2022 Progress Reports

Mike Davis Program for Advancing Golf Course Management

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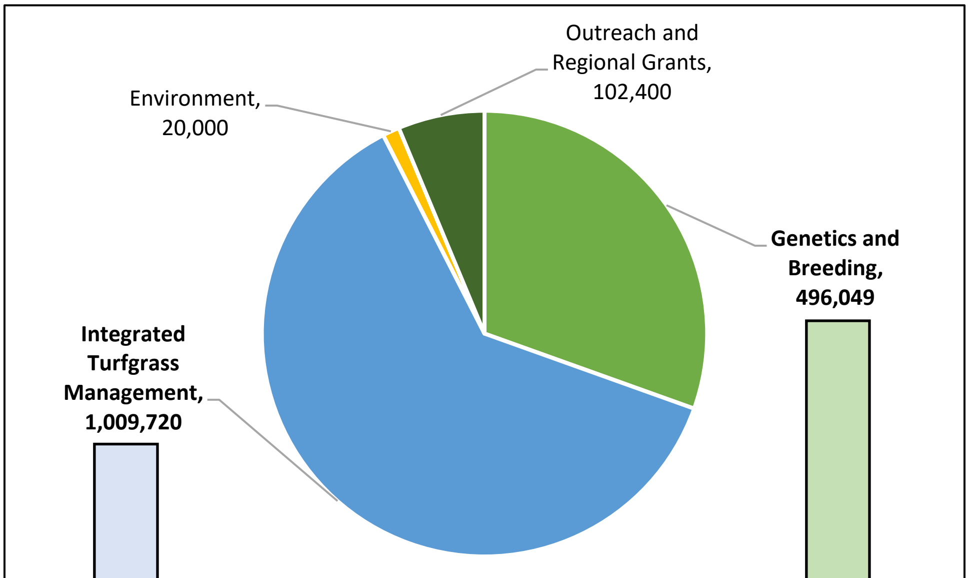
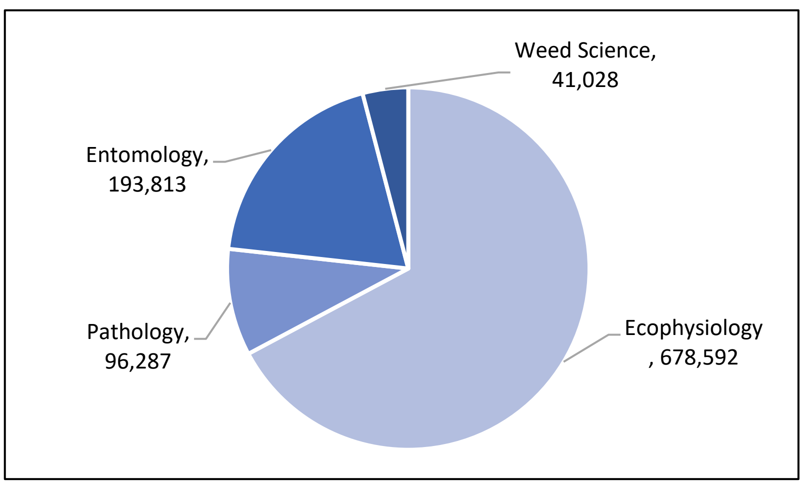


$1,628,169

Integrated Turfgrass Management



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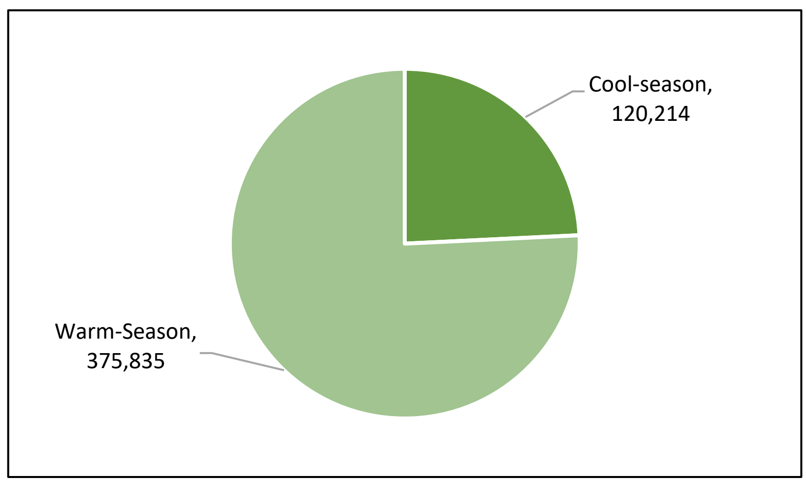
Genetics and Breeding



$496,049

USGA ID#: 2019-15-685

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

Project Leaders: David R. Huff and Christopher 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). COMPLETED.
Objective 2 (Month 1-18): Evaluate transgenerational retention of morphological characters and epigenetic signatures in subsequent generations of *Poa annua* mowed and unmowed. COMPLETED.
Objective 3 (Month 12-24): Use methods in genomic sequencing and bioinformatics to assemble genomes of *Poa annua* and its two diploid parental species Poa infirma and Poa supina. COMPLETED.
Objective 4 (Month 24-30): Align and map parental DNA sequences to the genome of allotetraploid *Poa annua*. COMPLETED.
Objective 5 (Month 18-36): Elucidate downstream transcriptional changes via RNA-seq analysis as a response to differential subgenome expression analyses during imposed mowing stress on clonal *Poa annua*. COMPLETED.
Objective 6 (Month 36 and beyond): Utilize the new genomic information to help guide the breeding of elite and stable cultivars of *Poa annua* for commercial release and use golf-course putting greens. COMPLETED.

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

Summary Points:

- Whole-genome resequencing of fifteen geographically distinct *Poa annua* ecotypes reveals large-scale chromosomal rearrangements resulting in extensive downsizing of repetitive DNA sequence.
- The genomic and transcriptomic resources generated for this work have helped guide the *Poa annua* breeding program at Penn State.
- Elite and stable cultivars of *Poa annua* are currently being evaluated on putting greens and fairways at 5 golf courses across the USA (PA, NY, NJ, NC, and CA).
- Additional funding is needed to elucidate the extent to which the observed chromosomal variation impacts turf quality in *Poa annua*.

Summary Text:

Rational and Methodology

Polyploidy, or whole genome duplication, is a re-occurring phenomenon in angiosperm evolution and plays an important role in shaping phylogenetic lineages with compounding ecological and molecular effects at each subsequent doubling. Upon polyploidization, duplicate chromosomes and genes are under immense pressure to purge excess copies and return to a more stable diploid-like state. The process of re-diploidizing occurs within the first few generations after polyploidy and is characterized by many changes to the genetic and genomic landscape including neofunctionalization and subfunctionalization of duplicate genes, genome fractionation, gene conversion, exchanges between homoeologous chromosomes, mobilization of repeat elements, and epigenetic reprogramming to name a few. The selective and passive forces that influence diploidization often lead to niche expansion and transgressive phenotypes.

We suspect that the recent polyploidization between diploids *Poa infirma* and *Poa supina* to form tetraploid *Poa annua* likely plays a role in *P. annua's* transgressive dwarfism and allows it to thrive under putting green heights (Huff, 2021). In collaboration with researchers at the USDA, we sequenced, assembled, and annotated chromosome-level genomes of *Poa infirma*, *Poa supina*, and *Poa annua* to shed light on the uncharacterized genetic and genomic components that might contribute to *Poa annua's* phenotypic novelty (Benson et al., 2021). We also conducted a subgenome-specific transcriptional analysis under putting green-style mowing to further narrow in on the metabolic pathways involved with regulating dwarfism on golf course putting greens (Benson & Huff, 2021). In 2022, we re-sequenced 13 geographically distinct genotypes and two additional elite breeding genotypes to explore intraspecific variation in *P. annua* at the whole-chromosome and DNA sequence level. Together, the 15 samples represent nine countries and four continents (**Fig. 1)**.

Results

Homoeologous exchanges and bursts of activity in transposable element contribute to genomic instability in polyploids, but do not provide a satisfying explanation for the observed 80% variation in DNA content between *Poa annua* ecotypes (Mowforth and Grime, 1989). In addition, cytological evaluation has demonstrated that some *P. annua* individuals have a karyotype that does not match that of it's diploid parents, leading some authors to hypothesize that major chromosomal rearrangements post-polyploidization could have led to novel karyotypes in the tetraploid (Koshy, 1968; Mao and Huff 2012; Benson *et al.,* 2021).

We identify remarkable variation in chromosome structure. The largest is a 224 Mbp deletion in the centromeric and pericentromeric of chromosome 1A of some samples (**Fig. 2**). The deletion at chromosome 1A amounts to 70% of the length of the reference chromosome. Coinciding with the deletion at 1A, is a duplication in the centromeric region of 1B. The duplication is up to 32 Mbp in length and contains the highest density of LTRs across the chromosome, suggesting that the duplicated region likely contains a centromere. The breakpoints at the 1A deletion and 1B duplication contain a handful of split and improperly paired reads. Reads at the breakpoints of the 1A deletion have pairing partners at the breakpoints of the 1B duplication. This suggests that the duplicated region at 1B most likely resides within the deleted region of 1A.

There are several possible modes of origin for the observed variation in karyotypes, and future cytological studies might help to resolve them. Perhaps the most parsimonious path to this karyotype is a two-step path, where (1) meiotic non-disjunction in polyploid *Poa annua* causes 1B to displace 1A (yielding an ABBB set of chromosome 1's), and subsequently (2) fertilization between a normal plant (AABB) and an aberrant plant (ABBB) ultimately leads to introgression of 1A whenever recombination occurs. This would cause most of the genic regions of the displacing 1B chromosome to return to a 1A-like state. That said, it is also possible that multivalents and "subgenome aneuploidy" may have contributed. Indeed, synaptic multivalents appear to occur more frequently in neo-allopolyploids (Xiong Z *et al.*, 2011). Multivalents and aneuploidy are associated with incorrect segregation and reduced fertility, so selection pressure would likely purge large-scale structural modifications if they did not lead to stable bivalents.

Large-scale chromosomal variation in *Poa annua,* a species that dominates the globe, is surprising given the implication on meiotic stability and potential loss of fertility. The extent to which large-scale chromosomal variation impacts breeders and weed scientists is an important question that needs to be addressed.

Future Expectations

This writeup represents the last report of this funded research project. We appreciate the level of support we've received and have hopefully been able to generate useful information for turfgrass geneticists, scientists, and end-users like golf course superintendents. The work funded by the USGA and outlined in our annual reports and enabled us to publish a paper that was recently selected as the "2022 Paper of the Year" in Crop Science by the Turfgrass Science (C05) Division (Benson, C., Q. Mao, and D.R. Huff. 2021). "Global DNA Methylation Predicts Epigenetic Reprograming and Transgenerational Plasticity in *Poa annua* L." https://doi.org/10.1002/csc2.20337

Our ultimate goal is to fully understand each of this project's genetic and genomic discoveries in order to better manipulate and enhance our breeding program to generate elite and stable *Poa annua's* for commercial use on golf course putting greens and fairways.

References:

Benson, C.W., Q. Mao, and D.R. Huff. 2020. Global DNA Methylation Predicts Epigenetic Reprogramming and Transgenerational Plasticity in *Poa annua* L. *Crop Science*, doi.org/10.1002/csc2.20337.

Benson, C. W. & Huff, D. R. 2021. Homoeolog gene expression and global DNA methylation in the allotetraploid subgenomes of golf course *Poa annua*. [Abstract]. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/134352

Benson, C. W., Huff, D. R., Bushman, B. S., Maughan, P. J., Jellen, E., & Robbins, M. D. 2021. Sequenced genomes of *Poa annua* and its diploid progenitors, *Poa infirma* and *Poa supina*, provide insight into the evolution of a versatile nascent allopolyploid [Abstract].

ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/134543

Huff, D. R. (2021) An ecological and evolutionary genomics perspective on the perenniality of polyploid annual bluegrass, *Poa annua* L. [Abstract]. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/135056

Mowforth, M. A., and J. P. Grime. 1989. Intra-Population Variation in Nuclear DNA Amount, Cell Size and Growth Rate in Poa Annua L. *Functional Ecology* 3, no. 3: 289–95. https://doi.org/10.2307/2389368.

Xiong, Z., Gaeta, R. T. & Pires, J. C. 2011. Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. *Proc. Natl Acad. Sci.* doi.org/10.1073/pnas.1014138108.

Huff, D. R. (2021) An ecological and evolutionary genomics perspective on the perenniality of polyploid annual bluegrass, *Poa annua* L. [Abstract]. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/135056

Koshy, T. K. Evolutionary Origin of Poa Annua L. in the Light of Karyotypic Studies. 1968. *Canadian Journal of Genetics and Cytology* 10, no. 1: 112–18. https://doi.org/10.1139/g68-015.

Mao, Qing, and David R. Huff. 2012. Evolutionary Origin of Poa Annua L. *Crop Science*. http://agris.fao.org/agris-search/search.do?recordID=US201500054965.

Figures:

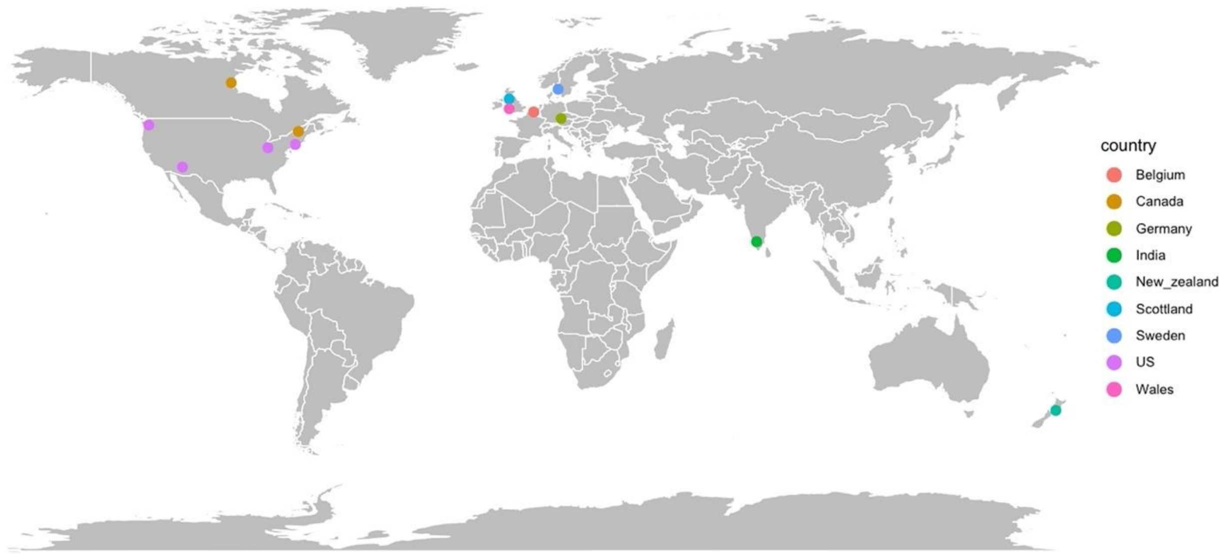
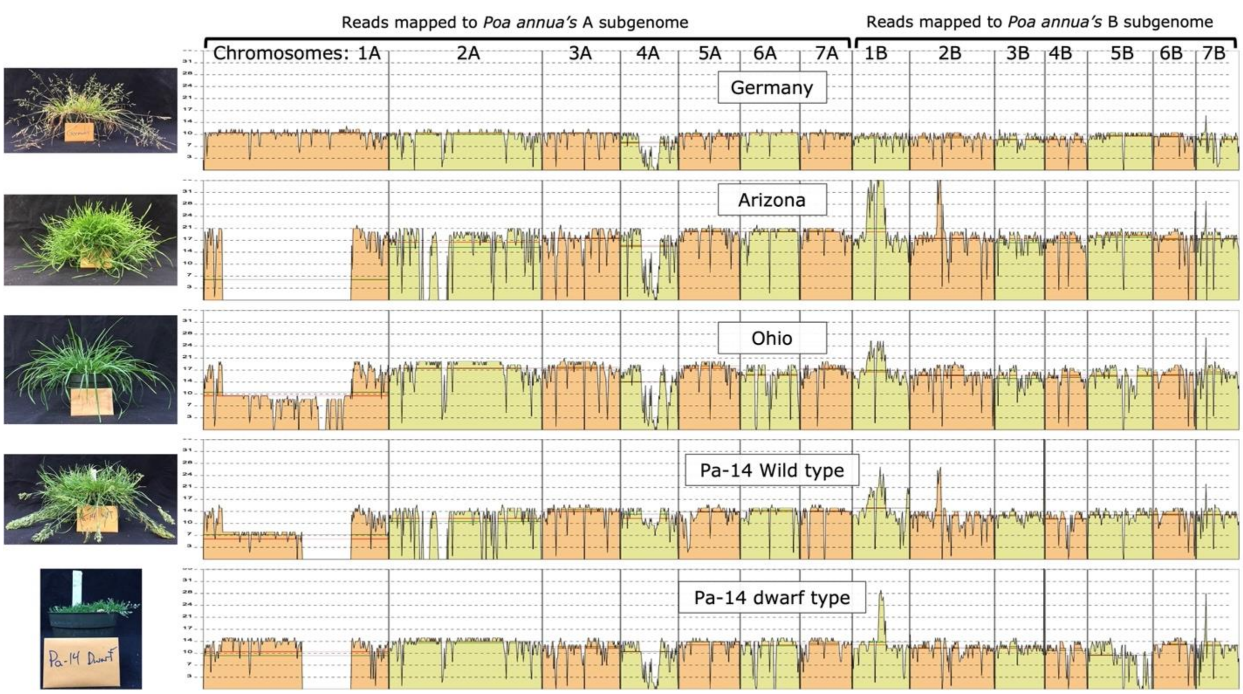


Fig. 1. Geographic distribution of 13 sequenced *Poa annua* genotypes. Two additional breeding line plants (PA-14_dwarf and Pa-14_WT) were also sequenced but not depicted on the map.



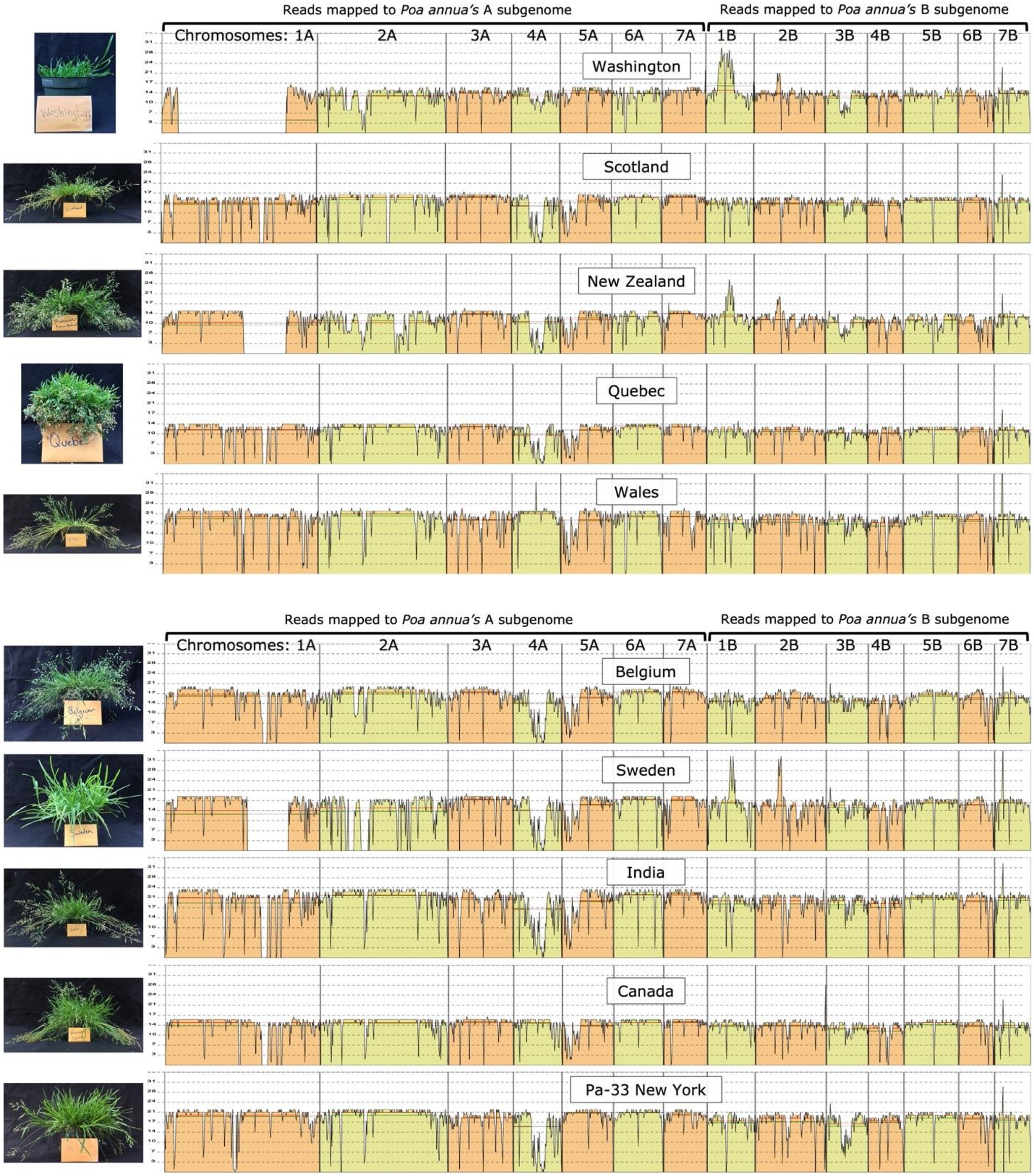


Fig. 2. Sample reads aligned to the *Poa annua* reference genome suggests large scale structural modification in *Poa annua*'s chromosomes. Chromosomes are ordered 1-7 for both subgenomes. 1/2X coverage, as observed in chromosome 1A of sample "Ohio", indicates a structural modification that occurs on a single chromosomal paring partner. All plants were germinated from seed except for breeding line plants (indicated by "Pa-"). Plants grown from seed were imaged 96 days after germination, except for "Ohio", which was imaged 27 days after germinating.

USGA ID#: 2022-01-744

Title: Transcriptome analysis of bentgrass germplasm using RNA-seq to identify novel targets for enhancement of dollar spot resistance in the field

Project Leaders: Dr. Brandon Horvath, Dr. Scott Warnke, Dr. Keenan Amundsen
Affiliation: The University of Tennessee, USDA, The University of Nebraska

Objectives:
The objectives of the proposed research are as follows:

- Evaluate differential response by bentgrasses with a range of known resistance to dollar spot via challenge inoculations.
- Evaluate the transcriptome of inoculated bentgrasses using RNA-seq to identify transcripts that are up and down regulated in response to infection.
- Compare the identified transcripts to the creeping bentgrass reference genome, and identify potential targets involved in resistance or susceptibility.
- Develop DNA markers to determine potential susceptibility of field-based plant tissue from a particular cultivar.

Start Date: 2021
Project Duration: 3 years
Total Funding: $99,180

Summary Points:

- Inoculation protocol optimized to yield reliable dollar spot infection. This was done by adjusting many different variables.
- Individual seedlings within cultivars did not differ wildly in dollar spot susceptibility. However, there were some perceived differences between cultivars emphasizing the merit of our RNAseq experiments
- Time points will be selected in the coming months after running several test inoculations and observing visual symptom appearance. We expect these time points to fall between 24 hpi and 72 hpi.

Summary Text:

Rationale

Dollar spot (*Clarireedia spp.*) is one of the most economically and environmentally devastating diseases in turfgrass systems. Roughly 43% of the projected total acres on golf courses in the United States are composed of C3 grasses (creeping bentgrass, annual bluegrass, and Kentucky bluegrass are most common), all of which are highly susceptible to dollar spot.[1] This pathogen is a stressor for superintendents because it aggressively kills turfgrasses, impacts the uniform roll of the green or field, and is expensive to treat. Due to the heavy reliance on fungicide applications, many dollar spot populations have developed resistance, limiting the ability to control large-scale disease outbreaks. Unfortunately, fungicide resistance is not easily reversible and will only become more widespread with time. Thus, there is a need for research that finds alternatives to fungicide control of dollar spot.

Our project is focused on one of the alternatives, genetic resistance. Genetic resistance involves harnessing the biology of creeping bentgrass (*Agrostis stolonifera*) by breeding new cultivars that are resistant to dollar spot. While creating new cultivars is the end goal, this project will advance our understanding of what creeping bentgrass genes could confer partial dollar spot resistance observed in the field. More specifically, we seek not only to characterize which genes are differentially expressed between creeping bentgrass and colonial bentgrass (*A. capillaris*) but also to examine how gene expression changes over the course of a dollar spot infection. Taken together, the results of this study will guide us in the creation of molecular markers to aid turfgrass breeders in selecting new creeping bentgrass varieties that are resistant to dollar spot.

Methodology

This study will evaluate dollar spot resistance of three creeping bentgrass cultivars ‘Declaration’, ‘Providence’, and ‘Crenshaw’ and one colonial bentgrass cultivar, ‘BCD’. A single seedling of each cultivar will be chosen and then clonally propagated on 80/20 sand/peat potting media using standard greenhouse management. Twelve clones of each cultivar will be evenly divided into control, post-inoculation sample time 1, post-inoculation sample time 2, and post-inoculation sample time 3 treatments (4 genotypes x 3 reps x 4 treatments = 48 samples).

The inoculum will be prepared by autoclaving Kentucky bluegrass (KBG) seed and then adding agar cubes of the *C. jacksonii* isolate ‘LWC10’. After incubating for 2-3 weeks, KBG seeds will be spread over the canopies of each cultivar which will then be bagged to maintain high humidity. Leaf tissue from control and treated plants will be collected and immediately frozen in liquid nitrogen at the three post-inoculation time points and stored at -80°C. Once all samples are collected, they will be sent to OmegaBioservices for RNA extraction and sequencing.

The sequencing data for each sample will be transferred to the Holland Computing Center at the University of Nebraska. Following established and successful protocols, the data will be checked for quality and then analyzed to determine any differentially expressed genes between cultivars. These genes will be mapped back to reference genomes of 'BCD' and 'Declaration' to identify sequence differences (single nucleotide polymorphisms, insertions or deletions, or simple sequence repeats) between 'BCD' and 'Declaration' for the creation of molecular markers.

Results and Future Expectations

Over the past months, our focus was on determining the best inoculation protocol that results in consistent dollar spot infection. Many factors were considered including the type of inoculum media, incubation time, amount of inoculum spread on the plant, relative humidity, etc. As a result, we settled on using sterilized Kentucky bluegrass seed as the inoculum media and letting it incubate for a minimum of 2 weeks. After this period, plants will be inoculated and sealed in individual plastic Ziploc bags with wet paper towels to increase relative humidity and maintain leaf wetness.

Once the inoculation protocol was established, we selected a single seedling of each cultivar to be used in RNAseq experiments. Creeping and colonial bentgrass are polyploid, meaning that individual seedling plants within a single cultivar can have a large range of dollar spot susceptibilities. Thus, our primary concern was selecting a seedling that best represented the average susceptibility of the cultivar to dollar spot. Therefore, we inoculated ten pots grown from individual seeds with dollar spot and used the Horsfall-Barratt scale to observe the disease severity at 24 hours post inoculation (hpi), 48 hpi, 72 hpi, and 96 hpi. We determined that there were only small differences between each seedling within a cultivar but found more notable differences when comparing cultivars. For example, Crenshaw's disease severity sharply increased at 72 hpi when compared to Declaration and Providence, suggesting that there are some genetic differences that lead to Crenshaw being more susceptible to dollar spot. This confirms the merit of our RNAseq experiments to determine what is happening transcriptionally that confers these differences.

The next step is to determine the tissue collection time points of the RNAseq experiments. This will be done by looking at previous literature and observing the time course of the dollar spot infection. After sampling time points are selected, a round of RNAseq will be completed and the data analyzed. The data from the first round will be informative for the proceeding rounds of RNAseq. We believe that there will be differential expression in hundreds to thousands of genes between cultivars and species.

Figure 1. After evaluation of many factors, the inoculation protocol will involve autoclaving Kentucky bluegrass (KBG) seed and then adding agar cubes of the *C. jacksonii* isolate ‘LWC10’. After incubating for 2-3 weeks, KBG seeds will be spread over the canopies of each cultivar.

Figure 2. After inoculation, plants will be sealed in individual plastic Ziploc bags with wet paper towels to increase relative humidity and maintain leaf wetness. Leaf tissue from control and inoculated plants of each cultivar will be collected and immediately frozen in liquid nitrogen for later RNA extraction and sequencing.

USGA ID#: 2022-04-747

Title: Selection and Evaluation of Shade Tolerance in Creeping Bentgrass (*Agrostis stolonifera*)

Stacy A. Bonos and Eric MacPherson

Rutgers University

Objectives:
- Identify traits that correlate with increased shade tolerance
- Identify creeping bentgrass cultivars that perform well under simulated foliar shade
- Select germplasm that has performed well under foliar shade in field studies
- Determine the inheritance of shade tolerance in creeping bentgrass

Start Date: 2022
Project Duration: three years
Total Funding: $90,000

Summary Points:
- Plant height and chlorophyll content are phenotypic traits correlated with increased shade tolerance and should be the primary data collected when looking at shade tolerance in creeping bentgrass
- Biomass and tiller number are not significantly different enough to allow for differentiation between shade tolerant and susceptible plants
- There is a significant amount of variation in shade performance across currently commercially available cultivars
  - Cultivars- L93XD, 007XL, and Oakley performed the best under shade and maintain consistent plant heights in sun and shade.
  - Cultivars- Penncross and Pin Up exhibited significant shade avoidance responses
  - Cultivars – Tyee, AU Victory and Penn A4 showed significant etiolation under shade.
- 40 genotypes were intercrossed for further inheritance studies.

Summary:

Shade stress and shade avoidance syndrome are major problems for grass growing under shade, and how that grass responds is species specific. Many golf courses have creeping bentgrass greens, tees, and fairways that experience shade during the day. In these areas bentgrasses are weaker, suffer from higher disease susceptibility, increased wear stress, and lower turf density (Settle 2008). Bentgrasses grown in shade also exhibit physiological symptoms of reduced tillering, decreased plant color, more vertical growth habit, longer and thinner leaves, and increased succulence leading to increased disease susceptibility (Casal 2012). Shade caused by vegetation greatly alters the spectrum of light reaching the grass below, by decreasing the amount of blue and red light, while allowing a larger proportion of far

red light through (Wherley *et al* 2005). This altering of the spectral composition is what elicits the shade symptoms (Ballare and Pierik 2017).

The most effective way for dealing with shade stress on golf courses is the removal of the trees/vegetation causing the shade (Dernoeden 2002). Removal of trees can greatly alleviate the shade stress, but significantly alters the playability and aesthetics of the course. Other shade management strategies involve high fungicide applications to prevent disease on the weakened grass, modified mowing schedules to reduce the increased vertical growth, and applications of the growth regulators to keep the grass low (Christians 2011). All these management strategies greatly increase the impact of the course through an increase in inputs and man hours.

This study aims to identify which cultivars perform the best under foliar shade, as well as correlated traits, in both controlled greenhouse and growth chamber settings, in addition to practical field settings. These traits can be utilized in future selections for improved shade tolerance in creeping bentgrasses. This study also aims to identify the heritability of shade tolerance in creeping bentgrass so that we can understand how shade tolerance is inherited. This will help to determine how much of the variation observed is due genetics and how much is due to the environment. Understanding this will help to optimize selection methods for shade tolerance.

Objective 1: Methodology for Greenhouse and Growth Chamber Studies

A greenhouse method to screen cultivars for shade tolerance would be useful to the industry because it can identify cultivars that are tolerant to shade and can provide golf course superintendents with options to improve the performance of putting greens in shaded sites. In order to identify cultivars that perform well under foliar shade both a replicated greenhouse and growth chamber study were carried out using identical methods. The entirety of the study utilizing both the greenhouse and growth chamber was repeated twice using the same methodology, including the measurements collected and shade stress simulations.

An initial greenhouse study of 31 commercial cultivars (Table 1) were grown under simulated foliar shade and full sun conditions. Simulated foliar shade was achieved using a photoselective film canopy with a neutral density of 0.3 allowing a ~50% transmittance and a reduction in Red:Far Red by ~33%, to simulate vegetative shading (Rosco E-Colour #209:.3 Neutral Density, PNTA). (Figure 1). Photosynthetically active radiation (PAR) was collected every 10 mins using a quantum light sensor (SQ-520: Quantum Sensor, Apogee Instruments). In addition to PAR, spectral composition measurements were taken periodically throughout using a spectroradiometer (LI-180, Li-Cor Biosciences).

The selected bentgrasses were seeded into soilless media in 4in^2 pots at a rate of 1lb per 1000 ft^2. There were 4 replicates of each cultivar in both light treatments, arranged in a completely randomized design and allowed to germinate for 2 weeks prior to the shade application. Plants were maintained at a height of 0.5in, being cut every 3 days. The plants were grown under their respective light treatment for 4 months to allow for adequate light stress.

The growth chamber experiment was conducted in the same manner as the greenhouse experiment. 41 cultivars were utilized in this experiment. The shade was applied using the same shade canopy as the greenhouse, and the same trait measurements (as described below) were taken in the same manner, for the same duration.

Table 1. Bentgrass cultivars and selections evaluated for shade tolerance under simulated shade in greenhouse and growth chamber experiments.

007	FMM-03	PFL-18
007 XL	GHE-08	PFM-08
777	HSN-08	Pin Up
Alpha	Kingdom	Piper
Armor	L93 XD	Piranha
AU Victory	LPD-15	S1
Barracuda	Luminary	Shark
Chinook	Macdonald	SMG-05
Coho	Match Play	Spectrum
Declaration	Nightlife	Tour Pro
Diplomacy	Oakley	Tyee
DLF AP 3084	PC2.0	V8
FEM-16	Penn A-4	VPN-12
Flagstick	Penncross	

Figure 1. Creeping bentgrass (*Agrostis stolonifera*) grown in the greenhouse under simulated shade. Simulated shade was created using a photoselective film with a neutral density of 0.3 allowing a ~50% transmittance, and a reduction in Red:Far Red to simulate vegetative shading (Rosco E-Colour #209:.3 Neutral Density, PNTA). Photosynthetically active radiation (PAR) was collected every 10 mins using a quantum light sensor (SQ-520: Quantum Sensor, Apogee Instruments). In addition to PAR, spectral composition measurements were taken periodically throughout using a spectroradiometer (LI-180, Li-Cor Biosciences).

Obj. 2 Trait Identification. Numerous traits were measured throughout the course of the greenhouse and growth chamber experiments. The traits measured were plant height, tiller number, height to tiller ratio, biomass, and chlorophyll concentration. Plant height was measured from media surface to leaf tip prior to trimming every three days. Tiller number (as a measure of shoot density) was conducted on three randomly selected plants per pot monthly. The tiller number and the corresponding plant height data were used to calculate height to tiller ratio. Biomass was calculated as a measure of dry weight of the grass clippings collected after every cutting and dried in an 80^{o}C oven across the duration of the experiment. Chlorophyll concentrations were established using a dimethyl sulfoxide extraction and resulting spectrophotometry at the conclusion of the experiment, according to the methodology of Hiscox and Israelstam (1979). Grasses were subjected to the shade conditions for 4 months to allow for adequate exposure to the shade stressor.

Greenhouse and Growth Chamber Results and Discussion

A two-way Analysis of Variance (ANOVA) and pairwise least squared means were used to identify which of the studied traits were significantly different amongst the cultivars in both experiments (Table 2). In the greenhouse study, there was significant variation amongst the cultivars and there were significant differences between full sun and foliar shade (Light condition, Table 2a.) When looking at the interaction between cultivar and light conditions, plant height and chlorophyll concentrations were significant. Height and chlorophyll concentration were also significant in the growth chamber study (Table 2b). However, there were some differences in cultivar responses between the two experiments (Figure 3ab). For example, 007XL exhibited larger plant heights under greenhouse shade and had lower plant heights under growth chamber shade. The plan in year 2 is to conduct another run of each experiment (greenhouse and growth chamber) to confirm results of the first runs of the growth chamber and greenhouse studies.

Some cultivars did perform similarly in both growth chamber and greenhouse experiments. Cultivars such as L93XD, Oakley, and 777, performed similarly under greenhouse and growth chamber conditions and also performed similarly between the full sun and shaded treatments (Figure 2ab). Little to no change in plant height between sun and shade indicates that cultivars perform similarly under both growing conditions. Cultivars such as Tyee, Au Victory, or PennA4 had a large positive change in height when grown under vegetative shade. Cultivars that exhibited large changes in height between shade and full sun, are cultivars that would require extra inputs such as mowing or growth regulators to maintain good quality. These would be examples of cultivars poorly adapted to shade (Figure 2ab).

Cultivars that have higher chlorophyll content in the shade should be able to collect the limited sunlight for photosynthesis and therefore should also be better adapted to shade stress. However, an extreme increase in cholorphyll content can increase the growth as well. Cultivars with a moderate increase in chlorophyll concentration show the most promise for shade tolerance; these include L93XD or 007XL (Figure 3ab).

Cultivars that show the best adaptation to shade should have consistent heights between shade and sun, as well as the ability to produce more chlorophyll under shade. A combination of these two traits should be focused on when looking to choose an appropriate cultivar to grow in shade.

We plan to continue these two experiments again in 2023 to confirm the results observed in 2022. Additionally, we will investigate more efficient ways to measure chlorophyll and investigate enzymes that are involved with chlorophyll degradation or synthesis to determine if there is a difference under shade conditions and whether there is a difference between cultivars for these two processes.

Table 2. Two-way ANOVA results for plant traits measured during the duration of both A. greenhouse experiment, and B. growth chamber experiment (plant height, tiller number, height:tiller ratio, biomass, and chlorophyll concentration). Variation was measured between cultivars themselves, between both lighting conditions, simulated shade vs full sun, and the variation between the interaction of cultivar and light condition. A. greenhouse. B. growth chamber. * significant at 0.05, ** significant at 0.01, *** significant at 0.001, NS not significant.

A. Greenhouse	**Plant Height**	**Tiller Number**	**Height:tiller ratio**	**Biomass**	**Chlorophyll Concentration**
Cultivar	***	**	***	NS	***
Light Condition	***	**	*	***	***
Cultivar:Light Condition	***	NS	NS	*	***

B. Growth Chamber	**Plant Height**	**Tiller Number**	**Height:tiller ratio**	**Biomass**	**Chlorophyll Concentration**
Cultivar	***	***	***	NS	***
Light Condition	***	NS	***	***	***

Cultivar:Light Condition	***	***	**	*	***

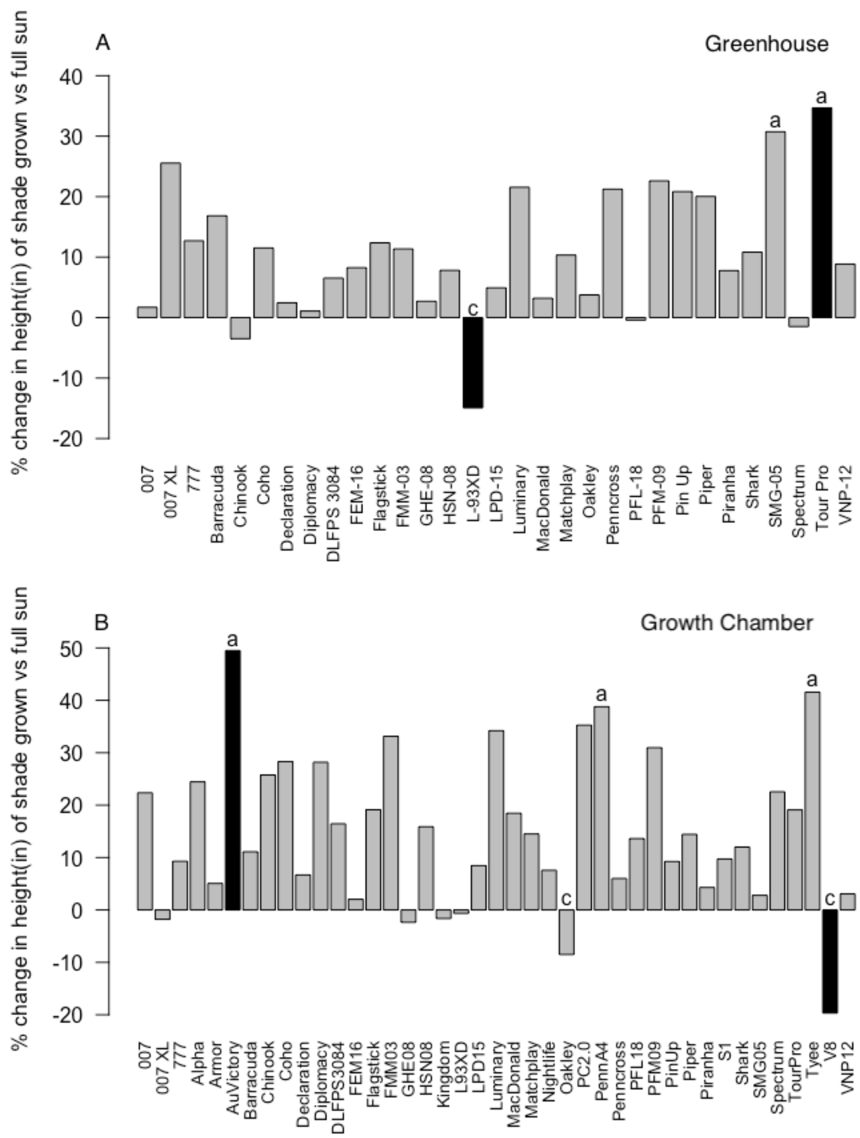


Figure 2. Percent change in plant height of creeping bentgrass cultivars and selections grown under simulated foliar shade versus full sun. Black shaded bars indicate cultivars with the highest and lowest means. Means were compared using all combinations of least squared means. a = significantly different from the lowest, not the highest; c = significantly different from the highest, not the lowest; no letter = not significantly different from either highest or lowest (p=0.05).

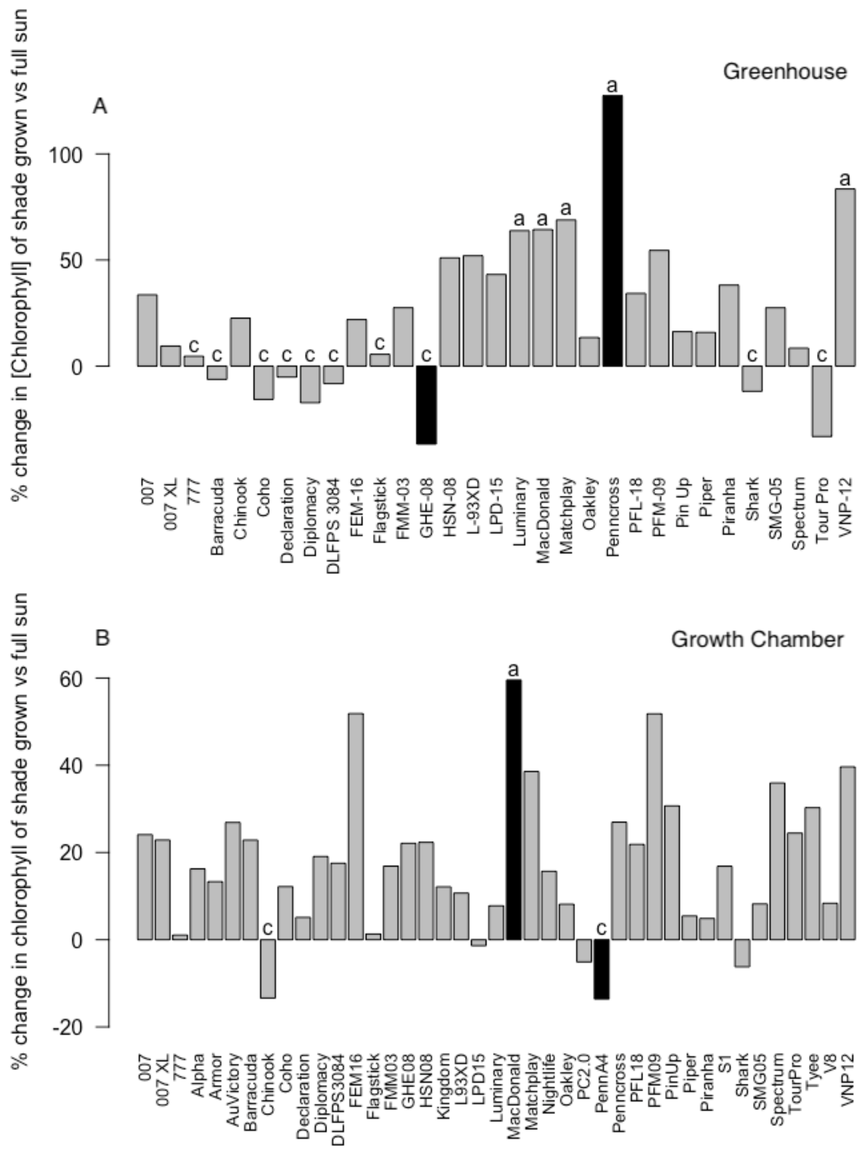


Figure 3. Percent change in chlorophyll concentration of creeping bentgrass cultivars and selections grown in simulated foliar shade versus full sun. Black shaded bars indicate cultivars with the highest and lowest means. Means were compared using all combinations of least squared means. a = significantly different from the lowest, not the highest; c = significantly different from the highest, not the lowest; no letter = not significantly different from either highest or lowest (p=0.05).

Obj. 3 – Inheritance of Shade Tolerance

329 elite creeping bentgrass genotypes showing favorable traits for turf quality including density, color growth habit etc. were planted in a randomized complete block design under shade of pecan trees at the Rutgers Adelphia Plant Science Research farm (Freehold, NJ)(Figure 4). Plants were arranged in a randomized complete block design on 1' spacing with

three replications in the spring of 2021 (n=987 plants). Light quality under the pecan trees averaged 181.6 µmol $m^{-2}s^{-1}$ in 2021 and 158.6 µmol $m^{-2}s^{-1}$ in 2022, with a Red:FarRed ratios of 0.742 in 2021 and 0.639 in 2022. Light quality in full sun averaged 1949.6 µmol $m^{-2}s^{-1}$ in 2021 and 1935.4 µmol $m^{-2}s^{-1}$ in 2022 with Red:FarRed ratios of 1.339 in 2021 and 1.296 in 2022. Turf quality and plant diameter measurements were taken monthly for two years (2021 and 2022) to characterize creeping bentgrass genotypes under shade. Data collection was completed in October 2022. Broad sense heritability calculations according to Koch et al (2015) are underway.

Figure 4. Creeping bentgrass selections grown under foliar shade provided by pecan trees at Rutgers Adelphia Plant Science Research Farm (Freehold, NJ).

Shade tolerant and susceptible clones were identified, and clones were moved to isolated crossing blocks. 22 creeping bentgrasses, with good performance (larger diameter and higher turf quality) under shade were chosen from the 329 plants planted. These 22 plants were then crossed in two polycross populations in 2022; one cross consisted of 9 early maturing plants, and the second consisted of 13 late maturing plants. 18 creeping bentgrass clones with poor shade tolerance (low turf quality and small diameter) were also identified and crossed in two polycross populations; one cross consisted of 9 early maturing plants, and the second consisted of 9 late maturing plants. Seeds were harvested from the crosses, and the parents were vegetatively propagated. Seedlings will be started from these crosses and planted with the parents in a randomized complete block design in a mowed-spaced plant nursery under shade in 2023. The performance of progeny plants compared to the parents will be utilized to calculate the narrow sense heritability of shade tolerance. This will provide more detailed information (heterosis, maternal, additive, and dominant effects) on the genetic control of shade tolerance.

Table 3. Average diameter measurements of the genotypes selected for inheritance crossing blocks from the 329 genotypes growing under foliar shade at Rutgers Adelphia Plant Science Research farm (Freehold, NJ)

Genotype	**2021 Avg Diameter (in)**	**2022 Avg Diameter (in)**	**Polycross Block**	**Genotype**	**2021 Avg Diameter (in)**	**2022 Avg Diameter (in)**	**Polycross Block**
PFM-07	4.9	5.6	Early Good Shade	**GHE-09**	2.0	2.5	Early Poor Shade
GHE-31	4.7	5.4	Early Good Shade	**FMM-06**	1.7	1.7	Early Poor Shade
HSN-07	4.7	5.8	Early Good Shade	**HSN-16**	2.2	3.6	Early Poor Shade
VNP-18	5.8	7.1	Early Good Shade	**FEM-14**	2.3	3.4	Early Poor Shade
PFM-16	5.2	6.5	Early Good Shade	**GHE-03**	2.8	3.4	Early Poor Shade
GHE-19	4.0	4.9	Early Good Shade	**FEM-20**	1.1	2.1	Early Poor Shade
HSN-14	5.3	6.7	Early Good Shade	**PFL-18**	1.0	1.0	Early Poor Shade
11545A	3.7	5.4	Early Good Shade	**HSN-01**	1.8	1.8	Early poor Shade
PFM09	4.3	6.3	Early Good Shade	**GHE-02**	2.9	3.8	Early PoorShade

H19TP 246-4	4.3	5.4	Late Good Shade	**SMG-03**	1.7	2.6	Late Poor Shade
H19TP 150-10	4.7	6.2	Late Good Shade	**11538C**	1.7	1.9	Late Poor Shade
LPD-11	5.1	6.1	Late Good Shade	**SMG-06**	2.0	3.5	Late Poor Shade
PFL-33	5.0	5.8	Late Good Shade	**11538B**	1.7	2.2	Late Poor Shade
H19TP 198-8	6.4	7.7	Late Good Shade	**LPD-05**	1.5	2.3	Late Poor Shade
11529C	5.5	7.1	Late Good Shade	**VNP-16**	1.5	1.6	Late Poor Shade
LPD-09	4.8	5.4	Late Good Shade	**SMG-09**	1.6	3.2	Late Poor Shade
SMG-17	5.3	7.3	Late Good Shade	**GHE-01**	2.0	2.9	Late Poor Shade
H19TP 208-12	4.5	5.1	Late Good Shade	**SMG-26**	1.8	2.2	Late Poor Shade
11528A	4.7	5.3	Late Good Shade				
PFL-09	5.0	6.7	Late Good Shade				
11528B	5.4	6.6	Late Good Shade				
SMG-11	5.1	5.9	Late Good Shade				

Another field trial of 30 commercial cultivars (many of them included in the greenhouse and growth chamber studies), was seeded at Rutgers Horticulture Farm 2 in North Brunswick, NJ. These cultivars were seeded in 3’ x 5’ plots at a rate of 1lb per 1000 ft^2 in a randomized complete block design with three replicates under natural foliar shade. These plots are currently being maintained as a putting green, and turf quality, disease ratings, NDVI, and digital images are being collected to monitor shade tolerance under a practical maintenance regimen. These data will be compared with data from greenhouse and growth chamber studies.

References

Ballaré, C. L., and Pierik, R. (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant, Cell & Environment*, **40**: 2530– 2543. doi: 10.1111/pce.12914.

Casal, J. (2012) Shade Avoidance. *The Arabidopsis Book* vol **10:** e0157. https://doi.org/10.1199/tab.0157

Dernoeden, P.H. (2000*) Creeping Bentgrass Management: Summer Stresses, Weeds and Selected Maladies* 21-24 Ann Arbor Press

Hiscox, J.D. and Israelstam, G.F. (1979) A Method for Extraction of Chlorophyll from Leaf Tissue without Maceration. Canadian Journal of Botany, 57, 1332-1334. http://dx.doi.org/10.1139/b79-163

Settle, D. (2008) Tree shade exacerbates physiological decline of greens during a Chicago summer, *On course* 9-12. http://www.specmeters.com/assets/1/7/TreeShadeAug08_OnCourse.pdf

Wherley, B.G., Gardner, D.S. and Metzger, J.D. (2005) Tall Fescue Photomorphogenesis as Influenced by Changes in the Spectral Composition and Light Intensity. *Crop Science*, **45**: 562-568. https://doi.org/10.2135/cropsci2005.0562

USGA ID: 2021-09-733

Title: Characterization of an antifungal protein from the fungal endophyte of strong creeping red fescue with activity against the dollar spot pathogen

Project Leaders: Faith C. Belanger and Bruce B. Clarke
Graduate Research Assistant: Patrick A. Fardella

Affiliation: Rutgers University

Objectives of the project: The overall goal of this project is to determine if the antifungal protein produced by the fungal endophyte of strong creeping red fescue could be used to inhibit dollar spot disease on creeping bentgrass.

Start Date: 2021

Project Duration: 3 years

Total Funding: $90,000

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. Our research indicates that it is likely involved in the dollar spot resistance observed in endophyte-infected strong creeping red fescue.

2. Active *E. festucae* antifungal protein was abundantly expressed in *Penicilllium chrysogenum* and was purified from the culture filtrate.

3. Foliar application of the purified *E. festucae* antifungal protein to endophyte-free strong creeping red fescue plants and creeping bentgrass plants protected them from severe dollar spot symptoms caused *by Clarireedia jacksonii*, the dollar spot pathogen.

Summary Text

Control of dollar spot disease on creeping bentgrass is a major problem for golf course managers who currently rely heavily on fungicide applications throughout the year. 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 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 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, designated *Efe*-AfpA. The gene for the antifungal protein 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 *Efe*-AfpA gene among *Epichloë* spp. suggested the *E. festucae* antifungal protein may be a component of the unique endophyte-mediated dollar spot resistance observed in strong creeping red fescue.

Our hypothesis is that *Efe*-AfpA is a factor in the well-documented disease resistance seen in endophyte-infected fine fescues in the field (Clarke et al., 2006). The ultimate goal of this research is to determine if *Efe*-AfpA can protect creeping bentgrass plants from dollar spot disease so that it could be used as an alternative or supplement to synthetic fungicides. Our results to date are described below and were recently published in a scientific journal (Fardella et al., 2022).

We have expressed *Efe*-AfpA in yeast, in bacteria, and in the fungus *Penicillium chrysogenum* (Tian et al., 2017; Fardella et al., 2020, 2021, 2022). Active antifungal protein was purified from all three systems, with the best system being *P. chrysogenum*. We are using an engineered strain of *P. chrysogenum* in which the gene for a similar antifungal protein, designated PAF, was deleted (Marx, 2004; Sonderegger et al., 2016). We previously reported that purified *Efe*-AfpA had activity in culture against *Clarireedia jacksonii*, the dollar spot pathogen. Since our last report, purified *Efe*-AfpA has been tested on plants inoculated with *C. jacksonii*.

We tested the purified protein on endophyte-free strong creeping red fescue plants, which are susceptible to dollar spot infection. Plants were inoculated with agar plugs of *C. jacksonii* and sprayed daily for 10 days with either water or with 100 $\mu g\ mL^{-1}$ of *Efe*-AfpA. As seen in Figure 1 below, the *C. jacksonii* inoculated plants had severe disease symptoms, observed as necrotic tillers surrounding the point of inoculation in the center of the plants. In contrast, the inoculated plants sprayed with *Efe*-AfpA had only a few minor leaf lesions. These results support the original hypothesis that *Efe*-AfpA is a major contributor to the dollar spot resistance seen in endophyte-infected strong creeping red fescue plants.

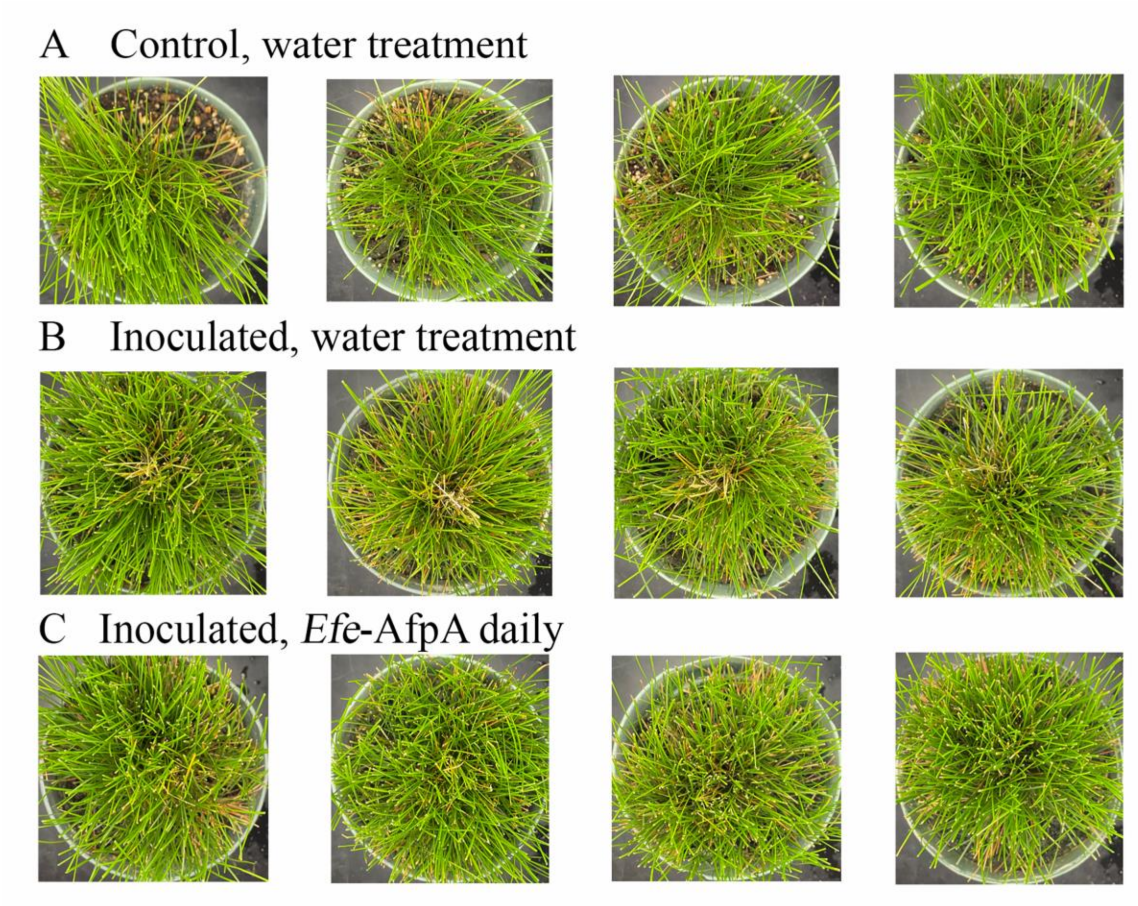


Figure 1. *Efe*-AfpA prevented severe symptoms of dollar spot disease when endophyte-free strong creeping red fescue plants were inoculated with *C. jacksonii*. Photos within a row are replicates of the labeled treatment.

Foliar application of *Efe*-AfpA to creeping bentgrass plants inoculated with *C. jacksonii* also resulted in reduced disease severity. Plants were inoculated with agar plugs of *C. jacksonii* and sprayed daily for 7 days with either water or with different concentrations of *Efe*-AfpA, with the most effective concentration being 100 μg mL^{-1}. As seen in Figure 2 below, the *C. jacksonii* inoculated plants had severe dollar spot symptoms, observed as necrotic sunken areas of the plants. In contrast, treatment with 100 μg mL^{-1} *Efe*-AfpA resulted in reduced disease severity.

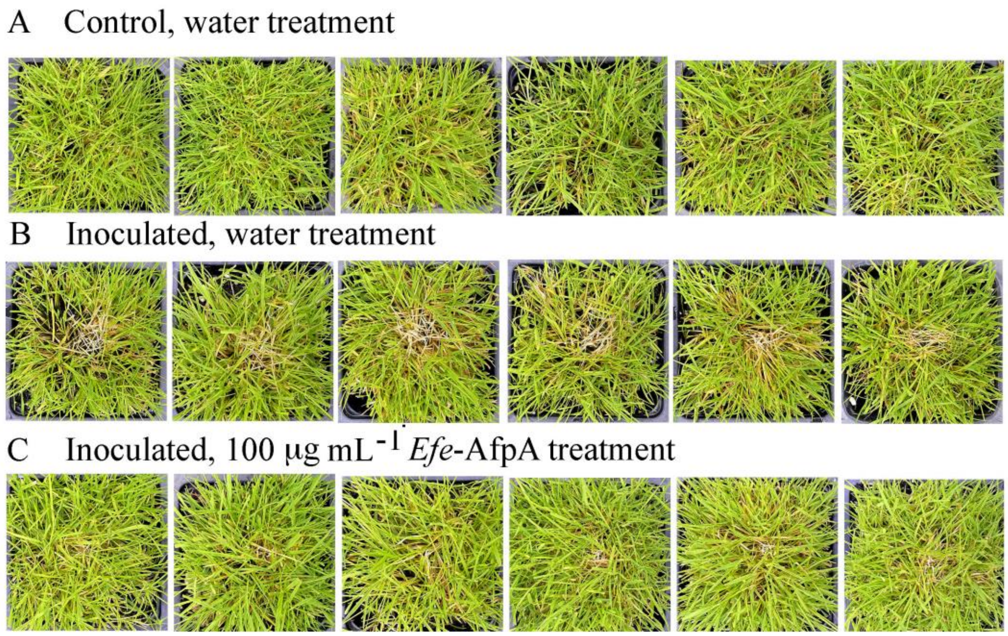


Figure 2. Foliar application with 100 μg mL^{-1} of *Efe*-AfpA reduced disease severity on *C. jacksonii*-inoculated creeping bentgrass. Photos within a row are replicates of the labeled treatment.

We also tested the effectiveness of fewer numbers of treatments with *Efe*-AfpA. As seen in Figure 3 below, two applications of 100 μg mL^{-1} *Efe*-AfpA, one at the time of inoculation with *C. jacksonii* and one at 48 hours after inoculation, were similar in effectiveness to daily applications.

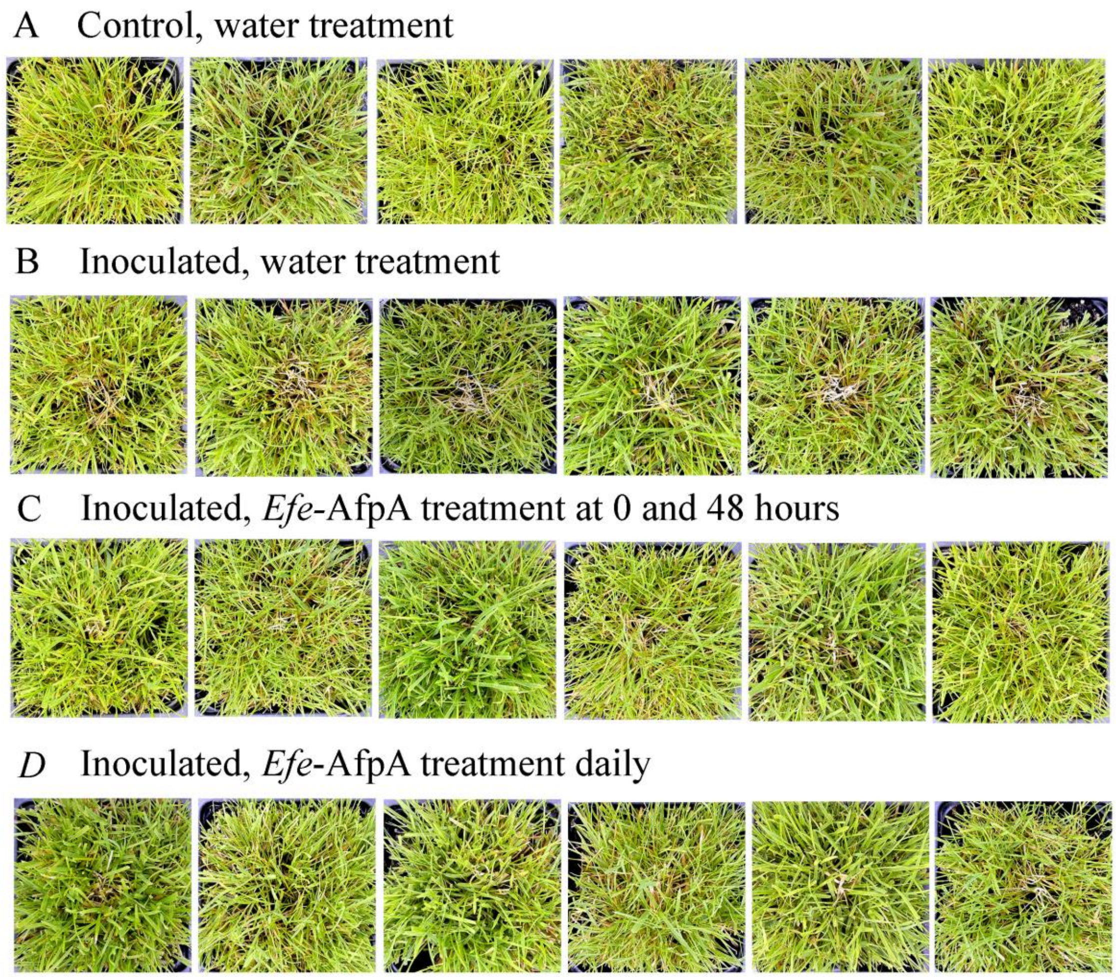


Figure 3. Foliar application with 100 μg mL^{-1} of *Efe*-AfpA on creeping bentgrass at C) the time of inoculation with *C. jacksonii* and 48 hours after inoculation provided similar reduction in dollar spot severity as D) daily applications. Photos within a row are replicates of the labeled treatment.

In summary, we determined that the likely mechanism underlying the endophyte-mediated dollar spot resistance observed in strong creeping red fescue (Clarke et al., 2006) is the production of the abundant *E. festucae* antifungal protein *Efe*-AfpA in the endophyte-infected plants. We expressed *Efe*-AfpA in *P. chrysogenum* and purified it from the culture filtrate. Foliar application of the purified protein reduced dollar spot disease severity on endophyte-free strong creeping red fescue plants and on creeping bentgrass plants. These results are promising regarding the potential of *Efe*-AfpA to be developed as an alternative or complement to fungicide use for dollar spot control on creeping bentgrass. The next step in this project is to test the efficacy of *Efe*-AfpA in reducing dollar spot severity in a field trial, which we are planning for the upcoming 2023 spring season.

References

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

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

Fardella, P., Wang, R., Luo S., Clarke, B.B., and Belanger, F.C. (2020) *Epichloë festucae* antifungal protein purification and gene knock-out. Proceedings of the Twenty-Ninth Annual Rutgers Turfgrass Symposium, January 10, 2020

Fardella, P., Clarke, B.B., and Belanger, F.C. (2021) Applications of the fungal endophyte *Epichloë festucae* antifungl protein *Efe*-AfpA. Proceedings of the Thirtieth Anniversary Rutgers Turfgrass Symposium, March 18, 2021

Fardella, P.A., Tian, Z., Clarke, B.B., and Belanger, F.C. (2022) The *Epichloë festucae* antifungal protein *Efe*-AfpA protects creeping bentgrass (*Agrostis stolonifera*) from the plant pathogen *Clarireedia jacksonii*, the causal agent of dollar spot disease. Journal of Fungi 8: 1097

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

Sonderegger C, Galgoczy L, Garrigues S, Fizil A, Borics A, Manzanares P, Hegedus N, Huber A, Marcos JF, Batta G, Marx F (2016) A *Penicillium chrysogenum*-based expression system for the production of small, cysteine-rich antifungal proteins for structural and functional analyses. Microbial Cell Factories 15:192

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

USGA ID#: 2022-08-751

Title: Evaluation and Breeding of Kentucky Bluegrass and Western Wheatgrass for Rapid Seed Germination, Salt Tolerance, and Turf Quality

Project Leader: Michael M. Neff
Affiliation: Washington State University

Objectives:

Objective 1: Screen Kentucky bluegrass and western wheatgrass for rapid germination and perform hybridization crosses.

Objective 2: Screen for salt tolerance in rapid germinating Kentucky bluegrass and western wheatgrass.

Objective 3: Perform turf trials for rapid germinating and salt-tolerant Kentucky bluegrass and western wheatgrass.

Start Date: 2022
Project Duration: 3 years
Total Funding: $83,202

Summary Points:

- Due to negotiations between Washington State University and the USGA, we were not able to initiate this project until the middle of July 2022. The delay in initiation of this project required an adjustment in our proposed timeline.

- We had previously screened Kentucky bluegrass and western wheatgrass accessions for rapid germination in 2018.

- From these seed germination screens, 182 Kentucky bluegrass accessions and 93 western wheatgrass accessions yielded sufficient seed to plant turf plots in the fall of 2022 (Objective 3).

- Over the winter of 2022 and spring/summer of 2023, we will screen the accessions planted in turf plots for salt tolerance. We will also screen additional accessions and progeny from previous hybridization crosses of Kentucky bluegrass and western wheatgrass for both rapid germination and salt tolerance.

Summary Text: Provide **800 to 1200 words** briefly outlining rational, methodology, results to date, and future expectations of the project.

Rational

This project focuses on the question of whether rapid seed germinating and salt tolerant Kentucky bluegrass (*Poa pratensis* L.) and western wheatgrass (*Pascopyrum smithii* P. A. Love) can be developed for golf courses in the Upper West, Mountain, Transition and Pacific regions of the United States. Since our new Washington State University Grass Breeding and Ecology Farm is located in Pullman WA, we surveyed a group of Inland Empire Golf Course Superintendents regarding problems that they would like to see addressed in research projects proposed to the USGA Turfgrass and Environmental Research Program. The superintendents are associated with the following: Prairie Falls Golf Course, Circling Raven Golf Course, Avondale Golf Course, Hayden Lake Country Club, Coeur d'Alene Resort and Palouse Ridge Golf Course. Two of the six surveyed asked us to address germination in Kentucky bluegrass as well as native grasses in no-mow areas (Prairie Falls and Avondale). An additional two asked us to address salt tolerance in general (Circling Raven and Palouse Ridge). Kentucky bluegrass is grown on 23% of U.S. golf courses and 49% of those in the Upper West/Mountain region. Western wheatgrass is a tall, drought tolerant, sod forming grass native to the Upper West/Mountain region. It is used for stabilization of land to prevent erosion including roadside plantings. Both grasses are known for having poor seed germination and stand/turf establishment.

Methodology

Objective 1: Screening Kentucky bluegrass and western wheatgrass for rapid germination and perform hybridization crosses.

We have previously screened four hundred and sixty-seven accessions of Kentucky bluegrass and sixty-three accessions of western wheatgrass from the USDA germplasm resources information network (GRIN, https://www.ars-grin.gov/). Both western wheatgrass and Kentucky bluegrass accessions were screened on KNO_3 soaked blotter papers at room temperature under AgroLED grow-lights for 24 hours/day. The top ten Kentucky bluegrass accessions were also screened as outlined in the AOSA rules for testing seeds (Association of Official Seed Analysts 2017) and transplanted in the field. Plants were grown on 2 ½ foot centers in a breeding nursery on one of the Grass Breeding and Ecology Farm research plots (Figure 1).

Figure 1. An example of a Kentucky bluegrass (foreground/center) and western wheatgrass (upper left) breeding/evaluation block with plants on 2 ½ foot centers. Photo was taken at the WSU Grass Breeding and Ecology Farm on April 20, 2021.

Forty of the sixty-three western wheatgrass accessions were planted in the field in 2016 and allowed to open pollinate, seed was collected in 2017 and 2018. Selections from the 2018 harvest were transplanted at the Grass Breeding and Ecology Farm in fall 2019. In spring 2020, sixteen western wheatgrass selections were polycrossed in the greenhouse and progeny were transplanted in fall 2020. The remaining western wheatgrass plants were left to open pollinate. Seed harvested in 2021 will be screened for germination and seed viability as outlined in the AOSA rules for testing seeds (Association of Official Seed Analysts 2017).

Objective 2: Screening for salt tolerance in rapid germinating Kentucky bluegrass and western wheatgrass.

Dr. Rebecca Brown from the University of Rhode Island previously developed a salt testing technique that she used to screen cool season grasses within the greenhouse (Brown *et al.*, 2011). This method relies on using a return pump to flood irrigate plants at regular intervals during the day to maintain soil moisture using a pre-determined salt solution. This technique is different than using top-down irrigation in that it does not cause leaf firing due to salt buildup on the leaf tissue and can more directly measure a plants' ability to withstand saline soil conditions. Our screening technique is a modified version in which plants are flood irrigated at pre-determined salt levels and screened for 4 weeks as turf pots. This will allow material harvested from Objective 1 to be cycled to the greenhouse that same year for salt testing. Rapid germinating lines would be established the fall after harvest in 4-inch cone-tainers as turf. Those cone-trainers will be maintained as turf and mowed by portable clippers.

Based on previous studies (Bushman 2016; Moxley 1978), Kentucky bluegrass and western wheatgrass will be subjected to four different salt rates (0, 6, 12 and 18 $dS \cdot m^{-1}$) to determine salinity tolerance. Once the 4 weeks are concluded, survivors would be sprigged into flats and used the following year as breeding lines for Objective 1. Salt tolerant Western Wheatgrass cultivars will be moved into field poly-crosses the following spring, Kentucky Bluegrass will be moved into paired crosses in the greenhouse. Seed from crossed cultivars will then be screened again for germination speed and seed dormancy and viability. WSU has the Plant Growth Facility which is a state-of-the-art greenhouse facility used for breeding small grains and grasses where the work will occur.

Aim 3: Performing turf trials for rapid germinating and salt-tolerant Kentucky bluegrass and western wheatgrass.

Turf trialing for the Kentucky bluegrass and western wheatgrass are currently taking place at the Grass Breeding and Ecology Farm in Pullman, Washington (Figure 2). Plants were established on 3' by 5' plots at a seeding rate of 2 pounds per thousand square feet for Kentucky bluegrass and 6 pounds per thousand square feet for western wheatgrass (Bunderson, 2009). We will use augmented block designs to maximize the entries from our nursery in Objective 1. Material from Objective 2 will not be directly moved to turf until it has been evaluated and improved using the process from Objective 1. Turf trials will be established yearly or bi-yearly depending on traits to be tested as well as the quantity of breeding lines available to produce a trail. Trails will be rated NTEP style with color, establishment, salt, quality and disease rated once monthly or as traits present themselves. The trials will be used to make evaluations of turf quality on all lines moving forward to return to Objectives 1 and 2 over time.

Results to Date

Due to negotiations between Washington State University and the USGA, we were not able to initiate this project until the middle of July 2022. The delay in initiation of this project required an adjustment in our proposed timeline. We had previously screened Kentucky bluegrass and western wheatgrass accessions for rapid germination in 2018. From these seed germination screens, 182 Kentucky bluegrass accessions and 93 western wheatgrass accessions yielded sufficient seed to plant turf plots in the fall of 2022. Over the winter of 2022 and spring/summer of 2023, we will screen the accessions planted in turf plots for salt tolerance. We will also screen additional accessions and progeny from previous hybridization crosses of Kentucky bluegrass and western wheatgrass for both rapid germination and salt tolerance.

Figure 2. An aerial view of the WSU Grass Breeding and Ecology Farm. Turf trials were planted in early fall 2022 in the tilled soil area in the mid-upper right-hand corner beneath the wing struts. Photo was taken June 29, 2022.

Future Expectations of the Project

The goal of this three-year project is to determine whether rapid seed germinating and salt tolerant Kentucky bluegrass and western wheatgrass can be developed for golf courses in in the Upper West, Mountain, Transition and Pacific regions of the United States. At the end of the third year, we expect to have completed our evaluation of Kentucky bluegrass and western wheatgrass accessions obtained from the USDA Plant Germplasm Introduction Testing Unit as well as varieties available from regional seed companies. We also expect to have advanced some of this material into pre-breeding trials. If we have succeeded in this goal, we will continue advance the highest quality genetic material into the final steps of breeding and will ultimately perform plant variety protection (PVP) trials with comparisons to other established Kentucky bluegrass and western wheatgrass varieties. Based on the results of those trials, we may submit a PVP application for any high-quality lines that we developed based on this research. We have experience with PVP trials and applications, having just completed one for a Kentucky bluegrass variety named ‘Matchless’ previously selected from ‘Kenblue’ by Drs. William Johnston and R.C. Johnson for dryland production in Eastern WA without the use of postharvest burning.

Literature Cited

Association of Official Seed Analysts. 2017. AOSA rules for testing seeds. Ithaca, N.Y.: Association of Official Seed Analysts.

Brown, R. N., Gorres, J., & Sawyer, C. (2011). Development of salt tolerant grasses for roadside use (No. FHWA-RIDOT-RTD-07-2A). Providence, RI: Rhode Island Department of Transportation.

Bunderson, Landon D, Johnson, Paul G, Kopp, Kelly L, Van Dyke, Adam. 2009. Tools for Evaluating Native Grasses as Low Maintenance Turf. *HortTechnology*. 19:626–632.

Bushman, B.S., Wang, L., Dai, X., Joshi, A., Robins, J.G., and Johnson, P.G. (2016). Responses of Tolerant and Susceptible Kentucky Bluegrass Germplasm to Salt Stress. *Journal of the American Society for Horticultural Science*. 141:449–456.

Moxley, M.G, Berg, W.A, Barrau, E.M. 1978. Salt tolerance of five varieties of wheatgrass during seedling growth. *Journal of Range Management*. 31:54–55.

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 Moss, Nathan Walker, and Mingying Xiang
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: February 1, 2016
Project Duration: 10 years
Total Funding: $500,000

Summary Points:

- OKC3920 bermudagrass was released in 2022 as a new cultivar for use on golf course putting greens in the US transition zone and beyond. OKC3920 has significantly improved freeze tolerance and high turfgrass quality at greens mowing heights as low as 3.2 mm.
- The greens-type bermudagrass mowing test established in 2021was carried out to characterize turfgrass performance related traits and ball roll distance under low mowing heights.
- The bermudagrass nursery established in 2017 was evaluated for drought resistance in the summer of 2022.
- A new greens-type mowing test was established in 2022 under the direction of Dr. Mingying Xiang, a new assistant professor in the department of Horticulture and Landscape Architecture. The test consisted of 13 experimental selections and two commercial cultivars (Tahoma 31®, and TifEagle).
- A large nursery of OKC3920 was planted in the summer of 2022. This nursery will be used to study best practices for managing OKC3920 for use on putting greens in the following two years.

Summary Text:

Bermudagrass is the most widely used warm-season turfgrass, covering up to 34% of all golf course turf acreage in the USA (Gelernter et al., 2017). Global warming has increased or been predicted to increase the use of turf-type bermudagrass in climates typically dominated by cool-season turfgrasses, however various challenges exist. Of these stresses, low temperatures in winter solely or combined with other undesirable environmental factors, such as shade or dehydration or traffic pose a major threat causing severe winterkill to bermudagrass stands. The long-term goal of the Oklahoma State University (OSU) grass breeding program is to develop 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 grant funded by the US Golf Association. Through multiple years of efforts, the OSU grass development team released OKC3920 as a new cultivar for use on putting greens of golf courses, especially in the US transition zone in 2022. This new cultivar is an interspecific F1 hybrid turf bermudagrass that has exhibited improved freeze tolerance over that offered by conventional ultradwarf cultivars (Figure 1). It has demonstrated high turfgrass quality that was equal to that of ultradwarf cultivars under mowing heights as low as 3.2 mm. OKC3920 has sufficient establishment characteristics, fine texture, early spring green up, and dark green color. We feel that OKC3920 is ready for on-site testing by golf course superintendents where high putting green quality turfgrass is needed and good management can be practiced in the US transition zone and southern states. OKC3920 bermudagrass must be vegetatively propagated due to its infertility.

Figure 1. Dr. Lakshmy Gopinath, a former graduate student, conducted a freeze tolerance test. She evaluated two green-type experimental genotypes (OKC0920 and OKC3920) and two commercially available bermudagrasses (‘Champion Dwarf’ and Tahoma 31® [‘OKC1131’] for freeze tolerance by subjecting them to 11 freezing temperatures (–4 to –14 ºC) under controlled environment conditions. The mean lethal temperature to kill

50% (LT50) of replicated clonal plants for each of the four genotypes was determined. OKC3920 had an LT50 of –8.1 °C, significantly better than that of 'Champion Dwarf' (–5.2°C), but not different from that of Tahoma 31 (–8.8°C) (Gopinath et al., 2021).

The greens-type bermudagrass mowing test established in 2021 was carried out to characterize turfgrass performance related traits and ball roll distance under low mowing heights. This test is part of Ryan Earp's thesis research [M.S. graduate student] (Figure 2). In the putting green trial, a randomized complete block design with a 15' by 5' plot size and 5 replicates was used. The test consisted of 12 OSU advanced selections, one genotype from University of Florida, one from Mississippi State University, and three commercial cultivars (Tahoma 31, Tifdwarf, and TifEagle). The data collected in 2022 included turfgrass quality under green-management conditions, ball roll distance, and rooting depth.

Figure 2. Graduate student, Mr. Ryan Earp (on the right), and Mr. Kenton Rasmussen, a research associate, measuring ball roll distances for a bermudagrass putting green plot at the Oklahoma State University Turf Research Center (OSU TRC), Stillwater, OK on September 9, 2022.

The OSU selections evaluated in this study demonstrated similar quality ratings compared to standards used, with 11x2, 15x9, OKC3920, and 19x19 in the same statistical group as 'TifEagle'. Additionally, OSU selections (OKC0920, OKC3920, and 11x2) had significantly deeper rooting depth compared to TifEagle across spring, summer, and fall collections. For ball roll measurements, weekly data was collected during the growing season. Standard entry

TifEagle had an average distance of 10 ft, while the OSU selections ranged from 7.9 to 8.7ft with entries OKC0805, 12x3, OKC0920, 19x19, and 11x2 averaging at least 8.5 ft.

Figure 3. Comparison of root lengths of 'TifEagle' in the left image and OKC3920 on the right demonstrating improved rooting depth of OSU selections maintained at 3.2 mm of mowing height at OSU TRC on October 12, 2022.

A replicated trial established at the OSU TRC in 2017 was continued this year. The trial included 35 OSU vegetatively-propagated experimental selections, four vegetatively-propagated commercial cultivars ('Astro', Latitude 36®, Tahoma 31® and TifTuf®), 11 seed-propagated experimental synthetics and two seed-propagated commercial cultivars ('Riviera' and 'Monaco'). We collected data for spring greenup, percent living cover, turf quality, and disease response. Irrigation to the trial was shut off in July of 2022 and left off, leaving the trial under ambient rainfall conditions through September 30th (Figure 4). The trial was then watered to promote recovery and to allow for assessment of recovery potential.

Figure 4. The drone image showing substantial variation in the bermudagrass entries in the 2017 nursery, responding to the drought conditions in the summer of 2022.

In 2022, a new greens-type mowing test was established at the OSU TRC under the direction of Dr. Mingying Xiang, a new assistant professor in the department of Horticulture and Landscape Architecture. The test consisted of 13 experimental selections and two commercial cultivars (Tahoma 31®, and TifEagle). The objective of the new trial is to evaluate turf quality and ball roll distance of the new experimental selections for putting green use. The test will be conducted from 2022 to 2025. More data will be reported in 2023.

A 7,000 sq. ft. putting green was established using OKC 3920 in 2022. The best management practices of this new putting green type bermudagrass will be determined in 2023 and 2024.

References Cited

1. Gelernter, WD, Stowell, LJ, Johnson, ME, and Brown, CD. 2017. Documenting trends in land-use characteristics and environmental stewardship programs on US golf courses. *Crop, Forage & Turfgrass Management*. DOI:10.2134/cftm2016.10.0066
2. Gopinath, L, Moss JQ, and Wu YQ. 2021. Quantifying freeze tolerance of putting green type bermudagrasses. *HortScience*. 56: 478-480. DOI:10.21273/HORTSCI15606-20

USGA ID#: 2020-11-716

Title: Expression profiling of host plants and *Ophiosphaerella* spp. during infection and colonization of diseased and asymptomatic hosts

Project Leader: Walker, N. R., D. Hagen, C. D. Garzon*, and Y. Wu

Affiliation: Oklahoma State University, 127 Noble Research Center, OSU, Stillwater, OK 74078. * C. Garzon is now employed at Delaware Valley University, Doylestown, Pennsylvania and is not involved in this project.

Objectives:

1. Use a bioinformatics approach to identify the gene(s) that are upregulated or downregulated during infection and colonization in warm temperatures not conducive for necrosis of the host tissues.
2. To use the same approach with several to asymptomatic hosts and non-disease hosts.
3. Conduct similar studies with Kentucky Bluegrass and *O. korrae* at cool and hot temperatures.

Start Date: January 2020 (funds released to the PI in July, 2020)
Project Duration: 2020-2023 (3 years with a one year no cost extension)
Total Funding: 85,792

Summary Points:

- Research efforts that were disrupted by the pandemic, were overcome in 2022
- RNA degradation and foreign contamination have been for the most part resolved.
- Creeping bentgrass RNA samples were sent to Novogene for sequencing on an Illumina HiSeq System produced an average of 78 million pair-end raw reads were yielded.
- Wheat samples it was found that 1857 genes were upregulated, and 66 genes were downregulated under inoculated condition compared with the non-inoculated control.
- High quality RNA extractions for bermudagrasses, and Kentucky bluegrass have been submitted for sequencing and future bioinformatic analysis. It is hoped that the data will be return to OSU in December 2022.
- Efforts with *Arabidopsis* were discontinued and modified to *Brachypodium distachyon*.

Summary Text:

Bermudagrass (*Cynodon dactylon*) and interspecific hybrids of bermudagrass (*C. dactylon* × *C. transvaalensis*) are the predominant turfgrass used for athletic, commercial, and residential urban ground cover in the southern United States. In regions where bermudagrasses enter a cold temperature induced dormancy during winter months, the disease spring dead spot (SDS) is the most devastating and important disease of this turfgrass (Figure 1). The disease is caused by three closely related fungi in the genus *Ophiosphaerella* (*O. herpotricha*, *O. korrae*, and *O. narmari*). In addition, *O. korrae* is the causal agent of necrotic ring spot of Kentucky bluegrass (*Poa pratensis*), a cool-season grass in the northern United States when the plants are exposed to elevated temperatures. To develop effective, durable bermudagrass cultivars that are resistant to the disease, a thorough understanding of how the pathogen induces necrosis of host tissues is necessary. Based on extensive gains in our understanding of the spring dead spot host/pathogen interaction and how they differ for resistant and susceptible cultivars (Figure 2), we are using a bioinformatics approach to identify the gene(s) in the fungus responsible for producing effectors of necrosis. Based on past research candidate necrotrophic-effector genes were identified in the fungal genomes, which were also found to be up-regulated *in planta*. Among these candidate genes, three were associated with pathogen-associated molecular pattern-triggered immunity. This implied that *Ophiosphaerella*-induced necrosis is the result of a plant basal defense mechanism. Expression profiling analysis of roots of susceptible bermudagrass cultivar Tifway infected with *O. herpotricha* demonstrated activation of plant innate immunity responses mediated by activation of jasmonic acid potentially resulting in hypersensitive response. The tolerant U3 biotype showed activation of basal defense response mediated by salicylic acid. This salicylic acid-mediated signaling could be involved in enhanced resistance to nutrient starvation and cold tolerance that allows the host to withstand pathogen infection and avoid organ death during periods of cold-temperature induced dormancy. The goal of this research is to mirror past studies to elevate the understanding of the SDS pathosystem to a level where bermudagrass breeding efforts can use known host specific disease resistance gene(s) to develop new cultivars with enhanced disease resistance.

Methodologies are similar for all studies where plants will then be incubated at various conducive and non-conducive temperatures with isolates of *Ophiosphaerella spp.* and total RNA will be extracted from roots by flash freezing in liquid nitrogen and preserved. Sequencing library preparation of RNA samples will be performed and sequenced using Illumina HiSeq System. Gene expression will be considered differentially expressed based on 5% false discovery rate and log fold change of two. The identities of fungal effector(s), and gene enrichment analyses from diseased plants at cool temperatures will be done using bioinformatics approaches like what was done previously.

Studies with bermudagrass and bluegrass have been completed and samples submitted for sequencing. Bentgrass samples results are under analysis, wheat data has been analyzed (Figure 2.) and *Arabidopsis* efforts were abandoned and another model plant species *Brachypodium distachyon* (Figure 3). This change was made primarily because infection of the plants by the fungus was inconclusive. Analysis will need to continue and will take next year to complete but it is expected that sequencing data will be available in late 2022.

Figure 1. Spring dead spot symptoms. Necrotic patches present on a golf course fairway in mid-May (left). Weed encroachment in a patch (right).

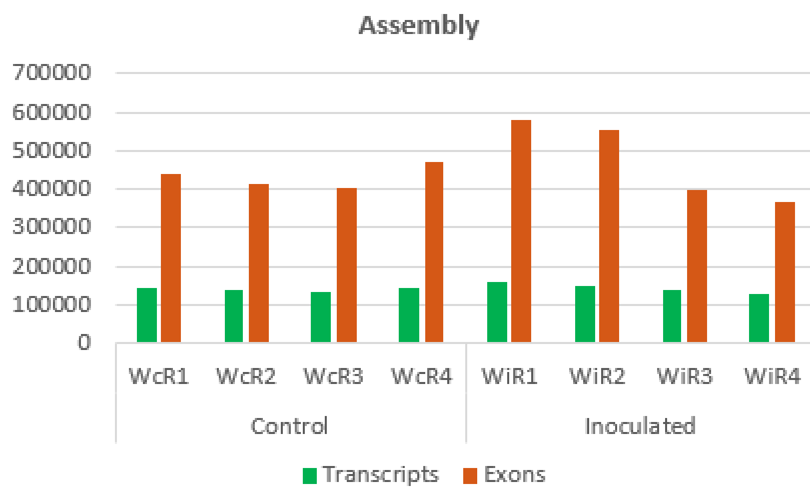


Figure 2. Transcriptome assembly of aligned reads across wheat reference genome.

Brachypodium distachyon

Figure 3. *Brachypodium distachyon,* non-inoculated on left, inoculated on the right.

USGA ID: 2016-38-608

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

Project leader: 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 10 years
Total funding $300,000

Summary Points
- Advanced lines and commercial cultivars of bermudagrass and zoysiagrass show separation for turfgrass quality and disease symptoms.
- A replicated trial of new bermudagrass hybrids was planted.
- Two new polycross blocks for *Z. matrella* and *Z. japonica* were established using non-winter dormant germplasm.
- Initial selections of non-dormant zoysiagrass lines were made from the 2021 spaced plant nursery.
- A new high density spaced plant nursery of zoysiagrass was established.

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. Most of the 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 improved disease resistance.

Fairway trials of bermudagrass and zoysiagrass were planted in 2019, each with 27 entries. The bermudagrass trial contains nine commercial cultivars and 18 experimental lines. The zoysiagrass trial contains two commercial cultivars and 25 experimental lines. For each trial, the plots are 9’ x 9’, planted in a randomized complete block design with three replications. In 2021, trials were rated for turf quality, and in the fall, clipping yields were collected from selected entries in the bermudagrass trial. Both trials are mowed twice per week at 1.3 cm and irrigated to prevent stress.

Bermudagrass

The nine commercial cultivars of bermudagrass include Tifway, TifTuf, Celebration, Latitude 36, NorthBridge, Tahoma 31, Landrun, Iron Cutter and Bimini. Visual ratings have been collected for several parameters throughout the study. Figure one shows turfgrass quality (TQ) ratings averaged across 2020, 2021, and 2022 and separated between summer and winter months. Summer months included April through November and winter included December through March. Only two entries, TifTuf and an experimental FB1630, averaged greater than six across the three years. During the winter months, three entries, FB1630, TifTuf, and FB1628 had average TQ values greater than five. While FB1630 rates well for turfgrass quality during the winter, when a freeze occurs it will quickly lose all color and turn brown. It does maintain dense cover, albeit brown. Conversely, TifTuf will hold green color even after several frosts but density decreases.

Bipolaris (*Bipolaris cynodontis*) is frequently observed in the bermudagrass trial (Figure 2). Many entries had acceptable levels of bipolaris leaf spot during both years, including: FB1628, 481-2, Celebration, FB1630, TifTuf, FB1903, 906, 19-12-2, 343-34, 9-6-8, and Tifway.

A new hybrid bermudagrass trial with 63 entries was planted in fall of 2023. One of the primary characteristics for advancing lines from this trial will be improved winter performance compared to industry standards.

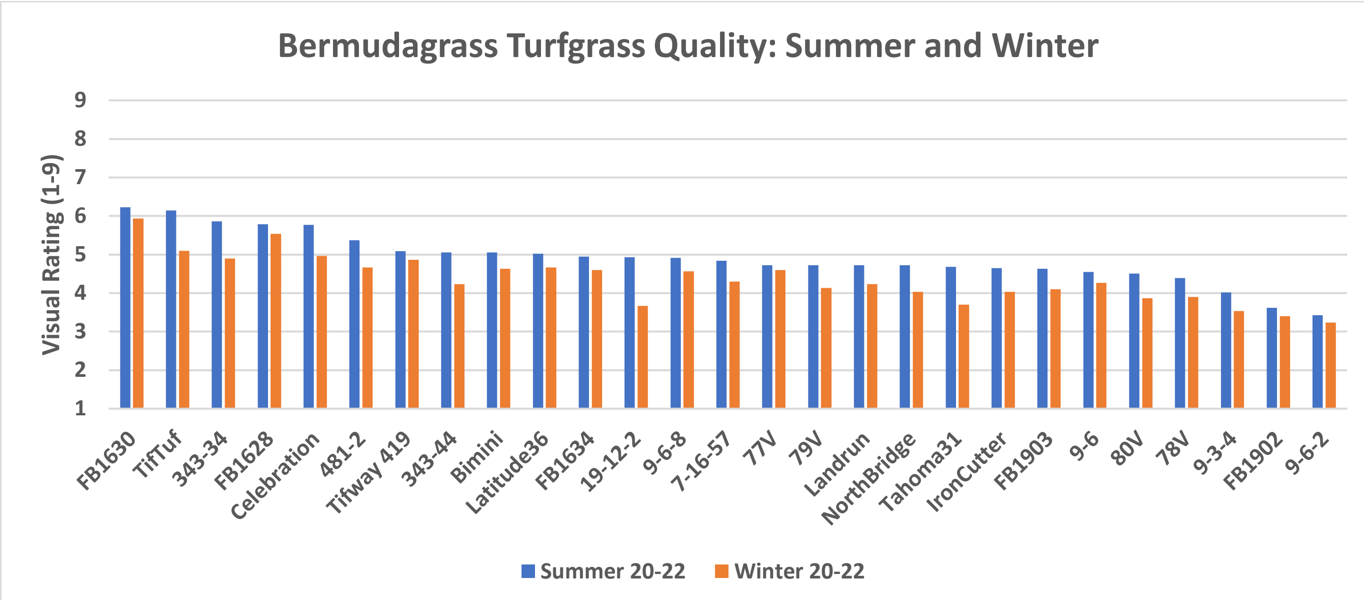


Figure 1. 2020, 2021, and 2022 average turfgrass quality ratings of 27 bermudagrass entries for summer and winter. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality.

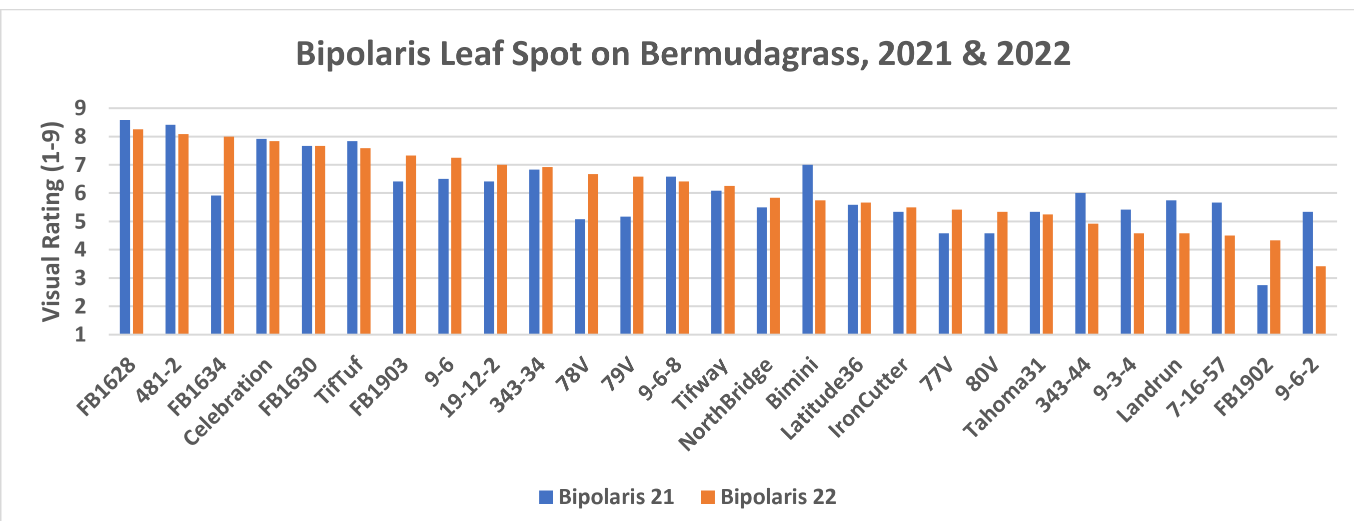


Figure 2. Average incidence of *Bipolaris cynodontis* on 27 entries of bermudagrass in 2021 and 2022. Disease was visually rated using a 1-9 scale, where 9 equals no disease and 1 = complete death of a plot from disease.

Zoysiagrass

The two commercial cultivars of zoysiagrass included in the 2019 fairway trial are Zeon and CitraZoy. Visual ratings were collected for several parameters throughout the study. Figure three shows turfgrass quality ratings averaged across

2020, 2021, and 2022 and separated between summer and winter months. Summer months included April through November and winter TQ values were determined by averaging December through March. The average turfgrass quality through the warmer growing seasons across years was ≥ 6 for most entries. Sixteen entries (including CitraZoy) had TQ values ≥ 5 when averaged across the three years for winter months; no entries rated 6.0 or better in the winter.

Dollar spot was rated three times in 2021 and four months in 2022 (Figure 4). When ratings were averaged across both years, 16 entries rated above six, while seven entries rated > 7. Bipolaris was evaluated once in 2021 and three times in 2022. Eighteen entries rated ≥ 6.0, 12 entries ≥ 7.0, and five entries ≥ 8.0. FZ1723, FAES1319, FZ1680, FZ1642, FZ1732, FZ1667, and FAES1329 were ≥ 7.0 for both dollar spot and bipolaris (Figure 4).

A new 2021 zoysiagrass spaced plant nursery was planted with 2,000 accessions. An initial group of 65 accessions were identified in late fall of 2022 for establishment, density, fall color retention, and lack of disease symptoms from the 2021 nursery. These selections will continue to be monitored in the field through the 2022-2023 winter and through the 2023 growing season. In 2022, a new spaced plant nursery was planted using a high-density planting scheme.

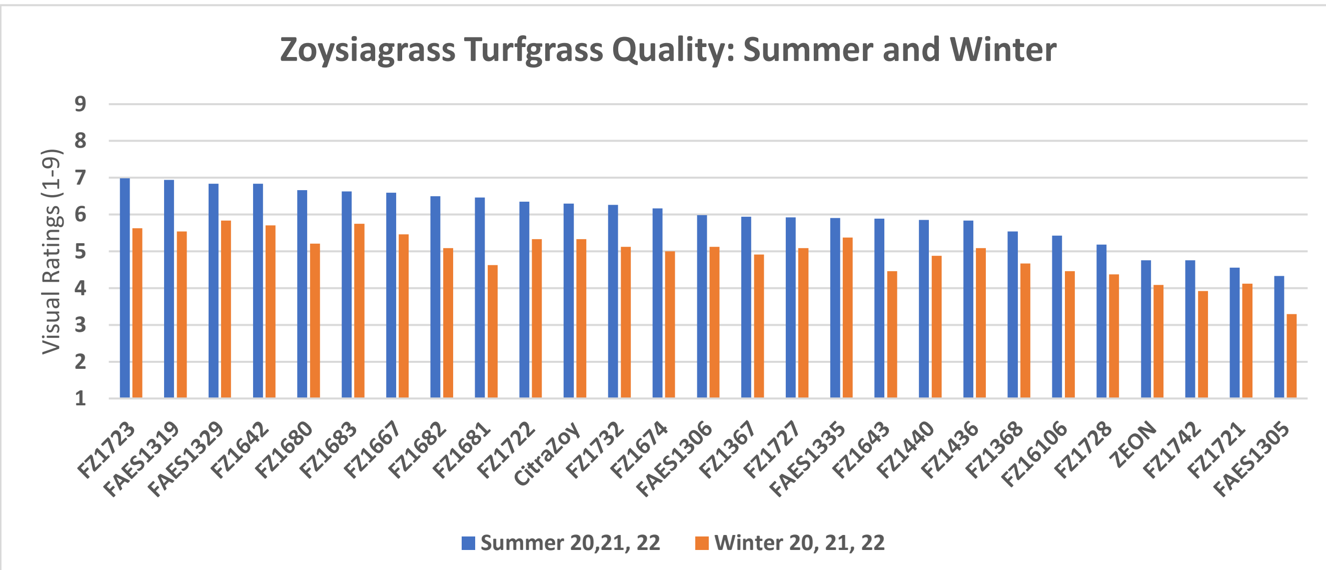


Figure 3. 2020, 2021, and 2022 average turfgrass quality ratings of 27 zoysiagrass entries for summer and winter. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality.

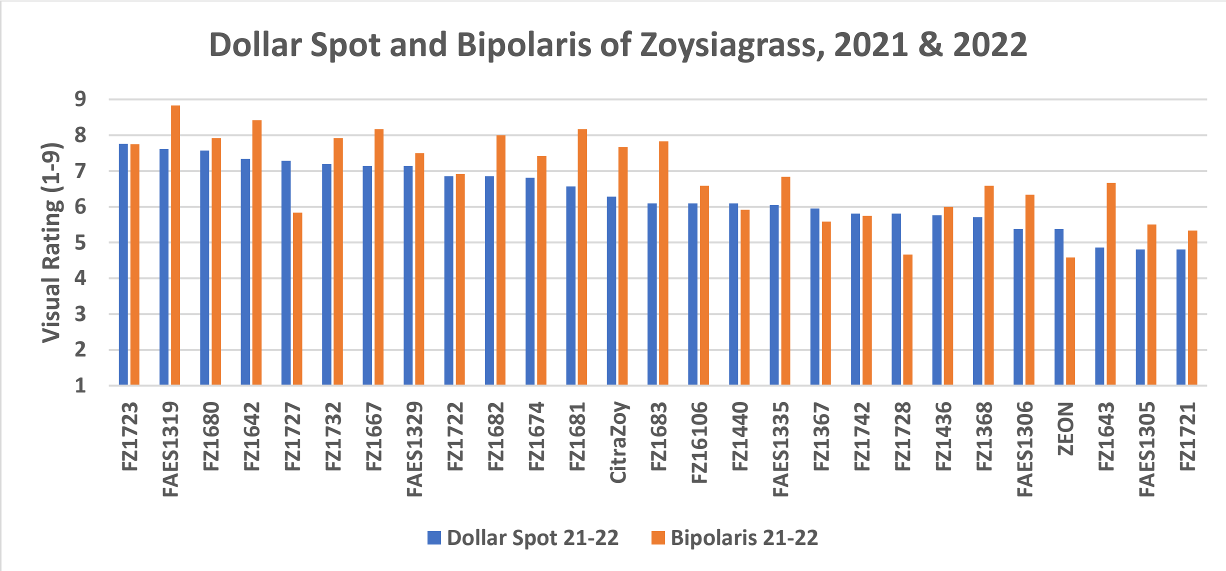


Figure 4. Average 2021 and 2022 incidence of dollar spot disease and bipolaris leaf spot on 27 entries of zoysiagrass. Disease was visually rated using a 1-9 scale, where 9 equals no disease and 1 = complete death of a plot from disease.

USGA ID#: 2017-21-631

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

Project leaders: Marta Pudzianowska, Christian S. Bowman, Adam J. Lukaszewski, 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 to reduce aggressiveness and improve turf quality and playability characteristics.

Start Date: 2017
Project Duration: 9 years
Total Funding: $450,000

Summary Accomplishments

- Patenting of the first two UCR-generated bermudagrass hybrids, UCR 17-8 and UCR TP6-3, was initiated in 2022.
- Established replicated trials of bermudagrass hybrids selected for fairways/sports fields and greens, and replicated test plots of kikuyugrass selected for fairways/sports fields, in various locations in CA and NV.
- New bermudagrass hybrids and kikuyugrass nurseries were established.
- Trials of shade tolerance and test plots of bermudagrass suitable for roughs and lawns were initiated in 2021.
- Evaluation of nurseries for drought and salinity stress trials with bermudagrass, zoysiagrass, St. Augustinegrass and seashore paspalum lines under the Specialty Crop Research Initiative (SCRI) project continues.
- Tests of bermudagrass and kikuyugrass for drought tolerance was initiated in 2019 and continued in 2022.
- Earlier DNA marker data were reanalyzed and combined with the cytological ploidy level verification, producing some species-specific markers for the collection entries.

Summary

Warm-season or C4 turfgrass species such as 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 the winter color retention and drought tolerance for Mediterranean and arid climates.

Since 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 is on genetics and genomics.

Bermudagrass

Evaluation and selection in nurseries established in earlier years continues in repeated cycles. New bermudagrass hybrids are generated routinely; the most recent set was generated in 2021 and planted in the nursery in July 2022. Parents with valuable traits, as judged by their own performance as well as that of their hybrids, are being used for pair-wise detached tiller crosses. Newly generated hybrids will be planted next spring in a new nursery.

In recent years, preselected bermudagrass hybrids are also being tested for their suitability for roughs/lawns. The most recent trial was planted in 2021. Twenty-two bermudagrass hybrids were planted in three replicates with UCR 17-8 and UCR TP6-3, as well as six cultivars (‘Bandera’, ‘Bullseye’ ‘Celebration’, ‘Midiron’ ‘Santa Ana’, ‘Tifway II’) added as checks. Plots are mowed once a week at 2”. Entries clearly vary in their quality and seedhead production, with UCRC180015, UCRC190307 and ‘Bandera’ showing the highest quality.

Two new replicated trials of bermudagrass hybrids were planted in August 2022. One includes 57 entries suitable for fairways/sports fields and the other with six entries suitable for greens. These entries were selected from our 2018 and 2019 nurseries. They will be compared with commercial checks and UCR 17-8 and UCR TP6-3.

Dry-down tests continued in 2022. This study includes 71 of our best performing hybrids and collection accessions identified in previous years, together with five commercial cultivars (‘Bandera’, ‘Celebration’, ‘Santa Ana’, ‘TifTuf’ and ‘Tifway II’) as checks. Trials were established in May 2019, in a completely randomized design with three replicates. As in 2020, the entries were subjected to two consecutive six-week dry-down periods followed by recovery periods. Several UCR entries outperformed commercial checks in both years, based on their average green (living tissue) coverage as determined by the digital image analysis. Year 2022 was the last one of the study. Five entries, UCRC180217, UCRC180557, UCRC180040, UCRC180146, and UCRC180229, have consistently remained among the top 10 performers since the first dry-down year in 2020, with two of the five, UCRC180217 and UCRC180557, remaining among the top five performers each year.

A trial planted in 2019 with hybrids from the 2014 nursery continued at the West Coast Turf farm in the Coachella Valley, Thermal, CA. Plots were mowed at 2” to evaluate their suitability for roughs and lawns. UCR BH 19-2, UCR BH 16-4, ‘Bandera’, ‘Tifway II’ and UCR BH 17-1 were the best performing entries. A similar trial in Carmel-by-the-Sea had to be terminated in 2021 due to water restrictions.

Based on performance in numerous trials, UCR 17-8 and UCR TP 6-3 were selected to be released as commercial cultivars. The patent submission process has been started by UCR. In April 2022 large, replicated test plots of UCR 17-8, UCR TP6-3, 'Santa Ana' and 'TifTuf' were established at UCR to test the performance of the two releases under various management practices such as fertilization and irrigation levels, traffic tolerance etc. The foundation blocks of both hybrids were planted in May 2022. These are being used to further expand the production area. First commercial quantities of UCR 17-8 and UCR TP6-3 are expected to become available in 2024.

Ploidy levels of the collection entries and of some hybrids were verified cytologically, identifying a large proportion of triploids. When these triploids were removed from the DNA marker analyses of the collection entries, species grouping improved considerably, and some species-specific markers were identified. It is expected that these markers will permit verification of the parentage of hybrids produced by open pollination of collection entries, and may help in the association mapping of various agronomic characteristics.

Kikuyugrass

Replicated trials of kikuyugrass hybrids selected from the nursery planted in 2019 were established in the spring of 2022. Forty experimental lines and cv. Whittet are being evaluated for establishment, turfgrass quality, winter color retention, color, texture and seedhead production. UCRK 190268, UCRK 190306 and UCRK 190280 have, so far, the highest overall quality and the finest texture. The kikuyugrass dry-down study continued in 2022. The study was planted in 2019 and conducted in a manner like that of the bermudagrasses. Thirty-eight accessions were selected based on their performance in a preliminary drought tolerance assessment, with 'Whittet' selections and 'AZ-1' serving as commercial checks. In 2022 only one drought cycle was applied, as the entries' response in the first cycle was poor, and recovery long. Generally, the drought tolerance of kikuyugrass appears lower than that of bermudagrass, but some variation among entries does exist. The best performing entries can be used in further breeding efforts to improve the drought tolerance of this species. A new nursery with 406 new kikuyugrass hybrids and progenies from self-pollinated hybrids was also established in 2022.

Pandemic restrictions made it impossible to bring in a specialist to continue the earlier efforts in androgenesis/microspore culture of kikuyugrass. At present these efforts are on hold until normal recruitment and international travel are possible again. The plan is to follow up on earlier efforts where microspore divisions were successfully initiated and calli formed.

Other species

In 2019, the UCR breeding program initiated cooperation with five warm-season grass breeding programs under the Specialty Crop Research Initiative (SCRI) funded by the National Institute of Food and Agriculture (NIFA). The project involves breeding programs of North Carolina State University (NCSU), Oklahoma State University (OSU), Texas A&M AgriLife (TAMUS), the University of Georgia (UGA), and the University of Florida (UF). Entries of four species (189 of bermudagrass, 216 of zoysiagrass, 125 of St. Augustine grass and 90 of seashore paspalum) were planted in June and July 2020. UCR is the testing site for the overall performance in the South-West US, as well as drought and salinity tolerance. Twenty of UCR hybrids are also evaluated in single spaced plant nurseries (SSPNs) across all testing locations. Like the previous year, dry-down was initiated in single spaced plant nurseries (SSPN) and in advanced drought trials in the summer 2022. Bermudagrass again showed the best performance under drought. One of UCR lines in SSPN showed very high quality under drought. Irrigation with water with electroconductivity at 4.4 dSm^{-1} was repeated in the salinity trial at the beginning of July 2022. Under salinity stress, the seashore paspalum entries showed higher turfgrass quality and lower leaf firing than the other species, while St. Augustinegrass had the lowest quality and the highest leaf firing. High variation in quality was observed among zoysiagrass and bermudagrass entries, which suggests that improvement of these two species through breeding efforts is possible. Plants in both studies are currently recovering, and drought and salinity tests will be repeated in 2023.

Figure 1. Bermudagrass entries in dry-down study after ca. 60 days without irrigation at UCR Agricultural Operations field in Riverside, CA. UCRC180557 in the bottom center of the picture. Photo taken on 12 August 2022.

Figure 2. Kikuyugrass lines establishing in replicated test plots at UCR Agricultural Operations field in Riverside, CA. Photo taken on 9 June 2022.

USGA ID#: 2017-11-621

Title: Development of Seeded Zoysiagrass Cultivars with Improved Turf Quality and High seed yields

Project Leaders: Ambika Chandra, Caydee Blankenship, A. Dennis Genovesi, Meghyn Meeks

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.

Summary Points:

- Evaluations are continuing for the 2021 Seeded Zoysia SPN
- Our collaboration with Johnston Seed Company in Enid, OK has resulted in identifying elite lines which have been used to form two synthetic and one recombination block in late 2021. The result of this work has encouraged us to produce a two-parent synthetic block which was sprigged in July 2022 in Dallas, TX in anticipation of producing enough seed yield to enter as a synthetic line in the 2024 NTEP Zoysiagrass Trial.
- A great effort was invested in summer 2022 which evaluated seedhead traits and yield components from the synthetic and recombination blocks planted in Dallas, TX; germination was similar between seed treated with and without chemical scarification; timing of harvest needs to be more closely evaluated to improve germination percentages.
- Our collaboration with Woerner Turf in southern Alabama has been fruitful as we continue to receive feedback about the strip trials and SPN with some potential elite lines.

Project updates:

- ***2017 Isolation Crossing Blocks*** – A proportion of the seed that was harvested in 2019 was scarified and germinated in 2020 to produce 15 to 30 progeny for each of 26 families. Progeny were transferred to 4” pots and planted 22 June 2021 as the 2021 Seeded Zoysia Space-Plant Nursery. Plots have been rated for establishment, quality, spring greenup, and seedhead development in the spring and summer of 2022. We intend to select genotypes in 2023 exhibiting desirable turfgrass characteristics and seedhead production traits in continuation of this project.
- ***2019 Isolation Crossing Blocks* -** As mentioned in the previous report, seed was not harvested from the 2019 Red Isolation Crossing Block in 2021 due to poor establishment and winter injury. However, seed that was harvested 27 May 2021 from the 2019 Yellow Isolation Crossing Block was processed to measure seed yield traits. Those seed are in storage for planting as time permits. We chose instead to expand our efforts in experimental synthetic seed lot development. The three parental lines from the 2019

Yellow Isolation Crossing Block with the best yield were TAES 6585-34, 6596-05, and 6596-22. Thus, in the spring of 2022, vegetative material from 6596-05 and 6585-34 were mass propagated in the greenhouse in preparation for planting a replicated synthetic isolation block which was sprigged on 28 July (Figure 1). Sprigging promotes faster establishment. We intend to evaluate seedhead production in spring 2023 and harvest enough seed for submission in the 2024 NTEP Seeded Zoysia Trial.

Figure 1. Dr. Dennis Genovesi shown sprigging one of two replications of the 2022 Synthetic Isolation block on 28 July. TAES 6596-05 and 6585-34 were planted 1 foot apart in an alternating pattern.

- ***Collaboration with Johnston Seed Company in Enid, OK*** – Out of the 23 parental lines that were sent to Oklahoma in 2018, we identified 4 elite lines (TAES 6596-05, 6585-34, 6086-21, and 6087-15) in early 2021 that demonstrated good seedhead production. Using these 4 elite lines, one early (TAES 6596-05 and 6086-21) and one late (TAES 6596-05, 6585-34, and 6087-15) flowering synthetic blocks were planted in late summer 2021 in both Enid, OK and Dallas, TX. Note, TAES 6596-05 is a common parent in both synthetic blocks. Out of the 535 coarse textured progeny which were also sent to Oklahoma in 2018, 8 elite lines were identified in early 2021. These genotypes were planted in a replicated and randomized recombination block in both locations in late summer 2021. Spring seedhead development was monitored in 2022 from both synthetics and the recombination block. About 4 weeks after emergence in Dallas, TX, seedheads were harvested by hand in replications from the early block on 10 May and from the late block on 27 May and 28 July. Seed were processed, and data was recorded for floral morphological traits and yield for each parental line. Germination tests were conducted in a growth chamber using seed from each parental line. Ninety seed were scarified while another 90 were not scarified to test the necessity of chemical scarification. Seed will be harvested again in 2023.
    - *Morphology and yield results* – Within the early synthetic block, most floral morphology and seed yield traits were similar between the two genotypes except the number of filled florets (Table 1) and the weight per 200 florets (Table 2)

which was higher for 6086-21. Within the late synthetic block harvested in May, 6585-34 had a short inflorescence length, narrow inflorescence diameter, fewer florets per inflorescence, but a greater percentage of filled florets and larger seed size (Table 1). This was also reflected in seed yield traits (Table 2). These traits were also like the July harvest data (Tables 1 and 2). Between 6087-15 and 6596-05, they are closely similar for most traits, however, 6596-05 has a shorter inflorescence length with more florets per inflorescence (Tables 1 and 2). No differences were significant between the 3 genotypes harvested from the recombination block.

- *Percent germination* – Percent germination with or without (water only) chemical scarification within each of the early and late synthetic blocks was compared within each genotype and broken down by the number days after treatment (Table 3). No germination was evident within the first 7 days. The greatest proportion was observed between 8 and 14 days followed by 15 to 21 days with minimal germination thereafter. Additionally, chemical scarification did not improve germination for most genotypes, except 6087-15 in the late synthetic block, despite slightly higher total percent germination from chemically treated seed. Further analysis determined no replication effect on total percent germination (data not shown). These results are interesting as we did not observe increase in germination percentage with chemical scarification. This needs to be investigated further.

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Table 1. Floral morphology of parental lines in the early and late synthetic blocks and recombination block harvested in Dallas, TX. Each entry within a block is replicated three times. Data presented here is across 15 inflorescence (subsamples) per replication making a total of 45 sub-samples.

Block	Genotype	Mean Inflorescence Length (mm)	Mean Inflorescence Diameter (mm)	Mean Florets / Inflorescence	Mean Filled Florets / Inflorescence	Mean Empty Florets / Inflorescence	Percent filled florets/ Inflorescence	Top Seed Size (mm)	Middle Seed Size (mm)	Bottom Seed Size (mm)
Early Synthetic (May harvest)	6086-21	23.3	1.5	26.0	13.3	12.8	50.8 a	2.8	2.8	3.1
	6596-05	23.0	1.5	26.2	11.6	14.7	42.9 b	3.0	2.9	3.1
Late Synthetic (May Harvest)	6087-15	30.2 a	1.4 b	19.7 b	10.0	9.7 b	50.0 b	2.9 c	2.9 b	3.1 b
	6585-34	25.8 b	1.4 b	17.7 c	11.4	6.3 c	65.1 a	3.5 a	3.4 a	3.6 a
	6596-05	25.4 b	1.5 a	24.9 a	12.0	12.9 a	47.7 b	3.1 b	2.9 b	3.2 b
Late Synthetic (July Harvest)	6087-15	16.5 a	-	23.0 a	7.1 ab	15.9 a	31.1	-	-	-
	6585-34	11.4 b	-	17.9 b	5.9 b	12.0 b	34.4	-	-	-
	6596-05	11.4 b	-	25.1 a	9.1 a	16.0 a	38.4	-	-	-
Recombination Block	7001-01	38.6 a	-	44.3	22.4	21.9	52.1	-	-	-
	7001-03	34.0 ab	-	41.3	22.7	18.6	54.8	-	-	-
	7004-02	33.0 b	-	41.0	21.2	19.9	51.0	-	-	-

Table 2. Floral seed yield of parental lines in the early and late synthetic blocks and recombination block harvested in Dallas, TX. Each entry within a block is replicated three times. Data presented here is across 15 inflorescence (subsamples) per replication making a total of 45 sub-samples.

Block	Genotype	Total florets	Total filled florets	Total empty florets	Percent filled[a]	Weight per 200 filled florets (mg)
Early Synthetic (May harvest)	6086-21	390.7	199.3	191.3	51.1	81.3 a
	6596-05	382.0	170.0	212.0	44.3	63.4 b
Late Synthetic (May Harvest)	6087-15	295.3 b	149.7	145.7 ab	50.9	64.6 b
	6585-34	260.7 c	173.0	87.7 b	66.5	84.8 a
	6596-05	372.0 a	178.7	193.3 a	47.9	75.0 ab
Late Synthetic (July Harvest)	6087-15	345.3 a	106.3	239.0	31.3	29.8 b
	6585-34	261.7 b	87.0	174.7	33.6	79.4 a
	6596-05	376.3 a	134.7	241.7	35.3	63.2 ab
Recombination Block	7001-01	664.3	336.0	328.3	51.7	66.8
	7001-03	620.0	341.0	279.0	55.6	54.6
	7004-02	615.7	317.7	298.0	51.6	42.5

a Total filled florets divided by total florets

Table 3. Percent germination recorded for each genotype either treated with or without chemical scarification.

Block	Genotype	Seed Treatment	Days after treatment: 0 to 7	8 to 14	15 to 21	22 to 28	29 to 35	36 to 42	Total Germination
			%						
Early Synthetic (May harvest)	6086-21	Scarified	0.0	21.1	2.2	0.0	0.0	0.0	23.3
		Not scarified	0.0	7.8	2.2	2.2	0.0	0.0	12.2
	6596-05	Scarified	0.0	17.8	4.4	0.0	2.2	4.4 a	28.8
		Not scarified	0.0	10.0	8.9	1.1	3.3	0.0 b	23.3
Late Synthetic (May harvest)	6087-15	Scarified	0.0	13.3 a	11.1 a	3.3	2.2	1.1	31.0
		Not scarified	0.0	2.2 b	0.0 b	5.6	3.3	6.7	17.8
	6585-34	Scarified	0.0	21.1	4.4	1.1	1.1	0.0	27.7
		Not scarified	0.0	21.1	2.2	0.0	1.1	0.0	24.4
	6596-05	Scarified	0.0	32.2	10.0	2.2	2.2	2.2	48.8
		Not scarified	0.0	20.0	11.1	4.5	4.4	6.7	46.7
Control	Zenith*	Scarified	0.0	19.3	2.6	0.4	0.0	0.0	66.7
		Not scarified	0.0	7.0	5.9	1.1	0.0	0.0	42.2

*Zenith seed was purchased locally and had been already scarified and ready for germination; therefore the scarified data represents a second scarification process.

- ***Collaboration with Woerner Turf in southern Alabama*** – Similar to our collaboration with Johnston Seed Company, 21 of 23 parental lines were sent to Woerner Turf in southern Alabama in March 2022. These were planted as plugs in strip trials (Figure 2). A progeny population of 520 was also sent that was derived from remnant seed harvested from the 2017 yellow isolation block in 2019. These progeny were planted as a single plugs in space plant nursery (Figure 2). All material sent to Alabama is being evaluated for establishment, quality, seedhead density, and seedhead color. Based on these traits, seed was harvested from 6 lines from the space plant nursery in summer 2022 and sent to Dallas to determine the total of number seedheads, yield and percent germination. These populations will continue to be evaluated to form another synthetic or recombination block in the future.
    - *Establishment* – As of August 2022, all the parental lines in the replicated strip trials were fully established. Several of the progeny in the space plant nursery were also fully established. Rate of establishment and quality data will be presented in the next annual report as more data is being collected.
    - *Yield and germination results* – The total number of inflorescences received for each of the 6 lines ranged from 151 to 548 (Table 4). The weight of processed seed also varied ranging from 0.28 g to 2.03 g. The greatest amount of germination was 20% from 7530-34 which also had the highest number of

inflorescences harvested. Low percent germination across all six lines is most likely due to harvest of immature seed. Small numbers of seed set is possibly due to a lack of pollen volume needed for out-crossing. However, the seedhead emergence and seedhead density of these lines look promising in this preliminary evaluation. Data will be collected again in spring 2023 to verify some of these results.

Figure 2. Seedhead development of parental line strip trials (left) and isolation block progeny (right) on 24 June 2022 at Woerner Turf in southern Alabama.

Table 4. Seedhead production, yield, and germination of 6 elite lines from Woerner Turf space plant nursery in 2022.

Genotype number	Total number of inflorescences	Weight of processed seed (g)	% Germination*
6599-47	151	0.28	3.3
6617-36	379	0.77	0.0
6618-37	327	2.03	13.3
7530-34	548	1.72	20.0
7531-46	423	1.16	10.0
7536-42	541	1.31	3.3

*Percent germination is based on 30 scarified seed.

USGA ID#: 2018-01-651, 2018-02-652, 2018-03-653, 2021-18-742f, 2021-18-742e, 2021-18-742d, 2021-18-742c, 2021-18-742b

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

Project Co-Leaders: Ambika Chandra[1], Jack Fry[2], Aaron Patton[3], Dani McFadden[2], Megan Kennelly[2], Dennis Genovesi[1], Meghyn Meeks[1], Ross Braun[2], Mike Richardson[4], Mike Goatley[5], Dan Sandor[5], John Sorochan[6], Kevin Kenworthy[7], and Jamie Bulhman[7]

Affiliation: Texas A&M AgriLife Research-Dallas[1], Kansas State University[2], Purdue University[3], University of Arkansas[4], Virginia Tech[5], University of Tennessee[6], University of Florida[7]

Objectives:

1. **Phase I (year 1): Completed** - 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 at Texas A&M AgriLife-Dallas in 2017/2018, and distributed across three test locations, Olathe, KS, West Lafayette, IN, and Dallas, TX, for evaluations.

2. **Phase II (year 2 and 3): Completed** - Field evaluation in 2018/2019/2020 in the form of non-replicated spaced plant nurseries (SPN) comprised of the newly generated progeny populations in Olathe, West Lafayette, and Dallas. The objective of Phase II was to identify those experimental hybrids with superior cold tolerance as well as excellent turfgrass quality.

3. **Phase III (year 4-6): In-progress** - A set of 65 hybrids (25 – Purdue, 20 - KSU and 20 – TAM AgriLife) were selected in fall of 2020 based on their superior performance in 2018/2019/2020. Entries were propagated into 11 18-cell trays in Dallas during the winter of 2020/2021. In year one, rate of establishment and winter survival were evaluated. In 2022, additional data were collected on many traits that are used to characterize turfgrasses such as rate of establishment, turf quality, spring green up, genetic color, leaf texture. In Olathe, KS, large patch disease was inoculated in the fall of 2022 and will be evaluated in 2023. In Dallas, TX, shade tolerance is being evaluated at 63% shade vs. full sun. Other trait evaluations beginning in 2023 include: hunting billbug, herbicide screening, and ball lie in West Lafayette, IN; divot recovery in Fayetteville, AR; mowing height in Blacksburg, VA; thatch and traffic tolerance in Knoxville, TN; water-deficit in Gainesville, FL; and cold screening and adaptability in Logan, UT. Principal component analysis and heat mapping were performed for establishment, turf quality, and spring greenup in 2022 using GraphPad Prism and JMP software (Figures 1 and 2).

Start Date: 2018
Project Duration: 6 years

Summary Points:

- In 2022, we continued the second year of evaluations for Phase III trials, which included the evaluation of 65 experimental genotypes, 4 elite genotypes, and 5 standards at Dallas, TX, Olathe, KS, West Lafayette, IN and five additional study locations throughout the year.

- Data collection in 2022 included rate of establishment (coverage), turf quality, spring green up, winter injury, leaf texture, genetic color, and fall color. Additional trait evaluations (large patch and hunting billbug tolerance, herbicide screening, ball lie, divot recovery, and cold-, drought-, shade-, and traffic-tolerance) will begin in 2023 after full establishment of the entries.

- Preliminary results suggest lines like TAES 6831-09, 6941-36, 6829-69, along with Emerald and DALZ 1701, showing early promise; however, at-least one to two more years of data is needed to observe better separation between experimental lines and identify superior line(s) across multiple traits and across diverse environments.

Summary Text:

As Phase II concluded, and Phase III began, sixty-five experimental lines were chosen among the three cooperating universities for ongoing evaluation. Progeny were collected from the field in autumn, 2020 and sent to Texas A&M AgriLife for propagation. In spring, 2021, these 65 experimental lines, 4 elite hybrids developed from a separate USGA-sponsored project (DALZ 1701, DALZ 1702, DALZ 1808, and DALZ 1818), and 5 standards were delivered to cooperative state universities. An establishment rating (rated visually on a 0 to 100% scale on which 0 = no establishment, and 100 = complete establishment) was taken at each site during the growing season in 2022 (Table 1; Figure 1). Range of coverage at each location in the summer of 2022 was as follows: KS, 10 to 100%; TN, 55 to 100%; IN, 0 to 83%; VA, 17 to 85%; TX 0 to 100%; FL 18 to 93%; and AR 53 to 100%. On average across seven locations, there were more than a dozen entries with rate of establishment similar to Palisades (78.4%). In Kansas and Indiana, winter injury occurred and decreased establishment rates for fine-textured experimental genotypes (primarily with *Z. pacifica* influence in their pedigrees) following the first winter after planting (Table 4).

When averaged across six locations (AR, FL, KS, TN, TX, and VA), 15 entries had a turfgrass quality ≥ 6. These included two commercial entries (Emerald and Palisades) and two top performing genotypes (DALZ 1701 and DALZ 1808; Figure 2). For spring greenup ratings across four locations, four entries, including DALZ 1702, exhibited an average rating > 7, and several other entries exhibited ratings ≥ 6 (minimum acceptable level). Figure 2 indicates promise for TAES 6831-09, DALZ 1702, and DALZ 1808 across multiple traits (establishment, turfgrass quality, and spring greenup). However, additional data collection is needed to see better separation between lines and to identify superior line(s) across traits and across diverse environments.

For winter injury, leaf texture, genetic color, and fall color trait evaluations, entries were combined and averaged within each TAES grouping. Top performers DALZ 1701, 1702, 1808, and 1818 were reported to have little to no winter injury in Olathe, Kansas (less than 2% winter injury); however, significant winter injury occurred in West Lafayette, IN on top-performing genotypes and commercial entries (Table 4). Experimental lines with *Z. pacifica* influence in their pedigrees exhibited finer leaf texture with greens-type potential (Table 4), darker genetic color, and better fall color (Table 5); however, these lines also experienced most winterkill in Indiana (up to 100%). This presents a challenge in developing cold-tolerant, green-types zoysia specifically for the transition zone.

Figure 1 shows the principal component analysis (PCA) of establishment, turfgrass quality, and spring greenup of genotypes in 2022 to better understand genotype by environment interaction. PCA biplots show entries that performed poorly across environments (away from the locations arrows) as well as entries that performed well across locations. For establishment, all locations except Florida were generally closely correlated and many top-performing genotypes along with standard entries performed well in this cluster of environments. For turfgrass quality, TX and FL (southern locations) were more correlated than KS (transition zone location). States TN and AR are closely correlated where Meyer and Palisades performed well. Emerald and Zeon were good performing lines in VA. DALZ 1701 and 1818 are located intermediate of locations, suggesting they performed well across the 6 locations.

In addition to evaluations under full sun conditions, a study was conducted in Dallas, TX where all experimental lines and commercial checks were planted in June 2021 as a single plugs (in three replications) under moderate artificial shade (63% shade). Figure 3 identifies entries exhibiting above minimum acceptable rating of 5.0 for turfgrass quality and shoot density in 2022. Several lines, including TAES 6831-09, 6941-36, 6829-69, DALZ 1701, Emerald, and Palisades placed in this upper right quadrant. Another one to two years of data is needed to fully understand the response and performance of these promising lines under shade conditions.

Collaborators at Texas A&M AgriLife, Kansas State University, and Purdue University met July 18th through 20th in Dallas, TX for an in-person update on this study (Figure 4). During the meeting, 2021 and early 2022 data at all locations were discussed, along with future plans for 2023 trait evaluations. In the summer of 2023, collaborators will meet in Olathe, KS for the next progress update. Beginning in 2023, initiation of additional trait evaluations will begin on genotypes once fully established. We expect there will be numerous, high quality, fine textured, cold-tolerant genotypes that will have the potential to become improved zoysiagrass cultivars in the transition zone and upper south.

Table 1. Establishment of experimental zoysiagrass genotypes across the states in replicated field trials in 2022, sorted by average establishment percent across seven locations.

		Establishment (%)[a]							
		AR	FL	IN	KS	TN	TX	VA	
Entry I.D.	Lineage[b]	15-Aug	3-Oct	16-Jul	27-Jul	4-May	11-May	3-Jul	Avg.
6844-36	[Zm x Zj] x [(Zj x Zp)/Zj])	98.3	71.7	78.3	96.7	91.7	90.0	58.3	83.6
DALZ 1702	(Zj x Zm)	87.7	85.0	50.0	100.0	98.3	95.0	68.3	83.5
DALZ 1808	(Zj)	93.5	73.3	73.3	100.0	95.0	100.0	45.0	82.9
6844-152	[Zm x Zj] x [(Zj x Zp)/Zj])	94.5	65.0	78.3	96.7	98.3	98.3	46.7	82.5
6940-15	(Zj x Zm)	97.9	73.3	75.0	86.7	100.0	95.0	38.3	80.9
6923-11	(Zj x Zm) x Zj)	87.8	86.7	73.3	73.3	100.0	100.0	43.3	80.6
6844-34	[Zm x Zj] x [(Zj x Zp)/Zj])	83.3	48.3	83.3	96.7	100.0	98.3	51.7	80.2
6924-44	(Zm x Zj) x (Zm x Zj)	97.2	51.7	81.7	90.0	96.7	95.0	41.7	79.1
6831-09	Zm x (Zm x Zj)	99.8	83.3	10.0	100.0	93.3	100.0	63.3	78.5
6844-04	[Zm x Zj] x [(Zj x Zp)/Zj])	98.6	53.3	68.3	96.7	91.7	80.0	60.0	78.4
Palisades	(Zj)	92.2	63.3	53.3	91.7	98.3	100.0	50.0	78.4
6910-172	Zj x (Zj x Zm)	92.4	66.7	45.0	93.3	86.7	98.3	65.0	78.2
6942-22	(Zj x Zp)	99.1	76.7	10.0	93.3	100.0	95.0	73.3	78.2
6830-11	(Zm x Zj) x Zm)	99.3	43.3	73.3	95.0	98.3	83.3	53.3	78.0
6830-56	(Zm x Zj) x Zm)	95.9	66.7	50.0	98.3	95.0	98.3	40.0	77.7
6844-104	[Zm x Zj] x [(Zj x Zp)/Zj])	95.9	53.3	65.0	95.0	85.0	98.3	48.3	77.3
6844-74	[Zm x Zj] x [(Zj x Zp)/Zj])	96.7	61.7	56.7	95.0	85.0	93.3	46.7	76.4
6844-128	[Zm x Zj] x [(Zj x Zp)/Zj])	93.9	55.0	56.7	83.3	85.0	95.0	61.7	75.8
6782-42	[(Zj x Zp)/Zj) x Zp]	85.7[c]	48.3	0	100.0	68.3	80.0	71.7	75.7
6844-141	[Zm x Zj] x [(Zj x Zp)/Zj])	98.5	46.7	68.3	95.0	83.3	96.7	41.7	75.7
6839-08	Zm x [(Zj x Zp)/Zj])	78.3	50.0	41.7	100.0	91.7	98.3	70.0	75.7
6844-190	[Zm x Zj] x [(Zj x Zp)/Zj])	98.6	50.0	68.3	91.7	85.0	90.0	45.0	75.5
6941-36	(Zj x Zm)	99.5	76.7	63.3	73.3	81.7	93.3	40.0	75.4
6844-202	[Zm x Zj] x [(Zj x Zp)/Zj])	99.0	66.7	51.7	95.0	81.7	90.0	40.0	74.9
DALZ 1701	(Zj x Zm)	83.5	76.7	56.7	86.7	88.3	100.0	30.0	74.6
6844-42	[Zm x Zj] x [(Zj x Zp)/Zj])	99.1	46.7	30.0	100.0	93.3	95.0	56.7	74.4
6844-31	[Zm x Zj] x [(Zj x Zp)/Zj])	90.4	46.7	48.3	81.7	88.3	91.7	73.3	74.3
6925-53	(Zm x Zj) x (Zm x Zj)	95.1	35.0	36.7	95.0	91.7	95.0	71.7	74.3
6844-53	[Zm x Zj] x [(Zj x Zp)/Zj])	90.0	58.3	10.0	100.0	86.7	88.3	85.0	74.0
Innovation	(Zm x Zj)	87.2	50.0	66.7	100.0	83.3	71.7	58.3	73.9
6829-20	[(Zj x Zp)/Zj] x Zm)	95.8	85.0	0	90.0	91.7	93.3	60.0	73.7
6844-150	[Zm x Zj] x [(Zj x Zp)/Zj])	99.9	53.3	26.7	98.3	86.7	80.0	70.0	73.6
6829-69	[(Zj x Zp)/Zj] x Zm)	95.9	90.0	0	100.0	76.7	100.0	50.0	73.2
6829-02	[(Zj x Zp)/Zj] x Zm)	95.1	70.0	0	100.0	91.7	93.3	60.0	72.9
6829-36	[(Zj x Zp)/Zj] x Zm)	76.4	56.7	60.0	90.0	95.0	78.3	51.7	72.6
Zeon	(Zm)	90.8	56.7	0	100.0	81.7	93.3	85.0	72.5
6830-02	((Zm x Zj) x Zm)	93.9	80.0	1.7	98.3	90.0	96.7	45.0	72.2
6924-47	(Zm x Zj) x (Zm x Zj)	91.4	33.3	58.3	98.3	76.7	93.3	53.3	72.1
6840-20	Zm x [(Zj x Zp)/Zj)	99.4	71.7	6.7	91.7	96.7	95.0	43.3	72.1
6844-147	[Zm x Zj] x [(Zj x Zp)/Zj])	83.6	51.7	38.3	96.7	90.0	93.3	50.0	71.9
6844-89	[Zm x Zj] x [(Zj x Zp)/Zj])	97.2	65.0	43.3	96.7	81.7	90.0	28.3	71.7
6830-39	(Zm x Zj) x Zm)	70.6	93.3	3.3	96.7	86.7	95.0	55.0	71.5
Emerald	(Zj x Zp)	99.1	73.3	1.7	96.7	88.3	91.7	46.7	71.1
6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]	93.2	41.7	51.7	95.0	83.3	80.0	51.7	70.9
DALZ 1818	(Zp x Zj) x Zj)	90.8	58.3	5.0	100.0	83.3	91.7	63.3	70.3
6782-75	[(Zj x Zp)/Zj) x Zp]	72.6	73.3	0	100.0	84.0	81.7	80.0	70.2
6835-33	(Zm x Zj) x Zm	94.4	81.7	5.0	91.7	95.0	88.3	35.0	70.2
6844-154	[Zm x Zj] x [(Zj x Zp)/Zj])	76.8	61.7	53.3	66.7	96.7	96.7	36.7	69.8
6789-52	[(Zj x Zp)/Zj] x Zp	83.4	90.0	0	93.3	96.7	98.3	26.7	69.8
6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	99.3	73.3	0	96.7	85.0	86.7	43.3	69.2
6933-11	(Zm x Zj) x (Zm x Zj)	97.9	41.7	0	100.0	76.7	93.3	73.3	69.0
6844-91	[Zm x Zj] x [(Zj x Zp)/Zj])	95.3	48.3	78.3	75.0	76.7	71.7	31.7	68.1
6924-66	(Zm x Zj) x (Zm x Zj)	98.7	18.3	20.0	98.3	95.0	88.3	55.0	67.7
Meyer	(Zj)	53.7	35.0	68.3	96.7	88.3	78.3	53.3	67.7
6828-77	Zm x [(Zj x Zp)/Zj]	99.5	60.0	0	91.7	71.7	80.0	66.7	67.1
6829-34	[(Zj x Zp)/Zj] x Zm)	98.1	70.0	1.7	80.0	86.7	93.3	35.0	66.4
6782-79	[(Zj x Zp)/Zj) x Zp]	71.2	65.0	0	100.0	58.7	95.0	71.7	65.9
6789-40	[(Zj x Zp)/Zj] x Zp	93.1	88.3	0	58.3	73.3	91.7	51.7	65.2
6782-104	[(Zj x Zp)/Zj) x Zp]	83.0	71.7	0	98.3	78.3	76.7	46.7	65.0
6828-53	Zm x [(Zj x Zp)/Zj]	94.0	53.3	1.7	88.3	80.0	98.3	38.3	64.8
6828-27	Zm x [(Zj x Zp)/Zj]	86.0	51.7	3.3	100.0	80.0	85.0	43.3	64.2
6787-18	[(Zj x Zp)/Zj] x Zp)	97.0	60.0	0	86.7	71.7	83.3	40.0	62.7
6910-157	Zj x (Zj x Zm)	86.7	50.0	5.0	86.7	73.3	100.0	36.7	62.6
6791-06	[(Zj x Zp)/Zj] x Zp)	93.4	60.0	0	73.3	76.7	98.3	30.0	61.7

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6784-17	[(Zj x Zp)/Zj] x Zp)	79.6	65.0	0	63.3	83.3	95.0	25.0	58.7
6828-56	Zm x [(Zj x Zp)/Zj]	93.5	60.0	0	61.7	76.7	83.3	31.7	58.1
6789-23	[(Zj x Zp)/Zj] x Zp	94.9	75.0	0	33.3	75.0	83.3	23.3	55.0
6785-22	[(Zj x Zp)/Zj] x Zp)	81.9	48.3	0	61.7	70.0	88.3	33.3	54.8
6785-19	[(Zj x Zp)/Zj] x Zp)	96.8	46.7	0	55.0	70.0	73.3	31.7	53.4
6786-02	[(Zj x Zp)/Zj] x Zp)	85.8	71.7	0	31.7	78.3	88.3	16.7	53.2
6783-03	[(Zj x Zp)/Zj] x Zp)	98.5	61.7	0	36.7	73.3	73.3	21.7	52.2
6792-44	[(Zj x Zp)/Zj] x Zp)	60.4	46.7	1.7	56.7	80.0	91.7	25.0	51.7
6782-120	[(Zj x Zp)/Zj) x Zp]	98.8	68.3	0	10.0	76.7	85.0	18.3	51.0
6787-20	[(Zj x Zp)/Zj] x Zp)	97.9	51.7	0	28.3	55.0	.	30.0	43.8

[a] Coverage was rated visually on a 0 to 100% scale on which 0 = no coverage, and 100 = complete coverage.
[b] Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Zmin: *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[c] Means across treatment replications (n = 3).

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Table 2. Turfgrass quality of experimental zoysiagrass genotypes across states in replicated field trials in the summer of 2022, sorted by average turf quality across six locations.

		Turf Quality[a]						
		AR	FL	KS	TN	TX	VA	
Entry I.D.	Lineage[b]	15-Aug	6-Sept	27-Jul	4-May	10-May	3-Jul	Avg.
6829-69	[(Zj x Zp)/Zj] x Zm)	6.7	6.3	6.0	7.0	8.7	6.0	6.8
6831-09	Zm x (Zm x Zj)	8.0	5.3	6.0	8.3	7.7	5.7	6.8
Emerald	(Zj x Zp)	7.0	5.0	6.0	8.0	8.0	6.0	6.7
6829-20	[(Zj x Zp)/Zj] x Zm)	7.3	4.7	5.7	8.0	8.0	5.7	6.6
DALZ 1702	(Zj x Zm)	7.3	5.7	5.7	8.3	6.7	6.0	6.6
6941-36	(Zj x Zm)	7.0	5.3	5.3	7.7	8.7	5.0	6.5
DALZ 1701	(Zj x Zm)	7.0	5.3	4.7	7.7	8.7	5.3	6.5
Zeon	(Zm)	7.3	3.7	6.0	8.3	7.0	6.7	6.5
6940-15	(Zj x Zm)	6.7	4.7	5.0	9.0	8.0	5.0	6.4
6942-22	(Zj x Zp)	6.7	4.7	5.3	8.7	7.3	5.7	6.4
6830-02	((Zm x Zj) x Zm)	6.3	5.0	5.7	8.3	7.7	5.0	6.3
6923-11	(Zj x Zm) x Zj)	7.0	5.3	5.3	8.7	5.3	6.0	6.3
DALZ 1808	(Zj)	7.0	4.3	6.0	7.3	6.7	6.3	6.3
6839-08	Zm x [(Zj x Zp)/Zj])	7.3	3.7	6.0	8.0	6.0	6.3	6.2
6830-39	(Zm x Zj) x Zm)	6.3	5.7	6.0	8.0	5.3	6.0	6.2
6789-52	[(Zj x Zp)/Zj] x Zp	7.0	5.0	5.0	8.0	7.3	5.0	6.2
6782-75	[(Zj x Zp)/Zj) x Zp]	4.0	5.0	7.0	7.3	7.0	6.0	6.1
6910-172	Zj x (Zj x Zm)	7.0	4.3	5.3	7.7	6.3	5.7	6.1
6844-31	[Zm x Zj] x [(Zj x Zp)/Zj])	6.3	3.7	6.3	7.7	6.0	6.3	6.1
6844-34	[Zm x Zj] x [(Zj x Zp)/Zj])	6.7	3.3	6.0	8.3	6.3	6.0	6.1
6829-02	[(Zj x Zp)/Zj] x Zm)	5.7	5.0	6.3	7.7	7.0	5.0	6.1
6844-104	[Zm x Zj] x [(Zj x Zp)/Zj])	6.7	4.0	5.7	8.0	6.7	5.7	6.1
6844-36	[Zm x Zj] x [(Zj x Zp)/Zj])	6.3	4.0	6.0	7.7	6.3	6.3	6.1
6840-20	Zm x [(Zj x Zp)/Zj)	6.3	4.7	5.3	8.3	6.7	5.3	6.1
6789-40	[(Zj x Zp)/Zj] x Zp	6.0	5.7	4.7	7.0	7.3	5.3	6.0
6844-147	[Zm x Zj] x [(Zj x Zp)/Zj])	7.3	3.3	5.7	7.7	5.7	6.0	6.0
6829-36	[(Zj x Zp)/Zj] x Zm)	6.7	4.3	5.7	7.7	5.7	5.7	6.0
DALZ 1818	(Zp x Zj) x Zj)	6.7	3.7	6.3	7.0	5.7	6.3	6.0
Palisades	(Zj)	7.0	3.7	5.7	8.0	6.7	5.0	6.0
6829-34	[(Zj x Zp)/Zj] x Zm)	5.3	5.0	4.7	7.3	8.3	4.7	5.9
6844-202	[Zm x Zj] x [(Zj x Zp)/Zj])	4.3	4.7	6.0	7.3	7.3	5.7	5.9
6784-17	[(Zj x Zp)/Zj] x Zp)	6.0	4.0	4.7	7.0	7.3	5.7	5.8
6910-157	Zj x (Zj x Zm)	7.0	2.7	5.3	7.0	7.7	5.3	5.8
6844-154	[Zm x Zj] x [(Zj x Zp)/Zj])	6.3	4.3	4.7	7.7	6.7	5.3	5.8
6782-104	[(Zj x Zp)/Zj) x Zp]	4.7	5.0	6.3	7.0	6.7	4.7	5.7
6787-18	[(Zj x Zp)/Zj] x Zp)	5.3	4.0	5.7	6.3	6.3	6.3	5.7
6844-152	[Zm x Zj] x [(Zj x Zp)/Zj])	8.0	3.0	5.7	8.3	4.3	4.7	5.7
6925-53	(Zm x Zj) x (Zm x Zj)	6.0	2.7	5.3	7.7	6.7	6.0	5.7
6844-150	[Zm x Zj] x [(Zj x Zp)/Zj])	5.3	3.7	7.0	7.3	5.0	5.7	5.7
6844-74	[Zm x Zj] x [(Zj x Zp)/Zj])	4.7	4.0	6.3	7.3	6.7	5.3	5.7
6844-42	[Zm x Zj] x [(Zj x Zp)/Zj])	7.0	3.3	6.0	7.7	5.0	5.3	5.7
6782-79	[(Zj x Zp)/Zj) x Zp]	4.7	3.7	6.3	7.0	6.3	5.7	5.6
6830-56	(Zm x Zj) x Zm)	6.7	4.3	5.3	7.7	4.7	4.7	5.6
6844-141	[Zm x Zj] x [(Zj x Zp)/Zj])	6.3	3.0	6.0	8.0	5.3	4.7	5.6
6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	5.3	5.0	6.3	7.0	4.7	5.0	5.6
6844-53	[Zm x Zj] x [(Zj x Zp)/Zj])	5.0	3.7	6.0	7.7	5.3	6.0	5.6
6835-33	(Zm x Zj) x Zm	6.7	5.0	5.0	7.7	4.0	5.0	5.6
6785-22	[(Zj x Zp)/Zj] x Zp)	5.0	3.7	5.7	7.0	6.3	5.3	5.5
6924-44	(Zm x Zj) x (Zm x Zj)	6.7	2.3	5.7	8.3	4.7	5.0	5.5
6844-190	[Zm x Zj] x [(Zj x Zp)/Zj])	5.0	3.0	5.0	7.3	6.3	5.7	5.4
6933-11	(Zm x Zj) x (Zm x Zj)	4.3	3.0	7.3	7.3	4.3	6.0	5.4
6844-04	[Zm x Zj] x [(Zj x Zp)/Zj])	4.0	4.3	5.3	8.0	4.7	6.3	5.4
6785-19	[(Zj x Zp)/Zj] x Zp)	4.7	3.3	5.0	6.7	6.3	5.7	5.3
6786-02	[(Zj x Zp)/Zj] x Zp)	4.7	4.0	4.7	7.3	7.3	4.0	5.3
6792-44	[(Zj x Zp)/Zj] x Zp)	5.7	3.3	5.0	7.3	7.0	3.7	5.3
6924-47	(Zm x Zj) x (Zm x Zj)	4.7	2.3	6.0	7.7	5.0	6.0	5.3
Meyer	(Zj)	7.0	3.0	6.0	7.0	3.3	5.7	5.3
6782-42	[(Zj x Zp)/Zj) x Zp]	3.0	3.3	6.7[c]	6.7	5.7	5.7	5.2
6782-120	[(Zj x Zp)/Zj) x Zp]	6.0	3.3	3.0	7.3	7.0	4.7	5.2
6783-03	[(Zj x Zp)/Zj] x Zp)	5.3	4.0	4.3	6.7	6.3	4.3	5.2
6789-23	[(Zj x Zp)/Zj] x Zp	4.7	4.3	4.0	7.3	6.0	4.7	5.2
6791-06	[(Zj x Zp)/Zj] x Zp)	3.7	3.7	4.7	7.3	7.0	5.0	5.2
6844-91	[Zm x Zj] x [(Zj x Zp)/Zj])	5.7	4.0	4.7	7.3	4.7	4.7	5.2
6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]	6.0	3.3	5.0	7.3	4.0	5.3	5.2

6844-89	[Zm x Zj] x [(Zj x Zp)/Zj])	6.3	4.0	6.0	7.7	2.7	4.7	5.2
Innovation	(Zm x Zj)	6.7	2.3	6.0	7.0	4.0	5.0	5.2
6844-128	[Zm x Zj] x [(Zj x Zp)/Zj])	6.0	4.3	4.3	7.3	3.3	5.3	5.1
6828-27	Zm x [(Zj x Zp)/Zj]	4.3	3.3	7.0	7.0	4.0	5.0	5.1
6828-53	Zm x [(Zj x Zp)/Zj]	6.0	3.3	5.0	7.0	3.7	4.7	5.0
6828-56	Zm x [(Zj x Zp)/Zj]	4.0	4.3	5.3	7.7	4.7	4.0	5.0
6828-77	Zm x [(Zj x Zp)/Zj]	4.0	3.7	6.0	7.3	2.7	6.0	5.0
6787-20	[(Zj x Zp)/Zj] x Zp)	5.0	4.0	3.7	6.3	.	5.3	4.9
6924-66	(Zm x Zj) x (Zm x Zj)	5.0	2.0	5.7	7.3	3.0	5.7	4.8
6830-11	(Zm x Zj) x Zm)	5.0	3.0	5.0	7.3	2.3	5.3	4.7

[a] Quality was rated visually on a 1 to 9 scale on which 1 = dead; 6 = minimally acceptable; 9 = optimum color, density, uniformity, and texture.
[b] Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Zmin: *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[c] Means across treatment replications (n = 3).

Table 3. Spring greenup of experimental zoysiagrass genotypes across states in replicated field trains in the late spring of 2022, sorted by average spring greenup across four locations.

		Spring greenup[a]				
		KS	TN	VA	TX	
Entry I.D.	Lineage[b]	5-May	4-May	18-May	1-April	Avg.
6782-75	[(Zj x Zp)/Zj) x Zp]	6.7	8.0	7.0	8.0	7.4
6782-42	[(Zj x Zp)/Zj) x Zp]	6.3[c]	9.0	6.7	6.7	7.2
6925-53	(Zm x Zj) x (Zm x Zj)	6.0	7.7	7.3	7.7	7.2
DALZ 1702	(Zj x Zm)	5.0	8.3	7.7	7.3	7.1
DALZ 1808	(Zj)	5.3	7.7	6.7	8.0	6.9
Palisades	(Zj)	5.7	7.7	7.0	6.7	6.8
6829-02	[(Zj x Zp)/Zj] x Zm)	5.0	8.3	6.3	7.3	6.7
6831-09	Zm x (Zm x Zj)	5.3	8.0	6.3	7.3	6.7
6829-20	[(Zj x Zp)/Zj] x Zm)	5.0	8.7	5.7	7.3	6.7
Zeon	(Zm)	3.7	8.7	7.7	6.7	6.7
Emerald	(Zj x Zp)	4.7	8.7	5.3	8.0	6.7
DALZ 1818	(Zp x Zj) x Zj)	4.3	7.7	7.0	7.3	6.6
6829-69	[(Zj x Zp)/Zj] x Zm)	4.3	8.0	5.3	8.3	6.5
6789-52	[(Zj x Zp)/Zj] x Zp	5.0	8.7	4.7	7.0	6.4
6787-18	[(Zj x Zp)/Zj] x Zp)	3.3	8.7	6.0	7.0	6.3
6791-06	[(Zj x Zp)/Zj] x Zp)	3.0	9.0	5.3	8.0	6.3
6941-36	(Zj x Zm)	3.7	7.7	5.3	8.3	6.3
6844-150	[Zm x Zj] x [(Zj x Zp)/Zj])	5.7	8.7	6.3	4.3	6.3
6830-02	((Zm x Zj) x Zm)	4.3	8.0	6.3	6.7	6.3
DALZ 1701	(Zj x Zm)	4.0	8.3	5.0	8.0	6.3
Innovation	(Zm x Zj)	6.0	8.0	6.3	4.7	6.3
6782-104	[(Zj x Zp)/Zj) x Zp]	4.3	8.0	5.0	7.3	6.2
6844-53	[Zm x Zj] x [(Zj x Zp)/Zj])	5.3	8.7	7.3	3.3	6.2
6844-36	[Zm x Zj] x [(Zj x Zp)/Zj])	5.3	8.0	6.3	5.3	6.2
6844-202	[Zm x Zj] x [(Zj x Zp)/Zj])	4.0	8.0	6.7	6.0	6.2
6782-79	[(Zj x Zp)/Zj) x Zp]	5.3	8.0	6.0	5.0	6.1
6835-33	(Zm x Zj) x Zm	5.0	8.0	5.7	5.7	6.1
6789-40	[(Zj x Zp)/Zj] x Zp	2.3	8.0	6.0	7.7	6.0
6844-152	[Zm x Zj] x [(Zj x Zp)/Zj])	4.3	8.0	4.7	7.0	6.0
6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]	5.7	8.7	5.7	3.7	6.0
6840-20	Zm x [(Zj x Zp)/Zj)	4.7	8.7	6.7	4.0	6.0
6933-11	(Zm x Zj) x (Zm x Zj)	6.0	8.3	6.0	3.7	6.0
6839-08	Zm x [(Zj x Zp)/Zj])	4.0	7.7	7.0	5.0	5.9
6844-104	[Zm x Zj] x [(Zj x Zp)/Zj])	5.3	8.0	6.3	4.0	5.9
6844-04	[Zm x Zj] x [(Zj x Zp)/Zj])	4.7	8.7	6.3	3.7	5.9
6923-11	(Zj x Zm) x Zj)	3.3	7.0	6.3	7.0	5.9
6910-157	Zj x (Zj x Zm)	2.7	8.0	5.3	7.0	5.8
6844-31	[Zm x Zj] x [(Zj x Zp)/Zj])	4.3	8.3	5.7	4.7	5.8
6942-22	(Zj x Zp)	4.0	8.0	5.0	6.3	5.8
6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	5.0	8.3	6.0	3.7	5.8
6828-77	Zm x [(Zj x Zp)/Zj]	4.7	8.7	6.7	3.0	5.8
6844-89	[Zm x Zj] x [(Zj x Zp)/Zj])	5.3	7.7	5.7	4.3	5.8
6785-19	[(Zj x Zp)/Zj] x Zp)	2.3	8.7	4.7	7.0	5.7
6844-154	[Zm x Zj] x [(Zj x Zp)/Zj])	3.3	7.3	4.7	7.3	5.7
6844-34	[Zm x Zj] x [(Zj x Zp)/Zj])	4.7	7.0	5.7	5.3	5.7
Meyer	(Zj)	4.7	7.7	7.0	3.3	5.7
6784-17	[(Zj x Zp)/Zj] x Zp)	2.7	8.0	4.3	7.3	5.6
6844-190	[Zm x Zj] x [(Zj x Zp)/Zj])	3.7	8.3	6.7	3.7	5.6
6786-02	[(Zj x Zp)/Zj] x Zp)	2.0	8.0	4.0	8.0	5.5
6829-34	[(Zj x Zp)/Zj] x Zm)	3.7	8.7	5.0	4.7	5.5
6828-27	Zm x [(Zj x Zp)/Zj]	4.0	8.3	5.0	4.7	5.5
6830-39	(Zm x Zj) x Zm)	4.3	7.7	6.0	4.0	5.5
6844-42	[Zm x Zj] x [(Zj x Zp)/Zj])	5.0	8.0	6.3	2.7	5.5
6783-03	[(Zj x Zp)/Zj] x Zp)	2.3	8.7	4.0	6.7	5.4
6910-172	Zj x (Zj x Zm)	4.3	8.0	5.3	4.0	5.4
6829-36	[(Zj x Zp)/Zj] x Zm)	4.0	7.7	7.0	3.0	5.4
6844-141	[Zm x Zj] x [(Zj x Zp)/Zj])	3.7	8.7	4.7	4.3	5.4
6924-47	(Zm x Zj) x (Zm x Zj)	4.0	7.7	5.3	4.7	5.4
6924-66	(Zm x Zj) x (Zm x Zj)	4.0	8.3	4.7	4.7	5.4
6785-22	[(Zj x Zp)/Zj] x Zp)	2.3	8.3	5.7	4.7	5.3
6792-44	[(Zj x Zp)/Zj] x Zp)	2.7	8.3	4.0	5.7	5.2
6844-91	[Zm x Zj] x [(Zj x Zp)/Zj])	3.7	8.0	4.7	4.3	5.2
6940-15	(Zj x Zm)	3.3	8.0	4.3	5.0	5.2
6828-53	Zm x [(Zj x Zp)/Zj]	3.3	8.0	5.3	4.3	5.2

6782-120	[(Zj x Zp)/Zj) x Zp]	1.7	9.0	2.3	7.3	5.1
6924-44	(Zm x Zj) x (Zm x Zj)	3.3	8.3	6.0	2.7	5.1
6844-74	[Zm x Zj] x [(Zj x Zp)/Zj])	3.7	8.7	4.0	4.0	5.1
6844-147	[Zm x Zj] x [(Zj x Zp)/Zj])	4.3	8.0	5.7	2.0	5.0
6828-56	Zm x [(Zj x Zp)/Zj]	3.3	8.0	4.7	4.0	5.0
6787-20	[(Zj x Zp)/Zj] x Zp)	2.0	8.0	4.7	.	4.9
6830-56	(Zm x Zj) x Zm)	2.7	7.7	3.7	5.3	4.9
6844-128	[Zm x Zj] x [(Zj x Zp)/Zj])	3.7	8.0	5.0	3.0	4.9
6830-11	(Zm x Zj) x Zm)	3.7	7.3	5.3	3.3	4.9
6789-23	[(Zj x Zp)/Zj] x Zp	2.0	8.0	3.3	5.3	4.7

[a] Spring greenup was rated visually on a 1 to 9 scale on which 1 = brown/straw/dead; 9 = dark green.
[b] Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Zmin: *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[c] Means across treatment replications (n = 3).

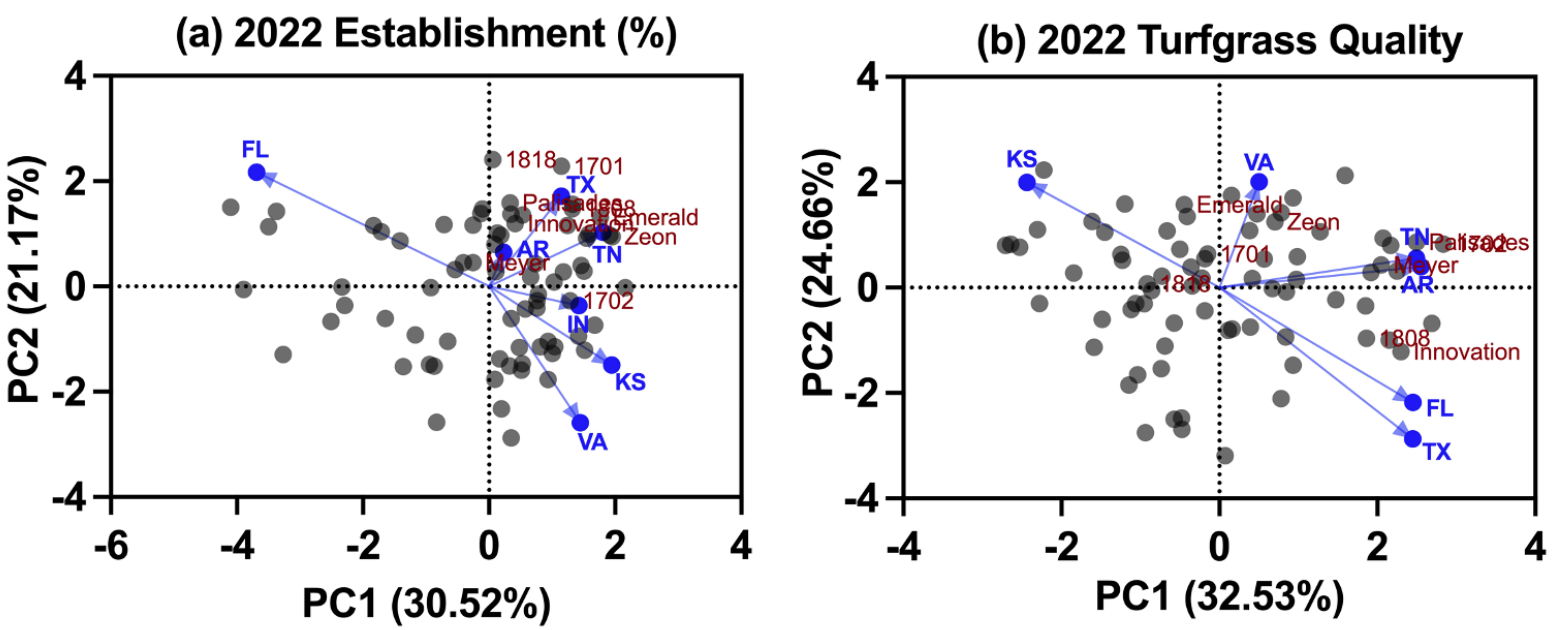

Fig. 1. Loadings (locations) and principal component plots from the analysis of turfgrass establishment across ≥ 7 locations (a), turfgrass quality across ≥ 6 locations (b), and spring greenup across ≥ 4 locations in 2022. The direction of the locations shows how much weight they have on each of the principal components (PC).

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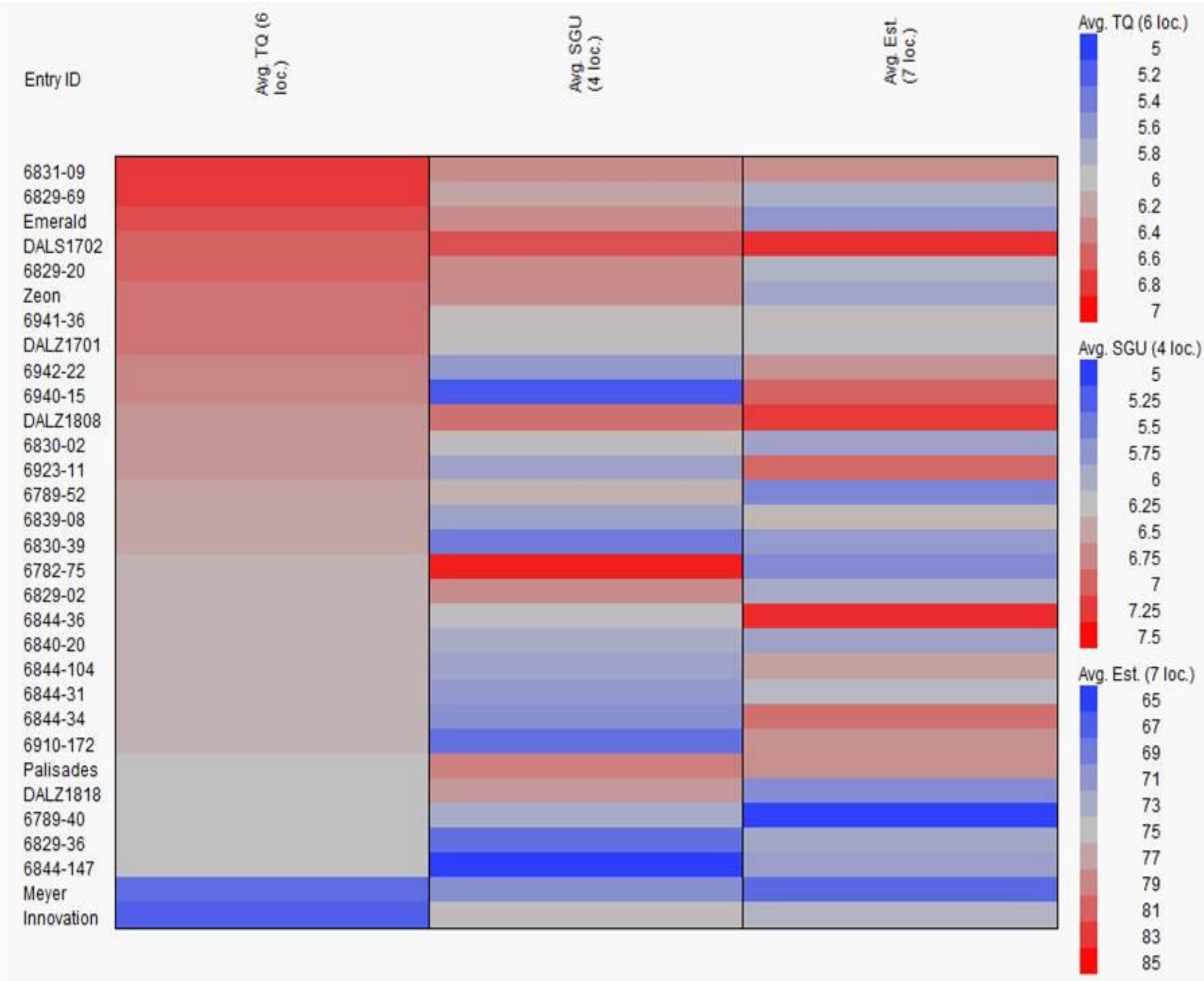

Fig. 2. "Heat map" of genotypes reflecting turfgrass quality, spring greenup, and establishment (%) across locations in 2022. Only entries with an average turfgrass quality of ≥ 6 plus commercial entries are presented.

Table 4. Winter injury and leaf texture of experimental families across states in replicated field trials in 2022.

		Winter injury[a]			Leaf texture[b]						
TAES Grouping	Lineage[c]	KS	IN	Avg.	AR	IN	KS	TN	TX	VA	Avg.
6787	[(Zj x Zp)/Zj] x Zp)	16.7	100	58.4	7.3	-	8.2	9.0	8.7	7.3	8.1
6791	[(Zj x Zp)/Zj] x Zp)	5	100	52.5	7.3	-	8.3	9.0	8.0	7.7	8.1
6783	[(Zj x Zp)/Zj] x Zp)	50	100	75.0	6.7	-	8.0	9.0	8.3	8.0	8.0
6786	[(Zj x Zp)/Zj] x Zp)	50	100	75.0	7.0	-	8.0	9.0	8.3	7.7	8.0
6785	[(Zj x Zp)/Zj] x Zp)	5.3	100	52.7	7.0	-	8.2	8.8	8.0	7.5	7.9
6784	[(Zj x Zp)/Zj] x Zp)	8.3	100	54.2	7.0	-	8.0	8.3	8.0	7.0	7.7
6789	[(Zj x Zp)/Zj] x Zp	14.7	100	57.4	7.6	-	7.2	8.9	8.1	6.8	7.7
6792	[(Zj x Zp)/Zj] x Zp)	11.7	100	55.9	6.7	-	8.0	9.0	8.0	6.7	7.7
Emerald	(Zj x Zp)	0	98.3	49.2	7.0	-	7.0	8.3	7.7	7.0	7.4
6782	[(Zj x Zp)/Zj) x Zp]	12.3[d]	100	56.2	6.7	-	5.9	8.9	8.2	5.7	7.1
6836	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	0	100	50.0	7.3	-	5.7	8.3	7.7	6.0	7.0
6839	Zm x [(Zj x Zp)/Zj])	0	88.3	44.2	6.0	7.7	7.0	7.3	7.3	6.7	7.0
6840	Zm x [(Zj x Zp)/Zj)	0	96.7	48.4	6.3	-	6.7	8.0	7.0	6.0	6.8
6828	Zm x [(Zj x Zp)/Zj]	3.3	100	51.7	6.9	-	5.7	7.8	7.3	5.8	6.7
6925	(Zm x Zj) x (Zm x Zj)	0	86.7	43.4	6.7	7.0	6.0	7.7	7.0	5.7	6.7
6942	(Zj x Zp)	0	98.3	49.2	6.3	-	6.3	7.7	6.7	6.7	6.7
Innovation	(Zm x Zj)	0	36.7	18.4	6.7	6.7	6.3	7.7	7.0	5.7	6.7
6923	(Zj x Zm) x Zj)	13.3	40	26.7	6.7	6.7	6.7	7.0	6.3	6.0	6.6
6933	(Zm x Zj) x (Zm x Zj)	0	100	50.0	6.3	-	6.3	8.0	6.3	6.0	6.6
6835	(Zm x Zj) x Zm	0	100	50.0	6.7	-	5.3	8.0	7.3	5.0	6.5
6924	(Zm x Zj) x (Zm x Zj)	2.2	42.8	22.5	6.4	5.3	7.0	7.3	6.2	6.8	6.5
6844	[Zm x Zj] x [(Zj x Zp)/Zj])	0.6	42.1	21.4	6.5	5.4	6.5	7.4	6.4	6.0	6.4
6941	(Zj x Zm)	0	61.7	30.9	6.3	5.0	8.0	6.3	5.3	7.7	6.4
Meyer	(Zj)	0	11.7	5.9	6.7	7.7	5.3	6.7	5.7	6.3	6.4
6919	[(Zm x Zp)/Zj] x [Zm x Zj]	0	18.3	9.2	6.3	6.0	6.3	7.0	6.3	6.0	6.3
DALZ 1701	(Zj x Zm)	1.7	70	35.9	6.7	5.7	6.0	7.3	6.0	5.7	6.2
DALZ 1818	(Zp x Zj) x Zj	0	100	50.0	6.0	-	6.0	7.0	6.0	6.0	6.2
6830	((Zm x Zj) x Zm)	0.8	67.1	34.0	6.8	2.7	6.2	7.8	7.3	5.8	6.1
6910	Zj x (Zj x Zm)	0.8	93.3	47.1	6.0	2.5	8.2	7.3	5.5	7.2	6.1
6940	(Zj x Zm)	4	53.3	28.7	6.3	5.3	6.3	6.7	6.0	6.0	6.1
6831	Zm x (Zm x Zj)	0	100	50.0	6.3	2.3	6.3	8.0	7.0	6.3	6.0
6829	[(Zj x Zp)/Zj] x Zm)	0.6	84.6	42.6	6.8	1.7	6.1	7.8	6.9	6.0	5.9
Zeon	(Zm)	0	100	50.0	7.0	3.3	-	7.7	7.7	3.7	5.9
DALZ 1702	(Zj x Zm)	0	90	45.0	6.3	6.0	4.7	7.3	5.7	4.7	5.8
DALZ 1808	(Zj)	0	56.7	28.4	6.7	5.3	4.3	6.3	6.0	5.3	5.7
Palisades	(Zj)	0.7	86.7	43.7	6.3	5.3	4.0	6.0	5.0	5.3	5.3

[a] Winter injury was rated visually on a 0 to 100% scale on which 0% = no winter injury; 100% = complete winter injury.
[b] Leaf texture was rated visually on a 1 to 9 scale on which 1 = coarse; 9 = fine.
[c] Zj: *Zoysia japonica*; Zm: *Zoysia* matrella; Zp: *Zoysia pacifica*; Zmin: *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[d] Means across treatment replications (n = 3).

Table 5. Genetic color and fall color retention of experimental zoysiagrass families across states in replicated field trials in 2022.

TAES Grouping	Lineage[c]	Genetic color[a]					Fall color[b]					
		AR	KS	VA	TX	Avg.	AR	KS	TX	UT	VA	Avg.
6785	[(Zj x Zp)/Zj] x Zp)	7.5	8.0	7.2	7.7	7.6	7.0	5.2	8.2	-	6.3	6.7
6786	[(Zj x Zp)/Zj] x Zp)	7.7	7.7	7.7	7.3	7.6	6.0	4.7	8.0	-	6.3	6.3
6783	[(Zj x Zp)/Zj] x Zp)	7	8.0	7.0	7.7	7.4	5.7	6.3	7.7	-	5.3	6.3
6784	[(Zj x Zp)/Zj] x Zp)	7.3	7.7	6.7	7.7	7.4	6.7	5.7	8.0	-	6.3	6.7
6787	[(Zj x Zp)/Zj] x Zp)	7.7	7.5	7.0	7.3	7.4	6.8	5.8	7.7	-	6.0	6.6
6791	[(Zj x Zp)/Zj] x Zp)	7.7	7.7	7.0	7.0	7.4	7.0	6.3	8.3	-	5.3	6.7
6792	[(Zj x Zp)/Zj] x Zp)	6.7	7.7	7.0	7.7	7.3	6.0	6.3	8.3	-	6.0	6.7
6789	[(Zj x Zp)/Zj] x Zp	7.4	7.3	6.6	7.6	7.2	5.9	3.9	8.1	-	5.4	5.8
6836	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	6.7	7.3	7.3	7.0	7.1	5.3	2.0	6.3	6.3	6.3	5.2
6933	(Zm x Zj) x (Zm x Zj)	7.7	6.3	6.7	7.7	7.1	6.3	2.0	4.3	-	5.3	4.5
DALZ 1701	(Zj x Zm)	7.0	6.7	7.0	7.3	7.0	4.7	2.0	7.7	8.7	4.7	5.6
6782	[(Zj x Zp)/Zj) x Zp]	7.1[d]	6.3	6.4	7.6	6.9	6.2	3.0	7.9	-	4.8	5.5
6840	Zm x [(Zj x Zp)/Zj)	6.0	7.3	7.0	7.3	6.9	5.0	2.3	7.0	6.0	5.7	5.2
6941	(Zj x Zm)	6.7	8.0	6.0	6.7	6.9	4.7	5.0	6.3	-	6.3	5.6
6828	Zm x [(Zj x Zp)/Zj]	7.6	7.1	6.7	5.7	6.8	5.4	2.0	6.0	6.7	4.9	5.0
6923	(Zj x Zm) x Zj)	6.0	7.3	8.0	5.7	6.8	3.3	3.7	5.7	-	5.3	4.5
Palisades	(Zj)	7.0	6.7	7.3	6.3	6.8	3.0	2.7	7.0	-	5.0	4.4
6844	[Zm x Zj] x [(Zj x Zp)/Zj])	6.9	6.4	6.5	6.9	6.7	3.7	2.7	6.0	6.0	5.3	4.7
6925	(Zm x Zj) x (Zm x Zj)	6.7	7.3	6.7	6.0	6.7	4.0	1.7	6.0	-	5.3	4.3
Emerald	(Zj x Zp)	7.3	6.3	6.3	7.0	6.7	4.3	2.0	8.0	-	4.7	4.8
6829	[(Zj x Zp)/Zj] x Zm)	6.8	6.5	6.3	6.7	6.6	4.9	2.9	7.0	5.8	5.2	5.2
6910	Zj x (Zj x Zm)	6.5	7.7	6.7	5.5	6.6	4.5	4.0	7.5	-	6.3	5.6
6924	(Zm x Zj) x (Zm x Zj)	6.6	6.9	6.6	6.4	6.6	3.3	2.4	5.8	6.0	4.8	4.5
6940	(Zj x Zm)	6.3	7.0	6.7	6.0	6.5	3.7	2.7	6.7	7.3	5.3	5.1
Meyer	(Zj)	6.7	6.0	6.7	6.7	6.5	3.7	3.3	5.0	7.3	5.0	4.9
6831	Zm x (Zm x Zj)	6.7	5.7	6.7	6.3	6.4	5.0	1.7	8.3	-	5.3	5.1
6839	Zm x [(Zj x Zp)/Zj])	6.7	5.7	6.3	6.7	6.4	3.3	2.0	6.7	-	5.3	4.3
6830	((Zm x Zj) x Zm)	6.8	6.4	6.1	5.8	6.3	4.3	2.7	6.3	6.5	5.5	5.1
6942	(Zj x Zp)	6.7	6.7	6.0	5.7	6.3	4.7	2.0	6.7	-	6.0	4.9
Zeon	(Zm)	6.7	6.0	6.0	6.3	6.3	4.3	2.0	7.0	-	4.0	4.3
6835	(Zm x Zj) x Zm	6.0	6.0	7.0	5.7	6.2	5.0	2.3	6.7	6.3	6.0	5.3
6919	[(Zm x Zp)/Zj] x [Zm x Zj]	6.7	6.3	5.3	6.3	6.2	3.0	2.3	7.0	-	4.7	4.3
DALZ 1818	(Zp x Zj) x Zj	6.7	5.7	5.7	6.3	6.1	4.3	3.0	6.3	-	5.3	4.7
Innovation	(Zm x Zj)	6.0	5.7	5.7	6.7	6.0	2.3	1.0	5.3	5.7	5.3	3.9
DALZ 1702	(Zj x Zm)	6.7	5.0	5.3	6.7	5.9	5.0	2.3	7.0	7.7	4.7	5.3
DALZ 1808	(Zj)	6.3	5.3	6.0	5.7	5.8	4.0	2.7	6.0	7.7	5.7	5.2

[a] Genetic color was rated visually on a 1 to 9 scale on which 1 = brown/straw/dead; 9 = dark green.
[b] Fall color retention was rated visually on a 1 to 9 scale on which 1 = brown/straw/dead; 9 = dark green.
[c] Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Zmin: *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[d] Means across treatment replications (n = 3).

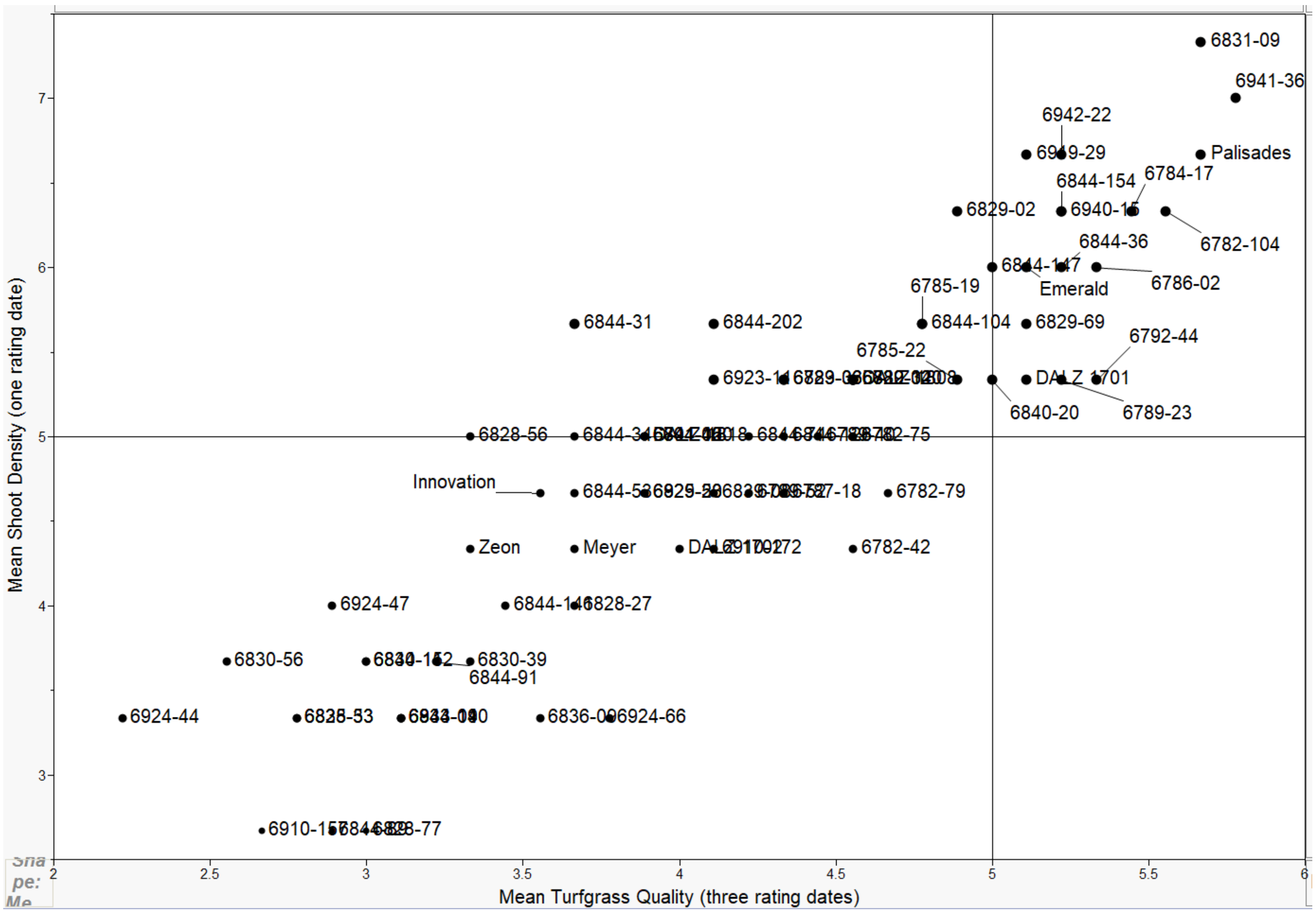

Figure 3. Mean turfgrass quality and shoot density of entries under moderate shade (63%) in Dallas, TX. Entries in the upper right quadrant exhibit above minimum acceptable ratings for both traits.

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Fig. 4. Collaborators from Texas A&M AgriLife, Kansas State University, and Purdue University examining genotypes during the progress update meeting held in Dallas, TX in July of 2022.

USGA ID# 2021-16-740

Title: Developing Stress Tolerant Zoysiagrasses as a Low-Input Turf for Golf Course Roughs

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

Collaborators: Evergreen Turf (Chandler, AZ), American Sod Farms (Escondido, CA), Pfau Indiana University Golf Course (Bloomington, IN), Lonnie Poole Golf Course (Raleigh, NC), Torrey Pines Golf Course (San Diego, CA), East Lake Golf Club (Atlanta, GA) and TPC Scottsdale (Phoenix, AZ).

Objectives: 1) Expand evaluation of zoysiagrass genotypes --previously selected for their drought tolerance and aggressiveness-- to larger areas to fully assess their performance under golf conditions, 2) develop materials with improved large patch tolerance through the identification of molecular markers associated with the trait, and 3) evaluate the performance of new experimental zoysiagrasses in warm-arid, warm-humid, transition zone climates.

Start date: 01/01/2021
Project duration: 5 years (01/01/2021-12/31/2025)
Total funding: $125,000

Summary points

- Nine experimental zoysiagrass genotypes that have exhibited excellent drought resistance and turf quality when managed with minimal inputs at multiple locations have been propagated to larger field plots for future distribution to three golf courses in IN, NC, and GA for demonstration and feedback from golf course superintendents and golfers.
- Evaluation of the Meyer x PI 231146 mapping population for large patch resistance has identified excellent segregation of disease response among individuals, which should facilitate our efforts to identify genomic regions controlling resistance.
- Preliminary evaluation new zoysiagrass hybrids has identified lines that appear very promising in terms of speed of establishment and stress tolerance.

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 germplasm available that has excellent stress and pest tolerance and fast establishment when managed with no inputs, but these materials are often discarded because current breeding efforts are more focused on “fairway” and “putting green” zoysiagrass. Our research team has evaluated zoysiagrasses for their performance and playability in multiple climates (warm-arid, warm-humid, transition zone) as a potential turfgrass for golf course roughs and other low-maintenance areas. Entries with superior drought tolerance, aggressiveness and color retention in combination with acceptable ball lie have been identified as part of those efforts.

For objective 1, nine experimental zoysiagrass genotypes were selected to be propagated alongside cultivar checks common to each state (i.e. Meyer, Zenith, Jamur, or Innovation) to be assessed in future on-site trials due to their drought resistance and aggressiveness observed at multiple locations (Braun et al., 2021). Therefore, 10 by 10 ft plots were established by either sodding or plugging in 2021 at the W.H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, IN; Lake Wheeler Turfgrass Field Lab, Raleigh NC; and Coastal Plain Experiment Station, Tifton, GA. As plot were not fully established this year, expansion of these blocks into golf course roughs had to be postponed. In summer 2023 at each site, zoysiagrass sod will be harvested from each plot and transplanted on a golf course rough area within each state (IN, NC, and GA). Following establishment, demonstration plots will be maintained with minimal inputs (fertilization, irrigation, or pest control) the following years, and mown as needed by the superintendent similar to their other primary rough areas. Golf professionals, members, and golf course superintendents at each course managing the demonstration plots will be interviewed once per year to receive feedback on turf quality, ball lie (acceptable and optimal), and other potential turf golfing or turf characteristics. In addition, ball lie will be measured for each entry using the method developed by Richardson et al. (2010).

For objective 2, 229 lines were developed from crosses of large patch (LP) susceptible Meyer by LP-resistant PI 231146. Plugs of all individuals were grown in Styrofoam and arranged in a Randomized Complete Block Design with two replications in a walk-in growth chamber. Large patch inoculations were performed by placing 8-10 *R. solani* infected rye grains in the crown region of each plant. Plant were kept at 20/18 C with >75% relative humidity to promote fungal growth. Disease severity was evaluated every three days for 33 days visually using the Horsfall-Barratt scale and also through digital imaging. Six runs of inoculations were completed. Wide segregation for LP response was observed across runs with some individuals showing better levels of resistance than PI 231146 (Figure 1). Results were very consistent across runs, especially for the best and worst performers (Figure 2). Additionally, differences in disease progression were observed between resistant and susceptible individuals, where there might be a 3-day delay in symptoms appearing and a 5-day delay in reaching the 10% threshold (Figure 3). For the genotyping component, Genotype-by-Sequencing (GBS) and Single Nucleotide Polymorphism (SNP) calling has been completed. Linkage mapping and QTL analysis are underway. We expect to have final results from this work by late spring.

Additionally, this population was planted at the W.H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, IN; and the Lake Wheeler Turfgrass Field Lab, Raleigh NC to evaluate their performance under field conditions. Data was collected on percent green cover (fall 2021 and 2022), winterkill, fall color, turf quality, texture and density. Significant variation was observed among genotypes for all traits. A large number of genotypes with superior percent green cover over Meyer and PI 231146 were observed (Figure 4). For winterkill, Meyer was the best genotype in Indiana, but not in North Carolina where several breeding lines performed better.

For objective 3, new crosses among zoysiagrass germplasm with excellent aggressiveness and persistence were performed in 2020. The resulting progeny lines were established in unreplicated field nurseries across North Carolina and Georgia in spring 2021. Plots were evaluated for speed of establishment, turf quality, and drought tolerance during 2021-2022. A few of these hybrids appear very promising in terms of speed of establishment and stress tolerance. Evaluation of these lines will continue through 2023 at which point, best performing lines will be advanced to replicated trials under low input conditions.

References

Richardson, M.D., D.E. Karcher, A.J. Patton, and J.H. McCalla, Jr. 2010. Measurement of golf ball lie in various turfgrasses using digital image analysis. Crop Sci. 50:730–736. doi:10.2135/cropsci2009.04.0233

Braun, R.C., S. Milla-Lewis, E. Carbajal, B.M. Schwartz, and A.J. Patton. 2021. Performance and playability of experimental low-input coarse-textured zoysiagrass in multiple climates. Grass Research, 1:10 1–12. https://doi.org/10.48130/GR-2021-0010

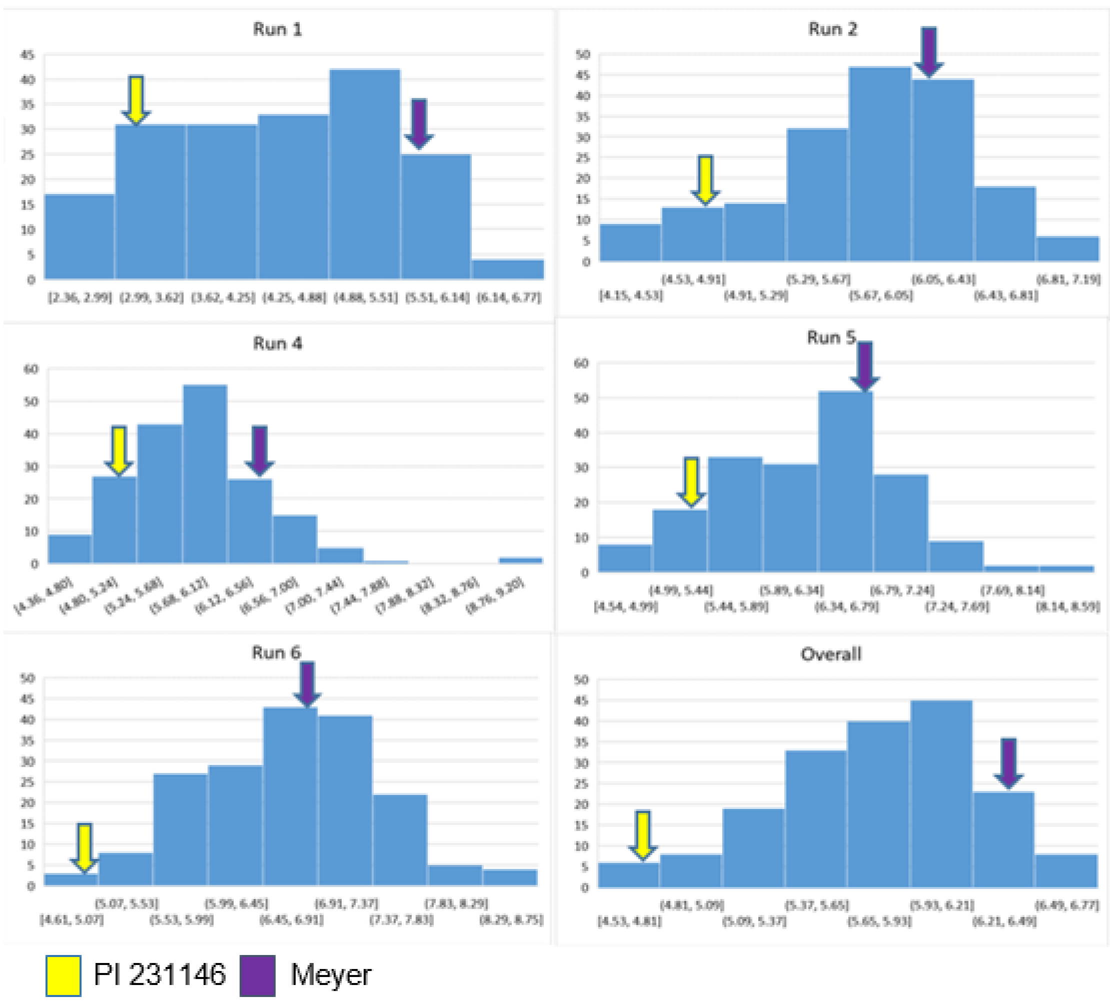

Figure 1. Distribution of LP response among the Meyer x PI 231146 mapping population across runs of evaluation. The purple and yellow arrows indicate values for susceptible Meyer and resistant PI 231146, respectively. The progeny exhibit normal distribution with some lines having more extreme response than either parent.

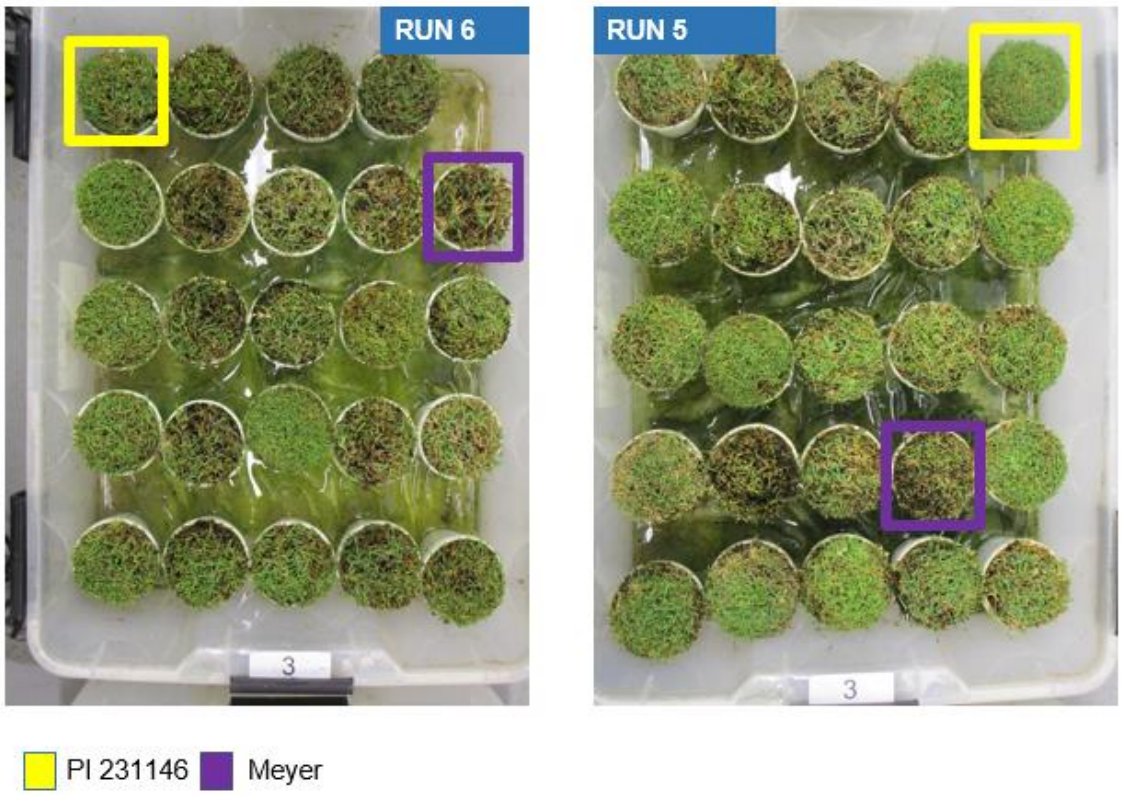

Figure 2. Examples of differences in levels of LP incidence among individuals from runs 5 and 6. Some plants remained unaffected with dense, green canopies, and some plants suffer severe canopy loss. Susceptible Meyer and resistant PI 231146 can be observed in the purple and yellow boxes, respectively.

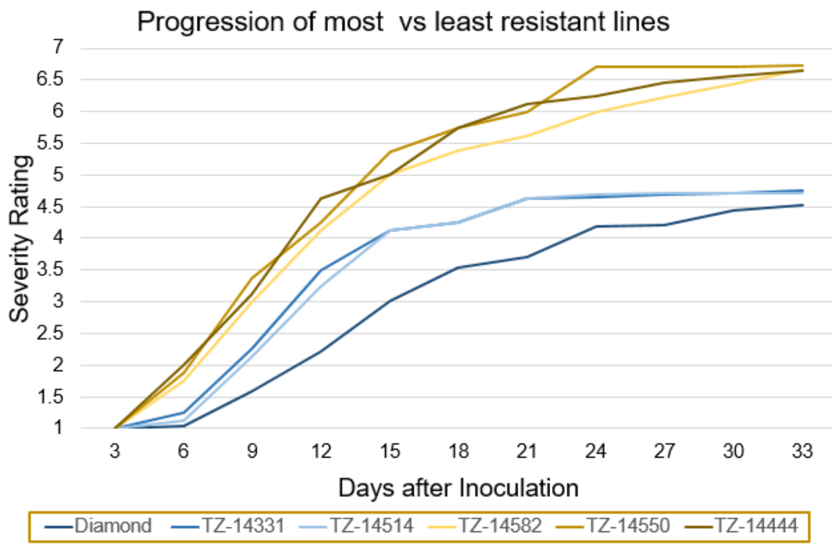

Figure 3. Average disease progression over 33 days across six runs of evaluation for the three most resistant genotypes as compared to the three most susceptible genotypes.

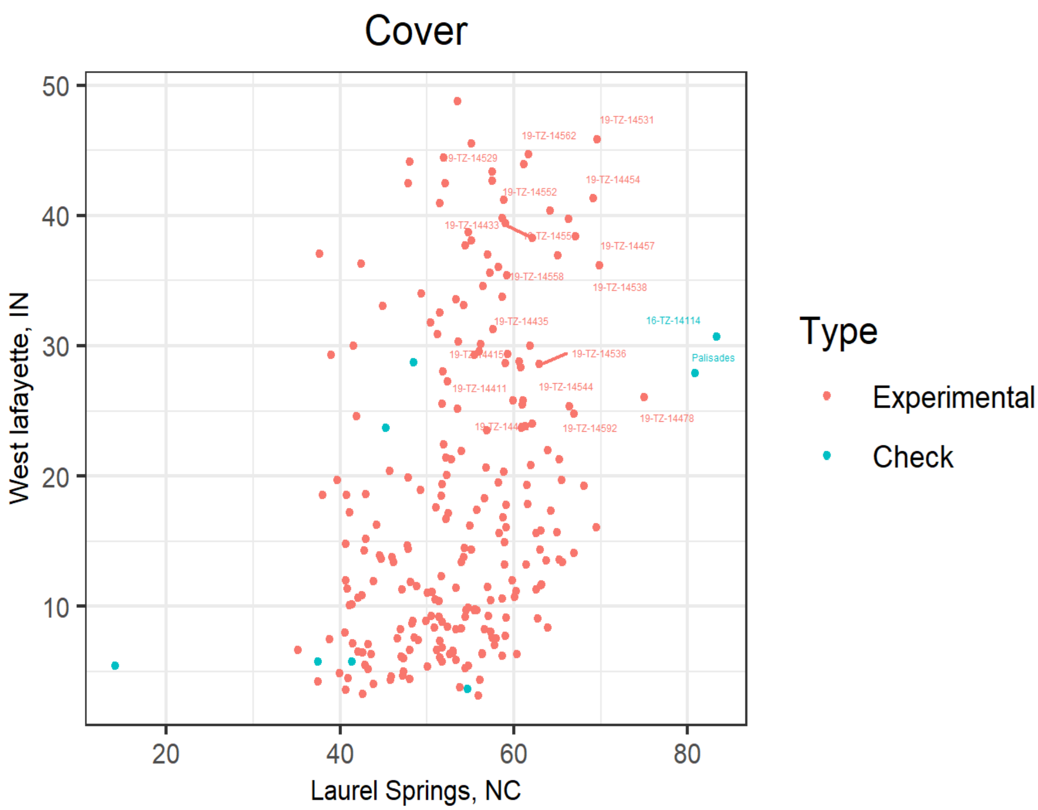

Figure 4. Percent green cover at West Lafayette, IN and Laurel Springs, NC two years after planting

USGA ID#: 2021-04-728

Title: Buffalograss Breeding and Development

Project Leader: Keenan Amundsen
Affiliation: University of Nebraska

Objectives:
Our primary objectives are to 1) optimize breeding schemes to improve their efficiency and reduce the cycle duration needed to release new buffalograss cultivars; 2) increase buffalograss yield and reduce production costs; and 3) continue to improve functional and visual quality of buffalograss cultivars through the application of classical and modern genetics and plant breeding techniques.

Start Date: 2021

Project Duration: 5 years

Total Funding: $150,000

Summary Points:
- Buffalograss selections were identified having rapid establishment rates
- Advanced buffalograss lines were established to be evaluated for sod strength and potential for use as vegetative cultivars
- Buffalograss breeding populations to overcome or minimize seed dormancy were advanced

Summary Text:

Buffalograss demand is on the rise, particularly in areas subjected to unpredictable and prolonged periods of high heat and drought. Buffalograss, known for its exceptional heat and drought tolerance, is highly stoloniferous and forms an exceptional sod, particularly when compared to other United States native grass species adapted for turf use. To address this increased demand for buffalograss, the turfgrass breeding program at the University of Nebraska-Lincoln is focused on increasing the production value of new vegetative and seeded buffalograss. Buffalograss germplasm, selections, and breeding lines are routinely evaluated for establishment rate, genetic color, timing of winter dormancy, canopy density, stolon internode length, and turfgrass visual and functional quality. As an example, 37 selections were evaluated along with two industry standard controls. Establishment rate was evaluated one and two months after planting, rated as percent plot coverage. At the early rating date, genotypes were different based on analysis of variance (P=0.003) and four lines had better establishment rates than the industry standards, and all remaining entries were as fast as the standard entries. By the second rating date, statistical differences were still observed (P=0.003) and 20 of the selections established as quickly as the faster establishing industry standard. Figure 1 is a dot plot representing the relative speed of establishment at the two rating dates; the samples in the upper right quadrant establish significantly faster than the others for both rating dates. Early and rapid establishment is important when growing buffalograss from seed and vegetative plugs as one tool to help minimize weed pressure.

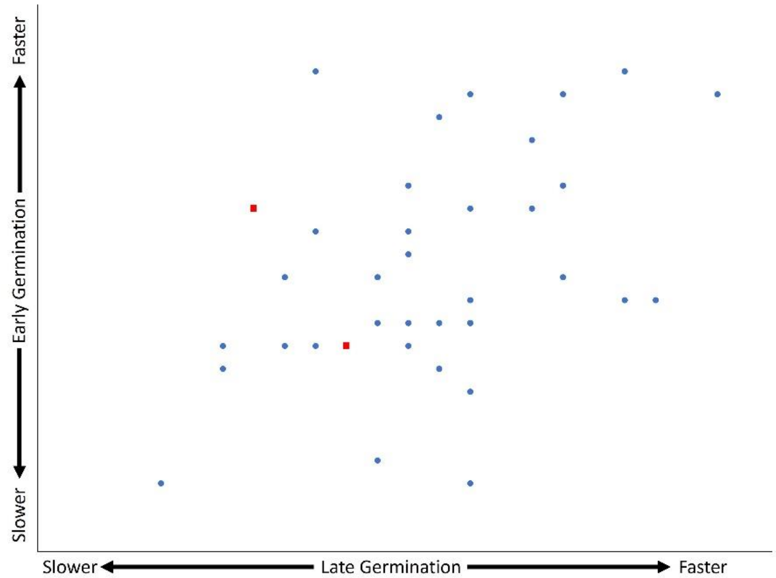

Figure 1. Germination rate of 39 buffalograss selections including 2 standard entries. Plot coverage was used as a measure of establishment rate one month (early) and two months (late) after planting. Red squares represent the industry standards.

Sod strength, unisexually female, establishment rate from plugs, and stolon numbers are some of the added evaluation criteria important for lines under consideration for vegetative buffalograss production, including those that will ultimately be used for sod or plugs. Reduced seed dormancy, high male and female inflorescence numbers, long culm length, germination rate, and yield are among the added evaluation criteria important for buffalograss seed production.

Advanced breeding lines additionally selected (in addition to the above criteria) for chinch bug resistance, leaf spot resistance, and shade tolerance were plugged at 1' spacing on May 16th, 2022. By June 27th, differences in establishment rate were observed and some plots were nearly filled in (Figure 2). By July 26th and through the rest of the growing season, the plots were 100% established and had exceptional turfgrass quality (Figure 3). These plots will be evaluated for sod strength and recovery after cutting over the next two years. Top performing lines will be entered into regional vegetative line evaluation trials or be tested for their combining ability as parents of seeded populations.

Figure 2. Advanced buffalograss selections one month after plugging

Figure 3. Fully established advanced buffalograss selections two months after planting

A typical approach taken by the University of Nebraska buffalograss breeding program to develop new seeded varieties relies first on selecting germplasm with desirable traits. Crossing blocks of those selections are established to develop separate buffalograss populations, often emphasizing specific traits such as resistance to chinch bugs, leaf spot disease, shade tolerance, and exceptional stand persistence. The derived populations are allowed to intermate, and an annual cycle of selection of top performing individuals followed by intermating is initiated. After a few generations, select male and female lines are propagated in the greenhouse to establish small seed production trials in the field in the next growing season, where the males surround the females (Figure 4). Seed is harvested and yield is compared among populations. Since buffalograss is a dioecious species, having separate male and female plants, production fields naturally segregate to a near 1:1 ratio by gender and are not as productive as species having perfect flowers.

Another complication for buffalograss seed production is seed dormancy. Buffalograss has evolved a seed dormancy mechanism to enable success in its primary growing region where temperatures and moisture availability fluctuate. In managed turf systems, supplemental irrigation can be used to support establishment and temperature issues can be somewhat mitigated by selection of appropriate seeding dates. Seed dormancy becomes a hindrance to successful establishment. Seed producers use potassium

Figure 4. Intermating population of buffalograss, with male selections shown in the right part of the image surrounding female selections.

nitrate followed by a stratification treatment to prime the seeds, increasing germination to >90%. The seed treatment comes at an added cost and seed dormancy can return if the seed is not stored correctly prior to planting, although the environmental conditions inducing this secondary dormancy are not known and are being studied. An initial recurrent phenotypic selection breeding scheme was used to overcome dormancy. Seeds were planted in the absence of a treatment and individuals germinating within 14 days were selected, grown to maturity, allowed to intermate, and the cycle repeated. Another approach taken was to dissect out germinating caryopses from each bur and advance. We then let those intermate and are currently advancing selections of top performing lines. Variability in early germination was observed among different populations, highlighting the potential for breeding to overcome dormancy. Many factors impact dormancy and the long-term benefit to this approach will be evaluated over the next several generations.

USGA ID#: 2021-11-735

Title: Seeded diploid buffalograss

Project Leader: Keenan Amundsen

Affiliation: University of Nebraska-Lincoln

Objectives: The primary goals for this project are to develop 1) seeded diploid buffalograss cultivars, and 2) a genomic reference framework to support buffalograss breeding research.

Start Date: 2021

Project Duration: 3 yrs

Total Funding: $44,080

Summary Points:
- Digital image analysis successfully documented buffalograss bur size relative to ploidy.
- Temperature treatments to characterize and understand the temporal response of winter dormancy are being tested.
- Seeded diploid buffalograss can persist with better color retention in areas lacking a hard winter.

Summary Text:

The variable response to the onset of winter among different ploidy levels of buffalograss is fascinating. Buffalograss occurs as a ploidy series and diploid, tetraploid, pentaploid, and hexaploid cultivars are often used in turf systems. In the central part of the Northern Great Plains, buffalograss most often occurs as hexaploid, and diploids are most often found in southern parts of the growing region. Higher ploidy buffalograss tends to have a stronger winter dormancy response allowing them to survive in northern climates, whereas the diploids do not have the same response and tend to be susceptible to winter injury. The lack in winter dormancy has historically created some limitations in the ability of the University of Nebraska turfgrass breeding program to develop diploid buffalograss cultivars, primarily since Nebraska is situated just north of the transition zone and desiccating winters and winter injury to diploid buffalograss is common.

Buffalograss winter dormancy response impacts turf quality and turf management decisions. When buffalograss enters dormancy, it ceases growing and loses its green color and the straw color negatively impacts visual quality. In northern parts of the buffalograss growing region, buffalograss often competes with cool-season grasses which have longer growing seasons and retain their green color longer (Figure 1). While dormant, buffalograss is not actively growing so there are concerns about its functional quality. For example, recovery from divot injury occurring while dormant would not occur until the subsequent spring when buffalograss resumes active growth; buffalograss is a highly stoloniferous species and fills in from damage relatively quickly. During the early stages of winter dormancy in the late fall and just before breaking dormancy in the spring, there are several benefits from a reduced

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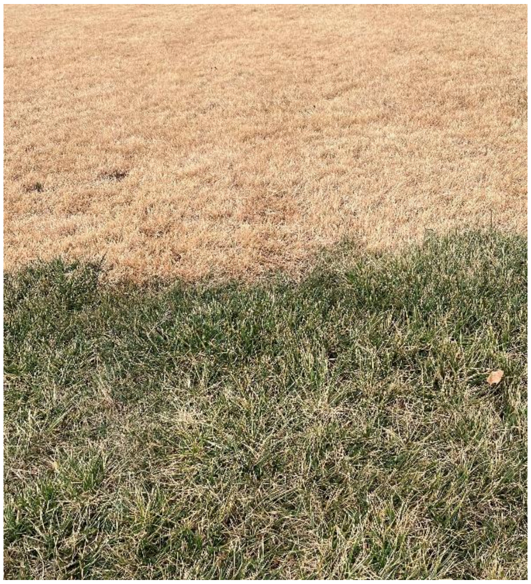


Figure 1. Neighboring tall fescue (foreground) and buffalograss (background) plots in fall 2022 after buffalograss has entered dormancy but while the cool-season species is transitioning.

management perspective. Since buffalograss is not actively growing while dormant, supplemental irrigation, fertility, pest control, and mowing, along with other common turf management practices are not necessary, reducing labor, equipment operating hours, and other inputs when compared with management of cool-season turfgrasses grown in comparable environments.

The University of Nebraska-Lincoln Turfgrass Breeding program has not previously focused efforts on diploid buffalograss development since it often does not persist in the region for the reasons mentioned above. It is important to note that diploid buffalograss is distinct from higher ploidy buffalograss and tetraploids and hexaploids thrive in the region and do not have the same winter injury problems. With the predicted and realized increased occurrence and duration of high heat and drought events, buffalograss is a natural turfgrass species choice to grow and manage. Diploid buffalograss may also be a good choice in parts of the country that do not have harsh winters, since the comparably weaker winter dormancy response helps it to maintain its green color longer than higher ploidy buffalograss.

Currently, the two main diploid buffalograss cultivars on the market are UC Verde and Density, and both are vegetative types. Vegetative types of buffalograss are sold as sod or plugs and seeded types are sold as burs. To our knowledge there are currently no seeded diploid buffalograss varieties available. We have been experimenting with methods to produce seeded diploid buffalograss. Diploid buffalograss has slightly finer leaf texture but is otherwise very similar to higher ploidy buffalograss, except for the winter dormancy response differences mentioned earlier and seed size. Buffalograss seed is sold as burs often containing 3-5 caryopses. Diploid buffalograss has smaller burs requiring different seed-cleaning procedures and often has, on average, 1-2 fewer caryopses. We have been selecting diploids with larger seed size (see our previous project report), but we also tested diploid seed size in comparison to tetraploid and hexaploid buffalograss. It is challenging to measure the buffalograss bur size, due to their irregular shape. We used imageJ software and a digital image analysis technique to measure the area occupied by seeds in an image as a proxy for seed size. Briefly, diploids, tetraploids, and hexaploids had average areas of 16.0, 17.2, and 20.8 units, respectively. Each measurement consisted of a random sample of 10 seeds and a cm ruler was used to normalize the size of each image. The images were captured, and area of the image occupied by the seeds was determined by imageJ. Three separate samples for each ploidy group were analyzed and as expected, seed size increased with higher ploidy levels, although the difference between diploid and tetraploid was not significant (Figure 2).

An important component of this project is to understand the genetics underlying the buffalograss winter dormancy response. To that end we are initiating a transcriptomics study to evaluate the genes that are differentially expressed between control grown plants and those maintained at just above freezing

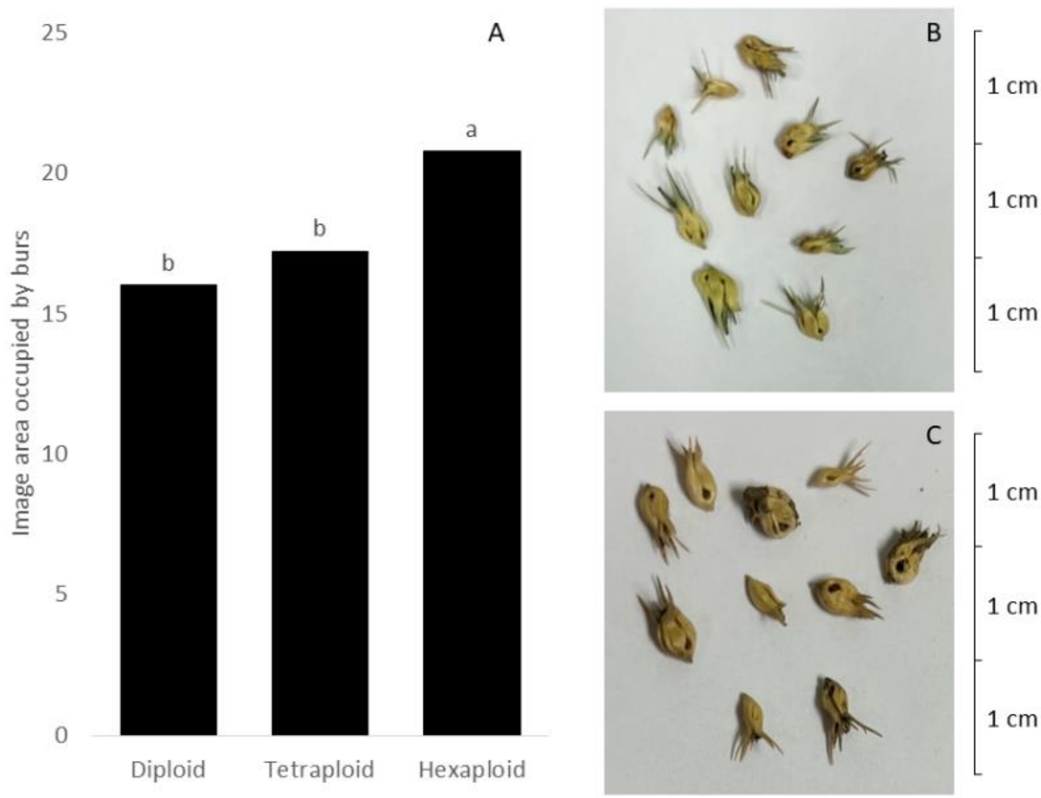


Figure 2. Different sizes of diploid, tetraploid, and hexaploid buffalograss burs. (A) The average area of images occupied by burs was determined using imageJ and used as a proxy for bur size. At least three separate bur samples (n=10) representing each ploidy level was measured. Means were separated by Fishers LSD, indicated by the letters. Relative size of diploid burs (B) and tetraploid burs (C) is shown, normalized based on a cm ruler.

temperatures and entering winter dormancy. We have identified experimental lines that differ in their dormancy responses; one has a strong winter dormancy response and enters winter dormancy quickly whereas the other essentially does not have a winter dormancy response. Both lines are currently being maintained in the greenhouse and we have initiated low temperature treatments to understand the temporal response in dormancy to optimize sample collection for the transcriptomics study (Figure 3).

Figure 3. Separate buffalograss lines that differ for their winter dormancy response, managed in the greenhouse.

During the final year of the project, we will complete the transcriptomics study and characterize genes involved in winter dormancy that explain the dormancy response differences between diploid and higher ploidy buffalograss. Ongoing work includes the continued development and advancement of seeded diploid populations.

USGA ID: 2016-04-554

Title: Development and Release of Turf-Type Saltgrass Variety

Project leader: Yaling Qian and Tony Koski

Affiliation: Colorado State University (CSU)

Objectives:

1. To establish field plots made up of progeny from elite parents and from seeds harvested from the third cycle of crossing block for advancement of saltgrass development.
2. To increase the materials (accessions) selected from the source nursery and the first and the second generation nurseries, further develop breeder's fields, and collect data and prepare document for release of elite vegetative saltgrass varieties.
3. Continue to evaluate several seeded lines for potential release; and collect data and prepare document for potential release of seeded saltgrass varieties.

Start date: 2016
Project duration: 3 years
Total funding: $90,000

Summary points:

1. To involve turf producers, two lines that were selected for potential release as vegetative cultivars were planted at a sod farm in CO.
2. Six parental lines with adequate commercial seed production yields at CSU research site were planted in a seed producer's field in OK for evaluation of seed production.
3. Both trials were unsuccessful, likely because saltgrass is a true halophyte, i.e., it grows more optimally under salinity conditions. The salinity requirement was not met both at the sod farm and seed producer's field.
4. Saltgrass increased fine root initiation and growth in response to 8 dS/m salinity treatment when compared to the control. Both growth chamber and field experiments demonstrated that saltgrass is a true halophyte that under non-saline conditions, saltgrass had lower shoot, root, and rhizome growth, therefore non-competitive against other species and weeds.
5. Saltgrass has value for use as turfgrass and as a remediation/revegetation plant in areas that commonly have soil salinity problems.

Background:

Water scarcity is an important issue in much of the arid western United States. Finding ways to conserve water, such as irrigating with non-potable water sources, are critical to turf industry. Another increasingly important issue in the arid West is the build-up of salts in the soil having a negative impact on turfgrass growth and plant community structure. Millions of acres of land are negatively impacted by salinity. Saltgrass is indigenous to western North America, it is adapted to specific niches of alkaline and saline soils. The use of saltgrass on landscapes and golf courses could help turf industry to conserve fresh water because of its tolerance to lesser quality water.

<u>To address objective 1:</u>

We established and evaluated field plots made up of progeny from elite parents and from seeds harvested from the third cycle of crossing block for saltgrass development. Specifically, seeds from previous crossing blocks were harvested. Individual seeds were stratified and germinated in the greenhouse. Single seed plant materials were propagated and material increased. Resulted single seed plugs were used to initiate field plots in the spring of 2017. Data was collected on turf quality, growth height, disease incidence, sex, and the number of seed heads per unit area. Data collected indicated that most of the lines did well during the second season, and thereafter. Five lines of third generation of saltgrass that showed the best turf quality were selected. The top lines with very few seedheads were selected as potential vegetative lines.

The top five female lines and 5 male lines with good seedhead production were also selected. Seed yield data was collected for these females. One line showed the highest seed production at about 1500 lb/acre.

It is important to note that all the work has been done at ARDEC-south research farm of CSU. At this site, the underground irrigation water has high salinity with total dissolved salts in the range of 2800 to 3200 mg/L. Soil salinity ranged from 3.5 to 5.5 dS/m, varying with seasons and locations.

<u>To Address Objective 2: Potential Vegetative Saltgrass Lines:</u>

We increased the materials selected from the source nursery and breeding nurseries. Among numerous saltgrass lines evaluated, two lines with distinguished different characters were selected for potential release as vegetative cultivars. Data on turf quality, disease incidence and growth were collected. One line had stronger rhizomes than the other. Both lines maintained an average turf quality rating above acceptable. One of the two lines was rust resistant. Without mowing, the plants had a 16 to 18 cm maximal height.

We increased these materials in the greenhouse and in the field. These potential vegetative saltgrass lines were further established in the field at ARDEC-south (as breeder's field) using sprigging establishment. Results indicated that saltgrass sprigged in May established adequate coverage in September with sprigging rates at > 270 bush/acre. The accumulated growing degree day requirements for saltgrass establishment generated in this study suggest that saltgrass is much slower in establishing than bermudagrass and buffalograss. Although labor and time intensive, it is feasible to establish saltgrass using sprigs.

Sod farm test: We contacted a sod producer in Colorado to evaluate vegetative saltgrass lines on the sod farm with the aim to scale up these lines for industry production. The deep rhizome growth characteristics of saltgrass makes conventional sod production for saltgrass difficult. Therefore, nettings and plastic sheets were used when sprigging saltgrass at the sod farm (alongside with bermudagrass test). However, the evaluation at the sod farm was not successful. The sod grower found saltgrass grew very slowly. It was much less aggressive when compared to bermudagrass.

To Address Objective 3: Potential Seeded Saltgrass Lines:

About 15-20 clones from the second-generation nursery with acceptable seed production were selected and increased. These saltgrass accessions were produced through several breeding cycles. Some of these increased materials were planted in the field in June 2017. The saltgrass accessions selected from previous nurseries were planted as open pollination crossing blocks to evaluate seed production. About 60% of all females produce seeds and 20% of females showed promise for further evaluation since those lines reached commercial seed production yields in our research plots.

On-site evaluation at a turfgrass seed company: Based on our field evaluations at the CSU research center, three pairs of males and females were selected for further evaluation at a seed producer's field in Oklahoma. The materials were propagated and increased in CSU greenhouse. About 6000 plugs were transported to the turfgrass seed company for on-site field evaluation of seed production via a material transfer agreement. These lines were planted to strips in three isolated fields. However, the on-site saltgrass seed production trial was unsuccessful. The parents clones did not grew well at the turf seed company site. Female lines produced seeds but acceptable seed yield was not achieved. This demonstrated that saltgrass seed yield trait was not stable and seed yield varied with environmental conditions.

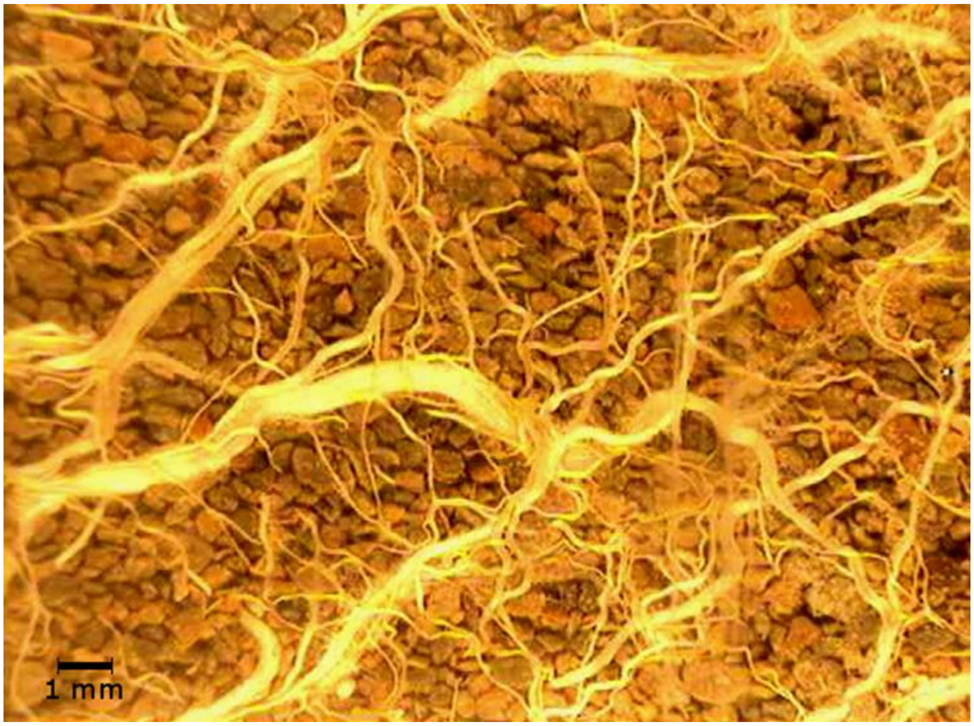
So, the question was that why saltgrass did not grow well both at the seed producer's field and the sod farm, despite these saltgrass lines grew well for years at the CSU research farm. One possible explanation/hypothesis is that saltgrass is a true halophyte. It is competitive with sustainable growth and quality under conditions at threshold salinity. However, its growth and production decline under non-saline conditions. Both the seed producer's field and the sod farm had non-saline soil and irrigation water. We conducted two experiments to test the hypothesis by evaluating shoot, root, and rhizome growth of saltgrass under different salinity treatments.

Experiment I and II: Saltgrass Shoot, Root, and Rhizome Growth under Different Salinity Levels.

Experiment I was conducted in environment-controlled growth chambers. Container-grown saltgrass was subject to control, 8, or 16 dS/m salinity treatments and minirhizotron systems were installed. Saltgrass increased fine root growth in response to 8 dS/m salinity treatment when compared to the control (Picture 1 and Figure 1). Root growth started to increase about 3 weeks after treatments began. In-growth root tubes placed in the containers showed trends of increasing rhizome growth under 8 dS/m salinity (Figure 2). Saltgrass new root initiation and rhizome growth are significantly declined under non-saline control (Figure 2).

Experiment II was conducted in the field. After saltgrass establishment, four salinity treatments were imposed on sandy soil by irrigation with waters at 2.0, 6.3, 12.5 and 18.8 dS m^{-1} salinity. Water was applied until the soil matric potential as measured by tensiometer reached to - 0.3 bar. Additionally, 15-25% excess water (above field capacity) was included in the irrigation water to maintain leaching fraction. Treatments continued for a year. Root and shoot growth of saltgrass were significantly enhanced as salinity increased from control to 18.8 dS/m. Compared to salinity treatments, the control had 10% and 58% lower clipping yield and root mass, respectively (Table 1).

Both experiments demonstrated that saltgrass is a true halophyte. It needs saline conditions for optimum growth. Under non-saline conditions, saltgrass had slower growth, is weak and non-competitive against other species and weeds. This presented major challenge for commercialize turf type saltgrass, because none of selected sod farm and seed production field present saline condition which is required for optimal saltgrass production.



Picture 1. Minirhizotron image showing healthy and active saltgrass roots when the container was irrigated with 8 dS/m saline water.

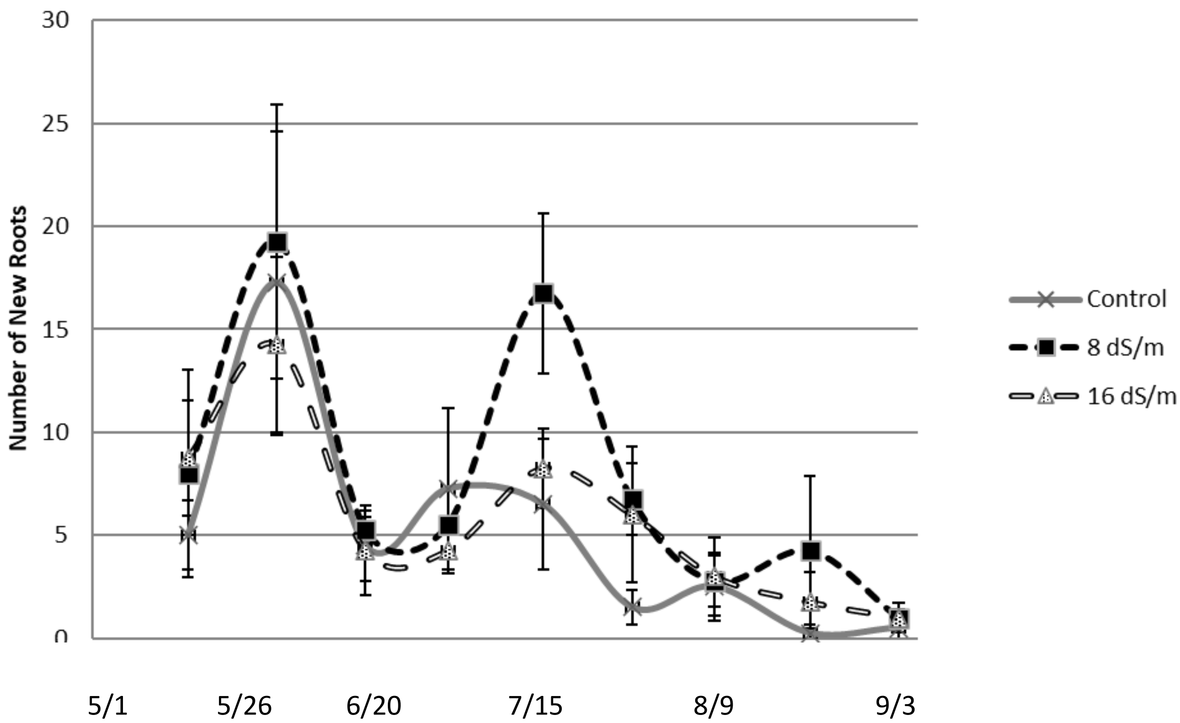


Figure 1. Saltgrass New Root Counts: Points are the number of roots produced between each measurement date at 15 cm depth treated with three levels of salinity. Control is freshwater. Error bars represent standard error.

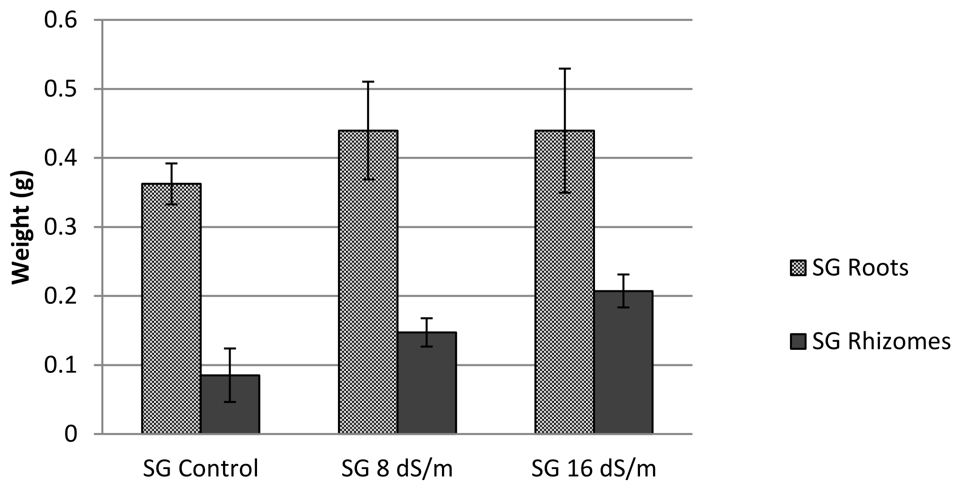


Figure 2. Average total weight for saltgrass(SG) roots and rhizomes produced in in-growth root cores in the growth chamber container study (n=12).

Table 1. Relative shoot, and root growth, root/shoot ratio, and turf quality of saltgrass subject to different levels of irrigation water salinity in the field experiment.

Salinity (dS/m)	Relative shoot growth (%)	Relative root growth (%)	Root to shoot ratio	Turf quality (1-9, 9 = best)
2.0 (control)	100.0 b	100.0 b	0.050 b	7
6.3	111.3 a	159.6 a	0.073 a	7
12.5	110.6 a	154.4 a	0.071 a	7
18.8	110.1a	160.6 a	0.074 a	7

Different letters indicate significant differences (*P* =0.05) among salinity treatments.

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

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

- Again in 2021, performance varied with turfgrass quality, percent living ground cover, weed invasion and canopy height measurements as the most important data collected.
- Turfgrass quality data from 2021 showed significant differences among entries and species, with 'XZ 14069' and'16-TZ-14114' zoysiagrass performing best over several test locations.
- 'XZ 14069', one of the highest performing entries in the trial, has been commercially released and is now called 'Lobo™'.

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.

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 stress
- 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 requests annual data by December 15th of each year, organizes, reviews and statistically analyzes submitted data, and publishes on the NTEP web site (www.ntep.org) in spring or summer of the following year.

Progress to Date

Ten (10) entries consisting of eight vegetatively-established and two seed-established entries were established in summer 2018. Species in the trial include multiple entries of bermudagrass, zoysiagrass, buffalograss, as well as one mixture entry consisting of buffalograss, curly mesquite and blue grama.

Turfgrass quality ratings collected in 2020 had 'Tifway' and 'Midirion' bermuda, both old standards, along with 'XZ 14069' zoysia as the most consistent performers, finishing in the top statistical group at seven of eight sites. Entries also performing well overall in 2020 included 'Meyer' and '16-TZ-14114' zoysia, along with 'FB 1628' bermuda.

Data collection in 2021 concentrated on turfgrass quality along with percent living ground cover, weed invasion and canopy height measurements between mowings. In year three, entry performance reflected the effects of reduced nitrogen and irrigation since establishment. Data from eight locations showed a shift in performance, particularly in the southeast U.S. Zoysia entries overall, performed better than the bermudagrasses in the southeast, presumably responding to the reduced nitrogen applications. 'XZ 14069' zoysia was a top performer at Citra, FL, Mississippi State, MS, Raleigh, NC, Stillwater, OK and College Station, TX. '16-TZ-14114' zoysia was also good at Raleigh, Stillwater and College Station. In those southeast U.S. sites, 'Meyer', the seventy-year-old standard cultivar, continued to perform admirably. Due to the success in this trial and other data, 'XZ 14069' has been commercially released by North Carolina State University and named 'Lobo™'. https://cals.ncsu.edu/crop-and-soil-sciences/news/nc-state-turfgrass-releases-lobo-zoysiagrass/

Standard cultivar 'Tifway' bermudagrass continued to produce good turfgrass quality at Jay, FL, Stillwater, OK and College Station, TX, finishing in the top statistical group at each site. 'Midirion' bermudagrass joined 'Tifway' as a top performer not only at College Station, but also at Las Cruces, NM and St. George, UT. 'FB 1628' bermuda was also a top performer at Las Cruces and St. George as higher pH soils in the western locations likely had a negative effect overall on zoysia performance. The only location with buffalograss as a top performer was St. George, UT, a site where that species is better adapted than the southeastern U.S.

Canopy height measurements were collected at several locations in 2019, 2020, and again in 2021. Data from 2021 was consistent with the previous years' data as zoysia entries, 'XZ 14069' and 'FAES 1322' frequently had the lowest canopy height between mowings.

Figure 1. Winter color differences of ‘Meyer’ (front), ‘Lobo’ (middle), and ‘Habiturf’ (rear) in Citra, FL.

Figure 2. An overview of the trial in Citra, FL. Notice the stark difference among entries.

USGA ID#: 2022-03-746

Title: A National Evaluation of Cool-Season Turfgrass Cultivar Water Use and Drought Tolerance under Golf Course Fairway Management

Project Leader: Kevin Morris
Affiliation: National Turfgrass Evaluation Program

Objectives: The objectives of the project are to evaluate cool-season species and cultivars under fairway management to 1) record the amount of water required to maintain a prescribed green cover, and 2) determine turfgrass quality and percent living ground cover under three replacement irrigation (ET_0) levels

Start Date: 2022
Project Duration: Three years
Total Funding: $120,000

Summary Points: Include 3-6 bullet points that summarize the findings of your project to date.

- In fall 2022, eighteen total entries, encompassing creeping and colonial bentgrass, hard fescue, chewings fescue, strong creeping red fescue and perennial ryegrass were submitted for the trial
- Five trial sites were established, two under rainout shelters and three in field sites with deficit irrigation (ET_0) levels
- A minimum percent green cover level during the drought 'season' will be utilized to determine the re-watering threshold at rainout shelter locations, thus allowing for a water use rate for each entry
- Three deficit irrigation levels, representing optimum, sub-optimum and severe restriction will be instituted at the deficit ET_0 sites
- Drought and deficit irrigation levels will be initiated when trial sites are mature, either summer 2023 or 2024.

Summary Text: Provide **800 to 1200 words** briefly outlining rational, methodology, results to date, and future expectations of the project.

In 2016, the USGA provided NTEP $500,000 to build rainout shelters and develop irrigation infrastructure at multiple locations to evaluate cultivars and experimental selections for water use and drought tolerance. Thirty-five total entries of Kentucky bluegrass and tall fescue were established and evaluated for three years at five rainout shelter locations and five deficit irrigation (ET_o) locations. In 2018, seventeen warm-season grass cultivars were established at ten locations (five rainout shelter, five deficit ET_o) with evaluations completed recently. With the completion of the cool-season trial, and because rainout shelter space limited evaluations to only Kentucky bluegrass and tall fescue, the time is now to evaluate additional cool-season species. Utilizing some of the current infrastructure funded by the USGA allows us to evaluate grasses for three years at six geographically appropriate locations (three rainout shelter, three deficit ET_o) under fairway management. We expected at least twenty paid entries of species such as bentgrass, fine fescue and perennial ryegrass to be submitted for the trial. Entry fees paid by sponsors are utilized to cover most costs, but additional funding from USGA and other sources is essential for trial success. Trial establishment in occur in fall 2022/spring 2023, with data collection for three years. Trial parameters are developed by an advisory committee consisting of USGA representatives, researchers and entry sponsors. Data is analyzed and published on the NTEP web site and uploaded into the NTEP database.

Data on percent green cover collected during the drought 'season', turfgrass quality and the amount of water needed by each entry to maintain a prescribed level of green cover will be available from each rainout shelter site. Data from the deficit ET_o sites will include percent green cover and turfgrass quality ratings during the deficit ET_o period. The data will document performance during drought periods applicable to eastern and western US turfgrass management conditions.

In fall 2022, twelve paid entries, encompassing creeping bentgrass, perennial ryegrass, hard fescue, chewings fescue and strong creeping red fescue were submitted for evaluation by sponsors. Added to the trial were six standard (unpaid) entries for a final total of eighteen entries. This was short of our budgeted number of entries, resulting in the need to drop one evaluation site from the trial. Therefore, five trial sites were established: rainout shelter sites in Amherst, MA and West Lafayette, IN measuring water needed to maintain green cover, and deficit irrigation sites in St. Paul, MN, Ft. Collins, CO and Logan, UT evaluating performance under three ET_o regimes.

Water use calculations will start within the rainout shelters in either summer 2023 or 2024, depending on trial maturity. To initiate the drought 'season', irrigation will be halted under the rainout shelter for approximately 100 days. As entries 'dry-down' and start to experience water stress, daily evaluation of green cover using digital image technology will determine which plots have reached the re-watering 'threshold' (the minimum acceptable percent green cover to be determined by the trial advisory committee). Using ½ - 1" of water, each plot reaching the threshold will be carefully re-watered. A daily record of plots that require water is totaled at the end of the drought 'season' to determine amounts of water required by each entry to maintain the threshold green level. Three years of data collection will provide water use rates under different climatic conditions.

Trials in St. Paul, MN, Ft. Collins, CO and Logan, UT, located in regions where summer drought is more common, will initiate irrigation levels when entries are mature. Daily evapotranspiration (ET) rates will be calculated and used to replace water lost by entries. Three irrigation regimes based on replacement ET_o will be determined by the advisory committee, representing optimum, sub-optimum and severely restricted (deficit) irrigation replacement levels. The irrigation regimes will be utilized for 100 -120 days over three years of data collection.

Results from this trial will document not only the amount of water needed to maintain a consistent green surface under a seasonal 'acute' drought period, but also cultivar performance under 'chronic' restricted irrigation replacement based on ET_o. This information is essential as golf course water use is being restricted, due to droughts and increased competition for limited water supplies due to development. Therefore, grasses that maintain high quality fairways with less water are needed for golf course superintendents, golf course owners, designers, architects, golfers and others that depend on the golf course industry for their livelihood, enjoyment and environmental protection.

2022 USGA/NTEP Cool-Season Fairway Water Use/Drought Tolerance Test
Entries and Sponsors

Entry No.	Name	Species	Sponsor
*1	Gladiator	Hard Fescue	Standard
*2	Navigator II	Strong Creeping Red	Standard
*3	Compass II	Chewings Fescue	Standard
4	PPG FRC 127	Chewings Fescue	Mountain View Seeds
*5	SR 4650	Perennial Ryegrass	Standard
6	Helios	Perennial Ryegrass	DLF USA
*7	Musket	Colonial Bentgrass	Standard
*8	Piper	Creeping Bentgrass	Mountain View Seeds
*9	Oakley	Creeping Bentgrass	Mountain View Seeds
*10	Piranha	Creeping Bentgrass	Mountain View Seeds
*11	AU Victory	Creeping Bentgrass	Mountain View Seeds
*12	007XL	Creeping Bentgrass	DLF USA
*13	Macdonald	Creeping Bentgrass	DLF USA
*14	777	Creeping Bentgrass	DLF USA
15	DLF-AP-3084	Creeping Bentgrass	DLF USA
*16	T-1	Creeping Bentgrass	Barenbrug USA
*17	Kingdom	Creeping Bentgrass	Barenbrug USA
*18	Penncross	Creeping Bentgrass	Standard

*COMMERCIALLY AVAILABLE IN THE USA IN 2022 OR ANY OTHER COUNTRY

Cooperators - 2022 National Cool-Season Fairway Water Use/Drought Tolerance Test

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AS OF 11/16/22

USGA ID#: 2022-14-757

Title: High throughput phenotyping of turfgrass photosynthetic and growth responses to drought

Project Leader: Dr. Emily-Merewitz Holm

Affiliation: Michigan State University

Start Date: Dec 1, 2021

Project Duration: 1 year

Total Funding: $29,648

Objectives:

1. To evaluate the efficacy of a dynamic lighting growth chamber system for continuous tracking of turfgrass photosynthetic health and growth responses to drought stress
2. To determine key photosynthetic parameters influenced during cool season turfgrass responses to drought
3. To identify which photosynthetic parameter may correlate with drought resistance in set of kentucky bluegrass and perennial ryegrass varieties

Summary Points (Preliminary):

- Kentucky bluegrass cultivars ‘Finnish line,’ ‘Jersey,’ Bordeaux, and ‘Rockstar’ and two perennial ryegrass cultivars, ‘Process’ and ‘Panthergills’, had higher relative water content during moderate drought stress compared to other varieties within the same species.
- The panel of 18 varieties chosen for this experiment exhibited sufficient variation in relative water content after 4 days of drought, which will be used to correlate to photosynthetic health parameters.
- Large dataset analysis for the multiple photosynthetic parameters taken hourly during the experiment is ongoing.

Rationale

Water conservation in the turfgrass industry may be enhanced by identifying drought resistant turfgrass cultivars for drought-prone areas or managerial strategies and by a better understanding of drought responses of important turfgrass species. Breeding for drought resistance is difficult and may be amplified by utilizing high throughput technologies. In the present study, we aimed to evaluate a small set of cool-season turfgrass cultivars of kentucky bluegrass (*Poa pratensis*) and perennial ryegrass (*Lolium perenne*) using an innovative high throughput chamber to measure in-depth photosynthetic parameters, referred to as a Dynamic Environmental Photosynthetic Imager (DEPI). The high intensity light and sensor programming allows for tracking of photosynthetic health multiple times in a day on all experimental plants simultaneously, which would not be possible with manual measurement of plants. The DEPI chamber also quantifies photoprotection associated parameters, for which little knowledge of these photosynthetic responses exists for turfgrass species. Experiments conducted by manually measuring fluorescent-based parameters like photochemical efficiency have illustrated their importance in stress responses of grass species and that varieties can have diversity in photosynthetic responses (Chai et al., 2010; Huang and Gao, 1999). This research will expand on our understanding of turfgrass stress responses by evaluating a comprehensive set of photosynthetic attributes simultaneously. These results will be informative for future physiological and breeding experiments.

Methodology

Plant material and growth conditions

A total of 18 turfgrass cultivars consisting of nine perennial ryegrass and nine Kentucky bluegrass cultivars were used for this study. A total of 72 pots were seeded with 8 replicates of each variety in an environmentally controlled greenhouse in East Lansing, MI. Once sufficiently established, plants were propagated and transferred to long cylindrical pots to allow for sufficient root development (Deepot tree containers, 6 cm diameter by 35 cm deep) with sandy loam soil (65.9% sand, 14.9% silt, 19.2% clay; Typic Hapludult). Once established with a canopy filling the soil surface, plants were transferred to a growth chambers (temperature 18°C/16 °C day/night, relative humidity 50%, photosynthetic photon flux density 440–570 μmol $m^{-2}s^{-1}$, photoperiod 12 h) for two weeks to establish, and then transferred to the DEPI chamber (Figure 1). Perennial ryegrass plants were evaluated in the DEPI chamber from July 14, 2022 through July 28, 2022 and repeated on Aug 21 through Sept 3, 2022. Kentucky bluegrass varieties were evaluated in the chamber from Aug 1, 2022 through Aug 14, 2022 and then repeated on Aug 21, 2022 to Sept 3, 2022. The DEPI chamber was maintained at optimum temperature (25°C/19°C day/night), with relative humidity 40/50% day/night, photosynthetic photon flux density 950 μmol $m^{-2}s^{-1}$, and photoperiod 14 h. Plants were allowed to acclimated to the DEPI chamber for 5 days prior to drought treatment.

Drought treatment

In the DEPI chamber, all plants were equally maintained for 5 d and fertilized once with half-strength nutrient solution (Hoagland and Arnon, 1950). Plants were trimmed to 1 inch and the initial soil moisture was equivalent for all plants. Drought stress was induced by withholding water on four randomly-selected replicate plants of each variety whereas, well-watered control plants were normally irrigated and maintained at 90% field capacity. Thus, among the eight plants per cultivar, four plants were subjected to drought stress (drought-treated plants) and four were maintained as well-watered control.

Measurements

Soil volumetric water content and leaf relative water content

Soil volumetric water content (SWC) was monitored daily using a soil moisture meter (TDR 100; Spectrum Technologies, Aurora, IL) with 7.6 cm probes. Leaf relative water content (RWC) was determined on leaf samples clipped from plants on day 0, 4, and 8 days of drought stress. Percent RWC was calculated using the following equation: RWC = [FW−DW] [TW−DW]×100, where FW, TW, and DW are fresh weight, turgid weight, and dry weight of leaf samples, respectively (Barrs and Weatherley, 1962).

High throughput measurement of photosynthetic parameters using DEPI

The DEPI chamber lighting system is programed to illicit various lighting states (such as dark adaption or light saturation) to captured fluorescent images that were used to calculate parameters including nonphotochemical quenching (NPQ), photosynthetic efficiency (ϕII), energy quenching (qE), and photoinhibition (qI). These parameters have been measured every 2 hours during the experiment and the data is currently being processed.

Statistical analysis and experimental design

The experimental design was a completely randomized block design within the growth chamber with cultivar and watering regime as factors. Analysis of variance (ANOVA) was performed following the General Linear Model, using GenStat 18th edition (http://www.genstat.co.uk). The ANOVA was performed by considering genotype by water regime as Treatment Structure, and Replication as Blocking (Nuisance terms). Preliminary cultivar–cultivar comparisons were conducted using Tukey Honestly Significant Difference test at the 0.05 P level, and potentially drought-tolerant candidates were identified based on high relative water content under drought stress.

Results

SWC and RWC were similar for all plants on day zero but decreased during drought stress treatment (Figures 2 and 3). However, the rate of decrease of RWC varied among cultivars. Two perennial ryegrass cultivars (‘Process’ and ‘Panthergills’) had significantly higher ($P < 0.05$) RWC (84 and 82%, respectively) than other cultivars (37–51%) on day 4 of drought stress. Similarly, four kentucky bluegrass cultivars (‘Finnish line’, ‘Jersey’, ‘Bordeaux’, and ‘Rockstar’) had significantly higher RWC (54–78%) than other cultivars (26–39%) in day 4. Perenial ryegrass ‘Furlong’ and KBG ‘Arc’ were among the lowest performing cultivars for RWC. By day 8, there was no significant difference in RWC among cultivars (Fig. 3). At present, we have captured chlorophyll fluorescence images using a DEPI chamber. This data is currently being processed to determine if various photoprotection associated parameters (NPQ, qE, qI) are traits for future stress screening of turfgrass species.

Future expectations for project

While all four originally proposed species were not able to be evaluated due to space limitations and space demands of researchers in the testing chambers, our evaluation of these two important turfgrass species, perennial ryegrass and kentucky bluegrass, will increase our understanding of photosynthetic health during drought stress. Upon completion of data analysis, we expect to be able to propose more in-depth studies using photoprotection based traits, to better understand their utility to the turfgrass industry.

Figure 1. Turfgrass plants in the dynamic environment photosynthetic imaging chamber that will be exposed to drought or well-watered conditions.

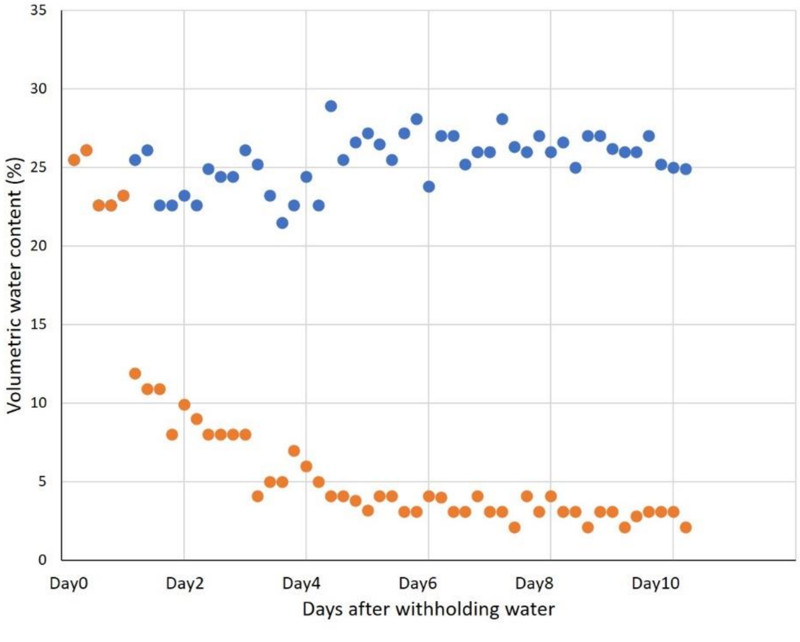


Figure 2. Soil volumetric water content (%) of turfgrass plant in pots treated with either well-watered (blue) or water completely withheld (orange) treatments.

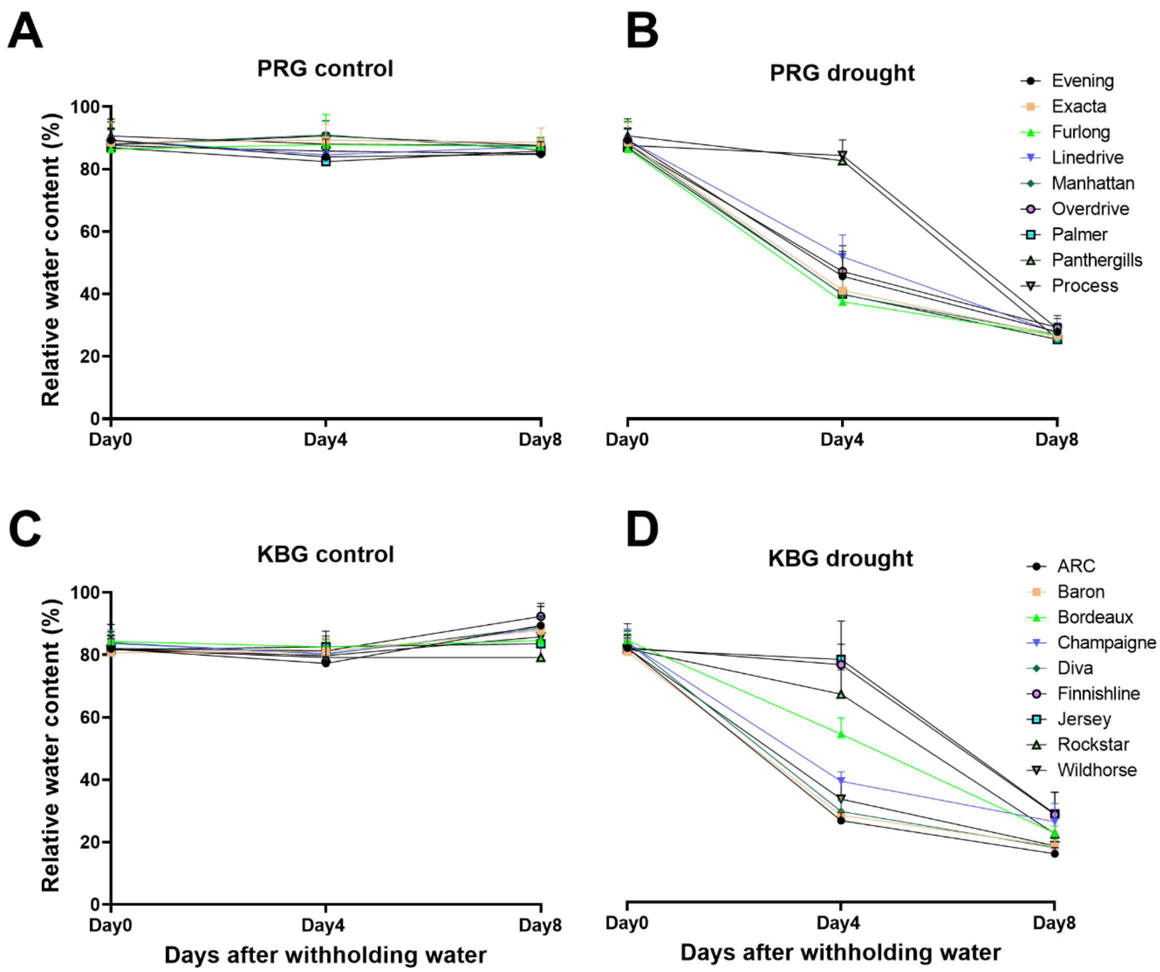


Figure 3. Relative water content of (A) perennial ryegrass during control conditions, (B) perennial ryegrass during drought stress, (C) kentucky bluegrass during control conditions, and (D) kentucky bluegrass during drought stress. Data represent the mean ± standard deviation of four independent biological replicates.

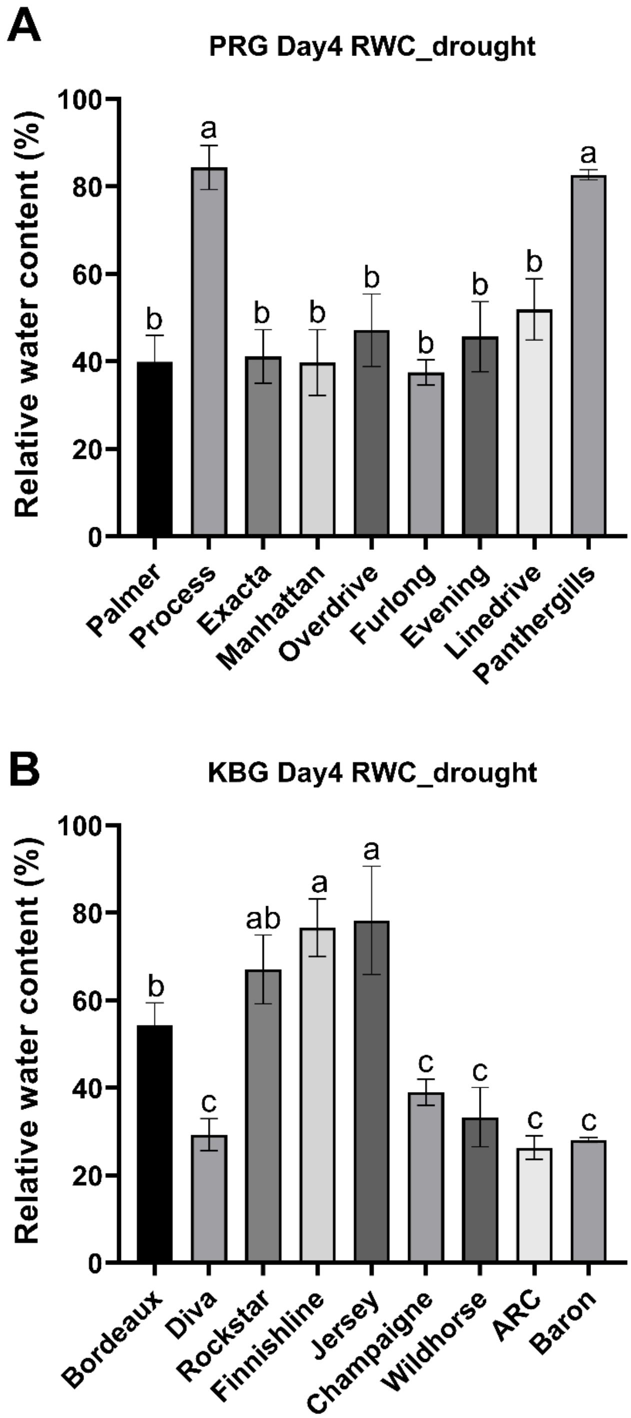


Figure 4. Relative water content in cold season turfgrass at four days under progressive drought stress. (A) perennial ryegrass cultivars, (B) kentucky bluegrass cultivars. Data represent the mean ± standard deviation of four independent biological replicates. Bars marked with the same letter are not significantly different (P<0.05), according to the Tukey HSD test.

References

Barrs, H.and Weatherley, P. A. (1862) Re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15 413 428.

Chai, X., F. Jin, E. Merewitz, and B. Huang. 2010. Growth and physiological traits associated with drought survival and post-drought recovery in perennial turfgrass species. J. Amer. Soc. Hort. Sci. 135(2):1-9.

Hoagland, D.R. and Arnon, D.I. (1950) The water-culture method for growing plants without soil University of California, College of Agriculture, Agricultural Experiment Station Berkeley, CA.

Huang, B. and H. Gao. 1999. Physiological responses of diverse tall fescue cultivars to drought stress. HortSci. 34: 897-901.

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:
- Black shade fabric (1 or 2 layers) had a strong effect on longwave and shortwave radiation in simulated shade structures.
- The addition of a blue lens to neutral shade fabrics (in order to reduce red light) had minimal effects on incidental shortwave or longwave radiation.
- There were minimal differences in net radiation among structures 36 to 72-inches tall.

<u>Rationale</u>

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, typically made from a polywoven material, 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 or R:FR ratio), include tree root competition for water and nutrients, or otherwise influence the energy balance differently than a shade fabric might. There has also been substantial interest in the effect of temporal shade versus perpetual shade.

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. As the academic community increases our understanding of turfgrass response to shade, there needs to be dedicated research in standardizing and validating methods used to evaluate shade tolerance.

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.

For objective 1, shade structures were built and tested using varying heights (18, 36, 48, and 72-inches), shade densities (1-layer versus 2-layers of 73% fabric), and shade materials (neutral or red selective). Measurements of net radiation, PPFD, temperature, and wind speed were collected over a period of 30 days under ambient and shaded conditions. Similar measurements were made under deciduous tree shade conditions (Fig. 1). Data were analyzed as a reduction from ambient conditions to normalize for day-to-day variability.

For objective 2, a spectroradiometer (Flame S, Ocean Optics) was used to measure light spectral properties under ten commercial lens filters under controlled conditions. Additional measurements under deciduous and coniferous trees were made during summer 2022. Data were analyzed to determine the R:FR ratio for each shade type and used to identify which methods most accurately simulate real world shade (Fig. 2 and 3).

For objective 3, an 8-week study was conducted at the OSU Horticulture Research Greenhouses. A seeded bermudagrass cultivar (Rio) was planted as a single seed within 4mm diameter cone-tainer filled with a soilless growing medium. Shade treatments included a control (no-shade), 40% black shade fabric (Greenhouse Megastore. Model #SC-BL40, Danville, IL), light blue polyester gel filter (LEE Filters, Burbank, CA), and a combination of the blue polyester gel filter and the black shade fabric. Evaluation of lens filters was reported on in the prior research summary. A light blue gel filter used was selected from the preliminary evaluation to best represent the targeted reduction in R:FR with minimal reduction in total PAR. Plants were 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 were made to determine if light quality is a critical factor when evaluating bermudagrass light requirements.

Results

Longwave, shortwave, and total net radiation varied with shade structure material and height (Table 1). Longwave and shortwave radiation had opposite responses to increasing shade severity. Longwave radiation increased under each shade structure type with multiple layers of fabric or red selective lens increasing values more than the single layer. Similarly, the shortest height resulted in the greatest increase in longwave radiation. This is similar to what is reported for tree canopies wherein shade during the night can moderate temperatures to some degree. Whether these temperature differences are physiological significant is not certain.

Shortwave radiation decreased under each structure type with two layers of neutral shade fabric resulting in the greatest decrease. This suggests minimal loss of short-wave radiation associated with the blue lens. Reductions in shortwave radiation generally decreased with increasing height of the structure. For example, going from an 18-inch to a 36-inch structure resulted in 15% more shortwave radiation reaching the surface. The biggest implication for this would be location of sensors for measuring PPFD. Sensors should be located as close to the actual surface as possible, or measurements may ignore diffuse radiation and appear artificially lower than what the turfgrass is subjected to.

Total net radiation was reduced by 50 to 120 Watts/m2 depending on shade material. Two layers of neutral shade fabric had the greatest reduction among treatments. Increasing height again decreased the severity of shade in comparison to the lowest height. Interestingly, there were little to no differences in structure height once reaching 36-inches.

Future Expectations

We reported on objectives 2 and 3 in previous summary reports. With this summary report, we have completed objective 1 and hope to move towards development of a final report and publication of results in a refereed journal. Future research efforts should continue to examine light quality response of warm-season grasses using various species, cultivars, and light regimes.

Table 1. Reduction in net radiation under various shade structure types.

Factor	Treatment	Longwave	Total	Shortwave
Shade Fabric		------- Reduction in Net Radiation-------		
	Neutral (1x)	-39.45A	84.56B	124.01B
	Neutral (2x)	-66.99B	120.48A	187.47A
	Neutral (1x)+ Reduced R	-55.94B	64.36C	120.31B
	Neutral (2x) + Reduced R	-62.15B	51.88C	114.03B
Height (inches)				
	18	-72.19B	90.26A	162.45A
	36	-52.31A	85.15AB	137.46AB
	54	-57.45AB	75.72AB	133.16AB
	72	-42.58A	70.15B	112.74B
p-values	*Shade Treatment*	***0.0089***	***<.0001***	***<.0001***
	Height	***0.0078***	*0.0892*	***0.0232***
	Interaction	*0.1147*	***0.0145***	*0.0907*

[a] Calculated as net radiation (W/m^2) under full sun conditions minus net radiation under shade.

Fig. 1 Setting up various micrometerological sensors under deciduous tree shade in Stillwater, OK.

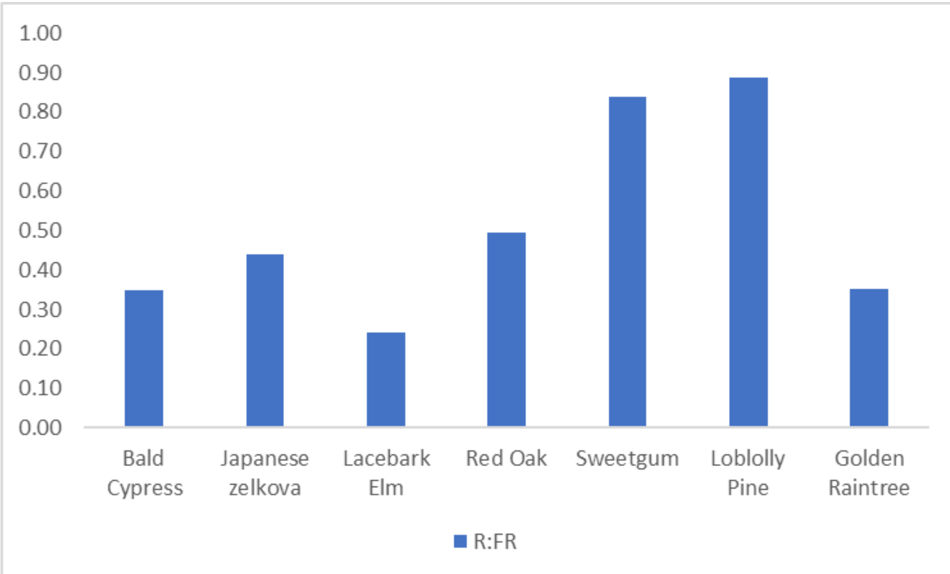


Fig. 2. Red to far red ratio (R:FR) of light transmitted through various trees in Stillwater, OK.

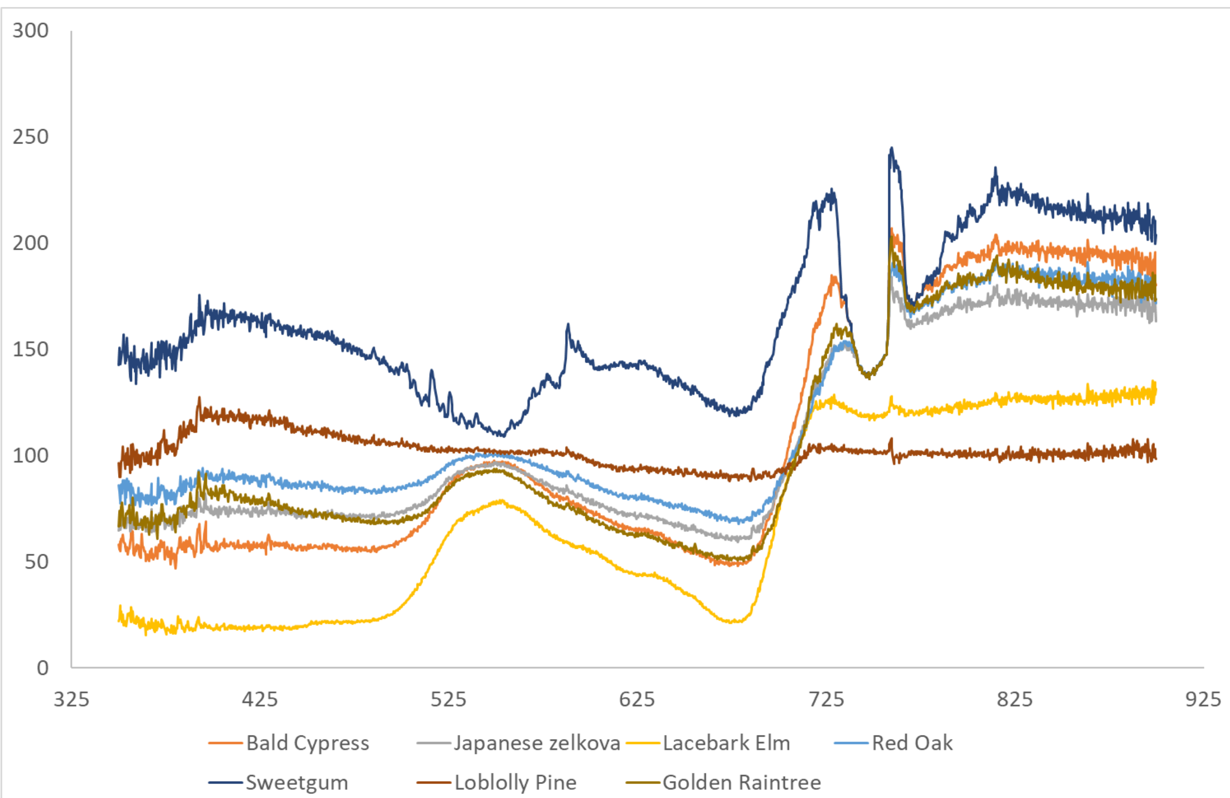


Fig. 3 Relative transmittance among various shade trees in Stillwater, OK.

USGA ID#: 2021-07-731

Title: Shade and water quality effects on efficacy of plant growth regulators
Project leaders: Michael Richardson[1], James Brosnan[2], and Aaron Patton[3]
Affiliations: [1]University of Arkansas, [2]University of Tennessee, [3]Purdue University

Objectives: There are two primary objectives associated with this project:

- Determine the effects of GDD-based application intervals of plant growth regulators on the shade tolerance and daily light requirements of an ultradwarf bermudagrass putting green.
- Determine the effects of divalent cations in the spray solution on the efficacy of trinexapac-ethyl when applied to ultradwarf bermudagrass

Start Date: 2021
Project Duration: 2 years
Total Funding: $60,078

Summary Points:

- Plant growth regulators (PGR) are commonly used on putting green turf to enhance performance to reduce the overall growth of the turf. In recent years, the application timing strategy has moved towards a growing-degree based model compared to calendar-based applications.
- PGRs have been shown to enhance plant performance in shaded environments, especially on warm-season grasses. A field study was conducted at two locations in 2021 and repeated in 2022 to compared different PGR application timings (calendar vs GDD) under 4 differing shade levels. At both locations, calendar-based applications of Primo Maxx have consistently produced higher quality under shaded conditions compared to a GDD application timing.
- Studies to investigate the effects of water quality on PGR efficacy were initiated in 2022, but the data has not been summarized to date.

Executive Summary:

Bermudagrass (*Cynodon spp.*) is one of the most important turfgrasses for southern and transition zone putting greens. While there are countless cultural and chemical practices that are utilized to effectively manage ultradwarf greens, plant growth regulators (PGRs) are often considered an essential component of putting green management. Plant growth regulators have been effectively used to reduce vertical growth, reduce mowing frequency, improve mowing quality, suppress seedheads, enhance stress tolerance, and reduce water use (Reicher et al., 2013). Trinexapac-ethyl, a common PGR, has been shown to enhance shade stress, especially in warm-season grasses (Qian and Engelke, 1999; Bunnell et al., 2005).

Historically, PGRs such as trinexapac-ethyl were applied on a calendar-based schedule. Over the last decade, a number of studies have documented that re-application of PGRs based on temperature-dependent, growing-degree-day (GDD) models is a more effective approach to maintain suppression and avoid surge growth (Kreuser and Soldat, 2011; Kreuser et al. 2017; Reasor et al., 2018). This approach to PGR use has been broadly-adopted in the golf course industry and has shown to be an effective strategy in both cool-season and warm-season putting greens.

All studies to date that have assessed the effects of PGRs on shade tolerance of warm-season turfgrasses have used calendar-based applications according to the manufacturer's label. As numerous labels are now describing application timings based on GDD models, the application frequency of the PGR is either increasing or decreasing during the growing season based on temperature conditions. While it is assumed that the suppressive effects of the PGR are more consistent across the growing season with GDD-based application timings, we have no information regarding how the change in application frequency might affect stress tolerance of the turf.

Study Methods: A study was conducted at the University of Arkansas (Fayetteville) and the University of Tennessee (Knoxville) during the 2021 and 2022 growing seasons to compare calendar vs GDD timings of two common PGRs under varying shade levels on an ultradwarf bermudagrass green (cv. TifEagle). Both sites were maintained using common cultural practices for the region, including frequent, light fertilization, routine topdressing, and irrigation to prevent drought stress. Both sites were maintained at a 0.125 inch height of cut. Four shade treatments and five plant growth regulator treatments were applied in a strip plot design across 4 replicate blocks per location. Treatment details are as follows:

Shade treatments used at both sites, along with the average daily light integral produced with each treatment. Shade was applied using various intensities of shade cloth (Bulk Shade Cloth, International Greenhouse Co., Danville IL).

Shade treatment	**Average daily light integral**
	(mol m^{-2} day^{-1})
Full sun (0% shade)	45.0
20% shade cloth	36.0
40% shade cloth	27.0
60% shade cloth	18.0

Plant growth regulator treatments, including application rates and timing.

Product (active ingredient)	**Application rate**	**Application interval**
Primo Maxx (trinexapac-ethyl)	3.0 fl oz / acre	7 days
	3.0 fl oz / acre	220 $GDD_{10°C}$ units †
Anuew (prohexadione calcium)	8.0 oz / acre	7 days
	8.0 oz / acre	120 $GDD_{10°C}$ units
Non-treated control		

† Growing-degree-day reapplication intervals based on the work of Reasor et al., 2018. All GDD calculations were based on a 10 °C base temperature

Plot were rated bi-weekly for turfgrass quality and digital images were also collected bi-weekly to assess turfgrass coverage. For this summary, only the turfgrass quality data are reported.

Results: Under the full sun and 20% shade treatments, all treatments produced acceptable turfgrass quality (Quality > 6.0) throughout the growing season (Figures 1 and 2). Under the 40 and 60% shade treatments, all treatments began to decline below acceptable levels of turfgrass quality in July and continued to decline for the remainder of the growing season. However, all PGR treatment combinations consistently improved turfgrass quality compared to the untreated control. It should be noted that a misapplication of all PGR treatments occurred early in the trial site at Arkansas, which caused significant bronzing to all plots and resulted in low quality numbers. The phytotoxicity lasted for almost a month, but the plots returned to more normal conditions for the last 8 weeks of the season.

There were no statistical differences in turfgrass quality for the Anuew treatments, suggesting that both calendar-based and GDD-based application intervals can be used to enhance shade tolerance of an ultradwarf green (Figures 1 and 2). However, the GDD interval for Primo Maxx experienced reduced quality in the 40 and 60% shade treatments compared to the calendar-based application in 3 of the 4 trials.

When compared to the calendar-based interval (every 7 days), the Primo-GDD treatments were generally applied on approximately 15-day intervals throughout the season, while the Anuew-GDD treatments were applied on approximately 8-day intervals. This extension of the interval for Primo Maxx appears to reduce its effectiveness with regard to shade tolerance of an ultradwarf putting green.

References

Bunnell, B. T., L. B. McCarty, and W. C. Bridges. 2005. 'TifEagle' bermudagrass response to growth factors and mowing height when grown at various hours of sunlight. Crop Sci. 45:575-581.

Kreuser, W. C., and D. J. Soldat. 2011. A growing degree day model to schedule trinexapac-ethyl applications on Agrostis stolonifera golf putting greens. Crop Sci. 51: 2228-2236.

Kreuser, W. C., J. R. Young, and M. D. Richardson. 2017. Modeling performance of plant growth regulators. Agric. Environ. Letters. 2:170001 [1-4].

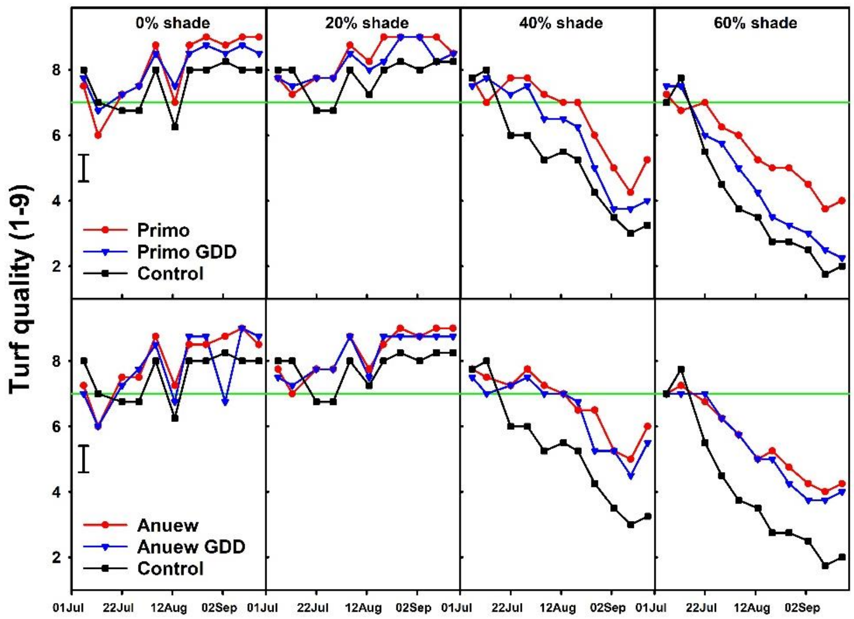
Qian, Y. L., and M. C. Engelke. 1999. Influence of Trinexapac-Ethyl on Diamond Zoysiagrass in a shade environment. Crop Sci. 39:202-208.

Reasor, E. H., J. T. Brosnan, J. P. Kerns, W. J. Hutchens, D. R. Taylor, J. D. McCurdy, et al. 2018. Growing degree day models for plant growth regulator applications on ultradwarf hybrid bermudagrass putting greens. Crop Sci. 58:1801-1807.

Reicher, Z. J., P. H. Dernoeden, and D. S. Richmond. 2013. Insecticides, fungicides, herbicides, and growth regulators used in turfgrass systems. In Stier, John C., Horgan, Brian P., and Bonos, Stacy A. (eds.) Turfgrass: Biology, Use, and Management. Madison, Wisconsin: American Society of Agronomy.

Figure 1. Turfgrass quality across the 2021 (top) and 2022 (bottom) growing seasons in Fayetteville AR for the various shade and PGR treatments. The green reference line represents a minimal acceptable quality rating of 7.0. Error bars represent the least significant difference (P=0.05) for comparing treatment means.

2021 results – Fayetteville AR



2022 results – Fayetteville AR

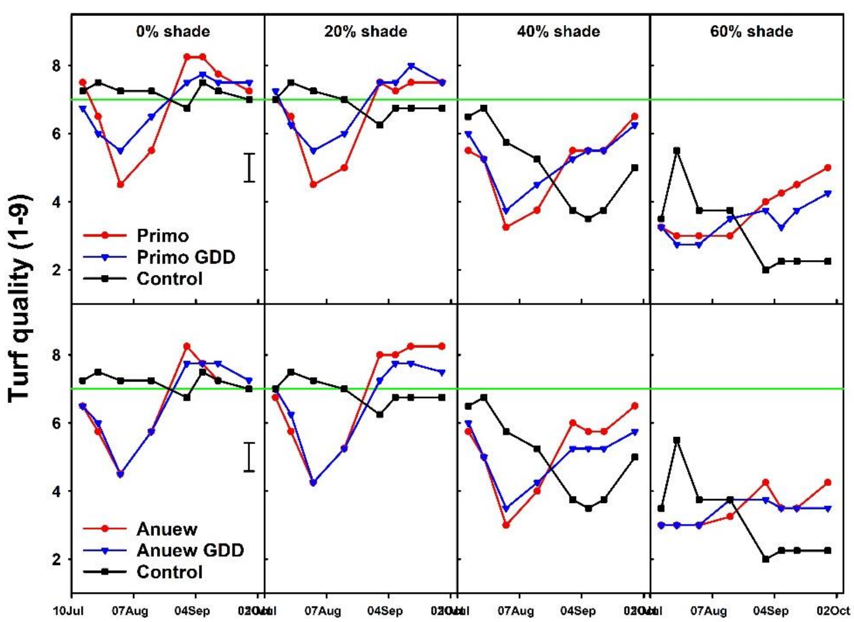
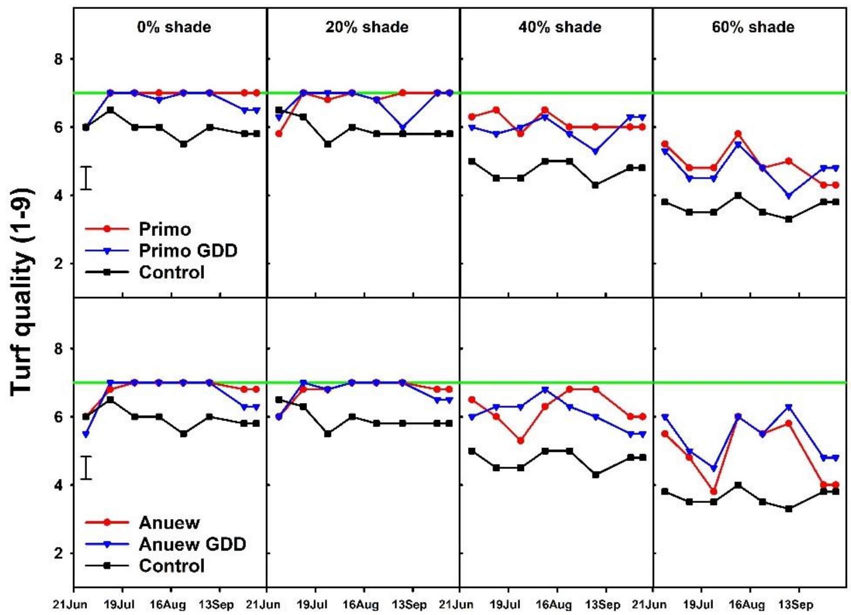
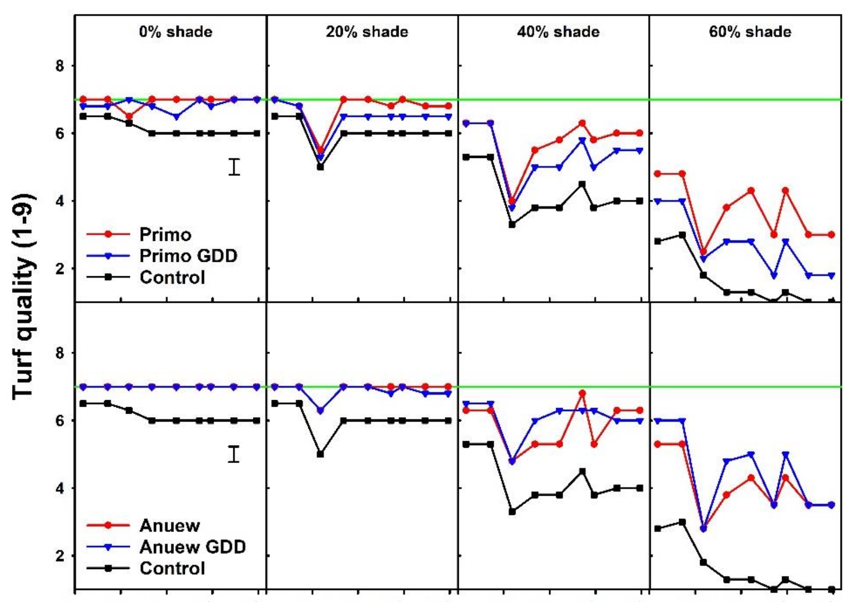


Figure 2. Turfgrass quality across the 2021 (top) and 2022 (bottom) growing seasons in Knoxville TN for the various shade and PGR treatments. The green reference line represents a minimal acceptable quality rating of 7.0. Error bars represent the least significant difference (P=0.05) for comparing treatment means.

2021 results – Knoxville TN



2022 results – Knoxville TN



USGA ID#: 2019-14-684

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

Project Leader: 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

This report presents the results of the second-year golf course trial conducted in 2022, addressing objective 3; to investigate the effects of plant-health products on the summer performance of annual bluegrass on putting green conditions.

Summary Points:

- The fungicides consisting of chlorothalonil, acibenzolar-S-methyl or plant-health enhancer had positive effects on improving the turf performance of *Poa*/bentgrass mixed greens throughout the summer.
- Seaweed extracts improved turf performance at the beginning and end of the summer when the putting green was well-irrigated during the summer months.
- The positive effects of the fungicide and seaweed extract biostimulants on improving the summer performance of Poa/bentgrass putting greens were consistent in the two-year trials.
- The effects of most amino acid and hormone treatments tested in this trial were inconsistent between the two putting greens, with the treatment of ethylene inhibitor combined with amino acids showing positive effects on turf quality on the putting green in late August when the putting green was well-irrigated.

Methodology:

<u>Location Description:</u>

Tamarack Golf Course is a two 18-hole course located in East Brunswick, NJ with putting greens originally established with creeping bentgrass, but now have a mixture of *Poa* and bentgrass. Trials were conducted on putting greens of two different holes on the West course; hole 4 and hole 17. Putting greens are mowed at 0.150'', well-irrigated and fertilized, with disease control. *P. annua* was not uniformly distributed on the Poa/Bent putting greens and was difficult to find uniformly distribution *Poa* as is typical in a real-world situation. A total of 3 replicate patches were selected for each treatment at each hole within this study. The replicate plots were arranged in three rows with 9 plots per row; one replicate per treatment.

This season was particularly dry resulting in a greater risk of drought. Of the two putting greens, the 4th green was well irrigated. The 17th green was overall drier and underwent a drought incident starting in early August due to a clog in one of the sprinkler heads. This resulted in ununiform dry spots on the 17th green during August that was most severe on August 16th before the sprinkler head was cleared.

<u>List of Regular Golf Course Spray Program</u>

Sprays were applied twice a month, with each spray including Plant Food products; 29-0-0, 8-27-5, Impulse, Micro Pack, Kelp 101 Manganese, Phosphite 30, Flo Thru A+ and Hydration A+ as well as Cutless, Primo Maxx and Par Pigment.

Fungicides were applied as follows;
4/12 - Honor and Proxy instead of Cutless
5/05 - Daconil Zn, Densicor and Proxy instead of Cutless
5/26 - Secure, Velista and back to Cutless from here out
6/10 - Lexicon
6/23 - Daconil Zn, Signature Xtra
7/12 - Navicon
7/26 - Secure, Signature
8/12 - Daconil Zn, Velista, Segway
8/24 - 26GT, Signature Xtra
9/09 - Daconil Zn, Tartan Stressguard
9/28 - Mancozeb, 26 GT
All fungicide rates were applied at the label minimum.
Insecticides and herbicides were also applied as a general control against pests and weeds.

<u>Four types of experimental treatments:</u>

Each treatment was applied by foliar spray every two weeks throughout the summer from June 2nd – August 26th at a carrier volume of 2 gal/1000 sq ft.

Fungicides with plant-health benefits

The fungicides consist of chlorothalonil, acibenzolar-S-methyl, or plant-health enhancer.

1) Fungicide 1: Daconil Action (3.5 fl oz/1000 sq ft) + A14658H (6.0 fl oz/1000 sq ft)
2) Fungicide 2: Daconil Action (3.5 fl oz/1000 sq ft) + A23728A (6.0 fl oz/1000 sq ft)

Seaweed extract biostimulants (SWE):

1) Seaweed Extract 1 (SWE-1): XP (6 oz/1000 sq ft) + Stress Rx (6 oz/1000 sq ft)

2) Seaweed Extract 2 (SWE-2): XP-N (6 oz/1000 sq ft) + Stress Rx (6 oz/1000 sq ft)

Amino acids and hormones:

1) Ethylene inhibitor: Aminoethoxyvinylglycine (AVG) at 25 µM solution
2) Amino acid/hormone mixture (RU): amino acid at 60 mM + hormone at 44 µM
3) AVG + RU: combination of treatments 1 and 2

Untreated control (Con): plants sprayed with water.

Measurements:

The following measurements were taken weekly. Turf quality (TQ) was visually rated. Normalized Difference Vegetation Index (NDVI), Stress Index (SI) and leaf area index (LAI) were evaluated using a multispectral radiometer (CropScan).

Summary of Results:

The data collected from each green was analyzed separately for treatment differences compared to the untreated control, considering the varying conditions of the two putting greens. The turf on the 17th green suffered from both heat stress and drought stress in the middle of summer due to irrigation malfunction. The 4th green was maintained well irrigated and the summer decline in turf performance was mainly due to heat stress.

Fungicide Treatments

Both fungicide treatments improved turf quality compared to the control. On the 4th green treated with either fungicide had significantly higher TQ during June and August (Figure 1A, Photo 1). On putting green 17 both treatments had relatively higher TQ at the beginning of the summer before the drought incident where TQ dropped for all treatments (Figure 1B).

Both fungicides had significantly higher NDVI compared to the control at putting green 4 (Figure 2A) while putting green 17 plots had relatively higher effects (Figure 2B).

SI, LAI, and canopy temperature were not consistently affected by either fungicide treatment when compared to the control. However, on putting green 17 both had lower stress levels on June 28th (Figure 3B), and on the 4th green, Fungicide-2 had higher LAI on July 5th (Figure 4A).

Seaweed Extract Treatments

Both seaweed extract treatments ("XP + Stress Rx" and "XP-N + Stress Rx") had a positive effect on TQ, both maintaining relatively higher TQ compared to the control throughout the summer with significant improvements in early July and late August. However, these significant results were only seen on putting green 4 (Figure 1C, Photo 2) and not on putting green 17 (Figure 1D). This was because of the dry spots caused by uneven watering on the 17th green.

On putting green 4, SWE-2 had a slightly positive effect on NDVI compared to the control with significant differences being measured on July 19th and August 2nd while SWE-1 only had relatively higher NDVI on these dates (Figure 2C). On putting green 17, SWE-2 had higher NDVI compared to the control throughout the summer (Figure 2D).

Early in the summer both SWE treatments had lower stress levels (Figure 3C and D) and greater LAI (Figure 4C and D) than the control. SWE-1 had significant differences on putting green 17 before drought stress caused a decline in performance and SWE-2 had significant effects on the 4th green for both SI and LAI.

Amino Acid + Hormone Treatments

Of the three treatments (“AVG”, “RU”, and “AVG + RU”) there were little effects on TQ throughout the summer, but all treatments were relatively higher than the control towards the end of summer on putting green 4. This is especially seen in the combination treatment, AVG + RU (Figure 1E). There were no effects on putting green 17 plots, likely due to uneven dry spots confounding the treatment effects (Figure 1F).

There were no significant effects on NDVI for the different treatments when compared to the control on either putting green (Figure 2E and 2F).

There were little to no effects on stress level or LAI on putting green 4 (Figure 3E and 4E). However, on putting green 17 treatments AVG and AVG + RU had significantly lower stress levels in early June (Figure 3F) and significantly higher LAI (Figure 4F).

Conclusions:

The fungicides consisting of chlorothalonil, acibenzolar-S-methyl or plant-health enhancer, and seaweed extract biostimulants were effective in promoting Poa performance in both field plots at the Rutgers research farm in 2020 and 2021 and the putting greens on the golf course under golf course putting-green conditions in 2021 and 2022. The fungicides with plant-health benefits and seaweed extract biostimulants can be incorporated into best practices in managing *Poa* putting greens to control summer decline and improving Poa summer performance on golf courses.

Amino acid and hormone treatments were more effective in summer 2021 when the putting greens were well irrigated and only exposed to summer heat stress than that in 2022 when Poa putting green was subjected to summer heat and drought stress due to irrigation malfunctions on the golf course. Amino acids and hormones tested in this trial may become less beneficial when Poa is exposed to severe stress due to the combined drought and summer heat. Better understanding and optimizing the application rates and intervals for amino acid and hormone treatments are needed for potential use in managing Poa putting greens that may suffer from the combined drought and heat stress during summer months.

Figure 1: Turf quality of mixed stand of *Poa*/creeping bentgrass putting greens as effected by different plant health products.

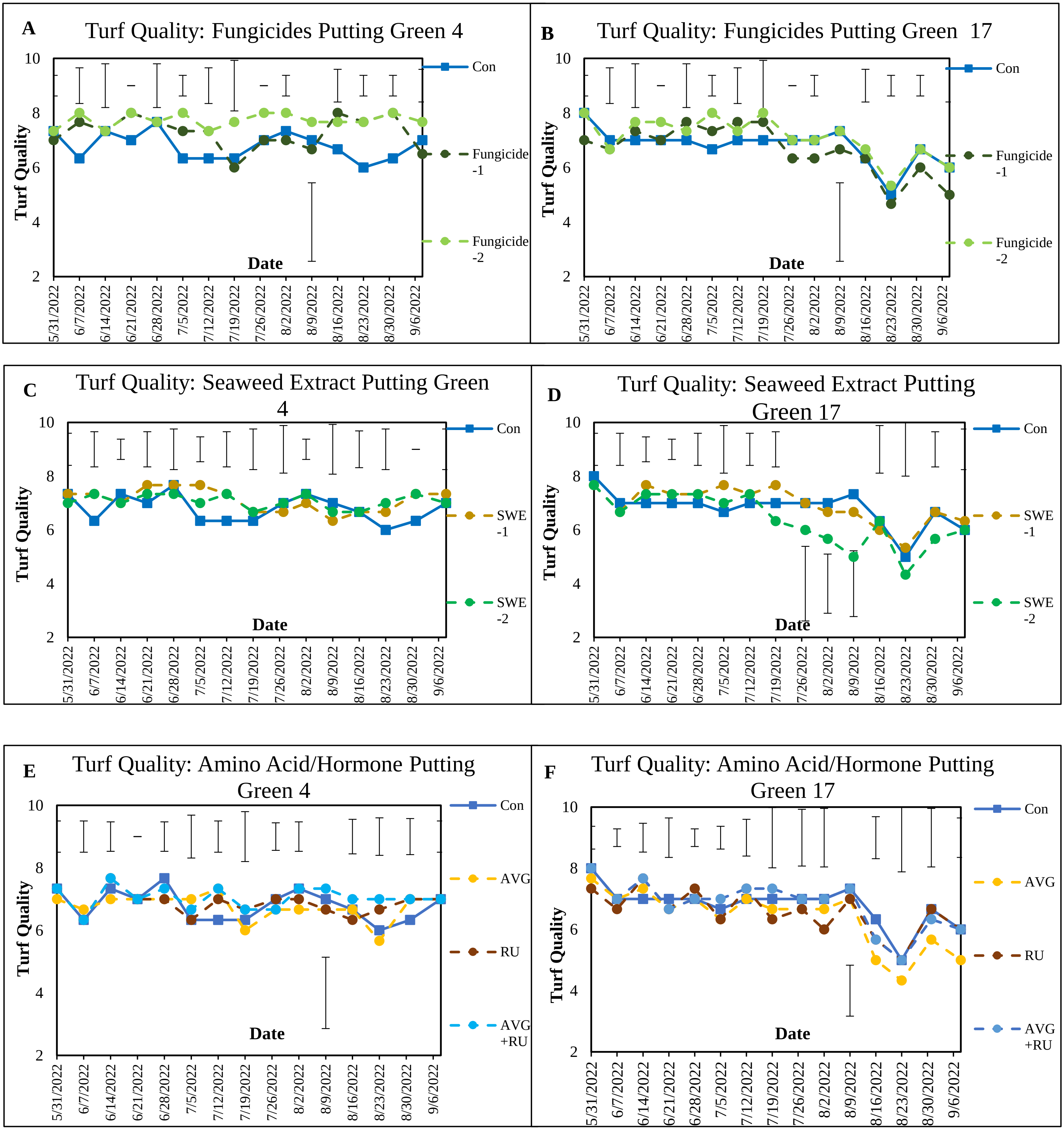


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Photo 1. Improved Poa summer performance on a putting green by application of fungicides with chlorothalonil, acibenzolar-S-methyl or plant-health enhancer

Photo 2. Improved Poa summer performance on a putting green by application of seaweed extract biostimulant

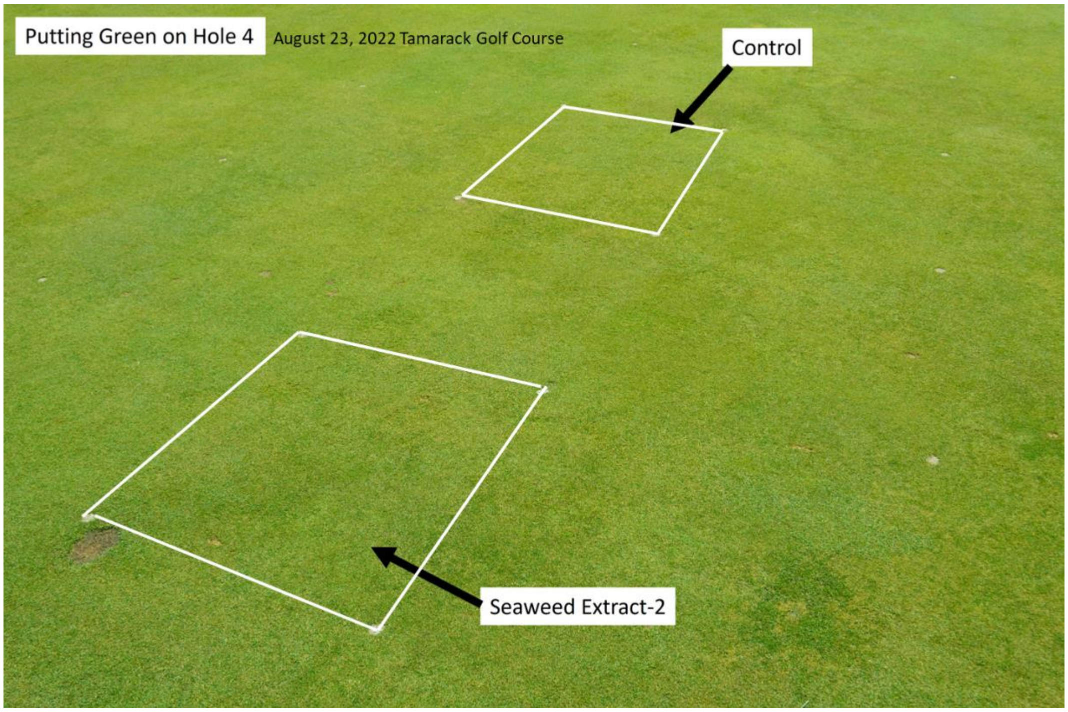


Figure 2: Normalized Difference Vegetative Index of *Poa*/creeping bentgrass putting greens as effected by different plant health products.

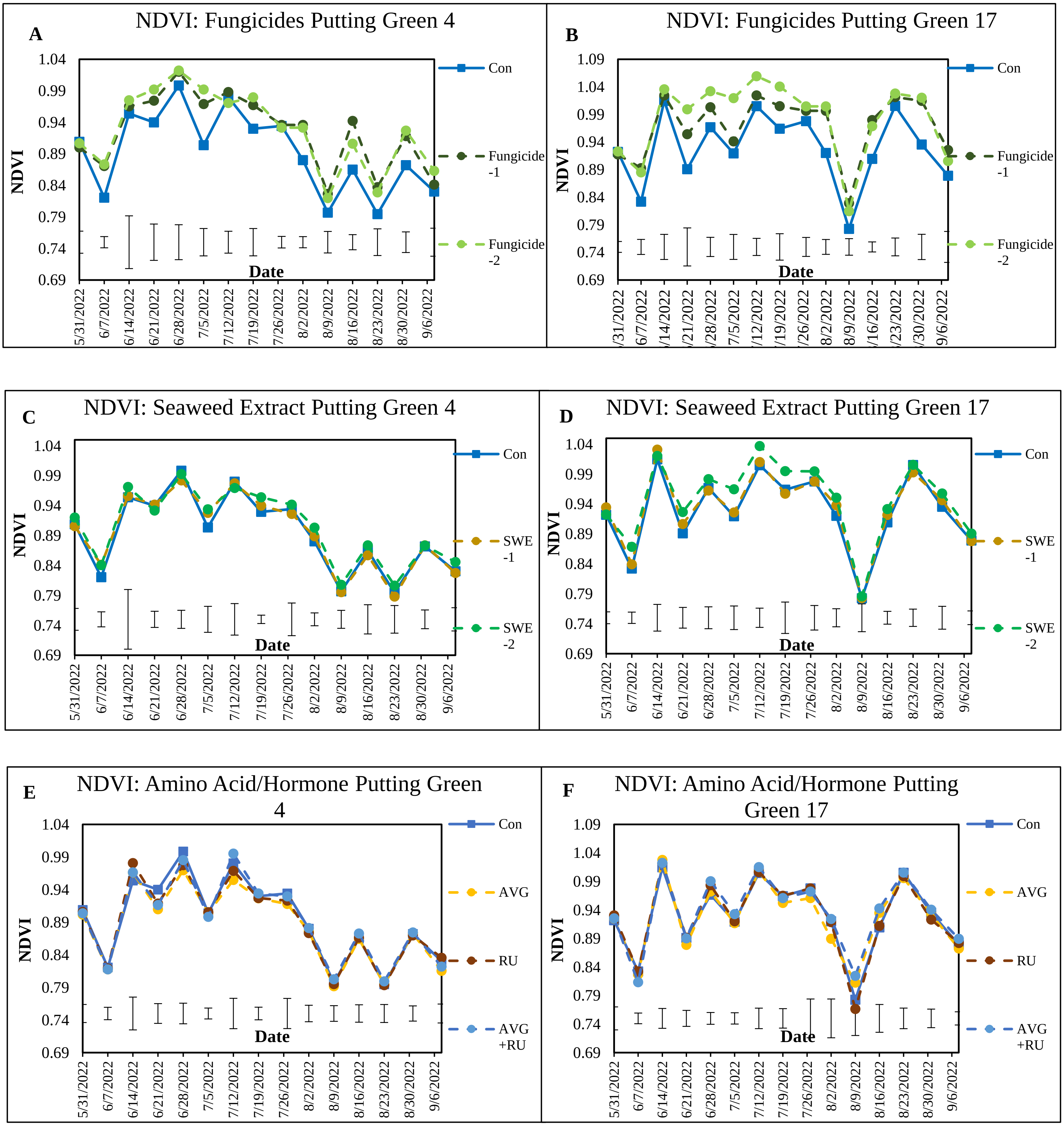


Figure 3: Stress Index of *Poa*/creeping bentgrass putting greens as effected by different plant health products. SI corresponds to stress of the plant.

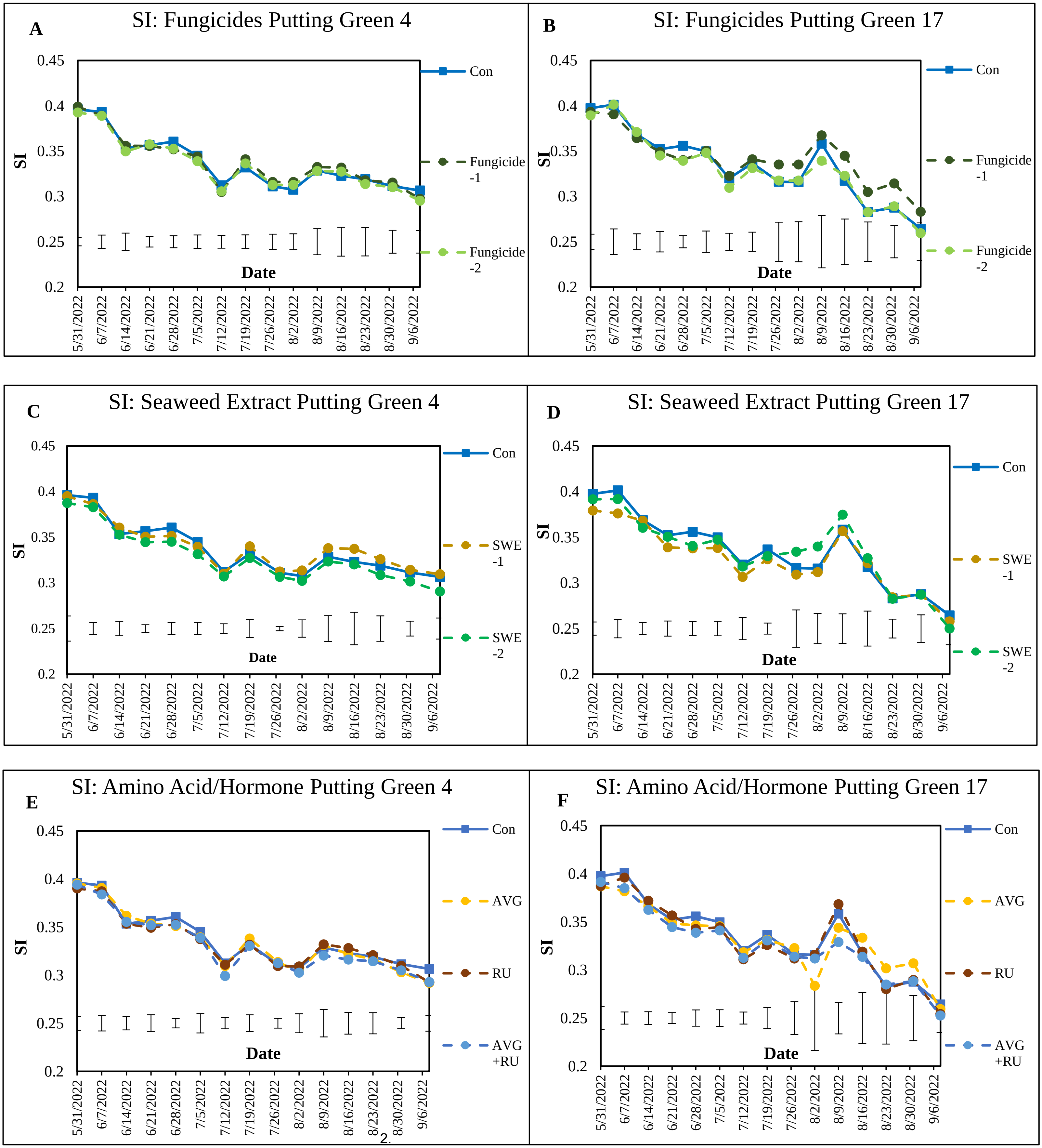
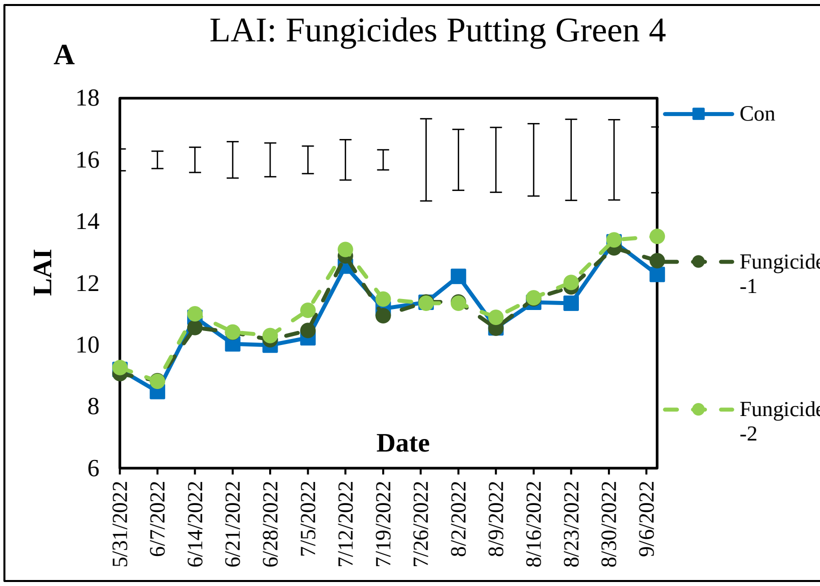
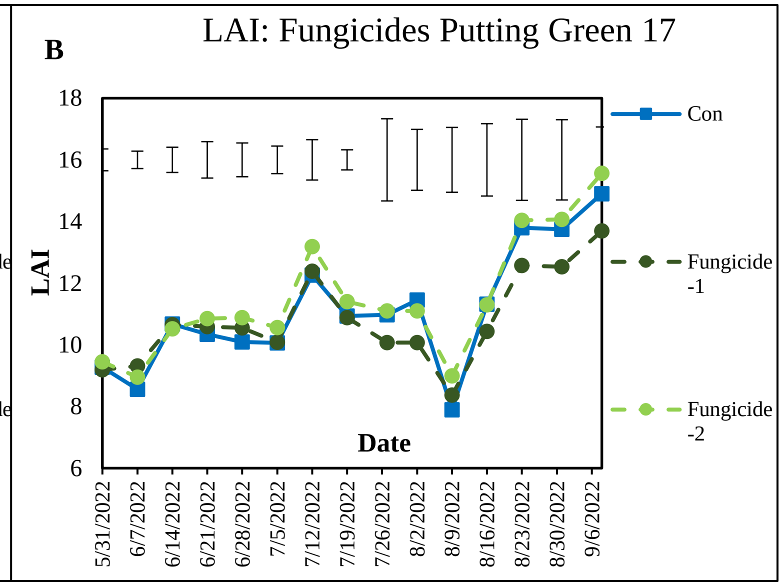
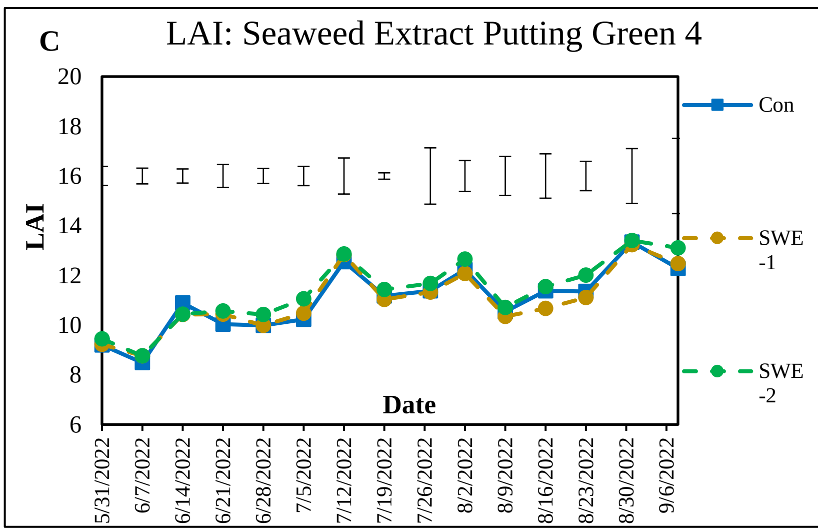
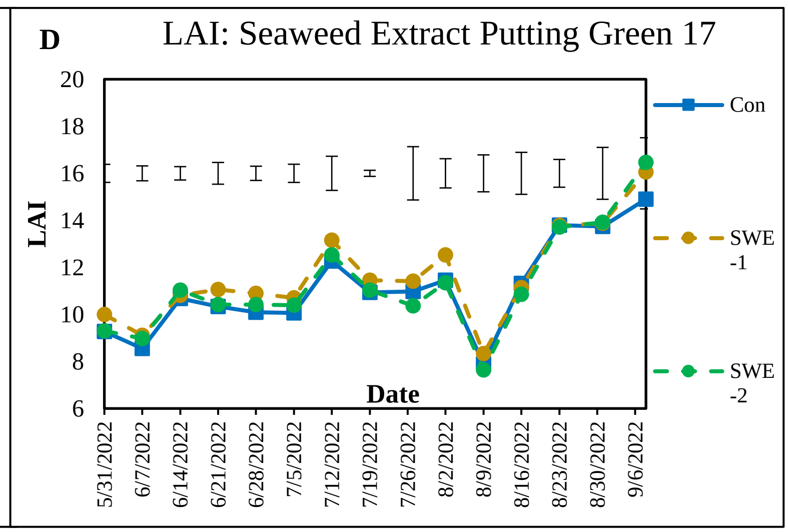


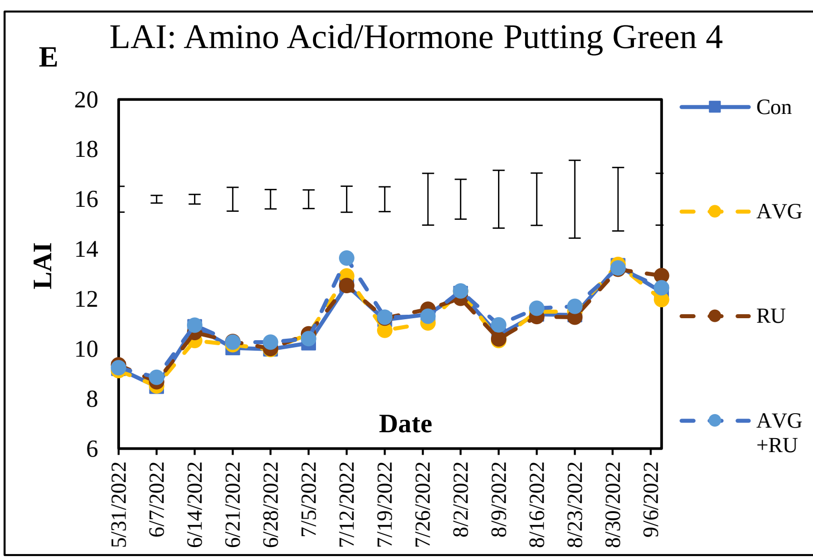
Figure 4: Leaf Area Index of *Poa*/creeping bentgrass putting greens as effected by different plant health products.

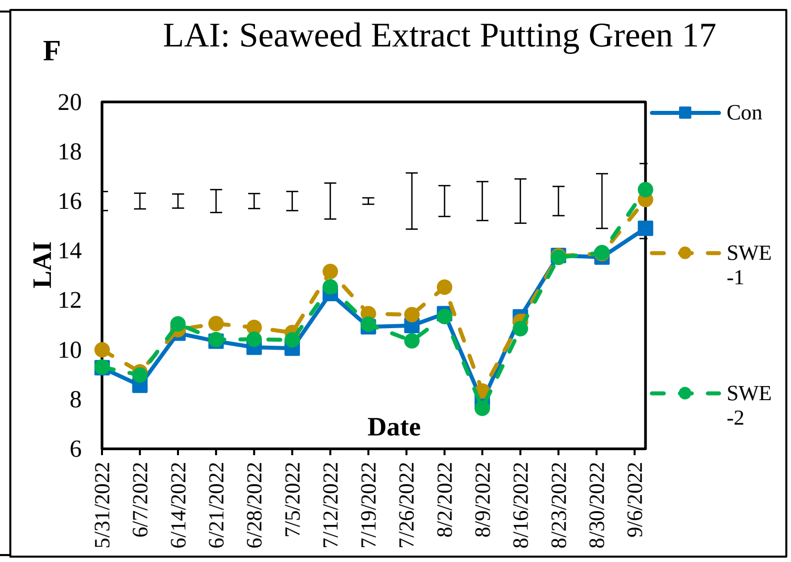












USGA ID#: 2020-06-711

Title: Timings and Rates of Proxy for Suppression of Annual Bluegrass Seed Heads on Putting Greens

Project Leader: Alec Kowalewski
Collators: Brian McDonald, Emily Braithwaite, and Clint Mattox

Affiliation: Department of Horticulture, Oregon State University

Objectives:

1. Will adding one application of Proxy applied October through February (along with traditional spring timing) improve annual bluegrass seed head suppression?
2. Will lower rates of Proxy applied with Primo during the summer improve annual bluegrass seed head suppression? (Note: Maximum annual Proxy amount is 30 fl. oz./yr.)

Start Date:
October 2020

Project Duration:
3 years, October 2020 to October 2023

Total Funding:
$30,000

Summary Points:

- Year 2 (2022) results determined that sequential applications of Proxy in January, February, March, April and May totaling 25 fl.oz./year (less than the annual max of 30 fl.oz/year) provided the greatest seedhead suppression.
- In both years (2021 and 2022) Proxy applied in January or February, and then March, April and May provided greater seedhead suppression than treatments applied in October, November, or December combined with March, April and May.
- These findings would suggest that late winter Proxy applications combined with spring applications will provide the greatest seedhead suppression in the Pacific Northwest.

Introduction:

Historically, seed head suppression of annual bluegrass with Proxy has been inconsistent. There may be several factors but clearly the weather and the timing of applications is a factor. One of the complications is that annual bluegrass is not one variety of one species, but rather is a diverse continuum of biotypes that react differently depending on many factors including the climate and the maintenance practices applied to it. Annual bluegrass initiates seed heads (flowers, inflorescences) in late fall or winter, well ahead of their emergence in spring. To make matters more complicated, research conducted in 1997 by Johnson and White found that annual biotypes do not require vernalization (a cooling period) to flower, while perennial biotypes do require vernalization. This research also determined that short days substituted for vernalization induced seed head formation in some biotypes. Considering these differences, monthly Proxy applications in the fall, winter, and spring could be necessary for annual bluegrass seed head suppression in areas of moderate climate.

Objectives:

3. Will adding one application of Proxy applied October through February (along with traditional spring timing) improve annual bluegrass seed head suppression?

4. Will lower rates of Proxy applied with Primo during the summer improve annual bluegrass seed head suppression (Note: Maximum annual Proxy amount is 30 fl. oz./yr.)

Materials and Methods:

A field trial was initiated in October 2020 at the Oregon State University Lewis-Brown Horticulture farm in Corvallis, OR. This project will conclude in the fall of 2023, after three consecutive years of data collection. Research is being conducted on a well-established annual bluegrass putting green with 12” of USGA sand over drain tiles and native soil.

Experimental design is be a randomized complete block design with four replications. Proxy timing treatments were initiated in October 2020 and are being applied with a CO_2-pressurized bicycle sprayer (Table 1, Image 1). Applications have been made to the October and November treatments and will be made to the December 2020 to August 2021 treatments. The plots are being cored annually in the fall with hollow tines on a 2” x 2” spacing. Fungicides will be applied year-round to prevent diseases. The plots are being fertilized every 2 weeks during the growing season and monthly during the winter. The plots are being mowed no higher than 0.125 inches during the growing season and 0.140 inches during the winter.

Response Variables:

Beginning in spring 2021, percent annual bluegrass seed head cover and turfgrass visual quality will be rated weekly from when seed heads first become easily visible (approximately April 1) through the end of the intense seed head period (approximately June 15), and then every 2 weeks during the remainder of the summer, and monthly thereafter. Other monthly response variables will include turfgrass heath measured with a FieldScout CM 1000 NDVI Chlorophyll Meter. Visual turfgrass quality will be rated using the National Turfgrass Evaluation Program (NTEP) scale of 1 to 9.

Year 1 findings:

- One early application of Proxy made any month from October to February increased seed head control vs. the standard program beginning in March and making three monthly applications (i.e., March, April, & May).

- A trend in the data, which was replicated across two groups (Trts 4 – 8 and Trts 9 – 13), showed that the best control of seed heads occurred when the early application of Proxy was made later (i.e., February) rather than sooner October to January February time frame. Conversely, when comparing the treatments receiving an early application, the worst seed head control occurred on plots when the early application was made in October and the seed head control improved as you made later and later applications (i.e., November, December, January, or February).

- There was some indication that summer Proxy applications (June, July, & August) at 3.3 fl. oz. per 1,000 ft^2 reduced seed heads. However, the percent seed heads were generally so low across the untreated plots that there was no difference in quality.

- The untreated plots had over 40% seed head cover at the peak on May 28th and the plot quality was unacceptable. The highest average seed head cover on any other treatment treated with Proxy only reached 5.3 percent, indicating that Proxy is still very effective at reducing seed head production (although not perfect) and improving plot quality.

- The growing degree day treatment (Trt 2) performed similarly to the other two treatments applied in February (Trts 8 & 13). It was started one week later than the other February treatments and subsequent treatments were made one week later as well. We thought a GDD treatment was a good idea and was added as the last treatment. However, after further analysis, the treatment was redundant as the 'Calhoun – Michigan State' growing degree day model has a 200 – 500 GDD range (32 base) which translates, in a normal year, to the first application being applied sometime between last week of January and the third week of February and we already have January and February treatments. In future, years, this treatment may be changed. For example, we could test multiple early applications (e.g., making two early applications in January and February), or possibly changing the application interval on one the other treatments.

Year 2 findings:

Note: Treatment 2 (growing degree day model) was eliminated in year 2, and going forward, because the other treatments overlapped the time period when the growing degree day model indicated a treatment needed to be made (i.e., the GDD model application was redundant). We replaced the GDD model with a winter application of Proxy made in both January and February (i.e., 2 apps), and like all other treatments, with subsequent apps made in March, April, and May.

- For the second year in a row, an early application of Proxy at 5.0 fl. oz./1,000 ft^2 in any month (October through February), combined with March, April, and May applications at the same rate, resulted in less seed heads than a sequence of three monthly applications in March, April, and May (Table 6).

- The trend from last year continued (with some subtle differences) which showed that the later the first application was made (i.e., nearer to March vs. in the fall), the better the control of seedheads (Table 2 & Table 6). For example, plots treated in February, March, April, and May had the lowest seed heads, with one exception, noted below. However, in year 2, January applications resulted in a little higher level of seed heads vs. the other months, which did not follow the pattern. Notes taken at the time the January application was made, indicated that there was a problem with one of the nozzles that was not noticed until the last plot was being sprayed. This nozzle problem was likely the reason for the higher level of seed heads. If in Year 3, the results mirror year 1, then will know that the nozzle problem in year 2 was the cause of higher seed heads from the January applications. The other interesting difference is that the treatments that received summer applications in year 1 (Trts 9 – 13) had fewer seed heads than the plots that did not receive summer applications, and the above mentioned trend did not exist for these treatments, mainly because the percent seed heads remained at a much lower level (i.e., all the timings performed well). Unfortunately, we were not able to make the summer applications in year 2 (see last bullet point, below), and will not be able to determine whether the summer application effect seed heads levels the next spring unless we extend the trial for another year.

- Treatment 2, the new treatment added this year, with Proxy applications made in January, February, March, April, and May had the lowest level of seed heads. The highest percentage of seed heads from this treatment throughout the trial occurred on June 8th and was only .05 percent. This compares favorably to the highest percentage of seed heads for plots sprayed in March, April, and May (Trt 3) of 12.50 percent, which occurred on April 22nd.

- There were very little differences in plot quality not related to a high percentage of seed heads. Low percentages of seed heads did not influence plot quality. The untreated plots averaged from 50 – 60 percent seed head cover in May and were rated as unacceptable from April 15th through June 8th (the last rating date). The Proxy applications made in the winter did lighten the turf color ½ to 1 rating value after treatment. However, when Primo was applied with Proxy (March – May), the color did not lighten.

- Year 2 experienced some environmental and chemical problems in mid and late June resulting in summer ratings and applications being untenable and the last rating date of June 8th. The trial area was heavily infected by a late season Microdochium patch outbreak in June (M. Patch season is normally over in June) as a result of the wettest spring on record. We sprayed the green on May 4th with Affirm (which is strong on M. Patch normally) and then sprayed it again with Affirm, Navicon, and Maxtima (M. Patch, anthracnose, yellow patch) on May 28th. In spite of these applications, the green got heavily infected with Microdochium patch. We sprayed the green again on June 14th with 26GT + Turfcide 400 + Densicor and on June 30th, with QuickSilver herbicide to control moss. The result of these last two applications damaged the healthy parts of the green slightly, but the patches that were infected with Microdochium patch were severely damaged. The green recovered at the end of summer after coring. We are not clear why these applications damaged the turf, and this green was one of three that were injured. We did a pilot study at the end of summer to try and duplicate the injury, but we were not successful.

Table 1: Thirteen different timing and rate (fl. Oz./1,000 ft^2) combinations of Proxy applications for annual bluegrass seed head suppression in Corvallis, OR fall 2020 to Spring 2023.

								Primo Included in these apps						
Trt #	1st App(s)	Subsequent Apps	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
1	untreated	Na												0
2	Jan & Feb	Mar, Apr, May				5	5	5	5	5				25
3	None	Mar, Apr, May						5	5	5				15
4	Oct	Mar, Apr, May	5					5	5	5				20
5	Nov	Mar, Apr, May		5				5	5	5				20
6	Dec	Mar, Apr, May			5			5	5	5				20
7	Jan	Mar, Apr, May				5		5	5	5				20
8	Feb	Mar, Apr, May					5	5	5	5				20
9	Oct	Mar, Apr, May	5					5	5	5	~~3.3~~	~~3.3~~	~~3.3~~	20
10	Nov	Mar, Apr, May		5				5	5	5	~~3.3~~	~~3.3~~	~~3.3~~	20
11	Dec	Mar, Apr, May			5			5	5	5	~~3.3~~	~~3.3~~	~~3.3~~	20
12	Jan	Mar, Apr, May				5		5	5	5	~~3.3~~	~~3.3~~	~~3.3~~	20
13	Feb	Mar, Apr, May					5	5	5	5	~~3.3~~	~~3.3~~	~~3.3~~	20

- Note: summer apps were not made due to turf injury from fungicide.

Table 2: Year 1 - Total Percent Seed Head from March 31st to September 10th, 2021

Trt #	1st App	Subsequent Apps	3/31 - 9/10 Sum	
1	Untreated	Untreated	343.0	
2	Feb	GDD - Started 1 week later	14.5	
3	None	Mar, Apr, May	28.4	
4	Oct	Mar, Apr, May	19.1	High --------> Low
5	Nov	Mar, Apr, May	18.3	
6	Dec	Mar, Apr, May	17.7	
7	Jan	Mar, Apr, May	13.6	
8	Feb	Mar, Apr, May	11.2	
9	Oct	Mar, Apr, May + Summer @ 3.3	19.0	High --------> Low
10	Nov	Mar, Apr, May + Summer @ 3.3	18.9	
11	Dec	Mar, Apr, May + Summer @ 3.3	15.3	
12	Jan	Mar, Apr, May + Summer @ 3.3	13.7	
13	Feb	Mar, Apr, May + Summer @ 3.3	12.1	

Table 3: Year 1 - Percent Seed Head Cover for the period 4/21 thru 5/5 (when most of the differences occurred).

Trt #	1st App	Subsequent Apps	Percent Seed Heads		
			04/21	04/29	05/05
1	Untreated	Untreated	27.4	33.4	33.6
2	Feb	GDD - Started 1 week later	1.8	1.5	1.1
3	None	Mar, Apr, May	4.0	5.3	3.0
4	Oct	Mar, Apr, May	3.5	4.0	2.5
5	Nov	Mar, Apr, May	2.5	2.8	3.3
6	Dec	Mar, Apr, May	1.8	3.0	2.5
7	Jan	Mar, Apr, May	1.3	1.3	1.8
8	Feb	Mar, Apr, May	1.3	1.1	1.4
9	Oct	Mar, Apr, May + Summer @ 3.3	4.8	2.5	2.8
10	Nov	Mar, Apr, May + Summer @ 3.3	2.5	3.3	3.1
11	Dec	Mar, Apr, May + Summer @ 3.3	1.5	2.0	3.0
12	Jan	Mar, Apr, May + Summer @ 3.3	1.3	1.1	1.5
13	Feb	Mar, Apr, May + Summer @ 3.3	1.0	0.7	0.7
		LSD @ .05	2.81	2.43	4.56

Table 3: Year 1 - Percent Seed Head Cover for the period 4/21 thru 5/5 (when most of the differences occurred). (Statistics calculated without the untreated).

			Percent Seed Heads		
Trt #	1st App	Subsequent Apps	04/21	04/29	05/05
2	Feb	GDD - Started 1 week later	1.8	1.5	1.1
3	None	Mar, Apr, May	4.0	5.3	3.0
4	Oct	Mar, Apr, May	3.5	4.0	2.5
5	Nov	Mar, Apr, May	2.5	2.8	3.3
6	Dec	Mar, Apr, May	1.8	3.0	2.5
7	Jan	Mar, Apr, May	1.3	1.3	1.8
8	Feb	Mar, Apr, May	1.3	1.1	1.4
9	Oct	Mar, Apr, May + Summer @ 3.3	4.8	2.5	2.8
10	Nov	Mar, Apr, May + Summer @ 3.3	2.5	3.3	3.1
11	Dec	Mar, Apr, May + Summer @ 3.3	1.5	2.0	3.0
12	Jan	Mar, Apr, May + Summer @ 3.3	1.3	1.1	1.5
13	Feb	Mar, Apr, May + Summer @ 3.3	1.0	0.7	0.7
		LSD @ .05	1.59	1.59	1.48

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Table 4: Year 1 - Turfgrass Quality for the period 4/21 thru 5/5 (when most of the differences occurred).

			Quality 1 – 9; 9 = best		
Trt #	1st App	Subsequent Apps	04/21	04/29	05/05
1	Untreated	Untreated	5.1	5.0	4.8
2	Feb	GDD - Started 1 week later	7.0	7.0	7.4
3	None	Mar, Apr, May	7.0	6.4	6.9
4	Oct	Mar, Apr, May	7.0	6.8	7.1
5	Nov	Mar, Apr, May	7.0	6.8	6.8
6	Dec	Mar, Apr, May	7.0	6.8	6.9
7	Jan	Mar, Apr, May	7.0	7.0	7.3
8	Feb	Mar, Apr, May	7.0	7.0	7.3
9	Oct	Mar, Apr, May + Summer @ 3.3	7.0	6.9	7.0
10	Nov	Mar, Apr, May + Summer @ 3.3	7.0	6.8	6.9
11	Dec	Mar, Apr, May + Summer @ 3.3	7.0	6.9	6.9
12	Jan	Mar, Apr, May + Summer @ 3.3	7.0	7.0	7.3
13	Feb	Mar, Apr, May + Summer @ 3.3	7.0	7.0	7.3
		LSD @ .05	0.099	0.274	0.376

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Table 5: Year 1 - Turfgrass Quality for the period 4/21 thru 5/5 (when most of the differences occurred). (Statistics calculated without the untreated.)

Trt #	1st App	Subsequent Apps	Quality 1 – 9; 9 = best		
			04/21	04/29	05/05
2	Feb	GDD - Started 1 week later	7.0	7.0	7.4
3	None	Mar, Apr, May	7.0	6.4	6.9
4	Oct	Mar, Apr, May	7.0	6.8	7.1
5	Nov	Mar, Apr, May	7.0	6.8	6.8
6	Dec	Mar, Apr, May	7.0	6.8	6.9
7	Jan	Mar, Apr, May	7.0	7.0	7.3
8	Feb	Mar, Apr, May	7.0	7.0	7.3
9	Oct	Mar, Apr, May + Summer @ 3.3	7.0	6.9	7.0
10	Nov	Mar, Apr, May + Summer @ 3.3	7.0	6.8	6.9
11	Dec	Mar, Apr, May + Summer @ 3.3	7.0	6.9	6.9
12	Jan	Mar, Apr, May + Summer @ 3.3	7.0	7.0	7.3
13	Feb	Mar, Apr, May + Summer @ 3.3	7.0	7.0	7.3
		LSD @ .05	ns	.285	.333

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Table 6: Year 2 - Percent Seed Head Cover for the period 3/16 thru 6/08.

Trt#	1st App(s)	Subsequent Apps	03/16	4/2	4/15	4/22	5/02	5/09	5/18	5/26	6/08	Avg
1	Untreated	Untreated	0.18	7.00	27.50	32.50	52.50	53.75	57.50	57.50	42.50	36.77
2	Jan & Feb	Mar, Apr, May	0.00	0.02	0.03	0.03	0.02	0.02	0.03	0.04	0.05	0.03
3	None	Mar, Apr, May	0.06	5.75	9.50	12.50	11.26	6.00	2.19	2.19	0.75	5.58
4	Oct	Mar, Apr, May	0.00	0.02	0.63	0.76	2.53	1.64	1.16	0.78	0.40	0.88
5	Nov	Mar, Apr, May	0.00	0.02	0.77	1.76	1.77	1.26	2.03	1.03	0.32	0.99
6	Dec	Mar, Apr, May	0.01	0.03	0.27	0.76	0.77	0.30	0.58	0.21	0.13	0.34
7	Jan	Mar, Apr, May	0.00	1.77	2.51	1.76	1.01	0.41	0.40	0.18	0.16	0.91
8	Feb	Mar, Apr, May	0.02	0.02	0.15	0.77	0.02	0.03	0.13	0.08	0.05	0.14
9	Oct	Mar, Apr, May + ~~Summer @ 3.3*~~	0.00	0.02	0.03	0.76	0.70	0.39	0.32	0.24	0.53	0.33
10	Nov	Mar, Apr, May + ~~Summer @ 3.3*~~	0.00	0.02	0.02	0.03	0.64	0.31	0.56	0.21	0.12	0.21
11	Dec	Mar, Apr, May + ~~Summer @ 3.3*~~	0.01	0.03	0.04	0.04	0.77	0.59	0.39	0.05	0.07	0.22
12	Jan	Mar, Apr, May + ~~Summer @ 3.3*~~	0.01	0.77	4.50	5.00	1.26	0.65	0.51	0.38	0.33	1.49
13	Feb	Mar, Apr, May + ~~Summer @ 3.3*~~	0.02	0.03	0.76	1.75	0.02	0.09	0.28	0.03	0.05	0.34
		LSD @ .05	**0.022**	**1.965**	**3.920**	**3.610**	**5.230**	**3.521**	**2.270**	**2.090**	**2.600**	
		p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Table 7: Year 2 - Percent Seed Head Cover for the period 3/16 thru 6/08 (Statistics without the Untreated Treatment Included)

Trt#	1st App(s)	Subsequent Apps	03/16	4/2	4/15	4/22	5/02	5/09	5/18	5/26	6/08	Avg
2	Jan & Feb	Mar, Apr, May	0.00	0.02	0.03	0.03	0.02	0.02	0.03	0.04	0.05	0.03
3	None	Mar, Apr, May	0.06	5.75	9.50	12.50	11.26	6.00	2.19	2.19	0.75	5.58
4	Oct	Mar, Apr, May	0.00	0.02	0.63	0.76	2.53	1.64	1.16	0.78	0.40	0.88
5	Nov	Mar, Apr, May	0.00	0.02	0.77	1.76	1.77	1.26	2.03	1.03	0.32	0.99
6	Dec	Mar, Apr, May	0.01	0.03	0.27	0.76	0.77	0.30	0.58	0.21	0.13	0.34
7	Jan	Mar, Apr, May	0.00	1.77	2.51	1.76	1.01	0.41	0.40	0.18	0.16	0.91
8	Feb	Mar, Apr, May	0.02	0.02	0.15	0.77	0.02	0.03	0.13	0.08	0.05	0.14
9	Oct	Mar, Apr, May + ~~Summer @ 3.3*~~	0.00	0.02	0.03	0.76	0.70	0.39	0.32	0.24	0.53	0.33
10	Nov	Mar, Apr, May + ~~Summer @ 3.3*~~	0.00	0.02	0.02	0.03	0.64	0.31	0.56	0.21	0.12	0.21
11	Dec	Mar, Apr, May + ~~Summer @ 3.3*~~	0.01	0.03	0.04	0.04	0.77	0.59	0.39	0.05	0.07	0.22
12	Jan	Mar, Apr, May + ~~Summer @ 3.3*~~	0.01	0.77	4.50	5.00	1.26	0.65	0.51	0.38	0.33	1.49
13	Feb	Mar, Apr, May + ~~Summer @ 3.3*~~	0.02	0.03	0.76	1.75	0.02	0.09	0.28	0.03	0.05	0.34
		LSD @ .05	**0.012**	**1.527**	**1.844**	**2.789**	**4.683**	**1.961**	**1.382**	**0.884**	**ns**	
		p-value	0.0000	0.0000	0.0000	0.0000	0.0021	0.0000	0.0406	0.0007	0.1914	

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Table 8: Year 2 – Turfgrass Quality (1-9; 9 = best) for the period 3/16 thru 6/08.

Trt#	1st App(s)	Subsequent Apps	03/16	4/2	4/15	4/22	5/02	5/09	5/18	5/26	6/08	Avg
1	Untreated	Untreated	7.25	6.50	5.38	5.38	4.75	4.75	4.63	4.50	4.75	5.32
2	Jan & Feb	Mar, Apr, May	6.50	6.38	7.00	7.00	7.50	7.50	7.50	7.50	7.00	7.10
3	None	Mar, Apr, May	7.00	6.38	6.13	6.38	6.50	6.88	7.25	7.13	7.00	6.74
4	Oct	Mar, Apr, May	6.25	7.00	7.00	6.88	7.13	7.50	7.38	7.38	7.00	7.06
5	Nov	Mar, Apr, May	6.75	6.88	6.88	6.88	7.25	7.38	7.38	7.25	7.00	7.07
6	Dec	Mar, Apr, May	6.75	7.00	7.00	7.00	7.38	7.50	7.50	7.50	7.00	7.18
7	Jan	Mar, Apr, May	6.88	6.75	6.75	6.88	7.50	7.50	7.50	7.50	7.00	7.14
8	Feb	Mar, Apr, May	7.00	6.63	7.00	7.00	7.50	7.50	7.50	7.50	7.00	7.18
9	Oct	Mar, Apr, May + ~~Summer @ 3.3*~~	6.00	6.88	7.00	6.88	7.50	7.50	7.50	7.50	7.00	7.08
10	Nov	Mar, Apr, May + ~~Summer @ 3.3*~~	6.63	7.00	7.13	7.00	7.50	7.38	7.50	7.50	7.00	7.18
11	Dec	Mar, Apr, May + ~~Summer @ 3.3*~~	7.00	7.00	7.00	7.00	7.38	7.50	7.50	7.50	7.00	7.21
12	Jan	Mar, Apr, May + ~~Summer @ 3.3*~~	6.88	6.75	6.50	6.50	7.38	7.50	7.50	7.50	7.00	7.06
13	Feb	Mar, Apr, May + ~~Summer @ 3.3*~~	6.88	6.63	7.00	6.88	7.50	7.50	7.50	7.50	7.00	7.15
		LSD @ .05	**0.351**	**0.340**	**0.283**	**0.359**	**0.476**	**0.126**	**0.192**	**0.180**	**0.115**	
		p-value	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

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Image 1 (Yr. 1): Two Untreated plots (left side) with excessive annual bluegrass seed heads on April 21st, 2021 showing unacceptable quality on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.

Image 2 (Yr. 1): Closeup of one Untreated plot with excessive annual bluegrass seed heads on April 21st, 2021 on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.

Image 3 (Yr. 2): Untreated plot (right) with excessive annual bluegrass seed heads on May 26th, 2022, on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.

Image 4 (Yr. 2): Turfgrass color lightening on Dec 16th, 2021, from Proxy applications made on Oct 15th and Nov 17th on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.

USGA ID#: 2021-14-738

Project Title: Environmental sensors for golf course greens to improve knowledge and management of winter stresses in cold climates

Project Leaders: Eric Watkins and Bryan Runck

Affiliation: University of Minnesota

Objective: (1) build and deploy sensor nodes on golf greens in cold climates to learn more about winter stress injury on golf courses; and (2) improve sensor node effectiveness through testing and observation.

Start Date: 2021

Duration: 2 years

Total Funding: $95,630

Golf course superintendents in the northern part of the U.S. are faced with the problem of winter damage risk every year; to date, few viable solutions have been developed by the turfgrass research community. This problem requires a large-scale, interdisciplinary approach. To that end, we have assembled a team of collaborators from across several institutions, representing a wide array of expertise, who desire to work together toward a common goal of providing tools that golf course superintendents can use to reduce winter stress injury. In this project, we have developed and deployed environmental sensor nodes on golf courses that to allow researchers to learn more about how turfgrasses die during winter. This information is driving additional research projects that can have a great impact on the golf course industry.

Methods

Ground sensors provided detailed measurements of what happened just above and below the soil surface on golf greens. Building on pilot work from the 2019-2020 and 2020-2021 winters, we placed in-situ sensing nodes on 35 golf greens in fall 2021. With additional funding from the United States Department of Agriculture, we increased the number of sensor nodes to 49 for 2021-2022. Locations for sensor installations were selected based on envirotyping mapping (Figure 1). This approach looks at environmental covariates from public geospatial datasets to create groups or "clusters" of golf course greens. From these clusters, the most representative courses were selected for node placement. This way, we could capture the widest range of environments where turf winter death might occur.

Sensor Nodes

In addition to envirotypes, we focused nodes on golf greens where winter damage has at some point been a problem. The sensing nodes measured important environmental parameters including soil moisture (METER TEROS 10) and temperature (DS18B20) at 3 depths (1.25 cm; 7.5 cm, 15 cm), air temperature, barometric pressure, relative humidity (Bosch BME280), O_2 (Apogee SO-110), and CO_2 (Sensirion SCD30; Figure 2) gas levels just below the soil surface, and photosynthetically active radiation (Apogee SQ-110). Calibration for all sensors was established in the Runck lab. Sensors are connected to a single node and are powered by solar power, with battery backup. The components of the current node design are shown in Figure 3. To ensure data collection system robustness, data is logged both locally to nonvolatile memory on the device and stored in real-time in the cloud. Each node consists of a custom power subsystem and board with a Particle Boron microcontroller that live telemeters data via 2G/3G or LTE cellular depending on the location. After traveling through the cellular network, data is routed through the Particle.io platform into a Postgres database associated with the UMN GEMS Sensing web user interface. The database is indexed on a tuple of unique node ID, latitude, longitude, and time making it functionally interoperable with other project data, and data is accessible through an easy-to-use web portal.

Additional Data Collection

For all courses on which sensor nodes are installed, we requested management records for each green. During late fall of each year, golf greens identified for sensor nodes were assessed by the host superintendent for turf health and species composition; the same assessment is done each spring so that before and after winter comparisons can be made. In addition, each superintendent collects snow depth information on each quadrant of the green, along with ice and water observations.

Data from sensors is stored in an integrated database along with other information obtained from golf course superintendent observations and measurements, drone flights, and satellite imagery. For almost 150 sites without sensors, golf course superintendents took before-winter and will take after-winter images to document changes due to winter stress. Using recently awarded funding from the USDA Specialty Crop Research Initiative, these data will be collected for the next three years and used for modeling, spatial data mining, and machine learning by collaborators at the University of Minnesota, leading to new knowledge about how turfgrasses are affected by winter stresses. In total, we have collected over 11,000,000 environmental data observations across sites to understand winterkill of cool-season turfgrasses.

Next Steps

We deployed 20 additional nodes in fall 2022 using funding from this project; this is in addition to nodes funded through other grants and organizations. Each year of deployment, we will learn which things the sensors do well and those things that need to be improved. A researcher in the Runck lab will continually optimize sensor function. One of our main areas of interest is to identify and improve a low-cost oxygen sensor that can accurately record oxygen levels under ambient conditions and under ice.

Summary Points

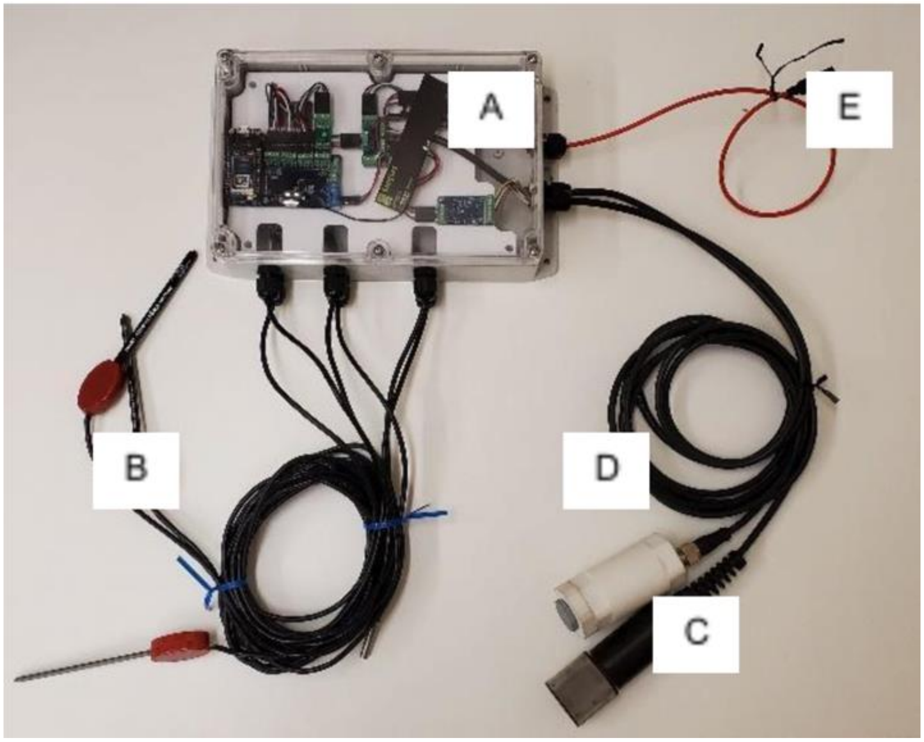
- Winter stresses are complex and more knowledge is needed to inform management recommendations and plant improvement efforts
- On-site environmental sensors can provide streams of useful data that can be used to develop winter injury prediction models
- Golf course superintendents are collecting weekly data that will be used in concert with weather data and satellite imagery to learn more about winter stress injury risk

- Funding from the USGA, combined with other sources, resulted in over 70 sensor nodes deployed on golf courses worldwide in fall 2022

Figure 1. Map of preliminarily recommended sites for sensor node deployment based on number of freezing days, total precipitation, and snow cover fraction, Figure credit Dr. Zhenong Jin.

Figure 2. Throughout the course of the project, we generated three versions of the carbon dioxide sensor system enclosure. Each version improved the responsiveness and data quality. From left to right v0, v1, v2. v0 used Sensiorion SGP30 CO2 equivalent; v1 and v2 used SCD Non-dispersive InfraRed Sensor (NDIR). Photo credit Bobby Schulz.

Figure 3. Sensors are connected to "nodes" that consist of a data logger and cellular modem. The current node (shown above) consists of (A) Logger and RGB light, (B) three soil moisture, and temperature (not shown), (C) O_2, (D) CO_2, (E) solar panel plugin. Nodes were installed by golf course superintendents across the United States, Canada, and Europe.



USGA ID#: 2020-03-708 (continued from 2019-01-671)
Title: Topdressing sand size effects on mat layer development during treatment years 5 - 7
Project leaders: James A. Murphy and Zhongqi Xu
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: 2020
Project duration: 3 years
Total funding: $161,163

Summary Points:
- Finer sands lowered field saturated hydraulic conductivity (K_{fs})compared to the medium-coarse sand. Pooled across both cultivation levels, K_{fs} dropped 1.8 in h^{-1} in plots topdressed with medium-fine sand and 2.5 in h^{-1} in plots topdressed with fine-medium sand. On average, plots that were cored cultivated twice per year increased K_{fs} 3.7 in h^{-1}.
- Under relatively dry conditions on 28 September 2022, surface hardness responded to sand size on non-cultivated plots where finer sands reduced surface hardness; there were no differences among sand size on plots that were cored twice per year. Similarly, surface hardness was greater for plots receiving a 100-lb of topdressing sand compared to 50-lb when there was no cultivation; however, there no effect of topdressing rate was observed when plots were cored twice per year. The strong drying effect of coring twice per year appeared to override any effect of topdressing sand size and rate.
- The effect of sand size and topdressing rate on surface water content frequently depended on the level of cultivation (interaction); finer topdressing sand increased surface water content when plots were not cored; however, this response was often not observed (no difference) on plots that were core-cultivated twice per year. Similarly, plots topdressed at the 50-lb rate often retained more surface water than plots topdressed at 100-lb; however, this response was often not evident on plots that were core-cultivated twice per year.
- Under non-cultivated conditions, the number of hand-watering events decreased as the topdressing sand size became finer. There were nearly 44 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand. The impact of sand size on the number of hand-watering events was much smaller under core-cultivated conditions; there were only 12 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand.
- Undisturbed core (3-inch diam.) samples of the mat layers will be removed from plots in late winter or early spring 2023 using the methods performed during the USGA ID#: 2019-01-671 grant. These cores will be used to evaluate the surface bulk density, pore size distribution, organic matter content, and sand size distribution of the mat layers. This data will conclude the grant activities of USGA ID#: 2020-03-708

Summary:

Sand topdressing of putting greens during the season is often avoided or applied at very low application rates (dusting) due to the potential of coarse sand particles interfering with play and dulling mower blades. Such topdressing practices may not keep pace with thatch accumulation in putting greens during the summer and could lead to problems associated with excess organic matter. Results from an ongoing trial (USGA ID#: 2016-06-556 and USGA ID#: 2019-01-671) indicate that a 0.05-mm topdressing sand (particles ≤ 0.5-mm) has diluted and modified thatch accumulation similar to that of the coarser, 1.0-mm topdressing sand (particles ≤ 1.0-mm). However, mat layer depth and surface wetness data suggest that differences among other treatment factors in this trial have intensified over time. In this project, we continued treatment applications and monitored turf and surface wetness responses for a 6th year. Data acquisition in the 7th (2022) year will be more intensive and destructive as was performed during the USGA ID#: 2019-01-671 grant, which evaluated the bulk density, pore size distribution and sand size distribution of the mat layer. Presuming negotiations with METER are productive, a dual head infiltrometer will be used to evaluate treatment effects on water infiltration in 2022.

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. Note mowing height was raised (3.2-mm) and frequency was reduced (every other day) during spring 2020 – in response to COVID-19 work restrictions and – returned to 2.8-mm and 5 to 6 days per week in June 2020 and continued through 2021.

The trial was a 3 x 2 x 2 factorially arranged randomized complete block design with four blocks. The factors were sand size (medium-coarse, medium-fine, fine-medium), quantity of mid-season topdressing (50- or 100-lb / 1,000-ft^2 every 10 to 14 days totaling ten applications from June through early October), and cultivation (non-cultivated or core cultivated plus backfilled in May and October). In 2020, the May cultivation treatment was re-scheduled to April to avoid work restrictions related to the COVID-19 pandemic as well as to reduce the time that coring holes were evident in late spring and early summer with cultivation in May; April cultivation was repeated in 2021. 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 used in this trial meets the USGA particle size recommendation for construction, whereas that of the medium-fine and fine-medium sands do not. The quantity of fine and very fine particles in the medium-fine and fine-medium sands exceed the USGA recommendations and these sands contain little to no coarse particles (Table 2).

Data collection during 2022 included visual ratings of turf quality and residual sand after topdressing, volumetric water content (VWC) of the surface 0- to 3-inch depth zone; Clegg soil impact values, Stimpmeter distances, normalized difference vegetation index (NDVI); and documentation of the number of hand-watering events and amount of water applied to individual plots.

Additionally, dual-head infiltrometers [SATURO | Automated Field Infiltrometer | METER Environment (metergroup.com), Pullman, WA] were used to measure field saturated hydraulic conductivity of eight treatment during August 2022. The treatment set include the non-topdressed control and three sand sizes applied at 100-lb per 1,000 sq ft every two weeks in the summer under both cultivation levels (non-cored and cored twice per year). Field saturated hydraulic conductivity data were analyzed using a 4 × 2 factorial randomized complete block design with 4 blocks.

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

Treatment no.	Sand size[a]	Topdressing rate during mid-season[b]	Cultivation[c]	Annual quantity of sand applied
		lb / 1,000 sq ft		lb / 1,000 sq ft
1	medium-coarse	50	none	1,200
2	medium-coarse	50	core + backfill	1,700
3	medium-coarse	100	none	1,700
4	medium-coarse	100	core + backfill	2,200
5	medium-fine	50	none	1,200
6	medium-fine	50	core + backfill	1,700
7	medium-fine	100	none	1,700
8	medium-fine	100	core + backfill	2,200
9	fine-medium	50	none	1,200
10	fine-medium	50	core + backfill	1,700
11	fine-medium	100	none	1,700
12	fine-medium	100	core + backfill	2,200
13	none	0	none	0
14	none	0	core + backfill	1,200

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

[b] Ten applications of topdressing applied every two weeks from June through early October. Topdressing at 50 lb per 1,000 sq ft represented a 'dusting' quantity (O'Brien and Hartwiger, 2003); whereas topdressing at 100 lb filled the surface thatch and lower verdure layers.

[c] Core cultivation to the 1.5-inch depth was performed twice a year (April/May and October) using 0.5-inch diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with medium-coarse sand at 600 lb per 1,000 sq ft. At the time of core cultivation, non-cultivated plots were topdressed with the respective sand at 400 lb per 1,000 sq ft to fill the verdure and surface thatch layers to the same extent as the cored and backfilled plots.

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

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

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

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

Results

Field Saturated Hydraulic Conductivity (K_{fs}): Both finer sands lowered K_{fs} compared to the medium-coarse sand (Table 3). Pooled across both cultivation levels, K_{fs} dropped 1.8 in h^{-1} in plots topdressed with medium-fine sand and 2.5 in h^{-1} in plots topdressed with fine-medium sand. Pooled across the 4 levels of sand size, plots that were cored cultivated twice per year increased K_{fs} 3.7 in h^{-1}.

Surface hardness: Contrasts of the non-topdressed controls to the pooled treatment effect typically indicates that topdressing lowers surface hardness compared to the non-topdressed control especially if plot are not core cultivated (Table 4). Comparison of the non-cultivated control to the cultivated control indicates that coring twice a year was enough to increase surface hardness.

The ANOVA indicated that surface hardness was strongly affected by the cultivation factor, but the effect was dependent on the sand size as well as the rate used to topdress plots (Table 4). Under relatively dry conditions on 28 September 2022, surface hardness responded to sand size on non-cultivated plots where finer sands reduced surface hardness; there were no differences among sand size on plots that were cored twice per year (Table 5). The surface hardness and VWC values on 28 September clearly indicated an inverse relationship between the two parameters. The strong drying effect that coring twice per year had on the plots also greatly increased surface hardness.

The topdress rate × cultivation interaction on 28 September indicated a similar effect of cultivation (Table 4). Surface hardness was greater for plots receiving a 100-lb of topdressing sand compared to 50-lb when there was no cultivation; however, there no effect of topdressing rate when plots were cored twice per year (Table 5). Again, the strong drying effect of coring twice per year appeared to override any effect of topdressing rate.

Under much wetter conditions on 7 October 2022, surface hardness also responded to sand size on non-cultivated plots where only the finest sand (fine-medium) reduced surface hardness compared to the coarser sands. There were no differences among sand sizes on plots that were cored twice per year on 7 October (Table 5).

The topdress rate × cultivation interaction on 7 October 2022 indicated the same response pattern reported for 28 September 2022; a higher surface hardness for 100-lb topdressing than 50-lb topdressing when plots were not cored, whereas there was not effect of rate on plots cored twice per year (Table 5).

Volumetric water content (VWC): Topdressing has reduced surface water retention compared to the controls and this effect was most pronounced for plots that were not core-cultivated (data not shown). Among the two controls, the core-cultivated frequently had dramatically lower surface water content (data not shown). Within the factorially arranged treatments,

Number of Hand-watering Events: For plots that were core-cultivated, the number of hand-watering events was lower on plots topdressed every two weeks compred to the non-topdressed control (Table 6). Topdressing had no effect on the number of hand-watering events for plots under non-cultivated conditions. There were 21 more hand-watering events on the core-cultivated control compared to the non-cultivated control.

Within the factorially arranged treatments, the effect of sand size on the number of hand-watering events depended on the level of cultivation (Table 6). Under non-cultivated conditions, the number of hand-watering events decreased as the topdressing sand size became finer (Table 7). There were nearly 44 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand. The impact of sand size on the number of hand-watering events was much smaller under core-cultivated conditions; there were only 12 fewer hand-watering events on the plots topdressed with fine-medium sand compared to plots topdressed with medium-coarse sand.

2023 Plan of Work

USGA ID#: 2020-03-708

- Undisturbed core (3-inch diam.) samples of the mat layers will be removed from plots in late winter or early spring 2023 using the methods performed during the USGA ID#: 2019-01-671 grant. These cores will be used to evaluate the surface bulk density, pore size distribution, organic matter content, and sand size distribution of the mat layers. This data will conclude the grant activities of USGA ID#: 2020-03-708

USGA ID#: 2023-01-768

- Under a new grant agreement, the controls, factorial treatment set will be continued for an 8th growing season in 2023. However, the cultivation factor will have hollow tine cultivation changed to solid tine cultivation.
- Data collection will include visual observations of turf quality and residual sand after topdressing, volumetric water content (VWC) of the surface 0- to 3-inch depth zone; Clegg soil impact values, Stimpmeter ball roll distances, normalized difference vegetation index (NDVI); and hand-watering quantification. We expect to acquire up to more dual head infiltrometers (SATURO) to assess water infiltration on a larger subset of plots during each of the next three growing seasons.

Table 3. Field saturated hydraulic conductivity (K_{fs}) response of the surface 0- to 5-cm depth zone to topdressing sand size and core cultivation on a 8-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone in New Brunswick NJ during August 2022.

	Field Saturated Hydraulic Conductivity [a]
ANOVA Factorial Source	*F* test
Sand Size (Size) [b]	*
Core Cultivation (CC) [c]	***
Size × CC	NS
Sand Size Main Effect	inch h^{-1}
None	5.0 ab
Medium-coarse	5.9 a
Medium-fine	4.1 b
Fine-medium	3.4 b
Cultivation Main Effect	
None	2.7 b
Twice per year	6.4 a

[a] Dual-head infiltrometer measurements made using four pressure cycles (pressure heads of 5- and 10-cm) with a 20-minute hold times within a 5-cm deep insertion ring. A 25-minute soak was performed before all measurements.

[b] Ten applications of topdressing applied every two weeks at 100 lb per 1,000 sq ft from June through early October; 100-lb filled the surface thatch and lower verdure layers.

[c] Core cultivation to the 1.5-inch depth was performed twice a year (April and October) using 0.5-inch diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with medium-coarse sand at 600-lb per 1,000 sq ft. At the time of core cultivation, non-cultivated plots were topdressed with the respective sand at 400-lb per 1,000 sq ft to fill the verdure and surface thatch layers to the same extent as the cored and backfilled plots.

Table 4. Surface hardness (Clegg Soil Impact Tester) and volumetric water content (VWC) at the surface 0- to 3-inch depth zone as affected by controls, topdressing sand size and rate, and core cultivation on a 8-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110-inch in New Brunswick NJ during 2022.

Orthogonal Contrasts	28 Sep. Clegg	28 Sep. VWC	7 Oct. Clegg	7 Oct. VWC
	G_{max}	%	G_{max}	%
Non-cultivated:				
Control versus	91.5	23.5	74.5	34.7
Pooled topdressing	80.0***	21.2*	70.0*	32.7^{NS}
Core cultivated:				
Control versus	96.6	12.5	80.8	22.2
Pooled topdressing [b]	92.1***	11.9^{NS}	82.7^{NS}	21.6^{NS}
Controls:				
Non-cultivated versus cultivated	**	***	***	***
ANOVA Factorial Source				
Sand Size (Size)	NS	***	**	***
Sand Rate (Rate)	NS	***	*	NS
Size × Rate	NS	NS	NS	NS
Core Cultivation (CC)	***	***	***	***
Size × CC	*	***	**	***
Rate × CC	**	**	*	NS
Size × Rate × CC	NS	NS	NS	NS

[a] Means within an orthogonal contrast followed by ***, ** or * letter indicate a significant difference at $P \leq 0.001$, 0.01, or 0.05, respectively.

[b] Controls did not receive topdressing except for the control that was core cultivated and coring holes were backfilled.

Table 5. Surface hardness (Clegg Soil Impact Tester) and volumetric water content (VWC) at the surface 0- to 3-inch depth zone as affected by the cultivation × sand size and cultivation × topdressing rate interactions on a 8-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110-inch in New Brunswick NJ during 2022.

Sand Size[a]	Cultivation[b]	28 Sep. Surface hardness	28 Sep. VWC	7 Oct. Surface hardness	7 Oct. VWC
		G_{max}	%	G_{max}	%
Medium-coarse	None	81.5 b	17.3 c	72.8 b	27.9 c
Medium-fine	None	80.8 bc	20.0 b	71.4 b	31.8 b
Fine-medium	None	77.8 c	26.5 a	65.8 c	38.3 a
Medium-coarse	Twice per year	91.6 a	11.5 d	82.4 a	20.3 e
Medium-fine	Twice per year	92.5 a	11.7 d	83.1 a	20.7 e
Fine-medium	Twice per yar	92.2 a	12.6 d	82.7 a	23.8 d
Topdress Rate[a]	Cultivation				
50-lb per 1000 sq ft	None	78.8 c	23.0 a	67.9 c	
100-lb per 1000 sq ft	None	81.2 b	19.5 b	72.1 b	
50-lb per 1000 sq ft	Twice per year	92.9 a	12.0 c	82.9 a	
100-lb per 1000 sq ft	Twice per year	91.3 a	11.8 c	82.6 a	
					ns

[a] Sand topdressing applied every two weeks from June through early October for ten applications; 100-lb filled the surface thatch and lower verdure layers.

[b] Core cultivation to the 1.5-inch depth was performed twice a year (April and October) using 0.5-inch diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with medium-coarse sand at 600-lb per 1,000 sq ft. At the time of core cultivation, non-cultivated plots were topdressed with the respective sand at 400-lb per 1,000 sq ft to fill the verdure and surface thatch layers to the same extent as the cored and backfilled plots.

Table 6. Number of hand-watering events during 2022 as affected by controls, topdressing sand size and rate, and core cultivation on a 8-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110-inch in New Brunswick NJ.

Orthogonal Contrasts	Hand-watering
	number of events
Non-cultivated:	
Control versus	79.5[NS]
Pooled topdressing	71.3
Core cultivated:	
Control versus	100.5*
Pooled topdressing [b]	88.1
Controls:	
Non-cultivated versus cultivated	**
ANOVA Factorial Source	*F* test
Sand Size (Size)	***
Sand Rate (Rate)	NS
Size × Rate	NS
Core Cultivation (CC)	***
Size × CC	***
Rate × CC	NS
Size × Rate × CC	NS

[a] Means within an orthogonal contrast followed by ***, ** or * letter indicate a significant difference at $P \leq 0.001$, 0.01, or 0.05, respectively.

[b] Controls did not receive topdressing except for the control that was core cultivated and coring holes were backfilled.

Table 7. Number of hand-watering events and the cumulative amount of water applied as affected by the sand size by cultivation interaction on a 8-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110-inch in New Brunswick NJ during 2021.

Sand Size[a]	Cultivation[b]	Hand-watering
		Number of events
Medium-coarse	None	92.0 a
Medium-fine	None	73.5 b
Fine-medium	None	48.5 c
Medium-coarse	Twice per year	92.8 a
Medium-fine	Twice per year	90.8 a
Fine-medium	Twice per year	80.8 b

[a] Sand topdressing applied every two weeks from June through early October for ten applications.

[b] Core cultivation to the 1.5-inch depth was performed twice a year (April and October) using 0.5-inch diameter hollow tines spaced to remove 10% of the surface area annually. Coring holes were backfilled with medium-coarse sand at 600-lb per 1,000 sq ft. At the time of core cultivation, non-cultivated plots were topdressed with the respective sand at 400-lb per 1,000 sq ft to fill the verdure and surface thatch layers to the same extent as the cored and backfilled plots.

Figure 1. SATURO dual-head infiltrometer used to measure field saturated hydraulic conductivity of plots topdressed with medium-coarse, medium-fine, and fine-medium sand and either not cultivated or hollow tine cultivated twice per year on a 8-year-old ‘Shark’ creeping bentgrass turf maintained as a putting green in North Brunswick, NJ.

Figure 2. Individidual plots were scouted daily for visual symptoms of wilt stress before water was applied via hand-watering. The date/time and amount of water applied were recorded for each event.

USGA ID#: 2020-14-719

Title: Long term effects of topdressing and cultivation on an annual bluegrass putting green

Lead Author: Chas Schmid
Project Leader: Alec Kowalewski
Collaborators: Ruying Wang, Emily Braithwaite, Brian McDonald, and Clint Mattox

Affiliation: Department of Horticulture, Oregon State University

Objectives:

1. Determining the optimum organic matter cultivation method and timing for annual bluegrass putting green turf
2. Determine optimum sand topdressing rate for organic matter management on annual bluegrass putting green turf; and if cultivation method or timing interact with sand topdressing rate

Start Date:
May 2020

Project Duration:
3 years, May 2020 to May 2023 (year 3 report)

Total Funding:
$30,000 ($10,000 per year)

Summary Points:

- No cultivation or sand topdressing (control) was the only treatment with unacceptable turfgrass quality by late August 2022; all other treatment maintained acceptable turfgrass quality.
- Sand topdressing at 100 lb/1,000 ft^2 every 14-d produced a firmer putting green surface compared to 50 lb/1,000 ft^2 rate.
- Cultivation in both spring and fall increased surface firmness compared to spring or fall alone, regardless of tine type (solid or hollow tine)
- Infiltration rate was significantly reduced in non-treated control and topdressing only treatments after 3 years
- No statistical difference in infiltration rate was observed between hollow tine and solid tine treatments or topdressing rates (50 or 100 lb/1,000 ft^2)

Introduction:

Hollow tine aerification and sand topdressing have been used on golf course putting greens for decades. These cultural practices are used to mitigate organic matter accumulation, provide rapid infiltration, and maintain firm playing conditions (Green et al., 2001; Stier and Hollman, 2003). In more recent years, superintendents and researchers have been exploring solid tine aerification and topdressing without aerification (Hempfling et al., 2014; Inguagiato et al., 2012; Wang et al., 2018). These practices are less intensive and minimize surface disruption, a frequent golfer complaint. Despite these recent trends, aerification and topdressing research on annual bluegrass putting greens in the Pacific Northwest, where 12 months of annual bluegrass growth can be expected, and long-term research on putting greens is minimal.

Materials and Methods:

A 5-year field trial was initiated in May 2020 at the OSU Lewis-Brown Horticulture Farm in Corvallis, OR. Research was conducted on a putting green that was built in 2009 by placing 12” of USGA spec sand over a silty clay loam soil with flat drainage. Turfgrass was established using sand-based annual bluegrass (*Poa annua*) sod (Bos Sod, Canada).

Experimental design for the trial is a randomized complete block design with four replications. Treatments are arranged in a 2 x 2 x 3 factorial, with two sand topdressing rates (50 and 100 lbs/1000ft^2), two tine types (hollow and solid tine), and three cultivation timings (spring, fall, and both spring and fall). A non-treated control (no cultivation, no sand topdressing) and two non-cultivated plots that received either 50 or 100 lb/1,000 ft^2 sand topdressing were also included in the analysis. Spring cultivation treatments were applied on 1 June 2020, 28 May 2021, and 2 Jun 2022; and fall cultivation treatments were applied 29 Sept 2020, 7 Oct 2021, and 14 Oct 2022. Sand topdressing treatments were applied every 2-wks during the summer from 15 June through 21 Sep 2020, 9 June through 22 Sept 2021, and 20 June through 27 Sept 2022.

Fungicides were applied year-round to prevent diseases including anthracnose (*Colletotrichum cereale*), yellow patch (*Rhizoctonia cerealis*), and Microdochium patch (*Microdochium nivale*). The plots were fertilized every 2 weeks during the growing season (spring, summer, and fall) at 0.2 lbs N/1000 ft^2, and at the same rate monthly during the winter. The plots were mowed no higher than 0.110 inches during the growing season and 0.140 inches during the winter.

Response Variables:

Visual turfgrass quality (TQ) was rated monthly throughout the year. Turfgrass quality used a 1 to 9 scale (9 = best, 5 = minimum acceptable, 1= dormant or dead turf) and took into account turf density, uniformity and evenness (playability), and overall appearance. Turfgrass health was measured with a FieldScout CM 1000 NDVI Chlorophyll Meter. Surface firmness was measured monthly using the FieldScout TruFirm meter, with 5 measurements collected within each plot. Percent volumetric water content was measure at the same time and location as surface firmness to determine if surface firmness differences were a result of a treatment response or soil moisture differences. Soil infiltration rates for each plot were collected on 26 May and 10 Aug 2020, 13 May 2021, and 19 Aug 2022 using a double ring (6” inner ring, 12” outer ring) falling head method similar to the methods described by Wander and Bollero (1999). One linear inch of water (450ml) was added to the inner ring on each plot and the time required to infiltrate 1 in. was recorded. This procedure was repeated for the second inch of infiltration. Soil samples for total organic matter were collected using methods described by Lockyer (2008) on 27 May 2020 prior to treatment initiation, and on 19 May 2021, and 24 May 2022; where soil samples are divided into depth increments of 0-0.8, 0.8-1.6, 1.6-2.4 in. (0-20, 20-40, and 40-60mm) and the verdure is not removed from the sample. Three soil samples were collected per plot using a 1.25" soil probe. Total organic matter was determined using loss on ignition (LOI) method described by Nelson and Sommers (1996).

Preliminary Findings:

Similar to the two previous years, few differences were observed between treatments with respect to turfgrass quality and NDVI during spring and mid-summer 2022 (data not shown). However, by August, TQ in the non-treated control plots reduced significantly due to Cyanobacteria (*Oscillatoria* sp.) outbreak, which was also observed in the previous season. On 25 Aug. 2022, the non-treated control plots were the only treatment with unacceptable TQ; all other combinations of cultivation and topdressing had acceptable quality (Fig. 1). Subtle differences were also observed between the cultivation and topdressing treatments on this date. The main effect of topdressing rate indicated that topdressing at the high rate (100 lb/1,000 ft^2), resulted in higher turfgrass quality compared to the low rate (50 lb/1,000 ft^2). The main effect of cultivation timing also influenced TQ on this date, with fall cultivation resulting in higher TQ compared to spring cultivation. The combination of spring and fall cultivation was not statistically different from either spring or fall cultivation alone (data not shown).

During 2022, the accumulation of 2 plus years of cultivation and topdressing treatments resulted in differences in soil physical properties and surface characteristics. Putting green surface firmness, measured with a Trufirm, was influenced by topdressing rate and cultivation timing during 2022 (Table 1). Sand topdressing at 100 lb/1,000 ft^2 resulted in a firmer surface compared to 50 lbs/1,000 ft^2 on 4 out of 5 ratings. Additionally, the combination of spring and fall cultivation resulted in a firmer putting green surface compared to spring or fall only cultivation on 4 and 3 out of the 5 ratings, respectively. On one rating in 2022, hollow tine cultivation produced a firmer surface compared to solid tine cultivation.

Statistical differences in soil infiltration rate were detected between treatments in 2022 (Fig. 2). The non-treated control and topdressing alone (both rates) resulted in significantly lower soil infiltration rates compared to all combinations of cultivation and topdressing. Visual observation of soil sample cores indicates a significant thatch layer has developed in non-treated control plots (Image 1), which is likely reducing infiltration rate. Similarly, soil cores from topdressing only treatments showed thatch layering, which was likely reducing the infiltration rates in these treatments. The treatment that produced the greatest infiltration rate was the combination of hollow tine cultivation in spring and fall, and sand topdressing at 100 lb/1,000 ft^2, which was also the only treatment that maintained an average infiltration rate above 6 in/hr (which is the USGA recommendation for infiltration rate for new greens construction). Cultivation timing also influenced infiltration rate in 2022, with fall only cultivation resulting in lower infiltration rates compared to spring alone and the combination of spring and fall (Table 2). Interestingly no statistical difference in infiltration rate was observed between hollow tine and solid tine treatments after almost three years. This result indicates that in the short-term, superintendents may implement solid tine cultivation to maintain infiltration rate, which reduces maintenance cost and recovery time compared to hollow tine cultivation.

References:

Green, R., L. Wu and G.J. Klein. 2001. Summer Cultivation Increases Field Infiltration Rates of Water and Reduces Soil Electrical Conductivity on Annual Bluegrass Golf Greens. HortScience. 36(4):776-779.

Hempfling, J.W., B.B. Clarke and J.A. Murphy. 2014. Anthracnose Disease on Annual Bluegrass as Influenced by Spring and Summer Topdressing. Crop Science. 55(1):437-443.

Inguagiato, J.C., J.A. Murphy and B.B. Clarke. 2012. Sand Topdressing Rate and Interval Effects on Anthracnose Severity of an Annual Bluegrass Putting Green. Crop Science. 52(3):1406-1415.

Lockyer, J. 2008. STRI – Testing for organic matter. Turfgrass Bulletin. Oct 2008. Issue 242.

Nelson, D.S., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: D.L. Sparks et al., editors, Methods of soil analysis, Part 3. SSSA Book Ser. 5. SSSA, Madison, WI. p. 961-1010.

Stier, J.C. and A.B. Hollman. 2003. Cultivation and Topdressing Requirements for Thatch Management in A and G Bentgrasses and Creeping Bluegrass. HortScience. 38(6):1227-1231.

Wander, M.M., and G.A. Bollero. 1999. Soil quality assessment of tillage impacts in Illinois. Soil Sci. Soc. Am. J. 63:961-971.

Wang, R., J.W. Hempfling, B.B. Clarke and J.A. Murphy. 2018. Seasonal and Annual Topdressing Effects on Anthracnose of Annual Bluegrass. Agronomy Journal. 110(6):2130-2135.

USGA. 2018. USGA recommendations for a method of putting green construction. United States Golf Association, Liberty Corner, N.J. (http://archive.lib.msu.edu/tic/usgamisc/monos/2018recommendationsmethodputtinggreen.pdf) Accessed Nov 21, 2022.

Table 1. Analysis of variance of surface firmness (TruFirm) response to topdressing rate tine type, and cultivation timing applied to annual bluegrass turf in Corvallis, OR during 2022. Please include a footnote that a greater TruFirm number represents a softer surface, I don't think "depth of travel" fully explains this.

Main effects	**Surface Firmness**				
	8-Jul	**18-Jul**	**1-Aug**	**15-Aug**	**30-Aug**
	Depth of travel (inches)†				
Topdressing Rate (T)‡					
50 lbs/M	630	623	623	617	631
100 lbs/M	624	614	605	604	612
Tine Type (TT)					
Hollow tine	625	616	615	604	616
Solid tine	630	620	613	618	627
Cultivation Timing (CT)					
Spring	630	624	627	616	631
Fall	632	623	616	615	624
Spring & Fall	620	607	600	600	608
$LSD_{(0.05)}$	-	10	20	14	18
Source of variation					
T	ns	*	*	*	*
TT	ns	ns	ns	*	ns
T x TT	ns	ns	ns	ns	ns
CT	ns	**	*	*	*
T x CT	ns	ns	ns	ns	ns
TT x CT	ns	ns	ns	ns	ns
T x TT x CT	ns	ns	ns	ns	ns

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability level; ns = not significant.

† Greater TruFirm number represents a softer putting green surface.

‡ Topdressing treatments applied every 14-day from June through September.

Table 2. Analysis of variance of infiltration rate in response to topdressing rate, tine type, and cultivation timing applied to annual bluegrass turf in Corvallis, OR on the 19 Aug 2022.

Main effects	Infiltration Rate	
	1st inch	2nd inch
	in/hr	
Topdressing Rate (T)[†]		
50 lbs/M	4.4	3.9
100 lbs/M	5.0	4.4
Tine Type (TT)		
Hollow tine	5.1	4.4
Solid tine	4.4	3.9
Cultivation Timing (CT)		
Spring	5.1	4.5
Fall	2.8	2.5
Spring & Fall	6.3	5.5
$LSD_{(0.05)}$	1.8	1.4
Source of variation		
T	ns	ns
TT	ns	ns
T x TT	ns	ns
CT	**	***
T x CT	ns	ns
TT x CT	ns	ns
T x TT x CT	ns	ns

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability level; ns = not significant.

[†] Topdressing treatments applied every 14-day from June through September

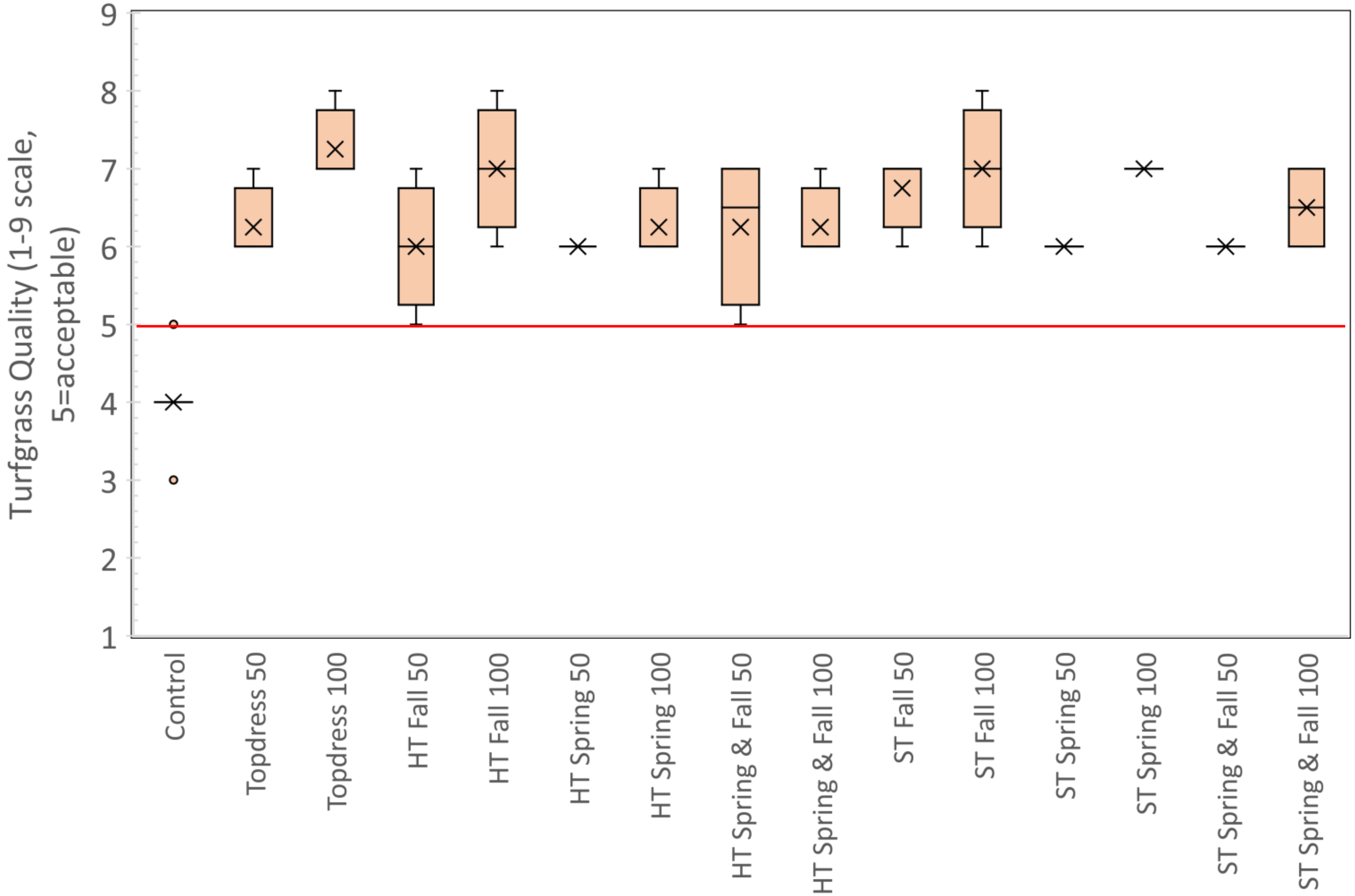


Figure 1. Box plot of cultivation and topdressing treatments effect on turfgrass quality of annual bluegrass putting green, collected 25 Aug 2022. HT= hollow tine, ST= solid tine; Spring and/or Fall refers to the timing of cultivation treatments; 50 or 100 at the end of treatment label refers to the summer topdressing rate in lbs /1,000 ft^2 applied every 14-d. Horizontal red line indicates the minimum acceptable turfgrass quality rating.

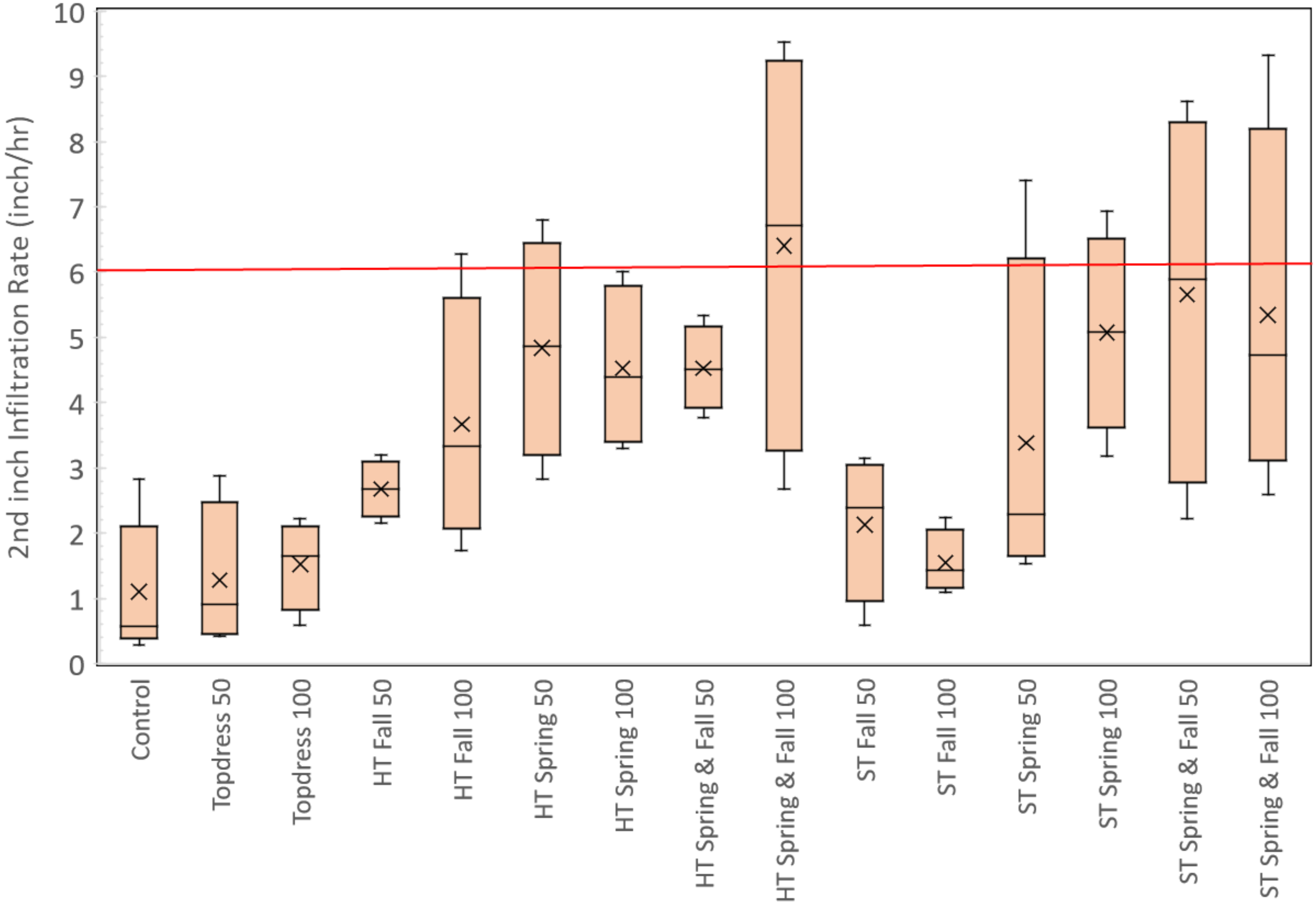


Figure 2. Box plot of cultivation and topdressing treatments effect on 2nd inch infiltration rate on an annual bluegrass putting green, collected 19 Aug 2022. HT= hollow tine, ST= solid tine; spring and/or fall refers to the timing of cultivation treatments; 50 or 100 at the end of treatment label refers to the summer topdressing rate in lbs /1,000 ft^2 applied every 14-d. Horizontal red line indicates the minimum infiltration rate (Ksat) for new putting green construction (6 in/hr; USGA staff 2018)

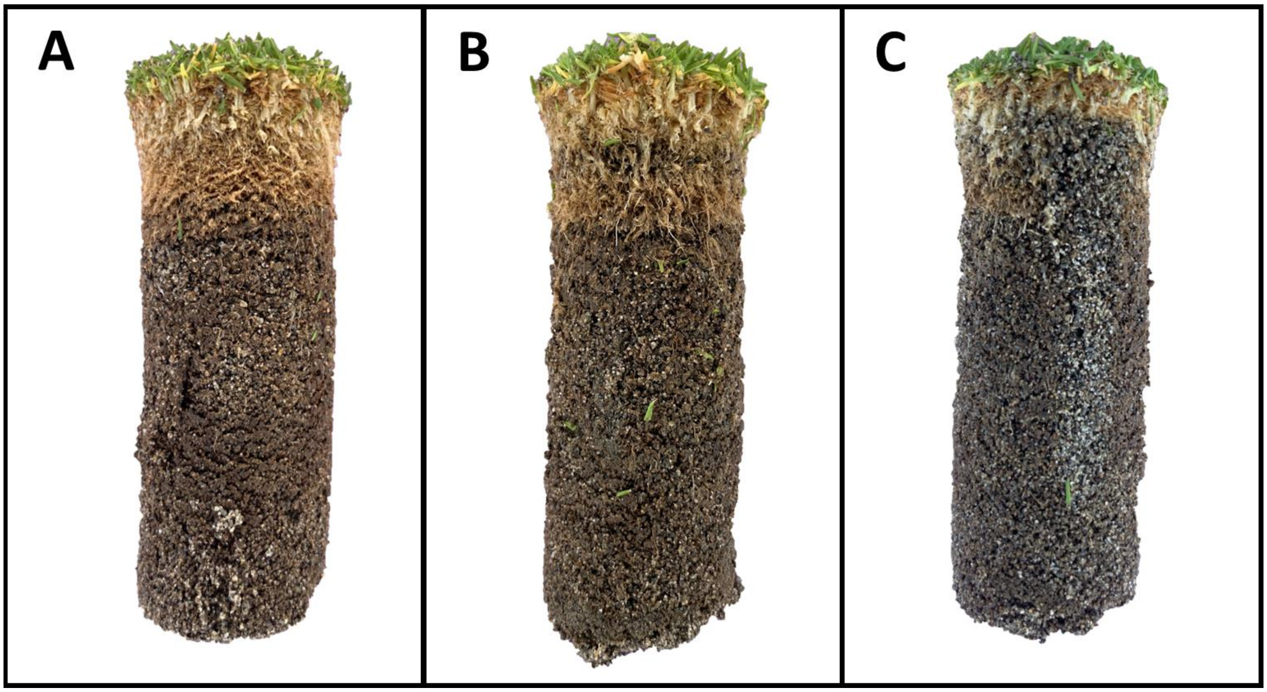


Image 1. Soil cores (1.25” diameter x 3.5” deep) from select cultivation and topdressing treatments, photographed on 24 May 2022; A) nontreated control (no cultivation, no topdressing) showing a thick thatch layer; B) topdressing at 50 lb/1,000 ft^2 with no cultivation showing a stratified thatch layer; C) hollow tine cultivation spring and fall, topdressing at 100 lb/1,000 ft^2.

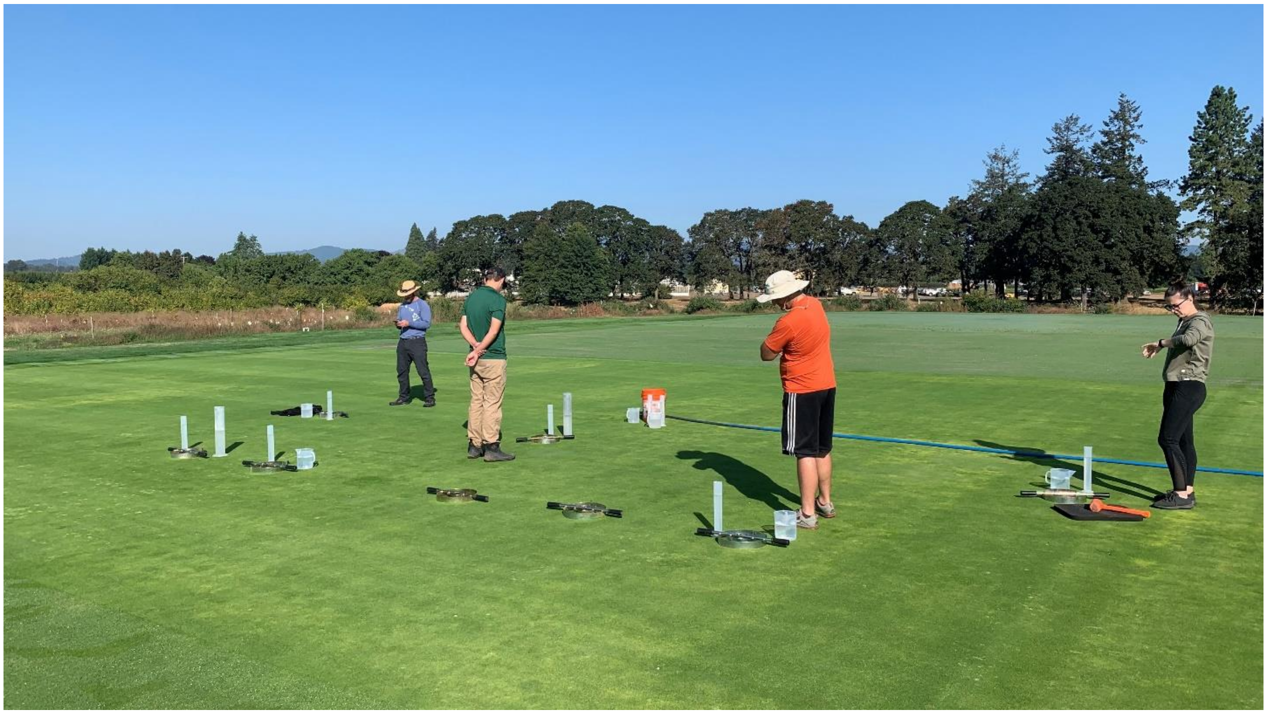


Image 2. Field measurement of infiltration rates on the cultivation and topdressing trial at Lewis-Brown Horticultural Farm in Corvallis, OR.

Image 3. Tools used to measure field infiltration rates

USGA ID#: 2022-10-753

Title: Influence of Topdressing on the Firmness and Soil Organic Matter Content of Bentgrass Putting Greens

Project Leader: Doug Soldat, Ph.D.
Affiliation: University of Wisconsin-Madison

Objectives: The objectives of this work are to 1) compare approaches for determining proper sand topdressing amounts, 2) understand how decisions about annual topdressing rate, application frequency, and sand particle size affect firmness, 3) compare the relationship among the major methods for measuring putting green surface firmness, and 4) validate the Ohio State Greens Organic Matter Management Tool.

Specific hypotheses include:

1. The USGA method will result in firmer putting surfaces than the PACE Turf and clipping volume methods.
2. Topdressing frequency (weekly vs. monthly) will not significantly influence putting green firmness on most measurement dates, allowing turfgrass managers to have the freedom to choose which topdressing schedules they prefer to use.
3. Finer topdressing sand will result in significantly greater surface firmness than sand that more closely matches the original root zone.
4. The Ohio State Greens Organic Matter Management Tool will prove useful in predicting changes in surface soil organic matter and therefore become a valuable tool for assisting turfgrass managers in their decision making for topdressing and cultivation events.

Start Date: 2022
Project Duration: 3 years
Total Funding: $96,749

Summary Points:
- This study is in its first year, so summary points below are likely to change as the research progresses.
- The four surface firmness methods tested were not well correlated with each other, suggesting that they are each measuring different aspects of surface firmness.
- The differences in annual sand application amounts in this study influenced turf quality and soil moisture but did not affect surface firmness.
- The two sand particle sizes tested had minimal impact on turfgrass quality or surface firmness.
- Application frequency (weekly vs. monthly) did not significantly influence turfgrass quality, soil moisture, or surface firmness.

Summary Text

Rationale: Sand topdressing of putting greens is one of the most important management practices for producing high quality playing surfaces. There is some consensus about the appropriate sand topdressing rate for putting greens, yet with recommendations ranging from

16-35 ft^3 per 1000 ft^2, there remains room for refinement. Identifying the proper annual topdressing rate is important for controlling the accumulation of soil organic matter over a period of years, but across a shorter temporal interval, topdressing decisions affect putting green performance and playability. Putting green firmness is one of the most important putting green performance characteristics. Unfortunately, the factors affecting firmness remain poorly characterized. The USGA developed and utilizes the TruFirm device for quantifying putting green firmness, the R&A uses the Clegg Impact Soil Tester, and the PGA Tour measures the depth of the depression created by a steel ball dropped from a height of 6 feet. Clearly, these international golf organizations value the surface firmness of putting greens, yet the research into the factors affecting surface firmness is remarkably thin. The majority of the information on putting green firmness exists in non-peer reviewed publications. A search for “putting green” AND “firmness” on the Turfgrass Information File turned up 16 hits in the refereed literature, of 301 total hits for that search. From the reviewed literature, surface firmness is related to soil water content, soil bulk density, and soil organic matter. This research seeks to quantify how topdressing rate, frequency, and particle size decisions affect surface firmness.

Materials and Methods: Three annual sand topdressing rates were evaluated and each was applied at either weekly or monthly intervals. In addition, two sand particles sizes were tested (properties in Table 1) in this RCBD three-factor factorial with four replications. The three topdressing rate methods include what we call the USGA method, the PACE Turf method, and the clipping volume method. The USGA method is based on the Whitlark and Thompson (2019) article that recommends 25-35 ft^3 per 1000 ft^2 for a golf course with a 30-week growing season. The bentgrass growing season in Wisconsin is approximately 26 weeks, 87% of Whitlark and Thompson’s example, so for this study that method will result in the application 26 ft^3/1000 ft^2 of sand. The PACE Turf Method relies on the use of the PACE Turf Growth Potential Model, which estimates cool-season grass growth based on weather or climate inputs. This model can be used to normalize sand topdressing to climate or weather data. The default spreadsheet uses data from Hempfling et al. (2017) to establish the maximum monthly sand application (300 lbs/1000 ft^2 / month). When the estimated growth decreases, the recommended monthly sand also decreases. For example, in New Brunswick, NJ, cool-season growth potential is nearly 100% in June and September. For these months, the PACE Turf method recommends ~300 lbs/1000 ft^2 per month. In October, the estimated growth potential is about 50%, so only about ~150 lbs/1000 ft^2 of sand is recommended. Using climate data from Madison, WI, the PACE Turf Method recommends 14 ft^3/1000 ft^2 of sand, substantially less than the USGA method. Finally, many turf managers are measuring clipping volume from their putting greens, while there is little research data to suggest how clipping volume could be used to guide topdressing, Dr. Micah Woods has suggested applying 1 mm (3.3 ft^3/1000 ft^2) of sand for each L/m^2 of fresh clippings collected; this method is referred to as the clipping volume method and will vary depending on the grass growth rate. All treatments received one annual hollow-tine core cultivation event in September 2022 where ~8 ft^3/1000 ft^2 of sand was used to fill the holes left by the tines.

Table 1. Particle size distribution of the two sands used.

Sand	Coefficient of Uniformity	V. Coarse	Coarse	Medium	Fine	V. Fine
		------------------------------	%	------------------------------		
Standard	2.0	0.4	31.5	56.6	9.1	1.5
Finer	2.5	0.1	22.7	42.9	26.8	7.3

We measured putting green surface firmness weekly using four devices:

1) Clegg Impact Soil Tester Hammer (2.25 kg hammer, flat bottom)
2) Clegg Impact Soil Test Hammer (0.5 kg hammer, round bottom)
3) Spectrum TruFirm
4) Precision USA Putting Green Firmness Meter

In addition to firmness measurements, we measured soil moisture, normalized difference red edge (NDRE), and visual turfgrass quality weekly during the growing season. Once a month, we measured ball roll distance and collected clippings from the plots. Soil organic matter was measured using loss on ignition prior to the trial initiation using a 2-inch diameter probe with five subsamples per plot. The sampling depths were 0-1, 1-2, 2-3, 3-4, and 4-5 inches, and the verdure and living roots were included in the sample. These measurements were repeated after the final topdressing event of the season. At this same time, verdure biomass and shoot density were quantified using three, 2-inch diameter subsamples from each plot.

Results:
In 2022, different amounts of sand were applied across the three methods for determining annual sand amount. The USGA method resulted in 22.5 ft^3/1000 ft^2, the PACE Turf 12.5 ft^3/1000 ft^2 and the Woods method resulted in 15.3 ft^3/1000 ft^2 of sand applied. In the following tables, different letters indicate statistical difference at the 0.05 confidence level. Additionally, all statistical analysis is from the last data collection event to allow for the biggest differences in sand application. For the purposes of brevity, only the main effects will be discussed. Annual application amount significantly influenced turfgrass quality, NDRE, and soil moisture, but did not influence surface firmness (Table 2). The most sand resulted in the greatest turf quality, but lowest NDRE, and soil moisture was found to be greatest in the method that applied the least sand, and lowest in the method that applied the most sand (Table 3). Surface firmness as measured by the Clegg 0.5 device detected significant differences in sand size with a value of 5.81 (softer) for fine sand and 6.03 (firmer) for medium sand. Interestingly, surface firmness measurements were not well correlated with each other (Table 4). The Spectrum TruFirm and the PGA method both measure the depth of a depression (deeper is softer), while the Clegg devices both measure the deceleration of a device striking the surface (smaller deceleration is softer), resulting in negative correlations between a Clegg device and the PGA and TruFirm devices. The TruFirm and PGA methods were positively correlated, and the Clegg devices were positively correlated. However, the greatest correlation among all devices was only found to be $r = 0.46$ ($r^2 = 0.21$). The PGA method and the Clegg 0.5 had an r of -0.01 ($r^2 = 0.01$), suggesting that these two methods were measuring very different properties. However, because this research is in its first year and is being conducted on the same initial surface, it is possible that better correlations among devices will be observed as differences in surface firmness accrue from the differential sand topdressing practices.

Table 2. ANOVA showing the impact of treatment factors on end-of-season putting green performance metrics in 2022. A bolded p-value indicates that the source of variation significantly affected the response variable (turf quality, NDRE, soil moisture, or surface firmness.

				Surface Firmness Method			
Source of Variation	**Turf Quality**	**NDRE**	**Soil Moisture**	**Clegg 2.25**	**Clegg 0.5**	**Spectrum TruFirm**	**PGA**
				p-value			
Block	0.615	**0.049**	**0.015**	0.478	**0.003**	**0.029**	0.125
Sand Size (sz)	0.203	0.519	0.839	0.163	**0.032**	0.521	0.512
Applic. Frequency (freq)	0.797	0.272	0.424	0.077	0.556	0.389	0.325
Applic. amount (amt)	**0.002**	**<0.001**	**<0.001**	0.253	0.756	0.567	0.082
sz*freq	0.797	0.488	**<0.001**	0.986	0.870	0.582	0.795
sz*amt	0.935	0.190	0.595	0.554	0.722	0.519	0.802
freq*amt	0.628	**<0.001**	0.281	0.400	0.690	**0.008**	0.589
freq*amt*sz	0.291	0.102	0.080	0.494	0.363	0.428	0.143

Table 3. End of season turf quality, NDRE, and moisture as affected by annual amount of sand topdressing applied during 2022.

Treatment Factor	Turf Quality	NDRE	Soil Moisture
Most sand (USGA)	5.8 A	0.330 B	25.2 C
Moderate Sand (Clipping Volume)	5.0 B	0.323 B	26.2 B
Least Sand (PACE Turf)	5.3 B	0.338 A	27.4 A

Table 4. Correlation coefficients (r) for the four methods of surface firmness used in the trial, n=1869.

Firmness Method	Clegg 2.25	Clegg 0.5	Spectrum TruFirm	PGA Method
Clegg 2.25	1.00	0.46	-0.32	-0.27
Clegg 0.5	0.46	1.00	-0.38	-0.10
Spectrum TruFirm	-0.32	-0.38	1.00	0.29
PGA Method	-0.27	-0.10	0.29	1.00

Future Work

We expect differences in soil organic matter, surface firmness, and turfgrass quality and visual characteristics to accrue as time passes and the soil properties change with differential sand applications. We will also compare the soil organic matter amounts to those predicted by the Ohio State University Greens Organic Matter Management Tool.

Graduate Student Travis Miller discussing the research and various surface firmness devices at the 2022 Wisconsin Turfgrass Field Day.

References:

Hempfling, J. W., C. J. Schmid, R. Wang, B. B. Clarke, and J. A. Murphy. 2017. Best management practices effects on anthracnose disease of annual bluegrass. Crop Sci. 57(2):602-610.

Whitlark, B., and C. Thompson. 2019. Light and frequent topdressing programs: A combination of field observations and recent research shed new light on the type of sand and quantity of topdressing needed to manage thatch and organic matter accumulation in putting greens. USGA Green Sec. Rec. 57(9):1-8.

USGA ID#: 2021-05-729

Title: Influence of Plant Growth Regulators on Core Cultivation Recovery Time of Annual Bluegrass Putting Green Turf

Project Leader: Chas Schmid
Collaborators: Emily Braithwaite, Brian McDonald, and Alec Kowalewski

Affiliation: Department of Horticulture, Oregon State University

Objectives:

1) Determine the effect of trinexapac-ethyl application timing on core cultivation recovery
2) Determine the effect of ethephon applications on core cultivation recovery time
3) Evaluate the effect of gibberellic acid (GA_3) on core cultivation recovery

Start Date:
March 2021

Project Duration:
3 years, March 2021 to Dec 2023 (year 2 report)

Total Funding:
$30,000 ($10,000 per year)

Summary Points:

- Spring applications of ethephon (Proxy) help annual bluegrass putting greens recover quicker from core cultivation.
- Trinexapac-ethyl (Primo Maxx) applied 400 or 200 GDD prior to cultivation improved recovery in the spring compared to TE applied 10 GDD prior to cultivation.
- Fall applications of ethephon (Proxy) to annual bluegrass putting greens slowed core cultivation recovery in one year and had no effect the other.
- Trinexapac-ethyl (Primo Maxx) timing had no effect on cultivation recovery time in the fall
- Increased turfgrass growth observed in gibberellic acid treatments initially increased the percent recovery compared to other treatments; however, scalping caused reduced turfgrass cover, and prolonged recovery time.

Introduction:

Organic matter (OM) management with core cultivation is one of the most important management practices for golf course putting greens, and one of the most disruptive to golfers.

Core cultivation is done in the spring and fall on actively growing cool-season turfgrasses to minimize recovery time (Beard, 1973). However, this is also the time of year when golf courses experience the most play. In a USGA report of the top 10 questions frequently asked by golfers, three questioned the need for core cultivation of putting greens (Maloy, 2002). Thus, there is a need to shorten recovery time post core cultivation to limit disruption to golfers.

Plant growth regulators (PGR) are commonly used on golf courses to manage vertical growth and to improve turf stand density (Beasley and Branham, 2007; Ervin and Koski, 1998; Fagerness and Yelverton, 2000). Golf course managers apply PGRs routinely on a calendar-based schedule or using growing degree day (GDD) models (Kreuser and Soldat, 2011) to limit post-inhibition growth enhancement (aka "rebound effect"). Plant growth regulator applications to cool-season turfgrasses are most commonly applied in the spring and fall, when shoot and root growth are maximal (Johnson, 1989). In contrast, there has been a trend with sports field managers to use post-inhibition growth enhancement to recover from events that damage turfgrass, such as concerts (Polimer, 2020). On golf courses, core cultivation is one of the most damaging events to occur, but no research currently exists that demonstrates the effect of PGRs on recovery from core cultivation or if plant hormones such as gibberellic acid can be used to reduce recovery time. It may be possible to use post-inhibition growth enhancement to decrease recovery time.

Materials and Methods:

A three-year field trial began March 2021 with evaluations ending October 2023. Research is being conducted on a sand-based annual bluegrass research green mowed at 0.110 inches. The trial was core cultivated in the spring on 1 May 2021 and 7 May 2022, and in the Fall on 28 Sept 2021 and 7 Oct 2022, respectively, using a Toro Procore 648 equipped with ½" I.D. hollow tines (2 x 2" spacing). A complete fertilizer (Anderson's 28-5-18) was applied 2-d prior to cultivation to all plots at a rate between 0.3 and 0.5 lbs N/1000 ft^2.

Treatments will be arranged as a randomized complete block design with four replications. Plot size for the trial will be 7' x 4' (28 ft^2). Treatments are listed below:

Treatments

1. Untreated control
2. TE[¥] applied 400 GDD[Ɏ] prior to cultivation
3. TE applied 400 GDD prior to cultivation + ethephon[↑]
4. TE applied 400 and 200 GDD prior to cultivation
5. TE applied 400 and 200 GDD prior to cultivation + ethephon
6. TE applied 400, 200 and 10 GDD prior to cultivation
7. TE applied 400, 200 and 10 GDD prior to cultivation+ ethephon
8. GA_3 applied 10 GDD prior to cultivation at 0.05 oz RyzUp /Acre
9. GA_3 applied 10 GDD prior to cultivation at 0.1 oz RyzUp/Acre

¥ All trinexapac-ethyl (TE) applications will be applied at 5.5 fl oz of Primo MAXX/Acre

ҰCumulative GDD model will be calculated as the summation of the daily mean air temperature (°C) with a base of 0°C following the most recent TE application.

↑All ethephon applications will be at 218 fl oz of Proxy/Acre 400 and 200 GDD prior to cultivation.

The first treatment application in the spring will be initiated at first sign of annual bluegrass plants in the “boot” stage, and subsequent applications and cultivation timing will be made based on a growing degree day model. Routine applications of trinexapac-ethyl will continue throughout the summer months on all plots (except non-treated control and GA plots) every 14 days.

Response Variables:

Digital photos will be taken at the same location in each plot, daily during the recovery period, using a lightbox to track recovery over time. Digital images will be analyzed using Turf Analyzer to determine percent recovery over time. Turfgrass clippings were collected 5 and 14 days post cultivation treatments to measure the amount of vertical growth (i.e. rebound effect). Soil samples were collected prior to the initiation of the study using methods described by Lockyer (2008) and analyzed using the loss on ignition method (Nelson and Sommers, 1996). Additional soil samples will be collected at the conclusion of the trial in Oct 2023 to determine the effect of PGRs on total organic matter. Response variables will be analyzed with analysis of variance (ANOVA) and the means separated using LSD at the 5% alpha level.

Preliminary Findings:

Results from the spring of 2021 and 2022 indicate that the main effect of ethephon had the greatest effect on cultivation recovery time (Fig 1). Percent recovery was greater in plots receiving ethephon plots that received no ethephon on 4 out of 7, and 7 out of 8 ratings in 2021 and 2022, respectively. This data suggests the seedhead suppression generated by ethephon applications results in improved lateral growth decreasing aerification recovery time. Plant growth regulator timing (GDD model) influenced cultivation recovery in the spring of 2021 and 2022 (Fig 2). In spring 2021, TE timing only influenced cultivation recovery on one rating, where the last app of TE applied 400 GDD prior to cultivation had greater percent recovery 5-d after cultivation compared to TE applied 10 GDD prior to cultivation. In 2022, the effect of TE timing was much more pronounced (4 out of 8 ratings), with the last application of TE 400 or 200 GDD prior to cultivation resulting in greater percent recovery than TE applied 10 GDD prior. These results indicate that TE can be applied 400 or 200 GDDs prior to a cultivation without inhibiting recovery; however, if TE is applied closer to cultivation (i.e. 10 GDD prior) recovery time will be increased. .

Results from the fall cultivation event indicated that the main effect of ethephon treatment influenced cultivation recovery time in 2021 but not 2022. In 2021, plots receiving ethephon were slower to recover than plots that received no ethephon (6 out of 11 ratings; Fig 3). This is concerning because recent research has suggested that fall applications of ethephon can be used to further mitigate annual bluegrass seedhead production (Reicher et al., 2022). Practitioners implementing fall applications of ethephon will likely see slower fall aerification recovery. Trinexapac-ethyl timing (GDD model) had no effect on cultivation recovery time in the fall of 2021 or 2022. The lack of statistical differences between treatments in the fall 2022 may be due to a chemical injury on the research green during late July to early August cause by an interaction of two pesticides. The pesticide injury may have caused a growth regulator effect to the turfgrass that influenced treatments. With that being said, it does appear that effect of PGRs on cultivation recovery in the fall is much less pronounced than the spring.

Interestingly, plots treated with GA at 0.05 or 0.1 oz/A initially had increased turfgrass growth and rapid recovery from core cultivation 2-6 days after cultivation in the spring of 2022 (Table 1). However, scalping from excessive turfgrass growth slowed recovery time overall. This result was similar to those observed in the spring and fall of 2021. In contrast, GA had no effect on cultivation recovery compared to the non-treated control in the fall of 2022. Further research is needed to better understand how GA applications influence cultivation recovery.

References:

Beard, J.B. 1973. Turfgrass science and culture. Prentice Hall, Englewood Cliffs, NJ.

Beasley, J.S. and B.E Branham. 2007. Trinexapac-ethyl and paclobutrazol affect Kentucky bluegrass single-leaf carbon exchange rates and plant growth. Crop Sci. 47:132-138.

Ervin, E.H, and A.J Koski. 1998. Growth response of *Lolium perenne* L. to trinexapac-ethyl. HortSci. 33:1200-1202.

Fagerness, M.J., and F.H. Yelverton. 2000. Tissue production and quality of 'Tifway' bermudagrass as affected by seasonal application patterns of trinexapac-ethyl. Crop Sci. 40:493-497.

Johnson, B.J. 1989, Response of tall fescue (*Festuca arundinacea)* to plant growth regular application dates. Weed Technol. 3:408-413.

Kreuser, W.C., and D.J. Soldat. A growing degree day model to schedule trinexapac-ethyl applications on *Agrostis stolonifera* golf greens. Crop Sci. 51:2228-2236.

Lockyer, J. 2008. STRI-testing for organic matter. Turfgrass Bulletin. 242:13-16.

Maloy, B. 2002. What are golfers thinking?: The top ten questions frequently asked of the USGA Green Section. USGA Green Sec. Rec. 40:13-16.

Nelson, D.S. and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. in: Sparks, D.L., et al., editors, Methods of Soil Analysis, Part 3. SSSA Book Ser. 5. SSSA, Madison, WI. p. 961-1010.

Polimer, B. (2020). Why every sports field manager should consider using PGRs (*SportsField Management*). Retrieved from https://sportsfieldmanagementonline.com/2020/05/11/why-every-sports-field-manager-should-consider-using-pgrs/11506/

Reicher, Z.J., M.D. Sousek, A.J. Patton, A. Van Dyke, W.C. Kreuser, J.C. Inguagiato, K.M. Miele, J. Brewer, S. D. Askew, A. Hathaway, T.A. Nikolai, **A. Kowalewski**, and B. McDonald. 2020. Adding a Late Fall Application of Proxy (Ethephon) Before Two Traditional Spring Applications Improves Seedhead Control of Annual Bluegrass. Crop Forage and Turfgrass Management. 2020:1-18. DOI:10.1002/cft2.20031.

Table 1. Plant growth regulator effect on core cultivation recovery time in the spring of 2022

Treatment	Last PGR app prior to cultivation	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT	13 DAT	15 DAT	17 DAT
	GDD	% Recovery							
Non-treated control		62	47	77	62	58	70	93	95
TE	400	61	45	73	63	56	67	92	95
TE + Ethephon	400	67	58	80	81	81	89	97	98
TE	200	62	43	77	53	52	64	95	96
TE + Ethephon	200	65	59	79	81	84	92	98	99
TE	10	59	35	68	38	33	46	87	89
TE + Ethephon	10	64	58	76	78	81	90	97	98
GA (0.05 oz/A)	10	80	77	101	77	70	71	94	95
GA (0.1 oz/A)	10	83	83	101	82	73	74	96	97
$LSD_{(0.05)}$		8	10	7	12	14	10	8	4

† DAC = days after core cultivation

‡ TE = Trinexapac-ethyl (Primo Maxx)

* GA = Gibberellic acid (RyzUp smartgrass®)

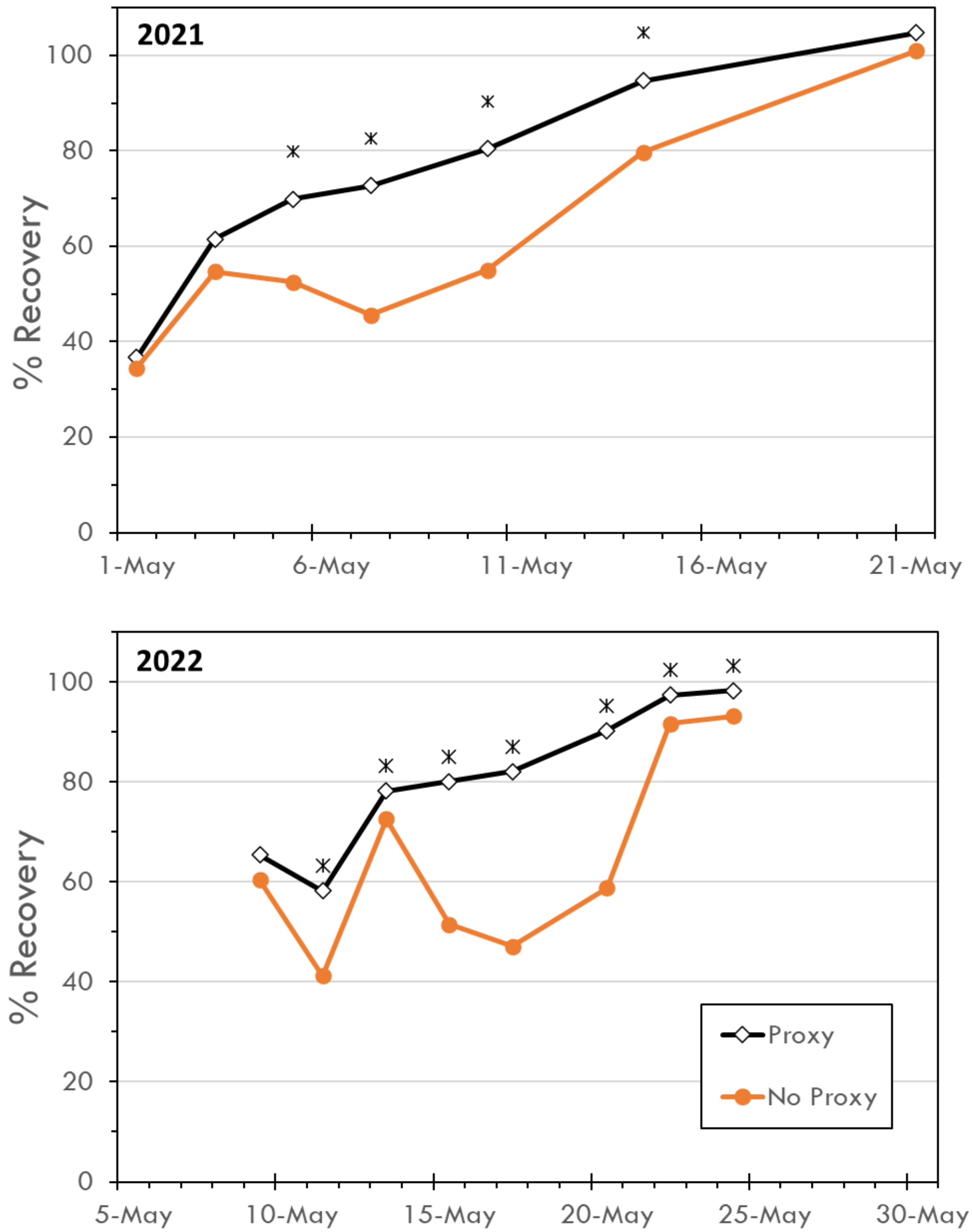


Figure 1. The main effect of ethephon (Proxy) on the percent recovery from core cultivation over time, in the spring of 2021 and 2022. Asterisk above a rating date indicates a significant difference between treatments.

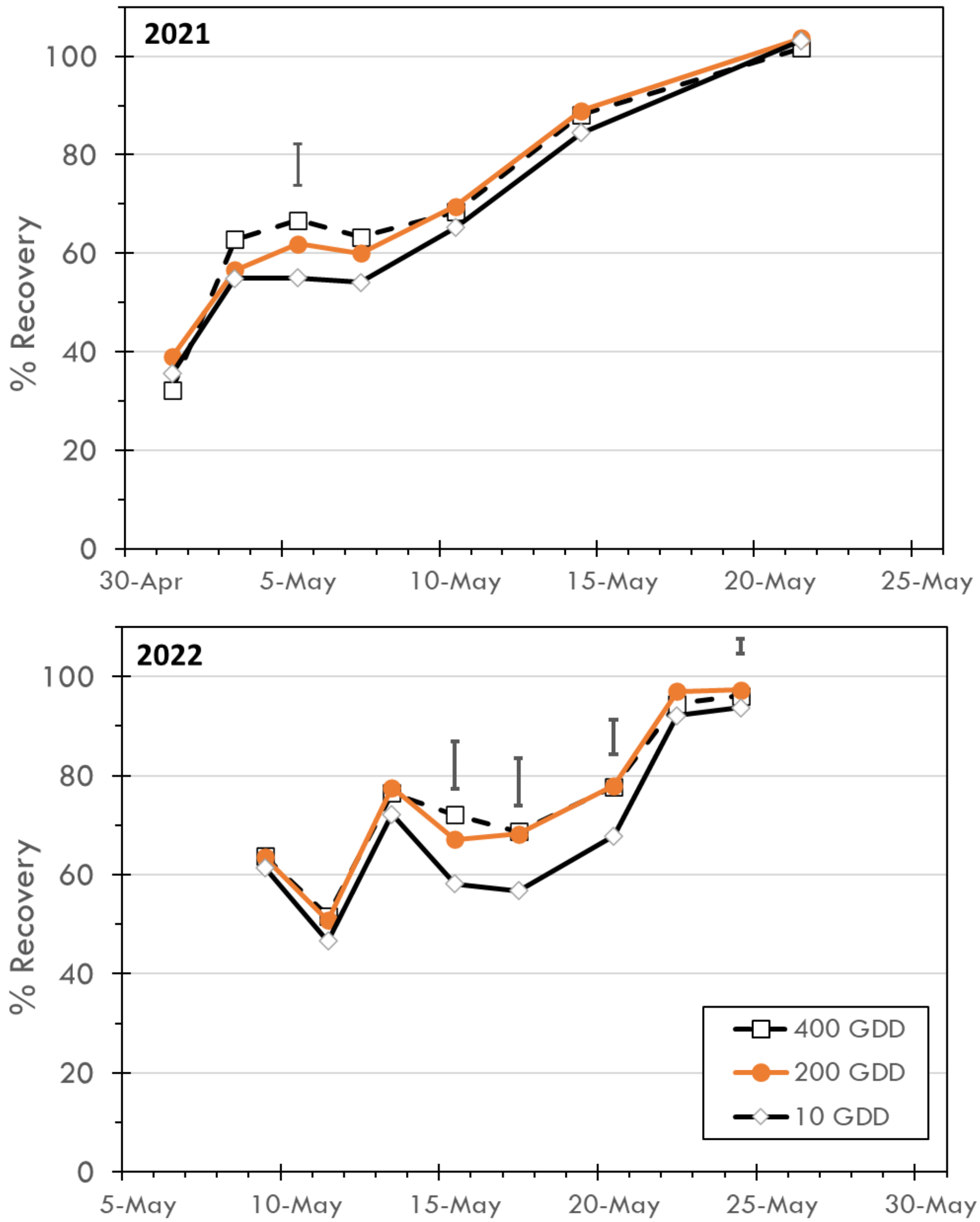


Figure 2. The main effect of PGR timing on the percent recovery from core cultivation over time, in the spring of 2021 and 2022. Asterisk above a rating date indicates a significant difference between treatments.

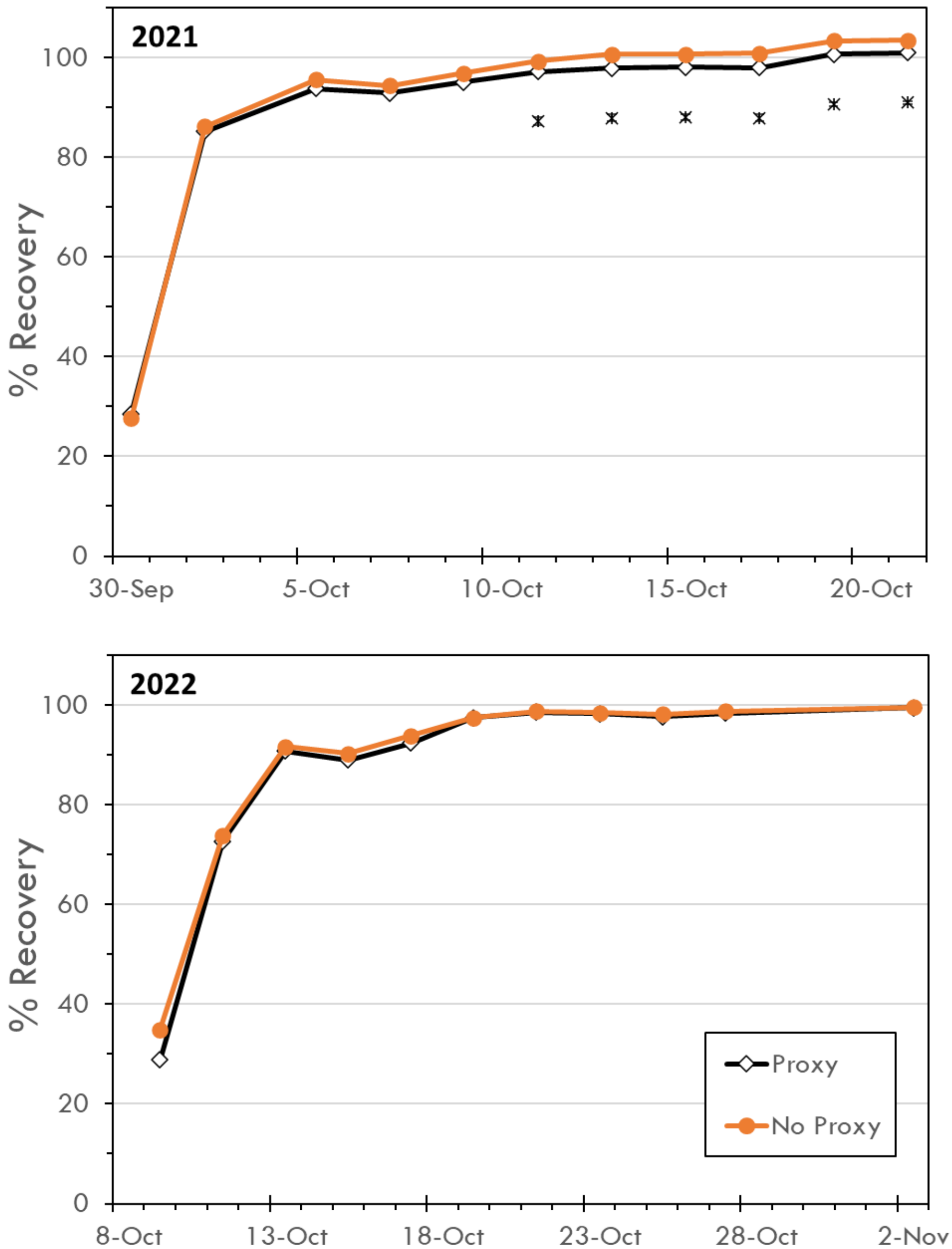


Figure 3. The main effect of ethephon (Proxy) on the percent recovery from core cultivation over time, in the fall of 2021 and 2022. Asterisk above a rating date indicates a significant difference between treatments.

Image 1. Visual differences in turfgrass quality and color between PGR treatments during core cultivation recovery, photographed 5 Oct 2021.

Image 2. Gibberellic acid treatments being applied to annual bluegrass putting green turf after a cultivation event, photographed 30 April 2021

USGA ID#: 2022-15-758

Title: Developing a standard for measuring organic matter in putting green soils

Project Leaders: Roch Gaussoin (University of Nebraska-Lincoln), Douglas Linde (Delaware Valley University), James Murphy (Rutgers University), Douglas Soldat (University of Wisconsin-Madison)

Objectives: The overall goal of the project was to develop a standard method for characterizing the surface organic matter of golf course putting greens. The specific objectives were to better understand: 1) How sample preparation (particularly verdure removal, grinding, sieving) affect the mean and variance of surface organic matter. 2) How core diameter affects the mean and variance of surface organic matter. 3) The number of samples required to adequately characterize the surface organic matter on a putting green. And 4) the minimum distance apart for each sub-sample on a putting green.

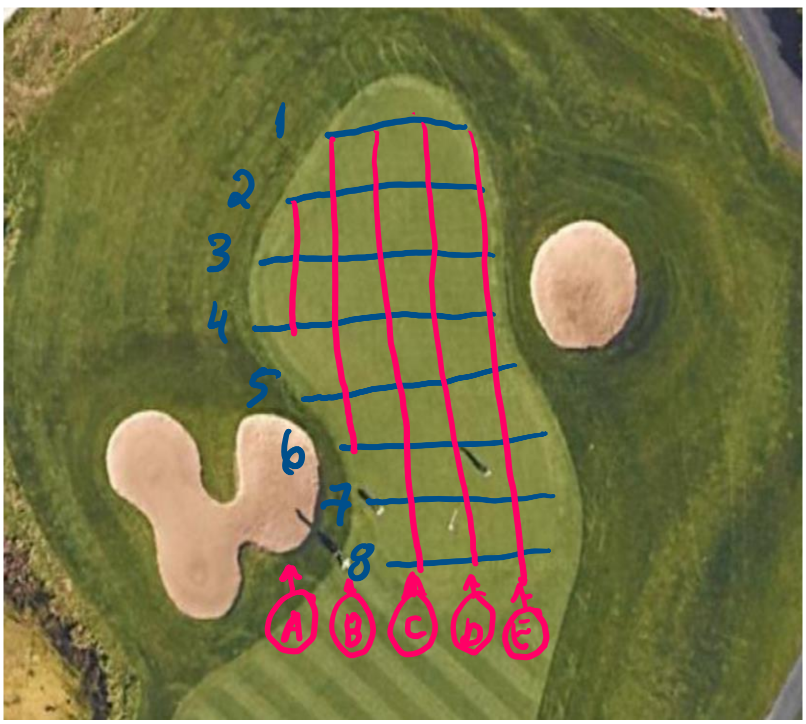
Start Date: 2022
Project Duration: 2 years
Total Funding: $56,284

Summary Points:

- Leaving verdure on increases surface organic matter levels. However, this difference is obscured if samples are ground and sieved (as is common at most soil testing labs). If the verdure is of interest, it is critical that the samples be ignited intact and not ground or sieved.
- Core diameters of 0.75 and 1.5 inches were compared. At one research site, larger cores led to lower mean surface organic matter. At another research site, the mean surface organic matter was identical regardless of diameter. Further investigation is required.
- For cores of 0.75 inches, only 5 to 8 samples were required to adequately characterize the surface organic matter of all 15 putting greens tested in three different states. When the larger cores were used (1.5-inch diameter), 4 to 5 samples were required.
- The spatial patterns of the data suggest that cores should be taken approximately 30 feet apart.

Summary Text: Managing soil organic matter is critical to the sustainable management of sand-based putting greens. While soil organic matter is commonly measured by practitioners and scientists, a review of the literature found that there is a need to create a standard method for sampling and measuring putting green organic matter to aid in interpretation of results. We set out to answer key questions about how sampling decisions affect the mean and variation of surface organic matter in putting greens. These questions included: 1) How sample preparation (particularly verdure removal, grinding, sieving) affects the mean and variance of surface organic matter. 2) How core diameter affects the mean and variance of surface organic matter. 3) The number of samples required to adequately characterize the surface organic matter on a putting green. And 4) the minimum distance apart for each sub-sample on a putting green.

To answer the first question, we collected 50 surface organic matter samples on a 3 m x 3 m grid from three sand-based research putting greens at the O.J. Noer Turfgrass Research Facility in Verona, WI, and 50 surface organic matter samples on a 3 m x 3 m grid from two sand-based putting greens from a golf course in Verona, WI. Cores were collected using both a standard 0.75-inch diameter soil probe, or a custom 1.5-inch diameter sampler. The cores were then processed in different ways (verdure off, verdure on, grinding and sieving the samples, or not grinding and sieving). After processing, all samples were dried at 105 C for 24 hours then ignited at 360 C for 2 hours in a muffle furnace. The results showed that leaving verdure on increased surface organic matter levels. However, this difference was obscured if samples were ground and sieved (as is common at most soil testing labs). If the verdure is of interest to the manager, it is critical that the samples be ignited intact and not ground or sieved. The larger core diameter (1.5 inch) resulted in significantly lower soil organic matter content for cores with verdure left on and ignited intact compared to the standard 0.75-inch diameter cores (5.93% vs. 6.21% surface organic matter).



For the second phase of the study, we collected surface organic matter samples from five putting greens from a single golf course in Nebraska, Wisconsin, and Pennsylvania. The samples were collected using a grid sampling method. In Nebraska, both 0.75- and 1.5-inch diameter cores were collected. In Wisconsin and Pennsylvania, only 0.75-inch diameter cores were evaluated. Verdure was not removed from these samples and intact cores were ignited at 360 for 2 hours following drying at 105 C. Each green had approximately 50 core samples and chi-squared analysis found that 5 to 8 samples using a 0.75-inch diameter probe were required to adequately characterize the mean surface organic matter (Table 1). The greens in Wisconsin and Nebraska had lower surface organic matter than the course in Pennsylvania, and required only 5 samples to characterize the true mean. The greens in Pennsylvania required 5 to 8 samples, suggesting that more samples will be required as soil organic matter increases. At the Nebraska site, 1.5 inch vs. 0.75-inch diameter cores were evaluated. Organic matter content from each core diameter was statistically similar but the number of samples to adequately characterize the mean differed slightly. For the larger core size 4 to 5 samples were required to adequately characterize the mean surface organic matter using the larger core compared to the 5 samples that were required when the standard 0.75-inch probe was used. However, 4 cores using a 1.5-inch

diameter core removes approximately 7 in^2 of material, while 5 cores from a 0.75 inch diameter core removes only 2.2 in^2 of material suggesting that a 0.75-inch diameter core is preferable to a larger core, due to lower surface disruption.

Finally, we were able to develop recommendations for how far apart samples should be taken by analyzing the spatial patterns of the data. While each putting green has a unique spatial pattern of soil organic matter, it appears that samples should be taken approximately 30 feet apart for best results.

There are several critical questions that need investigating. 1) Is 360C for 2 hours adequate to ignite organic matter from 0.75-inch diameter cores of warm season grasses which tend to have more surface organic matter associated with rhizomes and stolons. 2) We did not collect any data from warm season turf. 3) Does surface organic matter change seasonally which would make sampling time very important. We are currently analyzing samples collected monthly from the same location to determine how sampling monthly affects mean surface organic matter. We also wish to investigate the spatial patterns of soil organic matter that we collected to see how things like shade, traffic, and elevation may contribute to the patterns we observed.

Table 1. Number of samples required to adequately characterized the surface organic matter content on five putting greens from three golf courses in the USA.

Wisconsin		
Green	# Samples	Average OM
Chip	5	4.59
12	5	7.21
8	5	7.23
4	5	7.06
1	5	6.69

Pennsylvania		
Green	# Samples	Average OM
6	7	17.14
2	5	10.83
3	8	15.66
4	5	11.72
7	5	13.2

Nebraska		
Green	# Samples	Average OM
9	5	4.01
8	5	4.09
7	5	3.95
6	5	3.60
5	5	3.09

USGA ID#: 2022-13-756
Title: Variable-rate versus conventional nitrogen application methods to golf course fairways
Project Leader: Chase Straw, Briana Wyatt, and Julie Howe
Affiliation: Texas A&M University

Objectives:

1. Determine the relationship between vegetation indices, EC_a, and leaf tissue and soil nitrogen (N) status in a practical setting on golf course fairways.
2. Assess seasonal changes in vegetation indices and EC_a SSMUs within golf course fairways.
3. Compare variable-rate to conventional blanket N application methods on golf course fairways with respect to total N applied, turfgrass quality, and N volatilization.

Start Date: 2022
Project Duration: 4-years (2022-2025)
Total Funding: $90,000

Summary Points:

- Preliminary data collections using a Toro Precision Sense 6000 and EM-38 were conducted at two courses**:** The City Course at Briarcrest (Bryan, TX) and The Bearkat Course (Huntsville, TX).
- The Bearkat Course was selected for the study and preliminary data were used to group nine fairways into three similar groups of three (i.e., randomized complete block design) based on soil and turfgrass characteristics for Objective 3 in 2024 and 2025.
- A Draganfly Commander 2 unmanned aerial vehicle (UAV) with MicaSense Altum-PT multispectral camera capable of synchronized thermal images was purchased for use in the study because it was deemed a more practical means of vegetation indices data collection.

Summary Text:

Rationale

The golf course management industry continues to be under increasing pressure to reduce fertilizer use. Variable-rate fertilization is an area of precision turfgrass management (PTM) that may lead to reductions of fertilizer use and environmental impacts by identifying turfgrass and soil variability within a golf course for site-specific applications. Currently available GPS-equipped data acquisitions and maintenance equipment make variable-rate fertilization possible now. Moreover, fairways on golf courses have the most promise for input and environmental impact reductions through PTM and have shown to exhibit significant spatial and temporal variability with respect to turfgrass and soil characteristics. This has led to several suggestions for PTM on fairways; however, actual investigations into the efficacy of the proposed PTM strategies are scarce.

Sustainable golf course management of the future will rely on new technologies and management approaches to reduce golf course's overall environmental footprint, while maintaining turfgrass aesthetics and playability. Unfortunately, adoption of PTM is not widespread throughout the golf course management industry. The underlying reasons are complex, but contributing factors include lack of knowledge to translate data to management decisions and lack of research clearly showing the benefits of PTM. Therefore, research comparing novel, data-driven PTM approaches to conventional management approaches is needed to fully understand the benefits of PTM and entice its adoption. The objectives of this research are focused on developing prescription variable-rate granular N fertilization programs using a couple different data-driven approaches, and then comparing them to conventional blanket granular N applications on fairways.

Methodology

The goals for 2022 were to identify a golf course to conduct the study and begin Objectives 1 and 2. These goals were delayed due to a few reasons. First, the research team purchased a Draganfly Commander 2 (Draganfly Innovations Incorporated, Saskatoon, Saskatchewan, Canada) unmanned aerial vehicle (UAV) with MicaSense Altum-PT (MicaSense, Seattle, WA, USA) multispectral camera capable of synchronized thermal images in December 2021 (Figure 1). This technology will replace what was intended in the original proposal to collect vegetation indices [i.e., normalized difference vegetation index (NDVI) with a Toro Precision Sense 6000 (PS6000; The Toro Company, Bloomington, MN, USA) and normalized difference red edge (NDRE) with a handheld sensor], because it is deemed a more practical methodology for real-world scenarios. Unfortunately, the UAV and camera were not received until September 2022, and therefore has only been flown once to-date at the study locations. Second, there were calibration issues with our electromagnet induction (EMI) device that took three golf course surveys to correct, leading to only one usable survey in Oct 2022. Lastly, a key personnel member who was leading the project left Texas A&M in the middle of the year. The culminations of these delays led to requesting and receiving a no cost, 1-year extension for the project. Nevertheless, progress was still made in 2022.

Two golf courses were considered as potential locations for the study: The City Course at Briarcrest (Bryan, TX) and The Bearkat Course (Huntsville, TX). The City Course is a public golf course that has 'Tifway 419' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] and native soil (sandy loam classification) fairways. The Bearkat Course is also a public golf course that has 'TifSport' hybrid bermudagrass and native soil (sandy loam classification) fairways. Two surveys using the PS6000 were conducted at The City Course on 6 June and 7 July, while four surveys were conducted at The Bearkat Course on 26 May, 22 July, 14 Sept, and 26 Oct (Figure 1). All par 4 and 5 fairways at each golf course were considered during the surveys. The PS6000 is a mobile, multi-sensor device that is towed behind a utility vehicle and measures soil moisture (% volumetric water content), penetration resistance (MPa), NDVI, and electrical conductivity (EC_e; dS m^{-1}) while serpentining the length of a fairway. The result is hundreds of data points per fairway.

Additionally, an EM-38 (Geonics Ltd., Mississauga, Ontario, Canada) was used to survey apparent electrical conductivity (EC_a) at The Bearkat Course on 26 Oct The EM-38 is also a tow behind sensor that traverses a fairway and is a non-invasive means to determine EC_a by generating an electromagnetic field, which induces an electrical current in the soil that is related to the conductivity of that soil. There are two receivers on the EM-38 that provide EC_a data from depths of 1.0 and 0.5 m. All PS6000 and EM-38 data were georeferenced using an external GPS that provides sub-centimeter level locational accuracy.

Results to Date

The Bearkat Course was selected for use in the study because of logistical and agronomic reasons. All survey data that were gathered throughout the year were analyzed to calculate summary statistics for each individual par 4 and 5 fairway considered in the surveys. Spatial maps of all measured parameters were also generated from georeferenced point data in ArcMap using ordinary kriging (Figure 2). Nine fairways were ultimately selected for use in the study and grouped into three groups of similar fairways (i.e., a randomized complete block design with three treatments and three replications; treatments will consist of a 1) conventional blanket (control), 2) vegetation index-based variable-rate, and 3) soil available N- and vegetation index-based variable-rate N granular applications).

Future Expectations

- Continue to compile monthly or bi-monthly (depending on the measurement) spatiotemporal data on fairways in 2023 using the PS6000, UAV with MicaSense Altum-PT camera, and EM-38 to confirm experimental fairway groupings (i.e., replications) and assist in building prescription maps for variable-rate N applications in 2024 and 2025 (Objective 3).
- Create fairway vegetation index [e.g., NDVI, NDRE, green normalized vegetation index (GNDVI), and enhanced vegetation index (EVI)] and EC_a site-specific management units (SSMUs) in 2023 to collect tissue and soil samples from for comparison to leaf tissue and soil N content (Objective 1) and assess seasonal variations (Objective 2).
- Create a digital elevation map of all fairways so that topography can be considered in prescription maps for variable-rate N applications.
- Test GPS-guided spreader and preliminary prescription maps in fall 2023 on a fairway not being used in the study.

Figure 1. The Toro Precision Sense 6000 (left) and Draganfly Commander 2 with MicaSense Altum-PT (right) at Bearkat Course (Huntsville, TX) in fall 2022.

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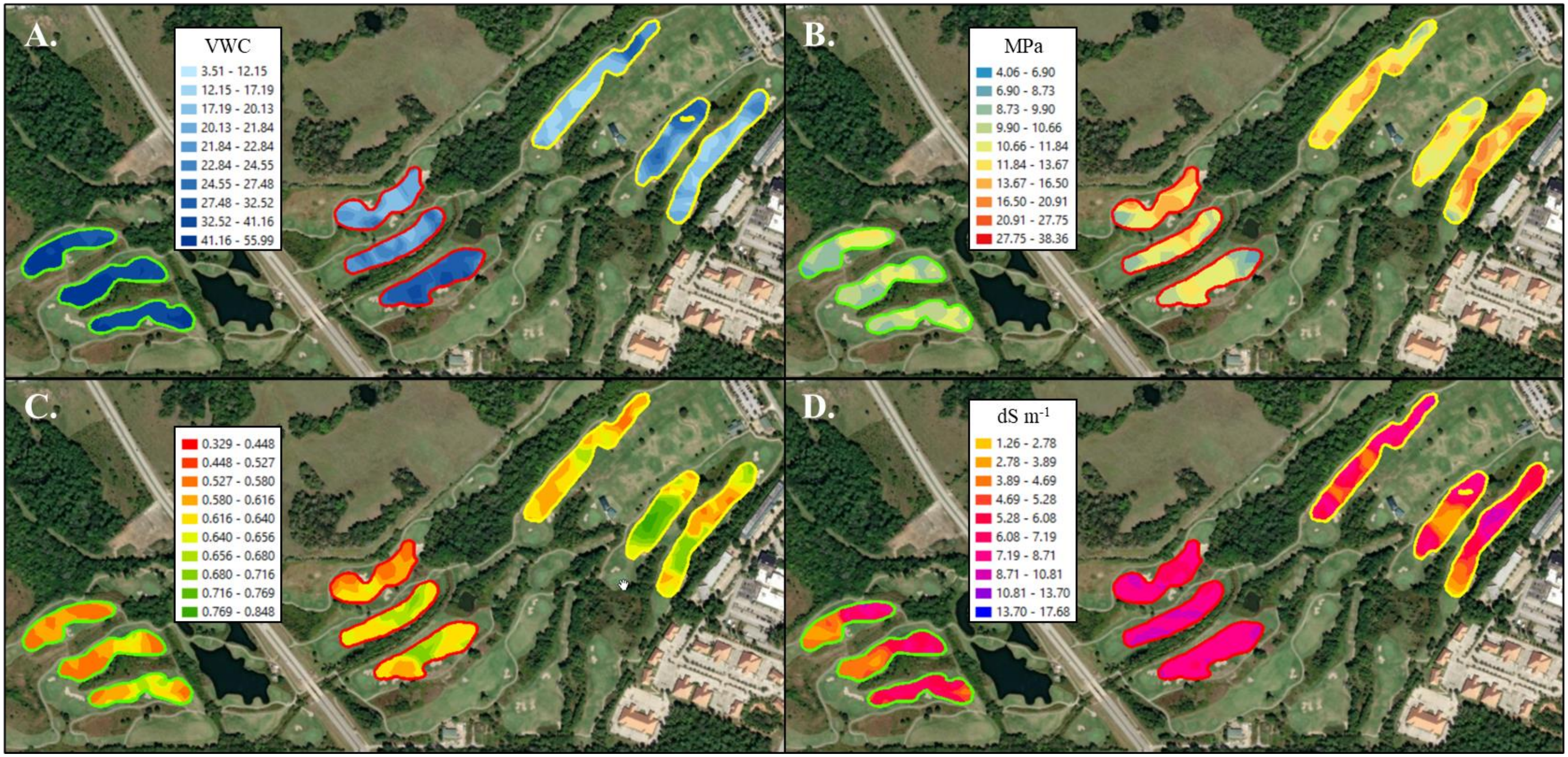

Figure 2. Spatial maps of data collected in fairways at Bearkat Course on 14 Sept. 2022 with the Toro Precision Sense 6000: A. soil moisture (volumetric water content; % VWC), B. penetration resistance (MPa), C. normalized difference vegetation index, and D. electrical conductivity (EC_e; dS m^{-1}). Data collected from this date and three other dates in 2022 were used to group fairways into similar groups of three (i.e., a randomized complete block design with three replications) for Objective 3. Fairways with the same color boundary are in the same group.

USGA ID#: 2019-30-700

Title: Advancing Precision Turfgrass Management (Previously, Mining GreenKeeper App Data to Quantify the Impact of Turf Research)

Project Leader: Roch Gaussoin, Michael Carlson

Affiliation: University of Nebraska-Lincoln

Objectives:

- Summarize Precision Turfgrass Management research and propose an agenda to promote adoption.
- Quantify the relationship between turfgrass canopy reflectance, growth rate, and visual quality.
- Quantify spatial and temporal variability of fairway turfgrass and test the accuracy of reflectance to monitor spatial and temporal variability of fairway growth.
- Develop and test novel remote sensing-based variable rate N (VRN) fertilizer application systems.

Start Date: 2019

Project Duration: Three Years

Total Funding:

Summary Points:

- A systematic review of precision turfgrass research reported only 6% of research focused on developing models or decision support systems.
- Growth rates to achieve ideal visual quality of 6 to 7 as determined by a NDRE sufficiency index ranged from 4.84 to 22.9 kg ha^{-1} d^{-1} in 2019 and from 6.05 to 17.8 kg ha^{-1} d^{-1} in 2020.
- Growth rate and canopy reflectance of creeping bentgrass fairways varied from May to September, whereas canopy reflectance-based site-specific management units reduced the variability of growth compared to the whole fairway suggesting that reflectance can be used to reduce variability of growth rate.

- A Threshold-based VRN system reduced processing time to create prescription VRN applications maps and total N applied.

Summary Text:

A Review of Precision Management for Golf Course Turfgrass

Precision turfgrass management (PTM) is a combination of methods and technologies proposed to increase the resiliency of golf courses by improving input efficiency while maintaining the function and aesthetics of the playing surface. However, there is no recent review describing the status of precision management in turfgrass. The objectives of this review were to 1) summarize peer reviewed research on precision technology for turfgrass management, 2) describe adoption of PTM based tools, and 3) propose an agenda of research priorities to advance and promote PTM adoption. Of the articles reviewed, 94% documented the accuracy of sensors to detect turfgrass performance and stressors before or during visual symptoms. Only 6% of the research reviewed focused on developing models or decision support systems to quantify the relationship among reflectance, nitrogen uptake, visual quality, biomass production, and irrigation which are required for precision management by golf course superintendents. Efficacy or value of using PTM methods and technologies have not been reported. Golf course superintendents lack of knowledge about PTM, and lack of quantification of benefits of PTM pose limitations to promote adoption. Increasing the adoption of PTM adoption will require research to focus additionally on: automating sensor data processing, quantifying costs, benefits and value of adopting PTM, and simplifying input applications in a PTM system. This review described the status of precision management in golf course turfgrass and shed light into the need for research to develop models and decision support tools for precision management of golf course turfgrass.

Monitoring Creeping Bentgrass (*Agrostis stolonifera* L.) Growth Rate and Quality using Normalized Difference Vegetation Index

Golfers expect golf course turfgrass to meet their desired function and aesthetic goals which requires timely inputs, specialized equipment and labor. Nitrogen fertilizer is an important driver of both turfgrass function and aesthetics. Monitoring turfgrass growth rate and visual quality are needed to increase nitrogen use efficiency and adopt precision turfgrass management methodologies. Remote sensing has been reported to quantify N fertilizer needs on lawn-height turfgrass and perennial ryegrass seed fields, whereas it has not been used to optimize visual quality and growth on creeping bentgrass fairways. the objectives of this study were to: 1) quantify the relationship between a normalized difference red edge (NDRE) sufficiency index, growth rate, and visual quality. Results from this study could support a remote sensing-based N recommendation tool for creeping bentgrass. A randomized complete block repeated measure design small-plot study was conducted from May to September in 2019 and 2020. The turfgrass received six different N treatments applied every 14 d throughout the study period each year. Turfgrass growth rate, visual quality, and normalized difference red edge (NDRE) vegetation index were measured from each plot about every 14 d. Creeping bentgrass growth rate, and NDRE exhibited linear responses to N rate that varied throughout the growing season. The visual quality of the turfgrass varied by N rate, whereas only varied among sampling dates in 2019. The growth rates that corresponded with ideal visual quality differed among years and sampling dates within years. The ideal growth rate to maintain visual quality ranged from 4.84 to 22.9 kg ha-1 d-1, in 2019 and 6.05 to 17.8 kg ha-1 d-1 in 2020. These results are the first report to quantify an ideal growth rate and visual quality by NDRE reflectance and could be used to create a N fertilizer decision support tool.

Spatial and Temporal Variability of Creeping Bentgrass (*Agrostis stolonifera* L.) Growth

Spatial and temporal monitoring of turfgrass aesthetics and function are important to the precision turfgrass management and increase the efficiency of nitrogen applications. Remote sensing can quantify turfgrass nitrogen needs and growth rate, spatial variability of golf course fairways, and delineate site-specific management units (SSMUs), whereas remote sensing has not been documented to delineate SSMUs for precision nutrient applications for growth rate management. The objectives of this study were to: i) quantify spatial and temporal variability of growth on creeping bentgrass fairways, ii) test the accuracy of reflectance to monitor temporal and spatial variability of fairway growth, iii) test the Straw and Henry (2018) method to delineate SSMUs to capture spatial and temporal variability of growth rate with remote sensing. The geo-referenced growth rate and normalized difference red edge vegetation index (NDRE) reflectance were measured from fairways 1, 5, and 7 at the Jim Ager Memorial Junior Golf Course in Lincoln, NE three times in 2020 and 2021. The mean, standard deviation, and coefficient of variation were quantified within each fairway and sampling date to document the variability of growth rate and NDRE, semivariograms were quantified to document the spatial variability. The growth rate and NDRE data were kriged using best-fit semiovariograms, and SSMUs were delineated for each fairway and sampling date based on the legend of the kriged NDRE maps. The mean, CV and SD of each SSMU were quantified to compare the to the whole fairway data and to the Straw and Henry (2018) method. Growth rate, the relationship between growth rate and NDRE, and semivariograms to quantify spatial variability varied among fairways and sampling dates. Sampling dates with significant relationships between growth rate and NDRE exhibited moderate to strong positive correlation coefficients ($r > 0.35$). The NDRE SD-based SSMUs reduced the growth rate SD by 45.7, 57.2, and 58.8% on average on fairways 1, 5, and 7, respectively, compared to the fairway SD. The SSMUs reduced the growth rate CV y 75.8, 86.7, and 74.9% on average on fairways, 1, 5, and 7, respectively, compared to the fairway CV. Non-destructive NDRE remote sensing can delineate SSMUs for precision nutrient management on creeping bentgrass fairways.

Remote Sensing Based Variable Rate Nitrogen Application Systems for Golf Course Fairways

Nitrogen (N) is the highest volume fertilizer applied on golf courses whereas nutrient restrictions are becoming more common requiring the increasing the efficiency of N on golf courses. Remote sensing based variable rate N (VRN) applications increase N use efficiency in cropping systems but have not yet been tested in golf course turfgrass management. In this paper, we proposed and tested active canopy sensor based VRN management for creeping bentgrass (Agrostis stolonifera L.) fairways. The objectives were to: 1) describe the components and implementation of active canopy sensor based VRN management fertilizer systems in turfgrass creeping bentgrass fairways, and 2) test impact of proposed VRN systems on total N use and turfgrass quality compared to a traditional fairway management. Combined between 2020 and 2021, three active canopy sensor based VRN management fertilizer systems were proposed to translate fairway reflectance measurements into a VRN management prescription based on normalized difference red edge vegetation index (NDRE) and normalized difference vegetation index (NDVI) monitoring for a period of 14 days: delta between day 1 and day 14 mean NDRE (“dNDRE”), slope of the linear trend of NDRE within 14 days (“Trend”), and fixed NDRE and NDVI thresholds (“Threshold”). Threshold based VRN systems reduced processing time, to analyze and create VRN prescription maps by 99% compared to the other VRN systems. The dNDRE and Trend systems exhibited similar increase in NDRE and visual quality after N application to the traditional N management. In contrast, NDRE Threshold based VRN system increased NDRE values after N applications by 6% and maintained visual quality. The Threshold based VRN systems exhibited high proportion of the fairway area with NDRE values above the optimal NDRE range (0.33 – 0.35). All VRN systems significantly reduced total N use compared to the traditional fairway management of 3.91 kg N ha-1 every 14 days for 10 weeks. The Threshold based system decreased total N applied by 10% more than the dNDRE and Trend based systems. Threshold based VRN systems were better suited to maintain visual quality, manage all fairways towards the same function and visual quality goal, and reduce total N applications.

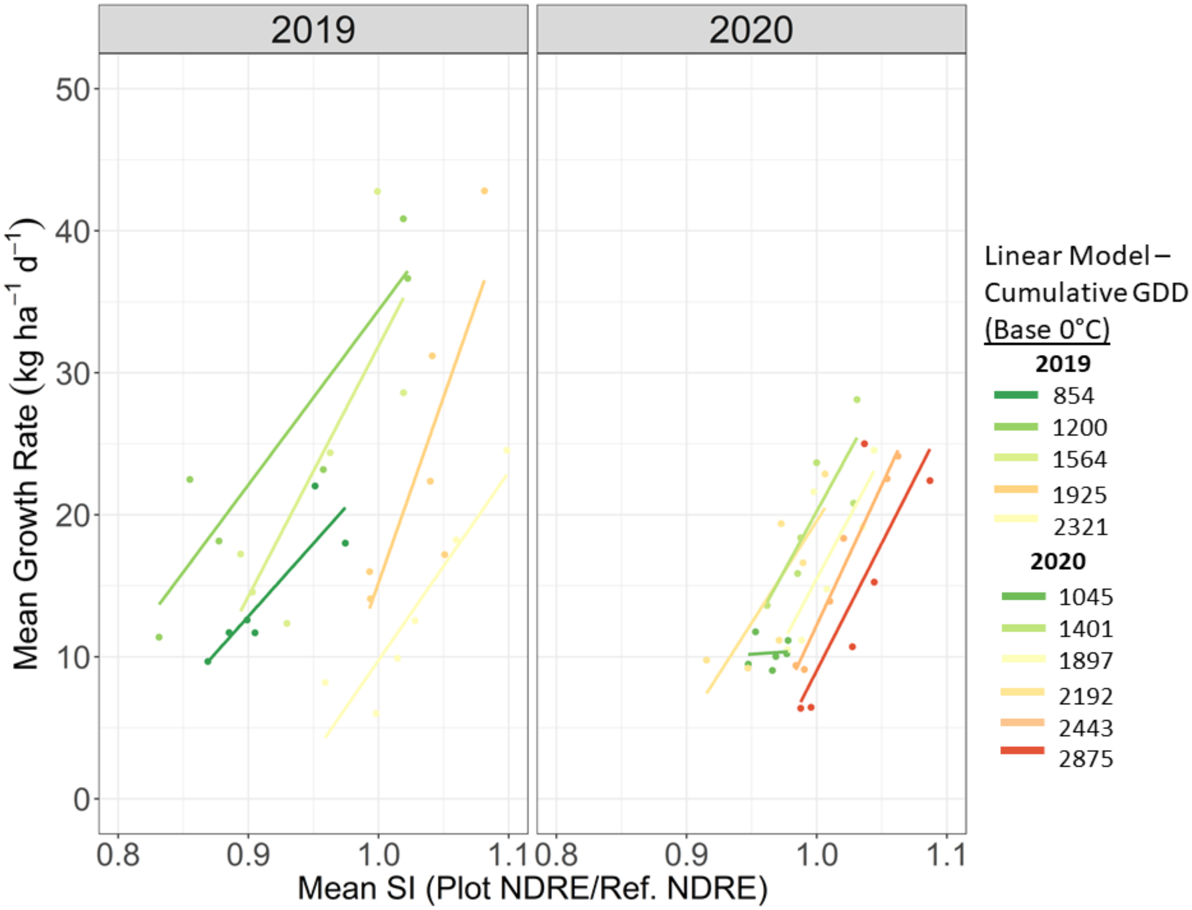

Figure 1. The mean growth rate (kg ha^{-1} d^{-1}) as a function of mean SI value (plot NDRE reference NDRE^{-1}) of 2019 and 2020. The data points and linear models are colored by cumulative GDD (base 0°C) of each sampling date.

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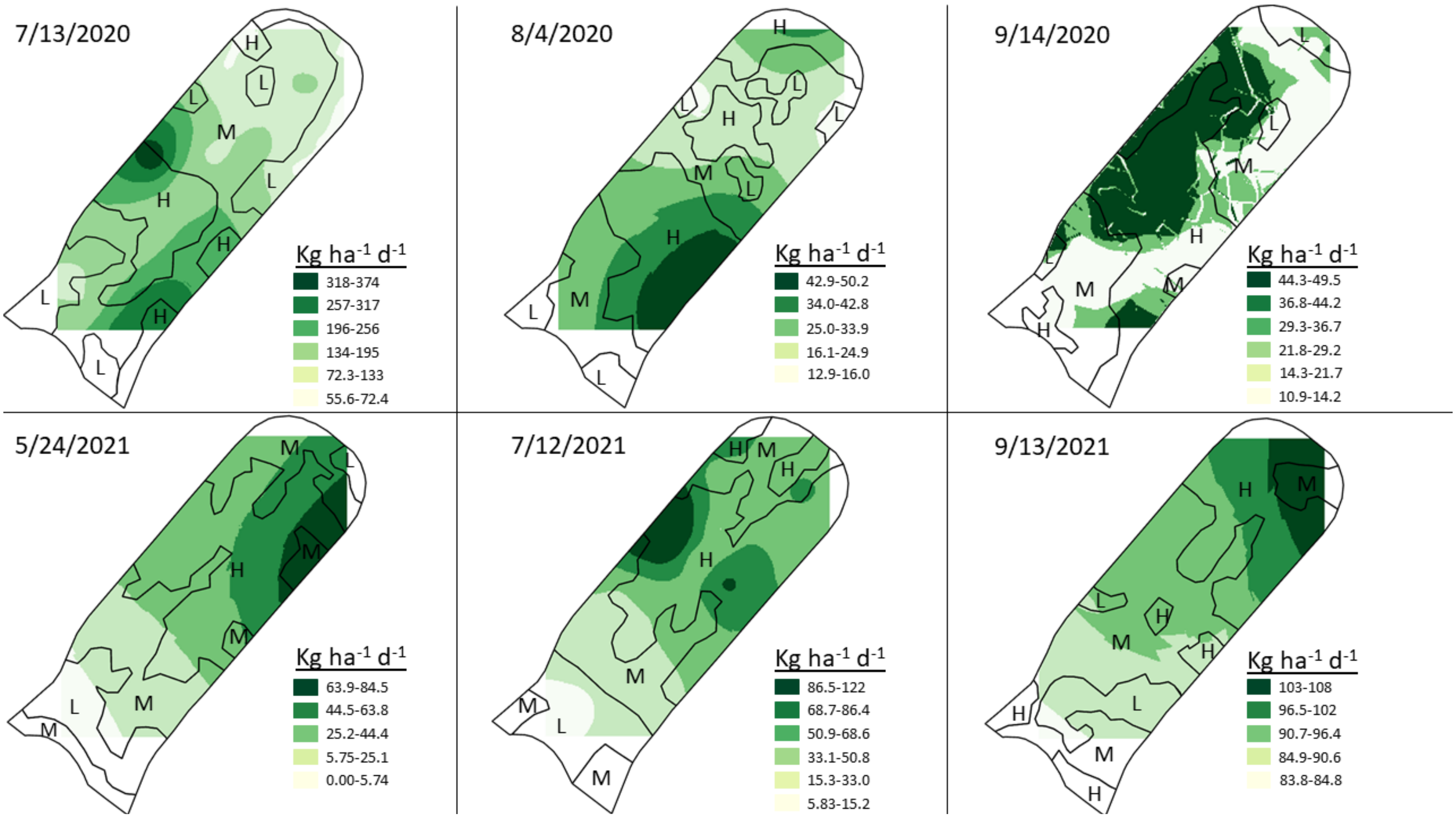


Figure 2. The standard deviation normalized difference red edge vegetation index (NDRE) legend delineated site-specific management units (SSMUs) on fairway 1 and the kriged growth rate (kg ha^{1} d^{-1}) among all sampling dates. The location of the SSMUs visually correspond with similar ranges of growth rates on fairway 1. Low, middle, and high SSMUs are denoted as L, M. and H, respectively.

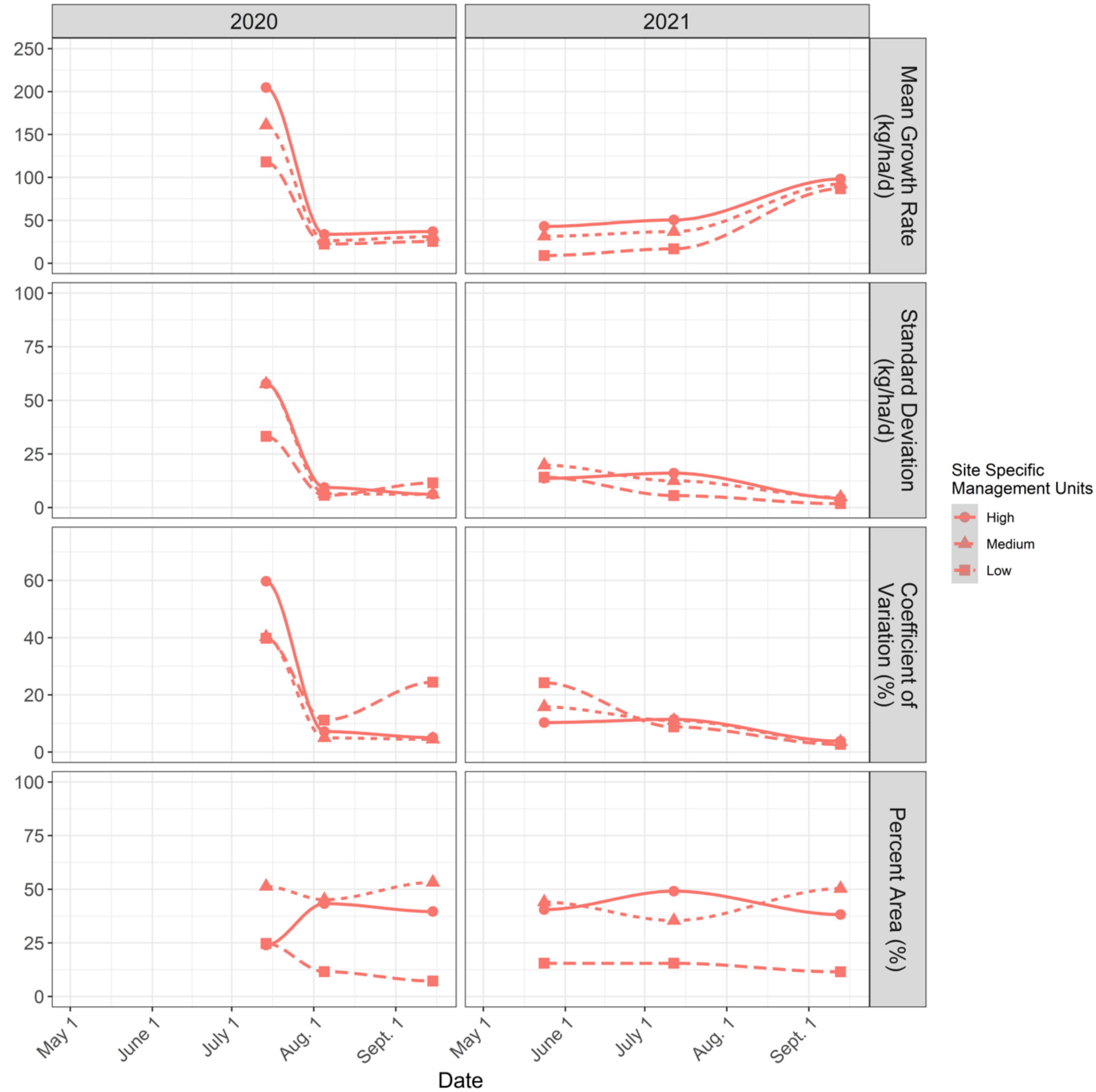


Figure 3. Mean growth rate (kg ha^{-1} d^{-1}), standard deviation of growth rate (kg ha^{-1} d^{-1}), and coefficient of variation of growth (%) of the high, medium, and low site specific management units on fairway 1 in 2020 and 2021. The shape of the data points and type of trend lines correspond with the different site specific management units. The fairways were sampled on 13 July 2020, 4 August 2020, and 14 September 2020, and in 2021 on 24 May, 12 July, and 13 September.

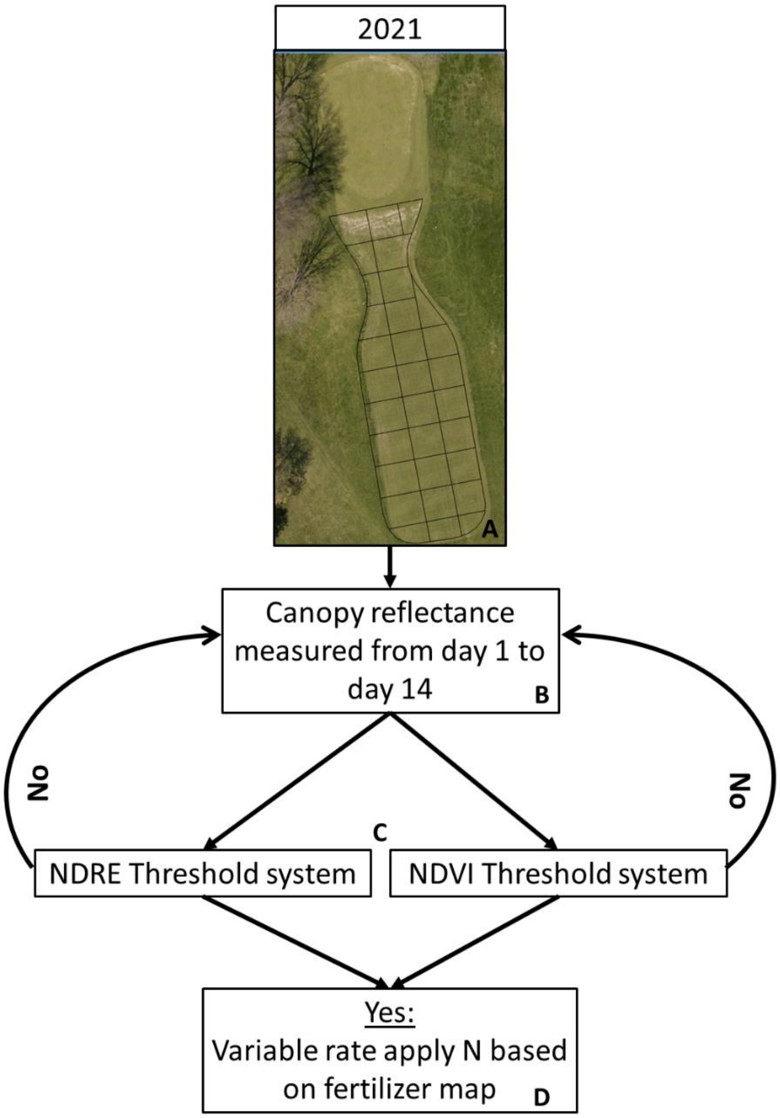


Figure 4. Flow chart active canopy sensor Threshold variable rate nitrogen (VRN) systems studied in 2021 at the Jim Ager Memorial Junior Golf Course in Lincoln, NE. A) Fairways were divided into 6.8 m^2 grid management units based on the width of the Arag-Toro sprayer boom width. B). Holland Scientific Crop Circle ACS-430 measured normalized difference red edge vegetation index (NDRE) and normalized difference vegetation index (NDVI) from the red, red edge, and near infrared portions of the electromagnetic spectrum from the fairway turfgrass canopy for 14 days. C). The NDRE and NDVI data were processed, interpreted, and nitrogen (N) fertilizer applications maps were produced using an automated R code. D). If the golf course superintendent desired N rate based on declining visual quality or function, and the Threshold system suggested the same, VRN applications were made with the Arag Ninja modified Toro sprayer otherwise the turfgrass did not receive N.

USGA ID#: 2022-02-745

Title: Tolerance of Sand-Capped Fairways to Deficit Irrigation and Simulated Traffic

Project Leaders: B. Wherley, B. Chang, C. Straw, and K. McInnes
Affiliation: Department of Soil and Crop Sciences, Texas A&M University

Background and Methodology
This project is being conducted at the Scotts Miracle-Gro Center for Lawn and Garden Research at Texas A&M University, College Station, TX on 8-year old 'Tifway' hybrid bermudagrass sand-capped fairway research plots. Half of the facility is sand-capped atop a fine sandy loam soil, while the other half is built atop clay loam soil. Thus, the entire project is treated as two separated studies (sandy loam study and clay loam study).

The current study was initiated on 6/20/22, and is arranged as a split-split-plot design, with capping depth (0 to 5 cm topdressed (TD), 5, 10, and 20 cm) as the whole plot, summer deficit irrigation level of 45 and 30% × Reference Evapotranspiration (ET_o) as the subplot. Irrigation subplots are further divided into monthly wetting agent sub-sub plots (Oars PS @ 0 or 6 oz per 1000 ft^2). Weekly traffic is imposed on all treatment plots using a golf cart, with 16 passes per week. A drone aerial image of the sand-capping study site is provided in Figure 1. During the study period, application of a 50% sulfur coated urea 21-7-14 fertilizer is applied at 1 lb. N per 1000 ft^2 every 6 weeks. Pelletized gypsum (applied at 40 lb per 1000 ft^2) is also applied monthly to all research plots.

For data collection, visual turf quality, visual percent firing, visual percent wilt, and percent green cover have been evaluated every two weeks of the 2022 season. Soil hydrophobicity was evaluated through water droplet penetration time testing conducted on 10/3/22 (sandy loam study- measured at 1 cm depth). Saturated hydraulic conductivity for both studies was also measured on 11/28/22. Divots were created using a simulated divot maker on 9/2/22, and recovery time has been evaluated weekly through light box image analysis. Soil moisture and salinity data are being collected using Toro Turfguard Sensors for the 5 and 18 cm depths within 10 and 20 cm capping treatments to better understand moisture dynamics as they relate to wilt/quality thresholds. These data continue to be collected, and comprehensive results will be made available in the final report.

Results to Date
For the clay loam subsoil study, percent green cover was primarily affected by capping depth and irrigation level during the initial season. Both capping depth and irrigation level also showed an interaction with week. In the beginning of the study, all plots maintained higher percent green cover (>90%), however, after deficit irrigation and simulated traffic were imposed, percent green cover decreased in all plots (Figure 2). With the exception of the 0 to 5 cm TD treatment, there was generally an inverse relationship between percent green cover and capping depth. Percent green cover of all plots rebounded during August, due to the first measurable rainfall received since May 2022. Even though there was a week x irrigation level interaction, the impact of irrigation level on percent green cover was consistent for most dates, so only the main effects are provided in this report. Interestingly, both deficit irrigation treatments supported >75% green cover during the summer period, with 45% x ET_o treatments mainaining 84% percent green cover and 30% x ET_o plots maintaining 78% green cover (Figure 3).

For the sandy loam study, overall trends were similar to that of clay loam, with few differences. There was again an interaction between irrigation level and week on percent green cover, but only the main effect of irrigation level is included in this report. Again, both deficit irrigation treatments supported >75% green cover across the season. Plots irrigated at 45% ET_o maintained 79% percent green cover while those receiving 30% ET_o showed 74% green cover, when averaged across rating dates (Figure 4). Similar to clay loam, percent green cover was decreased as capping depth increases, with significant difference found between capping depth 0 and 20 cm (Figure 4).

Divot recovery has been evaluated following divot creation in September. A significant main effect of capping depth on recovery rate was found for the clay loam study, with a range of 33.9 - 46.4% for all capping depths when data were pooled across date (Figure 6). With the exception of 0 to 5cm TD, divot recovery rate appears to be positively correlated with capping depth. Also, on the sandy loam study, there is a main effect of wetting agent on divot recovery rate, with 56% and 50% recovery observed for wetting agent-treated and non-treated plots, respectively (Figure 7).

Summary to Date

Results from the first season of this study reveal a few interesting findings. First, while wetting agent had minimal effect on visual turf quality as represented by percent green cover, application of monthly wetting agent reduced water droplet penetration time by ~90% for sandy loam study (Figure 5). Whereas prior studies (years 1-7) had only shown development of hydrophobicity on the deepest (20 cm) caps, the current study, now conducted under irrigation deficit, is showing it occurring in all treatments, but still progressively more severe with increasing capping depth (Figure 5). Another noteworthy and perhaps concerning observation has been the relatively low percent green cover noted in the 0 to 5 cm TD treatmewnt atop clay loam soil. Whereas this treatment had performed quite well (and similar to the 5 cm treatment) over the history of the study site, this observation may indicate that root development in underlying subsoil may be negatively impacted by elevated sodium, or perhaps, there may be accumulation of salts in the root zone due to deficit irrigation, limiting the downward movement of salts on this shallowest capping treatment. This will be more closely examined over the coming year. Data from the fall recovery period following resumption of 60% x ETo irrigation to all plots has been collected, and are currently being analyzed.

The 2022 data suggest the following:

1. Soil hydrophobicity is now evident in all capping depths, and continues to be worse with increasing capping depth.
2. Monthly application of wetting agent has been sufficient to mitigate soil hydrophobicity, based on WDPT testing. The impact of wetting agent on improving turf quality was minimal, but may drive differences in fall recovery, which is currently being investigated.
3. The 0 to 5 cm TD treatment has shown lower quality than in the past, which may suggest inhibition of root development into sodic subsoil, or increased root zone salinity due to limited ability to flush salts in this treatment.
4. Sand-capping atop clay loam provided somewhat improved overall quality compared to that atop sandy loam subsoil when irrigated at deficit irrigation levels.
5. Divot recovery rates increased as capping depth increased for the clay loam study, while application of wetting agent seems accelerated divot recovery for the sandy loam system.

Figure 1. Drone image of the Texas A&M sand-capping facility, College Station, TX

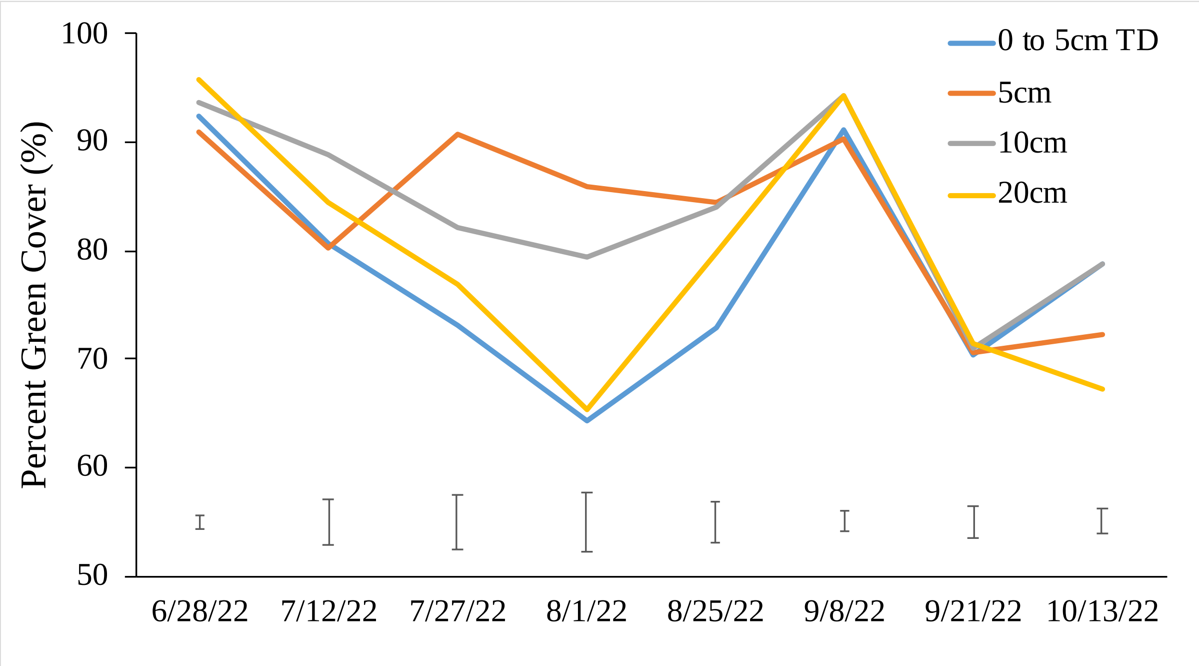


Figure 2. Main effect of sand-capping depth on percent green cover for clay loam study during the 2022 growing season. Data are pooled across deficit irrigation and wetting agent treatments. Bars represent Fisher's LSD at P<0.05.

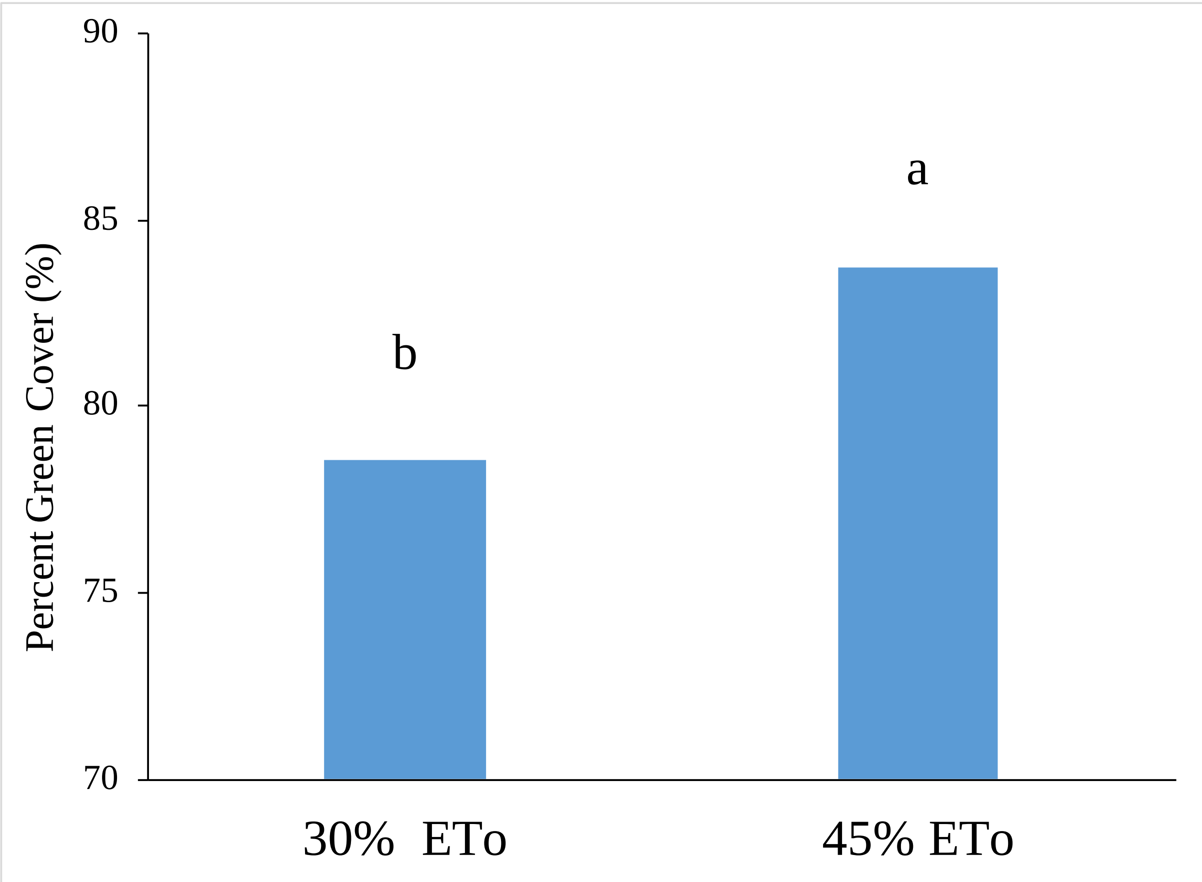


Figure 3. Main effect of irrigation level on percent green cover during the growing season for the clay loam study. Data are pooled across capping depth, wetting agent, and date. Bars with the same letter are not statistically different, based on Fisher's LSD at P<0.05.

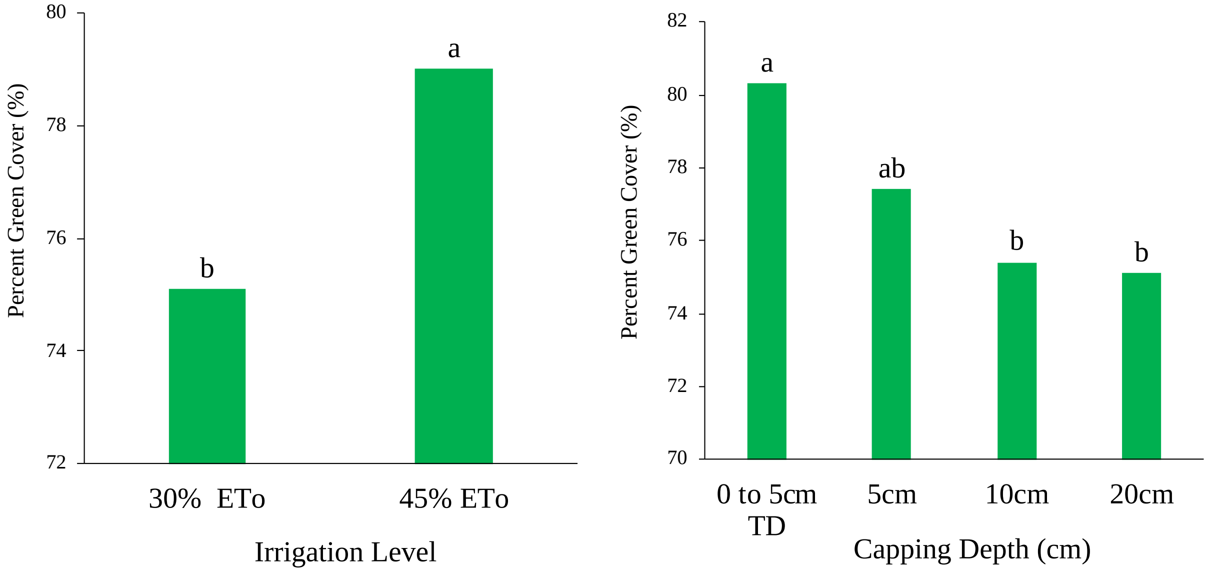


Figure 4. Percent green cover for sand-capped fairway plots (sandy loam study) as affected by deficit irrigation level (left) sand-capping depth (right). Percent green cover x irrigation data are pooled across rating date, capping depth, and wetting agent treatment. Percent green cover x capping depth data are pooled across rating date, irrigation level, and wetting agent treatment. Bars with same letter are not statistically different, based on Fisher’s LSD at $P<0.05$.

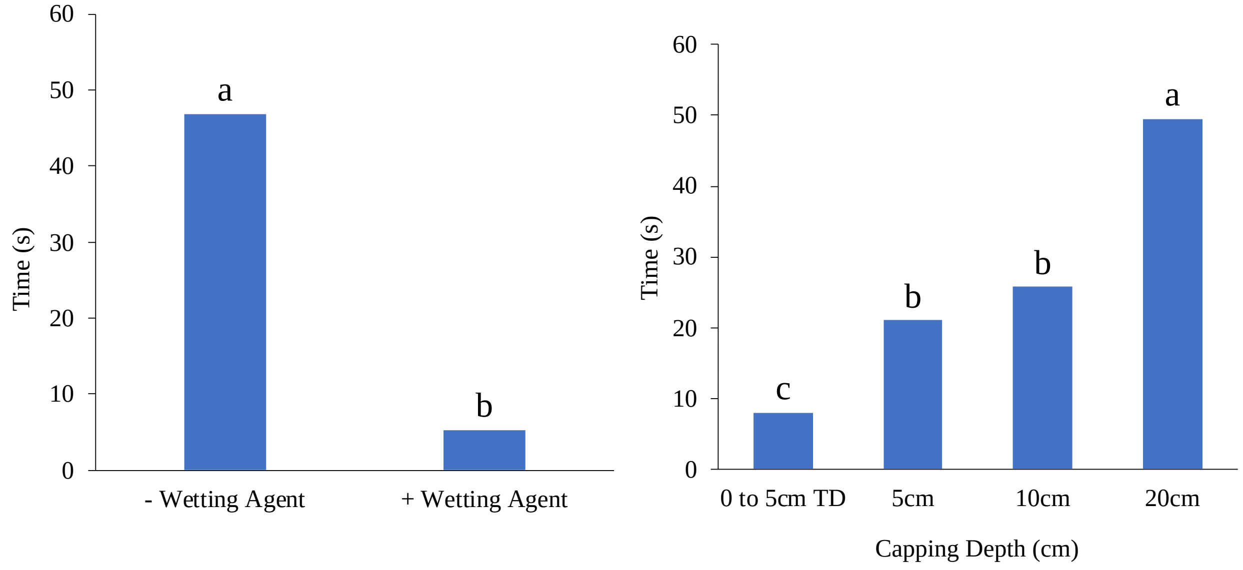


Figure 5. Main effect of wetting agent (upper left) and capping depth (upper right) on water droplet penetration time (hydrophobicity test) for the sandy loam study. Testing was performed at the 1 cm depth following four months of wetting agent treatment applications. Penetration time x wetting agent data are pooled across rating date, capping depth, and irrigation level. Penetration time x capping depth data are pooled across rating date, irrigation level, and wetting agent treatment. Bars with the same letter are not statistically different, based on Fisher's LSD at P<0.05.

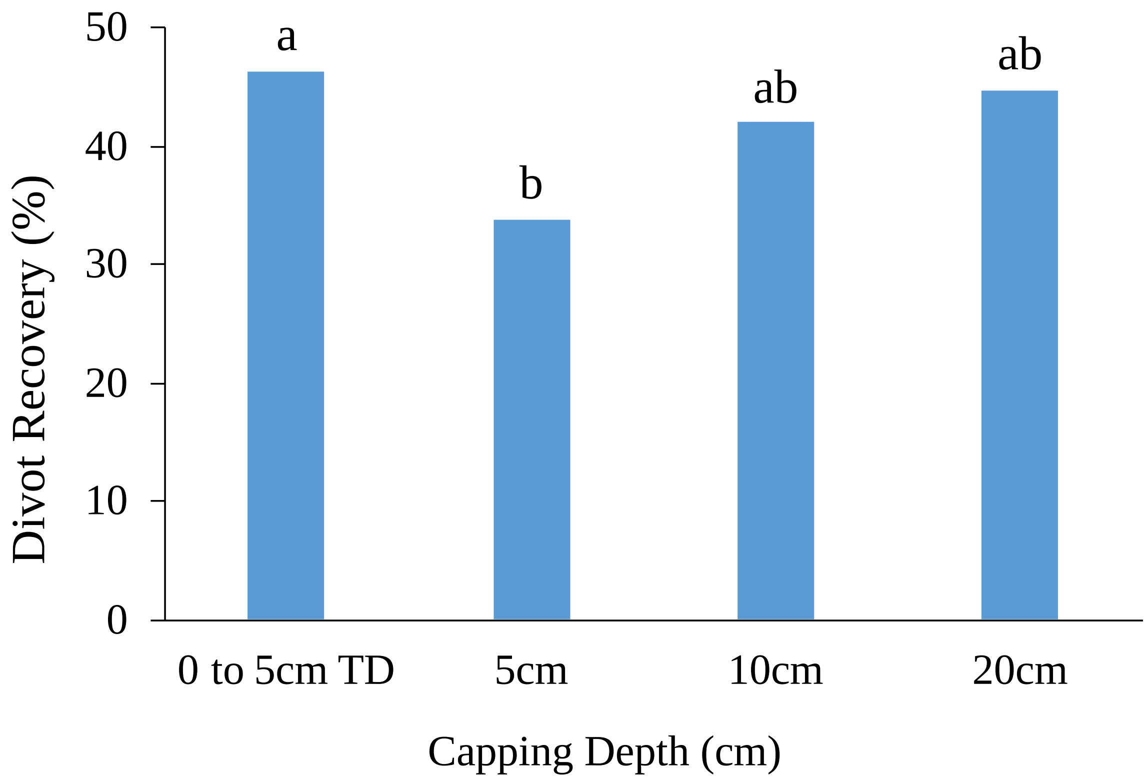


Figure 6. Main effect of capping depth on divot recovery rate (%) for the clay loam study. Data are pooled across rating date, irrigation level, and wetting agent treatment. Bars with the same letter are not statistically different, based on Fisher's LSD at P<0.05.

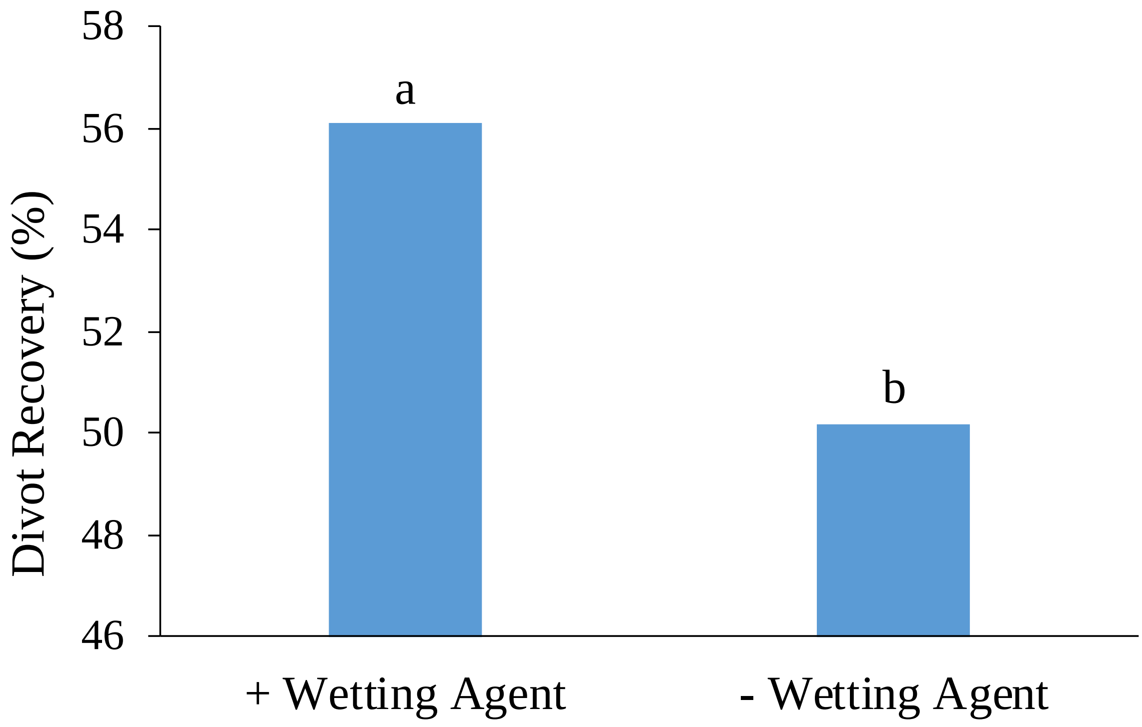


Figure 7. Main effect of wetting agent on divot recovery rate (%) for the sandy loam study. Data are pooled across rating date, irrigation level, and capping depth. Bars with the same letter are not statistically different, based on Fisher's LSD at P<0.05.

USGA ID: 2021-06-730 (continued from 2017-37-647)

Title: Towards Advancing Precision Irrigation on Golf Courses

Project Leaders: Chase Straw[1], Josh Friell[2], Ryan Schwab[3], and Eric Watkins[3]

Affiliation: [1]Texas A&M University, [2]The Toro Company, [3]University of Minnesota

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 (three-year duration + 1-year extension)

Total Funding: $204,876 (+$30,000 1-year extension)

Summary Points:

- During 2020, soil moisture sensor placement was finalized, the irrigation control system was rezoned, and the system was configured to record total water use by treatment, fairway, and soil moisture class.

- Irrigation treatments were initiated in 2020, and total water use was recorded throughout four independent runs during summer and fall 2020 and 2021 (two runs per year).

- Dry downs were conducted to determine the appropriate soil moisture thresholds for the SMS-based treatment prior to treatment applications each run.

- Precision Sense 6000 (PS6000) surveys were conducted twice weekly for each run in 2020, and weekly in 2021.

- Significantly less water was consumed on fairways using the SMS-based treatment. The ET-based approach used the most water of all treatments.

Rationale

The purpose of this research is to demonstrate that adoption of currently available SMS 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 implementing site-specific irrigation.

Progress to Date

Fairway Preparation

Nine fairways (six par 4s and three par 5s) at Edina Country Club in Edina, MN 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. Each grouping of three fairways is considered one replication in the study and each fairway within a grouping was assigned one of three irrigation treatments (i.e. randomized complete block design), which were initiated in 2020. Irrigation scheduling treatments for the study include:

1. SMS-based irrigation scheduling
2. ET-based deficit irrigation scheduling (3 times wk^{-1}, precipitation-adjusted, 60%-ET, K_c=0.98)
3. Traditional irrigation scheduling

Soil moisture sensor placement had been previously completed using two course surveys conducted with the Toro PS6000 in 2019. Those surveys provided field capacity-based segmentation and classification (low, moderate, or high soil moisture) of fairways that were assigned to the SMS-based treatment (Figure 1). Toro TurfGuard in-ground SMS were installed 22 Aug. 2019. One sensor was placed in each soil moisture class within each replication (Figure 1), for a total of nine sensors.

Threshold Determination

An initial dry down was conducted following 3.7 cm of precipitation from 27 May – 2 June 2020 on the SMS fairways, where irrigation was withheld to determine a lower threshold for triggering irrigation applications. The following dry downs prior to each run were conducted the morning after a rain event or blanket applications of irrigation on selected fairways. During the dry down, routine PS6000 surveys were conducted to monitor soil moisture and normalized difference vegetation index (NDVI) across all fairways. The dry down was planned to continue until substantial change was no longer observed in the recorded NDVI values, or the superintendent was no longer comfortable or observed wilt. No additional precipitation occurred during the dry down and PS6000 surveys were conducted on a total of five days throughout the process. Examples of the volumetric water content (VWC) and NDVI maps generated during the dry down are shown for fairway 5 in Figure 2. The TurfGuard sensor VWC values for all nine sensors were also monitored throughout the dry down and values were averaged from midnight to midnight on days those surveys were conducted. Mean NDVI values for each moisture class on each SMS treatment fairway were also calculated on those days. For all soil moisture classes on all fairways, the comfort limit of the superintendent based on observation was reached before notable features were identified in the TurfGuard VWC or PS6000 NDVI data. Once the dry down process was stopped, values from each of the nine TurfGuard sensors were recorded and used as the lower threshold triggers for the irrigation zones. This process was repeated for every run in the study (Table 1).

Table 1. Lower threshold soil volumetric water content (VWC) used for triggering soil moisture sensor-based irrigation treatment during the treatment periods in 2020 [22 June – 9 Aug. (Run 1) and 23 Aug – 16 Oct. (Run 2)] and 2021 [13 June – 8 Aug. (Run 1) and 16 Aug. – 10 Oct. (Run 2)].

Fairway	**Moisture Class**	**Lower Threshold Soil VWC (%)**			
		2020		2021	
		Run 1	Run 2	Run 1	Run 2
5	Low	18	18	20	29
	Moderate	30	29	30	26
	High	44	38	32	30
13	Low	19	19	22	25
	Moderate	26	26	25	29
	High	45	44	35	42
15	Low	22	24	14	22
	Moderate	21	21	28	20
	High	22	19	26	29

Irrigation Application & Data Collection

Prior to the initiation of treatments in 2020, the Toro Lynx central irrigation controller was configured to record water use for all irrigation heads, grouped by soil moisture class and fairway. Irrigation treatments were started for run 1 of 2020 on 22 June, following application of 2 mm of irrigation. For the SMS treatment, each time a sensor value dropped below the lower threshold, all irrigation heads assigned to that sensor (i.e., in that fairway and moisture class) were irrigated with 5 and 7.6 mm of water in 2020 and 2021, respectively. The ET-based treatment irrigation depth was calculated using data from an on-site Campbell Scientific T-107 weather station (Campbell Scientific, Logan, UT) and applied 3 times wk^{-1}. The superintendent was responsible for running the traditional scheduling treatment. For all treatments, the superintendent was allowed to run individual irrigation heads in critical areas, as needed. On 9 Aug., treatments were halted to allow the superintendent time for regular maintenance activities on the course. A second run of treatments began for the fall season of 2020 on 23 Aug., which ended on 16 Oct. During that time, the lower thresholds were reset, again based on the superintendent's input. In 2021, single runs were conducted in both summer (13 June – 8 Aug.) and fall (16 Aug. – 10 Oct.) following similar procedures as 2020.

Water use was recorded by the central controller for all applications across all treatments and total consumption was tracked over the course of each treatment run. A mobile application was created to record georeferenced visual quality ratings, taken by a researcher each run, of the fairways. Quality ratings were collected in subjective transitional quality areas in 2020 using a 1-3 scale (1 = poor, 2 = acceptable, 3 = good quality). The ratings were altered to a more objective approach in 2021, in which ratings were determined every 15 s using a 1-9 turfgrass quality scale during PS6000 surveys (Figure 3). In 2020, a PS6000 survey was conducted at least 2 times wk^{-1}; one of which all nine fairways were surveyed and the other only the three SMS-based treatment fairways were surveyed. This was reduced to one PS6000 survey wk^{-1} of all nine fairways in 2021.

Results and Analysis to Date

Total irrigated area and total water consumption for each of the treatment runs is presented in Table 2. Total irrigated area of the sprinkler heads used for each fairway was calculated using ArcGIS (ESRI, Redlands, CA), so that water consumption could be analyzed on a per-area basis. Since the water application amount is calculated by the controller based on the area of overlapping coverage within the fairway, areas outside the fairway that only received water from a single head received less water. Nonetheless, that area was included in the total area estimate because it was necessary to normalize the water use to the size of each fairway. The effect of this is to overestimate the total irrigated area, thus underestimating the applied depth for all treatments. For this analysis, we have assumed that all design inefficiencies due to coverage outside the fairway are proportional to the size of the fairway, and thus area-normalized water consumption is comparable across all fairways, treatments, and replications. Future analyses may more accurately estimate irrigated area using GIS tools to further assess effective coverage area. Furthermore, because the first run in 2020 was one week less than the other runs, water consumption was normalized to the length of the experimental run and expressed in units of depth per time (mm wk^{-1}). Water use data were analyzed using the *lme4* package in R (R Core Development Team, 2017) using a mixed effects model, where run and treatment were considered fixed effects and replicate within run and year were random effects. As no significant run × treatment interaction was present (Table 3), the data were combined by run for examination of treatment effects. Treatment means were calculated and separated by Fisher's protected least significant difference using the *emmeans* package in R.

Significant water savings were achieved using the SMS-based irrigation scheduling approach (Table 2). Due to the humid climate, as well as the professionalism and talent of the superintendent, the traditional scheduling approach used significantly less water than the ET-based irrigation treatment. This was despite accounting for precipitation and the use of a 60% ET_c deficit-based approach. The main effect for "Run" was also significant, where the mean applied irrigation depth in 2020 was 5.66 and 4.18 mm wk^{-1} for the first and second runs, respectively, and 8.76 and 4.88 mm wk^{-1} for the first and second runs in 2021, respectively. This difference is likely a reflection of the seasonal changes between the two runs and further exemplified by the differences in total ET_o, which was 25.9 and 18.6 cm during the first and second runs, respectively, in 2020 and 31.2 and 22.7 cm during the first and second runs, respectively, in 2021.

Table 2. Water use summary for both years of three irrigation treatments, including deficit-ET, traditional, and soil moisture sensor-based scheduling approaches.

			2020		2021		
Treatment	**Fairway**	**Irrigated Area (m^2)**	**Run 1 Irrigation Depth per Week (mm/wk)**	**Run 2 Irrigation Depth per Week (mm/wk)**	**Run 1 Irrigation Depth per Week (mm/wk)**	**Run 2 Irrigation Depth per Week (mm/wk)**	**Mean Irrigation Depth per Week (mm/wk)**
ET	3	14410	6.48	5.22	8.93	6.70	7.81 a†
	6	18303	8.02	7.09	10.21	7.41	
	8	24471	8.60	6.46	10.79	7.84	
Traditional	9	11973	5.60	3.70	7.25	4.26	5.57 b
	10	26439	6.70	3.82	8.79	5.02	
	14	15480	6.08	4.23	7.30	4.12	
SMS	5	16928	3.77	2.85	10.15	2.95	4.22 c
	13	21154	3.06	2.05	6.84	2.86	
	15	13467	2.63	2.20	8.53	2.78	

† Values within column followed by different letters are significantly different at the 95% confidence level

Table 3. Analysis of variance of water use data between soil moisture sensor, deficit-ET, and traditional irrigation scheduling. Replication within run and year were considered random effects.

Source	Num dF	Den dF	MSE	F	*Pr*(>F)
Run	1	4	64.49	45.73	0.002
Treatment	2	25	78.99	28.01	< 0.001
Treatment x Run	2	25	2.05	0.73	0.494

Future Expectations

Mobile app quality ratings and NDVI information from the PS6000 surveys will be analyzed in depth to determine the performance of each fairway, including uniformity (Figure 4). Further analyses will be conducted to evaluate the relationship between superintendent perception, water depletion, spatial variability, and turfgrass quality. The purpose of these analyses will be to determine whether acceptable and equal quality has been maintained for the SMS treatments, despite the significantly reduced water use. Findings will be written as a scientific article and submitted to a refereed journal in 2023.

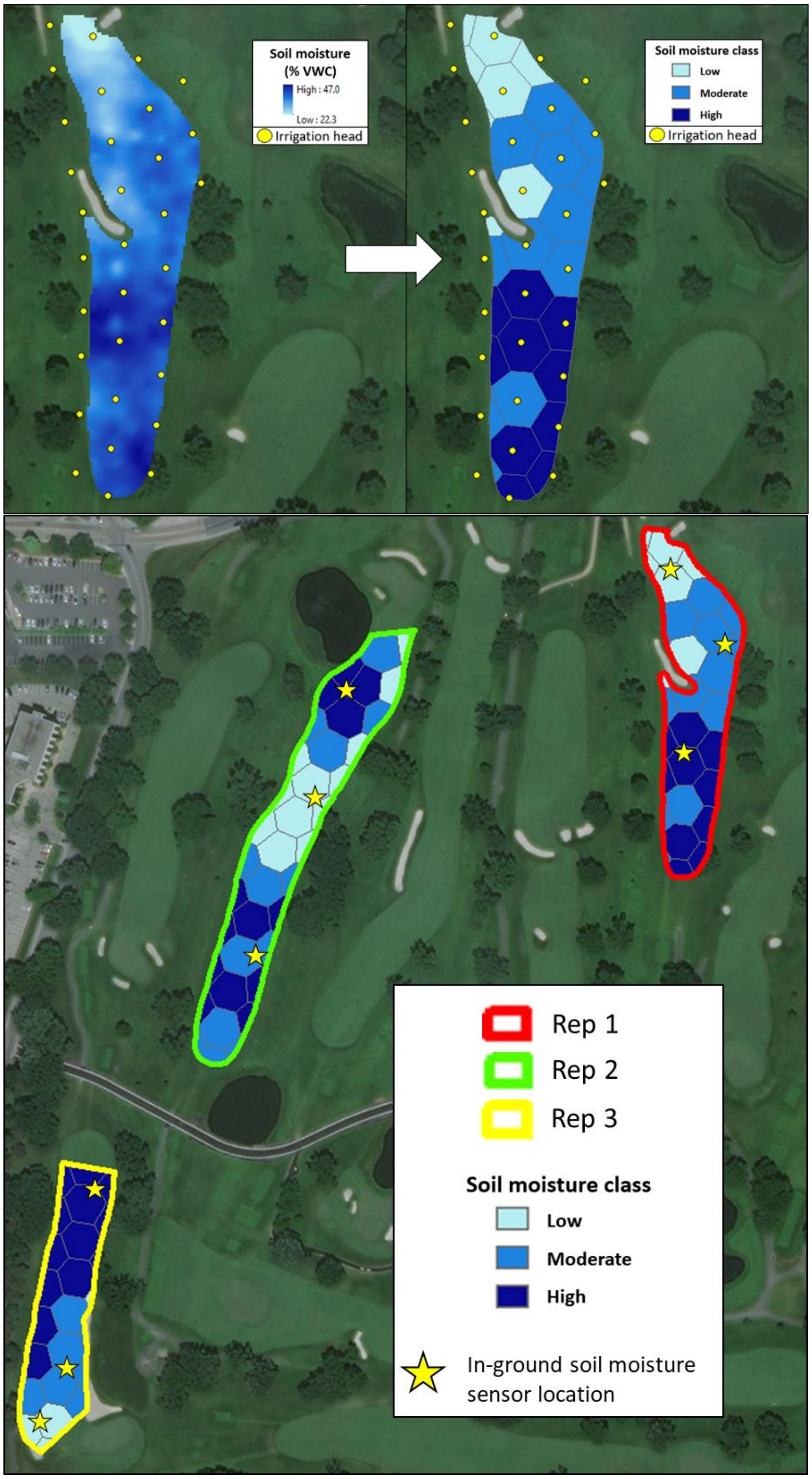


Figure 1. Top-left, percent volumetric water content (VWC) on one fairway in the soil moisture sensor treatment; top-right, soil moisture classes within delineated management zones on one fairway in the soil moisture sensor treatment; bottom, in-ground soil moisture sensor locations within each soil moisture class on the fairways receiving the SMS-based treatment.

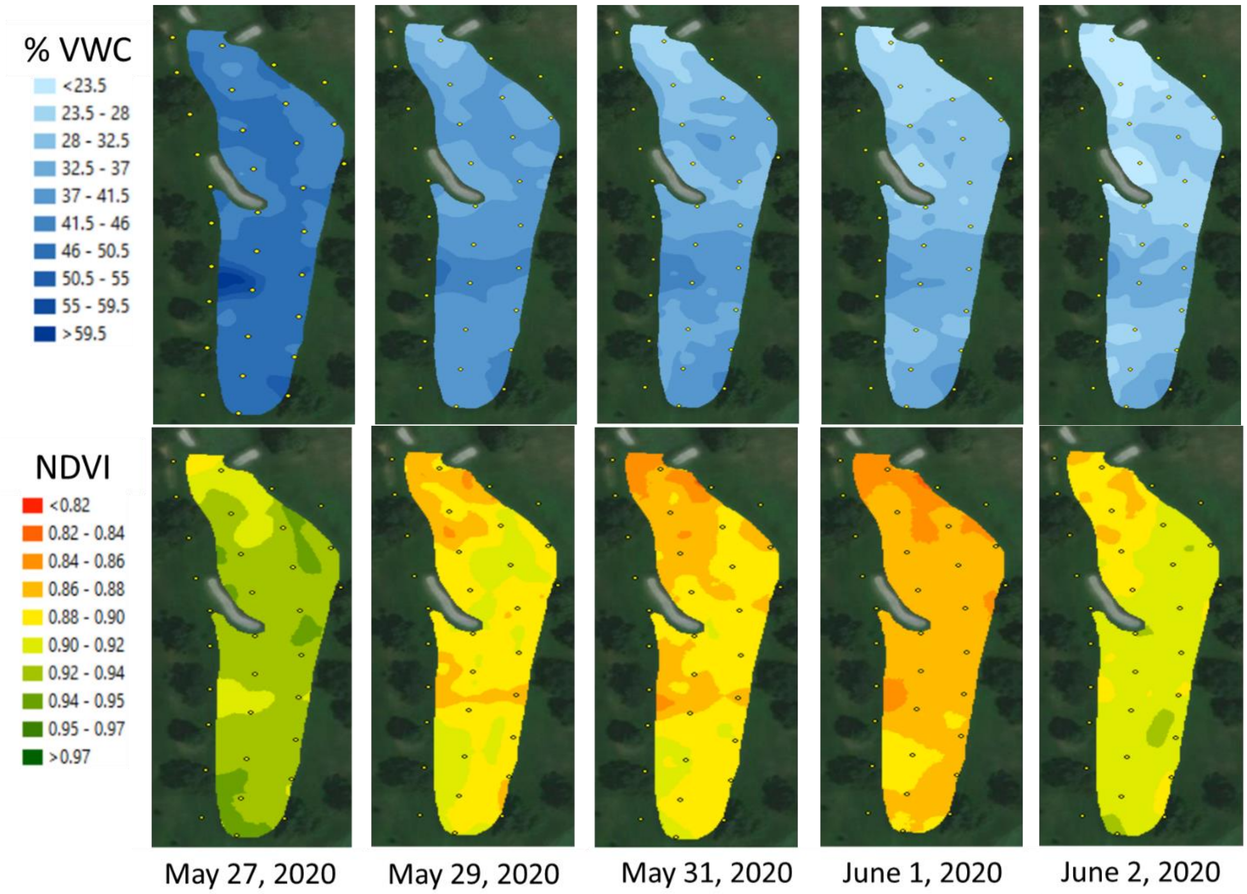


Figure 2. Kriged maps of volumetric water content (VWC) and normalized difference vegetation index (NDVI) on SMS-treatment fairway 5 over the course of a dry down event from May 27 – June 2, 2020 at Edina Country Club.

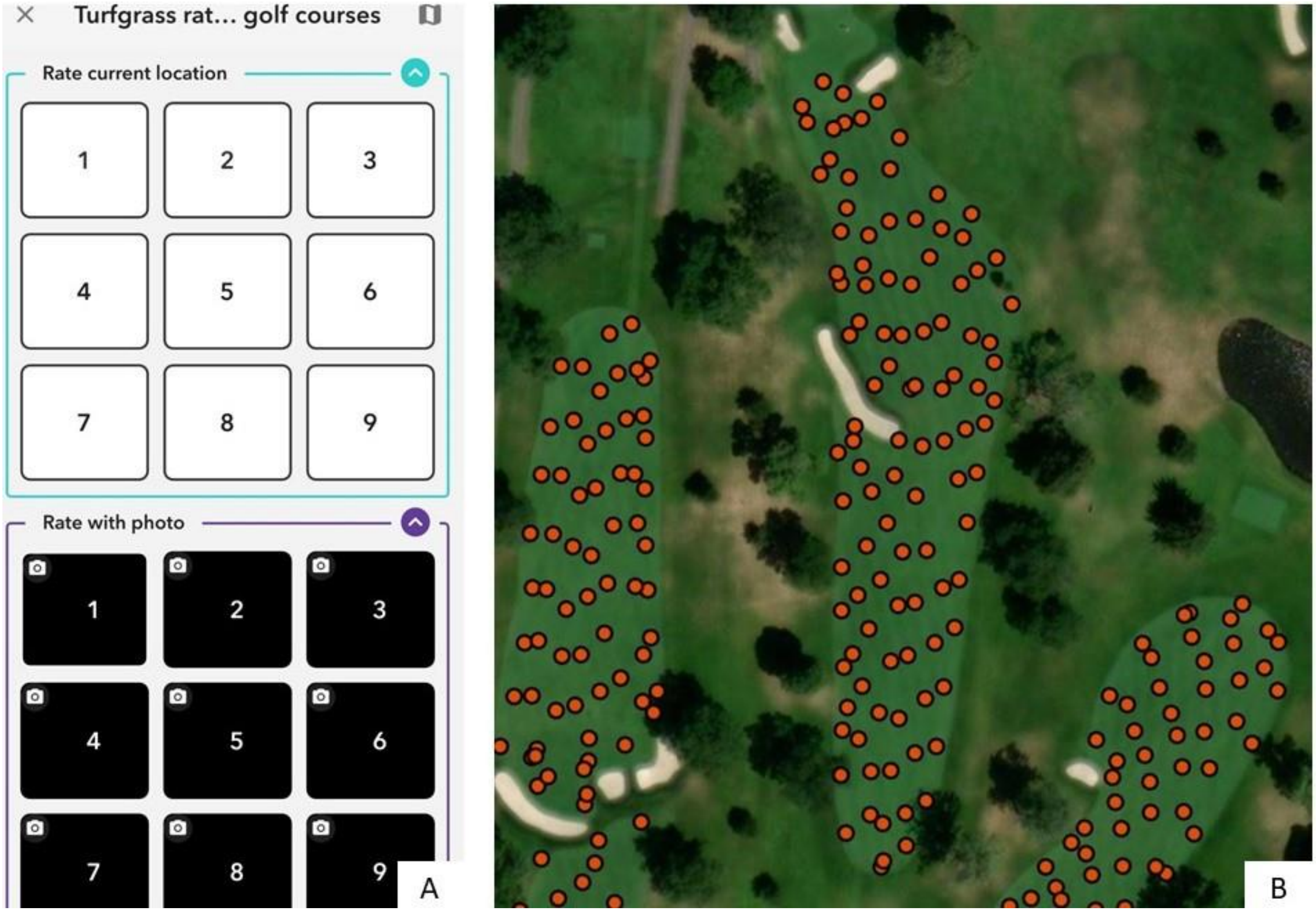


Figure 3. (A) The interface of an ArcGIS-based mobile application in which turfgrass quality ratings were collected. (B) Map of turfgrass quality rating data points on fairways taken on July 21, 2021 at Edina Country Club. Over 20,000 individual data points were recorded in 2021 on the 9 fairways that were part of this study.

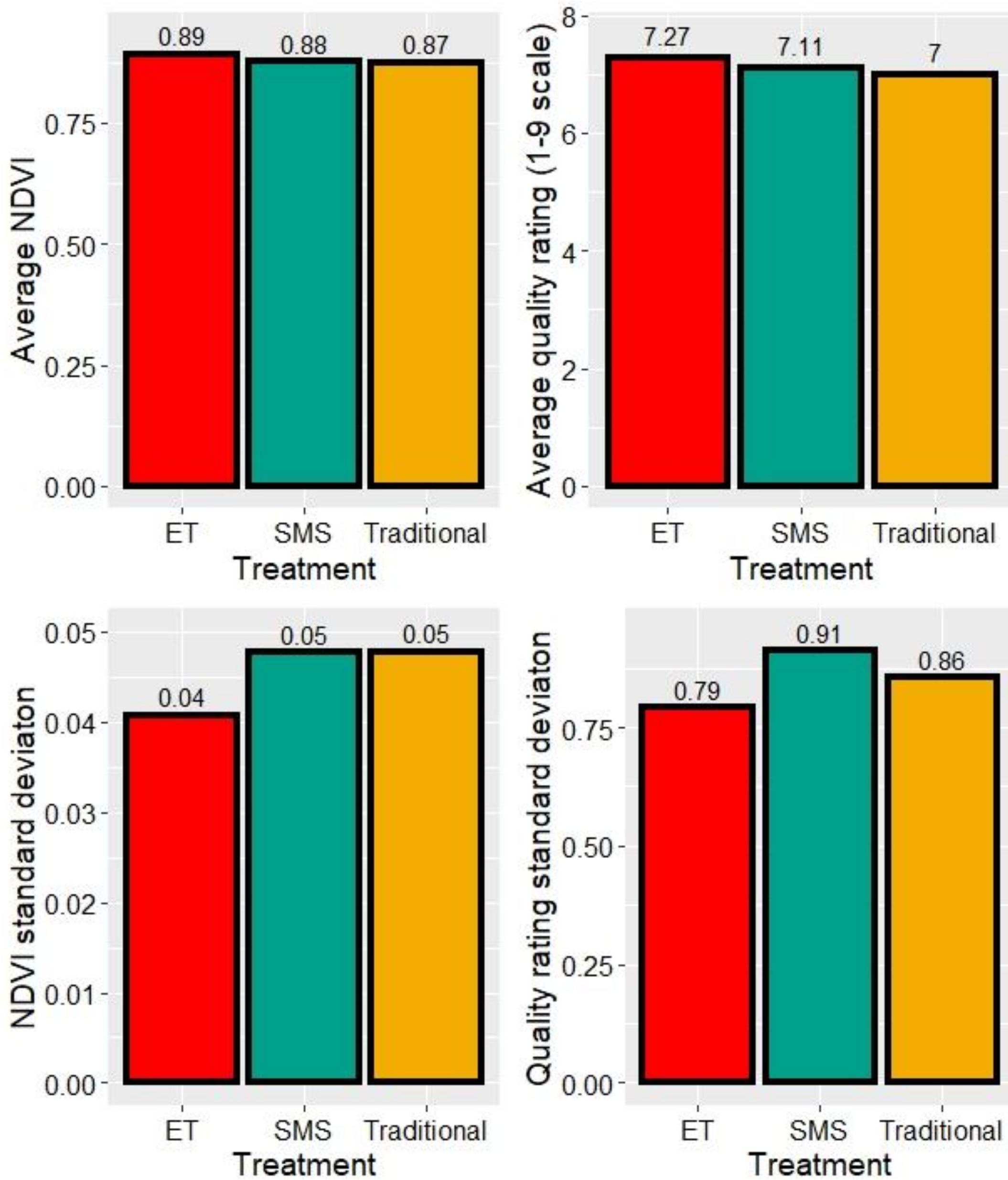


Figure 4. Average and standard deviation of the normalized difference vegetation index (NDVI) and turfgrass quality rating (1-9 scale) of each irrigation strategy treatment (ET = deficit evapotranspiration, SMS = soil moisture sensor, Traditional = superintendent preference) across all 2021 dates.

USGA ID#: 2021-15-739

Title: Determining Irrigation Thresholds to Optimize Water Use, Turf Health, and Playability

Project Leader: Josh Friell[1], Ryan Schwab[1], Eric Watkins[1], Kurt Spokas[2]
Affiliation: [1] University of Minnesota, [2] USDA-ARS

Objectives:

1) Evaluate measurement methods and devices that quantify physiological and physical responses of turfgrass swards during dry down events to determine their suitability for practical field use by superintendents

2) Determine appropriate PAW_{lt} values to optimize turf health and playability factors relative to water use on creeping bentgrass and Kentucky bluegrass fairways in cool, humid climates

3) Quantify the relationship between PAW_{lt} selection and long-term health of creeping bentgrass and Kentucky bluegrass fairways in cool, humid climates

Start Date: February 1, 2021
Project Duration: 3 years
Total Funding: $113,243.00

Summary Points:

- A second year of data was collected on drought susceptible and tolerant cultivars of Kentucky bluegrass and creeping bentgrass that were under a rainout shelter and subjected to well-watered or non-irrigated treatments. Cores of those plots were further evaluated in a greenhouse trial. Plots of each susceptible cultivar were compared under differing threshold-based irrigation treatments (Phase II).
- Canopy responses of creeping bentgrass field plots again indicated that despite declines in some easily-measured variables like percent green cover and normalized difference vegetation index, plants may not be experiencing physiological stress as measured by photochemical efficiency, FvFm. This relationship was not as consistent for Kentucky bluegrass during Year 2 or for either species in the greenhouse evaluation.
- Phase II study site soils were characterized for bulk density and soil water retention properties which showed a plant available water range (-1500 to -10 kPa) of 4.37 to 43.66% volumetric water content.
- Creeping bentgrass plots irrigated at higher moisture thresholds used significantly more water and remained significantly softer and greener; however, a moisture threshold of 45% plant available water reduced water use significantly while maintaining similar final firmness and canopy cover.

Phase 1 - Year 2

Treatments & Data Collection:

During the summer of 2022, methods for data collection used in Year 1 were repeated to obtain a second year of data. In short, two cultivars each of Kentucky bluegrass (*Poa pratensis* L.*)* and creeping bentgrass (*Agrostis stolonifera* L.) were maintained under an automated rainout shelter at the Turfgrass Research, Outreach, and Education Center on the University of Minnesota's Saint Paul Campus. Cultivars of each species were chosen to have reasonably contrasting drought tolerance. Tolerant cultivars were 'Prosperity' and 'Piranha' and susceptible cultivars were 'Shamrock' and 'Penncross' for Kentucky bluegrass and creeping bentgrass, respectively. Treatments consisted of two levels of irrigation: well-watered (100% ET_o replacement) and dry down (no irrigation).

Plots were maintained according to the same practices as during Year 1. Treatments were initiated and data was collected 3 times wk^{-1} beginning on 22 Jun and was reduced to 1 time wk^{-1} during a 24-d recovery period beginning on 10 Aug. Response variables are listed in Table 1. Detailed data collection methods as well as soil characterization and data analysis methods can be found in our 2021 update report.

Table 1. Measured visual, physiological, and playability responses of well-watered or dry down treated Kentucky bluegrass and creeping bentgrass fairway turf

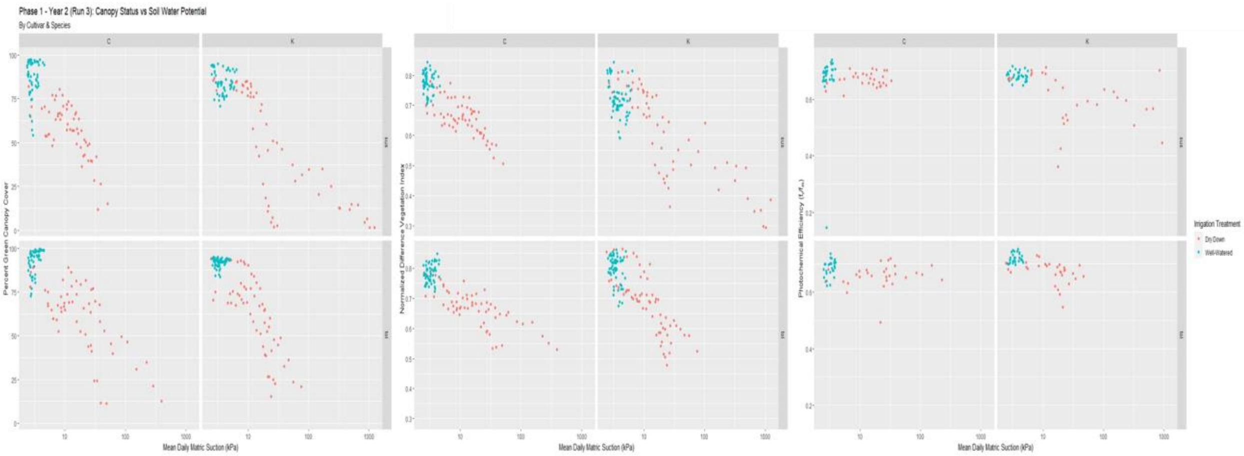
Measured response	**Tool**
Turfgrass quality	-
NDVI (plot scan)	RapidSCAN CS-45 (Holland Scientific, Inc., Lincoln, NE)
NDRE (plot scan)	RapidSCAN CS-45 (Holland Scientific, Inc., Lincoln, NE)
Green canopy cover	Sony RX1000 III digital camera (Sony Corp., Tokyo, Japan); 60.3 x 90.8 x 64.8 cm LED light box emitting 90 μmol m^{-2} s^{-1}; Turf Analyzer 1.0.4 (Karcher et al., 2017)
PAR absorptivity	IMAGING-PAM M-Series Maxi Version (Heinz Walz Co., Effeltrich, Germany)
Photochemical efficiency	IMAGING-PAM M-Series Maxi Version (Heinz Walz Co., Effeltrich, Germany)

Soil Moisture & Canopy Response:

Total ET_o depths during the treatment period was approximately 186 mm. At the start of the dry down period, soil moisture values for all plots were 39.1 – 43.5%. Following the dry down period, VWC values of the dry down plots were 7.8 – 13.6%. Although percent green canopy cover and normalized difference vegetation index declined with increasing soil matric suction, photochemical efficiency did not indicate that plots were experiencing physiological stress (Fig. 1).

Assuming a permanent wilt point of -1500 kPa soil water potential, the corresponding VWC was calculated from the soil moisture retention curve, determined previously, to be 7.5%. This VWC value was not achieved at the 5 cm depth during the dry down.

Figure 1. Percent green canopy cover, normalized difference vegetation index, and photochemical efficiency versus soil water potential for drought susceptible (sus) and tolerant (tol) creeping bentgrass (C) and Kentucky bluegrass (K) for two runs of the dry down experiment at St. Paul, MN on well-watered and non-irrigated plots during 2022.



As in the two experimental runs during Year 1, percent green canopy cover and NDVI declined with increasing matric suction indicating that these measures may be used as simple indicators of soil water status. Photosynthetic efficiency on creeping bentgrass did not appear to decline with increasing matric suction, consistent with Year 1 results. For Kentucky bluegrass, photosynthetic efficiency did appear to decline with increasing suction and further analysis of this difference from Year 1 results is left for future work.

Greenhouse Evaluation

During fall 2021, 25-cm diameter by 30-cm deep soil cores were collected from each of the Phase 1 plots for further evaluation in a greenhouse during winter 2021. Cores were maintained with shears and clipped 3-5 times wk^{-1} at a height of 1.3 cm and with appropriate nutrient, disease, and algae management. One CS655 soil moisture sensor (Campbell Scientific, Inc., Logan, UT) was installed in the side of each core and wired to a CR300 data logger. Sensors were placed 5.1 cm below the soil surface and inserted into the core such that the tines were centered across the diameter. Greenhouse atmospheric conditions were monitored using a HOBO MX2301A data logger (Onset Computer Corp., Bourne, MA). Treatments and design were identical to the Phase I field trial. Two trial runs were performed, beginning 11 Feb and 21 Mar with a recovery period in between, beginning on 7 Mar. During each trial run, total ET since the previous data collection date was determined by weighing the core and determining the mass lost since the previous measurement using an Optima OP-901A digital scale (Optima Scale, Rancho Cucamonga, CA) with a KNINE Outdoors KD-ZJ-50 tripod and winch (KNINE Outdoors, Ontario, Canada). Water was then added to cores in the well-watered treatment to replace 100% of the water mass lost. Visual and physiological canopy responses were measured three times per week during drydown. Green canopy cover was determined from digital images taken with a Sony RX1000 III digital camera and a 20.3 x 20.3 x 30 cm LED light box. All other responses were measured using the same equipment as in the Phase I field plots. The first trial run was stopped before reaching wilt point based on visual observation and soil moisture values to allow for recovery and the second was allowed to continue until wilt point was reached in the many of the cores as indicated by the CS655 sensors.

Figure 2. Percent green canopy cover, normalized difference vegetation index, and photochemical efficiency versus soil water potential for drought susceptible (sus) and tolerant (tol) creeping bentgrass (C) and Kentucky bluegrass (K) for two runs of the greenhouse dry down experiment at St. Paul, MN on well-watered and non-irrigated cores during 2022.

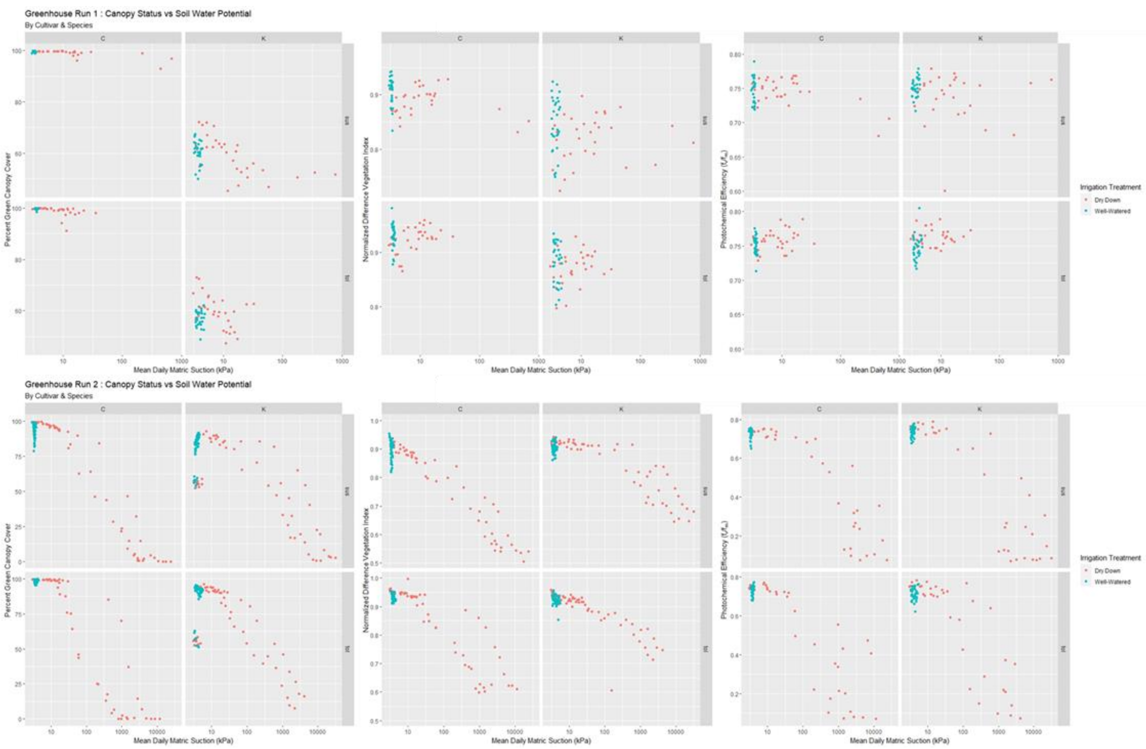
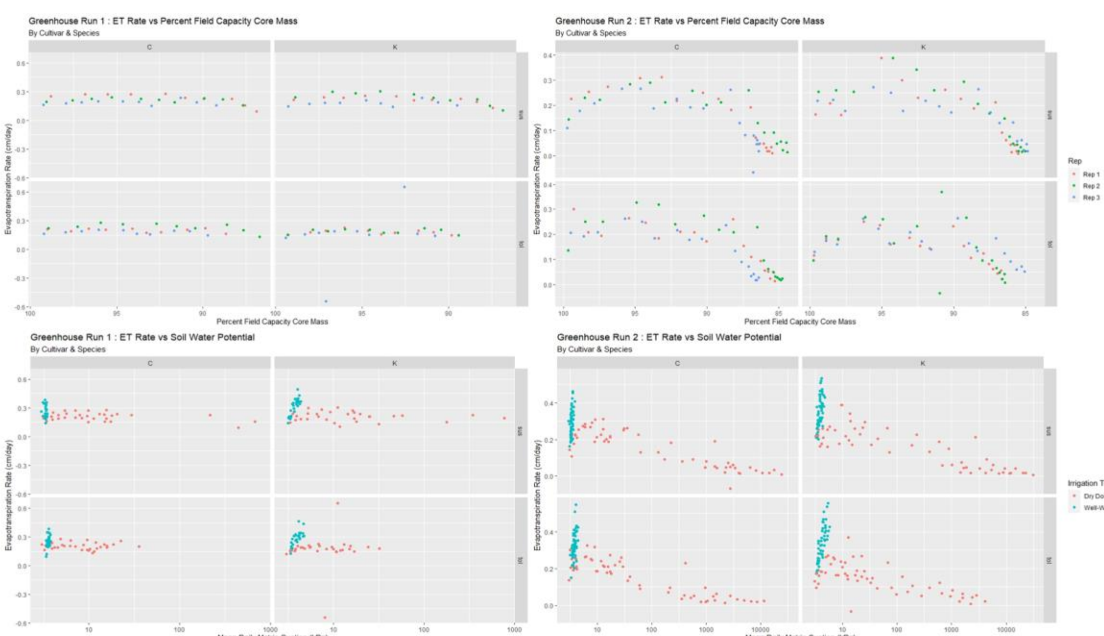


Figure 3. Calculated gravimetric ET rate versus soil water potential and core weight for drought susceptible (sus) and tolerant (tol) creeping bentgrass (C) and Kentucky bluegrass (K) for two runs of the greenhouse dry down experiment at St. Paul, MN on non-irrigated cores during 2022.



Phase 2, Year 1

Plot Establishment & Maintenance:

One cultivar each of Kentucky bluegrass (*Poa pratensis* L.*)* 'Shamrock' and creeping bentgrass (*Agrostis stolonifera* L.) 'Penncross' were established as individual plots (1.5 m x 1.5 m at the Turfgrass Research, Outreach, and Education Center on the University of Minnesota's Saint Paul Campus. The drought susceptible cultivar of each species from Phase I was chosen to ensure a sufficient drought stress response. All plots were seeded on 13 Aug 2021 at a rate of 7.3 g m^{-2}. During establishment, plots received 2.4 g N m^{-2}, 4.9 g P_2O_5 m^{-2}, and 1.6 g K_2O m^{-2} on 31 Aug and 27 Sep, 2021. Prior to initiation of treatments, plots received 2.4 g N m^{-2} and 2.4 g K_2O m^{-2} on 10 May 2022 and 2.4 g N m^{-2}, 4.9 g P_2O_5 m^{-2}, and 1.6 g K_2O m^{-2} on 18 May 2022. To discourage lateral movement of moisture between plots, aluminum barriers were installed in the soil to a depth of 12.7 cm. The research area was maintained as a golf fairway with little-to-no weed and disease tolerance. Weeds, including invasion of the edges of Kentucky bluegrass plots by creeping bentgrass, were either removed by hand or controlled with herbicide applications outside of data collection periods. Fungicides were applied preventatively during periods of high disease pressure. Plots were mowed 3 times wk^{-1} at 1.4 cm, always immediately prior to data collection. One CS655 soil moisture sensor was installed in each plot (Campbell Scientific, Inc., Logan, UT) and wired to a CR300 data logger. Sod was removed and sensors were installed horizontally in the center of each plot at a 5.1 cm depth before soil was repacked surrounding the sensor body and sod was replaced. Soil cores (5.1 cm diameter) were taken from each to a target depth of 15 cm from six arbitrary plots within the field study area for physical characterization

Treatments & Data Collection:

Treatments consisted of five levels of irrigation based on soil water thresholds levels of 15, 30, 45, 60, and 75% of the VWC range between wilt point and field capacity. Thresholds in units of percent volumetric water content were calculated from the soil water retention curve, assuming field capacity and permanent wilt point matric potential values were -10 and -1500 kPa, respectively. Three replications of each cultivar × irrigation treatment were arranged in a randomized complete block design. On 30 Jun 2022, 2.54 cm of irrigation were applied to plots prior to initiation of treatments to generate rootzone conditions near field capacity. Irrigation treatments were applied from 1 Jul – 30 Sep. Volumetric water content (VWC) was recorded every 15 minutes using the installed CS655 moisture sensors. Plots were irrigated by hand when the soil moisture content had dropped below the defined threshold for that treatment. At each irrigation application, 0.65 cm (0.25 in) of water were applied evenly to the plot using a hose-end flow meter (Model 825 Meter, Tuthill Corp., Burr Ridge, IL). The response of each plot to the irrigation treatments was quantified using the same tools as for the Phase I field plots and surface firmness was measured using a Clegg Impact Tester 0.5 kg model (Lafayette Instrument Co., Lafayette, IN). Visual and physiological responses were measured 3 times wk^{-1} with the exceptions of photochemical efficiency and surface firmness, which were measured once per week and whenever irrigation was applied in each plot.

Analysis & Results to Date:

Soil Characterization:

Bulk density of the collected soil cores ranged from 1.22 - 1.37 g ml^{-1}. The van Genuchten soil water retention model fit the combined sample data with a R^2 value of 0.976 and provided wilt point and field capacity values of 4.37 and 43.66% VWC, respectively.

Soil Moisture & Canopy Response:

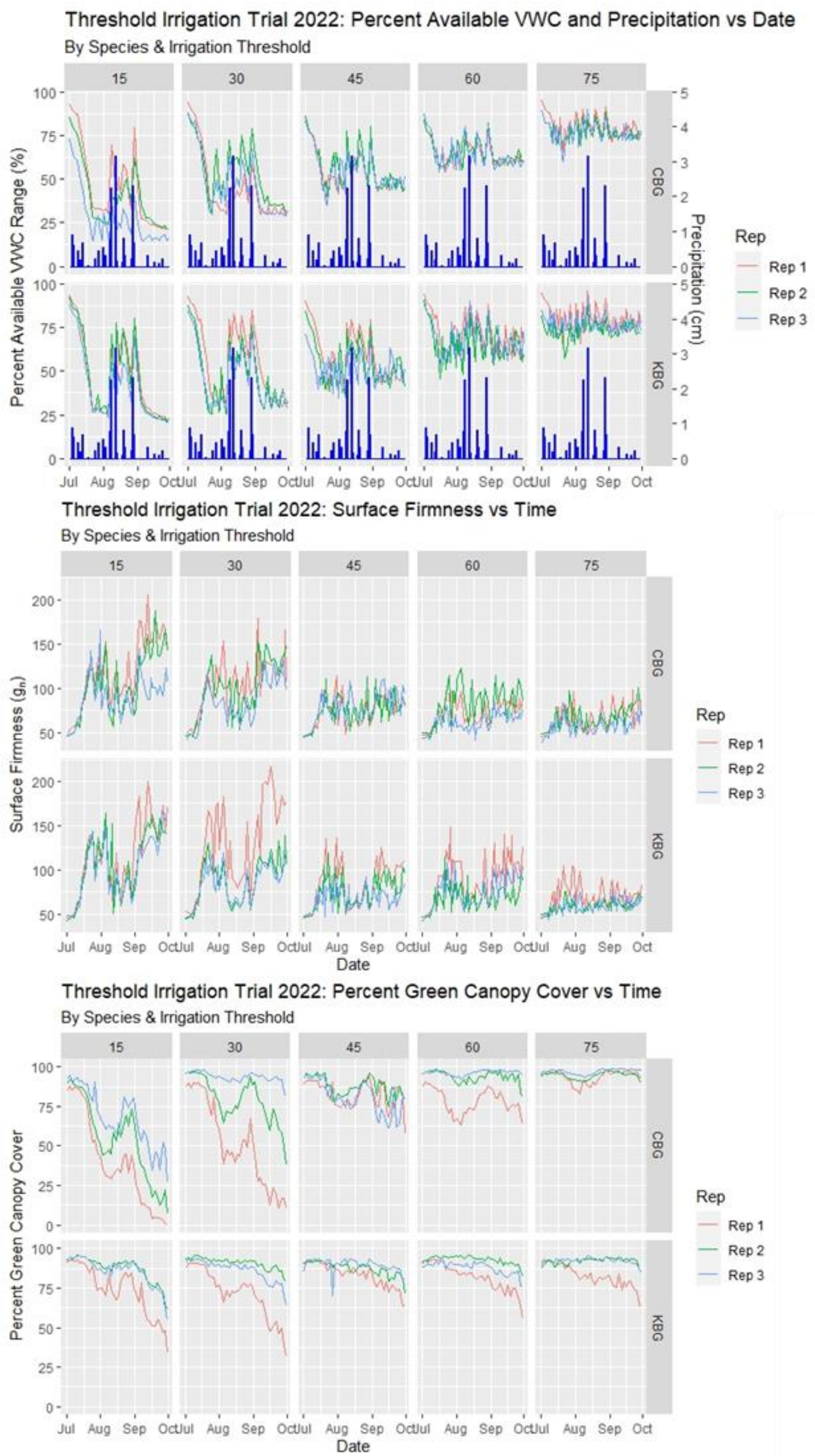
Total ET_o depth during the treatment period was approximately 29.5 cm. Total precipitation during the treatment period was 15.8 cm. At the commencement of the treatment period, soil moisture values for all plots were 32.4 – 41.8%. Following the treatment periods, soil moisture values for all plots were 26.9 – 33.3% (Fig 4).

Significant differences existed between the threshold treatments for total irritation water applied, final green canopy cover, and final surface hardness (Table 2). For green canopy cover, there was also a significant interaction between species and moisture threshold. Creeping bentgrass plots irrigated at higher moisture thresholds used significantly more water and remained significantly softer and greener; however, a moisture threshold of 45% plant available water reduced water use significantly while maintaining similar final firmness and canopy greenness. Further resolving the relationship between these and the other response variables is left for future analysis work.

Table 2. Comparison of moisture threshold treatment effects on total water use, resulting surface hardness, and percent green canopy cover for creeping bentgrass and Kentucky bluegrass fairway turf plots in St. Paul, MN.

Moisture Threshold	Total Water Use (L)	Group	Final Surface Hardness (g)	Group	Final Green Canopy Cover (%)			
					creeping bentgrass	Group	Kentucky bluegrass	Group
15	49.2	A	144.2	B	11.6	A	50.1	A
30	115.2	AB	125.8	B	43.4	B	58.6	A
45	235.2	ABC	94.4	A	72.3	C	71.6	A
60	284.2	CD	90.9	A	80.1	C	71.8	A
75	409.1	D	73.6	A	94	C	78	A

Figure 4. Top: Percent of available volumetric water content range and precipitation vs time; Middle: surface firmness in g_n as measured by Clegg impact hammer test vs time; Bottom: percent green canopy cover vs time on plots irrigated at different soil moisture thresholds.



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. Determine soil physical properties that lead to high variability of plant available water within golf course fairways
3. Expand soil moisture mapping protocol to include salinity measurement from TDR instrument

Start date 2018
Project duration 3 years + 1 Yr Extension to March 31, 2022
Total funding $95,618

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 or targeting inputs to areas of greatest need. Utilizing remote sensing data to improve turf management is a new area of study. However, research is needed to further elucidate the benefits of using technology in golf course management. The overall goal of this project is to evaluate spectral sensors in specific bands and acquire ground-truthed data to better understand spatial variability patterns and determine accuracy of drone imagery.

Methodology

UAV Flights and Ground-Truth Data Compilation. Drone flights were completed in summer 2018 (Rawls GC n = 5; Amarillo CC n = 3) and 2019 (Rawls GC n = 3; Amarillo CC n = 3) over two holes at each location. A complete flight included collecting geo-referenced imagery from four sensors [Red/Green/Blue (RGB); Red Edge (RE); NIR850 nm; and NIR970 nm). Stitched images were used to develop NDVI maps with the assistance of TurfScout (Dana Sullivan) working directly with PhD student (Juan Cantu). Soil samples and ground-based measurements were obtained from intersection points developed in Google Earth Path add-on within Google Earth. Soil samples (0-5 cm and 5-10 cm depths below turf thatch) were analyzed in the laboratory (texture, bulk density, organic matter, infiltration, plant available water, and thermal properties) along with measurements of soil compaction (0-5 cm and 5-10 cm), active NDVI (Turfscout Color Meter), and relative volumetric water content (VWC) with TDR at 3-inch (7.6 cm) depth (All instruments from Spectrum Technologies). Instrument data were obtained within 1-2 days of flight to overlay or correlate with analyzed drone images to validate any stress in fairways.

Expanding Soil Moisture Mapping Protocol for Salinity. With the extension of our funding availability, we have been testing the capacity to use soil moisture sensors to map soil salinity across golf course fairways (collaboration with Dr. Chase Straw, current Texas A&M University). We obtained GPS-Coordinate data from three golf courses in Lubbock between September and October 2021. Soil samples

(3-inch depth) were obtained for each 10th sample for laboratory soil EC determination (1:2 soil:water radi) to correlate the TDR bulk EC measurement to lab-based EC.

Results to Date

UAV Flights and Ground-Truth Data Compilation. We are working through stitched imagery to identify factors that will allow for appropriate alignment of images over multiple flights to assist with comparative analysis. Variability across fairways along with comparative drone-to-soil derived data comparisons will be completed (Fig. 1). There are some visual characteristics that appear to show improved NDVI on the right side of the fairway and better PAWC values (Fig. 1). Further analysis is needed to more specifically correlate aerial imagery and physical soil properties analyzed. A correlation analysis of PAW from Rawls Golf Course and Amarillo Country Club demonstrates key relationships among soil physical parameters measured at each golf course. The Rawls Golf Course had a negative correlation between bulk density and plant available water, but positive correlations with porosity, clay, and organic matter (Fig. 2). Amarillo Country Club had less significant correlations among physical soil parameters with only bulk density having a significant negative correlation with plant available water (Fig. 2). The negative correlation may indicate that higher traffic areas experiencing greater compaction of soil may be having a negative influence on plant available water that is also corresponding to poorer turfgrass growth when comparing NDVI from aerial images. Additionally, this may signal opportunities for site-specific aerification practices that could enhance water availability and growth of turfgrass in those regions experiencing higher bulk density measurements.

Expanding Soil Moisture Mapping Protocol for Salinity. Data analysis from Lubbock Country Club demonstrated a lack of agreement between bulk EC from the TDR 350 instrument and lab-based EC analysis (Fig. 3). However, the bulk EC appeared to be improving soil moisture content measurements from the TDR 350 with high correlation in the two values across a wide range of moisture and salinity measurements (Fig. 3). There was high variability in bulk EC across all fairways at each golf course with Lubbock Country Club ranging from 0.03-1.42 with a mean of 0.50. Because of the lack of correlation between instrument and lab-based EC, we developed soil moisture variability maps for the locations evaluated using the protocol developed by Dr. Straw and others at University of Minnesota (Fig. 4). The golf course superintendents in the area were highly interested in the outcomes of this work and the presentation of data on the specific irrigation zone basis. Data from one of the locations (Meadowbrook Golf Course) was also included in a recently accepted publication in *Agrosystems, Geosciences, and Environment* demonstrating sources of variability in soil moisture across a wide range of soils and climates in golf course fairways

Summary points

- The research expands our knowledge and understanding of NDVI variation within fairways by comparing ground-based physical soil characteristics and NDVI.
- Variability maps of laboratory measured plant available water were developed and correlated to key soil physical parameters.
- Moderate negative and positive correlations between plant available water and soil physical properties were documented.
- TDR 350 does not appear to provide effective EC measurements compared to lab-based EC.
- Accessibility to GPS-enabled soil moisture instruments and mapping procedures provide a valuable tool for superintendents to implement site-specific management practices for irrigation management.

Images

Figure 1. Red, green, blue image to compare NDVI and plant available water variability at Rawls Golf Course hole 14.

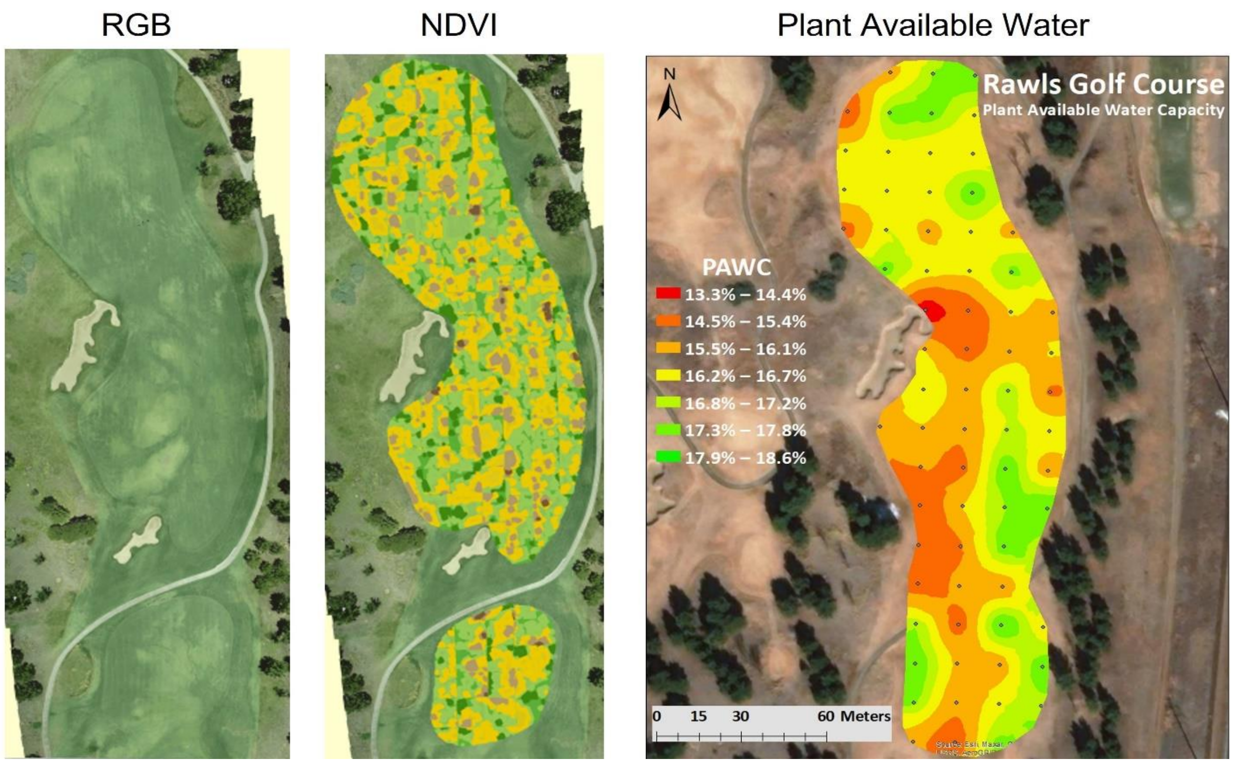


Figure 2. Correlation matrix demonstrating strength and direction of correlation of variables for the Rawls Golf Course and Amarillo Country Club fairways. All unmarked variable cells were significant (P< 0.05) in the analysis.

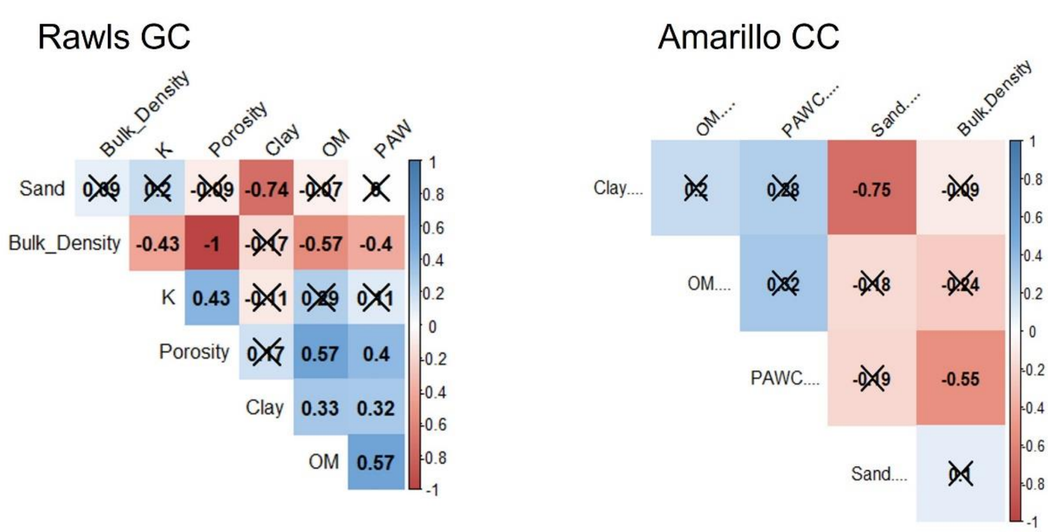


Figure 3. Scatter plot and regression line of bulk EC from TDR 350 and lab-based EC along with bulk EC and soil moisture content determined by TDR 350 at Lubbock Country Club.

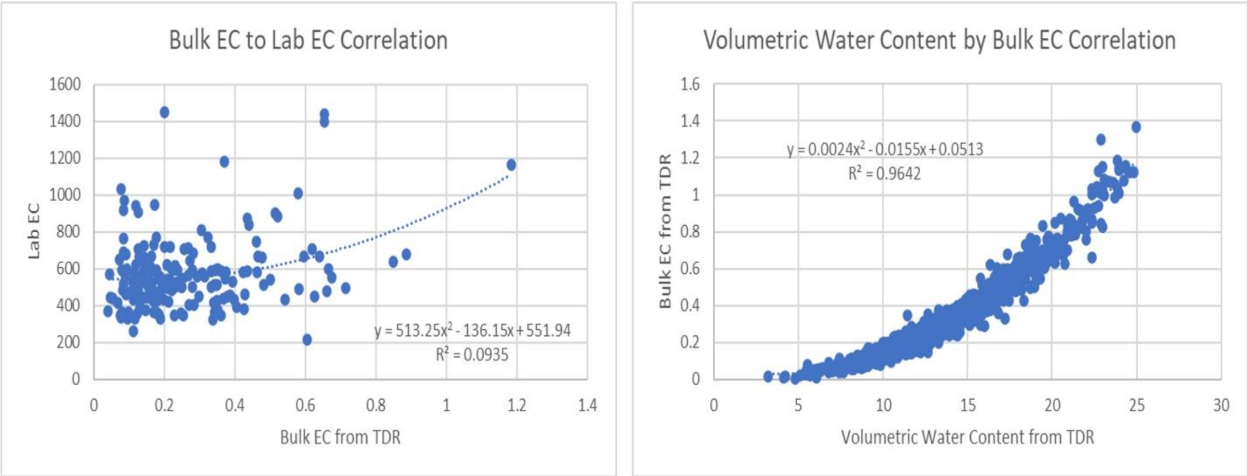
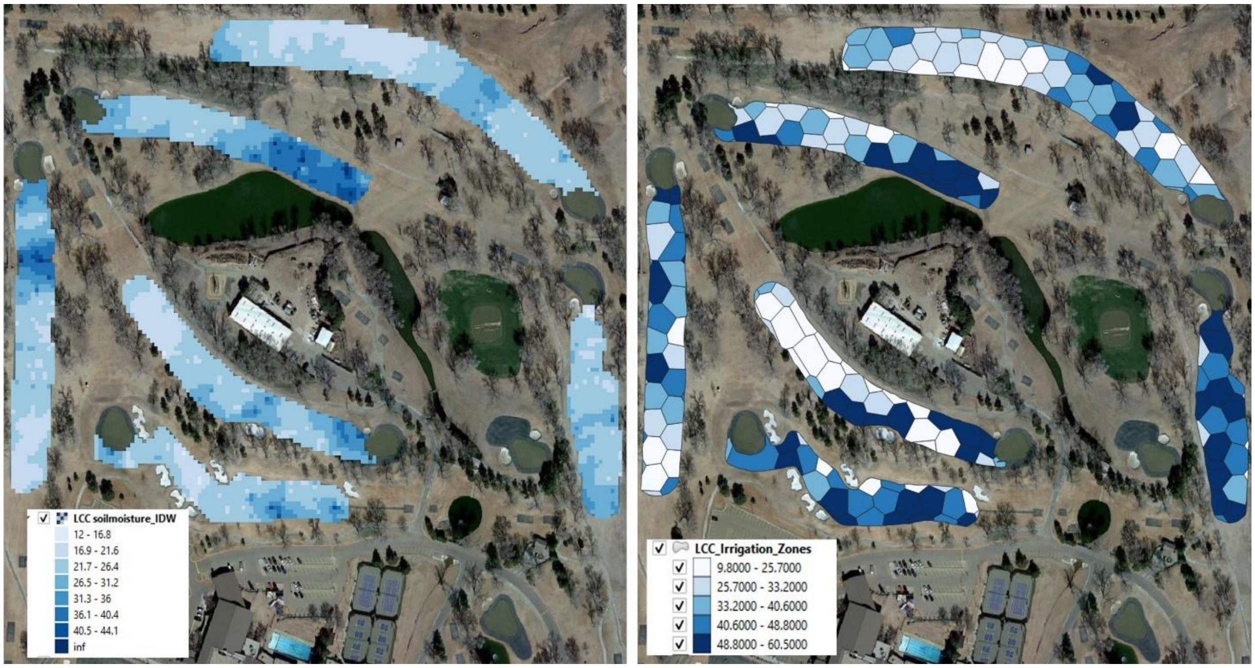


Figure 4. Soil moisture variability maps and soil moisture variability by irrigation zone developed using the soil moisture mapping protocol developed by Dr. Chase Straw and others at University of Minnesota.



USGA ID# 2022-07-750

Title: Optimizing Irrigation Strategies Through Remote Stress Detection

Project Leader: Dr. David McCall and Travis Roberson

Affiliation: Virginia Tech

Objectives:

1). Scaling early moisture stress detection to fairway growing conditions using canopy reflectance and aerial imagery

2). Mapping spatial variability across turfgrass systems to construct site-specific water management zones aerial imagery and ground validation

Start Date: January 2022

Project Duration: 3 years

Total Funding: $100,000

Summary Results (to date):

- Our data suggests that several vegetation indices (VI) collected through ground-based spectral data are influenced by atmospheric dew deposition, specifically popular VI such as the Normalized Difference Vegetation Index, the Normalized Green-to-Blue Index, the Water Band Index excluding the Visible Atmospheric Reflective Index.

- Creeping bentgrass consistently had more dew deposition on the surface that hybrid bermudagrass suggesting that it would be more critical to remove the dew from the surface before any form of spectral data collection is to occur.

- Removal of dew by means of rolling or light irrigation had no affect on the spectral data collected compared to leaving the dew on the surface unaffected, suggesting that minimal to no water on the surface does not diffuse or scatter solar radiation, thus affecting the spectral data collected.

Summary Text:

Objective 1: Water refracts light energy through scattering and diffusion (Nishita & Nakamae, 1994) as it contacts a water surface and this principle was examined for turfgrass with dew deposition as solar radiation incidence contacting the surface of creeping bentgrass (CBG) and Kentucky bluegrass managed areas (Madeira, Gillespie, & Duke, 2001). We hypothesized that because CBG and hybrid bermudagrass (HBG) have stark physiological growth and canopy structure differences, this would provide varying amounts of dew on the surface that could potentially influence the spectral data collected within the morning compared to afternoon events. The study is conducted on four separate locations consisting of two greens and fairway locations. The greens were grassed with ‘Champion’ HBG and ‘A1/A4’ CBG while the fairways were ‘Northbridge’ HBG and ‘L-93’ CBG. The CBG fairway location data were collected on September 20th, 2022, at the Pete Dye River Course while the data for the CBG green and HBG fairway

(Figure 1) and green were collected on September 21st, 2022, at the Country Club of Virginia in Richmond, Virginia. Dew deposition (Figure 2), volumetric water content (VWC: collected with a TDR-350 equipped with 1.5” probes), ground spectral data with a hyperspectral radiometer (HSR) and custom PVC stand equipped with a 5-degree field-of-view lens (Figure 3), and remotely sensed data (Figure 4., visual and thermal data collected at a 30-meter flight altitude) were collected in the morning and evening time at approximately 9:00 am and 2:00 pm, respectively. Treatments consisted of leaving the dew on the surface, rolling the dew, and light irrigation. A greens hand roller 3ft in length was used for rolling the plots and a flowerpot with 0.93 gallons of water was used to simulate ¼” of an irrigation cycle. From this study, we have first seen that with HSR data that the near-infrared bands are not reliable under limited lighting conditions such as early morning flight. This was observed from a 12-point running average of spectral ground measurements when no to limited dew on the plant surface for CBG fairway data (Figure 5). Furthermore, we have seen that across all locations, CBG had more dew deposition on the surface compared to HBG in all cases suggesting that more variability may be observed in spectral data collected during morning periods compared to HBG if no action is performed to dissipate the surface moisture. Lastly, three VI tested (NGRI: normalized green-to-blue index, NGRI: normalized green-to-red index, VARI: visible atmospheric reflective index) were tested and derived from the visible light region (VIS) and known the be correlated to moisture stress from small-plot research. (Hong, Bremer, & van der Merwe, 2019b). From this study we have seen that the blue wavelengths of light are incorporated into a VI calculation, the more variability that is associated with moisture on the plant surface (Figure 6). The VARI has all three key regions of VIS associated with it which lends it to being less associated with surface moisture and more related to soil moisture within this particular study. Investigating moisture stress across large acreage turfgrass areas requires extensive data sampling and occasionally requires experiments to be initiated during morning periods when the least amount of interruption occurs. This study proves the concept of surface moisture impacting spectral data that is likely to be collected for future field studies and shows the importance of ensuring some method is implemented to dissipate this surface moisture prior to data collection events.

Objective 2/3: Small plot research throughout many studies has shown the significance of visual and thermal data that is correlated with drought stress of many turfgrass species through data collected through ground-based mechanisms and aerial imagery (Badzmierowski, McCall, & Evanylo, 2019; Caturegli et al., 2015; Caturegli et al., 2020; Dettman-Kruse, Christians, & Chaplin, 2008; Hong, Bremer, & van der Merwe, 2019a; Hong et al., 2019b; Jiang & Carrow, 2005; Roberson et al., 2021; Suplick-Ploense, Alshammary, & Qian, 2011). However only a few research projects have attempted to map out volumetric water content spatially across turfgrass surfaces and make watering applications based on these collected data (Friell & Straw, 2021; Straw & Henry, 2018). No research has aimed at using spatially collected data related to aspects such as surface topography, soil texture, and consistently drought stressed areas to isolate the quantity and locations of wireless soil sensors across golf course fairways. Our research will be conducted across four golf course fairways where we will collect soil samples (similar layout to Figure 9) for soil texture and use digital surface models to isolate the known variability of each fairway. Furthermore, we plan to conduct several dry-down cycles across the fairways to ensure we consistently capture where areas of drought stressed vs. non-drought stressed areas are located. We will confirm this through ground truthing data where we will use surveying equipment (Emblid Reach) to collect sub-decimeter GPS points for each area that we collect VWC (Spectrum TDR 350), ground based spectral data (Spectral Evolution hyperspectral radiometer) and aerial remote sensing data (Mavic 2 Enterprise Advanced drone) for unsupervised classification of an orthomosaicked image across the golf course fairway being tested. Data from consecutive dry-down events will be used from one summer to construct management zones and generate site-specific management zones and group the irrigation heads together within the zone. The center point of these zones will determine where capacitive soil sensors (Figure 8: Spiio) will be installed for the two fairways designed to be managed with watering decisions made based on the output data from the sensors extracted by means of Spiio's web-based browser (Figure 9) and through the USGA's ET modeling designed to help facilitate water management applications for the golf course superintendent. The total amount of water will be compared between all fairways with and without soil sensor technology dictating when to make objective watering decisions across these large acreage areas. We hypothesize to see these methods will provide a more objective and feasible option to site-specifically water without assuming an entire fairway needs a complete and uniform irrigation application.

Figure 1. Illustration of treatments on 'North Bridge' fairway height-of-cut hybrid bermudagrass with three treatments of dew deposition removal (no removal, rolling, light irrigation) that were implemented prior to dew deposition, soil moisture, and light reflectance data collection.

Figure 2. Display showing how the dew deposition was collected with a rolling pin construction and medical grade absorbent paper towels that were adhesive around PVC pipe and rolled three times across a 12" linear path for a total of approximately 1/2 square foot sampled within each treatment.

Figure 3. Customized stand to hold 5-degree field of view camera for accurate hyper-spectral light reflectance data collection that simulates precise ground measurements in a remotely sensed fashion.

Figure 4. Mavic 2 Enterprise Advanced drone used for moisture stress research with a visible RGB (Red, Green, Blue) camera and FLIR on-board thermal camera.

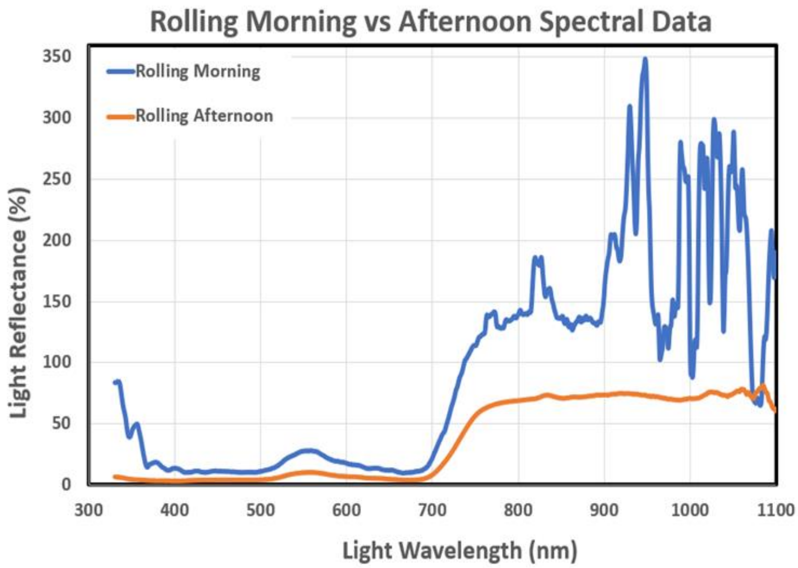


Figure 5. Comparison of ground hyperspectral data for a creeping bentgrass fairway location in Radford, Virginia between morning and afternoon collection events for rolling treatments with limited to no surface moisture present displaying the variation seen within the near-infrared regions of light.

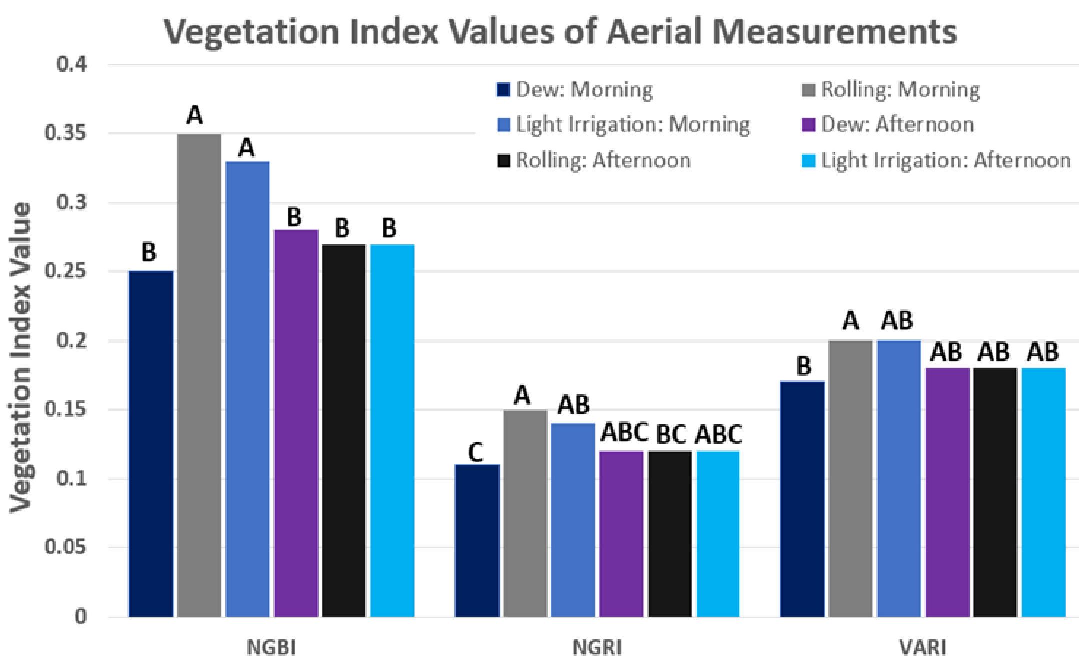


Figure 6. Comparison of three vegetation indices; normalized green-to-blue index, normalized green to red index and the visible atmospheric reflective index and their values associated with treatments of atmospheric dew deposition, rolling dew, and light irrigation collected in the morning and afternoon events on a creeping bentgrass fairway in Radford, Virginia.

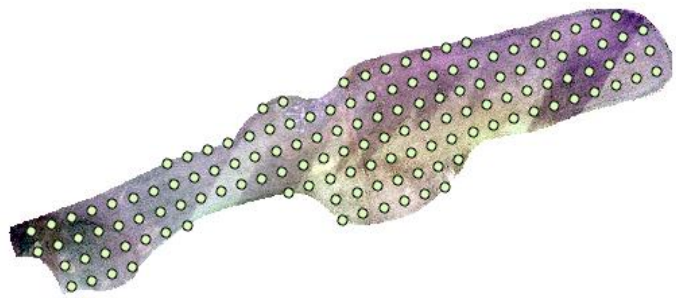


Figure 7. An example of spatial distribution of grid points where we will take soil samples (8 -meter x 8 -meter spacing) to ensure an accurate representation of soil texture analysis of each fairway for

Figure 8. Spiio capacitive soil sensor that will be used for determining large scale irrigation applications based on the soil moisture output for developed management zones through our remote sensing data.

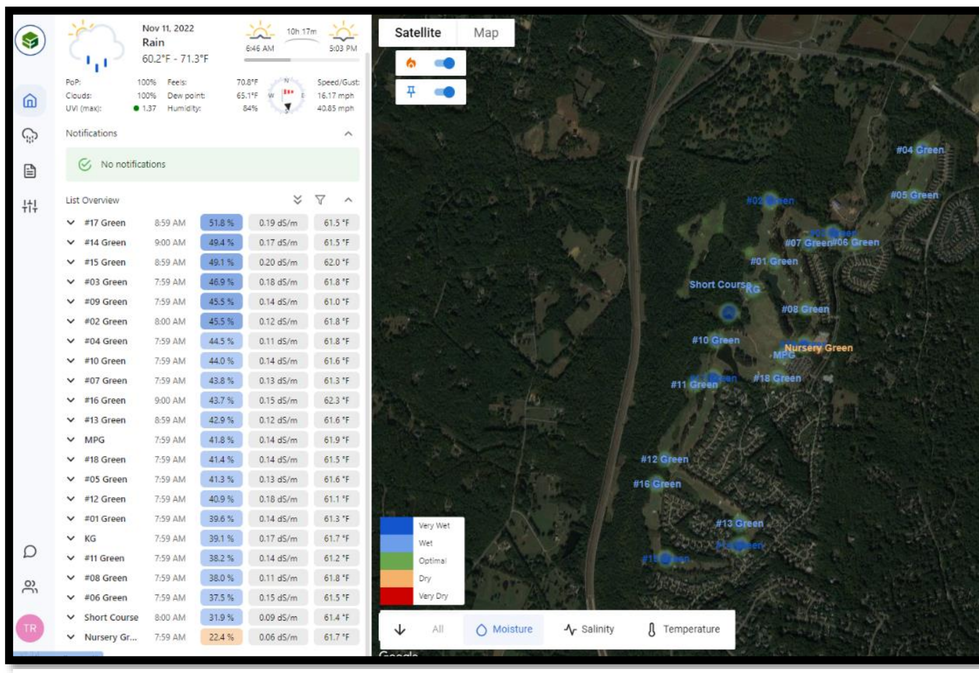


Figure 9. Spiio web browser interface that displays the soil moisture, salinity, and temperature data within the specific location of where the sensor is installed.

References:

Badzmierowski, M. J., McCall, D. S., & Evanylo, G. (2019). Using hyperspectral and multispectral indices to detect water stress for an urban turfgrass system. *Agronomy, 9*(8), 439.

Caturegli, L., Grossi, N., Saltari, M., Gaetani, M., Magni, S., Nikolopoulou, A. E., . . . Volterrani, M. (2015). Spectral reflectance of tall fescue (Festuca Arundinacea Schreb.) under different irrigation and nitrogen conditions. *Agriculture and Agricultural Science Procedia, 4*, 59-67.

Caturegli, L., Matteoli, S., Gaetani, M., Grossi, N., Magni, S., Minelli, A., . . . Volterrani, M. (2020). Effects of water stress on spectral reflectance of bermudagrass. *Scientific Reports, 10*(1), 1-12.

Dettman-Kruse, J. K., Christians, N. E., & Chaplin, M. H. (2008). Predicting soil water content through remote sensing of vegetative characteristics in a turfgrass system. *Crop Science, 48*(2), 763-770.

Friell, J., & Straw, C. (2021). Comparing ground-based and aerial data at field scale during dry down on golf course fairways. *International Turfgrass Society Research Journal*.

Hong, M., Bremer, D. J., & van der Merwe, D. (2019a). Thermal imaging detects early drought stress in turfgrass utilizing small unmanned aircraft systems. *Agrosystems, Geosciences & Environment, 2*(1), 1-9.

Hong, M., Bremer, D. J., & van der Merwe, D. (2019b). Using small unmanned aircraft systems for early detection of drought stress in turfgrass. *Crop Science, 59*(6), 2829-2844.

Jiang, Y., & Carrow, R. N. (2005). Assessment of narrow-band canopy spectral reflectance and turfgrass performance under drought stress. *HortScience, 40*(1), 242-245.

Madeira, A., Gillespie, T., & Duke, C. (2001). Effect of wetness on turfgrass canopy reflectance. *Agricultural and Forest Meteorology, 107*(2), 117-130.

Nishita, T., & Nakamae, E. (1994). *Method of displaying optical effects within water using accumulation buffer.* Paper presented at the Proceedings of the 21st annual conference on Computer graphics and interactive techniques.

Roberson, T. L., Badzmierowski, M. J., Stewart, R. D., Ervin, E. H., Askew, S. D., & McCall, D. S. (2021). Improving Soil Moisture Assessment of Turfgrass Systems Utilizing Field Radiometry. *Agronomy, 11*(10), 1960.

Straw, C. M., & Henry, G. M. (2018). Spatiotemporal variation of site-specific management units on natural turfgrass sports fields during dry down. *Precision Agriculture, 19*(3), 395-420.

Suplick-Ploense, M., Alshammary, S., & Qian, Y. (2011). Spectral reflectance response of three turfgrasses to leaf dehydration. *Asian Journal of Plant Sciences, 10*(1), 67.

USGA ID#: 2020-13-718

Title: Combined field irrigation trials and economic analysis to investigate water conservation determinants, water saving potential, and the return on investment of multiple water management technologies and strategies

Project Leader: Amir Haghverdi

Affiliation: University of California Riverside

Objectives: The overarching goal of this project is to develop and disseminate scientific knowledge, practical recommendations, and tools for efficient golf course irrigation and water management through field irrigation field research trials and economic analysis.

Start Date: 2020

Project Duration: 3 years (year 3 of 3)

Total Funding: $120,000

SUMMARY POINTS:

- Two irrigation research trials were conducted in southern California to investigate the response of hybrid bermudagrass (*Cynodon dactylon*) and buffalograss (*Buchloe dactyloides*) to a wide range of ET_o-based and soil moisture sensor (SMS)-based irrigation treatments.
- The smart SMS-based controller closely followed the programmed thresholds but the irrigation applications differed between years for the same treatments when converted to ET_o percentages.
- The hybrid bermudagrass NDVI values decreased across the treatments from year one to year two, which is attributed to salinity build-up in the root zone due to the application of recycled water.
- The smart ET-based controller showed an acceptable performance (4 to 10% overirrigation) compared to CIMIS ET_o values.
- Our findings indicate a significant increase in treated wastewater use between 2000 to 2015. Golf courses in Riverside, San Diego, Los Angeles, Orange, and San Bernardino, counties with the most golf courses, replaced surface potable water with treated wastewater, and in 2015 treated wastewater accounted for about 50% of all water use.
- Meter level analysis of water use by golf courses indicates that golf course potable water use is less than 0.5% of all potable water use in the sample water agencies.
- Our analysis indicates that water use was reduced by about 30% in 2017 compared to 2013 due to drought and demand management policies. This is comparable to the statewide mandate 25% reduction in urban water use.
- Our analysis indicates that, due to COVID-19, in April 2020, water use was reduced by approximately 80% compared to the same months in 2018 and 2019. However, in July 2020, water use started returning to the levels of 2018 and 2019 and was not statistically different from the 2018 and 2019 levels.

SUMMARY TEXT

Rational: Limited available water resources and frequent droughts in CA.

Methodology:

Irrigation trials: A hybrid bermudagrass ('Tifgreen 328', *Cynodon dactylon*) recycled irrigation research trial was implemented at UCANR SCREC in Irvine, CA, using a CS3550 smart controller plus TDT soil moisture sensors. A buffalograss ('UC Verde', *Buchloe dactyloides*) field research trial was established at UCR AES in Riverside, CA, using a Weathermatic SL4800 smart controller. Turfgrass performance and quality were monitored weekly using a handheld NDVI sensor.

Economic analysis:

Our approach was as follows:

1. Obtain aggregate county-level data on water use in the golf industry from the US geological survey (USGS) and analysis the trends in water use by county as well as the type of water (e.g., groundwater, surface water, recycled water).
2. Obtain and clean monthly water consumption from water agencies for the industrial water sector (which contains water meters associated with golf courses) from 2013 to 2020.
3. Use graphical and regression methods to analyze water use trends and evaluate golf course conservation achievements in the study period.
4. Analysis of the share of water use in the golf courses compared to other sectors.
5. Using regression methods, analyze the impact of COVID-19 on water use in golf courses, including how water use has transitioned following COVID-19.

For this study, in addition to the county-level golf course water use data from the USGS, we obtained individual meter-level water use data and property boundaries for a sample of golf courses. The study required significant involvement of individual water agencies to supply golf course-level water use. We initially contacted over twenty agencies to invite participation in the project. Three water agencies agreed to participate in the study in the time frame we identified. These agencies are located in Marin, Orange, and Riverside counties. Collaboration with these agencies lead to data on monthly water use at the individual golf course level in their service area from January 2013- December 2021. Furthermore, we also obtained agency-level water use by the residential and CII sectors from 2015-2021

Results:

Trial 1 at UCANR SCREC: We observed a good response from the Acclima smart controller to schedule irrigation based on the implemented thresholds. However, when the applied water was converted to percentages of ET_o (25-81%) substantial differences were observed between years. Table 2 shows the results from the statistical analysis for the NDVI. The irrigation levels. Frequency and their interaction significantly affected NDVI values ($p < 0.001$). NDVI for most treatments stayed below the acceptable quality range except for 75-100FC on-demand treatment, which had NDVI≥0.5 for half of the data collection period (Figure 1). The decline in the NDVI values was more apparent in the 3-day irrigation frequency treatments as the summer progressed in 2021. As shown in Figure 2, the soil salinity increased in the fall season, especially in the shallow soil depths (0-30cm), indicating the accumulation of salts due to high ET demand over summer. Soil salinity distribution at different depths varied with the irrigation season as spring collected samples showed significantly higher accumulation at the 0-15 cm depth, while 15- 30 cm depth had substantially higher accumulation in the fall. The infiltration rate measurements revealed no clear pattern across the treatments.

Trial 2 at UCR AES: The statistical analysis shows that irrigation level had a significant impact on NDVI but not the irrigation frequency restriction (Table 3). Figure 3 illustrates the changes in NDVI values over time across irrigation treatments for the buffalograss plots. The reduction in NDVI values is more pronounced for the severe deficit irrigation treatments. Overall, the Weathermatic controller showed an acceptable performance by overirrigation ranging from 4 to 10% compared with CIMIS ET_o values (Table 4).

Economic analysis & Water Use Trends:

We focused on the 31 golf courses in the sample and their meter level water use data and analyzed water use and conservation trends, yet in this case, we got back to January of 2013 given the availability of data. As indicated in Table 7, on average, sample golf courses consume approximately 8-acre feet (AF) of potable water per month. The sample golf courses have other water sources (e.g., recycled, groundwater, etc.), which are not included in this analysis. Water use has been declining in the sample golf courses since 2013, from approximately 9.33 AF per month in 2013 to about 6.73 AF per month in 2021.

Average water use has been declining in sample golf courses, with statistically significant results (Table 8). Focusing on our preferred specification in Column (3), regression results indicate that in 2015, when California was in a drought and the state implemented the drought conservation mandate policy, we see that golf courses reduced water use by about 4.7% compared to 2013 levels. This reduction continued in 2016 when water use was 14.8% lower than 2013 levels on average. After the drought, we observe that reductions in water use continued. Water use in 2019 decreased significantly again, likely due to 2019 being an extremely wet year. In 2020, we started to see the impacts of COVID-19 and observed a significant reduction. In 2020 water use was reduced by 40.7% compared to the levels in 2013.

In June 2014, the state made a voluntary request for all public water agencies to reduce water use by 20% relative to a 2013 baseline. As the drought lingered into 2015, the state then imposed a conservation mandate in June 2015 that required public water agencies to reduce their water use relative to 2013 by a percentage varying from 4% to 36%, depending on each agency's GPCD water use in 2013 to achieve an overall 25% reduction in statewide urban water use. With pushback from agencies following their drought contingency plans and feeling their situation did not warrant such draconian decreases, coupled with a slight reprieve from the drought in early 2016, the state rescinded the conservation mandate in June 2016. It replaced it with a self-certification period that lasted until May 2017.

We analyzed the impact of these policies on water consumption by the sample golf courses using water use in 2013 as the state-defined baseline. The sample golf courses reduced water consumption by about 20% during the voluntary period compared to 2013 levels (Figure 7). During the mandate, there was a further reduction in water use of approximately 22% compared to the 2013 levels. Although these results are not statistically significant in both periods. We observe reduction in water use during the self-certification period relative to the 2013 levels. Post drought, there was a slight rebound in water use, but it did not go back to 2013 levels. Water use post drought in sample golf courses was about 53% less than the 2013 levels.

Our results indicate that, on average, water use after controlling for weather variations was not reduced statistically significantly compared to 2018 and 2019 levels. We also explore the changes month-to-month compared to the average of the same months in 2018 and 2019 (Figure

8). Water use in these golf courses is not statistically different from levels in 2018 and 2019 for January to March. In April 2020, water use was reduced by approximately 80% compared to the same months in 2018 and 2019, respectively. In July 2020, water use started returning to the levels of 2018 and 2019 and was not statistically different from the 2018 and 2019 levels.

Table 1. Treatments for the soil moisture-based irrigation trial conducted at SCREC in Irvine, California.

Treatment	Lower limit	Upper limit	Watering days
T1	75%FC	FC	3days/week
T2	65%FC	FC	
T3	65%FC	FC-10%	
T4	55%FC	FC	
T5	55%FC	FC-20%	
T6	75%FC	FC+10%	
T7	75%FC	FC	7days/week
T8	65%FC	FC	
T9	65%FC	FC-10%	
T10	55%FC	FC	
T11	55%FC	FC-20%	
T12	75%FC	FC+10%	

- FC denotes the field capacity of the soil.

Table 2. Statistical analysis of the hybrid bermudagrass response in terms of NDVI values to irrigation treatments.

2020		2021	
Treatment	**NDVI**	**Treatment**	NDVI
55-80FC	0.41 a	55-80FC	0.28 a
55-100FC	0.45 b	55-100FC	0.27 a
65-90FC	0.50 c	65-90FC	0.42 c
65-100FC	0.47 bc	65-100FC	na
75-100FC	0.55 d	75-100FC	0.41 c
75-110FC	0.49 c	75-110FC	0.37 b
Frequency		**Frequency**	
3 d week^{-1}	0.47 a	3 d week^{-1}	0.32 a
On-demand	0.49 b	On-demand	0.38 b
Model effect		**Model effect**	
I	***	I	***
F	**	F	***
I x F	***	I x F	***
T	***	T	***
I x T	***	I x T	**
F x T	NS	F x T	NS
I x F x T	*	I x F x T	**

NS, ***, **, and * are non-significant or significant at $p \leq 0.001$, 0.01, and 0.05, respectively. Means sharing a similar letter are not significantly different, based on the Turkey's test at the significance level (α) = 0.05. I, F, and T in the table refer to irrigation levels, frequency, and time (i.e., repeated measures of visual rating each year over time), respectively.

Table 3. Statistical analysis of the buffalograss response in terms of NDVI values to irrigation treatments.

2021		2022	
Treatment	NDVI	**Treatment**	NDVI
37% ET_o	0.31d	39% ET_o	0.32a
49% ET_o	0.33d	52% ET_o	0.34a
62% ET_o	0.39c	65% ET_o	0.42ab
74% ET_o	0.46b	77% ET_o	0.47b
86% ET_o	0.48ba	90% ET_o	0.49bc
99% ET_o	0.51a	104% ET_o	0.51cd
Frequency		**Frequency**	
3 d $week^{-1}$	0.41a	3 d $week^{-1}$	0.43a
6 d $week^{-1}$	0.42a	6 d $week^{-1}$	0.42a
Model effect		**Model effect**	
I	***	I	***
F	NS	F	NS
I x F	NS	I x F	**
T	***	T	***
I x T	***	I x T	***
F x T	NS	F x T	NS
I x F x T	NS	I x F x T	NS

NS, ***, **, and * are non-significant or significant at $p \leq 0.001$, 0.01, and 0.05, respectively. Means sharing a similar letter are not significantly different, based on the Turkey's test at the significance level (α) = 0.05. I, F, and T in the table refer to irrigation levels, frequency, and time (i.e., repeated measures of visual rating each year over time), respectively.

Table 4. Summary of applied, targeted and programmed irrigation levels based on 81% irrigation efficiency for the buffalograss trial conducted at UCR AES in Riverside, California.

6 days per week watering days restriction - 2021						
Treatment ET_o	80%	70%	60%	50%	40%	30%
Programmed ET_o	99%	86%	74%	62%	49%	37%
Applied ET_o by Weathermatic	109%	94%	81%	67%	54%	41%
Overapplication	10%	8%	7%	5%	5%	4%
3 days per week watering days restriction - 2021						
Treatment ET_o	80%	70%	60%	50%	40%	30%
Programmed ET_o	99%	86%	74%	62%	49%	37%
Applied ETo by Weathermatic	106%	92%	79%	66%	53%	41%
Overapplication	7%	6%	5%	4%	4%	4%

6 days per week watering days restriction - 2022						
Treatment ET_o	80%	70%	60%	50%	40%	30%
Programmed ET_o	99%	86%	74%	62%	49%	37%
Applied ETo by Weathermatic	105%	90%	77%	65%	52%	39%
Overapplication	6%	4%	5%	3%	6%	6%
3 days per week watering days restriction - 2022						
Treatment ETo	80%	70%	60%	50%	40%	30%
Programmed ET_o	99%	86%	74%	62%	49%	37%
Applied ET_o by Weathermatic	104%	90%	77%	65%	52%	40%
Overapplication	5%	5%	5%	5%	5%	9%

Table 5. Participant Water Agencies and Data Availability.

Agency	County	Number of Golf Courses
Agency A	Marin	7
Agency B	Orange	9
Agency C	Riverside	15
Total	**-**	**31**

Notes: All the agencies provided monthly golf course-level water use data.

Table 6. Share of the golf courses' water use from total potable water production in the sample agencies' service area.

Year	Residential Share	CII Share	Sample Golf Courses Share
2015	87%	13%	0.38%
2016	84%	16%	0.44%
2017	84%	16%	0.47%
2018	87%	13%	0.48%
2019	86%	14%	0.42%
2020	89%	11%	0.38%
2021	87%	13%	0.38%
Average between 2015 to 2021	**86%**	**14%**	**0.43%**

Table 7. Summary statistics for the sample golf courses.

Year	Observations	Average Monthly Potable Water Consumption (AF)
2013	257	9.33
2014	271	10.08
2015	269	8.22
2016	295	8.75
2017	293	7.03
2018	282	6.87
2019	322	6.90
2020	326	6.73
Average	289	7.99

Table 8. Percent changes in water use over time compared to 2013 levels in the sample golf courses (Dependent variable= log of water use).

	(1)	(2)	(3)
2014	0.002	0.021	0.062
	(0.285)	(0.250)	(0.091)
2015	-0.182	-0.092	-0.047
	(0.287)	(0.252)	(0.091)
2016	-0.365	-0.338	-0.148
	(0.288)	(0.253)	(0.092)
2017	-0.606**	-0.546**	-0.283***
	(0.286)	(0.251)	(0.091)
2018	-0.614**	-0.468*	-0.118
	(0.289)	(0.254)	(0.093)
2019	-1.029***	-0.757***	-0.353***
	(0.289)	(0.255)	(0.094)
2020	-0.912***	-0.618**	-0.407***
	(0.286)	(0.252)	(0.094)
Observations	1,816	1,816	1,816
R^2	0.014	0.248	0.181
Month FEs	No	Yes	Yes
Golf Course FEs	No	No	Yes
Note:	*p **p ***p<0.01		

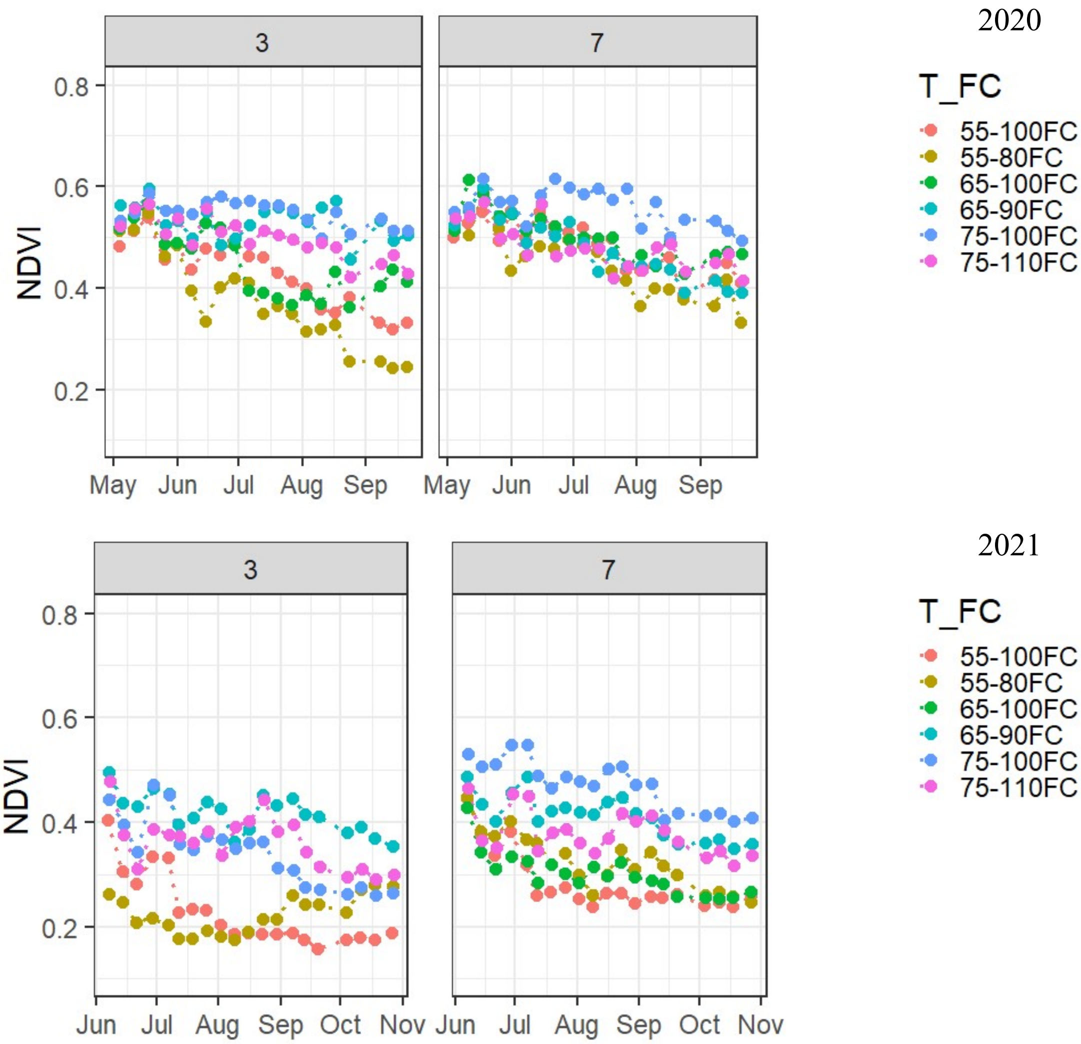


Figure 1. Changes in NDVI values of hybrid bermudagrass over time across the irrigation treatments for the restricted (3 d/week) and on-demand (7 day/week) irrigation treatments imposed in 2021.

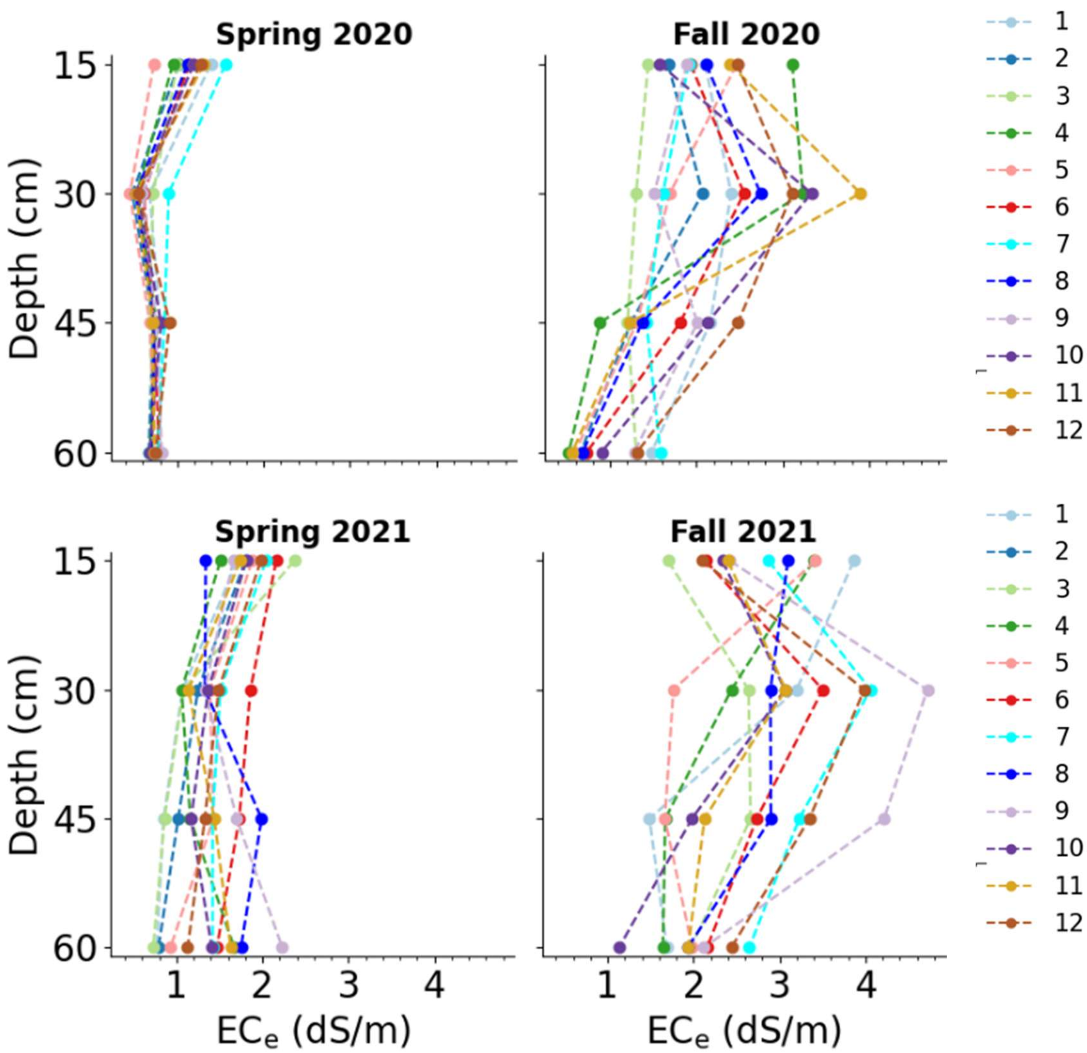


Figure 2. Soil salinity (EC_e) distribution in the soil profile from soil samples collected before (Spring) and after (Fall) of the summer irrigation season in 2020 and 2021 from hybrid bermudagrass plots located in Irvine, California. Legend at the right of the graphs represents the treatment number as shown in table 1.

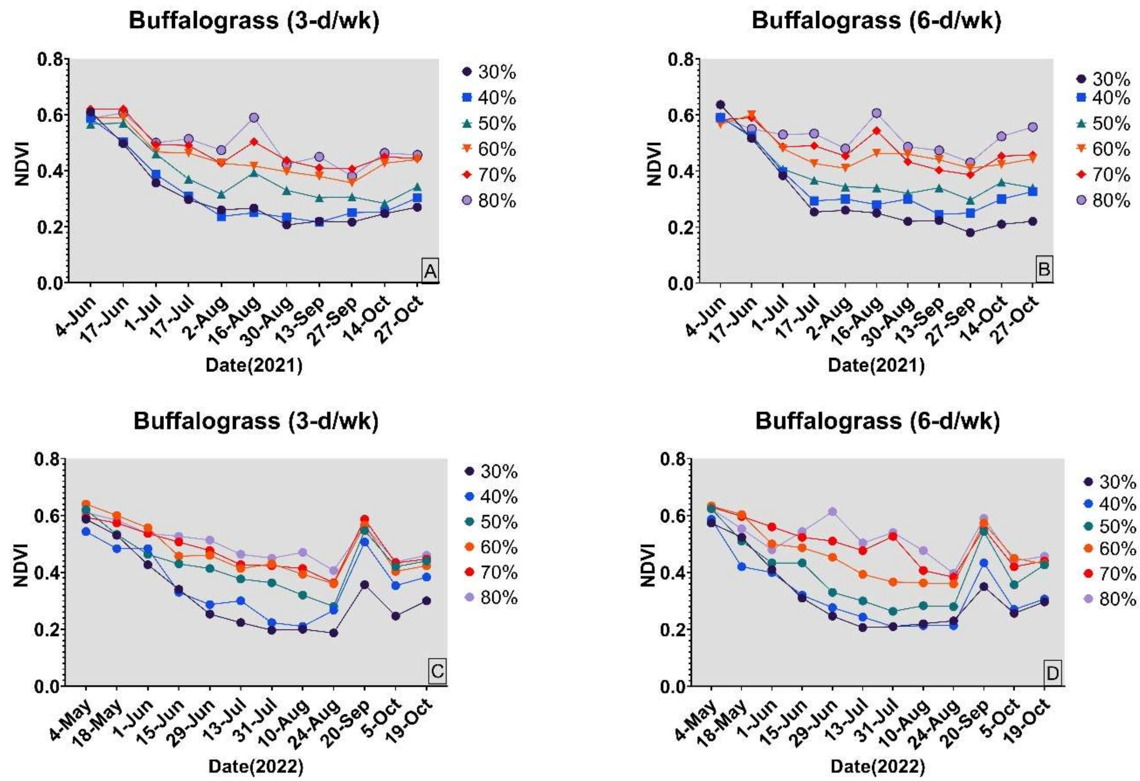


Figure 3. Changes in NDVI over time for the buffalograss irrigation trial conducted at UCR AES in Riverside, California.

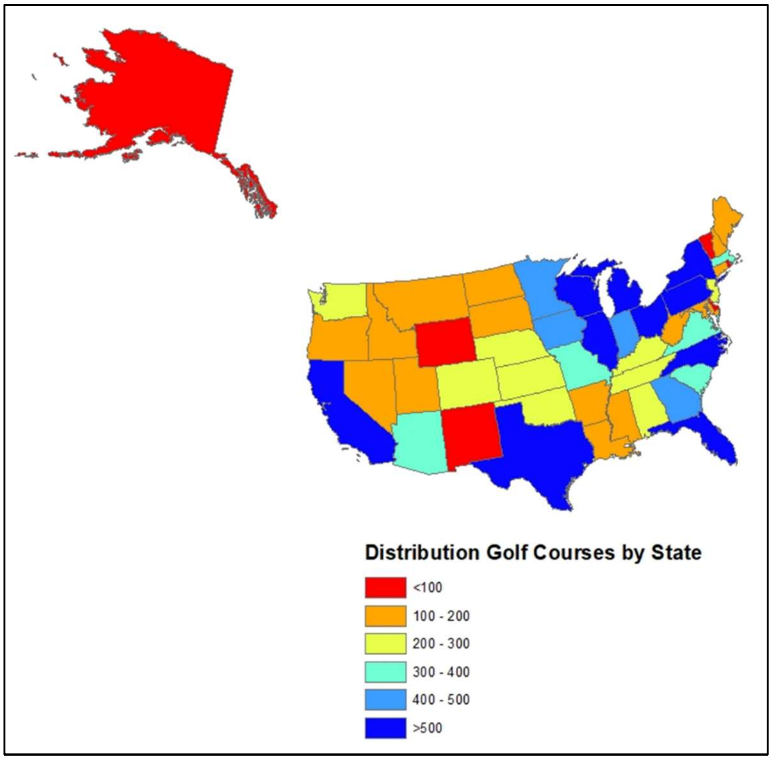


Figure 4. Distribution of the number of golf courses in the united states by state.

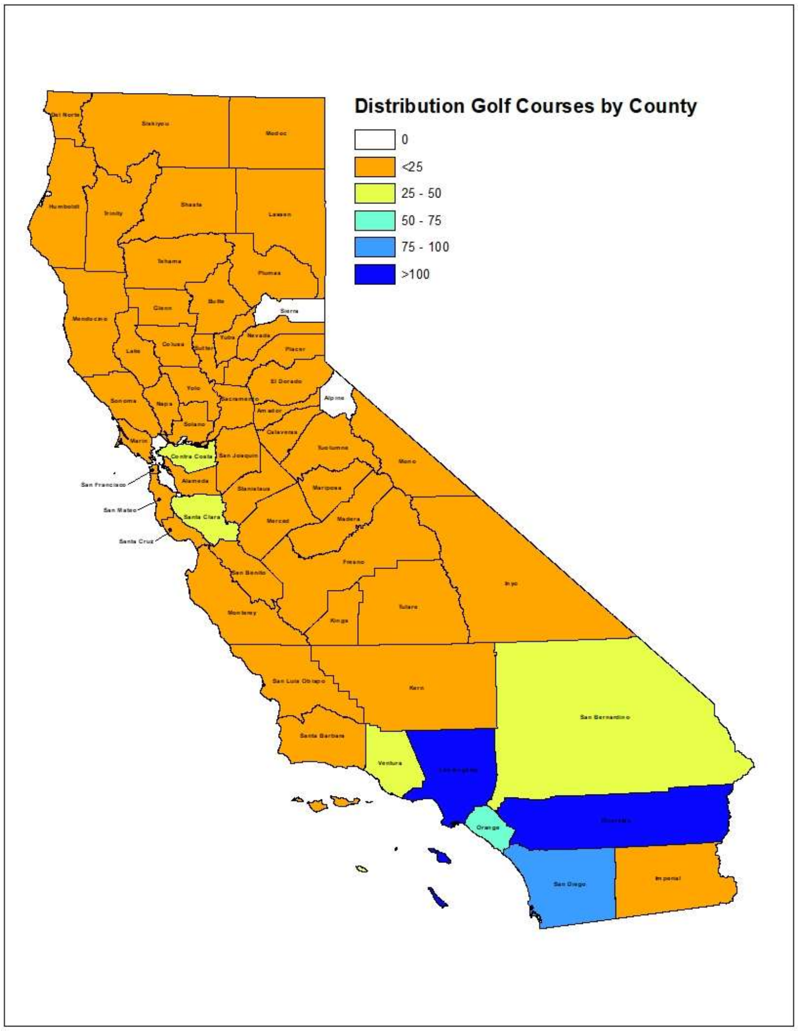
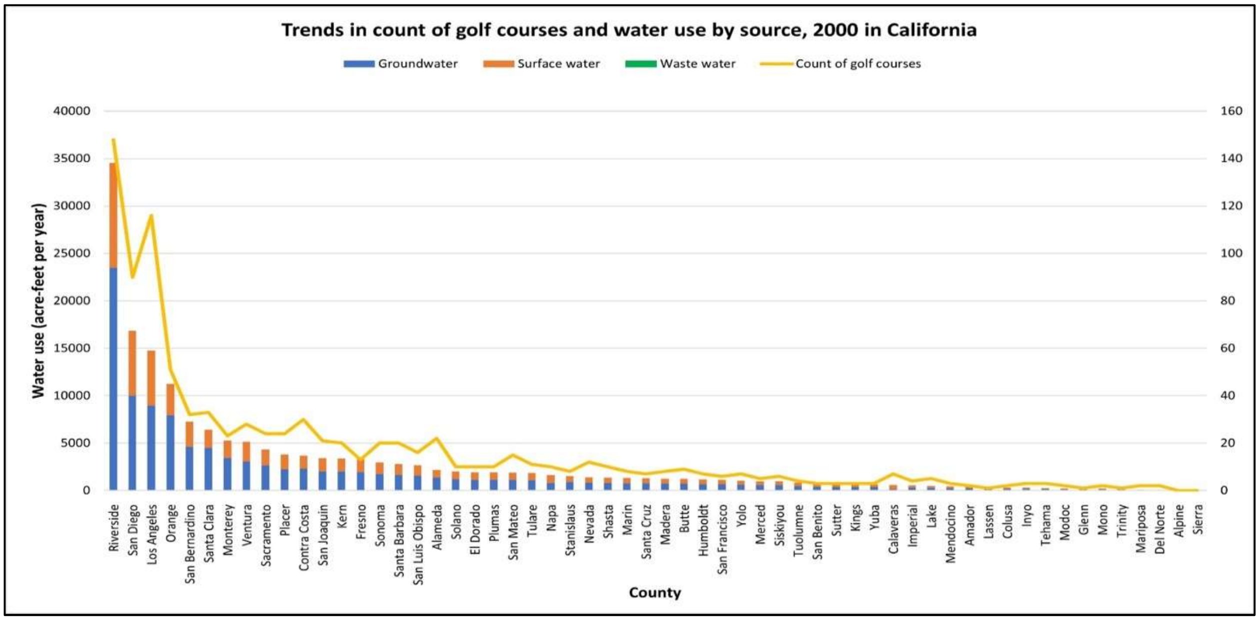
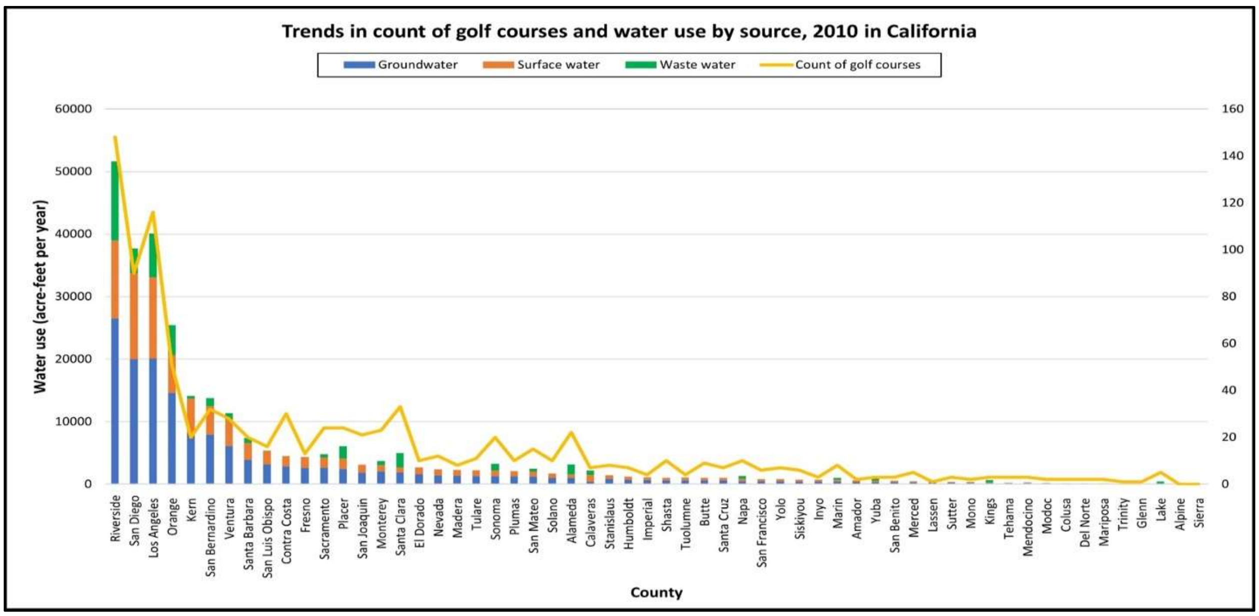


Figure 5. Distribution of the number of golf courses in California by county.



Trends in count of golf courses and water use by source, 2000 in California
Groundwater
Surface water
Waste water
Count of golf courses
Water use (acre-feet per year)
0
5000
10000
15000
20000
25000
30000
35000
40000
0
20
40
60
80
100
120
140
160
Riverside
San Diego
Los Angeles
Orange
San Bernardino
Santa Clara
Monterey
Ventura
Sacramento
Placer
Contra Costa
San Joaquin
Kern
Fresno
Sonoma
Santa Barbara
San Luis Obispo
Alameda
Solano
El Dorado
Plumas
San Mateo
Tulare
Napa
Stanislaus
Nevada
Shasta
Marin
Santa Cruz
Madera
Butte
Humboldt
San Francisco
Yolo
Merced
Siskiyou
Tuolumne
San Benito
Sutter
Kings
Yuba
Calaveras
Imperial
Lake
Mendocino
Amador
Lassen
Colusa
Inyo
Tehama
Modoc
Glenn
Mono
Trinity
Mariposa
Del Norte
Alpine
Sierra
County



Trends in count of golf courses and water use by source, 2010 in California
Groundwater
Surface water
Waste water
Count of golf courses
Water use (acre-feet per year)
0
10000
20000
30000
40000
50000
60000
0
20
40
60
80
100
120
140
160
Riverside
San Diego
Los Angeles
Orange
Kern
San Bernardino
Ventura
Santa Barbara
San Luis Obispo
Contra Costa
Fresno
Sacramento
Placer
San Joaquin
Monterey
Santa Clara
El Dorado
Nevada
Madera
Tulare
Sonoma
Plumas
San Mateo
Solano
Alameda
Calaveras
Stanislaus
Humboldt
Imperial
Shasta
Tuolumne
Butte
Santa Cruz
Napa
San Francisco
Yolo
Siskiyou
Inyo
Marin
Amador
Yuba
San Benito
Merced
Lassen
Sutter
Mono
Kings
Tehama
Mendocino
Modoc
Colusa
Del Norte
Mariposa
Trinity
Glenn
Lake
Alpine
Sierra
County

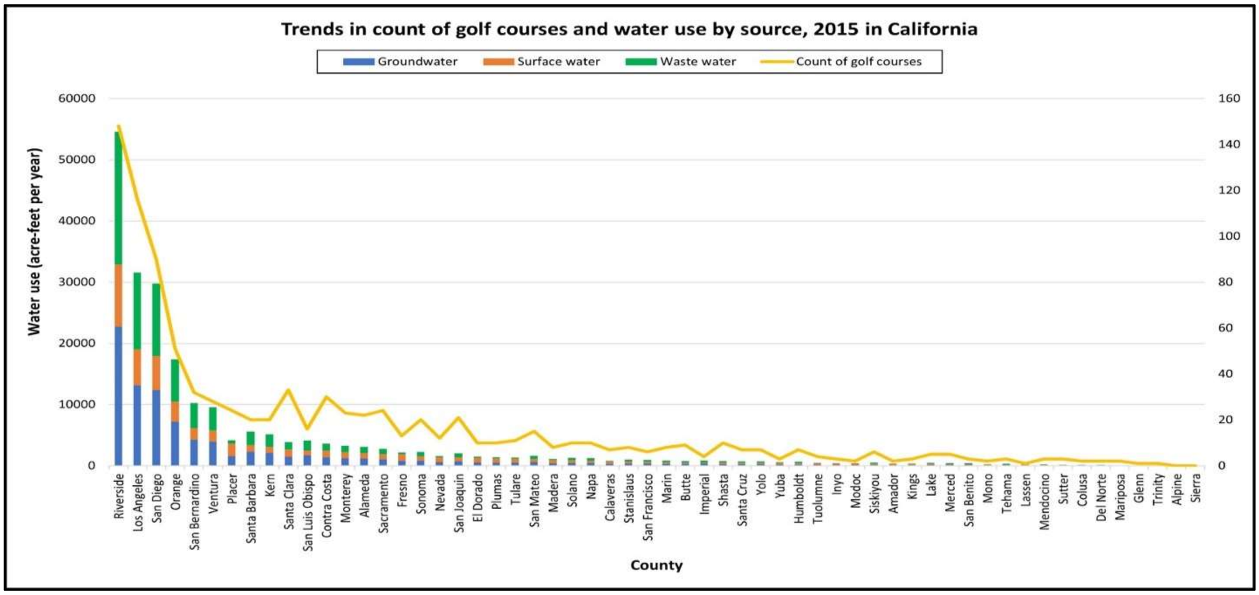


Figure 6. Water use trends and the number of golf courses in California by county and water source (2000-2015).

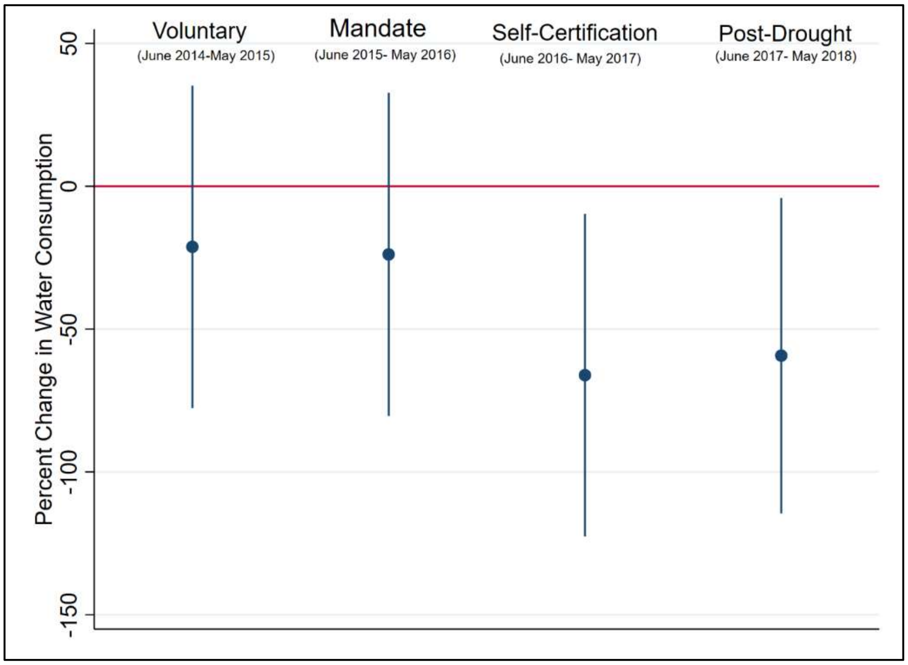


Figure 7. Percent changes in water use by drought policy period compared to 2013 levels in sample golf courses.

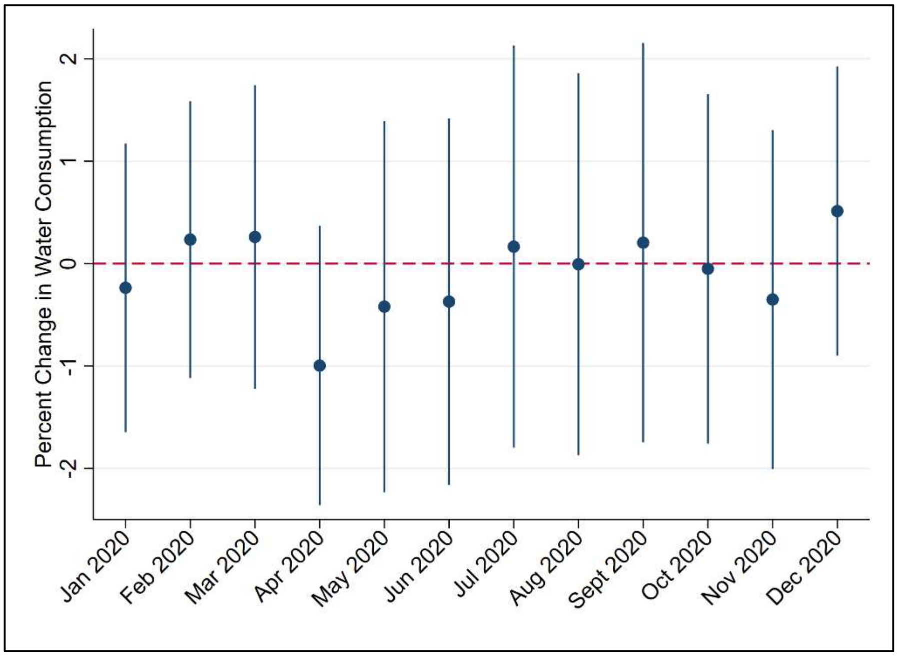


Figure 8. Percent changes in water use during COVID-19 (2020) compared to 2018 and 2019 levels in sample golf courses.

USGA ID#: 2021-03-727

Title: Characterizing Immune System Responses of Select Plant Health Products for Putting Greens

Principal Investigator(s): Erik Ervin[1], Charanpreet Kaur[1], Harsh Bais[1], Beth Guertal[2], and Mike Fidanza[3]
Affiliation: [1]University of Delaware; [2]Auburn University; [3]Penn State University

Objectives:
1. To determine morphological (quality, yield, root density) and physiological (chlorophyll, elemental) effects of seasonal plant health product programs on drought-stressed creeping bentgrass and ultra-dwarf bermudagrass greens.
2. To determine plant health product treatment effects on expression of pathogenesis-related genes in leaf and root tissue.
3. To determine the level of *Bacillus subtilis*-UD1022 colonization of roots via confocal microscopy and measurement of colony-forming units.

Start Date: 2021
Project Duration: 3 years
Total Funding: $132,277

Summary Points:

<u>Creeping Bentgrass Trial at Wilmington Country Club (Wilmington, DE)</u>

- Statistically significant differences were observed among the treatments for turf quality, clipping dry weight, root length, and chlorophyll content.
- No significant differences were observed among the treatments for soil volumetric water content (VWC).
- Acibenzolar-S-methyl, kelp extract, *Bacillus subtilis* UD1022, and fosetyl-Al had higher turf quality than trinexapac-ethyl, tank mix of all treatments, and control on most of the rating dates.
- Plots treated with *B. subtilis* UD1022 had significantly greater root length than control.
- Lower clipping yield was observed for trinexapac-ethyl and the tank mix of treatments.
- Laboratory and microscopic work regarding colonization of roots with *B. subtilis* UD1022 and upregulation of defense genes (or not) due to the treatments is yet to be completed.
- No dollar spot incident differences were observed across treatments due to high variability across replications (data not shown).

<u>Bermudagrass Trial (Auburn, AL)</u>

- Treatments varied significantly for soil VWC, clipping dry weight, root fresh weight, and shoot density.

- Clipping yield was lower for trinexapac-ethyl and the tank mix of treatments.
- Fresh root weight was lower due to fosetyl-Al and trinexapac-ethyl treatments.
- Trinexapac-ethyl reduced shoot density of Tifeagle only one measurement date, while there were no differences, relative to the control, for any of the other treatments.

Summary:

Loss of putting green quality on creeping bentgrass and ultra-dwarf bermudagrass is often associated with seasonal root decline, the prevention of which is a primary motivation for superintendents choosing to use so-called “plant health products” (PHP). Plant health products (PHP) application have a physiological effect that enhance a plant’s immunological defense system against various biotic and abiotic stresses. Some of these materials are EPA-registered active ingredients (e.g., acibenzolar-S-methyl, fosetyl-Al, trinexapac-ethyl), while others are registered as fertilizers (e.g., kelp extract, *Bacillus* spp., phosphite). For most, potential physiological effects have been documented in refereed journal articles. Rarely, however, are these materials studied on industry standard putting greens or in combination. Turfgrass consultants are often called to diagnose decline issues but are hard-pressed to determine primary causes due to confounding factors such as 3 to 10 ingredient tank-mixes.

Our target audiences are golf course superintendents, turfgrass consultants, and scientists. The overall goal of our research is to better understand why or why not one might choose to use certain plant health products, alone or in combination. If benefits are documented, our research may lead to more use of plant health products for maintaining stress-resistant putting greens and less use of pesticides. Improved drought resistance, if indicated, may result in improved superintendent confidence in their deficit irrigation practices. Less reliance on pesticides and water, while maintaining premier playing surfaces should result in measurable economic, environmental, and playability benefits.

Our trial was conducted at two locations on sand-based rootzones: creeping bentgrass (Tyee, 0.125 inch mowing height) at Wilmington Country Club, Delaware and an ultradwarf bermudagrass (TifEagle, 0.110 inch mowing height) green at Auburn University in Auburn, Alabama.

Our objectives are to determine morphological and physiological effects of seasonal plant health product programs, alone and in a five-way tank mix, on creeping bentgrass and to determine treatment effects on expression of pathogenesis-related genes. The treatments untreated control, acibenzolar-S-methyl (50% WSP), kelp extract (0-0-1), *Bacillus subtilis* UD1022, fosetyl-Al (80% WP), trinexapac-ethyl (11.3%), and five-product tank mix are applied at label rates every two weeks from May to October. All plots receive a uniform fertilizer program of 28-8-18 (soluble, with micronutrients) at 0.1 lb N/1000 ft^2 every 14 days. Fungicides are applied at 2-week rates every 3 weeks to result in some disease development and allowing data collection on treatment differences.

For the creeping bentgrass trial at Wilmington Country Club, seven treatments (Table 1) were applied every two weeks and plots were evaluated for turf quality. Clippings were collected for three days growth every two weeks to obtain dry weight (Figure 1). Leaf tissue subsamples were also collected for chlorophyll estimation. Statistically significant differences were observed among all the treatments for turf quality, clipping dry weight, root length, and chlorophyll content whereas no significant differences were observed among the treatments for soil volumetric water content (VWC). Acibenzolar-S-methyl, kelp extract, PGPR *Bacillus subtilis* UD1022, and fosetyl-Al had higher turf quality than trinexapac-ethyl, tank mix, and control treatments on most of the rating dates (Table 2). Clipping yield was lower for trinexapac-ethyl and tank mix of treatments possibly due to growth inhibiting effect of trinexapac-ethyl in both the treatments (Table 3). Chlorophyll a content was significantly lower due to trinexapac-ethyl on two of nine measurement dates (Table 4) while chlorophyll b was not significantly different (data not presented). Plant and soil nutrient contents were estimated at the beginning of the season and will be compared with end of the season samples (Table 5, 6).

Figure 1: Clipping collection for dry weight at Wilmington CC trial, July 8, 2022

Root samples (0.75-inch diameter, 6 inches deep) were collected at the beginning, middle, and end of the trial season. PGPR *B. subtilis* UD1022 showed increase in root length relative to the control for beginning and middle of the season while no significant differences were observed for root length at the end of the season (Fig. 2, 3). Subsamples have been stored at -80 C for pathogenesis related (PR)-gene expression and at 4 C for visualization of bacterial root colonization (confocal microscopy). UD1022 abundance in the rhizosphere will be determined by qPCR analysis. These lab analyses are scheduled to be completed in 2023.

For the bermudagrass trial (Auburn, AL), seven treatments were applied every two weeks. Soil VWC data were collected as an average of 5 readings per plot using a Spectrum brand volumetric soil moisture meter. Treatments varied significantly for soil VWC, clipping dry weight, root fresh weight, and shoot density. Clipping yield was consistently lower for trinexapac-ethyl and the tank mix of treatments (Table 8). Fresh root weight was lower, relative to the control, on August 2 due to fosetyl-Al, kelp extract and trinexapac-ethyl (Table 9). Shoot density was measured as number of shoots averaged over 2 samples that were 1 inch diameter x 6 inch deep cores. Only trinexapac-ethyl resulted in reduced shoot density at the September 15 measurement date (Table 10). Root samples are in sold storage and will be evaluated for root density using WinRhizo software in early 2023. Laboratory measurements for quantitative and qualitative estimation of UD1022 root colonization and estimation of PR-gene expression samples are in progress. The field trial will be resumed in mid-May in 2023.

Table 1. List of treatments applied to 2022 putting green trial.

Treatment	**Product Name**	**Concentration**
Control	…….	…….
Acibenzolar-S-Methyl (50% WSP)	Actigard (Syngenta)	0.3 g /1000 ft^2
Kelp Extract (0-0-1)	Guarantee Natural (Ocean Organics)	3 oz/1000 ft^2
PGPR *Bacillus subtilis*	UD1022	10^6 cfu/mL/1000 ft^2
Fosetyl-Al (80% WP)	Aliette (Bayer)	4 oz/1000 ft^2
Trinexapac-ethyl (11.3%)	Primo Maxx	0.125 oz/1000 ft^2
Tank mix	Treatments 2,3,4,5,6	

Table 2. 2022 Field Trial at Wilmington Country Club, DE – Turf Quality

Treatment	Date of Measurement					
	June 6	July 5	Aug 1	Aug 29	Sept 26	Oct 24
	Turf Quality (1 – 9 visual scale)					
Control	8.5a	7.8ab	7.5ab	5.8b	5.4b	6.0b
ASM	9.0a	8.3ab	8.3ab	8.0a	6.0ab	7.8a
Kelp Extract	9.0a	8.1ab	7.9ab	7.5a	6.1ab	7.8a
Bacillus subtilis UD1022	8.8a	8.4a	8.4a	7.6a	6.6a	7.8a
Fosetyl-Al	8.5a	8.3ab	8.3ab	7.1ab	5.8ab	8.0a
TE	8.3a	7.6b	7.6ab	5.8b	5.3b	6.0b
Tank Mix	6.5b	7.6b	7.4b	6.8ab	5.5ab	6.0b

Ratings are based on a scale of 1 – 9 (1 = poorest TQ, 9 = excellent turf, and 6 = minimum acceptable; P=0.05).

Figure 2. 2022 Field Trial at Wilmington Country Club, DE – Root Length

A) Spring 2022 B) Summer 2022

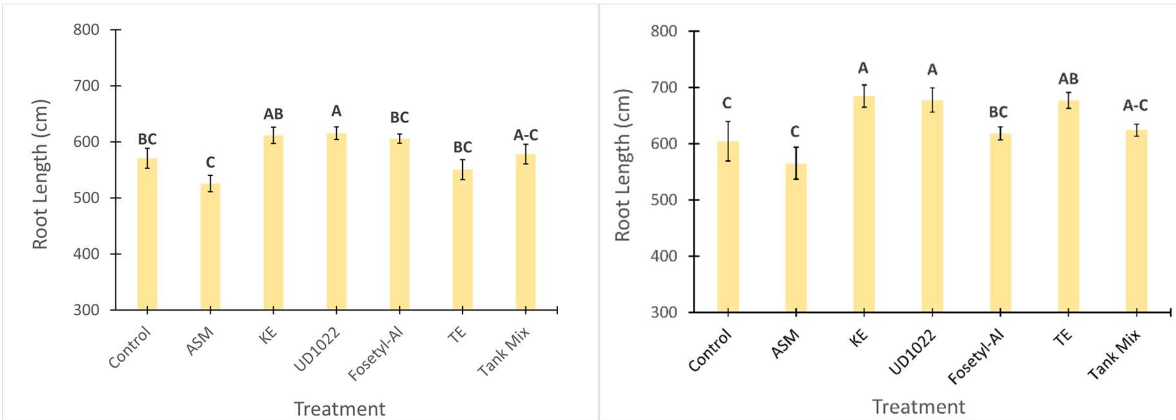


Fig. 1. Root length (cm) for A) Spring 2022 and B) Summer 2022. Means were separated using Fisher's LSD (α=0.05). Bars followed by same letter are not significantly different at α =0.05.

Figure 3: Qualitative view of Tyee bentgrass root length in July 2022 due to PHP treatments

Key: Control ASM Kelp UD1022 Fosetyl-Al Trinexapac ALL

Table 3. 2022 Field Trial at Wilmington Country Club, DE – Clipping Dry Weight

Treatment	Date of Measurement							
	May 23	June 8	June 28	July 11	July 28	Aug 9	Sept 30	Oct 17
	Clipping Dry Weight (g)							
Control	2.23a[x]	3.25abc	2.75bc	3.43ab	2.46	2.62	2.33	2.43c
ASM	2.71a	3.74a	3.07ab	3.32ab	2.23	2.51	2.53	2.78abc
Kelp Extract	2.47a	3.46ab	3.59a	3.40ab	2.25	2.60	1.95	3.50ab
Bacillus subtilis UD1022	2.16ab	3.74a	3.42ab	3.50ab	2.23	2.49	2.06	3.96a
Fosetyl-Al	2.16ab	4.02a	3.02ab	3.86a	2.06	2.45	2.26	2.74bc
TE	1.55bc	2.51c	2.14b	2.49c	2.43	2.63	2.79	2.51bc
Tank Mix	1.37c	2.80bc	2.83abc	2.99bc	2.08	2.49	2.28	2.42c

	Oct 30
Control	2.85
ASM	3.16
Kelp Extract	3.55
Bacillus subtilis UD1022	3.40
Fosetyl-Al	2.93
TE	2.37
Tank Mix	2.85

[x]Means accompanied by the same letter in a column are not significantly different at the P = 0.05 level. Dry weights were recorded after drying at 60°C for 24 hours.

Table 4. 2022 Field Trial at Wilmington Country Club, DE – Chlorophyll a

Treatment	Date of Measurement							
	May 23	June 8	June 28	July 11	July 28	Aug 9	Sept 30	Oct 17
	Chlorophyll a content (μg/ml)							
Control	13.32ab[x]	10.94	16.00abc	15.15	26.15b	16.26	31.33	17.23
ASM	12.08bc	8.39	17.31bc	16.27	26.70b	17.79	39.36	19.26
Kelp Extract	15.14a	11.21	16.38abc	17.51	31.48a	18.86	39.38	22.59
Bacillus subtilis UD1022	13.79ab	10.35	18.79a	16.54	27.97ab	19.53	37.96	21.02
Fosetyl-Al	12.29bc	8.95	14.36bc	16.68	26.13b	14.40	38.96	21.01
TE	10.18c	10.63	12.99c	17.82	23.90b	17.12	31.88	20.08
Tank Mix	13.59b	9.03	13.80bc	14.37	27.82ab	18.48	38.34	17.78

	Oct 30
Control	19.79
ASM	19.18
Kelp Extract	19.72
Bacillus subtilis UD1022	19.12
Fosetyl-Al	20.72
TE	21.78
Tank Mix	20.74

[x]Means accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

Table 5. 2022 Field Trial at Wilmington Country Club, DE – Soil nutrients (May)

Treatments	Water	OM by LOI	P	K
	pH	(%)	(mg/kg)	(mg/kg)
Control	5.4	0.3	59.7	31.0
ASM	5.4	0.4	60.3	26.7
Kelp Extract	5.3	0.4	73.7	30.8
Bacillus subtilis UD1022	5.4	0.4	58.1	28.8
Fosetyl-Al	5.4	0.4	64.5	28.9
TE	5.4	0.3	58.0	32.5
Tank Mix	5.4	0.5	66.1	29.5

Table 6. 2022 Field Trial at Wilmington Country Club, DE – Plant nutrients (May)

Sample	N	P	K	Al
	(%)	(%)	(%)	(ppm)
Control	3.817	0.477	1.875	539.8
ASM	3.857	0.483	1.843	485.3
Kelp Extract	4.010	0.478	1.912	397.0
Bacillus subtilis UD1022	3.709	0.450	1.776	509.9
Fosetyl-Al	3.402	0.435	1.492	905.7
TE	3.620	0.442	1.710	498.5
Tank Mix	3.492	0.472	1.482	1105.2

Table 7. 2022 TifEagle hybrid bermudagrass putting green field trial at Auburn, AL - Volumetric Soil Moisture

Treatment	Date of Measurement						
	July 18	July 26	Aug 2	Aug 8	Aug 15	Aug 29	Sept 15
	Percent soil moisture (%)						
Control	22.5a	10.6ab	15.8ab	9.2b	10.9ab	15.4	14.3
ASM + polysorbate 20	20.4ab	9.9ab	13.9ab	9.5b	11.2ab	14.6	13.3
Fosetyl-Al	21.9ab	9.8ab	16.7a	12.7a	11.6ab	16.2	14.5
Kelp Extract	18.9b	8.9b	12.5b	11.4ab	10.4b	14.0	14.0
Bacillus subtilis UD1022	21.6ab	9.8ab	14.9ab	11.0ab	10.7ab	15.1	14.6
TE	20.2ab	10.2ab	14.4ab	10.4ab	10.3b	14.0	13.1
Tank mix	22.7a	12.2a	15.0ab	12.5a	12.4a	15.9	13.8
	Sept 20	Sept 22	Sept 26	Oct 3	Oct 6	Oct 10	
	Percent soil moisture (%)						
Control	10.1	14.9	13.9	16.2	13.6 abc	14.2 b	
ASM + polysorbate 20	9.7	15.0	14.2	18.2	12.7 c	14.8 b	
Fosetyl-Al	11.0	15.0	16.9	17.2	16.8 a	20.8 a	
Kelp Extract	10.0	16.0	16.0	16.0	14.1 abc	16.4 ab	

Bacillus subtilis UD1022	11.1	15.5	16.7	15.7	16.3 ab	15.9 ab
TE	9.7	15.0	16.0	15.4	12.8 bc	15.6 ab
Tank mix	11.0	16.0	15.8	16.7	15.1 abc	14.6 b

Data collected as an average of 5 readings per plot using a Spectrum brand volumetric soil moisture meter.
All treatments applied biweekly.
Within each rating date means followed by the same letter are not significantly from each other via mean's separation at an alpha of 0.05.

Table 8. 2022 TifEagle hybrid bermudagrass putting green field trial at Auburn, AL - Clippings Dry Weight

Treatment	Date of Measurement							
	July 5	July 19	Aug 2	Aug 16	Aug 30	Sept 13	Sept 27	Oct 11
	Dry weight (grams per one mower pass)							
Control	8.2 a	8.4 a	3.5 a	4.7 ab	9.5 a	8.7 a	1.0 c	0.4 a
ASM + polysorbate 20	8.2 a	8.5 a	4.0 a	6.6 a	9.3 a	10.0 a	2.6 a	0.5 a
Fosetyl-Al	6.0 bc	9.5 a	3.7 a	5.2 ab	9.5 a	9.0 a	2.4 ab	0.7 a
Kelp Extract	6.5 ab	7.2 a	2.2 ab	5.2 ab	9.6 a	10.3 a	1.9 abc	0.5 a
Bacillus subtilis UD1022	7.7 ab	9.4 a	3.7 a	5.2 ab	10.0 a	8.3 a	2.2 abc	0.4 a
TE	4.3 c	4.2 b	1.2 c	3.4 c	6.7 b	7.9 a	1.1 bc	0.4 a
Tank mix	5.7 bc	4.8 b	2.2 bc	5.3 ab	6.8 b	9.2 a	1.6 abc	0.5 a

Within each rating date means followed by the same letter are not significantly from each other via mean's separation at an alpha of 0.05.

Table 9. 2022 TifEagle hybrid bermudagrass putting green field trial at Auburn, AL – Fresh Root Weight

Treatment	Date of Measurement			
	June 21	Aug 2	Sept 13	Oct 11
	Root fresh weight (g)			
Control	0.90	0.41 a	0.24	0.24
ASM + polysorbate 20	0.72	0.32 abc	0.16	0.24
Fosetyl-Al	0.66	0.26 c	0.19	0.19
Kelp Extract	0.96	0.27 bc	0.23	0.22
Bacillus subtilis UD1022	0.76	0.31 abc	0.22	0.20
TE	0.87	0.23 c	0.20	0.27
Tank mix	1.01	0.39 ab	0.21	0.28

Root weight = grams fresh weight washed, two-1 inch diameter cores, 6 inches deep.
Within each rating date means followed by the same letter are not significantly from each other via mean's separation at an alpha of 0.05.

Table 10. 2022 TifEagle hybrid bermudagrass putting green field trial at Auburn, AL - Shoot Density

Treatment	Date of Measurement		
	Aug 2	Sept 15	Oct 11
	Shoot number		
Control	51.5	36.6 ab	39.0 ab
ASM + polysorbate 20	47.6	33.4 bc	36.2 b
Fosetyl-Al	49.8	32.6 bc	41.3 ab
Kelp Extract	49.7	31.8 bc	41.8 ab
Bacillus subtilis UD1022	50.3	36.0 abc	45.9 a
TE	50.3	29.8 c	38.4 b
Tank mix	53.1	41.3 a	44.7 a

Shoot density = number of shoots per washed, two-1 inch diameter cores, 6 inches deep.
Within each rating date means followed by the same letter are not significantly from each other via mean's separation at an alpha of 0.05.

USGA ID#: 2020-05-710

Title: Turfgrass under effluent water irrigation: long-term data collection

Investigator: Yaling Qian and Yao Zhang

Affiliation: Colorado State University

Objectives: To collect and test soil properties and evaluate turfgrass on greens and fairways of golf courses irrigated with effluent water.

Start Date: 2020

Project Duration: 1 year

Total Funding: $10,000

Project Rational: Increasing demand on fresh water supplies in the arid and semi-arid western US and more stringent wastewater discharge standards have made effluent water a common water source for irrigating golf courses. The future of turfgrass industry needs effective strategies to manage irrigation waters containing higher levels of soluble salts and sodium absorption ratio (SAR), particularly in arid and semi-arid regions. Long-term evaluation of soil properties through field research is essential for the development of effective management strategies.

Experiment I

Methodology: This study leverages historical soil samples from 2 golf courses to examine long term impact of effluent water irrigation. The PI collected soil samples at the start of effluent water use for irrigation, then periodically thereafter. These soil samples were archived. In 2021 after 17 and 21 years of effluent water irrigation for Golf Course I and II, respectively, soil samples were collected again. Soil cores were collected 30 cm from the original sampling locations. Soil samples were taken at different depths at 0-20, 20-40, 40-60, 60-80, and 80-100 cm below soil surface. We conducted soil analysis for the following parameters: soil texture, soil pH, soil CEC, soil electrical conductivity, soil N and P content, soil ESP, and soil B, Cl, Fe, Mn, and Mg content.

Findings

Soil pH. Even though the average pH of effluent water leaving the water treatment plant was lower than fresh water, results from all facilities indicated that soil pH increased after effluent water irrigation. The observed pH increase was consistent across study sites. The degree of soil pH increase under effluent water irrigation was, in general, greater at deeper depths than at shallow soil depths on 2 golf courses where soils were sampled to 100 cm deep (Fig. 1 and 2). At Golf Course 1, pH did not show increase trend after the use of effluent water at 0-20 cm depth for the first 11 years. From 20 cm to 100 cm soil profile, soil pH significantly increased at each depth after effluent water irrigation (Fig. 1). At Golf Course II, soil pH in 2021 was greater than that in 2002 at each depth, with soil pH being greater at deeper depths than at shallow soil depths (Fig. 2). The soil pH increase may be partially due to the bicarbonate concentration in effluent water. At both sites, effluent water was stored in irrigation ponds.

During the storage, algae activity likely had increased water pH due the absorption of CO_2. There was significant linear relationship between the duration of effluent water irrigation and soil pH. Soil pH increased as the time of effluent water irrigation increased (Fig. 3).
A main implication of increasing soil pH is plant nutrient availability. The increase of soil pH can have effects on plant growth as the availability of certain nutrients in soil solution, especially Fe and Mn become less available. Results of analysis of variance test indicated that the soil extractable Fe and Mn concentration did not change significantly after 17 years of using effluent water (Table 1), although the effluent water contained small quantities of Fe and Mn, and effluent water could supply the soil with Fe and Mn if soil pH was in a proper pH range.

Soil Exchangeable Sodium Percentage (ESP).

At Golf course I, no increase in ESP from 2004 to 2009 and 2015 at surface 0-20 cm depth was observed (Fig. 4). However, the increase in ESP became significant from 20 cm to 100 cm along the soil profile; at 20-40cm, 40-60 cm, 60-80 cm, and 80-100 cm below soil surface, soil ESP increased 76%, 150%, 160%, and 205%, respectively, after 5 and 11 years effluent water irrigation. The degree of soil ESP increase was greater at deeper soil profile. However, soil ESP increased dramatically at the surface depths from 2015 to 2021 (Fig. 4). The changes along the soil profile reflect soil types and management that are conducive to Na leaching from the surface layer at this golf course. The course was built on alluvial sand deposits. Soils at this golf course are mostly sandy and drain well, and turf managers employed aggressive aeration and gypsum addition programs from 2004-2017. They generally aerate 1-2 times a year for fairways and apply about 1.5 to 2.0 Mg ha^{-1} yr^{-1} gypsum following aerification. Apparently, the aggressive soil aerification and gypsum addition plus the dominant presence of sandy soil effectively prevented a significant increase in soil ESP at the shallow soil depths (0-20 cm) from 2004 to 2015, however ESP at 40-60 cm, 60-80 cm, and 80-100 cm soil profile was more than doubled or tripled 11 years after effluent water irrigation. Due to pandemic and a change of turfgrass management, no aggressive gypsum applications were applied since 2018. We observed significant increase in soil ESP especially at 0-20 cm, 20-40 cm and 40 - 60 cm depths in 2021 (Fig. 4). The increase was the most significant at the surface 20 cm depth when no aggressive gypsum applications were applied. Our results indicated that effluent water irrigation increases soil ESP over time (Fig. 5). These results suggest that sodicity (as gauged by soil ESP) is a concern on the effluent water irrigated golf courses.

Electrical Conductivity (EC).

On the golf course I, the average soil EC over depths was 1.0, 0.7, 1.0, and 0.9 in 2004, 2009, 2015 and 2021, respectively (Table 1). There was no consistent significant difference in soil EC values 17 years after the start of effluent irrigation (Fig. 6). No clear trend of increasing soil salinity under effluent water irrigation was observed from 2004 to 2021 at golf course II and two parks (data not shown). However, soil EC increased with effluent water irrigation has been reported previously.

Experiment II:

Study Description. In this study, the soil chemical properties of greens and fairways on two golf courses that use either effluent water or fresh water were compared. Soil analyses of the two golf courses were compared because both courses had the same grasses and were managed by the same superintendent employing similar management practices. The main difference was that Golf course A transitioned to effluent water for irrigation while golf course B has always used fresh water. Another difference is that gypsum was applied granularly at 850 kg ha^{-1} yr^{-1} 2 years after the start of effluent water irrigation on Golf course A. Five years after the start of effluent water irrigation, additional gypsum was injected through the irrigation system. The total amount of gypsum applied in year 5 was 2242 kg ha^{-1}.

Over a 11-year period, a total of 238 soil samples of greens (104 from golf course A and 134 from golf course B) were collected and tested, and a total of 90 soil samples from fairways (45 from golf course A and 45 from golf course B) were collected and tested.

Findings:

Soil analyses showed that many changes occurred over time due to the use of effluent water irrigation. Starting 6 years of using effluent water, soil electrical conductivity increased but remained well below the critical threshold levels for turf , whereas the average soil pH increased by 0.42 units 1 to 7 years of effluent water irrigation on fairways and greens, respectively (Fig. 7). Compared to freshwater irrigation, effluent water irrigation increased soil pH. Both on fairways and greens, soil ESP increased by 2 to 5 times despite the application of gypsum (Fig. 8).

One unique finding is that the availability of micronutrients (Fe and Mn) decreased on greens irrigated with effluent water irrigation (Fig. 9). After the start of using effluent water irrigation, soil Fe concentration from greens of Golf Course A was significantly lower than greens of fresh water-irrigated Golf Course B in 2, 4, and 5 years after the start of effluent water irrigation, suggesting that iron availability is reduced under effluent water irrigation on greens (Fig. 9). Soil Mn concentration from greens of Golf Course A was significantly lower than greens of fresh water-irrigated Golf Course B at 4 to 6 years after effluent water irrigation in Golf Course A (Fig. 9). It is possible that the observed reductions in Fe and Mn availability were associated with soil pH increase caused by effluent water irrigation (Fig. 7). Soil pH plays an essential role in micronutrient availability. The available soil Fe and Mn were not observed to decrease on fairways. Although no turfgrass tissue analysis has been done, iron chlorosis was observed at several locations.

On the positive side, effluent contains N, which allowed the turf manager to reduce annual N application by 60 – 70 kg ha^{-1}. Soil P and K levels increased in the soil after using effluent water, which would be beneficial for turfgrass and potentially lower the fertilizer cost (data not shown).

Project Summary:

Both experiments demonstrated that despite the benefits of effluent water irrigation, there are concerns relating to soil ESP and soil pH increases. Since soil alkalinity would increase under effluent water irrigation, we recommend using acidifying fertilizers including ammonium sulfate and other sulfur-containing products to mitigate soil pH increase at the surface depth. However, it is often necessary to re-apply these substances to sustain the effect. In addition, it is difficult to prevent soil pH from increasing deep in the soil profile. The greater increase in soil pH deep in the soil would likely have a greater impact on deep rooted landscape plants such as shrubs and trees than shallow rooted plants such as grasses.

Under effluent water irrigation, soil ESP change was generally greater than salinity (EC), which provided reason for concern about possible long-term reductions in soil hydraulic conductivity, although measured

ESP were not high enough to result in short-term soil deterioration. However, urban landscapes may be more susceptible to the relatively high soil ESP due to the fact that urban landscapes are not subject to annual soil plowing and urban soil has a great compaction pressure from traffic. This project indicated that aerification and gypsum application can be used to reduce soil ESP in surface soil (0-20 cm), but it is less effective in reducing soil ESP at deeper soil depths.

As more landscape facilities switch to effluent water irrigation, landscape managers need to apply proactive management practices, such as applications of soil amendments that provide Ca to replace Na, improvement of drainage, adjustment of fertilization program, and selection and use more tolerant turfgrass and landscape plants to mitigate the negative impact and ensure continued success in effluent water reuse for landscape irrigation.

Summary Points:

- ✓ Effluent water contains nutrients which allowed the turf manager to reduce annual N application by 60 – 70 kg ha-1.
- ✓ Effluent water irrigation resulted in increases in soil ESP and soil pH.
- ✓ Gypsum application after aerification displaced sodium and reduced ESP at the surface depth (0-20 cm), but it is less effective in reducing soil ESP at deeper soil depths.
- ✓ The available micronutrients (Fe and Mn) decreased on greens irrigated with effluent water irrigation.

List of Figures:

Table 1. Mean soil chemical properties from Golf Course I at the initial (baseline) and 5, 11 and 17 years after effluent water irrigation (soils were sampled to 1 m).

Soil Parameter	Baseline	5 yr after	11 yr after	16 yr after
Cation exchange capacity (cmol, kg^{-1})	17.73	16.00	16.07	6.60
pH	7.28c	7.57b	7.56b	7.8a
Soil organic matter(%)	1.38	0.9	1.52	1.80
Electrical conductivity (dS m^{-1})	1	0.69	1.02	0.90
P (ppm)	11.19	8.68	10.24	7.45
NO3-N (ppm)	9.80a	5.08b	2.46b	4.93b
Ca (cmol, kg^{-1})	3.84	2.23	3.09	1.29
Mg (cmol, kg^{-1})	1.69	0.62	1.43	1.42
Mn (mg kg^{-1})	1.45	0.78	1.64	1.08
Fe (mg kg^{-1})	14.50	18.36	23.26	11.56
Boron (mg kg^{-1})	0.48	0.35	0.91	0.55
Exchangeable sodium percentage	2.34b	5.09ab	4.88ab	6.36a

Different letters indicate significant difference ($P \leq 0.05$) among years for each parameter.

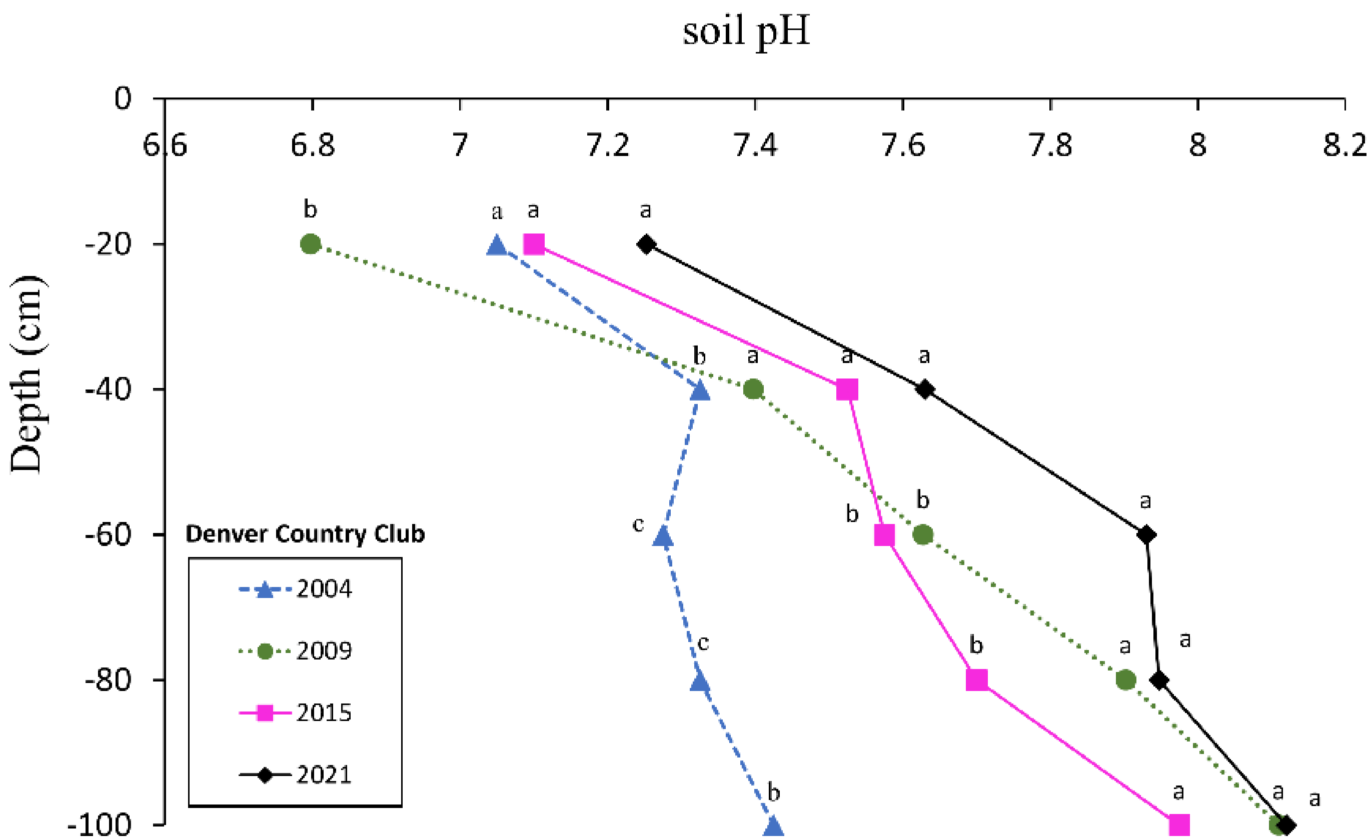


Fig. 1. Soil pH of 2004, 2009, 2015 and 2021 at Golf Course I.

Different letters indicate significant difference (P ≤ 0.05) among years at each depth. Each data is the mean of four replications.

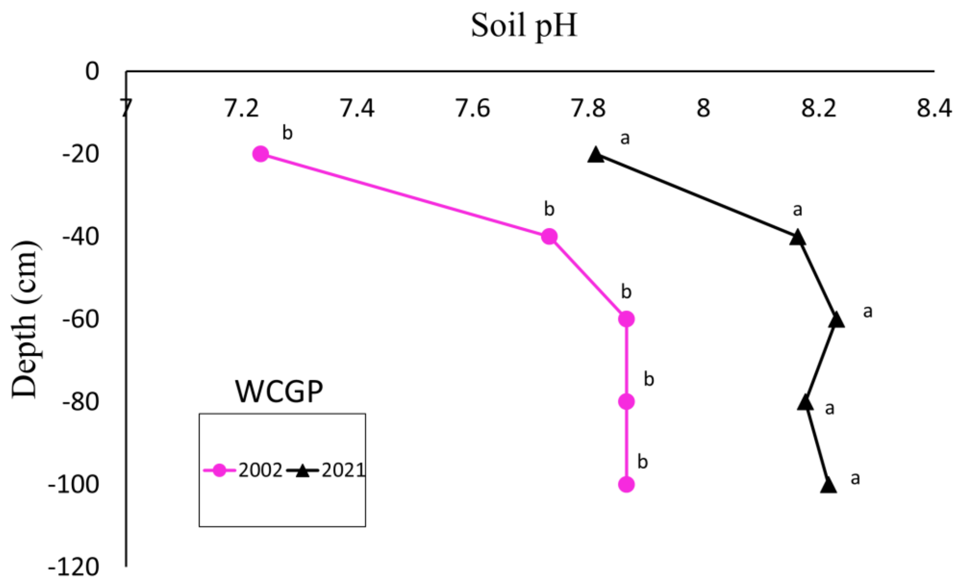


Fig. 2. Soil pH of 2002 and 2021 at Golf Course II. Each data is the mean of four replications.

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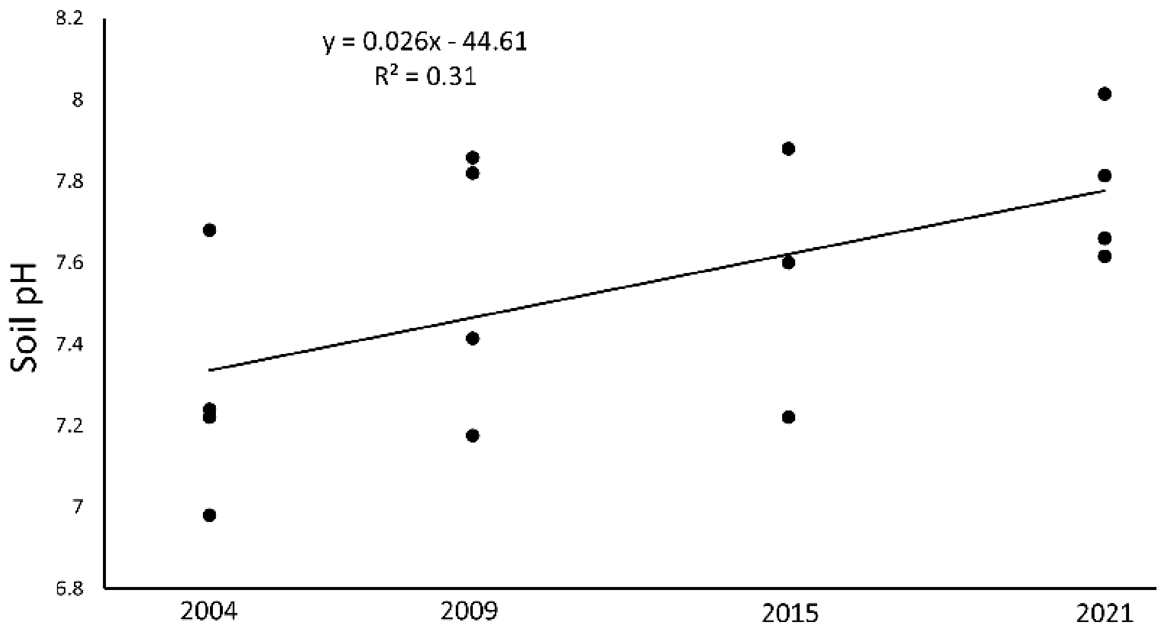

Fig. 3. Regression linear of the soil pH and years of effluent water irrigation at Golf Course I.

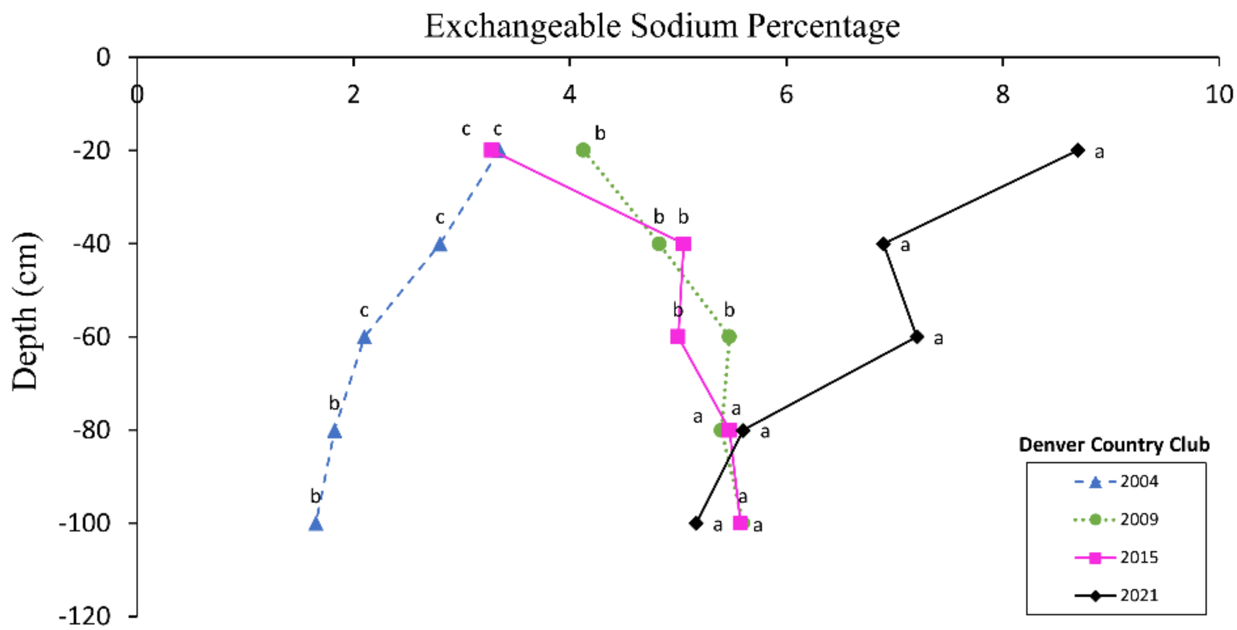

Fig. 4. Soil exchangeable sodium percentage (ESP) of 2004, 2009, 2015 and 2021 at Golf Course I.

Different letters indicate significant difference ($P \leq 0.05$) among years at each depth. Each data is the mean of four replications.

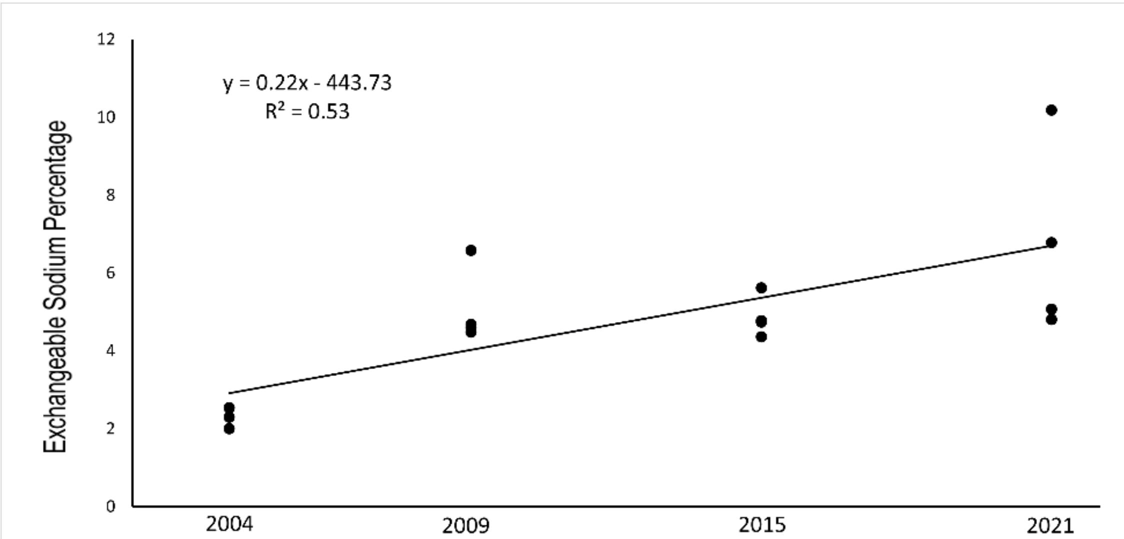

Fig. 5. Regression linear of the soil ESP of 2004, 2009, 2015 and 2021 at Golf Course I.

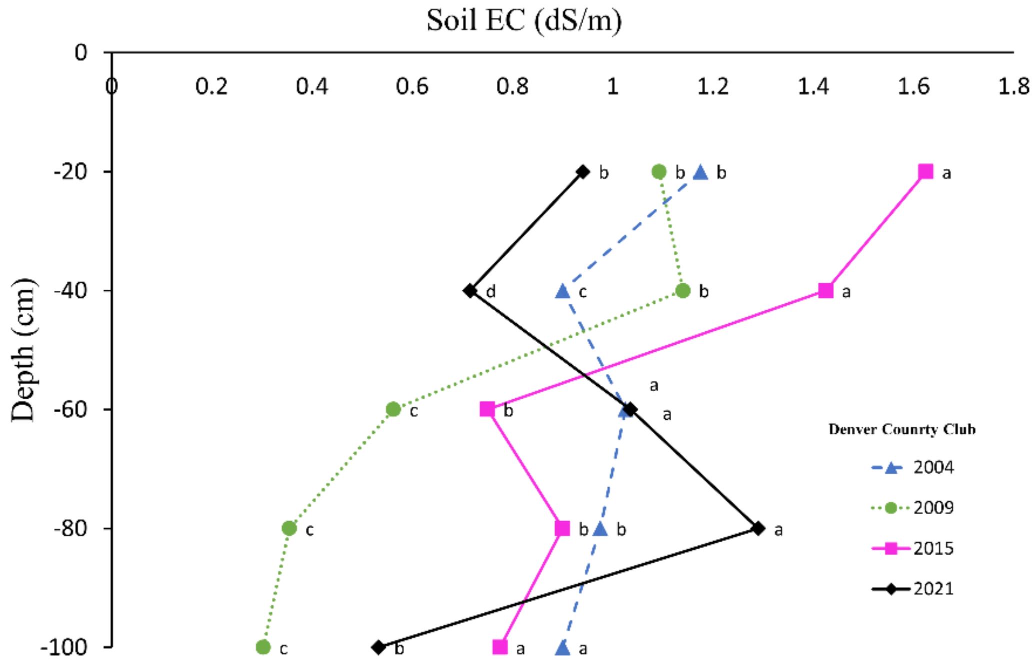

Fig. 6. Soil Electrical conductivity (EC) of 2004, 2009, 2015 and 2021 at Golf Course I.

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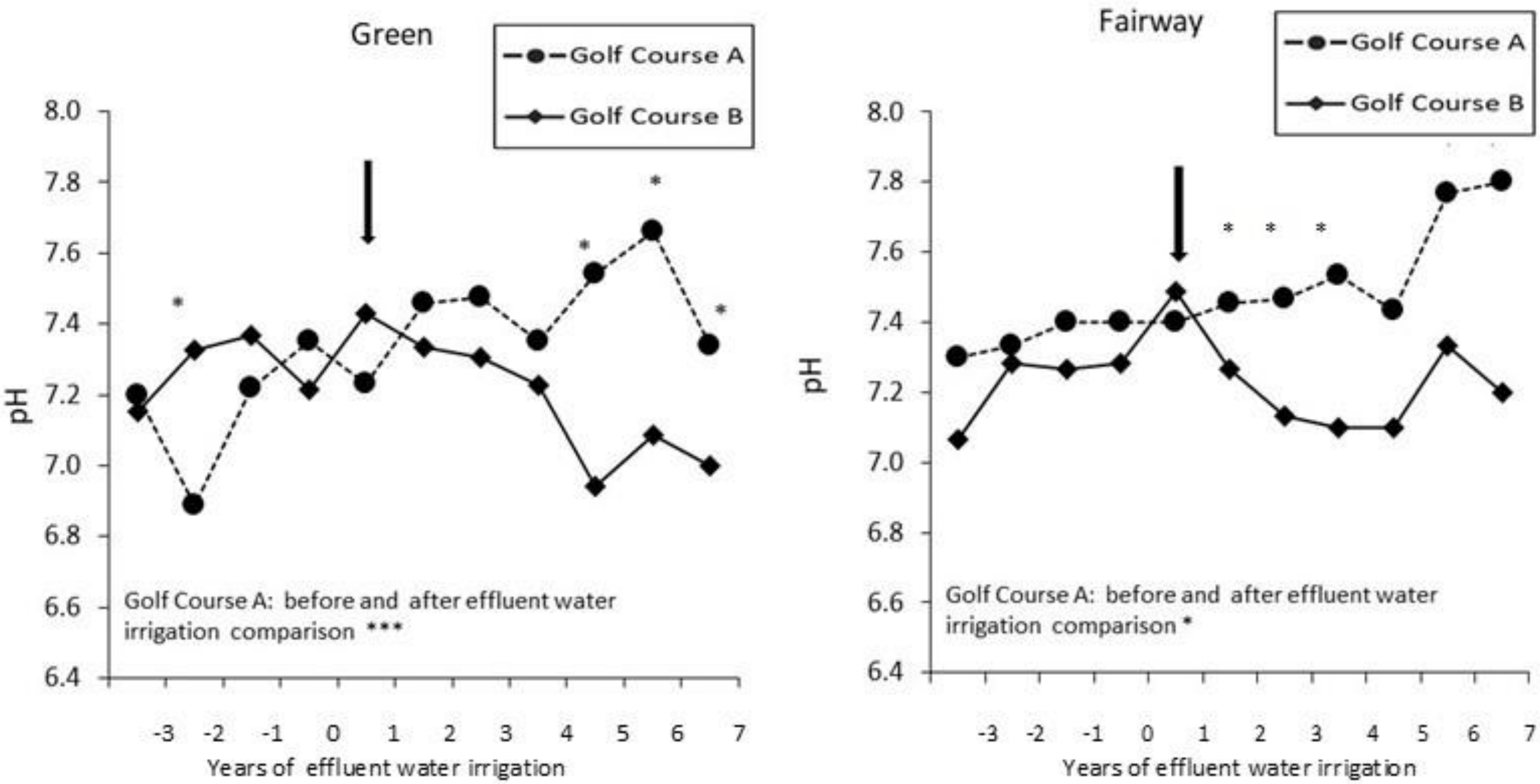

Fig. 7. Soil pH from putting greens and fairways of Golf Course A and Golf Course B.

* Significant at the 0.05 probability level.

Arrow indicates the year of beginning effluent water irrigation on Golf Course A.

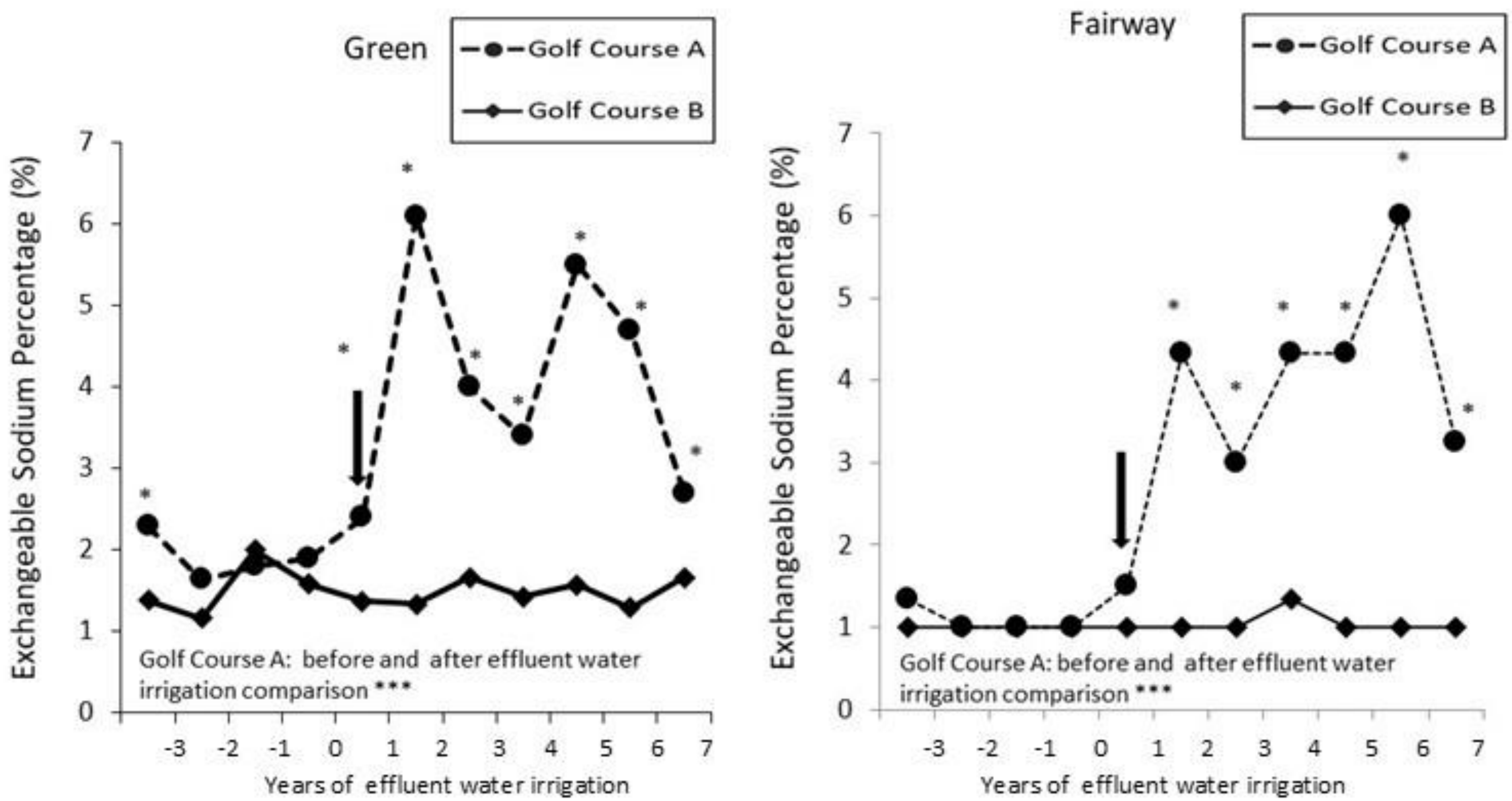

Fig. 8. Soil exchangeable Na percentage from Golf Course A and Golf Course B putting greens and fairways.

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

Arrow indicates the year of beginning effluent water irrigation on Golf Course A.

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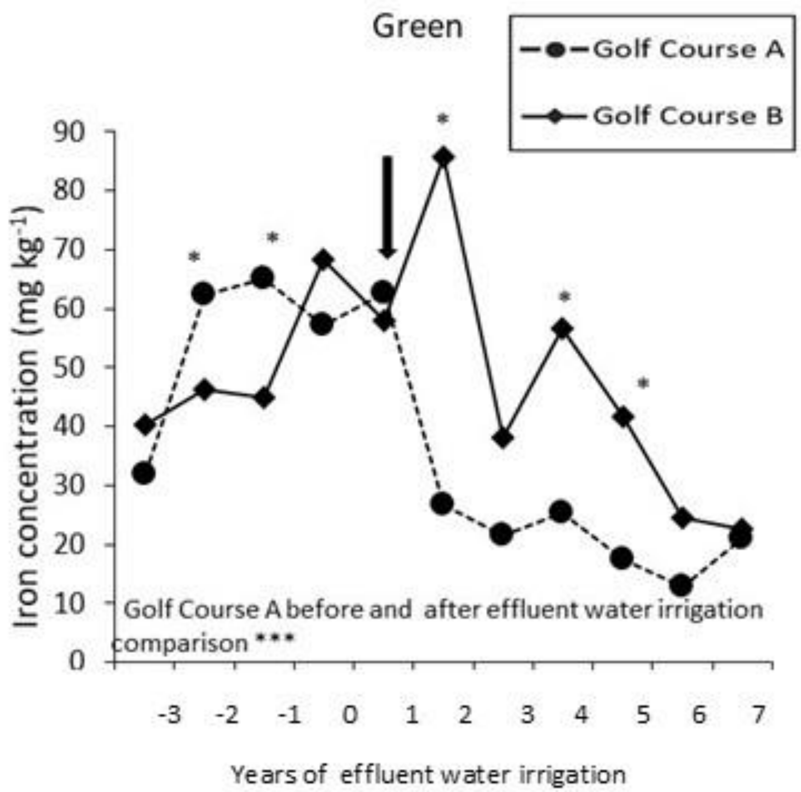

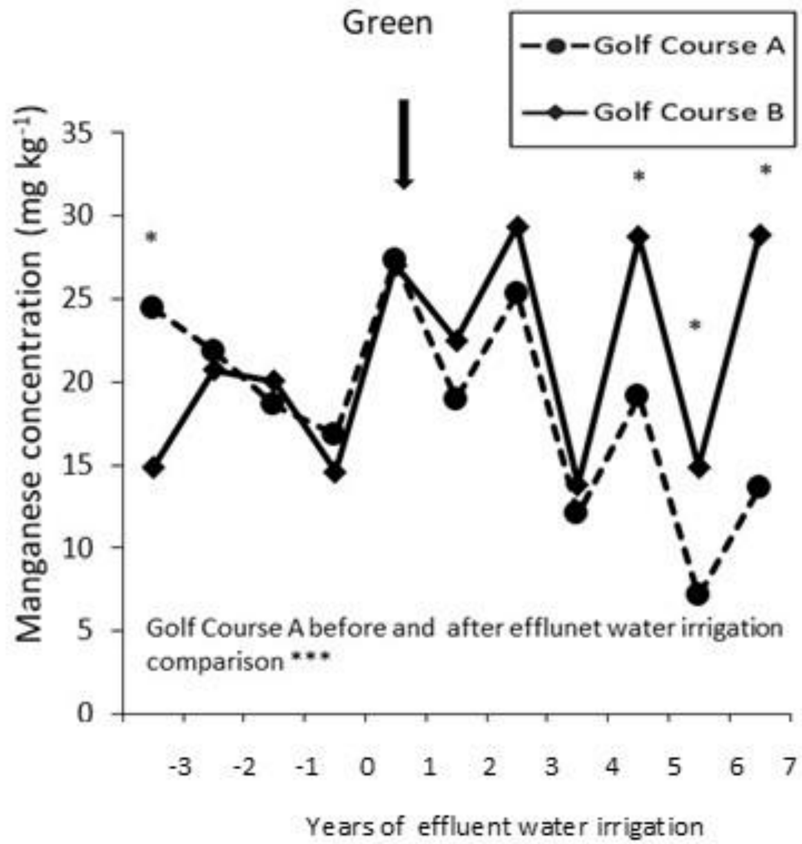

Fig. 9. Soil Fe and Mn concentration from Golf Course A and Golf Course B putting greens.
* Significant at the 0.05 probability level.
Arrow indicates the year of beginning effluent water irrigation on Golf Course A.

USGA ID#: 2020-04-709

Project Title: Effect of acidification on soil bicarbonate concentration, infiltration rate, and Kentucky bluegrass performance.

Project Leaders: Elena Sevostianova and Bernd Leinauer

Affiliation: New Mexico State University

Objectives:
1. Quantify changes of soil infiltration rates of rootzones irrigated with water high in Bicarbonates and treated with either N-pHuric acid or with WaterSOLV™ Curative.
2. Determine the effect of N-pHuric acid or WaterSOLV™ Curative on the level of bicarbonates and other chemical parameters in soil.
3. Determine the effect of irrigation water treated with N-pHuric acid or WaterSOLV™ Curative on the quality of Kentucky bluegrass.

Start Date: 2021
Project Duration: 3 years
Total Funding: $116,580.00

Summary Points:
- For both years, plots irrigated with water high in Bicarbonates to which N-pHuric acid was added had higher saturated hydraulic conductivity than plots receiving water only high in Bicarbonates.
- In the fall of 2022, highest levels of bicarbonates were found in plots irrigated with water high in Bicarbonates and in plots irrigated with water high in Bicarbonates to which WaterSOLV™ Curative was added.
- In the fall of 2021 and 2022, highest levels of soluble salts were found in the soil of plots irrigated with water high in Bicarbonates with N-pHuric acid. At depths of 10-20 and 20-30 cm, highest SAR was in plots having received water high in Bicarbonates and water high in Bicarbonates with N-pHuric acid.
- Turfgrass irrigated with water high in Bicarbonates with N-pHuric acid had lower tissue concentrations of Ca, but higher concentration of K, Cu, Na, and N_{total} compared to other treatments.
- First results on the effect on N-pHuric acid on Kentucky bluegrass quality were inconclusive. In August and November 2021 plots irrigated with water high in Bicarbonates and N-pHuric acid exhibited higher visual quality, but in August 2022 turfgrass quality was lower than on other plots.

Summary Text
Background and Rationale
Golf courses increasingly use poor quality water. Many of these sources, especially in arid regions, contain high levels of dissolved bicarbonates. Bicarbonate levels in irrigation water are measured along with sodium, calcium, and magnesium content of both soil and water, as these are believed to be the

major cause of soil physical problems such as low infiltration rate and reduction of plant rooting. Moreover, there is a concern that the deposit of calcium carbonate derived from irrigation water can seal soil pores over time, but this problem is separate from sodium-induced deterioration of soil physical conditions. While high levels of bicarbonates and sodium in irrigation water suggest that acidification is required, the question arises as to whether irrigation water acidification is necessary when ratios of Ca/Mg and HCO_3/CO_3 are high, but levels of sodium are low. Management practices that prevent the accumulation of calcite in soil have been described, however there is a lack of scientific evidence that such conditions occur in turf rootzones. While guidelines for interpretation of irrigation water quality consider many parameters and their interactions, such as EC and SAR, information about the effect on turfgrass performance of irrigation water high in bicarbonates is scarce.

Methods

A study was initiated at New Mexico State University's Turfgrass Salinity Research Center in Las Cruces, NM in November 2020. The soil at the site consisted of a sandy loam, a skeletal mixed thermic Typic Torriorthents, a sandy entisol typical for arid regions. The research area was seeded with Kentucky bluegrass "Barserati" in fall of 2020 and consisted of sixteen 2m by 2m plots arranged in a block design. Water treatments started on 15th June 2021. Irrigation water was prepared in four 1890L (500-gallon) tanks (Figure 1). Sodium bicarbonate and potassium bicarbonate was used to increase the level of bicarbonate to 500ppm in the irrigation water. Plots were irrigated 5 times per week at 80% of ETo by hand with the corresponding irrigation waters. A detailed list of ion concentrations in the irrigation waters is shown in Table 1. A control treatment received potable water only.

Figure 1. Set up of water treatments, four tanks (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative).

Four water treatments were used in the project:
Treatment 1. Potable water with low concentration of bicarbonates (200 ppm) was used as a control.
Treatment 2. Potable water with high concentration of bicarbonates (450-500ppm).

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Treatment 3. Potable water with high concentration of bicarbonates (450-500ppm) with N-pHuric acid added in the amount needed to adjust the pH to 6.5.
Treatment 4. Potable water with high concentration of bicarbonates (450-500ppm) with WaterSOLV™ Curative added following label recommendations.

Table 1. Chemical analysis of water samples collected from four water tanks.

Constituents	Treatment 1	Treatment 2	Treatment 3	Treatment 4
pH	8.2	8.6	7.9	8.5
EC, mmho/cm	0.78	1.3	1.31	1.27
Sodium, Na, ppm	62	116	129	124
Potassium, K, ppm	6	93	92	95
Sulfate, SO_4-S, ppm	37	38	80	38
Carbonate, CO_3, ppm	<1.0	9.2	<1.0	9.1
Bicarbonate, HCO_3, ppm	205	451	306	463
Total alkalinity, $CaCO_3$, ppm	173	382	253	392
Nitrate, NO_3-N, ppm	0.1	0.1	0.1	<0.1
Total nitrogen, N, ppm	0.1	0.3	42.1	0.3
Ammonium, NH_4-N, ppm	0.1	0.2	0.6	0.2
Total Phosphorus, P, ppm	0.5	0.21	0.7	0.43

During the growing season, plots receiving Control water, water high in Bicarbonates, and water high in Bicarbonates with Curative were fertilized with Urea in an amount to match the amount of nitrogen applied to water high in Bicarbonates with N-pHuric acid. At the end of the growing period, each plot received the same amount of nitrogen.

Soil samples were collected at the 10, 20, and 30 cm depths before and after the growing season (November 3, 2021, April 29, 2022, and September 29, 2022) and analyzed for bicarbonates (titration with 0.01N sulfuric acid), pH, EC (conductivity bridge), and SAR (plasma emission spectroscopy). Soil cores were collected on April 30, 3021, November 23, 2021, and October 27, 2022, and the saturated hydraulic conductivity (Ksat) was measured in the lab conditions using the KSAT (METER Group Inc., USA) (Figure 2). One photograph per plot was taken every other week (April 13 - October 27) to determine Dark Green Color Index (DGCI) using digital image analysis. Visual quality on a scale of 1 (brown, dead grass) to 9 (perfectly dark green, uniform grass) of the turf was evaluated every other week (February 22-November 29). Visual quality and DGCI data were averaged for each month and presented from June to November. For nutrient analysis plant tissue was collected on September 21, 2021, April 30, 2022, and September 14, 2022.

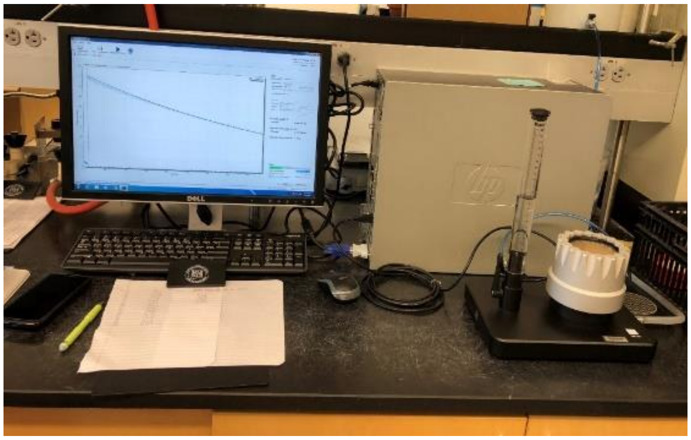

Figure 2. Measuring of Ksat using KSAT® (Meter Group, Inc. USA)

Each treatment was replicated four times. To test the effects of water treatments on Kentucky bluegrass quality, Ksat, soil parameters, DGCI, and ion concentration in plant tissue, 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.

2022 Results

Precipitation was higher in 2022 compared to 2021, reaching 253 mm and 162 mm, respectively.

Saturated hydraulic conductivity

P-values obtained from the corresponding ANOVA to examine differences in Ksat values of plots irrigated with differently treated water are listed in Table 2. When Ksat was analyzed for data averaged over 2021 and 2022, values were higher for plots irrigated with water high in Bicarbonates with N-pHuric acid than for plots irrigated with water high in Bicarbonates only (Table 2, Figure 3).

Table 2. Results of ANOVA testing the effect of date (April 30, 2021, November 23, 2021, and October 27, 2022) and four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative) on saturated hydraulic conductivity (Ksat) of soil.

	Ksat, 2021	Ksat, 2021 and 2022
Date	0.0002	0.0001
Water treatment	0.0573	0.0618
Date* Water treatment	0.0451	0.0666

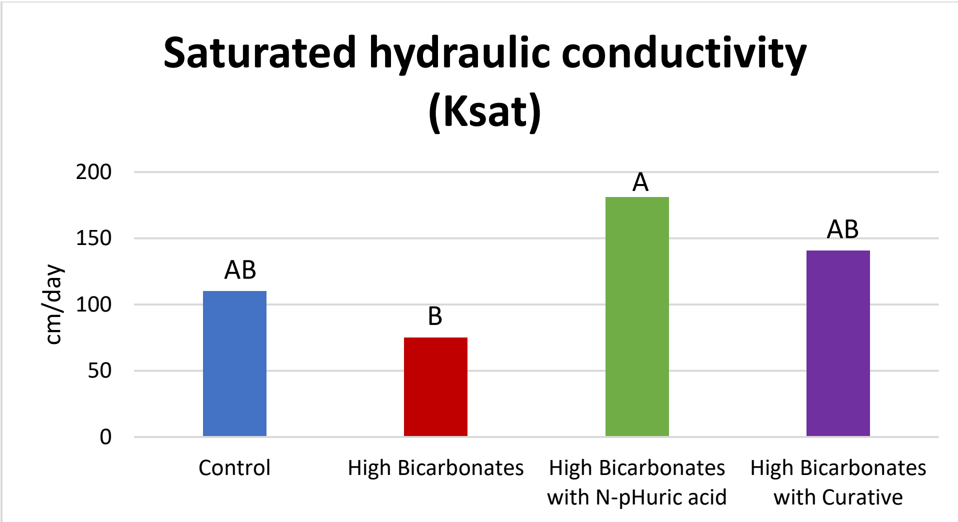

Figure 3. Saturated hydraulic conductivity of soil cores collected in the fall of 2022 from plots irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative).

Soil

P-values obtained from the corresponding ANOVA to examine differences in soil chemical parameters of plots irrigated with differently treated water are listed in Table 3. When HCO_3 data were pooled over three depths and analyzed separately for each sampling date, concentrations of HCO_3 were not different for plots irrigated with water high in Bicarbonates, water high in Bicarbonates with N-pHuric acid, and water high in Bicarbonates with Curative in spring 2022 (Figure 4). In the fall of 2022, plots irrigated with water high in Bicarbonates and water high in Bicarbonates with Curative had higher concentration of HCO_3 than soil irrigated with Control and with water high in Bicarbonates with N-pHuric acid. In the fall of 2022, soil electrical conductivity was greatest for plots irrigated with water high in Bicarbonates with N-pHuric acid (Figure 5). When Sodium Adsorption Ratios were pooled over sampling dates and analyzed separately for the three sampling depths, values were highest in plots irrigated with water high in Bicarbonates and water high in Bicarbonates with N-pHuric acid (Figure 6).

Table 3. Results of ANOVA testing the effect of four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative), sampling dates (November 2021, April 2022, and September 2022), soil depth (0-10, 10-20, and 20-30 cm) and their interactions on chemical characteristics of soil.

Effect	HCO_3 ppm	Mg Meq/L	NO_3 ppm	Na Meq/L	SAR	SO_4 ppm	EC	pH
Water treatment	<.0001	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Date	<.0001	0.5005	0.001	0.0005	<.0001	0.057	<.0001	<.0001
Date*water treatment	0.0002	0.0004	0.9636	<.0001	0.0009	0.0259	<.0001	0.0038

Depth	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.032	0.0001
Water treatment*Depth	0.1945	0.9032	<.0001	0.0323	0.0084	0.023	0.1144	0.3396
Date*Depth	0.0091	0.9181	<.0001	<.0001	<.0001	0.0032	<.0001	0.1
Date*Water treatment*Depth	0.8303	0.2847	0.173	0.011	0.1063	0.0123	<.0001	0.3014

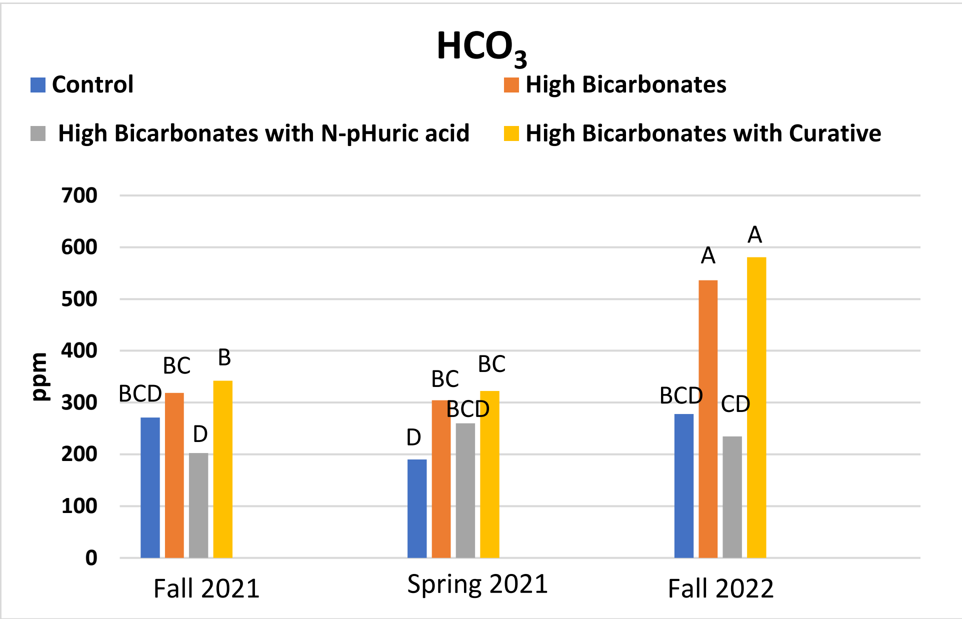

Figure 4. Bicarbonates (ppm) in soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative). Data are pooled over three depths (0-10, 10-20, and 20-30 cm).

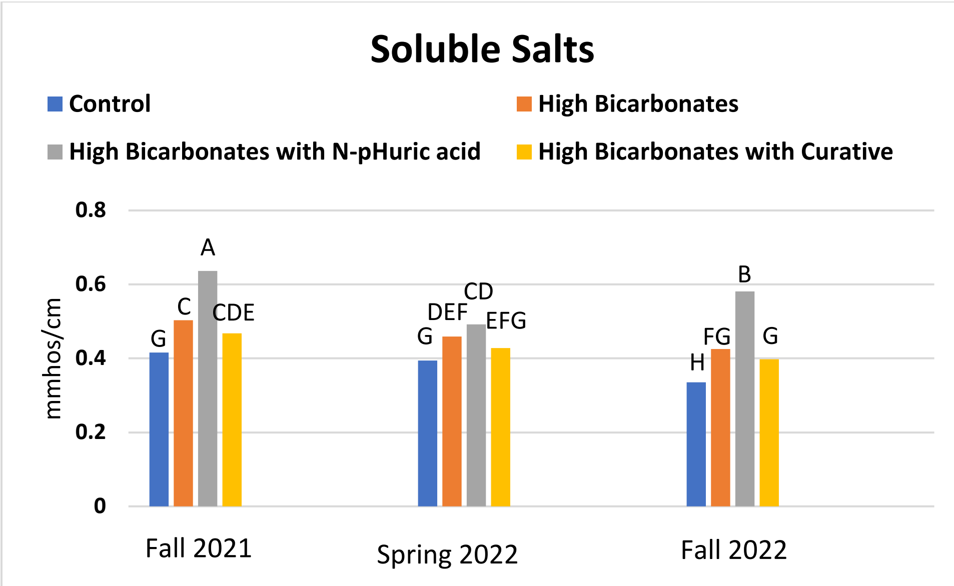

Figure 5. Soluble salts (mmhos/cm) in soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative). Data are pooled over three depths (0-10, 10-20, and 20-30 cm).

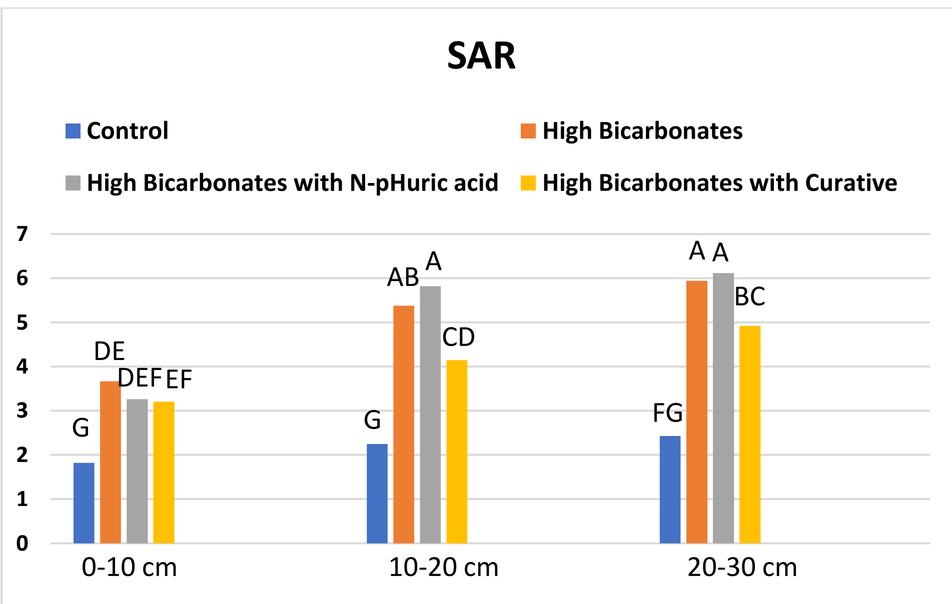

Figure 6. Sodium Adsorption Ratio (SAR) in soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative). Data are pooled over three depths (0-10, 10-20, and 20-30 cm).

Visual rating and DGCI

P-values obtained from the corresponding ANOVA to examine differences in visual rating and DGCI of Kentucky bluegrass irrigated with differently treated water are listed in Table 4. When visual quality and DGCI data were analyzed separately for each month, Kentucky bluegrass, irrigated with water high in Bicarbonates with N-pHuric acid exhibited higher visual quality than grass irrigated with other water treatments in August and November 2021 but lower visual quality than grass irrigated with water high in Bicarbonates in August 2022 (Table 5). Dark Green Color Index of Kentucky bluegrass, irrigated with water high in Bicarbonates with N-pHuric acid was higher than DGCI of grass irrigated with other water treatments in June and November 2021 and lower than DGCI irrigated with water high in Bicarbonates in August 2022 (Table 6).

Table 4. Results of ANOVA testing the effect of four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative), date (2021 and 2022) and their interactions on Dark Green Color Index (DGCI) and visual quality of Kentucky bluegrass.

Year	2021	2022	2021	2022
Effect	DGCI	DGCI	Visual quality	Visual quality
Water treatment	<.0001	0.006	<.0001	0.0002
Date	<.0001	<.0001	0.0014	<.0001
Date*Water treatment	0.0052	0.0432	0.0146	<.0001

Table 5. Quality (from 1=worst to 9=best) of Kentucky bluegrass irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative), in 2021 and 2022. Data are pooled over four replications and values represent an average of two readings.

Water treatment	June, 2021	July, 2021	August, 2021	September, 2021	October, 2021	November, 2021
	June, 2022	July, 2022	August, 2022	September, 2022	October, 2022	November, 2022
Control	7.8bc [ł¥]	7.3cd	7.6c	7.5cd	7.8bc	6.6d
	8.5ABC[§]	8.8AB	8.5ABC	8.8AB	9A	9A
High Bicarbonates	7.3cd	7.4cd	7c	7.6cd	7.4cd	6.6d
	8.6ABC	9A	8.9A	8.8AB	9A	9A
High Bicarbonates with N-pHuric acid	7.8bc	8.1bc	8.2b	7.9bc	8bc	8.8ab
	8.5ABC	9A	8.1BC	8.5ABC	9A	8.8AB
High Bicarbonates with Curative	7.3cd	7.1cd	7.5cd	7.8bc	7.6c	7.5c
	8.7A	9A	8.5ABC	8.8AB	9A	9A

[ł] Values followed by the same letter are not significantly different from one another (Fisher's protected LSD, α=0.05).

[¥] Lowercase letters denote differences between grass quality in 2021.

[§] Uppercase letters denote differences between grass quality in 2022.

Table 6. Dark Green Color Index (DGCI) of Kentucky bluegrass irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative), in 2021 and 2022. Data are pooled over four replications and values represent an average of two readings.

Water treatment	June, 2021 June, 2022	July, 2021 July, 2022	August, 2021 August, 2022	September, 2021 September, 2022	October, 2021 October, 2022	November, 2021 November, 2022
Control	0.45g [Ɨ¥]	0.45g	0.46fg	0.43ghi	0.47f	0.42i
	0.47F [§]	0.5ABC	0.5ABC	0.5BC	0.5C	0.53ABC
High bicarbonates	0.45g	0.45g	0.47f	0.43ghi	0.46fg	0.42i
	0.47F	0.6A	0.55AB	0.5ABC	0.5ABC	0.53ABC
High bicarbonates with N-pHuric acid	0.47f	0.45g	0.47f	0.44gh	0.48e	0.5d
	0.47F	0.5ABC	0.52CD	0.5D	0.5BC	0.53ABC
High bicarbonates with Curative	0.44gh	0.45g	0.44gh	0.42i	0.44gh	0.42i
	0.47F	0.5ABC	0.5ABC	0.5BC	0.5ABC	0.53ABC

[Ɨ] Values followed by the same letter are not significantly different from one another (Fisher's protected LSD, α=0.05).

[¥] Lowercase letters denote differences between grass quality in 2021.

[§] Uppercase letters denote differences between grass quality in 2022.

Plant tissue

P-values obtained from the corresponding ANOVA to examine differences in Kentucky bluegrass tissue irrigated with differently treated water are listed in Table 7. When data were pooled over three sampling dates and analyzed separately for each water treatment, Kentucky bluegrass irrigated with water high in Bicarbonates with N-pHuric had lower tissue concentrations of Ca, but higher concentration of Cu, K, Na, and N_{total} (Figure 7). Concentrations of Ca were greater in grass irrigated with Control water, but not different for grass irrigated with water high in Bicarbonates and water high in Bicarbonates with Curative.

Table 7. Results of ANOVA testing the effect of four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative), date (fall 2021, spring 2022, and fall 2022) and their interactions on nutrients in grass tissue.

	Ca	Cu	Fe	K	Mg	Mn	N_{total}	Na	P	S
Water treatment	<.0001	<.0001	0.0174	<.0001	0.3597	0.0874	<.0001	<.0001	<.0001	0.0003
Date	0.0009	<.0001	0.0004	0.6997	<.0001	0.002	0.0521	1	<.0001	<.0001
Date*Water treatment	<.0808	0.0666	0.2146	0.4504	0.0099	0.6467	0.0203	1	<.0001	0.004

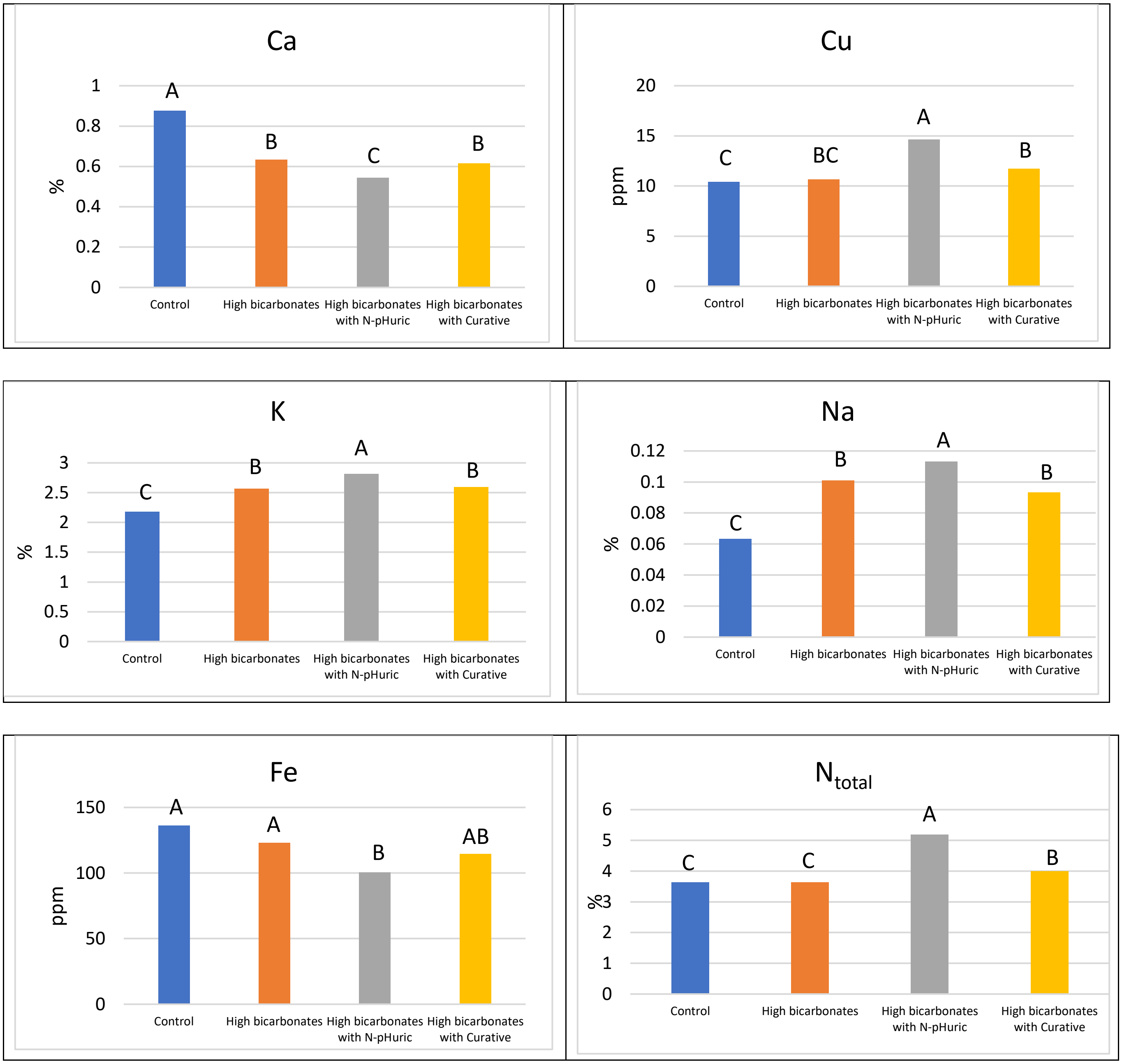

Figure 7. Nutrients in grass tissue irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with Curative). Data are pooled over date (fall 2021, spring 2022, and fall 2022) and four replications.

Future expectations of the project

As the change of bicarbonates as well as other chemicals in the soil most likely continues, it's effect on turfgrass quality and soil infiltration rates will be monitored

USGA ID#: 2021-02-726
Title: Bentgrass Cultivar and Autumn-applied Fungicide Timing Effects on Spring Suppression of Dollar Spot
Project Leaders: James A. Murphy, Bruce B. Clarke, Ning Zhang, Pingyuan Zhang, Glen Groben
Affiliation: Rutgers, The State University of New Jersey

Objectives:
Evaluate the 1) timing of autumn-applied fungicide, 2) disease tolerance in the host plant, and 3) antecedent inoculum load during autumn for effects on the onset, severity, and progress of dollar spot on bentgrass turf and the inoculum load during the subsequent growing season. 4) Based on results from preliminary trial 1, an additional objective was added to this research project: to evaluate the effect of fungicide chemistry on disease onset and progress.

Start Date: 2021
Project Duration: 2 years
Total Funding: $60,000

Summary Points:
1) Disease data from trials 1, 2, and 3 indicate that the onset and progress/severity of dollar spot during the subsequent growing season can be reduced by fungicide applied during the previous autumn (September, October, and November).
2) Tank mix applications of fluazinam + propiconazole in September and October had the greatest suppression of dollar spot during the subsequent growing season in trials 1 and 2.
3) Results in trial 3 suggested fluazinam was the key component in the suppression dollar spot in the fluazinam + propiconazole fungicide tank-mix.
4) Cultivar susceptibility greatly influenced dollar spot onset and suppression. 007 and Coho delayed the disease onset date for approximately 1 and 3 weeks, respectively, compared to Independence.
5) Quantification of pathogen DNA, using the qPCR method that we developed, indicated that the suppression of disease during 2022 was related to a lower pathogen inoculum load in the previous autumn.
6) Trials to address objectives 2, 3, and 4 will be continued in 2023.

Summary Text:
Results from trial 1 conducted in 2019-2021, as well as the first year of trial 2 in 2022, indicate that fungicide applied in September was associated with lower pathogen populations and lower disease severity the subsequent growing season. The onset date for dollar spot during 2022 was affected by fungicide timing and cultivar main effects. The average disease onset date of each cultivar across 12 autumn fungicide timings during 2022 was: 26 May for Independence, 3 June for 007, and 17 June for Coho. Therefore, 007 and Coho delayed the disease onset date for approximately 1 and 3 weeks, respectively, compared to Independence. Few differences in disease onset date were observed among fungicide timings in spring 2022. Fluazinam + propiconazole applied on Sep.-Oct.-Nov., Sep.-Oct., and at the 40% risk index delayed onset by 6 to 10 days compared to chlorothalonil applied in Sep.-Oct.-Nov. *C. jacksonii* inoculum load was lowest for Coho and no differences were detected between 007 and Independence in November 2021. Pearson correlations between AUDPC in 2022 and the *C. jacksonii* inoculum load on each cultivar in November 2021 indicated that the suppression of disease during 2022 was related to a lower pathogen inoculum load in the previous autumn. Trial 3 evaluated the impact of

applying propiconazole, fluazinam, and a tank-mix of fluazinam + propiconazole on the suppression of dollar spot during subsequent growing season. Results indicated that fluazinam (a contact fungicide) alone provided the same dollar spot suppression as the fluazinam + propiconazole tank-mix, while applying propiconazole alone was less effective.

Materials and Methods

Trial 1 (objective 1): This trial was conducted on '007' creeping bentgrass (*Agrostis stolonifera*) managed as fairway turf at 0.9-cm on a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) in North Brunswick, NJ (40°28' N, 74°25' W). Nine treatments: A tank mix of fluazinam and propiconazole once (three timings), twice (three timings), or thrice (one timing) in September, October and/or November in 2019 and 2020; chlorothalonil thrice (September, October, and November) and a non-treated control were applied from September to November 2019 and were arranged in a randomized complete block design with 4 replications. The trial was repeated on a different site in the same field during 2020-2021 using 6 blocks. Plot size was 0.91- by 1.5-m (3- by 5-ft). Full methodology and results were presented in the 2021 progress report.

Trial 2 (objectives 1 and 2): This trial was seeded in two separate but nearby locations on 8 September 2020 and 7 September 2021, respectively, and managed as a fairway turf at 0.9 cm on a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) in North Brunswick, NJ (40°28' N, 74°25' W.

Prior to the initiation of fungicide treatments, the trial locations were inoculated on 30 Mar. 2021 and 5 Apr. 2022 by uniformly distributing oats infested with *Clarireedia jacksonii* isolate NJDS003 and NJDS007 with a hand-shaker bottle at 1.34 g/m^2. Dollar spot was allowed to uniformly develop on plots and was then suppressed by periodic applications of chlorothalonil (Daconil Ultrex 82.5WG at 15.3 kg a.i. per ha) during summer. The 2021 trial location received chlorothalonil applications on 8 and 31 July, 14 Aug. and 10 Sep. 2021 and the 2022 trial location received applications on 22 July, 5 and 25 Aug. and 9 Sep. 2022. Dollar spot symptoms had not redeveloped before treatments were initiated in the 2021 location. However, small lesion centers were apparent at the initiation of fungicide treatments in the 2022 location.

A 3 $\times$ 12 factorially arranged randomized complete block design with 4 replications was used for this study. The cultivar factor has three levels (e.g., Coho, 007 and Independence) and represented low to high dollar spot susceptibility. The autumn-applied fungicide factor had twelve levels that included eight calendar-based treatments, two model-based treatments, one curative (threshold-based) treatment and a non-treated check. Fungicide timing treatments were initiated on 24 and 23 September for the 2021 and 2022 locations, respectively. Seven treatments received a tank mix of fluazinam and propiconazole once (three timings), twice (three timings), or thrice (one timing) in September, October and/or November in 2021 and 2022 (Table 1). An eighth treatment received chlorothalonil thrice (September, October, and November). Two model-based treatments used the Smith-Kerns dollar spot predictive model action risk index thresholds of 20 or 40%. Fungicide was applied whenever the risk index output was equal or higher than the action threshold and re-applied when the risk index action threshold was reached, but no sooner than 21-d since the previous application. The damage-based timing was applied within 24 hours after a dollar spot damage threshold of 314 mm^2 per 3-m^2 was observed on the respective cultivar; re-applications were made no sooner than 7 days after a previous application. All fungicides were applied from September through November. A tank mix of fluazinam (Secure 4.17SC) at 0.7 kg a.i. ha^{-1} (0.5 fl. oz. per 1,000 ft^2) and propiconazole (Banner MAXX 1.3MC) at 0.4 kg a.i. ha^{-1} (1.0 fl. oz. per 1,000 ft^2) was used for all treatments except the chlorothalonil (Daconil Ultrex 82.5WG) treatment, which was applied at 15.3 kg a.i. ha^{-1} (5 oz. per 1,000 ft^2).

Table 1. Fungicide and application timings to evaluate the effect of autumn fungicides applied in 2021 and 2022 on dollar spot onset and progress during the subsequent growing season on ‘Coho’, ‘007’, and ‘Independence’ creeping bentgrass in North Brunswick, NJ.

Treatments[1]	Number of Sprays[2]	Fungicide Timing (Date)[3]		
Non-treated control	0			
Calendar-based				
Sep.[4]	1	24 Sep./23 Sep.		
Oct.[4]	1		15 Oct./14 Oct.	
Nov.[4]	1			5 Nov./4 Nov.
Sep.-Oct.[4]	2	24 Sep./23 Sep.	15 Oct./14 Oct.	
Sep.-Nov.[4]	2	24 Sep./23 Sep.		5 Nov./4 Nov.
Oct.-Nov.[4]	2		15 Oct./14 Oct.	5 Nov./4 Nov.
Sep.-Oct.-Nov.[4]	3	24 Sep./23 Sep.	15 Oct./14 Oct.	5 Nov./4 Nov.
Chlorothalonil[5]	3	24 Sep./23 Sep.	15 Oct./14 Oct.	5 Nov./4 Nov.
Model-based[6]				
20% risk index	2/3	24 Sep./24 Sep.	15 Oct./17 Oct.	none/7 Nov.
40% risk index	2/0	24 Sep./none	15 Oct./none	none/none
Damage threshold - based[6, 7]				
Coho	1/1	none/24 Sep.	11 Oct./none	none/none
007	1/2	none/24 Sep.	11 Oct./none	none/1 Nov.
Independence	1/2	none/24 Sep.	9 Oct./none	none/1 Nov.

[1] Fungicide treatments initiated after the pre-treatment suppression of dollar spot on 10 September 2021 with fluazinam (Secure 4.17SC) at 0.7 kg a.i. per ha.

[2] Numbers listed to the left of the forward slash represent the number of applications in 2021 and to the right, the number of applications in 2022.

[3] Dates listed to the left of a forward slash represent the application date in 2021 and to the right, the applications date in 2022.

[4] Seven of the treatment timings (three single, three double, and one triple) were applied in September, October and/or November in both trial locations using a tank-mix of fluazinam and propiconazole (Banner MAXX 1.3ME) at 0.7 kg a.i. and 1.5 kg a.i. per ha, respectively.

[5] The eighth fungicide treatment consisted of chlorothalonil (Daconil Ultrex 82.5WG) at 15.3 kg a.i. per ha in September, October, and November in cultivars in the 2021 and 2022 trial locations.

[6] A tank-mix of fluazinam and propiconazole at 0.7 kg a.i. and 1.5 kg a.i. per ha, respectively, were applied for the risk index and damage-based timings for each cultivar in the 2021 and 2022 trial locations.

[7] Dollar spot damage threshold ≥ 314 mm^2 per 3-m^2.

The dollar spot infected area of each plot was measured every 1 to 7 days from September to November and May through the termination of trial 2 in 2022. Disease severity data was log_{10} transformed to correct for heteroscedasticity and used to calculate the area under the disease progress curve (AUDPC) for each disease outbreak. Then, continuous dollar spot outbreaks that shared similar ANOVA and statistical mean separations were combined to represent unique disease progress periods and used to calculate AUDPC for each period. Finally, each AUDPC period was analyzed using the mixed model in GLIMMIX, SAS version 9.4 (SAS Institute, Cary, NC) with fungicide treatment and cultivar as main effects. Significant main effects and interaction (fungicide treatment × cultivar) effects were retained in the final model. Means of main effects and interactions was separated using Fisher’s protected least significant difference at the 0.05 probability level. Disease data analysis is nearly completed for the 2021 trial location and in progress for the 2022 trial location.

For the molecular quantification of dollar spot, clippings were collected on 23 September 2021 for the pre-treatment population assessment and on 18 November 2021 at the end of the autumn-2021 treatments. Clippings were also collected on 17 May 2022 to assess the pathogen population just prior to dollar spot symptom development in the subsequent season; dollar spot developed on 21 May 2022. Clippings were collected using a Toro Greensmaster Flex 21 mower (The Toro Company, MN) equipped with a verti-cut reel set to cut 9-mm into the turf canopy. Plant tissues from each plot were grounded in liquid nitrogen with a mortar and pestle. A composite sample of 0.25 g of the ground tissue was used for DNA isolation using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. qPCR reactions were conducted following a previously established protocol (Groben et al 2020). Each sample was run with three technical replicates and each qPCR reaction included a positive DNA control isolated from a pure culture of *C. jacksonii* and a negative control of PCR grade water (Sigma-Aldrich, WY). A cycle threshold (Ct) value below 37 was considered positive for dollar spot (Groben et al 2020). The average Ct value for each collection date was analyzed separately using analysis of variance in R. The means of fungicide treatment was separated using Fisher's protected LSD test. Clippings were collected on 21 September 2022 for the pre-treatment population assessment and on 17 November 2022 at the end of the autumn-2022 treatments. Plant tissue is being processed and analyzed for the 2022 location of trial 2 and will be completed in 2023.

Trial 3 (objectives 2, 3, and 4): This trial was seeded in two locations: 8 September 2020 (2021 trial location) and 7 September 2021 (2022 trial location). Both areas were managed as a fairway turf at 0.9 cm on a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) in North Brunswick, NJ (40°28′ N, 74°25′ W). The plots in both trial locations were inoculated each spring to ensure uniform dollar spot colonization per the methods described for trial 2. Once dollar spot developed in the 2021 trial location chlorothalonil (Daconil Ultrex 82.5WG) was applied at 15.3 kg a.i. per ha to all plots on 8 and 31 July and 14 Aug. 2021 to suppress dollar spot symptoms prior to initiation of autumn fungicide treatments. Dollar spot symptoms developed when treatments were initiated on 24 Sep. 2021. A different fungicide program was used to suppress dollar spot prior to the initiation of autumn fungicide treatments in the 2022 trial location (see details below). Dollar spot symptoms developed when fungicide treatments were initiated on 23 Sep. 2022.

A 2 × 3 × 4 factorially arranged split-split plot design with 4 replications was used for this study. Antecedent inoculum load was the first factor arranged as the whole plot and had two levels: inoculated (1.34 g/m^2 of oat-infested inoculum on 10 September 2021 before initiation of autumn fungicide treatments) and non-inoculated. However, because no differences in the antecedent inoculum were observed in the 2021 location of this study, an alternate method to vary the antecedent inoculum load was used for the 2022 location. After inoculation of plots on 5 April 2022 (2022 location), two fungicide programs mimicking high- and low-budget dollar spot management were applied to the inoculum load whole plots. The high-budget program, that was expected to produce a lower inoculum load, rotated mefentrifluconazole at 0.7 kg a.i. ha^{-1} (Maxtima 3.34SC, 0.4 fl oz. per 1,000 ft^2) and fluazinam at 0.7 kg a.i. ha^{-1} every three weeks from 22 July to 2 Sep. 2022, resulting in three applications before the initiation of autumn fungicide timing treatments. The lower-budget program, expected to result in a higher inoculum load, applied chlorothalonil at 9.8 kg a.i. ha^{-1} as needed on a curative basis from 22 July to 9 Sep. 2022, which resulted in four applications at approximately a two-week application interval.

The second factor was cultivar, which had three sub-plot levels ('Coho', '007', and 'Independence' creeping bentgrass) representing a low to high range in disease susceptibility. The third factor was fungicide chemistry, which had four sub-sub-plot levels: a non-treated control, fluazinam applied at 0.7

kg a.i. ha^{-1}, propiconazole applied at 1.4 kg a.i. ha^{-1}, and a tank mix of fluazinam and propiconazole applied at 0.7 and 0.4 kg a.i. ha^{-1}, respectively. All fungicide chemistry levels were applied on 24 and 23 Sep., 15 and 14 Oct., and 5 and 4 Nov. in 2021 and 2022, respectively.

Disease data collection and analysis were the same method used for trial 2, except that the ANOVA structure used a 2 × 3 × 4 factorially arranged split-split plot design with 4 replications. The procedures for the molecular quantification of dollar spot in trial 3 were the same as those employed in trial 2. Autumn data and tissue analysis was completed for 2021 autumn and 2022 summer.

Data collection for the 2022 location in trial 3 is underway and will conclude in 2023.Clippings were collected on 21 September 2022 for the pre-treatment population assessment and on 17 November 2022 at the end of the autumn-2022 treatments. Plant tissue is being processed and analyzed for the 2022 location of trial 3 and will be completed in 2023. Data collection for the 2022 location in trial 3 has been completed and the analysis will be completed in 2023.

Results

Trial 2

Both the 20% and 40% risk index timings resulted in the same spray dates and numbers of applications as the Sep.-Oct. calendar-based timing in the 2021 trial (Table 1). However, the number and dates of applications for the model-based timings were unique in the 2022 trial (Table 1). The 20% risk index timing resulted in 3 applications, whereas the 40% risk index timing had no applications in 2022 (Table 1). Only one damage-based fungicide application was made on each of the three cultivars in the 2021 location, which was 4- to 6-d before the second calendar-based application on 15 October (Table 1). In the 2022 location, only one damaged-based application was made on Coho (24 Sep.), while two were applied on 007 and Independence (24 Sep. and 1 Nov.).

All fixed effects and the highest order interaction, cultivar × fungicide timing, affected autumn dollar spot disease response in 2021 measured as AUDPC and were included in the final mixed model (Table 2). Thus, differences among autumn-applied fungicide treatments are presented for each cultivar (Figures 1.1 and 1.2).

Table 2. Analysis of variance of area under disease progress curve (AUDPC) response from 24 Sep. to 24 Nov. 2021 as affected by 12 autumn-applied fungicide timings on three creeping bentgrass cultivars managed as fairway turf in North Brunswick NJ.

Source[a]	df	*F*	*P* of significant *F*
Cultivar (C)[b]	2	20.77	<.0001
Fungicide Timing (F)[c]	11	41.42	<.0001
C × F	22	3.69	<.0001

[a] Analyzed fixed effects (cultivar, autumn-applied fungicide treatments) and interactions using GLIMMIX in SAS.

[b] Coho, 007, Independence, creeping bentgrass.

[c] Twelve timings including eight calendar-based, two model-based, one damage -based and a non-treated control.

Regardless of the cultivar, the lowest disease severity during autumn 2021 was observed on fungicide timings that were applied on 24 Sep. 2021 (Figure 1.1). Moreover, the number of applications after 24 Sep. did not have a strong effect on autumn disease severity. Fungicide timings that initiated on and after 15 Oct. failed to reduced disease severity compared to the non-treated check on Coho and Independence, while a slight reduction was observed on 007 (Figure 1.1). The damage-based timings (one application in October) reduced disease severity compared to the non-treated check on all cultivars and, in fact, were among the timings with the lowest level of disease on Coho and Independence and nearly the lowest on 007 (Figure 1.1). Interestingly, the damaged-based timings only had one application made less than 7 days prior to the October calendar-based timing which did not perform as well as the damaged-based timings on 007 and Independence. This shows how important a few days can be with respect to dollar spot control.

No differences in autumn disease severity were observed among cultivars for timings that were initiated on 24 Sep. (Sep., Sep.-Oct., Sep.-Nov., Sep.-Oct.-Nov., 20% Risk index, 40% Risk index, and chlorothalonil) as well as the damage-based timing (Figure 1.2). However, Independence had more disease than Coho and 007 for calendar-based timings initiated on and after 15 Oct. (Oct., Nov., and Oct.-Nov.) and under the non-treated control condition. No difference in disease severity was observed between Coho and 007 for any fungicide timing (Figure 1.2).

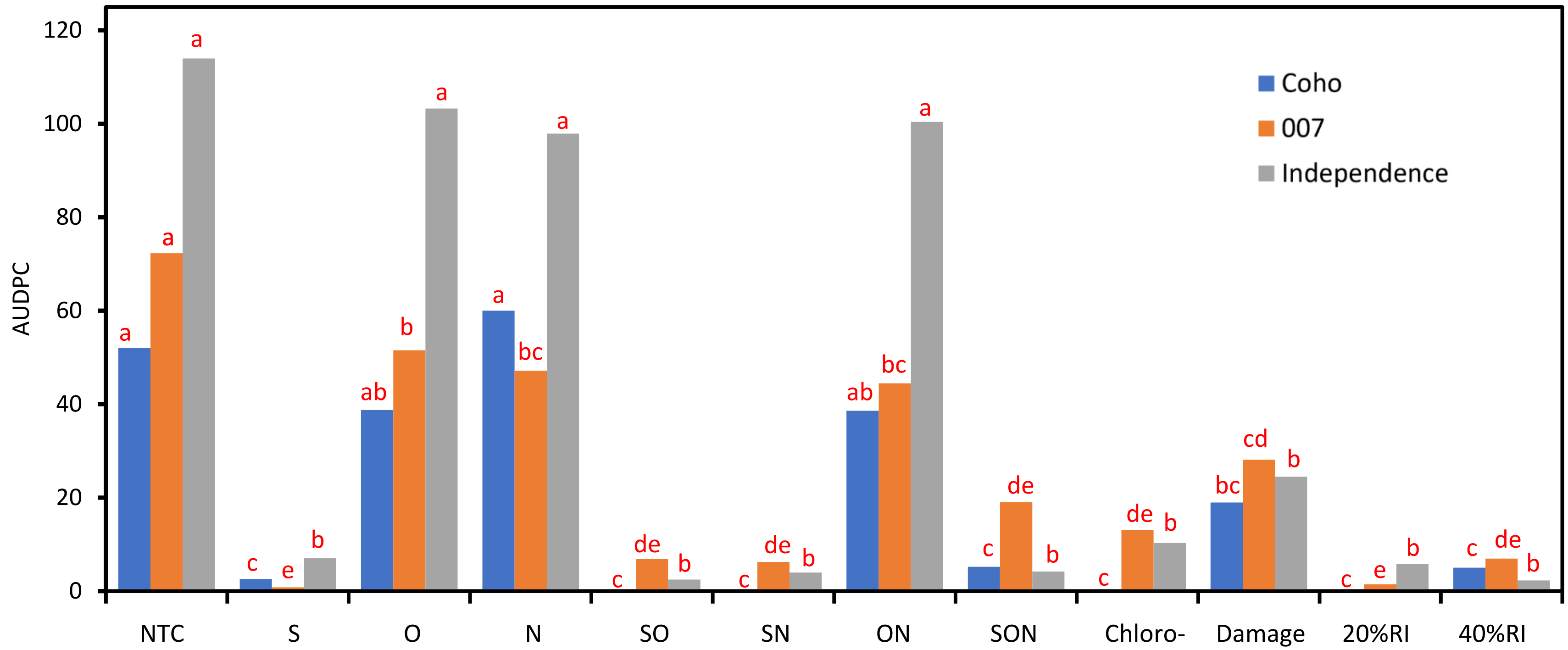

Figure 1.1 The area under disease progress curve (AUDPC) response from 24 Sep. to 24 Nov. 2021 as affected by autumn fungicide timing within a cultivar. Lower case letters indicate differences between fungicide timings within the same cultivar according to Fisher's protected LSD α=0.05. NTC: Non-treated control; S: September; O: October; N: November; SO: September + October; SN: September + November; ON: October + November; SON: September + October + November; Chloro-: Chlorothalonil; 20%RI: 20% risk index; 40%RI: 40% risk index; Damage: Dollar spot threshold ≥ 314 mm^2 per 3-m^2.

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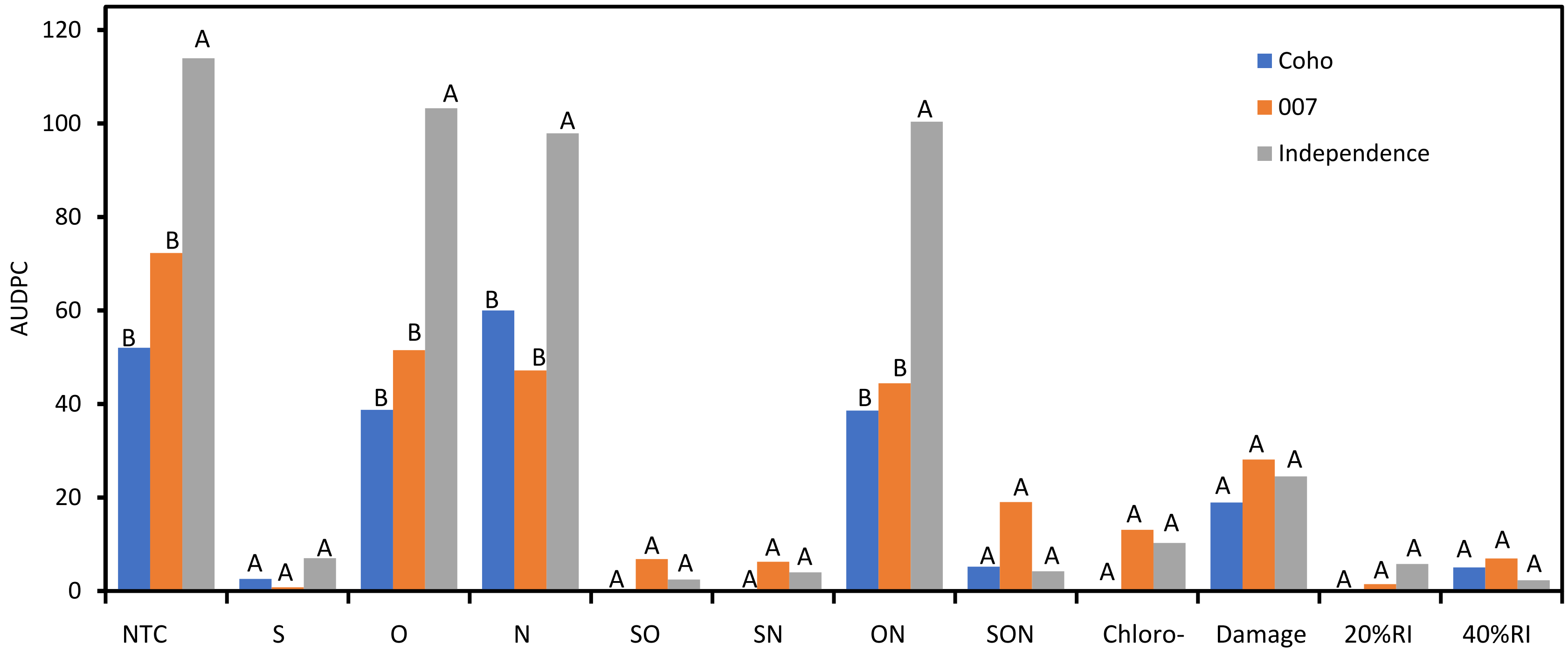


Figure 1.2. The area under disease progress curve (AUDPC) response from 24 Sep. to 24 Nov. 2021 as affected by cultivar within a given fungicide timing. Upper case letters indicate differences between cultivars within the same fungicide timing according to Fisher's protected LSD α=0.05. NTC: Non-treated control; S: September; O: October; N: November; SO: September + October; SN: September + November; ON: October + November; SON: September + October + November; Chloro-: Chlorothalonil; 20%RI: 20% risk index; 40%RI: 40% risk index; Damage: Dollar spot damage threshold ≥ 314 mm2 per 3-m^2.

The onset date for dollar spot during 2022 was affected by fungicide timing and cultivar main effects. Fungicide timing and cultivar did not interact (Table 3). The average disease onset date of each cultivar across 12 autumn fungicide timings during 2022 was: 26 May for Independence, 3 June for 007, and 17 June for Coho. Therefore, 007 and Coho delayed the disease onset date for approximately 1 and 3 weeks, respectively, compared to Independence. Few differences in disease onset date were observed among fungicide timings in spring 2022. Fluazinam + propiconazole applied on Sep.-Oct.-Nov., Sep.-Oct., and at the 40% risk index delayed onset by 6 to 10 days compared to chlorothalonil applied on Sep.-Oct.-Nov. (data not shown).

Table 3. Analysis of variance of the onset date for dollar spot in 2022 as affected by 12 fungicide timings in autumn 2021 applied to three creeping bentgrass cultivars managed as fairway turf in North Brunswick NJ.

Source[a]	df	*F*	*P* of significant *F*
Cultivar (C)[b]	2	128.4	<0.0001
Fungicide Timing (F)[c]	11	3.3	0.0149
C × F	22	1.8	0.49

[a] Analyzed fixed effects (cultivar, autumn-applied fungicide treatments) and interactions using GLIMMIX in SAS.

[b] Coho, 007, and Independence creeping bentgrass.

[c] Twelve timings including eight calendar-based, two model-based, one damage -based and a non-treated control.

Three unique disease progress periods were identified in trial 2 from 21 May to 15 July 2022. Disease onset was observed on Independence and 007 but not Coho from 21 May to 4 Jun (Table 4). No differences were observed among the 12 fungicide timings on Coho and 007 which had no and minor disease symptoms, respectively (Figure 2.1). Interestingly, a suppression of dollar spot symptoms was evident for most fungicide timings on Independence, except for the Nov. timing of fluazinam + propiconazole and the Sep.-Oct.-Nov. timing of chlorothalonil compared to the non-treated control. During this period of onset, Independence was the most diseased cultivar and differences between 007 and Coho were only observed for the Oct.-Nov. timing and for chlorothalonil applied at the Sep.-Oct.-Nov. timing (Figure 2.2).

Minor dollar spot symptom development was observed on Coho during 4 to 13 June 2022; whereas disease severity was moderate on 007 and greatest on Independence (Table 4). Fungicide timings that included a Sep. application tended to be among the treatments with the lowest disease severity, although the Oct.-Nov. timing was a notable exception and had the least disease during this period.

Disease symptoms were more advanced on all cultivars after 13 June 2022, yet dollar spot was far less developed on Coho than 007 or Independence; disease was most severe on Independence. Dollar spot suppression was observed for the fluazinam + propiconazole tank-mix applied at the Sep, Sep.-Oct., Sep.-Nov., Oct.-Nov., and Sep.-Oct.-Nov. timings, the 20% and 40% risk index action thresholds, and the damage-based threshold compared to non-treated control. It should be noted that the 20 and 40% risk index action threshold timings were applied on the same dates as the Sep.-Oct. timing and thus is not surprising that suppression of dollar spot was similar among these. Applying chlorothalonil at the Sep.-Oct.-Nov. timing did not suppress dollar spot compared to non-treated control. The individual Oct. and Nov. timings also had no effect on disease suppression compared to non-treated control.

Table 4. Analysis of variance of dollar spot severity calculated as area under disease progress curve (AUDPC) from 21 May to 4 June, 4 to 13 June, and 13 June to 15 July 2022 as affected by 12 fungicide timings in autumn 2021 on three creeping bentgrass cultivars managed as fairway turf in North Brunswick NJ.

ANOVA Source	AUDPC Periods		
	21 May to 4 June	4 June to 13 June	13 June to 15 July
	---------------------------------- probability of a significant *F* test --------------------------------		
Cultivar (C)	<0.0001	<0.0001	<0.0001
Fungicide (F)	0.0009	0.0086	<0.0001
C*F	0.0003	0.3658	0.3254
Cultivar	-------------------- AUDPC of Log_{10} transformed dollar spot infection area ------------------		
Coho	0.0 c	3.8 c	35.6 c
007	3.6 b	12.8 b	60.3 b
Independence	21.3 a	20.6 a	85.5 a
Fungicide[a]	-------------------- AUDPC of Log_{10} transformed dollar spot infection area ------------------		
Non-treated check	13.7 a	14.4 ab	77.4 ab
Chlorothalonil[b]	12.9 ab	13.9 abc	68.6 bc
Sep.	6.8 cde	11.9 abcde	58.4 cdef
Oct.	7.7 cde	13.6 abcd	70.9 abc
Nov.	11.0 abc	14.9 a	82.5 a
Sep.-Oct.	4.1 e	12.0 abcde	53.3 def
Sep.-Nov.	7.6 cde	10.6 cde	61.1 cde
Oct.-Nov.	8.5 bcde	9.0 e	48.1 ef
Sep.-Oct.-Nov.	5.1 de	12.2 abcde	46.5 f
20% risk index	5.9 de	10.4 de	49.4 ef
40% risk index	6.8 cde	11.9 bcde	45.4 f
Damage-area threshold	9.4 abcd	14.5 ab	64.1 cd

[a] Tank mix of fluazinam (Secure 4.17SC) and propiconazole (Banner MAXX 1.3MC) applied at 0.7 and 0.4 kg a.i. ha^{-1} (0.5 and 1.0 fl. oz. product per 1,000 sq. ft.), respectively, at the respective timing

[b] Chlorothalonil was applied as Daconil Ultrex 82.5WG at 13 kg a.i. ha^{-1} (5 oz. per 1,000 sq. ft).

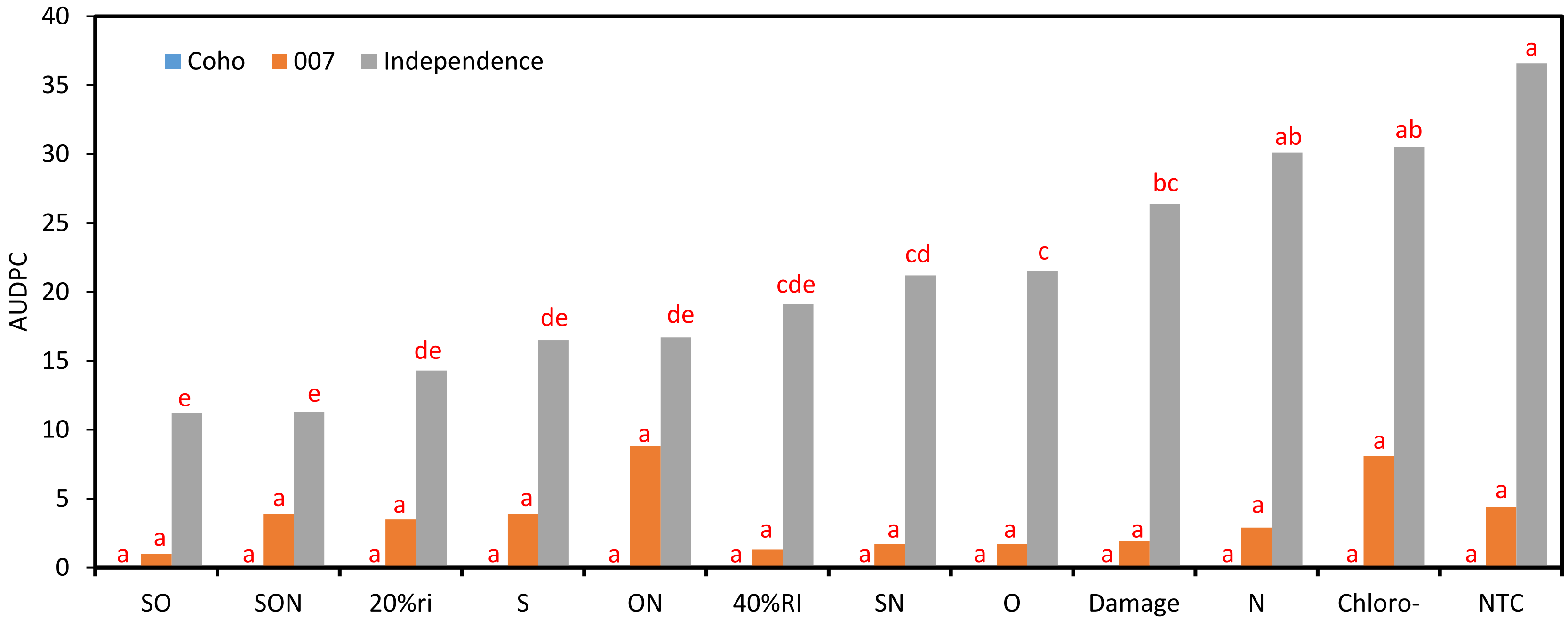


Figure 2.1. The area under disease progress curve (AUDPC) response from 21 May to 4 June 2022 to fungicide timing within a given cultivar. Lower-case letters indicate differences between fungicide timings within the same cultivar according to Fisher's protected LSD α=0.05. NTC: Non-treated control; S: September; O: October; N: November; SO: September + October; SN: September + November; ON: October + November; SON: September + October + November; Chloro-: Chlorothalonil; 20%RI: 20% risk index; 40%RI: 40% risk index; Damage: Dollar spot damage threshold ≥ 314 mm^2 per 3-m^2.

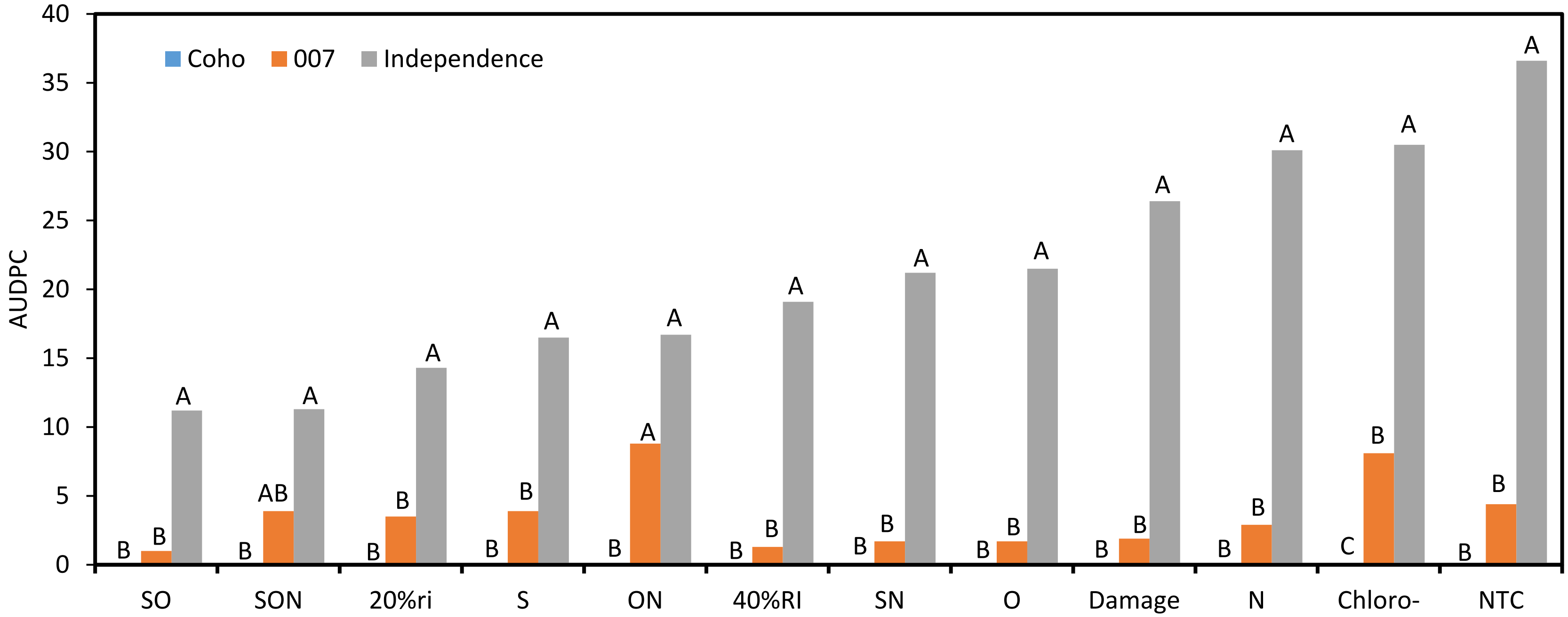


Figure 2.2. The area under disease progress curve (AUDPC) response from 21 May to 4 June 2022 to cultivar within a given fungicide timing. Upper-case letters indicate differences between cultivars within the same fungicide timing according to Fisher's protected LSD α=0.05. NTC: Non-treated control; S: September; O: October; N: November; SO: September + October; SN: September + November; ON: October + November; SON: September + October + November; Chloro-: Chlorothalonil; 20%RI: 20% risk index; 40%RI: 40% risk index; Damage: Dollar spot damage threshold ≥ 314 mm^2 per 3-m^2.

C. jacksonii inoculum load was lowest for Coho and no differences were detected between 007 and Independence in November 2021 (Table 5). Pearson correlations between AUDPC in 2022 and the *C. jacksonii* inoculum load on each cultivar in November 2021 indicated that the suppression of disease during 2022 was related to a lower pathogen inoculum load in the previous autumn (Figure 3).

Table 5. Analysis of variance of *C. Jacksonii* inoculum load sampled on 18 Nov. 2021 as affected by 12 fungicide timings applied in autumn 2021 on three creeping bentgrass cultivars managed as fairway turf in North Brunswick NJ.

Source[a]	df	*F*	*P* of significant *F*
Cultivar(C)[b]	2	14.2	<0.0001
Fungicide Timing (F)[c]	11	11.8	<0.0001
C × F	22	0.8	0.7165

[a] Analyzed fixed effects (cultivar, autumn-applied fungicide treatments) and interactions using GLIMMIX in SAS.

[b] Coho, 007, Independence, creeping bentgrass.

[c] Twelve timings including eight calendar-based, two model-based, one damage -based and a non-treated control.

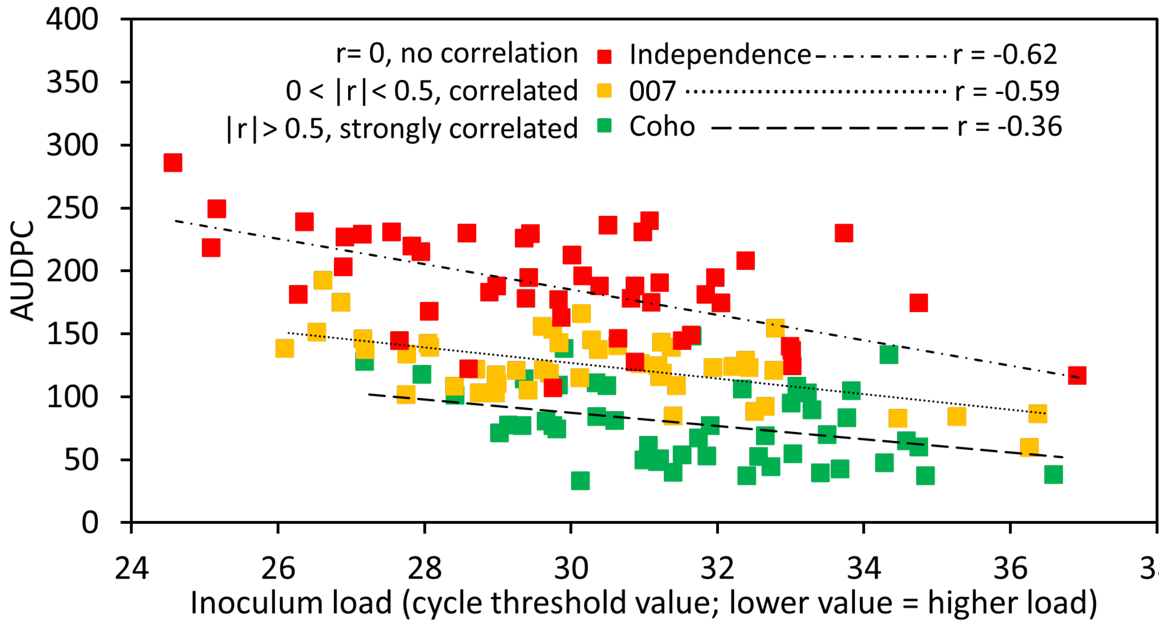


Figure 3. The Pearson correlation between *Clarireedia* spp. inoculum load (cycle threshold value, qPCR analysis Nov. 2021) and the AUDPC of three cultivars during 2022.

Trial 3

The analysis of variance of the area under disease progress curve (AUDPC) from 24 Sep. to 24 Nov. 2021 suggested that the inoculation factor (whole-plot level) had no effect on disease severity, whereas cultivar (sub-plot level), fungicide chemistry (sub-sub-plot level), and the cultivar × fungicide chemistry interaction significantly affected the disease response (Table 6). It is plausible that the existing pathogen population was well established during the growing season and was high enough that the inoculation applied on 11 September was not sufficient to notably increase disease severity. Accordingly, no differences were observed quantitatively on pathogen inoculum load based on qPCR analysis (sampled on 23 September, 18 November 2021, and 17 May 2022) between whole plots (data not shown).

Table 6. Dollar spot severity, area under disease progress curve (AUDPC) of $\log_{10}$ transformed data, as affected by two antecedent inoculum loads, three creeping bentgrass cultivars, and four fungicide chemistries arranged as a split-split plot design on turf managed as a fairway during autumn 2021 and 2022 in North Brunswick NJ.

Source[a]	df	*F*	*P* of significant *F*
AUDPC (24 Sep. to 24 Nov. 2021)			
Inoculation (I)[b]	1	0.24	0.6560
Cultivar (C)[c]	2	64.40	<0.0001
I × C	2	0.13	0.8771
Fungicide (F)[d]	3	283.77	<0.0001
I × F	3	0.49	0.6900
C × F	6	3.48	0.0056
I × C × F	6	1.60	0.1652
AUDPC (19 May to 27 June 2022)			
I[b]	1	0.08	0.8003
C[c]	2	297.26	<0.0001
I × C	2	1.04	0.3834
F[d]	3	53.05	<0.0001
I × F	3	0.39	0.7585
C × F	6	2.36	0.0424
I × C × F	6	1.01	0.4277
AUDPC (27 Jun3 to 29 July 2022)			
I[b]	1	0.00	0.9697
C[c]	2	90.84	<0.0001
I × C	2	0.57	0.5786
F[d]	3	63.43	<0.0001
I × F	3	0.33	0.8016
C × F	6	1.44	0.2163
I × C × F	6	0.89	0.5106

[a] Analyzed fixed effects and interactions using GLIMMIX in SAS.

[b] High antecedent inoculum load developed by inoculating at 1.34 g/m^2 on 10 September 2021.

[c] Coho, 007, Independence, creeping bentgrass.

[d] Fluazinam at 0.7 kg a.i. ha^{-1}, propiconazole at 1.4 kg a.i. ha^{-1}, a tank mix of fluazinam and propiconazole at 0.7 and 0.4 kg a.i. ha^{-1}, respectively, and a non-treated check which received no fungicide application during the treatment period

As expected, disease severity from 24 Sep. to 24 Nov. 2021 was greatest for the non-treated control for all cultivars (Figure 4, red letters). Propiconazole + fluazinam and fluazinam provided the best disease control within each cultivar while propiconazole was not as effective (Figure 4, red letters).

Disease response from 24 Sep. to 24 Nov. 2021 was least severe on Coho than 007 or Independence when these cultivars were treated with propiconazole, fluazinam, or no fungicide; however, disease severity was similar among Coho and 007 and both had significantly less disease than Independence when propiconazole + fluazinam was applied (Figure 4, black letters).

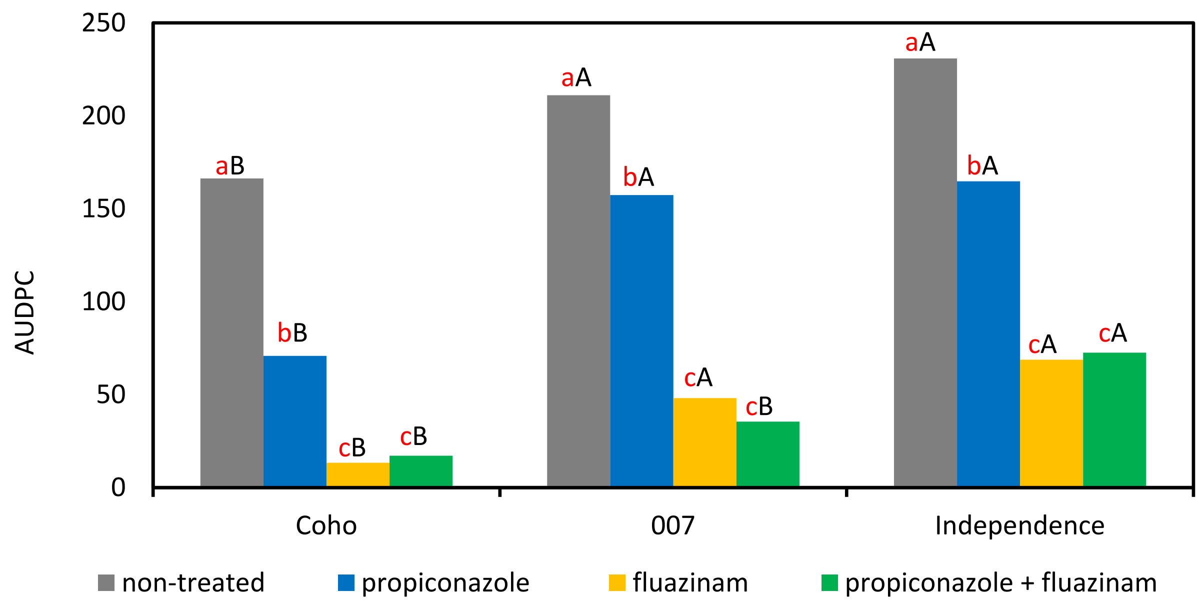


Figure 4. The area under disease progress curve (AUDPC) response 24 Sep. to 24 Nov. 2021 as affected by the cultivar × fungicide chemistries interaction. Lower case (red) letters indicate differences between fungicide chemistry levels within the same cultivar; Upper case (black) letters indicate differences between cultivars within the same fungicide chemistry treatment according to Fisher's protected LSD $\alpha=0.05$. Fungicide treatments were fluazinam at 0.7 kg a.i. ha^{-1}, propiconazole at 1.4 kg a.i. ha^{-1}, a tank mix of fluazinam and propiconazole at 0.7 and 0.4 kg a.i. ha^{-1}, respectively, and a non-treated check which received no fungicide application during the treatment period.

Two separate disease response periods were identified during 2022. Disease onset and early-stage symptom development occurred from 19 May to 27 June while more advanced symptom development was observed from 27 June to 29 July 2022 (Table 6). During both of these periods, dollar spot was least severe on Coho and 007 and both had less disease than Independence regardless of the fungicide chemistry level (Figures 5 and 6).

Fluazinam and fluazinam + propiconazole were the fungicide chemistry levels that provided the best disease suppression regardless of cultivar from 19 May to 27 June (Figure 5) and 27 June to 29 July (Figure 7) 2022. Propiconazole alone was not as good as other the fungicide chemistry levels but did consistently suppress dollar spot symptoms compared to the non-treated control on 007 and Independence from 19 May to 27 June (Figure 5) and 27 June to 29 July (Figure 7) 2022. Propiconazole alone did not suppress disease compared to the non-treated control on Coho from 19 May to 27 June 2022 (Figure 5) but did suppress disease symptoms on Coho from 27 June to 29 July 2022 (Figure 7).

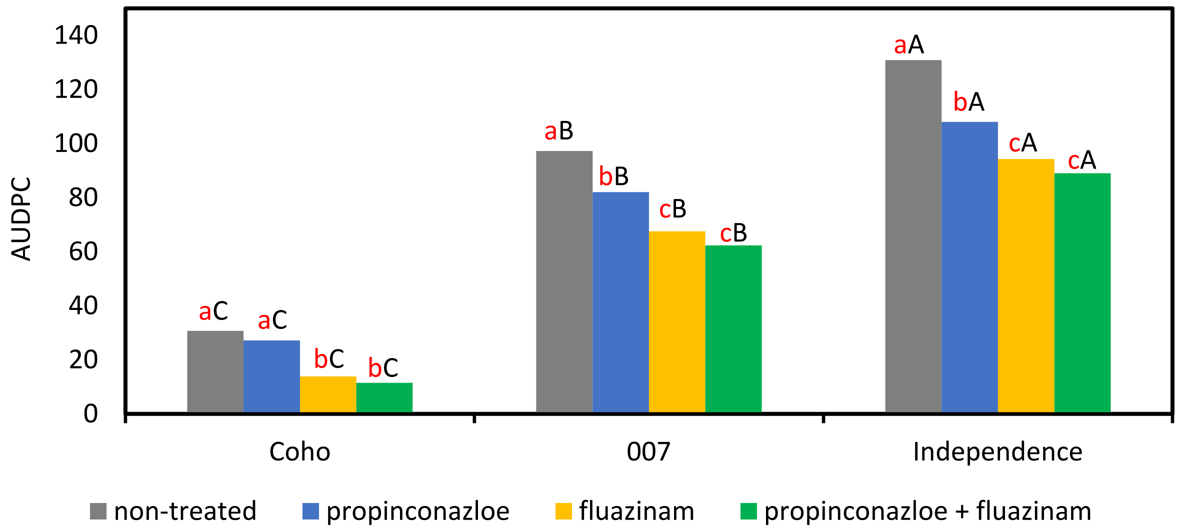


Figure 5. The analysis of variance of the area under disease progress curve (AUDPC) response from 19 May to 27 June 2022 as affected by the cultivar × fungicide chemistry interaction. Lower case (red) letters indicate differences between fungicide chemistry levels within the same cultivar. Upper case (black) letters indicate differences between cultivars within the same fungicide chemistry treatment. according to Fisher's protected LSD α=0.05. Fungicide treatments were fluazinam at 0.7 kg a.i. ha^{-1}, propiconazole at 1.4 kg a.i. ha^{-1}, a tank mix of fluazinam and propiconazole at 0.7 and 0.4 kg a.i. ha^{-1}, respectively, and a non-treated check which received no fungicide application during the treatment period

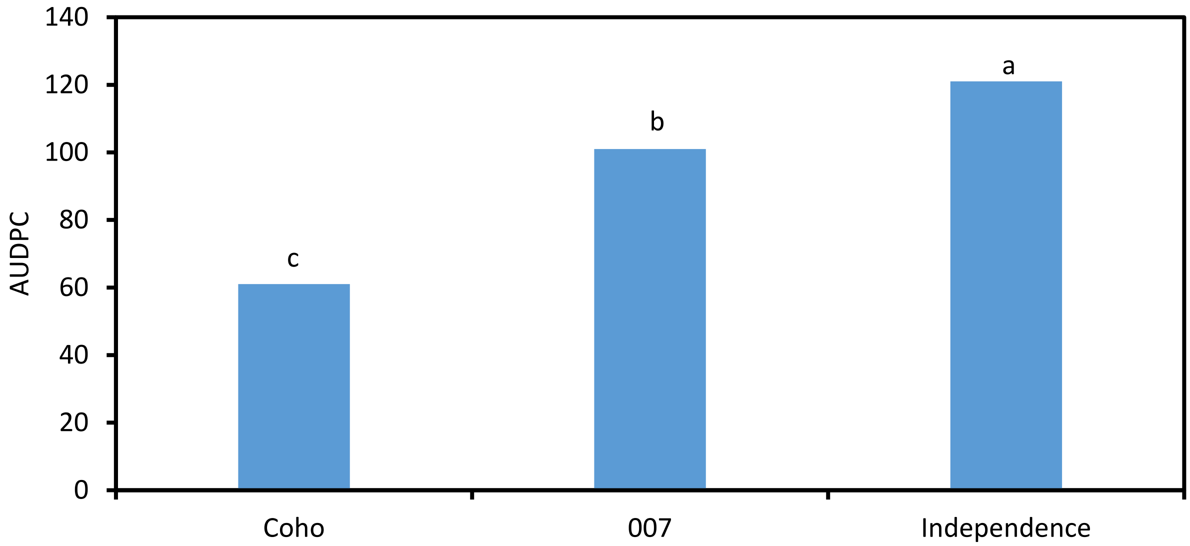


Figure 6. The area under disease progress curve (AUDPC) response from 27 June to 15 July 2022 as affected by the cultivar main effect. Lower case (black) letters indicate differences between cultivars according to Fisher's protected LSD α=0.05.

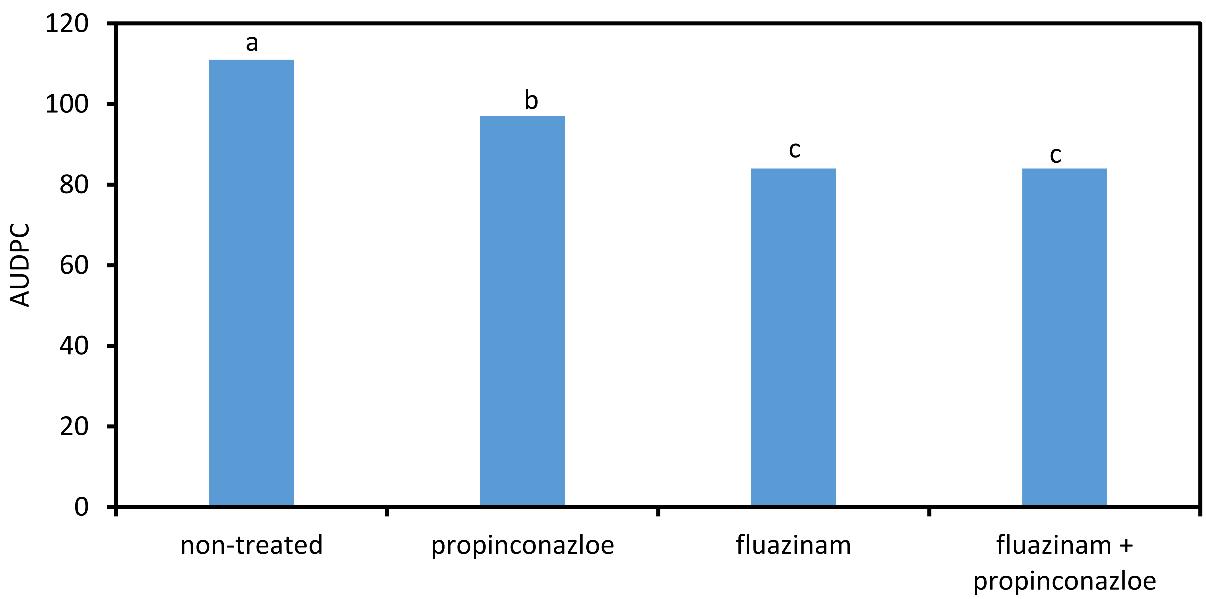


Figure 7. The area under disease progress curve (AUDPC) response from 27 June to 15 July 2022 as affected by fungicide chemistry main effect. Different lower-case letters indicate differences between fungicide chemistry levels according to Fisher's protected LSD α=0.05. Fungicide treatments were fluazinam at 0.7 kg a.i. ha^{-1}, propiconazole at 1.4 kg a.i. ha^{-1}, a tank mix of fluazinam and propiconazole at 0.7 and 0.4 kg a.i. ha^{-1}, respectively, and a non-treated check which received no fungicide application during the treatment period

Future Work

- The analysis of 2022 autumn disease response for the second locations of trials 2 and 3 is in progress.
- Disease data and foliar tissue will be collected for the second locations of trials 2 and 3 during the spring and early summer of 2023. This is expected to conclude trial 2.
- Due to the change in methodology to create two antecedent inoculum loads in trial 3, a third year (autumn-summer study cycle) would be needed to conclude trial 3. The third year would be conducted from autumn 2023 through summer 2024 using the same "inoculum method" as described for the 2022 location.
- Clipping samples were collected from trial 2 in July, September and November 2022 and will be analyzed to quantify the pathogen inoculum load to correlate with dollar spot disease symptom development. Similarly, clipping samples were collected from trial 3 (2021 location) in July 2022 and will be analyzed. Clipping samples were collected from trial 3 (2022 location) in July, August and September 2022 and will be analyzed.

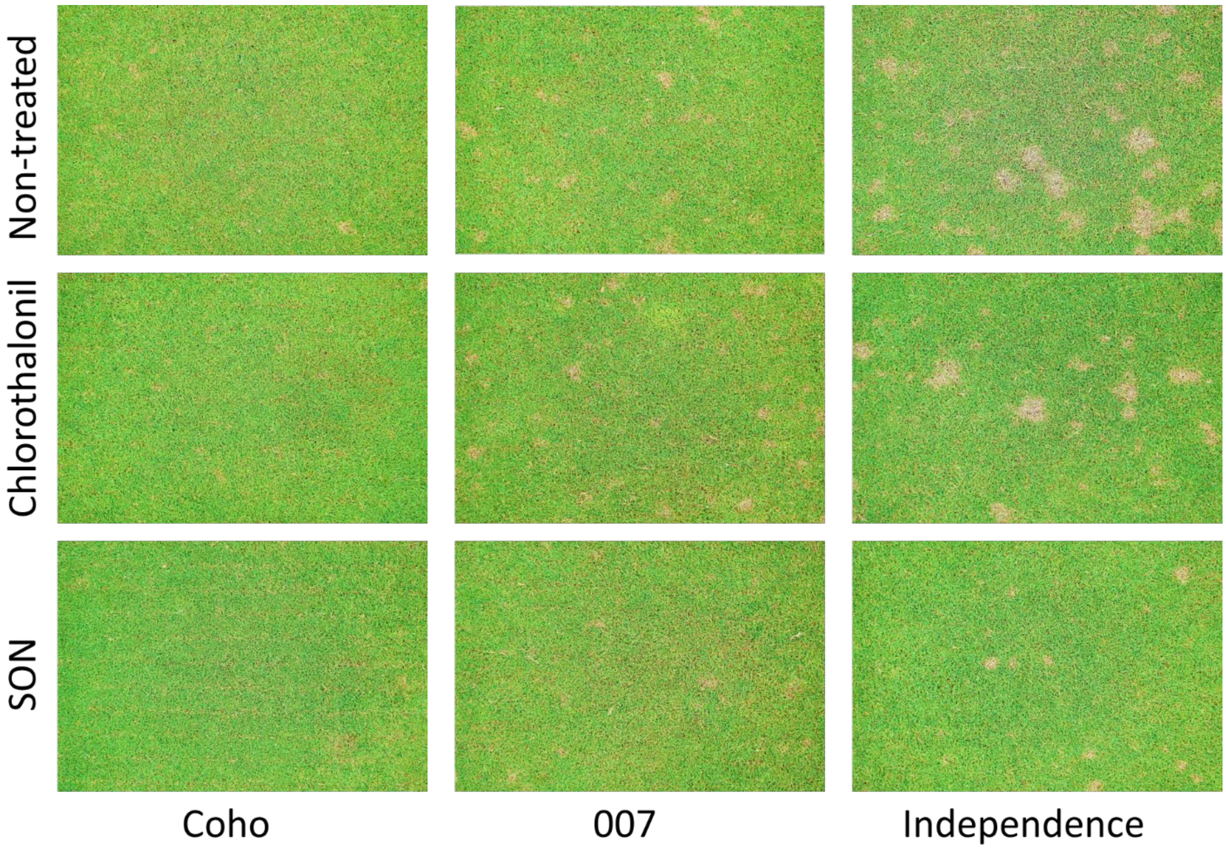


Figure 8. Disease severity on 12 Aug. 2022 for plots of Coho, 007, and Independence creeping bentgrass treated with either fluazinam + propiconazole (SON) or chlorothalonil in September, October, and November 2021 as well as the non-treated control.

USGA ID#: 2021-08-732

Title: Reduced Fungicide Usage Through Application Optimization

Project Leader: Bruce Branham
Affiliation: University of Illinois

Objectives: Determine if systemic fungicide efficacy can be increased through the use of activator adjuvants, which may enhance fungicide absorption into or coverage of turfgrass foliage. Evaluate dollar spot, *Clarireedia jacksonii*, (formerly *Sclerotinia homoeocarpa*) control with fungicide rates reduced by 33% to determine whether fungicide efficacy can be improved.

Start Date: January 2021
Project Duration: 2 years
Total Funding: $75,465

Summary Points:
- Of the four fungicides evaluated, only one, Velista (penthipyrad), showed a significant benefit from the addition of an adjuvant.
- Addition of urea ammonium nitrate (UAN) solution appeared to improve performance of each fungicide tested, although the effects were not dramatic
- Within trial disease variability obscures potentially positive results.
- A framework for understanding fungicide uptake and activity kinetics is needed to advance this area of research.

INTRODUCTION

Fungicides are an integral part of modern golf course turf management, particularly in the cool, humid region of the United States. Diseases can reduce turf quality at any time in the growing season and fungicides are applied frequently, and unlike other pest problems, throughout the entire calendar year. In the cool regions of the US, the last fungicide application is often made in November to prevent snow mold.

A key factor in the performance of systemic pesticides is their ability to move into the plant and translocate to the site of action. Pesticides must enter the plant by passing through a layer of epicuticular waxes, which are derived from very long-chained fatty acids, and present a significant barrier to pesticide penetration. Waxes are lipophilic substances and fungicide vary significantly in their lipophobicity. Thus some fungicides may diffuse through the epicuticular waxes and get inside the plant while other fungicides may diffuse into this wax layer, but remain there since they are highly lipophilic themselves. Even though the fungicide has been absorbed by the plant, it does not reach the site of activity within the plant and therefore does not provide fungicidal control. Laboratory evaluations of pesticide absorption and activity are complex, difficult, and don’t always align with activity observed in the field.

The increase in pesticide absorption with an appropriate adjuvant can be striking. For example, Green (1996) showed that inhibition of giant foxtail growth when treated with rimsulfuron varied from 13 % when the adjuvant concentration was 0.004 % to 94 % when the adjuvant concentration was increased to 0.1%. This wide variation in activity indicates how important adjuvant selection can be. Pesticide formulations are very complex mixtures and certainly some adjuvants are included. However, pesticide labels often call for additional adjuvants added at the time of application to improve activity.

MATERIALS and METHODS

These trials were conducted on the University of Illinois Golf Practice Facility on the University of Illinois campus. The site was a fairway established to L-93 creeping bentgrass and maintained at 1.2 cm height of cut. Fairway maintaince activities were typical for a golf course fairway in the cool, humid portion of the US.

Four separate fungicide trials were conducted. In each trial, the fungicide was applied at 2/3 of the full label rate of application combined with one of the five adjuvants shown in Table 1. The addition of urea ammonium nitrate (UAN) was included as a second factor in the stud, that is, each adjuvant was applied with or without UAN. Every trial included an untreated control and the full label rate of the fungicide evaluated, i.e. fungicide control. The fungicides evaluated were boscalid (Emerald), propiconazole (Banner), penthiopyrad (Velista), and metconazole (Tourney). Beginning on June 6th, initial applications of the four fungicide treatment combinations were applied. Boscalid and propiconazole were reapplied on 4-week intervals with the last applications applied on August 30th (4 applications total) while penthiopyrad and metconazole were reapplied every 3 weeks for a total of 5 applications. Dollar spot severity on Tourney treated plots was so severe by mid-July that a rescue application of Secure at 22 oz/A was applied on July 21st.

Dollar spot ratings were collected every two weeks beginning in July through September. Scalping ratings were also collected when scalping appeared to be treatment related.

RESULTS and DISCUSSION

Results in 2022 were disappointing (Tables 1 & 2). The adjuvant combinations tested did not provide a clear, significant benefit in dollar spot control. The comparison in these trials is to the control provided by the full label of the fungicide and part of the problem with this approach is that the full label rate usually gives good to excellent control. Matching that control based upon the statistical analysis was achieved by several adjuvant/UAN treatments, but there is no clear sign that any are superior to the full label rate. Compounding the analysis of the data, for Emerald, Tourney, and Velista, the 2/3 label rate performed equally well as the full label rate on all ratings dates (Table 1 and data not shown). Thus achieving meaningful separation of performance did not occur.

The data can also be analyzed as a factorial treatment design to better determine if there was any significance from these treatments. The results of the analysis for penthiopyrad (Velista) are shown in table 2. Adjuvant effects are better observed in the original data analysis, but the effect of UAN is more readily discernible from the factorial analysis. There were several instances where UAN significantly increased dollar spot control. Most apparent was with Velista and Tourney (data not shown) where the was a significant reduction in dollar spot on two separate rating dates due to UAN. This could be a nitrogen effect, as nitrogen fertilization is known to decrease dollar spot disease. However, the rates of nitrogen applied with UAN are quite low, 0.08 lbs N/M per application. Banner and Emerald received monthly applications, while Tourney and Velista received applications every 3 weeks. Since Tourney and Velista were the two fungicides that had two dates of significant UAN effect, and also received the equivalent of 0.1 lbs N/M per month, perhaps that was enough added nitrogen to affect dollar spot severity. Since most golf turf managers apply some form on nitrogen with regular fungicide applications, substituting UAN for other more costly forms of nitrogen may be an economically and agronomically sound strategy.

Finally, the combination of Velista +DynAmic + UAN provided dollar spot control as good as the full label rate of Velista on each rating date in 2022. The funding for these evaluations expires at the end of this year; however, we intend to look more closely at the effects of UAN and DyneAmic on the performance of Velista and Tourney in 2023.

Table 1. Dollar spot control, as a percent of plot coverage, from Velista as affected by various adjuvants with or without urea ammonium nitrate.

Trmt #	Treatment	Rate (oz prod/A)	7/6	7/20	8/4	8/19	9/1	9/23
1	Control		4.3 a	26	53 a	23 a	56 a	22 b
2	Velista	13	0.0 b	12	9 bc	1 b	9 b	64 a
3	Velista	8.7	1.5 b	14	12 bc	1 b	14 b	59 a
4	Velista + UAN	8.7 + 2.5 %	0.3 b	11	4 c	0 b	6 b	60 a
5	Velista + DyneAmic	8.7 +0.25 %	0.3 b	10	17 bc	0 b	14 b	65 a
6	Velista + Induce	8.7 + 0.5 %	0.8 b	14	28 b	3 b	14 b	73 a
7	Velista +MSO	8.7 + 0.5%	0.0 b	12	15 bc	1 b	13 b	75 a
8	Velista + Agri-Dex	8.7 + 0.75%	0.5 b	10	14 bc	0 b	14 b	74 a
9	Velista + Kinetic	8.7 + 0.125%	0.0 b	12	20 bc	2 b	14 b	73 a
10	Velista + DyneAmic + UAN	8.7 + 0.25 % +2.5 %	0.5 b	15	9 bc	0 b	8 b	63 a
11	Velista + Induce + UAN	8.7 + 0.5 % + 2.5 %	0.0 b	9	11 bc	0 b	2 b	64 a
12	Velista +MSO + UAN	8.7 + 0.5% + 2.5 %	0.3 b	10	8 bc	0 b	8 b	73 a
13	Velista + Agri-Dex + UAN	8.7 + 0.75% + 2.5 %	0.0 b	15	14 bc	1 b	3 b	66 a
14	Velista + Kinetic + UAN	8.7 + 0.125 + 2.5 %	0.5 b	15	19 bc	5 b	8 b	69 a
		LSD P=0.05	2.0	ns	21	12	15	21

Table 2. Probability of significance of main effects of adjuvants and UAN and their interactions on dollar spot control by Velista.

Factor main effects	7/6	7/20	8/4	8/19	9/1	9/23
Rep	0.1013	0.9737	0.6543	0.33	0.2790	0.8751
Adj	0.5895	0.9937	0.4651	0.3973	0.9381	0.3271
UAN	0.3181	0.8497	0.0933†	0.9670	0.0002	0.4376
Adj x UAN	0.2871	0.7889	0.8531	0.6759	0.8601	0.9943

† Significant at the 0.1 level of probability

USGA ID#: 2020-16-721

Title: Economic impact of take-all root rot on bermudagrass putting green management

Project Leader: Young-Ki Jo
Affiliation: Texas A&M University

Objectives: The objectives of the project is to develop a diagnostic protocol for *Gaeumannomyces* species associated with take-all root rot in bermudagrass and to remediate economic losses from the disease in bermudagrass putting greens.

Start Date: 2020
Project Duration: 3 years
Total Funding: $80,296

Summary Points:

- Set up the protocol of *Gaeumannomyces* species isolation from bermudagrass
- Set up the protocol of DNA-based identification of *Gaeumannomyces* species
- Set up the pathogenicity assay to determine virulence of *Gaeumannomyces* species
- Set up the rapid assay to determine virulence of *Gaeumannomyces* species

Summary Text:

Collection and isolation of *Gaeumannomyces* species from bermudagrass putting greens. The essential element for our program is to identify Texas golf courses with take-all root rot problems in their bermudagrass putting greens. Golf course superintendents were solicited and invited as collaborators. Five golf courses in Texas were initially selected. Superintendents of these golf courses expressed a committed interest in developing a new management program for take-all root rot in bermudagrass putting greens. Bermudagrass samples were collected from these collaborated golf courses. Infected stolons were surface-sterilized and plated on newly developed selective medium, potato dextrose agar (PDA) amended with streptomycin sulfate, mefenoxam, flutolanil, and iprodione. Plates were incubated at 25°C and were monitored for hyphae that curled back at the edges of fungal colonies, one of the typical cultural characteristics of *Gaeumannomyces* species. Hyphal tips were then transferred to PDA for isolation. Long-term storage of each isolate was achieved by keeping mycelial agar plugs in a 4-ml clear glass screw cap vial containing 1.5 ml of sterile distilled water. Vials were sealed with parafilm and kept in the dark at ambient temperature.

Development of molecular diagnosis of *Gaeumannomyces* species. Genomic DNA was extracted from each *Gaeumannomyces* isolate for the DNA-based diagnosis. Each isolate was grown on PDA at 25°C until petri dishes were entirely colonized. DNA isolation from harvested mycelium was conducted using ZYMO DNA Miniprep Kit. Internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using PCR with the previously-developed ITS1/ITS4 primer set. PCR was performed using Thermo Scientific Phire Plant Direct PCR Master Mix Kit. PCR amplicons were sent to Eton Biosciences for sequencing. In our initial screening, we found dominant isolates were determined as *Gaeumannomyces graminis* and *G. arxii.*

Establishment of in-planta assay for virulence of *Gaeumannomyces* species. For evaluating virulence of *Gaeumannomyces* species, the bermudagrass seedling pathogenicity assay has been developed. Bermudagrass seeds were surface sterilized in 1.2% NaClO for 10 minutes and rinsed 10 times with sterile distilled water. Seeds were pre-germinated in a petri dish containing two 7.5-cm filter papers moistened with 3 ml of sterile distilled water. 66-ml plastic cone-tainers were subsequently filled with sterile moistened vermiculite. At the depth of 3 cm from the top of each cone-tainer, five plugs (5-mm diameter) of actively-growing mycelium from each isolate were placed. Germinating seeds were added at the top of this layer, which were then covered with a final layer of vermiculite to fill to capacity. Prepared units were placed at a constant temperature of 25°C with a photoperiod of 16 h. All cone-tainers were watered daily to soil field capacity for the first week of incubation and once every other day thereafter. In 3 weeks after inoculation, take-all root rot symptoms could be observed. Significant differences of plant phenotypes such as shoot height and root length were noticed between inoculated plants with *Gaeumannomyces* species and uninoculated control plants (Fig.1).

Establishment of a rapid assay for virulence of *Gaeumannomyces* species.
Alongside the pathogenicity assay, a fast-screening method for pathogen virulence was developed. Each *Gaeumannomyces* isolate was grown on a PDA Petri dish amended with 100 µg/mL ampicillin to mitigate bacterial infection. Three Petri dishes were plated per isolate and one set of three PDA Petri dishes was kept without fungus as a control. These were incubated in darkness at 25°C, and the fungus was allowed to grow as normal. One week after the fungal isolates were plated, the PDA medium was carefully flipped into another dish using sterilized scalpels, so that the mycelium would be face down in the Petri dish. Ten germinated bermudagrass seeds were placed on the top of the medium. The Petri dishes were sealed with parafilm and placed in 12-h light-cycle incubation at 25°C for 7 days. The number of seedlings that survived and seedling height were recorded 4 days and 7 days post plating. This rapid assay provided similar results in virulence of *Gaeumannomyces* isolates (Fig. 2) compared with the pathogenicity assay.

Survey for golf course superintendents about bermudagrass putting management. We identified 4 golf course superintendents in Texas for collecting baseline information about take-all root rot (TARR).

Summary of TARR knowledge and experience.

- 2/4 have observed bermudagrass density loss on greens during fall and winter
- 3/4 are familiar with TARR symptoms
- 4/4 know TARR is associated with fungal pathogens
- 2/4 believe TARR negatively impacts green quality; and 2/4 are neutral
- 4/4 have used preventative fungicide applications to manage TARR
- 2/4 are somewhat satisfied with current TARR management programs
- 1/4 is extremely dissatisfied with current TARR management plans
- 4/4 are willing to send samples to the lab and collaborate with our project

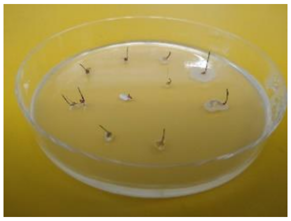
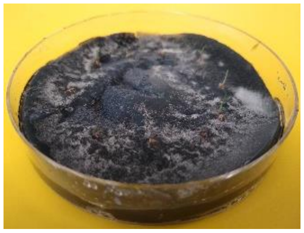
Future approaches and expectations. Upon the confirmation of infection by *Gaeumannomyces* species and their pathogenicity, we will develop and implement a more integrated management approach for golf course superintendents. Based on accurate diagnostics and detection of aggressive *Gaeumannomyces* species, proper management practices of take-all root rot will be able to be incorporated into current bermudagrass putting green maintenance.

We have experienced the difficulty in travels, field site visits and in-person meetings due to the Covid-19 pandemic in 2020 and 2021. In the last year (2023) of this project, we will invite at least 20 Texas golf course superintendents to participate in the survey and the implementation of an improved management program targeted for take-all root in bermudagrass putting greens. Turf samples will be collected from these golf courses. Causal *Gaeumannomyces* species will be isolated and identified. Also, their pathogenicity will be determined using the diagnostic methods developed in this study. The survey for Texas golf courses will measure economic impact of take-all root rot in the golf course management. Information we are interested in relevant to bermudagrass putting green management includes the rates and frequency of chemical applications (fertilizers, herbicides, fungicides, and insecticides) and labor cost for turf maintenance (mowing, irrigation, topdressing, and aeration). Finally, we will estimate potential economic impact of take-all root rot and its management on golf courses.

Figure 1. Pathogenicity of *Gaeumannomyces* isolates with bermudagrass seedlings. Uninoculated control plants are compared with *G. graminis* isolates (AM1 and BJN3) and one *G. arxii* (FZ3) isolate.

Gaeumannomyces isolates	Shoots	Roots
Control		
AM1		
BJN3		
FZ3		

Figure 2. Rapid assay of virulence of *Gaeumannomyces* isolates with bermudagrass seedlings. Uninoculated control plants are compared with *G. graminis* isolates (AM1 and BJN3) and one *G. arxii* (FZ3) isolate.

Gaeumannomyces isolates	Seedlings on a plate	Survivability
Control		8.7%
AM1		3.0%
BJN13		0.3%
FZ3		6.0%

USGA ID#: 2021-12-736

Title: Preservation of SDHI nematicide chemistry on golf turf

Project Leader: William T. Crow
Affiliation: University of Florida

Objectives: The objective of this research is to determine the long-term effects of applications of SDHI nematicide to turf, with particular regard to potential enhanced degradation and nematode resistance.

Start Date: 2021
Duration: 3 years
Total Funding: $107,604

Summary Points:

- Enhanced microbial degradation of fluopyram does not appear to be the cause of Indemnify failure.
- Nematode populations repeatedly exposed to fluopyram exhibit reduced sensitivity to the nematicide, indicating that resistance is the likely cause of observed Indemnify failure.

Summary Text:

Bermudagrass turf field plots were maintained at the UF Plant Science Research Unit in Citra FL where 5 plots each were treated with Indemnify 4 times per year for 4 years, 4 times per year for 1 year, or were untreated. Additionally, commercial golf course sites were identified where Indemnify had been applied repeatedly for a number of years and where it had never been used. Soil and nematodes from these plots and sites were used in this research.

Enhanced degradation:

Intact 2-inch-diameter and 6-inch-deep turf profiles were removed from the plots and fields and placed into a greenhouse for bioassay. The turf from the profiles was treated with either ½X, 1X, or 2X the maximum labeled rate of Indemnify, or left untreated, and maintained for time intervals of 1, 2, or 6 moths. After the specified time interval the turf was removed from the profiles, mixed and placed into small clay pots. Then tomato plants were transplanted into the pots and the soil was inoculated with southern root-knot nematode (*Meloidogyne incognita*), a species that was not already present in the turf soil but will infect tomato. After 6 weeks the tomato plants will be removed and the number of galls and egg masses on roots were counted. There were 5 replications of every treatment. If enhanced microbial degradation was occurring there would be greater nematode infection from the Indemnify when applied to soil originating from plots or locations where Indemnify had been used repeatedly than when applied to Indemnify 'virgin' soil.

Progress to-date – These experiments have been completed. The results indicate that no enhanced degradation of fluopyram was occurring.

Resistance:

Root-knot nematodes were collected from the small plots and fields described above. At least 25 juvenile nematodes from each plot or field were placed into microwells containing solutions of 0, 8, or 16 ppm of fluopyram. Nematode activity was observed after 1, 24, and 72 hours of exposure to determine how the nematodes were effected by fluopyram. If nematodes were straight and rigid they were considered impacted by the fluopyram, if they were moving or relaxed they were considered unaffected. If nematodes originating from plots and fields with a history of fluopyram use were less affected that nematodes originating from Indemnify 'virgin' locations it indicates development of resistance to fluopyram.

We attempted to conduct the above experiment on sting nematode but had a difficult time getting enough sting nematodes from the small plots and several of the field sites to conduct the experiment properly. Therefore, we collected sting nematode populations from 4 locations where Indemnify had been applied multiple times per year and the golf courses were not getting the good results they had at first, and from 4 Indemnify 'virgin' locations. We inoculated these sting nematodes onto bermudagrass in the greenhouse and treating the ones with a Indemnify history with Indemnify every 3 months. Once we have enough Indemnify exposed nematodes we will conduct the experiment described above with these 8 nematode populations.

Progress to-date – The root-knot nematode experiment is completed. The results indicate that root-knot nematodes coming from turf with a history of fluopyram use are less sensitive to fluopyram than the nematodes coming from Indemnify 'virgin' locations. This indicates the development of resistance to fluopyram in these nematodes. The sting nematode experiment will be conducted in 2023.

Metabarcoding:

Soil was collected from the small plot and golf course sites described above. Microbial and fungal DNA was be extracted from soil, amplified, and sequenced. The data indicated that soil microbial composition was not greatly impacted by fluopyram use

Progress to-date – Completed.

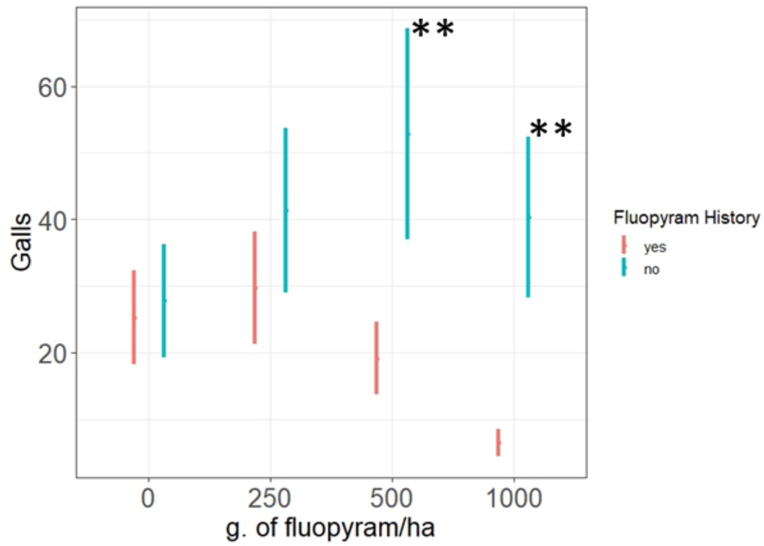


Figure 1. Results from the microbial degradation experiment indicates that nematode infection following fluopyram application was higher in soil originating from locations where Indemnify had never been used than from locations with a long history of fluopyram use. This is the opposite of the expected results if enhanced microbial degradation was occurring.

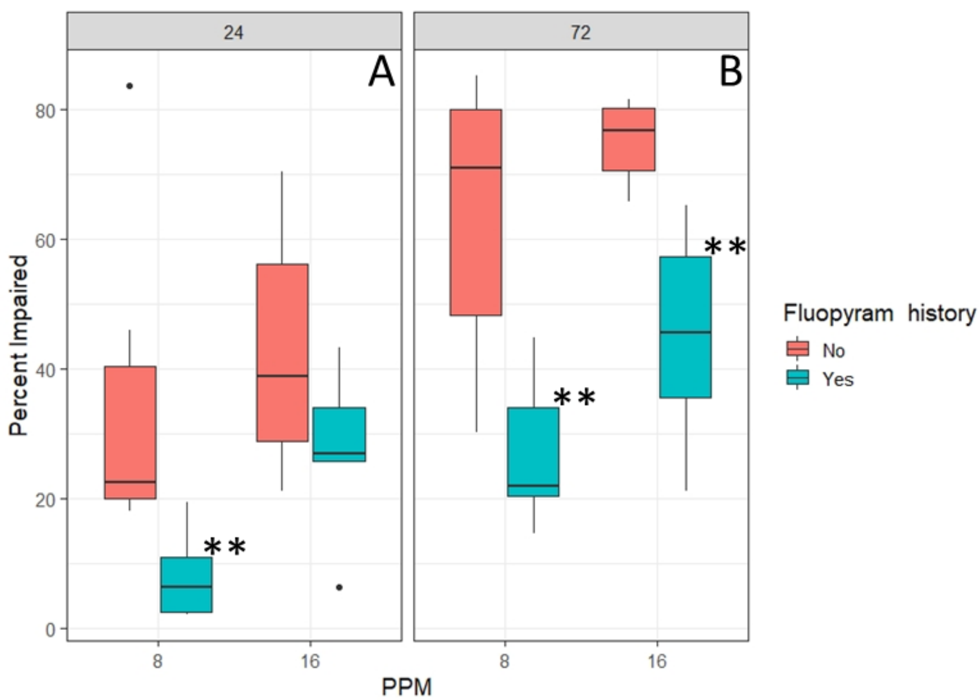


Figure 2. Results from the resistance experiment show that root-knot nematodes from golf courses with a history of Indemnify use are less sensitive to fluopyram than those from Indemnify 'virgin' locations. This indicates the development of resistance in these populations.

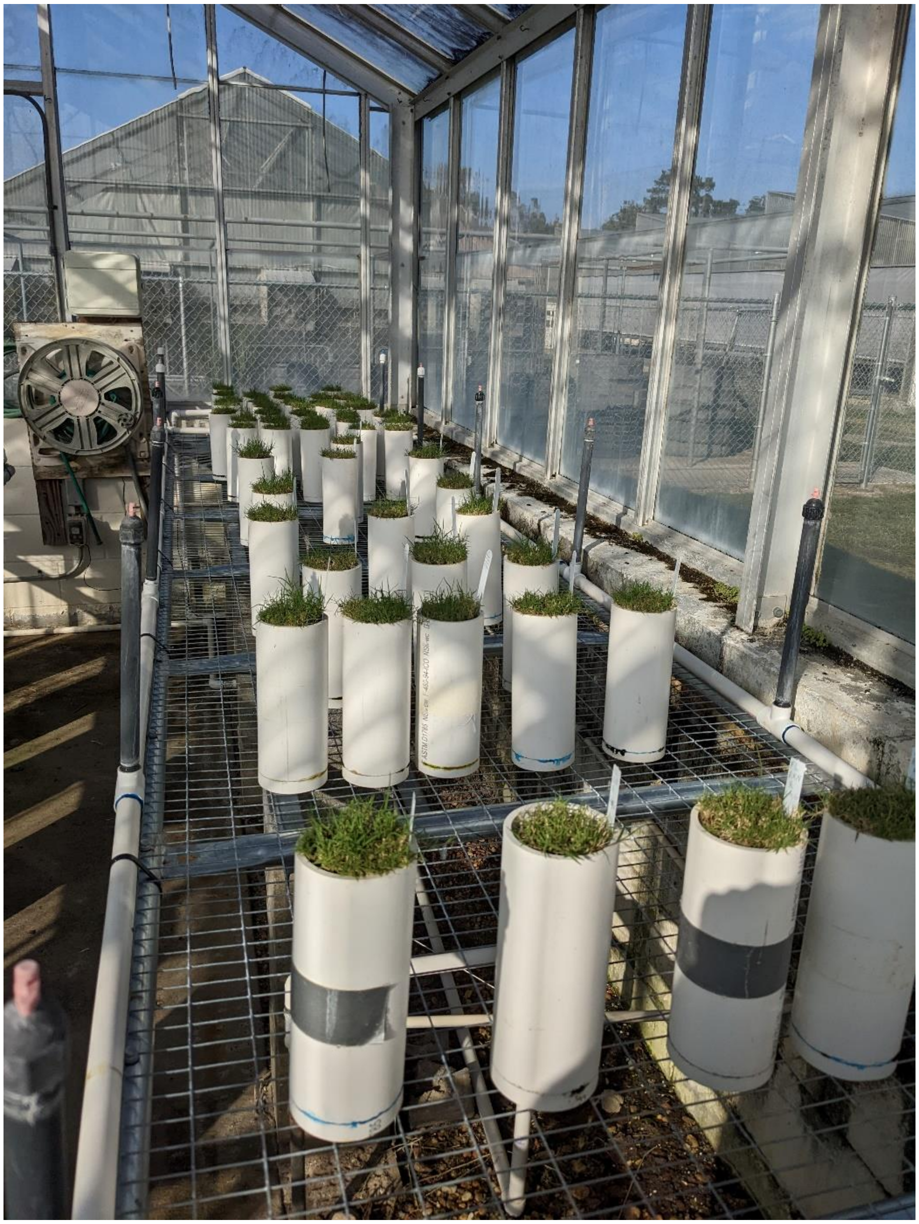


Figure 3. Sting nematode populations building up in the greenhouse for 2023 resistance experiment.

USGA ID#: 2022-12-755

Title: Use of endophytic microorganisms from a nematode-tolerant Bermudagrass cultivar as nematicidal biocontrol agents

Project Leader: Ulrich Stingl, Marco Schiavon, William T. Crow

Affiliation: University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS)

Objectives:

- To identify differences in microbiomes (bacteria and fungi) of two fairway bermudagrass cultivars with distinct susceptibility towards sting nematodes (preliminary data)
- To establish a culture collection of microorganisms from the endosphere and rhizosphere of a highly nematode-resistant turfgrass and from adjacent natural grass communities (completed, but extended to year 2 with modifications)
- To test cultures for ability to inhibit sting nematodes (completed, but extended to year 2)
- To identify lead strains that can be evaluated and tested for commercial application (ongoing)

Start Date: 2022

Project Duration: Two years

Total Funding: $116,655.69

Summary Points

- Preliminary data identified strong differences in fungal but not bacterial communities in nematode-tolerant TifTuf and nematode-susceptible Latitude 36 cultivars of Bermudagrass
- Isolated a total of 124 fungal endophytes from TifTuf, Latitude 36 and St. Augustine grass
- Used sequencing of ITS region to classify isolated fungal strains
- Cultured strains did not represent the most abundant endophytic fungi as identified with molecular methods (amplicon sequencing of ITS region)
- Tested supernatants of 13 representative strains for activity against sting nematodes
- Identified four lead strains that showed higher mortality than controls after three days of incubation, but higher replication is needed due to high variability (currently n=3)

Summary Text

Rational

All plants rely on associated microorganisms (microbiomes) to aid in acquiring nutrients, alleviating stress, and fighting pathogens (Vandenkoornhuyse et al., 2015). While microbiomes of traditional crops have been investigated to great extent for centuries, modern studies investigating microbiomes in turfgrass, and especially golf turf, are severely lacking behind thus hampering science-based efforts to make use of beneficial microbes to improve turf health (Stingl et al., 2022). In other plant systems, fungi and bacteria living inside the plant tissue ('endophytes') have shown to be very effective in protecting plants from pathogens (Sanchez-Canizares et al., 2017). In this project, we focus on analyzing fungi and bacteria associated with two fairway cultivars of bermudagrass that exhibit vastly distinct tolerance to nematode infections, with the goal of developing potential microbial biocontrol agents to suppress and treat nematode infections in golf turf. The project combines cultivation-independent high-throughput DNA sequencing of microorganisms (e.g. Rashid and Stingl 2015) associated with leaves and roots of these distinct cultivars with microbial cultivation and lab testing of efficiency of nematicidal fungi from the least susceptible one and from surrounding natural grasses. Our preliminary data indicated that especially fungal communities rather than bacterial differ between cultivars with different tolerance towards nematodes (Choi et al. 2022), therefore our current cultivation efforts are focused on endophytic fungi rather than bacteria.

Methodology

This project combines high throughput sequencing using Illumina amplicon sequencing of bacterial 16S rRNA genes and fungal Internal Transcribed Spacer (ITS) regions with traditional cultivation efforts to identify and isolate fungal strains that show high activity against sting nematodes. In this project year, we established a culture collection fungal endophytes using sterilization and isolation methods partly described in Choi et al. (2022). Briefly, grass leaves and roots are surface-sterilized, slit open, and placed on agar plates. After incubation and colonization of the agar plates by the endophytic cultures, the strains were transferred to new plates and identified with molecular tools (ITS sequencing, Zoll et al., 2016). The sequences were analyzed by comparison against public databases of sequences containing known fungal species and environmental samples, and the strains were grouped based on sequence similarity. Agar plugs in water and glycerol stocks (stored at -80°C) were prepared for long-term preservation of the strains. We also grew cultures of sting nematodes in turf pots in the greenhouse, extracted the nematodes, and established an assay to test the supernatant of spent fungal culture media of the tested fungal strains for bioactivity against sting nematodes. For this, each fungal culture was grown in a shaking incubator at a speed of 150 rpm for 7 days in liquid media at room temperature. The fungal cells were separated from culture supernatant by centrifugation at 4000 x g and the supernatant containing potential bioactive molecules was used for the following experiment. Each assay was conducted with three replicates and one control (uninoculated media) in 24-well microtiter plates. Mortality of the sting nematodes was checked under a microscope each day for three consecutive days. After three days, contamination of the assays by bacteria and fungi (presumably from the nematode preparations) caused issues and prevented longer incubation times.

Results to date

We have established a culture collection of 124 fungal endophytes that could be grouped into 13 different sequence types ('species') based on ITS sequencing (Table 1, Fig. 1). One representative of each species was used in a bioassay to identify bioactivity. Four strains had significantly higher mortality than the controls (Fig. 2).

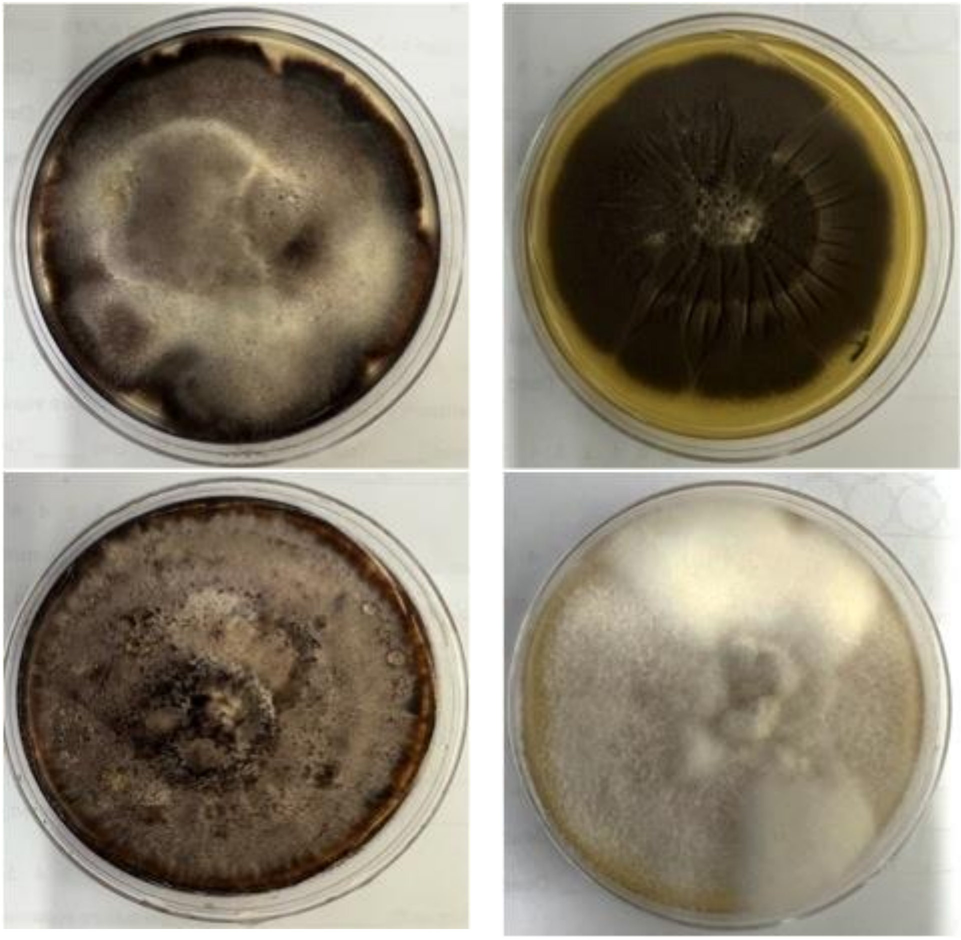


Fig. 1. Examples of cultures of fungal endophytes from turf grasses.

Those were two species of *Fusarium*, an unidentified endophyte, and a *Sordariomycetes* sp. A comparison with high-throughput sequencing data from these microbiomes (Fig. 4) showed that these species are not highly abundant in the grasses, indicating a (known) bias in cultivation, the great plate count anomaly (Staley and Konopka 1985).

Table 1. Taxonomic ID of the isolated fungal strains and percent mortality of sting nematodes in the bioassay.

Class	Top BLAST hit	% nt identity	Accession	% Mortality		
				Day 1	Day 2	Day 3
Agaricomycetes	*Agaricomycetes* sp. e50ss008	99.44	KM519352.1	11.8 ± 2.4	11.0 ± 2.9	12.7 ± 2.5
Dothideomycetes	*Dothideomycetes* sp. 11086	96.83	GQ153080.1	3.0 ± 5.3	16.7 ± 3.3	24.8 ± 31.3
	Phaeosphaeriaceae sp. Y124AA	94.39	MW791959.1	9.6 ± 8.3	11.1 ± 2.3	14.0 ± 1.6
	Phoma sp. CE_41	96.29	MT448833.2	9.2 ± 8.0	9.5 ± 8.3	28.8 ± 9.9
	Setophoma terrestris GLBRC342	96.89	OM106540.1	3.0 ± 5.3	19.0 ± 5.2	28.1 ± 0.8
	Uncultured *Pleosporales* 77584a6d	97.78	HG995901.1	12.0 ± 4.5	13.3 ± 6.9	15.3 ± 9.8
Sordariomycetes	*Fusarium concolor* NRRL 13459	99.81	GQ505763.1	11.2 ± 5.8	13.6 ± 2.7	13.4 ± 2.8
	Fusarium solani DE34	99.26	KY776027.1	11.1 ± 19.3	5.6 ± 9.6	49.3 ± 28.1

	Sordariales sp. GLBRC309	99.04	OM106507.1	13.0 ± 2.2	13.5 ± 4.9	16.5 ± 4.9
	Sordariomycetes sp. 282 FL0346	97.88	JQ760102.1	10.3 ± 2.1	17.8 ± 4.3	19.3 ± 9.5
Incertae sedis	*Gloeocercospora sorghi* NBRC 7268	96.93	LC063855.1	11.2 ± 2.1	11.7 ± 3.5	15.1 ± 6.2
	Uncultured fungus FICUS 186	96.46	JX174914.1	0.0 ± 0.0	23.3 ± 8.8	35.56 ± 33.6
Unknown	Fungal endophyte STRI:ICBG-Panama:TK1	96.15	KF436144.1	15.9 ± 2.0	17.4 ± 0.8	20.9 ± 1.8

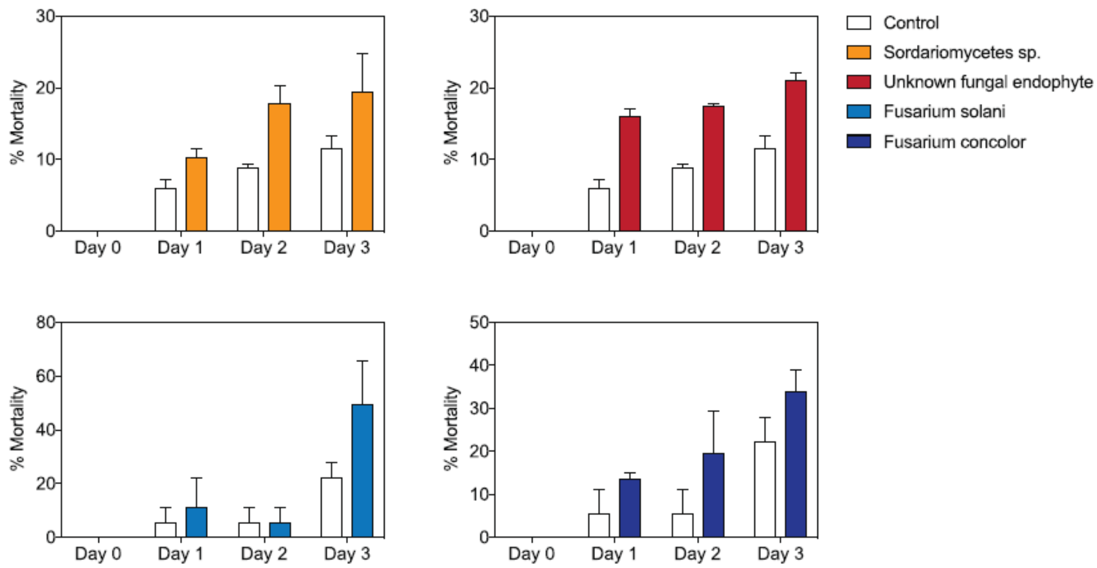


Fig.2. Mortality of sting nematodes for best-performing strains in bioassay compared to media controls.

Future expectations of the project

In the second year of the project, we will replicate our recently published amplicon sequencing approach to identify differences in fungal and bacterial microbiomes of a nematode-tolerant and a nematode-susceptible Bermudagrass cultivar (Choi et al 2022) in two seasons to test the variability and stability of microbiomes of these two cultivars (TifTuf and Latitude 36). This is important in order to understand the possible seasonality and stability of members of the microbiomes of nematode-tolerant cultivars of Bermudagrass. Although we reached our proposed number of cultures for this project, we will continue and improve our efforts in isolating fungal strains. We will modify the media composition in order to isolate more *abundant* fungi from the nematode-tolerant Bermudagrass as identified using amplicon sequencing. We will also use rhizosphere as inoculum to increase the number and the diversity of cultures. Although we identified several strains that showed bioactivity against sting nematodes, the difference between the best-performing strains and the control was only around 30% and we believe that we need to isolate better-performing strains for further studies, including greenhouse trials (outside the scope of this project). Also, the current bioassay only analyzes the mortality of adult sting nematodes and therefore would not identify bioactive compounds that might inhibit nematode development or would act on juvenile organisms. We hypothesize that bioactive molecules that inhibit the development of sting nematodes might be more specific, which would be highly desirable for a possible product. Therefore, we will develop and test another bioassay that will use extracted organic compounds, which will allow for longer incubation times.

References

Choi, C.J.; Valiente, J.; Schiavon, M.; Dhillon, B.; Crow, W.T.; Stingl, U. (2022) Bermudagrass Cultivars with Different Tolerance to Nematode Damage Are Characterized by Distinct Fungal but Similar Bacterial and Archaeal Microbiomes. *Microorganisms* 10 (2):457.

Rashid, M.; Stingl, U. (2015) Contemporary molecular tools in microbial ecology and their application to advancing biotechnology. *Biotechnology Advances* 33(8): 1755-1773.

Sanchez-Canizares, C.; Jorrin, B.; Poole, P.S.; Tkacz, A. (2017) Understanding the holobiont: the interdependence of plants and their microbiome. *Current Opinion in Microbiology* 38:188-196.

Staley, J.T.; Konopka, A. (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Reviews of Microbiology* 39:321-346.

Stingl, U.; Choi, C.J.; Dhillon, B.; Schiavon, M. (2022) The Lack of Knowledge on the Microbiome of Golf Turfgrasses Impedes the Development of Successful Microbial Products. *Agronomy* 12(1):71.

Vandenkoornhuyse, P.; Quaiser, A.; Duhamel, M.; Le Van, A.;Dufresne, A. (2015) The importance of the microbiome of the plant holobiont. *The New Phytologist* 206(4):1196-1206

Zoll, J.; Snelders, E.; Verweij, P.E.; Melchers, W.J.G. (2016) Next-Generation Sequencing in the Mycology Lab. *Current Fungal Infection Reports* 10 (2):37-42.

USGA-ID: 2020-08-713

Title: Long-term suppression of turfgrass insect pests with native persistent entomopathogenic nematodes.

Project leaders: Albrecht M. Koppenhöfer, Ana Luiza Sousa

Affiliation: Department of Entomology, Rutgers University, New Brunswick, NJ

Objectives: Isolate, characterize, and develop native persistent entomopathogenic nematodes for long-term insect pest suppression in golf course fairways and roughs.

Start date: 4-1-2019

Project duration: 3 years

Total funding: $29,865

Entomopathogenic nematodes (EPNs) have shown potential for the control of several important turfgrass insect pests. Most EPN research has focused on inundative applications and short-term effects. Studies in field crops have shown that inoculative applications of native EPN strains adapted to the local conditions and maintained to preserve their ability to persist in the environment, can effectively suppress pest populations for several years.

To obtain native persistent EPN strains, we surveyed during 2019 one fairway each at Pine Brook Golf Course (PB) and Howell Park Golf Course (HP) (Monmouth County, New Jersey). Most EPNs collected were *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. Mixes of isolates of each species were used to inoculate the field plots on one fairway each at PB and HP in early June 2020. These plots (20 m × 10 m) were half in the fairway, the other in the rough and were separated from each other by ≥ 10 m. Treatments were *H. bacteriophora*, *S. carpocapsae*, a 1:1 mixture of both species, all applied at a total of 1.25×10^9 infective juveniles/ha. Control plots were not treated. There were two replicates per treatment at each golf course. Samples of each kind were taken in each plot from a central 4 m × 4 m area in the rough and one in the fairway.

EPN populations in the plots were determined 1 week before application and 1, 4, 6, 13, 15, 25, and 28 months after application. Forty soil cores (7.5 cm × 2.5 cm diameter) were taken from each plot side (Fig. 1), mixed thoroughly, and a subsample of 120 grams placed into a plastic cup and baited with five waxworms for three consecutive 3-day baiting rounds. EPN-infected waxworms were collected and incubated to determine EPN species. EPN detection (i.e., number of infected waxworms) was highly variable. Generally, higher EPN numbers were detected in the rough vs. the fairway, particularly for *S. carpocapsae*. Numbers tended to be higher in the treated plots than the untreated plots for the species the plots were treated with, but this trend was stronger and more consistent with *S. carpocapsae* than with *H. bacteriophora* and the mix of the two species (Fig. 2). Numbers of *S. carpocapsae* declined strongly between early July and early October in 2022. This decline is likely related to the very dry and warm conditions during much of that period that was more likely to affect *S. carpocapsae* as it tends to be active closer to the soil surface than *H. bacteriophora*. A third EPN species, *Steinernema cubanum*, was also found regularly in many plots in both the fairway and the rough side (Fig. 2). Isolates of this species recently found elsewhere in New Jersey have shown high virulence to white grubs.

ABW populations were determined in mid-June 2020, 2021, and 2022. Thirty-two turf/soil cores (5.4 cm diameter × 3 cm depth) were taken from each plot side and extracted to record the number of life stages of ABW and any other insects. ABW densities were generally very low, but in all years significantly higher in the untreated fairway than in the untreated rough. In each year, numbers in the fairway were significantly lower in the plots treated with both EPN species (47% lower in 2020, 89% lower in 2021, 56% in 2022) than in the untreated plots (Fig. 3). No differences were detected in the rough. The only other insects found in significant numbers were larvae of the black turfgrass ataenius (BTA), numbers of which were not significantly affected by treatments.

Surface-active insect populations were determined in July and early September of 2020, 2021, and 2022 via soap flushes (Fig. 4, Table 1). In each plot side, two 30 cm × 30 cm areas were treated with 1,000 ml of 0.8% soap solution. Any insects found within 20 minutes were identified in the lab. Most common were BTA adults which occurred in higher numbers in the fairway where all EPN treatments significantly reduced their numbers (43-62%). ABW adults were more common in the fairway where *S. carpocapsae* (42%) and the *S. carpocapsae* + *H. bacteriophora* combination (74%) significantly reduced their numbers. Larvae of noctuid moths (cutworms, armyworms) were more common in the fairway where *S. carpocapsae* and the *S. carpocapsae* + *H. bacteriophora* combination significantly reduced their number. Other insects (sod webworm larvae, billbug adults, click beetle (Elaterid) adults) were found in number too low for meaningful analysis.

White grub populations were determined in late September 2020, 2021, and 2022. Sixteen turf/soil cores (10.5 cm diameter × 7.5 cm depth) were taken per plot side (Figure 5). Any soil insects found were identified in the lab. Sampling for white grubs showed a mix of oriental beetle followed by Japanese beetle and a few northern masked chafers. Due to the low densities, the three species were pooled. White grubs were more common in the rough than in the fairway. In the rough, densities were significantly lower in the plots treated with *H. bacteriophora* than in the untreated plots (88% lower in 2020, 50% in 2021, 71% in 2022); no treatment effect could be detected in the fairway (Fig. 6). BTA larvae were more common in the fairway than in the rough and tended to be lower in the plots treated with *S. carpocapsae*, but significantly so only in the rough. Numbers of other insect types detected were too low for meaningful analysis.

- Numbers of *S. carpocapsae* increased across the field plots, especially the fairway portion, following applications of lab reared mixes of native isolates but declined in summer of the third study year, likely due to unusually dry conditions.
- Numbers of *H. bacteriophora* tended to be higher in the treated plots through the first 13 months but thereafter numbers in the untreated plots increased to similar densities.
- ABW, white grubs, and BTA, albeit all in low densities, were present in the plots and likely contributed to EPN recycling.

Back to TOC

- ABW numbers in the fairway portion of the plots were significantly lower in the plots treated with the *S. carpocapsae* + *H. bacteriophora* combination in all three study years.
- Numbers of white grubs were significantly reduced in the rough portion of the plots treated with the *S. carpocapsae* + *H. bacteriophora* combination and especially with *H. bacteriophora* alone.

Table 1. Number of surface active inects (per 2 square feet sample) extracted with soapy water averaged across six sampling dates (July and September of 2020, 2021, and 2022).

Treatment[1]	**Grass type**	**ABW**[2]**, adults**	**BTA**[3]**, adults**	**Noctuid, larva**	**SWW**[4]**, larva**	**Billbug, adult**	**Elaterid, adults**
Utc	Fairway	4.8 a	35.8 a	2.0 a	1.0 a	0.5 a	1.3 a
Sc	Fairway	2.8 bc	16.8 bc	0.3 b	0.3 b	0.0 a	1.0 a
Hb	Fairway	3.3 ab	13.5 bcd	0.8 ab	0.8 ab	0.3 a	0.8 a
Sc+Hb	Fairway	1.3 c	20.5 b	0.5 b	1.0 a	0.5 a	0.5 a
Utc	Rough	0.5 c	4.0 cde	1.3 ab	1.0 a	0.3 a	1.0 a
Sc	Rough	0.3 c	3.8 de	0.0 b	0.0 b	0.0 a	0.0 a
Hb	Rough	0.3 c	1.5 e	0.3 b	0.3 b	0.0 a	1.3 a
Sc+Hb	Rough	0.0 c	3.5 e	0.3 b	0.5 ab	0.0 a	0.3 a

Means with the same letter within column are not significantly different ($P < 0.05$).
[1] Utc = untreated control, Sc = *S. carpocapsae*, Hb = *H. bacteriophora*
[2] Annual bluegrass weevil; [3] Black turfgrass ataenius; [4] Sod webworm

Fig. 1. Sampling of soil cores to determine populations of entomopathogenic nematodes.

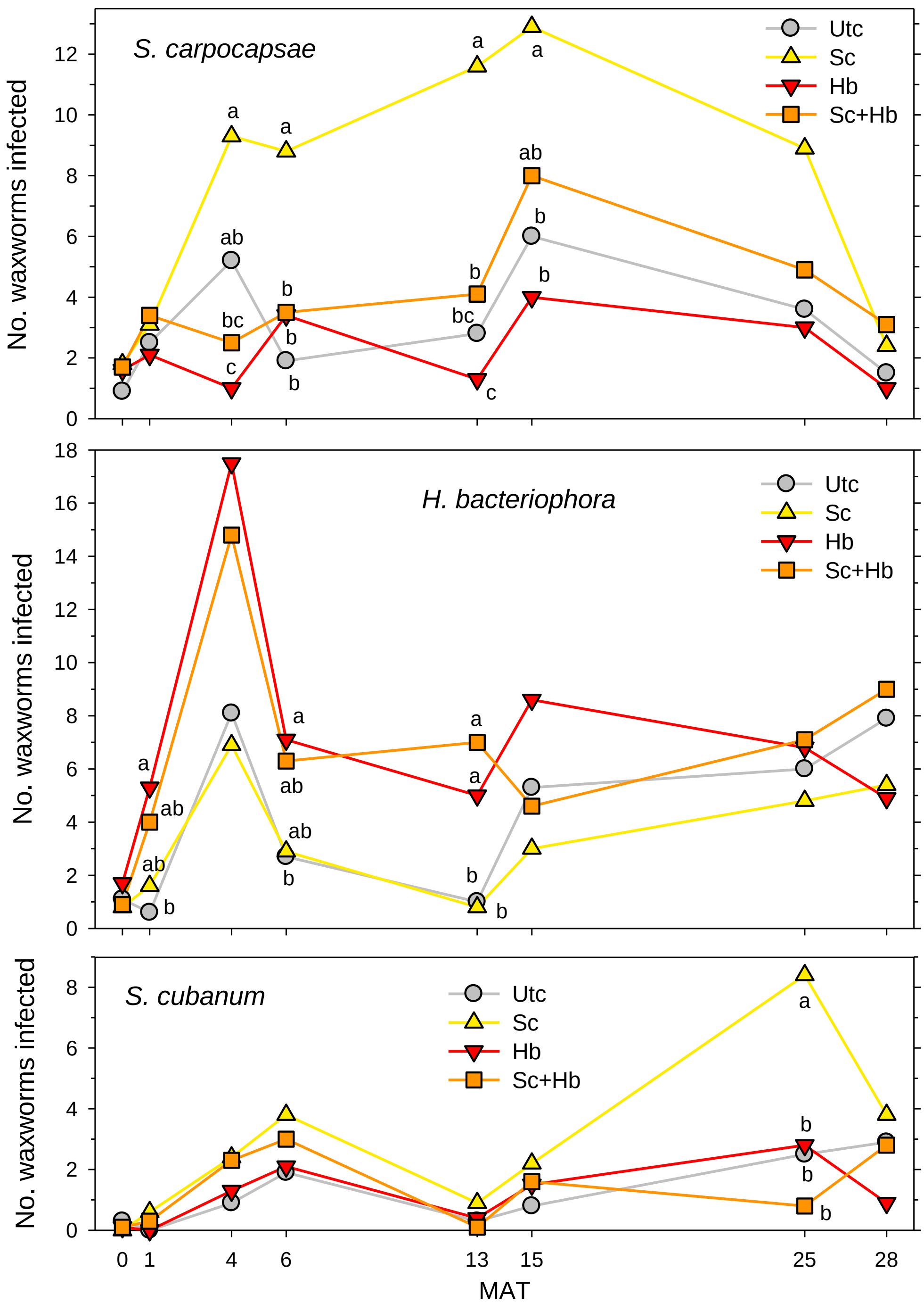


Fig. 2. Numbers of EPN-infected waxworms recovered per plot by baiting soil samples taken from plots extending from the fairway into the rough. Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc). A third species was regularly recovered that was identified as *Steinernema cubanum* or very closely related to it. Soil samples were taken 1 week before (0) and 1 to 28 months after application (MAT).

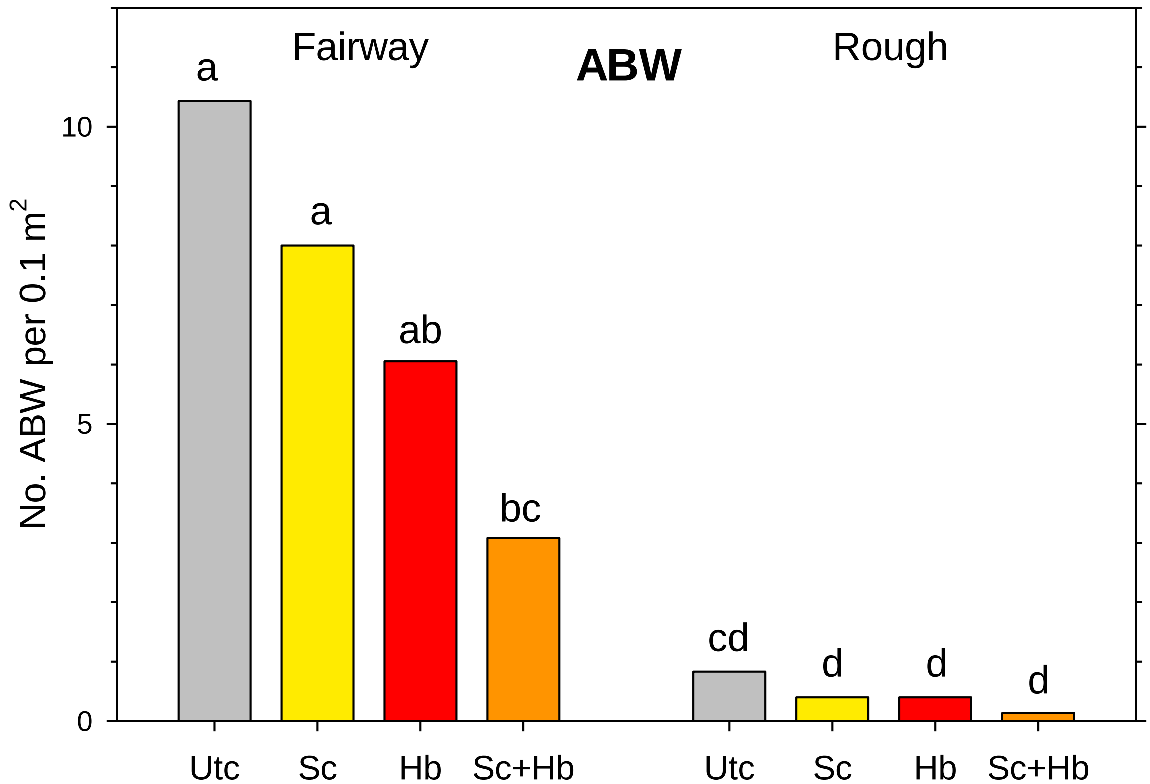


Fig. 3. Densities of annual bluegrass weevil developmental stages (L1 – teneral adults) in soil cores collected in mid-June (2020, 2021, and 2022; data combined) in plots extending from the fairway into the rough (data combined for both years). Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc).

Figure 4. Extraction of surface-active insects with soap solution.

Fig. 5. Taking cup cutter cores to determine populations of white grubs and other soil insects.

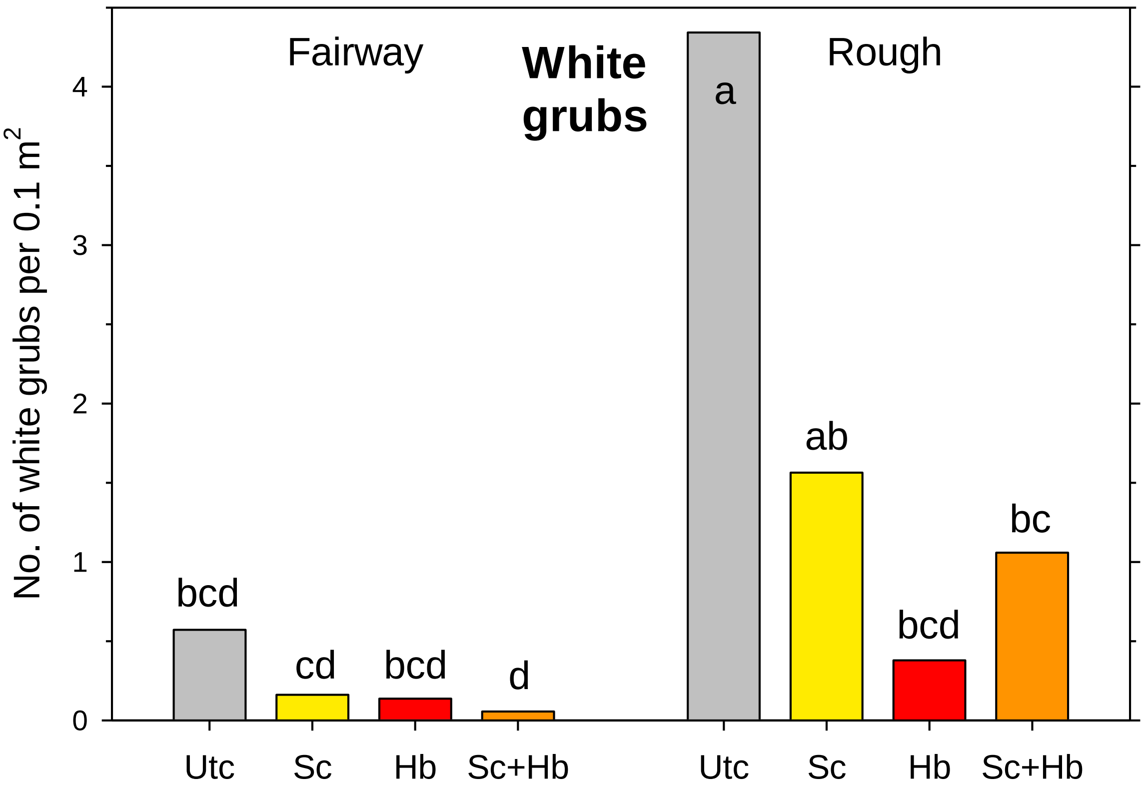


Fig. 6. Densities of annual white grub larvae in soil cores collected in late September (2020, 2021, and 2022; data combined) in plots extending from the fairway into the rough (data combined for both years). Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc).

Project ID: 2020-09-714
Project Title: Characterization of turfgrass plant induced defenses in response to annual bluegrass weevil feeding

Principal Investigator(s): Emily Merewitz-Holm[1]; Benjamin A. McGraw, Ph.D.[2]
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Objectives:
(1) Characterize creeping bentgrass and annual bluegrass tolerance to ABW herbivory
(2) Identify plant response mechanisms associated with ABW feeding
(3) Quantify nutritional composition of potential cool- and warm-season turfgrass hosts

Start Date: 2021 (*delayed one year due to COVID; original start 2020)
Project Duration: 2 years
Total Funding: $51,444

Summary Points:

i. Significantly more annual bluegrass weevil (ABW) larvae were recovered from annual bluegrass (ABG) than creeping bentgrass (CBG) at all observation periods (2^{nd}-4^{th} instar) in no-choice assays

ii. ABW herbivory did not significantly increase jasmonic acid (JA) in either turfgrass. However, CBG possessed greater JA concentrations constitutively than ABG, which may explain its tolerance to feeding damage.

iii. ABG was shown to have significantly higher total flavonoid content compared to CBG, perennial rye (Double Eagle), zoysiagrass (Zeon) and bermudagrass (Latitude 36)

Summary:

The annual bluegrass weevil (ABW) is considered to be a specialist of annual bluegrass (*Poa annua,* ABG). Over the last decade, multiple studies combined with field observations of recently invaded areas suggest that ABW may complete its life cycle in other cool-season turfgrasses. Creeping bentgrass (*Agrostis stolonifera,* CBG) is a commonly encountered species that ABW can develop within, though damage is rare and usually less severe compared to ABG. Furthermore, individual insects incur fitness costs (e.g., longer development, reduced body mass) when developing within CBG. This finding suggests CBG may elicit inducible defense mechanisms to counter ABW herbivory.

This project characterized CBG and ABG defense phytohormone response to ABW feeding at different life stages and within different tissue types to develop novel control strategies, such as improved (either resistant or tolerant) turfgrass cultivars. Additionally, we sought to profile the nutritional and

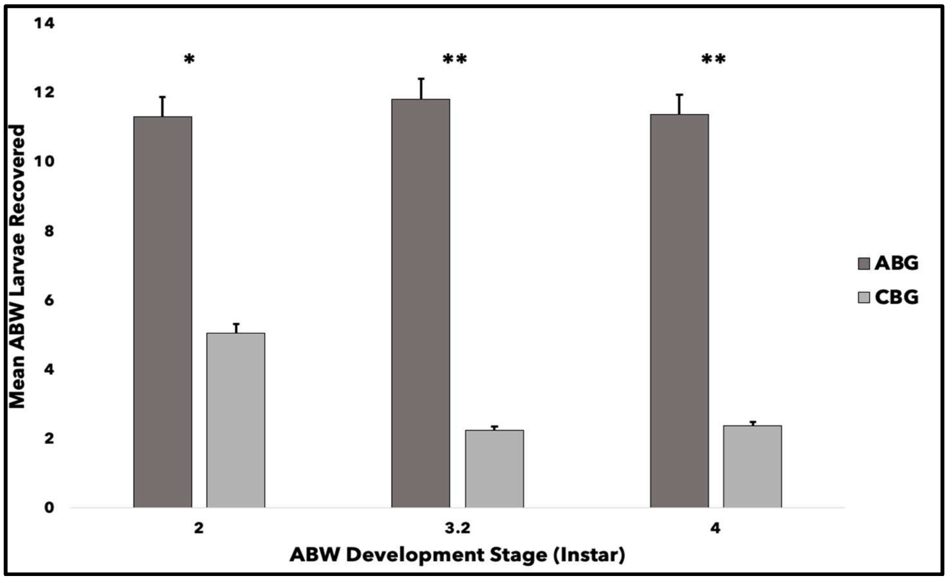
metabolic compositions of cool- and warm-season turfgrasses to gain insight on host plant selection. Identifying nutritional preferences will aid in determining whether ABW may exploit novel turfgrass hosts as it expands its distribution south and westward.

Objective 1: Characterize creeping bentgrass and annual bluegrass tolerance to annual bluegrass weevil herbivory

Methodology:
Turfgrass Growth and Maintenance: Plants were established from seed (*Agrostis stolonifera cv.* Penncross and wild-type *Poa annua*) in plastic growth containers (30 cm x 15 cm x 4 cm) in a greenhouse. Seedlings were grown on a pasteurized mixture of sandy loam soil and sand (3:1 ratio), watered as necessary, and clipped twice a week to maintain fairway-height conditions (13 mm).
Insects: ABW adults were vacuum collected from fairways, collars and tees on a golf courses (Harrisburg Country Club and Golf Course, Harrisburg, PA and Lancaster Country Club and Golf Course, Lancaster, PA) on April 13th, 2021and April 20th, 2022. Adults were assessed for viability, sexed, and kept separately in 840 ml plastic containers filled with sandy loam soil and sand (3:1 ratio) in an incubator (10 h light at 6°C:14 h dark at 4°C). Prior to bioassays, adults were placed in clear plastic containers (~500 ABW) with mesh lids and placed into an incubator (14 h light at 21°C:10 h dark at 14°C). Equal number of male and female ABW adults (10:10) were infested on pots and remained in turfgrass plugs for 7 days. Adult weevils were manually extracted from each of the treatment plugs over a 3-day period to avoid continuous oviposition. Larvae were heat extracted (40°C for 2 d) from cores, counted, and head capsules were measured to determine developmental age.

Results:
Greater densities of ABW larvae were found within ABG at all observation periods (between 2nd and 4th instar). Significantly more larvae were recovered from ABG at L 2.0 (11.3 vs. 5.1 per plug), L 3.2 (11.8 vs. 2.3) and L 4.0 (11.3 vs. 2.4 per plug) extraction timings (df =2; F=4.17; *P*=0.041)

Figure 1: Average (± standard deviation) annual bluegrass weevil larvae extracted from annual bluegrass and creeping bentgrass cores in no-choice bioassays in the greenhouse. Means were analyzed by Students t-test at the α= 0.05. Significant differences are presented by * (*P*<0.05), ** (*P*< 0.01), ***(*P*<0.001) and **** (*P*<0.0001).



Objective 2: Identify plant response mechanisms associated with ABW feeding

Methodology:

ABW were caged on CBG (c.v. Penncross) and ABG arenas in the greenhouse according to the methodologies described in Objective 1. Since the lifestage or the feeding location (stem borer vs. crown feeding stages) may influence host plant defense responses, phytohormone extractions were performed at three distinct larval stages (average age L 2.0, 3.2, and 4.0) corresponding to when the larvae were stem borers/early instars (~10 d after adult removal), early crown feeders (~15 d), and larger larvae/late crown feeders (~ 20 d). Before each extraction, 8 randomly selected untreated control pots were sampled for larvae, and age was determined based on head capsule width. Turfgrass leaf and stem tissues were manually removed until approximately 200 mg dry weight was acquired. Roots were separated at the crown of the plant and rinsed in water at room temperature to remove soil. Root tissue was dried on an paper towel to remove excess water from the washing step. Tissue samples were collected from four biological replicates per tissue type and from 8 technical replicates per grass type per larval development stage. During the collection process, tissue samples were stored in 5 mL centrifuge vials on dry ice, then flash frozen using liquid nitrogen, and transferred into a -80°C freezer for future phytohormone extractions. Hormone extraction and analysis were conducted using a modified crude extraction procedure reported by Liu et al. (2012).

Statistical analysis: Results are represented by the mean ± standard deviations. One-way analysis of variance (ANOVA) was used to determine significant differences between turfgrass species, tissue types and ABW larval development are expressed with HSD Tukey adjustments and defined by $P<0.05$. All statistical analysis was conducted using R studio freeware.

Results:

Salicylic acid (SA), the phytohormone associated with defense against pathogens and piercing-sucking insect damage, was found in significantly higher concentrations in the leaf and stem of ABG than CBG when analyzing the data combined for all tissue types and ABW larval stages (Table 1). Conversely, jasmonic acid (JA), the defense phytohormone associated with mechanical damage and chewing insect herbivory, was observed to be significant higher in CBG in the absence of ABW feeding. The higher constitutive levels of SA within ABG may suggest investment in SA at the expense of a JA, and explain ABG susceptibility to ABW damage. ABW herbivory did not appear to induce either phytohormone's production when combining all plant tissues in the analysis.

However, examining phytohormones within tissue types revealed dynamic changes. JA levels in both CBG and ABG significantly decreased in leaves and roots in response to ABW feeding (Table 2). CBG was shown to possess significantly higher JA concentrations in the stem compared to ABG, but neither species was shown to significantly increase JA production when ABW larvae fed in this part of the plant. CBG stems innately had higher JA concentrations than ABG, potentially providing evidence for CBG tolerance to ABW feeding. Furthermore, ABG was shown to have significantly greater concentrations of SA in leaves and stems compared to CBG. Investment in SA at the expense of JA within ABG leaf and stem tissues may explain ABW host plant preference of ABG and differences in tolerance between turfgrasses.

Future Directions:
We plan to assess ability to control ABW with JA applications to ABG in greenhouse and field, in addition to screening bentgrass cultivars for constitutive and inducible JA levels.

Table 1: Average (± standard deviation) salicylic acid (SA) and jasmonic acid (JA) concentrations (ng/g) from annual bluegrass (ABG) and creeping bentgrass (CBG) with (+) and without (-) annual bluegrass weevil (ABW) feeding. Treatments were analyzed by one-way analysis if variance (ANOVA) and means separated by HSD Tukey test.

Treatment	SA[1]	JA
ABG ABW-	245.3±326.2 **a**	20.0±24.7 **b**
ABG ABW+	199.5±257.1 **a**	22.6±27.3 **b**
CBG ABW-	81.4±103.8 **b**	37.8±45.7 **a**
CBG ABW+	43.96±41.3**b**	27.6±37.9 **ab**

[1]Means with the same letter within column are not significantly different from one another at the α = 0.05 level

Table 2: Average (± standard deviation) salicylic acid (SA) and jasmonic acid (JA) concentrations (ng/g) quantified from leaf, stem and root tissue annual bluegrass (ABG) and creeping bentgrass (CBG) with (+) and without (-) annual bluegrass weevil (ABW) feeding. Treatments were analyzed by one-way analysis if variance (ANOVA) and means separated by HSD Tukey test.

Treatment		SA[1]	JA
Leaf	ABG ABW-	439.3±389.2 **a**	19.2±23.7 **ab**
	ABG ABW+	306.1±306.1 **b**	12.9±18.1 **b**
	CBG ABW-	105.5±92.3 **c**	33.7±44.1 **a**
	CBG ABW+	61.6±51.4 **c**	10.9±8.3 **b**
Stem	ABG ABW-	273.2±287.5 **a**	33.2±28.8 **c**
	ABG ABW+	272.1±263.0 **a**	47.5±25.9 **bc**
	CBG ABW-	113.0±136.4 **b**	69.4±49.0 **a**
	CBG ABW+	53.8±30.3 **b**	65.6±44.1**ab**
Root	ABG ABW-	24.6±20.6 **a**	5.9±6.3 **ab**
	ABG ABW+	12.4±7.7 **b**	1.5±2.4 **b**
	CBG ABW-	24.6±24.5 **a**	9.3±9.8 **a**
	CBG ABW+	12.6±11.6 **b**	5.0±7.8 **b**

[1]Means with the same letter within column are not significantly different from one another at the α = 0.05 level

Figure 2: Average (± standard deviation) jasmonic acid concentrations (ng/g) quantified from leaf, stem and root tissue collected from annual bluegrass (ABG) and creeping bentgrass (CBG) with (+) and without (-) annual bluegrass weevil (ABW) feeding. Treatments were analyzed by one-way analysis if variance (ANOVA) and means separated by HSD Tukey test. Significant difference denoted by * (*P*<0.05), ** (*P*< 0.01), ***(*P*<0.001) and **** (*P*<0.0001). No significance difference is illustrated by ns.

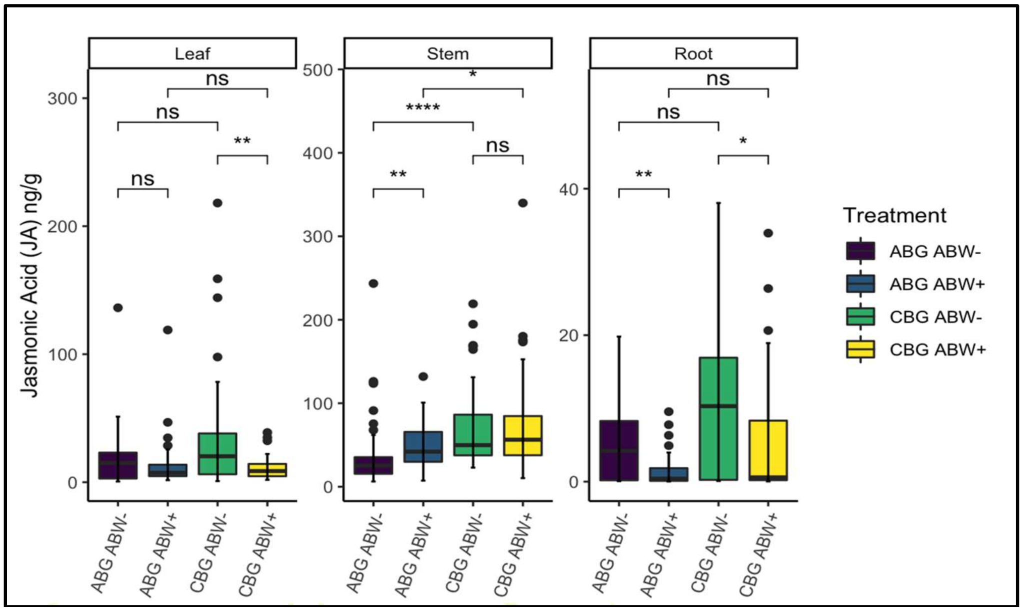
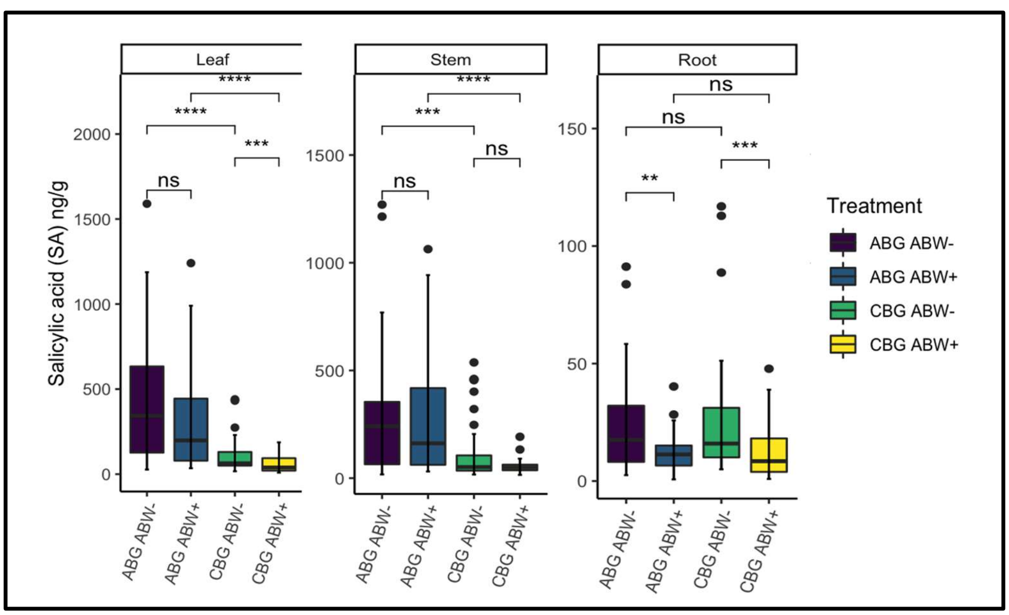


Figure 3: Average (± standard deviation) salicylic acid concentrations (ng/g) quantified from leaf, stem and root tissue collected from annual bluegrass (ABG) and creeping bentgrass (CBG) with (+) and without (-) annual bluegrass weevil (ABW) feeding. Treatments were analyzed by one-way analysis if variance (ANOVA) and means separated by HSD Tukey test. Significant difference denoted by * (*P*<0.05), ** (*P*< 0.01), ***(*P*<0.001) and **** (*P*<0.0001). No significance difference is illustrated by ns



Objective 3: Quantify nutritional composition of cool- and warm-season turfgrass
We assessed bioactive nutritional and pigment elemental differences between turfgrass species and cultivars (ABG; CBG c.v. Penncross; Penn-A4; *Lolium perenne*; *Zoysia matrella* and *Cynodon dactylon*) primarily to characterize nutritional differences between ABG and CBG, as well as assess the ability of warm-season turfgrasses to serve as novel hosts as the insect moves into new regions.

Methodology:
Proteins
Total protein concentration was estimated by using the Kjeldahl method, the standard Bicinchoninic Acid protocol suggested by the Association of Official Analytical Chemists (AOAC, 1980).
Carotenoids and Chlorophyll
Approximately 20ml of turfgrass extract from each species and cultivar was mixed with 1ml of ethyl acetate. The solution underwent centrifugation at 5000 rpm for approximately 10 min. Post supernatant and precipitant separation absorbance was measured between 400 and 700 nm using a Biotek Synergy HT microplate reader (BioTek Instruments, Highland Park, Winooski, VT, 05404). Carotenoid presence within turfgrass extract were calculated using the formula provided by Lichtentaler and Wellburn to calculate presence of carotene pigment (Lichtentaler and Wellburn, 1985).
Total Flavonoid Detection
A total of 1 ml of turfgrass extract and 4 ml of deionized water, followed by 0.3 ml of 5% (w/v) $NaNO_2$ after 5 min 0.6 mL of 10% (w/v) $AlCl_3$ was added to the solution. After an additional 5 min period 2 mL of 1 M NaOH followed by the addition of 2.4 mL of deionized water to dilute the solution and mixed to homogenize. Flavonoid content was measured via colorimetric assay established by Zhisehen et al. (1999). Turfgrass extract solution absorbance was measured at 510 nm against deionized water. Total flavonoid content was determined by milligram catechin equivalents per gram of sample dry weight (mgCE/gDW) was tested in triplicate.
Total Phenol Detection
Total phenolic content was determined by using the Folin-Ciocalteu (FC) reagent protocol (Singleton et al. 1999). Turfgrass extracts and gallic acid standards (1 ml) was transferred to a 25 ml volumetric flask containing 9 ml methanol (99.8%, HPLC). Samples (200 µl) were transfers to 10 ml test tubes, 1.5 ml of 10% FC reagent (w/v) was added to solution and shaken for 5 minutes in the dark. Additionally, 1.5 ml of 6% $NaCO_3$ (w/v) was added to the solution, diluted with 25 ml of deionized water and set aside for 90 minutes at room temperature before transferring the solution to a dark space to incubate at room temperature for an additional 2 hrs. Using a UV spectrophotometer, we measured the sample and gallic acid standards absorbance at 760 nm. All samples and standards were analyzed in triplicate (Qawasmeh et al. 2012).

Results:
Protein
Protein concentration differed significantly between turfgrass species (df= 5; F=21.74; $P>0.001$) and within tissue type per turfgrass species or cultivar (df= 2; F=764.84; $P>0.001$). CBG c.v. Penncross (X^2=770.7±122.2) and *Lolium perenne* (X^2=751.5±226.8) leaf protein content was significantly higher compared the remaining cool- and warm-season turfgrasses represented in this study.
Carotenoids and Chlorophyll
Total carotenoid concentrations were significantly higher (df= 5; F=41.24; $P>0.001$) amongst cool-season turfgrasses and cultivar (ABG, CBG c.v. Penncross and Penn-A4 and *L. perenne*) compared to the warm-season turfgrasses (*Z. matrella* and *C. dactylon*). CBG c.v. Penn-A4 had significantly higher total chlorophyll (df= 5; F=3.02; $P=0.014$) compared to *L. perenne*. No significant differences in chlorophyll content were found between the remainder of the assessed turfgrasses.
Total Phenolic Content

Our analysis demonstrates that concentration of total phenolic content differs significantly between turfgrass species (*df*= 5; *F*=21.74; *P>0.001*) and within tissue type per turfgrass species and cultivar (*df*= 2; *F*=764.84; *P>0.001*). Total phenolic content (mgGAE/L) was significantly higher within leaf tissue of CBG c.v. Penncross (X^2= 691.5± 330.3 mgGAE/L) and *L. perenne* (X^2= 817.3± 379.2 mgGAE/L) compared to ABG (X^2=8 394.7± 78mgGAE/L)*,* CBG c.v. Penn-A4 (X^2= 388.6±101.1 mgGAE/L), *Z. matrella* (X^2= 475.3± 78.8 mgGAE/L) and *C. dactylon* (X^2= 425.1±260.1 mgGAE/L*)*. No significant differences were observed between total phenolic content of stem- and root tissue of ABG and CBG c.v. Penncross, *L. perenne, Z. matrella* and *C. dactylon*.

Total Flavonoid Content

Total flavonoid concentration (mgQE/L) was significantly different between turfgrass species (*df*= 5; *F*=71.09; *P>0.001*) and per tissue type within each turfgrass species and cultivar (*df*= 2; *F*=1616.04; *P>0.001*). ABG total flavonoid concentration (mgQE/L) within both leaf- (X^2= 1423.6±598.8 mgQE/L) and stem-(X^2= 5.7±6.90 mgQE/L) were significantly higher compared to both CBG cultivars, *L. perenne* and the warm-seasoned turfgrasses. CBG c.v. Penn-A4 root express significantly higher flavonoid content (X^2= 5.7±5.4 mgQE/L) in contrast to the other turfgrasses

Future Directions:

We plant to assess ABW larval performance on the different turfgrasses in spring 2023. Additionally, we will characterize changes in nutritional composition of these turfgrasses throughout the growing season to help explain impacts on larval population dynamics and to assess the risk for ABW to develop on novel hosts.

Table 3: Quantification of bioactive nutritional elements and protein of cool- and warm-season turfgrasses tissue extract.

Nutritional Element	Turfgrass	Leaf[1,2]	Stem	Root
Protein (µg/ml)	*P. annua*	535.0±180.1 **b**	30.3± **d**	20.0± **d**
	A. stolonifera Penncross	770.7±122.2 **a**	75.5± **b**	48.0± **cd**
	A. stolonifera Penn-A4	469.5±55.6 **b**	193.6± **a**	97.0± **ab**
	L. perenne	751.5±226.8 **a**	57.4± **c**	104.1± **a**
	Z. matrella	265.2±50.15 **c**	54.5± **c**	45.1± **cd**
	C. dactylon	196.0±113.2 **c**	17.9± **d**	68.7 **bc**
Total Phenolic (mgGAE/L)	*P. annua*	394.7± 78.8 **b**	61.5±17.3 **d**	74.9± 18.7 **de**
	A. stolonifera Penncross	691.5± 330.3 **a**	60.9±12.9 **d**	84.7±7.9 **cd**
	A. stolonifera Penn-A4	388.6±101.1 **b**	83.9± 21.8 **a**	94.6±13.2 **bc**
	L. perenne	817.3± 379.2 **a**	72.8±8.1 **bc**	114.4±27.4 **a**
	Z. matrella	475.3± 78.8 **b**	67.3±15.7 **cd**	64.6±12.1**e**
	C. dactylon	425.1±260.1 **b**	82.6 ±9.1 **ab**	101.9±10.8 **b**
Total Flavonoid (mgQE/L)	*P. annua*	1423.6±598.8 **a**	5.7±6.90 **a**	1.7±0.74 **b**
	A. stolonifera Penncross	791.4±73.4 **b**	0.68±0.24 **c**	2.2±0.44 **b**
	A. stolonifera Penn-A4	468.7±39.3 **c**	2.9±0.94 **b**	5.7±5.4 **a**
	L. perenne	806.5±125.3 **b**	1.7±0.67 **bc**	2.1±0.85 **b**
	Z. matrella	439.± 73.8 **c**	0.93±0.10 **c**	0.8± 0.14 **b**
	C. dactylon	481.9± 210.1 **c**	0.80±0.22 **c**	1.7±1.06 **b**
Total Flavonoid/ Phenolic Ratio	*P. annua*	3.92±2.21 **a**	0.093±0.11 **a**	0.022±0.008 **b**
	A. stolonifera Penncross	1.36± 0.51 **b**	0.012±0.006 **b**	0.026±0.005 **b**
	A. stolonifera Penn-A4	1.27±0.30 **b**	0.036±0.010 **b**	0.060±0.056 **a**
	L. perenne	1.22±0.58 **b**	0.022± 0.008 **b**	0.020±0.011 **b**
	Z. matrella	0.96±0.31 **b**	0.014±0.003 **b**	0.013±0.004 **b**
	C. dactylon	1.36±0.68 **b**	0.010±0.009 **b**	0.017±0.011 **b**

[1]Mean value ± standard deviation One-way ANOVA, HSD Tukey adjusted α= 0.05

[2] Means with the same letter within column are not significantly different from one another at the α = 0.05 level

Table 3: Total carotenoid and chlorophyll (mg/100mL) concentration of turfgrass extracts.

Nutritional Element	Turfgrass	Leaf Tissue
Total Carotenoid (mg/100mL)	*P. annua*	15.2±0.76 **a**
	A. stolonifera Penncross	15.8±0.21 **a**
	A. stolonifera Penn-A4	15.4±0.54 **a**
	L. perenne	15.3±0.97 **a**
	Z. matrella	12.9±1.09 **b**
	C. dactylon	13.6±0.75 **b**
Total Chlorophyll (mg/100mL)	*P. annua*	44.80±2.03 **ab**
	A. stolonifera Penncross	44.91±0.15 **ab**
	A. stolonifera Penn-A4	45.39±0.38 **a**
	L. perenne	44.24±0.87 **b**
	Z. matrella	44.84±0.30 **ab**
	C. dactylon	44.57±0.27 **ab**

[1]Mean value ± standard deviation; One-way ANOVA, HSD Tukey adjusted $\alpha = 0.05$

[2] Means with the same letter within column are not significantly different from one another at the $\alpha = 0.05$ level

Reference:

AOAC. (1980). “Official Methods of Analysis,” 13th ed., Association of Official Analytical Chemists, Washington, DC.

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents.

Liu, H., Li, X., Xiao, J., & Wang, S. (2012). A convenient method for simultaneous quantification of multiple phytohormones and metabolites: application in study of rice-bacterium interaction. Plant methods, 8(1), 1-12.

Qawasmeh, A., Obied, H. K., Raman, A., & Wheatley, W. (2012). Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction between *Lolium perenne* and different strains of *Neotyphodium lolii*. Journal of agricultural and food chemistry, 60(13), 3381-3388.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 64(4), 555-559.

USGA ID#: 2022-09-752

Title: Engineering Turfgrass Rhizobacteria For Selective Control Of Fall Armyworm

Project Leader:
PI: John F. Beckmann
Co-PI: David Held
GRA Masters Student: Janiyah Cotton

Affiliation: Auburn University

Objectives:

Objective 1) Determine the best rhizobacterial chassis strain.

Objective 2) Equip rhizobacterial shuttle vectors with insect toxins.

Start Date: August 2022 (progress is results from first 4 months of the project)

Project Duration: 3 years (2022-2025)

Total Funding: $109,806

Summary Points:

- Endophytic Turfgrass microbe strains AP7, AP18, AP282 are susceptible to Ampicillin, Chloramphenicol, and Kanamycin antibiotics.
- Strain AP7 (putatively *Bacillus pumilus*) can be transformed with fluorescent marker plasmids
- We have Successfully identified a candidate vector plasmid for transformation.

Summary Text:

Rational: Turfgrasses are of economic importance to Golf courses. A major pest of turfgrass is the fall armyworm. We will develop cheap and residual controls for fall armyworm in turfgrass by engineering rhizobacterial frames to deliver customizable species-specific insect killing toxins to plant herbivores.

Plant growth promoting rhizobacteria (PGPR) are bacteria living in the rhizosphere. The PGPRs of turfgrass are *Bacillus* bacteria. *Bacillus subtilis* is also a model bacterium. These bacteria and their brethren will serve as our backbone for genetic engineering. The bacteria are capable of transient colonization of turfgrass foliage. Once in the foliage the bacteria would be imbibed by insect pest herbivores. Thereupon, delivery of a toxin would kill the herbivore. This technology will be transient and not permanently damage ecosystems because the Rhizobacteria we are working on transiently colonize turfgrass for ~12 weeks then die off.

Methodology:

Endophytic turfgrass strains were grown on standard LB media without antibiotics. Classical $CaCl_2$ transformations were performed by first growing overnight cultures in 5 ml LB. The next morning we inoculate 50 ml of fresh LB from the overnight culture. Cultures are shaken at 37ºC and 200 RPM within an Erlenmeyer flask. Using a spectrophotometer to measure optical density (OD) at 600nm we observe and track the growth of the cultures. When a culture reaches OD 600 of 0.5, we harvest the cells by centrifugation. Supernatant is removed. Pelleted cells are resuspended in 5ml of ice cold 50mM $CaCl_2$. Cells are iced for 30 minutes then pelleted by centrifugation. Supernatant is removed and the chemically competent cells are now resuspended in a final volume of 2 ml 50mM $CaCl_2$. For each transformation, 100 µl are transferred into an 1.7ml Eppendorf tube on ice. Miniprepped plasmid DNA (1 µl) is added to the competent cells and they are incubated on ice for 30 minutes. Cells are heatshocked at 42ºC for 45 seconds, then placed back on ice for another 2 minutes. LB media (1 ml) is then added to the tube and allowed to grow at 37ºC for 1 hour. Cells are then harvested by centrifugation and plated on LB media with the corresponding antibiotic.

Results to Date:

During the first fall semester of this project's duration we have begun the first phases of our two-objective project. Our overall goal is the selective control of fall armyworm (FAW).

We started with four strains three of which were from a characterized turfgrass endophyte blend (Coy et al., 2014). The strains in this blend are beneficial to bermudagrass, causing significantly greater root length, volume, surface area, and shoot weight (Groover et al., 2020); they can also transiently colonize bermudagrass.

The first step was to demonstrate that we could successfully culture these endophytes on LB media. All four strains grew on LB media well (**Figure 1A**). Strain DH44 grew slower than the others. After culture, we determined the strains' antibiotic susceptibility because transformations with plasmids requires antibiotic selection (**Figure 1B**). All four strains were susceptible to common antibiotics used in most plasmids including kanamycin, chloramphenicol, and ampicillin.

After confirming antibiotic susceptibility, the next step was to attempt initial $CaCl_2$ transformations with fluorescent and/or colored marker proteins. To date, only strain AP7 could be transformed with fluorescent magenta and purple chromoprotein plasmids with chloramphenicol selectable cassettes (**Figure 1C**). So far, we have only attempted classical $CaCl_2$ chemical transformation methods and we will look to expand to higher efficiency methodologies for the other strains (see below). However, the successful transformation of AP7 is promising in that we should be able to track its colonization with fluorescent microscopy.

Future Expectations of the Project:
In the future we will try more efficient methodologies of transformation including electroporation, other chemical transformation protocols, and conjugation. We expect that more efficient transformations will facilitate transformation of the other 3 endophytic strains.

We have successfully identified a vector capable of transforming endophytes. We still need to check the stability of this plasmid in endophyte cultures. In the next phase we will sequence this plasmid and insert Cry proteins to test their toxicity against Fall Army Worm. In addition, the current plasmids can be used for tracking the localization and replication of fluorescent magenta endophytic strain AP7. Downstream we will inoculate bermudagrass plugs, observe the bermudagrass for fluorescent bacteria using microscopy, and feed the turf foliage to fall armyworm (FAW). Guts of FAW will be dissected and investigated by microscopy to observe transformed bacteria.

Further steps of this method will be to inoculate bermudagrass, observe the bermudagrass for colored bacteria, feed the turf foliage (which should be colonized by transformed bacteria) to the FAW, guts of FAW will be dissected and screened for transformed bacteria successfully transitioning to the insect midgut.

We are currently working on sanger sequencing the magenta plasmid because the company would not provide us with the genome. Knowing the sequence of the magenta fluorescent protein gene, will allow us to be able to remove this gene and insert an insect toxin gene.

References:

Groover, W., Held, D., Lawrence, K., & Carson, K. (2020). Plant growth-promoting rhizobacteria: A novel management strategy for Meloidogyne incognita on turfgrass. Pest Management Science, 76(9), 3127–3138. https://doi.org/10.1002/ps.5867

Coy, R. M., Held, D. W., & Kloepper, J. W. (2014). Rhizobacterial Inoculants Increase Root and Shoot Growth in ‘Tifway’ Hybrid Bermudagrass. Journal of Environmental Horticulture, 32(3), 149–154. https://doi.org/10.24266/0738-2898.32.3.149

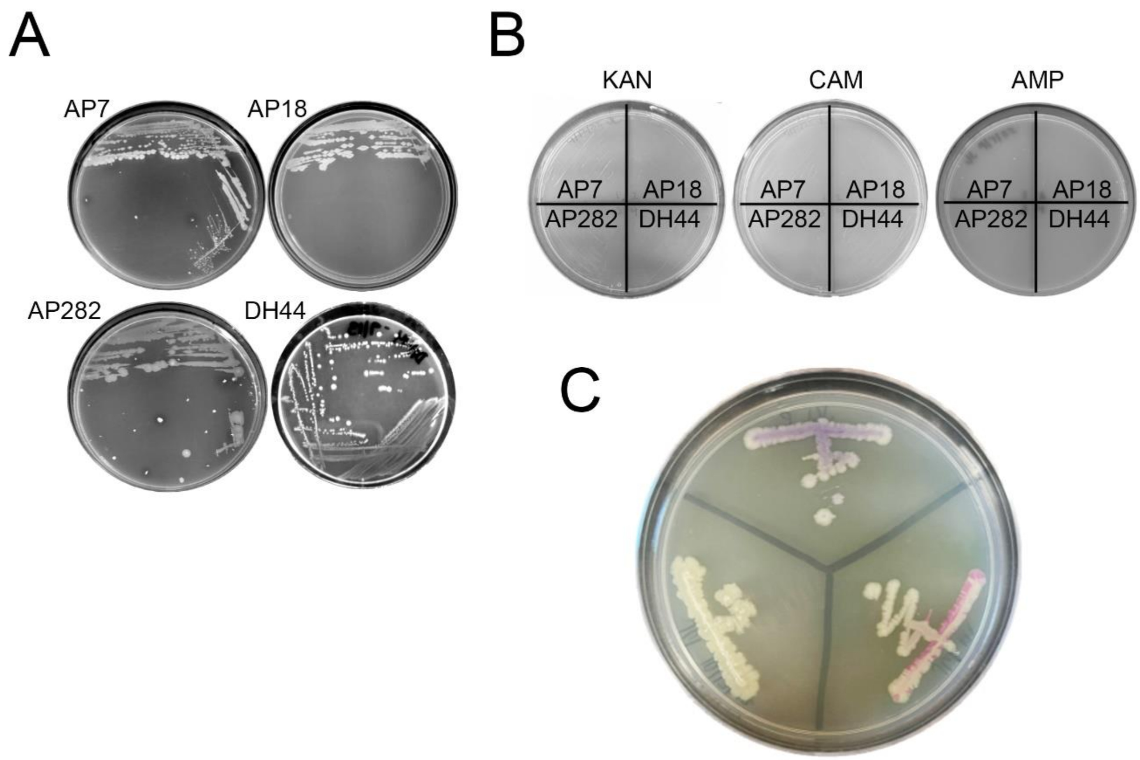


Figure 1. Transformations of turfgrass endophytes. **A.** Morphology or wild type turfgrass endophytes on LB media. AP7, AP18, AP282, DH44 were previously identified as turfgrass endophytes (Coy et al., 2014). **B.** Antibiotic susceptibility of turfgrass endophytes. The four endophytes are susceptible to three common antibiotics kanamycin (kan), chloramphenicol (cam), ampicillin (amp). **C.** Transformations of AP7 using fluorescent marker plasmids encoding purple chromoprotein (top), magenta fluorescent protein (bottom right), compared to wild type (bottom left).

USGA ID#: 2022-06-749

Project Title: Investigating white grub resistance in turf-type tall fescue

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Project Summary:
The complex of annual white grubs, including the larvae of Asiatic garden beetles, oriental beetles, Japanese beetles, and northern masked chafers, are the most destructive and widespread insect pests affecting turfgrass in the northeastern U.S. Golf course superintendents primarily rely on chemical measures for their control. Sole reliance on chemical control is a costly and not environmentally sustainable approach. Host plant resistance or increased tolerance to white grubs would be a valuable alternative. Increased tolerance to white grub feeding has been observed in tall fescue populations at the Rutgers Plant Science Research Farm in Freehold, NJ. Through the series of field and growth chamber trials proposed herein, we hope to gain a better understanding of the contributing factors to white grub resistance in turf-type tall fescue.

Research Objectives

The preliminary observations outlined above warrant more detailed investigations into the mechanisms for white grub tolerance in tall fescue (TF). The proposed research objectives include (i) determine if there is a preference for white grubs to lay eggs on certain TF populations and assess the ability of white grubs to survive among different TF populations, (ii) evaluate white grub feeding patterns for potential feeding preference on roots of different TF populations, (iii) compare TF populations for their ability to compensate for white grub root feeding, and (iv) assess TF populations for fungal endophytes to determine if there is an association with white grub feeding. Studies associated with these research objectives will be important in better understanding the interaction between white grubs and TF and progressing toward developing TF cultivars with tolerance to white grub damage, which would be a great contribution to the turfgrass industry.

Progress Report 2022

Objective 1: Determine differences in egg-laying and white grub survival among different TF populations.

A field trial was established at the Rutgers Adelphia Plant Science Research and Extension Farm in Freehold, NJ on August 24, 2022 to assess whether or not white grubs are attracted to certain TF cultivars more than others and also to investigate if eggs and larvae are able to survive on certain TF cultivars more than others. The trial included 16 TF cultivars (Table 1), which were selected based on differential grub responses in field observations during 2020. TF cultivars were seeded at a rate of 293 kg ha^{-1}. Plot sizes were 1.2 x 1.8 m, and the study was arranged in a RCB design with 5 replications per cultivar. Plot establishment was evaluated on September 13, 2022 and 3 subsequent turf quality ratings were taken from September through November (Table 1).

We utilized the most common white grub species in the region and in our 2020 observations. Oriental beetle, Japanese beetle, and northern masked chafer larvae were collected from turf sites at the Rutgers Plant Science Research and Extension Farm in Adelphia, NJ and Horticultural Farm 2 in North Brunswick, NJ from early to late October 2022 (Table 2). Some grubs were inoculated into the turf plots and the others were stored for rhizotron studies to commence in Winter of 2023. Turf plots were inoculated on October 28, 2022 (Figure 1) with 12 oriental beetle and 8 northern masked chafer larvae per turf plot. These numbers were based on the population densities of white grub species found during Fall grub collections (Table 2). Bird netting was used to cover the trial to prevent bird predation for 3 days to allow ample time for grubs to establish in the turf stand (Figure 2).

Plots will be evaluated for visual plant health monthly from April through October in 2023 and 2024. Egg count data will be collected from ten soil samples (10.2 cm diameter, 15.2 cm depth) in July 2023 and 2024. Ten samples will also be taken from each plot in September 2023, and white grubs will be identified, counted, and weighed from each sample. All eggs and grubs will be placed back into the respective plots after count and weight data have been collected. In September 2024, ten samples will be taken from each plot for white grub identification, count, and weight data. In addition, plant health data including visual root health and root length will be recorded, and root architecture will be assessed using WinRhizo root scanning software, from destructive soil samples taken from each plot. After these data have been collected, dry shoot and root weights will be recorded for each sample. Data will be analyzed with statistical analysis software, and conclusions will be made regarding the preference of white grubs for egg laying and survival characteristics.


Figure 1: Jennifer Halterman inoculating turf plots with grubs
Photo: Stacy Bonos

Objective 2: Evaluate white grub feeding patterns for potential feeding preference on roots of different TF populations.

Six rhizotron boxes made from polymethyl methacrylate (30.5 cm height x 30.5 cm width x 1.4 cm thickness) with perforated bottoms for water drainage were built in 2022 (Figure 3). Boxes were fit with a shelving compartment that can hold each rhizotron individually and can be separated to monitor water drainage per individual rhizotron to evaluate any nutrient runoff. We are currently in the process of building an irrigation system appropriate for the rhizotrons so they do not flood and shift seeds and/or tillers; this is anticipated to be completed by February 2023.

The rhizotron experiments will commence in the Spring of 2023. The rhizotrons will be filled with sterilized sand to a level of 0.5 cm from the top. One hundred seeds of 16 TF cultivars used for Objective 1 will be seeded into the rhizotrons, and a 0.25 cm deep layer of sand will be added over the seeds for moisture retention to promote germination. There will be three replications of each cultivar, and the study will be arranged in a completely randomized design. The seeded rhizotrons will be positioned in growth chambers with 12 h photoperiods, 60% relative humidity, and 21 C and 18 C daytime and nighttime temperatures, respectively. Plants will be watered daily and fertilized biweekly. Rhizotrons will be reorganized two times per week within growth chambers.


Figure 2: Grub trial with bird net protection.
Photo: Stacy Bonos

Weights of grubs will be recorded prior to placement in rhizotrons. Turfgrass plants will be visually evaluated for plant health weekly throughout the study. The study will continue for an additional 4 wks and the grubs will be reweighed to determine the change in weight. Turfgrass plants will be removed from the rhizotrons and cleaned of sand. Roots will be evaluated for visual root health and root length measurements. In addition, root architecture will be assessed using WinRhizo root scanning software.

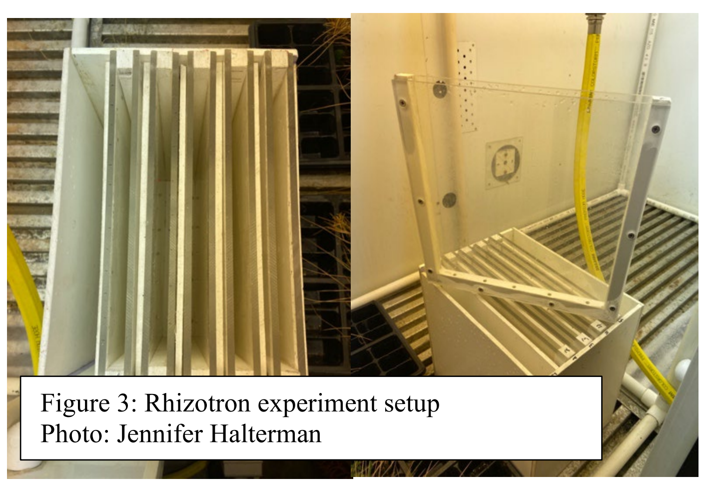


Figure 3: Rhizotron experiment setup
Photo: Jennifer Halterman

After all these data have been collected, dry shoot and root weights will be recorded for each unit. The trial will be conducted twice.

Objective 3: Compare ability of TF populations to compensate for white grub root feeding.

Another consideration is that certain TF cultivars are able to compensate for white grub feeding better than others. This could be due to tolerant cultivars having stronger root systems, being able to regenerate roots quicker, or having lower water requirements to sustain green vegetation. To explore these hypotheses, two trials will be initiated with trial 1 investigating root architecture among the TF cultivars under well-watered and drought stress conditions and trial 2 investing their root regeneration capacity. For this objective, oriental beetle and northern masked chafer grubs were collected and stored for growth chamber experiments (Figure 4). Rhizotrons have been built and have storage compartment as described in Objective 2, and rhizotron studies will be initiated in Spring 2023.

For trial 1, rhizotrons will be filled with sterilized sand to a level of approximately 0.5 cm from the top. One hundred seeds of the 16 TF cultivars used for Objective 1 will be seeded into the rhizotrons as described above. The study will be arranged in a completely randomized design with six replications (3 replications for well-watered treatments, 3 replications for drought stress treatments) of each cultivar. Environmental conditions will be the same as above. Plants will be watered daily and fertilized biweekly for a 5 wk establishment period. Rhizotrons will be randomly reorganized two times per week to increase homogeneity of environmental conditions within growth chambers. After the 5 wk establishment phase, the rhizotrons will be divided into a well-watered group and a drought stress group. Turfgrass plants will be visually evaluated for plant health weekly throughout the study. The study will continue for an additional 6 wks. At the end of the study, turfgrass plants will be removed from the rhizotrons and cleaned of sand. Roots will be evaluated for visual root health and root length measurements. In addition, root architecture will be assessed using WinRhizo root scanning software. After all these data have been collected, dry shoot and root weights will be recorded for each unit. The trial will be replicated two times.

For trial 2, the 16 TF cultivars will be as described in Objective 1. After the 3 wk establishment phase, the front panels of the rhizotron will be lifted and roots will be cut to a length of 0.3 cm to simulate white grub feeding damage. For the following 4 wk period, rhizotrons will continue to receive daily irrigation and biweekly fertilization. Turfgrass plants will be visually evaluated for plant health weekly throughout the study. At the end of the study, turfgrass plants will be removed from the rhizotrons and cleaned free of sand. Roots will be evaluated for visual root health and root length measurements. In addition, root architecture will be assessed using WinRhizo root scanning software. After all the data have been collected, dry shoot and root weights will be recorded for each unit. The trial will be conducted twice.

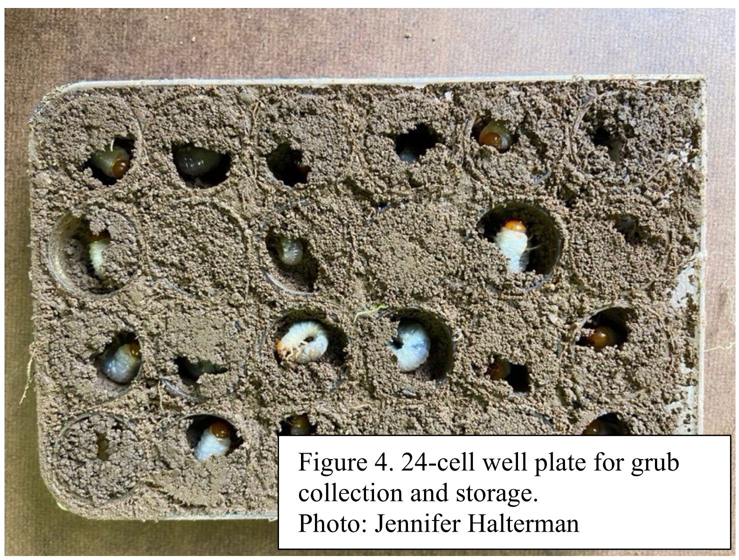


Figure 4. 24-cell well plate for grub collection and storage.
Photo: Jennifer Halterman

Objective 4: Assess TF populations for fungal endophytes to determine association with white grub feeding.

The 16 TF cultivars mentioned above will be evaluated for presence of fungal endophytes following the protocol by Bacon and White (1994). Ten tillers of each cultivar will be cut into 2-3 cm sections and epidermal cells will be gently scraped with a scalpel. Parenchyma tissue will be transferred onto clean glass slides. The tissue will be stained with aniline blue or rose Bengal and examined for the presence of atypical non-branching endophytic mycelia under the 40X objective of a compound light microscope (Figure 5). These observational data will be analyzed along with data from field and growth chamber trials to identify potential associations between endophyte presence and white grub tolerance in TF; this is anticipated to be completed May 2023.

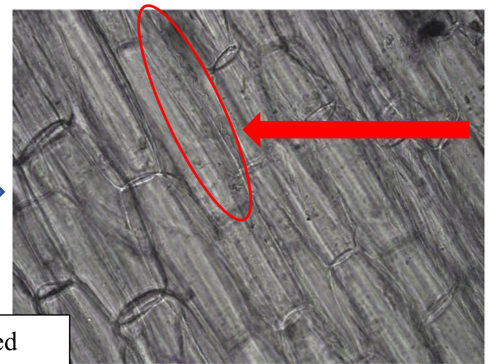


Figure 5. Left: Tillers collected from 2020 turf trials; Right: Endophytic fungi growing within plant cells. Photo: Jennifer Halterman

Table 1. Establishment and turf quality ratings of tall fescue cultivars seeded in August 2022 at the Rutgers Adelphia Plant Science Research and Extension Farm, Freehold, NJ.

			9=best
		Establishment	Turf Quality
		13-Sep	2022
Rank	Cultivar	2022	Avg.
1	Line Drive II	8.2	8.3
2	Bullseye LTZ	5.4	7.3
3	Degas	4.8	7.1
4	Thor	5.8	6.9
5	Technique	4.6	6.7
6	PPG TF-303	6.4	6.7
7	Padre II	6.2	6.5
8	Firenza II	4.4	6.5
9	Trinity	4.4	6.5
10	FireCracker SLS	4.4	6.3
11	Annapolis	5.2	6.3
12	Renegade DT	4.2	6.0
13	Bandit	5.2	5.9
14	Crossfire 3	2.0	4.3
15	GO-FNKY	2.6	3.9
16	Meridian	1.0	2.7
	CV=	19.3	12.1
	LSD at 5%=	1.1	0.9

Table 2. Grub species and number collected from turf sites at the Rutgers Plant Science Research and Extension Farm in Adelphia, NJ and Horticultural Farm 2 in North Brunswick, NJ from early to late October 2022.

Grub Species	Count
Northern masked chafer	810
Oriental beetle	1,136
Japanese beetle	303
Total	2249

References:

Bacon, C.W. and J.F. White. 1994. Stains, media, and procedures for analyzing endophytes. Biotechnology of endophytic fungi of grasses. 47:56.

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, Texas Tech University, The Davey Tree Expert Company

Objectives: (unchanged from 2021 report)

1. Organize our laboratory experiment on the effects of carfentrazone-ethyl (carfentrazone or CZ here) and light intensity on silvery-thread moss (STM, also *Bryum argenteum*) into a manuscript suitable for publication.
2. Initiate and complete field experiments on surfactants on the inhibition silvery-thread moss in experimental and working putting greens in Ohio.
3. Characterize the genetic diversity of silvery-thread moss in the United States using specimens from on- and off-golf courses by comparing to a newly assembled and annotated genome sequence.

Start date: January 1, 2019

Project duration: 3 years

Total funding: $119,991

Summary Points:

1. In order to understand (a) how golf greens become contaminated with STM (local adaptation vs. dispersal), and (b) how diverse the strains (genotypes) of STM are when compared against off-green (native) strains, we initiated a genetic approach consisting of sequencing the *Bryum argenteum* genome (RNA and DNA). Seventeen genotypes were sequenced over the last year.

2. Manuscript submitted on "The Effects of Carfentrazone Ethyl on the Growth and Survival of Bryum argenteum, the Silvery-Thread Moss". An abstract of this manuscript is given under Results to Date.

3. Experiment on the effectiveness of SDS (sodium dodecyl sulfide, a surfactant in soaps) is completed and the data are under preparation for a second manuscript. SDS was superior to carfentrazone in suppressing STM, with a dosage of 0.5% concentration for one minute reducing the photosynthetic capacity to levels associated with cell death (Fv/Fm <0.2). Shoot regeneration was suppressed to ~10% of controls (near complete shoot suppression), unlike the carfentrazone treatments. The treatment (0.5%) used in the lab is much less than the SDS concentration of Dawn® dishsoap (~20%).

5. Drench applications of Dawn dishsoap and SDS resulted in greater long-term reduction of STM compared to applications of carfentrazone in a research putting green at Hawks Nest Golf Course in Creston, OH. An experiment was initiated in 2022 to help discern whether asexual

reproduction from vegetative structures or sexual reproduction from spores is functioning as the primary dispersal mechanism.

Summary Text:

Rationale (unchanged from 2021 report)
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/companies (UNLV, Texas Tech U., Ohio State / Davey Tree) 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) on the growth response and photosynthetic health of STM; (2) determine the effect of carfentrazone 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

UNLV. During 2022, we submitted a manuscript on the effects of dosing STM with carfentrazone at different light intensities, and a second manuscript is in preparation on the effects of dosing STM with the surfactant SDS (sodium dodecyl sulfide). A pilot experiment on the ability of STM rhizoids to regenerate STM shoots after dosing with an SDS solution that kills STM shoots, was conducted as a prelude to a full experimental approach. Finally, the UNLV lab of Llo Stark is transitioning to an off-campus location, and key golf course STM collections and cultures will be preserved and relocated for future studies on the longevity of STM plants once discarded from the putting green enviroment.

Texas Tech. During 2022, efforts continued to produce sequences from two clones of STM, one male and one female, with the goal of assembling and annotating a new (to science) STM genome. We sequenced the RNA and DNA of both genotypes using "short-read" Illumina technology (NovaSeqSP6000), resulting in 84.3 Gbp (gigabase pair) of DNA and 12.6 Gbp of RNA sequences. To produce a more contiguous DNA assembly, we also used single-molecule sequencing of the female genotype. We used the Oxford NanoporePromethlON sequencer at the University of Connecticut to generate 66.9 Gbp of additional long-read data. We filtered the long-read data to remove contaminant sequences from fungi, bacteria, and viruses, and assembled using Flye to generate an initial assembly of 556 Mbp (megabase pairs) across 5,109 contiguous sequences (contigs). We used the short-read sequence data to filter and further remove overlapping contigs belonging to fungal genomes, and arrived at a final assembly of 350 Mbp across 2,735 contigs. We also produced Illumina short-read sequences from 17 genotypes of STM maintained in cell culture at TTU. Six of these genotypes are from golf courses, while the remainder are from nearby natural areas.

Davey Tree. Currently, it is unclear whether asexual or sexual reproduction is the primary dispersal mechanism that enables STM to infest golf course putting greens. Additionally, it is unknown whether a putting green infested with STM is comprised of a single genotype or multiple genotypes. The former is highly plausible if vegetative, asexual reproduction is serving as the primary dispersal mechanism while the latter is more likely if sexual reproduction via spores are responsible. Understanding which of these mechanisms is involved will elucidate which of the integrated weed management practices will be most critical for developing a long-term control strategy. In an attempt to better understand the mechanisms involved, samples of STM were collected from five separate putting greens from Hawks Nest Golf Course in Creston, OH. Within each putting green, three spatially separate STM infestations were identified and strategically sampled by collecting 1” diameter cores to a depth of 2”. Six random samples were collected from each infestation on the five putting greens (N = 90). The samples will be processed and analyzed to determine the genetic differences between the samples.

Results to date

UNLV. The abstract of our submitted manuscript (Greenwood, Raudenbush, Johnson and Stark) follows in slightly reduced form: This work examines multiple light regimes for carfentrazone ethyl (CZ) application when used to control *Bryum argenteum* (i.e., silvery-thread moss). We exposed samples to 0 ppm, 6 ppm, and 30 ppm concentrations of CZ for one hour followed by a 10-hour exposure to either a 1000, 1500, or 2000 of PAR (photosynthetically active radiation). We identified a relationship between PAR levels and effectiveness in suppression of moss growth and survival as well as a direct relationship between PAR + CZ in the control of sprouting of new shoots, protonemal growth, and photosynthetic efficiency (chlorophyll fluorescence). More intense light application improved the effectiveness of CZ. Our findings support CZ application during times of intense sunlight (high PAR) for maximal utility. A second manuscript is in preparation on the effects of SDS (sodium dodecyl sulfide) on STM. In summary, Both native (off-green) and green (on-green) STM were killed or nearly killed using a short exposure to 0.5% SDS (Fig. 1B of 2021 Annual Report). Nearly complete suppression of new shoot formation occurred following exposure to STM shoots to SDS (Fig. 1D of 2021 Annual Report). The rhizoid pilot experiment noted under Methodology resulted in a failure to demonstrate significant rhizoid regeneration even under control conditions, a puzzling result we are considering. Key golf course genotypes of STM were transferred to Stark’s off-campus lab during November of 2022. They will be retained as viable desiccated cultures/collections.

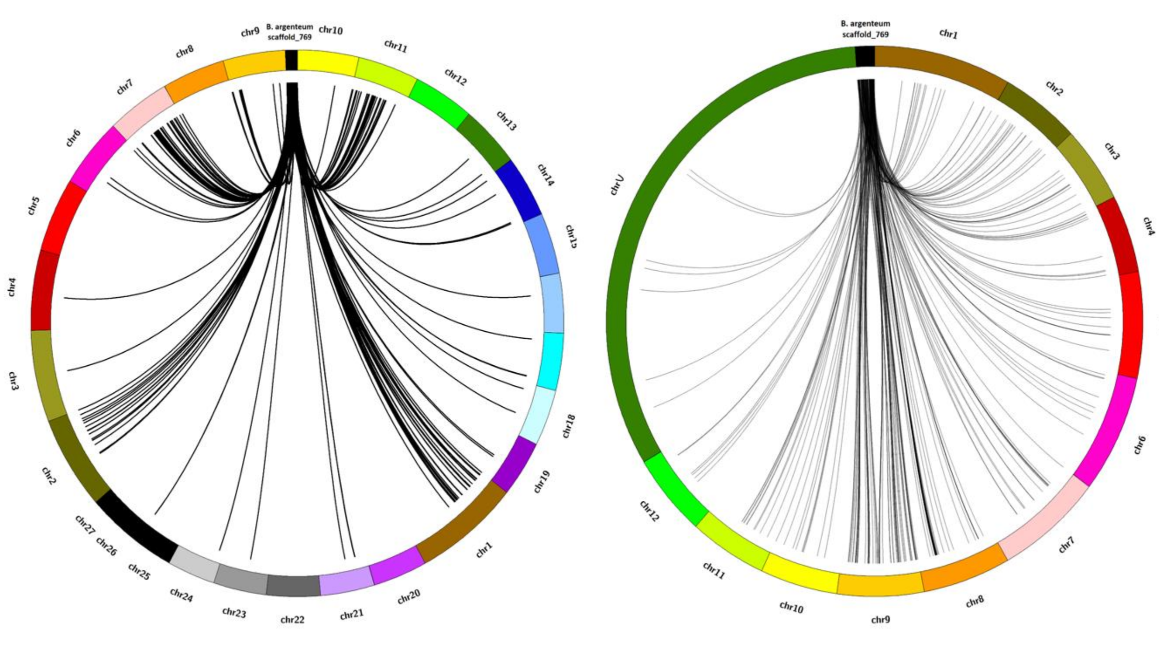
Texas Tech. The STM genome assembly contains 81.5% of all "universal" genes found in all land plant genes, and 95.7% of universal genes found in all green plants. Our initial genome assembly for STM suggests a large amount of genome evolutionary reorganization since the last common ancestor with the mosses *Ceratodon purpureus* and *Physcomitrium patens,* the closest relatives of STM with published genomes (**Figure 1**). This finding is surprising because previous results had indicated conserved genome structure between *C. purpureus* and *P. patens* despite 250 million years of evolution. We have now supplemented the genome assembly with additional sequencing of 17 STM genotypes, including samples of golf course and off-course origin. Our current assembly of the STM genome is in more than 1000 scaffolds (non-continuous genomic sequences separated by gaps of known length); we are currently awaiting a final round of genome sequencing that we anticipate will reduce the number of scaffolds to approach the number of chromosomes in the STM genome (11).

Davey Tree. Samples were recently collected and have not been analyzed for their genetic makeup, but results will be forthcoming. A manuscript is currently being drafted to report the results from a field study completed in 2020-2021 to evaluate the effects of chemical and cultural control of STM in creeping bentgrass putting greens.

Future expections for project

Our goals for the next phase of this project include getting both of the manuscript products of the research published, and to continue to address projects on STM that we were unable to complete during the timeline of this USGA project, but which are necessary to understanding the colonization process of STM on golf greens. To this end, we plan to identify the number and spatial distribution of genetically differentiated female clones present on five putting greens in an Ohio golf course. The genome sequence and set of reference individuals will yield valuable data on the relatedness of STM patches on golf courses and crystallize hypotheses for understanding how STM colonizes golf course putting greens.

Figure 1. Genome synteny (located on the same chromosome) analysis between one genome scaffold of *B. argenteum* (STM, in black at the top of each circle) and the genomes of two model moss species, *Physcomitrium patens* (left) and *Ceratodon purpureus* (right). Lines indicate areas of matching DNA sequence – this figure shows the genome of *Bryum argenteum* is highly reorganized compared with the genomes of the other model moss species.



USGA ID#: 2020-15-720

Title: Improving weed control and playability in naturalized fine fescue areas

Project Leader: Matthew T. Elmore, Phillip L. Vines, and Katherine H. Diehl

Affiliation: Rutgers, The State University of New Jersey

Objectives:
1. Identify the ideal herbicide application timing for deertongue grass control
2. Determine if frequent mowing provides deertongue grass control alone or in combination with an herbicide application
3. Evaluate various plant growth regulators in combination with two different fine fescue blends to determine effects on characteristics associated with playability in naturalized areas

Start Date: 2020
Project Duration: 3 years
Total Funding: $89,842

Summary Points:
- Glyphosate is extremely effective for deertongue grass control when applied at 175 GDD in springtime. Fluazifop was less effective than glyphosate. Fluazifop is a less effective option but with less risk of turfgrass injury. Fluazifop should be applied at 175 GDD or 25 CDD, but not in mid-summer for best efficacy.
- Glyphosate applied at 175 GDD was extremely effective for deertongue grass control and no fine fescue injury was observed in either experiment, but more research on glyphosate tolerance should be conducted before this treatment is recommended.
- Monthly mowing reduced deertongue and improved herbicide efficacy, especially fluazifop which was less effective than glyphosate without mowing.
- The less competitive hard fescue cultivar ‘Beudin’ had better playability than the more competitive cultivar Gladiator when both were in combination with ‘Quatro’ sheeps fescue in 2023.
- Ethephon + trinexapac-ethyl was more effective than other PGRs evaluated in improving playability of fine fescue areas in both years. Triclopyr improved playability in 2022.

Summary Text: 800-1200 words

2020 Efficacy Trial

The first phase of this research was completed in 2020 at Mendham Golf and Tennis Club (Mendham, NJ) to identify optimal timings for deertongue grass (*Dichanthelium clandestinum*) control in naturalized fine fescue areas. The conclusions of that experiment were largely presented in the 2021 report. This experiment determined that glyphosate is much more effective than fluazifop for deertongue grass control. Fine fescue injury was not observed at any time during the experiment (data not presented).

2021-2022 Efficacy Trial

In 2021, a trial building on the result of the aforementioned 2020 trial was initiated. The trial investigated glyphosate and fluazifop efficacy at two different application timings in a factorial with monthly mowing. Application timings were 175 GDD and 25 CDD, based on the 2020 experiment which found these to be effective timings. A more comprehensive explanation of methods can be found in the project proposal. For glyphosate only programs, glyphosate (560 g ha^{-1}) was applied at 175 GDD on May 13, 2021 and 25 CDD on September 27, 2021. Given the limited efficacy of single-application fluazifop programs observed as the trial was ongoing, a sequential fluazifop (280 g ha^{-1}) application was added to both treatments. The 175 GDD fluazifop treatment (May 13, 2021) was also treated with fluazifop at 25 CDD on September 27, 2021 and the fluazifop program initiated at 25 CDD was treated on September 27, 2021 and May 26, 2022. Mowing was initiated three weeks after the 175 GDD applications in each year and concluded in August 2022. A walk-behind push mower bench set to 6" was used.

Mowing-by-herbicide regimen interactions were significant on all rating dates in 2021 and 2022 for deertongue grass control (Table 1; Figures 1 and 2). In the absence of mowing, glyphosate applied at 175 GDD was the most effective treatment, providing 100% control in July and August 2022. Glyphosate applied at 25 CDD was less effective by July and August 2022, providing < 75% control. Efficacy of fluazifop regimens was similar at the conclusion of the experiment in August 2022. Efficacy of the 25 CDD fluazifop regimen was greater in June and July 2022 which we attribute to the 25 CDD regimen being treated more recently (May 2022) than the 175 GDD regimen (September 2021). Except for glyphosate applied at 175 GDD (which provided 100% control), mowing improved the efficacy of all herbicide treatments. From June to August 2022, all herbicide treatments provided similar >80% control in combination with mowing. In August 2022, all herbicides and the non-treated control provided similar (>90%) deertongue grass control in combination with mowing.

Assessments of deertongue grass cover revealed similar trends to control ratings, although the herbicide-by-mowing interaction was non-significant (Table 2). All glyphosate regimens resulted in ≤ 1% deertongue grass cover in August 2022 compared to 10 to 22% cover for fluazifop regimens without mowing and < 5% cover for fluazifop regimens with mowing.

A separate area for the 2022 experiment was established in autumn 2021, however this trial site was deemed unusable during the summer of 2022 after the spring herbicide applications for three reasons: 1) While planting the deertongue grass vegetative material in August 2021, the fine fescue was mostly killed due to the foot traffic that occurred during planting and the high heat, 2) The site was seeded with fine fescue in September 2022 to recover from traffic damage, but both fluazifop and glyphosate treatments applied at 175 GDD severely injured the fine fescue in 2022, and 3) Deertongue grass establishment was relatively poor. Thus the decision was made to terminate this trial in late summer 2022.

2022 Playability Trial

After establishment in 2020, a trial was initiated in 2021 to evaluate various herbicides and plant growth regulators in combination with fine fescue blends on fine fescue thinning (playability) and seedhead production. Results of the 2021 trial and assessment methods can be found in the previous report. A lodging, aesthetic, and density rating was conducted in 2022, but not 2021. See Table 3 footnote for more details.

Plant growth regulator (PGR)-by-cultivar blend interactions were not detected on any rating date in 2022. The effect of cultivar blend was significant only for clipping yield biomass.

Herbicides and traditional plant growth regulators (hereafter referred to collectively as PGRs) included indaziflam (Specticle FLO; 33 g ha^{-1}), ethofumesate (Prograss EC; 2.2 kg ha^{-1}), trinexapac-ethyl (Primo Maxx; 220 g ha^{-1}) + ethephon (Proxy; 3.8 kg ha^{-1}), and triclopyr (Turflon Ester Ultra; 1.12 kg ha^{-1}) with a non-treated included for comparison. Treatments were applied singly on May 11, 2022 at the first emergence of fine fescue seedheads. To encourage biomass production, N fertilizer was applied on April 15 at 25 kg N ha^{-1}. A second application was made on May 11, 2022 a 45 kg ha^{-1} to the entire site in combination with pendimethalin for preemergence annual weed control. Clopyralid was also applied to the entire site for broadleaf weed control.

The plant growth regulators ethephon + trinexapac-ethyl and triclopyr resulted in the best playability in June and August (Table 3, Figure 3). Ethofumesate improved playability compared to the non-treated control was but was less effective than ethephon + trinexapac-ethyl and triclopyr. Indaziflam did not improve playability. No treatments improved playability in October. Ethephon + trinexapac-ethyl and triclopyr also reduced seedhead count and lodging.

The Beudin + Quatro mixture resulted in 25% less clipping yield biomass than the Gladiator + Quatro mixture. The plant growth regulators ethephon + trinexapac-ethyl and triclopyr reduced clipping yield biomass by approximately 15% compared to the non-treated control. This trial will be repeated in 2023 on the same site.

Future Research Expectations:

Deertongue Efficacy Research

A similar concept will be applied to evaluate quackgrass (*Elymus repens*) control in 2023. Deertongue grass data will be evaluated in more detail to determine if the two different experiments conducted in 2020 and 2021 to 2022 can be published.

Fine Fescue Playability Research

It is possible that since this was year two of stand maturity, it allowed the different fine fescue mixtures to better express their differences. This was also the first year that biomass differences were apparent. To see if these differences continue, this trial will be repeated in 2023.

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Table 1. Effects of monthly mowing and herbicide applications on deertongue grass control in 2021 and 2022 in North Brunswick, NJ. Glyphosate (560 g ha^{-1}) and fluazifop (280 g ha^{-1}) were applied at 175 growing degree-days (GDD) on May 13, 2021 and at 25 cooling degree-days (CDD) on September 27, 2021. The 175 GDD fluazifop treatment (May 13, 2021) was also treated with fluazifop at 25 CDD on September 27, 2021 and the fluazifop program initiated at 25 CDD (September 27, 2021) was also treated on and May 26, 2022.

Mowing	**Herbicide**	**4 WAIT (June)**	**8 WAIT (July)**	**12 WAIT (August)**	**16 WAIT (October)**	**55 WAIT (June 2022)**	**59 WAIT (July 2022)**	**63 WAIT (August 2022)**
	Mowing							
		Deertongue grass control (%)						
Mowing	-	-[†]	68		81	90 a	90 a	94 a
No mowing	-	-	31		32	60 b	56 b	55 b
	Pr > F	-	*<0.001*	*<0.001*	*0.002*	*<0.001*	*<0.001*	*0.005*
	Herbicide							
-	Glyphosate 175 GDD	74 a[‡]	91 a	84 a	95 a	96 a	98 a	99 a
-	Fluazifop 175 GDD	59 b	68 b	60 b	74 b	63 b	61 bc	71 b
-	Glyphosate 25 CDD	-	-	-	39 c	93 a	84 a	83 ab
-	Fluazifop 25 CDD	-	-	-	41 c	88 a	79 ab	76 b
-	None	0 c	34 c	36 c	34 c	37 c	41 c	45 c
	Pr > F	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.005*
	*Herbicide * mowing*							
Mowing	Glyphosate 175 GDD	-	97 a	78 a	95 a	95 a	95 ab	99 a
	Fluazifop 175 GDD	-	65 cde	75 a	83 ab	83 abc	84 abc	91 ab
	Glyphosate 25 CDD	-	-	-	79 bc	100 a	96 a	98 a
	Fluazifop 25 CDD	-	-	-	83 ab	100 a	90 abc	93 a
	None	-	68 cd	73 a	69 c	74 c	83 abc	91 ab
No mowing	Glyphosate 175 GDD	-	85 b	91 a	95 a	96 ab	100 a	100 a
	Fluazifop 175 GDD	-	71 c	45 b	65 c	43 d	39 d	50 c
	Glyphosate 25 CDD	-	-	-	0 d	86 abc	73 bc	68 bc
	Fluazifop 25 CDD	-	-	-	0 d	75 bc	69 c	59 c
	None	-	0 f	0 c	0 d	0 e	0 e	0 d
	Pr > F	-	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*0.002*

[†]The first mowing treatment was conducted 4 weeks after the 175GDD herbicide application and effects were not borne out at the June 2021 rating. Thus they are not presented.

[‡]Means followed by the same letter are not significantly different according to Fisher's Protected LSD test; P=0.05.

Table 2. Effects of monthly mowing and herbicide applications on deertongue grass cover in June 22, 2022 in North Brunswick, NJ. Glyphosate (560 g ha^{-1}) and fluazifop (280 g ha^{-1}) were applied at 175 growing degree-days (GDD) on May 13, 2021 and at 25 cooling degree-days (CDD) on September 27, 2021. The 175 GDD fluazifop treatment (May 13, 2021) was also treated with fluazifop at 25 CDD on September 27, 2021 and the fluazifop program initiated at 25 CDD (September 27, 2021) was also treated on and May 26, 2022.

Mowing	**Herbicide**	**Grid count cover**	**Visual cover**
	Mowing		
Mowing	-	12	12
No mowing	-	2	3
	Pr > F	*0.18*	*0.10*
	Herbicide		
-	Glyphosate 175 GDD	1 b	1 b
-	Fluazifop 175 GDD	6 ab	7 ab
-	Glyphosate 25 CDD	0 b	1 b
-	Fluazifop 25 CDD	12 ab	13 a
-	None	17 a	15 a
	Pr > F	*0.04*	*0.01*
	*Herbicide * mowing*		
Mowing	Glyphosate 175 GDD	1	1
	Fluazifop 175 GDD	3	4
	Glyphosate 25 CDD	0	0
	Fluazifop 25 CDD	2	3
	None		
No mowing	Glyphosate 175 GDD	0	1
	Fluazifop 175 GDD	10	11
	Glyphosate 25 CDD	0	1
	Fluazifop 25 CDD	22	23
	None		
	Pr > F	*0.12*	*0.07*

Table 4. Effects of plant growth regulators and two cultivar blends on fine fescue seedhead production, playability, and biomass production in 2022 in North Brunswick, NJ.

Cultivar	PGR	Playability June	Playability August	Playability October	Seedheads	Biomass	Aesthetic	Lodging	Density
			1 to 9†		#‡	g per plot¥	1 to 9¶		%
Cultivar blend									
Beudin + Quatro	-	5.5	5.2	4.9	24	284 b	5.6	5.1	89
Gladiator + Quatro	-	5.4	4.3	5.1	24	388 a	6.1	5.8	92
	Pr > F	*0.81*	*0.17*	*0.60*	*0.97*	*0.04*	*0.53*	*0.49*	*0.06*
Plant growth regulator (PGR)									
-	ethofumesate	5.3	4.8 b	5.3	27 ab	365 a	5.3 b	4.9 b	91 ab
-	indaziflam	3.7	3.1 c	4.6	31 a	365 a	7.3 b	3.0 b	96 a
-	ethephon + trinexapac-ethyl	7.5	6.3 a	5.3	21 bc	305 b	4.8 a	7.9 a	87 bc
-	triclopyr	7.4	6.3 a	5.4	17 c	293 b	4.8 a	8.0 a	82 c
-	None	3.4	3.1 c	4.4	24 abc	350 ab	7.1 b	3.4 b	96 a
	Pr > F	*< 0.001*	*<0.001*	*0.37*	*0.01*	*0.056*	*< 0.001*	*< 0.001*	*0.003*
*Cultivar * PGR*									
Beudin + Quatro	ethofumesate	5.0	5.0	5.0	33	334	4.8	4.0	91
	indaziflam	3.8	3.5	4.3	30	320	7.0	2.5	98
	ethephon + trinexapac-ethyl	8.3	7.0	5.3	18	255	5.3	8.8	81
	triclopyr	7.0	6.5	6.0	17	250	4.5	7.8	75
	None	3.5	2.5	3.8	21	260	7.5	7.8	97
Gladiator + Quatro	ethofumesate	5.5	4.5	5.5	21	397	5.8	5.8	90
	indaziflam	3.5	2.8	5.0	33	408	7.5	8.3	94
	ethephon + trinexapac-ethyl	6.8	5.5	5.3	24	354	5.3	7.0	93
	triclopyr	7.8	6.0	4.8	17	337	5.0	8.3	89
	None	3.3	3.8	5.0	26	441	6.8	4.3	95
	Pr > F	*0.36*	*0.86*	*0.14*	*0.15*	*0.18*	*0.55*	*0.28*	*0.47*

†1 (least playable) to 9 (most playable) scale. Playability was measured by estimating how easily a golfer would be able to play a ball from the plot.

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¶Aesthetic a subjective assessment [1 (worst) to 9 (best) scale] that scored plots with a high number of seedheads of typical length, and long leaves of fine fescue higher than plots with fewer, shorter seedheads and shorter fescue leaves. It did not take into account playability and was assessed on 15 July 2022. Lodging [1 (completely lodged) to 9 (no lodging) scale] was a subjective assessment that took into account the severity of both seedhead and leaf lodging; assessed on 15 August 2022.
‡The number of fine fescue culms per square foot on 29 June 2022. The mean of three counts per plot is presented.
¥Biomass was collected as the clipping yield collected by making a pass down the center of each plot with a rotary mower bench set to 3”. Clippings were dried prior to weighing.
§Means followed by the same letter are not significant different according to Fisher’s Protected LSD test; P=0.05.

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Figure 1. A non-treated plot (left) compared to a plot treated with glyphosate at 175 GDD (right), photographed on August 31, 2022 showing excellent deertongue grass control.

Figure 2. A non-mowed plot treated with fluazifop at 175 GDD (left) and a mowed plot treated with fluazifop at 175 GDD (right) photographed on August 31, 2022


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Figure 3. Fine fescue playability trial on July 27, 2022 showing the non-treated plot (left) and ethephon + trinexapact-ethyl (right).

USGA ID#: 2020-10-715

Project Title: Golf course biodiversity project: Facilitating abundance and biodiversity of at-risk taxa on golf course ecosystems

Project Leaders: [1]Joe Milanovich, Ph.D, [1]Martin Berg, Ph.D, [2]Seth Magle, Ph.D., [2]Liza Lehrer, M.S.

Affiliation: [1]Loyola University Chicago, [2]Lincoln Park Zoo

Objective: The objectives of this research are to: (1) conduct an analysis of the effectiveness of newly constructed pollinator gardens within golf courses to increase abundance and diversity of diurnal and nocturnal invertebrate pollinators, (2) examine the usefulness of placing bat boxes within golf courses to facilitate the abundance and diversity of bats, and (3) quantify whether additions of coarse woody debris in golf course ponds can measurably increase abundance and biodiversity of macroinvertebrates.

Start Date: 2020
Duration: 3 years
Total Funding: $60,000

Summary:

- Best management practices including 25 m^2 pollinator gardens, Rocket booster bat boxes, and addition of coarse woody debris were constructed at 5 golf courses in Cook, DuPage, McHenry, and Lake Counties, IL.
- Sampling of bats, macroinvertebrates, and diurnal and nocturnal pollinators was conducted between June to September within golf courses with BMPs and 5 courses without.
- Identification, enumeration, and analysis of data is currently ongoing.

Diurnal and nocturnal pollinators: At 10 sites (5 experimental and 5 control) we quantify abundance and diversity of diurnal pollinators within the non-turfgrass areas (control sites) or within the planted pollinator gardens (experimental sites) once per month between May to September 2020, 2021, and 2022. We did this by creating two plots per course and surveying each plot for two consecutive 10 min surveys – for a total of 160 total pollinator surveys. During each 10 min survey we categorized pollinators into 9 functional groups and noted abundance of each group (Fukase and Simons, 2016). Nocturnal pollinators were sampled at each site at one of the plots (within the pollinator garden for experimental courses) once per month between May to September using a 32 Watt light trap with a 365 Quantum Black Light from Bioquip, Inc. Light traps were turned on at 1030 pm and operated until 0630 am. Contents were collected the following morning and stored in 70% ETOH. In 2021, data collection of pollinators at one non-experimental site was removed.

Our result to date show diurnal pollinators were not significantly different between experimental (garden) and control (no garden) sites in 2020, 2021 or 2022 (Two-way ANOVAS, $P < 0.05$; Fig. 1); however, several taxa were significantly higher in abundance in 2022 (Fig. 1). For example, large bees, large fly's, and other taxa were found in greater numbers across all sites in 2022 compared to 2020 and 2021 (Fig. 1). Sampling was altered in 2021 at two sites unexpectedly, as one course removed and moved a pollinator garden (and subsequent bat box) in June 2021 and pollinator sampling at one site did not occur due to restricted access of project personnel. To date, we processed and identified 63 nocturnal pollinator samples across all 10 sites.

Across treatment (n = 37) and control (n = 26) sites between 2020 and 2021 our results indicate nocturnal pollinator diversity was significantly different between control (H = 1.89) and treatment (H = 1.25) sites.

Macroinvertebrate assessment: Between May to September 2020-2022, we collected 3 macroinvertebrate samples at each site (experimental and control) once per month using a 20 cm diameter 80 µm mesh plankton net attached to a 74 µm mesh bucket and a D-frame dip net (500 µm mesh) across a 0.3 m (linear) area. Samples (150 total) were immediately stored in 70% ETOH and are currently being analyzed. In addition, at each site we collected a suite of water quality variables using a YSI multiprobe. All of these samples were taken from the same locations examined during the 2016-2019 USGA study, and from 2020.

To date, 80 macroinvertebrates samples have been sorted and 54 of those have been identified and enumerated. A total of 14 classes/orders, 42 families, and 62 genera were collected during this study. Post-nMDS cluster analysis using maximum silhouette width revealed similar results in taxa and FHG relative density analyses (Figs. 2 and 3) grouping GC2 and GC5 together (group 1) and GC1, GC3, GC6, and GC4 together (group 2). In both cases these groups cluster together along NMDS axis 1 with group one on the left and group 2 on the right. Weighted average calculations of taxa relative densities for each pond illustrated that *Caenis* sp. and *Orthotrichia* sp. most influenced the clustering of group 1,whereas *Coenagrion* & *Enallagma* sp., *Callibaetis* sp., *Menetus* sp., *Turbellaria*, *Physa* or *Physella* sp., *Probezzia*/*Bezzia*/*Palpomyia* spp., *Bithynia tentaculatum*, *Culicoides*/*Stilobezzia*/*Ceratopogon* spp., *Cipangopaludina* sp., *Euhirudinea*, *Fossaria* sp., *Gyraulus* sp., *Hyallela* sp., *Hydrobiidae*, *Ischnura* sp., *Libellula* sp., and *Sphaeriidae* most influenced the clustering of group 2. In the FHG cluster analysis weighted average calculations of FHG relative densities revealed that climbers most influenced clustering of group 1, whereas sprawlers, burrowers, and clingers most influenced clustering of group 2. The two-way

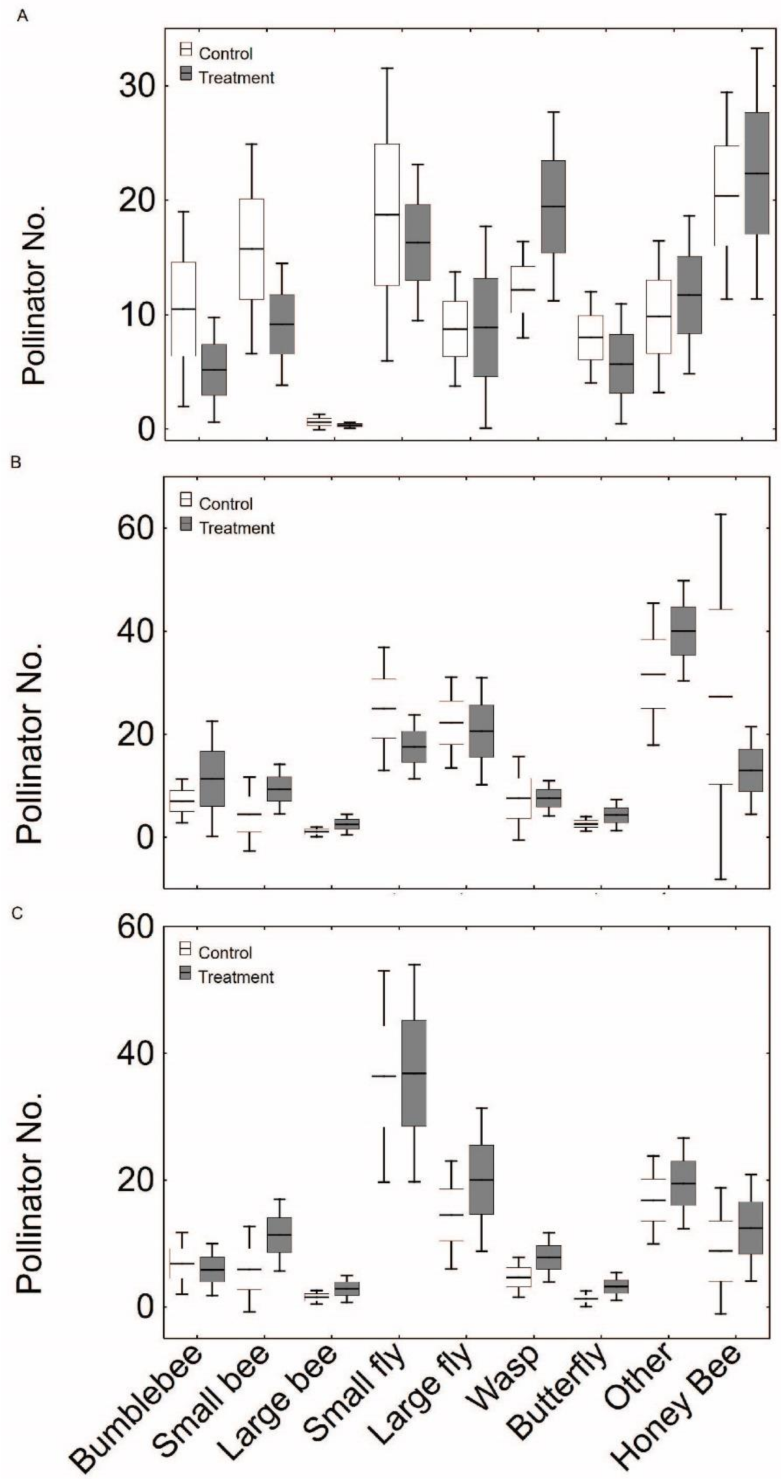

Figure 1.. Mean number of pollinator individuals per taxa from sampling plot, and surveys for sites with or without gardens in 2020, 2021, and 2022. Line = mean; box = SE, and whiskers = 95% CI.

ANOVA revealed that there was no significant interaction between pond and month with respect to Simpson's index of diversity (p = 0.279). Simpson's index of diversity did not differ across months (p = 0.162), but it did differ across ponds (p < 0.001; Fig. 4).

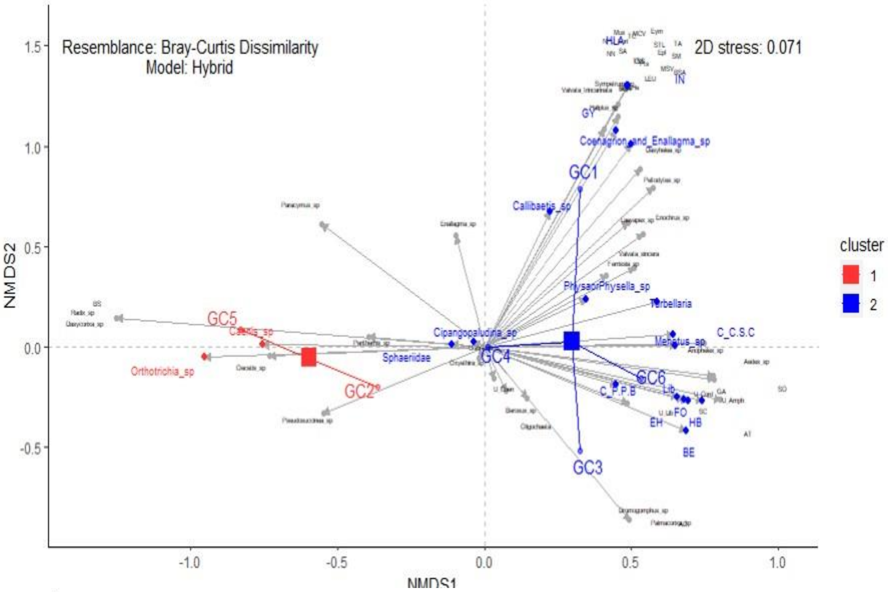

Figure 2. nMDS plot of Aquatic Invertebrate Taxonomic Community Structure of relative average densities (no/m2) over the sampling period in 2020. A Bray-Curtis dissimilarity matrix was generated and used in a hybrid (local and global) model. Pairwise differences were then best fitted in 2-D space resulting in a good illustration of underlying gradients in the data (stress = 0.071). Cluster analysis revealed maximum similarity between ponds occurring with two groups (1 – red, 2 – blue). Taxa in different colors contribute most to their respective groups. Abbreviations of all taxa as in Table 1. Golf course abbreviations denoted in study area map.

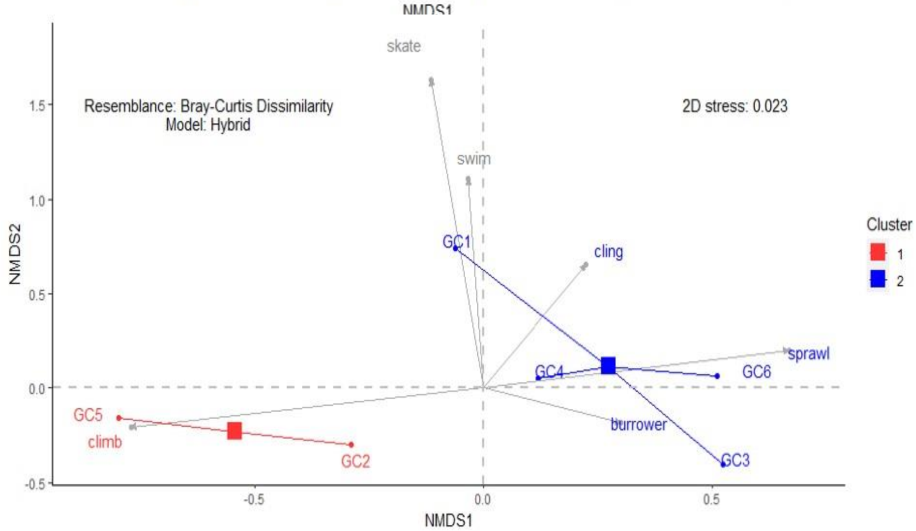

Figure 3. nMDS plot of Aquatic Invertebrate FHG Community Structure of relative average densities (no/m^2) over the sampling period in 2020. A Bray-Curtis dissimilarity matrix was generated and used in a hybrid (local and global) model. Pairwise differences were then best fitted in 2-D space resulting in a good illustration of underlying gradients in the data (stress = 0.023). Cluster analysis revealed maximum similarity between ponds occurring with two groups (1 – red, 2 – blue). FHGs in different colors contribute most to their respective groups. Golf course abbreviations denoted in study area map.

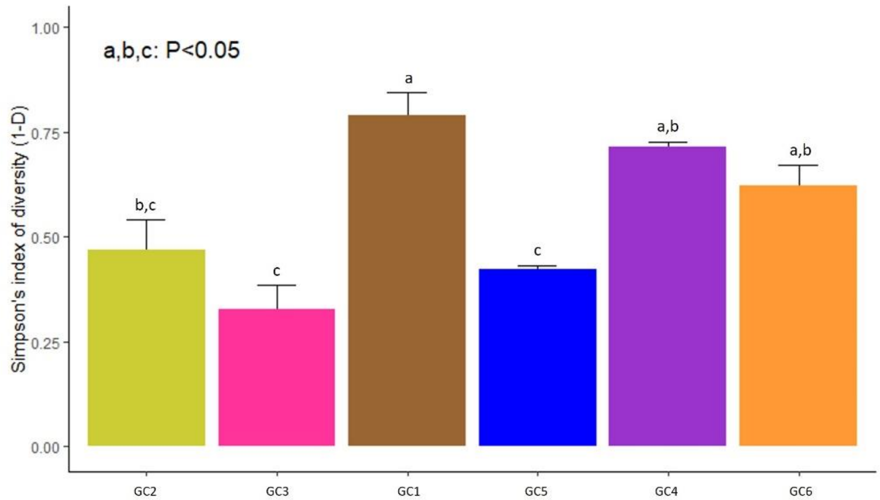

Figure 4. Simpson's Diversity of the Aquatic Invertebrate Communities of each pond (N = 54) in 2020. Letters above each bar indicate sites with significantly different Simpson's index of diversity as determined by a post-hoc Tukey multiple comparisons of means test. Error bars represent standard error.

Bat assessment:
Bat boxes at 4 experimental sites (see above for details on altered sampling) were inspected approximately once per month May - September. During our checks in July, we observed evidence of bat occupancy at Village Links and White Deer Golf Courses. We did not observe occupancy during subsequent visits to these sites in August and September but this is encouraging.

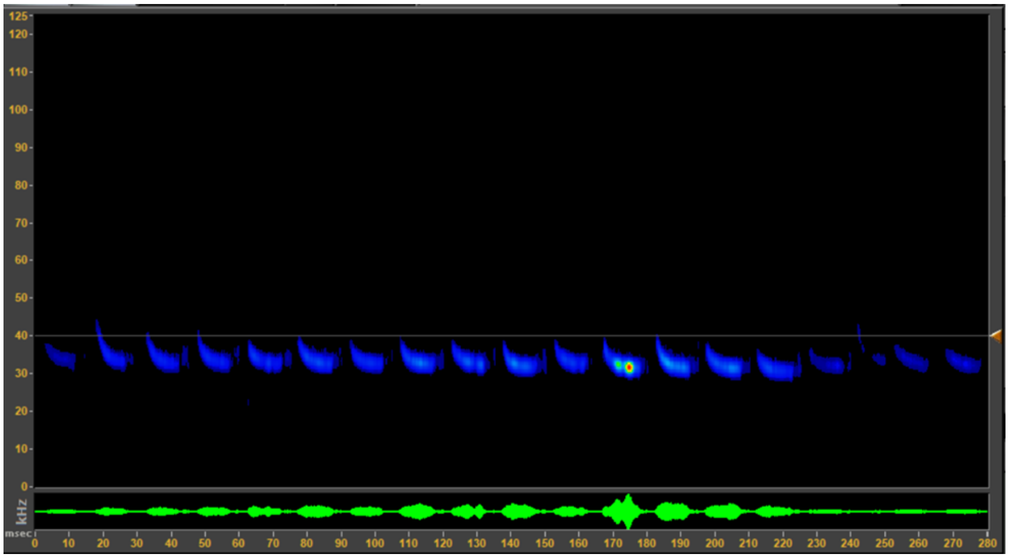

Figure 5. Spectrogram of an Eastern red bat call (Lasiurus borealis) recorded at Bryn Mawr Country Club on 28 June 2021 at 9:37 PM. Spectrograms can be identified to species based on unique characteristics

Between June 16 and June 24, 2022, we deployed acoustic recorders (SM4BATFS; Wildlife Acoustics) equipped with ultrasonic microphones (SMM-U2) 8 of 10 courses (4 experimental and 4 control). Each golf course was surveyed for 7 nights from sunset to sunrise. Due to equipment failures at White Deer and Schaumburg Golf Clubs, we deployed recorders at these sites for an additional sampling week, July 1- July 8. June and July are considered to be the peak season for bat activity in this area, thus samples from two different periods within a week of one another are considered equivalent. Acoustic recorders were placed adjacent to pollinator gardens at experimental sites. At control sites, we placed acoustic recorders in one of the sampled pollinator habitats identified at each course, as described for the pollinator sampling. Recorders were programmed to record when triggered by ultrasonic noise in the environment surrounding the detector. After deployment, call files were scrubbed and processed using a bat call processing software (SonoBat v. 4.45 Midwest; Arcata, CA). Calls considered in the summary were based on those that met rigorous quality standards, resulting in 2307 call files. We visually confirmed any SonoBat classifications of rare species by examining the call spectrogram for key characteristics known for that species (see Fig. 5 for example). Across sites we detected a diversity of species, including the big brown bat, silver-haired bat, Eastern red bat, hoary bat, and evening bat. We did not detect activity by the three species considered to be under conservation risk due to white-nose syndrome, including the little brown bat, tricolored bat, and the Northern long-eared bat.

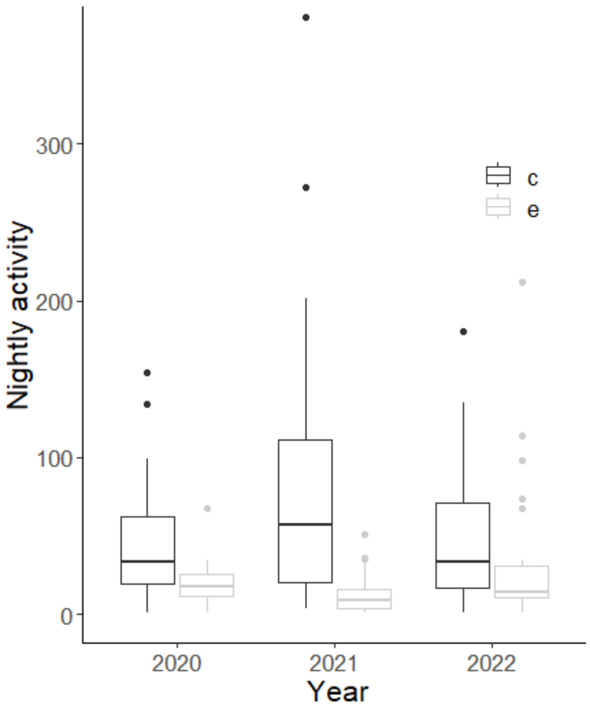

Figure 6. Nightly bat activity based on call recordings with all species combined, grouped by year and treatment (C= Control; E= Treatment).

Using general linear models and AIC, we considered models examining combinations of the variable treatment (Control or Experimental) and year (2020, 2021, 2022) on nightly bat activity. The top model included an interaction term between treatment and year. Surprisingly, bat activity was lower at experimental sites than at control sites, and this effect was most pronounced in 2021 (Figure 6; Table 1), where we observed a higher amount of activity overall. Examining the same models for each species revealed a similar effect. The benefits of the pollinator gardens for bats may not have been fully realized in the three-year study. Future analyses will examine local site characteristics that might be influencing this pattern.

Table 1. AIC models examining the relationship between nightly bat activity (all species combined), treatment (control or experimental), year (2020, 2021, 2022), and the interaction between treatment and year (treatment * year), ordered by lowest ΔAIC.

Model	df	AIC	ΔAIC
Treatment* Year	6	6871.049	0.0000
Treatment + Year	4	7440.898	569.8494
Treatment	2	7567.619	696.5707
Year	3	9069.739	2198.6908
Intercept	1	9129.443	2258.3943

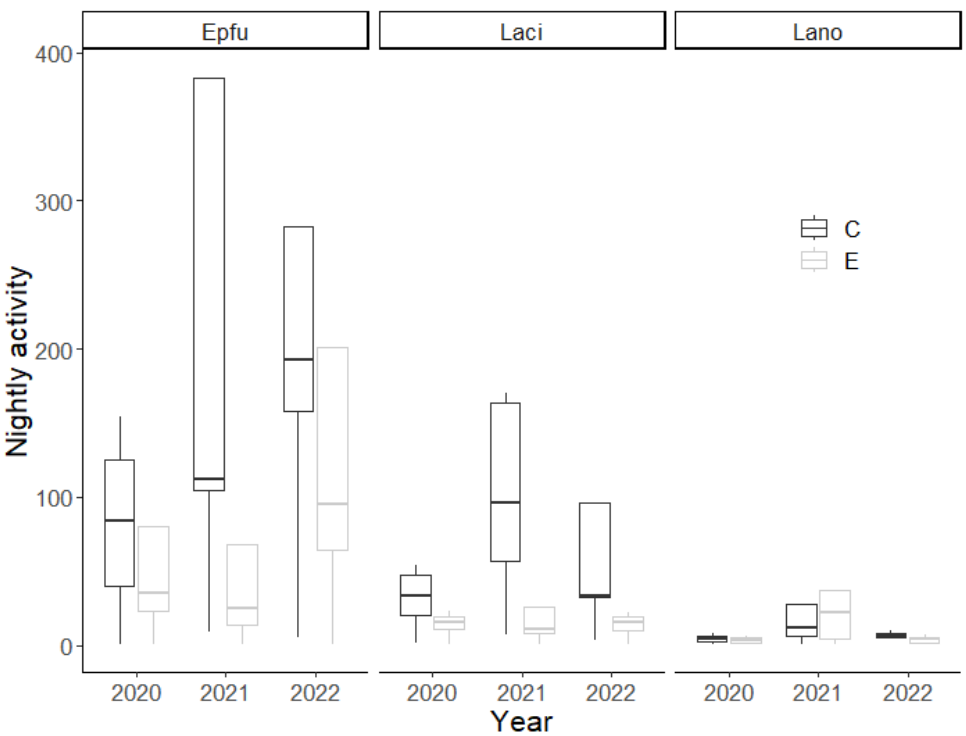

Figure 7. Nightly bat activity by species based on call recordings for the three most common species detected (Epfu= Big brown bat, *Eptesicus fuscus*; Lano= silver-haired bat, *Lasionycteris noctivagans*; Laci= hoary bat, *Lasiurus cinereus*), grouped by year and treatment (C= Control; E= Experiment). Individual species demonstrated the same patterns as when all species were combined.

USGA ID#: 2019-21-691

Advancement of Five Elite Zoysiagrass Hybrids in the 2019 Zoysiagrass NTEP

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Background: The USGA has sponsored 5 experimental entries from Texas A&M. Namely, DALZ 1701, 1707 and 1808 selected for cold-hardiness and large patch resistance in the transition zone, and DALZ 1802 and 1807 selected for winter color retention in Riverside, California. These genotypes have also rated highly for establishment and turfgrass quality in previous multilocation and environment experiments. Due to their overall performance in earlier trials, these were advanced to the 2019 Zoysiagrass NTEP (further referred to as 2019 NTEP in this report).

Experimental #	**Advanced to 2019 NTEP as**	**Lineage**
TAES 6095-83	DALZ 1701	(*Z. matrella* x *Z. japonica*) x *Z. japonica*
TAES 6099-145	DALZ 1808	*Z. japonica* x *Z. japonica*
TAES 6119-179	DALZ 1707	*Z. japonica* x (*Z. matrella* x *Z. japonica*)
TXZ 463	DALZ 1807	(*Z. minima* x *Z. matrella*) x *Z. pacifica*
TXZ 488	DALZ 1802	*Z. matrella* x *Z. pacifica*

Data reported here reflects performance in 2021 from 15 of the 20 NTEP trial locations (standard and ancillary). This included locations in the north (Columbia, MO; West Lafayette, IN; and College Park, MD) transition zone (Stillwater, OK; Fayetteville, AR; Manhattan, KS; Knoxville, TN), southwest (Riverside, CA), and southeastern (Auburn, AL; Griffin, GA; Gainesville, FL; Jay, FL; and Ft. Lauderdale, FL) regions. Data was also provided from Raleigh, NC (transition zone; traffic tolerance) and Dallas, TX (southcentral; drought). Trial management differed at each location.

Trials reported under Schedule A (College Park, MD; Columbia, MO; Manhattan, KS; Auburn, AL; Griffin, GA; Ft. Lauderdale, GA; and Dallas, TX) were mowed at a height between 0.5” and 1.0” to reflect conditions like golf courses tees and fairways. Trials under Schedule B (West Lafayette, IN; Stillwater, OK; Fayetteville, AR; Knoxville, TN; Raleigh, NC; Riverside, CA; Gainesville, FL; and Jay, FL) were mowed between 1.5” and 2.0” like homeowner conditions. Both maintenance levels were restricted on monthly nitrogen inputs (< 0.33 lb N/1,000 ft^2/ month) and irrigation (to prevent dormancy or drought stress), and fungicides were only applied on a curative basis. Turfgrass quality ratings will be reported by maintenance level or ancillary trial conditions. All other data provided in this report has combined standard and ancillary trial data for traits such as spring greenup, winter survival, fall color, and seedhead density. No data was reported for divot recovery, sod strength, shade tolerance or resistance to large patch, billbugs, or zoysiagrass mites. To rank overall performance, a turfgrass performance index was tallied for each event an entry placed in the top statistical group among all 39 entries and commercial cultivars as determined by Fisher’s LSD.

Turfgrass quality – Turfgrass quality was rated numerous times in each location. The means across all months evaluated in the 2019 NTEP report are presented. The three intermediate-textured cold-hardy lines (DALZ 1701, 1707, and 1808) were top performers in most locations (higher TPI) under low mowing heights (Schedule A) compared to commercial cultivars (Table 1). However, means were below acceptable for most entries including cultivars in the southeastern locations of Alabama and Georgia. For the two hybrids selected for winter color retention in California (DALZ 1802 and 1807), turfgrass quality under low mowing height management was generally not acceptable in locations that reported data from the north, transition zone, or southeast except for in Fort Lauderdale, FL where they were both top performing genotypes (Table 1). This is interesting as both genotypes have dwarf-like growth habits.

Table 1. Mean turfgrass quality of 5 elite zoysiagrasses compared to commercial cultivars in 2021 under golf course management conditions (low mowing height = 0.5" to 1.0", Schedule A).

Entry	Mean Turfgrass Quality (Schedule A - 2021)						TPI
	North		Transition	Southeast			
	College Park, MD	Columbia, MO	Manhattan, KS	Auburn, AL	Fort Lauderdale, FL	Griffin, GA	
DALZ 1701	7.7 a	6.8 a	7.2 a	5.6 a	7.2	5.5 a	5
DALZ 1707	8.0 a	5.2	7.1 a	4.4 a	7.4 a	5.4	4
DALZ 1808	8.0 a	5.8 a	7.3 a	5.1 a	7.3 a	5.5 a	6
DALZ 1802	2.5	1.6	1.6	5.2 a	7.4 a	5.0	2
DALZ 1807	2.1	2.2	1.7	3.5	7.5 a	5.0	1
Emerald	7.9 a	7.3 a	7.1 a	5.6 a	6.9	5.1	4
Empire	7.0	5.5 a	6.5	5.5 a	7.5 a	6.0 a	4
Meyer	6.6	3.0	6.2	2.6	7.5 a	5.0	1
Zeon	8.0 a	6.8 a	7.0 a	5.8 a	6.8	5.3	4
LSD	0.6	2.0	0.7	1.9	0.6	0.6	
C.V.	6.1	34.5	8.1	24.4	4.9	6.3	

Under a higher mowing height regime, DALZ 1701 (TPI of 4) performed most similarly to Emerald which had the highest quality in five of the eight reported locations, primarily those in the northern, transition zone and southeastern locations (Table 2). In Tennessee, neither of the five elite DALZ lines or cultivars were top performers though means were generally greater than acceptable. Under simulated traffic conditions in North Carolina means for presented entries were either at or below acceptable with DALZ 1808 having a higher mean. In California, DALZ 1802 and 1807 outperformed the cold-hardy lines and cultivars.

Table 2. Mean turfgrass quality of 5 elite zoysiagrasses compared to commercial cultivars in 2021 under home lawn management conditions (mowing height = 1.5" to 2.0", Schedule B).

Entry	Mean Turfgrass Quality (Schedule B - 2021)								TPI
	North	Transition				Southwest	Southeast		
	West Lafayette, IN	Stillwater, OK	Fayetteville, AR	Knoxville, TN	Raleigh, NC (Traffic)	Riverside, CA	Gainesville, FL	Jay, FL	
DALZ 1701	8.4 a	5.9	7.1 a	7.0	5.2	5.9	6.0 a	6.4 a	4
DALZ 1707	8.8 a	6.5 a	6.8	6.9	6.0	5.6	5.2	6.4 a	3
DALZ 1808	8.3 a	5.8	6.9 a	7.4	6.4	5.4	5.9 a	5.8	3
DALZ 1802	1.0	5.8	6.8	6.0	5.6	6.8 a	4.2	6.2 a	2
DALZ 1807	1.0	5.3	3.9	6.4	5.4	6.5 a	3.8	6.0 a	2
Emerald	8.3 a	6.5 a	7.3 a	6.4	5.7	5.8	5.9 a	6.4 a	5
Empire	8.2 a	5.5	6.5	7.2	6.2	4.9	4.5	6.0 a	2
Meyer	5.9	5.9	6.1	5.8	5.9	3.8	3.5	5.8	0
Zeon	7.8	6.1 a	7.5 a	6.9	5.7	6.0	4.6	6.3 a	3
LSD	0.6	0.4	0.6	1.2	1.2	0.8	1.0	0.8	
C.V.	6.8	4.7	5.3	11.2	12.4	8.4	11.1	8.7	

In Dallas, TX the trial was managed under Schedule A, however data is presented separately due to ancillary drought trial conditions (Table 3). Before initiating drought in July 2021, only DALZ 1701 and 1808 had mean turfgrass quality (average of two months) that was as high as Emerald and Empire. After

6 d of no supplemental water, DALZ 1701 and 1808 were top performers along with Emerald, Empire, and Zeon. Neither of the presented entries were top performers after 21 d although only DALZ 1701 had an acceptable quality rating. Two rainfall events occurred at 24 and 25 d totaling 1.42”. This promoted a brief recovery period which was reflected at 35 d with an increase in mean quality. At this time, only DALZ 1701 was a top performer. After another 14 d of no supplemental irrigation, DALZ 1701 had the highest mean quality (6.7) compared to other presented entries though none were top performers. DALZ 1802 and 1807 did not perform well under drought conditions. Following an 18-d recovery period, DALZ 1701, Emerald, Empire, and Zeon were top performers. Overall, DALZ 1701 had a higher TPI compared to other presented cold-hardy lines and commercial cultivars.

Table 3. Mean turfgrass quality of 5 elite zoysiagrasses and 4 cultivars under non-stress, drought, and recovery conditions in 2021.

Entry	Mean Turfgrass Quality (2021)						TPI
	Non-stress†	Drought‡				Recovery	
		6 d	21 d	35 d	49 d	18 d	
DALZ 1701	6.7 a	7.3 a	6.0	7.3 a	6.7	8.0 a	4
DALZ 1707	5.8	6.3	4.7	6.3	5.7	7.0	0
DALZ 1808	7.0 a	8.0 a	4.7	5.7	5.3	6.0	2
DALZ 1802	4.0	6.3	5.3	6.0	5.7	6.3	0
DALZ 1807	2.2	3.5	3.5	3.5	3.5	4.5	0
Emerald	6.3 a	7.3 a	5.7	6.3	6.3	7.7 a	3
Empire	7.5 a	7.7 a	5.3	7.0	7.0	8.3 a	3
Meyer	2.5	2.0	1.0	1.0	1.7	1.3	0
Zeon	6.2	7.7 a	5.7	7.0	6.3	8.3 a	2
LSD	1.9	2.0	1.9	1.8	1.6	1.9	
C.V.	29.3	16.9	21.2	17.8	16.7	17.6	

† Non-stress is the average of means from April and June 2021 prior to initiating drydown on July 9, 2021.
‡ Drought was initiated on July 9, 2021 and was suspended on September 10, 2021.

Spring greenup and winter survival – Spring greenup was rated five times in northern and transition zone locations (Table 4) as well as five times in southern locations (Table 5). In the northernmost location of Indiana, the elite cold-hardy hybrids greened up similarly to the cold-hardy cultivar, Meyer which was greater than Emerald and Empire but also like Zeon. Means for DALZ 1802 and 1807 were not reported in this location due to poor survival in previous years. In the transition zone, DALZ 1701, 1707, and 1808 were top performers along with Emerald and Zeon in Oklahoma, Kansas, and Tennessee. Although mean greenup was low in North Carolina, only DALZ 1808 was a top performer among the cold-hardy hybrids. DALZ 1802 and 1807 were also top performers in the transition zone, but not in Kansas. Across southern locations, DALZ 1701 outperformed other presented hybrids and cultivars having higher spring greenup in all five reported locations. All five hybrids were top performers in California along with Emerald.

Table 4. Mean spring greenup of 5 elite zoysiagrasses and cultivars across northern and transition zone locations in 2021.

Entry	Spring Greenup					TPI
	North	Transition				
	West Lafayette, IN	Stillwater, OK	Manhattan, KS	Knoxville, TN	Raleigh, NC	
DALZ 1701	6.7 a	6.0 a	7.3 a	8.3 a	2.3	4
DALZ 1707	7.0 a	4.7 a	6.7 a	8.0 a	2.3	4
DALZ 1808	6.0 a	5.3 a	6.0 a	8.0 a	3.7 a	5
DALZ 1802	.	4.5 a	1.0	7.7 a	3.7 a	3
DALZ 1807	.	5.5 a	1.0	8.0 a	3.7 a	3
Emerald	5.7	5.0 a	7.0 a	8.3 a	3.0	3

Empire	5.0	3.7	4.7	7.3 a	2.0	1
Meyer	7.0 a	5.0 a	8.0 a	6.3	1.7	3
Zeon	6.0 a	5.0 a	7.0 a	8.7 a	1.3	4
LSD	1.0	1.7	2.1	2.3	1.0	
C.V.	15.6	27.6	25.8	18.2	23.2	

Table 5. Mean spring greenup of 5 elite zoysiagrasses and cultivars across southern locations in 2021.

Entry	Spring Greenup					TPI
	Southcentral	Southeast			Southwest	
	Dallas, TX	Auburn, AL	Gainesville, FL	Jay, FL	Riverside, CA	
DALZ 1701	4.7 a	8.7 a	7.3 a	5.3 a	6.3 a	5
DALZ 1707	3.7	8.3 a	6.0	4.3	6.0 a	2
DALZ 1808	4.3	6.3	6.3	4.7	6.3 a	1
DALZ 1802	1.3	6.3	6.0	6.0 a	7.0 a	2
DALZ 1807	1.5	5.7	6.3	5.0	6.7 a	1
Emerald	3.7	8.7 a	6.3	6.3 a	6.7 a	3
Empire	5.0 a	6.7	5.7	5.0	5.0	1
Meyer	1.3	8.3 a	3.7	5.3	3.7	1
Zeon	3.7	9.0 a	6.0	5.3	5.3	1
LSD	1.3	1.2	1.0	1.5	1.5	
C.V.	26.3	10.3	9.1	17.6	15.9	

Winter weather brought an unprecedented duration of below freezing temperatures and snow to Dallas, TX in February 2021. While great losses were expected, most of the 39 entries survived. However, Meyer, DALZ 1802, and 1807 experienced a high percentage of winterkill (Table 6). Meyer, however, has not historically performed well in this location and was not expected to have high survival. Contrastingly, the cold-hardy hybrids, Emerald, Empire, and Zeon experienced very little loss and were top performers among all entries. Survival across the northern and transition zone locations varied. DALZ 1701 was the only cold-hardy hybrid to have higher survival in the four reported locations. DALZ 1707 had lower survival in Arkansas and DALZ 1808 had marginally lower survival in Oklahoma. Both DALZ 1802 and 1807 did not survive well in Missouri but were top performers in the transition zone. Overall, DALZ 1701 and Zeon had the greatest survival which was closely followed by DALZ 1707, 1808 and Empire.

Table 6. Winter survival of 5 elite zoysiagrasses and 4 cultivars in 2021.

Entry	Percent Winterkill	Percent Living Cover in Spring				TPI
	Dallas, TX	Columbia, MO	Stillwater, OK	Fayetteville, AR	Knoxville, TN	
DALZ 1701	8.3 a	91.7 a	85.0 a	50.0 a	93.7 a	5
DALZ 1707	0.0 a	58.3 a	88.7 a	36.7	92.0 a	4
DALZ 1808	10.0 a	80.0 a	77.3	53.3 a	92.0 a	4
DALZ 1802	65.0	23.3	85.0 a	38.3 a	85.7 a	3
DALZ 1807	88.0	31.7	85.0 a	45.0 a	90.3 a	3
Emerald	6.7 a	81.7 a	76.0	36.7	93.3 a	3
Empire	1.7 a	61.7 a	84.3 a	36.7	86.0 a	4
Meyer	80.0	25.0	81.7 a	38.3 a	70.0	2
Zeon	1.7 a	70.0 a	86.7 a	41.7 a	94.3 a	5
LSD	21.9	42.3	9.9	16.3	22.2	
C.V.	48.1	61.3	7.3	25.8	15.8	

Fall color retention – Fall color retention was rated six times across northern and transition zone locations (Table 7) and five times in southern locations (Table 8). Among the cold-hardy hybrids, DALZ 1701 and 1707 retained their color better than Empire and Meyer across all northern and transition zone locations, but were like Emerald and Zeon in Oklahoma, Kansas, and Arkansas. DALZ 1802 and 1807 also retained their color across transition zone locations except in Kansas. In southern locations, DALZ 1802 was a top performer next to Empire.

Table 7. Fall color retention in northern and transition zone locations in 2021.

Entry	Fall Color Retention						TPI
	North	Transition					
	West Lafayette, IN	Stillwater, OK		Manhattan, KS	Fayetteville, AR	Raleigh, NC	
	Oct	Oct	Nov	Nov	Oct	Nov	
DALZ 1701	7.7 a	8.7 a	4.3	6.3 a	6.7	6.7 a	4
DALZ 1707	7.0 a	8.3 a	4.0	5.7 a	5.3	6.3 a	4
DALZ 1808	6.7 a	7.7	4.3	4.7 a	5.3	5.7	2
DALZ 1802	.	9.0 a	6.5 a	1.0	8.0 a	7.0 a	4
DALZ 1807	.	9.0 a	6.5 a	1.0	7.7 a	6.0	3
Emerald	6.3	8.3 a	4.7	5.7 a	6.0	5.7	2
Empire	5.7	7.0	4.0	4.0	6.0	6.0	0
Meyer	5.7	7.0	2.7	3.0	4.3	3.7	0
Zeon	6.3 a	9.0 a	3.7	6.0 a	5.3	5.7	3
LSD	1.3	0.8	0.9	1.9	1.1	1.2	
C.V.	11.3	5.6	13.7	21.8	10.5	12.6	

Table 8. Fall color retention in southern locations in 2021.

Entry	Fall Color Retention					TPI
	Southeast				Southwest	
	Auburn, AL		Griffin, GA		Riverside, CA	
	Sept	Dec	Sept	Oct	Dec	
DALZ 1701	4.0	2.7	6.3 a	6.7 a	5.3	2
DALZ 1707	2.7	2.0	6.3 a	6.3 a	4.7	2
DALZ 1808	3.3	1.7	6.3 a	6.3 a	4.3	2
DALZ 1802	9.0 a	2.3	6.3 a	6.3 a	8.7 a	4
DALZ 1807	9.0 a	3.0	6.3 a	6.0	8.0 a	3
Emerald	5.3	2.7	6.7 a	6.3 a	3.7	2
Empire	8.3 a	6.0 a	6.3 a	6.3 a	4.3	4
Meyer	3.0	1.0	6.3 a	6.3 a	3.0	2
Zeon	6.7	4.3	6.0	6.0	5.3	0
LSD	1.0	0.9	0.8	0.8	1.8	
C.V.	12.0	16.1	7.9	7.7	18.6	

Seedhead density – Seedhead density ratings occurred a total of 11 times across locations and occurred either during spring or fall or both seasons depending on the location (Table 9). DALZ 1808 and 1701 had the lowest seedhead density (≥ 7 times) along with Emerald and Zeon compared to other presented entries.

Table 9. Seedhead density of elite hybrids and cultivars.

Entry	Seedhead Density											
	North		Transition		Southcentral			Southeast		Southwest		TPI
	West Lafayette, IN		Stillwater, OK	Manhattan, KS	Dallas, TX			Jay, FL		Riverside, CA		
	Spring	Fall			April	May	Oct	April	Sept	Spring	Fall	
DALZ 1701	8.3 a	8.3 a	7.0 a	4.3	5.7	8.3 a	4.3	1.7 a	1.3 a	8.0 a	5.3	7
DALZ 1707	7.7	9.0 a	7.0 a	2.3	1.7	8.3 a	9.0 a	1.3	1.7 a	8.3 a	1.3	6
DALZ 1808	9.0 a	8.3 a	7.0 a	6.3	8.0 a	9.0 a	9.0 a	2.0 a	2.0 a	2.3	1.0	8
DALZ 1802	.	.	7.0 a	9.0 a	9.0 a	2.7	1.3	1.3	1.3 a	7.3	6.7	4
DALZ 1807	.	.	7.0 a	9.0 a	9.0 a	9.0 a	8.5 a	1.3	1.0 a	2.0	3.7	6
Emerald	9.0 a	9.0 a	7.0 a	7.7	9.0 a	9.0 a	8.7 a	2.3 a	2.0 a	1.7	1.3	8
Empire	8.7 a	5.7	5.7	5.3	6.7	2.7	6.3	2.7 a	1.3 a	8.0 a	2.3	4
Meyer	7.7	9.0 a	7.0 a	3.7	6.0	8.0 a	9.0 a	2.0 a	1.3 a	5.3	1.0	6
Zeon	9.0 a	9.0 a	7.0 a	7.7	9.0 a	9.0 a	8.7 a	2.3 a	2.7 a	1.7	5.0	8
LSD	1.1	1.2	0.8	1.2	1.2	1.3	1.7	2.8	2.8	1.5	2.5	
C.V.	5.4	10.3	7.9	9.9	9.9	13.4	17.7	53.4	48.5	16.4	35.2	

Traffic tolerance – Traffic tolerance was simulated by mechanical means in North Carolina between the months of September and November in 2021 (Table 10). Hybrids did not perform statistically as well as cultivars. However, DALZ 1701 retained at least 75.7% cover and DALZ 1802 retained at least 81.7% cover after several months.

Table 10. Percent green cover of experimental entries and commercial cultivars because of mechanical traffic in Raleigh, NC in 2021.

Entry	Percent Green Cover						TPI
	Initial	30-Sep	9-Oct	16-Oct	26-Oct	2-Nov	
DALZ 1701	99.0 a	93.3	92.3	89.0	83.0	75.7	1
DALZ 1707	99.0 a	90.0	87.3	81.7	76.7	68.0	1
DALZ 1808	99.0 a	88.3	84.0	80.0	74.0	65.0	1
DALZ 1802	99.0 a	95.0 a	95.0 a	93.3	89.7	81.7	3
DALZ 1807	95.0	86.7	83.3	79.3	73.3	65.0	0
Emerald	99.0 a	90.0	99.0 a	99.0 a	99.0 a	99.0 a	5
Empire	99.0 a	91.7	99.0 a	99.0 a	99.0 a	99.0 a	5
Zeon	99.0 a	85.0	99.0 a	99.0 a	99.0 a	99.0 a	5
Meyer	95.3	85.0	95.3 a	95.3 a	95.3 a	95.3 a	4
LSD	1.9	5.2	6.0	5.2	5.7	6.1	
C.V.	1.0	3.3	4.1	4.0	4.8	5.7	

Overall turfgrass performance – All traits considered, the maximum potential TPI was 63. DALZ 1701 had the highest TPI of 41 followed by Emerald (38), DALZ 1808 and Zeon (34) and DALZ 1707 (30). DALZ 1701 offers high turfgrass quality under different mowing heights and greater performance during drought and drought recovery than tested cultivars. Additionally, it offers higher spring greenup in southern locations, greater winter survival, and longer fall color retention in more northern regions than the tested commercial cultivars.

Table 11. Total turfgrass performance index for all traits.

Entry	Turfgrass quality			Spring Greenup		Winter Survival	Fall Color Retention		Seedhead Density	Traffic Tolerance	Total TPI
	Schedule A	Schedule B	Drought	Northern/Transition	Southern		Northern/Transition	Southern			
DALZ 1701	5	4	4	4	5	5	4	2	7	1	41
DALZ 1707	4	3	0	4	2	4	4	2	6	1	30
DALZ 1808	6	3	2	5	1	4	2	2	8	1	34
DALZ 1802	2	2	0	3	2	3	4	4	4	3	27
DALZ 1807	1	2	0	3	1	3	3	3	6	0	22
Emerald	4	5	3	3	3	3	2	2	8	5	38
Empire	4	2	3	1	1	4	0	4	4	5	28
Meyer	1	0	0	3	1	2	0	2	6	5	20
Zeon	4	3	2	4	1	5	3	0	8	4	34

USGA ID#: 2022-18-761

Title: Soil Moisture Sensing for Golf Course Water Conservation – A Case Study

Project Leader and Co-Leaders: Bernd Leinauer[1], Tom Egelhoff[2] and Ciro Velasco-Cruz[1]

Affiliation: [1]New Mexico State University, Department of Extension Plant Sciences and [2]The Club at Las Campanas, Santa Fe, NM

Objectives: 1) To determine soil moisture variability on 2 fairways
2) To evaluate possible water savings when irrigating golf course fairways and subsurface-drip irrigated tee boxes using SMS compared to ET scheduling

Start Date: 2022
Project Duration: The project is expected to run for three years.
Total Funding: $30,000

Summary Points:

1) An irrigation audit was conducted on fairways #2 and #7 during summer of 2022. Low Quarter Distribution Uniformity was determined at 0.68 on #2 and 0.51 on #7.
2) Irrigation averaged 0.21” (Min 0.09”, Max 0.32”) on #2 and 0.16” on #7 (Min 0.05”, Max 0.36”).
3) From May 1 to August 10, 2022, soil moisture was recorded on two fairways using a Pogo Pro. Values ranged from a minimum of 0% to a maximum of 64.5% on #2 and from 0% to 65.9% on #7.
4) Areas that consistently exhibited volumetric soil moisture values of less than 20%, approximately 35%, and greater than 40% were selected and marked as dry, moist, and wet, respectively. Toro Turfguard soil moisture sensors will be installed into those areas.
5) Due to material supply chain issues and resulting manufacturing problems, delivery of Toro Turfguard wireless soil moisture sensors was delayed, and sensors are expected to be installed in spring of 2023.

Study

Few studies have demonstrated the water conservation potential of soil moisture sensor (SMS) -based irrigation scheduling on golf course fairways. Information is lacking on the effects of SMS-based irrigation scheduling on irrigation water use and turfgrass quality in arid or semi-arid environments on heavily trafficked turfgrass areas, such as golf course fairways. A three-year field study has been initiated in 2022 to investigate water use of two golf course fairways that are irrigated based on SMS readings compared to traditional Evapotranspiration (ET) based irrigation.

Methods and Results

Two fairways, #2 (Photo 1) and #7, were selected from the Sunset golf course at The Club at Las Campanas in Santa Fe, NM. Both fairways are par 4 and cover 121,000 ft^2 (#2) and 82,000 ft^2 (#7). Fairways were established in 1999 with ‘Penn Trio’ creeping bentgrass. Mowing has been conducted at 0.4” (10 mm) twice per week with clippings returned. Both fairways are aerated once per year in April and dethatched with a verti-cutter Model VC60 (First Products Inc., Tifton, GA) in September. The fairways received approximately 10 g N/m^2 (2 lbs N/1000 ft^2) annually. The surfactant ‘Revolution’ and plant growth regulators ‘Primo’ (ai Trinexapac-ethyl) and ‘Legacy’ (ais Flurprimidol and Trinexapac-ethyl) were applied monthly at label rate. Weed control was achieved on a curative basis with the herbicide Trimec Bent (ais Dicamba, 2,4-D, MCPP) and Merit (ai Imidacloprid) was applied at label rate for insect control. Sand topdressing was conducted six times during the growing season (April to October). Fairways are irrigated predominately from Toro DT34 and DT35 heads installed within the center and from RainBird 750 heads around the perimeter (Photo 2).

Photo 1. Fairway #7 of the Sunset Course at The Club at Las Campanas, Santa Fe, NM.

Photo 2: Sprinkler head placement on Fairway #2 (app.pogoturfpro.com).

Irrigation Audit

Irrigation audits were conducted on May 17, 2022, on fairway #7 and on August 1, 2022, on fairway #2 (Photo 3). The audits were conducted as part of a normal irrigation cycle which lasted 12 minutes for each fairway. Seventy-six catch cans were placed on fairway #2 and 80 on fairway #7 resulting in approximately 1 cup/1600 ft^2 on a grid of 50 x 30 ft (fairway #2) and 1 cup/1000 ft^2 on a grid of 50 ft x 20 ft (fairway #7).

Photo 3. Tom Egelhoff (Agronomist, The Club at Las Campanas, Santa Fe, NM) places catch cans for an irrigation audit on fairway #2.

The audit revealed a Low Quarter Distribution Uniformity of 0.68 on #2 and 0.51 on #7. Precipitation amount averaged 0.21” (Min 0.09”, Max 0.32”) on #2 and 0.16” on #7 (Min 0.05”, Max 0.36”). Summary results of the audits are shown in Table 1 and Figure 1. The length of the box of each chart represents the distance between the 25th and 75th percentiles (the interquartile range). The symbol inside the box denotes the group mean and the horizontal line represents the group median. The vertical lines (called whiskers) extending from the box indicate the group minimum and maximum values. Data points on top of or below the whiskers can be considered outliers.

Table 1. Precipitation analysis of 2 Fairways of the Sunset Course at The Club at Las Campanas, Santa Fe, NM. N denotes number of catch cans, and Std Dev and lqDU represents standard deviation and low quarter Distribution Uniformity, respectively, for each fairway.

Fairway	N	Mean	Median	Minimum	Maximum	Std Dev	lqDU
		---------------------inches----------------					
#2	76	0.21	0.21	0.09	0.32	0.05	0.68
#7	80	0.16	0.16	0.05	0.36	0.06	0.51

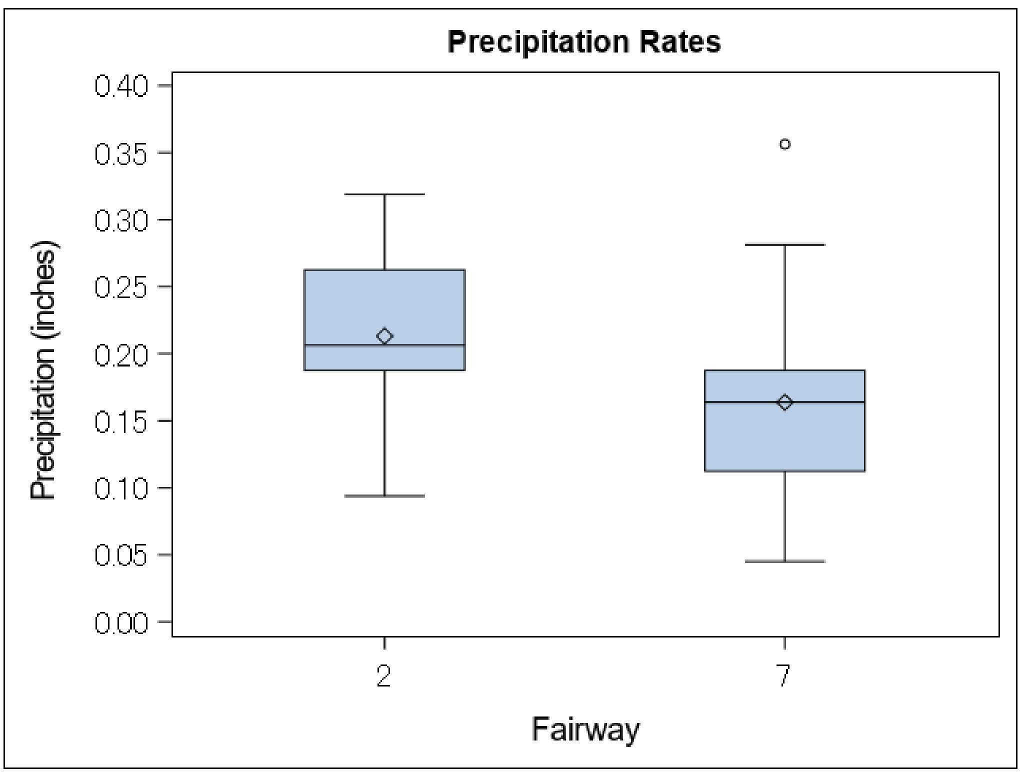

Figure 1. Box and whisker chart of precipitation amounts collected during irrigation audits on fairway #2 and #7 of the Sunset Course at The Club at Las Campanas, Santa Fe, NM. Bars display the result of amounts collected in 76 (fairway #2) and 80 catch cans (fairway #7).

Soil Moisture Measurements

From May 1 to August 10, 2022, soil moisture measurements (Pogo Pro, Stevens Water Monitoring Systems, Portland, OR) at depths of 0 – 5.7 cm were recorded on 31 days for Fairway #2 and on 36 days for Fairway #7. The complete data set including all sampling information is listed in Appendix 1.

Moisture readings were taken predominately between 5:30 and 6:00 am on Fairway #2 and between 6:20 and 7:00 on Fairway #7. Occasionally, moisture readings on #7 were taken during the afternoon. Rainfall of greater than 0.05” occurred on 7 days during the investigative period, reaching amounts as high as 1.34” and 1.64”. Sampling points ranged from 60 to 188 on Fairway #2 and from 9 to 251 on Fairway # 7. Soil moisture ranged from a minimum of 0% to a maximum of 64.5% on #2 and from 0% to 65.9% on #7.

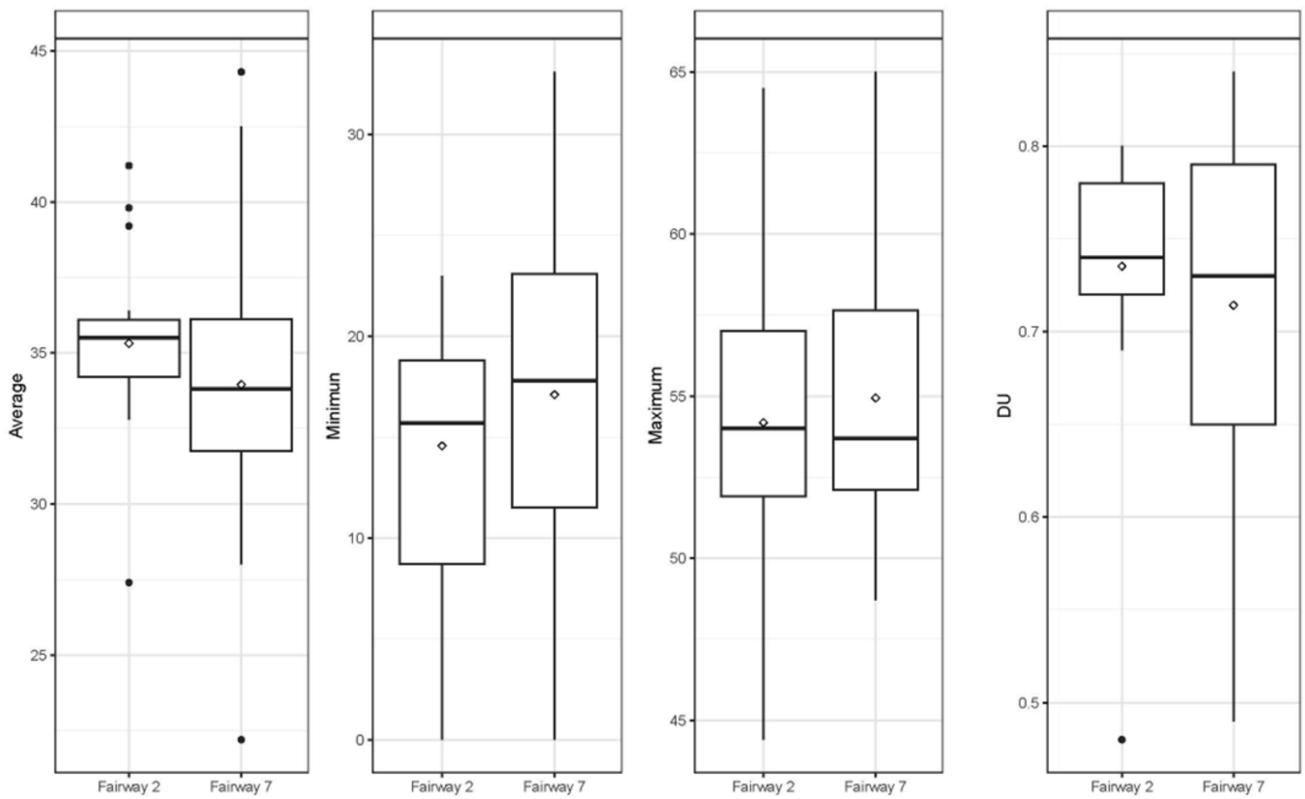

Figure 2. Box and whisker plots of average, minimum, and maximum soil moisture readings and of soil moisture Distribution Uniformity (DU) of fairways #2 and #7 of the Sunset Course at The Club at Las Campanas, Santa Fe, NM. Plots summarize the values displayed in Appendix 1 and exclude days for which a rainfall event was recorded during 24 hours prior to sampling.

From June 6 to June 15 no rainfall occurred, and this time period was used to select areas for the placement of Turfguard soil moisture sensors. Areas that consistently exhibited volumetric soil moisture values of less than 20%, approximately 35%, and greater than 40% were selected and marked as dry, moist, and wet, respectively (Photos 4 and 5).

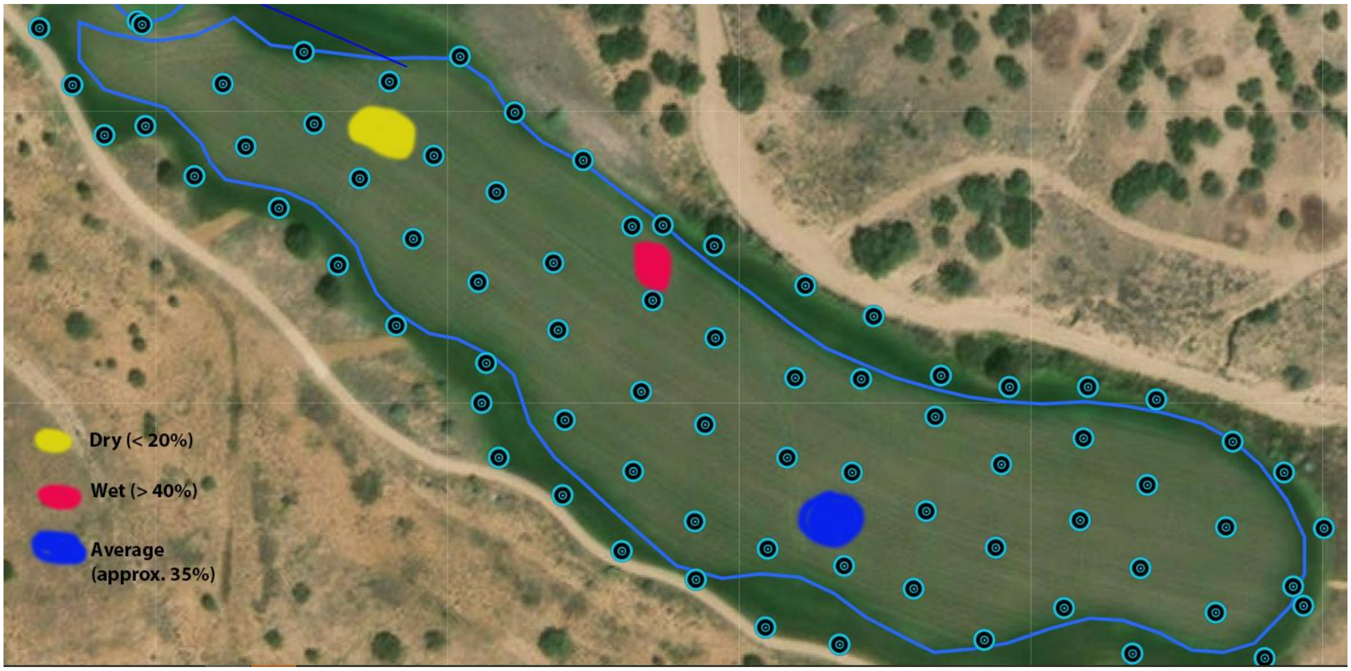

Photo 4. Sprinkler heads and locations of Turfguard soil moisture sensors on fairway #2 at the Sunset course of The Club at Las Campanas, Santa Fe, NM.

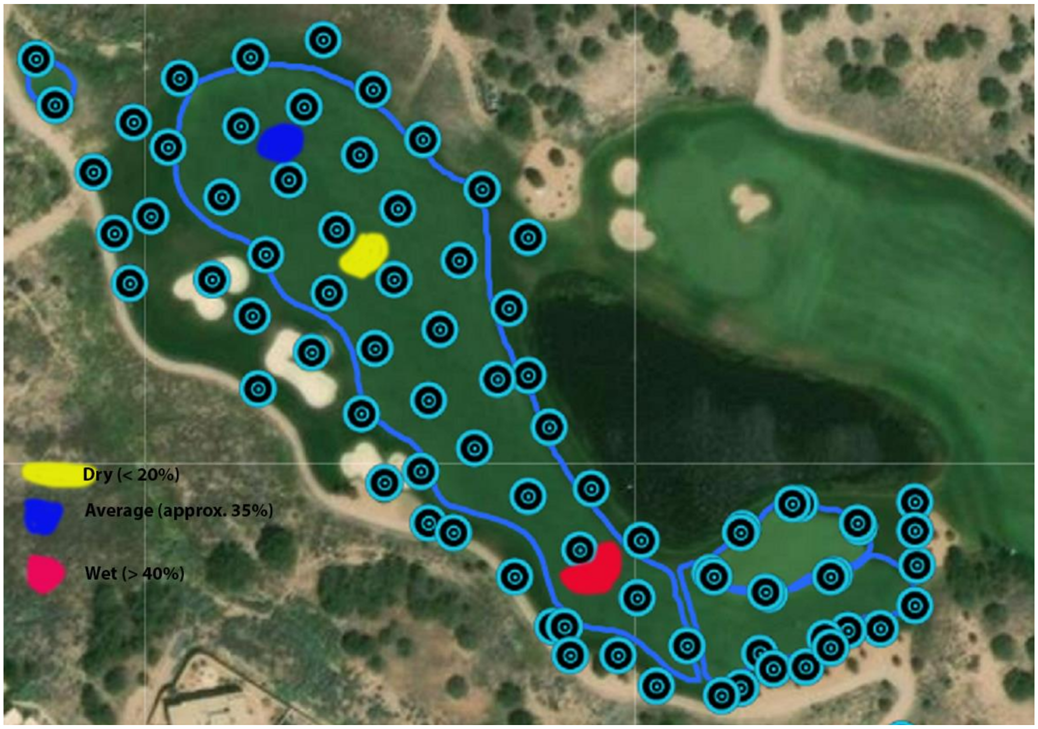

Photo 5. Sprinkler heads and locations of Turfguard soil moisture sensors on fairway #7 at the Sunset course of The Club at Las Campanas, Santa Fe, NM.

Future Expectations

Due to material supply chain issues and resulting manufacturing problems, delivery of Toro Turfguard wireless soil moisture sensors was delayed. Base station, signal repeater, and sensors will be tested and are expected to be installed in spring of 2023. Soil moisture readings from inground sensors in fairway #7 will be recorded and will determine whether or not irrigation will be applied. Irrigation on the control fairway #2 will be applied based on ET replacement values determined by on-site weather stations. Moreover, this study will be combined with a new project starting in Spring of 2023 to investigate the accuracy of several new remote soil moisture sensing technologies (Project Title "Remote Soil Sensing of Fairways for Irrigation Water Conservation).

Appendix 1.

Soil moisture information based on readings collected on Fairway #2 and Fairway #7 of the Sunset Course at The Club at Las Campanas, Santa Fe, NM. Precipitation values indicate rainfall amount within 24 hours prior to soil moisture measurements.

		#2							#7							
#		Time	Data Points	Avg	DU	Min	Max		Time	Data Points	Avg	DU	Min	Max		Precip
1	1-May								12:01 PM	251	22.2	0.49	2.8	53.4		
2	3-May								1:33 PM	208	29.5	0.62	11.4	57.5		
3	5-May								1:21 PM	169	29	0.63	7.5	52.8		
4	9-May								9:31 AM	128	29.2	0.64	11.8	54.3		0.01
5	10-May	1:30 PM	60	27.4	0.48	6.7	44.4									
6	13-May								1:58 PM	141	28	0.65	0	65		
7	24-May								2:25 PM	117	30.5	0.61	10.9	59.8		
8	6-Jun	7:46 AM	149	32.8	0.72	8.4	45.9		9:04 AM	90	33.6	0.65	4.2	63		
9	7-Jun	6:29 AM	142	35.3	0.71	7.9	55.3		7:21 AM	104	31.9	0.65	9.2	52.1		
10	8-Jun	6:09 AM	129	33.5	0.7	12.3	52.7		7:05 AM	120	32.5	66	10.7	57.4		0.03
11	9-Jun	6:05 AM	107	34.3	0.69	8.3	51.5		6:50 AM	83	33.1	0.68	12.3	52.7		
12	12-Jun	5:38 AM	85	36.4	0.78	15.7	58.6		6:23 AM	9	34	65	18.9	50.8		
13	13-Jun	5:53 AM	103	36.1	0.74	18.8	58.5		6:37 AM	59	32.1	69	14.8	57.5		
14	14-Jun	5:50 AM	87	35.7	0.73	14.1	57		6:32 AM	55	32.8	0.63	13.2	53.1		
15	15-Jun	5:51 AM	162	34	0.69	10.3	53.8		6:52 AM	94	31.7	0.71	16.7	58.9		
16	16-Jun	5:54 AM	147	34.8	0.73	13	60.1		6:52 AM	94	31.3	0.68	12.8	55.4		0.03
17	17-Jun	5:38 AM	183	34.2	0.74	8.7	53.1		6:42 AM	104	34.1	0.76	22.8	55.5		
18	20-Jun	5:56 AM	175	39.8	0.78	20.7	64.5		7:06 AM	111	38.3	0.77	22.4	58.3		
19	21-Jun	5:54 AM	126	37.4	0.79	19.9	56.2		6:51 AM	81	35.7	0.72	0	53.4		0.18
20	22-Jun	5:52 AM	126	39.2	0.72	0	56.6		6:44 AM	125	44.3	0.84	33.1	61.6		
21	23-Jun	5:48 AM	121	39.9	0.78	20.4	58.7		6:33 AM	113	40.2	0.8	25.2	57.6		0.33
22	24-Jun	5:40 AM	147	34.9	0.77	20.9	52.3		6:35 AM	266	35.2	0.75	13.6	53.3		
23	27-Jun	5:55 AM	148	42.1	0.79	20.6	61.3		6:47 AM	85	49	0.8	32.1	65.9		1.34
24	28-Jun	5:55 AM	142	39.6	0.8	26.5	57.8		6:52 AM	101	43.5	0.81	23.9	56.4		1.64
25	29-Jun	5:50 AM	127	35.3	0.78	22.2	51.6		6:53 AM	63	41.3	0.79	24.1	57		0.02
26	30-Jun	5:49 AM	118	33.1	0.72	14.2	51.9		6:42 AM	158	35.6	0.79	21.3	49.2		
27	1-Jul	5:37 AM	107	35.5	0.78	20.2	47.4		6:20 AM	119	38.5	0.82	26.8	52.1		
28	5-Jul	5:57 AM	119	36.5	0.78	21.5	56.2		6:37 AM	136	37	0.81	24.1	51.9		0.07
29	6-Jul	9:48 AM	79	36.2	0.78	23	52.5		10:25 AM	50	35.9	0.76	23.2	50.9		
30	7-Jul	5:52 AM	140	36	0.77	17.9	58.1		7:09 AM	103	36.2	0.8	24.6	51		0.01
31	8-Jul	5:38 AM	91	35.2	0.75	18.7	54		6:23 AM	101	36.2	0.82	24.8	48.7		
32	11-Jul	5:49 AM	155	35.8	0.77	17.8	56		6:49 AM	95	35.1	0.79	19	54		
33	12-Jul	5:48 AM	91	33.1	0.73	15.5	50.9		6:27 AM	81	33.6	0.78	22.8	51.3		
34	13-Jul	6:05 AM	158	41.2	0.79	17.1	59.2		7:13 AM	102	42.5	0.8	29.3	54		
35	14-Jul	5:51 AM	97	33.2	0.77	21.6	50.6		6:32 AM	82	35.6	0.77	22.7	50.3		0.43
36	15-Jul	5:53 AM	188	35.5	0.8	21	56.3		7:05 AM	123	39.7	0.79	27.2	57.7		
37	10-Aug								7:57 AM	133	41.9	0.79	6.5	54.1		0.12

USGA ID#: 2021-19-743

Title: NC1208 Dollar Spot Cultivar Project

Project Leaders: Paul Koch, Stacy Bonos, Nancy Dykema, Geunhwa Jung, John Kaminski, Megan Kennelly, Richard Latin, James Murphy, Joe Vargas

Affiliation: University of Wisconsin – Madison, Kansas State University, Michigan State University, Penn State University, Rutgers University, University of Massachusetts - Amherst

Objective: The primary goal of this project is to combine multiple cultural practices (dew removal and biocontrol) with varying levels of host resistance to determine the level of dollar spot suppression that can be achieved in the absence of fungicides.

Start Date: 2021
Project Duration: 3 years
Total Funding: $36,000

Summary Points:

- Plots at each of the research facilities were planted in the late summer and fall of 2021. Coho, Shark, and Penncross were planted at each location to represent high, medium, and low levels of dollar spot resistance.
- Each research plot was inoculated with *Clarireedia jacksonii* in the spring of 2022 to produce a consistent level of dollar spot across the experiemental area. Treatments that included regular dew removal and the use of Zio biological fungicide were initiated in May of 2022 and continued through August.
- All of the locations had significant annual bluegrass encroachment within the plot. Dollar spot development was also extremely high at all of the research locations, likely as a result of the spring inoculation. This made rating the disease within each treatment difficult.
- Each location reported significantly less disease on the Coho plot compared to Shark and Penncross, but limited differences in the dew removal and biocontrol treatments within each cultivar.
- Locations implemented various forms of annual bluegrass control during 2022 (Poacure, Velocity, etc) to limit annual bluegrass infestation for 2023.

Summary Text:

Rationale: Numerous cultural practices have demonstrated varying levels of dollar spot suppression, but they rarely suppress dollar spot to the point where fungicides can be eliminated or significantly reduced. Most of these studies have taken place on creeping bentgrass cultivars such as Penncross that are highly susceptible to dollar spot. However, numerous new bentgrass cultivars have been developed in recent years with varying levels of dollar spot resistance. Even the most highly resistant cultivars still typically experience some dollar spot during high pressure times, but combining cultural practices with improved host resistance may provide an effective strategy for suppressing dollar spot while significantly reducing or eliminating fungicide usage.

Methods: In this study we are comparing 3 levels of host resistance (high, medium, low) combined with daily dew removal and the use of biocontrols for their ability to suppress dollar spot at seven different institutions around the country. The institutions hosting research sites are Kansas State University, Michigan State University, Penn State University, Rutgers University, University of Massachusetts – Amherst, University of Wisconsin – Madison, and a USGA Research Facility in Pinehurst, NC. Research sites were seeded with Coho (high resistance), Shark (medium), and Penncross (low) in late summer and fall of 2021 and the initiation of treatments scheduled for spring of 2022. The experimental design was a split-split plot with cultivar as the main plot and dew removal and biocontrol as the subplots (Figure 1). Each plot was inoculated with a local isolate of *Clarireedia jacksonii* in the spring of 2022 to provide consistent dollar spot development across all research sites. Dew removal began in mid May and ended on approximately September 1st with a goal of removing dew 5 times per week prior to 7 AM. Zio was chosen as the biological fungicide and was applied every 14 days beginning in mid-May and running through the end of August.

Results: Two problems made assessing the treatment impacts difficult in 2022. First, significant annual bluegrass encroachment across all the locations altered the genetic makeup of the various cultivars and made rating difficult. Second, the spring inoculation led to high levels of dollar spot and made discerning differences between treatments difficult. Despite those difficulties, some interesting results were observed. Coho clearly had less dollar spot compared to the other two cultivars (Figure 2). In addition, regular rolling did periodically lead to lower dollar spot severity compared to non-rolled plots. No impact of Zio was observed in the 2022 results. The full results from each location is available upon request, but the Wisconsin summary report is posted at the following site: https://tdl.wisc.edu/wp-content/blogs.dir/42/files/Interactive%20Pages/2022_Summer/Reports/UWHostResistance_2022.pdf

Future plans: Annual bluegrass control was implemented at numerous locations with the goal of having more 'pure' bentgrass stands in 2023. Wisconsin applied Poacure, Michigan State applied Velocity, and Rutgers applied Prograss, and Penn State applied Xonerate. Inoculation will not be needed again in 2023 so we anticipate more representative levels of dollar spot to occur. Dew removal and Zio applications will begin at each location shortly prior to regular dollar spot development, which will likely be in mid to late May for most sites. Dollar spot and turf quality ratings will be collected every 2 weeks in the same manner they were collected in 2022.

Figure 1. Experimental research area at the OJ Noer Turfgrass Research Facility in Verona, WI on August 8th, 2022. Only the first replication is painted and labeled in this picture.

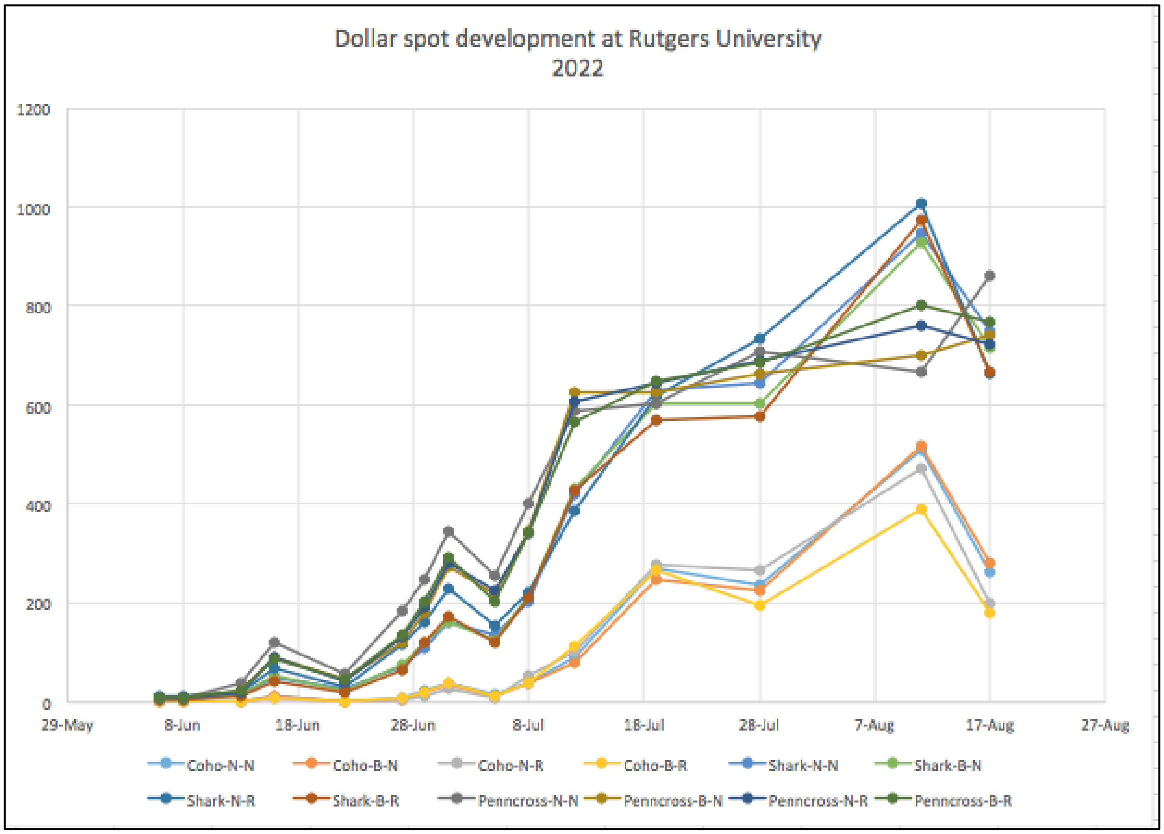

Figure 2. Results from Rutgers University demonstrating strong dollar spot resistance of Coho bentgrass but limited impact of the dew removal and Zio treatments.

USGA ID#: 2022-16-759

Project Title: Evaluate toxicological response of field populations of mole crickets to fipronil

Principal Investigator: David Held, Alumni Professor, Auburn University
Amanda Vinson, Lab Technician
Gracie Cotter, Research Assistant

Objective: To evaluate the responses of field collected tawny and southern mole crickets to doses of fipronil in the lab.

Start date: Jan. 1, 2022
Project Duration: 1 year

Total funding: $6,000

Summary Points:

- Field-collected adult southern mole crickets from Riviera CC, Miami FL (Spring 2022) and tawny mole cricket nymphs from Mobile AL (Fall 2022) received topical applications of technical grade fipronil in acetone in the laboratory. Despite insecticide applications, staff at both locations reported outbreaks of mole crickets.
- All southern mole crickets from Florida were killed by the 1000 ppm rate within 72 hours of treatments and about half of the test insects were killed by the 10 ppm rate at 96 hours. Similarly, 1000 ppm was enough to kill 100% of tawny mole crickets from a single population in Alabama in 48 hours. After 96 hours, 100% of tawny mole crickets from that population treated topically with 5 ppm were dead. It is challenging to relate field application rates to ppm of fipronil in solution applied directly to the insect.
- There is little evidence to support a concern for resistance to fipronil at either site we sampled. If fipronil is applied at label rate, there would be 260 micrograms of fipronil per sqft. That is 26x the highest contact dosage that provided 100% mortality in the lab experiment. The breakthroughs in treatments at both sites may be due to treatment timing, application rates, or re-infestation after application. Lighted driving ranges and golf courses are attractive to mole crickets in flight. At best, fipronil provides 4 to 6 months of residual control when applied at peak egg hatch.

Summary Text: Mole crickets have the metabolic enzymes needed to detoxify insecticides. However, insecticide resistance is not documented in mole crickets, and therefore resistance management approaches are not strongly considered for mole crickets. The objectives have implications for product stewardship and mitigation of losses for golf and sports turf, homeowners, and municipalities. Past work in my lab has outlined methodologies to assess populations suspected to be resistant to fipronil. These laboratory assays provide dose response data for field collected mole crickets independent of application or environmental factors. With relatively few new soil insecticides being brought to market, this work can inform efforts to foster stewardship of turfgrass insecticides.

Methods: On 28 February 2022, a sample of adult southern mole crickets was collected from five locations on the Riviera Country Club, Coral Gables FL. On 19 September 2022, a sample of tawny mole cricket nymphs with wing pads were collected from the driving range of the Springhill College Golf Course, Mobile AL. In brief, the mole crickets were collected by the soap flush method, provided a rinse after surfacing, then placed into moistened sand in a cooler. Mole crickets were transported to the lab and held in 16 oz cups with moistened sand and fed mealworms before the experiment began. Some insects were lost during transfer and while in captivity. In the insecticide bioassay, 10 µl of a solution of technical grade fipronil in acetone

was applied to the ventral side of the mole cricket. Mole crickets were treated one of these concentrations of fipronil (0, 1, 5, 10, 100, 1000 ppm) and controls (0 ppm) received 10 µl of acetone. At least four insects from each site (Florida and Alabama) were treated with each concentration per replicate (3 replicates, n=12 insects per concentration). Post application mole crickets were returned to their cup with sand and monitored for mortality every 24 h for at least 4 days. Mole crickets were provided freeze dried mealworms as food during the post-treatment evaluation period.

Results:

Adult Southern Mole Crickets, Florida population. After 96 hours, only 1 southern mole cricket in the control (0 ppm fipronil) treatment died. Mortality of southern mole crickets after 24 hour was only observed in the 10 and 1000 ppm treatments. After 96 hours, all southern mole crickets from Florida were treated topically once with 1000 ppm fipronil, and 58% or more of those treated with 10 or 100 ppm were dead. After 96 hours, only 2 and 3 southern mole crickets were dead in the 1 and 5 ppm concentrations respectively.

Tawny Mole Cricket Nymphs, Alabama population. At 24 and 48 hours, 3 mole crickets in the control (0 ppm fipronil) treatment had died. Mortality increased to 6 and 8 dead at 72 and 96 hours, respectively. All concentrations 5 ppm and greater caused 100% mortality at or before 96 hours, but only 1000 ppm reached 100% mortality after 48 hours.

Conclusions:

Insecticide susceptibility is relative. Data from lab experiments are best interpreted relative to other populations of the same species and compared only to insecticides with the same mode of action. A greater concentration of fipronil was required to kill the adult southern mole crickets than the tawny nymphs in these lab experiments. This could be due to differences in species and not just site-specific management practices. Tawny mole crickets are more difficult to maintain in captivity after field collection than southern mole crickets. The higher loss in the control treatment may be due to a combination of handling mortality and susceptibility to the acetone solvent. Previous lab work (Yu 1982 Pest. Biochem. Physiol. 17:170-176) suggests that tawny mole crickets are more susceptible to insecticides than southern mole crickets.

Our lab also has data for tawny mole crickets that can be used for comparison. In March and April 2017, the principal investigator on this project collected and assayed adult tawny mole crickets from two Alabama golf courses. In the lab experiment, 100 ppm of fipronil in acetone applied topically was sufficient to kill 100% of tawny adults within 48 to 96 hours from both golf courses. Concentrations between 10 and 50 ppm were had 0 to 25 % mortality after 96 hours. Based on this, a one-time exposure to 100 ppm of fipronil was consistently toxic to tawny mole crickets.

From these laboratory assays and the principal investigator's experience with mole cricket management, there is little evidence to support a concern for resistance to fipronil at either site that was sampled. If fipronil is applied at label rate, there would be 260 micrograms of fipronil per sq foot of treated area. That is 26x the highest contact dosage that provided 100% mortality in the lab experiment. The breakthroughs in treatments at both sites may be due to treatment timing, application rates, mixed species populations, or re-infestation after application. At best, fipronil provides 4 to 6 months of residual control when applied at peak egg hatch. Timing of application to 'peak egg hatch' as indicated on insecticide labels can be challenging given the potentially wide oviposition window. In past field samples, we have recovered 2nd to 9th instars (nymphs with wing pads) from soap slushes in August (Held and Cobb 2016, ANR-176). Eggs laid in late July may not be exposed to a lethal soil concentration if applications were made in March or April. Cumming et al. (2006) reported a 120 d fipronil residue in turfgrass cores of 0.047 ug per g of soil which was sufficient to kill 60% of the tawny nymphs (instar or size not reported) confined in treated soil cores for up to 10 days. Given the differences in insecticide susceptibility previously reported, it is also plausible that a single rate application of fipronil to turfgrass may selectively kill or reduce one species of mole cricket allowing another to persist. Previous work in the NE US (Cowles et al. 1999, J Econ Entomol, 92:427-434) has demonstrated this phenomenon for mixed species populations of white grubs. There are no field data to support this phenomenon with mole crickets, but it is a possible explanation for the treatment breakthroughs noted on the sampled golf courses and others that have previously contacted the principal investigator.

USGA ID#: 2022-19-762

Title: Effects of foot and cart traffic during frost on annual bluegrass and creeping bentgrass putting greens and fairways

Project Leaders: Alec Kowalewski, Brian McDonald, Cole Stover and Zach Hamilton

SUMMARY

Intial research conducted at Oregon State University would suggest that foot traffic applied to an annual bluegrass putting green covered in frost would not produce noticeable turf injury or reductions in plant health. However, cart traffic applied to a creeping bentgrass fairway did produce signficant turf injury and reductions in turf health [normalized differnce vegitaion index (NDVI)] quantified using a FieldScout CM 1000 NDVI Meter.

OBJECTIVES

1. Evaluate the effects of foot traffic on a sand-based annual bluegrass putting green during periods of frost.

2. Evaluate the effects of foot traffic on a native soil annual bluegrass putting green during periods of frost.

3. Evaluate the effects of cart traffic on a creeping bentgrass fairway duiring a period of frost.

MATERIALS AND METHODS

Foot traffic during frost was applied to two separate annual bluegrass putting greens maintained at a 0.14" height of cut. The first was a sand-based putting green, while the second was a native soil putting green. Foot traffic was applied in the morning on the following days when frost was present (Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022). Air temperature and soil moisture measurements were taken on days when frost was present, and traffic was applied (Table 1).

Experimental design was a randomized complete block with four replications. Foot traffic treatments applied to each green were as follows:

- No traffic
- 6 steps on frosted turf – traffic produced by 2 golfers putting
- 12 steps on frosted turf – traffic produced by one foursome (4 golfers) putting
- 24 steps on frosted turf – traffic produced by two foursome (8 golfers) putting ~ 15 minutes of golf
- 48 steps on frosted turf – traffic produced by four foursomes (16 golfers) putting ~ 30 minutes of golf

- 48 steps on turf after frost thawed

Table 1: Weather data collected on the days frost covered annual bluegrass putting greens at the OSU Lewis-Brown Horticulture Farm in Corvallis, OR, 2022. Foot traffic was applied on the days frost was present.

Weather data	6-Feb	7-Feb	12-Feb	13-Feb	22-Feb	23-Feb	24-Feb	25-Feb
Low Air Temp (F)	26	28.1	26.9	26.1	28.5	22.6	20.6	20.1
Time of low temp	4:45 AM	3:15 AM	7:15 AM	7:30 AM	7:15 AM	7:15 AM	1:45 AM	6:00 AM
Time to above 32 (F)	8:15 AM	8:15 AM	8:30 AM	8:30 AM	8:15 AM	10:00 AM	9:45 AM	8:30 AM
High Air Temp (F)	49.8	57.8	61	58.6	42.3	39.2	43.6	48.1
Time of high temp	4:15 PM	3:45 PM	3:30 PM	4:00 PM	3:30 PM	3:15 PM	3:45 PM	3:15 PM
Traffic Applied	7:30 AM	7:15 AM	7:15 AM	8:30 AM	7:08 AM	7:34 AM	7:50 AM	8:15 AM
VWC @ 1.5" of sand-based green	44.1	43.2	42.8	42	47	45.2	48.3	33.3
VWC @ 1.5" of native soil green	45.1	43.1	40.6	38.9	45.6	39.5	45.7	32

Cart traffic was applied to bentgrass maintained at fairway height (0.5”). Cart traffic was applied in the morning on two consecutive days with frost (Feb 12 and Feb 13, 2022). Air temperature and soil moisture measurements were taken on days when frost was present, and traffic was applied (Table 2).

Experimental design was a randomized complete block design with three replications. Cart traffic treatments were as follows:

- No traffic
- 1 pass with cart on frosted turf
- 1 pass with cart on unfrosted turf
- 7 passes with cart on frosted turf
- 7 passes with cart on unfrosted turf

Table 2: Weather data collected on the days frost covered a creeping bentgrass fairway at the OSU Lewis-Brown Horticulture Farm in Corvallis, OR, 2022. Cart traffic was applied on the days frost was present.

Weather data	6-Feb	7-Feb
Low Air Temp (F)	26.9	26.1
Time of low temp	7:15 AM	7:30 AM
Time to above 32 F	8:30 AM	8:30 AM
High Air Temp (F)	61	58.6
Time of high temp	3:30 PM	4:00 PM
Traffic Applied	7:15 AM	8:30 AM
VWC @ 1.5" of a native soil fairway	31.7	29.3

RESPONSE VARIABLES:

The plots were rated for traffic injury (1 – 3 scale, with 1 = no injury) and normalized difference vegetation index (NDVI) values were recorded. Data were subjected to Analysis of Variance (ANOVA) and differences were determined by LSD at the 5 percent alpha level.

RESULTS

Objective 1: Effects of foot traffic on a sand-based annual bluegrass putting green during periods of frost

Foot trafffic applied to a sand-based annual bluegrass putting green on days with frost did not produce injury or reduce NDVI values in Corvallis, OR (Table 3 and 4).

Table 3: Effects of foot traffic combined with frost on traffic injury (1 to 3 scale, 1 = no injury) on a sand-based annual bluegrass green in Corvallis, OR. Frost was present and foot traffic was applied on the mornings of Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022.

	6-Feb	8-Feb	10-Feb	12-Feb	22-Feb	24-Feb	25-Feb
Foot traffic treatment	Traffic injury (1 to 3, 1 = no injury and 3 = extreme injury)						
No Traffic	1	1	1	1	1	1	1
6 steps frost‡	1	1	1	1	1	1	1
12 steps frost	1	1	1	1	1	1	1
24 steps frost	1	1	1	1	1	1	1
48 steps frost	1	1	1	1	1	1	1
48 steps after frost	1	1	1	1	1	1	1
LSD@ .05	ns	ns	ns	ns	ns	ns	ns

‡Foot traffic with frost was applied early in the morning on Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022, while foot traffic without frost was applied in the day on these same days.
ns = no significant differences at a 0.05 levele of probability.

Table 4: Effects of foot traffic combined with frost on normailized difference vegitation index (NDVI) on a sand-based annual bluegrass green in Corvallis, OR. Frost was present and foot traffic was applied on the mornings of Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022.

	6-Feb	8-Feb	10-Feb	12-Feb	22-Feb	24-Feb	25-Feb
Foot traffic treatment	Normalized difference vegetation index (NDVI)						
No Traffic	84.0	83.8	83.3	80.5	81.5	80.0	80.8
6 steps frost‡	84.3	84.0	82.8	80.0	82.5	81.5	81.3
12 steps frost	84.0	83.5	82.8	81.0	82.0	81.3	80.3
24 steps frost	83.8	83.3	82.5	80.3	82.3	81.0	81.0
48 steps frost	84.5	84.0	83.0	81.0	82.3	81.8	81.0

48 steps after frost	84.8	84.0	83.0	78.0	83.0	81.8	81.8
LSD@ .05	ns	ns	ns	ns	ns	ns	ns

‡Foot traffic with frost was applied early in the morning on Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022, while foot traffic without frost was applied in the day on these same days.

ns = no significant differences at a 0.05 levele of probability.

Objective 2: Effects of foot traffic on a native soil annual bluegrass putting green during periods of frost

Foot trafffic applied to a native soil annual bluegrass putting green on days with frost did not produce injury or reduce NDVI values in Corvallis, OR (Table 5 and 6).

Table 5: Effects of foot traffic combined with frost on traffic injury (1 to 3 scale, 1 = no injury) on a native soil annual bluegrass green in Corvallis, OR. Frost was present and foot traffic was applied on the mornings of Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022.

	6-Feb	8-Feb	10-Feb	12-Feb	22-Feb	24-Feb	25-Feb
Foot traffic treatment	Traffic injury (1 to 3, 1 = no injury and 3 = extreme injury)						
No Traffic	1	1	1	1	1	1	1
6 steps frost‡	1	1	1	1	1	1	1
12 steps frost	1	1	1	1	1	1	1
24 steps frost	1	1	1	1	1	1	1
48 steps frost	1	1	1	1	1	1	1
48 steps after frost	1	1	1	1	1	1	1
LSD@ .05	ns	ns	ns	na	ns	ns	ns

‡Foot traffic with frost was applied early in the morning on Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022, while foot traffic without frost was applied in the day on these same days.

ns = no significant differences at a 0.05 levele of probability.

Table 6: Effects of foot traffic combined with frost on normailized difference vegitation index (NDVI) on a native soil annual bluegrass green in Corvallis, OR. Frost was present and foot traffic was applied on the mornings of Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022.

	6-Feb	8-Feb	10-Feb	12-Feb	22-Feb	24-Feb	25-Feb
Foot traffic treatment	Normalized difference vegetation index (NDVI)						
No Traffic	84.0	83.8	83.3	80.5	81.5	80.0	80.8
6 steps frost‡	84.3	84.0	82.8	80.0	82.5	81.5	81.3
12 steps frost	84.0	83.5	82.8	81.0	82.0	81.3	80.3
24 steps frost	83.8	83.3	82.5	80.3	82.3	81.0	81.0
48 steps frost	84.5	84.0	83.0	81.0	82.3	81.8	81.0
48 steps after frost	84.8	84.0	83.0	78.0	83.0	81.8	81.8
LSD@ .05	ns	ns	ns	ns	ns	ns	ns

‡ Foot traffic with frost was applied early in the morning on Feb 6, 7, 12, 13, 22, 23, 24 and 25, 2022, while foot traffic without frost was applied in the day on these same days.
ns = no significant differences at a 0.05 levele of probability.

Objective 3: Effects of cart traffic on a creeping bentgrass fariway duiring a period of frost

Cart traffic, 1 or 7 passes, applied when frost was present produced singifcant injury to the bentgrass fairway on all observation dates (Feb 12 to March 1, 2022), and reduce NDVI values on Feb 15, 2022 only (Table 7 and 8). The high level of cart traffic (7 passes) without frost produced some turf injury when repeatedly applied and compared to the no traffic treatment on Feb 25 and Mar 1, 2022 (Table 7). Seven passes with the cart during frost resulted in the greatest reduction in NDVI values, followed by 1 pass with the cart during frost (Table 8). Cart traffic (1 or 7 passes) without frost resulted in NDVI comparable to the no traffic treatment.

Table 7: Effects of cart traffic combined with forst on traffic injury (1 to 3 scale, 1 = no injury) on a native soil creeping bentgrass fairway in Corvallis, OR. Frost was present and cart traffic was applied on the mornings of Feb 12 and 13, 2022.

	12-Feb		13-Feb		15-Feb		25-Feb		1-Mar	
Cart traffic treatment	Traffic injury (1 to 3, 1 = no injury and 3 = extreme injury)									
No Traffic	1.0	b‡	1.0	c	1.0	a	1.2	c	1.0	c
1 pass with frost Ɏ	2.0	a	2.0	b	2.0	a	1.5	b	1.5	b
1 pass without frost	1.0	b	1.0	c	1.0	a	1.0	c	1.0	c
7 passes with frost	2.3	a	2.7	a	3.0	a	2.2	a	2.0	a
7 passes without frost	1.0	b	1.0	c	1.5	a	1.5	b	1.5	b

Ɏ Cart traffic with frost was applied early in the morning on Feb 12 and 13, 2022, while cart traffic without frost was applied in the day on these same days.

‡Means followed by the same letter are not statistically different according to Fisher's least protected significant difference test at P≤ 0.05.

Table 8: Effects of cart traffic combined with forst on normailized difference vegitation index (NDVI) on a native soil creeping bentgrass fairway in Corvallis, OR. Frost was present and cart traffic was applied on the mornings of Feb 12 and 13, 2022.

	12-Feb		13-Feb		15-Feb	
Cart traffic treatment	Normalized difference vegetation index (NDVI)					
No Traffic	72.0	a	80.0	a	76.0	a
1 pass with frost	72.3	a	78.0	a	71.0	ab
1 pass without frost	73.0	a	76.0	a	73.3	a
7 passes with frost	69.0	a	71.0	a	66.0	b
7 passes without frost	71.3	a	76.0	a	75.7	a

Ɏ Cart traffic with frost was applied early in the morning on Feb 12 and 13, 2022, while cart traffic without frost was applied in the day on these same days.

‡Means followed by the same letter are not statistically different according to Fisher's least protected significant difference test at P≤ 0.05.

USGA ID#: 2021-17-741
Title: Golf ball reaction and soil strength study

Project Leader: Jackie Guevara, Jake Kilby, Ryan Bearss, Evan Rogers, Thomas O. Green, James R. Crum, John N. Rogers III

Affiliation: Michigan State University

Objectives: Determine golf ball reaction from simulated inbound golf shots on various rootzone mixtures

Start Date: 2021
Project Duration: 2 years
Total Funding: $34,800

Summary Points:

- #28 sand had the lowest volumetric water content and produced the highest ball reaction.
- #28 sand with 15% silt+clay had the highest volumetric water content and produced the lowest ball reaction.
- It was observed that as soil moisture levels decreased, ball reaction increased while pitch mark depth decreased.
- These observations suggest that a golf ball landing on a drier soil (particularly with a coarser material) creates a shallower pitch mark resulting to a longer ball bounce and roll.

Summary Text:

Rationale

Golf course playability and the golfer experience improves when golfers can bounce their golf shots up onto putting greens and when golf shots bounce and roll from tee shots. While soil moisture and surface organic matter levels influence inbound golf ball reaction, the rootzone components also play a role; one that is not well understood in the golf industry. For over half a century, golf courses have enjoyed the benefits of sand topdressing fairways and approaches or even placing a sand “cap” over poorly drained soils to yield drier conditions. However, field observations have determined that such a scenario does not necessarily (and even unlikely) yield a firm surface that delivers good golf ball reaction. We aim to offer a better guidance on how to improve fairway/approach drainage characteristics, offer a rootzone with adequate aeration porosity and yet still deliver a firm, stable surface that yields good ball reaction. Moreso this study may give insight on improving golf shots by allowing golfers the option of bouncing shots to gain distance while placing them nearer target putting green.

Methodology

The experimental site was constructed in the summer of 2000 to assess the effects of natural and artificial soil enhancements on strength of an athletic field rootzone when subjected to simulated traffic. To prepare for the golf ball reaction study and soil strength study, the site was renovated in the summer of 2020 and reseeded with an 80% Kentucky bluegrass and 20% perennial ryegrass mix and has since been maintained as a simulated golf course fairway. Treatment plot dimensions were 10 feet by 16 feet.

The experimental design was a randomized complete block design (RCBD) with three replications. The two factors were rootzone mixtures and days after irrigation (DAI). The seven rootzone mixtures were TDS 2150 sand, # 28 sand (control), #28 sand + 7% silt+clay, #28 sand + 9% silt+clay, #28 sand + 15% silt+clay, #28 sand + Profile and #28 sand + ZeoPro. Treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (based on #28 sand rootzone plot). In the second year of the study, the 1st dry down cycle ran from June 16th until June 21st while the 2nd dry down cycle ran from August 15th until August 20th.

Volumetric water content was measured using a time domain reflectometry device 300 (Spectrum Technologies, Aurora, IL). Surface firmness (depth of impact) was measured using USGA TruFirm. In 2022, the newer version of TruFirm was used during the 1st dry down cycle while the older version of TruFirm provided by USGA was used during the 2nd dry down cycle. To simulate the launch conditions of a 6-iron club, a modified pitching machine at setting #12 (speed of 84, spin of 100 [top wheel control dial = 2.5, bottom wheel control dial = 8]). Ball reaction (i.e., ball bounce and roll) and pitch mark depth were measured after each 3-ball launch series.

All analyses were performed in R (R Core team, 2022. Analysis of Variance (ANOVA) was analyzed using the ‘lmer’ and ‘car’ packages. When a significant difference was detected ($P < 0.05$), means were separated using Least Significance Difference (LSD) pairwise comparison.

2022 Results and Discussion

The rootzone mixture influenced the volumetric water content and ball reaction in both dry down cycles (Table 1). #28 sand (control) had the lowest volumetric water content and produced the highest ball reaction. Whereas #28 sand with 15% silt+clay had the highest volumetric water content and produced the lowest ball reaction (Figures 1a & b). Coarse particles (e.g., sand) have a low water holding capacity; hence #28 sand had a low volumetric water content. Finer soil particles (e.g., silt and clay) have a higher water-holding capacity than sand. As a result, #28 sand with the highest percentage of silt and clay (15% silt+clay) had the highest volumetric water content.

The volumetric moisture content, ball reaction, and ball bounce data from each day of the dry down cycle (days after irrigation) followed noticeable trends. As anticipated, volumetric water content decreased towards the end of the dry down cycle. Mean volumetric water content started from 30.8% and dropped to 15.6% for the 1st dry down cycle while it started from 30.8% and dropped to 20.2% for the 2nd dry down cycle (Figure 2a). It was also observed that as soil moisture levels decreased, ball reaction increased while pitch mark depth decreased (Figure 2a, b & c). The lowest ball reaction and highest pitch mark depth were during the start of a dry down cycle. Meanwhile, the highest ball reaction and lowest pitch mark depth were observed towards the end of a dry down cycle (Figure 2b & c). These observations suggest that a golf ball landing on drier soil (particularly with a coarser material) creates a shallower pitch mark leading to a longer ball bounce and roll.

The newer version of the TruFirm was used to measure surface hardness (depth of impact) in the 1st dry down cycle (June). A significant interaction between rootzone mixture and DAI on surface firmness (depth of impact) was found (Table 1). For the three rootzone mixtures (TDS2150 sand, #28 sand + 7% silt+clay, and #28 sand + 15% silt+clay), a higher depth of impact (softer surface) was observed at 0 DAI compared to subsequent days (Figure 3). For two rootzone mixtures (#28 sand + 9% silt+clay and #28 sand + Profile), no differences in surface hardness between DAI were observed. For one rootzone mixture (#28 sand + ZeoPro), a softer surface was observed at 0 DAI and became firmer for the following days until it became softer yet again at 5 DAI. The older version of the TruFirm provided by USGA was used in the 2nd dry down cycle (August). The dry down cycle (days after irrigation) did not influence surface firmness (Table 1). Due to the inconsistencies of the TruFirm data, it was difficult to make conclusions based on these observations. It is recommended to research the reliability of TruFirm as a testing device for surface firmness in the future.

Table 1. Analysis of variance and effect of rootzone and days after irrigation on ball reaction, surface firmness, pitch mark depth, and volumetric water content for both dry down cycles (June and August) at East Lansing, MI in 2022.

Source of variation	Volumetric moisture content		Surface firmness		Ball reaction		Pitch mark depth	
	Jun	Aug	Jun	Aug	Jun	Aug	Jun	Aug
Rootzone (R)	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001
Days after irrigation (D)	<0.001	NS	<0.001	NS	<0.001	<0.001	<0.001	<0.001
R x D	NS	NS	<0.05	NS	NS	NS	NS	NS

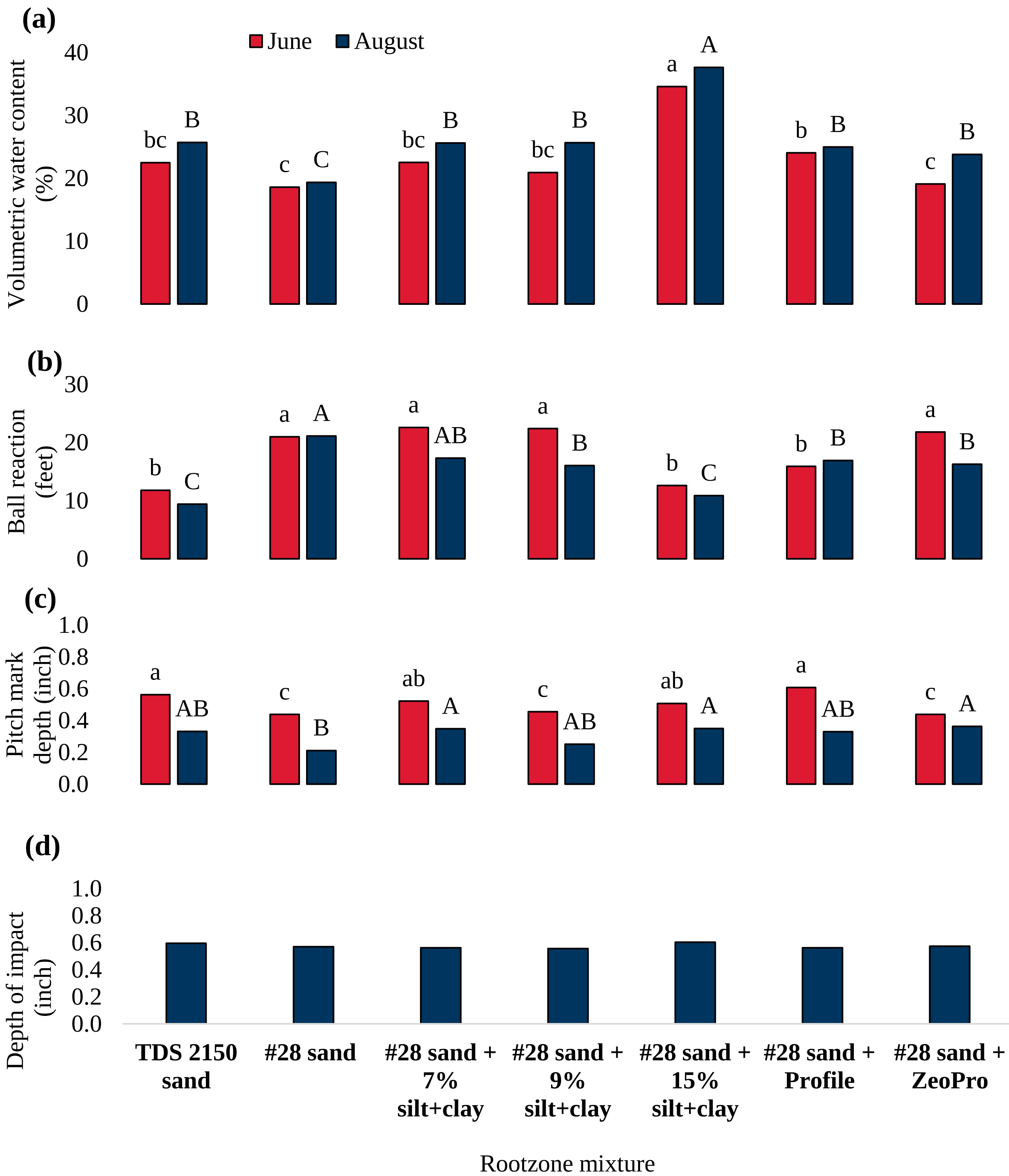

Figure 1. (a) Volumetric water content, **(b)** ball reaction, **(c)** pitch mark depth and **(d)** depth of impact of seven rootzone mixtures (TDS 2150 sand, # 28 sand, #28 sand + 7% silt+clay, #28 sand + 9% silt+clay, #28 sand + 15% silt+clay, #28 sand + Profile and #28 sand + ZeoPro) for two dry down cycles (June and August) at East Lansing, MI in 2022. Means followed by the same letter do not statistically differ according to Fisher's protected least significant difference (LSD) at P = 0.05. Mean comparison for the June (1st dry down cycle) was shown in lowercase letters and for August (2nd dry down cycle) was shown in uppercase letters.

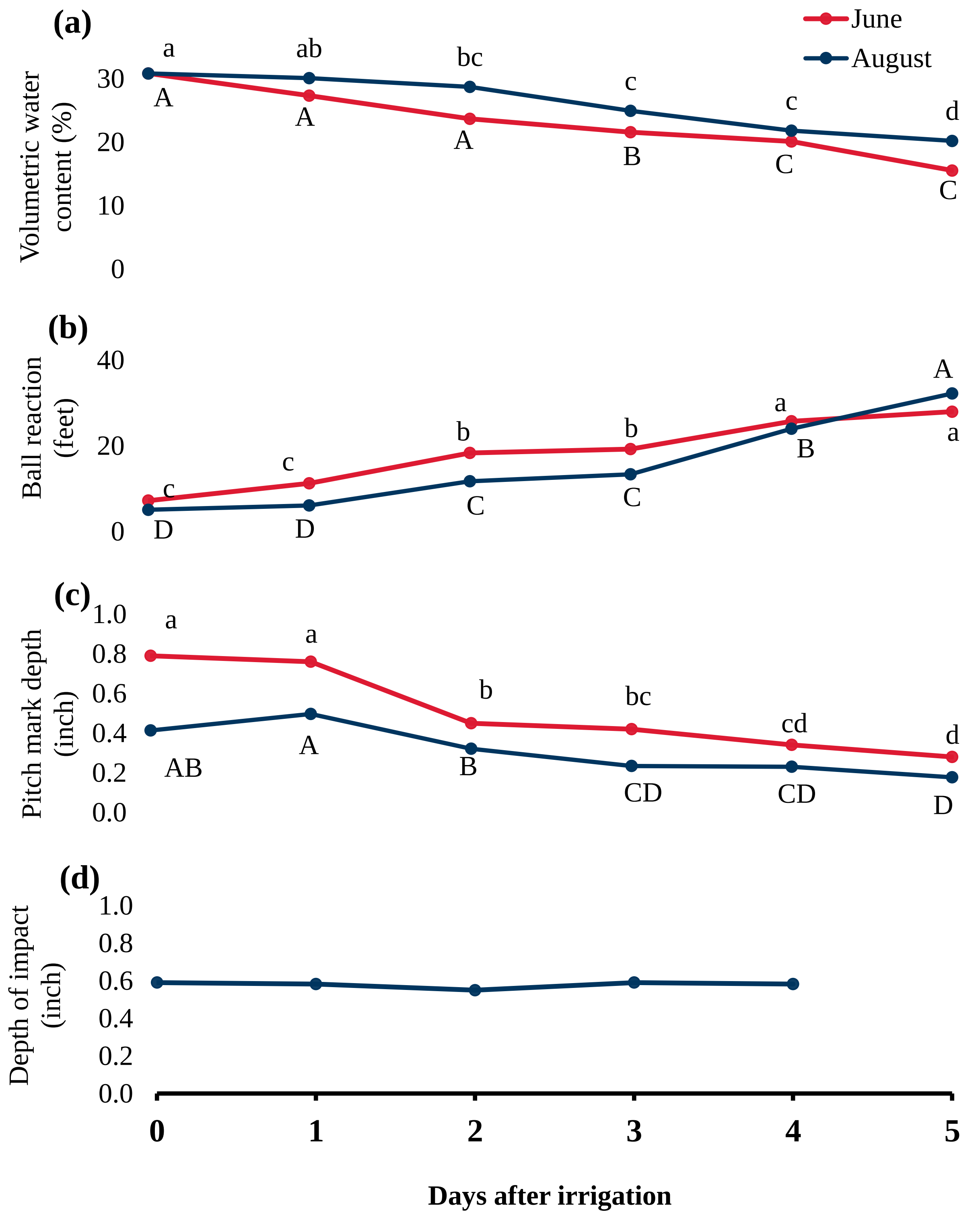

Fig 2. (a) Volumetric water content, **(b)** ball reaction, **(c)** pitch mark depth and **(d)** depth of impact of 0, 1, 2, 3, 4 and 5 days after irrigation for two dry down cycles (June and August) at East Lansing, MI in 2022. Means followed by the same letter do not statistically differ according to Fisher's protected least significant difference (LSD) at P = 0.05. Mean comparison for the June (1st dry down cycle) was shown in lowercase letters and for August (2nd dry down cycle) was shown in uppercase letters.

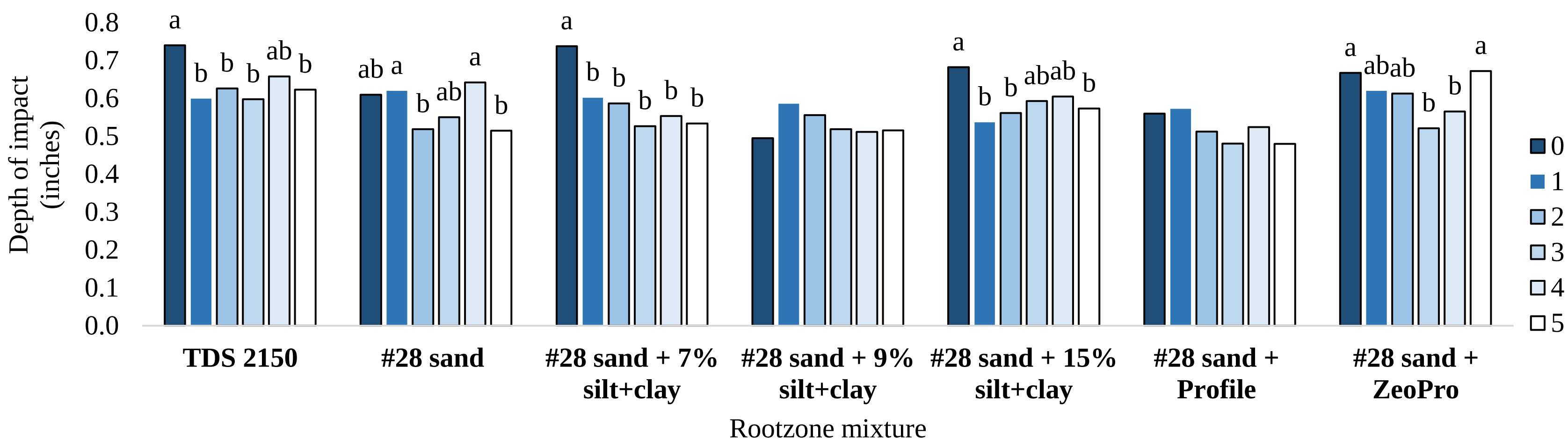

Figure 3. Surface firmness (depth of impact) of rootzone mixture and days after irrigation from June 16th until June 21st (1st dry down cycle). A lower depth of impact indicates a firmer surface.

Supplementary Table 1. A list of different rootzone mixtures used in the treatment plots to study golf ball reaction and soil strength in 2021 at East Lansing, MI.

Treatment	Percent retained [z]							
	Size class (mm)							
	FG (2.0)	VCoS (1.0)	CS (0.5)	MS (0.25)	FS (0.1)	VFS (0.05)	Silt (0.002)	Clay (<0.002)
TDS 2150 sand	0.0	0.0	2.4	64.0	32.9	0.1	0.5	0.0
#28 sand	1.4	13.7	26.0	42.5	14.1	0.4	1.0	0.9
7% silt + clay [y]	1.5	13.0	22.4	38.5	15.9	1.7	3.9	3.1
9% silt + clay	1.5	12.2	21.6	36.6	16.5	2.3	5.7	3.6
15% silt + clay	1.1	10.2	18.9	33.5	17.5	3.7	10.6	4.4
Profile [x]	1.0	11.2	24.8	44.8	14.1	0.8	1.6	1.8
ZeoPro [w]	0.5	9.6	25.0	43.4	17.8	0.8	2.0	0.9

[z] The retained percentage is the weight of soil particles in each size class defined by the United States Department of Agriculture (USDA) as being fine gravel (FG), very coarse sand (VCoS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS), silt and clay.
[y] Well-graded sand (#28) was used as a base and mixed on a volume basis with a sandy loam soil to produce the sand-soil mixes.
[x] Profile rootzone mixture consisted of 75% well-graded sand (#28), 20% porous ceramic product manufactured from illite clay and amorphous silica, and 5% sphagnum peat by volume.
[w] ZeoPro rootzone mixture consisted of 80% well-graded sand (#28), 10% clinoptilolite, and 10% sphagnum peat by volume.
The treatment plots were irrigated to field capacity and allowed to dry down to near wilting point; soil moisture thresholds were assessed using the control plot with #28 sand rootzone.
The study site at East Lansing, MI was first constructed in the summer of 2000 as a simulated athletic field plotted with different rootzone mixtures. It was renovated in the summer of 2020 as a simulated golf fairway plotted with different rootzone mixtures.

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Supplementary Table 2. Analysis of variance and effect of rootzone and days after irrigation on ball reaction, surface firmness, pitch mark depth, and volumetric water content in 2022 at East Lansing, MI.

Treatment	June 2022 (1st dry down cycle)				August 2022 (2nd dry down cycle)			
	Volumetric moisture content	Depth of impact	Ball reaction	Pitch mark depth	Volumetric moisture content	Depth of impact	Ball reaction	Pitch mark depth
	%	inch	feet	inch	%	inch	feet	inch
Rootzone (R)								
TDS 2150	22.5 bc		11.8 b	0.56 a	25.7 b	0.595	7.2 d	0.33 ab
#28 sand	18.6 c		21.0 a	0.44 c	19.4 c	0.569	16.4 a	0.21 b
#28 sand + 7% silt+clay	22.5 bc		22.6 a	0.52 ab	25.6 b	0.562	13.8 ab	0.35 a
#28 sand + 9% silt+clay	20.9 bc		22.4 a	0.45 c	25.7 b	0.556	12.7 abc	0.25 ab
#28 sand + 15% silt+clay	34.6 a		12.6 b	0.51 ab	37.6 a	0.604	9.5 cd	0.35 a
#28 sand + Profile	24.1 b		15.9 b	0.61 a	25.0 b	0.562	12.6 abc	0.33 ab
#28 sand + ZeoPro	19.1 c		21.8 a	0.44 c	23.8 b	0.574	12.1 bc	0.36 a
LSD	4.3		4.9	0.11	2.6		4.1	0.13
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001
Days after irrigation (D)								
0	30.8 a		7.2 c	0.79 a	30.8 a	0.590	5.1 d	0.41 ab
1	27.3 ab		11.3 c	0.76 a	30.1 a	0.582	6.1 d	0.50 a
2	23.7 bc		18.4 b	0.45 b	28.7 a	0.550	11.7 c	0.32 b
3	21.6 c		19.3 b	0.42 bc	24.9 b	0.569	13.4 c	0.23 cd
4	20.1 c		25.8 a	0.34 cd	21.8 c	0.582	24.0 b	0.23 cd
5	15.6 d		28.0 a	0.28 d	20.2 c	-	32.2 a	0.18 d
LSD	4.0		4.5	0.1	2.5		3.8	0.12
p-value	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.001	<0.001
R x D	NS	<0.05	NS	NS	NS	NS	NS	NS

NS, nonsignificant at the 0.05 probability level.
Within columns, means followed by the same letter are not significantly different according to Fisher's protected least significance difference (p = 0.05).

Supplemental Figure 1. A photograph showing the golf ball launcher used in the ball reaction and soil strength study in 2022 at East Lansing, MI.

Supplemental Figure 2. Measuring ball reaction (aggregate golf ball bounce and roll) and pitch mark depth during the ball reaction and soil strength study in 2022 at East Lansing, MI.

Supplemental Figure 3. Measuring volumetric water content using time domain reflectometry device 300 during the ball reaction and soil strength study in 2022 at East Lansing, MI.

USGA ID#: 2022-17-760

Title: Exploring divot resistance and recovery of new turfgrasses for use on driving range tees

Project Leader: Maureen Kahiu and James T. Brosnan, Ph.D.
Affiliation: University of Tennessee, Knoxville

Objectives: Evaluate divot resistance and recovery of new turfgrass cultivars used on golf course driving range teeing surfaces.

Start Date: 2022
Project Duration: One Year
Total Funding: $5,000

Rationale and methodology

The American Society of Golf Course Architects (ASGCA) recommends 0.15 to 0.20 ft^2 of useable tee space per round played (ASGCA, 2020). However, there is scant information regarding appropriate sizing of driving range tee surfaces that have received increased use during the COVID-era. Moreover, several new hybrid bermudagrass (*C. dactylon* x *C. transvaalensis*) and creeping bentgrass (*Agrostis stolonifera*) cultivars have been released into the golf market with little known about how these selections respond to divot stress.

Research was conducted at the University of Tennessee during summer 2022 to evaluate divot resistance and recovery of two hybrid bermudagrasses [‘Latitude 36’ (L36) and ‘Tifway’ (TIF) and creeping bentgrass (‘L-93XD’). All turfgrass stands were established on silt loam and maintained at a height of 1.3 cm with a fairway reel mower. Preemergence herbicides were applied in spring 2022 to control infestations of summer annual weeds in these test sites.

The D.I.V.O.T. apparatus developed by Brosnan et al. (2013) was used to create uniform divots in this study. This instrument consists of a weighted pendulum and pitching wedge head that strikes the teeing surface with 531 J of impact energy. A total of 100 divots were created on each turfgrass surface and evaluated for six weeks.

Divot resistance and recovery were evaluated on each surface using methods similar to Jones et al. (2014). Divot resistance was assessed via measurements of divot volume immediately after divots were created. The weight of sand needed to fill (and level) each divot scar was used to calculate divot volume via the following equation:

$$\textit{Divot Volume } (cm^3) = \textit{Weight of sand } (g) * (1\ cm^3 / 1.51\ (g))$$

Divot recovery was evaluated via visual assessments of turfgrass cover within the divot scar, as well as via digital image analysis of turfgrass coverage within the divot scar.

Descriptive statistics were used to compare divot resistance among the three turfgrasses evaluated in this experiment. For divot recovery, data were fit to a sigmoidal non-linear regression model in GraphPad Prism that was used to estimate the days required to achieve 25, 50, 75, and 95% recovery. Confidence intervals (95%) were used to compare recovery estimates among turfgrasses in this experiment.

Results

Divot resistance varied among turfgrass with mean divot volume on L36 (341 cm^3) and TIF (342 cm^3) measuring less than L93-XD (750 cm^3). Despite similar mean values, standard deviation assessments showed more variability in divot volume on TIF compared to L36.

Significant differences in divot recovery were detected among turfgrasses (Table 1). Twenty-eight days were required to achieve 95% recovery on L36 compared to 44 days for TIF; this recovery benchmark was not reached on L-93XD during this experiment.

An equation was developed to assist golf course superintendents in estimating the amount of ground space required on driving range tee surfaces based on the recovery rate of the turfgrass planted. This equation is presented below.

Ground Space Needed = Depth Hitting Area is Moved per Week* Frequency Hitting Area is Moved per Week * Weeks to 95% Recovery.

An example of how superintendents could use this equation is as follows. If golfers use a one meter section of the turfgrass surface on a driving range tee and the superintendent repositions the hitting area three times per week, 33% percent less space would be required for driving range tees established to L36 compared to TIF.

Future work

The experiment will be repeated in the summer of 2023 to confirm these results. Future research evaluating divot resistance and recovery at different times of the year is warranted.

Summary Points:

- Divot resistance of both hybrid bermudagrasses was greater than creeping bentgrass in this experiment. Additionally, divot volume was more variable on Tifway than Latitude 36 hybrid bermudagrass.

- Divot recovery was faster on Latitude 36 than Tifway in this study. Only 28 days were required to reach 95% recovery on Latitude 36 compared to 44 days on Tifway.
- As much as 33% percent less space is needed on driving range tees established to Latitude 36 compared to Tifway.

Table 1. Days required to achieve different recovery benchmarks following the creation of divots on 24 May 2022 in Knoxville, TN. Values in parentheses represent 95% confidence intervals for each estimate.

	Recovery Estimates (Days)			
Turfgrass species	**25%**	**50%**	**75%**	**95%**
Latitude 36	9.48 (9.25 to 9.69)	12.77 (12.59 to 12.96)	17.22 (16.91 to 17.54)	28.44 (27.36 to 29.61)
Tifway	13.53 (13.24 to 13.83)	18.62 (18.31 to 18.93)	25.62 (24.97 to 26.29)	43.78 (41.61 to 46.12)
L93-XD	-	-	-	-

Figure 1. Divots created with D.I.V.O.T apparatus in Knoxville, TN on 24 May 2022. Note the pitching wedge head at the end of the weighted pendulum apparatus.

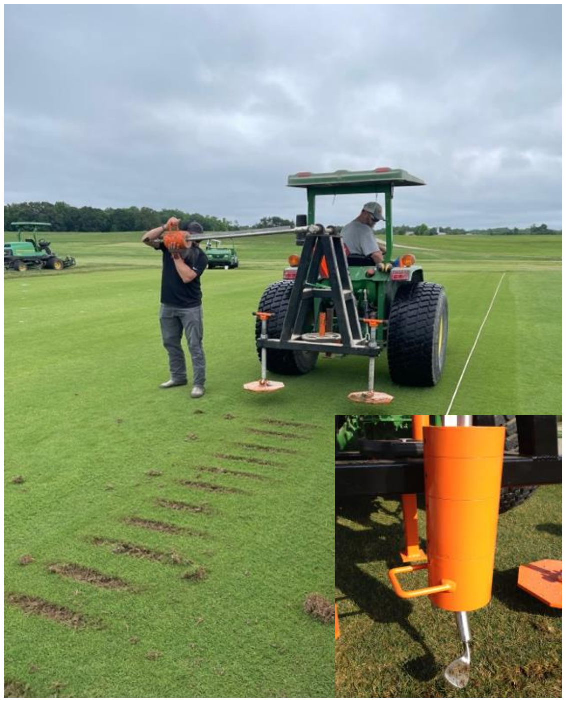
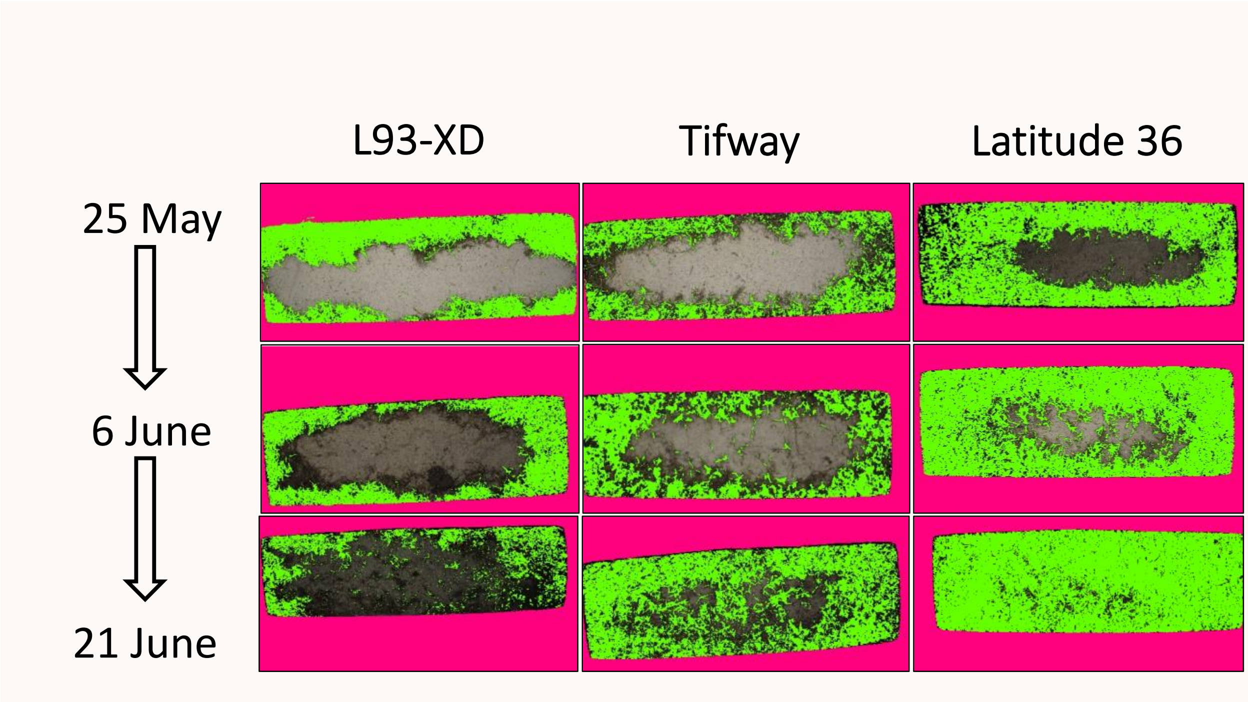


Figure 2. Divots created and filled with sand on day of study initiation in Knoxville, TN on 24 May 2022.

Figure 3. Digital image analysis photos of divot recovery throughout a four-week period in 2022. Divots were created on three different turfgrass stands: L-93XD creeping bentgrass, Tifway hybrid bermudagrass, and Latitude 36 hybrid bermudagrass.



References

1. American Society of Golf Course Architects. (2020) Designs on a better golf course: Practical answers to common questions for green committees. ASGCA Foundation, Brookfield, Wisconsin.

2. Brosnan, J.T., Hart, W.E., Thoms, A.W. and Sarten, J.R. (2013) A new apparatus to evaluate turfgrass divot resistance Intl. Turf. Res. J. 12 619 624

3. Jones, P.A., J.T. Brosnan, G.K. Breeden, J.J. Vargas, B.J. Horvath, and J.C. Sorochan. (2014) Effect of preemergence herbicides on hybrid bermudagrass divot resistance and recovery. HortScience. 49:1449-1453.

USGA ID#: 2021-01-725

Title: Revision, promotion and funding of the National Turfgrass Research Initiative (NTRI)

Project Leader: Kevin Morris
Affiliation: National Turfgrass Federation

Objectives: This project seeks recognition for turfgrass research, and requests recurring federal funding for critical turfgrass research needs.

Start Date: 2021
Project Duration: Three years
Total Funding: $120,000

SUMMARY POINTS

- As a result of past advocacy efforts and the FY20 $3,000,000 federal appropriation, the USDA-ARS has organized a 'Turfgrass Consortium', consisting of multiple federal turfgrass scientists conducting genetics, genomics, water conservation and ecosystem services research
- With results from the Turfgrass Stakeholder Summit II, the National Turfgrass Research Initiative (NTRI) has been reviewed and is being updated
- National Turfgrass Survey language and authorization funding has been submitted to Congress for inclusion in the 2023 Farm Bill
- A request has been made for a $20,000,000 Congressional authorization of the first turfgrass-specific competitive federal grant program, the Sustainable Turfgrass Research Initiative (STRI) in the 2023 Farm Bill
- The National Turfgrass Federation website update is in progress, for launch in early 2023

SUMMARY TEXT

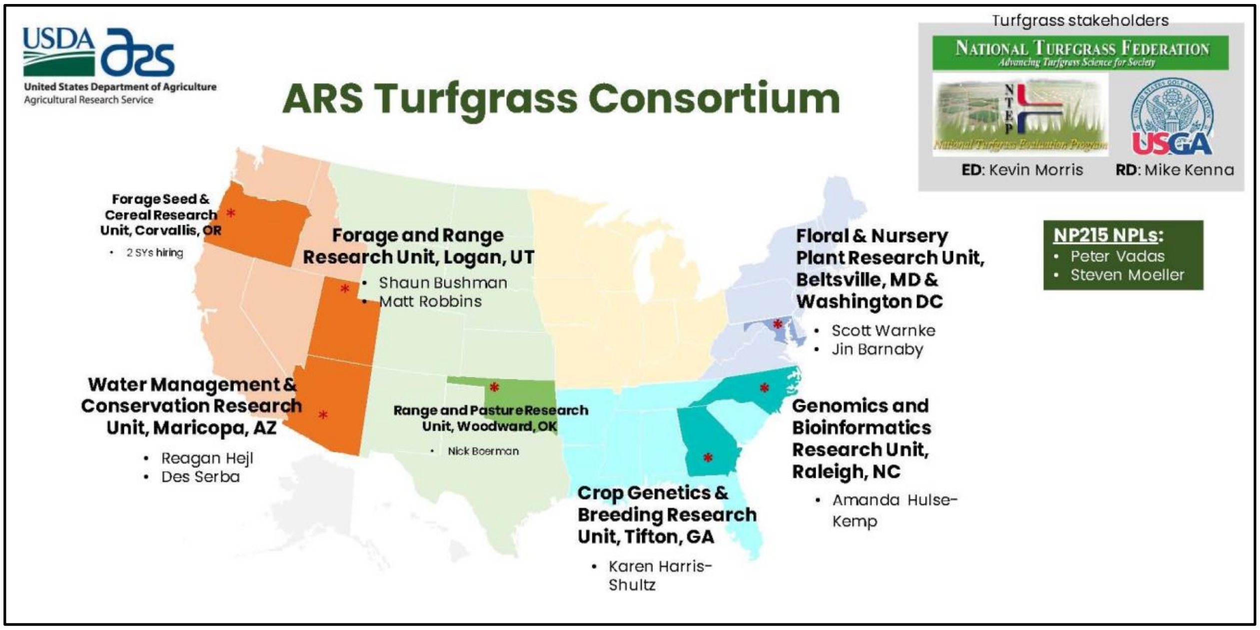
Turfgrass is an estimated $60 billion, 60 million-acre industry in the U.S., making turfgrass the third largest agricultural crop in the U.S. by acreage. However, the turfgrass industry faces serious challenges such as water shortages, concerns about pesticide use, fertilizer restrictions and economic issues. Research is needed to help golf overcome these challenges and thrive over the next 25-30 years, but *recurring* federal government turfgrass research funding falls far below research funding for other comparably sized agricultural industries, averaging less than $1,000,000 annually. The National Turfgrass Research Initiative (NTRI) was developed in 2004 as a joint strategic plan between turfgrass industry stakeholders and USDA-ARS to document research needs and help secure funding. NTRI now needs updating, refocusing and subsequently, additional funding.

National Turfgrass Federation (NTF) efforts have led to a recurring Congressional increase of $3,000,000 for turfgrass research within USDA-ARS (enacted in December 2019 for Fiscal Year 2020), the first step in confronting the golf industry's needs. The ARS funding builds on the existing ARS turfgrass effort by adding federal scientists and staff, focusing on long-term genomics, water conservation and ecosystem services studies.

To further these efforts and build on our success, this project addresses turfgrass' biggest challenges by 1) surveying the entire turfgrass industry to document its size and scope, 2) identifying, prioritizing and documenting national research needs, 3) seeking more federal recognition and support of turfgrass research to address prioritized needs, 4) seeking non-traditional federal and non-governmental organization (NGO) funding sources for research, and 5) publicizing this effort and successes to engage stakeholders and inform the public. These steps, taken in conjunction with the research conducted by USDA-ARS over the next several years, will have a significant positive impact on solving the challenges faced by the turfgrass industry.

A 2017 turfgrass stakeholder workshop developed a priority list of research and resulted in USDA-ARS committing new funding in FY19 ($225,000) to sequence the genomes of turfgrasses at several locations. The ARS federal funding initiated in December 2019 was led to four new positions and hires: a geneticist and an agronomist at Maricopa, AZ working on developing improved water conserving germplasm as well as management systems for the desert southwest U.S., a bioinformaticist at Logan, UT to analyze genetic information generated by ARS geneticists, and a geneticist at Beltsville, MD working on water saving and sustainable germplasm for the eastern U.S. These scientists, as well as researchers at St. Paul, MN, Madison, WI and Tifton, GA will conduct extensive, long-term research on turfgrass genomics, water conservation and ecosystem service maximization.

The ARS researchers have now organized into a 'Turfgrass Consortium', which allows them to collaborate and develop their research plans. The Consortium, after meeting via Zoom during the pandemic, met for the first time in-person on November 8th. Discussions included increased effort and planning on advances in their genome construction research, which will likely result in new genomes sequenced within the next 12-18 months. This funding will contribute foundational information to aid the development of improved heat, cold, drought, disease and insect resistant grasses. The Consortium is currently based on genetics and genomics research. However, the group is also organizing a multi-location ecosystem services project. The ecosystem services effort will add locations and personnel at St. Paul, MN, as well as a collaborative effort with researchers at the University of Wisconsin, Madison. It is exciting that the ARS turfgrass research consortium is expanding beyond its original purpose to include ecosystem services projects.



In fall 2020, NTF partnered with the Foundation for Food and Agriculture Research (FFAR), a non-profit established by Congress in the 2014 Farm Bill, to conduct the Turfgrass Stakeholder Summit II. Participants identified priority research needs, and those needs are being summarized and included in the updated National Turfgrass Research Initiative (NTRI). The summit presentations, needs prioritization and other info can be found here: https://www.nationalturfgrassresearchinitiative.info/. When the NTRI is finalized, it will be available at www.turfresearch.org. The summit also served as a 'Convening Event' for FFAR, developing innovative research programs that can be funded utilizing a 1:1 match of dollars from FFAR and industry.

The number one priority item determined by Stakeholder Summit II participants was the need for a National Turfgrass Survey, which will not only document acreage, scope and economic value of this crop, but also justify the need for increased federal research funding. On two separate occasions, we have applied for a $1,000,000 USDA, Agricultural Marketing Service (AMS), Specialty Crop Multi State grant to conduct a turfgrass survey that will cover the U.S. not only nationally, but regionally and by state as well. Being unsuccessful thus far, we are now relying on inclusion of turfgrass survey language in the upcoming Farm Bill.

We have decided not to request additional ARS funding until we can show significant results from the initial $3,000,000. In the meantime, we focused our efforts on the National Park Service (NPS) for funding. Since NPS is a well-known, well respected federal agency that most Americans recognize, encouraging and aiding turfgrass improvement at highly visible NPS sites, especially those at crowded, damaged locations will raise the awareness of functional turf's importance among the general public. We submitted our plan for a new NPS appropriation in FY22 but our funding was not granted. We will continue this effort but by requesting the shifting of internal, existing NPS funding to turfgrass improvement.

To address the research needs identified by participants at the Turfgrass Summit and documented in the NTRI, we are requesting inclusion of a new research initiative in the upcoming Farm Bill. The Farm Bill is a very important piece of legislation that is developed, debated and enacted by Congress every five years. We are proposing, along with inclusion of a

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National Turfgrass Survey authorization, the *Sustainable Turfgrass Research Initiative (STRI)*. STRI will address critical needs such as environmental protection, consumer health and athlete safety, developing solutions such as sustainable turfgrasses and management systems, and consequently increasing innovation. For *STRI*, we are requesting an annual *authorized* funding level, not to exceed $20,000,000 per fiscal year. An *authorization* does not guarantee funding, therefore if Farm Bill language and authorization is achieved, annual *appropriations* will be requested for both STRI and the National Turfgrass Survey.

In 2022, a new NTF website framework, necessary to the promotion of NTRI and our new funding request, is under development. Finishing the NTRI update is critical to the launch of the new website, which will happen in early 2023. Having a new website, updated NTRI and promotion activity is essential to the success of our efforts in 2023.

USGA ID#: 2022-05-748

Title: Stratification of Herbicide Resistance in Soil: An Ecological Glimpse into Changes in Poa annua Herbicide Resistance by Soil Depth

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Objectives: 1) Determine if the frequency of glyphosate resistance in Poa annua varies by soil depth, and 2) Determine the depth of remediation (i.e. fraise mowing or sod removal) required to remove herbicide resistance from a turf site once resistance develops.

Start Date: 01/01/2022
Project Duration: 2 years (01/01/2022-12/31/2023)
Total Funding: $22,081

Summary points
- Viable annual bluegrass seed is found predominantly in the upper 15 mm of soil on golf course fairways.
- A higher percentage of resistant plants were found in soil layer close to the surface.
- Additional testing on other sampled golf courses will help us refine recommendations on how best to mechanically remove herbicide resistant weeds via fraise mowing.

Summary Text:
Annual bluegrass (*Poa annua* L.) is a common turf weed of warm- and cool-season turfgrasses across the world. A reliance on herbicides to control annual bluegrass has resulted in many populations evolving resistance to preemergence and postemergence herbicides, particularly where warm-season turfgrasses are grown. Annual bluegrass populations with herbicide resistance Have developed across the US, with glyphosate resistance among the most common sites of action (enolpyruvylshikimate-3-phosphate synthase (EPSPS, WSSA Group 9). Through an assessment of four glyphosate resistant *Poa annua* populations on golf courses in the Southeast US, we hope to gain new information that will help superintendents determine the depth of remediation (i.e. fraise mowing or sod removal) required to remove herbicide resistance from a turf site once resistance develops. With this baseline knowledge, future work could also focus on how the depth of hollow-tine aerification and verticutting could influence the population dynamics of *Poa annua*.

Preliminary Test:
In preliminary testing conducted between August 2021 and February 2022, six soil cores from a golf course in Knoxville, TN were assessed to determine if the frequency of glyphosate resistant annual bluegrass plants varies by depth in the soil profile. All soil cores were sectioned into six layers (0-5, 5-10, 10-15, 15-30, 30-50, 50-100 mm) (Figure 1). The sectioned layers were placed into greenhouse trays to germinate the seedbank. Annual bluegrass plants from each layer were transplanted into separate containers when they reached approximately 1.5 cm in height. All plants

were initially sprayed with 840 g ha^{-1} glyphosate (24 fl oz/A of a 41% formulation) at the 5-6 tiller stage and rated 21 days after treatment. All plants were subjected to the second screening with a higher rate of glyphosate (1680 g ha^{-1}, 48 fl oz/A of a 41% formulation). All glyphosate treatments included the addition of 0.25% (v/v) nonionic surfactant and 2.0% sprayable ammonium sulfate (w/w).

The results of preliminary screening revealed that the percentage of resistant annual bluegrass plants was the highest in the shallowest layer of the soil cores (0-5 mm) (Table 1 and Figure 2). However, 72% of annual bluegrass seeds, regardless of susceptibility to glyphosate, were found in the upper 15 mm of the soil (Figure 3).

Table 1. Percentage of glyphosate susceptible annual bluegrass plants found in various depths of the soil cores, sampled from a golf course in Knoxville TN.

Depth	Plants[a]	Susceptible
mm	no.	%
0-5	69	12 a
5-10	93	20 ab
10-15	38	31 bc
15-30	35	40 c
30-50	31	42 c
50-100	9	56 c
P-value		0.0006

[a]Number of plants obtained across all 6 soil cores

Figure 1. Sectioning the soil core obtained on a golf course in Knoxville, TN, by using a serrated knife and measuring tape.

Collections:
Following the preliminary test, 25 cup cutter plugs taken to a 6” depth were obtained in August 2022 from fairways with a history of glyphosate resistant annual bluegrass at four different golf courses: two golf courses in TN and two golf courses in NC. The cores were wrapped with aluminum foil and sectioned into soil layers as described above. Section areas were de-aggregated by hand, dried on a greenhouse bench, and stored in bags until germination.

Recent Progress:
The study was initiated in December 2022, using 10 soil cores from the same golf course located in Knoxville TN. The methodology for the experiment was similar to preliminary testing with a couple of exceptions. First, trays with sectioned layers were placed into the germination chamber instead of the greenhouse bench, with the goal to achieve more favorable conditions for annual bluegrass germination. The germination chamber was set to 19/10°C (day/night) with 8-hr photoperiod. Second, in a goal to distinguish susceptible from resistant plants more accurately annual bluegrass plants were sprayed earlier, at 2-3 tiller stage. Spray rates remained 840 g ha^{-1}, and 1680 g ha^{-1}, with an addition of 0.25% (v/v) 2.0% ammonium-sulfate (w/w). However, plants were treated and rated in 14-day intervals instead of 21-day intervals. Third, in addition to visual rating of plants as susceptible or suspected resistant, digital images of each plant were taken.

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Across all 10 soil cores and all examined depths, 188 annual bluegrass plants were successfully germinated. Green cover and percentage of susceptible plants did not statistically differ by depth in the soil profile. However, 85% of the annual bluegrass plants were obtained from the upper 15 mm in the soil profile with more resistant plants found per soil layer close to the surface (Figure 2).

Next Steps:
Due to space restrictions in the growth chamber another cycle of the experiment has been initiated on May 8th 2023, using the remaining five soil cores obtained in Knoxville, TN. Additionally, 15 soil cores from each of the two sampled golf courses located in Raleigh, NC, and High Point, NC, will be examined using the same methodology.

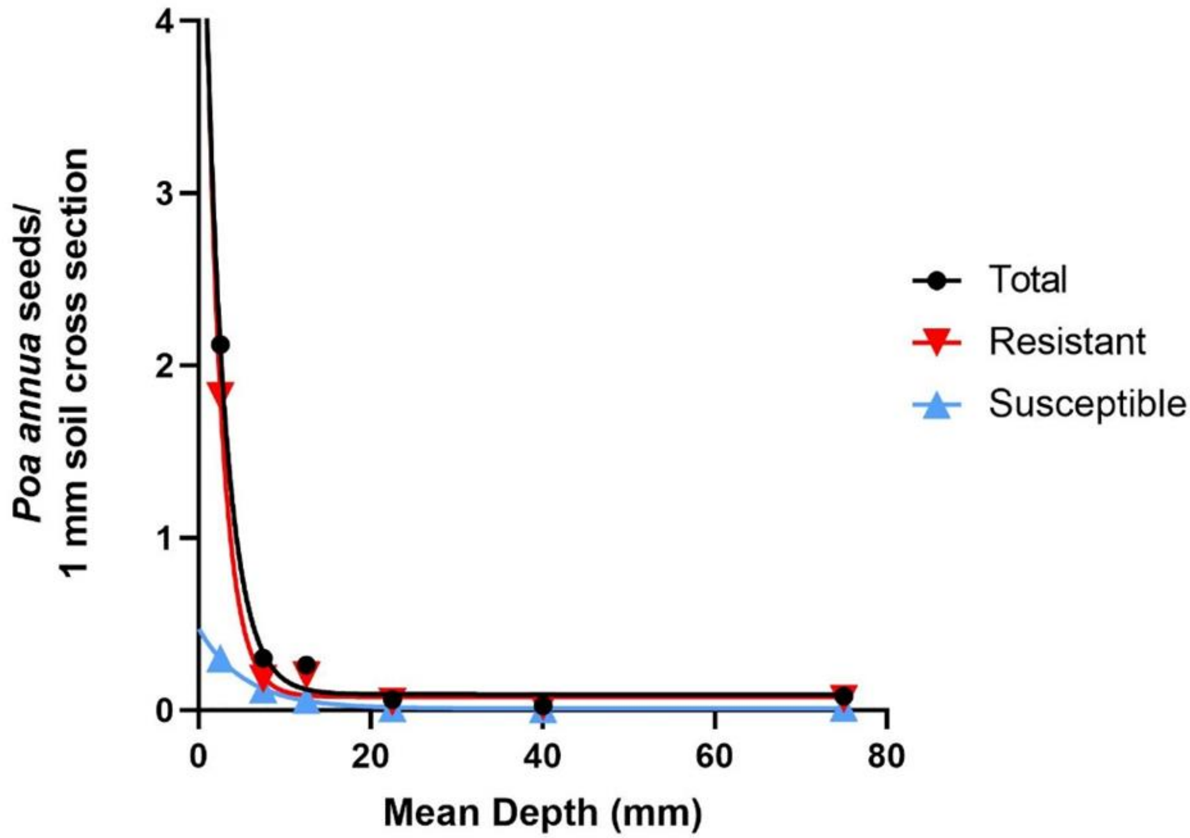

Figure 2. The number of annual bluegrass seeds from a 1 mm soil cross section of a golf course cup cutter (11 cm-diameter) at various depths in the soil profile. Determined based on ten soil cores, obtained from a golf course in Tennessee in 2022.