USGA Davis

2023 Progress Reports

Mike Davis Program for Advancing Golf Course Management



2023 USGA Davis Program Progress Reports

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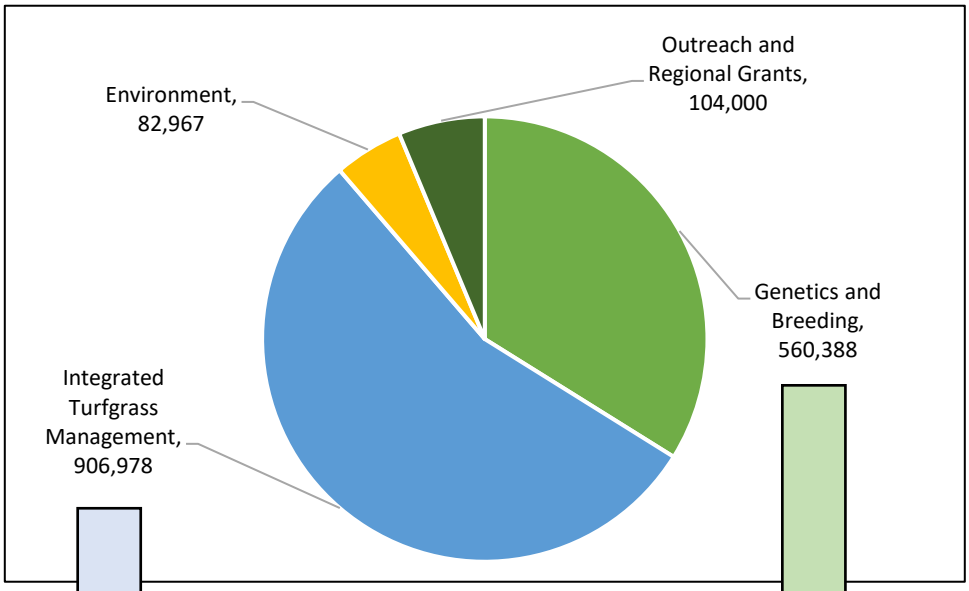
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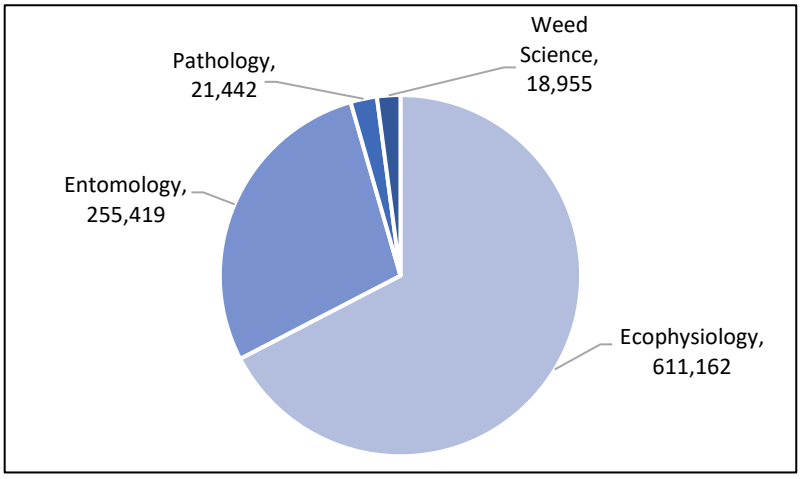
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Davis Program Grants - 2023



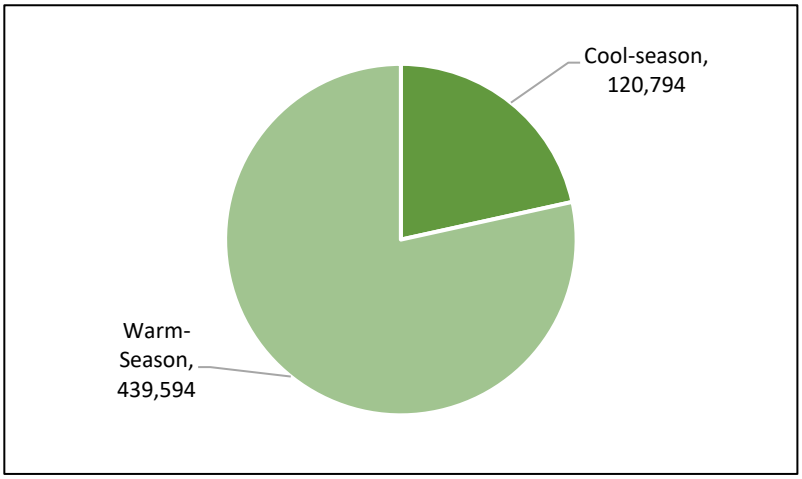
$1,654,333

Integrated Turfgrass Management



$906,978

Genetics and Breeding



$560,338

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 involved in the dollar spot resistance observed in endophyte-infected strong creeping red fescue.

2. 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.

3. The purified *E. festucae* antifungal protein also had activity in culture against other important plant pathogenic fungi. It may be possible to develop the antifungal protein as a complement biofungicide or alternative to synthetic fungicides used in crop protection from disease.

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 practices. We pursued 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. Our research identified an abundant antifungal protein produced by *E. festucae* that we have now confirmed as the mechanism behind the observed endophyte-mediated dollar spot resistance in strong creeping red fescue. In the future it may be possible to develop the antifungal protein as a biofungicide for control of dollar spot on creeping bentgrass. Below we summarize our research on the antifungal protein.

In 1989, strong creeping red fescues and Chewings fescues infected with the endophyte *E. festucae* were observed to have resistance to dollar spot disease at the Rutgers Plant Science Research Farm in Adelphia, NJ. Fine fescue plants not infected with *E. festucae* did not exhibit resistance (Clarke et al., 2006). In contrast to the endophyte-infected fine fescues, *Epichloë* spp. infected tall fescue and perennial ryegrass do not exhibit endophyte-mediated disease resistance. Moreover, endophytes have not been identified in bentgrass spp. Determining the mechanism behind the field phenomenon of *E. festucae*-mediated dollar spot resistance in fine fescues has been the focus of our research.

The first step was to carry out a comparative transcriptomic analysis on endophyte infected and endophyte free strong creeping red fescue by using the SOLiD-SAGE molecular sequencing method. The resulting sequence data showed that the fungal endophyte genes for secreted proteins were abundant, and the second most abundant fungal transcript (6.34% of mapped fungal tags) was for a predicted small secreted, cysteine rich protein with similarity to an antifungal protein from the fungus *Penicillium chrysogenum* (Ambrose and Belanger, 2012).

Only five of the sixteen *Epichloë* spp. for which whole genome sequences are currently available have a gene similar to the *E. festucae* antifungal protein gene. The limited existence of an antifungal protein gene among *Epichloë* species and the unique nature of the endophyte-mediated disease resistance in *F. rubra* suggested that the antifungal protein may be a factor in the disease resistance. We therefore focused on characterizing the antifungal protein.

We expressed the antifungal protein in yeast, bacteria, and the fungus *Penicillium chrysogenum* (Tian et al., 2017; Fardella et al., 2020, 2021, 2022). The purified antifungal protein from all three systems was active in culture against the dollar spot pathogen, *Clarireedia jacksonii*. The *P. chrysogenum* system was the easiest to use and yielded the greatest quantity of antifungal protein. Purified antifungal protein from this system was assayed on dollar spot infected plants and was found to significantly reduce the symptoms of infection (Figures 1 and 2).

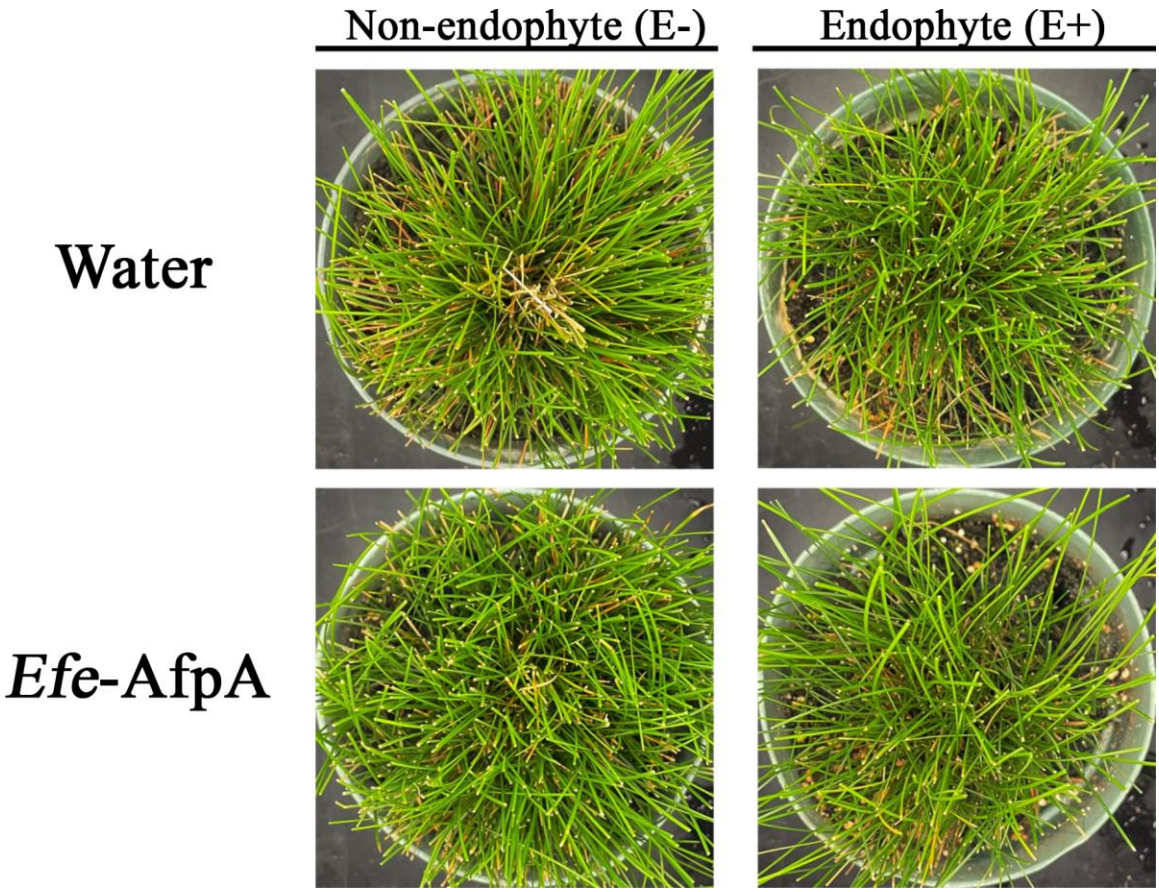


Figure 1. The *E. festucae* antifungal protein reduced dollar spot disease symptoms on non-endophyte (E-) strong creeping red fescue. Both endophyte infected (E+) and E- strong creeping red fescue was inoculated with a plug of *C. jacksonii*. The pots were then sprayed with either water or 100 μg mL^{-1} antifungal protein, daily for 10 days. As expected the endophyte infected plants were not infected with the dollar spot pathogen.

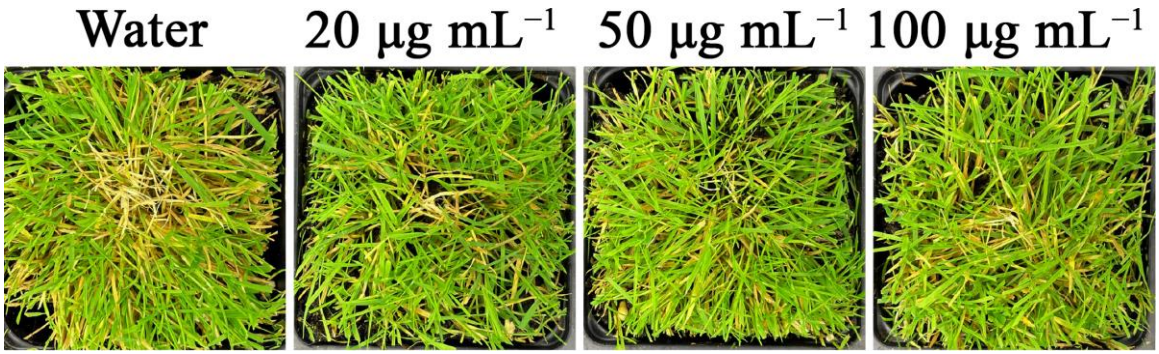


Figure 2. The *E. festucae* antifungal protein reduced dollar spot disease symptoms on creeping bentgrass inoculated with a *C. jacksonii* mycelial plug. Pots were treated with either water or increasing concentrations of antifungal protein (20, 50, and 100 μg mL^{-1} antifungal protein) daily for 7 days.

In the summer of 2023, a small field test of the antifungal protein was carried out on creeping bentgrass naturally infected with the dollar spot fungus. The results were inconclusive since some of the control plots did not show symptoms of the disease. In the future it will be important to pursue additional field testing of the antifungal protein.

The antifungal protein was also assayed against a variety of plant pathogenic fungi in addition to the dollar spot fungus. In culture the antifungal protein had activity against fungi that cause economically important diseases such as Botrytis blight (*Botrytis cinerea)*, Fusarium head blight (*Fusarium graminearum)*, and gray leaf spot/rice blast (*Pyricularia oryzae)* (Fardella et al. 2023). An example is shown below in Figure 3.

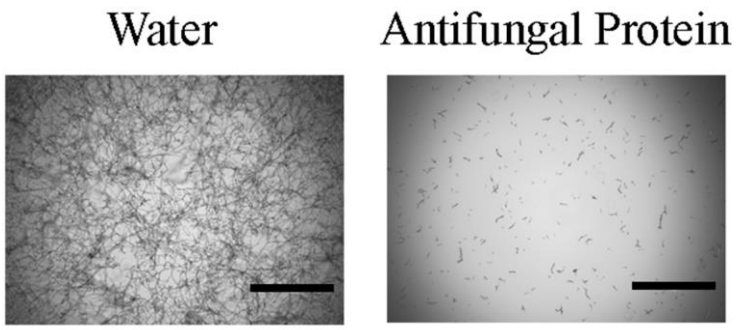


Figure 3. The *E. festucae* antifungal protein also inhibits *Botrytis cinerea* conidial growth (right image). *B. cinerea* conidia were treated with water or 0.6 μg mL^{-1} of the antifungal protein. Photographs were taken after 30 hours of incubation at room temperature. Bars are 750 μm.

In summary, we determined that the 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 in the endophyte-infected plants. We expressed the antifungal protein 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. The antifungal protein also had inhibitory activity against several other plant pathogenic fungi in culture. These results are promising regarding the potential of the antifungal protein to be developed as an alternative biofungicide or complement to synthetic fungicide use for dollar spot control on creeping bentgrass and perhaps against other plant pathogens on turfgrass and other hosts. A review of our research on the antifungal protein has been submitted for publication (Fardella, 2023).

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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.
- Four time points were selected (48, 60, 72, 96 hours post inoculation) by observing the time course of infection and symptom development. Uninoculated controls were added at the 48 and 96 hours as well.
- Colonial bentgrass has 3x as many differentially expressed genes when comparing control and inoculated samples.
- More genes are upregulated in response to dollar spot than down regulated.

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 (USGA, 2021). 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 (Goodman

and Burpee 1991). 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 (Devries et al., 2008). 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 evaluated dollar spot resistance of three creeping bentgrass cultivars 'Declaration' and 'Crenshaw' and one colonial bentgrass cultivar, 'BCD'. A single seedling of each cultivar was chosen randomly after a seedling selection experiment and then clonally propagated on 80/20 sand/peat potting media using standard greenhouse management. 18 clones of each cultivar were evenly divided (3 reps each) into two non-inoculated controls sampled at 48 & 96 hours post inoculation (hpi) and four inoculated groups sampled at 48, 60, 72, and 96 hpi (Figure 1).

The inoculum was prepared by adding 60 ml of DI water to an Erlenmeyer flask containing 40 g of Kentucky bluegrass seed. The Kentucky Bluegrass was then be autoclaved on a liquid 30 cycle and allowed to cool overnight. 20-25 potato dextrose agar cubes from a pure culture of *Clarireedia jacksonii* isolate 'LWC-10' was added to the flask. The flask was then shaken once a day until it reached maturity (~21 days). After inoculum matured, all bentgrass plants will be placed in gallon sized Ziploc bags along with three wet paper towels. The canopy of each plant was misted 5 times with a spray bottle of DI water before spreading 2.5 g of inoculum on each pot except those marked non-inoculated controls. Bags were then be sealed, inflated, and all plants were be placed in a growth chamber, which was set to have daytime temperature of 26 °C, nighttime temperature of 16 °C, and a DLI ~5 mol/m^2/day (12-hour light/dark cycle).

Leaf tissue from control and treated plants were collected and immediately frozen in liquid nitrogen at the four post-inoculation time points and stored at -80°C. Once all samples were collected, they were sent to OmegaBioservices for RNA extraction and sequencing. Once the data was returned, it was checked for quality leading to low quality reads and adapters being trimmed. Dollar spot RNA was removed from each file. De novo transcriptomes were assembled for each cultivar separately before reads were mapped and counted by Salmon. Differential expression analysis and creation of volcano plots were performed in RStudio.

Results and Future Expectations

In the previous report, a reliable inoculation protocol was established, and a seedling selection experiment was run. Since that point much has been accomplished. First, the tissue collection time points were established through observing the time course of infection and when

certain symptoms started to present themselves. We decided on sampling uninoculated controls at 48 and 96 hpi and sampling inoculated plants at 48, 60, 72, and 96 hpi. From here, two experimental runs were conducted, and RNA was sequenced. Data for both runs has been processed and cleaned using the above protocol, however only data from the first run has been analyzed in RStudio.

The data we received had both excellent quality and sequencing depth. From this point, I will focus on the first experimental run since the other runs data is still being analyzed. Using RStudio, differential expression analysis was performed on our data set. We were most interested in the gene expression differences of our control and inoculated samples in order to isolate genes that are involved with pathogen identification and resistance. As a result, volcano plots were created in order to get a zoomed-out view of what is happening in each of the cultivars (Figure 2). When looking at the plots two things are evident. First, in response to the pathogen each cultivar has more genes upregulated than down regulated (right of the two vertical dotted lines). Second, the colonial cultivar, 'BCD', which is more resistant in the field to dollar spot, had nearly 3x as many differentially expressed genes than both creeping cultivars 'Crenshaw' and 'Declaration', 3,179 genes to 991 genes and 1,255 genes, respectively. As a result, this difference warrants further investigation into what the functionality of the genes are and whether some of these genes are unique to 'BCD'. This will be accomplished through gene ontology analysis and blasting one set of genes against the others to see what matches and what's unique.

Additionally, the other main objective of this study was to look at how gene expression changes over the time course of the infection period. Therefore, to demonstrate this concept the top 5 differentially expressed genes for each cultivar were plotted in Figure 3 with respect to time. A similar trend is seen as above that over the time course of the infection each gene steadily increases in expression.

The next steps of this project are mainly to characterize the functionality of the differentially expressed genes in order to find some potential gene targets that are important in resistance to dollar spot. Additionally, analysis of the second run of RNAseq will allow us to confirm our findings by showing its repeatability. Lastly, when gene targets are identified in 'BCD' that potentially confer resistance molecular markers can be created in order to begin to breed new cultivars that are more resistant to dollar spot.

Images:

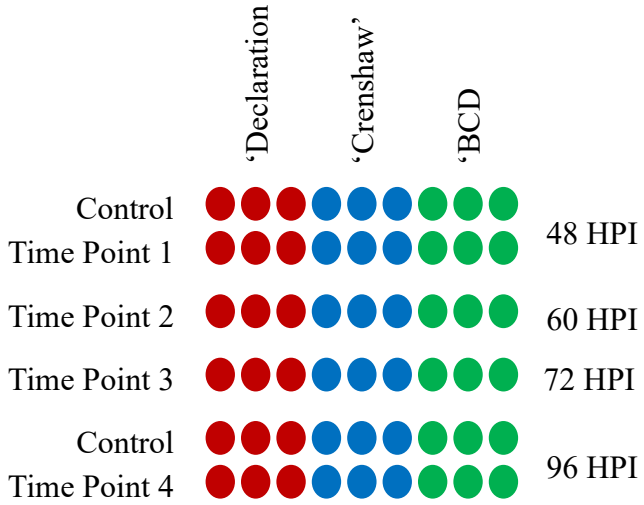


Figure 1: Design of RNAseq experiment. The top labels include two creeping bentgrass cultivars ('Declaration' and 'Crenshaw') and one colonial cultivar (BCD). The 48- & 96-hour timepoint have a non-inoculated control.

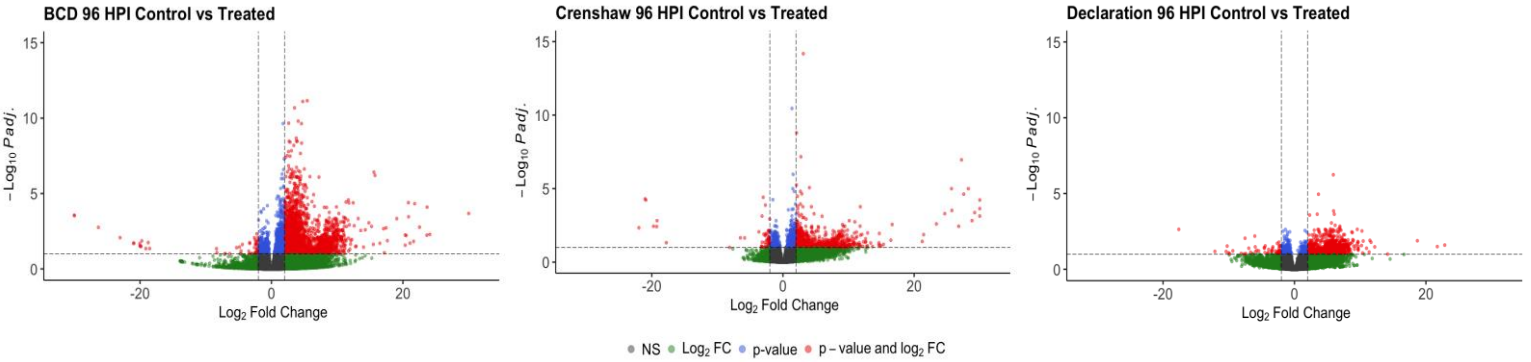


Figure 2: Volcano plots for each cultivar/species at 96 hours post inoculation: BCD (left), Crenshaw (middle), Declaration (right). The horizontal line designates an alpha value of 0.1 with any genes above being significantly different from control. The vertical lines show what genes fall outside a Log_2 fold change of 2.

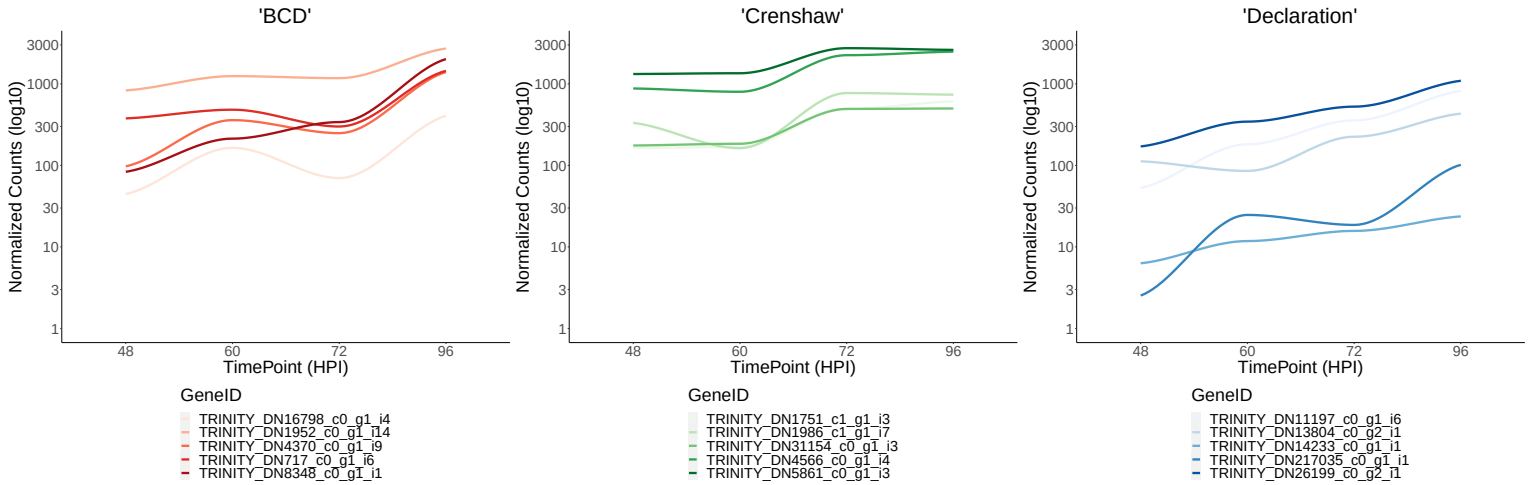


Figure 3: Top 5 Differentially Expressed Genes plotted over time. Left pane is the colonial bentgrass cultivar 'BCD', middle pane is creeping bentgrass cultivar 'Crenshaw', and right pane is the creeping bentgrass cultivar 'Declaration'. Each graph is the normalized read counts graphed over time of infection.

USGA ID#: 2022-04-747

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

Project Leaders: Stacy A. Bonos, Ph.D. and Eric MacPherson
Affiliation: Rutgers University

Objectives:

- Identify traits that correlate with increased shade tolerance.
- Identify creeping bentgrass cultivars that perform well under simulated foliar shade.
- 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 remain phenotypic traits correlated with increased shade tolerance following second greenhouse trial.
- Biomass again shows no significance between shade tolerant and susceptible plants.
- Commercial cultivars exhibit a wide range of variation in response to foliar shade in the green house and growth chamber.
    - Cultivars- L93XD, Chinook, Diplomacy, and Oakley exhibited the best shade tolerance under simulated shade.
    - Cultivars- Penncross, PinUp, Tour Pro, AuVictory exhibited significant shade avoidance responses, and therefore lacked shade tolerance.
- Significant differences in cultivar response were also observed under field shaded plots.
    - Cultivars- Piranha, Spectrum, Diplomacy, Oakley, and Piper, had the best quality ratings throughout the year under natural shade at putting green height.
- 2,880 progeny from controlled crosses selected from tolerant and susceptible parents were planted under intense foliar natural shade for heritability estimation in 2024 and 2025.
- Broad-sense heritability was calculated from 987 creeping bentgrass clones grown under foliar shade. The heritability estimate was $H^2 = 0.54$.

Summary:

Shade stress and shade avoidance syndrome are major issues affecting shaded golf greens. 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 shade symptoms (Ballare and Pierik 2017).

The most effective way of dealing with shade stress on golf courses is the removal of the trees/vegetation causing the shade. 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 grasses from vertically (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 creeping bentgrass cultivars that perform the best under foliar shade, as well as to identify correlated traits in both controlled greenhouse and growth chamber settings and 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 second run of simulated foliar shade evaluation was conducted under greenhouse conditions in the same manner as previously described and summarized below. Forty-one cultivars were grown under simulated foliar shade conditions provided by a photoselective film, which reduced light intensity by ~50% and Red:FarRed ratio by ~33% (Rosco E-Colour #209:.3 Neutral Density, PNTA). Light intensity and spectral compositions were taken periodically throughout the duration of the experiment.

The selected cultivars were seeded into 4 in^2 pots containing soilless media at a rate of 1lb per 1000 ft^2. They were replicated 4 times in both the simulated shade and full sun conditions. The pots were arranged in a randomized design, and maintained at a height of 0.25 in, for a duration of 4 months.

A second growth chamber study was also initiated in the same manner and is still ongoing at the time of this writing. At the conclusion of this experiment, two runs under greenhouse conditions and two runs under growth chamber conditions will be completed. Our goal is to publish and present this research at the International Turfgrass Research Conference in Japan in 2025. We hope to submit for publication in the spring of 2024.

Objective 2: Trait Identification

Several traits were evaluated for both trials; height, from media surface to leaf tip; tiller number on three randomly selected plants per pot; height to tiller ratio from two previous traits; biomass, collected from grass clippings and allowed to dry in an 80^{o}C oven across the duration of the experiment; and lastly chlorophyll concentration, using a dimethyl sulfoxide extraction according to the methodology of Hiscox and Israelstam (1979).

Greenhouse second trial results

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 (Table 1). 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 1.) When looking at the interaction between cultivar and light conditions, plant height and chlorophyll concentrations were significant. These results were consistent with the first run of the greenhouse experiment.

Some cultivars performed similarly in both greenhouse experiments. Cultivars such as L93XD, Chinook, Diplomacy, and Oakley, exhibited little to no change in plant height between sun and shade indicating that these cultivars perform similarly under both growing conditions (Figure 1 – 2022 data only). Cultivars such as Declaration, Nightlife, and S1, had a large positive change in height when grown under foliar 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 1).

Cultivars that have higher chlorophyll concentration 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 chlorophyll concentration can increase vertical growth as well. Cultivars with a moderate increase in chlorophyll concentration show the most promise for shade tolerance; these include Oakley, Piper, and Piranha (Figure 2).

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.

A second trial under growth chamber conditions is currently underway.

Table 1. Two-way ANOVA results for plants traits measured during the duration of greenhouse experiment (height, tiller number, Height:Tiller Ratio, Biomass, Chlorophyll Concentration). Variation was measured between cultivar, light condition, and cultivar light interaction.

2022	**Height**	**Tiller Number**	**Height:Tiller**	**Biomass**	**Chlorophyll Concentration**
Cultivar	***	***	***	NS	***
Light Condition	***	***	***	**	***
Cultivar: Light Condition	***	*	***	NS	***

*Significant at 0.05
**Significant at 0.01
*** Significant at 0.001
NS, Not Significant

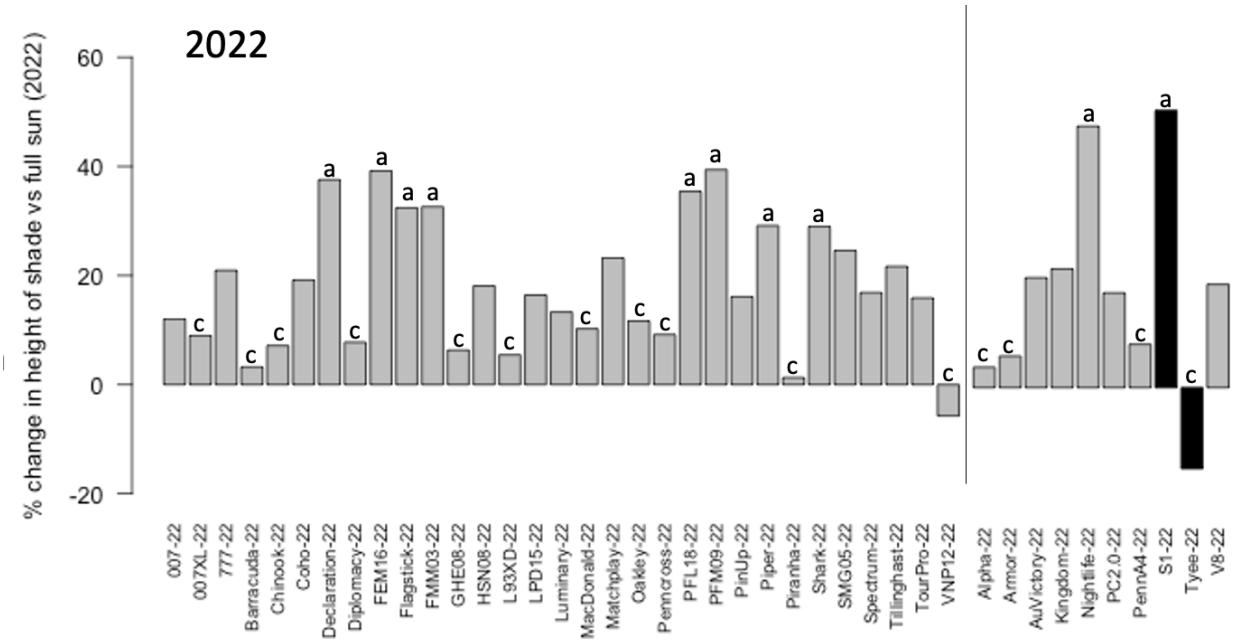


Figure 1. Percent change in plant height of creeping bentgrass cultivars and selections grown under simulated foliar shade versus full sun under greenhouse conditions in 2022. Black shaded bars indicate cultivars with the highest and lowest means. Means were compared using all the combinations of least 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).

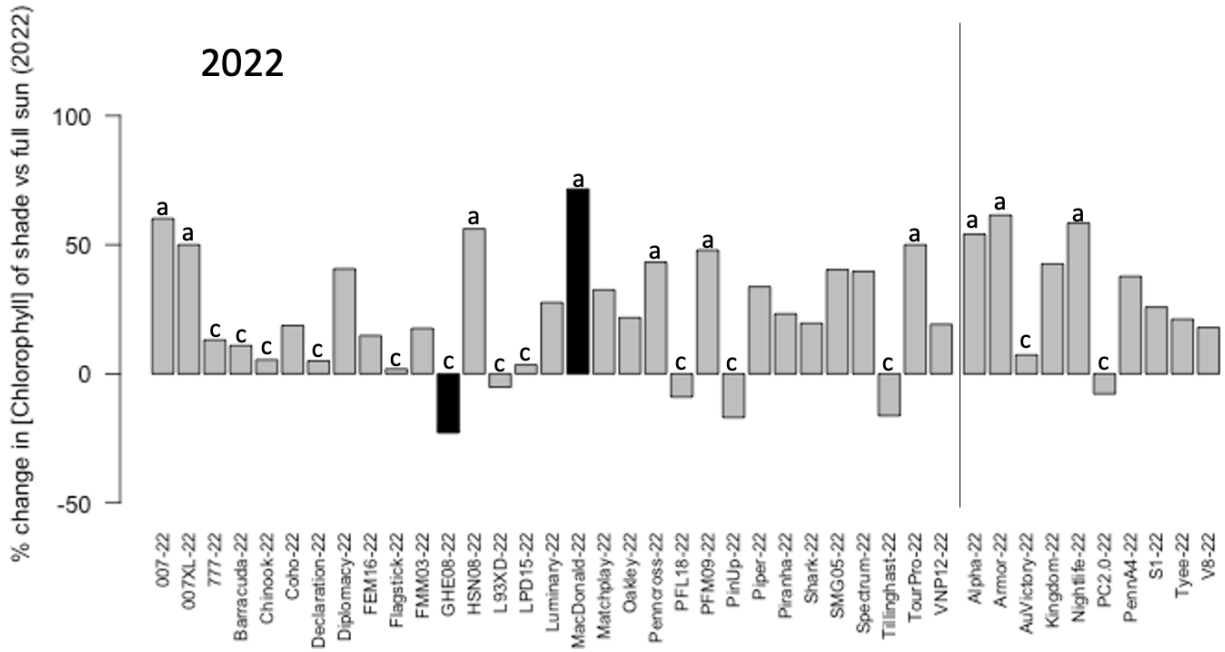


Figure 2. Percent change in chlorophyll concentration of creeping bentgrass cultivars and selections grown under simulated foliar shade versus full sun under greenhouse conditions in 2022. Black shaded bars indicate cultivars with the highest and lowest means. Means were compared using all the combinations 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).

Field Trial

A field trial with 30 commercial cultivars was seeded into 3' x 5' plots at Rutgers Horticulture Farm 2 in North Brunswick, NJ. The field is managed as a putting green, mown at 0.110 inches. Turf quality ratings are made monthly on a 1-9 scale with 9 being the best and 1 the worst. The field is under shaded conditions provided by nearby trees from leaf out in May until leaf fall in November. The shade provides a 70% reduction in PPF, and a 40% reduction in Red:FarRed compared to full sun.

An ANOVA was conducted, and means were separated using Fisher's LSD. Cultivars that have rated the highest in turf quality are Piranha, Spectrum, Luminary, Diplomacy, Oakley, and Piper. These cultivars have also demonstrated good shade traits in both the above greenhouse and growth chamber studies (Figures 1&2). Cultivars such as V8, Tyee, Tour Pro had the poorest quality in shaded plots, which was consistent with observations in the greenhouse and growth chamber studies (Figures 1&2).

Table 2. Performance of creeping bentgrass cultivars in a putting green trial under foliar shade established in Nov 2021 at Rutgers Horticulture Research Farm 2.

	Cultivar	**Quality**
1	Piranha	6.94
2	Spectrum	6.72
3	Luminary	6.50
4	Diplomacy	6.22
5	Oakley	6.17
6	Piper	6.00
7	007XL	5.94
8	Barracuda	5.94
9	Chinook	5.89
10	L-93XD	5.72
11	MacDonald	5.72
12	777	5.61
13	Tour Pro	5.56
14	Tyee	5.44
15	Coho	5.39
16	Matchplay	5.33
17	007	5.17
18	AuVictory	5.11
19	Shark	5.06
20	PennA4	4.89
21	S1	4.89
22	Declaration	4.83
23	Kingdom	4.61
24	PC2.0	4.28
25	Nightlife	4.17
26	Flagstick	4.00
27	Penncross	3.89
28	Alpha	3.50
29	Armor	3.39
30	V8	2.83
	LSD @ 0.05	0.5

Objective 3- Inheritance of shade tolerance in creeping bentgrass

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). 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 158.6 µmol $m^{-2}s^{-1}$ in 2022, with a Red:FarRed ratio of 0.639 in 2022. Light quality in full sun averaged 1935.4 µmol $m^{-2}s^{-1}$ in 2022 with Red:FarRed ratio of 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 November 2022.

Broad-sense heritability was then calculated using a best linear unbiased predication model in R, with corresponding ANOVA. Data was standardized before processing. The resulting broad-sense heritability was $H^2 = 0.54$. A broad-sense heritability of 0.54 suggests that that 54% of the variation can be attributed to genes expressed in the plant vs. environmental effects. A high broad-sense heritability would indicate a strong genetic effect and an increased ability to select shade tolerant plants. A heritability estimate of 0.54 is lower than what has been observed for disease resistance to dollar spot and salt tolerance in creeping bentgrass. And indicates that it may be more challenging to select for shade tolerance compared to other traits.

A population 22 creeping bentgrasses with good performance under shade were crossed in two polycross populations. A population of 18 creeping bentgrass with poor performance under shade were also crossed in two polycrosses. The resulting seeds were collected from each plant and germinated in greenhouse flats. 96 progeny plants were then selected from each parent plant (n=2880) (Figure 3) in each population and planted under foliar shade provided by pecan trees at Rutgers Adelphia Research Farm. These progeny will be compared to the parent genotypes to estimate narrow-sense heritability. The narrow-sense heritability estimate is indicative of the additive genes that can be transferred to the progeny and will help to refine the best selection procedures for shade tolerance.

Table 3. Analysis of variance (ANOVA) of creeping bentgrass genotypes grown under foliar shade at Rutgers Adelphia Plant Science Research Farm in 2021 and 2022.

Source	df	MS	*F*-value	P>*F*
Genotype	328	8.3	20.436	<0.0001
Rep	2	239.07	588.661	<0.0001
Genotype x Rep	656	3.6	9.27	<0.0001
Residuals	7895	0.41		
Heritability	H^2=0.54			

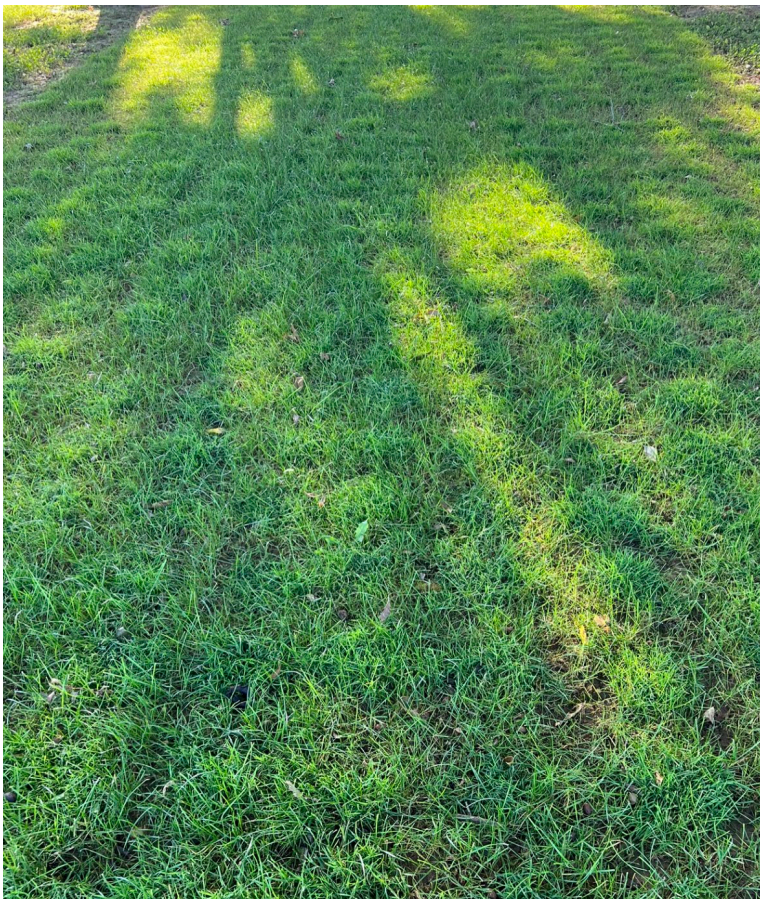


Figure 3. Creeping bentgrass progeny from 30 parents (n =2880) planted under shade provided by pecan trees at Rutgers Adelphia Plant Science Research Farm (Freehold, NJ) in 2023. These plants will be used to calculate narrow-sense heritability.

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:

- The top germination lines of Kentucky Bluegrass have been advanced for hybridization and selection in breeding plots at the WSU Grass Breeding and Ecology Farm.

- One hundred and seventy-six high germination lines of Kentucky Bluegrass with the highest seed yield have been subjected to salt tolerance screens with 60 surviving at a rate of 12 $dS \cdot m^{-1}$.

- All one hundred and seventy-six lines described above have been planted as turf trials along with additional selections and varieties from our breeding program.

- 'Matchless' a Kentucky Bluegrass variety selected for dryland production in Eastern WA without the use of postharvest burning was awarded Plant Variety Protection (PVP) status (PV #202200523) and is currently licensed and in production.

- Ninety-three Western Wheatgrass selections were planted as turf trials though none were successfully established, despite being previously selected for high germination.

- Given the failure of Western Wheatgrass for turf establishment, with the approval from USGA, we would like to shift this evaluation and breeding effort to the two progenitors of Western Wheatgrass, Streambank Wheatgrass and Beardless Wildrye, both native plants in the Pacific Northwest. We would also like to include a non-native wheatgrass, Intermediate Wheatgrass. These three plants still meet many of the research requests from Inland Empire Golf Course Superintendents.

Summary Text:

Rational

This project focuses on the question of whether rapid seed germinating and salt tolerant Kentucky Bluegrass (*Poa pratensis*) and Western Wheatgrass (*Pascopyrum smithii*) can be developed for golf courses in the Upper West, Mountain, Transition and Pacific regions of the United States. 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 and Results to Date

Objective 1: Screening Kentucky Bluegrass and Western Wheatgrass for rapid germination and perform hybridization crosses.



Figure 1. An example of a Kentucky Bluegrass breeding/evaluation block with plants on 2 ½ foot centers. Photo was taken at the WSU Grass Breeding and Ecology Farm on July 2, 2023.

Kentucky Bluegrass:
Two hundred and ninety-six Kentucky Bluegrass selections were planted in the fall of 2022 and harvested in 2023 (Figure 1). In addition, eighty plants were selected for paired crosses, forty-five of which were harvested for seed. Each of these lines were screened for percent germination on KNO_3 soaked blotter papers in a Hoffman environment chamber/seed germinator at 25°c with 16 hours/day light. Germinated seeds were counted at 5, 10 and 15 days. The forty lines with the highest viability will be screened for the rate of germination as outlined in the AOSA rules for testing seeds (Association of Official Seed Analysts 2017) and transplanted in the field. Another one hundred and fifty-five plants that were harvested in 2022 are still being cleaned and inventoried. In addition, another one hundred and thirty-three selections from previous crosses were harvested in 2023 and are currently being cleaned and inventoried.

One of the varieties used in these trials, 'Matchless', is the product of breeding efforts from this program. 'Matchless' was previously selected from 'Kenblue' by Drs. William Johnston and R.C. Johnson for dryland production in Eastern WA without the use of postharvest burning. In 2023, 'Matchless' was awarded Plant Variety Protection (PVP) status (PV #202200523) and is currently licensed and in production. Matchless rates well for germination, salt tolerance and turf quality.

Western Wheatgrass:
Advanced lines of western wheatgrass were rated in the field based on color, maturity, texture, and potential turf quality. Of these, twenty-nine were transplanted into three blocks for polycrossing. Due to challenges associated with establishing western wheatgrass turf (see Objective 3), we have also begun evaluating the hybridization progenitors for this species as well as an additional non-native wheatgrass.

Other Wheatgrasses:
Western Wheatgrass arose as a hybrid of Streambank Wheatgrass (*Elymus lanceolatus*) and Beardless Wildrye (*Leymus triticoidies*). These species are native to the Pacific Northwest. Fifty-two accessions of Streambank Wheatgrass, thirteen accessions of Beardless Wildrye and three accessions of hybrids between the two were planted into the field. We also planted eighteen accessions of Intermediate Wheatgrass (*Thinopyrum intermedium*). Intermediate Wheatgrass is planted for erosion control and has high food value for animals.

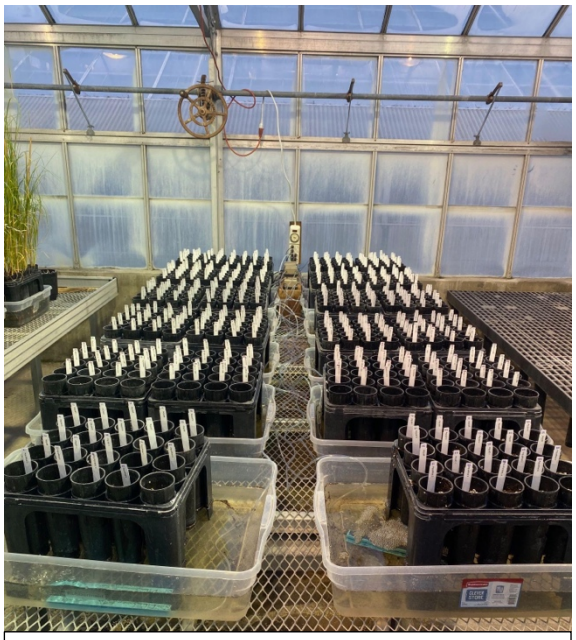


Figure 2. Salt tolerance analysis.

Objective 2: Screening for salt tolerance in rapid germinating Kentucky Bluegrass and Western Wheatgrass.

Our salt tolerance analysis uses a modified version of the protocol describe in Brown *et al.* (2011) in which plants are flood irrigated at pre-determined salt rates (0, 6, 12 and 18 $dS \cdot m^{-1}$) and screened for 4 weeks as turf pots (Bushman 2016; Moxley 1978)(Figure 2).

Kentucky Bluegrass:
The one hundred and seventy-six highest yielding lines to date were evaluated for salt tolerance at a rate of 12 $dS \cdot m^{-1}$. Of these, the sixty lines that had the highest survival will be further screened for salt

tolerance at 18 $dS \cdot m^{-1}$. All the one hundred and seventy-six highest yielding lines were also planted in turf trials (see Objective 3).

Western Wheatgrass:
Due to challenges associated with establishing Western Wheatgrass turf (see Objective 3) we have postponed salt-tolerance screening for this species.

Objective 3: Performing turf trials for rapid germinating and salt-tolerant Kentucky Bluegrass and Western Wheatgrass.

Kentucky Bluegrass:
The one hundred and seventy-six highest yielding Kentucky Bluegrass selections that were evaluated for salt tolerance at a rate of 12 $dS \cdot m^{-1}$ in Objective 2 were also planted as turf trials at the WSU Grass Breeding and Ecology Farm (Figure 3). These trials include additional selections from 'Matchless' and three low-vernalization selections from our current breeding program that are also in PVP trials. Plants were established on 3' by 5' plots at a seeding rate of 2 pounds per thousand square feet for Kentucky Bluegrass. We use augmented block designs to maximize the entries from our nursery. Turf trials will continue to 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 are 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.



Figure 3. Kentucky Bluegrass turf trials. The high pressure of rust this fall provided an excellent opportunity to rate differences in disease resistance. Photo was taken at the WSU Grass Breeding and Ecology Farm on October 23, 2023.

Western Wheatgrass:
Ninety-three Western Wheatgrass selections were planted as turf trials at a seeding rate of 6 pounds per thousand square feet as per Bunderson (2009). Unfortunately, none became

established. With this in mind, we have begun evaluating other wheatgrasses as described in Objective 1.

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.

Kentucky Bluegrass:

At the end of the third year, we expect to have completed our evaluation of Kentucky Bluegrass 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 established Kentucky Bluegrass. Based on the results of those trials, we will submit a PVP application for any high-quality lines that we developed based on this research.

Western Wheatgrass and other Wheatgrasses:

Our research to date suggests that Western Wheatgrass is not a good choice for golf course plantings. With this in mind, and pending approval from USGA, we would like to shift this evaluation and breeding effort to the two progenitors of Western Wheatgrass, Streambank Wheatgrass and Beardless Wildrye, both native plants in the Pacific Northwest. We would also like to include a non-native wheatgrass, Intermediate Wheatgrass. These three plants still meet many of the research requests from Inland Empire Golf Course Superintendents.

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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, 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:

- A US Plant Patent application was filed in 2023 for ‘OKC3920’ bermudagrass. Last year, the genotype was released as a new cultivar for use on golf course putting greens in the US transition zone.
- A large ‘OKC3920’ putting green nursery was established to study the effects of Proxy growth regulator (Ethephon, 2-chloroethyl phosphonic acid) on seedhead suppression. The study indicated that applying Proxy at a 0.5x rate in the first week of May and another spray two weeks later was fully effective in suppressing seedheads throughout the growing season this year.
- One interspecific F1 hybrid (*Cynodon dactylon* x *C, transvaalensis*), ‘15x9’, survived the last winter at the mowing height of 3.2 mm (1/8”) without protective cover while ‘TifEagle’ and ‘Tifdwarf’ were fully winterkilled.
- The bermudagrass nursery established in 2017 was continued to collect data for turfgrass performance in 2023.
- A new nursery consisting of 52 clonal genotypes with ‘TifTuf’ and ‘Astro’ as checks, and five seeded experimental synthetics with ‘Monaco’ as a check was established in the summer of 2023.
- The greens-type mowing test established in 2022 was carried out under the direction of Dr. Mingying Xiang, to collect data on turfgrass quality and ball roll distance.

Summary Text:

Bermudagrass is the most widely used turfgrass on US golf courses, covering up to 32% of all golf course turf acreage (Shaddox et al., 2023). The 2023 USDA plant hardiness zone map indicates that many locations in the US are experiencing warmer average annual minimum temperatures. Many areas are approximately one-half zone warmer in winter (https://planthardiness.ars.usda.gov/) as compared to the map published in 2012. Climate warming has increased or is predicted to increase the use of bermudagrass in climates typically dominated by cool season turfgrasses creating various challenges for turfgrass managers. Unseasonably warm temperature spikes in winter, cold spikes in spring, along with low temperatures in winter, solely or combined with other undesirable environmental factors, such as shade or dehydration or traffic, pose a major winterkill threat 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 greens-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. This new cultivar is an interspecific F1 hybrid turf bermudagrass that has exhibited improved freeze tolerance over that offered by conventional ultradwarf cultivars (Gopinath et al., 2021). In 2023, Oklahoma State University filed a patent application with the US Patent and Trademark Office to protect the intellectual property ‘OKC3920’ bermudagrass.

‘OKC3920’ produces some seedheads in May, at times resulting in undesirable turf quality. One experiment has been conducted to develop a solution for suppressing seedheads in ‘OKC3920’ bermudagrass. A research putting green was sprigged with ‘OKC3920’ bermudagrass in June 2022. The rootzone comprised 80% sand and 20% shredded sphagnum peat moss. The objective of this study was to assess the impact of four plant growth regulators (PGR) at different application rates on ‘OKC3920’ bermudagrass seedhead suppression. The experimental design follows a two-way factorial design. Each plot measures 5 by 5 ft. Plant growth regulators included trinexapac-ethyl (PrimoMaxx; Syngenta Crop Protection, Inc., Greensboro, NC), ethephon (Proxy; ENVU, Research Triangle Park, NC), prohexadione calcium (Anuew; Nufarm, Alsip, IL), and Paclobutrazol (Trimmit; Syngenta Crop Protection, Inc., Greensboro, NC). The second treatment factor involved application rates: 0.5X, 1X, and 2X label rates, applied every 2 weeks from April 28 to June 31. In 2023, seedheads emerged in late May. Our data indicated that two applications of Proxy at any rate (0.5X, 1X, or 2X) during the study resulted in over 90% seedhead control (Figure 1).



Figure 1. Comparison of a control plot (no Proxy application) for ‘OKC3920’ putting green (on the left) with a plot treated at 0.5X with Proxy (on the right) on May 30th, 2023.

The 2021 greens-type bermudagrass mowing test was continued to evaluate winter survival, turfgrass performance related traits and ball roll distance under low mowing heights. The test consisted of 12 OSU advanced selections, one genotype from the University of Florida, one from Mississippi State University, and three commercial cultivars (‘Tahoma 31’®, ‘Tifdwarf’, and ‘TifEagle’). A randomized complete block design with a 15’ by 5’ plot size and five replicates was used. The testing plots were not covered throughout last winter. One genotype ‘15x9’ greened up earlier than many others in April with more than 90% green coverage in May while ‘Tifdwarf’ and ‘TifEagle’ were fully winterkilled in the test (Figure 2).



Figure 2. The plots were not covered throughout the winter between 2022 and 2023. ‘15x9’ bermudagrass greened up well while ‘TifEagle’ was fully winterkilled at the Oklahoma State University Turf Research Center (OSU TRC), Stillwater, OK. The image was taken on April 23, 2023.

A replicated trial established at the OSU TRC in 2017 was evaluated for spring greenup in 2023 (no winter kill was noted) and left in place under lower maintenance conditions than in prior years to hold the germplasm for later use. This trial was under the direction of Dr. Dennis Martin. 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’). Results from the analysis of entry responses to drought in summer of 2022 suggested that entry ‘OSU1101’ has improved drought resistance in terms of leaf firing resistance and higher normalized difference vegetative index (NDVI) compared to many other entries but was similar to that of ’TifTuf’. Additional testing of ‘OSU1101’ and up to six other experimental lines is justified in 2024.

A greens-type mowing test was established in 2022 at the OSU TRC under the direction of Dr. Mingying Xiang. 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. In the 2023 growing season, we observed that one experimental selection, ‘20x10’ demonstrated ball roll distance comparable to ‘TifEagle’.

In the summer of 2023, a new trial was established at the OSU TRC under the direction of Dr. Dennis Martin. This test consists of 52 clonal experimental genotypes with ‘TifTuf’ and ‘Astro’ as checks, and five seeded experimental synthetics with ‘Monaco’ as a commercial standard cultivar (Figure 3). The experimental design for the test is a RCBD with three replications. The objective of the test is to evaluate establishment vigor, sod tensile strength, regrowth vigor post sod harvest, turfgrass quality and its components, fall color, spring green up, winterkill, seedhead prolificacy, and diseases. Mowing height in 2023 was 1.5-inches with strip-plot mowing at 0.5-inch (typical fairway) and 1.5 inch (medium rough) mowing heights planned in spring 2024.



Figure 3. New clonal and seeded bermudagrass replicated field trial established in 2023 at Oklahoma State University. The image was taken on October 19, 2023.

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1. Shaddox T.W., Unruh J.B., Johnson M.E., Brown C.D., and Stacey G. 2023. Turfgrass use on US golf courses. HortTechnology. 33(4): 367-377. https://doi.org/10.21273/HORTTECH05238-23
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USGA ID#: 2023-09-776

Title: Developing new bermudagrass cultivars combining improved drought resistance, cold hardiness and high turf quality

Project Leaders: Yanqi Wu, Mingying Xiang, and Shuhao Yu
Affiliation: Oklahoma State University

Objectives:

1. To make crosses between cold-hardy parents and drought-resistant parents in common bermudagrass
2. To identify true hybrids in the progeny populations from the target crosses
3. To evaluate and select cold-hardy and drought-resistant progeny in field trials

Start Date: January 1, 2023
Project Duration: 3 years
Total Funding: $109,161

Summary Points:

- Four single crosses between cold-hardy and drought-resistant common bermudagrass parents were established and seedheads were harvested in 2023.
- Putative hybrids from two previous crosses were evaluated for spring green-up and drought resistance.

Summary Text:

Bermudagrass is the most extensively used turfgrass on golf courses in the U.S. transition zone, covering approximately 45% of the turf acreage (Shaddox et al., 2023). Winterkill is a major threat to growing bermudagrass in transitional climates. The OSU turfgrass breeding program has focused on improving turf bermudagrass cold hardiness since the 1980's. The program released cold hardy cultivars 'Midlawn' and 'Midfield' collaborated with Kansas State University in 1991, 'Yukon' in 1996, 'Riviera' in 2000, 'Patriot' in 2002, Latitude 36 and 'NorthBridge' in 2010, 'Tahoma 31' in 2017, and 'OKC3920' in 2022. However, none of these cultivars has the drought resistance level comparable to some drought-resistant cultivars used in the southern regions. Accordingly, our long-term goal is to breed, test, and develop interspecific hybrid cultivars improved in drought resistance, cold hardiness, and high turfgrass quality.

Four single crosses between cold-hardy and drought-resistant common bermudagrass parents were established in a field nursery on the Oklahoma State University Agronomy Farm this year (Figure 1). By September 2023, all the parental plants flowered. Mature seedheads were hand harvested on each parent separately in November post a few heavy frosts. These seedheads will be threshed and seeds will be cleaned this winter. Seeds from the crosses will be germinated at the beginning of 2024. Progeny plants will be grown in individual containers in a greenhouse.

Leaf tissue samples will be collected to identify true hybrids using simple sequence repeat (SSR) markers. We will plant true hybrids in a replicated nursery in the summer of 2024.



Figure 1. Four single crosses between cold-hardy and drought-resistant common bermudagrass parents were established in the summer of this year (the image taken on July 29, 2023).

We made two single crosses in previous years. Putative hybrid progeny from the crosses have been evaluated in two single-space planted nurseries, one established in 2021 and another in 2022. We evaluated spring green-up, turf quality components, and drought resistance in the nurseries in 2023. Some progeny plants had very good spring green-up ratings (Figure 2). These plants will be genotyped with SSR markers this winter and true hybrids will be planted in the same nursery as described above in 2024.



Figure 2. Multiple progeny plants from two crosses between cold-hardy and drought-resistant common bermudagrass parents spring greened up well (the image taken on May 7, 2023).

References Cited

1. Shaddox, T. W., J. B. Unruh, M. E. Johnson, C. D. Brown, and G. Stacey. 2023. Turfgrass use on US golf courses. HortTechnology. 33(4): 367-377. https://doi.org/10.21273/HORTTECH05238-23

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 now 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:

- RNA degradation and foreign contamination have been resolved.
- All host plant RNA samples were sent to Novogene for sequencing on an Illumina HiSeq platform and generated an average of 78 million pair-end reads (~156 million each).
- High quality RNA extractions for wheat, Bermudagrasses, bentgrass, and Kentucky bluegrass were sequenced and have undergone bioinformatic analysis.
- Sequences from *Brachypodium distachyon* have also been obtained and have undergone bioinformatic analysis.

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, we have used a genomics and bioinformatics approach to identify the gene(s) in the fungus responsible for producing effectors of necrosis. Methodologies are similar for all studies where plants where incubated at various conducive and non-conducive temperatures with isolates of *Ophiosphaerella spp.* and total RNA was extracted from roots by flash freezing in liquid nitrogen and preserved. Sequencing library preparation of

RNA samples was performed and sequenced using the Illumina HiSeq platform. Genes were considered differentially expressed based on 5% false discovery rate and log fold change of two. The identities of fungal effector(s), and gene ontology and pathway enrichment analyses from diseased plants at cool temperatures was performed using bioinformatics approaches as done previously. The goal of this research is to mirror prior studies, leveraging new and old datasets, to elevate the understanding of the SDS pathosystem to a level where Bermudagrass breeding efforts can utilize known host-specific disease resistance gene(s) to develop new cultivars with enhanced disease resistance.

All studies with bermudagrass, wheat, bentgrass, *Brachypodium distachyon*, and bluegrass have been completed and samples sequenced. Briefly, for Bermudagrass, A total of 297 annotated genes were found to be differentially expressed, including 147 upregulated and 150 downregulated genes in inoculated vs non-inoculated roots of U3 (tolerant) biotype. For Tifway 419 (susceptible), a total of 862 annotated genes were found to be differentially expressed, including 165 upregulated and 697 downregulated genes in inoculated vs non-inoculated roots. The tolerant U3 biotype exhibited an increase in gene expression responsible for the synthesis of cysteine-rich receptor-like protein kinases, while Tifway 419 showed a decrease in this expression (Figure 2). These observations imply that U3 biotype resembles a pattern-triggered immunity defense mechanism or a potential symbiotic relationship with *O. korrae*. Conversely, the reaction of the susceptible cultivar Tifway 419 to the fungal infection indicates a response in effector-triggered susceptibility, which is mediated by the accumulation of jasmonic acid and may trigger tissue necrosis.

For Kentucky bluegrass, A total of 2836 annotated genes were found to be differentially expressed, including 1416 upregulated and 1420 downregulated genes in inoculated vs non-inoculated roots incubated at warm temperatures. For roots inoculated at cool temperatures, a total of 1712 annotated genes were found to be differentially expressed, including 1281 upregulated and 431 downregulated genes. At warm temperatures, there was an increase in gene expression involved in the synthesis of jasmonic acid and genes involved in the detection of pathogen-associated molecular patterns (Figure 3). At cool temperatures, the expression of genes involved in the synthesis of leucine-rich repeat receptor-like proteins, salicylic acid synthesis, and cysteine-rich receptor-like protein kinases were upregulated following *O. korrae* infection.

Efforts will continue to analyze these data and work towards identifying genes and pathways most likely driving host defense and susceptibility.



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

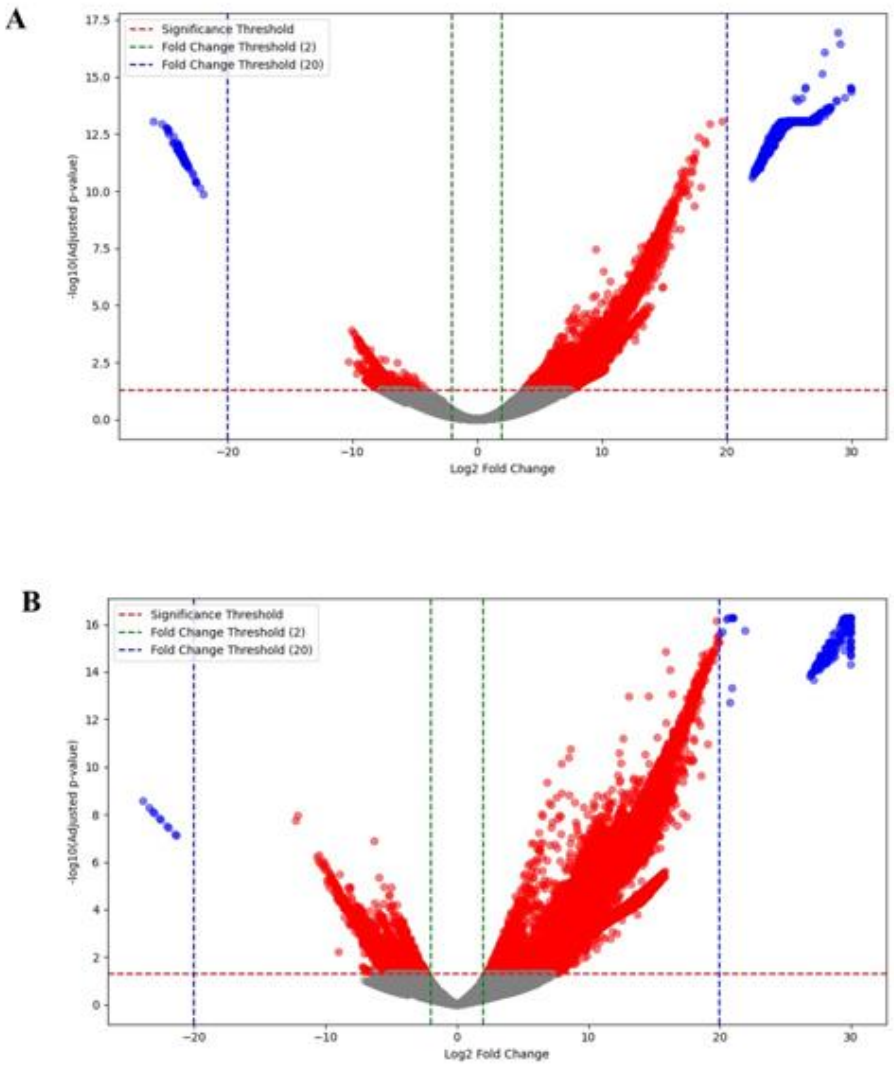


Figure 2. Transcriptomic analysis. Volcano plots showing the differentially expressed genes and the highly differentially expressed genes in red and blue respectively, gray dots indicate no significant differential expression between inoculated vs non -inoculated condition. (A) U3 biotype and (B) Tifway.

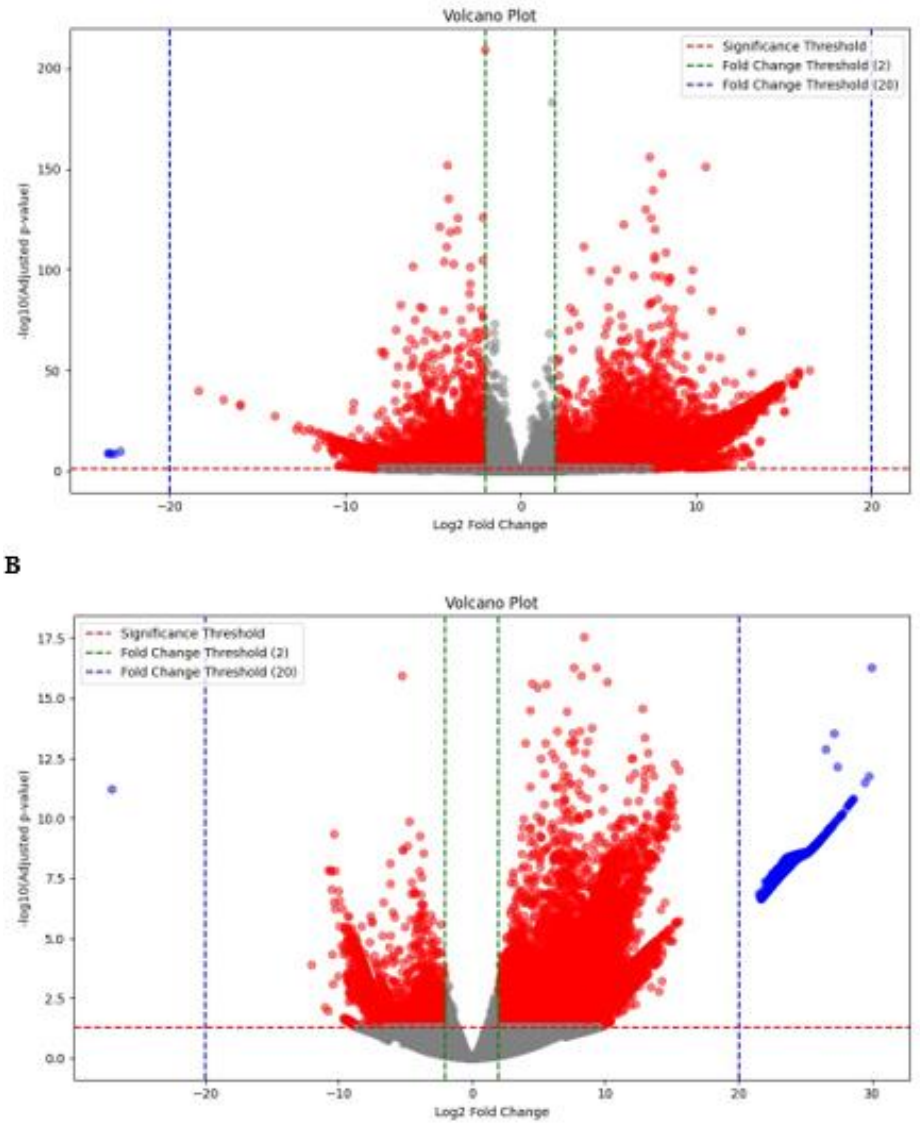


Figure 3. Volcano plots showing the differentially expressed genes and the highly differentially expressed genes in red and blue respectively, gray dots indicate no significant differential expression between inoculated vs non-inoculated conditions. (A) incubated at warm temperatures (B) incubated at cool temperatures.

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.
- A 2,100 plant spaced plant nursery of zoysiagrass was planted.
- A 1,000 plant spaced plant nursery of bermudagrass was planted.
- First selections were made from the 2021 zoysiagrass spaced plant nursery.
- Second selections were made from 2017 zoysiagrass spaced plant nursery.

In Florida, most rounds of golf are played through the winter months justifying the need for improved cultivars of bermudagrass and zoysiagrass to sustain playability through colder periods.

Bermudagrass

2019 Bermudagrass Fairway Trial

In 2019, 27 entries of bermudagrass were planted in a randomized complete block design with three replications. Since establishment, the mowing height has been 1.3 cm. In 2023, it was irrigated to prevent stress. Nine commercial cultivars are included: 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 for summer and winter months through 2013. Summer months included April through October and winter included January – March 2023 and November – December 2023. Freezing temperatures did not occur in late 2023. Overall, the turf quality of the trial was lower in 2023 compared to previous years. This is the result of three consecutive days of hard freezes in late December 2022 followed by another 2-day freezing event two weeks later. TifTuf and an experimental, FB1628 were the most consistent entries in the trial.

TifTuf, Celebration, and three experimental entries (FB1628, FB1630, and 343-34) were the only entries to average TQ above five across all months and years of rating from 2020-2023 (Figure 2). Several entries produced an average color > 6.0 through 2020 – 2023; however, Celebration, FB1630, FB1628, 9-6, and FB12634 were in the top statistical group (Figure 3).

2022 Bermudagrass Trial

A new hybrid bermudagrass trial with 63 entries was planted in fall of 2022 with two replications in a randomized complete block design. The trial includes TifTuf, Bimini, and Celebration as commercial checks and is mowed at 5.0 cm. Visual turfgrass quality was rated from this trial in the second half of 2023 (Figure 4). Only lines with an average TQ value of 3 or greater were included in the graph. TifTuf and FB1628 were among the best performing entries.

2023 Spaced Plant Nursery

A new nursery of 1,000 plants was planted on 5’ centers in the fall of 2023. Another 1,500 plants will be planted in spring 2024.

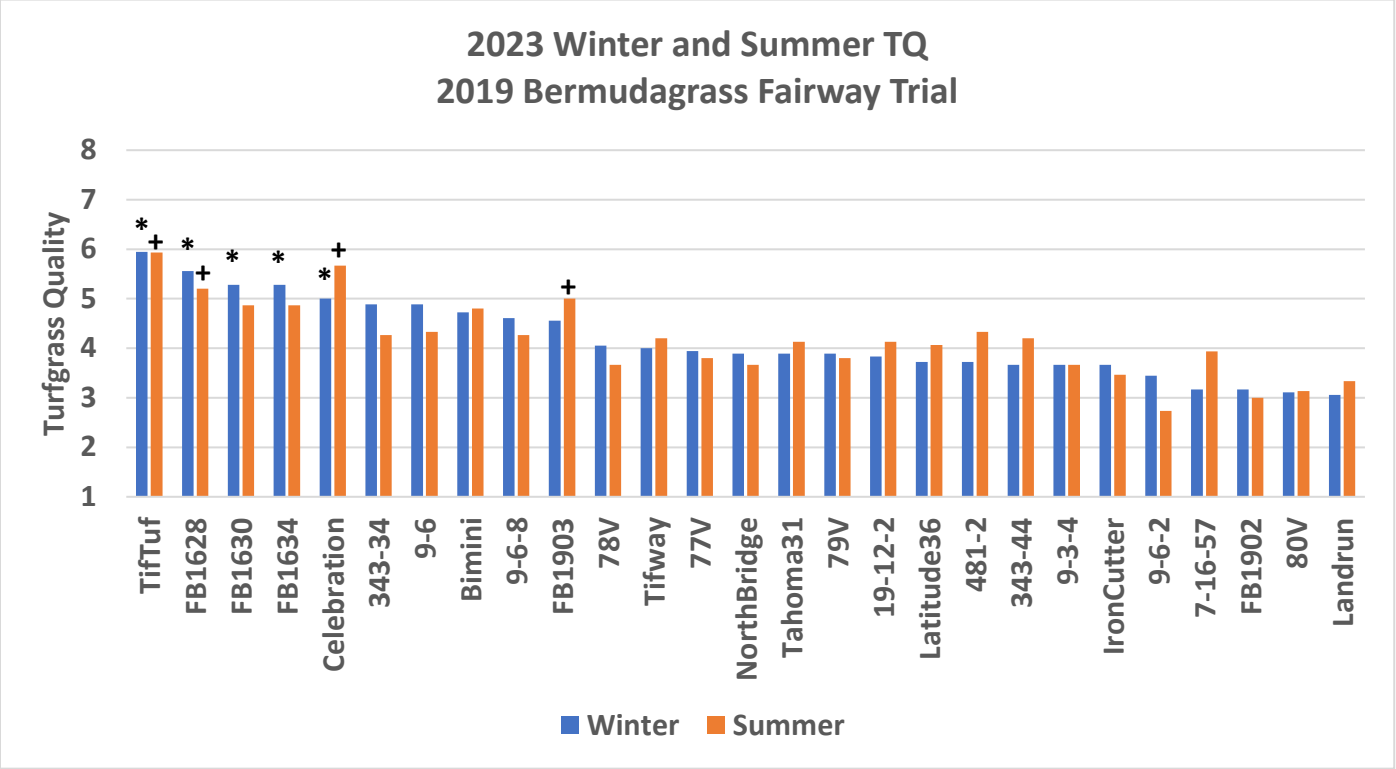


Figure 1. Average winter and summer turfgrass quality ratings of 27 bermudagrass entries in 2023. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality. “*” indicates entries in the top statistical group during winter months. “+” indicates entries in the top statistical group during warmer months.

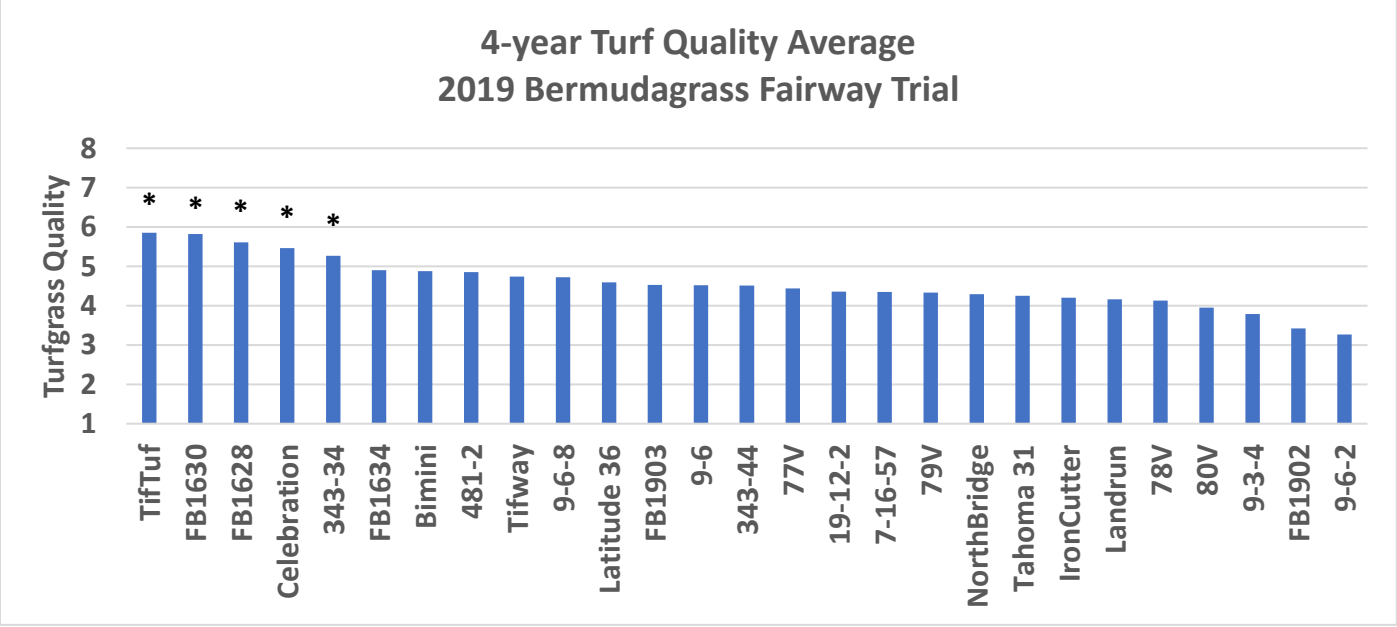


Figure 2. Average four-year turfgrass quality ratings of 27 bermudagrass entries from 2020 through 2023. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality. “*” indicates entries in the top statistical group.

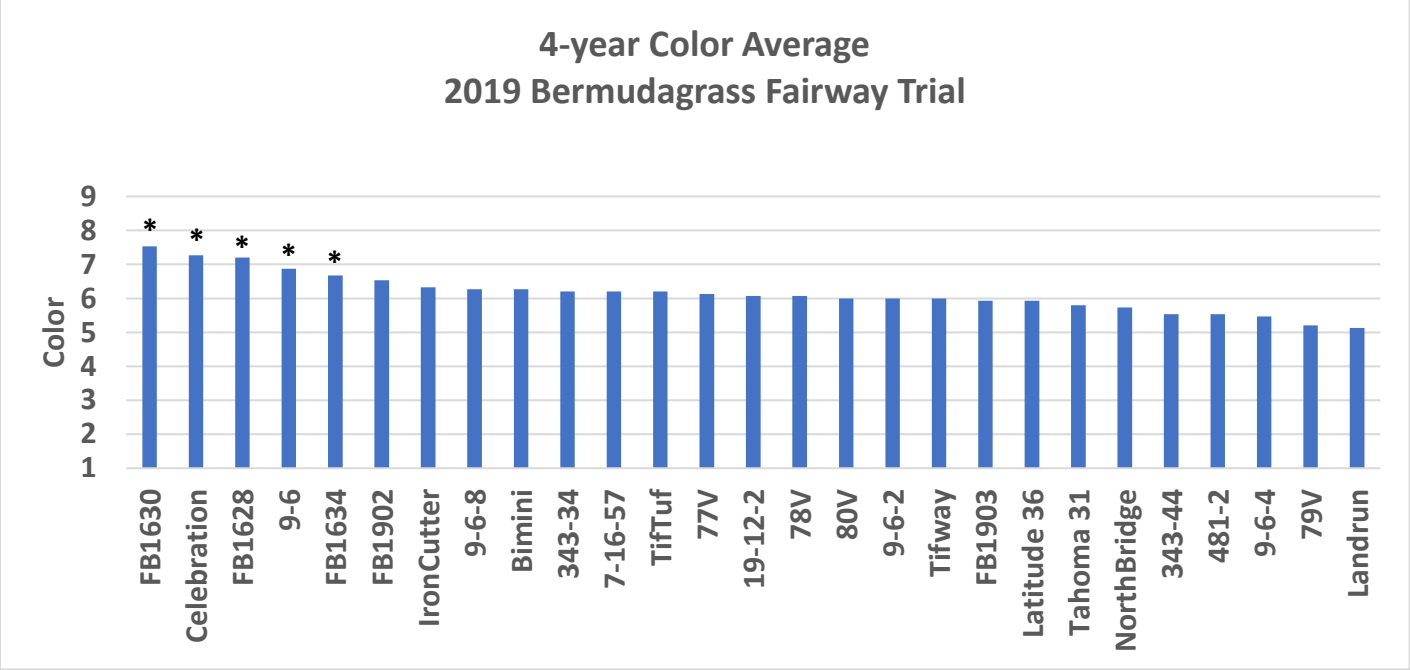


Figure 3. Average four-year color ratings of 27 bermudagrass entries from 2020 through 2023. Color was visually rated using a 1-9 scale, where 9 = dark green and 1 = brown. "*" indicates entries in the top statistical group.

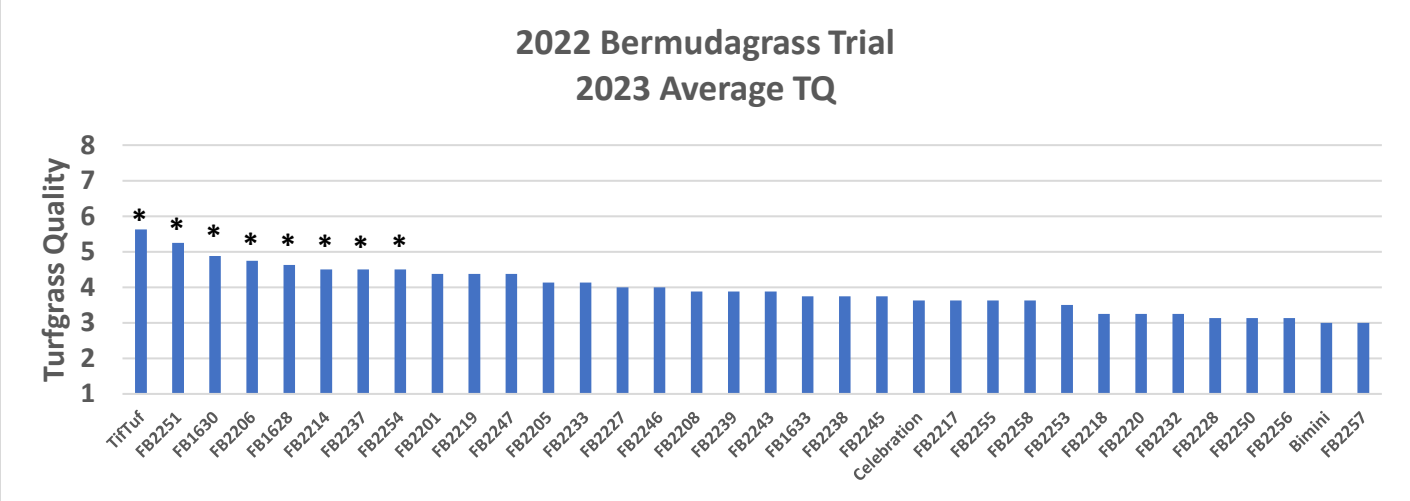


Figure 4. Average turfgrass quality of bermudagrass entries in 2023. There were a total of 63 entries in the trial. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality. "*" indicates entries in the top statistical group.

Zoysiagrass

2019 Zoysiagrass Fairway Trial

In 2019, 27 entries of zoysiagrass were planted in a randomized complete block design with three replications. Since establishment, the mowing height has been 1.3 cm. In 2023, it was irrigated to prevent stress. Two commercial cultivars, Zeon and Emerald, were included as controls. Visual ratings have been collected for several parameters throughout the study. Figure 5 shows turfgrass quality (TQ) ratings for summer and winter months. Summer months included April through October and winter included January – March 2023 and November – December 2023. Freezing temperatures

did not occur in late 2023. Four entries were the most consistent in 2023: FZ1667, FZ1680, FZ1642, and recently named Brazos™ (FAES1319). Lines are being identified for increase.

Eight entries were in the top statistical group, but Brazos, FZ1723, FZ1642, FZ1667, FZ1680, and FZ1683 were the only entries to average TQ above six across all months and years of rating from 2020-2023 (Figure 6). Several entries produced an average color of 6.0 or greater through 2020 – 2023; however, FZ1667, FZ1642, FZ1680, and Brazos were in the top statistical group (Figure 7).

2023 Zoysiagrass Spaced Plant Nursery

In July 2023 a new spaced plant nursery of 2,100 zoysiagrass breeding lines was planted on 3' centers (Figure 8).

2021 Zoysiagrass Spaced Plant Nursery

Throughout 2023, approximately 80 individual plants were selected based on winter color, and responses to drought, disease, and tropical sod webworms. In 2024, plugs will be collected and transplanted to a second nursery with replications.

2017 Zoysiagrass Spaced Plant Nursery

In 2021, the initial selections (120 plants) from the 2017 zoysiagrass spaced plant nursery were transplanted into a second nursery on 5' centers. Throughout 2023, this field was evaluated for turfgrass quality, winter color, and persistence under scalping. In 2024, 20 lines will be selected and propagated for further testing in larger plots.

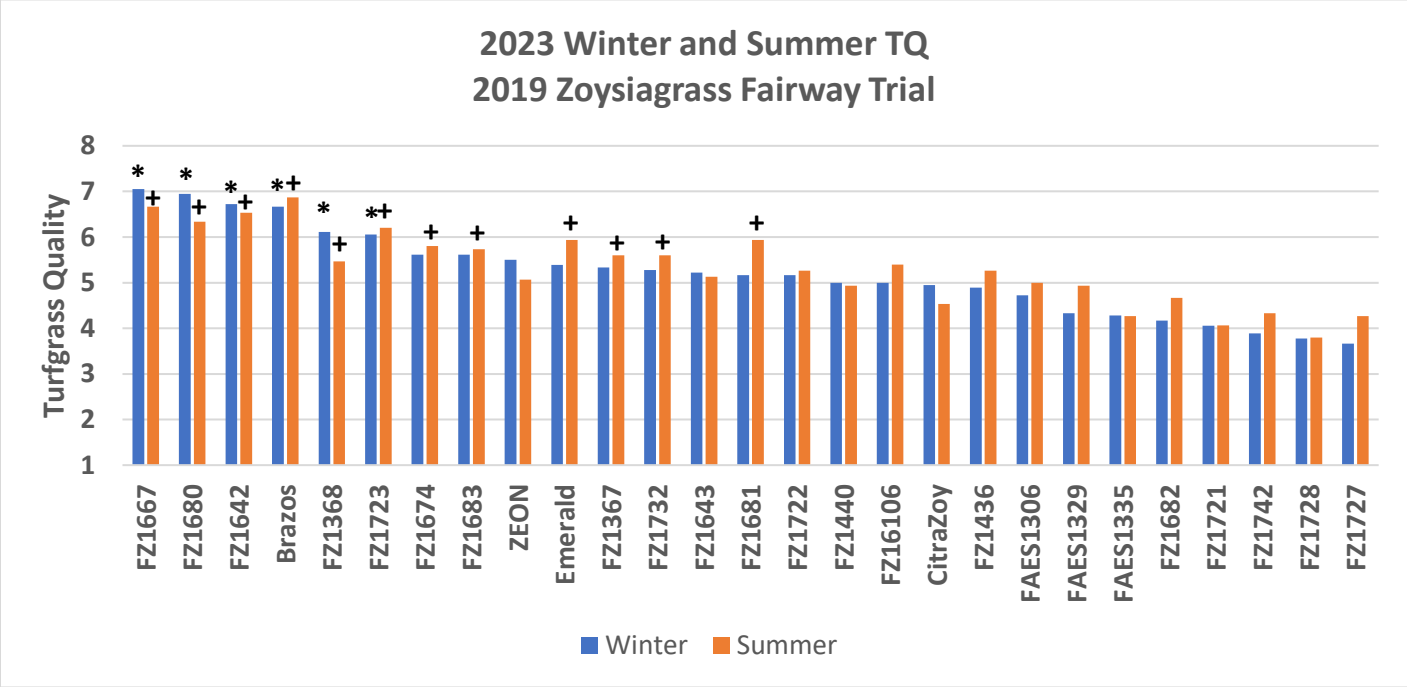


Figure 5. 2020, 2021, and 2022 average turfgrass quality ratings of 27 zoysiagrass entries for summer and winter. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable zoysiagrass quality. "*" indicates entries in the top statistical group during winter months. "+" indicates entries in the top statistical group during warmer months.

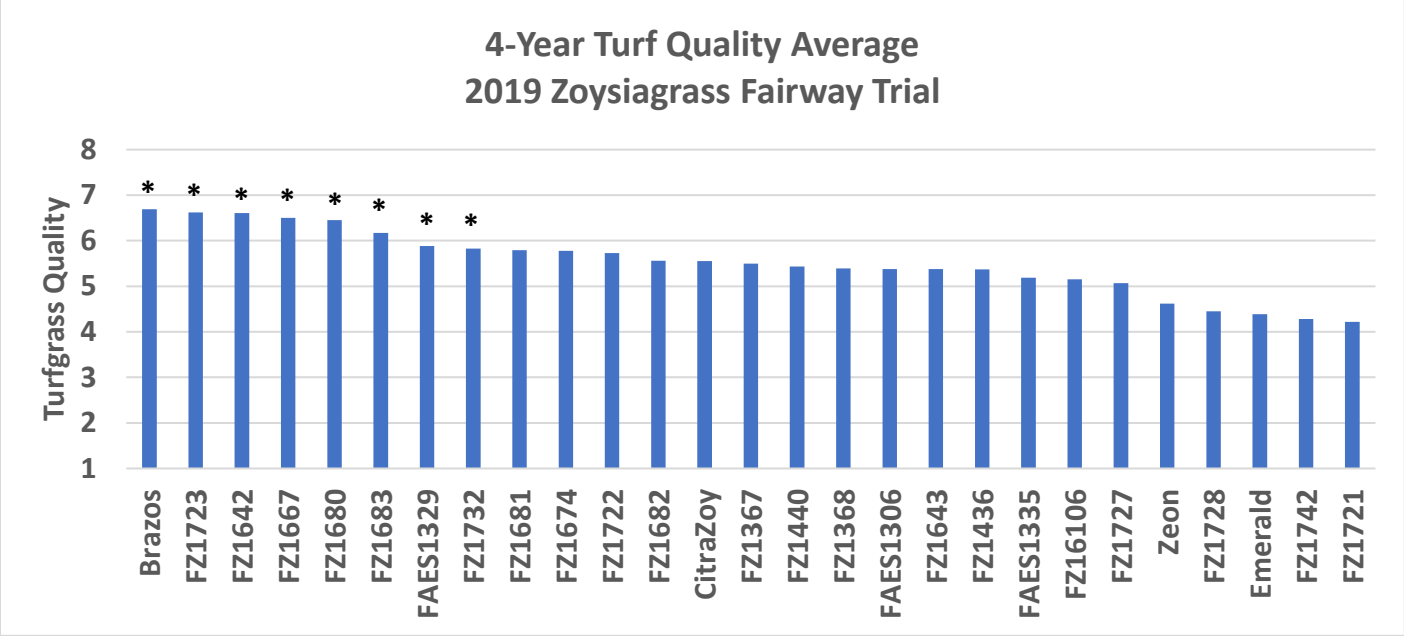


Figure 6. Average four-year turfgrass quality ratings of 27 zoysiagrass entries from 2020 through 2023. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable zoysiagrass quality. "*" indicates entries in the top statistical group.

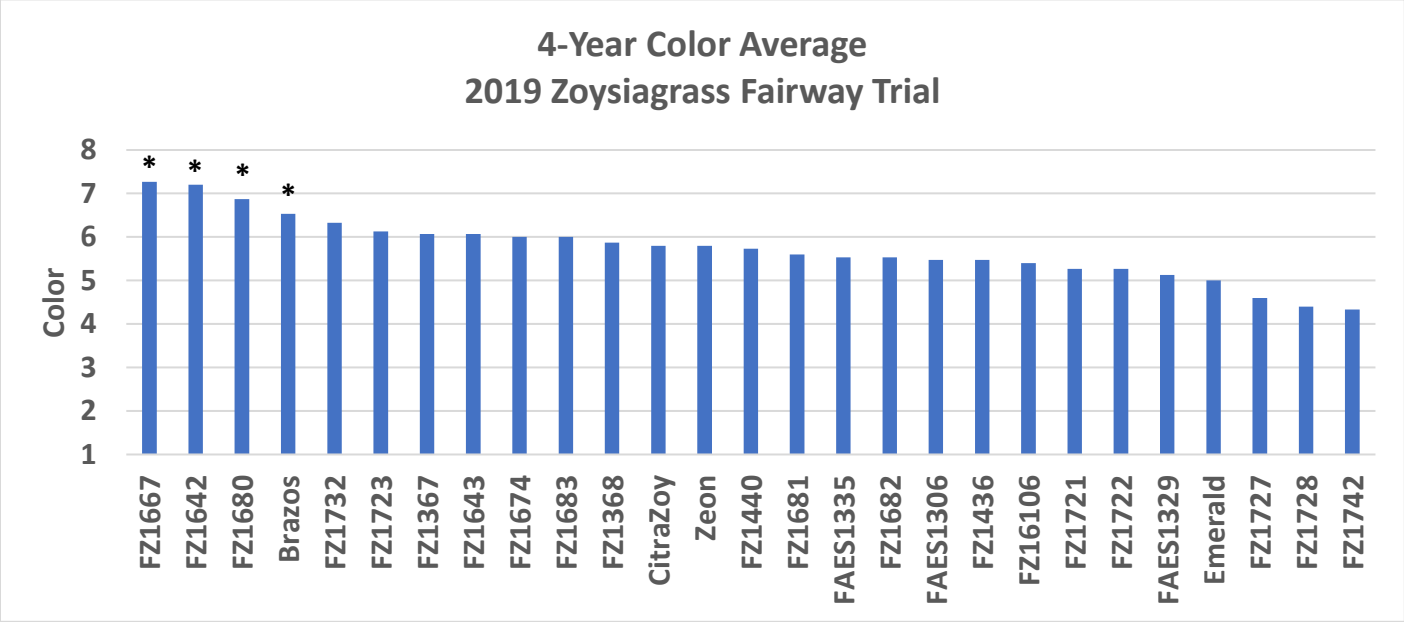


Figure 7. Average four-year color ratings of 27 zoysiagrass entries from 2020 through 2023. Color was visually rated using a 1-9 scale, where 9 = dark green and 1 = brown. "*" indicates entries in the top statistical group.



Figure 8. Zoysiagrass spaced plant nursery planted in July 2023. Picture taken November 2023.

USGA ID#: 2017-11-621

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

Project Leaders: Ambika Chandra, A. Dennis Genovesi, and 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:

- Evaluation of the 2021 Seeded Zoysia SPN has been completed with the identification of nine experimental lines selected for further testing following five cycles of phenotypic recurrent selections.
- Seed was harvested from early (two parent) and late synthetic (three parent) varieties in 2023 at Dallas, TX and Enid, OK with Johnston Seed Company (JSC). In addition, seed was harvested in Dallas from three lines originally identified by JSC as elite seed producing lines.
- Our collaboration with Woerner Farms (WF) in southern Alabama has led to the identification of seven seed parents adapted to their region. Seed lots were collected from these seven lines and percent germination was determined.
- A new Spaced Plant Nursery (SPN) consisting of 1152 hybrid progeny (emphasizing Meyer and cold tolerant zoysiagrass germplasm like DALZ 1701and KSUZ 0806 as parental lines) was planted in Dallas in 2023 for future evaluation with potential adaptation to the southern and transition zones.
- Reciprocal crosses between elite germplasm using a diallel mating design are currently being made for a new 2024 seeded SPN which will allow us to identify elite lines with ability to serve as both maternal and paternal seeded parental lines.

Project updates:

- ***2021 Spaced Plant Nursery*** – Seed harvested in 2019 from the 2017 Spaced Plant Nursery 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 Spaced Plant Nursery consisting of 663 experimental entries. Plots have been rated for establishment, quality, spring greenup, and seedhead development in the spring and summer of 2022 and 2023. Selections were made 23 August 2023 for parental lines exhibiting desirable turfgrass characteristics and seedhead production traits consistent with

the goals of this project. *A total of nine lines were selected representing five cycles of phenotypic selection.*

- ***Collaboration with Johnston Seed Company (JSC) in Enid, OK –*** Out of the 23 intermediate-textured parental lines (cycle four of phenotypic recurrent selection) sent to JSC in Oklahoma in 2018, four elite lines (TAES 6596-05, 6585-34, 6086-21, and 6087-15) were identified in 2021 with good seedhead production traits. Using these 4 elite lines, **one early flowering synthetic (TAES 6596-05 and 6086-21) and one late flowering synthetic (TAES 6596-05, 6585-34, and 6087-15) 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. Anthesis began on April 13, 2022, for all three blocks. On April 19, 2022, the average seedhead number per plug and average tallest seedhead height per plug were recorded (Table 1). Seedhead number and height are important measurements for estimating yield potential and harvest feasibility, respectively. TAES 6596-05 produced between 43 and 46 seedheads per plug with an average height between 9.3 and 9.8 cm. TAES 6086-21 produced more seedheads but at a shorter height. TAES 6585-34 produced a similar number of seedheads as 6596-05 but were slightly taller. TAES 6087-15 produced the fewest seedheads, and this is because it flowered much later than the other two parental lines in the late synthetic. TAES 6087-15 also produces red versus yellow-colored inflorescences which were easy to distinguish between genotypes. In 2023, the anthesis started earlier than in 2022 around April 4. Seed were harvested three times for the early synthetic (5/16, 6/20, and 8/3) and once for the late synthetic (8/3).

  Additionally, out of 535 medium-coarse textured progeny (cycle 1 of phenotypic recurrent selection) 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 traits were measured in 2022 and harvests occurred in 2023 (5/16 and/or 6/20) for four genotypes (7001-01, 7006-01, 7004-01, and 7013-01) (Table 2). TAES 7001-03 and 7004-01, had poor winter survival in 2022. **Six of the eight genotypes produced very robust-sized seedheads with large seed (Fig. 1)**. TAES 7004-03 produced the most seedheads of 30 per plug. TAES 7004-03, 7006-01, and 7013-01 produced the tallest seedheads ranging from 14.7 to 17.3 cm.





Figure 1. Robust seedhead development observed in the recombination block.

Table 1. Seedhead traits of the early and late synthetic blocks in April 2022.

Textural class	Block	Genotype	Average Seedhead #/plug	Average Tallest Seedhead Height per plug (cm)
Intermediate to fine	Early Syn	6596-05	42.5	9.8
		6086-21	60.2	8.7
	Late Syn	6596-05	45.7	9.3
		6585-34	45.5	10.7
		6087-15	28.0	11.8
Medium coarse	Rec. block	7001-01	4.7	12.2
		7001-03	9.7	11.5
		7004-01	0.0	0.0
		7004-02	27.7	13.0
		7004-03	30.3	14.7
		7006-01	13.0	16.5
		7013-01	24.0	17.3
		7013-02	4.7	6.0

Table 2. Seed weight and estimated yields from 2023 field harvests.

Textural class	Block/Genotypes	Date	Actual Yield (g)	Total Yield (g)	Harvested area (ft^2)	Estimated Yield (lb per acre)
Intermediate to fine	Early Syn	16-May	71.37	177.98	224	78.4
		20-Jun	71.93		224	
		3-Aug	34.68		224	
	Late Syn	3-Aug	38.11	38.11	196	17.4
Medium coarse (Rec. block)	7001-01	16-May	9.22	20.87	12	74.1
		20-Jun	11.65		12	91.5
	7006-01	16-May	37.57	37.57	12	300.6
	7013-01	16-May	18.16	18.16	12	143.7
	7004-01	20-Jun	20.08	20.08	12	161.2

Early and late synthetics with intermediate to fine leaf texture have *Z. matrella* influence in their lineage whereas the recombination block lines with medium-coarse leaf texture are of pure *Z. japonica* lineage, which helps explain higher estimated seed yields in the lines from the recombination blocks. Commercially available zoysiagrass (Zenith and Compadre) are both *Z. japonica* types. While Diesburg (2000) reported commercial production yields in the range of 100 to 600 pounds per acre for zoysia, these estimates seem high considering that 'Riviera' bermudagrass (the warm-season species with the highest yields) has averaged 150 lbs/acre over a 15-year period (J. T. Lamle with Johnston Seeds in Enid, OK (personal communication)

For the early synthetic block, we suspect poor yield may be due to the inability to harvest seedheads below the turf canopy as well as prolonged flowering that made it difficult to determine maturity resulting in three harvests over the summer. All genotypes harvested from the JSC recombination block produced red/dark red seedheads and flowered at relatively the same time. Among these, 7006-01 produced the greatest amount

from a single harvest and had the most promising yield potential. TAES 7006-01 also notably had the greatest visual abundance. Seed obtained from all three crossing blocks will be scarified and sowed into 1) a five-replication trial including Zenith and Compadre, and 2) a seed increase block. We plan to conduct germination tests over the 2023/2024 winter.

- ***Collaboration with Woerner Farms in southern Alabama*** – Similar to our collaboration with JSC, 21 of 23 parental lines (cycle 4 intermediate texture) were sent to WF in southern Alabama in March 2022. All material sent to Alabama is being evaluated for establishment, quality, seedhead density, and seedhead color. Based on these traits, seed was harvested on 23 June 2023 from nine lines in the space plant nursery and sent to Dallas for cleaning, seed weight, and percent germination. Seed yield and percent germination is presented in Table 3. Low seed set and germination percentages observed could be due to a number of factors (e.g. – seed harvest when still immature, possible inbreeding depression, limited opportunity for cross pollination in the nursery, high self-incompatibility).

Table 3. Seedhead production, yield, and germination of 9 elite lines from Woerner Farms space plant nursery in 2022.

Intermediate texture Genotype	Actual Yield (g)	Area harvested (ft^2)	Estimated yield (lb per acre	100 seed count weight (mg)	% Germination
6588-49	13.18	35	34.8	31	4.95
6610-30	16.78	35	47.9	51	20.41
6618-31	18.54	35	52.3	34	12.87
6618-37 (east)	18.68	35	52.3	36	14.00
6618-37 (west)	8.78	35	26.1	37	11.11
7539-50	9.01	16	52.3	36	14.00
7542-34	8.47	16	52.3	39	14.43
7542-49	2.61	16	17.4	37	8.08
7553-44	3.97	16	21.8	49	26.00

*Percent germination is based on 100 scarified seed

- ***Hybrid seeded zoysia approach*** - Zoysiagrass is an outcrossing species that has evolved a method of reproduction that promotes heterozygosity by virtue of its protogynous flowers and pollination by windblown pollen. In order to maximize seed yields we are investigating the utility of crossing elite lines arising from our ongoing recurrent selection program to self-fertile lines like ‘Meyer’, ‘Diamond’ and other germplasm lines to promote self-compatibility. This season (2023) we created 43 paired hybrid combinations for field trials leading to the planting of an SPN on 26 July 2023 comprised of 1152 hybrid progeny here at the Dallas Center (Fig. 2).



Figure 2. 2023 Seeded SPN progeny and nursery photographed on 10/20/2023, nearly 3 months after planting.

- ***Reciprocal crosses*** between elite medium-coarse germplasm lines in are currently being made in a diallel mating design for SPN testing. As crosses are being processed, floret number, estimated seed set, and percentage germination are being recorded. Germinated progeny will be planted in the summer of 2024 and evaluated for desirable phenotypic traits such as progeny uniformity, quality, and disease resistance, as well as anthesis timing, and abundance. This will allow us to identify compatible parental lines for future synthetics.

References:

Diesburg, K. L. 2000. Expanded germplasm collections set the stage for increased zoysiagrass breeding for turf use. Diversity 16(1):49-50).

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], Meghyn Meeks[1], Dennis Genovesi[1], Ross Braun[2], Mike Richardson[4], Mike Goatley[5], Dan Sandor[5], John Sorochan[6], Taylor Williams[6], Kevin Kenworthy[7], Paul Johnson[8], Kelly Kopp[8], Paul Harris[8], and Megan Kennelly[2]

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], Utah State University[8]

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 2016/2017 and distributed across three test locations for evaluation in 2017: Olathe, KS, West Lafayette, IN, and Dallas, TX.

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 and excellent turfgrass quality.

3. **Phase III (years 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 18-cell trays in Dallas during winter 2020/2021 and distributed to eight participating trial locations across the United States. Replicated trials were planted in late summer of 2021. In year one, the 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, and leaf texture. In 2022-2023, sites began evaluating individual traits including: shade tolerance in Dallas, TX; 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. Results from these evaluations are included herein.

Start Date: 2018
Project Duration: 6 years

Summary Points:

- In 2023, we continued the third year of evaluations for Phase III trials, which included the evaluation of 65 experimental genotypes, four elite genotypes, and five standards at Dallas, Olathe, West Lafayette, and five additional study locations throughout the year.

- Data collection in 2023 included turf quality, spring green up, winter injury, leaf texture, genetic color, and fall color. Additional trait evaluations [divot recovery (AR), cold (UT, IN, KS)-, drought (FL), and traffic-tolerance (TN)] were initiated in 2023 after full establishment of the entries. Genotype testing under shade was performed in TX from 2021-2023.

- Based on total TPI and ancillary trial performance, the 2023 analysis suggests TAES 6782-75, 6782-104, 6789-23, 6792-44, 6831-09, 6839-08, 6844-31, 6844-104, 6844-150, 6844-202, and 6941-36 performed well; along with DALZ 1701 and Emerald. At least two more years of data are needed to

observe better separation among experimental lines and identify superior line(s) across multiple traits and across diverse environments.

- Currently, all three years of data from all locations is being analyzed with a goal of making selection of top 10 lines from this set of 65 experimental lines. These elite selected lines will need to be further evaluated in order to identify varieties with commercial potential.

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 Research in Dallas for propagation. In spring, 2021, these 65 experimental lines, four elite hybrids developed from a separate USGA-sponsored project (DALZ 1701, DALZ 1702, DALZ 1808, and DALZ 1818), and five standards were delivered to eight cooperative state universities (Table 1). Due to cooperator, Dr. Lee Miller, leaving University of Missouri at the start of this study, no data was collected and shared from this trial location.

In 2023, when averaged across five to seven locations (AR, FL, IN, KS, TN, TX, and UT), 10 entries had summer turfgrass quality ≥ 6, which included one commercial entry (Emerald) and one top-performing genotype (DALZ 1701; Table 2). Genetic color was rated at six locations (AR, IN, KS, TN, TX, and UT) during the summer months; several had average genetic color > 7.0 across all, or several locations (Table 3). Entries with *Z. pacifica* lineage in TAES families 6782-6789, 6791, 6792 and 6840 were reported to have finer leaf texture ratings for the same six locations (Table 4). Spring greenup rated in 2023 indicated that top-performing genotypes DALZ 1701, 1702, and 1808 had a quicker spring greenup compared to other entries when averaged across locations (Table 5). Statistical analysis was performed for each trait and when an entry ranked in the top statistical "a group" it received one point. The sum of "a group" points is defined as the entries total TPI (Turfgrass Performance Index) score. Data from 2023 suggest lines like TAES 6782-75, 6782-104, 6789-23, 6792-44, 6831-09, 6839-08, 6844-31, 6844-104, 6844-150, 6844-202, and 6941-36 show promise across many locations for turf quality, genetic color, spring greenup, winter injury, and overall stress tolerance to ancillary evaluations (Table 6). Many of the top performing genotypes (DALZ 1701, 1702, 1808, and 1818) and commercial entries (Emerald, Innovation, Meyer, and Palisades) had high TPI scores in all evaluations across locations in 2023.

At specific locations, experiments were conducted to evaluate performance under shade (TAMU) and drought (U Florida), recovery from divots (U Ark), and mechanical trafficking damage (U. Tenn.). Under 63% shade conditions in Dallas, TX, 18 entries had a turf quality of 6 or above (Table 7) with TAES 6782-104 and 6941-36 ranking as superior performers (Figure 1). In Gainesville, Florida a 7-day dry down period was initiated from May 1st to May 8th, 2023. Green cover (on a 0-100% scale) was used to evaluate turf performance during the drought period, and for seven days following dry-down when irrigation was resumed. On the final day of drought conditions (15 May), 11 entries had > 40% green cover including DALZ 1702 and Emerald (Table 8). TAES 6941-36 was the only top-ranking performer under both shade and drought and demonstrated resistance to wilting.

Divot recovery was investigated in Fayetteville, AR beginning on September 1, 2023 when damage was initiated. Divots were simulated using a modified edger (5cm by 10cm divot size) and backfilled with topdressing sand. Ratings on September 1st and September 8th were insignificant ($P > 0.05$). However, on September 19th, most entries had significantly recovered from divot injury. Forty-four of the 74 entries had > 70% divot recovery including DALZ 1701 and DALZ 1702 (Table 9). In August of 2022 and 2023, traffic tolerance was evaluated in Knoxville, TN. Traffic tolerance was simulated with a 4-ft wide modified Cady Traffic Simulator. One pass with the trafficker is equivalent to 57 cleat marks per square foot. Five simulated traffic events were initiated starting in August and applied weekly, for a total of 30 total games each year. Pictures were taken after each 10 games and were analyzed using Turf Analyzer software. Many entries tolerated traffic after 20 simulated games in 2022 and 2023 (Table 10) as evident by mean percent green cover of 64.5% (2022) and 62.9% (2023). TAES 6941-36 demonstrated high resistance to 20 and 30 games across years compared to other lines.

Although the full multiple location and year analyses are still on-going, preliminary results suggest that there is not one genotype that performs well across all locations and environments. However, there seems to be a separation between the transition zone and the southern climatic zone. Using the 2023 USDA plant hardiness map, analyses across six climatic zones ranging from 6a to 9a revealed that in the coldest zone (6a), TAES 6844-202 and 6844-150 were consistently top performers demonstrating enhanced cold/freezing tolerance. These two also were top ranking performers in 6b, 7a and 7b, but not further south. Several other lines also did well in zones 6b to 7b including TAES 6782-75, 6933-11, 6844-104, and 6782-104. TAES 6941-36 was the only line to perform well from 7a to 9a demonstrating southern adaptability as well as drought resistance, shade tolerance, traffic tolerance, and divot recovery. TAES 6941-36 was derived from a cross between cold tolerant *Z. japonica* and *Z. matrella* and closely resembles Palisades in leaf texture. Further analysis is currently being conducted to finalize selections for future testing.

Table 1. Texas Agricultural Experiment Station (TAES) family lineage summary

TAES Family	Lineage
DALZ 1701	(Zm x Zj) x Zj
DALZ 1702	(Zm x Zj) x Zj
DALZ 1808	Zj x Zj
DALZ 1818	(Zp x Zj) x Zj)
6782	[(Zj x Zp)/Zj) x Zp]
6783	[(Zj x Zp)/Zj] x Zp)
6784	[(Zj x Zp)/Zj] x Zp)
6785	[(Zj x Zp)/Zj] x Zp)
6786	[(Zj x Zp)/Zj] x Zp)
6787	[(Zj x Zp)/Zj] x Zp)
6789	[(Zj x Zp)/Zj] x Zp
6791	[(Zj x Zp)/Zj] x Zp)
6792	[(Zj x Zp)/Zj] x Zp)
6828	Zm x [(Zj x Zp)/Zj]
6829	[(Zj x Zp)/Zj] x Zm)
6830	((Zm x Zj) x Zm)
6831	Zm x (Zm x Zj)
6835	(Zm x Zj) x Zm
6836	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]
6839	Zm x [(Zj x Zp)/Zj])
6840	Zm x [(Zj x Zp)/Zj)
6844	[Zm x Zj] x [(Zj x Zp)/Zj])
6910	Zj x (Zj x Zm)
6919	[(Zm x Zp)/Zj] x [Zm x Zj]
6923	(Zj x Zm) x Zj)
6924	(Zm x Zj) x (Zm x Zj)
6925	(Zm x Zj) x (Zm x Zj)
6933	(Zm x Zj) x (Zm x Zj)
6940	(Zj x Zm)
6941	(Zj x Zm)
6942	(Zj x Zp)
Emerald	(Zj x Zp)
Innovation	(Zm x Zj)
Meyer	Zj
Palisades	Zj
Zeon	Zm

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.

Table 2.	2023 Turfgrass Quality (1-9)*							
	AR	FL	IN	KS	TN	TX	UT	
Entry I.D.**	17-Jul	3-Jul	21-Jul	19-Jul	6-Jun	20-Jul	7-Jul	Average
6782-104	5.7	3.0	.	8.0	8.3	7.3	.	6.7
6789-23	7.0	3.0	.	3.7	8.3	8.3	.	6.2
6844-104	7.0	3.0	7.0	7.7	7.7	5.7	6.0	6.2
DALZ 1701	7.3	3.7	5.7	5.3	7.3	7.0	8.0	6.2
6782-79	6.3	3.7	.	6.7	8.0	7.0	.	6.1
6782-75	5.7	3.3	.	7.0	7.7	7.0	.	6.1
6941-36	7.0	4.3	5.7	4.7	6.7	7.3	.	6.0
6844-36	7.3	3.0	5.7	7.7	7.3	5.3	6.3	6.0
6839-08	5.7	5.0	5.7	7.7	7.7	5.0	.	6.0
Emerald	7.3	3.7	3.7	6.7	7.0	7.3	.	6.0
6785-22	6.7	2.7	.	4.3	7.7	7.3	.	5.9
6787-18	7.3	3.7	.	5.0	7.3	6.3	.	5.9
DALZ 1808	6.7	4.0	6.3	6.3	6.7	5.0	7.0	5.9
6844-74	5.7	3.0	6.7	6.0	8.7	6.3	.	5.9
6933-11	6.3	2.7	.	7.7	8.3	4.3	.	5.9
Palisades	7.0	3.3	5.3	6.3	6.3	7.3	.	5.9
6789-40	6.0	4.7	.	3.7	7.7	6.7	.	5.8
6940-15	7.3	4.0	6.0	5.0	6.7	5.3	7.0	5.8
Innovation	6.0	3.0	6.7	7.0	7.0	6.0	6.3	5.8
6784-17	6.7	3.3	.	3.7	8.0	6.7	.	5.8
6829-34	7.7	4.0	.	5.0	7.3	6.0	.	5.8
6830-39	6.7	3.7	.	6.7	7.3	6.0	.	5.8
6844-202	6.0	2.0	6.0	7.7	7.7	5.7	.	5.8
6844-154	7.0	3.3	5.7	4.7	7.0	5.0	7.0	5.8
DALZ 1702	7.0	3.3	5.0	6.0	7.0	5.7	7.3	5.7
6782-120	6.7	3.7	.	2.0	7.7	7.7	.	5.7
6785-19	6.7	3.3	.	2.0	7.0	8.0	.	5.7
6829-36	6.7	4.0	6.3	7.0	7.0	5.0	5.3	5.7
6844-04	5.7	3.3	6.0	6.3	8.3	6.0	.	5.7
6844-150	5.7	3.3	4.7	7.3	7.3	5.0	7.7	5.6
6923-11	6.7	2.7	6.0	6.3	6.7	5.3	.	5.6
6840-20	6.7	2.7	3.4	6.7	7.3	7.7	3.3	5.6
6789-52	6.7	3.7	.	6.3	7.0	5.3	.	5.6
6829-20	6.7	3.7	.	6.3	7.3	5.0	.	5.6
6844-31	6.7	2.7	5.3	6.3	7.7	5.7	5.7	5.5
6844-141	5.7	2.3	6.0	6.7	7.7	4.3	6.0	5.5
6792-44	6.3	3.3	3.1	4.0	7.7	6.0	.	5.4
6844-152	6.0	3.3	5.0	6.7	6.7	4.0	8.0	5.4
6830-11	5.3	3.7	6.0	6.7	7.7	4.3	.	5.4
6844-34	5.7	3.0	6.3	6.7	7.0	5.0	.	5.4
DALZ 1818	7.0	2.7	3.3	6.3	7.0	6.3	.	5.4
6844-53	5.7	3.0	3.9	6.7	8.0	6.3	5.7	5.4
6910-172	7.0	2.7	4.7	5.7	6.0	6.7	.	5.3
6924-66	6.3	3.0	4.3	6.3	7.7	4.3	.	5.3
6942-22	3.0	4.0	4.9	5.3	7.0	7.0	.	5.3
Meyer	5.7	2.0	5.3	7.3	7.3	5.3	5.3	5.3
6782-42	3.7	2.3	.	7.0	7.3	5.3	.	5.3
6786-02	6.3	2.3	.	2.3	8.0	6.3	.	5.3
6829-69	5.7	4.3	1.9	6.3	7.7	6.7	.	5.3
Zeon	7.0	4.0	2.9	5.7	7.3	5.3	.	5.3
6844-147	7.0	2.3	5.0	7.0	7.0	5.0	3.0	5.3
6844-128	6.0	2.7	6.3	5.3	7.0	5.0	4.7	5.2
6844-190	5.0	4.0	6.0	5.0	7.0	4.7	5.3	5.2
6791-06	6.7	2.7	.	3.7	7.3	6.0	.	5.2
6829-02	6.3	3.0	.	7.0	7.3	4.7	1.0	5.1
6831-09	7.0	2.3	3.7	6.0	7.7	4.0	.	5.1
6844-42	6.7	3.0	5.0	6.0	7.0	4.3	.	5.1
6919-29	5.7	2.7	5.3	5.0	7.7	4.3	.	5.1
6836-09	6.7	2.3	1.9	7.0	7.7	5.0	5.3	5.1
6783-03	4.3	3.3	.	2.7	7.0	6.0	.	5.0
6924-44	6.0	2.3	5.7	7.0	7.3	3.7	.	5.0
6925-53	5.3	2.7	4.0	7.3	7.0	4.0	.	4.9
6924-47	5.7	2.7	4.3	6.3	6.7	5.0	4.3	4.8
6787-20	6.3	3.0	.	2.0	8.0	.	.	4.8
6835-33	6.7	3.3	2.9	7.0	6.3	4.0	5.7	4.8
6910-157	6.3	3.3	3.4	4.7	6.3	6.0	.	4.8
6830-02	7.3	3.3	1.9	7.3	7.3	4.7	2.0	4.8
6844-89	4.7	2.7	5.3	6.7	7.3	3.7	.	4.8
6844-91	5.0	3.0	4.7	5.0	7.7	3.7	4.7	4.7
6830-56	5.0	3.3	5.3	5.7	7.3	4.3	4.0	4.7
6828-27	5.0	2.3	1.9	8.0	8.0	5.0	1.7	4.6
6828-53	6.7	3.3	2.9	6.0	7.0	4.0	3.0	4.6
6828-77	4.3	2.7	.	6.7	7.3	3.0	1.0	4.1
6828-56	3.3	3.0	.	5.0	7.3	3.3	2.0	4.1

* Turfgrass quality rated on a 1 to 9 scale (1 = lowest quality; 6 = minimum acceptable quality; and 9 = maximum quality)
** Texas Agricultural Experiment Station (TAES) entry I.D.
Means highlighted in green are best performers, those in yellow are moderate, those in red are poorest.

Table 3.	2023 Genetic Color (1-9)*						
	AR	IN	KS	TN	TX	UT	
Entry I.D.**	17-Jul	24-Jul	12-Jun	5-Aug	25-Aug	25-Aug	Average
6785-22	7.3	.	7.7	8.7	8.7	.	8.1
6782-104	8.0	.	7.0	8.3	8.3	.	8.1
6782-75	7.7	7.1	7.3	8.7	8.7	.	8.0
6784-17	7.3	.	7.3	8.7	8.0	.	7.9
6789-23	7.0		5.7	8.7	9.0	.	7.8
6787-18	7.3	.	6.7	8.7	7.7	.	7.7
6836-09	7.0	7.3	7.7	8.7	7.7	8.0	7.7
6783-03	6.7	.	8.0	8.3	7.3	.	7.7
6789-40	5.7	7.0	7.0	8.7	9.0	.	7.6
6791-06	7.3	7.3	7.7	7.7	8.3	.	7.6
6792-44	7.0	7.0	7.3	8.3	8.0	.	7.6
6828-53	6.3	7.0	8.0	8.3	8.0	8.3	7.6
6844-36	8.0	.	6.3	7.7	7.7	7.3	7.5
6844-31	7.3	7.5	6.3	8.7	7.7	7.0	7.5
6789-52	7.0	6.7	6.7	9.0	8.0	.	7.4
6933-11	5.7	8.0	7.0	8.0	8.0	.	7.4
6844-104	7.0	.	7.7	8.3	7.7	6.7	7.4
6830-02	7.3	7.3	7.0	7.7	7.3	8.0	7.4
DALZ 1818	7.7	.	5.7	7.7	8.0	.	7.3
6925-53	6.7	.	7.0	7.7	7.7	.	7.3
6924-66	6.7	8.0	6.7	7.7	7.3	.	7.3
6782-42	5.0	.	6.7	8.7	8.0	.	7.3
Zeon	7.3	8.0	5.7	8.7	7.3		7.3
6844-141	6.3	7.7	6.7	7.7	7.3	7.7	7.3
6840-20	6.7	7.3	6.3	7.3	7.7	8.0	7.3
6941-36	6.3	6.3	7.7	7.3	8.3	.	7.3
Emerald	6.7	6.3	6.3	8.0	8.3	.	7.3
6785-19	7.0	.	3.3	8.7	8.7	.	7.3
Palisades	7.0	.	7.0	7.0	7.7		7.3
6844-42	7.3	8.1	5.0	8.3	7.7	.	7.2
6829-02	6.0	7.3	6.0	8.0	7.7	8.0	7.2
DALZ 1701	7.0	.	6.3	7.7	7.7	6.7	7.2
6844-04	6.3	7.0	6.3	7.7	8.0	.	7.2
6828-27	6.0	7.3	7.0	7.7	7.3	8.3	7.2
6830-11	6.7	7.3	6.3	7.7	8.0	.	7.2
6844-150	5.7	8.1	6.3	8.3	7.0	7.3	7.2
6782-79	6.7	.	5.7	8.3	8.3	.	7.1
6829-34	6.7	6.7	7.0	7.7	7.3	.	7.1
6830-39	6.3	8.0	5.7	8.3	7.7	.	7.1
6910-157	6.7	8.0	6.3	7.7	8.0	.	7.1
6923-11	6.0	7.3	6.7	7.0	8.0	.	7.1
6844-53	6.3	.	5.3	8.0	8.3	7.7	7.1
6844-89	5.7	7.3	6.3	9.0	7.3	.	7.1
6828-56	5.0	7.3	7.3	7.7	7.0	8.3	7.1
6829-20	6.3	8.0	6.0	7.7	7.3	.	7.1
6844-147	6.7	.	6.0	8.0	7.3	7.0	7.1
6844-128	6.3	8.0	6.3	8.0	7.7	6.3	7.1
6839-08	6.3	7.5	5.7	8.3	7.0	.	7.0
6829-69	6.0	7.3	5.0	8.3	8.3	.	7.0
6844-34	5.3	8.1	6.7	7.0	7.7	.	7.0
Meyer	6.7	7.7	6.3	8.3	7.0	5.7	7.0
6831-09	6.3	6.7	6.0	8.0	7.0	.	6.9
6844-74	5.7	.	6.0	7.7	7.7	.	6.9
6844-152	7.0	7.7	6.3	7.7	7.3	6.0	6.9
6844-154	6.7	.	7.3	7.3	7.3	5.7	6.9
6924-47	7.0	.	6.3	8.0	7.7	6.3	6.9
6782-120	7.7	.	1.0	8.3	8.7	.	6.9
6910-172	6.0	.	6.3	7.3	7.7	.	6.9
6828-77	6.0	6.3	6.3	8.3	6.7	7.7	6.9
6919-29	6.3	6.3	6.0	7.7	7.0	.	6.8
6844-91	5.3	7.0	6.3	7.7	7.3	7.0	6.8
6829-36	6.3	6.9	6.3	8.0	7.0	6.3	6.8
6924-44	5.3	7.3	7.0	8.7	6.7	.	6.8
6787-20	7.3	7.9	3.3	8.3	.	.	6.7
6835-33	6.7	7.0	6.0	9.0	7.7	5.0	6.7
6844-202	6.3	6.7	5.0	8.0	7.0	.	6.7
6942-22	3.7	7.0	6.3	7.7	8.0	.	6.7
6786-02	6.3	.	3.3	7.7	7.7	.	6.7
Innovation	6.0	6.3	5.3	7.7	8.0	5.7	6.6
6844-190	5.7	.	5.3	7.7	7.3	6.7	6.6
6940-15	6.3	7.7	6.0	7.7	7.0	4.3	6.6
6830-56	4.7	8.0	6.3	7.7	7.7	6.0	6.5
DALZ 1702	6.7	.	5.0	7.3	7.0	6.0	6.5
DALZ 1808	6.7	.	5.3	6.7	7.3	5.3	6.4

* Genetic color visually rated on a 1 to 9 scale (1 = brown/straw/dead; 9 = dark green)
** Texas Agricultural Experiment Station (TAES) entry I.D.
Means highlighted in green are best performers, those in yellow are moderate, those in red are poorest.

Table 4.	2023 Leaf Texture (1-9)*						
	AR	IN	KS	TN	TX	UT	
Entry I.D.**	17-Jul	21-Jul	23-Aug	5-Aug	24-Aug	25-Aug	Average
6787-18	8.0	.	7.7	9.0	8.0	.	7.9
6789-40	7.0	.	8.0	9.0	8.0	.	7.9
6787-20	6.7	.	8.0	9.0		.	7.9
6784-17	7.7	.	8.0	8.7	7.0	.	7.7
6786-02	6.7	.	8.0	9.0	7.7	.	7.7
6789-23	7.0	.	8.1	8.7	7.3	.	7.6
6783-03	6.3	.	8.0	9.0	7.7	.	7.5
6791-06	6.7	.	8.0	8.3	7.7	.	7.5
6785-22	6.3	.	8.0	9.0	7.3	.	7.5
6785-19	6.3	.	8.0	8.7	7.3	.	7.5
6792-44	7.0	5.9	8.0	9.0	7.3	.	7.4
6782-104	6.7	.	6.0	9.0	7.7	.	7.3
6782-120	6.3	.	.	8.7	7.3	.	7.3
6789-52	7.0	.	6.3	9.0	7.0	.	7.2
6782-42	6.3	.	5.7	9.0	7.3	.	7.1
6782-75	6.3	.	5.3	9.0	7.7	.	7.1
Emerald	7.3	8.0	7.0	8.0	6.0	.	7.1
6840-20	6.3	7.5	6.3	8.0	6.0	8.7	7.0
6836-09	6.3	.	6.0	8.0	6.3	9.0	6.9
6835-33	6.7	7.5	5.3	8.7	5.7	8.7	6.9
6828-53	6.0	7.0	7.0	8.0	6.0	8.7	6.9
6782-79	6.0	.	5.3	8.7	7.7	.	6.9
6830-02	7.0	.	6.0	7.7	6.0	8.3	6.8
6830-39	6.7	.	5.7	8.7	7.0	.	6.8
6828-56	6.0	.	6.7	7.7	5.7	8.7	6.8
6829-02	6.7	.	6.3	7.7	5.7	8.1	6.7
6829-34	6.7	.	8.0	7.3	6.0	.	6.7
6844-150	5.3	7.7	6.7	7.7	6.0	8.3	6.7
6831-09	6.3	7.0	6.7	8.0	5.7	.	6.6
6844-53	6.3	7.5	6.0	7.3	5.7	7.7	6.6
6844-104	6.7	6.7	6.7	7.3	5.3	7.3	6.6
6942-22	6.7	7.0	6.7	7.0	5.7	.	6.5
6839-08	6.0	7.3	7.0	7.3	5.3	.	6.5
6910-157	7.0	6.5	8.0	7.0	5.3	.	6.5
6830-56	6.0	7.0	7.3	7.3	5.0	7.7	6.5
Zeon	7.0	8.0	4.0	7.7	6.0	.	6.5
6828-27	5.3	7.0	6.0	7.3	5.7	8.0	6.4
6844-147	6.7	6.3	6.7	7.7	5.0	7.7	6.4
6844-31	6.3	6.7	7.0	7.7	5.0	7.0	6.4
6844-152	6.7	6.3	6.3	7.7	5.0	7.0	6.4
Innovation	6.0	6.0	6.3	7.7	5.7	7.3	6.4
6844-89	5.7	6.0	6.7	8.0	6.0	.	6.3
6844-36	6.7	6.3	6.7	7.0	5.3	6.7	6.3
6829-20	7.0	.	6.3	7.7	5.3	.	6.3
6844-154	6.3	6.3	8.0	6.7	5.0	6.7	6.3
6844-141	6.3	7.0	6.3	7.3	5.0	6.7	6.2
6844-190	6.0	6.3	7.3	7.3	4.7	7.0	6.2
6829-69	6.3	7.0	5.3	7.7	5.3	.	6.2
6844-202	6.0	6.3	6.7	8.0	5.0	.	6.2
6924-44	5.7	6.3	7.3	7.3	5.0	.	6.2
6924-66	6.0	6.3	7.7	7.3	5.0	.	6.2
6925-53	6.0	7.3	6.3	7.3	5.0	.	6.2
6829-36	6.0	6.0	6.0	7.7	5.0	7.0	6.2
6924-47	5.7	6.7	7.0	7.0	5.0	7.0	6.2
6844-74	6.3	6.3	6.3	7.0	5.3	.	6.2
6828-77	5.3	.	6.3	7.0	5.0	7.0	6.1
6830-11	6.0	6.0	7.7	7.0	5.0	.	6.1
6844-34	6.0	6.0	7.0	7.3	5.0	.	6.1
6844-42	6.3	6.3	5.7	7.3	5.3	.	6.1
DALZ 1701	6.7	6.0	6.7	7.0	5.0	6.3	6.0
DALZ 1818	7.0	6.0	6.3	6.7	5.0	.	5.9
6933-11	5.7	.	6.0	7.3	5.3	.	5.9
6923-11	6.7	5.7	5.7	7.0	5.3	.	5.9
6919-29	5.3	6.0	6.0	7.0	5.3	.	5.8
6844-91	5.3	6.0	6.3	6.7	4.7	6.7	5.8
6844-04	5.3	5.7	6.3	7.0	5.3	.	5.8
DALZ 1702	6.7	5.7	5.0	7.0	5.0	6.0	5.8
DALZ 1808	6.7	5.7	5.3	6.7	5.0	6.0	5.8
6910-172	6.7	5.0	8.0	6.3	4.0	.	5.7
6941-36	6.7	5.0	8.0	6.3	4.0	.	5.7
6940-15	6.7	5.0	6.7	6.7	4.0	5.7	5.6
6844-128	6.3	5.0	7.0	6.0	5.0	5.0	5.5
Meyer	5.3	5.7	7.0	6.3	4.0	5.7	5.4
Palisades	5.7	4.0	5.0	6.0	3.0	.	4.4

* Leaf texture visually rated on a 1 to 9 scale (1 = coarse; 9 = fine)
** Texas Agricultural Experiment Station (TAES) entry I.D.
Means highlighted in green are best performers, those in yellow are moderate, those in red are poorest.

Table 5.	2023 Spring Greenup (1-9)*						
	FL	IN	KS	TN	TX	UT	
Entry I.D.**	13-Feb	27-May	8-May	20-Apr	20-Mar	11-May	Average
6782-75	6.7	.	7.0	5.0	6.0	.	6.0
6782-104	6.0	.	7.0	4.3	6.7	.	5.9
6942-22	7.0	6.3	6.7	5.3	5.7	.	5.8
Emerald	5.0	4.3	5.0	7.3	7.3	.	5.7
6829-69	6.0	.	6.7	8.0	4.7	.	5.6
DALZ 1702	6.7	4.7	7.0	8.3	3.3	4.7	5.5
6782-79	6.0	.	7.7	5.3	5.7	.	5.4
6792-44	6.3	4.5	3.7	7.0	5.0	.	5.2
DALZ 1808	5.3	4.7	8.0	8.7	4.7	3.0	5.2
6789-23	4.7	.	3.3	7.0	5.7	.	5.2
6787-18	5.3	.	5.3	6.0	6.3	.	5.2
DALZ 1701	5.0	4.3	7.0	8.0	4.7	4.3	5.2
6844-152	2.7	5.0	7.7	8.3	4.7	6.0	5.1
Meyer	2.3	5.7	7.3	9.0	3.7	6.3	5.1
Zeon	5.3	.	6.7	6.7	4.7	.	5.1
6791-06	6.0	.	1.7	6.3	6.3	.	5.1
6941-36	5.3	3.0	5.7	8.0	5.7	.	5.1
6844-36	4.0	4.7	7.0	8.7	3.7	5.7	5.1
6782-42	6.3	.	7.7	4.0	3.0	.	5.1
6829-36	2.3	6.3	7.3	8.7	3.0	6.3	5.0
6844-128	2.3	5.7	6.0	9.0	3.3	6.3	5.0
6844-154	3.7	4.7	5.0	9.0	4.0	6.0	5.0
6844-150	3.3	6.0	7.3	8.7	3.0	5.3	5.0
6829-20	7.3	.	6.7	6.0	2.3	.	4.9
DALZ 1818	3.0	5.0	6.7	8.0	5.0	.	4.9
6844-04	2.0	6.7	6.7	8.7	3.3	.	4.9
6844-202	2.3	5.7	8.0	8.3	4.0	.	4.9
6785-22	6.0	.	3.0	6.7	5.3	.	4.9
6789-52	6.7	.	6.3	5.3	3.0	.	4.9
6925-53	4.7	3.7	7.7	7.7	3.3	.	4.8
6844-104	2.0	5.3	7.7	8.7	3.0	5.0	4.8
6940-15	4.0	5.3	5.3	8.3	4.3	4.0	4.8
6785-19	5.7	.	1.3	6.7	7.0	.	4.8
6933-11	4.7	.	7.3	7.3	2.3	.	4.8
Innovation	3.3	5.7	7.0	9.0	2.0	5.3	4.8
Palisades	4.7	4.7	6.7	6.7	2.3	.	4.6
6830-11	2.0	7.3	5.7	8.7	2.7	.	4.6
6919-29	2.0	6.3	5.0	9.0	3.7	.	4.6
6784-17	5.0	.	4.7	6.0	3.3	.	4.6
6831-09	5.3	3.7	6.3	5.3	4.3	.	4.6
6789-40	6.0	.	1.7	6.0	5.0	.	4.5
6830-39	6.0	.	7.7	6.0	1.7	.	4.5
6836-09	4.3	.	7.0	7.7	3.3	2.0	4.5
6844-74	2.0	6.3	5.3	9.0	3.0	.	4.5
6844-89	3.0	6.0	6.0	7.3	3.3	.	4.5
6829-02	4.7	.	7.0	7.3	3.7	1.0	4.4
6787-20	5.3	.	1.0	7.0	.	.	4.4
6830-02	6.0	.	7.3	7.0	2.7	1.3	4.4
6839-08	2.0	6.0	5.3	7.0	4.3	.	4.4
6844-91	2.3	4.3	6.0	9.0	2.7	5.3	4.4
6924-47	2.3	4.0	6.7	8.3	3.0	5.0	4.4
6782-120	6.0	.	1.0	3.7	4.7	.	4.3
6844-31	2.0	4.0	6.3	8.0	4.3	4.7	4.3
6829-34	6.3	1.9	5.3	7.0	2.7	.	4.3
6923-11	4.0	4.7	8.0	5.3	2.3	.	4.3
6844-141	2.3	5.0	6.0	6.3	3.0	5.3	4.2
6924-44	3.0	5.7	7.0	6.0	2.3	.	4.2
6844-34	4.0	6.0	3.3	7.3	2.7	.	4.2
6828-27	5.0	3.6	6.3	8.0	2.3	1.3	4.1
6783-03	6.7	.	1.3	3.7	6.0	.	4.1
6844-53	2.0	3.8	7.0	8.0	2.7	3.3	4.0
6910-157	6.3	3.8	4.3	5.3	3.0	.	4.0
6844-190	2.7	4.0	4.0	8.0	2.3	5.7	4.0
6844-42	2.0	4.7	6.3	7.0	2.7	.	3.9
6924-66	2.3	3.3	4.3	8.7	3.0	.	3.9
6840-20	3.7	3.3	7.7	7.3	2.3	1.7	3.9
6786-02	6.0	.	1.0	4.3	4.7	.	3.9
6844-147	2.0	4.3	6.0	7.7	2.7	3.0	3.9
6830-56	3.0	5.0	5.0	7.3	2.0	3.7	3.9
6835-33	3.3	2.8	8.0	5.3	2.7	3.0	3.8
6910-172	3.0	3.7	4.3	7.7	2.3	.	3.7
6828-77	5.0	.	7.3	6.0	1.3	1.0	3.7
6828-56	4.3	.	5.7	7.0	2.3	1.3	3.6
6828-53	4.0	2.6	7.7	5.0	1.3	1.3	3.3

* Spring greenup visually rated on a 1 to 9 scale (1 = brown/straw/dead; 9 = dark green)
** Texas Agricultural Experiment Station (TAES) entry I.D.
Means highlighted in green are best performers, those in yellow are moderate, those in red are poorest.

Table 6. Turf Performance Index (TPI) based on observational ratings in 2023*

Entry I.D.**	Turf Quality (6 max)	Genetic Color (5 max)	SGU (5 max)	Winter Injury (max 2)	Stress tolerance** (max 15)	Total TPI (max 33)
Emerald	6	5	4	2	14	31
6942-22	5	5	5	2	14	31
6844-202	6	5	4	2	13	30
6941-36	5	5	5	2	13	30
DALZ 1702	6	4	5	2	13	30
6844-104	6	4	4	2	13	29
6782-75	5	5	3	1	15	29
6940-15	5	5	4	2	13	29
DALZ 1808	6	4	5	2	12	29
6782-104	5	4	3	1	15	28
6840-20	6	5	3	2	12	28
Palisades	6	4	3	2	13	28
6792-44	5	5	4	1	12	27
6789-40	4	5	3	0	15	27
6831-09	6	5	5	1	10	27
6782-79	5	4	4	1	13	27
6829-69	5	5	4	1	12	27
6844-36	6	4	4	2	11	27
6844-128	5	5	4	2	11	27
6923-11	6	5	3	2	11	27
Meyer	6	5	4	2	10	27
6844-04	6	5	4	2	10	27
6787-18	4	4	4	0	14	26
6839-08	6	5	4	2	9	26
6844-147	6	4	3	2	11	26
6844-34	6	5	2	1	12	26
6782-42	4	4	3	1	14	26
6789-52	5	5	4	1	11	26
6829-34	4	5	4	1	12	26
6829-36	5	5	4	2	10	26
6830-11	5	5	3	2	11	26
6844-150	6	5	4	2	9	26
6844-152	5	5	4	2	10	26
6844-154	5	4	4	1	12	26
DALZ 1701	5	4	4	2	11	26
Zeon	6	5	4	1	10	26
6829-02	4	5	3	1	13	26
6829-20	5	5	3	1	12	26
6844-74	6	4	4	2	10	26
DALZ 1818	6	4	4	2	10	26
6844-91	4	5	3	2	12	26
6785-22	4	4	3	0	14	25
6791-06	4	5	3	0	13	25
6828-53	5	5	2	2	11	25
6844-31	6	5	4	2	8	25
6844-141	5	5	4	2	9	25
6844-53	6	4	3	2	10	25
6910-157	5	5	4	1	10	25
6828-27	5	5	3	1	11	25
6830-02	4	5	3	1	12	25
6784-17	4	4	3	0	13	24
6789-23	4	4	2	0	14	24
6782-120	4	3	2	0	15	24
6830-39	5	5	3	1	10	24
Innovation	6	5	3	2	8	24
6836-09	4	5	3	1	11	24
6910-172	5	4	2	1	12	24
6783-03	3	4	2	0	14	23
6785-19	4	3	3	0	13	23
6786-02	4	3	2	0	14	23
6830-56	5	4	3	2	9	23
6924-44	5	5	3	2	8	23
6828-56	3	5	2	1	12	23
6925-53	5	4	4	2	8	23
6933-11	4	5	2	1	11	23
6919-29	4	5	4	1	9	23
6828-77	3	5	2	1	12	23
6844-89	4	5	4	2	7	22
6924-66	5	5	3	2	7	22
6844-42	5	5	3	2	7	22
6924-47	5	3	4	2	7	21
6844-190	4	4	2	2	8	20
6835-33	5	5	3	1	6	20
6787-20	3	3	2	0	11	19

* Turf performance index (TPI) represents the number of times an entry occurred in the top statistical group based across all locations (a higher number is desirable)
** Stress tolerance refers to entries in the top statistical group for ancillary evaluations (shade, drought, traffic, and divot recovery).
Means highlighted in green are best performers, those in yellow are moderate, those in red are poorest.

Table 7.	Shade Tolerance* in Dallas, TX			
	TQ	Color	Texture	SGU
Entry I.D.**	20-Jul	25-Aug	24-Aug	20-Mar
6782-104	8.0	8.7	7.3	5.3
6792-44	7.7	8.0	7.0	5.0
6785-19	7.0	8.7	7.0	3.3
6785-22	7.0	8.0	7.0	3.3
6789-23	7.0	8.7	7.0	5.3
6840-20	7.0	7.7	6.0	1.7
6783-03	6.7	8.0	6.7	2.7
6829-02	6.7	7.7	6.0	3.0
6782-120	6.3	8.7	6.7	6.3
6784-17	6.3	8.0	7.0	4.0
6786-02	6.3	8.3	7.0	3.7
6789-40	6.3	8.3	7.7	4.0
6844-154	6.3	7.0	5.0	2.3
6941-36	6.3	7.7	4.0	3.0
6782-42	6.0	8.3	7.3	4.3
6787-18	6.0	8.3	7.0	3.0
6933-11	6.0	8.0	5.3	2.3
6942-22	6.0	7.3	6.0	4.0
Emerald	6.0	8.0	6.0	5.0
6782-75	5.7	8.7	7.0	5.3
6844-147	5.7	7.3	5.0	1.7
6923-11	5.7	7.7	5.0	1.3
DALZ 1808	5.3	7.0	5.0	2.3
6831-09	5.3	7.7	6.0	2.3
6844-104	5.3	7.0	6.0	2.0
6844-141	5.3	7.7	5.0	1.3
6844-202	5.3	7.0	5.3	1.0
6844-36	5.3	8.0	5.7	2.0
6919-29	5.3	7.7	5.3	1.7
6924-66	5.3	7.7	5.0	2.0
6940-15	5.3	7.0	4.3	2.3
Palisades	5.3	7.7	3.0	2.7
DALZ 1701	5.0	8.0	4.7	3.0
DALZ 1818	5.0	7.7	4.7	1.7
6782-79	5.0	6.7	6.7	2.3
6839-08	5.0	7.3	6.0	2.0
6844-74	5.0	7.7	5.7	1.3
6791-06	4.7	7.3	7.0	5.3
6828-27	4.7	6.7	5.7	2.3
6828-56	4.7	7.0	6.0	1.0
6829-34	4.7	7.3	5.7	2.7
6830-02	4.7	7.0	6.0	2.3
6836-09	4.7	7.7	6.0	2.7
6844-128	4.7	6.7	4.3	2.3
6844-190	4.7	7.0	5.0	1.0
6910-172	4.7	7.0	4.0	1.0
Zeon	4.7	7.0	6.0	2.3
DALZ 1702	4.3	7.0	5.0	3.7
6789-52	4.3	7.3	6.7	3.0
6829-20	4.3	7.0	5.3	2.3
6829-69	4.3	7.0	5.7	2.7
6830-39	4.3	6.7	6.0	1.3
6844-31	4.3	7.7	5.3	1.0
Innovation	4.3	7.3	5.7	1.0
6829-36	4.0	6.7	5.7	1.3
6830-11	4.0	7.0	5.0	1.3
6844-04	4.0	8.0	5.0	2.0
6844-150	4.0	7.3	5.3	1.0
6844-34	4.0	7.0	5.0	1.7
6844-42	4.0	7.0	5.3	1.0
6844-91	4.0	7.0	5.0	1.0
6925-53	4.0	7.7	5.3	2.0
Meyer	4.0	7.0	4.0	1.7
6828-53	3.7	7.0	5.7	1.0
6828-77	3.7	6.7	6.0	1.3
6844-152	3.7	6.3	5.7	1.7
6844-53	3.7	7.0	5.7	1.3
6910-157	3.7	6.0	5.0	1.0
6924-47	3.7	6.0	5.0	1.3
6844-89	3.3	7.0	5.7	1.3
6830-56	2.7	5.3	5.0	1.3
6835-33	2.7	5.7	6.0	1.7
6924-44	2.7	5.7	5.7	1.0

* Shade tolerance evaluated at 63% shade in Dallas, TX.
** Texas Agricultural Experiment Station (TAES) entry I.D.
TQ = Turf quality visually rated on a 1-9 scale (1 = lowest quality; 6 = minumum acceptable quality; 9 = maximum quality)
Color = Genetic color visually rated on a 1-9 scale (1 = brown/straw/dead; 9 = dark green)
Texture = Leaf texture visually rated on a 1-9 scale (1 = coarse; 9 = fine)
SGU = Spring greenup visually rated on a 1-9 scale (1 = brown/straw/dead; 9 = dark green)

Table 8.	Drought tolerance in Gainesville, FL			
	Green cover (%)			
	Drought Ratings		Recovery Ratings	
Entry I.D.	4-May	8-May	11-May	15-May
6829-69	93.3**	30.0	80.0	86.7
6942-22	86.7	60.0	85.0	86.7
6941-36	83.3	53.3	78.3	85.0
6829-34	81.7	33.3	71.7	81.7
6840-20	83.3	50.0	76.7	81.7
DALZ 1702	93.3	40.0	66.7	78.3
6789-40	85.0	30.0	71.7	78.3
6829-36	76.7	13.3	61.7	76.7
6830-02	86.7	18.3	53.3	76.7
6940-15	81.7	40.0	60.0	76.7
6782-75	63.3	50.0	68.3	73.3
6829-02	71.7	21.7	60.0	71.7
DALZ 1701	66.7	6.7	56.7	70.0
DALZ 1808	73.3	8.3	46.7	70.0
Emerald	75.0	46.7	66.7	70.0
6844-202	68.3	25.0	49.6	68.3
6828-56	58.3	25.0	61.7	66.7
6844-154	60.0	10.0	45.0	66.7
6787-20	68.3	43.3	60.0	65.0
6782-104	50.0	35.0	53.3	63.3
6783-03	66.7	43.3	60.0	63.3
6782-79	61.7	43.3	58.3	61.7
6784-17	56.7	36.7	55.0	61.7
6786-02	63.3	15.0	40.0	61.7
6787-18	46.7	18.3	50.0	61.7
6829-20	65.0	50.0	51.7	61.7
6844-104	60.0	15.0	46.7	61.7
6782-120	63.3	36.7	50.0	60.0
6789-52	76.7	25.0	46.7	60.0
6844-36	53.3	6.7	40.0	60.0
6789-23	58.3	30.0	51.7	58.3
6828-77	53.3	18.3	43.3	58.3
6830-39	81.7	5.0	20.0	58.3
6836-09	52.4	18.6	39.3	58.2
6910-172	55.0	16.7	46.7	56.7
6844-152	55.0	15.0	46.7	55.0
Zeon	50.0	8.3	40.0	55.0
6828-27	51.7	25.0	40.0	53.3
6844-34	53.3	18.3	45.0	53.3
6923-11	50.0	3.3	30.0	51.7
6828-53	56.7	13.3	40.0	50.0
6844-74	48.3	11.7	26.7	50.0
6782-42	46.7	30.0	38.3	48.3
6830-11	46.7	21.7	38.3	48.3
6830-56	63.3	5.0	33.3	48.3
6844-53	51.7	26.7	35.0	48.3
6785-22	43.3	30.0	40.0	46.7
6791-06	40.0	26.7	41.7	46.7
6839-08	40.0	5.0	26.7	46.7
6844-128	50.0	21.7	33.3	46.7
6844-91	53.3	13.3	46.7	46.7
6924-44	41.7	8.3	43.3	46.7
Palisades	51.7	23.3	45.0	46.7
DALZ 1818	46.7	11.7	26.7	45.0
6910-157	40.0	21.7	35.0	45.0
6831-09	53.3	5.0	43.3	43.3
6844-04	48.3	11.7	40.0	43.3
6844-150	36.7	5.0	20.0	41.7
6933-11	38.3	23.3	38.3	41.7
6844-89	51.7	10.0	26.7	40.0
6785-19	40.0	16.7	33.3	38.3
6792-44	31.7	13.3	30.0	38.3
6844-141	36.7	5.0	23.3	38.3
6844-147	40.0	18.3	36.7	38.3
6844-190	33.3	15.0	33.3	38.3
Innovation	38.3	10.0	31.7	38.3
6844-31	36.7	10.0	16.7	36.7
6844-42	36.7	1.7	26.7	36.7
6919-29	36.7	11.7	26.7	36.7
6924-47	31.7	5.0	30.0	30.0
6925-53	20.0	3.3	15.0	26.7
Meyer	58.3	13.3	23.3	25.0
6835-33	43.3	1.7	15.0	16.7
6924-66	6.7	1.7	6.7	8.3

* Experimental plots were irrigated on the morning of 1 May and then withheld for
7 days. Green cover was visually rated on a percent scale (0% = no green cover; 100% = complete green cover of plot) on 4-May (3 days without rainfall) 8-May (7 days without rainfall); 11-May (3 days following resumed irrigation); and 15-May (7 days following resumed irrigation).
** Entries highlighted in green occured in the top statistical group within each rating date.

Table 9.	Divot Recovery (%)* in Fayetteville, AR		
	Recovery (%)		
Entry I.D.	1-Sep	8-Sep	19-Sep
6844-34	36.8	40.4	82.9**
6844-190	35.7	32.2	81.1
6828-53	39.3	44.1	78.9
6784-17	37.6	43.5	78.6
6919-29	43.9	48.7	78.3
6924-44	39.8	44.2	77.9
6844-89	41.5	42.8	77.4
6941-36	36.9	44.3	77.0
6844-91	32.4	39.3	76.9
6828-77	33.5	36.3	76.1
6844-147	33.3	35.5	75.8
6844-31	28.8	35.8	75.3
6829-69	29.2	36.8	75.2
6782-75	34.0	41.7	74.8
6844-74	39.3	44.4	74.8
6910-172	36.8	42.4	74.8
6783-03	38.8	46.4	74.4
DALZ 1818	42.8	48.2	74.2
6923-11	40.2	43.3	74.1
6844-04	36.8	41.9	73.9
6844-36	34.9	39.2	73.9
6789-52	38.6	40.4	73.9
DALZ 1702	39.8	40.9	73.8
6844-150	41.3	41.6	73.7
DALZ 1701	35.1	40.5	73.3
6910-157	36.4	41.1	73.3
6925-53	40.5	43.3	73.0
6835-33	44.1	43.2	72.8
6844-42	31.5	33.9	72.7
6829-08	36.7	36.0	72.2
6785-22	38.8	43.9	71.8
6924-66	32.7	37.3	71.6
6844-202	37.5	37.0	71.4
Palisades	39.2	39.8	71.4
6924-47	44.9	45.7	71.4
6933-11	34.9	37.6	71.3
6831-09	24.2	44.5	71.2
6828-27	43.3	45.9	70.9
6829-34	32.5	44.2	70.7
6844-104	35.0	39.4	70.7
6791-06	36.7	39.4	70.5
6844-152	38.2	43.9	70.5
Emerald	37.1	43.5	70.4
6844-141	38.6	46.3	70.3
Zeon	35.2	41.7	69.8
6830-02	42.0	47.1	69.6
6839-08	38.8	42.5	69.3
6782-79	34.7	44.8	69.2
Meyer	36.9	41.4	69.0
6828-56	34.8	37.2	68.8
6829-36	39.8	44.9	68.8
6786-08	41.4	43.2	68.6
6782-42	37.3	39.6	67.8
6782-120	34.4	36.0	67.7
6940-15	29.6	35.6	67.6
6844-128	35.1	37.9	67.2
6840-20	41.4	42.9	67.2
DALZ 1808	35.8	42.6	67.2
6829-20	40.2	41.2	66.9
6782-104	39.1	41.3	66.1
6830-11	34.8	35.3	65.0
6830-39	33.6	43.5	64.9
6792-44	40.5	40.4	64.8
6836-09	35.3	38.3	63.8
6789-40	24.5	27.5	63.4
6844-154	37.8	38.8	63.0
6830-56	39.1	35.4	62.9
6942-22	34.7	37.8	62.3
Innovation	41.1	38.1	61.7
6785-19	34.3	42.1	59.6
6787-20	27.9	35.9	58.7
6787-18	38.2	44.1	57.9
6789-23	30.1	35.8	46.9
6844-53	28.5	27.8	43.6

* Standardized divots (5 cm by 10 cm) were cut from each plot on 9/1/23 using a modified edger (Fry et al., 2008) and then backfilled with topdressing sand. Recovery was monitored for each divot by collecting digital images weekly, beginning on the day of injury and continuing until full recovery (100% coverage). A total of three divots for each plot were analyzed independently for turf coverage. (Trappe et al., 2011; Karcher and Richardson, 2013)
** Entries highlighted in green occured in the top statistical group for the 9/19/23 rating date. Means across treatment replications (n=3) on 9/1/23 and 9/8/23 were insigificant ($P > 0.05$).

Table 10.	Traffic Tolerance* in 2022-2023 Evaluated in Knoxville, TN							
	Green Cover (%)**							
	0 Games		10 Games		20 Games		30 Games	
Entry I.D.	2022	2023	2022	2023	2022	2023	2022	2023
DALZ 1702	90.7***	95.3	80.3	76.9	71.0	64.6	40.2	37.9
DALZ 1701	97.2	94.9	88.4	76.5	81.5	64.2	36.4	34.2
DALZ 1808	96.9	90.4	79.9	71.9	69.9	59.6	34.4	32.2
DALZ 1818	98.0	94.8	82.3	76.4	72.9	64.1	42.5	40.3
6782-104	98.1	94.7	86.4	76.3	76.8	63.9	34.6	32.4
6782-120	96.9	91.5	82.1	73.1	72.7	60.7	42.4	40.2
6782-42	94.7	91.9	80.1	73.5	69.8	61.1	42.6	40.4
6782-75	97.9	91.8	83.4	73.3	72.8	61.0	41.3	39.1
6782-79	96.1	93.7	80.1	75.3	69.1	62.9	42.5	40.2
6783-03	95.6	91.6	82.0	73.2	71.6	60.8	43.4	41.2
6784-17	93.1	92.2	56.7	73.7	44.4	61.4	50.8	48.6
6785-19	96.5	95.5	81.2	77.1	70.5	64.7	41.8	39.6
6785-22	98.0	93.7	85.6	75.3	76.3	62.9	43.5	41.3
6786-02	97.3	94.3	81.2	75.9	70.5	63.5	45.1	36.2
6787-18	96.9	95.1	83.0	76.7	73.7	64.3	46.8	44.6
6787-20	97.1	96.3	85.2	77.9	76.2	65.5	50.4	48.2
6789-23	99.1	94.2	91.8	75.8	85.0	63.5	38.7	36.4
6789-40	97.0	95.7	78.2	77.2	66.4	64.9	48.3	46.1
6789-52	93.2	94.1	52.5	75.7	38.1	63.4	33.2	31.0
6791-06	97.8	95.0	83.6	76.5	73.1	64.2	36.4	34.2
6792-44	98.8	90.2	87.4	71.8	77.7	59.4	59.2	57.0
6828-27	93.5	93.5	73.3	75.1	61.7	62.7	26.5	24.3
6828-53	95.2	96.2	75.4	77.8	63.6	65.4	53.1	50.9
6828-56	94.2	95.7	69.4	77.3	57.2	64.9	40.9	38.6
6828-77	95.8	94.7	82.5	76.3	73.3	64.0	38.7	36.5
6829-02	98.4	96.0	89.1	77.6	81.8	65.2	42.0	39.8
6829-20	94.8	94.8	80.0	76.4	69.2	64.1	34.7	32.5
6829-34	97.3	94.0	84.8	75.5	75.6	63.2	41.7	39.5
6829-36	95.0	94.2	57.1	75.7	46.0	63.4	44.4	42.2
6829-69	99.0	94.3	85.8	75.8	77.4	63.5	33.5	31.3
6830-02	94.3	94.6	71.5	76.2	59.7	63.8	36.4	34.2
6830-11	94.4	93.5	72.8	75.0	61.9	62.7	38.8	36.6
6830-39	91.1	95.4	67.9	77.0	55.5	64.6	37.3	35.1
6830-56	94.7	94.6	80.5	76.1	71.8	63.8	47.7	45.5
6831-09	93.6	92.1	53.8	73.7	41.1	61.4	35.0	32.8
6835-33	87.8	93.6	48.1	75.2	35.0	62.8	44.8	42.5
6836-09	96.5	96.3	81.9	77.9	70.7	65.5	44.6	42.4
6839-08	89.3	91.4	61.6	73.0	50.5	60.6	39.9	37.6
6840-20	93.1	92.9	61.1	74.5	48.3	62.1	29.4	27.2
6844-04	89.9	94.2	74.7	75.8	64.3	63.4	31.1	28.8
6844-104	94.8	92.1	75.4	73.7	63.6	61.3	38.0	35.8
6844-128	91.8	94.5	64.8	76.1	53.5	63.7	41.3	39.0
6844-141	94.1	95.1	70.2	76.7	59.7	64.3	39.4	37.1
6844-147	96.2	94.9	69.8	76.5	59.5	64.1	37.7	35.4
6844-150	97.4	92.2	87.1	73.7	79.3	61.4	42.3	40.1
6844-152	84.8	93.0	64.8	74.6	54.0	62.2	35.3	33.0
6844-154	94.0	94.0	66.4	75.6	56.1	63.2	50.4	48.2
6844-190	86.3	96.2	68.1	77.8	56.4	65.4	49.3	47.0
6844-202	94.6	94.5	80.4	76.1	71.9	63.7	33.9	31.6
6844-31	92.6	95.0	70.9	76.6	59.2	64.2	51.5	49.3
6844-34	90.1	94.8	68.9	76.4	56.8	64.1	39.1	36.8
6844-36	92.4	95.5	73.2	77.1	62.8	64.7	37.2	35.0
6844-42	91.8	93.9	61.3	75.5	49.0	63.1	45.8	43.5
6844-53	97.3	94.3	82.6	75.9	73.0	63.5	48.3	46.1
6844-74	96.7	95.4	83.0	77.0	74.2	64.6	39.4	33.8
6844-89	91.6	90.5	40.2	72.1	27.9	59.7	31.1	28.8
6844-91	92.3	95.3	76.2	76.9	67.3	64.5	41.8	39.6
6910-157	96.0	95.2	78.3	76.8	68.8	64.4	44.5	42.2
6910-172	97.0	88.9	86.4	70.5	78.3	58.1	42.7	40.5
6919-29	91.6	93.5	78.2	75.1	69.0	62.7	42.9	40.7
6923-11	92.4	96.0	72.5	77.6	61.0	65.3	46.4	44.1
6924-44	92.8	94.0	47.1	75.6	35.6	63.2	34.7	32.5
6924-47	93.2	91.0	64.8	72.5	53.0	60.2	31.5	29.2
6924-66	90.6	91.8	57.0	73.4	43.1	61.1	46.5	44.2
6925-53	93.6	92.3	80.9	73.9	71.9	61.6	36.9	34.7
6933-11	96.8	94.1	82.3	75.7	72.2	63.3	33.5	31.3
6940-15	95.9	92.7	84.2	74.2	75.9	61.9	39.7	37.4
6941-36	98.4	93.1	93.9	74.7	89.9	62.3	52.5	50.2
6942-22	93.7	92.0	76.5	73.6	66.3	61.3	22.2	20.0
Emerald	95.6	87.5	71.4	69.1	59.1	56.7	28.4	26.2
Innovation	92.1	93.6	67.3	75.2	55.3	62.8	43.7	41.5
Meyer	95.3	90.4	78.1	72.0	67.7	59.6	45.1	42.9
Palisades	95.7	89.4	79.1	71.0	70.7	58.6	22.5	20.3
Zeon	96.7	94.5	74.8	76.1	63.5	63.7	42.5	40.3
Average	94.7	93.7	74.8	75.2	64.5	62.9	40.4	38.1

* Traffic tolerance was simulated with a 4-ft wide modified Cody Traffic Simulator. One pass with the trafficker is equivalent to 57 cleat marks per square foot. Five simulated traffic events were initiated in August of 2022 and 2023 and applied weekly, for a total of 30 total games each year.
** Pictures were taken after each 10 games and analyzed using Turf Analyzer Software to evaluate green cover.
*** Entries highlighted in green occured in the top statistical group within each column. Means across treatment replications (n=3) for traffic tolerance after 30 games in both years were insigificant (P > 0.05).

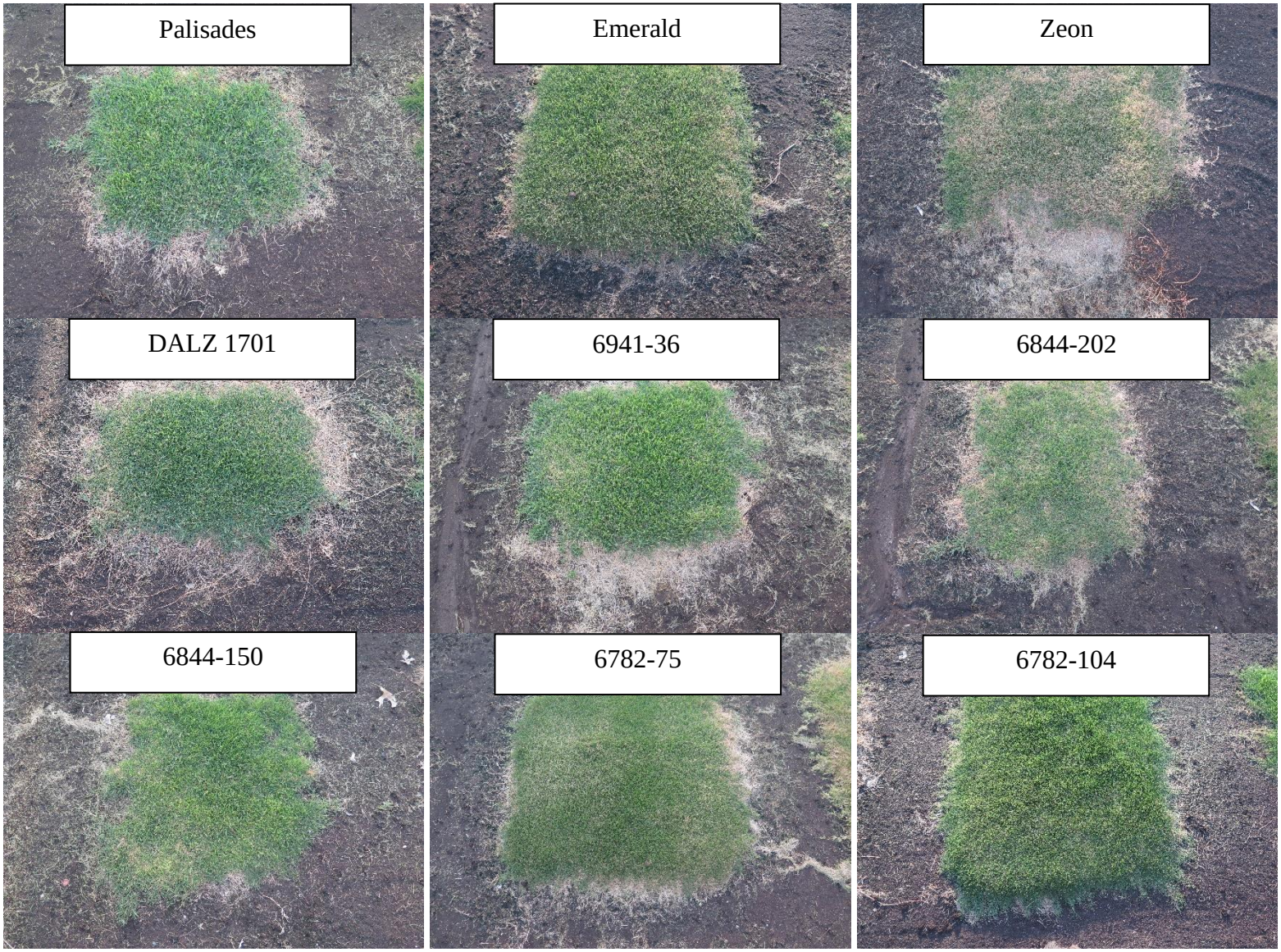


Figure 1. Turfgrass quality of some cultivars and elite lines photographed under 63% shade on August 24, 2023 (791 days after planting under shade) in Dallas, TX.

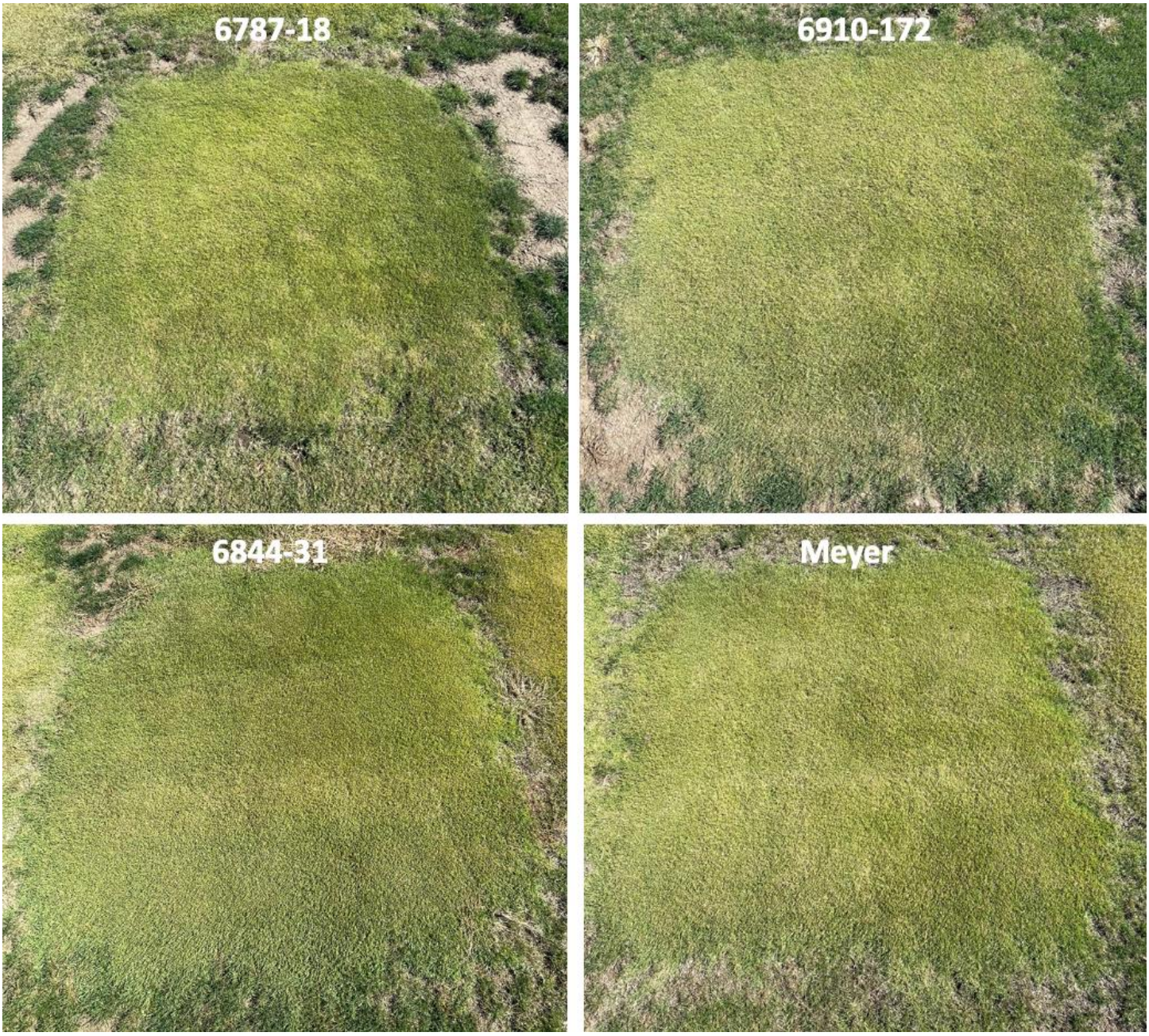


Figure 2. Experimental plots on October 16, 2023 in Olathe, KS.

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). In 2021, 10 by 10 ft plots were established by either sodding or plugging 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. In North Carolina sod from these blocks was harvested in August and planted in a golf course rough (chosen in consultation with Superintendent Brian Green) at Lonnie Poole. A total of five experimental lines (16-TZ-13685, 09-TZ-54-9, XZ 14070, and XZ 14070) and three commercial checks (Lobo, Zeon and Meyer) were plnated in 8 ft x 5 ft plots with 1.5 alleys in between (**Figure 1**). 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 evaluated for LP through atificial inoculations in growth chambers. Disease severity was evaluated every three days for 33 days visually using the Horsfall-Barratt scale and through digital imaging. Six runs of inoculations (with two reps each) were completed. Values for final severity, final digital image analysis percent incidence (DIAPI), and area under the disease progress curve (AUDPC) are presented here. High levels of variation for LP response were observed across runs of evaluation. Results were consistent across runs, especially for the best and worst performers. Resistant checks Diamond and PI 231146 were the top performers, but several of the experimental lines exhibited good levels of resistance, especially in comparison to checks Zeon and Meyer (**Figure 2**). To identify genomic regions controlling LP resistance, a high-density linkage map was constructed based on 2,556 SNPs. Using this Meyer × PI 231146 linkage map, 34 total significant QTL were identified across chromosomes 1, 2, 4, 5, 6, 7, 11, 12, 18, 19 and 20 (**Figure 3**). Across all runs, 13 of the QTL were identified for AUDPC, 11 for DIAPI, and 11 for severity. When looking at regions of overlap across traits, these QTL coalesced into 13 resulting regions: QTL 4.1, had shared association with all three traits; QTL 11.2 was associated with both AUDPC and DIAPI; QTL 19.1 shared association between AUDPC and Severity; QTL 1.1, 7.1, 11.1 and 18.1 were solely associated with AUDPC; QTL 1.2, 6.1, and 12.1 were specific to Severity; and QTL 2.1, 5.1, and 20.1 were specific to DIAPI (**Figure 3**). Specific QTL of interest included 4.1, 6.1, 11.2 and 12.1 due to not only the overlap across traits but also the high percentage of the phenotypic variance these regions explained. These final 13 QTL regions were searched to identify candidate genes associated with disease response. Annotation revealed gene functions relating to four distinct components of the plant pathogen interaction process: pathogen recognition either through the pattern-triggered immunity (PTI) or effector triggered immunity (ETI) response pathways, regulation of the Ca2+ hormonal pathway, ethylene (ET) and jasmonic acid (JA) pathway regulators, and pathogenesis-related (PR) proteins. These QTL appear to harbor strong candidate genes that could

later be implemented in marker-assisted selection for the development of LP resistant zoysiagrass cultivars

For objective 3, this population was planted at the W.H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, IN; the Lake Wheeler Turfgrass Field Lab, Raleigh NC; the Coastal Plain Experiment Station, Tifton, GA; and the Georgia Mountain Research and Education Center in Blairsville, GA to evaluate their performance under field conditions. Because of limitations in the availability of vegetative stock, plots were established from either 1-2 plugs in two replications in late June of 2020. That year, plots were evaluated for speed of establishment only. In 2021, plots were evaluated for speed of establishment, winterkill, turf quality, and end of year coverage at all locations, and for early spring green up, mid-spring green up, leaf texture, canopy density, and fall color in NC only. When looking at speed of establishment, several breeding lines significantly outperformed Palisades, the top performer among checks. For winter survival traits, Meyer was the top performer for winterkill but not for spring green-up. A few experimental lines were identified as having superior performance for both traits combined. When looking at aesthetics, several experimental lines showed promising performance across turf quality and fall color (**Figure 4**). Combined analysis through fall 2023 identified lines with superior performance across locations. These will be propagated over winter in order to produce materials for on-farm trials.

Additionally, 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 2021-2023. In North Carolina, 43 breeding lines with superior performance for lateral growth, drought tolerance, winter survival and turf quality were identified after three years of evaluation at the Lake Wheeler Turf Field lab (Raleigh), the Sandhills Research Station (Jackson Springs), and the Upper Mountain Research Station (Laurel Springs). These lines will be propagated over winter for establishment of replicated field trials in spring 2024.

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. Establishment of demo plots on rough area at Lonnie Poole golf course (Raleigh, NC).

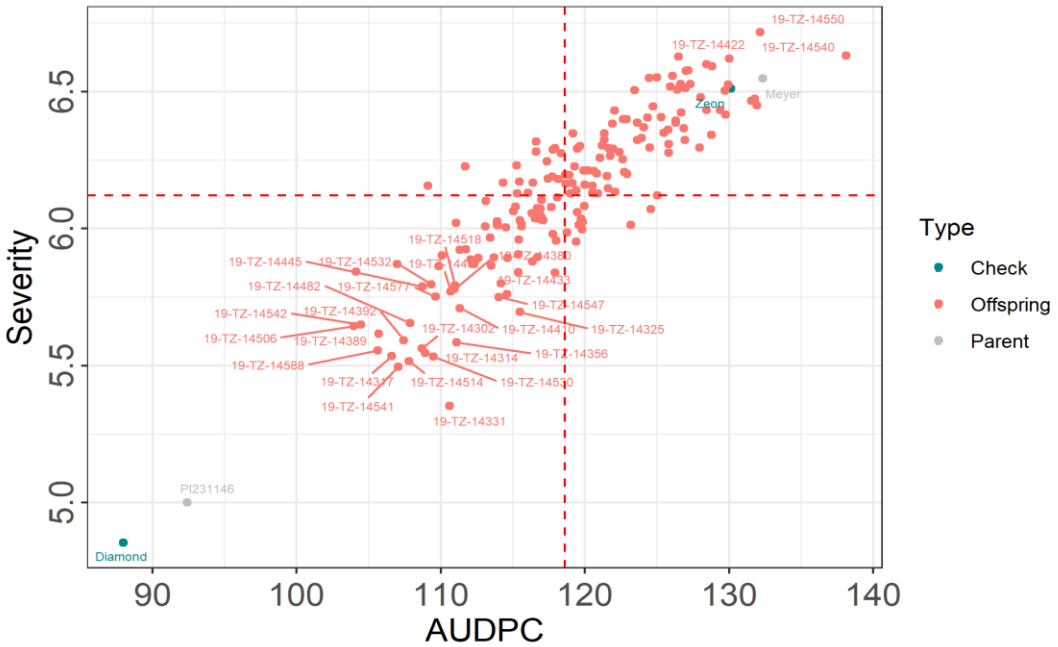


Figure 2. Means across runs for the Meyer x PI 231146 population evaluated for large patch (LP) response under controlled environmental conditions. Disease severity versus Area Under the Disease Progress Curve. The lower the value the more resistant the genotype

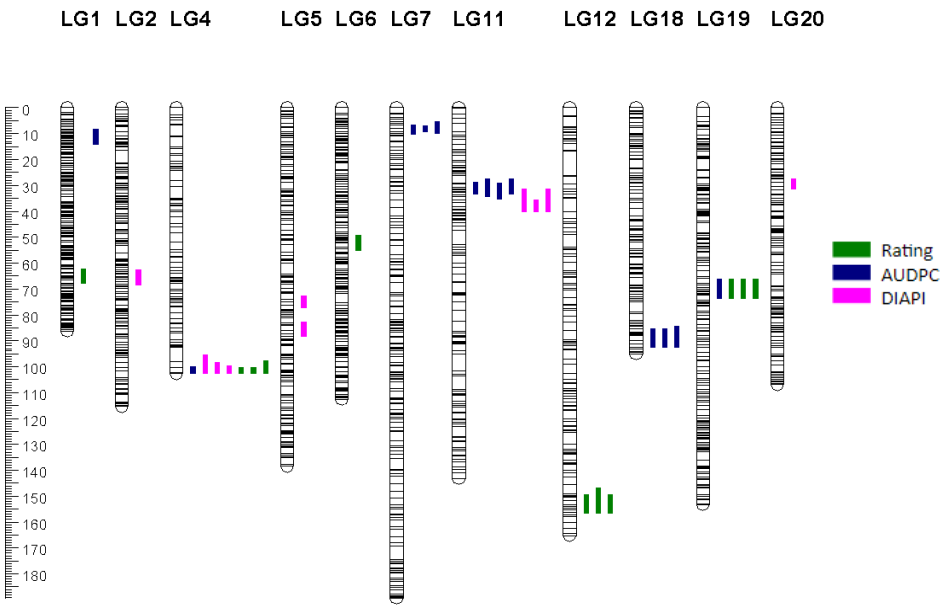


Figure 3: Chromosomal position of QTL associated with final disease severity (Rating, green), area under the disease progress curve (AUDPC, blue) and digital image analysis percent incidence (DIAPI, pink). Physical distances (kbp) were determined by aligning markers to the Zoysia japonica "Nagirizaki" reference genome.

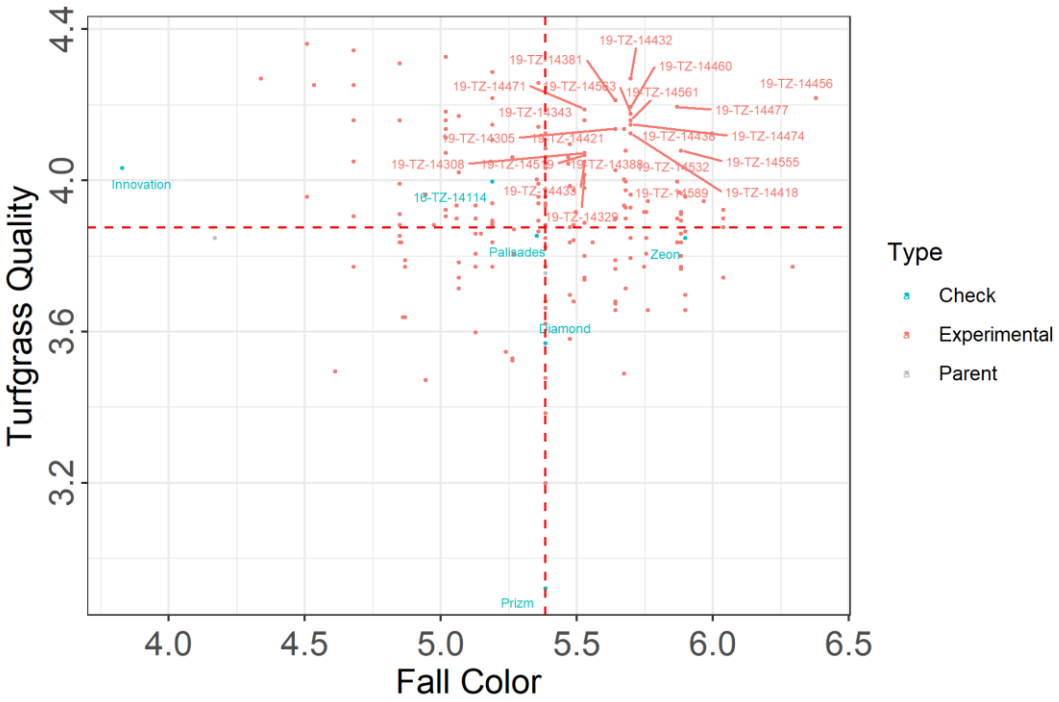


Figure 4. Mean performance for fall color versus turf quality. While turf quality was taken at both locations, fall color was evaluated at West Lafayette, IN only.

USGA ID#: 2021-04-728

Title: Buffalograss Breeding and Development
Project Leader: Keenan Amundsen
Affiliation: University of Nebraska-Lincoln

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:
- A late season high nitrogen fertility application increases seed yield and biomass in seed production fields.
- New breeding lines have exceptional sod and recovery characteristics
- Experimental seeded populations are high yielding and were selected for their abiotic and biotic stress tolerance

Summary Text: Buffalograss is an important turfgrass species because of its exceptional drought tolerance and high quality with minimal supplemental fertility and irrigation input requirements. Additionally, there are few pests that damage buffalograss within its primary growing region. Chinch bugs are the most damaging pest to buffalograss and only cause problems when numbers are high, and they are not managed. Chinch bug pressure was high throughout the region in 2023. In addition to managed turf stands, chinch bugs can negatively impact seed and sod production, reducing quality and yield (Figure 1). Since buffalograss is a



Figure 1. High chinch bug pressure in a buffalograss production field, causing significant damage. Chinch bugs are circled.

dioecious species, populations naturally segregate for gender, typically approaching a 1:1 ratio of females to males, reducing yield potential in seed production. Buffalograss yields are relatively low since buffalograss produces burs and not many burs are produced per plant. To maximize yield, experiments were initiated to test fertility applications that promote flowering and increased yields. A mature buffalograss stand (NE-BFG-07-01) was managed with 80 lbs of N/acre with supplemented applications of urea, ammonium suflate, monoammonium phosphate, and/or lime applied June 21st. Plots treated with ammonium sulfate and urea had a darker green color at the end of the season. Plots were harvested in late July and biomass and seed yield data was collected. Plots receiving an additional 60# N per acre yielded on average 62% more seed and 66% more biomass compared to non-treated control, plots receiving lime only or 200 lbs/acre monoammonium phosphate (Figure 2). Another added cost for seed production is the



Figure 2. Late season nitrogen applications increase seed yield and biomass. From left to right, in late June, the plots received 1) 60 lbs N/acre from urea, 200 lbs/acre 11-52-0, and 300lbs/acre pelletized lime; 2) control plots with no supplemental fertility; 3) 60 lbs N/acre from ammonium sulfate; 4) 60 lbs N/acre from urea; 5) 300 lbs/acre pelletized lime; and 6) 200 lbs/acre 11-52-0.

post-harvest potassium nitrate and stratification seed treatment to break seed dormancy. Fourteen advanced breeding populations derived from crossing blocks designed to fix shade tolerance, leaf spot resistance, false smut resistance, chinch bug tolerance, and stand persistence were allowed to intermate and advanced. All plots were established from seed that was not treated with potassium nitrate with a goal of reducing seed dormancy in subsequent populations. Top performing males and females for establishment rate from each population were also selected and advanced in separate crossing blocks to intermate, promoting rapid establishment in future populations.

For seeded buffalograss, establishment rate, impacted by seed dormancy and early seedling vigor, and seed yield are important factors for advancing buffalograss lines for production trial evaluations. Vegetative types are additionally rated for sod strength and recovery, impacted by the duration of the shock response following sod cutting and stolon production. Advanced breeding lines (n=24) were established in 2022 and evaluated for spring green-up, visual quality, sod strength (measured on June 6th and July 10th), green-up post sod cutting (13 days post cutting), and recovery (20 days post cutting) in 2023. Commercial cultivars 'Prestige' and 'Legacy' were used as standard check plots. Compared to the standard entries, 7 lines performed better for visual quality, 3 for spring green-up, 2 for green-up post sod cutting, 20 better than 'Prestige' for sod strength (1 was better than 'Legacy'), and 7 lines performed as well as the industry checks for recovery (Figure 3). Many of the new buffalograss breeding lines held up well after cutting, another important



Figure 3. Sod strength a recovery evaluated on 24 advanced buffalograss breeding lines. A single plot is marked and the image was taken 43 days after the first sod cutting (right strip in plot) and 9 days after the second sod cut center strip in plot). Early and late recovery is observed.

trait for sod production (Figure 4).



Figure 4. A strip of sod taken from an experimental buffalograss line with exception quality and sod strength.

In the coming evaluation year, we have plans to continue our plant breeding pipeline to advance breeding populations while also selecting material that can be economically produced while maintaining exceptional turfgrass quality. Our advanced lines will be increased to produce enough seed or vegetative material to support regional evaluation and production trials and data collection for PVP and patent applications as appropriate.

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 years
Total Funding: $44,080

Summary Points:
- This project is addressing a need for seeded diploid buffalograss lines
- Diploid buffalograss has an extended growing season compared to hexaploid buffalograss
- Seeded diploid buffalograss is ideally suited for drought-impacted areas without a hard frost

Summary Text: Buffalograss is a mixed ploidy species with diploids, tetraploids, and hexaploids common throughout the U.S. Great Plains region. It is a warm-season species and survives winter in northern regions through a dormancy mechanism. In general, the winter dormancy response is stronger in higher ploidy buffalograss lines and diploids are considered to have a weak response. Buffalograss with a strong dormancy response will start entering dormancy following the first hard frost and not break dormancy until soil temperatures warm in the spring. While dormant, buffalograss turns straw colored but does not require management inputs. There is a tradeoff with the low management input requirements to maintain a quality buffalograss stand and the reduced quality while dormant in the early spring and late fall. Diploid lines do not have a strong dormancy response, maintain their green color later into the fall, and are ideally suited for turf applications in regions that do not experience harsh winters. Currently the only commercially available buffalograss diploids are vegetative types. Buffalograss is sold as vegetative plugs or sod and seed. There are advantages to both types (vegetative or seeded types) depending on the turf application and expectations. Buffalograss is a dioecious species and male buffalograss flowers develop on a relatively long culm, extending above the turf canopy, while female flowers develop on shorter culms often hidden by the turf canopy. Clonal vegetative female lines would avoid male flower expression and contribute to reduced mowing frequency. Seeded lines are more genetically diverse contributing to better abiotic and biotic stress tolerance.

One of the goals of this project is to develop seeded diploid buffalograss lines (Figure 1) and develop genomic resources for understanding differences in dormancy mechanisms among buffalograss with different ploidy levels. A preliminary study was designed to compare changes in gene expression in diploids and higher ploidy lines when exposed to low, non-freezing

temperatures. Plants were placed in a growth chamber maintained at 4°C. The plants were monitored for onset of dormancy and at which time, tissue would have been collected for an RNA-seq study. However, even after 8 wks at the low temperature treatment, the plants did not enter dormancy, so the experiment was terminated. The results suggest that a freezing event such as a hard frost is necessary to induce dormancy. Experiments are underway to determine optimal conditions to induce dormancy, supporting further experiments to identify genes important for the trait that can be used as breeding targets.



Figure 1. A high-yielding, fine textured diploid buffalograss line.

In addition to its winter dormancy response and compared to other turfgrass species, buffalograss has minimal water requirements making it ideally suited to regions that experience severe and/or prolonged drought. In drought affected areas or for turfgrass managers that want to reduce irrigation inputs, and in areas that do not have a cold enough winter to induce dormancy, diploid buffalograss is an apt species. Our goal is to develop diploid buffalograss populations to serve those regions and to offer seeded diploids which are not currently available. We have been developing distinct diploid populations through several rounds of selection for yield and turfgrass quality. Diploid buffalograss has finer leaf texture and smaller bur size, which complicates seed production. Two of the best performing populations yielded

16.1 and 18.2 lbs of seed per 500 sq. ft. during the 2022 growing season (Figure 2). That amount of seed exceeds expectations and allows for establishment of large breeder blocks that will produce enough seed useful for regional evaluation and yield trials, anticipated in 2025.



Figure 2. Isolated seed increase plot of an elite diploid buffalograss population.

USGA ID#: 2023-11-778

Title: New discoveries and methods for complex data analysis

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

Objectives: The objectives of the project are to 1) use existing genomic and transcriptomic data to identify elements with variable responses to different conditions; 2) develop and publish workflows for genomic, transcriptomic, and fine resolution computational biology methods; and 3) test and validate the methods on other published data from turfgrass species; and 4) train a student with interest in the turfgrass industry to become a leading expert in computational biology methodologies

Start Date: 2023
Project Duration: 3 years
Total Funding: $153,970

Summary Points:
- DNA sequencing technologies are readily available to study turfgrass genomes, but tools for analyzing the complex data are still needed.
- GeneCNN was developed for gene prediction in complex sequenced genomes.
- GeneCNN successfully predicted 3,015 coding sequences within a 10 million nucleotide buffalograss genome fragment
- GeneCNN performed as well as conventional gene prediction tools

Summary Text: Turfgrasses are fascinating because of their uniform appearance coupled with their complex underlying diversity. The amount of diversity complicates genetic and genomic studies. There has been a significant increase in the amount of molecular genetic and genomic data available to study turfgrasses, particularly as costs of modern DNA sequencing technologies lower and the technology becoming more accessible to turfgrass genetics researchers. An industry goal is to develop sequenced reference turfgrass genomes for the many species used by the industry. Reference genomes are useful for identifying structural variation, developing large numbers of molecular markers, characterizing important genes, supporting genomic selection and other modern breeding methodologies, and many other applications. With increased adoption of sequencing technologies comes large amounts of data that needs processing. It is tempting to use off-the-shelf tools for analyzing the data, accessible to researchers without bioinformatics training, but many important discoveries are overlooked. As an example, commonly used gene prediction tools, a key step in genome sequencing, use Hidden Markov Models (HMM), which are probabilistic models that can predict outcomes based on observable processes with unobservable states. The Markov process assumes independence which may be violated in gene prediction, since gene location and structure are influenced by several factors. The models are robust and work well, but there may also be a

better approach. Graduate student Michael Morikone, supported by this project, recently developed GeneCNN, a tool for coding sequence prediction in newly sequenced genomes built on convolutional neural networks (CNN). A CNN is a machine learning algorithm, useful for deep learning, often used in image analysis. In collaboration with Dovetail Genomics (Scotts Valley, CA) and Dr. Dave Huff at Penn State University, a diploid buffalograss genome was sequenced and assembled. A comparison of GeneCNN to other gene prediction tools was done on a 10 million nucleotide fragment from the largest buffalograss scaffold. GeneCNN predicted 3,015 coding sequences, BRAKER3 predicted 2,068 genes, AUGUSTUS predicted 1,865 genes, and Fgenesh predicted 2,303 genes within the 10 million nucleotide sequence fragment. Table 1 shows the number of coding sequences uniquely predicted by GeneCNN that were not represented by the other tools when pairwise comparisons were made. The unique sequences were used to search the non-redundant nucleotide collection of sequences housed by the National Center for Biotechnology Information. A minimum of 77.8% of the GeneCNN unique coding sequences were present in the nr database. The GeneCNN predicted coding sequences in common with other tools are summarized in Table 2. The number of predicted coding sequences by the other tools in common with the 3,015 predicted coding sequences by GeneCNN ranged from 1,220 to 2,537. Together these data suggest that this first-generation tool is successfully predicting coding sequences and is useful for gene prediction in newly sequenced genomes lacking a reference. Gene start and stop sites along with intron and exon boundaries will be important to include in future evolutions of GeneCNN. Michael Morikone's project addressed objectives 2-4 and I am pleased to report that he successfully defended his PhD in November, 2023. Over the next year our lab will begin comparing the multiple published data sets to identify data signatures associated with certain conditional responses of turfgrasses to support future turfgrass cultivar and germplasm development efforts.

Table 1. GeneCNN predicted coding sequences not found by other gene prediction tools.

Tool compared to GeneCNN	GeneCNN uniquely predicted genes	Unique in database	Unique not in database
AUGUSTUS	1,535	1,233 (80.3%)	302 (19.7%)
BRAKER3	1,089	895 (82.2%)	194 (17.8%)
Fgenesh	478	372 (77.8%)	106 (22.2%)

Number of coding sequences predicted by GeneCNN (n=3,015) that were not predicted by AUGUSTUS, BRAKER3, or Fgenesh. GeneCNN unique sequences were used to search the NCBI nr database.

Table 2. GeneCNN predicted coding sequences (n=3,015) supported by other gene prediction tools

Gene prediction tool and combinations	Number of predicted genes in common with GeneCNN
AUGUSTUS	1480
BRAKER3	1940
Fgenesh	2537
AUGUSTUS, BRAKER3	1249
AUGUSTUS, Fgenesh	1434
BRAKER3, Fgenesh	1786
AUGUSTUS, BRAKER3, Fgenesh	1220

USGA ID#: 2023-02-769

Title: Evaluation of Saltgrass for Turfgrass Characteristics under Drought and Heat Stresses

Project Leader: Desalegn D. Serba
Affiliation: USDA-ARS, Maricopa, AZ

Objectives:

1. To characterize desert saltgrass (*Distichlis spicata*) accessions under different level of deficit irrigation in Maricopa, AZ field conditions.
2. To identify the most drought-and heat tolerant saltgrass genotypes with good turfgrass characteristics for use as a turfgrass in a drought/salinity stressed landscapes,
3. To identify desirable traits and reproductive characteristics for complementing desirable traits in a genotypic background through hybridization.

Start Date: 5/1/2023
Project Duration: 3 years
Total Funding: $40,866.67

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

- Field established a total of 78 saltgrass accessions in a Split-Plot design with two replication.
- Deficit irrigation treatment of 80, 60, and 40 % ET replacement will start on May 1st, 2024.
- Evaluation for color, quality, and stress tolerance will continue for the next two years using visual assessment and proximal sensing devices that collect spectral and image data.

Summary Text:

Rationale and Methodology

Recent megadroughts and a substantial spike in temperature in southwestern USA have strained the availability of fresh water for irrigation. This consequential situation is attributable to natural and anthropogenic climate change and mandates water conservation through reduced landscape irrigation requirements. Development and adoption of drought-tolerant turfgrass varieties that can survive under harsh environmental stresses such as native grasses with low-maintenance requirements and are adaptable to the desert southwest will provide significant benefits in irrigation water conservation by golf courses, sport fields, and home lawns. Saltgrass, Distichlis spicata (L.), is a euhalophyte native grass of the arid and semi-arid regions that has shown to tolerate full strength seawater level soil salinity and dry conditions that exclude other plants. Use of saltgrass on golf course fairways and roughs as well as sport fields could help conserve significant amount of potable water while maintaining attractive turf with excellent playing conditions. Roughs and fairways constitute the larger proportion of the golf courses. Adapted

and evergreen native grasses with low maintenance requirement such as inland saltgrass will be an ideal product to conserve water resources and offset significant expenses for golf courses. This project is aimed at identifying drought and heat tolerant saltgrass genotypes with good aesthetic value. A total of 78 saltgrass accessions (62 accessions obtained through Material Transfer Research Agreement with USGA and 16 accessions obtained from Germplasm Resource Information Network (USDA-GRIN)) were transplanted into the field in Maricopa on August 30, 2023. A bermudagrass and a zoysiagrass commercial cultivars were included as checks. The experiment was laid out in Split-Plot design with two replications. Necessary agronomic practices are being conducted for the establishment of the plots. Three irrigation levels (80, 60, and 40 % evapotranspiration (ET) replacement) are assigned to the main plots and the genotypes were assigned to the sub-plots. Four plug were planted at a square 0.4 m apart in center of $2.25 m^{-2}$ (1.5 m x 1.5 m) sub-plot size. In this first year, all the plots will be established under 80% ET replacement irrigation. Rating for greening up will start March 1st, 2024. The deficit irrigation treatment will start May 1st, 2024 and the plots will be evaluated on a bi-weekly basis for color, quality, and stress tolerance for the next two years using visual assessment and proximal sensing devices that collect spectral and image data.

Results and Future Expectations

It is expected that significant genetic variation exists among the genotypes with respect to establishment rate, aesthetic value, and in response to deficit irrigation. It is also expected that significant genotype-by-irrigation treatment interaction that complicate the selection will be encountered. This project is expected to identify drought and heat tolerant genotypes with good turfgrass characteristic for use as a turfgrass in a drought/salinity stressed landscapes and other desirable traits.

USGA ID: 2021-13-737

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

Project Leader(s): Brian Schwartz

Affiliation: University of Georgia

Objectives:

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

Start Date: 2021

Project Duration: 3 years (2021 – 2023)

Total Funding: $28,500

Summary Points:

1. 12-TG-101, an interspecific triploid hybrid bermudagrass (*Cynodon transvaalensis* × *C. dactylon*), was released from the University of Georgia's College of Agricultural & Environmental Sciences during the fall of 2021 and named 'Tif3D'.
2. 'Tif3D' generally has superior vigor to 'TifEagle' and 'Mach 1' during, or immediately following, periods of stress and is darker green than other ultradwarf bermudagrasses.
3. 'Tif3D' was planted at (9) golf course evaluation sites over the course of 2021, 2022, and 2023 in Florida, Georgia, and Louisianna within the scope of this research. Observations on establishment at different sprig rates, regional adaptation under low (0.125") to very low (0.075") mowing, and disease incidence were made.

Summary Text:

'Tif3D' was selected from Taylor's Creek Golf Course at Fort Stewart in Georgia, home of the U.S. Army's 3rd Infantry Division and has been compared to 'Tifgreen', 'Tifdwarf', 'Champion', 'MiniVerde', and 'TifEagle' on the University of Georgia Tifton Campus since 2012. It is generally darker green and more uniform than these cultivars during, or immediately following, many environmental (drought, foliar disease, and temperature fluctuations during the spring and fall) or mechanical (mower scalping, verticutting, and hollow-core aeration) stresses. 'Tif3D' was highly adapted to the lower-intensity management conditions of the research trials in Tifton. Therefore, we further tested it at on-site golf course research putting green trials beginning in 2015, largely against 'TifEagle' and 'Mach 1'. Over years and locations, 'Tif3D' consistently outperformed these cultivars with its dense surface, eventually leading to its release at the request of several golf course superintendents.

Table 1. Summary of USGA supported putting green trials planted at golf courses in Florida, Georgia, Louisiana, and South Carolina from 2021 – 2023.

Golf Course	Year Planted	Location	Golf Course Superintendent
UGA Golf Course	2021	Athens, Georgia	Scott Griffith
Palmetto Golf Club	2021	Aiken, South Carolina	Mark Swygert
Augusta National Golf Course	2021	Augusta, Georgia	Brent Seyer
TPC Sawgrass	2021	Ponte Vedra Beach, Florida	Jeff Plotts
Everglades Club	2022	Palm Beach, Floria	Brian Birney
Pine Tree Golf Club	2022	Boynton Beach, Florida	Joe Pantaleo
PGA Golf Club	2023	Port St. Lucie, Florida	Christopher Sykes
Belleair Country Club	2023	Belleair, Florida	Andy Neiswender
Southern Trace Country Club	2023	Shreveport, Louisiana	Graham Kornmeyer

2021

UGA Golf Course – This over 20,000 ft^2 test area of 'Tif3D' is used as a putting and chipping green for the UGA golf team. An estimated 80+ GA bushels per 1000 ft^2 planting rate was used, which is over twice the normal rate. Sprigs were not cut-in and rolled to maintain the shape of the final surface grade. During this grow-in, we compared normal irrigation against a misting irrigation that ran more frequently. After six weeks we decided there was not an establishment advantage where the sprigs were being misted and that the nozzles were in the way of other agronomic practices such as fertilizing, rolling, and mowing. This putting green established nicely, but we took note of an immediate organic layer forming, likely due to the heavy sprigging rate. In November of 2022 we began to notice dark spots scattered throughout the whole green. This was alarming, so we took many plugs from dark and light areas, grew them out in the greenhouse, and also performed tissue nutrient and genetic tests. After a few weeks in the greenhouse, the plugs equilibrated and differences were no longer obvious. DNA fingerprinting was negative, but 3x levels of Mn were found in the tissue of the dark green plants. The spots faded during the winter and have not been observed since. This green has many swells and can be challenging to mow. We have observed grain on the slopes on this green, and where mower patterns are hard to rotate. Scott keeps this green at speeds between 11 and 13 on the stimp meter for the golf team and continues to watch it in-case UGA decides to renovate the bentgrass greens on his golf course.



Palmetto Golf Club – ‘Tif3D’, ‘TifEagle’, and ‘Mach 1’ were planted on the “Pro” practice green, and there were enough ‘Tif3D’ sprigs leftover to plant an approach for chipping. This green has shade pressure, and ‘TifEagle’ and ‘Mach 1’ lost canopy density. ‘Tif3D’ performed well on the green and several of the local Pros commented that the approach played well. Mark renovated his greens in 2023 and commented that he would have seriously consider ‘Tif3D’ had it been available.

Augusta National Golf Course – There may, or may not, be a putting green trial at this location.

TPC Sawgrass – A 5,000 ft^2 ‘Tif3D’ green was established using a ~120 GA bushels per 1000 ft^2 planting rate without cutting-in to determine if faster establishment was possible for renovations in environments like on the 17^{th} hole. Interestingly, ‘Tif3D’ established in a “normal” 7 to 8 weeks, but almost immediately afterwards it was overseeded with Jeff’s “secret blend” for the winter. Similarly to the UGA Golf Course test green, a layer formed and rooting has been stunted on this green, which is in direct contrast to the results of the 2017 TPC Sawgrass putting green trials where the performance of 12-TG-101 was impressive. My takeaway from this trial can be summed up from a discussion Jeff and I had where he pointed out “Son, I’m glad your grass is doing great at Olde Florida, but I need it to perform here for me!” I agree with him wholeheartedly, and think this statement sums up the impact this USGA research project has had on our breeding program.



2022

Everglades Club – A low-input, 'Tif3D' test green was sprigged at a normal, 40 GA bushels per 1000 ft^2 planting rate without cutting-in next to an older 'TifEagle' nursery on the driving range. It is mowed regularly at greens height with thatch reducing maintenance as time allows. 'Tif3D' is performing well under these conditions. Brian plans to renovate soon, and has concluded that 'Tif3D' is as least as good as 'TifEagle', but that a greens renovation will make or break his career. I respect his honesty when it comes to taking a chance on a new putting greens cultivar.

Pine Tree Golf Club - Joe Pantaleo has trialed many grasses over the years and agreed to grow-in a putting green of 'Tif3D' when he sprigged his new 'TifEagle nursery. This green established quickly in 2022, but was under drought stress during 2023 during the dry season. In this trial, 'Tif3D' and 'TifEagle' have performed similarly when stressed, although the dark green color of 'Tif3D' was apparent.

2023

PGA Golf Club – A practice green of 'Tif3D' in Port St. Lucie, FL is heavily used for chipping and putting. It was established at a standard sprigging rate of 40 GA bushels per 1000 ft^2, with a slow-release fertility program consisting of one application at planting intended to span the grow-in. After week 4, a quick release source of nitrogen and other elements were applied weekly to supplement the grow-in. The 'Tif3D' responded and was established by week 9. This green has 'Trinity' zoysiagrass collars that create a striking color contrast. 'Tif3D' has performed very well once transitioned to a standard maintenance program. Chris has recently applied Fusilade + Recognition to control 'Tif3D' encroachment into the 'Trinity' with success.



Belleair Country Club – In 2023, we provide 'Tif3D' and 'TifEagle' sprigs to plant a practice green that is shaded in the morning. 'Tif3D established faster on this putting green, and has shown resilience to scalping and dry conditions. The environment on this coastal test site is taxing and will include water quality issues and seashore paspalum encroachment.



Southern Trace Country Club - We planted a 5,000 ft^2 practice green of 'Tif3D' in the late summer of 2023. This green grew-in and was playable by week 9, but shortly after, cold weather moved in for the winter. Graham noted that 'Tif3D' held green color longer than an older 'Mach 1' chipping green nearby. This spring he updated us that there was significant winter damage on the golf course, but that the 'Tif3D' had no winter kill and was greening-up.

Ms. Makayla Riley is currently pursuing a B.S. in Environmental Horticulture on the Turf & Environmental track. Makayla has taken ownership of several zoysiagrass greenhouse trials and has been instrumental in propagating plant materials for the USGA sponsored research trials, as well as for those planted at the UGA Tifton Campus.


ABAC
16-T-4

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:

- In fall 2022 and spring 2023, eighteen total entries, encompassing creeping and colonial bentgrass, hard fescue, chewings fescue, strong creeping red fescue and perennial ryegrass were established at five sites, two under rainout shelters and three in field sites with deficit irrigation (ET_0) levels
- A minimum percent green cover threshold 75% was utilized during the drought 'season' to determine re-watering at the rainout shelter locations, thus allowing for a water needs rate for each entry
- Three deficit irrigation levels, representing high (optimum - 80% ET_o), medium (sub-optimum - 65% ET_o) and low (severe restriction - 50% ET_o) were instituted at the three deficit ET_0 sites
- Due to slow establishment, data was collected and submitted from three of the five locations in 2023
- The top bentgrasses required 22-31% less water than the highest water using bentgrasses, and 33-56% less water than the fine fescue entries under the rainout shelter in West Lafayette, IN.
- Under both the medium (65% ET_o) and low (50% ET_o) irrigation replacement levels at St. Paul, MN, several bentgrass entries had the highest quality, while at Ft. Collins, CO, several fine fescue entries outperformed most bentgrass entries under medium and low irrigation levels.

Summary Text:

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 of Kentucky bluegrass and tall fescue for water use and drought tolerance. Thirty-five total entries 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, it was time 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 shelters, 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, but only received twelve paid entries. Added to the trial were six standard (unpaid) entries for a final total of eighteen entries. Entry fees paid by sponsors are utilized to cover most costs, therefore, we had to eliminate one trial location. Thus, 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.

Trial establishment was in fall 2022/spring 2023, with data collection for three years. Trial parameters were 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 are collected from each rainout shelter site. Data from the deficit ET_o sites 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.

Water use calculations start within the rainout shelters in either summer 2023 or 2024, depending on trial maturity. To initiate the drought 'season', irrigation is 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 determines which plots have reached the 75% green cover re-watering 'threshold', the minimum acceptable percent green cover 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.

Deficit irrigation sites calculate and replace water lost based on daily evapotranspiration (ET) rates. Three irrigation regimes based on replacement ET_o: 80, 65 and 50% are utilized at each site, 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.

Data was collected and submitted in 2023 from St. Paul, MN, Ft. Collins, CO (ET_0 sites) and West Lafayette, IN (shelter site). Since establishment was not sufficient by the summer of 2023, no data was submitted by Logan, UT (ET_0) and Amherst, MA (shelter) sites.

Under the rainout shelter at West Lafayette, IN, several of the bentgrass, and the two perennial ryegrass entries required less water than all the fine fescues to maintain 75% green cover throughout the drought 'season'. The creeping bentgrass entries with the highest quality and least water used include 'Piranha' (135.3 mm, 5.3 inches), '777' (152.7 mm, 6.0 inches), 'AU Victory' (156.7 mm, 6.1 inches), 'Oakley' (156.7 mm, 6.1 inches) and 'T-1' (161.0 mm, 6.3 inches). The colonial bentgrass cultivar 'Musket' and perennial

ryegrass ‘SR 4650’ also delivered high quality with less water than most other entries: 152.3 mm (6.0 inches) and 148.3 mm (5.8 inches), respectively. The highest water using entries, ‘PPG FRC 127’ and ‘Compass II’ chewings fescue, and ‘Navigator II’ strong creeping red fescue needed 232 - 305 mm (9.3 - 12 inches) of water to maintain 75% green cover. Despite the differences in water use in 2023, almost all the bentgrass entries performed statistically equal, and only ‘Piranha’ demonstrated a significant *statistical* difference in water use over all fine fescue entries.

In the first year of reduced irrigation at St. Paul, MN, the bentgrass entries, overall, provided the highest quality. Several entries delivered a mean turfgrass quality rating of 7.0 or higher, which is the NTEP threshold for higher quality turf. Under both the medium (65% ET_o) and low (50% ET_o) irrigation replacement levels, ‘007XL’, ‘MacDonald’, ‘DLF-AP-3084’, ‘777’, ‘Oakley’ and ‘Piper’ creeping bentgrasses finished in the top statistical group. Entries with the poorest performance under all three replacement levels include ‘Navigator II’ strong creeping red fescue, ‘Helios’ and ‘SR 4650’ perennial ryegrass and ‘Penncross’ creeping bentgrass.

Finally, in Ft. Collins, CO, data revealed several of the fine fescues outperforming many of the bentgrasses, and in particular, the best performing entries from St. Paul and West Lafayette. ‘PPG FRC 127’ chewings fescue, ‘Navigator II’ strong creeping red fescue and ‘DLF-AP-3084’ creeping bentgrass scored highest under 50% ET_o irrigation replacement, with ‘Gladiator’ hard fescue, ‘Helios’ perennial ryegrass, and ‘Oakley’, ‘Penncross’ and ‘AU Victory’ creeping bentgrass finishing in the top turf quality statistical group. In turf quality ratings collected under 65% ET_o irrigation replacement, ‘Navigator II’, ‘Compass II’, ‘PPG FRC 127’ and ‘SR 4650’ were the only entries finishing in the top statistical group. In all the turf quality data, even under optimum irrigation replacement (80% ET_o), very few 7.0+ ratings were collected.

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.



Figure 1. Plots at Purdue University on 6.1.23 at initiation of drought (top) and on 7.16.23 after 45 days of drought (bottom).

USGA ID#: 2022-14-757

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

Project Leader: Dr Emily Holm

Affiliation: Michigan State University, Plant Soil and Microbial Science Department

Objectives: the objective of this study was to evaluate the efficacy of modern and novel-to-turfgrass high-throughput growth chambers equipped with DEPI sensors to real-time track turfgrass photosynthetic and growth responses to drought stress in various cool-season turfgrass species.

Start Date: January 1, 2022 – June 30, 2023 (no cost extension added)

Project Duration: 1 year

Total Funding: $29,648

Summary Points:

- Kentucky bluegrass and perennial ryegrass contrast in drought resistance and chlorophyll fluorescence parameters reveal novel insight into resistance mechanisms in each species.
- Plant non-photochemical protection strategies were activated by drought stress in both grass species.
- Measurements of non-photochemical quenching parameters indicated drought responses earlier than quantum efficiency.
- Non-photochemical parameters are good, early indicators of drought stress incidence in grass species.

Summary Text: Provide 500 to 1200 words briefly outlining rational, methodology, results to date, and future expectations of the project.

Kentucky bluegrass (*Poa pratensis L.;* drought resistant) and perennial ryegrass (*Lolium perenne L.*; drought sensitive) are economically important grass species contrasting in drought stress resistance. This study aimed to determine the optimal chlorophyll fluorescence parameters to indicate drought incidence and whether the parameters differentiate intraspecies and interspecies variation in drought stress. For each species, nine cultivars were exposed to well-watered or drought (water withheld) conditions in growth chambers containing high throughput photosynthetic imagers to track real-time responses of maximal quantum efficiency of photosystem II (F_v/F_m), quantum yield of photosystem II (Φ_{II}), non-photochemical quenching (NPQ), energy dependent quenching (qE), and photoinhibition quenching (qI; Cruz et al., 2016). The experiment was repeated for a total of two experiments. Soil water content (SWC) was evaluated with time domain reflectometry and relative water content (RWC) of leaf tissues were evaluated using a standard fresh, turgid, and dry weight method (Barrs and Weatherley, 1962).

Prior to drought treatment (day 0), soil water content was equivalent in all pots. Due to drought treatment, leaf RWC decreased in all turfgrass cultivars, but the rate of decrease varied among the cultivars. On day 4 after drought treatment, three perennial ryegrass cultivars, ‘Process’, ‘Panthergills’, and ‘Linedrive’ had higher RWC ($P < 0.05$; 51– 84.3%) compared to the other perennial ryegrass cultivars (averaging 41.8%) among which ‘Furlong’ and ‘Palmer’ had the lowest values (Figure 1). On day 8, there was no significant difference among cultivars. Among the Kentucky bluegrass cultivars, ‘Finishline’, ‘Jersey’, ‘Bordeaux’ and ‘Rockstar’ had significantly higher RWC (54–78%) than other cultivars (averaging 31.2%) among which ‘ARC’, ‘Diva’, and ‘Baron’ had the lowest values at day 4. On day 8, ‘Jersey’ (28.25%) and ‘Finishline’ (28.4%) had a higher RWC than other cultivars (averaging

16.8%). Overall, major differences in RWC between cultivars were found on day 4 which represents mid-term drought stress.

Photochemical efficiency indicators such as Φ_{II} and photoprotection-associated parameters (NPQ, qE, qI) have been underexplored in turfgrass species compared to F_v/F_m and are potentially effective traits to reveal stress incidence. Measurements of F_v/F_m often does not exhibit differences in response to genotype or environmental influences until later stages of drought (Wang et al., 2003) and requires dark adaption for measurement. The NPQ parameters can have a more dynamic response to stress conditions than F_v/F_m (Mutka and Bart, 2015). Drought stress influenced all photosynthetic health parameters that were measured (Figures 2 and 3). In this study, F_v/F_m values decreased below control levels due to drought stress starting around 90 hr in perennial ryegrass and 140 hr in Kentucky bluegrass. Among perennial ryegrass cultivars, 'Evening Shade', 'Process', and 'Panthergills' had higher values compared to 'Furlong' and 'Palmer' at most time points. Kentucky bluegrass 'Jersey', 'Champaign', 'Bordeaux', and 'Finish Line' had higher F_v/F_m values than 'ARC', 'Baron', and 'Diva' at most time points. NPQ parameters which itself is measured, and includes qE and qI as components, were more dynamic and responsive to drought stress than F_v/F_m.

Real time tracking of NPQ associated parameters allowed for differentiation of drought stages which we define as early drought, mid-drought and late drought. These stages occurred at different times in each species. We found early drought to occur up to 48 hr, mid-drought as 72–120 hr, and late drought as 148–240 hr in perennial ryegrass. In Kentucky bluegrass, early drought is 96 hr, mid-drought 100–180 hr, and late drought stress 190–240 hr. Varietal differences within a species were also clearly observed using these parameters. The more drought sensitive cultivars of Kentucky bluegrass (based on early loss of RWC, 'ARC', 'Baron', and 'Diva') exhibited an upregulation of NPQ mechanisms (NPQ, qE, and qI) during day 7 (about 150 hr) followed by lowest values of NPQ towards the end of the experiment (Fig. 2). A loss of photoinhibition-related energy dissipation (qI) indicates more extensive metabolic damage to photosystem II compared to plants able to maintain higher levels of qI during severe stress. Drought-resistant cultivars or species have delayed response to drought stress with higher values of photoprotection traits, especially NPQ. Photoprotection in relation to soil and plant water status observed in this study is important for *early detection of drought or other abiotic or biotic stress* in turfgrass fields and should be more widely used to study stress resistance mechanisms in turfgrass and other grass species. Field research evaluating these parameters and determinations of nonphotochemical protection strategies in other turfgrass species is warranted. These results indicate important stress tolerance protection mechanisms for grass species and will broadly impact basic and applied grass research as a non-destructive phenotyping tool.

Publication statement – These results have been accepted for publication in *Crop Science*. Please refer to this publication for additional details.

Literature Cited

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Figure 1: Soil volumetric water content for (A) perennial ryegrass and (B) Kentucky bluegrass and leaf relative water content for (C) perennial ryegrass and (D) Kentucky bluegrass cultivars in pots in a growth chamber treated with either well-watered (control) or drought condition. Dashed lines represent values for well-watered control conditions. Differences among cultivar means were detected using Fisher's least significant difference (LSD) test at $P \leq 0.05$. Vertical bars indicate LSD values.

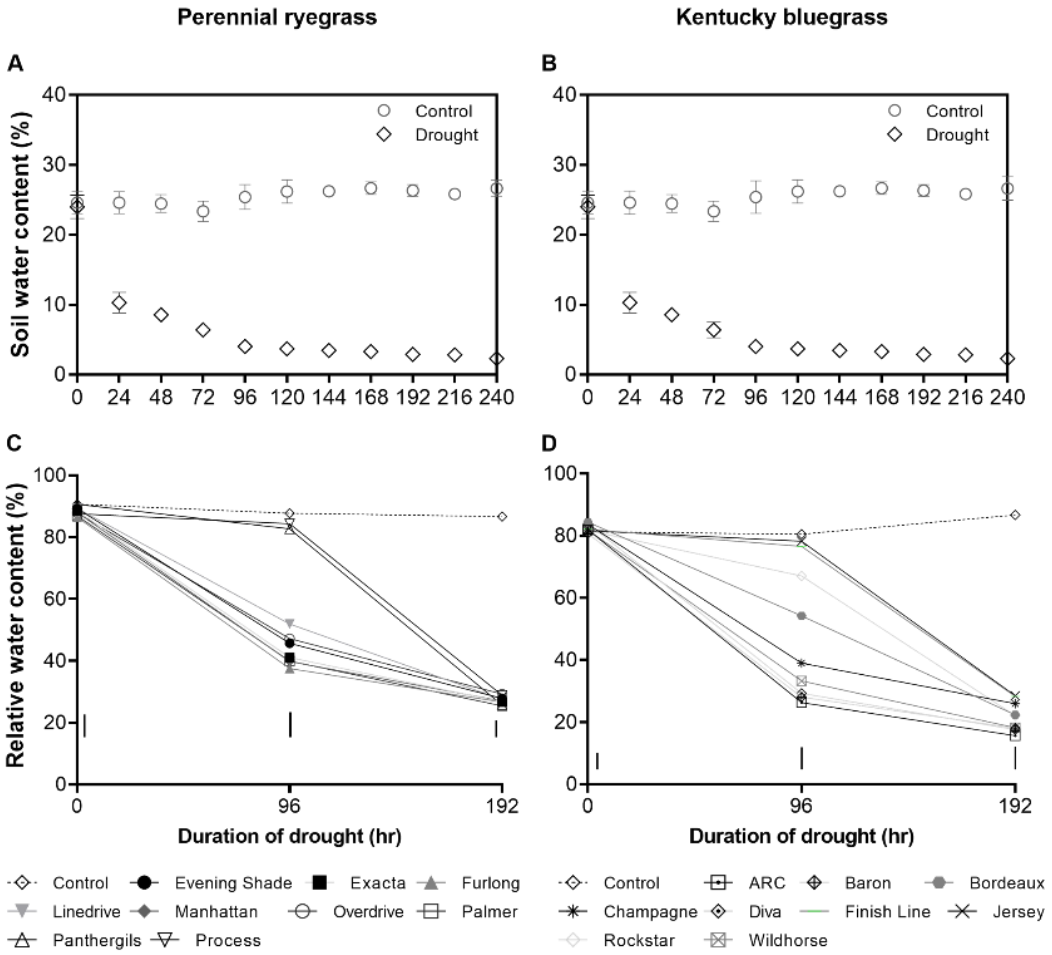


Figure 2: Photochemical efficiency (Fv/Fm) quantum yield of photosystem II (Φ_{II}), and non-photochemical quenching (NPQ) of perennial ryegrass (left) and Kentucky bluegrass (right) cultivars during well-watered (control) and drought treatment in a growth chamber for 10 days (240 hr). Dashed lines indicate the mean for well-watered controls and solid lines indicate mean for drought-treated plants. Vertical bars indicate Fisher's least significant difference between cultivars on respective days ($P \leq 0.05$).

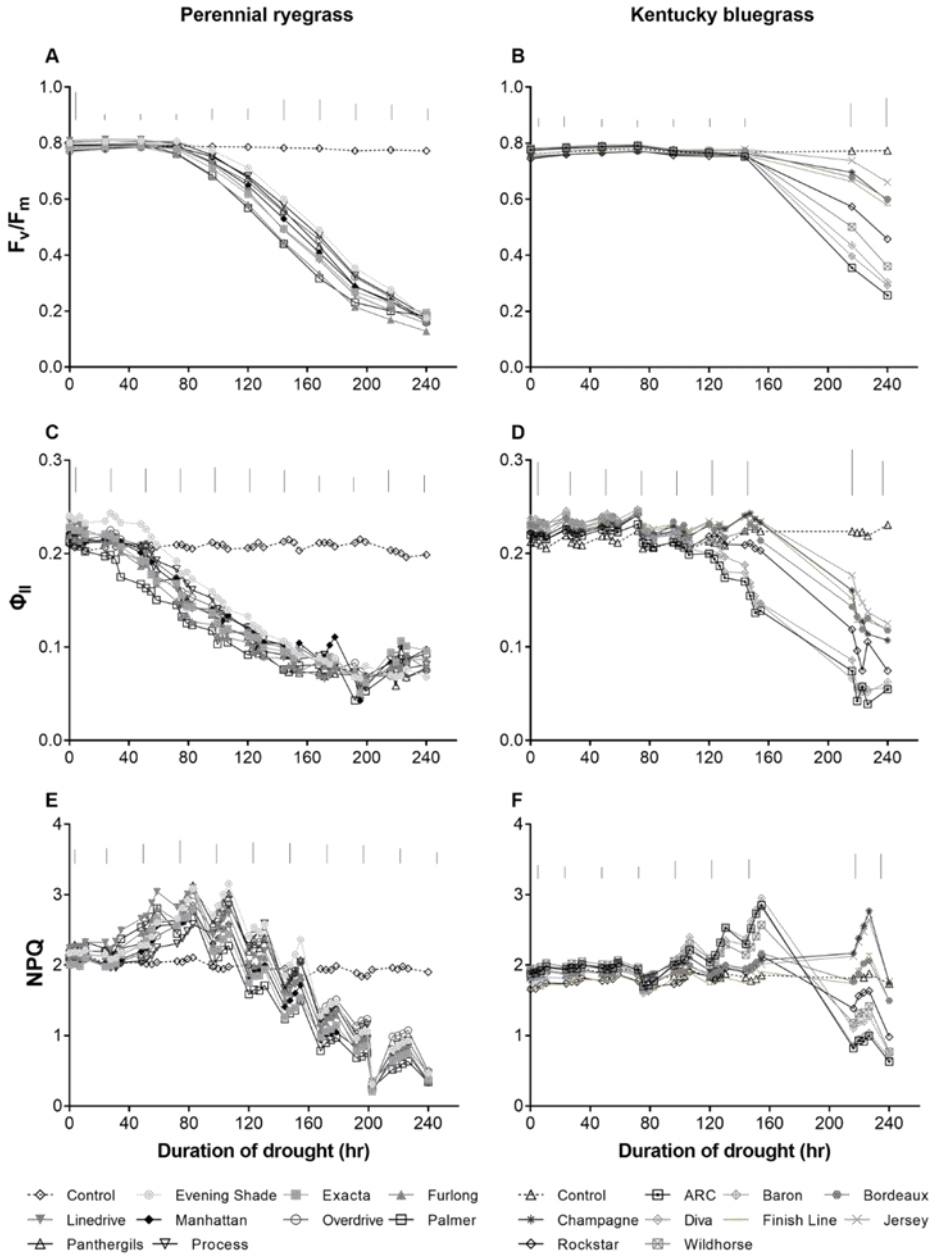
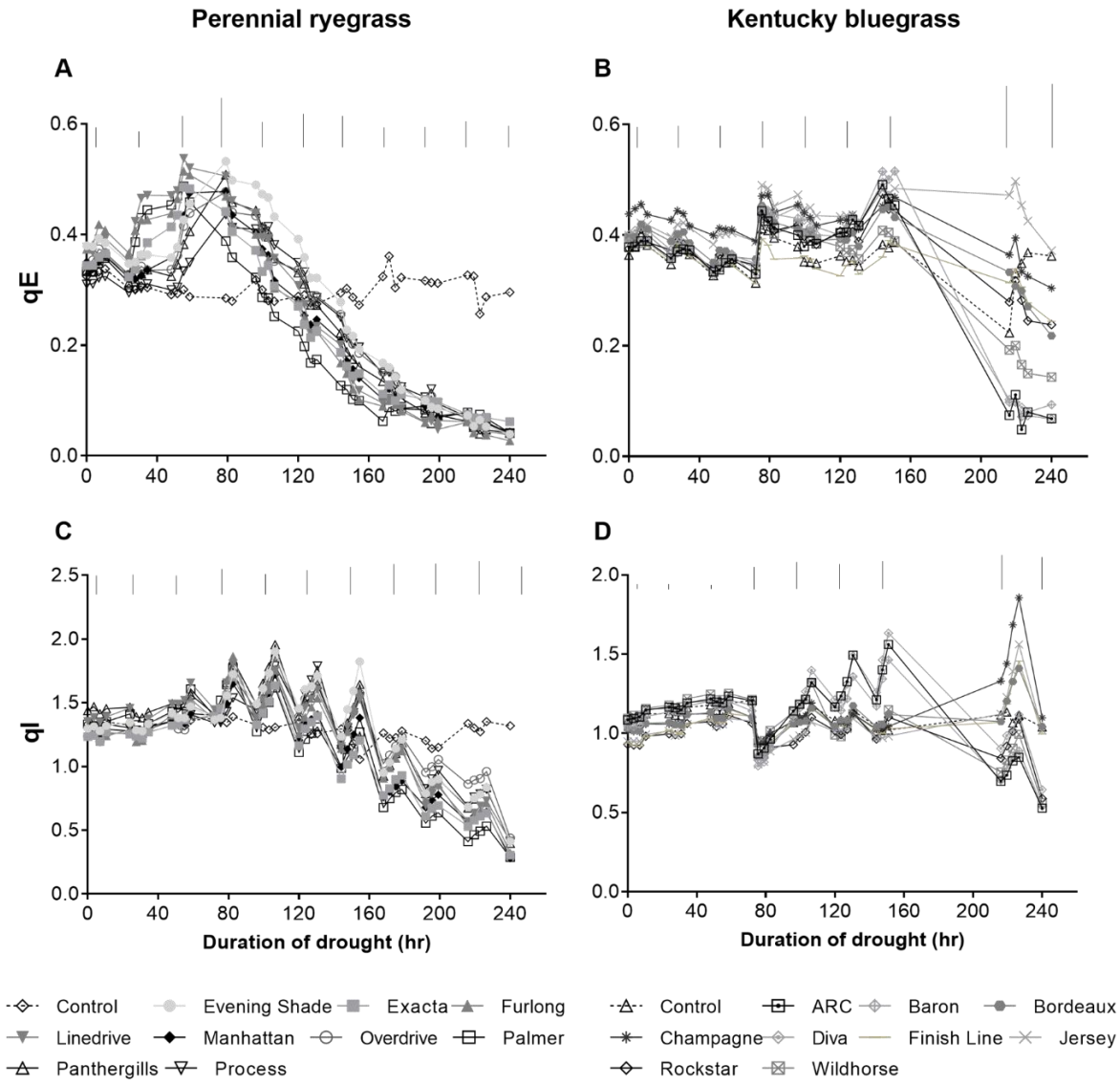


Figure 3: Photosynthetic energy dependent quenching (qE) in perennial ryegrass (A) and Kentucky bluegrass (B), and photoinhibition associated quenching (qI) in perennial ryegrass (C) and Kentucky bluegrass (D) during well-watered (control) and drought treatment for 10 days (240 hr) in a growth chamber. Dashed lines indicate the mean for well-watered controls and solid lines indicate mean for drought-treated plants. Vertical bars indicate Fisher's least significant difference between cultivars on respective days ($P \leq 0.05$).



USGA ID#: 2023-06-773

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

Project leaders: Stacy Bonos and Mark LaBarge

Affiliation: Rutgers University

Objectives:

- Publish data on superior bermudagrass and zoysiagrass germplasm for fairways in the northern transition zone
- Conduct an environmental assessment comparing bermudagrass, zoysiagrass and creeping bentgrass
- Conduct an economic analysis including return on investment calculator if fairways are converted to warm-season grasses
- Develop a decision-making framework to aid superintendents in determining if zoysiagrass or bermudagrass is a viable option for their course environment

Start Date: 2023
Project Duration: Three years
Total Funding: $90,000

Summary Points:

- Bermudagrass and zoysiagrass can be vegetatively established from sprigs in New Jersey with establishment possible in one growing season for certain accessions.
- There are significant differences in establishment, turfgrass quality, and fall color retention between bermudagrass and zoysiagrass accessions grown in New Jersey.
- Bermudagrass with the fastest establishment were Rio, Monaco, and Hollywood.
- Zoysiagrass with the fastest establishment were Empire, FZ 1727, and DALZ 1701.
- The largest decrease in fall color retention was from November 17 to December 1, 2022 with all plots being dormant by December 1, 2022.

Summary Text:

Rationale

Climate change is causing more extreme temperature fluctuations with variable conditions in the transition zone. High temperatures negatively affect the performance of creeping bentgrass and result in increased inputs for adequate playability. Utilization of cold-hardy zoysiagrass and bermudagrass in the transition zone can provide an option for superintendents to potentially save money, resources, and have less of an impact on the environment. We are evaluating the performance of zoysiagrass and bermudagrass germplasm and their potential use for fairways in the northern transition zone compared to creeping bentgrass.

Methodology

Table 1. Bermudagrass and Zoysiagrass Selections

Bermudagrass	Type	Zoysiagrass	Type
Tahoma 31	Vegetative	Meyer	Vegetative
Latitude 36	Vegetative	Emerald	Vegetative
Patriot	Vegetative	Zeon	Vegetative
Northbridge	Vegetative	Empire	Vegetative
Tiftuf (DT-1)	Vegetative	Lobo	Vegetative
Tifway	Vegetative	Gateway	Vegetative
Iron Cutter	Vegetative	Innovation	Vegetative
Landrun	Vegetative	KSUZ 1201	Vegetative
Yukon	Seeded	DALZ 1701	Vegetative
Rio	Seeded	DALZ 1808	Vegetative
Monaco	Seeded	FAES 1319	Vegetative
Hollywood	Seeded	FZ 1732	Vegetative
OKS 2011-1	Seeded	FZ 1727	Vegetative
OKC 1876	Vegetative		
MSB 1042	Vegetative		
FB 1628	Vegetative		

Zoysiagrass and bermudagrass cultivars and selections (Table 1) with proven cold hardiness (according to NTEP reports and collaboration with plant breeders) were identified as promising entries to establish in New Jersey. The selected germplasm was received via mail from the provider listed (Table 2) and was the result of advice from numerous sources and germplasm availability. Each selection was expanded into 20 greenhouse flats in a soilless media. Supplemental greenhouse lighting was used to encourage growth with a day length of 16 hours, a daytime temperature of 30-32 °C, and a nighttime temperature of 21-24 °C. All flats were watered daily, fertilized weekly, and trimmed as needed.

A new 10,000 sq ft field space was chosen for the study and soil samples were collected from the area in a cross pattern at a depth of 4 inches. The samples were aggregated and sent to the Rutgers soil testing lab for analysis. The soil test reported a pH of 4.88 and recommended addition of dolomitic limestone at a rate of 225 lb/1000 sq ft. Heavy metals were not found in high abundance in the soil samples. The area was treated with 225 lbs/1000 sq ft of dolomitic limestone in accordance with the soil test. The area was worked with the following tractor implements to provide a surface suitable for sprigging: disk harrow, spring-tooth harrow, land leveler/grader, and cultipacker.

After field preparation, selections were manually washed and torn apart to obtain vegetative material for sprigging. Vegetative material was measured in 5-gallon buckets for volumetric consistency and placed in 50-gallon contractor bags with air holes for respiration. Each bag contained 10 gallons of vegetative material (sprigs) and each selection required 3 bags, 1 for each replicated field plot. Upon completion, bags were placed in a seed storage room at 15.5 °C. Figure 1 exemplifies the process of preparing sprigs for planting. Field plots measured 7 ft by 14 ft for a total of 98 sq ft. A sprigging rate of approximately 500 US bushels/acre was chosen which correlates to 10 gallons/98 sq ft plot. Some selections did not provide enough vegetative material and can be seen in Table 3.

The prepared field was sprigged on June 30, 2022 (Figure 2). Each bag of sprigs was watered before being taken to the field to prevent drying out and potential sprig death. The field was fertilized with a granular 10-10-10 fertilizer at a rate of 0.75 lbs N/1000 sq ft. Each bag of sprigs was evenly distributed by hand over the plot area and sprigs were kept moist throughout the process with a portable water tank. After sprigs were evenly distributed in each plot, the field

Table 2. Germplasm Collection and Collaborators (Z = Zoysiagrass and B = Bermudagrass)

Provider	Affiliation	Selections Provided
Dr. Brian Schwartz	University of Georgia	Meyer (Z) Zeon (Z) Tiftuf (DT-1) (B) Tifway (B)
Dr. Kevin Kenworthy	University of Florida	FAES 1319 (Z) FZ 1732 (Z) FZ 1727 (Z) FB 1628 (B)
Dr. Ambika Chandra	Texas A&M University	Innovation (Z) KSUZ 1201 (Z) DALZ 1701 (Z) DALZ 1808 (Z)
Dr. Susana Mila-Lewis	NC State University	Lobo (Z)
Dr. Yanqui Wu	Oklahoma State University	Tahoma 31 (B) Latitude 36 (B) Patriot (B) OKC 1876 (B) Northbridge (B)
Dr. Hongxu Dong	Mississippi State University	MSB-1042 (B)
Dr. Leah Brilman	DLF/Seed Research of Oregon	Yukon (B)
Mr. John Lamle	Johnston Seed	Ironcutter (B) Landrun (B) Rio (B) Monaco (B) OKS2011-1 (B) Hollywood (B)
Mr. Roberto Gurgel	Sod Solutions	Empire (Z)
Graham Simmons	Woerner Farms	Emerald (Z)
Adam Russel	Mountain View Seeds	Gateway (Z)

was rolled twice with a Gandy three gang-roller weighing 1,766 lbs to improve soil to sprig contact. A sandy loam topdressing (of native soil) was applied at a rate of ¼ inch. The field was treated with Ronstar Flo (Oxadiazon) at a rate of 3 lbs ai/acre. Finally, above ground irrigation was setup and 3,900 gallons of water was applied after sprigging.

The field layout was established in a Randomized Complete Block Design with 3 replications. Plots were fertilized weekly for the first 4 weeks of establishment with a 22-0-12 granular fertilizer at a rate of 0.8 lbs N/1000 sq ft. During establishment, plots were watered 2-3 times daily to keep sprigs moist for one month after sprigging (June 30 to July 30, 2022) and gallonage was recorded. Approximately 76,000 gallons of irrigation water were used in the first month of establishment with a total of 1.14 inches of rain during the period. After the first month, plots were watered as needed to keep non-established sprigs moist and promote growth. From July 30 to September 1, 2022, approximately 21,500 gallons of irrigation water was used with 9.39 inches of rainfall. Plots were first mowed on August 4, 2022 (approximately 1 month after sprigging). Plots were mowed an additional 12 times with the height of cut being decreased successively until reaching 1 inch on September 21, 2022. Plots were mowed with a reel mower at 0.5 inches in early spring (while dormant) to improve spring green-up, reduce thatch, and reach the desired fairway height of cut.

In addition to data being collected on irrigation and maintenance, visual ratings and image data were collected for the field trial. From July 7 to October 6, 2022, visual establishment ratings were taken weekly on a 0-10 rating scale representing percent green cover. Turfgrass quality ratings, on a 1-9 scale, were taken monthly starting on August 26, 2022 (approximately 2 months after sprigging) until the plots entered winter dormancy. In addition, fall color retention ratings

Table 3. Washed Sprig Amounts

Cultivar/ Experimental Selection	Type	Sprig Amount (gallons/ plot)
Tahoma 31	Vegetative	7
Patriot	Vegetative	7
OKS 2011-1	Seeded	8
OKC 1876	Vegetative	6
MSB 1042	Vegetative	7
FB 1628	Vegetative	8
All Others	Vegetative/ Seeded	10

were recorded on a weekly basis from September 30 to December 1, 2022 on a 1-9 scale according to NTEP protocol. Digital image data was collected using a Canon PowerShot G15 with a 20 x 24 inch lightbox with 4 florescent bulbs. Images were taken on August 5, 2022 (approximately 1 month after sprigging) and then weekly from September 23 to December 1, 2022. In addition to lightbox images, a full plot image was taken using the same camera to provide an accurate representation of the entire 98 sq ft plot. Lightbox images were processed using turf analyzer software. Visual ratings were statistically analyzed using SAS 9.4 to perform an ANOVA test. The adjusted means and LSD values can be seen for 2022 ratings in Table 4. Non-linear regression using equations 1 and 2 (Karcher et al, 2008; Sandor et al, 2021) was performed using GraphPad Prizm (version 10.1) to determine the time each entry requires to reach 90% turfgrass coverage and results can be seen in Figure 3.

Results

Bermudagrasses established quicker than zoysiagrasses (Figures 3-4 and Table 4). Within the bermudagrasses, Rio, Monaco, and Hollywood had the quickest establishment. It is interesting to note that the plots with quickest establishment were [typically] seeded cultivars. Most plots filled in eventually but as you can see from Figure 5, several plots did not establish well even by October 20, 2022 (Meyer, Emerald, Gateway, KSUZ 1201, and Zeon [all zoysiagrasses]). Empire, FZ 1727 and DALZ 1701 had the quickest establishment among the zoysiagrasses. The top nine selections based on turfgrass quality ratings were all bermudagrass. The best performing bermudagrass and zoysiagrass (based on turfgrass quality) were FB 1628 and Empire, respectively. Four out of the five top performers for fall color retention were zoysiagrass. The best bermudagrass and zoysiagrass for fall color retention were FB 1628 and FZ 1732, respectively. It was observed that all plots were dormant by December 1, 2022 with the largest decrease in color retention from November 17 to December 1, 2022. No selections reached 100% winter kill but some selections were at or above 70% winter kill including Hollywood, Empire, FZ 1732, and FZ 1727. The most desirable characteristic for the region is extended fall color retention with limited winter kill which was achieved by DALZ 1701, Tiftuf (DT-1), Tahoma 31, and Ironcutter which can be seen in figure 6.

We will continue to evaluate turf quality, spring green up, winter kill, and disease resistance. This trial will also be used to identify the best performing bermudagrass and zoysiagrass for NJ climactic conditions. The best performing selections will be utilized for the additional objectives of the study.

Table 4. Average Visual Ratings for 2022 in order of highest turfgrass quality.

ID	Turfgrass Quality	Establishment	Fall Color Retention	Winter Kill
FB 1628	7.3	6.9	6.6	6.3
Tiftuf (DT-1)	7.1	6.7	6.2	2.0
MSB 1042	6.7	7.8	4.9	5.0
Latitude 36	6.3	7.5	4.9	3.0
Landrun	6.3	8.7	4.1	3.3
Tifway	6.3	5.9	6.3	5.3
Iron Cutter	6.2	7.4	4.9	2.0
Patriot	6.1	8.2	4.1	4.0
Northbridge	6.0	7.9	4.6	2.3
Empire	6.0	5.2	5.8	7.3
FZ 1732	6.0	4.5	6.8	7.7
DALZ 1808	6.0	4.4	5.5	3.0
OKS 2011-1	5.8	8.2	4.2	6.7
OKC 1876	5.8	5.1	6.3	6.7
FZ 1727	5.7	4.8	6.4	8.0
Tahoma 31	5.7	6.4	5.0	2.0
Lobo	5.7	4.3	6.4	5.3
DALZ 1701	5.6	5.1	6.0	2.0
Rio	5.2	9.1	3.1	5.0
Monaco	4.9	9.1	3.3	5.7
Yukon	4.2	4.8	4.2	4.0
Hollywood	3.9	8.8	4.2	9.0
FAES 1319	3.2	2.4	6.3	6.3
Innovation	3.0	2.7	4.6	2.3
Meyer	2.3	1.7	5.3	1.7
Emerald	2.0	1.2	5.2	1.0
Gateway	1.1	0.7	2.2	1.7
KSUZ 1201	0.9	0.5	1.7	0.0
Zeon	0.7	0.4	0.5	0.0
lsd at 5%=	**1.04**	**0.98**	**1.5**	**2.2**

Future Expectations

A high and low maintenance fairway trial will be established from sod in the summer of 2024 to compare between zoysiagrass, bermudagrass, and creeping bentgrass under fairway management in NJ. We will select the top 2 zoysiagrass and bermudagrass entries from Study 1 and the top 2 creeping bentgrass selections from previous trials conducted in NJ. Meyer will be used as the zoysiagrass standard, Iron cutter as the bermudagrass standard, and 007 as the creeping bentgrass standard. After initial establishment, comparisons between species will include mowing frequency, turf quality, and disease/weed/pest susceptibility.

An environmental assessment will be conducted on each species using data collected from maintenance inputs, Field Use Environmental Impact Quotients, nutrient loss modelling, and irrigation requirements. An economic analysis (and subsequent cost models) will be conducted using data collected on maintenance inputs, interviews with golf course superintendents, and current pricing for materials and labor. These analyses will be used to make comparisons between species and provide useful information for golf course managers in the region. The top performers of each species and standards will also be tested in a drought trial to determine the irrigation requirements of each species needed for adequate turf quality. Lastly, a survey of golfers in the region will be designed, conducted, and statistically analyzed to determine if there is a preference on fairway turfgrass species. The survey will consist of several short questions to assess whether golfers prefer a certain species and if they are more likely to consider playing a course that is more environmentally friendly.



Figure 1. Sprigging of Bermuda and Zoysiagrass selections: Left image depicts a sprig washing station, center image depicts a sprig tearing station, right image depicts bagged sprigs in a seed storage room.



Figure 2. Hand-sprigging bermudagrass and zoysiagrass.

Equation 1

$$\text{Days to 90\% coverage} = Days\ K - \left[\frac{1}{slope} * \log\left(\frac{K}{100-K}\right)\right]$$

Equation 2

$$\text{Turfgrass Coverage (\%)} = \frac{100}{1+10^{[(Days90-X)*slope]}}$$

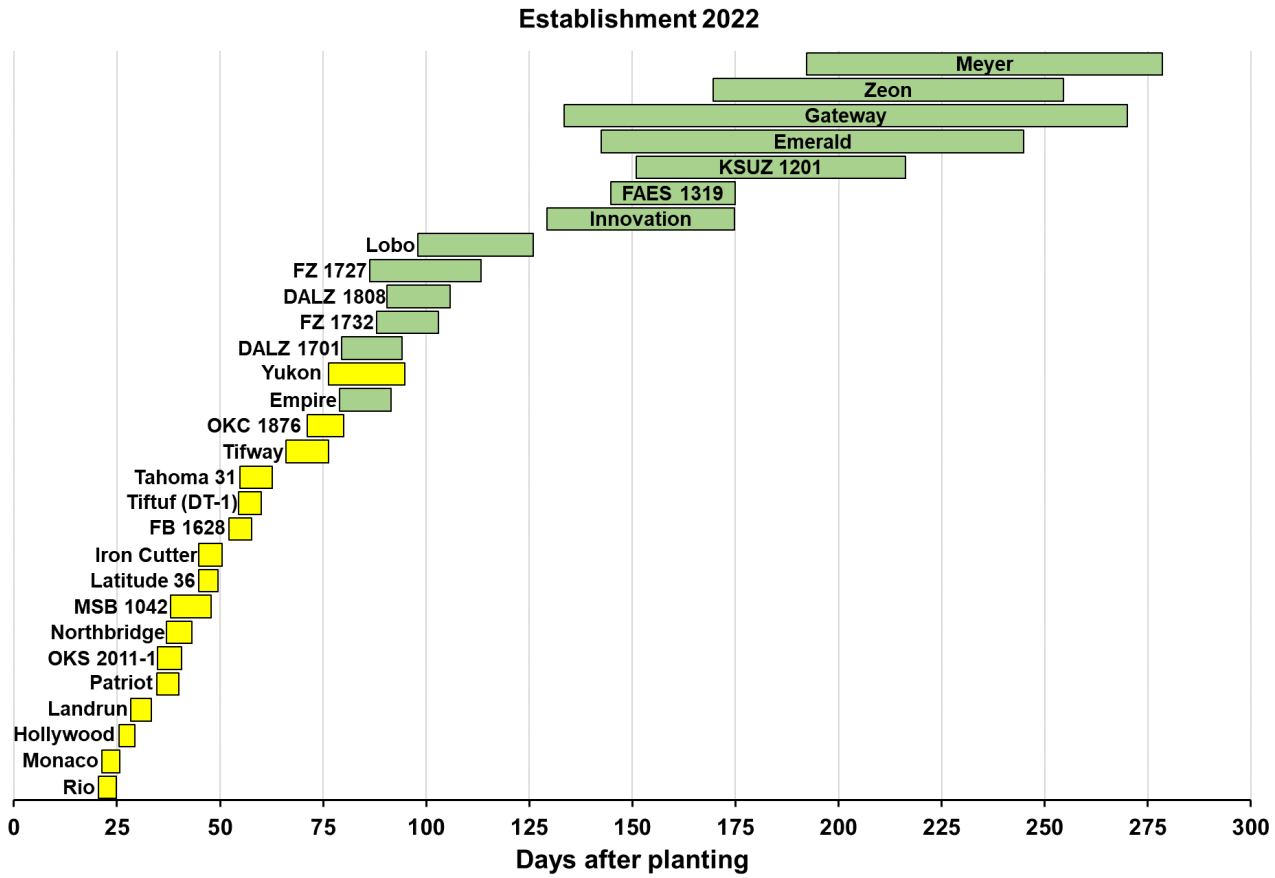


Figure 3. Visual establishment ratings analyzed with non-linear regression showing days to reach 90% turfgrass coverage with non-overlapping bars being significantly different. Bermudagrass is represented with yellow bars and zoysiagrass is represented with green bars.



Figure 4. Establishment of warm-season grasses in NJ. Green plots represent bermudagrass plots with quick establishment. Photo taken July 20, 2022.



Figure 5. Drone image of entire field taken on October 20, 2022.

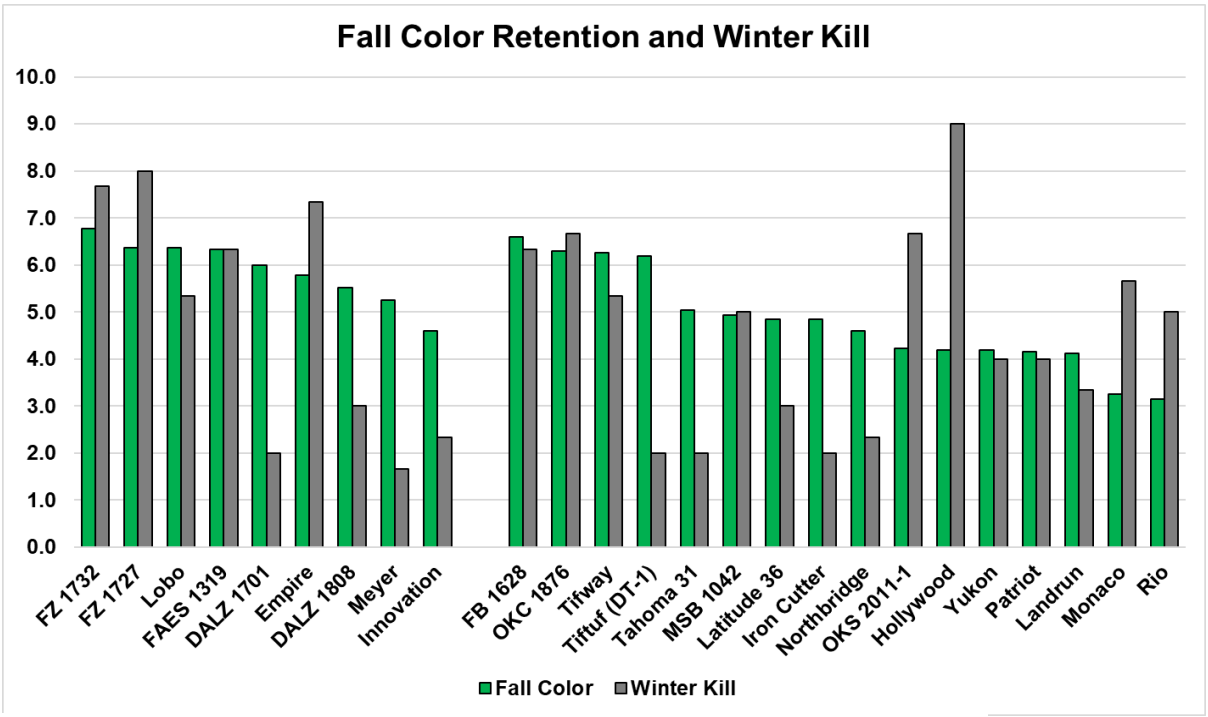


Figure 6. Comparison of Fall Color Retention and Winter Kill visual ratings.

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USGA ID#: 2023-10-777a

Title: Developing management tools for new greens-type zoysiagrasses

Project Leaders: Michael Richardson and John McCalla, University of Arkansas, Ambika Chandra and Chase Martin, Texas A&M AgriLife Research, and Jim Brosnan and Greg Breeden, University of Tennessee-Knoxville

Background: Traditionally, creeping bentgrass (*Agrostis stolonifera* L.) and hybrid bermudagrass (*Cynodon dactylon* (L.) Pers x *C. transvaalensis* Burtt-Davy) have been planted on golf course putting greens throughout the transition zone, with the popularity of grass selection being cyclical with climatic trends and genetic advances. Zoysiagrass (*Zoysia* spp. Willd.) is a perennial, C4 plant that is also adapted for use in the transitional and warm climatic zones of the United States and its use on golf greens, while still limited, is of interest to golf courses in the region. Historically, 'Diamond' [*Z. matrella* (L) Merr] was the only viable cultivar for golf courses choosing to use a zoysiagrass on their putting surface but research from Clemson University determined ball roll speeds were unacceptable for tournament use (Stiglbauer et al., 2009). More recently, three *Z. matrella* cultivars ['Prizm', 'M85', and 'Trinity'] (Douget et al., 2017) and 'Lazer', the first interspecific hybrid developed between Diamond and *Z. minima* (ecoptype TAES 5194-5) (Chandra et al., 2020) have been released for use on golf greens. For this project, we will focus on Lazer and Prizm zoysiagrass as these two new and improved cultivars are now commercially available and are being tested and utilized on several golf courses in the United States.

Compared to *Cynodon* spp., zoysiagrass has many positive traits such as reduced fertilizer needs (Kussow et al., 2012, Christians, et al., 2017), enhanced cold tolerance, excellent salinity tolerance (Qian et al., 2000), and good shade tolerance (Qian and Engelke, 1999; Walton, 2022); however, high seedhead production (McCullough et al., 2017) and lack of herbicide options for greens (W. Bowling, personal communication, May 16, 2022) are causes of concern for superintendents and may limit early adoption. Diamond is a prolific seedhead producer in the fall and spring seasons (McCullough et al., 2017) and early observations indicate that seedhead production can be significant on Lazer in the spring season (Chandra, unpublished and Richardson, unpublished). Seedhead suppression research using plant growth regulators (PGRs) has recently been investigated in *Z. japonica* (Brosnan et al., 2012, Patton et al., 2018), but the efficacy and timing of application should be further investigated in these new hybrid cultivvars.

Currently, there are no herbicides that are labeled for zoysiagrass golf green use and most herbicides that are labeled for zoysiagrass do not even differentiate between *Z. japonica* and *Z. matrella* species. Currently, golf course superintendents planting zoysiagrass on golf greens can only rely on information regarding fairway tolerance to herbicide applications, which may not be appropriate. For example, preliminary work conducted at the University of Tennessee and University of Arkansas highlighted that both Prizm and Lazer are sensitive to applications of fluazifop + triclopyr that are regularly used for bermudagrass suppression in fairways and roughs (Walton, Richardson, Carr, and Brosnan, unpublished; McElroy and Breeden, 2006). Both cultivars were also sensitive to sulfentrazone, a common active ingredient used to control *Cyperus* and *Kyllinga* species, whereas foramsulfuron was safe on Prizm but injurious to Lazer (Walton, Richardson, Carr, and Brosnan, unpublished). In a trial at Arkansas, Lazer zoysiagrass was sensitive to the PGRs, trinexapac-ethyl and prohexadione calcium, when applied at label rates (Walton and Richardson, unpublished). Although some of the phytotoxicity associated with PGR use can be reduced via treatment at lower rates, there is no information on current PGR labels that addresses rates or timing of application to zoysiagrass putting greens.

Objectives: This project is a multi-site effort among three universities with the following objectives:

1. Determine the tolerance of Lazer and Prizm zoysiagrass to various chemicals commonly used for managing putting greens, including plant growth regulators, herbicides, and fungicides.
2. Determine the effects of herbicides and plant growth regulators on seedhead suppression on Lazer and Prizm zoysiagrass (*the seedhead suppression work was initiated prior to dormancy in the fall of 2023 and no data has been collected, so will not be reported here*)

Research Methods: Pesticide tolerance work was performed on Lazer zoysiagrass at the Texas A&M Agrilife Center in Dallas TX (32.988673, -96.766987) and the Milo Shult Agricultural Research and Extension Center in Fayetteville AR (36.100674, -94.173790). Tolerance was evaluated on Prizm zoysiagrass at the East Tennessee AgResearch and Education Center in Knoxville TN (35.902185, -83.957604). Trials at all locations were established as a randomized complete block with four replications with individual plots sizes ranging from 8-9 ft^2 at each location. Trial areas were maintained under typical putting green management practices, with a 0.125 inch height of cut, fertilization at 0.25 lb. N/1000ft^2/growing month, and irrigated applied as needed.

Chemical applications were made on 18 July 2023 in Knoxville, on 1 August 2023 in Dallas, and on 24 July 2023 in Fayetteville. Applications were made at the 1x and 2x labeled rates (Table 1) to take into account overlaps and/or application errors that might occur in the field. Products were applied according to label instructions, with adjuvants included if specified on the label (Table 1). All applications were made using a spray volume of 40 gallon / acre volume using CO_2 as the propelant. Data collected include visual turfgrass quality and phytotoxicity (% injury) at 3, 7, 14, 21, and 28 days after treatment or until injury has recovered to the acceptable threshold of <10%. For brevity, only the phytoxocity data will be reported here.

Results and discussion

Visual phytotoxicity, including chlorosis and other discoloration of the plots, was observed at all locations with some treatments (Figure 1 and Table 1). Treatments that produced unacceptable phytotoxicity included Fusilade II + Turflon Ester and Speedzone EW. With both treatment combinations, the 2X rate caused significantly more injury than the 1X rate (Table 1). Although the Fusilade II + Turflon Ester treatment caused significant injury at all locations, the Speedzone EW treatment caused more injury on Lazer in Dallas than it did at the other two locations (Table 1). It is unclear at this time why these differences between locations may have occurred; however, higher air temperatures in Dallas may have contributed to these results.

None of the other treatments caused injury that was considered unacceptable (Table 1). It was expected to potentially see some injury with Primo Maxx since this was observed in earlier trials at Arkansas on Lazer zoysiagrass (Walton, 2022). However, no injury was observed at any of the three locations in the current trial (Table 1). The fungicides included in the trial, Densicor and Banner Maxx, are both demethylation inhibitor (DMI) fungicides and older chemistries such as Banner Maxx (active ingredient = propiconazole) have been know to cause phytotoxicity and growth regulation on some turfgrass species, while newer chemistries such as Densicor (active ingredient = prothioconazole) reported to cause less phytotoxicity. With both fungicides, no injury was observed across the three locations (Table 1).

One observation that is worth noting is the Recognition + Fusilade II treatments (Table 1 and Figure 1). Recognition is a new product from Syngenta that includes a sulfonylurea herbicide (trifloxysulfuron-sodium) and a proprietary safener (metcamifen) that has been shown to greatly enhance the safety of Fusilade II (fluazifop-P-butyl) and allows Fusilade II to be applied at much higher rates (up to 24 fl oz / acre) to several turfgrass species, including zoysiagrass, St. Augustinegrass, and kikuyugrass. This product combination also had excellent safety in the current trial and could be an important part of controlling bermudagrass contamination in zoysiagrass putting greens.

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Figure 1. Representative phytoxocitiy from several treatments in Fayetteville AR on 31 July 2023 (7 days after treatment)



Untreated control



Fusilade II (8 fl oz/A) + Turflon Ester (64 fl oz/A)



Speedzone EW (8 pt/A)



Fusilade II (24 fl oz/A) + Recognition (3.9 oz/A)

Table 1. 'Phytotoxicity of herbicide, plant growth regulators, and fungicides applied to zoysiagrass putting greens at three locations

				Dallas TX (cv. Lazer)						Fayetteville AR (cv. Lazer)						Knoxville TN (cv. Prizm)					
Trt	Treatment		Rate	8/8/23		8/15/23		8/29/23		7/31/23		8/7/23		8/21/23		7/26/23		7/31/23		8/15/23	
No.	Name	Rate	Unit	7DAT		14DAT		28DAT		7DAT		14DAT		28DAT		8DAT		13DAT		28DAT	
				phytotoxicity (0-100%)																	
1	Untreated			0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
2	Revolver	26.2	fl oz/a	0.0	e	0.0	e	0.0	e	0.5	c	3.0	cde	0.0	c	0.0	b	0.0	d	0.0	b
3	Revolver	52.4	fl oz/a	0.0	e	0.0	e	0.0	e	0.0	c	1.0	ef	0.0	c	0.0	b	0.0	d	0.0	b
4	Katana *	3.0	oz wt/a	0.0	e	0.0	e	0.0	e	0.5	c	2.5	c-f	0.5	c	0.0	b	0.0	d	0.0	b
5	Katana *	6.0	oz wt/a	0.0	e	0.0	e	0.0	e	1.0	c	0.5	ef	0.5	c	0.0	b	0.0	d	0.0	b
6	Speedzone EW	4.0	pt/a	15.0	d	12.5	d	7.5	d	1.8	c	1.8	def	0.5	c	0.0	b	0.0	d	0.0	b
7	Speedzone EW	8.0	pt/a	43.8	b	37.5	b	20.0	b	7.3	b	8.0	b	2.0	c	1.3	b	0.0	d	0.0	b
8	Kerb	15.0	fl oz/a	0.0	e	0.0	e	0.0	e	0.5	c	2.3	c-f	0.5	c	0.0	b	0.0	d	0.0	b
9	Kerb	30.0	fl oz/a	0.0	e	0.0	e	0.0	e	0.5	c	0.5	ef	0.0	c	0.0	b	1.3	cd	0.0	b
10	Poa Cure	0.6	fl oz/1000 ft2	0.0	e	0.0	e	0.0	e	0.5	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
11	Poa Cure	1.2	fl oz/1000 ft2	0.0	e	0.0	e	0.0	e	0.5	c	0.5	ef	0.0	c	0.0	b	0.0	d	0.0	b
12	Ronstar G	3.0	lb ai/a	0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
13	Ronstar G	6.0	lb ai/a	0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
14	Tribute Total **	3.2	oz wt/a	0.0	e	0.0	e	0.0	e	1.0	c	2.3	c-f	0.0	c	0.0	b	0.0	d	0.0	b
15	Tribute Total **	6.4	oz wt/a	0.0	e	0.0	e	0.0	e	3.0	c	5.0	c	0.0	c	1.3	b	3.8	b	0.0	b
16	Recognition *	2.0	oz wt/a	0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
	Fusilade II	12.0	fl oz/a																		
17	Recognition *	3.9	oz wt/a	0.0	e	0.0	e	0.0	e	2.5	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
	Fusilade II	24.0	fl oz/a																		
18	Fusilade II *	4.0	fl oz/a	21.3	c	20.0	c	16.3	c	6.3	b	4.5	cd	8.5	b	1.3	b	2.5	bc	0.0	b
	Turflon Ester	32.0	fl oz/a																		
19	Fusilade II *	8.0	fl oz/a	71.5	a	69.0	a	60.5	a	40.0	a	45.0	a	35.0	a	28.8	a	13.8	a	10.0	a
	Turflon Ester	64.0	fl oz/a																		
20	Arkon	52.5	g ai/ha	0.0	e	0.0	e	0.0	e	1.8	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
21	Arkon	105.0	g ai/ha	0.0	e	0.0	e	0.0	e	0.0	c	0.5	ef	0.0	c	0.0	b	0.0	d	0.0	b
22	Primo Maxx	3.0	fl oz/a	0.0	e	0.0	e	0.0	e	1.0	c	1.0	ef	0.0	c	0.0	b	0.0	d	0.0	b
23	Primo Maxx	6.0	fl oz/a	0.0	e	0.0	e	0.0	e	1.0	c	1.0	ef	0.5	c	0.0	b	0.0	d	0.0	b
24	Densicor	8.5	fl oz/a	0.0	e	0.0	e	0.0	e	2.5	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
25	Densicor	17.0	fl oz/a	0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
26	Banner Maxx	88.0	fl oz/a	0.0	e	0.0	e	0.0	e	0.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
27	Banner Maxx	176.0	fl oz/a	0.0	e	0.0	e	0.0	e	1.0	c	0.0	f	0.0	c	0.0	b	0.0	d	0.0	b
LSD P=0.05				*2.6*		*1.9*		*3.1*		*3.1*		*3.0*		*4.2*		*1.4*		*2.1*		.	
Treatment Prob(F)				0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		1	

Means followed by same letter or symbol do not significantly differ (P=.05, LSD).

* - treatment also included a nonionic surfactant at 0.25% v/v

** - treatment also included methylated seed oil at 0.5% v/v

USGA ID#: 2023-10-777c

Title: Developing Management Tools for New Greens-Type Zoysiagrasses

Project Leader: John C. Sorochan, Ph.D., Kyley H. Dickson, PhD., and Taylor Williams
Affiliation: University of Tennessee, Knoxville

Objectives: To identify cold-hardy tees-to-green cultivars of zoysiagrass for the transition zone

Start Date: 2021
Project Duration: Three Years
Total Funding: $15,000

Summary Points:

- Fourteen weeks after planting, entry 6785-22 was slowest to establish (23.3% green coverage), compared to a 45% average percent green coverage of all other plots.
- After 30 simulated traffic events, the variety with poorest traffic tolerance was experimental entry 6942-22 (21.08% green coverage). Palisades, a check plot, had a similar wear tolerance (21.4%) after 30 simulated traffic events.
- The best traffic tolerance after 30 simulated traffic events, was held by experimental plot 6792-44 (58.08% green coverage)

Summary Text:

Zoysiagrass (*Zoysia* spp.) is one of the most commonly used turfgrass species in the southern United States. It is commonly used for golf course tees, fairways, and greens as it often provides more shade tolerance than bermudagrass (*Cynodon* spp.). Several zoysiagrasses are maintained between 0.100" – 1" and provide a consistent, aesthetically pleasing playing surfaces for golf courses. The objective of this study was to evaluate traffic effects on zoysiagrass that could potentially be used from tee to green.

Tennessee is located in the transition zone, making it necessary to conduct zoysiagrass research. Zoysiagrasses used for putting greens often do not require as much nitrogen as bermudagrass or creeping bentgrass putting greens and can provide similar green speeds.

Research was conducted at the East Tennessee Research and Education Center in Knoxville, TN, at the University of Tennessee summer 2021 – fall 2023 to evaluate traffic tolerance of 74 Zoysiagrass varieties (64 experimental lines, 4 elite hybrids, and 5 standards). The 5 standards were Meyer, Innovation, Zeon, Emerald, and Palisades. Plots were plugged into a Sequatchie silt loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) on June 24, 2021. Establishment rates were tracked after planting, resulting in entry 6785-22 having the slowest time to 90% green coverage. Plots were mown at 0.75" with a fairway reel mower for the duration of this experiment. It was important to keep blades sharp in order to provide a high

quality of cut, due to the rigidity of the zoysiagrass leaf blades. Dull blades often produce a gray appearance due to the leaf tips becoming frayed. Finale, a non-selective herbicide was used to separate plots monthly as lateral growth caused plots to grow together. A pre-emergent herbicide, Ronstar G, was applied during grow-in to control weed populations during summer months. 4-Speed XT, a post-emergent herbicide, was applied July 8, 2022 and July 10, 2023 to eliminate broadleaf weed populations. Overhead irrigation was applied as necessary to prevent wilt. Five nitrogen applications (0.5 lbs N/1000 ft^2) were made each year May – September.

A modified Cady Traffic Simulator was used to simulate traffic. This trafficker is equipped with steel plates and studs to resemble cleats worn by NFL football players. Each pass with the trafficker is equivalent to 57 cleat marks per square foot, which is the average traffic intensity between the 40 - yard lines and between the hashes. Five simulated traffic events were applied weekly, for a total of 30 simulated traffic events each year. Traffic was initiated August 23, 2022 after each plot had established to at least 90% green coverage. Traffic was applied the following year, beginning August 21, 2023.

Results to date indicate all zoysiagrass plots fell below 60% green coverage after 30 simulated traffic events. After 30 simulated traffic events, the variety with poorest traffic tolerance was experimental entry 6942-22 (21.08% green coverage). Palisades, a check plot, had a similar wear tolerance (21.4%) after 30 simulated traffic events. The best traffic tolerance after 30 simulated traffic events, was held by experimental plot 6792-44 (58.08% green coverage).

Future expectations for the project include zoysiagrass plots becoming very compacted, which will adversely affect plant health over time. All plots have received a total of 60 simulated traffic events to date, without any cultivation, such as aerification.

Figure 1. A lightbox was used to capture images for digital image analysis monthly and after each ten games of simulated traffic. Image from April 20, 2023.



Figure 2. A modified Cady Traffic Simulator was used to apply five simulated traffic events each week until thirty games were reached during August 2022 and August 2023.



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 consistently produced higher quality under shaded conditions compared to a GDD application timing (data reported in 2023).
- Studies to investigate the effects of water quality on PGR efficacy were initiated in 2023. Trinexapac-ethyl (Primo Maxx) effectively suppressed the growth of TifEagle bermudagrass when mixed with various divalent cations. None of the divalent cations caused a reduction in Primo Maxx efficacy indicating that golf course superintendents can effectively tank-mix macronutrients and micronutrients with trinexapac-ethyl without reducing PGR efficacy.

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.

Golf course superintendents are also curious about the effect of Ca, Mg, Mn, Zn, and Fe on the efficacy of trinexapac-ethyl applications used to regulate growth on golf course putting greens. It is well-known that hard water with high concentrations of Ca, Mg, and Fe can antagonize efficacy of certain herbicides (Patton et al., 2016) but mineral effects on PGRs has received limited investigation. Superintendents often tank-mix micronutrients such as Fe, Mn and Zn with their PGR application. All of these minerals (Ca, Mg, Mn, Zn, and Fe) are divalent cations with chemistry capable of reducing the effectiveness of many PGRs and herbicides.

Study Methods:

A greenhouse study was conducted at the Purdue University (West Lafayette, IN) to determine the influence of Ca, Mg, Fe, Zn, and MN on the efficacy of trinexapac-ethyl for regulating growth of TifEagle bermudagrass. TifEagle was established in greenhouse pots (1050 cm^3 volume) filled with USGA topdressing sand. Pots were established using stolons in the greenhouse to provide 100% turfgrass coverage before initiating experiments. Plots were irrigated using an overhead irrigation system with nutrients delivered weekly using a complete nutrient solution. Plots were mowed at 5 mm using electric clippers (Premier clippers and shears, Washington, IA) and were maintained at 29/23 C day/night temperature under conditions of natural lighting supplemented with metal halide bulbs. After establishment, plots were treated with seven treatments (Table 1) on July 31, 2023 and arranged in a completely randomized design with six replications.

Treatment solutions were mixed in the following order: deionized water added first, cations added to the solution next for treatments 2-6, and the plant growth regulator added last in treatments 1-6 with bottles agitated after each addition. Treatments were applied in an enclosed spray chamber (Generation III research track sprayer, DeVries Manufacturing, Hollandale, MN) at 215 L ha^{-1} via an 8004 EVS noozle (TeeJet, Wheaton, IL). Mowing was withheld for 44 d after application. Growth was qualified 3 times per week (Monday, Wednesday, and Friday) with a modified turf height tester. At the end of the study, clippings were harvested and dried at 60 C for 72 h and weighed. Data were analyzed with SAS version 9.4 (SAS Institute Inc.) utilizing the GLM procedure.

Table 1. Treatment combinations tested in this study. Trinexapac-ethyl was supplied as Primo Maxx.

Trt no.	Treatment	Treatment Description	Rate
1	Primo Maxx	Primo Maxx	3 fl ozA
2	Primo Maxx + Ca	Primo Maxx	3 fl ozA
		$CaCl_2$	881 mg/L
3	Primo Maxx + Mg	Primo Maxx	3 fl ozA
		$MgSO_4$	722 mg/L
4	Primo Maxx + Mn	Primo Maxx	3 fl ozA
		Brandt EDTA chelate	2 qt/A
5	Primo Maxx + Zn	Primo Maxx	3 fl ozA
		Agrisolution Citric-Che Zinc	1 qt/A
6	Primo Maxx + Fe	Primo Maxx	3 fl ozA
		$FeSO_4$	0.5 lb/1000 ft^2
7	Control	Deionized water	

Results:

The nontreated control pot had more growth than pots treated with Primo Maxx at all measured dates Figure 1). Within Primo Maxx treated pots, Primo Maxx alone had the highest cumulative growth, whereas the Primo Maxx combine with iron (ferrous sulphate) had the lowest mean growth (Figure 1) although there were few differences between these treatments (Figure 2).

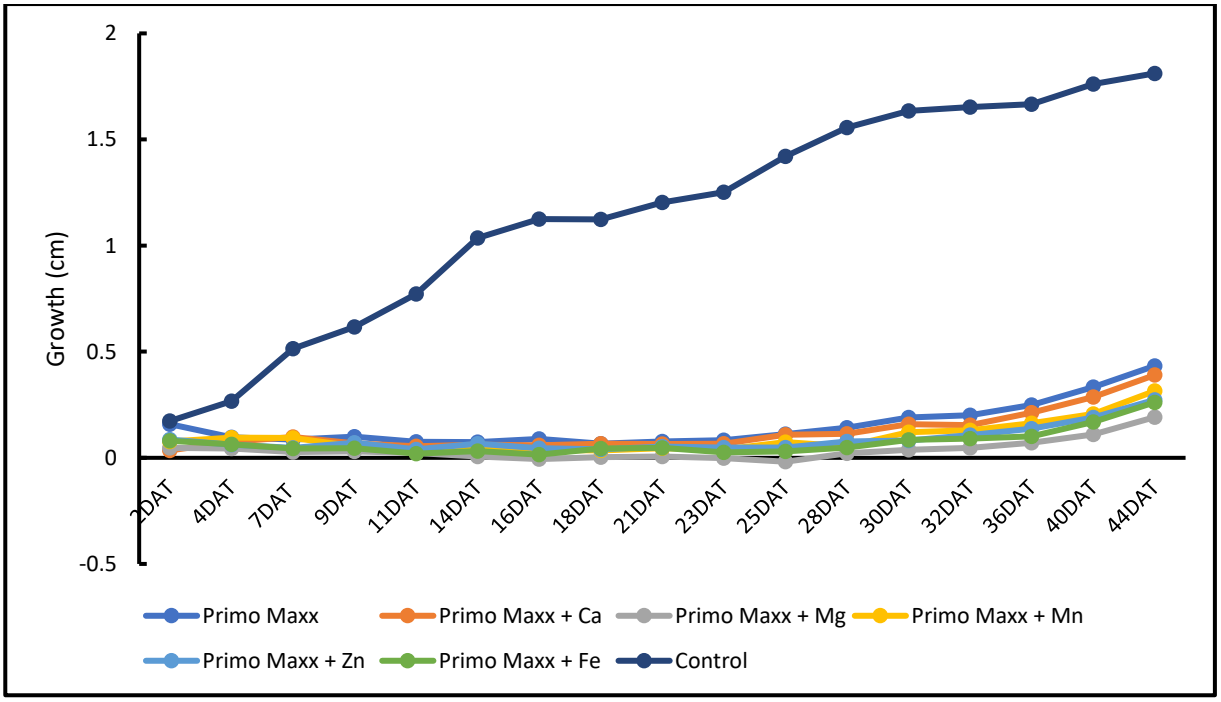


Figure 1. Growth of TifEagle bermudagrass treat with trinexapac-ethyl (Primo Maxx) combinations with various divalent cations.

The control pots also had the highest clipping weight compared to pots treated with trinexapac-ethyl plant growth regulators combine with various divalent cations. Across a 44-day period after treatment, pot treated with Primo Maxx alone or in combination with various divalent cations suppress clipping yield by 67-75% (Figure 2). The highest level of clipping yield suppression was observed in Primo Maxx + Fe followed by Primo Maxx + Mg and Primo Maxx + Zn. Primo Maxx + Ca had the lowest clipping yield suppression.

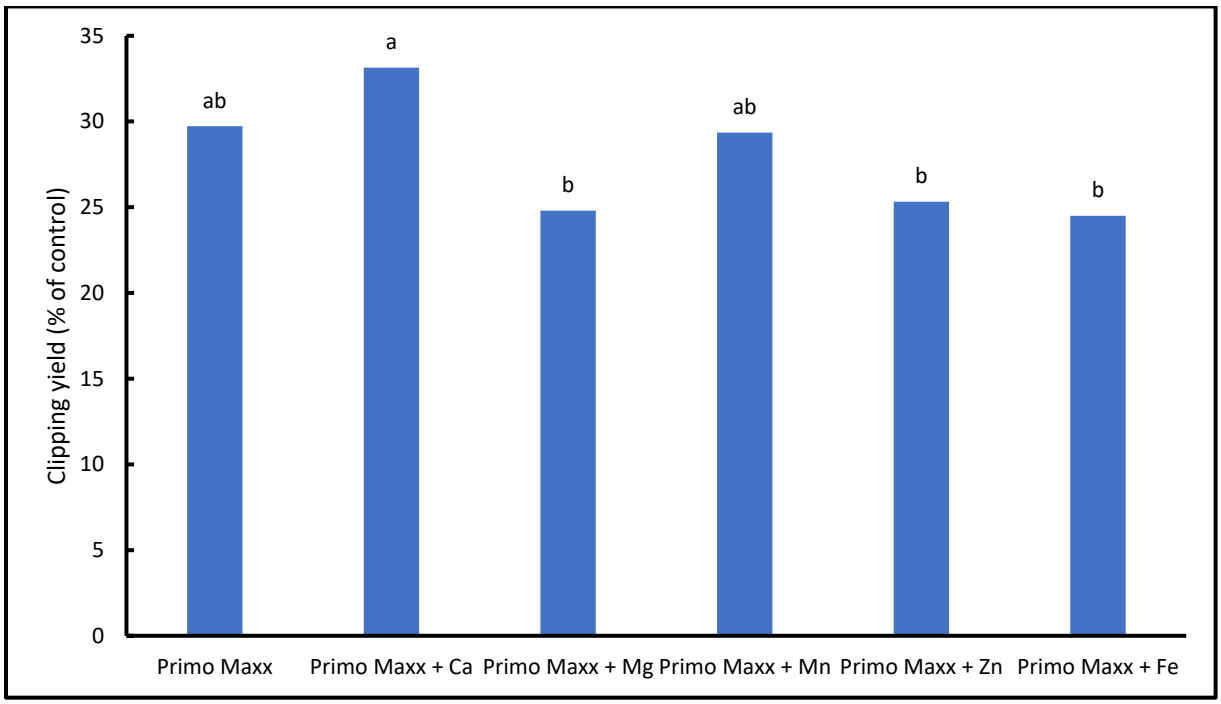


Figure 2. Effect of trinexapac-ethyl (Primo Maxx) combinations with various divalent cations on clipping yield of TifEagle bermudagrass. Means are the percentage of the control. Letters represent statistical difference at p=0.05.

Conclusion

The plant growth regulator trinexapac-ethyl (Primo Maxx) effectively suppressed the growth of TifEagle bermudagrass when mixed with various divalent cations. None of the divalent cations caused a reduction in Primo Maxx efficacy indicating that golf course superintendents can effectively tank-mix macronutrients and micronutrients with trinexapac-ethyl without reducing PGR efficacy.

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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:

- Years 2 & 3 (2022 & 2023) results determined that sequential applications of Proxy in January, February, March, April, and May totaling 25 fl. oz. per year (less than the annual max of 30 fl. oz. per year) provided the greatest seedhead suppression. Note: in Year 1, this treatment was not included as part of the trial.

- In year 3, the treatment mentioned above caused unacceptable yellowing in March, which lasted about 3 – 4 weeks, as a result of the January and February applications (see Image 5, below). This yellowing did not occur in year 2; further investigation is needed.

- In all years (2021, 2022, 2023) the timing of the first application of Proxy applied later (i.e., closer to March), and then followed by March, April and May applications provided greater seedhead suppression than treatments applied earlier (i.e., in October, November, or December) combined with March, April and May applications. The differences from changing the timing of the first application were smaller vs. comparing no early application against making an early application. Note: in Year 3, the October applications were not as effective as in the prior two years.

- 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 are factors. 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 was concluded in the fall of 2023, after three consecutive years of data collection. Research was conducted on a well-established annual bluegrass putting green with 12” of USGA sand over drain tiles and native soil.

The trial was arranged as a randomized complete block design with four replications on plots that measured 5 feet wide by 4 feet long. Proxy timing treatments (Table 1) were initiated in October 2020 and were applied with a CO_2-pressurized bicycle sprayer at 30 psi using TeeJet XR80015 flat fan nozzles. The plots were cored annually in the fall with ½ inch inside diameter hollow tines on a 2” x 2” spacing using a Toro 648 aerifier and holes filled with sand meeting USGA specs (Fazio Brothers Sand Co. Inc., Vancouver, WA). Fungicides were applied year-round to prevent diseases. The plots were fertilized every two weeks during the growing season and as needed during the winter. The plots were mowed at 0.110 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 was 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. Other monthly response variables will include turfgrass health 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 at 5.0 fl. oz. per 1,000 ft^2 made in any month from October to February combined with the standard program of applications made in March, April, and May, increased seed head control vs. the standard program alone (apps made only in 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., January or February) rather than sooner (e.g., October thru December). However, the difference in control from the timing of the first application was smaller versus the difference between making a first application at any time and only applying Proxy in March, April, and May.

- 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 percent 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. However, a plot with 5 percent seed head cover is slightly lower in quality than a plot with 2 percent or less seed head cover, but superintendents may interpret these values differently (i.e., 2 percent is still too high).

- The growing degree day (GDD) treatment (Trt 2) performed similarly to the other two treatments applied in February (Trts 8 & 13). The GDD treatment was initiated 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, thus making the treatment redundant. In Years 2 and 3, this treatment was changed to an early application being made in both January and February combined with the standard program of March, April, and May applications.

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 therefore, will not be able to determine whether this summer application effect occurs in Year 3.

- 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.

- During the summer growing period, there were very little differences in plot quality which were not related to a high percentage of seed heads. In general, we did not

interpret a low percentage of seed head cover (< 1 or 2 percent) to negatively influence plot quality. However, individual golf course superintendents may have a different standard. 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. The lighter turf color in winter resulting from Proxy applications could be mitigated with applications of iron or higher nitrogen rates.

- 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 meaning we did not get any injury.

Year 3 findings:

Note: in Year 3, we continued the new treatment (# 2) from Year 2 where Proxy applications are made in both January and February (i.e., 2 apps), followed by apps made in March, April, and May.

- For the third 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 fewer seed heads than a sequence of three monthly applications in March, April, and May (Tables 9 & 10). These early applications resulted in a 50 to 70 percent reduction in seed heads from approximately 5 percent which occurred on plots treated only in March, April, & May. Note: 5 percent seed head cover is high enough to lower the quality of the plots.

- The trend from the prior two years, which showed that the later the first application is made (i.e., nearer to March vs. in the fall), the better the control of seedheads, continued (Tables 2, 6, & 9). For example, plots treated in February, March, April, and May had the

lowest seed heads with the exception of those plots (Trt 2) that also received an application in January (i.e., they received an application in January, February, March, April, and May).

- In Year 3 only, one of the two sets of October apps (Trt 4) did not perform as well as in the last two years, as it had similar seed head numbers as the plots treated only in March, April, and May. However, the other set of October apps (Trt 9) had a 50% reduction in seed heads vs. the plots treated only in March, April, and May.

- In general, in all years, the differences in seed heads from the timing of the first application (November vs. January, for example) has been much smaller than the difference between making a first application combined with a March, April, and May application versus only applying in March, April, and May. This means that making an early application is much more important than the timing of that application. However, if you are attempting to get the best control, or are not getting satisfactory control, making the first application in February has provided the lowest seed heads every year in the last three years, with the exception of making two early applications in January and February, but that treatment led to some unacceptable yellowing in Year 3 (see below).

- Treatment 2, the new treatment added in Year 2, with Proxy applications made in January, February, March, April, and May, had the lowest level of seed heads for the second straight year. It was roughly 50% better than applications made in February, March, April, and May. But the real reduction was only 0.70 percent, changing from approximately 1.4 percent to 0.70 percent. In other words, there are diminishing returns from this application. Additionally, in Year 3, this treatment caused unacceptable yellowing of the plots in March which lasted 3 – 4 weeks (Image 5). This yellowing did not occur in Year 2 so further investigation is needed.

- Plot quality was slightly reduced with fall and winter applications of Proxy because of Proxy's tendency to lighten turf color (some describe it as a lime green), or slightly yellow the turf color. In the fall and winter, this effect could be mitigated with iron or higher nitrogen rates. In the summer, adding Primo Maxx to the application will improve color. Plot quality in the spring and early summer was negatively impacted by the seed heads. The plots with no Proxy applications had unacceptable turf quality in all years. The plots treated with Proxy only in March, April, and May had acceptable turf quality, but the high number of seed heads did reduce the quality by ½ to a full point during the seed head growth period (roughly, late April through early June).

- Summer applications of Proxy were unnecessary as the percent seed head numbers were so low as not to warrant treatments. By mid-July, almost all the seed heads were gone.

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

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).

			Percent Seed Heads		
Trt #	1st App	Subsequent Apps	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

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

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.)

			Quality 1 – 9; 9 = best		
Trt #	1st App	Subsequent Apps	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

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	

* Summer apps were not made because of turfgrass injury.

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	

* Summer apps were not made because of turfgrass injury.

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	7.3	6.5	5.4	5.4	4.8	4.8	4.6	4.5	4.8	5.3
2	Jan & Feb	Mar, Apr, May	6.5	6.4	7.0	7.0	7.5	7.5	7.5	7.5	7.0	7.1
3	None	Mar, Apr, May	7.0	6.4	6.1	6.4	6.5	6.9	7.3	7.1	7.0	6.7
4	Oct	Mar, Apr, May	6.3	7.0	7.0	6.9	7.1	7.5	7.4	7.4	7.0	7.1
5	Nov	Mar, Apr, May	6.8	6.9	6.9	6.9	7.3	7.4	7.4	7.3	7.0	7.1
6	Dec	Mar, Apr, May	6.8	7.0	7.0	7.0	7.4	7.5	7.5	7.5	7.0	7.2
7	Jan	Mar, Apr, May	6.9	6.8	6.8	6.9	7.5	7.5	7.5	7.5	7.0	7.1
8	Feb	Mar, Apr, May	7.0	6.6	7.0	7.0	7.5	7.5	7.5	7.5	7.0	7.2
9	Oct	Mar, Apr, May + ~~Summer @ 3.3*~~	6.0	6.9	7.0	6.9	7.5	7.5	7.5	7.5	7.0	7.1
10	Nov	Mar, Apr, May + ~~Summer @ 3.3*~~	6.6	7.0	7.1	7.0	7.5	7.4	7.5	7.5	7.0	7.2
11	Dec	Mar, Apr, May + ~~Summer @ 3.3*~~	7.0	7.0	7.0	7.0	7.4	7.5	7.5	7.5	7.0	7.2
12	Jan	Mar, Apr, May + ~~Summer @ 3.3*~~	6.9	6.8	6.5	6.5	7.4	7.5	7.5	7.5	7.0	7.1
13	Feb	Mar, Apr, May + ~~Summer @ 3.3*~~	6.9	6.6	7.0	6.9	7.5	7.5	7.5	7.5	7.0	7.2
		LSD @ .05	**0.35**	**0.34**	**0.28**	**0.36**	**0.48**	**0.13**	**0.19**	**0.18**	**0.12**	
		p-value	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

* Summer apps were not made because of turfgrass injury.

Table 9: Year 3 – Percent Seed Head Cover for the period 3/17 thru 7/21.

Trt #	1st App(s)	Subsequent Apps	03/17	04/25	05/12	05/19	05/26	06/03	06/17	07/03	07/21	Avg
1	Untreated	Untreated	0	2.75	31.25	41.25	41.25	42.50	37.50	26.25	3.50	25.2
2	Jan & Feb	Mar, Apr, May	0	0.01	0.64	0.64	0.39	0.02	0.02	0.02	0.01	0.2
3	None	Mar, Apr, May	0	0.06	4.75	4.75	4.51	1.78	0.46	0.01	0.02	1.8
4	Oct	Mar, Apr, May	0	0.02	5.01	5.01	5.01	0.49	0.34	0.02	0.02	1.8
5	Nov	Mar, Apr, May	0	0.02	2.75	2.63	3.25	0.43	0.58	0.02	0.02	1.1
6	Dec	Mar, Apr, May	0	0.01	2.63	1.85	1.85	0.65	0.24	0.03	0.02	0.8
7	Jan	Mar, Apr, May	0	0.01	1.26	1.77	1.77	0.23	0.07	0.02	0.01	0.6
8	Feb	Mar, Apr, May	0	0.01	0.76	1.39	1.26	0.15	0.09	0.02	0.02	0.4
9	Oct	Mar, Apr, May + Summer @ 3.3	0	0.02	2.13	2.31	2.28	0.68	0.20	0.01	0.01	0.8
10	Nov	Mar, Apr, May + Summer @ 3.3	0	0.01	1.64	2.51	2.40	0.40	0.16	0.02	0.01	0.8
11	Dec	Mar, Apr, May + Summer @ 3.3	0	0.01	2.26	2.14	2.14	0.13	0.12	0.01	0.02	0.8
12	Jan	Mar, Apr, May + Summer @ 3.3	0	0.01	2.26	2.03	2.27	0.15	0.06	0.01	0.01	0.8
13	Feb	Mar, Apr, May + Summer @ 3.3	0	0.01	0.64	1.26	0.89	0.07	0.05	0.01	0.01	0.3
		LSD @ .05	**nv**	**0.20**	**2.92**	**4.10**	**4.18**	**3.86**	**4.97**	**6.37**	**0.40**	

Table 10: Year 3 – Percent Seed Head Cover for the period 3/17 thru 7/21 (without untreated plots).

Trt #	1st App(s)	Subsequent Apps	03/17	04/25	05/12	05/19	05/26	06/03	06/17	07/03	07/21	Avg
2	Jan & Feb	Mar, Apr, May	0	0.01	0.64	0.64	0.39	0.02	0.02	0.02	0.01	0.2
3	None	Mar, Apr, May	0	0.06	4.75	4.75	4.51	1.78	0.46	0.01	0.02	1.8
4	Oct	Mar, Apr, May	0	0.02	5.01	5.01	5.01	0.49	0.34	0.02	0.02	1.8
5	Nov	Mar, Apr, May	0	0.02	2.75	2.63	3.25	0.43	0.58	0.02	0.02	1.1
6	Dec	Mar, Apr, May	0	0.01	2.63	1.85	1.85	0.65	0.24	0.03	0.02	0.8
7	Jan	Mar, Apr, May	0	0.01	1.26	1.77	1.77	0.23	0.07	0.02	0.01	0.6
8	Feb	Mar, Apr, May	0	0.01	0.76	1.39	1.26	0.15	0.09	0.02	0.02	0.4
9	Oct	Mar, Apr, May + Summer @ 3.3	0	0.02	2.13	2.31	2.28	0.68	0.20	0.01	0.01	0.8
10	Nov	Mar, Apr, May + Summer @ 3.3	0	0.01	1.64	2.51	2.40	0.40	0.16	0.02	0.01	0.8
11	Dec	Mar, Apr, May + Summer @ 3.3	0	0.01	2.26	2.14	2.14	0.13	0.12	0.01	0.02	0.8
12	Jan	Mar, Apr, May + Summer @ 3.3	0	0.01	2.26	2.03	2.27	0.15	0.06	0.01	0.01	0.8
13	Feb	Mar, Apr, May + Summer @ 3.3	0	0.01	0.64	1.26	0.89	0.07	0.05	0.01	0.01	0.3
		LSD @ .05	**nv**	**0.014**	**2.787**	**ns**	**ns**	**0.830**	**ns**	**ns**	**0.009**	
		p-value	**na**	**0.00**	**0.0371**	**0.0661**	**0.080**	**0.0123**	**0.237**	**0.359**	**.0145**	

Table 11: Year 3 – Plot Quality – October thru April

Trt #	1st app (s)	Subsequent Apps	10/20	11/18	12/16	01/23*	02/21	03/17	04/25
1	Untreated	Untreated	7.0	7.0	6.0	7.8	6.8	7.5	7.0
2	Jan & Feb	Mar, Apr, May	7.0	7.0	6.3	7.6	6.5	5.3	7.0
3	None	Mar, Apr, May	7.0	7.0	6.5	7.9	6.8	7.3	7.0
4	Oct	Mar, Apr, May	7.0	6.0	6.0	7.5	6.0	6.9	7.0
5	Nov	Mar, Apr, May	7.0	6.8	7.0	7.0	6.4	6.9	7.0
6	Dec	Mar, Apr, May	7.0	6.8	6.3	7.5	6.4	7.0	7.0
7	Jan	Mar, Apr, May	7.0	6.9	6.5	7.8	6.8	7.0	7.0
8	Feb	Mar, Apr, May	7.0	6.9	6.5	7.5	6.3	6.9	7.0
9	Oct	Mar, Apr, May + Summer @ 3.3	7.0	6.0	6.0	7.4	6.3	7.0	7.0
10	Nov	Mar, Apr, May + Summer @ 3.3	7.0	7.0	7.0	7.1	6.3	7.0	7.0
11	Dec	Mar, Apr, May + Summer @ 3.3	7.0	7.0	6.0	7.0	6.3	7.0	7.0
12	Jan	Mar, Apr, May + Summer @ 3.3	7.0	7.0	6.5	7.8	6.9	6.9	7.0
13	Feb	Mar, Apr, May + Summer @ 3.3	7.0	6.9	6.4	7.6	6.8	6.9	7.0
		nv	**nv**	**0.24**	**0.54**	**0.28**	**0.34**	**0.26**	**nv**
		na	na	0.000	0.002	0.000	0.000	0.000	na

* 0.50 lbs. of nitrogen per 1,000 sq. ft. was applied on 12/9 which greatly improved turf quality on January 23rd.

Table 12: Year 3 – Plot Quality – May thru August

Trt #	1st app (s)	Subsequent Apps	05/12	05/19	05/26	06/03	06/17	07/03	07/21	08/21
1	Untreated	Untreated	5.4	5.4	5.4	5.5	5.6	6.0	7.0	7.5
2	Jan & Feb	Mar, Apr, May	7.8	7.5	7.6	7.5	7.5	7.5	7.3	7.5
3	None	Mar, Apr, May	7.0	7.0	7.0	7.0	7.3	7.5	7.0	7.5
4	Oct	Mar, Apr, May	7.4	7.1	7.1	7.4	7.1	7.5	7.1	7.5
5	Nov	Mar, Apr, May	7.4	7.0	7.1	7.4	7.4	7.5	7.3	7.5
6	Dec	Mar, Apr, May	7.3	7.3	7.3	7.4	7.4	7.5	7.1	7.5
7	Jan	Mar, Apr, May	7.6	7.4	7.4	7.5	7.5	7.5	7.1	7.5
8	Feb	Mar, Apr, May	7.8	7.4	7.4	7.5	7.5	7.5	7.0	7.5
9	Oct	Mar, Apr, May + Summer @ 3.3	7.0	7.1	7.0	7.4	7.5	7.5	7.0	7.5
10	Nov	Mar, Apr, May + Summer @ 3.3	7.4	7.4	7.3	7.3	7.4	7.5	7.0	7.4
11	Dec	Mar, Apr, May + Summer @ 3.3	7.4	7.1	7.3	7.5	7.5	7.5	7.0	7.5
12	Jan	Mar, Apr, May + Summer @ 3.3	7.3	7.3	7.1	7.4	7.5	7.5	7.0	7.5
13	Feb	Mar, Apr, May + Summer @ 3.3	7.8	7.5	7.6	7.4	7.5	7.5	7.1	7.4
		LSD @ .05	**0.42**	**0.37**	**0.36**	**0.31**	**0.22**	**0.28**	**ns**	**ns**
		p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.3403	0.4685



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.



Image 5 (Yr. 3): Turfgrass yellowing on Mar 22nd, 2023, from Proxy applications made on Jan 21st and Feb 27th on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.



Image 6 (Yr. 3): Two Untreated plots (left side) with excessive annual bluegrass seed heads May 12th on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.



Image 7 (Yr. 3): High quality plot with very few Poa seed heads from Proxy applications made Feb 27th, Mar 16th, and Apr 21st (photo taken May 12th) on an annual bluegrass putting green located at Lewis-Brown Farm in Corvallis Oregon.

USGA ID#: 2023-15-782

Title: Influence of nitrogen rate on growing degree day models for plant growth regulator reapplication interval on annual bluegrass putting greens

Project Leader: Chas Schmid
Co-leader: Alec Kowalewski

Affiliation: Department of Horticulture, Oregon State University

Objectives:

1) Develop a growing degree day model for PGR (trinexapac-ethyl and prohexadione-Ca) application intervals on annual bluegrass putting greens
2) Determine if nitrogen rate has an influence on a growing degree day model for annual bluegrass putting greens

Start Date: May 2023

Project Duration: 3 years, May 2023 to Dec 2023 (year 1 report)

Total Funding: $90,000 ($30,000 per year)

Summary Points:

- Preliminary trends suggest reapplication intervals for PGR varied by month of application, with PGRs applied to annual bluegrass under stressful environmental conditions during July resulting in longer reapplication intervals
- N rate appears to impact the reapplication interval for PGRs, with the lower rate of N having longer reapplication intervals.
- Peak suppression provided by trinexapac-ethyl ranged from 30 to 52% reduction in growth depending on N rate and month.
- Peak suppression provided by prohexadione-Ca ranged from 31 to 52% reduction in growth depending on N rate and month.

INTRODUCTION

Plant growth regulators (PGR) are typically applied to golf course putting greens on a calendar-based schedule, but recently growing degree-day (GDD) models for PGR reapplication interval have been developed to provide season-long growth suppression; including models for applications of trinexapac-ethyl (TE; Primo Maxx 1ME, Syngenta) (Kreuser et al., 2011) and paclobutrazol (Trimmit 2SC, Syngenta) on creeping bentgrass (Agrostis stolonifera L.) putting greens (Kreuser et al., 2018), and TE and paclobutrazol on ultradwarf hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] putting greens (Reasor et al. 2017). However, no previous research has attempted to develop a GDD model for PGRs on annual bluegrass (ABG) putting greens. Instead, superintendents managing ABG putting greens have

relied on GDD models developed for creeping bentgrass as a baseline for reapplication intervals. Thus, development of GDD models for PGR reapplication interval on ABG turf is needed.

The effect of N rate on ABG putting green turf quality, disease susceptibility, and playability has been well documented (Hempfling et al., 2017; Inguagiato et al. 2008; Schmid et al. 2017), but little is known about the effect of N rate on PGRs applied to annual bluegrass. Specifically, if N rate influences PGR reapplication interval for ABG putting greens. It is possible that greater rates of N may require a more frequent (< GDD) reapplication interval compared to lower rates. Further research is needed to determine if GDD models for PGRs should be adjusted based on N rates.

MATERIALS AND METHODS

Site Description and Maintenance

This three-year field trial was initiated in June 2023. The trial is being conducted on a sand-based annual bluegrass research green constructed using the United States Golf Association rootzone specifications (USGA, 2018). The research green is managed to simulate conditions found on golf course putting greens in the Pacific Northwest. Pesticides (except DeMethylation Inhibitors) were applied preventatively to control common disease and insect pests. An onsite Campbell Scientific weather station was used to measure daily air temperatures, which were used to calculate cumulative GDD using methods described by McMaster and Wilhelm (1997), with a base temperature of 0°C and the stipulation that if the daily mean temperature is less than the base temperature, then the GDD for that day is set to zero.

Treatment Design

The treatment design for the trial was adapted from those described by Kreuser et al. (2017) and Reasor et al. (2018). The experiment was designed as 2 by 2 factorial in a randomized complete block design with four replications. Factors include PGR type, either a single application of TE at 0.125 fl oz 1000ft^{-2} or prohexadione-Ca (PH; Anuew, Nufarm) at 0.05 fl oz 1000ft^{-2}, and N rate, either 0.075 or 0.15 lb N 1,000 ft^{-2} every 7-d throughout the trial period (June through September). Multiple runs of the experiment were conducted in 2023, with applications starting on June 13, July 13, and August 15 to plots where no previous PGR application had been made within that season. Two sets of nontreated control plots were included per replication to improve the accuracy of calculating relative clipping yield based on recommendations from Kreuser et al. (2017). Plot size for the trial is 3 x 10 ft (30 ft^2). Plant growth regulator treatments were applied with a CO_2-pressured backpack sprayer using a carrier volume of 2.0 gal 1,000 ft^{-2}.

Data Collection

Clipping collection methods were adapted from those described by Reasor et al. (2018), with clippings collected three times per week (Monday, Wednesday, and Friday) until ≈600 GDD; at which point, clippings were collected once per week until the effects of TE are no longer detectable (≈1000 GDD). Clipping collection and general plot mowing were done using an electric walk-behind greens mower (eFlex 2120, Toro Company), with bench height set at 0.110 inches. The entire plot area was mowed six days per week (except Saturday), including on days when clippings had been collected from treated plots. Sand topdressing was withheld from the trial area to prevent contamination in clipping samples. Any debris was removed from collected clippings prior to weighing.

Statistical Analysis

Relative clipping yield was compared to GDD using an amplitude-dampened sine wave regression model (Kreuser et al. 2017). Data from June, July, and August applications for each PGR and N rate combination were analyzed separately to determine their effect on relative growth suppression and reapplication interval. The nonlinear regression function in SigmaPlot 15 (Systat Software, 2023) was used to fit the amplitude-dampened sinewave model, which is defined by Eq. [1].

$$\text{Relative clipping yield } (\text{g g}^{-1}) = 1 + \text{A} * e^{\left(\frac{GDD}{\text{D}}\right)} * \sin\left(2\pi * \frac{GDD}{\text{B}} + \pi\right) \qquad [1]$$

Where A is the magnitude of the suppression and rebound growth stages, D is the amplitude decay coefficient, GDD is the cumulative GDD since the last PGR application, and B is the period of the model in GDD.

PRELIMINARY RESULTS

Sine wave regression models identified significant relationships between relative clipping yield and GDD for all PGR (TE and PH) and N rate (0.075 and 0.15 lb N 1,000 ft^{-2} wk^{-1}) combinations, with R^2 values ranging from 0.575 to 0.873 (Fig. 1, 2, 3, and 4). Table 1 lists the reduction in growth at peak suppression (%; compared to untreated control), and the cumulative GDD at peak suppression and for reapplication interval. Peak suppression values ranged from 30 to 47% and 28 to 52% reduction in growth compared to untreated plots for TE and PH, respectively (Table 1). No obvious trend in peak suppression values were observed between N rates treatments or month of application during 2023. The cumulative GDD for reapplication of TE and PH varied based on month of application. In June and August of 2023, reapplication interval for PGR ranged from 177 to 275 GDD and 206 to 246 GDD, respectively, (averaged over PGR type and N rate; Table 1). In contrast, the reapplication interval for PGRs during July was greater, ranging from 291 to 366 GDD (averaged over PGR type and N rate). These results suggest that applications of PGRs made to annual bluegrass under stressful

environmental conditions during late summer can result in PGRs taking longer to achieve peak suppression, and thus extending the reapplication interval. Additionally, N rate appears to impact the reapplication interval for PGRs, with the lower rate of N having greater cumulative GDD (longer) between reapplications during July and August of 2023. Further data is needed to confirm these results and provide recommendations for PGR reapplication intervals for annual bluegrass putting greens.

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Table 1. Percentage of peak suppression and the cumulative growing degree days at peak suppression determine using an amplitude-dampened sinewave model of annual bluegrass putting green clipping yields in response to plant growth regulators (PGRs) and nitrogen (N) rates during three months in 2023.

PGR[†]	N rate[‡]	Month	Peak suppression	Peak suppression	Reapplication interval[§]
			%	cumulative $GDD_{0°C}$	
Trinexapac-ethyl	Low	June	30	146	189
		July	52	282	366
		August	40	170	221
Trinexapac-ethyl	High	June	33	212	275
		July	37	224	291
		August	47	159	206
Prohexadione-Ca	Low	June	52	136	177
		July	40	282	366
		August	28	190	246
Prohexadione-Ca	High	June	31	168	218
		July	42	243	316
		August	49	159	206

[†] Trinexapac-ethyl (Primo) applied at 0.125 fl oz 1,000ft^{-2} or Prohexadione-Ca (Anuew) at 0.05 fl oz 1,000ft^{-2}; single applications.

[‡] Nitrogen applied at either a low rate of 0.075 lb N 1,000 ft^{-2} or a high rate of 0.15 lb N 1,000 ft^{-2} every 7-days

[§] Reapplication interval calculated by multiplying the cumulative $GDD_{0°C}$ at peak suppression by 1.3

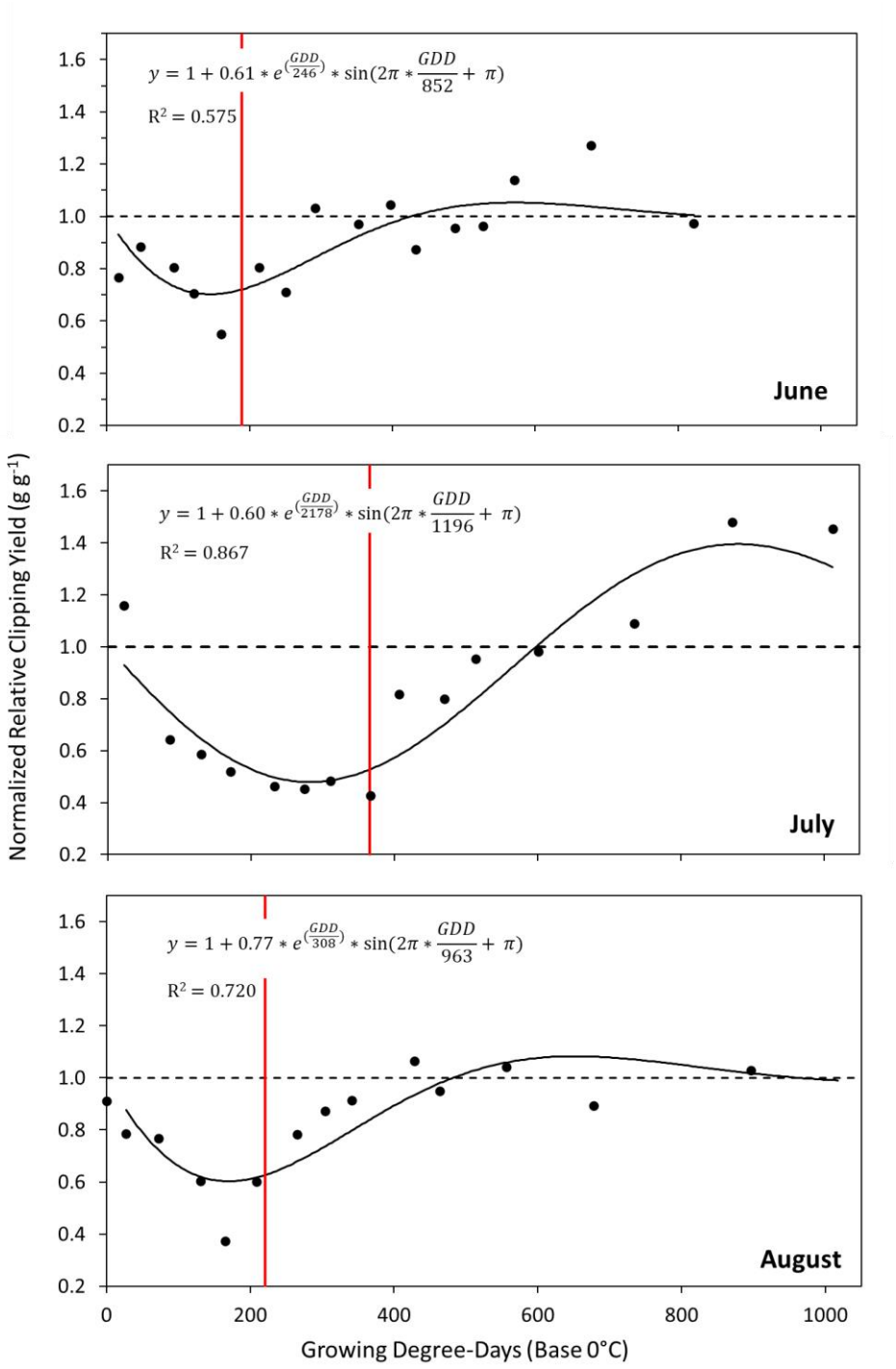


Figure 1. Normalized relative clipping yield of annual bluegrass putting green turf in Corvallis Oregon during June, July, and August, respectively, after a single application of trinexapac-ethyl and weekly applications of nitrogen at 0.075 lb N/1,000 ft^2 (Low N). Equation and R^2 values are from the sinewave model used to calculate relative growth suppression. Vertical red line indicates reapplication interval.

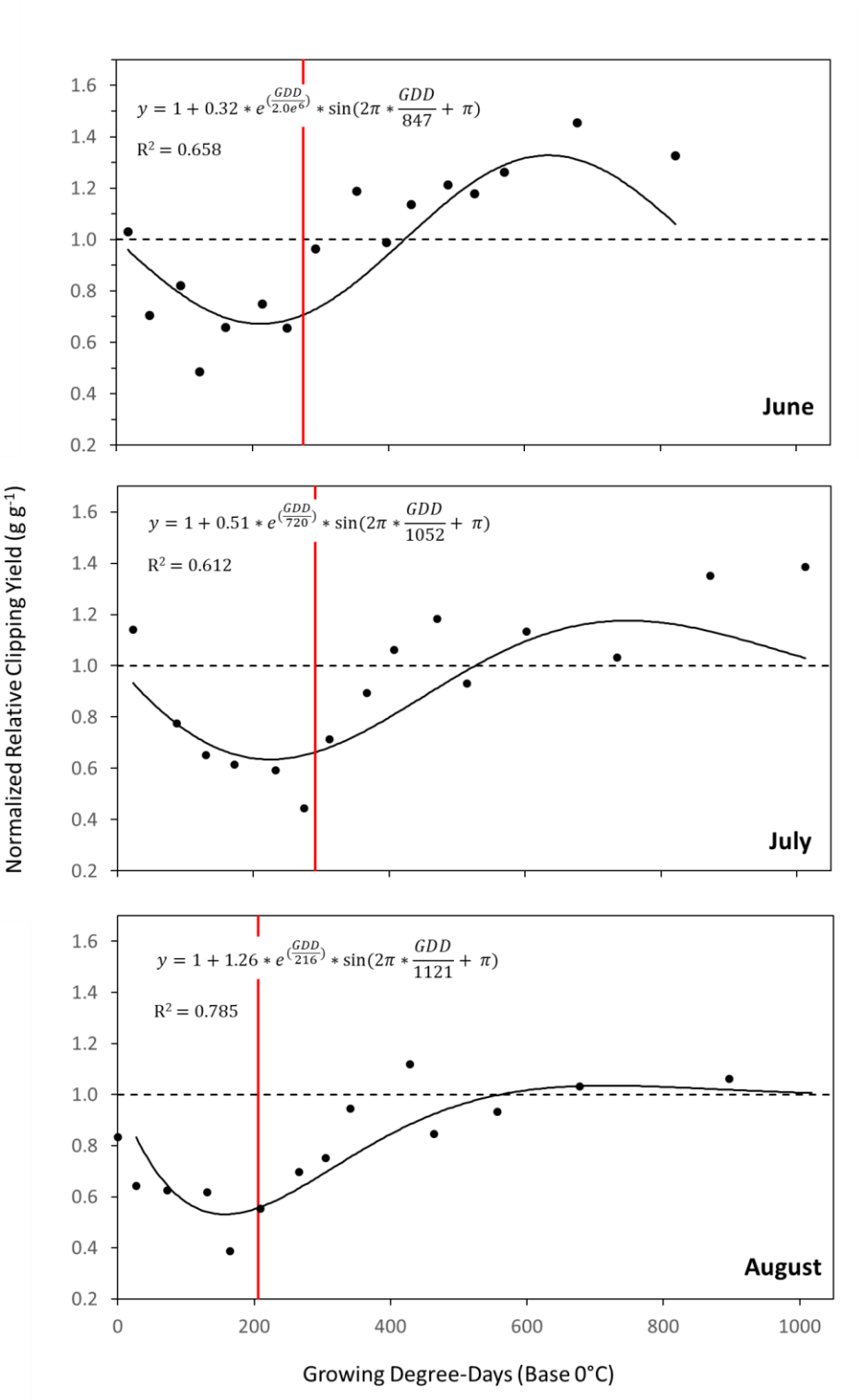


Figure 2. Normalized relative clipping yield of annual bluegrass putting green turf in Corvallis Oregon during June, July, and August, respectively, after a single application of trinexapac-ethyl and weekly applications of nitrogen at 0.15 lb N/1,000 ft^2 (High N). Equation and R^2 values are from the sinewave model used to calculate relative growth suppression. Vertical red line indicates reapplication interval.

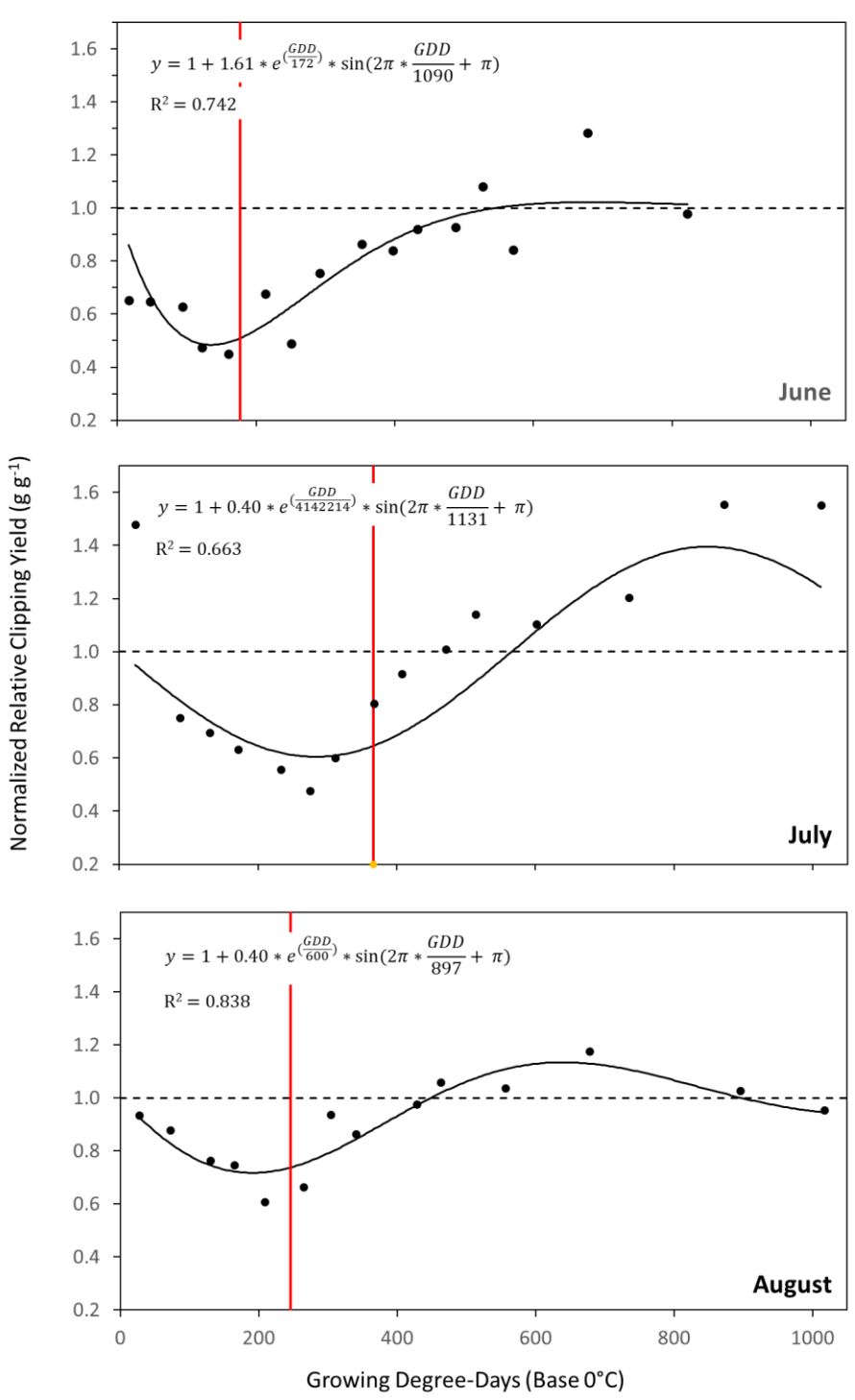


Figure 3. Normalized relative clipping yield of annual bluegrass putting green turf in Corvallis Oregon during June, July, and August, respectively, after a single application of Prohexadione-Ca and weekly applications of nitrogen at 0.075 lb N/1,000 ft^2 (Low N). Equation and R^2 values are from the sinewave model used to calculate relative growth suppression. Vertical red line indicates reapplication interval.

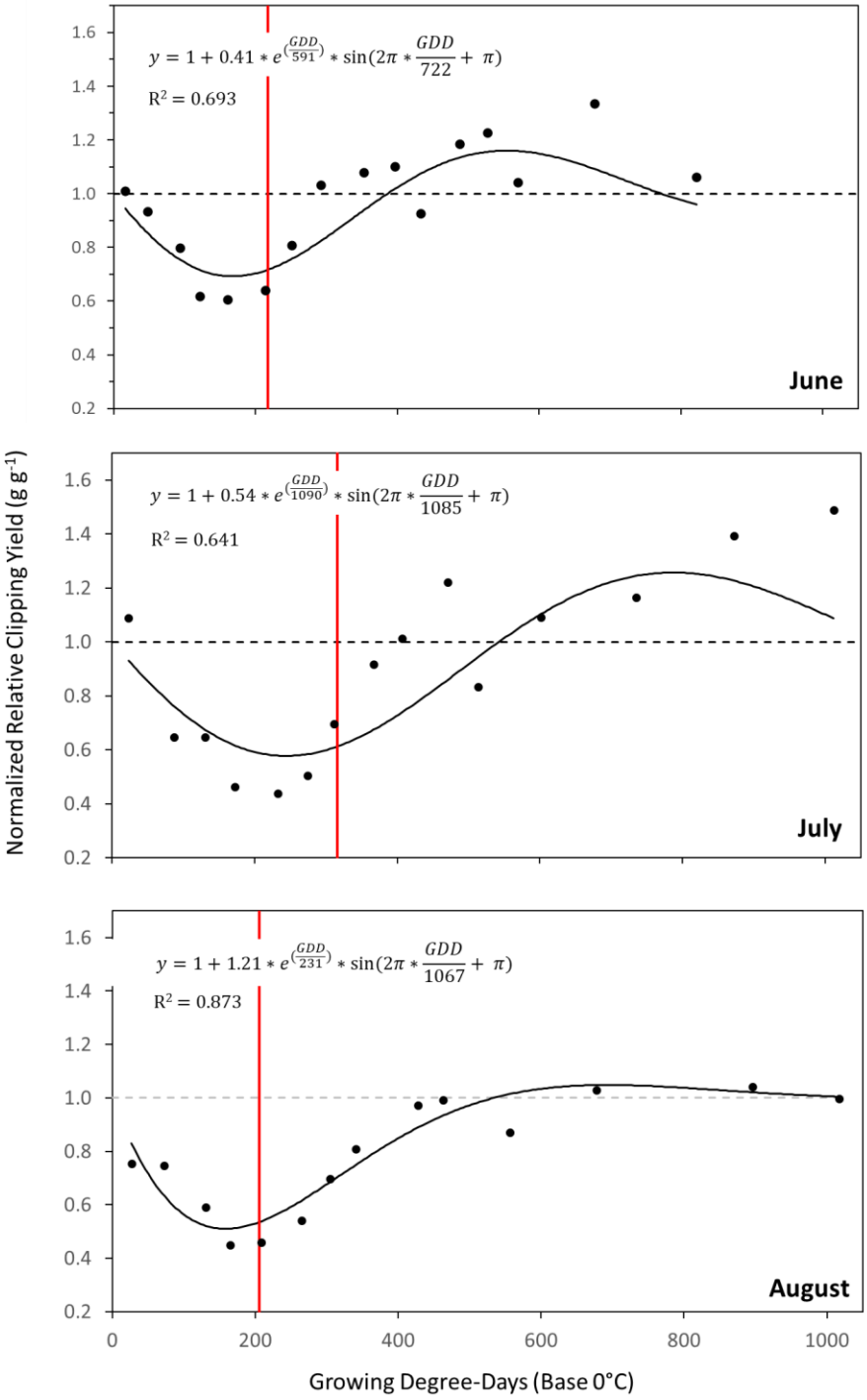


Figure 4. Normalized relative clipping yield of annual bluegrass putting green turf in Corvallis Oregon during June, July, and August, respectively, after a single application of Prohexadione-Ca and weekly applications of nitrogen at 0.15 lb N/1,000 ft^2 (High N). Equation and R^2 values are from the sinewave model used to calculate relative growth suppression. Vertical red line indicates reapplication interval.



Figure 5. Turfgrass leaf clipping samples collected with an electric greens mower equipped with custom clipping collection bucket on an annual bluegrass research green in Corvallis, Oregon. (Photo credit: C. Schmid)



Figure 6. Custom clipping collection bucket improves the efficiency of leaf clipping collection and reduces the amount of error. (Photo credit: C. Schmid)

USGA ID#: 2019-17-687a

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

Project Leaders: Michelle DaCosta[1] and Eric Watkins[2]
Affiliation: University of Massachusetts[1], University of Minnesota[2]

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

Norwegian Institute of Bioeconomy Research[3]

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

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

Summary Points:

- We evaluated a set of 12 creeping bentgrass cultivars for differences in seedling vigor and establishment in response to low temperatures and variable light intensities.
- Exposure of creeping bentgrass seedlings to freezing temperatures temporarily inhibited photosynthesis and growth of some cultivars to a greater extent than others, with cultivars such as Piranha, Declaration, T1, and Penn A4 showing highest tolerance to freezing events.
- Creeping bentgrass cultivars differ in their overall seedling vigor when grown under lower nutrient availability and at lower temperatures.
- Based on replicated field experiments in Minnesota and Norway, using shade cloths to achieve 50 to 90% reductions in light intensity increased photochemical efficiency and growth of creeping bentgrass seedlings during low temperatures typical of spring months.
- Different creeping bentgrass cultivars did not significantly vary in their overall establishment rate, but synthetic permeable cover decreased the time needed to achieve 50% turf coverage during spring establishment.

Summary Text

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

Our research aimed to evaluate factors affecting spring re-establishment of creeping bentgrass, the most widely used turfgrass on golf course greens and fairways in the northern U.S. The objectives were to assess the genetic variability among creeping bentgrass cultivars for post-germination seedling vigor, particularly interactions with low temperatures and variable light intensities typical of spring plantings at northern latitudes. In addition, we examined the effectiveness of chemical priming agents and biostimulants to enhance seedling vigor at low temperatures.

This research will help adjust plant selection and management practices for golf course superintendents to utilize more effective strategies to enhance re-establishment success in the spring months. A unique aspect of our research is establishing an international collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO) and the Scandinavian Turfgrass and Environment Research Foundation (STERF). We have collaboratively defined our research objectives to explore potential barriers more broadly and identify solutions for successful spring re-establishment in northern climates.

We selected 12 creeping bentgrass cultivars representing a range of cold germination or spring green-up traits as identified in our previous research at the University of Massachusetts (UMass) and the University of Minnesota (UMN). Both locations used seed from the same lots shipped by the breeders. We used two general approaches to address our main questions, including testing in climate-controlled growth chambers to simulate early spring stresses as well as field locations at UMass, UMN, and Norway to achieve natural stress conditions.

Controlled Environment Experiments

Although creeping bentgrass is known to have excellent freezing tolerance at the mature plant stage, we do not have a good understanding of whether exposure to freezing temperatures at the early seedling stage impacts overall plant vigor and establishment rate for this species. Therefore, our first sets of climate-controlled experiments were designed to test the post-germination seedling cold tolerance of the 12 bentgrass cultivars exposed to simulated overnight freezing temperatures. Creeping bentgrass seedlings were germinated and grown in pots filled with USGA soil at 15°C (59°F) for three weeks and then exposed to -5°C (23°F) for 8 hours during the dark period. Plants were then recovered at 15°C for three days, and visual quality was used to assess presence of injury symptoms such as leaf discoloration and wilting. Photosynthetic efficiency based on chlorophyll fluorescence imaging was used as an additional screening tool to detect potential damage to

photosynthetic machinery in response to freezing events. Following freezing at -5°C, the cultivars with the highest visual quality and photosynthetic efficiency included Piranha, Declaration, T1, and Penn A4, while some visual injury was observed for cultivars Barracuda, Memorial, Independence, and Luminary (Figure 1). We found that the level of post-freezing injury was worse when seedlings were returned to conditions simulating a higher daytime light intensity, which negatively impacted the photosynthetic efficiency (Figure 2). Although it is important to note that the seedlings recovered following freezing, the data suggest that exposure to freezing temperatures at the seedling stage may temporarily inhibit photosynthesis and growth of some cultivars to a greater extent than others, especially in combination with higher light intensities.

To gain a better understanding of the interactions of low temperatures and light intensity on seedling establishment, optimization of growth chamber conditions was first required to allow for different light treatments within a single growth chamber. With a maximum chamber light intensity of 800 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), we utilized different shade cloth combinations to achieve variable levels of light intensity reduction while minimizing any effects on canopy temperature underneath the shade cloths. In early spring, light intensity in the field generally peaks around 1,500 µmol m^{-2} s^{-1}, and while our unshaded treatments were about half of this, 800 µmol m^{-2} s^{-1} is well above the amount of light intensity needed to promote efficient photosynthesis in creeping bentgrass. The same 12 creeping bentgrass cultivars were grown for three weeks at 15˚C under a light intensity of approximately 800 µmol m^{-2} s^{-1} PPFD. Using shade cloth, we then exposed plants to combination treatments of low or high temperatures (10 or 20˚C) and three light intensity levels (0, 50%, and 90% reduction of full light intensity in the chamber, 800 µmol m^{-2} s^{-1} PPFD).

The different temperature and light treatment combinations resulted in visible changes in creeping bentgrass seedling growth and development (Figure 3). Under the highest light intensity (0% light reduction, 800 µmol m^{-2} s^{-1} PPFD), the leaves of most creeping bentgrass cultivars exposed to the low temperature of 10˚C exhibited a purple coloration. This response is generally associated anthocyanin pigment accumulation, and is considered a photoprotective mechanism at low temperatures. Cultivars such as ‘Memorial’, ‘Piranha’, and ‘007’ produced the most anthocyanin pigment at 10˚C under 800 µmol m^{-2} $s^{-1,}$ while ‘Penncross’ and ‘Penn-A4’ accumulated the lowest amounts. At this same low temperature of 10˚C, creeping bentgrass seedlings exposed to a 90% reduced light intensity (approximately 100 µmol m-2 s-1 PPFD) exhibited increased growth and higher photochemical efficiency. For cultivars such as Penncross and Memorial, photochemical efficiency levels were the same at 20 and 10˚C, regardless of light intensity. This suggested that covering creeping bentgrass seedlings with 50 to 90% shade cloth before a cold temperature event could decrease the potential for low-temperature photoinhibition. To confirm these findings, the effectiveness of light reduction treatments in field environments was also tested, as described below.

Next we tested whether cultivars responded differently to nutrient availability at the early seedling stage. Seeds of each cultivar were established in petri dishes using an agar-based medium. Two nutrient treatments included a non-amended agar (low nutrient) and a nutrient-amended agar (one-quarter strength Hoagland solution). We maintained the petri dishes with seeds vertically in racks in a growth chamber at either 15°C or 25°C. Seed germination and seedling growth was monitored for approximately three weeks. As expected, nutrient availability significantly impacted seedling growth at the lower temperature of 15°C, with higher nutrient availability resulting in the highest shoot and root growth across all cultivars (Figure 4). Differences in seedling vigor were observed, particularly at low nutrient availability, with the highest shoot and root growth observed for the cultivars Barracuda, Declaration, Luminary, Penncross, Penn A-4, Proclamation, and T-1.

For cultivars exhibiting lower growth rates at low nutrient availability (e.g., 007, Independence, Memorial, and Piranha), the availability of nutrients was critical to achieve higher root and shoot growth rates at the lower temperature of 15°C.

Field Experiments

Field experiments were designed to evaluate the effects of creeping bentgrass cultivar and covering treatments on spring establishment. Bare soil plots were seeded with the 12 bentgrass cultivars when average soil temperatures reached approximately 10°C (8 April, 2021 in South Deerfield, MA and 16 April, 2021 in St. Paul, MN). Following seeding, two covering treatments were applied, which included either a no cover or Evergreen 'Radiant' permeable cover. Covers remained on plots until a minimum of 50% turfgrass cover was achieved.

The use of a permeable cover decreased the time to achieve 50% turfgrass cover by approximately 7 to 12 days, depending on location and regardless of cultivar (Figure 5). This was due to higher soil temperatures achieved under the permeable cover, which were approximately 4 to 5°C higher on average compared to plots with no cover. However, following removal of covers and by the end of the trial assessment period (early June), there were no differences in the turfgrass percent cover based on the use of a covering treatment. Compared to a covering treatment, the effect of creeping bentgrass cultivar did not contribute to large differences in spring establishment rates.

An additional set of experiments was conducted to examine whether difference chemical priming compounds and biostimulants could be used to enhance creeping bentgrass seedling vigor in early spring plantings. Field trials were conducted in 2021 and 2022 at UMass and with collaborators in Landvik, Norway. 'Luminary' creeping bentgrass was seeded when soil temperatures reached 10°C. Treatments were then applied weekly following seedling emergence, and included products containing different formulations of urea, chitin, silicon, salicylic acid, glycine betaine, seaweed extracts, and growth regulators such as gibberellic acid and trinexepac-ethyl. Among the 12 treatments tested over two years and two locations, we did not identify any specific biostimulant or chemical priming compound that consistently enhanced early spring establishment.

Lastly, one of the important findings from this research is the identification of important light intensity interactions for early spring plantings. Based on the initial controlled environment studies, a preliminary field trial was conducted at UMN to test the effects of different shade cloth combinations to achieve 50 or 90% light intensity reductions. We seeded the cultivars Memorial, Penncross, Penn-A4, and Barracuda into pots and then placed them into large plastic tubs filled with USGA soil to buffer soil temperature effects in the field. Similar to growth chamber studies, shade treatment of either 50% or 90% light reduction improved seedling growth and photochemical efficiency compared to plants exposed to full light intensity, with similar responses across the different bentgrass cultivars (Figure 6). This result suggested that reducing light intensity at low temperatures typical of spring plantings could decrease the potential for low-temperature photoinhibition in creeping bentgrass, particularly in more northern climates. Additional research is underway to better understand these light responses and to determine best practices to translate this information for golf turf management.

Figure 1. Response of creeping bentgrass seedlings grown at 15°C (59°F) for three weeks and then exposed to -5°C (23°F) for 8 hours during the dark period. Plants were recovered at 15°C under low or high light intensity for three days, and visual quality was used to assess presence of injury symptoms such as leaf discoloration and wilting.

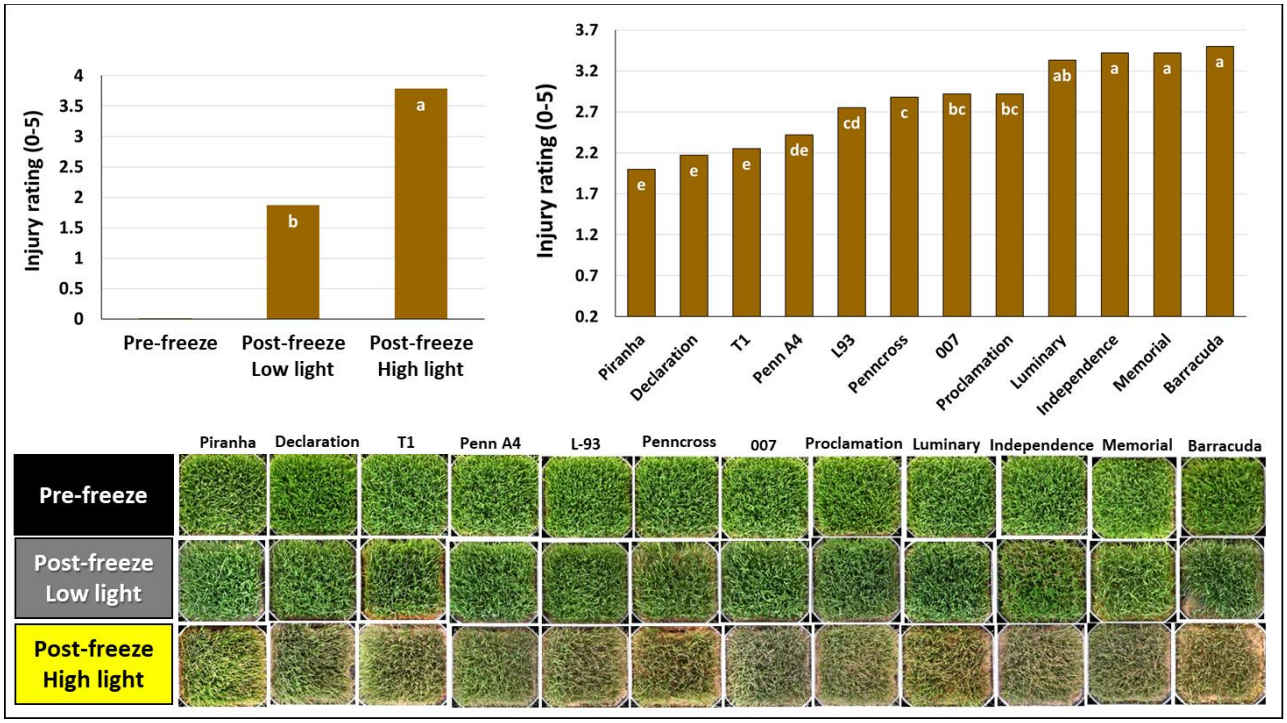


Figure 2. Chlorophyll fluorescence imaging of creeping bentgrass seedlings in response to a simulated overnight freezing event at -5°C (23°F). Images represent measurement of the percentage of photochemical efficiency pre-freezing, and then post-freezing under low or high light conditions. A higher photosynthetic efficiency is indicated by a higher amount of red and orange pixel colors. In contrast, lower photochemical efficiency is indicated by colors of green to dark blue, indicating post-freezing damage to photosynthetic machinery.

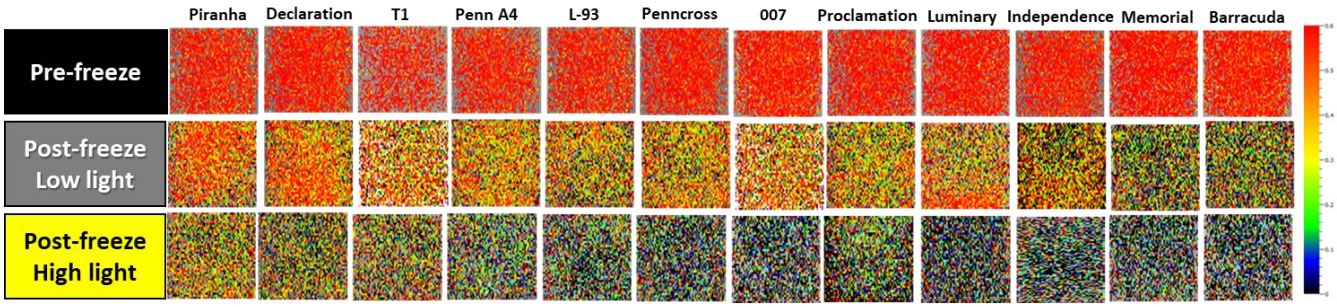


Figure 3. Creeping bentgrass seedlings exposed to combination treatments consisting of high or low temperatures (20 or 10˚C) and three light intensity levels (800, 400, and 100 µmol m^{-2} s^{-1} PPFD). We used light-intensity treatments with shade cloths to reduce 0 to 90 % light. The top panel photos show plants at seven days of treatment. The bottom panel highlights the growth changes for one cultivar, 'Memorial' creeping bentgrass.

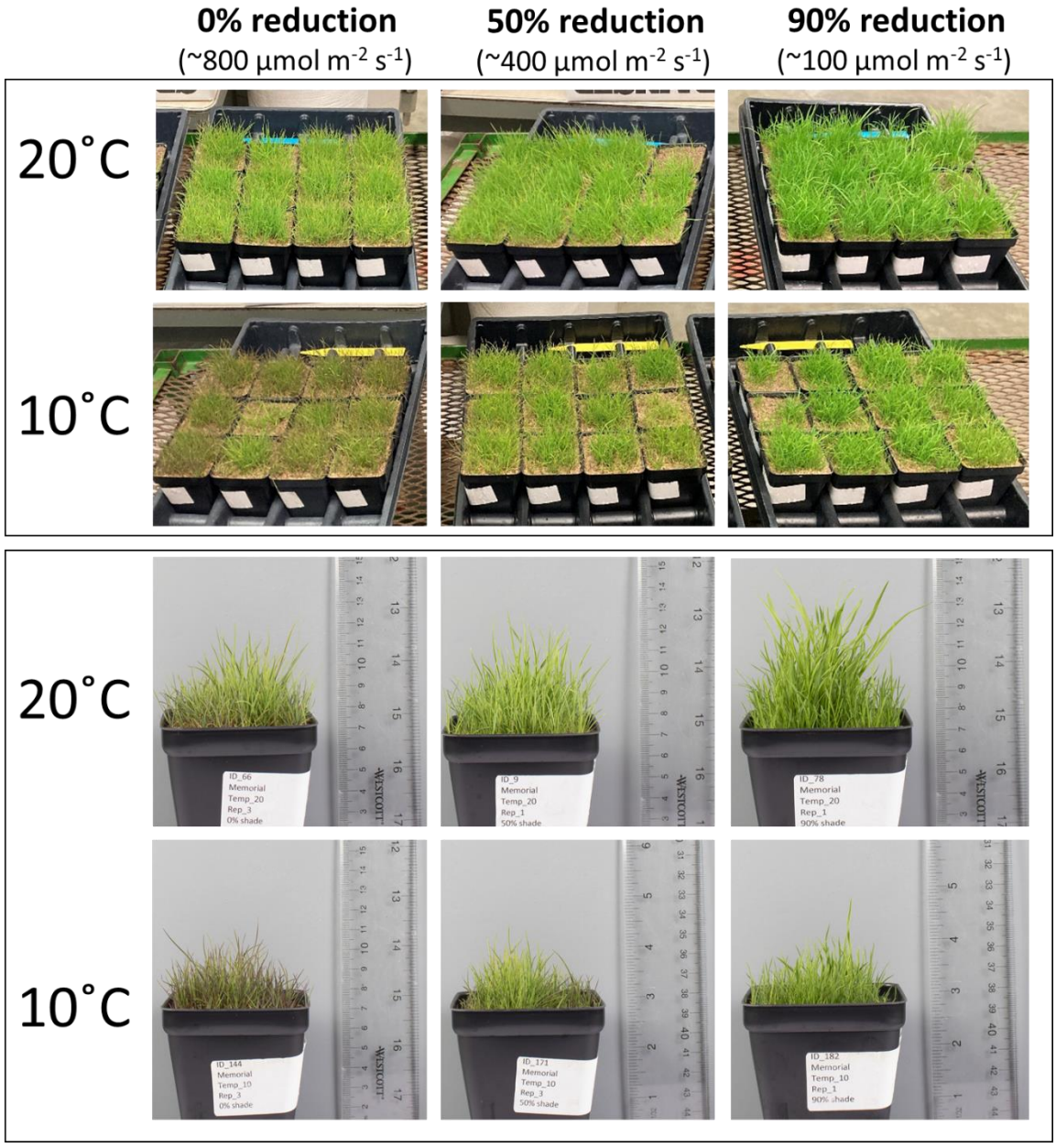


Figure 4. Controlled environment experiments using petri dish assays to assess the seedling vigor of different creeping bentgrass cultivars when grown at 15°C (59°F). Seed were sown into agar media amended with either low nutrient (top two rows) or high nutrient solution (bottom two rows).

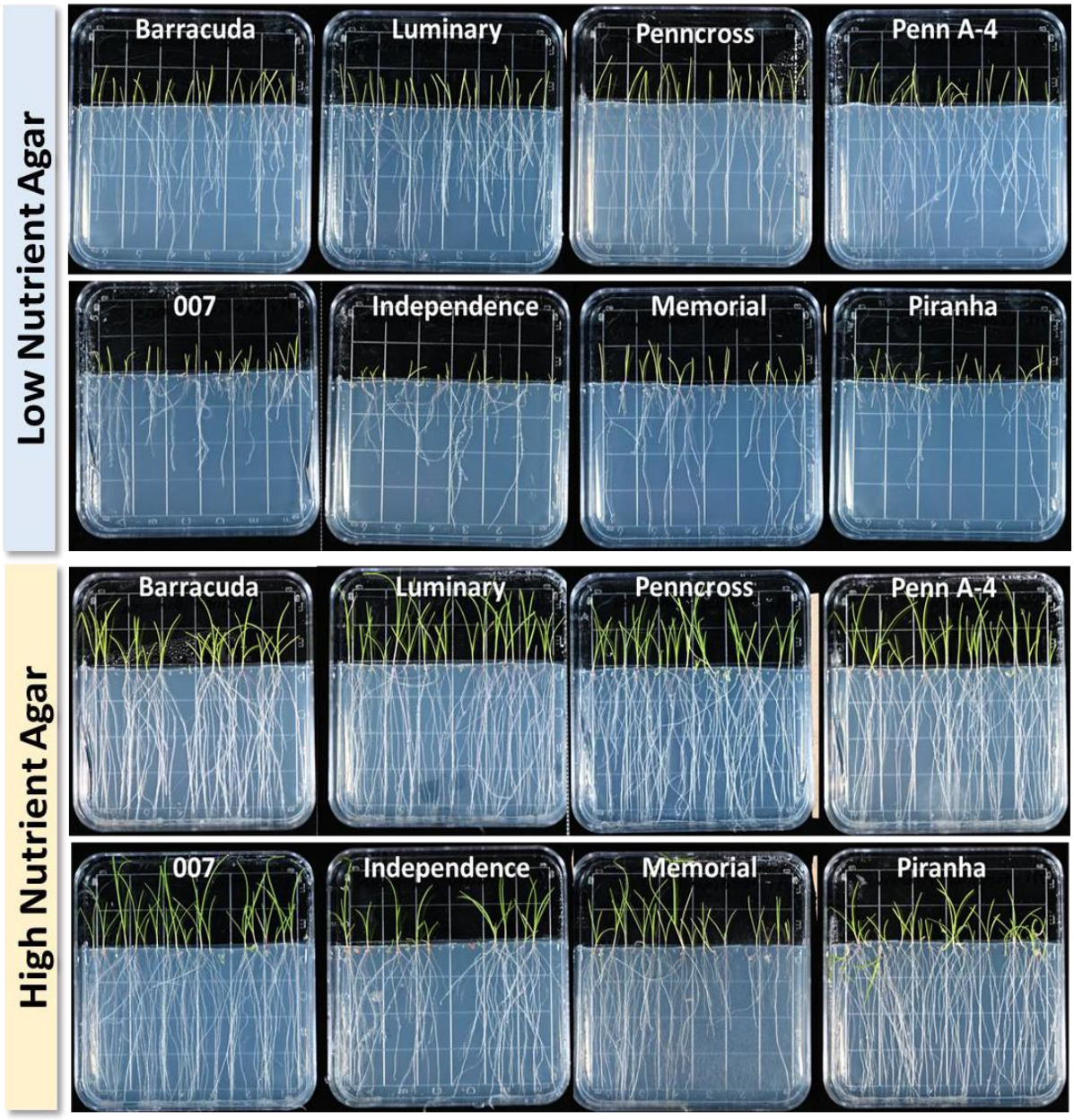


Figure 5. Field trials at UMass (South Deerfield, MA) and UMN (St. Paul, MN) testing the effects of creeping bentgrass genetics and use of a permeable cover on spring establishment. The top panel shows the effects of cover treatments on increasing time to 50% green cover, and bottom panel includes overhead plot images during the trial period at UMN.

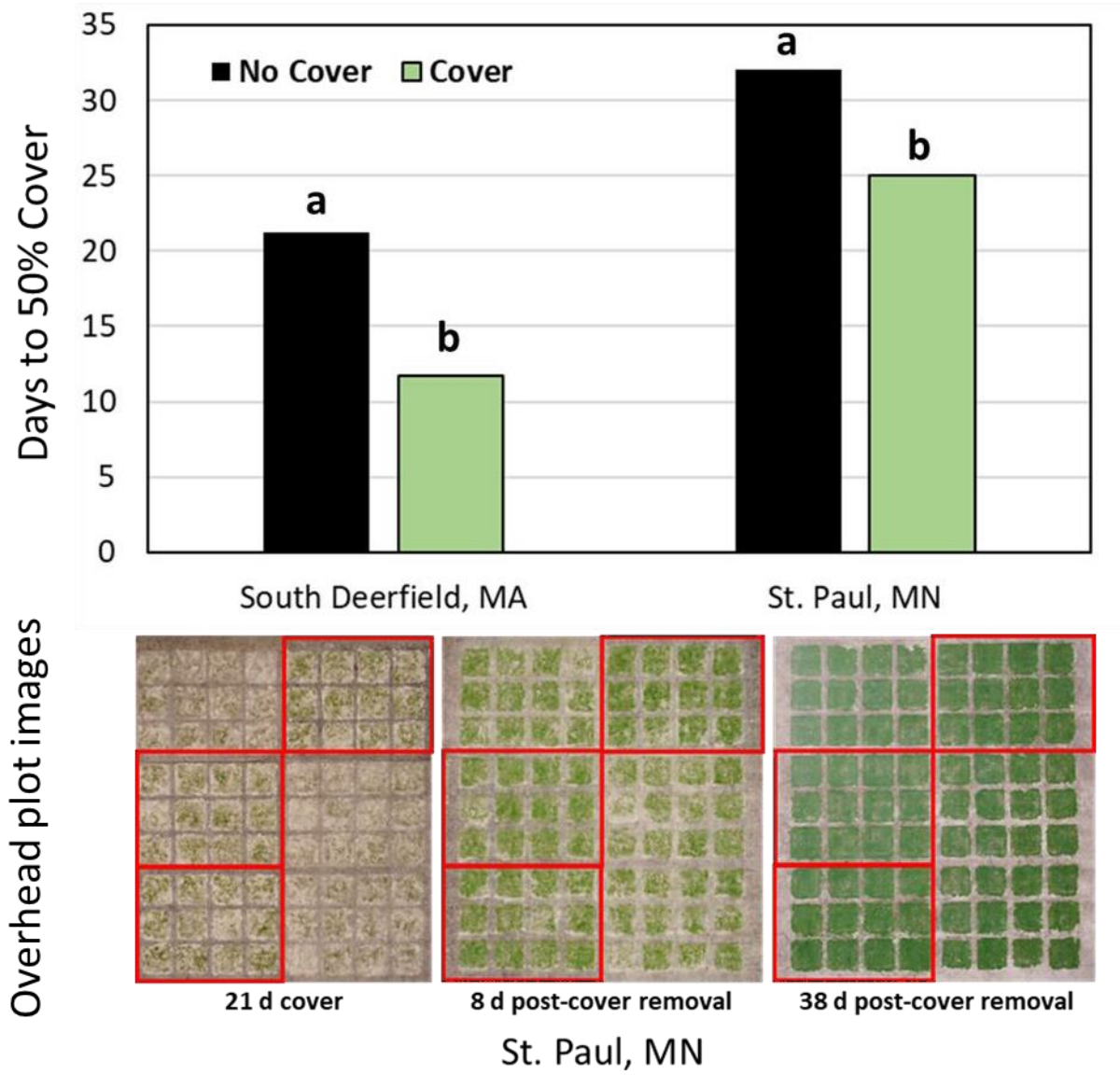
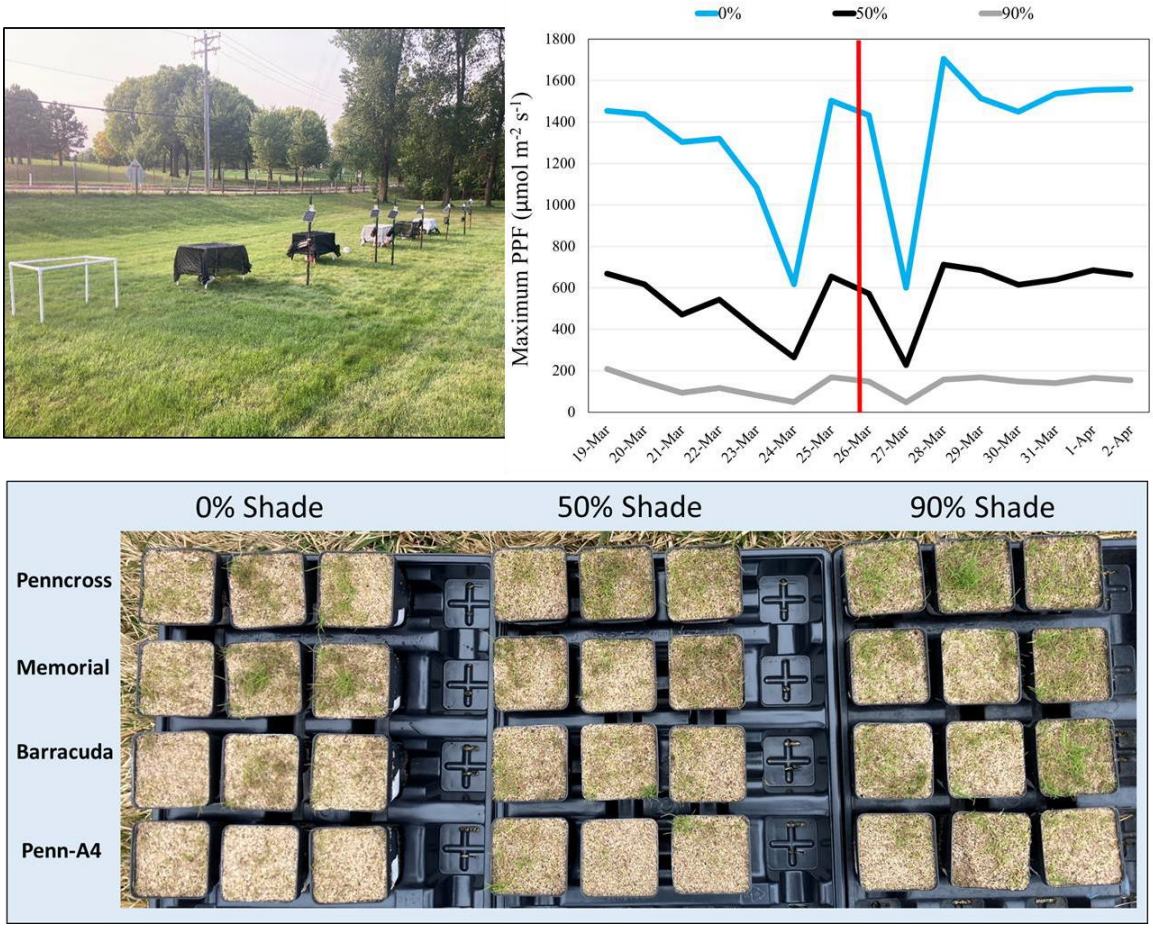


Figure 6. We conducted shade trial field experiments at St. Paul, MN, in March 2021. Shade cloths provided 0, 50%, and 90 % light intensity reduction. The bottom panel shows the effects of shade treatment on the seedling establishment of four creeping bentgrass cultivars, with the 90% shade treatment resulting in the highest turfgrass percent cover.



USGA ID#: 2023-05-772

Title: Effects of Equipment Traffic on Turfgrass During Frost Conditions

Project Leader: Alec Kowalewski

Project collaborators: Chas Schmid, Brian McDonald, Cole Stover, Zach Hamilton

Affiliation: Oregon State University

Objectives:
- Evaluate the effects of daily winter cart traffic applied to a creeping bentgrass, annual bluegrass, and perennial ryegrass fairway.
- Evaluate the effects of daily winter rolling applied to an annual bluegrass putting green.

Start Date: 2023
Project Duration: 3 years (2023, 2024 and 2025)
Total Funding: $30,000 ($10,000 per year)

Summary Points:
- Cart traffic and rolling applied after mornings with freezing soil temperatures resulted in unacceptable turfgrass quality.
- Cart traffic and rolling applied in the morning and afternoon reduced turfgrass quality compared to no traffic or rolling on days when the morning soil temperature was below freezing.
- When morning soil temperatures are below freezing, traffic and rolling applied in the morning resulted in greater turfgrass quality reductions than traffic or rolling applied in the afternoon.
- Soil temperature was the greatest predictor of traffic injury and suggests that cart traffic and rolling should be avoided on days when soil temperatures are below freezing.

Background:

Cool-season (C_3 carbon fixation) turfgrass species such as annual bluegrass (*Poa annua*), creeping bentgrass (*Agrostis stolonifera*) and perennial ryegrass (*Lolium perenne*) are prominent on golf courses in northern climates (Bertrand et al., 2013; Tompkins et al., 2000; Tompkins et al., 2004). Classically, winter injury is associated with freezing temperatures and snow cover, or extremely cold and dry conditions with no snow cover. These conditions lead to turfgrass injury in a variety of ways such as direct freeze damage, crown hydration, desiccation, anoxia, and snow mold. In 2022 a USDA-SCRI grant in the amount of $8 million was award to Dr. Eric Watkins at the University of Minnesota and collaborators from 7 other universities in the United States, and Norway, to explore solutions to crown hydration, anoxia, and freezing temperatures (University of Minnesota Department of Horticultural Science, 2022). A cool weather concern not included in this exploration is the effects of traffic during frost conditions.

Frost is a concern on northern and transition zone golf courses in the U.S. during the fall, winter and spring (Ackerson, et al., 2015), as well as places with moderate winter conditions like the coastal Pacific Northwest and southern U.S which allow for continued play year-round. These areas alone account for 10,004 golf courses according to the 2017 Golf Course Environmental Profile (GCSAA, 2017). This concern has become more of an issue recently with the surge in golf since the COVID pandemic, and the high demand for tee times, even during winter months. The United States Golf Association (USGA) currently has conservative recommendations with respect to frost delays, suggesting significant delays on mornings with frost to avoid turfgrass damage; but it is not well understood what causes turfgrass injury, and the environmental conditions necessary for damage (USGA, 2015; USGA 2018; USGA Green Section Record, 2021). Current recommendations are to delay the start of golf until after the frost has melted. However, there has been significant pushback from golfers who are skeptical as to whether these delays are truly necessary. It is also poorly understood how turfgrass species, mowing height, and source of traffic (foot traffic, cart traffic or maintenance equipment) affect turfgrass injury during frost. Frost delays translate to significant reductions in revenue and valuable maintenance time which is often early in the morning prior to golfer arrival.

In response to these questions, a series of preliminary studies were conducted at Oregon State University, Corvallis, OR in February of 2022 at the request of USGA Agronomy and Research staff. Results from these initial explorations determined that foot traffic, equivalent to 16 golfers on a putting green, applied during eight frost events in February did not produce visual annual bluegrass damage, or reductions in normalized difference vegetation index (NDVI) values. Findings from this initial work determined that as little as one pass of cart traffic during a frost event produced noticeable damage on a creeping bentgrass fairway. Considering these intial results, further exploration into the effects of golf cart, and golf maintenance equipment traffic, during frost is warranted. Therefore the objectives of this project are the following: Evaluate the effects of daily winter cart traffic applied to a creeping bentgrass, annual bluegrass, and perennial ryegrass fairway, and rolling applied to an annual bluegrass putting green.

Year 1 Results:

Effects of daily winter cart traffic on creeping bentgrass

On the creeping bentgrass fairway, frost was observed on January 10, 11, 16, 30 and 31, and daily from February 1 to 17, 2023. On these dates frost was observed at 8:00 am and not 1:00 pm. Frost events were followed by reductions in creeping bentgrass quality when cart traffic was applied at 8:00 am and 1:00 pm (Figure 1; Image 1 and 2). On a few events in mid-February when the bentgrass was showing improved turfgrass quality, the afternoon traffic resulted in higher turf quality than the morning traffic. In late February no differences were observed between the 8:00 am and 1:00 pm traffic treatments. Correlation analysis between turfgrass quality and environmental factors determined that morning soil temperature was the most effective parameter for predicting bentgrass cart traffic injury (Figure 2). Specifically, when morning soil temperatures are less than 32°F, traffic applied in the morning or afternoon will result in bentgrass injury at unacceptable levels. The presence or absence of frost,

afternoon soil temperature, morning or afternoon surface temperature, and morning or afternoon volumetric water content were poorly correlated with turfgrass quality and thus not good predicters of cart traffic injury.

Effects of daily winter cart traffic on annual bluegrass

On the annual bluegrass fairway, frost was observed daily from January 30 to February 17, 2023. On these dates frost was observed at 8:00 am and not 1:00 pm. Frost events were followed by reductions in annual bluegrass quality when cart traffic was applied at 8:00 am and 1:00pm (Figure 3; and Image 3 and 4). From January 25 to Feb 13 the 8:00 am cart traffic regularly reduced annual bluegrass turfgrass quality compared to the 1:00 pm cart traffic. Again, correlation analysis determined that morning soil temperature was the most effective parameter for predicting annual bluegrass cart traffic injury (Figure 4). Again, when morning soil temperatures are less than 32°F, traffic applied in the morning or afternoon will result in unacceptable injury.

Effects of daily winter cart traffic on perennial ryegrass

On the perennial ryegrass fairway, frost was observed on January 16, and January 30 to February 17, 2023. On these dates frost was observed at 8:00 am and not 1:00 pm. Frost events were followed by reductions in perennial ryegrass quality when cart traffic was applied at 8:00 am and 1:00 pm (Figure 5). From January 25 to Feb 13 the 8:00 am cart traffic regularly reduced perennial ryegrass turfgrass quality compared to the 1:00 pm cart traffic. Again, correlation analysis determined that morning soil temperature was the most effective parameter for predicting perennial ryegrass cart traffic injury; however, with perennial ryegrass, unacceptable injury occurred when traffic was applied at morning temperatures ≤ 35°F (Figure 6).

Effects of daily winter rolling on annual bluegrass

On the annual bluegrass putting green, frost was observed on January 9 and 10, and daily from January 30 to 17, 2023. On these dates frost was observed at 8:00 am and not 1:00 pm. Frost events were followed by reductions in annual bluegrass quality when rolling was applied at 8:00 am and 1:00pm (Figure 7; Image 5 and 6). From January 25 to Feb 17 the 8:00 am rolling regularly reduced annual bluegrass turfgrass quality compared to the 1:00 pm rolling. Again, correlation analysis determined that morning soil temperature was the most effective parameter for predicting annual bluegrass rolling injury (Figure 8). With annual bluegrass, unacceptable injury occurred when rolling was applied at morning temperatures ≤ 32°F. .

Future Research:

Morning and afternoon cart traffic treatments will be applied to the creeping bentgrass, annual bluegrass and perennial ryegrass fairways again from January 2 to February 28, 2024, and 2025. Rolling applications on the annual bluegrass putting green will also be repeated. In 2024 and 2025 daily morning and afternoon foot traffic will be applied to the creeping bentgrass, and perennial ryegrass fairways (in the preliminary trial, foot traffic on these species was not

evaluated). Weather data will be collected in 2024 and 2025, and compared/correlated with turfgrass injury.

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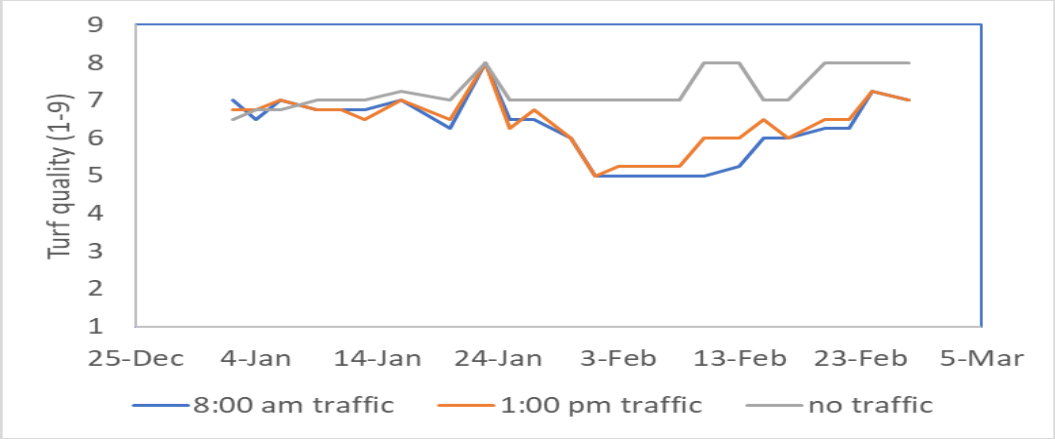


Figure 1: Creeping bentgrass fairway turf quality affected by cart traffic applied at 8:00 am or 1:00 pm over time in Corvallis, OR from Jan 2 to Feb 28, 2023.

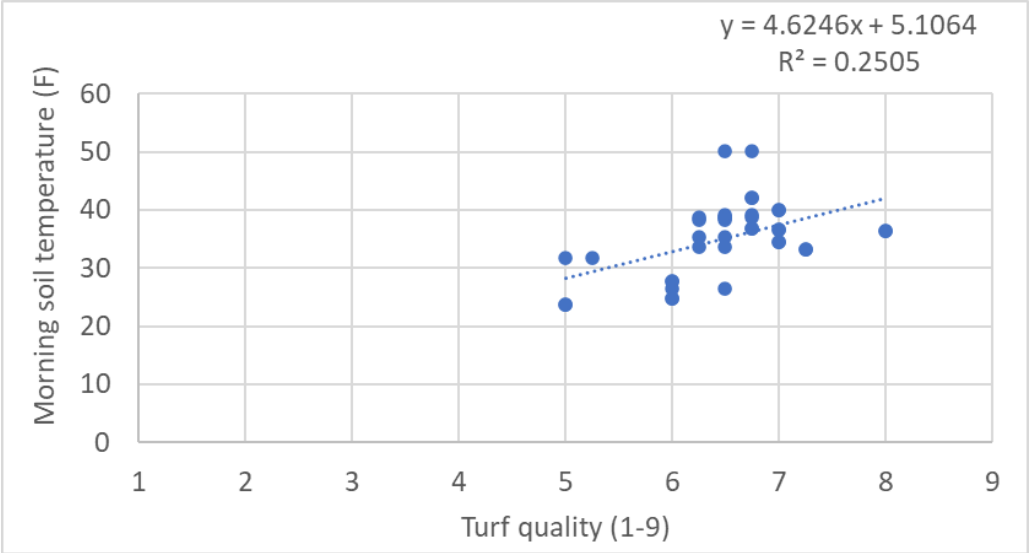


Figure 2: Relationship between morning soil temperature and creeping bentgrass fairway turf quality when traffic was applied at 8:00 am or 1:00 pm (control treatment excluded) in Corvallis, OR from Jan 2 to Feb 28, 2023.

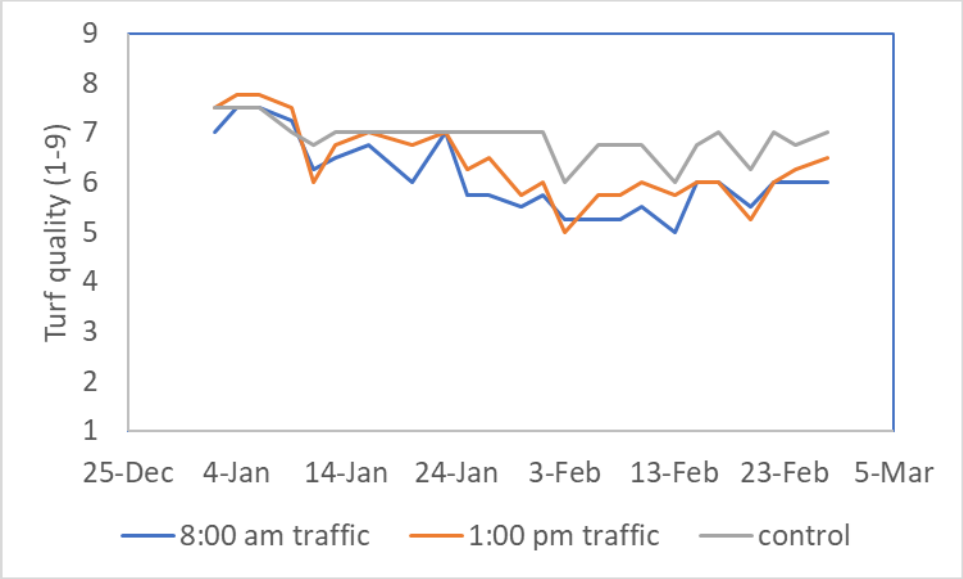


Figure 3: Annual bluegrass fairway turf quality affected by cart traffic applied at 8:00 am or 1:00 pm over time in Corvallis, OR from Jan 2 to Feb 28, 2023.

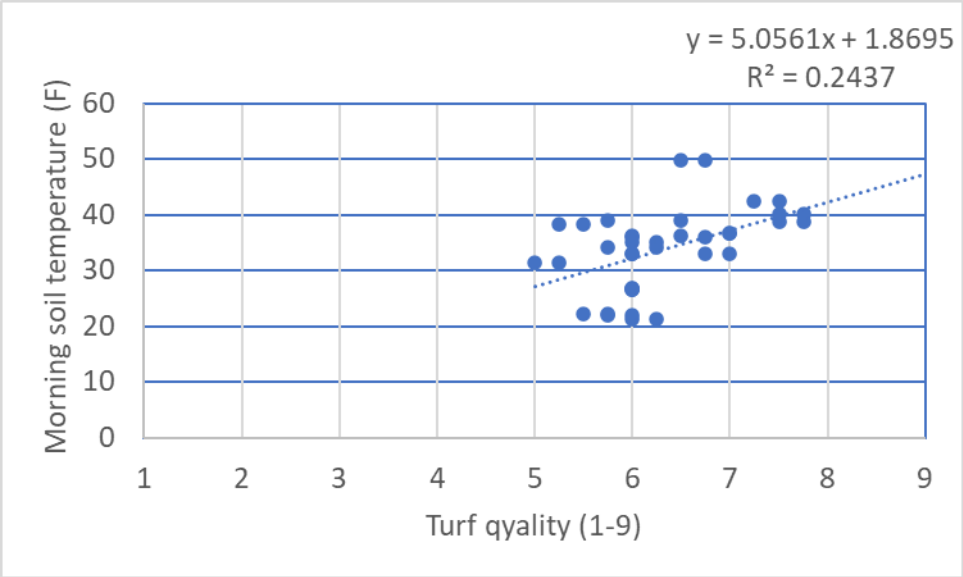


Figure 4: Relationship between morning soil temperature and annual bluegrass fairway turf quality when traffic was applied at 8:00 am or 1:00 pm (control treatment excluded) in Corvallis, OR from Jan 2 to Feb 28, 2023.

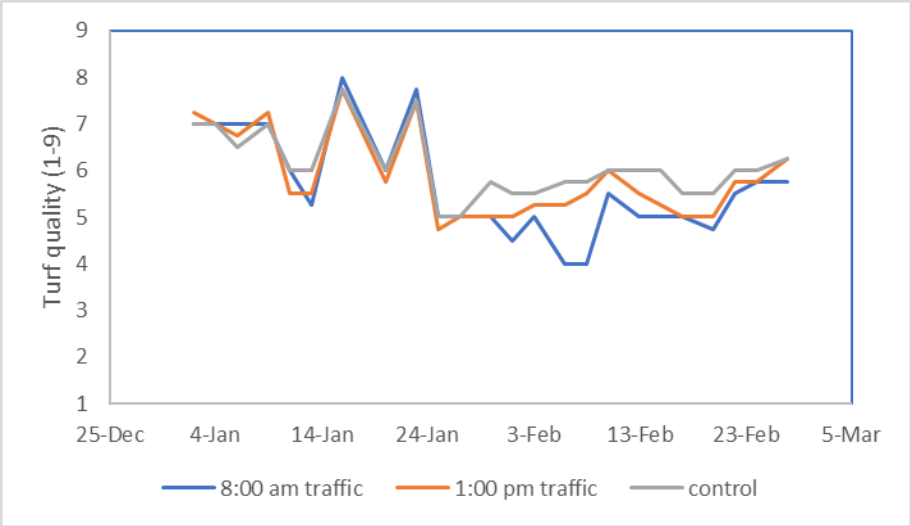


Figure 5: Perennial ryegrass fairway turf quality affected by cart traffic applied at 8:00 am or 1:00 pm over time in Corvallis, OR from Jan 2 to Feb 28, 2023.

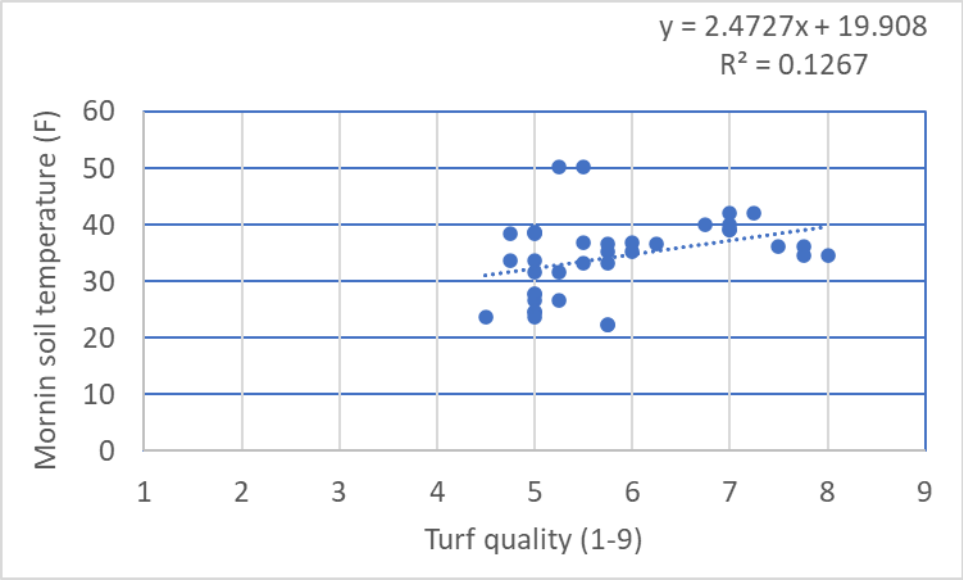


Figure 6: Relationship between morning soil temperature and perennial ryegrass fairway turf quality when traffic was applied at 8:00 am or 1:00 pm (control treatment excluded) in Corvallis, OR from Jan 2 to Feb 28, 2023.

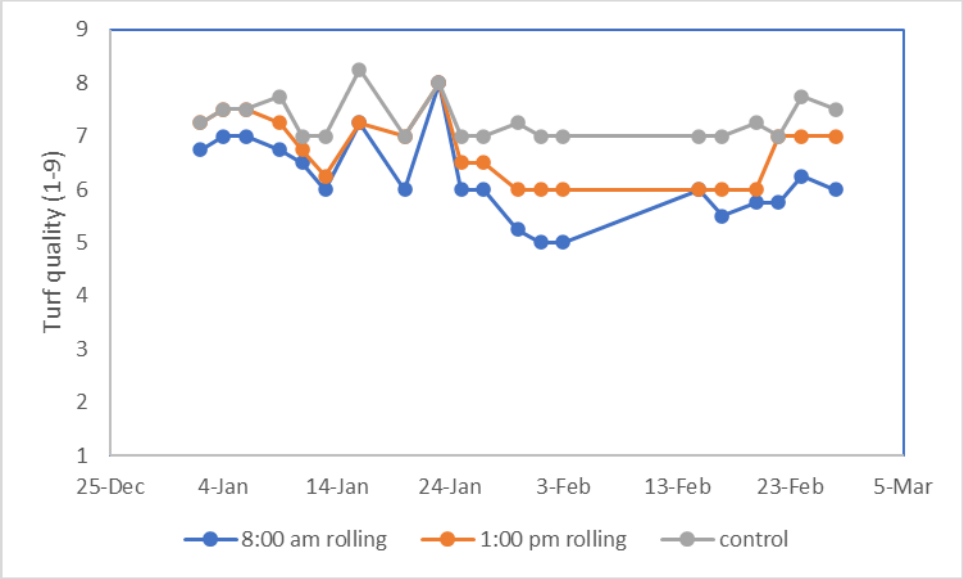


Figure 7: Annual bluegrass putting green turf quality affected by rolling applied at 8:00 am or 1:00 pm over time in Corvallis, OR from Jan 2 to Feb 28, 2023.

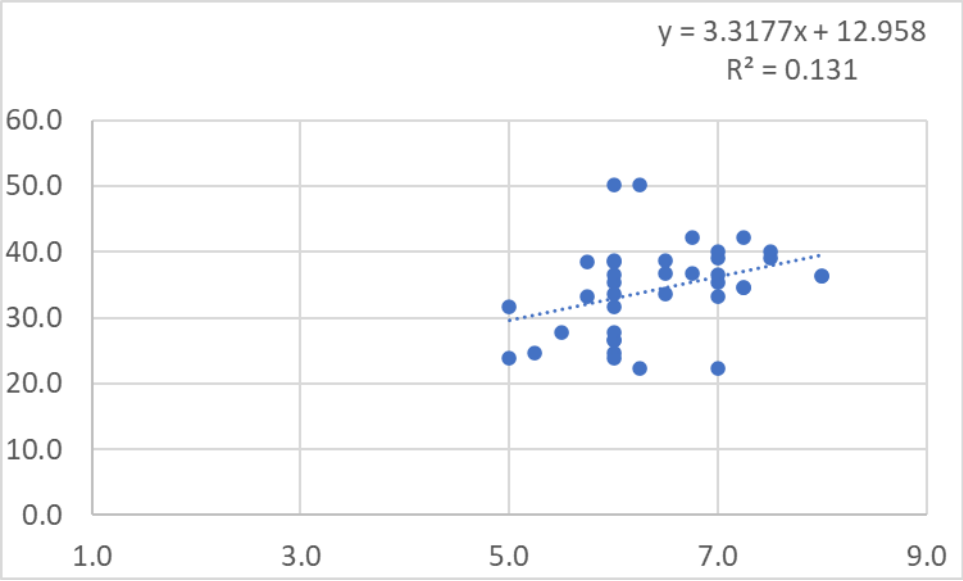


Figure 8: Relationship between volumetric water content and annual bluegrass putting green turf quality when rolling was applied at 8:00 am or 1:00 pm (control treatment excluded) in Corvallis, OR from Jan 2 to Feb 28, 2023.



Image 1: Moring cart traffic on a frost covered creeping bentgrass fairway in Corvallis, OR on February 1, 2023.



Image 2: Morning cart traffic (left), afternoon cart traffic (middle), and no cart traffic (right) on a creeping bentgrass fairway in Corvallis, OR on February 6, 2023.



Image 3: Morning cart traffic applied to an annual bluegrass fairway in Corvallis, OR on January 3, 2023.



Image 4: Morning cart traffic (left), afternoon cart traffic (middle), and no cart traffic (right) on an annual bluegrass fairway in Corvallis, OR on February 6, 2023.



Image 5: Rolling an annual bluegrass green in the morning in Corvallis, OR on January 3, 2023 (left) and February 1, 2023 (right).



Image 6: Morning rolling (left), afternoon rolling (middle), and no rolling (right) on an annual bluegrass green in Corvallis, OR on February 6, 2023.

USGA ID#: 2023-12-779

Title: Mitigating Bentgrass Summer Decline Utilizing Endophytic Plant Growth-Promoting Bacteria

Project Leader: Bingru Huang, Ning Zhang, and William Errickson

Affiliation: Rutgers University

Objectives:

1.) Determine efficacy of plant growth promoting bacteria (PGPB) inoculants on improving heat stress tolerance in creeping bentgrass under field conditions
2.) Optimize dosage or concentration of inoculants for most effective heat stress mitigation in creeping bentgrass

Start Date: 2023

Project Duration: 2 years

Total Funding: $95,120

This report presents the results of the first-year field trial conducted in 2023, addressing objectives 1 and 2; to investigate the effects of PGPB inoculants on the summer performance of creeping bentgrass under field conditions.

Summary Points:

- Inoculation with two novel strains of PGPB resulted in improved turf quality, reduced canopy temperature, improved reflectance indices, and enhanced root growth of creeping bentgrass under heat stress conditions
- Combining two strains of PGPB was more effective at improving heat stress tolerance of creeping bentgrass than either strain individually.
- Increasing the application rate relative to greenhouse and growth chamber trials was more effective at improving heat stress tolerance in field conditions.

Methodology:

This project was carried out in sand-based putting green plots established with creeping bentgrass (cv. 007) at the Rutgers University turfgrass research farm. The turf plots were managed following typical practices of fertility and pest management for putting greens in the northeast region during the growing season. The field experiment was performed during the months of June through September 2023 and will be repeated in 2024.

Bacteria inoculation and application

Two novel strains of growth promoting *Paraburkholderia aspalathi* ‘WSF23’ and ‘WSF14’, and a commercially available microbial product, Quantum Growth (Green Earth Ag and Turf LLC), containing *Bacillus* spp. were applied to the plots every two weeks at varying concentrations

listed below. A 0.01% humic acid solution was also added to the *P. aspalathi* and non-inoculated control treatments. All treatments were applied using a backpack sprayer in a carrier volume of 2 gallons /1000 ft^2 and were watered in with 0.25" of water to saturate the root zone immediately following application.

Experimental design

The experiment was set up as a complete randomized design. Each treatment was repeated in four 3 x 4 ft plots.

Bacteria inoculant treatments:

1) Non-inoculant Control
2) Quantum Growth
3) 'WSF23' (1.0 x 10^7 cfu/mL)
4) 'WSF23' (1.5 x 10^7 cfu/mL)
5) 'WSF23' (2.0 x 10^7 cfu/mL)
6) 'WSF14' (1.0 x 10^7 cfu/mL)
7) 'WSF14' (1.5 x 10^7 cfu/mL)
8) 'WSF14' (2.0 x 10^7 cfu/mL)
9) 'WSF23 +WSF14' (1.0 x 10^7 cfu/mL)
10) 'WSF23 +WSF14' (1.5 x 10^7 cfu/mL)
11) 'WSF23 +WSF14' (2.0 x 10^7 cfu/mL)

Evaluation of turfgrass heat tolerance and bacteria colonization

Turf performance and physiological health were evaluated biweekly during the hottest months of July-September or peak heat season in NJ. Turf performance was visually rated on a scale of 1 to 9. Thermal images of turf plots were also taken to evaluate canopy temperature using an infrared thermal imaging camera. An unmanned aerial vehicle (UAV) outfitted with a multispectral imaging camera that measures canopy reflectance in the range of 400 to 1000 nm wavelengths was used to calculated various vegetation and physiological indices, including photochemical reflectance index (PRI) = (R531 − R570)/(R531 + R570) and plant senescence reflectance index (PSRI) = (R680 − R500)/R750. A handheld ground-based multispectral radiometer was also used to collect reflectance data for normalized difference red edge (NDRE) = (R750 – R705)/(R750 + R705), and normalized difference vegetation index (NDVI) = (R800 − R680)/(R800 + R680). Roots were collected and washed free of soil to quantify rooting characteristics (length, surface area, and biomass). Bacteria were also be isolated from roots to quantify the colonization level and stability using qPCR analysis.

Summary of Results:

The most effective treatment among all 11 treatments is the combination of two bacteria strains (WSF23 +WSF14) at the highest does tested in this trial, which significantly improved performance and root growth compared to the untreated control and the commercial product, Quantum Growth. For better clarity of comparisons, the data for those three treatments are summarized below and presented in the figures.

Turf quality

Inoculation with WSF23+14 at the 2.0x concentration resulted in greater turf quality of creeping bentgrass during summer stress conditions, comparable to the effects of Quantum Growth. Inoculated plots were able to maintain higher turf quality throughout peak heat stress and showed significant differences on 8/16 and 8/30 (Fig. 1).

Canopy temperature

Canopy temperature was reduced to the greatest degree with inoculation by WSF23+14 at the 2.0x concentration. Significantly lower canopy temperature was observed with inoculation on 8/23 (Fig. 2).

Multispectral image analysis

Reflectance parameters collected by the unmanned aerial vehicle demonstrated trends for improved heat tolerance in plots inoculated with WSF23+14 at the 2.0x concentration. The PRI was greater and the PSRI was lower in inoculated plots on the 8/24 flight and data collection date (Fig. 3, 4). Reflectance parameters collected with the ground-based MSR unit also demonstrated similar trends, with higher NDRE and NDVI values observed in creeping bentgrass plots inoculated with WSF23+14 (2.0x) (Fig. 5, 6).

Root characteristics

Inoculation with WSF23+14 at the 2.0x concentration enhanced root growth of creeping bentgrass during summer stress. Inoculated plants demonstrated greater root length (Fig. 7), root surface area (Fig. 8), root volume (Fig. 9), and root dry weight (Fig. 10) compared to non-inoculated plants, with the effects especially pronounced on the September sampling date.

Conclusions

Heat stress tolerance of creeping bentrgrass was improved by inoculation with a combination of two novel strains of plant growth promoting bacteria in field conditions. The consortium of *P. aspalathi* WSF23 and WSF14 was most effective at improving heat tolerance when the highest application rate was used (2.0×10^7 cfu/mL). Inoculation resulted in the enhancement of turf quality, reduced canopy temperature, improved PSRI, PRI, NDRE, and NDVI reflectance metrics, and greater root development evident in root length, surface area, volume, and dry weight. The *P. aspalathi* treatments performed comparably or better than the commercial Quantum Growth inoculant for the parameters that were measured. The trial will be conducted again in the same field plots in 2024. We anticipate that multiple years or times of applications of the consortium of P. aspalathi WSF23 and WSF14 could be more effective to improve turf performance during summer months. Based on these encouraging results, *P. aspalathi* WSF23 and WSF14 have potential for further research and development into commercially viable inoculants to improve summer performance of cool season turfgrass.

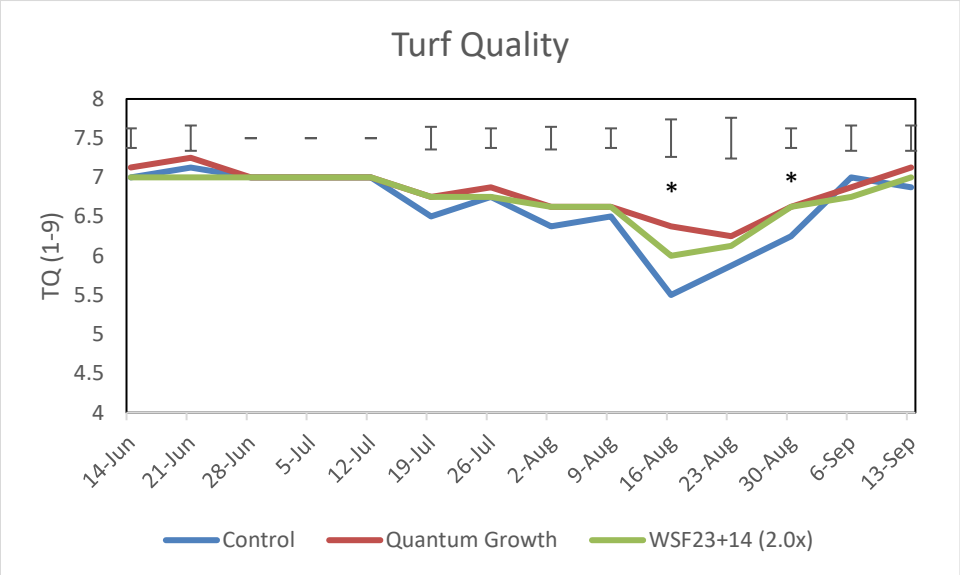


Figure 1: Turf quality of creeping bentgrass with inoculation treatments under summer heat stress conditions. * Indicates significance at p≤0.05.

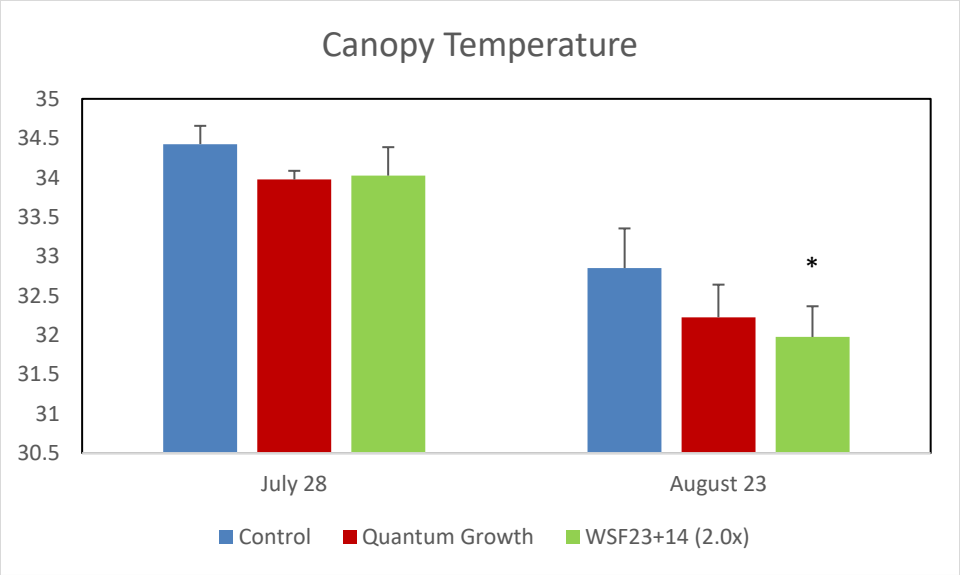


Figure 2: Canopy temperature of creeping bentgrass with inoculation treatments under summer heat stress conditions. * Indicates significance at p≤0.05.

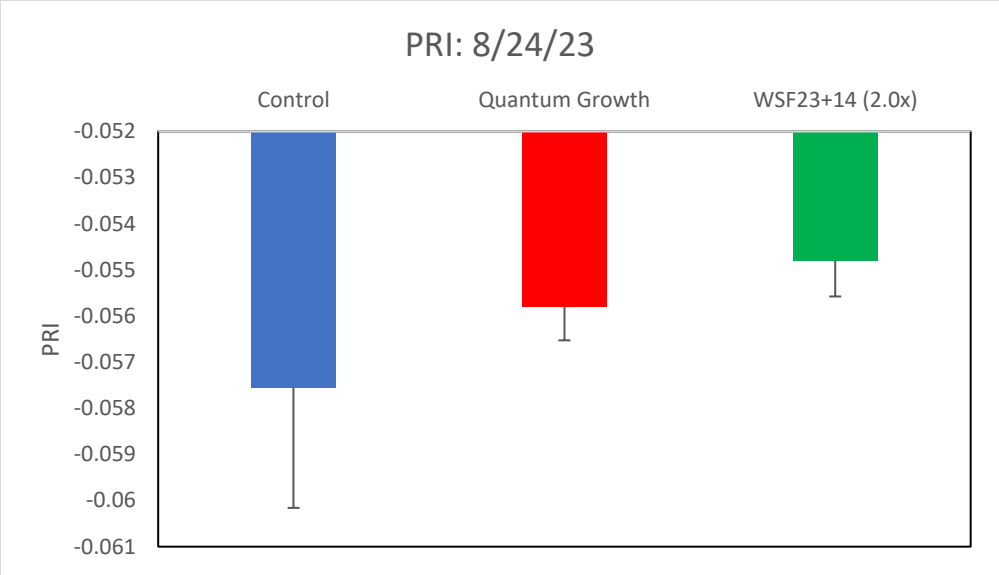


Figure 3: Photochemical reflectance index (PRI) of creeping bentgrass with inoculation treatments under summer heat stress conditions. Greater PRI indicates higher stress tolerance.

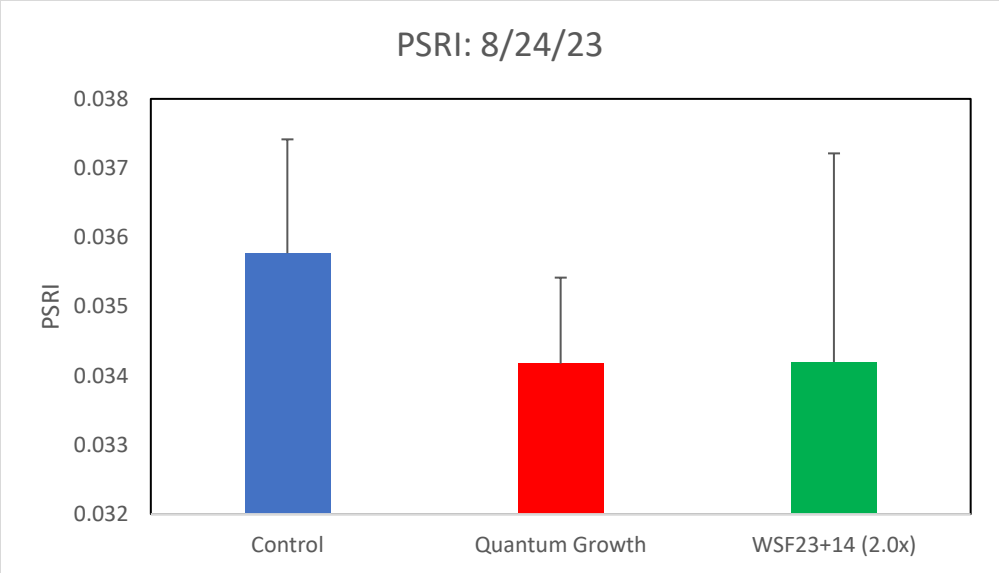


Figure 4: Plant senescence reflectance index (PSRI) of creeping bentgrass with inoculation treatments under summer heat stress conditions. Lower PSRI indicates reduced stress damages.

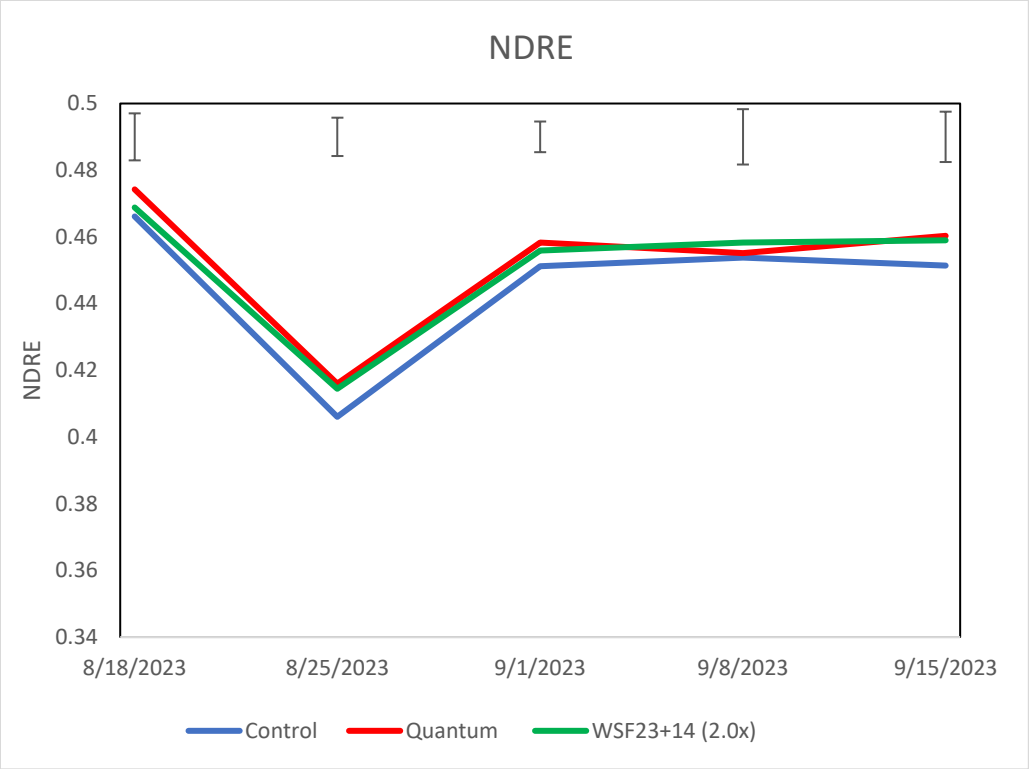


Figure 5: Normalized difference red edge (NDRE) of creeping bentgrass with inoculation treatments under summer heat stress conditions. Greater NDRE indicates higher stress tolerance.

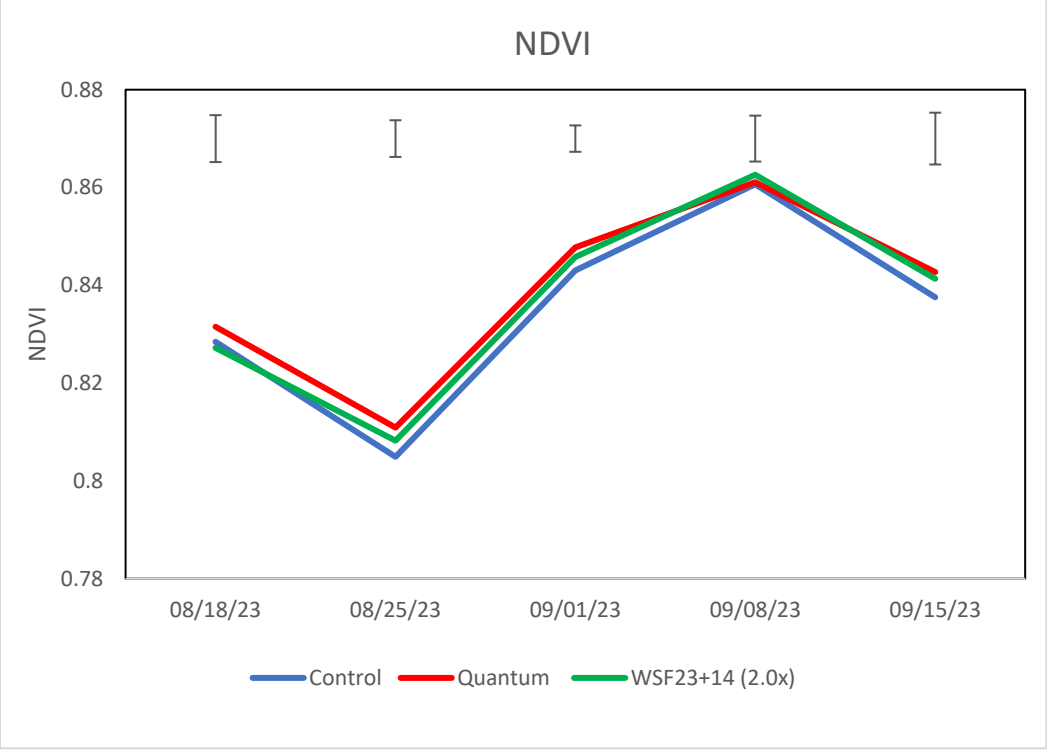


Figure 6: Normalized difference vegetation index (NDVI) of creeping bentgrass with inoculation treatments under summer heat stress conditions. Greater NDVI indicates higher stress tolerance.

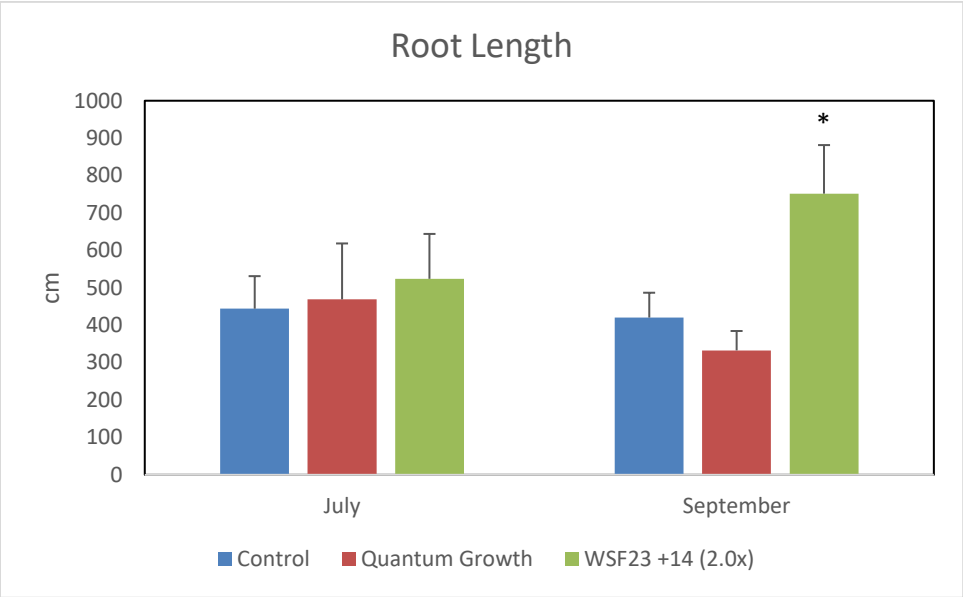


Figure 7: Root length of creeping bentgrass with inoculation treatments under summer heat stress conditions. * Indicates significance at p≤0.05.

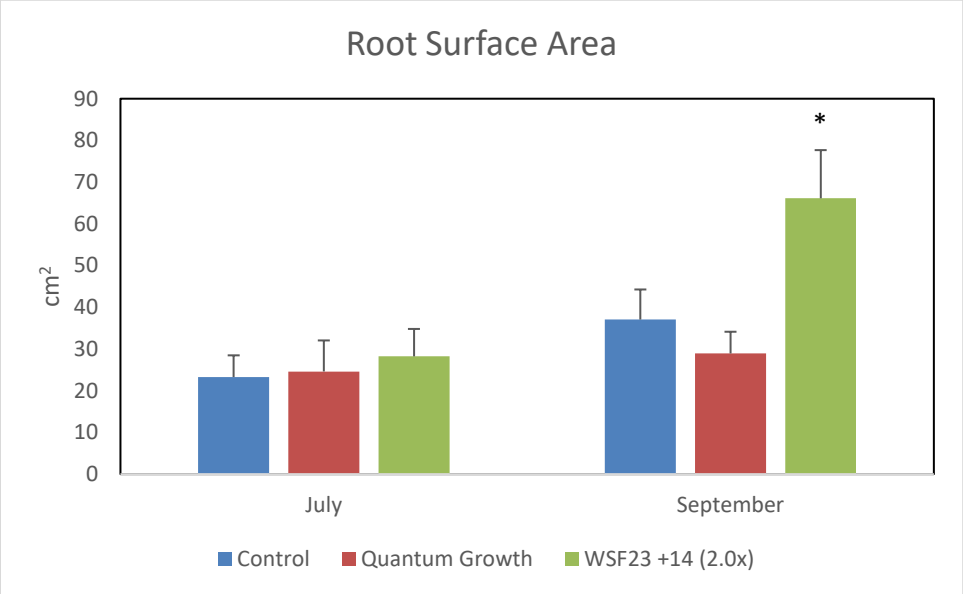


Figure 8: Root surface area of creeping bentgrass with inoculation treatments under summer heat stress conditions. * Indicates significance at p≤0.05.

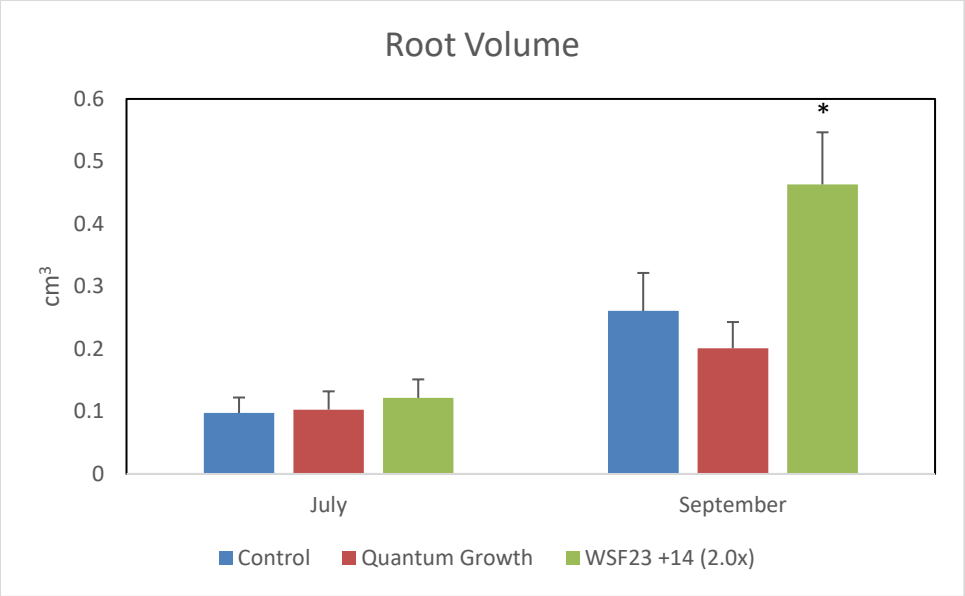


Figure 9: Root volume of creeping bentgrass with inoculation treatments under summer heat stress conditions. * Indicates significance at $p \leq 0.05$.

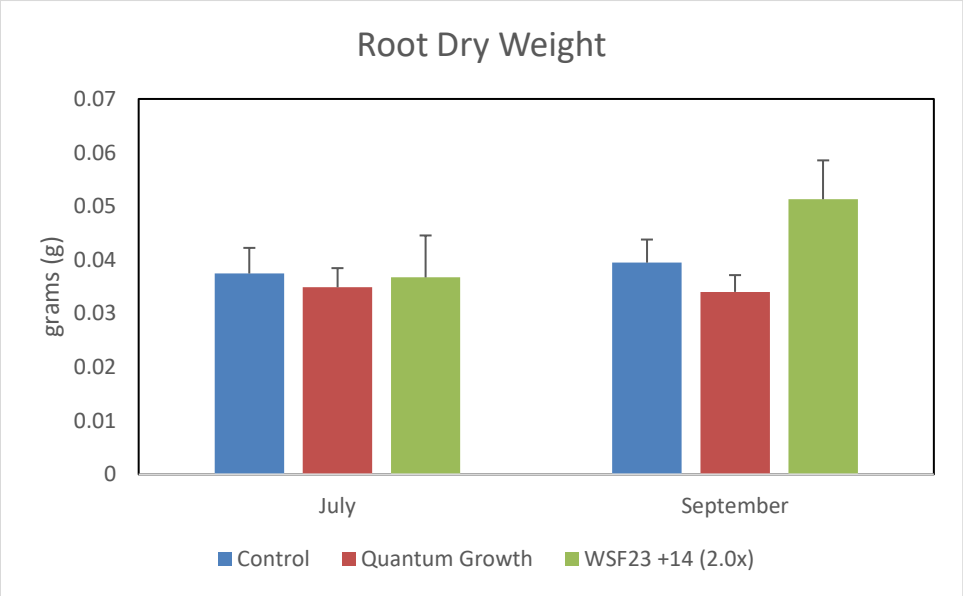


Figure 10: Root volume of creeping bentgrass with inoculation treatments under summer heat stress conditions.

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:

- Firmness devices that applied more force were better correlated with moisture, color and visual quality whereas lower force devices were better correlated with surface organic matter.
- Higher annual sand application volume led to an increase in visual quality but a decrease in surface firmness and surface organic matter.
- Standard sized sand resulted in a slightly firmer surface than the finer sand despite the higher coefficient of uniformity of the finer sand.
- 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), 23 N force
2) Clegg Impact Soil Test Hammer (0.5 kg hammer, round bottom), 5 N force
3) Spectrum TruFirm, 5 N force
4) Precision USA Putting Green Firmness Meter (aka PGA method), 11 N force

In addition to firmness measurements, we measured soil moisture, normalized difference vegetation index (NDVI), soil surface strength, and visual turfgrass quality weekly during the growing season. Once a month, we measured ball roll distance and collected clippings from the plots. Surface 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:
These results reflect the second year in a three-year study. We found that annual sand volume had the largest impact on overall putting green performance compared to the other main effects of application frequency (weekly vs monthly) and sand particle size (standard vs finer). When the annual volume of sand was increased, visual quality increased, surface firmness decreased, and surface organic matter decreased (Table 2). The decrease in organic matter in response to increasing sand application was expected, but we were surprised to see that surface firmness was decreased with increasing topdressing rate despite lower surface organic matter. Also surprising was the finer sand (with a lower CU) resulted in a less firm surface (6.13 g for the standard sand vs 6.04 g for the finer sand). We expected the increased coefficient of uniformity from the finer sand size would lead to an increase in surface firmness. The frequency of sand application (weekly vs. monthly) had no impact on any of our measurements.

We observed interesting differences among the four firmness devices tested and the putting green performance criteria. The Clegg 2.25 kg had the highest impact force (23 N), followed by the PGA method (11 N) and the Clegg 0.5 kg and Spectrum Trufirm (both 5 N). The two devices with the greatest force had stronger correlations with visual quality, NDVI, and soil moisture when compared to the two lower force devices which were both poorly correlated with quality, NDVI, and moisture. However, the opposite was true for surface organic matter, and the lower force devices were more strongly correlated to this property compared to the two higher force

devices. The PGA method was intermediate in force, and had a better correlation with surface organic matter than the Clegg 2.25 kg. This result suggests that important differences exist among the most popular surface firmness devices.

Table 2. Turfgrass quality, surface firmness, and surface organic matter as affected by annual topdressing amount. Means within columns followed by a different letter are statistically significantly different at the 0.05 level.

Annual Sand Rate	Turfgrass Visual Quality	Surface Firmness with Clegg 2.25	Surface Organic Matter, 0-1 inch
$ft^3/1000\ ft^2$	1-9, 9=best	g (greater is firmer)	%
26	5.95 A	6.08 B	4.1 B
18	5.70 B	6.01 B	5.4 AB
14	5.61 B	6.18 A	5.9 A

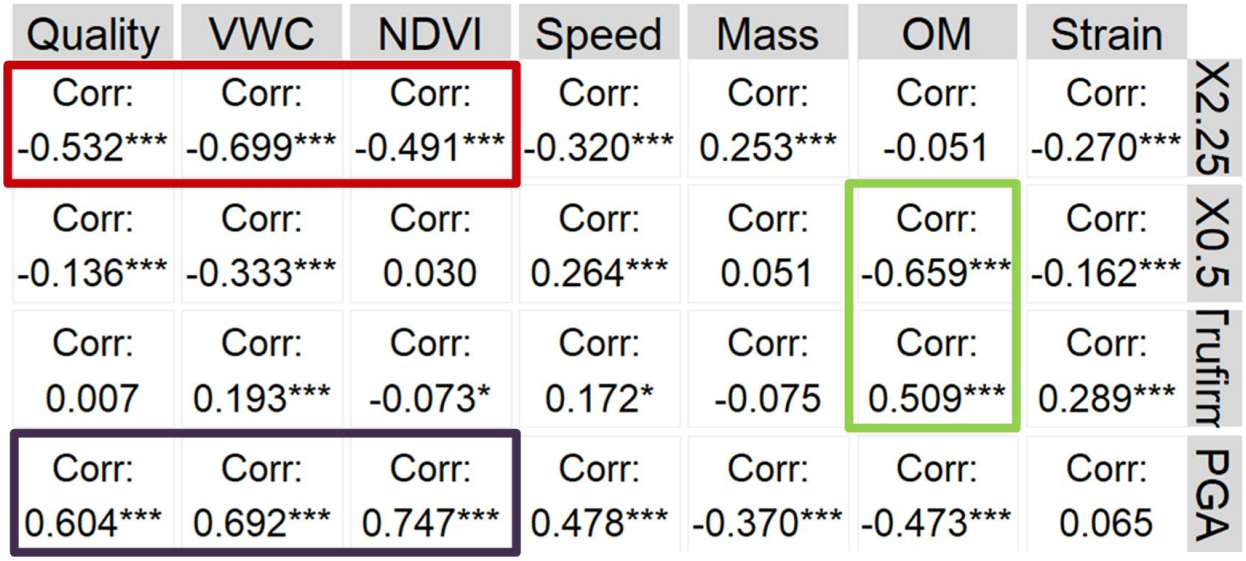


Figure 1: Relationships between the firmness measurement devices and putting green performance criteria. Highlighted in red is the relationship between Clegg 2.25 kg device and turfgrass quality, volumetric water content (VWC) and n NDVI. Highlighted in purple is the relationship between PGA method and quality, VWC and NDVI. Highlighted in green is the relationship between Clegg 0.5 kg and Trufirm and surface organic matter content. Values closer to 1 or -1 indicate stronger relationships, close to 0 indicates no relationship.

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#: 2023-01-768 (altered continuation from 2020-03-708 and 2019-01-671)

Title: Switching to Solid Tine Cultivation after Seven Years of Hollow Tine Cultivation on Plots Topdressed with Three Sand Sizes

Project leader: James A. Murphy
Affiliation: Department of Plant Biology, Rutgers University

Objectives: Continue to assess the

1) long-term effects of topdressing with sand dominated by medium or fine sand particles and
2) impact of switching to solid tine cultivation and backfilling holes with medium-coarse sand on turf performance and the physical properties at the surface of a putting green root zone.

Start date: 2023
Project duration: 3 years
Total funding: $145,557

Summary Points:

- We continued application of treatments comprised of topdressing with three sands differing in particle size distribution. Each of the three sands were applied ten times during the growing season at two rates: 50 or 100 lb per 1,000 sq ft, a 3 x 2 factorial combination of topdressing treatments. We also continued with two levels of a cultivation factor but changed the cultivated level from hollow tine to solid tine cultivation. A ProCore® 648 setup with mini tine heads (2x5 staggered holder; 5 tines per plate) and equipped with ⅝-inch diam. solid tines was used to apply the solid tine cultivation treatment.
- Undisturbed core (3-inch diam.) samples of the mat layers were removed from plots in April 2023 using the methods performed during the USGA ID#: 2019-01-671 grant. Determination and analyses of the surface bulk density, pore size distribution, organic matter content, and sand size distribution of the mat layers is underway. These data will conclude the grant activities of USGA ID#: 2020-03-708 and set the benchmark for data to be collected during the grant activities of USGA ID#: 2023-01-768.
- Seven years of hollow tine cultivation has clearly maintained a greater surface bulk density compared to non-cultivated plots; bulk density was as much as 24% greater than the non-cultivated plot. Moreover, topdressing sand size has no detectable effect on bulk density when hollow tine cultivation was also practiced on plots; whereas bulk density did respond to topdressing sand size when coring was not practiced on plots. Bulk density was greater when topdressing used a coarser sand size on non-cultivated plots.
- As bulk density increased in 2023 with either hollow tine cultivation or topdressing with coarser sand on plots not receiving core cultivation, surface hardness increased. Future measurements with the USGA GS3 device should provide more insight on potential ramifications of these surface responses to playability.
- Non-cultivated plots retain much more water in the surface than cultivated plots. Similarly, topdressing with finer sand results in greater water retention in the mat layer than coarser sand. Greater water retention with finer topdressing sand was greatly muted by cultivation; however, topdressing with the fine-medium sand still results in more water being retained albeit to a smaller degree.

- Two cultivation trials were initiated on creeping bentgrass maintained as putting green turf to compare ½-inch i.d. hollow tine, solid tine cultivation, and a non-treated control.

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. Topdressing with finer sand enhances incorporation greatly reducing the concerns of interference, which could enable superintendents to keep pace with thatch accumulation in putting greens during the summer and reduce problems associated with excess organic matter. Results from a seven-year field trial (USGA ID#: 2016-06-556 and USGA ID#: 2019-01-671) indicate that a finer 0.05-mm topdressing sand (particles ≤ 0.5-mm) has diluted and modified thatch accumulation much like that of a coarser 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.

Grant funding from the USGA was provided to continue a revised version of this long-term topdressing project, in which we continued applications of topdressing treatments but one of the levels in the cultivation factor was switched from hollow tine to solid tine cultivation.

This new funding also supports the initiation of two complementary trials to compare hollow and solid tine cultivation. The objectives of these complementary trials were to directly compare hollow and solid tine cultivation for effects on thatch modification and associated changes in surface physical properties including surface water retention, water infiltration, organic matter content, and pore size distribution of the mat layer.

Materials and Methods

Long-term Topdressing Trial

The long-term topdressing trial was initiated in May 2016 on a 19-month-old 'Shark' creeping bentgrass grown on a sand-based root zone.

The experimental design of the trial was a 3 x 2 x 2 factorially arranged randomized complete block design with four blocks. During 2023, the sad factors remained as before: sand size (medium-coarse, medium-fine, fine-medium) and 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). One level of the cultivation factor, however, was changed in 2023; plots were either non-cultivated or cultivated with ⅝-inch diam. tines in May and October. Controls (no mid-season topdressing) at each level of cultivation were also included for comparisons resulting in 14 total treatments (Table 1).

The medium-coarse sand used in this trial meets the USGA particle size recommendation for construction, whereas that of the medium-fine and fine-medium sands do not (Table 2). 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.

Mowing – except during COVID-19 restrictions – was performed with a 2.8-mm bench setting 5 to 6 days per week from late April through October. Fertilization was applied every 1 o 2 weeks at rates of 0.1 to 0.3 lb of N per 1,000 sq ft and achieved an annual total of ~2.7 lb N per 1,000 sq ft. Irrigation was applied at no more than 80% ET_o. During select periods irrigation by handheld hose was initiated on an individual plot when the plot exhibiting transient wilt stress. Each handheld hose watering event for the gallons or water applied. Pests were preventively managed with pesticides.

Data collection during April 2023 included removing undisturbed core (3-inch diam.) samples of the mat layers of each plot using the methods performed during the USGA ID#: 2019-01-671 grant.

Determination and analyses of the surface bulk density, pore size distribution, organic matter content, and sand size distribution of the mat layers is underway. These data will conclude the grant activities of USGA ID#: 2020-03-708 and set the benchmark to contrast with data collected during the grant activities of USGA ID#: 2023-01-768.

Data collection during 2023 also 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, we contracted with a geospatial consultant to develop a UAV (DJI Mavic 3 Multispectral drone) to collect remote sensing data from the trial. Statistically analyses of most data are in progress.

Complementary Cultivation Trials

The two complementary cultivation trials were initiated on creeping bentgrass maintained as putting green turf during 2023. Mowing was performed with a 2.8-mm bench setting 5 to 6 days per week from late April through October. Fertilization was applied every 1 to 2 weeks at rates of 0.1 to 0.3 lb of N per 1,000 sq ft. Irrigation was applied after at no more than 80% ET_o once the turf had dried sufficiently after rain.

One trial, initiated on 30 June 2023, applied four treatments: non-cultivated control, ½-inch i.d. hollow tine cultivation, ½-inch diam. solid tine cultivation, and ⅝-inch diam. solid tine cultivation. All treatments were replicated four times in randomized complete block design. All treatments were reapplied a second time in this trial on 11 October 2023.

The second complementary trial was initiated on 18 October 2023 and three treatments were applied: non-cultivated control, ½-inch i.d. hollow tine cultivation, and only ⅝-inch diam. solid tine cultivation. All treatments were replicated four times in randomized complete block design.

All cultivated treatments were applied with a ProCore® 648 setup with mini tine heads (2x5 staggered holder; 5 tines per plate) equipped with the respective tines and depth setting to reach 2-inches below the turf surface. The tine spacing affected ~5% of the turf surface area with each treatment application. All cultivation tine holes were backfilled with medium-coarse sand at 600 lb per 1,000 sq ft. At the same time cultivation treatments were applied, non-cultivated plots were topdressed with the medium-coarse sand at 400 lb per 1,000 sq ft to fill the verdure and surface thatch layers to the same extent as plots that were solid tine cultivated and backfilled with sand.

Data collection during 2023 included visual ratings of turf quality, healing of tine holes, residual sand after topdressing, normalized difference vegetation index (NDVI), VWC of the surface 0- to 3-inch depth zone, and Clegg soil impact values. Additionally, dual-head infiltrometers [SATURO | Automated Field Infiltrometer | METER Environment (metergroup.com), Pullman, WA] were used to measure field saturated hydraulic conductivity of the four treatment cultivation trial during August 2023. Finally, the USGA GS3 device was used after October treatment application to assess the distance, trueness, and smoothness of ball rolls and drop tests to evaluate firmness. Statistically analyses of most data are in progress.

Results

Long-term Topdressing Trial

As mentioned, analyses of most data are underway. However, analyses of surface bulk density and VWC (measured with a Troxler model 3440 moisture density gauge) of the turf surface collected before treatment initiation in 2023 indicated these parameters were affected by all three treatment factors.

The most dramatic response of surface bulk density to treatments was observed in the interaction of the sand size and cultivation factors (Table 3). Seven years of hollow tine cultivation has clearly maintained a greater surface density compared to non-cultivated plots; bulk density was as much as 24% greater than the non-cultivated plot. Moreover, topdressing sand size has no detectable effect on bulk density when coring was also practiced on plots whereas bulk density did respond to topdressing sand size when coring was not practiced on plots. Bulk density was greater when topdressing used a coarser sand size on non-cultivated plots.

As bulk density increased with either hollow tine cultivation or topdressing with coarser sand on plots not receiving core cultivation, surface hardness increased (Table 3). Future measurements with the USGA GS3 device should provide more insight on potential ramifications of these surface responses to playability.

Plots receiving the greater sand topdressing rate (100-lb) have a higher bulk density (1.18 g/cm^3) than the plots topdressed with sand at 50-lb (1.15 g/cm^3). This effect was also evident before the second solid tine cultivation treatment application on 25 Oct. 2023; bulk density of plots topdressed at 100-lb increased to 1.21 g/cm^3 while the plots topdressed at 50-lb was 1.17 g/cm^3. Although small in magnitude, this effect on bulk density was associated with a changes in volumetric water content and surface hardness measured with a Clegg Soil Impact Tester (Table 3).

Volumetric water content measured with the Troxler moisture density gauge in April and October 2023 demonstrates the large difference in water retention between non-cultivated and cultivated plots (Table 3). Non-cultivated plots retain much more water in the surface than cultivated plots. Similarly, topdressing with finer sand results in greater water retention in the mat layer than coarser sand. This greater water retention response with finer sand was greatly muted by cultivation; however, topdressing with the fine-medium still resulted in more water being retained albeit to a smaller degree under cultivation.

Complementary Cultivation Trials

Statistical analyses of data are in progress. See report on GS3 for treatment means of parameters collected with the USGA GS3 device.

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	Solid tine + backfill	1,700
3	medium-coarse	100	none	1,700
4	medium-coarse	100	Solid tine + backfill	2,200
5	medium-fine	50	none	1,200
6	medium-fine	50	Solid tine + backfill	1,700
7	medium-fine	100	none	1,700
8	medium-fine	100	Solid tine + backfill	2,200
9	fine-medium	50	none	1,200
10	fine-medium	50	Solid tine + backfill	1,700
11	fine-medium	100	none	1,700
12	fine-medium	100	Solid tine + backfill	2,200
13	none	0	none	0
14	none	0	Solid tine + 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] Solid tine cultivation to the 2-inch depth was performed twice a year (May and October) using ⅝-inch diameter solid tines spaced to affect 10% of the surface area annually. Solid tine holes were backfilled with medium-coarse sand at 600 lb per 1,000 sq ft. At the time of solid tine 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 plots that were solid tine cultivated and backfilled with sand.

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.

Table 3. Topdressing sand size and cultivation interaction effects on surface bulk density, volumetric water content, and Clegg surface hardness on a 'Shark' creeping bentgrass turf maintained at 0.11-inch mowing height in North Brunswick, NJ.

		25 May 2023[a]		25 Oct. 2023[b]		25 May 2023
Topdressing Sand Size	Hollow Tine Cultivation	Bulk Density[c]	Gravimetric Water Content[c]	Bulk Density[c]	Volumetric Water Content[c]	Clegg Surface Hardness
		g/cm^3	%	g/cm^3	%	G_{max}
Medium-coarse	None	1.10	26.4	1.14	30.0	73.1
Medium-fine	None	1.07	29.8	1.11	34.0	72.9
Fine-medium	None	1.02	39.3	1.05	40.2	68.4
Medium-coarse	Twice/year	1.27	15.4	1.30	18.7	77.0
Medium-fine	Twice/year	1.26	16.4	1.30	18.6	77.6
Fine-medium	Twice/year	1.26	18.4	1.28	22.7	78.6
	$LSD_{0.05}$	0.029	2.6	0.03	3.2	3.1

[a] Measurements on 25 May 2023 were before the initiation of solid tine cultivation and topdressing treatments in 2023.

[b] Measurements on 25 Oct. 2023 were before the second application of solid tine cultivation in 2023.

[c] A Troxler Road Reader model 3440 moisture density gauge set in backscatter mode was used to collect bulk density and water content data.

2023 Plan of Work

USGA ID#: 2020-03-708

- Analyses of data from undisturbed core (3-inch diam.) samples removed from mat layers in April 2023 will be completed and conclude the grant activities of USGA ID#: 2020-03-708

USGA ID#: 2023-01-768

- Treatments for all trials will continue as will data collection for 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); hand-watering quantification, dual head infiltrometers (SATURO) to assess water infiltration, and measurements with the GS3 will be made during 2024.
- Additionally, we anticipate contracting with a geospatial consultant to deploy a UAV (DJI Mavic 3 Multispectral drone) to collect remote sensing data from the trial (dependent on approval of funding from another grant).

USGA ID#: 2022-15-758a

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.
- Approximately 5 to 10 individual cores from a putting green are necessary to minimize the standard deviation. Putting greens with typical levels of surface organic matter (~5%) required approximately 5 cores, while putting greens with higher levels (>10%) required about 10 cores.
- A core diameter of 0.75 inches was sufficient for surface organic matter characterization and a larger 1.5 inch diameter core did not reduce the number of samples required to minimize the standard deviation but increased surface disturbance.
- The spatial patterns of the data suggest that individual samples 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 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) The number of samples required to adequately characterize the surface organic matter on a putting green; 3) How core diameter affects the mean and variance of surface organic matter; and 4) the minimum distance apart for each sub-sample on a putting green.

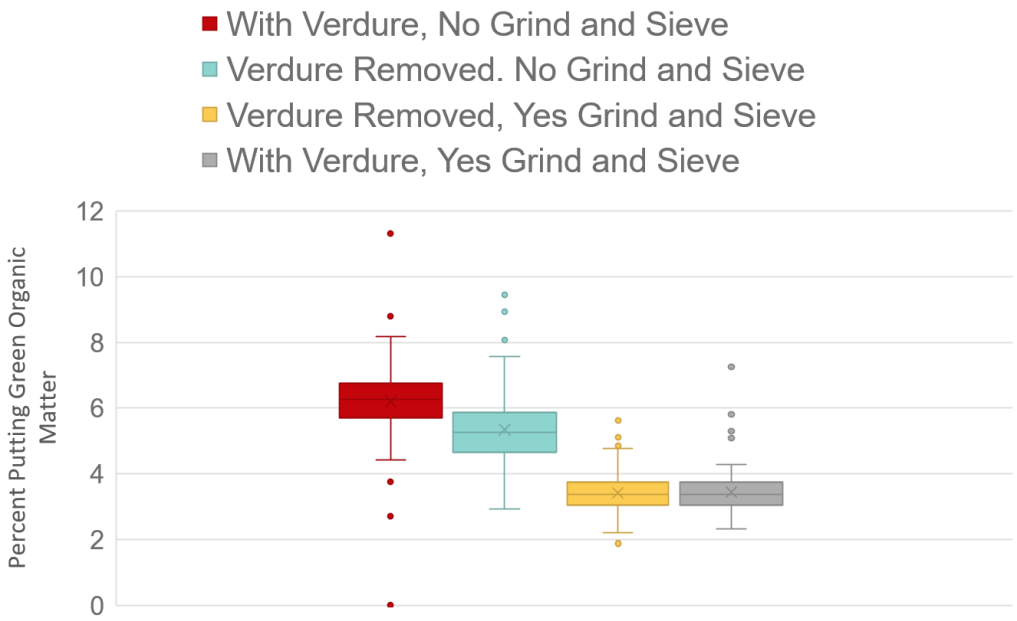


Figure 1. Grinding and sieving samples at the lab reduces the surface organic matter content and obscures the effect of the verdure removal. The greatest surface organic matter content was found when samples were ignited intact with no verdure removal.

To answer the question about sample processing, 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. 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 degrees C for 24 hours then ignited at 360 degrees 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.

To answer the question about how many samples are required to adequately characterize the surface organic matter of putting greens 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 degrees C for 2 hours following drying at 105 degrees C. Each green had approximately 50 core samples and derivative analysis found that 5 to 10 samples using a 0.75-inch diameter probe were required to adequately characterize the mean surface organic matter of all putting greens tested (Figures 2).

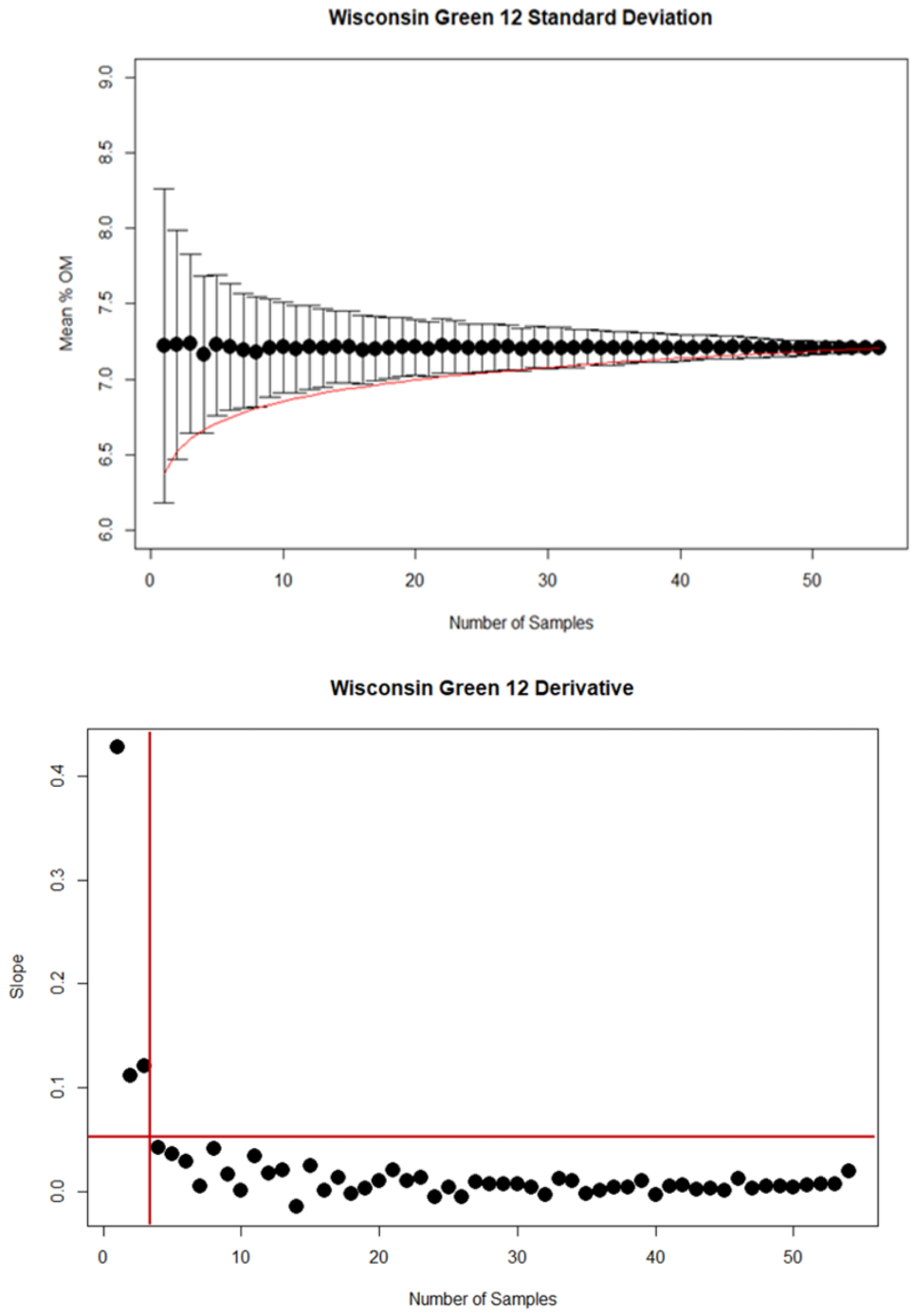


Figure 2. A representative relationship for one of the putting greens tested that shows how the standard deviation changes as the number of samples is increased (top). Derivative analysis (bottom) of the standard deviation trendline (curved red line in top figure) shows that four samples was required to minimize the standard deviation according to Cate-Nelson analysis in the bottom figure (straight red lines).

At the Nebraska site, 1.5 inch vs. 0.75-inch diameter cores were evaluated to answer the question about the importance of sample diameter. Organic matter content from each core diameter was statistically similar as well as the number of samples needed to characterize the means. However, 5 cores using a 1.5-inch diameter core removes approximately 9 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 of the 15 putting greens we tested had a unique spatial pattern of surface organic matter distribution, in all cases samples taken approximately 30 feet apart yielded the most accurate results.

We are continuing to investigate questions about how surface organic matter changes throughout the season. Preliminary results suggest that highest organic matter values are seen at the beginning and the end of the season and organic matter values during the summer. However, data collection and analysis is continuing. We also are investigating the effect of ignition temperature (360 C vs 440 C vs 500 C), warm season surface organic matter levels, and are also investigating the influence that sand-filled core holes and recently applied topdressing have on a the %OM of a composite sample from a putting green.

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 (final report)

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

Summary Points:

- Spring applications of ethephon (Proxy) reduced core cultivation recovery time in 2 out of 3 years, indicating that spring seedhead control programs with ethephon should continue through cultivation events.
- Timing of trinexapac-ethyl (TE; Primo Maxx) had an inconsistent effect on cultivation recovery in the spring but withholding TE applications within 200 GDD prior to cultivation generally decreased recovery time.
- Trinexapac-ethyl and ethephon application in the fall had little effect on cultivation recovery; Turfgrass managers can continue PGR programs during fall cultivation without fear of increasing recovery time.
- Gibberellic acid treatments caused excessive growth/scalping and should be avoided on annual bluegrass putting greens.

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 when golf courses experience the most play. 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 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 (i.e. rebound effect) 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 November 2023. Research was 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, 7 May 2022, and 13 May 2023; and in the fall on 28 Sept 2021, 7 Oct 2022, and 13 Oct 2023, 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 were arranged as a randomized complete block design with four replications. Plot size for the trial was 7' x 4' (28 ft^2). Treatments are listed below:

Treatments

1. Untreated control
2. TE[¥] applied 400 GDD[ɣ] 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 applied at 5.5 fl oz of Primo MAXX/acre

[ɣ] Cumulative GDD model 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 at 218 fl oz of Proxy/acre 400 and 200 GDD prior to cultivation.

The first treatment application in the spring was initiated at the first sign of annual bluegrass plants in the "boot" stage, and subsequent applications and cultivation timing were made based on a growing degree day model.

Response Variables:

Digital photos were taken at the same location in each plot, every 2 days during the recovery period, using a lightbox to track turfgrass recovery over time. Digital images were analyzed using Turf Analyzer to determine percent recovery over time (Image 1). 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 were analyzed with analysis of variance (ANOVA) and the means separated using LSD at the 5% alpha level.

Results:

Results from the three-year trial indicate that in spring, the main effect of ethephon had the greatest effect on cultivation recovery time (Fig 1). Percent recovery was greater in plots receiving ethephon compared to plots that received none, on 4 out of 7, and 7 out of 8 ratings in 2021 and 2022, respectively. However, ethephon had no effect on cultivation recovery in the spring of 2023. This data suggests the seedhead suppression generated by ethephon applications results in improved turfgrass growth, decreasing cultivation recovery time. Trinexapac-ethyl timing (GDD model) influenced cultivation recovery in the spring of 2021 and 2022, but not during 2023 (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 compared to TE applied 10 GDD prior. These results indicate that PGR timing did not have a consistent effect on cultivation recovery. One possible explanation for this could be inconsistent rebound growth following PGR application in the spring. Initial data from a PGR GDD model trial on annual bluegrass (USGA ID#: 2023-15-782) indicates that environmental conditions can have an impact on whether a rebound effect is observed after a single application of TE. In addition, peak rebound growth was observed between 570 and 881 GDD, thus rebound growth may occur after the turfgrass has already recovered from cultivation.

Results from the fall cultivation event indicated that the main effect of ethephon treatment influenced cultivation recovery time in 2021 but not 2022 or 2023. In 2021, plots receiving ethephon were slightly slower to recover than plots that received no ethephon (6 out of 11 ratings; Fig 3). However, during 2022 and 2023 ethephon applications had no effect on cultivation recovery. The 2021 results are 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 prior to cultivation could see slower fall aerification recovery. Results on proxy timing research conducted in western Oregon (USGA ID#:2020-06-711) found that January and February proxy

applications followed by Mar, Apr, and May applications provided the greatest seedhead suppression. Suggesting that proxy can successfully be applied in the winter months after green have recovered from aerification. Trinexapac-ethyl timing (GDD model) had no effect on cultivation recovery time in the fall of 2021, 2022, or 2023. Together, these results suggest that PGRs (TE and ethephon) effect on cultivation recovery in the fall is much less pronounced than in the spring and turfgrass managers should continue routine PGR application prior to cultivation without fear of delaying recovery.

Gibberellic acid treatments caused rapid turfgrass growth, followed by scalping after the first mow which reduced turfgrass quality and delayed cultivation recovery, regardless of GA rate (Image 2). Because of this, GA applications to annual bluegrass putting greens are not recommended.

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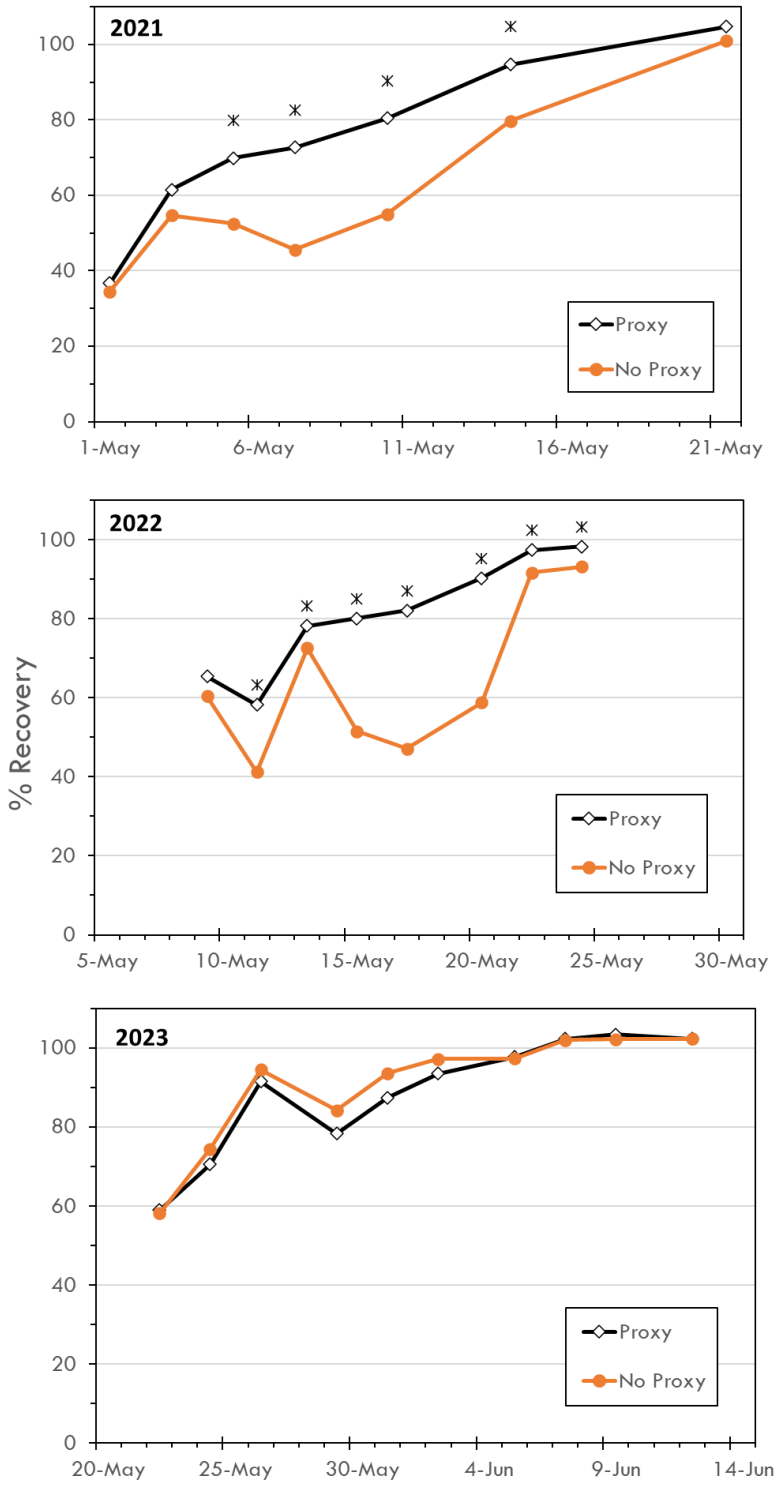


Figure 1. The main effect of ethephon (Proxy) on the percent recovery from core cultivation on annual bluegrass turf, in the spring of 2021, 2022 and 2023. Asterisk above a rating date indicates a significant difference between treatments.

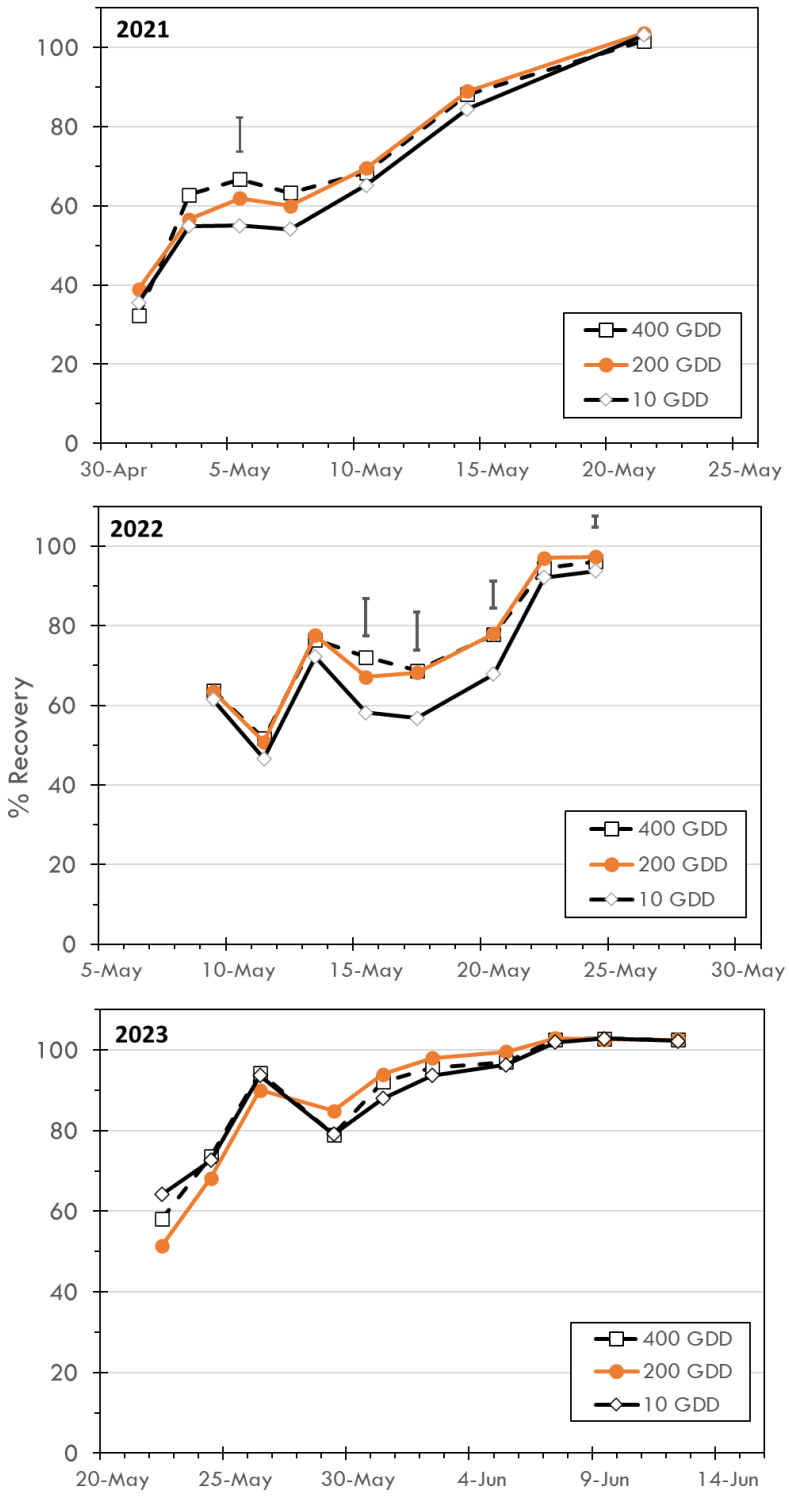


Figure 2. The main effect of PGR timing on core cultivation recovery on annual bluegrass turf, in the spring of 2021, 2022 and 2023. Bars above a rating date represent the Fisher's protected LSD value and indicates a significant difference between treatments.

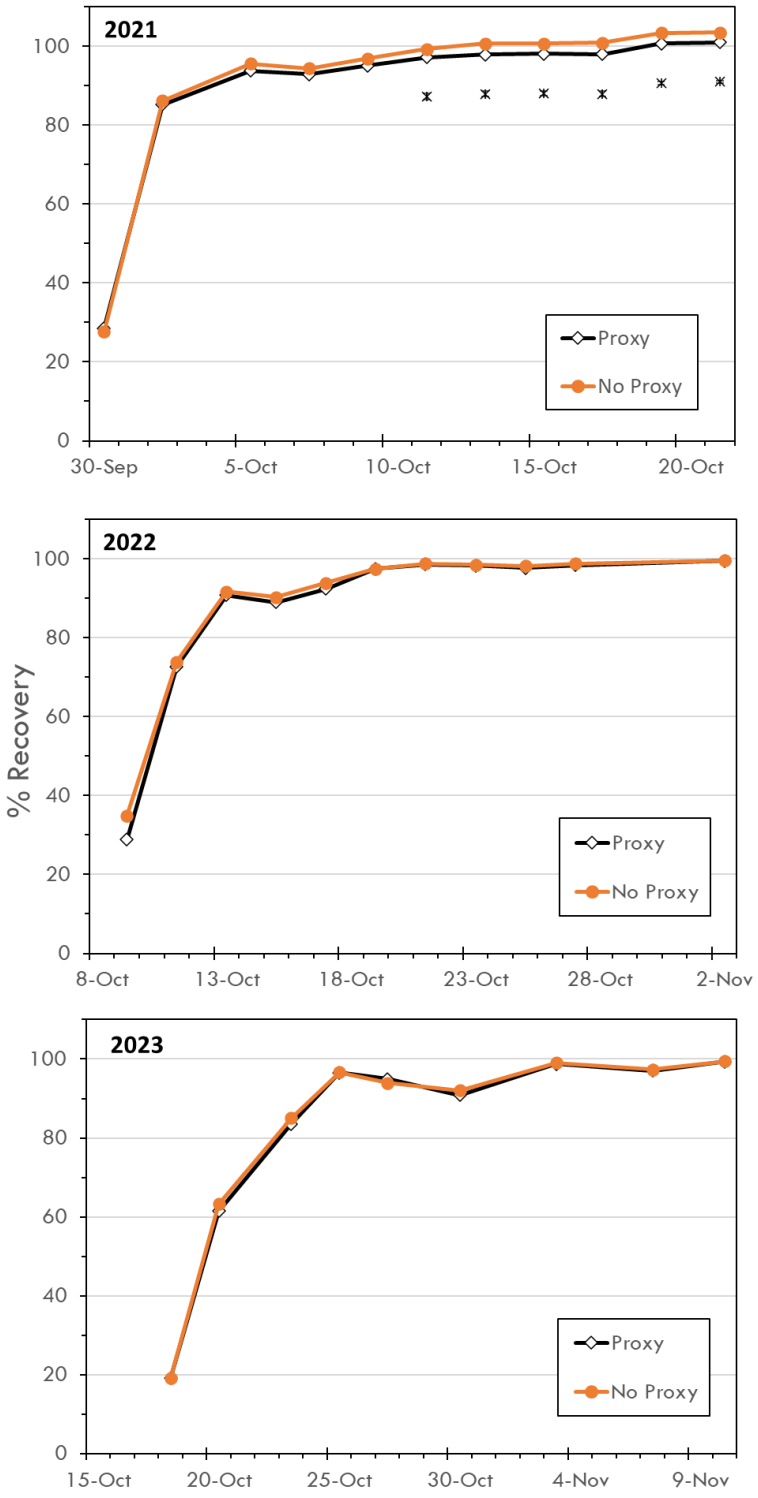


Figure 3. The main effect of ethephon (Proxy) on the percent recovery from core cultivation on annual bluegrass turf, in the fall of 2021, 2022, and 2023. Asterisk below a rating date indicates a significant difference between treatments.

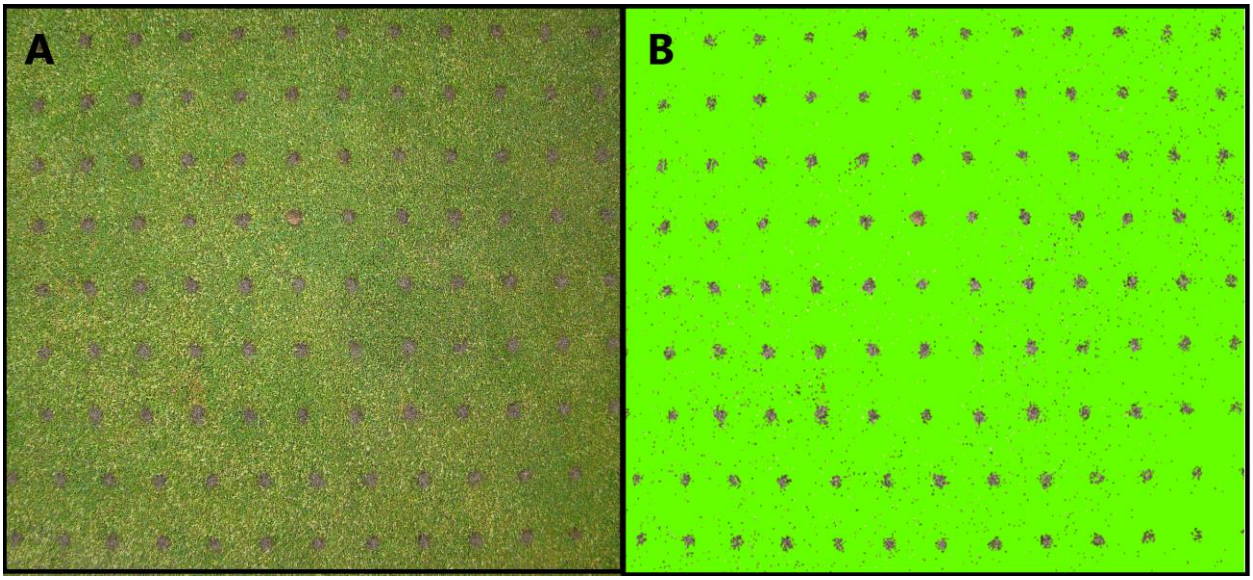


Image 1. Light box image of a core cultivated plot (A) and TurfAnalyzer image of percent green cover, which is used to calculate the cultivation recovery (B)



Image 2. Reduction in turfgrass quality in a gibberellic acid treated plot (left) compared to a plot that received trinexapac-ethyl 10 GDD prior to cultivation treatments (right), photographed 20 Oct 2023

USGA ID#: 2022-13-756

Title: Variable-rate versus conventional nitrogen application methods to golf course fairways

Project Leader: Chase Straw, Madan Sapkota, 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 three golf facilities: The City Course at Briarcrest (Bryan, TX), The Bearkat Course (Huntsville, TX), and The Club at Carlton Woods (The Woodlands, TX).
- The Club at Carlton Woods was selected for Objective 1, featuring two courses with sand-capped fairways, one course was hybrid bermudagrass and the other zoysiagrass. Variability in apparent electrical conductivity (EC_a) within these fairways was affected by turfgrass and soil attributes. However, the varying relationships observed within and between courses highlight the need for further research (Objective 1).
- Different vegetation indices were closely linked to soil moisture and, to some extent, penetration resistance, rather than distinctly indicating their ability to detect spatial variability in tissue N (Objective 1).
- The Bearkat Course was selected for Objectives 2 and 3. 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.
- Aerial imagery and ground data acquired during 2022 and 2023 are currently undergoing processing to evaluate seasonal variations (Objective 2) and to generate variable-rate prescription N fertility maps (Objective 3).

Summary Text:

Rationale

Precision turfgrass management (PTM) offers a solution for reducing fertilizer use on golf courses by identifying turfgrass and soil variability for targeted applications, potentially

lessening environmental impact. Current technology enables variable-rate fertilization, especially on fairways, which exhibit significant variability in turfgrass and soil characteristics. Limited investigation into these strategies exists, yet future sustainable management demands innovative approaches to decrease environmental impact while maintaining course quality. Despite this need, PTM adoption remains limited due to unclear benefits and decision-making knowledge. Comparative research between data-driven PTM and traditional methods is crucial for understanding and promoting PTM's advantages. This study aims to develop and compare variable-rate N fertilization programs on fairways with a conventional blanket strategy.

Methodology

Objective 1: This objective was broken down into two sub-objectives. Data were collected on April 12, 2021, at The Club at Carlton Woods in The Woodlands, TX, focusing on two sand-capped hybrid bermudagrass and two sand-capped zoysiagrass fairways. A tow-behind DUALEM-1S (D-1S; Dualem Inc., Milton, ON, Canada) with an external GNSS receiver measured georeferenced EC_a (mS m^{-1}) data at a 0.5 m depth. A Toro Precision Sense 6000 (PS6000; The Toro Company, Bloomington, MN) was used to collect ground soil moisture (% volumetric water content), penetration resistance, and normalized difference vegetation index (NDVI) from numerous georeferenced locations within each fairway. For each site, a sampling grid (62-137 samples, depending on fairway size) was created and imported into a handheld GNSS device. This guided the collection of soil moisture and EC_a at a 12.2 cm depth using a FieldScout TDR 350 (Spectrum Technologies, Aurora, IL, USA), elevation with the handheld GNSS receiver, a single soil core for organic layer depth (sub-objective 1), and three soil cores per location for clippings analyzed for tissue N using combustion (sub-objective 2).

Moreover, for the second sub-objective, images were captured from 120 m using either a Quantum F90+ or Matrice 200 unmanned aerial vehicle (UAV) with a MicaSense Altum multispectral camera (MicaSense Inc., Seattle, WA, USA). Pix4D processed images into orthomosaics and raster maps for four VIs: NDVI, green NDVI (GNDVI), normalized difference red edge (NDRE), and enhanced vegetation index (EVI). For both sub-objectives, ground data underwent ordinary kriging to produce raster maps. Sampling grid points were used to extract pixel values, and spatial correlations assessed relationships between variables ($\alpha = 0.05$).

Objective 2 and 3: The study location is the Bearkat Course in Huntsville, TX. It is a public golf course that has 'TifSport' hybrid bermudagrass and native soil (sandy loam classification) fairways. Four surveys using the PS6000 were conducted in 2022 on 26 May, 22 July, 14 Sept, and 26 Oct. In 2023, four additional surveys were conducted on 25 May, 27 July, 7 Aug, and 9 Oct. Aerial images were captured using a Dragonfly Commander 2 UAV (Dragonfly^{TM} Innovations Inc, Saskatoon, Saskatchewan, Canada) equipped with MicaSense Altum-PT multispectral camera (MicaSense Inc., Seattle, WA, USA) on the Oct 2022 date and all dates in 2023.

Additionally, an EM-38 (Geonics Ltd., Mississauga, Ontario, Canada) was used to survey apparent electrical conductivity (EC_a) via electromagnetic induction at the course in Oct both years. The EM-38, also towed behind a vehicle, generates an electromagnetic field to determine EC_a non-invasively by inducing an electrical current in the soil. All PS6000 and EM-38 data were georeferenced using an external GPS, ensuring sub-centimeter level locational accuracy.

Results to Date

Objective 1: The first sub-objective aimed to explore the relationship between EC_a and turfgrass and soil characteristics in the sand-capped fairways of the two golf courses at The Club at Carlton Woods. A positive correlation and statistically significant relationship (three out of four fairways) was found between EM-38 EC_a and soil moisture from both PS6000 (r=0.13 to 0.63) and handheld TDR 350 (r=0.20 to 0.62). Negative correlations existed between EM-38 EC_a and penetration resistance, while positive correlations were observed with EM-38 EC_a and organic matter depth; however, the strength of correlation was low and not statistically significant. Notably, NDVI and EM-38 EC_a had significant positive relationships in zoysiagrass fairways but showed mixed relationships in bermudagrass fairways. Elevation had a negative relationship with EM-38 EC_a in three fairways. Spatial maps of each parameter suggested that EM-38 EC_a variability in sand-capped fairways is influenced by turfgrass and soil characteristics. However, the inconsistent relationships within and between golf courses highlight the need for further research.

The second sub-objective explored the spatial relationship between turfgrass tissue N content and several vegetation indices (VIs) in the sand-capped fairways of the two golf courses at The Club at Carlton Woods. The VIs included NDVI, GNDVI, NDRE, and EVI. It was found that almost all VIs ($NDVI_{UAV/Ground,}$ $GNDVI_{UAV}$, $NDRE_{UAV}$, and EVI_{UAV}) showed significant and positive relationships with each. However, the relationships between VIs and tissue N were consistently weak and non-significant. Tissue N did not display any significant relationship with soil moisture or penetration resistance at any site. The general relationship between VIs and soil moisture was significant and positive on the fairways, while the general relationship between VIs and penetration resistance was not significant and negative on the fairways, except for one zoysiagrass fairway. Therefore, the results suggested that VIs were highly interrelated, with no clear distinction in their ability to detect spatial variability in tissue N. Instead, they were more closely associated with soil moisture and, to some extent, penetration resistance (Figure 1 and 2).

Objective 2 and 3: Survey data collected throughout 2022 at the Bearkat Course underwent analysis to calculate summary statistics for each par 4 and 5 fairway. Spatial maps were generated using ordinary kriging in ArcMap. From this analysis, nine fairways were chosen for the study and organized into three groups of similar fairways. This arrangement followed a randomized complete block design with three treatments and three replications (Figure 3). The treatments will include a 1) conventional blanket (control), 2) VI-based variable-rate, and 3) modeling-based variable-rate N granular applications.

For objective 2, three out of the nine fairways will be randomly selected for data analysis to establish site-specific management units (SSMUs), capturing both spatial and temporal variations within each fairway. Processing of the 2023-collected images is currently underway, intended for use in both Objectives 2 and 3. The second treatment for Objective 3 will employ NDVI-based variable rate-N applications, as it's the most used VI, and no differences were detected between it and other VIs in Objective 1. Additionally, secondary data such as elevation from the digital elevation model and soil moisture and penetration resistance from the PS6000 will be integrated with NDVI through data fusion using a principal component analysis and cluster analysis approach. The resulting data will facilitate the creation of model-based prescription maps for variable-rate N applications.

Future Expectations

- Generate fairway vegetation index-based SSMUs (NDVI) to evaluate seasonal variations (Objective 2) and craft variable-rate N prescription maps (Objective 3).
- Develop a digital elevation map encompassing all fairways to integrate topography, NDVI, soil moisture, and penetration resistance into modeling-based variable-rate N application prescription maps (Objective 3).
- Test GPS-guided spreader and preliminary prescription maps in early spring 2024 on a fairway not included in the study.
- Initiate treatment applications for Objective 3 in spring 2024.

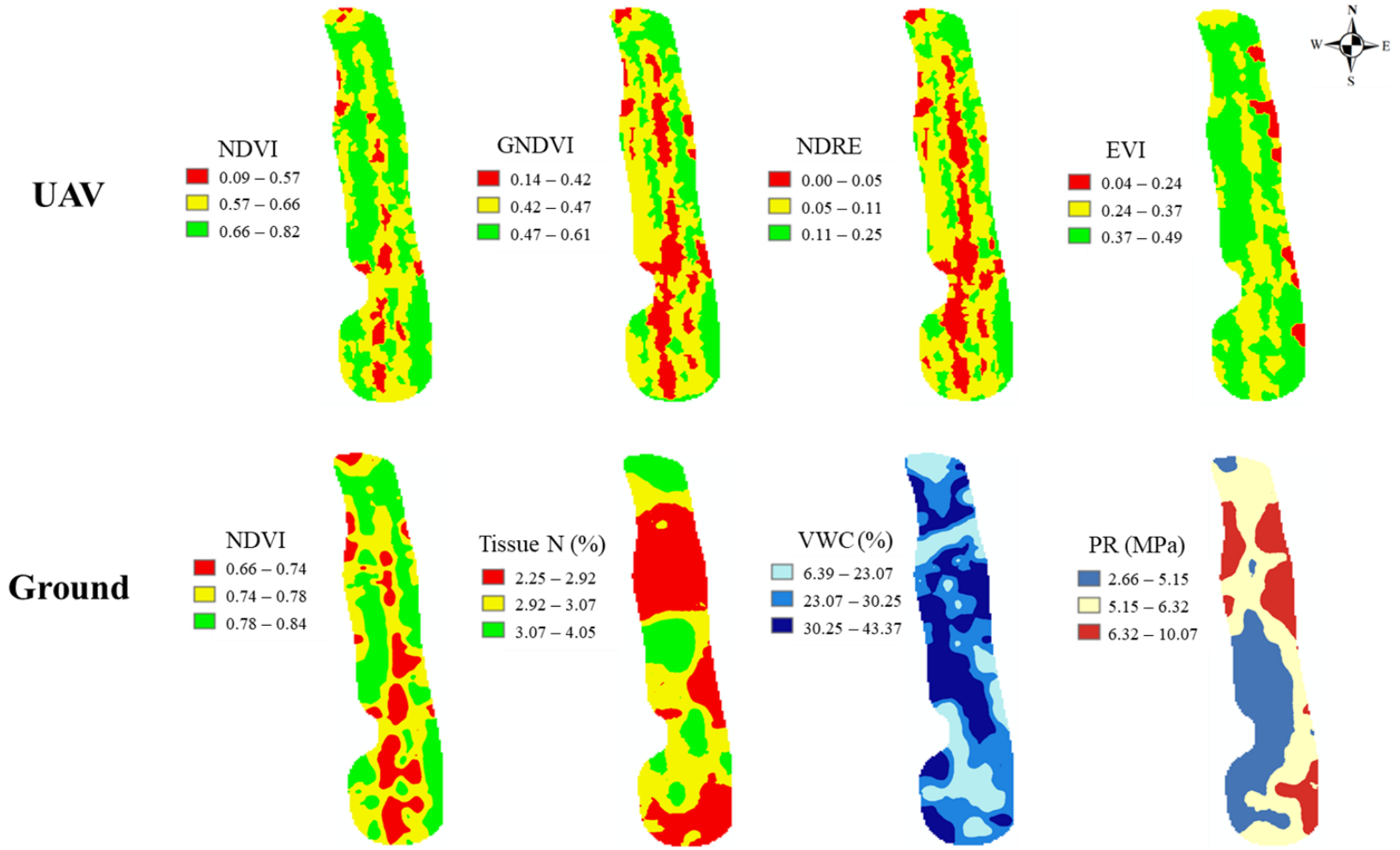


Figure 1. Spatial maps of variables collected from bermudagrass fairway 2 at The Club at Carlton Woods, The Woodland, TX.

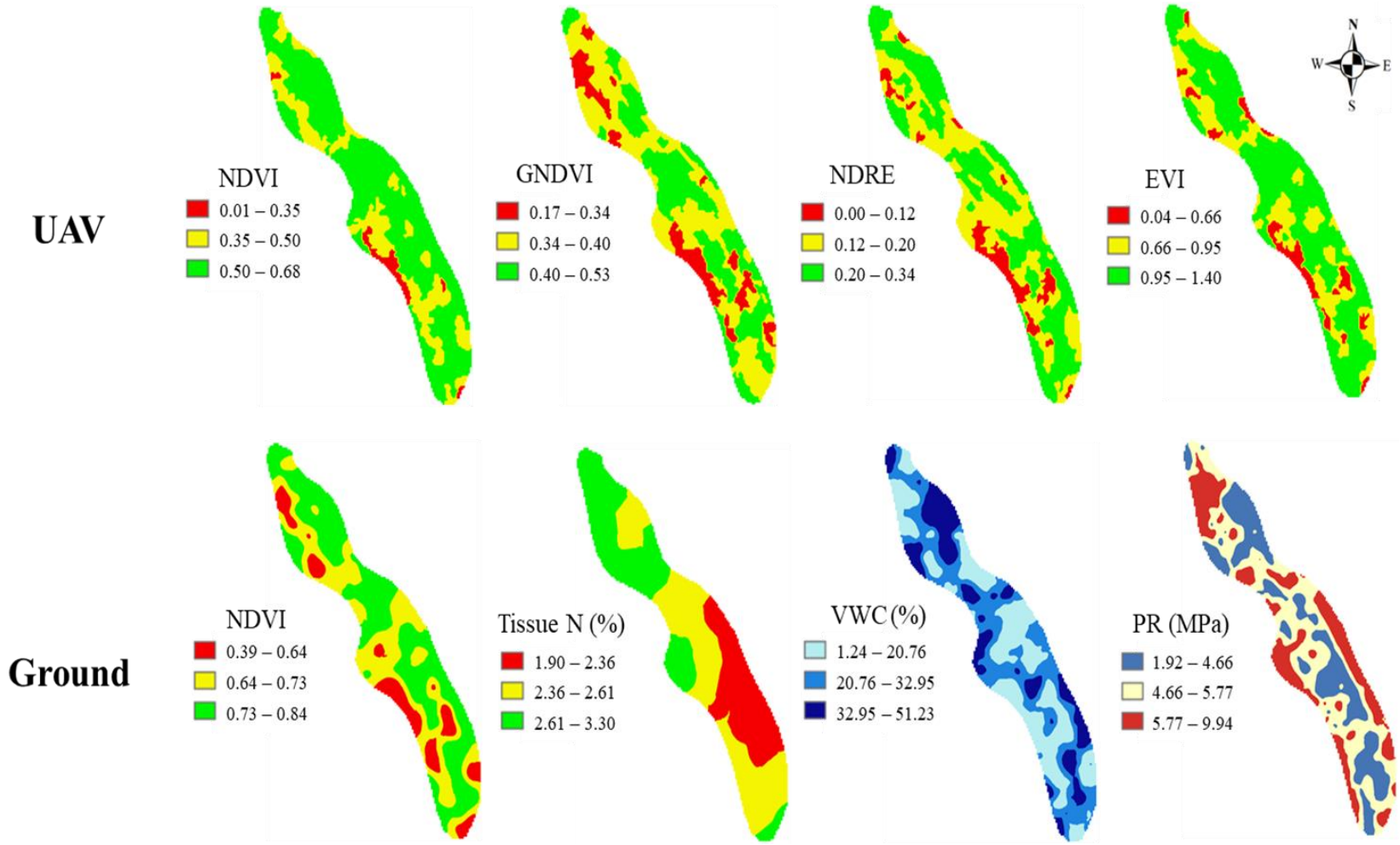


Figure 2. Spatial maps of variables collected from zoysiagrass fairway 8 at The Club at Carlton Woods, The Woodlands, TX.

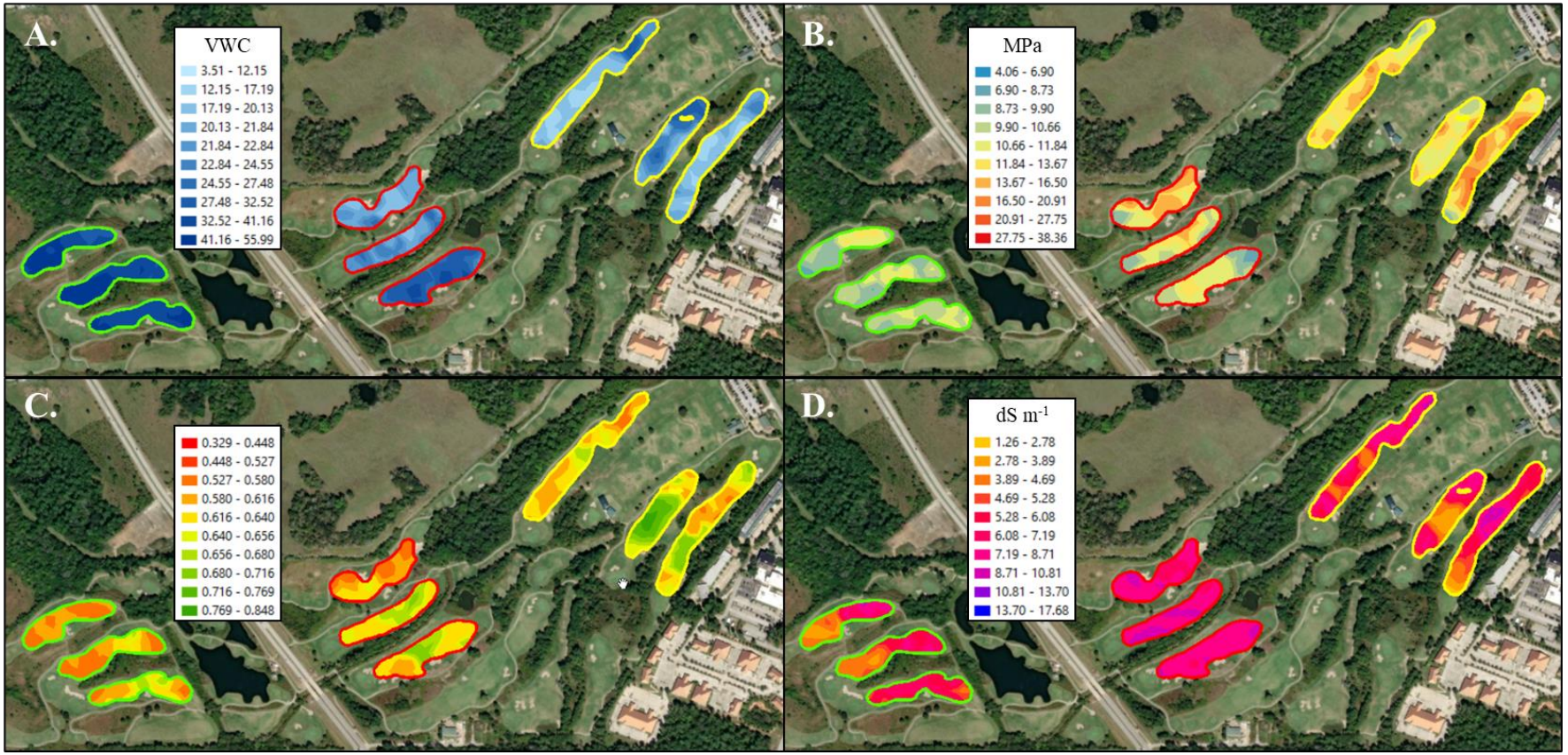


Figure 3. Spatial maps of data collected in fairways at Bearkat Course on 14 Sept. 2022 with the Toro Precision Sense 6000: A. soil moisture (volumetric water content; % VWC), B. penetration resistance (MPa), C. normalized difference vegetation index, and D. electrical conductivity (EC_e; dS m^{-1}). 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: 2017-17-627

Title: *Satellite-Based Estimation of Actual Evapotranspiration of Golf Course Cool Season Turf*
Project Leader: Lawrence Hipps
Affiliation : Utah State University

Original Project Objectives

1. *Quantify daily and seasonal evapotranspiration (ET) and energy balance of irrigated turfgrass of a golf course using eddy covariance measurements. Use findings to test currently used simplistic approaches such as reference ET.*

2. *Use several remote sensing-based models to estimate ET and validate their performance against ground-based measurements.*

3. *Combine measurements with theoretical knowledge to determine the response of ET to variations in weather and climate. Use this knowledge to develop a physically based model to estimate ET for the periods between satellite overpasses.*

Executive Summary
During the 2023 season, eddy covariance measurements of ET and energy balance of the site continued. Also, six unmanned aircraft flights were conducted to collect very high spatial resolution images. Because of the very large snowpack in the mountains of northern Utah during the winter of 2022 – 2023, irrigation water was not limited as greatly as during 2021 and 2022.

A major effort was made during 2023 to quantify turfgrass quality. This involved several approaches including: visual quality ratings; radiation data from the drones; and a handheld NDVI Turf Color Meter. A procedure was developed to correlate the high-resolution remote sensing data with turf quality at a high spatial resolution. A model was constructed to estimate turfgrass quality from the high- resolution remote sensing data. The result is high spatial resolution maps of turfgrass quality over the entire golf course. The remote sensing ET model was also used to generate spatial maps of ET, as had been done for 2022. The combined result is a set of maps for both ET and turf quality.

The relationship between ET and turf quality was variable depending on time of year and the different functional parts of the golf course. The strongest relationship was for times and regions that had a large spatial range of ET and turf quality. Turfgrass quality was also related to reference ET for fairways, tees, greens, driving range and rough.

To promote site-specific irrigation, management zones for ET and turf quality were quantified. Sprinker head locations were documented, and zones were classified by the size of the ET.

The Open ET (https://openetdata.org/) platform outputs ET estimates from six different remote sensing models. We tested the accuracy of all of these models against our measured ETa values. While several models reasonably estimated ET, the Disalexi model was the most accurate. Our findings over several years indicate that spatial distribution of ET can be adequately predicted by remote sensing ET models.

Summary of Activities 2023

Graduate Student Activities

A graduate student, Karem Meza Capcha conducts her main research on this project. Her responsibilities include monitoring the data each day using the telecommunications system connected to the weather station; coordinating with the golf course superintendent for our visits and activities at the site; arranging for him to clean the sensors when needed; and conducting all turfgrass quality measurements at the golf course. She also has a major responsibility for analyzing the ground-based data for actual ET and energy balance, scheduling and flying the USU drone remote sensing flights, getting the satellite data, and running the remote sensing ET models. She published her first paper in the Irrigation Science Journal (*https://doi.org/10.1007/s00271-023-00899-y).*

I. Measurements of ET and Energy Balance

Research is conducted at the Eagle Lake Golf Course near Layton, UT, about 25 miles north of Salt Lake City. The Eddy Covariance (EC) system consists of a CSAT-3 sonic anemometer (Campbell Scientic Inc., Logan, UT, USA) and an LI-7500 open-path infra-red gas analyzer (Li-Cor Biosciences, Lincoln, NE, USA) mounted at a height of 2.0 m. These instruments were operational from May through October 2023 and were sampled at a rate of 20 Hz. An image of the station and instruments is shown in Figure 1b.

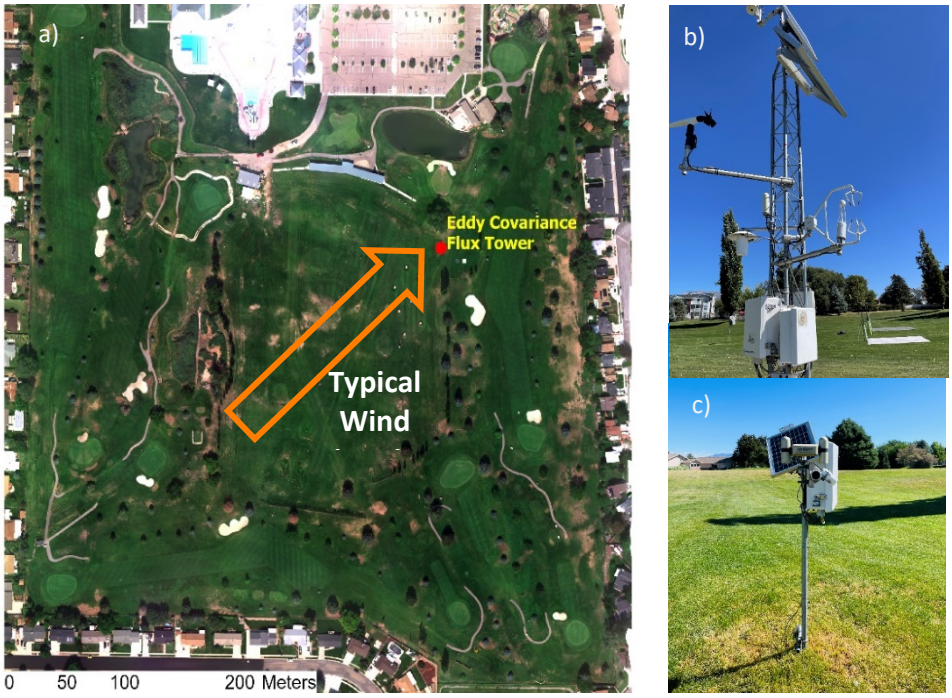


Figure 1. a) Map of Eagle Lake Golf Course and surrounding area, with the eddy covariance flux tower marked with a red circle, b) eddy covariance flux tower, and c) surface energy balance system.

The location of the eddy covariance and energy balance measurements on the golf course did not change in 2023 and are denoted in Figure 1a. The most common wind direction from the SW is also represented.

Net Radiation and Soil Heat Flux

The net radiation and soil measurements were at the same site as the previous year, about 20 m east of the eddy covariance tower. Note that this site was irrigated enough to be green, while the upwind regions including the driving range had brown regions due to water limitations during the middle part of the summer. Net radiation measurements were made with a SN-500 net radiometer (Apogee Instruments Inc., Logan, UT, USA) at a height of 1.45m (Figure 1c).

Soil water content was measured at two depths 0.04 m and 0.1 m using TDR-310H probes (Acclima, Inc., Meridian, ID). Soil heat flux was measured with two soil heat flux plates (REBS, Seattle, WA, USA) buried at 0.06 m, two averaging thermocouples at 0.015 m and 0.025 m. Soil measurements were made at the same location as the net radiation (Figure 1c).

II. Irrigation Issues Due to Drought 2022 and Irrigation 2023

Because of the near-record winter snowpack in the mountains of norther Utah, the availability of irrigation water was not limited as greatly as in 2021 and 2022 (Figure 2a). So, the golf course was more uniformly green in 2023 (Figure 2b).

(a)  **(b)** 

Figure 2. Drone image of the Eagle Lake Golf Course and surrounding areas in (a) July, 2022, and (b) July, 2023.

II. Remote Sensing Data

A rotary wing UAS DJI Matrice 600 Pro (DJI Technology Inc., Shenzhen, China) carrying an Altum PT camera (AgEagle Sensor Systems, Wichita, KS, USA) was used in 2023. The cameras have five high-resolution multispectral bands (blue, green, red, red edge, and near-infrared) and an integrated long-wave thermal infrared (TIR) sensor (based on the FLIR Lepton) which was aligned with the multispectral sensors. The imaging campaigns took place over six days to acquire spectral and thermal information (Table 1). The campaigns were carried out during midday considering bright sky conditions and the Federal Aviation Administration (FAA) regulations for urban study locations. In Table 1, overpass time (MDT), unmanned aircraft system (sUAS) altitude, multispectral and thermal pixel resolution, and weather conditions are listed for the overpass dates.

Table 1. Dates, times, flight height, spatial resolution, and weather to capture images with UAS

Date	Time (MDT)		UAS Elevation	UAS Spatial	Weather
	Launch	Landing	(m) AGL	resolution (cm)	
16-Jun-23	12:30 p.m	13:00 p.m	60	3.0	Sunny
28-Jul-23	12:30 p.m	13:00 p.m	60	3.0	Sunny
9-Aug-23	12:30 p.m	13:00 p.m	60	3.0	Sunny
26-Aug-23	12:30 p.m	13:00 p.m	60	3.0	Sunny
7-Sep-23	12:30 p.m	13:00 p.m	60	3.0	Sunny
16-Sep-23	12:30 p.m	13:00 p.m	60	3.0	Sunny

III. Turfgrass Quality Measurements and Prediction

The visual turfgrass quality (TQ) ratings and ground NDVI data were acquired simultaneously with unmanned aircraft system (UAS) data. NDVI data were measured on 16 June, 28 July, 9 and 26 August, and 7 and 16 September 2023, with the handheld TCM 500 NDVI Turf Color Meter (Spectrum Technologies Inc., Plainfield, IL) as an indicator of turfgrass visual quality assessment. This device utilizes an internal light source to measure the reflectance of red (660 nm) and near-infrared (850 nm) spectra. It then calculates the Normalized Difference Vegetation Index (NDVI) using the formula NDVI = [(R850 - R660)/(R850 + R660)], which is a scale ranging from 0 to 1. Values closer to 1 indicate a green and dense turfgrass canopy. A total of five hundred ten NDVI measurements were collected on 9 fairways, 15 tees, 9 greens, driving range, and rough areas, and five measurements per sample were recorded and averaged on a 1.0 m x 1.0 m sampling area. Box plots were made to represent the distribution of NDVI data.

Visual TQ ratings were collected using subjective considerations from The National Turfgrass Evaluation Program (NTEP) evaluation guidelines. The visual assessment integrates turfgrass color, density, uniformity, and leaf texture and rates the turfgrass stand on a scale of 1 to 9, where 9 indicates an ideal turfgrass stand, 6 is the minimum acceptable quality and 1 indicates the turfgrass is dormant or dead. Turfgrass quality was assessed at the same time as NDVI measurements, and a coefficient of determination was conducted to quantify the correlation between visual TQ ratings and NDVI measurements.

Spectral Indices (SIs) were selected using the Awesome Spectral Indices (ASI) catalog (https://github.com/awesome-spectral-indices/awesome-spectral-indices). This catalog contains 231 spectral indices and is divided into 8 groups, which mostly represent specific application domains namely: vegetation, water, burn, snow, urban, radar, soil, and kernel indices (Montero et al. 2023). The six multispectral bands and one thermal band were used to compute the selected SIs.

The machine learning method RF regression was utilized as a base learner in this study to generate a TQ prediction model for the dataset. The RF method was chosen among different ML approaches as it is an efficient technique for analyzing linear and non-linear relationships. The predictors included eighty-five variables, comprising six multispectral bands, one thermal band, and seventy-nine SIs. A response variable was the TQ based on NDVI–TCM 500 measurements. The input data was split randomly into training (70%) and testing (30%).

Results of Remote Sensing Model for Turfgrass Quality

The mean ground TQ-based NDVI was 0.74, 0.73, 0.71, 0.63, and 0.52 in greens, fairways, tees, driving range, and rough areas, respectively (Figure 3a). The lowest ground TQ ratings were observed

for rough areas on July 28, and August 9, 2023, which were drier than the other four dates. Objective measurements such as NDVI are being adopted more often for their reliable and repeatable estimation of quality, however, TQ based on a 1 -9 scale is still important when evaluating turf performance. TQ based on a 1 -9 scale was correlated positively with ground TQ-based NDVI with R^2 equal to 0.93 (Figure 3b).

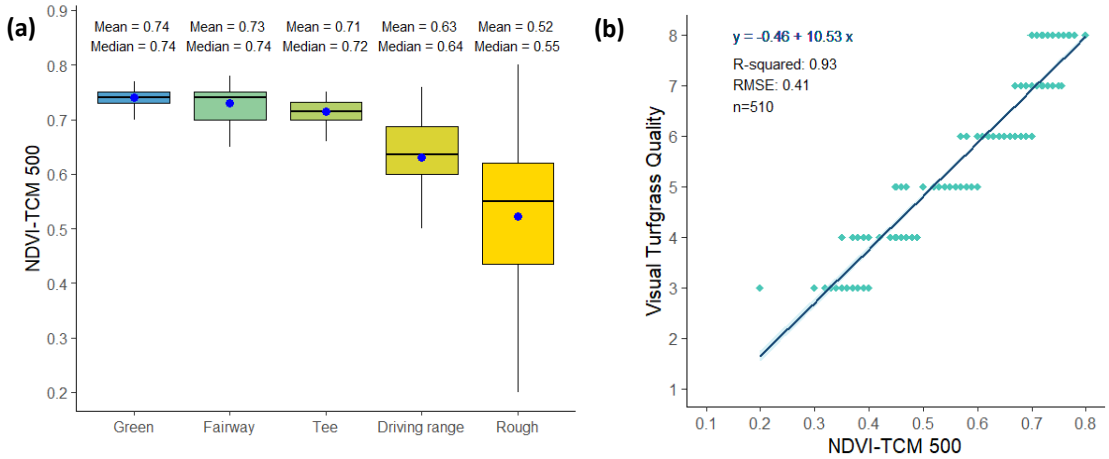


Figure 3. (a) Box plots showing the distribution of the sampling NDVI using the TCM 500 sensor in the greens, fairways, tees, driving range, and rough areas, the blue dots show the average, and (b) Relationship between visual TQ and TQ based on NDVI.

The RF regressor model with all predictors was selected to investigate the relative importance of input variables for TQ prediction. According to the Gin importance analysis (Figure 4a), the Blue Normalized Difference Vegetation Index (BNDVI), Simple Ratio Index (SR), and Red Green Blue Vegetation Index (RGBVI) were shown to be more significant in predicting TQ. Figure 4b shows the scatter plot showing the relationship between the observed and predicted TQ for test and train data, reaching an R^2 of 0.98 and 0.85, respectively.

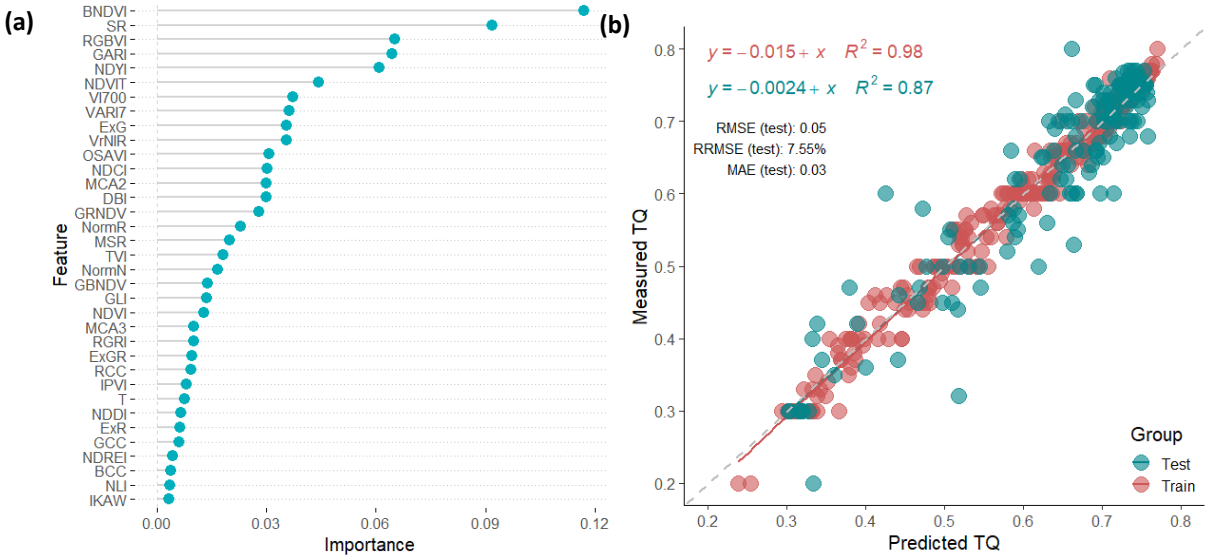


Figure 4. (a) Gini importance of RF regression using the six spectral bands, one thermal band, and seventy-nine SIs based on UAS information and (b) Testing and training data, the relation between the measured TQ and the estimated TQ calculated by RF.

The BNDVI, SR, and RGBVI indices were the most important predictors for turfgrass quality modeling (Figure 5).

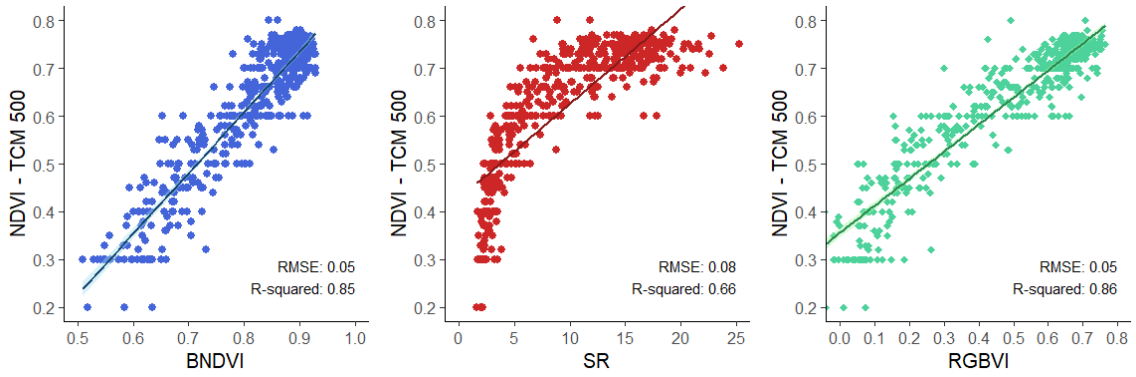


Figure 5. Linear regression between NDVI-TCM 500 and a) BNDVI, b) SR, and c) RGBVI.

IV. Spatial Variability of Actual Evapotranspiration and Turfgrass Quality

Spatial variability of actual evapotranspiration and turfgrass quality

Figure 6 illustrates the spatial distribution of turfgrass quality-based random forest (TQ-RF) and ETa maps in the study area. Descriptive analyses were performed to verify the spatial variability of the TQ-RF and ETa.

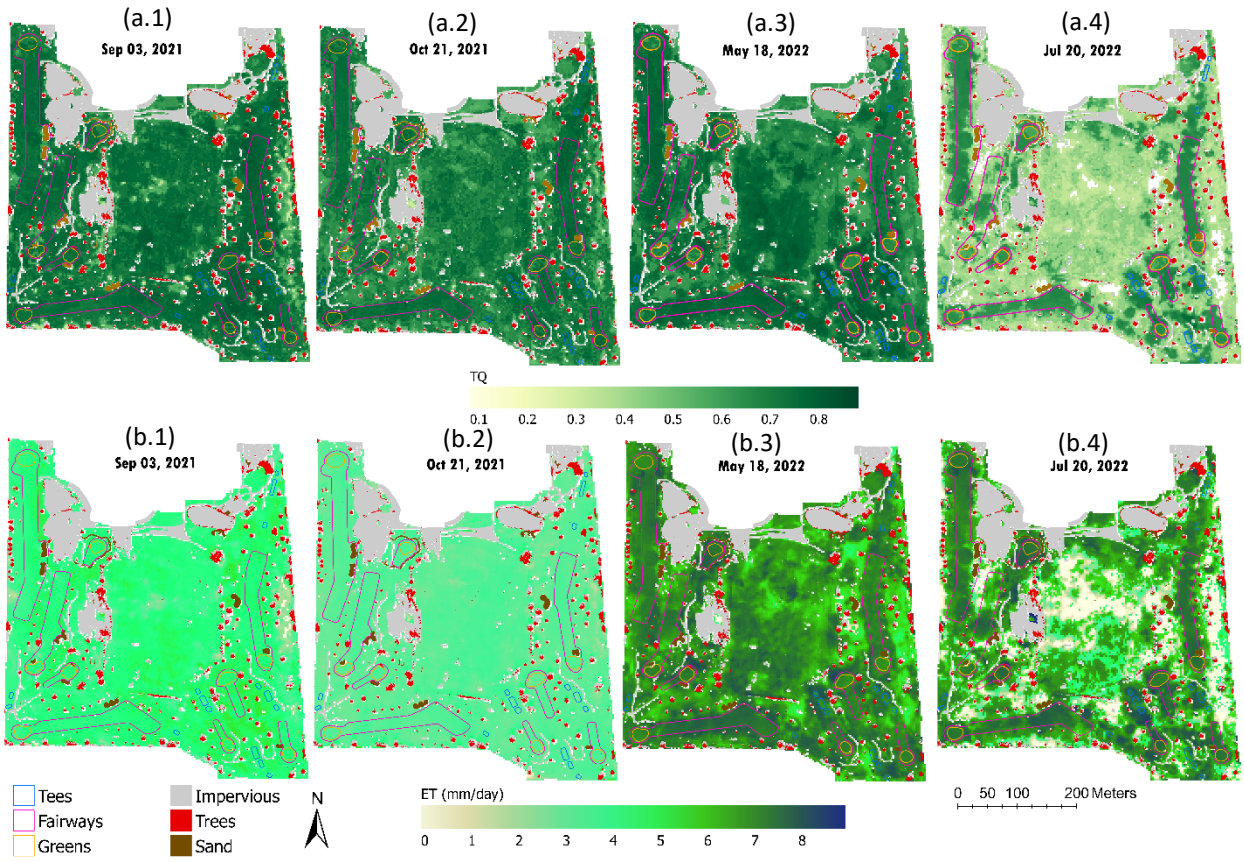


Figure 6. (a) Spatiotemporal distribution of the estimated turfgrass quality using RF model and (b) actual evapotranspiration using the TSEB model in each 3 × 3 m.

Relationship of Turfgrass Quality with Actual Evapotranspiration

Linear regressions between TQ-RF and ETa are shown in Figure 7. The relationship between TQ-RF and ETa was found to be significant on fairways, greens, tees, driving range, and roughs during the dates of May 18, and July 20, 2022. The TQ increased with increasing ETa rates, the linear model was a better predictor of TQ at fairways (R^2=0.74), tees (R^2=0.66), and roughs (R^2=0.75) than greens (R^2=0.25) and driving range (R^2=0.36) on July 20, 2022. The linearity of the TQ-ETa relationship on fairways indicates that any decline in ET resulted in a reduction in TQ-RF, this relation is for spring and summer days. The TQ-ETa regressions for September 03 and October 21, 2021, showed a low correlation, since very little change occurred in TQ (0.6 - 0.75) over a narrow range of ETa (2.5 - 4.6 mm/day).

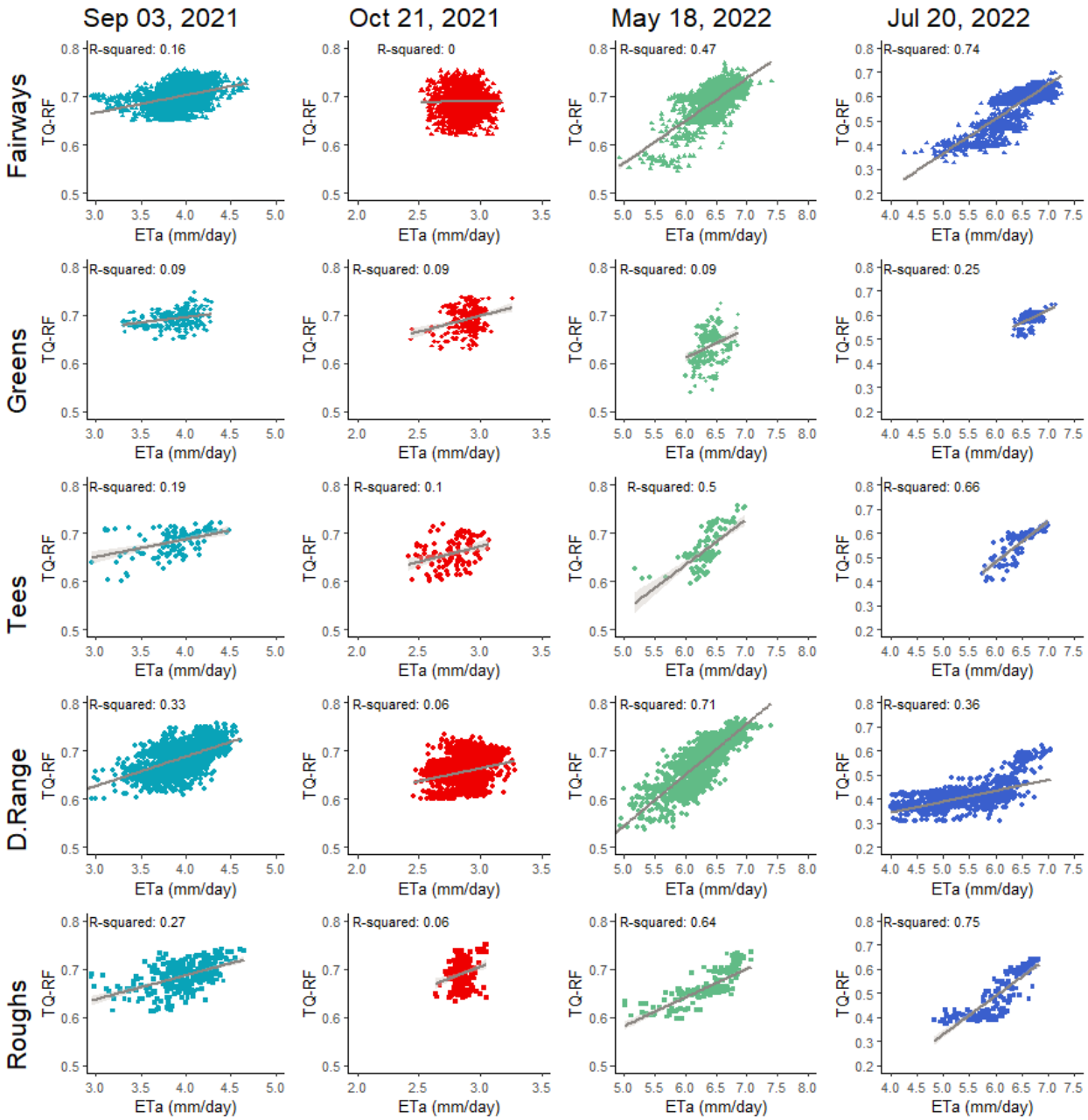


Figure 7. Linear regression relationship between ETa and TQ on fairways, greens, tees, driving range, and roughs during the dates of September 03 and October 21, 2021, and May 18, and July 20, 2022.

V. Precision irrigation for turfgrass management

Reference ET fraction and turfgrass quality

ET fraction (ETf) is synonymous with the commonly used short grass-based crop coefficient (K_c), this was introduced by Allen et al. (2013).

$$ET_f = \frac{ET_a}{ET_r}$$

Where, ET_a=daily actual evapotranspiration, ET_r= daily short grass reference evapotranspiration. The daily actual evapotranspiration was estimated using the TSEB model and UAS information. The daily reference evapotranspiration was acquired from the weather station located at the research location, which is part of the Utah State Climate Center network of weather stations (https://climate.usu.edu). A model was developed to quantify the decrease in TQ in relation to the ET replacement. Exponential, linear, and power regressions models were performed between TQ-RF and ET_f. The selection of the regression model was based on residuals, R^2, and RMSE to evaluate the goodness of fit.

Results of the relationship between TQ-RF and ETf

TQ as a function of reference evapotranspiration fraction (ETf) is shown in Figure 8. Spatial TQ and ETf information on May 18, 2022, and July 20, 2022, were selected to find the best performance. Strongly supported results were observed when modeling using an exponential function with R2 equal to 0.8. The graphs (Figure 8) show the relationship between TQ and ETf, the division of golf course areas was made based on priority areas for irrigation in summer (Figure 8a), non-priority areas for irrigation in summer under drought scenarios (Figure 8b), and all golf course areas for irrigation (Figure 8c). These graphs show how the TQ-RF decreased as ETf decreased.

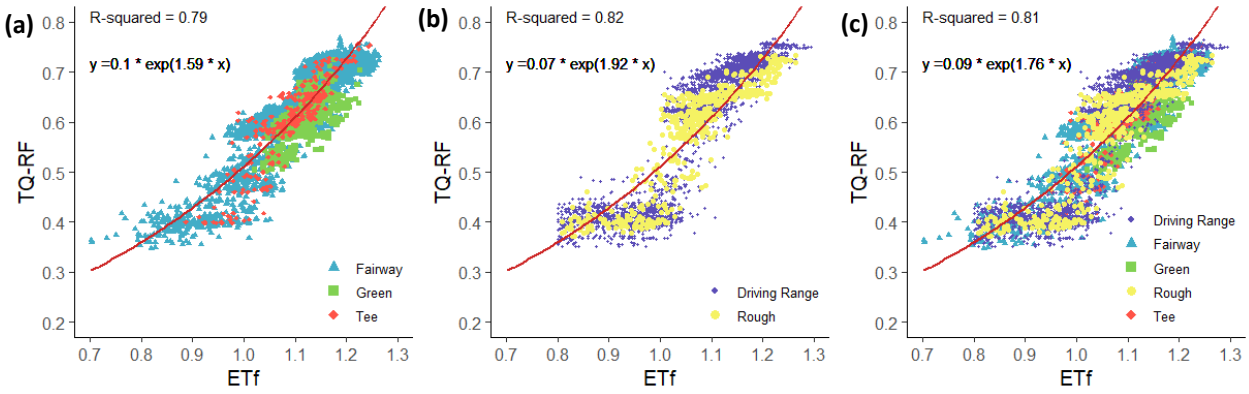


Figure 8. Coefficient of determination (r^2) and exponential function between TQ and ETf on (a) fairways, greens, and tees (b) driving range and tees, (c) fairways, greens, tees, driving range, and roughs.

For fairways, greens, and tees, the composed turfgrass decreased about 10 % in quality with an irrigation schedule providing up to a 100% ET_r (Figure 7a). For driving range, fairways, greens, roughs, and tees, the composed turfgrass decreases about 10 % in quality with an irrigation schedule providing up to 97% (Figure 7c). The large decrease of TQ-RF with a decrease in ETa extended to greater ETf.

Classifying ETa and TQ management zones for site-precision irrigation

ETa and TQ site-specific management zone (SSMZ) were classified using the "*Natural Breaks*" classification method in ArcGIS Pro software. Four interval legend classifications were calculated to display spatial variability in the maps. For the irrigation management zone delineation, sprinkler heads were assigned to ETa classes, as a result of their surrounding ETa values. A shapefile of points, which includes the irrigation head locations (340 rotors), was created to create boundaries. The "*Thiessen Polygons"* tool was used to divide the area covered by the input point feature into proximal zones in the Analyst Toolbox, ArcGIS Pro. The mean value of all pixels within each Thiessen Polygon was calculated for each associated ETa map using the *Zonal Statistics* tool in the Spatial Analyst Toolbox (Straw et al. 2022).

Results of classifying ETa and TQ management zones for site-precision irrigation

The ETa site-specific management zones (SSMZ), and histograms are shown in Figure 9. Each irrigation head was associated with a specific TQ-RF and ETa classification, based on their surrounding ETa values, respectively. The values range within each high ETa SSMZ classification increased during summer and decreased during fall. The high ETa SSMZ classification on July 20, 2022 (5.7 - 7.3 mm/day) was greater than May 18, 2022 (6.4 - 6.9 mm/day), and the high ETa SSMZ classification on September 03, 2021 (4.0 - 4.5 mm/day) was greater than October 21, 2021 (2.9 - 3.6 mm/day).

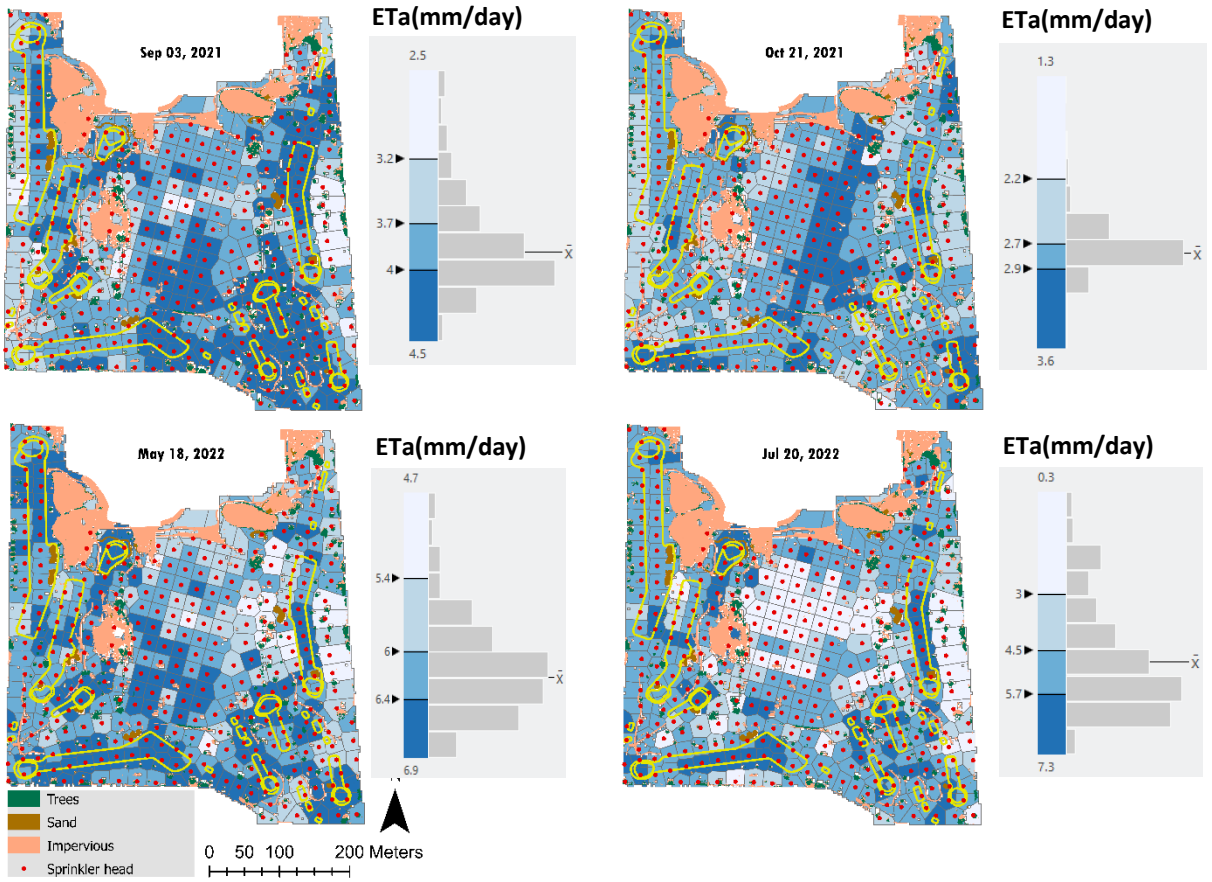


Figure 9. Maps showing the ETa site – specific management zones on golf course. Yellow lines are fairways, greens, and tees boundaries.

VI. Comparison of ETa-EC with Open ET models

The Open ET (https://openetdata.org/) platform outputs ET estimates from six different remote sensing models. We tested the accuracy of all of these models against our measured ETa values using RMSE, r^2, and residuals.

Results of Comparison of ETa-EC with Open ET models

The linear regression plots showed the ability of the Disalexi model using satellite imagery to estimate the spatial variation of daily actual ET for well and partially-irrigated urban turfgrass, which is useful for landscape irrigation management, especially in drought conditions.

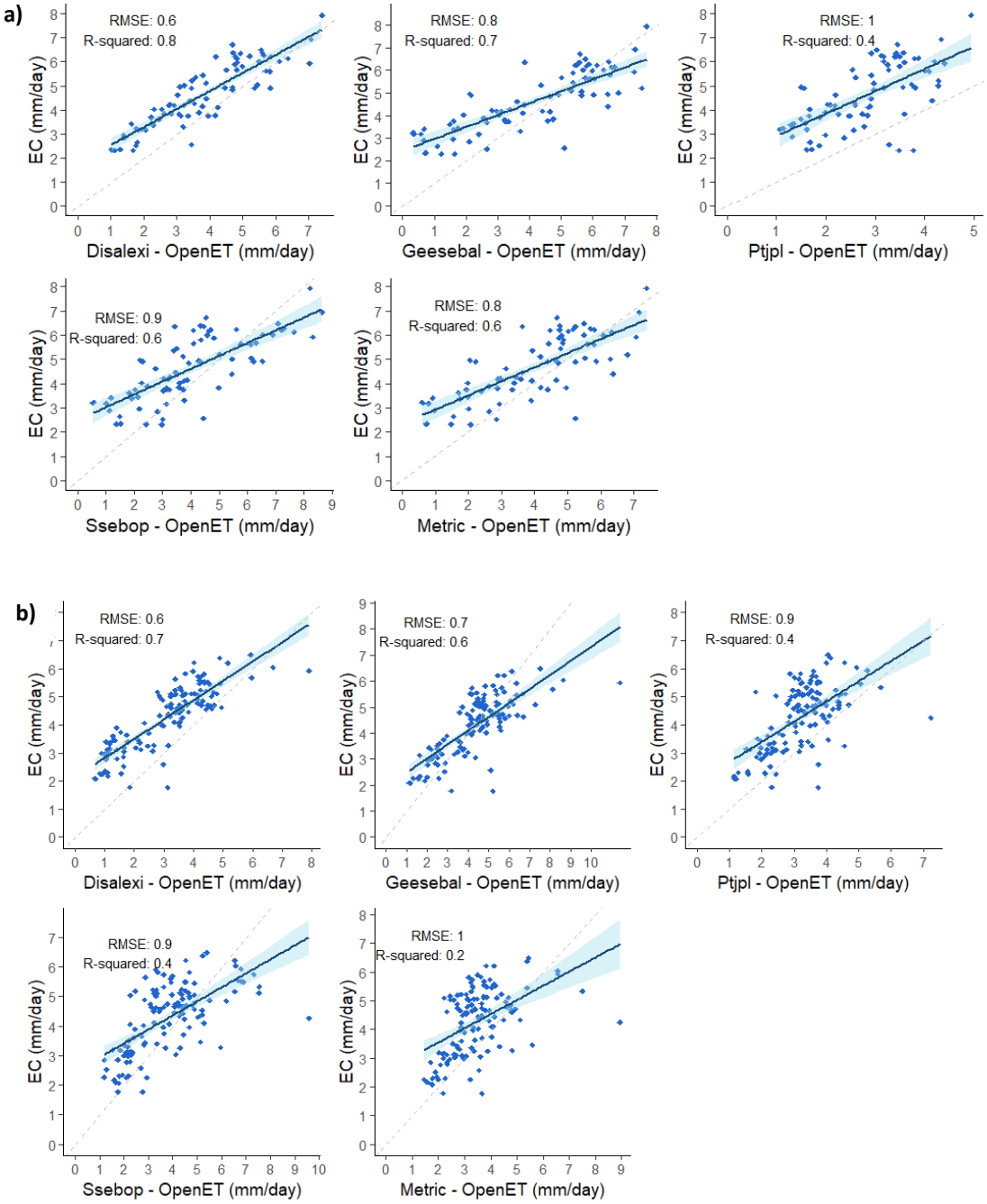


Figure 10. OpenET - satellite remote sensing methods vs eddy covariance measurements in a)2021 and b)2022

Plan of Work 2024

Investigate How to Package Information and Methods for Users
This was not in the original objectives of the project. But we feel it is important to consider how the advanced information about water use from this project could be provided to golf courses in the future. This is a bigger issue than we can address here, but we can at least work with our golf course partner, to find out what they would like as products, and what they might be willing to do to take some ownership of the methodology.

The response of turfgrass ET to physiological and climate factors
We will assess the responses of stomatal resistance and ET to climate variations during three summer seasons (2021, 2022, and 2023), taking into account both well-irrigated and partially irrigated scenarios. The goal is to document the relative role of available energy and saturation on the ET of the turfgrass, and how it may change with variations in weather conditions. In addition, it is useful to determine how the turfgrass stomatal resistance responds to saturation deficit. Since the increases in saturation deficit are largely due to increases in temperature, these findings will help understand how turfgrass Et might respond to warmer temperatures.

References

Straw C, Bolton C, Young J, et al (2022) Soil moisture variability on golf course fairways across the United States: An opportunity for water conservation with precision irrigation. Agrosystems, Geosciences and Environment 5:. https://doi.org/10.1002/agg2.20323

Montero D, Aybar C, Mahecha MD, et al (2023) A standardized catalogue of spectral indices to advance the use of remote sensing in Earth system research. Sci Data 10:197. https://doi.org/10.1038/s41597-023-02096-0

USGA ID#: 2021-15-739

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

Project Leader: Josh Friell, Ryan Schwab, Eric Watkins
Affiliation: University of Minnesota

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 cultivars of Kentucky bluegrass and creeping bentgrass subjected to irrigation treatments based on differing threshold-based irrigation treatments.
- Plots irrigated at higher moisture thresholds used significantly more water and remained significantly softer and greener; however, a threshold of 45% provided similar surface firmness and green canopy cover as the 75% and 60% treatments, but did not significantly reduce water use. Although the water reduction for that threshold was not statistically significant, it still represented a 25% reduction from the 75% treatment, which is practically meaningful.
- Canopy NDVI declined (15% threshold), increased (45, 60, or 75% threshold) or did not change (30% threshold) at a statistically significant rate over time and the interaction of time with irrigation threshold was significant.

Phase 2, Year 2

Plot Maintenance & Treatments:

A second year of data was collected from the plots described in our 2022 update report, which were established prior to Phase 2, Year 1 (Figure 1). Briefly, Kentucky bluegrass (*Poa pratensis* L.*)* 'Shamrock' and creeping bentgrass (*Agrostis stolonifera* L.) 'Penncross' were maintained 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. Prior to initiation of treatments, plots received 2.4 g N m^{-2} and 2.4 g K_2O m^{-2} on 26 Apr 2023. 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.

Treatments again 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 25 Jun 2023, 2.5 cm of irrigation was applied to plots prior to initiation of treatments to generate rootzone conditions near field capacity. Irrigation treatments were applied from 26 Jun – 30 Sep 2023. 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 was applied evenly to the plot using a hose-end flow meter (Model 825 Meter, Tuthill Corp., Burr Ridge, IL). The response of each plot canopy and soil to the irrigation treatments was quantified using the same tools as in the previous year. Device repairs prohibited collection of photosynthetic efficiency and PAR absorptivity data for dates after 8 Aug. Similarly, surface firmness was not collected on several dates due to Clegg device malfunctions.

Analysis & Results to Date:

Data Analysis

Significant rainfall of 13.2 cm during the final week of treatments created conditions not representative of the rest of the treatment period. Therefore, for the purposes of this report, analysis was limited to data prior to 23 Sep 2023 and conditions on 22 Sep were used as an indication of long-term impact of treatment effects. Analysis of variance was conducted on those data in R with subsequent means separation using the *emmeans* and *cld* package implementation of Tukey contrasts. A repeated measures analysis was performed using the *lme4* package to estimate the effect of irrigation treatment on NDVI over time.

Soil Moisture & Canopy Response:

Total ET_o depth during that period was 30.5 cm and total precipitation was 26.6 cm. At the commencement of the treatment period, soil moisture values for all plots were 33.3 – 41.9%. As of 22 Sep, soil moisture values for all plots were 10.2 – 38.3% (Fig 2).

Data collected as of 22 Sep 2023 showed significant differences between the threshold treatments for total cumulative irrigation water applied up to that date as well as final green canopy cover and final surface hardness as measured on that date (Table 1). Unlike Year 1, there was no significant interaction between species and moisture threshold for green canopy cover. Plots irrigated at higher moisture thresholds used more water and remained softer and greener. A moisture threshold of 30% plant available water significantly reduced water use compared to a 75% threshold but created a firmer surface and less green canopy cover. Interestingly, a threshold of 45% provided similar surface firmness and green canopy cover and did not significantly reduce water use. Although the water reduction for that threshold was not statistically different than the 75% threshold treatment it still represented a 25% reduction, which is practically meaningful. Interestingly, the 60% threshold treatment used numerically, but not significantly,

less water than the 45% threshold treatment, which differed from 2022 results. Further resolving the relationship between these and the other response variables over time is left for future analysis work.

To assess the long-term impact of the irrigation threshold selection, a model was built using the *lmer* function and included a term for species as well for main and interaction effects of irrigation treatment and days since treatment initiation. Random intercepts were allowed for each plot. The analysis of NDVI response showed that the effect for days and its interaction with irrigation threshold treatment were both statistically significant. This indicated that the rate at which NDVI changed over time was different between threshold treatments. Marginal interaction effect sizes ranged from -0.0012 to 0.0168 day^{-1}. The slope over time for the 15% threshold treatment was the only negative value, which indicated it was the only treatment for which the NDVI declined over time at a rate significantly different from zero. The slope over time for the 30% threshold treatment had a value of 0.0003, but was not significantly different from zero indicating that it did not change over time. All other slopes were positive and significantly different from zero indicating that for the 45, 60, and 75% thresholds, NDVI increased over time. Additional analyses of this type are planned to evaluate the effect of each treatment over time on other response variables.

Table 1. 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 on 22 Sep 2023.

Moisture Threshold	Total Water Use (L)	Group	Final Surface Hardness (g)	Group	Final Green Canopy Cover (%)	
15	123	A	139.3	C	34.4	A
30	164	A	105.8	BC	55.0	AB
45	301	AB	83.8	AB	78.3	BC
60	255	AB	86	AB	70.4	BC
75	392	B	54.6	A	85.9	C



Figure 1. Creeping bentgrass (CBG) and Kentucky bluegrass (KBG) fairway turf plots maintained at different soil moisture thresholds (15, 30, 45, 60, or 75 % plant available water) on 3 Aug in St. Paul, MN.

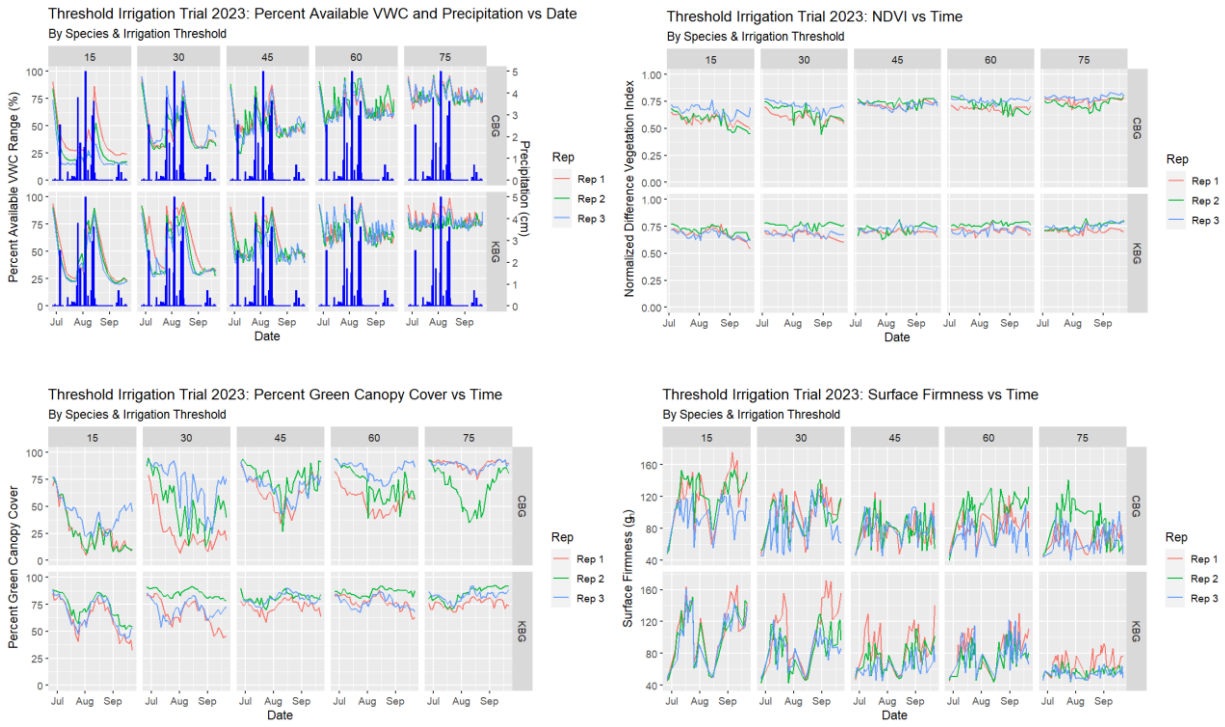


Figure 2. Top-Left: Percent of available volumetric water content range and precipitation vs time; Top-Right: NDVI vs time; Bottom-Left: percent green canopy cover vs time on plots irrigated at different soil moisture thresholds; Bottom-Right: surface firmness in g_n as measured by Clegg impact hammer test vs time for creeping bentgrass and Kentucky bluegrass fairway turf plots maintained at different soil moisture thresholds in St. Paul, MN.

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 9-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 separate 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 2 inch topdressed (0 TD), 2, 4, and 8 inch) 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), applied June through October. Weekly traffic is imposed on all treatment plots using a golf cart, with 16 passes per week (Figure 1). During the study period, application of a 50% sulfur coated urea 21-7-14 fertilizer was applied at 1 lb. N per 1000 ft^2 every 6 weeks. Pelletized gypsum (applied at 40 lb per 1000 ft^2) has also been applied monthly to all research plots.

For data collection, visual turf quality, visual percent firing, visual percent wilt, and percent green cover were rated evaluated monthly over the 2022 and 2023 seasons. Soil hydrophobicity was evaluated through water droplet penetration time testing conducted in October of both seasons (measured at 1 cm depth). Saturated hydraulic conductivity was also measured at the end of both study years. Divots were created using a simulated divot maker in early September both years, with recovery evaluated. Soil moisture and salinity data were also 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. Data for the project have all been collected, and are currently being analyzed, with the full set of comprehensive results and final report to be provided at the conclusion of the project.

2023 Season Summary
The 2023 study period was one of the hottest and dryest on record for College Station, with daily highs during the majority of the June 15 through October 1 deficit irrigation period exceeding 100°F, and evapotranspiration exceeding 0.26"/day. Less than 0.1" rainfall occurred from June 15- Sept. 13th. Approximately 0.75" of precipitation was received during late September, coinciding with the resumption of full (0.6 x ETo) irrigation levels to plots on October 1.

ANOVA showed an Irrigation level x date interaction for turf quality (data not shown). While there were no differences between the 0.45 and 0.3 x ETo treatments on the initial June rating dates, quality fell to 3.5 out of 9 for the 0.3 x ETo level by late August, and to a low of 4.5 out of 9 for the 0.45 x ETo level (Figure 2). Neither of the deficit irrigation levels supported acceptable (6) quality from late-June through September, but both recovered to acceptable levels by late October, following 3-4 weeks of recovery.
There was also a capping depth x date interaction for turf quality in the study (Figure 3). No differences were detected due to capping depth on the initial two rating dates. However, by mid-July, there was an inverse relationship between capping depth and turf quality, with the topdressed 0" treatment producing the highest quality, and the 8" capping depth producing the poorest quality. Interestingly, the TD 0" treatment produced minimally acceptable quality on 3 of the 7 rating dates during the study period, The 2" capping depth had also rebounded to acceptable quality by the final rating date, but the 4 and 8" capping depths were noticeably slower to recover from summer drought stress.

There was also an irrigation level x wetting agent interaction on turf quality during the study. As such, wetting agent application led to significantly improved turf quality (5.9 vs. 5.5) at the 0.45 x ETo level, but there was no benefit in quality due to wetting agent noted at the 0.3 x ETo level, as both treatments averaged quality of 5.4 (Figure 4).

Despite observed benefits of wetting agent on turf quality at the 0.45 x ETo irrigation level, effects of wetting agent on water droplet penetration time (WDPT) (1 cm depth) were more pronounced at the 0.3 than the 0.45 x ETo level (Figure 5). Wetting agent application reduced WDPT from 110 to less than 10 sec in the 0.3 x ETo treatment, and reduced WDPT from ~37 to 12 sec at the 0.45 x ETo level. Interestingly, although there was a capping depth main effect on WDPT in past years, there was no significant effect of depth on WDPT for either soil in 2023 (P = 0.09 for clay loam, 0.7 for sandy loam).

In late October 2022, subsoil (0-2") samples were removed from plots for sodium adsorption ratio analysis. Samples were removed only from the 4 and 8" capping depth plots within the 0.45 x ETo plots. The data showed that despite continued pelletized gypsum applications (10 lbs./1000 sq. ft. monthly from June through October, SAR values indicate highly sodic soil conditions in the subsoils underlying sand-capped plots. Subsoil SAR values ranged from 12 for 8" cap depth to 15 for 4" cap depth treatments, which suggests that, although the shallower capping depth is producing higher turf quality due to more favorable soil water relations, it may also be leading to more rapid deterioration of subsoils.

Root samples were collected for the sand-cap as well as 0-12" subsoils in plots during November 2023. These samples are currently being rinsed and oven dried for determining root dry mass within the sand-cap and subsoil root zones. These data will be compared to those distributions observed early on from the site (2016) to determine how root development has been affected by long term exposure to these sodic subsoils. Divots were also created in plots on August 1, with subsequent biweekly imaging of plots and determination of percent recovery. Data are currently being analyzed for inclusion in final report. The comprehensive data set for the two-year project will be provided in a final report in the coming 4-6 weeks.

The 2023 data suggest the following:

1. During 2023, none of the sand-capped treatments sustained acceptable turf quality during the deficit irrigation study period, and there was an inverse relationship with capping depth and turf quality, suggesting that sand-capped turf may be more sensitive to deficit irrigation than turf managed on native soil.

2. Although monthly application of wetting agent applications mitigated soil hydrophobicity, its effects on turf quality were more pronounced at the 0.45 x ETo irrigation level. No significant benefit of wetting agent on turf quality was observed at the 0.3 x ETo irrigation level. There was also not a significant effect of capping depth on WDPT as had been noted in earlier years of the study.

3. Sodium adsorption ratio analysis of upper subsoils beneath sand layers suggests sodic soil conditions, despite regular gypsum applications. The rate of accumulation appears to be more rapid with shallower capping depths.

4. By late October, 1-month into the recovery phase, all treatment plots showed good signs of recovery, with shallower capping depths (0 TD and 2") achieving acceptable quality (6.5) by the final rating date.



Figure 1. Image of the sand-capped Tifway bermudagrass fairway deficit irrigation study at Texas A&M University Turfgrass Field Laboratory, College Station, TX. Image taken July 20,2023.

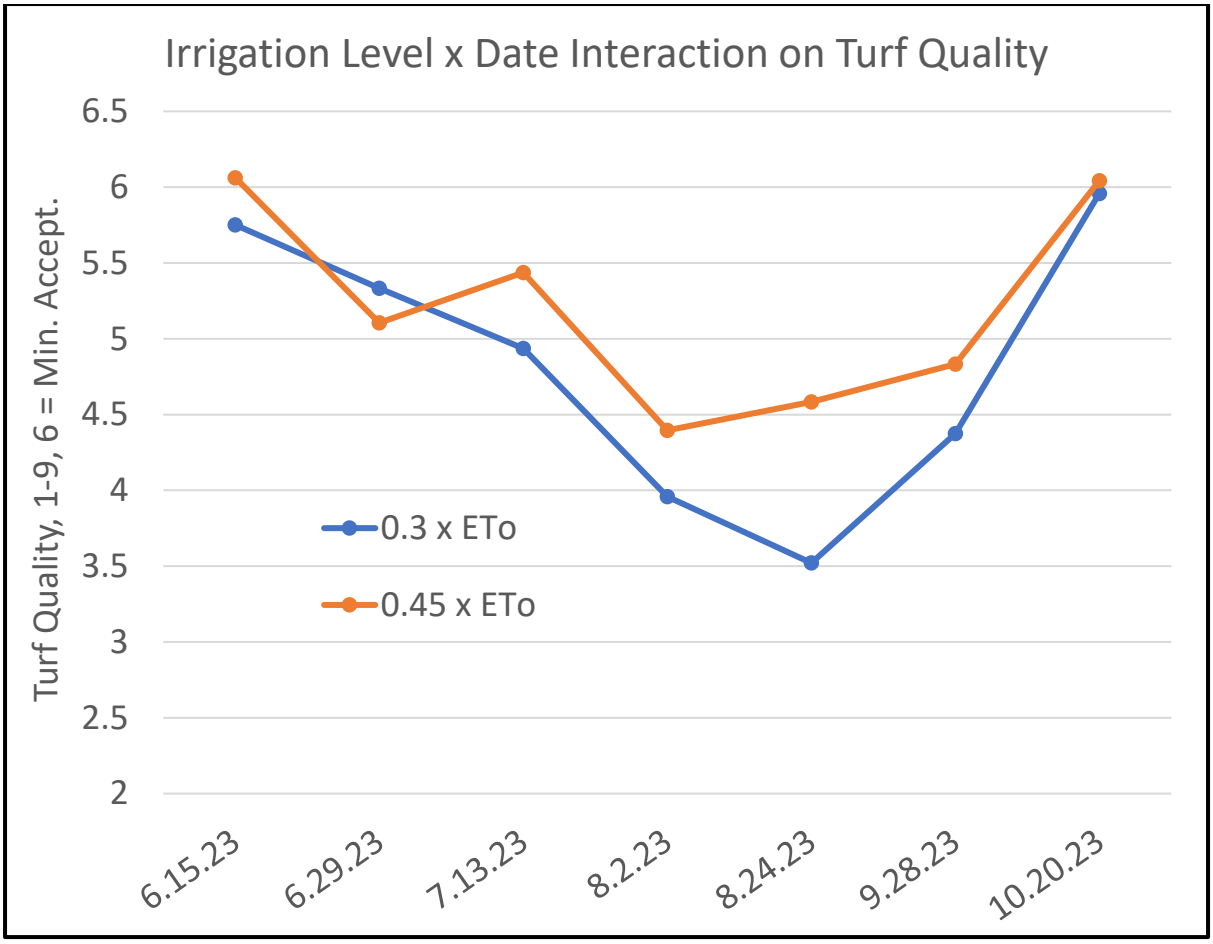


Figure 2. Deficit irrigation level x date interaction on visual turf quality for clay loam study during the 2023 season. Full irrigation levels (0.6 x ETo) were resumed to all plots beginning October 1. Data are pooled across capping depth and wetting agent treatments.

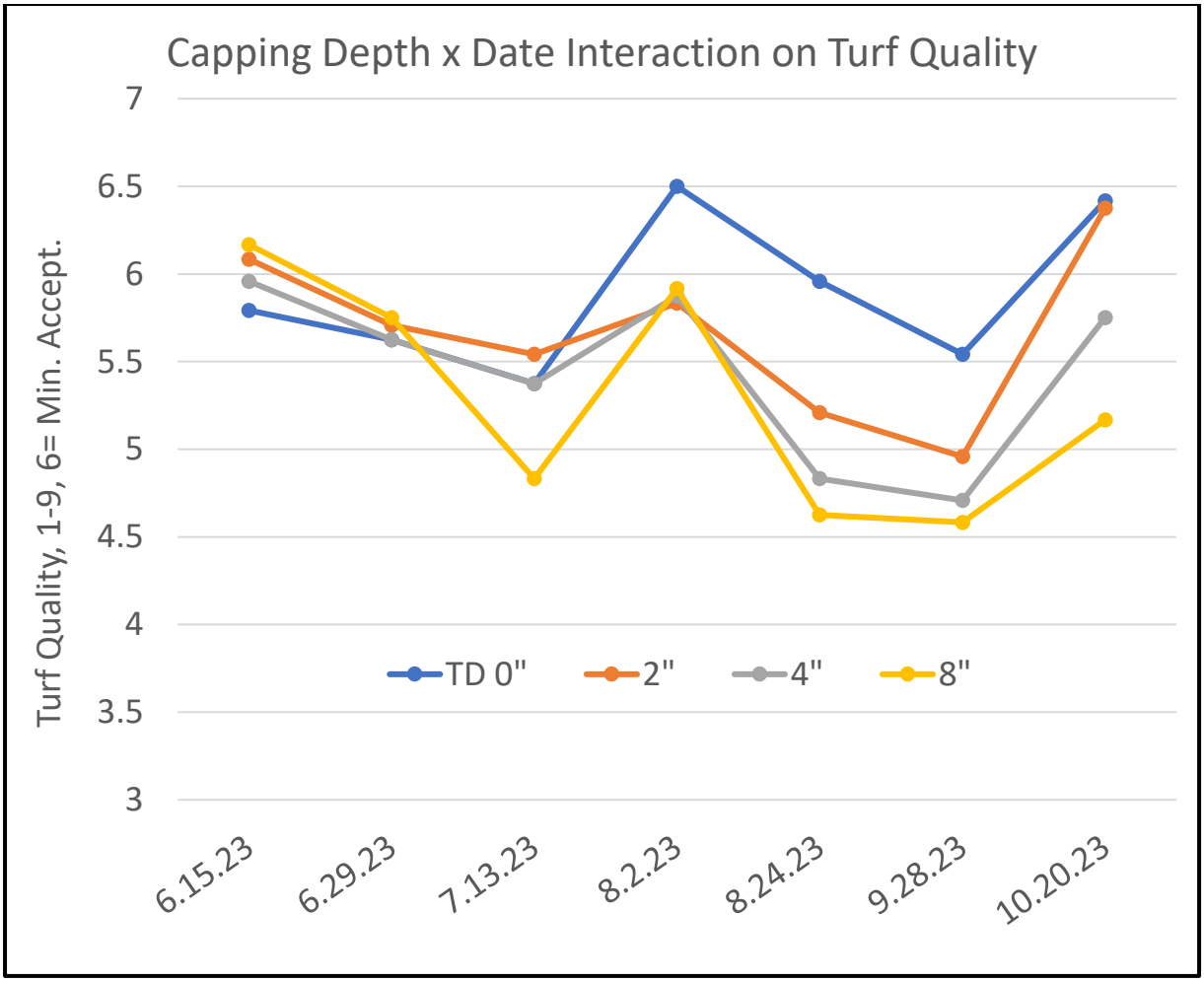


Figure 3. Capping depth x date interaction for turf quality on the sandy loam subsoil for the 2023 season. Full irrigation levels (0.6 x ETo) were resumed to all plots beginning October 1. Data are pooled across irrigation level and wetting agent treatment.

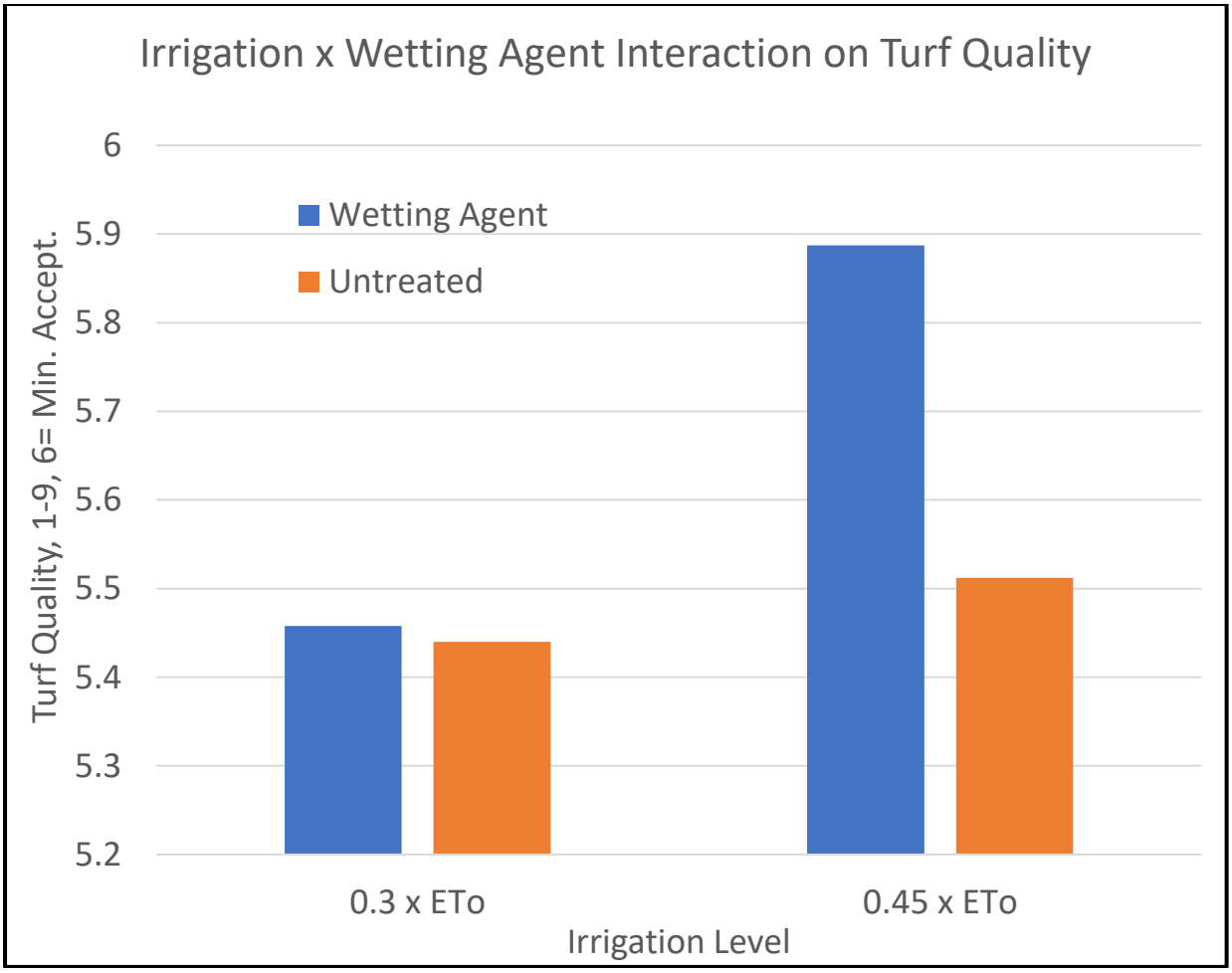


Figure 4. Irrigation Level x Wetting Agent interaction on Turf Quality for the 2023 season. Data are pooled across rating date and sand-capping depth.

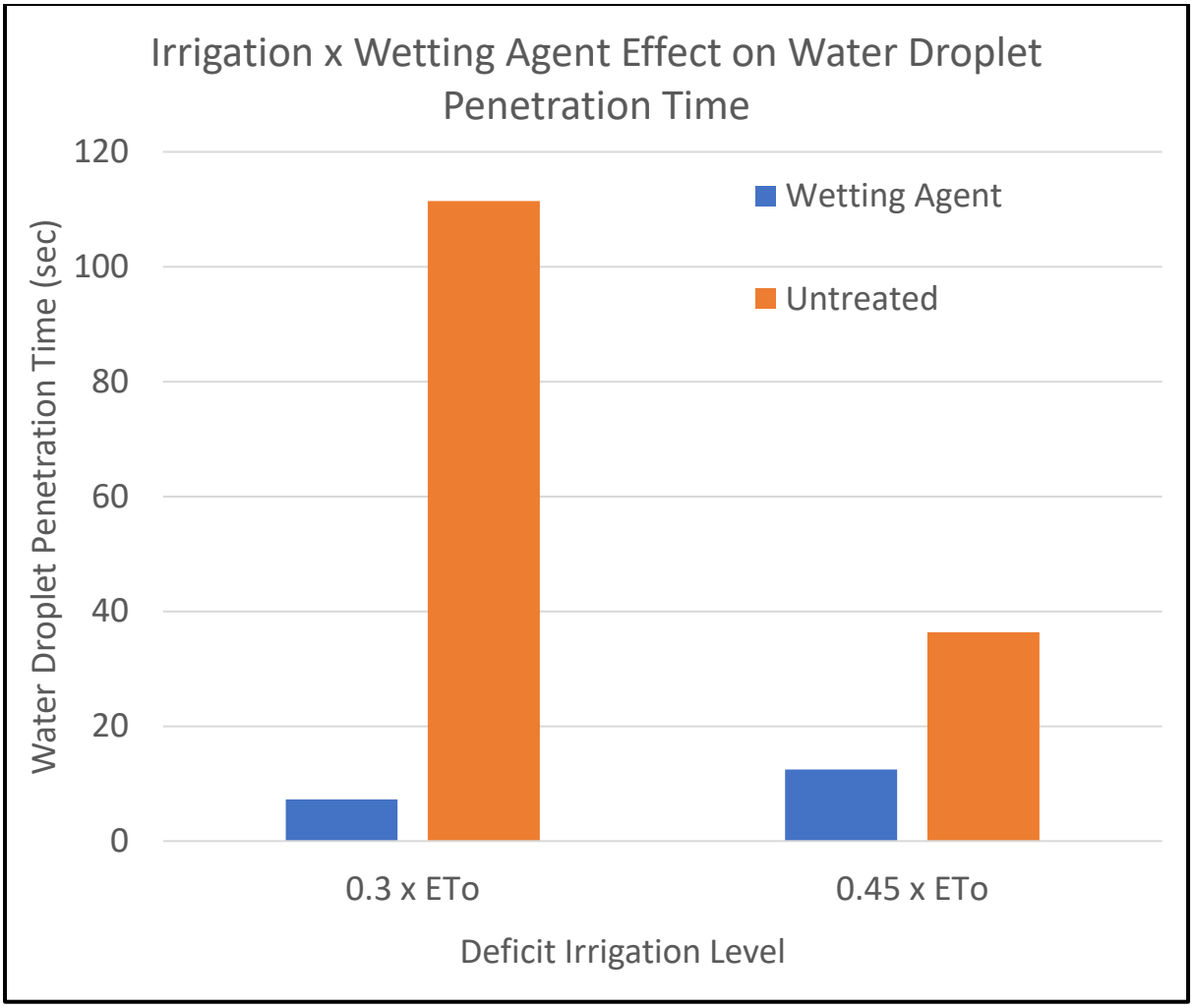


Figure 5. Irrigation x Wetting Agent effect on water droplet penetration time during the 2023 season on the clay loam subsoil. Data are pooled across capping depth and date.

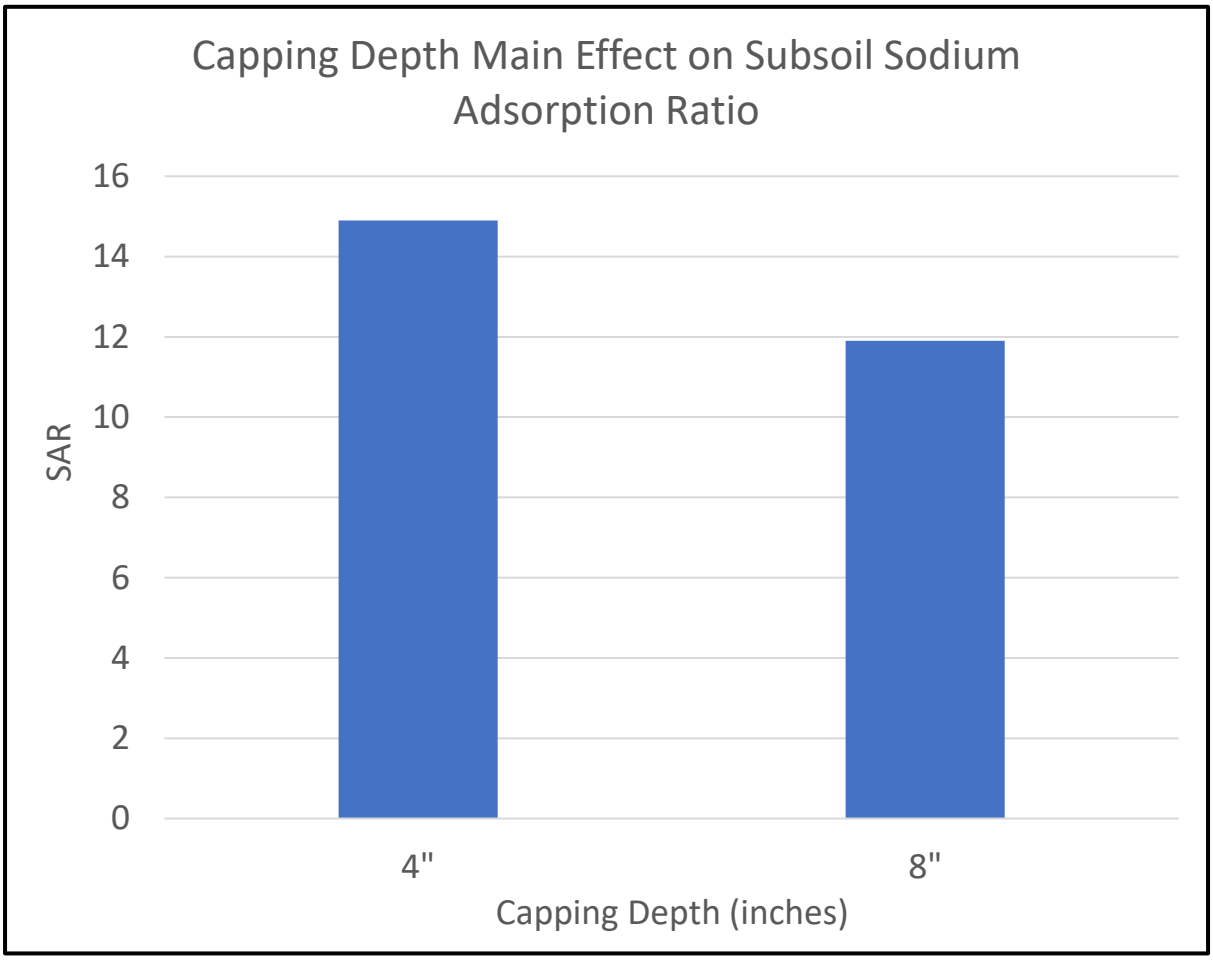


Figure 6. Main effect of sand-capping depth on subsoil (0-2") sodium adsorption ratio (SAR). Soil samples were removed from plots October 20, 2023. Data are for the 45% x ETo irrigation level on the clay loam study.

USGA ID#: 2022-07-750

Title: Optimizing Irrigation Strategies Through Remote Stress Detection

Project Leader: Dr. David McCall and Travis Roberson
Affiliation: Virginia Tech

Original Objectives:

- **Objective 1:** Scaling early moisture stress detection to fairway growing conditions using canopy reflectance and aerial imagery.
- **Objective 2:** Mapping spatial variability across turfgrass systems to construct site-specific water management zones using aerial imagery and ground validation.

Explanation: The original research objectives were intended to be accomplished Summer 2023; however, several factors impacted our choice to shift our focus finalizing the updated research objectives (see below). We performed multiple sampling events manually across a range of 4 – 8 fairways between May – September 2023. In all instances, using spatial analysis to compare our field sampled TDR350 soil moisture data with pixel data extracted from corresponding drone flights, there were no statistical differences. We concluded that these observations were not necessarily due to a representation of the data where this is a plausible outcome, but rather from instances that did not provide the adequate environment for accurate testing. Across the sampling events, rainfall occurred at inopportune times when trying to conduct the field experiments. Furthermore, not having full control over the irrigation scheduling led to similar instances. Lastly, after going through several data sampling events, we realized we needed another method to capture representative moisture data through a method that provides larger spatial sampling and in a more rapid fashion. For these reasons, we intend to sample to address Objectives 1 and 2 above using a TurfRad by TerraRad Tech (Figure 14). We will use this technology for frequent sampling across multiple fairways in comparison with drone flights to investigate the ability for constructing moisture management zones derived from the sampled data.

Updated Objectives:

1) **Estimating Golf Course Irrigation Efficiency Using Thermal Aerial Imagery**
    a) Utilizing remote sensing through thermal aerial imagery to determine the distribution uniformity of a bermudagrass green and tee area
    b) Determining the maximum allowable flight time for collecting thermal imagery post application irrigation events to identify potential irrigation distribution issues.
    c) Validate thermal data within field research acquired through aerial imagery is consistent with ground validation by way of a thermal camera.

Start Date: 2022
Project Duration: 3 years
Total Funding: $100,000

Summary Results:
Objective 1:
Introduction: Distribution uniformity and net precipitation rates are vital to performing an irrigation audit and determining base irrigation schedules. Performing an irrigation audit is a well-established method for determining the efficiency of the distribution of an irrigation system for lawns, landscapes, golf courses, sports fields, etc. Audits also help to determine the inefficiencies of an irrigation system

and minimize the loss of plant quality due to lower than expected applications of water of a given area (Dukes, Haley, & Hanks, 2006). However, these audits require proper training on how to effectively capture the total irrigation water caught over a spatial area and is a time-consuming process (Kieffer & O'Connor, 2007). Furthermore, irrigation distribution uniformity tests of fairway locations segmented by either low, medium, or high traffic zones along with greens and tees on golf course acreage have shown to not be main influential factor regarding soil moisture variability of the tested area (Kieffer & Huck, 2008; Miller, Dukes, & Pressler, 2014). Other factors contributing to the low correlation between soil moisture status and catch can volumes were documented to be from permanent soil features such as texture, structure, existing topography; along with the pre-existing soil moisture status at the time of audit initiations that can make post application irrigation (PAI) variability. Both Kieffer and Miller attempted to use soil moisture distribution uniformity (SMDU), which is the same calculation as traditional DU values, as a method of enhancing the speed of data collection to estimate irrigation distribution uniformity but found it to be unsuccessful. The ability to document the surface conditions directly after an irrigation event through aerial thermal imagery has not been focused on, rather only the underground soil responses have been investigated.

Materials and Methods:

Distribution Uniformity: Our research was conducted on four ultradwarf bermudagrass greens and hybrid bermudagrass tees at Independence Golf Club in Midlothian, Virginia. The greens varieties were as follows: two 'G-12', 'Tif-Eagle', and an experimental from the University of Georgia and all tee locations were 'Midlawn'. Greens were mown at approximately 0.125" while tee locations were mown at 0.450". Data collected at each location during each experimental run were soil moisture before and after irrigation, catch can volumes PAI, thermal imagery immediately after PAI. All Orbit-BHyve (Figure 1) catch cans used were spaced on a 3m x 3m spacing for all locations and a range of 47 – 94 catch cans that were used for this study with a throat opening of approximately 17.83in^2. All locations encompassed a total area of 2927ft^2 – 7061ft^2. All greens locations had two, five-minute irrigation cycles that applied a total of 0.25" of irrigation, while all tee locations were on one, twenty-minute irrigation cycle that delivered approximately 0.5" of irrigation through Toro 760 model irrigation heads. The range of catch can volume was 60ml – 150ml for all locations. Immediately after each irrigation event, a Mavic 3T drone (Figure 2) was used to document the thermal temperature of the turfgrass canopies for all locations and no location was done on the same day due to limitations and time constraints that were required for experimental run. The Mavic 3T drone is equipped with a dual 48MP RGB and 640 x 512 DJI thermal cameras but only thermal is investigated within this study. All flights were approximately 43 – 98 seconds in flight time at a flight altitude of 200ft and collected 58 – 129 images within these pre-determined flight paths were a 90% overlap which a high image overlap is required for thermal image acquisition. A Field Scout Soil Moisture TDR (Figure 2) equipped with 1.5" probes were used to document the soil moisture state before and after PAI to investigate previous trends established within literature. The 1.5" probes were determined to be the best selection of soil moisture depth analysis to document the soil moisture near the surface of the canopy being tested. All collected images were uploaded to Thermoconverter version 1.8 to convert thermal images from a .JPEG to. RJPEG and then uploaded to Pix4D version 4.8.4 and once orthomosaic were stitched at an accuracy of 0.04 cm or less of error, the thermal orthomosaic was exported to QGIS version 3.30.3 for further analysis. Within QGIS for all locations, the base thermal orthomosaic was used to create a shape file of multi-points (Figure 3) to identify within the image where each catch can was located. The order sampled was documented for each location to ensure the order sampled for ground data was the same as the thermal pixel data sampled within each image. Each location the before and after orthomosaic for PAI was sampled to compare the canopy surface temperature difference and ensure differentiation was detected. After, sampling points established, then a polygon was established with a 4.93ft x 3.92ft (Figure 4) that was

centered around each catch can sampling point that sampled approximately 363 pixels that acquired an average thermal pixel value. These polygon sizes were determined by features within the QGIS application and being less than half the distance between catch cans within the field so no pixel was resampled within the data extraction. The average thermal pixel values were extracted and exported for JMP v16 analysis. All data were used for a multivariate and bivariate analysis to determine relationships between thermal mean (TM) pixel values to catch can amounts (CCA) along with soil moisture before and after for correlation coefficient of determination.

Aerial Timeframe Assessments: The next step based on our findings from the DU study was to determine how long of a flight time actually exists in capturing the potential use of thermal aerial imagery within a real-world application. The same locations listed above were used to prove this concept and provide clarity if our flight times were adequate enough for our data collected and for future research projects. Four plexiglass panels that were approximately 0.25" in thickness were placed randomly on each location prior to initiating irrigation cycles of the same runtimes mentioned before and a blue film adhered to them to mitigate any magnification effects that might be observed with transparent plexiglass panels (Figure 5). The same Mavic 3T drone and flight paths were used from the DU study to limit variation. Immediately after PAI, the plexiglass panels were carefully removed to ensure no water that was on the surface coated the area that was under the plexiglass protecting it from irrigation water, these areas of interest were deemed as the untreated checks from water. After the initial image acquisition directly subsequent of PAI, one drone flight at 10-minute intervals were acquired for approximately one hour after PAI to document the thermal changes throughout this temporal period. All images acquired were handled using the same software mentioned within the DU study. The thermal pixel data were extracted similar to the DU study within the QGIS but within the initial flight deemed as the 0min timeframe, the plexiglass areas were visible enough to manually create a polygon over these areas and a directly adjacent, matched pair sampling area using the same polygon to sample the same number of pixels comparing the non-irrigated vs. irrigated areas over time (Figure 6). All matched pair polygon areas were sampled from green to tee for the green locations and tee to green for tee locations and the same polygons were used for each subsequent flight location since the high accuracy of orthomosaic stitched using real-time kinematic ground control points allowed for images to align with derived polygons from initial flights. With sampled polygons, an average of 36 pixels were sampled for all locations and the average thermal data were exported to JMP v16 for data analysis. The first step was to analyze the overall differences over time for the non-irrigated vs irrigated areas and compare to minimum threshold when all locations had significant differences between the two treatments. Next, we wanted to determine the average flight time for each location area, green and tee, using a prediction profiler which was done so through the Gaussian process model with a sensitivity indicator and prediction profiler to identify the horizontal asymptote and the associated timeframe at which it is located.

Thermal Aerial vs. Ground Validation: The last component to our study was to determine how consistent and accurate the DJI thermal lens within the Mavic 3T is compared to ground validated data acquired by means of a FLIR 650sc (Figure 7). This supplementary study was conducted on a one 'Midlawn' hybrid bermudagrass fairway and 'G12' ultradwarf bermudagrass green's location. At each location, a randomized complete block design was initiated with four treatments: untreated surface temperature, plexiglass 2ft x 3ft, charcoal shingles 2ft x 3ft, and 2 gal of water applied over a 2ft x 3ft framed area equating to 0.49" of irrigation (Figure 7). The Mavic 3T drone hovering at an altitude of 200 ft and capturing images every 5 seconds all treatments were initiated and subsequent thermal images were taken with the FLIR 650sc camera and all ground images were aligned by timestamp with the aerial photos taken. FLIR 650sc images were processed using the ResearchIR software and the DJI Thermal

Analysis tool for Mavic 3T images. All data were imported to JMP v16 for bivariate analysis to estimate the correlation between ground vs aerial thermal data.

Results and Discussion:

Distribution Uniformity: The multivariate analysis (Figure 8), shows that the average Thermal_mean compared to catch can water had a moderate correlation ((mL) (r = 0.3191)) and similar to the same relationship with Red_mean (Figure 8). Furthermore, it is interesting that the soil moisture values have a weak correlation to catch can water amount as well which is congruent with other reports in literature that irrigation applications do not directly influence the inherent variability of soil moisture seen within turfgrass systems. Moreover, looking at the relationship of thermal mean (TM) by catch can amounts (CCA) for greens and tee's locations (Figure 9). There were moderate to strong relationships for all greens locations with a decrease in thermal values based on average pixel values as the catch can amounts increased in water volume collected. These same trends were observed with tee locations as well but not as strong with a weaker to moderate relationship. The data were more spread out in the tee locations and we theorize this for a few reasons. One, the greens locations required a lower runtime based on Irrigation Association recommendations that could have led to lower soil moisture saturation and more variability introduced spatially for a strong correlation. Moreover, these runtimes were also split into cycles due to initial attempts that led to puddling issues when collecting data. Furthermore, the longer runtimes on tee locations could have masked the overall canopy temperature at the time of irrigation applications due to soil saturation as reported in literature when documenting soil moisture changes. Lastly, the distribution uniformity was lower for greens locations (DU range = 0.76 – 0.84) compared to tee locations (DU range = 0.86 – 0.90) suggesting that the higher the DU value the lower the correlation due to everything being treated effectively with the same amount of irrigation water. Further investigation would be required to prove this observed concept.

Aerial Timeframe Assessment: The results to date show that there is a period that may be longer than is theorized to collect thermal imagery directly after a post application irrigation event. We are observing through our data that all locations of greens and tees had a significant difference in thermal canopy temperature differences between non-irrigated vs irrigated treatments (Figure 10). This suggests that within a 20-minute timeframe after an irrigation cycle of 10 minutes or longer could be collected. This becomes useful information when considering trying to determine the irrigation efficiency or in-efficiencies for large acreage areas. With the higher image overlap required for thermal image acquisition, flight times increase the larger the area trying to map. However, a 20-minute flight time allows for most fairways to be mapped depending on size. Elucidating this out beyond the treatment differences, we wanted to know how long we would have based on locations, or height of cut, differences and by the evaporative effect seen from water when applied to a surface. Figure 11 shows how for one green location the irrigated replications sampled indicate a heating and then cooling effect on the grass surface. This quadratic relationship indicates a peak or asymptote area we theorized as a fixed point to treat all locations the same to get an average maximum flight time based on grass density, mowing height, and other soil properties observed for each location. The Gaussian process model and prediction profiler (Figure 12) was used to predict the time associated with the asymptote area for each location giving an average maximum flight time of 40.62 minutes for greens and 26.1 minutes for tee locations. Overall, the higher flight time associated with the greens locations is most likely associated with the density of the grass limiting the amount of water infiltrating into the surface and more water being left of the surface taking a longer time to evaporate.

Thermal Aerial vs Ground Validation: Our data suggest that based on fairway and green locations that an $r^2 \geq 0.87$ is observed between the thermal ground sampled values and the thermal aerial values.

Overall, this suggests that the data we collected is congruent and consistent with data that would theoretically be collected on the ground as compared with a drone at a 200 ft flight altitude. On average, between both locations, there was an average of 0.3 °F between the aerial and ground thermal values.

Images and Figures:



Figure 1. Orbit BHyve irrigation catch can used to collect irrigation volume for distribution uniformity tests.



Figure 2. Field Scout TDR 350 used to document soil moisture status before and after irrigation applications on the left and DJI Mavic 3T on the right used to collect thermal aerial imagery immediately after irrigation events.

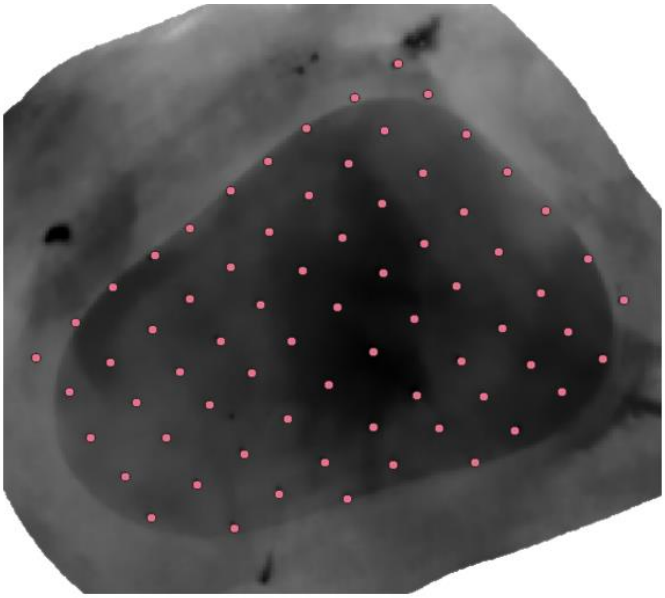


Figure 3. 'G-12' ultradwarf bermudagrass green post application irrigation with catch cans identified with multi-points through manual sampling.

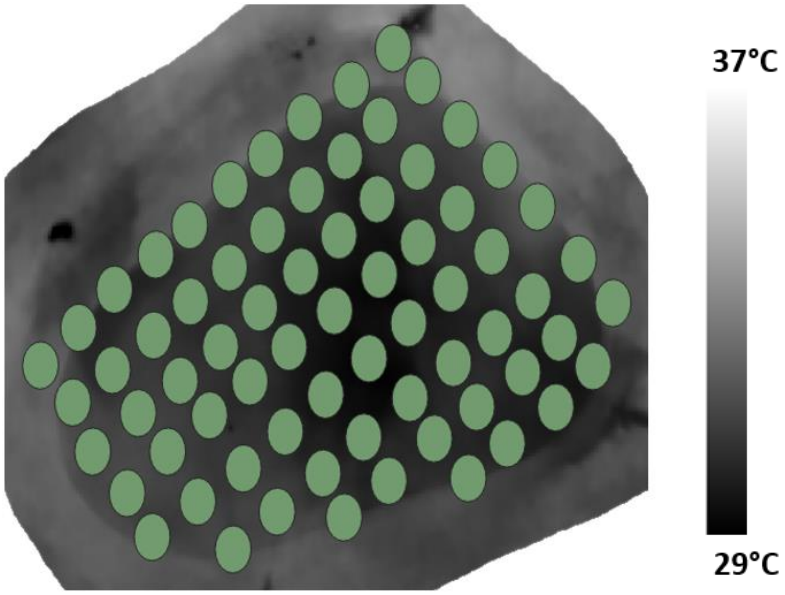


Figure 4. Polygons established by the 'buffer' feature within QGIS to take extract an average thermal pixel value centered around each catch can within the image directly correlated to measured water volumes within the field.

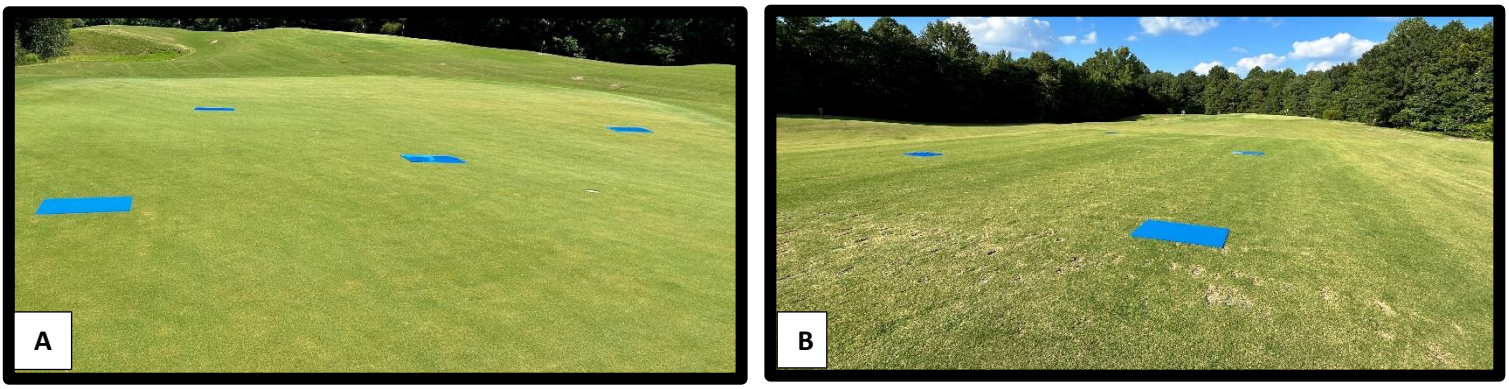


Figure 5. 'Tif-Eagle' ultradwarf bermudagrass green (A) and 'Midlawn' sand capped tee box (B) used for timeframe study and a visual representation of how the plexiglass panels were placed at these locations.

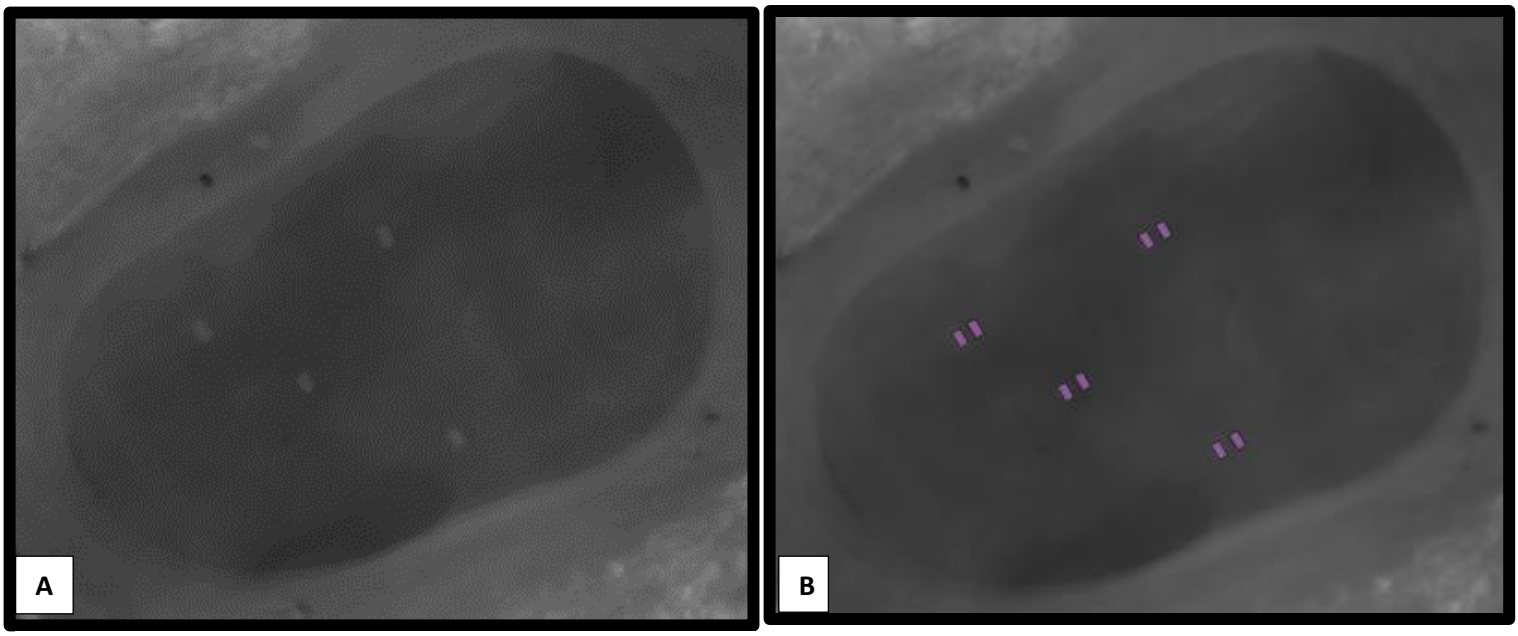


Figure 6. (A) Orthomosaic for 'Tif-Eagle' ultradwarf bermudagrass green with plexiglass areas visible immediately PAI and removal of plexiglass panels and (B) polygons used to sample pixel areas within matched pair areas of interest.



Figure 7. Mavic 3T, FLIR 650sc, shingles, plexiglass, watering cans, spatial watering template, and experimental layout for 'G12' ultradwarf bermudagrass location.

Correlation Table All Green and Tee Locations						
	Thermal_mean	Catch Can Water (mL)	Red_mean	Green_mean	Blue_mean	Soil Moisture (SM)
Thermal_mean	-	0.3191	0.2848	-0.2715	-0.2498	0.1369
Catch Can Water (mL)	0.3191	-	0.3274	0.0692	0.173	0.0845
Red_mean	0.2848	0.3274	-	0.7469	0.7778	-0.0891
Green_mean	-0.2715	0.0692	0.7469	-	0.9125	-0.2374
Blue_mean	-0.2498	0.173	0.7778	0.9125	-	-0.1575
Soil Moisture	0.1369	0.0845	-0.0891	-0.2374	-0.1575	-

Figure 8. correlation table of catch can amounts compared to visual and thermal imagery for distribution uniformity project

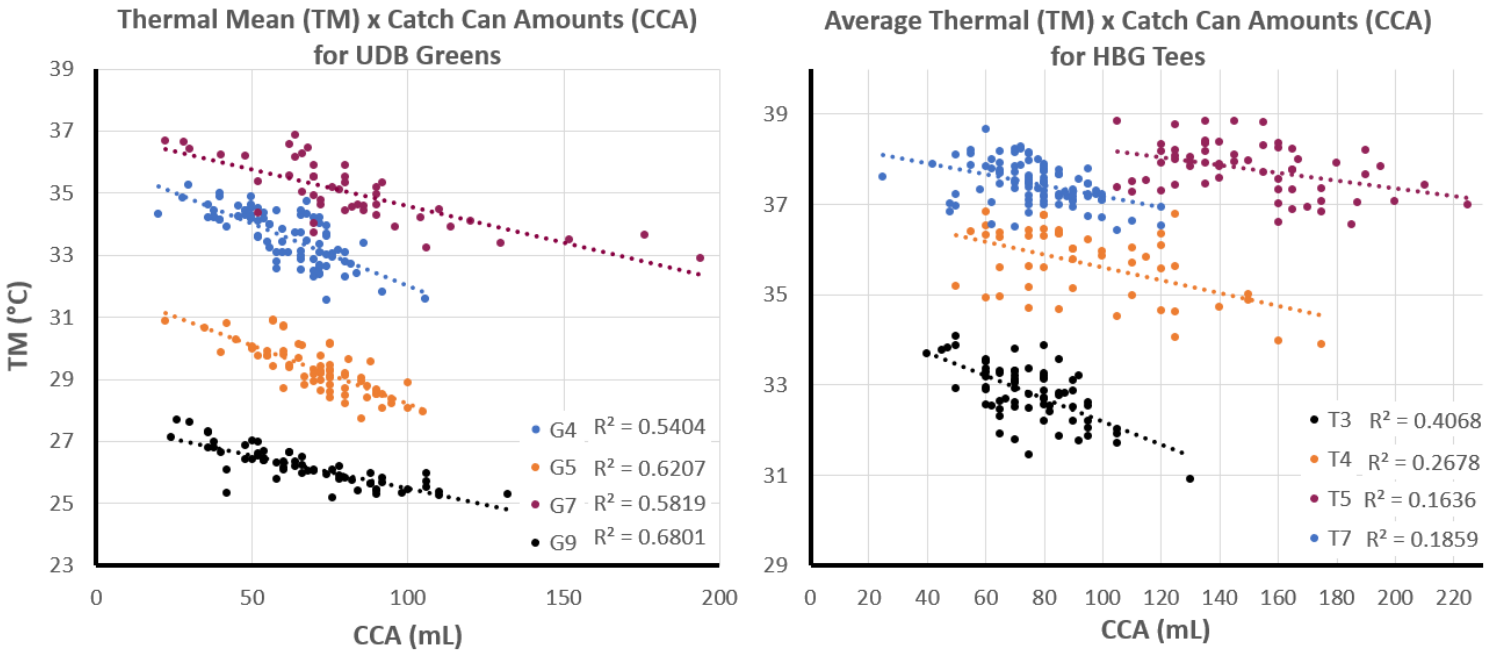


Figure 9. bivariate analysis of thermal mean values (TM) by catch can amounts (CCA) separated by ultradwarf bermudagrass greens (UDB) and hybrid bermudagrass (HBG) tee's locations.

	Greens				Tees			
TAI (min)	G3	G4	G5	G7	T3	T4	T5	T7
0	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*
20	*	*	*	*	*	*	*	*
30	*	*	*	NS	*	*	*	*
40	*	*	*	NS	NS	*	*	*
50	*	*	*	NS	NS	*	NS	*

* significant at P = 0.05

Figure 10. Overall significance of runtimes comparing non-irrigated plots to irrigated plots as the water evaporates.

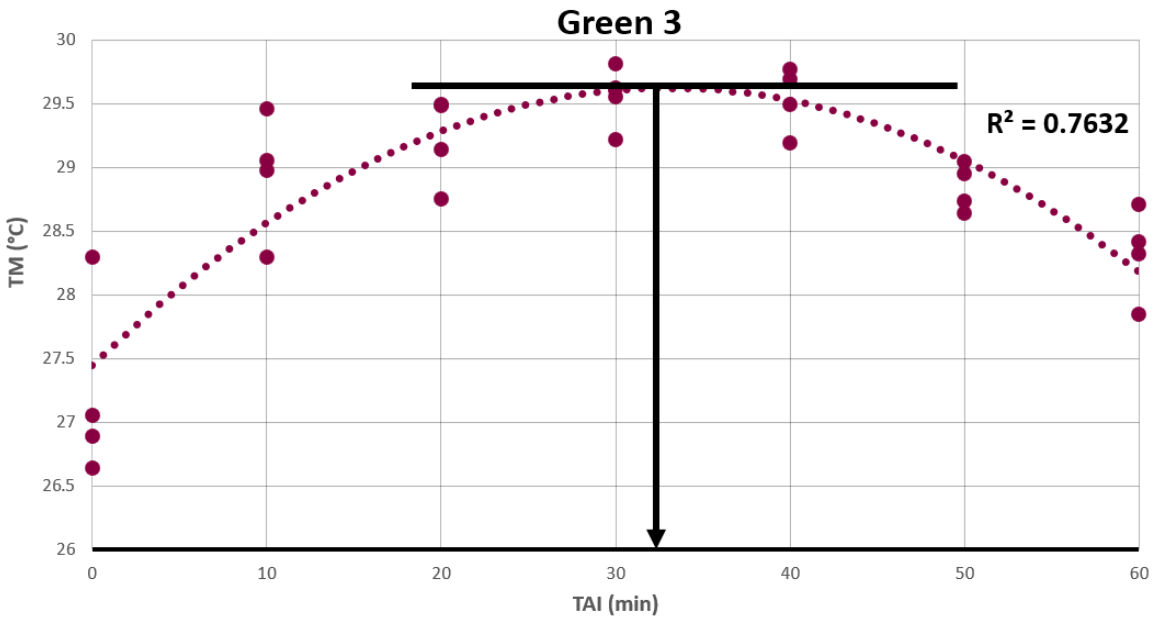


Figure 11. 'G12' ultradwarf bermudagrass location showing the temperature increase after an irrigation application and then the cooling effect seen through the evaporative effects of water.

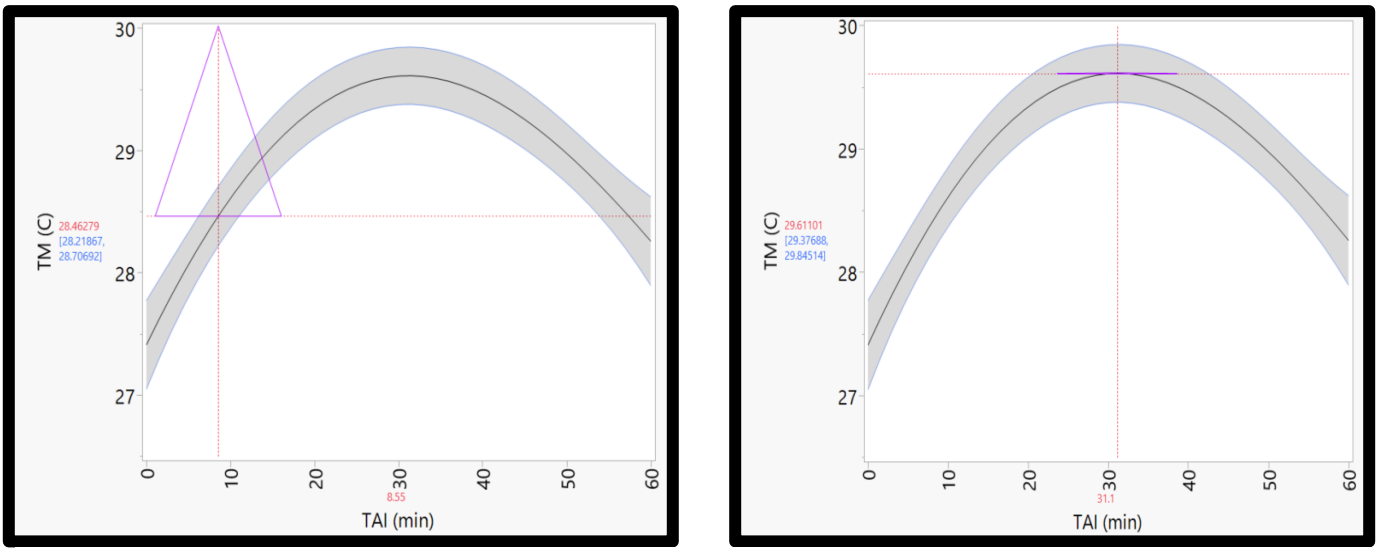


Figure 12. The Gaussian process model with the prediction profile that indicates when a zero slope occurs and the timeframe associated at that point.

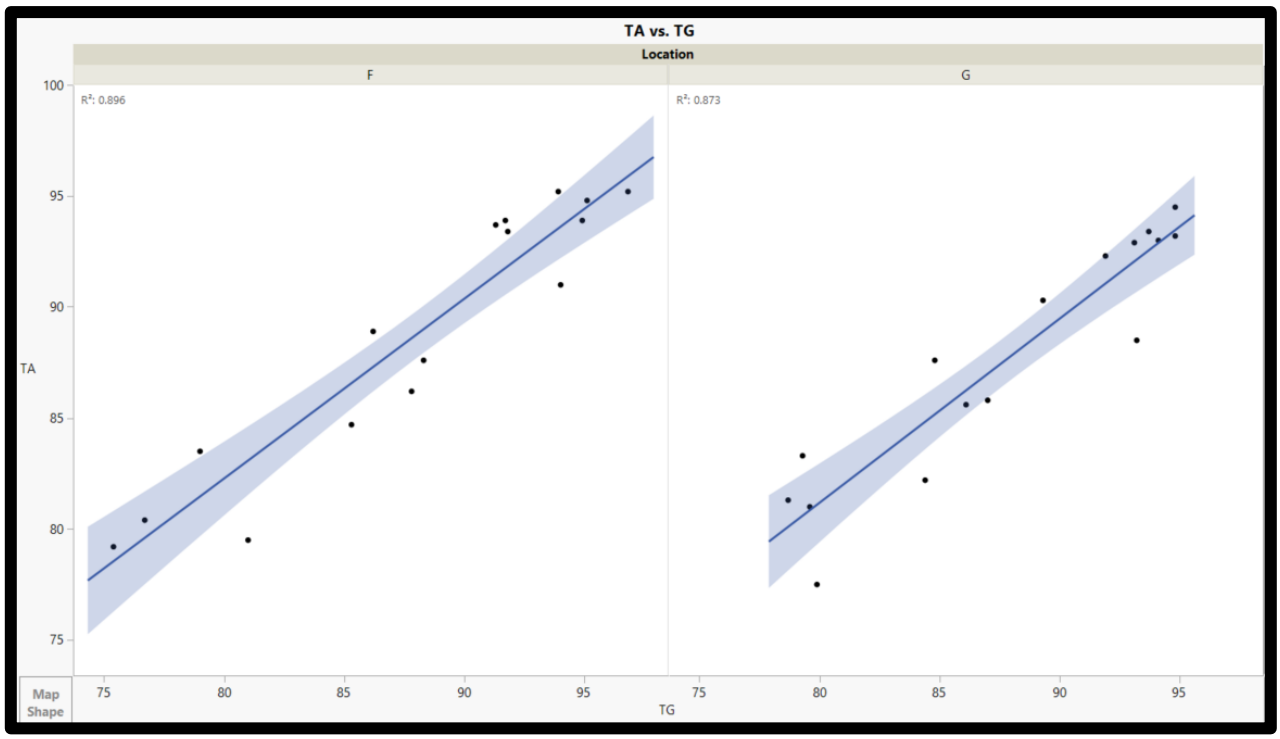


Figure 13. Bivariate analysis of thermal aerial (TA) compared to thermal ground (TG) data in degrees F and separated by Fairway (F) and Green (G) locations.



Figure 14. TurfRad on a custom mount bolted to a gator used to traverse the fairway areas for sampling soil moisture.

References:

Dukes, M. D., Haley, M. B., & Hanks, S. A. (2006). *Sprinkler irrigation and soil moisture uniformity.* Paper presented at the Proceedings.

Kieffer, D. L., & Huck, M. (2008). A comparison of fairway distribution uniformity computed with catch can data and with soil moisture data from three sampling depths. *29th Annual Irrigation Show. California, 2*(4).

Kieffer, D. L., & O'Connor, T. S. (2007). Managing soil moisture on golf greens using a portable wave reflectometer. *Proc. 2007 Int. Irrig. Show. Dec*, 9-11.

Miller, G., Dukes, M., & Pressler, N. (2014). Golf course irrigation systems' distribution uniformity affects soil moisture variability. *European Journal of Horticultural Science, 79*(3), 135-141.

USGA ID#: 2023-03-770

Title: Remote Soil Sensing of Fairways for Irrigation Water Conservation

Project Leader and Co-Leaders: Bernd Leinauer[1], Borys Drach[2], Tatiana Kardashina[2], Tom Egelhoff[3], and Ciro Velasco-Cruz[1]

Affiliation: New Mexico State University, [1]Department of Extension Plant Sciences and [2]Mechanical and Aerospace Engineering[2]; The Club at Las Campanas, Santa Fe, NM

Objectives: 1) Determine accuracy of remote moisture sensing technologies
2) Evaluate technologies for irrigation scheduling of golf course fairways and select the most accurate to irrigate one fairway for at least one season

Start Date: 2023
Project Duration: The project is expected to run for three years.
Total Funding: $208,102

Summary Points:

1) Measurements taken with TDR 350 show a significant and strong correlation with volumetric soil moisture (θ_V) determined by loss of weight.
2) Generally, both satellite and turfRad underestimate soil moisture when compared to TDR 350
3) Moisture readings determined by Sentinel-1A satellite or turfRad showed either no or only a weak relationship with the PS 6000 or the TDR 350.
4) Based on data collected in this study, none of the tested technologies can currently be used for irrigation scheduling to conserve water as they all lack the necessary accuracy.

Study

Several studies have demonstrated the water conservation potential of soil moisture sensor (SMS) -based irrigation scheduling on turfgrass areas. However, a barrier to the adoption of such technology has been the perception that too many measurement points (either by a hand-held device or from in-ground sensors) are needed to accurately determine soil moisture over a large area. Recently, alternatives to hand-held or in-ground SMS sensors have been introduced. These include Cosmic Ray Neutron Sensing (CRNS) devices placed either on a vehicle or outside of turfgrass areas. Active microwave remote sensing from a satellite orbiting the earth using Synthetic Aperture Radar (SAR) penetrates approximately 3-5 cm into the ground and has been used to estimate surface soil moisture on large landscapes and agricultural fields. The SAR sensors transmit electromagnetic pulses and record the backscattered energy from the earth's surface. A more recent advancement allows data to be collected at a resolution as high as the 10 m scale. More recently portable radiometers (PoLRa) operating at a wavelength of 21 cm (L-Band) that can be mounted on a fairway reel-mower have also shown promising results in estimating soil moisture to a depth of 5 to 10 cm. Nonetheless, information is lacking on the accuracy of these remote operating soil moisture sensors, their potential for irrigation scheduling and subsequently its impact on water use and turfgrass quality. A three-year field study has been initiated in 2023 to investigate accuracy and feasibility of remote SMS to schedule irrigation of golf course fairways and its water conservation potential when compared to traditional Evapotranspiration (ET) based irrigation.

Methods

Location and Maintenance

Two fairways, #2 (121,000 ft^2) and #7 (82,000 ft^2) (Figure 1) were selected from the Sunset golf course at The Club at Las Campanas in Santa Fe, NM. Both fairways are par 4 and were established in 1999 with 'Penn Trio' creeping bentgrass. The fairways were mowed at 0.4" (10 mm) twice per week with clippings returned. Both fairways are aerated once per year in April and de-thatched 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' (as Flurprimidol and Trinexapac-ethyl) were applied monthly at label rates. 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.



Figure 1: Fairway #2 (left) and #7 (right) (courtesy of Tom Egelhoff).

Soil Moisture Equipment

Soil moisture sensing equipment included the Toro Precision Sense 6000 (PS6000) (The Toro Company, Bloomington, MN), Sentinel 1A satellite (Karuna Technology UG, Wiesbaden, Germany), turfRad (TerraRad Tech, Zürich, Switzerland), Cosmic Ray Neutron Sensor (CRNS) (Silverside Detectors Inc., Waltham, MA), and TDR 350 (Spectrum Technologies, Aurora, IL). At the end of the study period, the Rover (Hydro Sphere Inc., Tucson, AZ), a portable CRNS sensor was added to the study. In addition, soil cores were collected to determine volumetric soil moisture θ_V (kg kg^{-1}) by loss of weight. More detailed information on the soil moisture measurements used in the study is provided in Table 1.

Table 1. Remote soil moisture sensing equipment used to determine accuracy of measurements compared to reference values obtained with either the Precision Sense or the TDR sensor.

						
Sensor	Precision Sense	Sentinel-1A	TurfRad	Cosmic Ray Neutron Sensor	Rover	TDR 350
Sensor Type	Capacitance	Synthetic Aperture Radar (SAR, Active microwave)	Passive reflectance	Stationary neutron detector	Mobile (portable) neutron detector	Time Domain Reflectometer
Wave-length	unknown	C-Band (4 – 8 cm)	L-Band (21 cm)	n/a	n/a	unknown
Soil depth	10 cm	Approx. 5 cm†	Approx. 5 cm†	Unknown (depends on soil moisture)		7.5 cm
Data points	470 / 930‡	850 / 1300	12,000 / 18,000	2 / 2	2045 / 12,880	150 – 400

‡First number indicates data points on fairway #7, second number on #2.
†Information provided by manufacturer

Data collection started on 16 May 2023 and ended on 15 November 2023. Measurements collected with the PS6000 were intended to be used as reference points against which all other measurements would be compared. Unfortunately, on 18 July the clutch broke and the unit became non-operational. From 6-August until the end of the study we used the values collected with the TDR 350 as our reference numbers.

Data collection occurred as often as golf course operations allowed and when the satellite was positioned over Las Campanas. The Sentinel-1A Satellite crossed Las Campanas at either 7 am or 7 pm. TurfRad, TDR 350, and θ_V (kg kg^{-1}) measurements were taken either between 6 and 8 am or between 6 and 8 pm. Satellite derived soil moisture data were originally scheduled to be provided by two satellites, Sentinel-1A and 1B. Unfortunately, power failure on board of Sentinel-1B led to the loss of data transmission and the European Space Agency announced the end of the mission for Sentinel-1B in August of 2022. This resulted in less available satellite data than originally planned.

Two stationary Cosmic Ray Neutron Sensors were placed on the perimeter of each fairway. Measurement areas extended in a circular pattern with a radius of about 130 m from the sensor (Figure 2). Soil moisture was recorded continuously until 13 August of 2023 at which time all nodes stopped working due to power failures and limited storage capacity of on-board computers. Data collection and processing was performed by Silverside Detectors.

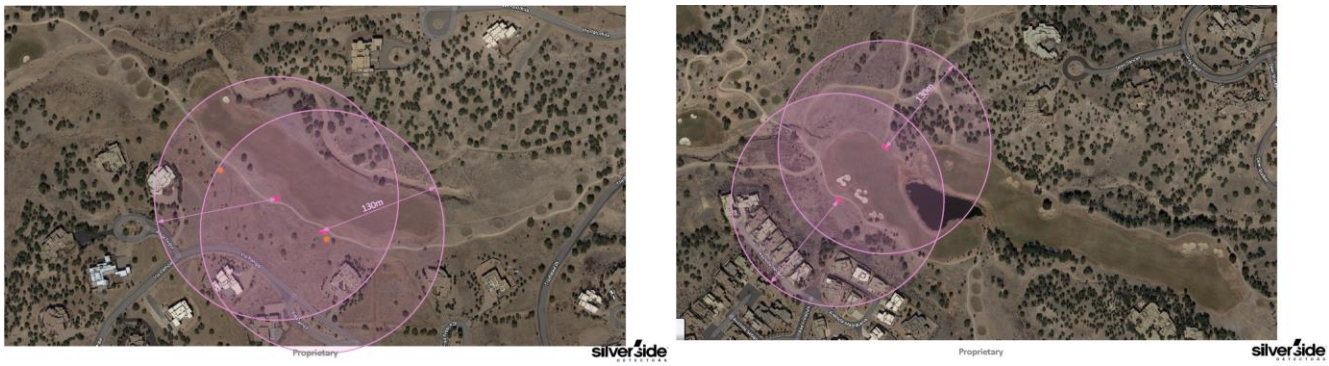


Figure 2. Cosmic Ray Neutron Sensor locations and measurement areas at Fairway #2 (left) and Fairway #7 (right). Images provided by Silverside Detectors.

On November 15, the last sampling date of the growing season, the Rover, a portable neutron detector was added to the study (Table 1). The unit was placed on the back of a utility vehicle and operated at the same speed as the PS 6000.

Soil samples were collected several times between 18 July and 16 September. Approximately 20 soil cores per fairway, 5 cm in diameter and 10 cm in depth, were taken randomly, weighed, dried for 24 hours at 105 °C, weighed again and volumetric soil moisture was calculated. TDR 350 readings were taken at the same locations. Subsequently, θ_V (kg kg^{-1}) and TDR 350 values were compared to one another. A detailed list of types of data and dates at which soil moisture readings were collected and/or provided is listed in Appendix 1.

In order to investigate the accuracy of the GPS coordinates of the TDR 350, locations of the TDR measurements were compared to locations established by a Topcon Hiper V RTK system (Topcon Positioning Systems, Inc. Livermore, CA) and to a Garmin eTrex® 22x (Garmin Ltd., Olathe, KS). According to the unit's manual, the Hiper V determines locations with an accuracy of 3 to 5 mm.



Figure 3. Comparing GPS coordinates determined by the TDR 350 (left) to coordinates established by a Topcon Hiper V RTK, a professional grade surveying equipment.

Data Analysis

Soil moisture readings collected by the PS6000 and the TDR 350 were defined as the reference method to which the Satellite and turfRad measurements were compared. Soil moisture was measured at numerous locations on two golf fairways (Figures 4 and 5). Linear regression was used to compare θ_V to TDR 350 measurements. Satellite and turfRad readings varied across time and locations did not align with measurements taken by the TDR 350 and PS6000. Consequently, we implemented a Geoadditive

model (Kamman and Wand, 2003) to predict both turfRad and satellite at the PS6000 and TDR 350 locations. The number of knots needed for the turfRad method was a subset of the sampling locations due to the large number of measurement sites (Figures 4 and 5). To avoid location duplication, the coordinates of the knots were jittered using a white noise with standard deviation equal to 0.000001. The spatial covariance structure and the mean function used for this analysis were chosen based on the mean squared prediction error (MSPE) of the dataset collected on July 18, 2023. The isotropic Matern structure and a smoothing parameter of $\nu = 0.5$ minimized the MSPE. The statistical analysis was performed using the R software (version 4.3.0) with a user defined function coded in C++ 17. As an assessment of simple association, the corresponding coefficient of determination values (r2= R2), the slope of the regression line, the p-value to indicate whether it's significantly different from zero, and the mean squared error (MSE) are reported.

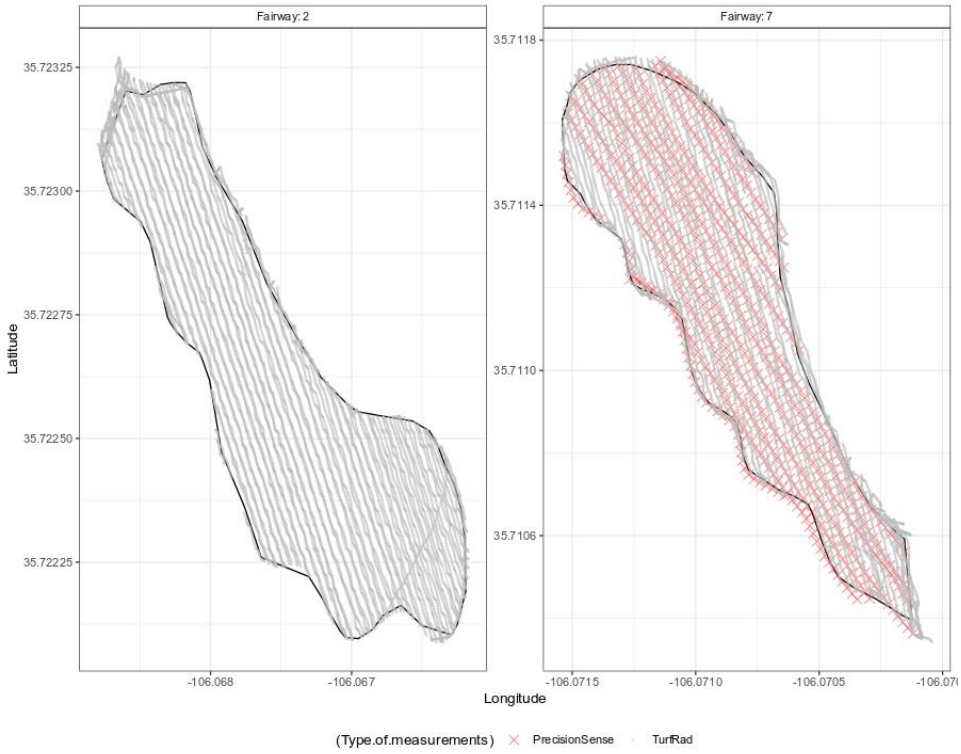


Figure 4. Geolocation points for soil moisture measurements conducted on 16 May. Grey symbols indicate turfRad locations on Fairway #2 and #7 and red crosses highlight Precision Sense locations on Fairway #7.

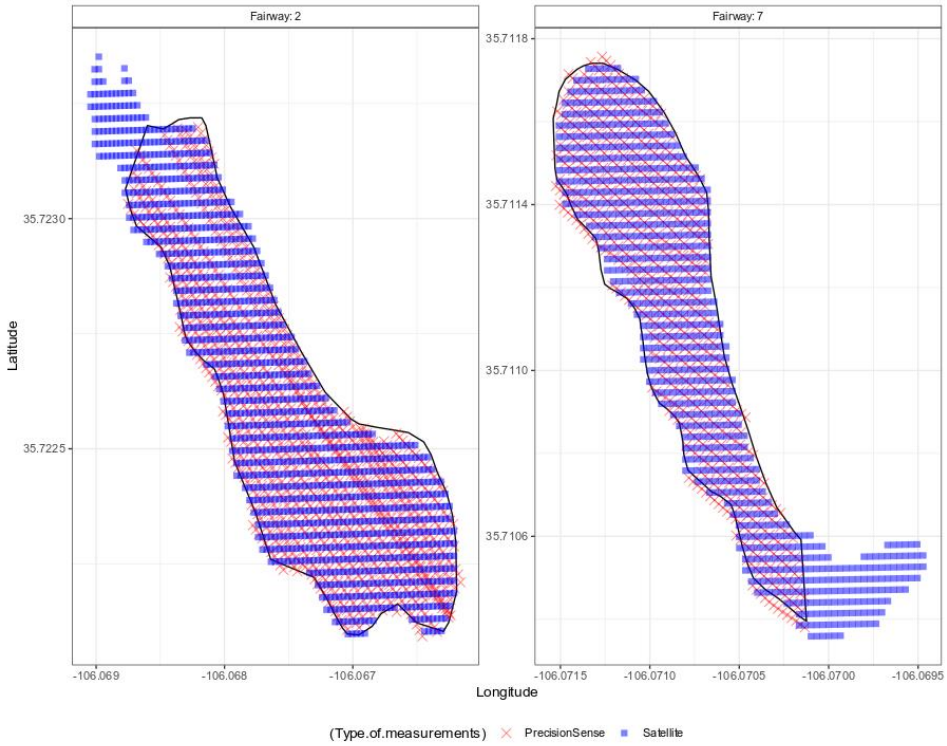


Figure 5. Geolocation points for soil moisture measurements conducted on 19 June. Blue squares indicate Sentinel-1A satellite and red crosses highlight Precision Sense locations.

Results

Irrigation Audit

Irrigation audits were conducted on 17 May 2022, on fairway #7 and on 1 August 2022 on fairway #2. 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).

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 2 and Figure 6.

Table 2. 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

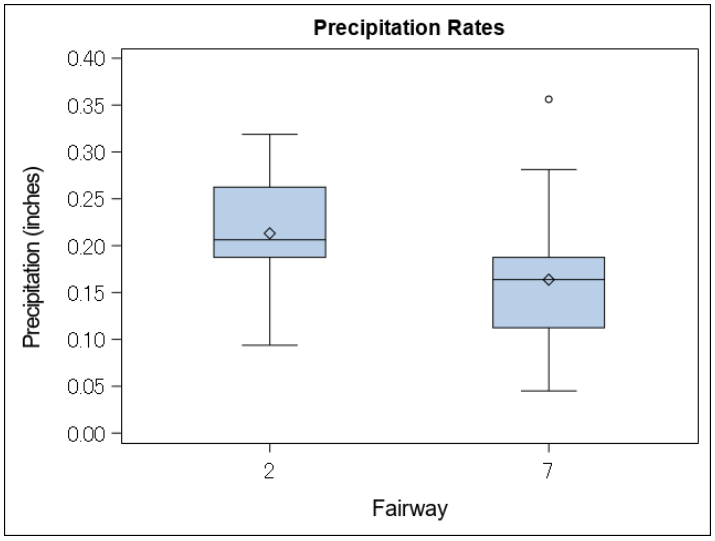


Figure 6. Box and whisker chart of precipitation amounts collected during irrigation audits on fairway #2 and #7 of the Sunset Course at The Club at Las Campanas, Santa Fe, NM. Bars display the result of amounts collected in 76 (fairway #2) and 80 catch cans (fairway #7). The length of the box of each 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.

Soil Moisture Measurements

Soil moisture measurements taken with TDR 350 show a significant and strong correlation with θ_V (kg kg^{-1}) determined by loss of weight (Figure 7). These results confirm our decision to use the TDR 350 as a reference or the standard against which remote sensing technologies can be compared.

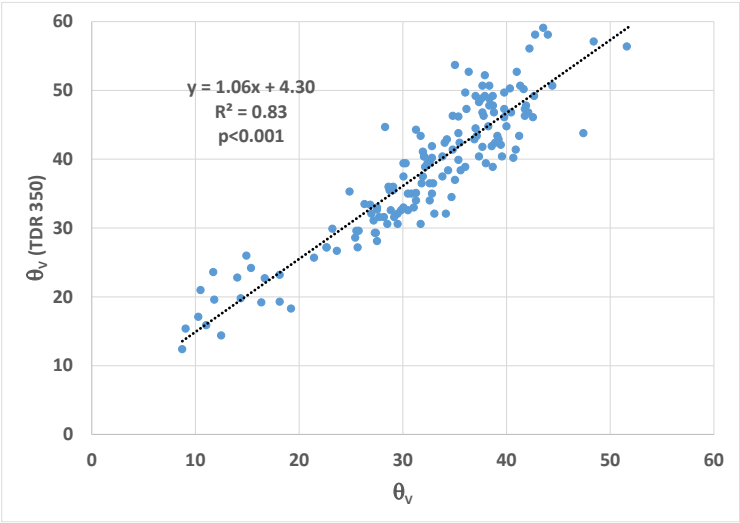


Figure 7. Relationship between volumetric soil moisture determined gravimetrically (θ_V, kg kg^{-1}) and TDR 350 values.

Moisture readings showed either no (June 7) or a very weak relationship between Sentinel-1A and PS 6000 on both fairways. Linear regression R^2 values ranged from 0 to 0.045 and MSE from

19.815 to 113.016. Except for June 7, measurements using the PS6000 showed a much wider moisture range on each fairway than satellite values (Figures 10 and 11).

Linear regression analysis of values collected on May 17 indicates a moderate relationship between turfRad and PS6000, with an even stronger relationship on Fairway 7 than on Fairway 2 (Figure 9). Of all the sampling days, 17 May was the only day on which such a moderate relationship was documented. On all other days there was either no or a very weak relationship between measurements taken by the turfRad and the PS6000 (Figures 9 and 11). Linear regression R^2 values ranged from 0 to 0.132 and MSE from 20.38 to 109.82. Except for data collected on 7 June (Figure 11), satellite and turfRad measurements showed a narrower moisture range on both fairways than the PS6000 (Figures 9 and 11).

When soil moisture determined by the satellite and turfRad were compared to the TDR 350, both remote sensing systems underestimated soil moisture (Figures 12 and 13). Similar to the data comparison between turfRad, Sentinel-1A and the PS 6000, the satellite and turfRad also showed a narrower moisture range on both fairways when compared to the TDR 350.

A significant but weak correlation between soil moisture data determined with the turfRad and TDR 350 could be observed for both fairways with R^2 values ranging from 0.089 to 0.364. A significant relationship of moisture values between the satellite and the TDR 350 was evident on only three out of six measurement events. R^2 values ranged from 0.001 to 0. 143.

An assessment of the accuracy of the GPS coordinates displayed on the TDR 350 revealed that on fairway #7 a difference between 1 m and up to 9 m can exist between the location shown on the TDR and the exact location determined by a professional grade surveying equipment (Figure 8). Distance differences were less pronounced on fairway #2, ranging from approximately1 m to 3 m. Differences between the hand-held Garmin unit and the surveying unit were similar on both fairways and ranged from approximately 10 cm to 2.7 m (Figure 8).

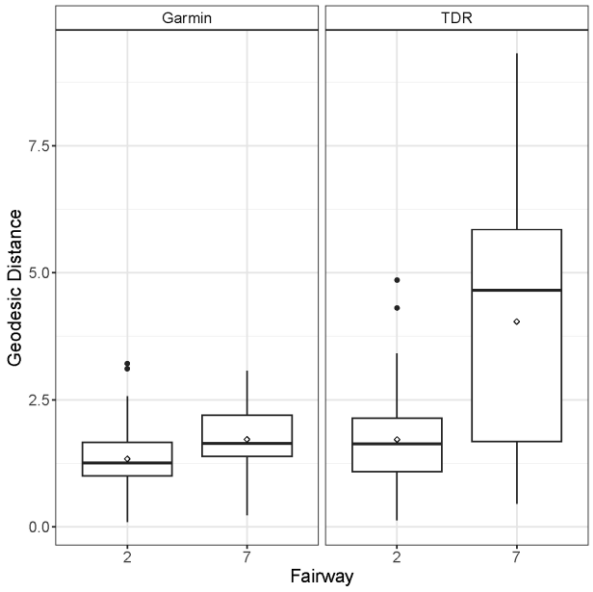


Figure 8. Distance difference (m) on two fairways between GPS coordinates determined by a Topcon Hiper V RTK system and the Garmin eTrex® 22x (left) and between the Topcon Hiper and the TDR 350 (right). Box plots indicate median (line inside the box), average (data point inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

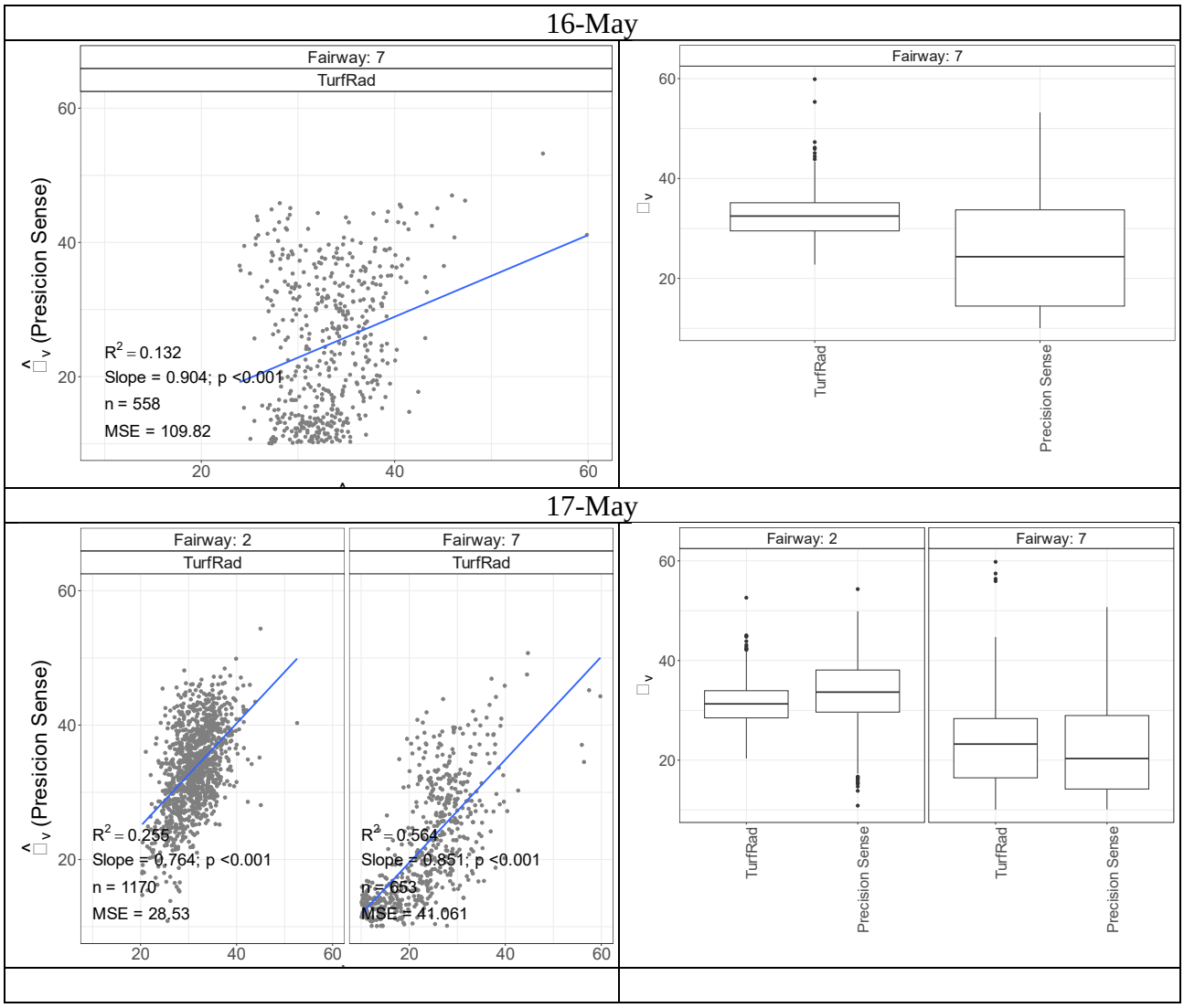


Figure 9. Volumetric soil moisture values collected on Fairway #7 on May 16 and on Fairways #2 and #7 on May 17 with a portable radiometer (turfRad) installed on a fairway mower and compared to values collected with a capacitance sensor mounted on a mobile sampling device (Precision Sense PS6000). Linear regression graphs (left column) highlight relationship of volumetric soil moisture (θ_V = $_V$) measured with PS6000 and TurfRad. Box plots (right column) indicate median (line inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

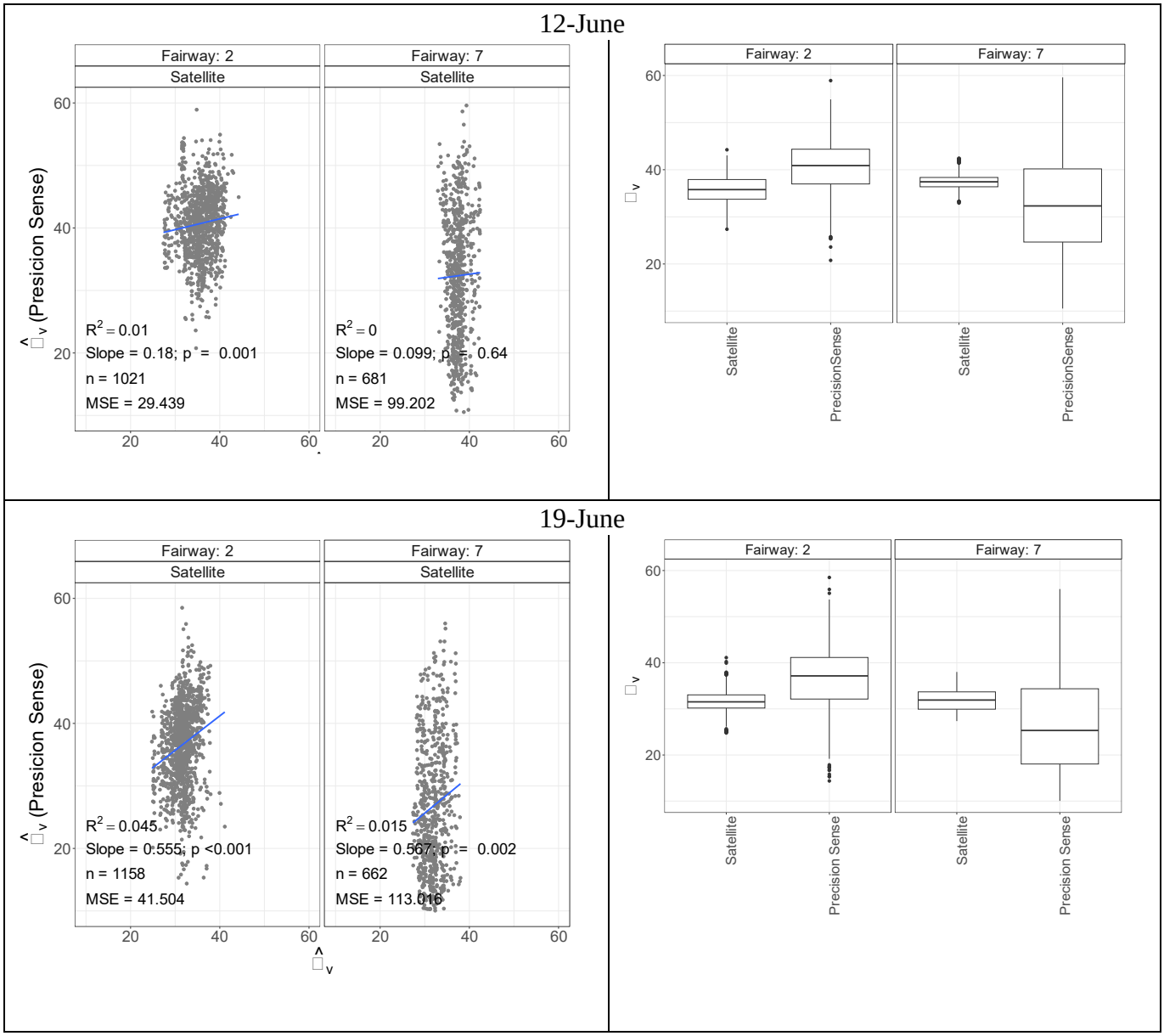


Figure 10. Volumetric soil moisture values collected on Fairways #2 and #7 on 12 June and 19 June with Sentinel-1A satellite and compared to values collected with a capacitance sensor mounted on a mobile sampling device (Precision Sense PS6000). Linear regression graphs (left column) highlight relationship of volumetric soil moisture (θ_V = $_V$) measured with PS6000 and turfRad. Box plots (right column) indicate median (line inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

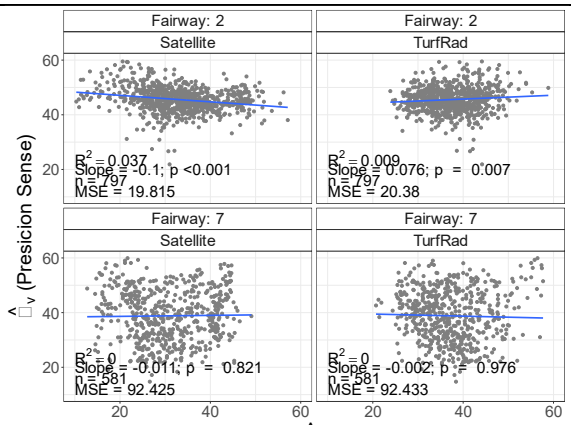
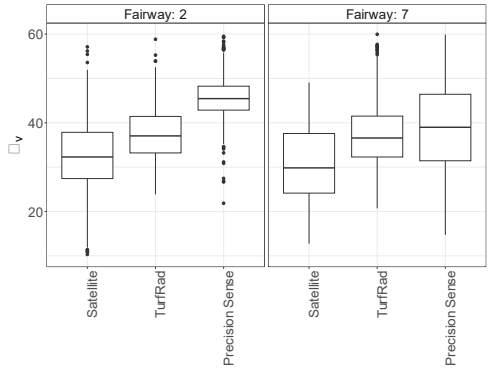
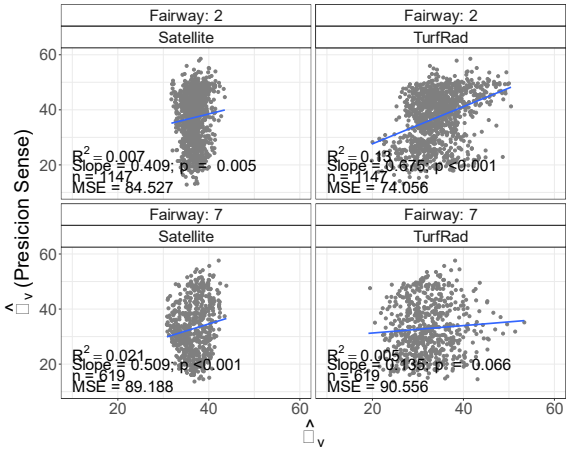
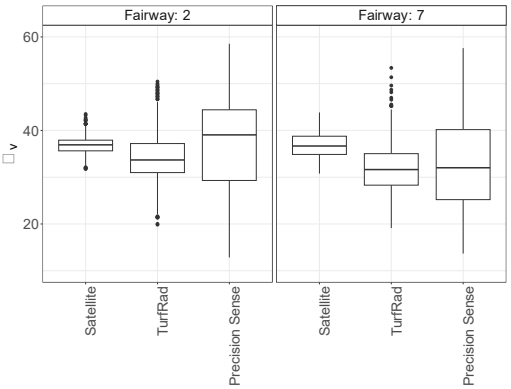
Date		
7-Jun		
13-Jul		

Figure 11. Volumetric soil moisture values collected on Fairways #2 and #7 on 7 June and 13 July with a portable radiometer (turfRad) installed on a fairway mower and with the Sentinel-1A satellite and compared to values collected with a capacitance sensor mounted on a mobile sampling device (Precision Sense PS6000). Linear regression graphs (left column) highlight relationship of volumetric soil moisture (θ_V = $_V$) measured with PS6000 and turfRad and with PS6000 and Sentinel-1A. Box plots (right column) indicate median (line inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

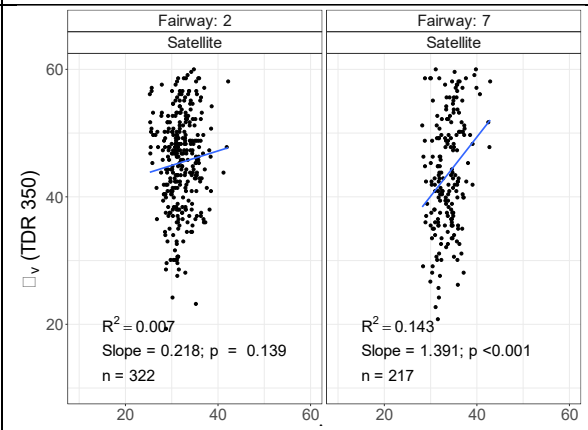
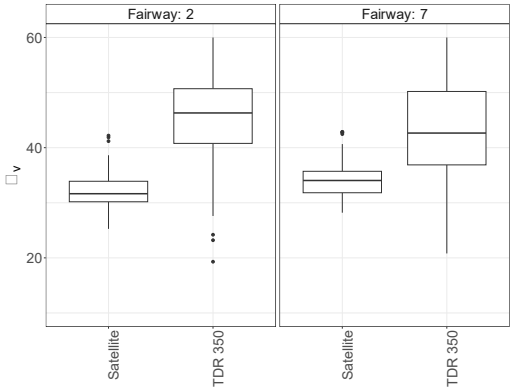
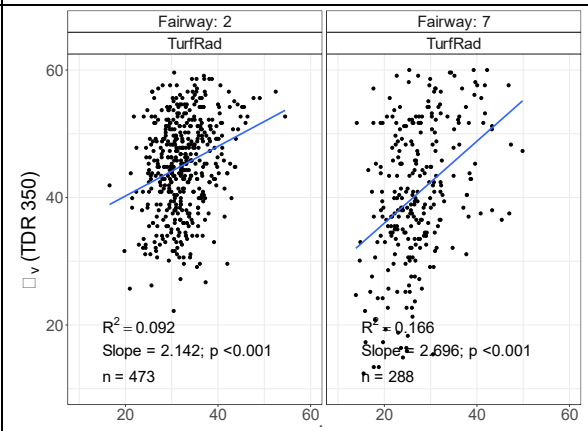
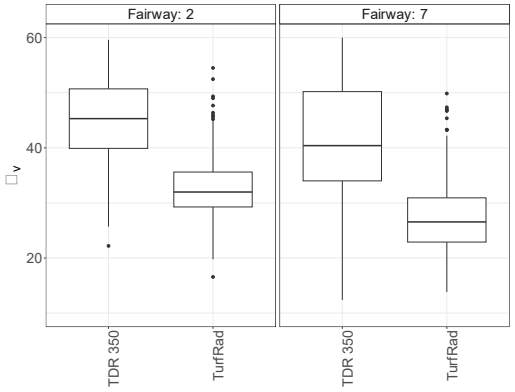
Date		
6-Aug		
7-Aug		

Figure 12. Volumetric soil moisture values collected on Fairways #2 and #7 on 6 and 7 August with the Sentinel-1A satellite and with a portable radiometer (turfRad) installed on a fairway mower and compared to values collected with the TDR 350. Linear regression graphs (left column) highlight relationship of volumetric soil moisture (θ_V = $_V$) measured with TDR 350 and Sentinel-1A and turfRad and with TDR 350. Box plots (right column) indicate median (line inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

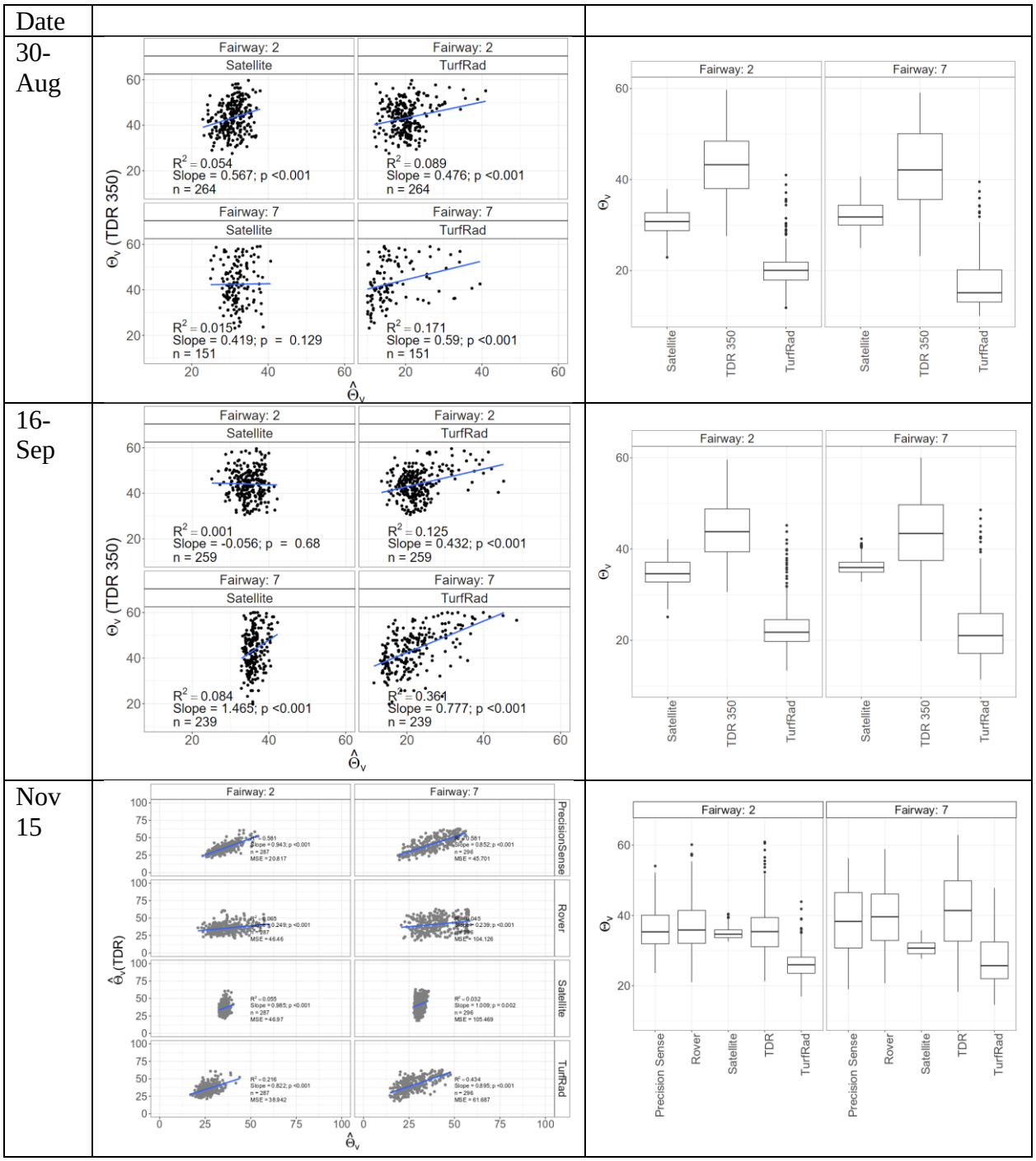


Figure 13. Volumetric soil moisture values collected on Fairways #2 and #7 on 30 August, 16 September, and 15 November with the Sentinel-1A satellite, a portable radiometer (turfRad), the PS 6000, and a portable neutron detector (Rover) and compared to values collected with the TDR 350. Linear regression graphs (left column) highlight relationship of volumetric soil moisture (θ_V) measured with TDR 350 and remote sensing units. Box plots (right column) indicate median (line inside the box), the 25th and 75th percentiles (length of box) and minimum and maximum (vertical lines extending from the box) values. Data points on top of or below the lines can be considered outliers.

Conclusions and Future Expectations

Remote sensing technologies such as turfRad or Rover show some promise in tracking soil moisture easily and with sufficient frequency to be helpful for irrigation scheduling. Data are uploaded and made available to the golf course superintendent almost immediately after measurements are taken and can assist in timely irrigation decisions. However, more studies are needed to improve the accuracy of measurements and to determine if different mathematical models should be used to estimate soil moisture based on reflectance values on turf areas. The soil moisture values estimated from the models used in our study are not sufficiently accurate for them to be useful for scheduling irrigation with the aim to conserve water. These models were developed using data collected from agricultural fields and general landscape areas and not turfgrass areas. Satellite data appear to be impractical for the purpose of irrigation scheduling. Data were either not available at all or after a significant delay, and values lacked the necessary accuracy when compared to the Precision Sense or the TDR 350 measurements, the current industry standard.

Soil moisture based on remote sensing technologies are all model estimates and come inherently with some degree of error. Aside from this uncertainty, two other sources of errors may contribute to the measurement discrepancies that were observed in our study. First, we assumed that GPS coordinates displayed on each unit describe the exact location. However, as Figure 7 shows, this is not the case for the TDR 350 and the extent of discrepancies varied even between fairways. We were not able to assess the location accuracies for the satellite, the turfRad, or the Rover. It is reasonable to assume that their measurement locations also included a certain degree of inaccuracy. The second source of error is the non-uniformity of the irrigation system. Low quarter Distribution Uniformity on Fairway #7 reached only a 0.51. That, in combination with a considerable difference in GPS coordinates for any given location, can result in a significant difference in moisture content when kriging is used to estimate moisture at a location that has not been measured. Considering all these sources of errors, we cannot be sure that measurements which were compared in the linear regression model, were taken at the same location.

Our second objective in this study was to select the best performing technology and use it to schedule irrigation in 2024. At this point, none of the tested technologies can be used for such a task as they all lacked the necessary accuracy. Instead, during 2024 we will test the accuracy of the GPS coordinates of all units by limiting soil measurements to areas in which the perimeter has been clearly defined by professional grade surveying equipment. We will also work on developing a better model to predict soil moisture using reflectance values for the turfRad and the Rover derived from a golf course fairway.

Appendix: Dates when soil moisture was collected and/or provided

	Precision Sense		Gravimetry		TDR 350		Satellite		TurfRad				
	Fwy 2	Fwy 7	Fwy 2	Fwy 7	Fwy 2	Fwy 7	Fwy 2	Fwy 7	Fwy 2	Fwy 7			
16-May		X							X	X			
17-May	X	X							X	X			
19-May							X	X	X	X			
25-May							X	X					
26-May							X	X					
29-May	X	X							X	X			
31-May							X	X	X	X			
7-Jun	X	X					X	X	X	X			
12-Jun	X	X					X	X					
16-Jun	X	X							X	X			
19-Jun	X	X					X	X					
20-Jun	X	X							X	X			
26-Jun	X	X							X	X			
30-Jun	X	X											
3-Jul	X	X							X	X			
10-Jul	X	X											
13-Jul	X	X					X	X	X	X			
14-Jul	X	X							X	X			
18-Jul	X	-	X	X			X	X	X	X	**Clutch broke**		
6-Aug			X	X	X	X	X	X					
7-Aug			X	X	X	X			X	X			
11-Aug							X	X	X	X			
18-Aug					X	X	X	X					
22-Aug							X	X	X	X			
23-Aug							X	X	X	X			
30-Aug			X	X	X	X	X	X	X	X			
11-Sep					X	X	X	X					
15-Sep			X	X	X	X							
16-Sep			X	X	X	X	X	X	X	X			
10-Oct					X	X	-	-	X	X	**Satellite data not provided**		
7-Nov	X	X											
15-Nov	X	X			X	X	X	X	X	X	**+ Rover (Hydro Sphere)**		

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:

Creeping Bentgrass Trial at Wilmington Country Club (Wilmington, DE)

- Statistically significant differences were observed among the treatments for turf quality, clippings yield, and chlorophyll content.
- No significant differences were observed among the treatments for soil volumetric water content (VWC).
- Turf quality was significantly different for three out of six rating dates.
- Acibenzolar-S-methyl, kelp extract, PGPR *Bacillus subtilis* UD1022, and fosetyl-Al had higher turf quality than trinexapac-ethyl and tank mix treatments on most of the rating dates.
- Total chlorophyll content was significantly different for two out of six rating dates.
- No significant differences were observed among the treatments for root characteristics for spring and summer 2023.
- Bacterial abundance in the soil (measured as colony forming units) was significantly increased by UD1022 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 has a physiological effect that enhances 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 one might not 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 is being 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 were: 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. Clipping samples were collected every two weeks for chlorophyll estimation and yield. Statistically significant differences were observed among all the treatments for turf quality (Table 2) and total chlorophyll content (Table 3) whereas no significant differences were observed among the treatments for soil volumetric water content (VWC) (Table 5). Acibenzolar-S-methyl, kelp extract, PGPR *Bacillus subtilis* UD1022, and fosetyl-Al had higher turf quality than trinexapac-ethyl and tank mix treatments on most of the rating dates (Table 2). Chlorophyll content was significantly different for two out of six rating dates (Table 3) with PHP treated plants having significantly higher chlorophyll content as compared to the control. Kelp extract, PGPR *Bacillus subtilis* UD1022, and fosetyl-Al had higher clippings yield than control and trinexapac-ethyl containing treatments towards the end of the season (Table 4).

Root samples (0.75-inch diameter, 6 inches deep) were collected at the beginning, middle, and end of the trial season. No significant differences were observed in root characteristics for beginning and mid-season samples for 2023 (data not presented). Root samples for 2023 end of the season have been harvested and will be evaluated for root parameters using WinRhizo root analysis software. Laboratory measurements for estimation of PR-gene expression for samples is in progress.

Root colonization potential of UD1022 was determined using serial dilution plating technique and number of viable cells was estimated as colony forming units (CFU). Significantly higher bacterial colonization was observed among the UD1022 treated plots as compared to the non-treated control (Fig 2,3).

Fig. 1 Creeping bentgrass trial site at Wilmington Country Club, DE



Table 1. List of treatments applied to 2023 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. 2023 Field Trial at Wilmington Country Club, DE – Turf Quality

Treatment	Date of Measurement					
	5-Jun	3-Jul	31-Jul	28-Aug	25-Sep	23-Oct
	Turf quality (1-9 visual scale)					
Control	5.62c[x]	6.50	7.13	6.75	7.50ab	5.75a
ASM	7.50a	7.75	7.63	7.25	7.50ab	5.62ab
Kelp Extract	7.62a	7.63	7.75	7.88	7.87a	6.25a
Bacillus subtilis UD1022	7.50a	7.88	7.63	7.63	7.87a	6.12a
Fosetyl-Al	7.25ab	7.75	7.88	7.13	8.00a	6.25a
TE	5.87c	6.50	7.00	6.38	7.12b	4.62bc
Tank Mix	6.37bc	7.25	7.00	6.75	7.50ab	4.50c

Ratings are based on a scale of 1 – 9 (1 = poorest TQ, 9 = excellent turf, and 6 = minimum acceptable)

Table 3. 2023 Field Trial at Wilmington Country Club, DE – Total Chlorophyll

Treatment	Date of Measurement					
	5-Jun	3-Jul	31-Jul	28-Aug	25-Sep	23-Oct
	Total chlorophyll content (µg/ml)					
Control	11.02	4.48e[x]	9.48f	20.32	4.28	11.09
ASM	8.66	12.17bc	12.81e	26.07	4.97	8.57
Kelp Extract	8.71	10.51cd	15.17d	25.50	9.40	14.02
Bacillus subtilis UD1022	10.12	19.14a	19.96ab	35.01	7.34	12.60
Fosetyl-Al	10.08	13.32b	20.51a	32.44	7.28	12.12
TE	12.80	9.28d	18.62c	38.72	3.59	11.00
Tank Mix	9.77	12.93b	19.34bc	22.13	4.23	13.18

[x]Means accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

Table 4. 2023 Field Trial at Wilmington Country Club, DE – Clippings yield

Treatment	Date of Measurement					
	5-Jun	3-Jul	31-Jul	28-Aug	25-Sep	23-Oct
	Clippings yield (g)					
Control	3.25	3.44	3.47	4.17abc[x]	2.78b	1.31
ASM	3.74	3.67	4.13	3.88bc	3.01b	1.97
Kelp Extract	3.93	3.92	4.15	4.64ab	3.85a	1.44
Bacillus subtilis UD1022	3.74	4.30	4.22	5.10a	3.69a	1.49
Fosetyl-Al	4.02	4.11	4.27	4.67ab	3.84a	1.74
TE	3.51	3.18	3.89	3.26bc	2.96b	1.09
Tank Mix	3.30	3.16	3.93	4.19abc	2.67b	1.30

[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 5. 2023 Field Trial at Wilmington Country Club, DE – VWC

Treatment	Date of Measurement					
	5-Jun	3-Jul	31-Jul	28-Feb	25-Sep	23-Oct
	Volumetric Water Content					
Control	15.67	15.41	19.57	15.55	20.95	16.02
ASM	15.42	14.85	18.75	10.87	20.07	17.90
Kelp Extract	16.90	19.13	17.87	18.10	23.13	21.15
Bacillus subtilis UD1022	16.27	18.55	19.35	15.27	23.45	19.32
Fosetyl-Al	17.75	18.43	19.97	18.35	24.05	20.10
TE	11.50	14.64	15.30	13.82	22.65	17.65
Tank Mix	15.20	16.11	17.25	18.82	23.13	18.10

[x]Means accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

Fig. 2 Root colonization potential of UD1022 was determined using serial dilution plating technique and number of viable cells was estimated as colony forming units (CFU).

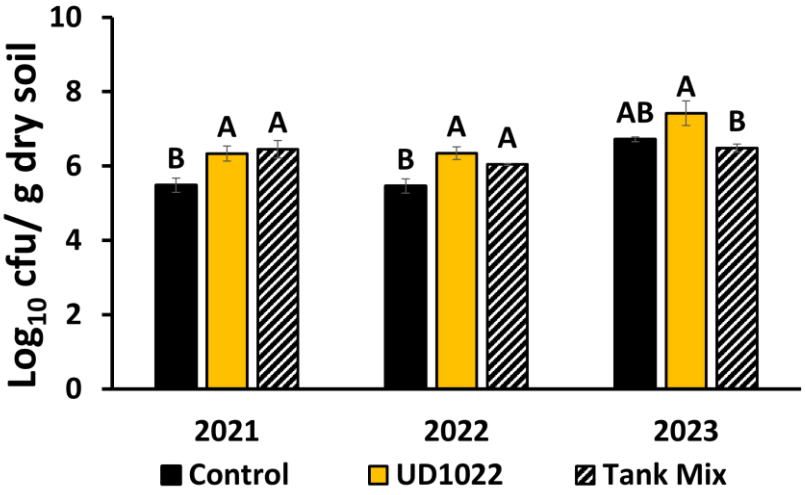
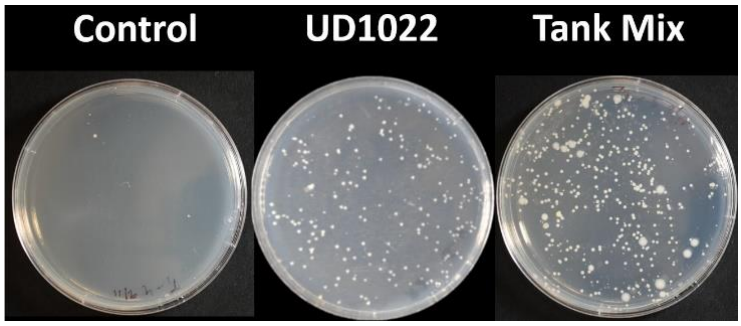


Fig. 3 Soil serial dilution petri plates showing bacterial abundance as CFUs (Plate dilution = 10^{-5}).



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 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 bicarbonate levels and other chemical parameters in the rootzone.
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:

- Over a three-year study period, irrigation with water high in bicarbonates resulted in a decreased saturated hydraulic conductivity compared to the control treatment.
- In 2022, plots irrigated with water high in bicarbonates and with water high in bicarbonates to which WaterSOLV™ Curative was added exhibited the highest bicarbonate levels at depths of 10 cm and 20 cm. By spring 2023, plots irrigated with high bicarbonates and WaterSOLV™ Curative displayed a higher bicarbonate level at a depth of 30 cm than soil irrigated with Control water.
- After three years, Kentucky bluegrass irrigated with water high in bicarbonates plus N-pHuric acid demonstrated lower visual quality compared to Kentucky bluegrass irrigated with all other treatments (Control water, water high in bicarbonates, and water high in bicarbonates with WaterSOLV™ Curative).
- The decline in Kentucky bluegrass visual quality coincided with nutrient imbalances.

Summary Text

Background and Rationale

Golf courses are increasingly reliant on suboptimal water sources, particularly in arid regions, many of which exhibit elevated levels of dissolved bicarbonates. The assessment of bicarbonate levels in irrigation water, in conjunction with the examination of sodium, calcium, and magnesium content in both soil and water are believed to be primary contributors to soil physical issues, including diminished infiltration rates and restricted plant root development. Furthermore, concerns have been raised regarding the potential formation of calcium carbonate deposits from irrigation water, leading to the gradual sealing of

soil pores. It is important to note that this issue is different from the deterioration of soil physical conditions induced by sodium.

There is a lack of scientific evidence that irrigation water acidification is efficient when bicarbonate and sodium levels are elevated. Specifically, questions arise regarding the necessity for acidification when the ratios of Ca/Mg and HCO_3/CO_3 are high, yet sodium levels remain low. While management practices aimed at averting the accumulation of calcite in the soil have been proposed, a lack of scientific evidence if such conditions even occur in turf rootzones. Despite existing guidelines for interpreting irrigation water quality, considering many parameters and their interactions, such as Electrical Conductivity (EC) and Sodium Adsorption Ratio (SAR), information about the effect on turfgrass performance when irrigated with water high in bicarbonates is scarce. Addressing this gap is crucial for advancing our understanding of the interplay between water quality and turf health.

Methods

A study was initiated at the Turfgrass Salinity Research Center of New Mexico State University in Las Cruces, NM, in November 2020. The soil composition at the site consisted of the Bluepoint sandy loam, characteristic of arid regions. The research area, sown with Kentucky bluegrass variety "Barserati" in the fall of 2020, comprised of sixteen 2m by 2m plots arranged in a completely randomized block design. Irrigation with the different sources of water started on June 15, 2021, utilizing four 1890L (500-gallon) tanks (Figure 1). Sodium bicarbonate and potassium bicarbonate were employed to elevate the bicarbonate level to 500ppm in the irrigation water. Plots were manually irrigated five times per week at 80% of the reference evapotranspiration (ETo) with the respective irrigation waters. Table 1 presents a comprehensive list of ion concentrations in the irrigation waters. A control treatment received potable water only.

Figure 1. Four water tanks containing local tap water (Control treatment), water high in Bicarbonates, water high in Bicarbonates to which N-pHuric acid was added, and water high in Bicarbonates to which WaterSOLV™ Curative was added.



Four water treatments were used in the project:
Treatment 1: Local potable tap water with a low concentration of bicarbonates (200 ppm) served as the control treatment ('Control water').
Treatment 2: Potable water with a high concentration of bicarbonates (450-500ppm).
Treatment 3: Potable water with a high concentration of bicarbonates (450-500ppm) with N-pHuric acid added to adjust the pH to 6.5.
Treatment 4: Potable water with a high concentration of bicarbonates (450-500ppm) with WaterSOLV™ Curative ('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

Throughout all three growing seasons, control plots and plots receiving water high in bicarbonates, and water high in bicarbonates with Curative were fertilized with Urea to match the nitrogen applied to water high in bicarbonates with N-pHuric acid. At the end of the growing period, each plot received an equal amount of nitrogen.

Soil samples were collected at depths of 10, 20, and 30 cm before and after the growing season (November 3, 2021; April 29 and September 29, 2022; April 20 and September 13, 2023). Analyses included bicarbonates (titration with 0.01N sulfuric acid), pH, electrical conductivity (EC), and sodium absorption ratio (SAR) using plasma emission spectroscopy. Soil cores were collected on April 30, 2021, November 23, 2021, October 27, 2022, and October 4, 2023, and the saturated hydraulic conductivity (Ksat) was measured in laboratory conditions using the KSAT (METER Group Inc., USA) (Figure 2).



Figure 2. Measurement of saturated hydraulic conductivity using KSAT® (Meter Group, Inc. USA)

One photograph per plot was taken every other week (April - October) 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 - November). Visual quality and DGCI data were averaged monthly and presented from June to November. For nutrient analysis, plant tissue samples were collected in September 2021, April and September 2022, and in May and September 2023.

Each treatment was replicated four times. To assess the effects of water treatments on Kentucky bluegrass quality, Ksat, soil parameters, DGCI, and ion concentration in plant tissue, data underwent 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.

2021-2023 Results

Total precipitation for the years 2021, 2022, and the estimated value for 2023 were recorded as 155.9 mm, 199.4 mm, and 104.9 mm, respectively.

Saturated hydraulic conductivity

P-values, obtained from the corresponding Analysis of Variances to examine variations in saturated hydraulic conductivity (Ksat) values among plots irrigated with different water treatments, are listed in Table 2 and mean values are highlighted in Figure 3. When Ksat data were averaged over all sampling dates, there were no significant differences in values between Control water (162 ppm), water high in bicarbonates with N-pHuric acid (183 ppm), and water high in bicarbonates with Curative (134 ppm). However, Ksat values were lower for plots irrigated solely with water high in bicarbonates (78.7 ppm) compared to those irrigated with Control water or water high in bicarbonates with N-pHuric acid (data are not shown)

Table 2. P-values obtained from ANOVA testing the effect of date (Spring 2021, Fall 2021, Fall 2022, and Fall 2023) and four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative) on saturated hydraulic conductivity (Ksat) of soil.

	2021	2021 and 2022	2021, 2022, and 2023
Date	0.0002	0.0001	0.008
Water treatment	0.0573	0.0618	0.0064
Date* Water treatment	0.0451	0.0666	0.0485

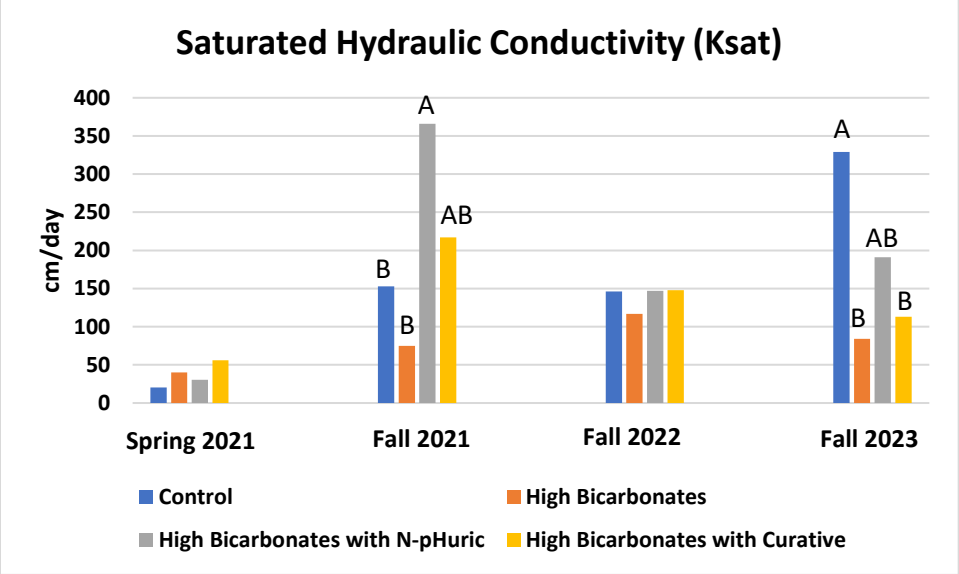


Figure 3. Saturated hydraulic conductivity of soil cores collected in 2021-2023 from plots irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative).

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. Upon completion of the third year of the study, individual analyses of bicarbonate (HCO_3) concentrations at varying sampling depths revealed no significant differences at 10 cm and 20 cm (Figure 4). However, at a depth of 30 cm, plots irrigated with Control water and water high in bicarbonates with N-pHuric acid exhibited lower HCO_3 concentrations compared to soil irrigated with water high in bicarbonates (Figure 4).When Sodium Adsorption Ratios were pooled over sampling dates and analyzed separately for the three sampling depths, plots irrigated with any of the treatments, excluding control plots, exhibited higher SAR values (Figure 5).When data were pooled over three years, plots irrigated with Control water had lower EC values than those irrigated with water high in bicarbonates with N-pHuric acid. Additionally, plots irrigated with Control water demonstrated lower pH levels compared to plots irrigated with any other treatment (Table 4).

Table 3. P-values obtained from ANOVA results testing the impact of four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative), sampling dates (November 2021, April and September of 2022, April and October of 2023), soil depth (0-10, 10-20, and 20-30 cm) and their interactions on soil chemical characteristics.

Effect	HCO_3	Mg	Na	SAR	EC	pH
Water treatment	<.0001	0.0687	0.0001	<.0001	<.0012	<.0001
Date	<.0001	0.0001	0.0070	<.0001	<.0001	<.0001
Date*water treatment	0.0003	0.0008	<.2364	0.0422	<.0048	0.0060
Depth	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
Water treatment*Depth	0.0046	0.3984	<.0001	<.0001	0.0544	0.0160
Date*Depth	0.0060	0.7078	<.0001	<.0001	<.0001	<.0001
Date*Water treatment*Depth	0.5042	0.0881	0.0142	0.0193	<.0083	0.0056

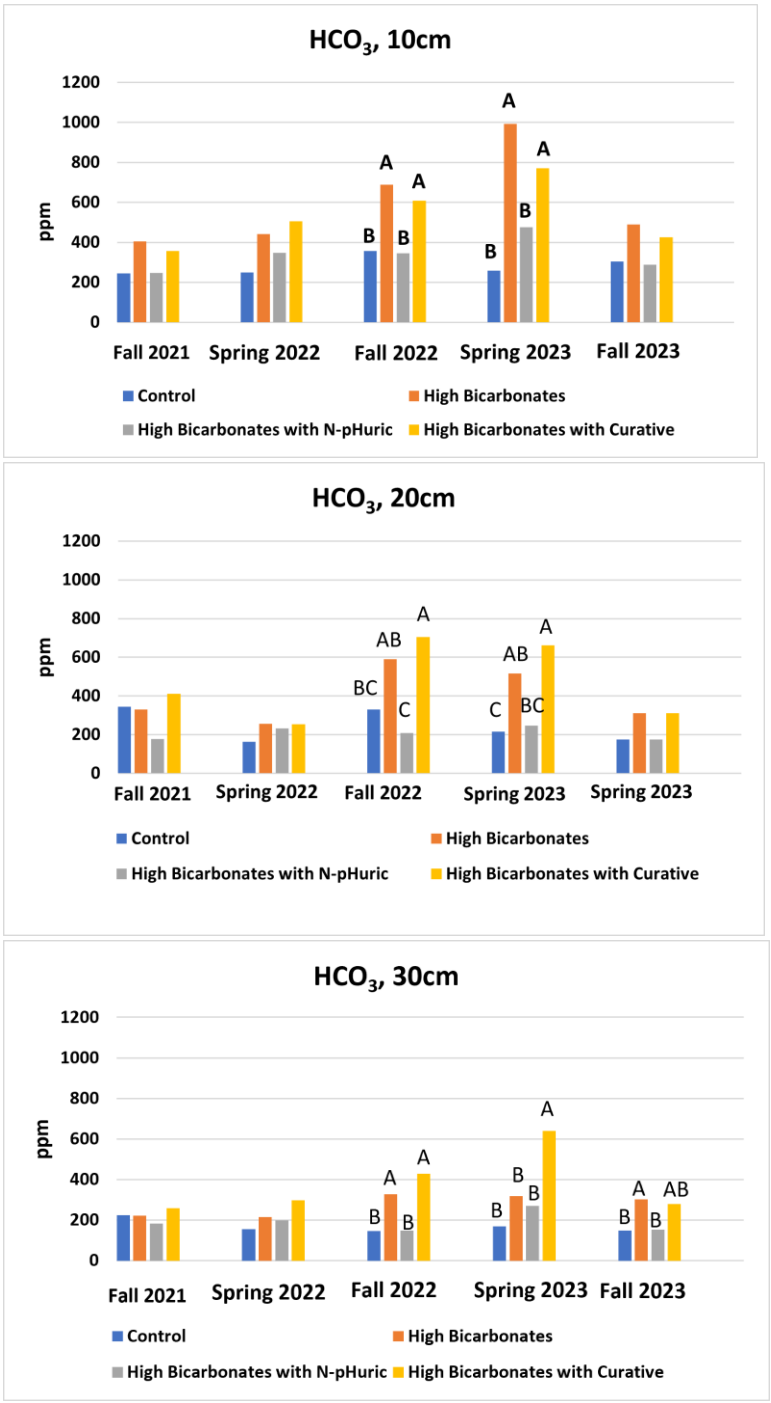


Figure 4. Bicarbonates concentrations (ppm) in soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative) at three depths (0-10, 10-20, and 20-30 cm).

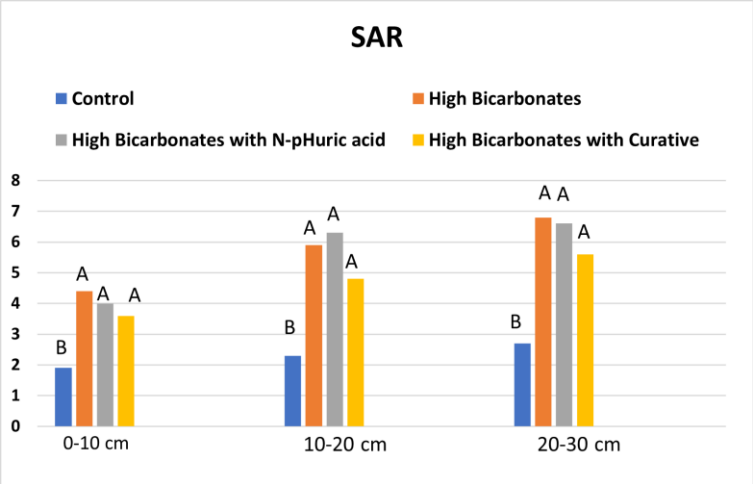


Figure 5. Sodium adsorption ratio (SAR) in soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative) at three depths (0-10, 10-20, and 20-30 cm).

Table 4. Electrical conductivity (EC), sodium adsorption ration (SAR), and pH of soil irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative). Data are pooled over 3 depths and 5 sampling dates.

Treatment	EC, (mmhos/cm)	SAR	pH
Control	0.62B	1.9B	8.1C
Bicarbonates	0.82AB	4.4A	8.7A
Bicarbonates with N-pHuric acid	0.95A	4.0A	8.4B
Bicarbonates with WaterSOLV™ Curative	0.75AB	3.6A	8.6A

Visual rating and DGCI

P-values obtained from the corresponding ANOVA to examine differences in visual rating and DGCI of Kentucky bluegrass irrigated with different water treatments are listed in Table 5. When visual quality and DGCI data were analyzed separately for each month, it was observed that Kentucky bluegrass, irrigated in 2023 with water high in bicarbonates with N-pHuric acid exhibited lower visual quality compared to grass irrigated with alternative water treatments (Table 6). Furthermore, the DGCI of Kentucky bluegrass, irrigated with water high in bicarbonates with N-pHuric acid, was lower in the fall of 2023 than the DGCI of grass irrigated with other water treatments (Table 7).

Table 5. P-values obtained from ANOVA testing the effect of four water treatments in 2021, 2022, and 2023 (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative), and their interactions on Dark Green Color Index (DGCI) and visual quality of Kentucky bluegrass.

Effect	DGCI	Visual quality
Water treatment	0.2218	0.31
Date	<.0001	<.0001
Date*Water treatment	<.0001	<.0001

Table 6. 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 WaterSOLV™ Curative), in 2021, 2022, and 2023.

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
	June, 2023	July, 2023	August, 2023	September, 2023	October, 2023	November, 2023
Control	7.8a[¥]	7.3ab	7.a	7.5a	7.8a	6.6a
	8.5A[§]	8.8A	8.5A	8.8A	9A	9A
	8.8A[α]	8.4A[α]	8.6A[α]	8.9A[α]	8.7A[α]	.
High bicarbonates	7.3a	7.4ab	7a	7.6a	7.4a	6.6a
	8.6A	9A	8.9A	8.8A	9A	9A
	8.8A[α]	7.6AB[α]	7.8AB[α]	8.1A[α]	8.0A[α]	.
High bicarbonates with N-pHuric acid	7.8a	8.1a	8.2a	7.9a	8a	8.8a
	8.5A	9A	8.1A	8.5A	9A	8.8A
	8.8A[α]	7.0B[α]	6.9B[α]	6.0B[α]	7.0B[α]	.
High bicarbonates with Curative	7.3a	7.1b	7.5a	7.8a	7.6a	7.5b
	8.7A	9A	8.5A	8.8A	9A	9A
	8.5A[α]	7.9AB[α]	8.3A[α]	8.5A[α]	8.3A[α]	.

[I] Values followed by the same letter in a column are not significantly different from one another (Fisher's protected LSD, α=0.05).

[¥] Lowercase letters in a column denote differences between grass quality in 2021.

[§] Uppercase letters in a column denote differences between grass quality in 2022.

[α] Uppercase letters in a column denote differences between grass quality in 2023.

Table 7. 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 WaterSOLV™ Curative), in 2021 and 2022.

Water treatment	June, 2021	July, 2021	August, 2021	September, 2021	October, 2021
	June, 2022	July, 2022	August, 2022	September, 2022	October, 2022
	June, 2023	July, 2023	August, 2023	September, 2023	October, 2023
Control	0.45a [ǂ¥]	0.49b	0.46a	0.44a	0.47a
	0.47A[§]	0.54A	0.54A	0.53A	0.53A
	0.5A[α]	0.6A[α]	0.57A[α]	0.5A[α]	0.5A[α]
High bicarbonates	0.45a	0.49b	0.47a	0.44a	0.46a
	0.48A	0.55A	0.55A	0.54A	0.54A
	0.51A[α]	0.58AB[α]	0.55A[α]	0.48A[α]	0.5A[α]
High bicarbonates with N-pHuric acid	0.47a	0.54a	0.47a	0.45a	0.48a
	0.47A	0.53A	0.52A	0.51A	0.53A
	0.48A[α]	0.55B[α]	0.48B[α]	0.45B[α]	0.49A[α]
High bicarbonates with WaterSOLV™ Curative	0.44a	0.48b	0.45a	0.42a	0.45a
	0.5547A	0.55A	0.55A	0.53A	0.54A
	0.5A[α]	0.57AB[α]	0.54A[α]	0.48AB[α]	0.51A[α]

[ǂ] Values followed by the same letter in a column are not significantly different from one another (Fisher's protected LSD, α=0.05).

[¥] Lowercase letters in a column denote differences between grass quality in 2021.

[§] Uppercase letters in a column denote differences between grass quality in 2022.

[α] Uppercase letters in a column denote differences between grass quality in 2023.

Plant tissue

P-values obtained from the corresponding ANOVA, used to examine differences in Kentucky bluegrass tissue irrigated with various water treatments, are listed in Table 8. When data were pooled over four sampling dates and analyzed separately for each water treatment, it was observed that Kentucky bluegrass irrigated with Control water exhibited a higher concentration of Ca but lower concentrations of K and Na. Conversely, the concentration of N_{total}, P, and S was higher in tissue of Kentucky bluegrass irrigated with water high in bicarbonates with N-pHuric acid (Table 9).

Table 8. P-values obtained from ANOVA testing the effect of four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative), date (fall 2021, spring and fall 2022, and spring 2023) and their interactions on nutrients in grass tissue.

	Ca	Cu	Fe	K	Mg	N_{total}	Na	P	S
Water treatment	<.0001	.0002	0.5958	<.0001	0.1016	<.0001	0.0033	.0006	0.0031
Date	0.0009	<.0001	<.0001	0.3530	<.0001	<.0001	0.0898	<.0001	<.0001
Date*Water treatment	0.0344	0.1563	0.1849	0.0470	<.0001	0.0079	<.0001	<.0001	0.0006

Table 9. Nutrients in Kentucky bluegrass tissue irrigated with four water treatments (Control, high Bicarbonates, high Bicarbonates with N-pHuric acid, and high Bicarbonates with WaterSOLV™ Curative). Data are pooled over date (fall 2021, spring and fall 2022, and spring 2023) and four replications.

Water treatment	Ca (%)	Fe (ppm)	K (%)	N_{total} (%)	Na (%)	P (%)	S (%)
Control	0.83A	165A	2.1C	3.3B	0.07B	0.47A	0.4AB
High bicarbonates	0.58B	136A	2.5B	3.3B	0.1A	0.42A	0.39B
High bicarbonates with N-pHuric acid	0.51B	176A	2.9A	4.9A	0.11A	0.36B	0.46A
High bicarbonates with WaterSOLV™ Curative	0.57B	145A	2.5B	3.5B	0.1A	0.4A	0.39B

Future expectations of the project

As the change in bicarbonate levels, EC, SAR, and pH in the soil is expected to continue, monitoring is imperative to understand the impact of all these parameters on turfgrass quality and soil infiltration rates. Investigating the conditions for the accumulation of soil carbonates when irrigation water contains elevated bicarbonate levels can provide insights into the likelihood of changes in soil infiltration rates as well as predict the dynamic of changes in soil chemical and physical parameters.

USGA ID#: 2021-08-732

Title: Reduced Fungicide Usage Through Application Optimization

Project Leader: Bruce Branham
Affiliation: University of Illinois

Objectives: Determine if systemic or contact 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 reduced fungicide rates reduced by 33% to determine if fungicide efficacy has been improved.

Start Date: January 2021
Project Duration: 2 years
Total Funding: $75,465

Summary Points:

- Both DyneAmic, an adjuvant, an UAN (urea ammonium nitrate solution), a nitrogen source, had instances of improved dollar spot suppression; however, a lack of consistent improvement in dollar spot suppression is problematic.
- Using UAN modestly improved dollar spot suppression when applied with systemic fungicides.
- Replacing commonly used nitrogen sources with UAN as a tank mix partner may improve pesticide performance while reducing unit cost of nitrogen
- A better approach to screening adjuvant-fungicide combinations than brute force field testing is needed.

Introduction

Turfgrass diseases in the humid region of the United States represent the most significant and consistent pest problem faced by golf turf managers. Fungicide applications are made regularly throughout the growing season and conclude with a late fall fungicide application to prevent snow mold. Dollar spot, while readily controlled, is certain to occur every year in the cool, humid region of the US.

A key factor in the performance of systemic pesticides is the uptake into the plant and the translocation to the site of action. Pesticides must traverse the plant cuticle, after first moving through a layer of epicuticular waxes, which are derived from very long-chained fatty acids, presenting a significant barrier to pesticide penetration. Waxes are lipophilic substances and pesticides vary significantly in their lipophobicity. Thus, some pesticides may diffuse through the epicuticular waxes and get inside the plant while other pesticides may diffuse into the cuticle and remain there since they are highly lipophilic themselves. Thus, measuring pesticide uptake into plants is challenging since a pesticide may move into the cuticle, but go no

further. One way to measure uptake is to apply a known quantity of the pesticide to the leaf surface. Additionally, a pesticide may move through the cuticular layer but not translocate to the site of action within the plant. The point here is that laboratory evaluations of pesticide movement 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. Once an adequate level of pesticide activity has been achieved, the incentive for pesticide manufacturers to spend additional research dollars to optimize activity is marginal at best.

In 2021 and 2022, we studied five different adjuvant chemistries, but did not find striking differences in fungicide activity. In 2023, we included the most promising of the adjuvants, a product with the trade name DyneAmic. This reduction in the number of treatments allowed us to examine a second nitrogen fertilizer adjuvant, ammonium sulfate, AMS.

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 and maintained at 1.2 cm height of cut. The fairway was established to L-93 creeping bentgrass with little weed contamination. Maintenance practices were typical of a well-conditioned golf course.

Two levels of adjuvants (DyneAmic or no adjuvant) and three levels of nitrogen adjuvants (no nitrogen, UAN, or AMS) were applied with each fungicide. Each fungicide was applied at 2/3 of the full label rate of application along with each nitrogen source, with and without DyneAmic (Table 1). Each trial included an untreated control and the full label rate of the fungicide being evaluated. The fungicides evaluated were boscalid (Emerald), propiconazole (Banner), penthiopyrad (Velista), and metconazole (Tourney). Dates of application are shown in Table 2.

RESULTS and DISCUSSION

Results in 2023 were similar to results observed in previous years. That is, glimmers of improved control but not consistently throughout the summer. An important point to remember is that fungicides are all unique and one should not expect that they will all respond the same to an adjuvant or nitrogen source addition.

For Emerald and Banner, there was only one rating date when significant differences were observed (Table 3). On that 8/16 rating, both fungicides were losing control with dollar spot ratings ranging between 16 and 63%. For Emerald, the 2/3 rate showed worse control than the full label rate, although these differences weren't statistically significant (Table 3). We expected to see less control from lower rates, but that hasn't always occurred.

On 8/16, adding UAN to either fungicide at the reduced fungicide rate yielded dollar spot suppression equal to that of the full label rate (Tables 3 and 4). Adding DyneAmic improved dollar spot suppression compared to no adjuvant, again this is for both fungicides. AMS showed no significant reduction in dollar spot suppression compared to no nitrogen source (Table 4).

For Banner only, there was an improvement in dollar spot suppression on 8/16 from the addition of DyneAmic and UAN. For Emerald alone, the addition of DyneAmic plus AMS increased dollar spot suppression compared to Emerald alone (Table 3).

Results for Tourney and Velista showed significant dollar spot suppression results on two evaluation dates, 8/2 and 8/16 (Table 5). When analyzing the data as a factorial, the only significant difference, other than the main effect of fungicide, was an interaction between adjuvant and fungicide (Table 6). On 8/2, adding DyneAmic increased dollar spot suppression for Tourney, but not for Velista. As mentioned, it should be expected that each fungicide may respond differently to a particular adjuvant or nitrogen source. However, because the overall level of dollar spot amongst the Velista treatments was quite low, between 1 and 5 percent on 8/2, no significant difference can be expected (Table 5).

On both the 8/2 and 8/16 evaluations, none of the Velista combinations were statistically different than either rate of Velista alone (Table 5). DyneAmic + UAN showed good dollar spot suppression, at each evaluation date, that combination was as good as the full rate of Velista alone. For Velista, both AMS and UAN seemed to provide some contribution to dollar spot suppression. Similarly, for the 8/16 evaluation, none of the Tourney combinations were statistically different than either rate of Tourney alone (Table 5).

SUMMARY

Results of the three years of this investigation indicate have been consistently inconsistent. Really significant gains in dollar spot suppression have not been realized. However, two important points can be articulated. First, it appears that UAN does have some ability to increase the activity of certain fungicides. This is shown in the data on Emerald and Banner (Table 4) and hinted at in the data for Velista and Tourney (Table 5). Results from previous years also several instances where UAN provided significant increases in dollar spot suppression. Since most golf turf managers include a small amount of nitrogen in scheduled fungicide applications, substituting UAN for products more commonly used by turf managers may increase length/level of dollar spot suppression. UAN is most likely less expensive than current products used by golf turf managers. Further, UAN is a liquid form making it easier to use than those who apply small amounts of urea in their tank mix applications since urea must be dissolved prior to adding to the spray tank.

DyneAmic also has shown evidence of increased dollar spot suppression, particularly in combination with UAN. However, here a blanket recommendation shouldn't be made. Tourney

showed excellent control when DyneAmic + UAN were mixed with fungicide in both 2023 and 2022. This combination may improve the performance of Tourney on a consistent basis.

I still believe there is value in looking for methods to improve fungicide performance, but our results show that field testing is a difficult way to find consistent performance improvement. The variability in dollar spot occurrence, even over relatively short distances, further increases the difficulty of this task.

Table 1. Description of each adjuvant used in the fungicide evaluations.

Adjuvant Name	Description	classification
DyneAmic	methyl esters of C16-C18 fatty acids, polyalkyleneoxide modified polydimethylsiloxane, alkylphenolethoxylate	NIS/Organosilcone
UAN	Urea ammonium nitrate	28% nitrogen solution
AMS	Ammonium sulfate solution	8% Nitrogen

Table 2. Dates of Fungicide application

Fungicide	Application dates	Interval
Banner & Emerald	June 8, July 6, Aug 3, Aug 31	4 weeks
Velista & Tourney	June 8; June 29, July 20th, Aug 10, Aug 31	3 weeks

Table 3. Dollar spot control, as a percent of plot coverage, from Emerald and Banner as affected by adjuvants, UAN, or AMS

			Visual % Dollar spot			
Trmt #	Treatment	Rate (oz prod/A)	7/19	8/2	8/16	9/1
1	Control		22	15	63 a	59
2	Emerald	5.7	5	6	29 bc	43
3	Emerald	3.8	5	16	46 ab	40
4	Emerald + UAN	3.8 + 2.5% v/v	6	9	26 bc	45
5	Emerald + AMS	3.8 + 20 gm/L	19	15	51 ab	45
6	Emerald + DyneAmic	3.8 + 0.25% v/v	12	10	45 ab	49
7	Emerald + DyneAmic + UAN	3.8 + 0.25 % + 2.5 % v/v	9	18	25 bc	48
8	Emerald + DyneAmic + AMS	3.8 + 0.25 % v/v + 20 gm/L	7	7	16 c	33
9	Banner	44	6	6	45 ab	60
10	Banner	29	13	15	64 a	51
11	Banner + UAN	29 + 2.5% v/v	9	14	38 abc	55
12	Banner + AMS	29 + 20 gm/L	8	5	41 abc	64
13	Banner + DyneAmic	29 + 0.25% v/v	7	11	40 abc	53
14	Banner + DyneAmic + UAN	29 + 0.25 % + 2.5 % v/v	5	5	30 bc	54
15	Banner + DyneAmic + AMS	29 + 0.25 % v/v + 20 gm/L	6	12	40 abc	49
			NS	NS	26.3	NS

Table 4. Factorial Analysis of adjuvant, nitrogen, and fungicide source for August 16th rating for Emerald and Banner fungicides.

Source	% Dollar spot
Adjuvant	
None	44 a
DyneAmic	33 b
Fertilizer	
None	49 a
UAN	30 b
AMS	37 ab

Table 5. Dollar spot control, as a percent of plot coverage, from Tourney and Velista as affected by adjuvants, UAN, or AMS

			Visual Dollar Spot %			
Trmt #	Treatment	Rate (oz prod/A)	7/19	8/2	8/16	9/1
1	Control		18	13 ab	63 a	68
2	Velista	13	2	3 cd	18 bcd	49
3	Velista	8.7	2	2 d	11 d	43
4	Velista + UAN	8.7 + 2.5 % v/v	1	5 cd	11 d	46
5	Velista + AMS	8.7 + 20 gm/L	5	2 d	16 bcd	41
6	Velista + DyneAmic	8.7 +0.25 % v/v	3	5 cd	15 bcd	43
7	Velista + DyneAmic + AMS	8.7 + 0.25 % v/v + 20 gm/L	1	1 d	14 cd	54
8	Velista + DyneAmic + UAN	8.7 + 0.25 % + 2.5 %v/v	1	2 d	14 cd	35
9	Tourney	12	2	7 bcd	20 bcd	65
10	Tourney	8	5	8 bcd	29 bcd	40
11	Tourney + UAN	8 + 2.5 % v/v	7	10 abc	30 bc	50
12	Tourney + AMS	8 + 20 gm/L	6	15 a	33 b	66
13	Tourney + DyneAmic	8 + 0.25 % v/v	2	4 cd	26 bcd	55
14	Tourney + DyneAmic + UAN	8 + 0.25 % + 2.5 % v/v	2	2 d	18 bcd	55
15	Tourney + DyneAmic + AMS	8 + 0.25 % v/v + 20 gm/L	6	1 d	24 bcd	49
		LSD (P=0.05)	NS	7.5	18.6	NS

Table 6. Adjuvant by fungicide interaction for Dollar spot rating on August 2nd

Source	% Dollar spot	
Adjuvant	Velista	Tourney
None	3 a	11 a
DyneAmic	2.7 a	2.3 b

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: 4 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.
- Measured the economic impact of take-all root rot on Texas golf course.

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. The 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.

1. We identified 4 golf course superintendents in Texas for collecting baseline information about take-all root rot (TARR) in 2022.

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.

2. We extended our survey to 48 Texas golf course superintendents to collect feedback about TARR management in 2023.

General Information

- The majority of survey responders are superintendents of golf courses.
- They primarily maintain private, 18-hole golf courses in Harris and Montgomery counties.
- Tif Eagle, Mini Verde, and Champion are the top three bermudagrass varieties of putting green.

Fungicide Sprays

- Most golf courses spend between $100,000 and $200,000 on all chemical controls. A few spend over $400,000. Little change has occurred in budgets over the past three years.
- Estimated annual budgets devoted to putting green management are fairly evenly distributed across all points. Most are from 11% - 50% of their budgets.
- The largest number of golf courses spend 11-20% of their budgets annually on fungicides, but 5 responders spend 66-80%.
- A majority of golf courses apply fungicides multiple times per month.
- Observation of fungicide efficacy reduction is limited, with the largest number of respondents saying, “Might or Might Not”; 4 responders answering “Definitely Yes,” and 4 responders answering “Definitely Not.”

Turfgrass Density

- The majority of golf courses have not seen significant declines in their turfgrass density.
- Those that do notice losses observe this primarily in Winter and Spring.
- Bermudagrass is noted for the grass with most problems maintaining density.

TARR Knowledge

- A significant majority of responders are familiar with TARR symptoms; only 3 responders were not.
- A majority of responders believe that TARR significantly impacts green quality.

TARR Management

- The majority of respondents have taken steps to manage or mitigate TARR on their putting greens. Four indicated having no plan.
- A significant number of plants rely on or employ fungicide applications. Just over half also use improved soil quality, improved local drainage, and adjusted mowing heights.
- The majority of respondents are “Extremely” or “Somewhat” satisfied with their current TARR management plans. Only 1 responded as “Dissatisfied.”

Conclusion:

Turf samples were collected from different golf courses throughout Texas. Subsequently, causal *Gaeumannomyces* species were isolated and identified. Also, their pathogenicity was followed to determine virulence using the diagnostic methods developed in this study.

Through this project, we have developed and implemented a more integrated management approach for golf course superintendents. Based on accurate diagnostics, we can detect aggressive *Gaeumannomyces* species. Upon the confirmation of infection by *Gaeumannomyces* species and their pathogenicity, proper management practices of take-all root rot can be incorporated into superintendent's 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 for the first two years, 2020 and 2021. Since this project was extended to 2024, we could invite up to 40 Texas golf course superintendents to participate in the survey and conveyed the implementation of an improved management program targeted for take-all root in bermudagrass putting greens.

The survey for Texas golf courses indicated there is significant economic impact of take-all root rot for the golf course management. Most superintendents are aware of prevalent TARR infection in bermudagrass and are concerned about potential damage or reduced density of green quality due to TARR. However, most did not confirm TARR infection in their greens by professional diagnosticians. Currently they heavily rely on fungicide applications. We estimate potential economic impact of TARR as its management cost on Texas golf courses, ranging $50,000 to $100,000 per golf course.

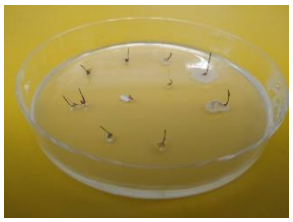
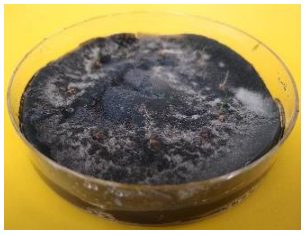


Dr. Jo was presenting the new finding about take-all root rot disease management in the bermudagrass putting green at the South Texas Golf Course Superintendent Association meeting in January 2024. Meeting attendees voluntarily participated in our survey.

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%

Project ID: 2023-04-771

Project Title: Characterizing surfactant toxicity and synergistic activity with pyrethroids in managing resistant annual bluegrass weevil populations

Project Leader: Benjamin A. McGraw, Ph.D.
Affiliation: Pennsylvania State University

Address: Dept. of Plant Sciences, 243 ASI Building, University Park, PA 16802

Objectives:

1. Identify ABW toxic surfactant classes and pyrethroid synergists
2. Characterize the detoxification enzyme expression after treatment with surfactants

Start Date: 2023
Project Duration: 1 year
Total Funding: $26,795

Summary Points:

1. Three surfactant classes (alkylpolyglucoside, organosilicone, and an anionic blend) showed insecticidal activity on their own and in combination with a pyrethroid (bifenthrin) against adult annual bluegrass weevil (ABW) in dose-response petri dish assays.
2. One surfactant class (block copolymer + alkylpolyglucoside) showed no insecticidal activity on its own against ABW but had a significant increase in mortality when in combination with bifenthrin.
3. All combinations of surfactants with bifenthrin showed an additive effect in mortality of ABW, but no synergistic combinations were observed.
4. Overall, P450 activity did not appear to be influenced by bifenthrin exposure. Only ABW treated with the combination of the block copolymer + alkylpolyglucoside and bifenthrin showed a significant reduction in P450 activity when compared to the high dose of bifenthrin.

<u>Summary</u>:

Rationale:

The annual bluegrass weevil (ABW) is a significant pest in eastern North America, with spring populations being the most damaging. Turfgrass managers typically combat these pests using broad-spectrum insecticides as the weevils move from overwintering to playing surfaces. Without treatment, ABW's long egg-laying period results in multiple larval stages that are difficult to control, as early larvae and pupae are protected from chemicals. Since the 1990s,

pyrethroid insecticides have been preferred for their effectiveness and low toxicity to non-target species. However, resistance to pyrethroids is increasing, leading to higher use of insecticides and greater costs.

Resistance is suspected to be at least partly due to enhanced detoxification by the weevils (Ramoutar et al. 2009). Surfactants, typically used for water management in turfgrass, may similarly reduce enzyme activity and restore the effectiveness of pyrethroids. This research aimed to identify specific surfactants that enhance pyrethroid toxicity and suppress detoxification in ABW, potentially offering a new management strategy.

Objective 1: Identify ABW toxic surfactant classes and pyrethroid synergists

Methodology:

Insects: Overwintering adults were collected from leaf litter and tall grass bordering fairways at the Pine Meadows Golf Complex in Lebanon, PA (40°25'01.6"N 76°25'30.4"W) in November of 2022. Weevils were collected by submerging their overwintering substrate in buckets of warm water to extract the adults as they floated to the surface. The weevils were transported back to the laboratory, where they were placed in 840 mL clear plastic cups filled with 500 g moist (5% v/w) sand and maintained in a growth chamber (10 h light at 6 °C:14 h dark at 4 °C) until use in experiments (up to two weeks following collection). Weevils from this location were previously evaluated to have moderate resistance to bifenthrin.

Surfactant Dose-Response Assay: Thirteen commercial surfactants, covering anionic and non-ionic types, were evaluated for their effectiveness in turfgrass management, following the Fidanza et al. (2020) chemical categorization. Bioassays were performed with adult weevils in 875 cm^3 containers, using four replicates for each treatment and repeating the experiment twice. The surfactants were applied at a 3% volume-to-volume ratio with a CO_2 backpack sprayer at approximately 28 psi, delivering 800 L/ha. Post-application, containers were sealed and brought indoors. Weevils were then placed in a Petri dish with filter paper and *Poa annua* leaves for sustenance. Weevil survival was checked after 24 and 48 hours by prodding. Mortality rates were normalized using arcsine transformation and tested for significant differences using ANOVA with Tukey's HSD test. The type of interaction between the surfactant and pyrethroid—synergistic, additive, or antagonistic—was determined using a χ^2 test (Finney 1964; Koppenhöfer & Kaya 1998; McVay et al. 1977).

Results:

Three surfactant subclasses—alkylpolyglucoside, organosilicone, and di-sulfosuccinate—were effective alone and when combined with a pyrethroid in enhancing weevil mortality (Fig. 1). Alkylpolyglucoside and organosilicone significantly increased mortality compared to the untreated control when used with a pyrethroid ($F_{29, 208} = 2.7$; $p < 0.0001$). The block copolymer was ineffective alone but improved mortality when combined with bifenthrin. None of the treatments showed statistically significant differences when compared to the low dose of pyrethroid alone. All combinations tested in this study had an additive effect, implying that the insecticidal activity of the surfactant-pyrethroid combinations was not affected by any

antagonistic interactions. However, no significant interaction was observed between any of the surfactants and the low dosage of pyrethroid ($\chi 2 \leq 0.17$; df = 1; $p \geq 0.64$).

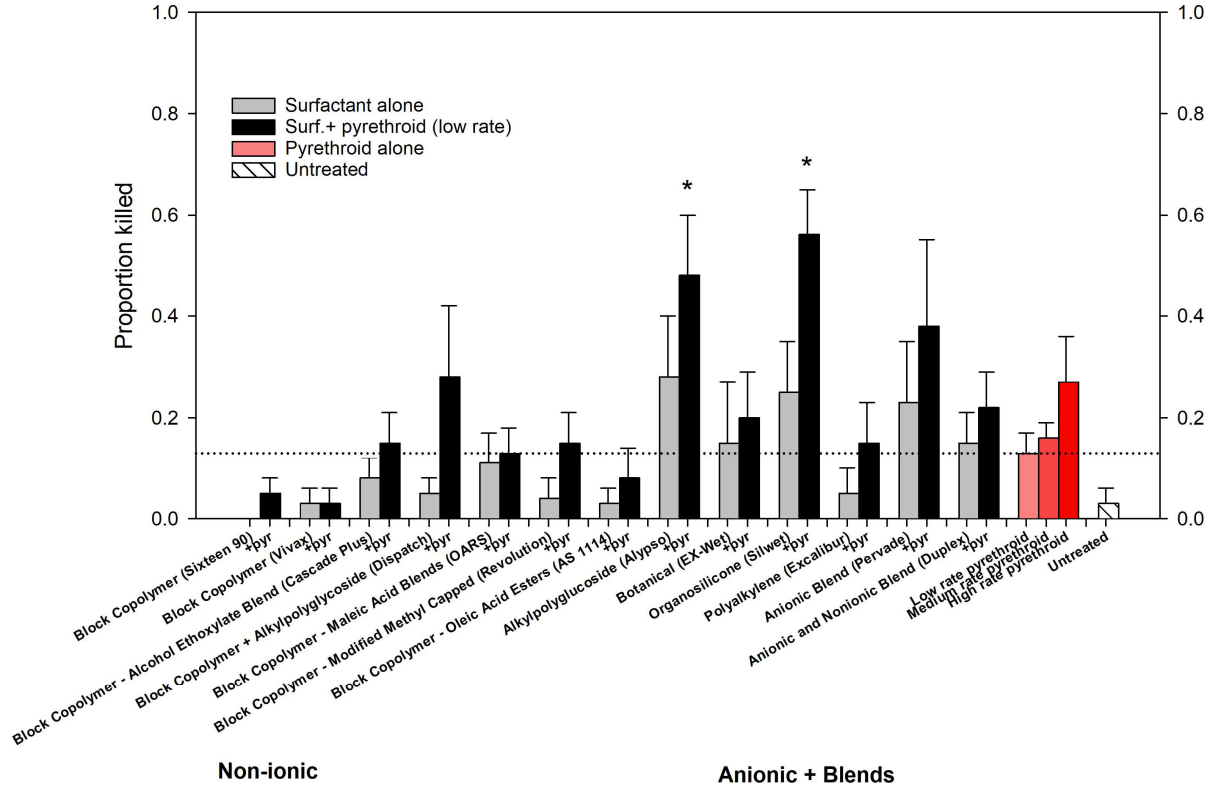


Figure 1: Proportion of adult *Listronotus maculicollis* that were killed 48 hours after treatment with a surfactant alone (gray bars) and in combination with a low rate of pyrethroid (at 115 mg AI/m^2)(black bars). Bifenthrin (low =115 mg AI/m^2, medium = 346 mg AI/m^2, and high rate = 3459 mg AI/m^2)(red bars) and an untreated control (water)(white bar) are also included. The bars labeled with an asterisk (*) denote a statistically significant increase in mortality compared to the low rate bifenthrin control.

Objective 2: Characterize the detoxification enzyme expression after treatment with surfactants

Methodology:

The effect of the four classes of surfactants that exhibited elevated control when combined with pyrethroids (block copolymer + alkylpolyglucoside, alkylpolyglucoside, organosilicone, anionic blend) on detoxification enzyme activity were examined using the same methodologies as those used in the dose-response assay. Treatments (12) included each of the four surfactants alone, the surfactants in combination with a low dosage of a pyrethroid, three bifenthrin concentrations (115, 346, and 3459 mg AI/m^2), and an untreated control. Each experiment was replicated three times.

After 24 hours, ABW were rinsed in ice-cold 0.1 M phosphate buffer, pH 7.5 (PBS) to remove contaminants. Groups of five weevils of each treatment were homogenized together with 500 µL

of 0.1 M PBS in 1.5 mL microcentrifuge tubes via electric mortar and pestle. Homogenates were then centrifuged at 10,000 RCF for 15 minutes and the resulting supernatant was used for protein and enzyme assays. The total concentration of protein in each sample was measured via a Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) using the microplate procedure as described by the manufacturer. A reference standard curve was plotted using varying concentrations of albumin at 562 nm in a microplate reader.

The amount of P450 activity in each sample was measured as the demethylation of NADPH using an Invitrogen P450 Demethylation Fluorescent Activity Kit (Thermo Scientific, Waltham, MA, USA) using the microplate procedure as described by the manufacturer. A reference standard curve was plotted using a serial dilution of the provided formaldehyde standard. One-way ANOVA was used to compare enzyme activities across treatments and means were separated by Tukey's HSD test. The P450 activity of each sample was normalized using that sample's protein concentration.

Results:

There was a significant reduction in P450 activity in ABW treated with a combination of block copolymer + alkylpolyglucoside and a low dosage of pyrethroid when compared to weevils treated with the high dosage of bifenthrin ($F_{11, 143} = 2.01$; $p = 0.03$) (Fig. 2). No significant differences in P450 activity were found between the different surfactants or between their combinations with a low dosage of pyrethroid. There were no significant differences in P450 activity between weevils treated with a surfactant alone and those treated with the surfactant in combination with a low rate of pyrethroid. Finally, no significant differences were observed between weevils treated with surfactants and those treated with any of the pyrethroid controls.

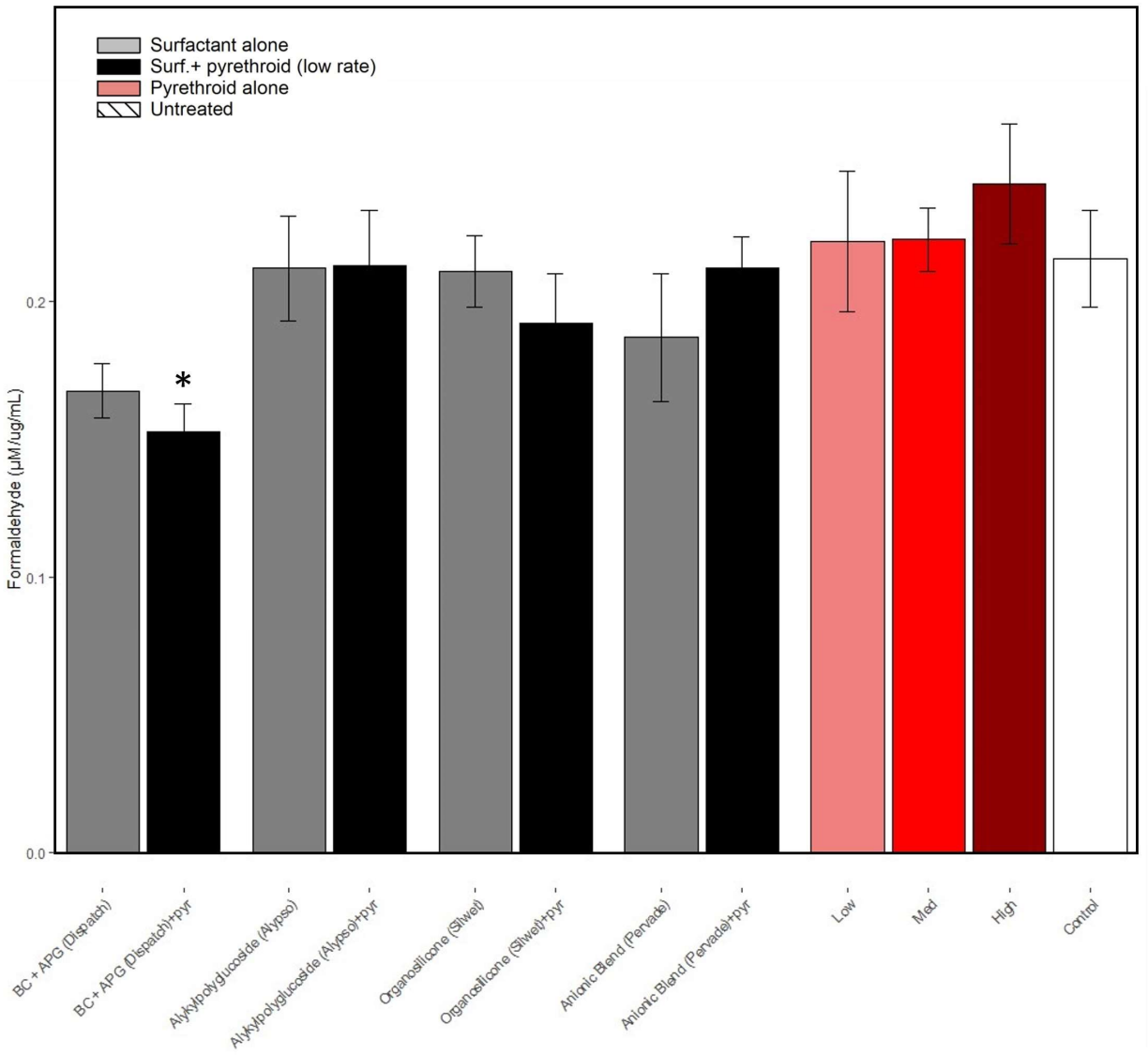


Figure 2: The concentration of formaldehyde produced as a proxy for the enzymatic activity of cytochrome P450 enzymes of adult *Listronotus maculicollis* collected from the Pine Meadows Golf Complex (Lebanon, PA) treated with either a surfactant alone (block copolymer + alkylpolyglucoside, alkylpolyglucoside, organosilicone, anionic blend), a surfactant in combination with a low dosage of a pyrethroid (Talstar Pro, 115 mg AI/m^2), control (H_2O), a pyrethroid alone (low (115 mg AI/m^2), medium (346 mg AI/m^2), or high dosage (3459 mg AI/m^2), or untreated (H_2O). The bars labeled with an asterisk (*) denote a statistically significant decrease in cytochrome P450 activity compared to the high pyrethroid control group.

Future Directions:

Future studies could explore how surfactants increase insecticide efficacy against weevils by examining their impact on weevil anatomy and behavior, and by testing their compatibility with various insecticides.

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Project ID: 2023-08-775

Project Title: Comparisons of early-season broad spectrum insecticide and novel insect growth regulator applications on non-target insect populations on golf courses

Principle Investigator: Benjamin A. McGraw, Ph.D.

University: Pennsylvania State University

Adress: Dept. of Plant Sciences, 243 ASI Building, University Park, PA 16802

Objectives:

(1) Determine the effect of novaluron applications on soil-dwelling and surface-active arthropod and predator populations.
(2) Determine the effect of novaluron applications on consumption of sentinel prey.
(3) Determine the effect of novaluron applications on predatory insect behavior, using Turfgrass ant (*Lasius* neoniger) as a model organism.

Start Date: 2023

Project Duration: 2 years

Total Funding: $74,050

Summary Points:

i. Soil-dwelling and surface-active arthropod abundance in novaluron-treated turf was not significantly different from untreated controls.
ii. Insect populations in all treatments recovered to pre-application levels after 14 days.
iii. No significant differences were observed in arthropod predation between insecticide treatments.
iv. Novaluron did not impact ant mounding behavior whereas bifenthrin and a bifenthrin-clothianidin significantly reduced ant mounding for a period of 14 days.

Summary:

Insect Growth Regulators (IGRs) are a class of insecticides that target the endocrine system to disrupt normal growth and development of immature insects. IGRs are regarded as being relatively safe to applicators compared to most insecticides on the market which generally affect nervous system targets common to both humans and insects. Despite this general mechanism, many view IGRs as selective, reduced-risk insecticides that are compatible with IPM programs and less toxic to beneficial insects than traditional chemistries. However, few studies are available to support these claims.

Novaluron, a benzoylphenyl urea IGR, inhibits chitin biosynthesis in developing immature insects leading to abortive molting. Suprado (Control Solutions, Pasadena, TX) demonstrates superior control of annual bluegrass weevil (ABW)(McGraw et al. in review) and has already been widely used in the Northeast for control of first-generation larvae. The impact of extensive adoption, both in area and throughout the season, of IGR insecticides on non-target and beneficial insects in the golf course environment has not been considered. Novaluron is known to be broadly toxic to several orders of insects, including Lepidoptera (moths and butterflies), Coleoptera (beetles), Heteroptera (true bugs), and Diptera

(flies)(Ishaaya et al. 1996). Additionally, adult insects that contact or ingest novaluron do not perish, though egg development, fecundity, and egg hatch may be reduced (Kostyukovsky et al. 2006; Alyokhin 2008; Catchot et al. 2020). Laboratory studies conducted on ABW support these findings (McGraw et al., in review). Not surprisingly, these same effects that have been observed on pests have also been documented on several beneficial insect species (Cutler et al 2006; Cabrera et al. 2017, He et al. 2018, Santorum et al. 2019).

Objective 1: Determine the effect of novaluron applications on soil-dwelling and surface-active arthropod and predator populations.

Methodology:

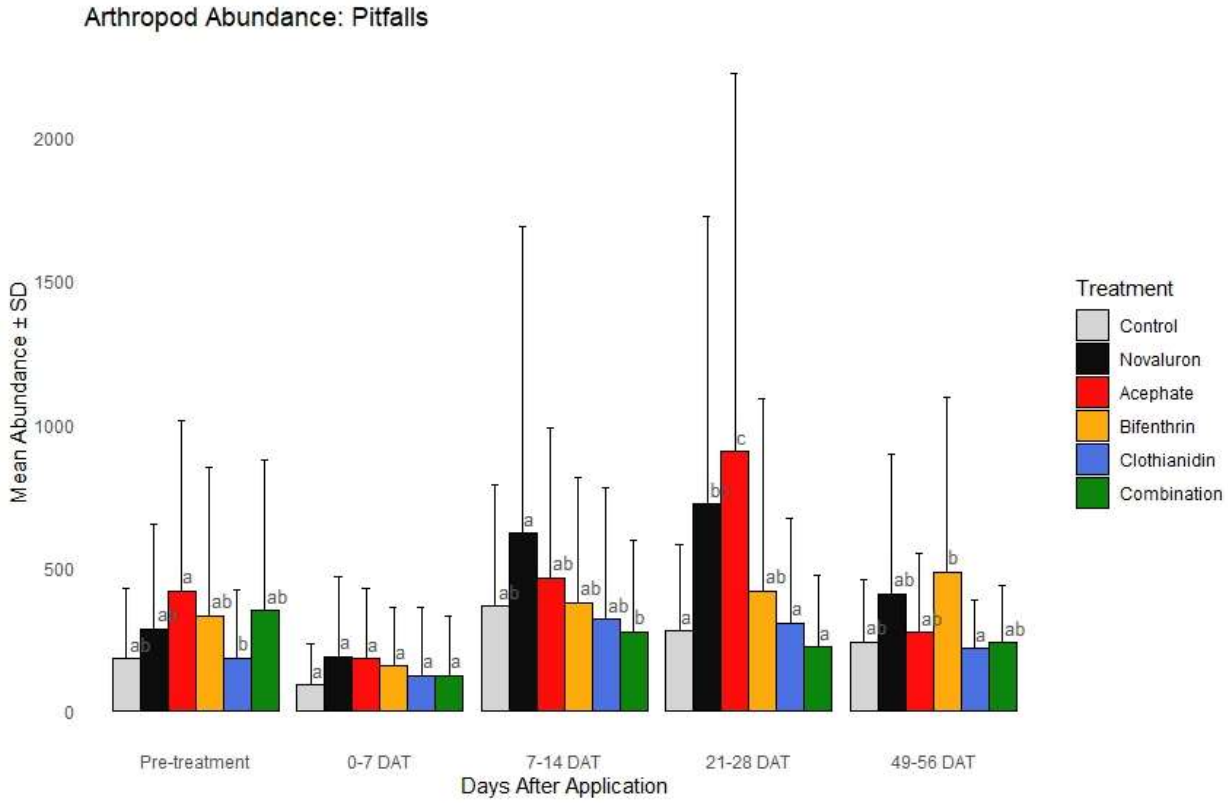
Two golf courses, Mountain View Country Club (40.7837,-77.7586) and Toftrees Golf Resort (40.8225,-77.9018) in State College, Pennsylvania were selected to test the impact of IGRs and conventional insecticides on beneficial arthropod abundance. Both golf courses were chosen because of their limited insecticide applications. Fairways each site consisted of primarily of *Poa annua* and *Agrostis stolonifera*. Treatments were arranged in a complete random block. Plots (3m × 3m with 1m borders) were replicated five times at each site. Treatments included an untreated control, novaluron (Suprado), bifenthrin (Talstar), clothianidin (Arena 50 WDG), bifenthrin + clothianidin combination product (Aloft LC), and acephate (Acephate 97 UP) at highest labeled rates. Treatments were chosen to represent common products used to control ABW and other turfgrass insect pests. All treatments were applied using a backpack CO_2-pressurized sprayer at a rate of 402L/ha (86 gal/ac), followed by 2.5mm (0.1”) of irrigation.

Surface-active arthropod sampling: To assess the effect of insecticide application of ground dwelling arthropods, five permanent pitfall traps (50mL centrifuge tubes, Corning) were installed. Pitfalls (150 in total) were installed in an X pattern in each plot, with one trap being in the exact center of the plot and the other four 1m extending diagonally from the center. Pitfalls were filled with 10mL of 20% propylene glycol solution to prevent degradation of insect specimens. Pitfalls were installed then replaced 7 days prior to application and 7, 14, 28, and 56 days after application to track arthropod abundance over time. Sampling began on May 9th, 2023. In the event of significant rain forecasts, pitfalls were replaced early, and specimens were combined. Once collected, insect specimens were with 70% ethanol and combined at the plot level for identification.

Ground-dwelling arthropod sampling: Within each plot two randomly selected turf cores were extracted from the center of the plot using a tubular turfgrass plugger (6cm diameter × 6.35cm deep) one hour before application and 7, 14, 28 and 56 days following application. Once at the lab turf cores were placed upside down on Berlese funnels and incubated at 40°C in a darkened climate-controlled incubator between 48 and 72 hours, or until soil was desiccated. Specimens that were extracted fell into a small cup of 70% ethanol then combined by plot and stored for later identification.

Results – Surface-active arthropods: Our model indicated that novaluron did not have a significant effect on surface-active arthropod abundance ($z=1.608$, $p=0.107$). However acephate, bifenthrin and the bifenthrin+clothianidin did have a significant effect on arthropod abundance ($z=3.03$, $p=0.002$ | $z=2.17$, $p= 0.03$ | $z=2.38$, $p=0.02$, respectively) (Fig. 1). Pairwise comparisons revealed several statistical differences within sampling periods. At 7-14 DAT surface-active arthropod abundance was significantly higher in novaluron treated areas compared to bifenthrin+clothianidin combination treated areas ($z=2.876$, $p=0.0464$). At 21-28 DAT arthropod abundance in novaluron treated areas was significantly greater than untreated controls, clothianidin, and bifenthrin+clothianidin treated areas ($z=-3.63$, $p=0.004$ | $z=3.201$, $p=0.02$ | $z=4.50$, $p=<0.001$ respectively).

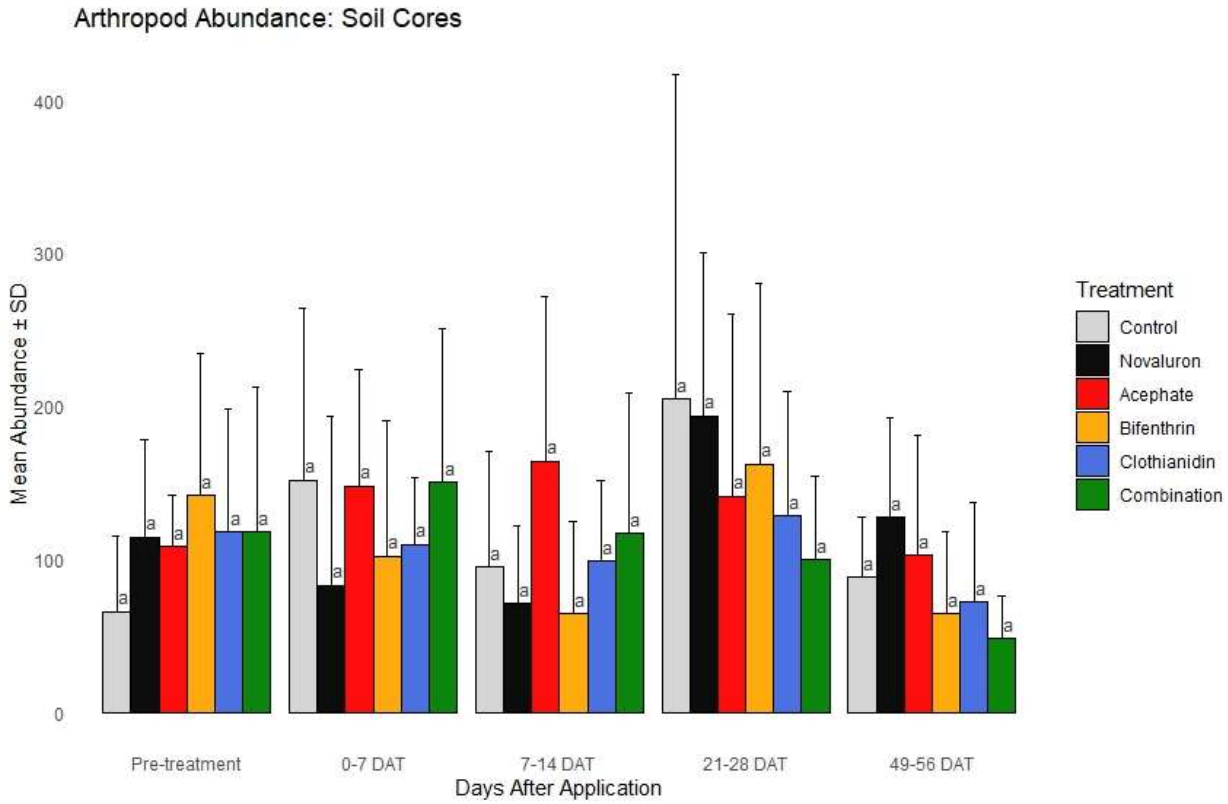
Figure 1: Mean surface-active arthropod captures (± standard deviation) quantified from each insecticide treatment group across each sampling period. Treatments were analyzed using Tukey pairwise comparisons using the *lsmeans* package. Bolded letters represent results from Tukey pairwise comparisons, groups that share the same letter are not statistically significant from each other, α = 0.05.



Results – Ground-dwelling arthropods:

Our model indicated that novaluron did not have a significant effect on ground-dwelling arthropod abundance (z=1.274, p=0.202). In addition, the areas treated with neurotoxic insecticides did not impact ground-dwelling arthropod abundance significantly (Fig. 2). The sampling period between 21-28 DAT did significantly impact arthropod abundance (z=2.600, p=0.009) and there were marginally significant effects at 0-7 DAT (z=1.933, p=0.053). Using pairwise comparisons we observed that there were not significant effects of insecticide treatment on ground-dwelling arthropod abundance across any of the sampling periods (Figure 2).

Figure 2: Mean ground-dwelling arthropod captures (± standard error) quantified from each insecticide treatment group across each sampling period. Treatments were analyzed using Tukey pairwise comparisons using the *lsmeans* package. Bolded letters represent results from Tukey pairwise comparisons, groups that share the same letter are not statistically significant from each other, $\alpha = 0.05$.

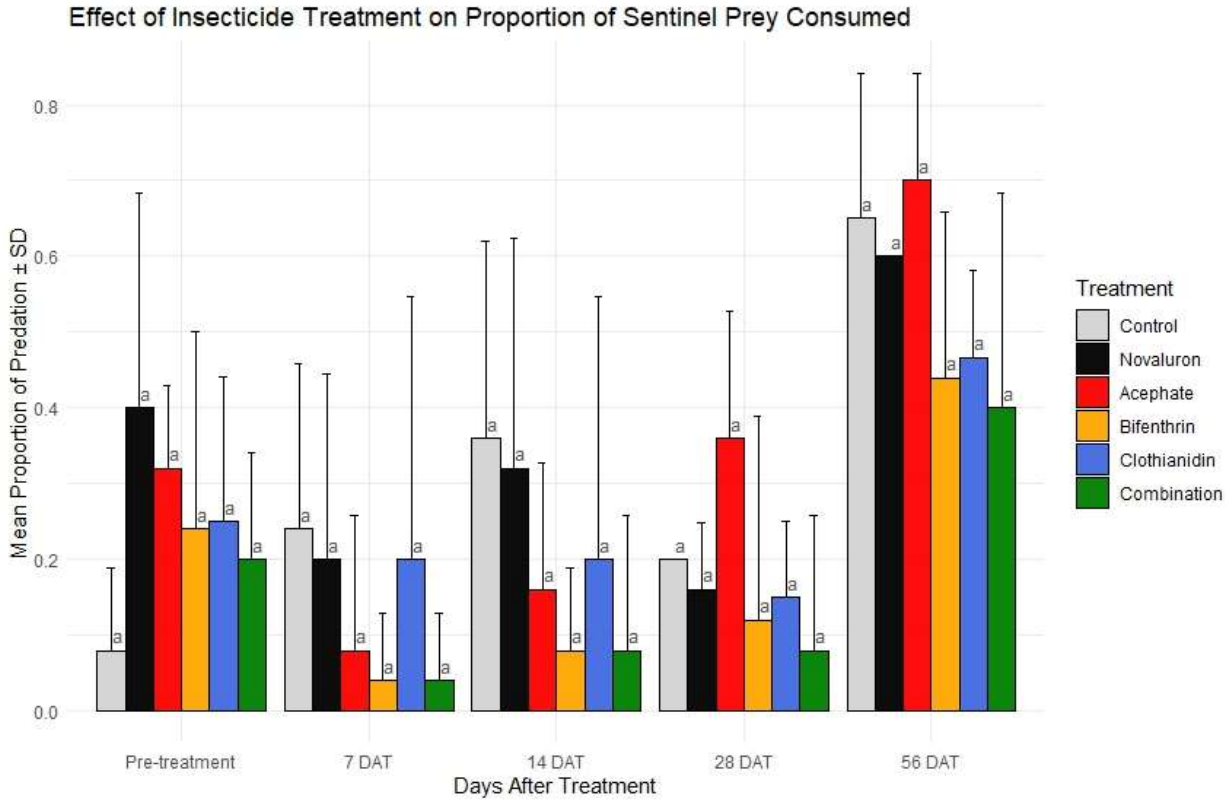


Objective 2: Determine the effect of novaluron applications on consumption of sentinel prey.

A trial designed to assess the effect of novaluron on predator behavior was held at the Joseph E. Valentine Turfgrass Research Center (40.8119,-77.8665). Insecticides had not been applied within the study area for over 3 years. Plots (3m × 3m with 1m borders) were replicated five times. The same treatments and application methods as those in Objective 1 were used. Sentinel prey (3rd-instar *Agrostis ipsilon* larvae) were pinned through the second to last abdominal segment into the turf in an X pattern, with one being in the exact center of the plot and the other four being pinned 1m from the center diagonally. A vertebrate exclusion cage was then placed over the caterpillar to exclude predation by birds and other mammals. After caterpillars had been left in the field for 24 hours their fate was assessed in situ. Fates included: dead, alive, partially predated, or wholly predated. Caterpillars were deployed at 7, 14, 28, and 56 days after application. Sampling began on May 10th, 2023.

Results: Treatment and sampling period both had significant effects on the proportion of sentinel prey consumed (df=5, F=3.40, p=0.007 | df=4, F=12.19, p<0.001 respectively). However, pairwise comparisons between each treatment at each sampling date did not reveal any significant differences when p-values were adjusted for a family of six estimates. Consumption of sentinel prey was highly variable for the duration of the experiment with consumption increasing significantly at 56 DAT (Figure

Figure 3: Mean proportion of predation (± standard deviation) quantified from each insecticide treatment group across each sampling period. Treatments were analyzed using Tukey pairwise comparisons using the *lsmeans* package. Bolded letters represent results from Tukey pairwise comparisons, groups that share the same letter are not statistically significant from each other, α = 0.05.



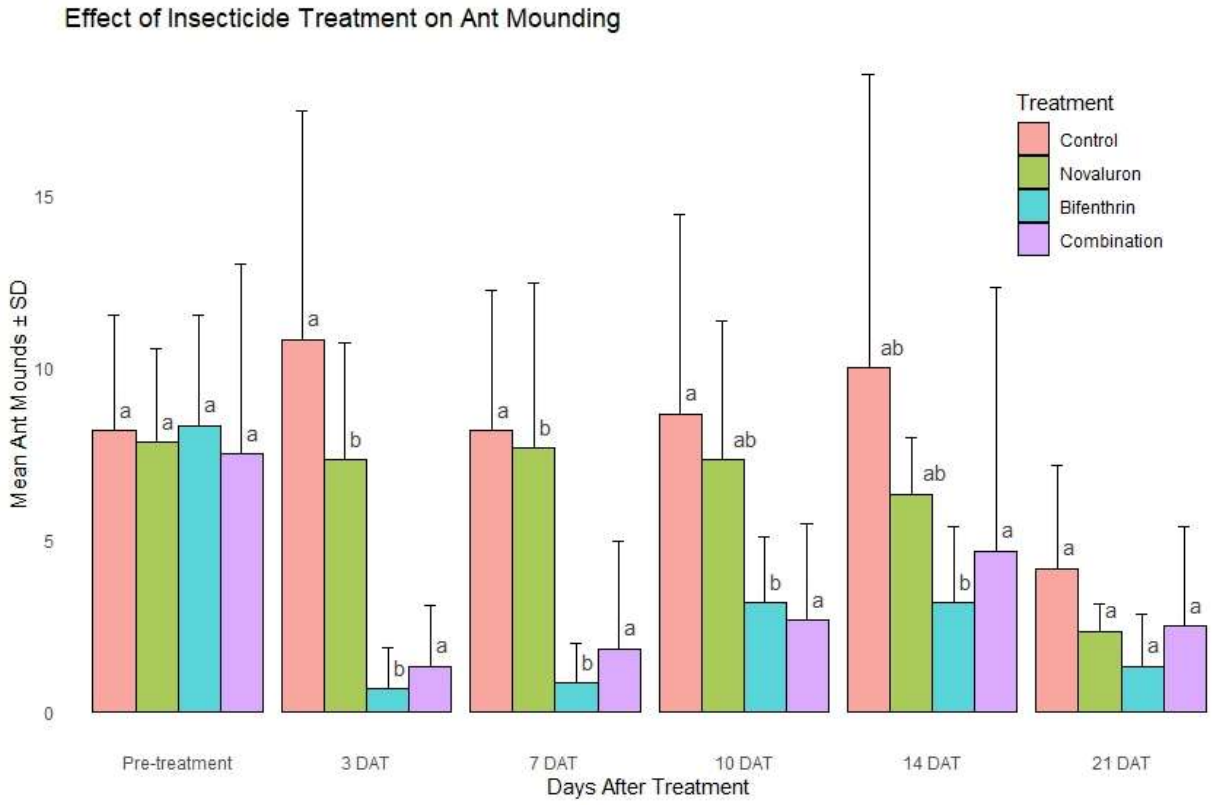
Objective 3: Determine the effect of novaluron applications on predatory insect behavior, using Turfgrass ant (*Lasius* neoniger) as a model organism.

Three research greens were selected; two at the Penn State Landscape Management Research Center (40.8240,-77.8550) and one at the Joseph E. Valentine Turfgrass Research Center (40.8119,-77.8665). Greens were surrounded by rough cut turf and had high amounts of ant mounding activity. Plots (4m × 4m) were laid out with 2.5m of the plot extending into the rough and 1.5m extending into the green where active ant mounding was occurring separated by at least a 1m untreated border region. Plots were arranged based on pre-application mound counts and blocked as necessary to avoid overlapping colonies and to balance mound counts between treatments. Each treatment was replicated six times. Plots received one of four insecticide treatments: untreated controls, novaluron (Suprado), bifenthrin (Talstar), and a combination product of bifenthrin+clothianidin (Aloft LC). Insecticide was applied at field rate for control of turfgrass ant and all treatments were applied using a backpack CO_2-pressurized sprayer at a rate of 402L/ha (86 gal/ac), followed by 2.5mm (0.1") of irrigation. We returned to the study greens 3, 7, 10, 14, and 21 days after treatment (DAT) to count active ant mounds. An ant mound was recorded as active if there were ants moving in and out of it's entrance.

Results: Significant effects of treatment, sampling date, and the interaction of treatment and sampling date (df=3, F=24.54, p<0.001 | df=5, F=8.49, p<0.001 | df= 15, F=1.191, p=0.03 respectively). When conducting pairwise comparisons it was revealed that at 3 and 7 DAT ant mounding was significantly reduced by both bifenthrin and bifenthrin+clothianidin. At 10 and 14 DAT ant mounding in the bifenthrin+clothiainidin treatments was significantly reduced to both untreated controls and novaluron treated areas. By 21 DAT ant mounding was reduced overall and there were no significant differences

between treatments. Novaluron did not significantly impact ant mounding in comparison to controls at any sampling period (Figure 4).

Figure 4: Effect of insecticide treatment on ant mounding behavior (± standard deviation) over several sampling dates. Treatments that share the same letter are not statistically significant from each other.



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Project ID: 2023-16-783

Project Title: Effects of moisture management on annual bluegrass weevil movement, oviposition, larval survival, and turfgrass damage

Project Leader: Benjamin A. McGraw, Ph.D.
Affiliation: Pennsylvania State University

Address: Dept. of Plant Sciences, 243 ASI Building, University Park, PA 16802

Objectives:

1. Determine the effect of irrigation treatment on ABW oviposition behavior in choice assays
2. Assess egg laying, larval survival and fitness, and turfgrass damage in various irrigation replacement treatments
3. Determine the effect of soil moisture on adult ABW movement and spatial distribution of larvae

Start Date: 2023
Project Duration: 2023-2024 (Two years)
Total Funding: $76,762

Summary Points:

1. Egg viability, as determined by 1st instar larval densities, was significantly lower in moisture stressed plants compared to fully saturated cores.
2. Larval survival was significantly impacted by moisture stress as fewer larvae were extracted after 30 days
3. Female host plant preference, as determined by behavioral tracking software, was not influenced by moisture-stress treatment in laboratory assays.

Summary:

Rationale:

Female insect choice in selecting a host can significantly impact their offspring, leading to reduced growth, increased predation risk, and decreased reproductive success (Awmack & Leather, 2002). This selection pressure is especially strong for herbivorous insects with relatively immobile offspring like the annual bluegrass weevil (ABW), a destructive pest on short-mown turfgrasses in eastern North America (Vittum 2020). ABW's oviposition behavior remains poorly understood, though larval damage is often associated with *Poa annua* (McGraw & Koppenhöfer, 2010), a plant that is often associated with moist soils.

The effect of plant moisture on ABW behavior, fecundity, and larval survival remains unexplored. It stands to reason that ABW is attracted to and performs best in well-irrigated turf as the genus Listronotus is a semi-aquatic group of weevils (O'brien 1981), and ABW has only transitioned to feeding on short-mown turfgrass recently in evolutionary terms (Cameron and Johnson 1971).

The proposed study seeks to determine the connection between soil moisture and 1) ABW movement into short-mown areas, 2) oviposition site selection, 3) larval survival and 4) turfgrass damage expression. The study will combine multiple approaches including video and direct observation in laboratory bioassays, fecundity and larval survivorship assessments in greenhouse pot studies, and controlled field studies assessing adult movement, larval spatial distribution, and turfgrass damage relative to water status. Findings from these studies will assist in forecasting ABW feeding damage in spring and developing best management practices for culturally manipulating populations through irrigation during times when moisture is limiting.

Objective 1: Determine the effect of irrigation treatment on ABW oviposition behavior in choice assays

ABW adults were collected from the Lancaster Golf Course (Lancaster, PA) fairways by utilizing a reverse leaf blower vacuum. Adults were sexed in the laboratory and females and males were separated and placed into plastic containers with damp paper towels to provide a moist environment for the adults. Containers were kept at 20°C and 11L:13D photoperiod in an incubator (Percival Scientific, IA). *Poa annua* cores were obtained from a fairway height plot enclosed by pitfall traps at the Landscape Management Research Center (University Park, PA). Soil-turf cores (6 cm diameter × 5 cm depth) were taken to the laboratory and immersed in water for 10 minutes within a plastic box to obtain different moisture treatments (Griffin-Nolan et al. 2023) and to remove insects. Cores were placed on a screen to remove excess water. Once dripping has stopped, cores were weighed, and their initial weight was recorded (fully-saturated weight; 100% treatment). Cores were held in greenhouse conditions to dry within plastic containers (Figure 1) and water was added to replicates weight every 24 hours to obtain four moisture treatments (100%, 85%, 75%, and 65%).

Observational arenas were created to observe female movement and behavior towards moisture treatments (Figure 2). Four soil cores were arranged equidistant from one another within a plastic container. A single female was placed into the center of the arena then observed by camera directly above the arena for 10 mins. Each observational run involved using a new female and turf grass cores. Additionally, the arenas were rotated to remove any potential directional bias. Behavioral metrics were obtained through EthoVision software and analyzed using analysis of variance.

<u>*Results*</u>: Female *L. maculicollis* did not display any significant difference in host plant choice based on moisture treatment.

Objective 2: Assess egg laying, larval survival and fitness, and turfgrass damage in various irrigation replacement treatments

To determine egg survival and larval survivorship in different soil moistures, three moisture treatments were obtained (100%, 85%, 65%) using the same methodologies as above. Two female and two male ABW were placed into an arena and placed into the greenhouse for 7 days to provide time for mating and oviposition. After the oviposition period, a subset of cores was removed from the incubator, placed on Berlese funnels in a darkened incubator (40^{o} C), and larvae were heat extracted over 72 hrs. The number of 1st instar larvae provided an estimate of egg viability. The remaining cores were held in the growth chamber for of 30 days after which cores were placed on Berlese funnels and larvae were extracted to determine larval survival.

Results:

Egg viability, as determined by the average number of first-instar larvae present in Berlese extractions after 7 days, was significantly greater in saturated (100% moisture) cores than in cores experiencing moisture stress (65% moisture)(df = 4; F=3.32, P = 0.05)(Figure 3). A similar declining trend was observed in larval survival after 30 days. Significantly higher larval survival was observed different between moisture treatments (X^2=7.27, P = 0.03). Significantly more larvae were recovered at higher moisture levels.

Objective 3: Determine the effect of soil moisture on adult ABW movement and spatial distribution of larvae

Due to time constraints, field experiments will be conducted Spring 2024.

Future directions

Future directions will focus on conducting extended behavioral analyses on both female and male ABW to identify effect of time in host choice based on moisture level. Additional experiments on egg and larval survival will be conducted to determine if observed trends persist and will identify whether oviposition rate significantly differs from egg survival. Field experiments in moisture manipulation will enable us to examine whether moisture level affects ABW spatial distribution.

Figures



Figure 1: Modified plastic container with mesh fabric (front), and initial plastic containers with screened plastic covers (back).

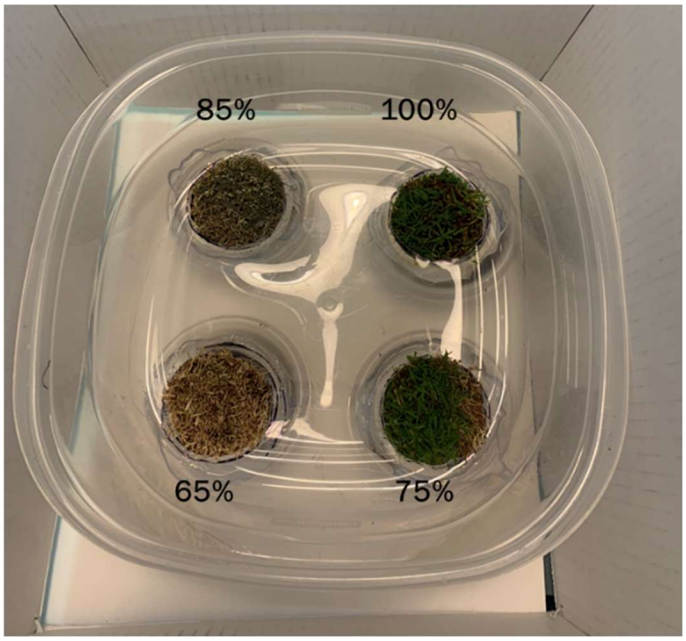


Figure 2: Ethovision arena with different moisture zones.

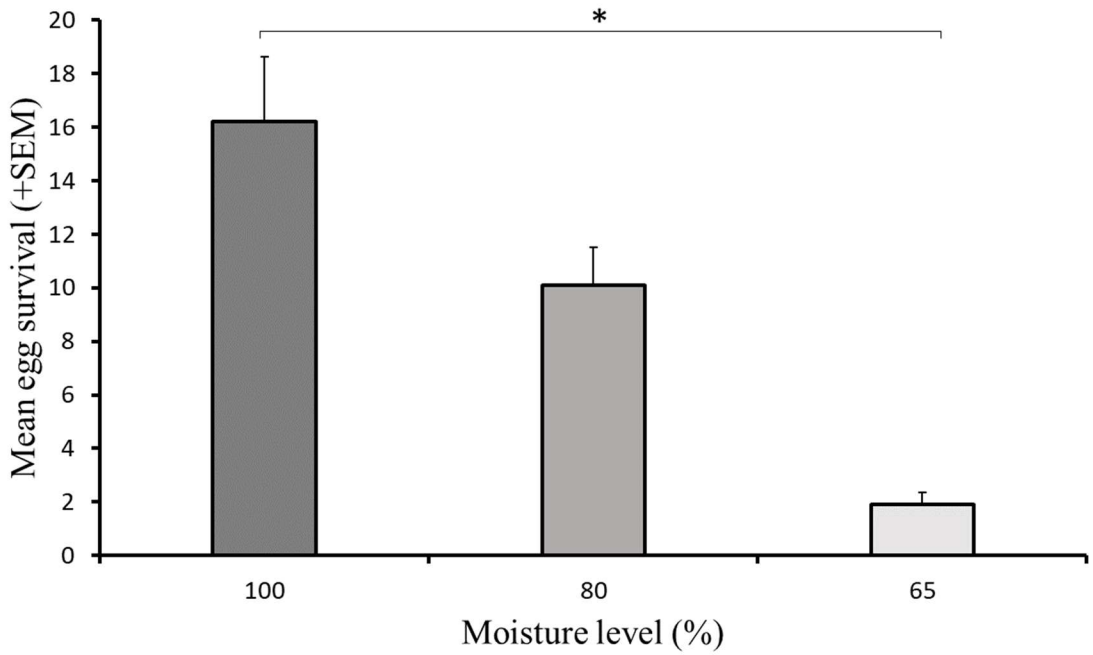


Figure 3: Mean egg survival (+SEM) in *Poa annua* soil cores within moisture levels, 100%, 80%, and 65%, respectively.

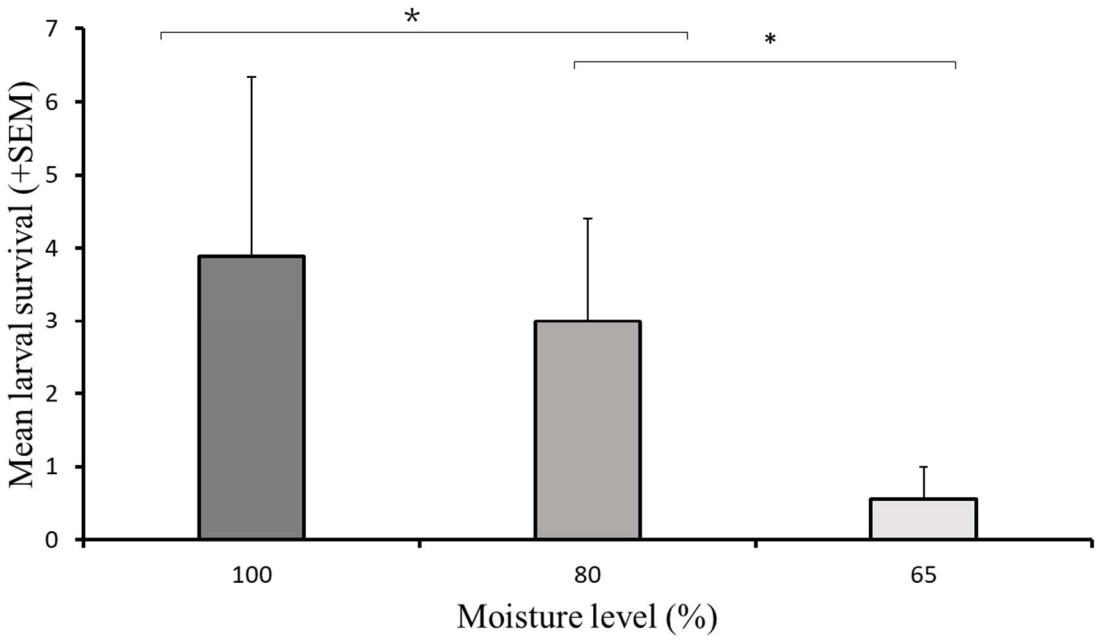


Figure 4: Mean larval survival (+SEM) in *Poa annua* cores within moisture levels, 100%, 80%, and 65%, respectively.

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USGA-ID: 2023-14-781

Title: Silicon to induce resistance to annual bluegrass weevil in annual bluegrass and creeping bentgrass.

Project leaders: Albrecht M. Koppenhöfer, Ana Luiza Sousa, Tarikul Islam, Matthew S. Brown

Affiliation: Department of Entomology, Rutgers University, New Brunswick, NJ

Objectives: To reinforce annual bluegrass and creeping bentgrass resistance to the annual bluegrass weevil using silicon fertilization

Start date: 2023-1-1

Project duration: 2 years

Total funding: $53,270

The annual bluegrass weevil (ABW, *Listronotus maculicollis*) is the most destructive and difficult-to-manage insect pest of short-mown golf course turf in the eastern United States and southeastern Canada with severe insecticide resistance issues. There is a need for alternative control methods to promote effective and sustainable ABW management. In this context, strengthening the resistance of plants using external agents such as silicon (Si) fertilization has the potential to be adopted in IPM programs for ABW control. Si fertilization, either in the soil or as a topical application, can induce/increase plant resistance to herbivorous insects. Si is readily absorbed as silicic acid by plant roots and transported via the xylem to stems and leaves. Si deposition in leaf tissues as amorphous silica gel or phytoliths ($SiO_2.H_2O$) can form a mechanical barrier to herbivory, which can impair the ability of insects to chew and digest leaf tissues. Insect mandibles may be eroded due to the abrasiveness of silicified tissues, resulting in impaired feeding ability and retarded growth due to starvation.

Grasses are hyper-accumulators of Si, and Si fertilization has been shown to enhance the resistance of several poaceous crops including rice, wheat, and sugarcane to insect herbivores. Si accumulation has various functions in this family, such as mechanical stability, disease, insect and herbivore resistance, drought tolerance, facilitation of light interception and alleviation of problems caused by deficiency or excess of nutrients. Both warm and cool season turfgrasses can accumulate substantial amounts of Si in tissues and Si fertilization can improve turfgrass wear tolerance, heat stress tolerance, disease resistance, photosynthetic capacity, growth and establishment, root propagation, and overall turf quality.

A previous study with creeping bentgrass and black cutworms as test subjects demonstrated minor negative effects of Si on larval growth. Furthermore, the density of the intercostal Si deposition in leaf sheaths in pasture grasses was found to be inversely related to the oviposition preference of the Argentine stem weevil, *Listronotus bonariensis*. Given that stem and leaf silicification has been viewed as both a constitutive and quantitatively inducible anti-herbivore defense of grasses, we hypothesize that Si fertilization induces turfgrass resistance to ABW oviposition and larval development.

In our greenhouse experiments, creeping bentgrass (CBG) (cv. 007) and wildtype annual bluegrass, *Poa annua*, were grown for at least 2 months in pots before Si fertilization. Si was provided in two forms: Wollastonite (Vansil® W-10; Vanderbilt Mineral LLC) which is calcium silicate powder containing 24% Si and AB Yellow (ReXil Agro BV), a liquid fertilizer based on stabilized, bioavailable silicic acid (2.5%) and a mixture of micronutrients. Treatments in all greenhouse experiments consisted of 1) Control (lime); 2) Wollastonite at label rate (1,221 kg/ha) once at 7 days before adult release; 3) Wollastonite at 1.5x label rate once at 7 days before adult release; 4) Silicic acid foliar spray at 0.8 L/ha at 7 days before adult release followed by 1 L/ha a week later.

To test the effect of Si on ABW oviposition, cores of greenhouse-grown grass were fitted into 120-ml plastic vials. Seven days after the Si application, 2 male and 2 female ABWs were released per vial for 1 week to allow for egg-laying. Then the plugs were examined under a dissecting microscope to determine the number of eggs deposited. To determine larval development and survival, after Si treatment application ABW adults (4 males and 4 females per container) were caged in containers with the treated grass for 1 week. After another 4 weeks, the number of ABW larvae per cup and their stages were determined by standard saltwater extraction and examination under a dissecting microscope. Greenhouse experiment were conducted twice.



Fig. 1. Greenhouse experiment pots with grass.

A field experiment was performed at Rutgers Horticultural Farm No. 2 with a mix of *P. annua* and CBG maintained as a fairway and naturally infested with ABW. Plots consisted of 30.5 x 30.5 cm turf areas separated from each other by 30.5 cm. Into each plot, 4 cores (10.8 cm diam. x 2.5 cm depth) of *P. annua* or CBG 'Luminary' were implanted in early April. Plots were treated with lime (control), and 1x (1,221 kg/ha), 2x, and 4x of the wollastonite label rate. For evaluation, one core per replicate was extracted in mid-May, late May, and mid-June. ABW stages were saltwater-extracted from the cores and counted, and developmental stages were determined under a dissecting microscope. Grasses were further allowed to grow for another 10 days to get soil-free grass clippings for Si analysis.

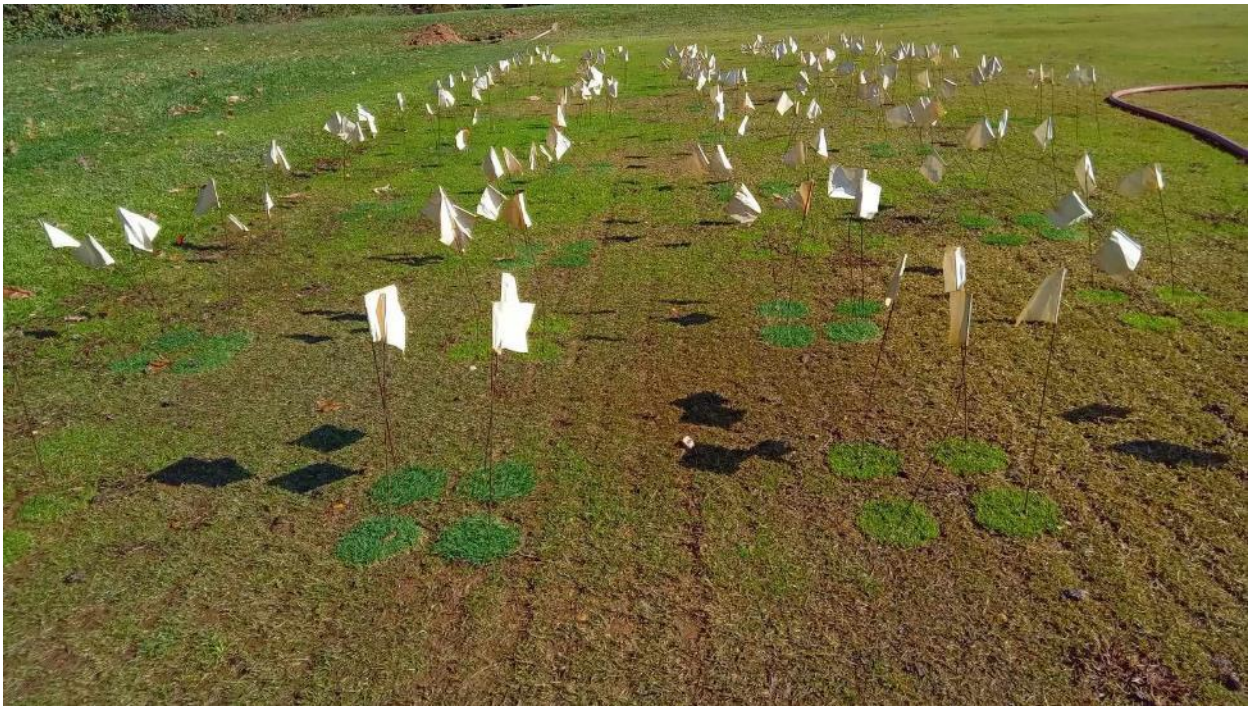


Fig. 2. Implanted turf cores in the field.

Results to date

In the greenhouse experiments, Si fertilization had a stronger suppressive effect on ABW biology in CBG than in *P. annua*. In *P. annua*, Si fertilization reduced egg-laying by 12-37% (Fig. 3a) and the number of larvae developing by 0-35% (Fig. 3b); developmental speed was unaffected. In CBG, Si fertilization reduced egg-laying by 22-37% and larval numbers by 68-85% and slowed larval development (Figs. 3a and 3b).

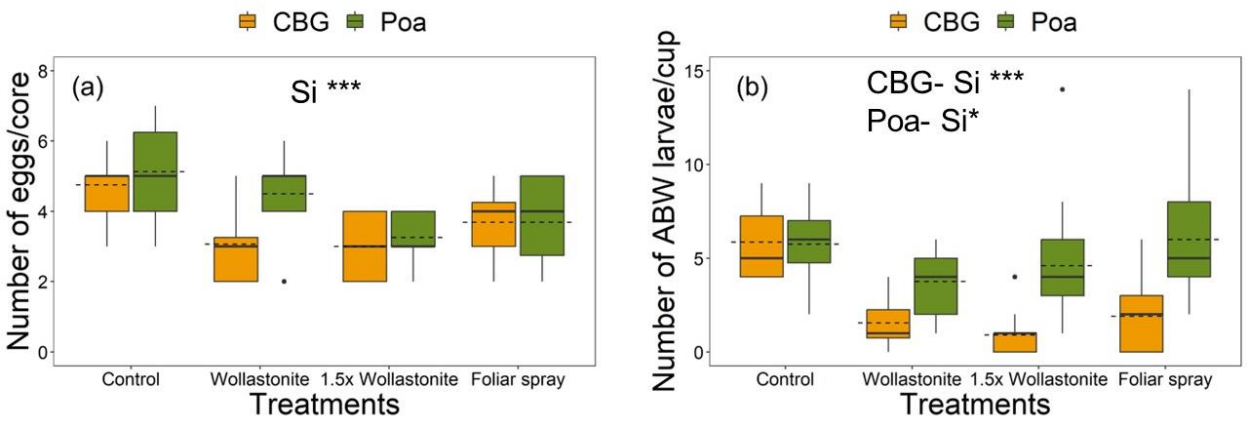


Fig. 3. Effects of different Si treatments on (a) ABW oviposition and (b) larval density under greenhouse conditions. Dashed lines represent mean values. Treatment means were compared using one-way ANOVAs. Asterisks indicate the level of statistical significance (*$p < 0.05$, ***$p < 0.001$) at 95% confidence intervals. Creeping bentgrass (CBG), annual bluegrass (Poa).

In the field, Si supplementation increased Si concentrations in the grass clippings regardless of grass species. The mean Si concentration rose from 0.37-0.47% in control plots to 1.54–2.48% in wollastonite-treated plots (Fig. 4a). ABW populations was significantly higher in *P. annua* than in CBG plots but were no significantly affected by Si fertilization (Fig. 4b).

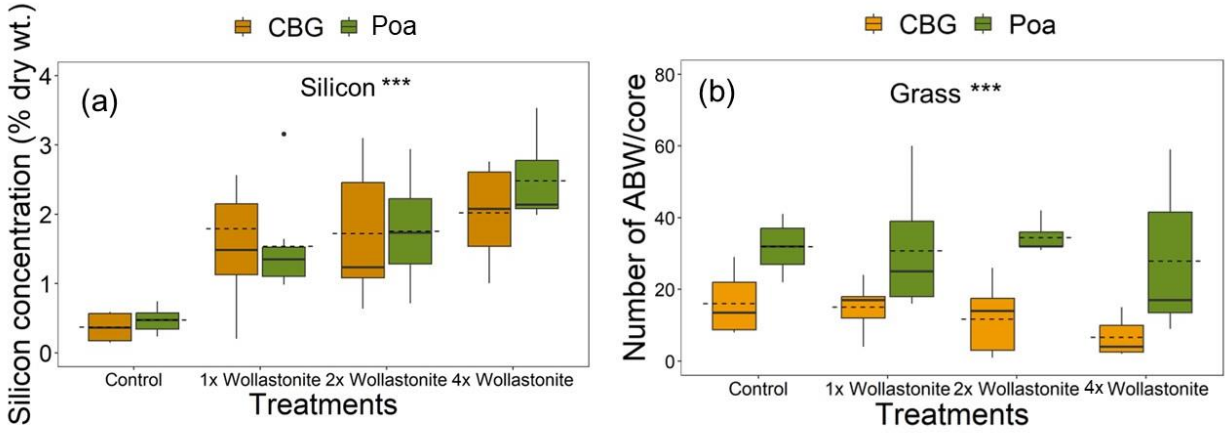


Fig. 4. (a) Total Si concentrations (% dry weight) in grass clippings and (b) effects of different Si treatments on ABW population density under field conditions. Dashed lines represent mean values. Treatment means were compared using two-way ANOVAs. Asterisks indicate the level of statistical significance (***$p < 0.001$) at 95% confidence intervals. Creeping bentgrass (CBG), annual bluegrass (Poa).

We are planning to conduct another greenhouse experiment using the same wollastonite rates used as in the first field experiment. Based on the results, we will select wollastonite rates for

the second field experiment to be conducted in spring 2024. Turf cores for the second field experiment were implanted in October 2023 to allow grasses to be better established than in the first experiment. Our observations so far suggest that Si fertilization negatively affects ABW performance and biology more strongly on CBG compared to *P. annua*. Hence, Si fertilization it enhances CBG resistance to ABW and it could assist CBG to outgrow *P. annua* in mixed CBG-*P. annua* fairways.

- Silicon supplementation reduced ABW egg laying, larval survival, and population density on annual bluegrass and creeping bentgrass.
- Silicon suppressed ABW performance more strongly on creeping bentgrass.
- Silicon may support bentgrass to outgrow annual bluegrass in mixed stands of turfgrasses.

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

Project Duration: 3 years (2022-2025)

Total Funding: $109,806

Summary Points:

- We have successfully synthesized and cloned the putative *Lepidopteran* killing gene *cry1F* into a shuttle vector plasmid for downstream bacterial transformations.

- We have successfully development a robust bioassay testing killing efficacy of bacterial strains against Fall Armyworm (FAW).

- After screening over 30 bacterial strains from an Auburn plant growth promoting rhizobacterial (PGPR) database we have detected natural killers of FAW.

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.

PGPRs represent a sustainable and eco-friendly approach to enhance plant growth, improve turfgrass health and yields, while potentially mitigating the adverse impacts of conventional synthetic insecticides. Understanding the intricate mechanisms of PGPR-plant interactions is crucial for harnessing their full potential in sustainable turfgrass production. PGPRs can enhance nutrient availability through the solubilization of phosphates and the production of plant growth hormones. Additionally, PGPR can fix atmospheric nitrogen, making it accessible to plants in a usable form. Furthermore, they stimulate the plant's defense responses by inducing systemic resistance against pathogens, known as induced systemic resistance (ISR).(Bano & Muqarab, 2017; Kloepper et al., 2004; Pineda et al., 2010). As research progresses, the integration of PGPR into turfgrass management systems holds the promise of a more resilient and sustainable future.

PGPRs live in the rhizosphere. The PGPRs of turfgrass are *Bacillus* bacteria. *Bacillus subtilis* is also a model bacterium. Our research implements two redundant strategies to limit damage by FAW in turfgrass systems. The first approach is to engineer turfgrass PGPRs to kill FAW and the second approach is to screen PGPR libraries for natural killers of FAW. Thus, PGPRs serve as both a backbone for genetic engineering and a natural source of potential insecticidal activity. Some PGPRs 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.

One notable potent killer of FAW is *Bacillus thuringiensis* (BT), a pathogenic microbe of caterpillars. BT expresses crystalized toxin proteins, also known as Cry proteins, on plasmids. When tested against four *Lepidopteran* species, toxins Cry1F and Cry1D showed the highest toxicity in FAW, but Cry1F had an overall higher toxin activity in all four species when compared to Cry1D (Bohorova et al., 1997). Expressing the *cry1F* gene in a turfgrass PGPR that can transiently colonize both turfgrass foliage as well as FAW’s midgut could also be a possible solution. Engineering an insecticidal bacterium specific to FAW opens the door to engineering bacteria to target other major pests in different systems.

Methodology:

Bioassays: Our research goal is to both find natural strains and engineer GMO strains of rhizobacteria that kill Fall Armyworm. Thus, it was necessary to develop a bioassay with controls capable of measuring FAW death. For the initial screen, PGPR strains are grown in Tryptic Soy Broth (TSB) at 37ºC, for 72 hours to allow for growth and potential production of any secondary metabolites. 1oz plastic cups are filled halfway with artificial FAW diet and left to solidify. Once solidified, the entire surface of the diet is inoculated with 50µl of the desired strain. A single 1st instar larva is placed on top of the diet and covered with a lid. The cups are then put in rearing trays and placed in a

growth chamber set at 27ºC with a 14 hour day :10 hour night photo light period. FAW are monitored daily until death or pupation.

Genetic Engineering of Potential Killing Plasmids: To build a transferable FAW killing plasmid construct, we first ordered a synthesized *cry1F* gene from genscript. Next, using restriction enzymes we cut out, purified, and ligated this gene into our shuttle vector (pMag). From there, we transformed this shuttle vector into *E. coli* for long-term storage. Mini-preps were performed to extract plasmids for cloning. Cloning success was confirmed by DNA sequencing via whole plasmid sequencing at Plasmidsaurus.

Results to Date:

Developing Bioassay Controls: Because this research is ongoing an important step is developing robust controls for FAW bioassays. The desired controls include negative (non-killing controls) and positive (killing controls). As a first positive control we experimented with a commercial BT strain which significantly reduced the life span of FAW when compared to a TSB negative control (**Figure 1**).

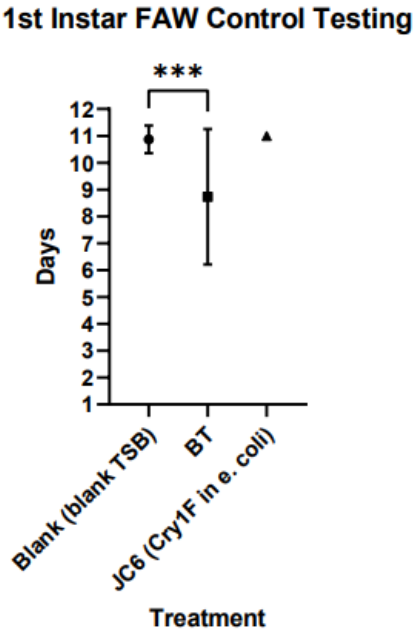


Figure 1. Graph showing mortality results of control treatments. Blank (Blank TSB) serves as negative control. BT serves as positive control and shows statistically significant killing of FAW. The JC6 treatment is *E. coli* transformed with a construct containing the *cry1F* gene.

After identifying a robust positive control we began intensive bioassay screening of PGPRs from the Auburn PGPR databases for natural insecticidal activity against FAW. To date, we have screened over 30 PGPRs and our rate of screening is increasing. **Figure 2** shows first round data from some of these screened strains. We caution that the data is preliminary and needs more replication – although we are excited to report some interesting results.

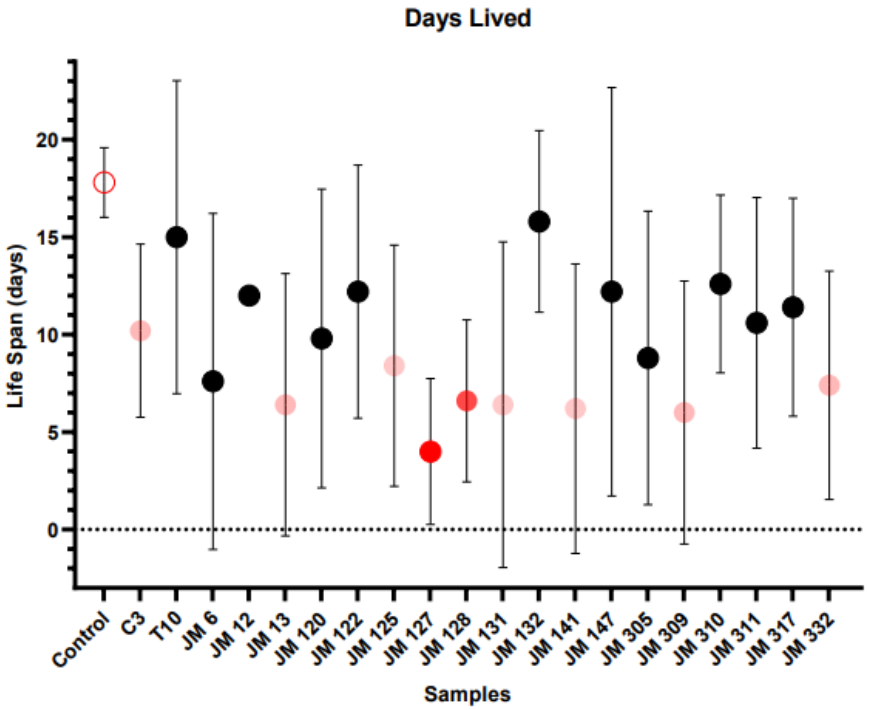


Figure 2. Mortality of FAW. The red circle outline is negative TSB control without killing. All other circles received diverse PGPR strain treatments. Black circles show strains with minimal to no effect on killing and/or too great of variation to be consistent. Light red circles indicate potential killers with higher standard deviations. Deep red circles indicate potential killing strains with statistically significant results when compared against the TSB negative control. Dots are means from 3 replicates with 5 larvae each.

In summary, we have successfully developed a positive and negative control (Figure 1) and developed a bioassay for quick screening of PGPR strains for killing activity. These results have led to the potential discovery of 2 FAW killing strains (JM127, JM128). Though we caution that these results need to be replicated. Importantly, our technique is improving, and we expect to screen 50-100 more quickly in early 2024.

Developing a Transferable *cry1F* Expressing, FAW Killing Plasmid: In an alternative redundant approach to manage FAW in turfgrass, we sought to engineer a plasmid carrying a transgene capable of killing FAW. Using restriction enzyme cloning, we have successfully integrated the *cry1F* gene into our shuttle vector **(Figure 3).**

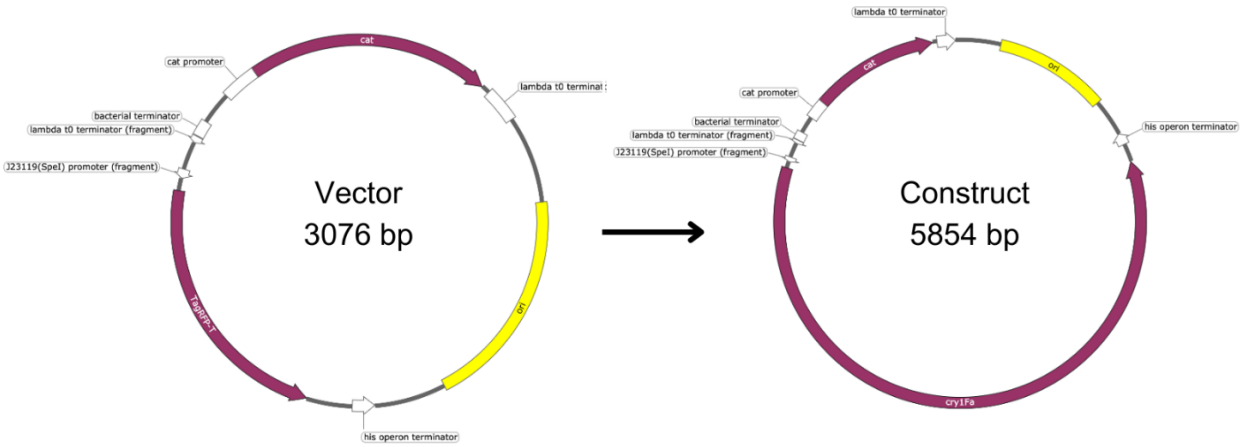


Figure 3. Plasmid map of shuttle vector before and after replacement the of florescent marker gene (*tagRFP-T*) with a Cry gene (*cry1Fa*).

The goal of constructing this plasmid is to transform it into turfgrass colonizing rhizobacterial strains from Blend 20 (AP7, AP18, AP282) which we prior discussed in our previous report from 2022. To date, these strains have yet to be successfully transformed with our new construct. We are working toward developing more efficient transformation protocols involving triparental and birental conjugation methods.

Future Expectations of the Project:

In the future we will continue to develop and explore more efficient methodologies of transformation for PGPR strains as well as continue screening of rhizobacteria for FAW killing activity. We expect to end the final year of the project with a sprayable and/or injectable formulation that can be applied in turfgrass systems. We are close to achieving that goal.

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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.
- Root-knot nematode populations repeatedly exposed to fluopyram exhibit reduced sensitivity to the nematicide, indicating that resistance is the likely cause of observed Indemnify failure.
- Sting nematode resistance experiment will be completed in February 2024.

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.

Root-knot nematode 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.

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.

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.

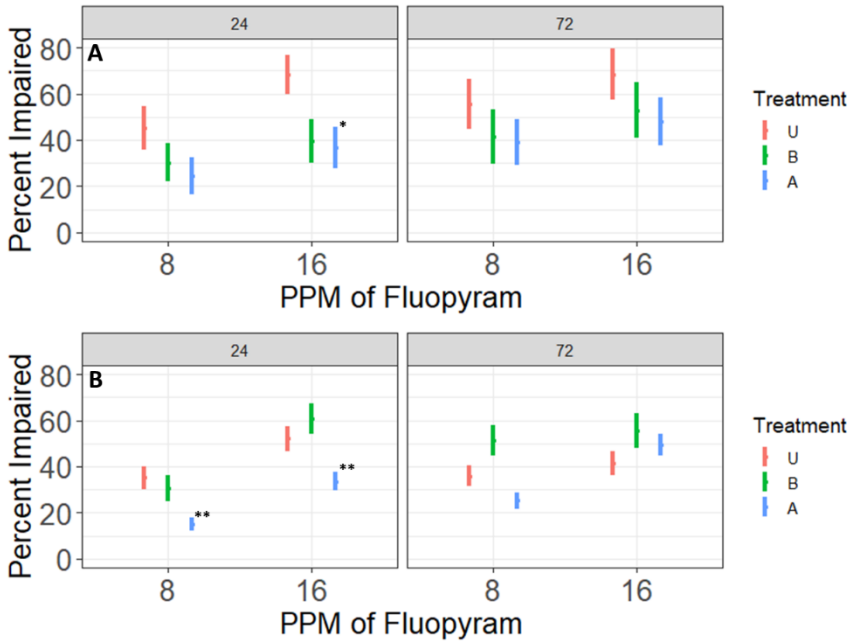
Progress to-date – Completed.

Sting nematode resistance:

Sting nematodes are harder to collect for in-vitro studies than are root-knot nematodes. For in-vitro studies healthy nematodes are needed. Normally sting nematodes are extracted from soil using centrifugal flotation methods, but after being centrifuged the nematodes do not behave normally. To collect healthy sting nematodes from soil a modified Barmann method is used, but recovery of sting nematodes from field soil using this method is low, and not enough nematodes were collected using this method to conduct in-vitro experiments. Fortunately, sting nematodes grown on bermudagrass in pure sand in greenhouse culture do extract easily by this method.

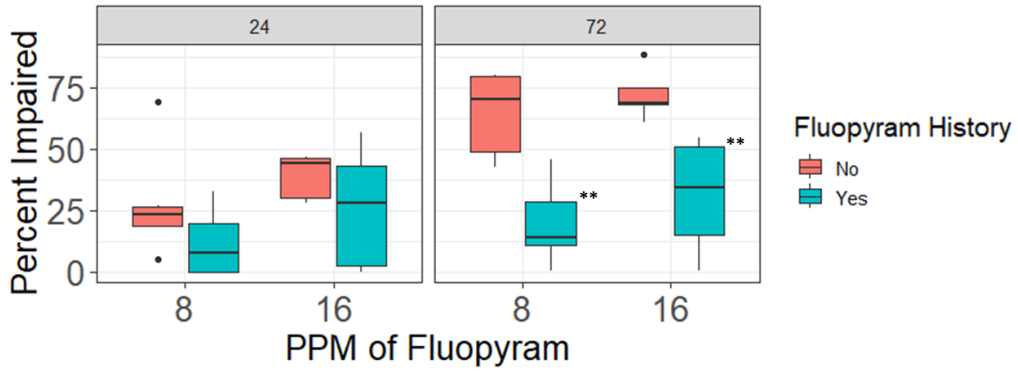
Sting nematodes were collected from four golf course locations with long-term Indemnify use that were suspected of having resistance to fluopyram and four locations that had never used fluopyram products. These sting nematodes were extracted from the field soil using a Baermann method and inoculated onto 'Latitude 36' bermudagrass growing in pure sand in a greenhouse on the UF campus. These nematodes were maintained for a year to build up large enough numbers to conduct resistance experiments. Sting nematodes from the Indemnify-use locations were treated with Indemnify 4 times per year to maintain fluopyram exposure and the nematodes from no-Indemnify use locations remained unexposed. In January 2024 the sting nematodes from the greenhouse cultures will be collected and exposed to fluopyram in in-vitro tests as described for root-knot nematode. As soon as this is completed we will sent USGA a final project report in February.

Figure 1. Results from in vitro testing of root-knot nematodes collected from small plots response to fluopyram after 24 and 72 hours of exposure to 8 or 16 PPM. Panel A is from the first trial and Panel B is from the repetition. Treatment refers to the fluopyram history of the plots: Treatment A had a four year history and B had a one year history of four applications of 500 g fluopyram/ha per year, U had no previous fluopyram history.



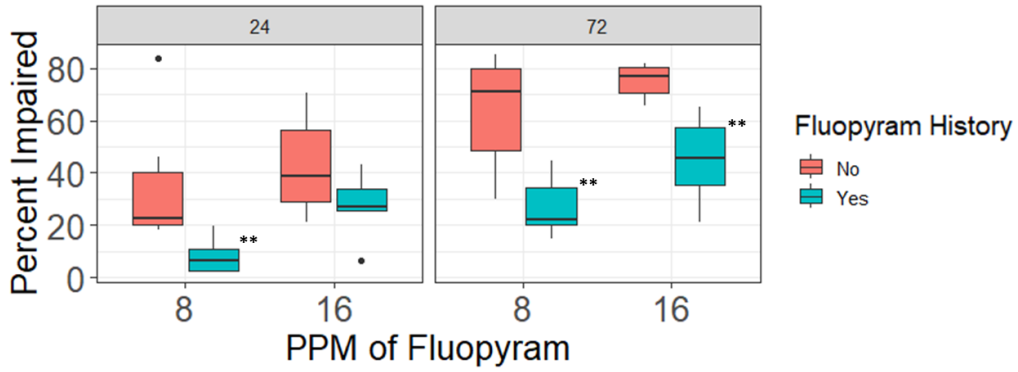
*,**Different from treatment U at the specified fluopyram rate $P \leq 0.10$, $P \leq 0.05$.

Figure 2. Results from first in vitro testing of root-knot nematodes collected from golf courses response to fluopyram after 24 and 72 hours of exposure to 8 or 16 PPM. "Yes" indicates prior history of fluopyram use and "no" indicates no history of fluopyram use.



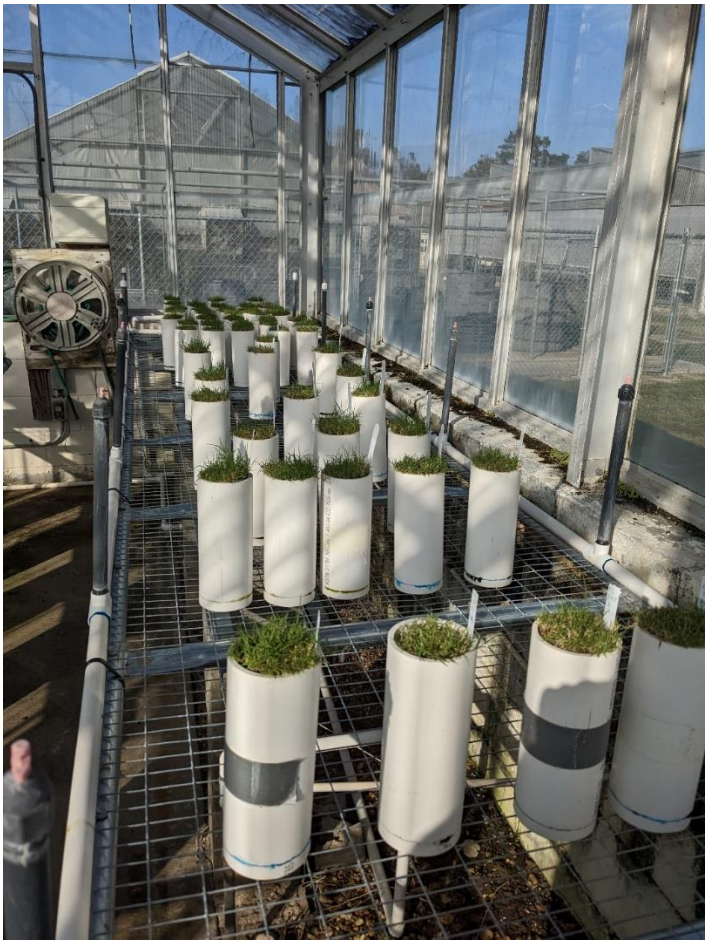
*,**Different from treatment U at the specified fluopyram rate $P \leq 0.10$, $P \leq 0.05$, respectively.

Figure 3. Results from second in vitro testing of root-knot nematodes collected from golf courses response to fluopyram after 24 and 72 hours of exposure to 8 or 16 PPM. "Yes" indicates prior history of fluopyram use and "no" indicates no history of fluopyram use.



*,**Different from treatment U at the specified fluopyram rate $P \leq 0.10$, $P \leq 0.05$, respectively.

Figure 4. Sting nematode populations building up in the greenhouse for sting nematode resistance experiment to be conducted in January, 2024.



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 test cultures for ability to inhibit sting nematodes (completed, but extended)
- To identify lead strains that can be evaluated and tested for commercial application (ongoing, seven lead strains identified so far)

Start Date: 2022
Project Duration: Two years
Total Funding: $116,655.69

Summary Points
- Variability and stability of microbiomes in a nematode-tolerant and nematode-susceptible Bermudagrass cultivar is currently being tested. For this, samples of soil, root- and leaf endosphere have been taken, DNA has been extracted, and the extracts are currently being sequenced. These samples will be compared to samples from 2022 and will provide valuable information that is essential for developing microbial products in turf.
- 30 additional fungal endophytes were isolated from Bermudagrasses and identified by molecular methods. They were grouped into 7 distinct taxa and were tested for bioactivity against sting nematodes. Four strains showed significant nematicidal activities (Table 1 and Figure 1). Around 30 additional cultures were isolated, but have not yet been identified by molecular methods.
- Based on previous data, we modified our bioassay and extended it to 7 days.
- Extracts from the fungal strain *Colletotrichum* sp. showed strong nematicidal activity. To identify its bioactive compounds, we re-grew the strain and extracted organic compounds. This extract is currently being analyzed by HPLC-MS at the lab of a collaborator. Identifying these compounds provides insides into the mode of action of these bioactive fungi and can provide novel nematicides.

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 bacteria 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 expended our culture collection of 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 154 fungal endophytes that could be grouped into 20 different sequence types (‘species’) based on ITS sequencing. One representative of each species was used in a bioassay to identify bioactivity. A total of seven strains (including four isolated in 2023) showed significantly higher mortality for sting nematodes than the controls (Table 1). The newly isolated active strains belong to three different classes of fungi.

Table 1. Taxonomic ID of additional fungal endophytes isolated in 2023 displaying strong nematicidal activities against sting nematodes. All experiments were done in triplicates.

Phylum	Class	Top BLAST hit	% nt identity	Accession	Host plants	Isolation Source	% Mortality at Day 7	
							control	treatment
Ascomycota	Dothideomycetes	*Curvularia aeria*	99.91	OQ657945.1	TifTuf, Latitude 36	Leaves, Roots	36.1 ± 3.5	57.8 ± 11.1
		uncultured Pleosporales fungus	98.91	HG995490.1	TifTuf, Latitude 36	Leaves, Roots	36.1 ± 3.5	71.7 ± 7.6
	Sordariomycetes	*Colletotrichum* sp.	100	ON154502.1	Latitude 36	Leaves	36.1 ± 3.5	71.3 ± 6.6
Basidiomycota	Agaricomycetes	*Ceratobasidium* sp.	99.88	ON231659.1	TifTuf	Roots	25.5 ± 3.0	43.3 ± 9.3

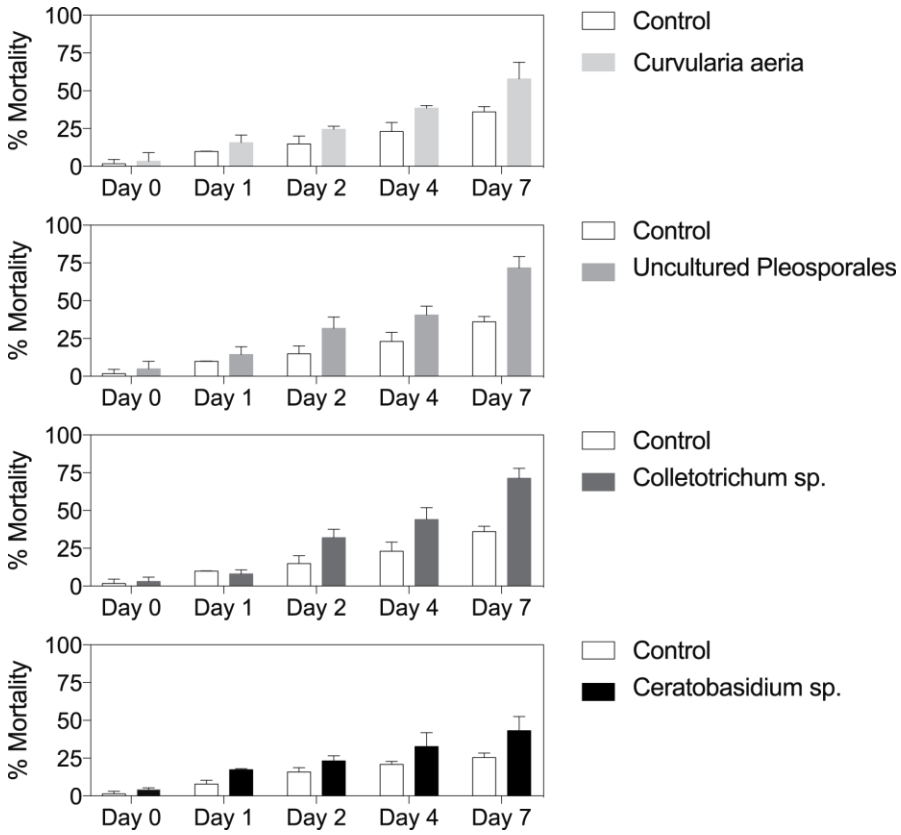


Figure 1. Mortality rate of sting nematodes against additional fungal endophytes over 7 day incubation.

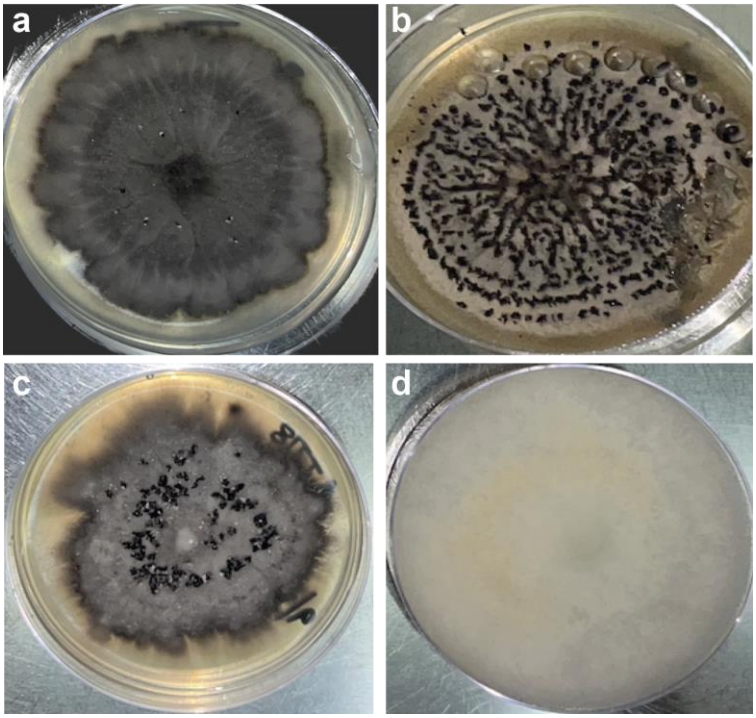


Figure 2. Cultures of endophytic fungi displaying nematicidal activities against sting nematodes.
a. *Curvularia aeria*, **b.** uncultured Pleosporales, **c.** *Colletotrichum* sp. **d.** *Ceratobasidium* sp.

Future expectations of the project

In the second year of the project, we replicated 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) 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. These samples are currently being sequenced. We will process the data and final results will be available for the final report.

We identified seven bioactive endophytic fungi during this project so far. While we will still test more strains until the end of the project, we will try to identify the chemical structure of the bioactive molecules produced by these fungi. We are currently testing this with one strain but hope to extend this to all bioactive strains.

The bioassay has several limitations. Fungal and bacterial contaminations from the sting nematode preparations are able to grow on spent media from our test strains. We will retest all seven bioactive strains in a modified bioassay that will use organic extracts rather than spent media. We expect that this will result in better data.

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USGA ID#: 2023-07-774

Title: Understanding Pacific Northwest Turfgrass Plant-Parasitic Nematode Communities to Improve Management Efficiency

Project Leaders: Hannah Rivedal[1], Emily Braithwaite[2], Alec Kowalewski[2], Inga Zasada[3]
Affiliation: [1]USDA-ARS Forage Seed and Cereal Research Unit; [2]Oregon State University Department of Horticulture; [3]USDA-ARS Horticultural Crops Disease and Pest Management Unit

Objectives:

1) Determine plant-parasitic nematode (PPN) genus and species presence across 60 golf courses in California, Oregon, and Washington (20 per state) using traditional morphological examination, and PPN community metabarcoding.
2) Evaluate survey timing (winter, spring, summer, and fall) and collection method (soil, thatch, tillers) for the most accurate detection of PPN associated with annual bluegrass, creeping bentgrass, and fine fescue putting greens.
3) Develop action thresholds and integrated pest management programs for PPN of putting greens in the PNW region.

Start Date: January 2023
Project Duration: 3 years
Total Funding: $65,326

Summary Points:

- PPN were recovered from all surveyed golf courses, at every time point, regardless of reported health of the putting green surface by superintendent.
- Fifteen unique genera were identified across California, Oregon, and Washington, with multiple species reported within each genus.
- Trends were observed between reported health of putting green (moderately healthy to indicator greens routinely stressed by biotic or abiotic factors), and the overall abundance of PPN counts per 100cc of soil, with the highest counts seen on routinely stressed greens.
- *Meloidogyne* spp. (root knot nematode) were present in 77 to 100% of courses and had variable populations, with counts per green ranging from 4 to over 3,900 juvenile nematodes per 100 cc of soil.

Summary

***Rationale*:**

Plant-parasitic nematodes (PPN) are important pests affecting turfgrass health, playability, and maintenance. In recent years, nematodes have increasingly become a topic of

interest for golf course managers in the Pacific Northwest (PNW) region. The distribution and species of PPN in California, Oregon, and Washington has not been extensively surveyed, though recent reports indicate that PPN are causing significant damage to cool-season putting greens managed in all three states. PPN are difficult to quickly diagnose and require expensive nematicides and management strategies to treat. In the PNW, nematode thresholds have not been established for many turfgrass PPN, leading to mis-timed control applications, and a less sustainable management strategy for turfgrass PPN. Metabarcoding, a technique that uses next generation sequencing (NGS) of a genetic region of identification for a group of organisms, is a powerful tool to help understand the community of pathogens associated with a cropping system. In conjunction with morphological identification methods, metabarcoding of the PPN community could reveal new species of concern and indicate PPN species of importance that are more difficult to recover using traditional methods. This project aims to establish baseline information on PPN in the PNW to improve detection strategies, associate specific PPN with identifiable symptoms, and implement management/action thresholds for PNW golf course superintendents.

***Methodology*:**

Objective 1 and 2: To determine PPN population dynamics throughout the PNW, we established a golf green survey in May 2023. The survey began with 10 courses from California, 20 courses from Oregon, and 20 courses from Washington. Courses will be sampled through fall of 2025. Additional participants from California will be added in the spring of 2024. In each year, sampling is conducted at four time points, February, May, August, and November, to capture temporal changes in PPN populations. At each course, 6 greens of varying turf health are surveyed. Golf greens are walked in a zig-zag fashion, collecting 20 samples per green. Soil and plant samples are bulked by green before nematode extraction. Turfgrass quality is rated visually using a 1 to 9 scale (9 = best, 6 = minimally acceptable, and 1 = dead or dormant). Ratings integrate turfgrass density and uniformity, disease severity, and overall visual appeal. In conjunction with visual ratings, we are collecting information on overall management practices, prior spray records, and other environmental factors that could influence the health of the putting green, to develop more detailed action threshold levels. Nematodes are extracted from samples using a Baermann funnel and mist chamber extractor for 3 days. Extracted nematodes are morphologically identified with a microscope.

Individual nematodes of PPN genera and species of interest are being selected for nucleic acid extraction and molecular identification of barcoding regions. These individuals will be used as positive controls for the development of metabarcoding community analysis procedures.

Objective 3: Data collection is still under way for initial action threshold determinations and will be available in year 2 or 3 of study. In the interim, we have prepared educational PowerPoints that have been presented to golf course superintendent groups, like the Northwest Turfgrass Association, to provide general information on PPN and explain the scope of our research.

Results to date: Across California, Oregon, and Washington, 15 genera of PPN were identified. On average, Oregon had courses with the most detected genera, with more than 5 genera detected at 10 of 20 courses. California had the lowest diversity of genera on average and had only 1 of 10 courses with more than 5 detected genera. *Meloidogyne* (root knot nematode)

species are widely prevalent and have a broad range in population size, from 4 to over 3,900 juveniles per 100cc of soil. *Meloidogyne* species were present in 77% of California courses, 95% of Washington courses, and 100% of Oregon courses. Molecular confirmation of *Meloidogyne* species is underway. We have identified multiple species within most genera, including a new *Heterodera* (cyst nematode) species and others that will be confirmed with molecular identification tools. We did note that reported healthy greens had lower PPN abundance counts compared to reported unhealthy greens which had higher overall PPN abundance counts. Based on initial findings, PPN are much more widespread than previously thought and the distribution of genera throughout the region is high.

Future Expectations: In upcoming years of the study, we will continue PPN survey and identification efforts. In addition, we will begin molecular identification and barcoding efforts of PPN communities. We will start relating damage symptoms to PPN community members as we gather more sampling information. We will regularly report to golf course superintendents through extension presentations and publications.

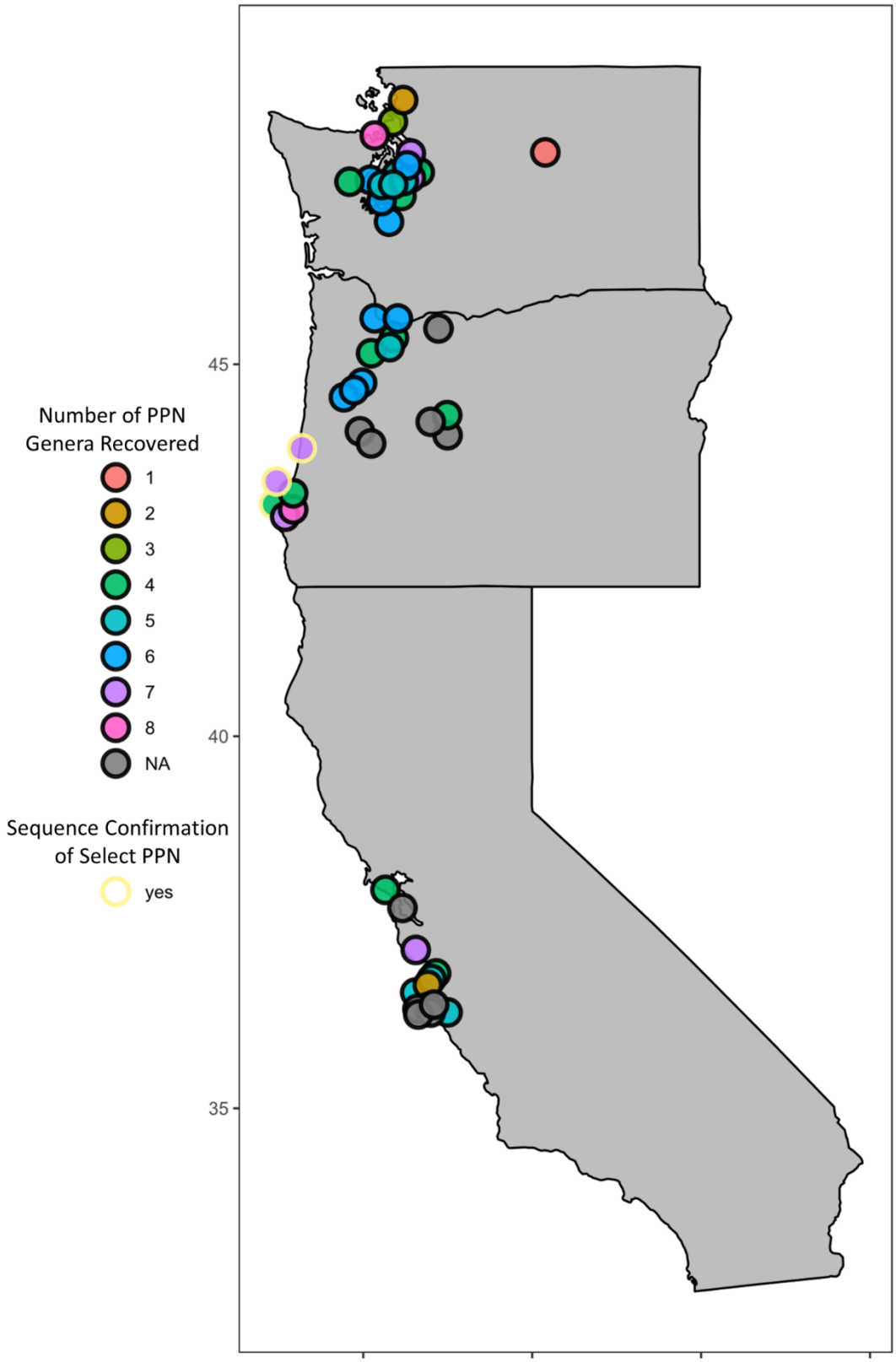


Figure 1. Locations of 2023 plant-parasitic nematode survey as of August 2023. Dot color indicates the number of recovered genera as determined by visual examination. The dots with a yellow outline have had individual nematodes evaluated with molecular identification methods.

Table 1. Percentage of total sampled courses per state with positive recovery of plant-parasitic nematodes.

Plant-parasitic nematode genera	**Survey state**		
	California*	Oregon†	Washington‡
Spiral nematode *Helicotylenchus sp.*	100%	94%	95%
Root-knot nematode *Meloidogyne sp.*	77%	100%	95%
Cyst nematode§ *Heterodera sp.* *Punctodera sp.*	8%	29%	15%
Ring nematode *Criconema sp.*	15%	76%	50%
Stunt nematode *Tylenchorhynchus sp.*	15%	59%	70%
Root-lesion nematode *Pratylenchus sp.*	8%	47%	45%
Lance nematode§ *Hoplolaimus sp.* *Rotylenchus sp.*	0%	12%	15%
Sheathoid nematode *Hemicycliophora sp.*	0%	15%	12%
Stubby root nematode§ *Trichodorus sp.* *Paratrichodorus sp.*	46%	88%	80%
Pin nematode *Paratylenchus sp.*	15%	12%	35%
Dagger nematode *Xiphinema sp.*	0%	6%	0%
Pacific shoot-gall nematode *Anguina pacificae*	38%	0%	0%

*A total of 10 courses from California, sampled in August 2023

† A total of 20 courses from Oregon, sampled in August 2023

‡ A total of 20 courses from Washington, sampled in August 2023

§ Identification to the specific genus for these nematodes requires molecular sequencing for confirmation

USGA ID#: 2022-06-749

Project Title: Investigating white grub resistance in turf-type tall fescue

Principal Investigator(s): Stacy A. Bonos, Albrecht Koppenhöfer, Phillip L. Vines, and Jennifer L. Halterman

Affiliation: Rutgers, The State University of New Jersey

Research Objectives

(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 preferences on roots of different TF populations
(iii) Compare TF populations for their ability to compensate for white grub root feeding
(iv) Assess TF populations for their ability to compensate for white grub root feeding
(v) Assess TF populations for fungal endophytes to determine if there is an association with white grub feeding

Start Date: August 24, 2022
Project Duration: 3 years
Total Funding: $56,746.00

Summary Points:

- Grubs were collected, counted, identified and weighed from turf plots and lightbox images were taken for visual assessment within 12 hours of collection.
- Turf plots were visually rated on a 1-9 scale, with 9 being the best, monthly from May to October.
- Individual genotypes for growth chamber experiments and endophyte testing have been cloned out, grown in the greenhouse and grub feeding experiments in the growth chamber are underway. Grubs were collected for growth chamber experiments.
- Endophyte results were tested for each cultivar, with only 2 cultivars testing endophyte positive based on microscopy methods.

Summary Text:

The complex of annual white grubs is the most destructive and widespread insect pest affecting turfgrass in the northeastern U.S. Golf course superintendents primarily rely on chemical measures for control which is costly and not an environmentally sustainable approach. Increased tolerance to white grub feeding has been observed in tall fescue populations at the Rutgers Plant Science Research Farm in Freehold, NJ. Through a series of field and growth chamber trials, we hope to gain a better understanding of the contributing factors to white grub resistance in tall fescue as a better alternative to chemical treatments.

Previously, we established a field trial at the Rutgers Adelphia Plant Science Research and Extension Farm in Freehold, NJ including 15 TF cultivars and 1 perennial ryegrass (PR) cultivar and turf quality ratings were taken throughout the growing season. For the growth chamber studies, 64 rhizotron boxes were made and pilot studies were performed to identify the best protocol moving forward.

Plots were evaluated for visual health quality ratings throughout the growing season and grub count data was collected from 8 cup cutter plugs per turf plot (Figure 1). Furthermore, lightbox photos were taken

prior to grub collection for quantitative visual health (Figure 2). Bullseye LTZ, Firenza II, and Line Drive II (PR) had the best turf quality while Crossfire 3, GO-FNKY, and Meridian had the poorest turf quality (Table 1). A Kruskal-Wallis test was performed to identify the mean total of grubs per cultivar. Firecracker SLS, Renegade DT, and Bandit had the most grubs while GO-FNKY, Meridian, and Bullseye LTZ had the least (Table 2. A total 2,237 grubs were collected for growth chamber experiments this Winter (Table 3;). Individual genotypes from the field were collected and are actively growing for cloning to perform growth chamber experiments (Figure 3). Lastly, 20 individual genotypes were tested for endophyte using a protocol by Bacon and White (1994). Endophyte results were unexpectedly poor with the maximum amount of endophyte being 20% in a single cultivar (Table 4).

The next step in this process is to evaluate each of the 20 individual genotypes per cultivar in growth chamber experiments with grubs in rhizotrons (Figures 4 and 5), drought studies, and simulated grub feeding in conetainers. These studies will involve collecting data on root and shoot biomass and root and shoot length measurements. The next field season will consist of continued visual health quality ratings and grub collecting at different variable timepoints to evaluate egg laying and larval instar development.

Tables and Figures:

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			9=best
			Turf Quality			Establishment
			2022-2023	2022	2023	13-Sep
Rank	Cult		Avg	Avg	Avg	2022
5	6	Bullseye LTZ	7.0	7.3	6.7	5.4
12	11	Firenza II	6.7	6.5	7.0	4.4
1	16	Line Drive II	6.7	8.3	5.0	8.2
2	14	PPG TF-303	6.6	6.7	6.6	6.4
4	8	Thor	6.6	6.9	6.2	5.8
9	3	Technique	6.5	6.7	6.3	4.6
8	9	Degas	6.4	7.1	5.8	4.8
3	12	Padre II	6.4	6.5	6.3	6.2
6	2	Annapolis	6.4	6.3	6.4	5.2
11	10	Trinity	6.1	6.5	5.8	4.4
10	7	FireCracker SLS	6.0	6.3	5.8	4.4
13	5	Renegade DT	5.8	6.0	5.5	4.2
7	4	Bandit	5.7	5.9	5.5	5.2
15	13	Crossfire 3	4.2	4.3	4.1	2.0
14	15	GO-FNKY	3.6	3.9	3.4	2.6
16	1	Meridian	3.2	2.7	3.7	1.0
		LSD at 5%	0.8	0.9	0.8	1.1
		CV	10.5	12.1	11.2	19.3

Table 2. Kruskall-Wallis test and one-way analysis of variance performed on grub count data to determine mean total grubs per cultivar.
One-Way Analysis of Variance

Cult		Mean Total
1	Meridian	1.8
2	Annapolis	2.8
3	Technique	2.8
4	Bandit	4.2
5	Renegade DT	5.2
6	Bullseye LTZ	2
7	FireCracker SLS	5.4
8	Thor	3.6
9	Degas	3
10	Trinity	2.2
11	Firenza II	2.4
12	Padre II	2.8
13	Crossfire 3	3.2
14	PPG TF-303	4
15	GO-FNKY	1.6
16	Line Drive II	3.4

Kruskal-Wallis Test

Chi-Square	DF	Pr>ChiSq
18.4938	15	0.2376

Table 3. Total grubs collected for growth chamber experiments.

Grub Species	Count
Northern masked chafer	869
Oriental beetle	792
Japanese beetle	576
Total	2237

Table 4. Endophyte testing results for 15 TF cultivars and 1 PR cultivar. Testing was conducted on 20 genotypes per cultivar.

		% Endophyte
1	Meridian	0%
2	Annapolis	0%
3	Technique	5%
4	Bandit	0%
5	Renegade DT	20%
6	Bullseye LTZ	0%
7	FireCracker SLS	0%
8	Thor	0%
9	Degas	0%
10	Trinity	0%
11	Firenza II	15%
12	Padre II	0%
13	Crossfire 3	0%
14	PPG TF-303	0%
15	GO-FNKY	0%
16	Line Drive II	0%

Figure 1. Grub collecting on September 19, 2023 at Adelphia Research and Extenstion Farm, Freehold NJ. Pictured from left to right: Jennifer Halterman, Olivia Wright, Mason Orrei, Mark Labarge, Eric Macpherson, and Ryan Earp.



Figure 2. Lightbox image examples for quantitative analysis of turf quality of tall fescue plots established in 2022 at the Adelphia Plant Science Research and Extension Farm.

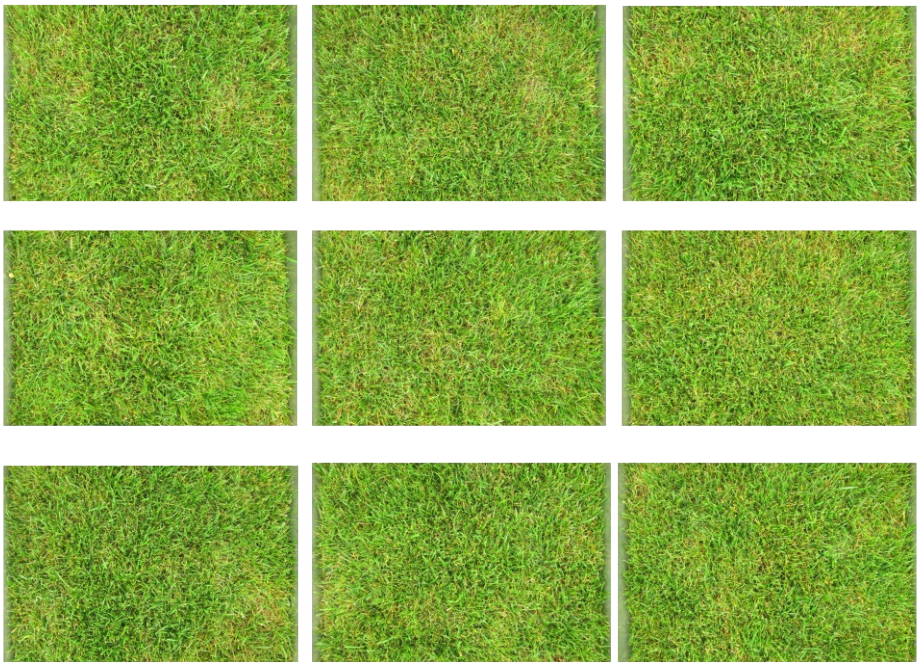


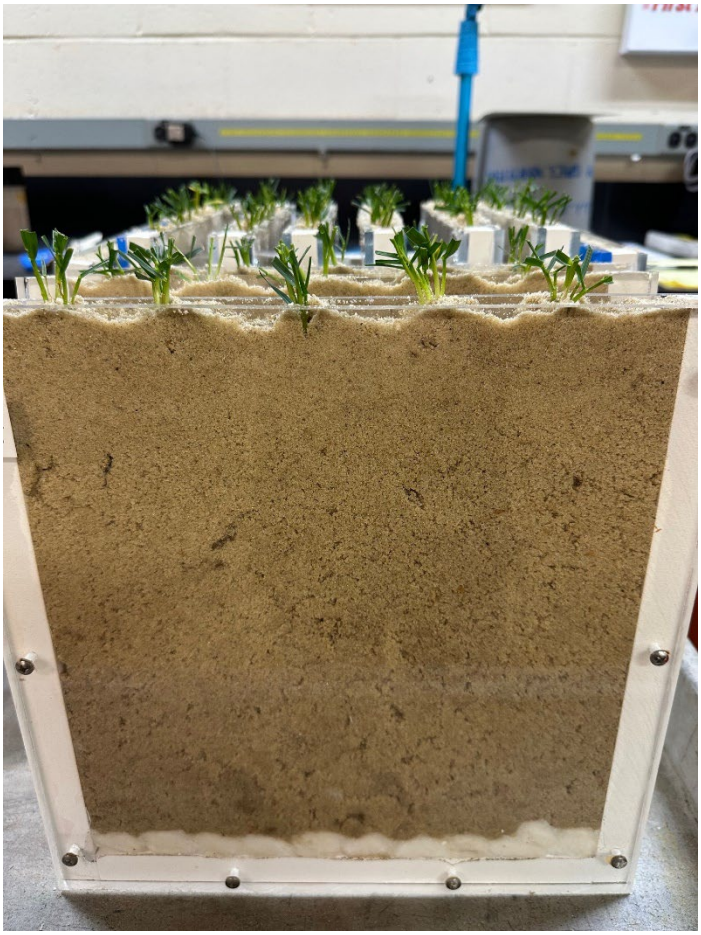
Figure 3. Individual tall fescue genotypes in conetainers for growth chamber experiments and individual genotypes in 48-cell flat bottoms for endophyte testing. A total of 20 individuals were selected per cultivar for endophyte testing and grub feeding studies.



Figure 4. Fully assembled and built rhizotrons for grub feeding experiments.



Figure 5. Plants established in mini-rhizotrons prior to adding grubs.



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# 2022-05-748

Title: Stratification of Herbicide Resistance in Soil: An Ecological Glimpse into Changes in Poa annua Herbicide Resistance by Soil Depth

Project Leaders: Aaron Patton[1], Vera Vuković[1], Jim Brosnan[2], Travis Gannon[3]
Affiliation: [1]Purdue University, [2]University of Tennessee, [3]North Carolina State University

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, but it is present deeper than 50 mm.
- 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 four 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.

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 disaggregated by hand, dried on a greenhouse bench, and stored in airtight bags until germination.

Recent Progress:
The study was initiated in December 2022, using 16 soil cores from the same golf course located in Knoxville TN, and 15 soil cores from each of additional three locations (Bean Station, TN; High Point, NC; and Raleigh, NC). 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 and green cover of each plant was determined with digital image analysis (ImageJ, v. 1.48v, National Institutes of Health, Bethesda, MD 20892).

<u>Study location 1</u>: Knoxville, TN
Across all 16 soil cores and all examined depths, 718 annual bluegrass plants were successfully germinated (Table 2). Green cover did not statistically differ by depth in the soil profile. The percent of glyphosate resistant annual bluegrass varied by soil depth. Overall, the percent of glyphosate resistant plants from 0 to 30 mm ranged from 26 to 45%. However, resistant accessions were found in the deepest examined soil layer (50-100 mm) as well.

The data from this location indicates that removal of upper 15 mm of the soil by fraze mowing or sod removal should decrease annual bluegrass soil seedbank significantly since 86% of the plants germinated from the upper three soil layers (Figure 2). The sampling location at this site was on a slope so it was not practical for the golf course to perform cultivations where the soil was collected. As such, we were surprised to find glyphosate resistant *Poa annua* so deep in the soil profile. An example of glyphosate resistant annual bluegrass plant found in the upper soil increment is shown in Figure 3.

Table 2. The mean green cover and the percent of resistant annual bluegrass germinated from various depths in soil profile in golf course, Knoxville, TN.

Depth	Plants[a]	Green cover	Resistant
mm	no.	cm^2	%
0-5	365	1.8	45 a
5-10	182	1.7	40 ab
10-15	73	1.2	27 bc
15-30	49	1.5	26 c
30-50	40	0.8	35 abc
50-100	9	2.2	44 abc
P-value		0.1920	0.0192

[a]Number of plants obtained from each layer of the soil core.
[b]Means followed by a common letter within column do not differ according to Fisher's protected LSD at α=0.05.

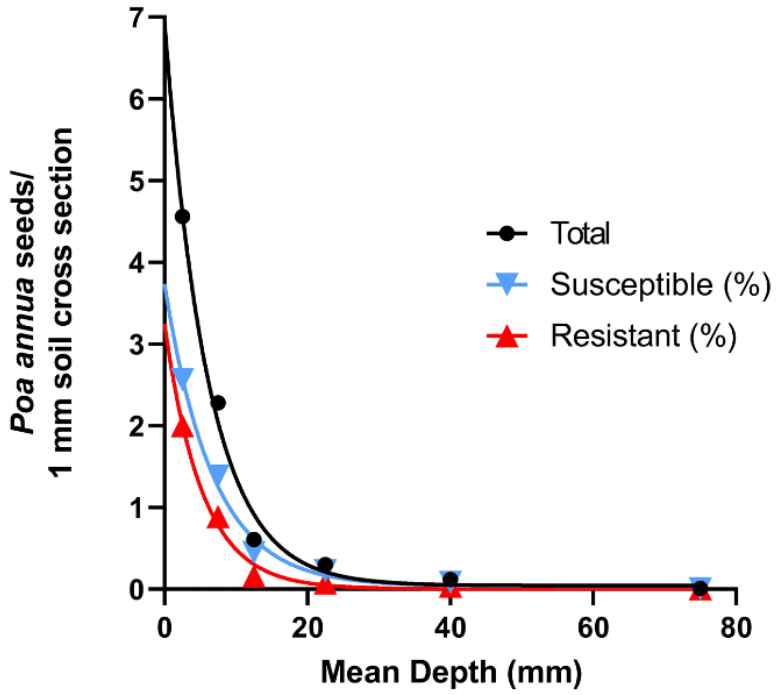


Figure 2. The number of annual bluegrass seeds per 1 mm soil cross section by mean depth in the soil profile based on 16 soil cores obtained from a golf course in Knoxville TN, in 2022.

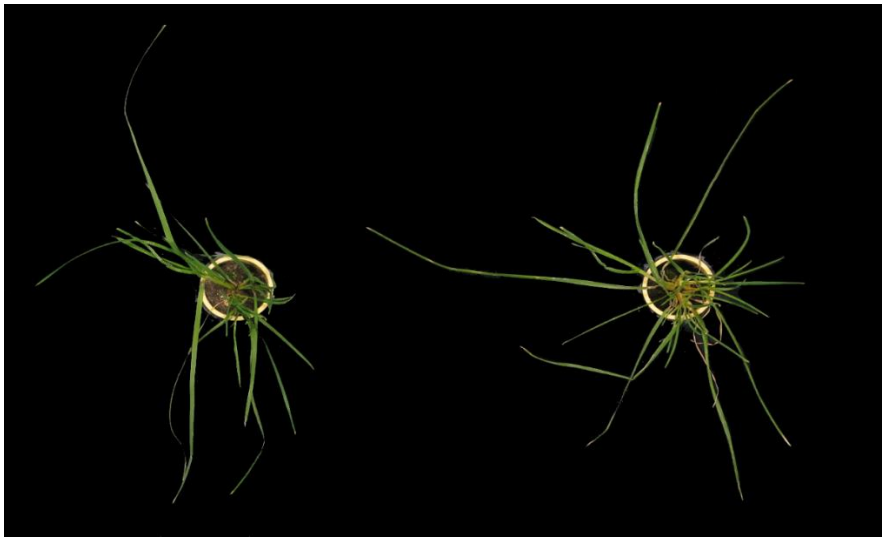


Figure 3**.** Annual bluegrass plant found in 0 to 5 mm soil depth at golf course in Knoxville TN, after sequential application of 840 (left) and 1680 (right) g glyphosate ha^{-1} in two weeks intervals.

<u>Study location 2:</u> Raleigh, NC

Across 14 soil cores obtained in Raleigh, NC, only 80 plants germinated. The highest number (32) of plants at this site germinated from 15-30 mm soil layer. There was no significant difference detected in green cover and percent of the resistant plants by soil depth when analyzed by ANOVA although there is a decrease in resistance by soil depth (Figure 3) when analyzed by regression.

Table 3. The mean green cover and the percent of resistant annual bluegrass germinated from various depths in soil profile in golf course Raleigh, NC.

Depth	Plants[a]	Green cover	Resistant
mm	no.	cm^2	%
0-5	4	2.17	75
5-10	11	2.14	57
10-15	11	1.86	59
15-30	32	1.12	55
30-50	15	0.65	45
50-100	7	0.34	43
P-value		0.2953	0.8107

[a]Number of plants obtained from each layer of the soil core.
[b]Means followed by a common letter within column do not differ according to Fisher's protected LSD at α=0.05.

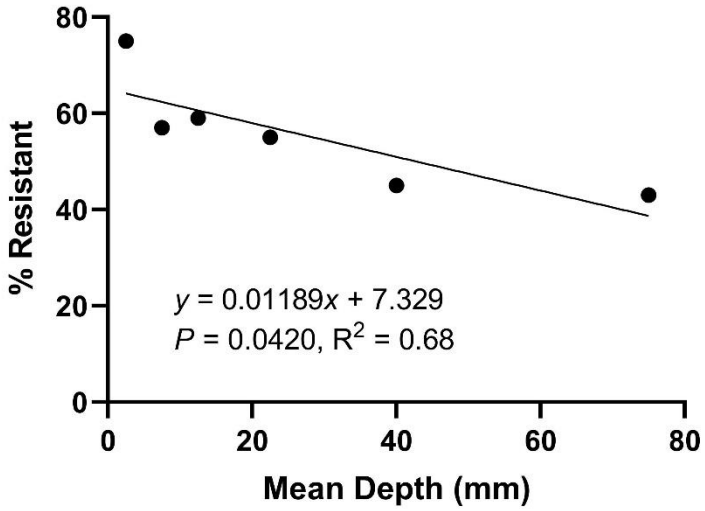


Figure 3. The percentage of annual bluegrass seeds resistant by depth in Raleigh, NC.

<u>Study locations 3 and 4:</u> Bean Station, TN and High Point, NC

We attempted to germinate annual bluegrass seed using soil cores collected at two additional locations (Bean Station, TN and High Point, NC) both in the greenhouse conditions (27/21°C day/night, with 14-hour photoperiod) and at two different regimes in the growth chamber (25/15°C day/night 8 h photoperiod and 19/10°C day; 8 h photoperiod). Further 4 or 6 g of activated charcoal powder (Charcoal Green® Soil D•Tox™, Crawford, NE) was added to the soil layers in order to minimize the possibility of potential herbicide residue affecting the germination of plants. However, no plants germinated from samples obtained at these two locations regardless of environmental settings despite a past history of problems with herbicide resistant annual bluegrass.

Next Steps:

Due to space restrictions in the growth chamber assessment of soil cores was initiated in several cycles. Assessment of the last soil core from Raleigh, NC will be finalized in December 2023. Soil texture analysis and physical properties of the soil will be determined for samples from Raleigh, NC and Knoxville, TN.

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

Final Summary Points:

- 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 at the end of the season in 2021. There was a non-significant tendency for Beudin + Quatro to exhibit better playability in summer 2023.
- Beudin + Quatro produced 25% less clipping biomass than Gladiator + Quatro when plots were mowed once annually in autumn in two of three years.
- Ethephon + trinexapac-ethyl was more effective than other PGRs evaluated in improving playability of fine fescue areas in all three years. Improvements in playability were not consistent throughout the year and were less pronounced in some years.
- Triclopyr slightly reduced density of Beudin + Quatro in 2022, but otherwise there were no density reduction or phytotoxicity from any of the PGR/herbicide treatments evaluated in three years of the experiment. The same herbicide/PGR treatments were applied to the same plots in three consecutive years to evaluate compounding effects.

Summary Text:

2023 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 and 2022 trial and assessment methods can be found in the previous report. Since the results of the 2021 and 2022 trials were not conclusive, a third run of the trial was conducted in 2023 on the same site. 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 23, 2023 at the first emergence of fine fescue seedheads. Data collected are presented in Table 1 with the exception of phytotoxicity ratings which are not presented as none was observed.

Lodging
Plant growth regulator (PGR)-by-cultivar blend interactions were detected only for lodging (Table 1). Regardless of herbicide treatment, Gladiator + Quatro lodged less than Beudin + Quatro. This is different than 2022 where lodging was similar for both cultivars. The PGR-by-cultivar interaction was caused trinexapac-ethyl + ethephon increasing lodging for Beudin + Quatro but there was no effect of PGR for Gladiator + Quatro.

Playability
The plant growth regulators ethephon + trinexapac-ethyl and triclopyr resulted in the best playability in August. PGR did not affect playability on any other date in 2023, although there was a non-significant trend for ethephon + trinexapac-ethyl to improve playability in June and July, but not November. No other treatments improved playability on any other date.

Clipping Yield
The Beudin + Quatro mixture resulted in 25% less clipping yield biomass than the Gladiator + Quatro mixture which is exactly the same reduction measured in 2022. Unlike 2022 when the PGR ethephon + trinexapac-ethyl and triclopyr reduced clipping yield biomass, PGR did not affect biomass in 2023. PGR also did not affect biomass in 2021. We speculate the reason for this difference between years is that N fertilizer was applied to the site in 2022 and may have stimulated vegetative growth that exacerbated PGR effects.

Aesthetic
Aesthetic was assessed in July after seedhead maturity but before lodging occurred. There was a strong non-significant tendency for Gladiator + Quatro to have a better aesthetic than Beudin + Quatro. While playability of Gladiator + Quatro was poorer, the seedheads seedheads were longer and there appeared to be more of them which improved aesthetics. This trend was much less significant and less pronounced in 2022.

Injury and Density
All treatments had similar turfgrass density when it was evaluated in August 2023. No phytotoxicity was observed at any time after PGR application (data not presented).

Future Research:
Fine Fescue Playability Research
The importance of ethephon + trinexapac-ethyl timing and PGR effects in fine fescues growing on low and high fertility soils should be investigated. Non-chemical means such as clipping removal, aggressive raking, or sandcapping should be evaluated.

Table 1. Effects of plant growth regulators and two cultivar blends on fine fescue seedhead production, playability, and biomass production in 2023 in North Brunswick, NJ.

Cultivar	PGR	Playability				Biomass	Aesthetic	Lodging	Density
		June	July	August	November				
		1 to 9[†]				g[¥]	1 to 9[¶]		%
Cultivar blend									
Beudin + Quatro	-	6.4	5.5	5.1	4.1	208 b	5.1	7.4 a	98
Gladiator + Quatro	-	5.2	4.4	4.5	3.7	275 a	6.7	3.7 b	98
	Pr > F	*0.18*	*0.12*	*0.26*	*0.41*	*0.01*	*0.06*	*0.001*	*0.98*
Plant growth regulator (PGR)									
-	ethofumesate	6.0	4.4	4.5 bc[§]	4.4	244	6.8	5.3	100
-	indaziflam	5.3	4.9	4.6 bc	3.6	223	6.1	5.0	97
-	ethephon + trinexapac-ethyl	6.3	5.5	5.2 ab	4.0	255	5.4	5.6	97
-	triclopyr	5.9	5.0	5.5 a	4.0	238	5.4	6.2	98
-	None	5.5	4.8	4.3 c	3.5	250	5.7	5.5	99
	Pr > F	*0.36*	*0.29*	*0.03*	*0.16*	*0.33*	*0.43*	*0.35*	*0.21*
*Cultivar * PGR*									
Beudin + Quatro	ethofumesate	6.3	4.3	4.5	3.5	202	5.9	7.8 ab	100
	indaziflam	6.0	6.0	5.3	3.5	207	4.8	6.3 bc	98
	ethephon + trinexapac-ethyl	6.8	6.5	5.1	4.3	209	4.0	8.3 a	98
	triclopyr	6.6	5.3	6.3	4.5	208	5.5	7.8 ab	98
	None	6.3	5.3	4.5	3.5	217	5.4	6.8 ab	100
Gladiator + Quatro	ethofumesate	5.8	4.5	4.5	3.5	239	7.8	2.8 e	100
	indaziflam	4.5	3.8	4.0	3.8	239	7.4	3.8 de	97
	ethephon + trinexapac-ethyl	5.8	4.5	5.3	3.8	301	6.8	3.0 de	98
	triclopyr	6.3	4.8	4.8	3.5	267	5.4	4.6 cd	99
	None	4.8	4.3	4.1	3.5	282	6.0	4.3 de	99
	Pr > F	*0.33*	*0.08*	*0.17*	*0.15*	*0.37*	*0.36*	*0.04*	*0.90*

[†]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.

¶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 10 July 2023. 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 22 August 2023.

¥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.

USGA ID#: 2023-17-784

Title: Cloaking and Controlling Quackgrass in Golf Course Turf

Project Leaders: Quincy Law[1], Deying Li[1], Esther McGinnis[1], Nathan Haugrud[2], and Joseph Ikley[1]
Affiliation: [1] North Dakota State University and [2] Syngenta

Objectives:

- Evaluate grasses for their ability to visually cloak quackgrass for golf course superintendents and Extension Master Gardeners.
- Identify grass species that compete with quackgrass to minimize its spread.
- Screen herbicides for their efficacy on quackgrass.
- Investigate the interaction between nitrogen fertilization and the chemical control of quackgrass.

Start Date: 2023
Project Duration: 3 years
Total Funding: $19,775

Summary Points:

- This research suffered two major setbacks. First, the field experimental area suffered extensive winterkill damage after the Fall 2022 seeding and plugging. Spring 2023 re-seeding was unsuccessful.
- The second setback involves the departure of the principal investigator, Quincy Law, from NDSU, without sufficient support to continue the project at NDSU.

Summary Text:

Rational

Quackgrass (*Elymus repens* L.) is a perennial, rhizomatous grass species that invades turf, landscape, and cropland areas. Quackgrass has been identified in all 50 US states (Werner and Rioux, 1977), but it is especially problematic in the upper Midwest and Northeast United States. Due to its invasive nature and difficulty to control, the species has been designated as a noxious weed in 25 US states and four Canadian provinces (Klein, 2011). While quackgrass is a sod-forming grass that can successfully colonize established turf, it does not form a dense monoculture and results in playability issues on golf courses. Further, the fast growth, coarse leaf texture, upright leaf architecture, and blue-green color of the species create an aesthetic nuisance for improved turf and can increase mowing requirements and related emissions.

To date, no cultural or biological control practices in turf have been identified, and few to no selective chemical control options exist, especially in mixed swards. Given that turfgrass selection is an important factor for preventing weed infestation in general (Christians et al.,

2016), it should be explored specifically for quackgrass. Further, in the situations in which quackgrass is more of an aesthetic issue than one of playability (e.g., in no-mow areas of golf courses), grasses that cloak (i.e., hide) quackgrass would be of great benefit. Coupling the difficulty to control quackgrass with the consideration of reducing pesticide use provides merit for investigating the concept of cloaking weeds in aesthetic scenarios.



Figures 1 and 2. A near quackgrass monoculture patch on a golf course fairway in Fargo, ND from standing (1) and close up (2).

Chemical control options should also be explored for situations in which quackgrass does interfere with play. There are a number of herbicides labeled for quackgrass control, although the specific active ingredients and even rates depend on the desired species. For example, fluazifop is labeled at 6 and 16 oz A^{-1} for tall fescue and fine fescue, respectively. Thus, similar to row crops, turfgrass selection should be guided in part by the control options available when a pernicious weed problem exists. Previous research has demonstrated synergy between a sulfonylurea urea (SU) herbicide (nicosulfuron) and urea-ammonium nitrate for quackgrass control (Bruce et al., 1996), so SU herbicide research for quackgrass control should also investigate nitrogen synergism.

Methodology

Field Research

Turf species listed in Table 1 were seeded in September 2022. Unfortunately, the field experimental area suffered extensive winterkill damage after the Fall 2022 seeding and plugging. Spring 2023 re-seeding was unsuccessful.

Table 1. Turfgrass species proposed for the field experiment.

Common name	Scientific name	Reason for selection
Quackgrass	*Elymus repens*	Non-treated check
Tall fescue	*Schedonorus arundinaceus*	Leaf texture
Kentucky bluegrass	*Poa pratensis*	Golf course commonality
Sheep fescue	*Festuca ovina*	Color
Red fescue	*Festuca rubra*	Allelopathic trait(s)
Junegrass	*Koeleria macrantha*	Low input, native
Buffalograss	*Bouteloua dactyloides*	Color, drought tolerance, native
Creeping bentgrass	*Agrostis stolonifera*	Golf course commonality, color
White clover	*Trifolium repens*	Previous research

Greenhouse Research

Greenhouse research is tentatively planned for 2024. The greenhouse trial will be a randomized complete block design with six blocks and a factorial arrangement including eight herbicides/rates and two nitrogen fertilization rates (0 and 0.5 lb N 1000 ft^{-2}), which will be run twice. Table 2 lists proposed herbicide treatments.

Table 2. Herbicide treatments to evaluate for the chemical control of quackgrass.

Active ingredient(s)	Product rate	Notes
Non-treated		
Fluazifop	6 oz A^{-1}	Labeled rate for tall fescue
Fluazifop	16 oz A^{-1}	Labeled rate for fine fescue
Metsulfuron	0.5 oz A^{-1}	Kentucky bluegrass, fine fescue
Sethoxydim	48 oz A^{-1}	Fine fescue
Sulfosulfuron	2 oz A^{-1}	Kentucky bluegrass
Glyphosate	48 oz A^{-1}	Nonselective
Fluazifop + metsulfuron + sethoxydim	16 + 0.5 + 48 oz A^{-1}	Fine fescue

In September 2024, at the conclusion of the greenhouse trial and after field plots had been evaluated, the best-performing herbicide and nitrogen treatment safe on each respective species, as determined in the greenhouse trial, will be applied to the field plots. Plots will then be rated in May, June, and July of 2025 for quackgrass cover. Combining turfgrass selection (a cultural practice) with nitrogen fertilization (another cultural practice) and herbicide application (a chemical control) will demonstrate the importance of integrated pest management.

Results to Date

Other than limited winterkill data collected in Spring 2023, few results have been produced. This is largely due to difficulty establishing field plots in Fall 2022 and Spring 2023. Our expenditures for this project total $515.52 so far, all for undergraduate labor transplanting quackgrass plugs.

Future Expectations of the Project

Future expectations of this project depend on the project's ability to be transferred to a new institution (i.e., Iowa State University, where Quincy Law aims to complete the work). Should this project be transferred, the research objectives will be addressed.

References Cited

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USGA ID#: 2023-13-780

Title: Renovating out-of-play areas to conservation habitat: Effects of seeding time and method on plant establishment and ecosystem services

Project Leader: Douglas S. Richmond and Ryan R. Beard
Affiliation: Purdue University

Objectives: The overarching goal of this research is to understand the economic, aesthetic and ecological outcomes stemming from the establishment of native, prairie vegetation on golf courses. In support of this goal, the objectives of this Project are to 1) compare the effects of seeding time and seeding method on plant community establishment and 2) quantify ecosystem services by characterizing resulting pollinator communities.

Start Date: 2022
Project Duration: 3 Years
Total Funding: $83,628

Summary Points:

- Neither seeding time, nor seeding method influenced establishment of the conservation planting during the first year of the study.
- Although a relatively low number of plant species from the seed mixture were detected during 2023, those species comprised between 18 and 29% of the plant community.
- Fall drill seeding provided a higher density of flower blooms during the following summer but the number of blooms present during fall was similar across all seeding treatments.

Summary Text

Rational:
Efforts to improve the sustainability of golf courses focus largely on practices that reduce inputs and increase the ecosystem services provided. Among these efforts, conversion of out-of-play areas for conservation purposes has received increased attention (Barden 2016, USGA Green Section 2017, Vohden 2020). The underlying reasons include a combination of economic (Janke et al. 2021), aesthetic (Turo & Gardiner 2019), and ecological (Sexton & Emery 2020) considerations, all of which must be balanced against the unique constraints and goals of golf course staff, their clientele, and the surrounding community. As such, success often hinges on the ability of golf course staff to effectively carry out the conversion process and accurately communicate expected outcomes to stakeholders. At present, there is virtually no road map to guide such efforts or characterize expected outcomes.

Methodology:
Three out-of-play areas on the Kampen-Cosler Golf Course at Purdue University totaling 6.4 acres were “burned-down” using a combination of broadleaf and grassy weed herbicides in early November, 2021. Repeated herbicide applications and mowing continued throughout the 2022 growing season to minimize weed competition during the prairie establishment phase which began in the fall of 2022. At that time, plots within each out-of-play area were established and randomly assigned one of four different seeding treatments: 1) Fall (2022) dormant seeding using a broadcast spreader, 2) Fall (2022) dormant seeding using a seed drill, 3) Spring (2023) seeding using a broadcast spreader, and 4) Spring

(2023) seeding using a seed drill (Fig. 1 A & B). Composition of the seed mixture is detailed in Table 1.

To characterize resulting plant communities, a 1.0 m point intercept frame was placed at six evenly spaced locations along a diagonal sampling transect across each plot. Ten of the sampling pins were engaged at each sampling location for a total of 60 data points for each plot. Each plant coming into contact with a pin was identified to species and all flower blooms associated with those plants were counted. Sampling was repeated twice in 2023 (Summer, Fall).

Pollinator abundance and diversity, was assessed using a combination of active and passive sampling. Active sampling was performed using a hand-operated, battery powered vacuum suction device. The operator walked a random transect across each location for 5 minutes collecting all pollinators within 1 meter of the transect. Collection was performed on clear, calm days over the course of one week during July, August, and September. Sampling was performed in the morning and again during the afternoon of each day. Passive sampling was facilitated using blue vane traps. Three traps were set at canopy height at each location, and the contents of each trap was collected at the end of each day. Passive trapping was performed for one week coinciding with each active sampling period.

Results to Date:
Neither seeding time, nor seeding method appeared to have much influence on establishment of the conservation planting during the first year of the study. Although a relatively low number of plant species from the seed mixture were detected during 2023 (Fig. 2), those few species comprised between 18 and 29% of the plant community present in renovated plots (Fig. 3). Fall drill seeding provided a higher density of flower blooms during the summer (23/linear meter), but the number of blooms present during fall was similar across all seeding treatments (4-7/linear meter) (Fig. 4).

A total of 1,273 pollinator specimens (mostly bees) were collected during 2023 with the majority (910) being passively collected in blue vane traps. These specimens have been curated and pinned for identification to species level.

Future Expectations:
The coming season will provide additional opportunities to characterize plant communities resulting from the seeding treatments, and the impact of renovation on pollinator abundance and diversity. Plant communities will be surveyed three times in the coming season (Spring, Sumer, Fall) and pollinator communities will be synchronized to these sampling timeframes. Additional selective herbicide applications (Clethodim or Plateau) and mowing to reduce cool-season grass populations may be implemented during the spring of 2024.

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Table 1. Composition of the prairie seed mixture planted in conservation areas on the Kampen Cosler golf course at Purdue University and approximate seeding rates for each species.

Scientific Name	Common Name	Seeding rate (g)/ha
Andropogon gerardii	Big bluestem grass	35.03
Asclepias syriaca	Common milkweed	210.16
Asclepias tuberosa	Butterfly weed	210.16
Avena sativa	Oats	28021.26
Bouteloua curtipendula	Side-oats grama	420.32
Carex bicknellii	Copper-shouldered oval sedge	70.05
Coreopsis lanceolata	Sand coreopsis	210.16
Dalea candida	White prairie clover	280.21
Dalea purpurea	Purple prairie clover	490.37
Echinacea pallida	Purple coneflower	280.21
Elymus canadensis	Canada wild rye	1120.85
Juncus tenuis	Path rush	70.05
Koeleria macrantha	June grass	70.05
Monarda fistulosa	Wild bergamot	70.05
Monarda punctata	Horse mint	70.05
Panicum virgatum	Switch grass	35.03
Pycnanthemum tenuifolium	Slender mountain mint	17.51
Rudbeckia hirta	Black-eyed susan	420.32
Schizachyrium scoparium	Little bluestem	2802.13
Silphium laciniatum	Compass plant	35.03
Sorghastrum nutans	Indian grass	35.03
Sporobolus heterolepis	Prairie dropseed	70.05
Symphyotrichum novae-angliae	New-England aster	52.54
Symphyotrichum oolentangiense	Sky-blue aster	17.51
Verbena stricta	Hoary vervain	35.03
Vernonia fasciculata	Common ironweed	70.05
Zizia aurea	Golden alexanders	140.11
Total		35359.32



Figure 1. Drill seeding (A) and broadcast seeding (B) a low-profile prairie seed mixture during fall 2022.

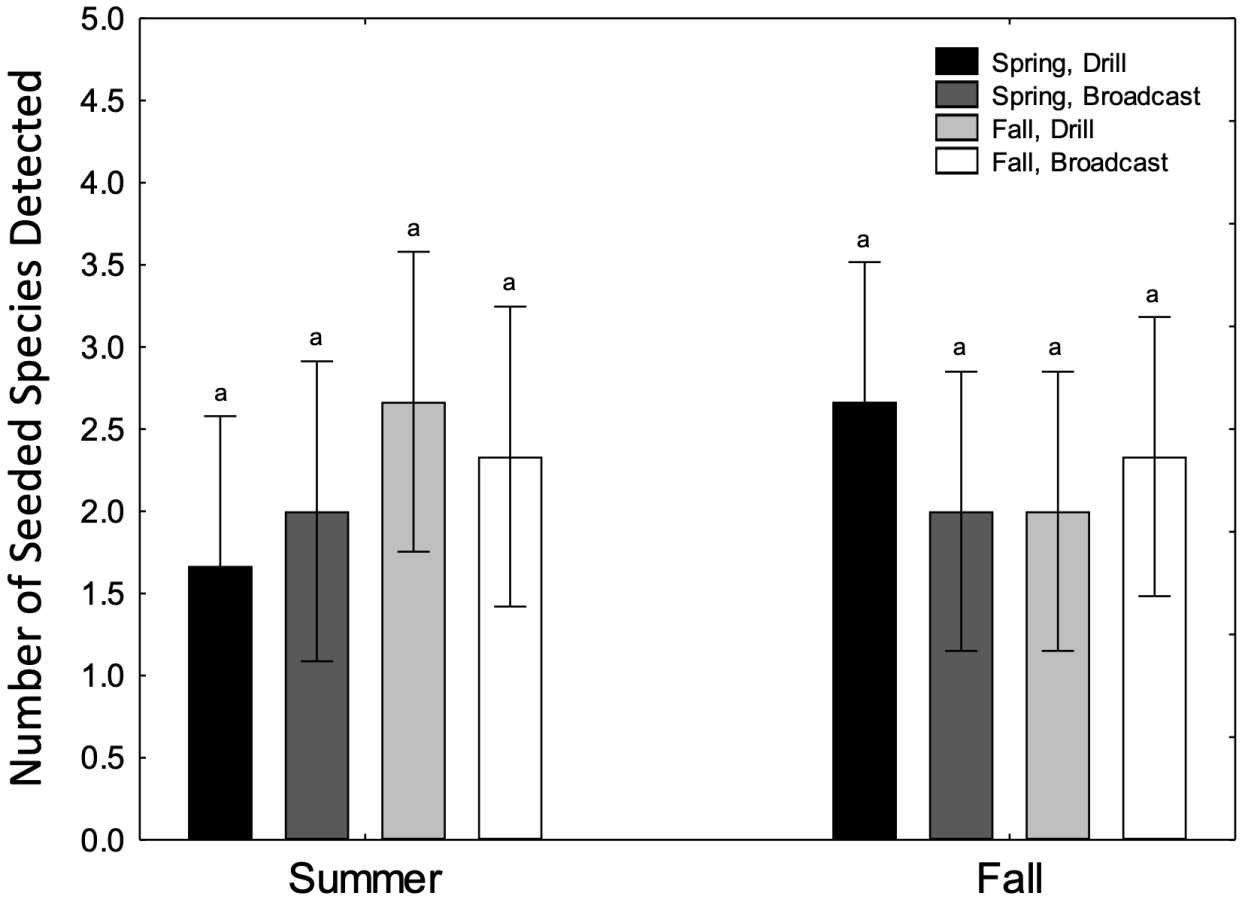


Figure 2. Number seeded species detected during summer and fall following fall or spring seeding of a low-profile prairie seed mixture using either a seed drill or broadcast spreader.

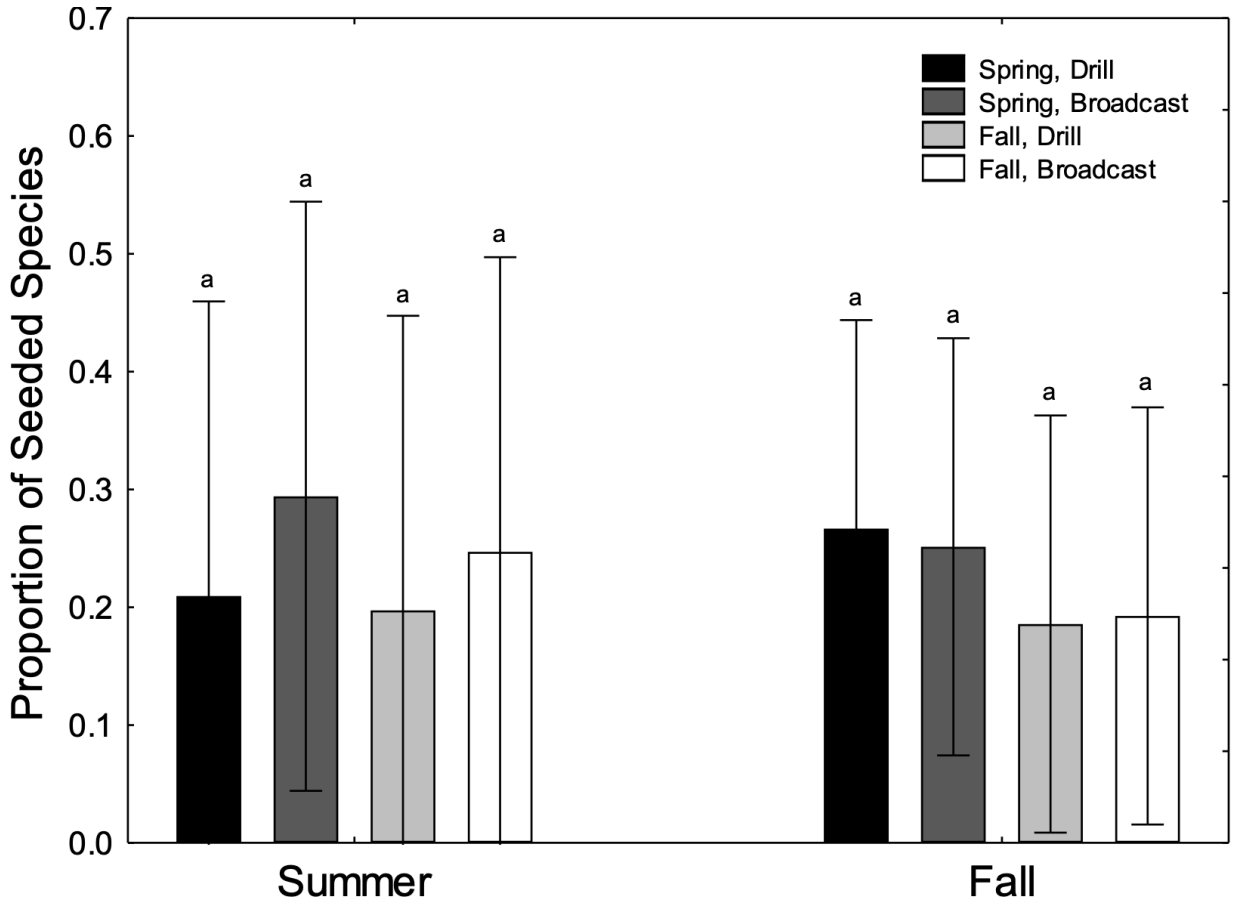


Figure 3. Proportion of the plant community comprised of seeded species following fall or spring seeding of a low-profile prairie seed mixture using either a seed drill or broadcast spreader.

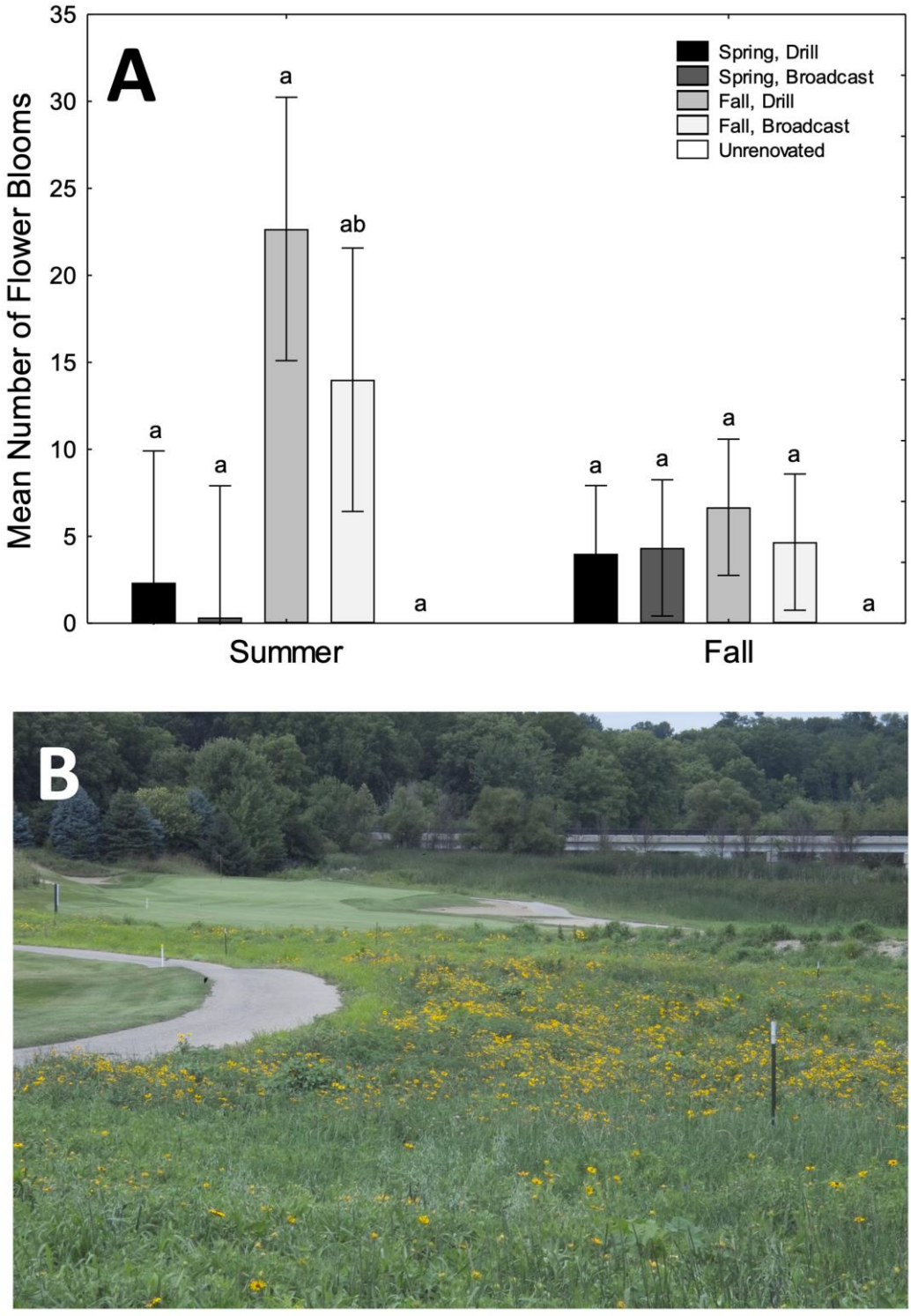


Figure 4. (A) Number of flower blooms/linear meter as detected using a pin frame following fall or spring seeding of a low-profile prairie seed mixture using either a seed drill or broadcast spreader and (B) blooming plants (primarily black-eyed Susan) during summer following fall seeding using a seed drill.

USGA ID#: 2022-20-763

Title: Longitudinal Analysis of US Golf Course Value

Project Leader: J. Aaron Hipp, PhD, and Kyle S. Bunds, PhD
Affiliation: Department of Parks, Recreation, and Tourism Management and Center for Geospatial Analytics, NC State University

Objectives: The objective of the project was the development of a spatio-temporal mapping platform producing a time-series (2000 - 2020) golf course value proposition for Wake County, NC, and Los Angeles County, CA:

A. Literature and project review of value proposition evidence related to US golf courses
B. Engage in conversation with select golf courses to identify their perceived value propositions and community value
C. Collate proposed parameter list for review by the USGA and partners based on Steps A and B
D. Collate data sources and identify availability for agreed upon parameters
E. Integration of draft in time-series GIS
F. Refinement of data and mapping platform for potential presentation at 2023 US Open

Start Date: 2023
Project Duration: 12 months (with 3 month no-cost extension)
Total Funding: $85,611

Summary Points:

- The project utilizes data (parameters) from nine broad categories of sustainability reflecting people, profit, and planet.
- Temporal and spatial data varied by parameter and by county, with some data covering 2000 – 2022 and other data being a single time point (2020).
- One-mile buffers were created around all golf courses and parameter data was compared between within one-mile of a golf course and outside of one-mile of a golf course.
- In general, any differences between within/outside the one-mile golf course buffer, held steady from 2000 – 2022, meaning any differences seen with the 2000 data general held across time – not growing or lessening in variation.

Summary Text:

A. Literature and project review of value proposition evidence related to US golf courses

The team conducted an academic and gray literature review using the terms “golf course*” AND “sustain*” or “golf course*” AND “ecosystem service*”. A total of twenty articles were reviewed. In addition, a Google Alert, “golf AND sustainability” was created to capture any new articles or news relevant to the aims of this project. The articles were annotated for information including year; US

geographic region; measures, operationalization, and data sources related to sustainability and ecosystem services; analyses; primary results; limitations.

B. Engage in conversation with select golf courses to identify their perceived value propositions and community value

The team met with golf professionals from three separate golf courses, a former city manager with public golf courses under their purview, and the board for First Tee of the Triangle. We also engaged with the faculty within NC State's Professional Golf Management Program. In these meetings we separately asked those attending to speak to the value proposition they perceive as well as to respond to the draft list created from the literature review in Step A.

C. Collate proposed parameter list for review by the USGA and partners based on Steps A and B

The team presented a list of 32 unique data parameters to the US Golf Association team. Several of these parameters included subcategories. For example, the US Census Data included multiple data points such as Median Household Income; population density; specific age group absolute numbers, etc.

D. Collate data sources and identify availability for agreed upon parameters

The final dataset includes nine broad categories:

Parameter Category	**Specific Categorization**	**Years of Data Available**
Land Use	Five distinct categories of land use	2001, 2006, 2011, 2016, 2021
Land Surface Temperature	Temperature in Fahrenheit	2000, 2005, 2010, 2015, 2020
Parcel-level Land Value	Taxable value of parcels	Wake: 2002, 2007, 2012, 2017, 2022 LA: 2006, 2021
Greenness	Normalized Digital Vegetation Index	2000, 2005, 2010, 2015
Air Pollution	Particulate Matter 2.5 and Nitric Oxcide	2000, 2005, 2010, 2015
US Census	Household income, educational attainment, race, ethnicity, homeownership rate, and total population	2000, 2010, 2015, 2020
Flood Zones	100yr Flood Zones	Wake: 2022 LA: 2023
CDC PLACES (Health)	Depression, physical activity, obesity, heart disease	2020
Stream Flow	Average monthly discharge (cubic feet per second)	2000, 2005, 2010, 2015, 2020

E. Integration of draft in time-series GIS

The team has created and shared Tableau data dashboards with the US Golf Association. The dashboards present the majority of the data outlined in the table above. The data is shown for each year and each parameter. There are separate Tableaus for Los Angeles and Wake counties.

F. Refinement of data and mapping platform for potential presentation at 2023 US Open

All data have been zipped into a folder to share with the US Golf Association. A parameters document has been drafted outlining data refinement decisions and original websites for the data. The primary task during the no cost extension (1/5/24 – 4/5/24) is the creation of a StoryMap to help tell the story of the data and present data that did not visualize well within Tableau.

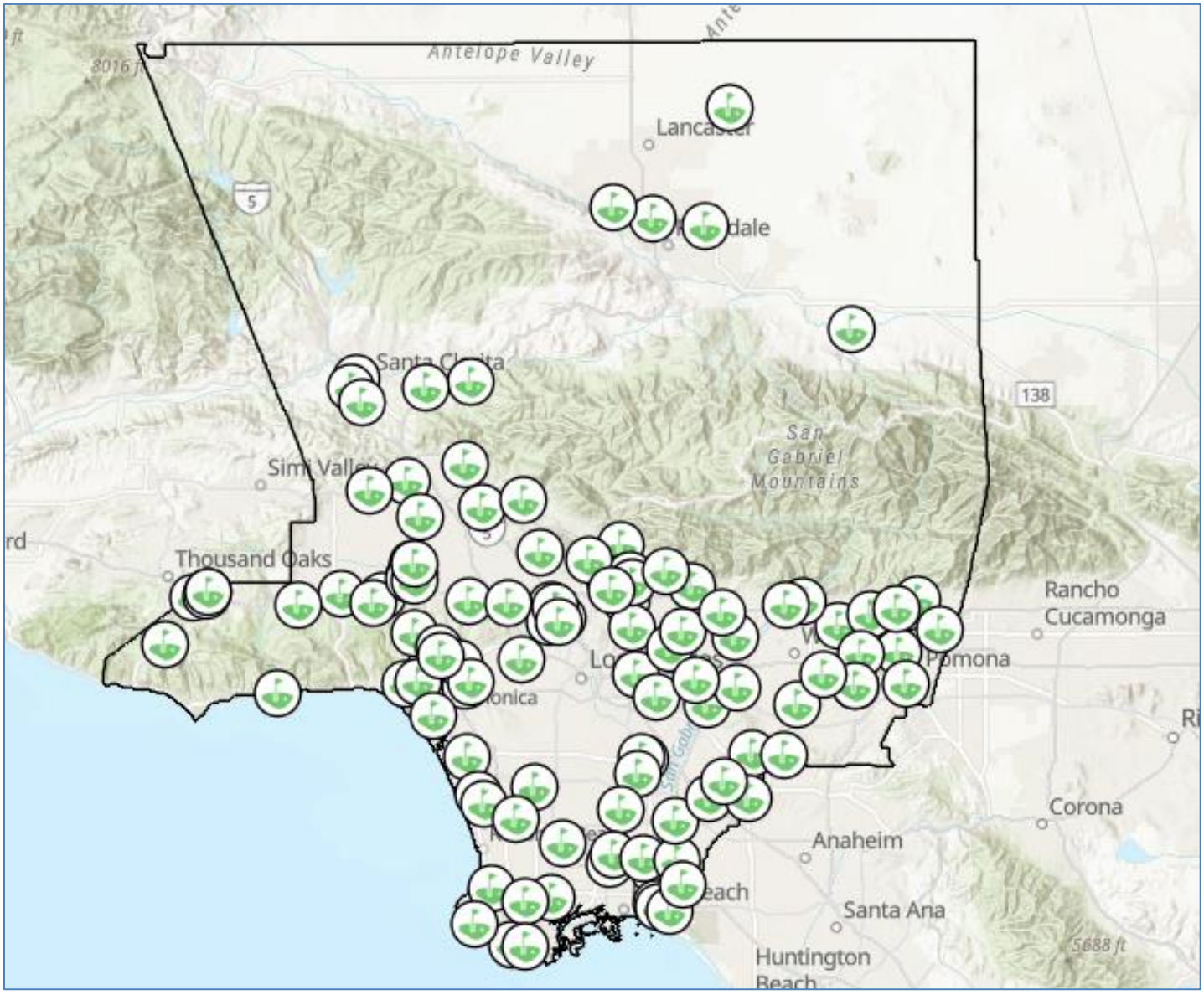


Image 1: Overview of golf course locations (2022) in Los Angeles County, CA.

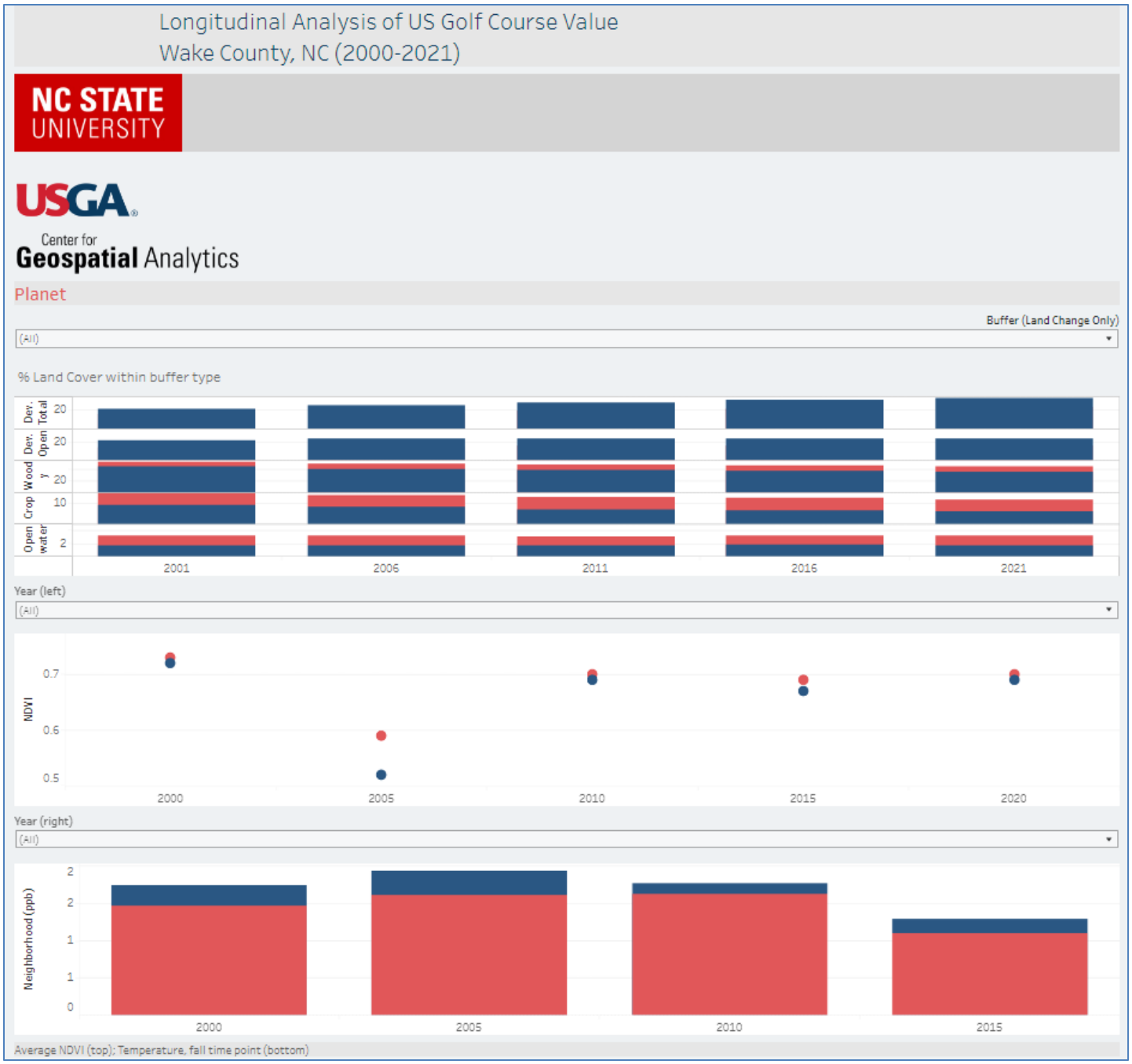


Image 2: Draft Tableau dashboard of Wake County, NC, Data.

USGA ID#: 2022-22-765

Title: Potential CO_2 Capture via Enhanced Weathering by Basaltic Sand Spreading on Golf Courses in the U.S.

Project Leader: Qiyu Zhou
Affiliation: North Carolina State University

Objectives:

1. Understanding the factors that affect basalt grain dissolution rate and intensity
2. Estimating the overall CO_2 emissions associated with the operational activities of basaltic sand including rock mining, crushing and grinding, transportation, and application
3. Assessing net CO_2 sequestration potential of enhanced basalt weathering on a typical 18-hole golf course.

Start Date: 10/1/2022
Project Duration: 1 year
Total Funding: $5,000

Summary Points:

1. Temperature, soil pH and basalt grain size significantly affect CO_2 sequestration potential
2. Substituting the currently used topdressing sand source, silica sand, with basaltic sand on putting greens located in warm region (≥ 25°C) and soil pH ≥ 8 could results in CO_2 sequestration of a minimum of 0.4 Mt $CO_{2(eq)}$ yr^{-1}
3. For the golf course is located in warm region (≥ 25°C) and soil pH ≥ 8, light and frequent basaltic sand application on maintained turfgrass area including putting greens, fairways, tees, practice areas, and roughs could result in CO_2 sequestration of approximately 12 Mt $CO_{2(eq)}$ yr^{-1}
4. Transportation of basalt material is a major source of CO_2 emissions, substantially diminishing its sequestration potential.
5. Instead of using USGA suggested sand particle size (0.05 to 1 mm), using reduced basaltic sand (≤50 μm) guarantees CO_2 sequestration in warm region, and reduced basaltic sand (≤20 μm) guarantees CO_2 sequestration in cold region.

Rational and Methodology:

Global warming poses a significant threat to society. Besides limiting CO_2 emissions, effective CO_2 sequestration practices are needed to meet the long-term goal of carbon neutrality in the atmosphere. Enhanced weathering has been recognized as a negative emissions technology, involving the application of ground silicate minerals to land surfaces. This practice results in the removal of CO_2 from the atmosphere as the silicate mineral is weathered by weak acid, which forms in the atmosphere through the interaction of CO_2 and H_2O. As the silicate minerals weather, they release byproducts, including HCO_3^- which will eventually be stored in the ocean for thousands of years, achieving carbon sequestration. Basalt is one of the most promising minerals due to its cost-effectiveness, fast dissolution rate, and lower levels of harmful elements. Given that topdressing using quartz-dominant sand is a routine cultural practice, this project aims to explore the potential CO_2 sequestration benefits of substituting quartz sand with

basaltic sand, taking into consideration the life cycle of enhanced basalt weathering associated with basalt rock mining, grinding, transportation, and application on golf courses. These goals are achieved by reviewing existing research.

Basalt dissolution rate

The CO_2 sequestration potential of enhanced basalt weathering is determined by the basalt grain dissolution rate. The first part of this study aims to explore enhanced basalt weathering using particle sizes of 0.05, 0.15, 0.25, 0.5, and 1 mm, as suggested by the UGSA for topdressing. The study focuses on golf courses located in both cold and warm region, where the annual average temperatures are approximately 7 or 25 °C, and soil pH ranges from 6 to 8.5. Figure 1 provides an example of basalt grain dissolution speed. Generally, the required dissolution time decreases as the basalt grain size decreases. The reaction time increases roughly 4-5 times as the temperature drops from 25 to 7 °C. As the pH increases or decreases, the basalt grain dissolution rate will likely increase, and the required time for basalt to dissolve will decrease accordingly (data not shown).

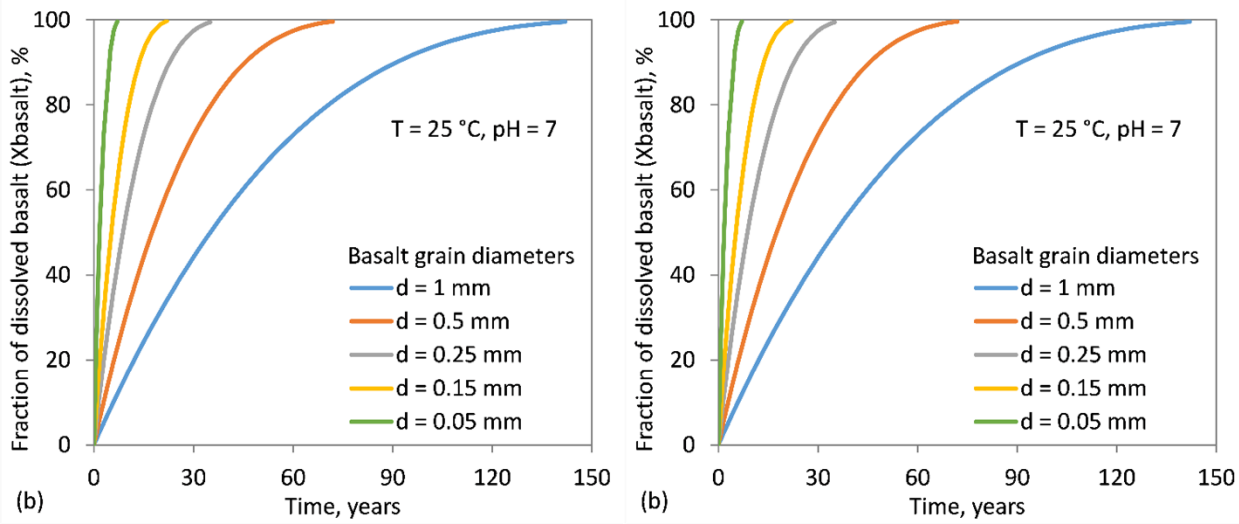


Figure 1. The relationship between dissolution reaction rate of sizes of basalt grain and time. a: when basalt dissolution process at T = 7 °C and pH =7, dissolution rate value is 3.34×10^{-10} mol m^{-2} s^{-1} (Gislason & Oelkers, 2003). b: when basalt dissolution process at T = 25 °C and pH = 7, dissolution rate value is computed as 1.27×10^{-9} mol m^{-2} s^{-1} (Gislason & Oelkers, 2003).

CO_2 Emission during Basalt Mining, Comminution, Transportation, and Application

To produce a final product with a particle size ranging from 0.05 to 1 mm, basalt minerals undergo a general mining and grinding process, also known as comminution. These processes typically involve CO_2 emission and could potentially compromise the CO_2 sequestration potential of enhanced weathering. In the U.S., diesel and electricity are the two primary energy sources used for basalt mining and comminution. Table 1 presents the overall energy requirements and corresponding CO_2 emissions during the entire mining and comminution process to obtain the final basalt product with a particle size of 0.05 to 1 mm.

Table 1. Overall energy requirement and corresponding CO_2 emissions in mining and comminution of basalt a final product of 0.05 to 1 mm in particle size.

Process stage	Activities	Energy	Energy use	Data source	CO_2 emissions
			kWh tonne^{-1} basalt		kg $CO_{2(eq)}$ tonne^{-1} basalt
Basalt extraction	Drilling	Diesel	1.52	(DOE, 2007)	0.39
	Blasting	Diesel	0.12	(DOE, 2007)	0.03
	Digging	Diesel	2.49	(DOE, 2007)	0.63
	Dewatering	Diesel	0.64	(DOE, 2007)	0.16
	Ancillary operations	Diesel	0.91	(DOE, 2007)	0.23
Materials handling	Transfer	Diesel	2.78	(DOE, 2007)	0.70
	Load haul dump, conveyors, pumps	Diesel	0.08	(DOE, 2007)	0.02
Processing	Crushing and grinding (to ≤ 50 mm)	Electricity	0.80	(DOE, 2007)	0.31
	Separation	Electricity	0.38	(DOE, 2007)	0.15
Rock comminution	Crushing and grinding (to ≤ 1 mm)	Electricity	8.38	(Metso, 2021) (Rosado et al., 2017)	3.25
Total			18.10		5.87

CO_2 Emissions due to Transportation and Application on Golf Courses in the U.S.

Following rock comminution, the crushed basalt product is ready for transport to golf courses using heavy-duty trucks, which predominantly rely on diesel as their energy source in the U.S. Table 2 presents the potential CO_2 emissions due to transportation from the basalt quarry to the golf course field, assuming that trucks transport the basalt material from the quarry to the golf course and return empty from the golf course to the quarry. Moreover, this study assumes that basalt topdressing will be applied every three weeks during a 30-week golf season in a cold region (7 °C), considering turfgrass dormancy during winter months and golf course closure. For a warm region (25 °C), the study assumes a 52-week golf season and an application rate of (14.4 ± 4.8) tonne of basalt per hectare for each topdressing event, following the USGA guidelines.

Table 2. CO_2 emissions due to transport basalt materials from quarry to golf course field.

Distance between quarry and golf course	CO_2 emissions[a]
km	kg $CO_{2(eq)}$ ha^{-1} topdressing $event^{-1}$
10	43.0 (28.7 – 57.4)
50	215.2 (143.4 – 286.9)
100	430.3 (286.9 – 573.8)
150	645.5 (430.3 – 860.7)
200	1075.9 (717.2 – 1434.5)

[a] CO_2 emissions due to transportation and application was estimated based on crushed basaltic sand application rate at (14.4 ± 4.8) tonne ha^{-1} per each topdressing event.

Potential Net Carbon Sequestration of Basalt Spreading on Golf Courses

Two spreading scenarios were considered in this study: 1) green spreading, where basaltic sand is only applied on golf course putting greens; 2) broadcast spreading where basaltic sand is applied on all maintained turfgrass areas of the golf course, including putting greens, fairways, tees, practice areas, and roughs.

Due to the overwhelmingly high CO_2 emissions caused by the transportation of basalt to golf courses from different distances of quarries, the application of basaltic sand on golf courses located in cold region always results in negative carbon capture (data not shown). Both green spreading (Figure 6a) and broadcast spreading (Figure 6b) would also result in negative carbon capture in warm region, implying that the major CO_2 emissions from transportation and application would offset and eventually surpass the potential CO_2 sequestration via enhanced basalt weathering.

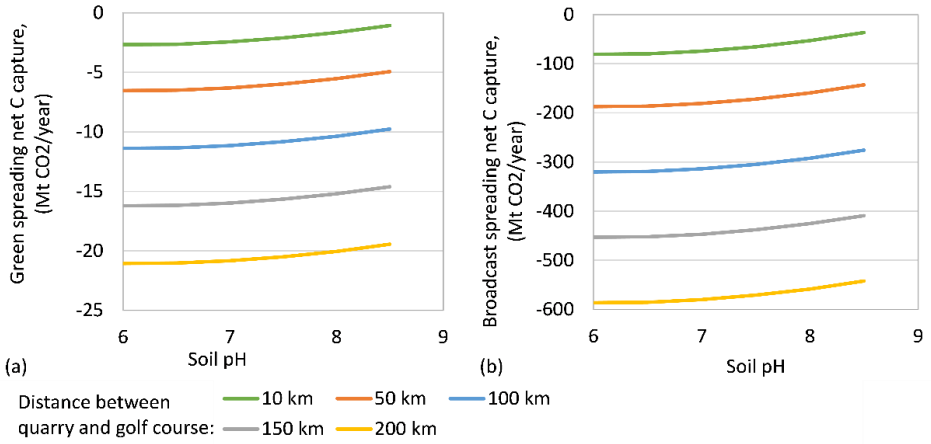


Figure 2. Net CO_2 sequestration on golf courses located in warm region (T = 25 °C) and pH ranges 6 to 8.5, considering all CO_2 emissions produced by basalt mining, grinding, comminution, transportation, and application. a) Green spreading: Basaltic sand is only applied to the putting greens on the golf course; b) Broadcast spreading: Basaltic sand is applied to the maintained turfgrass areas of a golf course, including putting greens, fairways, tees, practice areas, and roughs.

However, it is worth noting that CO_2 emissions already exist when using quartz-dominated sand as topdressing material, which is a common turfgrass management practice on golf courses. Figure 3 explores the additional benefits when substituting quartz-sand with basaltic sand on golf course at warm region. If we exclude the existing CO_2 emissions caused by basalt material transportation and application, and only consider the CO_2 emissions from basalt mining, grinding and comminution, there was positive net CO_2 sequestrations when spreading basaltic sand using two application strategies on the golf courses when the environment favors. As the soil pH was less than 8 and the basalt dissolution rate decreased, there was negative C capture and no additional benefits if substituting the quartz-dominant sand with basaltic sand. When the soil pH increased to 8.5, basalt enhanced weathering could remove 0.42 Mt $CO_{2(eq)}$ yr^{-1} from the atmosphere following green spreading; and remove 11.56 Mt $CO_{2(eq)}$ yr^{-1} from atmosphere following broadcast spreading.

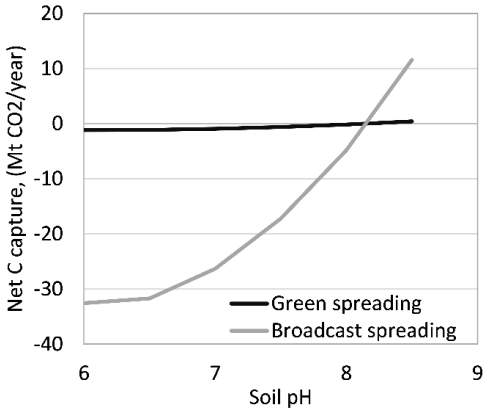


Figure 3. Net CO_2 sequestration on golf course that located at warm region (T = 25 °C) and soil pH ranges 6 to 8.5 by basalt enhanced weathering of green spreading or broadcast spreading, with the consideration of CO_2 emissions produced by basalt mining, grinding and comminution.

CO_2 Sequestration Potential with Reduced Size of Basalt Grain

This study also explored the potential of using finer size of basaltic sand on golf course roughs which covers approximately 19.4 ha, and application rate is at 14.4 14.4 tonne basalt ha^{-1} application $event^{-1}$, which is approximately 4833 tonne basalt $year^{-1}$ applied on the golf course in a warm region with a 52-week growing season and approximately 2794 tonne basalt $year^{-1}$ applied on the golf course in a cold region with a 30-week growing season.

Due to the slow dissolution rate of basalt and its limited CO_2 capture ability in cold region, using basalt powder finer than 20 μm could entirely offset and surpass the overall CO_2 emissions cause by mining, grinding, comminution, transportation (assuming a distance between quarry and golf course of less than 200 km), and application. Moreover, for the golf courses in warm region, choosing to use basalt powder finer than 50 μm could complete offset and surpass the overall CO_2 emissions (Figure 4).

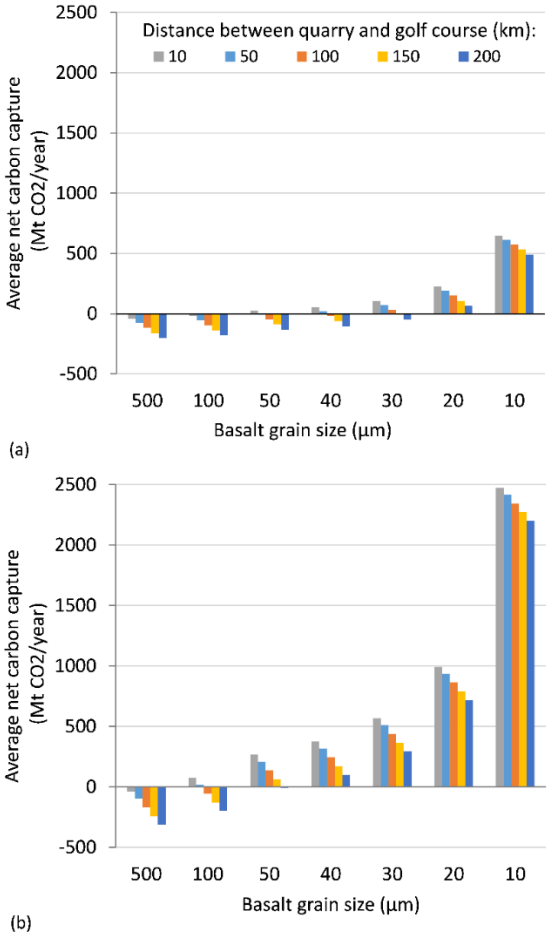


Figure 4. Estimated average net CO_2 sequestration potential on the roughs of 18-hole golf course with application of seven different sizes of basalt grain and five different distances between quarry and golf course. The net CO_2 sequestration potential was analyzed with the consideration of all potential CO_2 emissions including mining, grinding, rock comminution, transportation and application on a golf course's roughs. a) application on the golf course at cold region (T = 7 °C), application rate was at 14.4 tonne basalt ha^{-1} application $event^{-1}$ with 10 topdressing events; b) application on the golf course at warm region (T = 25 °C), application rate was at 14.4 tonne basalt ha^{-1} application $event^{-1}$ with approximately 17 topdressing events.

USGA ID#: 2023-19-786

Title: Climate change vulnerabilities of U.S. golf courses and potential adaptation opportunities

Project Leaders: Laura Hilberg and Lara Hansen
Affiliation: EcoAdapt

Objectives:

Objective 1 (Year 1–2): Complete a review of climate change projections and existing scientific literature (both peer-reviewed and gray literature) to identify the primary climate change impacts that will affect golf courses, including:

- Sea level rise (e.g., inundation of low-lying properties or access to those properties, increased salinity that impacts water quality);
- Warmer temperatures and more heat waves (e.g., changes in suitability for existing turfgrass, increased heat stress for staff/managers and users, and associated changes in patterns of demand);
- Changes in precipitation patterns and flooding/drought (e.g., changes in water availability and quality, impacts to turfgrass); and
- Climate-driven increases in disease, insect pests, weeds, etc.

Objective 2 (Year 1–2): Design and conduct a survey of golf course owners, superintendents, users, and other critical stakeholders to identify existing concerns, including climate impacts already being observed as well as non-climate stressors that might interact with climate change (e.g., water availability/quality issues, invasive plants and introduced pests/disease).

Objective 3 (Year 2): Identify 4–6 golf courses representing a range of geographic regions (e.g., Northeast, Southeast, Gulf, Midwest, Southwest, Northwest) and hold focus groups or interviews to elicit more information about climate change concerns and observations as well as potential adaptation strategies.

Objective 4 (Year 2–3): Compile literature review and survey/stakeholder input into a comprehensive report outlining general vulnerabilities for U.S. golf courses, summarizing the most important concerns, and providing a case study for each region. The report will include information about priority concerns for course superintendents and suggested adaptation strategies.

Objective 5 (Year 3): Create a website with visualizations to provide managers with information about potential climate impacts that could affect their golf courses.

Start Date: 2023
Project Duration: 3 years
Total Funding: $150,000

Summary Points:

We have identified and cataloged ~125 articles related to the impacts of climate change on golf courses, representing about half of the keyword combinations we plan to search.

- Results of the literature survey to date show more publications on the topic of climate change and golf in recent years, indicating an increasing interest in the issue.
- We have designed an outreach plan to undertake a survey to elicit additional information from key stakeholders (e.g., golf course owners, superintendents, users, etc.) related to existing concerns, including climate impacts already being observed and non-climate stressors that might interact with climate change (e.g., water availability/quality issues, invasive plants, and introduced pests/disease).

Summary Text:

Rationale

The primary objective of this project is to assist the golf community in the United States in gaining a better understanding of the consequences of climate change on golf and its facilities, identify opportunities to decrease adverse impacts, and improve the golf community's ability to sustain site functionality and the overall experience of the participants.

Methodology

In Year 1 of this project, our team is conducting a comprehensive review of the scientific literature to identify climate change impacts that may affect golf courses in the US, such as rising sea level, increasing temperatures and heatwaves, changes in precipitation patterns leading to flooding and drought, as well as climate-driven increases in disease, pests, and weeds. We are using a targeted literature review approach that involves searching for a predefined set of keywords as well as a "snowballing" technique wherein results of the predefined searches identify additional sources or keywords by examining the references in the set of articles collected during the initial literature review.

We defined 24 combinations of keywords prior to beginning the literature search (Table 1), chosen because they cover a range of topics related to the impacts of climate change on golf courses (e.g., turfgrass and sea level rise, golf course and erosion, turfgrass and hurricane, golf course and water availability, golfers and allergies, golf course maintenance and climate change). Throughout this process, we are also noting examples of golf courses with documented impacts of climate change (e.g., flooding, drought, fire) in popular press articles or reports that could be used as case studies or future targeted outreach.

Table 1. Keyword combinations used during the literature review process.

Topic Area	Keywords
Sea level rise	Turfgrass and sea level rise
	Turfgrass and saltwater intrusion
	Golf courses and sea level rise
	Golf course and saltwater intrusion
	Golf course and erosion
Warmer temperatures and more heat waves	Turfgrass and climate change
	Turfgrass and heat
	Golf course and heat stress
Changes in precipitation patterns and flooding/drought	Turfgrass and hurricane
	Turfgrass and extreme weather events
	Turfgrass and flooding/drought
	Turfgrass and water quality
	Golf course and flooding/drought
	Golf course and water availability and climate change
Climate-driven increases in disease, insect pests, and weeds	Golf and climate change
	Golf courses and climate change
	Turf grass pests and climate change
	Turf grass disease and climate change
	Golf course weeds and climate change
	Golf course invasive species and climate change
Golf course management and use	Golf course maintenance and climate change
	Golfers and allergies and climate change
	Golfers and heat
	Golfers and bad weather

We are cataloging the search results to capture information about each article we find, including the keywords utilized, the geographic locations of the studies, publication dates, and the specific impacts addressed in the articles. We are also documenting the outcomes and any recommendations mentioned in the studies.

We are also in the process of drafting a survey to be distributed to golf course owners, superintendents, users, and other stakeholders. The survey will be comprised of roughly 20 multiple-choice and short-answer questions to gather their insights into: 1) level of

concern about the impacts of climate change, 2) observed and anticipated climate impacts of greatest concern, 3) observed and anticipated actions taken to reduce the impacts of climate change, and 4) perceived obstacles or challenges in addressing the impacts of climate change on the golf industry. The survey will also ask participants for basic demographic information. This survey will be distributed in Spring 2024 through various means (e.g., social media, USGA monthly newsletter, Golf Journal, and outreach by state and regional golf associations).

Results

We have identified ~125 articles from the scientific literature to date and are in the process of cataloging them. These are related to the impacts of climate change on turfgrasses and the function of golf courses as a whole and represent 13 of the 24 pre-defined keyword combinations we plan to search (52%, four of those are complete and the rest are still in progress) (Figure 1). The majority of the articles that we have reviewed thus far are relatively recent (published between 2018 and 2023) (Figure 2), suggesting that interest in the impact of climate change on the golf industry is growing. Among the articles we reviewed, we noted a significant focus on turfgrass breeding and topical applications of plant growth regulators (e.g., melatonin, citric acid, humic acid) as potential methods for enhancing tolerance to different environmental factors, such as increasing salinity. Additionally, we found that there is a growing concern regarding the transition of golf courses from carbon sinks to carbon sources, which has prompted discussions about the environmental impact of fossil-fuel-intensive maintenance practices. The review also highlighted a growing interest in using local native species and planting warm-season turfgrasses as part of golf course management practices due to the potential decrease in the viability of cool-season grasses under changing climate conditions.

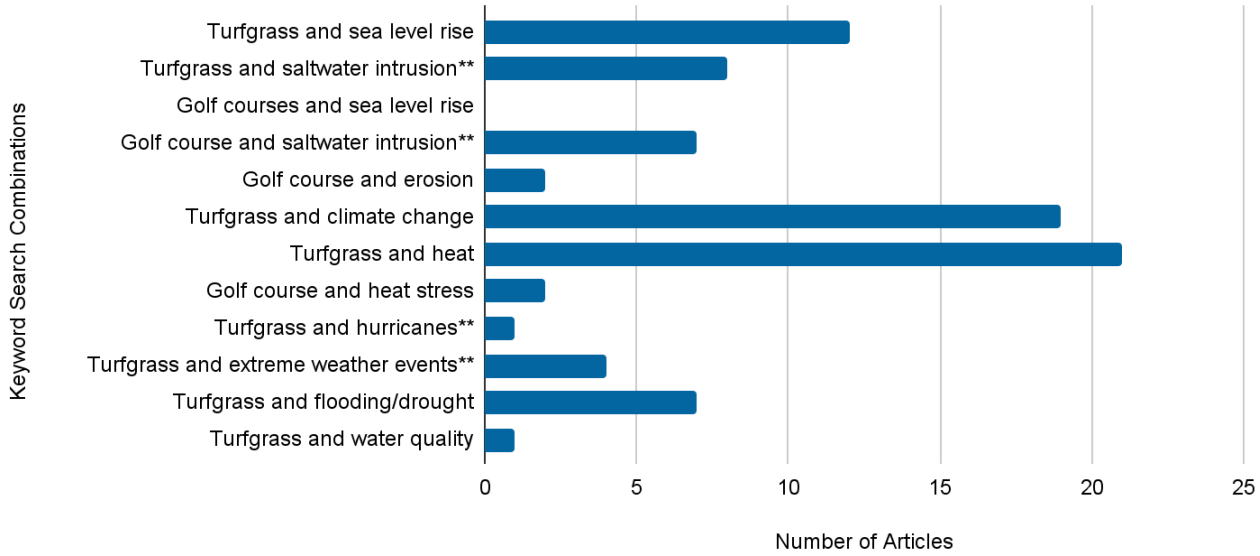


Figure 1. Number of articles identified and cataloged by keyword search. Asterisks (**) denote the keyword combinations for which we have completed the search; all others are in progress, and more articles will likely be added.

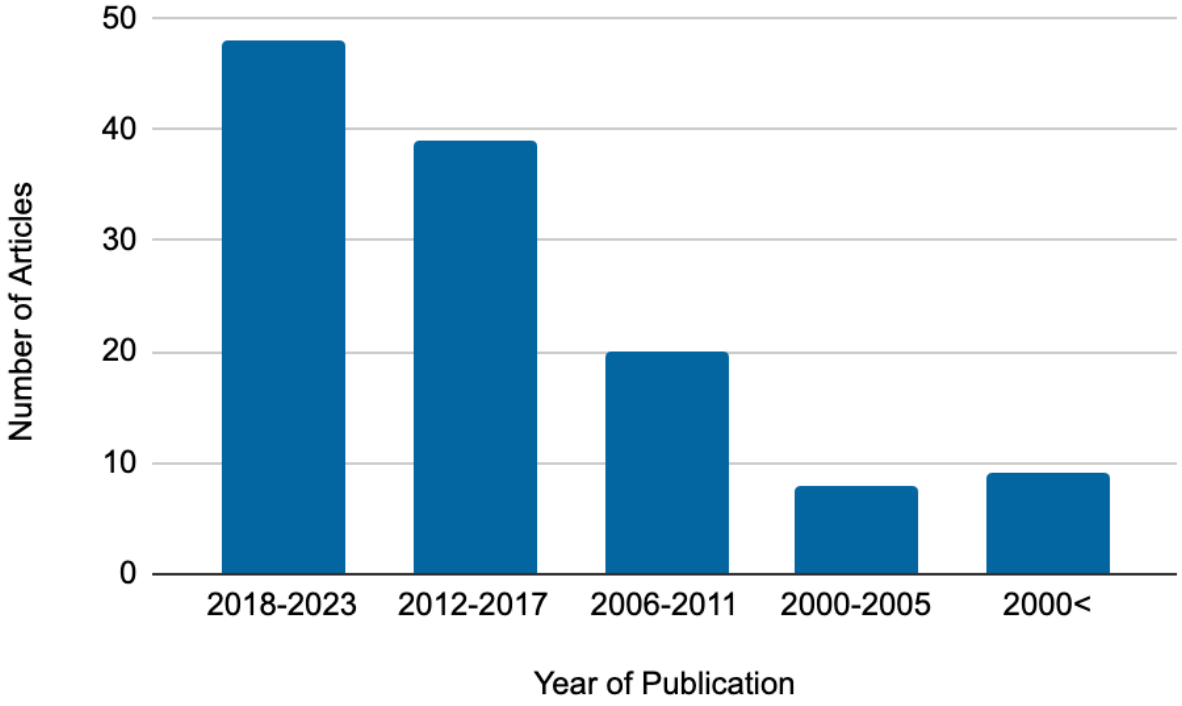


Figure 2. Publication date range for articles cataloged in this literature review.

Future Expectations

Over the next several months, we plan to complete the literature search and catalog all articles identified for review. Our next search areas include potential climate change impacts on golf course management and use and climate-driven increases in pests, diseases, and weeds. Simultaneously, we are completing the development of our survey, which will be released in the spring of 2024 (coinciding with Earth Month in April). We hope to receive responses from all geographic regions of the country to provide us with representative data over the course of several months, allowing for data analysis in the late summer and fall. During this period, we will also be convening a series of virtual focus groups to elicit additional input in a small group format. Upon their completion, we will begin compiling all the information we have gathered into a final report and website.

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

- The USDA-ARS Turfgrass Consortium has grown to a dozen federal turfgrass scientists conducting genetics, genomics and water conservation. A new effort of ecosystem services research is expanding within the Consortium
- Using feedback from the Turfgrass Stakeholder Summit II, the National Turfgrass Research Initiative (NTRI) was recently updated
- National Turfgrass Survey language and $2,000,000 in authorization funding has been submitted to Congress for inclusion in the 2023 Farm Bill
- A $20,000,000 Congressional authorization of the first turfgrass-specific competitive federal grant program has been requested for inclusion in the 2023 Farm Bill

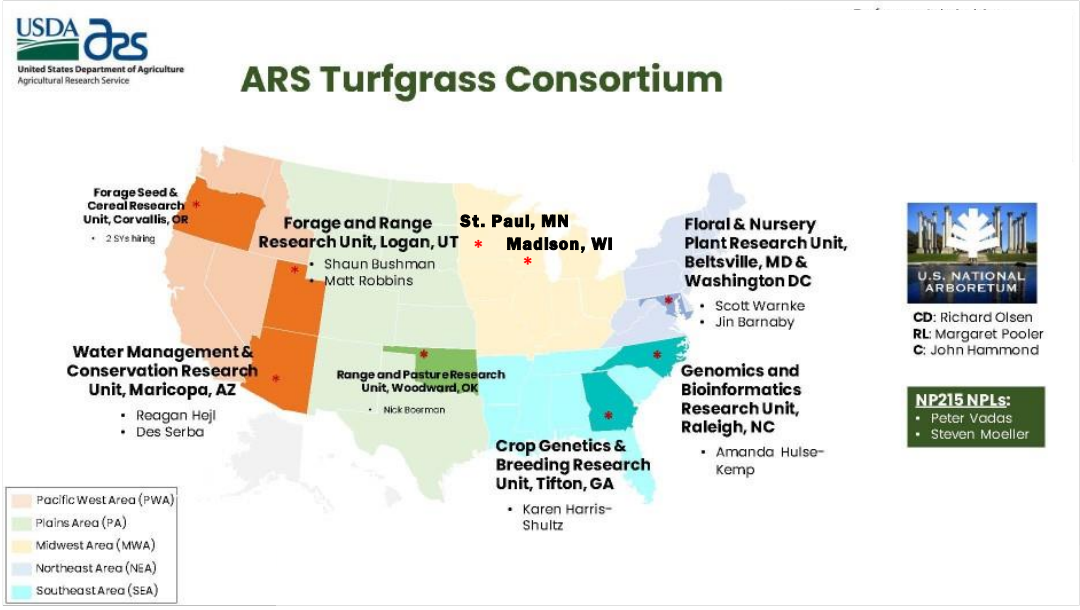
SUMMARY TEXT

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 ARS federal funding resulted in four new positions and hires. The ARS researchers are now organized into a 'Turfgrass Consortium', allowing them to collaborate and develop their research plans. The Consortium is conducting genome construction research, which will result in new genomes sequenced within the near future (Turfgrass genome sequencing from the USDA-ARS turfgrass consortium)). This funding will contribute

foundational information to aid the development of improved heat, cold, drought, disease and insect resistant grasses. The Consortium is currently focused on genetics and genomics research but is also organizing a multi-location ecosystem services project. The ecosystem services effort includes personnel at St. Paul, MN, Maricopa, AZ and Beltsville, MD, as well as a collaborative effort with researchers at the University of Wisconsin, Madison. In addition, the ARS turfgrass research consortium is expanding to add research at Corvallis, OR (grass seed production) and Woodward, OK (plant genetics).

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.



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 have been 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/. The NTRI is finalized and will be available soon 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 document acreage, scope and economic value as well as justify the need for increased federal research funding. On two separate occasions, we have unsuccessfully 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.

Our efforts in 2023 have include the following: 1) turfgrass survey language in the upcoming Farm Bill as well as $2,000,000 for the USDA, National Agricultural Statistics Service to conduct the survey, 2) a national turfgrass research initiative funded at $20,000,000 annually and 3) $5,000,000 annual funding for the National Park Service (NPS) turfgrass management and improvement strategy. We submitted our plan for a new NPS appropriation in 2023 but our funding was not included in appropriations. We hope that favorable language released by the Senate will be included in any final appropriations bill. This language would give NPS a chance to request turfgrass management funding in their FY25 budget request. The following is our proposed Farm bill language:

Turfgrass Economic Value and Utilization survey

(1) In General, starting no later than one year after enactment of this Act, the [USDA] Secretary shall conduct as a follow on study to the census of agriculture, under Section 2 of the Census of Agriculture Act of 1997 (7 U.S.C. 2204g) a national statistical census of turfgrass; to be conducted once every five years by USDA's National Agricultural Statistics Service to compile, analyze, and distribute information data on the following:

(A) Total number of acres (newly established and maintained)
(B) Total industry employment and broken down by segment
(C) Turfgrass equipment and related commercial retail sales
(D) Turfgrass maintenance expenses
(E) Regional and national economic impacts of the turfgrass industry
(F) Statistics documenting the change in number of turfgrass acres and its effects on the environment
(G) Potential areas where turfgrass would aid in addressing climate change and brownfields issues
(H) Other measurements of innovative applications of turfgrass and sod as determined by the Secretary

(2) AUTHORIZATION OF APPROPRIATIONS:—There is authorized to be appropriated to carry out this subsection $2,000,000 for the period of fiscal years 2024 through 2028, and once every five years thereafter.

National Turfgrass Research Initiative (NTRI)

There is established in this Act a National Turfgrass Research Initiative (NTRI) which the Secretary of Agriculture may make competitive grants for fundamental and applied research, extension, and education to address turfgrass and sod sciences (as defined under Section 1404 of the National Agricultural Research, Extension, and Teaching Policy Act of 1977) a competitive grants program with authorization for appropriations up to $20,000,000 per year for Fiscal Year 2024 to Fiscal Year 2028 to address the following priority areas:

(A) Drought and heat tolerance for water savings
(B) Pest resistance and pesticide reduction
(C) Nutrient and resource use efficiency
(D) Climate Change and turfgrass impacts
(E) Athlete safety and consumer health concerns
(F) Biodiversity to increase insect and wildlife habitat

In seeking proposals for research grants, NTRI proposals will seek the widest participation of qualified individuals in the Federal Government, colleges and universities, state agricultural experiment stations, and the private sector. The Secretary of Agriculture (or USDA Chief Scientist) shall allocate funds based on competitive peer review criteria.

Eligible recipients: The Secretary may make grants to carry out research, extension, and education under NTRI based on competitive criteria to the following:

(A) State agricultural experiment stations
(B) Colleges and Universities
(C) University research foundations
(D) Other research institutions and non-profit organizations
(E) Federal agencies
(F) National laboratories
(G) Private organizations, corporations, or individuals

A new Farm Bill has not been passed by Congress. The current Farm Bill has just been extended through September 2024. We are hopeful Congress will pass the new Farm Bill early next year. We will continue our advocacy efforts through 2024 as we watch the process. We also plan on resubmitting our USDA turfgrass survey grant proposal in case our turf survey language and funding is not included in the new legislation.

USGA ID#: 2019-21-691

Advancement of Five Elite Zoysiagrass Hybrids in the 2019 Zoysiagrass NTEP

Ambika Chandra, Meghyn Meeks and Dennis Genovesi
Texas A&M AgriLife Research-Dallas, 17360 Coit Road, Dallas, TX 75252

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 five genotypes have also ranked high for establishment and turfgrass quality in previous multilocation and environment experiments. Due to their overall performance in earlier trials, they 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 2022 from 12 of the 20 NTEP trial locations (standard and ancillary). Standard locations are in the northern (West Lafayette, IN), transition zone (Stillwater, OK; Manhattan, KS; Knoxville, TN; Raleigh, NC), southwestern (Riverside, CA), and southeastern (Auburn, AL; Gainesville, FL; Jay, FL; and Ft. Lauderdale, FL) regions. Data were also provided from Raleigh, NC (transition zone; traffic tolerance), Dallas, TX (southcentral; drought), and College Station, TX (shade). Trial management differed at each location.

Trials reported under Schedule A (Manhattan, KS; Auburn, AL; Ft. Lauderdale, FL; 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; Knoxville, TN; Raleigh, NC; Riverside, CA; Gainesville, FL; and Jay, FL) were mowed between 1.0" and 2.5" 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.

Establishment data was presented in previous reports and will not be reported here. Turfgrass quality ratings will be reported by maintenance level or ancillary trial conditions. All other data provided in this report have combined standard and ancillary trial data for traits such as spring greenup, winter survival, fall color, and seedhead density. No data were reported for divot recovery, sod strength, billbugs, or zoysiagrass mites. To rank overall performance, a turfgrass performance index was tallied for each event when 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 on a 1 to 9 scale (1=brown/dead, 6 = minimum acceptable, 9 = excellent). The means across all months evaluated in the 2019 NTEP report are presented. Under low mowing height (Schedule A) management, only three locations reported data in 2022 as compared to six locations in 2021, due to plots being damaged by severe by winter conditions. The three intermediate-textured cold-hardy lines (DALZ 1701, 1707, and 1808) were top performers (higher TPI) in the Manhattan, KS (transition) and Ft. Lauderdale, FL

(southeast) and most comparable to Empire (Table 1). 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 (Table 1).

Table 1. Mean turfgrass quality of 5 elite zoysiagrasses compared to commercial cultivars in 2022 under golf course management conditions (low mowing height = 0.5" to 1.0", Schedule A).

Entry	Mean Turfgrass Quality (Schedule A - 2022)			TPI (3)
	Transition	Southeast		
	Manhattan, KS	Auburn, AL	Fort Lauderdale, FL	
DALZ 1701	7.1 a	6.0	6.5 a	2
DALZ 1707	7.2 a	6.2	5.9	1
DALZ 1808	7.6 a	6.2	6.6 a	2
DALZ 1802	1.6	5.9	6.2	0
DALZ 1807	1.6	4.9	5.7	0
Emerald	7.1 a	6.8 a	6.3	2
Empire	6.8 a	5.6	6.9 a	2
Meyer	7.0 a	4.2	5.6	1
Zeon	7.3 a	6.8 a	5.6	2
LSD	1.2	0.8	0.7	
C.V.	13.1	8.6	6.6	

Under higher mowing height regimes, DALZ 1701, 1707, and 1808 performed most similarly to Emerald (Table 2, TPI of 3). Meyer, the cold tolerant cultivar, was a top performer in the northern location of West Lafayette, IN, which was statistically better than DALZ 1701, 1707, and 1808. DALZ 1802 and 1807 were not tolerant to extreme cold due to *Z. pacifica* influence in these pedigrees. In the transition zone location of Stillwater, OK, Emerald and DALZ 1802 had the highest mean quality although others except Meyer were above acceptable. In Tennessee, all five elite DALZ lines and some cultivars were top performers having higher quality than Empire. Under simulated high traffic conditions in Raleigh, NC, quality for DALZ 1701, 1707, and 1802 was greater than Emerald, and like Empire. In the southwest location of Riverside, CA, all five elite lines performed similarly to Zeon, which was greater than Emerald, Empire, and Meyer, but all quality means were below an acceptable score (6). In the southeast location of Gainesville, FL, most entries were below acceptable except DALZ 1808 and Emerald. In Jay, FL, performance was generally better than Gainesville, where DALZ 1802 was a top performer. DALZ 1802 (TPI of 5) and the three cold-hardy hybrids (TPI of 3) were higher performers than Empire (1), Meyer (2), and Zeon (2) under higher mowing heights.

Table 2. Mean turfgrass quality of 5 elite zoysiagrasses compared to commercial cultivars in 2022 under home lawn management conditions (mowing height = 1.0" to 2.5", Schedule B).

Entry	Mean Turfgrass Quality (Schedule B - 2022)							TPI (7)
	North	Transition			Southwest	Southeast		
	West Lafayette, IN	Stillwater, OK	Knoxville, TN	Raleigh, NC (traffic)	Riverside, CA	Gainesville, FL	Jay, FL (large patch)	
DALZ 1701	6.3	6.8	5.5 a	7.2 a	5.8 a	5.3	6.3	3
DALZ 1707	5.8	6.4	5.9 a	6.6 a	5.8 a	4.2	6.5	3
DALZ 1808	6.0	6.8	5.9 a	5.0	5.8 a	6.0 a	6.0	3
DALZ 1802	1.0	7.4 a	5.2 a	6.1 a	5.8 a	4.4	7.2 a	5
DALZ 1807	1.0	6.5	5.9 a	4.9	5.7 a	3.2	5.5	2
Emerald	5.5	7.3 a	6.0 a	5.8	5.5	6.1 a	6.6	3
Empire	5.0	6.2	5.1	6.3 a	5.3	5.0	6.9	1
Meyer	7.2 a	5.7	5.4 a	3.1	4.6	3.9	4.6	2
Zeon	5.5	6.5	5.8 a	5.9	5.6 a	4.4	6.5	2
LSD	0.9	0.3	1.0	1.3	0.6	1.1	0.5	
C.V.	14.4	2.8	10.8	14.5	6.7	13.1	5.2	

Drought tolerance - 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 2022, DALZ 1701 was the only hybrid with mean turfgrass quality (average of two months) above acceptable but lower than Emerald and Empire. However, after 14 d and 29 d of no supplemental water, the quality of DALZ 1701, Emerald, and Empire was similar. At 6 d and 14 d after returning irrigation, DALZ 1701 and Empire had similar or greater quality than Emerald. Twelve days into another drought cycle, Empire retained higher quality which was not statistically different from DALZ 1701 or Emerald. All three entries were in the top statistical group 5 d after resuming irrigation. Overall, DALZ 1701 was the DALZ hybrids with a TPI of 3, between Emerald (2) and Empire (5).

Table 3. Mean turfgrass quality of 5 elite zoysiagrasses and 4 cultivars in Dallas under non-stress, drought, and recovery conditions in 2022.

Entry	Mean Turfgrass Quality (2022)							TPI (7)
	Non-stress†	Cycle 1‡				Cycle 2§		
		Drought		Recovery		Drought	Recovery	
		14 d	29 d	6 d	14 d	12 d	5 d	
DALZ 1701	6.3	6.0	3.7	7.0 a	6.0 a	6.0	6.7 a	3
DALZ 1707	4.3	3.0	2.0	4.7	4.0	4.0	5.3	0
DALZ 1808	4.0	3.3	1.7	4.0	3.3	3.3	4.3	0
DALZ 1802	5.0	4.0	2.0	5.7	5.0	4.3	5.7	0
DALZ 1807	4.7	3.3	2.7	4.0	3.7	4.0	4.7	0
Emerald	7.3	6.0	3.3	8.0 a	5.3	6.3	7.7 a	2
Empire	7.8 a	6.3	3.3	8.0 a	6.7 a	6.7 a	8.0 a	5
Meyer	1.3	1.0	1.0	1.0	1.7	1.0	1.7	0
Zeon	6.5	4.7	2.0	6.0	5.7 a	4.7	7.0 a	2
LSD	1.3	1.8	1.2	2.4	2.4	2.1	2.5	
C.V.	22.7	28.6	31.5	27.2	30.9	30.1	27.3	

† Non-stress is the average of means from May and June 2022 prior to initiating drought.
‡ The first drought cycle was initiated on July 13, 2022 and was suspended on July 12, 2022 to start recovery.
§ The second drought cycle was initiated on July 29, 2022 and was suspended on August 10, 2022 to start recovery.

Shade tolerance – Shade tolerance was evaluated in College Station, TX from June through September 2022 (Table 4). Percent green cover was digitally captured from 0 to 100 % and turfgrass quality was averaged across dates of collection. DALZ 1808 and 1802 retained the highest amount of green cover like Emerald, Empire, and Zeon (≥65.0%). However, quality was below minimum acceptable (6) for all entries and cultivars presented. DALZ 1808, 1802, Emerald, Empire and Zeon all had TPIs of 5.

Table 4. Shade tolerance rated as green cover and turfgrass quality in College Station, TX.

Entry	Percent Cover				Turfgrass Quality	TPI (5)
	Jun	Jul	Aug	Sep	Mean	
DALZ 1701	76.7 a	38.3	38.3 a	38.3	3.8	2
DALZ 1707	63.3 a	50.0 a	33.3	46.7	3.4	2
DALZ 1808	81.7 a	56.7 a	61.7 a	76.7 a	4.7 a	5
DALZ 1802	68.3 a	63.3 a	68.3 a	81.7 a	4.3 a	5
DALZ 1807	53.3	43.3 a	56.7 a	53.3	3.9 a	3
Emerald	75.0 a	63.3 a	71.7 a	73.3 a	4.5 a	5
Empire	75.0 a	58.3 a	38.3 a	65.0 a	4.3 a	5
Meyer	63.3 a	35.0	33.3	35.0	3.0	1
Zeon	90.0 a	83.3 a	65.0 a	73.3 a	5.2 a	5
LSD	32.8	40.8	44.3	31.5	1.5	
C.V.	20.3	36.8	41.0	30.6	19.8	

Traffic tolerance – Traffic tolerance was simulated by mechanical means in North Carolina between the months of September and November in 2022 (Table 5). Percent green cover was recorded weekly. Hybrid DALZ 1802 performed better than other presented entries and cultivars with the greatest percent green cover retention over time (TPI of 5 out of 9). However, few statistical differences were observed for experimental hybrids compared to check cultivars.

Table 5. Percent green cover of experimental entries and commercial cultivars because of mechanical traffic in Raleigh, NC in 2022.

Entry	Percent Green Cover									TPI (9)
	12-Sep	15-Sep	22-Sep	24-Sep	6-Oct	13-Oct	20-Oct	27-Oct	3-Nov	
DALZ 1701	97.0 a	95.7 a	92.3	86.3	72.7	53.3	41.7	31.7	23.3	2
DALZ 1707	97.0 a	95.7 a	89.7	83.0	67.7	51.7	41.7	35.0	26.7 a	3
DALZ 1808	92.3	89.0	83.0	76.0	59.0	45.0	41.0	31.7	20.0	0
DALZ 1802	97.0 a	94.7	90.0	84.0	68.7	61.7 a	51.0 a	45.0 a	36.7 a	5
DALZ 1807	93.0	90.7	84.7	78.3	60.7	46.0	35.0	26.7	18.3	0
Emerald	95.7	94.7	89.0	83.0	69.3	50.0	41.7	31.7	20.0	0
Empire	97.7 a	96.3 a	91.3	84.0	67.3	49.3	38.3	28.3	15.0	2
Zeon	96.3	94.0	88.0	80.7	66.7	45.0	35.0	26.7	16.7	0
Meyer	93.3	91.3	85.7	78.3	61.0	45.0	38.3	28.3	20.0	0
LSD	2.3	2.9	4.0	5.8	8.3	11.6	11.8	11.9	12.4	
C.V.	1.4	1.9	2.7	4.0	7.0	12.1	15.0	19.2	29.5	

Spring greenup and winter survival– Spring greenup was rated from 1 to 9 (1=brown/dormant, 9 = completely green) in four transition zone (Table 6) and six southern (Table 7) locations. In most transition zone locations, the three cold-hardy entries had similar spring greenup except in Stillwater, OK where DALZ 1808 had faster greenup which was also better than Empire, Meyer, and Zeon but like Emerald. DALZ 1802 was slow to greenup across the transition zone, except in Knoxville, TN where it was like other entries. DALZ 1807 had a surprising greenup like DALZ 1808 and Emerald in Stillwater, OK, and was better than other entries in Raleigh, NC. Spring greenup for DALZ 1707 and 1808 were some of the highest mean ratings in Riverside, CA, and like Zeon.

Table 6. Mean spring greenup of 5 elite DALZ zoysiagrasses and 4 cultivars across northern and transition zone locations in 2022.

Entry	Spring Greenup				TPI (4)
	Transition				
	Stillwater, OK	Manhattan, KS	Knoxville, TN	Raleigh, NC	
DALZ 1701	3.0	6.3 a	7.3 a	1.3	2
DALZ 1707	2.7	6.7 a	8.3 a	3.0	2
DALZ 1808	4.3 a	6.3 a	8.0 a	2.7	3
DALZ 1802	2.5	1.0	7.3 a	1.0	1
DALZ 1807	4.5 a	1.0	8.3 a	4.7 a	3
Emerald	3.7 a	5.7	8.0 a	2.0	2
Empire	3.0	5.3	5.3	2.0	0
Meyer	3.0	7.3 a	7.7 a	3.3	2
Zeon	2.7	6.7 a	8.3 a	1.7	2
LSD	1.1	1.2	2.1	1.1	
C.V.	23.7	19.8	16.5	38.5	

Table 7. Mean spring greenup of 5 elite DALZ zoysiagrasses and 4 cultivars across southern locations in 2022.

Entry	Spring Greenup						TPI (6)
	Southcentral		Southeast			Southwest	
	Dallas, TX	College Station, TX	Auburn, AL	Gainesville, FL	Jay, FL	Riverside, CA	
DALZ 1701	7.0 a	3.3	5.0	5.7 a	5.7 a	5.3	3
DALZ 1707	5.3	2.7	5.3	3.3	5.3 a	6.0 a	2
DALZ 1808	3.7	2.0	6.3	6.3 a	6.0 a	6.7 a	3
DALZ 1802	5.3	2.7	6.3	4.0	5.0	4.3	0
DALZ 1807	5.7	3.0	4.0	4.3	5.0	5.0	0
Emerald	8.0 a	2.3	3.7	5.7 a	5.3 a	4.7	3
Empire	6.7 a	2.3	5.3	4.3	5.0	4.3	1
Meyer	1.3	2.0	1.7	3.3	5.7 a	2.7	1
Zeon	7.7 a	3.7	2.3	4.7	4.7	6.0 a	2
LSD	2.3	3.5	0.9	1.5	0.9	1.4	
C.V.	26.3	38.8	12.3	19.2	10.7	19.1	

Environmental conditions were variable during the 2021-2022 winter, where some locations were much colder than others which was reflected in spring greenup ratings. Winterkill was rated in Manhattan, KS (transition zone) and Dallas, TX (southcentral), and percent living green cover was recorded in West Lafayette, IN (northern) and Knoxville, TN (transition zone) (Table 8). For comparison, Meyer was the cold and freeze tolerant commercial check which had very little winterkill in Kansas and high living green cover in Indiana and Tennessee. DALZ 1701, 1707, and 1808 also had low winterkill in Kansas and high spring greenup like Meyer (Table 6), but lower living cover than Meyer in Indiana where greenup was not reported. Contrastingly, DALZ 1802 and 1807 both had high winterkill in Kansas and no living cover in Indiana (Table 8) which was reflected in very low spring greenup ratings (Table 6). All five entries were reported to have high percent living cover (Table 8) and greenup like Meyer in Knoxville, TN (Table 6) In Dallas, TX, Meyer does not perform well in general and therefore, had a much higher winterkill rating than in the northern and transition zone locations (Table 8). DALZ 1701, 1807, Emerald, Empire, and Zeon, were reported to have lower winterkill (Table 8) as compared to Meyer and DALZ 1808 which was reflected in higher spring greenup ratings (Table 6).

Table 8. Winter survival of 5 elite DALZ zoysiagrasses and 4 cultivars in 2022.

Entry	Winterkill†		Percent Living Cover (Spring)		TPI (4)
	Manhattan, KS	Dallas, TX	West Lafayette, IN	Knoxville, TN	
	%	1 to 9	%		
DALZ 1701	8.3 a	7.3 a	56.7	81.7 a	3
DALZ 1707	0.0 a	6.3	71.7	70.0 a	2
DALZ 1808	0.0 a	4.3	56.7	76.7 a	2
DALZ 1802	83.3	6.3	0.0	83.3 a	1
DALZ 1807	80.0	8.0 a	0.0	83.0 a	2
Emerald	33.3	8.3 a	49.0	41.2	1
Empire	10.0 a	7.3 a	36.7	41.3	2
Meyer	6.7 a	3.7	99.0 a	81.0 a	3
Zeon	15.0 a	8.7 a	51.7	30.0	2
LSD	18.9	2.4	19.3	39.4	
C.V.	21.5	25.0	49.6	38.7	

†Winterkill was rated as a percentage of green cover lost from 0 to 100% in Manhattan, KS, but was rated from 1 to 9 in Dallas, TX where 1 = complete loss and 9 = no loss.

Overall, DALZ 1701 exhibited winter survival (TPI of 3) and spring greenup like Meyer (TPI of 2) in northern and transition zone locations but better survival in Dallas, TX and higher spring greenup (TPI of 3) across southern locations where it is most like Emerald (TPI of 3). DALZ 1808 had a similar combined greenup and survival TPI (8) as DALZ 1701 but had better greenup in Oklahoma and higher winterkill in Dallas, TX. DALZ 1707 (combined TPI of 6) performed similarly to Empire across locations. DALZ 1802 and 1807 were both negatively impacted by extremely cold temperatures and are not suitable for northern locations or widely across the transition zone.

Fall color retention – Fall color retention was rated from 1 to 9 (1 =brown/dormant, 9 = completely green) eight times across northern and transition zone locations (Table 9) and five times in southern locations (Table 10). DALZ 1707 (TPI of 6) and 1802 (TPI of 5) were top performers more frequently than other hybrids in northern and transition locations, although most fall color retention ratings are not statistically different. DALZ 1701 (TPI of 4) was most like Empire (TPI of 4) in northern and transition locations, and like Emerald (TPI of 2) in southern locations. In the southern locations, however, DALZ 1802 (TPI of 5) retained color better than other hybrids, particularly in Alabama and California. DALZ 1807 (TPI of 3) also had high color retention in California. Both DALZ 1802 and 1807 were selected in California for high fall/winter color retention and are continuing to perform as expected.

Table 9. Fall color retention in northern and transition zone locations in 2022.

Entry	Fall Color Retention								TPI (8)
	North	Transition							
	West Lafayette, IN	Stillwater, OK	Manhattan, KS	Knoxville, TN			Raleigh, NC		
	Oct	Nov	Oct	Sept	Oct	Nov	Nov	Dec	
DALZ 1701	6.0	7.0	3.3 a	5.7 a	5.3 a	5.0	7.7 a	3.7	4
DALZ 1707	6.3 a	7.0	3.7 a	6.0 a	5.3 a	5.3 a	8.0 a	4.0	6
DALZ 1808	5.3	6.0	4.3 a	5.7 a	4.7	4.7	5.7	3.3	2
DALZ 1802	.	9.0 a	.	5.7 a	5.7 a	5.7 a	7.3 a	4.3	5
DALZ 1807	.	7.0	.	5.7 a	5.0 a	4.7	6.3	6.0 a	3
Emerald	5.7	7.0	3.0	5.3 a	5.3 a	4.7	6.3	4.0	2
Empire	6.3 a	7.0	2.0	5.7 a	5.3 a	5.3 a	7.0	3.3	4
Meyer	5.0	6.7	3.3 a	5.3 a	5.3 a	5.0	4.0	2.0	3
Zeon	6.0	7.0	2.3	5.0	4.7	5.0	6.3	2.7	0
LSD	0.9	0.5	1.1	0.9	0.8	0.5	1.1	1.4	
C.V.	8.4	4.3	27.9	9.8	9.0	6.3	9.6	24.3	

Table 10. Fall color retention in southern locations in 2022.

Entry	Fall Color Retention					TPI (5)
	Southeast	Southcentral		Southwest		
	Auburn, AL	Dallas, TX	College Station, TX	Riverside, CA		
	Sept	Nov	Oct	Nov	Dec	
DALZ 1701	7.3	5.0 a	4.7 a	5.7	2.0	2
DALZ 1707	6.0	5.0 a	5.0 a	6.0	3.3	2
DALZ 1808	4.3	4.7 a	6.3 a	5.0	2.0	2
DALZ 1802	9.0 a	5.3 a	7.0 a	8.0 a	6.7 a	5
DALZ 1807	7.7	5.3 a	4.7 a	7.0 a	5.3	3
Emerald	7.0	6.0 a	7.0 a	5.0	3.0	2
Empire	7.3	3.7	4.3 a	4.7	2.3	1
Meyer	6.0	4.0 a	3.3	3.0	1.0	1
Zeon	7.3	5.7 a	6.3 a	5.3	3.0	2
LSD	0.8	2.5	4.5	1.2	1.1	
C.V.	8.3	20.7	40.6	12.6	18.9	

Disease Resistance- Two diseases were rated on a 1 to 9 scale (1=completely susceptible, 9 = completely resistant/no evidence) (Table 11). Resistance to large patch (*Rhizoctonia solani*) was rated twice in fall 2022 in Jay, FL. Resistance to dollar spot (*Sclerotinia homeocarpa*) was rated once in Raleigh, NC. Among the cold-hardy hybrids, DALZ 1808 (TPI of 2) exhibited greater resistance to large patch like Meyer as compared to other entries tested. DALZ 1802 and 1807 (TPI of 0) were like Emerald, Empire, and Zeon with greater susceptibility. DALZ 1701 (TPI of 1) and Empire had high resistance to dollar spot compared to other entries and cultivars presented.

Table 11. Resistance to large patch and dollar spot in 2022.

Entry	Large Patch†		Dollar Spot Resistance‡	TPI (3)
	Oct	Nov		
DALZ 1701	3.7	6.0	7.3 a	1
DALZ 1707	2.0	4.0	6.0	0
DALZ 1808	6.0 a	6.7 a	5.3	2
DALZ 1802	1.0	2.7	3.7	0
DALZ 1807	2.0	3.7	5.7	0
Emerald	1.0	2.3	5.7	0
Empire	1.7	3.0	7.3 a	1
Meyer	7.0 a	8.3 a	5.0	2
Zeon	1.0	3.7	5.7	0
LSD	1.8	2.0	1.3	
C.V.	49.4	32.2	12.6	

† Large patch was rated in Jay, FL.

‡ Dollar spot was rated in Raleigh, NC.

Seedhead density – Seedhead density was rated from 1 to 9 (1=very dense, 9 = none) in two transition and four southern locations for a total of nine times (Table 12). In Oklahoma, most ratings were similar among presented entries and cultivars with no evidence of seedheads when compared to Empire which had a heavy density. In Kansas, DALZ 1808 and 1802 had the least number of seedheads compared to others. DALZ 1701, Emerald, and Empire had fewer seedheads than Meyer and others in Gainesville, FL. Seedheads were very dense in Jay, FL with no observable differences. In Dallas, seedheads were rated in early spring (April), late spring (May) and mid-summer (Jul). Seedhead density was highest in April in general, but lowest (higher rating) for DALZ 1802, Meyer, and Zeon whereas the three cold-hardy hybrids, as well as DALZ 1807, Emerald, and Empire were similar. This trend was repeated in May, but DALZ 1807 had fewer seedheads than before. Most seedheads were no longer evident in July except for Empire which was moderately dense. In California, Empire and Meyer had the highest seedhead density in spring while DALZ hybrids had little to none. In fall, however, only DALZ 1808 and 1802 had statistically higher evidence of expression. Overall, Empire had the highest seedhead expression as is shown by a lower TPI (2) as compared to DALZ 1707 and 1808 (4), DALZ 1701 and 1807 (5), and DALZ 1802, Emerald, and Zeon (6).

Overall turfgrass performance – All traits considered, the maximum potential TPI was 68 (Table 13). DALZ 1802 and 1701 had the highest TPI (33 and 32, respectively), followed by DALZ 1808 and Emerald (28), DALZ 1707 (27), Empire and Zeon (25), and finally Meyer (21). DALZ 1701 offers high turfgrass quality under different mowing heights, turfgrass quality retention during drought, high spring greenup in southern locations, winter survival, longer fall color retention in more northern areas, and resistance to dollar spot. DALZ 1802 produces good quality under higher mowing heights, is tolerant to shade and traffic, and offers greater fall color retention than tested cultivars but is best suited for warmer climates, particularly in the southwestern location of Riverside, CA.

Table 12. Seedhead density of DALZ elite hybrids and cultivars.

Entry	Seedhead Density									TPI (9)
	Transition		Southeast		Southcentral			Southwest		
					Dallas,TX			Riverside, CA		
	Stillwater, OK	Manhattan, KS	Gainesville, FL	Jay, FL	Apr	May	Jul	Spring	Fall	
DALZ 1701	9.0 a	6.3	7.0 a	1.3	6.3	7.3	8.0 a	8.3 a	8.7 a	5
DALZ 1707	9.0 a	5.0	1.3	1.0	4.7	5.0	9.0 a	8.3 a	9.0 a	4
DALZ 1808	9.0 a	9.0 a	1.0	1.0	4.7	7.3	9.0 a	7.0 a	2.7	4
DALZ 1802	9.0 a	8.7 a	1.3	1.0	9.0 a	9.0 a	9.0 a	9.0 a	6.7	6
DALZ 1807	9.0 a	6.3	1.3	1.3	6.7	8.0 a	9.0 a	9.0 a	9.0 a	5
Emerald	9.0 a	7.3	5.7 a	1.0	4.3	8.3 a	9.0 a	9.0 a	9.0 a	6
Empire	2.0	7.7	5.0 a	2.0	5.7	6.3	5.3	5.0	9.0 a	2
Meyer	9.0 a	5.3	2.0	1.0	8.0 a	9.0 a	9.0 a	5.7	9.0 a	5
Zeon	9.0 a	7.7	1.0	1.0	7.7 a	9.0 a	9.0 a	9.0 a	9.0 a	6
LSD	0.8	1.1	2.7	1.0	2.2	1.4	1.5	2.1	2.3	
C.V.	6.6	8.3	41.2	38.2	16.3	10.5	12.5	17.2	17.6	

Table 13. Total turfgrass performance index for all traits.

Entry	Turfgrass quality (22)				Traffic Tolerance (9)	Spring Greenup (10)		Winter Survival (4)	Fall Color Retention (12)		Disease resistance (3)	Seedhead Density (9)	Total TPI (68)
	Schedule A	Schedule B	Drought	Shade		Northern/ Transition	Southern		Northern/ Transition	Southern			
DALZ 1701	2	3	3	2	2	2	3	3	4	2	1	5	32
DALZ 1707	1	3	0	2	3	2	2	2	6	2	0	4	27
DALZ 1808	2	3	0	5	0	3	3	2	2	2	2	4	28
DALZ 1802	0	5	0	5	5	1	0	1	5	5	0	6	33
DALZ 1807	0	2	0	3	0	3	0	2	3	3	0	5	21
Emerald	2	3	2	5	0	2	3	1	2	2	0	6	28
Empire	2	1	4	5	2	0	1	2	4	1	1	2	25
Meyer	1	2	0	1	0	2	1	3	3	1	2	5	21
Zeon	2	2	2	5	0	2	2	2	0	2	0	6	25

USGA ID#: 2021-19-743f

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.
- Treatments that included regular dew removal and the use of Zio biological fungicide were initiated in May of 2022 and continued through August 2022 and then trial was repeated in May through August of 2023.
- Annual bluegrass control was initiated at each location and all locations reported significant reductions in the amount of annual bluegrass present.
- In 2022 the primary issue across locations was too much dollar spot which overwhelmed the treatments. In 2023 the primary issue across most locations outside of Rutgers was too little dollar spot as a significant drought lessened dollar spot severity across multiple regions.
- As in 2022, the clearest result across locations in 2023 was the increased dollar spot resistance observed with Coho compared to Shark and Penncross. Within each cultivar there were minimal statistical differences between the dew removal and biocontrol treatments.

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. 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. In both 2022 and 2023 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 (Figure 1). Rhapsody was chosen as the biological fungicide and was applied every 14 days beginning in mid-May and running through the end of August.

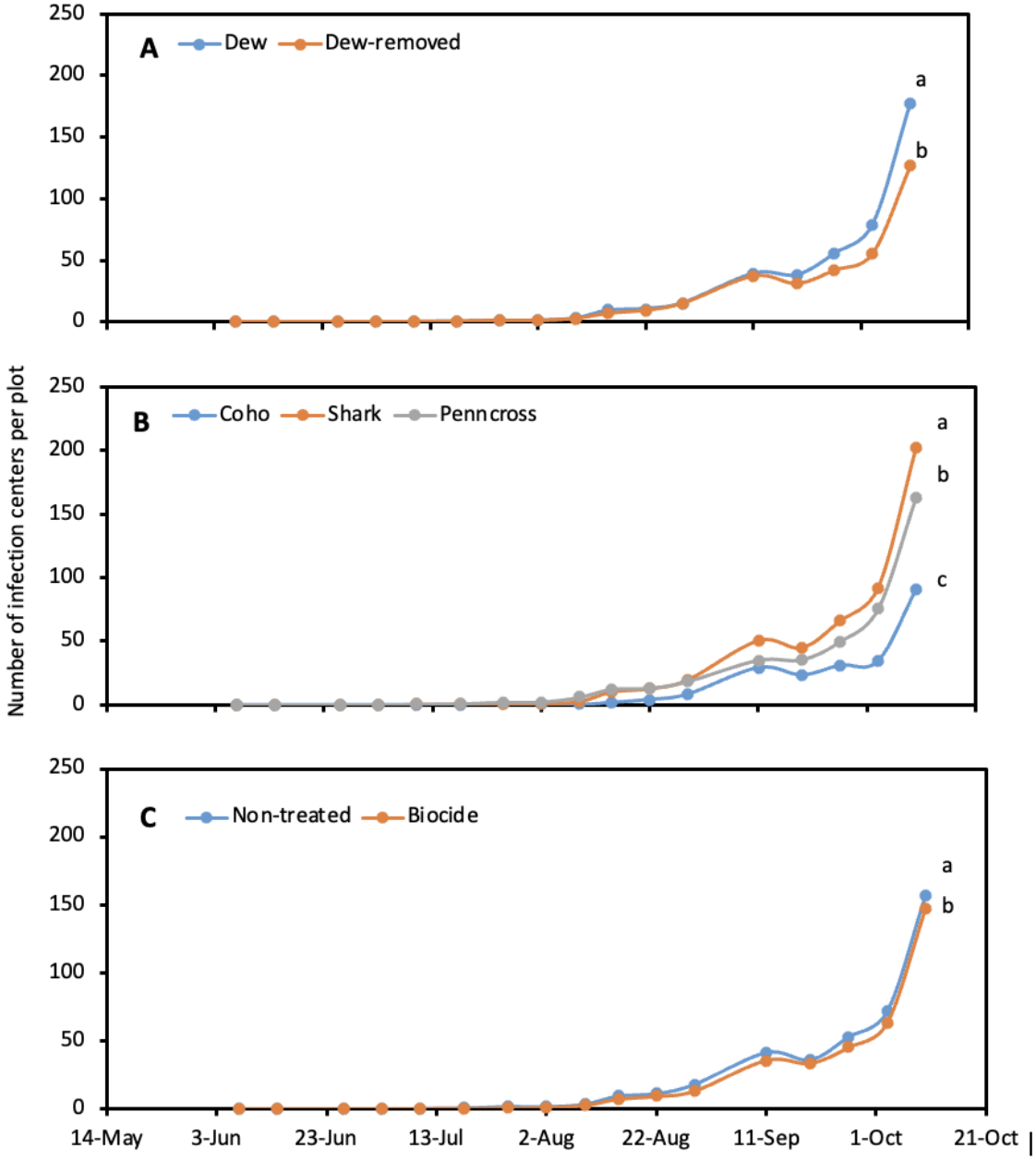
Results: The annual bluegrass suppression efforts were largely successful at each location and much less annual bluegrass was visible across the plots compared to 2022. Though an in-depth data analysis has not yet been conducted, the preliminary results clearly show that cultivar had the largest influence on dollar spot severity with Coho clearly more resistant to dollar spot compared to Shark and Penncross. Within cultivar the results of the dew removal and biocontrol were less clear. At Wisconsin, Michigan State, Kansas, and Massachusetts there were mostly only non-significant differences. At Rutgers where the disease pressure was higher there was a modest reduction in dollar spot on plots where the dew was removed (Figure 2). At Pinehurst only the biocontrol was tested within cultivar and there was only one instance where it provided statistical reductions in dollar spot compared to the non-treated control. Summary reports from each location are available upon request but the report from Wisconsin is available at the following website: https://tdl.wisc.edu/wp-content/blogs.dir/42/files/Interactive%20Pages/2023_Summer/Reports/UWDSHostResistance_2023.pdf.

Future plans: The data collected in the two field seasons so far has indicated that dew removal and biocontrol provide minimal additional dollar spot suppression beyond the resistance of the cultivar. Given the very different results from 2022 and 2023 it may be prudent to conduct this study for a 3rd field season in 2024, which we recognize would be beyond the end of the funding provided with this project. The NC1208 will make this decision at their annual meeting in late January.

Figure 1. Experimental research area following dew removal in East Lansing, MI in 2023.



Figure 2. Results from Rutgers University in 2023 demonstrating strong dollar spot resistance of Coho bentgrass but modest impact of the dew removal and biocontrol treatments.



USGA ID#: 2022-21-764

Title: Environmental and economic comparison between Capillary Hydroponic System and Variable Depth Rootzone to promote sustainable putting green management

Project Leaders: Dr. T.A. Nikolai, Mike Rabe, Dr. W. Zhang, Dr. K. Frank, & Dr. B. Horgan
Affiliation: Michigan State University

Objectives:

1. Determine differences in the amount of irrigation water used between the Capillary Hydroponic System (CHS) and the Variable Depth Rootzones (VDR).
2. Determine the uniformity of soil moisture retention in the CHS and VDR.
3. Gather data including green speed, surface firmness, clipping yield, pest observations, nutrient holding capacity, organic matter build-up, and drainage water nutrient content.

Start Date: 2023
Project Duration: 2-years
Total Funding: $29,876.56

Summary Points:

- CHS greens required significantly less irrigation than VDR.
- Weather conditions impact irrigation differences between the systems.
- The CHS and VDR resulted in insignificant differences regarding green speed, surface firmness, visual appearance, clipping yield, and organic matter content in the first year of the study.

Rationale

United States golf courses use approximately 2.27 trillion liters of water per year for irrigation and water cost is significant (e.g., $1,068/Million L in the pacific region) (Gelernter et al., 2015 Shaddox et al., 2022). The need for water conservation may be further amplified by climate change (Scott et al., 2018). Furthermore, turfgrass quality and playability of golf courses must be maintained or even improved when adopting any new water and nutrient conservation practices.

Methods and Materials

Christopher Wilczynski, American Society of Golf Course Architects, drafted 6 greens with identical undulations and slopes of 1.5, 3, and 5%. Each green was constructed 11 m x 11 m separated by a concave buffer zone 6 m wide east to west and 3.5 m wide north to south to negate the possibility of runoff or overhead irrigation drifting from one green to another. All six research greens were constructed with the same sand conforming to USGA specification.

The site was seeded with T1 creeping bentgrass (*Agrostis stolonifera*) on 15 June 2022 and was grown in with overhead irrigation. Construction included block style irrigation system with heads on each corner of each green. Each Capillary Hydroponic System (CHS) includes a waterproof liner divided into 2 equal sections with a 55-gallon control basin buried beside the green connected to feed pipes that protrude to the middle of each section. The control system

pumps water back and forth between sections every four hours and the control basin also houses an adjustable water table. The Variable Depth Rootzone (VDR) greens diverges from United States Golf Association (USGA) recommendations by decreasing the rootzone depth at the top of slopes (8–10 inches) and increasing it in low lying areas (14-16 inches).

Maintenance practices include mowing six times per week at 3 mm, rolling 3 times per week, and sand topdressing and foliar fertility. The greens had sufficient density to initiate irrigation treatments on 21 June 2023.

Prior to study initiation irrigation triggers were determined by combining visual observations and volumetric moisture content (VMC) measurements with a TDR-350. Greens displayed visible wilting at 7% VMC, therefore 8%VMC became the irrigation trigger. VDR returned 80% relative daily evapotranspiration via overhead irrigation while CHS system was met by setting auto fill when the water table fell below 20 cm below the surface of the lowest spot (Figure 1). CHS greens only received overhead irrigation following sand topdressing, fertility, and fungicide applications receiving the same volume of irrigation as VDR on those occasions.

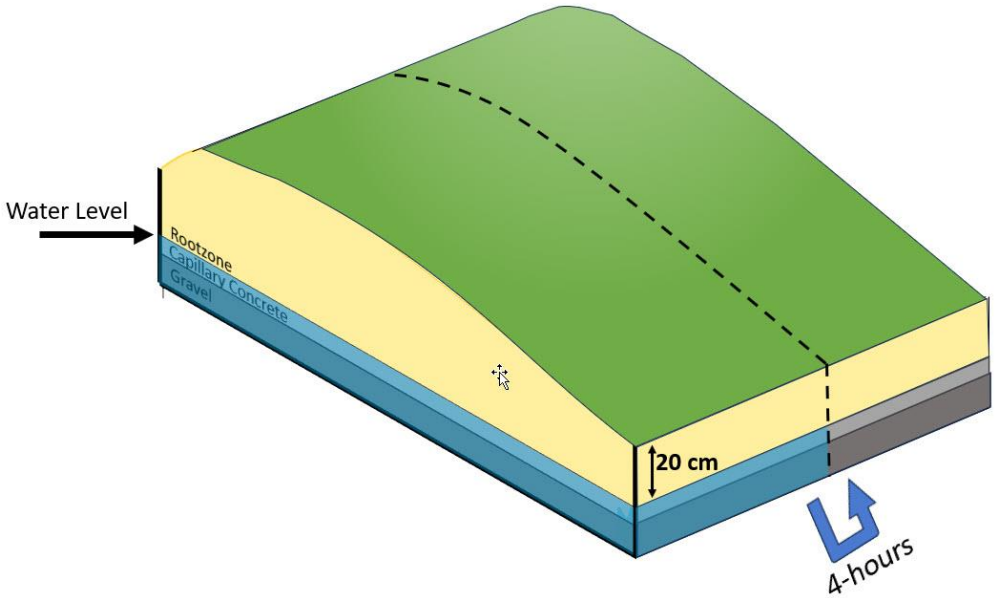


Figure 1 A cross section of the Capillary Hydroponic System

Each green was constructed with a drainage line and following significant rain events samples were collected and analyzed. When pests were observed data was collected and curative applications were made. Soil tests were conducted by collecting nine 19 mm diameter cores 7.5 cm deep and clippings yield was measured by drying and weighing clippings from a single pass with a Toro Flex 2100 in the identical location spanning elevation changes on each green.

Other data collection included green speed measurements with a USGA Stimpmeter, surface firmness on six locations on each green with a USGA TruFirm, volumetric moisture content monitored weekly at two depths, 7.5 cm, and 20 cm, with a handheld TDR 350 across the greens surface in a 1.5 m x 1.5 m grid to map uniformity of water distribution.

Results to Date

CHS greens required 60% less irrigation water to prevent surface wilting compared to TDR greens over a ten-week period in summer 2023 (Figure 2). Within that time period three 11-day intervals were selected to represent three weather conditions:

1. low precipitation (0.14 cm/day) and high Relative Potential Evapotranspiration (RPET) (0.31 cm/day) resulting in CHS greens needing 44% less irrigation than the VDR greens
2. high precipitation (0.44 cm/day) and high RPET (0.31 cm/day) resulting in CHS greens using 76% less water from irrigation than the VDR greens
3. high precipitation (0.77 cm/day) and low RPET (0.25 cm/day) CHS greens required 83% less water for irrigation than the VDR greens

There was no significant difference regarding soil chemical analysis or organic matter content, clipping yield, green speed, or surface firmness. On a couple of occasions CHS greens resulted in better quality and color but the differences were not meaningful. VDR consistently demonstrated a more uniform volumetric moisture content compared to the CHS and there were some differences in nutrient content in the drainage water, but more data is required to make any conclusions.

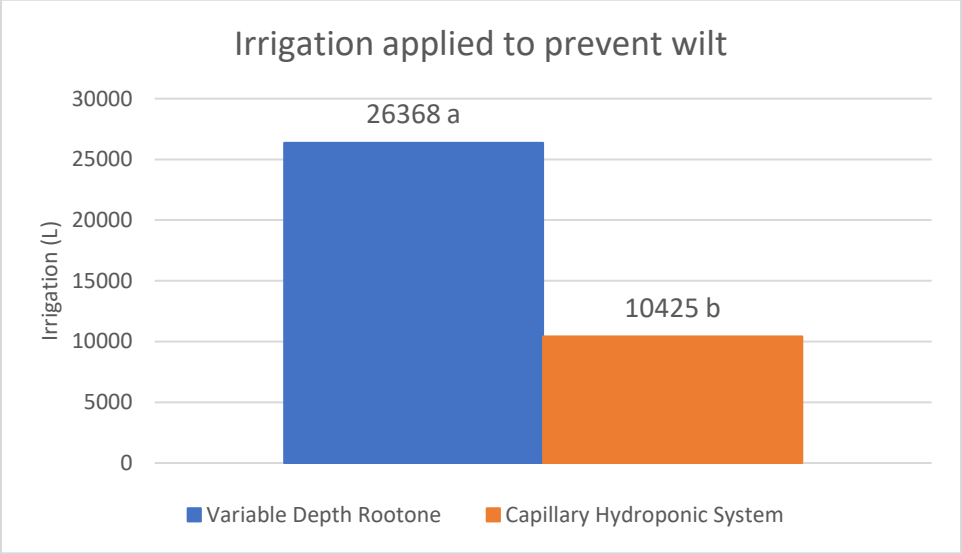


Figure 2. Irrigation applied to Variable Depth Rootzone and Capillary Hydroponic System research greens between June 21 - September 21, 2023. Means with same letter are not significantly different (α=0.05).

Future expectations

In year one the most significant difference between CHS and VDR is the amount of irrigation water applied. It seems logical to hypothesize that greens with identical rootzone particle size and surface contours that the green receiving more than twice as much irrigation would result in greater organic matter build up, but that was not the case. In year two we expect the turfgrass density to increase as plants mature and this may lead to a difference in organic matter/thatch build up. If this is the case, we expect differences in surface firmness, pests, clipping yield, and

soil chemical tests. However, it could be that since we are maintaining the CHS and VDR greens at a minimal amount of irrigation, to maintain 8% VMC, that there will be no changes in organic matter content and therefore very little meaningful difference between the systems going forward other than the amount of irrigation water applied, which could change as the grass matures. As the study progresses, we will attempt to calculate the amount of water that is actually used by the greens to assist in our understanding of the systems, and we will also generate an economic comparison between the systems.

Literature Cited

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Scott, D., Rutty, M., & Peister, C. (2018). Climate variability and water use on golf courses: optimization opportunities for a warmer future. Journal of Sustainable Tourism, 26(8), 1453-1467. doi:10.1080/09669582.2018.1459629

Shaddox, T.W., Unruh, J.B., Johnson, M.E., Brown, C.D. & Stacey, G. 2022 Water use and management practices on U.S. golf courses *Crop Forage Turfgrass Manage.* 8 e20182 https://doi.org/10.1002/cft2.20182

USGA ID#: 2022-24-767

Title: Measuring golf course irrigation acreage in the Phoenix Active Management Area

Project Leader: Arizona Alliance for Golf

Objectives: Provide accurate measurement of 154 golf courses to determine the acer-feet of water applied per acre in a highly sensitive water use environment

Start Date: 2022

Project Length: 1-year

Total Funding: $65,000

Turf Survey Methodology:

CourseVision provided an “irrigated turf survey” of golf properties in Maricopa County, AZ. This survey was done between October 2022 and April 2023 using:

- High resolution (3” pixel or better) aerial photos of the properties from 2022.
- A library of historical aerial photos images for reference.
- Standard geographic information system (GIS) tools for area measurements.
- Property boundary data based on Maricopa County parcel lines.

The properties were surveyed by creating detailed areas of turf measurement using GIS tools and recording these results in an Excel spreadsheet. Each area of the property was also screen captured to provide a visual record of the turf areas.

For purposes of this survey, the following guidelines were used to determine turf that was in the irrigation coverage area:

- The measurement encompasses all irrigated turf (golf, practice, and clubhouse) within the property boundaries.
- Turf includes all tees, fairway, rough, approaches, greens, and clubhouse lawns.
- Bunkers are included in turf acreage totals.
- Water features / creeks surrounded by or touching turf are included in the turf total.
- Lakes/ponds are not included in turf totals, but were captured separately.

- Cart paths with turf on both sides are included in the turf total.

Finding Bullet Points:

- 154 courses were measured from updated aerials beginning in 2022
- Average facility turf acreage is 110 acres
    - Average 18 hole facility turf acreage is 93.3 acres (71% of facilities)
- Average acre per hole 5.0
- Average acre-feet used per facility: 590 in 2022
    - Average acre-feet used per 18 hole facility in 2022: 486.67
- Average acre-feet per acre: 5.41 in 2022
- Water sources used as an average from 2018-2022: 45% ground water, 22% effluent, 16% CAP (California-Arizona Project), 6% other

Summary: The Arizona Department of Water Resources (ADWR) determines individual golf course water allocations with a progression of legislative required Management Plans. The MP's apply to golf courses within 5 Active Management Areas (AMA). The State is currently operating under the 4th MP with the 5th MP beginning 1/1/2025. The MP's requires each individual facility to submit an annual report stating total turf acres, low water landscape planted acres, water surface acres and water use. These are mostly self-reported statistics. In 2020, the ADWR began public discussions on specific requirements for the 5th MP. With golf course water use becoming such an important conversation point for the State of Arizona, the Arizona Alliance for Golf (AAG) was established to work in partnership with the State to help develop a fair and equitable plan that provides necessary water to maintain reasonable golf course conditions but to also continue looking for ways to conserve water. This discussion continued for a couple years with a final plan adopted in 2022. In working with the ADWR, the AAG found that much of the State's property data did not accurately measure each facility total irrigation coverage so as a result, water use was not correctly measured (as acre-feet used per irrigated acre). The AAG received funding from the USGA, GCSAA and Arizona Junior Golf to accurately measure individual facilities in the Phoenix AMA. This encompasses over 154 facilities on one of the most visible and important golf regions on the country. The completion of the measuring process found inconsistent course size data and resulted in inaccurate water use statistics for a number of facilities. The AAG corrected the data and

with the addition of the water use numbers submitted by the facilities to the State, was able to determine more accurate water use for the Phoenix AMA.

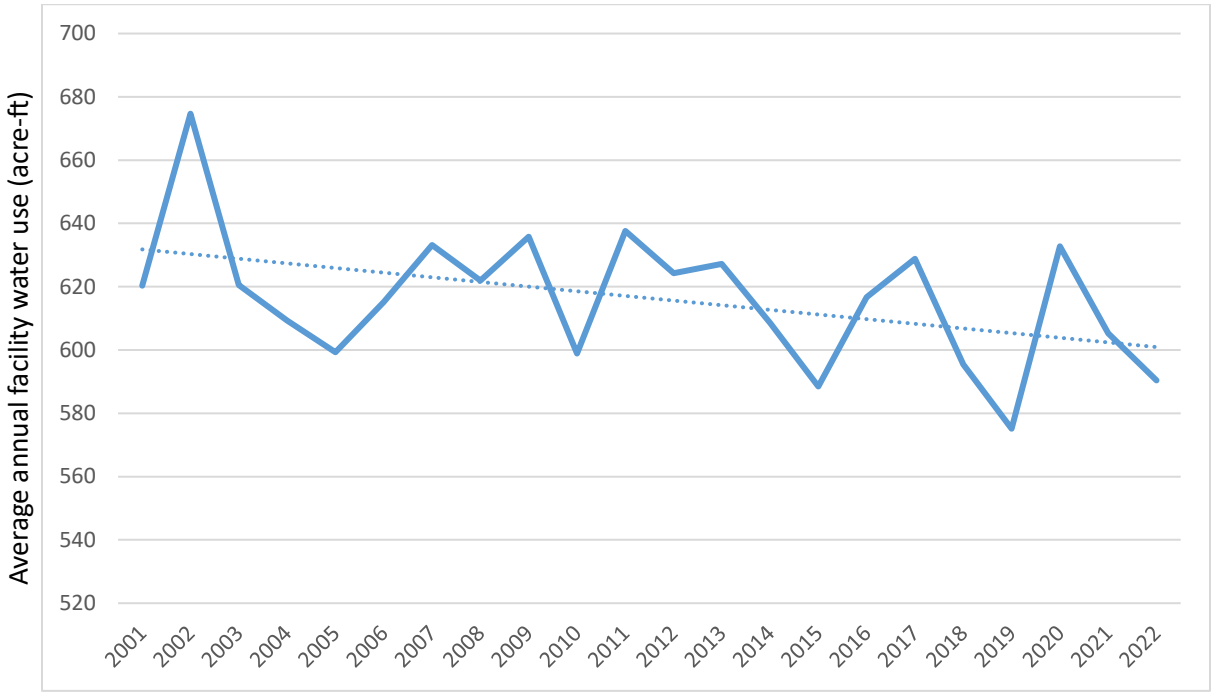


Figure 1. Average annual water use per golf course facility in the Phoenix Active Management Area from 2001 to 2022. For perspective, from 2018-2022, the average facility irrigated approximately 110 acres of turfgrass, yielding an average per acre water use rate of 5.76 acre-ft over the period.

USGA ID#: 2024-13-823

Title: Evaluation of gypsum and fertilizer products for enhanced bermudagrass performance under salinity conditions in sandy soils

Project Leaders: Alejandra Sierra and Marco Schiavon
Affiliation: Fort Lauderdale Research and Education Center UF-IFAS

Start Date: December 2023
Duration: 1 year
Total Funding: $10,000

OBJECTIVE:

1. Evaluate the effect of two fertilizers (ammonium sulphate and calcium nitrate) used in combination with gypsum or by themselves, to counteract the effect of Na hazard and bicarbonates in Florida sandy soils.

TREATMENTS:

Trt No.	Treatment Code	Product Description
1	CAMS	Control ammonium sulfate
2	CCaNO3	Control calcium nitrate
3	SAMS	Salt + ammonium sulfate
4	SCaNO3	Salt + calcium nitrate
5	GAMS	Gypsum + ammonium sulfate
6	GCaNO3	Gypsum + calcium nitrate
7	SGAMS	Salt + gypsum + ammonium sulfate
8	SGCaNO3	Salt + gypsum + calcium nitrate

MATERIALS AND METHODS:

The experiment was conducted on a mature stand of 'Celebration' bermudagrass located at the University of Florida's Fort Lauderdale Research and Education Center (26.0840° N, 80.2372° W). Table salt (NaCl) was dissolved in water and applied at a rate of 5 lb NaCl/1000 ft^2 and fertilizers were applied at a rate of 0.5 lb N/1000 ft^2. Salt and fertilizer were applied every two weeks, alternating between each other, for a total of four applications each. Initial salt treatment was applied on December 6, 2023, while fertilizer treatments were initially applied on December 13, 2023. Gypsum was applied at a rate of 230 lb gypsum/1000 ft^2 and divided in two applications at mid-point (December 27, 2023) and prior to completion of the study (January 24, 2024). Area was irrigated after each treatment application. Four replications of 5' x 5' plots in a split-split-split design were used for the trial, with salt serving as a main plot, gypsum as a sub-plot and fertilizer treatment as a sub-sub-plot (Figure 1). Turf was mowed two times per week at 0.58 in with clippings returned. Area was irrigated daily to prevent drought stress. Weather data was retrieved from FAWN weather station locate 1000 ft away from the experiment.

Data was collected weekly. Turf quality was measured on a 1-9 scale with 9=dark green dense turf, 1=dead/brown turf, and 6=minimally acceptable turf. Normalized difference vegetation index was assessed using a handheld sensor. Percent green cover and DGCI were assessed through digital image analysis from one image taken per plot. Turf injury was measured on a scale from 0 to 100% where 0% = nonvisible, 100% = dead plant, volumetric water content and electrical conductivity (EC) data were collected starting after the first salt and fertilizer applications and were measured at a 3-in depth using a time domain reflectance sensor and were analyzed as an average of three readings per plot. Soil cores were collected prior to trial initiation and upon completion. Data were subject to statistical analysis using SAS to determine treatment effect at $P<0.05$.

Main plot	Salt	No Salt
	No Salt	Salt
	No Salt	Salt
	Salt	No Salt

Sub-plot	Gypsum	No Gypsum	Gypsum	No Gypsum
	Gypsum	No Gypsum	No Gypsum	Gypsum
	No Gypsum	Gypsum	Gypsum	No Gypsum
	No Gypsum	Gypsum	Gypsum	No Gypsum

Sub-sub-plot	CaNO3	AMS	AMS	CaNO3	AMS	CaNO3	CaNO3	AMS
	AMS	CaNO3	CaNO3	AMS	CaNO3	AMS	CaNO3	AMS
	CaNO3	AMS	CaNO3	AMS	AMS	CaNO3	AMS	CaNO3
	AMS	CaNO3	AMS	CaNO3	CaNO3	AMS	AMS	CaNO3

Figure 1. Treatment distribution.

METEOROLOGICAL:

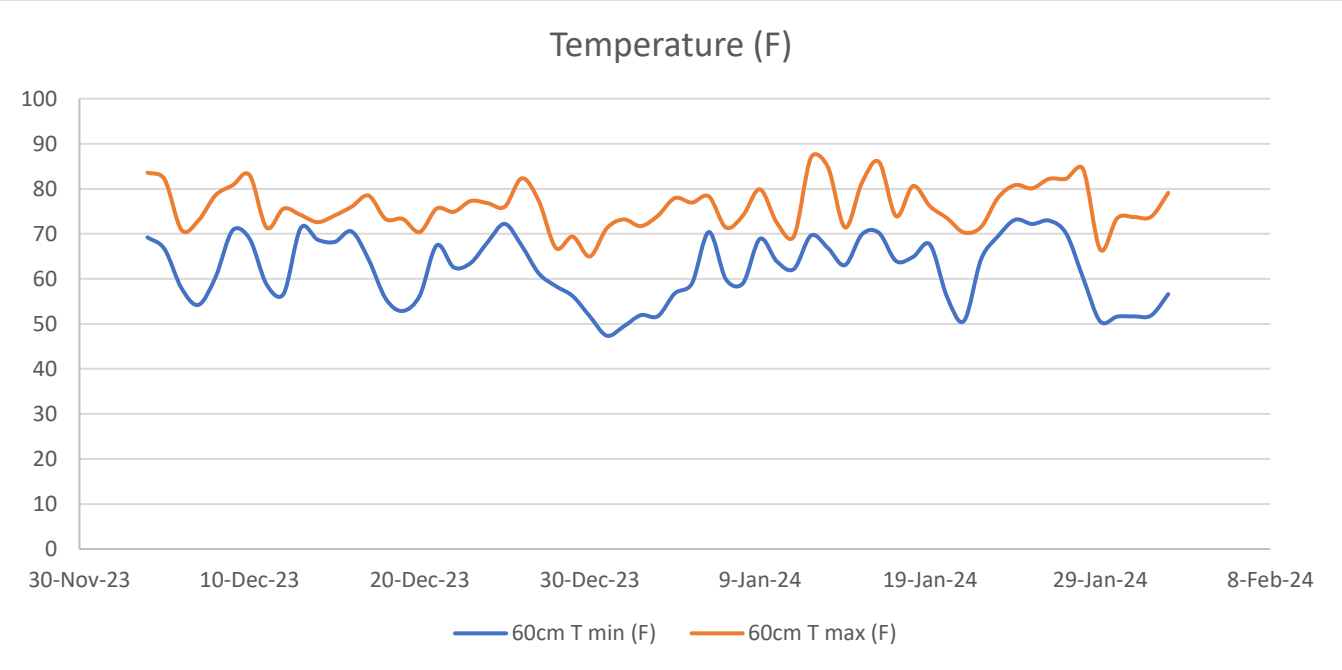


Figure 2. Minimum and maximum temperature during reported trial period.

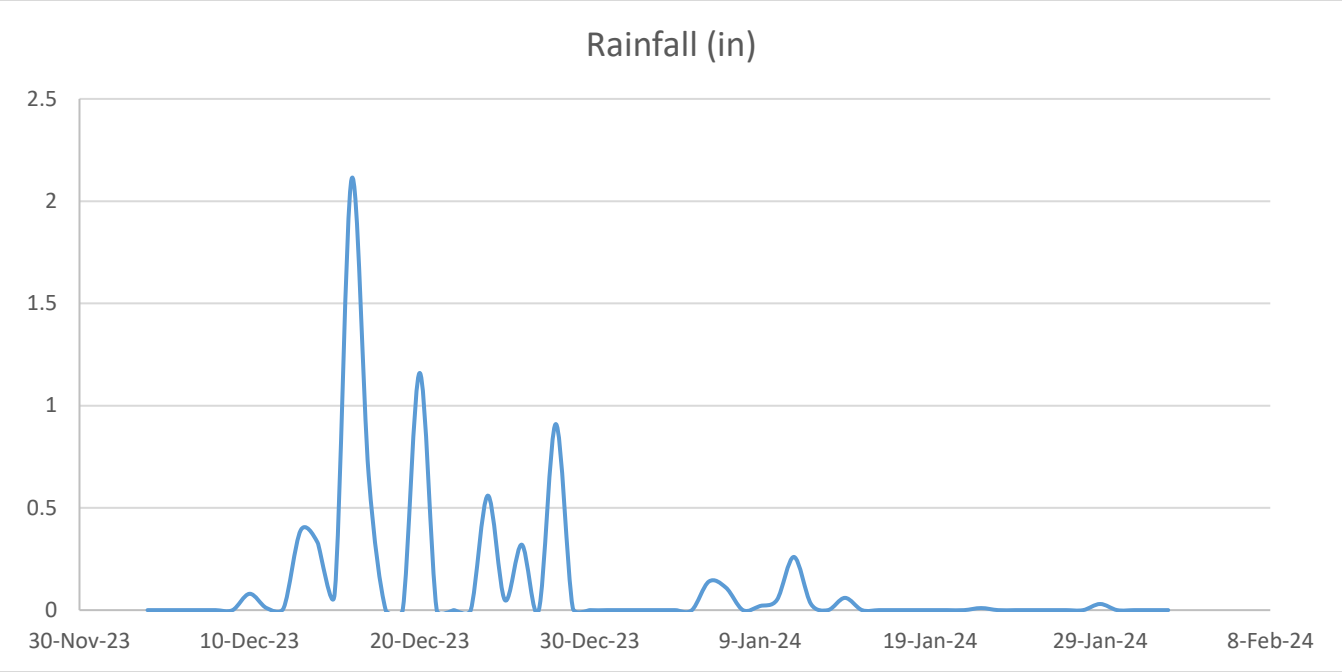


Figure 3. Precipitation during reported trial period.

RESULTS:

Gypsum did not affect turfgrass performance during the trial period. Plots fertilized with AMS showed a higher quality than plots fertilized with calcium nitrate after fertilization started, however no differences were found toward the end of the trial (Figure 4). Salt had a negative effect on turfgrass quality, reducing it below minimal acceptable threshold (6) until January and quality when salt was applied to bermudagrass was consistently lower (Figure 5). A similar trend was observed for NDVI, were plots with salt applied yielded lower NDVI than plots without salt application (Figure 6). After the second salt application, percent cover of plots that received no salt was consistently higher than plots applied with salt (Figure 7). Treatments had no effect on DGCI. Salt caused turfgrass injury, especially after the first and second application, however, it seems that turfgrass adapted to salinity conditions as turfgrass injury from salt declined in the second half of the experiment (Figure 8). Volumetric water content was affected by the interaction between salt and gypsum applications. Throughout the experiment, plots that received salt and no gypsum had a similar VWC to plots that received no salt nor gypsum; however, after the last salt application, VWC of plots where salt was applied increased (Figure 9). Salt applications aided gypsum in maintaining a higher VWC (Figure 9). A similar trend was observed for electrical conductivity with plot that received gypsum regardless of salt application, showing higher electrical conductivity than plots that received no gypsum (Figure 10).

Treatments had no effect on P, Mg and Ca concentration (Table 1). Potassium concentration and SAR were higher in plots applied with salt (Table 2). The interaction between salt and fertilizer affected Na concentration (Table 3). Plots receiving both salt and AMS had the highest Na concentration, followed plots where salt and $CaNO_3$ were applied. No differences were found among fertilizer treatments that received no salt. Gypsum application affected soil pH, soil EC and bicarbonates concentration (Table 4). Application of gypsum lowered pH and bicarbonate concentrations by the end of the trial, but increased EC. Soil pH and EC were also affected by fertilizer source (Table 5). Plots fertilized with AMS showed a lower pH compared to plot fertilized with $CaNO_3$, but higher EC. Soil

salinity levels remained under dangerous threshold for bermudagrass growth, hence turfgrass injury was caused by direct application of salt to the leaves. Results suggest that gypsum application in sandy soils brings little to no benefits to turfgrass and soil, and fertilization alone can help bermudagrass withstand salt injury. More research is needed to investigate long term effects of salt application on bermudagrass fairway to simulate worst case scenarios, and to assess effect of precipitation on soil properties. Pictures of experiment progression are attached at the end of this report.

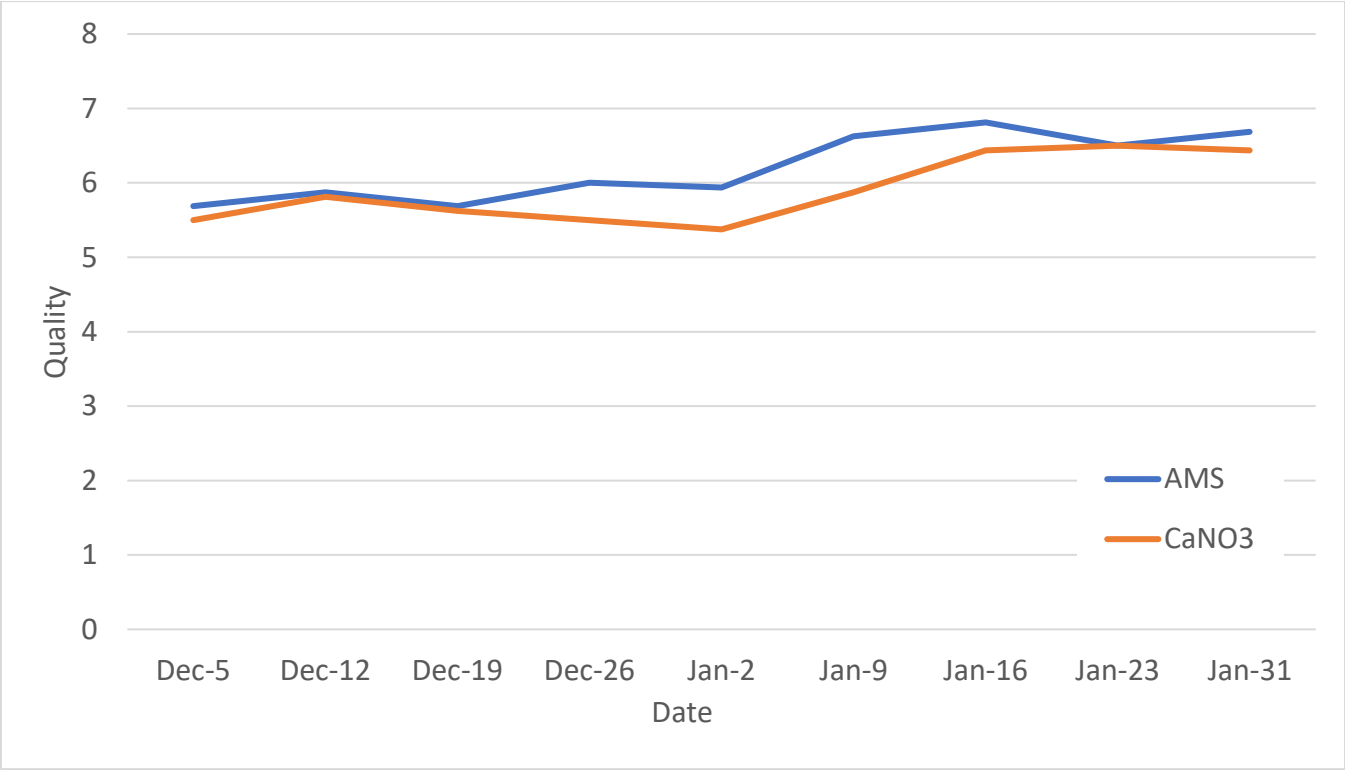


Figure 4. Effect of fertilizer on turfgrass quality.

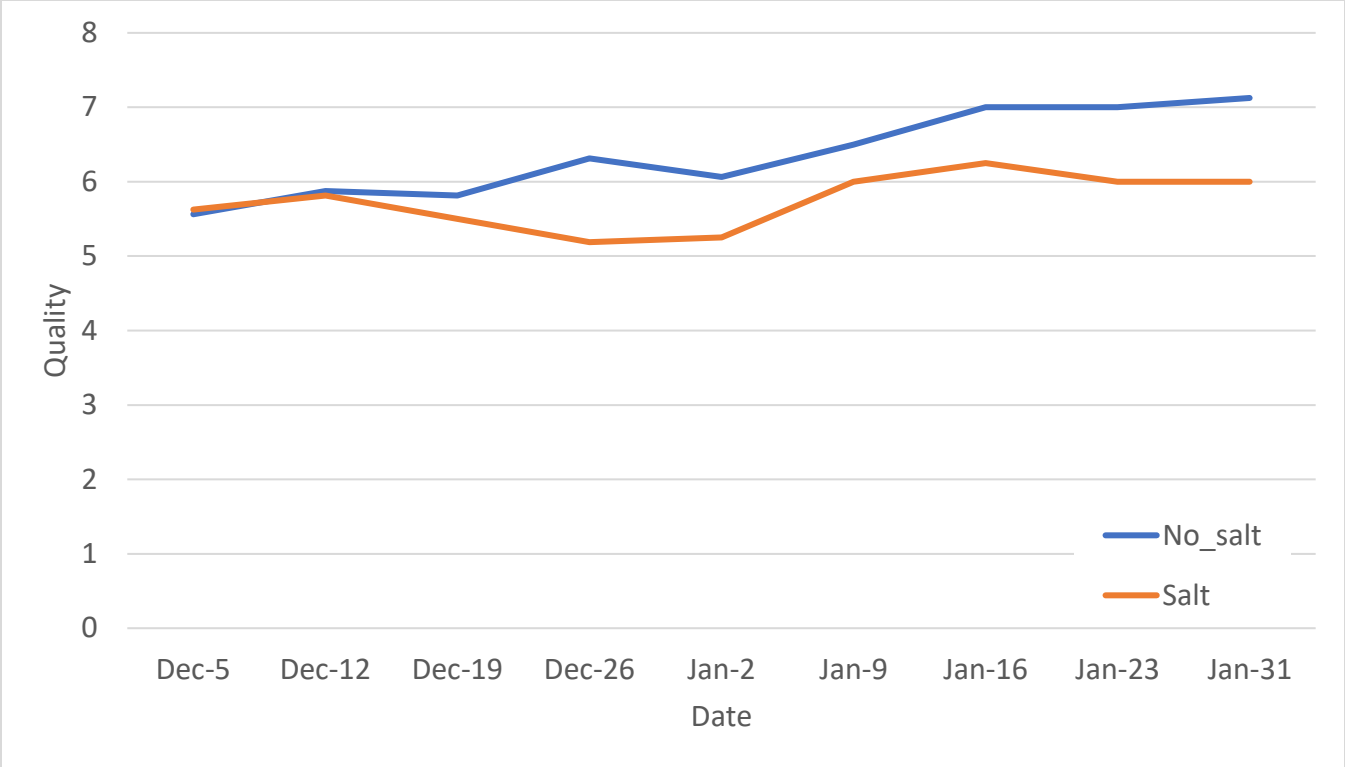


Figure 5. Effect of salt on turfgrass quality.

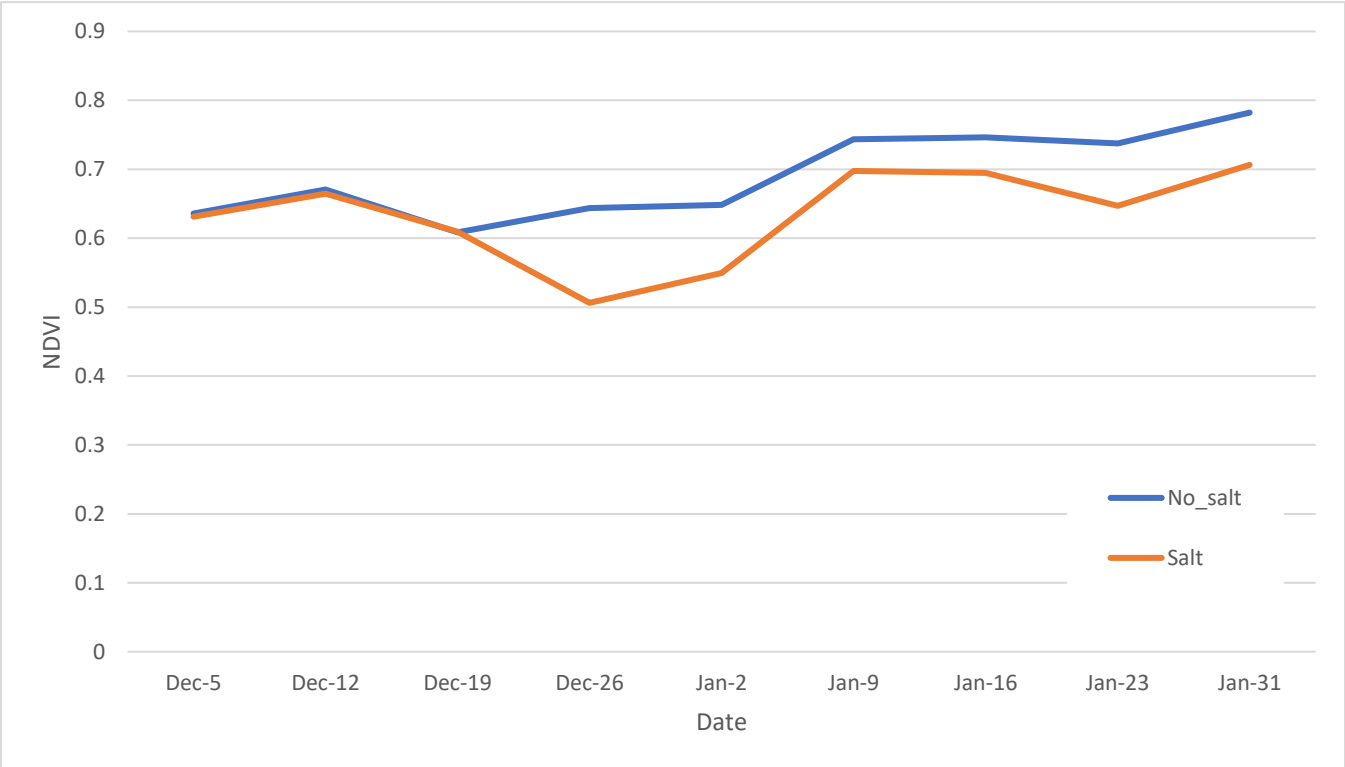


Figure 6. Effect of salt on turfgrass NDVI.

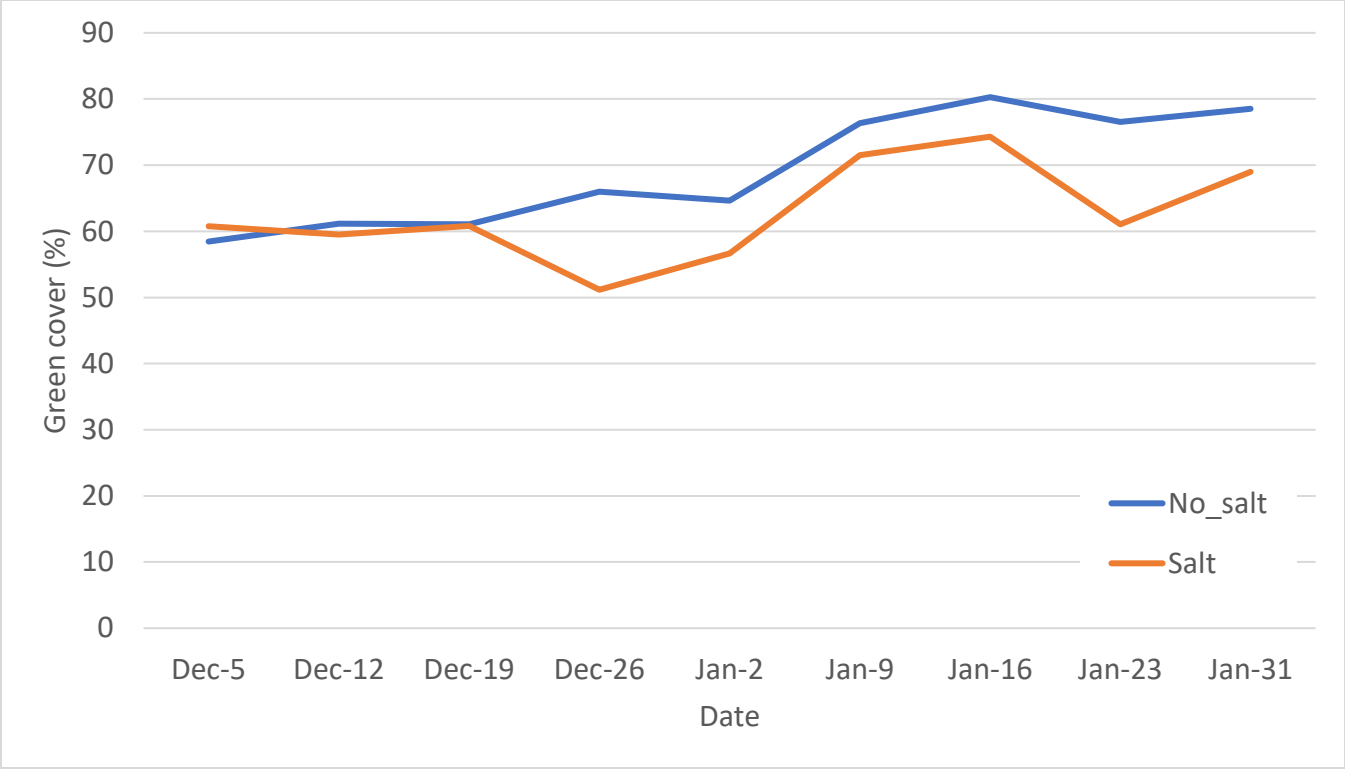


Figure 7. Effect of salt on percent green cover.

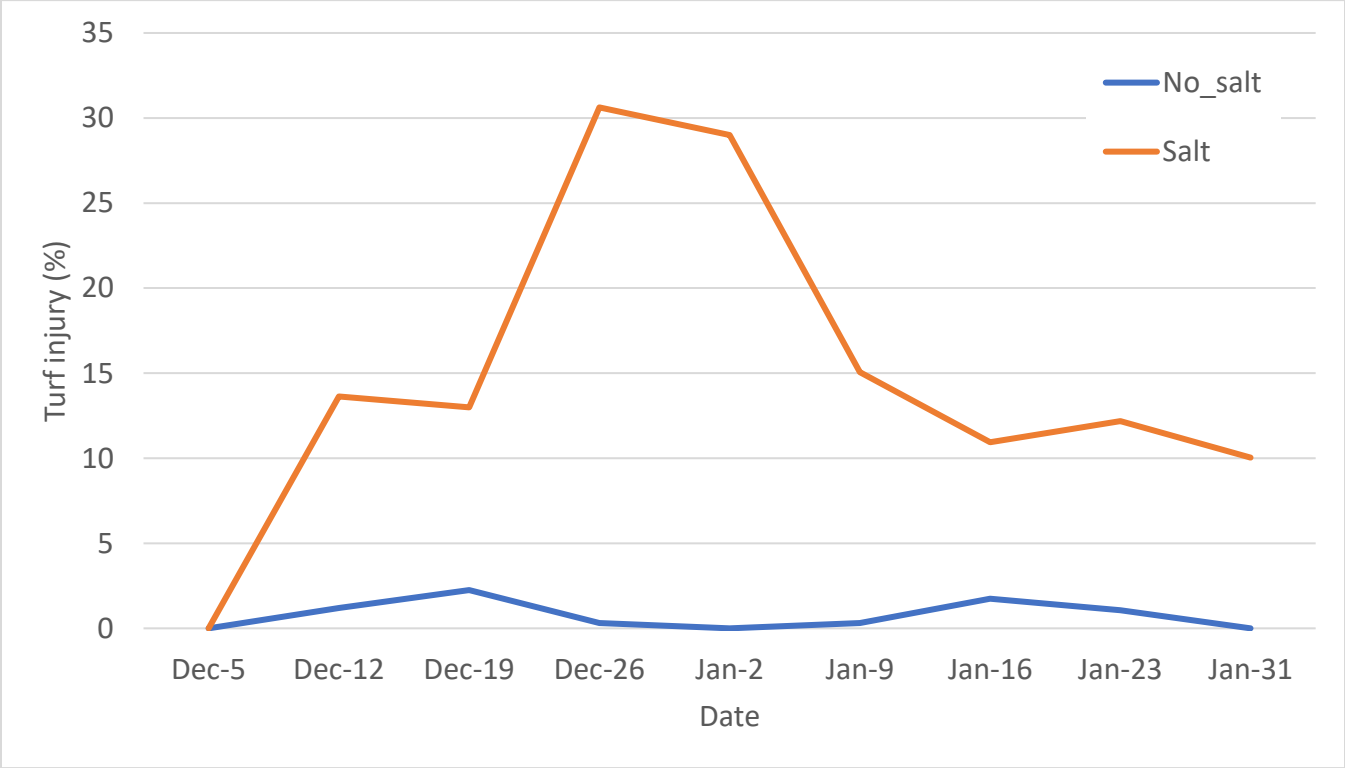


Figure 8. Effect of salt on turfgrass injury.

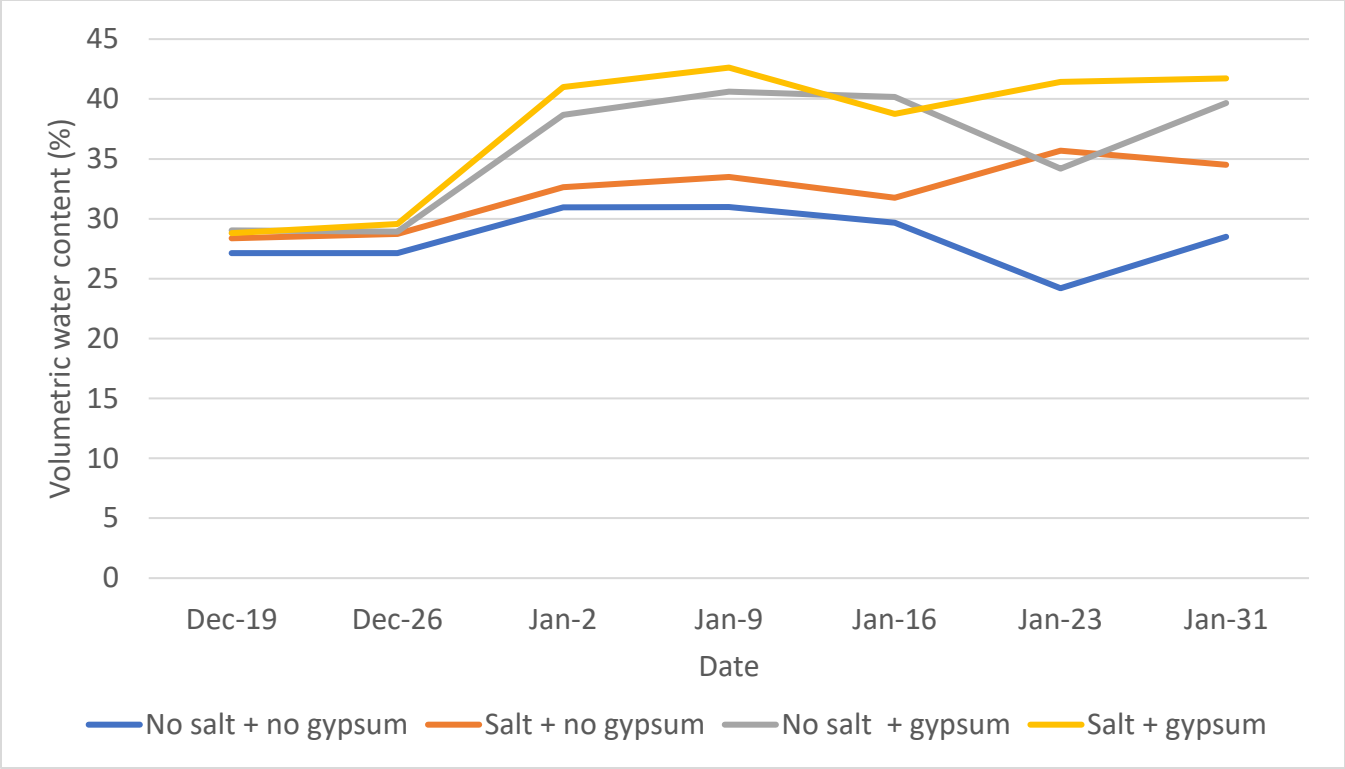


Figure 9. Effect of salt and gypsum on soil volumetric water content.

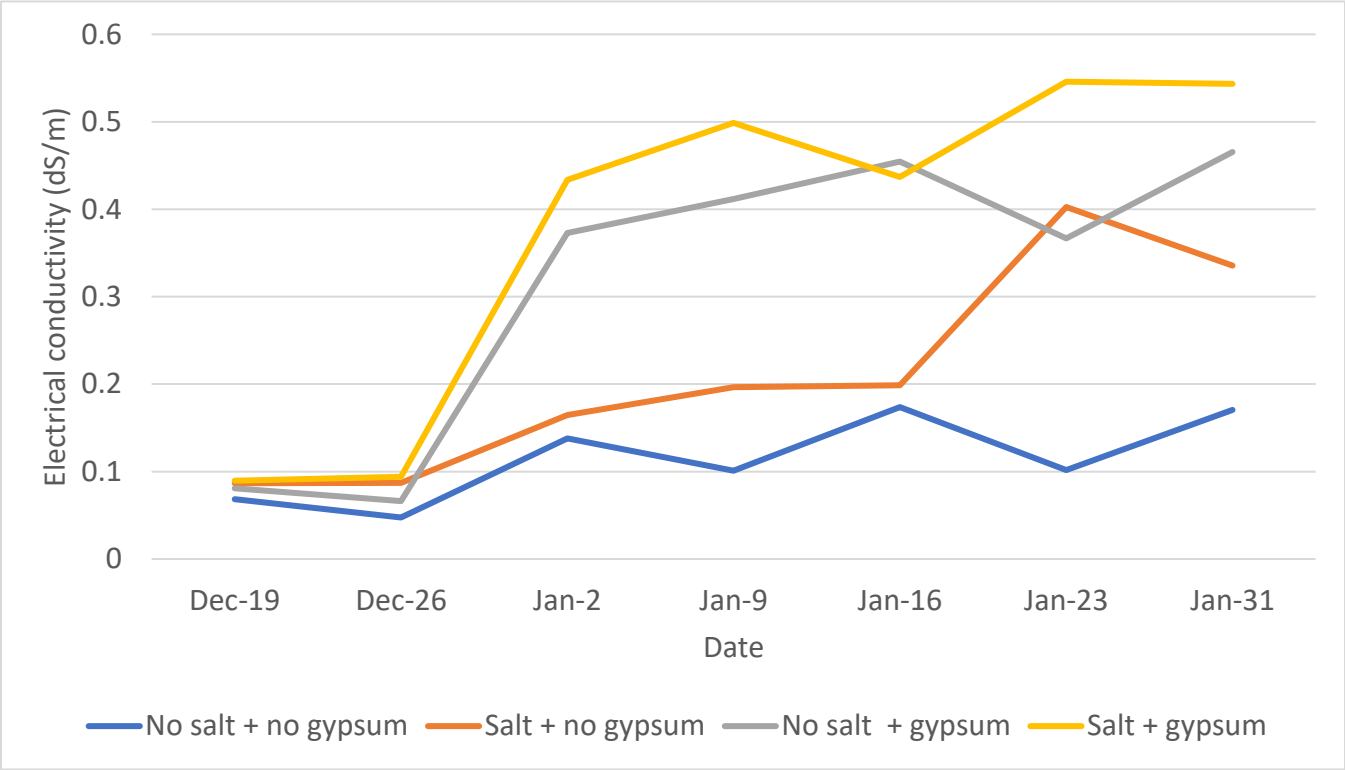


Figure 10. Effect of salt and gypsum on soil electrical conductivity.

Table 1. Average concentration of P, Mg and Ca (lb/a) before treatment application and after treatment application.

	P (lb/a)		Mg (lb/a)		Ca (lb/a)	
Treatment	Pre-trial	Post-trial	Pre-trial	Post-trial	Pre-trial	Post-trial
AMS	199.0	214.5	77.0	79.8	4368	4411
CaNO3	218.0	246.5	74.0	79.8	4004	4554
Gypsum+AMS	208.0	216.3	73.0	84.5	4083	4692
Gypsum+$CaNO_3$	237.0	259.8	72.0	83.8	3949	4485
Salt+AMS	244.0	266.5	79.0	82.0	4103	4385
Salt+CaNO3	227.0	225.0	85.0	81.8	4455	4636
Salt+Gypsum+AMS	246.0	259.0	70.0	83.3	4111	4629
Salt+Gypsum+CaNO3	194.0	203.3	67.0	80.8	3912	4547

Table 2. Salt effect on K concentrations (lb/a) and Sodium Absorption Ratio (SAR).

Treatment	K (lb/a)		SAR	
	Pre-trial	Post-trial	Pre-trial	Post-trial
No salt	34.5	21.0 b	0.11	0.16 b
Salt	37.3	27.4 a	0.09	0.43 a
Significance	**N/A**	***	**N/A**	***

Table 3. Effect of salt and fertilizer treatments on Na concentration.

Treatment	Na (lb/a)	
	Pre-trial	Post-trial
No salt		
AMS	69	270 c
CaNO3	60	279 c
Salt		
AMS	62	1582 a
CaNO3	64	1388 b
Significance	**N/A**	**

Table 4. Effect of gypsum on soil pH, electrical conductivity, and bicarbonates.

Treatment	pH		EC (dS/m)		Bicarbonates (meq/L)	
	Pre-trial	Post-trial	Pre-trial	Post-trial	Pre-trial	Post-trial
Gypsum	7.1	6.6 b	0.07	0.63 a	1.53	1.16 b
No gypsum	7.1	6.9 a	0.07	0.22 b	1.37	1.36 a
Significance	**N/A**	***	**N/A**	***	**N/A**	***

Table 5. Effect of fertilizers on soil pH and electrical conductivity.

	pH		EC (dS/m)	
Treatment	Pre-trial	Post-trial	Pre-trial	Post-trial
AMS	7.1	6.7 b	0.07	0.47 a
CaNO3	7.1	6.9 a	0.07	0.39 b
Significance	**N/A**	**	**N/A**	**

PICTURES OF PROGRESS

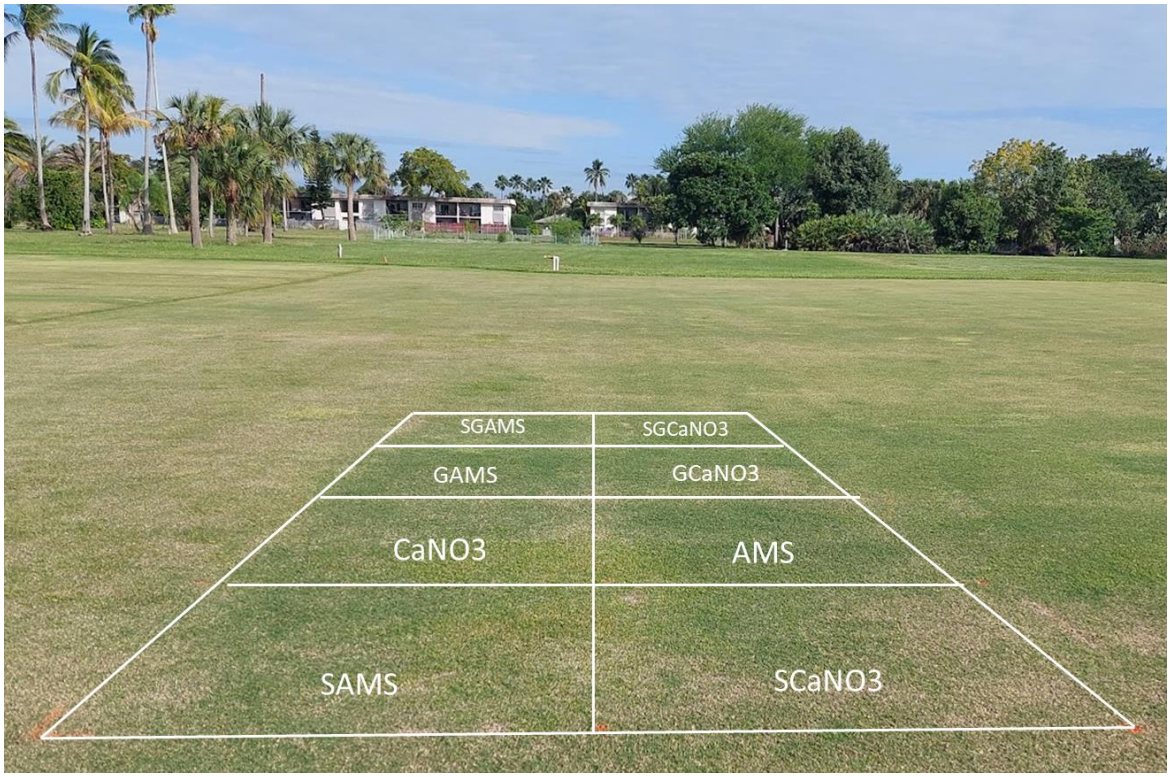
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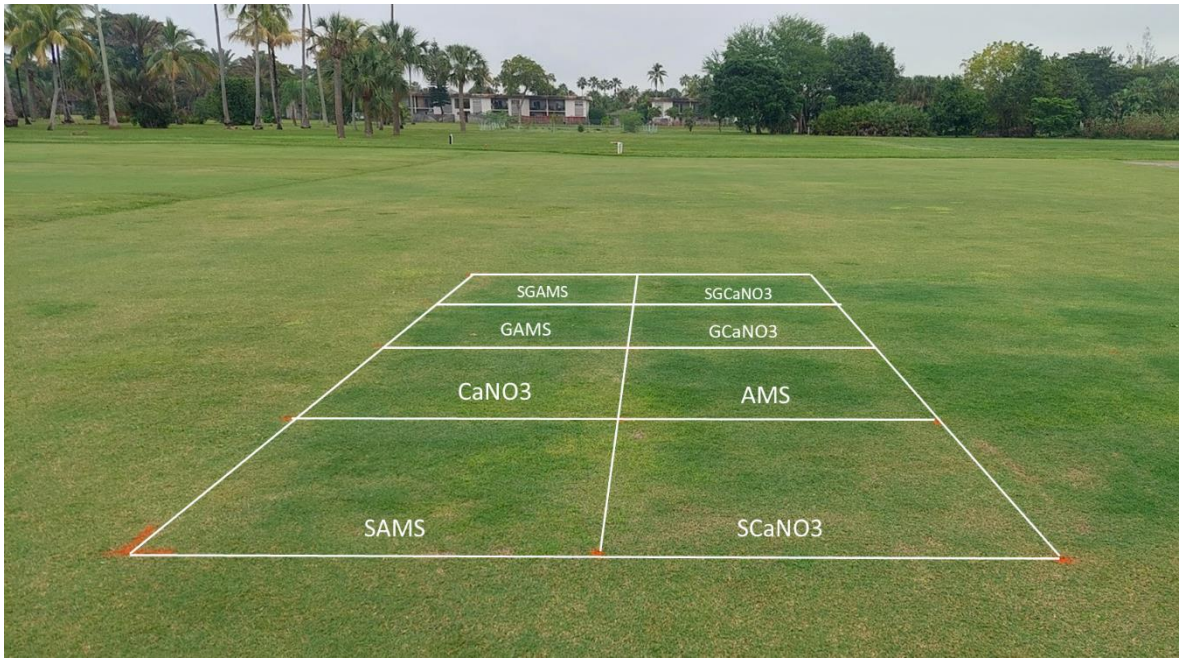
December 26,2023 (before gypsum application)



January 4, 2024 (after gypsum application)



January 11, 2024



February 2, 2024



USGA ID#: 2023-39-806

Title: Does the Right Divot Pattern Actually Increase Recovery?

Project Leaders: Aaron Patton and Naba Amgain
Affiliation: Purdue University

Objective: Quantify the impact of three common divot patterns on available tee space, divot mix needs, and divot recovery.

Start date: 7/1/2023
Project duration: 2 years (01/01/2023-12/31/2024)
Total funding: $10,000

Summary points

- Divot pattern impacts available tee space, divoted turf percent and divot recovery.
- Although scattered pattern had a higher area of impact and a higher percentage of divoted turf, this divot type was fastest to recover. This pattern would work well when sufficient driving range tee space is available.
- Concentrated divots had a lower area of impact and percentage of divoted turf, but required the most time to recover.
- The linear divot pattern appears to be the best divot pattern option as it had a lower area of impact and divoted turf than scattered pattern and a higher recovery rate than concentrated pattern. This pattern would work best when driving range tee space is limited which is likely the case for most.

Summary Text:

Golfers warm-up and practice on driving range tees by hitting various clubs causing severe damage through the creation of multiple divots. Golf course superintendents shift hitting positions on the range tee to provide a fresh surface for the golfers while simultaneously allowing the recovery of the damaged portion of the tee. Divots can be of different shapes and sizes depending on the club used and the divot pattern used by the golfer. Golf courses commonly post signage related to the preferred divot pattern (Figure 1, Table 1). The linear divot pattern is preferred and recommended by the USGA because it “removes the least amount of turf” and “promotes quick recovery.” However, neither a removal of less turf or a faster recovery has been documented beyond anecdotal accounts. Faster divot recovery is very important for golf courses to use driving range tee space efficiently.

Table 1. Description of divot patterns shown in Figure 1.

Divot pattern	Description
scattered	A scattered divot pattern removes the most amount of turf because a full divot is removed with every swing. Scattering divots results in the most turf loss and uses up the largest area of a tee stall. This forces the golf facility to rotate tee stalls most frequently and often results in an inefficient use of the tee.
concentrated	A concentrated divot pattern removes all turf in a given area. While this approach does not necessarily result in a full-sized divot removed with every swing, by creating a large void in the turf canopy there is little opportunity for timely turf recovery.

linear	The linear divot pattern involves placing each shot directly behind the previous divot. In so doing, a linear pattern is created and only a small amount of turf is removed with each swing. This can usually be done for 15 to 20 shots before moving sideways to create a new line of divots. So long as a minimum of 4 inches of live turf is preserved between strips of divots, the turf will recover quickly. Because this divot pattern removes the least amount of turf and promotes quick recovery, it is the preferred method.

Descriptions from McClellan, 2014.

We conducted an experiment in 2023 at the W.H. Daniel Turfgrass Research and Diagnostic Center in West Lafayette, IN on a 'Penncross' creeping bentgrass fairway mown at 0.5". The site was established in 2012 and received 2 lbs N/1000 sq. ft (applied over three applications annually). The site was also treated with fungicide to ensure 100% green turf to quantify divot injury and recovery using digital image analysis.

Three divot patterns were created by four single digit handicap golfers. Each golfer struck 10 full shots with a sand wedge (54-56 degrees). The treatments were arranged in randomized complete block design with three replications of each divot pattern. In total, there were 39 plots [3 blocks × (4 golfers x 3 divot patterns, plus a nontreated plot)] (Figure 2). Each plot was randomly assigned a golfer and pattern. Golfers were instructed to hit 10 shots from each plot per the prescribed pattern. Each golfer hit 90 total sand wedges (3 reps, 3 patterns, 10 golf balls for each plot) on August 2, 2023 to initiate the experiment.



Concentrated divots	Scattered divots	Linear divots

Figure 1. Divot pattern treatments produced by golfers in this experiment.

Divots were filled with sand, and the volume of sand required to fill the divots was recorded. The total area of divots was quantified with digital image analysis on Day 0. Following day 0, digital images of each plot were collected with a light box twice per week to quantify recovery. The light box captured an image with a surface area of 40 by 55 cm. Images were taken before mowing to avoid interference from fresh grass clippings. Data collections were continued until each divot pattern was filled in or the end of the growing season.

Each block contained a non-treated check plot with no divots. A check plot was used to determine the maximum green coverage potential of a plot (accounts for plot injury that is not a divot, i.e., disease or scalping).

The percentage of each plot divoted (turf removed via divots) was calculated as

$$\% \text{ divoted turf} = 100 \times \frac{(check - divot)}{check} \qquad \text{Eq.1}$$

Where check is the % green cover in the non-treated plot and divot is the % green cover in the plot with divots.

Divot recovery was calculated as:

$$\% \text{ recovery} = 100 \times \frac{(d_0 - d_i)}{d_0} \qquad \text{Eq.2}$$

where d_0 is the percent of turf in the image removed by divots (divoted) on day 0 and d_i is the percent of turf in the image removed by divots (divoted) on day i (i^{th} observation). Digital image analysis was done with Turf Analyzer.

The total teeing area made unavailable for other golfers was calculated for each divot pattern in each plot. This was done on day 0 immediately after shots by using a rectangle frame around each area of divots, allowing for a 2” border of green grass. A 2-inch border was chosen based on half the distance recommended between linear divot patterns. The “area made unavailable” was used as a proxy to determine how much tee space would be occupied or created by each pattern. All data were analyzed with SAS version 9.4 (SAS Institute Inc.) utilizing GLIMMIX procedure.



Figure 2. A photo of one of the golfers in the study during treatment initiation.

Day 0 results: The results revealed that a linear divot pattern had the lowest percentage of divoted turf, whereas a scattered pattern had the highest percentage of divoted turf. A scattered divot pattern resulted in less teeing area being available compared to linear and concentrated divot patterns (Table 1). The amount of sand required to fill divots was not significantly different, but the concentrated divot patterns had numerically higher sand requirements compared to scattered and linear divot patterns.

Table 1. Effect of divot patterns on percentage of divoted turf, area impacted, and divot mixes.

Divot Pattern	% Divoted turf	Area impacted (cm^2)	Divot mix needed (cm^3)
Concentrated	36 ab	19.9 b	723.3 a
Linear	29 b	22.9 b	608.3 a
Scattered	37 a	30.5 a	625.5 a

Recovery: In the beginning, scattered divot patterns had a higher area of divoted turf (Figure 2). Due to faster recovery, divoted turf from the scattered pattern decreased over time and was fully recovered at 54 DAT, which was faster than linear and concentrated divot patterns (Figure 3).

The recovery rate of individual plots was higher for scattered divot patterns followed by linear and concentrated patterns (data not shown). Scattered divots recovered 50% in 19 days, linear divots recovered 50% in 27 days, 8 days after scattered divots. Concentrated divots recovered 50% in 34 days, which is 15 days later than scattered divots and 7 days slower than the linear divot pattern. At 54 DAT, divots in scattered plots recovered fully, linear divots were recovered 95%, and concentrated divots were recovered 92%.

Divot pattern impacts available tee space, divoted turf percent and divot recovery. Although scattered pattern had a higher area of impact and a higher percentage of divoted turf, this divot type was fastest to recover. This pattern would work well when sufficient driving range tee space is available. Concentrated divots had a lower area of impact and percentage of divoted turf, but required the most time to recover. The linear divot pattern appears to be the best divot pattern option as it had a lower area of impact and divoted turf than scattered pattern and a higher recovery rate than concentrated pattern. This pattern would work best when driving range tee space is limited which is likely the case for most.

We plan on repeating this experiment in 2024 to confirm our preliminary results and learn more about divot recovery. We conducted this experiment without adding seed to our mixtures. Adding seed would affect the results and help encourage faster recovery of tee boxes.

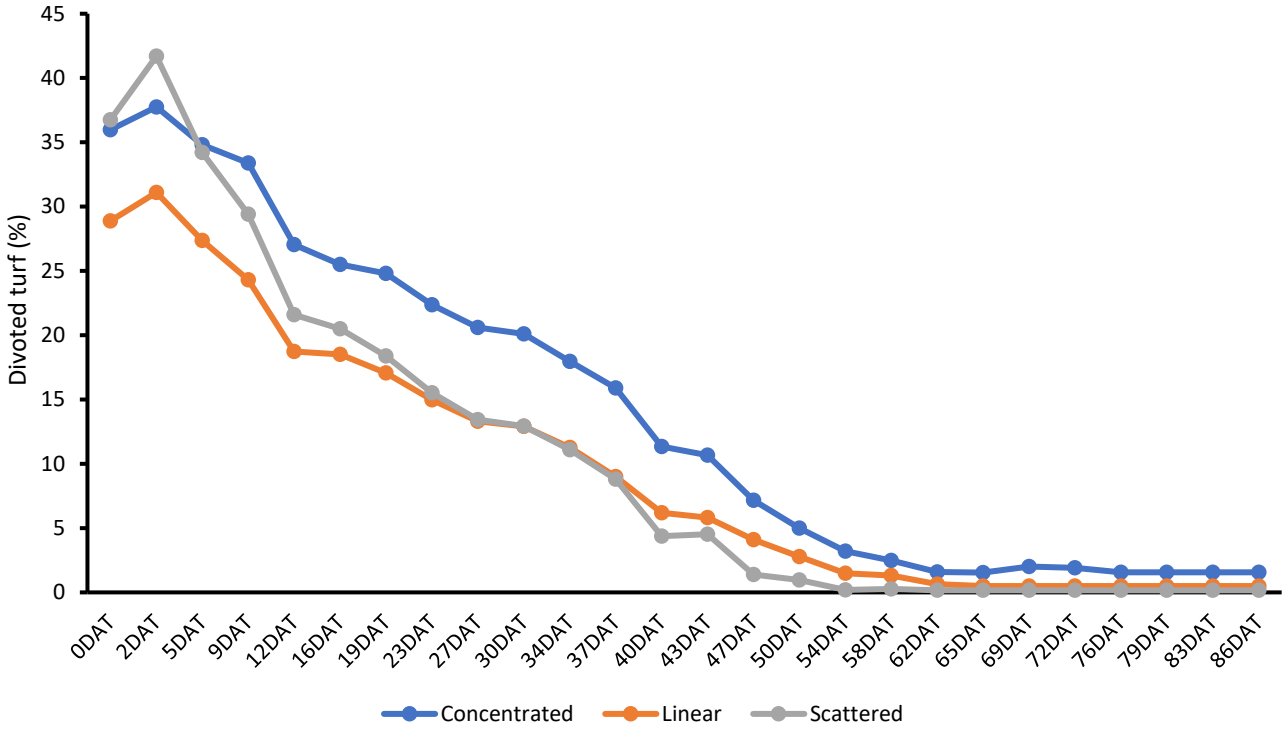


Figure 2. Effect of divot patterns on the percent turf filled with divoted turf.

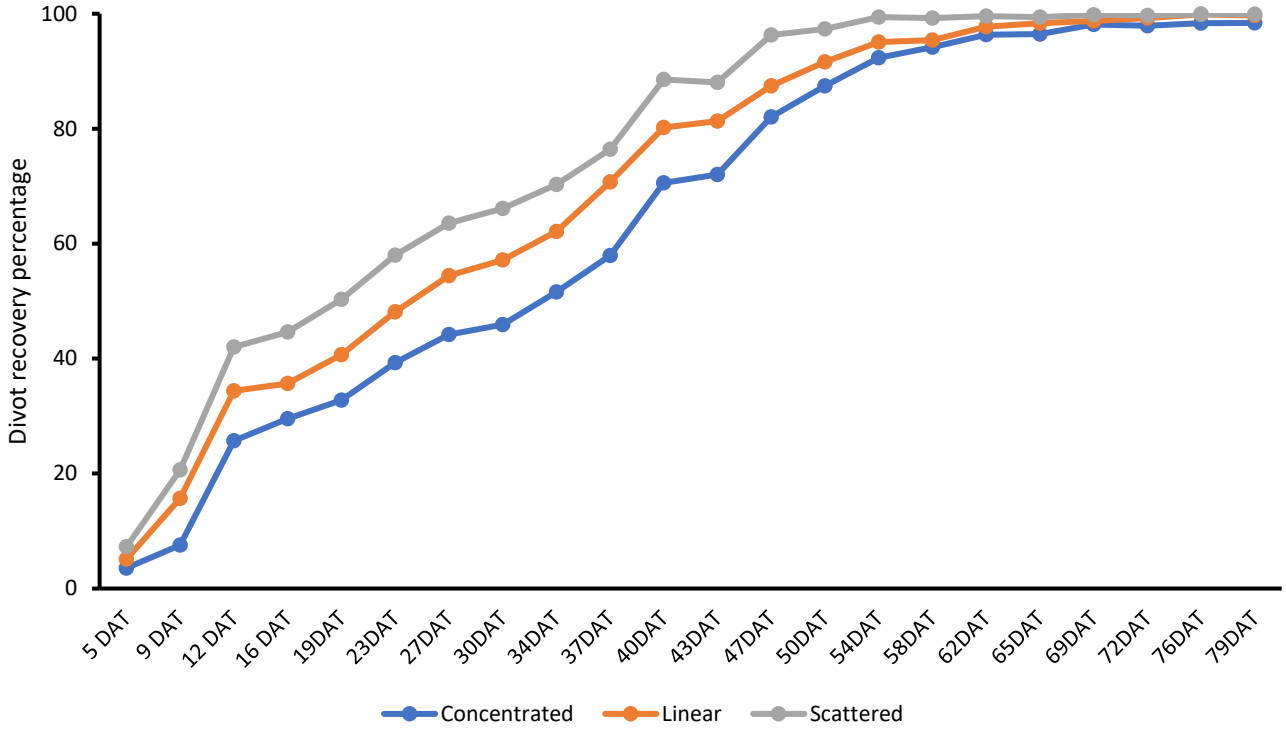


Figure 3. Effect of divot patterns on divot recovery.

References:

1. McClellan, T.A. (2014). Does Your Divot Pattern Help Or Hurt The Golf Course? Available at: https://www.usga.org/course-care/2014/09/does-your-divot-pattern-help-or-hurt-the-golf-course-21474873114.html