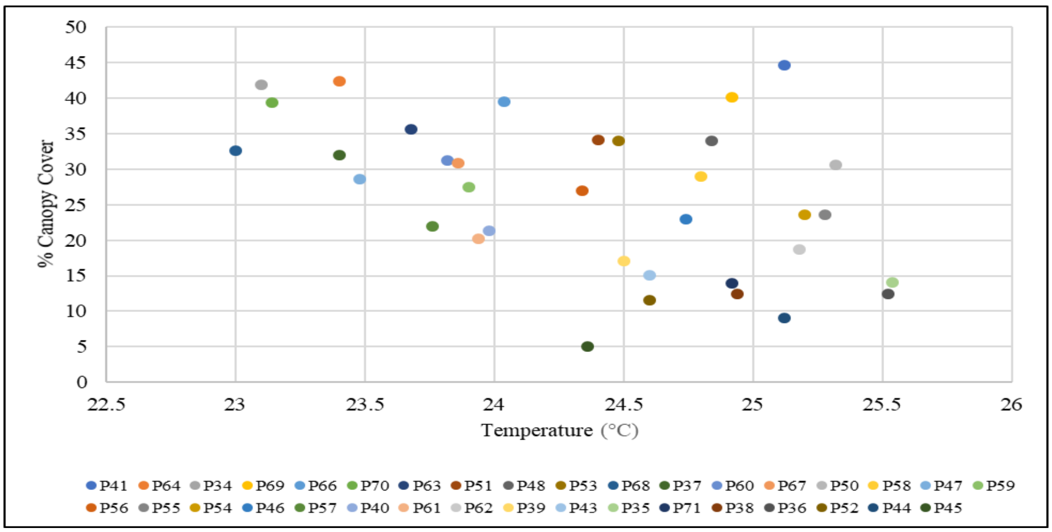
We have conducted two trials in 2019 to address objective 1 and 2.

<u>Trial I: Evaluation of heat performance for Poa ecotypes collected from northeast states</u>
A collection of Poa ecotypes from northeast states, including NJ, NY, VT, NH, and ME was examined to determine variability in Poa heat tolerance. A total of 35 ecotypes from this collection were planted in plastic containers (20 cm deep and 5 cm in diameter) filled with sand in the greenhouse at Rutgers University. Uniform-sized plants (5 containers for each ecotype) were transferred to a controlled-environment growth chamber and subjected to heat stress at day/night temperature of 35/30 ^{o}C for 18 days. Other environmental conditions of the chambers were controlled at 14-h photoperiod and 680 µmol photon m^{-2} s^{-1} photosynthetically active radiation at the canopy level. Plants were watered twice a day to maintain adequate soil water content and fertilized weekly with half-strength Hoagland's nutrient solution to supply adequate nutrition during the heat-stress period.

Heat performance of different ecotypes was examined by evaluating canopy cover and canopy temperature. At 6, 12, and 18 d of heat stress, regular photos and thermal images were taken to evaluate canopy cover and canopy temperature, respectively, using imaging analysis programs.

Canopy cover and canopy temperature declined during heat stress to different levels across 35 ecotypes of Poa collected from the northeast states, suggesting there existed variations in Poa performance in response to heat stress. However, whether the phenotypic variations in different ecotypes in heat responses is due to genetic variations or differences in environmental conditions where the ecotypes were collected are yet unclear. It is worth noting that there was a general negative relationship between canopy cover and canopy temperature (Fig. 1), indicating that Poa ecotypes with greater canopy density could be better in heat tolerance by maintaining lower canopy temperature through active transpirational cooling. Further research will determine physiological traits associated with heat tolerance by comparing heat-tolerant to heat-sensitive ecotypes or cultivars of Poa.

Fig. 1. Poa Heat Screening - Relationship between Temperature and Canopy Cover at 18 Days of Heat Stress



<u>Trial II: Effects of plant health products on Poa heat tolerance</u>

Poa sods (2'' in diameter) were collected from putting green plots in Rutgers turfgrass research farm, and transplanted into plastic containers (20 cm deep and 5 cm in diameter) filled with sand. Plants were established in a greenhouse for 3 weeks. Uniform-sized plants were transferred to a controlled-environment growth chamber.

Three types of plant-health products were examined for their effects on Poa heat tolerance. The following chemical treatments were applied by foliar spray of plants: 1) untreated control: plants were sprayed with water in the same volume as the chemical treatments (10 mL) to saturate turf canopy; 2) biostimulants: amino acids (AA) (60 mM); seaweed-extracts (SWE) - SWEA (6 fl oz), SWEB (6 fl oz), SWEC (6 fl oz); 3) Plant growth regulators (PGRs): Primo Maxx (trinexapac-ethyl) (0.1 fl oz), Proxy (ethephon) (5 fl oz), Primo Maxx + Proxy (PP); 4) Fungicides: Signature

XTRA StressGard (2.0 fl oz) (sig), Daconil Action (2 fl oz) (DacAc), and Appear II (3 fl oz) (AppII).

All treatments were applied at 8 days prior to the initiation of heat stress, the day before the initiation of heat stress, and every 7 days during heat stress. Each treatment had 6 replicates (containers).

Plants (6 containers for each chemical treatment) were subjected to heat stress at day/night temperature of 35/30 ºC. Non-stress control plants were maintained in a growth chamber with temperature controlled at 22/17 ºC. Other environmental conditions of growth chamber were controlled at 14-h photoperiod and 680 µmol photon m^{-2} s^{-1} photosynthetically active radiation at the canopy level. Plants were watered twice a day to maintain adequate soil water content and fertilized weekly with half-strength Hoagland's nutrient solution during the heat stress period.

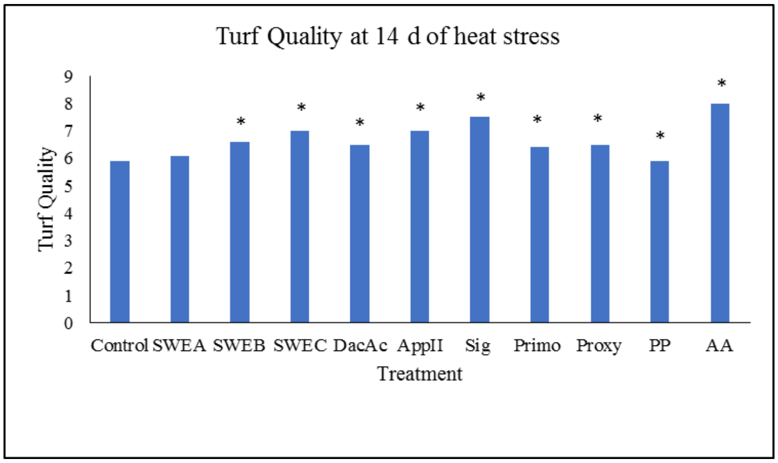
Effects of different plant-health products on Poa performance under heat stress were examined by evaluating canopy cover and canopy temperature. Regular photos and thermal images were taken to evaluate canopy cover and canopy temperature, respectively, using imaging analysis programs.

All treatments, except the combined Primo and Proxy treatment, improved turf quality (Fig. 2), maintained greener turf (Fig. 3), and higher leaf membrane stability (shown as lower EL) (Fig. 4) in Poa plants exposed to 14 d of heat stress. The combined Primo and Proxy treatment applied at the rates recommended for seedhead control suppressed Poa growth under normal temperature and heat stress.

The results from the controlled-environment trial suggested the potential application of those plant-health products tested in this experiment for improving heat performance of Poa under heat stress. Leaf and root samples were collected and the analysis of leaf samples for carbohydrate content and root samples for the determination of root length, surface area, and volumes are in progress.

The effective treatments for improving Poa heat performance will be further tested in field trials in 2020.

Fig. 2. Turf quality as affected by different treatments for Poa exposed to heat stress. Treatments marked with * were significantly different from the control at p = 0.05.



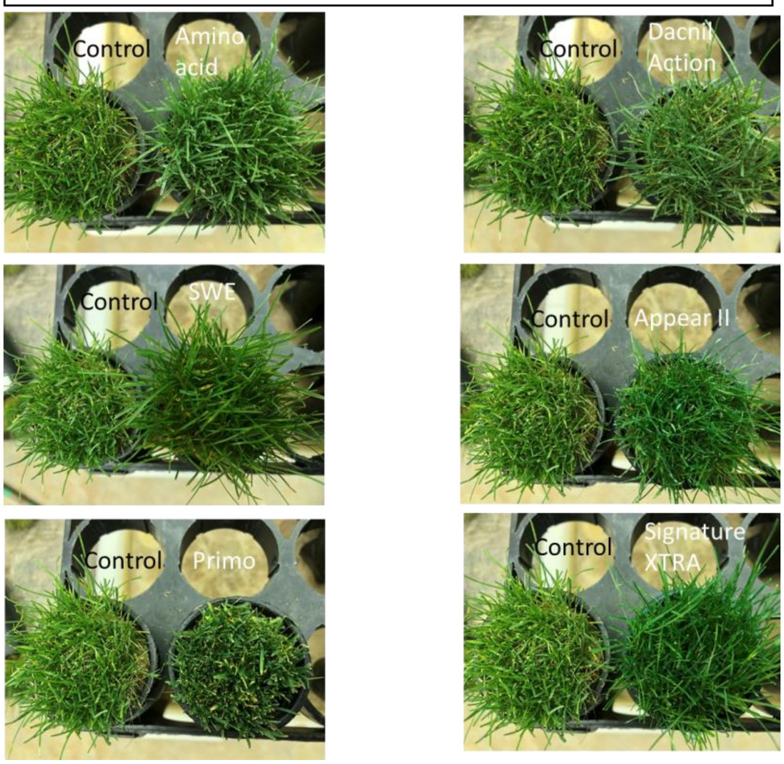


Fig. 3. Dark green color index (DGCI) as affected by different treatments for Poa exposed to heat stress. Treatments marked with * were significantly different from the control at p = 0.05.

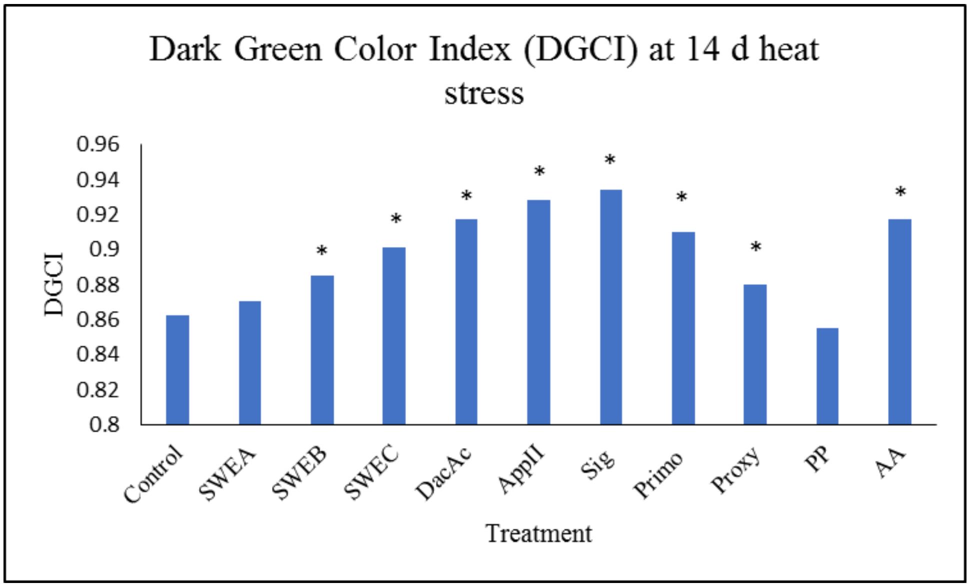
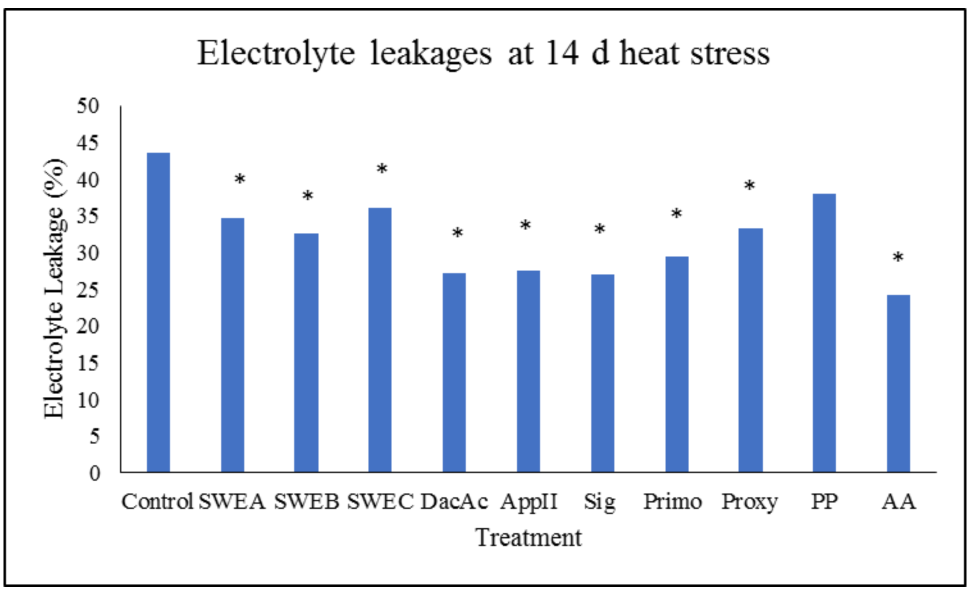


Fig. 4. Leaf eelectrolyte Leakage as affected by different treatments for Poa exposed to heat stress. Treatments marked with * were significantly different from the control at p = 0.05.



USGA ID#: 2019-10-680

Title: Building a Better Growth Model to Optimize Nitrogen Applications to Bentgrass Putting Greens

Project Leader: Doug Soldat
Affiliation: University of Wisconsin-Madison

Objectives:

The objectives of this research are to 1) investigate the effects of weather variables, footwear traffic, nitrogen application rate, soil moisture content and soil organic matter on creeping bentgrass growth at putting green; and 2) build an accurate growth model for creeping bentgrass growth that can be useful for making weekly management decisions.

Start Date: 2019
Project Duration: 3 years
Total Funding: $84,830

Summary Points:

1. Bentgrass growth rate is negatively correlated to soil moisture on sand root zones.
2. Bentgrass growth rate is negatively correlated to traffic level, but across the range of typical traffic levels, this factor is not as significant as others.
3. Annual bentgrass growth on sand greens with different soil organic matter levels varies substantially.
4. Management practices and weather both play highly significant roles in controlling bentgrass growth
5. A machine learning approach to accurately predict bentgrass growth appears feasible.

Summary Text:

Creeping bentgrass is one of the most common grass species used on golf course putting greens in the US. Nitrogen fertilization has a strong influence on bentgrass growth, and conversely growth the difference between actual and desired growth can be used to determine nitrogen a reasonable nitrogen fertilizer rate. However, an accurate method to help turf mangers to make reasonable nitrogen application decisions does not exist. There is a need to understand the factors that influence creeping bentgrass growth and then use that knowledge to build a growth model that could be used for bentgrass nitrogen management.

Methods
To investigate the interactions among soil, turfgrass, environment and management practices, this study will use four 'Focus' bentgrass sand putting greens that vary in soil organic matter content and quality. The study was initiated in summer 2018 and expanded in 2019. Generally, N fertilizer treatments were applied at 0, 0.1 and 0.2 lbs N/1000 square feet every two weeks, footwear traffic was applied by walking on the green wearing golf shoes. In 2018, traffic was maintained at high, medium and low level which represent the golf course received 3600 rounds/week, 1800rounds/week and 0 rounds/week; in 2019, traffic levels were decreased and maintained at 1400 rounds/week, 700 rounds/week and 0 rounds/week. In 2018, treatments were included 3 nitrogen application rates and 3 foot traffic levels with three replications on two of greens. In 2019, we investigated the effect of soil moisture content on bentgrass growth.

Treatments were maintained at high (25-27% volumetric water content), medium (18-20% volumetric water content) and low (8-13% volumetric water content) moisture levels during periods without precipitation. Bentgrass clipping yield was collected about 4 times a week in both years. Soil moisture content was measured before clipping collection.

To build the bentgrass growth model, several different weather factors were selected as input variables, including air temperature, evapotranspiration, relative humidity, precipitation and wind speed. Weather data were either used from an onsite weather station and online weather data (Weather Underground). Soil samples were collected each month for analyzing potentially mineralizable nitrogen, which is method to estimate the fraction of soil organic matter that can be easily converted to plant available nitrogen. Turf visual quality was measured every two weeks and NDRE was measured prior to each clipping collection event. The growth model was built with the “scikit-learn” random forest package from Python.

Preliminary results

Impact of management practices on bentgrass growth:

Figure 1 shows that growth overall is greatest on the plots contain high soil moisture content, and grass on the medium and low soil water content plots produce significantly lower clipping yields. Lowest levels of soil moisture (8-13%) led to decreased visual quality and localized dry spots. Treatments receiving relatively high traffic levels (3600 rounds/week) produced lower clipping yields than the plots receiving lower traffic. However, the difference in traffic was not significant across more realistic levels of traffic. Least surprisingly, bentgrass fertilized with higher levels of nitrogen produced significantly higher yields. However, there is too much variability among these management variables to make an accurate prediction of bentgrass clipping production. However, these findings and relationships are very useful in a machine learning approach to build a model that can better predict bentgrass growth.

Model performance:

Both 2018 and 2019 data were used for building the random forest model. However, we built several models based on different numbers of variables input, in search of a simple and accurate model. The full model includes the entire suite of growth variables including soil moisture content, NDRE, traffic level, N application rate and weekly weather data (min, max and average of air temperature, precipitation, evapotranspiration, wind speed and min, max and average of relative humidity). We also tested two sets of simplified models by using subsets of the input: 1. without NDRE and soil moisture content input; 2. using only weekly weather data input. The reduced models focus on the variables that most easily available or obtained by the end user. Figure 2 shows the correlation between predicted clipping and actual clipping data for each model, and Table 1 shows the statistical performance of each model. These results suggest that excellent growth predictions can be made from readily available and easily obtained data, although using only weather data appears to be far less accurate than including management information and soil factors.

Figure 3 shows the most important variables for each model. Generally, the management practices include N application rate, irrigation practice or soil moisture content and traffic are very important, and the key weather variables include relative humidity, evapotranspiration, and air temperature. Moreover, NDRE, as appears to increase model accuracy. The single decision tree (Figure 4) created based on the most important factors in the full model explain how the important variables drive bentgrass growth rate. Generally, NDRE, which reflects plant chlorophyll content, is highly correlated with bentgrass growth. Management practices, such as N application rate and traffic level could result in different growth rate and play an essential role in controlling bentgrass yield production. Our analysis shows weather data are as important factors as management practices. However, models built with only weather data input do not perform as well as the other models.

These models currently use root zone as a categorical variable, which is not ideal for widespread use. The next phase of the research will be to quantify and characterize the impact of root zone and soil organic matter so that the model can be better parameterized and eventually used by golf course managers.

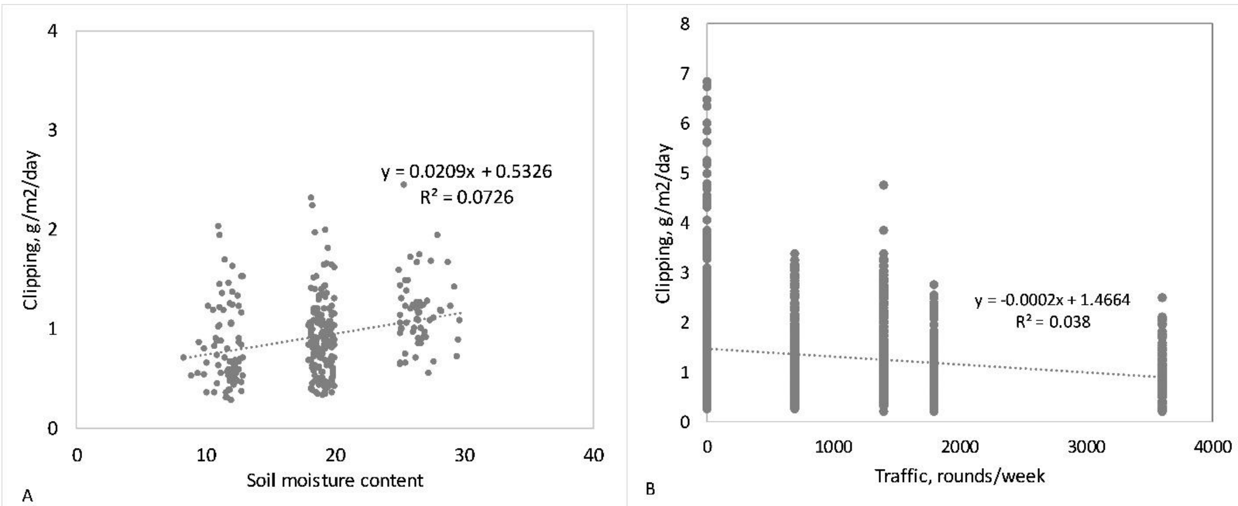


Figure 1. *The impact of management practices on bentgrass growth. The effect of soil moisture content on bentgrass growth (A); the effect of footwear traffic on bentgrass growth (B).*

Table1. *Comparison of performance of each model*

Input variables	R^2	**RSME**
All variables	0.72	0.3678
Without NDRE, and soil moisture content	0.75	0.3926
Only weather data	0.61	0.4578

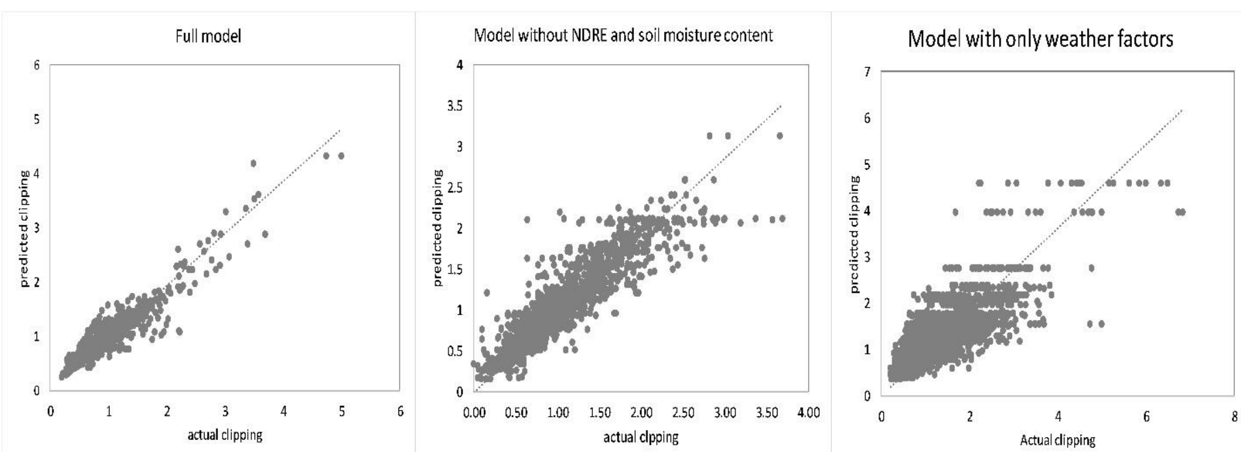


Figure 2. *The scatter plot of predicted clipping and actual clipping from full model and reduced models.*

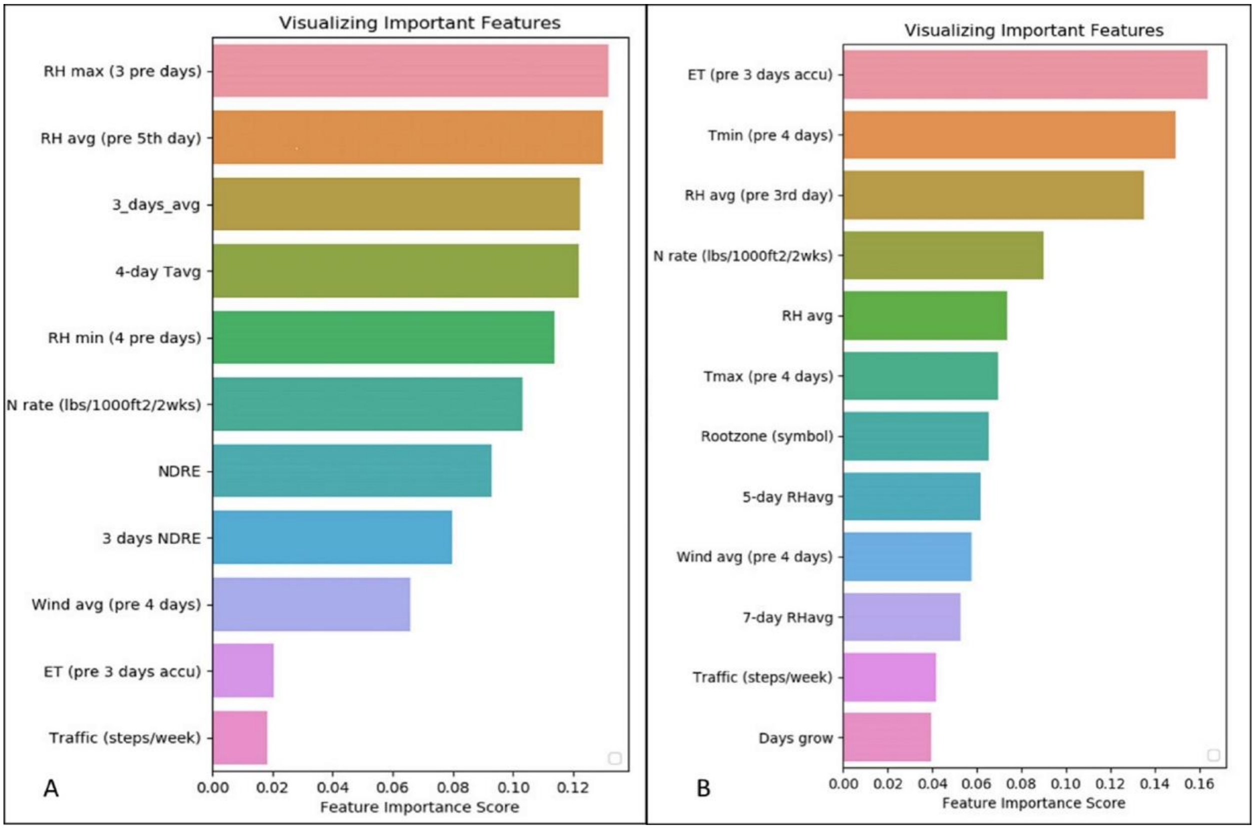


Figure 3 *. Important features for two sets of prediction models (A) Full model; (B) model without NDRE and soil moisture content*

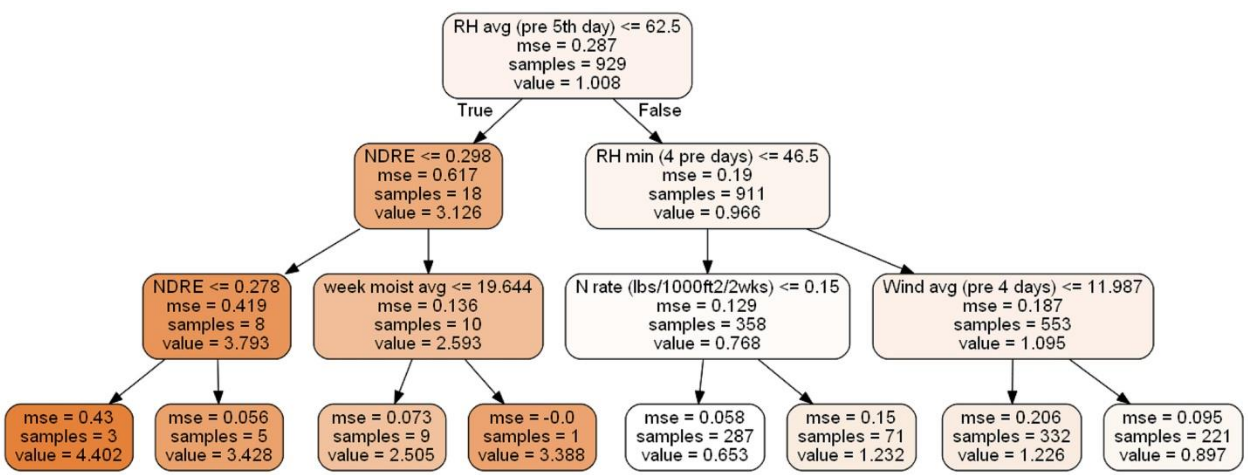


Figure 4*. A single decision tree built by top important features in the full model*

USGA ID#: 2017-15-625

Title: Modeling GA Production Improves Prediction of Turf Growth and PGR Performance

Project Leader: William C. Kreuser
Affiliation: University of Nebraska-Lincoln

Objectives:

1. Quantify the rate of GA production across a range of environmental and management factors in growth chambers and field plots.
2. Use statistical models to create mathematical models to predict GA production.
3. Pair GA production models with PGR GDD models to accurately estimate clipping yield suppression and rebound.
4. Fully integrate the results into an easy to access web-app, GreenKeeper App.

Start Date: 2018
Project Duration: 3 years
Total Funding: $88,160

Summary Points:

- Clipping yield as a proxy for GA production is being used to determine the peak growth and GA production of four cool-season (creeping bentgrass, Kentucky bluegrass, perennial ryegrass and tall fescue) and two cool season (buffalograss and zoysiagrass) turfgrasses. Preliminary results have determined that peak clipping yield, as thus GA production for Kentucky bluegrass and creeping bentgrass was found at 77 F, whereas peak GA production of perennial ryegrass was recorded at 70 F.
- This research is currently ongoing.

Summary Text:

Measuring GA production with growth as a proxy to help model PGR suppression
Turfgrass growth is regulated by GA production, and by using clipping yield as a proxy for GA production this study aims to determine how temperature and nitrogen treatments effect cool- and warm-season turfgrass GA production. Understanding the peak GA production of turfgrass systems will improve the modeling and application of PGRs for growth suppression. Plugs of buffalograss, creeping bentgrass, Kentucky bluegrass, perennial ryegrass, tall fescue and zoysiagrass were pulled from the East Campus Turfgrass Research Plots in Lincoln, NE. Plugs were immediately placed into conetainers in a 75 F greenhouse to acclimatize for one month. During the re-growth in the greenhouse the plugs were fertilized with sprayer grade urea at 0.1 lb N M^{-1}. After one month in the greenhouse, the plugs were placed into different growth chambers set at 45, 59, 77 and 95 F, the different temperature treatments. These plugs were fertilized for two weeks more with 0.1 lb N M^{-1} and mowed weekly, while on the third week in

the growth chamber the plugs were not fertilized but still mowed. Starting on week four in the growth chamber the plugs were fertilized with different N treatments: 0, 0.125 and 0.500 lb N M^{-1} for five weeks. Clippings were collected from each plug once a week starting on the week five and sampled until week nine in the growth chamber. Clippings were dried in a 140 F oven and dry biomass was recorded. An additional replication of this study is currently on going.

The quantification of GA production of these turfgrass was difficult to accomplish because of the location of where GA is found in turf. Because of the difficulty to measure the GA production, the growth of the plant (i.e. clipping yield) is currently being used as proxy for GA production.

Preliminary results have determined that the peak clipping yield of creeping bentgrass and Kentucky bluegrass is at 77 F, whereas peak clipping yield for perennial ryegrass is found at 70 F (Fig. 1). All three species recorded their lowest clipping yield at 95 F, but the relative decrease different among species. The peak GA production varies between species and is different depending on the temperature.

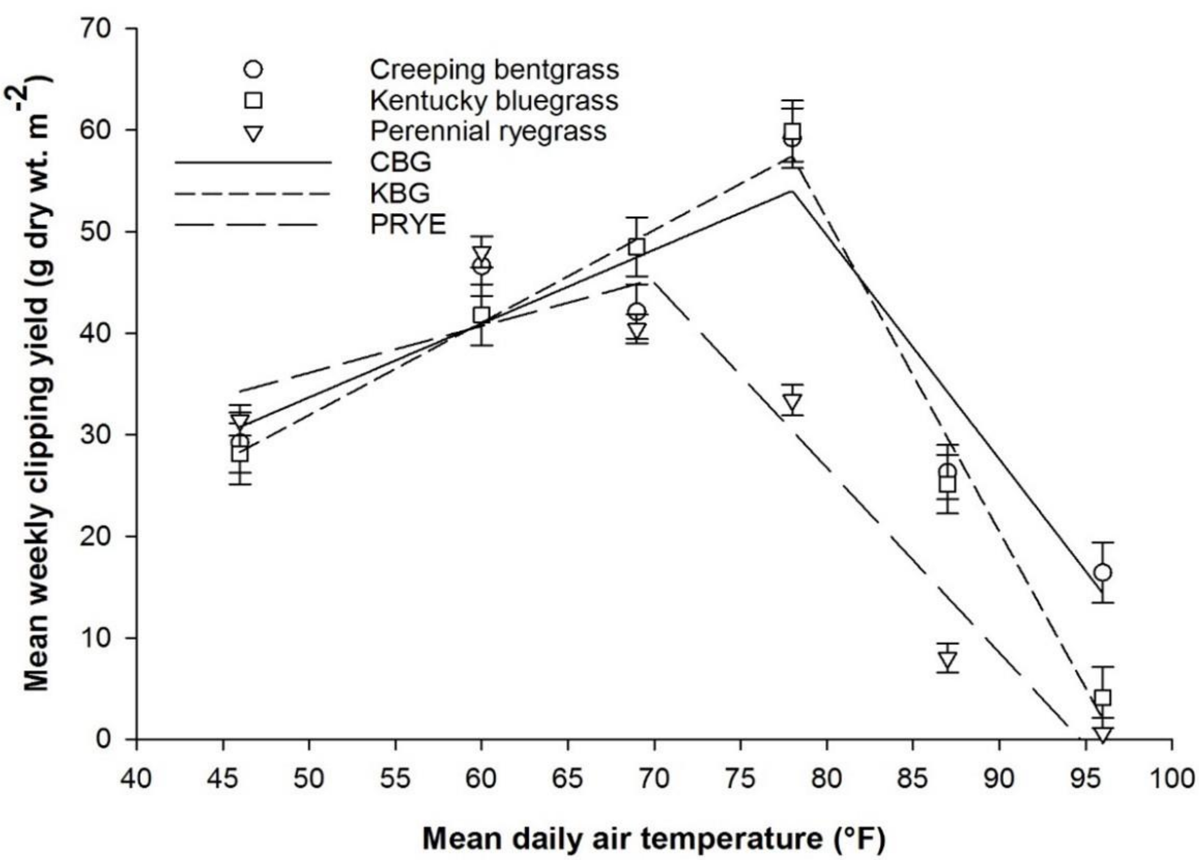


Figure 1. The mean weekly clipping yield of cool-season grasses grown in growth chambers at 45, 59, 77 and 95 F. The plotted lines on this graph visualize the peak growth of these species and peak GA production.

USGA ID#: 2018-09-659

Title: Growing Degree Day Models to Guide PGR Application Rates

Project Leaders: William C. Kreuser
Affiliation: University of Nebraska-Lincoln

Objectives:

1. Develop PGR GDD models for various PGR active ingredients and application rates on cool- and warm-season greens and fairways.
2. Quantify and correlate PGR metabolite levels to different points on the GDD models from the first objective.
3. Calculate critical PGR levels to sustain suppression and base temperatures for PGR metabolism.
4. Integrate GA production results and growth potential models from our 2016 USGA grant to account for physical removal of PGRs during mowing.
5. Use field research from objective one and lab research from objective two to develop a "flipped" PGR algorithm for application rate selection.

Start Date: 2018
Project Duration 3 years
Total Funding: $120,000

Summary Points:

- Developed PGR GDD models for three cultivars of ultradwarf bermudagrass putting greens in NC, MS and TN with our collaborators. Peak suppression for prohexadione-calcium ranged from 50-45% with ideal re-application intervals of 120-160 GDD (base 10C). Peak suppression ranged from 49-62% with re-application intervals ranging from 216-300 GDD (base 10C) for trinexapac-ethyl. Warm-season results were published in Crop Science. Results have been added to GreenKeeper App.
- Developed PGR GDD models for paclobutrazol applications on creeping bentgrass putting greens. Clipping yield suppression ranged from 29-62% of the non-treated control depending on application rate. The ideal re-application interval ranged from 269-302 (base 0C) and model R^2 values ranged from 0.41 to 0.86. Results have been added to GreenKeeper App.
- "Flipped" PGR models were tested to estimate the amount of PGR remaining in the plant when the PGRs were applied prior to their ideal re-application interval. A half-life approach model was used to schedule PGR application rate. The models tested resulted in an intensification of clipping yield suppression and increase phytotoxicity overtime. This indicates the models were too aggressive. A different PGR degradation model will be evaluated in 2020.

Summary Text:

Development of a "Flipped" PGR Model
The goal is to create a PGR model that guides managers with the correct partial PGR application rate when a follow-up application is made prior to the ideal re-application interval. This will limit the stacking effect and intensification of growth suppression that has been documented in our past research. This experiment was started on a creeping bentgrass golf fairway mowed at 0.400 inches. The ten-treatments included a non-treated control for normalization and a 3x3 factorial of three PGRs (trinexapac-ethyl, paclobutrazol and prohexadion-Ca; called TE, PC PH) applied weekly at either the standard rate or two different fractions of the standard rate depending on GDD accumulation. There were three replicates. The equation used to estimate PGR degradation was based on the half-life equation:

Replacement PGR rate (oz/A) = Full PGR Rate – (Full PGR Rate*(0.5)^(Current GDD/half-life in GDD))

The full rates for the TE, PC and PH were 7, 16 and 11 oz/acre, respectively. The tight and long estimated half-lives for the TE, PC and PH were 116/175 GDD, 160/240 GDD and 140/210 GDD. Clippings were collected several times each week to determine if clipping yield suppression was static of intensifying over time.

The experiment was concluded after four weeks because of the clipping yield suppression intensifies over the three applications for all treatments except for the trinexapac-ethyl with the 175 GDD half-life (Fig. 1). This lead to strong phytotoxicity (Fig. 2) and greater than 80% clipping yield suppression for all other treatments relative to the non-treated control. Future research will evaluate other degradation models (i.e. linear) and different proposed half-life coefficients. This research is important because it will help with variable rate sprayers and minimize PGR-induced collar decline.

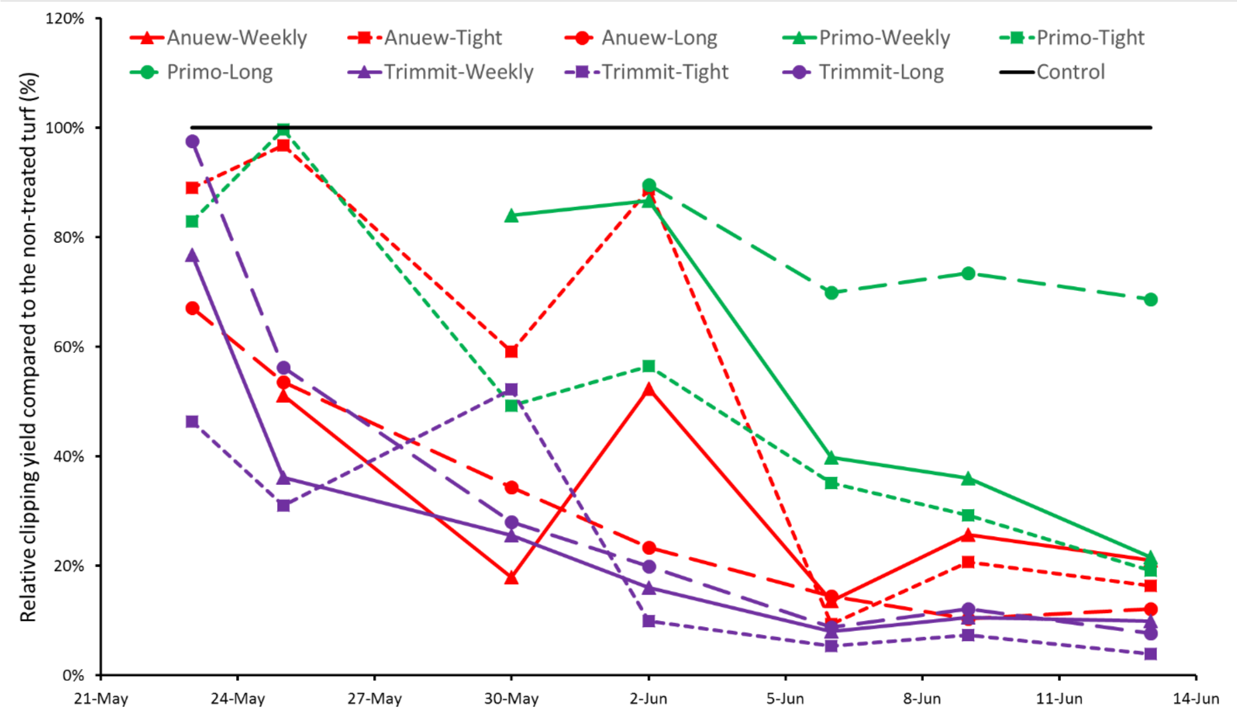


Figure 1. The impact of different PGR re-application models on the relative clipping yield suppression of a creeping bentgrass fairway mowed at 0.400 inches.

Figure 2. The impact of different PGR re-application models on the turfgrass quality of a creeping bentgrass fairway mowed at 0.400 inches.

USGA ID#: 2019-30-700

Title: Mining GreenKeeper App Data to Quantify the Impact of Turf Research

Project Leader: Bill Kreuser, PhD
Affiliation: University of Nebraska-Lincoln

Objectives:

1) Condense data from two USGA funded research grants into a new unified PGR guidance model.
2) Build that model into GreenKeeper App
3) Monitor changes in PGR use and turfgrass performance through data analytics and surveys.

Start Date: 2019
Project Duration: 2 years
Total Funding: $55,000

Summary Points:

- Collected data from a creeping bentgrass green treated with various combinations of PGRs validate PGR degradation models generated in USGA grant 2018-09-659.
- Clipping yield results are still be analyzed and the non-linear regression models are being optimized with a streamlined user interface to increase adoption of new models.
- Contracting with a web-developer to create the front and back-end changes required to integrate these models into GreenKeeper and assess impact of this USGA funded research project.

Summary Text:

For the past twelve years, our lab has been creating models to predict the duration and performance of plant growth regulators (PGRs) using growing degree day (GDD) model. To date, we have over 570 different models that are specific to grass species, active ingredient, application rate and even mowing height. Our recent USGA-funded research shows PGRs are a likely source of golf course collar decline because these products are more efficacious at collar/fairway mowing height than putting green mowing height. While our understanding of these commonly applied products has increased over the past decade, the increasing complexity to implement these models has grown. Added complexity can severely limit implementation. We needed a tool to help managers easily use the research.

A web-based, decision-support tool called GreenKeeper (GreenKeeperApp.com) was developed at the University of Nebraska. The initial development goal was to house and drive the various PGR GDD models with automated weather data retrieval. Users simply select the PGR and GreenKeeper tells them how it is working at their course. Over time, the feature sets within GreenKeeper grew to include sprayer mixing instructions, pesticide and fertilizer recordkeeping, soil testing, management and visualization of weather and performance data (clipping volume, green speed, soil water content, etc.), and the Smith-Kerns Dollar Spot forecast model.

Closely mining the PGR use data in GreenKeeper suggests that 70% of PGR applications within GreenKeeper's application records used GDD-based models instead of traditional – inefficient – calendar-based intervals. Those data can be further refined by region and economic impact can be calculated. The PGR research funded by past USGA grants can further improve the PGR GDD information provided by GreenKeeper. Instead of treating every PGR as an individual application, the newly created and complex models can account for combination applications and PGR residual effects from past applications.

In 2016, we started validating the PGR stacking models developed from the 2016 and 2017 USGA grants: i) Modeling GA Production Improves Prediction of Turf Growth and PGR Performance, and ii) Growing Degree Day Models to Guide PGR Application Rates. Various rates of prohexadione-Ca, trinexapac-ethyl and paclobutrazol were applied in combination to a 'V-8' creeping bentgrass putting green during the fall of 2019 (Fig. 1). The clippings yield data were then normalized to the non-treated control and modeled with sinewave non-linear regression. The model coefficients of amplitude and period were then fitted to experimental PGR degradation models developed in past USGA grants (Fig. 2). Once validation and model optimization are complete, this new PGR GDD tracking (v.2.0) will be integrated into GreenKeeper so managers can be alerted to potential problems and synergism when mixing different PGR active ingredients or when PGRs are applied too frequently. This dashboard will clearly warn managers of potential problems and guide lower application rates to minimize the risk of collar decline or PGR over-regulation. We have started to hire a team of developers to integrate this model and create the user interface in 2020. A beta test version is expected to be released in the summer of 2020.

Figure 1. The subtle color changes of the creeping bentgrass putting green following applications of PGR combinations at various rates.

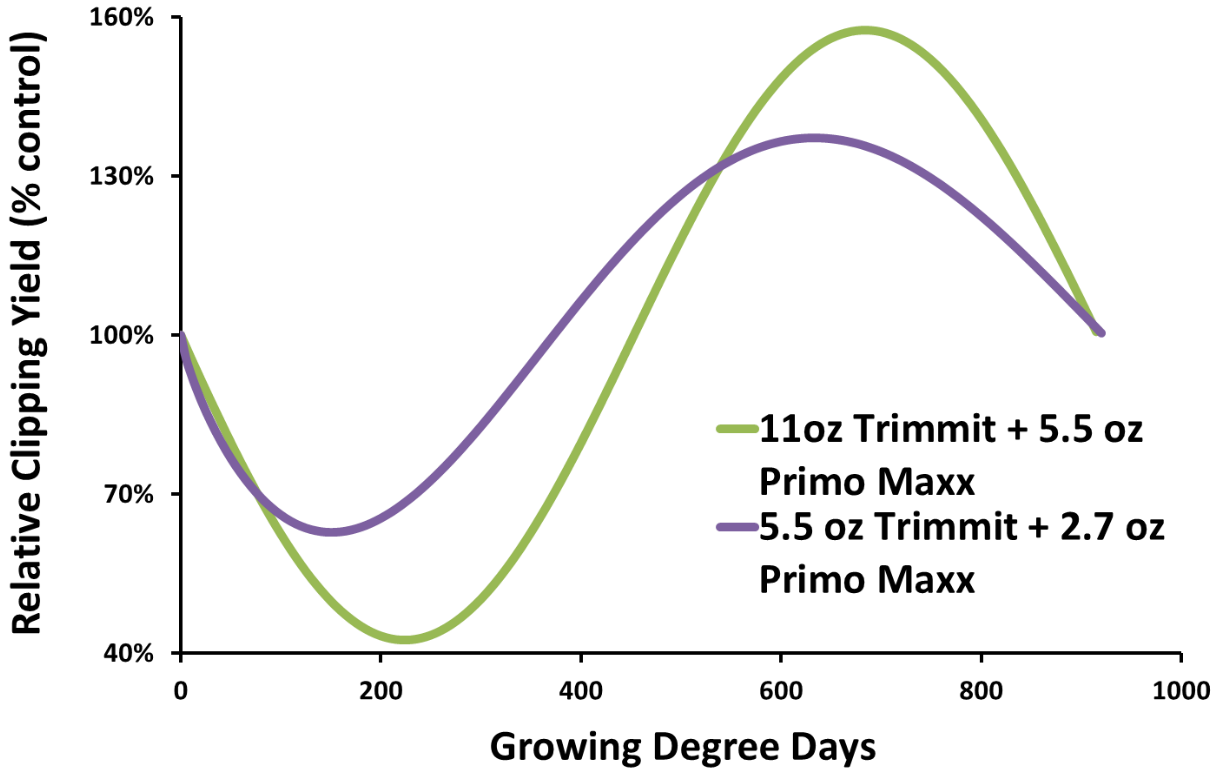


Figure 2. Mixture of trinexapac-ethyl (Primo Maxx) and paclobutrazol (Trimmit 2SC) change the amplitude and period of the sinewave regression model (relative yield = amplitude*sin(2π*GDD/period+ π)+1.

USGA ID#: 2016-17-567

Title: Assessment of Topdressing Sands and Associated Cultural Practices used to Manage Ultradwarf Bermudagrass Greens

Project Leaders: K. McInnes and B. Wherley
Affiliation: Texas A&M University, College Station, Texas

Start Date: 2016
Project Duration: 4 years
Total Funding: $85,501

Summary Points:

- Infiltration rates of putting green surfaces were less than expected from the particle size distribution of the sand alone near the surface.
- For the sand in the surface 1 inch of putting greens, the three way interaction OM:GMD2:e-Cu on infiltration rate was significant at the 0.001 level, and when combined with OM alone, 60% of the variability in infiltration rate was explained.
- For the sand used to topdress putting greens, the three way interaction OM:GMDtds2:e-Cu,tds on infiltration rate was not statistically significant, and when combined with OM alone, about 10% of the variability in infiltration rate was explained.
- Principal Component Analysis showed OM, GMD2, and e-Cu acted on the first two principal components in opposing directions. The first two principal components explain 90% of the variability in the factors.

Summary Text:

An additional thirteen golf courses were sampled since our 2018 report. This sampling involved 39 infiltration measurements and 108 core samples (540 subsamples from cores). Infiltration rates, apparent total porosity, and apparent capillary porosity of the putting greens were measured in addition to sieve analyses of particle size distribution of sand in the putting green profile and of sand used for topdressing.

For all measurements, a 15-cm diameter permeameter was used to test in situ infiltration rates and near-surface water retentions in the putting greens. The permeameter was 30 cm in total height and is inserted into a green so that half is below the surface. In operation, fifteen cm of water is added to the permeameter and allowed to infiltrate then a second 15 cm depth of water is added and allowed to infiltrate. During the second run, infiltration rate was determined from the recorded change in depth of water in the permeameter with time. After this second aliquot had infiltrated, the surface water content (0 to 3 inch and 0 to 6 inch) was measured for one hour to estimate the effective capillary porosity after drainage. Then, three cores from the surface of the green to the drainage gravel were removed for analyses. The cores were split into subsamples of 0 to 1, 1 to 2, 2 to 3, 3 to 6, 6 to 12 inch depth. If the depth to gravel exceeded 12 inches, that layer was collected and analyzed in addition. The surface 3 layers had organic matter content estimated by loss on combustion. Particle size characteristics were determined from standard sieve analysis.

The Krumbein and Monk (1943) equation was used to relate the saturated hydraulic conductivity Ksat from standard particle size analysis of pure sand. The estimation predicts Ksat of a sand to be proportional to the square of the square of the geometric mean diameter GMD2 of its particles and to an exponentiation of the sand's coefficient of uniformity Cu.

$$Ksat \propto GMD2 \cdot e\text{-}Cu$$

Arya et al. (2010) measured the paricle sized distributions and saturated hydraulic conductivity of several sands used on golf courses. For sands meeting USGA recommendation for particle size distribution and having similar Cu, their data show that

$$Ksat \cong 11 + 670 \cdot GMD2,$$

where Ksat has units [in h-1] and GMD2 has units [mm2]. In data used in our 2018 report, all putting green samples fell below this estimate. This was true for the additional thirteen couses sampled in the past year. Presumably these differences arose from the influence of organic matter in clogging of hydraulically active pores. As well known, particle size of sand is not the only factor controlling infiltration rate. For example, less than 1% of the variability in infiltration rate on greens could be explained by variation in particle size (GMD2), whereas 14% of the variability could be explained by the amount of organic matter (OM). However, near 60% of the variability in infiltration rate could be explained by adding a three way interaction term OM:GMD2:e-Cu to OM as factors (Table 1). Interestingly, only about 10% of the variability in infiltration rate could be explained when the GMDtds2 and e-Cu,tds of the topdressing sand were substituted for those same properties of the surface 1 inch of sand in the green (Table 2). The interaction of OM, GMD2, and e-Cu also can be seen in a Principal Component Analysis of the three variables where the first two principal components explain 90% of the variability in the factors (Tables 3 and 4). A biplot showed that the three factors act on the first two principal components in opposing directions (Figure 1). This explains, in part, the lack of significant correlations between infiltration rate and any one of the three single factors. Addition of apparent total porosity, as estimated by maximum observed volumetric water content (MaxVWC), as a factor in the Principal Component Analysis showed this new factor acted in the same direction on the biplot as OM, indicating a positive correlation between the two factors (Figure 2).

A plot of the GMD of the sand found in the top 1 inch of the putting greens with the GMD of the sand used for topdressing suggested that larger particles in the topdressed sands were being removed with cultural practices such as mowing and verticutting (Figure 3). On closer inspection of the GMD of the sand in layers 3 to 6 and 6 to 12 inches, it appears that many of the courses were constructed with sand finer than that currently being used for topdressing.

We are continuing to analyze data and hope to sample a couple more courses that are using fine sand to topdress before our final report at the end of January 2020.

References

Arya, L.M., J.L. Heitman, B.B. Thapa, D.C. Bowman. 2010. Predicting saturated hydraulic conductivity of golf course sand from particle-size distribution. Soil Sci. Soc. Am. J. 74:33-37.

Krumbein, W.C., G.D. Monk. 1943. Permeability as a function of the size parameters of unconsolidated sand. T. Am. I. Min. Met. Eng. 151:153-163.

Table 1. Multiple linear regression coefficients of the model I~OM+OM:GMD2:e-Cu. Organic matter OM (loss on combustion), geometric mean diameter squared GMD2, and the base of the natural logarithm scale e raised to the negative of the coefficient of curvature Cu were all for the surface 1 inch of the putting greens sampled.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-3.81	4.03	-0.946708	0.357
OM	-1.18	0.542	-2.17	0.0444 *
OM:GMD2 :e-Cu	213	45.9	4.64	0.000236 ***

Significance codes: 0.0001 '***', 0.001 '**', 0.01 '*'
Multiple R-squared: 0.62, Adjusted R-squared: 0.58

Table 2. Multiple linear regression coefficients of the model I~OM+OM:GMDtds2:e-Cu,tds. Geometric mean diameter of topdressing sand squared GMDtds2 and the base of the natural logarithm scale e raised to the negative of the coefficient of curvature Cu were for the topdressing sand.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	3.92	6.25	-0.628	0.538
OM	0.711	0.527	1.34	0.197
OM:GMDtds2 :e-	10.2	18.1	0.563	0.581

Significance codes: 0.0001 '***', 0.001 '**', 0.01 '*'
Multiple R-squared: 0.16, Adjusted R-squared: 0.06

Table 3. Importance of principal components of variable controlling infiltration rate of putting greeen sampled:

	PC1	PC2	PC3
Standard deviation	1.2665	1.0535	0.53483
Proportion of Variance	0.5347	0.3699	0.09535
Cumulative Proportion	0.5347	0.9046	1.00000

Table 4. Correlations (-1 to 1) between principal components PC1, PC2, and PC3 with OM, GMDSQ, and EXP(-Cu).

	PC1	PC2	PC3
OM	-0.630	0.484	-0.607
GMDSQ	0.734	0.116	-0.669
Exp(-Cu)	-0.253	-0.867	-0.428

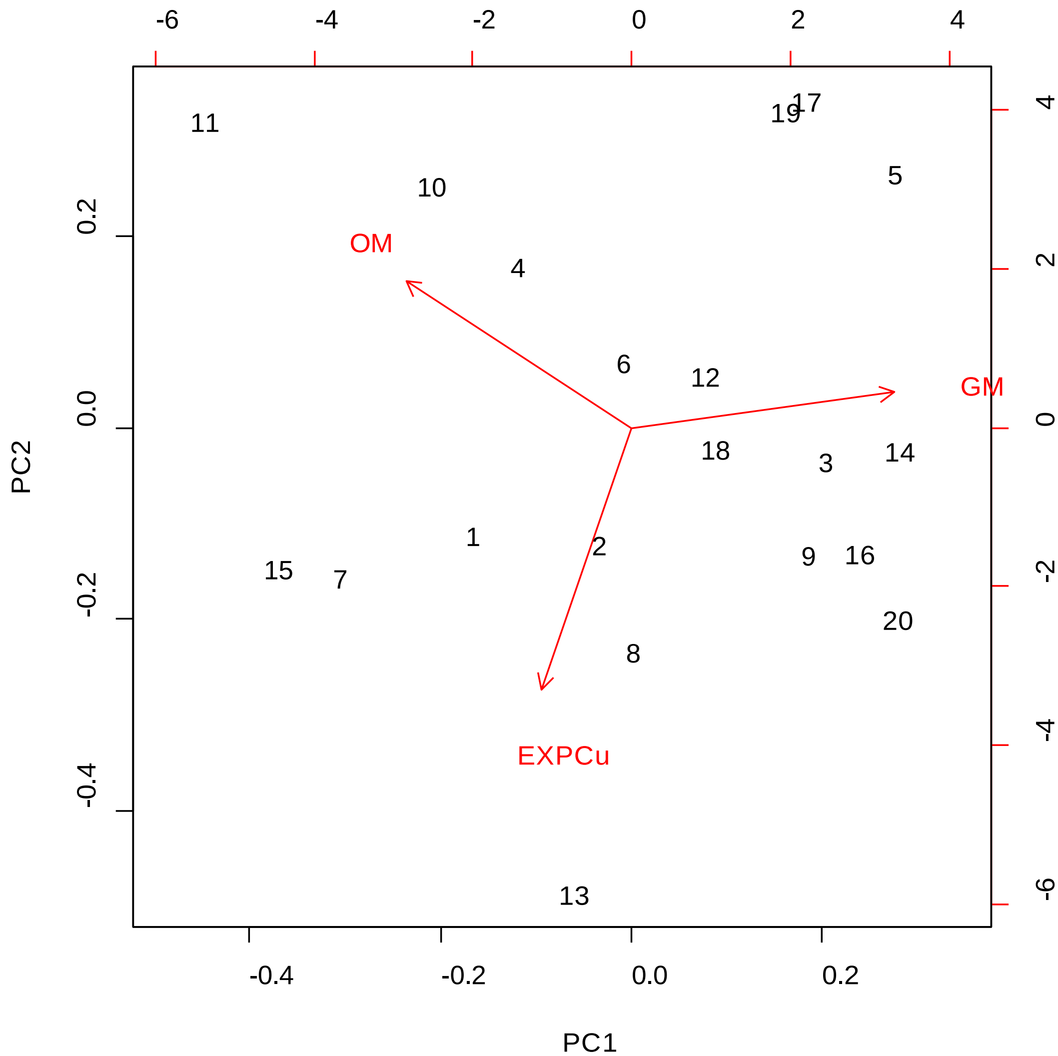


Figure 1. Biplot of Principal Component Analysis of variables controlling infiltration rate of putting greens sampled. Organic matter OM (loss on combustion), geometric mean diameter of sand squared GMD2 (GMDSQ), and the base of the natural logarithm scale e raised to the negative of the coefficient of curvature Cu (EXPCu) were all for the surface 1 inch of the putting greens sampled.

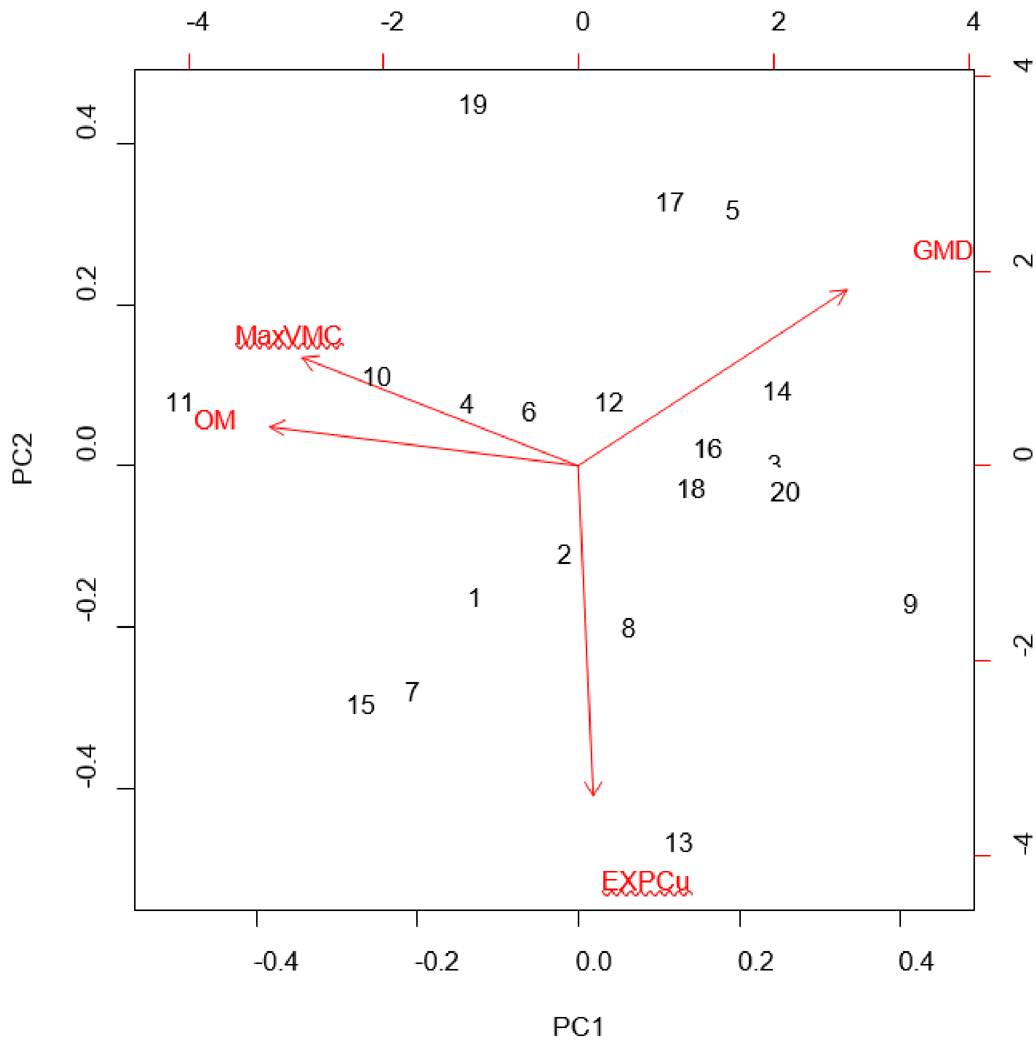


Figure 2. Biplot of Principal Component Analysis of variables controlling infiltration rate of putting greens sampled. Organic matter OM (loss on combustion), maximum volumetric water content (MaxVMC), geometric mean diameter of sand squared GMD2 (GMDSQ), and the base of the natural logarithm scale e raised to the negative of the coefficient of curvature Cu (EXPCu) were all for the surface 1 inch of the putting greens sampled.

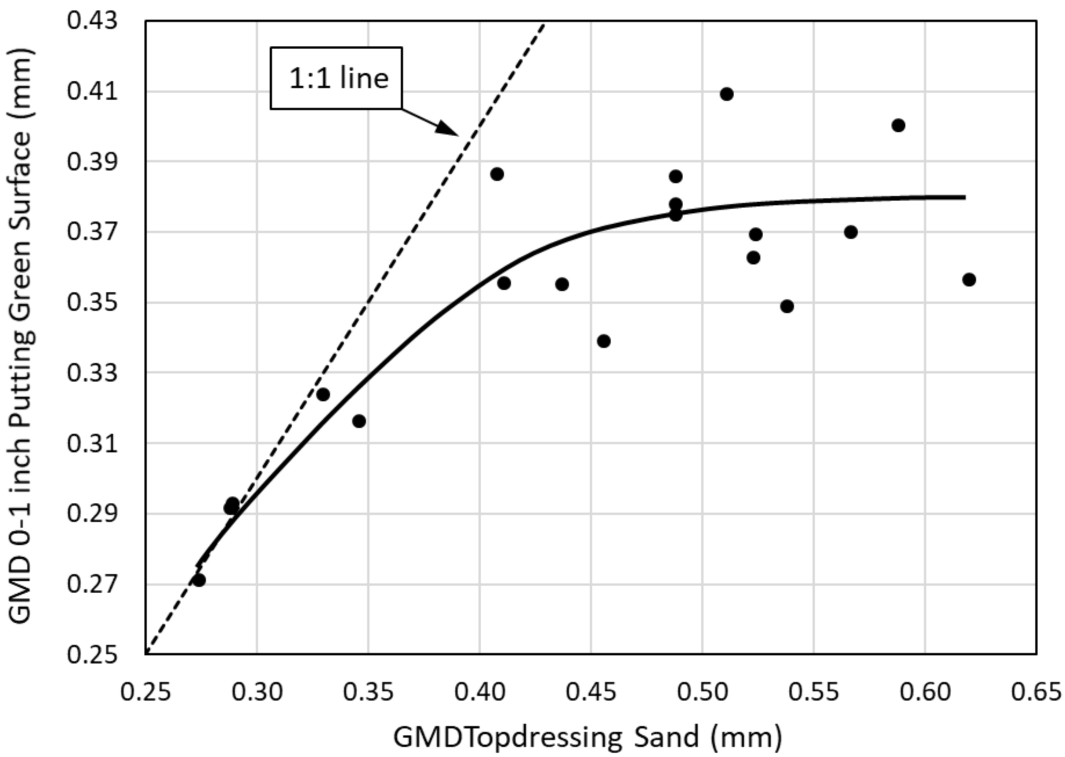


Figure 3. Geometric mean diameter GMD of sands found in the top 1 inch of putting greens compared to the GMD of the topdressing sands currently used by the course sampled.

USGA ID#: 2019-01-671

Title: Physical Properties of Creeping Bentgrass Mat Layers Formed with Differing Sand Sizes

Project Leaders: James A. Murphy and Hui Chen
Affiliation: Department of Plant Biology, Rutgers University

Objectives:

1) Determine the effects of eliminating coarse particles from topdressing sand (subsequently increasing the quantities of medium, fine and very fine particles) on the performance of creeping bentgrass maintained as putting green turf.
2) Assess the impact of core cultivation and backfilling holes with medium-coarse sand to ameliorate the potential negative effects of finer-textured topdressing sands on turf performance and the physical properties at the surface of a putting green root zone.

Start Date: 2019
Project Duration: 1 year
Total Funding: $40,000

Summary Points:

- Topdressing with medium-fine and fine-medium sands increased the fineness of sand within the mat layer, which explains the slower water infiltration of plots topdressed with sands containing a greater amount of fine sand.
- The much greater fine sand content in the mat layer of plots topdressed with fine-medium sand explains the greater capillary porosity measured in the mat layer of those plots as well as the wetter plots surfaces (VWC) observed during 2019.
- Core cultivation was capable of offsetting the effects of topdressing with finer sands. Water infiltration rate was increased by core cultivation and backfilling with a medium-coarse sand. Additionally, the surface wetness of plots was significantly drier on plots that were core cultivated.
- The organic matter concentration (% by weight) was decreased (diluted) by topdressing; however, mass-content of organic matter (kg/m^2) in the mat layer was either increased or unchanged by topdressing. Core cultivation reduced both the concentration and mass-content of organic matter in the mat layer.
- Vegetative cover of plots (normalized difference vegetation index [NDVI]) was lower on core cultivated plots compared to non-cultivate during 2019. Similarly, plots topdressed at 100 lb/1,000-ft^2 had lower NDVI values than plots topdressed at 50 lb/1,000 ft^2 during 2019. Thus, vegetative cover was lower under more aggressive management of thatch accumulation with core cultivation and topdressing.

Summary Text:

Sand topdressing of putting greens during the season is often avoided due to the potential of coarse sand particles interfering with play and dulling mower blades. This project evaluated the effect of topdressing sand size on the performance of putting green turf.

Materials and Methods

The trial was initiated in May 2016 on a 19-month-old 'Shark' creeping bentgrass maintained at 2.8-mm on a sand-based root zone. A 3 x 2 x 2 factorially arranged randomized complete block design with four replications included the factors of sand size (medium-coarse, medium-fine, fine-medium), quantity of mid-season topdressing (50- and 100-lb / 1,000-ft^2 every 10 to 14 days from June through October), and cultivation (non-cultivated or core cultivated plus backfill in 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 sand met USGA recommendations for construction; whereas the fine sand content of the medium-fine and fine-medium sands exceeded USGA recommendations and contained little to no coarse particles (Table 2).

Data collection included: clippings to determine the quantity and particle size distribution of sand collected during mowing; falling-head infiltration rate; surface hardness (Clegg Soil Impact Tester, 0.5-kg and 2.25-kg missiles); surface firmness (penetration depth); mat layer thickness, organic matter content (loss on ignition), pore size distribution, and sand particle size distribution; volumetric water content (VWC) of the surface 0- to 38-mm and 0- to 76-mm depth zones; visual rating of turf color, density and quality; and normalized difference vegetation index (NDVI).

Results

Infiltration Rate (Table 3)

Infiltration rate was strongly affected by sand size and cultivation (Table 3). Plots topdressed with medium-coarse and medium-fine sand had greater infiltration rates than plots topdressed with fine-medium sand. Infiltration rate was greater in core cultivated plots compared to non-cultivated. Additionally, the 3rd inch of water infiltration indicated that sand size had a greater effect on infiltration of non-cultivated plots than core cultivated plots in both 2018 and 2019 (Table 4).

Mat-layer thickness and organic matter concentration (Table 5)

Topdressing increased the mat layer depth and promoted organic matter accumulation (mass-content, kg/m^2) in the mat layer, while the diluting effect of adding sand to the mat layer reduced the organic matter concentration [% by weight] compared to non-topdressed controls (Table 5). Similar responses to the pooled topdressing effect were observed on core cultivated plots; however, it was apparent that core-cultivation plus backfilling holes with sand masked the effects of topdressing.

Mid-season topdressing rate and cultivation were the factors that influenced development of the mat layer in topdressed plots (Table 5). Plots topdressed at 100 lb/1,000-ft^2 developed a thicker mat layer depth while reducing the organic matter concentration [% by weight] compared to plots topdressed at 50 lb/1,000-ft^2 (Table 6). Greater organic matter accumulation (mass-content, kg/m^2) occurred in plots topdressed at 100 lb./1,000-ft^2 than 50 lb/1,000-ft^2 in 2017 and 2018, but not 2019. Core cultivated plots reduced mat layer depth and decreased organic matter accumulation (mass-content, kg/m^2) compared to non-cultivated plots in 2018 and 2019. There was a substantial reduction of the organic matter concentration (% by weight) in core cultivated plots compared to non-cultivated plots in all years measured.

Mat-layer sand size distribution (Table 7)

Among topdressing treatments, topdressing sand size affected the sand size distribution within the mat layer, and the effect of sand size depended on the cultivation factor (Table 7). The sand size distribution within the mat became finer as the topdressing sand increased in fine sand content (Table 8). Plots that were core cultivated and backfilled with medium-coarse sand reduced the tendency to increase the fineness of sand in the mat layer of plots topdressed with sand containing more than 20% fine sand. The fine sand content of plots topdressed with fine-medium sand and have not been cultivated was 37.3% (by weight) in May 2019; core cultivation and backfilled lowered the fine sand content to 25.5%.

Mat-layer pore size distribution (Table 9)

Porosity with the mat layer was affected by all factors (Table 9). Total porosity was reduced as topdressing rate increased and core cultivated was practiced on plots. Air-filled porosity was reduced by topdressing with fine-medium sand and increased in plots that were core cultivated. Capillary porosity (water retention) was reduced as the rate of topdressing increased. Capillary porosity increased as the fineness of topdressing sand increased; however, only the fine-medium sand decreased capillary porosity when plots were core-cultivated and backfilled with coarse-medium sand (Table 10).

Normalized difference vegetation index (NDVI; Figures 1-2)

The NDVI (vegetative cover) of creeping bentgrass turf was frequently lower for plots topdressed at 100 lb/1,000-ft^2 compared to 50 lb/1,000 ft^2 throughout 2019 (Figure 1). Similarly, the NDVI of creeping bentgrass turf was lower on core cultivated plots compared to non-cultivated plots throughout 2019 (Figure 2).

Volumetric water content (Figures 3-9)

Under non-cultivated conditions, the pooled-topdressing effect reduced surface wetness (VWC at the 0 to 3.0-inch depth) compared to the non-topdressed control (Figure 3). However, under core cultivated conditions, this drying effect of topdressing was observed less often probably because the core cultivation treatment was highly effective at lowering VWC throughout the year (Figure 4).

Plots topdressed with medium-fine sand at 100 lb/1,000 ft^2 occasionally had a lower VWC than plots that received 50 lb/1,000 ft^2 in 2019 (Figure 5). A similar effect was observed on 14 of 70 dates in 2018 (data not shown). Surface wetness of plots topdressed with either medium-coarse or fine-medium sand was not affected by topdressing rate (Figures 6 and 7).

The effect of sand size on surface wetness also depended on the cultivation factor during 2019. Without core cultivation, topdressing with either medium-coarse or medium-fine sand produced a substantially drier surface compared to plots topdressed with fine-medium sand (Figure 8). However, these differences among sand sizes were less prominent when plots were core cultivated due to core cultivation being highly effective at reducing surface VWC (Figure 9).

Table 1. Individual treatment combinations of sand size, topdressing rate, and cultivation as well as two controls (no mid-season topdressing) being evaluated on a 'Shark' creeping bentgrass turf grown on a sand-based rootzone.

	Factors in the Experiment			
Treatment No.	Sand Size†	Topdressing Rate during Mid- Season‡	Cultivation¶	Annual Quantity of Sand Applied
		lbs. / 1,000 sq. ft.		lbs. / 1,000 sq. ft.
1	Medium-coarse	50	Non-cored	1,200
2	Medium-coarse	50	Core + Backfill	1,700
3	Medium-coarse	100	Non-cored	1,700
4	Medium-coarse	100	Core + Backfill	2,200
5	Medium-fine	50	Non-cored	1,200
6	Medium-fine	50	Core + Backfill	1,700
7	Medium-fine	100	Non-cored	1,700
8	Medium-fine	100	Core + Backfill	2,200
9	Fine-medium	50	Non-cored	1,200
10	Fine-medium	50	Core + Backfill	1,700
11	Fine-medium	100	Non-cored	1,700
12	Fine-medium	100	Core + Backfill	2,200
13	None	0	Non-cored	0
14	None	0	Core + Backfill	1,200

†, First-mentioned size class represent the predominant size fraction in the sand.

‡, Topdressing applied every two weeks from 10 June through 12 October (10 applications). Topdressing at 50 lbs. per 1,000 sq. ft. represented a 'dusting' quantity (O'Brien and Hartwiger, 2003); whereas, topdressing at 100 lbs. filled the surface thatch and lower verdure layers.

¶, Core cultivation to the 1½-inch depth was performed twice a year (10 May and 2 November) using ½-inch diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with 600 lbs. per 1,000 sq. ft. of medium-coarse sand. At the time of coring, non-cultivated plots were topdressed with the respective sand size at 400 lbs. per 1,000 sq. ft. to fill the surface thatch and verdure layers to the same extent as on 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.

	Particle Diameter (mm)/Size Class†				
Topdressing Sand Size	2.0-1.0 V. Coarse	1.0-0.5 Coarse	0.5-0.25 Medium	0.25-0.15 Fine	0.15-0.05 V. Fine
	------------------------------- % retained (by weight) -------------------------------				
Medium-coarse	0	33.8	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‡	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

† 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

‡ Size distribution of sand in 45 core samples of the mat layer collected before the initiation of treatments in May 2016.

Table 3. Infiltration rate as affected by topdressing with three sand sizes applied at two rates on creeping bentgrass turf maintained at 2.8-mm during 2018 and 2019.

	2018			2019		
Source of Variation	1st inch of Water	2nd inch of Water	3rd inch of Water	1st inch of Water	2nd inch of Water	3rd inch of Water
Sand Size (SS)	***	***	***	***	***	***
Topdressing Rate (TR)	ns	ns	ns	ns	ns	ns
SS*TR	ns	ns	ns	ns	ns	ns
Core Cultivation (CC)	***	***	***	***	***	***
SS*CC	ns	ns	*	ns	ns	*
TR*CC	ns	ns	ns	ns	ns	ns
SS*TR*CC	ns	ns	ns	ns	ns	ns
Main Effects						
Sand Size	--------------- inches/hour ---------------					
Medium-coarse	10.7	6.7	6.4	12.6	8.8	8.5
Medium-fine	8.3	6.1	5.8	11.5	8.5	8.1
Fine-medium	6.0	3.9	3.6	8.0	5.6	5.5
$LSD_{0.05}$	1.9	0.9	1.0	2.1	1.2	1.0
Topdress Rate						
50 lbs./1,000-ft^2	7.8	5.5	5.3	10.3	7.4	7.1
100 lbs./1,000-ft^2	8.8	5.6	5.2	11.1	8.0	7.6
Cultivation						
Non-cultivated	5.8	4.1	3.8	7.8	5.1	5.0
Core Cultivated	9.8	7.0	6.7	13.6	10.2	9.7

* Significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; *** significant at $p \leq 0.001$; NS: nonsignificant

† A falling-head infiltration rate was measured three times per plot using one double-ring falling-head infiltrometer (20-inch diameter outer ring and 12-inch diameter inner ring) in August 2018 and 2019. One-inch of water was applied to the inner and outer rings and the time required to infiltrate all water in the inner ring was recorded, after which the time to infiltrate a second- and then a third-inch of water in the inner ring were also recorded.

Table 4. Infiltration rate affected by the sand size by topdressing rate and sand size by core cultivation interactions in August 2018 and 2019.

Interacting Factors		Water Infiltration†	
Sand Size	Mid-season Topdressing Rate	3rd inch of Water	
Cultivation	Sand Size	2018	2019
Non-cultivated	Medium-coarse	5.1	6.7
Non-cultivated	Medium-fine	4.1	5.8
Non-cultivated	Fine-medium	2.3	2.5
Core Cultivated	Medium-coarse	7.8	10.3
Core Cultivated	Medium-fine	7.4	10.3
Core Cultivated	Fine-medium	4.9	8.5
	$LSD_{0.05}$	1.4	1.4

† Falling-head infiltration rate was measured three times per plot using one double-ring falling-head infiltrometer (20-inch diameter outer ring and 12-inch diameter inner ring) in August 2018 and 2019. One-inch of water was applied to the inner and outer rings and the time required to infiltrate all water in the inner ring was recorded, after which the time to infiltrate a second- and then a third-inch of water in the inner ring were also recorded.

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Table 5. Mat layer depth, organic matter and sand mass-content, and organic matter concentration by weight in May 2017, 2018 and 2019 as affected by sand size, topdressing rate and core cultivation on a creeping bentgrass turf maintained at 2.8-mm.

	2017 Mat Layer[¶]				2018 Mat Layer				2019 Mat Layer			
		Mass-content				Mass-content				Mass-content		
Orthogonal Contrasts	Depth[†]	OM[‡]	Sand	OM	Depth	OM	Sand	OM	Depth	OM	Sand	OM
	mm	---- kg/m^2 ----		% (kg/kg)	mm	---- kg/m^2 ----		% (kg/kg)	mm	---- kg/m^2 ----		% (kg/kg)
Non-cultivated:												
Pooled-topdressed	17.2*	0.98*	11.1*	6.7	20.4*	1.21*	15.4*	5.9	30.0*	1.59	27.1	6.1
vs. Non-topdressed	15.9	0.83	9.3	8.5*	13.2	0.92	7.5	8.6*	21.4	1.57	11.5	13.4*
Core Cultivated:												
Pooled-topdressed	16.9*	0.86*	14.2*	5.7	19.3*	0.96*	20.2*	4.6	27.2*	1.40	27.1	4.9
vs. Non-topdressed	15.0	0.80	13.9	6.8*	15.9	0.82	19.4	5.1*	23.0	1.37	20.3	6.5*
Source of Variation	-------------------------------- probability of significant *F* test -------------------------------------											
Sand Size (SS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Topdress Rate (TR)	***	**	***	***	***	***	***	***	***	ns	ns	***
SS*TR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Core Cultivation (CC)	ns	ns	ns	***	*	***	***	***	***	***	ns	***
SS*CC	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns
TR*CC	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns
SSS*TR*CC	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns

* Significant at p≤0.05; ** significant at p≤0.01; *** significant at p≤0.001; ns: not significant

¶ Forty-five core samples (1-inch diam.) of the mat layer were collected from across the trial area in May 2016 before treatment initiation. Four core samples (3-inch diam.) of the mat layer were collected from each plot in May 2017, 2018, and 2019. The thickness, organic matter content (loss on ignition) and sand particle size distribution of core samples were measured

† Average mat layer depth was 6.3-mm at the initiation of treatments in May 2016.

‡ Average organic matter mass-content of mat layer was 0.82-kg/m^2 at the initiation of treatments in May 2016.

Table 6. Mat layer depth, organic matter and sand mass-content, and organic matter concentration by weight responses in May 2017, 2018 and 2019 to mid-season topdressing rate and core cultivation main effects on a creeping bentgrass turf maintained at 2.8-mm.

	2017 Mat Layer[¶]				2018 Mat Layer				2019 Mat Layer			
		Mass-content				Mass-content				Mass-content		
Main effect	Depth [†]	OM[‡]	Sand	OM Conc.	Depth	OM	Sand	OM Conc.	Depth	OM	Sand	OM Conc.
	mm	---- kg/m^2 ----		% (kg/kg)	mm	---- kg/m^2 ----		% (kg/kg)	mm	---- kg/m^2 ----		% (kg/kg)
Topdress Rate												
50 lbs./1,000-ft^2	16.5	0.90	12.9	6.5	18.8	1.06	18.2	5.6	27.5	1.59	25.0	6.0
100 lbs./1,000-ft^2	17.7	0.94	15.2	6.0	20.9	1.11	21.3	5.0	29.8	1.58	29.2	5.1
$LSD_{0.05}$	0.6	0.03	0.7	0.2	0.9	0.05	0.7	0.2	0.7	ns	0.7	0.2
Cultivation												
Non-cultivated	17.2	0.98	13.9	6.7	20.4	1.21	19.4	5.9	30.0	1.78	27.1	6.1
Core Cultivated	16.9	0.86	14.2	5.7	19.3	0.96	20.2	4.6	27.2	1.39	27.1	4.9
$LSD_{0.05}$	ns	ns	ns	0.2	0.9	0.05	0.7	0.2	0.7	0.06	ns	0.2

¶ Forty-five core samples (1-inch diam.) of the mat layer were collected from across the trial area in May 2016 before treatment initiation. Four core samples (3-inch diam.) of the mat layer were collected from each plot in May 2017, 2018, and 2019. The thickness, organic matter content (loss on ignition) and sand particle size distribution of core samples were measured.

† Average depth of the mat layer was 6.3-mm at the initiation of treatments in May 2016.

‡ Average organic matter mass-content of the mat layer was 0.82-kg/m^2 at the initiation of treatments in May 2016.

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Table 7. Particle size distribution of sand within the mat layer of creeping bentgrass turf in May 2017, 2018, and 2019 as affected by sand size, topdressing rate and core cultivation.

	2017 Sand Size Distribution†				2018 Sand Size Distribution				2019 Sand Size Distribution			
Source of Variation	Very Coarse	Coarse +Medium	Fine	Very Fine	Very Coarse	Coarse +Medium	Fine	Very Fine	Very Coarse	Coarse +Medium	Fine	Very Fine
	---------- probability of significant *F* test ----------											
Sand Size (SS)	ns	***	***	***	ns	***	***	***	ns	***	***	***
Topdress Rate (TR)	ns	*	ns	***	ns	ns	ns	ns	**	ns	ns	ns
SS*TR	ns	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns
Core Cultivation (CC)	ns	***	***	***	ns	***	***	**	**	***	***	***
SS*CC	ns	***	***	***	ns	***	**	**	ns	***	***	***
TR*CC	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SSS*TR*CC	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at p≤0.05; ** significant at p≤0.01; *** significant at p≤0.001; ns: not significant

† Four core samples (3-inch diam.) of the mat layer were collected from each plot in May 2017, 2018 and 2019; organic matter was removed (loss on ignition) and sand particle size distribution measured. Very Coarse represents 1-2 mm; Coarse represents 0.5-1 mm; Medium represents 0.25-0.5 mm; Fine represents 0.25-0.15 mm; Very Fine represents 0.15-0.05 mm

Table 8. Particle size distribution of sand within the mat layer of creeping bentgrass turf in May 2017, 2018, and 2019 as affected by the interaction of sand size and topdressing rate, and sand size and core cultivation.

Interacting Factors		2017 Sand Size Distribution†				2018 Sand Size Distribution				2019 Sand Size Distribution			
Sand Size	Topdressing Rate	Very Coarse	Coarse +Medium	Fine	Very Fine	Very Coarse	Coarse +Medium	Fine	Very Fine	Very Coarse	Coarse	Fine	Very Fine
	lbs./1,000-ft^2	----------	----------	----------	----------	----------	% by weight	----------	----------	----------	----------	----------	----------
Med.-coarse	50	3.2	75.5	17.9	3.3	2.1	79.5	15.9	2.5	1.4	81.3	15.0	2.3
Med.-coarse	100	3.6	76.3	16.9	3.2	2.9	82.2	13.0	1.9	1.3	83.0	13.6	2.1
Med.-fine	50	3.2	71.8	21.2	3.7	2.2	76.1	18.9	2.8	1.6	75.6	19.2	3.6
Med.-fine	100	2.9	72.3	21.2	3.6	2.1	76.6	18.8	2.5	1.3	76.6	19.4	2.7
Fine-med.	50	3.6	62.0	26.6	7.7	2.3	62.2	28.2	7.3	1.4	58.3	30.4	9.9
Fine-med.	100	3.1	57.5	29.7	9.6	1.5	58.8	30.8	8.8	1.0	56.0	32.4	10.6
	$LSD_{0.05}$	ns	1.1	1.2	0.6	ns	3.1	ns	ns	ns	ns	ns	ns
Sand Size	Cultivation												
Med.-coarse	Non-cultivated	3.2	76.2	17.4	3.2	1.9	81.2	14.8	2.1	1.4	81.6	14.7	2.3
Med.-coarse	Core Cultivated	3.6	75.7	17.4	3.3	3.0	80.6	14.1	2.3	1.2	82.7	13.9	2.1
Med.-fine	Non-cultivated	3.1	70.0	22.9	4.0	2.4	73.7	20.9	3.0	1.7	73.6	21.6	3.2
Med.-fine	Core Cultivated	3.0	74.1	19.5	3.4	1.9	79.0	16.7	2.4	1.4	81.6	14.7	2.3
Fine-med.	Non-cultivated	4.0	54.1	31.4	10.5	2.0	52.9	35.0	10.1	1.3	48.2	37.3	13.1
Fine-med.	Core Cultivated	2.7	65.5	25.0	6.9	1.7	68.2	24.0	6.0	1.1	66.1	25.5	7.3
	$LSD_{0.05}$	ns	3.1	1.2	0.6	ns	3.1	1.8	1.5	ns	3.9	2.6	1.6

† Four core samples (3-inch diam.) of the mat layer were collected from each plot in May 2017, 2018 and 2019; organic matter was removed (loss on ignition) and sand particle size distribution measured. Very Coarse represents 1-2 mm; Coarse represents 0.5-1 mm; Medium represents 0.25-0.5 mm; Fine represents 0.25-0.15 mm; Very Fine represents 0.15-0.05 mm.

Table 9. Pore size distribution within the mat layer of creeping bentgrass turf in May 2019 as affected by sand size, topdressing rate and core cultivation.

	2019		
Source of Variation	Total Porosity	Air-filled Porosity	Capillary Porosity
Sand Size (SS)	*	***	***
Topdressing Rate (TR)	***	ns	***
SS*TR	ns	ns	ns
Core Cultivation (CC)	***	***	***
SS*CC	ns	ns	*
TR*CC	ns	ns	ns
SS*TR*CC	ns	ns	ns
Main Effects			
Sand Size		%	
Medium-coarse	60.1	15.9	44.2
Medium-fine	60.1	15.1	45.1
Fine-medium	60.8	12.6	48.2
$LSD_{0.05}$	0.6	0.8	0.9
Topdress Rate			
50 lbs./1,000-ft^2	61.6	14.5	47.1
100 lbs./1,000-ft^2	59.1	14.6	44.5
Cultivation			
Non-cultivated	61.7	12.6	49.0
Core Cultivated	59.0	16.4	42.6

* Significant at p≤0.05; ** significant at p≤0.01; *** significant at p≤0.001; NS: nonsignificant

† Total porosity was calculated by [1 – (bulk density/particle density)] *100%; air-filled porosity was determined by weight loss at 30-cm tension and capillary porosity was determined by (total porosity – air-filled porosity).

Table 10. Pore size distribution of sand within the mat layer of creeping bentgrass turf in May 2019 as affected by the interaction of sand size and core cultivation

Interacting Factors				
Sand Size	Mid-season Topdressing Rate	Total Porosity	Air-filled Porosity	Capillary Porosity
Cultivation	Sand Size		%	
Non-cultivated	Medium-coarse	61.3	14.4	46.8
Non-cultivated	Medium-fine	61.2	13.0	48.2
Non-cultivated	Fine-medium	62.5	10.4	52.1
Core Cultivated	Medium-coarse	58.9	17.3	41.6
Core Cultivated	Medium-fine	59.1	17.1	41.9
Core Cultivated	Fine-medium	59.1	14.8	44.3
	$LSD_{0.05}$	ns	ns	1.3

† Total porosity was calculated by [1 – (bulk density/particle density)] *100%; air-filled porosity was determined by weight loss at 30-cm tension and capillary porosity was determined by 100% - air-filled porosity

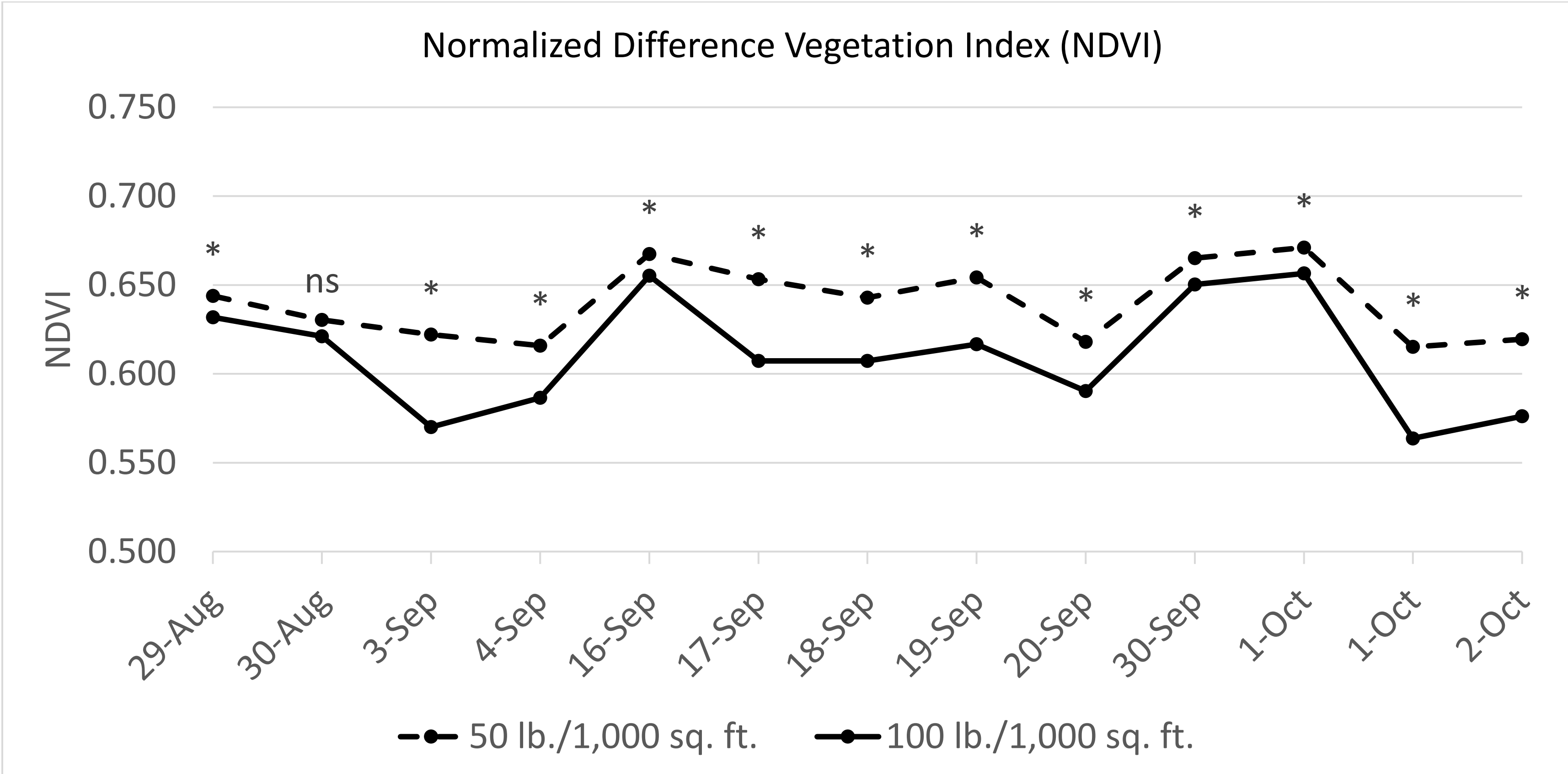


Figure 1. Effect of topdressing rate on normalized difference vegetation index (NDVI) of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. NDVI measured using RapidSCAN CS-45 (Holland Scientific) every 14 to 28 days. Core cultivation performed on 16 May and 23 October 2019.

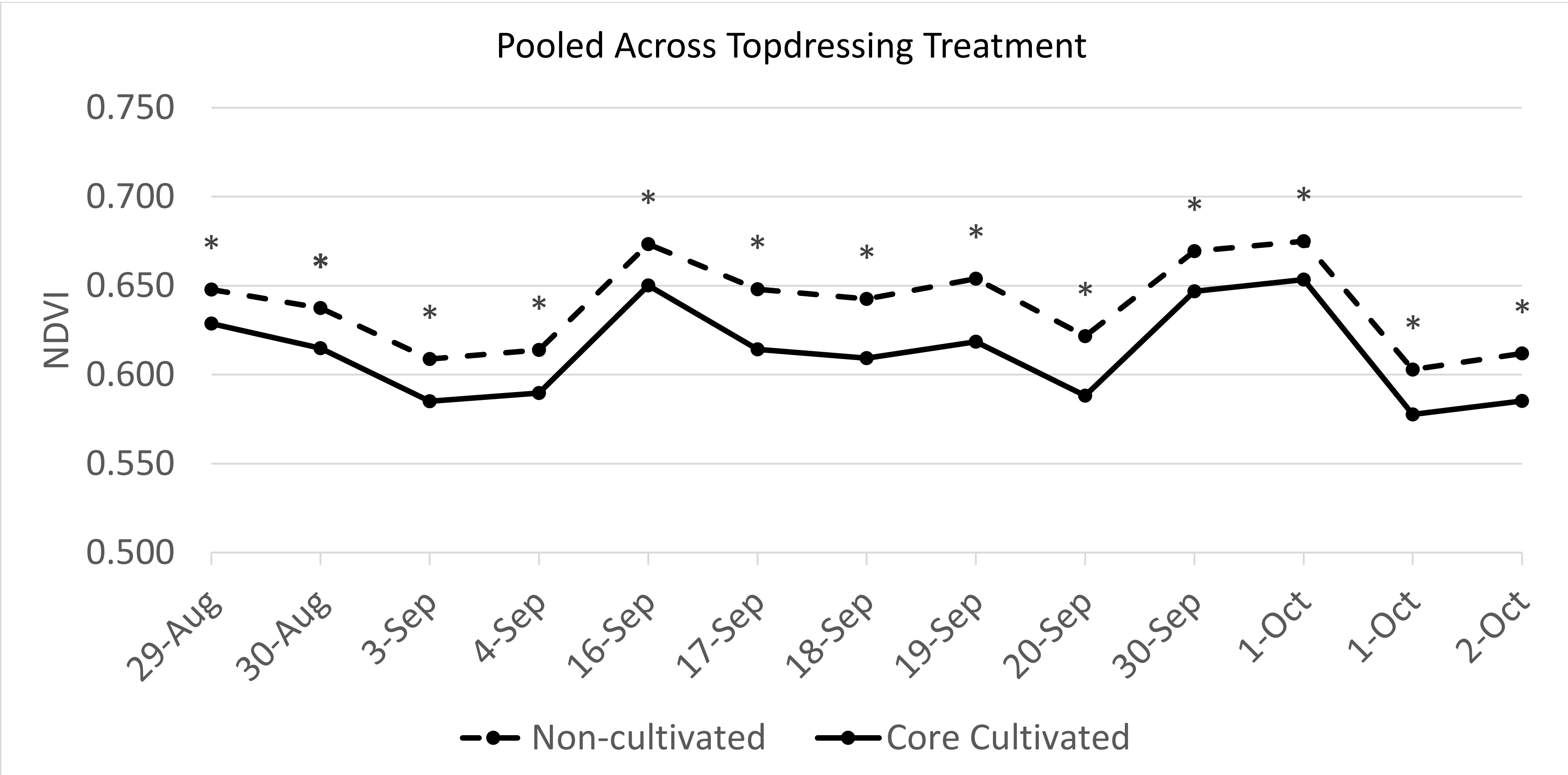


Figure 2. Effect of core cultivation on normalized difference vegetation index (NDVI) of a ‘Shark’ creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. NDVI measured using RapidSCAN CS-45 (Holland Scientific) every 14 to 28 days. Core cultivation performed on 16 May and 23 October 2019.

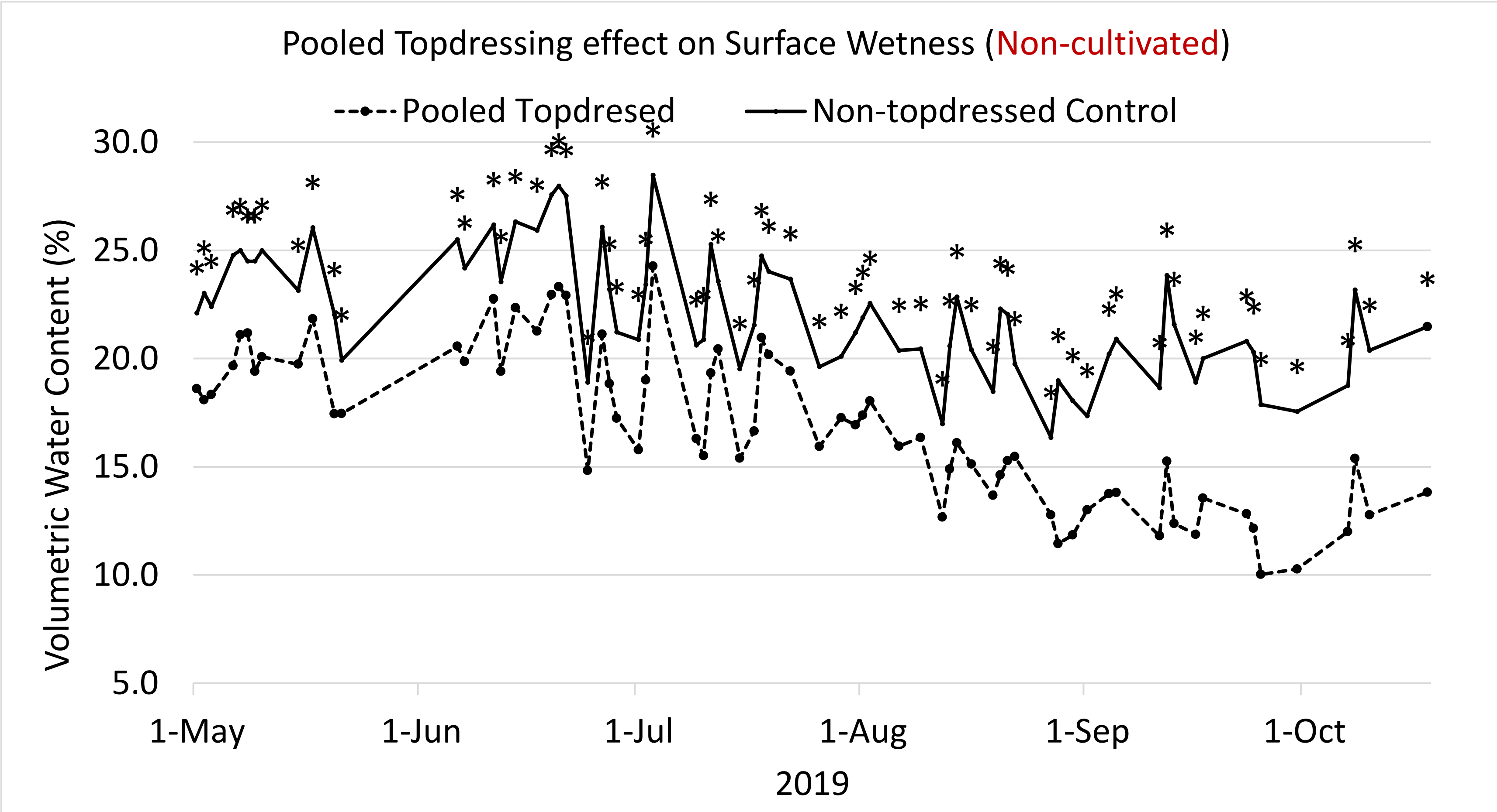


Figure 3. The volumetric water content of the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm for the pooled topdressing effect compared to the non-topdressed control under non-cultivated conditions during 2019 in North Brunswick, NJ. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content.

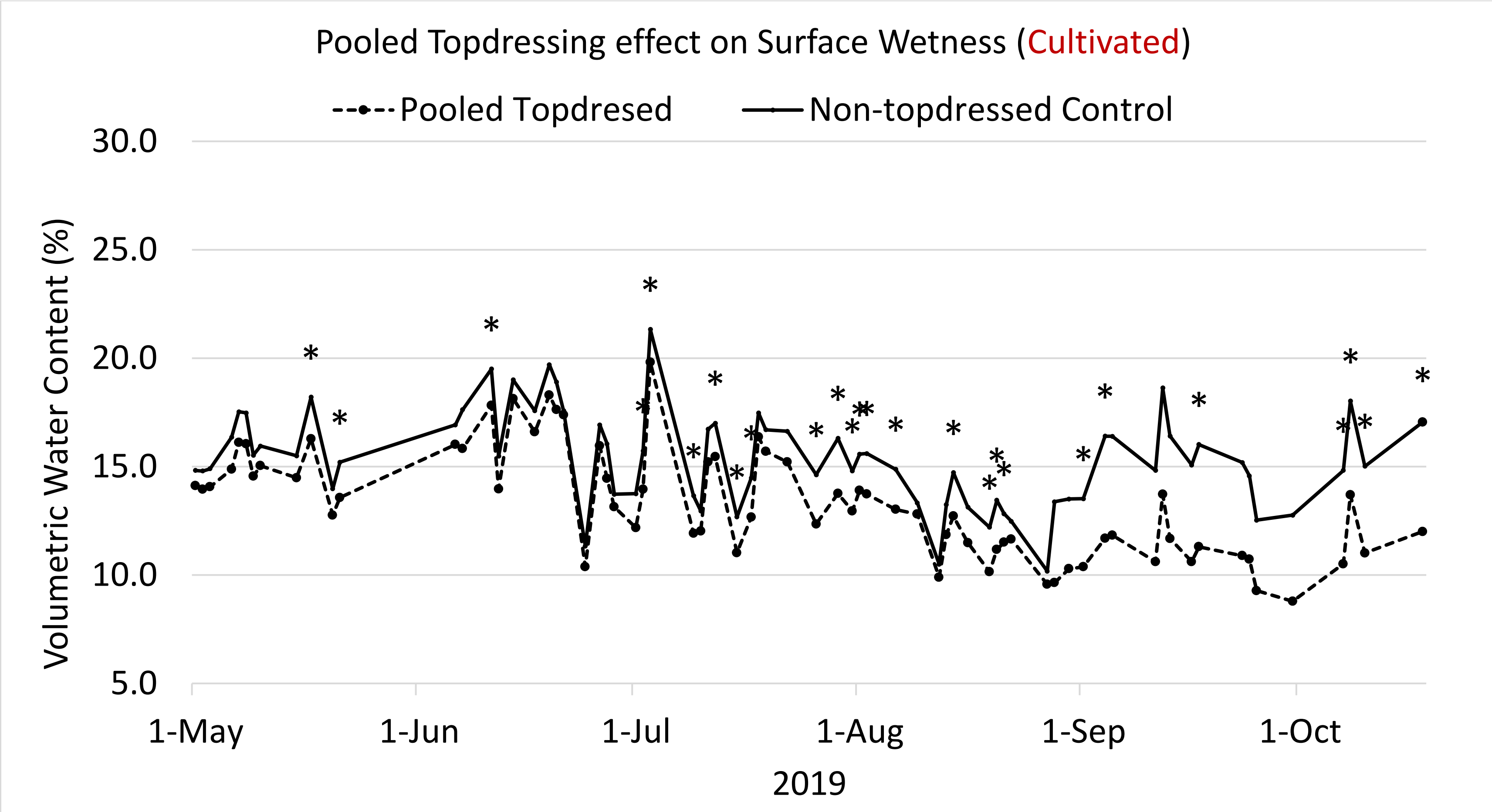


Figure 4. The volumetric water content of the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm for the pooled topdressing effect compared to the non-topdressed control under core cultivated conditions during 2019 in North Brunswick, NJ. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content. Core cultivation performed on 16 May and 23 October 2019.

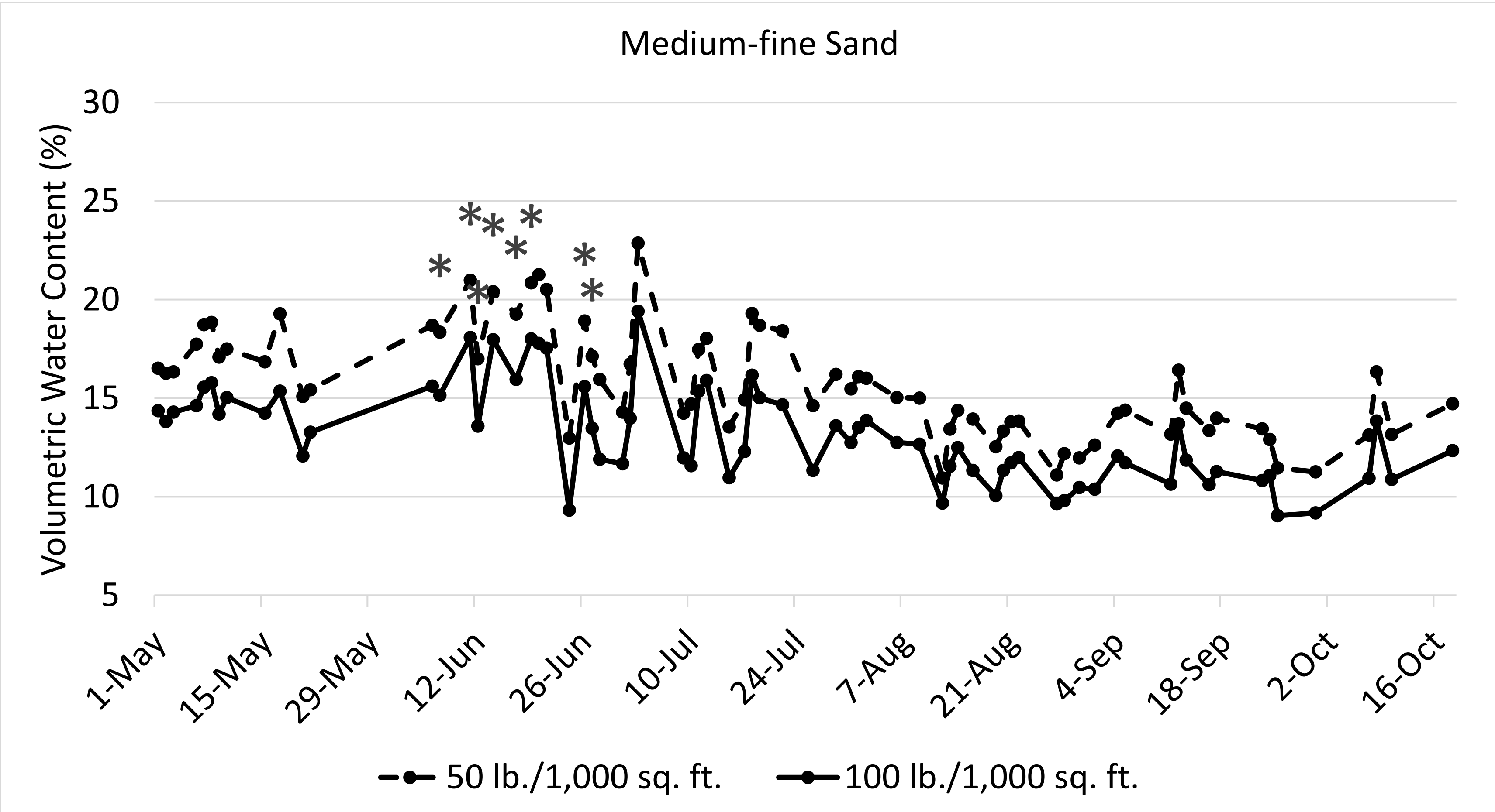


Figure 5. The effect of topdressing rate with medium-fine sand on volumetric water content at the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content.

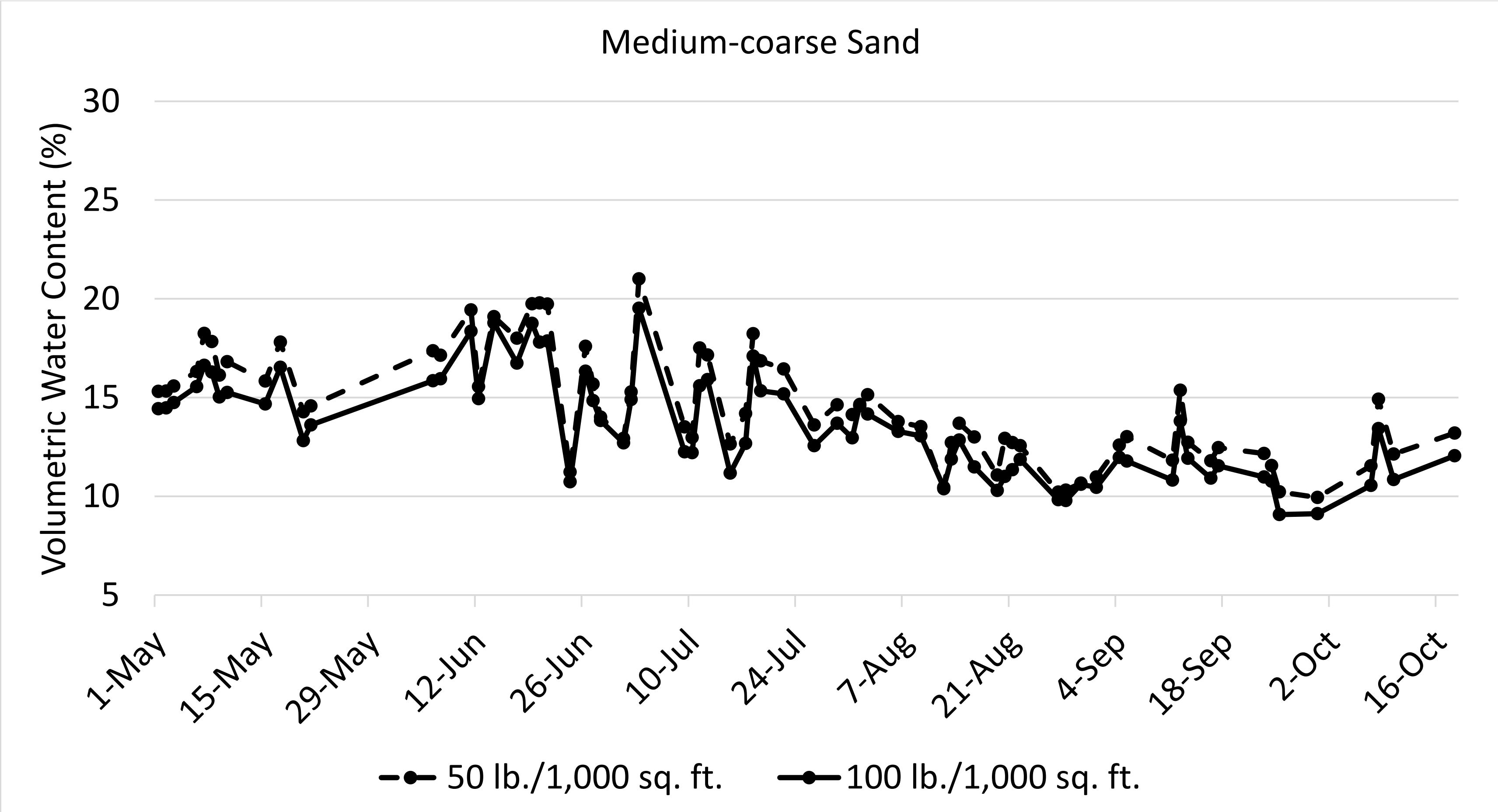


Figure 6. The effect of topdressing rate with medium-course sand on volumetric water content at the 0- to 3.0-mm depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content.

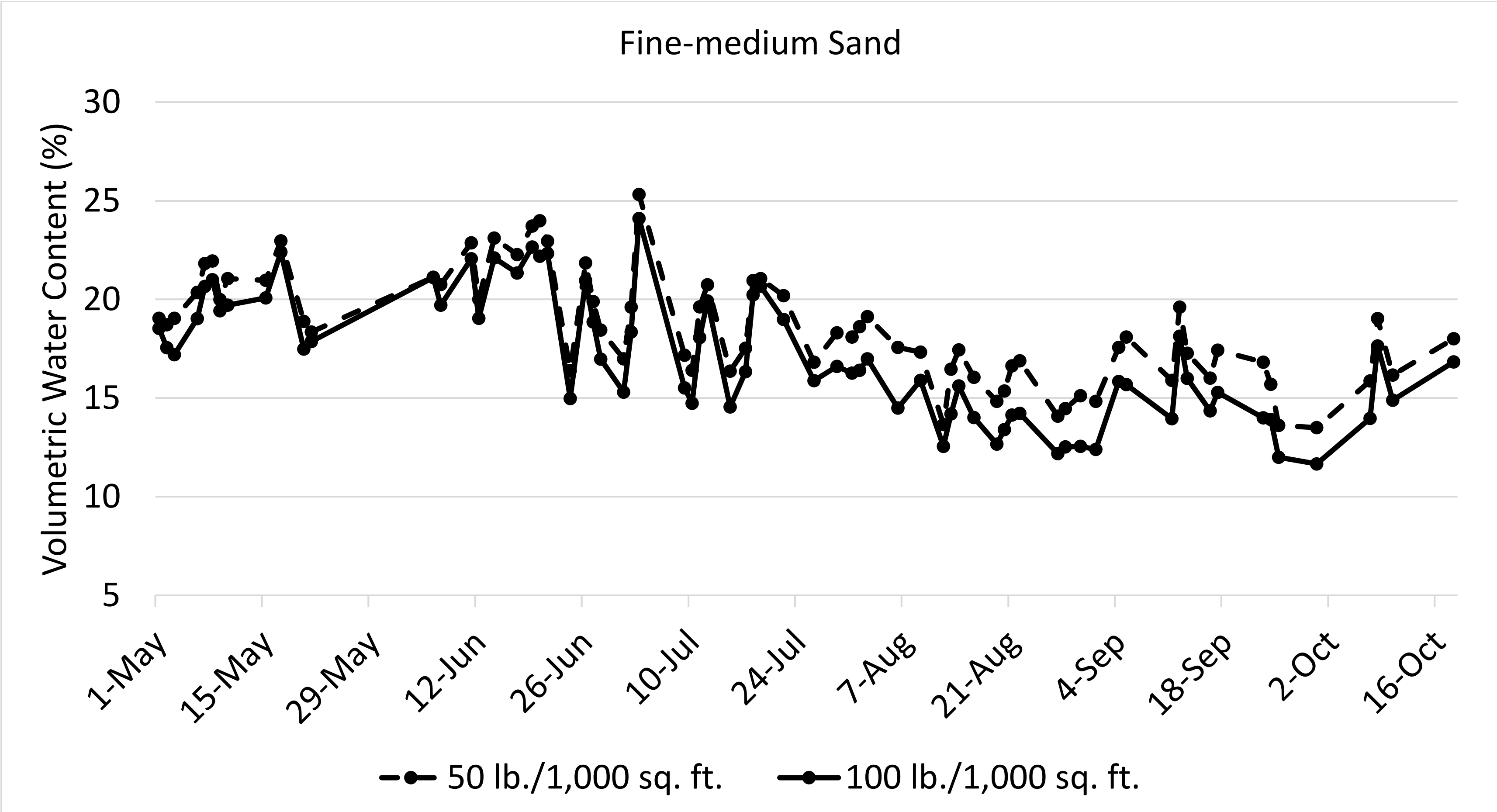


Figure 7. The effect of topdressing rate with fine-medium sand on volumetric water content at the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content.

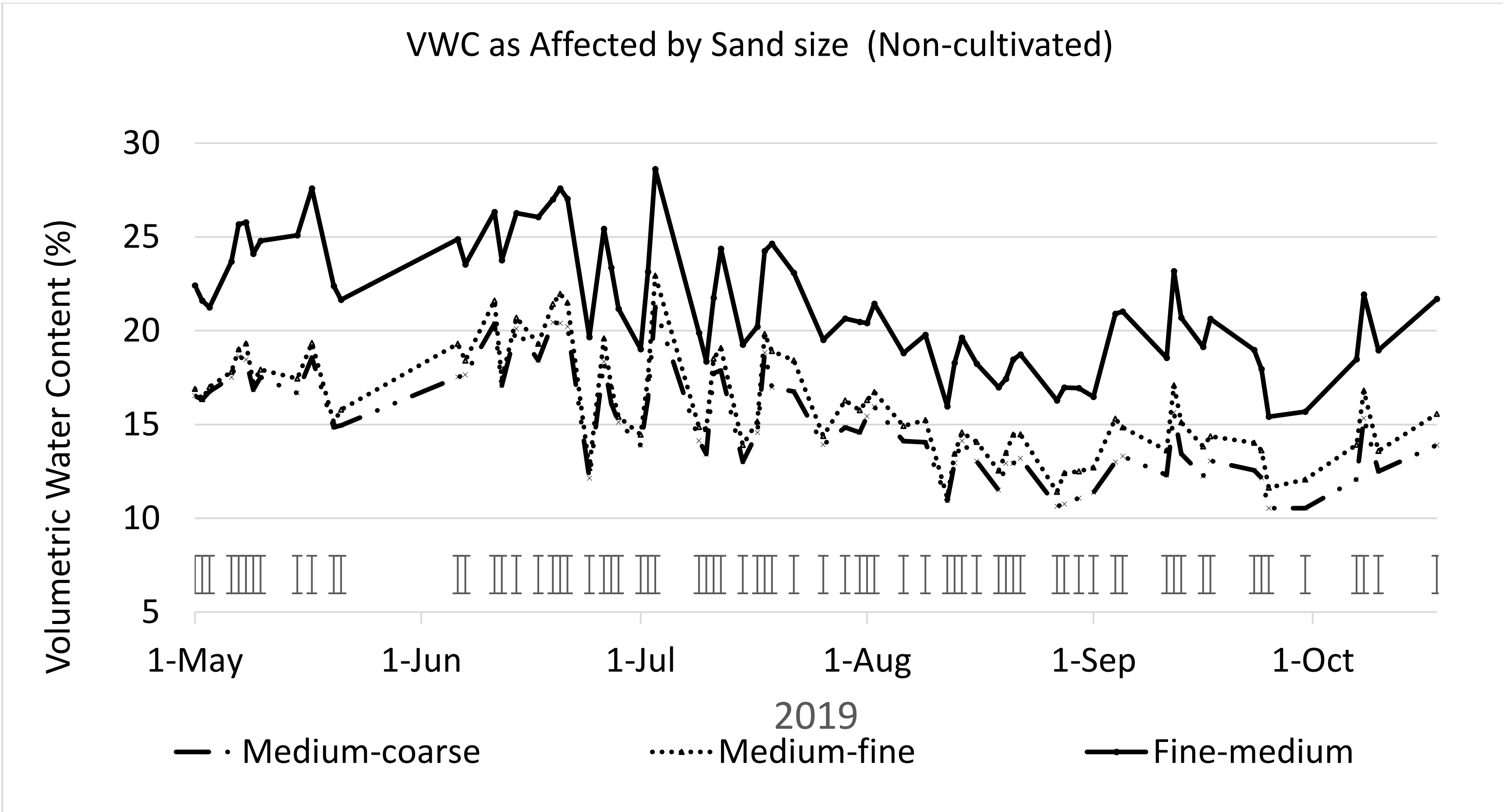


Figure 8. The sand size effect under non-cultivated conditions on volumetric water content at the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content.

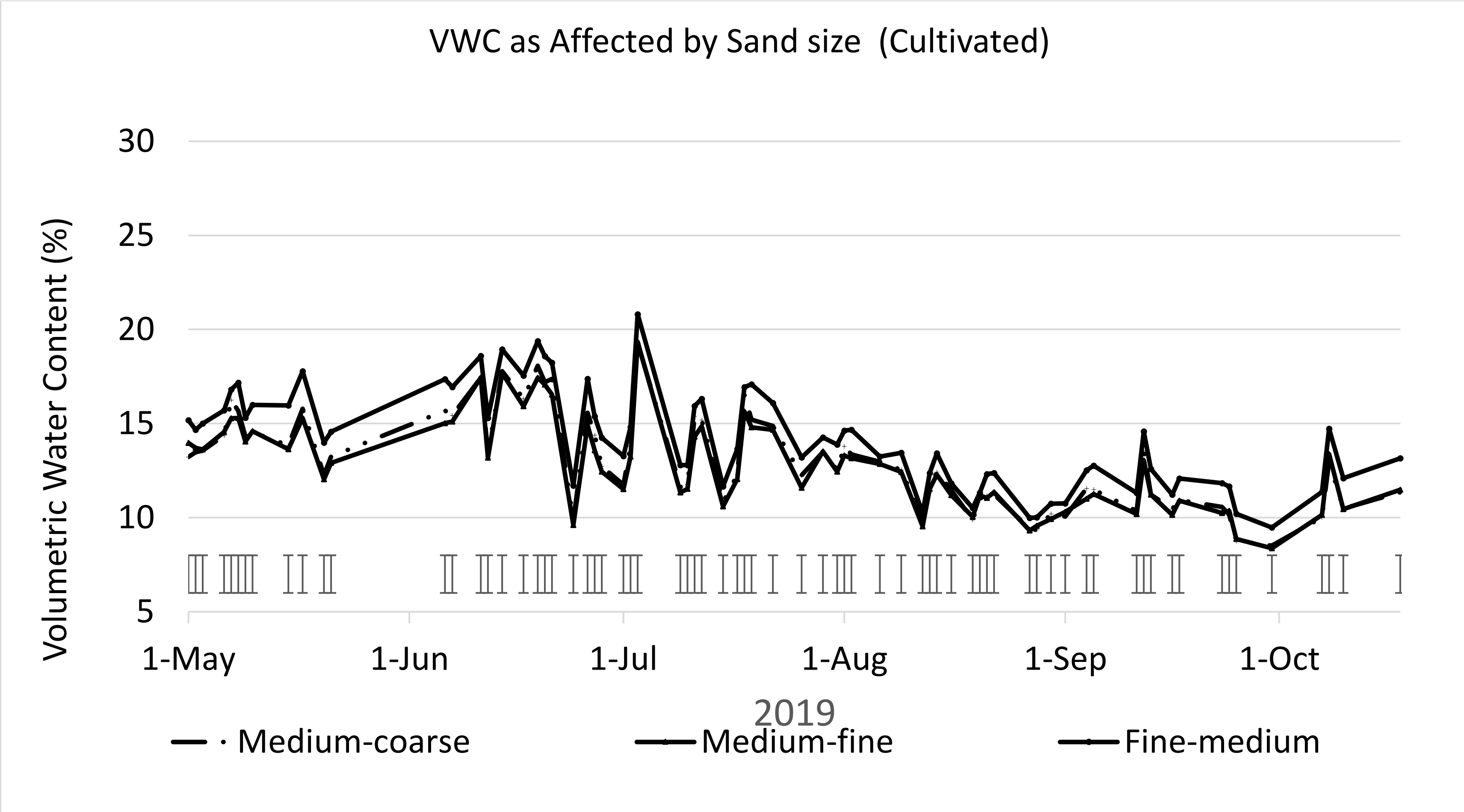


Figure 9. The sand size effect under core cultivated conditions on volumetric water content at the 0- to 3.0-inch depth zone of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2019. Field Scout™ TDR 300 Soil Moisture Meter was used to measure volumetric water content. Core cultivated on 16 May and 23 October 2019.

USGA ID#: 2018-08-658

Project Title: Long-Term Dynamics and Management Requirements of Sand-Capped Fairways

Project Leaders: Benjamin Wherley, Will Bowling, Kevin McInnes, Tony Provin, and Chrissie Segars

Affiliation: Texas A&M University, College Station, TX

Start Date: 2018
Project Duration: 3 years
Total Funding: $101,386

Summary Points:

- Although traditional soil physical testing methods would likely suggest use of a 20 cm sand-cap based on the particle size distribution of this sand, the highest overall turf quality levels continue to be associated with sand-capping treatment depths of 5 and 10 cm (7 and 6.9 out of 9, respectively). The 20 cm capping depth continues to produce lower turf quality levels through years 4 and 5 (6.1 out of 9) (Fig. 1)
- The highest soil volumetric water contents within the upper sand-cap (0-7.5 cm depth) are associated with topdressed over time (TD 5 cm) treatments (~29% VWC). The 5 and 10 cm capping depths exhibit intermediate soil moisture levels (~19% VWC), while the 20 cm capping depth supports the least moisture (~13%). The data suggest that wetting agents should not be necessary for shallower capping depths (5 and 10 cm), but significantly improve soil moisture within 20 cm sand-capping depths (Fig. 2)
- Water droplet penetration time (WDPT) tests performed at the 1.3 cm depth indicate that surface hydrophobicity is primarily a concern only for 20 cm sand-capping depth treatments. No hydrophobicity was observed in wetting-agent treated plots, while WDPT ranged from 80 to 140 seconds in the absence of wetting agent (Fig. 3).
- Subsoil sodium adsorption ratio (SAR) increases during summer months due to elevated Na in irrigation water (~300 ppm) and decreases during the fall and winter months due to natural rainfall. While the 100 lb. single annual gypsum application was most effective at initially reducing subsoil SAR during the 2018 season, it provided similar SAR reductions to the 10 lbs. monthly gypsum treatment through the 2019 season. Both gypsum treatments offered only marginal reductions in subsoil SAR by the end of the second (2019) season, suggesting that other strategies may need to be considered for impacting subsoil Na levels. (Fig. 4)
- Over the initial 2 seasons of the study, cultural management treatments have had only marginal impacts on overall turf quality. During late summer and fall of both years, there has been a trend (non-significant) towards improved turf quality in all cultural management treatments relative to the untreated control. (Fig. 5)
- Surface organic matter levels for the 0-5 cm depth measured at the end of the 2018 season showed a trend of decreasing organic matter with increasing cultural management intensity at the end of the initial season. Percent organic matter ranged from 5.2% (untreated), 5.1% (verticutting), 4.8% (aeration), to 4.7% (verticutting + aeration) at the end of the first season. (Fig. 6)

Summary Text:

As golf course irrigation water quality continues to decline, sand-capping of golf course fairways is increasing. This study is evaluating long-term (years 4-6) changes in turf performance, soil physical properties, and cultural management requirements of sand-capped fairway plots originally established in 2014. The project is being conducted at the Texas A&M Turfgrass Field Laboratory, College Station, TX, on 5-year old 'Tifway' bermudagrass sand-capped fairway research plots. Four replicated capping depth treatments have been constructed on both subsoils, including native soil topdressed at a depth of 2.5 cm sand per year resulting in a 5 cm sand-cap at the initiation of this project, as well as capping depths of 5 cm, 10 cm, and 20 cm at consturction. A split-plot design is being utilized to assess sand-cap cultural management practices addressing surface organic matter accumulation, hydrophobicity, and subsoil sodicity issues arising from elevated Na and bicarbonates in the local water source.

The fine sandy loam subsoil study focuses on subsoil sodicity and surface hydrophobicity management, specifically evaluating effects of wetting agent applications for mitigating surface hydrophobicity and gypsum application treatments for mitigating subsoil sodium accumulation, as well as the interaction of the two treatments on moving gypsum deeper into the profile. Within each capping depth, whole plots consist of wetting agent (Oars PS) applied at either 0 or 6 oz/ 1000 sq. ft., with gypsum (VerdeCal G applied at either 0 or 10 lbs./ 1000 sq. ft. monthly or as a single annual application at 100 lbs./1000 sq. ft) as the subplot treatment. Measurements including turf quality, soil volumetric water content, infiltration rates, water droplet penetration times, and subsoil (0-2.5 cm) sodium adsorption ratio are being monitored within treatments across capping depths during the course of the season. Root biomass within sand and subsoil will be evaluated at the end of the project.

The clay loam subsoil study focuses on surface organic matter management, specifically focusing on secondary cultural regimes for managing surface organic matter. Whole plots consist of sand-cap depth (Topdressed 5 cm, 5 cm, 10 cm, and 20 cm), with subplots consisting of either no secondary cultural management, verticutting, core aeration, verticutting + core aeration performed twice annually. Measurements including turf quality, percent green cover, surface firmness, and surface infiltration rates are being monitored during the course of the season. Percent organic matter for the 0-5 cm sand-cap depth is being determined via combustion analysis and loss on ignition method at the end of each season.

At the end of the final (2020) season, saturated hydraulic conductivity and water release curve data (water to air-filled porosity) will also be developed for select treatments in order to better understand and characterize physical changes that have occurred over time with various treatments and cultural management inputs.

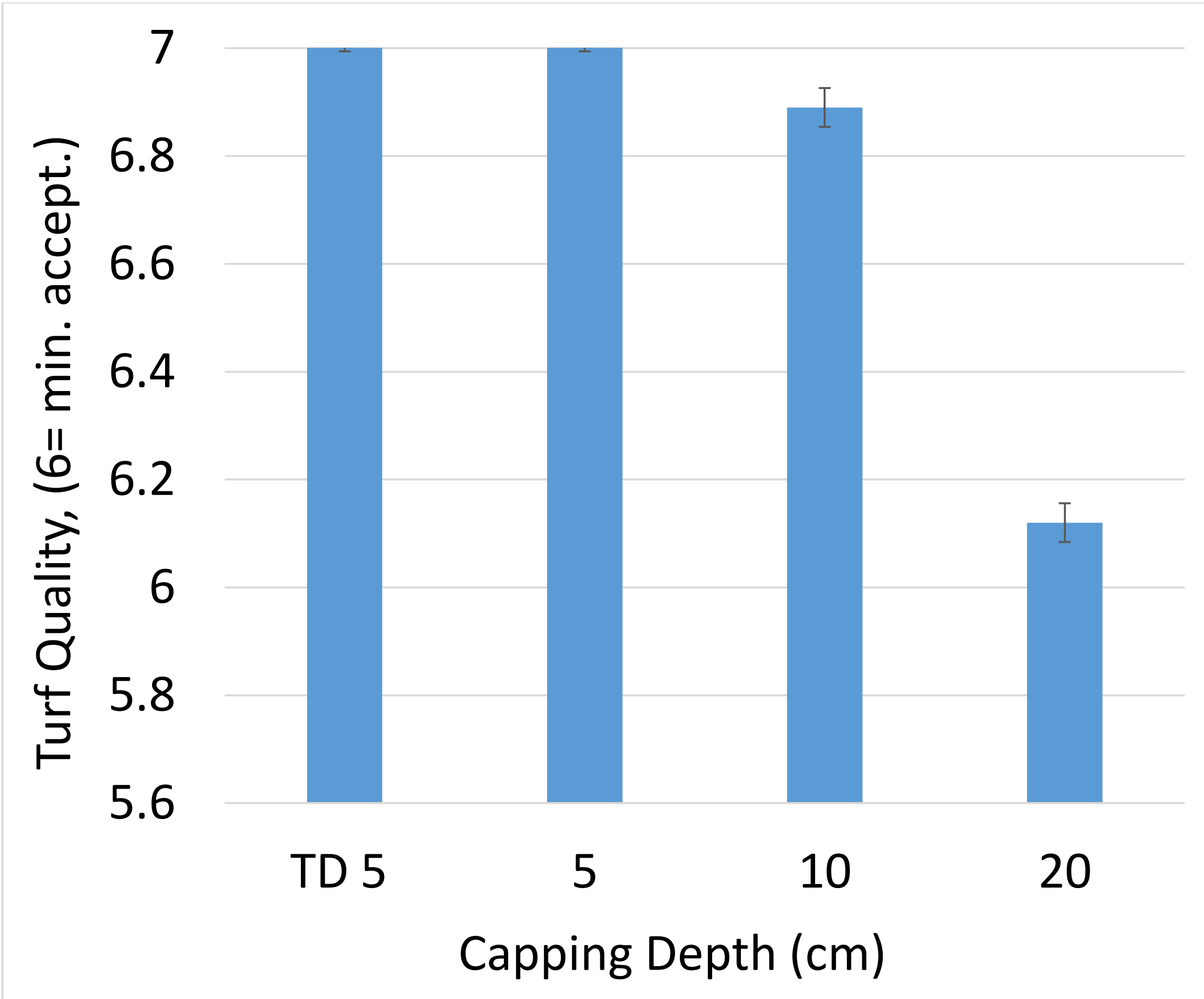


Figure 1. Effect of sand-capping depth on overall visual turf quality. Data are averaged across 2018 and 2019 rating dates. Error bars denote Fisher's LSD (0.05).

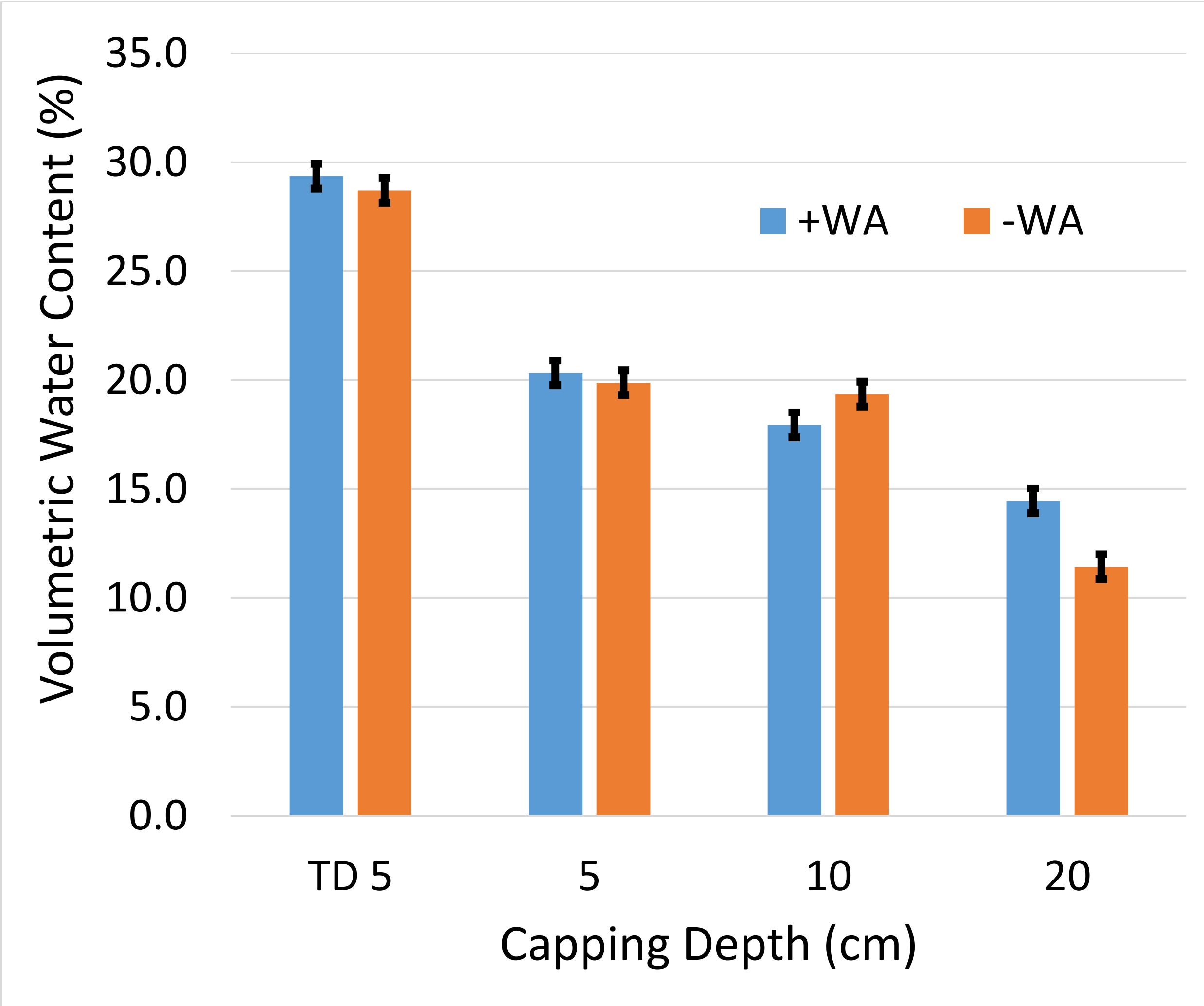


Figure 2. Effect of sand-capping depth and wetting agent on volumetric water content for the 0-7.6” sand-cap depth atop the fine sandy loam subsoil. Data are averaged across measurements from the 2018 and 2019 seasons. Error bars denote Fisher’s LSD (0.05).

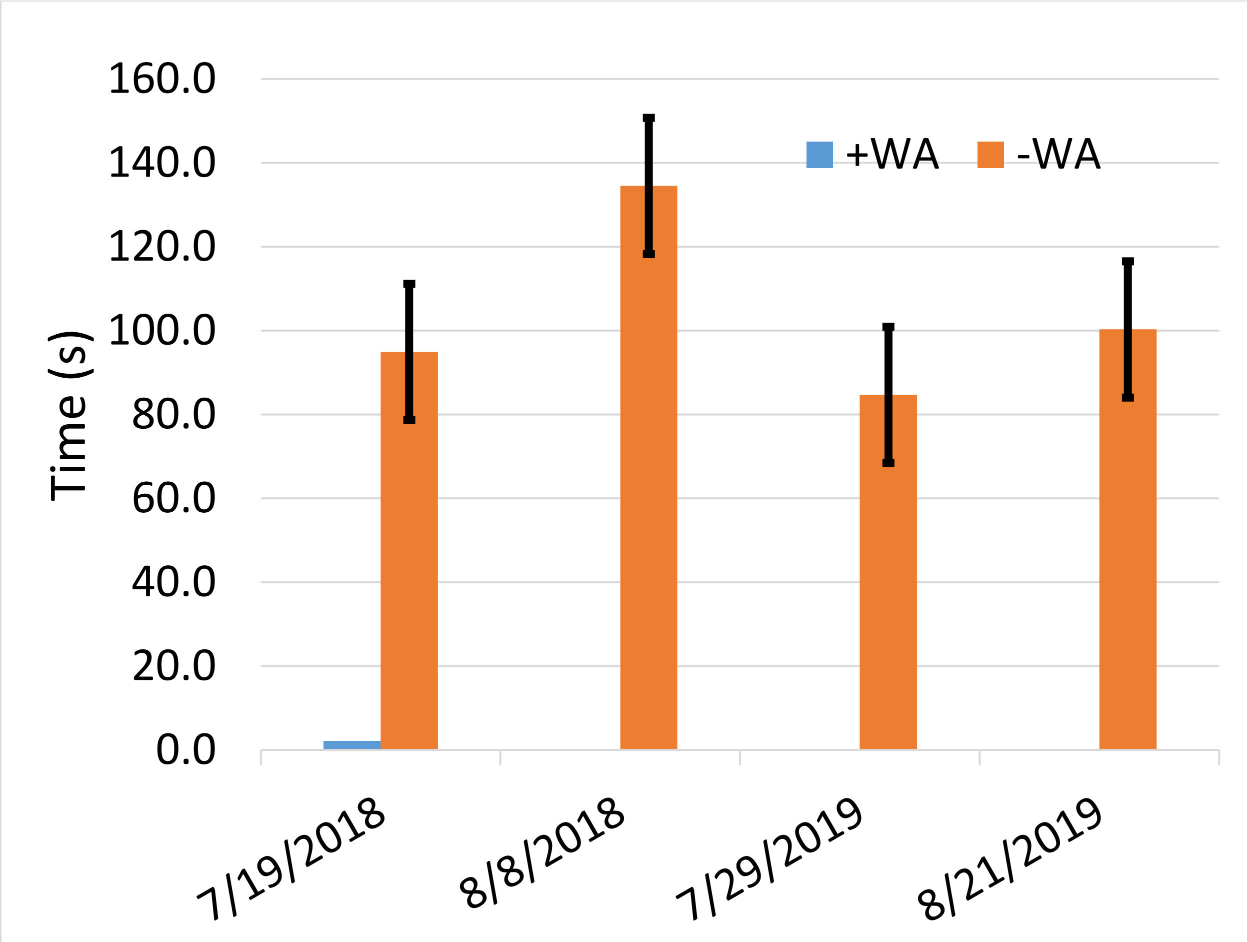


Figure 3. Water Drop Penetration Time measured at the 1.3 cm depth for the 20 cm sand cap treatments atop the fine sandy loam subsoil. Data are for July and August 2018-2019 measurement dates. Error bars denote Fisher's LSD (0.05).

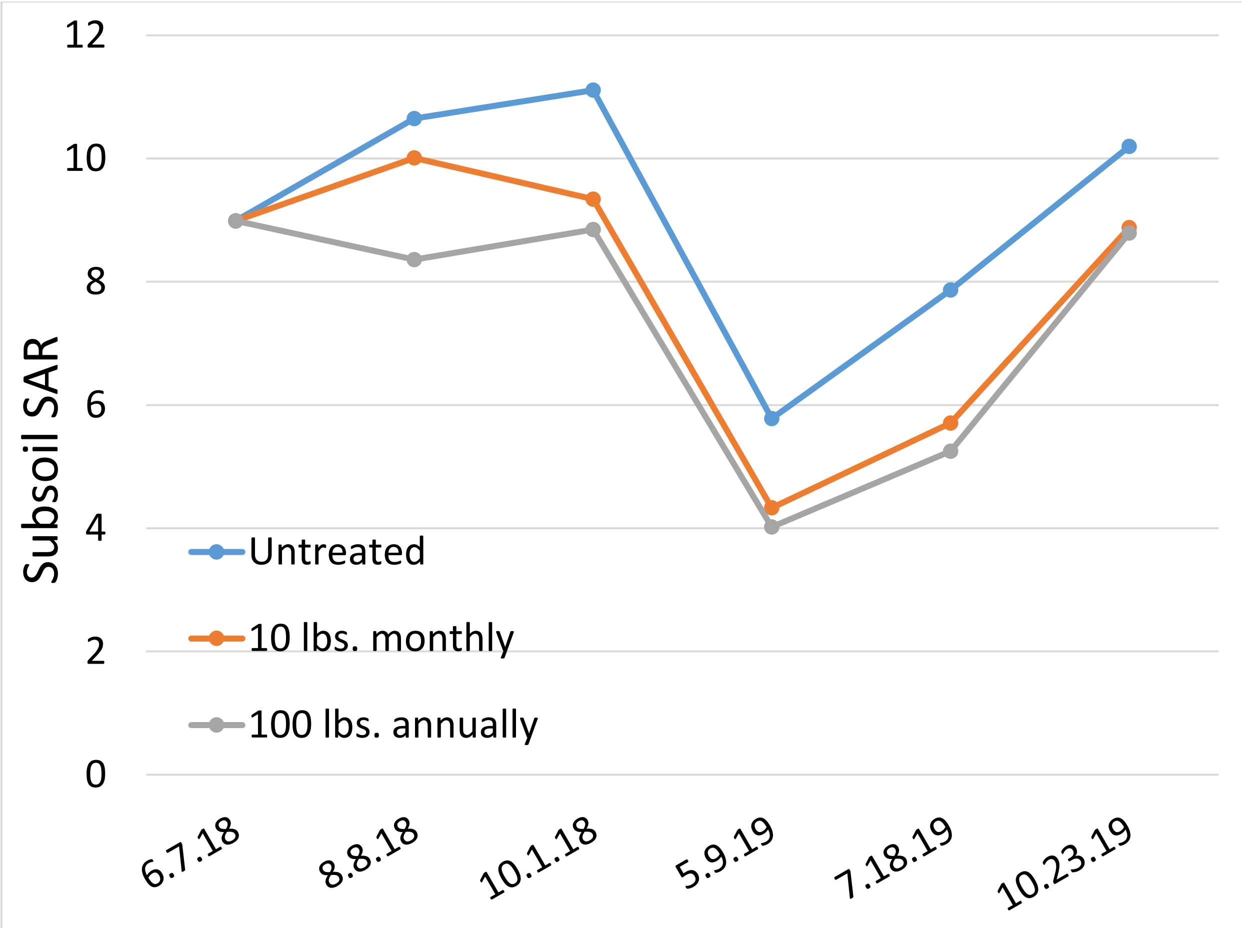


Figure 4. Effect of gypsum application regime (Untreated, 10 lbs./1000 sq. ft. monthly, and 100 lbs. one time annually) on subsoil sodium adsorption ratio as measured at the 0-2.5 cm subsoil depth. Data are averaged across sand-capping depth treatments.

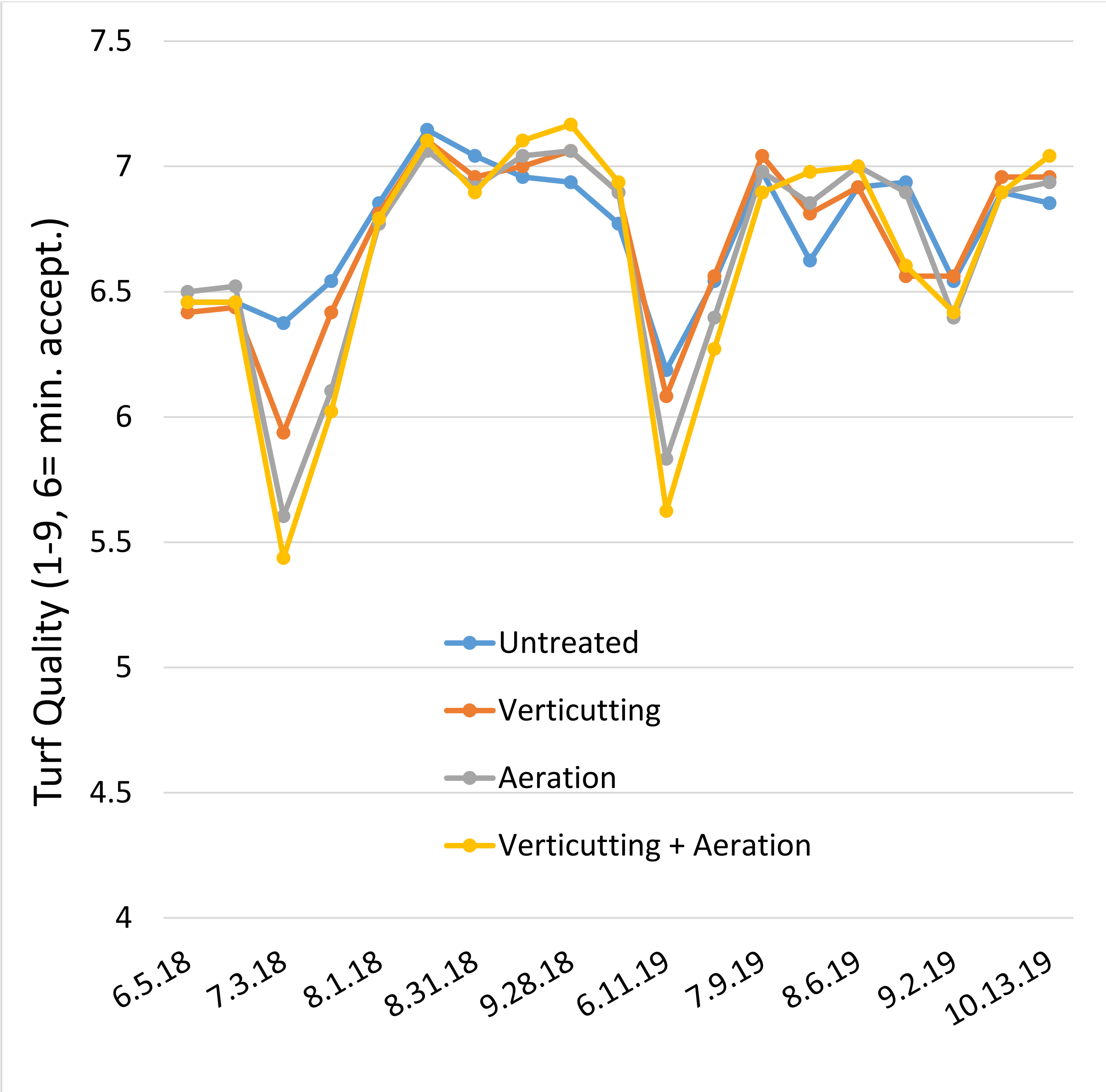


Figure 5. Effect of cultural management treatments on turf quality during 2018 and 2019 rating dates. Means are averaged across 10 and 20 cm capping depth treatments.

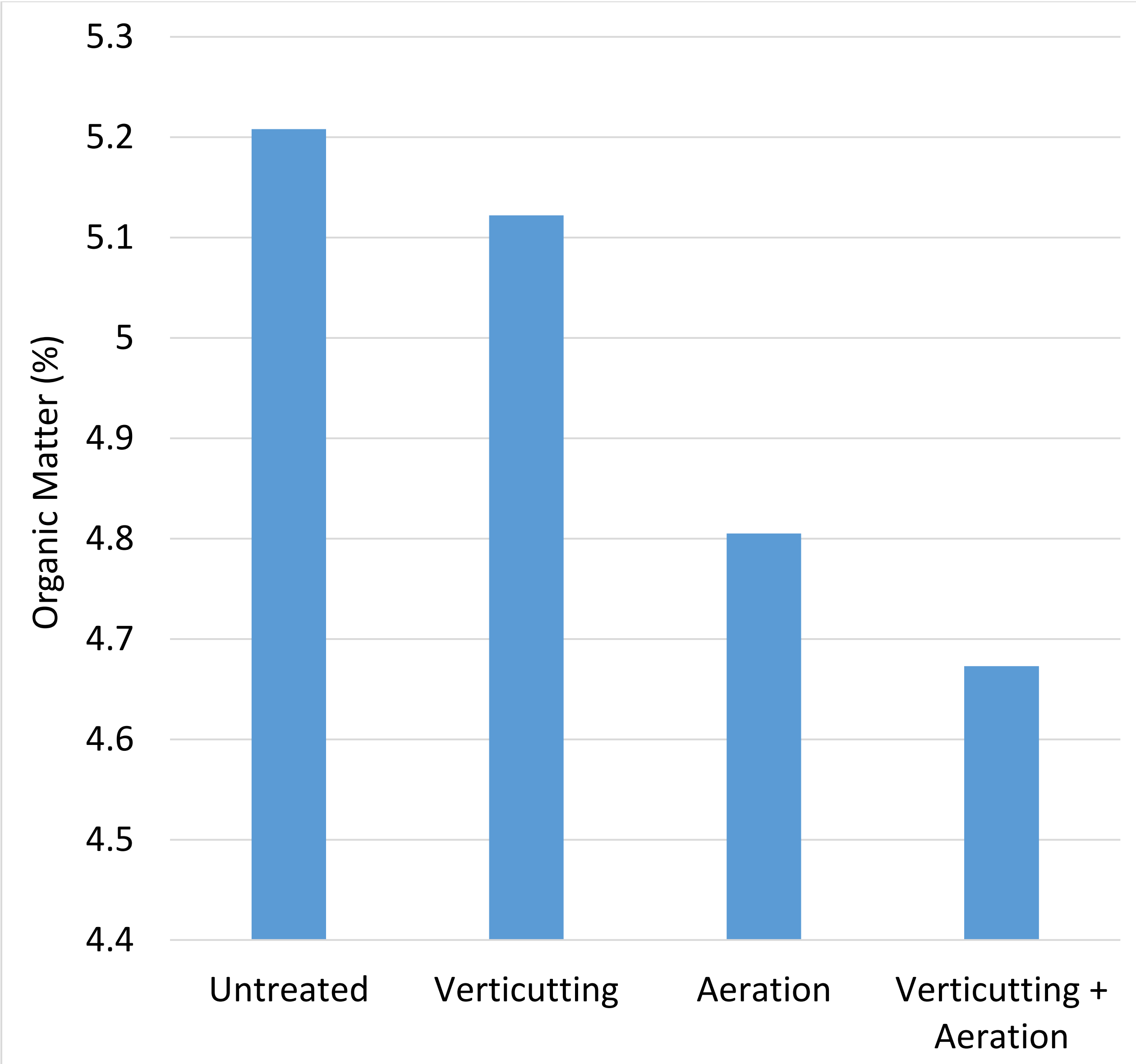


Figure 6. Effect of cultural management treatments on sand-cap surface organic matter (0-5 cm depth). Measurements were obtained at the end of the 2018 season. Percent organic matter was determined using the loss on ignition method. Means are pooled over 10 and 20 cm capping depth treatments.

USGA ID#: 2018-10-660

Title: Evaluation of Hollow Tine Core Aerification Recycling on Sand-Based Putting Greens Performance and Playability

Project Leaders: Adam Thoms, Nick Christians, and Ben Pease
Affiliation: Iowa State University

Objectives:

1. To compare the performance and playability of sand-based putting green plots that are either:
    a. subjected to hollow tine aerification, all of the cores are removed, and topdressing is applied;
    b. subjected to hollow tine aerification, the cores are verticut, chopped cores are drug back in, excess organic matter is removed, and additional topdressing is added as needed;
    c. subjected to hollow tine aerification, cores are processed through the Core Recycler, and additional topdressing sand is added as needed.
2. Conduct an economical comparison for each of the three treatments listed in objective 1, and determine the amount of sand saved by recycling cores.

Start Date: 2018
Project Duration: 2
Total Funding: $10,000

Summary Points:

- Hollow tine core recycling with the Wiedenmann Core Recycler was compared to traditional hollow tine aeration core removal and replacement with fresh sand and verticutting hollow tine cores and dragging back in the chopped up cores.
- A creeping bentgrass putting green meeting USGA spec rootzone was aerated on 2” by 2” spacing with 3/4” tines on 30 Aug. 2018 and 23 August 2019 at which time treatments were applied. All treatments were fully recovered from aeration within two weeks of aeration, with no negative effects of recycling cores on putting green recovery. Day of percent cover was lower on plots with cores either recycled or verticut.
- No differences were present between treatments for soil organic matter, and there were no increases in soil organic matter during the study. This indicates no negative effects from returning hollow tine cores to the putting green after two years.
- Few differences were found between treatments for the variables measured including porosity, water infiltration rates, and bulk density. This indicates that the Wiedenmann Core Recycler or verticutting hollow tine cores can provide a way to return sand into the putting green surface without negative effects of recycling on the rootzone characteristics and performance.

Summary Text:

One of the most overlooked aspects of putting green performance is the management of a rootzone, especially those that are sand-based. It is often difficult to find a sand source with desirable sand particle characteristics, especially after hollow tine aerification. Many golf courses will pay more for the cost of trucking than the actual sand used to fill aeration holes. Hollow tine aerification and topdressing is the best way to remove organic matter from the rootzone without having to remove all of the grass and sand present. This practice will also often benefit the health of the turfgrass roots. The addition of all new topdressing sand after hollow tine aerification can be costly and will strain budgets. The result is many golf courses under budget constraints will reduce the frequency of hollow tine aerification. Many lower budget golf courses will take this a step farther and skip hollow tine aerification and topdressing which can result in the formation of layers, or the golf course superintendent will try to recycle the cores back into the putting green by dragging the cores back into the rootzone.

Sand containing organic matter has been used on golf course putting greens for topdressing in the past. This has included reincorporating aeration cores back into the rootzone. Hollow tine core cultivation and soil reincorporation into the thatch did not affect thatch amounts significantly but did increase bulk density (Danneberger and Turgeon, 1986; Hurto et al., 1980) and water holding capacity (Hurto et al., 1980). Rieke and Murphy (1989) provide a great review of cultivation methods and studies done to date, but do not mention any devices like the modern Core Recycler machine. Current research is lacking to provide data on how hollow tine core recycling will impact a creeping bentgrass putting green.

This study investigated recycling hollow tine aerification cores as compared to traditional methods of complete removal of aerification cores and topdressing with new sand. Hollow tine core recycling is being compared by the Wiedenmann Core Recycler (which has brushes and rotating screens allowing sand to be separated from the organic matter) to verticutting hollow tine aerification cores on a putting green. These treatments were compared to hollow tine aerification with removal of all the cores. This research project was conducted at the Iowa State University Horticulture Research Station in Ames, Iowa on a ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.) putting green established over a rootzone that was constructed to meet USGA putting green specifications. The plots were maintained to optimize turfgrass health with proper fertilization and minimize turfgrass disease, weed, and insect pressure. The creeping bentgrass was mowed six times a week at 0.125” height of cut with a reel mower to simulate a golf course putting green. Additional water was applied through irrigation to minimize turfgrass stress. The study was conducted in a randomized complete block design with three replications.

Before aerification each year, each plot was evaluated for organic matter in the rootzone, water infiltration, total soil pore space, percent turfgrass cover, green speed, surface hardness with the TruFirm. Hollow tine aerification was applied on a 2” by 2” spacing with ¾” tines on 30 August 2018 and 23 August 2019. Plots were then subjected to one of the three treatments explained earlier. Additional topdressing sand conforming to USGA putting green specifications was added to the plots to ensure the aerification holes were filled. After treatments were applied plots were tracked weekly for recovery (percent green cover with digital image analysis), green speed, volumetric soil moisture, and surface hardness. After turfgrass recovery plots were tested for soil organic matter, water infiltration, and total porosity.

Data were analyzed in Proc Mixed in SAS. Means were separated at the 0.05 level of significance with Fishers LSD. Treatments were repeated over two years. Soil organic matter levels and soil porosity did not differ between treatments after any rating date in either year of the study. Soil organic matter was 5% before treatments applied and the levels were reduced on the core recycler and traditional treatments: 4.9% for the core recycler and traditional treatments 4.7%, and 5.6% for the verticutting and dragging of the cores treatment but none of these were significantly different. Turfgrass green cover increased over time after aerification as expected and all treatments were fully recovered from aeration by week two. Percent green cover only differed between treatments on the day of aerification with traditional aerification (38%) having greater cover than verticutting corers and dragging them in (27%) and core recycling (27%) cover, and the first week after aeration when the traditional aeration methods had a higher percent green cover (90%) than the verticutting and dragging of cores method (85%), but not the core recycler treatments (87%). There were no differences in percent green cover on any rating date between core recycler and verticutting core treatments. This data indicates that more sand is left on the surface after a recycling event as compared to traditional aerification, however the sand is not present on the surface by one week after aerification. No differences were found between treatments for soil moisture, green speed, green hardness, sand in the clippings, or water infiltration during either year. It was found that the core recycler saved about 60% sand compared to traditional aerification, while verticutting and dragging cores back in was similar. Sand meeting USGA specifications delivered to the research station was \$39 ton^{-1}. On average a golf course has 6 acres of tees and greens, and with the aerification described above there would a need for 401 tons of sand to fill the holes, which would result in \$15,639 but with recycling 60% of the sand back it would only be \$6,255. Overtime this would be a great savings for the course.

Figure 1. Wiedenmann Core Recycler used to separate sand from organic matter in the top portion of a hollow tine core.

Figure 2. Hollow tine aeration cores on the left before being processed by the Wiedenmann Core Recycler, and on the right after passing through the core recycler.

Figure 3. Creeping bentgrass putting green hollow tine aerification core study (Traditional treatment removed as described above) in Ames, IA.

USGA ID#: 2017-05-615

Title: Solvita® Soil Test Kits to Categorize Golf Course Fairway Responsiveness to Nitrogen Fertilization

Project Leader: Karl Guillard
Affiliation: University of Connecticut

Objectives:

1. Determine if Solvita Soil CO2-Burst and Soil Labile Amino N tests are correlated to fairway creeping bentgrass quality and growth responses.
2. If test results are correlated to bentgrass fairway turf responses in Objective 1, then categorize the responsiveness to N fertilization as a function of Solvita soil test results in relation to a Standard fertilizer treatment.

Start Date: 2017
Project Duration: 3 years
Total Funding: $90,000

Summary Points:

- Compost and organic fertilizer rates have produced a wide range of SLAN and SCB test concentrations in fairway creeping bentgrass plots.
- SLAN and SCB test concentrations respond linearly to compost and organic fertilizer rates.
- Fairway creeping bentgrass growth and quality responses are moderately to strongly correlated to SLAN and SCB test concentrations.
- Trafficked plots generally had lower response values than the No-Traffic plots, but trend responses across compost and organic fertilizer rates were generally similar for most variables.
- Binary logistic regression generated curves to estimate the probability that compost and organic fertilizer rate responses would equal or exceed that of the Standard fertilizer treatment.
- The SCB test produced better binary logistic regression model fits than the SLAN test.
- The SCB and SLAN tests show potential for estimating the mineralization potential of fairway creeping bentgrass soils, and these may be used to guide N fertilization.
- The 2019 results suggest that fairway creeping bentgrass soils can be categorized with Solivta tests as to their probability of equaling or exceeding the response of a standard N treatment.

Summary Text:

Need for the Study:

The ability to predict the N mineralization potential of any turfgrass site and its expected response to N fertilization would be a valuable tool in nutrient management. Turfgrass soils often accumulate organic matter over time, and this increases their mineralization potential. However, assessing this mineralization potential is not routine due to the lack of mineralization tests offered with many labs, cost of the tests, and the long-term requirements (a week to months) of these tests for reliable results. Solvita and Woods End Laboratories offers two tests that have been developed to rapidly measure the biologically-active C and N fractions in soil organic matter: the Soil CO2-Burst (SCB) and Soil Labile Amino Nitrogen (SLAN) test kits. These tests measure labile C and N fractions are correlated to soil microbial activity, and therefore, the Solvita soil tests should be able to estimate the mineralization potential of turfgrass soils. An estimate of the mineralization potential should help guide N fertilization.

Methods:

The study site is located in Storrs, CT, and was initiated in August, 2017. The experiment was set out as a split-block design with traffic (with/without) as the horizontal factor and compost (10 rates, in 0.25-lb increments from 0 to 2.25 lbs available N per 1000ft2) as the vertical factor with three replicates. Compost was incorporated into the 0 to 4-inch soil profile by rototilling prior to seeding. After compost incorporation, creeping bentgrass ('13M') was seeded into the study site and managed as a fairway. During the late fall, an organic fertilizer (Suståne all natural 5-2-5) is applied to the plots at the same rates as the initial incorporated compost rates. In addition to the organic treatments, a standard fertilizer regime treatment with 0.2 to 0.25 lbs N 1000ft-2 was applied approximately every 21 days as liquid urea. Traffic is applied to half of the plots with a cart-traffic simulator three times a week during the growing season. Bentgrass response measurements (NDVI, percentage green cover, Dark Green Color Index [DGCI], visual quality, visual color, visual density, and clippings yield) and soil samples are collected monthly from May through November from each plot. Soil samples are analyzed using the Solvita SCB and SLAN tests. Data were statistically analyzed using analysis of variance to determine treatment effects (fertilizer rates, traffic, and the fertilizer rate × traffic interaction) on the mean bentgrass quality and growth responses and soil SCB and SLAN concentrations. Fairway bentgrass responses were correlated to SLAN and SCB concentrations within and across traffic treatments. Binary logistic regression was applied to determine the probability of bentgrass fairway responses that would be equal to or exceeding the responses from the standard N fertilization practice across the Solvita soil test values for each of the traffic treatments.

2019 Results:

Traffic effects were significant for NDVI, DGCI, visual quality, visual color, visual density, percentage green cover, and clipping yields. In these cases, No-Traffic treatment responses were significantly greater than where traffic was applied (Table 1). Fertilizer treatment effects were significant for all variables (Table 1). Averaged across traffic treatments, all responses were linear and significant in relation to fertilizer N rate ($P < 0.001$) (Fig. 1).

Correlations between fairway bentgrass responses in relation to SLAN and SCB concentrations were all highly significantly with moderate to high *r* values across the traffic treatments (SLAN $r = 0.439$ to 0.917; SCB $r = 0.454$ to 0.882) (Table 2). There was no difference in *r* values between traffic treatments, except for SLAN DGCI and SCB Cover where Traffic *r* value was higher than the No-Traffic *r* value. Scatter plots and correlations pooled across traffic treatments are shown in Fig. 2.

Binary logistic regression was applied to determine the probability of organic compost and fertilizer plot responses producing responses that were equal or greater than the response of the Standard fertilizer treatment with respect to the SLAN and SCB concentrations. Probability curves are shown in Figs. 3 and 4 for Traffic and No-Traffic plots.

For SLAN concentrations, visual color was not modeled well in the No-Traffic plots, whereas NDVI, visual color, and percent green cover were not modeled well in the Traffic plots. Averaged across all variables, there would be a ≥ 67% chance that fairway bentgrass responses would equal or exceed the responses of the Standard fertilizer treatment when SLAN concentrations were ≥ 353 and ≥ 446 mg kg-1 for non-trafficked and trafficked plots, respectively (Table 3). Removing the variables with poor model fits, the corresponding SLAN concentrations would be ≥ 329 and ≥ 313 mg kg-1, respectively.

For SCB concentrations, probability curves for all variables in both No-Traffic and Traffic treatments were modeled relatively well. Averaged across all variables, there would be a ≥ 67% chance that fairway bentgrass responses would equal or exceed the responses of the Standard fertilizer treatment when SLAN concentrations were ≥ 164 and ≥ 171 mg kg-1 for non-trafficked and trafficked plots, respectively (Table 3).

Future Expectations:

It is anticipated that with more data, we will be able to produce a reliable table of SLAN and SCB concentrations and associated probabilities of responses being equal to or exceeding the response of the Standard fertilizer treatment of 0.2 to 0.25 lbs N 1000ft-2 applied approximately every 21 days. This could then assist the superintendent in guiding fertilization based on their risk tolerance. An example is shown in Table 4 and Fig. 5.

The goal of using the Solvita tests to guide N fertilization for turfgrasses would be to recommend a specific amount of N needed for optimum response for any specific SLAN-N or SCB–C concentration. Following the concepts presented in Table 4 and Fig. 5, it could be suggested that fairway creeping bentgrass with SLAN-N or SCB–C concentrations that fall below the P = 0.33 cutoff receive the full currently-recommended N rate; fairway creeping bentgrass with SLAN-N or SCB–C concentrations that fall between the P = 0.33 and the P = 0.67 cutoffs receive ⅔ to ½ of the currently-recommended N rate; those with SLAN-N or SCB–C concentrations that fall between the P = 0.67 and the P = 0.90 cutoffs receive ½ to ⅓ of the currently-recommended N rate; and those with SLAN-N or SCB–C concentrations that fall above the P = 0.90 cutoff receive little to no additional N fertilization. This would assume that optimum conditions for mineralization would be present across the growing season. Another approach to using the P values to guide N fertilization is for superintendents to apply (1 – P) × the full rate of N fertilization, where P is the probability of equaling or exceeding the Standard fertilizer treatment response based on the SLAN-N or SCB–C concentration.

Table 1. Mean Solvita soil test concentrations and fairway creeping bentgrass quality and growth responses, with analysis of variance *P* values for traffic and N rate treatment effects. 2019 results.

	SLAN	CO_2-Burst	NDVI	DGCI	Visual Quality	Visual Color	Visual Density	Cover	Sum Monthly Clippings Yields
Traffic	mg kg^{-1}	mg L^{-1}			----------1-9; 9 best --------			% green	g m^{-2}
No	256	128	0.692	0.486	6.7	6.9	6.9	85	41.5
Yes	256	130	0.682	0.471	6.0	6.3	6.1	80	19.2
Treatment†									
0	227*	105*	0.660*	0.461	4.7*	5.3*	5.1*	73*	17.7*
0.25	239	119	0.672*	0.467	5.1*	5.7*	5.5*	79	23.1
0.5	239	120	0.684	0.473	5.7*	6.0*	5.8*	82	26.2
0.75	247	124	0.685	0.476	6.0*	6.1*	6.2*	83	26.2
1	251	125	0.684	0.476	6.4*	6.4*	6.4*	83	26.1
1.25	256*	133*	0.692	0.482	6.4*	6.8*	6.6	84	34.6
1.5	261*	138*	0.692	0.487	6.6	6.7*	6.8	85	34.7
1.75	271*	141*	0.696	0.487	7.0	7.1*	7.1	86	39.2*
2	282*	142*	0.698	0.488	7.3	7.3	7.3	86	37.3*
2.25	300*	149*	0.702	0.491*	7.6	7.7	7.5	86	41.0*
Standard	242	122	0.694	0.475	7.1	7.7	7.3	82	28.1
					AOV *P*-values				
Traffic	0.8509	0.5117	0.0294	0.0084	0.0017	0.0036	0.0087	0.0078	0.0023
Treatment	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0114	<0.0001
T × T	0.0110	0.1523	0.4701	0.5830	0.3369	0.9595	0.5400	0.3264	0.0306

†Compost and organic fertilizer rates (lbs N per 1,000ft^2); Standard treatment is liquid urea at approximately 0.2 lbs N per 1,000ft^2 every 21 days.
* Significantly different from the Standard treatment ($P < 0.05$)

Table 2. Correlation coefficients (*r*) and *P* values for No-Traffic and Traffic plot responses in relation to Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO2-Burst (SCB) concentrations, and *P* values for the difference between traffic treatment *r* values for each variable. 2019 results.

SLAN	No-Traffic		Traffic		
Variable	*r* value	*P* value for *r*=0	*r* value	*P* value for *r*=0	*P* value for difference between traffic treatments *r* values
NDVI	0.721	<.0001	0.784	<.0001	0.5879
DGCI	0.600	0.0003	0.843	<.0001	0.0485
Quality	0.828	<.0001	0.885	<.0001	0.4269
Color	0.903	<.0001	0.917	<.0001	0.7666
Density	0.803	<.0001	0.830	<.0001	0.7659
Cover	0.439	0.0145	0.698	<.0001	0.1495
Yield	0.756	<.0001	0.776	<.0001	0.8620

SCB	No-Traffic		Traffic		
Variable	*r* value	*P* value for *r*=0	*r* value	*P* value for *r*=0	*P* value for difference between traffic treatments *r* values
NDVI	0.731	<.0001	0.876	<.0001	0.1154
DGCI	0.680	<.0001	0.862	<.0001	0.0841
Quality	0.821	<.0001	0.882	<.0001	0.4022
Color	0.793	<.0001	0.870	<.0001	0.3528
Density	0.757	<.0001	0.869	<.0001	0.2125
Cover	0.454	0.0109	0.764	<.0001	0.0585
Yield	0.846	<.0001	0.811	<.0001	0.6753

Table 3. Concentrations of Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO2-Burst (SCB) concentrations of equaling or exceeding the response of the Standard fertilizer treatment at a selected probability of *P* = 0.67. 2019 results.

P = 0.67	SLAN-N, mg kg^{-1}	
Variable	No-Traffic	Traffic
NDVI	308	599
DGCI	352	338
Visual Quality	373	313
Visual Color	499	620
Visual Density	396	327
Percent Green Cover	244	655
Clipping Yields	302	273
Mean	353	446

P = 0.67	SCB-C, mg L^{-1}	
Variable	No-Traffic	Traffic
NDVI	142	180
DGCI	139	145
Visual Quality	177	161
Visual Color	197	198
Visual Density	209	224
Percent Green Cover	121	147
Clipping Yields	164	141
Mean	164	171

Table 4. Estimated probabilities for Soil CO2- Burst test concentrations across both traffic treatments with all variables. 2019 results.

SCB-C, mg L^{-1}	Probability of equaling or exceeding response from Standard fertilizer treatment
50	0.05
75	0.11
100	0.20
125	0.35
150	0.53
175	0.71
200	0.84
225	0.92
250	0.96
275	0.98
300	0.99

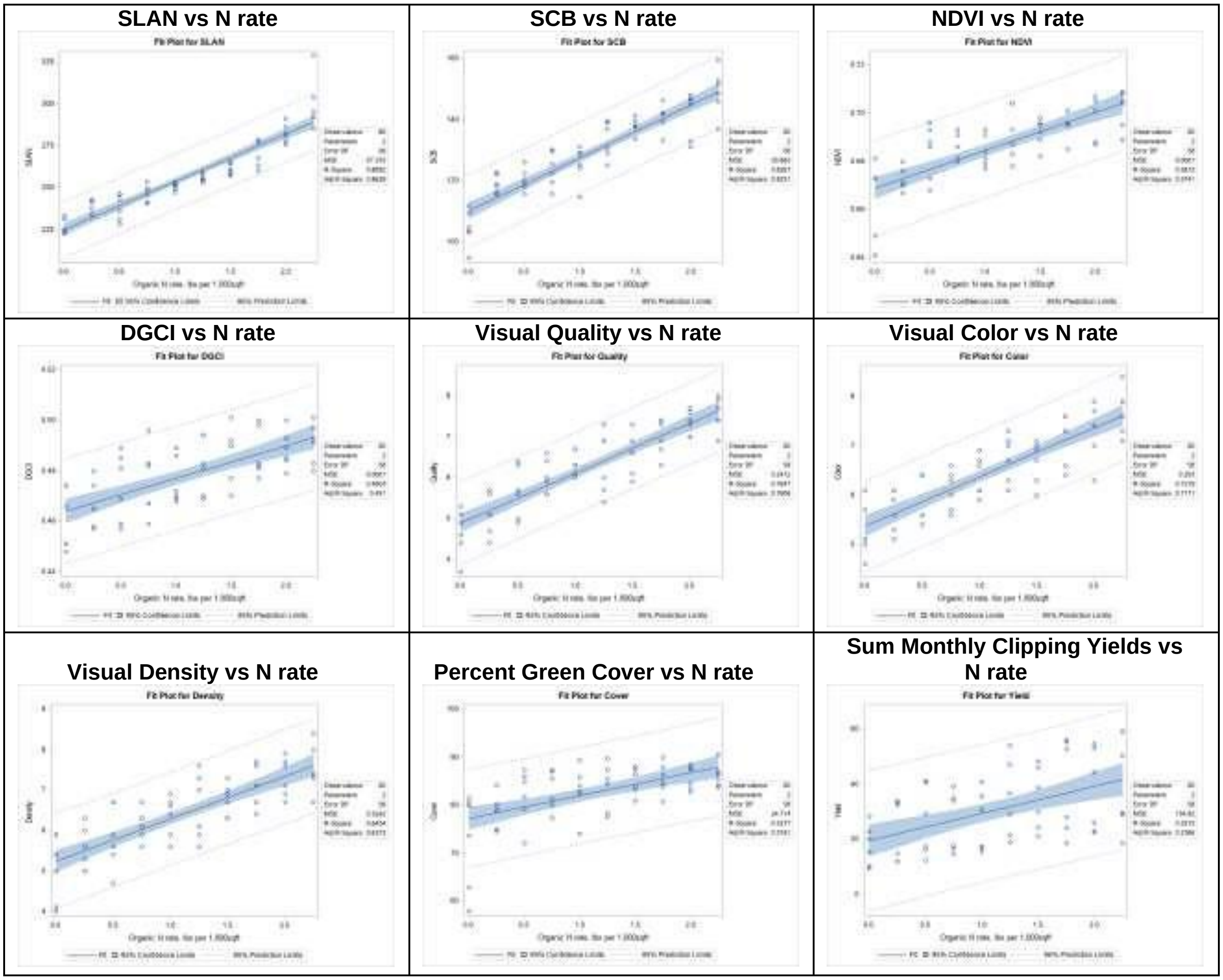
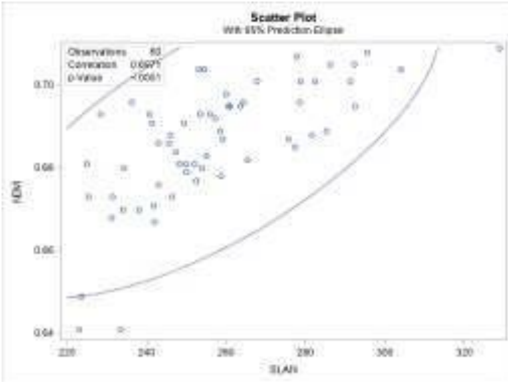
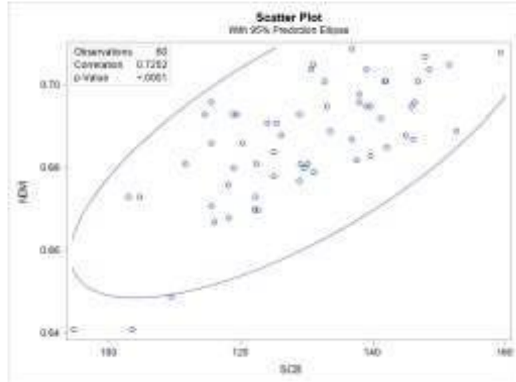


Figure 1. Fairway creeping bentgrass responses (Soil Labile Amino Nitrogen [SLAN], Soil CO_2- Burst [SCB], Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and monthly clippings yields) in relation to organic fertilizer (initial compost followed by yearly Suståne applications) N rates for 2019. Since there were few traffic × treatment interactions, responses are averaged across Traffic and No-Traffic plots.

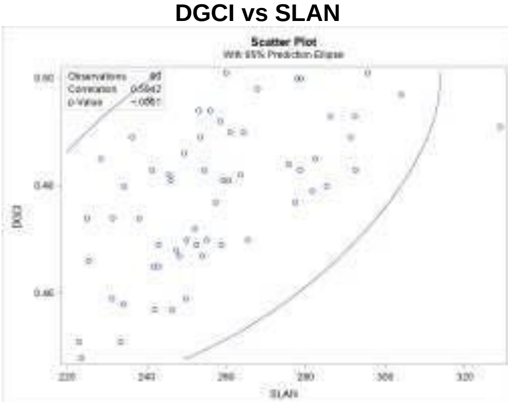
NDVI vs SLAN



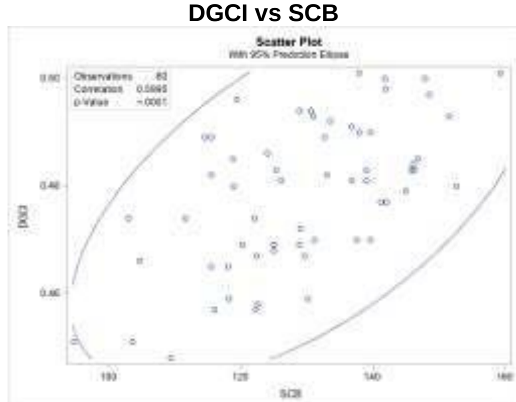
NDVI vs SCB



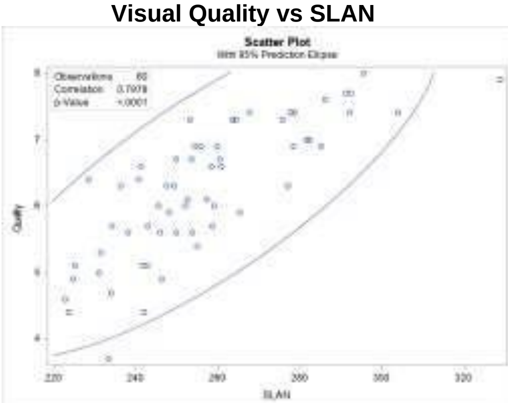
DGCI vs SLAN



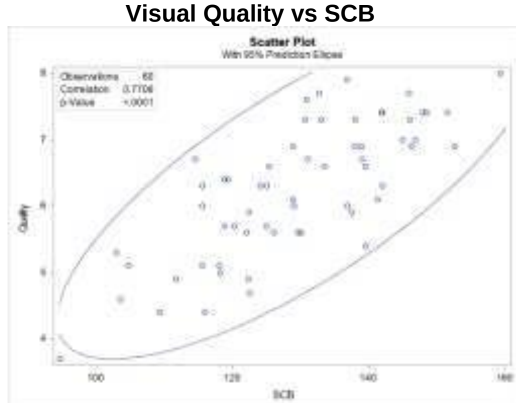
DGCI vs SCB



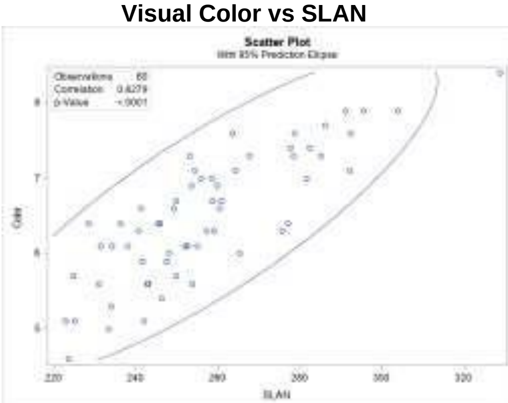
Visual Quality vs SLAN



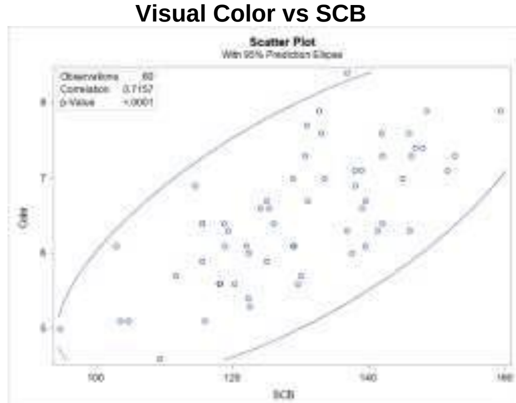
Visual Quality vs SCB



Visual Color vs SLAN



Visual Color vs SCB



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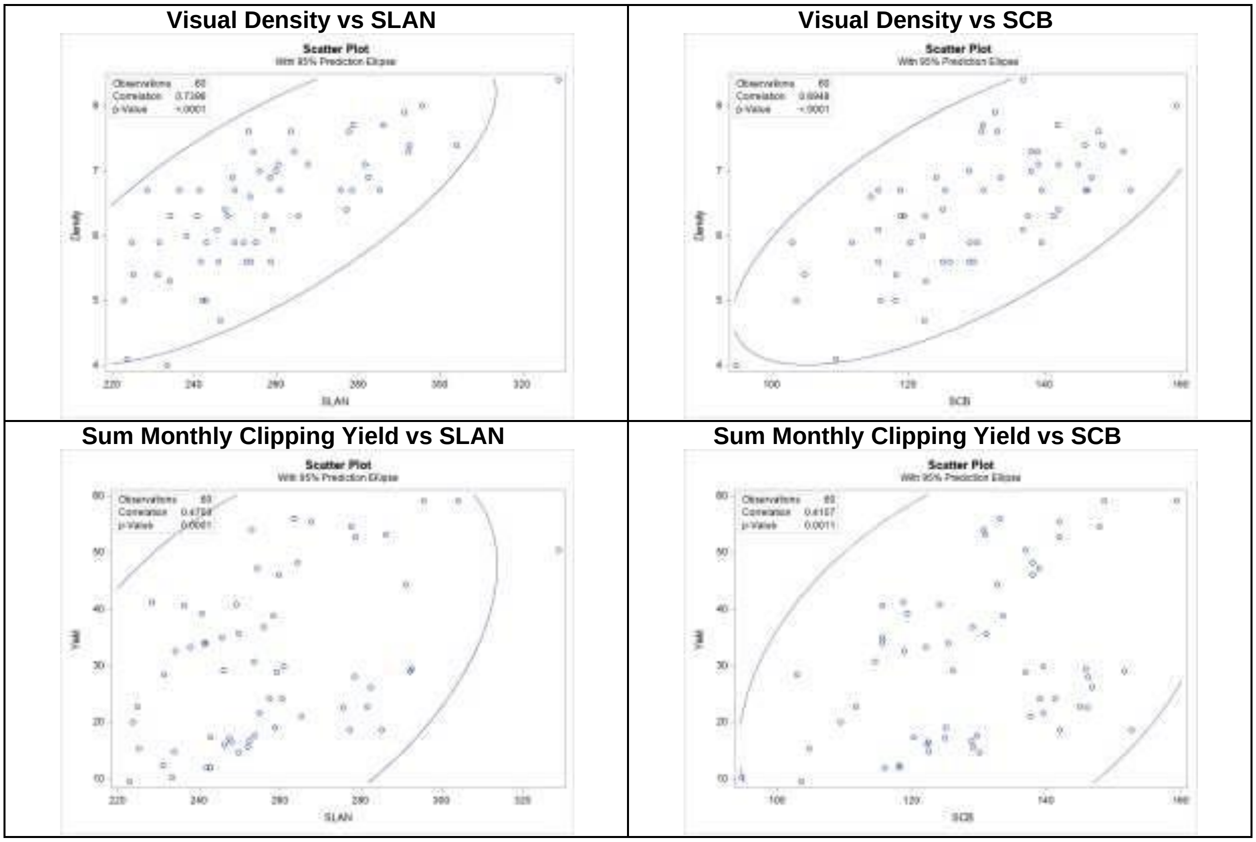


Figure 2. Correlations between fairway creeping bentgrass responses (Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and monthly clippings yields) in relation to Soil Labile Amino Nitrogen (SLAN) and Soil CO2-Burst (SCB) concentrations averaged across sampling dates and traffic treatments plots. The ellipses represent the 95% prediction space.

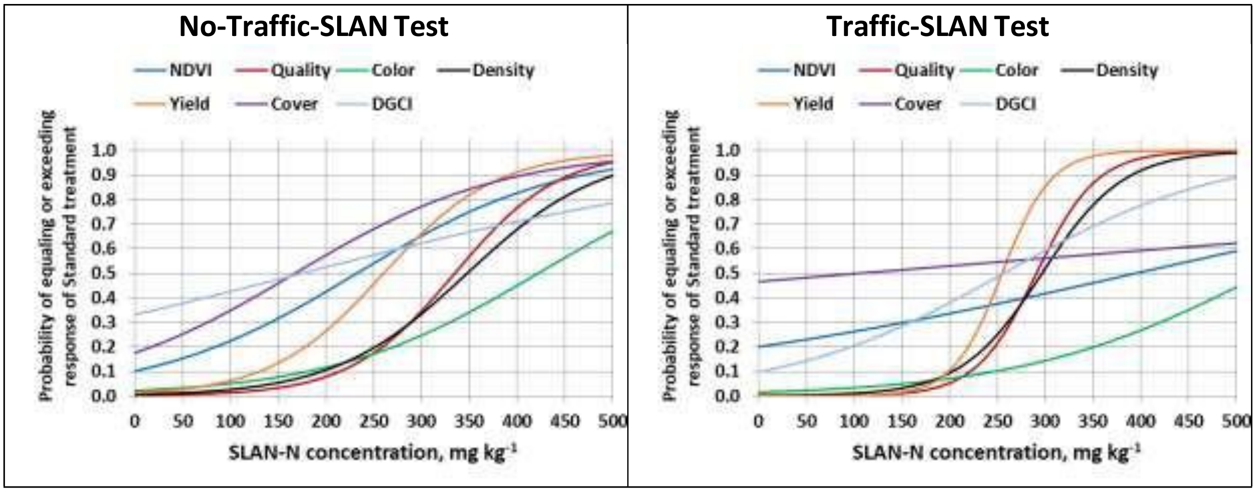


Figure 3. Probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft2 every 21 days) in relation to the Solvita SLAN-N concentrations for the No-Traffic and Traffic plots. 2019 results pooled across all sampling dates.

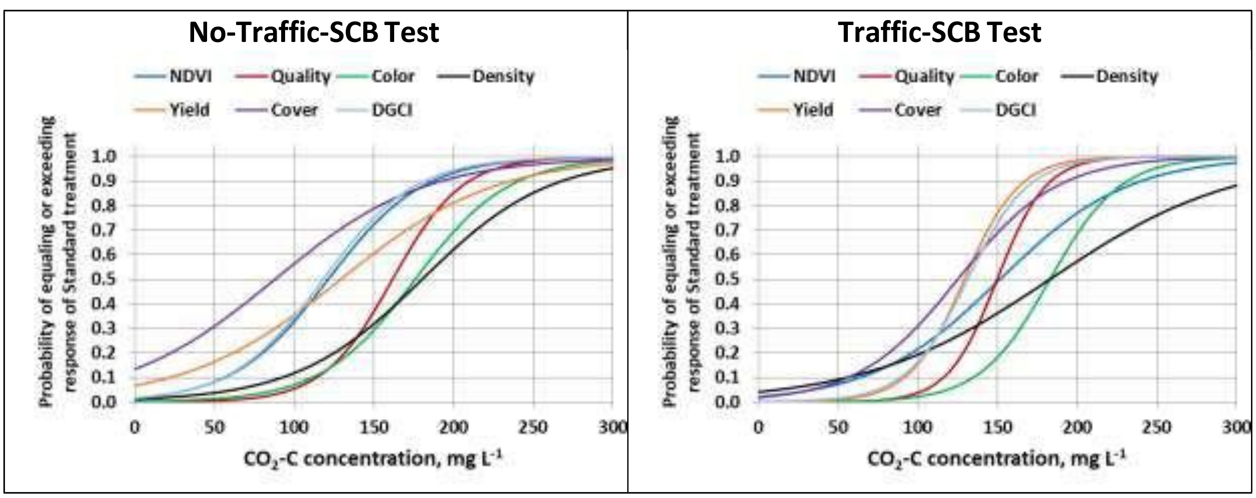


Figure 4. Probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft2 every 21 days) in relation to the Solvita SCB-C concentrations for the No-Traffic and Traffic plots. 2019 results pooled across all sampling dates.

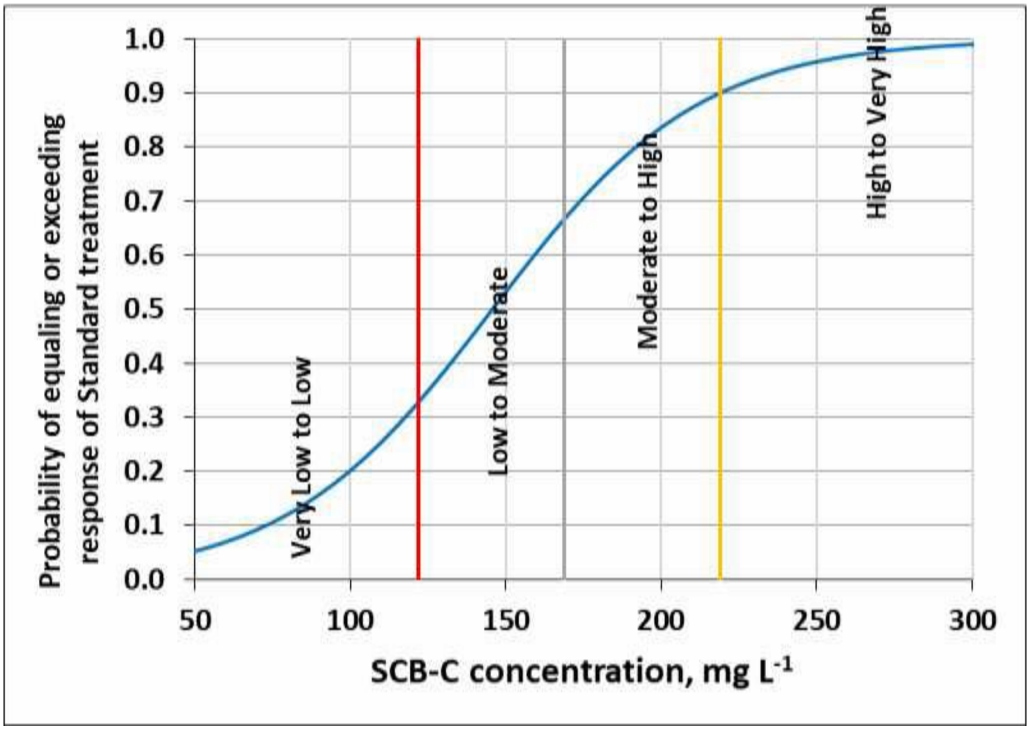


Figure 5. Probability curve representing all variables combined across both traffic treatments and categories of fairway creeping bentgrass responses that would be equal to or greater than the responses obtained from the Standard fertilizer treatment in relation to Solvita Soil CO2-Burst (SCB)–C concentrations. The red, gray, and yellow vertical lines indicate *P* values of 0.33, 0.67, and 0.90, respectively, obtained from Table 4. 2019 results.

USGA ID#: 2015-35-550

Title: National Evaluation of Turfgrass Water Use and Drought Resistance

Project Leader: Kevin Morris
Affiliation: National Turfgrass Evaluation Program (NTEP)

Start Date: 2016
Project Duration: 5 years
Total Funding: $500,000

Summary Points:

- Thirty-five total entries in the Cool-Season Water Use and Drought Resistance trial were planted in fall 2016/spring 2017. Five locations measure water used over a 100-day period under a rain exclusion shelter, and five locations induce drought by restricting ET_o replacement.
- All ten locations collected data in 2018, with differences in drought response noted among entries. In some instances, two-fold differences in water used under rain exclusion shelters were recorded among entries.
- The 40% ET_o replacement level did not result in acceptable turf at Riverside, CA location in 2018, while the 40% ET_o replacement level at Las Cruces, NM resulted in acceptable performance by some entries but with no statistical differences.
- Greater potential for acceptable turf quality under the lowest deficit reduction level was noted at Loga, UT, Fort Collins, CO and St. Paul, MN.
- Changes in trial protocol and statistical analysis resulted in greater statistical power and differences among entries than in 2017.
- A warm-season water use/drought trial with seventeen entries was planted at ten locations in summer 2018, but no data was collected under the rain exclusion locations in 2019.

Summary Text:

This project identifies turfgrass cultivars that deliver high quality turf while using significantly less water. Established at multiple locations nationwide, this project: 1) measures the actual amount of water required to maintain a prescribed level of quality or green cover, and 2) documents the performance of cultivars under varying levels of reduced evapotransporation (ET_o) levels.

Rain exclusion shelters are used to simulate 100-day drought periods in higher rainfall regions. Under the rain exclusion shelters we measure the amount of water needed to maintain a prescribed level of green cover, rate turfgrass quality as well as evaluate recovery from drought when irrigation is resumed.

The drier climate ET_o-based sites evaluate performance at three deficit irrigation levels for 100-120-day periods. Data recorded includes percent green cover over time, turfgrass quality and recovery rate after adequate irrigation is applied. The ET_o-based locations allow us to determine the minimum level of deficit irrigation appropriate for, and thus the water savings from each entry.

In separate trials, we are collecting three years of data on cool-season and warm-season turfgrass entries at ten trial locations each. This data will be used to develop and apply U.S. EPA WaterSense (http://www3.epa.gov/watersense/) certification (or another certification organization) label to grasses that qualify.

The cool-season trial entries submitted include nineteen tall fescues, fifteen Kentucky bluegrasses and one perennial ryegrass. In fall 2016 and spring 2017, these entries were established at ten locations, with five sites in higher rainfall regions utilizing a rain exclusion shelter, and five sites in low rainfall regions where irrigation is applied based on varying degrees of deficit ET replacement (40, 60 and 80% ET_o replacement). Difficulties and delays in obtaining rain exclusion shelters, as well as developing irrigation infrastructure resulted in delayed plantings at some locations.

Of the ten locations planted, six were able to collect at some data on drought response and recovery in 2017 (we agreed that the remaining four locations did not have test plots that were fully mature, and therefore not ready to apply drought stress). The locations that did not simulate drought in 2017 (Logan, UT; St. Paul, MN; Ft. Collins, CO; Amherst, MA), initiated drought treatments in 2018.

The six cool-season trial locations that initiated drought treatments in 2017 include Fayetteville, AR, College Park, MD, Griffin, GA and West Lafayette, IN (rain exclusion shelter sites); and Riverside, CA and Las Cruces, NM (deficit ET_o replacement sites).

Due to little or no statistical differences noted at the rain exclusion sites in 2017, a few tweaks were made to trial protocol and analysis. After consulting with our trial cooperators, the percent green threshold for re-watering was changed to 65% (from 50%) for 2018. Cooperators felt this change would more accurately reflect a homeowner's desire to maintain a consistent green lawn, as 50% showed too much brown (loss of color) and in some cases, did not allow for recovery from water lost in the plant and soil profile. Also, a change to the statistical analysis procedure was suggested to better reflect performance.

Rain exclusion shelter data from the southern-most cool-season sites in 2018 (Griffin, GA and Fayetteville, AR) showed a large range in water needed to maintain 65% green (i.e. 4.3 – 72 mm at Fayetteville, 123 – 262.7 mm at Griffin, GA)) but with no statistical differences among entries. Possibly, the higher summer heat load at these sites masks the differences in drought tolerance.

Data from the Mid-Atlantic (College Park, MD) and Midwest rain exclusion locations (West Lafayette, IN) had much greater statistical significance in 2018 with tall fescues generally maintaining green cover with less water than Kentucky Bluegrasses. However, significant differences were also noted within species at both sites. For example, the lowest water-consuming tall fescue in Indiana (DLFPS 321/3678) used only 50.6% (161 mm) of the water used by highest water-consuming tall fescue (LTP-SYN-A3, 317.7 mm). A similar result was seen for Kentucky Bluegrass at the two locations with the lowest water-consuming bluegrass at College Park, MD (BAR PP 110358, 165 mm) using only 61.5% of the water needed by the highest-consuming bluegrass in 2018 (Dauntless, 275 mm).

The ET_o-based site at Riverside, CA saw significant stress under 40% ET_o replacement as plots recovered from 2017 damage. No entry provided acceptable turf quality under 40% ET_o during the 120-day deficit irrigation period at this location in 2018. The 60% ET_o replacement level also saw some significant grass loss while very few statistical differences were noted among tall fescue entries. Statistical differences did occur among many Kentucky bluegrass entries during days 50-63 of the dry down period. A couple Kentucky bluegrass entries did not perform well at the 80% ET_o replacement level, hence they may not be not adapted to the southern California climate. There are still several Kentucky bluegrasses that show promise for irrigation reduction in a desert climate.

In 2017, significant differences in drought resistance and turf quality were noted among entries at Las Cruces, NM as well as differences in recovery from drought. Data from the 40% ET_o level in 2018 showed some entries delivering acceptable turf quality and performance throughout the trial period, albeit with little to no statistical significance. The 60% ET_o deficit level did show significant turf quality entry differences toward the end of the 2018 drought period (100-120 days), with greater differences noted among Kentucky bluegrass entries than tall fescues.

Three locations (Logan, UT, Fort Collins, CO and St. Paul, MN) collected their first data from this trial in 2018. With more favorable summer conditions for cool-season grasses, these locations have a greater potential for our lowest ET_o level to deliver acceptable turf quality. For instance, under 40% ET_o at Logan, UT, tall fescues outperformed Kentucky bluegrass with some entries maintaining acceptable turf quality for up to 95 days. At Fort Collins, CO, significant differences were noted among tall fescue and Kentucky bluegrass entries under 40% ET_o, but none outperformed the perennial ryegrass entry.

Finally, the St. Paul, MN site adjusted its irrigation levels to 0, 25 and 75% ET_o conforming to local conditions and needs. Late spring rains in 2018 led to little early drought stress at the 0% ET_o deficit replacement level, but by the end of the 120-day period, differences were notable. Many Kentucky bluegrasses held their turf quality for the first 40 days of drought under 0% ET_o but declined as expected in the remaining 80 days. The tall fescues in general showed little statistical differences, but some entries maintained good turf quality well into the drought period.

The warm-season version of this trial was established in summer 2018 at ten trial locations (five rain exclusion shelter, five using ET_o -based deficit irrigation). This trial consists of seventeen entries: eleven bermudagrasses, five zoysiagrasses and two buffalograsses. The rain exclusion shelters and deficit irrigation infrastructure were installed but it was decided to withhold initiating drought treatments at the shelter sites until 2020, allowing additional time for establishment of entries.

Deficit irrigation levels (ET_o based sites) were set at 30, 45 and 60% ET_o replacement for 2019. We are just now receiving data from the sites that initiated treatments in 2019, but thus far results are encouraging. After approximately 130 days of deficit irrigation, several entries have shown enhanced drought tolerance in at a least a few locations.

Figure 1. Representative quality of a buffalograss cultivar (***left***), 'TifTuf' hybrid bermudagrass (***middle***), and a zoysiagrass cultivar (***right***) in Riversdide, CA after irrigating at 30% ET for 140 days.

USGA ID#: 2017-01-611

Project Title: Effects of deficit irrigation and rootzone depth on water use and drought resistance of warm-season fairways

Project Leaders: Charles Fontanier and Justin Moss
Affiliation: Oklahoma State University

Objectives:

1. *Quantify water use of key turfgrasses as affected by deficit irrigation practices.*
2. *Evaluate the drought resistance of key turfgrasses as affected by rootzone depth.*
3. *Assess the effects of traffic on turfgrasses under drought stress caused by deficit irrigation programs.*

Start Date: 2017
Number of Years: 3
Total Funding: $90,000

Summary Points:

- Field plots for a warm-season fairway deficit irrigation study show differences among cultivars regarding drought resistance and irrigation water requirements.
- Several cultivars of bermudagrass were able to maintain acceptable appearance in a relatively wet year with less than 25mm of supplemental irrigation and an effective K_c of 0.18.
- An automated golf cart trafficker and irrigation system has been installed, but excessive rainfall throughout 2019 limited implementation.

Summary Text:

Background and Rationale
Water used for turf irrigation has been considered the number one restriction to advancement of the game of golf in many regions of the United States. In some cases, reduction of total irrigated acreage can be utilized for immediate water savings. A more feasible approach for many superintendents is to reduce the quantity of water applied to the irrigated footprint. Fairways represent on average 38% of irrigated acreage on a golf course and are often irrigated in excess of turf minimum requirements (Lyman, 2012). Research aimed at developing targeted water conservation programs for fairway irrigation could create meaningful water savings in some regions of the country. In mesic climates, irrigation should be applied as a supplement to rainfall and not in place of rainfall.

Modern irrigation practices typically rely on reference ET as calculated from meteorological data to estimate evaporative demand of the atmosphere. Warm-season turfgrass water use is then estimated as the product of reference ET and a crop coefficient of 0.6 to 0.7. Irrigation can then be scheduled to replace soil water lost through ET. Applying irrigation at volumes less than ETc is a common water conservation practice which attempts to maintain a target turf quality while reducing irrigation volumes. Many turfgrasses will demonstrate acceptable turf quality under deficit irrigation, although the severity of the program that sustains acceptable turf performance varies with species, cultivar, and soil/rooting properties (Feldhake et al., 1984; Poudel, 2010;

Wherley et al., 2014). Research aimed at measuring the interactions of turf performance, plant water use rates, soil moisture content, rootzone depth, and traffic is warranted.

Methods

Completed: A field experiment is being conducted at the Turfgrass Research Center in Stillwater, OK, to measure turf water use rates as affected by cultivar and deficit irrigation program. Eight fairway-type grasses (U-3, Celebration, Tifway, Latitude 36, TifTuf, Meyer, PremierPro, and OSU1403) were established from plugs in small plots as a randomized complete block design with three replications. Grasses were planted in June 2017 and allowed to fully establish under non-limiting irrigation in Year 1. Plots have been mowed three times per week at 0.5-inches during the growing season.

During summer 2018 and 2019, cultivar main plots were split into four irrigation levels (25, 50, 75, and 100% of ET_o x 0.7 or ET_c). Irrigation was hand-applied once per week using a nearby weather station to estimate reference ET. To assess how cultivar performance varies under the presence of restrictive rootzones, lysimeters (8-in and 12-in) were installed within the same plots (Fig. 1). Measurements of turf performance were conducted weekly using turf quality ratings (NTEP methods) and NDVI (Rapid Scan, Holland Scientific). During winter 2017-18, access tubes were installed for measurement of soil moisture using a soil profile sensor (PR2, Delta-T Devices). Volumetric water content was measured at 3, 4, 8, 12, and 16-inch depths twice per week. Differences in moisture content between measurement dates will be used to estimate ET rates over the course of a typical irrigation interval.

A second experiment is being conducted to study the effects of cart traffic on irrigation water requirements of common fairway turfgrasses. Small plots were established in summer 2018 from plugs (TifTuf, U-3, Latitude 36, Celebration, Tifway, OKC1403, and OKC1221) or sod (Meyer) as a randomized complete block design having three replications. The plots are unique in that they were planted as a pie wedge around a central point. A small–scale center pivot irrigation system has been designed such that it creates a radial gradient irrigation system (RaGIS) moving from near the center (wet) to the outer edge (dry) (Fig. 2). The pivoting arm is also designed to simulate golf cart traffic associated with the turning of wheels. Construction and installation of the RaGIS was completed in June 2019 with soil moisture sensors (TDR-315-L, Acclima) have been installed to monitor volumetric water content to the 10-inch depth. Initial irrigation and traffic treatments were applied briefly in August before rainfall halted the study.

To Be Completed: For experiment 1, a complete data analysis will occur during winter 2019-20. Preliminary results are reported below. For experiment 2, a full year of traffic and irrigation treatment will be applied in 2020 to meet project objectives.

Early Results

For the first objective, minimal irrigation was required during May or June 2019 due to frequent rainfall (Fig. 1). Calculation of daily ET_a rates was limited to three dates in July due to additional timely rainfall. When soil moisture was not limiting, K_c's ranged from 0.6 to 0.7 but further estimation was not feasible due to rainfall (data not presented). NDVI was affected by genotype on nearly every date, typically due to lower density of 'U-3' (Fig. 2). During July when soil moisture began to be depleted, NDVI was affected by the genotype by treatment interaction. Similar to the prior report, 'Meyer' and 'OKC1403' showed greater reductions in NDVI with diminishing soil moisture of the 25% ET_c treatment (Fig. 2).

The RaGIS applied a diminishing amount of water from the center to the outer edge of the circular plot as desired. Over a 10-day period in August, the RaGIS was used to apply traffic and differential irrigation resulting in measureable differences in soil surface moisture content, while moisture in the lower rootzone remained similar among treatments. Results suggest the RaGIS is functioning properly but longer duration study is required to separate cultivar responses.

Future Expectations

Results from experiment 1 will be compiled for submission of a manuscript to a peer-reviewed journal and the USGA Green Section Record. A full year of data will be collected for experiment 2 and reported on in 2020.

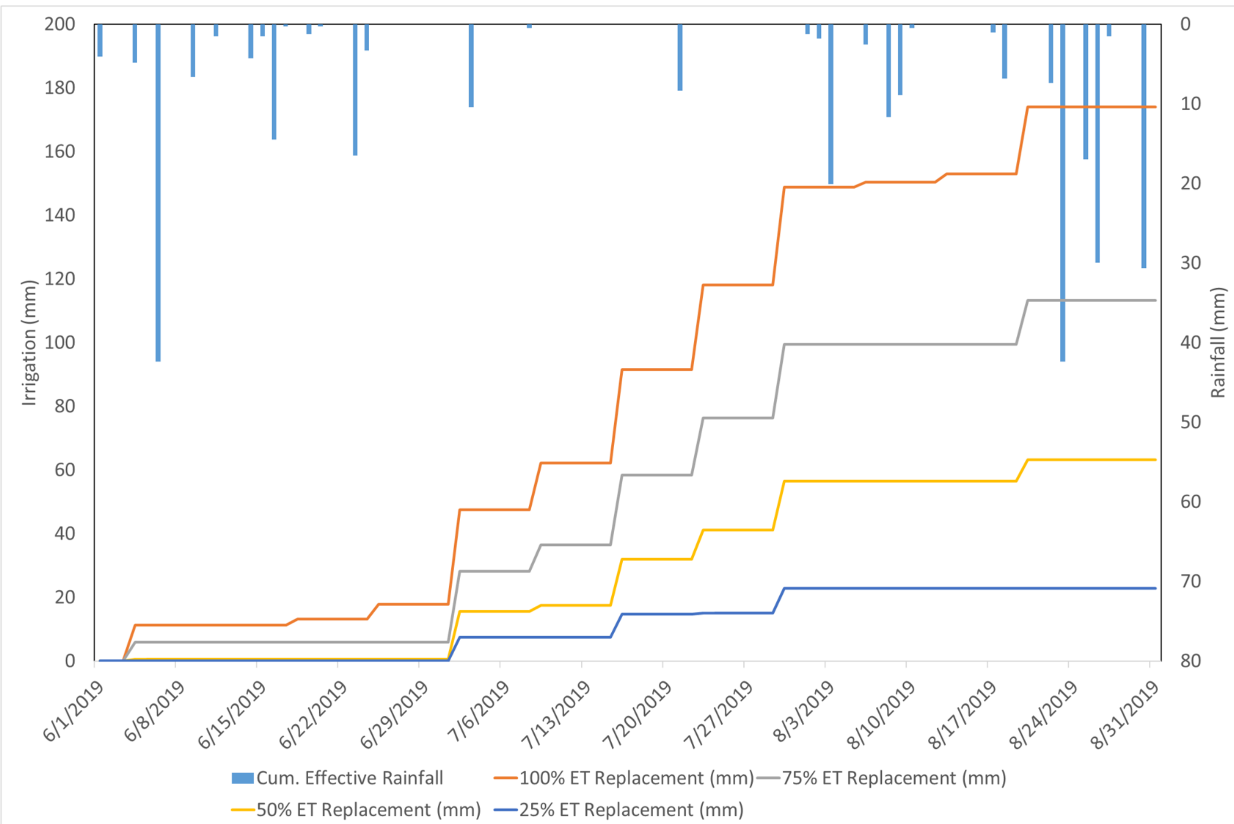


Figure 1. Cumulative irrigation applied for experiment 1 and rainfall occurrence for the 2019 growing season.

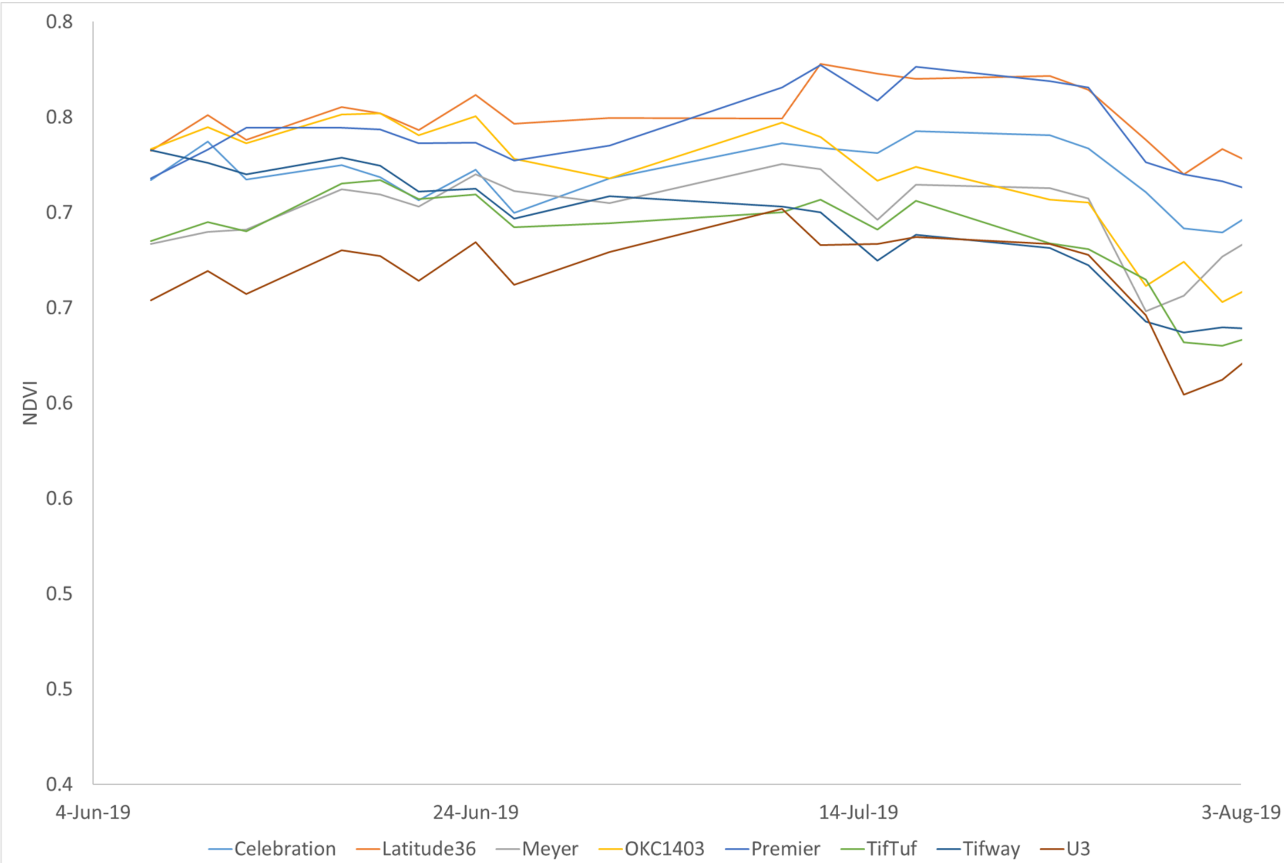


Figure 2. Normalized difference vegetation index (NDVI) of 8 turfgrasses irrigated at 25% ET_c during the 2019 growing season.

Figure 3. Overview of the RaGIS applying traffic to plots in August 2019.

USGA ID#: 2017-17-627

Title: Satellite-Based Estimation of Actual Evapotranspiration of Golf Course Cool Season Turf

Project Leader: Lawrence Hipps
Affiliation: Utah State University

Objectives:

1. *Conduct eddy covariance measurements of evapotranspiration (ET) and energy balance of a golf course over multiple years to document daily and seasonal water use values. Use findings to test currently used simplistic approaches such as reference ET.*
2. *Use remote sensing based models to estimate ET, and validate their performance against ground- based measurements.*
3. *Combine measurements with theoretical knowledge to determine the response of ET to variations in weather and climate. Use this knowledge to develop a physically based model to estimate ET for the periods between satellite overpasses.*

Start Date: 2017
Project Duration: 3 Years
Total Funding: $89,862

Summary Text:

Measurements of ET
The research site is at the Eagle Lake Golf Course near Layton, UT, which is about 25 miles north of Salt Lake City. The general region around the course is a mixture of urban, residential, and some agriculture. The golf course has been cooperative, and even asked us to add a weather station to run all year. Key research measurements include typical weather data, as well as: available radiation energy, heat flow into/out of soil, soil moisture and the transport (flux) of heat and water vapor (ET) from the surface. Fluxes of heat and water are determined from the eddy covariance technique, which is the gold standard. An image of the station is shown in Figure 1.

Figure 1. Image of the eddy covariance station on the golf course.

There had been an issue during the summer of 2017, in that secondary source irrigation water resulted in salt deposits on the lens of the water vapor sensor. Even with numerous cleanings, some data were not usable, and the quality of the results was compromised. In 2018, sprinklers were modified to nearly eliminate this problem, allowing better ET measurements from spring to fall. Various additional corrections result in hourly, daily and seasonal water use for the site.

ET Estimates from Remote Sensing Models
Landsat overpasses occur at approximately 2 week intervals, and were checked to verify they were cloud free and usable. The *Triangle Method Model* is used to determine ET for each overpass. Images are obtained for a much larger region surrounding the golf course. Different bands of the image allow estimation of normalized surface temperature (T*) and fractional vegetation cover (Fr) for each 30 meter element or pixel of the surface. When all the values of temperature and Fr from the Landsat image are plotted, the resulting cloud of points resembles a distorted triangle. Documenting the “cold” and “hot” edges, allows one to determine the fraction of available energy used in evaporation, or *evaporative fraction* (EF). A diagram showing how the calculation is made for each location is shown in Figure 2.

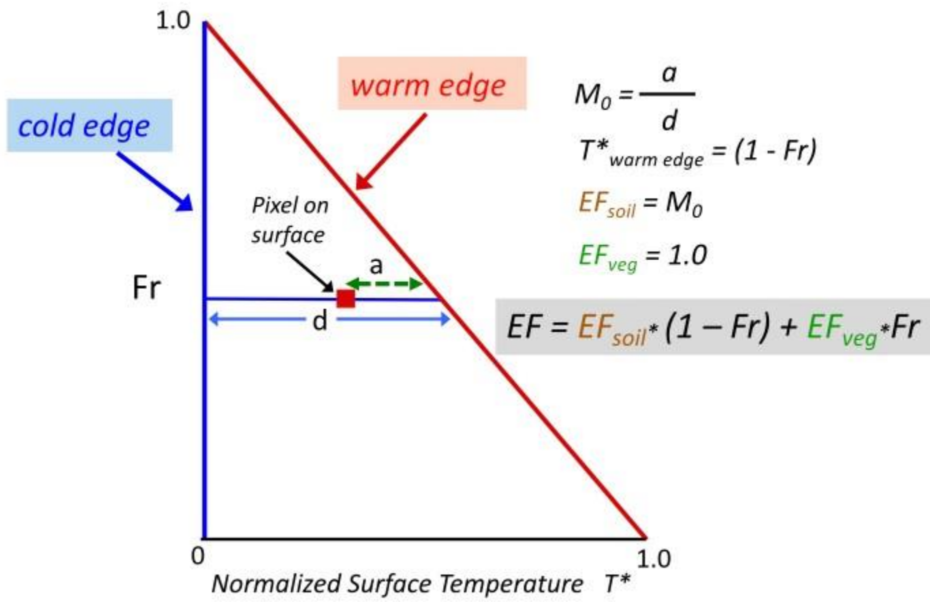
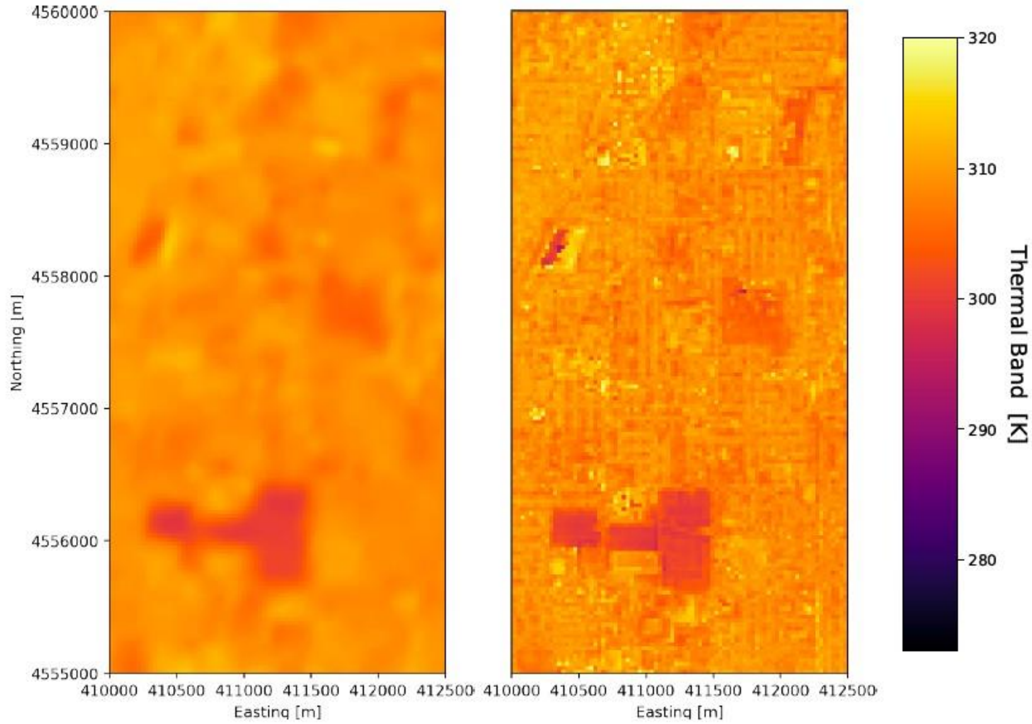


Figure 2. Diagram of how the EF determined from location of any pixel.

Sharpened Thermal Images
The actual spatial resolution of the Landsat temperature fields is ~100 m. A procedure is supplied in the Google Earth Engine (GEE) to estimate the resolutions down to 30 m, which is utilized by most users. However, it is a fairly crude algorithm. Using a more sophisticated and published approach from the USDA-ARS (Gao et al., 2012), leads to a much sharper image and more accurate data to use in the methodology. Figure 3 illustrates an example of the improved thermal sharpening resulting from the Gao et al. algorithm.



a. Original GEE interpolation b. Approach of Gao et al. (2012)

Figure 3. Example of thermal image sharpened to 30 m.

Results of Triangles From Images

An example of an actual triangle for a Landsat overpass is shown below in Figure 4, including the cold and warm edges. The lobes on the lower right corner of the points, are the result of urban surfaces such as concrete, asphalt etc. These have larger thermal storage properties than vegetated or bare soil, and do not physically relate to the triangle shape used to determine ET. Hence, they were not considered in fitting the warm edge line. Actual triangles like this allow the calculations for EF to be done for each pixel, as described above in Figure 2.

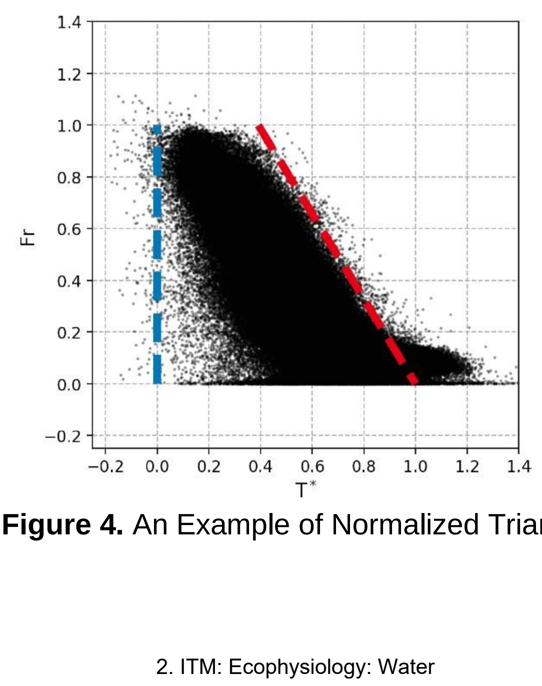


Figure 4. An Example of Normalized Triangle.

For each overpass, a map of both t EF and ET can be generated for the region described by the triangle. Figure 5 shows an example of the spatial distribution of EF values resulting from one image. On the right is a magnification of the golf course and bordering areas. Since EF is the ratio of actual ET to available energy, the net radiation minus the soil heat flow is later used to calculate actual ET.

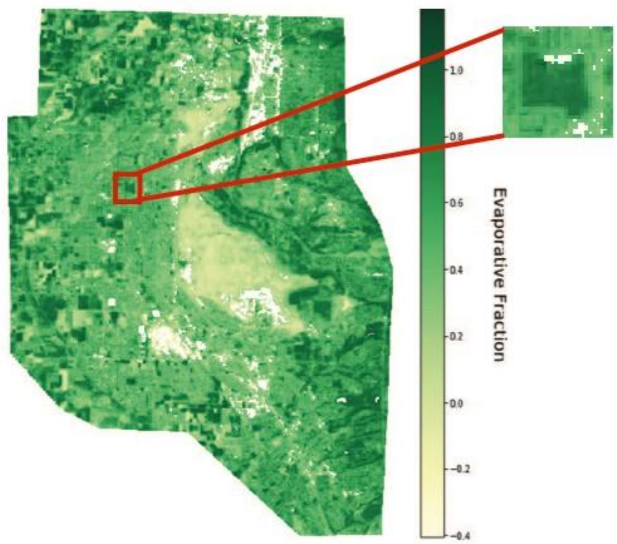


Figure 5. Example of evaporative fraction over region, with magnification of golf course.

The white areas on the edges of the golf course represent either buildings or paved surfaces. When examined carefully, some spatial variability is present in the EF even inside the golf course. The larger EF values are associated with larger ET.

The same procedure was used to generate both EF and actual ET values for 10 cloud-free overpasses. Figure 6 depicts the measured and model ET values for the golf course site for all 10 cases.

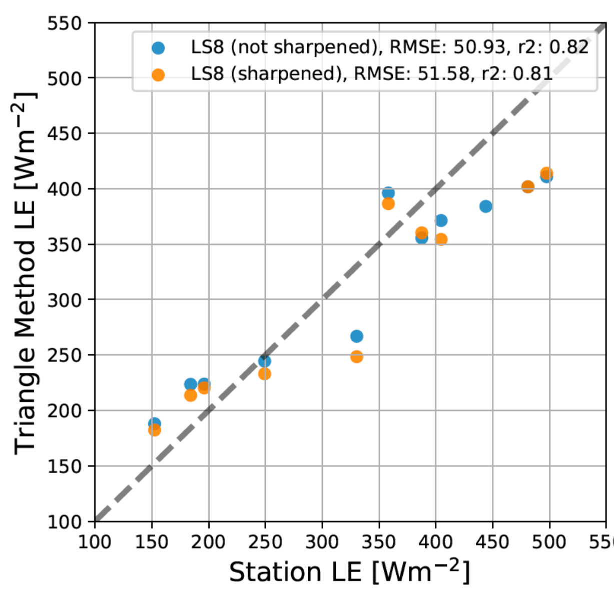


Figure 6. Model vs. measured ET values for original and sharpened surface temperatures.

These show model vs. measured ET at time of the over pass. The units of Watts per square meter represent the energy used in evaporation. These can easily be converted to inches of water per time. Agreement is good, and within the 10 -15% uncertainty of the ET measurements much of the time. However, the Triangle Model estimates are biased low at the highest ET values.

Seasonal Changes in ET From Eddy Covariance

Energy Balance Closure

These results were obtained by forcing the energy equation to balance. In other words, the sum of available energy (net radiation minus energy flow into the soil) is set equal to the sum of the energy used in the fluxes of heat and water vapor from the surface.

$$R_{net} = H + LE + G \quad (1)$$

Where R_{net} is net available radiation at the surface (sources minus losses), G is soil heat flux, H is heat flow between the surface and air above, and LE is energy used in evaporating water (ET). Generally, eddy covariance values for H and LE are lower than available energy (R_{net} – G). One approach is to add to the H and LE values to “force the above equation to be balanced. This issue is currently under discussion in the meteorological research community, with some proposing that the imbalance is not real, but due to the fact that soil heat flow is slower and not in phase with the other “fast” terms. Since the triangle method assumes the above equation is balanced, we decided to force the closure as just noted.

The daily total of ET determined from the eddy covariance tower is shown below in Figure 7 for the 2018 season. Some wind direction issues for several days in early July led to a gap in results. Gap filling techniques will be used to fill in this days.

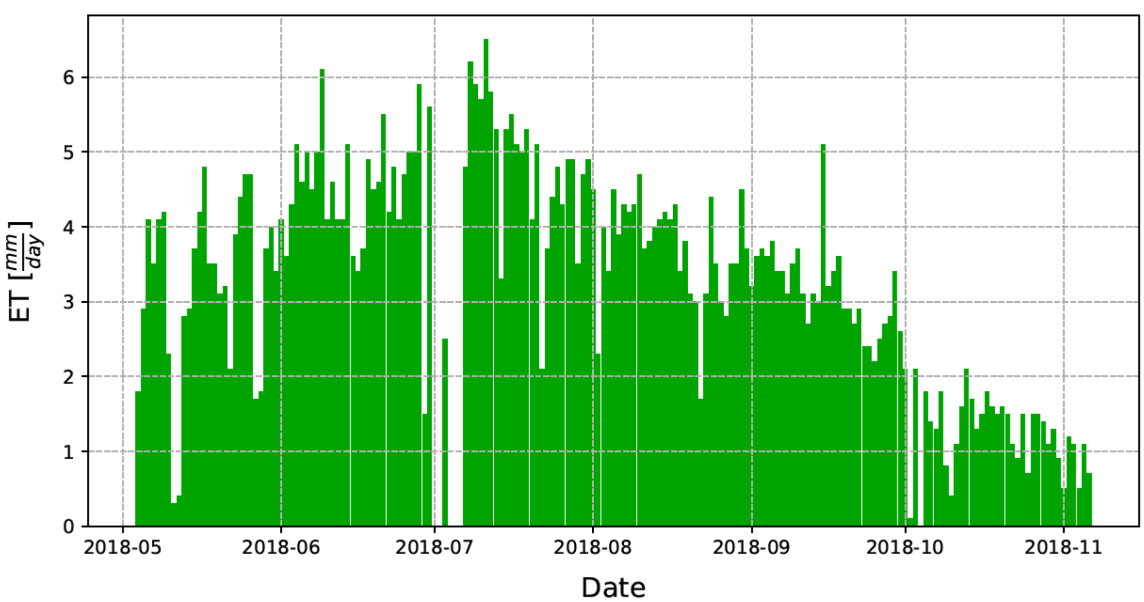


Figure 7. Daily ET measured by the eddy covariance system at the tower.

Note that the daily values vary over time with changes in weather conditions. However typically the daily totals are between 3 and 5 mm day-1 (0.12 to 0.20 inches day-1). Occasionally, the values exceed 6 mm day-1, which is quite large considering the few credible current estimates that are available about turf water use.

Future Work

- Examine more carefully, the daily, weekly and seasonal ET values for the site. Quantify which environmental factors play the most critical roles in ET. Key properties examined will be soil moisture, radiation, air temperature and humidity, as well as wind and any transport of heat from warmer and urban surroundings.

- MODIS satellite images are more frequent -- useable ones as often as 3-4 days per week if skies are clear. But they are more spatially coarse (250 m, and 1,000 m for temperature). It is worth the effort to test to see how well these images can be used in the triangle method to estimate ET for the golf course at much more frequent intervals.

- Test the accuracy of the commonly used Reference ET model. This is an empirical approach, that simply determines a “reference” value for ET from the weather data, and then multiplies it by a “black box” value. Our initial analyses suggests it has problems due to the value of that constant changing from day to day.

- The Penman Monteith ET model can combined with a stomatal conductance model to allow ET to be simulated at hourly intervals. Such an approach incorporates the plants physiological responses to changes in soil water and/or weather conditions. This will be tested to determine if it can yield improvements in ET estimation from weather data.

Reference

Gao, F., W. P. Kustas, and M. C. Anderson. 2012: A data mining approach for sharpening thermal satellite imagery over land. *Remote Sensing*, 4 (11), 3287-3319, doi:10.3390/rs4113287.

USGA ID#: 2017-36-646

Title: Soil Moisture Sensor Irrigation Scheduling in Bermudagrass [*Cynodon dactylon* (L.)] Fairways.

Project Leader: Priti Saxena, Robert Green, Eudell Vis and Valerie Mellano
Affiliation: California State Polytechnic University, Pomona, CA.

Objectives:

1) Analyze the performance of SMS systems to apply less irrigation and result in water savings by bypassing irrigation events when soil moisture is adequate. 2) Evaluate SMS capability to maintain bermudagrass quality. 3) Compare SMS performances against standard irrigation scheduling. 4) Evaluate turfgrass quality, density and color.

Start Date: 2017
Project Duration: 3 years
Total Funding: $45,000

Summary Points:

- Effects of three soil moisture sensor treatments were found to apply lesser amount of water on bermudagrass plots than controlled plots, and therefore, effectively reducing the number of irrigation cycles.
- Controlled plots irrigation is based on ETcrop, previous 2-d CIMIS ETo (adjusted for precipitation), monthly warm-season turfgrass Kc, full run time multiplier (RTM) based on individual plot irrigation system distribution uniformity, low quarter (DU_{LQ}), and individual plot precipitation rate.
- The duration of the study (April – October) is based on growing season of bermudagrass in southern California, which concluded that soil moisture sensors are effective in conserving water than control while maintaining acceptable turfgrass quality**.**

Summary Text:

Water conservation is the main objective of this study and Soil moisture sensors (SMS) on turfgrass plots, maintained as fairways of Southern California would be an excellent irrigation management tool; with the potential to conserve water and reduce daily water use. Reducing a, irrigation application per valve on a golf course could significantly save on water and energy cost and assisting superintendents in scheduling irrigation by assessing soil moisture accurately and quickly at the root zone.

Automated soil moisture sensor systems contribute significantly to maintaining adequate moisture in root zone by overriding irrigation scheduling or bypassing unnecessary irrigation cycles (Cardenas-Lailhacar, 2007). To maintain such moisture levels in fine texture soil, the accurate monitoring of moisture content is imperative along with the use of SMS. Additionally, healthy turfgrass is the result of a balance between soil not too wet or not too dry by applying the appropriate amount of irrigation. The goal of this study is to identify SMS systems that could reduce the number of irrigation cycles or amount of water applied while maintaining acceptable turfgrass quality as compared to traditional time-based irrigation scheduling on fairways.

Hybrid bermudagrass [*C. dactylon* (L.) Pers. × *C. transvaalensis* (Burtt-Davy)] GN-1 plots (each 10 ft. × 10 ft. [3m × 3m]) were sodded in 2002 and separated by 3 ft. (0.91 m) in all directions at Center for Turf, Irrigation and Landscape Technology (CTILT), California State Polytechnic University, Pomona, CA. Soil moisture sensors from three different sources (Toro, Rain Bird and tucor) were installed and compared to a control treatment (no SMS). The control plots were irrigated based on the ET value collected from CIMIS station #78. Irrigation amount is based on ET_{crop}, previous 7-d CIMIS ET_o (adjusted for precipitation), monthly warm-season turfgrass K_c, full run time multiplier (RTM) based on individual plot irrigation system distribution uniformity, low quarter (DULQ), and individual plot precipitation rate. Plots are individually scheduled once per week. Total weekly irrigation run time will be equally divided over five irrigation days per week.

Each plot is individually zoned and has a rotating nozzle sprinkler at each of the four corners. The experiment were laid in complete randomize design with three replication. Bermudagrass plots were maintained at the height of ½" and mowed twice a week during. The clippings were collected to measure the dry weight, weekly. The plots were double cut in opposing directions using a Tru-Cut walk behind reel mower. Glyphosate was spot-sprayed utilizing a Solo® 3-gallon backpack sprayer at a rate of 1 oz/ gallon to eradicate broadleaf weeds between rows. SpeedZone Southern® Boradleaf Herbicide was applied on the plots @ 1.5 oz /1000 sq. ft for the post emergent control of broadleaf weeds. The plots were verticutted and Urea was applied @ 1 lb N/100ft^2 in fall 2019.

Main study Data Collection would be conducted during April 1st – October 31st, 2018 and 2019). The study would be started with all plots at similar water content (field capacity). Data collection includes runtime (minutes) for each plot and treatment average (by week, month, season), irrigation applied for each plot, amount of saved applied irrigation (actual amount and as a percentage of no SMS control treatment), visual turfgrass quality, density and color ratings, clipping yield and soil salinity (bulk electrical conductivity). The results so far obtained showed that run times of soil moisture sensors were lesser than that of control plots in all replications. ThThe results will provide irrigation applied for all the treatments, along with water savings for the SMS treatments during three years of study. The SMS response comparisons will also be reported. The bermudagrass turfgrass quality ratings are acceptable (6-7) for a fairway in Southern California based on NTEP rating (1-9).

Image 1: Installation of Rain Bird Integrated sensor system in Bermudagrass plots.

Image 2: Mowed bermudagrass plots.

USGA ID#: 2017-37-647

Title: Encouraging adoption of precision irrigation technology through on-course application and demonstration of water savings

Project Leaders: Chase Straw[1], Josh Friell[2], and Eric Watkins[1]
Affiliation: [1]University of Minnesota, [2]The Toro Company

Objectives:

1. Quantify response of turf and course conditions to changes in plant available water.
2. Quantify changes in water consumption between soil moisture sensor (SMS)-based, evapotranspiration (ET)-based, and traditional irrigation scheduling.

Start Date: 2018
Project Duration: 3 years
Total Funding: $204,876

Summary Points:

- The study was moved from BCCC to ECC due to unforeseen golf course renovations at BCCC
- Two course surveys were completed at ECC with the PS6000 under varying soil moisture conditions to gain an understanding of soil moisture variability at the study site
- Nine fairways were identified for use in the study (randomized complete block design; three treatments and three replications)
- Irrigation zones were delineated, and then soil moisture classes within each zone were calculated, on fairways receiving the soil moisture sensor-based treatment
- In-ground SMS were installed within each soil moisture class on the fairways receiving the SMS-based treatment
- One catch can irrigation audit was conducted on all fairways used in the study

Summary Text:

Rationale

The purpose of this research is to demonstrate that adoption of currently available soil moisture sensor and mapping technologies can provide golf course superintendents with appropriate, actionable information that can result in significant water and cost savings relative to ET-based and traditional irrigation scheduling methods. Additionally, since this is the first on-course application of soil moisture sensor and mapping technologies, we expect that the knowledge gained will assist in creating practical protocols for their use in implementing site-specific irrigation.

Progress to Date

The study was initiated in 2018 at Brackett's Crossing Country Club (BCCC; Lakeville, MN). The golf course had substantial damage coming out of the 2018-2019 winter. As a result, they decided to close in the fall for major renovations to tees, fairways, and greens. This unfortunate circumstance led to the need to move the study to another golf course. In June, the study was proposed to the Edina Country Club (ECC; Edina, MN) Green Committee, and then ultimately approved later that month. The study was initiated in July 2019 at ECC with the goal for the year being to efficiently apply knowledge gained in the previous year and eliminate or minimize any time lost.

Two course surveys were conducted July 11 and 15 using the Toro Precision Sense 6000 (PS6000) to measure hundreds of georeferenced soil moisture (i.e. % volumetric water content) data points. Approximately 9 cm of rainfall occurred prior to the first survey, so it was conducted under near-saturated conditions. No additional rainfall or irrigation occurred between the first and second survey. The second survey was most informative because soil moisture at the time was likely the best representation of field capacity. Soil moisture at field capacity has a stable pattern of spatial variation that can be strongly correlated with other stable soil properties (e.g. soil texture).

All spatial methods and analyses to-date were conducted in ArcMap. Nine fairways (six par 4's and three par 5's) were selected for use in the study and placed into similar groups of three based on size, soil moisture descriptive statistics, and spatial maps of soil moisture variability (Figure 1). Ordinary kriging was used to interpolate PS6000 data and produce the soil moisture spatial maps, which were raster maps comprised of 1 m^2 pixels. Each grouping of three fairways is considered a replication in the study (Figure 1). Three irrigation scheduling treatments were assigned using a randomized complete block design and will be initiated in 2020. Irrigation scheduling treatments for the study include:

1. SMS-based irrigation scheduling
2. ET-based irrigation scheduling
3. traditional irrigation scheduling

The remainder of 2019 focused on creating irrigation management zones and installing in-ground soil moisture sensors within the three fairways that will receive SMS-based irrigation treatments. A GPS receiver on the PS6000 was used to georeference irrigation head locations for all nine fairways. Irrigation management zones for the fairways receiving SMS-sensor based irrigation were delineated around each irrigation head using Thiessen polygons. Soil moisture raster maps from the two course surveys were used to determine average soil moisture values within each irrigation management zone. Irrigation management zones were classified into one of three soil moisture classes ("low," "moderate," or "high" soil moisture) using Jenks natural breaks (Figure 2).

Toro TurfGuard in-ground SMS were installed August 22. One sensor was placed in each soil moisture class within each replication (Figure 3), for a total of nine sensors. Placement was made within the largest cluster of a classification or where it was believed to be most representative of the classes average soil moisture, while also considering location effects (e.g. slope, shade). Where zone classification differed between surveys, highest consideration was given to the soil moisture classification maps created from the second survey during in-ground SMS installation, since it was likely the best representation of field capacity. Sensors were installed so that the top and bottom tines were at soil depths of 5 and 18 cm, respectively. Soil moisture is measured from the in-ground sensors every 5 minutes and can be monitored at any time using Toro SiteVision software.

Next, dry downs were attempted to determine upper and lower soil moisture limits for each in-ground sensor. In order to initiate the dry downs, the superintendent was asked to completely turn off irrigation within fairways receiving the SMS-based treatment. Due to rainfall the weeks following in-ground soil moisture sensor installation, a sufficient dry down was not achieved. Ideally, during a dry down, soil moisture values at each sensor corresponding to field capacity (defined in this study as the stable value following a saturating irrigation or precipitation event after excess water has drained) and permanent wilting point (defined in this study as the value at which wilt becomes apparent, NDVI values begin to decline significantly, or the superintendent feels that we have reached his comfort limit) will be used to calculate plant available water (field capacity minus permanent wilting point). Considering a sufficient dry down was not achieved, 50% field capacity will be used as initial estimates for permanent wilting point within each soil moisture class. Once treatments are initiated, the calculated plant available water will be used to determine a threshold that triggers irrigation within each soil moisture class. NDVI will also be measured during dry downs with the PS6000. A UAV will be used to measure NDVI as well, in addition to canopy temperature.

Finally, one catch can irrigation audit was conducted on all fairways included in the study to quantitatively define the relationship between the programmed water application and the true depth of irrigation applied. Catch can audits were conducted on September 16 (rep 1 fairways), 19 (rep 3 fairways), and 23 (rep 2 fairways). Irrigation heads were programmed to make one full rotation during the audits (i.e. runtime of 4 minutes and 10 seconds per head). Irrigation depth data were interpolated for visual assessment of irrigation distribution uniformity (Figure 4). The data will ultimately be useful in identifying the influence of run-time on soil moisture values around individual heads within soil moisture classes after an irrigation event, in order to adjust their command as needed. Correlation coefficients were determined between catch can amount data from the one irrigation audit and volumetric water content data from the two course surveys and there were no significant relationships on any of the fairways (data not presented).

Future Expectations

Once irrigation resumes in 2020, visual observations will be made ensuring that heads are rotating properly and correct nozzles are installed. Any necessary adjustments will be made at that time. The nozzle for a given head will be checked within the irrigation system software (Toro Lynx Central Control), to ensure that it matches what is found on the course. This is important because water consumption between treatments will be determined by the irrigation system, and the reported water use is influenced by nozzle type. We will conduct another catch can irrigation audit to account for any changes made after the previous audit. Additionally, we will attempt dry downs in the fairways receiving the SMS-based treatment to continue identifying the soil moisture values for field capacity, permanent wilting point, and plant available water, and adjust our estimated values accordingly.

We will then apply the knowledge gained to compare SMS-based, ET-based, and traditional irrigation scheduling methods for the remainder of the 2020 growing season. For the three fairways receiving the SMS-based treatment, the valve-in-head sprinkler control will be used to schedule individual heads in each soil moisture class within each fairway to run together (e.g. all heads that are located in the low soil moisture class and within the same fairway will run together, all heads in the moderate soil moisture class will run together, etc.). Irrigation will only be allowed within a soil moisture class once the plant available water has been reduced by 50% (as measured by the SMS associated with that soil moisture class). When irrigation is allowed, the applied depth will be the lesser of 1) the total forecasted ET before the next forecasted rain event, or 2) the amount required to return the soil moisture to 75% of total plant available water (adjustments made as necessary). For the three fairways receiving the ET-based scheduling treatment, we will take a deficit irrigation approach and apply 70% of reference ET every three days (adjustments made as necessary). Finally, for the three fairways receiving the traditional irrigation treatment, we will ask the superintendent to irrigate as he usually would, taking into account any information that would typically be used.

Once irrigation scheduling treatments are initiated, total water consumption will be recorded for each treatment, where totals will be quantified and compared on an area basis. Soil moisture variability will be monitored periodically with the PS6000. Noticeable issues with the irrigation system (leaks, clogged nozzle, head doesn't rotate properly, etc.) will be made throughout the study on all fairways, as necessary. NDVI will be measured regularly by a UAV, and to a lesser extent the PS6000, to evaluate turfgrass quality between treatments. We hypothesize that by implementing mobile sensor and geographic information system technologies to properly place in-ground SMS, golf courses can realize the greatest water savings among the evaluated irrigation scheduling methods.

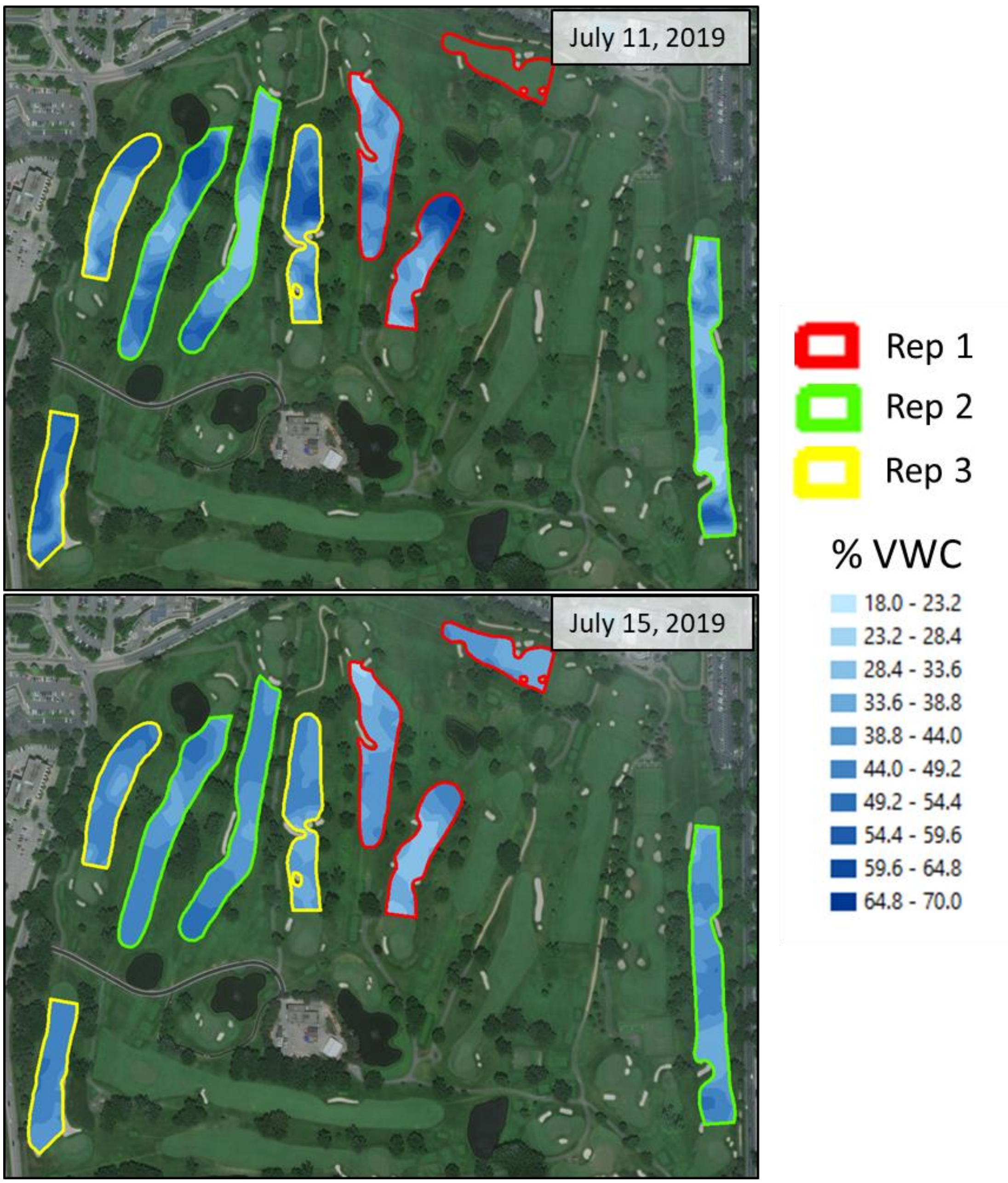


Figure 1. Soil moisture maps from July 11 and 15, 2019, where data were collected under saturated and approximate field capacity conditions, respectively. The nine fairways selected for use in the study were placed into similar groups of three based on their size, soil moisture (% volumetric water content; VWC) descriptive statistics, and spatial maps of soil moisture variability. Each grouping of three fairways is considered a replication in the study (i.e. randomized complete block design with three replications).

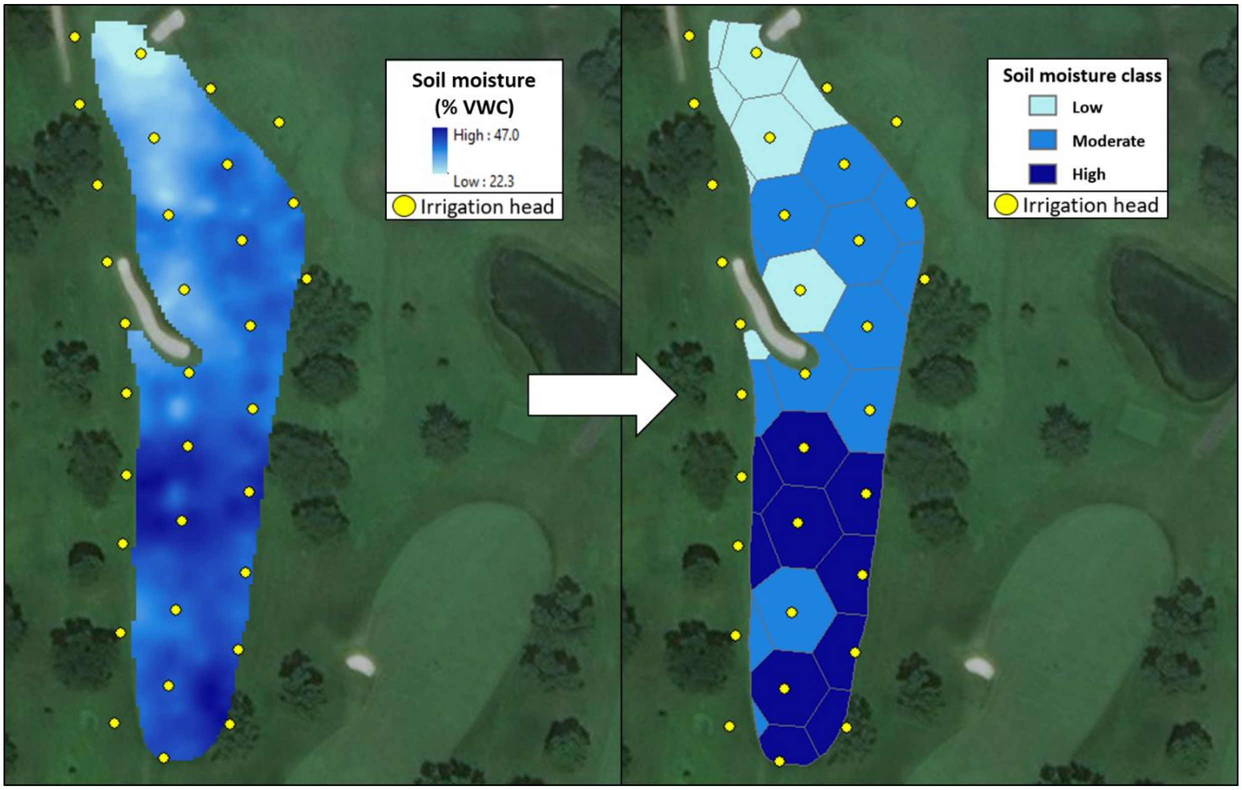


Figure 2. Soil moisture map [left; % volumetric water content (VWC)] and soil moisture classes within delineated management zones (right) on one fairway receiving the SMS-based treatment.

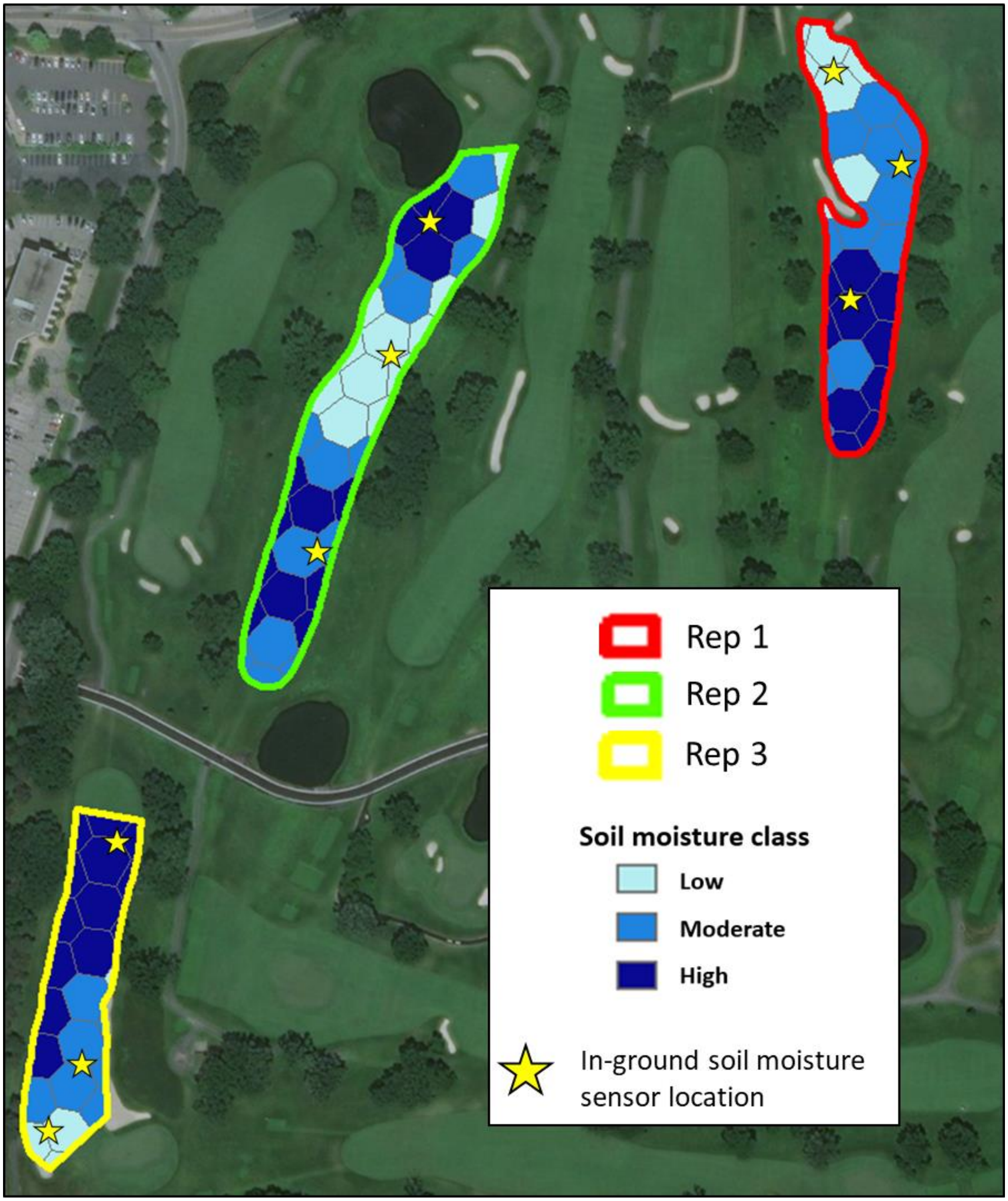


Figure 3. In-ground soil moisture sensor locations within each soil moisture class on the fairways receiving the SMS-based treatment.

Figure 4. Applied irrigation depth from catch can audits conducted on September 16 (rep 1 fairways), 19 (rep 3 fairways), and 23 (rep 2 fairways). Irrigation heads were programmed to make one full rotation during the audits (i.e. runtime of 4 minutes and 10 seconds per head).

USGA ID#: 2017-38-648

Project Title: Comparison of Irrigation Scheduling Methods

Project leaders: W. Dyer[1], D. Bremer[1], A. Patrignani[2], J. Fry[1], J. Hoyle[1] and J. Friell[3]
Affiliation: [1]Dept. Horticulture and Natural Resources, Kansas State Univ.; [2]Dept. Agronomy, Kansas State Univ.; [3]Toro Co.

Objectives:

1) Determine quantitative turf canopy responses to plant available water from in-situ soil moisture sensors
2) Compare SMS-based irrigation scheduling to traditional irrigation and ET-based irrigation scheduling

Start Date: 2017
Project Duration: 4 years
Total Funding: $129,733

Summary Points:

- Irrigation threshold was determined based on the fraction of plant available water at which the turfgrass showed sign of water stress.
- Integrating soil moisture and canopy information from in-situ sensors has the potential to improve the timing and amount of irrigation events during the growing season.
- Soil moisture sensor-based irrigation saved the most water compared to traditional frequency-based irrigation and deficit irrigation (60% ET) approaches.

Summary Text:

Soil Lab Research (Determining Plant Available Water)

Plant available water (PAW) is defined as the equivalent depth of water available for plant uptake within a layer of soil of specified thickness (Ochsner 2019). In our research we determined PAW utilizing site-specific soil properties to characterize soil texture and model the relationship between matric water potential and volumetric water content (VWC). After utilizing the HYPROP and WP4C instruments (Meter Group), a soil water retention curve was generated using the van Genuchten model (van Genuchten 1980). Upper and lower limits were set at 10% air-filled porosity and -1500 kPa (Figure 1). The difference between the upper and lower limit quantifies the PAW, which is an indicator of the soil's storage capacity. Next, the hydrometer method was used to determine particle size analysis for each plot resulting in an average of 12% ± 3.3 sand and 38% ± 2.3 clay. Silt loam was the determined soil texture classification.

Greenhouse Research (Determining Irrigation Threshold)

A pilot experiment was conducted during spring 2019 on 'Meyer' zoysiagrass (*Zoysia japonica L.)* in the greenhouse to force a dry down period to monitor the turf canopy response to VWC. Four undisturbed soil core monoliths (20 cm diameter x 38 cm height) were taken from the research site and placed in PVC tubes. A time-lapse camera was mounted above the soil core monoliths to collect images of the turf canopy. Soil moisture sensors (CS655, Campbell Sci.) were placed horizontally at a 10 cm depth to collect VWC. Air temperature and relative humidity sensors (CS215, Campbell Sci.) monitored atmospheric conditions within the greenhouse. Soil core monoliths were initially well watered, and thereafter a dry down period was enacted for 40 days. Results indicate an average decrease in VWC by 15% during the dry down period, and green cover of the canopy decreased by 50%. The onset of turf canopy stress was first detected at 0.70 fraction of plant available water (Figure 2). Information gained from this pilot experiment allowed us to set irrigation thresholds to incorporate into the field research.

Field Research (Comparing Irrigation Techniques)

Field research was conducted from June through September 2019 on 'Meyer' zoysiagrass (*Zoysia japonica L.*) at the Rocky Ford Turfgrass Research Center in Manhattan, KS. The objective was to integrate soil moisture and canopy information from in-situ sensors to determine turf canopy responses to soil water deficits and improve the timing and amount of irrigation events during the growing season. Treatment layout consisted of a Latin square design (8m x 8m plot sizes) comprising of four various irrigation treatments: a traditional frequency-based irrigation, a deficit irrigation based on reference ET (ETo), an irrigation based on soil moisture sensor (SMS) information, and a check treatment of zero irrigation (i.e., precipitation only). Soil moisture sensors (CS655, Campbell Sci. and TurfGuard, Toro) were placed horizontally at 10 cm depth in the center of each of plot. Eight normalized difference vegetation index (NDVI) sensors (SRS, Meter Group) were utilized to continuously monitor the turf canopy, and eight infrared radiometers (SI-111, Apogee) monitored canopy temperature of the turf. Weather data were collected from an on-site Kansas Mesonet weather station to track air temperature, relative humidity, and precipitation throughout the growing season. Total precipitation between the months of June and October resulted in an above average amount totaling 800 mm, compared with the 30-year normal precipitation during the same period. However, a dry period at the end of July had a pronounced soil water deficit decline (Figure 3). This was the only period the threshold for initiating irrigation was met. Green canopy cover had declined slightly (by 5%) when VWC reached the irrigation threshold, and green cover continued to decline within the non-irrigated (check) plots (by 12% at the end of the dry period). Green canopy cover and VWC remained well above the irrigation threshold for the remainder of the season in all treatments. NDVI measurements also captured the decline in turf canopy during the dry period, especially within the check treatments, declining from 0.83 to 0.73. Precipitation received and cumulative irrigation applied to each treatment during the 2019 season are depicted in Figure 4. Total water applied to each of the treatments resulted in 187 mm applied to the traditional frequency-based irrigation, 119 mm applied to the 60% ET treatment and 37 mm applied to the SMS treatment. Thus, the SMS based irrigation approach applied 80% less water than the traditional frequency-based irrigation treatment and 44% less water than the 60% ET-based irrigation treatment.

By determining the upper and lower limits of plant available water based on soil physical properties and the fraction of plant available water at which turfgrass shows sign of water stress, we were able to apply a proper threshold for initiating irrigation. Significant water savings were achieved by utilizing this approach, while still maintaining turfgrass quality. Research will continue through the 2020 growing season and future research will look to incorporate forecasted reference evapotranspiration, which could have even further implications for water savings within turfgrass systems.

References

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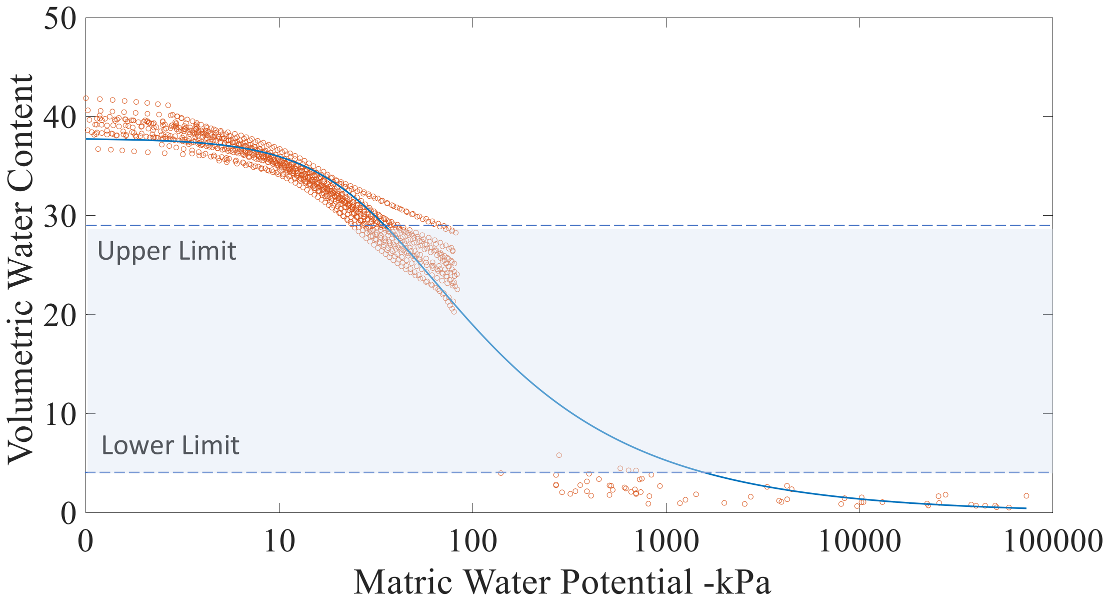

Figure 1. Moisture release curve depicting the relationship between matric water potential and volumetric water content in the turfgrass root zone.

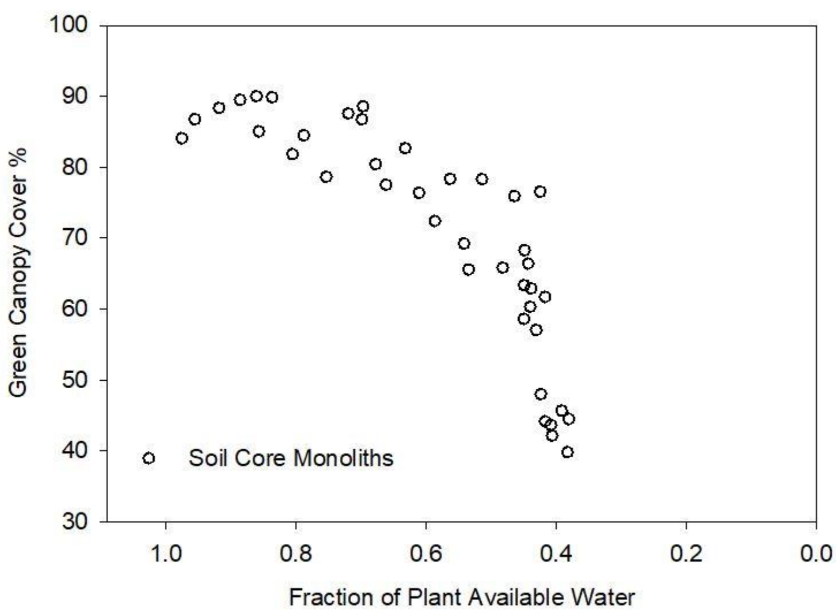

Figure 2. Average relationship between green canopy cover and fraction of plant available water.

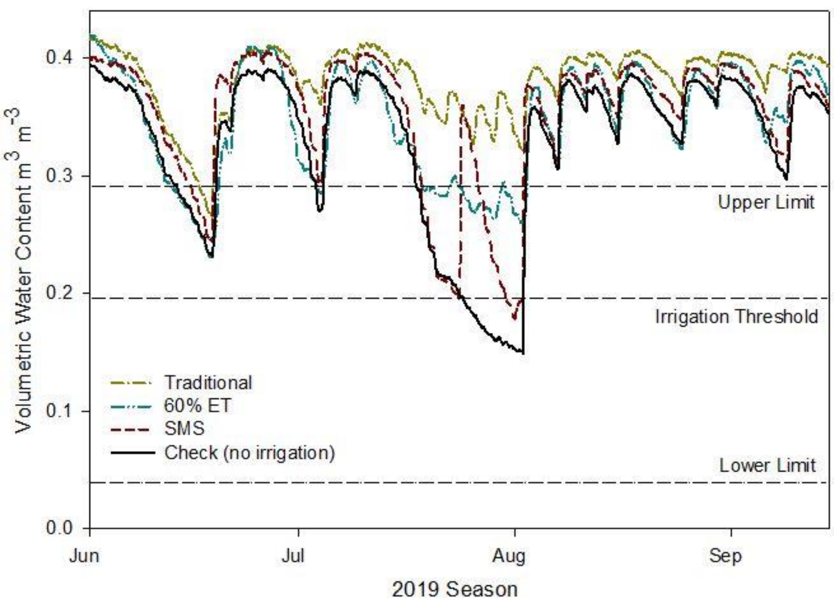

Figure 3. VWC at a10 cm depth throughout the 2019 growing season showing upper and lower limits of available water.

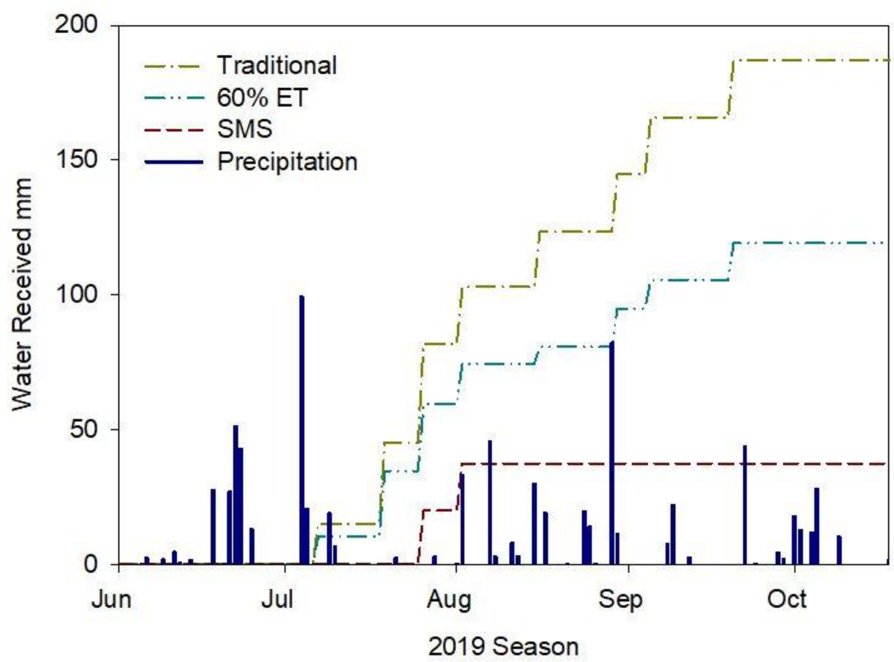

Figure 4. Precipitation and cumulative irrigation during the 2019 season.

USGA ID#: 2018-04-654

Title: Enhancing Water Conservation through Remote Sensing Technology on Golf Courses

Project Leader: Dr. Joseph Young, Dr. Sanjit Deb, Dr. Glen Ritchie, Dr. Wenxuan Guo, Eduardo Escamilla, Juan Cantu, and Dr. David McCall[2]

Affiliation: Texas Tech University and Virginia Tech University[2]

Objectives:

1. Ground-truth spectral sensory data from a UAV to specifically recognize water-deficit stress
2. Evaluate/Compare satellite-based NDVI to ground based measurements of soil properties (NDVI, compaction, water content, infiltration etc.).
3. Optimize the best technology for ease of transfer to the golf industry and quantify water savings

Start Date: 2018
Project Duration: 3 years
Total Funding: $95,618

Summary Points:

- The spectral sensors collecting images in this research do not appear to improve our ability to identify drought stress in warm- or cool-season turfgrass fairways.
- Soil physical properties and water infiltration rates have high spatial variability within a fairway.
- Preliminary results show slight overestimation of soil moisture from field calibrations of TurfGuard Sensors.
- Correlating soil physical properties to NDVI from drone imagery will be a novel approach to assist with soil moisture variability and may enhance site-specific management designations.
- Comparing high-resolution drone imagery to low-resolution satellite imagery will improve recommendations and usefulness for superintendents adopting technology.

Summary Text:

Rationale

Water conservation strategies continue to be developed and tested throughout the golf industry. Agricultural producers have effectively incorporated remote sensing technology into maximizing yield while reducing inputs or targeting inputs to areas of greatest demand. Utilizing remote sensing data to improve turf management is in its infancy, but there are many current research studies working to demonstrate the benefits of site-specific management practices or pesticide applications. The overall goal of this project is to test various spectral sensors collecting images of golf course fairways to improve recognition of drought stress signatures on cool- and warm-season turf species.

Methodology

UAV Flights and Data Compilation. Weather conditions continued to challenge completion of more frequent flights, but full flight data were obtained from two holes of a warm-season golf course (Rawls Golf Course n = 5) and three holes of a cool-season golf course (Amarillo Country Club n = 3). A complete flight included collecting geo-referenced imagery from four sensors [Red/Green/Blue (RGB); Red Edge (RE); NIR850 nm; and NIR970 nm). All images were compiled and stitched into a single image per golf course fairway flown and analyzed in Blue Marble Global Mapper GIS software. Analysis consisted of NDVI calculations using RE/NIR850/NIR970 sensor with RGB images.

Image 1*. Toro TurfGuard Sensor Installation.*

Ground-truth Data with Flight. A GPS grid-point map was developed in Google Earth Path add-on within Google Earth. Soil samples were obtained at each intersection (texture, bulk density, organic matter, infiltration, water retention and thermal properties) along with measurements of soil compaction (0-5 cm and 5-10 cm), NDVI, and relative volumetric water content (VWC) with TDR at 3 inch (7.6 cm) depth (All instruments from Spectrum Technologies). Instrument data collection was targeted within 1-2 days of flight to overlay or correlate with analyzed drone images to validate stress in fairways.

Field Calibration of Soil Moisture Sensor. Field calibrations of the Toro soil moisture sensors were performed at two distinct depths (5 and 10 cm) at 12 locations at each golf course. Soil sensor locations (sand-based tees and native soil fairways) were chosen to represent the variability of soil physical properties within the study area. Using a standard greens cup cutter, a cylindrical soil plug and column (8 in. depth) was removed (Image 1). A TurfGuard sensor was carefully inserted laterally 2 inches below grade with probes inserted completely into the soil and then backfilled and topped with the remaining soil and soil plug. Continuous VWC measurements with the sensors were then obtained, and gravimetric-based soil water content were determined at different times and under different soil moisture regimes (wet and dry).

Results to Date/Future Expectations

UAV Flights and Data Compilation. All four sensors were used for flights over the same two fairways used in 2018. Additional fairways were not flown in 2019 as originally planned because of time required for ground-based measurements in each fairway. Flight images from both locations have been stitched and analyzed using an NDVI formula. Preliminary results from these flights appear similar to descriptions from 2019, but we will be working to obtain numerical values around ground-based measurement points to assist with correlations to instrument or soil parameters. The comparison to soil parameters will be incredibly beneficial and a novel approach compared to current literature.

Ground-truth Data with Flight. Ground measurements were obtained in close proximity to 1-2 flights at each location again in 2019. Soil sampling and analysis were completed with spatial variability maps developed for bulk density, field capacity, permanent wilting point, and unsaturated water infiltration (Figure 1). Recent work has developed spatial variability maps and described high variability within golf course fairways, but we have not seen any information depicting variability in the soil physical properties and water characteristics measured in this study (Table 1). Each soil sample and instrument measurement was obtained from a specific GPS coordinate, so we are looking to incorporate these ground-based data into correlations with NDVI measured images from sensors flown on the drone. Additionally, we will be obtaining satellite imagery (Sentinal) of these fairways to contrast variability in resolution with imaging techniques (drone flight vs satellite). This information may provide recommendations for golf course superintendents to use free applications to develop variability maps.

Calibration of Soil Moisture Sensor. There were numerous challenges with sensor communication in our laboratory, so field calibration techniques were pursued in 2019. Initial comparisons of the soil moisture sensor versus actual VWC indicated that the sensor tends to overestimate actual water content. Linear calibration equations comparing actual VWC and TurfGuard sensor VWC at two soil depths are being developed for both golf courses (Figure 2). Additionally, a variety of calibration equations widely used for dielectric soil moisture sensors are being analyzed. Any soil texture specific variation will be determined using a least–squares optimization approach. This is critical when comparing measured VWC from different soil textures across a golf course. Understanding this information will assist us in determining if the sensor could effectively help golf course superintendents schedule irrigation more efficiently than previous methods.

Figure 1. Spatial variability maps for soil bulk density, volumetric soil moisture content (field capacity and permanent wilting point), and nonsaturated infiltration from mini-disk infiltrometer in the upper 2 inch soil profile from a single golf course fairway.

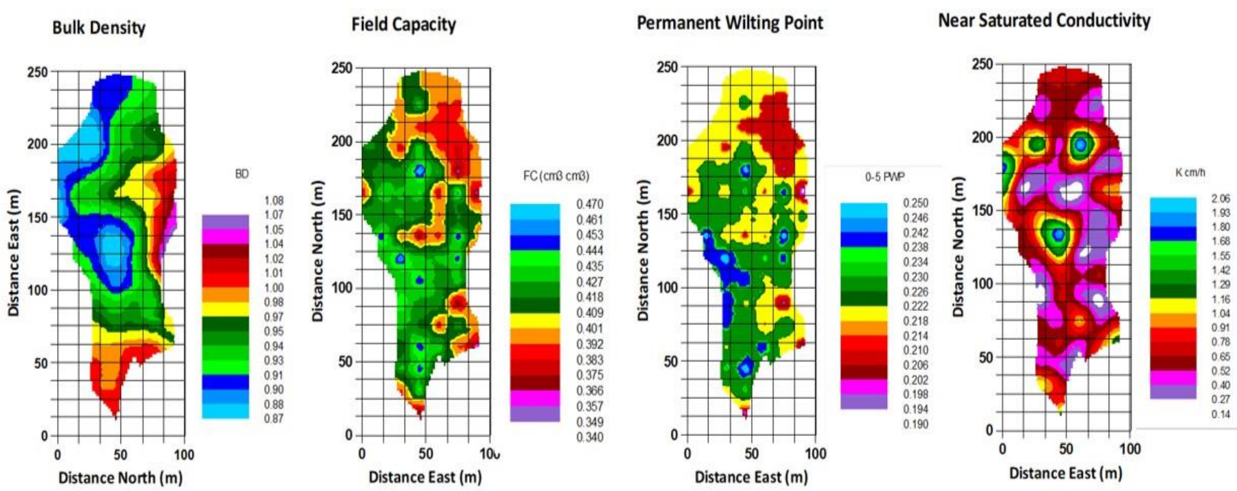

Figure 2. Linear regression equation comparing actual volumetric soil moisture content to TurfGuard sensor soil moisture from sand-based tee boxes and native soil fairways. Measured VWC was determined from soil samples collected in close proximity to sensor (Gravimetric Water Content x bulk density)

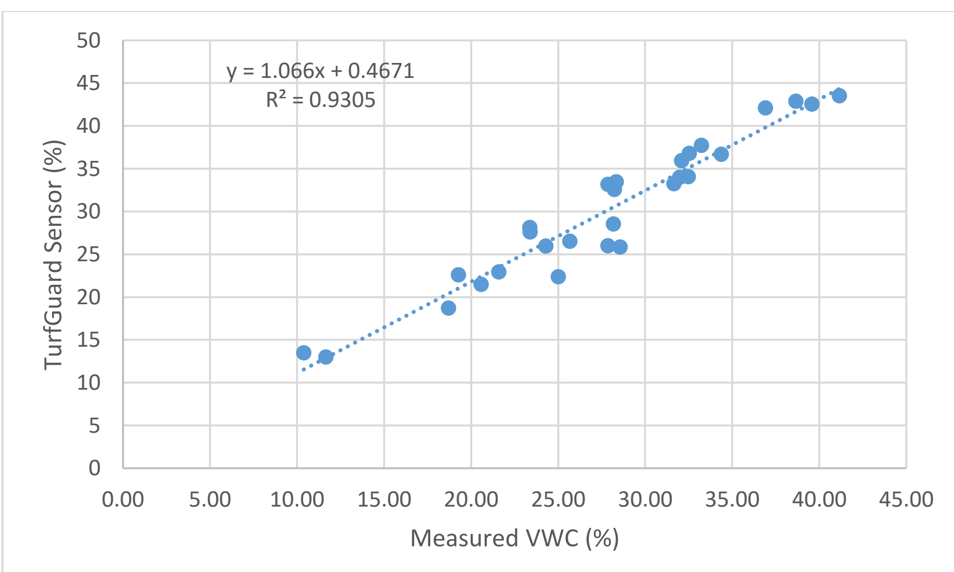

Table 1. Correlation matrix of soil physical parameters and water characteristics from a single fairway evaluated in this research.

	NDVI	**SC 7.5 cm (Kpa)**	**SC 10.0 cm (Kpa)**	**K cm/h (2 cm suction)**	**Bulk Density (0-5cm)**	**FC**	**0-5 pwp**	**Bulk Density (5-10cm)**	**FC**	**5-10 pwp**
NDVI	1									
SC 7.5 cm (Kpa)	-0.41**	1								
SC 10.0 cm (Kpa)	-0.33**	0.88**	1							
K cm/h (2 cm suction)	-0.06	0.28*	0.23*	1						
Bulk Density (0-5cm)	-0.23*	0.13	0.19	-0.34**	1					
FC	0.17	-0.22	-0.24*	-0.36**	-0.23	1				
PWP 0-5cm	0.21	-0.30*	-0.27*	-0.27*	-0.17	0.87**	1			
Bulk Density (5-10cm)	-0.25*	0.09	0.14	-0.21	0.45**	-0.02	0.08	1		
FC	0.10	-0.13	-0.19	0.00	-0.33**	0.41**	0.32**	0.06	1	
PWP 5-10cm	0.25*	-0.24*	-0.30**	-0.25*	-0.44**	0.66**	0.47**	0.01	0.76**	1

USGA ID#: 2018-05-655

Title: Data-Driven Irrigation Scheduling Techniques for Managing Sand-Capped Fairways

Project Leader: Benjamin Wherley, Reagan Hejl, Kevin McInnes, and B. Grubbs

Affiliation: Texas A&M University, College Station, TX

Objective:

Evaluate feasibility and determine best management practices for irrigation of sand-capped fairways when irrigating based on various data-driven scheduling techniques.

Start Date: 2018
Project Duration: 3 years
Total Funding: $97,000

Summary Points:

- Year 1 results indicate National Oceanic and Atmospheric Administration forecasted reference evapotranspiration (NOAA FRET) is a reliable predictor of actual reference evapotranspiration (ET_o).
- There was a trend towards greater water use with SMS and wilt-based irrigation scheduling in comparison to the ET based scheduling approaches, which could be a function of the approach taken to determine irrigation amount.
- Wilt-based soil moisture thresholds appear to be higher during mid-summer and lower during early-summer and fall, suggesting that different thresholds might need to be used at different times of the year when using SMS-based irrigation scheduling.

Summary Text:

Background

With current strains on water resources and with the increasing trend of capping degraded golf fairways with sand, research toward efficient methods for irrigation management on sand-capped soil is needed. Reference ET has proven to be an effective means of predicting irrigation requirements, however, access to locally representative weather station data is often a barrier for implementation. The recent availability of open-access NOAA FRET data provides ET data regardless of proximity to a weather station. This potentially offers superintendents another tool for scheduling irrigation. Unfortunately research is lacking on how accurately FRET values predict actual on-site weather-station based ET_o at a given location. In-ground soil moisture sensors (SMS) are another potential technology for aiding in golf course fairway irrigation scheduling, but these have been underutilized, largely due to soil heterogeneity present in most native soil systems. SMS may offer promise in sand-capped fairways due to the higher level of soil textural and depth uniformity across these systems. The objectives of this study were to evaluate turf performance and overall water use during the growing season using various irrigation scheduling techniques as well as determine whether the critical moisture threshold at which wilt occurs changes by season for application in SMS based systems.

Methodology

The 3-year study was initiated in 2018 with the construction of a 10,000 ft^2 sand-capped facility and establishment of Latitude 36 Bermudagrass (*Cynodon dactylon* L. Pers. x C. *Transvaalensis* Burtt-Davy). The 7" deep sand-cap was constructed from a medium coarse construction sand atop a fine sandy loam subsoil at the site. Various irrigation scheduling treatments were initiated in June 2019 with the following techniques and scheduling approaches:

1) Wireless SMS
   - Irrigation is applied based on 75% allowable depletion. Field capacity and permanent wilting was based on evaluation of wilt in relation to soil moisture in late May. (Fig. 1)

2) On-site Penman-Monteith ET_o
   - Plots are irrigated twice weekly based on the previous 3-day (Monday – Wednesday) or 4-day (Thursday – Sunday) on-site ET_o cumulative values multiplied by the warm-season turfgrass crop coefficient (0.6 x ET_o).
   - Effective rainfall is accounted for in calculating irrigation requirements.

3) NOAA Forecasted ETo
   - Plots are irrigated twice weekly based on split applications of total weekly FRET values multiplied by the warm-season turfgrass crop coefficient (0.6 x ET_o).
   - Effective rainfall is accounted for in calculating irrigation requirements.

4) Visual Wilt-based approach
   - Wilt-based plots are irrigated back to field capacity with 2.3 cm of water once a given plot expresses 50% wilt.

Irrigation treatments are arranged in a randomized complete block design with 4 replicates and individual plot size of 21 ft x 21 ft. Plots are mowed 2-3 times per week at 0.5" height and fertilized with 0.75 lb N/ 1000 ft^2 every 3-4 weeks from May through September. Wetting agent (Aquatrols Revolution) is applied at the label rate every month during the growing season. Turf quality of plots is evaluated weekly using a 1-9 scale, with minimum quality = 5. Bi-weekly digital image analysis is performed using Turf Analyzer software (Green Research Services, LLC, Fayetteville, AR) (Karcher et al., 2017). Water usage is determined by utilizing a water meter installed at the valve of each plot. Toro Turf Guard ® Wireless SMS are placed in each plot to monitor volumetric water content (VWC). Each sensor has 2 sets of probes that monitor VWC (%) at the upper portion of the sand-cap (3") as well as the upper portion of the underlying subsoil (8"). In Visual Wilt-based plots, an additional sensor is positioned at a deeper depth for gaining greater spatial resolution, monitoring volumetric water content at the 6" sand-cap depth as well as deeper within the subsoil (11"). Data are subjected to ANOVA using the GLM procedure of SPSS (IBM, Inc.). Where appropriate, mean comparisons have been performed using Tukey's HSD ($P \leq 0.05$).

Results to Date

In the 2019 season, all irrigation scheduling techniques were successful in maintaining acceptable turf quality with no significant differences detected between scheduling techniques (Figure 2). There was an observed 32% higher water use with the SMS-based scheduling strategy in comparison to onsite ET_o (Figure 3), however, this could be due to the approach taken to calculate irrigation return. The critical moisture threshold for wilt (VWC at which wilt was observed) changed on a monthly basis during the 2019 season (based on measurements taken in the upper portion (3" depth) of the sand-cap (Figure 4). The critical moisture threshold was lowest in early and late season months (~2%) and peaked in July (6.5%). However, this change in critical threshold for wilt was not observed for sensor readings obtained within the lower portions of the sand-cap or subsoil. Over the final year of the project, we intend to examine root development and distribution within the sand-cap and subsoil in relation to each of the irrigation scheduling methods.

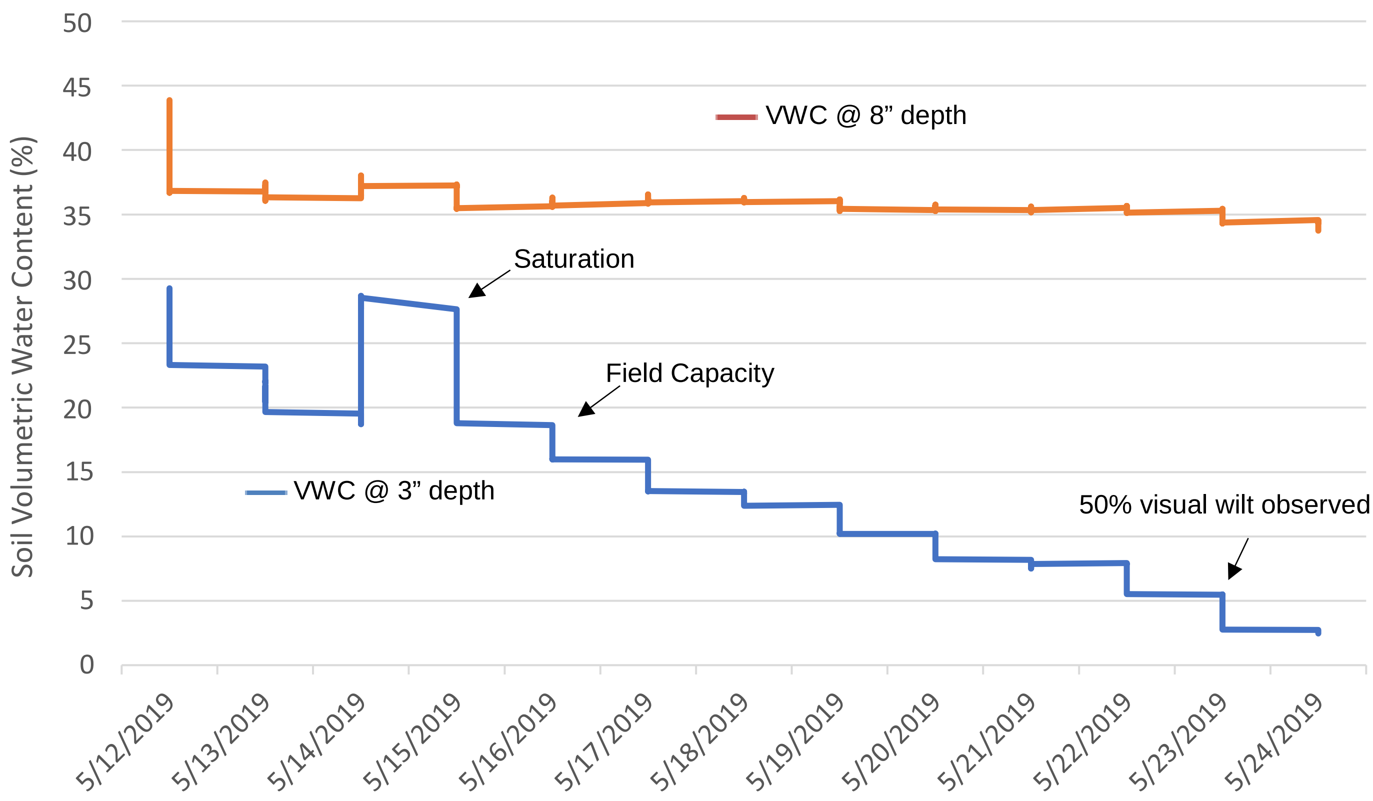

Figure 1. Dry-down soil moisture profile at 3 and 8 inch sensor depths for the wireless soil moisture sensor-based plots used for determining field capacity and wilt endpoints. Data were collected during late May 2019.

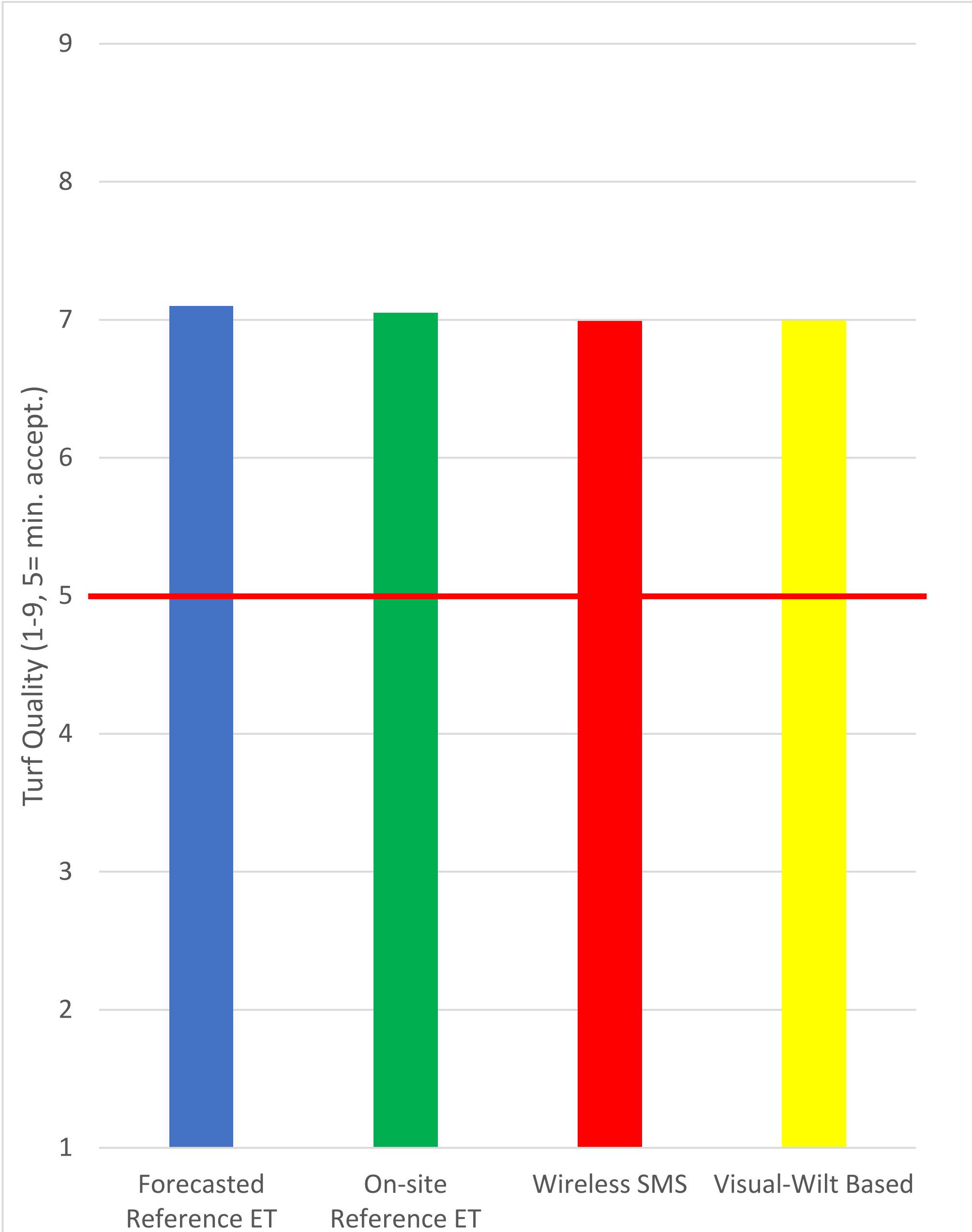

Figure 2. 2019 Visual turf quality as affected by irrigation scheduling treatment. Data were pooled across all rating dates. Horizontal red line indicates minimally acceptable turf quality.

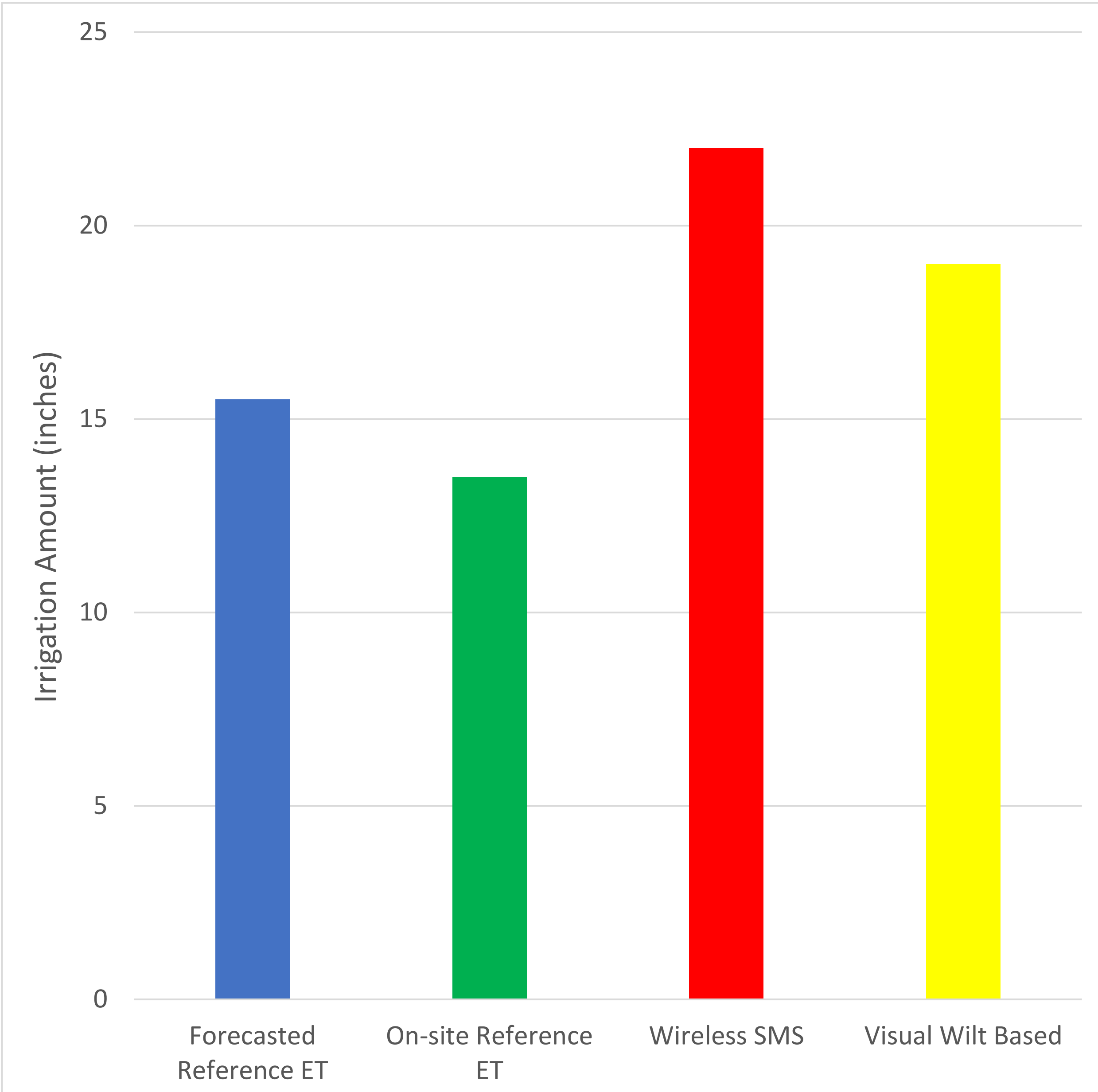

Figure 3. 2019 seasonal water use for each irrigation scheduling treatment. Data were derived from difference between water meter readings at trial initiation and end of season.

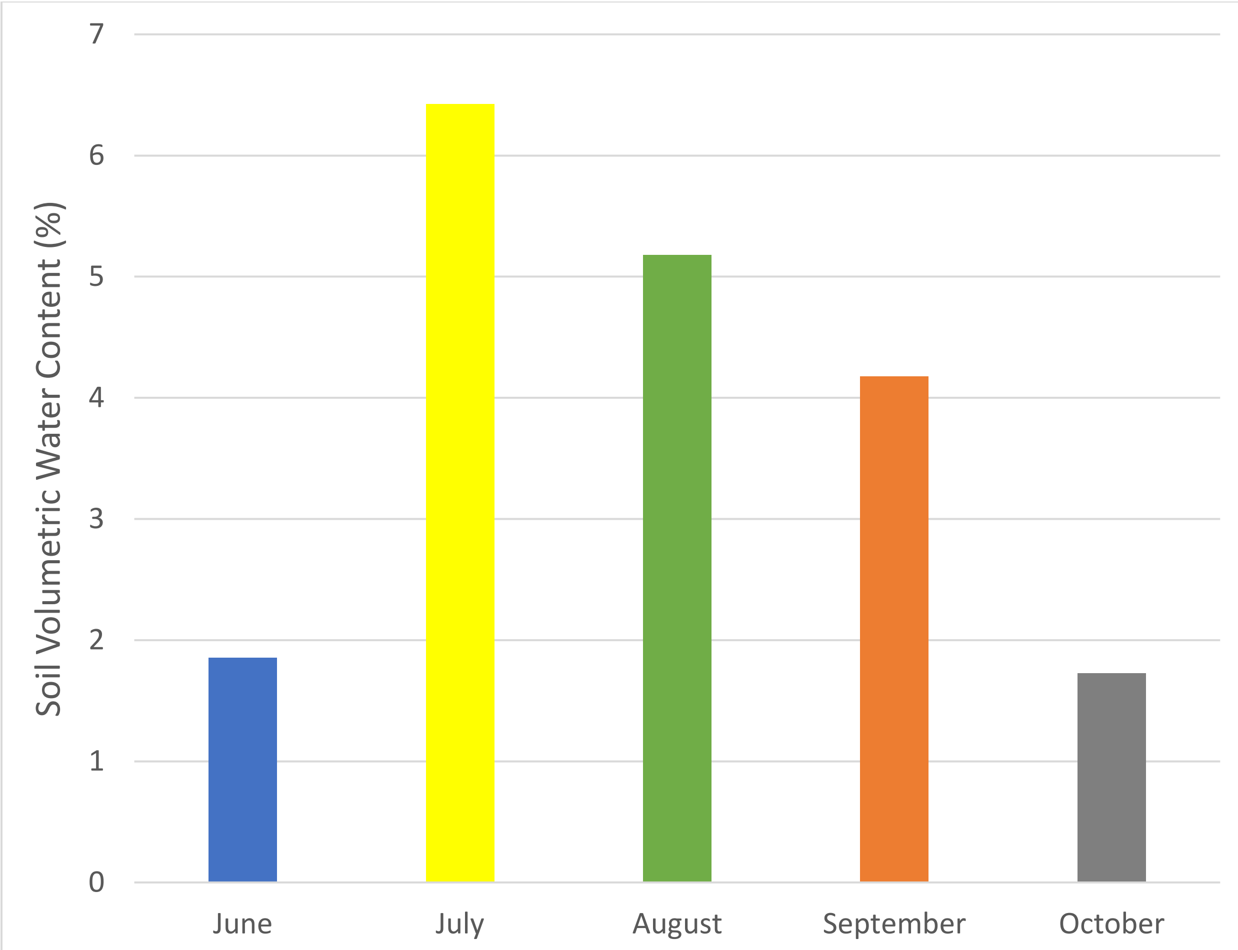

Figure 4. Year 1 soil volumetric water content thresholds (3” depth) at which wilt occurred during each month of the 2019 growing season. Measurements were made using the Toro Turf Guard® wireless soil moisture sensor.

Figure 5. Image of the sand-capping irrigation facility at Texas A&M University, College Station, TX. The facility contains 16 independently irrigated zones and is composed of Latitude 36 bermudagrass atop a 7” sand-cap.

USGA ID#: 2018-06-656

Title: Enhancing Site-Specific Turf Irrigation Management and Developing Turf Deficit Irrigation Strategies Using Soil Moisture Sensors, Smart ET-Based Irrigation Controllers, and Remote Sensing

Project Leader: Dr. Amir Haghverdi
Affiliation: Department of Environmental Sciences, University of California Riverside

Objective:

The overarching goal of this project is to develop recommendations for water conservation and turf irrigation best management practices in inland Southern California.

Start Date: 2018
Project Duration: 2 years
Total Funding: $49,491

Summary Points:

- An irrigation research trial was conducted in 2018 and 2019 at UCR Agricultural Experiment Station in inland Southern California to study the response of tall fescue (*Festuca arundinacea*), and bermudagrass (*Cynodon dactylon*) turfgrass species to multiple irrigation treatments.
- The irrigation treatments were autonomously regulated by Weathermatic smart irrigation controller, which showed a promising agreement with ET data collected from a nearby CIMIS data.
- The catch can test estimation of the precipitation rate was substantially lower than the precipitation rate measured with a precise flow meter. This finding suggests that in irrigation research projects the precipitation rate should not be scheduled based on a catch can test.
- Our results (three years; 2017-2019) indicate an irrigation application of approximately 110% ET_{ref} and 80% ET_{ref} for tall fescue and bermudagrass species as a minimum to maintain an accepted turf quality over summer months in inland Southern California. Assuming the irrigation efficiency of 80% for the sprinkler system, the estimated minimum plant factor for the tall fescue and bermudagrass species, therefore, are 0.88 and 0.64 for the study area.

Summary Text:

1. Introduction:

Implementation of smart irrigation technologies offers to improve water use efficiency by maintaining the soil water status at the active root zone within a predefined desired range. Advancements in smart irrigation technologies have led to affordable smart irrigation controllers. Much of the scientific research in recent years on the application of landscape irrigation technologies, including the use of smart controllers, has been done in humid regions (mainly in Florida and North Carolina) wherein the main focus has been to avoid over-irrigation when rainfall is abundant. Currently, information is lacking about the application of smart irrigation technologies leading to turf water conservation and deficit irrigation strategies in arid regions such as inland Southern California.

2. Material and Methods:

2.1. Deficit Irrigation Trial

A total of 72 irrigation research plots were established at the University of California, Riverside Agricultural Experiment Station in Riverside, California. The plots were planted to sod tall fescue (*Festuca arundinacea*), and bermudagrass (*Cynodon dactylon*) in 2017 and for several months afterward were under full irrigation for root development and grass establishment. We followed standard cultural practices to maintain the plots (i.e., control weeds, pests, fertilizers, mowing) throughout the experiment. For each species, 36 plots were organized in a factorial complete randomized block design to impose 12 irrigation treatments replicated 3 times. The 12 irrigation treatments consisted of 6 irrigation levels ranging from full to multiple deficit irrigation scenarios and 2 irrigation frequencies. The irrigation treatments for both species are listed in Table 1.

Each plot was irrigated by 4 quarter-circle (pop-up heads) sprinklers, all four controlled by a common solenoid valve for independent control of each plot. A Weathermatic Smartline SL 4800 smart irrigation controller was installed and used to autonomously impose all irrigation treatments. The smart controller was connected to an SLW5 wireless Weathermatic weather sensor and an SLFSI-T20 Weathermatic flow sensor to continuously monitor weather conditions and irrigation applications to the plots. In January 2019, we replaced the Weathermatic flow sensor with a Badger Meter Recordall Turbo 2” flowmeter to more accurately monitor the water applied to each zone.

The uniformity and application rate of the system was determined by a catch can test following the standard protocol (ANSI/ASABE S626). Plots were irrigated between midnight and early morning to minimize evaporative losses and wind drift. In addition, total daily irrigation run time was divided into several irrigation applications to avoid runoff. We used California DWR’s procedure (equation 1) outlined in the model water efficient landscape ordinance to calculate irrigation water requirement.

$$IWR = \frac{PF \times ET_{ref}}{IE} \quad (1)$$

where *IWR* is the irrigation water requirement, *PF* is plant factor, ET_{ref} is reference evapotranspiration and *IE* is the irrigation efficiency. We programmed irrigation treatments in the smart controller at the beginning of the experiment. The controller calculated the ET_{ref} using temperature data collected by its weather sensor.

The performance and reliability of the ET-based smart irrigation controller for efficient autonomous turf irrigation management were evaluated against independent reliable daily ET_{ref} data collected from a nearby CIMIS (California Irrigation Management Information System) weather station.

2.2. Root Zone Soil Water Dynamics

A total of 24 plots (2 blocks of 12 plots each) were instrumented with soil moisture sensors in two configurations: (a) 24 soil moisture sensors: 12 Acclima TDT sensors (Acclima Inc., Meridian, ID) and 12 Decagon MPS6 water potential sensors (Decagon Inc., Pullman, WA) installed side-by-side, 4 inches deep, to determine the dynamic of soil water potential and soil water content under bermudagrass irrigation treatments. (b) 48 Watermark soil potential sensors (Irrometer Co., Riverside, CA) were installed in each plot at 4 depths from 5-30 inches to understand the tall fescue's root water uptake patterns across the treatments. Data loggers will store sensor data every 30 minutes over the course of this experiment.

2.3. Gound-based and UAV-based Remote Sensing

The Trimble GreenSeeker handheld NDVI sensor and MicaSense RedEdge multispectral drone-mounted camera were used to collect remote sensing data. The handheld data were collected for a total of 18 times in 2018 (May 1, 2018 – September 24, 2018) and 16 times in 2019 (June 1, 2019 – October 19, 2019). To eliminate the plot edge effect and avoid interference between adjacent plots, adequate borders (2-3 feet) were considered and measurements were taken at the center of each plot. The drone data were collected 3 times in 2018 and 4 times in 2019. The data were analyzed to determine the response of turfgrass to the deficit irrigation treatments and potential drought injury.

3. Results & Discussion:

3.1. Deficit Irrigation Trial

Figures 1 and 2 summarize the results of the study (i.e., NDVI values) in 2018 and 2019 for the bermudagrass plots. Figures 3 and 4 depict the same results for the tall fescue plots. Figure 5 depicts the cumulative irrigation applications by the Weathermatic smart controller for both species and the 12 irrigation treatments (i.e., 6 irrigation levels and 2 watering days) in the years 2018 and 2019 against the optimum irrigation application based on CIMIS station ET_{ref} data. Our findings show a good agreement between the irrigation treatments autonomously regulated by Weathermatic smart irrigation controller and ET data collected from the nearby CIMIS data.

Figure 6 summarizes the response of both species to a wide range of irrigation scenarios in 2017, 2018 and 2019. Turfgrass images are also included for two NDVI levels for each species. The results indicate an irrigation application of approximately 110% ET_{ref} and 80% ET_{ref} for tall fescue and bermudagrass species as a minimum to maintain an accepted turf quality over summer months in inland Southern California. Assuming the irrigation efficiency of 80% for the sprinkler system, the estimated minimum plant factor for the tall fescue and bermudagrass species, therefore, are 0.88 and 0.64 for the study area. Our results indicate no water-saving potential due to restricting the watering days to only a few days a week. On-demand irrigation using the smart controller (i.e., allowing the controller to irrigate the plots every day if needed) often yielded a higher turf quality and lower drought injury in particular for the tall fescue plots.

The 2018 catch can test estimation of the precipitation rate was substantially lower than the precipitation rate measured in 2019 with a precise flow meter. This finding suggests that the precipitation rates calculated using a catch can test may not be reliable to schedule irrigation and therefore should not be used in irrigation research projects.

3.2. Root Zone Soil Water Dynamics

Figures 7-10 show the daily average soil moisture data collected in the years 2018 and 2019 across the treatments. For the tall fescue plots, the soil moisture levels of the top two sensors (8 and 13 inches deep) show a constant decrease in both years for low and medium irrigation treatments. For treatments receiving higher irrigation levels, this reduction was less significant with some fluctuations indicating the wetting and drying cycles. The bottom two sensors (17 and 25 inches deep) show no sign of water uptake for higher irrigation levels but showed a gradual decline in soil moisture for high deficit irrigation treatments. This result suggests that the tall fescue plots under more severe deficit irrigation treatments may have developed deeper roots to uptake water from deeper layers, but this did not prevent drought injury due to these treatments. The in-situ soil water retention collected from bermudagrass plots show a wide range of variation depending on the irrigation treatment. Considering 80% as the minimum water application to maintain the quality of bermudagrass, it seems the soil moisture in the top layer can go as low as roughly 20 % with soil tension of 100 kPa (pF = 3).

3.3. Drone and Ground-based Remote Sensing:

We explored the utility of drone-based multispectral imagery to study the response of the two turfgrass species to a wide range of irrigation treatments. The results were promising. There is a good agreement between the NDVI data collected using the handheld sensor and drone-based NDVI values (Figures 1-4).

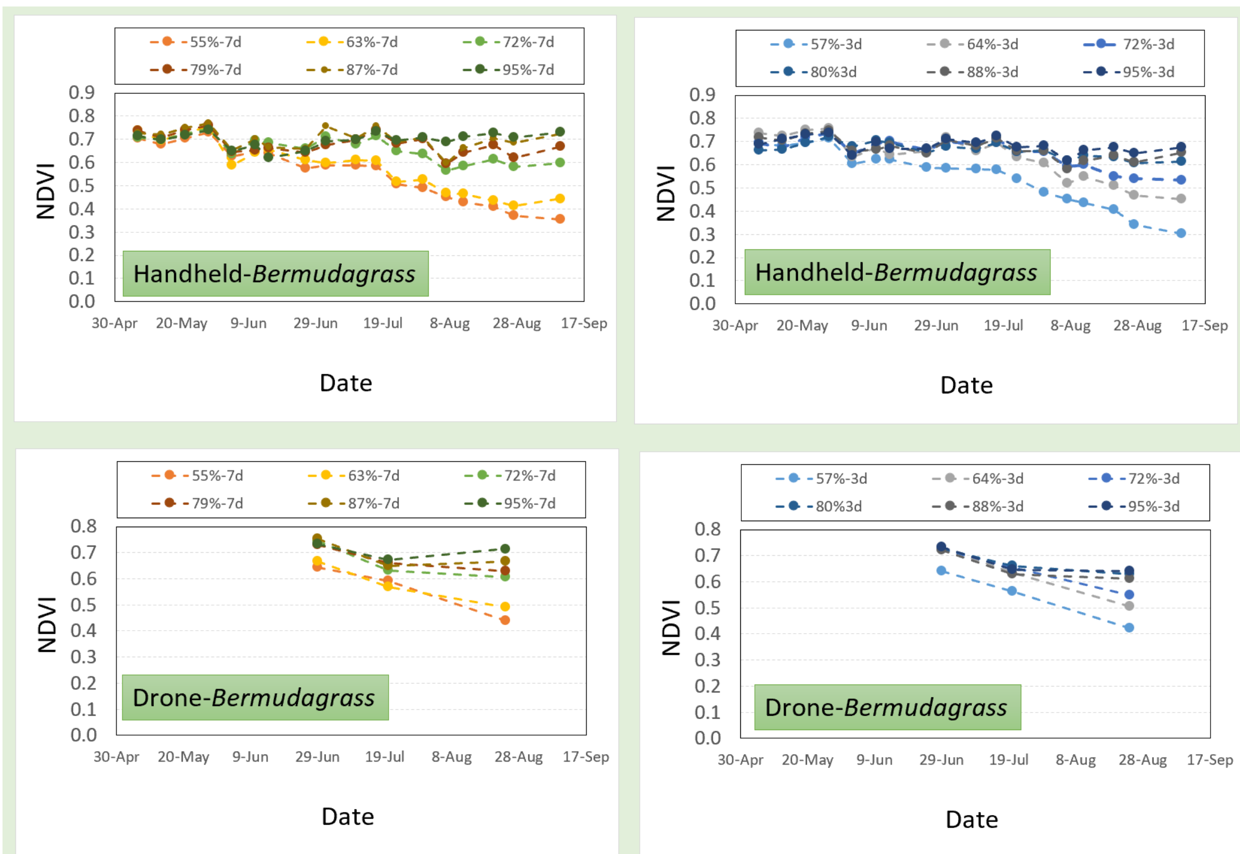

Figure 1. The response of bermudagrass turfgrass species to 6 irrigation levels and 2 irrigation frequencies (watering days) in 2018. The NDVI data were collected using Trimble GreenSeeker sensor (handheld) and Micasesnse Rededge Multispectral camera (drone-mounted).

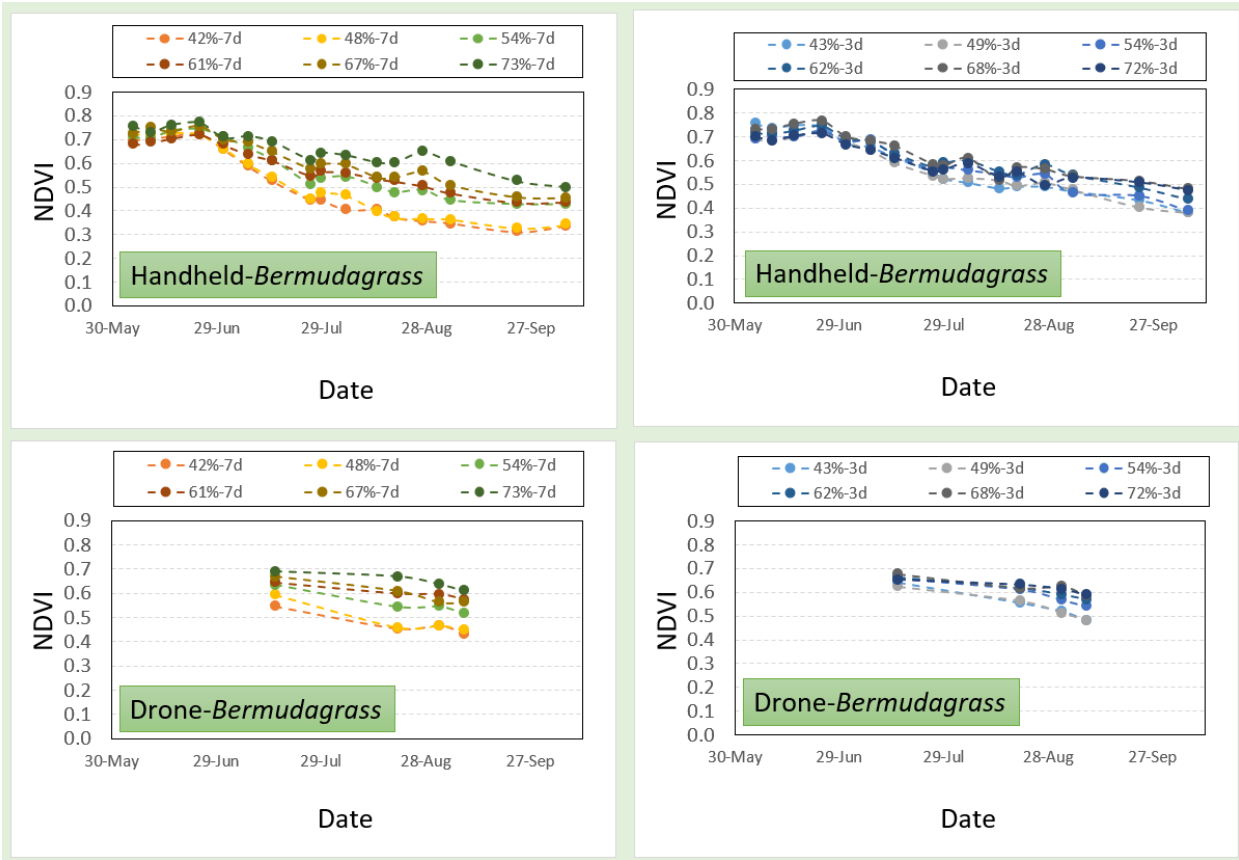

Figure 2. The response of bermudagrass turfgrass species to 6 irrigation levels and 2 irrigation frequencies (watering days) in 2019. The NDVI data were collected using Trimble GreenSeeker sensor (handheld) and Micasesnse Rededge Multispectral camera (drone-mounted).

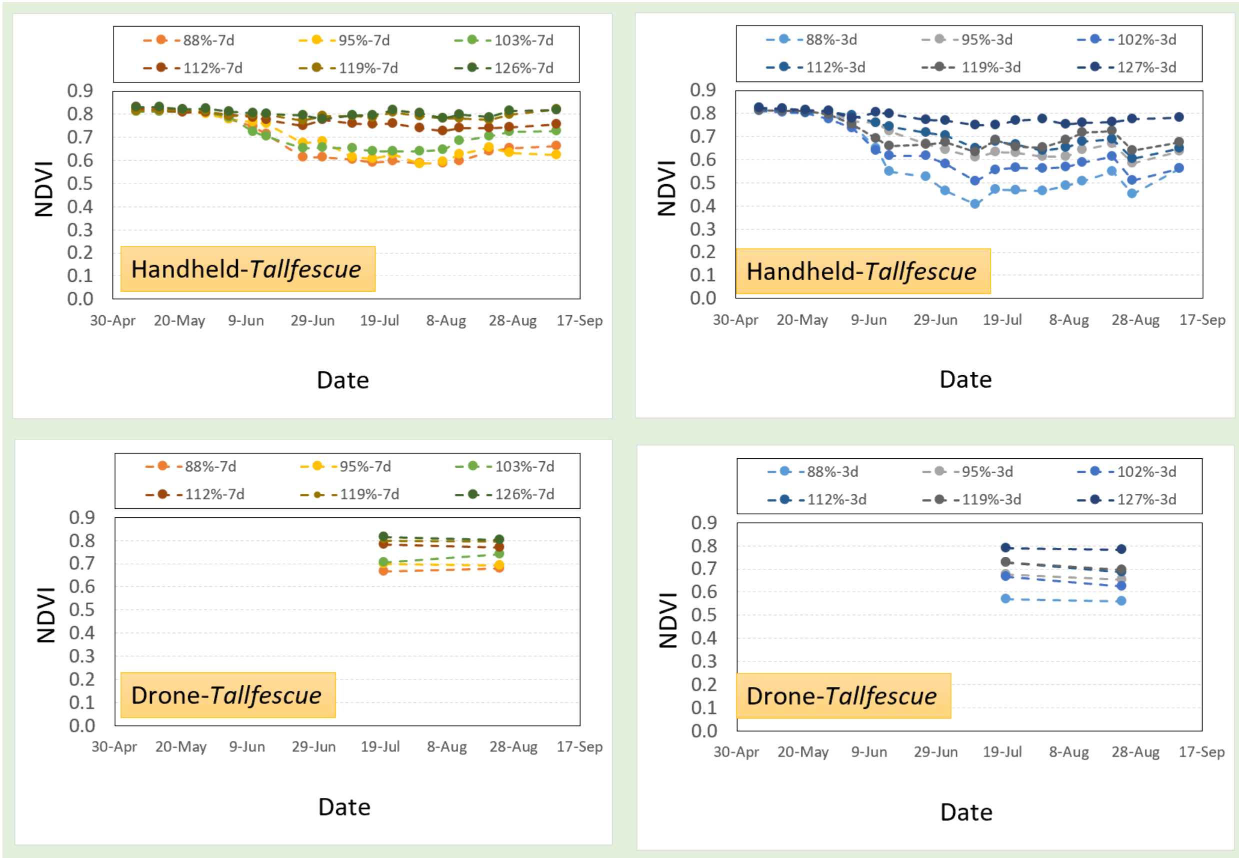

Figure 3. The response of tall fescue turfgrass species to 6 irrigation levels and 2 irrigation frequencies (watering days) in 2018. The NDVI data were collected using Trimble GreenSeeker sensor (handheld) and Micasesnse Rededge Multispectral camera (drone-mounted).

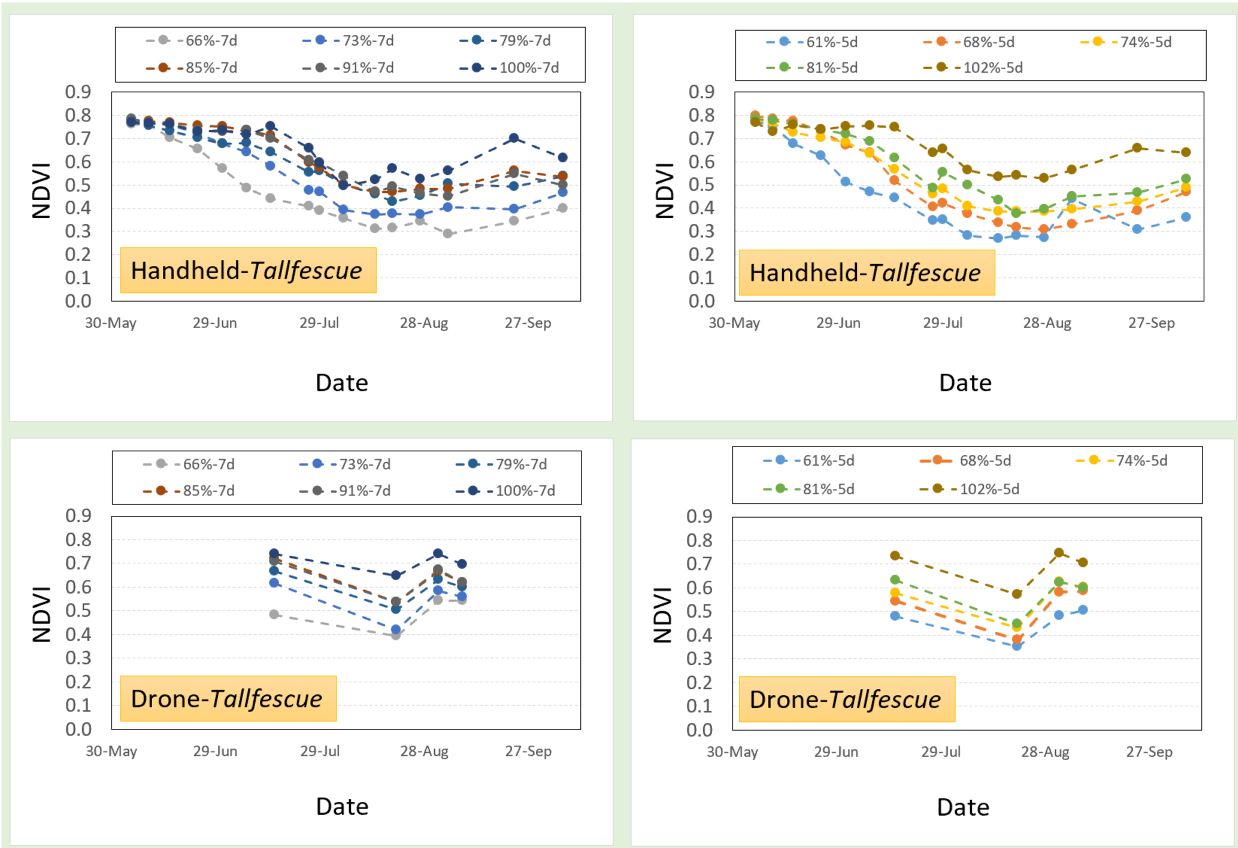

Figure 4. The response of tall fescue turfgrass species to 6 irrigation levels and 2 irrigation frequencies (watering days) in 2019. The NDVI data were collected using Trimble GreenSeeker sensor (handheld) and Micasesnse Rededge Multispectral camera (drone-mounted).

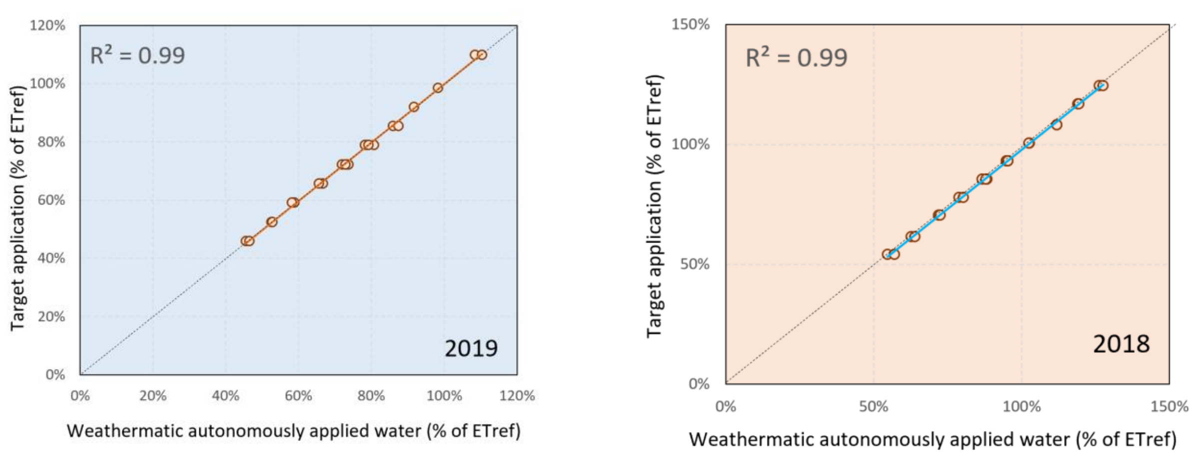

Figure 5. Performance of Weathermatic smart irrigation controller for autonomous full/deficit irrigation scheduling of tall fescue and bermudagrass turfgrass species.

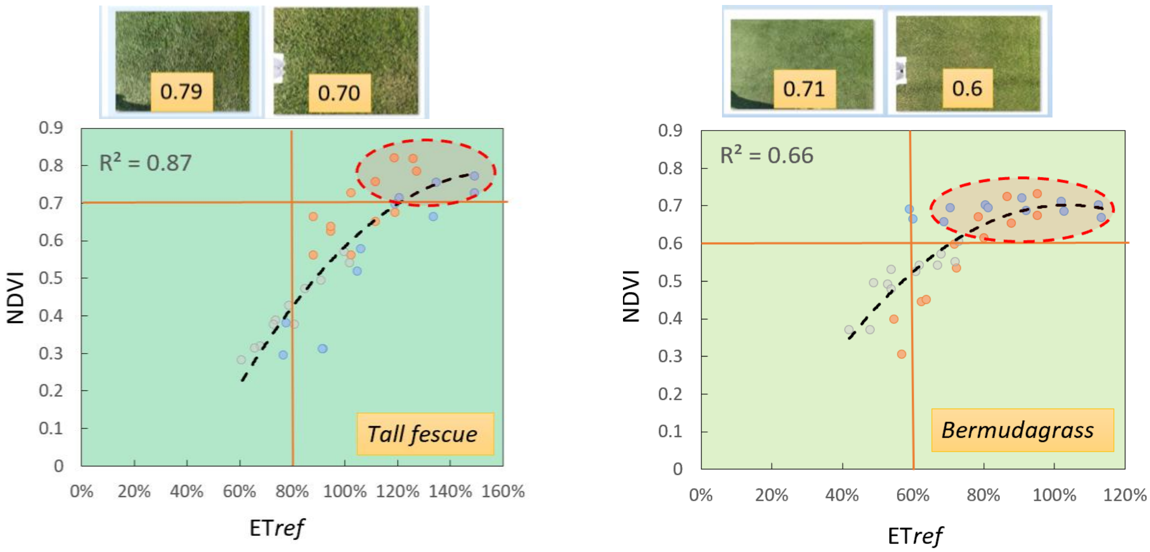

Figure 6. Top: Examples of turfgrass images and their respective NDVI values. **Bottom**: The response (NDVI values) of tall fescue and bermudagrass turfgrass species to 6 irrigation levels and 2 irrigation frequencies (watering days) in the years of 2017 (60 days since the beginning of the experiment), 2018 (132 days since the beginning of the experiment), and 2019 (79 days since the beginning of the experiment).

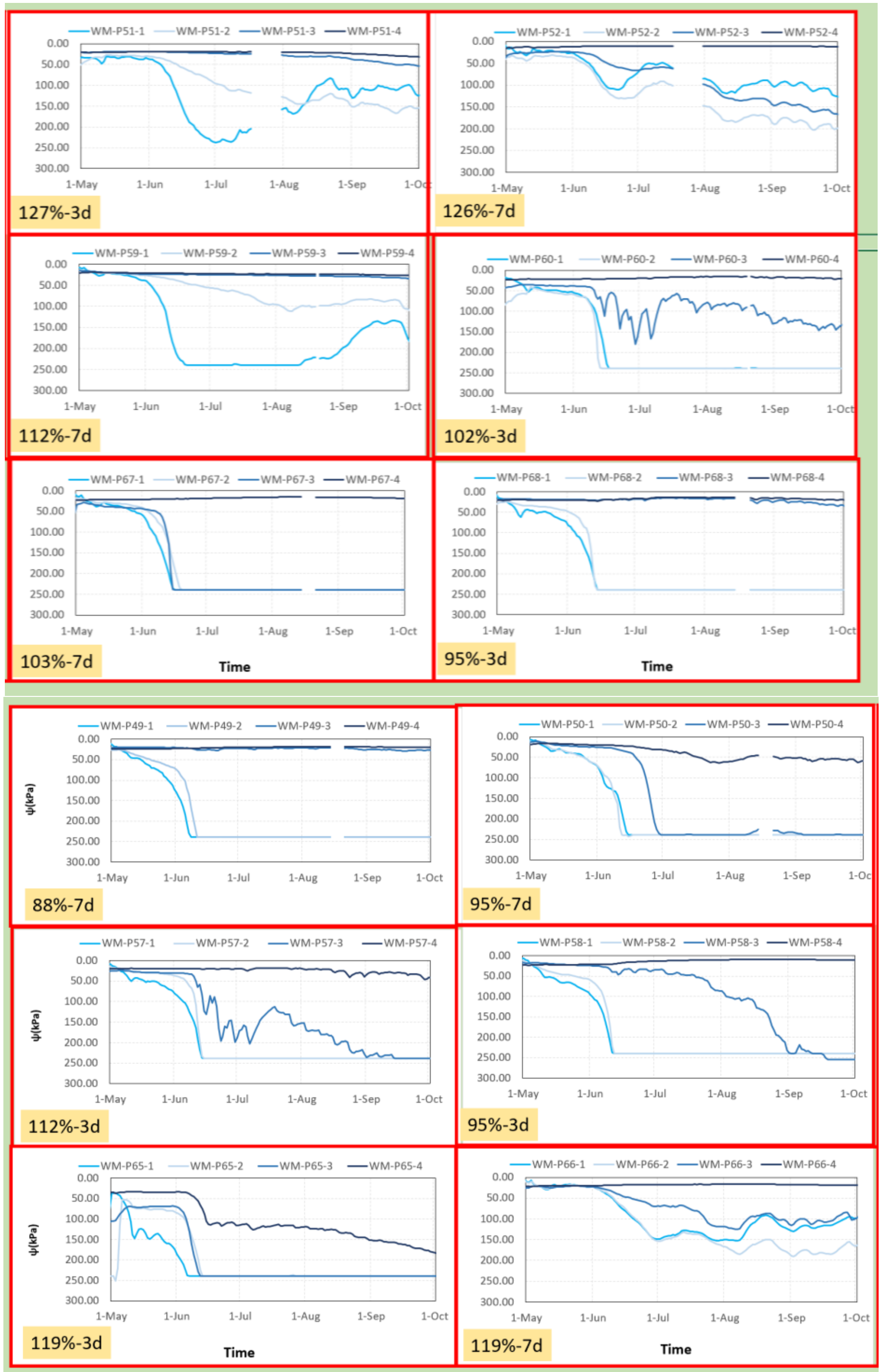

Figure 7. The dynamics of soil tension (kPa) tall fescue turfgrass plots under 6 irrigation levels and 2 irrigation frequencies (watering days) in 2018. Sensors were installed at depth approximately 8, 13, 17, and 25 inches.

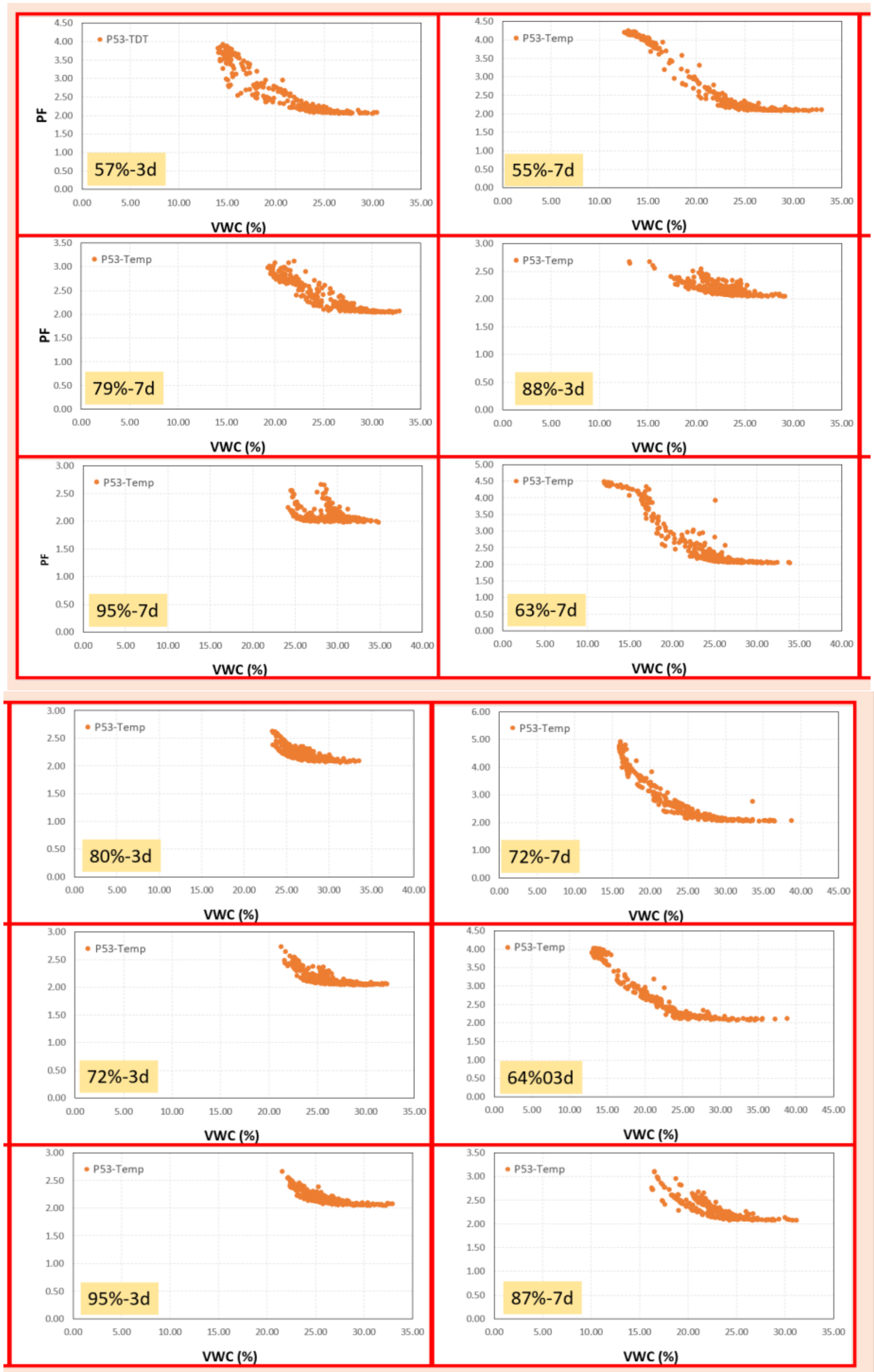

Figure 8. The in-situ water retention curves of bermudagrass turfgrass plots under 6 irrigation levels and 2 irrigation frequencies (watering days) in 2018. The x-axis is the volumetric water content and y-axis is the pF value (logarithmic transformation of soil suction in cm of water).

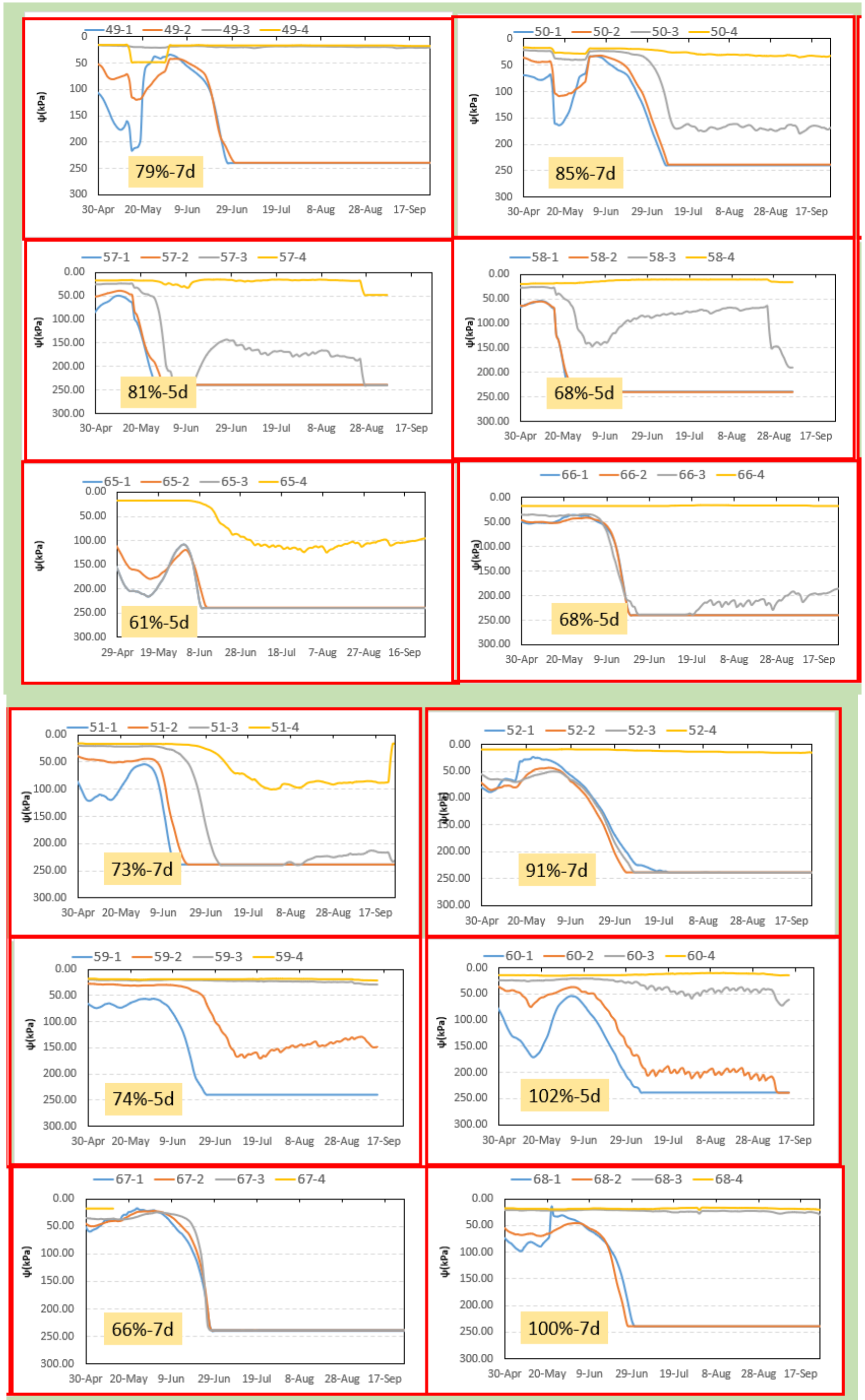

Figure 9. The dynamics of soil tension (kPa) tall fescue turfgrass plots under 6 irrigation levels and 2 irrigation frequencies (watering days) in 2019. Sensors were installed at depth approximately 8, 13, 17, and 25 inches.

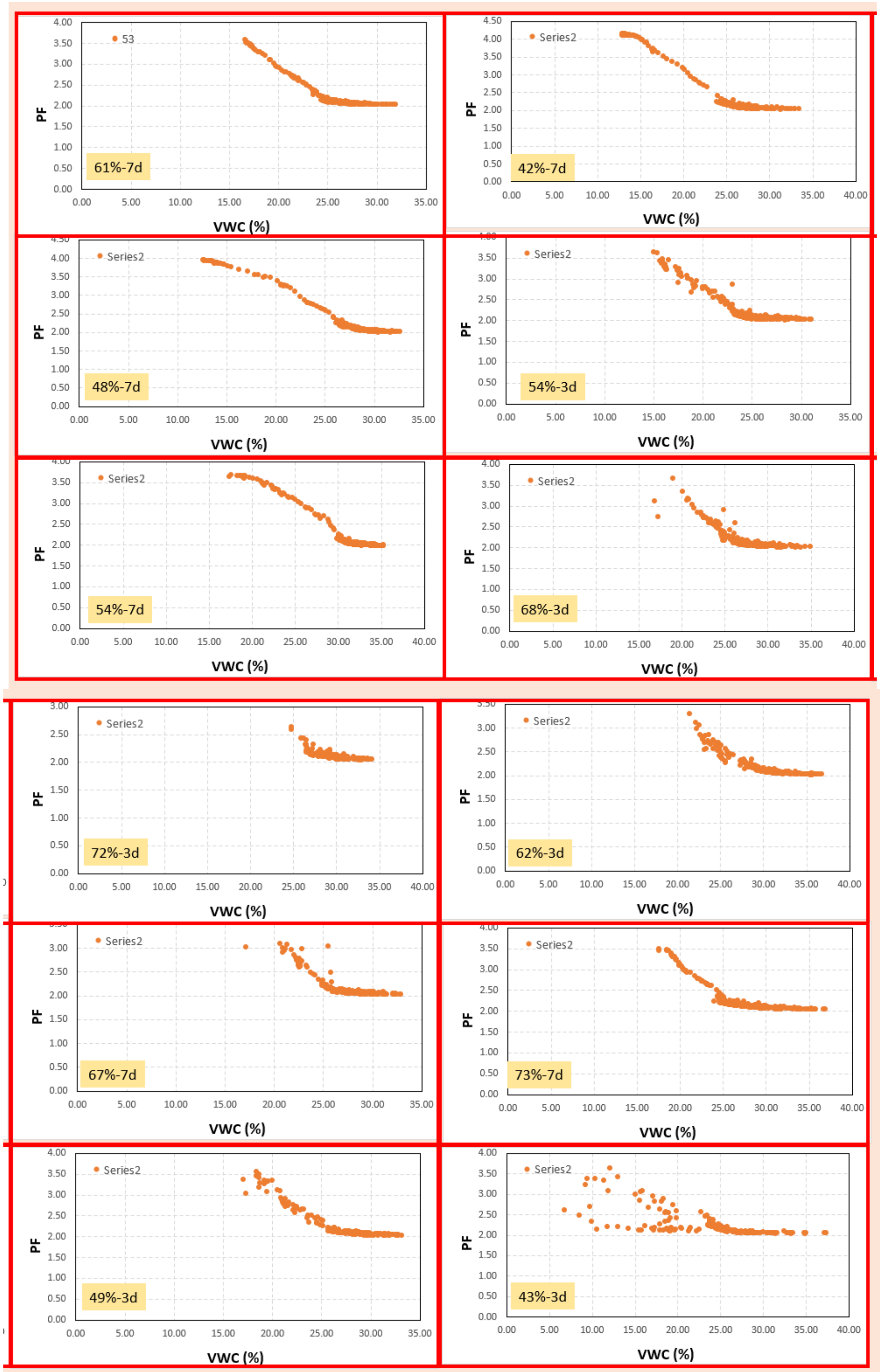

Figure 10. The in-situ water retention curves of bermudagrass turfgrass plots under 6 irrigation levels and 2 irrigation frequencies (watering days) in 2019. The x-axis is the volumetric water content and y-axis is the pF value (logarithmic transformation of soil suction in cm of water).

Figure 11. PhD student is flying a drone to collect multispectral data (left) and undergraduate students are visiting the research plots (right).

Table 1. Irrigation treatments imposed in years 2017, 2018 and 2019.

2017 (July 9, 2017 – October 5, 2017)

Species: *Tall fescue*
78%, 92%, 106%, 121%, 135%, 149% (5 days per week)
77%, 92%, 105%, 119%, 134%, 150% (3 days per week)
Species: *Bermudagrass*
59%, 69%, 81%, 91%, 102%, 113% (5 days per week)
60%, 71%, 81%, 92%, 103%, 113% (3 days per week)

2018 (May 1, 2018 – September 22, 2018)

Species: *Tall fescue*
88%, 95%, 103%, 112%, 119%, 126% (7 days per week)
88%, 95%, 102%, 112%, 119%, 127% (3 days per week)
Species: *Bermudagrass*
55%, 63%, 72%, 79%, 87%, 95% (7 days per week)
57%, 64%, 72%, 80%, 88%, 95% (3 days per week)

2019 (June 1, 2019 – October 19, 2019)

Species: *Tall fescue*
61 %, 68%, 74%, 81%, 102% (5 day per week)
66 %, 73%, 79%, 85%, 0.91%, 100% (7 day per week)
Species: *Bermudagrass*
43%, 49%, 54%, 62%, 68%, 72% (3 day per week)
42%, 48%, 54%, 61%, 67%, 73% (7 day per week)

USGA ID#: 2019-12-682

Title: Surfactants for water conservation and their impact on soil health

Project Leaders: Matteo Serena, Omololu John Idowu, Will Bosland, Bernd Leinauer
Affiliation: Extension Plant Sciences Department, New Mexico State University

Objectives:

i. To evaluate the effects of natural and market available chemical surfactants on the physical, chemical and biological soil health indicators in turfgrass under both sufficient and deficit irrigation in an arid environment
ii. To identify suitable minimum data set (a suite of soil measurements) that can be used for turfgrass soil health assessment and to develop soil health indexes from these measurements that will be related to turfgrass performance for bermudagrass and Kentucky bluegrass
iii. To assess the effects of natural and market available chemical surfactants on turfgrass quality under deficit irrigation
iv. Incorporate our findings into best turfgrass management practices

Start Date: 2019
Project Duration: 2 years
Total Funding: $29,290.00

Summary Points:

1) Soil surfactants did not affect soil biological parameters Total Biomass, Arbuscular Mycorrhizal Biomass, Total Bacteria Biomass, Total Fungi Biomass, Fungi Bacteria Ratio, and Diversity Index for both Kentucky bluegrass and bermudagrass.
2) Soil biological parameters did not correlate with turfgrass performance parameters such as visual quality, percent coverage, or DGCI and NDVI.
3) When data were averaged over all sampling dates and ET replacement levels, Kentucky Bluegrass plots treated with Revolution had highest percent green coverage. Bermudagrass plots treated with Revolution had highest quality, cover, and NDVI.
4) Bermudagrass plots that received Revolution exhibited the most uniform soil moisture distribution.

Summary Text:

Soil surfactants have been used in regular turfgrass maintenance programs to increase irrigation efficiency, because their use has been shown to increase uniformity and improve the moisture retention in the root zones (Alvarez et al., 2016; Kostka and Bially, 2005; Leinauer et al., 2001; Leinauer et al., 2010; Leinauer and Devitt, 2013; Mitra et al., 2005). Due to their ability to weaken the surface tension, wetting agents permit the penetration of water not only into repellent rootzone areas but also into the meso and micropores of soils. Thus, in addition to offering remediation of LDS and hydrophobic soil conditions, soil surfactants may also help to reduce irrigation requirements by improving water use efficiency. Several studies have documented improved turfgrass performance under drought or decreased irrigation when soil surfactants were applied (Alvarez et al., 2017; Cisar et al., 2000; Kostka, 2005; Kostka et al., 2007). Currently, 94% of golf courses in the United States have incorporated soil surfactants into their regular maintenance protocols (Gelernter et al., 2015).

While the benefits of surfactants in combating LDS and soil hydrophobicity have been well documented, the effects of long-term use of surfactants on overall soil health have not been studied. Golf course superintendents have started to report changes in the soil physical properties after long-term application of surfactants, such as decreased drainage, increasing anaerobic soil conditions, and lower turf quality.

Soil health or soil quality is “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (Doran & Parkin, 1996). To fulfill these requirements, healthy soils usually integrate physical, chemical, and biological attributes of the soil (Idowu et al., 2008). The interactions of soil chemical, physical, and biological properties often determine how effectively the soil functions in areas of nutrient retention and release, partitioning of rainfall into runoff and infiltration, moisture retention and release, resistance to environmental degradation, and buffering environmental pollutants (Karlen et al. 1997). The assessment of soil health is not based on the magnitude of any single soil parameter, but rather relies on selected soil measurements (soil health indicators) to quantify management-induced changes (Arshad & Coen, 1992; Doran & Parkin, 1994). A suite of soil measurements that best describe changes in response to management practices constitutes the minimum data set (MDS), and the MDS vary with soil health management goals (Andrews et al., 2002). Currently, there is no documentation on the MDS that can be used to holistically assess soil health of turfgrass in arid environments. Most of the previous studies have only focused on specific soil aspects, without any integration of the physical, chemical and biological attributes of the soil. Due to environmental concerns, chemicals applied to turfgrass systems are receiving increased scrutiny, and the impacts of these chemical need to be related to soil health.

Study

A study has been conducted at New Mexico State University in Las Cruces, New Mexico in 2019 to investigate the effects of repeated applications of commonly available chemical and natural soil surfactants on soil health, irrigation water requirement, and turfgrass quality (Figure 1). The study was initiated in 2018 and included four non-ionic wetting agents which were compared against an untreated control on ‘Princess 77’ bermudagrass (*Cynodon dactylon* L.)and ‘SR 2100’ Kentucky bluegrass (*Poa pratensis* L.).

Figure 1. Soil health study sites at New Mexico State University. Bermudagrass site shown at the left and Kentucky bluegrass at the right.

The study included the following surfactants:
1) a modified methyl capped block co-polymer (trade name Revolution)
2) an alkyl polyglycoside (trade name Dispatch)

3) a natural wetting agent derived from *Yucca schidigera* (trade name Therm X-70) and 4) a rhamnolipid biosurfactant (trade name ZONIX). Rhamnolipids are glycolipids (two rhamnoses conjugated to fatty acid chains) produced by *Pseudomonas aeruginosa*. Their high surface activity has been reported not only for emulsifiers and detergents but also when applied to agricultural and horticultural soils (Ali et al., 2017; Renfro, 2013; Yang, 2008).

Turfgrass performance was evaluated monthly by means of visual quality ratings, Digital Image Analysis (Coverage, Hue, Saturation, Brightness, and Dark Green Color Index), and Normalized Difference Vegetation Indices (NDVI) by means of a Greenseeker. Soil biological indicators included the permanganate oxidizable carbon (Weil et al., 2003), soil organic matter using the Walkley-Black method (Nelson and Sommers, 1982) and the soil microbial community using the phospholipid fatty acid (PLFA) analysis (Buyer and Sasser, 2012). Phospholipid fatty acid analysis provides information on the amount of gram positive and gram negative bacteria; the amount of arbuscular mycorrhiza fungi and the total fungi; and the amount of anaerobes and actinomycetes. Soil physical measurements included saturated hydraulic conductivity, bulk density, dry aggregate size distribution, wet aggregate stability, and soil moisture retention characteristics.

Plots were mowed three times per week at a height of 1.2 cm (1/2") by means of a reel mower with clippings returned. A pre-emergence herbicide, Barricade 4L (Prodiamine @ 21 oz/A) was applied in mid-March and in mid-June. The insecticide Acelepryin (Chorantraniliprole @ 12oz/A) was applied in mid-June for white grub control. Fertilization consisted of a total of 20 g N, 4 g P_2O_5, and 8 g K_2O m^{-2} for both grasses. Fertilizer was applied monthly from April to September on bermudagrass and in March, April, May, August, September, and October on Kentucky bluegrass. Iron fertilization was applied 3 times during the growing season by means of Six Iron™ (12-0-0) which was added to the spray tank at 6oz/1000sqft during July and August.

The field experiment was laid out in a completely randomized block design with two levels of irrigation (75% and 45% ETos on bermudagrass and 90% and 65% ETos on Kentucky bluegrass) as the block treatment and surfactants at the plot level. Each treatment combination was replicated four times.

Results

Bermudagrass

Analysis of variance revealed that the interaction between surfactants, ET, and sampling dates had a significant effect on DGCI and NDVI (Table 1). The interaction between surfactants and sampling dates affected percent green cover, quality, and moisture uniformity (Table 1). When data were averaged over both irrigation levels and all sampling dates bermudagrass plots treated with Revolution had highest quality, cover, and NDVI. Bermudagrass plots also exhibited the most uniform soil moisture distribution. However, surfactants did not affect soil biological parameters Total Biomass, Arbuscular Mycorrhizal Biomass, Total Bacteria Biomass, Total Fungi Biomass, Fungi Bacteria Ratio, and Diversity Index (Table 4).

Kentucky Bluegrass

Similar to bermudagrass, ANOVA also revealed for Kentucky bluegrass that the interaction between surfactants, ET, and sampling dates had a significant effect on DGCI, NDVI, and percent green cover (Table 1). When data were averaged over all sampling dates and ET replacement levels, plots treated with Revolution had highest percent green coverage. The interaction between surfactants and sampling dates affected soil moisture content (Table 1). Soil surfactants did not affect visual quality and moisture uniformity, neither as main effect nor as interactions with ET and sampling dates. Similar to bermudagrass, surfactants also did not affect soil biological parameters (Table 4).

Conclusion

Surfactant treatments had no effect on soil biological parameters measured for either Kentucky bluegrass or bermudagrass. First year results indicate that surfactants do not positively or negatively influence the soil microbial community, regardless of the type of surfactant applied (organic or synthetic). However, we feel that longer term investigations are warranted to verify if this trend holds if surfactants are applied over several years.

Table 1. Probability values obtained from ANOVA, testing the effects of surfactants, irrigation replacement based on evapotranspiration for short grass (ET), sampling month (Date), and their interactions on percent green cover (Cover), Dark Green Color Index (DGCI) (both parameters determined by means of digital image analysis), volumetric soil water content (moisture), Normalized Difference Vegetation Index (NDVI), visual turfgrass quality (Quality), and soil moisture uniformity of 'Princess 77' bermudagrass (*Cynodon dactylon* L.) and 'SR 2100' Kentucky Bluegrass (*Poa pratensis* L.).

	Cover	DGCI	Moisture	NDVI	Quality	Uniformity
Effect			Bermudagrass			
Block	0.2151	0.2991	0.4291	0.6619	0.1545	0.3421
Surfactant	0.0008	0.1081	0.0025	0.0556	0.005	0.0016
ET	0.0134	0.0463	0.0014	0.0269	0.0042	0.1013
ET*Surfactant	0.0619	0.1345	0.1428	0.3619	0.6671	0.0833
Date	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Surfactant*Date	0.0021	0.0061	0.0009	0.0054	0.0005	0.003
ET*Date	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
ET*Surfactant*Date	0.4456	0.0096	0.791	0.0357	0.5067	0.1092
			Kentucky Bluegrass			
Block	0.32701	0.2583	0.5234	0.7856	0.7742	0.2327
Surfactant	0.0128	0.6077	0.1	0.305	0.1454	0.681
ET	0.0037	0.0047	0.0448	0.0079	0.0069	0.1273
ET*Surfactant	0.0169	0.1098	0.8099	0.257	0.4998	0.7659
Date	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Surfactant*Date	<.0001	0.0078	0.0008	0.039	0.2255	0.818
ET*Date	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
ET*Surfactant*Date	<.0001	0.0012	0.6933	0.0031	0.315	0.1803

Table 2. Percent green cover (Cover), Dark Green Color Index (DGCI), soil moisture content (Moisture), Normalized Difference Vegetation Index (NDVI), visual turfgrass quality (Quality), and soil moisture uniformity (Uniformity) of 'Princess 77' bermudagrass as affected by different soil surfactants. Values are averaged over 11 sampling dates and two irrigation levels (45% or 75% of reference evapotranspiration for short grass).

Surfactant	Cover	DGCI	Moisture	NDVI	Quality	Uniformity
Control	92.5B[¶]	0.4311	17.9A	0.6343B	5.1C	3.4AB
Dispatch	92.6B	0.4300	16.0C	0.6348B	5.4BC	3.9A
Revolution	95.7A	0.4390	17.7AB	0.6593A	5.8A	2.4C
Rhamnolipids	91.9B	0.4268	16.0BC	0.6297B	5.2BC	3.2B
Yucca	93.3B	0.4336	14.5C	0.6395AB	5.4AB	3.5AB

[¶]Values followed by the same letter in each column are not significantly different according to LSD (0.05).

Table 3. Percent green cover (Cover), Dark Green Color Index (DGCI), soil moisture content (Moisture), Normalized Difference Vegetation Index (NDVI), visual turfgrass quality (Quality), and soil moisture uniformity (Uniformity) of 'SR 2100' Kentucky bluegrass as affected by different soil surfactants. Values are averaged over 11 sampling dates and two irrigation levels (55% or 75% of reference evapotranspiration for short grass).

Surfactant	Cover	DGCI	Moisture	NDVI	Quality	Uniformity
Control	89.4B[¶]	0.4581	18.8	0.6692	5.3	3.6
Dispatch	89.6B	0.4596	17.2	0.6515	4.9	3.4
Revolution	96.7A	0.4738	21.1	0.6977	5.9	3.5
Rhamnolipids	90.1B	0.4587	18.4	0.6690	5.3	3.9
Yucca	86.9B	0.4612	19.0	0.6673	5.3	3.7

[¶]Values followed by the same letter in each column are not significantly different according to LSD (0.05).

Table 4. Probability values obtained from ANOVA, testing the effects of surfactants and irrigation replacement based on evapotranspiration for short grass (ET) and their interactions on Arbuscular Mycorrhizal Biomass, Diversity Index, Fungi:Bacteria Ratio, Total Bacteria Biomass, Total Biomass, and Total Fungi Biomass of 'Princess 77' bermudagrass (*Cynodon dactylon* L.) and 'SR 2100' Kentucky Bluegrass (*Poa pratensis* L.).

	Arbuscular Mycorrhizal Biomass	Diversity Index	Fungi: Bacteria	Total Bacteria Biomass	Total Biomass	Total Fungi Biomass
			Bermudagrass			
Block	0.3904	0.4657	0.3346	0.9462	0.8342	0.6067
Surfactant	0.6285	0.2383	0.3317	0.7363	0.8082	0.6026
ET	0.7468	0.3277	0.477	0.8956	0.9824	0.9922
ET*Surfactant	0.976	0.3812	0.8522	0.7935	0.8049	0.9034
			Kentucky Bluegrass			
Block	0.4329	0.201	0.4772	0.4576	0.4993	0.383
Surfactant	0.6775	0.4081	0.5223	0.2642	0.1615	0.5247
ET	0.4456	0.4127	0.813	0.6769	0.658	0.5471
ET*Surfactant	0.2267	0.0356	0.1977	0.3121	0.3193	0.2002

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USGA ID#: 2019-13-683

Title: Kentucky bluegrass fairway establishment and drought tolerance under plant growth-promoting microorganisms (PGPMs) application

Project Leader: Qi Zhang
Affiliation: North Dakota State University

Objectives:

- Determine the efficacy of PGPMs on Kentucky bluegrass establishment and
- Quantify the effects of PGPMs on drought tolerance and recovery of Kentucky bluegrass fairway

Start Date: 2019
Project Duration: 3 years
Total Funding: $13,412

Summary Points:

- No data were collected from the field trials in 2019 due to poor establishment caused by abnormal wet and cold weather conditions and a breakout of barnyard grass and thistle. A one-year extension has been requested and granted for this project. The field trials will be re-established in 2020.
- One greenhouse experiment has been conducted to determine the efficacy of PGPMs on Kentucky bluegrass establishment under salinity.
  - ‘Kenblue’ was taller than ‘Moonlight’. It also had a higher tissue biomass but lower specific root length when comparing to ‘Moonlight’.
  - Salinity inhibited Kentucky bluegrass seedling growth but enhanced specific root length.
  - Limited differences were observed among the PGPM products applied in the current study.
- Another greenhouse experiment to determine the efficacy of PGPMs on Kentucky bluegrass establishment under drought is currently ongoing.

Summary Text:

The rhizosphere is the soil region largely influenced by plants through rhizodeposition of exudates and metabolites, providing rich carbon sources and colonization structures to soil microorganisms. Microorganisms in turn may have deleterious, neutral, or beneficial effects on plants. It has been well documented that plant growth-promoting microorganism (PGPM), including arbuscular mycorrhizal fungi and plant growth-promoting bacteria, help improve yield and stress tolerance in field crops by influencing resource acquisition (e.g. water and nutrient), modulating plant hormone levels, regulating source-sink relations and energetic metabolism, and inducing systemic resistance.

In turfgrass, the beneficial effects of PGPM are mainly observed in perennial ryegrass, tall fescue, and fine fescues. Limited information is available on the relationships between PGPM and other important turfgrass species, such as Kentucky bluegrass. The objective of this research is to determine the efficacy of commercially available PGPM products on Kentucky bluegrass establishment under stress and non-stress conditions.

Field trial – Excessive snow accumulation in the winter of 2018 and the long and cold spring of 2019 delayed field preparation. Although Kentucky bluegrass was seeded in mid-June, strong storms which occurred a few days after seeding might had scattered the seeds. Exceptional wet weather in the summer of 2019 prevented regular mowing and herbicide applications in the research plots, resulted in a breakout of barnyard grass and thistles. By the end of the growing season (late September), about 20 – 30% of the research plots was covered with turf. Therefore, no data were collected from the field trial in 2019. A one-year extension has been requested and granted for this project. The field trials will be re-established and studied in 2020 and 2021.

Greenhouse experiments – Two greenhouse experiments were conducted in 2019 to determine the efficacy of PGPMs on Kentucky bluegrass establishment under salinity (Experiment 1) and drought (Experiment 2). Six commercially available PGPM products were included in the greenhouse experiments (Table 1).

Table 1. Six commercially available plant growth-promoting microorganism (PGPM) products included in the present study.

Product name	Active ingredient	Application rate (product/acre)
Serenade (Bayer CropScience LP, Research Triangle Park, NC)	*Bacillus subtilis* strain QST713 (1.34%)	4 qt
BotaniGard 22WP (BioWorks, Inc., Butte, MT)	*Beauveria bassiana* strain GHA (22.0%)	11 lbs
RootShield PLUS+ WP (BioWorks, Inc., Victor, NY)	*Trichoderma harzianum* Rifai strain T-22 (1.15%) + *Trichoderma virens* strain G-41 (0.61%)	65 lbs
Companion (Growth products, White Planins, NY)	*Bacillus subtilis* strain GB03 (4.26%)	1.5 lbs
Nortica 10WP (Bayer Experimental Science, Research Triangle Park, NC)	*Bacillus firmus* strain 1-582 (10.0%). It also contains N (14%), K_2O (21%), and chloride (51%)	50 lbs
Molt-X (BioWorks, Inc., Victor, NY)	*Azadirachtin* (3.0%)	10 oz

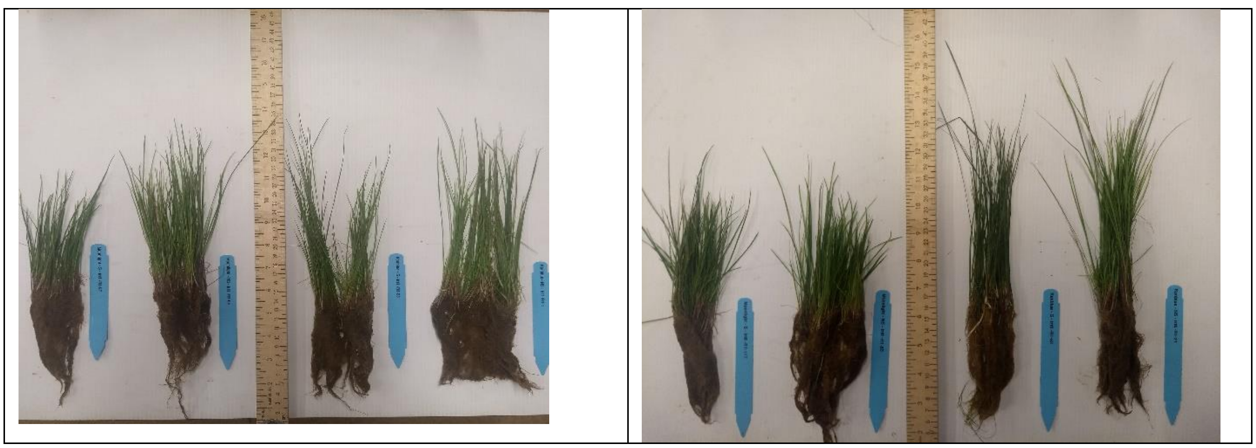
<u>*Experiment 1 – salinity.*</u> 'Kenblue' and 'Moonlight' were seeded at 3 lbs pure live seeds /1,000 sq. ft in 4 x 4 inch pots filled with a topsoil:sand mixture (1:1, v:v). A starter fertilizer, 18-24-5, was applied at 1 lbs N/1,000 sq. ft at seeding. The PGPM products were applied at seeding and once weekly from week (wk) 3 to wk 6, except Nortica (at seeding and once at week 4). Pots were saturated with tap water from the bottom after seeding to minimize soil disturbance and kept in a tub filled with tap water (1-inch depth) from wk 1 to wk 2 to ensure good germination. Turf pots were moved out of the tub at wk 3 and hand-watered with tap water or NaCl at 6 dS m^{-1} three times weekly (Mon., Wed., and Fri.) from wk 3 to wk 6. Turfgrass was harvested when the experiment was terminated at wk 6. The experimental design was a 2 (cultivar) x 2 (salt condition) x 7 (6 PGPM products + 1 no PGPM application control) factorial design with the experimental unit (pot) arranged in a RCBD with four replicates. Data were collected on plant height, shoot and root dry weight, root length, root to shoot dry weight ratio, and specific root length (i.e. root length / root dry weight). Data were analyzed using PROC MIXED and means were separated with Fisher's protected least significant difference at $P \leq 0.05$.
'Kenblue' outperformed 'Moonlight' in plant height and tissue biomass (Table 2). Higher specific root length of 'Moonlight' compared to 'Kenblue' is due to its lower root dry weight. Salinity stress inhibited turfgrass growth, except specific root length. A higher reduction in root dry

weight (836.5 vs. 574.6 mg) than in root length (15.9 vs. 13.6 cm) resulted in increased specific root length under the saline condition. No differences were observed in PGPM products, except plant height and shoot dry weight (picture).

Table 2. Kentucky bluegrass seedling growth as affected by three main factors, cultivar, salt, and plant growth-promoting microorganism (PGPM) products.

Treatment	Height (cm)	Shoot dry weight (mg)	Root dry weight (mg)	Root length (cm)	Root /shoot dry weight ratio (%)	Specific Root length (cm/g)
Cultivar						
Kenblue	12.7a[z]	1547.3a	816.0a	14.6a	50.4a	17.8b
Moonlight	11.4b	1079.4b	594.4b	14.9a	53.6a	26.8a
Salt (NaCl, dS m^{-1})						
0	12.7a	1435.4a	836.5a	15.9a	56.1a	19.7b
6	11.4b	1191.4b	574.0b	13.6b	47.9b	24.8a
PGPM products						
Control (no PGPM)	13.7a	1518.5a	785.2a	14.3a	50.0a	19.5a
BotaniGard 22WP	11.6ab	1317.1ab	693.8a	14.3a	51.3a	22.8a
Companion	12.7ab	1090.4b	650.2a	15.0a	58.0a	24.6a
Molt-x	10.5b	1082.2b	621.5a	14.3a	53.0a	25.0a
Nortica 10WP	12.5ab	1430.3a	756.4a	16.7a	52.5a	22.6a
RootShield PLUS+ WP	11.9ab	1313.6ab	678.9a	13.7a	50.1a	21.1a
Serenade	11.4ab	1441.4a	750.5a	14.6a	49.2a	20.3a

[z]Means followed by the same letter within each main factor are not significantly different at $P \leq 0.05$.



‘Moonlight’ and ‘Kenblue’ Kentucky bluegrass without PGPM application (left) and treated with Nortica (right). The seedlings were either non-salt-treated (NS) or treated with NaCl at 6 dS m^{-1} (S).

Experiment 2 - drought

‘Kenblue’ and ‘Waterworks’ Kentucky bluegrass were seeded and maintained as described in Experiment 1. So were the PGPM applications. Half of the pots were hand watered with tap water three times weekly (Mon., Wed., and Fri.) from wk 3 to wk 6 (i.e. non-drought -treatment). Irrigation of the other half of the pots were applied only when leaf wilting was observed (i.e. drought stress treatment) (once weekly). The experiment design and data collection and analysis are identical to Experiment 1. This experiment is currently ongoing.

USGA ID#: 2017-16-626

Title: Quantifying the effects of iron sulfate and phosphorous acid applications on the surface pH and the suppression of Microdochium patch.

Project Leader: Alec Kowalewski
Affiliation: Oregon State University

Objectives:

The third year of this three-year project focused on exploring the effects that iron sulfate and phosphorous acid applications were having on the pH of the leaf surface post application and also to explore the effects of an acidifying agent on the suppression of Microdochium patch.

Start Date: 2016 – 2018 (two field trials took place); 2018 – 2019 growth chamber studies
Project Duration: 3 years
Total Funding: $30,000

Summary Points:

- Leaf surface pH was affected by iron sulfate and phosphorous acid applications.
- Sulfuric acid applications lowered the leaf surface pH, although these applications did not suppress Microdochium patch compared to a control.
- Iron sulfate applied at 2.0 lbs. per 1000 ft^2 suppressed Microdochium patch regardless of the presence of phosphorous acid.

Summary Text:

A two-year field trial focusing on the use of 5 rates of iron sulfate either with or without phosphorous acid resulted in all iron sulfate and phosphorous acid applications suppressing Microdochium patch. It was observed that these treatments were noticeably decreasing the pH of the spray suspensions compared to the water control, with some spray suspensions having a pH as low as 2.2. Previous work has shown that *Microdochium nivale*, the pathogen responsible for Microdochium patch, has a reduced growth below pH 5.5 and will not grow below a pH of 2.5. It was hypothesized that the pH may be responsible for the suppression of Microdochium patch. A growth-chamber experiment focusing on quantifying the leaf surface pH of the same 10 treatments as the field study was performed (treatments listed in Table 1).

Ten cup-cutter sized turfgrass pots were made by removing sod from four blocks on a USGA recommended particle size sand research green and placing the sod into 4-inch diameter PVC irrigation couplers with the bottoms capped with geotextile. One pot per block was randomly assigned and the designated treatment was applied using a backpack sprayer calibrated to apply 2 gallons / 1000 ft^2. Pots were randomly placed in a growth chamber set to 39 degrees Fahrenheit with a 10-hour daylight. Pots were not irrigated because drought stress was not observed throughout the trial. At multiple increments starting at 6 hours post application up to 17 days post application, 1 ml of de-ionized water was placed on the surface of the pot and pH was quantified using a Field Scout pH meter held in place with a support designed for this purpose. The pots were re-randomly assigned a location in the growth chamber following each pH reading. Two trial runs took place and treatments significantly decreased the pH compared to the water control during the 17-day trial (Table 1).

Table 1: Effects of treatments on the pH of the surface of *Poa annua* pots originating from a putting green maintained at 0.110 inches. Measurements followed by different letters indicate significant differences according to Tukey's HSD at an alpha = 0.05.

Treatment	Run 1			Run 2		
	pH Spray Suspension	+ 6 days $P < 0.001$	+ 17 days $P < 0.001$	pH Spray Suspension	+ 6 days $P < 0.001$	+ 17 days $P < 0.001$
0.075 lbs. H_3PO_3 / M	2.3	4.01 bc	4.41 b	2.3	4.93 ab	4.76 b
0.25 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	2.2	3.69 c	4.18 bc	2.3	4.16 c	4.22 bc
0.5 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	2.2	3.80 bc	4.04 bc	2.2	4.26 bc	4.54 bc
1.0 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	2.2	3.49 c	4.09 bc	2.2	4.19 c	4.28 bc
2.0 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	2.2	3.52 c	3.70 c	2.2	3.91 c	4.10 c
Non-treated Control	6.5	5.29 a	5.32 a	6.7	5.61 a	5.58 a
0.25 lbs. $FeSO_4$ / M	3.5	4.37 b	4.43 b	3.5	4.28 bc	4.74 b
0.5 lbs. $FeSO_4$ / M	3.1	3.96 bc	3.87 bc	3.1	4.15 c	4.76 b
1.0 lbs. $FeSO_4$ / M	3.0	3.85 bc	4.05 bc	2.9	4.23 c	4.48 bc
2.0 lbs. $FeSO_4$ / M	2.9	3.38 c	3.73 c	2.8	3.85 c	4.34 bc

After this initial growth chamber experiment, a new experiment was designed to explore how an acidifying agent would affect the suppression of Microdochium patch in a growth chamber setting. Sulfuric acid was chosen as the acidifier because sulfuric acid can be formed when iron sulfate dissociates in water. Three rates of sulfuric acid were chosen based on preliminary results that provided a range of surface pH that included the surface pH observed from the iron sulfate and phosphorous acid treatments in the first experiment (treatments listed in Table 2).

The pots were prepared in an identical manner to experiment 1 and arrangement in the growth chamber was also completely randomized and re-randomized following each pH measurement. The temperature in the growth chamber for the second experiment was set to 35 degrees Fahrenheit at night and 51 degrees during the day (10 hours) because these temperatures reflected the averages for February in Corvallis, OR, when Microdochium patch pressure is considered very high. After the initial pH reading, 6 hours after treatment, three cores of *Microdochium nivale* were placed mycelium side down onto the surface of each pot. The pots were covered with a plastic container and sealed using Glad Wrap in order to retain a high relative humidity. After 24 hours, all of the containers were saturated with water, indicating that humidity was near 100%. Once a week, the containers were opened, pH of the surface was measured following the protocol in experiment one, the expansion of Microdochium patch was recorded with a camera, 50 ml of water was added to the container to maintain high humidity, and the containers were re-sealed.

The pH of the surface of the pots showed that the sulfuric acid treatments resulted in a range of pH measurements 6 hours post application from 2.9 to 4.2 in run 1 and 3.1 to 4.1 in run 2. The highest rate of sulfuric acid (0.5% H_2SO_4) resulted in the lowest pH. Each run of the second experiment lasted for three weeks because it took about 14 days before Microdochium patch symptoms developed on the pots. The experiment continued for a third week to allow the Microdochium patch symptoms to expand. After three weeks, there was no difference in Microdochium patch severity between the three sulfuric acid treatments and the control in either year indicating that the lower leaf surface pH did not result in suppression of Microdochium patch. In both runs, 2.0 lbs of FeSO4 / M suppressed Microdochium patch compared to the control regardless of the presence or absence of phosphorous acid (Table 2 & Figure 1).

Table 2: Effects of treatments on the pH of the surface of *Poa annua* pots originating from a putting green maintained at 0.110 inches. Measurements followed by different letters indicate significant differences according to: †Tukey's HSD or ‡Dunn's pairwise comparisons, at an alpha = 0.05.

	Run 1			Run 2		
	pH Spray Suspension	pH + 6 hours	Microdochium patch (mm^2) + 21 days	pH Spray Suspension	pH + 6 hours	Microdochium patch (mm^2) + 21 days
0.05% H_2SO_4	2.0	4.23 b†	632.5 ab‡	2.0	4.09 bc†	1787.3 ab†
0.125% H_2SO_4	1.7	3.61 c	976.0 a	1.6	3.69 cd	1304.8 ab
0.5% H_2SO_4	1.4	2.93 d	501.1 ab	1.4	3.07 e	1789.2 ab
Water Control	7.0	4.89 a	492.8 ab	7.0	4.89 a	2025.5 a
2.0 lbs. $FeSO_4$ / M	2.6	3.53 c	0.6 cd	2.7	3.59 d	0.0 b
0.075 lbs. H_3PO_3 / M	2.1	3.73 c	106.4 bc	2.2	4.20 b	309.0 ab
2.0 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	1.9	3.33 cd	0.0 d	1.8	3.43 de	0.0 b

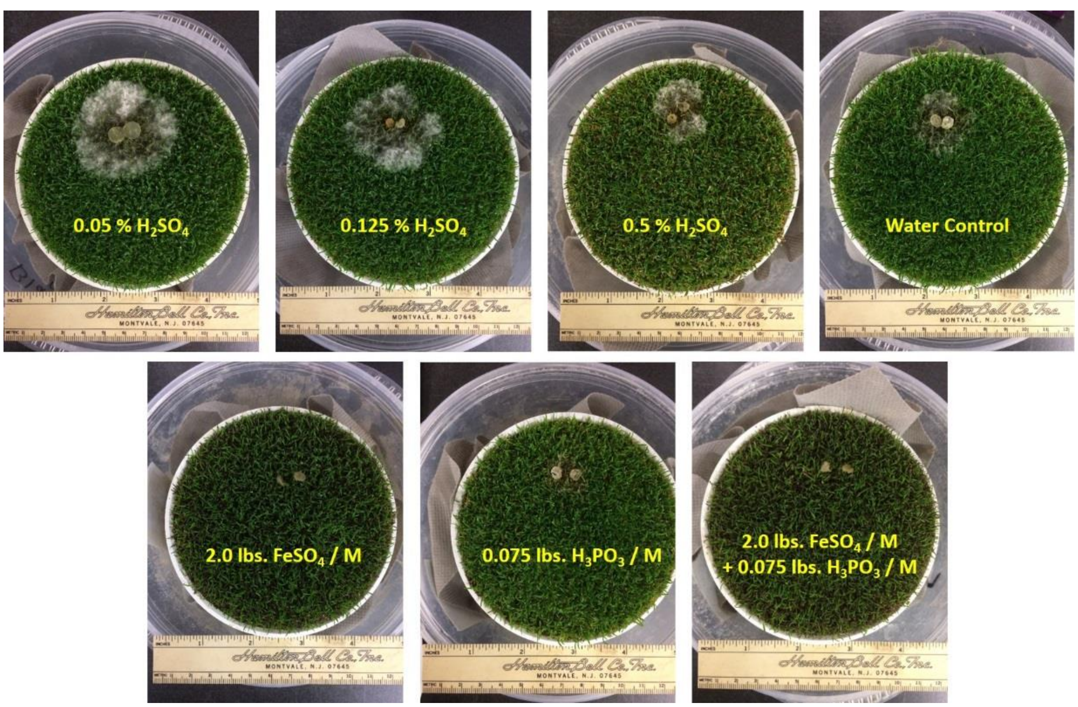


Figure 1: Microdochium patch symptoms + 21 days after treatment.

Future expectations of the project:

Currently, a scientific journal article is being drafted for publication and it is the intention of the authors to also write a trade journal article about this experiment. These results are being shared at state, national, and international meetings.

USGA ID#: 2017-18-628

Title: Effects of Nitrogen Source & Rate, Phosphate Rate, and Potassium Rate on Microdochium

Project Leader: Alec Kowalewski
Project Member: Brian McDonald, Emily Braithwaite, Clint Mattox

Affiliation: Oregon State University

Objectives:

The objective of this research is to evaluate the effects of fall through spring monthly applications of N (0 and 0.1 lbs N per 1,000 sq ft), P (0 and 0.025 lbs per 1,000 sq ft), and K (0 and 0.1 lbs P per 1,000 sq ft), on Microdochium patch development within an annual bluegrass putting green.

Start Date: 2017
Project Duration: 3 years
Total Funding: $30,000

Summary Points:

- In year 1, the high rate of N resulted in more disease, but in year 2, the low rate of N had more disease.
- In year 1, potassium applied at a rate of 0.1 lbs N per 1,000 sq ft reduced percent disease when compared to treatments that did not receive K.
- In both years, the main effect of P rate and the interactions between N, P and K were not significant.
- A third year of research is required to see if trends over time can be repeated.

Summary Text:

Research on primary nutrient nitrogen (N), phosphorus (P) and potassium (K) ratios have suggested that maintaining the proper balance of the nutrients is critical to disease mitigation. However, research on N, P, and K ratios relevant to annual bluegrass and Microdochium patch is not available.

The objective of this research is to evaluate the effects of fall through spring monthly applications of N (0 and 0.1 lbs N per 1,000 sq ft), P (0 and 0.025 lbs per 1,000 sq ft), and K (0 and 0.1 lbs P per 1,000 sq ft), on Microdochium patch development within an annual bluegrass putting green.

A two year field research trial was initiated in September 2017 with the second year completed in May of 2019. The trial was located on a 100% USGA sand putting green which was constructed in 2009 at the Lewis-Brown Horticulture Farm, Corvallis, OR. Experimental design is a 2 by 2 by 2 factorial randomized complete block design with four replications; factors include nitrogen rate, phosphorus rate, and potassium rate. To reduce the disease pressure, all treatments received monthly applications fall through spring of phosphorous acid (Duraphite 12 applied at 3.7 kg H_3PO_3 ha^{-1} in year 1 and 7.4 kg H_3PO_3 ha^{-1} in year 2) and sulfur (Sulfur DF applied at 12 kg S ha^{-1}). Traditional fungicides were not applied to this experiment, except for summer anthracnose control.

In both years, N applied alone resulted in more disease. In year 1, the high rate of N resulted in more disease (Figure 1), but in year 2, the low rate of N had more disease (Figure 2). In year 1, potassium applied at a rate of 0.1 lbs N per 1,000 sq ft reduced percent disease when compared to treatments that did not receive K. In both years, the main effect of P rate and the interactions between N, P and K were not significant.

Future Research:

In October 2019 (year three) the nitrogen, phosphorus and potassium rates were initiated again. Treatments will be applied until April 2020. As previously done, all treatments will receive monthly applications of phosphorous acid (Duraphite 12 applied at 3.7 kg H_3PO_3 ha^{-1} in year 1 and 7.4 kg H_3PO_3 ha^{-1} in year 2) and sulfur (Sulfur DF applied at 12 kg S ha^{-1}) to minimize disease pressure without traditional fungicides.

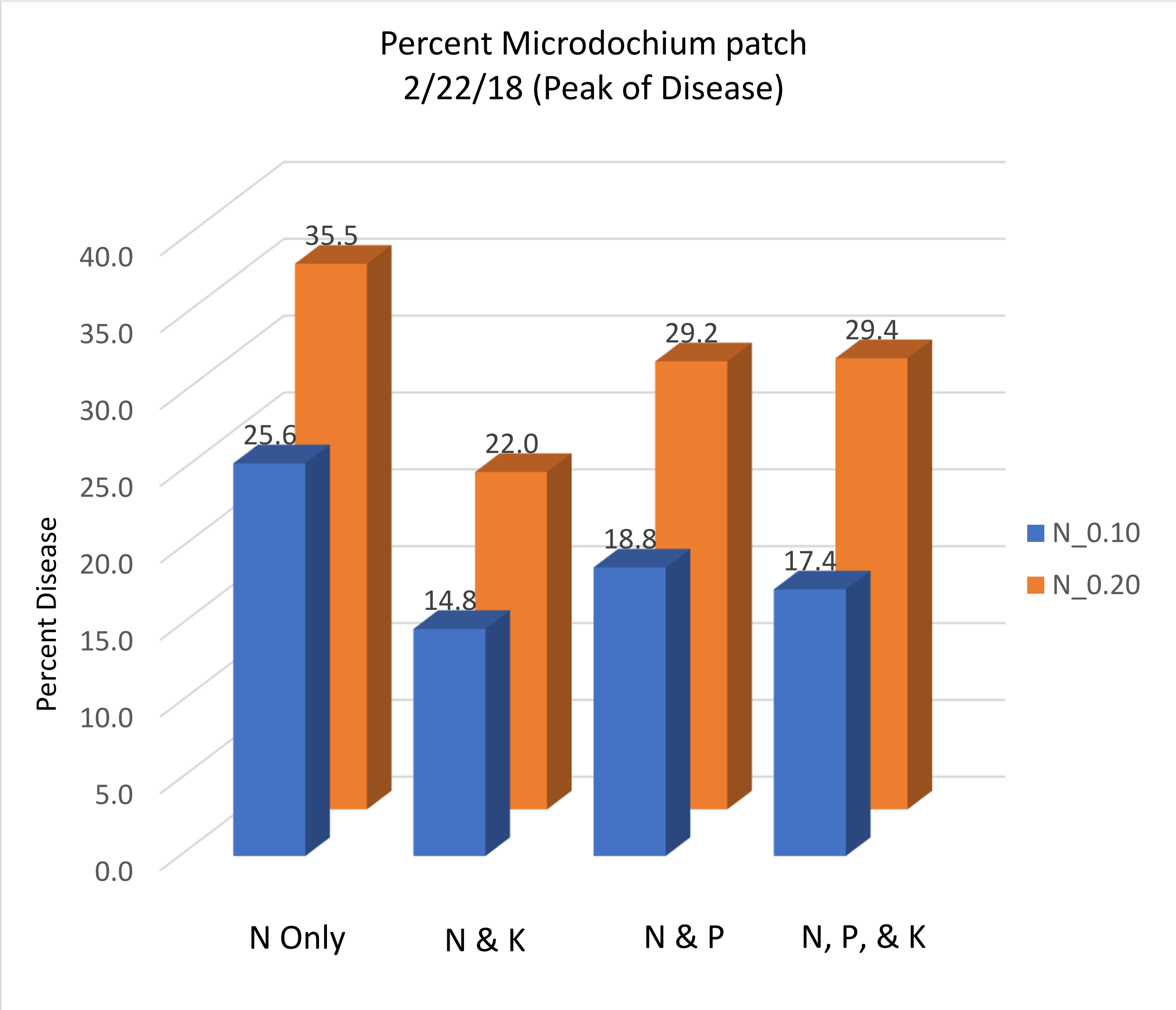


Figure 1: Percent disease observed at the peak of disease in year 1 (2/22/2018); Nitrogen (N) rates include 0.1 and 0.2 lbs N per 1,000 sq ft applied every four weeks; Phosphorus rates included 0.0 and 0.025 lbs P per 1,000 sq ft every four weeks; Potassium (K) rates included 0.0 and 0.1 lbs K per 1,000 sq ft applied every four weeks; Fertilizer was applied at these rates from October 2017 to April 2018.

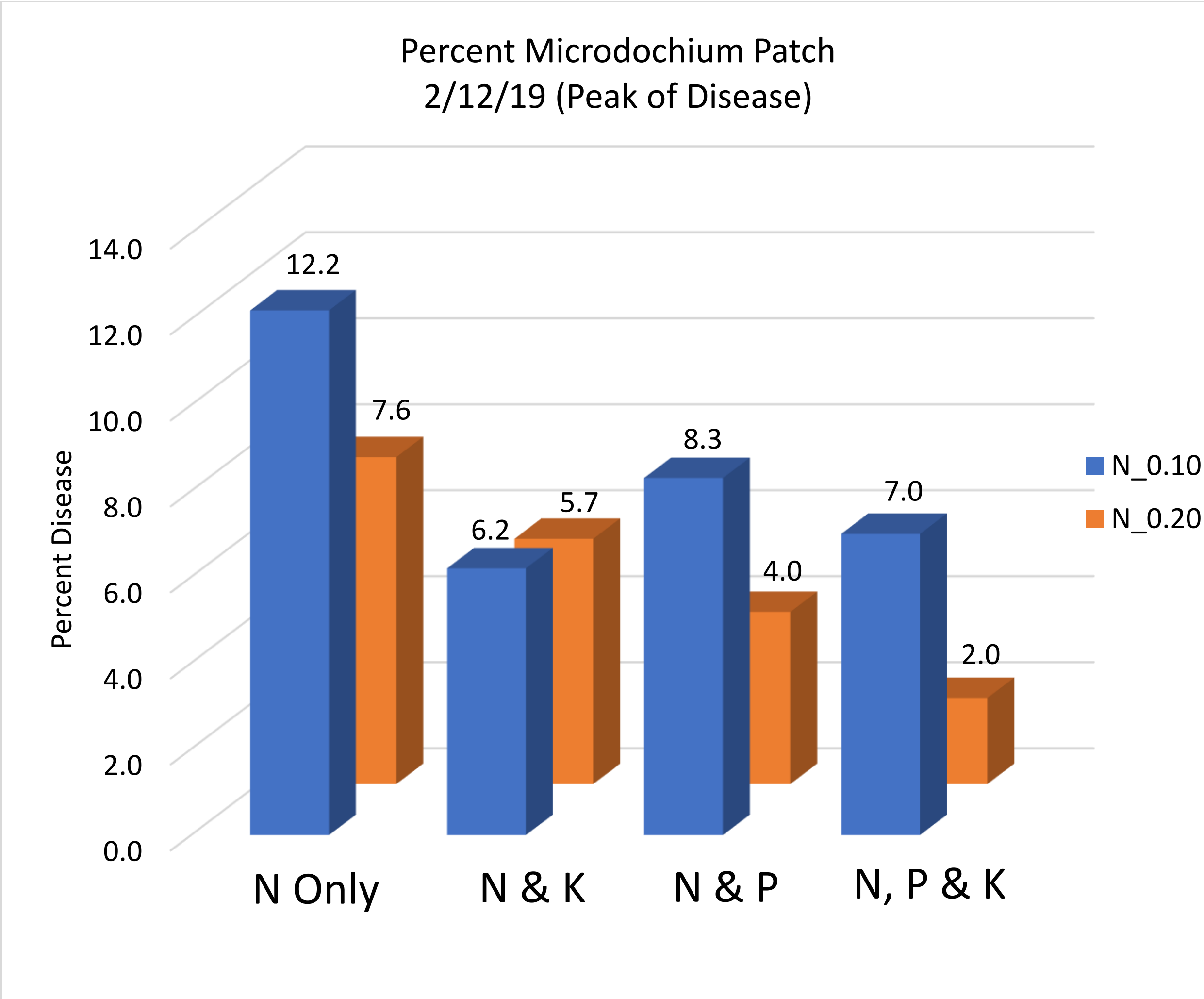


Figure 2: Percent disease observed at the peak of disease in year 2 (2/12/2019); Nitrogen (N) rates include 0.1 and 0.2 lbs N per 1,000 sq ft applied every four weeks; Phosphorus rates included 0.0 and 0.025 lbs P per 1,000 sq ft every four weeks; Potassium (K) rates included 0.0 and 0.1 lbs K per 1,000 sq ft applied every four weeks; Fertilizer was applied at these rates from October 2018 to April 2019.

USGA ID#: 2019-02-672

Title: Comparing iron sulfate versus chelated iron for the suppression of Microdochium patch on annual bluegrass putting greens in the absence and presence of phosphorous acid

Project Leader: Alec Kowalewski
Affiliation: Oregon State University

Objectives:

The objective of this experiment is to compare the effects of iron sulfate versus chelated iron in the presence or absence of phosphorous acid on the suppression of Microdochium patch and turfgrass thinning.

Start Date: 2019
Project Duration: 3 years
Total Funding: $30,000

Summary Points:

- All treatments suppressed Microdochium patch compared to the non-treated control except for 0.1 lbs. Fe/M applied alone as DTPA.
- Less than 1% Microdochium patch was observed when any rate of iron regardless of source was applied in combination with phosphorous acid compared to the non-treated control with 45% disease.
- No statistical differences in turfgrass thinning as measured by percent green cover were observed between iron treatments in combination with phosphorous acid.

Summary Text:

Figure 1. Overview of the plots on 24th of January 2019 in Corvallis, OR.

The first-year applications began the 4th of September 2018 and were applied every two weeks until the 9th of April 2019. The second-year applications began on the 6th of September 2019 and are ongoing. In the first year, Microdochium patch symptoms began to appear in late September and disease pressure was high with an average Microdochium patch percentage of 45% in the non-treated control plots on the 24th of January 2019 rating date (Figure 1 and Table 1). Replicated golfer traffic representing 73 golf rounds a day was applied to the plots five days a week by walking over the trial with golf shoes. First year results indicate that all treatments significantly suppressed Microdochium patch except for the 0.1 lbs. of iron applied as chelated iron in the absence of phosphorous acid. There was no difference in suppression of Microdochium patch by the iron sources when applied alone at 0.1 lbs. iron per 1000 ft^2, although iron sulfate suppressed Microdochium patch more than chelated iron when applied alone at 0.2 lbs. iron per 1000 ft^2 (1.8% versus 25% Microdochium patch respectively). All treatments that included phosphorous acid suppressed Microdochium patch to the same level as the fungicide control treatment. Less than 1% Microdochium patch was observed when any rate of iron regardless of source was applied in combination with phosphorous acid. This is in comparison to an average Microdochium patch percentage of 3.3 % when phosphorous acid was applied alone.

Percent green cover was assessed using the TurfAnalyzer program with the following parameters: hue 60 to 360, saturation 10 to 100, and brightness 0 to 100 (Figure 2). Concerning percent green cover, no statistical differences were observed between the different iron sources or rates when applied in combination with phosphorous acid. Visually, turfgrass thinning was not apparent on the chelated iron treatments, although thinning was observed on the iron sulfate treatments. Surprisingly, the chelated iron treatments did not provide a positive green cover response. Soil test analyses are ongoing and may reveal some indication why the response was not apparent.

Turfgrass quality ratings were taken using the National Turfgrass Evaluation Program (NTEP) ratings from 1 to 9 with a 6 or greater considered to be acceptable. Only 0.2 lbs. of iron applied as iron sulfate in combination with phosphorous acid and the fungicide control resulted in a higher turfgrass quality rating than the non-treated control. The 0.2 lbs. of iron sulfate applications in combination with phosphorous acid suppressed Microdochium patch, although visible turfgrass thinning resulted in turfgrass quality ratings that were not acceptable. Only the fungicide control resulted in acceptable turfgrass quality ratings.

Future expectations of the project:

This experiment is ongoing and will continue to assess Microdochium patch percentage, percent green cover, and turfgrass quality. In addition, soil test analyses are ongoing and results will be included in future trial updates as well as in a future manuscript publication, a trade journal article, field days, in addition to future state, national, and international presentations.

Table 1. Letter diagram of effects of iron sources in the combination or the absence of phosphorous acid on percent Microdochium patch, percent green cover, and turfgrass quality.

	< - - - - - - - - - - - - - - - 24th of January 2019	- - - - - - - - - - - - - - - - >	
	% Microdochium patch†	Percent Green Cover‡	Turf Quality§
0.1 # Fe/M as $FeSO_4$	20.0% b¶	81.8% cd¶	5.00 abc#
0.1 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.4% c	94.6% ab	5.00 abc
0.2 # Fe/M as $FeSO_4$	1.8% c	87.6% bc	5.00 abc
0.2 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.0% c	91.6% ab	5.75 ab
0.1 # Fe / M as DTPA	32.5% ab	71.3% e	4.00 bc
0.1 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.7% c	95.0% ab	5.00 abc
0.2 # Fe/M as DTPA	25.0% b	77.4% de	4.00 bc
0.2 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.1% c	94.8% ab	5.25 abc
0.075 lbs. H_3PO_3 / M	3.3% c	93.6% ab	5.00 abc
Fungicide Control	0.0% c	97.8% a	7.25 a
Not-treated Control	45.0% a	43.7% f	3.25 c

† Percent Microdochium patch assessed through visual ratings from 0 to 100%

‡ Percent green cover assessed using the TurfAnalyzer program using the parameters: hue 60 to 360, saturation 10 to 100, and brightness 0 to 100.

§ Turf quality rating assessed using the NTEP scale from 1 to 9 with a rating of 6 or greater considered acceptable for putting green turf.

¶ Mean differences in the same column followed by the same letter are not significantly different according to Dunn's test (alpha ≤ 0.05).

Means in the same column followed by the same letter are not significantly different according to Tukey's HSD (alpha ≤ 0.05).

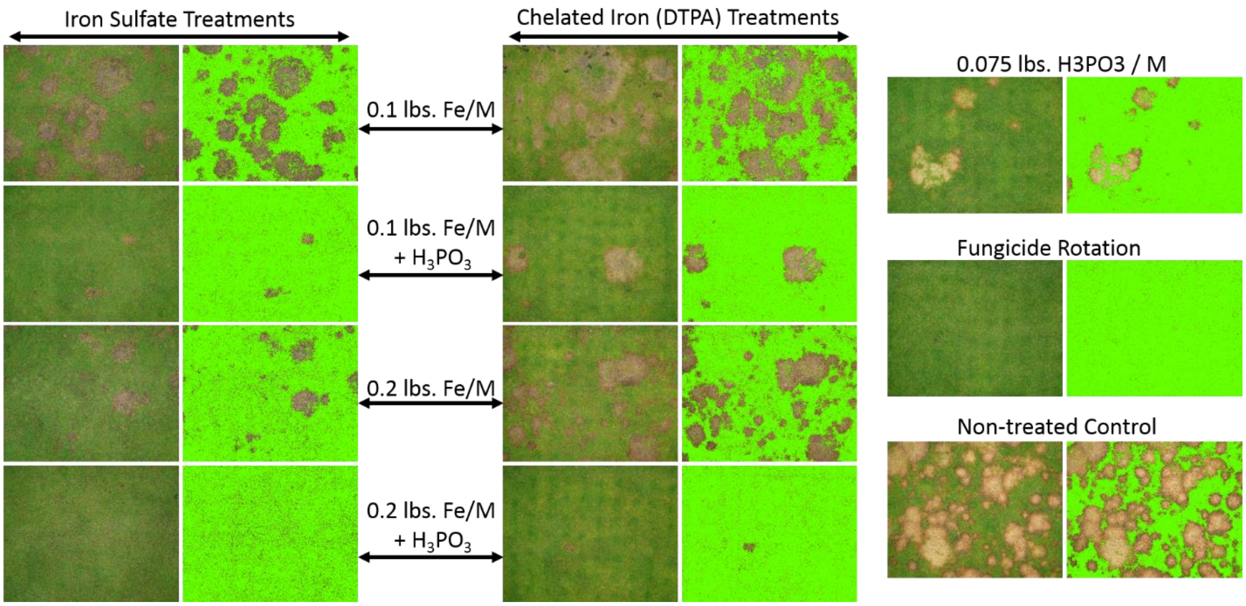


Figure 2. Percent green cover assessed on 24th of January 2019 of iron source treatments either in the combination or the absence of phosphorous acid in addition to phosphorous acid applied along, a fungicide rotation, and a non-treated control. Percent green was assessed using the TurfAnalyzer program using the parameters: hue 60 to 360, saturation 10 to 100, and brightness 0 to 100. The original photos are followed by overlays of pixels that met the parameters for percent green cover.

USGA ID#: 2019-03-673

Title: Quantifying the long-term effects of alternative Microdochium patch management techniques on sand-based annual bluegrass putting green performance over multiple seasons

Project Leader: Alec Kowalewski
Affiliation: Oregon State University

Objectives:

The objective of this experiment is to observe the long-term impacts of winter applications of alternatives to traditional fungicides on summer putting green performance, soil fertility, and winter turfgrass quality.

Start Date: September 2018
Project Duration: Year 1 of 3 (year 2 was initiated in fall 2019)
Total Funding: $30,000

Summary Points:

- All treatments reduced Microdochium patch severity to less than 5% compared to the control.
- There was some evidence that anthracnose severity was influenced by winter treatments.
- There was no evidence that winter treatments affected summer putting green speeds, NDVI ratings or water infiltration.

Summary Text:

Applications began on the 4th of September 2018 and took place every two weeks until the 9th of April 2019 for a total of 16 applications. Second-year applications began on the 6th of September 2019 and are ongoing. Microdochium patch was visible on plots by late September and disease pressure was high with an average of 40% Microdochium patch in the non-treated control plots on the 24th of January 2019 (Figure 1 and Table 1).

Figure 1. Overview of the Microdochium patch disease pressure on 8 February 2019 in Corvallis, OR.

All treatements reduced Microdochium patch severity to ≤ 2% with the exception of sulfur or phosphorous acid applied alone every two weeks. Mineral oil applied in combination with phosphorous acid every two weeks in September, October, November, and April with a sulfur and phosphorous acid combination applied December through March as well as a mineral oil applied in combination with phosphorous acid rotated every four weeks with a sulfur and phosphorous acid combination suppressed Microdochium patch to levels equivalent to a fungicide rotation.

In order to provide abiotic stress to the plots, replicated golfer traffic representing 73 golf rounds a day took place five days a week by walking over the trial with golf shoes. No abiotic damage was observed from the mineral oil treatments, however some turfgrass thinning was visible on the iron sulfate treatments, reducing turfgrass quality on those plots.

Table 1. Letter diagram of effects of treatments on percent Microdochium patch, percent anthracnose, and turfgrass quality.

		<----- 24th of January 2019 ------->		<---- 19th of September 2019 ---->	
Treatment #	Treatment Description	% Microdochium patch	Turf Quality	% Anthracnose	Turf Quality
1	0.25 # S / M + 0.075 lbs. H_3PO_3 / M	2.0% ab†	5.0 ab†	8.0% ab†	5.8 a†
2a (Sep, Oct, Nov, Apr)	8.5 oz. Civitas Turf Defense + 0.075 lbs. H_3PO_3 / M	0.3% b	6.8 a	1.4% ab	6.3 a
2b (Dec - Mar)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M				
3a (in 4-wk rotation)	8.5 oz. Civitas Turf Defense + 0.075 lbs. H_3PO_3 / M	0.3% b	6.8 a	0.3% ab	6.8 a
3b (in 4-wk rotation)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M				
4a (in 4-wk rotation)	8.5 oz. Civitas Turf Defense	0.9% ab	5.4 ab	1.9% ab	5.3 a
4b (in 4-wk rotation)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M				
5	0.5 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	0.6% ab	5.0 ab	10.0% a	4.8 a
6	1.0 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	0.5% ab	5.0 ab	2.6% ab	6.0 a
7	0.25 # S / M	4.3% ab	5.0 ab	0.3% ab	6.8 a
8	0.075 lbs. H_3PO_3 / M	3.8% ab	5.0 ab	5.3% ab	5.0 a
9	Fungicide Control	0.3% b	6.3 a	0.0% b	7.3 a
10	Non-treated Control	40.0% a	3.0 b	0.3% ab	6.0 a

† Mean differences in the same column followed by the same letter are not significantly different according to Dunn's test (alpha ≤ 0.05).

At the conclusion of the Microdochium patch treatments, soil samples were collected on the 8th of May 2019 from a 0" to 1" depth with the verdure removed and also from a 1" to 3" depth. Soil analysis results for the 1" to 3" depth are reported in table 2. Of note, only treatment 1 (0.25 # S / M + H_3PO_3 every two weeks) resulted in a lower pH compared to the non-treated control, even though treatment 1 and 7 received 4 lbs. S / M / year and treatments 2,3, and 4 received 2 lbs. S / M / year. Treatments 1,3,4 and 7 did have a higher ppm S compared to the non-treated control. No differences were observed for Cu, Fe, or K. Soil analyses results for the top 1" are still pending. Perhaps differences for Cu from the mineral oil pigment and Fe from the $FeSO_4$ applications will be observed in the top 1" soil samples.

The green was hollow-tine aerified on the 21st of May with cores collected on each individual plot to avoid contamination between treatments. During the spring and summer, the green was fertilized with Andersons 28-5-18 plus micronutrients.

In June, July, and August, half of the plots were sprayed with a fungicide as a split-plot in order to protect half of the plot to quantify putting green characteristics and the non-treated side was used to observe any winter treatment effects on anthracnose. Water infiltration, greenspeeds, and NDVI were collected monthly starting in July. Results for the 19 September 2019 rating date are reported in table 2. Putting green characteristic date were collected from the fungicide treated site and no significant differences in these metrics were observed.

On the non-fungicide treated split-plot, differences among the winter treatments regarding anthracnose were observed with the 0.5 lbs. $FeSO_4$ / M + 0.0075 lbs. H_3PO_3 / M every two weeks resulting in more anthracnose compared to plots that received fungicides in the winter (Table 1). There is some prior evidence that winter sulfur applications may increase the risk of anthracnose and some anecdotal evidence that this is also the case for iron sulfate treatments.

Table 2. Letter diagram of effects of treatments on putting green characteristics and soil fertility.

		<---- 19th of September 2019 ---->				<---- Mehlich III (ppm) ---->					
Treatment #	Treatment Description	H_2O Infiltration[†]	Greenspeed[‡]	NDVI[§]	pH	Cu	Fe	K	Mn	P	S
1	0.25 # S / M + 0.075 lbs. H_3PO_3 / M	6.5 a[¶]	9.3 a[¶]	44.0 a[††]	6.42 b[††]	1.3 a[††]	157.3 a[††]	26.8 a[††]	27.6 a[††]	13.1 ab[††]	11.6 ab[††]
2a (Sep, Oct, Nov, Apr)	8.5 oz. Civitas Turf Defense + 0.075 lbs. H_3PO_3 / M	8.0 a	8.9 a	43.7 a	6.50 ab	1.3 a	168.7 a	25.3 a	26.6 ab	12.5 ab	7.6 bcd
2b (Dec - Mar)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M										
3a (in 4-wk rotation)	8.5 oz. Civitas Turf Defense + 0.075 lbs. H_3PO_3 / M	5.5 a	9.3 a	44.5 a	6.55 ab	1.4 a	171.6 a	29.1 a	24.3 abc	14.9 ab	8.9 abc
3b (in 4-wk rotation)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M										
4a (in 4-wk rotation)	8.5 oz. Civitas Turf Defense	10.8 a	9.5 a	41.5 a	6.48 ab	1.4 a	196.0 a	30.2 a	23.2 abcd	15.4 a	9.7 abc
4b (in 4-wk rotation)	0.25 # S / M + 0.075 lbs. H_3PO_3 / M										
5	0.5 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	19.5 a	9.6 a	41.2 a	6.61 ab	1.2 a	169.3 a	24.3 a	22.8 abcd	14.8 ab	5.2 cd
6	1.0 lbs. $FeSO_4$ / M + 0.075 lbs. H_3PO_3 / M	8.0 a	9.2 a	40.0 a	6.69 ab	1.0 a	117.0 a	24.3 a	18.2 d	9.5 b	5.6 cd
7	0.25 # S / M	16.8 a	9.8 a	42.4 a	6.50 ab	1.3 a	168.5 a	28.7 a	27.5 a	11.4 ab	13.4 a
8	0.075 lbs. H_3PO_3 / M	3.3 a	9.2 a	43.8 a	6.62 ab	1.2 a	142.8 a	23.1 a	21.8 bcd	12.4 ab	3.2 d
9	Fungicide Rotation	4.3 a	8.9 a	43.2 a	6.56 ab	1.2 a	152.3 a	23.2 a	21.7 bcd	9.9 b	3.1 d
10	Non-treated Control	8.8 a	9.2 a	41.2 a	6.73 a	1.1 a	160.9 a	27.2 a	20.9 cd	13.8 ab	3.1 d

[†] H_2O infiltration readings are recorded using a Turf-Tec Infiltrometer for 5 minutes
[‡] Greenspeed is an average of 3 ball roll distances in each direction using the "2X" notch on the USGA Stimpmeter to accommodate a plot size of 8 feet in length.
[§] NDVI ratings are a means of 5 readings using a FieldScout CM 1000 NDVI Meter.
[¶] Mean differences in the same column followed by the same letter are not significantly different according to Dunn's test (alpha ≤ 0.05).
[††] Means in the same column followed by the same letter are not significantly different according to Tukey's HSD (alpha ≤ 0.05).

Future expectations of the project:

In order to better quantify putting green characteristics throughout the trial, the strip-plot fungicide application that took place over the summer months is going to be continued throughout the year. The objective is to be able to quantify green speeds, water infiltration, and NDVI without damage from Microdochium patch. In addition, replicated golfer traffic is only going to take place on the fungicide strip-plot applications because any abiotic damage observed will be able to be quantified without regards to disease. This experiment will continue for at least two more full years for a total of three years of data collection. One advantage of this long-term trial is to observe the effects of these treatments over multiple years on putting green characteristics and their influence on summer disease. Preliminary results are going to be shared at field days and at state, national, and international meetings. At the conclusion of the experiment, a scientific manuscript will be published followed by a trade journal article.

USGA ID#: 2018-20-670

Title: *Sclerotinia homoeocarpa* epidemiology and resistance development as measured through improved molecular detection techniques

Project Leaders: Paul Koch[1], Bruce Clarke[2], Jim Murphy[2], Geunhwa Jung[3], Ning Zhang[2]

Affiliation: [1]Department of Plant Pathology, University of Wisconsin – Madison; [2]Department of Plant Biology and Pathology, Rutgers University; [3]Stockbridge School of Agriculture, University of Massachusetts - Amherst

Year 2 Summary Points:

- Dollar spot sampling sites were continued in New Jersey, Wisconsin, and Massachusetts and sampled repeatedly throughout the 2019 growing season.
- Two assays were developed for quantifying the dollar spot fungus in field samples. The first is a 'digital droplet PCR (ddPCR)' developed at Wisconsin that is new to Plant Pathology and represents a potentially significant advancement for the detection of fungal DNA in field samples. The second is a more common 'quantitative PCR (qPCR)' assay developed at Rutgers and Massachusetts that will provide an assay that nearly any research laboratory would be able to perform without the specialized equipment required of ddPCR. The qPCR methodology developed at Rutgers consistently detects all dollar spot (*Clarireedia)* species present in bentgrass shoot and thatch samples at very low levels, and does not result in false positives.
- The ddPCR protocol developed at Wisconsin was found to be highly sensitive and repeatable in Year 1, however the samples collected in 2019 have yet to be analyzed using due to staff turnover. The samples collected at Wisconsin in 2019 will be analyzed in late 2019 and early 2020.
- A rapid PCR-based assay was developed at Massachusetts that has effectively identified SDHI resistance in a variety of isolates collected from the Northeaster US and Japan.
- Two field studies were initiated at Wisconsin to test 1) the fungal population response to various fungicides with different modes of action and 2) the spatial distribution of the fungus over a small (1 m x 1 m) area in both symptomatic and non-symptomatic conditions. For the first study, samples were collected weekly from the first week of May through the last week of October, and a total of 288 samples were collected. For the second study, samples were collected in mid-May and again in August and totaled 216 samples. The DNA has been extracted from all samples in the first study but extractions are still ongoing in the second study.
- A subsample number of 8 to 16 one-cm diameter x two-cm deep cores were needed to attain a consistent and accurate representation of the pathogen population from a 6 m^2 turf plot in the field.
- The upper ~0.5-cm depth of individual sample cores, which contained lower leaf sheaths, the crown, and upper thatch appeared to be the best portion (subsample) of the core from which to isolate pathogen DNA for turf that had a relatively deep (~2.5 cm) thatch layer.
- qPCR analysis of samples containing leaf sheaths, crowns and 0.5-cm of surface thatch was able to detect a higher pathogen population on Independence creeping bentgrass (highly susceptible cultivar) compared to Declaration (resistant cultivar) when foliar symptoms were present (28 August 2019).

  - However, homogenizing entire core samples that had a ~2.5-cm thick thatch layer resulted in the pathogen not being detected, presumably because DNA was diluted to the point of non-detection using qPCR.
- In a spatial distribution study, the dollar spot pathogen was present at low levels throughout the entire plot before disease was visible.
- A trial was initiated in fall 2019 to test the hypothesis (using the newly developed qPCR methodology) that fall fungicide applications reduce pathogen population and delay disease onset the next spring.

Objective 1: Develop one or more molecular methods to effectively quantify *S. homoeocarpa* in the field and assess the fungal response to host genotypes, and cultural and chemical practices.

Year 2 Goal: Use the effective detection techniques developed in year 1 to conduct field studies in years 2 and 3.

Wisconsin Update:

Field sampling: Two field-sampling plots were established in May of 2019 at the OJ Noer Research and Education Facility in Madison, WI on a 'Penncross' creeping bentgrass stand maintained under fairway conditions. One study researched the impacts of Daconil WeatherStik, Xzemplar, and a non-treated control on the dollar spot population. The study design was a RCND with 4 replications, and 8 subsamples were conducted from within each plot on a weekly basis, pooled using liquid nitrogen, and the DNA extracted using Qiagen DNA extraction kits. Samples were collected weekly from the first week of May through the last week of October, resulting in a total (post-pooling) of 288 samples. The second study researched the spatial distribution of the fungus during both a non-symptomatic period (May) and a post-symptomatic period (August). The experimental area for this study was a 300 by 300 cm grid made up of 36 individual 50 x 50 cm plots. Three of these plots were randomly selected for sampling in May and August, and within each plot a total of 36 samples were collected at evenly spaced 10 cm intervals. The DNA has been extracted from all the samples collected in the fungicide experiment, though due to the departure of a postdoctorial researcher and the need to train a new MS student none of the samples have been analyzed using ddPCR. The DNA has not yet been extracted from all of the samples collected in the spatial distribution study, though they should be complete by the end of the calendar year.

Year 3 Goals: The first and most immediate goal will be to analyze the samples collected in 2019 using ddPCR. This will be complete by the end of January 2020. Following the results we will meet with the co-PIs to discuss alterations to the field experiments for year 3, but the studies conducted in year 2 will largely be replicated in year 3.

Rutgers Update:
Development of new qPCR TaqMan Primers and Probe to detect *Clarireedia* species

The multiple sequence alignment used in Salgado-Salazar et. al 2018 was scanned manually for candidate primers and probe locations. It was determined that ITS1 was the best candidate region for *Clarireedia* genus specific TaqMan probe and primers because of a large *Clarireedia* specific insertion in this region (see designed primers and probe sequence characteristics; Table 1).

Primer,Probe	Sequence	Length	Tm	GC Content
Forward: ITS1	TCCGTAGGTGAACCTGCGG	19	59.5 °C	63.2 %
Reverse: DS_ITS1_R	ACGACACTGACAATTCAGAG	20	52.3 °C	45.0 %
Probe: DS_ITS1_Probe	FAM-GGAGGGCGTGAGGCTGTC-Iowa Black FQ	19	63.6 °C	73.7%

Table 1. *Clarireedia* specific TaqMan probe and primer characteristics.

The TaqMan system design at Rutgers was tested against 31 fungal isolates and one oomycete isolate including the target *Clarireedia* species, fungi phylogenetically closely related to *Clarireedia*, other turfgrass pathogens, and common environmental fungal species isolated from turfgrass (Table 2). The qPCR system was able to consistently detect all four *Clarireedia* species and had no negative control issues (Fig 1). The qPCR system can clearly distinguish Clarireedia from other fungal species with the *Clarireedia* isolates cycle threshold ranging from 12.42 to 16.98, while the non-target isolates mean cycle thresholds were greater than 38.15, well above the minimum cycle detection of 37 cycles that we determined for *Clarireedia* species.

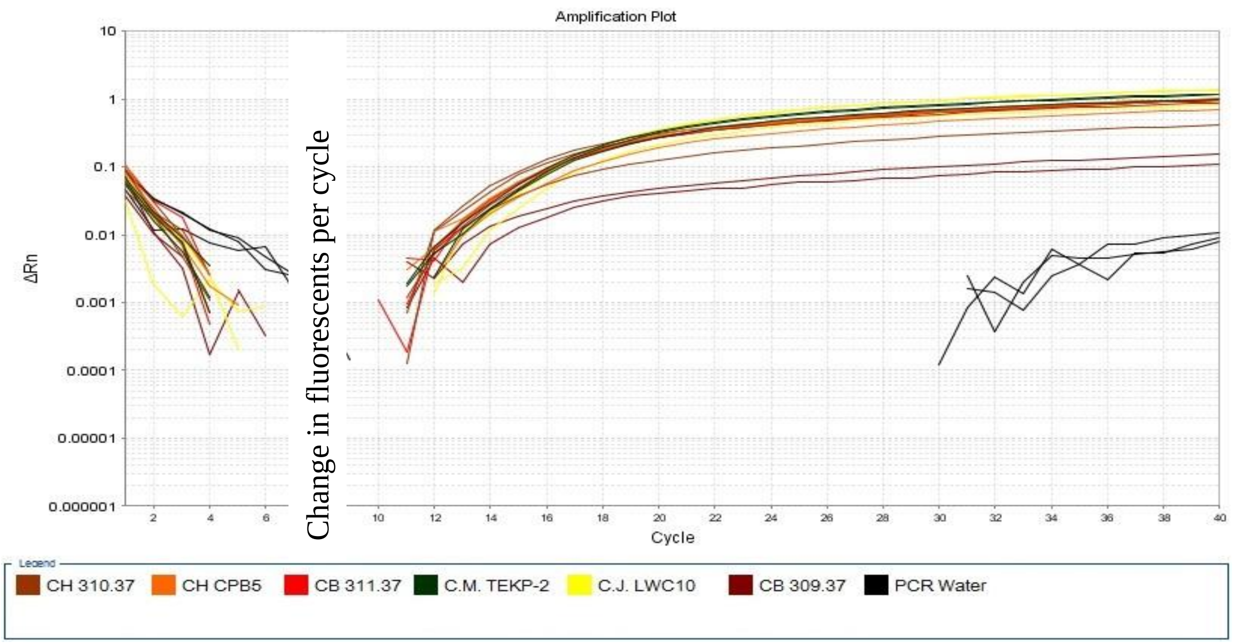


Figure 1. Amplification plot of TaqMan system using *Clarireedia* pure culture isolates and a negative control (PCR water). Quantification was preformed using a StepOnePlus Real-Time PCR System in triplicate.

We ran a 10 fold serial dilution of a *Clarireedia* spp. isolate to create a standard curve that can be used to convert cycle thresholds to DNA concentrations (Fig 2). The method can detect *Clarireedia* DNA concentrations ranging from 3.8(10^{-8}) to 3.8(10^{-14}) grams.

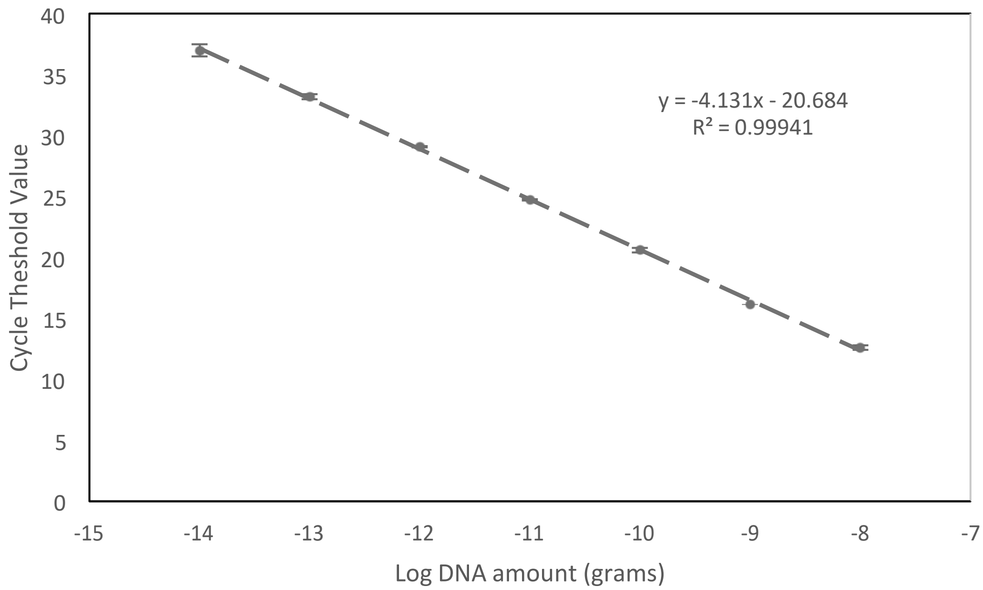


Figure 2. qPCR standard curve of the log of *Clarireedia jacksonii* input genomic DNA concentrations versus the corresponding cycle threshold value. The detection limit for *Clarireedia* was determined to be a Ct of 37 or higher.

Isolate Name	Taxon	Source	Host	Isolate Type[a]	Storage Location	qPCR Mean Ct value (standard deviation)[b]	Detection[c]
ROS16-151	*Cadophora* sp.	NJ, USA	Festuca arundinacea	Environmental	Rutgers	40.144	-
CBS 309.37	*Clarireedia bennettii*	United Kingdom	N/A	Target	USDA	16.416 (0.698)	+
CBS 311.37	*Clarireedia bennettii*	United Kingdom	N/A	Target	USDA	13.666 (0.187)	+
CBS 310.37	*Clarireedia homeocarpa*	United Kingdom	N/A	Target	USDA	13.338 (0.648)	+
CPB-5	*Clarireedia homeocarpa*	United Kingdom	*Festuca rubra*	Target	USDA	13.815 (0.443)	+
LWC10	*Clarireedia jacksonii*	NC,USA	*Agrostis stolonifera*	Target	Rutgers	14.443 (0.421)	+
RCCPG-1	*Clarireedia jacksonii*	NC, USA	*Agrostis stolonifera*	Target	Rutgers	16.981 (0.157)	+
MAFF 235854	*Clarireedia jacksonii*	Japan	*Agrostis stolonifera*	Target	Rutgers	14.059 (0.584)	+
SH80	*Clarireedia jacksonii*	Canada	*Agrostis stolonifera*	Target	Rutgers	16.527 (0.328)	+
RE18G-38	*Clarireedia jacksonii*	NC, USA	*Agrostis stolonifera*	Target	Rutgers	12.422 (0.805)	+
D19	*Clarireedia jacksonii*	OH, USA	*Poa pratensis*	Target	Rutgers	15.438 (0.413)	+
DRR9	*Clarireedia monteithiana*	Dominican Republic	*Paspulum vaginatum*	Target	Rutgers	15.378 (0.141)	+
RB19	*Clarireedia monteithiana*	MS, USA	*Cyodon dactylon x transvaalensis*	Target	Rutgers	16.181 (0.392)	+
TEKP-2	*Clarireedia monteithiana*	HI, USA	*Paspulum vaginatum*	Target	Rutgers	13.943 (0.173)	+
MAFF 236938	*Clarireedia monteithiana*	Japan	*Cyodon dactylon*	Target	Rutgers	14.647 (0.241)	+
247J	*Colletotrichum cereale*	NJ, USA	*Poa pratensis*	Turf Pathogen	Rutgers	39.487 (0.155)	-
M11	*Cortinarius avreilolius*	NJ, USA	Mushroom	Environmental	Rutgers	41.142 (0.266)	-
ROS16-1	*Fusarium oxysporum*	NJ, USA	*Festuca arundinacea*	Environmental	Rutgers	39.962 (1.024)	-
CBS 774.95	*Lambertella corni-maris*	Croatia	*Cornus mas*	Closely Related	USDA	40.000	-
ROS16-109	*Leptodontidium* sp.	NJ, USA	*Festuca arundinacea*	Environmental	Rutgers	39.366 (3.435)	-
ROS16-55	*Magnaporthiopsis meyeri-fectucae*	NJ, USA	*Festuca arundinacea*	Turf Pathogen	Rutgers	39.147 (1.753)	-
251a	*Microdochium* sp.	NJ, USA	*Poa annua*	Turf Pathogen	Rutgers	38.579 (1.091)	-
ROS16-121	*Pleosporales* sp.	NJ, USA	*Festuca arundinacea*	Environmental	Rutgers	39.706 (0.665)	-
ROS16-140	*Pyrenochaetopsis* sp.	NJ, USA	*Festuca arundinacea*	Environmental	Rutgers	38.905 (2.17)	-
128J	*Pythium* sp.	NJ, USA	*Agrostis stolonifera*	Turf Pathogen	Rutgers	40.280 (1.501)	-
AF14	*Rhizoctania solanii*	NJ, USA	*Panicum virgatum*	Turf Pathogen	Rutgers	41.078 (0.906)	-
IA	*Rhizoctania* sp.	IA, USA	*Panicum virgatum*	Turf Pathogen	Rutgers	40.310	-
CBS 341.62	*Rustroemia firma*	France	N/A	Closely Related	USDA	38.174 (1.592)	-
ROS16-149	*Sacrocladium strictum*	NJ, USA	*Festuca arundinacea*	Environmental	Rutgers	40.602	-
CBS 112.17	*Sclerotinai minor*	Netherlands	*Lactus sativa*	Closely Related	USDA	38.342 (1.605)	-
CBS 111.17	*Sclerotinia matthiolae*	Switzerland	*Matthiola vallesiaca*	Closely Related	USDA	38.155 (2.872)	-
M14	*Thelephora terrostus*	NJ, USA	Mushroom	Environmental	Rutgers	39.076 (2.869)	-

Table 2. Isolate name, taxon name, source, host, isolate type, storage location, qPCR results and detection for the isolates used in the development of this assay.

[a] Environmental - Isolates found in the turfgrass system, Target - Isolates that the primers were designed to amplified, Turf Pathogen - Isolates that cause other turfgrass diseases, Closely Related - Isolates that are phylogenetically closely related to the target isolates

[b] Cycle Thresholds for single technical replicate. Other technical replicates were undetected.

[c] Reactions with no fluorescents or mean Ct values >37 are considered negative

Results from Year 1 Field Sampling Experiment

A field-sampling study was established in May of 2018 at Hort Farm No. 2 in North Brunswick, NJ on 'Barracuda' creeping bentgrass maintained under fairway conditions. One half of the 6 m x 6 m plot was inoculated in May with 50 grams oats infested with two isolates of *Clarireedia* jacksonii isolates provided by Rutgers and the other half of the plot remained non-inoculated. A modified sampler (hollow golf club shaft) provided by Rutgers was used to obtain one cm diameter x two cm deep cores containing plant tissue and soil from the plots. Samples were taken weekly in May then once per month from June through October 2018. At each sampling date, 62 cores were collected (31 from each half) from the plot and immediately frozen at -20°C until DNA extraction could occur.

Pooling experiment

DNA was isolated from cores (bulked in the following numbers: 16, 8, 4, 2, and 1) to determine how many cores needed to be combined to get a consistent representation of *Clarireedia* in the field. We analyzed only the non-inoculated thatch samples for this experiment (Fig 3). In general DNA amplification of the pooled samples was low in the beginning of May, steadily built up to the highest pathogen population in August, and then rapidly declined in September. The single core sample did not amplify pathogen DNA for three dates, did not follow visual disease development in the field, and amplification was extremely variable for the other sampling dates. Combining two or four cores performed better, amplifying *Clarireedia* DNA for all sampling dates and generally following the disease in the field; however, variability was still a problem. Eight and Sixteen cores were the most consistent but both had one date in which they did not amplify DNA. From this study we suggested to take somewhere between 8 and 16 cores are needed to get the most consistent and accurate representation of the pathogen population in the field.

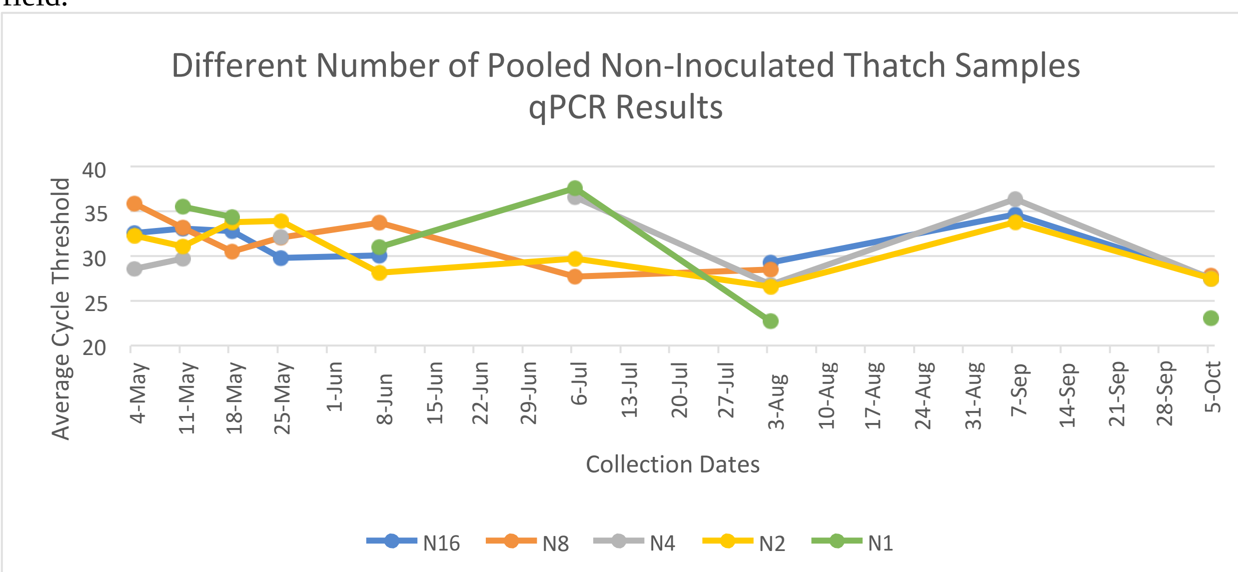


Figure 3. qPCR cycle threshold result from pooling different number of non-inoculated thatch cores on different collection dates over the growing season

Amplification of DNA in Leaf and Thatch Layers

The Rutgers group decided to separate non-inoculated and inoculated cores into a leaf and thatch layer to see if there were differences in pathogen abundance between the two layers. We analyzed the 16 combined set of samples only because they gave us the best results from the pooling experiment (Fig 4). The results showed that the leaf layer had consistently higher pathogen populations and was more consistently detected compared to the thatch layer.

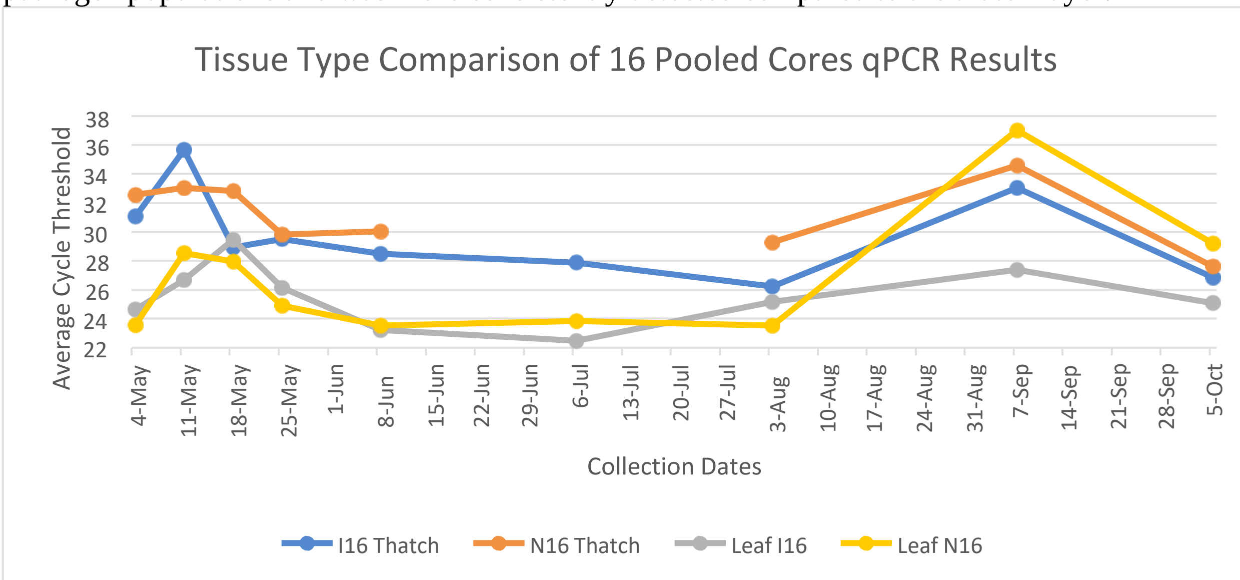


Figure 4. qPCR cycle threshold results from non-inoculated (N) and inoculated (I) leaf and thatch samples using 16 pooled cores from different collection dates over the growing season.

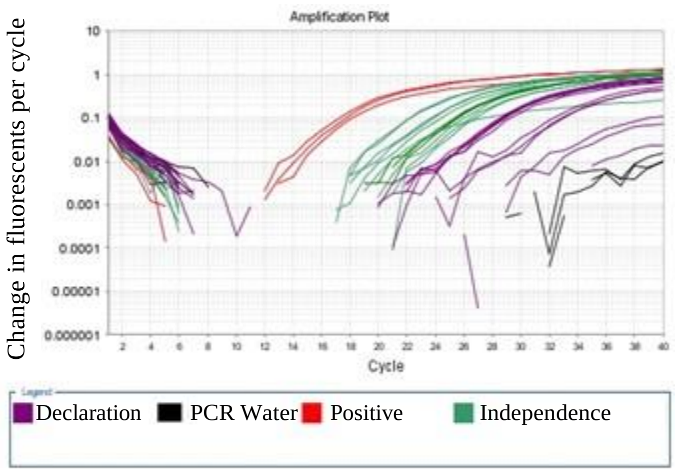
Cultivar Experiment: Five-year old field plots of two different creeping bentgrass cultivars Independence (a dollar spot susceptible cultivar) and Declaration (a dollar spot tolerant cultivar) were sampled weekly throughout the season to test the hypothesis that different cultivars have different pathogen populations that can be detected using our TaqMan qPCR method. Eight samples were taken from a randomly chosen 0.09 m^2 area inside each 0.91 m by 1.52 m plot and replicated four times. Sampling started on 1 May and disease ratings of the entire plot and the 0.09 m^2 area were collected along with the samples. Samples were collected almost every Monday, Wednesday and Friday for the month of May. Once disease developed (30 May) samples were taken weekly until 28 August. Collection dates were 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, and 24 May for asymptomatic samples and 30 May, 5, 12, 19, and 26 June, 3, 10, 17, 24, and 31 July, and 7, 14, 21, and 28 August for symptomatic samples. The eight cores taken from each plot were homogenized using liquid nitrogen then a portion (0.25 g) of the homogenized sample was used for DNA isolation. The samples that were homogenized included samples taken on 1, 8, 15, 22, and 30 May, 5, 12, and 26 June, 3, 10, 17, 24, and 31 July, and 7, 14, and 21 August. The eight samples from 8/28 were homogenized using only leaf tissue. The DNA samples were analyzed using the TaqMan qPCR method developed by Rutgers.

I1[a]	Ct	I2[a]	Ct	I3[a]	Ct	D1[b]	Ct	D2[b]	Ct	D3[b]	Ct
I1 5/1	ND	I2 5/1	ND	I3 5/1	ND	D1 5/1	ND	D2 5/1	ND	D3 5/1	ND
I1 5/1	ND	I2 5/1	ND	I3 5/1	ND	D1 5/1	ND	D2 5/1	ND	D3 5/1	ND
I1 5/1	ND	I2 5/1	ND	I3 5/1	ND	D1 5/1	ND	D2 5/1	ND	D3 5/1	ND
I1 5/8	ND	I2 5/8	ND	I3 5/8	ND	D1 5/8	32.764	D2 5/8	ND	D3 5/8	ND
I1 5/8	ND	I2 5/8	33.89	I3 5/8	ND	D1 5/8	33.092	D2 5/8	ND	D3 5/8	ND
I1 5/8	ND	I2 5/8	ND	I3 5/8	ND	D1 5/8	32.9	D2 5/8	ND	D3 5/8	ND
I1 5/15	ND	I2 5/15	ND	I3 5/15	ND	D1 5/15	ND	D2 5/15	ND	D3 5/15	ND
I1 5/15	ND	I2 5/15	ND	I3 5/15	ND	D1 5/15	ND	D2 5/15	ND	D3 5/15	ND
I1 5/15	ND	I2 5/15	ND	I3 5/15	ND	D1 5/15	ND	D2 5/15	ND	D3 5/15	ND
I1 5/22	ND	I2 5/22	ND	I3 5/22	28.902	D1 5/22	ND	D2 5/22	ND	D3 5/22	ND
I1 5/22	ND	I2 5/22	ND	I3 5/22	29.207	D1 5/22	ND	D2 5/22	ND	D3 5/22	ND
I1 5/22	ND	I2 5/22	ND	I3 5/22	28.948	D1 5/22	ND	D2 5/22	ND	D3 5/22	ND
I1 5/30	ND	I2 5/30	ND	I3 5/30	ND	D1 5/30	ND	D2 5/30	ND	D3 5/30	ND
I1 5/30	ND	I2 5/30	ND	I3 5/30	ND	D1 5/30	ND	D2 5/30	ND	D3 5/30	ND
I1 5/30	ND	I2 5/30	ND	I3 5/30	ND	D1 5/30	ND	D2 5/30	ND	D3 5/30	ND
I1 6/5	ND	I2 6/5	ND	I3 6/5	ND	D1 6/5	ND	D2 6/5	ND	D3 6/5	ND
I1 6/5	ND	I2 6/5	ND	I3 6/5	ND	D1 6/5	ND	D2 6/5	ND	D3 6/5	ND
I1 6/5	ND	I2 6/5	ND	I3 6/5	ND	D1 6/5	ND	D2 6/5	ND	D3 6/5	ND
I1 6/12	ND	I2 6/12	ND	I3 6/12	ND	D1 6/12	ND	D2 6/12	ND	D3 6/12	ND
I1 6/12	ND	I2 6/12	ND	I3 6/12	ND	D1 6/12	ND	D2 6/12	ND	D3 6/12	ND
I1 6/12	ND	I2 6/12	ND	I3 6/12	ND	D1 6/12	ND	D2 6/12	ND	D3 6/12	ND
I1 6/19	26.854	I2 6/19	24.325	I3 6/19	36.429	D1 6/19	ND	D2 6/19	ND	D3 6/19	28.121
I1 6/19	26.02	I2 6/19	24.249	I3 6/19	ND	D1 6/19	ND	D2 6/19	ND	D3 6/19	27.575
I1 6/19	27.156	I2 6/19	24.791	I3 6/19	ND	D1 6/19	ND	D2 6/19	ND	D3 6/19	28.517
I1 6/26	ND	I2 6/26	ND	I3 6/26	ND	D1 6/26	ND	D2 6/26	ND	D3 6/26	ND
I1 6/26	ND	I2 6/26	ND	I3 6/26	ND	D1 6/26	ND	D2 6/26	ND	D3 6/26	ND
I1 6/26	ND	I2 6/26	ND	I3 6/26	ND	D1 6/26	ND	D2 6/26	ND	D3 6/26	ND
I1 7/3	ND	I2 7/3	ND	I3 7/3	ND	D1 7/3	36.185	D2 7/3	ND	D3 7/3	ND
I1 7/3	ND	I2 7/3	ND	I3 7/3	ND	D1 7/3	34.104	D2 7/3	ND	D3 7/3	ND
I1 7/3	ND	I2 7/3	ND	I3 7/3	ND	D1 7/3	ND	D2 7/3	ND	D3 7/3	ND
I1 7/10	ND	I2 7/10	ND	I3 7/10	ND	D1 7/10	ND	D2 7/10	ND	D3 7/10	ND
I1 7/10	ND	I2 7/10	ND	I3 7/10	ND	D1 7/10	ND	D2 7/10	ND	D3 7/10	ND
I1 7/10	ND	I2 7/10	ND	I3 7/10	ND	D1 7/10	ND	D2 7/10	ND	D3 7/10	ND
I1 7/17	ND	I2 7/17	ND	I3 7/17	ND	D1 7/17	29.112	D2 7/17	ND	D3 7/17	ND
I1 7/17	ND	I2 7/17	ND	I3 7/17	ND	D1 7/17	28.976	D2 7/17	ND	D3 7/17	ND
I1 7/17	ND	I2 7/17	ND	I3 7/17	ND	D1 7/17	28.956	D2 7/17	ND	D3 7/17	ND
I1 7/24	25.167	I2 7/24	ND	I3 7/24	32.044	D1 7/24	ND	D2 7/24	28.323	D3 7/24	27.952
I1 7/24	24.586	I2 7/24	ND	I3 7/24	32.836	D1 7/24	ND	D2 7/24	28.305	D3 7/24	27.703
I1 7/24	ND	I2 7/24	32.38	I3 7/24	32.863	D1 7/24	ND	D2 7/24	28.157	D3 7/24	27.322
I1 7/31	ND	I2 7/31	ND	I3 7/31	ND	D1 7/31	ND	D2 7/31	28.292	D3 7/31	31.409
I1 7/31	ND	I2 7/31	ND	I3 7/31	ND	D1 7/31	ND	D2 7/31	28.469	D3 7/31	31.009
I1 7/31	ND	I2 7/31	ND	I3 7/31	ND	D1 7/31	ND	D2 7/31	28.099	D3 7/31	31.006
I1 8/7	27.808	I2 8/7	31.513	I3 8/7	ND	D1 8/7	ND	D2 8/7	ND	D3 8/7	24.23
I1 8/7	27.188	I2 8/7	30.984	I3 8/7	ND	D1 8/7	ND	D2 8/7	ND	D3 8/7	23.504
I1 8/7	27.282	I2 8/7	30.972	I3 8/7	ND	D1 8/7	ND	D2 8/7	ND	D3 8/7	23.756

Figure 5. qPCR results of the cultivar experiment.

[a] I1, I2 and I3 are the qPCR result using DNA isolated from the Independence cultivar replication 1, 2, and 3, respectfully [b] D1, D2 and D3 are the qPCR result using DNA isolated from the Declaration cultivar replication 1, 2, and 3, respectfully

The qPCR results from the cultivar experiment were able to amplify a total of 49 out of 270 qPCR reactions and not detection (ND) 221 qPCR reactions. The reason why the qPCR results were so poor was because the thatch layer in this field was approximately 2.5 cm in depth and, when the eight cores were homogenized, the majority of the ground sample was thatch which diluted pathogen DNA in the samples. Analysis of only leaf tissue from the 28 August sampling resulted in detection of the pathogen in all samples (24 out of 24 qPCR reactions). The results indicated a difference in the pathogen population between the two cultivars. Independence (the more susceptible cultivar), had a higher pathogen population than Declaration (the more tolerant cultivar) (Fig 6). Unfortunately, we homogenized all the samples we took weekly from 1 May to 21 August so we can't re-isolate DNA using just the leaf tissue from these samples. We plan to analyze the remaining asymptomatic samples (3, 6, 10, 13, 17, 20, and 24 May) for pathogen DNA in the leaf tissue to test the hypothesis that there are differences in pathogen populations between the two cultivars before disease symptoms are seen. This experiment will be repeated in 2020.



Positive 15.694618
Negative ND
I1 8/28 [a] 24.40357
I2 8/28 [a] 23.383947
I3 8/28 [a] 21.147797
I4 8/28 [a] 21.837378
D1 8/28[b] 26.828161
D2 8/28[b] 30.514933
D3 8/28[b] 34.910202
D4 8/28[b] 26.56707

Figure 6. Amplification plot of TaqMan system using DNA isolated from leaf samples collected on 28 August, a pure culture of *Clarireedia jacksonii* (positive control), and PCR water (a negative control). The average cycle thresholds for the samples (right of graph). The detection limit for *Clarireedia* was determined to be a Ct of 37 or higher. Quantification was preformed using a StepOnePlus Real-Time PCR System in triplicate.

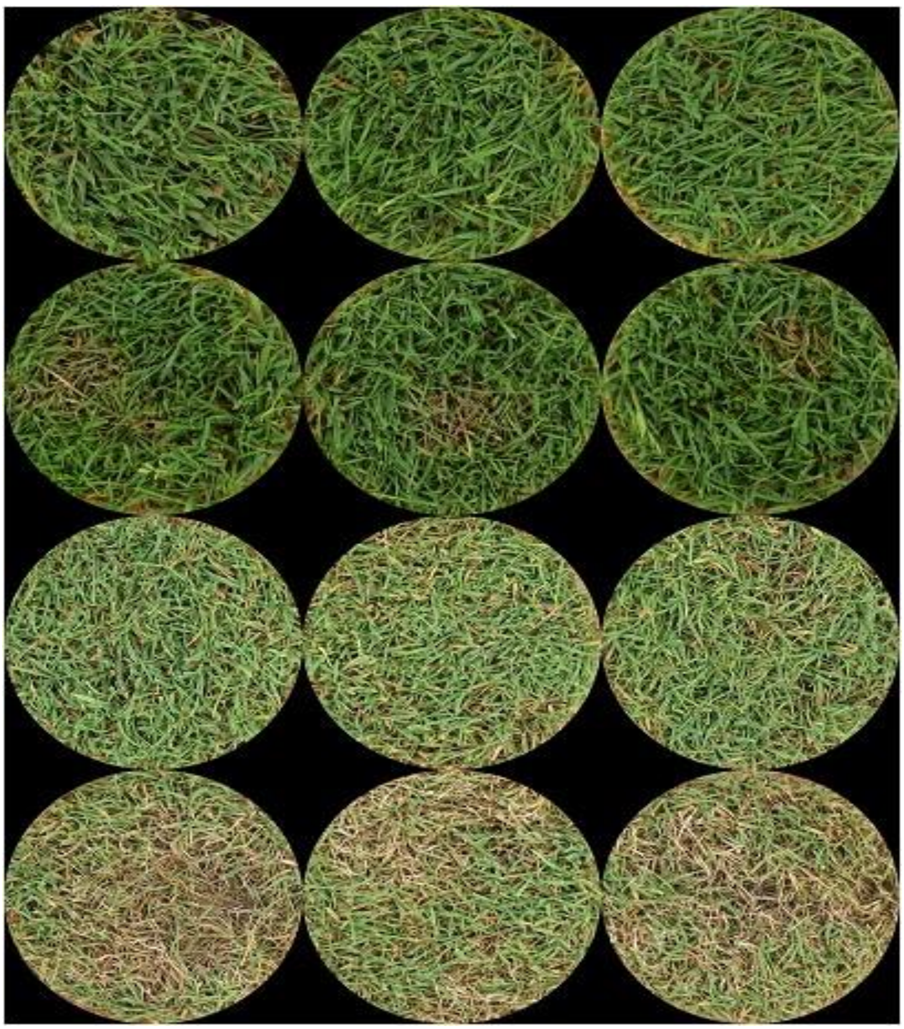
[a] I1, I2, I3 and I4 are the qPCR result using DNA isolated from the Independence cultivar replication 1, 2, 3, and 4, respectfully

[b] D1, D2 and D3 are the qPCR result using DNA isolated from the Declaration cultivar replication 1, 2, 3, and 4, respectfully

Environmental Sample Validation: We evaluated the effectiveness of the TaqMan primers and probe on field cores (environmental samples) taken to quantify the abundance of the pathogen in foliar tissue, thatch and soil layers during different levels of disease severity in the field. Cores (7.6 cm diameter x 7.6 cm deep) were taken from a stand of 'Independence' creeping bentgrass with varying amount of diseased leaf tissue: no visible symptoms (-), slightly diseased (+), and severely diseased (++). Three cores with no visible disease (-) and slightly diseased (+) samples were taken on 19 June 2019. Another set of three cores of no visible disease (-) and severely diseased (++) were taken on 16 August 2019. Photos of the 12 cores can be seen in Fig 7.

The pathogen was consistently detected in the foliar tissue; *Clarireedia* was positively detected in 11 of 12 cores (Table 3). The cycle thresholds (pathogen population) observed for foliar tissue were consistent with the severity of the visual symptoms observed; the lowest pathogen populations were detected when there were no visual disease symptoms on the samples, a higher pathogen population was found on slightly diseased samples, and the highest pathogen population was observed in foliar tissue of severely diseased samples. Interestingly, cores with no visual disease had a higher pathogen population in the foliar when sampled in August compared to June.

The detection of pathogen populations in the thatch and soil layers was inconsistent; *Clarireedia* was positively detected in only five and seven out of twelve samples reached the cycle threshold for thatch and soil layer, respectfully. Moreover, the pathogen populations were low in the thatch and soil layers and were not consistent with the visual symptoms on the cores; slightly diseased samples had a higher pathogen population than the severely diseased samples. The qPCR technique was also unable to detect the pathogen from either the thatch or soil layers from cores with no visual disease.



June - No Disease (-)

June – Slightly Diseased (+)

August - No Disease (-)

August - Severely Diseased (++)

Figure 7. Environmental 7.6 cm diameter cores with different intensities of dollar spot disease

Sample	Disease Severity	Collected	Foliar	Thatch[a]	Soil[a]	Positive Control[b]	Negative Control[a,b]
1	-	6-19-19	36.330 (0.942)	ND	ND		ND
2	-	6-19-19	33.905 (0.415)	ND	39.154 (1.692)		ND
3	-	6-19-19	33.793 (0.332)	37.960 (0.624)	ND		ND
4	+	6-19-19	21.079 (0.165)	30.280 (0.023)	36.316 (0.367)	15.536	
5	+	6-19-19	21.683 (0.113)	30.548 (0.497)	32.682 (0.319)	15.485	
6	+	6-19-19	22.152 (0.171)	34.203 (0.264)	34.280 (0.352)	15.522	
7	-	8-16-19	29.296 (0.508)	ND	ND		ND
8	-	8-16-19	31.508 (0.267)	ND	ND		ND
9	-	8-16-19	38.020	ND	ND		ND
10	++	8-16-19	18.414 (0.806)	ND	35.909 (0.903)	16.274	
11	++	8-16-19	18.921 (0.217)	36.086 (0.156)	36.660 (1.388)	16.995	
12	++	8-16-19	17.981 (0.292)	ND	36.211 (0.753)	16.397	

Table 3. qPCR mean cycle thresholds of the environmental sample foliage, thatch, and soil layers (standard deviation). The detection limit for *Clarireedia* was determined to be a Ct of 37 or higher.

[a] ND stands for Not Detected.
[b] Technical replicate cycle thresholds value

The data shows that the soil and thatch layers inconsistently detected the pathogen population and at very low levels. This indicated that the pathogen is not widely distributed in the soil or thatch layer and primarily lives in the foliar layer. It was interesting to see that the foliar tissue with no visual disease from June and August had different pathogen populations, with the August sample having a higher pathogen population. This suggests that the foliar tissue can tolerate a certain pathogen population before disease symptoms are observed. Our data can't pinpoint the exact pathogen population when visual symptoms occur, but if would appear to be between a mean cycle threshold of 29.269 and 22.151. More data is needed to determine whether the approximate cycle threshold for foliar symptoms is cultivar specific or varies with different treatments.

Spatial Distribution: Groups at Rutgers and the University of Wisconsin ran concurrent spatial distribution projects to see how the pathogen is distributed in the field before and after dollar spot disease develops. The design included 25, 60 cm by 60 cm plots within a large stand of Independence creeping bentgrass. Samples were collected before disease was visible on 1 May 2019 and on 24 August 2019 when disease was severe (Fig 8). Each 60 cm by 60 cm plot was fitted with a 50 cm by 50 cm grid with 36 evenly spaced intersects (10 cm apart). A core sample (1 cm in diameter x 2.5 cm deep) was collected at each intersect. A 5 cm unsampled border was placed around the grid to avoid sampling the same spot twice between neighboring plots. DNA was isolated from the leaf and thatch layers of each individual core. To date, qPCR analysis has only been completed for the first replication of the cores taken on 1 May (no disease) and 24 August (severely diseased cores) (Table 4). The results indicate that dollar spot pathogen is present at low levels throughout the entire plot. When disease developed, the pathogen population increased dramatically causing a lower cycle threshold as seen in the cycle threshold result of the 4,3 August (Fig 8). The molecular method result corresponds very well with the after sampling picture; the clearly diseased samples had lower cycle thresholds. The remaining two replications will be processed and photos taken of individual cores to correlate disease intensity and the qPCR results.

Sample	Average May Ct	August Avg Ct
0,0	32.86586	Not Detected
0,1	36.161453	25.25122
0,2	31.215263	26.35146
0,3	Not Detected	30.526
0,4	37.179573	33.57336
0,5	37.623226	33.83912
1,0	31.426346	35.28621
1,1	38.00367	26.44865
1,2	35.877373	35.67071
1,3	29.891277	29.10096
1,4	34.38374	27.09596
1,5	27.872078	33.55903
2,0	31.765799	33.1535
2,1	36.611237	35.11998
2,2	31.271355	29.8335
2,3	33.762054	23.46434
2,4	33.369053	25.85931
2,5	36.510098	32.28109
3,0	33.85456	32.73354
3,1	33.542255	26.92315
3,2	35.403027	32.0234
3,3	31.439627	31.59448
3,4	33.01104	30.2365
3,5	31.97905	37.13607
4,0	Not Detected	35.13628
4,1	34.741955	33.22849
4,2	33.839695	36.33553
4,3	Not Detected	23.7961
4,4	30.26793	31.42788
4,5	33.76984	34.37654
Negative	Not Detected	Not Detected
Positive	13.690976	14.0886

Table 4. qPCR mean cycle threshold results of the first replication of the samples collected on 5/1/19 and 8/24/19. Sample corresponds with the location of the intersection in Figure 8. The detection limit for *Clarireedia* was determined to be a Ct of 37 or higher

Figure 8. Top- Photo taken after sampling the 36 evenly separated (10 cm) intersection of the 5/1/19 first replication. Bottom- After photo of the samples taken on 8/24/19 of the first replication with the sample labels that correspond with Table 4

Fall Fungicide Experiment: Empirical and some experimental observations suggest that fall applications of fungicides can delay disease onset the next spring. A trial was initiated to test the hypothesis that fall fungicide applications reduce the pathogen population and delay disease onset the next spring. Specifically, we are looking at the impact of the timing of fall fungicides applications, the number of applications, and fungicide mode of action on the dollar spot response the next spring. The trial was established in September 2019 on a one-year old stand of 007 creeping bentgrass that had not received fungicide control for several months. Plots were 0.91 m by 1.52 m and replicated 4 times using a randomized complete block design. The ten treatments to be evaluated in the study are summarized in Table 5. A tank mixture of fluazinam (Secure 4.2SC) + propiconazole (Banner MAXX 1.3MC) applied at 0.8 kg a.i. ha^{1} (0.5 fl. oz product 1,000 ft^{2}) and 0.49 kg a.i. ha^{1} (1.0 fl. oz product 1,000 ft^{2}), respectfully, will be applied on a 21 day interval, to test the effect of fungicide timing and number of applications on delaying disease onset in the spring (treatments 1-9). Chlorothalonil (Daconil Ultrex 82.5WG) will be applied at 12.6 kg a.i. ha^{1} (5.0 fl. oz. product 1,000 ft^{2}) on a 21 day interval to determine if fungicide mode of action has an effect on delaying disease onset the following spring (treatment 10).

	Treatment Description					
Treatment Number	Initial Spray[1]	Number of Sprays	End Date	Tank Mix Fungicide Timing[2]		
1	none	0	Non-treated			
2	Sep. 10	0	Non-treated			
3	Sep. 10	1	Nov. 5			Nov. 5
4	Sep. 10	2	Nov. 5		Oct. 15	Nov. 5
5	Sep. 10	2	Nov. 5	Sep. 24		Nov. 5
6	Sep. 10	3	Nov. 5	Sep. 24	Oct. 15	Nov. 5
7	Sep. 10	1	Oct. 15		Oct. 15	
8	Sep. 10	2	Oct. 15	Sep. 24	Oct. 15	
9	Sep. 10	1	Sep. 24	Sep. 24		
				Chlorothalonil Timing[3]		
10	Sep. 10	3	Nov. 5	Sep. 24	Oct. 15	Nov. 5

Table 5. Fall fungicide treatment and application schedule

[1] fluazinam (Secure 4.2SC) applied at 0.8 kg a.i. ha^{1} (0.5 fl. oz product 1,000 ft^{2})
[2] Tank mix of fluazinam (Secure 4.2SC) + propiconazole (Banner MAXX 1.3MC) applied at 0.8 kg a.i. ha^{1} (0.5 fl. oz product 1,000 ft^{2}) and 0.49 kg a.i. ha^{1} (1.0 fl. oz product 1,000 ft^{2}), respectively
[3] Chlorothalonil (Daconil Ultrex) applied on 24 Sept., 15 Oct., and 5 Nov. at 12.6 kg a.i. ha^{1} (5.0 fl. oz. product 1,000 ft^{2}).

Ten, 1 cm diameter x 2.5 cm deep cores were collected for all the treatments (1-10) on 23 September (after the initial spray) and 11 November (after the final spray) to quantify the pathogen populations at the beginning and end of the treatment period. Samples were also collected on 14 October and 4 November for treatments 1, 6 and 10 to determine the effects of repeated fungicide applications on dollar spot populations. Samples will also be collected from all the treatments (1-10) in the spring before disease symptoms appear. Cores will be taken from a randomly chosen 0.09 m^2 area inside each 0.91 m by 1.52 m plot. Samples will be sectioned into the leaf layer and thatch (0.5 cm) and DNA of *Clarireedia* will be analyzed using the qPCR system developed at Rutgers to determine the effect of treatment on the pathogen population. The qPCR results will be used to potentially predict which plots will get dollar spot symptoms first in the spring (lowest mean cycle threshold) and which plots will have delayed dollar spot development (highest mean cycle threshold).

Finalizing Sampling Protocol: Based on our preliminary data, *Clarireedia* appears to be most consistently detected in foliage compared to thatch and soil tissue. To confirm this hypothesis, we plan to take 1 cm diameter x 2.5 cm deep cores in 2020 and analyze segments (foliage, surface thatch [consisting of lower leaf sheaths, the crown, and upper thatch], and lower thatch) over the course of the growing season to determine where *Clarireedia* is most consistently detected. Once this is determined, we will concentrate on this zone for future pathogen population studies.

Year 3 Goals We plan to finish analyzing the core samples collected in 2019 from the spatial distribution, cultivar, and fall fungicide projects. We will also be continuing the cultivar and fall fungicide experiment in 2020.

Massachusetts Update:

Field sampling and storage of samples From June to September 2019, thatch samples from fairway turf mixed bentgrass and *Poa annua* were collected from ammonium sulfate treated/untreated area at the Joseph Troll Turf Research Center in S. Deerfield. Additionally, dollar spot infected (DSI) thatch and leaf samples were collected from putting greens. Collected samples were stored in liquid nitrogen for transport to our lab on the UMass Amherst campus. Upon arrival, the samples were stored at -80 degrees.

Two genes of interest, elongation factor alpha (EF1α) and actin gene (*Shact*), were selected for *C. homoeocarpa* based on the information provided in Allen-Perkin et al. (2018) and Hulvey et al. (2012). Primers for these genes were selected based on these references and the annealing temperature for both genes was determined to be 60°C. Total DNA was extracted from each sample using a DNeasy PowerSoil Kit and prepared for qPCR with QIAGEN QuantiTect SYBR Green RT-PCR Master Mix. All extracted DNA samples were analyzed in technical triplicates for each gene using a QIAGEN Rotor-Gene Q RT-PCR cycler. A nuclease-free water blank was used as a negative control. Cycler parameters for qPCR were are as follows: hold of 95 °C for 5 min, 40 cycles of 95 °C for 5 s, and 60 °C of combined annealing and extension for 10 s. For compassion with DNA, total RNA of leaf samples was extracted using QIAGEN RNeasy Plant Mini Kit. Total RNA of thatches was extracted from each sample using QIAGEN RNeasy PowerSoil Kit. All the RNA samples were reverse transcripted using QuantiTect Reverse Transcription Kit. Each cDNA sample was standardized by adjusting Ct value of *Sh*act is around 31. All extracted RNA samples were analyzed in technical triplicates for each gene using a QIAGEN Rotor-Gene Q RT-PCR cycler. A nuclease-free water blank was used as a negative control. Cycler parameters for qPCR were as follows: hold of 95 °C for 5 min, 40 cycles of 95 °C for 5 s, and 60 °C of combined annealing and extension for 10 s. Critical threshold (Ct) values were recorded at a threshold value set to 0.02.

Figures 9a and 9b show the fold change of *C. homoeocarpa* inoculum and expression between leaves and thatches. Error bars were used to represent the standard deviation of replicates.

Figure 9a

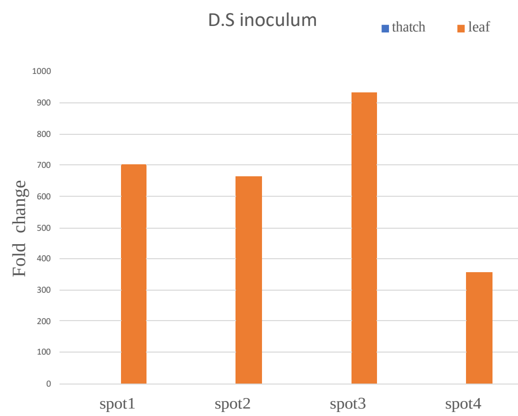


Figure 9b

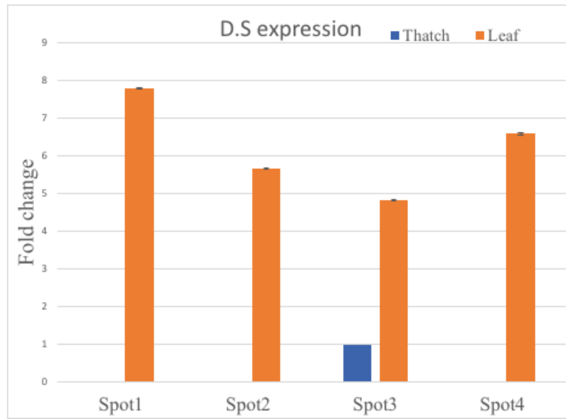


Figure 9. a) Graph of average Ct values obtained through *ShEF1a* gene analysis from dollar spot infected (DSI) thatch and leaf samples on four collection locations. B) Graph of average Ct values fold change obtained through *ShEF1a* and *Shact* genes analysis from dollar spot infected (DSI) thatch and leaf samples on four collection locations.

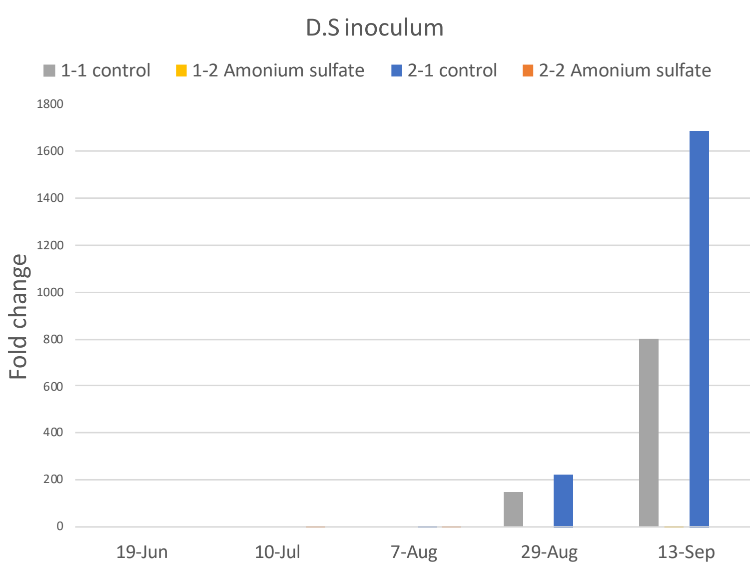


Figure 10. Graph of average Ct values obtained through ***ShEF1a* gene** analysis from thatch samples on five collection dates.

Results:

In general, there are more than four hundred times *C. homoeocarpa* inoculum in leaves than in thatches and the expression of EF1a is five times higher in leaves than in thatches. There is a significant correlation between *C. homoeocarpa* inoculum and expression. The variation between samples from different spots may have been due to sampling variations and leaf residue in thatches.

Additionally, according to Figure 10, dollar spot inoculum increased from the end of August. And the ammonium sulfate treatment inhibited the increase of dollar spot compared with untreated samples.

Objective 2: Determine impact of fungicide class and number of applications on the development of fungicide resistance in *S. homoeocarpa* populations through *in vitro* assays and molecular techniques.

Year 2 Goal: Develop an effective molecular technique for detecting fungicide resistance (five SDHI mutations) within *S. homoeocarpa* populations that can be used in field studies in years 2 and 3 of the grant.

Massachusetts Update:

In vitro sensitivity assays of *S. homoeocarpa* field resistant and sensitive isolates in Table 6 were carried out for 4 SDHI active ingredients at a single discriminatory concentration (1,000 ug ml^{-1}). Discriminatory concentrations were selected based on preliminary screening results that showed consistent growth differences between sensitive and resistant isolates. Fungicide-amended PDA (Potato dextrose agar medium) plates and non-amended PDA plates were inoculated in the center with Agar plugs (5 mm diameter) from fungal colonies of field resistant isolates previously collected from several golf courses and Rutgers University. These isolates are listed in Table 6.

After three days of incubation, two perpendicular diameters of mycelial growth were measured using a 16EX digital caliper (Mahr). Relative mycelial growth (RMG) % values of each isolate were then calculated by dividing the diameter of colonies grown on SDHI amended PDA by the diameter of those grown on non-amended PDA and multiplying by 100.

For rapid and accurate detection of SDHI mutations in resistant field isolates, we have developed a molecular diagnostic system that uses a PCR-based molecular marker known as CAPS (Cleaved Amplified Polymorphic Sequence) and dCAPS (derived Cleaved Amplified Polymorphic Sequence) for 5 SDHI mutations. The primers and the restriction enzymes for each SDHI mutation used for this study were in Table 7. An example of this molecular diagnostic test is included in Figure 11, which displays the banding pattern of PCR amplicons of field isolates with five different SDHI mutations after having been digested with specific restriction enzymes.

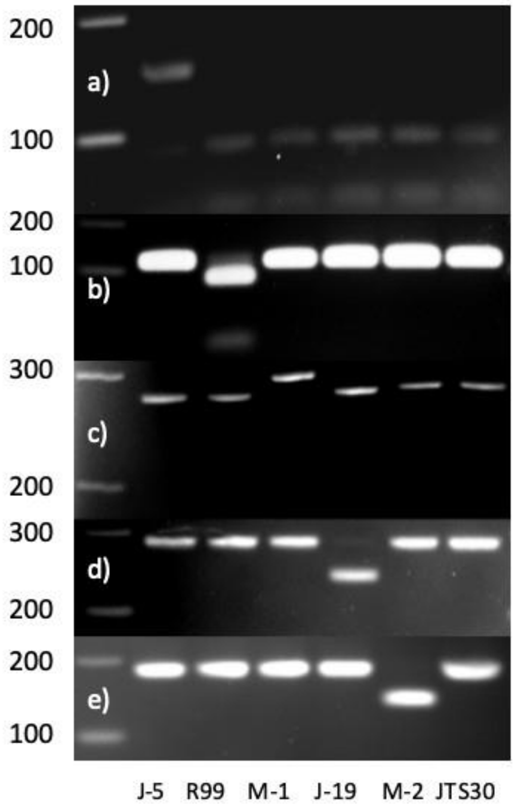


Figure 11. **Marker detection analysis of *ShSdhB* or *ShSdhC* gene mutations using representative resistant (J-5, M-1, J-19, M-2, R99) and sensitive (JTS30) isolates.** (a) CAPS analysis for detection of B-H267Y mutation using restriction enzyme Tsp45I, (b) dCAPS analysis for detection of B-H267R mutation using restriction enzyme Hpy99I, (c) dCAPS analysis for detection of C-G91R mutation using restriction enzyme SmaI, (d) dCAPS analysis for detection of C-G150R mutation using restriction enzyme AvaII, (e) dCAPS analysis for detection of B-L181 silent mutation using restriction enzyme BsmAI. All digested PCR products were electrophoresed on 3% agarose gel. The first lane is 100 bp DNA ladder (New England Biolab), and following lanes are reference isolates.

Table 6. *Sclerotinia homoeocarpa* strains used in this study.

No.	Name	Description	Origin	Source
1	JT30	Fungicide sensitive strain	The Joseph Troll Turf Research Center, South Deerfield, Massachusetts	Popko et al., 2017
2	J-5	SDHI resistant strain (only pyridine-carboxamide class) harboring H267Y mutation in *ShSdhB* gene	Takehara Country Club, Hiroshima, Japan	Popko et al., 2017
3	J-19	SDHI resistant strain harboring G150R mutation in *ShSdhC* gene	Takehara Country Club, Hiroshima, Japan	Popko et al., 2017
4	M1	SDHI resistant strain harboring G91R mutation in *ShSdhC* gene	The Misquamicut Club, Westerly, Rhode Island	Popko et al., 2017
5	M2	SDHI insensitive strain harboring Silent mutation in *ShSdhB* gene (CTT to CTC) at codon 181	The Misquamicut Club, Westerly, Rhode Island	Popko et al., 2017
6	HRI11	Multidrug-resistant (MDR) strain harboring M853T mutation in *Shxdr1* gene	Hickory Ridge Golf Club, Amherst, Massachusetts	Sang et al., 2018
9	CT45	Dicarboximide resistant strain harboring I366N mutation in *Shos1* gene	Wethersfield Country Club, Wethersfield, Connecticut	Sang et al., 2017
10	R99 to -231	SDHI resistant strain (only pyridine-carboxamide class) harboring H267R mutation in *ShSdhB* gene	Rutgers University, New Brunswick, New Jersey	This study

Table 7. Primers and associated annealing temperatures and restriction enzymes for each dCAPS and CAPS analysis, and the sizes of products after digestion.

Target mutation	Restriction enzyme	Products (bp) after RE digestion	Annealing temp (℃)	Primer names	Primer sequence (5'-3')
ShB-H267Y	MseI	Wild-type: 159 Mutant: 28, 131	72	F_dCAPS_SdhB_H267Y	GCAAGTCCTCGAGCAGTTGAGAATTGTTTNN
				R_dCAPS_SdhB_H267Y	TGGAACAGCGAAGAATACCTGGGACCAG
ShB-H267R	Hpy99I	Wild-type: 177 Mutant: 29, 88	72	F_dCAPS_SdhB_H267R	GACAACAGCATGAGCTTGTACAGACGTC
				R_dCAPS_SdhB_H267R	TTAAAAAGCCATCTCCTTCTTGATCTCCGCAATCGC
ShC-G91R	SmaI	Wild-type: 19, 277 Mutant: 296	72	F_dCAPS_SdhC_G91R	CCGCGCTAAACCGCATCCCG
				R_dCAPS_SdhC_G91R	AGCACTGGTCACACTCAACCCCACAAT
ShC-G150R	AvaII	Wild-type: 23, 243 Mutant: 266	72	F_dCAPS_SdhC_G150R	CGCATCCCAAGCCAAATGTCTCGGTC
				R_dCAPS_SdhC_G150R	CGCACCTCACCATCTACCAGCC
ShB-L181	BsmAI	Wild-type: 186 Mutant: 41, 145	72	F_dCAPS_SdhB_L181	TCAATTCTACAAACAGTACAAATCAATCAAGCCGTGTCT
				R_dCAPS_SdhB_L181	AGGTATTCTTCGCTGTTCCACCAGTACGAAGG
ShMDR1-M853T	NlaIII	Wild-type: 81, 90 Mutant: 171	69	F_CAPS_xdr1_M853T	GGCAACGATGCCAATTCACC
				R_CAPS_xdr1_M853T	GTTCATATGCAGCTCCGGGT
Shos1-I366N	MluCI	Wild-type: 140, 197 Mutant: 337	71	F_CAPS_os1_I366N	GAGAGACCTGCTCAGGGTGA
				R_CAPS_os1_I366N	GGTCCACCATGGAGTTGATGGT

USGA ID#: 2019-09-679

Title: Using the Smith-Kerns Dollar Spot Model for precision management of dollar spot on golf course fairways

Project Leaders: Paul Koch[1], Kurt Hockemeyer[1], Josh Friell[2], Walker Olson[2]
Affiliation: [1]Department of Plant Pathology, University of Wisconsin – Madison; [2]Toro Company, Bloomington, MN

Objective:

Schedule dollar spot fungicide applications in different areas of a golf course based on site conditions using weather monitoring stations and the Smith-Kerns Dollar Spot Model.

Start Date: 2019
Project Duration: 2 years
Total Funding: $20,000

Summary Text:

The trial consisted of three treatments in a randomized complete block design with 4 replications and an individual plot size of 6 x 10 feet. The treatments were a non-treated control, a traditional calendar-based fungicide program, and fungicide applications scheduled using the Smith-Kerns Dollar Spot Model. The trial was replicated at 3 nearby sites: the 7th hole at University Ridge GC in Madison, WI, the 18th hole at University Ridge, and the OJ Noer Turfgrass Research Facility located on land adjacent to University Ridge.

As stated above in the objective, we placed a weather sensor with the Smith- Kerns dollar spot model preprogrammed inside it adjacent to each plot. These weather sensors were to then transmit to the cloud and to a desktop computer and allow us to time fungicide applications for each site individually. We partnered with Josh Friell and Walker Olson of The Toro Company to develop beta versions of these weather sensors. The first version of sensors was based around a LoRa radio connection. The lithium battery powered sensors would collect temperature and humidity data along with a timestamp and periodically send their data to a base station through the radios. The base station would receive the data and pass it through a Wi-Fi connection to the cloud. However, these sensors were never successful in communicating with the base station. The problem was that in order for LoRa communication to work, they need a good line of site. To get this, the base station was moved on top of the facility, which helped quite a bit in terms of the LoRa communication, but caused the unit to be outside of Wi-Fi reception. The result was a system which could not effectively send its collected data to the cloud.

The second version of sensors was based around a 4G LTE module. This remedied the connection problem, as each sensor had a direct link to the cloud via its cellular connection. A solar panel was added to each sensor as the power consumption of the cellular modem can get to be quite high. There was also a GPS module added to give the geo-location of each device. The added complexity and the fact that these were all hand-wired contributed to one of the devices going completely silent after about a month of use. The remaining sensors exhibited an issue where the humidity readings would be saturated at 100% after rain events for long periods of time (2-3 days) before finally drying out. This resulted in only about a month's worth of meaningful data before the sensors were rendered ineffective.

The third version of sensors is currently being developed to address these issues. They will continue to use the same 4G LTE module for connectivity. The rest of the design will be refined and put onto a printed circuit board to reduce the amount of wiring and causes for problems. As for the temperature and humidity sensors, alternatives will be assessed, as well as different options for warding off condensation such as internal heater settings, enclosure modifications, etc. The code will be continually updated for efficiency and robustness. Finally, conformal coating will be added to the electronics to help with water-proofing, and other enclosure modifications may be done to make the device more rugged and less susceptible to failure in poor weather. We expect these sensors to be fully operational for the start of the 2020 growing season.

Due to the season-long problems with the sensors we were unable to use the sensors to time fungicide applications at each site based on the model. As a result, the dollar spot model numbers for the Noer were used to time applications for all 3 sites and there were no differences in fungicide usage between the 3 sites (Tables 1 through 3). However, because of consistently conducive conditions for dollar spot development in Madison during the summer of 2019, there were no differences in fungicide usage between a traditional calendar program and using the Smith-Kerns model.

When dollar spot severity between the 3 locations was compared, it was clear that conditions were more conducive for dollar spot on the 18th hole relative to the other two sites (Table 4). This was evident even though the location on the 18th hole was inadvertently sprayed with a fungicide in June. This strongly suggests that the central premise of this small study, that dollar spot pressure is different throughout a golf course due to microclimates and should be treated in a more precise manner, is valid and that finding an effective means to measure those microclimates and apply fungicide over small areas can provide significant reductions in overall fungicide usage for dollar spot management.

Year 2 Goal:

The primary goal is to ensure that the new weather sensors are accurately measuring the microclimate weather conditions and effectively communicating the dollar spot model numbers. The shortcomings of the first two versions of the sensors were described above and we have full confidence that the new sensors will be effective. The design of the field study will be exactly the same as Year 1, but we will use the new weather sensors to time fungicide applications for each site individually and report any possible fungicide savings at the end of 2020.

Table 1. Mean number of dollar spot infection centers per treatment at the OJ Noer Turfgrass Research and Education Facility in Madison, WI in 2019.

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jul 10	Aug 21	Sep 18
1		Non-treated control			45.0a	76.8a	123.5a
2	Standard Program	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 oz/1000 ft2 2 fl oz/1000 ft2 4 fl oz/1000 ft2 0.5 oz/1000 ft2 0.5 fl oz/1000 ft2 0.26 fl oz/1000 ft2 0.31 fl oz/1000 ft2 4 fl oz/1000 ft2 2 fl oz/1000 ft2	May 28 Jun 25 Jul 16 Jul 30 Jul 30 Aug 13 Sep 10 Oct 8 Oct 22	0.0b	11.8b	1.0b
3	Smith-Kerns model: Standard	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 oz/1000 ft2 2 fl oz/1000 ft2 4 fl oz/1000 ft2 0.5 oz/1000 ft2 0.5 fl oz/1000 ft2 0.26 fl oz/1000 ft2 0.31 fl oz/1000 ft2 4 fl oz/1000 ft2 2 fl oz/1000 ft2	28 day 21 day 14 day 14 day 28 day 28 day 14 day 14 day	1.0b	0.0b	0.8b
				LSD P=.05	24.31	27.7	32.6

[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Table 2. Mean number of dollar spot infection centers per treatment at University Ridge 7 fairway in Madison, WI in 2019.

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jul 10	Aug 21	Sep 5
1		Non-treated control			13.0-	110.5a	176.3a
		Emerald	0.18 oz/1000 ft2	May 28			
		Banner Maxx	2 fl oz/1000 ft2	Jun 25			
		Interface	4 fl oz/1000 ft2	Jul 16			
		Velista	0.5 oz/1000 ft2	Jul 30			
2	Standard Program	Secure	0.5 fl oz/1000 ft2	Jul 30	2.0-	12.5b	33.8b
		Xzemplar	0.26 fl oz/1000 ft2	Aug 13			
		Pinpoint	0.31 fl oz/1000 ft2	Sep 10			
		26 GT	4 fl oz/1000 ft2	Oct 8			
		Banner Maxx	2 fl oz/1000 ft2	Oct 22			
		Emerald	0.18 oz/1000 ft2	28 day			
		Banner Maxx	2 fl oz/1000 ft2	21 day			
		Interface	4 fl oz/1000 ft2	14 day			
		Velista	0.5 oz/1000 ft2	14 day			
3	Smith-Kerns model: Standard	Secure	0.5 fl oz/1000 ft2		1.3-	6.8b	31.0b
		Xzemplar	0.26 fl oz/1000 ft2	28 day			
		Pinpoint	0.31 fl oz/1000 ft2	28 day			
		26 GT	4 fl oz/1000 ft2	14 day			
		Banner Maxx	2 fl oz/1000 ft2	14 day			
				LSD P=.05	13.34	79.96	114.23

[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Table 3. Mean number of dollar spot infection centers per treatment at University Ridge 18 fairway in Madison, WI in 2019.

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jul 10	Aug 21	Sep 5
1		Non-treated control			0.0-	38.3a	51.5-
2	Standard Program	Emerald	0.18 oz/1000 ft2	May 28	0.0-	0.0b	8.3-
		Banner Maxx	2 fl oz/1000 ft2	Jun 25			
		Interface	4 fl oz/1000 ft2	Jul 16			
		Velista	0.5 oz/1000 ft2	Jul 30			
		Secure	0.5 fl oz/1000 ft2	Jul 30			
		Xzemplar	0.26 fl oz/1000 ft2	Aug 13			
		Pinpoint	0.31 fl oz/1000 ft2	Sep 10			
		26 GT	4 fl oz/1000 ft2	Oct 8			
		Banner Maxx	2 fl oz/1000 ft2	Oct 22			
3	Smith-Kerns model: Standard	Emerald	0.18 oz/1000 ft2	28 day	0.0-	0.0b	8.3-
		Banner Maxx	2 fl oz/1000 ft2	21 day			
		Interface	4 fl oz/1000 ft2	14 day			
		Velista	0.5 oz/1000 ft2	14 day			
		Secure	0.5 fl oz/1000 ft2				
		Xzemplar	0.26 fl oz/1000 ft2	28 day			
		Pinpoint	0.31 fl oz/1000 ft2	28 day			
		26 GT	4 fl oz/1000 ft2	14 day			
		Banner Maxx	2 fl oz/1000 ft2	14 day			
				LSD P=.05	NA	22.89	64.33

[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Table 4. Mean number of dollar spot infection centers on non-treated controls in all 3 locations.

	Jul 10	Aug 21	Sep 18
OJ Noer	45.0	76.8	123.5
7 Fwy	13.0	110.5	69.0
18 Fwy	0.0	38.3	264.0

USGA ID#: 2019-04-674

Project Title: Biology and Management of Pythium Root Rot in Golf Course Putting Greens

Project Leaders: James P. Kerns
Affiliation: North Carolina State University

Objectives:

1. Determine the distribution and prevalence of pathogenic root-infecting *Pythium* species in golf course putting greens.
2. Assess aggressiveness towards mature turfgrass plants of *Pythium* species associated with Pythium root rot.
3. Determine *in vitro* sensitivity of *Pythium* species collected to various fungicides.
4. Develop a quantitative PCR assay to detect *Pythium* species in turfgrass roots.

Start Date: 2019
Project Duration: 3 years
Total Funding: $81,250

Summary Points:

- Isolate of *Pythium* species during summer months is challenging as most of the isolates recovered were non-pathogenic species such as *Pythium torulosum*.
- Based on the limited data we collected this year, we hypothesize that *Pythium* infection precedes symptom development in creeping bentgrass.
- *P. torulosum* growth was only inhibited by cyazofamid, fluazinam, and etridazole.
- *In vitro* sensitivity varied among *Pythium* species, but all were extremely sensitive to cyazofamid.

Summary Text:

Samples exhibiting symptoms of Pythium root rot that were submitted to the NC State Turfgrass Diagnostic Lab were selected for isolation of *Pythium* species. Affected roots were washed for at least 3 hours and plated on semi-selective and non-selective media. After 24 hours of incubation in the dark, candidate hyphae were transferred from the aforementioned plates to a fresh petri plate containing water agar to obtain a pure culture. Out of 125 isolates collected, 88 were identified as *Pythium torulosum* and the remaining were identified as either *Pythium vanterpoolii (8), P. irregulare (5), P. aphanidermatum (1), or P. volutum* (1). All of the isolates collected except for *P. torulosum* were extremely aggressive when placed on creeping bentgrass seedlings. The pathogenic *Pythium* species were primarily collected during May and June which is early in terms of symptom expression. Of the 125 isolates collected, 22 were collected from ultradwarf bermudagrass putting greens. Fifteen of the isolates collected were *P. vanterpoolii,* 4 were *P. torulosum,* and 4 were *P. arrhenomanes.* Identifications were conducted by extracting the ITS regions and amplifying using ITS 4 and 5 primers in PCR. Sequences were aligned and generated sequences were compared using GenBank's BLAST and the Oomycete Gene Table database. Sequence identification was corroborated with molecular characteristics such as oospore/oogonia dimensions and ornamentations, antheridia characteristics and colony morphology. We will continue isolation efforts and will construct a phylogenetic tree once we have collected isolates from next year.

Given the challenges associated with isolation, we plan to establish permanent plots at the Lake Wheeler Turfgrass Research and Education Lab in Raleigh, NC for sampling purposes. We will sample from 8 replicate plots that will remain untreated throughout the course of the spring and summer. We plan to commence sampling in February and sample every three weeks throughout 2020 to help establish a clear picture of the species associated with this disease. We will also bury soil temperature and soil moisture probes at this location to see if we can correlate these factors to Pythium root rot development.

Sensitivity of *Pythium* isolates vary dramatically to fungicides. All isolates tested were extremely sensitive to cyazofamid and etridiazole. Most the isolates we collected were insensitive to propamocarb, which is the first report of insensitivity to this chemistry. The non-pathogenic speices, *P. torulosum*, was only sensitive to cyazofamid, fluazinam and etridiazole. It grew readily on the other fungicides we tested, which may explain why it is so prevalent during our summer sampling strategy. Certain species like *P. vanterpoolii,* were highly sensitive to QoIs. This is similar to what Kerns and Tredway document with *P. volutum*. We will continue to screen isolates for sensitivity as we collect them. This portion will continue into 2021.

We have not started pathogenicity work with the isolates we have collected, but that will begin in January of 2020. The quantitative PCR assay development will start in Fall of 2020 and will be completed in spring of 2021. In addition to this work, we started a preventive fungicide trial in spring of 2019. Applications of cyazofamid were made starting in March, April, May, June or July and subsequent monthly applications were applied after the initial application. The product was applied at 0.45 floz/1000 sq ft and applications were irrigated immediately after application with 1/8 inch of water. We found that preventive applications have to start no later than May 1 in order to be effective. In other words, soil temperatures could not exceed 72^{o}F in order to prevent Pythium root effectively. Applications in June and July were not effective in preventing Pythium root rot. This further supports our hypothesis that infection precedes symptom development. In fact, infection could occur as creeping bentgrass rooting is at its height in March, April and May.

Justification:
Creeping bentgrass, annual bluegrass and hybrid bermudagrass are the most commonly used turf species for putting greens due to their high plant density and tolerance for low mowing (5). During the summer months, heat and physiological stress makes creeping bentgrass and annual bluegrass management difficult. Impairment of root function by soilborne plant pathogens, particularly *Pythium* species, is a significant problem that leads to the decline of creeping bentgrass and annual bluegrass during summer stress periods (12). On the other hand, bermudagrass struggles during the fall, winter and spring when light levels are low and temperatures are suboptimal (12). Approximately 116 species are members of the *Pythium* genus (2), and many have been found in association with creeping bentgrass or annual bluegrass roots. Only a few studies have investigated *Pythium* species associated with bermudagrass roots.

To further complicate matters, there are two distinctly different *Pythium* root diseases. Root rots caused by *Pythium* species are associated with overly wet soils, which is normally a result of high organic matter content or poor drainage in sand-based putting greens (1, 12). As the name implies, Pythium root rots cause a distinct root necrosis, associated with brown or even black roots. Pythium root rots have been associated with various different *Pythium* species, and therefore disease development is highly moisture dependent and mostly temperature independent (1).

Pythium root dysfunction is a very different disease than traditional Pythium root rot. As opposed to Pythium root rot, symptoms of Pythium root dysfunction do not include a distinct root necrosis and are much more difficult to discern from healthy roots. Infected roots are shorter, lack root hairs, and are only slightly tan or buff compared to normal roots (6). Unlike root rot, Pythium root dysfunction is more prevalent on younger bentgrass greens (< 5 years) and in well-drained soil profiles. Pathogen infection by root dysfunction causing species also seems to be temperature dependent. Infection occurs during the spring and fall, which makes the bentgrass more susceptible to decline during periods of heat stress (8). Oospores of the pathogen are also only produced in quantity during the infection period, resulting in difficult diagnosis of this disease when symptoms are present.

For fungicide selection, it is critical to know which Pythium root disease and corresponding species is present. Pythium root rots are best controlled preventively with alternate applications of mefenoxam, cyazofamid, or propamocarb. Control once symptoms develop is difficult and typically relies on short interval re-applications of ethazole followed by one of the active ingredients listed above. Although fungicides are commonly applied to golf course putting greens, cost associated with these products are increasing and reducing the sustainability of putting green management. It is not uncommon for golf course superintendents to spend $10,000 a month when their turf starts to suffer (personnel communication with golf course superintendents who use our diagnostic services). Understanding the etiology of root rot will lead to reduced applications and lower costs associated with putting green management.

Pythium root dysfunction (PRD) is not controlled by traditional Pythium fungicides, but instead pyraclostrobin and cyazofamid have been found most effective (8). In addition, fungicides targeted for Pythium root dysfunction must be applied preventively in the fall and spring during the infection period, well before symptoms arise. Since the recommendations for Pythium root dysfunction are well supported with research, it is likely that this has led to distributors, salesmen and turf managers clinging to these recommendations for all soil-borne Pythium issues. This disease is a classic example of how understanding etiology and epidemiology of disease reduced costs associated with management. When PRD was first observed in NC, it was mistakenly identified as take-all patch and golf course superintendents were applying fungicides throughout the summer months to no avail. Once we discovered that the pathogen, *Pythium volutum,* was active when soil temperatures were between 55 and 75^{o}F we were able to manage the disease with three targeted fungicide applications applied when soil temperatures were conducive (8).

Although Pythium diseases are major issues for numerous crops, including turfgrass, the research community does not focus on these organisms. Research targeting pathogenicity and epidemiology of oomycete diseases are focused on *Phytophthora* species or downy mildews. These organisms are easy to manipulate and typically develop on above ground plant structures. *Pythium* species however remain associated with roots and cultivation of these organisms can be challenging. A recent manuscript demonstrated that 40% of roots collected from herbicide-terminated winter rye were colonized with *Pythium volutum* (4). Yet, the authors were not able to isolate *P. volutum* with traditional cultivation (plating using semi-selective media). The authors developed an amplicon sequence isolation method directly from roots. If this was available for turfgrass it would allow diagnosticians to accurately demonstrate PRR or PRD and more importantly identify the species that is most prevalent. A tool such as this could revolutionize our understanding of the population dynamics of *Pythium* in golf course putting greens and could possibly lead to regional fungicide programs tailored to the species present in a given area.

Considerable confusion exists among golf superintendents, diagnosticians, and researchers on the type of Pythium root diseases that are most prevalent. As a result, improper selection or timing of controls has led to an abundance of Pythium root disease outbreaks in recent years. Since 2008, the Turfgrass Disease Diagnostic Lab at NCSU has received 5,250 golf course putting green samples. Of those 30% (1,575) were diagnosed as Pythium root rot, which is significant considering that over half of the samples we receive are typically diagnosed with various abiotic problems.

Experimental Design:

Distribution: Approximately 200 root samples from soil profiles of healthy and poorly performing putting greens from North Carolina and neighboring states will be acquired through submissions to the diagnostic lab or through course visits. Roots will be analyzed microscopically for the presence of *Pythium* oospores. *Pythium* species will be isolated with selective culture media and a baiting technique (7). Speciation of *Pythium* in culture will be conducted by traditional morphological means and with sequencing of the internal transcribed spacer (ITS) region of DNA (11). For morphological identification, isolates will be transferred to sterile water containing 15 to 20 1.5 cm pieces of autoclaved tall fescue. These cultures will be incubated at room temperature under constant light to induce sporangia and oospore formation. Characteristics such as oospore diameter, number of antheridia, oospore wall thickness and shape will be recorded for identification. Molecular analysis will be performed by extracting genomic DNA and the ITS region will be amplified in two directions using ITS 4 and 5 primers. The amplicons will be sequenced at the Duke University Sequencing Facility. Existing sequences for *Pythium* species will be collected from the Pythium genome database (http://pythium.plantbiology.msu.edu) and will be used for phylogenetic analysis with the isolates we collect. In addition, DNA will be extracted from root tissue, and identification will be attempted via selective amplification and sequencing of Pythium ITS sequences (13).

Aggressiveness Assessment: A greenhouse or growth chamber study will be initiated to assess the aggressiveness of *Pythium* species on established 'Penn A-1' and 'Penncross' creeping bentgrass. Only species previously determined by Abad et al. (1) as highly aggressive or moderately aggressive will be used in this study. Bentgrass will be seeded in cone-tainers in the greenhouse and inoculated as conducted in previous studies (1, 10). Once creeping bentgrass plants reach 6 weeks old, the roots will be severed at 5 cm depth. The remaining soil will be smoothed and grass leaf blade cultures containing various *Pythium* species will be placed on the soil. The grass plug with 5 cm of roots will be placed on top of the infested soil. The inoculated plants will be subjected to high heat and over irrigation to stimulate disease development. The plants will be trimmed daily to simulate normal putting green mowing practices. Disease severity and turf quality will be assessed visually, and digital image analysis will be utilized as an objective measure of aggressiveness.

In vitro sensitivity: As isolates are collected from samples submitted to the Turfgrass Diagnostic Lab, those isolates will be screened for in vitro sensitivity to the 11 fungicides (Segway, Banol, Subdue MAXX, Insignia, Heritage, Stellar, Terrazole, Signature, Appear, Fame, and Daconil Action) that list Pythium blight, Pythium root dysfunction, or Pythium root rot on their labels. This work will be accomplished by amending water agar with 0, 0.001, 0.01, 0.1, 1 and 10 ppm of each fungicide. Each concentration will be replicated in triplicate and the entire study will be repeated twice. Sensitivity will be determined by measuring the radial diameter of the colony in two perpendicular directions. This data will be converted to LD_{50} values using log probit transformation and linear regression in SAS.

Amplicon sequence isolation assay: Cup-cutter cores will be collected from a creeping bentgrass and bermudagrass putting green at the Lake Wheeler Turfgrass Research and Education Center. Cores will be collected in March, April, May, June and July and the roots will be washed free of soil and freeze dried. After freeze drying the roots will be pulverized and DNA will be extracted using a DNeasy Plant Mini Kit. DNA extracts will be analyzed using the methods presented by Bakker et al. 2017 (4). Fungal DNA will be analyzed as well using the same method. The number of roots with positive isolation of DNA will be reports as well the number of open reading frames. The monthly data will be presented as to see if *Pythium* species vary at different times of the year.

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Table 1. *In vitro* sensitivity of *Pythium* species (number of isolates) to commercially available fungicides.

Pythium species	Fungicides[x]									
	EC_{50} Concentrations µg ml^{-1}									
	cyazofamid	fluazinam	etridiazole	azoxystrobin[y]	fluoxastrobin[y]	pyraclostrobin[y]	mefenoxam	chlorothalonil	propamocarb	fluopicolide
***P. aphanidermatum* (2)**										
P. aph	9.895 a[z]	0.380 de	0.439 def	>10 a	>10 a	>10 a	0.074 e	3.390 de	>10 a	6.640 b
P. aph2	0.035 d	0.559 de	2.310 a	>10 a	>10 a	>10 a	0.226 e	3.094 def	>10 a	>10 a
***P. irregulare* (1)**										
P. irr	4.098 b	>10 a	0.755 d	0.9354 b	3.336 b	0.643 b	0.202 e	>10 a	>10 a	>10 a
***P. arrhenomanes* (2)**										
WRGC5	0.039 d	0.198 de	1.368 c	>10 a	>10 a	>10 a	3.116 b	8.907 ab	1.141 c	0.956 d
Sedgefield	0.004 d	0.110 e	0.518 def	>10 a	>10 a	>10 a	0.204 e	1.212 efg	>10 a	>10 a
***P. vanterpoolii* (6)**										
RBR	0.012 d	0.237 de	0.241 ef	0.0608 c	0.06 c	0.271 b	1.965 bcd	9.137 ab	>10 a	>10 a
P1	0.058 d	0.267 de	0.799 d	>10 a	>10 a	>10 a	>10 a	0.997 fg	>10 a	>10 a
Lambert	0.031 d	0.292 de	1.945 ab	0.1637 c	0.116 c	0.06 b	0.485 e	3.501 d	>10 a	>10 a
DMC15	0.044 d	0.241 de	0.775 d	.0733 c	0.116 c	0.047 b	2.547 bc	7.615 bc	>10 a	>10 a
DMC22	0.026 d	0.212 de	0.642 de	.0904 c	0.113 c	0.047 b	0.618 e	>10 a	6.468 b	3.341 c
Pinehurst	0.074 d	0.432 de	1.287 c	>10 a	>10 a	>10 a	>10 a	6.276 c	>10 a	>10 a
***P. ultimum* var. *ultimum* (1)**										
P. ult	0.367 d	3.01 c	0.383 def	0.1284 c	0.163 c	0.139 b	>10 a	7.491 bc	>10 a	>10 a
***P. volutum* (1)**										
OC6	0.002 d	0.058 e	1.341 c	0.0431 c	0.095 c	0.041 b	1.833 cd	0.678 g	>10 a	>10 a
***P. torulosum* (4)**										
LW1	0.098 d	0.819 d	1.532 bc	>10 a	>10 a	>10 a	>10 a	7.173 bc	>10 a	>10 a
LW5	0.042 d	0.195 de	0.223 ef	>10 a	>10 a	>10 a	>10 a	1.935 defg	>10 a	>10 a
LW10	0.056 d	0.210 de	0.532 def	>10 a	>10 a	>10 a	>10 a	1.729 defg	>10 a	>10 a
LW12	0.045 d	0.212 de	0.257 ef	>10 a	>10 a	>10 a	>10 a	2.08 defg	>10 a	>10 a
***P. vexans* (1)**										
Ed-mum-27	>10 a	5.229 b	0.526 def	0.1649 c	0.263 c	0.92 b	0.3576 e	0.127 g	>10 a	3.966 c
***P. myriotylum* (1)**										
Ed-mum-22	1.078 c	0.591 de	0.148 f	0.076 c	0.058 c	0.403 b	0.168 e	>10 a	6.407 b	>10 a

[x] Commercial formulations of fungicides.
[y] SHAM (50 µg ml^{-1}) was added with fungicides to reduce alternative oxidase pathway.
[z] Values followed by the same letter within a column are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

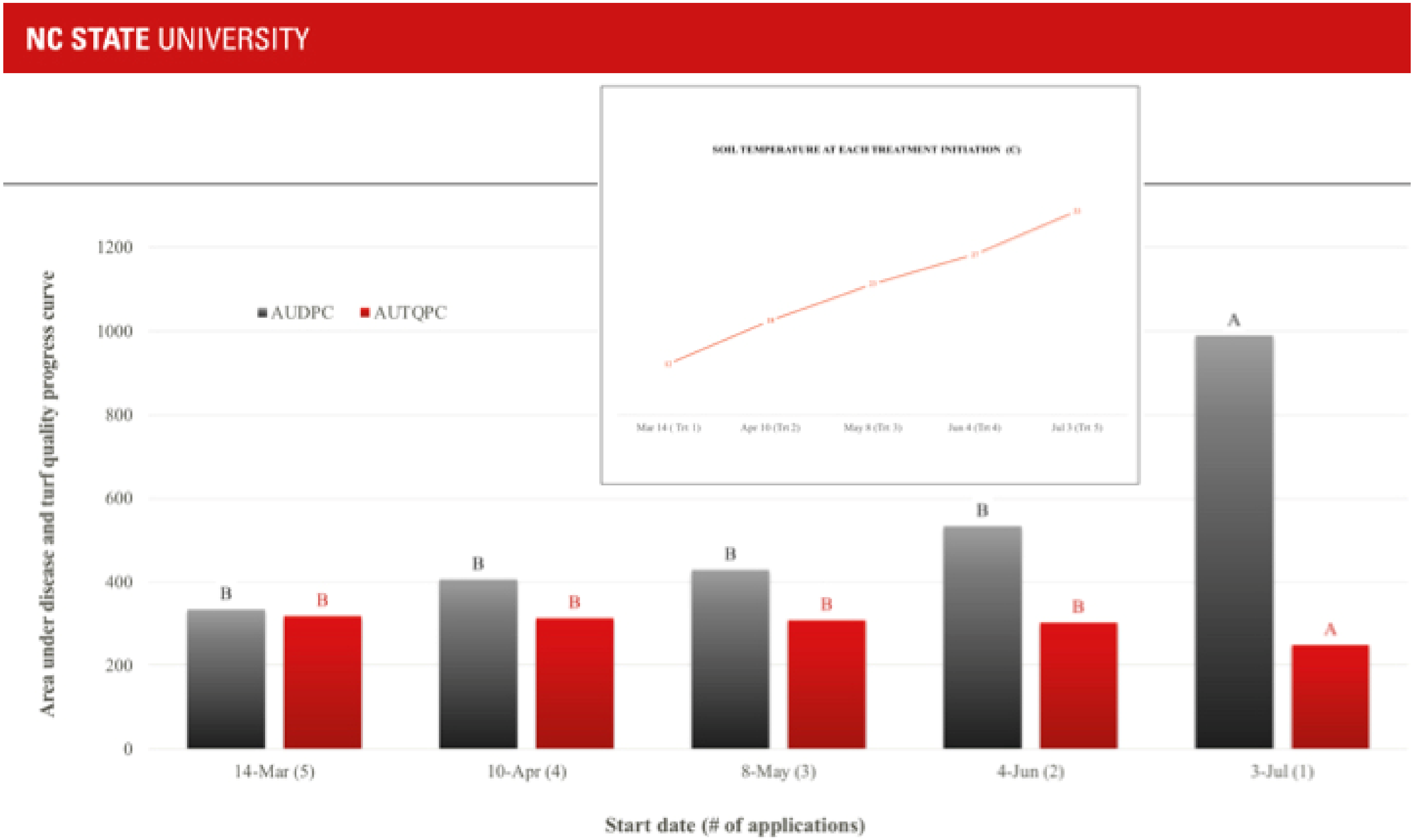


Figure 1. Efficacy of preventative cyazofamid applications for Pythium root rot in creeping bentgrass. Applications started in either March, April, May, June or July and were re-applied monthly until August. All applications were irrigated immediately with 1/8 inch of water and cyazofamid was applied at 0.45 floz/1000 ft^2.

USGA ID#: 2018-12-662

Project Title: Investigating Factors Affecting Fungicide Performance on Golf Course Turf

Project Leaders: James P. Kerns, Travis W. Gannon, Cameron M. Stephens
Affiliation: North Carolina State University

Objectives:

1. Determine the influence of post application irrigation and mowing timing on fungicide movement on a golf course putting green
2. Evaluate the *in vitro* fungicide sensitivity of organisms causing take-all root rot on ultradwarf bermudagrass putting greens

Summary Points:

- Delaying mowing events did not alter the amount of fungicide removed in turfgrass clippings as little fungicide was detected within the clippings.
- It is difficult to move fungicide past the remaining above ground vegetation (RAV) regardless of irrigation treatment.
- Irrigating immediately resulted in greater fungicide movement past the RAV.
- Small differences in fungicide movement could have a dramatic impact in potential efficacy of these compounds in the field.
- Multiple (n=5) ectotrophic root infecting fungi (ERI) were routinely isolated from take-all root rot symptomatic root and stolon tissue.
- ERI fungi were evaluating in *in vitro* fungicide assays and demethylase inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides suppressed mycelial growth more when compared to succinate dehydrogenase inhibitor (SDHI) fungicides.

Start Date: 2018
Project Duration: 2 years
Total Funding: 34,800

Summary Text:

Fungicide fate and performance are influenced by post application management practices (i.e. post application mowing timing and irrigation timing). Work conducted by Dr. Gannon and Dr. Jefferies found up to 34% of azoxystrobin was removed in tall fescue clippings following a single mowing event one day after application. However, the environmental fate and efficacy of fungicides as influenced by post application management practices has not been evaluated on golf course putting greens. Therefore, the environmental fate of pyraclostrobin, triadimefon, and penthiopyrad following various post application irrigation and mowing treatments were evaluated on a putting green constructed to USGA specifications and planted with ‘A-1’ creeping bentgrass. Plots were treated with a single application of the aforementioned fungicides and irrigated either immediately (0h; 0 hour) or 6 hours (6h; 6 hour) after fungicide application with 0.25” (0.64cm) of water. Plots were then mowed at either 0, 1, or 3 days after treatment (DAT). Daily mowing resumed after 3 DAT. Cores were harvested using a standard 4.25” cup cutter and dissected into 4 subsections: remaining above ground vegetation (RAV; verdure/thatch), 0-1” (0-2.5cm), 1-2”(2.5-5.1cm), and 2-3” (5.1-7.6cm) depths. All samples were homogenized using a Fitz Mill and dry ice then analyzed using high performance liquid chromatography-mass spectrometry. To date, a full dataset from a single year has been analyzed for pyraclostrobin, triadimefon, and penthiopyrad and data is presented as a percent of applied. Second year samples are currently being processed.

Pyraclostrobin recovered in turfgrass clippings at 0, 1, and 3 DAT did not exceed 2.5% of total fungicide applied. The movement and distribution of pyraclostrobin over time following different irrigation and mowing treatments is illustrated in Figure 1. Delaying mowing events post pyraclostrobin application did not significantly influence the amount of fungicide removed with clippings. A large amount of fungicide remained bound in the RAV. However, we did start to see significant differences between irrigation treatments at and past 1 DAT at the 0-1" depth. We detected a greater amount of fungicide in the 0-1" and 1-2" depth when plots where irrigated immediately compared to plots that were irrigated 6 hours after application. We only had detected pyraclostrobin the 2-3" depth at 14 DAT when plots were irrigated immediately. It is also important to notice that in later DATs (5, 7, 14 DAT) we observed a greater total amount of pyraclostrobin in plots irrigated immediately compared to plots irrigated 6 hours after treatment. This may have implications in residual disease control with this compound.

Triadimefon behaved similarly to pyraclostrobin. The movement and distribution of triadimefon over time following different irrigation and mowing treatments is illustrated in Figure 2. Less triadimefon was removed in turfgrass clippings when compared to pyraclostrobin. This may be due to the physiochemical properties of triadimefon (low K_{oc}=300 and high Ks=70). There were less significant differences between irrigation treatments in the RAV and 0-1" depth when compared to pyraclostrobin. However, detection in the 1-2" depth occurred earlier and in greater quantity than pyraclostrobin. Detection in the 2-3" depth was only present at 14 DAT when plots were irrigated immediately.

Post application irrigation timing greatly influenced penthiopyrad movement. The movement and distribution of penthiopyrad over time following different irrigation and mowing treatments is illustrated in Figure 3. We saw significantly less fungicide bound in the RAV and more fungicide moving into the 0-1" depth when plots were irrigated immediately compared to plots irrigated 6 hours after penthiopyrad application. Similar to the other compounds, fungicide was detected in the 1-2" and 2-3" depth earlier and in greater amounts when plots were irrigated immediately. I also wanted to note that for all three fungicides evaluated fungicide recovery was >90% at 0 DAT which confirms the methodology used in this experiment.

Post application irrigation timing, and to a lesser extent mowing timing, can influence fungicide movement through the soil profile and fungicide removal with clippings. Delaying mowing events did not result in less fungicide removed in clippings, but may influence the downward movement and distribution of product into the RAV and soil layers. The current data suggests the majority of fungicide is retained in the RAV regardless of post application irrigation timing. However, irrigating immediately following fungicide application can move fungicide into deeper soil depths where target soil-borne pathogens reside.

A total of four (n=4) different organisms (*Gaeumannomyces graminis* (Gg), *Gaeumannomyces graminicola* (Ggram), *Candidacolonium cynodontis* (Cc), and *Magnaportheopsis cynodontis* (Mc)) across three genera associated with take-all root rot on ultradwarf bermudagrass greens were evaluated in *in vitro* fungicide sensitivity assays. Take-all root rot organisms were evaluated using *in vitro* fungicide sensitivity assays against 10 fungicides from 3 different chemical classes (Figure 4). Fungicide concentration were 0, 0.01, 0.1, 1.0, and 10 ppm. Experimental units were incubated in constant darkness at 25°C and two-way colony diameter was measured on day 3 (Gg and Ggram) and day 6 (Cc and Mc). The effective concentration resulting in a 50% reduction of growth compared to the control (EC_{50}) and relative mycelial growth (RMG) were determined using the probit procedure. Take all root rot organisms differed in fungicide sensitivity. All isolates evaluated against succinate dehydrogenase inhibiting fungicides consistently had the highest EC_{50} and RMG values. The lowest EC_{50} and RMG values were observed when isolates were grown on azoxystrobin-amended media. Differences in fungicide sensitivity within and across chemical classes provide novel insight into biological factors of take all-root rot organisms (Figure 4).

Current data suggests that small difference in fungicide movement may make a large impact in potential field efficacy. For example, an *in vitro* evaluation of Gg on pyraclostrobin-amended media showed that 1 ppm is the concentration required to completely inhibit fungal growth, regardless of incubation temperature (Figure 5). If we convert the percent of applied data from a sample core collected on 14 DAT to ppm we are able to deliver an efficacious amount of fungicide (1.1ppm) to the 0-1" depth when irrigated immediately (0 hour) and only .5 ppm when waiting to irrigate until 6 hours after application.

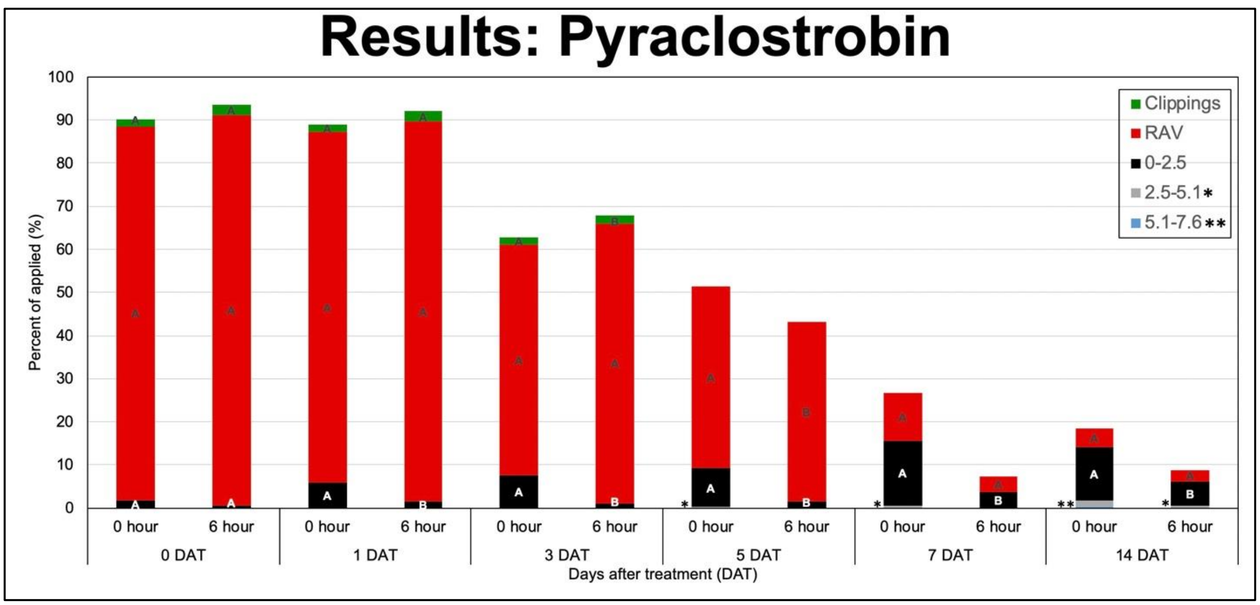


Figure 1. Influence of post application irrigation timing on pyraclostrobin movement on a golf course putting green. Means followed by the same letter between irrigation treatments within each day after treatment at each individual depth are not statically different at P<0.05 according to Fischer's Protected Least Significant Difference Test. Soil depths are expressed in centimeters.

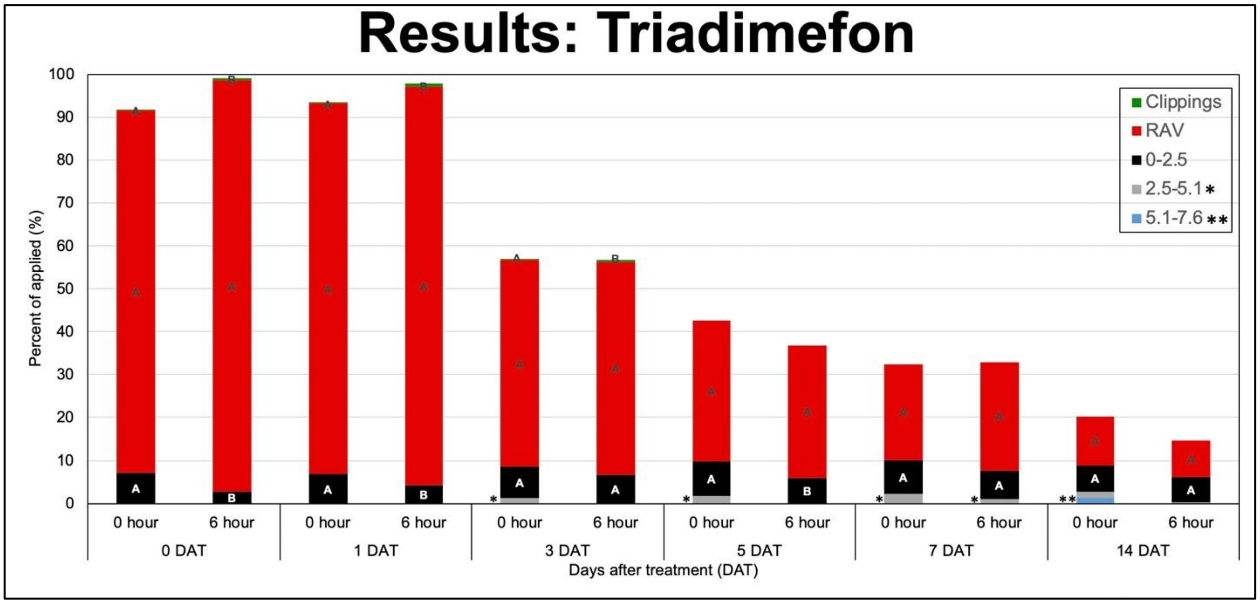


Figure 2. Influence of post application irrigation timing on triadimefon movement on a golf course putting green. Means followed by the same letter between irrigation treatments within each day after treatment at each individual depth are not statically different at *P*<0.05 according to Fischer's Protected Least Significant Difference Test. Soil depths are expressed in centimeters.

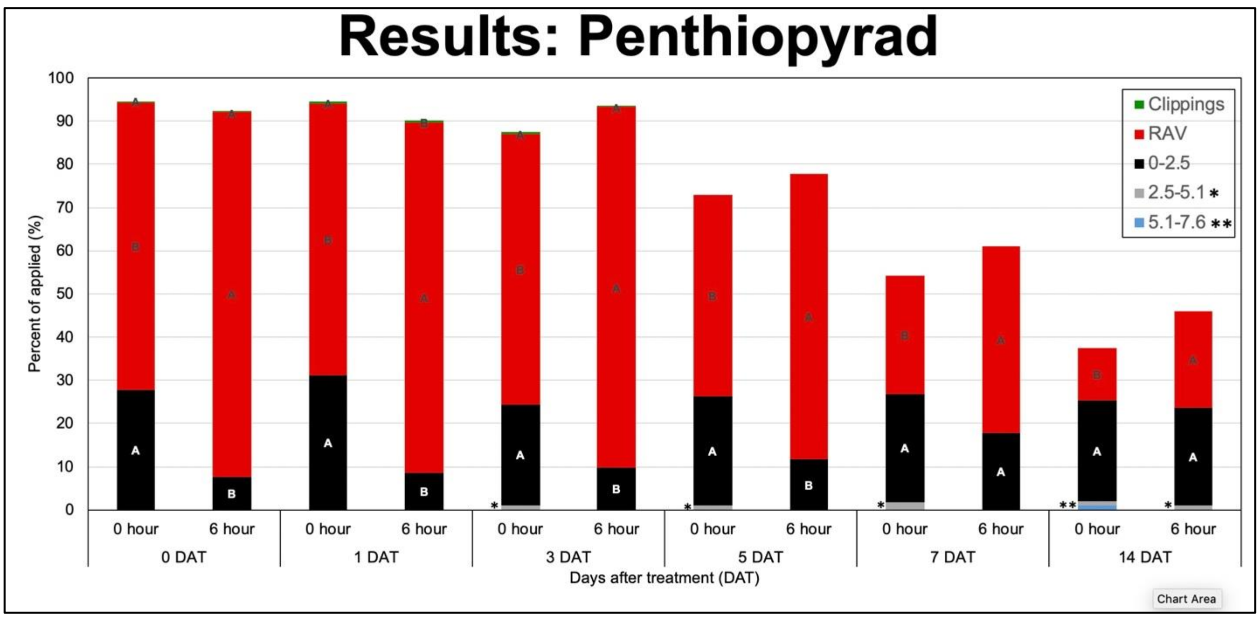


Figure 3. Influence of post application irrigation timing on penthiopyrad movement on a golf course putting green. Means followed by the same letter between irrigation treatments within each day after treatment at each individual depth are not statically different at *P*<0.05 according to Fischer's Protected Least Significant Difference Test. Soil depths are expressed in centimeters.

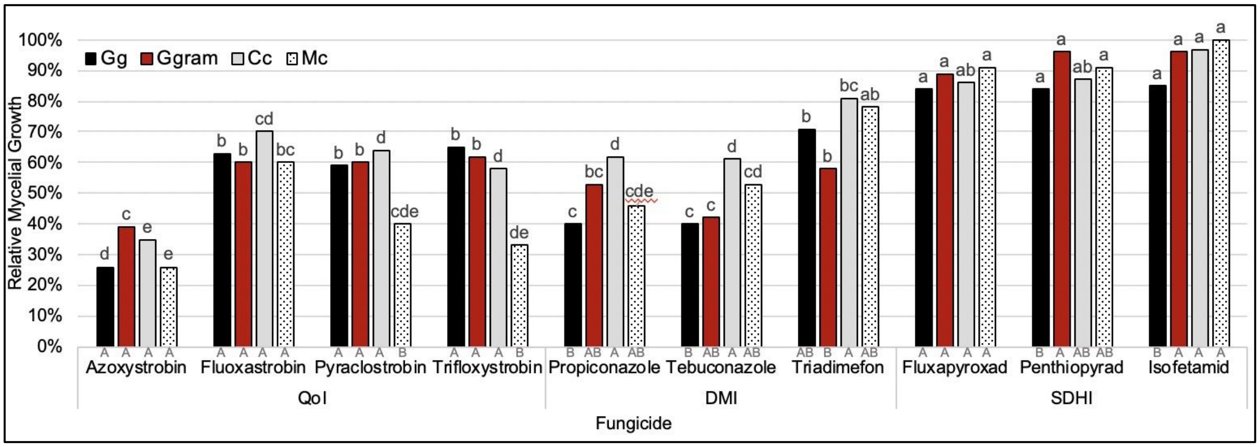


Figure 4 Relative mycelial growth of take-all root rot organisms on fungicide-amended potato dextrose agar. Means followed by the same lowercase letter above each bar across fungicides are not statically different at *P*<0.05 according to Fischer's Protected Least Significant Difference Test. Means followed by the same capital grey letter at the bottom of each bar are not statically different within each fungicide at *P*<0.05 according to Fischer's Protected Least Significant Difference Test. *Gaeumannomyces graminis* (Gg), *Gaeumannomyces graminicola* (Ggram), *Candidacolonium cynodontis* (Cc), and *Magnaportheopsis cynodontis* (Mc) were evaluated in this assay.

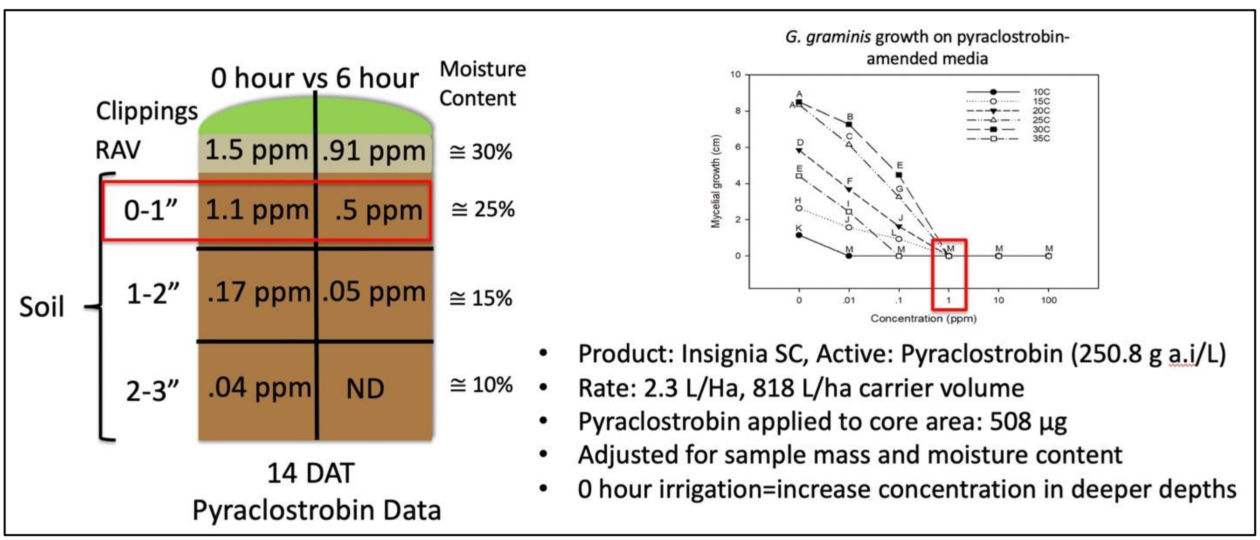


Figure 5. Pyraclostrobin residue data from a sample core collected at 14 days after treatment. Percent of applied data converted to ppm at each individual depth. *In vitro* evaluation of *Gaeumannomyces graminis* on pyraclostrobin-amended media demonstrating that 1ppm completely inhibits fungal growth.

USGA ID#: 2017-08-618

Title: Biological Control of Annual Bluegrass Weevil with novel Formulation Types and Application Systems for Entomopathogenic Fungi: Microsclerotia-based formulations and Hydrogels

Project leaders: Albrecht M. Koppenhöfer[1], Olga S. Kostromytska[1], Shaohui Wu[1], Ann E. Hajek[2]

Affiliation: [1]Department of Entomology, Rutgers University, New Brunswick, NJ; [2] Dept. Entomology, Cornell University, Ithaca, NY

Objectives:

The goal is to develop a granular formulation of microsclerotia of *Metarhizium brunneum* F52 as an effective and viable biological control option for ABW. Specifically, we want to determine: 1. Compatibility of formulation with commonly used golf course fungicides. 2. Efficacy of formulation against ABW adults and externally feeding larvae. 3. Effect of hydrogels on efficacy and persistence of formulation and compatibility with golf course turfgrass.

Start Date: 2018
Project Duration: 2 years
Total Funding: $38,976

Summary Points:

- *M. brunneum* microsclerotia are compatible with the turf fungicides iprodione and chlorothalonil but the fungicide propiconazole has a suppressive effect on *M. brunneum* spore production.
- *M. brunneum* microsclerotia alone did not significantly suppress ABW larvae in field experiments.
- Hydrogels had no significant effect on the performance of *M. brunneum* microsclerotia and their combinations with imidacloprid.
- A liquid conidia-based formulation provided significant control of ABW larvae.
- Combinations of imidacloprid with microsclerotial and conidial formulations of *M. brunneum* always provided additive control of ABW larvae, and the highest control was achieved in imidacloprid combinations with the high rate of conidia (70%).

Summary Text:

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a serious and expanding pest of short-mown golf course turf in eastern North America with demonstrated ability to develop resistance to a range of insecticide modes of action. These widespread resistance issues warrant the development of alternative control methods. While products based on the conidial spores of entomopathogenic fungi have thus far given unreliable control of ABW adults and larvae in the field, the use of fungal microsclerotia may improve economy and efficacy of fungus-based products. Some entomopathogenic fungi including *Metarhizium brunneum* naturally form microsclerotia in soil, which serve as survival structures. Applied microsclerotia granules produce infective conidial spores over several weeks, thus prolonging the residual effect of the fungus application. The addition of hydrogels may improve conidia production from microsclerotia in soil because hydrogels can hold large volumes of water when moistened and slowly release this retained water over time. This stabilizes soil moisture to the advantage of plants, fungi and other organisms.

In previously reported laboratory and greenhouse experiments, we had shown that the fungicides chlorothalonil and iprodione were compatible with *M. brunneum* F52 applied as microsclerotia but that propiconazole reduced conidia production by around 50%. Combinations of propiconazole + trifloxystrobin and metconazole + pyraclostrobin were incompatible with the fungus in the laboratory experiment.

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

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

Fig. 1. Late spring damage caused by ABW larvae along fairway edge.

Fig. 2. *Metarhizium brunneum* F52-infected ABW adult.

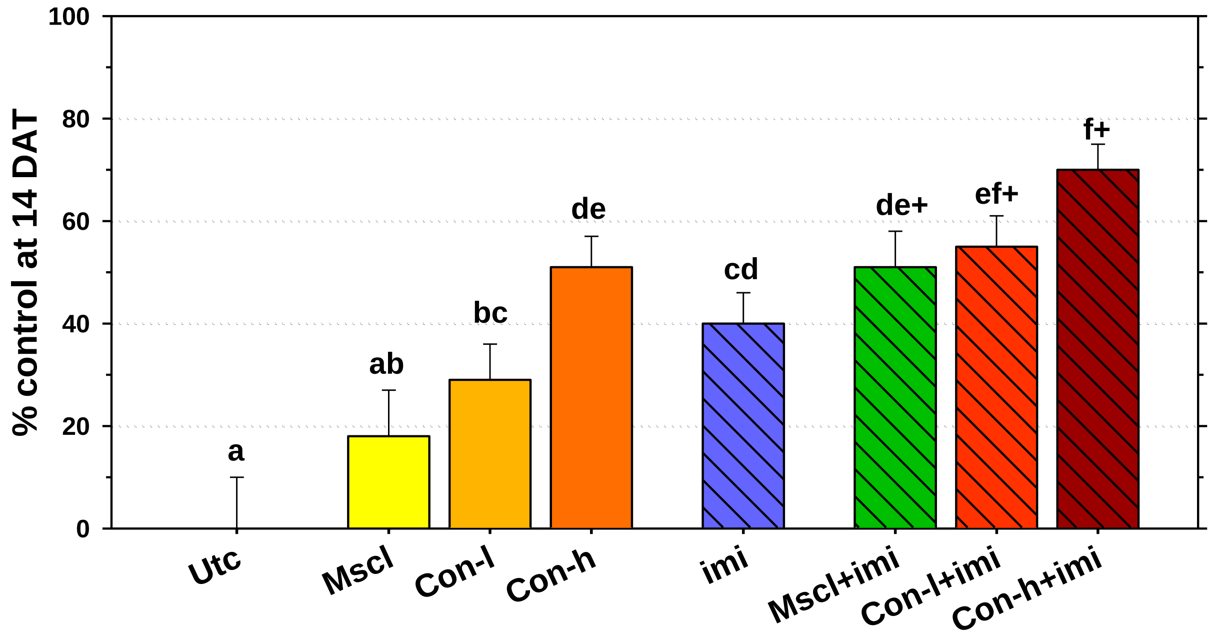


Fig. 3. Mean percent control (± SEM) of ABW immature stages in golf course fairways treated with *Metarhizium brunneum* F52 in a granular formulation of microsclerotia (Mscl) and a low (Con-l) and high rate (Con-h) of a liquid formulation of conidia, the insecticide imidacloprid (imi), and the combinations of *M. brunneum* with imidacloprid. Means with same letter inside do not differ significantly (P = 0.05).

USGA ID#: 2018-13-663

Title: Biorational Control of Annual Bluegrass Weevil Adults and Larvae with Petroleum-Derived Spray Oils and Soil Surfactants

Principal Investigator(s): Benjamin A. McGraw, Ph.D.[1], Steven Alm, Ph.D.[2], Albrecht Koppenhöfer, Ph.D.[3]

Affiliation: [1]Pennsylvania State University; [2]University of Rhode Island; [3]Rutgers University

Objectives:
(1) Determine effects of soil surfactant and Petroleum-Derived Spray Oil (PDSO) rate, soil moisture, and post-spray irrigation on adult annual bluegrass weevil (ABW) control.
(2) Determine effects surfactant and PDSO rate, soil moisture, and post-spray irrigation on larvae control.
(3) Determine effects of insecticide resistance on the efficacy of surfactant and PDSO applications.
(4) Determine effect of adult ABW canopy-surface activity on product efficacy

Start Date: 2018
Project Duration: 2 years
Total Funding: $60,000

Summary Text:

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is the most difficult to control turfgrass insect pest in eastern North America. Superintendents have traditionally relied on synthetic insecticides for ABW management, primarily using broad-spectrum adulticides (pyrethroids, chlorpyrifos) to control overwintered adults in spring prior to oviposition. Recent increases in the development of pyrethroid- and multiple-resistant populations has created a dire need to develop alternative controls, especially for adult management. Preliminary laboratory trials suggested that oils and petroleum-derived spray oils (PDSOs) are capable of controlling adults, and that mortality is achieved quickly. These studies also determined that high levels of moisture are necessary to improve the products' contact activity. This report provides a summary of the experiments conducted under the research objectives, including seven field trials conducted between 2018-19 in three states.

Objectives 1: Effects of surfactant and PDSO rate, soil moisture, post-spray irrigation on adult control.

Rate

The effect of product rate and post-spray irrigation on adult mortality in Petri assays. Label rates of both the surfactant (Silwet, Helena Chemical Co.) and PDSO (Civitas, Suncor Energy Inc.) caused moderate to high adult mortality (> 80% control), with most mortality occurring within 24 hrs. Applications of Civitas, but not of Silwet resulted in significant increases in mortality with time. Additionally, rate was a significant factor in Civitas treatments and there was a weak effect of application water volume at 3 and 24 h.

Phytotoxicity was observed with increasing surfactant rate in RI and PA field trials. Therefore, Silwet was removed from further field studies. No issues were observed with either 8.5 or 17 fl oz/M (label rates) of Civitas in the field. However, field trials have shown similar trends as laboratory trials in that little improvement is observed with increasing rates of Civitas. In 2018, rate led to numerical improvements, but not statistical differences in adult control on two application dates (Figure 1). Two sequential applications (back-to-back weeks) provided the highest level of suppression, though not significantly different from one application. Similar results were observed in 2019, though split applications of the low label rate of Civitas provided statistically significant reductions of adults on the second date. Additionally, this treatment had the greatest reductions on future development stages (larvae, pupae) (Figure 2).

Soil moisture and post-spray irrigation

Soil moisture significantly affected Civitas and Silwet efficacy in greenhouse studies. For both products, 50% moisture had only very limited mortality (< 5%) which was significantly lower than for the higher moisture levels (100 and 150%). For Civitas, mortality at 150% soil moisture was significantly higher than at 100%.

We sought to determine whether adult control could be improved in the field by irrigating prior to application and increasing carrier volume or post application irrigation. Counter to our hypotheses, adult control was reduced under increasing carrier volumes (1 to 4 gal/M) and irrigation scheduling (0 – 0.1”) when soils were saturated. However, these differences between treatments were not statistically significant. The addition of a “sticker” adjuvant to Civitas in the following year’s trial also did not improve adult control.

Objective 2: Determine effects surfactant and PDSO rate, soil moisture, and post-spray irrigation on larvae control.

Larval susceptibility to surfactants and PDSOs was assessed in RI and NJ. Neither Civitas rate, carrier volume, post-application irrigation, or the interaction between the variables provided more than 10% reductions in larvae compared to the untreated controls. Silwet appeared to provide high levels of control (92%) when applied at high rates (5 fl oz./M) to late-instar larval populations. However, this effect was not observed in controlled greenhouse studies or the following year’s trial.

Objectives 3: Determine effects of insecticide resistance on the efficacy of surfactant and PDSO applications.

Civitas and Silwet were tested against a bifenthrin-susceptible and a 95x bifenthrin-resistant population in Petri dish assays. No differences in the susceptibility to Silwet and Civitas were observed between populations. Additionally, several field studies in NJ have demonstrated that adult control with Civitas alone or in combination with a pyrethroid may provide moderate adult control. In 2018, adult densities in a 55x resistant population were significantly suppressed in all treatments with no differences among treatments (Civitas 50%, pyrethroid (Talstar) 44%, Civitas + Talstar 67%) (Table 1). Additionally, Civitas (48%) and the Civitas + pyrethroid (Talstar) combination (59%) but not Talstar (4%) significantly reduced larvae.

Objective 4: Effect of adult ABW canopy-surface activity on product efficacy.

The effect of adult surface activity on product efficacy was assessed in growth chamber studies. Adults were held on turf cores at a constant temperature (10, 17, or 25^{o} C) for 24 hr. After the acclimation period, turf cores were removed, photographed, immediately treated, and then placed back into the incubator. A significant correlation between surface activity and temperature was observed, but the lowest average activity was observed at 17^{o} C, or what was predicted to be the optimum for ABW surface activity based on previous research. Mortality was very low in all treatments, with Silwet providing the highest control (12.5 -13%).

<u>Summary Points (2018- 2019)</u>:

1. Both surfactants (Silwet, Helena Chemical Co.) and PDSOs (Civitas, Suncor Energy Inc.) can cause moderate to high adult mortality. Mortality was observed shortly after application (< 3 hrs), with most mortality occurring within 24 hrs.
2. Neither products reduced larvae in the greenhouse or field. Phytotoxicity was observed with Silwet at 9 oz./M and thus it was abandoned from future field studies.
3. Moisture level significantly affected Civitas and Silwet efficacy in the laboratory. However, manipulating soil moisture in the field through pre- and post-irrigation and/or altering carrier volume did not improve adult control.

4. No differences in susceptibility to surfactants or PDSOs were detected between pyrethroid resistant and susceptible populations
5. Civitas rate has not been a significant factor in field trials. Split or sequential applications in back-to-back weeks has provided the greatest reductions of adults and their future developmental stages.

Table 1. Densities of pyrethroid-resistant (55x) annual bluegrass weevil adults (per ft^2 ± SE; % control) at 2 DAT and of ABW larvae and pupae on 7 June, 2018 (per ft^2 ± SE; % control) in a golf course fairway treated when adults had reached peak densities (4 May).

Treatment/ Formulation	Rate (fl oz/ 1,000 ft^2)	Application dates	Adult densities/ft^2 [a]	No. of stages per ft^2 (± SE) (% control) [b]
UTC	---	---	1.2 ± 0.3 a	48.6 ± 10.1 a
Civitas	8.5	4 May	0.6 ± 0.1 b (50)	25.2 ± 5.2 b (48)
Talstar	0.1 lb ai/ac	4 May	0.7 ± 0.1 b (44)	46.8 ± 10.5 a (4)
Civitas + Talstar	8.5 0.1 lb ai/ac	4 May 4 May	0.4 ± 0.1 b (67)	19.8 ± 5.4 b (59)

[a] ANOVA: $F = 6.00$; df = 3, 19; $P = 0.0097$.
[b] ANOVA: $F = 5.29$; df = 3, 19; $P = 0.0149$.

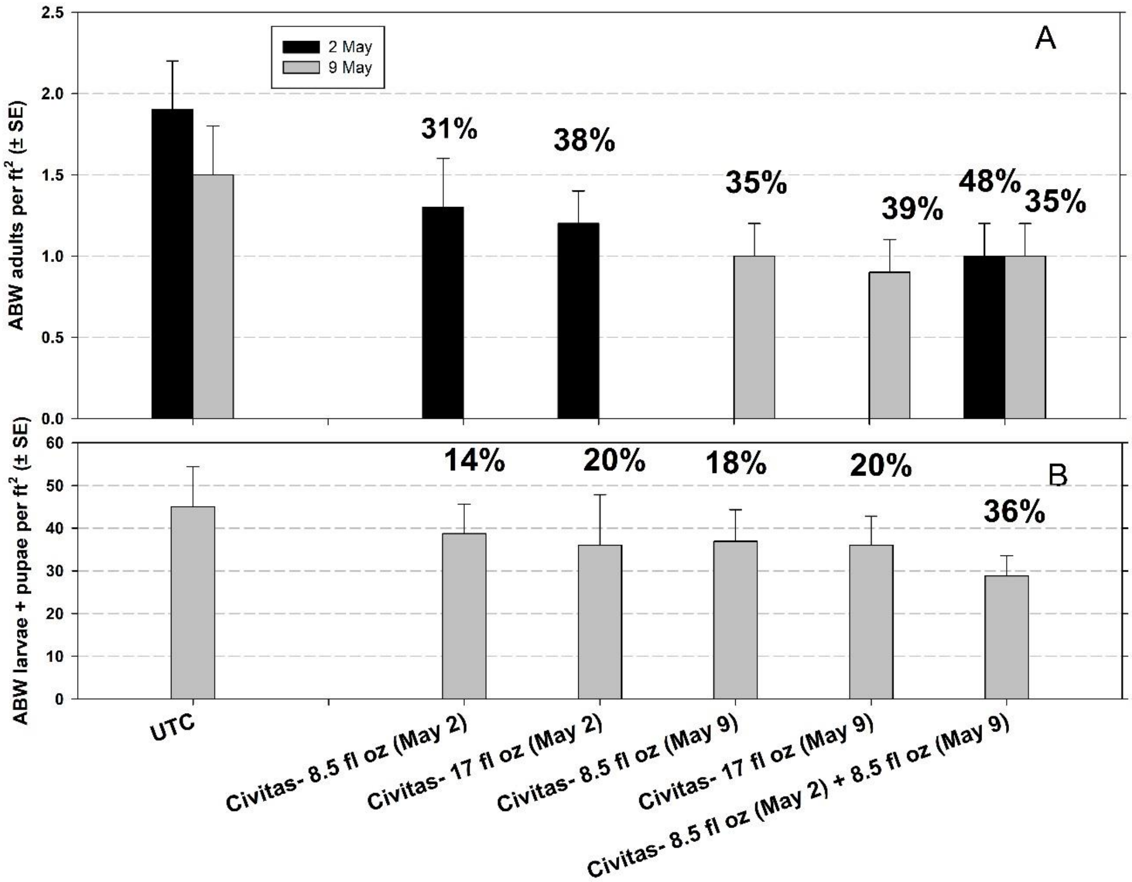


Figure 1. **(A)** Densities of annual bluegrass weevil adults (per ft^2 ± SE; % control) at 2 days after treatment (DAT) after first (2 May 2018) and second application (9 May). Percentages above the bar indicate control relative to the untreated checks on the same evaluation date. **(B)** Densities of ABW larvae and pupae on 5 June 2018 (per ft^2 ± SE; % control) in a golf course fairway treated when adults had reached peak densities (2 May) and/or 1 week later (9 May). Percentages above the bar indicate control relative to the untreated checks. No significant differences were observed.

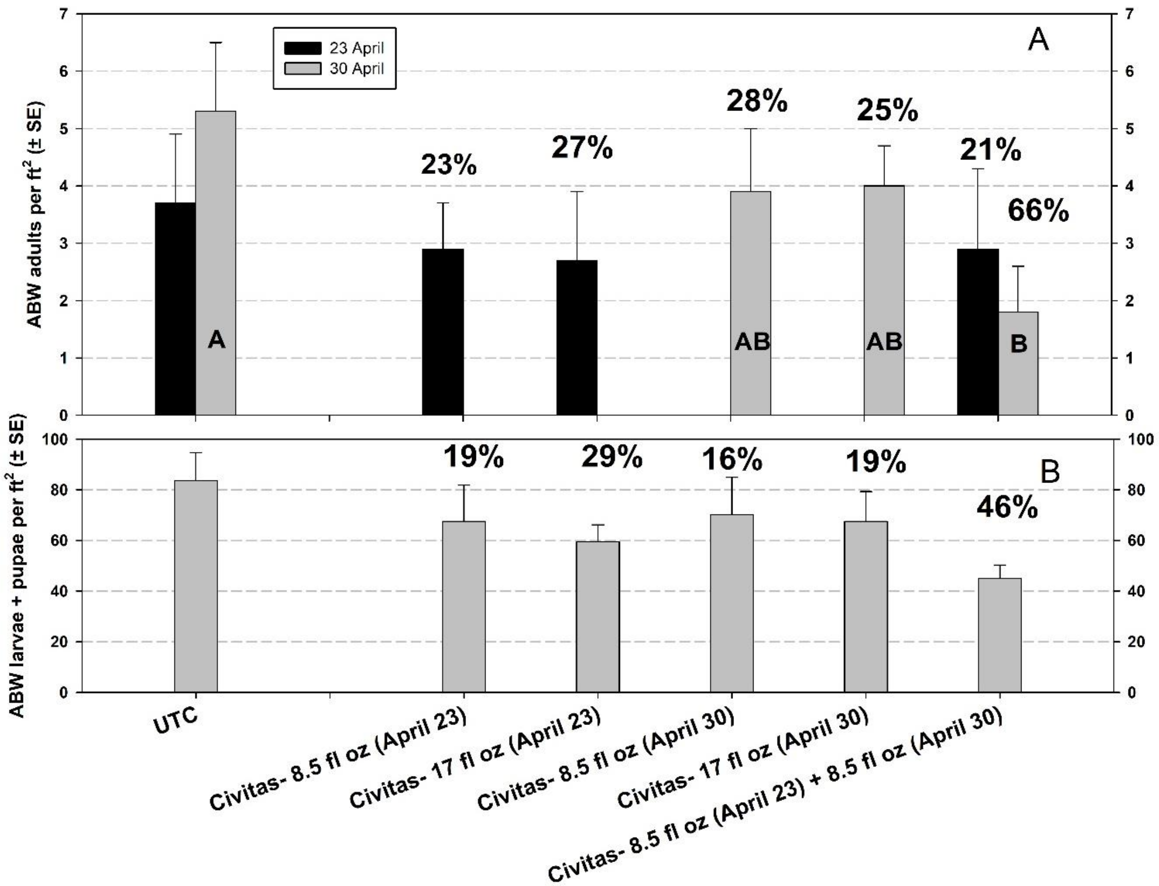


Figure 2. **(A)** Densities of annual bluegrass weevil adults (per ft^2 ± SE; % control) at 2 days after treatment (DAT) after first (23 April 2019) and second application (30 April). Percentages above the bar indicate control relative to the untreated checks on the same evaluation date. **(B)** Densities of ABW larvae and pupae on 11 June 2019 (per ft^2 ± SE; % control) in a golf course fairway treated when adults had reached peak densities (23 April) and/or 1 week later (30 April). Percentages above the bar indicate control relative to the untreated checks. Letters inside bars indicate significant differences between treatments. Treatments with the same letters are not significantly different from one another at α = 0.05 level.

Figure 3. Annual bluegrass weevil adult covered in a petroleum-derived spray oil (Civitas; Suncor Corporation).

USGA ID#: 2019-06-676

Title: Understanding and optimizing sampling methods for the Annual Bluegrass Weevil

Project Leaders: Albrecht M. Koppenhöfer
Affiliation: Department of Entomology, Rutgers University, New Brunswick, NJ

Objectives:

The goal is to optimize the use and predictive power of sampling/monitoring methods for ABW adults. Specifically, we will determine the effect of temperature and mowing height on the percentage of adults detected (1) in the clippings of a mower, (2) by vacuuming with a leaf blower, and (3) by soap flushing. In addition, (4) we will determine the effect of water volume applied and detergent concentration on the extraction efficiency of the soap flushing method.

Start Date: 2019
Project Duration: 2 years
Total Funding: $19,910

Summary Points:

- Adult ABW recovery in mower clippings from a green was 15% without and 24% with a brush attached in front of the mower.
- Mower clippings from a fairway only recovered 0.2% of ABW adults.
- Vacuuming with a leaf blower recovered only 4.5% of adults from a fairway but 31% from a green.
- Soap-flushing with 500 ml of water containing 0.4% dish washing detergent recovered applied twice recovered 83% of adults from a fairway within 20 minutes.

Summary Text:

The annual bluegrass weevil (ABW) is the most important and difficult to control insect pest of short-mown golf course turf in eastern North America. Golf course superintendents have relied on synthetic insecticides for ABW management, but excessive insecticide use has led to widespread insecticide resistance to insecticides from several classes. It can be expected that overuse of any remaining effective synthetic insecticides will desensitize ABW to these compounds as well. Ultimately, golf course superintendents have to delay resistance development by applying control products only when and where necessary. That requires monitoring and sampling methods that are easy enough to use and fit into their busy schedules while still having a high predictive power. Currently available monitoring methods monitor the adult or the larval stage.

The quickest and most likely to be used monitoring methods involve sampling adults by vacuuming, soap flushing, or clippings examination. However, various factors are likely to influence the efficiency of these methods, particularly that of vacuuming and clippings examination including temperature and mowing height. The method least likely to be affected by environmental conditions and mowing height is soap flushes where water mixed with liquid dish washing detergent is applied to a specified area which irritates the adult to the surface and up the grass blades where they can be counted. For soap flushing, the effect of water volume and concentration of the detergent on extraction efficiency has yet to be examined.

In the first year of study, sampling methods were examined under warm conditions to allow for the optimization of extraction methods. Color-marked adults were released into turf plots about 1 hr before extractions started to allow the adults to settle in and distribute naturally (Fig. 1). In lab observations it had been found that the color powder adhered for several days to the adults without interfering with their behavior. In all experiments, adult recovery was tested in areas consisting of mix stands of annual bluegrass and creeping bentgrass mown at fairway (9 mm) and greens (3 mm) heights. After the plots were either mown or vacuumed, adult ABW were extracted from the plots with soap flushes. 500 ml water with 0.4% lemon scented dish washing detergent was distributed within a 30.5 x 30.5 sampling square at 0 and 5 min and adults collected for 20 min.

Recovery of adults in mower clippings from a Toro flex 21"mower was significantly affected by mowing height. Adults were recovered only sporadically in the fairway, and significantly more adults were recovered from the green (Fig. 3). However, the total number recovered from clippings and soap extraction was about twice as high from the fairway as from the green. Adults clearly dispersed more quickly from the release area on the green and some may have left the sampling area before sampling started. Relative to the total recovery, recovery from the clippings was only 0.2% from the green irrespective of attachment of a brush in front of the mower basket. At fairway height, significantly more adults were recovered with the brush (24% of total recovery) than without the brush (15%).

Recovery of adults by vacuuming (Fig. 2) was also significantly affected by mowing height. Adult recovery did not differ significantly between the treatments with one or two passages with the vacuum. But significantly more adults were recovered from the green than the fairway (Fig. 4). As in the mowing experiment, about half as many adults were recovered in total (including soap extraction) from the green as from the fairway, whether plots were vacuumed or not before soap extraction. Soap flushing alone recovered 83% of adults from the fairway but only 42% from the green. This was likely again because of faster dispersal of the adults out of the sampling area on the green. Relative to the total recovery, recovery by vacuuming was 4.5% from the fairway and 31% from the green.

Preliminary experiments indicated soap extraction efficiency increased with soap concentration and water volume used. Additional experiments in 2020 will further investigate these effects and also examine the effect of temperature on the efficiency of the sampling methods.

Fig. 1. Color marked ABW adults released into a fairway height creeping bentgrass plot.

Fig. 2. Vacuuming of ABW adult with a leaf blower next to plots from which adults are being extracted with soap flushes within sample squares.

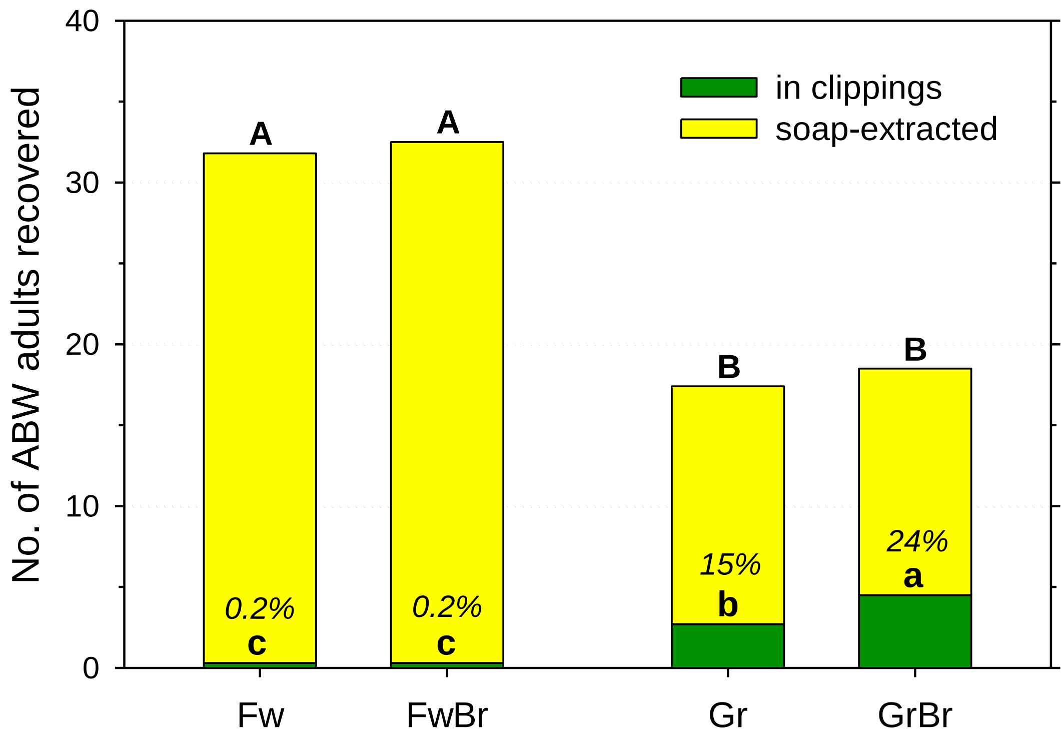


Fig. 3. Recovery of marked ABW adult from areas mown at fairway height (Fw) and greens height (Gr) using a mower with (Br) or without a brush attached to the mower basket. Letters indicate significant differences between the number of adults recovered from mower clippings (lower case) and from clippings and ensuing soap extraction combined (capital). Percentages within bars are percentages of recovery in clippings relative to total recovery.

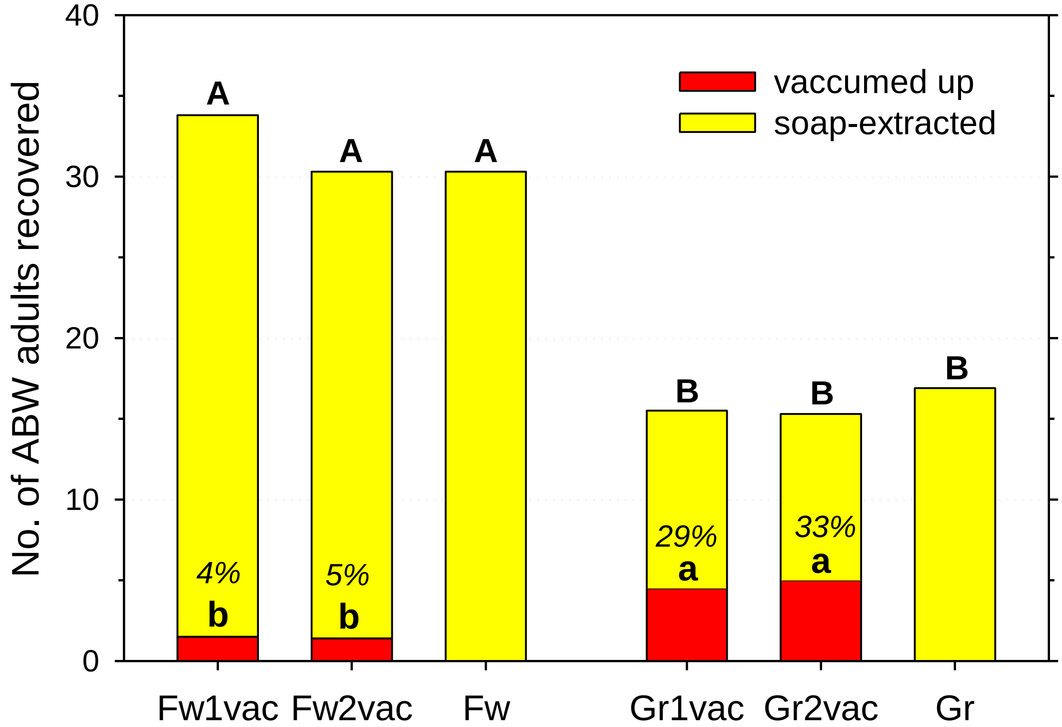


Fig. 4. Recovery of marked ABW adults from areas mown at fairway height (Fw) and greens height (Gr) by no, one (1vac) or two (2vac) passages with a with a vacuum followed by soap-extraction. Letters indicate significant differences between the number of adults recovered by one or two vacuum passages (lower case) and by vacuuming and ensuing soap extraction combined (capital). Percentages within bars are percentage recovery by vacuum relative to total recovery.

USGA ID#: 2018-07-657

Title: Developing Methods to Diagnose Herbicide Resistance in Goosegrass

Project Leader: James T. Brosnan, Ph.D. and José J. Vargas
Affiliation: University of Tennessee

Objective:

Develop diagnostic assays to screen mature goosegrass plants from golf course turf for resistance to various pre- and postemergence herbicides.

Start Date: 2018
Project Duration: 2 years
Total Funding: $78,800

Summary Points:

- Golf course superintendents have few means of confirming herbicide resistance in goosegrass in a timely manner leaving them little guidance regarding proper management in-season.
- Goosegrass biotypes suspected to be resistant to oxadiazon and foramsulfuron were collected from golf courses in hopes of developing new assays for detecting resistance to herbicidal inhibitors of PPO and ALS.
- Efforts to develop new assays were not successful. This suggests that poor performance of PPO and ALS inhibiting herbicides in the field may be due to other factors than resistance, particular those pertaining to the edaphic and atmospheric environment surrounding goosegrass at application.

Summary Text:

Once a problem in only the southernmost regions of the United States, goosegrass (*Eleusine indica*) has become a troublesome weed throughout the entire southern region, and has moved north into the transition zone and mid-Atlantic regions of the United States. Limited herbicide options for selective goosegrass control in these areas has driven selection pressure for goosegrass biotypes resistant to several different herbicidal modes of action, which serves to compound the problem of controlling goosegrass on golf courses. A two-year projected was initiated at the University of Tennessee in 2018 to develop diagnostic assays to screen mature goosegrass plants from golf course turf for resistance to various pre- and postemergence herbicides.

Protoporphorinogyen Oxidase (PPO) Inhibiting Herbicides: An electrolyte leakage assay developed for agronomic weeds was refined for use in screening mature goosegrass plants from golf courses for resistance to the PPO inhibiting herbicide oxadiazon. Thirty-two leaf segments of goosegrass biotypes suspected to be resistant and susceptible to oxadiazon were placed in plant tissue culture boxes (Magenta GA-7, Bioworld, Dublin, OH) filled with 100 mL of a solution containing the following: sucrose (2%), 1mM 2-(N-morpholino)ethane-sulfonic acid buffer (MES), a non-ionic surfactant (0.25%) and 100 µM of oxadiazon. Eight cucumber (*Cucumis sativus*) leaf segments (1 cm2) were included in similar plant tissue culture boxes as a positive control for comparison. This design provided 32 cm of cut leaf tissue (per species/biotype) in contact with herbicide-buffer solution. Electrolyte leakage was quantified by measuring electrical conductivity of the buffer-herbicide solution inside each plant tissue culture box before inclusion of leaf segments and then on four-hour intervals thereafter until 16 hours had elapsed. Initial pilot experiments suggested no benefit to incubating boxes in darkness prior to collecting electrical conductivity data nor collecting data beyond an exposure period of 16 hours. Data were collected using a conductivity cell (PYPC12S. Sartorius. Bohemia, NY) affixed to a electrochemistry meter (Model 250. Denver Instrument. Bohemia, NY) and expressed as change in electrical conductivity (ΔEC) over time. This experiment was replicated seven times during 2018 with data from each subjected to regression analysis in Prism (v7.0d). Interestingly, minimal increases in ΔEC where observed when exposing both goosegrass biotypes to oxadiazon, whereas ΔEC values for cucumber increased dramatically (Image 1). This response suggests that oxadiazon may not be the ideal herbicide to use in developing a diagnostic assay to screen goosegrass for resistance to PPO inhibitors. Additionally, this response also suggests that poor oxadiazon performance observed by golf course superintendents may be due to factors other than PPO resistance, such as enhanced soil degradation or site-specific environmental variability (i.e., rainfall, application timing, etc).

Acetolactate Synthase (ALS) Inhibiting Herbicides: A biotype of goosegrass with putative resistance to ALS inhibiting herbicides was collected during 2018. Our objective was to confirm ALS-resistance in this biotype during 2019 such that it could be used as a resistant-standard in developing an agar-based assay to screen for ALS resistance in goosegrass. Dose-response experiments were conducted at the University of Tennessee (Knoxville, TN) during summer of 2019 comparing this putative-resistant (PR) goosegrass to a biotype known to be susceptible (S) to foramsulfuron. Goosegrass was surface seeded into 8.9 cm3 greenhouse pots filled with Sequatchie silt loam soil amended with calcined clay in a 4:1 soil-to-clay ratio. After establishment, pots were thinned to contain three plants and treated with foramsulfuron at 0, 5.5, 11, 22, 44, 88, 176, 352 g ha-1 using an enclosed spray chamber calibrated to deliver 215 L ha-1. Plants were at a three-tiller growth stage when herbicide was applied. Goosegrass control was visually assessed 63 days after treatment using a 0 (i.e., no control) to 100% (i.e., plant death) scale. Additionally, aboveground biomass was harvested at the soil line, dried in a forced-air oven at 55 C, and weighed. Treatments were arranged in a randomized complete block design with four replications; the experiment was conducted twice during the summer of 2019. Data from each experiment were subjected to log-logistic regression analysis in Prism (v 8.0) to determine the relationship between PR and S goosegrass in response to increasing doses of foramsulfuron. Few differences in visual control were detected among biotypes and those present were inconsistent across experimental runs. Moreover, no differences in aboveground biomass were detected among PR and S goosegrass treated foramsulfuron at rates of 5.5 to 352 g ha-1. Our findings indicate that PR goosegrass is susceptible to foramsulfuron and not suitable for use as a resistant-standard in an agar assay.

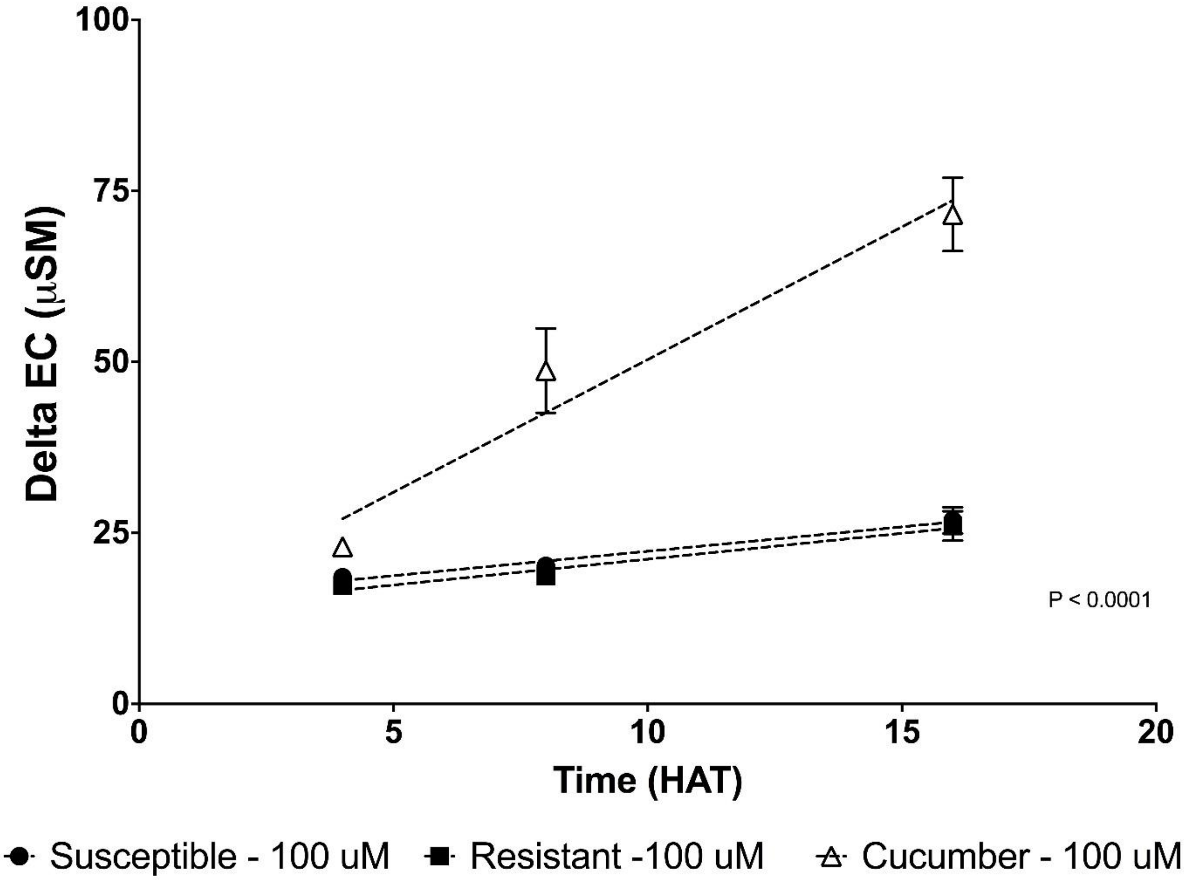


Figure 1. Change in electrical conductivity (ΔEC) from 0 to 20 hours after treating susceptible or resistant goosegrass biotypes and cucumber with oxadiazon.

USGA ID#: 2018-17-667

Title: Creeping Bentgrass Injury Potential from Carfentrazone-Ethyl Following Treatment with Bensulide

Project leader: Roch Gaussion and Zane Raudenbush
Affiliation: University of Nebraska-Lincoln and Ohio State University ATI

Objectives:

1) Determine the safe-application interval for carfentrazone-ethyl at 2.0, 3.3, or 6.7 fl oz/A on creeping bentgrass previously treated with bensulide.
2) Determine if irrigation timing following bensulide application is the true culprit of reports of injury.
3) Determine the duration of injury following applications that result in injury.

Start Date: 2018
Project Duration: 2 years
Total Funding: $20,000

Summary Points:

- Results indicate superintendents can apply carfentrazone-ethyl 14 days after a spring application of bensulide.
- If superintendents wish to apply carfentrazone-ethyl within 30 days of a bensulide treatment, then we recommend treating small “test areas” to determine if injury is likely to occur.
- Applying carfentrazone-ethyl within 7 days of bensulide will potentially cause injury.
- Phytotoxicity caused by an application of carfentrazone-ethyl was transient and caused no lasting effects on turfgrass quality.

Summary Text:

Carfentrazone-ethyl (Quicksilver® T&O) is the most commonly used herbicide for controlling silvery-thread moss (*Bryum argenteum* Hedw.) in creeping bentgrass (*Agrostis stolonifera* L.) putting greens. The herbicide exhibits strong selectivity as creeping bentgrass rapidly metabolizes carfentrazone-ethyl following an application. However, creeping bentgrass injury has been reported if carfentrazone-ethyl is applied soon after treatment with bensulide (Bensumec™ 4LF). Bensulide is a preemergent herbicide applied in the spring and early fall for crabgrass and annual bluegrass control in putting greens, respectively. The pesticide labels for Bensumec and Quicksilver do not restrict the use of these two products on the same turfgrass site; however, the general recommendation is withhold the application of carfentrazone-ethyl for 45 days following an application of bensulide. Currently, superintendents who apply bensulide in the spring months for crabgrass control are hesitant to use carfentrazone-ethyl for silvery-thread moss control due to the issues discussed previously. This is undesirable because silvery-thread moss is extremely competitive during the spring when temperatures are moderate and precipitation is plentiful. Therefore, our research aimed to determine the safe interval for an application of carfentrazone-ethyl following treatment with bensulide.

In the first year of this research, bensulide was applied at 7.3 fl oz/1000ft^2 in the springtime to creeping bentgrass putting greens in Creston, OH and Lincoln, NE. Following the application of bensulide, carfentrazone-ethyl was applied at three labeled rates (2.0, 3.3 and 6.7 fl oz/A) at 0, 1, 3, 7, 14, 21, 35, 49, or 63 days after treatment (DAT) with bensulide. A minor amount of phytotoxicity was observed when the highest rate of carfentrazone-ethyl was applied at 0, 1, and 3 days after bensulide, but the effects were short-term and undetectable by 7 DAT. The study was repeated 2019, however, the carfentrazone-ethyl 3.3 fl oz/A rate was eliminated and a rate of 13.4 fl oz/A was included in the study to simulate an overapplication from excess boom overlap while also providing inference about the role of herbicide metabolism as it relates to phytotoxicity.

In 2019, field studies were conducted from May-August on putting greens built to USGA specifications containing ‘007’ and ‘Alpha’ creeping bentgrass in Creston, OH and Lincoln, NE, respectively. Putting greens were mowed 6 d wk^{-1} at 0.125 inches and received regular topdressing and fertilizer applications. Plots were irrigated as needed to prevent drought stress and fungicides were applied preventatively to control disease. Treatments were evaluated using a 2 (preemergence herbicide) × 3 (carfentrazone-ethyl rate) × 8 (carfentrazone-ethyl timing) factorial treatment structure in a randomized complete-block design. Preemergence herbicide levels were 1) Bensulide (Bensumec 4LF) at 7.3 fl oz/1000ft^2 irrigated with 0.5 inches of water immediately after application, and 2) untreated. Levels of carfentrazone-ethyl rate were 2.0, 6.7 and 13.4 fl oz/A. Carfentrazone-ethyl timing levels were achieved by applying each rate at 0, 1, 3, 5, 7, 10, 14 or 21 days after treatment with bensulide. Treatments were applied to 3 × 5 ft. plots using single nozzle, CO_2-powered sprayer with a spray volume of 80 gal/A (Figure 1). Bensulide was applied on May 2 in Ohio and Nebraska. Turfgrass visual quality (1 = necrotic, dead turf; 6 = minimum acceptable quality; 9 = optimum turf quality) was rated weekly throughout the growing season when applications were complete. Normalized difference vegetation index (NDVI) measurements were recorded weekly using a hand-held active crop canopy meter (CS-45; Holland Scientific, Lincoln, NE).

Results indicated at 7 DAT, an application of carfentrazone-ethyl following spring-applied bensulide did not significantly reduce turfgrass visual quality or NDVI in Nebraska, regardless of carfentrazone application rate or timing. In Ohio, plots treated with carfentrazone-ethyl at 2.0 and 6.7 fl oz/A had no reductions in turfgrass quality or NDVI at 7 DAT at any application timing. Interestingly, plots treated with 13.4 fl oz/A of carfentrazone-ethyl had reductions in quality at 7 DAT when applied 0, 1, 3 and 5 days after treatment with bensulide (Fig 2); no injury was observed in the absence of bensulide. By 14 DAT, the initial reduction in quality was no longer detectable. No reduction in turfgrass quality was observed in Ohio for any application rate of carfentrazone-ethyl applied 7 days after bensulide or later. These results indicate superintendents can apply carfentrazone-ethyl 14 days after spring-applied bensulide with minimal concerns of phytotoxicity; however, the differences in response to creeping bentgrass treated with 13.4 fl oz/A of carfentrazone in Ohio and Nebraska indicate potential differences in sensitivity between creeping bentgrass cultivars. If superintendents wish to apply carfentrazone within 30 days of a bensulide treatment, then we suggest treating small test plots on several putting greens to determine if injury will occur. In our research, injury from an application of carfentrazone was detectable within 1-2 days following the application; therefore, superintendents should feel confident to treat all putting surfaces with carfentrazone if no injury was observed in the test plots. Lastly, injury from the application of carfentrazone was short-lived and nearly undetectable 7 days after treatment.

Figure 1. Catch-cups positioned in untreated plots to confirm 0.5 inches of irrigation were applied immediately following application of bensulide in Creston, OH on May 2, 2019.

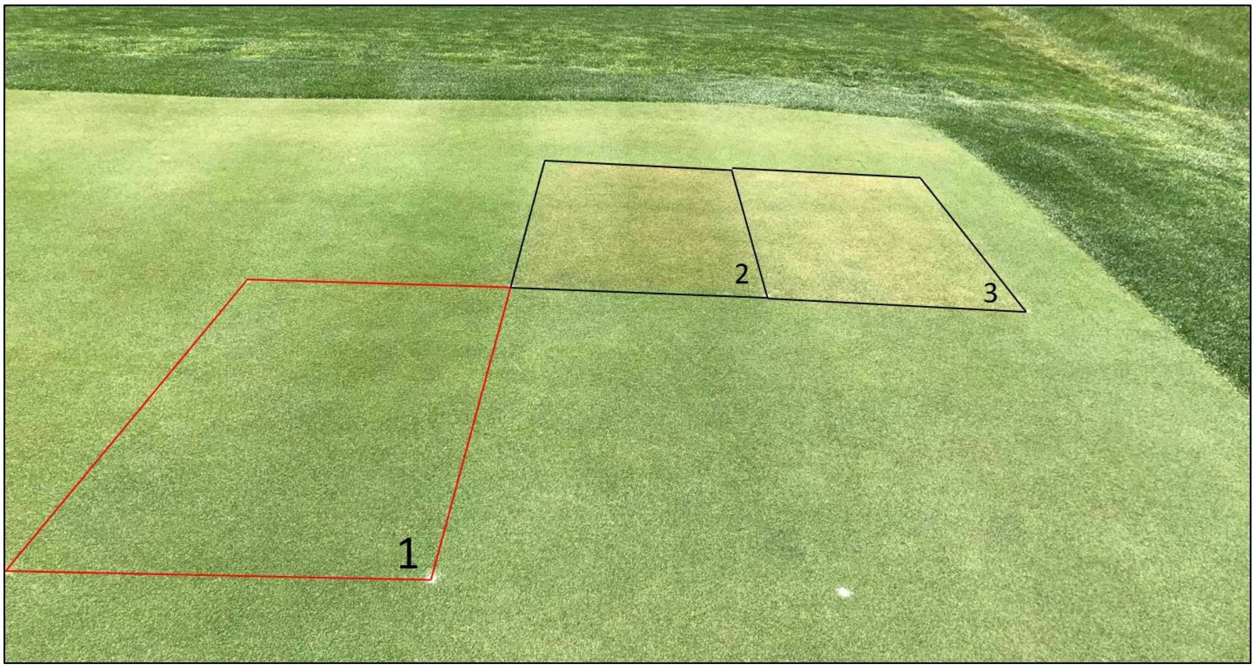


Figure 2. Herbicide injury symptoms on '007' creeping bentgrass (*Agrostis stolonifera*). Plots descriptions indicated by number in the bottom right corner: 1) 13.4 fl oz/A of carfentrazone-ethyl applied to untreated plot on May 2; 2) 13.4 fl oz/A of carfentrazone-ethyl applied 0 days after bensulide (May 2); 3) 13.4 fl oz/A of carfentrazone-ethyl applied 3 days after bensulide (May 5). Photo captured on May 8.

USGA ID#: 2019-07-677

Title: Progress toward solving the silvery-thread moss issue in cool-season putting greens

Project Leaders: Llo Stark, Zane Raudenbush, Matthew Johnson, Joshua Greenwood
Affiliation: University of Nevada Las Vegas, Ohio State University, Texas Tech University

Objectives:

1. Using carfentrazone and a surfactant, determine the concentration, exposure time, and light environment required for lethality of STM.
2. Determine if lab testing is consistent with field conditions of putting greens in Ohio.
3. Investigate the possibility of resistant strains of STM by genetic analyses.

Start Date: 2019
Project Duration: 3 years
Total Funding: $119,991

Summary Points:

1. Lab experiments using STM (silvery-thread moss) treated with Carfentrazone (CZ) at different light levels suggest that the moss is most vulnerable to CZ applied in full sunlight.

2. STM from both golf greens and from native habitat is very sensitive to the surfactant sodium docecyl sulfate (SDS): even a one minute exposure at 0.5% concentration killed the moss.

3. Common anionic surfactants suppressed silvery-thread moss growth in experimental golf course putting greens.

4. DNA was extracted from a male and a female clone of *Bryum argenteum* from Kentucky for long-read genome sequencing.

Summary Text:

Rationale
Silvery-Thread Moss (STM, *Bryum argenteum*) is an undesirable weedy species that has colonized golf greens across the USA and has proven difficult to erradicate. Our group of four researchers (Stark, Raudenbush, Johnson, and Greenwood) from three institutions (UNLV, Ohio State U., Texas Tech U.) initiated lab and field studies to (1) test the effectiveness of a surfactant-based product (*Dawn Ultra* dishsoap) and a moss suppressant on the market (*Quicksilver*, known as Carfentrazone-ethyl, CZ here) on the growth response and photosynthetic health of STM; (2) determine the effect of CZ at different light intensities; (3) determine the effect of a single known surfactant (sodium dodecyl sulfate, SDS, ($CH_3(CH_2)_{11}SO_4Na$)) on moss growth in both putting green and laboratory settings; and (4) isolate high quality DNA for sequencing the genome of this moss.

Methodology
In the lab, we dosed the moss cultures with two concentrations of CZ (5× and 25× suggested exposures, based on pilot experiments, one hour exposure) at three different light intensities (1000, 1500, 2000 PAR, Photosynthetic Active Radiation, 10 hour exposure). The moss cultures were derived from both putting green isolates and native field isolates and cultured to single clonal lines several months old. A second lab experiment consisted of dosing moss cultures with a one-minute exposure to a 0.25 and 0.50% solution of the surfactant SDS (these exposures based on pilot experimental results). Response variables for both experiments included chlorophyll fluorescence 24 hours post-exposure (maximum photochemical efficiency of dark-adapted photosystem II, or F_v/F_m, an indication of current plant health and stress level) and regrowth observations conducted daily for 25 days (capacity of plants to regenerate), and these data were compared to untreated control mosses.

In the field on a nursery putting green at the Sharon Club golf course in Sharon Center, OH having a current STM infestation, a completely randomized design with four replications was used to evaluate three treatments: solution of *Dawn Ultra* dishsoap, CZ applied at 6.7 fl. oz./acre, and untreated control. Treatments were applied on 8 July 2019 and rated 30 days post-treatment using deviations from initial moss cover and a rating grid with 330 intersections; a count was recorded if a moss colony was located beneath a grid intersection. In a second field experiment on a practice green (creeping bentgrass) at Hawks Nest Golf Course in Creston, OH with a current moss infestation, surfactant effects were tested as follows. Both *Dawn Ultra* dishsoap and the isolated surfactant SDS were applied as a drench on 25 July 2019 and general visual observations recorded.

In order to address our objective of assessing potential resistance of the moss to CZ and surfactants, we need to sequence the genome of *Bryum argenteum* and statistically compare genomes from putting green isolates with native (off-green) isolates. Using two single clone isolates (a male and a female) from native habitats from Kentucky, *Bryum argenteum* gametophyte tissue was ground with liquid nitrogen using a mortar and pestle. High molecular weight DNA was extracted using the modified Doyle and Doyle CTAB/chloroform method in a 15 mL tube with minimal agitation to preserve large DNA fragments. Genomic DNA was preciptated using isopropyl alcohol and eluted in 25 µL 0.1 M Tris-EDTA pH 8. This method resulted in 2.75 µg of DNA, half of which was submitted to RTL Genomics (Lubbock, Texas) for sequencing.

Results to date
1. High light intensity (2000 PAR, equivalent to full sunlight during summer) increases the effectiveness of CZ relative to partially shaded conditions (1000 and 1500 PAR). This result suggests that golf course superintendents should perform applications of CZ on clear days. Although CZ applications in the lab inhibited regrowth rates of new shoots and protonema of the moss, CZ (as *Quicksilver*) ultimately was not effective in killing moss samples. No differences in response were noted between golf course and native isolates, so the data were pooled (Figure available upon request).

2. After a one minute dose of 0.50% SDS for both putting green and native isolates of STM, moss shoots were killed (**Figure 1**). High magnification observations using a compound microscope revealed the lysing of individual cells. Interestingly, golf course isolates of the moss were more resistant to SDS at the lower concentration (0.25% SDS), and golf course isolates had higher levels of control chlorophyll fluorescence than the native isolates.

3. In the field experiment at Sharon Club, both *Dawn Ultra* and *Quicksilver* (CZ) produced a significant reduction in percent cover relative to the controls 30 days post-exposure (**Figure 2**). No differences in moss cover were reported for plots treated with *Dawn* Ultra and *Quicksilver*; however, we note that a significant amount of necrotic moss tissue was observed in plots treated with *Dawn Ultra* while a majority of the moss was recovering in plots treated with *Quicksilver* at 30 days post-exposure. This indicates that the mosses are able to regenerate on a delayed basis after treatment with *Quicksilver*.

4. In the field experiment at Hawks Nest GC, creeping bentgrass injury increased as the concentration of the surfactants increased. All products and concentrations severely injured moss shoots within 1 day of application (**Figure 3**). No differences in symptomology or injury were observed between the two formulations of *Dawn Ultra* dishsoap.

5. High-molecular weight DNA was successfully extracted from two accessions of *Bryum argenteum* from a wild population in Kentucky (Univ. of Kentucky Lexington campus) that included one female clone and one male clone (**Figure 4**). The DNA was submitted for genome sequencing using the long-read method of Pacific Biosciences; funds were used to pay for one library preparation and two SMRT cells.

Future expections for project
Dawn Ultra contains several surfactants and their concentrations are trade secrets. Future research aims to determine if specific surfactants can provide the greatest moss suppression and be readily available to turfgrass managers. Our recommendations will be based on both field putting green and lab experiments. Future research will evaluate SDS concentrations at various application timings throughout the growing season and their effectiveness at controlling silvery-thread moss. Data for genome assembly is expected in early 2020, and will provide context for accurate genotyping of wild and golf course populations.

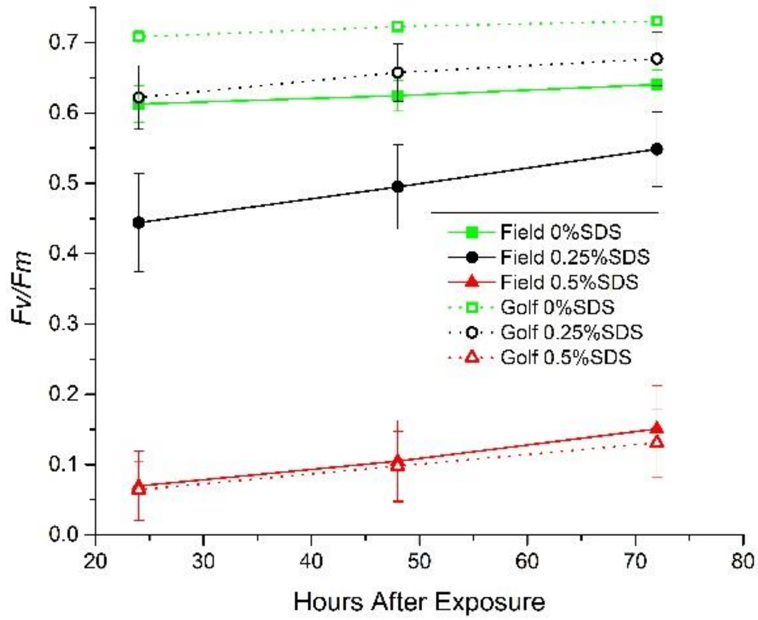


Figure 1. Effect of surfactant (SDS) at three concentrations (0, 0.25, 0.50%) on golf course and native isolates of the moss *Bryum argenteum*, assessed by chlorophyll fluorescence (*Fv/Fm*) daily following exposure for three days.

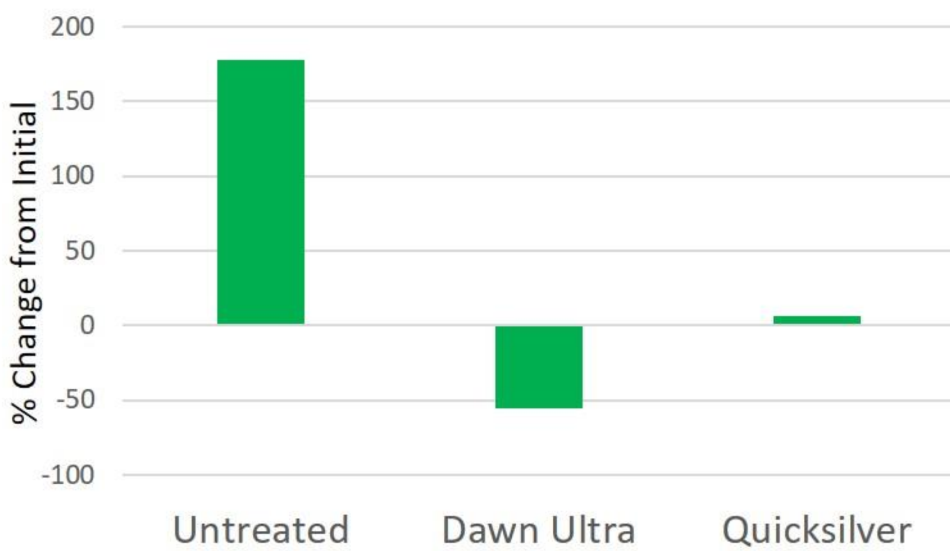


Figure 2. Effects of *Dawn Ultra* and *Quicksilver* on silvery-thread moss cover in a creeping bentgrass putting green.

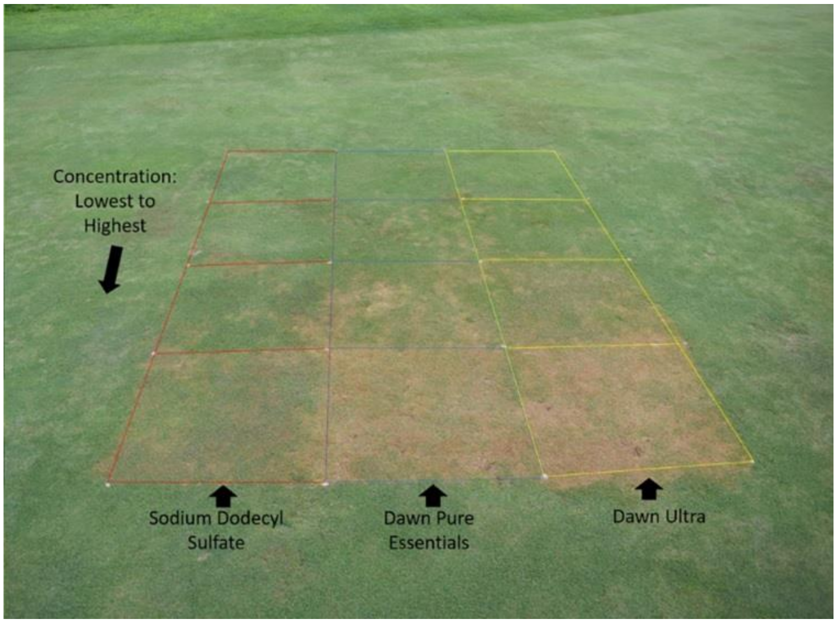


Figure 3. Injury to creeping bentgrass following an application of sodium dodecyl sulfate (SDS) and two *Dawn* dishsoap products.

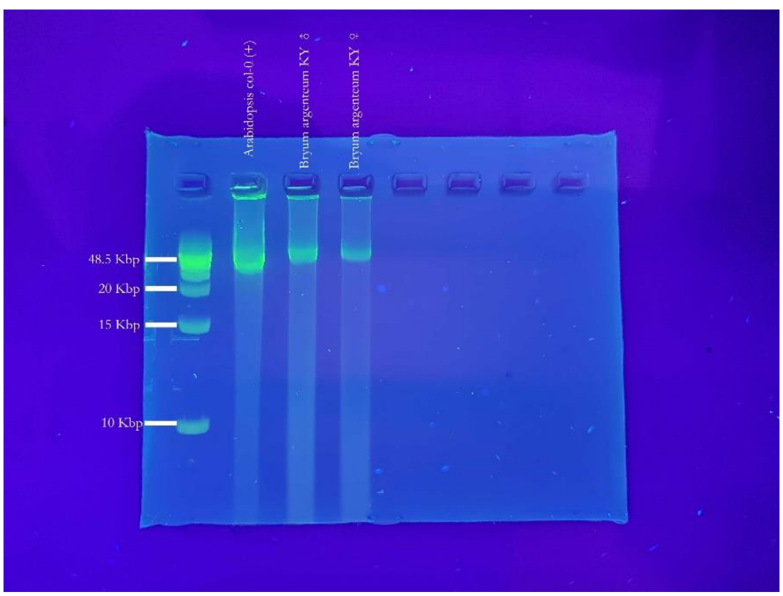


Figure 4. High Molecular Weight DNA extraction from two *Bryum argenteum* tissue cultures and a positive control from *Arabidopsis thaliana*. Ladder (left) shows size of DNA in kilobasepairs.

3. ENVIRONMENT

USGA ID#: 2014-14-503

Title: Water treatment and remediation using a bioreactor

Project Leader: Klaus Doelle
Affiliation: State University of New York, College of Environmental Science and Forestry

Objectives:

1. Investigate if bacteria cultures of a constructed wetland can remediate pharmaceutical compounds in waste water.
2. Investigate the best treatment sequence for pharmaceutical removal.
3. Investigate a treatment sequence for the removal and degradation of pharmaceutical, chemical, and organic compounds in various wastewater types.

Start Date: 2014
Project Duration: 5 years
Total Funding: $59,995

Summary Points:

- Designed, installed and operated a laboratory benchtop bioreactor and large scale bioreactor system
- Both the laboratory benchtop bioreactor and large scale bioreactor system can meet a waste water treatment plants effluent discharge limit.
- Tested large scale bioreactor system can treat, assuming year-round 24/7 operation 8,760,000 gallons or 26.8 acre feet of waste water yearly for irrigation purpose use on golf courses.

1. Introduction:

The increasing needs for water resources for residential, commercial and industrial use accelerate the depletion of the water resources. Reuse of the effluent water before and after treatment can becomes an effective solution to the shortage of the water resources. Currently golf courses require on average of 48.2 acre feet to 386.2 acre feet of water for irrigation purpose annually. In spite of using fresh water or well water, needed irrigation water might come in the future from: **(i)** storm runoff from impervious surfaces captured in retention ponds, **(ii)** high flow (flood) water diversion into storage ponds, **(iii)** secondary or tertiary effluent from a Waste Water Treatment Plant (WWTP), **(iv)** grey water, and **(v)** treated or raw water from a local public water supply distribution systems.

In this study we have evaluated the use of municipal waste water (MWW) using a Laboratory Benchtop Bioreactor System (LBBS) and a large-scale bioreactor system (LBS) arrangement.
A laboratory benchtop biotower was designed and tested with wastewater in the laboratory. At the same time a large scale biotower was designed, installed, started-up and tested with municipal wastewater at the Village of Minoa Clean Water Educational Research Facility (CERF) located at the Village of Minoa Waste Water Treatment plant (WWTP).

2. Materials and Testing:

For this study pre-clarified municipal wastewater was used. 30ml samples were collected from the influent and effluent and tested with a HACH DR900 spectrophotometer and Hach COD TNTplus Vial Test (3-50.0mg/L), TP TNT Reagent Set (1-100.0mg/L) and NH_4-N TNT Reagent Set (0.4-50.0mg/L).

3. Laboratory Benchtop Bioreactor System:

A Laboratory Benchtop Biorreactor System (LBBS) was designed according to Figure 1. The LBBS was started up and the function ability tested before tests with WW were conducted.

For starting up the LBBS cow manure at a consistency of 12% was diluted 100 times with tap water. 10 liter of the diluted cow manure (CM-suspension) was put into the reservoir (a) and den pumped with pump (b) at a rate of 0.5 l/min trough pipe (c) to the distributer (e). The flow was regulated with valve (d). The distributer (e) trickled the CM-suspension onto the growth media (g) were bacteria contained in the cow manure started to grow removing the contaminants contained in the CM-suspension. After the CM-suspension made its way through the growth media (g) it was transferred back with pipe (h) into the reservoir (b). The LBBS was operated in this way for 3 weeks till the growth media was covered with a brown coat of bacteria.

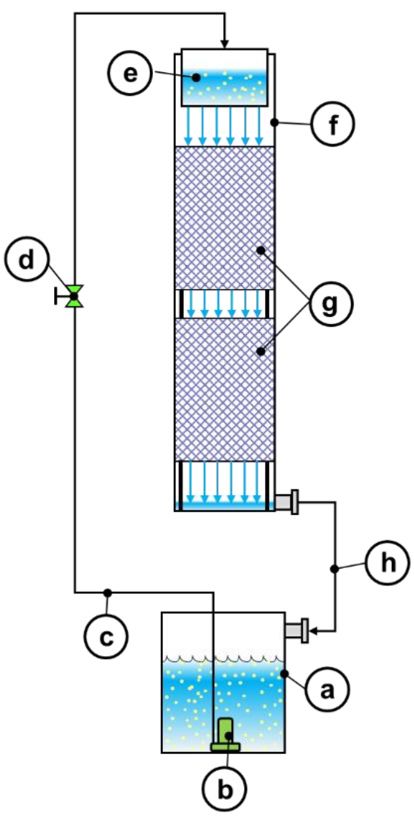


Figure 1: Laboratory Biotower; a) Reservoir, b) Pump, c) Feed Pipe, d) Valve, e) Distributor f) Reactor Vessel, g) Growth Media, h) Return Pipe.

3.1. Results:

Figure 2 showed that under different flow rates in the LBBS, Chemical Oxygen Demand (COD), Total Phosphorus (TP) and Nitrogen ammonia (NH_3-N) concentration decreased dramatically at first 8-10 hours. At flow rate 0.6 l/min, COD concentration dropped from 55 to 7mg/l, TP dropped from 1.6 to 0.3mg/l and NH_3-N dropped from 9.2 to 0.4 mg/l. At a flow rate 0.8 L/min, COD concentration dropped from 130 to 32mg/l, TP dropped from 5.4 to 0.5 mg/l and NH_3-N dropped from 12.5 to 8 mg/l and NH_3-N dropped from 7.6 to 0.3 mg/l. At a flow rate 1.5 L/min the COD concentration dropped from 57 to 11mg/l, TP dropped from 3.7 to 0.6 mg/l and NH_3-N dropped from 11.5 to 0.6 mg/l. After 10 hours. , their concentration kept at a very low level. In this case, bioreactor significantly reduced COD, Total phosphorus and Nitrogen ammonia content in wastewater at different flow rates.

Also, as the flow rate increased, the COD percentage reduction decreased as shown in Table 1. This may be due to a shorter residence time of the wastewater in the bioreactor. Microorganisms might not have enough time to degrade the COD. Also, insufficient oxygen supply in the tower might have an effect on the microorganisms, inhibiting an effectively degradation of COD. N reduction was found to be between 94% and 96%, whereas P reduction was between 69% and 87%.Overall, depending on the daily WW supply the LBBS was able to achieve effluent permit levels of TP of 0.8 mg/l, NH_3-N of 0.5 mg/l, and COD <4 mg/l.

Table 1. COD, TP and NH_3-N reduction at different flow rate.

Flow rate(L/min)	COD reduction percentage	N reduction percentage	P reduction percentage
0.6	87%	96%	81%
0.8	78%	94%	87%
1	70%	96%	69%
1.5	81%	95%	84%

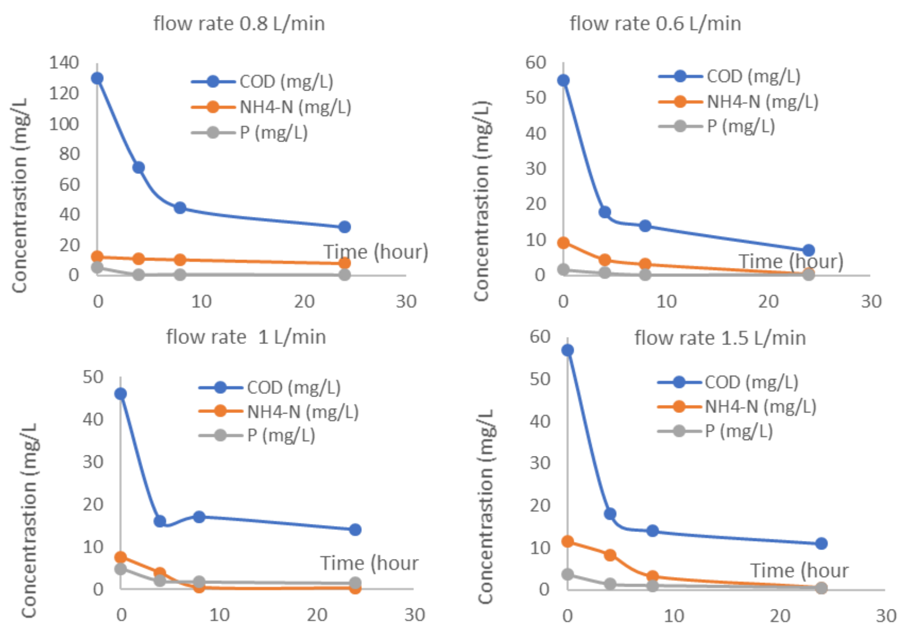


Figure 2: COD, TP and NH_3-N concentration at different time under different flow rate.

4. Large-Scale Bioreactor System:

A large-scale bioreactor system (LBS) was designed according to Figure. 3 for testing the removal of compounds contained in the waste water. The LBS was installed at CERF located at the Village of Minoa wastewater treatment plant.

The LBS features a 4 foot diameter and 13 foot above ground tank with a volume of 1150 gallons, that holds the bacteria growth media, and an underground recirculation tank with 4 foot diameter and 4 foot tall tank holding 375 gallons of waste water. Both tanks were installed by the Minoa Department of Public works (DPW) and CERF personnel according to local regulations. The tanks were built from commercial available concrete manhole structures. The Feed pipe was connected to the WWTP primary clarifier discharge tank, and the effluent pipe was connected to the primary clarifier discharge tank of the WWTP to make sure that no wastewater (treated and untreated) can exit the plant.

The LBS was started up using diluted cow manure diluted with municipal wastewater as an inoculum, in accordance to the laboratory test unit described above. Once the underground tank was filled with the start-up inoculum (SUI) the SUI was pumped with pump (b) at a constant rate of 40 gal/min trough pipe (c) to the distributer (e) which tricked the inoculum equally onto the growth media (e) contained in tank (f). The flow was regulated with valve (d). The distributer (e) trickled the CM-suspension onto the growth media (g) were bacteria contained in the cow manure and wastewater inoculum started to grow removing the contaminants contained in the inoculum suspension. After the suspension made its way through the growth media (e) it was transferred back with pipe (g) into the underground reservoir (a). The airflow into the LBS was controlled by valves (j). The LBS was operated this way for 3 weeks till the growth media was covered with a brown coat of bacteria, replacing the inoculum periodically every 4 to 6 days. After the inoculum phase the LBS was fed with pre-clarified wastewater from the clarifier tank at a rate of 2,000 gallons per day (83.3 gal/h). The treated wastewater was discharged from the underground tank at the same rate as the feed rate of the reactor to the primary clarifier discharge tank for further treatment. The feed rate to the LBS was increase after 2 weeks to 4,000 gal/d (166.66 gal/h), 8000 gal/h (333.33 gal/h), 12,000 gal/h (500 gal/d), and 16,000 gal/d (666.66 gal/h) respectively.

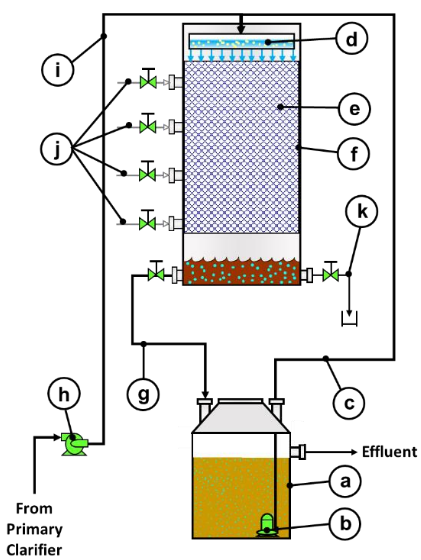


Figure 3: Large Scale Biotower at CERF; a) Reservoir, b) Variable Speed Recirculating Pump, c) Feed Pipe, d) Distributor, e) Growth Media f) Reactor Vessel, g) Return Pipe h) Feed Pump, i) Feed Pipe, j) Air Supply Valve, k) Drain Pipe.

4.1. Results:

Table 2 shows the reduction of COD and NH_3-N achieved with the LBS at a feed rate of 2,000 gal/d and 16,000 gal/d. COD reduction varied between 55.2% and 91.6%. $_{NH3-}$N reduction varied between 91.8 and 99.3 %. The variation between the individual feed rates can be explained with the different influent concentration of COD and NH_3-N. The study also showed that the LBS can produce an effluent that meets effluent standards of a WWTP of NH_3-N of 0.5 mg/l, and COD <4 mg/l.

Table 1: COD and NH_4-N reduction at different flow rate.

Flow rate(gal/d)	Influent COD [mg/l]	Effluent COD [mg/l; %]]	Influent NH_3-N [mg/l]	Effluent NH_3-N [mg/l, %]]
2,000	145	65; 55.2	21.6	0.2; 99
4,000	120	10; 91.6	18.5	0.6; 96.8
8,000	115	42; 63.5	30.2	1.8; 94.0
12,000	140	37; 73.6	28.8	0.2; 99.3
16,000	125	22, 82.4	22	1.8; 91.8
24,000	125	22; 82.4	22	1.8; 91.8

Temperature might also play a role in the degradation of COD and NH3-N. However the influent temperature of the waste water to the LBE is constant at 55°F. Operation of the LBS is possible at temperatures above 68°F and as low as 20°F as shown in Figure 4.

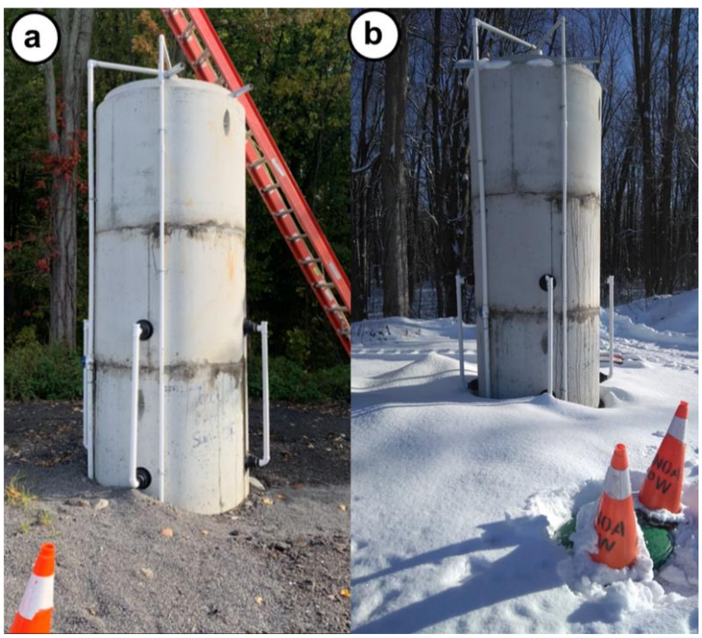


Figure 4: Large Scale LBS at CERF; a) operating at 70°F, b) operating at 20°F.

5. Conclusion:

In this study it is shown that a LBBS can reduce COD between 70%-80% COD, over 90% TP and over 80% of Nitrogen ammonia under flow rate at 0.6, 0.8, 1.0, 1.5 L/min flow rate in 10 hours. A designed LBS system is able to treat up to 24,000 gallons per day achieving a COD and NH_3-N reduction between 55.2% and 91.6%, and 91.8 and 99.3 % respectively. Both the LBBS and LBS systems can meet a WW effluent discharge of NH_3-N of 0.5 mg/l, and COD <4 mg/l.

In addition, one LBS as tested with 154 cuft of growth media can treat, assuming year-round 24/7 operation 8,760,000 gallons or 26.8 acre feet of waste water yearly for irrigation purpose use on golf courses. This represents approximately 55% of the average 48.2 acre feet minimum yearly irrigation needs of a golf course.

If designed with a 10 ft inside diameter 177 acre feet of waste water can be treated yearly for irrigation purpose which is approximately 46% of the maximum average irrigation requirement, based on 384 acre foot, for golf course irrigation annually.

USGA ID#: 2017-27-637

Project Title: Examining the Response of Golf Course Lentic Ecosystems to Insecticide and Nutrient Additions Using Survey and Experimental Approaches

Project Leaders: Joseph Milanovich, Ph.D. and Martin Berg, Ph.D.

Affiliation: Loyola University Chicago

Objective:

The objectives of this research were to: 1) conduct a survey to quantify water quality and biotic communities of lentic turfgrass ecosystems across 25 courses within the Chicago Metropolitan area, and 2) use data from survey efforts to inform an experimental design to mechanistically examine whether additions of pesticides and/or nutrients (nitrogen and phosphorus) have measurable impacts on turfgrass lentic water quality and ecosystem communities.

Start Date: 2017
Duration: 3 years
Total Funding: $82,053

Summary Points:

Golf courses in the United States have long been considered to play a significant role in maintaining and enhancing local biodiversity – particularly when the adjacent landscape is dominated by anthropogenic land-use (e.g., urbanization, agriculture). In the face of global change, managed areas that can harbor native biodiversity are crucial for supplying source populations to adjacent areas and for maintaining ecological processes and ultimately, ecosystem integrity. During the first two years of our study, we quantified water quality and chemistry, algal concentrations, and micro-and macroinvertebrate and amphibian diversity and density across 25 golf course lentic ecosystems (herein ponds) and compared those to the same parameters in ponds located within adjacent forest preserves (n = 30; 15 permanent ponds with fish and 15 fishless ephemeral ponds). In addition, we implemented a mesocosm-based experiment investigating the influence of pesticide and nutrient additions on aquatic ecosystems. During the third year of our study, we implemented a mesocosm-based experiment investigating the combined influence of pesticide and nutrient additions on aquatic ecosystems. In short, are results are summarized below.

<u>Year 1 Summary Points</u>

- Concentrations of analytes (fungicides, insecticides and herbicides) measured in golf course ponds were low and infrequently detected for 8/10 analytes examined. Azoxystrobin, a fungicide, was the most widespread analyte measured.
- Amphibian diversity was low across the region and was similar in golf course ponds and ponds in forest preserve habitats.
- Concentrations of algae (both green [Chlorophyta and Charophyta] and blue-green [Cyanobacteria] algae) were similar across golf course and forest preserve ponds.

- Water quality measures were measurably different between golf course ponds and ephemeral wetlands, but not between golf course ponds and permanent forest preserve ponds containing fish.
- These results suggest golf course ponds provide similar aquatic ecosystems to more natural, forested ecosystems. Further examination is required to fully examine the degree to which these ecosystems are similar or different.
- Initial results suggest macro- and microinvertebrate diversity is similar in golf course ecosystems compared to natural forest preserve ponds/ephemeral wetlands

<u>Year 2 Summary Points</u>

- Azoxystrobin had no measurable, consistent influence on aquatic organisms (biofilms or amphibians)
- Nitrogen and N+P additions led to an increase in size and development rate of American toads

<u>Year 3 Summary Points</u>

- Azoxystrobin had no measurable, consistent influence on aquatic organisms (biofilms or amphibians)
- Nitrogen and N+P additions led to an increase in size and development rate of American toads
- The combination of these compounds had similar effects as when stand alone.

Summary Text:

Year 1: 2017-2018

Water quality/chemistry and algae: We examined concentrations of 10 analytes (name, minimum detection limit [ppb]) within each of the 25 course ponds and seven accessible course inflows (e.g., courses with accessible wells or lotic systems filling course ponds) in April and August 2017: Azoxystrobin (0.50), Bifenthrin (0.20), α-Chlordane (0.20), γ-Chlordane (0.20), Chlorpyrifos (0.20), Cypermethrin (1.00), Oxadiazon (0.50), cis-Permethrin (1.30), trans-Permethrin (1.30), Fenvalerate (0.30). Azoxystrobin, α-Chlordane, and Oxadiazon were the only compounds above the detectable limit in any of the 25 courses or course inflows in August (Table 1), and no detections occurred in April samples. Nearly 30% of course inflows we examined (3/7) had detectable compounds that were likely contributing to the concentrations found in course ponds. Concentrations of chlorophyll *a* were only significantly different between ephemeral ponds and forest preserve/golf course sites in August (Fig. 1), whereas concentrations of phycocyanin (blue/green algae) were not measurably different between any pond type. Water quality variables taken with a YSI multi-probe meter show significant differences between golf course and ephemeral wetlands, but not between golf courses and forest preserve ponds (Fig. 2). Water chemistry values, i.e., nitrate, ammonium and phosphorus show ephemeral wetlands have higher levels of nitrogen and phosphorus compared to golf course and forest preserve ponds in April and June (Fig. 3). These data suggest the water quality and chemistry of golf course ecosystems are similar to adjacent permanent, fish-containing forested lentic systems in managed forest preserves.

Biotic assessment: We collected 495 macroinvertebrate and 495 microinvertebrate samples from April to August 2017 (3 or 5 each month/site) to quantify micro (zooplankton) and macroinvertebrate diversity and density. We plan to process and identify macro-and microinvertebrates from 660 (330) total samples, which would equate to two samples per site per month. To date, we processed over 450 macroinvertebrate samples for identification and identified/quantified abundance and diversity for 125 macroinvertebrate samples and 75 microinvertebrate samples. We are on schedule to have all samples identified by the end of summer 2020. *These data will help elucidate the degree to which golf course ponds harbor biodiversity compared to adjacent systems considered more natural*.

To date, our results show species diversity of macroinvertebrates on golf course lentic ecosystems during April 2017 (Shannon's H'=2.06) is lower, but similar to, permanent, fish-filled (H'=2.19) and ephemeral, fishless adjacent ecosystems (H'=2.37); however, these represent an incomplete dataset of 20 golf courses, 11 ephemeral and 10 permanent, fish-filled sites (Fig. 4). In August 2017, Shannon's H'(1.92) is lower, but similar to, permanent, fish-filled (H'=2.07) and higher than ephemeral, fishless adjacent ecosystems (H'=1.74). Our results indicate microinvertebrate diversity in April and August 2017 was also variable, but was more similar across lentic ecosystem types than macroinvertebrates (Figs. 5 and 6).

Year 2: 2017-2018

Experimental mesocosms: We designed a mesocosm-based study to investigate the influence of the fungicide azoxystrobin, and nitrogen and phosphorus additions on aquatic ecosystems. In spring/summer 2018, we implemented a randomized experiment with 9 treatments (High/Low fungicide, nitrogen (N), phosphorus (P), N+P, and control) – see Table 2 for mesocosm treatment concentrations. Each treatment was then replicated with 4-6 mesocosms using organisms from three trophic levels: amphibians (American toads and Leopard frogs), dragonfly nymphs (*Pantala* and *Leucorrhinia*), and biofilms for a total of 120 mesocosm units. Our results suggest chlorophyll *a* and phycocyanin concentrations did not differ across treatments (Fig. 7), but American toads raised in low concentrations of azoxystrobin were smaller compared to control or high concentration mesocosms (Fig. 8). We believe this may be a result of a decreased, though not significant, amount of food resources. We also found that high nutrient levels, particularly nitrogen (Fig. 9) and N+P (Fig. 11 resulted in larger toads at metamorphosis, but low phosphorus concentrations led to smaller toads (Fig. 10).

Year 3: 2018-2019

Experimental mesocosms: We designed a mesocosm-based study to investigate the combination of the fungicide azoxystrobin, and nitrogen and phosphorus additions on aquatic ecosystems. In spring/summer 2019, we implemented a randomized experiment with 15 treatments (High/Low fungicide, nitrogen (N), phosphorus (P), N+P, fungicide/N, fungicide/P, funcicide N+P, and control) – see Table 2 for mesocosm treatment concentrations. Each treatment was then replicated with 4 mesocosms using organisms from twp trophic levels: amphibians (American toads) and biofilms for a total of 120 mesocosm units. Our results suggest American toads raised in low or high concentrations of azoxystrobin or N, P, and N+P reached metamorphosis significantly faster than control animals and were significantly larger, but did have measurably different survival (Fig. 12). The combination of azoxystrobin, N, P and N+P in High and Low concentrations showed similar trends to single additions with size of treatment toads being significantly larger, development time being significantly faster, and no measurable influence on survival compared to controls (Fig. 12). Data regarding chlorophyll *a* and phycocyanin concentrations are still being analyzed.

In addition, we examined the influence of N, P, N+P and azoxystrobin concentrations on dragonfly nymphs (*Pantala* and *Leucorrhinia*) survival (number of days alive) and mass (starting minus ending) across a 30-day period using 10 L plastic containers. Dragonflies were fed black worms *ad libitum* after being exposed to treatment water. Water was changed with control water after the initial treatment inoculation. We found all nymphs survived the full 30 days in single combination doses, and although mass varied across treatments it was not significantly different (Fig. 13). In combined treatments, variation existed for mass and survival, however the results were not significant (Fig. 14).

Future expectations:

- By spring 2020, we expect to submit a peer-reviewed manuscript detailing the comparison of water quality and chemistry of golf course and adjacent forest preserve ecosystems.
- We expect to have all micro-and macroinvertebrate samples enumerated and will begin construction of a manuscript detailing the biodiversity comparison of invertebrates and vertebrates across golf course and forest preserve (permanent and ephemeral) ponds by summer 2021. This manuscript will include undergraduate and graduate student authors.
- Construction of an M.S. thesis and manuscript detailing mesocosm data from 2018 and 2019 is underway and we plan to submit a manuscript detailing the work by winter 2020. This manuscript will include undergraduate and graduate student authors.
- Metamorphic toads raised in this experiment were placed in outdoor enclosures at Loyola University Chicago LUREC campus. These data will help determine the long-term influence of nutrient additions on anuran survival. An M.S thesis and manuscript detailing this work is expected to be submitted during Summer 2021. This manuscript will include undergraduate and graduate student authors.
- Metamorphic toads are also undergoing CT scanning to examine whether nutrient or azoxystrobin additions alter the development or morphology of anurans. This manuscript is led by undergraduate authors.
- In addition, the experimental set up and data were used to conduct an additional experiment investigating the influence of glyphosate and the compounds it is combined with during application on larval anuran fitness and morphology. This manuscript will include undergraduate and graduate student authors.

Table 1. Concentrations of analytes within golf course ponds and course inflows. Mean, range, and sample size (n = number of courses where each analyte is found).

Analyte	Within course ponds				Course inflows			
	n	Mean	Std. error	Range	n	Mean	Std. error	Range
Azoxystrobin	11	2.21	1.05	0 –24.39	2	0.45	0.90	0 – 2.29
Bifenthrin	0	0	–	–	0	0	–	–
α-Chlordane	2	0.14	0.12	0 – 2.81	1	0.09	0.47	0 – 0.63
γ-Chlordane	0	0	–	–	0	0	–	–
Chlorpyrifos	0	0	–	–	0	0	–	–
Cypermethrin	0	0	–	–	0	0	–	–
Oxadiazon	2	0.16	0.11	0 – 2.22	1	0.11	0.52	0 – 0.77
cis-Permethrin	0	0	–	–	0	0	–	–
trans-Permethrin	0	0	–	–	0	0	–	–
Fenvalerate	0	0	–	–	0	0	–	–

Table 2. Concentrations of Azoxystrobin, nitrogen, phosphorus, and N+P found in wetlands across 55 lentic ecosystems sampled and the concentrations used in the 2018 mesocosm study. MEAS = mean values from systems; Tx = treatment concentrations (Low or High) in mesocosm studies.

Systems	Azoxystrobin (ppb)		Phosphorus (μg/L)		Nitrogen (μg/L)	
	MEAS	T_x	MEAS	T_x	MEAS	T_x
Golf course	2.1 (L), 24 (H)		58		252	
Permanent-FP	NA	1.4 (L), 14.3 (H)	43	99 (L), 1700 (H)	421	263 (L), 7350 (H)
Ephemeral	NA		300		57	

Figure 1. Box plots (line = mean, box = SE, and whiskers = 95% CI) of *in situ* chlorophyll *a* and phycocyanin concentrations within sites across months. Different upper case letters suggest statistical significance (p≤0.05) within months using one-way ANOVAs and Tukey multiple comparison tests.

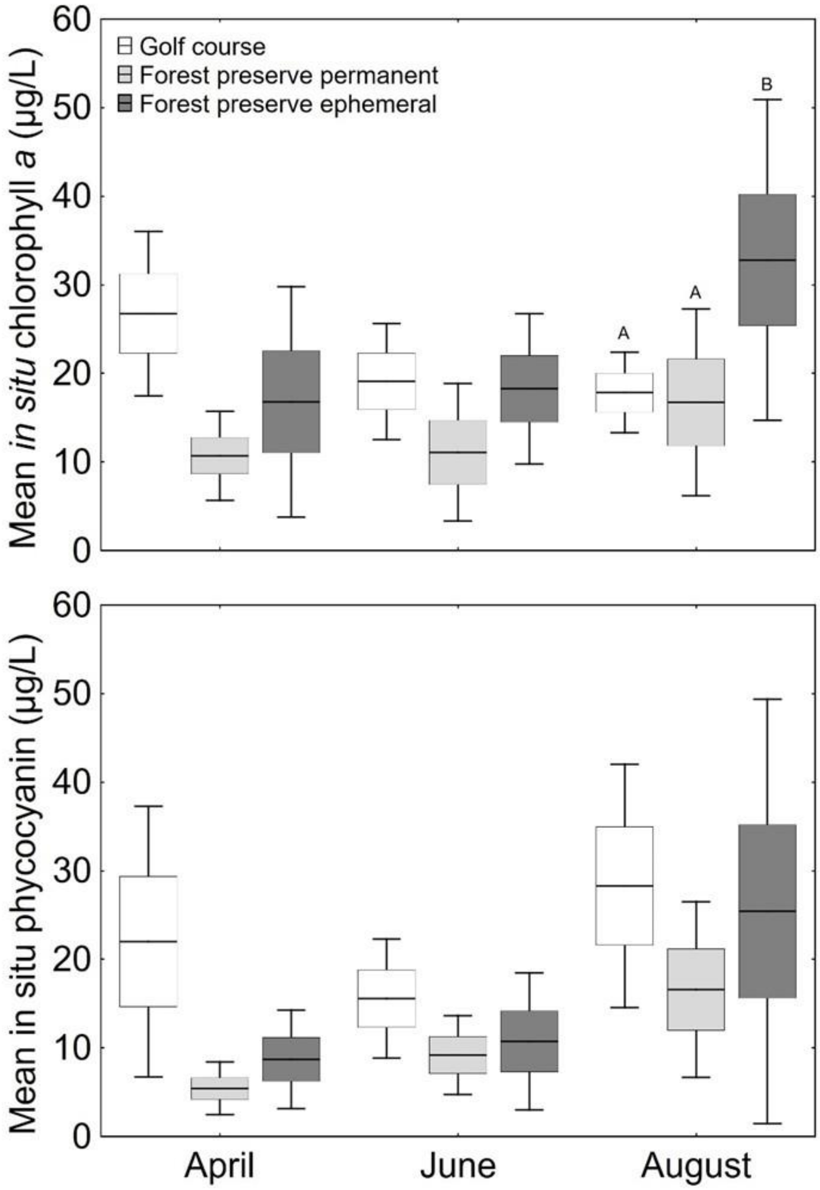


Figure 2. Box plots (line = mean, box = SE, and whiskers = 95% CI) values for water quality parameters within site types and across months (DO = dissolved oxygen, ORP = oxidation reduction potential). Different upper case letters represent statistical significance (p≤0.05).

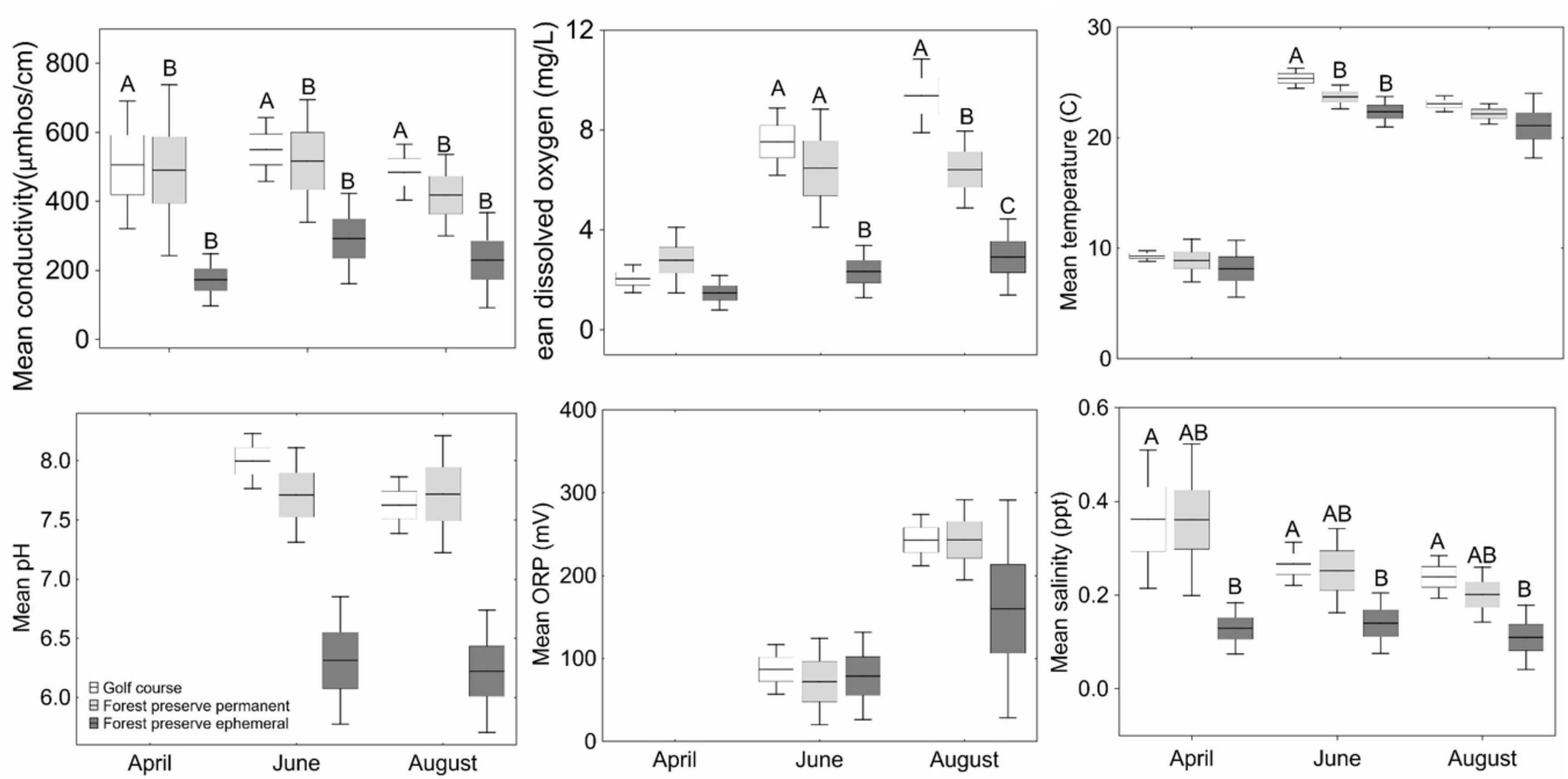


Figure 3. Box plots (line = mean, box = SE, and whiskers = 95% CI) of phosphorus, ammonium, and nitrate + nitrite values within sites and across months. Different upper-case letters indicate statistical significance ($p \leq 0.05$) within months using Tukey HSD test.

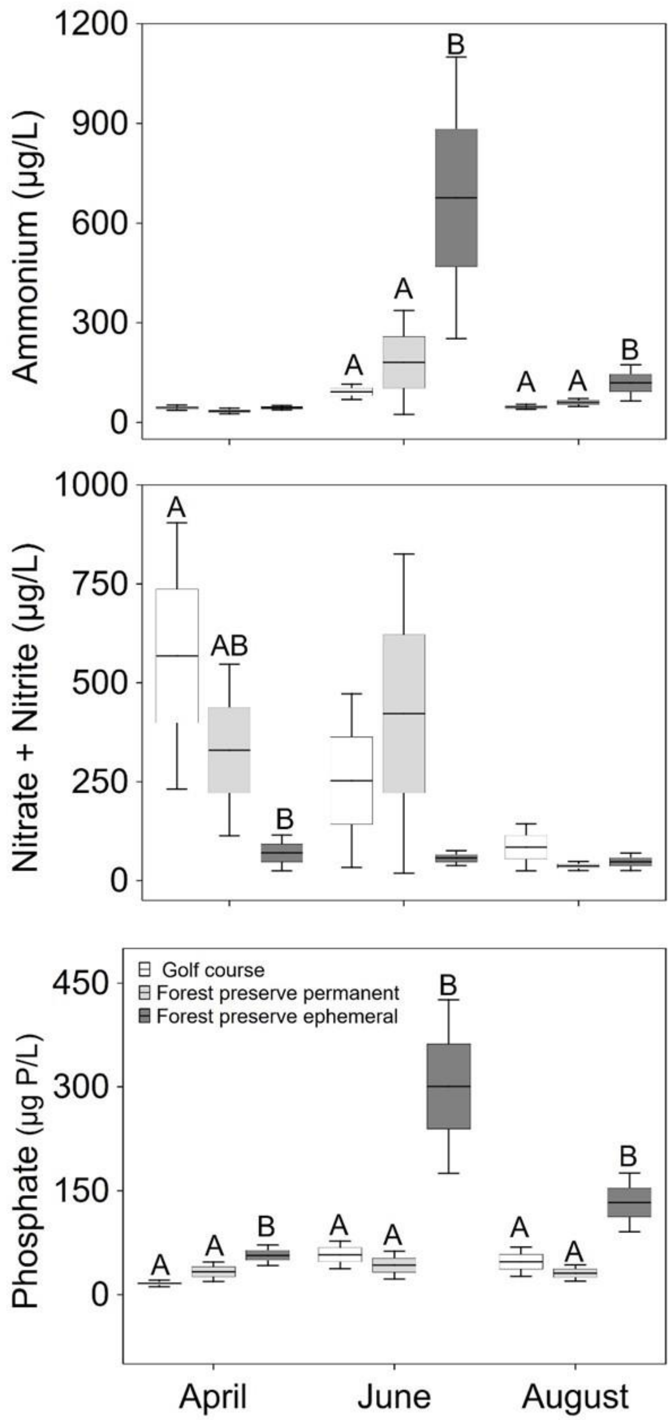
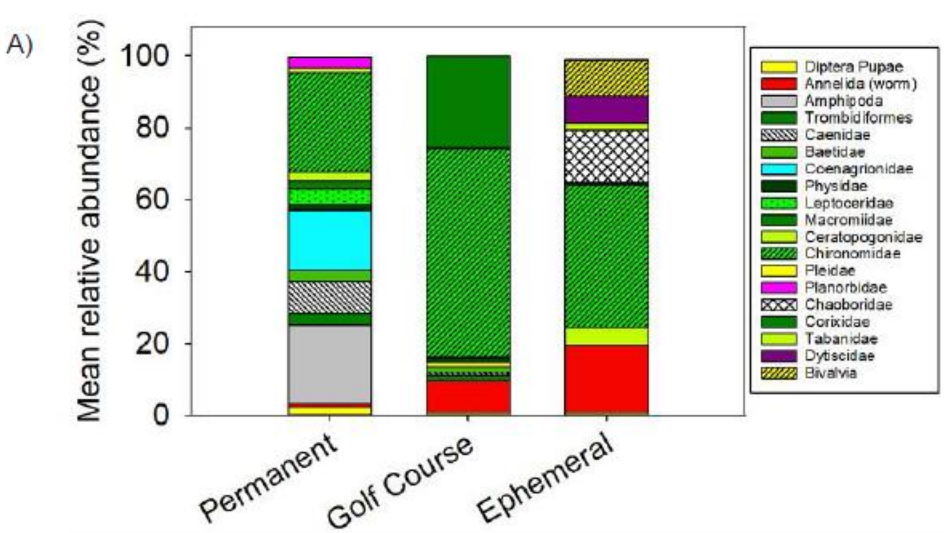


Figure 4. A) Mean relative abundance of macroinvertebrate families in permanent, fish-filled forest preserve or golf course ponds, and ephemeral wetlands in August 2017. Only taxa representing over 1% of relative abundance across all sites are presented. B) Non-metric Multidimensional Scaling (nMDS) results for macroinvertebrate community structure across treatments in August 2017.



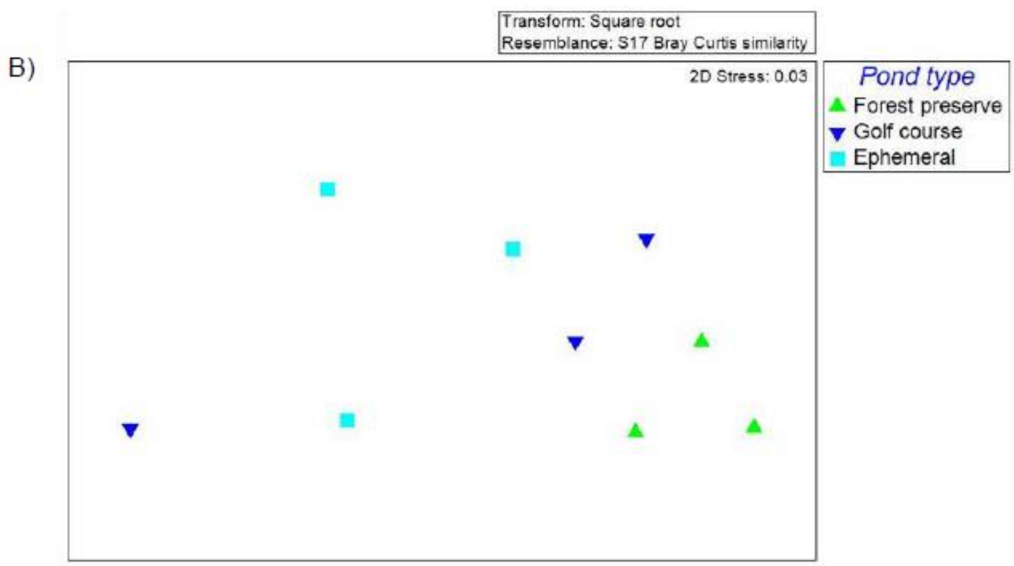


Figure 5. A) April and B) August mean relative abundance (%) of Cladocera, and C) April and D) August mean relative abundance (%) of Copepoda across golf course, ephemeral, and permanent, fish-filled lentic ecosystems.

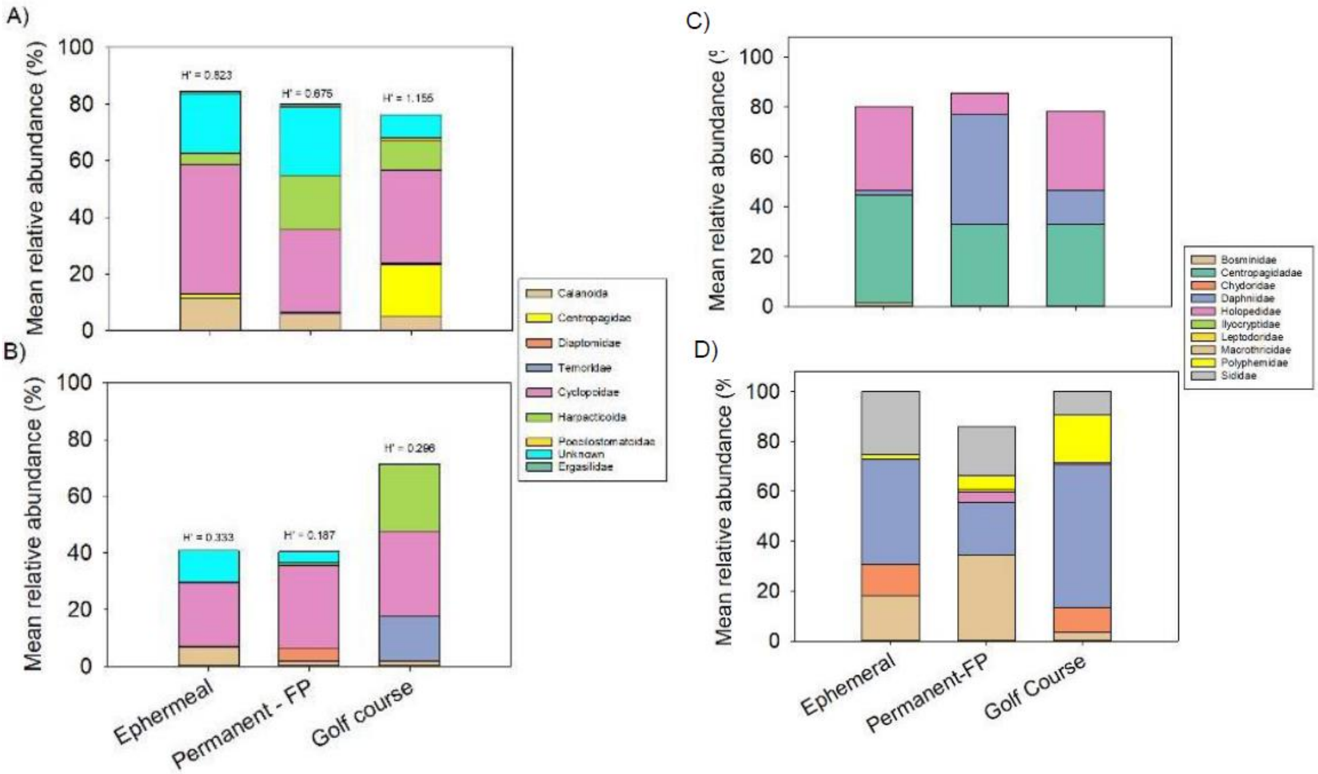


Figure 6. Non-metric Multidimensional Scaling results for: A) April and B) August Cladocera, and C) April and D) August Copepoda across golf course, ephemeral, and permanent, fish-filled lentic ecosystems.

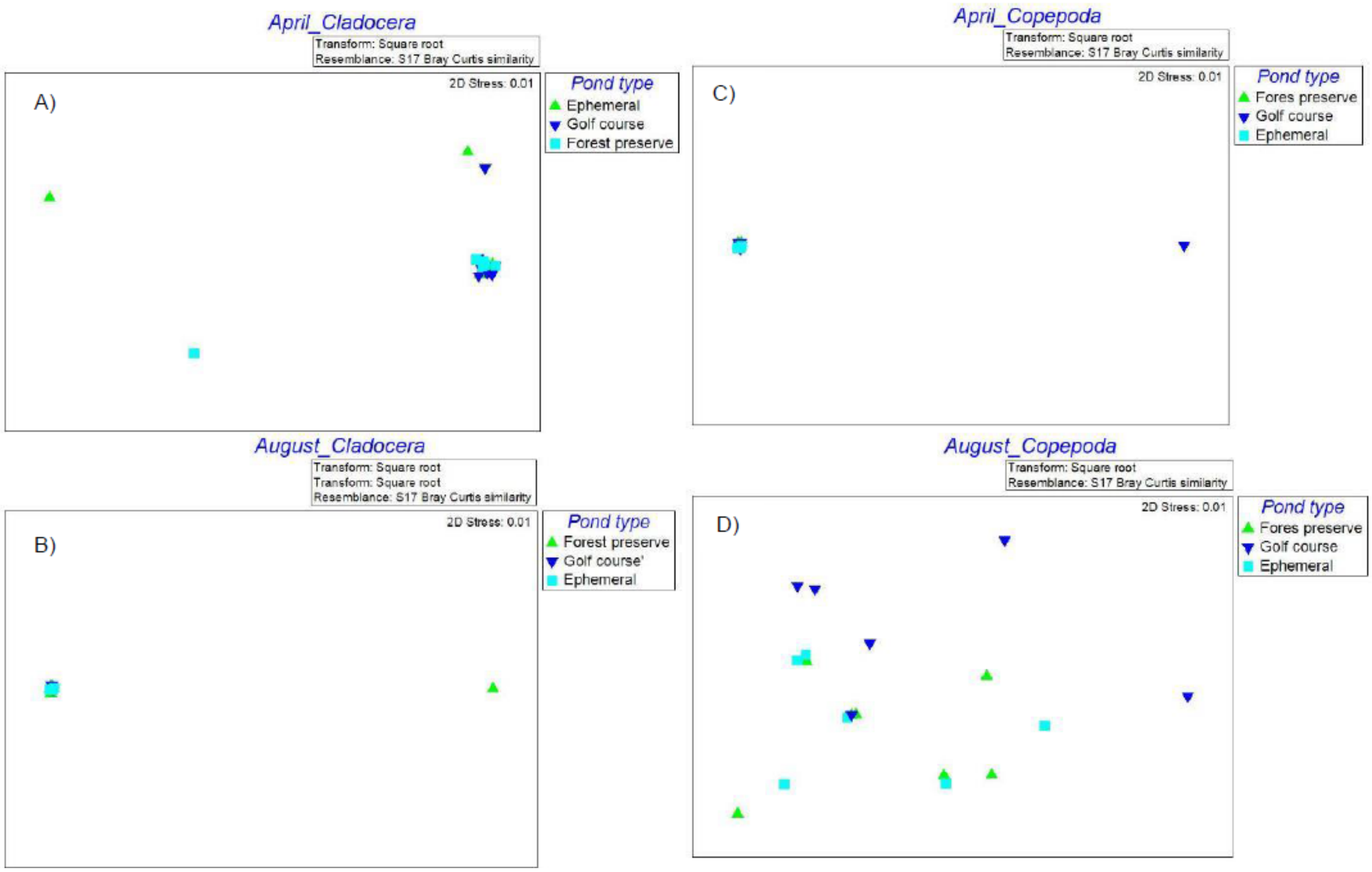


Figure 7. Box plots (line = mean, box = SE, and whiskers = 95% CI) values for *in situ* chlorophyll *a* (µg/L) and phycocyanin (µg/L) concentrations across fungicide and nutrient treatments in mesocosm settings from 2018.

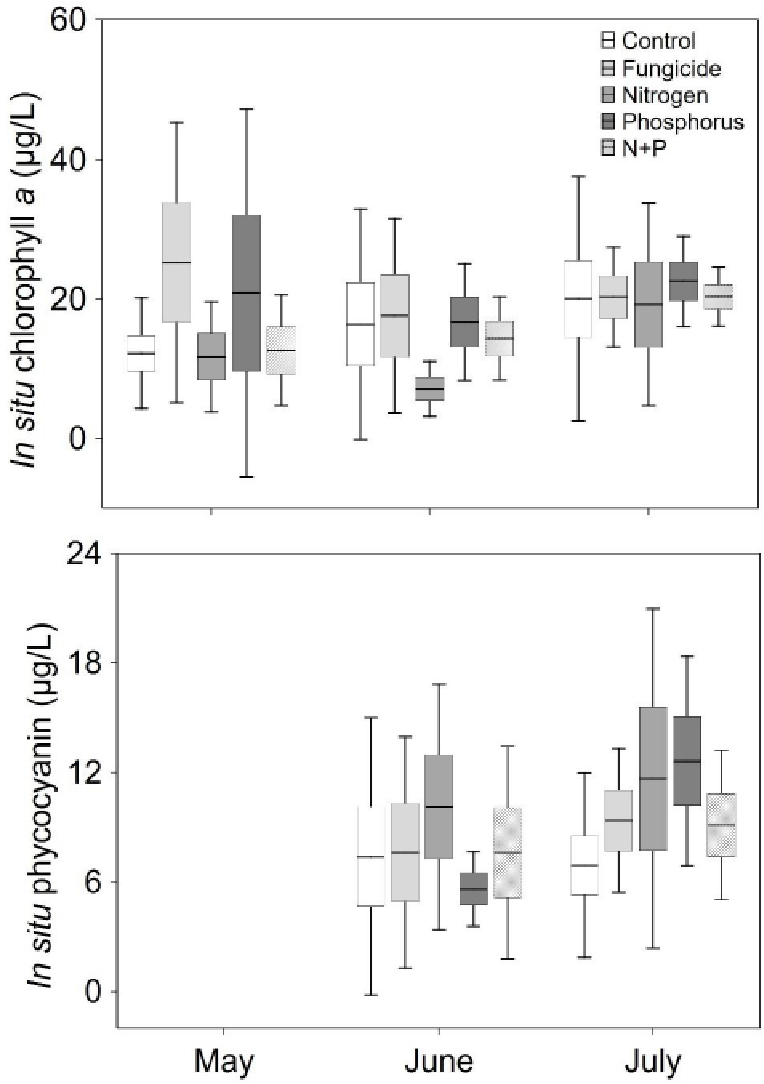


Figure 8. Box plots (line = mean, box = SE, and whiskers = 95% CI) of survival, days to metamorphosis and mass of American toad and leopard frog larvae raised in control or low/high fungicide treatments in a mesocosm setting. Different upper-case letters indicate statistical significance ($p \leq 0.05$) within months using Tukey HSD test.

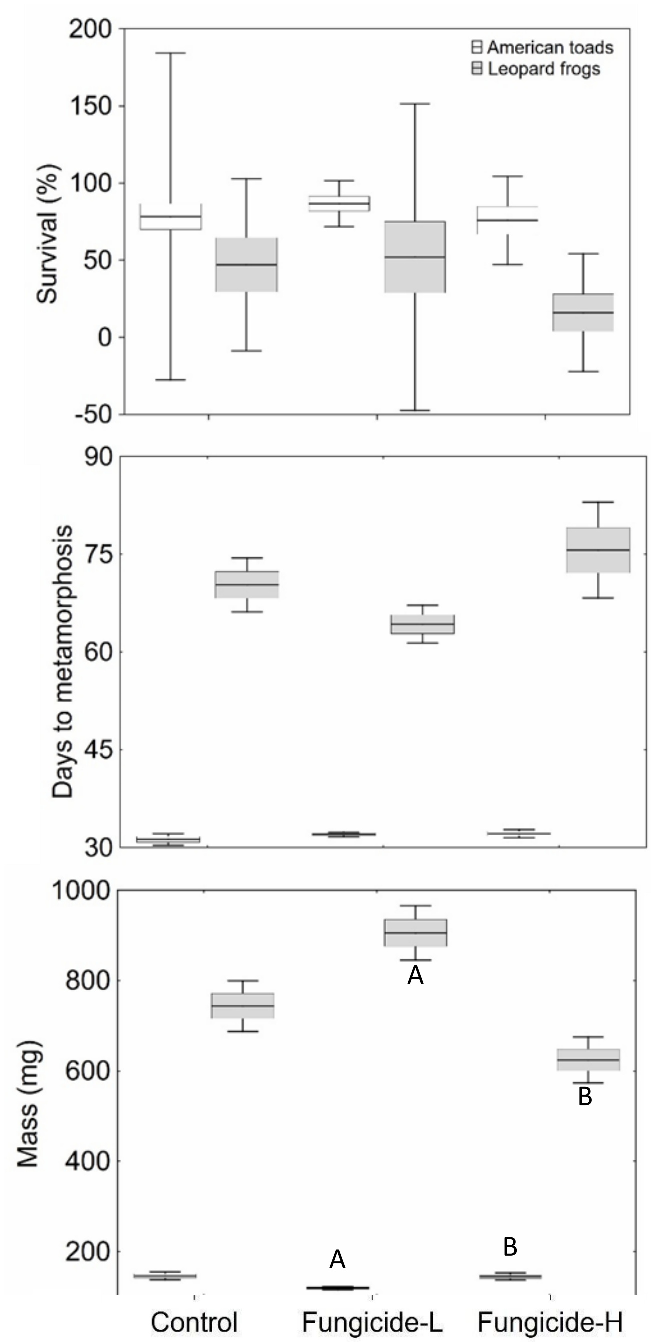


Figure 9. Box plots (line = mean, box = SE, and whiskers = 95% CI) of survival, days to metamorphosis and mass of American toad larvae raised in control or low/high nitrogen treatments in a mesocosm setting. Different upper-case letters indicate statistical significance (p ≤ 0.05) within months using Tukey HSD test.

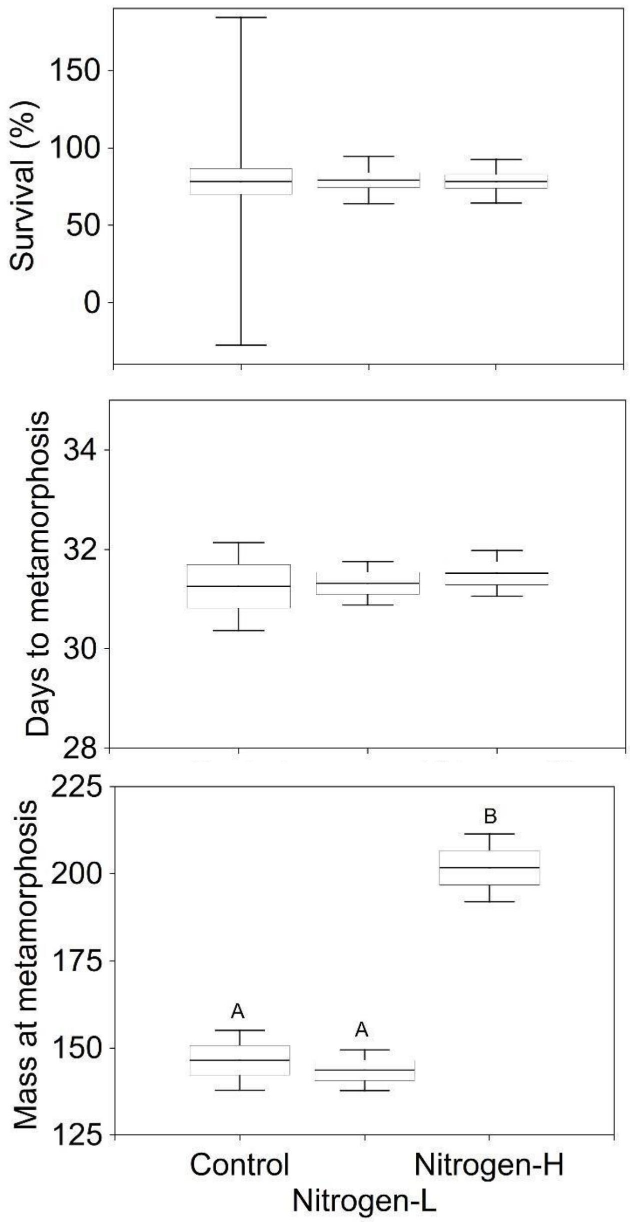


Figure 10. Box plots (line = mean, box = SE, and whiskers = 95% CI) of survival, days to metamorphosis and mass of American toad larvae raised in control or low/high phosphorus treatments in a mesocosm setting. Different upper-case letters indicate statistical significance (p ≤ 0.05) within months using Tukey HSD test.

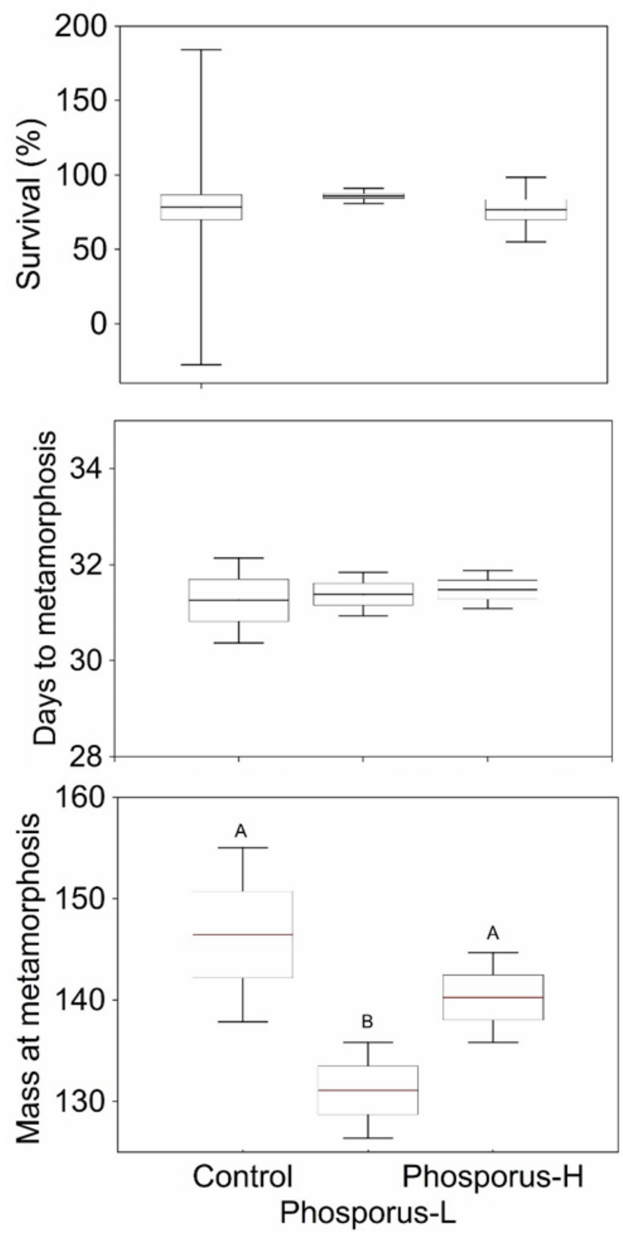


Figure 11. Box plots (line = mean, box = SE, and whiskers = 95% CI) of survival, days to metamorphosis and mass of American toad larvae raised in control or low/high nitrogen + phosphorus treatments in a 2018 mesocosm setting. Different upper-case letters indicate statistical significance ($p \leq 0.05$) within months using Tukey HSD test.

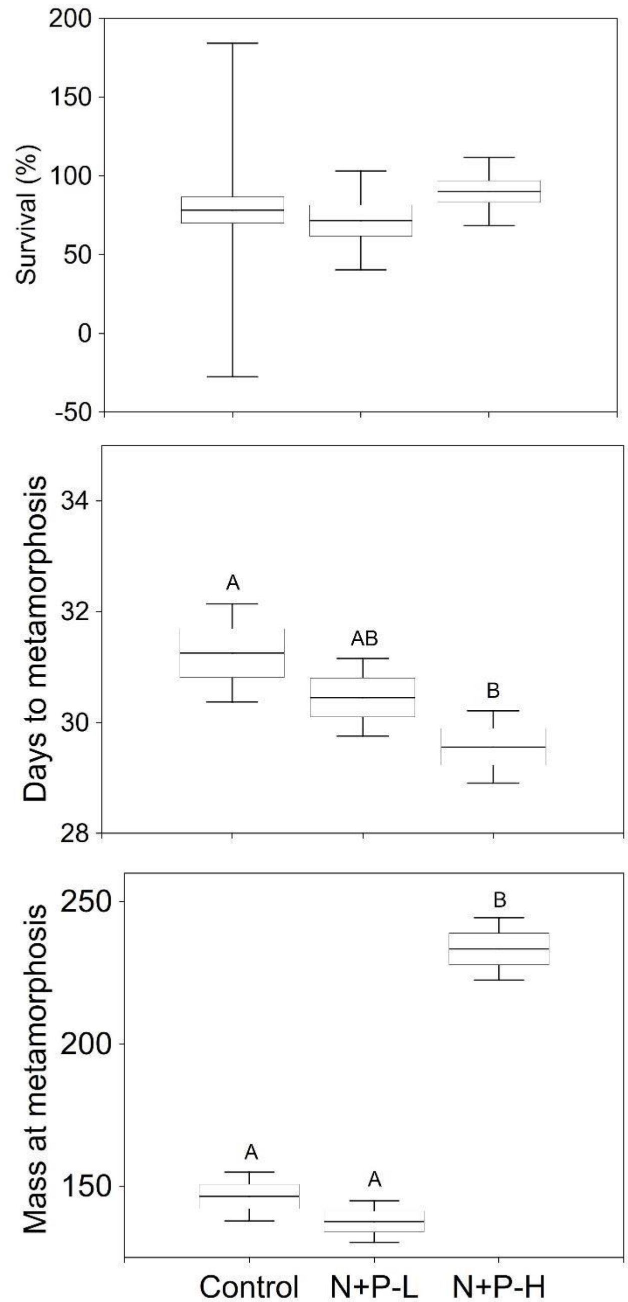


Figure 12. Box plots (line = mean, box = SE, and whiskers = 95% CI) of survival, days to metamorphosis and mass of American toad larvae raised in control or low/high nitrogen, phosphorus, N+P and fungicide treatments in a 2019 mesocosm setting. Different upper-case letters indicate statistical significance ($p \leq 0.05$) within months using Tukey HSD test.

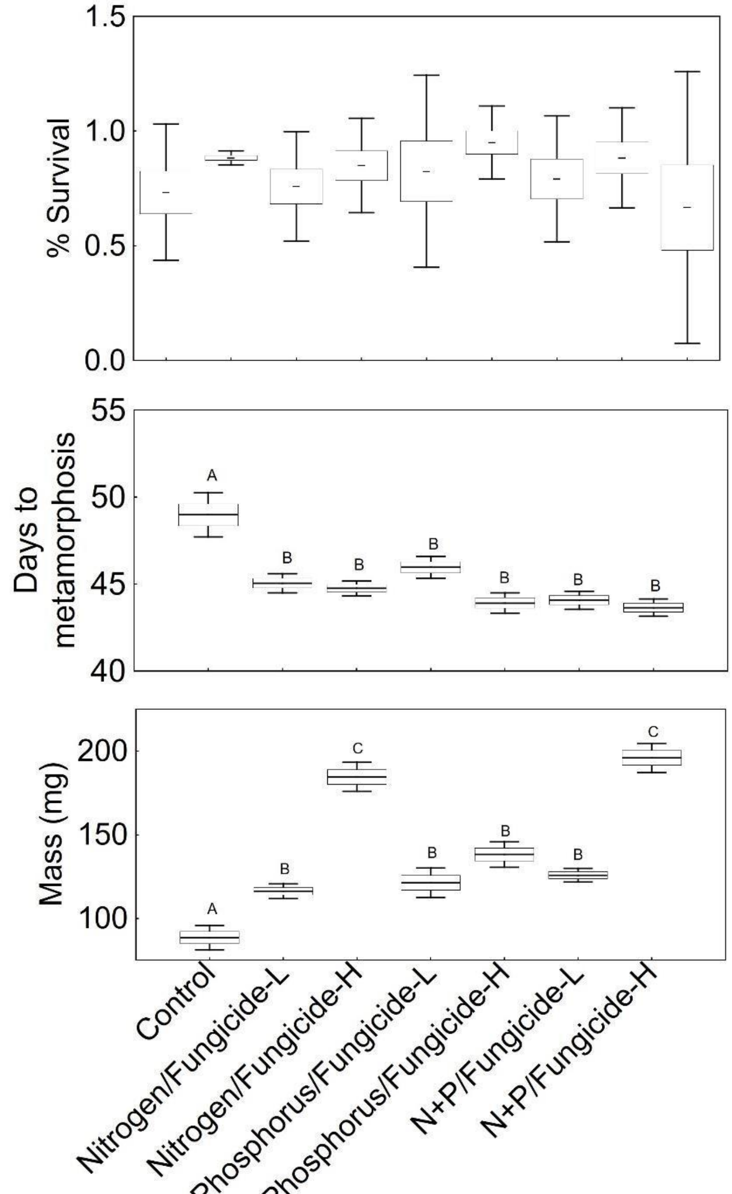


Figure 13. Box plots (line = mean, box = SE, and whiskers = 95% CI) difference in starting and ending mass of dragonfly nymphs raised in control or low/high nitrogen + phosphorus or fungicide treatments in a 2019 mesocosm setting.

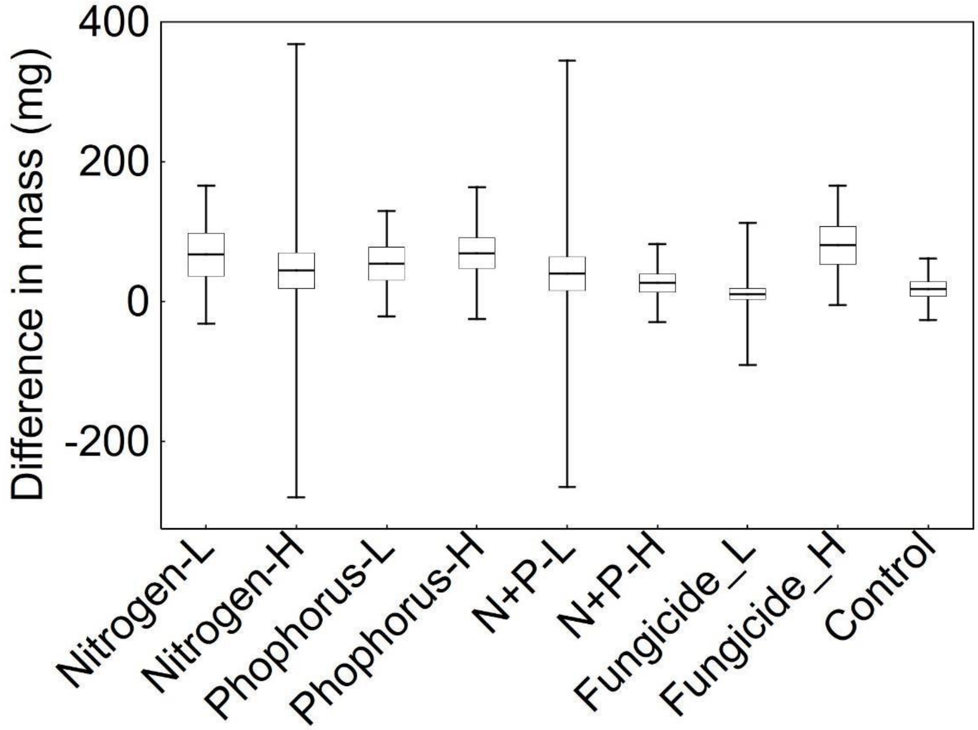
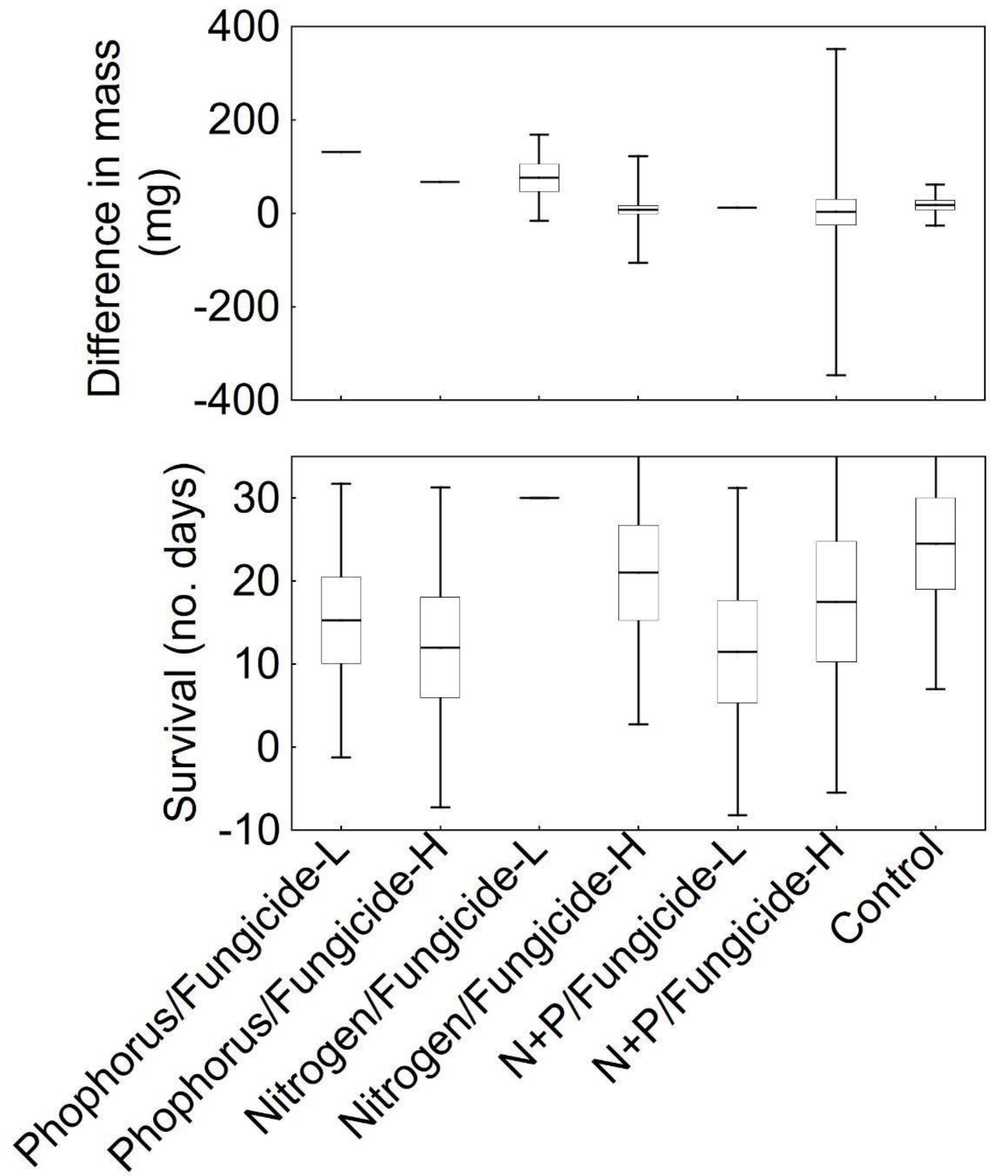


Figure 14. Mean (line), standard error (box) and range (whiskers) difference in starting and ending mass of dragonfly nymphs raised in control or the combination of low/high nitrogen + phosphorus or fungicide treatments in a 2019 mesocosm setting.



USGA ID#: 2018-22-649

Title: Monarchs in the Rough

Project Leader: Marcus B. Gray, MS
Affiliation: Audubon International

Objectives:

Establishment of an additional 500 acres of monarch butterfly habitat on golf courses within a 28-State Project Area by November 2020.

Start Date: 2018
Project Duration: 2 years
Total Funding: $100,000

Summary Points:

- Monarchs In The Rough is now the largest coordinated pollinator conservation initiative for golf courses.
- For the life of the effort, nearly 800 acres has been committed to butterfly stewardship.
- We have allocated resources for 310/500 acres and are on track for successful completion of grant deliverables.

Summary Text:

Monarchs in The Rough (MITR) was established through a partnership between Audubon International and the Environmental Defense Fund to establish new, high-quality habitat for monarch butterflies on golf courses across North America. The program connects and supports superintendents and other golf course staff as they plan, install, and manage habitat projects for the monarch butterfly on their courses.

Monarchs In The Rough (monarchsintherough.org) has now grown to 568 courses, representing 45 U.S. States, Puerto Rico, 6 Canadian Provinces and 2 Mexican States. To date, 792 acres (more than a square mile [640 acres]), have been committed to habitat establishment by each MITR course planting at least 1 acre for the migratory species. This truly continental effort is a network of reserves working toward the same goal: recovering America's favorite insect.

Outcomes:
As outlined in our proposal for funding to USGA, MITR has successfully brought the golf industry to monarch conservation - Golf Course Management Teams. Superintendents, General Managers, Owners, Management Companies, Builders and others have really taken the initiative under their wing, making MITR is the leading pollinator program for the golf industry.

Thanks to USGA's early and continued support via partnerships and funding, Audubon International was also able to demonstrate MITR's scalability to the National Fish and Wildlife Foundation, Syngenta, Wadsworth Golf Charities and United States Fish & Wildlife Service, NJ Field Office.

Objectives Accomplished:
The total support from USGA is $100,000 in cash match toward NFWF's $150,000. The scope of the grant is to create 500 new acres of monarch habitat by November 2020. Partners have committed the 250 acres sponsored by NFWF and are now working on the 250 underwritten by USGA. To date, 60 golf courses have been provided an acre's worth of milkweed and regionally-appropriate wildflower seed ($22,800) and technical assistance for planting and management. Also, marketing activities that included promotional outreach, how-to tutorials and provided examples from the field were delivered through:

- Gateway Chapter of PGA, St. Louis, Missouri.
- Lightning Round Learning to 300 people, Golf Industry Show, San Diego, California.
- Golf Course Naturalized Area Management Class in collaboration with Jim Skorulski (USGA) and Dave Kaplow (Eco-Management), New England Turfgrass Foundation Show, Providence, Rhode Island.
- Television appearance on 17 news stations across the country.
- Provided slides and guidance for talks by Kimberly Erusha (USGA) and Kat Findlay (Audubon International Program Specialist).
- Television appearance on *Virginia Home Grown* (PBS in Richmond, Virginia) to discuss planting for butterflies and scaling up to landscape level conservation.
- Podcast with ConserveTheWild (conservewild.org) airing in October 2019.
- Assisted others such as BlackHawk CC hosted their own events highlighting MITR (WI Golf Courses on Par with Pollinators).
- Pheasants Forever/Quail Forever in MO held youth pollinator events using seed we provided and consultation with Audubon International. In AR and other states, that organization continues to promote our program with golf courses in their coverage area. Members of the general public often request information to pass out to their local golf properties.
- Over 20 articles and news segments have come out about MITR in Golf Course Management, TurfNet, Golf Central Magazine, Golf Course Industry, USGA Green Section Record, GCSAA, Mother Nature Network, Green Matters, US Fish & Wildlife Service and local outlets in areas where courses are conducting the work (i.e., Ann Arbor, MI). Many pieces may be found on the 'Learn' tab of monarchsintherough.org.

Monitoring of planting success has begun through a participant survey, self-reporting from Superintendents and ground-truthing working with the Monarch Joint Venture. The participant survey conducted by Audubon International revealed that the scope of habitat enhanced has gone well beyond the initial support provided through multiple grants and contributions. In the survey, an additional 160 acres were planted by respondents on their own. When you apply the ratio of responses to all the participants, the acreage involved is likely much higher. The figure also does not include golf course personnel that have been learning about pollinator needs through Monarchs In The Rough marketing and implementing separate activities. This is a wonderful development because the expectation was that golf courses would be inspired to increase plantings moving forward once their employees became comfortable with installing and maintaining native plantings out-of-play. The high visibility of Monarchs In The Rough projects has served the role of being demonstration sites which encourage golfers and other property visitors to change their practices at home. The influence of Monarchs In The Rough is already reaching beyond the boundaries of courses and driving broader community involvement to save butterflies as demonstrated through personal conversations with Superintendents in areas without dedicated funding to cover their expenses learning about the program and being inspired to undertake projects themselves.

Next steps:
The program is looking for another 190 acres of butterfly plantings across 28 U.S. States in the next year. Eligible areas include all States West of Mississippi River plus IL, IN, MI, OH, PA & WI. An additional award of $10,000 will be used to create an additional 15 acres in New Jersey (comprehensive mix of milkweed and native wildflower mix) in that state from the US Fish & Wildlife Service's Partners for Fish & Wildlife Program. This agreement sets the precedent for pursuing similar funding in other states. There are approximately 15,000 golf courses in the United States comprising an estimated 2.3 million acres. Increasingly, golfers want sustainability actions where they play. Diverse natural areas improve water quality, reduce inputs, build soil health, bolster beneficial insects that control turf pests, provide cover for birds and otherwise add to the aesthetics of golf properties. There is a myriad of outreach and education opportunities facilitated by these plots including Master Naturalist groups, birders, butterfly watchers, collegiate course field trips, guest lectures and local community understanding of how golf courses are maintained.

Marcus Gray plans to attend the Monarch Joint Venture Partners Meeting in Arizona and is slated to provide a Monarchs In The Rough update and serve on a panel concerning shovel ready projects. An upcoming seminar at the Golf Industry Show in Orlando (Step by Step: Establishing and Managing Native and Naturalized Roughs) will cover in-depth details of converting existing turf and non-turf areas to vegetation beneficial to the environment generally, in addition to butterflies, while considering the needs of golf course operations.

Figure 1. To date, 60 golf courses have been provided an acre's worth of milkweed and regionally-appropriate wildflower seed ($22,800) and technical assistance for planting and management.

USGA ID#: 2019-22-692

Title: Community Values of Golf Courses: From the Minneapolis-St. Paul Region to US cities - Phase 2

Project Leader: E. Lonsdorf[1], and B. Horgan[2]
Affiliation: [1]University of Minnesota; [2]Michigan St. University

Objectives:

1. Adapting newly developed models of urban ecosystem services to multiple cities in the United States
2. Applying these new models towards evaluating the benefits nature provides people around golf courses in urban areas

Start Date: 2019
Project Duration: 1 year
Total Funding: $159,470

Summary Points:

- Land use context of courses varies considerably among the cities
- There are a higher density of courses more generally in Phoenix, many of which are integrated into housing developments
- Golf courses in these highly-urbanized areas, in all six cities, stand out in the maps as areas of high retention for P in landscapes that are generally less retentive.
- The metro areas also vary strongly in the intensity and pattern of the urban heat island

Summary Text:

Nature's contributions to people, e.g. ecosystem services, support human systems around the world from agriculture to coastal resilience. With more than half the world's population living in urban areas (Elmqvist et al. 2019), most of people's potential to receive ecosystem service benefits occurs in cities. However, a straightforward, replicable approach to quantifying multiple urban ecosystem services has yet to emerge; and while there has been an increasing interest in integrating ecosystem services into urban planning decisions, uptake remains limited. Golf courses represent a substantial part of urban areas in the United States, comprising just over 9,300 km^2 from approximately 14,200 courses as of 2015.

Given their common occurrence in urban areas and the potential pressures to develop them, golf courses provide an important case study to evaluate urban ecosystem services in a changing environment. The goal of our work is to apply newly developed models of urban ecosystem services to address the question: how do golf courses support nature's benefits to the surrounding community in urban areas? And how do these benefits vary across different cities in the United States? The overarching goal is to provide an approach that allows one to answer this question in any urban area in the United States.

Methodology

To address the contribution of golf courses to ecosystem services in urban areas, we applied newly developed tools provided by the Natural Capital Project's Urban InVEST

(Integrated Valuation of Ecosystem Services and Tradeoffs) tool. We applied the models of Urban Cooling, Stormwater Retention and Pollinator Habitat to six metro areas in the United States that span a variety of social and ecological contexts: San Francisco Bay, Phoenix, Dallas-Ft. Worth, Minneapolis-St. Pau, Atlanta and Philadelphia (Figure 1a). We identified golf courses in each metro area using Open Street Maps (Figure 1b).

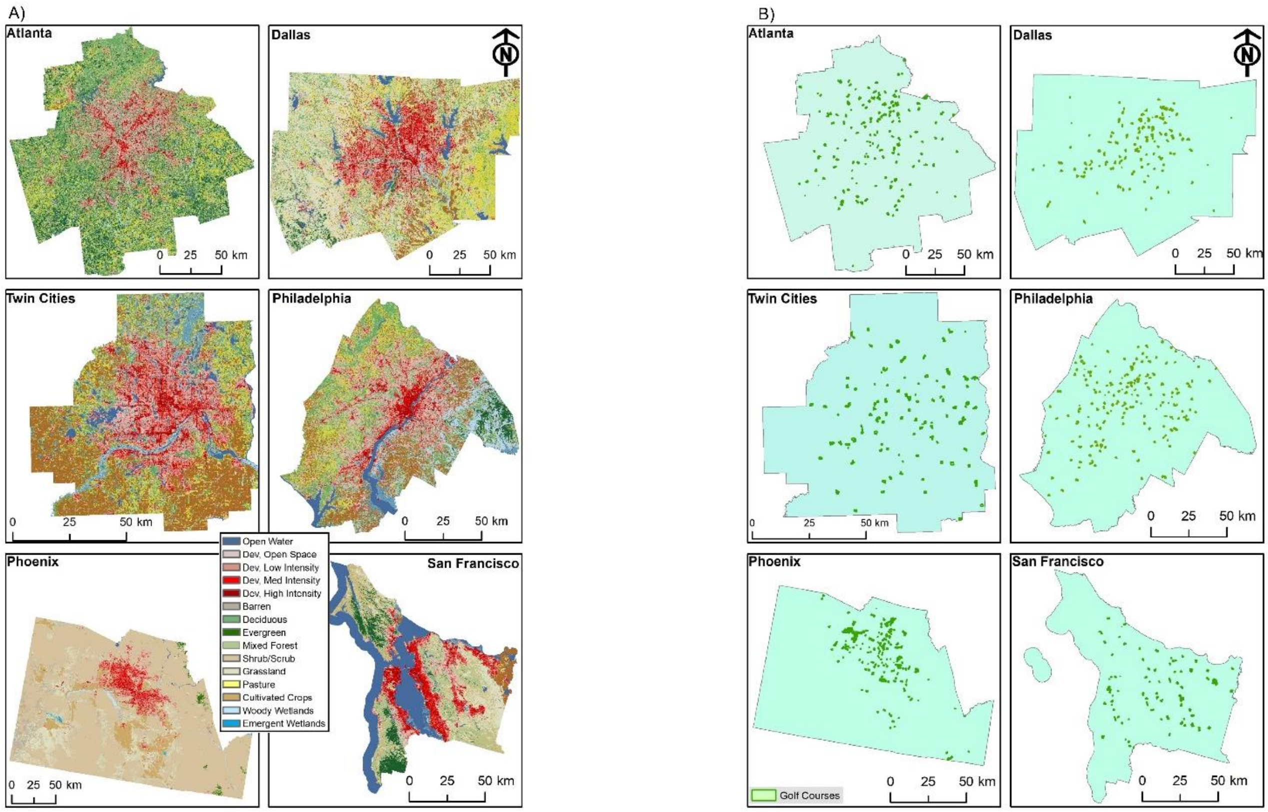


Figure 1. Land cover (a) and golf courses (b) maps for selected areas in the six case study cities. Land cover data are 30 m resolution. Land cover Source: 2016 National Land Cover Database (https://www.mrlc.gov/data). Golf course source: Open Street Map (https://www.openstreetmap.org)

To evaluate the contribution of golf courses, we used a marginal value approach. We defined a golf course's marginal value as the change in a landscape's total ecosystem service value for a given service in response to a change in the golf course. We have developed an ArcGIS tool to automate this process, called the "wallpaper" approach as it identifies and replicates common urban land cover patterns, e.g. urban residential, suburban residential, city park. We replace a current golf course with one of these patterns and re-evaluate the ecosystem services. We've gathered necessary data to run models on all six cities, including identifying golf courses described on Open Street Maps. We are ready to run the marginal analyses for each city and will be doing so soon.

Stormwater Retention: The InVEST Stormwater Retention model predicts the partitioning of mean annual rainfall into the volume retained (infiltrated into the ground), the volume exported from the landscape as runoff, and the masses of nitrogen and phosphorus associated with these two primary fluxes. The model also predicts the upper limit of potential groundwater recharge (of the volume infiltrated, this is the amount that is not intercepted by plant roots and potentially percolates to deeper aquifers). The various water fluxes simulated by this model are a function of climate (annual rainfall and evapotranspiration), land cover/land use (30m-resolution LandSAT), and soil hydrologic group (SSURGO), with reductions in retention for cells located near roadways and high-impervious land cover -- surfaces that are often directly connected to the storm drain networks. Nutrient fluxes are determined from stormwater runoff concentrations specific to the various land cover/land use types, per various literature sources.

The Stormwater Retention model was first tested in the Twin Cities Metropolitan Area, where results were compared to annual volume and to loads (mass) of nitrogen and phosphorus observed at 18 stream and storm drain monitoring sites. Results of the testing were encouraging (R^2 = 0.66 for volume, R^2 = 0.60 for total phosphorous load, R^2 = 0.60 for total nitrogen load; not shown). The model has since been applied to all six case study cities to predict baseline retention of water, N, and P across the landscapes (see Figure 2 for total phosphorous retention ratio maps). Next steps will be to assess the change in these retention parameters as a function of change in land use on the courses to (for example) natural, single-family residential, and industrial/commercial land uses.

Urban Cooling: Urban heat mitigation is a priority for many cities that have undergone heat waves in recent years. Vegetation can help reduce the urban heat island by providing shade, modifying thermal properties of the urban fabric, and increasing cooling through evapotranspiration. The InVEST urban cooling model calculates an index of heat mitigation based on shade, evapotranspiration, and albedo, as well as distance from cooling islands (e.g. parks). The index is used to estimate a temperature reduction by vegetation.

Pollinator Model: We applied the InVEST pollination model (v3.3.0) to evaluate the consequences of each urban typology for pollinators, across all golf courses. The model provides a spatially explicit index of pollinator habitat quality and has worked reasonably well to predict observed bee abundance on pollinator-dependent crops (Lonsdorf et al. 2009, Kennedy et al. 2013) and other empirical results across the US (Koh et al. 2016), including in urban areas (Davis et al. 2017).

Results to date

Observationally, a few patterns emerged from this baseline application. The land use context of courses varies considerably among the cities, such as a higher density of courses more generally in Phoenix, many of which are integrated into housing developments, with the Twin Cities, San Francisco, and Philadelphia having perhaps a larger number of courses surrounded by medium- and high-intensity urban land cover. The courses in these highly-urbanized areas, in all six cities, stand out in the maps as areas of high retention for P (i.e. high amount of P generated in runoff but also a high amount retained) in landscapes that are generally less retentive. A more rigorous marginal analysis will allow us to quantify this pattern for each city.

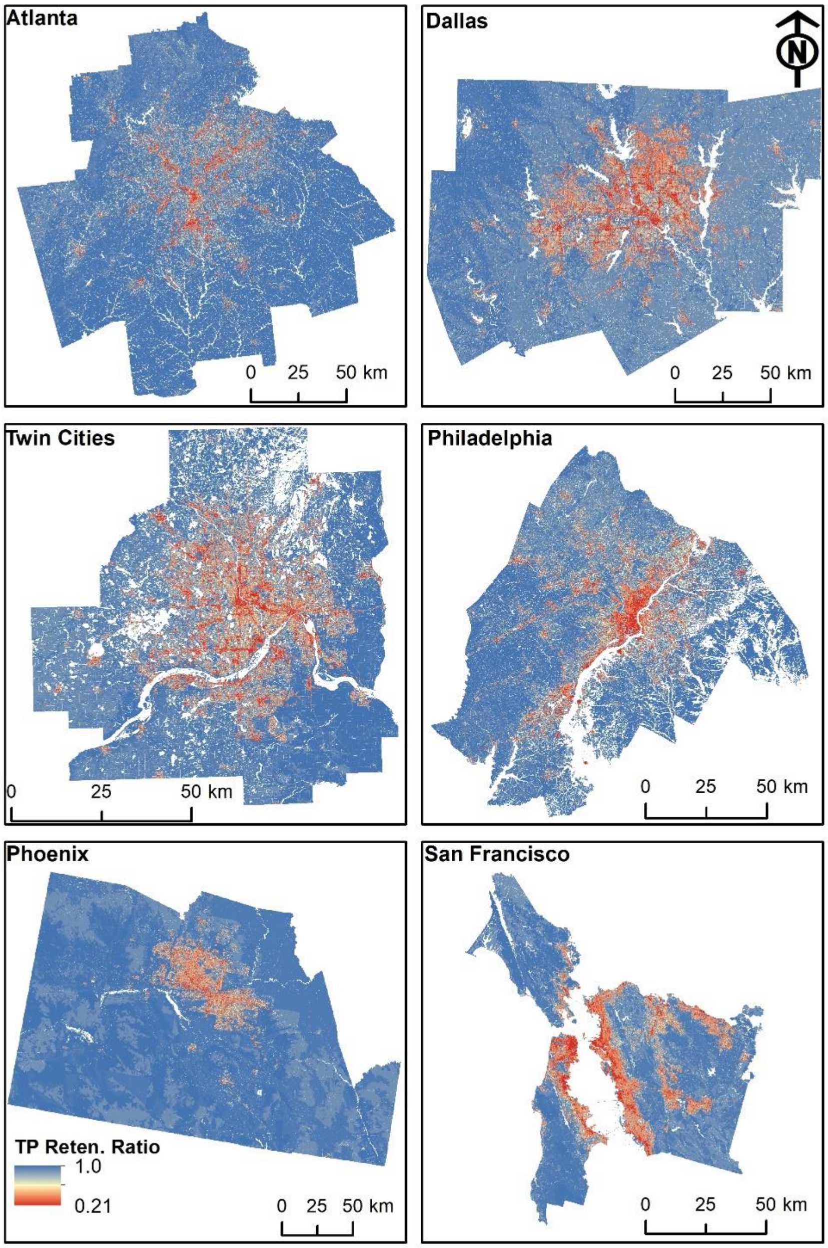


Figure 2. Baseline Annual Total Phosphorus (TP) retention ratio for selected areas in the six case study cities, with golf course parcels shown in green outlines. TP retention ratio = (annual TP retained) / (annual TP exported + annual TP retained), such that 1 = complete retention, 0 = no retention. Baseline scenario shown (golf courses with parameters associated with golf courses). Map scale is 1:300,000 for all sub-maps.

Urban heat islands tended to vary in intensity and size (Figure 3a) with Dallas having the largest and broadest UHI and San Francisco having very strong temperature differences due to land use. It will be interesting to evaluate how context affects the impact of golf courses on cooling in these different cities.

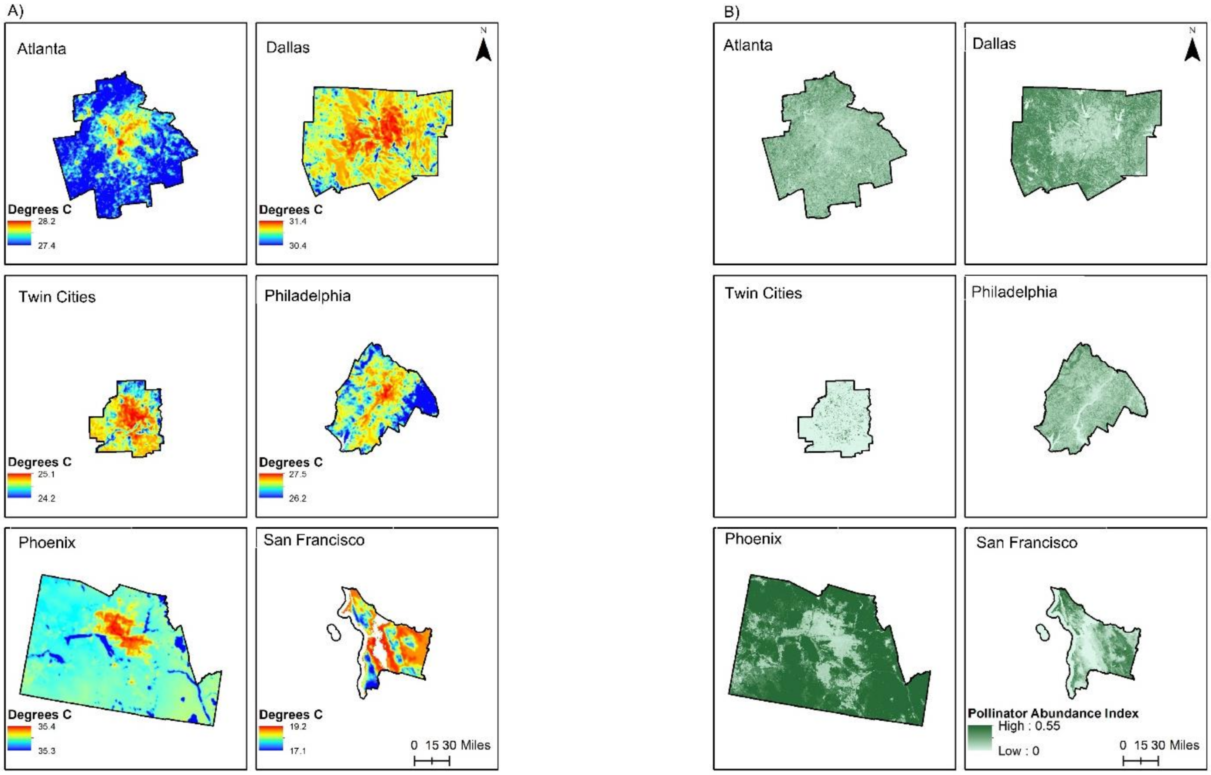


Figure 3. Baseline results of urban heat island (a) and pollinator habitat (b) for each of the six cities. Temperature ranges are set independently so colors depicting temperature are relative to the range for each city. The color gradient depicting pollinator habitat quality is the same across the six cities.

Future expectations

We have nearly completed setting up baseline analyses for each of the six metro areas. Our next step is to evaluate the change in services provided by each golf course in each city, i.e. the marginal valuation. We hope to leverage and generalize the insights from the application to golf courses to urban planning more generally and develop interactive tools that provide opportunities for communicating the benefits golf courses deliver to the surrounding landscape and community. We will also be looking to include aspects of social information to evaluate issues related to equity with respect to the distribution of ecosystem services in urban areas.

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USGA ID#: 2018-19-669

Title: Simulation of Nitrous Oxide Emissions and Carbon Sequestration in Zoysiagrass Fairway Turf Using the DAYCENT Model

Project Leaders: Mu Hong[1], Yao Zhang[2], Ross Braun[3], and Dale J. Bremer[1]
Affiliation: [1]Kansas State University; [2]Colorado State University; [3]Purdue University

Objectives:

1. Calibration of the DAYCENT model to predict emissions of nitrous oxide (N_2O), a greenhouse gas (GHG) implicated in climate change, from fairway zoysiagrass;
2. Prediction of long-term impacts of N fertilization and irrigation management practices on N_2O emissions and C sequestration in fairway zoysiagrass; and
3. Estimation of long-term impacts of N fertilization and irrigation management on GHG inventories by estimating energy (e.g. mowing and irrigation) expenses associated with turfgrass maintenance.

Start Date: 2018
Project Duration: 2 years
Total Funding: $76,812

Summary Points:

- The DAYCENT model was parameterized and calibrated for zoysiagrass with data from (Braun and Bremer, 2018a).
- N_2O flux measurements are well modelled by DAYCENT daily outputs.
- Field measurements of soil moisture, N_2O emissions, and soil organic nitrogen of zoysiagrass from an earlier study (Lewis and Bremer 2013) will be used to validate the model.

Summary Text:

Nitrous oxide (N_2O) and carbon dioxide (CO_2) are important greenhouse gases that have been implicated in global climate change, and therefore should be monitored in turfgrass systems, which have large land coverage and are typically fertilized with nitrogen (N) and irrigation (Braun and Bremer, 2018b).

A previous USGA-funded study at K-State revealed significant effects of N fertilizer type and irrigation management on N_2O emissions in zoysiagrass over two years and C sequestration over three years (Braun and Bremer, 2018a, 2019). The acquisition of these data provides a unique opportunity to calibrate the DAYCENT model for zoysiagrass turf; specifically, to predict long-term impacts of N fertilization and irrigation management on N_2O emissions and C sequestration. Such model development is important because continuous long-term measurements are expensive and time consuming. DAYCENT is a powerful model developed and used widely to predict GHG fluxes in agricultural lands. DAYCENT has been applied to C3 but not C4 turfgrasses such as zoysiagrass, which must be calibrated separately because of different physiological characteristics (Zhang et al., 2013a).

In the current project the DAYCENT model was parameterized for ‘Meyer’ zoysiagrass using the method of Zhang et al. (2013b). Simulated aboveground and belowground biomass was adjusted by aboveground and belowground C allocation parameters and shoot and root death rates based on field biomass samples and Patton et al. (2007)’s research on ‘Meyer’. Leaf lignin content was set at 6% (Hale et al.,2009; Hamido et al., 2016). Lignin content of roots was set to 24.3% based on lab analysis of zoysiagrass root and rhizome of 10-cm depth samples using the method of Sluiter et al. (2008). Model monthly outputs of average leaf and root C/N ratio are compared with C/N ratio of field samples analyzed with the method of Braun and Bremer (2019). Therefore, minimum aboveground C/N ratio was set between 9.6 and 25, and belowground C/N was set at 45 (Golubiewski, 2006).

Information on weather, soil, and management practices was required for model simulations. Daily maximum/minimum temperatures and precipitation were obtained from an on-site weather station (http://mesonet.k-state.edu). Two soil cores at upper 30-cm depth were taken at the study site to determine average soil texture (14.4% sand, 62.5% silt, and 23.1% clay), from which field capacity and wilting point are calculated (Saxton et al., 2006). The soil pH is 7.0.

Previous land use was simulated as Konza prairie for 4000 years to reach equilibrium, followed by cropland, then turfgrass with high maintenance since the 1970s, and finally the experimental site of Braun and Bremer (2018a) since 2000. Turfgrass management practices of fertilization, irrigation, and mowing were scheduled in the model. Urea was modeled as the input of NH_4^+ into soil. Polymer-coated urea (90-day release) was modeled as 45 events of NH_4^+ input with 1-day interval (Salman et al., 1989). Irrigation during the growing season outside the summer experimental periods (when N fertility and irrigation treatments were applied) were scheduled as weekly inputs of 2.5-cm water. Mowing was treated as a harvest event in the model that returns the harvested biomass as litter (clippings), which was adjusted by a field sample and research of Trappe et al. (2011) on ‘Meyer’ (Zhang et al. 2013a).

The authors have already measured soil moisture, N_2O emissions, soil organic nitrogen, soil organic carbon in a simulated ‘Meyer’ zoysiagrass fairway in a field setting under an automated rainout shelter that allowed for precise control of irrigation at the Rocky Ford Turfgrass Research Center near Manhattan, KS (Braun and Bremer, 2018a, 2019). The DAYCENT model was calibrated by adjusting critical water flow for leaching of minerals (0.1 cm H_2O per day). Results show measured and daily simulated N_2O fluxes are well matched under different irrigation and fertilization practices (Fig. 1). The Pearson’s *r* across all management treatments is 0.67 (P<0.00001). There is still room for model improvement. The model may slightly underestimate N_2O fluxes from unfertilized zoysiagrass fairway during the growing season.

Thereafter, the calibrated DAYCENT model will be validated by comparing simulation outputs with field measurements of soil moisture, N_2O emissions, and soil organic nitrogen measured from zoysiagrass in an earlier study at another site of the Rocky Ford Turfgrass Research Center (Lewis and Bremer, 2013). This validation will justify the following long-term simulation of impacts of irrigation and N-fertilization practices on N_2O emissions and carbon sequestration in zoysiagrass turf.

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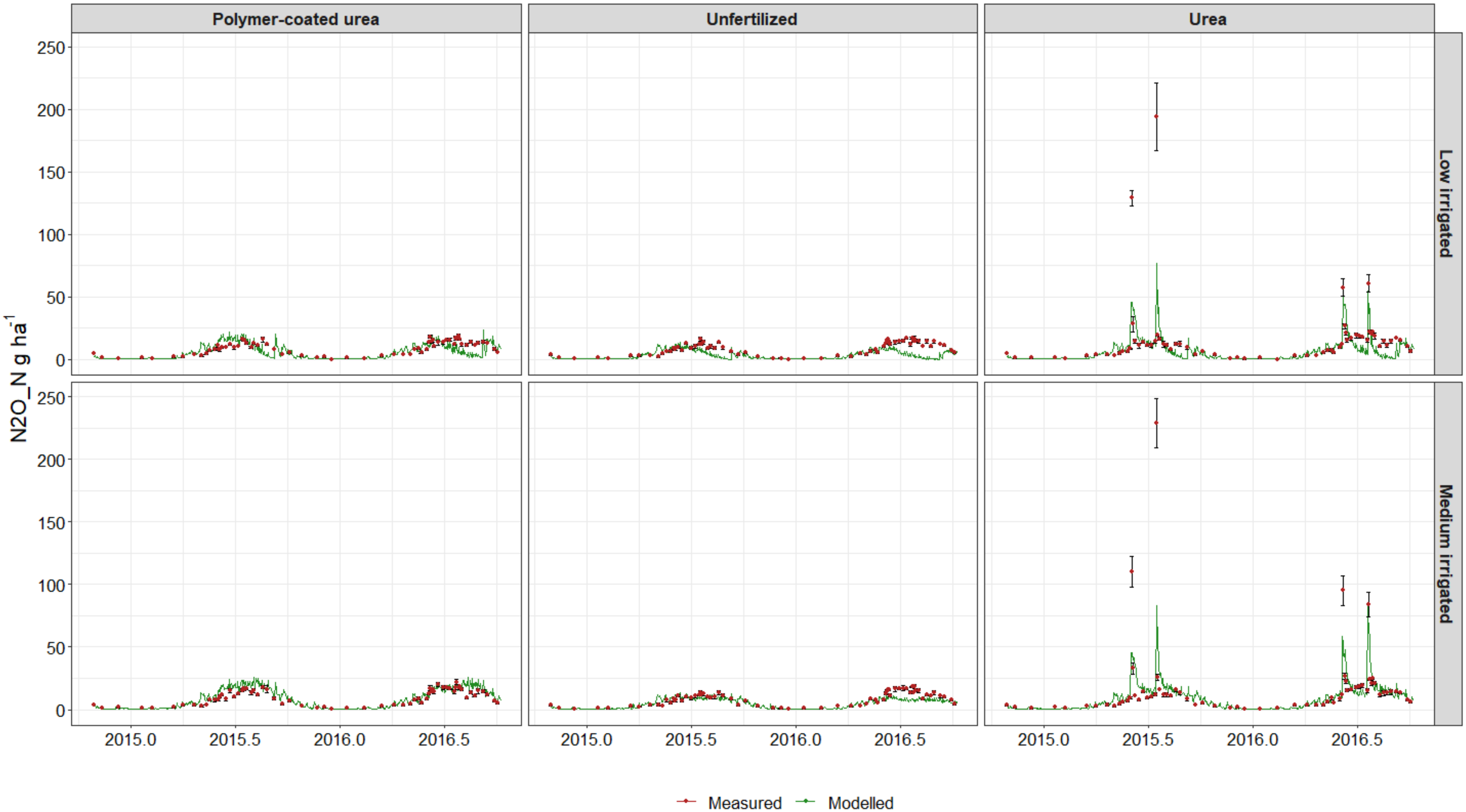

Figure 1. Measured and daily DAYCENT-simulated nitrous oxide (N2O) under low/medium irrigation and polymer-coated urea/urea/no fertilization.

4. REGIONAL GRANTS

USGA ID#: 2017-35-644

Title: Winter Survival of Experimental Bermudagrasses in the Upper Transition Zone

Project Leaders: Mingying Xiang[1], Jack Fry[2], and Yanqi Wu[3]

Affiliation:
[1]Department of Botany and Plant Sciences, University of California, Riverside; Previous graduate research assistant, Department of Horticulture and Natural Resources, Kansas State University.
[2]Department of Horticulture and Natural Resources, Kansas State University, Kansas State University;
[3]Department of Plant and Soil Sciences, Oklahoma State University

Summary Text:

Rationale: Winter survival is the limiting factor in developing and selecting new bermudagrass cultivars for use in the transition zone. The objectives of this project were to compare new, experimental bermudagrasses to existing cultivars for winter survival in Kansas.

Methods: On 19 July, 2016, vegetative plugs of 60 new bermudagrass progeny along with standards cultivars Latitude 36, NorthBridge, TifTuf, Tifway, and Patriot were planted at the Rocky Ford Turfgrass Research Center in Manhattan, KS. Bermudagrass progeny came from the turfgrass breeding program at Oklahoma State University. Plots measured 4 ft. by 4 ft. and were arranged in a randomized complete block design with three replicates. The soil type was a silty clay loam (fine, smectitic, mesic, Aquertic Argiudoll) with a pH of 7.3. Plots were mowed three times per week at 5/8". Nitrogen from urea was applied twice during the summer to provide 1 lb. of N at each application. Ronstar was applied in April to prevent annual grassy weeds, and Trimec was applied at the same time to remove broadleaves. The first freezing temperature occurred on 10 Nov., 2016, 27 Oct., 2017 and 15 Oct., 2018, and bermudagrasses started to lose color. After 25 Dec., there were 5 days during the 2016-2017 winter, 17 days during the 2017-2018 winter and 13 days during the 2018-2019 winter on which the low temperature was < 10 °F; the lowest temperatures in each winter occurred on 6 Jan 2017 (-2.1°F), 1 Jan. 2018 (-9 °F), and 30 Jan and 4 March 2019 (-1.9 °F).

Data were collected on spring green up on a 1 to 9 scale (1 = brown; 9 = completely green) in 2017, 2018, and 2019. Data were analyzed using PROC Mixed, and results are presented in Table 1.

Results:

- Progeny showed a wide range of variability in cold hardiness (Table 1; Fig. 1 and 2).
- None of the progeny in the top statistical group in 2017 appeared in that group in 2018 or 2019. Progeny in the top statistical group in 2018 and 2019 were OSU 1337, OSU 1406, OSU 1433, OSU 1629, OSU 1666, OSU 1673, OSU 1675, and OSU 1682.
- Tifway exhibited poor spring green up in 2017, 2018, and 2019. In each year, other improved cultivars, including Latitude 36, NorthBridge, and Tahoma 31were inferior in spring green up compared to some experimental progeny.

Table 1. Spring green up of bermudagrass selections and standard cultivars from 2017 to 2019 in Manhattan, KS.

	Spring green up[z]			
Entry	4/5/17	5/12/18	5/1/19	Mean
OSU1257	5.0	3.3	4.3	4.2
OSU1310	4.7	1.5	3.7	3.3
OSU1318	4.0	2.3	4.0	3.4
OSU1337	4.5	4.3	5.0	4.6
OSU1402	4.8	2.8	5.0	4.2
OSU1403	4.0	2.7	4.0	3.6
OSU1406	3.7	5.0	4.7	4.4
OSU1408	1.7	2.0	3.7	2.4
OSU1409	4.2	2.8	3.7	3.6
OSU1412	3.5	2.2	3.0	2.9
OSU1415	3.3	1.1	2.7	2.4
OSU1417	3.7	1.1	2.3	2.4
OSU1418	4.7	1.0	1.3	2.3
OSU1420	4.3	1.6	4.0	3.3
OSU1423	5.8	2.1	4.3	4.1
OSU1425	5.2	1.5	2.7	3.1
OSU1433	4.8	4.3	4.7	4.6
OSU1435	2.5	2.8	5.3	3.6
OSU1439	5.2	2.7	5.7	4.5
OSU1601	2.3	3.2	4.3	3.3
OSU1603	5.0	1.3	1.8	2.7
OSU1604	6.7	3.2	3.7	4.5
OSU1605	4.0	2.0	4.3	3.4
OSU1606	3.7	1.7	3.3	2.9
OSU1607	5.3	2.3	3.3	3.7
OSU1610	4.2	1.8	3.3	3.1
OSU1611	6.3	1.8	3.0	3.7
OSU1612	3.0	1.1	2.3	2.1
OSU1614	2.8	3.0	3.0	2.9
OSU1615	3.3	1.5	3.0	2.6
OSU1616	3.3	1.1	1.3	1.9
OSU1617	7.5	2.3	4.0	4.6
OSU1620	3.8	3.5	4.3	3.9
OSU1625	5.7	3.5	5.7	4.9
OSU1628	4.7	3.7	3.7	4.0
OSU1629	5.8	4.7	6.0	5.5
OSU1631	3.8	3.0	2.7	3.2
OSU1634	3.7	2.2	2.7	2.8
OSU1636	4.5	4.0	4.7	4.4
OSU1639	3.5	3.0	2.7	3.1
OSU1640	3.2	2.0	3.3	2.8
OSU1641	4.0	3.3	4.0	3.8
OSU1644	3.3	2.7	3.0	3.0
OSU1645	5.2	2.0	4.0	3.7
OSU1649	3.7	4.8	3.0	3.8
OSU1656	4.8	5.2	4.0	4.7
OSU1657	4.0	3.7	5.7	4.4

OSU1662	4.8	3.0	3.3	3.7
OSU1664	4.2	3.7	4.3	4.1
OSU1666	3.8	5.7	5.7	5.1
OSU1669	3.7	1.8	3.3	2.9
OSU1673	3.0	5.3	4.7	4.3
OSU1674	4.7	2.8	3.7	3.7
OSU1675	3.0	4.2	4.7	3.9
OSU1680	2.8	3.5	3.7	3.3
OSU1682	4.0	4.3	4.7	4.3
OSU1687	2.2	4.3	3.3	3.3
OSU1691	1.3	2.0	2.7	2.0
OSU1695	2.8	3.7	2.7	3.1
OSU1696	3.3	3.5	4.0	3.6
TifTuf	2.8	1.2	3.3	2.5
Latitude 36	4.7	1.7	4.0	3.5
NorthBridge	3.8	2.0	3.8	3.2
Patriot	2.0	2.2	3.0	2.4
Tahoma 31	NA[y]	NA	3.7	3.7
Tifway	2.5	1.0	1.0	1.5
LSD[x]	1.2	1.5	1.9	0.9

[z]Spring green up was rated visually on a 1 to 9 scale (9 = completely green); results are averaged over two rating dates and three replicates.
[y]NA, Data not available, as Tahoma 31 was planted in 2018.
[x]To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value (*P* < 0.05).

Figure 1. Overhead photos of new bermudagrass progeny and standard cultivars taken above a single, representative plot on 28 May, 2018 in Manhattan, KS.

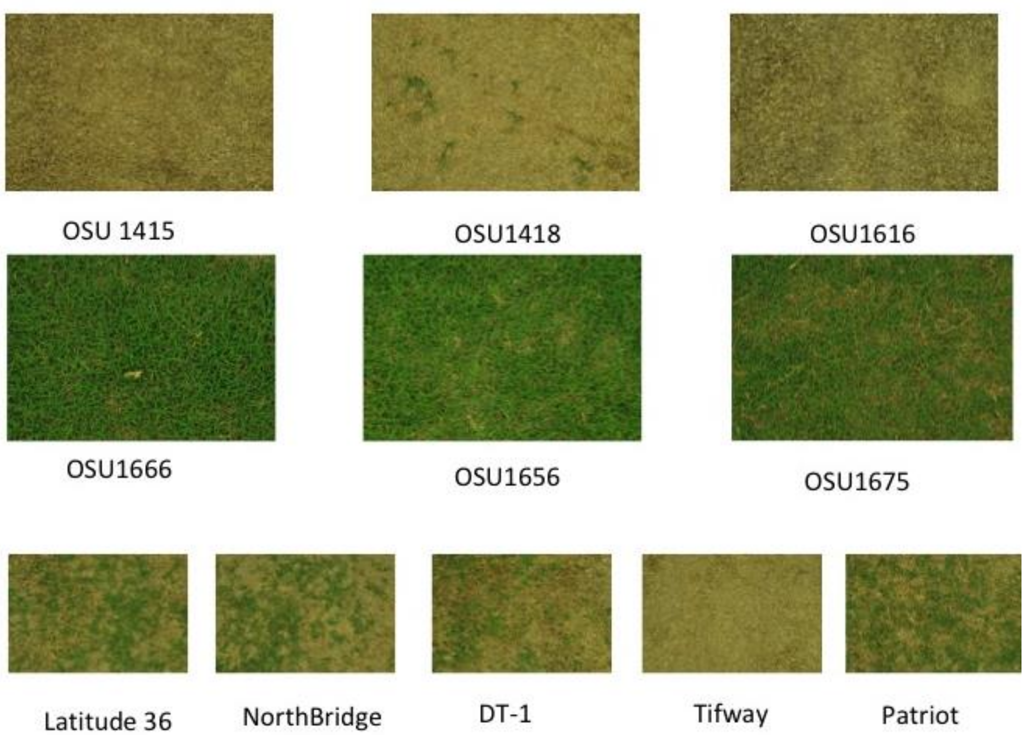

Figure 2. Variability in spring green up among bermudagrass cultivars and experimental progeny on 3 May, 2018.

USGA ID#: 2019-21-691

Title: Advancement of Five Elite Zoysiagrass Hybrids in the 2019 Zoysiagrass NTEP

Project Leader: Ambika Chandra
Affiliation: Texas A&M AgriLife Research-Dallas, 17360 Coit Road, Dallas, TX 75252

Start Date: 2019
Project Duration: 5 years
Total Funding: $25,000

Summary Text:

Background:
Cold Hardy/Large Patch Disease Tolerance: Since its initiation in 2012, significant progress has been made to develop cold hardy and large patch disease tolerant zoysiagrass hybrids as part of the collaborative project between Texas A&M AgriLife Research, Kansas State University and Purdue University, funded by the United States Golf Association. The Texas A&M AgriLife – Dallas breeding team has developed 2,858 new hybrids in 2011/2012 by crossing selected parental lines exhibiting large patch tolerance, fine or intermediate leaf texture, good turfgrass quality and cold hardiness. These hybrids were tested at three locations (Dallas, TX; Manhattan, KS and West Lafayette, IN) from 2012 to 2014 (2 yr. of turfgrass quality and winter recovery data). The 60 best hybrids underwent more extensive testing at nine locations across a wide range of environments for another 3 years. In 2018, the 10 best of the 60 hybrids were chosen based on their spring green up, winter injury, monthly turfgrass quality, large patch tolerance, and percentage establishment across all nine locations. The top three of these top 10 hybrids will be entered into 2019 Zoysiagrass NTEP.

Experimental # (tested as)	Advanced to 2019 NTEP as
TAES 6095-83	DALZ 1701
TAES 6099-145	DALZ 1808
TAES 6119-179	DALZ 1707

Winter color Retention and Performance in UC-Riverside: A total of 218 zoysiagrass hybrids were planted at UC-Riverside in the fall of 2016. Based on the data from 2016 to 2018, top performers for winter color retention and turfgrass quality are as follows:

Experimental # (tested as)	Advanced to 2019 NTEP as
TXZ 463	DALZ 1807
TXZ 488	DALZ 1802

Year 1 (2019-2020):

A total of 20 18-cell trays for each of the five elite hybrids were propagated at Texas A&M AgriLife-Dallas in 2018/2019. Propagated materials were delivered to NTEP headquarters in Beltsville, MD on June 5, 2019. These five elite hybrids will be tested as part of the 2019 National Zoysiagrass NTEP at 20 test locations including 11 standard and 9 ancillary locations. Hybrids will be tested for their overall performance at standard locations as well as for traits like drought, shade, large patch, billbug, traffic tolerance, divot recovery and sod strength at the ancillary locations. Out of these 20 test sites, 11 are in the transition zone or north of the transition zone [KS, MO, IN (x2), MD, VA, NC, TN, AR (x2), OK] and should provide a good platform to screen these hybrids for their cold hardiness. The trial was planted at all NTEP test sites in the of summer 2019 with either 6 ft x 6 ft or 7 ft x 7 ft plot size replicated three times. One tray of each of these hybrids were supplied to each test site where the 3 in x 3 in plugs were divided into four 1-1/2 in mini-plugs for planting on approximately one-foot centers making it a total of twenty-four (24) 1.5 in plugs per plot. Texas A&M-Dallas is one of the ancillary locations and will evaluate these hybrids under drought stress conditions. We anticipate receiving the first data set from the NTEP in 2021.

USGA ID#: 2019-31-701

Title: On-course evaluation of new zoysiagrass hybrids

Project Leaders: James H. Baird, Marta Pudzianowska, and Pawel Petelewicz
Affiliation: University of California, Riverside (UCR)

Objective:

1. Evaluate advanced zoysiagrass lines from Texas A&M and commercial cultivars for adaptation and performance on golf course fairways in Northern California.

Start Date: 2019
Project Duration: 2 years
Total Funding: $4,000

Summary Points:

- Twenty zoysiagrass genotypes including 16 experimental lines from Texas A&M and 4 commercial cultivars were established from plugs on two golf courses in Northern California in July 2019.
- Establishment of all zoysiagrass genotypes was slower than expected, with an average of less than 32% cover 130 days after planting. Cool temperatures, especially average low temperatures, were most likely a limiting factor for turf establishment.
- Despite less than ideal establishment, there was considerable variation among the genotypes in terms of growth, texture, and color.

Summary Text:

Studies were initiated on July 24 and 25, 2019 at Meadow Club, Fairfax (Marin County) and Napa Golf Course, Napa (Napa County), respectively. Sod of existing cool-season turf (ryegrass, annual bluegrass) was removed from fairway areas on both golf courses in preparation for planting. Plant material arrived as plugs or was divided into plugs and planted in 5 x 5 ft plots (no alleys) with 3 replications per entry at each location. A total of 20 zoysiagrass genotypes were planted including 16 experimental lines from Texas A&M, 2 standard commercial cultivars ('Innovation' and 'Meyer'), and 2 local standard commercial cultivars developed by UCR ('El Toro' and 'De Anza'). Both studies received irrigation as necessary and 2 lbs N/M during in 2019. Turf was evaluated visually for: ground cover (0-100%); quality (1-9, 9 = best); and green color (1-9, 9 = darkest). Experimental design was a randomized complete block. Data were subjected to analysis of variance and means separated using Fisher's Least Significant Difference Test.

Average monthly temperatures for both locations are provided in Table 1. Cool temperatures, especially low temperatures, most likely were responsible for minimal growth and establishment of zoysiagrass in 2019 (Table 2). Since plant material was provided in different sized cells/containers, plots were not planted with the same size or number of plugs. Nevertheless, ground cover ranged from only 20-28% and 17-32% in Fairfax and Napa, respectively, >4 months after planting. Visual quality was evaluated in December and was largely based on texture, cover, and fall color retention (Table 3). Lower ratings of some genotypes in Napa may have been caused by injury from application of indaziflam for annual bluegrass control. Although the first year of establishment is typically not the best predictor of fall/winter color retention or spring greenup, differences in green color were observed among the genotypes in December (Table 4). Evaluations will continue in 2020 with more emphasis on increasing fertility to help expedite establishment of the genotypes.

Table 1. Average monthly high and low temperatures (F) at Meadow Club, Fairfax, CA and Napa Golf Course, Napa, CA from July to November 2019.

Month (2019)	Meadow Club		Napa Golf Course	
	Avg.High (F)	Avg. Low (F)	Avg.High (F)	Avg. Low (F)
July	80.3	50	78.5	54.2
August	82	52.8	82.1	55.1
September	78.3	52.5	81.4	50.3
October	74.3	41.6	77.2	40.6
November	66.5	35	66.7	37.4

Table 2. Percent visual living ground cover of sixteen experimental zoysiagrasses and four commercial standards at 131 days after planting (DAP) in Fairfax, CA and 132 DAP in Napa, CA.

	Ground Cover*	
Entry	Meadow Club, Fairfax CA 3 Dec. 2019 131 DAP** (0-100%)	Napa Golf Course, Napa CA 3 Dec. 2019 132 DAP (0-100%)
DALZ 1308	23.3	28.3 abc***
DALZ 1309	23.3	31.7 a
DALZ 1701	21.7	20.0 def
DALZ 1702	25.0	25.0 bcd
DALZ 1703	28.3	25.0 bcd
DALZ 1707	21.7	25.0 bcd
DALZ 1802	26.7	31.7 a
DALZ 1807	28.3	31.7 a
DALZ 1808	25.0	25.0 bcd
DALZ 1809	20.0	16.7 f
DALZ 1810	23.3	18.3 ef
DALZ 1811	31.7	23.3 cde
DALZ 1812	25.0	16.7 f
DALZ 1813	23.3	25.0 bcd
DALZ 1814	22.5	25.0 bcd
DALZ 1815	23.3	28.3 abc
De Anza	20.0	23.3 cde
Diamond	26.7	30.0 ab
El Toro	23.3	28.3 abc
Innovation	21.7	21.7 def

* Living ground cover was rated visually on a scale of 0 to 100%.

** DAP – days after planting. Plugs were planted on 25 July in Fairfax, CA and 24 July 2019 in Napa, CA.

*** Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 3. Turfgrass visual quality of sixteen experimental zoysiagrasses and four commercial standards at 131 days after planting (DAP) in Fairfax, CA and 132 DAP in Napa, CA.

	Turfgrass Quality[*]	
	Meadow Club, Fairfax CA	Napa Golf Course, Napa CA
	3 Dec. 2019	3 Dec. 2019
	131 DAP[**]	132 DAP
Entry	(1-9)	(1-9)
DALZ 1308	7.3 abc[***]	5.0 cd[***]
DALZ 1309	7.0 bcd	5.7 bc
DALZ 1701	6.7 cde	5.7 bc
DALZ 1702	7.0 bcd	6.0 abc
DALZ 1703	7.0 bcd	6.0 abc
DALZ 1707	6.3 de	6.0 abc
DALZ 1802	7.7 ab	7.0 a
DALZ 1807	8.0 a	6.7 ab
DALZ 1808	7.0 bcd	6.0 abc
DALZ 1809	6.0 e	4.3 d
DALZ 1810	7.0 bcd	5.0 cd
DALZ 1811	6.7 cde	6.0 abc
DALZ 1812	6.3 de	4.3 d
DALZ 1813	6.7 cde	6.0 abc
DALZ 1814	7.0 bcd	5.7 bc
DALZ 1815	7.7 ab	6.3 ab
De Anza	7.5 abc	6.0 abc
Diamond	7.7 ab	6.7 ab
El Toro	6.3 de	6.0 abc
Innovation	6.0 e	4.3 d

[*] Turfgrass quality was rated visually on a scale of 1 to 9, where 9 = highest quality, 6 = minimum acceptable quality and 1 = lowest quality.
[**] DAP – days after planting. Plugs were planted on 25 July at Fairfax, CA and 24 July 2019 in Napa, CA.
[***] Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 4. Turfgrass visual color of sixteen experimental zoysiagrasses and four commercial standards at 131 days after planting (DAP) in Fairfax, CA and 132 DAP in Napa, CA.

Entry	Turfgrass Color*	
	Meadow Club, Fairfax CA 3 Dec. 2019 131 DAP** (1-9)	Napa Golf Course, Napa CA 3 Dec. 2019 132 DAP (1-9)
DALZ 1308	4.7 de***	4.3 ef***
DALZ 1309	5.0 cde	5.0 cdef
DALZ 1701	4.3 ef	5.0 cdef
DALZ 1702	5.0 cde	4.3 ef
DALZ 1703	4.7 de	5.0 cdef
DALZ 1707	4.7 de	6.0 abc
DALZ 1802	6.7 a	6.7 a
DALZ 1807	6.3 ab	6.7 a
DALZ 1808	4.7 de	5.0 cdef
DALZ 1809	3.3 fg	4.0 f
DALZ 1810	4.7 de	5.0 cdef
DALZ 1811	5.0 cde	4.7 def
DALZ 1812	3.0 g	4.0 f
DALZ 1813	4.3 ef	5.3 bcde
DALZ 1814	5.5 bcd	6.0 abc
DALZ 1815	6.3 ab	6.3 ab
De Anza	6.0 abc	6.0 abc
Diamond	6.3 ab	6.7 a
El Toro	4.7 de	5.7 abcd
Innovation	2.7 g	4.0 f

* Turfgrass color was rated visually on a scale of 1 to 9, where 9 = darkest green color, 6 = minimum acceptable green color and 1 = brown.

** DAP – days after planting. Plugs were planted on 25 July in Fairfax, CA and 24 July 2019 in Napa, CA.

*** Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Figure 1. Zoysiagrass genotypes at Meadow Club in Fairfax, CA. August 2019.

Figure 2. Zoysiagrass genotypes at Napa Golf Course, CA. August 2019.

Figure 3. Zoysiagrass genotypes at Meadow Club in Fairfax, CA. December 2019.

Figure 4. Zoysiagrass genotypes at Napa Golf Course, CA. December 2019.

USGA ID#: 2019-32-702 (Purdue); 2019-34-704 (KSU)

Title: On-course Evaluations of New Zoysiagrass Hybrids

Project Leaders: Jack Fry[1], Megan Kennelly[1], Manoj Chhetri[1], Aaron Patton[2], Ross Braun[2], Ambika Chandra[3], and Dennis Genovesi[3]

Affiliation: Kansas State University[1], Purdue University[2] Texas A&M AgriLife Research-Dallas[3]

Cooperators: Scott Johnson, Shadow Glen Golf Club, Olathe, KS; Brad Pugh, Country Club of Terre Haute, Terre Haute, IN, and Randy Brehmer, The Fort Golf Course, Indianapolis, IN.

Objectives:

Evaluate replicated field trials comprised of elite zoysiagrass hybrids at multiple environments in the transition zone with the objective to select experimental hybrids that have comparable/superior cold tolerance to Meyer, but finer texture, and improved large patch tolerance.

Start Date: 2019
Project Duration: 2 years
Total Funding: $6,000 ($4,000 Purdue; $2,000 KSU)

Summary Points:

- Ten experimental zoysiagrass hybrids that demonstrated cold hardiness, good quality, and large patch tolerance were planted as plugs at Shadow Glen Golf Club, Olathe, KS on 17 June 2019, The Country Club of Terre Haute, Terre Haute, IN on 2 July 2019, and The Fort Golf Resort, Indianapolis, IN on 2 July 2019.
- On 27 Sept. 2019 (102 days after planting), coverage ranged from 63 to 93% in Olathe, KS.
- On 30 Aug. 2019 (59 days after planting), coverage ranged from 25 to 39% in Terre Haute, IN and from 18 to 35% in Indianapolis, IN.
- Performance of hybrids will be evaluated throughout the 2020 growing season.

Summary Text:

Expansion studies were established on driving ranges or nursery areas at Shadow Glen Golf Club, Olathe, KS on 17 June 2019, The Country Club of Terre Haute, Terre Haute, IN on 2 July 2019, and The Fort Golf Course, Indianapolis, IN on 2 July 2019. Plots in KS were prepared by removing the existing Kentucky bluegrass (*Poa pratensis*) using a sod cutter. Soil was added back to each plot and leveled smooth. Each plot measured 6 ft x 6 ft with 2 ft alley between plots. Plots at the two IN sites were prepared by treating the area with glyphosate to kill the existing vegetation and then dead vegetation was mowed at a height of 1 inch or lower to remove debris. Twenty-four plugs were planted in each plot to evaluate ten experimental zoysiagrass hybrids that have demonstrated good quality, cold tolerance, and tolerance to large patch. At all the sites, two commercial cultivars, 'Meyer' and 'Innovation,' were also planted using twenty-four plugs at IN sites, and 12 larger plugs were used per plot in KS. For that reason, these cultivars were not included in the ground cover data analysis for Olathe, KS. Ronstar G was applied immediately after planting at 100 lbs product per acre at all sites. The study was set up as randomized complete block with four replicates in Olathe, KS and three replicates at IN sites. Percent living ground cover was rated visually on a scale of 0-100% on 27 Sept. 2019 [102 days after planting (DAP)] in Olathe, KS, and 30 Aug. 2019 (59 DAP) at IN sites following NTEP guidelines (Morris and Shearman, 2000). Data were subjected to analysis of variance and means separated using Fisher's Least Significant Difference Test.

Olathe, KS coverage ranged from 63% to 93% on 27 Sept. 2019. The genotypes DALZ 1703, DALZ 1812, and DALZ 1808 had significantly more ground coverage than DALZ 1707 and DALZ 1809 in Olathe, KS (Table 1). At Terre Haute, IN, coverage ranged from 25 to 39% on 30 Aug. 2019 (59 DAP), with no statistical differences among entries, while in Indianapolis, IN, coverage ranged from 18 to 35% on 30 Aug. 2019. Similar to Olathe, KS results, the genotypes DALZ 1812 and DALZ 1808 were two of the faster establishing entries (Table 1). In addition, the two previously mentioned genotypes, DALZ 1812 and DALZ 1808, along with DALZ 1703, DALZ 1810, and DALZ 1701 had significantly more ground coverage than both commercial standards (Meyer and Innovation) (Table 1).

References:

Morris, K. N., & Shearman, R. C. 1998. NTEP turfgrass evaluation guidelines. In NTEP turfgrass evaluation workshop, Beltsville, MD. p. 1-5.

Acknowledgements:

We would like to thank Scott Johnson, CGCS at Shadow Glen Golf Club, Olathe, KS; Brad Pugh, CGCS at the Country Club of Terre Haute, Terre Haute, IN, and Randy Brehmer, CGCS at The Fort Golf Course, Indianapolis, IN for hosting and managing a zoysiagrass expansion site.

Table 1. Percent living ground cover of ten experimental zoysiagrasses and two commercial standards at 102 days after planting (DAP) in Olathe, KS and at 59 DAP at both IN sites.

	Ground cover†		
	Olathe, KS 27 Sept. 2019 (102 DAP)	Terre Haute, IN 30 Aug. 2019 (59 DAP)	Indianapolis, IN 30 Aug. 2019 (59 DAP)
Entry	-----% -----	-----% -----	-----% -----
DALZ 1703	92.7 a‡	33.3	29.0 abc
DALZ 1812	89.3 ab	33.0	35.0 a
DALZ 1808	81.7 abc	38.3	34.0 ab
DALZ 1811	76.7 bcd	25.0	26.7 bcd
DALZ 1701	75.0 bcd	31.0	28.3 abc
DALZ 1702	74.3 bcd	37.3	25.0 cde
DALZ 1813	71.7 cd	30.0	23.3 cde
DALZ 1810	70.0 cd	33.3	28.3 abc
DALZ 1707	65.0 d	28.3	24.0 cde
DALZ 1809	63.3 d	30.0	25.0 cde
Meyer	--§	29.0	18.7 e
Innovation	--	29.3	20.7 de

† Living ground cover was rated visually on a scale of 0 to 100%. Plugs were planted on 17 June 2019 at Olathe, KS and on 2 July 2019 at both Indiana sites.
‡ Means within a column followed by the same letter are not statistically different at $P \leq 0.05$ according to Fisher's protected least significant difference test.
§ 'Meyer' and 'Innovation' were not included in the Olathe, KS analysis because of using fewer plugs/plot during establishment.

Figure 1: Ten experimental zoysiagrasses, and Meyer and Innovation, were planted from plugs at Shadow Glen Golf Club, Olathe, KS on June 17, 2019. Plots showing some variability in ground cover 102 days after planting on September 27, 2019.

USGA ID#: 2019-33-703

Title: Zoysiagrass Establishment Trial in the Low Desert Southwest

Project Leaders: Kai Umeda and Worku Burayu
Affiliation: University of Arizona

Objectives:

Evaluate and compare the adaptation and performance of Zoysiagrass cultivars in the low desert southwest United States.

Start Date: 2019
Project Duration: 2 years
Total Funding: $2,000

Summary Text:

Rationale
Maintaining green turfgrass year around is a goal for the low desert southwest where warm season dormant bermudagrass is overseeded with a cool season annual grass in the winter. In recent years, winter survival of warm season zoysiagrasses has been improved such that green color retention has been observed for some cultivars.

Methods
A field study of 16 new zoysiagrass introductions, along with 2 commercial cultivars, and 2 bermudagrass hybrids was established at the Wigwam Golf Club in Litchfield Park, AZ. The experimental plots for each turfgrass cultivar measured 8 ft by 8 ft and were arranged in a randomized complete block design with 3 replicates. Zoysiagrass plugs and bermudagrass sod strips were planted on two dates: 11 July and 02 August 2019 (Table 1). The site of the plots is irrigated with overhead sprinklers and water was applied multiple times per day until the plugs were adequately established. The grasses were regularly evaluated during the summer for plug survival and overall performance and appearance that included color, density, and vigor. This first-year report especially emphasizes survival and establishment. The survival and performance ratings (1 to 9) were evaluated following the procedures developed by National Turfgrass Evaluation Program (1 = poor and 9 = excellent).

Results
Overall, most of the zoysiagrasses and the 2 bermudagrasses established very well (Figure 1). One cultivar, DALZ1814 did not survive in this planting under the low desert conditions and DALZ1815 struggled to establish (Figure 1). There was also variation in the general performance in establishment (Figure 3). Bermudagrass cultivars TifTuf and Tifway 419 demonstrated excellent performance as compared to many cultivars of zoysiagrasses. The zoysiagrass cultivars DALZ1810, DALZ1813, DALZ1812, DALZ1809, DALZ1703, DALZ1808, DALZ1309, DALZ1308, and DALZ1811 appeared somewhat less comparable to the bermudagrasses but improved over the commercial Innovation and Diamond cultivars at about 2 months after planting. The DALZ 1702, DALZ1707, DALZ1701, DALZ1807, DALZ1802, and DALZ1815 were less vigorous and dense relative to the commercial cultivars. The spread and breadth of surface coverage was negligible or minimal for the zoysiagrass cultivars.

Table 1. Zoysiagrasses and bermudagrasses planted and evaluated in the low desert Arizona (2019)		
Variety	Planting date	Notes
DALZ1701	11 July	24 plugs
DALZ1702	11 July	24 plugs
DALZ1703	11 July	24 plugs
DALZ1707	11 July	24 plugs
DALZ1808	11 July	24 plugs
DALZ1812	11 July	24 plugs
DALZ1810	11 July	24 plugs
DALZ1811	11 July	24 plugs
DALZ1809	11 July	24 plugs
DALZ1813	11 July	24 plugs
Innovation	11 July	24 plugs
DALZ 1802 (Pacifica 1)	02 August	48 plugs
DALZ 1807 (Pacifica 2)	02 August	48 plugs
DALZ 1814 (Minima 1)	02 August	48 plugs
DALZ 1815 (Minima 2)	02 August	48 plugs
Diamond	02 August	48 plugs
Tifway 419	02 August	sod strip
TifTuf	02 August	sod strip
DALZ 1309	11 July	48 plugs
DALZ 1308	11 July	48 plugs

Figure 1. Establishment of zoysiagrasses, 08 August (upper) and 26 November (lower) at Wigwam Golf Club in Litchfield Park, AZ after plantings on 11 July and 02 August 2019.

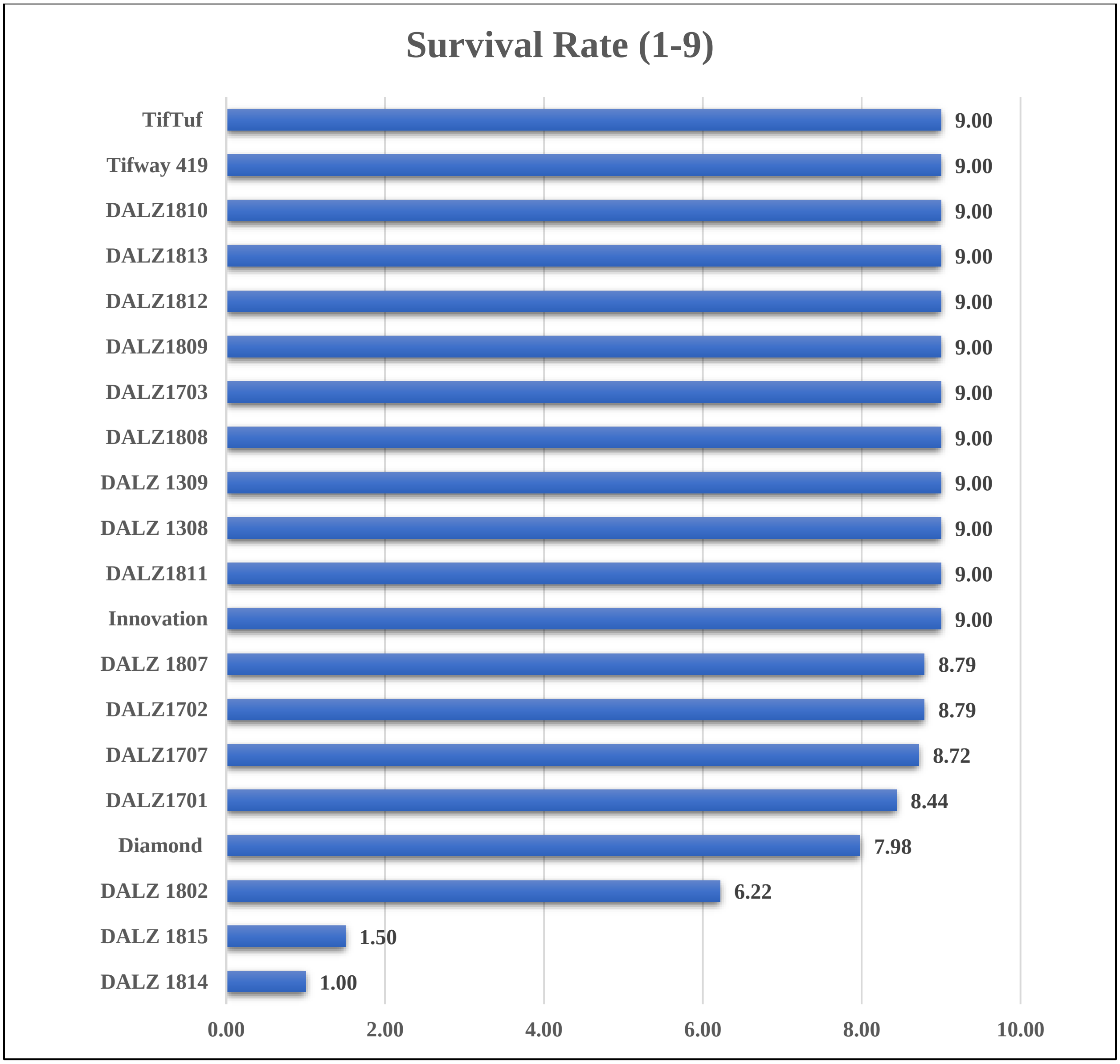

Figure 2. The survival rate of zoysiagrasses at Wigwam Golf Club in Litchfield Park, AZ in 2019.

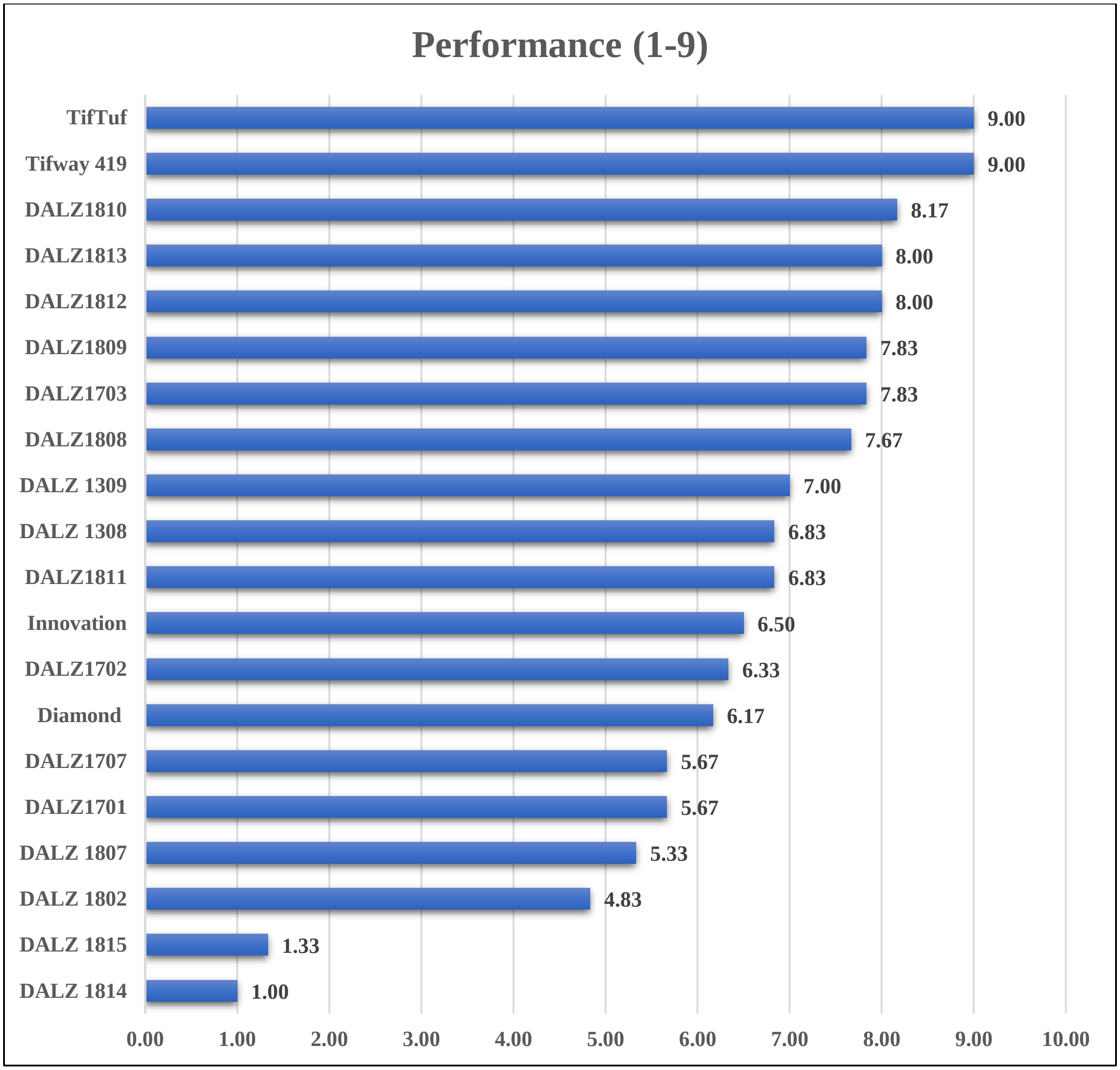

Figure 3. Vigor, density, and color performance of zoysiagrasses on 15 October 2019 after plantings on 11 July and 02 August 2019 at Wigwam Golf Club in Litchfield Park, AZ.

USGA ID#: 2019-20-690

Title: Chemical Priming to Improve Annual bluegrass Responses to Ice Stress

Projects Leaders: Kevin Laskowski and Emily Merewitz Holm
Affiliation: Michigan State University

Objectives:

1) Evaluate whether chemical priming and Primo applications influence winter survival and spring green up rates in Michigan in 2017 and 2018 (field experiment)
2) Determine whether chemical priming and Primo applications affects annual bluegrass performance under no ice and ice stress conditions (freezer experiment)

Start Date: 2019
Project Duration: 2 years
Total Funding: $20,000

Summary Points:

- Treatment of annual bluegrass in the summer and fall seasons with CIVITAS, PRIMO+CIVITAS, JA, and PRIMO+JA may improve recovery from prolonged ice encasement conditions.
- The chemical treatments that improved ice encasement recovery may have altered annual bluegrass fatty acid contents, such as increasing the unsaturated fatty acid, linoleic acid.
- The CIVITAS+PRIMO and PRIMO treated annual bluegrass had the highest turfgrass quality during the summer and fall treatment periods.

Summary Text:

Priming of plants means that a given treatment makes plants more prepared to take on a subsequent stress. Information from controlled research studies available on priming chemicals for turfgrass species in response to abiotic stress is lacking. Plant priming with salicylic acid (SA) and jasmonic acid (JA) could potentially boost the systemic acquired resistance (SAR) or induced systemic resistance (ISR) pathways, respectively. Both JA and SA are either already in turf products or have potential to be in turf products. These are two pathways that are primarily associated with plant defense of biotic stress but are also involved in promoting tolerance to abiotic stresses. CIVITAS Pre-M1xed is also said to have an ISR stimulating effect on plants. In our previous work funded by the USGA, we have found that this CIVITAS product was beneficial to annual bluegrass survival of ice cover. CIVITAS treated plants had a higher level of the fatty acid linolenic acid, a precursor to JA, than control plants (Laskowski et al, 2018). In that same study, PGRs such as Primo showed some evidence of decreasing ice tolerance of annual bluegrass; however, not on all days measured. This study aims to determine whether priming of annual bluegrass with CIVITAS Pre-M1xed, SA, and JA in combination with PGR treatment improves or inhibits winter survival and spring green-up under natural field conditions and ice stress tolerance in simulated controlled conditions.

All chemical treatments began on 27 June through 4 August of 2017 and 2018 being applied every two weeks, then on 30 October 2017 and 2018 based on CIVITAS program recommendations for use in the summer and fall months. All treatments were applied with a pressure-calibrated backpack sprayer (63.3-gal a^{-1} at 275 kPa) equipped with four flat fan nozzles (DG8002 DS, Teejet Technologies, Wheaton, IL.). The treatments were:1) Control 2) Primo (0.125 fl oz/1000ft^2) 3) CIVITAS Pre-M1xed (8 fl oz/1000ft^2) 4) JA (2mM) 5) JA (0.5mM) 6) SA (20µM) 7) SA (10µM) 8) CIVITAS Pre-M1xed + Primo (8 fl oz/ 1000ft^2 + 0.125 fl oz/1000ft^2) 9) JA + Primo (2mM + 0.125 fl oz/1000ft^2) 10) JA + Primo (0.5mM +0.125 fl oz/ 1000ft^2) 11) SA + Primo (20µM +0.125 fl oz/1000ft^2) and 12) SA + Primo (10µM +0.125 fl oz/1000ft^2). Commonly measured turf evaluation parameters were measured in the field on all plots including turf quality, the dark green color index (DGCI), normalized difference vegetation index (NDVI).

After two years of data CIVITAS or CIVITAS + Primo had the greatest turf quality when compared to the untreated control. On several dates and for turf quality, NDVI and DGCI, CIVITAS and Primo, JA and Primo, and SA and Primo treatment combinations had improved values compared to control plots and Primo alone (only for DGCI). For recovery from ice treatments, after 40 d of ice, ABG treated with CIVITAS, CIVITAS+PRIMO, had significantly higher recovery than all other treatments. JA, SA, and PRIMO+SA had significantly higher recovery than untreated controls. After 80 d under ice, CIVITAS, PRIMO+CIVITAS, JA, and PRIMO+JA had greater recovery when compared to the untreated controls. Less significant differences were determined following just low temperature conditions; however, after 80 d of low temperature CIVITAS treated ABG had significantly greater recovery than all other treatments.

Means for fatty acid analysis for both years and ice and no ice treatment are pooled due to no significant differences between those two factors. Fatty acid analysis indicates after 0 d of -4^{o}C treatment, SA and Primo+JA treated ABG had lower steric acid contents when compared to the untreated control (Table 1.). After 20 d, CIVITAS treated ABG had lower palmitic fatty acid (saturated) content and greater linoleic acid (unsaturated) content when compared to the untreated control (Table 2.). After 40 d JA, CIVITAS, PRIMO + CIVITAS and PRIMO + JA treated ABG had lower palmitic acid when compared to the untreated control (Table 3.). CIVITAS treated ABG had greater linoleic acid when compared to the untreated control while PRIMO and SA treated ABG had lower linoleic acid when compared to the untreated control. After 80 d, SA, JA, and PRIMO + CIVITAS treated ABG had lower palmitic acid content when compared to the untreated control (Table 4.). Primo treated ABG had lower linoleic acid when compared to the untreated control.

Literature Cited

Laskowski, K., K. Frank, and E. Merewitz. 2018. Chemical Plant Protectants and Plant Growth Regulator Effects on Annual Bluegrass Survival of Ice Cover. Journal of Agronomy and Crop Science. *Under Review*.

Figure 1. Turfgrass quality (1–9 scale with 1 (poor) and 9 (best), with 6 being acceptable) of annual bluegrass under chemical priming treatments in 2017 and 2018. Bars represent Fisher's protected least significant difference at P ≤ 0.05 for the comparison of means on each date.

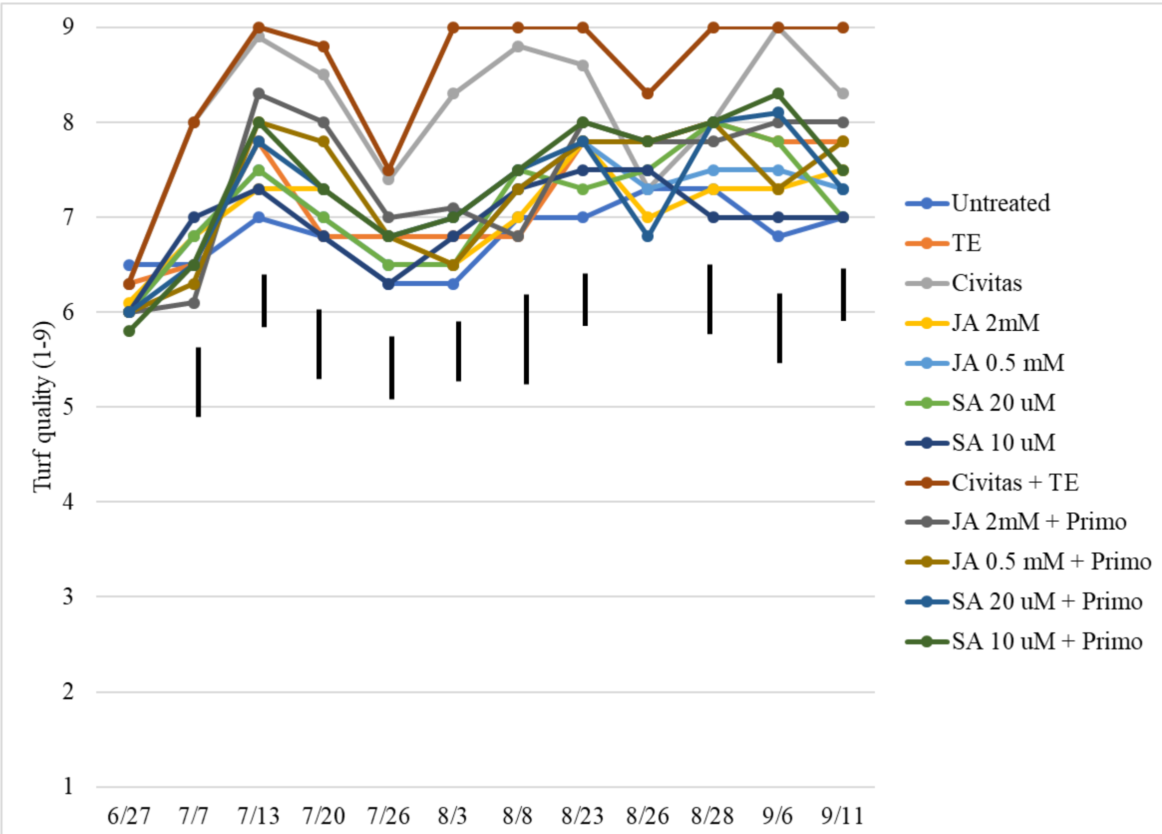

Figure 2. Normalized difference vegetation index of annual bluegrass under chemical priming treatments in 2017 and 2018. Bars represent Fisher's protected least significant difference at P ≤ 0.05 for the comparison of means on each date.

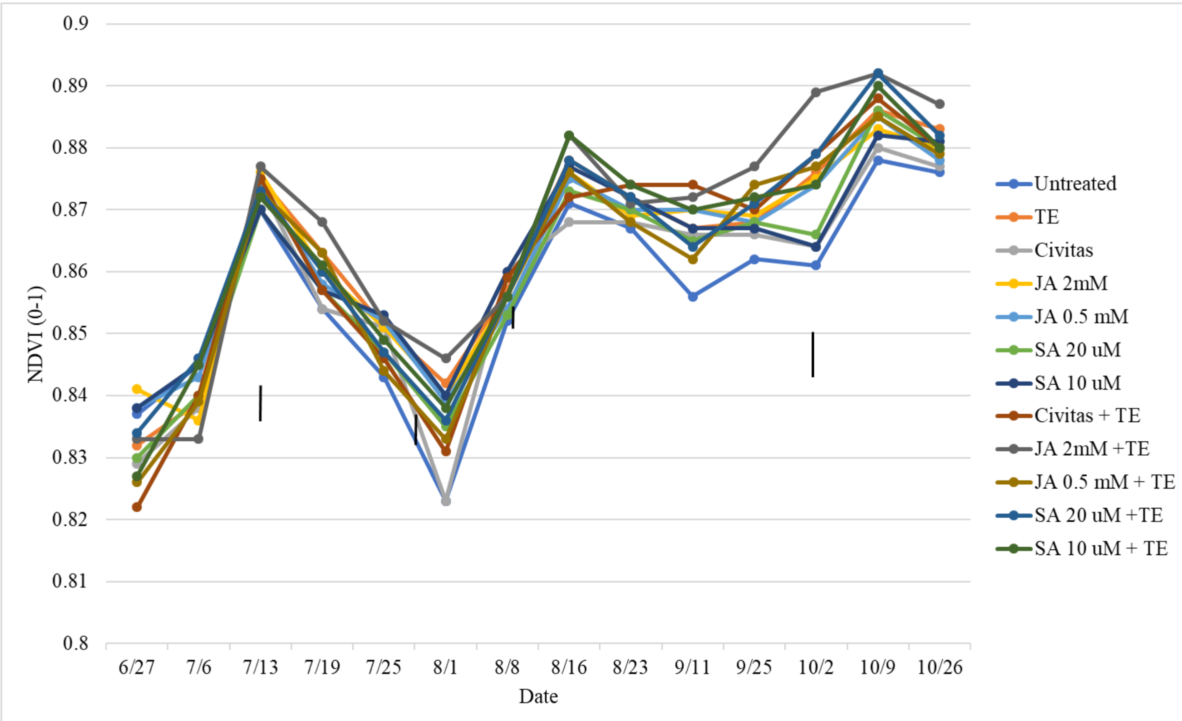

Figure 3. Dark green color index of annual bluegrass under chemical priming treatments in 2017 and 2018. Bars represent Fisher's protected least significant difference at P ≤ 0.05 for the comparison of means on each date.

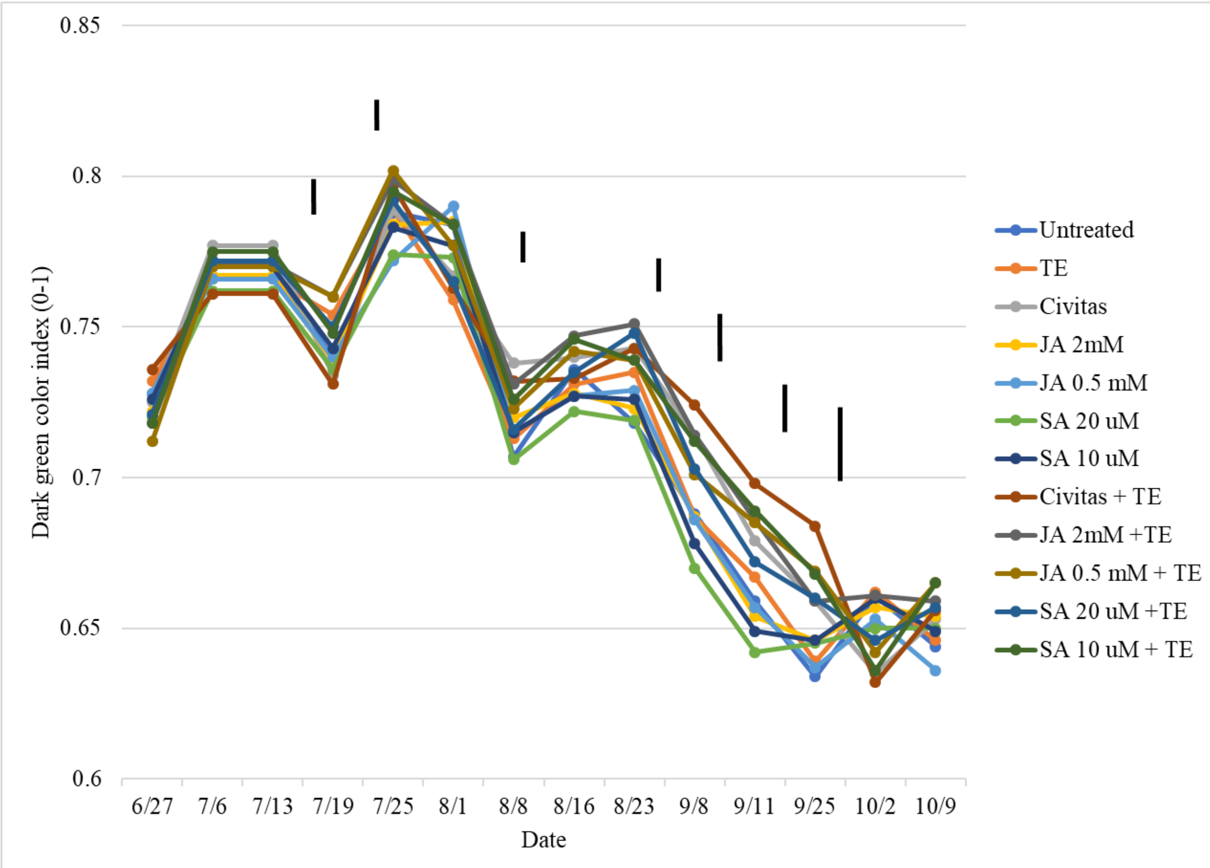

Figure 4. Percent recovery of annual bluegrass under chemical priming treatments in 2017 and 2018 after 0, 20, 40 and 80 days after initial growth under ice cover. Bars with different letters are significantly different (P ≤ 0.05) due to treatment within a given day.

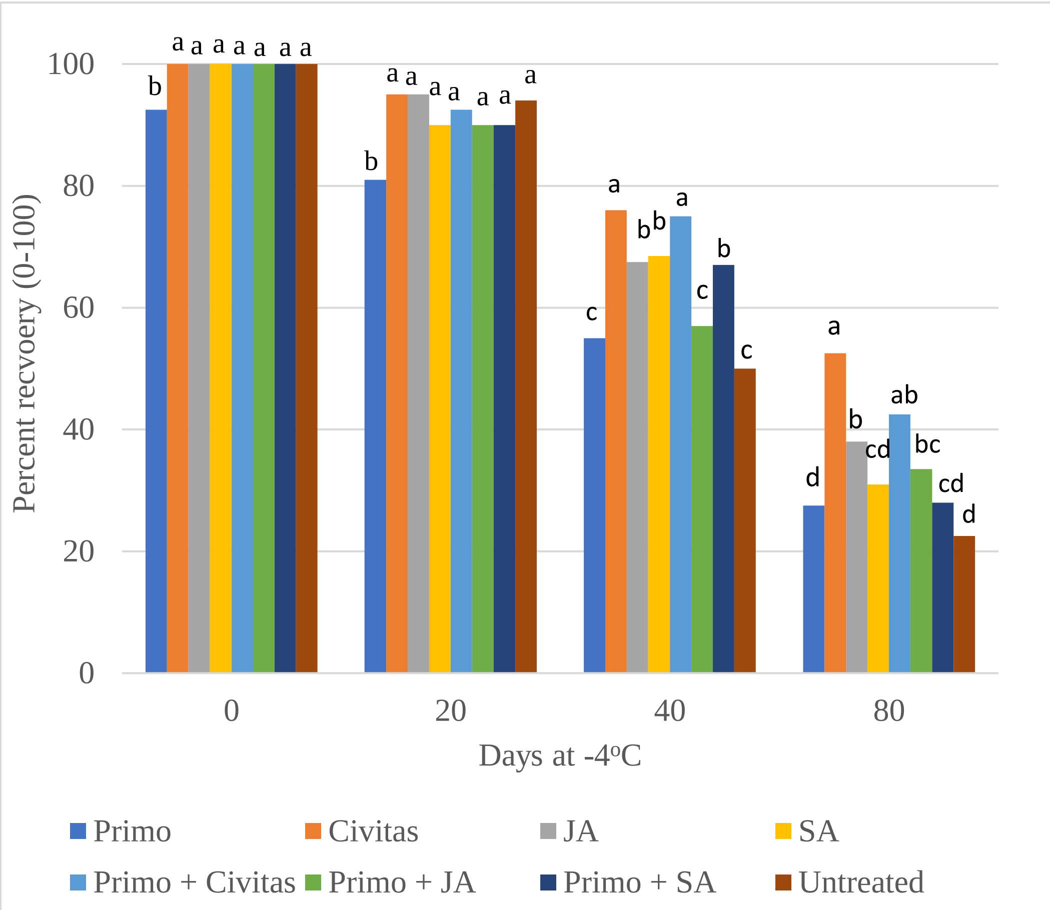

Figure 5. Percent recovery of annual bluegrass under chemical priming treatments in 2017 and 2018 after 0, 20, 40 and 80 days after initial growth under no ice cover. Bars with different letters are significantly different (P ≤ 0.05) due to treatment within a given day.

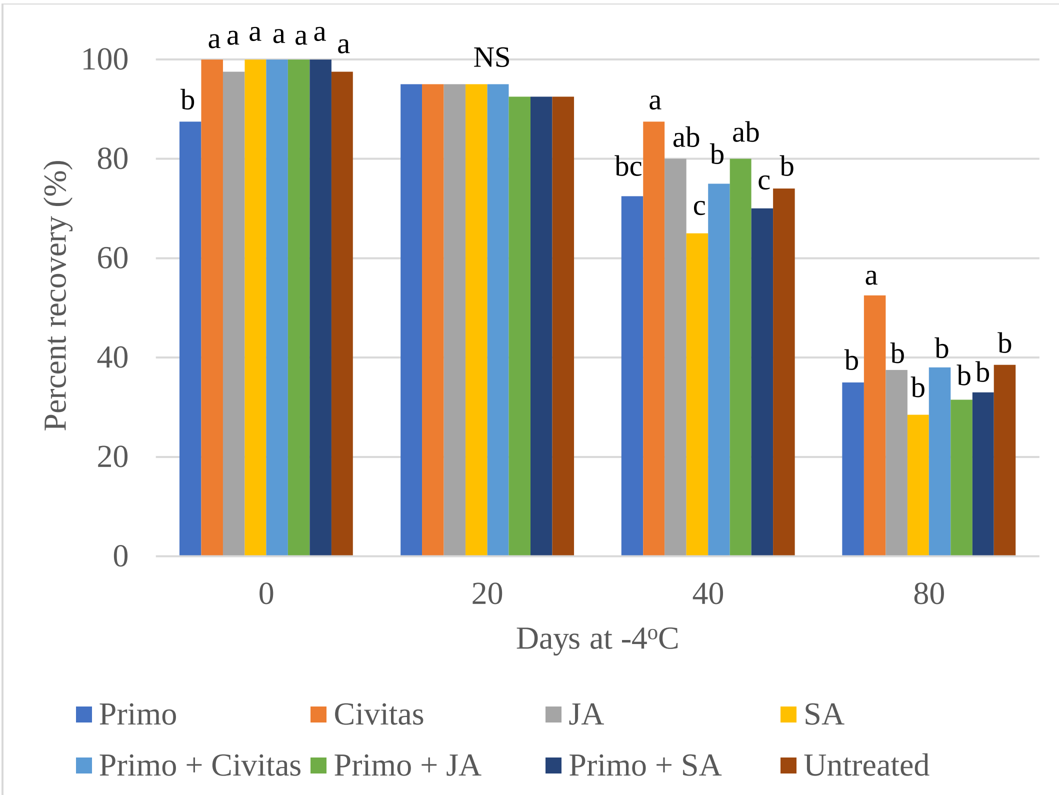

Table 1. Changes in fatty acid contents of crown tissue of annual bluegrass (*Poa annua* exposed to 0 days in a low temperature growth chamber (-4 °C) under chemical priming treatments. Means from both 2017 and 2018 are pooled together. Ice and no ice cover treatment means are pooled together. Within each column for each fatty acid, means followed by the same letter are not significantly different ($P \leq 0.05$). Columns with no letters indicate no significant differences among chemical treatments.

	Crown tissue Fatty Acids					
	Molar percentage (mol %)					
	saturated		unsaturated			
	16:0	18:0	16:1	18:1	18:2	18:3
	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid
Primo	44.5a	22a	1.4b	2.3	19.5	14
SA	41.4ab	17.6c	1.9ab	2.1	20.7	16.7
JA	34.3cd	22.8a	2a	2.3	24	13.5
Civitas	37.8bc	22.8a	2.1a	3.3	24	14.9
Primo + Civitas	44ab	19.3abc	1.4b	3.2	24.3	15.7
Primo + SA	42ab	18.3bc	2.3 a	3.3	20	15.9
Primo + JA	31.8d	16.3c	2ab	2.3	24	13.5
Untreated	39.5abc	21ab	1.9ab	2.8	19.8	15.8
LSD	5.78	3.61	0.64	NS	NS	NS

Table 2. Changes in fatty acid contents of crown tissue of annual bluegrass (Poa annua exposed to 20 days in a low temperature growth chamber (-4 °C) under chemical priming treatments. Means from both 2017 and 2018 are pooled together. Ice and no ice cover treatment means are pooled together. Within each column for each fatty acid, means followed by the same letter are not significantly different ($P \leq 0.05$). Columns with no letters indicate no significant differences among chemical treatments.

	Crown tissue Fatty Acids					
	Molar percentage (mol %)					
	saturated		unsaturated			
	16:0	18:0	16:1	18:1	18:2	18:3
	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid
Primo	42.8a	22.8	1.9	1.9	17.8d	13
SA	42a	21.8	1.7	3.7	21.3abcd	16.8
JA	42a	19.3	3	3.1	19bcd	15.5
Civitas	34b	18.5	1.9	3.2	24.5a	16.8
Primo + Civitas	40.5a	22.3	1.8	3.5	22.8ab	16.5
Primo + SA	42.5a	20.5	2.2	3.2	21.8abc	16
Primo + JA	37.5b	18.5	2.7	3	17.5d	14.5
Untreated	40.5a	22.8	2	3.1	18.3cd	13
LSD	2.74	NS	NS	NS	3.94	NS

Table 3. Changes in fatty acid contents of crown tissue of annual bluegrass (Poa annua exposed to 40 days in a low temperature growth chamber (-4 °C) under chemical priming treatments. Means from both 2017 and 2018 are pooled together. Ice and no ice cover treatment means are pooled together. Within each column for each fatty acid, means followed by the same letter are not significantly different (P ≤ 0.05). Columns with no letters indicate no significant differences among chemical treatments.

	Crown tissue Fatty Acids					
	Molar percentage (mol %)					
	saturated		unsaturated			
	16:0	18:0	16:1	18:1	18:2	18:3
	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid
Primo	45.8a	24.8	1.8bc	2.4c	12.5d	11.9
SA	41.5bc	21.3	1.9bc	2.9abc	12.7d	13.6
JA	40.7c	18.4	1.3c	3.4ab	15.2bcd	13.4
Civitas	38c	25.8	3.3a	2.7bc	19a	13.8
Primo + Civitas	40.3c	19.5	2.3b	3.6a	17.2ab	13.7
Primo + SA	41bc	19.8	1.8bc	3.5ab	13.3cd	14
Primo + JA	40c	23.8	1.6bc	3.4ab	14.3bcd	15.1
Untreated	44.3ab	24.3	1.7bc	2.4c	16.1abc	14.7
LSD	3.54	NS	0.75	0.89	3.28	NS

Table 4. Changes in fatty acid contents of crown tissue of annual bluegrass (Poa annua exposed to 80 days in a low temperature growth chamber (-4 °C) under chemical priming treatments. Means from both 2017 and 2018 are pooled together. Ice and no ice cover treatment means are pooled together. Within each column for each fatty acid, means followed by the same letter are not significantly different (P ≤ 0.05). Columns with no letters indicate no significant differences among chemical treatments.

	Crown tissue Fatty Acids					
	Molar percentage (mol %)					
	saturated		unsaturated			
	16:0	18:0	16:1	18:1	18:2	18:3
	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid
Primo	43.5a	28.5a	1.7	2.6	10.8c	10.8
SA	36c	21.8b	2.8	3.1	17.9ab	15
JA	37c	20.7bc	2.2	2.6	19.6a	16.2
Civitas	37.8bc	18c	2	2.5	20a	14.8
Primo + Civitas	35.8c	20.5bc	1.4	3.3	20.5a	14.2
Primo + SA	44.5a	21.3b	2.5	3	19.3a	15.9
Primo + JA	38.8bc	21.3b	2	2.2	15.1b	13.7
Untreated	41.8ab	22.8b	1.6	2.5	17.3ab	15.1
LSD	4.52	3.12	NS	NS	3.71	NS

USGA ID#: 2019-18-688

Title: Evaluating plant growth regulators and the soil surfactant Revolution® to alleviate drought stress in bermudagrass

Project Leader: Matteo Serena, Elena Sevostianova, Bernd Leinauer,
Affiliation: New Mexico State University

Objectives:

To evaluate the effect of six plant growth regulators (PGR) alone and in combination with the soil surfactant Revolution® on turfgrass quality and soil moisture content of bermudagrass irrigated at two different evapotranspiration (ET) replacement rates

Start Date: 2019
Project Duration: 2
Total Funding: $10,000

Summary Points:

- Revolution applied alone increased turfgrass quality at both ET replacement rates. When PGR were applied alone, only Cutless and Legacy resulted in higher turfgrass quality compared to the non-treated control.
- Generally, PGRs in combination with Revolution did not improve turf quality compared to the non-treated control. When Trimmit, Cutless and Legacy were combined with Revolution, quality was lower than with PGR alone.
- Turf height was greatest on control plots when irrigated at 75% ET
- Irrigation level (45% vs 75% ET) had no effect on turf height when PGRs were applied alone or in combination with Revolution
- Plots treated with Revolution had a more uniform moisture distribution than non-treated plots.

Summary Text:

The use of plant growth regulators (PGRs) and soil surfactants is a common practice in the golf industry. Previous research has demonstrated that applying the PGR trinexapac-ethyl (TE) under drought conditions improved bermudagrass turf quality (Schiavon et al., 2014). Additionally, soil surfactants have been shown to increase turfgrass quality under reduced water conditions (Cisar et al., 2000, Leinauer et al., 2007, Schiavon et al., 2014, Alvarez et al., 2016). More recently, the effects on bermudagrass quality of combining TE and a soil surfactant have been compared to those observed when each products was applied separately to determine if there was increased benefits under drought conditions (Serena et al., 2018; Schiavon et al., 2019). However, for the most part there were no differences among plots receiving TE alone and those receiving TE+Revolution (Serena et al., 2018; Schiavon et al., 2019). This could indicate the larger benefit of the PGR vs the PGR+ soil surfactant in decreasing the symptoms of drought related stress. To date, TE is the only PGR that has been evaluated for its beneficial role in drought tolerance, either alone or in combination with a soil surfactant. A turfgrass trial was conducted at New Mexico State University to study the effects of several PGRs, applied alone and in combination with Revolution®, on turfgrass performance parameters and soil moisture of drought stressed ‘Princess 77’ bermudagrass.

Material and Methods

The study was conducted at the New Mexico State University Saline Research Center in Las Cruces (NM) (arid, 1265-m elevation) from May to November 2018 to 2020. Plots were mowed three times per week at a height of 1.2 cm (1/2”) by means of a reel mower with clippings returned. Irrigation audits were conducted two times during the course of the study and provided data necessary to calculate irrigation system’s run times. During the spring of 2019, irrigation to the entire area was applied at 100% ET. Plots received the daily equivalent of 1/7 of the total weekly ET. Irrigation treatments included 45% (drought stressed) and 75% ET (unstressed control) from May to October. Chemical treatments were applied using a calibrated CO2 backpack boom sprayer (Bellspary Inc. Opelousas, LA) at 544 L ha-1 using three flat nozzles (XR1103VS Teejet) and operating at 482 KPa.

The following plant growth regulators were included in the study:

1. Untreated Control (Control)
2. PrimoMaxx 11oz/A (TE)
3. Trimit 32oz/A (Pac)
4. Primo 11oz/A + Trimit 8oz/A (TE+Pac)
5. Anuew 15oz/A (ProHex)
6. Cutless 25oz/A (Ful)
7. Musketeer 20oz/A (TE+Pac+Ful)
8. Legacy 15oz/A (TE+Ful)

Visual turf quality was assessed on a scale of 1 to 9. A rating of 1 represented extremely poor, dead, or no turf and 9 indicated a perfect, exceptional green and uniform plot. A rating of 6 was considered minimally acceptable appearance. Digital image analyses (percent green cover and DGCI) were evaluated following methods described by Richardson et al. (2001). Normalized Difference Vegetation Indices (NDVI) was determined with a GreenSeeker Hand HeldTM Optical Sensor Unit Model 505 (NTech, Ukiah, CA). Chlorophyll content, and soil moisture content was recorded twice per month starting 1 week after the first treatment application. Volumetric soil moisture content at depths of 0 to 10 cm was measured by means of a TDR 350 soil moisture meter (Field Scout TDR 350 Probe, Spectrum Technologies, Inc., Aurora IL). Irrigation was withheld for 24h before each measurement. Nine readings per plots were collected and averaged for moisture content. Moisture uniformity was calculated as standard deviation of the 9 readings. Lower values indicate a more uniform moisture distribution in the rootzone of each plot. Turf growth was determined by measuring the height of the turfgrass canopy with a Prism Gauge (Turf-Tec International, Tallahassee, FL). Turf growth was subsequently recorded as the difference between a measurement immediately after and three days after mowing.

The study was laid out in a completely randomized block with ET as the whole block treatment. PGRs alone or tank mixed with Revolution® (6 oz/M) were applied on randomly assigned plots (1.5 x 1.5 m) within each block. To test the effects of surfactants on turfgrass quality, cover, NDVI, DGCI, soil moisture uniformity and turf height, data were subjected to an analysis of variance (ANOVA) using SAS Proc Mixed followed by multiple comparisons of means using Fisher's LSD test at the 0.05 probability level.

Results

Statistical analysis indicated a significant interaction between pgr and surfactant for color (p=0.0016), cover (p=0.0195), NDVI (p=0.0380), and quality (p=0.0004) (Figure 1). Revolution increased turfgrass quality of the untreated control plot. However, when combined with other PGRs (TE, TE+Pac, TE+Pac+Ful) there was either no difference to the control or control plots showed greater visual quality (Pac, Ful, TE+Ful) (Fig. 1). Similar findings were noted for color, percent green turf cover and NDVI (Fig. 1). Additionally, the interaction of pgr and ET level was significant for turf growth (p=0.0297). The control plot exhibited a significant higher growth when irrigated at 75% when compared to 45% ET (Fig. 2). Surfactant had a statistically significant effect on soil moisture uniformity (p <.0001). When plots were treated with surfactant the moisture uniformity was lower that the untreated plots (3.1 vs. 3.9).

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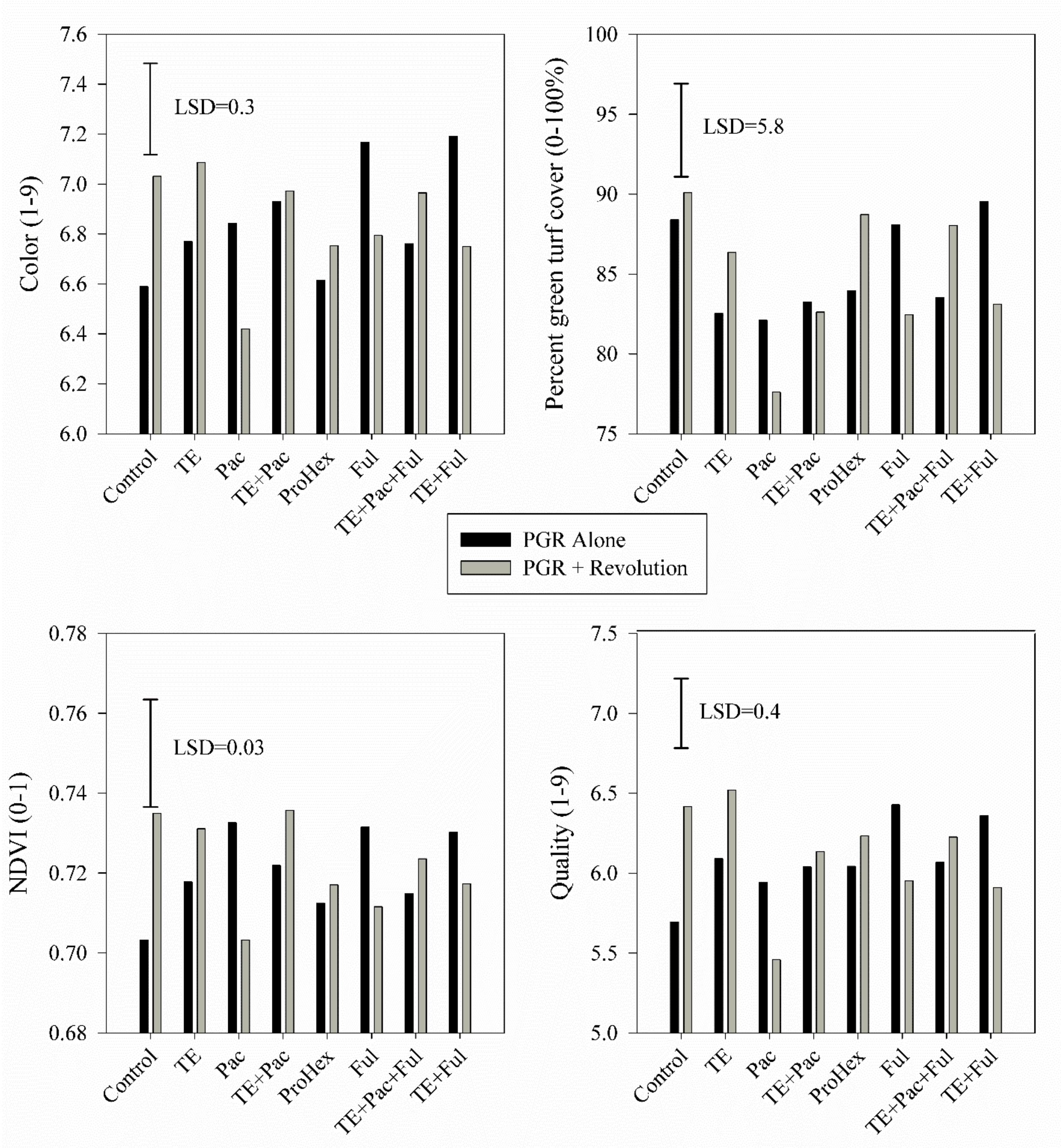

Figure 1. Bermudagrass color, percentage green turf cover, normalized difference vegetation index (NDVI) and quality, as affected by pgr [PrimoMaxx 11oz/A (TE), Trimit 32oz/A (Pac), Primo 11oz/A + Trimit 8oz/A (TE+Pac), Anuew 15oz/A (ProHex), Cutless 25oz/A (Ful), Musketeer 20oz/A (TE+Pac+Ful), Legacy 15oz/A (TE+Ful)]and soil surfactant Revolution. Values represent an average of 2 ET levels (45% and 75%), 14 sampling (bi-weekly over seven months) and four replications.

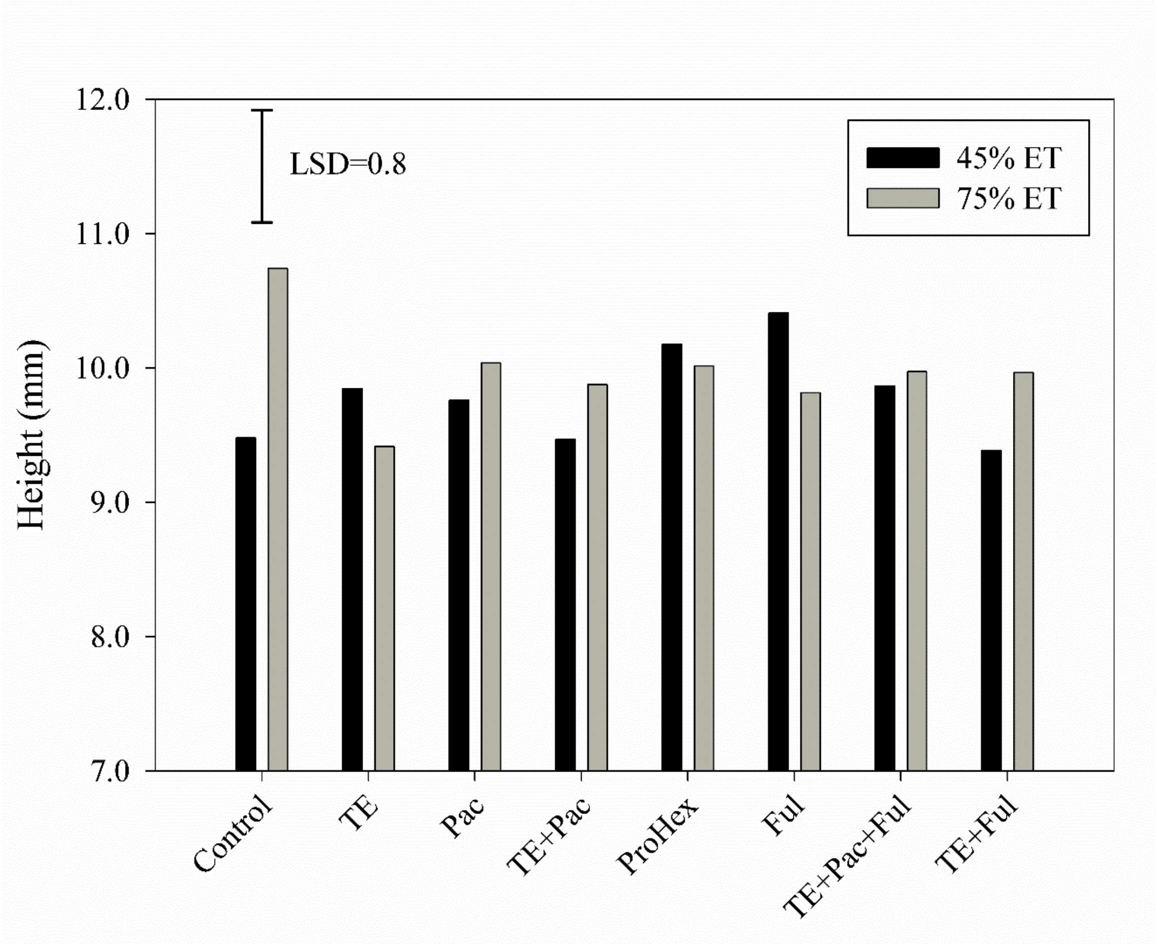

Figure 2. Bermudagrass growth (height) as affected by pgr [PrimoMaxx 11oz/A (TE), Trimit 32oz/A (Pac), Primo 11oz/A + Trimit 8oz/A (TE+Pac), Anuew 15oz/A (ProHex), Cutless 25oz/A (Ful), Musketeer 20oz/A (TE+Pac+Ful), Legacy 15oz/A (TE+Ful)]and ET level (45% and 75%) Values represent an average of 2 surfactant treatments (with and without Revolution), 20 sampling (weekly over seven months) and four replications.

USGA ID#: 2019-19-689

Title: Investigating infiltration rate and soil bicarbonate changes after sulfuric acid treatment of the irrigation water

Project Leader: Elena Sevostianova, Bernd Leinauer, and Matteo Serena
Affiliation: New Mexico State University

Objectives:

To investigate a relationship between irrigation water with increased levels of bicarbonates and associated soil infiltration and hydraulic conductivity rates using four methods of measurement.

Start Date: 2019
Project Duration: 6 months
Total Funding: $5,000

Summary Points:

- When pooled over water treatments, infiltration and unsaturated hydraulic conductivity rates determined by smaller diameter infiltrometer (Figure 1) and Mini Disk Infiltrometer (Figure 2) were lower at the end of the study (November) compared to the beginning (June). Infiltration rates measured by the larger diameter infiltrometer (Figure 1) did not differ between the beginning and the end of the study.
- Infiltration rates (measured with large diameter infiltrometer) were higher when irrigation water was amended with sulfuric acid compared to potable irrigation water or irrigation water high in bicarbonates after 6 months of irrigation.
- Unsaturated hydraulic conductivities determined by means of a Mini Disk Infiltrometer did not differ among water treatments at the end of the study. Hydraulic conductivity measured by KSAT (Figure 2) was lower in containers irrigated with water high in bicarbonates compared to containers irrigated with potable water or water treated with sulfuric acid.
- Soil irrigated with water high in bicarbonates had numerically but not statistically lower infiltration rates when measured with the smaller diameter infiltrometer.
- The type of infiltrometers influenced measurements but border effects may have affected our results. Generally, field experiments are needed to investigate possible differences in infiltration/hydraulic conductivity rates after irrigation over several growing periods.

Summary Text:

Many sources of irrigation water contain high levels of dissolved bicarbonates, which, along with sodium, calcium, and magnesium, are believed to be the major cause of soil physical problems such as low infiltration rates and reduced rooting. In arid climate zones, there is a concern that the deposit of calcium carbonate derived from irrigation water can seal soil pores over time. Such a problem is different from sodium-induced flocculation and deterioration of soil physical conditions. Acidification (e.g. with sulfuric acid) is one of the most common management practices used to decrease bicarbonate levels in irrigation water. However, the beneficial effect of amending irrigation water has been documented only for some chemical but not for physical properties. If irrigation with water containing high levels of bicarbonates is not negatively affecting soil infiltration rates or grass performance, the application of sulfuric acid can be reconsidered. Unfortunately, common measurements of infiltration rates have limitations. When the standard double ring infiltrometer is used in the field, factors such as surface vegetation, extent to which the soil has been compacted, soil layers (strata) and cracks / root channels can disturb the measurements.

A study was conducted in a controlled-environment greenhouse at New Mexico State University in Las Cruces, NM, during the summer and fall of 2019. Containers measuring 10 cm in diameter and 50 cm in depth were prepared and placed on the greenhouse floor to allow for free draining water to exit through holes in the bottom of each column. Each container was filled with a 5 cm layer of small gravel and a local air-dried loamy sand that was passed through a 2 mm sieve. Containers were packed to equal weights. Irrigation was applied to each column three times per week to maintain field capacity with one of three different water qualities:

1. tap water (control), pH = 7.8; HCO_3 =164-170 ppm
2. water with high level of bicarbonates (HCO_3 = 400 ppm)
3. water with high level of bicarbonates (#2) plus sulfuric acid. The irrigation water had a pH of 6.5 and HCO_3 of 76-78ppm after sulfuric acid was added.

To measure soil infiltration rates, two double ring infiltrometers (Turf-Tec International, Tallahassee, FL) were used: a smaller with an inner ring of 6 cm and an outer ring of 12 cm and a larger with an inner ring of 15 cm and an outer ring of 30 cm. Soil hydraulic conductivity was measured by a Mini Disk Infiltrometer (Decagon Devices, Inc., USA) and a KSAT® device (Meter, Germany). The Mini Disk Infiltrometer is a tension infiltrometer with adjustable suction and measures the unsaturated hydraulic conductivity of the medium it is placed on at different applied tensions. The KSAT® measures the hydraulic conductivity, Ks, of saturated soil cores using the Darcy equation.

Measurements using the double-ring infiltrometers and the Mini Disk Infiltrometer were made in the beginning (June), and at the end of the study (November). Measurements with the KSAT were conducted on soil cores that were removed from the container. Therefore, data could only be collected at the end of the study (November). The experimental design of the study was completely randomized with four replications per treatment.

The ANOVA revealed that the two-way interactions between water treatments and sampling months did not affect the infiltration rate or hydraulic conductivity readings (Table 1). Water treatments did not affect infiltration rates and hydraulic conductivities of smaller infiltrometers and the Mini Disk Infiltrometer (Table 1). However, water treatments affected readings of the larger infiltrometer and KSAT. Both infiltration rates and hydraulic conductivities were higher in the beginning and at the end of the study for the smaller infiltrometer and the Mini Disk Infiltrometer.

The lower infiltration rates at the end of the study indicate that either all micropores were filled with water which slowed down water movement or that the finer soil particles, such as silt and clay migrated to the lower part of the columns. However, a difference in soil texture between different depths of the columns (determined by the feel method) could not be established. Therefore, particle migration as a reason for the lower infiltration rate may be ruled out, especially given the short duration of the experiment. We hypothesize that the lower infiltration rates at the end of the study were due to water movement in a completely saturated column which did not occur at the beginning of the study.

When the larger diameter infiltrometer was used, the comparatively small diameter of the soil columns may have caused possible inaccurate measurements due to preferential flow. Regardless, at the end of the study we did not observe any depositions of carbonate in the soil at any depth of the column. Our findings confirm those of Obear et al (2016), who also did not observe a reduction of infiltration rates and calcium carbonate depositions after irrigation with water high in bicarbonate.

Reference:

Obear, GR., P Barak, and D.J Soldat. 2016. Soil inorganic carbon accumulation in sand putting green soils II: acid-base relationships as affected by water chemistry and nitrogen source. Crop Sci. 56:1-11. doi: 10.2135/cropsci2015.06.0342

Table 1. Results of ANOVA testing the effect of three water treatments (Trt) and sampling dates (June and November) on soil infiltration and soil hydraulic conductivity rates using larger- and smaller diameter infiltrometers, Mini Disk infiltrometer, and KSAT. Measurements for KSAT were taken on soil cores only in November.

	Larger infiltrometer	Smaller infiltrometer	Mini Disk infiltrometer	KSAT
Month	ns†	***	***	
Trt	*	ns	ns	*
Month*Trt	ns	ns	ns	

*Significant F test at the 0.05 level of probability.

***Significant F test at the 0.001 level of probability.

† ns, not significant at the 0.05 probability level.

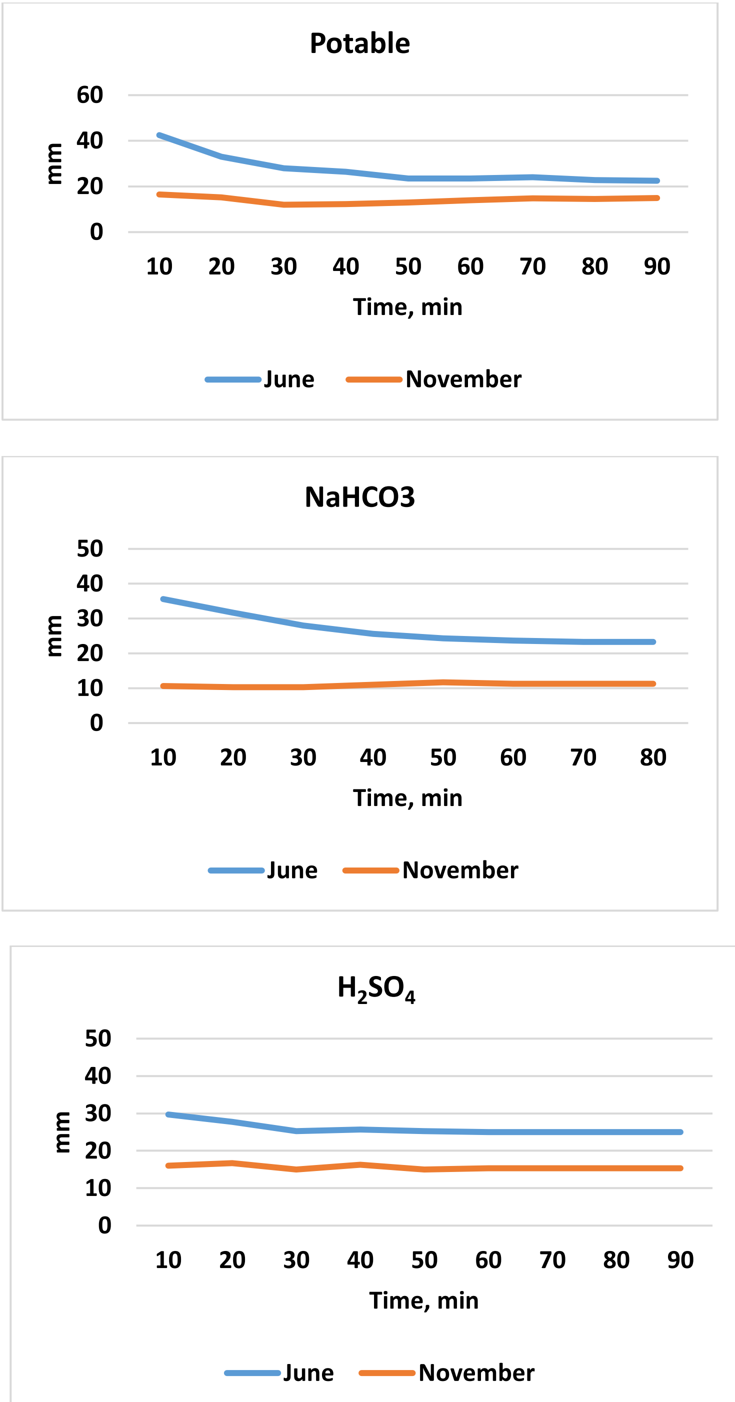

Figure 1. Infiltration rates measured by smaller diameter infiltrometers in the beginning (June) and at the end (November) of the study for containers irrigated with potable water (top), water high in bicarbonates (middle), and water treated with sulfuric acid (bottom). Values represent an average of four readings.

Figure 2. Small (left) and large (right) infiltrometer used in the study.

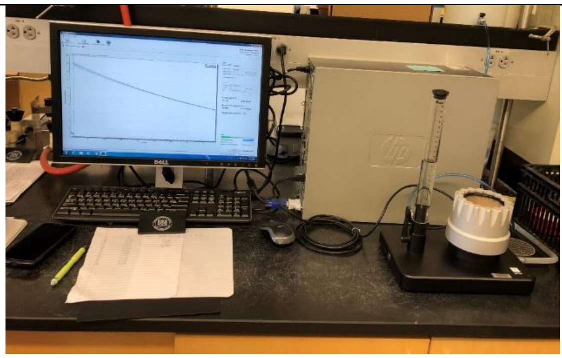

Figure 3. Mini Disk Infiltrometer (left) and KSAT (right)

USGA ID#: 2019-27-697

Title: Detection of Blue-Green Algae and Cyanotoxins in Irrigation Water and Potential Impact on Putting Green Health

Project Leader: Gerald L. Miller, Clayton A. Rushford, and Rebecca L. North
Affiliation: University of Missouri – Columbia

Objective:

- Determine the concentration of microcystin in irrigation systems supplied by a variety of water sources

Start Date: 2019
Project Duration: 1 year
Total Funding: $3,000

Summary Points:

- In most samples, microcystin concentrations are well below the low health risk range (8 µg/L) for recreational ambient water quality according to the Environmental Protection Agency.
- Microcystin was present in 2/3 of irrigation samples, but perhaps not at concentrations that would be inhibitory to creeping bentgrass growth.
- Concentrations of additional cyanotoxins are being evaluated

Summary Text:

Creeping bentgrass (*Agrostis stolonifera*) is a cool season turfgrass used on putting greens throughout the transition zone. During hot summer months frequent irrigation is required to meet evapotranspiration requirements and maintain turfgrass quality. Many golf courses utilize natural water bodies for irrigation sources, potentially creating a dissemination pathway for waterborne organisms including pathogens to invade putting greens. In Missouri, cyanobacteria, also known as blue-green algae (e.g., *Oscillatoria* and *Nostoc* spp.), have been detected in natural water bodies and are known invaders of bentgrass putting green surfaces. Approximately 120 different cyanobacteria species produce potentially toxigenic chemicals in water bodies (Chapman and Foss, 2019). Nearly half of them, including several *Phormidium*, *Oscillatoria* and *Nostoc* spp. produce microcystins, a cyanotoxin that is hazardous to humans and can inhibit plant development. Other potentially harmful cyanotoxins produced by cyanobacteria include cylindrospermopsins, anatoxins, and saxitoxins.

In addition, surface cyanobacteria occurrence and associated toxin development can cause turf thinning on bentgrass greens, and have been associated with both yellow spot and black layer formation. Irrigation water has not been investigated as a potential source of infestation, nor have cyanotoxins in golf irrigation sources been assessed for potential limitation of bentgrass growth. The objective of this study is to obtain preliminary data on the levels of cyanotoxins in irrigation water, laying the groundwork for future investigation into assessment of cyanobacteria and cyanotoxins in irrigation sources and their impact on putting green health.

Water samples were collected in July of 2019 from ten golf courses in Missouri and Kansas. These locations utilize various water sources for irrigation, including retention ponds, wells, a lake, and municipal water. Samples (5 ml) were collected directly from irrigation heads, near irrigation intakes, and from the epilimnion of exposed water sources (N=27). Water was collected and consolidated from multiple irrigation heads using Pyrex baking dishes. A Van Dorn sampler was used to collect samples near the irrigation intake. The epilimnion was sampled with a 0.5 m deep sampling tube. Water samples were stored in Qorpak™ amber borosilicate sample vials and sealed with polytetrafluoroethylene-lined caps. Vials underwent three freeze/thaw cycles to lyse cells and release stored cyanotoxins. Microcystin concentrations were quantified using Abraxis® ELISA kits. Cylindrospermopsin, anatoxin and saxitoxin concentrations will be quantified in the future using similar Abraxis® ELISA kits designed for each toxin.

July samples were analyzed first due to the assumption that warmer temperatures may cause cyanobacteria blooms and resultant higher cyanotoxin levels. April and October samples have also been collected and stored for future investigation. Microcystin concentrations from July samples are reported in Table 1. Locations with unexposed water sources, such as wells and municipal water, did not have detectable levels of cyanotoxins.

The EPA has recommended an 8 µg/L microcystin concentration as a threshold for recreational water use. Except for one water source that is also used as an agriculture retention pond, all quantified microcystin concentrations in this study were considered a low health risk to humans by this standard. Interestingly, the water sample taken from the irrigation heads at the golf course (WC – listed in Table 1 below) fed from the high microcystin containing source did not show the same elevated microcystin concentration.

The impact of microcystin on plant growth has been documented on a wide variety of crops (Machado et al. 2017), but not on turfgrasses. In most cases, exposure to high microcystin levels (>100 µg/L) in plant experiments decreased seed germination, growth and photosynthesis, and promoted oxidative stress. Microcystin levels >100 µg/L are very high and extremely rare in natural water bodies.

In some agriculture irrigation systems heavily contaminated with microcystin in countries such as Tunisia, Spain, China, and Morocco, microcystin concentration values range from 4 – 50 µg/L up to 760 µg/L. In this study, the highest observed microcystin concentration was 8.53-8.65 µg/L, found in the epilimnion and irrigation intake from a large agricultural pond (9.6 ha) that also serves as a golf course irrigation source. Microcystin levels from all other samples averaged 0.38 µg/L, with nine samples falling below the detection limit.

At the low, and perhaps ecologically relevant, microcystin concentrations found in our samples, most studies in plant-soil systems indicate no deleterious effects on plant health, and in some cases the low concentrations may even accelerate plant development (Machado et al. 2017). While this is encouraging, the effect of multiple cyanotoxins and a potential additive deleterious effect on plant growth has not been well studied. In addition, an intensive putting green system is not managed like a farming system, and the constant mowing and limited fertility utilized to promote a playable surface do not encourage the accumulation of plant biomass and yield. Future research assessing even low cyanotoxin levels on bentgrass health in a putting green system is warranted. This study provides evidence that the ecologically relevant level is most likely below 1 μg/L.

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Table 1. Microcystin concentrations

Site code (Water source)	Location on course	Microcystin concentration (µg/L)
SF (Pond)	Irrigation head	0.38
	Irrigation intake	0.54
	Epilimnion	0.44
CCMO (Pond)	Irrigation head	Below detection limit
	Irrigation intake	0.2
	Epilimnion	Below detection limit
WC (Pond)	Irrigation head	0.34
	Irrigation intake	8.65
	Epilimnion	8.53
SA (Pond)	Irrigation head	0.16
	Irrigation intake	0.26
	Epilimnion	0.28
DAL (Pond)	Irrigation head	0.23
	Irrigation intake	0.84
	Epilimnion	0.92
LED (Pond)	Irrigation head	Below detection limit
	Irrigation intake	0.42
	Epilimnion	0.32
HS (½ pond & ½ well)	Irrigation head	Below detection limit
	Irrigation intake	Below detection limit
	Epilimnion	0.22
AG (well)	Irrigation head	Below detection limit
	Irrigation intake	Below detection limit
LQ (lake)	Irrigation head	0.19
	Irrigation intake	0.28
	Epilimnion	Below detection limit
STLCC (municipal water)	Irrigation head	Below detection limit

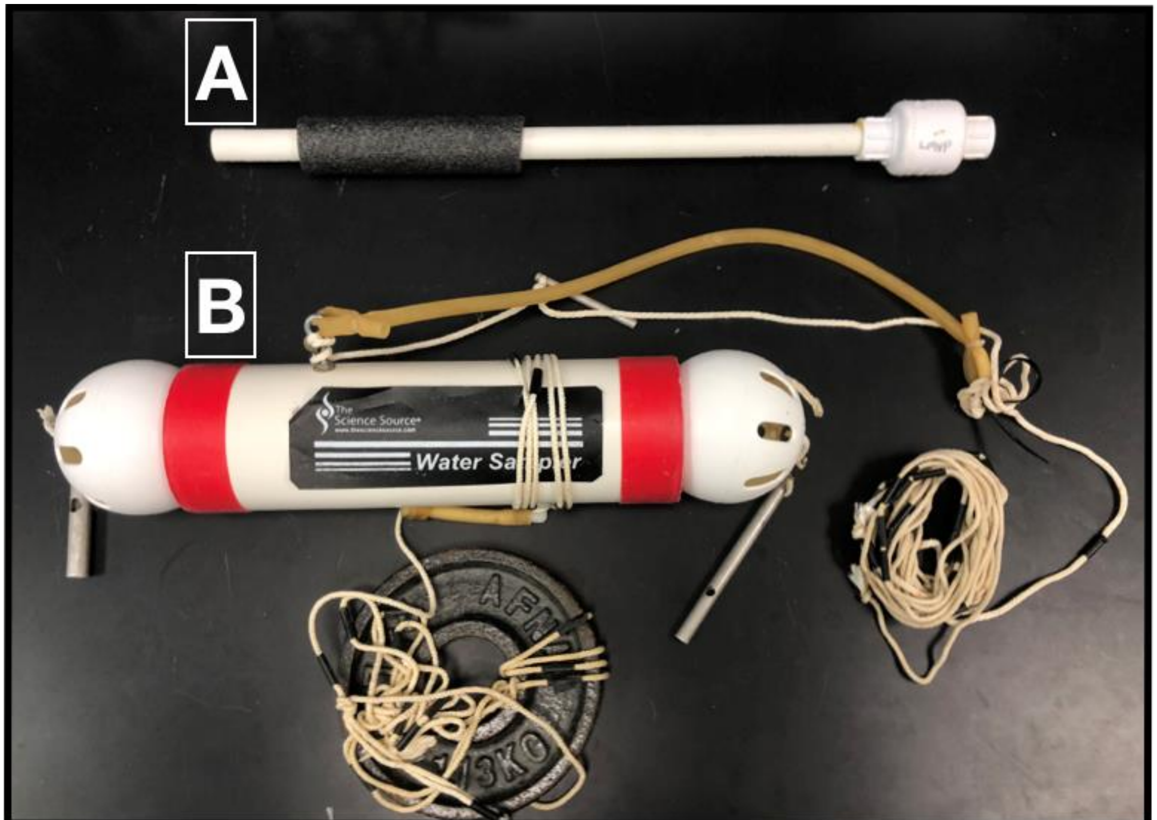

Figure 1: Sampling Instruments

A. Simple valve sampler for sampling the top 0.5 m (epilimnion) of retention ponds used for irrigation sources.
B. Van Dorn sampler used to sample water close to irrigation intake of retention ponds.

Figure 2. Sampling golf irrigation water from near the intake of a retention pond.

USGA ID#: 2019-26-696

Title: Evaluating the Competitiveness of Various Bentgrass Cultivars Against Annual Bluegrass

Project Leader: Matthew Elmore, Ph.D.
Affiliation: Rutgers University

Objectives:

1. Determine if certain cultivars of bentgrass (*Agrostis* spp.) are more competitive than others against annual bluegrass (*Poa annua* L.) in a simulated golf course fairway.
2. Determine the relative vegetative spread of various bentgrass cultivars into annual bluegrass.

Start Date: 2019
Project Duration: 1 year
Total Funding: $5,000

Summary Points:

- Eight bentgrass cultivars were vegetatively established into an annual bluegrass fairway by installing 8 by 90 cm strips in October 2018.
- By August 2019, no bentgrass cultivar provided more than 15% bentgrass cover at a 1” distance from the original edge of establishment.
- Certain bentgrass cultivars will likely demonstrate better encroachment into annual bluegrass than others over time, but it is too early for these differences to be apparent.

Summary Text:

The objective of this research was to evaluate the vegetative spread of various creeping bentgrass cultivars in simulated fairway of annual bluegrass. This research was conducted at the Rutgers Horticulture Farm No. 2 in North Brunswick, NJ. Seven creeping bentgrass (*Agrostis stolonifera* L.) cultivars and one colonial bentgrass (*Agrostis capillaris* L.) cultivar were seeded at 100 kg PLS ha^{-1} on 23 October 2017 to 1.2 by 1.2 m plots in a nursery treated with dazomet on 13 October 2017. During creeping bentgrass establishment from seed, an adjacent creeping bentgrass/annual bluegrass fairway was prepared by selectively controlling creeping bentgrass with sequential applications of fluazifop at 0.18 kg ha^{-1} in September and October 2017 and May and August 2018. One year after seeded establishment in the nursery, each cultivar was vegetatively established to 1.0 by 1.0 m plots into the aforementioned annual bluegrass fairway on 20 October 2018 (Figure 1). Each plot was established by cutting four 8 by 90 cm strips (2.5 cm depth) of a particular cultivar from the bentgrass nursery and installing them in the annual bluegrass fairway in parallel, spaced 15 cm apart.

Creeping bentgrass cultivars were selected to represent a gradient from of low density, older cultivars to higher density, modern cultivars and consisted of Penncross, L93, 007, Shark, Luminary, Pirhana, and Flagstick. Puritan colonial bentgrass was also included. The eight bentgrass cultivars were arranged in an RCBD design with four replications. An annual bluegrass only plot was included for comparison. We characterize the annual bluegrass on this site as a weak perennial (*Poa annua* var. *annua*). It survives the summer under careful management but does not exhibit characteristics of the reptans variety [*Poa annua* var. *reptans* (Hauskins) Timm.].

To maintain annual bluegrass quality, this fairway was irrigated to prevent wilt, treated with insecticides, fungicides, and wetting agents preventatively, and was mowed thrice weekly at 1.0 cm using a triplex reel mower. Granular nitrogen fertilizer was applied at 38 kg ha^{-1} on 8 May and 14 October 2019 to promote annual bluegrass growth. Nitrogen was applied foliarly at 5 to 10 kg ha^{-1} from June through September as needed to maintain fullness of turf cover. Trinexapac-ethyl was applied at 100 g ha^{-1} at 380 GDD intervals (Base temperature 0 °C; W. Krueser unpublished data) from 6 June to 15 October.

In August 2019 the decision was made to impose simulated traffic across the entire experiment to simulate high traffic areas on a golf course fairway where annual bluegrass is often most problematic. Traffic events consisted of 6 passes of a smooth pavement roller (1.7 metric ton tandem roller, Model RD11A, Wacker Neuson, Germany) followed by 6 passes of the Rutgers wear simulator (Bonos et al. 2001) applied weekly unless soil conditions were too saturated. There were 7 traffic events imposed from 30 August to 6 November 2019.

Annual bluegrass and creeping bentgrass cover was evaluated in July, August, October and December using a grid count. The grid contains 40 intersects each directly over the centerline of the originally installed bentgrass strip and at 1, 2, and 3” from the edge of the strips to evaluate percent bentgrass cover at various distances from the original vegetative planting (Figure 2). Data were subjected to ANOVA and Fisher’s Protected LSD in ARM (α=0.05).

L93 had less bentgrass cover at the centerline of each strip than other cultivars in both July and October (Table 1). In August, Penncross, Shark, and Pirhana had more bentgrass cover at both 1” and 2” from the edge of the original bentgrass strip than most other cultivars. Anecdotally, we observed that Penncross and L93 tend to produce a thinner canopy than other cultivars and we suspect that annual bluegrass may colonize these cultivars more than others during autumn 2019 and spring 2020 from ‘seed rain’ from spring 2019.

Future Research Expectations: We expect differences in creeping bentgrass cultivar encroachment into annual bluegrass will become more apparent during the 2020 growing season. We also expect to observe annual bluegrass encroachment into the bentgrass strips from spring 2019 ‘seed rain’ and hypothesize that cultivars with a thinner canopy may be more susceptible to this encroachment.

References:

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

Table 1. Percent bentgrass cover as determined by a grid intersect count at 0, 1, and 2 inches from the centerline of bentgrass strips vegetatively established into a simulated annual bluegrass fairway in North Brunswick, NJ. Grid counts were conducted in July, August, and October 2019.

	July			August			October		
	--------------inches from bentgrass strip ------------								
Variety	0	1	2	0	1	2	0	1	2
	-------------------bentgrass cover %--------------------								
Penncross	95	4	0	100	11	2	94	9	3
L93	96	2	0	100	8	0	88	10	2
007	100	0	0	100	4	0	94	7	1
Shark	100	4	0	100	10	2	94	11	3
Luminary	100	5	0	100	8	1	96	11	1
Pirhana	100	1	0	100	13	3	97	11	2
Puritan	100	1	0	100	4	0	87	11	3
Flagstick	100	1	0	100	7	0	95	9	1
$LSD_{0.05}$	*3*	*NS*	*NS*	*NS*	*6*	*2*	*6*	*NS*	*NS*

Figure 1. Bentgrass (*Agrostis* spp.) strips being established in a simulated annual bluegrass (*Poa annua*) fairway at Rutgers Horticulture Research Farm No. 2.

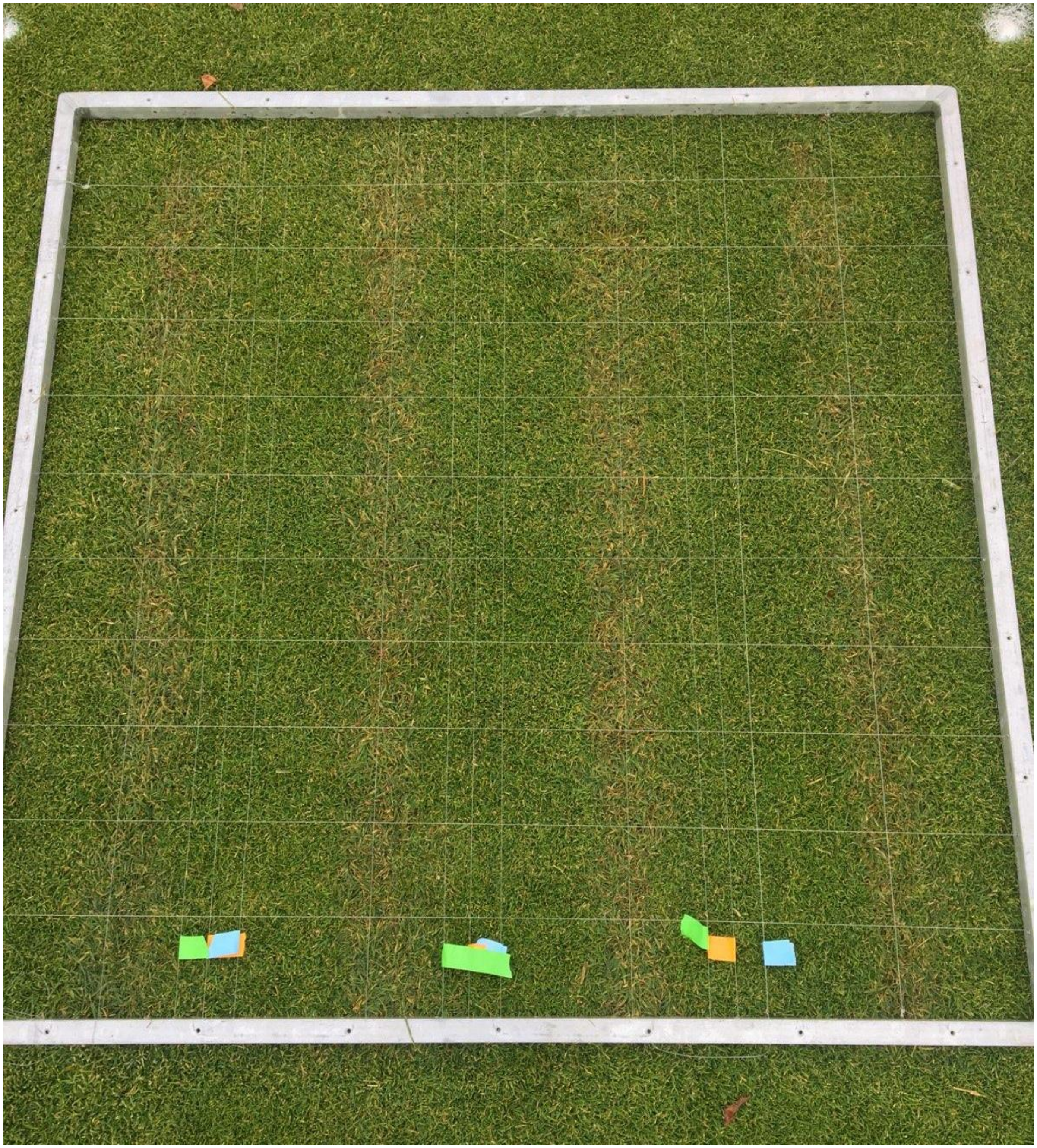

Figure 2. Grid used for intersect counts with 40 intersects each placed directly over the centerline of the originally installed bentgrass strip and at 1, 2, and 3” from the edge of the strips. Photo from November 2019.\

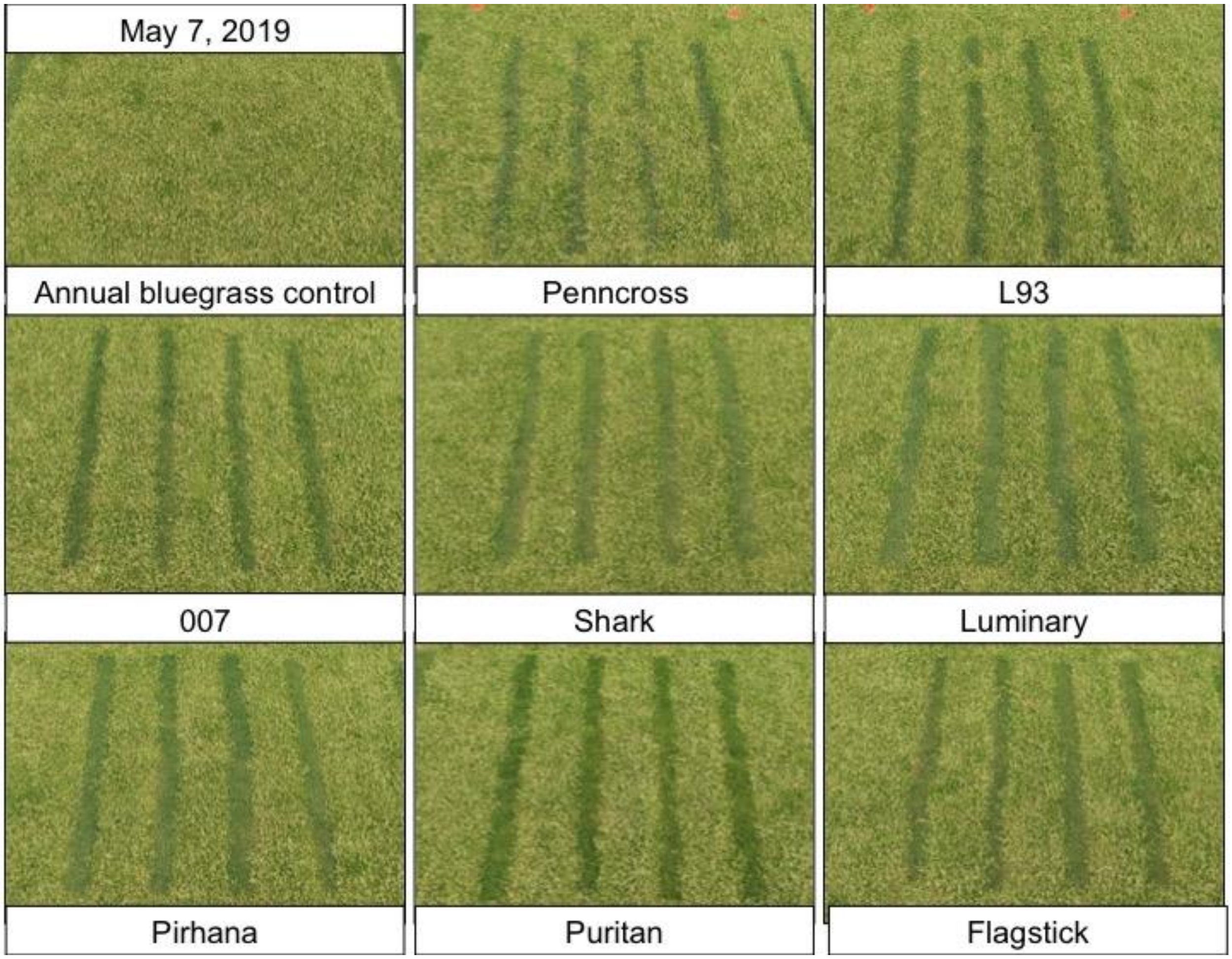

Figure 3. Various bentgrass (Agrostis spp.) cultivars seven months after establishment from vegetative strips (8 by 90 cm) in a simulated annual bluegrass (*Poa annua*) fairway at Rutgers Horticulture Research Farm No. 2. These photos were taken during annual bluegrass seedhead shatter on 7 May 2019.

Figure 4. Various bentgrass (*Agrostis* spp.) cultivars nine months after establishment from vegetative strips (8 by 90 cm) in a simulated annual bluegrass (*Poa annua*) fairway at Rutgers Horticulture Research Farm No. 2. These photos were taken on 24 July 2019.

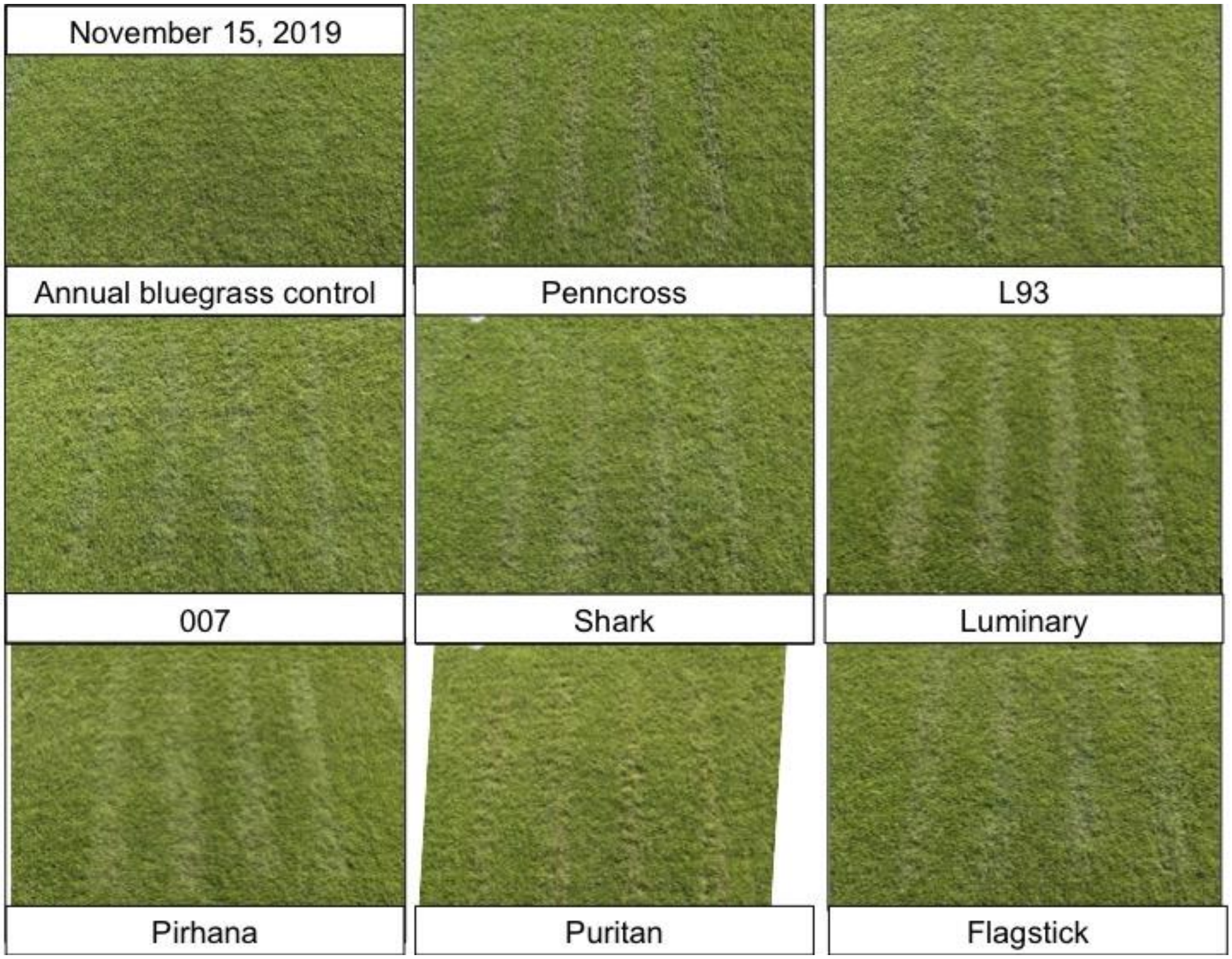

Figure 5. Various bentgrass (*Agrostis* spp.) cultivars on year after establishment from vegetative strips (8 by 90 cm) in a simulated annual bluegrass (*Poa annua*) fairway at Rutgers Horticulture Research Farm No. 2. These photos were taken on 15 November 2019 after most of the simulated traffic events occurred.