USGA Davis

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Mike Davis Program for Advancing Golf Course Management

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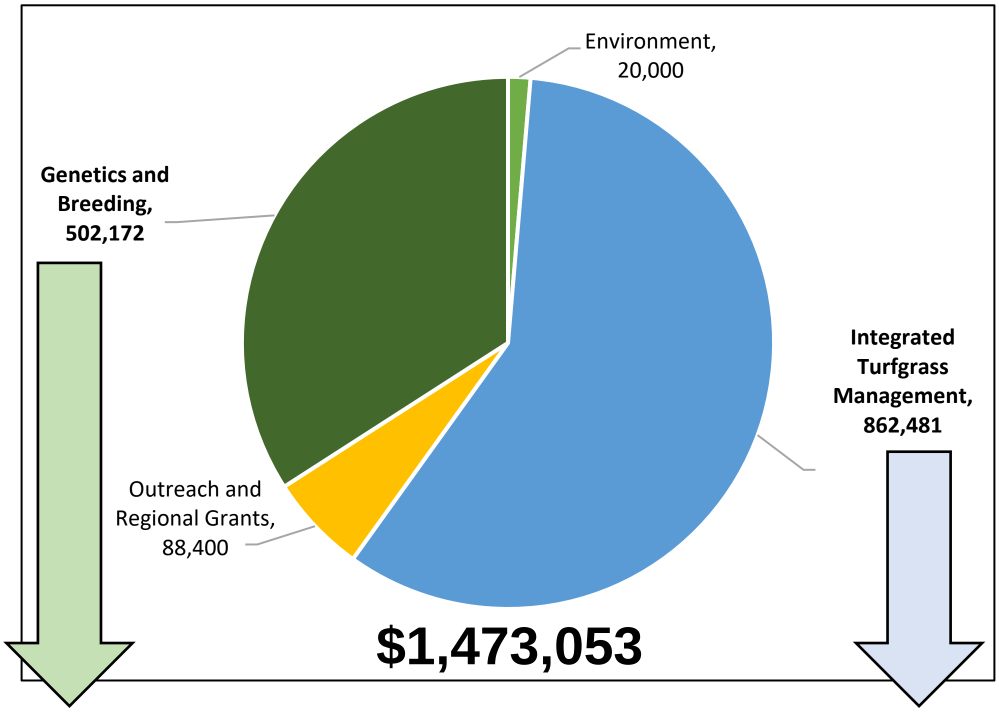
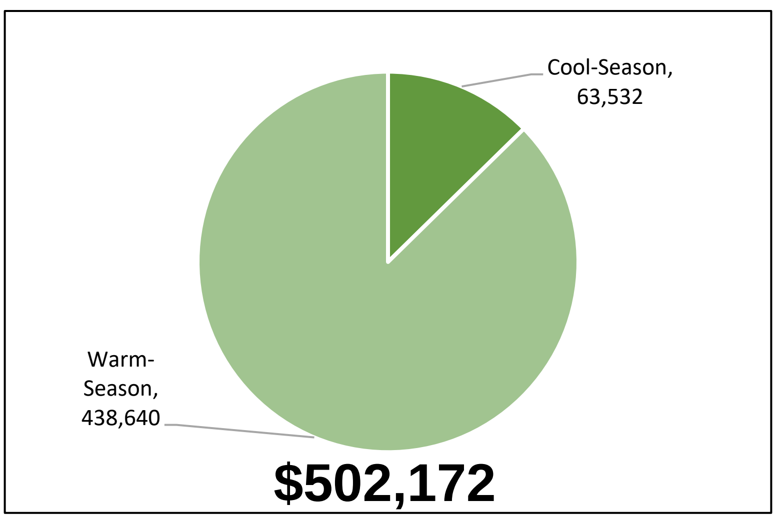
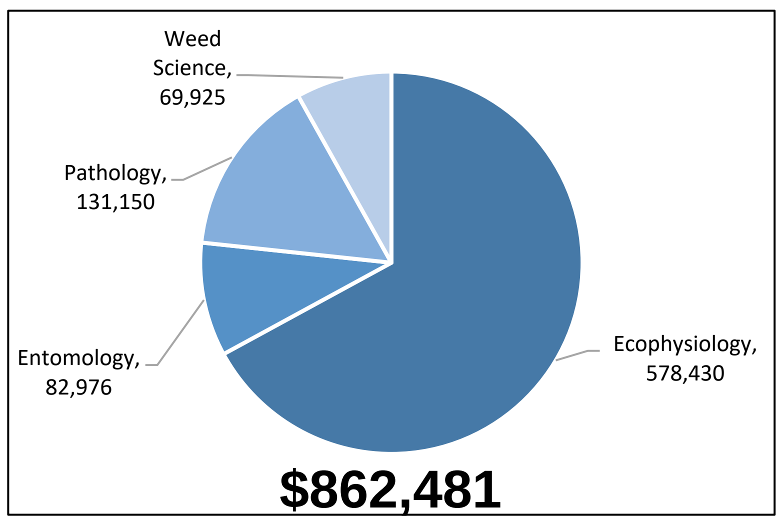
Davis Program Grants - 2021



Genetics and Breeding



Integrated Turfgrass Management



1983-01-001

Annual Report – 2021

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

William A. Meyer, Phillip L. Vines,
and Stacy A. Bonos, Rutgers
University

Objectives:

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

Start Date: 1982
Project Duration: Continuous
Total Funding: $ I0,000 per year

As of October 30,2020 over 1,600 promising turfgrasses and associated endophytes were collected in Serbia, Croatia, Italy, The Netherlands, and Austria. These were evaluated in the spring of 2021 in the Netherlands and over 588 had seed produced in the summer of 2021 and were evaluated in New Jersey starting in fall 2021. Over 12,108 new turf evaluation plots, 128,101 spaced-plant nurseries plants, and 34,282 mowed single-clone selections were established in 2021 in New Jersey.

Over 7828 seedlings from intra- and inter-specific crosses of Kentucky bluegrass were screened for promising hybrids under winter greenhouse conditions, and the superior plants were put into spaced-plant nurseries in the spring. Over 20,069 tall fescues, 9,490 Chewings fescues, 624 hard fescues, 6,672 strong creeping red fescue 38,716 perennial ryegrasses, and 3,948 bentgrasses were also screened during the winter in greenhouses, and superior plants were put in spaced-plant nurseries. Over 145 new intra- and inter-specific Kentucky bluegrasses were harvested in 2021.

The following crossing blocks were moved in the spring of 2021: five hard fescues (227 plants), five Chewings fescues (293 plants), ten strong creeping red fescues (372 plants), twenty-three perennial ryegrasses (1173 plants), twenty tall fescues (1078 plants), seven creeping bentgrasses (227 plants), and four colonial bentgrasses (251 plants).

The breeding program continues to make progress breeding for disease resistance and improved turf performance. New promising named and released perennial ryegrass varieties in 2021 were Fiesta Cinco, Palace II, Umpqua, Umatilla, Stellar 4GL, Spike GLS, Apex and Siletz . The new tall fescues are Raceway, Firehawk SLT, Raptor LS, Spyder LS, Avenger III, Genius, Honeymoon, Firenza II, Manzanita, Valsetz, Tough and Talladega II . One creeping red fescue named Simmons. Three hard fescues, Beacon II, Tenacious and Sword II. The new Kentucky bluegrasses were Acoustic and Jersey.

Summary Points

- Continued progress was made in obtaining new sources of turfgrass germplasm. These sources are being used to enhance the Rutgers breeding program.
- Modified population backcrossing and continued cycles of phenotypic and genotypic selection combined with increasing sources of genetic diversity in turfgrass germplasm has resulted in the continued development and release of top performing varieties in the NTEP
- Eight perennial ryegrasses, twelve new tall fescues, one creeping red fescue, two Kentucky bluegrasses, three hard fescues were named and released in 2021
- Published 5 referred journal articles and 18 non-referred journal articles in 2021.
- Twenty-four US Plant Variety Protection Certificates Issued and 13 applications in 2021.

Refereed Publications 2020/2021

1. Qu, Yuanshuo, Edwin J. Green, Stacy A. Bonos, and William A. Meyer, 2021. Evaluation of recurrent selection for drought tolerant tall fescue (Festuca arundinacea) using rain-out shelters.” Journal of Agronomy and Crop Science. https://onlinelibrary.wiley.com/doi/10.1111/jac.12570

2. Tate, Trent, S.A. Bonos, W.A. Meyer. 2021. Breeding and evaluation of fine fescues for increased tolerance to mesotrione. HortTechnology 31(3): 315-326. https://doi.org/10.21273/HORTTECH04772-20

3. Vines, P.L., R.M. Daddio, J. Luo, R. Wang, N. Zhang, B.B. Clarke, W.A. Meyer, and S.A. Bonos. 2021. Pyricularia oryzae incites gray leaf spot disease on hard fescue (Festuca brevipila). International Turfgrass Society Research Journal 21:1-6. https://doi.org/10.1002/its2.17

4. Bonos, S.A., E.N. Weibel, J. Honig, J. A. Murphy, L. H. Chappell and W. A. Meyer. 2021. Divot recovery of cool-season turfgrass species and mixtures in low maintenance fairways. International Turfgrass Society Research Journal, 21: 1-14. DOI:10.1002/its2.27itsrj.

5. Qu, Y., E. J. Green, P. L. Vines, S. Wu, S. A. Bonos, and W. A. Meyer. 2021. Evaluation and genetic analysis of red thread (Laetisaria fuciformis) prevalence in tall fescue (Festuca arundinacea). International Turfgrass Society Research Journal 21:1-10. DOI:10.1002/ITS2.20

Abstracts

1. Di, R., S. A. Bonos, and W. A. Meyer. 2021. Application of CRISPR-gene editing and tissue culture to improve 'Crenshaw' creeping bentgrass p. 12. *In* Proceedings of the 30th Rutgers Turfgrass Symposium. March 18, 2021 (*Virtual Meeting*).
2. Wu, S., A. L. Grimshaw, Y. Qu, P. L. Vines, E. N. Weibel, W. A. Meyer, and S. A. Bonos. Inheritance of summer patch disease resistance in hard fescue (*Festuca brevipila* Tracey). p.46. *In* Proceedings of the 30th Rutgers Turfgrass Symposium. March 18, 2021 (Virtual Meeting).
3. Wu, D.S., A.L. Grimshaw, H.Y. Qu, P.L. Vines, E. N. Weibel, W.A. Meyer and S.A. Bonos. 2020. Inheritance of summer patch disease resistance in hard fescue. In Agronomy Abstracts, ASA, CSSA, SSSA Annual Meeting Nov. 7-9, 2020 (Virtual Meeting).

Technical Reports

1. Weibel, E.N., T.J. Lawson, J.B. Clark, J.A. Murphy, B.B. Clarke, W.A. Meyer and S.A. Bonos. 2021. Performance of bentgrass cultivars and selections in New Jersey turf trials. 2020 Rutgers Turfgrass Proceedings 52:1-24.
2. Wu, S. P. L. Vines, R. F. Bara, D. A. Smith, R. M. Daddio, S. A. Bonos, and W. A. Meyer. 2021. Performance of fine fescue cultivars and selections in New Jersey turf trials, 2020. Rutgers Turfgrass Proceedings. 52:25-46.
3. Wright, O.K., R. F. Bara, P. L. Vines, S. A. Bonos, R. M. Daddio, D. A. Smith, E. N. Weibel, J. A. Murphy, and W. A. Meyer. 2021. Performance of Kentucky bluegrass cultivars and selections in New Jersey turf trials, 2020 Rutgers Turfgrass Proceedings. 52: 47-92.
4. Jennifer Halterman, Ronald F. Bara, Dirk A. Smith, Ryan M. Daddio, Phillip L. Vines, Stacy A. Bonos, and William A. Meyer. 2021. Performance of perennial ryegrass cultivars and selections in New Jersey turf trials, 2020 Rutgers Turfgrass Proceedings. 52:93-121.
5. Daddio, R.M. P. L. Vines, R. F. Bara, D. A. Smith, S. A. Bonos, and W. A. Meyer. 2021. Performance of tall fescue cultivars and selections in New Jersey turf trials. 2020 Rutgers Turfgrass Proceedings 52: 123-148.

Figure 1. Reaction of perennial ryegrasses to gray leaf spot disease.

Figure 2. Reaction of four species of fine fescue to gray leaf spot disease.

USGA ID#: 2019-15-685

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

Project Leaders: David R. Huff and Christopher W. Benson
Affiliation: Pennsylvania State University

Objectives:

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

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

Summary Points:

- In contrast to other allopolyploids, the subgenomes of *Poa annua* closely mirror their respective diploid ancestors with remarkable subgenome stability.
- We observed no transcriptional difference between annual and perennial biotypes of *Poa annua*.
- Transcriptional analysis of *Poa annua* under mowing suggests that the subgenomes have partitioned primary metabolic activity with fine-tune growth response from each of the subgenomes.
- Gene silencing of *Poa annua's infirma* subgenome mediated by brassinosteroid signaling may play an important role in stabilizing elite cultivars of *Poa annua* for commercial release and use on golf course putting greens.

Summary Text:

Rational and Methodology

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

We suspect that the recent polyploidization between diploids *Poa infirma* and *Poa supina* to form tetraploid *Poa annua* likely plays a role in *P. annua's* transgressive dwarfism and allows it to thrive under putting green heights (Huff, 2021). In collaboration with researchers at the USDA, we sequenced, assembled, and annotated chromosome-level genomes of *Poa infirma*, *Poa supina*, and *Poa annua* to shed light on the uncharacterized genetic and genomic components that might contribute to *Poa annua's* phenotypic novelty (Benson et al., 2021). We also conducted a subgenome-specific transcriptional analysis under putting green-style mowing to further narrow in on the metabolic pathways involved with regulating dwarfism on golf course putting greens (Benson & Huff, 2021). Our ultimate goal is to incorporate those discoveries into our breeding program and generate elite and stable *Poa annua's* for commercial use on golf course putting greens.

Results

The chromosome-level scaffolds contain between 97-98% conserved embryophyte orthologs, indicating high contiguity with minimal assembly errors. Our *de novo* gene annotates were similar in quality, with conserved orthologs present between 89-96% in our amino acid sequence. The *Poa annua* genome assembled into 14 chromosomes totaling 1.78 gigabases in size and containing 76,659 genes. The *Poa infirma* and *Poa supina* genomes each assembled into seven chromosomes totaling 1.13 and 0.64 gigabases with 39,420 and 37,935 genes, respectively. The subgenomes of *Poa annua* are 95% identical in sequence and 98% identical to their respective parental species, suggesting that most of the mutational variation between subgenomes happened during the speciation divergence of *Poa supina* and *Poa infirma* and pre-date the polyploidization event that formed *Poa annua.* Syntentic relationship between blocks of orthologous genes validate this result and indicate that the subgenomes of *Poa annua* strongly resemble their progenitor genomes (Fig. 1).

We conducted an RNA-seq experiment on clonally propagated *Poa annua's* to further elucidate the gene regulatory relationships between treatment (mowed vs unmowed), biotype (annual vs perennial), and subgenome (*P. annua's infirma* subgenome vs *P. annua's supina* subgenome). A stringent mapping pipeline was used to assign 20 million reads per sample to their corresponding subgenome with low ambiguity. Interestingly, subgenome designation seems to have a far greater influence on gene expression than treatment or biotype. The impact of treatment on gene expression is visible but subtle, while the impact of biotypes appears nested and was eliminated as a variable in subsequent analysis to avoid potentially spurious associations (Fig. 2).

Gene ontology and functional classification of differentially expressed genes suggest that the subgenomes have largely partitioned the primary metabolic activity of the plant and point toward a more harmonious relationship between *Poa annua's* diploid subgenomes than has been described in other neoallopolyploids, such as *Brassica napus* (Xiong *et al.*, 2011) or *Tragopogon miscellus* (Chester *et al.*, 2012). When comparing the effect of treatment (mowed vs unmowed), we observe several functional enrichments of interest. The most noticeable enrichments are transcripts involved in gene silencing and brassinosteroid signaling of *Poa annua's infirma* subgenome under golf course-style mowing. These genes occupy central hubs in our ontological network and are the sole links between other functionally enriched classes of genes, suggesting that they may have a regulatory role in dwarfism that is upstream of other metabolic pathways and would make good marker candidates for application in our breeding program (Fig. 3).

Future Expectations

With USGA resources provided during the previous two years of funding, we discovered that *Poa annua* modifies its epigenetic landscape by acquiring DNA methylation under stress of mowing. We observed that DNA methylation acquired through mowing was at least partially heritable from one generation to the next and that those methylation marks correlated with increased dwarfism in offspring plants (Benson et al., 2020). We have since incorporated those findings into our breeding program using novel methods to help stabilize the dwarfism phenotype and expect to submit experimental cultivars for variety trials in 2022.

After consultation with researchers at Penn State University and collaborators at the USDA, we have decided to temporarily sideline the epigenetic aspect of this project and focus on the impact of polyploidy on dwarf-type *Poa annua*. The link between epigenetic restructuring and polyploidization is well established in plants and current technological advancements allow us to make larger strides toward our breeding goals by studying polyploidy. With this final year of funding, we have generated chromosome-level genome assemblies for *Poa annua* and its diploid progenitors and identified several candidate genes involved in dwarfism specifically through gene silencing of *Poa annua's infirma* subgenome through brassinosteroid signaling. We have found that these genes play a central role in connecting other metabolic pathways and likely influence the stability of the greens-type phenotype. We plan to publish these genome sequences and their gene annotations in 2022 and anticipate that they will be a valuable resource for *Poa annua* researchers and the scientific community as a whole due to their exceptional quality and contiguity. We will continue to elucidate the uncharacterized gene silencing pathways and incorporate discoveries into our preexisting breeding program for the generation of commercially available greens-type *Poa annua*.

References:

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

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

Benson, C. W., Huff, D. R., Bushman, B. S., Maughan, P. J., Jellen, E., & Robbins, M. D. 2021. Sequenced genomes of *Poa annua* and its diploid progenitors, *Poa infirma* and *Poa supina*, provide insight into the evolution of a versatile nascent allopolyploid [Abstract]. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/134543

Chester, M. et al. 2012. Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). *Proc. Natl Acad. Sci.* doi.org/10.1073/pnas.1112041109.

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

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

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

Figures:

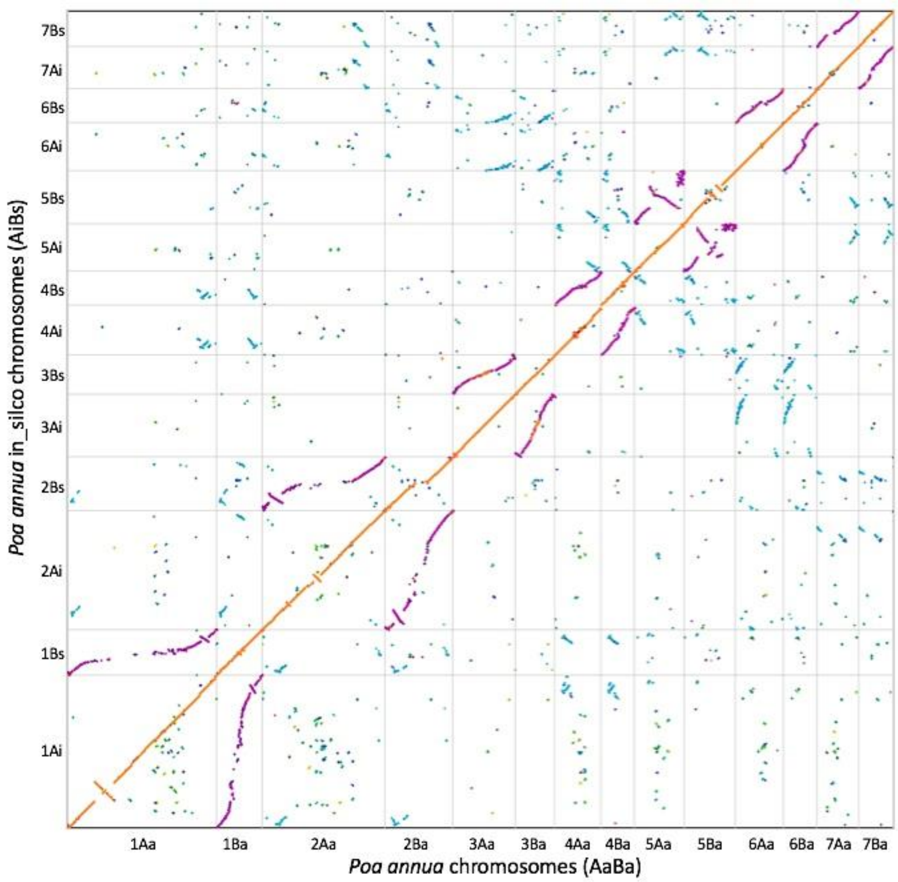


Fig. 1. Syntenic dotplot depicting structural variation between *Poa annua* (AaBa) and its diploid progenitor subgenomes (AiBs). Orange dots show structural changes that occurred after polyploidization (<50,000 years ago). Purple dots show structural variations that occurred between parental species divergence and polyploid hybridization (between 6 million years ago and 50,000 years ago), and teal dots date to an ancient polyploidy event (*Rho*, 86-95 million years ago). Note the size expansion of *Poa annua's infirma* subgenome indicated by the S-shaped curve of the largest purple alignments.

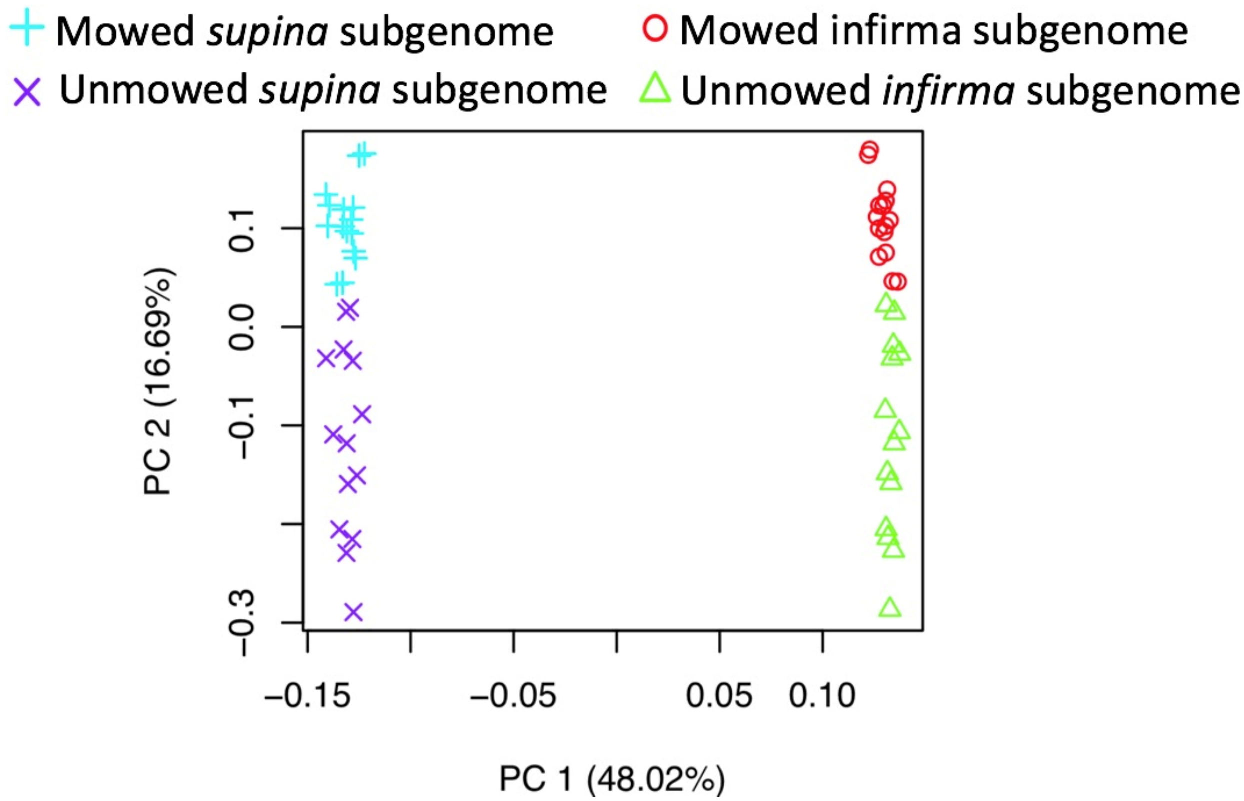


Fig. 2. Principal component analysis (PCA) of the transcript expression profiles of 14 *Poa annua* genotypes under putting green mowing height. The expression profiles of greens-type and annual-types were found to be nested (data not shown), indicating that biotype seemed not to influence gene expression; hence, biotypes as a factor were removed from the above PCA to prevent spurious associations.

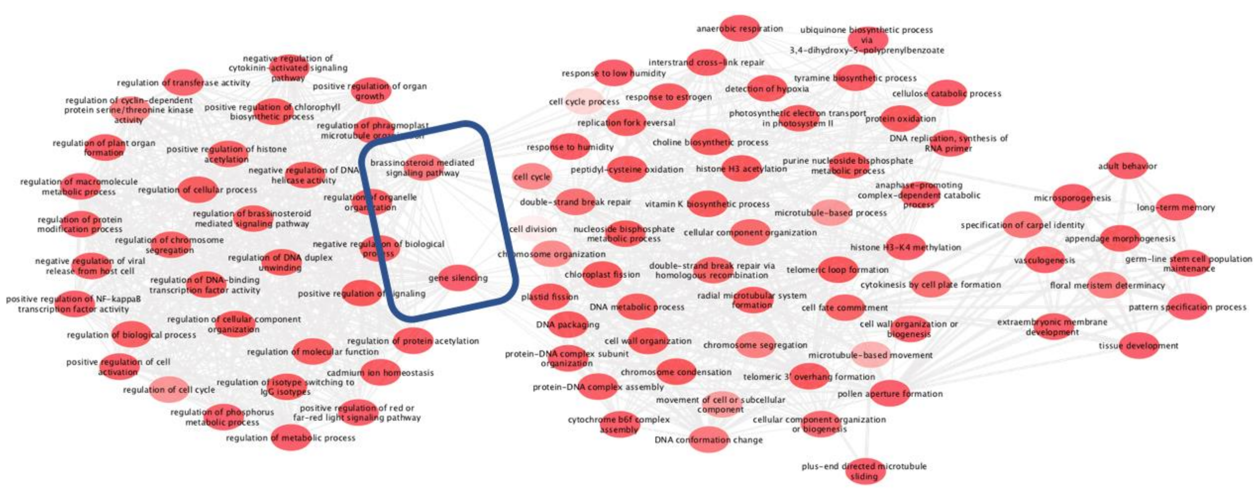


Fig. 3. Interaction plot of gene ontologies identified as being upregulated in *Poa annua's infirma* subgenome under mowing. The blue box circumscribes the two central hub ontologies that appear to play an important role in silencing *Poa annua's* more wildtype/annual subgenome under golf course-style mowing stress.

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

Affiliation: Rutgers University

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

Start Date: 2021

Project Duration: 3 years

Total Funding: $90,000

Summary Points:

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

2. The activities of the *E. festucae* antifungal protein produced in yeast, bacteria, and in the fungus *Penicilllium chrysogenum* were assessed for antifungal activity.

3. The *P. chrysogenum* expression system was the best for recovery of highly active *E. festucae* antifungal protein. However, the antifungal protein also had activity against *P. chrysogenum*, which reduced the yields of the protein that could be recovered. To overcome this issue we developed a new protocol for growing the fungus that resulted in high yields of the antifungal protein.

4. We have developed a reliable method for inoculating creeping bentgrass plants in the greenhouse with the dollar spot fungus. We have started to test foliar sprays containing the antifungal protein onto dollar spot infected plants to determine if this could be a new method for disease control.

Summary Text

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

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

Our hypothesis is that *Efe*-AfpA is a factor in the well-documented disease resistance seen in endophyte-infected fine fescues in the field (Clarke et al., 2006). The ultimate goal of this research is to determine if *Efe*-AfpA can protect creeping bentgrass plants from dollar spot disease so that it could be used as an alternative or supplement to synthetic fungicides. We have been focused on developing expression systems and purification methods for producing large amounts of the antifungal protein for testing in direct application to plants. Our results to date are described below.

We have expressed *Efe*-AfpA in yeast, in bacteria, and in the fungus *Penicillium chrysogenum* (Tian et al., 2017; Fardella et al., 2020, 2021). Active antifungal protein was purified from all three systems, with the best system being *P. chrysogenum*. We are using an engineered strain of *P. chrysogenum* in which the gene for a similar antifungal protein, designated PAF, was deleted (Marx, 2004; Sonderegger et al., 2016). An unexpected result from expression of *Efe*-AfpA in *P. chrysogenum* was the discovery that *Efe*-AfpA had activity against *P. chrysogenum*. Although highly active antifungal protein could be recovered from *P. chrysogenum*, the yields were variable and in some cases lysis of the fungal cells was observed. This result was unexpected because the *P. chrysogenum* antifungal protein PAF is very similar in sequence to the *E. festucae* antifungal protein and under the same growth conditions that we were using PAF does not have activity against *P. chrysogenum*. To confirm the impression that *Efe*-AfpA had activity against *P. chrysogenum*, the activity was quantitatively determined by measuring growth of conidia of *P. chrysogenum*. Even at the low concentration of 5 $\mu g\ mL^{-1}$ of *Efe*-AfpA, growth of *P. chrysogenum* conidia was dramatically inhibited relative to growth of the PAF-treated sample (Fig. 1). Microscopy of the treated samples is shown in Fig. 2. The *P. chrysogenum* conidia germinated and grew in the water treated and PAF treated samples, but there was almost no growth in the *Efe*-AfpA treated sample.

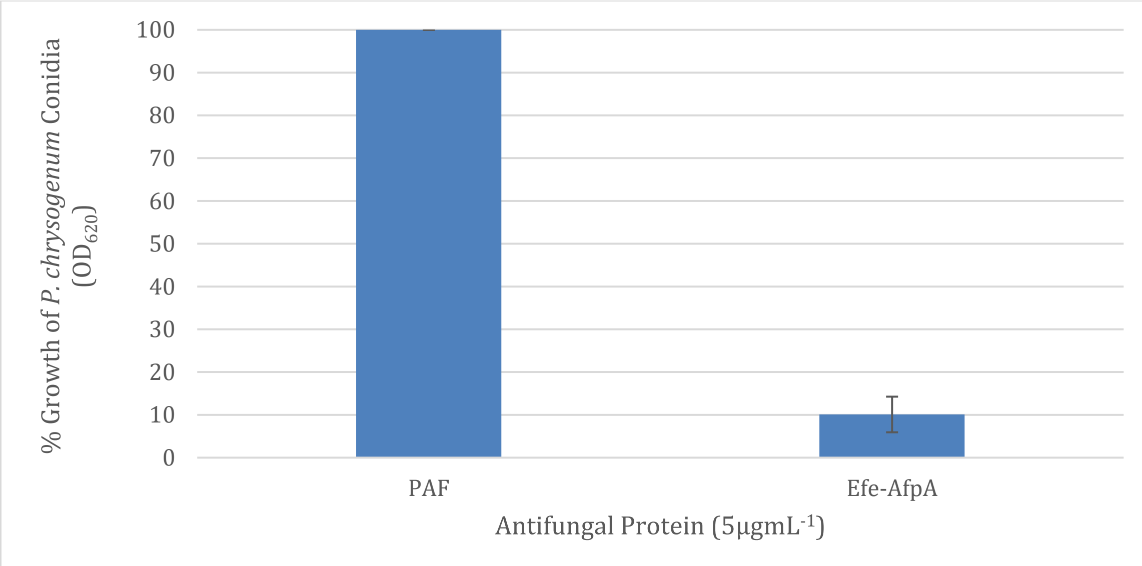


Figure 1. Percent Growth of PAF and *Efe*-AfpA-Treated *Penicillium chrysogenum* conidia. Percent growth was calculated by measuring the change in absorbance of $2x10^6$ conidia mL^{-1} of *Penicillium chrysogenum* after treatment with either water, the *P. chrysogenum* antifungal protein PAF, or the *Epichloë festucae* antifungal protein *Efe*-AfpA at a concentration of 5µg mL^{-1}. The data presented are the percent growth of the treated samples relative to the growth of the water control. OD_{620} absorbance was measured at 0hr and 24hr. Standard deviation represented by error bars.

A. **B** **C**

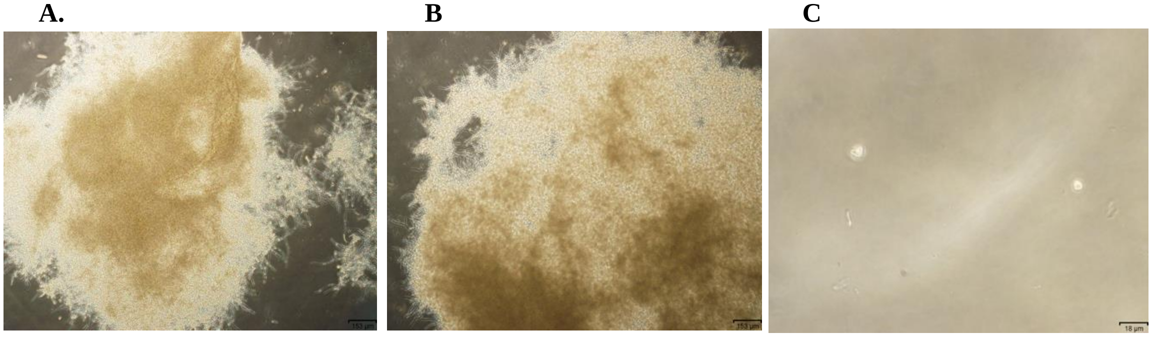


Figure 2. Microscopy of PAF and *Efe*-AfpA Treated *Penicillium chrysogenum* conidia. A. *Penicillium chrysogenum* mycelium after water treatment. **B.** *Penicllium chrysogenum* mycelium after PAF treatment. **C.** *Penicllium chrysogenum* mycelium after *Efe*-AfpA treatment. All samples were observed under brightfield microscopy.

These results necessitated development of a new protocol for growth of *P. chrysogenum* expressing *Efe*-AfpA to avoid the toxic effects of the antifungal protein on *P. chrysogenum*. A successful protocol was developed that paired growth of the fungus for 48 hours in a high nutrient medium that did not induce expression of the antifungal protein followed by transfer of the fungal mycelium to a low nutrient medium for 48 to 72 hours. In the high nutrient medium, the fungus grew rapidly generating a large biomass of mycelium. In the low nutrient medium the antifungal protein was expressed and secreted to the surrounding medium from the large biomass, resulting in high yields of active protein. *Efe*-AfpA was purified from the culture medium using a combination of cation exchange and size exclusion filtration (Fig. 3).

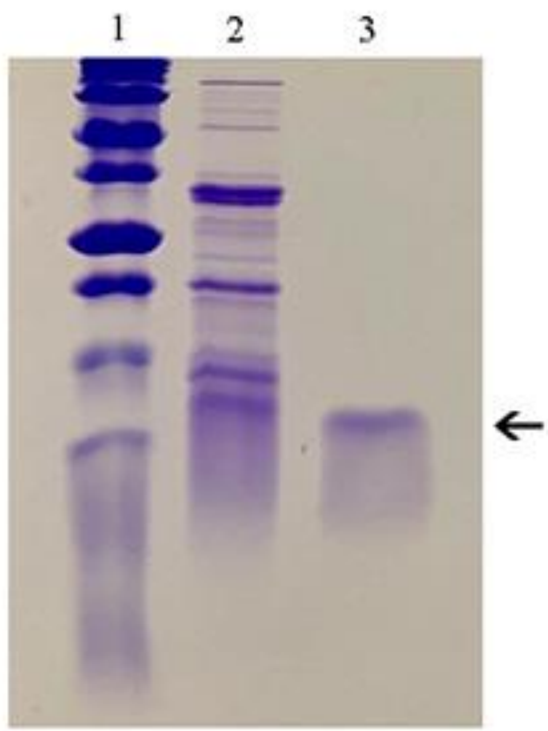
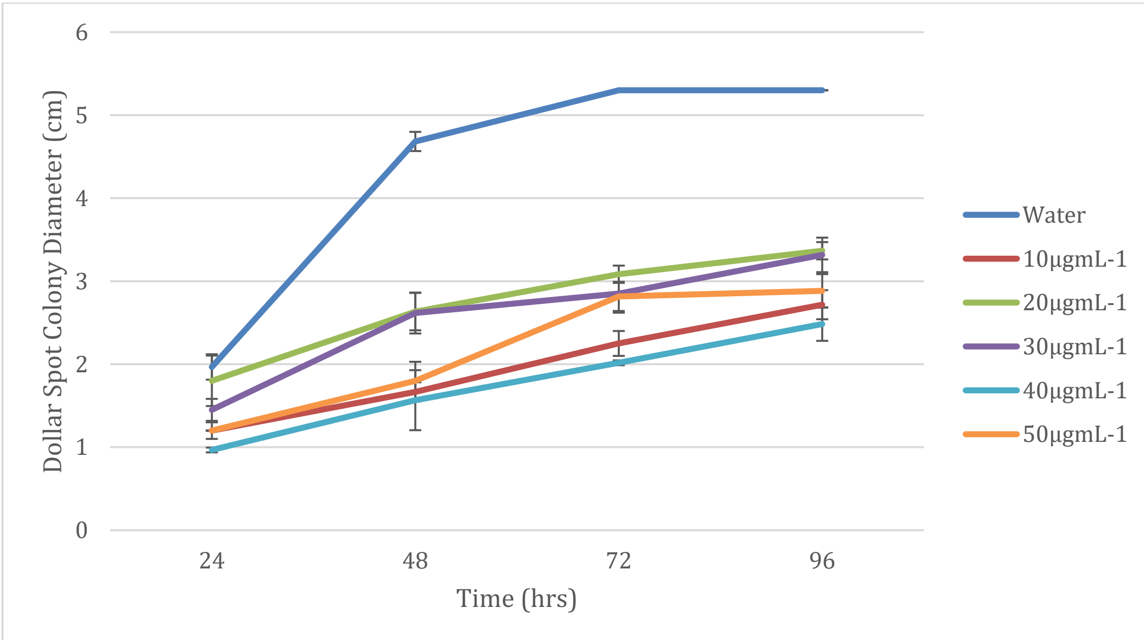


Figure 3. SDS polyacrylamide gel of purification of *Efe*-Afp produced in *P. chrysogenum*. Lane 1, Bio-Rad Precision Plus Protein Dual Xtra Standards; Lane 2, Crude supernatant of *Penicillium chrysogenum* expressing Efe-AfpA; Lane 3, purified *Efe*-AfpA.

We also purified PAF from *P. chrysogenum* using an engineered overexpressing strain (Marx 2004; Sonderegger et al., 2016). Since the amino acid sequences of PAF and the *E. festucae* antifungal protein are very similar it was of interest to determine if PAF could be used to inhibit the dollar spot fungus. In several different types of laboratory assays we found that PAF did not inhibit the dollar spot fungus, whereas *Efe*-AfpA did inhibit the pathogen. Incorporation of *Efe*-AfpA into agar plates resulted in inhibition of growth of the dollar spot fungus. In contrast, incorporation of PAF at the same concentrations did not inhibit growth of the dollar spot fungus (Fig. 4).

A



B

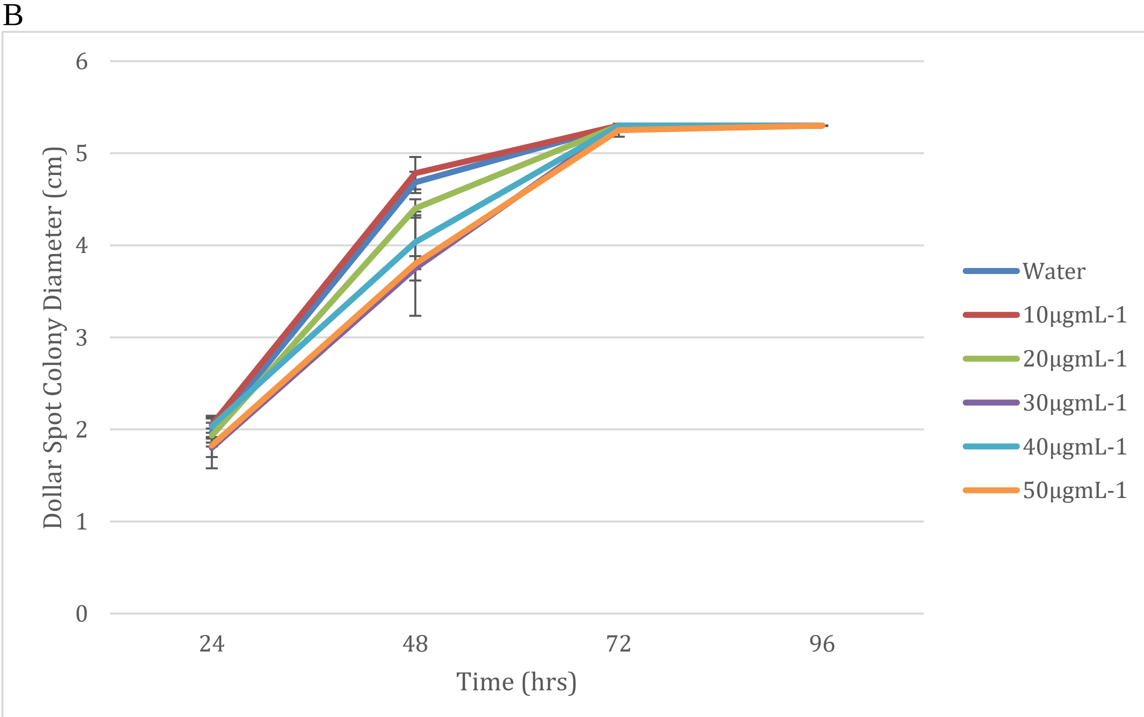


Figure 4. Comparison of the Effects of *Efe*-AfpA and PAF on the Growth of the Dollar Spot Fungus. A. Colony growth of the dollar spot fungus on PDA amended with water (control) or increasing concentrations of the *Epichloë festucae* antifungal protein *Efe*-AfpA. **B.** Colony growth of the dollar spot fungus on PDA amended with water (control) or increasing concentrations of the *Penicillium chrysogenum* antifungal protein

PAF. Colony diameters measured in centimeters. Standard deviation represented by error bars.

Fig. 5 shows representative petri plates after 96 hours of incubation. The control and the PAF amended plates show growth of the dollar spot fungus to the edges of the plate, whereas the Efe-AfpA amended plate has significantly reduced growth.

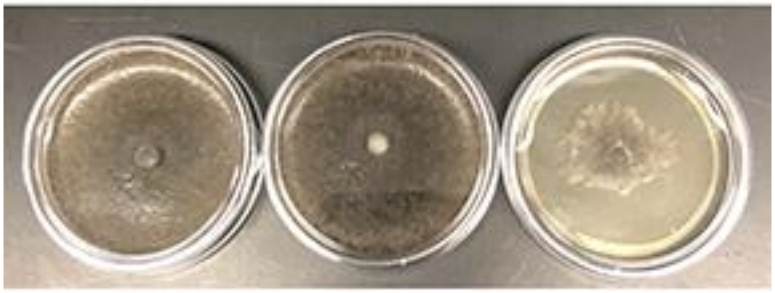


Figure 5. Growth of the dollar spot fungus on PDA plates amended with water, 20 μg mL^{-1} PAF, or 20 μg mL^{-1} *Efe*-AfpA.

The next step in this project is to test the purified *E. festucae* antifungal protein on creeping bentgrass plants inoculated with the dollar spot fungus. We have established a greenhouse inoculation protocol that reliably results in dollar spot disease on creeping bentgrass. We are now preparing to test if foliar application of *Efe*-AfpA to dollar-spot inoculated creeping bentgrass plants will result in reduced disease. If the purified protein is effective, this could provide an additional method for control of dollar spot and could reduce fungicide inputs in the field.

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USGA ID#: 2016-01-551

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

Yanqi Wu, Dennis Martin, Justin Quetone Moss, and Nathan Walker
Oklahoma State University

Objectives:

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

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

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

Developing putting green-type bermudagrass cultivars is an important component of the current research grant funded by the US Golf Association. The 2018 established putting green mowing trial, including 19 OSU experimental selections and three commercial cultivars ('Champion Dwarf', 'TifEagle', and 'Sunday'), was terminated this year. On the basis of data collection in the past four years, one genotype '11x2' that had decent performance under low mowing heights was selected for further evaluation. This test was uncovered this winter to evaluate freeze tolerance and spring green up in the field. Entries, '11x18' and '34x14' exhibited early spring greenup and excellent winter survival in the spring even with several days of uncommon freezing temperatures that occurred in February 2021. Accordingly these two genotypes were selected for further evaluation.

The 2019 putting green mowing test encompassing 16 OSU experimental selections and four cultivars (Champion Dwarf, TifEagle, 'Mini Verde', and Sunday) was continued in 2021. Several genotypes showed excellent spring greenup, high density and coverage under low mowing heights (around 4.5 mm) as compared with the commercial standard cultivars in that test. Entries '3x23', '5x23', '15x9', '19x9', and '34x20' were selected for further testing.

In 2021, a new greens-type bermudagrass mowing test was established to characterize turfgrass performance related traits and ball roll distance under low mowing heights. This test is part of M.S. graduate student, Ryan Earp's thesis research (Figure 1). In the putting green trial, a randomized complete block design with a 15' by 5' plot size and 6 replicates was used. The test consisted of 12 OSU advanced selections, one genotype from University of Florida, one from Mississippi State University, and three commercial cultivars (Tahoma 31, Tifdwarf, and TifEagle). The test was planted in May and fully filled in in early September in 2021. Initial rooting depth data indicated all OSU experimental selections had deeper roots than TifEagle. Ball roll distances of OSU experimental selections ranged from 7.5 to 8.5 feet while commercial cultivars rolled from 8.0 to 9.5 feet. More data will be collected in 2022.

In 2017, 2018, and 2019 replicated plots of several experimental greens-type selections and two standards (Champion Dwarf and TifEagle) were established for testing disease resistance. In 2018 the 2017-planting was infested with root-knot nematodes (*Meloidogyne marylandi*), leaf spot (*Bipolaris cynodontis*), and the causal agent of root-decline of warm-season grasses (*Gaeumannomyces graminis*) the three most common diseases in the region on ultradwarf bermudagrasses. The 2019 planting was infested in 2020 and the 2017 and 2018 established plots were evaluated for nematode reproduction, leaf spot and root decline severities.

A replicated trial established at the OSU TRC in the summer of 2017 was continued this year. The trial included 35 OSU vegetatively-propagated experimental selections, four vegetatively-propagated commercial cultivars ('Astro,' 'Latitude 36®,' Tahoma 31® and 'TifTuf®'), 11 seed-propagated experimental synthetics and two seed-propagated commercial cultivars ('Riviera' and 'Monaco'). We collected data for spring greenup, percent living cover, turf quality, and disease response. Irrigation to the trial was shut off in July of 2021 onward, leaving the trial under ambient rainfall conditions.

On April 29th Dr. Dennis Martin conducted a diagnostic and germplasm collection visit to a small rural 9-hole golf facility that had seeded Riviera bermudagrass to their push-up style putting greens approximately one decade before. The greens were converted to bermudagrass from bentgrass due to inadequate labor and budget to maintain creeping bentgrass. Riviera had functioned suitably for the needs of the membership on the low-input greens prior to the severe winter conditions of February 2021 (Figure 2). Promising segregates were collected from the surviving Riviera for reincorporation into the OSU breeding and development program.

Summary Points

- Three OSU experimental clones in the 2018 greens-type mowing test and five OSU experimental clones in the 2019 test were selected for further testing.
- A new greens-type mowing test was established to characterize turfgrass performance related traits and ball roll distances in 2021.
- Disease resistance testing of green-type bermudagrass selections was continued.
- One lawn-type mowing test established in 2017 was continued this year.

Figure 1. A drone image of a new greens-type bermudagrass mowing test established at the OSU Turf Research Center, Stillwater, OK in the summer 2021.

Figure 2. Severe winter conditions caused extreme selection pressure on low budget Riviera seeded bermudagrass putting greens in February 2021. OSU team members collected winter-hardy segregates out of Riviera in late April for possible use in germplasm improvement in the future.

USGA ID#: 2017-14-624

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

Principal Leaders: Charles Fontanier and Yanqi Wu

Affiliation: Oklahoma State University

Objectives:

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

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

Summary Points:

- 75 bermudagrass genotypes have been screened for shade tolerance under greenhouse conditions with the top 20 genotypes and industry checks planted in the field for further evaluation.
- The majority of seeded entries and several clonal selections did not survive their first winter in the field trial.
- An additional 95 genotypes were screened for shade tolerance under greenhouse conditions with plans to plant in the field in 2021.

<u>Background and Rationale</u>

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

Methods

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

In June 29, 2018, the top 20 performing genotypes were then planted as 2.5-inch plugs in a field study alongside industry standard cultivars (Patriot, Riley's Super Sport, TifGrand, Latitude36) and five seeded populations from the OSU breeding program. Plots measured 3-ft by 3-ft and each treatment combination was replicated 3 times. The study site was split into two environments: heavily shaded versus non-shaded. The heavily shaded site was characterized by evergreen trees along the western edge and deciduous trees along the southern and eastern edge of the space. Plots were mowed weekly at 1.5-inches using a rotary mower, and fertilizer was applied monthly at 0.5 lb N per 1000 square feet. Plots were evaluated for percent green coverage from 2018 to 2020. Once the majority of plots were fully covered, visual ratings of turfgrass quality and spring green up were collected monthly.

An additional set of 95 plants from the OSU breeding program were selected and planted in conetainers using sprigs. The plants were evaluated in the greenhouse for a rapid screening of shade response similar to the one conducted previously. The top 17 performers from this second greenhouse trial plus three standard cultivars were planted in the field in 2021. Plants will continue to be evaluated beyond this project.

Results

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

In the most recent greenhouse screening trial, variation in response to shade stress was again evident. Data related to turf quality, leaf elongation rate, leaf angle, and biomass were collected in Oct 2019 and again on a second run in August 2020. Preliminary analysis of these data show entries ‘27x2, ‘577’, and ‘15x16’ as having excellent maintenance of shoot dry weight as compared to all other entries. In some cases, grasses having excellent shoot dry weight maintenance did not have maintain green coverage or turf quality to the same degree (and vice versa). This was seen in entry ‘#29’ which had excellent green coverage and quality but had a nearly 60% reduction in shoot dry weight under shade. However, several entries including ‘28x19’ showed above average green coverage and shoot dry weight suggesting enhanced shade tolerance compared to other entries. The top 17 entries from this later greenhouse screening were planted under field conditions in 2021 and will be evaluated under moderate shade in a similar manner as the other site (Figure 1).

In the field study, most entries that had survived were deemed to be sufficiently established by the end of 2020 (Figure 2). Visual ratings from 2021 showed few differences in spring green up among entries, although one of the seeded-types (OKS2) had the highest numerical value (3.5) which was greater than six other entries including ‘Patriot’ (Table 1). Summer turfgrass quality ranged from 1 (all plots from an entry died) to 7.0 for ‘Riley’s Super Sport’, OSU1439, and OKS4. Turfgrass quality for all entries declined in the fall due to continued shade stress and fall armyworm predation, although ‘Riley’s Super Sport’ demonstrated the highest numerical turfgrass quality. The top performing entry from greenhouse trials (‘2014-4x2’) was among the worst performing entries under field conditions. In contrast, OSU1439 did well in both greenhouse and field trials, suggesting greenhouse screening can be useful but requires careful validation in field. These findings also reinforce evidence that dwarfism alone may not convey shade resistance under more complex growing conditions as seen in the field.

Future Expectations and Early Conclusions

This project has concluded, and a final report will be developed after more comprehensive analysis of the data can be conducted. Preliminary findings suggest only moderate correlation between greenhouse and field studies, likely due to cold temperature stress and poor establishment of some entries under field conditions. Entries such as OSU1439 have shown multiple years of good turfgrass quality in comparison to industry standards for shade tolerance and may have promise if other traits (eg, drought resistance, traffic tolerance) can be confirmed. Seeded-types continue to perform similarly to the standards but in no cases was there consistent improvement in shade response.

Figure 1. Top performers from most recent greenhouse trials planted for field evaluation in 2021.

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Figure 2. Field trial under heavy shaded environment showing variation in coverage among entries.

Table 1. Visual ratings of field plots in 2021 after three years of shade stress in Stillwater, OK.

Entry	Spring Green Up April 10	Turf Quality July 23	Turf Quality September 13
Riley's Super Sport	2.5abc[z]	7.0a	5.0a
Latitude 36	3.0ab	6.5ab	4.5ab
TifGrand	2.0abc	5.5abc	2.5ab
Patriot	1.0c	2.0cd	1.0b
2014-4x2	1.0c	2.0cd	1.5ab
2014-3x1	2.0abc	3.0abcd	1.5ab
OSU1418	2.0abc	3.0abcd	2.5ab
2014-2x11	1.0c	1.0d	1.5ab
OSU1414	2.0abc	4.5abcd	2.0ab
OSU1247	2.5abc	5.5abc	4.0ab
2014-13x19	1.5bc	3.5abcd	2.0ab
OSU1117	2.5abc	5.0abcd	3.5ab
OSU1217	2abc	4.5abcd	2.5ab
2014-12x1	2abc	3.0abcd	2.0ab
2014-24x4	2abc	3.5abcd	1.5ab
OSU1403	1.5bc	3.5abcd	2.0ab
OKC-C20	2.0abc	2.5bcd	1.0b
2014-9x5	2.5abc	3.5abcd	2.0ab
OSU1337	2.5abc	6.5ab	3.5ab
OSU1156	2.5abc	4.0abcd	2.5ab
OSU1439	3.5a	7.0a	3.5ab
OSU1257	2.0abc	4.0abcd	3.5ab
2014-18x2	1.5bc	6.5ab	4.5ab
2014-3x3	3.0ab	4.5abcd	3.0ab
Riviera	2.5abc	4.0abcd	2.0ab
OKS1	2.0abc	3.5abcd	3.0ab
OKS2	3.5a	5.5abc	4.0ab
OKS3	2.5abc	4.5abcd	3.0ab
OKS4	3.0ab	7.0a	4.5ab
OKS5	2.0abc	3.0abcd	2.0ab

[z]Means followed by the same letter are not significantly different ($p \leq 0.05$)

USGA ID#: 2020-11-716

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

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

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

Objectives:

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

Start Date: January 2020 (funds released to the PI in July, 2020)
Project Duration: 2020-2024 (3 years with a one year no cost extension)
Total Funding: 85,792

Summary Points:
- A Ph.D. student was recruited and started on the project in late spring of last year.
- Research efforts were still disrupted by the pandemic, critical training/learning opportunities for the student were cancelled and it took until the summer of 2021 for full research efforts to be continued.
- Several Bioinformatics Workshops have been completed by the student.
- RNA degradation and foreign contamination have been an issue but typical at the beginning of these studies.
- High quality RNA extractions are soon to be sent for complete sequencing and 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 (Figure 2), we are using a bioinformatics approach to identify the gene(s) in the fungus responsible for producing effectors of necrosis. Based on past research candidate necrotrophic-effector genes were identified in the fungal genomes, which were also found to be up-regulated *in planta*. Among these candidate genes, three were associated with pathogen-associated molecular pattern-triggered immunity. This implied that *Ophiosphaerella*-induced necrosis is the result of a plant basal defense mechanism. Expression profiling analysis of roots of susceptible bermudagrass cultivar Tifway infected with *O. herpotricha* demonstrated activation of plant innate immunity responses mediated by activation of jasmonic acid potentially resulting in hypersensitive response. The tolerant U3 biotype showed activation of basal defense response mediated by salicylic acid. This salicylic acid-mediated signaling could be involved in enhanced resistance to nutrient starvation and cold tolerance that allows the host to withstand pathogen infection and avoid organ death during periods of cold-temperature induced dormancy. The goal of this research is to mirror past studies to elevate the understanding of the SDS pathosystem to a level where bermudagrass breeding efforts can use known host specific disease resistance gene(s) to develop new cultivars with enhanced disease resistance.

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

Studies with bermudagrass, bentgrass, Kentucky bluegrass, wheat and *Arabidopsis* species were started but have been plagued with low quality RNA yields and often foreign fungal contamination (Figure 3). This is typical for students starting these types of studies and usually is overcome quickly; however, the pandemic has made advancing this research challenging. Studies have continued and will take several years to complete but it is expected that sequencing data will be available in early 2022.

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

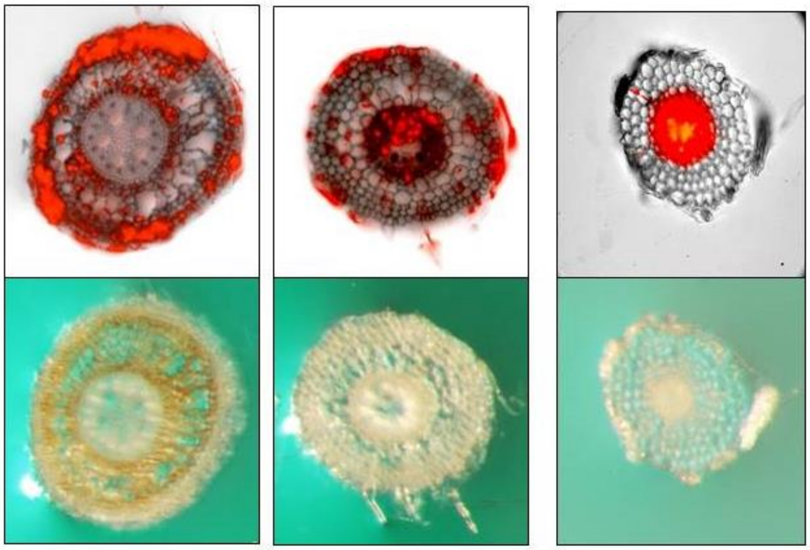

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Figure 2. Colonization of a spring dead spot susceptible bermudagrass and cortical necrosis by *Ophiosphaerella korrae* (left), a tolerant bermudagrass (Center) exhibiting vascular colonization by *O. korrae* and no necrosis, and *O. korrae* colonization of a grass which does not produce disease (right). Pictures by F. Flores.

Date of submission: 11/15/2021

Source:

WI: Wheat inoculated with *Ophiosphaerella korrae*, **WC:** Wheat control, **WOK:** *O. korrae* only, **BC:** Bentgrass control, **BI**: Bentgrass inoculated with *O. korrae*, **BOK:** *O. korrae* only, **KC:** Kentucky bluegrass control, **KI:** Kentucky bluegrass inoculated with *O. korrae*, **KOK:** *O. korrae* only.

Standard for Illumina sequencing: RIN ≥ 6.3; Concentration ≥ 20 ng/uL

Source	Sample ID	260/230	260/280	ng/uL nanodrop	RIN	ng/uL Bioanalyzer	Standard for Illumina
WI	D0	0.07	1.98	26.2	5.8	21	N
WI	D1	1.26	2	33	7.2	28	Y
WI	D3	0.26	1.67	16.4	6.1	13	N
WI	D4	0.43	1.91	20.4	5.5	12	N
WI	D5	1.49	1.89	26.7	2.9	21	N
WC	E1	0.65	1.85	18.2	7.7	14	Y, ?
WC	E2	1.7	1.99	49.3	7.5	37	Y
WOK	F3	1.63	0.69	19.9	N/A	2	N
BC	41	0.68	1.68	16.9	N/A	9	N
BC	42	1.25	1.27	19.3	N/A	6	N
BC	43	1.03	1.14	16.9	N/A	7	N
BI	51	1.03	1.96	17.8	7.5	12	Y,?

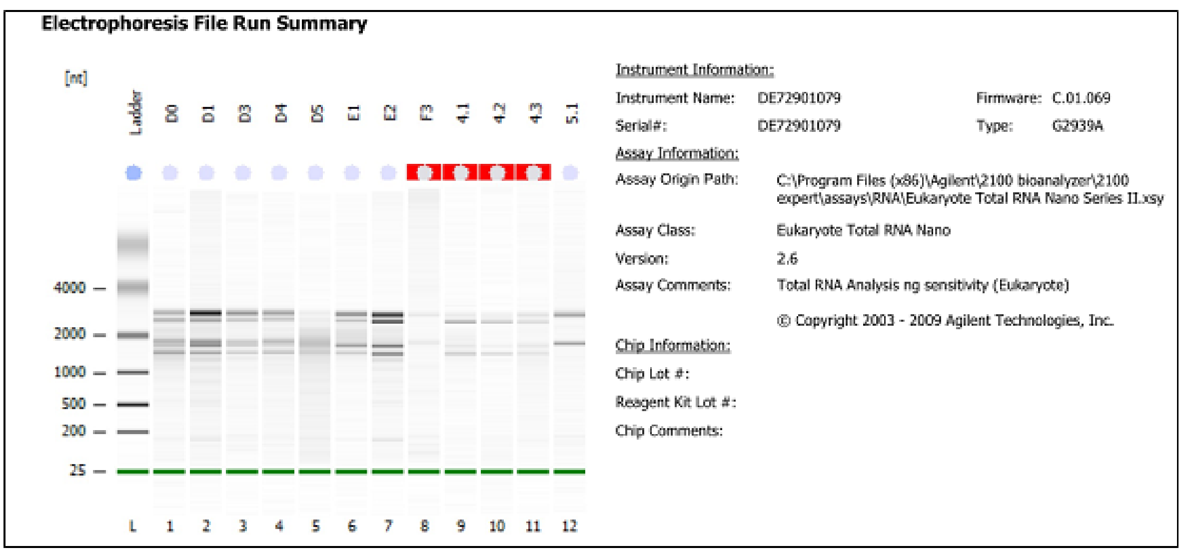

Figure 3: Recent Bioanalyzer results for a subsample of recently extracted RNA from a variety of samples. High quality samples highlighted in yellow.

USGA ID#: 2016-34-604

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

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

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

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

Summary Points:
- We evaluated cool temperature green color retention in a large set of bermuda and zoysia grass germplasm lines.
- We found that, for both species, the average color retention value of the included check cultivars was greater than the average color retention value of the germplasm populations.
- We also identified germplasm populations from both species that possessed greater cool temperature color retention than the cultivar with the highest value.

Summary Text:

Water is rapidly becoming the most important turfgrass management issue. Turfgrass irrigation requires large inputs of water during the hotter summer months. The use of warm-season grasses, including bermudagrass and zoysiagrass in hotter areas can result in substantial irrigation savings during the summer months. Yet, these same grasses tend do lose color and quality during the cooler winter months, at which time a common practice is to overseed with cool-season grass, such as perennial ryegrass. This overseeding results in improved cover and quality of the winter turf but comes at the cost of additional wintertime irrigation. A potential solution is the development of bermudagrass and zoysiagrass cultivars that possess increased cool temperature color retention. This approach would maintain turfgrass quality and simultaneously reduce the need for wintertime irrigation.

We received bermudagrass and zoysiagrass germplasm populations and check cultivars from the University of Florida, Oklahoma State University, and Texas A&M University. We cloned the plants to produce sufficient material for a replicated growth chamber cool temperature evaluation. We placed the cloned plants into 50 cell (141 cm^3) plant trays for the randomized complete block design (3 complete blocks). We allowed plants to grow for one month in the plant trays prior to being placed in the growth chambers for the cool temperature evaluation. We acclimated the plants to the growth chambers for one week at 25 °C. We the conducted the cool temperature evaluations by decreasing the temperature from 25 °C to 4 °C over the course of 12 weeks. Thus, from cloning to study completion took approximately five to six months. While the bermudagrass clones were in the growth chamber, we cloned and prepared the zoysiagrass genotypes. The evaluation continued with alternating bermudagrass and zoysiagrass runs until three runs were completed for each species. With work stoppages and slowdowns for the 2019 US government shutdown and the COVID-19 epidemic, it will take us four and a half years to complete three runs of the study for both species – species were evaluated separately. We have now completed all but the final run for the bermudagrass.

We weekly took digital images of each plant tray using a digital camera and a customized light box. We processed these images by “cutting” the image of each individual cell from the overall picture and converted each image to Dark Green Color Index ratings using the Turf Analyzer software. We then use the ASReml-R package of R to analyze the resulting data. Because we still have additional an additional run of the bermudagrass germplasm, this is still a preliminary analysis.

We found a range of Dark Green Color Index ratings between 3.47 and 5.73 among the zoysiagrass populations and 4.94 and 7.41 among the bermudagrass populations. The included cultivars possessed greater color retention than the germplasm populations (4.64 vs. 4.51 for the zoysiagrass and 6.26 vs. 6.09 for the bermudagrass). The bermudagrass apparently possesses greater color retention than the zoysiagrass, but because they were run in separate experiments there exists no statistical way to compare them. Three zoysiagrass germplasms possessed greater color retention than ‘Zorro’ (5.10), which was the zoysiagrass cultivar with the greatest color retention. One bermudagrass germplasm possessed greater color retention than ‘Chilly Verde’, which was the bermudagrass cultivar with the greatest color retention. For both species, several germplasm populations possessed color retention values similar to those of the cultivars with the greatest values.

The next step in this research is to complete the data collection, amalgamation, and analysis of the growth chamber evaluations. Following that step, we will initiate a gene expression study of the five greatest and lowest color retention entries for both species. We will then publish a final analysis in a peer-reviewed journal.

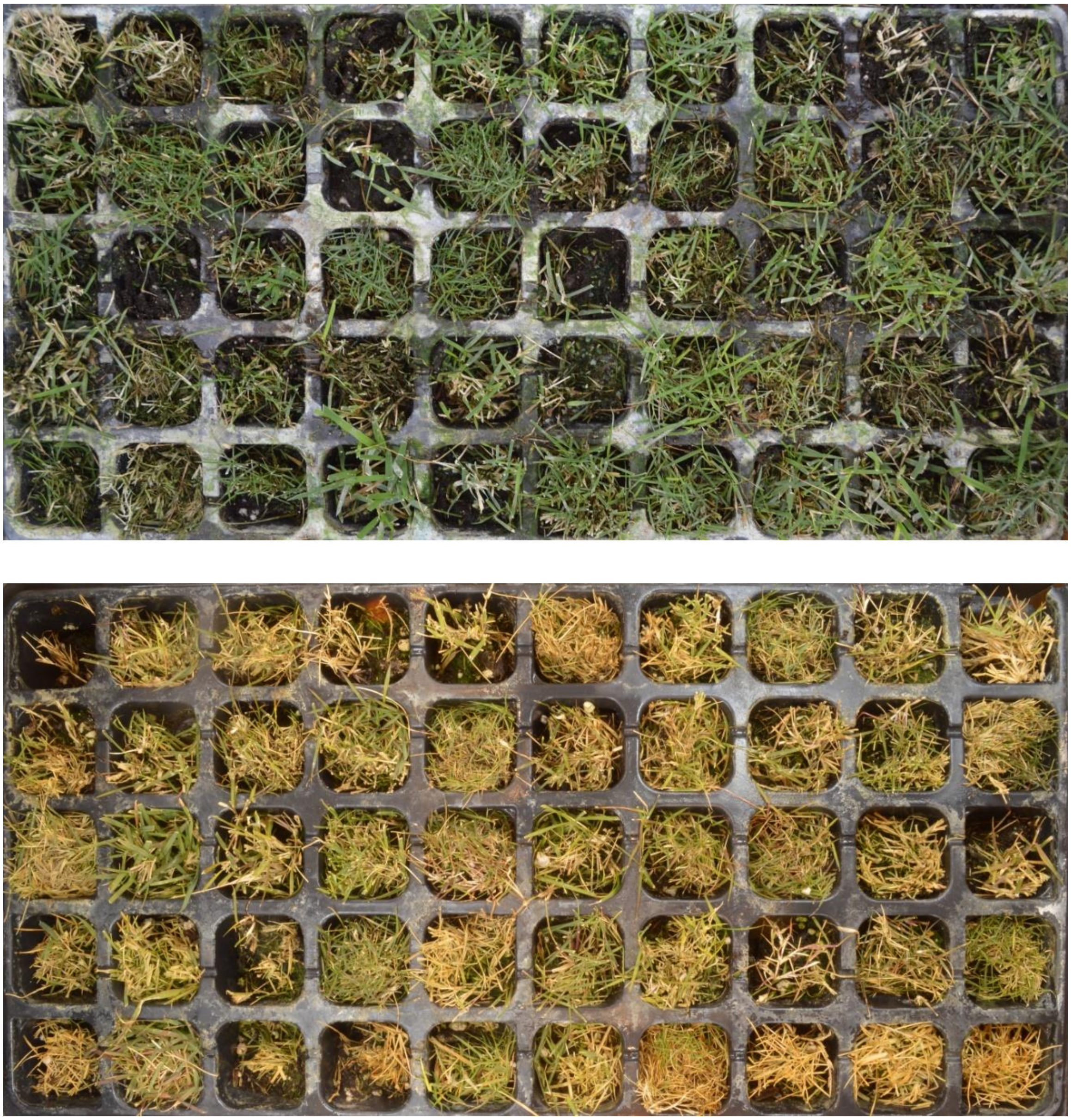

Figure 1. Digital images of trays containing 50 individual genotypes (clones) at different time points in the cool-season color retention evaluation.

Table 1. Summary of bermudagrass and zoysiagrass Dark Green Color Index ratings for cool temperature color retention based on growth chamber analysis of decreasing temperature.

	Bermudagrass	Zoysiagrass
High Accession Mean	7.41	5.73
Low Accession Mean	4.94	3.47
High Cultivar Mean	6.96	5.10
Low Cultivar Mean	5.71	4.17
Overall Accession Mean	6.09	4.51
Overall Cultivar Mean	6.26	4.64

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 5 years
Total funding $150,000

Summary Points
- Advanced lines and commercial cultivars of bermudagrass and zoysiagrass show separation for turfgrass quality, disease symptoms, and clipping yield during fall/winter (bermudagrass only).
- 100 lines of zoysiagrass selected in 2020 for winter performance were planted in a replicated trial
- Seed was harvested from crossing blocks planted with non-winter dormant germplasm

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

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

Bermudagrass

The nine commercial cultivars of bermudagrass were 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 ratings averaged across 2020 and 2021 and separated between summer and winter months. Summer months included March through November and winter included December through February. Turfgrass quality among commercial cultivars through the warmer growing seasons averaged greater than six for only two cultivars: TifTuf and Celebration. Averages ≥ 6 were produced from three experimental lines: FB1630, FB1628 and 343-34. Only two experimental entries averaged above five for turfgrass quality during the cooler months (FB1630 and FB1628). All commercial cultivars rated below five during the winter. FB1630 and Celebration maintain density during the cooler months (data not shown); however, both grasses can exhibit complete loss of color with heavy frosts. In contrast, TifTuf and Bimini will both hold color in the presence of a heavy frost but will lose density. FB1628 maintains color better than FB1630 and Celebration and density better than TifTuf.

Bipolaris (*Bipolaris cynodontis*) is frequently observed in the bermudagrass trial (Figure 2). Most entries had acceptable levels of disease. Entries with ratings ≥ 7 were 481-2, FB1628, FB1630, TifTuf, Celebration, 19-12-2, Bimini and FB1634.

In the fall of 2021, clippings were collected from selected lines in the bermudagrass study, including the nine commercial cultivars, FB1628, FB1630, 343-34, and 481-2 (Figure 3). Clippings were collected weekly from 8 November to 13 December from a strip in each plot measuring 1.4 m x 0.5 m at a mowing height of 1.3 cm. The collected clippings were dried at 50^{0} C and weighed. On 8 November the data violated the assumptions of Tukey's HSD, hence means were not different despite the large range in clippings collected. Differences in clipping dry weights were found for other weekly harvests. In the final reported harvest, Bimini, 481-2, 343-34, FB1628, TifTuf, and FB1530 were in the highest statistical group. Clipping weights, as an indication of entries with more active fall and winter growth are aligned with several entries exhibiting good turf quality in the winter. These data provide good evidence of differences between bermudagrass entries for their ability to grow and maintain turfgrass quality during cooler periods and time of year when more rounds of golf are played in Florida.

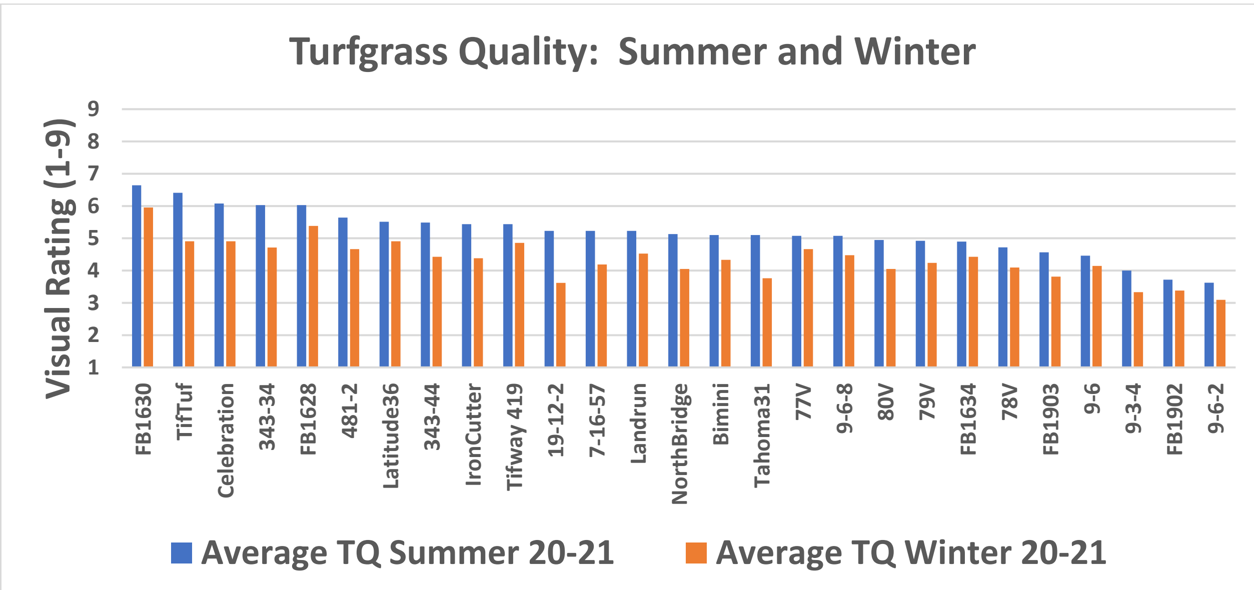

Figure 1. 2020 and 2021 average turfgrass quality ratings of 27 bermudagrass entries for summer and winter. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality.

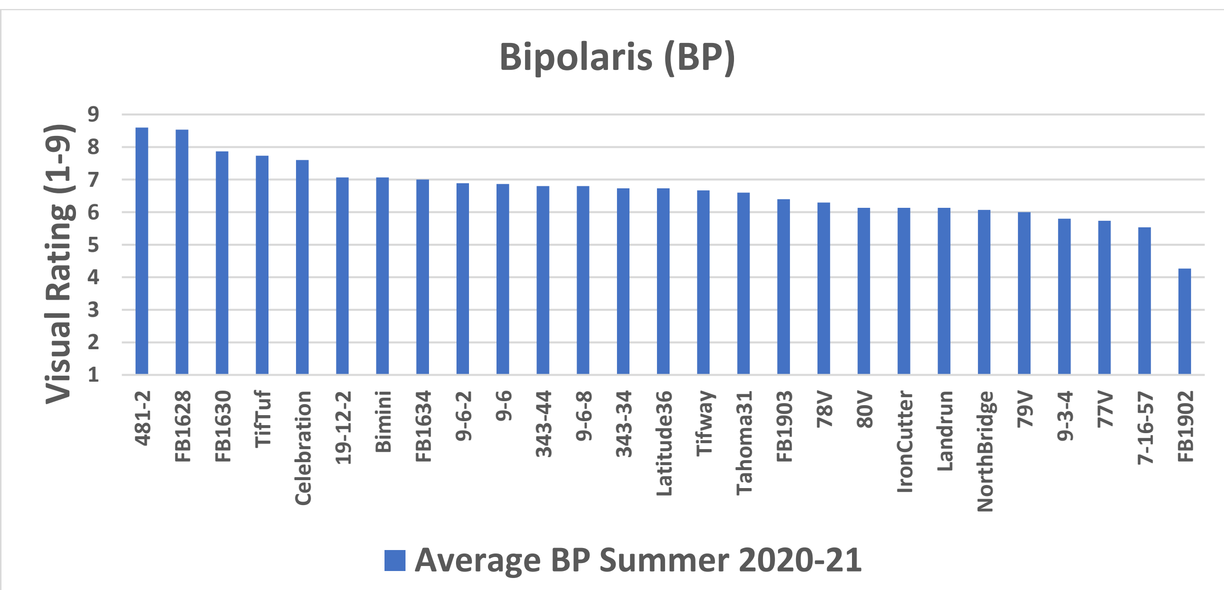

Figure 2. Average incidence of *Bipolaris cynodontis* on 27 entries of bermudagrass. Disease was visually rated using a 1-9 scale, where 9 equals no disease and 1 = complete death of a plot from disease.

Figure 3. Harvesting and bagging of clippings from selected bermudagrass entries mowed at 1.3 cm.

Table 1. Clipping Dry Weight Comparisons of Selected Bermudagrass Entries (grams)

Entries	11/8/2021		11/15/2021		11/22/2021		11/29/2021		12/7/2021		12/13/2021	
FB1630	4.67	a*	1.77	ab	1.52	a	0.80	bcd	1.16	abc	1.80	a
TifTuf	7.66	a	2.22	a	1.10	ab	1.58	a	1.70	a	1.55	ab
FB1628	5.47	a	1.72	abc	0.92	ab	1.17	abc	1.33	ab	1.10	abc
343-34	3.37	a	1.60	abcd	1.48	a	1.46	ab	1.07	abc	1.02	abc
481-2	2.88	a	1.17	abcde	1.18	ab	0.82	bcd	0.84	abc	0.98	abc
Bimini	2.22	a	0.98	bcde	0.71	ab	0.62	cd	0.61	bc	0.87	abc
Tifway	1.35	a	1.08	bcde	0.93	ab	0.63	cd	0.56	bc	0.83	bc
FB1634	0.92	a	0.59	de	0.72	ab	0.33	d	0.48	bc	0.56	c
Celebration	0.54	a	0.59	de	0.79	ab	0.29	d	0.46	bc	0.55	c
Tahoma31	0.55	a	0.67	cde	0.52	ab	0.42	cd	0.62	bc	0.40	c
IronCutter	0.92	a	0.71	bcde	0.53	ab	0.35	d	0.27	c	0.29	c
NorthBridge	0.54	a	0.45	e	0.39	b	0.26	d	0.66	bc	0.25	c
Landrun	0.80	a	0.53	e	0.38	b	0.25	d	0.20	c	0.24	c
Latitude36	0.87	a	0.42	e	0.46	ab	0.29	d	0.24	c	0.21	c

*Means within a column followed by different letters are significantly different (P<0.05)

Zoysiagrass

The two commercial cultivars of zoysiagrass were Zeon and CitraZoy. Visual ratings were collected for several parameters throughout the study. Figure three shows turfgrass quality ratings averaged across 2020 and 2021 and separated between summer and winter months. Summer months included March through November and winter included December through February. Turfgrass quality through the warmer growing seasons averaged for both years was ≥ 6 for most entries. Four experimental entries were ≥ 7: FAES1329, FZ1723, FAES1319, and FZ1642. For the winter months, no entries had average acceptable ratings; although, 15 entries (including CitraZoy) were ≥ 5.

Fifteen entries rated ≥ 6 for incidence of Dollar Spot (*Sclerotinia homoeocarpa*) (Figure 4). Many of these entries rated higher than seven indicating good potential for development of zoysiagrass cultivars with improved disease resistance for use in Florida.

Additional activities related to the objectives were the selection of 100 zoysiagrass lines from a spaced plant nursery that was planted in 2017. Selections were based on winter color retention, turf quality, and persistence. These selections were screened in the greenhouse for drought tolerance and planted in a replicated study. A new 2021 zoysiagrass spaced plant nursery was planted with 2,000 accessions.

Seed was harvested from both bermudagrass and zoysiagrass crossing blocks. This seed will be germinated for new spaced plant nurseries in 2022.

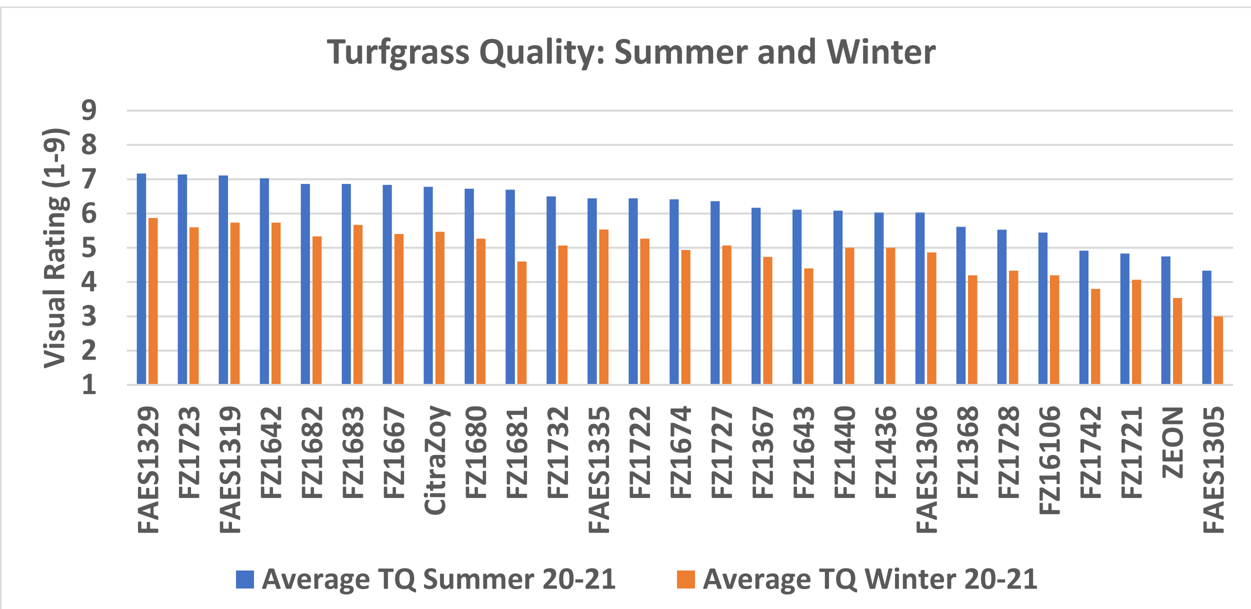

Figure 4. 2020 and 2021 average turfgrass quality ratings of 27 zoysiagrass entries for summer and winter. Turfgrass quality was visually rated using a 1-9 scale, where 9 = dark green, healthy, uniform turf, 1 = dead plot, and 6 = acceptable bermudagrass quality.

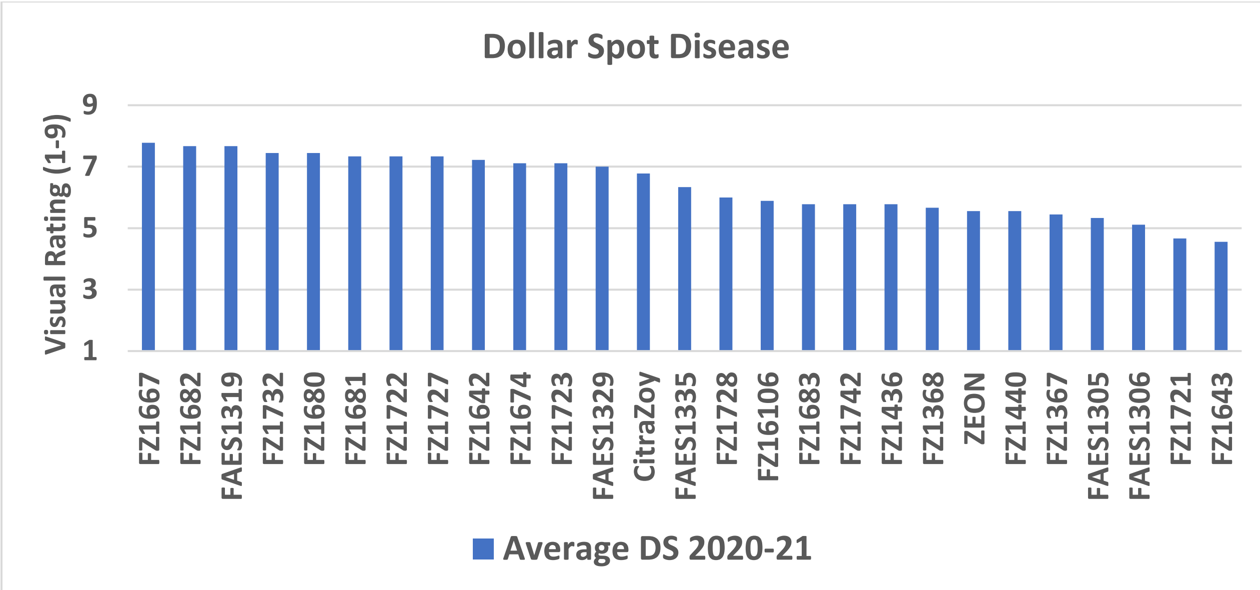

Figure 5. Average incidence of dollar spot disease on 27 entries of zoysiagrass. Disease was visually rated using a 1-9 scale, where 9 equals no disease and 1 = complete death of a plot from disease.

USGA ID#: 2017-21-631

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

Project leaders: Adam J. Lukaszewski, Marta Pudzianowska, Christian S. Bowman, and James H. Baird

Affiliation: University of California, Riverside

Objectives:

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

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

Summary Points

- Demonstration plots of UCR 17-8 and UCR TP6-3 bermudagrasses were established. Later they were used to start demonstration/test plots at four golf courses in Southern and Northern California, and at the California State University, Fullerton softball field.
- New shade tolerance study was established in 2021, including bermudagrass and kikuyugrass hybrids selected from 2018 and 2019 nurseries.
- Trial with 22 bermudagrass hybrids for suitability for roughs and lawns was established in July 2021.
- Drought and salinity stress trials were initiated in July 2021 for bermudagrass, zoysiagrass, St. Augustinegrass and seashore paspalum lines under the Specialty Crop Research Initiative (SCRI) project.
- Evaluation of the best UCR performers (#17-8, TP6-3, BF2 and #10-9) and seven commercial cultivars continues at the Napa Golf Course in Northern California to test performance under regular fairway traffic and maintenance.
- Testing bermudagrass and kikuyugrass for drought tolerance was initiated in 2019 and continued in 2021.

Summary

Warm-season or C4 turfgrass species including bermudagrass, zoysiagrass, and kikuyugrass are much better adapted to heat, drought, and salinity compared to cool-season grasses, but they go dormant during winter months making them less desirable choices for lawns, athletic fields, and golf courses. Clear differences in winter color retention, drought tolerance, and water use

efficiency exist among warm-season grasses, and within individual species, which indicates that genetic improvements are possible. Our objectives are to develop improved genotypes of these three species with emphasis on the winter color retention and drought tolerance for Mediterranean and arid climates.

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

Bermudagrass

Evaluation and selection in nurseries established in 2018, 2019 and 2020 continues. The best performing hybrids from 2018 and 2019 nurseries, selected in previous years, were planted in July 2021 under artificial shade. Thirty-three hybrids of bermudagrass were selected for this trial, they will be compared to five commercially available cultivars and two UCR hybrids, 17-8 and TP6-3. The latest nursery, planted in 2020, is currently under evaluation. Parents with valuable traits are being used for detached tiller crosses. Generated hybrids will be planted next spring in a new nursery.

After selection under fairway mowing height, 2018 and 2019 bermudagrass nurseries were subjected to another screening, this time for suitability for roughs and lawns. Twenty-four hybrids have been selected so far, and planted in July 2021 in test plots with 'Bandera', Bullseye', 'Midiron', 'Santa Ana', 'Tifway II', UCR TP6-3 and UCR 17-8 serving as checks. Plots are establishing, evaluation under 2 in mowing height will start in 2022.

Dry-down tests continued in 2021. This study includes 71 of the best hybrids and collection accessions identified in previous years, together with five commercial cultivars ('Bandera', 'Celebration', 'Santa Ana', 'TifTuf' and 'Tifway II') as checks. Plots were established in May 2019, in a completely randomized design with three replicates. As in 2020, entries were subjected to two consecutive dry-down periods followed by recovery periods. Several UCR entries outperformed commercial checks in both years, based on their average green (living tissue) coverage as determined by digital image analysis. Two accessions, UCRC180557 and UCRC180229, have remained among the top 5 performers since last year.

The fairway study planted in 2019 at the Napa Golf Course in Napa, CA continued in 2021. Study includes four top performing in previous trials UCR hybrids: 17-8, TP6-3, BF2 and 10-9, and seven cultivars: 'Bandera', 'Celebration', 'Latitude 36', 'Santa Ana', 'Tifway II', 'TifTuf' and 'Tahoma 31' (added later). Plots (20 x 12 ft) were placed on 2 fairways and maintained like the rest of the fairway. Plots were evaluated for their performance under regular golf course traffic and management, and for winter color retention. The best performing entries were 'Latitude 36' and 17-8, followed by TP6-3 and 'Santa Ana'. 'Celebration' had the lowest quality. UCR 17-8, UCR TP6-3, 'Santa Ana', 'TifTuf' and UCR BF2 had good winter color retention. Entries varied in seedhead production, with 'TifTuf' and 'Santa Ana' producing seedheads more intensively than the other three entries. The lowest seedhead production was in 'Latitude 36', UCR BF2 and UCR 17-8. Entries also varied in uniformity, with UCR 17-8 and 'Latitude 36' showing the highest scores, and 'Tahoma 31' and 'Celebration' the lowest.

A trial planted in 2019 including hybrids from 2014 nursery was continued at West Coast Turf farm in Coachella Valley, Thermal, CA and at Santa Lucia Preserve, Carmel-by-the-Sea, CA.

Plots were mowed at 2 inches to evaluate their suitability for roughs and lawns. UCR BH 19-2 and 'Bandera' were the best performing entries in both locations. All of the evaluated entries performed better in Coachella Valley than in Carmel-by-the-Sea. Due to water restrictions imposed on Santa Lucia Preserve golf course, plots did not receive water in the summer, which resulted in significant quality reduction and intensive seedhead production.

Based on performance in previous and ongoing studies, UCR 17-8 and UCR TP 6-3 were selected to be released as commercial cultivars. Demonstration plots of both entries were established in May 2021, with three mowing heights (0.5, 0.875 and 1.5 in), to show their performance at 2021 UCR Turfgrass and Landscape Field Day. In October 2021 they were utilized as source of sod for testing/demonstration plots at two golf courses in Southern California: The Farms at Rancho Santa Fe near San Diego and Wilshire Country Club, Los Angeles; and two in Northern California: Yocha Dehe Golf Club at Cache Creek, Brooks and Cinnabar Hills Golf Club, San Jose. Sod of UCR TP6-3 was also laid in November on a softball field of California State University, Fullerton.

Kikuyugrass

Evaluation of kikuyugrass hybrids planted in 2019 continued. Seventeen hybrids with 'Whittet' as a check were planted in August 2021 in a new shade trial. Plots are establishing, and evaluation will start in 2022. The kikuyugrass dry-down study also continued in 2021. The study was planted in 2019 and performed in a manner similar to that of the bermudagrasses. Thirty-eight accessions were selected based on their performance in a preliminary drought tolerance assessment, with 'Whittet' selections and 'AZ-1' serving as commercial checks. In 2021 only one drought cycle was applied, due to poor response of kikuyugrass entries in the first cycle and their prolonged recovery. Generally, the drought tolerance of kikuyugrass is lower than that of bermudagrass, but some variation among entries does exist. The best performing entries can be used in further breeding efforts to improve the drought tolerance of this species.

Occurrence of grey leaf spot in the summer of 2020 and 2021 allowed to evaluate susceptibility of collection accessions and their hybrids. Variation among hybrids appeared to be wider than that of parental populations, suggesting presence of different loci/alleles among collection entries, and a chance to stack these loci/alleles in the hybrids.

Other species

In 2019, the UCR breeding program established cooperation with five warm-season grass breeding programs under the Specialty Crop Research Initiative (SCRI) funded by the National Institute of Food and Agriculture (NIFA). The project involves breeding programs of North Carolina State University (NCSU), Oklahoma State University (OSU), Texas A&M AgriLife (TAMUS), the University of Georgia (UGA), and the University of Florida (UF). The lines of four species (189 lines of bermudagrass, 216 of zoysiagrass, 125 of St. Augustine grass and 90 of seashore paspalum) were planted in June and July 2020. UCR is the testing site for the overall performance, drought and salinity tolerance. Twenty of UCR hybrids are also evaluated in single space plant nurseries (SSPNs) across all testing locations. In July 2021 dry-down was initiated in SSPN and in advanced drought trials. Bermudagrass showed the best performance under drought. Irrigation with water of electroconductivity EC=4.4. dSm^{-1} was initiated in salinity trial at the beginning of July. The best performer under high salinity was seashore paspalum, the only species showing increase of quality when watered with saline water. The most intensive leaf firing was observed in zoysiagrass. Bermudagrass exhibited relatively low leaf firing, but also excessive seedhead production. Plants in both studies are currently recovering, and drought and salinity will be initiated again in 2022.

Figure 1. Bermudagrass entries in Specialty Crops Research Initiative (SCRI) project after ca. two months without irrigation at UCR Agricultural Operations field in Riverside, CA. Photo taken on 7 September 2021.

Figure 2. Zoysiagrass lines in a salinity trial in Specialty Crops Research Initiative (SCRI) project at UCR Agricultural Operations field in Riverside, CA. Photo taken on 28 October 2021.

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:

- Seed lots harvested in 2019 from the 2017 Isolation Blocks were scarified and germinated during the winter of 2019-2020. Due to reduced work times of staff because of social distancing imposed by COVID-19 pandemic, the newly produced progeny population (662) could not be planted as planned in 2020. They were instead held over in the greenhouse until the summer of 2021 when they were planted 22 June. A total of 24 new selections (12 red and 12 yellow) were made from the 2017 Isolation Blocks and the 2015 SPN and were planted in 2019 Isolation Crossing Blocks (Red vs Yellow). Two of the red seed parents did not grow well in the isolation block and neighboring entries grew into those plots so the functional number of red seed parents is 10 while the yellow seed parents remain at 12. Seed from the 2019 Yellow Isolation Crossing Block was harvested 27 May 2021. The seed from the 2019 Red Isolation Crossing Block was not harvested since it was negatively impacted by the winter. It will be re-evaluated in 2022.
- Our collaboration with Johnston Seeds has proven to be productive. Their evaluation of our germplasm and seed parents for cold hardiness in Enid, OK has led to the identification of 5 parental lines to be used in two synthetic/polycross nurseries and 8 coarse textured lines for recombination in an isolation block. These nurseries were planted in both Enid, OK and in Dallas.
- A new collaboration was initiated with Woerner Farms in 2021 with the transfer of 21 of our advanced seeded parental lines and a progeny population consisting of 520 genetically distinct individuals.

Introduction:

Zoysiagrass (*Zoysia* spp.) is a warm season, perennial grass with several redeeming traits that contributes to it being used on golf courses and home lawns. Zoysiagrass creates a uniform, dense, low-growing, high-quality turf with excellent heat, drought, pest and wear tolerance. It has a lower level of maintenance requirements as well as shade tolerance as compared to other turfgrasses making zoysiagrass a desirable alternative for turfgrass managers (Murray and Morris, 1988). Most cultivars are vegetatively propagated since they offer a higher quality and more uniform turf than seeded varieties. Seeded varieties such as ‘Zenith’ and ‘Compadre’ are relatively less expensive to establish when compared to their vegetative equivalents but lack the finer texture of some of the best vegetative type lines like ‘Innovation’. Speed of establishment has been shown to be faster with seeded zoysiagrass varieties when planted at appropriate densities (Patton et al., 2006). Availability of seed can also be a limiting factor. The focus of this research project is the development of multi-clone synthetic varieties that exhibit leaf textures finer than Zenith or Compadre with seed yields that meet the production goals needed to make it profitable to produce. Since the inception of the project in 2010, our breeding strategy has been to utilize the classical plant breeding method known as phenotypic recurrent selection. Our approach has been to alternate between Spaced Plant Nurseries (SPN) for progeny selection, and isolation crossing blocks to promote outcrossing and recombination. This strategy should allow for the gradual accumulation, over multiple generations, of desirable alleles affecting both seed yields and finer leaf texture. Thus far we have plant materials in different nurseries that are the result of 4 or 5 generations of recurrent selection.

Progress update:

In 2017, we began our fourth cycle of recurrent selection with the planting of 23 advanced lines identified from 1,750 progeny planted in the 2015 SPN. Those 23 lines were planted in isolation blocks, Red #1 (7 entries), Red #2 (9 entries) and Yellow (7 entries), based on their seedhead color, seedhead density, height of inflorescence exertion and texture. They were planted late in 2017 (9/26/17) so they were allowed to grow in during 2018. Seedheads were harvested in May of 2019 and stored until they could be processed during the winter of 2019-2020 to produce a clean seed product. The seed was scarified with 30% NaOH (Yeam et. al., 1985) and seed germinated on either filter paper or in potting mix to produce seedlings for planting in the field in 2020. Those plans had to be modified due to a reduction in hours in the lab and greenhouse because of the social distancing protocols brought on by the COVID-19 pandemic. Our goal of producing 25 seedlings growing in a 4” pot from each of the 26 families was accomplished. However, their development was too immature for planting in the field for adequate establishment prior to the onset of fall and winter in 2020. Our revised plan for field planting in the spring of 2021 was accomplished on 22 June.

In 2019 two Isolation Blocks were planted on 15 August. Parental line selections were made both from the 2017 Isolation Blocks and the 2015 SPN resulting in 12 red seedhead types being planted in one isolation block and 12 yellow seedhead types in the other isolation block. Five of our best red seeded parents were chosen from the 2017 Isolation Blocks to be combined with six new selections from the 2015 SPN and one of our Cold Hardy/Large Patch Tolerant advanced lines from another USGA sponsored project. Also, we chose two of our best yellow seeded parents from the 2017 Isolation Blocks to be combined with 10 new selections from the 2015 SPN. Of the 12 red seeded parents, two failed to thrive and were overgrown by neighboring plots resulting in a reduction in red seed parents to 10. All 12 yellow seeded parents expanded vegetatively to fill their plots. Seed from the 2019 Yellow Isolation Crossing Block was harvested 27 May 2021 and measurements for seedhead density, seed per seedhead and seedhead height were taken (see Table 1). The lines that had the best performance from a seed production perspective were 6585-34, 6596-05 and 6596-22. That seed will be processed in the spring of 2022 for germination and the development of another spaced plant nursery. It should be noted that seedheads were not harvested from the 2019 Red Isolation Crossing Block due to poor flowering. This was presumably due to winter injury incurred during February of 2021. We will reevaluate the nursery for seedhead production in 2022.

Johnston Seeds has extensive experience with seeded Bermudagrass production which lead us to trying and leverage that experience and apply it to seeded zoysiagrass. Our collaboration with Johnston Seed (Enid, OK) was initiated in 2018 with the transfer of vegetative material from our most advanced parental lines and 535 progeny from 16 coarse textured families. Data collected by Dr. Kevin Kenworthy at Johnston Seed Co. in 2019 and 2020 has enabled us to identify five parental lines for use in two synthetic/polycross nurseries. One of those nurseries will have two parents (TAES 6596-05 and 6086-21) and the other nursery will make use of three parents (TAES 6596-05, 6585-34 and 6087-15). The first polycross nursery has been identified based on its flowering date as the “early seed set nursery” allowing harvest in June and the other polycross nursery identified as the “later seed nursery” with an estimated harvest date of July. These two polycrosses were planted in both Enid, OK and Dallas, TX in 2021 for evaluation of seed yields and other performance characteristics. The objective of this combined effort is to produce enough seed to enter one or both in the 2025 NTEP. Furthermore, an evaluation of the 535 progeny in the coarse textured spaced plant nursery led to the identification of 8 lines for use in a recombination isolation crossing block planted both in Enid OK and Dallas, TX in 2021. In 2021 we further expanded our collaborative efforts by partnering with Woerner Farms in the evaluation of our advanced seeded zoysia germplasm in the unique environmental conditions found in southern Alabama. Twenty-one of our advanced seed parents will be grown there to evaluate seed yields. In addition, we have produced a population of 520 seedlings each growing in a 4” pots (Figure 1) derived from remnant seed harvested from our 2017 Isolation Crossing Blocks. These same seed lots were used to create the 2021 spaced plant nursery planted in Dallas on 22 June. We anticipate the identification of seed parents that are uniquely adapted to the two different environments.

Figure 1. Seeded zoysiagrass progeny growing in 4" pots to be evaluated by Woerner Farms in southern Alabama.

Table 1. Seedhead parameters for the 2019 Yellow Isolation Block

Entry	TAES#	Inflorescence/ft^{2a}	Seed/Inflorescence[b]	Seed/ft^{2c}	Seedhead height[d]
		#			in
1	6585-34	336.7	23.5 ab	7912.45	6.5 bc
2	6588-49	228.3	19.7 d	4497.51	3.7 e
3	6596-05	657.3	23.2 abc	15,249.36	6.8 abc
4	6596-22	342.3	25.3 a	8660.19	4.0 de
5	6606-14	286.0	21.5 bcd	6149.00	6.2 bc
6	6606-47	459.0	23.7 ab	10,878.30	6.0 bc
7	6609-44	292.3	21.3 bcd	6225.99	8.7 a
8	6609-47	394.7	25.1 a	9906.97	5.5 cde
9	6610-30	403.7	18.8 d	7589.56	5.7 cd
10	6611-18	348.7	18.7 d	6520.69	6.3 bc
11	6612-10	254.3	19.0 d	4831.70	5.3 cde
12	6618-37	271.0	20.3 cd	5501.30	8.2 ab
LSD		ns	3.03	n/a	1.93
CV			17.3		19.0

[a]Number of inflorescence per square feet measured using a 12” x 12” string lattice laid on the plot in each of 3 reps.
[b]Number of seed per inflorescence where number of florets on four inflorescence per rep for 3 reps.
[c]Number of seed per square feet determined by multiplying inflorescence/ft^2 by seed/inflorescence.
[d]Height of the seedhead determined by measuring seedhead height using a ruler in each of 3 reps.

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

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Objectives:

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

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

3. **Phase III (year 4-6): In-progress -** A set of 65 hybrids (25 – Purdue, 20 - KSU and 20 – TAM AgriLife) was selected in fall of 2020 based on their superior performance in 2018/2019/2020. Entries were propagated into 11 18-cell trays in Dallas during the winter of 2020/2021. In year one, rate of establishment and winter survival will be evaluated. Additional data will be collected on many traits that are used to characterize turfgrasses such as rate of establishment, turf quality, spring green up, genetic color, leaf texture. Tolerance to large patch disease will be evaluated in Olathe, KS and hunting billbug in West Lafayette, IN.

Start Date: 2018
Project Duration: 6 years

Summary Points:

- In 2021, the initiation of Phase III, which included 65 experimental genotypes, 4 elite genotypes, and 5 standards were planted at Dallas, TX, Olathe, KS, West Lafayette, IN and five additional study locations throughout the summer and early fall of 2021.

- Coverage data were collected at the eight locations in the fall of 2021, and many experimental genotypes had a rate of coverage that was the same as or better than the elite experimental lines and standard entries. Additional data collection will begin in 2022 after full establishment of the entries.

Summary Text:

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

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

TAM AgriLife, KSU and Purdue University have been working on a USGA-funded project since 2012 where the main objective is to incorporate large patch (*Rhizoctonia solani* AG 2-2LP) disease tolerance, along with cold hardiness and improved quality, into new transition zone adapted zoysiagrasses. In 2018, the top ten hybrids with intermediate leaf texture (out of over 2,800) exhibiting large patch tolerance and cold hardiness were selected for advanced evaluations by the three collaborating institutions and five golf courses. This project is approaching completion and four elite lines originating from this research are included in this evaluation as well.

For the current project, we have planted new crosses between these intermediate texture types with cold hardiness available to us from the earlier projects and under-utilized and finer-textured *Zoysia* species (*Z. pacifica, Z. minima and Z. pauciflora*) available in our germplasm collection. The focus of this project is to investigate how these cold hardy zoysiagrasses with quality suitable for golf course fairways, tees, and putting greens adapt to regions throughout the United States. In addition to cold hardiness and turfgrass quality, experimental hybrids will also be evaluated for large patch (Kansas, Missouri), herbicide screening, ball lie, and billbug tolerance (Indiana), divot recovery (Arkansas, Virginia), thatch accumulation and traffic tolerance (Tennessee), water-deficit effects (Florida), and shade tolerance (Texas).

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 TAM AgriLife for propagation. In spring, 2021, these 65 experimental lines, 4 elite hybrids developed from a separate USGA-sponsored project (DALZ 1701, DALZ 1702, DALZ 1808, and DALZ 1818), and 5 standards were delivered to cooperative state universities listed in in Table 1; photos of a few planting sites are presented in Fig. 1. Arkansas is also included in evaluation, and will provide additional information soon. An establishment rating (rated visually on a 0 to 100% scale on which 0 = no establishment, and 100 = complete establishment) was taken at each site at the end of the 2021 growing season (Tables 2 and 3). Range of coverage at each location was as follows: KS, 30 to 100%; TN, 27 to 77%; IN, 30 to 55%; MO, 38 to 96%; VA, 17 to 48%; TX (full sun) 27 to 88%; TX (shade), 25 to 75%. Planting was done on 30 August in FL, and little spread had occurred by the time plots were rated. Many experimental lines have established the same as or better than elite experimental lines and standard entries. Beginning in 2022, initiation of data collection will begin on genotypes once fully established. We expect there will be numerous, high quality, fine textured, cold-tolerant genotypes that will have the potential to become improved zoysiagrass cultivars in the transition zone and upper south.

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Table 1. Site information for replicated field trials planted in the summer of 2021.

	KSU (Olathe, KS)	Univ. of Tennessee (Knoxville, TN)	Purdue (West Lafayette, Indiana)	Univ. of Missouri (Columbia, MO)	Virginia Tech. (Blacksburg, VA)	TAM AgriLife (Dallas, TX)	Univ. of Florida (Gainesville, FL
Planting Date	17 June	17 June	21 July	15 June	18 June	24 June	30 August
Soil Type	Oska-Martin Silty Clay Loam	Sequatchie Silt Loam	Silty Clay Loam	Mexico Silt Loam	Groseclose-Urban Land Complex Loam	Silty Clay Loam	Candler Sand
Fertilizer	6 July Andersons 16-0-4 (0.75 lb N/1000 ft^2)	Monthly 46-0-0 (1lb N/1000 ft^2)	21 July Shaw's starter 6-24-24 (0.25 lb N/1000ft^2 [1lb P_2O_5) 17-Aug Shaw's 24-0-22 (1lb N/1000ft^2)	24 June, 9 July Thrive 13-13-13 (1 lb N/1000 ft^2)	18 July 10-10-10 (1.0 lb N/1000 ft^2)	22 July, 15-Sept Harrells 25-5-10 (1lb N/1000 ft^2)	27-Oct 15-0-15 (1 lb N/1000 ft^2)
Pesticides	17 June Ronstar 2 G (4.5 lbs/1000 ft^2)	17 June Ronstar 2 G (4.5 lbs/1000 ft^2) Late Sept. & mid Oct. Fusilade (3.4mL/1000 ft^2) + Turflon Ester (21.7 mL/1000 ft^2)	21 July Ronstar (0.069 lb ai/1000 ft^2) 2-Nov Trimec 992 (1.5 fl oz/1000 ft^2) 3-Nov Princep (21.7 mL/1000 ft^2)	23 Sept Speedzone (1.5 fl oz/1000 ft^2) + Dismiss NXT (6.8 mL/1000 ft^2) 30-Sept Turflon Ester (21.7 mL/1000 ft^2) + Fusilade II (3.4 mL/1000 ft^2)	18 June Ronstar 2 G (4.5 lbs/1000 ft^2) Mid-Aug. Acclaim Extra (19 mL/1000 ft^2) + Turflon Ester (21.7 mL/1000. ft^2)	24 June Andersons 5-0-15 with Oxadiazon (4.5 lbs/1000 ft^2) (no slow release N)	None
Irrigation	Daily for 2 weeks after planting 1" weekly thereafter	Daily during establishment Once every 4 days after establishment	July-August 4-6 times weekly Sept-Nov 1-2 times weekly	1" in June for two weeks post-planting 1" in August	Daily during the first 2 weeks Weeks 3-4 4-5 times/wk Hand watered to prevent wilt after wk 4	1"/week for full sun treatments 0.5"/week for shade treatments	4x/week
Mowing	None	Maintained at 7/8" with reel mower	Late Aug-Oct 1.75" weekly	1.5" HOC in August through September	None	1" weekly starting 13-July	None

Table 2. Coverage of experimental zoysiagrass genotypes across states in replicated field trials in the fall 2021.

Entry Number	Entry I.D.	Lineage[a]	Kansas	Tennessee	Indiana	Missouri	Virginia	Texas (full sun)	Texas (63% shade)
			Coverage (%)[bc]						
			Days after planting						
			120	120	82	143	145	129	129
1	6782-42	[(Zj x Zp)/Zj) x Zp]	91.7[d]	26.7	30	96	31.7	28.3	25
2	6782-75	[(Zj x Zp)/Zj) x Zp]	88.3	32	31.7	89.3	46.7	35	33.3
3	6782-79	[(Zj x Zp)/Zj) x Zp]	96.7	30	35	94.3	35	61.7	40
4	6782-104	[(Zj x Zp)/Zj) x Zp]	95	31.7	30	88.3	21.7	26.7	40
5	6782-120	[(Zj x Zp)/Zj) x Zp]	30	31.7	30	48.3	15	35	33.3
6	6783-03	[(Zj x Zp)/Zj] x Zp)	30	34	31.7	56.7	20	33.3	26.7
7	6784-17	[(Zj x Zp)/Zj] x Zp)	35	42	33.3	70	21.7	70	60
8	6785-19	[(Zj x Zp)/Zj] x Zp)	36.7	41.3	31.7	38.3	16.7	30	35
9	6785-22	[(Zj x Zp)/Zj] x Zp)	28.3	23.3	31.7	53.3	25	45	43.3
10	6786-02	[(Zj x Zp)/Zj] x Zp)	31.7	41.3	35	46.7	18.3	41.7	46.7
11	6787-18	[(Zj x Zp)/Zj] x Zp)	58.3	27.7	30	76.7	28.3	31.7	31.7
12	6787-20	[(Zj x Zp)/Zj] x Zp)	30	26.7	30	41.7	16.7	x	x
13	6789-23	[(Zj x Zp)/Zj] x Zp	31.7	28	31.7	60	18.3	53.3	33.3
14	6789-40	[(Zj x Zp)/Zj] x Zp	35	30.3	30	70	31	45	40
15	6791-06	[(Zj x Zp)/Zj] x Zp)	30	36.7	30	50	25	45	38.3
16	6792-44	[(Zj x Zp)/Zj] x Zp)	33.3	34	31.7	48.3	21.7	50	35
17	6829-34	[(Zj x Zp)/Zj] x Zm)	38.3	40	33.3	45	20	55	40
18	6910-157	Zj x (Zj x Zm)	38.3	36.7	36.7	50	21.7	55	31.7
19	6910-172	Zj x (Zj x Zm)	73.3	42.3	41.7	80	31.7	81.7	46.7
20	6941-36	(Zj x Zm)	36.7	47.7	38.3	56.7	20	65	41.7
21	6844-154	[Zm x Zj] x [(Zj x Zp)/Zj])	41.7	61.3	38.3	66.7	23.3	65	46.7

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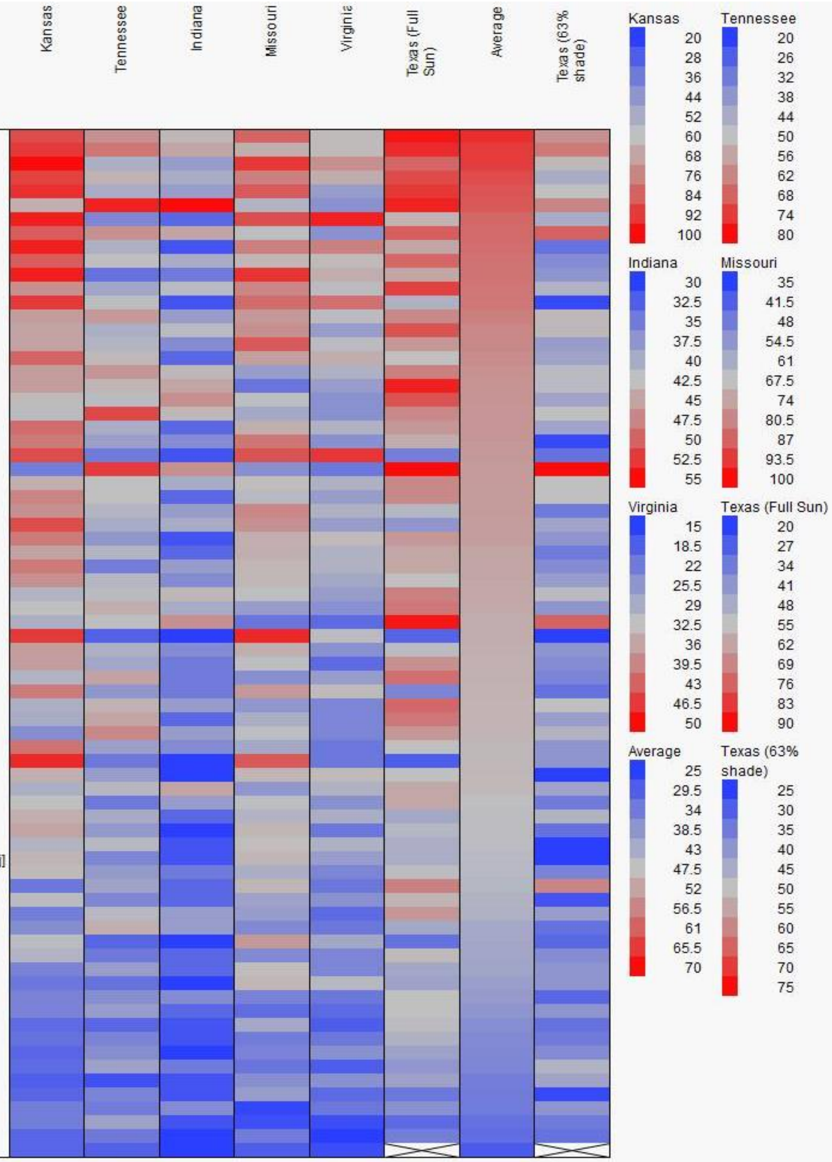
22	6844-91	[Zm x Zj] x [(Zj x Zp)/Zj])	36.7	32.3	36.7	36.7	21.7	40	40
23	6830-56	(Zm x Zj) x Zm)	68.3	45	41.7	78.3	26.7	78.3	51.7
24	6844-190	[Zm x Zj] x [(Zj x Zp)/Zj])	60	31.7	38.3	66.7	25	61.7	35
25	6940-15	(Zj x Zm)	55	48.3	43.3	66.7	26.7	70	48.3
26	6844-128	[Zm x Zj] x [(Zj x Zp)/Zj])	58.3	35	33.3	58.3	25	58.3	28.3
27	6844-31	[Zm x Zj] x [(Zj x Zp)/Zj])	68.3	46.7	36.7	88.3	31.7	65	41.7
28	6844-147	[Zm x Zj] x [(Zj x Zp)/Zj])	76.7	50	33.3	65	26.7	68.3	50
29	6829-36	[(Zj x Zp)/Zj] x Zm)	70	45.3	36.7	71.7	25	53.3	40
30	6844-141	[Zm x Zj] x [(Zj x Zp)/Zj])	78.3	32.7	38.3	70	30	61.7	38.3
31	6924-47	(Zm x Zj) x (Zm x Zj)	78.3	40.7	36.7	83.3	25	60	26.7
32	6844-152	[Zm x Zj] x [(Zj x Zp)/Zj])	70	52.3	45	46.7	26.7	86.7	48.3
33	6924-66	(Zm x Zj) x (Zm x Zj)	83.3	51.7	33.3	75	35	55	43.3
34	6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]	65	43.7	33.3	63.3	29.3	46.7	46.7
35	6844-34	[Zm x Zj] x [(Zj x Zp)/Zj])	91.7	64.7	45	71.7	33.3	85	61.7
36	6830-11	(Zm x Zj) x Zm)	73.3	46.7	38.3	80	30	51.7	35
37	6924-44	(Zm x Zj) x (Zm x Zj)	61.7	48.7	46.7	66.7	25	78.3	43.3
38	6942-22	(Zj x Zp)	70	58.3	38.3	76.7	31.7	68.3	51.7
39	6839-08	Zm x [(Zj x Zp)/Zj])	85	50.3	40	70	33.3	75	38.3
40	6925-53	(Zm x Zj) x (Zm x Zj)	53.3	47.3	45	55	30	61.7	43.3
41	6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	63.3	34.7	31.7	70	26.7	48.3	25
42	6844-53	[Zm x Zj] x [(Zj x Zp)/Zj])	91.7	49	31.7	85	41.7	50	26.7
43	6844-150	[Zm x Zj] x [(Zj x Zp)/Zj])	78.3	38.3	35	76.7	33.3	36.7	33.3
44	6829-02	[(Zj x Zp)/Zj] x Zm)	81.7	44.3	33.3	71.7	30	65	43.3
45	6828-53	Zm x [(Zj x Zp)/Zj]	53.3	53	38.3	55	23.3	75	50
46	6844-104	[Zm x Zj] x [(Zj x Zp)/Zj])	55	56.3	35	53.3	26.7	73.3	36.7
47	6828-56	Zm x [(Zj x Zp)/Zj]	38.3	40	33.3	68.3	23.3	46.7	40
48	6830-02	((Zm x Zj) x Zm)	70	59	43.3	56.7	30	70	48.3
49	6844-36	[Zm x Zj] x [(Zj x Zp)/Zj])	65	50	40	68.3	30	68.3	50
50	6835-33	(Zm x Zj) x Zm	41.7	54	38.3	51.7	21.7	46.7	33.3
51	6828-27	Zm x [(Zj x Zp)/Zj]	68.3	39.3	30	70	21.7	51.7	33.3
52	6828-77	Zm x [(Zj x Zp)/Zj]	65	40	30	70	33.3	55	25
53	6840-20	Zm x [(Zj x Zp)/Zj)	60	54	40	56.7	25	71.7	40

54	6933-11	(Zm x Zj) x (Zm x Zj)	96.7	45.3	31.7	81.7	40	61.7	33.3
55	6830-39	(Zm x Zj) x Zm)	58.3	71.7	43.3	60	25	68.3	50
56	6844-04	[Zm x Zj] x [(Zj x Zp)/Zj])	55	47.7	31.7	68.3	30	48.3	25
57	6844-202	[Zm x Zj] x [(Zj x Zp)/Zj])	55	31.7	33.3	51.7	23.3	56.7	38.3
58	6844-74	[Zm x Zj] x [(Zj x Zp)/Zj])	80	40.7	36.7	60	21.7	55	40
59	6829-69	[(Zj x Zp)/Zj] x Zm)	93.3	44.3	38.3	88.3	26.7	83.3	50
60	6923-11	(Zj x Zm) x Zj)	36.7	73.3	46.7	53.3	21.7	90	75
61	6844-42	[Zm x Zj] x [(Zj x Zp)/Zj])	100	45	38.3	93.7	38.3	75	51.7
62	6831-09	Zm x (Zm x Zj)	88.3	60	43.3	86.7	33.3	88.3	58.3
63	6789-52	[(Zj x Zp)/Zj] x Zp	51.7	56	33.3	61.7	23.3	71.7	41.7
64	6844-89	[Zm x Zj] x [(Zj x Zp)/Zj])	70	42.3	35	66.7	20	66.7	38.3
65	6829-20	[(Zj x Zp)/Zj] x Zm)	68.3	46.7	33.3	71.7	30	61.7	35
66	6095-83	(DALZ1701 = Zj x Zm)	53.3	49.7	46.7	46.7	20	88.3	65
67	6095-101	(DALZ1702 = Zj x Zm)	90	53	40	81.7	35	80	45
68	6099-145	(DALZ 1808 = Zj)	85	60	45	66.7	25	76.7	65
69	6109-87	(DALZ 1818 = (Zp x Zj) x Zj)	78.3	38.7	31.7	71.7	33.3	65	40
70	Meyer	(Zj)	61.7	39.3	35	61.7	23.3	53.3	36.7
71	Innovation	(Zm x Zj)	88.3	44	40	78.3	26.7	41.7	43.3
72	Palisades	(Zj)	65	77	55	63.3	25	86.7	60
73	Zeon	(Zm)	96.7	35	33.3	90	48.3	58.3	45
74	Emerald	(Zj x Zp)	73.3	47.3	36.7	70	28.3	55	41.7
LSD[e]			18.0	15.0	4.8	18.9	9.2	14.5	13.7

[a] Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
[b]Dates for planting and coverage rating: KS, 17 June and 15 Oct.; TN, 17 June and 15 Oct.; IN, 21 July and 11 Oct.; MO, 15 June and 5 Nov.; VA, 18 June and 10 Nov.; TX, 24 June and 1 Nov.
[c] Coverage was rated visually on a 0 to 100% scale on which 0 = no coverage, and 100 = complete coverage.
[d] Means across treatment replications (n = 3).
[e] LSD, least significant difference between means within a column based on Fisher's LSD at $P = 0.05$.

Table 2. "Heat map" of genotypes reflecting coverage at the end of the first growing season. Genotypes near the top of the table in red are those which demonstrated the most rapid coverage across locations.

EntryNum	EntryID	Lineage
62	6831-09	Zm x (Zm x Zj)
35	6844-34	[Zm x Zj] x [(Zj x Zp)/Zj]
61	6844-42	[Zm x Zj] x [(Zj x Zp)/Zj]
67	6095-101 (DALZ 1702)	(Zm x Zj) x Zj
59	6829-69	[(Zj x Zp)/Zj] x Zm
72	Palisades	Zj
73	Zeon	Zm
68	6099-145 (DALZ 1808)	Zj x Zj
54	6933-11	(Zm x Zj) x (Zm x Zj)
39	6839-08	Zm x [(Zj x Zp)/Zj]
3	6782-79	[(Zj x Zp)/Zj] x Zp
19	6910-172	Zj x (Zj x Zm)
42	6844-53	[Zm x Zj] x [(Zj x Zp)/Zj]
38	6942-22	Zj x Zp
23	6830-56	(Zm x Zj) x Zm
27	6844-31	[Zm x Zj] x (Zj x Zp)/Zj]
33	6924-66	(Zm x Zj) x (Zm x Zj)
48	6830-02	(Zm x Zj) x Zm
32	6844-152	[Zm x Zj] x [(Zj x Zp)/Zj]
37	6924-44	(Zm x Zj) x (Zm x Zj)
55	6830-39	(Zm x Zj) x Zm
44	6829-02	[(Zj x Zp)/Zj] x Zm
31	6924-47	(Zm x Zj) x (Zm x Zj)
2	6782-75	[(Zj x Zp)/Zj] x Zp
60	6923-11	(Zm x Zj) x (Zj x Zj)
49	6844-36	[Zm x Zj] x [(Zj x Zp)/Zj]
28	6844-147	[Zm x Zj] x [(Zj x Zp)/Zj]
36	6830-11	(Zm x Zj) x Zm
71	Innovation	Zm x Zj
69	6109-87 (DALZ 1818)	[(Zj x Zp)/Zj] x Zj
65	6829-20	[(Zj x Zp)/Zj] x Zm
30	6844-141	[Zm x Zj] x [(Zj x Zp)/Zj]
74	Emerald	Zj x Zp
25	6940-15	Zm x Zj
53	6840-20	Zm x [(Zj x Zp)/Zj]
66	6095-83 (DALZ 1701)	(Zm x Zj) x Zj
1	6782-42	[(Zj x Zp)/Zj] x Zp
29	6829-36	[(Zj x Zp)/Zj] x Zm
64	6844-89	[Zm x Zj] x [(Zj x Zp)/Zj]
46	6844-104	[Zm x Zj] x [(Zj x Zp)/Zj]
43	6844-150	[Zm x Zj] x [(Zj x Zp)/Zj]
45	6828-53	Zm x [(Zj x Zp)/Zj]
63	6789-52	[(Zj x Zp)/Zj] x Zp
21	6844-154	[Zm x Zj] x (Zj x Zp)/Zj]
58	6844-74	[Zm x Zj] x [(Zj x Zp)/Zj]
4	6782-104	[(Zj x Zp)/Zj) x Zp
52	6828-77	Zm x [(Zj x Zp)/Zj]
40	6925-53	(Zm x Zj) x (Zm x Zj)
24	6844-190	[Zm x Zj] x [(Zj x Zp)/Zj]
34	6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]
51	6828-27	Zm x [(Zj x Zp)/Zj]
56	6844-04	[Zm x Zj] x [(Zj x Zp)/Zj]
41	6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]
70	Meyer	Zj
7	6784-17	[(Zj x Zp)/Zj] x Zp
26	6844-128	[Zm x Zj] x (Zj x Zp)/Zj]
20	6941-36	Zj x Zm
50	6835-33	(Zm x Zj) x Zp
11	6787-18	[(Zj x Zp)/Zj] x Zp
57	6844-202	[Zm x Zj] x [(Zj x Zp)/Zj]
47	6828-56	Zm x [(Zj x Zp)/Zj]
14	6789-40	[(Zj x Zp)/Zj] x Zp
18	6910-157	Zj x (Zj x Zm)
17	6829-34	Zm x (Zj x Zm)
13	6789-23	[(Zj x Zp)/Zj] x Zp
16	6792-44	[(Zj x Zp)/Zj] x Zp
15	6791-06	[(Zj x Zp)/Zj] x Zp
10	6786-02	[(Zj x Zp)/Zj] x Zp
9	6785-22	[(Zj x Zp)/Zj] x Zp
6	6783-03	[(Zj x Zp)/Zj] x Zp
22	6844-91	[Zm x Zj] x [(Zj x Zp)/Zj]
8	6785-19	[(Zj x Zp)/Zj] x Zp
5	6782-120	[(Zj x Zp)/Zj] x Zp
12	6787-20	[(Zj x Zp)/Zj] x Zp



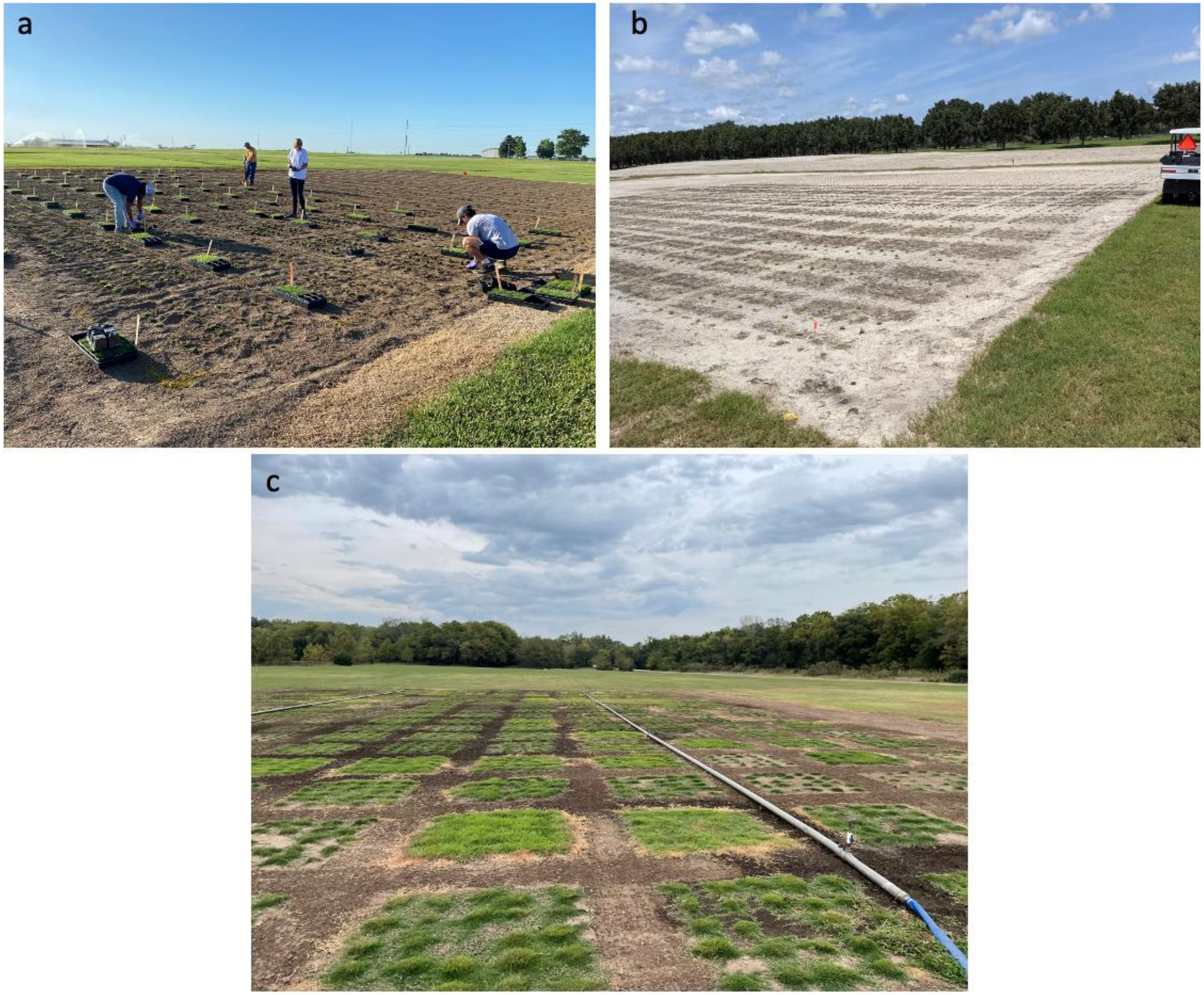


Figure 1. Zoysiagrass genotypes being planted on 15 June in Columbia, MO (Image a); Completed zoysiagrass planting on 30 August in Gainesville, FL (Image b); View of zoysiagrass genotypes 15 weeks after planting in Olathe, KS (Image c).

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 in the autumn of 2020 or summer of 2021 to be assessed in future on-site trials due to their drought resistance and aggressiveness observed at multiple locations (Braun et al., 2021). In addition, cultivar checks (i.e. Meyer, Zenith, Jamur, or Innovation) common to each state are included. Therefore, twelve (10 by 10 ft) plots were established by either sodding or plugging in 2020 or 2021 at the W.H. Daniel Turfgrass Research and Diagnostic Center, West Lafayette, IN; Lake Wheeler Turfgrass Field Lab, Raleigh NC; and Coastal Plain Experiment Station, Tifton, GA. In summer 2022 at each site, zoysiagrass sod will be harvested from each plot and transplanted on a golf course rough area within each state (IN, NC, and GA). Following establishment in 2022, demonstration plots will be maintained with minimal inputs (fertilization, irrigation, or pest control) the following years, and mown as needed by the superintendent similar to their other primary rough areas. Golf professionals, members, and golf course superintendents at each course managing the demonstration plots will be interviewed once per year to receive feedback on turf quality, ball lie (acceptable and optimal), and other potential turf golfing or turf characteristics. In addition, ball lie will be measured for each entry using the method developed by Richardson et al. (2010).

For objective 2, 229 lines were developed from crosses of large patch (LP) susceptible Meyer by LP-resistant PI 231146. Plugs of all individuals were grown in Styrofoam cups filled with calcined clay for 2-3 months until fully established. At the time, cups were arranged in a Randomized Complete Block Design with two replications in a walk-in growth chamber at the NC State Phytotron. Large patch inoculations were performed by placing 8-10 *R. solani* infected rye grain in the crown region of each plant. Plant were kept at 20/18 C with >75% relative humidity to promote infection. Disease severity was evaluated every three days for 21 days using the Horsfall-Barratt scale and digital imaging. Two runs of inoculations have been completed two date. Excellent segregation for LP response has been observed across runs (Figure 1). Additionally, differences in disease progression have been observed between resistant and susceptible individuals (Figure 2). A third run of inoculations will be started in January 2022. For the genotyping component, DNA extractions from all inviduals have been completed. Genotype-by-Sequencing (GBS) library preparation is underway and samples should be sent for sequencing by the end of the year. We expect Single Nucleotide Polymorphism (SNP) calling and linkage map construction to be complete by Mid March.

For objective 3, new zoysiagrass lines were produced from crosses last spring. They were established in unreplicated field nurseries across North Carolina and Georgia. Plots were evaluated for speed of establishment, turf quality, and drought tolerance during 2021. A few of these hybrids appear very promising in terms of speed of establishment and stress tolerance.

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

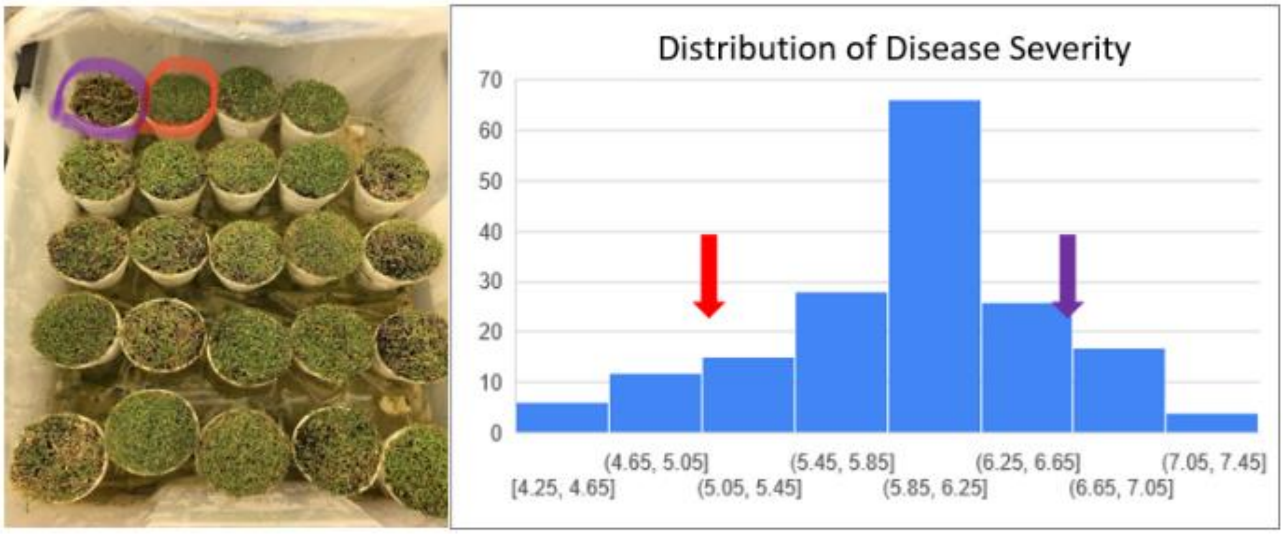


Figure 1. Excellent segregation for LP response has been observed across two runs of evaluation. Meyer (purple) is consistently susceptible and PI 231146 (red) resistant. The progeny exhibit normal distribution with some lines having more extreme response than either parent.

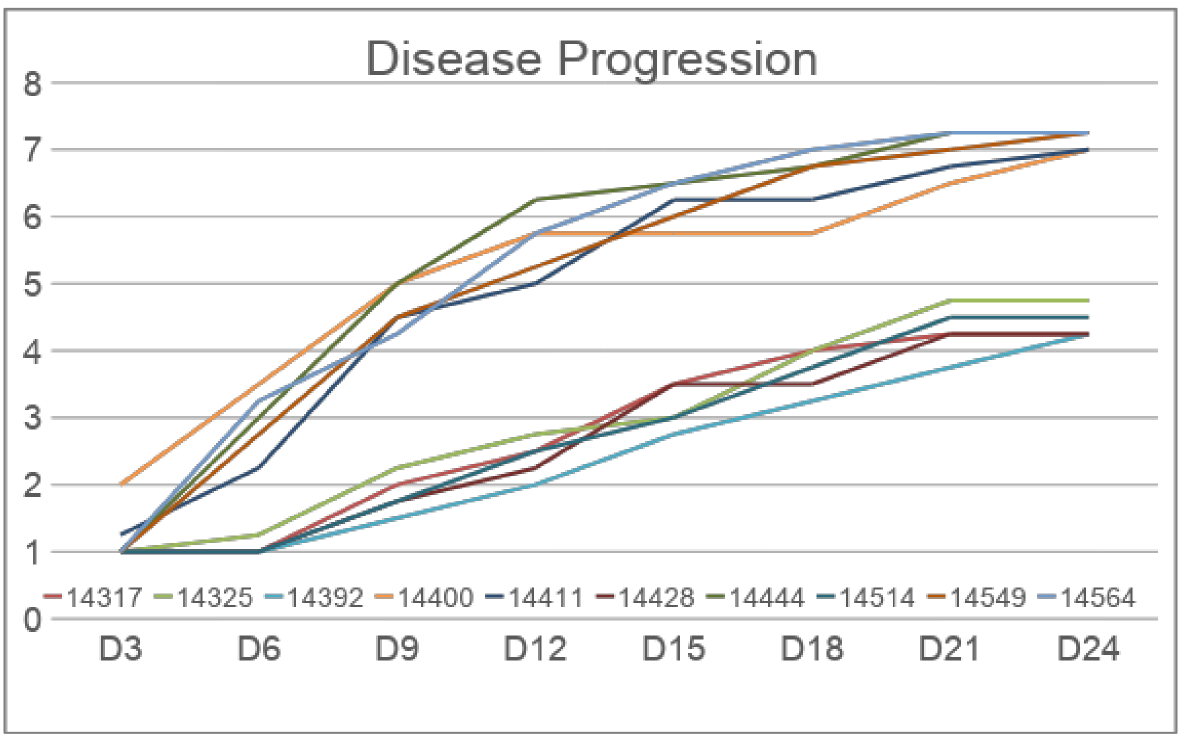


Figure 2. Average disease progression (over 24 days) across two runs of evaluation for the top 5 (most resistant) and bottom 5 (most susceptible) lines.

USGA ID#: 2021-04-728

Title: Buffalograss Breeding and Development

Project Leader: Keenan Amundsen
Affiliation: University of Nebraska

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

Start Date: 2021

Project Duration: 5 years

Total Funding: $150,000

Summary Points:
Superior female and male parental lines were identified to support our buffalograss breeding efforts.
New breeding lines have more rapid establishment compared to contemporary cultivars.
Advanced buffalograss lines have improved late-season color retention.

Summary Text: Buffalograss [*Buchloë dactyloides* (Nutt.) Engelm. syn *Bouteloua dactyloides* (Nutt.) Columbus] is a dioecious, perennial, and sod forming grass species native to the central Great Plains of the United States. Buffalograss is recognized, compared to other turfgrass species, for its relatively low water, fertility, mowing, and pesticide requirements, making it highly successful in low-input turf systems. In collaboration with the USGA, the University of Nebraska-Lincoln has been developing buffalograss cultivars suitable for the golf industry since the mid-1990s. Early focus of the breeding program was to improve turfgrass quality and production traits. The primary focus of the breeding program now is to develop buffalograss with improved turf quality, quicker establishment, and high seed yields compared to contemporary buffalograss cultivars. Another focus of our program is to work closely with seed and sod producers to increase adoption of buffalograss among turfgrass managers. The goals of this project are to focus our breeding efforts by optimizing breeding schemes and reducing the time needed to release new buffalograss cultivars, increase seed yield, and improve visual and functional turf quality.

Buffalograss is available predominantly as vegetative plugs and seed. It can be relatively slow to establish and as a warm-season species that is planted in the spring, it is often competing with summer annual weeds during establishment. Our previous research has demonstrated that dormant seeding can help to get a jumpstart on establishment and reduce the impact of weed pressure. In addition, there are several herbicides that can be safely used during buffalograss establishment also reducing the impact of weed competition. From a plant breeding perspective, one of the primary goals of our buffalograss breeding program is to increase the establishment rate for new cultivars which will reduce weed pressure and the amount of time necessary to obtain a mature turf. Figure 1 shows the lateral spread of experimental buffalograss lines one month after establishment of plugs that were initially planted at a 1 ft spacing; there are clear differences in the lateral spread among the 39 lines evaluated in that study. Establishment rate was evaluated one and two months after planting and differences were observed between accessions based on an analysis of variance, supporting the visual observations. A similar evaluation of 59 experimental buffalograss breeding lines showed differences based on analysis of variance for establishment rate, stolon width (measured with a caliper), and stolon count; traits associated with improved establishment. Based on a principal component analysis, the first two principal components explained 90% of the variability in these accessions for establishment rate, stolon width, and stolon count (Figure 2). Out of the three traits, the number of stolons and establishment rate explained most of the variability among the lines.

Buffalograss survives winters through a winter dormancy response and color retention during the onset of winter dormancy is an important buffalograss trait. Buffalograss is straw-colored when dormant and has poor visual quality compared to cool-season grasses that are still green in the early spring and late fall. Care is needed when selecting lines with good color retention to prevent selection of lines that are also more susceptible to winter injury. Some buffalograss lines that do not have a strong winter dormancy response will keep their green color late into the fall but are more susceptible to winter injury. Figure 3 is an image of two buffalograss studies that was taken in the fall when buffalograss was entering winter dormancy. Dormant straw-colored lines and lines with good color retention are present in both studies, showing variability for color retention among these experimental breeding lines. The study in the foreground represents new lines that have not undergone selection and are being evaluated for several different traits. The study in the background represents entries that have gone through different rounds of selection through our plant breeding pipeline. There are more green lines in the background study compared to the foreground study, demonstrating our ability to breed for color retention. More research is needed to determine if any of the lines with better color retention are also more susceptible to winter injury.

We are also evaluating the turf performance of lines derived from pairwise crosses to determine estimated breeding values and combining ability of parental lines to focus our breeding efforts on the most successful breeding pairs (Figure 4). Since buffalograss is a dioecious species, it is important to simultaneously develop superior female and male parental lines while also evaluating their progeny. The estimated breeding values shown in Figure 4 are derived from the turf performance of the progeny and their mean is normalized to zero. In this analysis, buffalograss parental lines that contribute more to higher quality progeny have more positive values. We identified 13 superior female and two superior male parental lines that have normalized estimated breeding values greater than zero. Pairwise crosses have been made between these female and male lines and we are propagating the seed in the greenhouse to begin advancing those populations.

Our goals for the next year are to advance our breeding populations and take advantage of developing genetic and genomic resources for buffalograss to determine if we can improve the efficiency of our breeding pipelines. We are planning to also perform experiments to measure seed yield for our elite buffalograss breeding lines to determine if they are suitable for large scale production.

Figure 1. Establishment of buffalograss from plugs planted at a 1 ft spacing. The image was taken one month after planting. The upper left plot represents a buffalograss line that does not aggressively spread by stolons, the lower left plot represents a line that is intermediate for spreading by stolons, and the bottom right image represents a line that spreads more aggressively by stolons.

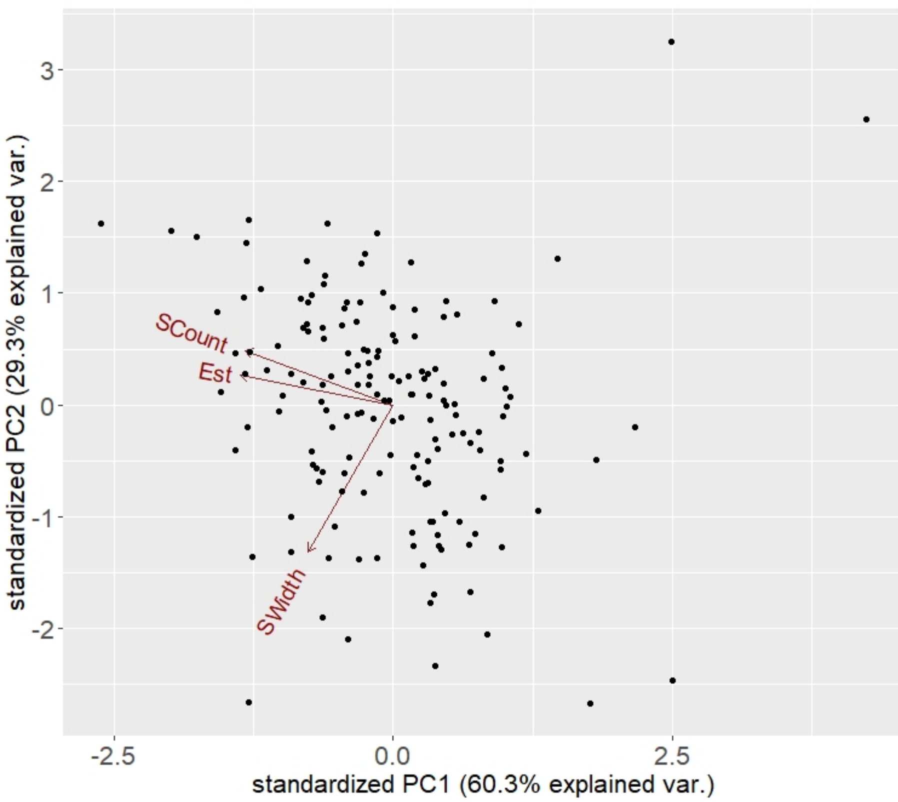


Figure 2. Principal coordinate analysis for establishment rate (Est), stolon count (SCount), and stolon width (SWidth) for 59 experimental buffalograss lines. The first two principal components explain 89.6% of the variability among these lines.

Figure 3. Evaluation studies of buffalograss that are entering winter dormancy. The buffalograss study in the foreground was designed to evaluate wild buffalograss accessions for turf characteristics. The buffalograss study in the background was designed to evaluate advanced breeding selections. There is improved color retention in the advanced breeding lines.

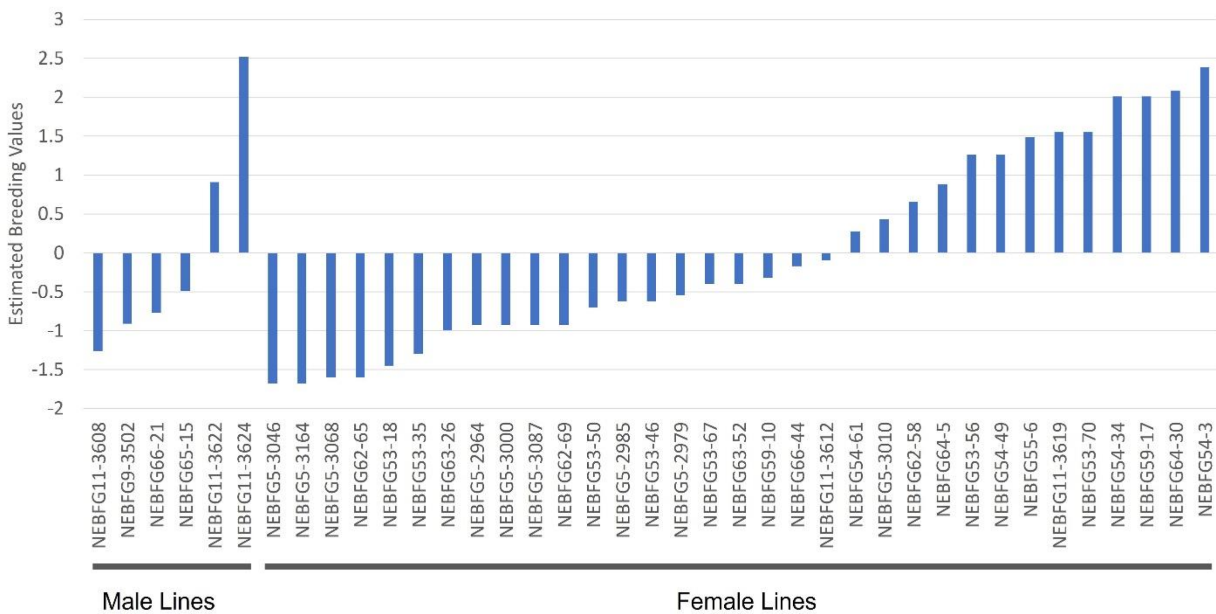


Figure 4. Estimated breeding values for selected male and female parental lines. The mean estimated breeding values are normalized to zero. Lines with positive breeding values contribute to better performing progeny.

USGA ID#: 2021-11-735

Title: Seeded diploid buffalograss

Project Leader: Keenan Amundsen

Affiliation: University of Nebraska-Lincoln

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

Start Date: 2021

Project Duration: 3 yrs

Total Funding: $44,080

Summary Points:
In collaboration with Dr. David Huff and Chris Benson at Penn State University, a diploid buffalograss genome was sequenced.
Diploid buffalograss breeding populations have increased seed size and seed weight.
Distinct diploid buffalograss populations are being developed to satisfy a market need for seeded diploid buffalograss varieties.

Summary Text:

Buffalograss is a warm-season, dioecious, sod-forming stoloniferous grass species native to the Great Plains of the United States. Buffalograss is ideally suited for use as turf, but it is most recognized for its exceptional heat, cold, and drought tolerance. It has few pests of economic importance and is an exceptional choice for low-input turf stands. In contrast to many warm-season grasses used for turf, the majority of buffalograss cultivars have a strong winter dormancy response and do not suffer from winter injury. Buffalograss is a polyploid species with reports of diploid, tetraploid, pentaploid, and hexaploid accessions. Prior research, analyzing the ploidy level of a buffalograss collection showed that diploids accessions in the collection originated from the southern Great Plains, tetraploids originated from the western parts of the region, and hexaploids were distributed throughout the region (Johnson et al., 2001). Buffalograss distribution based on ploidy is likely related to its winter dormancy response since diploid buffalograss, in general, does not enter winter dormancy, while higher ploidy buffalograss does. We sampled a collection of 56 buffalograss genotypes and found a similar distribution with previous reports where ploidy was statistically different based on geocentric latitude of the genotype origins.

The winter dormancy response of buffalograss is interesting from a turfgrass management perspective since supplemental management while dormant is not necessary, significantly reducing inputs and management associated costs compared to cool-season turfgrasses that are actively growing in the early spring and late fall. Since diploid buffalograsses do not have a winter dormancy response, they tend to keep their green color later into the fall (Figure 1), but they are also slow to green up in the spring and can suffer from winter injury (Figure 2). In regions not impacted by harsh winters, diploid buffalograsses are appealing because they will keep their green color for most of the year. 'Density' and 'UC Verde' are examples of commercially available vegetative diploid buffalograss cultivars but there is a noticeable absence of seeded diploids available on the market. Vegetative and seeded cultivars each have their own advantages and disadvantages. Compared to seeded varieties clonal material has less genetic diversity, is available as plugs or sod, can fix a specific gender in a turf stand. A lack of genetic diversity contributes to a more uniform turf stand than seeded varieties, but there is also a potential for stand loss if the clone is susceptible to a specific stress. Vegetative plugs can be easily delivered anywhere, but seed is less expensive and easier to transport and store prior to establishment. Female flowers develop in the turf canopy, while male flowers develop above the turf canopy. Vegetative lines can be unisexually female, contributing to increased uniformity and reduced mowing needs compared to seeded varieties that are comprised of both male and female genotypes.

We began developing independent populations of diploid seeded buffalograss to create more flexibility on the market for diploid buffalograss. Developing diploids has been an interesting challenge since we cannot maintain diploid buffalograss in the field to facilitate advancement of breeding lines due to Nebraska's climate and winter injury. By covering plants in the winter and working with cooperators in warmer climates, we are successfully breeding for seeded diploids. Initial diploid buffalograss genotypes (133) were screened for turf quality and seed production traits. Breeding populations were developed to maintain diversity and increase seed yield. One high yielding population derived from a selection of 16 genotypes consistently outperforms the others for seed yield, producing 6x more seed. Another complication when working with diploid buffalograss is the seed size, which is comparatively small. Seed is screened and larger seed is selected when advancing generations (Figure 3). This process has contributed to seed that more readily germinates and is easier for our producers to harvest and process. We are currently increasing germplasm in the greenhouse from distinct F3 populations to facilitate early planting for seed increases next growing season.

Gene expression studies enable us to identify genes to distinguish genotypes and help us understand conditional responses, or how plants respond to certain environmental conditions or stress. We have previously conducted RNA-seq studies to understand how buffalograss responds to chinch bugs and leaf spot, and to differentiate male and female buffalograss. Those prior studies relied on syntenic relationships among grasses, using published genomes of other species as a reference or de novo strategies in the absence of a buffalograss genome. Sequenced genomes help resolve genome dynamics and support marker assisted plant breeding strategies among other things, but buffalograss lacks these essential genomic resources. A part of this project was to sequence and assemble a diploid buffalograss genome to support subsequent genetics research. At the time this project was awarded, a separate and fortuitous opportunity arose to collaborate with Dr. David Huff and Christopher Benson at Penn State University to coordinate efforts towards a sequenced diploid buffalograss. A high-quality buffalograss genome was assembled with all ten chromosomes represented, ranging in size from 27.6 to 42.7 Mb (Huff et al., 2021). With the sequenced buffalograss genome, we plan to revisit the previous RNA-seq studies to better understand buffalograss-specific conditional responses; apply resequencing and high-resolution genetic mapping methodologies to identify genomic regions important for conditional responses; develop more robust markers to support variety discrimination; and do comparative genomics studies to better understand the evolutionary history of buffalograss.

References

Huff, D. R., Benson, C. W., Amundsen, K., & Morikone, M. (2021) A Fully Annotated, High Quality Genomic Sequence of Buffalograss [Abstract]. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/135041

Johnson, PG, KE Kenworth, DL Auld, TP Riordan. 2001. Distribution of buffalograss polyploid variation in the southern Great Plains. Crop Science 41:909-13.

Figure 1. A segregating population of buffalograss representing different ploidy levels entering winter dormancy. The greener plots are diploid buffalograss lines and exhibit later onset of winter dormancy.

Figure 2. A segregating population of buffalograss representing different ploidy levels during spring green-up. The straw-colored plots are diploid buffalograss lines and are slower to green up in the spring.

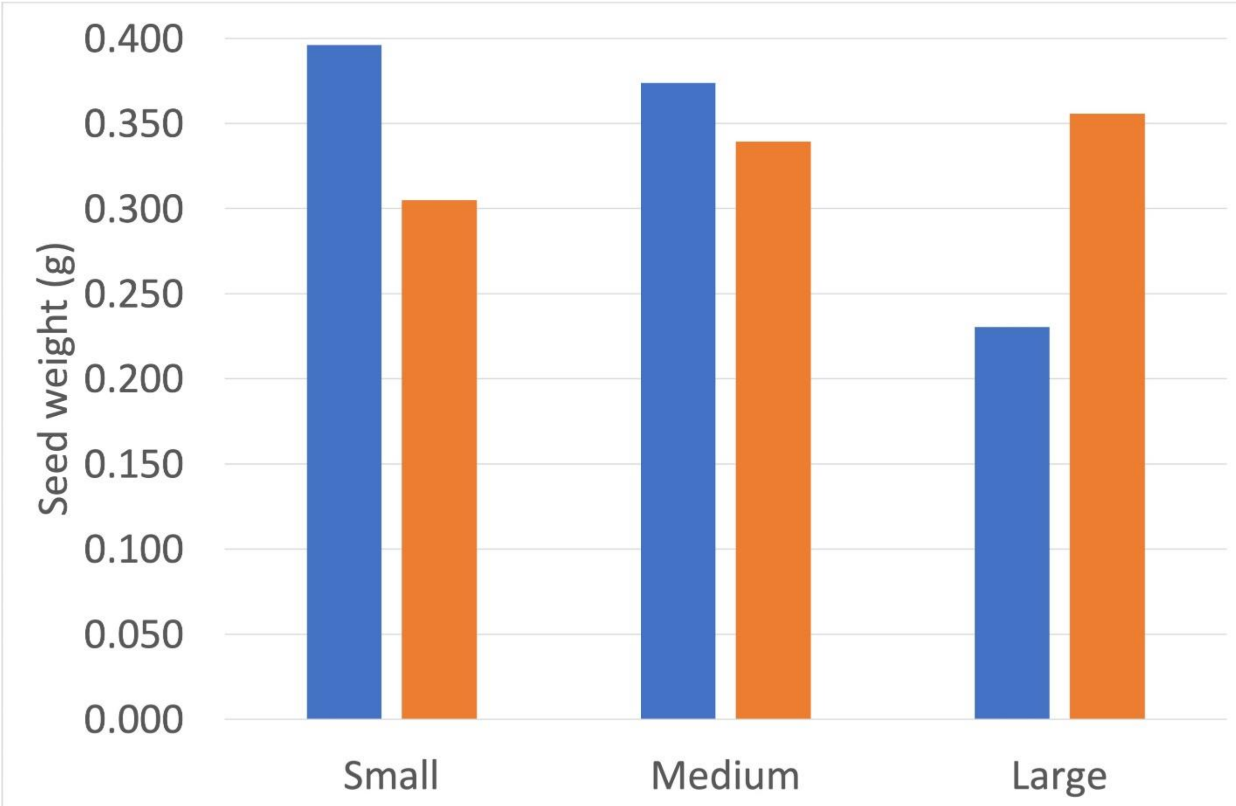


Figure 3. Distribution of 100 seed weight following gravity size separation. Blue bars represent seed weights for F1 seed for small, medium, and large sized seed, whereas the orange bars represent seed weight distribution by size for F3 seed. Seed is increasing in size for later buffalograss generations following selection.

USGA ID: 2018-15-665

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

PROJECT LEADER:

Kevin Morris, Executive Director
National Turfgrass Evaluation Program (NTEP)
BARC-West, Bldg. 005, Rm. 307
Beltsville, MD 20705

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

START DATE: 2018

PROJECT DURATION: Three years

TOTAL FUNDING: $45,000

SUMMARY TEXT

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

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

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

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

Location and Number of Trial Sites

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

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

Trial Specifics and Protocols

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

Management protocol during establishment

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

Management protocol after establishment period

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

Data Collection and Publication

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

1. Percent establishment every 14 days until plots are fully established
2. Percent living ground cover of planted species in spring to assess winter survival
3. Spring greenup ratings in years two through five
4. Turfgrass quality ratings each month throughout the growing season
5. Percent living ground cover of planted species monthly throughout each growing season
6. Percent grassy and broadleaf weed encroachment two times per year (excluding planted species)
7. Canopy height measurements monthly just prior to mowing (average of three locations in each plot)

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

Progress to Date

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

Data from the first year after planting (2019) reflected mainly establishment rate. 2020 data is the latest information available as 2021 data is only just being received by NTEP. Turfgrass quality ratings were collected at eight locations from the southeast U.S. to the western U.S. (Utah). Two experimental entries, ‘XZ 14069’ zoysiagrass, ‘FB 1628’ bermudagrass and a standard, well-known commercial entry, ‘Tifway’ bermudagrass, were the only grasses to finish in the top turfgrass quality statistical group at six or more locations. ‘XZ 14069’ and ‘Tifway’ were the only entries finishing in the top statistical category of both Location Performance Index (LPI) groups (four locations in each LPI grouping).

‘XZ 14069’ also finished with the highest overall turfgrass quality rating in three of the five southern and southeast U.S. locations, indicating its adaptability across the southern region. ‘FB 1628’ was a top performer as well in the southeast and southern U.S., as well as in Utah, only faltering in Citra, FL. Specific characteristics and ratings exhibited by these grasses include high living ground cover ratings at most locations, which most likely led to low percent weed invasion ratings for both entries. The exception was increased weed invasion in ‘FB 1628’ at Citra, which probably lowered overall turf quality ratings. In addition, ‘XZ 14069’ showed significantly better spring greenup at two locations, probably leading to higher quality scores, while ‘FB 1628’ genetic color ratings were the highest of any entry. ‘XZ 14069’, ‘FB 1628’ and ‘Tifway’ showed the highest leaf texture scores at Stillwater, OK.

As mentioned above ‘Tifway’ is still performing well in this trial, despite being commercialized almost 60 years ago. ‘Tifway’ was a top performer at all locations except Raleigh, NC and Citra, FL while ‘Midiron’ bermudagrass, another commercially available entry, was a top statistical group performer at five locations. Entries such as ‘ASC-117’, a seed established bermudagrass, ‘Cody’ buffalograss and ‘Habiturf’, a mixture of native grasses did not perform well overall, and particularly in the southeast U.S. locations. Data from 2021 and future years of testing will hopefully further separate these entries and give us additional information on the best and newest grasses for warm-season golf course roughs.

SUMMARY POINTS

- Performance has varied since planting in 2018 as the establishment rate in the first year of the trial was the key factor influencing early turf quality ratings.
- Turfgrass quality data from 2020 showed significant differences among entries and species, with ‘XZ 14069’ zoysiagrass and bermudagrasses ‘FB 1628’ and ‘Tifway’ performing best over several test locations.
- Factors such as percent living ground cover, low weed invasion, early spring greenup and finer leaf texture contributed to the higher turf quality ratings.
- Not all cultivars within a species have performed similarly in this trial thus far, with ‘XZ 14069’ outperforming other zoysiagrass entries at many locations.

Figure 1. Entries in Fresno, CA.

Figure 2. Entries in the low-input golf course rough trial at Citra, FL, with ‘Habiturf’ in the upper right corner.

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: $9,500 to date

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.
2. 12-TG-101 generally has superior turf uniformity to 'TifEagle' 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.
3. 12-TG-101 has a very dark green leaf that should distinguish it from other ultradwarf cultivars, including 'TifEagle', 'MiniVerde', and 'Champion'.
4. 12-TG-101 has equal putting green speeds as 'TifEagle', the most widely used ultradwarf bermudagrass cultivar in the world.

Summary Text:

Bermudagrasses can be found on golf course putting greens throughout the southern United States. Before genetic improvement of these species began, greens were planted from seed which provided golfers with very inconsistent putting surfaces (Burton, 1977). Modern expectations of putting greens include consistent and fast ball roll, which can be accomplished by growing a uniformly smooth, short and dense turf canopy. In 1946, the United States Golf Association's Green Section supported research at Tifton, GA in collaboration with the United States Department of Agriculture – Agricultural Research Services to create new grasses for golf courses. The annual $500 USGA grant allowed the USDA-ARS to accumulate turf-type bermudagrass germplasm through the creation of lower growing genotypes with higher canopy density from the forage breeding efforts in addition to the collection of golf course adapted selections (Burton, 1991).

'Tifgreen'(2*n*=3*x*=27) was an improved triploid interspecific hybrid bermudagrass developed by crossing *Cynodon transvaalensis* (2*n*=2*x*=18) and a *C. dactylon* (2*n*=4*x*=36) selection that demonstrated good putting green characteristics on the Charlotte Country Club in North Carolina (Burton, 1964). 'Tifgreen' has been reported to be a pollen and seed sterile cultivar. A few years after its release, several off-types were identified on golf greens planted with 'Tifgreen' and sent back to the Tifton USDA-ARS research program for analysis. It was determined that these off-types were not contaminations from seed, but vegetative mutations (Burton and Elsner, 1965). One of these off-types was eventually released as 'Tifdwarf' (1964), which proved to be no more genetically stable than 'Tifgreen'. In the years following there were numerous 'Tifgreen' derived cultivars released from multiple institutions, including 'Pee Dee 102' (1968), 'Champion Dwarf' (1987), 'MiniVerde' (1992), 'Floradwarf' (1995), 'TifEagle' (1997), 'MS-Supreme' (1997), and 'Mach 1' (2018).

'Tifgreen' was planted on the putting greens at Taylor's Creek Golf Course at Fort Stewart in Georgia during 1961. Fifty-one years later on 13 April 2012, 155 presumed mutants of the original 'Tifgreen' bermudagrass were collected from the home of the U.S. Army's 3rd Infantry Division (O'Brien, 2012). 12-TG-101 was one of 14 selections from the 13th green, which was plagued with shade and poor air movement (Figure 1).

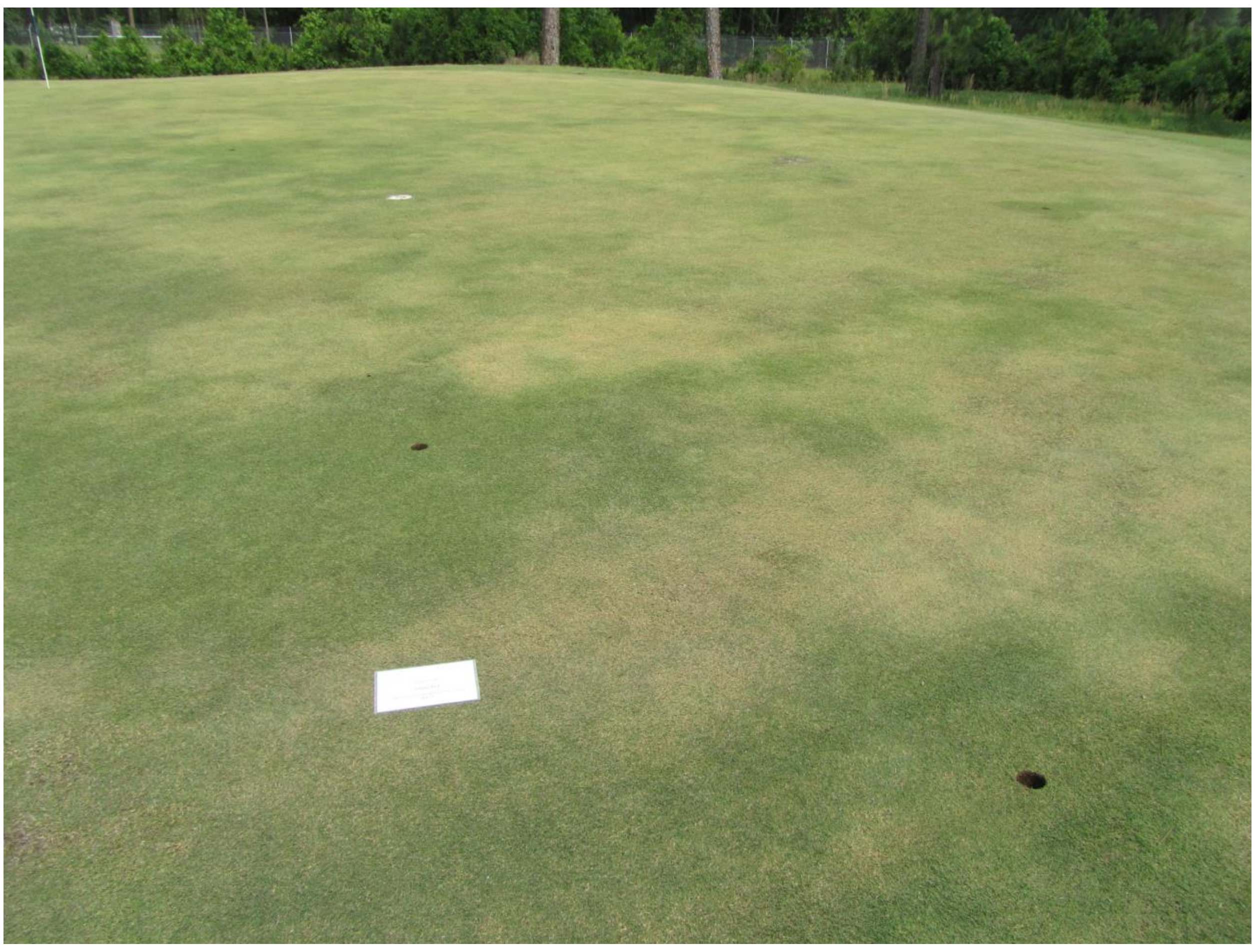


Figure 1. Samples were collected from the darker green, low-growing 'Tifgreen' mutants on the 13th putting green at Taylor's Creek Golf Course at Fort Stewart in Georgia during 2012.

Two identical, replicated field trials were established in 2013 and 2014 at the University of Georgia Tifton Campus where inflorescence, morphological, and turfgrass performance observations were recorded on these 155 putative somatic mutants for comparison with the bermudagrass cultivars 'Tifgreen', 'Tifdwarf', 'Champion Dwarf', 'MiniVerde', and 'TifEagle'. 12-TG-101 was highly adapted to the management conditions of the research trials in Tifton, although they are notably of lower-intensity than what is possible on golf courses with more resources. Therefore, 12-TG-101 was selected to be planted in 10 on-site golf course research putting green trials in the Southeastern United States from 2015 through 2019 for evaluation of turf color, turf uniformity, and putting green speeds against 'TifEagle' under standard maintenance protocols not possible on the Coastal Plain Experiment Station. The golf courses that hosted these trials were the Country Club of Columbus in Columbus, GA (2015); The Landings Club in Savannah, GA (2015); Valdosta Country Club in Valdosta, GA (2016); Atlanta Country Club in Marietta, GA (2016); Big Canoe Golf Course in Jasper, GA (2017); TPC Sawgrass in Ponte Vedra Beach, FL (2017); Streamsong Golf Resort in Bowling Green, FL (2018); East Lake Golf Club in Atlanta, GA (2019); Olde Florida Golf Club in Naples, FL (2019); and The Meadows Country Club in Sarasota, FL (2019).

Table 1. Summary of putting green performance of 12-TG-101 bermudagrass compared to TifEagle bermudagrass measured between 2015 – 2021 on 10 golf courses in the Southeastern United States[1].

Genotype	Year of release	Turf color[2]	Turf uniformity[3]	Green speed[4]
		visual rating	visual rating	ft
12-TG-101	2021	7.6 a[5]	7.6 a	9.4 a
TifEagle	1997	6.4 b	6.9 b	9.2 a

[1]Field trials planted between 2015 – 2019 at the Country Club of Columbus, The Landings Club, Valdosta Country Club, Atlanta Country Club, Big Canoe Golf Course, TPC Sawgrass, Streamsong Golf Resort, East Lake Golf Club, Olde Florida Golf Club, and The Meadows Country Club.
[2]Turf color was visually rated on a 1 to 9 scale with 1 = yellow, 6 = acceptable, and 9 = dark green.
[3]Turf uniformity was visually rated on a 1 to 9 scale with 1 = least, 6 = acceptable, and 9 = most. Putting green surface leaf density, leaf width, leaf canopy distribution, leaf orientation, leaf mowing quality, and weed encroachment were all taken into consideration for the comprehensive turf uniformity visual rating.
[4]Green speeds were determined by measuring the distance golf balls rolled when released from an inclined plane called a Stimpmeter (United States Golf Association, 1979).
[5]Least squares means within columns followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$).

Data presented in Table 1 were analyzed by restricted maximum likelihood using PROC MIXED in SAS 9.4 (Littell et al., 2006). A Kenward–Rodgers adjustment was applied to correct the denominator degrees of freedom, ensuring appropriate standard errors and F statistics for each model. Multiple covariance structures were tested and the Bayesian's Information Criterion indicated that Autoregressive (1) was the best fit. Fixed effects included genotype, year of trial maturity, and season of year (winter, spring, summer, and fall). Each golf course trial was considered a replicate and was designated as a random effect. Other random effects were sprig source (research farm or sod farm), existing cultivar being managed on the golf course greens ('TifEagle', 'MiniVerde', 'Tifdwarf', or bentgrass), and the subsoil of the research greens (new profile or no-till profile). Means were compared using the LSMEANS procedure with Tukey–Kramer adjustment ($P < 0.05$). Differences were considered significant at $P < 0.05$. No genotype × year of trial maturity, or genotype × season of year interactions were detected (data not shown). Across the golf course putting green performance trials, 12-TG-101 was visually darker green and more uniform than 'TifEagle', but had the same green speeds as determined with a USGA Stimpmeter (Table 1).

Figure 2. Different genotypic response to mower scalping on adjacent golf greens plots of 12-TG-101 (A) and 'TifEagle' (B) after a 2-month (A/B) and 5-month (C/D) sustained period of lower agronomic maintenance intensity at the Olde Florida Golf Club in Naples, FL. Trial planted on 18 September 2019.

Although 'TifEagle' remains the most utilized and genetically stable ultradwarf bermudagrass cultivar across the world, advances in golf putting green uniformity and ball roll consistency are always needed, especially after mechanical injury (Figure 2), or when disease pressures are high (Figure 3).

Figure 3. Different genotypic response to a foliar patch disease on adjacent plots of 12-TG-101 (A) and 'Mach 1' (B) at East Lake Golf Club in Atlanta, GA on 9 June 2021 after hollow-core aeration in late May 2021. Trial planted on 1 May 2019.

12-TG-101 has been tested for broad adaptation in areas of the Southeastern United States where warm-season grasses are grown on golf putting greens and should also benefit those in regions of the world where 'TifEagle' has previously been successful. A 5,400 ft^2 Breeder Field and a 1-acre Foundation Field of 12-TG-101 have been established (Figure 4). Planting of sprig and sod production fields is set for early 2022, with a limited supply of 12-TG-101 predicted to be available for sale to consumers in late 2022 or early 2023.

Figure 4. The 1-acre George Seed Development Foundation Field of 12-TG-101 located at Pike Creek Turf in Adel, GA. Field planted on 14 May 2021.

USGA ID#: 2019-10-680

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

Project Leaders: Doug Soldat, Ph.D. and Qiyu Zhou (Ph.D. Student in Soil Science)
Affiliation: University of Wisconsin - Madison

Objectives: The objectives of this research were to 1) investigate the combined effect of management practices, weather and soil characteristics on creeping bentgrass response; 2) develop a machine learning growth model for creeping bentgrass that can accurately estimate a short-term turfgrass growth rate; 3) test the feasibility of using the developed machine learning growth prediction model to improve N management by investigating creeping bentgrass growth response and corresponding N fertilizer usage to the machine learning model based N fertilization plan and other commonly used N fertilization strategies.

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

Summary Points:
1. Temperature, relative humidity and evapotranspiration were the key weather factors for estimating bentgrass growth. Foot traffic, nitrogen rate and soil moisture were weakly correlated with bentgrass growth. However, model accuracy substantially increased when these variables were included.
2. A data-driven statistical model using the machine learning random forest algorithm can accurately predict bentgrass yield. However, the model was only effective for the location where the model was built, suggesting that individual golf courses need to build customized growth prediction models to manage nitrogen adaptively. This can be accomplished by collecting and recording clipping volume for at least one year.
3. The machine learning random forest algorithm appears to be very helpful for guiding N application decisions, and when it was used over a two-year period on two different root zones, it resulted in acceptable turfgrass performance with about 50% less N fertilizer usage than the method that recommended the most N fertilizer (PACE Turf method) and about 30% less fertilizer than the traditional way that golf course superintendents schedule N applications.

Summary Text:
The N cycle on golf course sand-based putting greens is relatively simple. The lone significant N input is from N fertilizer and the lone significant N output is mainly from clipping removal. Therefore, a turfgrass manager could simply make N application decisions by relying on a good estimation of clipping removal of N. Instead of only relying on temperature to estimate turfgrass growth rate, such as PACE Turf Growth Potential (GP) model, a series of machine learning random forest (RF) models were developed based on several categories of growth factors, including weather data, management practices (irrigation, foot traffic and fertilization) and soil. The RF models better predicted turfgrass clipping removal compared with the PACE Turf GP model. Moreover, after investigating creeping bentgrass growth and overall nitrogen fertilizer use response to four N fertilization plans including machine learning RF model, as well as Pace Turf GP model, university recommendation and spectral reflectance determined, the RF model saved up to 50% N fertilizer input, and the turfgrass performance was still satisfactory.

METHODS

To investigate the interactions among soil, turfgrass, environment and management practices, this study was conducted on two 'Focus' crepping bentgrass sand putting greens that varied in soil organic matter content and quality. The study was conducted from 2018 to 2021 at the O. J. Noer Turfgrass Research and Education Facility, Madison WI. In 2018 and 2019 (the work in 2018 was done prior to funding from WGCSA), we investigated the factors that influenced daily bentgrass growth and used the collected data to develop a growth prediction model. These factors include 1) foot traffic rates, which were maintained at 0, 700, 1400, 1800, 3600 rounds wk^{-1}; 2) irrigation plan (expressed as soil moisture content) that maintained soil moisture at high (25-27% volumetric water content), medium (18-20% volumetric water content), and low (8-13% volumetric water content) moisture levels in the top 3 inches; 3) N fertilization rates, that included 0, 0.1 and 0.2 lbs N 1000 ft^{-2} 2 wks^{-1}. Turf visual quality was measured every two weeks and NDRE was measured before each clipping collection event. All clipping data collected from 2018 and 2019 were used to build a series of machine learning growth models. To develop the growth prediction models, several different weather factors were selected as input variables, including air temperature, evapotranspiration, relative humidity, precipitation and wind speed. Weather data were used from online weather data (Weather Underground). Moreover, soil moisture content, historical nitrogen rate, walking traffic level and proximal sensing data (NDRE) were used to develop the model. The growth model was built with the "scikit-learn" RF package from Python. To validate the model, we used 90% of the total data to train the model during the training process and the remaining 10% of data was used to evaluate the model performance.

In 2020 and 2021, to test the ability of a soil CO_2 burst, which was used to present the plant available N released from the soil, on estimating soil N supply on sand-based putting green soils and whether can be used as growth factor when building the growth prediction model, we collected soil samples on four different root zones with soil organic matter content with 1.0%, 0.9%, 0.6%, and 0.4% in the top 4 inches. Soil was collected from 0-4 inches every three weeks during growing season. Soil CO_2 burst was estimated with the flush of CO_2 following rewetting of dried soil (1-day incubation at ≈50% water-filled pore space and 25°C).

Moreover, the developed model was validated and put to use by making nitrogen application decisions. Nitrogen application decisions were made according to biweekly accumulated predicted growth multiply leaf tissue nitrogen content. We compared the model with three other nitrogen management methods that include 1) the Pace Turf GP model which estimates growth (and therefore N use) based on air temperature; 2) the experience method where 0.2 lbs N 1000 ft^{-2} 2wks^{-1} were applied every other week, and 3) a modification of the experience method where N was applied at 0.2 lbs N 1000 ft^{-2} 2wks^{-1} only if the treatment fell below a spectral reflectance (NDRE) threshold. Turfgrass growth response and corresponding N fertilizer usage were compared among these four N fertilization plans. In each year's study, clippings were collected about three times a week, turf visual quality was measured every two weeks and NDRE was measured before each clipping collection event.

RESULTS

Impact of management practices and soil CO_2 burst on creeping bentgrass growth

Bentgrass growth rate overall was greatest on the plots containing the highest soil moisture content. Research plots receiving relatively high traffic levels produced significantly lower clipping yields than the plots receiving lower traffic. The difference in growth caused by traffic was not significant across more realistic levels of traffic. Least surprisingly, bentgrass fertilized with higher levels of nitrogen produced higher yields. However, individually, all of these relationships were fairly weakly correlated with bentgrass growth and using these factors as model inputs will not accurately predict bentgrass growth rate. Soil CO_2 burst was only weakly correlated with creeping bentgrass clipping yield and bentgrass N uptake on four different root zones (Figure 1). Soil CO_2 burst was not used as a variable input when developing the machine learning growth prediction model. Instead categorized root zone was recognized as a variable input.

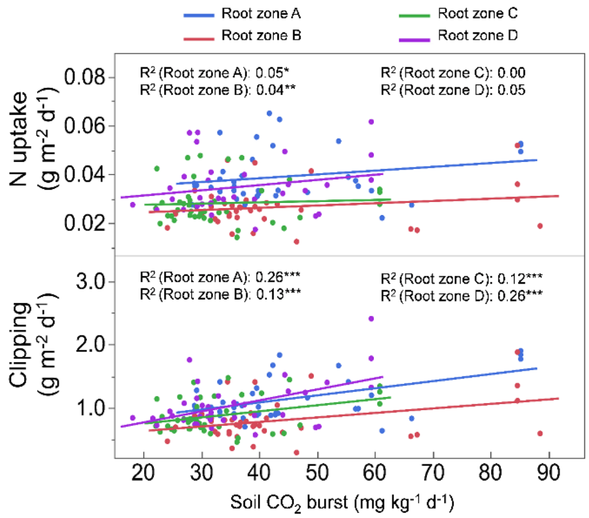


Figure 1. . correlation among soil CO_2 burst, creeping bentgrass clipping yield and N removal from 2020 and 2021 on four root zones. R^2 values followed by *: significant at $p \leq 0.05$; followed by **: significant at $p \leq 0.01$; followed by ***: significant at $p \leq 0.001$.

Random Forest Models Performance

We built a series of RF models using different input data, in search of a model that was both simple and accurate. The complete model included the entire suite of growth variables including soil moisture content, NDRE, traffic level, N application rate and weekly weather data (min, max and average of air temperature, precipitation, evapotranspiration, wind speed and min, max and average of relative humidity). We also tested two sets of simplified models by using subsets of the input: 1. A simplified RF model with historical N rate record, traffic intensity and weather data; and 2. A simplified RF model with only weather data. These simplified models focused on the variables that are most easily available or obtained by the end-user. Figure 2 showed the complete RF model that included the entire suite of variables had the best performance on the validation dataset which had an average R^2 of 0.64. The simplified RF model has an average R^2 of 0.57 on the validation dataset. The weather-only RF model only contained weekly weather data inputs and had an average R^2 of 0.46 on the validation dataset. These results suggest that accurate and reasonably precise growth predictions can be made from readily available and easily obtained data, although using only weather data appears to be far less precise than models including management information and soil factors. However, all the machine learning models performed better than Pace Turf GP model ($R^2 = 0.01$).

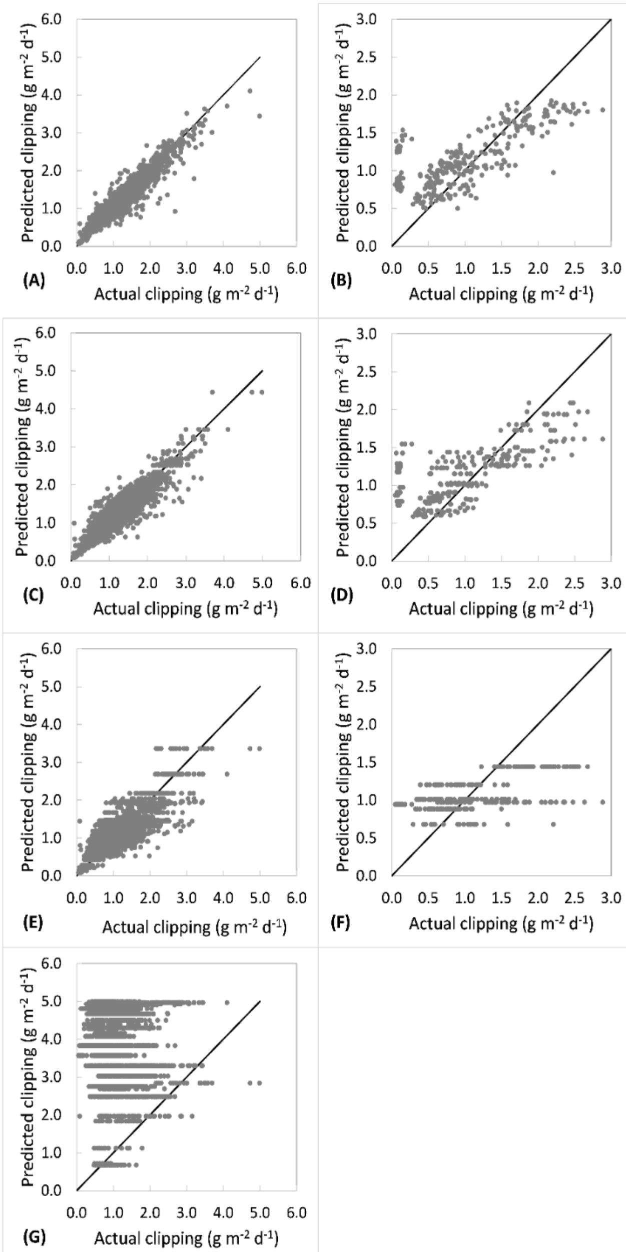


Figure 2. Scatter plot of model performances with (a) complete RF (RF) model with all variables inputs on training dataset; (b) complete RF model with all variables inputs on validation dataset; (c) simplified RF model with historical N rate record, traffic intensity and weather data on training dataset; (d) simplified RF model with historical N rate record, traffic intensity and weather data on validation dataset; (e) simplified RF model with only weather data input on training dataset; (f) simplified RF model with only weather data input on validation dataset; (g) PACE Turf GP model.

The machine learning RF models that were built based on the data collected from the University of Wisconsin-Madison OJ Noer Facility in 2019 and 2020 were used to predict the clipping yield on the bentgrass putting greens from a golf course located in Minnesota, USA. Since the golf course only had the access to limited variables (including historical N fertilization rate and weather), we used the simplified RF model to make predictions. The simplified RF model which was built based on the data collected from the Wisconsin research putting greens performed poorly with an R^2 of 0.03 (Fig. 3b). The

PACE Turf GP model also had relatively low prediction accuracy (R^2 =0.05) on the turfgrass clipping production (Fig. 3c). However, a customized RF model based on the Minnesota data predicted clipping yield well with an R^2 of 0.74 (Fig. 3a). While we failed to create a universal statistical bentgrass yield prediction model, we have demonstrated that it is possible to build accurate and precise growth models with local clipping data and readily available input variables like weather data and N fertilization rate.

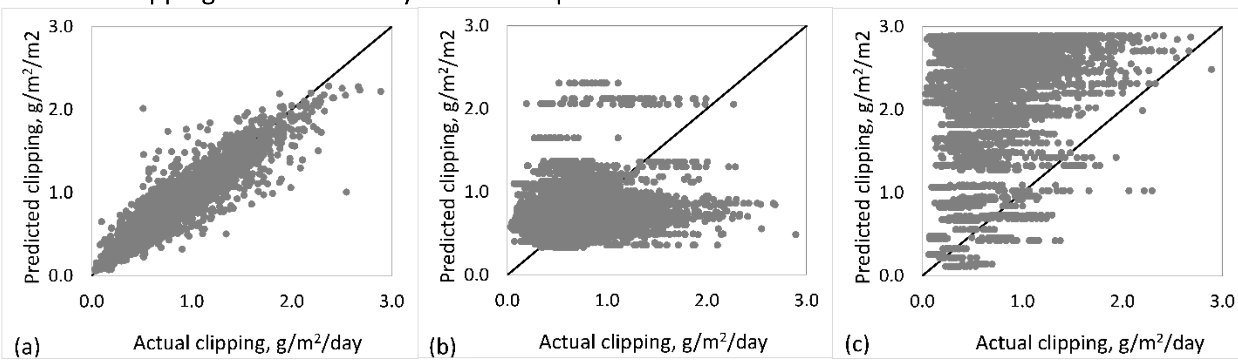


Figure 3. Scatter plot with (a) random forest (RF) model performance on the golf courses that model was built on the on-site clipping data; (b) RF model built with clipping data collected from Madison, Wisconsin, USA perform on the clipping data collected from golf course from Minnesota, USA; (c) PACE Turf GP model

Lastly, Table 1 presents the two-year average creeping bentgrass clipping yield, NDRE and turf quality response to four N treatments, as well the two-year cumulative N fertilizer usage, clipping yield and N use efficiency (NUE). There was no significant difference in clipping yield, NDRE and turfgrass quality between the two root zones at UW-Madison research facility. Turfgrass that received N treatments followed by the PACE Turf GP model produced significantly higher clipping yield, NDRE readings and turf visual quality. The experience-based model produced the second greatest clipping yield, NDRE and turf quality, followed by ML-RF and NDRE-based N fertilization plans. NUE was highest on both root zones following the NDRE-based N fertilization plan, which was near 100%. NUE was around 45% on the root zones following ML-RF model N plans, and was about 34% following N fertilization plan with PACE Turf GP model. NUE was lowest when using experience-based method, which was around 26%. The PACE Turf GP model and experience-based strategies were not able to recommend different amounts of N on different root zones, while the ML-RF model and NDRE methods were able to account for differing root zone properties. The experience-based method resulted in 32% less N fertilizer than the PACE Turf GP method, and the ML-RF model applied 52% and 49% less N fertilizer on root zone A and B respectively than the PACE Turf GP method. The NDRE-based method resulted in 72% and 75% less N fertilizer on root zone A and B respectively.

While precision N management remains elusive and there are aspects to it that remain subjective, we have demonstrated that a machine learning approach can be of assistance to turfgrass managers for making decisions about putting green fertilization. Decisions based on data will help to improve fertilizer use efficiency without sacrificing playing surface quality. The next steps will be to incorporate these statistical models into decision support tools that will be easy for end users to create and manipulate.

Table 1. Two-year average creeping bentgrass clipping yield, NDRE and turfgrass quality response to four Nitrogen (N) application strategies on two putting greens. Turf quality scaled from 1 to 9 where 1 represents completely dead turf, 6 represents the minimally acceptable quality, and 9 represents a perfect or ideal turfgrass quality.

Root zone ID	N app. strategies	Clipping ($g\ m^{-2}\ d^{-1}$)	NDRE	Turf quality	Sum of N fertilizer ($kg\ ha^{-1}\ 2yrs^{-1}$)	Sum of clipping ($g\ m^{-2}\ 2yrs^{-1}$)	NUE$^{\mu}$ (%)
A	Pace Turf GP$^{\Theta}$	1.63 a	0.328 a	7.6 a	281	303	33.9
	Experience$^{\beta}$	1.38 b	0.315 b	7.4 ab	190	194	27.1
	ML-RF approach$^{\alpha}$	1.20 c	0.302 c	7.2 b	136	222	46.2
	NDRE-based$^{\Gamma}$	1.02 d	0.277 d	6.1 c	80	259	97.1
B	Pace Turf GP$^{\Theta}$	1.62 a	0.326 a	7.5 a	281	301	34.2
	Experience$^{\beta}$	1.32 b	0.318 b	7.4 ab	190	181	25.4
	ML-RF approach$^{\alpha}$	1.17 c	0.306 c	7.2 b	142	221	45.2
	NDRE-based$^{\Gamma}$	0.96 d	0.282 d	6.2 c	70	247	106.6

$^{\Theta}$ Pace Turf GP model-guided N application strategy

$^{\beta}$ Traditional N application plan

$^{\alpha}$ Machine Learning Growth Model (RF model)-guided N application strategy

$^{\Gamma}$ Turfgrass vegetative index (NDRE) determined N application strategy

$^{\mu}$NUE, nitrogen use efficiency, calculated by (N uptake by plant-N uptake by plant from no N fertilizer plot)/two-year N fertilizer applied

USGA ID#: 2019-11-681

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

Principal Leaders: Charles Fontanier

Affiliation: Oklahoma State University

Objectives:

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

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

Summary Points:

- Black shade fabric reduced bermudagrass productivity to a larger degree than the blue shade lens (reduced R:FR ratio).
- Reduced R:FR ratio increased leaf size and length but responses were linearly related to PPF more so than light quality.
- Evaluation of the R:FR response in warm-season turfgrasses may require additional genotypes including both shade sensitive and resistant entries.

Rationale

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

The currently accepted method for developing minimum light requirements for turfgrasses involves calculation of the daily light integral (DLI) for the accumulated photosynthetically active radiation (PAR). This is an improvement over historical recommendations based on ‘hours of sunlight', but the results may be limited due to the variation in ‘types of shade’ that exist in the real world. Furthermore, the scientific community has not established a standard design for shade research which has resulted in variation in how investigators simulate shade. As the academic community increases our understanding of turfgrass response to shade, there needs to be dedicated research in standardizing and validating methods used to evaluate shade tolerance.

Methods
The proposed research has three primary objectives: characterize the energy balance under real world and simulated shade, characterize the light spectrum under real world and simulated shade, and conduct a bioassay of the bermudagrass shade response to varying light quality.

Shade structures will be built and tested using varying heights (e.g., 1, 2, 3, 10-ft tall), shade densities (e.g., 50%, 80% shade), and shade materials (neutral or red selective). Sensors will be installed to measure net radiation, wind speed, ambient temperature, relative humidity, and surface temperature. Data will be collected under shaded and non-shaded conditions for each shade type to determine their effect on microclimatic conditions including long-wave radiation and evaporative demand. Similar measurements will be made under real-world shade conditions varying in tree species and shade severity.

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

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

Results
In the greenhouse trials, a significant species by treatment interaction occurred for tiller counts, leaf counts, leaf length, and leaf area. Shade of any type reduced leaf counts in each species in a similar manner, whereas tiller count was more strongly reduced by shade in bermudagrass (Table 1). Leaf length increased with increasing shade for perennial ryegrass, but no pattern was observed for bermudagrass. Because there was no obvious R:FR response, variables were analyzed using correlation to determine if there was a linear response to light intensity. For most variables, morphological responses were strongly correlated with DLI, with leaf count, tiller count, and leaf length each having an r = 0.99 (Table 2).

As previously reported, initial evaluation of light filters under indoor conditions resulted in selection of a light blue product having a R:FR ratio of 0.71. After several months in the greenhouse, filters were only producing a R:FR ratio of 0.99 (Figure 1). Although this was still a reduction in R:FR, it was certainly less than we had hoped and likely diminished the potential for a response. Additional work on longevity of these filters is needed or future projects may require indoor or growth chamber environments.

Future Expectations

Activities related to objectives 1 and 2 were again delayed due to staffing shortages and continued uncertainty related to COVID-19. Expectations are that these objectives will be accomplished in summer 2022 with construction of shade structures scheduled for early 2022.

The responses of both turfgrass species used in this study were largely attributed to the differences in PPF. Whether this lack of response to R:FR is true or an artifact of deteriorating filters over time is unclear. These studies were also conducted on a single cultivar of bermudagrass, and we hypothesize that evaluation of grasses having a range of shade tolerance may contribute to better information related to this trait. Additional studies are under way to evaluate R:FR responses of varying genotypes of bermudagrass and St. Augustinegrass (Figure 2).

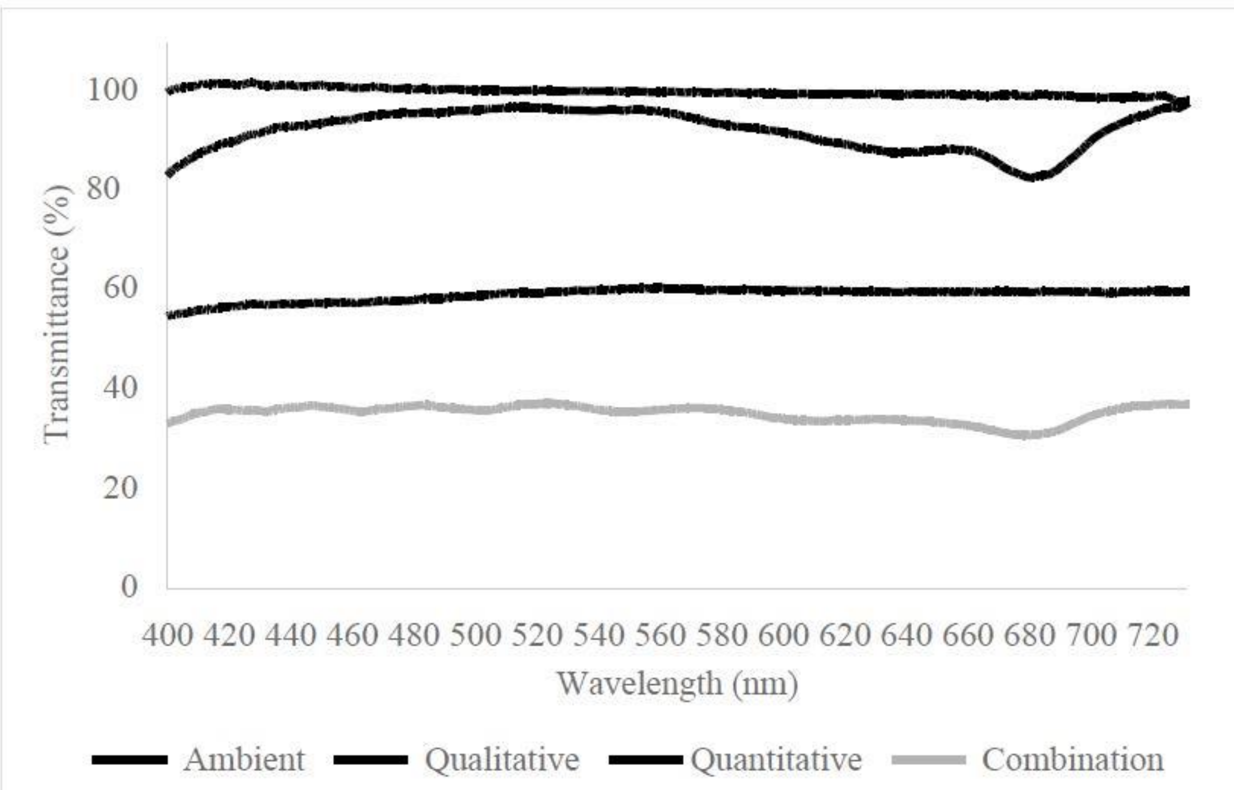


Fig. 1 Relative transmittance of selected shade treatments used in greenhouse trials.

Fig. 2 Indoor trials using selective filters to evaluate R:FR ratio responses in St. Augustinegrass and bermudagrass clones.

Table 1. Interaction between species and shade treatment for tiller count.

Species	Treatment	Tiller Count	Leaf Count
Bermudagrass	Ambient	4.61a[z]	99a
	Quantitative	1.82c	52bc
	Qualitative	3.14b	67b
	Combination	1.32c	33cd
Perennial Rye	Ambient	2.34bc	35cd
	Quantitative	2.03bc	23d
	Qualitative	2.14bc	28d
	Combination	1.09c	18d

[z]Means within a column followed by same lowercase letter are not significantly different at 0.05 significance level.

Table 2. Correlation (r) between daily light integral (DLI) and specific leaf area (SLA), leaf dry weight (DW), shoot DW, root DW, leaf count, leaf length, leaf area, and tiller count of bermudagrass grown in greenhouse conditions.

Variable	r
SLA	-0.89
Leaf DW	0.62
Shoot DW	1.00
Root DW	0.99
Leaf Count	0.99
Leaf Length	-0.99
Leaf Area	-0.95
Tiller Count	0.99

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 to compared different PGR application timings (calendar vs GDD) under 4 differing shade levels. At both locations, calendar-based applications of Primo Maxx produced higher quality under shaded conditions compared to a GDD application timing.
- Studies to investigate the effects of water quality on PGR efficacy will be initiated in Year 2 of the study and the shade trials will be repeated in Year 2.

Executive Summary:

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

Historically, PGRs such as trinexapac-ethyl were applied on a calendar-based schedule. Over the last decade, a number of studies have documented that re-application of PGRs based on temperature-dependent, growing-degree-day (GDD) models is a more effective approach to maintain suppression and avoid surge growth (Kreuser and Soldat, 2011; Kreuser et al. 2017; Reasor et al., 2018). This approach to

PGR use has been broadly-adopted in the golf course industry and has shown to be an effective strategy in both cool-season and warm-season putting greens.

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

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

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

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

Plant growth regulator treatments, including application rates and timing.

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

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

Plot were rated bi-weekly for turfgrass quality and digital images were also collected bi-weekly to assess turfgrass coverage. For this summary, only the turfgrass quality data from Arkansas are reported, although similar results were observed at the Tennessee location.

Results: Under the full sun and 20% shade treatments, all treatments produced acceptable turfgrass quality (Quality > 7.0) throughout the growing season (Figures 1 and 2). Under the 40 and 60% shade treatments, all treatments began to decline below acceptable levels of turfgrass quality in July and continued to decline for the remainder of the growing season. However, all PGR treatment combinations improved turfgrass quality compared to the untreated control.

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

When compared to the calendar-based interval (every 7 days), the Primo-GDD treatments were applied on approximately 15-day intervals throughout the season, while the Anuew-GDD treatments were applied on approximately 9-day intervals. This extension of the interval for Primo Maxx appears to reduce its effectiveness with regard to shade tolerance of an ultradwarf putting green. These trials will be repeated in the 2022 season to determine if this trend is continued.

References

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

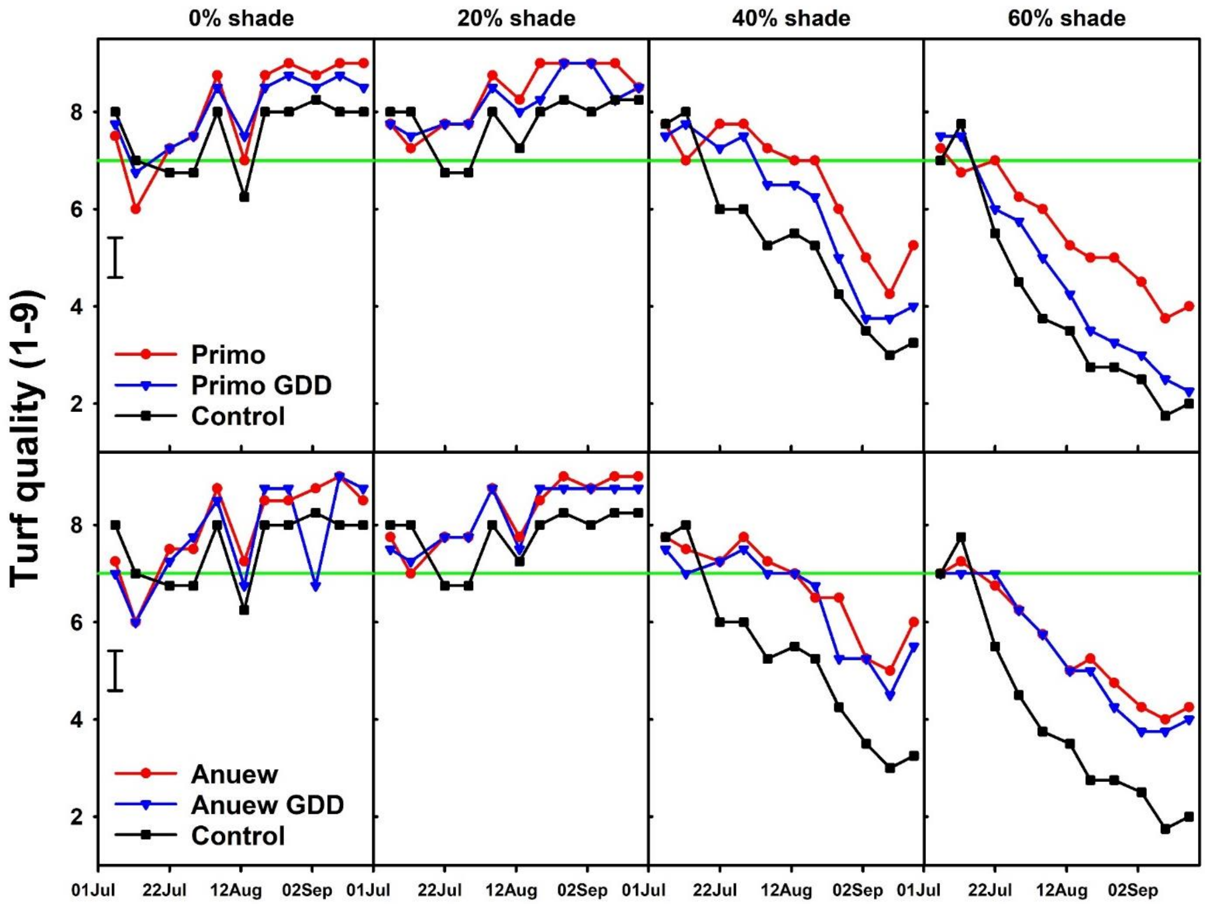


Figure 2 – Drone photo of two replicates from the Arkansas site on August 31, 2021. The % shade treatments are identified to the left and right of the photo and the PGR treatments are identified across the top of the image.

USGA ID#: 2019-13-683

Title: Kentucky bluegrass fairway establishment and drought tolerance under plant growth-promoting microorganisms (PGPMs) application

Project Leader: Qi Zhang
Affiliation: North Dakota State University

Objectives:
- Determine the efficacy of PGPMs on Kentucky bluegrass establishment
- Quantify the effects of PGPMs on drought tolerance and recovery of Kentucky bluegrass fairway

Start Date: 1/1/2019
Project Duration: 3 yrs
Total Funding: $13,412

Summary Points:
- 'Moonlight' had higher Normalized Difference Vegetation Indices and Leaf Area Index than 'Kenblue' 60-days after seeding (DAS), although both cultivars performed similarly at 30 DAS.
- 'Kenblue' had higher values in growth indices (such as tissue biomass) than 'Waterworks' under drought stress when evaluated under a greenhouse setting. However, 'Waterworks' and 'Barserati' had lower stress indices than 'Kenblue' when subjected to drought under the field condition. The discrepancy might be due to the growing condition, parameter quantified, and the timing of the evaluation.
- PGPM had limited effects on Kentucky bluegrass establishment and drought responses.

Summary Text:
The rhizosphere is the soil region largely influenced by plants through rhizodeposition of exudates and metabolites, providing rich carbon sources and colonization structures to soil microorganisms. Microorganisms in turn may have deleterious, neutral, or beneficial effects on plants. It has been well documented that plant growth-promoting microorganism (PGPM), including arbuscular mycorrhizal fungi and plant growth-promoting bacteria, help improve yield and stress tolerance in field crops by influencing resource acquisition (e.g. water and nutrient), modulating plant hormone levels, regulating source-sink relations and energetic metabolism, and inducing systemic resistance. In turfgrass, the beneficial effects of PGPM are mainly observed in perennial ryegrass, tall fescue, and fine fescues. Limited information is available on the relationships between PGPM and other important turfgrass species, such as Kentucky bluegrass, which rarely form symbiotic relationships in nature. The objective of this research is to determine the efficacy of commercially available PGPM products (Table 1) on Kentucky bluegrass establishment and drought tolerance enhancement.

<u>Experiment 1 – drought responses under a greenhouse setting.</u> 'Kenblue' and 'Waterworks' were seeded at 3 lbs pure live seed (PLS)/1,000 sq. ft in plastic pots in a greenhouse. After two weeks of germination under optimal conditions, half of the pots was hand-watered three times weekly from week 3 to 6 (i.e. non-drought) and the other half was saturated only when leaf

wilting was observed (i.e. drought stress treatment). The PGPM products were applied at seeding and once weekly from week 3 to 6, except Nortica (at seeding and week 4). When the experiment was terminated at week 6, data were collected on shoot and root dry weight (SDW and RDW), root length (RL), and plant height (PH). The experimental design was a 2 (cultivar) x 2 (condition) x 7 (PGPM treatment) factorial combination, arranged in a RCBD with 4 replicates. ‘Kenblue’ outperformed ‘Waterworks’ in all growth indices, except RL and SRL (Table 2). Drought condition inhibited turfgrass growth with the highest reduction in RDW (50%). The higher reduction in RDW in ‘Waterworks’ compared to ‘Kenblue’ resulted in a higher SRL in ‘Waterworks’. Similarly, plants under drought had a higher SRL than the non-stressed plants due to a higher reduction in RDW than in RL.

Experiment 2 – Field establishment. ‘Kenblue’ and ‘Moonlight’ Kentucky bluegrass were seeded at 1 lbs PLS/1,000 sq. ft on May 21, 2021. A breakout of barnyard grass occurred shortly after seeding, resulting in unacceptable turfgrass coverage/performance. Thus, the trial was re-established at 3 lbs PLS/1,000 sq. ft on July 28, 2020 to achieve quick turf coverage and suppress weeds. All PGPM products were applied at seeding and once weekly thereafter, except Nortica (5 days before seeding and once a month thereafter) and RootShield PLUS WP (at seeding and once a month thereafter). The experiment was setup as a split-plot design with the whole-plot being cultivar arranged in a RCBD with 4 replicates and sub-plot being PGPM treatments. Remote sensing data were collected with MSR16 (CropScan, Inc., Rochester, MN) at approximately 30, 60, and 90 days after seeding (DAS). Two Normalized Difference Vegetation Indices (NDVI) (NDVI1 = ($R$760-$R$710)/($R$760+$R$710); NDVI2 = ($R$950-$R$660)/($R$950+$R$660)) and Leaf Area Index (LAI = $R$950/$R$660) were calculated. This experiment was repeated in 2021.

No differences were observed in any remote sensing indices between the cultivars 30 DAS in both years (Tables 3 and 4). ‘Moonlight’ had higher values in the remote sensing indices than ‘Kenblue’ on all other evaluation dates, except 90 DAS in 2020. PGPM differences were only detected on the 90 DAS in NDVI1 in 2020, in which Molt-x was higher than Nortic, Serenade, and the control.

Experiment 3 – Drought evaluation under the field condition. ‘Kenblue’, ‘Baserati’, and ‘Waterworks’ Kentucky bluegrass were seeded at 1 lbs PLS/1,000 sq. ft on May 21, 2021. Similar to the establishment trial (Experiment 2), a breakout of barnyard grass occurred and the research area had to be re-established (Aug. 25, 2020). Drought condition was not induced in 2020 due to late establishment. Drought stress was applied in 2021 by withholding irrigation from June 24 to Sept. 23. Irrigation was applied on Aug. 6 as turf quality fell below acceptable and continued until Aug. 16 to help turfgrass recovery. The experimental design and data collection were identical to Experiment 2, except the data collection frequency. Two stress indices, Stress Index 1 ($R$710/$R$810) and Stress Index 2 ($R$710/$R$760) were calculated.

PGPM treatments were not applied in 2020 as drought stress was not induced. No differences were observed in any remote sensing indices among the cultivar (data not shown). ‘Kenblue’ had higher values in Stress Index 1 than ‘Barserati’ and ‘Waterworks’ on all evaluation dates, except Aug. 9 (Table 5). ‘Barserati’ performed similarly as ‘Waterworks’ until Aug. 23, but showed a higher Stress Index 1 in the later evaluation dates. The same trend was observed in Stress Index 2 (data not shown). No differences were observed in PGPM products.

Table 1. Six commercially available plant growth-promoting microorganism (PGPM) products included in the present study.

Product name	Active ingredient	Application rate (product/acre)
Serenade (Bayer CropScience LP, Research Triangle Park, NC)	*Bacillus subtilis* strain QST713 (1.34%)	4 qt
BotaniGard 22WP (BioWorks, Inc., Butte, MT)	*Beauveria bassiana* strain GHA (22.0%)	11 lbs
RootShield PLUS^{+} WP (BioWorks, Inc., Victor, NY)	*Trichoderma harzianum* Rifai strain T-22 (1.15%) + *Trichoderma virens* strain G-41 (0.61%)	65 lbs for the first 2 applications and 21.7 lbs thereafter
Companion (Growth products, White Planins, NY)	*Bacillus subtilis* strain GB03 (4.26%)	1.5 lbs
Nortica 10WP (Bayer Experimental Science, Research Triangle Park, NC)	*Bacillus firmus* strain 1-582 (10.0%). It also contains N (14%), K_2O (21%), and chloride (51%)	50 lbs at the first application and 17.5 lbs thereafter
Molt-X (BioWorks, Inc., Victor, NY)	*Azadirachtin* (3.0%)	10 oz

Table 2. Kentucky bluegrass seedling growth as affected by three main factors, cultivar, drought condition, and plant growth-promoting microorganism (PGPM) products.

Treatment	Height (cm)	Shoot dry weight (g)	Root dry weight (g)	Root length (cm)	Root /shoot dry weight ratio (%)	Specific Root length (cm/g)
Cultivar						
Kenblue	13.8a[z]	1.21a	0.69a	15.4a	55.5a	26.0b
Waterworks	12.6b	1.12b	0.53b	16.0a	46.4b	34.7a
Condition						
Non-drought	14.5a	1.37a	0.82a	17.0a	59.7a	21.9b
Drought	11.9b	0.96b	0.41b	14.4b	42.2b	38.8a
PGPM products						
Control (no PGPM)	12.8a	1.01c	0.51c	15.4a	49.2ab	38.0a
Serenade	12.7a	1.05bc	0.57bc	15.2a	53.1ab	30.8ab
BotaniGard 22WP	13.5a	1.22ab	0.69a	15.7a	54.7a	25.7b
RootShield PLUS^{+} WP	13.0a	1.13bc	0.61a-c	15.8a	51.9ab	31.8ab
Companion	13.7a	1.34a	0.67ab	17.2a	49.1ab	28.3ab
Nortica 10WP	13.5a	1.22ab	0.59a-c	15.4a	46.5b	31.5ab
Molt-x	13.4a	1.19a-c	0.64ab	15.4a	52.3ab	26.4b

[z]Means followed by the same letter within each main factor are not significantly different at $P \leq 0.05$.

Table 3. Remote sensing indices of Kentucky bluegrass evaluated 30 – 90 days after seeding (DAS) as affected by cultivar and plant growth-promoting microorganism (PGPM) products in 2020.

	30 DAS			60 DAS			90 DAS		
Treatment	NDVI1[y]	NDVI2	LAI	NDVI1	NDVI2	LAI	NDVI1	NDVI2	LAI
Cultivar									
Kenblue	0.328a[z]	0.661a	5.082a	0.480b[z]	0.823a	10.591a	0.487b	0.843b	11.991b
Moonlight	0.342a	0.637a	4.692a	0.512a	0.819a	10.415a	0.558a	0.859a	13.577a
PGPM products									
Control	0.325a	0.637a	4.655a	0.471b	0.813a	9.657a	0.526a	0.850a	12.697a
Serenade	0.317a	0.629a	4.444a	0.477b	0.800a	9.087a	0.505a	0.840a	11.770a
BotaniGard 22WP	0.333a	0.649a	4.927a	0.504ab	0.830a	11.173a	0.526a	0.854a	13.261a
RootShield PLUS^{+} WP	0.359a	0.675a	5.429a	0.509ab	0.834a	11.347a	0.520a	0.850a	12.499a
Companion	0.357a	0.672a	5.327a	0.507ab	0.827a	11.079a	0.514a	0.848a	12.534a
Nortica 10WP	0.320a	0.635a	4.683a	0.484b	0.806a	9.559a	0.521a	0.849a	12.495a
Molt-x	0.333a	0.644a	4.655a	0.520a	0.839a	11.619a	0.546a	0.868a	14.232a

[z]Means followed by the same letter within each main factor are not significantly different at $P \leq 0.05$.

[y]Normalized Difference Vegetation Index (NDVI) 1 ((NDVI1 = ($R$760-$R$710)/($R$760+$R$710)) and 2 ((NDVI2 = ($R$950-$R$660)/($R$950+$R$660)) and Leaf Area Index (LAI = $R$950/$R$660), with R being reflectance and the number being the wavelength (nm).

Table 4. Remote sensing indices of Kentucky bluegrass evaluated 30 – 90 days after seeding (DAS) as affected by cultivar and plant growth-promoting microorganism (PGPM) products in 2021.

	30 DAS			60 DAS			90 DAS		
Treatment	NDVI1[y]	NDVI2	LAI	NDVI1	NDVI2	LAI	NDVI1	NDVI2	LAI
Cultivar									
Kenblue	0.433a[z]	0.762a	8.506a	0.404b	0.750b	7.335b	0.399b	0.761b	7.553b
Moonlight	0.451a	0.759a	8.078a	0.509a	0.811a	10.400a	0.501a	0.816a	10.187a
PGPM products									
Control	0.447a	0.773a	8.543a	0.457a	0.788a	8.875a	0.453a	0.794a	8.954a
Serenade	0.485a	0.808a	9.733a	0.480a	0.807a	10.081a	0.459a	0.797a	9.378a
BotaniGard 22WP	0.467a	0.788a	9.246a	0.481a	0.808a	9.688a	0.468a	0.805a	9.736a
RootShield PLUS^{+} WP	0.399a	0.715a	6.600a	0.457a	0.780a	8.584a	0.446a	0.787a	8.680a
Companion	0.446a	0.762a	6.355a	0.461a	0.785a	8.945a	0.457a	0.794a	9.128a
Nortica 10WP	0.445a	0.757a	8.053a	0.454a	0.775a	8.561a	0.430a	0.774a	8.083a
Molt-x	0.402a	0.721a	7.513a	0.404a	0.719a	7.339a	0.438a	0.770a	8.131a

[z]Means followed by the same letter within each main factor are not significantly different at $P \leq 0.05$.

[y]Normalized Difference Vegetation Index (NDVI) 1 ((NDVI1 = ($R$760-$R$710)/($R$760+$R$710)) and 2 ((NDVI2 = ($R$950-$R$660)/($R$950+$R$660)) and Leaf Area Index (LAI = $R$950/$R$660), with R being reflectance and the number being the wavelength (nm).

Table 5. Stress Index 1 (*R*710/*R*760, *R* being reflectance and the number being the wavelength, nm) of Kentucky bluegrass under drought as affected by cultivar and plant growth-promoting microorganism (PGPM) products in 2021. Drought stress was induced by holding irrigation from June 24 to Sept. 23. Irrigation was reapplied from Aug. 6 – 16 due to severe drought stress.

Treatment	July 1	July 13	Aug. 9	Aug. 11	Aug. 13	Aug. 23	Aug. 30	Sept. 9	Sept. 17
Cultivar									
Barserati	0.233b[z]	0.241b	0.321a	0.349b	0.352b	0.281b	0.272b	0.289b	0.289b
Kenblue	0.293a	0.321a	0.313a	0.447a	0.439a	0.350a	0.336a	0.349a	0.345a
Waterworks	0.230b	0.238b	0.316a	0.333b	0.332b	0.263b	0.250c	0.259c	0.256c
PGPM products									
Control	0.253a	0.261a	0.303a	0.372a	0.372a	0.295a	0.284a	0.303a	0.302a
Serenade	0.244a	0.269a	0.281a	0.382a	0.383a	0.305a	0.290a	0.301a	0.296a
BotaniGard 22WP	0.244a	0.263a	0.331a	0.371a	0.371a	0.291a	0.280a	0.296a	0.294a
RootShield PLUS$^+$ WP	0.249a	0.266a	0.310a	0.372a	0.369a	0.299a	0.288a	0.299a	0.294a
Companion	0.255a	0.272a	0.329a	0.378a	0.373a	0.301a	0.288a	0.294a	0.293a
Nortica 10WP	0.253a	0.265a	0.325a	0.369a	0.368a	0.297a	0.289a	0.299a	0.301a
Molt-x	0.265a	0.271a	0.340a	0.389a	0.383a	0.299a	0.284a	0.300a	0.297a

[z]Means followed by the same letter within each main factor are not significantly different at $P \leq 0.05$.

USGA ID#: 2019-14-684

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

Bingru Huang and James Murphy

Rutgers University

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

Start Date: 2019

Project Duration: four years

This report describes the results for the second year of a field study in the university research farm and the first year of a golf course study conducted in 2021, addressing objective 3; to investigate effects of plant-health products on summer performance of annual bluegrass on putting green conditions.

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

- Results from 2021 are consistent with those of 2020, where Daconil Action, Appear II, Daconil Action + Appear II, and Daconil Action + Appear II + Primo effectively enhanced *Poa* performance during summer, but unlike 2020 Signature XTRA was not as effective.
- The combined treatment of Daconil Action + Appear II or Daconil Action + Appear II + Primo was more effective in improving *Poa* summer performance than each individual treatment alone.
- Seaweed Extract A had positive effects on *Poa* health and quality in the field trial in the university research farm.
- Of the plant growth regulators (PGRs) Primo alone had no significant effects on Pao summer performance while Proxy alone and the combination of Proxy and Primo had adverse effects during summer months.
- In the golf course trial Seaweed-based Extract C, Daconil Action + Appear II + Primo, and Daconil Action + A23728A + Primo had the greatest effect on enhancing *Poa*/Bent mixed greens throughout the summer
- Amino acid treatments enhanced turf performance in the summer in both trials.

Methodology for 2021 Field Trial

This experiment was conducted on Rutgers University research plots managed under putting green conditions.

The field sites at the research farm (Field #18 E South) were established with mixed biotypes of *P. annua* originally collected from Rutgers University Golf Course and Plainfield Country Club. *Poa* turf is maintained at a cutting height of 0.125 inches with adequate irrigation and fertilization, as well as curative and preventive programs for disease control. The maintenance program applied included Brown Ring Patch, Dollar Spot, Anthracnose, and Summer Patch control (Prostar, Secure, Medallion + Banner Maxx, Prostar, Torque, Affirm + Emerald, Maxtima + Heritage TL, Maxtima + Prostar) and fertilizer (0.1 N).

Three types of plant-health products were examined for their effects on *Poa* heat tolerance. The following chemical treatments were applied by foliar spray to field plots:
1) untreated control: plants were sprayed with water (2 gal/1000 ft^2); 2) biostimulants: amino acids A (60 mM /1000ft^2), amino acids B (.44 uM / 1000 ft^2), amino acids A + B (amino acid C); seaweed-based A (12 fl oz / 1000 ft^2), seaweed-based B (15 fl oz / 1000 ft^2); 3) Plant growth regulator: Primo Maxx (trinexapac-ethyl) (0.1 fl oz / 1000 ft^2), Proxy (ethephon) (2 fl oz / 1000 ft^2), Primo Maxx + Proxy; 4) Fungicides: Signature XTRA StressGard (4.0 fl oz / 1000 ft^2) (Sig), Daconil Action (3.5 fl oz / 1000 ft^2) (DacAc), Appear II (6 fl oz / 1000 ft^2) (AppII), Daconil Action + Appear II (DAppII), Daconil Action + Appear II + Primo (DAIIP).

All treatments were applied to 3'x 4' field plots (5 replicates each) every 14 days between June 3rd to August 31st.

The following measurements were taken weekly based on weather conditions. Turf quality (TQ) was visually rated. Regular photos were taken to measure the percent green canopy cover and dark green color index (DGCI) using imaging analysis programs. Canopy temperature was measured by taking thermal pictures and using FLIR image analysis software to calculate the change in canopy temperature.

Treatment effects on different parameters were determined by analysis of variance procedure according to the general linear model (GLM) procedure of the SAS program. Significant effect of each individual treatment was compared to the untreated control at $p = 0.05$.

Summary of Results

Poa plots with application of Daconil Action, Appear II, Daconil Action + Appear II, and Daconil Action + Appear II + Primo had significantly higher turf quality (Fig. 1A), and also maintained greener canopy cover (Fig. 2A). Of these fungicides, Daconil Action, Daconil Action + Appear II, and Daconil Action + Appear II + Primo also had significantly higher DGCI (Fig. 3A) throughout August.

Application of Primo alone had little to no significant effect on any of the parameters examined in this experiment. *Poa* treated with Proxy alone and the combination of Proxy and Primo had significantly lower turf quality (Fig. 1B), and relatively lower DGCI levels (Fig. 3B).

Treating *Poa* with biostimulants had mixed results. Seaweed Extract A plots had relatively higher TQ throughout the study and had significant increases towards the end of the summer (Fig. 1C). However, TQ for all three amino acid treatments had inconsistent effects throughout the study, fluctuating between having relatively higher TQ to lower TQ compared to the control (Fig. 1C). Additionally, Seaweed Extract B and Amino Acid C both maintained greener canopy cover (Fig. 2C), while both Seaweed Extract treatments and Amino Acid B maintained significantly higher DGCI towards the end of summer (Fig. 3C).

Treatments had mixed effects on canopy temperature throughout the summer months. Daconil Action + Appear II, and Daconil Action + Appear II + Primo maintained cooler canopies toward the end of the summer (Fig. 4A). Seaweed Extract A and Seaweed Extract B maintained cooler temperatures in the beginning and again at the end of the study compared to untreated control (Fig. 4C).

Methodology for 2021 Golf Course Trial

This study was conducted at Tamarack Golf Course, a two18-hole course located in East Brunswick with putting greens made up of a mixture of *Poa* and bentgrass. Trials were conducted on putting greens of two different holes on the West course; hole 4 and 17. Putting greens were mowed at 0.150'', well-irrigated and fertilized, and under disease control. *Poa* was not uniformly distributed on the *Poa*/Bent putting greens. In respect to this, greens were examined and patches with relatively more uniform *Poa* were selected for treatments. *Poa* patches on the putting greens were not arranged adjacent to each other unlike uniform research field plots. A total of 6 replicate patches were selected for each treatment, three at hole 4 and three at hole 17.

The following treatments were applied by foliar spray to field plots: 1) untreated control: plants were sprayed with water (2 gal/1000 ft^2); 2) Seaweed-based Extract C (12 fl oz/1000 ft^2); Seaweed-based Extract D (12 fl oz/1000 ft^2) + wetting agent + a granular seablend (4.2 lbs/1000 ft^2 applied in May and end of August); 3) Daconil Action (3.5 fl oz) + Appear II (6.0 fl oz) + Primo (0.125 fl oz); 4) Daconil Action (3.5 fl oz) + A23728A (6.0 fl oz) + Primo (0.125 fl oz); 5) Amino Acid C (same concentration as field trial); 6) Amino Acid D (25uM/1000 ft^2); 7) Amino Acid C + Amino Acid D (Amino Acid E).

Each treatment was applied once every 2 weeks from June 7th to August 26th.

The following measurements were taken weekly based on weather conditions from June 7th to September 6th. Turf quality (TQ) was visually rated. Normalized Difference Vegetation Index (NDVI), Stress Index (SI) and leaf area index (LAI) was evaluated using a multispectral radiometer (CropScan).

Data analysis was conducted following the same procedure used for the field trial above.

Summary of Results

Seaweed-based Extract C, Seaweed-based extract D, Daconil Action + Appear II + Primo, Daconil Action + A23728A + Primo, Amino Acid D, and Amino Acid E showed significant increases in TQ (Fig. 5).

All treatments had relatively higher NDVI, LAI, and lower stress levels throughout the summer. On August 16th, Seaweed-based Extract C, Daconil Action + Appear II + Primo, and Daconil Action + A23728A + Primo showed significant increases in NDVI (Fig. 6), LAI (Fig. 7), and significantly lower stress levels (Fig. 8).

Of the amino acid treatments, Amino Acid D had significantly higher NDVI on August 16th (Fig. 6), and both Amino Acid C and Amino Acid D plots had significantly higher LAI (Fig. 7) and lower stress levels (Fig. 8) at the end of the summer.

The golf course study will be replicated in summer 2022 to see if effects are consistent across two years.

Fig. 1. Turf quality of *Poa* putting green as affected by different treatments.

"*" indicate treatments significantly different from the control at p = 0.05.

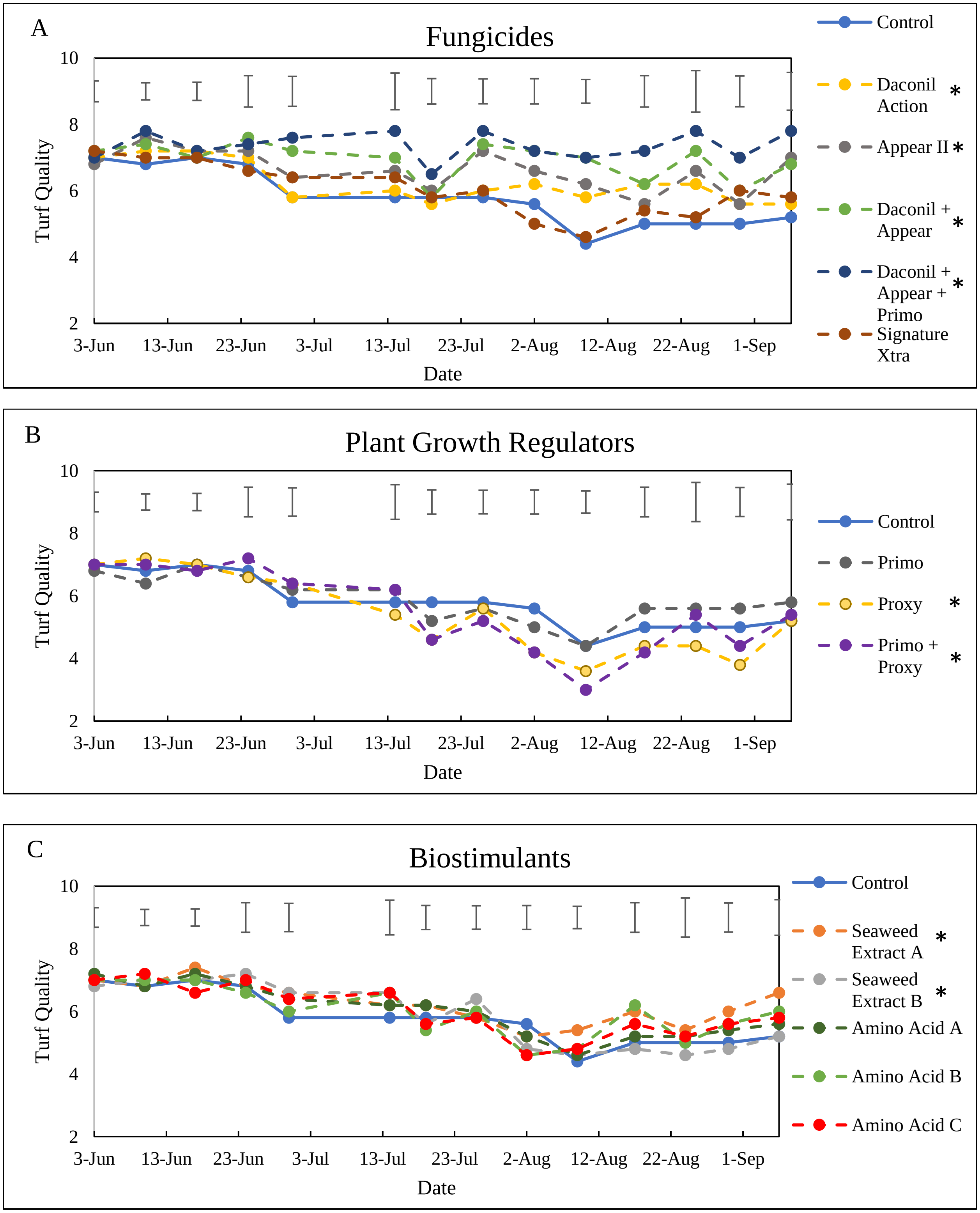


Fig. 2. Canopy cover of *Poa* putting green as affected by different treatments.
"*" indicate treatments significantly different from the control at p = 0.05.

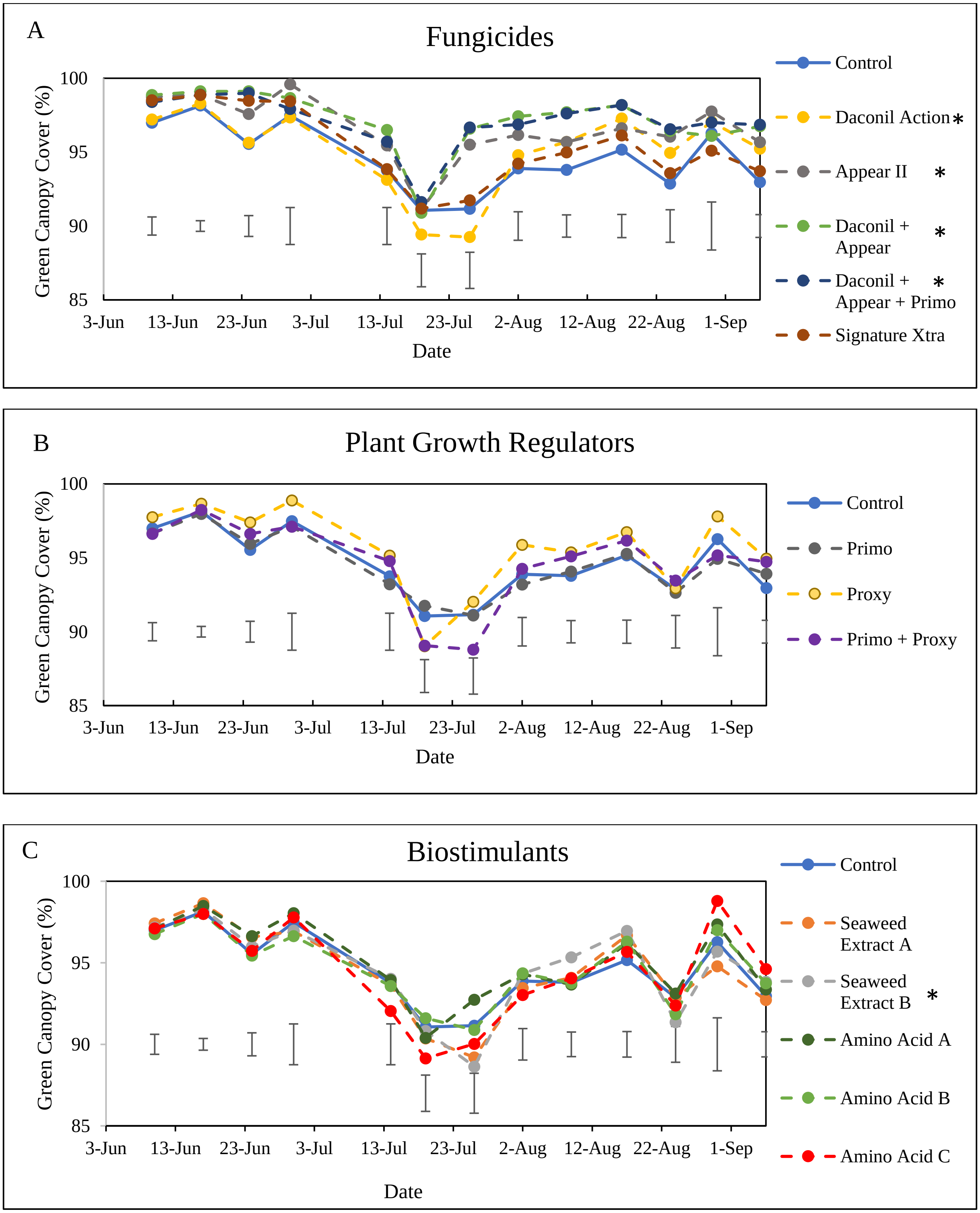
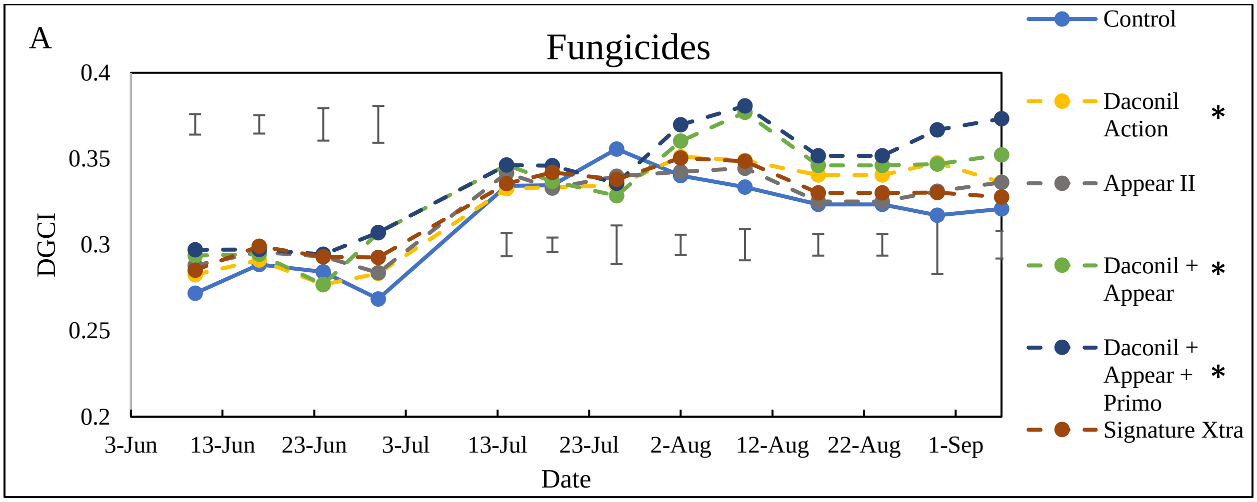
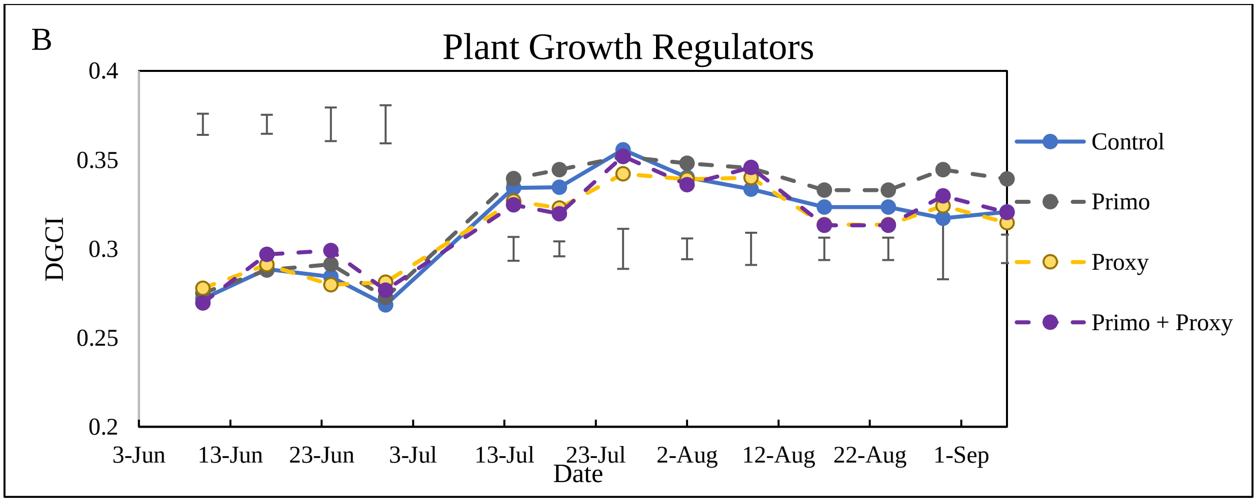


Fig. 3. Dark Green Color Index (DGCI) of *Poa* putting green as affected by different treatments. “*” indicate treatments significantly different from the control at p = 0.05.





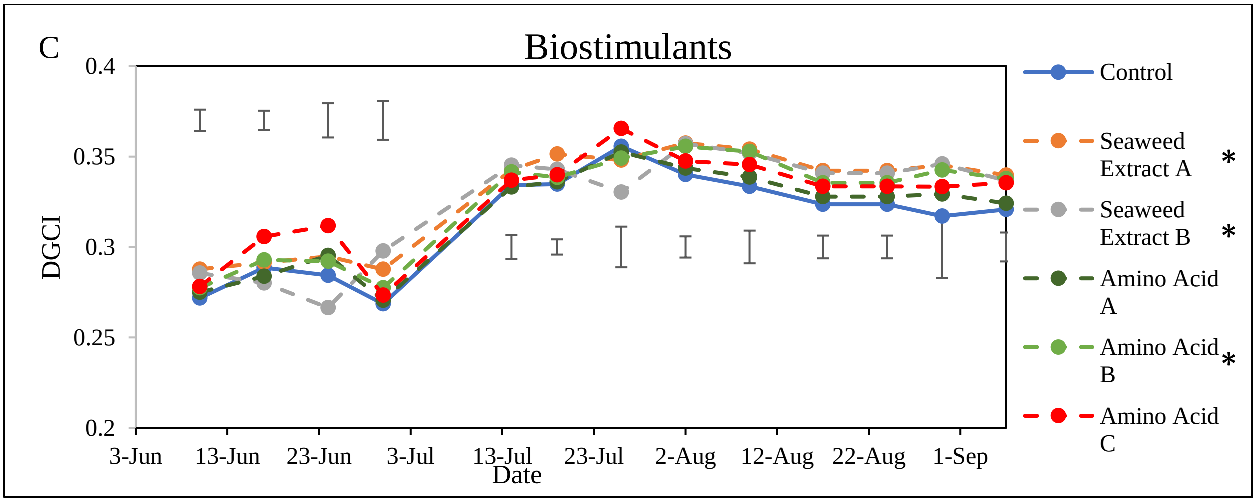
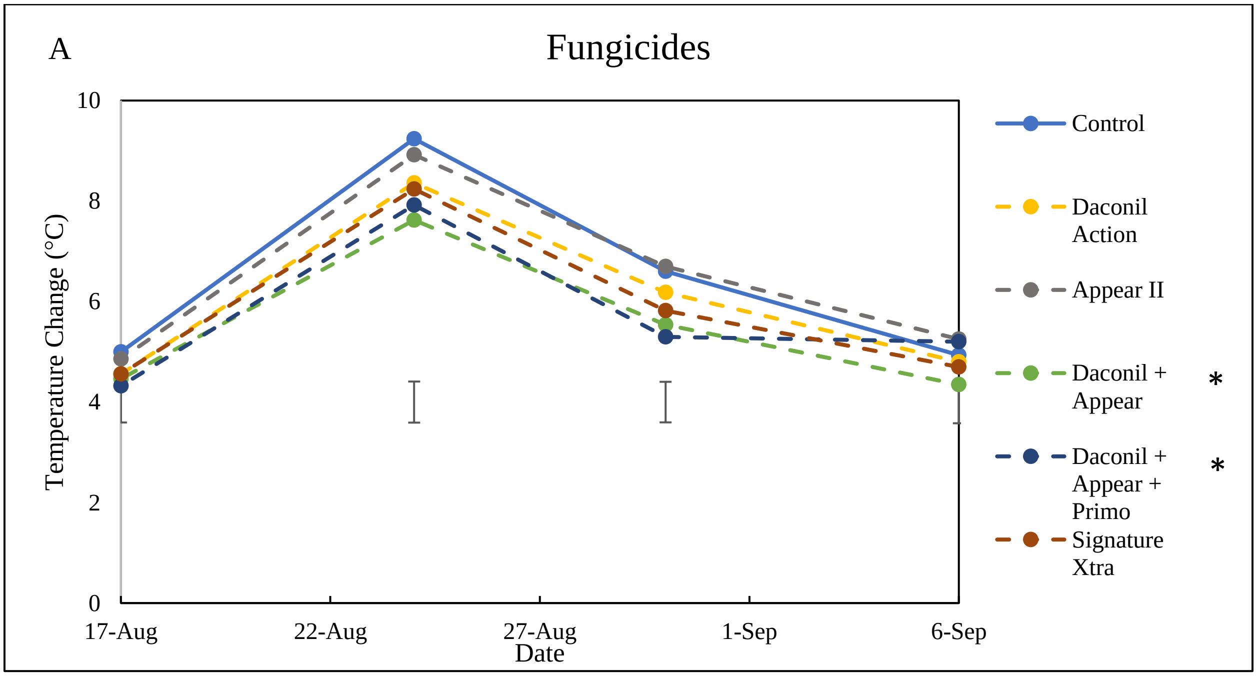


Fig 4. Change in canopy temperature of *Poa* putting green as affected by different treatments. "*" indicate treatments significantly different from the control at p = 0.05.



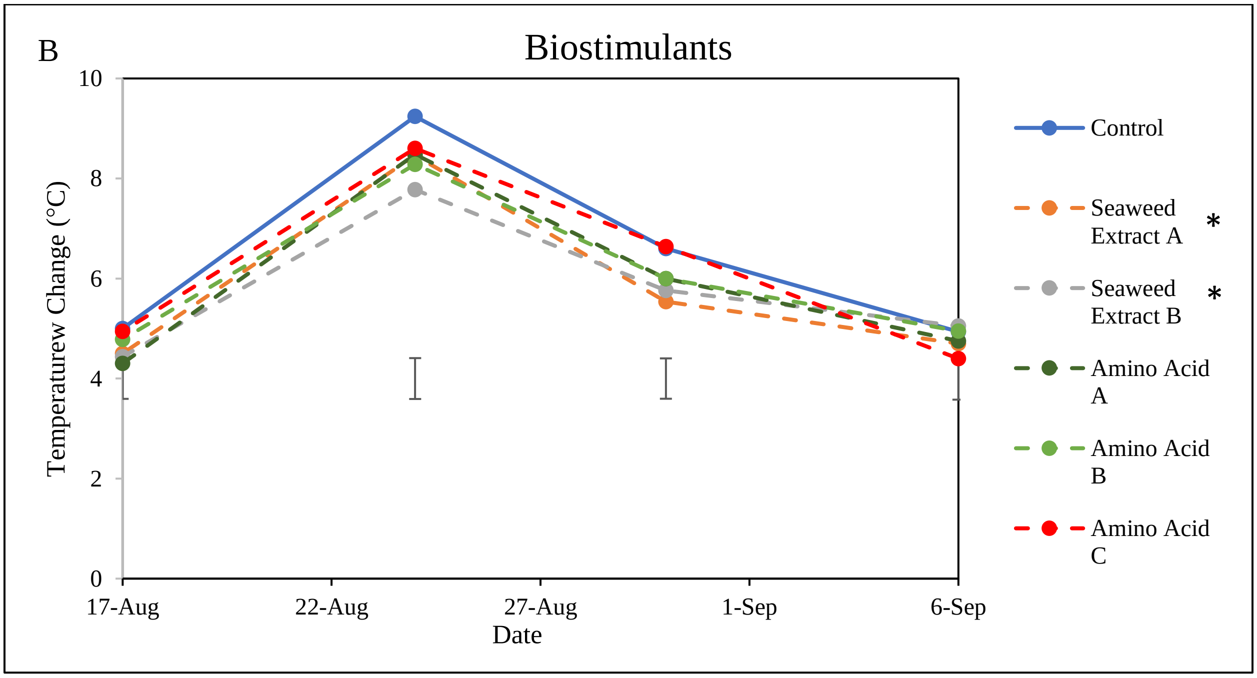


Fig 5. Turf quality of mixed *Poa*/Bent putting green on the golf course as affected by different treatments.

"*" indicates treatments significantly different from the control at p = 0.05.

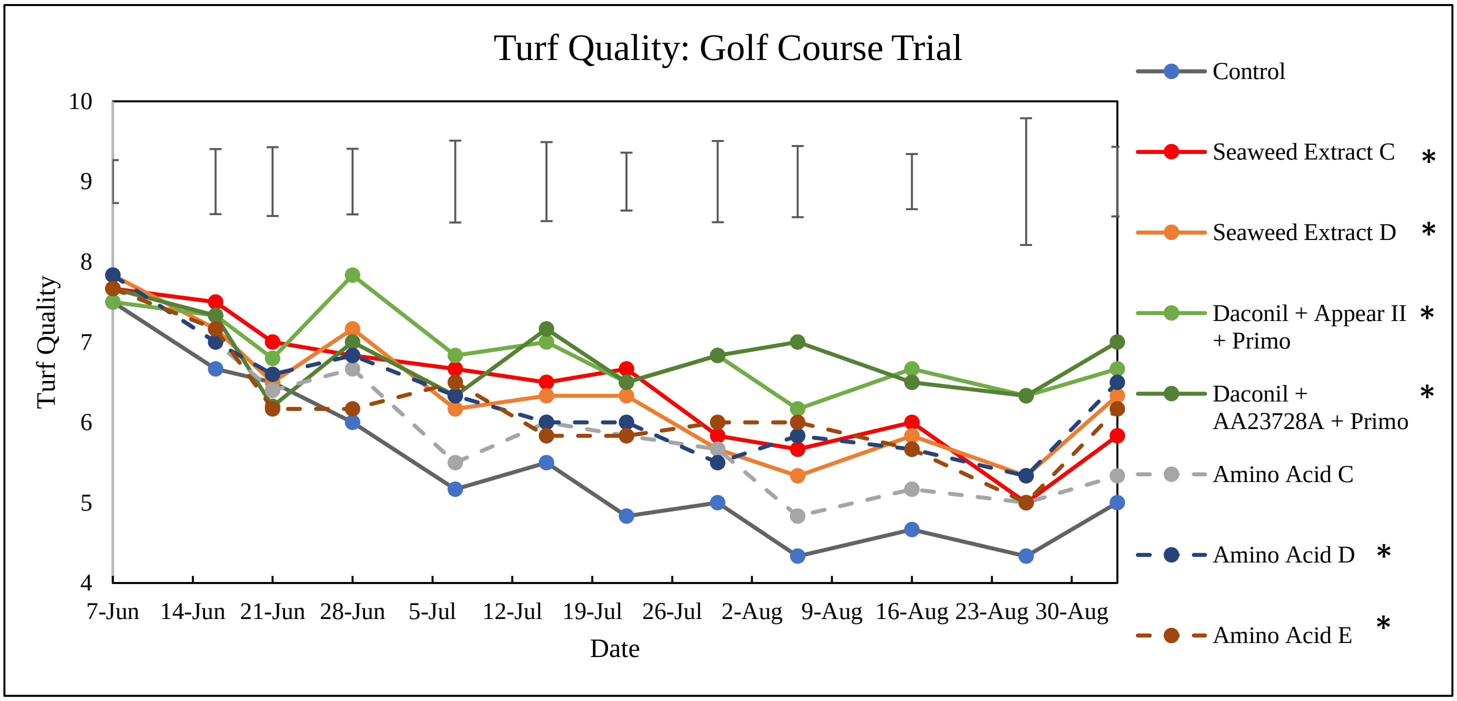


Fig 6. NDVI of mixed *Poa*/Bent putting green on the golf course as affected by different treatments. "*" indicates treatments significantly different from the control at p = 0.05.

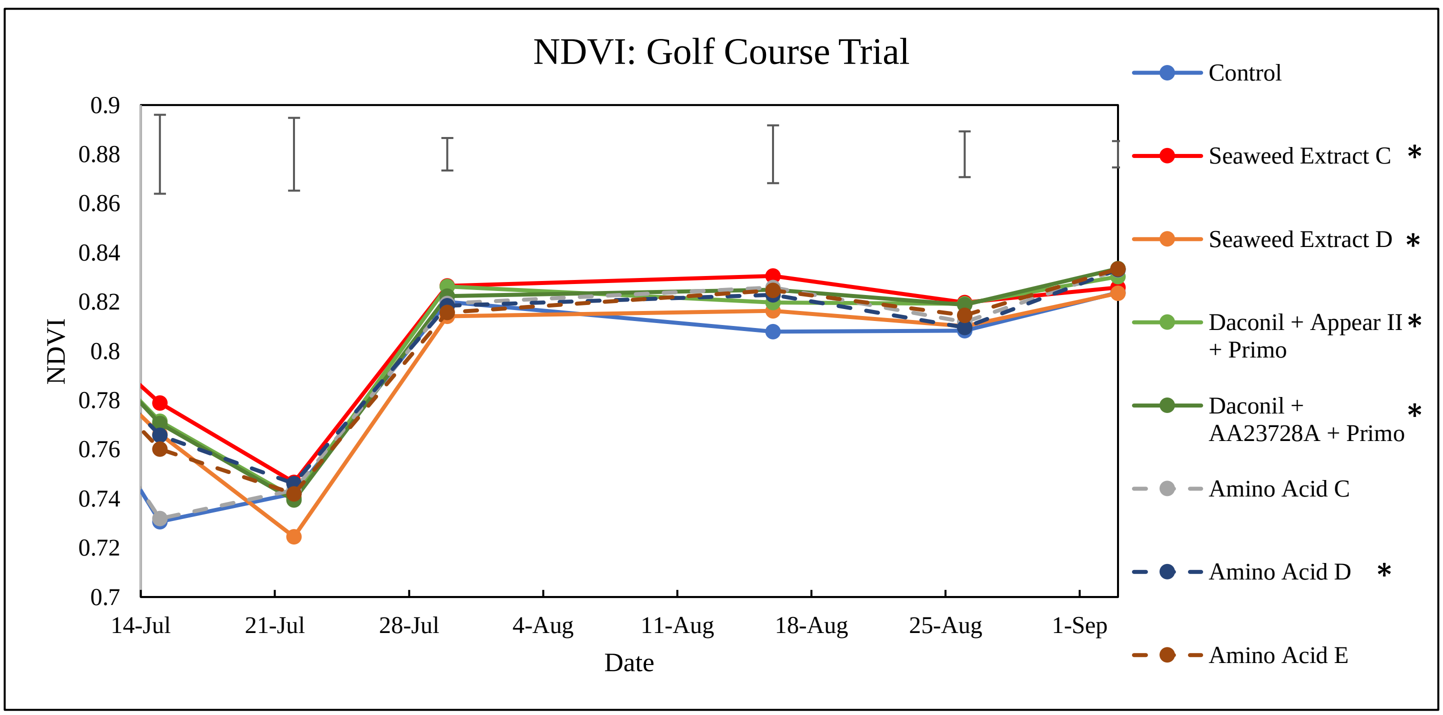


Fig 7. LAI of mixed *Poa*/Bent putting green as affected by different treatments. “*” indicates treatments significantly different from the control at p = 0.05.

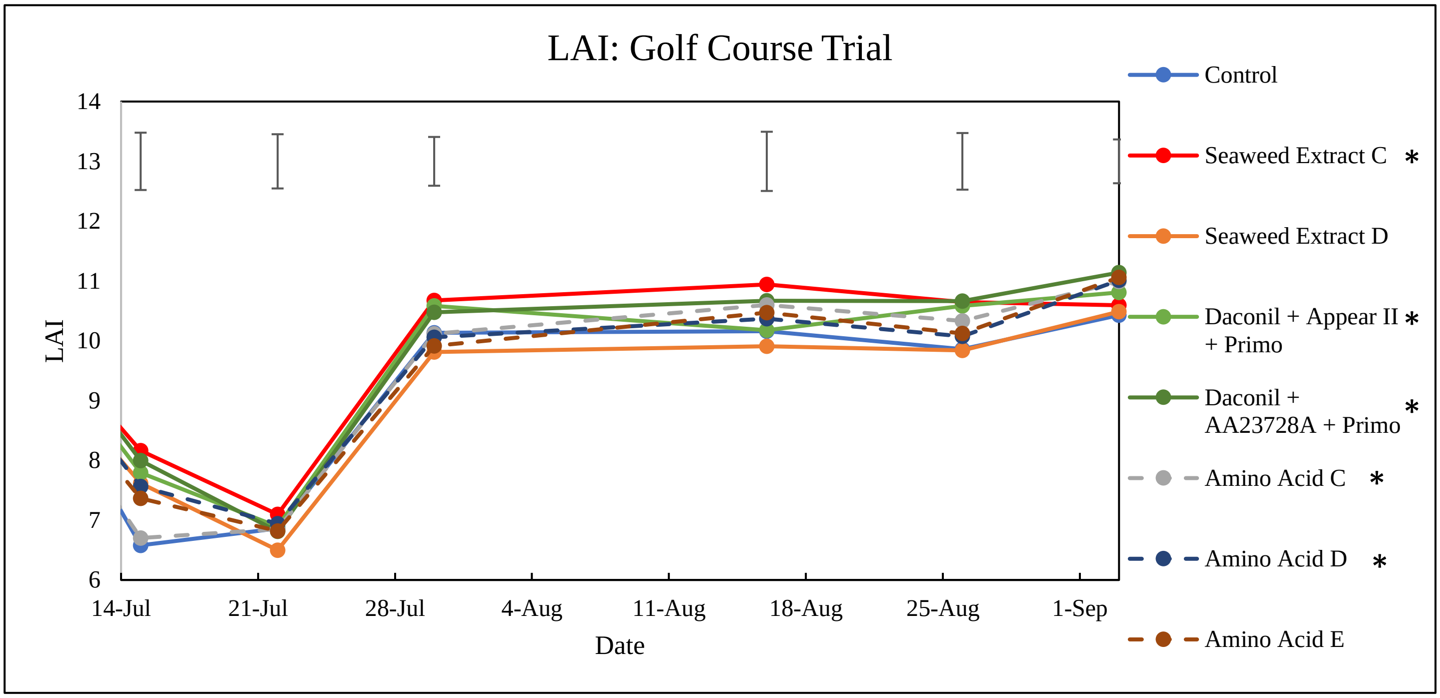
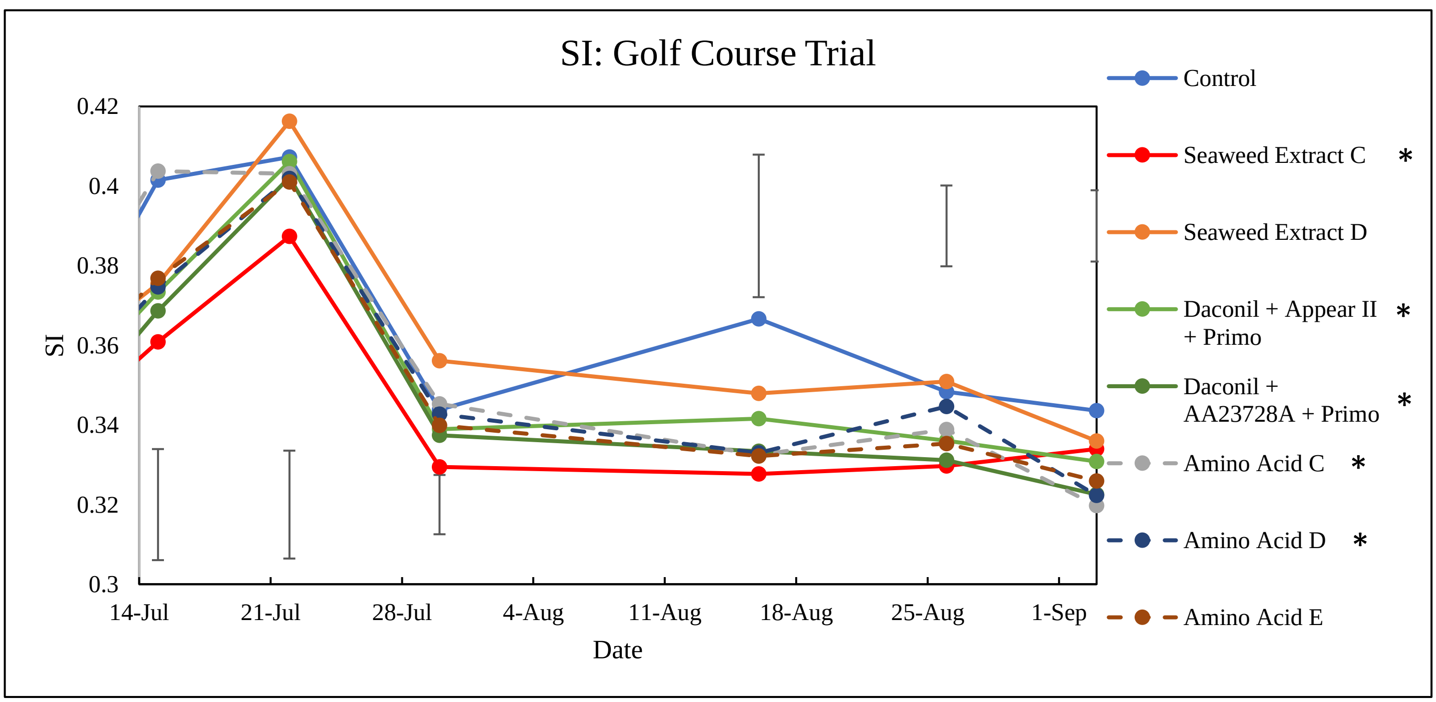


Fig 8. SI of mixed *Poa*/Bent putting green as affected by different treatments. “*” indicates treatments significantly different from the control at p = 0.05



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, shoot dry weight (SDW), and chlorophyll content. Plant growth promoting rhizobacteria *Bacillus subtilis* UD1022 had better turf quality than trinexapac-ethyl treatment.
- Lower SDW was observed for trinexapac-ethyl and tank mix of treatments possibly due to growth inhibiting effect of trinexapac-ethyl in both the treatments.
- Chlorophyll content was higher with the tank mix of treatments and lower for fosetyl-Al and untreated control at the end of the season.

Creeping Bentgrass Trial at Penn State Berks Campus (Reading, PA)

- Plots treated with UD1022 had better turf quality (area under turf quality progress curve) than the untreated control while turf quality was highest for plots treated with the tank-mix of treatments.
- No statistically significant differences were detected among the treatments for Normalized Difference Vegetation Index (NDVI) and soil volumetric water content (VWC).
- Plots treated with UD1022 had less dollar spot incidence (area under disease progress curve) than the untreated control. Best dollar spot control was observed in plots treated with the tank-mix of treatments.

Bermudagrass Trial (Auburn, AL)

- The tank mix of treatments consistently showed superior performance throughout the trial period for visual color and quality.

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). PHP application does not result in direct lethality to a pest; rather, their application has a physiological effect that boosts a plant’s immune defense system against various stresses. Some of these materials are EPA-registered active ingredients (e.g., acibenzolar-S-methyl, fosetyl-Al, trinexapac-ethyl), while others are registered as fertilizers (e.g., kelp extract, *Bacillus* spp., phosphite). For most, potential physiological effects have been documented in refereed journal articles. Rarely, however, are these materials studied on industry standard putting greens or in combination. Turfgrass consultants are often called to diagnose decline issues but are hard-pressed to determine primary causes due to confounding factors such as 3 to 10 ingredient tank-mixes.

Our target audiences are golf course superintendents, turfgrass consultants, and scientists. The overall goal of our research is to better understand why or why not one might choose to use certain plant health products, alone or in combination. If benefits are documented, our research may lead to more use of plant health products for maintaining stress-resistant putting greens and less use of pesticides. Improved drought resistance, if indicated, may result in improved superintendent confidence in their deficit irrigation practices. These results support Strategic Initiatives 1 and 2 of this call. 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 three locations on sand-based rootzones: creeping bentgrass (Penntrio, 0.5 inch mowing height) at Penn State Berks campus near Reading, Pennsylvania, creeping bentgrass (Tyee, 0.125 inch mowing height) at Wilmington Country Club, Delaware and an ultradwarf bermudagrass (TifEagle, 0.110” mowing height) green at Auburn University in Auburn, Alabama (Fig. 1). Treatments were applied every 14 days during the designated trial period. All plots received a uniform fertilizer program of 28-8-18 (soluble, with micronutrients) at 0.1 lb N/1000 ft^2 every 14 days.

For creeping bentgrass trial at Wilmington Country Club, seven treatments were applied every two weeks (Table 1) and plots were evaluated for turf quality. Clippings were collected for three days growth every two weeks to obtain shoot dry weight (SDW). Leaf tissue subsamples were also collected for chlorophyll estimation. Statistically significant differences were observed among all the treatments for turf quality, SDW, and chlorophyll content.

UD1022 had better turf quality than trinexapac-ethyl treatment (Table 2). Lower SDW was observed for trinexapac-ethyl and tank mix of treatments towards the end of the season possibly due to growth inhibiting effect of trinexapac-ethyl present in both the treatments that restricts

vertical growth (Table 3). Chlorophyll content was higher with the tank mix of treatments and lower for fosetyl-Al and untreated control at 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. Subsamples have been stored at -80 C for Pathogenesis-related (PR)-gene expression and at 4 C for visualization of bacterial root colonization (confocal microscopy) and plating on agar for determining Bacillus UD1022 colony forming units. Root subsamples have also been harvested and stored for WinRhizo root density analysis.

For the trial at Penn State Berks Campus, plots were evaluated for Turf Quality, NDVI, VWC, and dollar spot. NDVI and VWC were measured using a hand-held GreenSeeker and a FieldScout TDR with 7.62 cm rods, respectively taking an average of 3 readings per plot. Dollar spot ratings were taken based on number of active infection centers (i.e., foci) per plot. A light box was used to take images for digital image analysis (Fig. 3).

Plots treated with UD1022 had better turf quality (area under turf quality progress curve) than untreated plots (Table 5). More dollar spot activity in untreated plots also contributed to lower turf quality. Better turf quality was observed for UD1022 as compared to untreated but was statistically similar to several other treatments (Fig. 2). Plots treated with the tank-mix of acibenzolar-S-methyl + fosetyl-Al + kelp extract + UD1022 + trinexapac-ethyl showed higher turf quality. No statistically significant differences were observed among the treatments for NDVI and VWC (Table 6 and 7). Although initially the purpose of this field trial was to impose drought stress and evaluate any treatment effects, the rootzone never “dried-down”.

Dollar spot ratings were similar to turf quality with UD1022 treatment showing lower dollar spot incidence than the untreated control (Table 8.). Dollar spot incidence was best controlled by the tank mix of acibenzolar-S-methyl + fosetyl-Al + kelp extract + UD1022 + trinexapac-ethyl.

For the bermudagrass trial (Auburn, AL), seven treatments were applied every two weeks and plots were evaluated for visual color and quality. The tank mix of treatments consistently showed superior performance throughout the trial period and was in the top statistical group on all rating dates for visual color and visual quality (Table 9, 10). Root samples have been harvested and will be evaluated for UD1022 root colonization (via colony forming units) and root density using WinRhizo root analysis software.

Laboratory measurements for quantitative and qualitative estimation of UD1022 root colonization, estimation of PR-gene expression, and root density for 2021 samples is in progress. The field trial will be resumed in mid-May in 2022.

Fig.1 Creeping bentgrass trial site at A) Wilmington Country Club, DE and B) Penn State Berks Campus, PA.

Table 1. List of treatments applied to 2021 putting green trial.

S. No	Treatment	Product Name	Application rate
1	Untreated Control	…….	…….
2	Acibenzolar-S-Methyl (50% WSP)	Actigard (Syngenta)	0.3 g /1000 ft^2
3	Kelp Extract (0-0-1)	Guarantee Natural (Ocean Organics)	3 oz/1000 ft^2
4	Plant Growth Promoting Rhizobacteria	*Bacillus subtilis* ‘UD1022’	10^6 cfu/mL in 2-gal water/1000 ft^2
5	Fosetyl-Al (80% WP)	Aliette (Bayer)	4 oz/1000 ft^2
6	Trinexapac-ethyl (11.3%)	Primo Maxx	0.125 oz/1000 ft^2
7	Treatments 2, 3, 4, 5, and 6	5-product tank-mix	….....

Table 2. 2021 Field Trial at Wilmington Country Club, DE – Turf Quality

Treatment	Date of Rating							
	June 1	**June 16**	**July 12**	**July 21**	**Aug 6**	**Aug 23**	**Sept 8**	**Sept 13**
	Turf Quality (1 – 9 visual scale)							
1. Untreated Control	5.6a[x]	5.6	4.0	3.6b	5.3ab	5.0ab	4.9	6.3
2. Acibenzolar-S-Methyl	5.9ab	5.6	4.8	3.9ab	5.5a	5.4a	5.0	6.3
3. Kelp Extract	6.1a	6.0	4.8	3.8ab	5.8a	5.6a	5.6	6.5
4. *Bacillus subtillis* (UD 1022)	5.9ab	5.6	4.5	4.1a	4.8abc	5.1ab	5.5	6.0
5. Fosetyl-Al	5.5ab	6.0	4.8	4.6a	4.0bc	4.8ab	4.5	5.7
6. Trinexapac-ethyl	5.3b	5.1	3.8	3.4b	3.8c	4.5ab	5.0	6.5
7. Combo of treatments 2-6	5.2b	5.6	4.4	4.0ab	4.0bc	4.0b	4.6	5.5
	Sept 27	**Oct 5**	**Oct 12**	**Oct 21**				
	Turf Quality (1 – 9 visual scale)							
1. Untreated Control	6.9ab	6.9ab	7.4	7.3				
2. Acibenzolar-S-Methyl	6.6abc	7.3ab	8.0	8.0				
3. Kelp Extract	7.4a	7.5a	7.8	8.0				
4. *Bacillus subtillis* (UD 1022)	7.3ab	7.5a	7.8	8.0				
5. Fosetyl-Al	6.8bc	6.8ab	7.1	7.1				
6. Trinexapac-ethyl	6.5abc	6.4b	6.6	7.0				
7. Combo of treatments 2-6	6.0c	6.3b	6.8	6.8				

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

Table 3. 2021 Field Trial at Wilmington Country Club, DE – Shoot Dry Weight

Treatments	**Aug 30**	**Sept 14**	**Sept 28**	**Oct 12**	**Oct 25**
	Shoot Dry Weight (g)				
1. Untreated Control	3.7[x]	4.0	4.1	4.3abc	5.2bcd
2. Acibenzolar-S-Methyl	4.4	3.6	4.0	4.3abc	6.3ab
3. Kelp Extract	4.5	3.5	4.3	4.5ab	7.2a
4. *Bacillus subtillis* (UD 1022)	4.6	3.8	4.4	5.1a	6.1abc
5. Fosetyl-Al	4.3	4.0	3.7	3.8abc	6.0abc
6. Trinexapac-ethyl	3.7	4.4	3.7	2.3c	4.0d
7. Combo of treatments 2-6	3.4	3.8	3.3	3.0bc	4.4cd

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

Dry weights were recorded after drying at 60°C for 24 hours.

Table 4. 2021 Field Trial at Wilmington Country Club, DE – Chlorophyll content

Treatments	Aug 30	Sept 14	Sept 28	Oct 12	Oct 25
	Chlorophyll content (mg/ml)				
1. Untreated Control	16.6b[x]	16.1	20.26bc	19.9	17.8de
2. Acibenzolar-S-Methyl	15.5b	15.6	23.2abc	18.6	24.4ab
3. Kelp Extract	18.5a	15.8	19.3c	21.6	21.5bc
4. *Bacillus subtillis* (UD 1022)	16.4b	18.4	20.3bc	20.3	22.4abc
5. Fosetyl-Al	15.9b	18.5	19.3c	21.8	16.1e
6. Trinexapac-ethyl	16.1b	14.6	26.7a	24.4	20.4cd
7. Combo of treatments 2-6	15.3b	13.7	23.6ab	23.1	25.1a

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

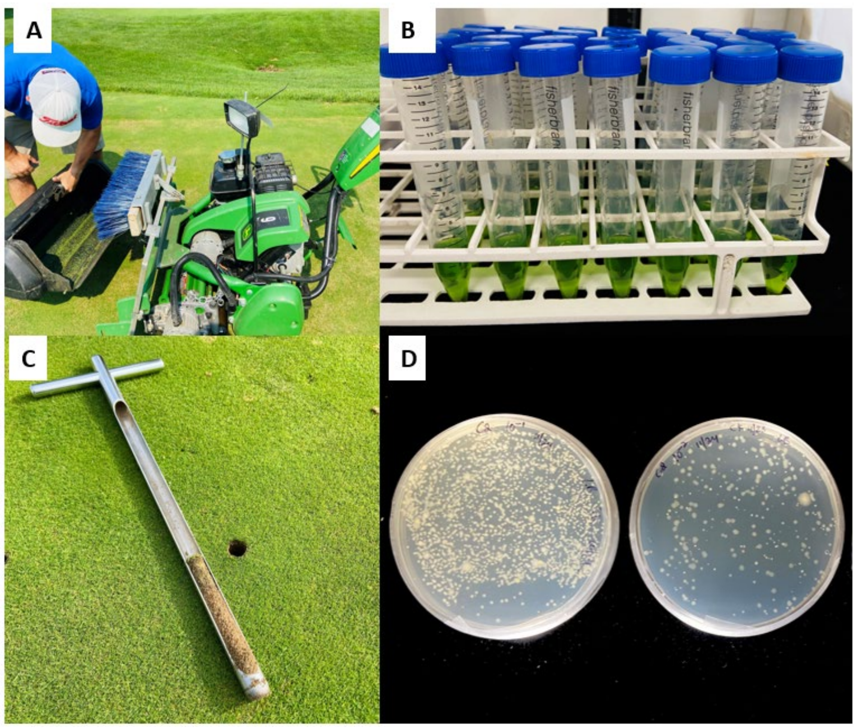


Fig. 2. A) Clippings collection, B) chlorophyll extraction, C) root sampling up to 15 cm depth, and D) quantification of UD1022 colonization on roots by plating on agar for determining colony forming units

Table 5. 2021 Field Trial at Penn State Berks Campus (Reading, PA) – Turf Quality

		5/15/2021 TurfQuality		6/15/2021 TurfQuality		7/15/2021 TurfQuality		8/15/2021 TurfQuality		9/15/2021 TurfQuality		9/15/2021 AUTQPC	
No.	Treatment												
1	Untreated Control	7.0	a	7.2	a	7.0	a	6.0	c	4.8	e	801.5	e
2	Acibenzolar-S-Methyl + polysorbate 20	7.2	a	7.7	a	8.0	a	6.5	bc	6.0	cd	883.4	abc
3	Fosetyl-Al	7.2	a	7.8	a	7.5	a	6.8	ab	6.8	ab	896.5	ab
4	Kelp Extract	7.3	a	7.3	a	7.5	a	6.0	c	5.5	d	837.3	de
5	*Bacillus subtillis* (UD 1022)	7.0	a	7.7	a	7.5	a	6.3	bc	5.8	cd	857.8	bcd
6	Trinexapac-ethyl	7.0	a	7.7	a	7.5	a	6.3	bc	6.3	bc	865.6	bcd
7	Acibenzolar-S-Methyl	7.0	a	7.5	a	7.8	a	7.5	a	7.0	a	917.2	a
	+ polysorbate 20												
	+ Fosetyl-Al												
	+ Kelp Extract												
	+ Bacillus subtillis (UD 1022)												
	+ Trinexapac-ethyl												
8	Chlorothalonil	7.2	a	7.7	a	7.2	a	6.2	bc	5.8	cd	845.1	cd
LSD P=.05		0.37		0.40		0.57		0.68		0.64		42.8813	
Standard Deviation		0.22		0.23		0.33		0.40		0.37		25.1772	
CV		3.04		3.08		4.43		6.22		6.13		2.91	
Replicate Prob(F)		0.5352		0.0414		0.2942		0.3855		0.8507		0.9792	
Treatment Prob(F)		0.4098		0.0589		0.0857		0.0071		0.0001		0.002	

Note – all treatments last applied on July 26, 2021.

Table 6. 2021 Field Trial at Penn State Berks Campus (Reading, PA) – NDVI

No.	Treatment	5/15/2021 NDVI		6/15/2021 NDVI		7/15/2021 NDVI		8/15/2021 NDVI	
1	Untreated Control	0.727	a	0.737	a	0.763	a	0.757	a
2	Acibenzolar-S-Methyl + polysorbate 20	0.713	a	0.743	a	0.763	a	0.753	a
3	Fosetyl-Al	0.727	a	0.770	a	0.753	a	0.763	a
4	Kelp Extract	0.743	a	0.753	a	0.770	a	0.783	a
5	*Bacillus subtillis* (UD 1022)	0.727	a	0.740	a	0.757	a	0.750	a
6	Trinexapac-ethyl	0.737	a	0.773	a	0.763	a	0.763	a
7	Acibenzolar-S-Methyl	0.730	a	0.747	a	0.770	a	0.747	a
	+ polysorbate 20								
	+ Fosetyl-Al								
	+ Kelp Extract								
	+ Bacillus subtillis (UD 1022)								
	+ Trinexapac-ethyl								
8	Chlorothalonil	0.727	a	0.777	a	0.780	a	0.757	a
LSD P=.05		0.034		0.037		0.027		0.052	
Standard Deviation		0.020		0.022		0.016		0.031	
CV		2.73		2.86		2.04		4.00	
Replicate Prob(F)		0.8147		0.0366		0.0013		0.2234	
Treatment Prob(F)		0.8955		0.1681		0.5944		0.7681	

Note – all treatments last applied on July 26, 2021.

Table 7. 2021 Field Trial at Penn State Berks Campus (Reading, PA) – VWC

No.	Treatment	5/15/2021 % VWC		6/15/2021 % VWC		7/15/2021 % VWC		8/15/2021 % VWC	
1	Untreated Control	39.0	a	24.0	a	23.6	a	27.7	a
2	Acibenzolar-S-Methyl + Polysorbate 20	42.1	a	22.8	a	21.9	a	29.2	a
3	Fosetyl-Al	38.5	a	22.3	a	23.1	a	25.8	a
4	Kelp Extract	38.3	a	25.6	a	25.0	a	29.2	a
5	*Bacillus subtillis* (UD 1022)	39.9	a	22.9	a	24.1	a	26.5	a
6	Trinexapac-ethyl	43.0	a	22.6	a	26.6	a	30.3	a
7	Acibenzolar-S-Methyl	39.3	a	21.5	a	25.5	a	27.9	a
	+ Polysorbate 20								
	+ Fosetyl-Al								
	+ Kelp Extract								
	+ Bacillus subtillis (UD 1022)								
	+ Trinexapac-ethyl								
8	Chlorothalonil	37.0	a	22.9	a	26.6	a	25.5	a
LSD P=.05		7.22		4.89		8.25		7.81	
Standard Deviation		4.24		2.87		4.85		4.59	
CV		10.73		12.36		20.03		16.37	
Replicate Prob(F)		0.0001		0.0001		0.0001		0.0001	
Treatment Prob(F)		0.5288		0.4751		0.6357		0.9584	

Note – all treatments last applied on July 26, 2021.

Table 8. 2021 Field Trial at Penn State Berks Campus (Reading, PA) – Dollar Spot

No.	Treatment	7/30/2021 DS foci		8/15/2021 DS foci		8/30/2021 DS foci		9/15/2021 DS foci		9/15/2021 AUDPC	
1	Untreated Control	2.0	a	12.7	a	19.7	a	36.0	a	805.2	a
2	Acibenzolar-S-Methyl + Polysorbate 2(	1.0	a	5.0	bc	9.3	b-e	20.3	de	392.8	bcd
3	Fosetyl-Al	1.3	a	5.0	bc	6.0	cde	9.7	fg	258.5	def
4	Kelp Extract	0.0	a	6.3	bc	10.0	bcd	28.0	bc	477.2	bc
5	*Bacillus subtillis* (UD 1022)	0.7	a	2.3	c	10.3	bcd	31.3	ab	452.3	bc
6	Trinexapac-ethyl	1.3	a	5.7	bc	6.3	cde	15.3	ef	319.3	cde
7	Acibenzolar-S-Methyl	0.7	a	2.3	c	3.0	e	6.7	g	141.3	f
	+ Polysorbate 20										
	+ Fosetyl-Al										
	+ Kelp Extract										
	+ Bacillus subtillis (UD 1022)										
	+ Trinexapac-ethyl										
8	Chlorothalonil	0.0	a	2.3	c	4.3	de	11.7	fg	196.7	ef
LSD P=.05		1.64		4.70		6.57		6.70		172.70	
Standard Deviation		0.96		2.76		3.86		3.94		101.40	
CV		109.76		51.77		42.01		19.22		25.61	
Replicate Prob(F)		0.0273		0.0012		0.0119		0.1528		0.0008	
Treatment Prob(F)		0.3672		0.0058		0.002		0.0001		0.0001	

Note – all treatments last applied on July 26, 2021; fungicide last applied on June 1, 2021.

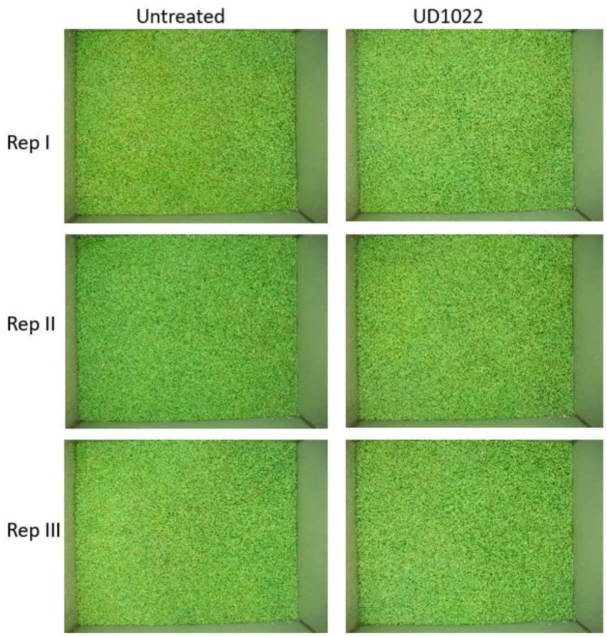


Fig. 3. Light box digital photos taken on August 10, 2021. Visually, plots treated with UD1022 had better turf density and sometimes better turf color versus untreated; this effect was subtle but noticeable.

Table 9. Relative visual color of a TifEagle hybrid bermudagrass putting green, Auburn, AL, 2021 as affected by various biological/fungicide/growth regulator treatments. All treatments applied biweekly.

Treatment	Date of Rating							
	July 6	July 20	July 23	July 27	July 30	Aug 6	Aug 14	Aug 21
	Relative Color (1 – 9 visual scale)							
1. Untreated Control	6.5 ab	7.0 ab	5.8 b	6.0 ab	6.3 ab	6.0 ab	6.3 ab	4.8 b
2. Acibenzolar-S-Methyl + Polysorbate 20	6.8 ab	7.5 a	6.0 b	5.8 ab	6.0 b	5.0 b	5.3 b	5.0 b
3. Fosetyl-Al	5.8 ab	6.0 ab	6.0 b	5.5 b	5.8 b	5.0 b	4.3 b	5.0 b
4. Kelp Extract	5.5 b	5.3 b	4.8 c	5.3 b	4.8 c	5.0 b	5.0 b	5.0 b
5. *Bacillus subtillis* (UD 1022)	6.3 ab	6.3 ab	6.3 ab	6.0 ab	6.3 ab	5.5 ab	6.3 ab	5.5 b
6. Trinexapac-ethyl	6.0 ab	6.3 ab	6.3 ab	5.3 b	5.5 b	4.8 b	4.8 b	4.8 b
7. Combo of treatments 2-6	7.3 a	7.0 ab	7.8 a	7.5 a	7.8 a	6.8 a	7.8 a	7.3 a
	Aug 25	Sept 3	Sept 13	Sept 25	Oct 6	Oct 15	Oct 21	
	Relative Color (1 – 9 visual scale)							
1. Untreated Control	6.0 b	5.8 a	6.0 ab	5.3 a	4.5 a	4.8 c	4.8 ab	
2. Acibenzolar-S-Methyl + Polysorbate 20	6.8 ab	5.5 a	6.8 ab	5.8 a	5.0 a	6.0 b	5.8 a	
3. Fosetyl-Al	5.5 b	5.0 a	6.0 ab	4.8 a	3.5 ab	4.8 b	4.5 ab	
4. Kelp Extract	6.0 b	5.0 a	5.5 b	5.5 a	4.3 ab	5.3 bc	5.0 ab	
5. *Bacillus subtillis* (UD 1022)	7.0 ab	5.5 a	6.0 ab	5.8 a	4.8 a	5.8 bc	5.0 ab	
6. Trinexapac-ethyl	6.5 ab	5.8 a	5.8 ab	5.3 a	2.8 b	5.3 bc	4.0 b	
7. Combo of treatments 2-6	8.0 a	6.5 a	7.5 a	6.5 a	4.5 a	7.5 a	6.0 a	

Within each rating date means followed by the same letter are not significantly from each other via mean's separation at an alpha of 0.05.

Table 10. Relative visual quality of a TifEagle hybrid bermudagrass putting green, Auburn, AL, 2021 as affected by various biological/fungicide/growth regulator treatments. All treatments applied biweekly.

Treatment	Date of Rating							
	July 6	July 20	July 23	July 27	July 30	Aug 6	Aug 14	Aug 21
	Relative Quality (1 – 9 visual scale)							
1. Untreated Control	5.8 a	6.3 ab	6.5 a	5.8 ab	6.3 ab	5.5 ab	5.8 ab	5.8 a
2. Acibenzolar-S-Methyl + Polysorbate 20	4.8 a	5.8 ab	5.8 a	5.8 ab	5.5 bc	5.5 ab	5.5 b	6.0 b
3. Fosetyl-Al	4.5 a	5.3 ab	5.3 a	5.8 ab	5.5 bc	5.0 b	5.8 ab	5.8 b
4. Kelp Extract	4.8 a	4.8 b	5.3 a	5.0 b	5.0 c	5.8 ab	5.5 b	5.5 b
5. *Bacillus subtillis* (UD 1022)	5.3 a	6.0 ab	6.0 a	5.5 ab	5.8 abc	5.8 ab	5.8 ab	5.8 b
6. Trinexapac-ethyl	5.3 a	6.0 ab	5.8 a	5.3 ab	6.0 abc	5.0 b	5.5 b	5.8 b
7. Combo of treatments 2-6	5.3 a	6.5 a	6.0 a	6.3 a	6.8 a	6.5 a	7.0 a	7.3 a
	Aug 25	Sept 3	Sept 13	Sept 25	Oct 6	Oct 15	Oct 21	
	Relative Quality (1 – 9 visual scale)							
1. Untreated Control	5.8 b	6.0 ab	6.5 ab	5.3 b	4.0 ab	6.0 ab	4.8 a	
2. Acibenzolar-S-Methyl + Polysorbate 20	6.5 ab	5.5 ab	6.0 ab	5.5 b	4.5 ab	5.5 b	5.3 a	
3. Fosetyl-Al	5.5 b	5.3 ab	5.8 ab	5.5 b	4.3 ab	5.3 b	4.8 a	
4. Kelp Extract	5.5 b	5.0 b	5.5 b	5.0 b	3.5 ab	4.8 b	4.8 a	
5. *Bacillus subtillis* (UD 1022)	6.0 b	5.5 ab	6.3 ab	5.5 b	4.5 ab	6.0 ab	5.3 a	
6. Trinexapac-ethyl	6.3 b	5.8 ab	6.0 ab	4.8 b	3.3 b	5.8 ab	4.3 a	
7. Combo of treatments 2-6	7.8 a	6.5 a	7.3 a	6.3 a	4.8 a	7.3 a	5.3 a	

Within each rating date means followed by the same letter are not significantly from each other via mean’s separation at an alpha of 0.05.

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:

- Confirmation of funding was received in late winter 2019. Therefore, initiation of research was delayed until October 2020.
- Initial fall treatments were applied in October and November 2020, applications will be continued in the winter and spring of 2021.
- Seed head suppression differences will not be visible until spring 2021. I short supplemental update can be sent to the USGA at this time.

Introduction:

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

Objectives:

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

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

Materials and Methods:

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

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

Response Variables:

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

Preliminary Findings:

The treatments were initiated in October 2020 and will be continued in 2021. Plots treated with Proxy in October and November had a lighter green color than untreated plots (Image 1). This is a well-documented turfgrass response, and often the reason Primo Maxx is applied with Proxy. Primo Maxx tends to darken turfgrass color, offsetting the light color produced by Proxy applications. Seed head emergence data collection will begin in spring 2021.

Year 1 findings:

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

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

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

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

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

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						
1st App	Subsequent Apps	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
untreated	Na												
GDD Model	Estimated					5	5	5	5				20
None	Mar, Apr, May						5	5	5				15
Oct	Mar, Apr, May	5					5	5	5				20
Nov	Mar, Apr, May		5				5	5	5				20
Dec	Mar, Apr, May			5			5	5	5				20
Jan	Mar, Apr, May				5		5	5	5				20
Feb	Mar, Apr, May					5	5	5	5				20
Oct	Mar, Apr, May	5					5	5	5	3.3	3.3	3.3	30
Nov	Mar, Apr, May		5				5	5	5	3.3	3.3	3.3	30
Dec	Mar, Apr, May			5			5	5	5	3.3	3.3	3.3	30
Jan	Mar, Apr, May				5		5	5	5	3.3	3.3	3.3	30
Feb	Mar, Apr, May					5	5	5	5	3.3	3.3	3.3	30
Note: GDD Model Timings are estimated													

Table 2: 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: Percent Seed Head Cover for the period 4/21 thru 5/5 (when most of the differences occurred).

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

Table 3: 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: 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: 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

Image 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: 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.

USGA ID#: 2019-17-687

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

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

- A set of 12 creeping bentgrass cultivars were evaluated for differences in seedling vigor and establishment in response to low temperatures and variable light intensities.
- Exposure of creeping bentgrass seedlings to freezing temperatures was shown to temporarily inhibit photosynthesis and growth of some cultivars to a greater extent than others, with cultivars such as for L-93, Memorial, Proclamation, and T-1 showing higher seedling sensitivity to freezing events.
- Creeping bentgrass cultivars different in their overall seedling vigor when grown under lower nutrient availability at 15°C
- Based on replicated field experiments in Minnesota and Norway, the use of 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 the use of a synthetic permeable cover decreased the time 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 for species such as annual bluegrass (*Poa annua*) and creeping bentgrass (*Agrostis stolonifera*). 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, and sub-optimal light intensity and spectral composition typical of early spring plantings can delay seed germination, diminish establishment vigor, and increase competition to weeds and summer stress.

The overall goal of our research is to evaluate factors affecting spring re-establishment of creeping bentgrass, which is the most widely used turfgrass on golf course greens and fairways in the northern U.S. The specific objectives are to evaluate the genetic variability among creeping bentgrass cultivars for post-germination seedling vigor, particularly interactions with low temperatures and variable light intensities typical of spring plantings at northern latitudes. These data will help adjust plant selection and management practices for golf course superintendents to utilize more effective strategies to enhance re-establishment success in spring months. A unique aspect to our research is the establishment of an international research collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO) and the Scandinavian Turfgrass and Environment Research Foundation (STERF). We have collaboratively defined our research objectives to more broadly explore potential barriers and identify solutions for successful spring re-establishment in northern climates.

In Year 3, we conducted controlled environment and field experiments to examine the post-germination cold tolerance and seedling vigor of 12 creeping bentgrass cultivars exposed to low temperatures. Creeping bentgrass seedlings were grown at 15°C (59°F) 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 genetic differences among cultivars for seedling cold tolerance based on presence of injury symptoms (e.g. leaf discoloration, 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. Following freezing at -5°C, the cultivars with the highest visual quality and photochemical efficiency included Barracuda, Penncross, and Penn A-4, moderate damage was observed for 007, Declaration, Luminary and Piranha, and the highest damage was observed for L-93, Memorial, Proclamation, and T-1 (selected cultivars from each grouping are shown in Figure 1). Although most plants recovered following freeze tests, 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, although the physiological basis for these differences has not been investigated.

To examine genetic variation in seedling vigor at low temperature, seeds of each cultivar were established using an agar medium in petri dishes. Two nutrient treatments were established, which included a non-amended agar (low nutrient) and nutrient

amended agar (one quarter strength Hoagland solution). The petri dishes with seeds were maintained vertically in racks and maintained in a growth chamber at 15°C and light intensity of 500 µmol m^{-2} s^{-1} PPFD. Seedling germination and growth were monitored for three weeks. Nutrient availability had the biggest impact on seedling growth at 15°C, with higher nutrient availability resulting in the highest shoot and root growth across all cultivars (Figure 2). 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 addition of nutrients in the agar medium was critical to achieve higher root and shoot growth rates at 15°C.

Previous controlled environment experiments at UMN determined that reductions in light intensity through use of shade cloths could improve the establishment of creeping bentgrass seedlings exposed to cold temperatures. In Year 3, field studies were replicated at UMN (St. Paul, MN) and two NIBIO sites (Landvik and Trom Norway) using shade cloths to achieve 0, 50, or 90% light intensity reductions and four bentgrass cultivars (Penncross, Memorial, Barracuda and Penn A-4) (Figure 3). 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. This suggested that reduction of 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.

Lastly, field studies were replicated at UMN (St. Paul, MN) and UMass (South Deerfield, MA) 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 in South Deerfield and 16 April in St. Paul). Following seeding, two covering treatments were applied that included 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 significantly decreased the time to achieve 50% turfgrass cover by approximately 7 to 12 days depending on location and regardless of cultivar (Figure 4). This was due to higher soil temperatures achieved under the permeable cover, which on average was approximately 4 to 5°C 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 permeable covering treatment (Figure 4).

Compared to the use of a covering treatment, the effect of creeping bentgrass genetics did not contribute to large differences in spring establishment rates. However, there were some small statistical differences among cultivars, mainly with Penn A-4, T-1, and Independence being slower to establish compared to other cultivars at the UMass field site, and Independence and T-1 at the UMN field site. In the final year of the project, field experiments will be repeated in spring 2022 at UMass and Norway to confirm whether use of chemical priming compounds may impact creeping bentgrass seedling establishment.

Figure 1. Visual quality and chlorophyll fluorescence responses of three-week old creeping bentgrass seedlings grown at 15°C (59°F) and exposed to a simulated overnight freezing event at -5°C (23°F). Images are shown for four creeping bentgrass cultivars exhibiting different responses to freezing, including Memorial (sensitive), 007 (moderately sensitive), Penncross (tolerant) and Barracuda (tolerant). Two representative pots per cultivar are included in the images. Right-hand panels are based on chlorophyll fluorescence imaging, with a higher percentage of photochemical efficiency represented by colors of red and orange and lower photochemical efficiency with colors of yellow, green to dark blue. Lower photochemical efficiency post-freezing indicates potential inhibition or damage to photosynthetic machinery.

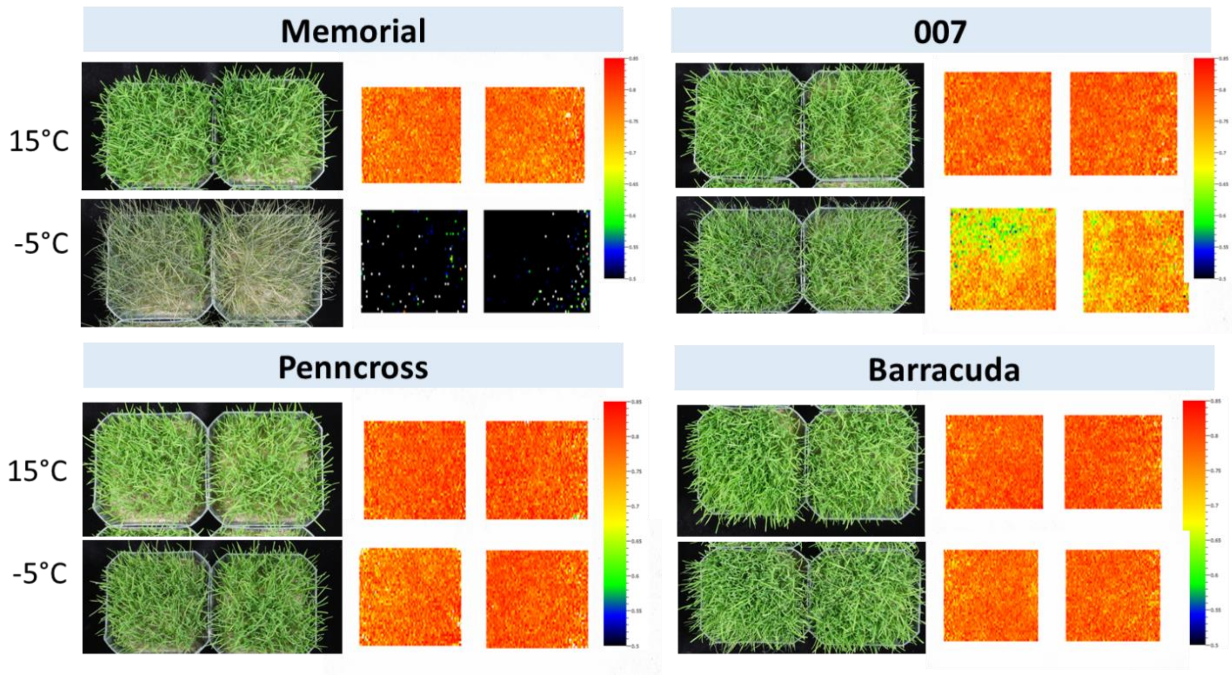


Figure 2. Controlled environment experiments using petri dish assays to assess the seedling vigor of different creeping bentgrass cultivars when grown at 15°C (59°F). Seed were sown into agar media amended with either low nutrient (top two rows) or high nutrient solution (bottom two rows).

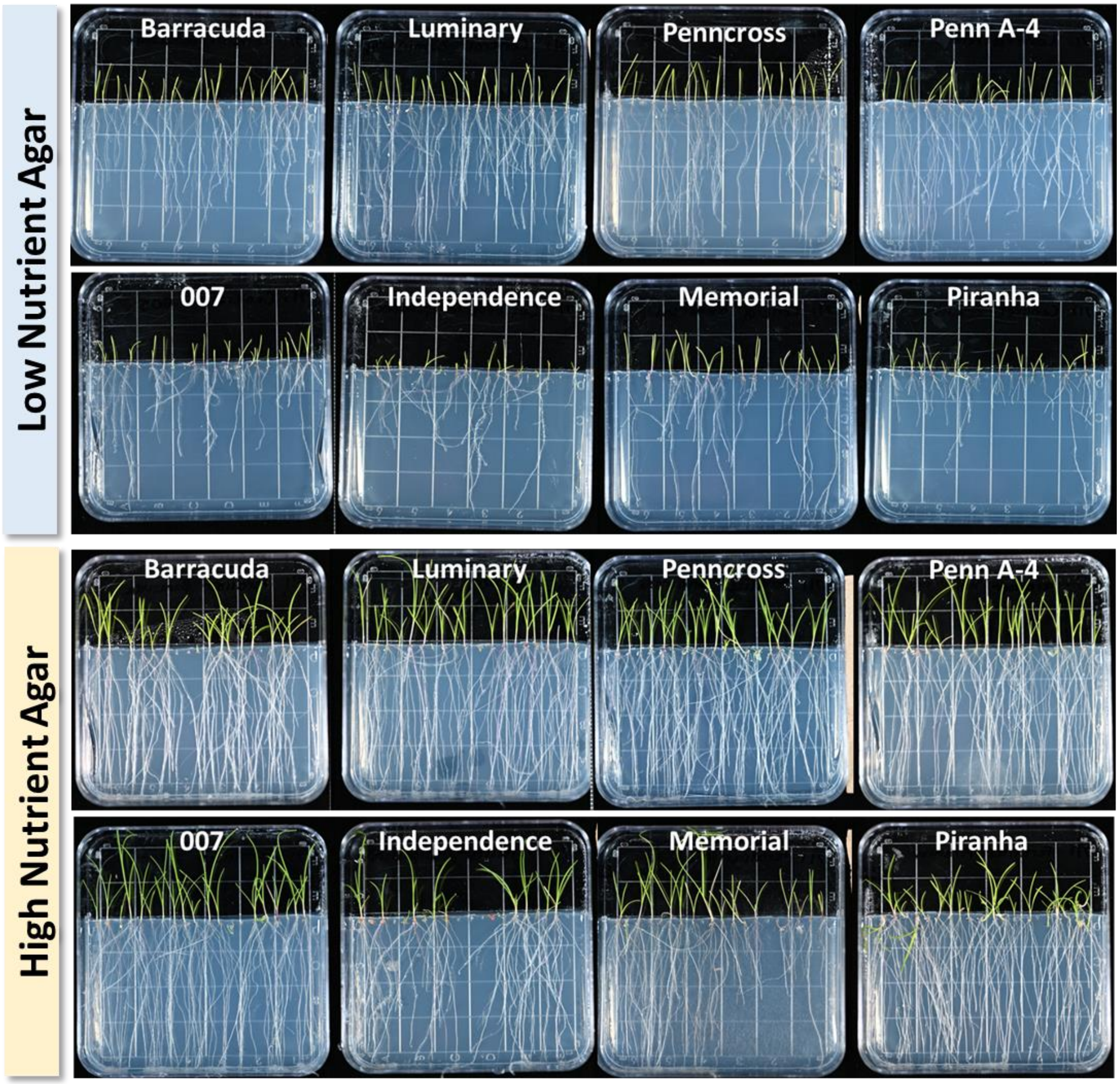


Figure 3. Shade trial field experiments conducted at St. Paul, MN in March 2021. Different light intensity treatments were established with shade cloths to achieve 0, 50%, and 90 % light intensity reduction. Bottom panel shows the effects of shade treatment on the seedling establishment of four creeping bentgrass cultivars, with the 90% shade treatment resulting in the highest turfgrass percent cover.

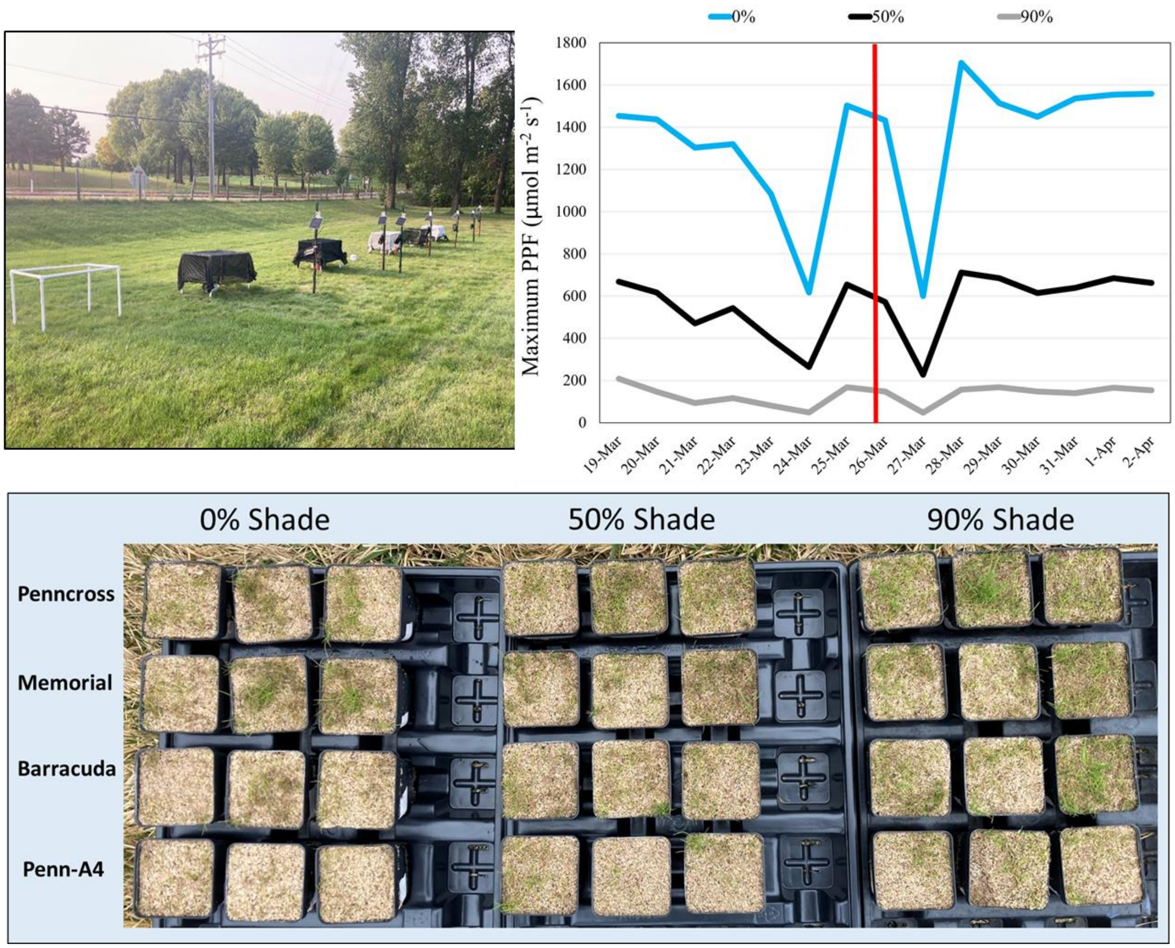
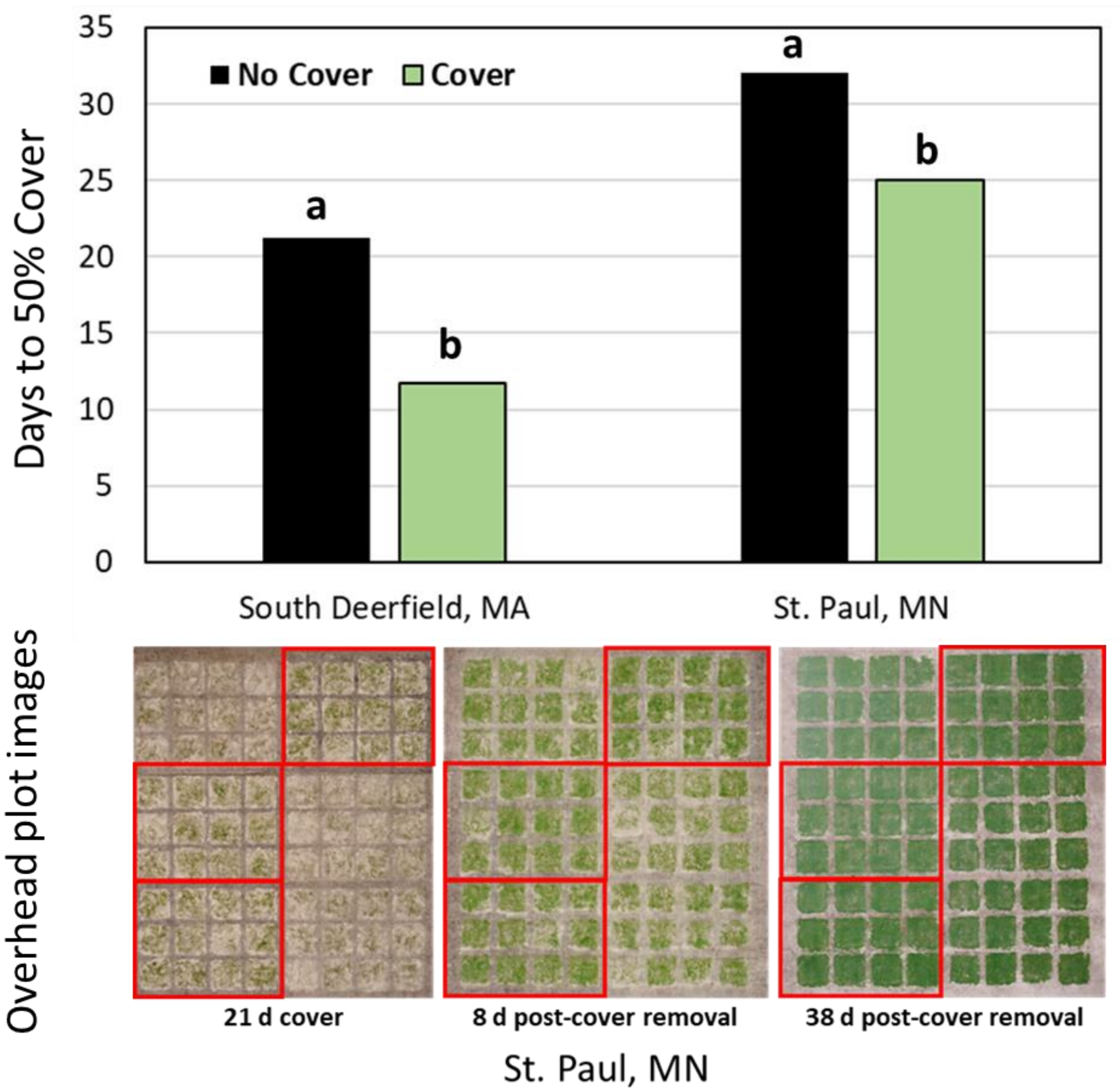


Figure 4. Field trials at UMass (South Deerfield, MA) and UMN (St. Paul, MN) testing the effects of creeping bentgrass genetics and use of a permeable cover on spring establishment. The top panel shows the effects of cover treatments on increasing time to 50% green cover, and bottom panel includes overhead plot images during the trial period at UMN.



USGA ID#: 2019-20-690

Title: Effects of Soil Water Content on Physiological Parameters Associated with Annual Bluegrass Cold Acclimation and Winterkill

Project Leader: Emily (Merewitz) Holm
Affiliation: Michigan State University

Objectives: To evaluate the effects of soil water content and growth regulator treatments on annual bluegrass physiological parameters associated with cold acclimation and regrowth following winter stress

Start Date: 2019
Project Duration: 2 years (Covid delay)
Total Funding: $5000.00

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

- Chemical treatments tested had no significant influence on normalized difference vegetative index of annual bluegrass in the fall
- Ice encasement stress resulted in less recovery compared to annual bluegrass plants that were exposed to low temperature only
- Ongoing data analysis is occurring to investigate interactions of chemical treatment and soil water content on annual bluegrass winter stress survival. Traits influenced during acclimation by these treatments are also currently being evaluated.

Rationale

Annual bluegrass is widespread on northern golf course putting greens and is known to be sensitive to winterkill, particularly due to ice encasement. Climate change may cause ice encasement to become more frequent due to potentially less snow cover, more frequent temperature fluctuations to cause snow melt and refreeze into ice layers, and due to severe storms. How soil moisture content influences annual bluegrass survival of winterkill stress and whether plant growth regulators can be used to alleviate potential overly wet or overly dry falls is not well investigated. Thus, the objective of this study is to determine if low, optimal, or high soil moisture content in the fall influences cold acclimation or winterkill of annual bluegrass and to determine if the application of plant growth regulators influencing plant ethylene content (to stimulate via ethephon or reduce via ethylene inhibitors such as aminoethoxyvinylglycine, AVG) has an impact on the winter survival of annual bluegrass. The results from this study should help to elucidate new winter preparatory management strategies with plant growth regulators and potential pros and cons of wet, dry, or optimal levels of soil water content in the fall.

Methodology

Field Conditions. This study was conducted during the Fall of 2019 to Spring 2020 and again from Fall of 2021 to Spring 2022 on two different annual bluegrass fields at the Michigan State

University Hancock Turfgrass Research Center in East Lansing, Michigan. In 2019, a field (57 x 33 ft) was divided into 72 individual plots each measuring 4' X 5' with an 18" buffer strip on each side to limit the amount of water that could get into the shelter due to blowing rain. In mid-September the plots were covered with a static rainout shelter structure that was covered with 10 mil polyethylene film with the lower three feet on the long edges being left uncovered to allow for air flow. In 2021, three fields each measuring 60' X 60' and were then divided into 20 or 24 plots each. One block was divided into 20 plots each at 10.5' X 6' with 1' buffer strips on each side. Another block was divided into 20 plots each at 12' X 6' with 1' buffer strips on each side. A third block was divided into 24 plots each at 12' X 6' with 1' buffer strips on each side. The blocks were separated by 12' of buffer strips on each side to allow for different soil moisture levels. All the plots were maintained at putting green height of 1/8 inch and were top dressed with sand weekly. All plots received equal and standard rates of fertilization and pesticides (on a preventative and curative basis).

Watering Treatments. During late summer and fall, automated irrigation was withheld, and any irrigation was done by hand to regulate the amount of water applied to the individual plot to maintain soil moisture at either 8%, 12 % or 20% soil moisture content. The plots were blocked by soil moisture and were irrigated according to the moisture block assigned and the moisture contents of the plots prior to irrigation. Soil moisture was measured with a time domain reflectometry instrument using 1.5 in probes (FieldScout TDR 100 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, Il.). When needed water was applied to increase the soil moisture to get it to the desired moisture level. Precipitation was restricted from the low irrigation plot by the rainout shelter in 2019 and by placement of tarps in 2021.

*Chemical Treatments. C*hemical treatments began on 7 Oct in 2019 and 6 Oct in 2021 and were applied weekly for six weeks. Foliar chemical treatments were as follows (a) ethephon (Proxy) at a rate of 7.96 L/ha, (b) Civitas at a rate of 40.6 L/ha, (c) ReTain (AVG) at a rate of 335 g/ha and (d) an untreated control. The plots were blocked by target soil moisture at rates of 8% (low), 12% (med), or 20% (high) soil moisture content. Within each of these blocks, five replicate plots of the four treatments were completely randomized.

Measurements. From September to Nov in both years, weekly measurements of normalized difference vegetation index (NDVI) were made with a multispectral radiometer (CropScan, Inc, Rochester, MN). Percent recovery following controlled winter stress treatment was determined by moving half of a core sample to the greenhouse for regrowth. The percent recovery was calculated by manual counting of all regrowing tillers over the total number of tillers present in a core sample.

Controlled Winter Stress Treatments - On November 22nd, 5 core samples from each plot were removed and placed into 4" pots and filled with soil. All the samples were moved inside to the low temperature growth chamber set at -3 degree Celsius with a light level of 200μmol $m^{-2}s^{-1}$ and a day length period of 10 hours to simulate a typical Michigan winter day on 18 Dec 2019. The samples were allowed to acclimate at this temperature for approximately two and a half weeks and ice encasement began on January 8th, 2020. At the start of the ice encasement process, the temperature inside the chamber was lowered to -5 C. To develop the ice, the samples designated for ice encasement were misted with deionized water until the samples were covered with ice at a depth of approximately 1.27 cm.

Results and Discussion

Soil water content was maintained at or near the target values in 2019 and 2021 (Figure 1). The rainout shelter was damaged in a severe windstorm in Dec of 2019. Therefore, the experiment was moved to a different field. Without the rainout shelter, soil moisture content was more variable and higher in 2021. NDVI did not show significant differences across date or chemical treatment (Figure 2). Other values that were obtained in 2021 such as leaf area index and chlorophyll content are currently being analyzed. For 2021, plant leaf, crown, and root samples were acquired and preserved at -80 ºC to evaluate physiological parameters during cold acclimation and plant growth regulator treatment. These samples will be tested in the laboratory during the winter of 2022. Recovery following controlled low temperature or low temperature with ice encasement of annual bluegrass plots averaged about 30% for all treatments (Figure 3 and 4). Recovery following ice encasement was observed to be less than for those plants that experienced only low temperature. Statistical analysis for these results is ongoing and we are still acquiring data for plant cores that were just taken in Dec 2021. These plants are currently in the low temperature growth chamber receiving winter treatment. A growth chamber study is currently being conducted to further investigate the interaction between soil water content and winter stress tolerance of annual bluegrass. This growth chamber study allows for better control of soil moisture content and other external factors but does not have plant growth regulator treatments as a factor.

Figure Captions

Figure 1 – Soil water content (%) of experimental annual bluegrass plots receiving high, medium, or low water inputs during the fall acclimation period for years 2019 and 2021.

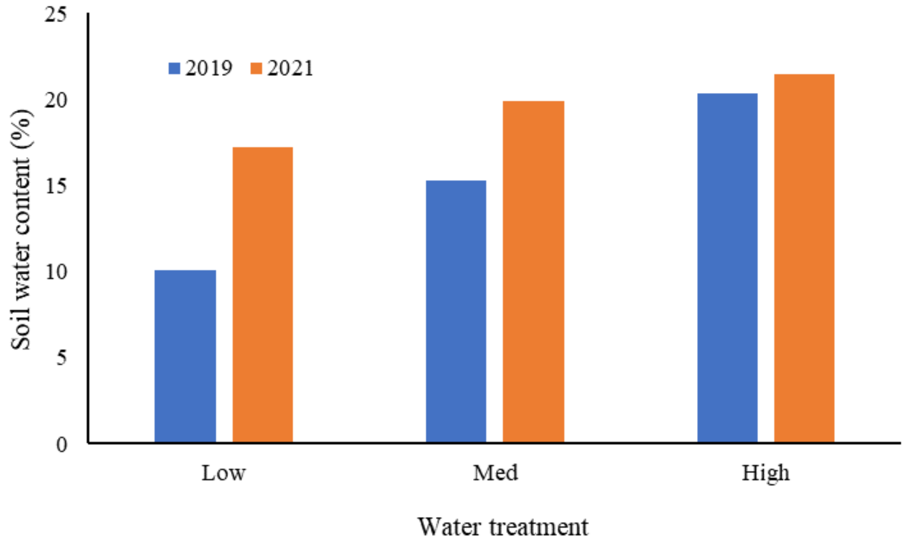
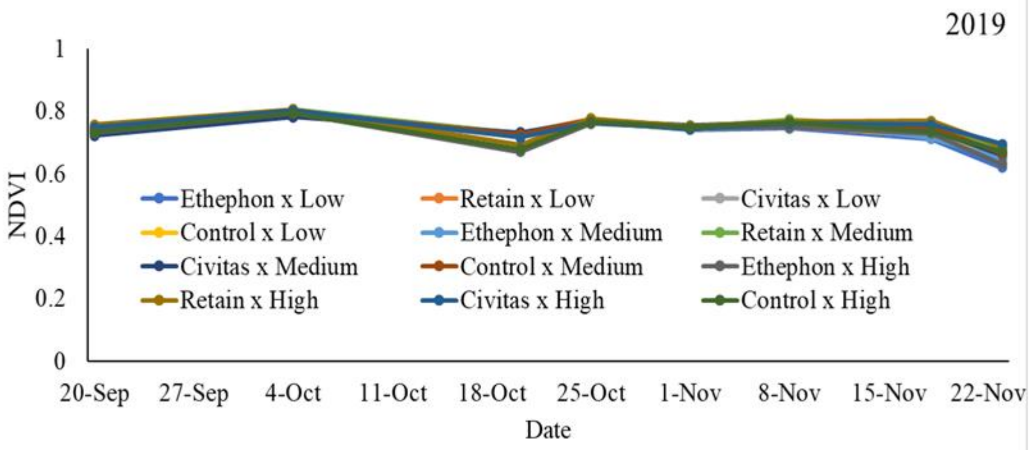


Figure 2 – Normalized vegetative difference index (NDVI) of annual bluegrass plots exposed to varying levels of soil water content and plant growth regulator treatment in 2019 and 2021.



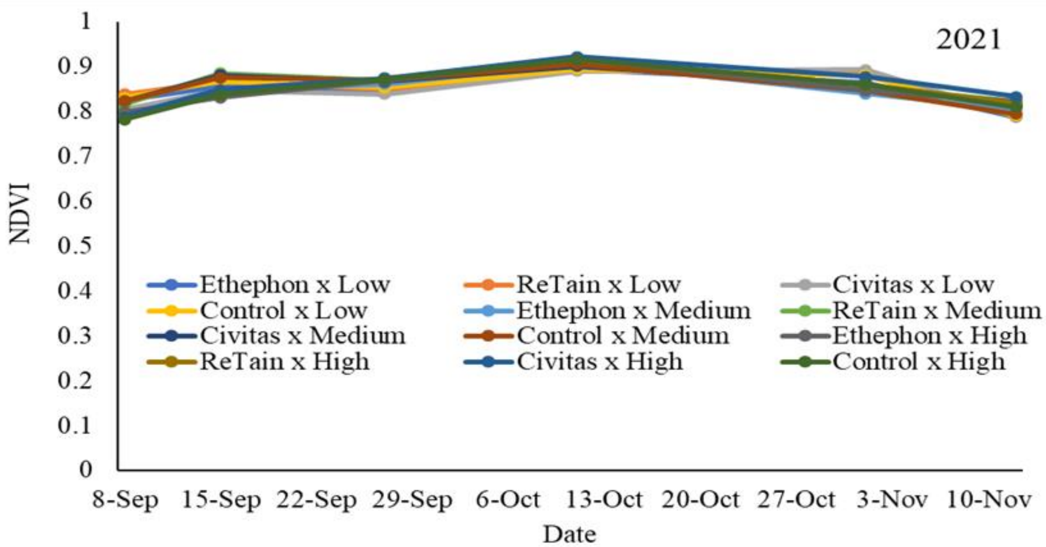


Figure 3 – Recovery of annual bluegrass plants averaged across all chemical treatments following winter stress treatments in a low temperature growth chamber. Low = 8%, Med = 12%, High =20% soil water content.

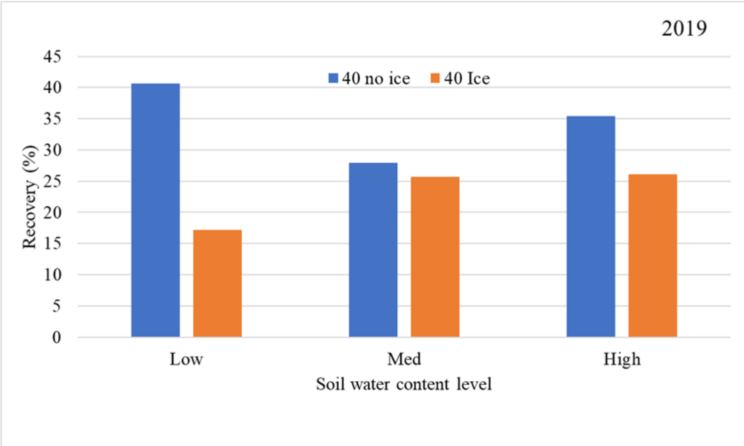
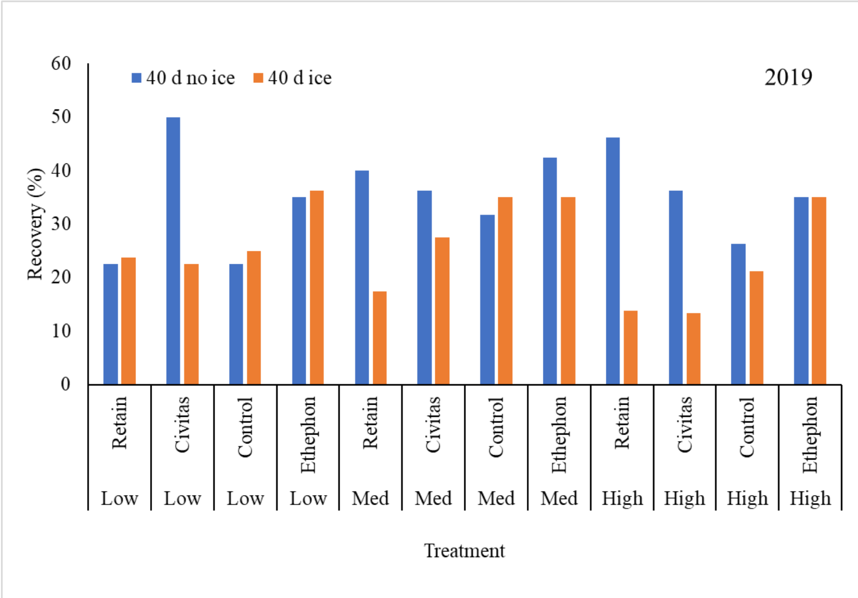


Figure 4 – Recovery of annual bluegrass plants exposed to various plant growth regulators and soil water content in the fall acclimation period following winter stress treatments in a low temperature growth chamber. Low = 8%, Med = 12%, High =20% soil water content. Plants were moved from winter stress treatment to the greenhouse for recovery after 40 d of ice or no ice.



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USGA ID#: 2021-14-738

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

Project Leaders: Eric Watkins and Bryan Runck

Affiliation: University of Minnesota

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

Start Date: 2021

Duration: 2 years

Total Funding: $95,630

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

Methods

Ground sensors will provide detailed measurements of what is happening just above and below the soil surface on golf greens. Building on pilot work from the 2019-2020 and 2020-2021 winters, we placed in-situ sensing nodes on 35 golf greens in fall 2021. Locations for sensor installations were selected based on envirotyping mapping. This approach looks at environmental covariates from public geospatial datasets to create groups or "clusters" of golf course greens. From these clusters, the most representative courses were selected for node placement. This way, we could capture the widest range of environments where turf winter death might occur.

Sensor Nodes

In addition to envirotypes, we focused nodes (see Figures 1 and 2) on golf greens where winter damage has at some point been a problem. The sensing nodes measure important environmental parameters including soil moisture (METER TEROS 10) and temperature (DS18B20) at 3 depths (1.25 cm; 7.5 cm, 15 cm), air temperature, barometric pressure, relative humidity (Bosch BME280), O_2 (Apogee SO-110), and CO_2 (Sensirion SCD30) gas levels just below the soil surface; and photosynthetically active radiation (Apogee SQ-110). Calibration for all sensors was established in the Runck lab. Sensors are connected to a single node and are powered by solar power, with battery backup. To ensure data collection system robustness, data is logged both locally to nonvolatile memory on the device and stored in real-time in the cloud. Each node consists of a custom power subsystem and board with a Particle Boron microcontroller that live telemeters data via 2G/3G or LTE cellular depending on the location. After traveling through the cellular network, data is routed through the Particle.io platform into a Postgres database associated with the UMN GEMS Sensing web user interface. The database is indexed on a tuple of unique node ID, latitude, longitude, and time making it functionally interoperable with other project data, and data is accessible through an easy-to-use web portal (Figure 3).

Additional Data Collection

For all courses on which sensor nodes are installed, we will obtain soil and management records for each green. During November 2021, golf greens identified for sensor nodes were assessed by the host superintendent for turf health and species composition; the same assessment will be done each spring so that before and after winter comparisons can be made. In addition, each superintendent has agreed to collect snow depth information on each quadrant of the green, along with ice and water observations.

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

Next Steps

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

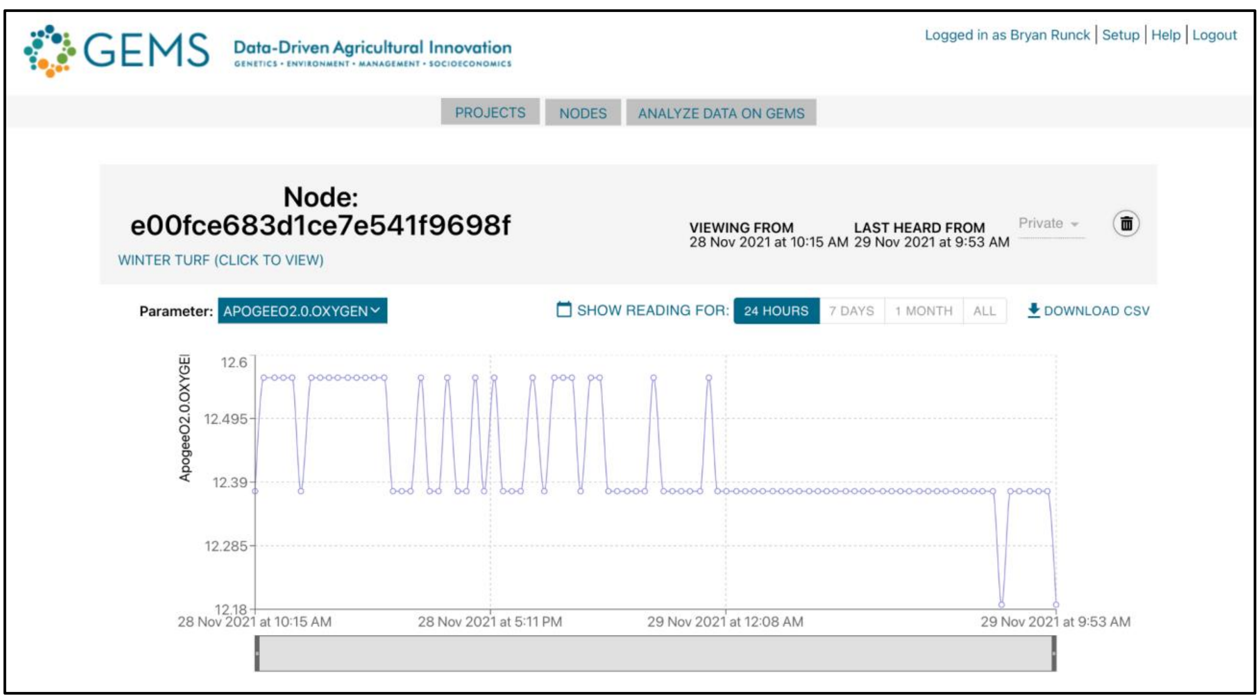
Summary Points

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

Figure 1. An up-close picture of the sensor "node" deployed near Biwabik, Minnesota that telemeters data from each sensor back to central servers at the University of Minnesota. Photo credit: Andrew Hollman

Figure 2. Sensor nodes installed and buried under an inch of ice at the University of Minnesota's Turfgrass Research, Education and Outreach Center. Photo credit: Andrew Hollman.

Figure 3. GEMS Sensing user interface and easy-to-access data portal.



USGA ID#: 2017-05-615

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

Project Leader: Karl Guillard assisted by graduate student Brendan Noons

Affiliation: University of Connecticut

Objectives of the Project:

Objective 1: Determine if Solvita Soil CO_2-Burst and Soil Labile Amino N tests are correlated to fairway creeping bentgrass quality and growth responses.

Objective 2: If test results are correlated to bentgrass fairway turf responses in Objective 1, then categorize the responsiveness to N fertilization as a function of Solvita soil test results in relation to a Standard fertilizer treatment.

Start Date: August 2017

Project Duration: 3 years; no-cost extension for another 2 years

Total Funding: $90,000

Summary Points

- Compost and organic fertilizer rates have produced a wide range of SLAN and CO2B test concentrations in fairway creeping bentgrass plots.
- SLAN and CO2B test concentrations respond linearly to compost and organic fertilizer rates.
- Fairway creeping bentgrass growth and quality responses are strongly correlated to SLAN and CO2B test concentrations.
- Trend responses across compost and organic fertilizer rates were generally similar between trafficked and non-trafficked plots for most variables.
- Binary logistic regression generated curves to estimate the probability that compost and organic fertilizer rate responses would equal or exceed that of the Standard fertilizer treatment.
- The CO2B test produced better binary logistic regression model fits than the SLAN test.
- The CO2B and SLAN tests show potential for estimating the mineralization potential of fairway creeping bentgrass soils.
- The 2021 results suggest that fairway creeping bentgrass soils can be categorized with Solvita tests as to their probability of equaling or exceeding the response of a standard N treatment.
- The Solvita SLAN and CO2B tests have potential to guide N fertilization of creeping bentgrass fairways.

Summary

Need for the Study:
The ability to predict the N mineralization potential of any turfgrass site and its expected response to N fertilization would be a valuable tool in nutrient management. Turfgrass soils often accumulate organic matter over time, and this increases their mineralization potential. However, assessing this mineralization potential is not routine due to the lack of mineralization tests offered with many labs, cost of the tests, and the long-term requirements (a week to months) of these tests for reliable results. Solvita and Woods End Laboratories offer two tests that have been recently developed to rapidly measure the biologically-active C and N fractions in soil organic matter: the Soil CO_2-Burst (CO2B) and Soil Labile Amino Nitrogen (SLAN) test kits. These tests measure labile C and N fractions that are correlated to soil microbial activity, and therefore, the Solvita soil tests should be able to estimate the mineralization potential of turfgrass soils. An estimate of the mineralization potential should help guide N fertilization.

Methods:
The study site is located in Storrs, CT, and was initiated in August, 2017. The experiment was set out as a split-block design with traffic (yes or no) as the horizontal factor and compost (10 rates, in 0.25-lb increments from 0 to 2.25 lbs available N per 1000ft^2) as the vertical factor with three replicates. Compost was incorporated into the 0 to 4-inch soil profile by rototilling prior to seeding. After compost incorporation, creeping bentgrass ('13M') was seeded into the study site and managed as a fairway. During the bentgrass grow-in period during the late fall of 2017, an organic fertilizer (Suståne all natural 5-2-4) was applied to the plots at the same rates as the initial incorporated compost rates. In addition to the organic treatments, a Standard fertilizer regime treatment with 0.2 to 0.25 lbs N 1000ft^{-2} was applied approximately every 21 days as liquid urea. The fall of 2017 was used as the establishment period. Full implementation of the treatments and data collection commenced in 2018 and continued in 2019, 2020, and 2021 with fall applications of Suståne organic fertilizer.

In 2021, traffic was applied with a cart-traffic simulator three times a week during the growing season. Bentgrass response measurements (NDVI, percentage green cover, Dark Green Color Index [DGCI], visual quality, visual color, visual density,) and soil samples were collected monthly from May through November from each plot. Clippings yield was collected monthly from June through November for each plot. Soil samples were analyzed using the Solvita CO2B and SLAN tests. Data were statistically analyzed using analysis of variance to determine treatment effects (fertilizer rates, traffic, and the fertilizer rate × traffic interaction) on the mean bentgrass quality and growth responses and soil CO2B and SLAN concentrations. Mean fairway bentgrass responses were correlated to mean SLAN and CO2B concentrations within and across traffic treatments. Binary logistic regression was applied to determine the probability of bentgrass fairway responses from the compost-organic fertilizer plots that would be equal to or exceed the responses from the Standard N fertilization plots across the Solvita soil test values for each of the traffic treatments.

2021 Results:

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

Compared with the Standard treatment, concentrations of SLAN were significantly lower at the non-fertilized 0 lbs N per 1,000ft^2 rate, but significantly greater once the N rate reached ≥ 1.5 lbs N per 1,000ft^2 from compost-organic fertilizer (Table 1). Concentrations of CO2B were not significantly different than the Standard at the non-fertilized 0 to 0.5 lbs N per 1,000ft^2 compost-organic fertilizer rates, but significantly greater than the Standard treatment once the compost-organic fertilizer N rates reached ≥ 0.75lbs N per 1,000ft^2. NDVI was significantly less than the Standard treatment at the 0 and 0.5 lbs N per 1,000ft^2 compost-organic fertilizer rates, but were significantly greater than the Standard treatment once the compost-organic fertilizer N rates reached ≥ 1.5 lbs N per 1,000ft^2. Response of DGCI from the Standard treatment was greater than the 0 and 0.5 lbs N per 1,000ft^2 compost-organic fertilizer treatments, but was significantly lower than the highest compost-organic fertilizer rate of 2.25 lbs N per 1,000ft^2. Visual quality, color, and density ratings were significantly lower than the Standard treatments from compost-organic fertilizer rates 0 to 0.5 lbs N per 1,000ft^2, but were significantly higher at the highest compost-organic fertilizer rates of 2.0 and 2.25 lbs N per 1,000ft^2. Percent green cover of the Standard treatment was significantly greater than the 0 to 0.5 lbs N per 1,000ft^2 compost-organic fertilizer treatments, but not different from compost-organic fertilizer rates ≥ 0.75 lbs N per 1,000ft^2. The 0, 0.25, and 1.0 lbs N per 1,000ft^2 compost-organic fertilizer rates were significantly lower than the Standard treatment for clippings yield, whereas the compost-organic fertilizer 2.25 lbs N per 1,000ft^2 rate produced significantly greater clipping yields than the Standard treatment.

Correlations between fairway bentgrass compost-organic fertilizer responses in relation to SLAN and CO2B concentrations were all highly significantly with high *r* values across the traffic treatments (SLAN $r = 0.727$ to 0.904; CO2B $r = 0.620$ to 0.914) (Table 2). There was no significant difference in *r* values between traffic and non-traffic treatments for SLAN concentrations and from all but one variable (visual density) for CO2B (Table 2). Scatter plots and correlations pooled across traffic treatments are shown in Fig. 2.

Since there were strong correlations between Solvita soil test concentrations and fairway creeping bentgrass responses, binary logistic regression was applied to determine the probability of compost-organic fertilizer plot responses that were equal to or greater than the response of the Standard fertilizer treatment with respect to the SLAN and CO2B concentrations. Probability curves are shown in Figs. 3 and 4 for Traffic and No-Traffic plots.

For SLAN concentrations, probability curves for all variables in both No-Traffic and Traffic treatments were modeled relatively well. When all variables were combined, there would be a

≥ 67% chance that fairway bentgrass responses would equal or exceed the responses of the Standard fertilizer treatment when SLAN concentrations were ≥ 260 and ≥ 252 mg kg^{-1} for non-trafficked and trafficked plots, respectively (Table 3).

For CO2B concentrations, probability curves for all variables in both No-Traffic and Traffic treatments were modeled relatively well. When all variables were combined, there would be a ≥ 67% chance that fairway bentgrass responses would equal or exceed the responses of the Standard fertilizer treatment when SLAN concentrations were ≥ 131 and ≥ 133 mg kg^{-1} for non-trafficked and trafficked plots, respectively (Table 3).

Future Expectations:
With each year of treatment imposition, we are observing better correlations and model fits of the data. We attribute this to more mineralization of the compost and organic fertilizer additions. The data are suggesting that reliable tables could be produced of SLAN and CO2B concentrations and associated probabilities of responses being equal to or exceeding the response of the Standard fertilizer treatment of 0.2 to 0.25 lbs N $1000ft^{-2}$ applied approximately every 21 days for our soils and climate conditions. This could assist the superintendent in guiding fertilization based on their risk tolerance. An example is shown in Tables 4 and 5, and Fig. 5.

Based on the selected response categories shown in Fig. 5, it is suggested that when SLAN-N and CO2B–C concentrations are associated with $P \leq 0.33$, there is a very low to low probability of obtaining a response equal to or greater than the Standard fertilizer treatment response. When SLAN-N and CO2B–C concentrations are between $P = 0.33$ and $P = 0.67$, there is a low to moderate probability of obtaining a response equal to or greater than the Standard fertilizer treatment response. When SLAN-N and CO2B–C concentrations are between $P = 0.67$ and $P = 0.90$, there is a moderate to high probability of obtaining a response equal to or greater than the Standard fertilizer treatment response. And when SLAN-N and CO2B–C concentrations are at $P \geq 0.90$, there is a high to very high probability of obtaining a response equal to or greater than the Standard fertilizer treatment response.

The goal of using the Solvita tests to guide N fertilization for turfgrasses would be to recommend a specific amount of N needed for optimum response for any specific SLAN-N or CO2B–C concentration. Following the concepts presented in Tables 4 and Fig. 5, it could be suggested that fairway creeping bentgrass soils with SLAN-N or CO2B–C concentrations that fall below the $P = 0.33$ cutoff receive the full currently-recommended N rate; fairway creeping bentgrass soils with SLAN-N or CO2B–C concentrations that fall between the $P = 0.33$ and the $P = 0.67$ cutoffs receive ⅔ to ½ of the currently-recommended N rate; those with SLAN-N or CO2B–C concentrations that fall between the $P = 0.67$ and the $P = 0.90$ cutoffs receive ½ to ⅓ of the currently-recommended N rate; and those with SLAN-N or CO2B–C concentrations that fall above the $P = 0.90$ cutoff receive little to no additional N fertilization. This would assume that optimum conditions for mineralization would be present across the growing season. Another approach to using the P values to guide N fertilization is for superintendents to apply $(1 - P) \times$ the full rate of N fertilization, where P is the probability of equaling or exceeding the Standard fertilizer treatment response based on the SLAN-N or CO2B–C concentration. An example of this is presented in Tables 4 & 5.

The results from 2021 suggest that the Solvita soil test results are well correlated to fairway creeping bentgrass responses. We have had three years of consistently strong data to show the potential of these new tests in predicting fairway mineralization and use in guiding N fertilization. If this holds true across different years, soils, and climates, golf course superintendents will have new tools to be able to easily and quickly assess the mineralization potential of any fairway on their course. These tests will be site-specific and will give the superintendent an objective guidance for N fertilization. Using a more site-specific, objective means to guide N fertilization will maintain optimum turf quality and function, while reducing fertilizer costs, reducing turf loss due to certain N-related diseases, reducing the risk of water pollution caused by N losses from excess fertilizer, and reducing the greenhouse gas emission footprint (especially with N_2O) of the golf course by not applying N when it has a low probability of response due to high mineralization potential, or not applying the full rate of N when mineralization potential is moderate. The value of using the Solvita soil tests also would be seen on fairway areas where mineralization potential is low, and where they could benefit from an optimal N fertilizer rate. An additional advantage of the Solvita soil tests is that they could be conducted on-site by the superintendent with a full test kit, if desired, without the need to send samples to a laboratory.

Table 1. Mean Solvita soil test concentrations and bentgrass quality and growth responses, with analysis of variance *P* values. 2021 results.

	SLAN	CO_2-Burst	NDVI	Visual Quality	Visual Color	Visual Density	DIA† Cover	DIA DGCI	Sum Clippings yield
Traffic	mg kg^{-1}	mg kg^{-1}		----------1-9; 9 best --------			% green		g m^{-2}
No	247.3	119.7	0.677	6.3	6.7	6.5	89.5	0.495	43.4
Yes	243.3	121.2	0.667	5.9	6.1	6.0	82.3	0.471	30.5
Treatment‡									
0	216.7*	99.9	0.638*	4.5*	4.7*	4.7*	73.0*	0.447*	25.0*
0.25	230.8	107.8	0.645*	4.6*	5.1*	4.8*	77.1*	0.454*	28.6*
0.50	232.9	111.4	0.657*	5.4*	5.6*	5.6*	82.8*	0.468*	30.6
0.75	237.9	119.9*	0.667	5.9	6.3	6.2	87.7	0.486	34.6
1.00	245.9	120.7*	0.673	6.1	6.4	6.1	84.2	0.479	29.6*
1.25	245.9	124.2*	0.673	6.4	6.6	6.5	87.8	0.489	39.4
1.50	253.6*	131.3*	0.684*	6.6	7.0	6.9	90.2	0.499	42.2
1.75	258.8*	129.6*	0.686*	6.9	7.0	6.9	91.1	0.499	41.0
2.00	265.1*	134.2*	0.693*	7.3*	7.4*	7.3*	90.6	0.501	44.1
2.25	272.2*	140.1*	0.703*	7.4*	7.5*	7.2*	92.6	0.508*	52.2*
Standard	238.6	106.2	0.670	6.3	6.7	6.5	88.2	0.486	38.9
					AOV p-values				
Traffic	0.2258	0.3939	0.0230	0.0017	0.0287	0.0078	0.0463	0.0266	0.0178
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
T × T	<.0001	0.0072	0.4647	0.3234	0.7027	0.4346	0.2400	0.7615	0.0449

* Significantly different from the Standard treatment ($P < 0.05$)

†DIA, Digital Image Analysis

‡Compost and organic fertilizer rates of available N (lbs per 1000ft^2); Standard treatment is liquid urea at 0.2 lbs N per 10000ft^2 every 21 days.

Table 2. Correlation coefficients (*r*) and *P* values for No-Traffic and Traffic plot responses in relation to Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO2-Burst (CO2B) concentrations, and *P* values for the difference between traffic treatment *r* values for each variable. 2021 results.

SLAN	No-Traffic		Traffic		
Variable	*r* value	*P* value for *r*=0	*r* value	*P* value for *r*=0	*P* value for difference between traffic treatments *r* values
NDVI	0.892	<.0001	0.894	<.0001	0.9744
DGCI	0.787	<.0001	0.838	<.0001	0.5595
Quality	0.833	<.0001	0.897	<.0001	0.3144
Color	0.791	<.0001	0.904	<.0001	0.1039
Density	0.794	<.0001	0.877	<.0001	0.2773
Cover	0.754	<.0001	0.809	<.0001	0.5849
Yield	0.755	<.0001	0.727	<.0001	0.8043

CO2B	No-Traffic		Traffic		
Variable	*r* value	*P* value for *r*=0	*r* value	*P* value for *r*=0	*P* value for difference between traffic treatments *r* values
NDVI	0.838	<.0001	0.876	<.0001	0.5788
DGCI	0.708	<.0001	0.859	<.0001	0.1148
Quality	0.730	<.0001	0.880	<.0001	0.0835
Color	0.704	<.0001	0.873	<.0001	0.0680
Density	0.687	<.0001	0.914	<.0001	0.0059
Cover	0.697	<.0001	0.811	<.0001	0.2979
Yield	0.620	0.0001	0.750	<.0001	0.3366

Table 3. Concentrations of Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO2-Burst (CO2B) concentrations of equaling or exceeding the response of the Standard fertilizer treatment at a selected probability of *P* = 0.67. 2021 results.

P = 0.67	SLAN-N, mg kg^{-1}	
Variable	No-Traffic	Traffic
NDVI	255	254
DGCI	267	258
Visual Quality	248	243
Visual Color	252	249
Visual Density	255	242
Percent Green Cover	267	266
Clipping Yields	279	252
Mean	260	252

P = 0.67	CO2B-C, mg L^{-1}	
Variable	No-Traffic	Traffic
NDVI	127	137
DGCI	134	154
Visual Quality	123	123
Visual Color	125	127
Visual Density	129	121
Percent Green Cover	146	153
Clipping Yields	137	130
Mean	131	133

Table 4. Recommended N rate based on SLAN concentrations and the probability of those concentrations equaling or exceeding the response of the Standard N treatment across both traffic treatments for all variables combined using logistic regression output for 2021.

Apply (1 – *P*) × Standard rate of N For Example: Standard N rate = 0.2 lbs N/1,000ft^2 approx. every 21 d		
SLAN-N, mg kg^{-1} soil	Probability	Suggested rate of N to apply
≤ 50	0.000	0.20
100	0.000	0.20
150	0.005	0.20
200	0.082	0.18
250	0.594	0.08
300	0.960	0.01
≥ 350	0.997	0.00

Table 5. Recommended N rate based on CO2B concentrations and the probability of those concentrations equaling or exceeding the response of the Standard N treatment across both traffic treatments for all variables combined using logistic regression output for 2021.

Apply (1 – *P*) × Standard rate of N For Example: Standard N rate = 0.2 lbs N/1,000ft^2 approx. every 21 d		
CO2B-C, mg L^{-1}	Probability	Suggested rate of N to apply
≤ 50	0.015	0.20
75	0.063	0.19
100	0.232	0.15
125	0.573	0.09
150	0.857	0.03
175	0.964	0.01
200	0.992	0.00
225	0.998	0.00
≥ 250	1.000	0.00

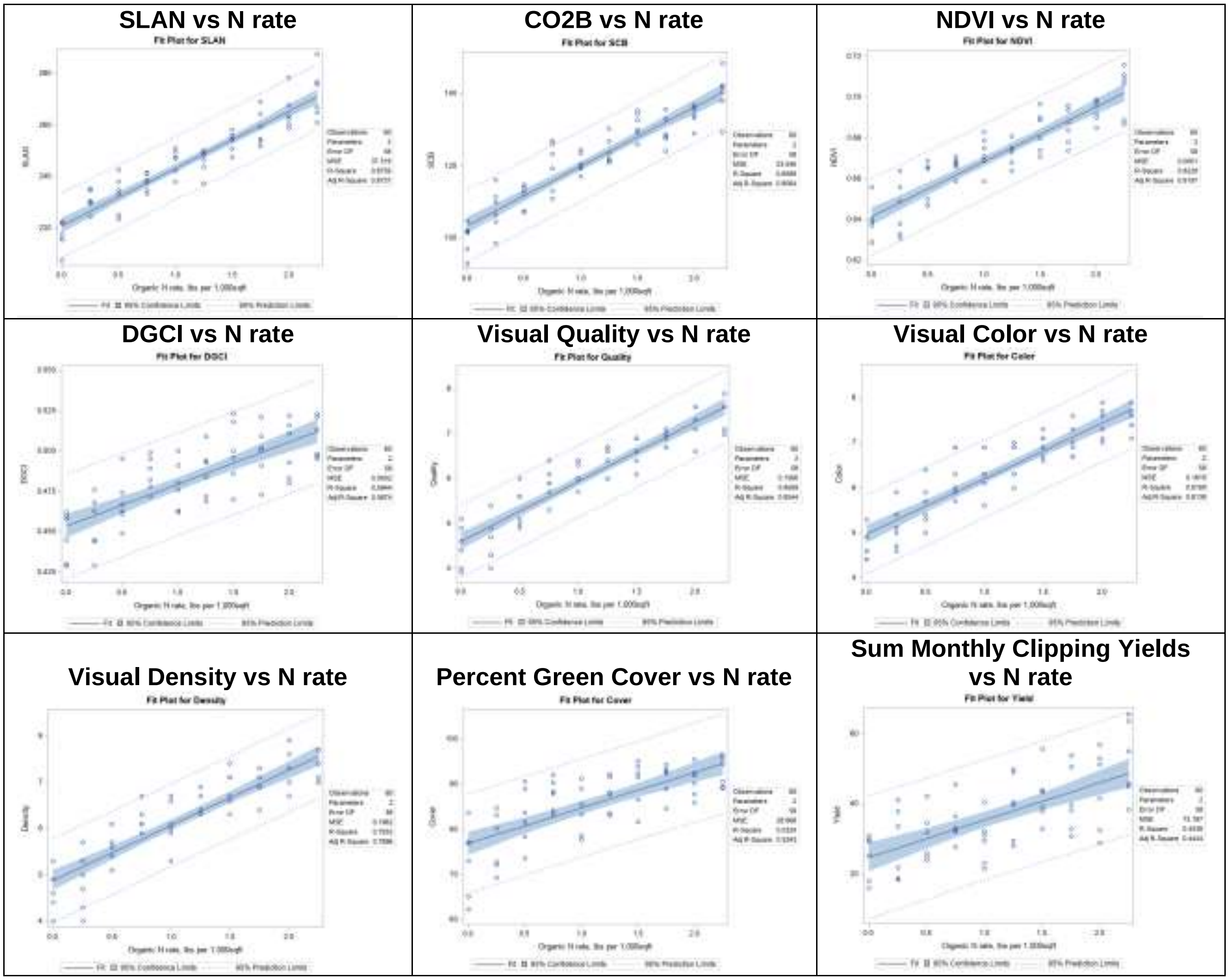
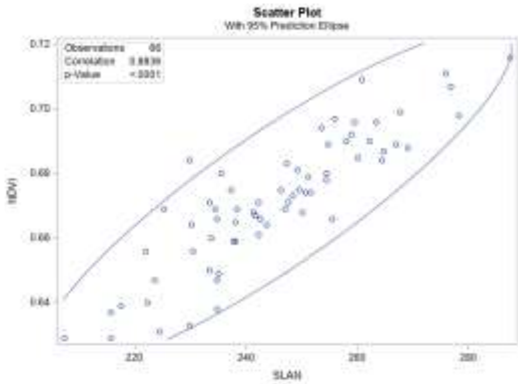
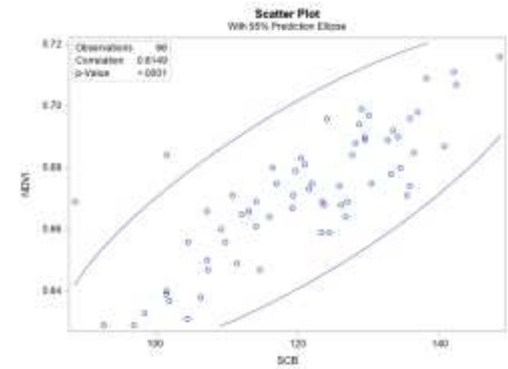
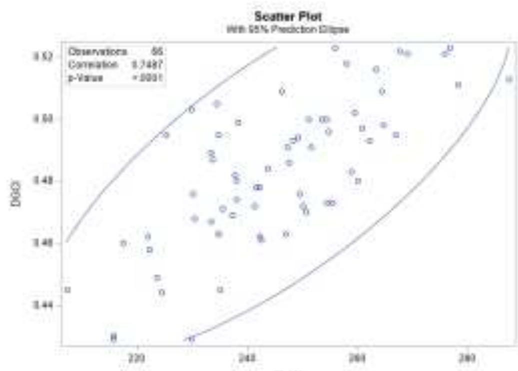
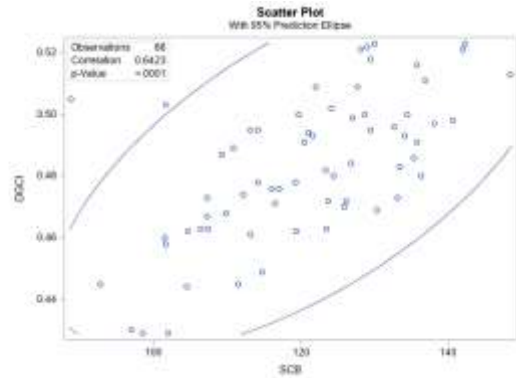
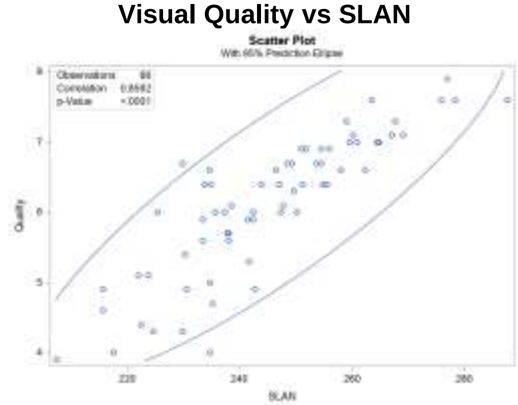
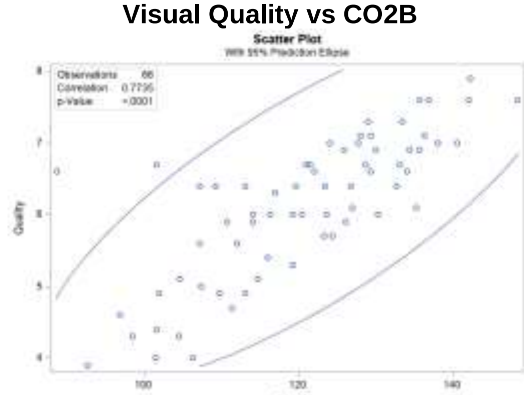
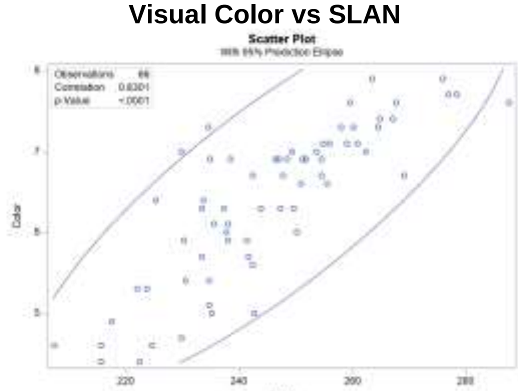
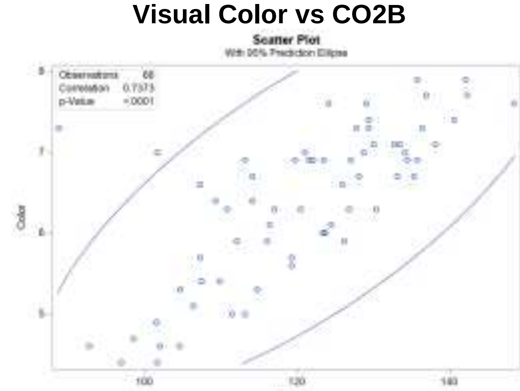


Figure 1. Fairway creeping bentgrass responses (Soil Labile Amino Nitrogen [SLAN], Soil CO_2-Burst [CO2B], Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and clippings yields) in relation to compost-organic fertilizer (initial compost followed by yearly Suståne applications) N rates for 2021. Since there was only one significant traffic × treatment interaction (for SLAN), responses are averaged across Traffic and No-Traffic plots.

NDVI vs SLAN	**NDVI vs CO2B**
	
DGCI vs SLAN	**DGCI vs CO2B**
	
Visual Quality vs SLAN	**Visual Quality vs CO2B**
	
Visual Color vs SLAN	**Visual Color vs CO2B**
	
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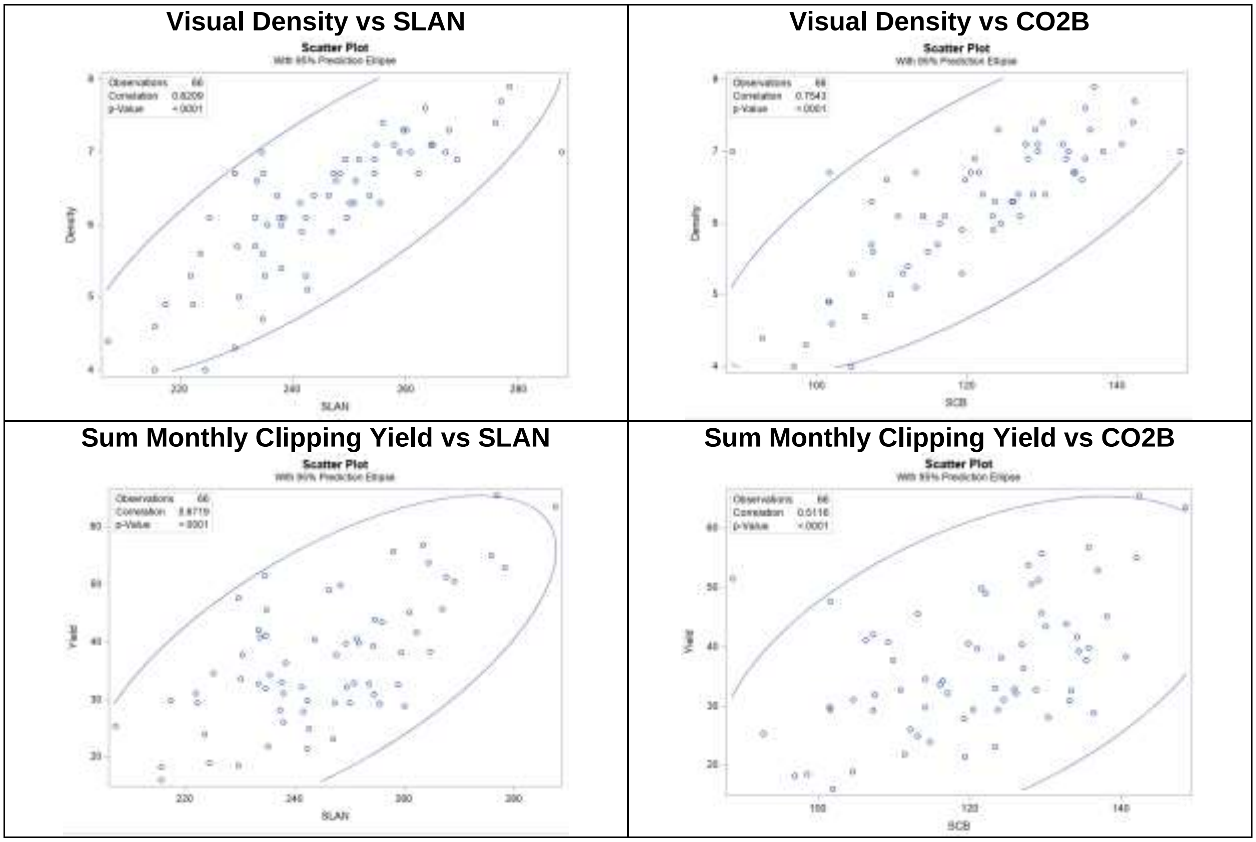


Figure 2. Correlations between fairway creeping bentgrass responses (Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and sum of monthly clippings yields) from the compost-organic fertilizer plots in relation to their respective Soil Labile Amino Nitrogen (SLAN) and Soil CO_2-Burst (CO2B) concentrations averaged across sampling dates and traffic treatments plots for 2021. The ellipses represent the 95% prediction space.

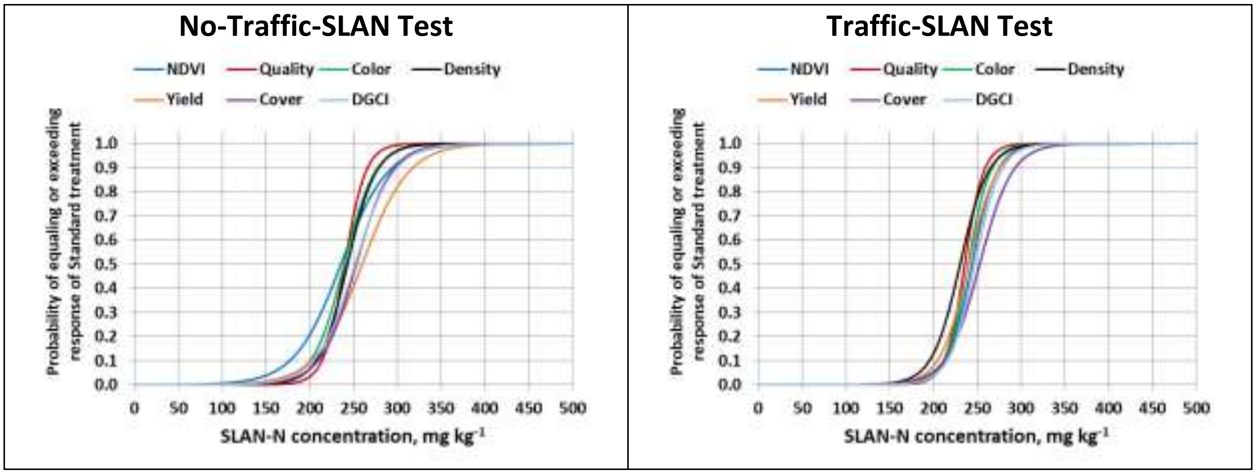


Figure 3. Compost-organic fertilizer probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft^2 every 21 days) in relation to the Solvita SLAN-N concentrations for the No-Traffic and Traffic plots. 2021 results pooled across all sampling dates.

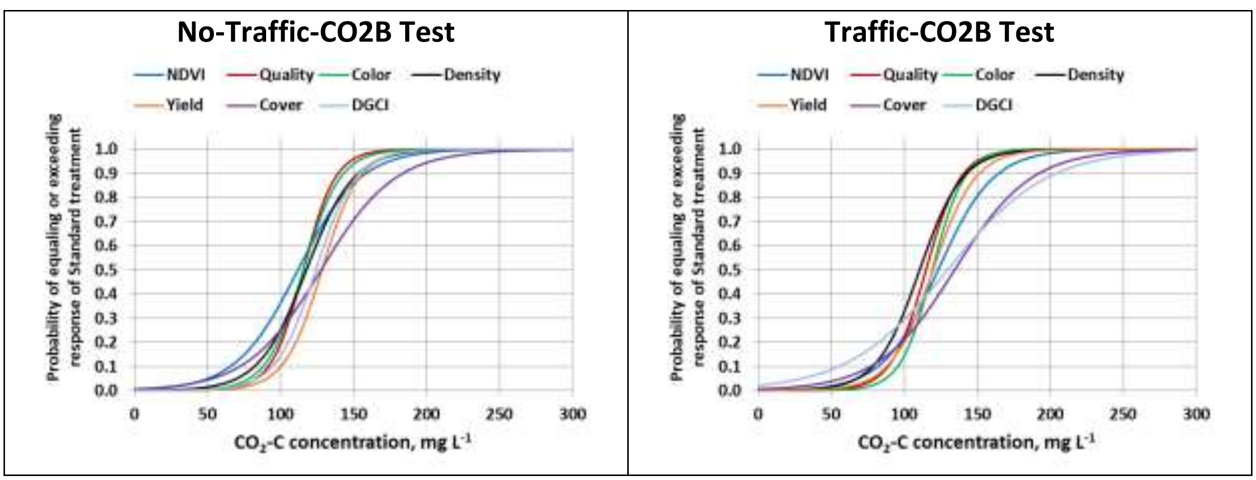
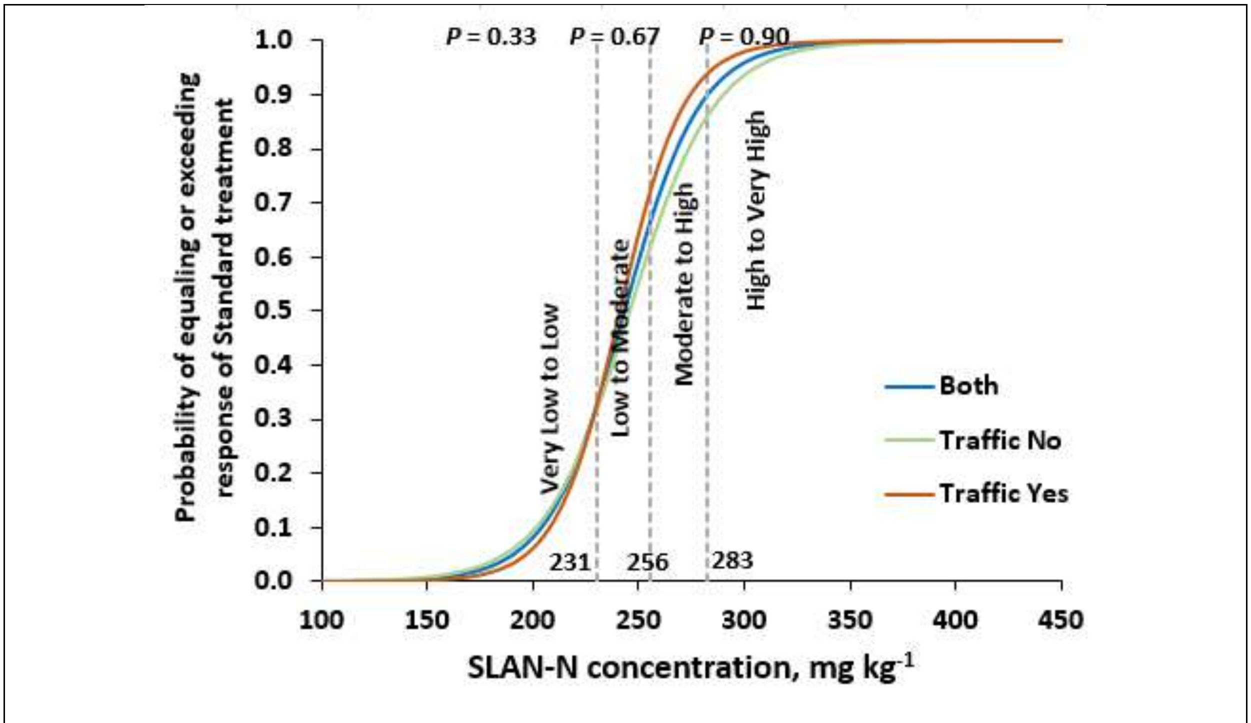


Figure 4. Compost-organic fertilizer probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft^2 every 21 days) in relation to the Solvita CO2B-C concentrations for the No-Traffic and Traffic plots. 2021 results pooled across all sampling dates.



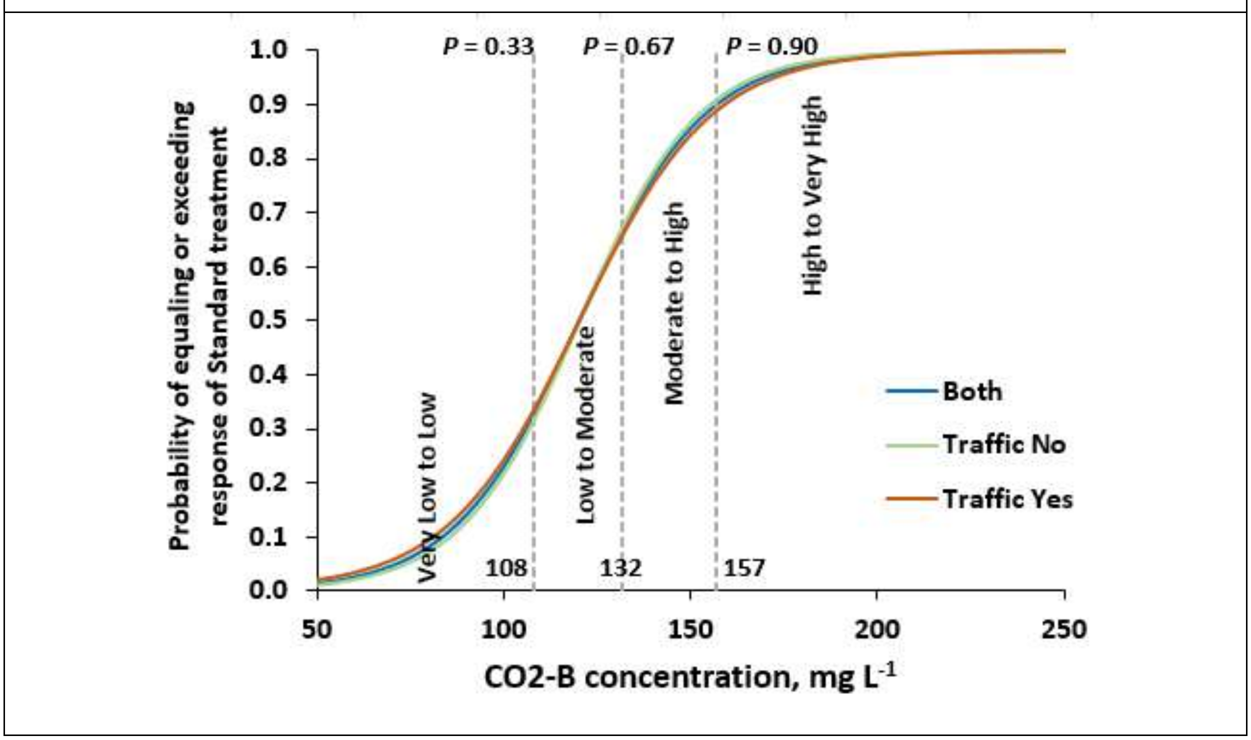


Figure 5. Compost-organic fertilizer probability curve representing all variables combined for both traffic treatments and categories of fairway creeping bentgrass responses that would be equal to or greater than the responses obtained from the Standard fertilizer treatment in relation to Solvita Soil Labile Amino-Nitrogen (SLAN)–N and CO_2-Burst (CO2B)–C concentrations. The gray vertical lines indicate *P* values of 0.33, 0.67, and 0.90 obtained from the equations used for values in Tables 4 & 5. 2021 results.

USGA ID#: 2019-30-700

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

Project Leader: Roch Gaussoin, Michael Carlson

Affiliation: University of Nebraska-Lincoln

Objectives:

- Develop a prescription nitrogen fertilizer decision support system based on reflectance for creeping bentgrass fairways.
- Develop a method to create site-specific nitrogen management units on creeping bentgrass fairways using reflectance measurements.
- Document the nitrogen use and changes in variability from an automated reflectance threshold-based variable rate nitrogen fertilizer program.

Start Date: 2019

Project Duration: Three years

Total Funding: $55,000

Summary Points:

- The relationship among visual quality, growth rate, and NDRE of creeping bentgrass is linear and varies throughout the year.
- Guided machine learning will be used to create a decision support system tool to determine the rate of nitrogen fertilizer needed to meet visual quality and growth rate goals.
- Spatial variability of creeping bentgrass fairways were quantified throughout the summer from reflectance measurements to create site-specific management units for precision nutrient management.
- Variable rate nitrogen fertilizer application based on reflectance thresholds were demonstrated on a working golf course in Lincoln, NE. The NDVI variable rate application reduced variability and nitrogen applied by 30% compared to a constant and NDRE variable rate application methods.
- Further research is needed to validate the nitrogen fertilizer decision support system and to integrate the models into a user-friendly software for use by golf course superintendents.

Summary Text:

Reflectance-Based Prescription Nitrogen Fertilizer Model Development

Reflectance measurements have been documented to discriminate the nitrogen status of turfgrass, whereas minimal work has been performed to create prescription nitrogen fertilizer models using reflectance. The goal is to create a prescription nitrogen fertilizer model for creeping bentgrass fairway-height turfgrass to meet aesthetic and function goals. Data to develop the models were collected from two randomized complete block design studies during 2019 and 2020 on creeping bentgrass managed as a fairway. The turfgrass was mowed at 0.375 inch height of cut and diseases and weeds were sprayed to not influence data collection. The two studies varied by irrigation rates where one study received 80% replacement ET irrigation, and the other received 0% and 40% replacement ET irrigation in 2019 and 2020, respectively. Turfgrass visual quality and growth rate were altered in each study with nitrogen applications ranging from 0 to 0.4 lb N M^{-1} every 14 d. Turfgrass biomass, visual quality, and reflectance were collected and measured every 14 d from June through August in 2019 and June through September in 2020. These data were used to create the prescription fertilizer models.

Initial results indicate that the relationship among visual quality, growth rate, and NDRE reflectance exhibit a linear relationship where the slope varies throughout the data collection periods (Fig. 1). The growth rate and visual quality generally increased with increasing N rates in the 80% ET study, whereas the increase in growth rate and visual quality was not as common in the non-irrigated study. These initial results will be written into a paper to characterize how reflectance can be used to model visual quality and growth rate to make fertility management decisions. Further data analysis will be conducted this winter to develop a decision support tool to determine in nitrogen fertilizer is required and if required what application rate will achieve a desired visual quality and growth rate. The decision support system tool will be developed using guided machine learning which could be used by superintendents to apply nitrogen fertilizer more precisely on golf course fairways.

Reflectance-based nitrogen fertility site-specific management unit method development

Historically golf course fairway fertility is applied at constant rates throughout the fairway regardless of any spatial variability of aesthetics or function. Minimal work has been published in how to reduce variability of fairways with increased micromanagement of nitrogen fertilizer applications. Site-specific management units group together areas on fairways that respond to inputs in similar manners that can be managed as a single unit which could help reduce the complexity of precision turfgrass management. Reflectance measurements can accurately measure variability of turfgrass nitrogen, aesthetics, and function without disrupting play and the labor needed to collect destructive samples. The goal of this study is to develop a method to create site-specific management units for nitrogen fertilizer applications based on reflectance measurements.

The study was a completely randomized, repeated measure design and executed on three fairways at the Jim Ager Memorial Junior Golf Course in Lincoln, NE. Turfgrass biomass, visual quality, visual percent annual bluegrass ratings, and reflectance were measured once a month in 15 ft^2 grids on each fairway in May, July, and September in 2020 and 2021. Turfgrass biomass was collected from a single mower unit from the fairway mower (Fig. 2). Quality (1-9., 9=best quality) and percent annual bluegrass ratings were based on visual estimates, and reflectance was measured using a geo-referenced Holland Scientific Crop Circle ACS-430 active sensor. Data analysis is currently being performed to determine how site-specific management units can be developed using reflectance and may change throughout the peak growing season. This research is important as it can help superintendents with increased precision and less complexity because of the homogeneity of the site-specific management units. Further work will be needed to determine how to automate the creation of site-specific management units for nitrogen management from reflectance data and quantify how plastic the units are throughout the growing season.

Documenting prescription nitrogen applications on creeping bentgrass fairways

Nitrogen fertilizer application rates on golf courses are currently decided on historical application rates, changes in biomass volume collected, or calendar-based applications. These methods do not account for spatial variability of aesthetics or function. Fairways are the largest fertilized area on golf courses and maintained for specified aesthetic and function goals. The goal of this study was to develop an automated method to determine variable rate fertilizer application rates to increase nitrogen use efficiency on creeping bentgrass fairways.

This study was performed at the Jim Ager Memorial Junior Golf Course in Lincoln, NE in 2021 on all nine of the creeping bentgrass fairways. The study was a completely randomized, repeated measure design with three replications. Fairways were split into three groups based on the nitrogen application treatments: constant rate, NDRE-threshold, and NDVI-threshold. Constant rate fairways received nitrogen at 0.08 lb N M^{-1}. The NDRE- and NDVI-threshold fairways received variable rate nitrogen applications based on reflectance thresholds related to visual quality ratings determined from previous work. Reflectance values throughout each fairway that were within the ideal range received nitrogen at 0.08 lb N M^{-1}, reflectance values below the ideal range received 50% more N, and reflectance values above the ideal range received 50% less N. A geo-referenced Holland Scientific Crop Circle ACS-430 sensor measured NDRE and NDVI values on fairways to make prescription fertilizer recommendations and quantify changes in variability. ArcGIS Pro was used to create 20 ft^2 grided shapefiles for each fairway to match the size of the sprayer boom. The prior two-weeks reflectance data before N applications and shapefiles were used in an automated program to create prescription fertilizer shapefiles. The amount of nitrogen applied on each fairway was quantified from the prescription maps, and variability of fairways were quantified using NDRE reflectance of the data two weeks before and after N application.

Nitrogen fertilizer was only applied once in 2021 as the growth of the fairways did not warrant any applications. This was confirmed based on the fertilizer program, and from the suggestion of the superintendent. The NDVI-based thresholds applied around 30% less N on the fairways than the constant rate and NDRE-based threshold fertilizer method for the single N application. The NDVI treatments exhibited around 30% decrease in variability after N application, whereas the constant rate and NDRE treatments exhibited around 22% decrease (Fig. 3). Further research is needed to determine how to choose reflectance thresholds for N fertilizer applications, and what other vegetation indices may be better suited to determine N needs of the turfgrass.

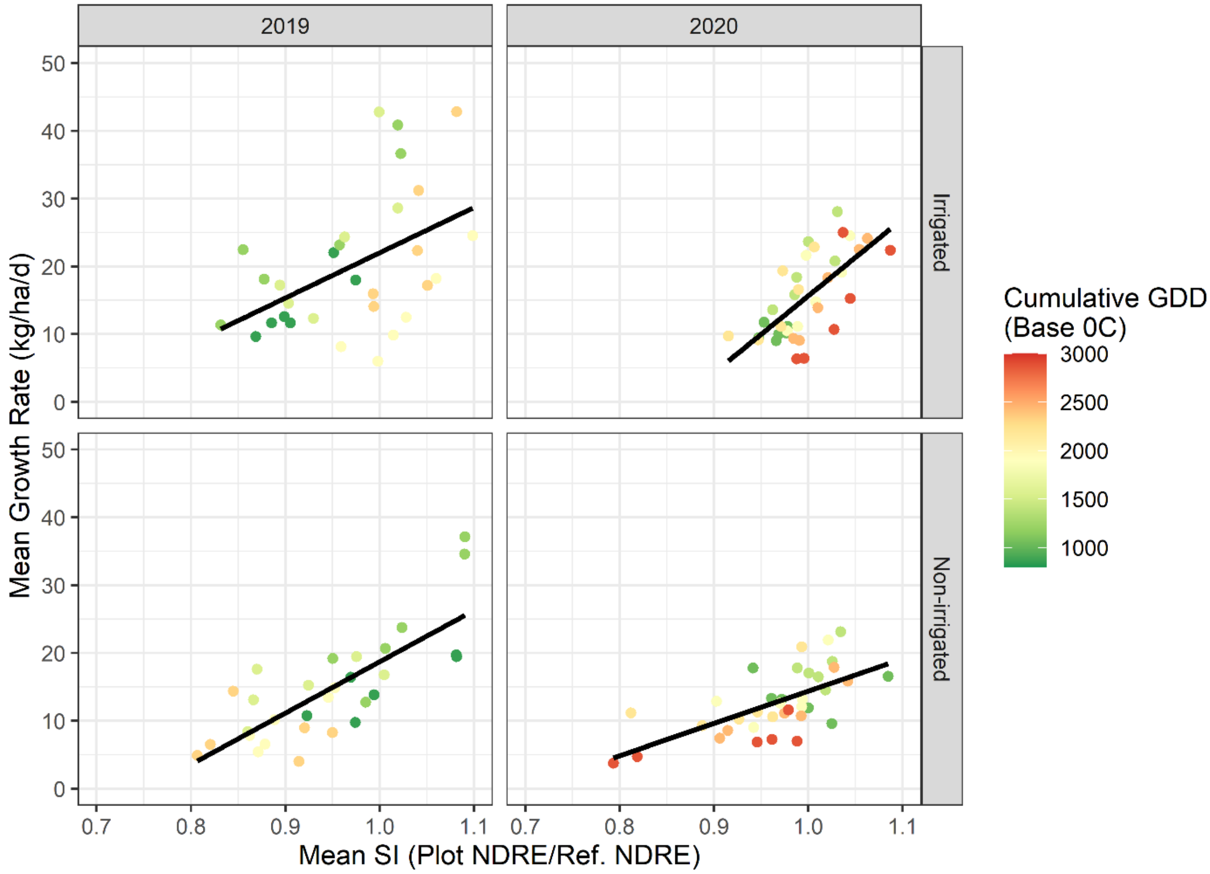


Figure 1. Mean growth rate linear response from a sufficiency index (SI) value based on NDRE reflectance throughout the data collection period in both irrigated and non-irrigated studies. These figures show that growth rate response to SI value varies throughout the growing season and that machine learning may be beneficial to determine accurate fertilizer application models.

Figure 2. Turfgrass biomass collection on number 5 fairway at the Jim Ager Memorial Junior Golf Course in Lincoln, NE in 2020. Biomass was collected to quantify growth rate for site-specific management unit creation.

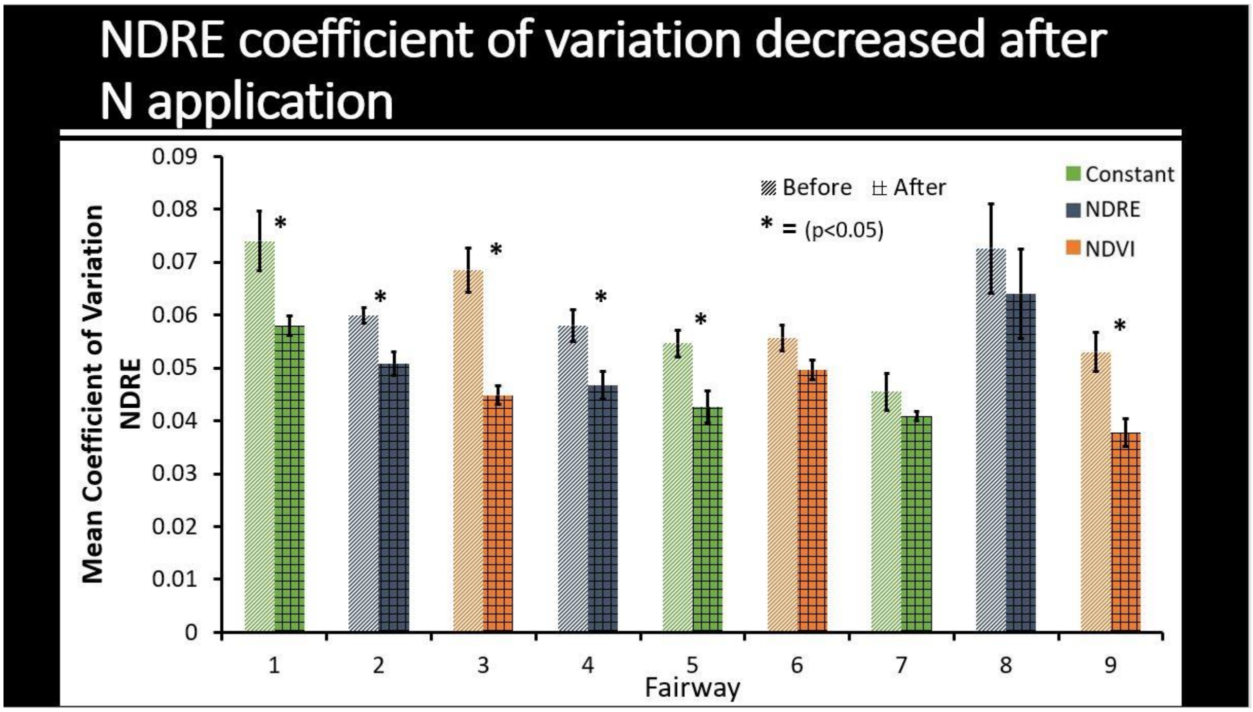


Figure 3. Significant changes in coefficient of variation of NDRE for individual fairways before and after N application on May 5, 2021. Constant rate and NDRE treatments reduced on average around 22% after N application, whereas NDVI treatments reduced coefficient of variation by 30% on average.

USGA ID#: 2020-03-708 (continued from 2019-01-671)
Title: Topdressing sand size effects on mat layer development during treatment years 5 - 7
Project leaders: James A. Murphy and Zhongqi Xu
Affiliation: Department of Plant Biology, Rutgers University

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

Start date: 2020
Project duration: 3 years
Total funding: $161,163

Summary Points:
- Control, topdressing, and cultivation treatments were continued for a 6th growing season. The May cultivation treatment was re-scheduled for 6 April to avoid severe work restrictions in 2020; this April timing was continued in 2021.
- Topdressing with all sand sizes produced acceptable turf quality; however, turf quality was often better on plots topdressed with finer sands and non-cultivated plots. Better turf quality was attributed to greater water availability in plots topdressed with finer sands and the greater turf cover (NDVI) throughout the growing season on non-cultivated plots.
- Greater water retention in plots topdressed with finer sands was associated with reduced surface hardness. However, this effect for finer sands was often not observed under cultivated conditions. Plots treated with core cultivation were typically the hardest surfaces.
- The procedure developed in 2020 to document the frequency and quantity of hand-watering was used in 2021. The method was further refined to distinguish between plots that require full plot watering versus spot watering. Preliminary analysis of these data indicated that plots with lower water retention require more irrigation to correct drought stress of the entire plot or localized dry spots within plots.

Summary:

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

was performed during the USGA ID#: 2019-01-671 grant, which evaluated the bulk density, pore size distribution and sand size distribution of the mat layer. Presuming negotiations with METER are productive, a dual head infiltrometer will be used to evaluate treatment effects on water infiltration in 2022.

Materials and Methods

The trial was initiated in May 2016 on a 19-month-old 'Shark' creeping bentgrass maintained at 2.8-mm on a sand-based root zone. Note mowing height was raised (3.2-mm) and frequency was reduced (every other day) during spring 2020 – in response to COVID-19 work restrictions and – returned to 2.8-mm and 5 to 6 days per week in June 2020 and continued through 2021.

The trial was a 3 x 2 x 2 factorially arranged randomized complete block design with four blocks. The factors were sand size (medium-coarse, medium-fine, fine-medium), quantity of mid-season topdressing (50- or 100-lb / 1,000-ft^2 every 10 to 14 days totaling ten applications from June through early October), and cultivation (non-cultivated or core cultivated plus backfilled in May and October). In 2020, the May cultivation treatment was re-scheduled to April to avoid work restrictions related to the COVID-19 pandemic as well as to reduce the time that coring holes were evident in late spring and early summer with cultivation in May; April cultivation was repeated in 2021. Controls (no mid-season topdressing) at each level of cultivation were also included for comparisons resulting in 14 total treatments (Table 1).

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

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

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

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

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

The medium-coarse sand used in this trial meets the USGA particle size recommendation for construction, whereas that of the medium-fine and fine-medium sands do not. The quantity of fine and very fine particles in the medium-fine and fine-medium sands exceed the USGA recommendations and these sands contain little to no coarse particles (Table 2).

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]				
	2.0-1.0	1.0-0.5	0.5-0.25	0.25-0.15	0.15-0.05
Topdressing Sand Size	very coarse	coarse	medium	fine	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.

Data collection during 2021 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 hand-watering quantification. A recently acquired dual head infiltrometer [SATURO | Automated Field Infiltrometer | METER Environment (metergroup.com), Pullman, WA] was field tested on border areas of plots in 2021.

Results

Analysis of data collected during 2021 is in progress.

Dual head Infiltration: Preliminary tests indicate time requirement for dual-head infiltrometer will be less than 4-hours. Moreover, this method greatly improves acquisition of field saturated hydraulic conductivity of plots. Thus, up to 7 devices will be needed to collect water infiltration from one block of treatments (14) in a single day (two runs of 7 plots in the morning and afternoon). We are in negotiating with METER to purchase up to 6 devices.

Visual Turf Quality: Similar to previous years, visual ratings in 2021 indicated that treatments generally had acceptable quality scores (≥ 5) throughout much of the growing season (data not shown). Both topdressing and cultivation affected turf quality; quality was better on plots that were topdressed and plots topdressed with finer sized sand often had better quality than plots topdressed with medium-coarse sand. Visual quality was frequently poorer on plots that were core cultivated, due to the slow healing of open coring holes.

Residual sand after topdressing: Sand size and the rate of topdressing have the greatest influence on the persistence of residual on the turf surface. Medium-coarse lingered on the surface longer than medium-fine and fine-medium sands especially when applied at the 100-lb per 1,000 sq ft.

Volumetric water content (VWC): Water retention at the surface 0- to 3-inch depth zone has become more strongly affected by treatment with time. Topdressing reduced surface water retention compared to the controls and this effect was pronounced for plots that were not cultivated (Table 3). Among the controls, core cultivation dramatically reduced surface water content as well (Table 3). All three factors frequently influenced surface water retention in 2021 and the effects of sand size and topdressing rate depended on the level of cultivation (interacted) (Table 3). As topdressing sand became increasingly finer among the three sands, surface water retention increased; however, this response effect was often not evident for plots that were cultivated (Table 4). Similarly, plots topdressed at the 50-lb rate often retained more surface water than plots topdressed at 100-lb; however, this response was often not evident on plots that were cultivated (Table 4).

Clegg soil impact values: Similar to observations of the volumetric water content data, as topdressing sand became increasingly finer among the three sands, surface hardness decreased (data not shown). However, this response of surface hardness to sand size was often not evident for plots that were cultivated, and cultivated plots were typically harder than non-cultivated. Plots topdressed at the 100-lb rate were often harder than plots topdressed at 50-lb; however, there often was not a response to sand size on plots that were cultivated.

Stimpmeter distances: Topdressing and core cultivation treatments typically had small (< 6 inches) or no effects on ball roll distance (data not shown).

Normalized difference vegetation index (NDVI): During 2021, NDVI data was collected nearly once per week. NDVI frequently respond to the cultivation factor with cultivated plots have a lower NDVI than non-cultivated plots. This response is consistent with visual quality data, which indicated poorer quality of cultivated plots compare to non-cultivated. Cultivated plots typically have a thinner turf canopy than non-cultivated and thus lower NDVI values.

Hand-watering quantification: Preliminary analysis of hand-watering data indicated that plots with lower water retention at the 0- to 3-inch surface depth required more watering to correct for drought stress of the entire plot and/or localized dry spots within plots.

2022 Plan of Work

Control, topdressing, and cultivation treatments will be continued for a 7th growing season in 2022; the spring cultivation treatment will be applied in early April 2022. Data collection during 2022 will include 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 ball roll distances, normalized difference vegetation index (NDVI); and hand-watering quantification. We expect to acquire up to 6 more dual head infiltrometers (SATURO) to assess water infiltration on treated plots during 2022. Finally, undisturbed core samples of the mat layers will be removed from plots in either late fall 2022 or early spring 2023 using the methods performed during the USGA ID#: 2019-01-671 grant to evaluate the bulk density, pore size distribution, organic matter content, and sand size distribution of the mat layers.

Table 3. Response of volumetric water content at the surface 0- to 3-inch depth zone to controls, topdressing sand size and rate, and core cultivation on a 7-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110oinch in New Brunswick NJ during 2020.

Orthogonal Contrasts	13 July	14 July	15 July	16 July	17 July
	---------------------------------- volumetric water content (%) / probability of significance ----------------------------------				
Non-cultivated:					
Control versus	34.3***	34.3***	31.4***	27.8***	26.8***
Pooled topdressing	28.3	27.6	23.7	20.4	19.9
Core cultivated:					
Control versus	21.7*	22.0*	18.0 NS	14.5*	14.4 NS
Pooled topdressing [b]	19.1	18.6	15.1	12.0	12.9
Controls:					
Non-cultivated versus cultivated	***	***	***	***	***
ANOVA Factorial Source					
Sand Size (Size)	***	***	***	***	***
Sand Rate (Rate)	**	***	***	***	***
Size × Rate	NS	Ns	NS	NS	NS
Core Cultivation (CC)	***	***	***	***	***
Size × CC	***	***	***	***	***
Rate × CC	NS	NS	NS	*	*
Size × Rate × CC	NS	NS	NS	NS	NS

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

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

Table 4. Response of volumetric water content at the surface 0- to 3-inch depth zone to the sand size by cultivation and the topdressing rate by cultivation interactions on a 7-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.110-inch in New Brunswick NJ during 2021.

Sand Size	Cultivation	13 July	14 July	15 July	16 July	17 July
		-- volumetric water content (%) --				
Medium-coarse	None	25.0	24.2	19.8	17.0	16.2
Medium-fine	None	27.0	26.0	22.2	18.7	18.8
Fine-medium	None	32.9	32.5	29.0	25.5	24.6
Medium-coarse	Twice/yr	18.3	17.7	14.4	11.5	12.9
Medium-fine	Twice/yr	18.3	18.6	15.0	11.8	12.6
Fine-medium	Twice/yr	20.5	19.6	15.9	12.8	13.4
	LSD	1.8	1.9	2.1	1.4	1.8
Topdress Rate	Cultivation					
50-lb	None	n.a.	n.a.	n.a.	22.0	21.5
100-lb	None	n.a.	n.a.	n.a.	18.8	18.2
50-lb	Twice/yr	n.a.	n.a.	n.a.	12.7	13.4
100-lb	Twice/yr	n.a.	n.a.	n.a.	11.4	12.5
	LSD				1.2	1.4

USGA ID#: 2020-14-719

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

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

Affiliation: Department of Horticulture, Oregon State University

Objectives:

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

Start Date:
May 2020

Project Duration:
3 years, May 2020 to May 2023 (year 2 report)

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

Summary Points:

- Combination of cultivation (solid or hollow) and sand topdressing improved turf quality and reduced disease incidence after two years
- Higher rates of sand topdressing (100 lbs 1000ft^{-2}) subtly improved turf quality after two years compared to a lower rate (50 lbs 1000ft^{-2})
- Yellow patch and Cyanobacterial incidence were greater in plots that received no cultivation (non-treated control and topdressing only).

Introduction:

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

Materials and Methods:

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

Experimental design for the trial is a randomized complete block design with four replications. Treatments are arranged in a 2 x 7 factorial, with two sand topdressing rates (50 and 100 lbs 1000 sq ft^{-1}) and 7 cultivation treatments (hollow tine (HT) spring, fall, and both spring and fall; solid tine (ST) spring, fall, and both spring and fall; and a non-cultivated plot that received sand topdressing). A non-treated control (no cultivation, no sand topdressing) was also included in the analysis. Spring cultivation treatments were applied on 1 June 2020 and 28 May 2021, and fall cultivation treatments were applied 29 Sept 2020 and 7 Oct 2021. Sand topdressing treatments were applied every 2-wks during the summer from 15 June through 21 Sep 2020, and 9 June through 22 Sept 2021.

Fungicides were applied year-round to prevent diseases including anthracnose, yellow patch, and Microdochium patch. The plots were being fertilized every 2 weeks during the growing season (spring, summer, and fall) at 0.2 lbs N 1000 sq ft^{-1}, and at the same rate monthly during the winter. The plots are mowed at 0.110 inches during the growing season and 0.140 inches during the winter.

Response Variables:

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

Preliminary Findings:

As expected, few differences between response variables were observed during 2020. It is likely that several years of cultivation and sand topdressing treatments are required to see differences in soil physical properties and putting greens surface characteristics. With that said, statistical differences in TQ were observed between treatments in 2020. The main effect of cultivation treatment influenced TQ in Aug, Sept, Oct, and Nov of 2020, with spring cultivation treatments (HT spring, HT spring & fall, ST spring, ST spring & fall) generally resulting in greater TQ rating than fall cultivation treatments and topdressing only. Interestingly, this trend continued through the Nov TQ rating, with HT spring, and ST spring plots having the greatest TQ. Further research is needed to confirm this response. The main effect of sand topdressing rate had no effect on TQ during 2020. Neither main effect of topdressing rate or cultivation treatment had an effect on yellow patch severity in the fall of 2020 (table 1); however, all combinations of topdressing rate and cultivation treatments reduced yellow patch severity compared to the non-treated control (no cultivation, no topdressing; data not shown). No statistical difference in soil infiltration rate (collected on 10 Aug 2020) was detected between either cultivation treatments, sand topdressing rates, or the interaction between the two factors, which was not surprising since half of the cultivation treatments (fall treatments) had not been applied at the time of sampling. Orthogonal contrast between spring cultivation treatments (HT spring, HT spring & fall, ST spring, and ST spring & fall) and all other treatments indicate spring cultivation had greater infiltration (or less of a reduction in infiltration rates) than plots that didn't receive spring cultivation. These results indicate that the method used to determine infiltration rate is adequate to detect treatment differences. No statistical difference was observed between cultivation and topdressing treatments with respect to NDVI or surface firmness.

During 2021, differences in TQ between treatments became more apparent. Topdressing rate had a significant effect on TQ on 4 of the 5 rating dates in 2021, with topdressing applied at 100 lbs $1000ft^{-2}$ increasing turf quality compared to a rate of 50 lbs $1000ft^{-2}$ (Table 2). Cultivation treatment had an effect on TQ on 2 July 2021, with the fall hollow tine cultivation treatment having a greater TQ than any other cultivation treatment or topdressing alone. The non-treated control plot had the lowest TQ rating of all treatments, on all rating dates in 2021. This response was particularly evident late summer when a cyanobacteria (*Oscillatoria* sp.) infestation reduced TQ in the non-treated control plots (Fig 1). No statistical difference in soil infiltration rate or surface firmness characteristics (Trufirm) were detected between either cultivation treatments, sand topdressing rates, or the interaction between the two factors during 2021.

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Table 1. Analysis of variance of the turf quality and yellow patch response to topdressing rate and cultivation treatment applied to annual bluegrass turf in Corvallis, OR during 2020.

	Turf Quality					Yellow Patch
Main effects	Jul	Aug	Sept	Oct	Nov	Severity
Topdressing rate (T)†				1-9 scale		
50 lbs/M	6.1	6.1	5.8	6.0	5.9	1.9
100 lbs/M	6.4	5.9	5.9	6.3	5.8	1.6
Cultivation (C)‡						
HT§ Spring	6.3	6.2	5.9	5.9	6.6	2.1
HT Fall	6.5	5.5	5.8	6.5	5.0	1.4
HT Spring & Fall	6.1	6.3	6.3	6.3	5.6	1.3
ST Spring	6.1	6.0	6.0	6.3	6.4	1.8
ST Fall	6.6	6.0	5.6	6.1	5.5	1.6
ST Spring & Fall	6.0	6.6	6.3	6.4	5.8	1.4
Topdress only	6.4	5.4	5.3	5.4	6.0	2.8
$LSD_{(0.05)}$	-	0.7	0.6	0.6	0.6	0.9
				ANOVA		
Source of variation						
T	ns	ns	ns	ns	ns	ns
C	ns	**	*	*	***	*
T x C	ns	ns	ns	ns	ns	ns
CV (%)	9.3	10.8	10.8	9.7	9.6	48.8

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

† Topdressing treatments were applied from 15 June to 21 Sept 2020.

‡ Cultivation treatments were applied in the spring on 1 June 2020 and in the fall on 29 Sept 2020.

§ HT=hollow tine; ST=solid tine.

Table 2. Analysis of variance of the turf quality ratings in response to topdressing rate and cultivation treatment applied to annual bluegrass turf in Corvallis, OR during 2021.

Main effects	2021 Turf Quality				
	2-Jul	15-Jul	6-Aug	25-Aug	17-Sep
Topdressing rate (T)†	1-9 scale				
50 lbs/M	6.3	6.3	6.2	6.2	6.1
100 lbs/M	6.8	6.6	6.5	6.6	6.8
Cultivation (C)‡					
HT Spring	6.5	6.5	6.5	6.5	6.4
HT Fall	7.4	6.8	6.3	6.1	6.0
HT Spring & Fall	6.6	6.5	6.3	6.8	6.9
ST Spring	6.4	6.0	6.3	6.5	6.8
ST Fall	6.6	6.6	6.4	6.1	6.0
ST Spring & Fall	6.3	6.5	6.6	6.8	7.1
Topdress only	6.1	6.0	6.3	6.1	5.8
Non-treated control§	4.9	5.0	4.5	4.9	4.4
$LSD_{(0.05)}$	0.6	--	--	--	--
	ANOVA				
Source of variation					
T	**	*	ns	*	**
C	**	ns	ns	ns	ns
T x C	ns	ns	ns	ns	ns

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

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

‡ Cultivation treatments were applied in the spring and fall on the 28 May and 7 Oct 2021, respectively; HT=hollow tine; ST=solid tine.

§ Non-treated control was not included in the factorial analysis, but is present for comparison only

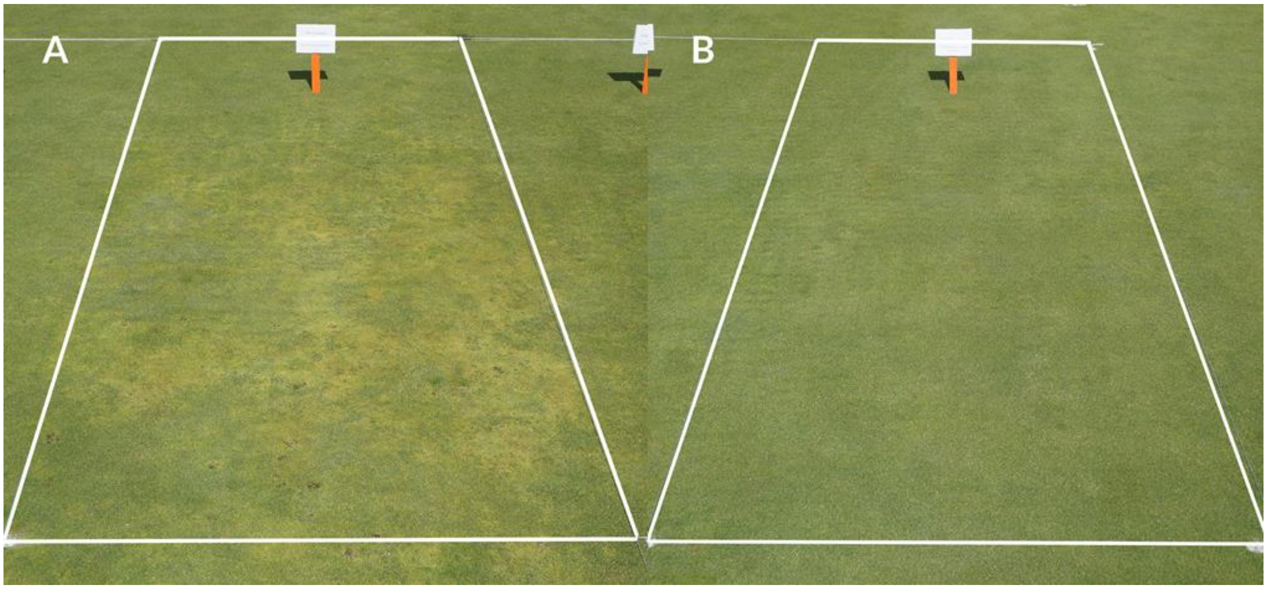

Figure 1. Reduced turfgrass quality in the non-treated control (A) as a result of cyanobacteria (*Oscillatoria* sp.) outbreak, compared to the spring solid tine cultivation treatment with 100 lbs 1000ft^{-2} of topdressing sand applied every 14-days (B). Photo taken on 26 Aug 2021.

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 if ethephon treatments applied in the spring for annual bluegrass seedhead control influence cultivation recovery time
3) Evaluate the effect of gibberellic acid (GA_3) on core cultivation recovery

Start Date:
March 2021

Project Duration:
3 years, March 2021 to Dec 2023 (year 1 report)

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

Summary Points:

- Spring applications of ethephon (Proxy) help annual bluegrass putting greens recover quicker from core cultivation, whereas fall applications slow recovery
- Increased turfgrass growth observed in Gibberellic acid treatments initially increased the percent recovery compared to other treatments; however, scalping caused reduced turfgrass cover, and increased recovery time.
- Trinexapac-ethyl (Primo Maxx) timing had little effect on cultivation recovery time in 2021, regardless of season.

Introduction:

Organic matter (OM) management with core cultivation is one of the most important management practices for golf course putting greens, and one of the most disruptive to golfers. Core cultivation is done in the spring and fall on actively growing cool-season turfgrasses to minimize recovery time (Beard, 1973). However, this is also the time of year when golf courses experience the most play. In a USGA report of the top 10 questions frequently asked by golfers,

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three questioned need for core cultivation of putting greens (Maloy, 2002). Thus, there is a need to reduce 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 (Krueser and Soldat, 2011) to limit post-inhibition growth enhancement (aka "rebound effect"). Plant growth regulator applications to cool-season turfgrass are most commonly applied in the spring and fall when shoot and root growth are maximal (Johnson, 1989). In contrast, there has been a trend with sports field managers to use post-inhibition growth enhancement to recover from events that damage turfgrass, such as concerts (Polimer, 2020). On golf courses, core cultivation is one of the most damaging events to occur, but no research currently exists that demonstrates the effect of PGRs on recovery from core cultivation or if plant hormones such as gibberellic acid can be used to reduce recovery time. It may be possible to use post-inhibition growth enhancement to decrease recovery time.

Materials and Methods:

This three year field trial began March 2021 with evaluations ending October 2023. Research is being conducted on a sand-based annual bluegrass research green maintained at 0.110 inches HOC. Irrigation will be applied to provide moderately moist soil to encourage recovery from cultivation treatments. The trial was core cultivated in the spring and fall on 1 May and 28 September 2021, respectively, using a Toro Procore 648 equipped with ½" I.D. hollow tines (2 x 2" spacing). A complete fertilizer (Anderson's 28-5-18) was applied 2-d prior to cultivation to all plots at a rate of 0.3 and 0.5 lbs N/1000 ft^2 in the spring and fall, respectively.

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

Treatments

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

[¥] All trinexapac-ethyl (TE) applications will be applied at 5.5 fl oz of Primo MAXX/Acre ᵞCumulative GDD model will be calculated as the summation of the daily mean air temperature (°C) with a base of 0°C following the most recent TE application.

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

Treatments will be applied with a CO2-pressured backpack sprayer equipped with 4 TeeJet flat fan 80015 nozzles applied at 35 psi using a carrier volume of 1.8 gallons per 1,000 sq. ft. The first treatment application in the spring will be initiated at first sign of annual bluegrass plants in the “boot” stage, and subsequent applications and cultivation timing will be made based on a growing degree day model. Routine applications of trinexapac-ethyl will continue throughout the summer months on all plots (except non-treated control and GA plots) every 14 days.

Response Variables:

Digital photos will be taken at the same location in each plot, daily during the recovery period, using a lightbox to track recovery over time. Digital images will be analyzed using Sigmascan to determine percent recovery over time. Plots were periodically rated for visual turf quality and turf color throughout the growing season, and intensively rated for the 10-14 day period post core cultivation, in both spring and fall. Turfgrass leaf clippings were collected 5 and 14 days post cultivation treatments to measure the amount of vertical growth (i.e. rebound effect). Soil samples will be collected at the conclusion of the study to determine the effect of PGRs on total organic matter. 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. Response variables will be analyzed with analysis of variance (ANOVA) and the means separated using LSD at the 5% alpha level.

Preliminary Findings:

Results from the spring of 2021 indicate that the main effect of ethephon treatments had the greatest effect on cultivation recovery time, with plots receiving ethephon recovering quicker than plots that received no ethephon (Fig. 1). The main effect of TE timing was only significant on one date in the spring of 2021, 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 (data not shown). Results from the fall cultivation event also indicated that the main effect of ethephon treatment had the greatest effect on cultivation recovery time; however, during this season plots receiving ethephon were slower to recover than plots that received no ethephon (Fig 1). Trinexapac-ethyl timing had no effect on cultivation recovery time in the fall of 2021.

Interestingly, plots treated with GA at 0.05 or 0.1 oz/A initially had increased turfgrass growth (Fig 2) and rapid recovery from core cultivation 3-5 days after cultivation (Table 1 & 2; Fig 3). However, scalping from excessive turfgrass growth slowed recovery time overall. A higher rate of nitrogen (0.5 lbs N/ 1000 ft^2 in Fall compared to 0.3 lbs N/ 1000 ft^2 in spring) applied prior to the fall cultivation event seem to reduce the negative effect of the GA treatments long-term. It may be possible to limit the negative effect of excessive turfgrass growth by mowing more frequently and/or applying higher rates nitrogen prior to GA applications. Further research is needed to better understand how PGR applications influence cultivation recovery

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Table 1. Plant growth regulator effect on core cultivation recovery time in the spring of 2021

Treatment	**Last PGR app prior to cultivation**	**1 DAT**	**3 DAT**	**5 DAT**	**7 DAT**	**10 DAT**	**14 DAT**	**21 DAT**
	GDD				% Recovery			
Non-treated control		38	54	54	50	56	83	103
TE	400	32	61	61	55	58	81	100
TE + Ethephon	400	32	65	72	71	79	95	104
TE	200	42	54	52	44	57	83	104
TE + Ethephon	200	36	60	72	76	82	95	103
TE	10	36	50	44	37	50	75	99
TE + Ethephon	10	36	60	66	71	80	94	108
GA (0.05 oz/A)	10	45	86	88	81	77	92	100
GA (0.1 oz/A)	10	42	93	86	82	66	77	93
$LSD_{(0.05)}$		-	13	12	13	12	10	-

† DAC = days after core cultivation

‡ TE = Trinexapac-ethyl (Primo Maxx)

* GA = Gibberellic acid (RyzUp smartgrass®)

Table 2. Plant growth regulator effect on core cultivation recovery time in the fall of 2021

Treatment	**Last PGR app prior to cultivation**	**2 DAT**	**4 DAT**	**6 DAT**	**9 DAT**	**13 DAT**	**17 DAT**
	GDD			% Recovery			
Non-treated control		30	73	88	95	95	99
TE	400	25	69	84	96	97	101
TE + Ethephon	400	34	76	88	95	96	98
TE	200	30	74	87	95	96	100
TE + Ethephon	200	29	77	88	95	96	99
TE	10	27	72	88	96	97	102
TE + Ethephon	10	23	69	81	91	93	97
GA (0.05 oz/A)	10	54	91	94	98	97	99
GA (0.1 oz/A)	10	54	92	89	95	93	96
$LSD_{(0.05)}$		12.3	8	8	4	4	2

† DAC = days after core cultivation

‡ TE = Trinexapac-ethyl (Primo Maxx)

* GA = Gibberellic acid (RyzUp smartgrass®)

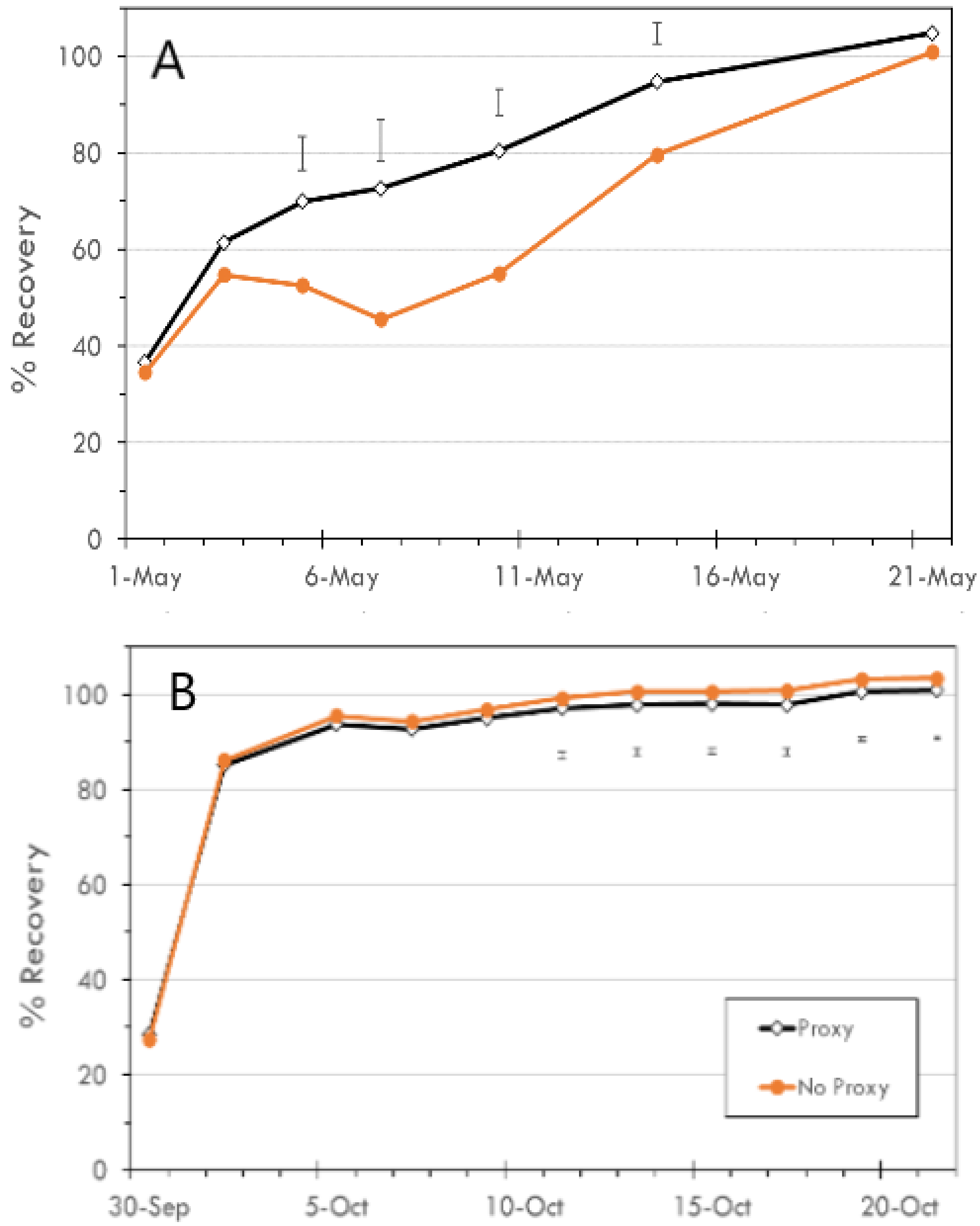


Figure 1. The main effect of ethephon (Proxy) on the percent recovery from core cultivation over time, in the spring (A) and fall (B) of 2021. Error bars above or below a rating date indicates a significant difference between treatments. The size of the error bar represents the LSD value.

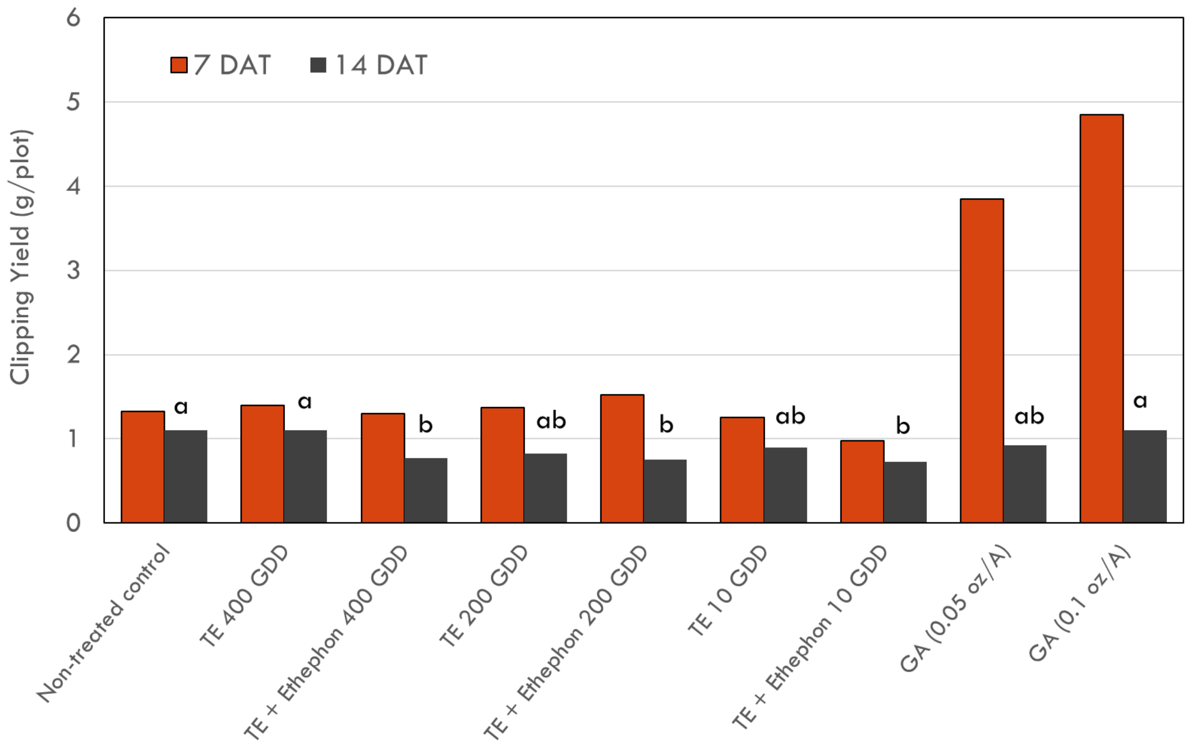
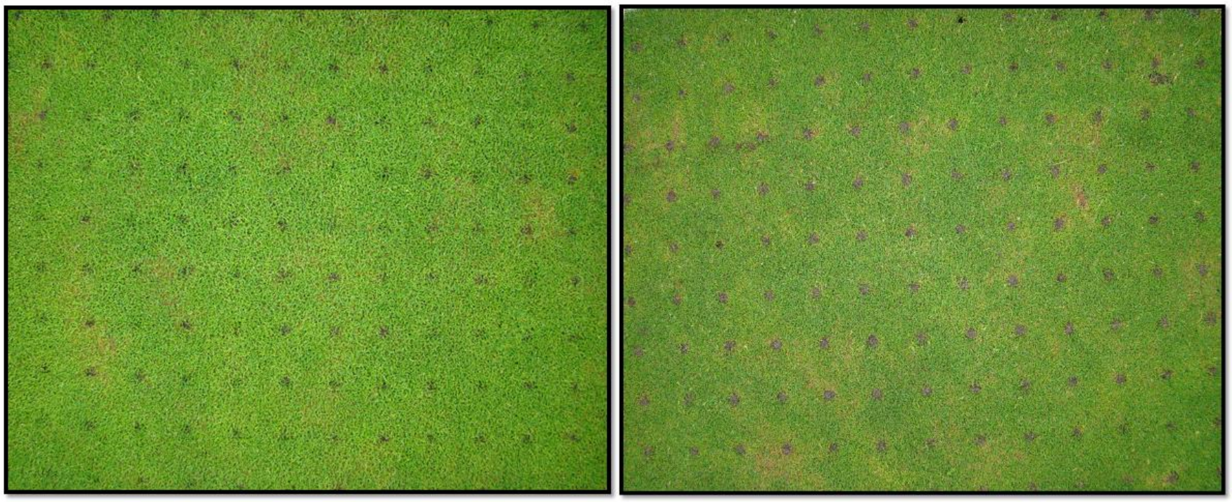


Figure 2. The effect of PGR treatments on clipping yield, 7 days after cultivation and 14 days after cultivation in the spring of 2021. Upper-case letters indicate differences between treatments 7 day after cultivation and lower-case letter indicate differences between treatments 14 days after cultivation.



GA at 0.1 oz/A Non-treated control

Figure 3. Cultivation recovery 4 days after fall coring event in plots treated gibberellic acid (GA) at 0.1 oz/A 10 GDD prior to cultivation (L) and the non-treated control (R).

USGA ID#: 2020-12-717

Title: Challenges in developing a simple, practical method for organic matter content determination by superintendents

Project Leaders: Roch Gaussoin, Ph.D.
Affiliation: University of Nebraska - Lincoln

Start Date: 2019
Project Duration: 2 years
Total Funding: $10,000

Summary Points:

- Samples containing pre-determined organic matter (OM) content were successfully created, as verified by Loss on Ignition (LOI).
- Methods using hydrogen peroxide adapted from Leifeld and Kogel-Knabner (2001) proved to be excessively time-consuming and complicated for the practical use intended in this study.
- A series of methods were tested to develop a correction factor that might be used in concert with less complicated methods.
- Regression models based on data of the best attempt indicated excessive variation measuring OM content of standardized samples.
- We conclude that a rapid, practical, inexpensive, and reliable method to test OM content on golf courses but superintendents or their designee cannot be achieved using hydrogen peroxide and readily available equipment.

Summary Text:

The objective of this study was to develop a rapid, practical, inexpensive, and reliable method to test organic matter (OM) content on golf courses using hydrogen peroxide (H_2O_2). Such a method would allow superintendents to test OM locally and reduce time to results and financial cost associated with sending samples to a laboratory. Hydrogen peroxide has been used since the 1920s to degrade OM in soil samples (Robinson, 1922). Since then, others have successfully used H_2O_2 in several modified methods (Mikutta et al., 2005). Using H_2O_2, a low cost widely available chemical solvent, has several benefits. Most laboratory protocols require a 30% solution of H_2O_2, we evaluated lower concentration H_2O_2 solutions readily available at a pharmacy or online.

Samples of a pre-determined OM percentage were created to ensure any tested method were verified before sampling from putting greens of unknown OM content. Soil cores were obtained from a research putting green (average OM-11%) at the John Seaton Anderson Turfgrass Research Facility near Mead, NE. Cores were left to air dry for multiple days so that sand was easily removed from OM. Samples were placed in a recirculating water bath at room

temperature where soil particles sank to the bottom, leaving OM floating on the surface. Organic matter was skimmed from the water surface and placed in an oven at 60°C for at least 24h until reaching a constant weight. Dry OM was shredded in a food processor (PowerPro, Black & Decker, Towson, MD) to ensure shredded OM could pass through a 2 mm sieve. The extracted OM was combined with pre-sieved (2 mm) dried sand to create an OM:sand ratio of 3% w/w. Organic matter content measured via loss on ignition (LOI) confirmed OM content. This blend was used in all subsequent H_2O_2 assays. Upon reviewing methods summarized by Mikutta, et al. (2005), we used H_2O_2 analysis methods (Methods 1 and 2, Table 1) adapted from Kunze & Dixon (1986) and Leifeld & Kogel-Knabner (2001) for initial analysis using 30% H_2O_2. This method proved to be time-consuming and labor intensive for the practical outcome intended in this study. Subsequently, we evaluated methods using 3% H_2O_2 onward while altering the amount of additional solution added and heating time in the oven (Methods 3, 4, and 5) (Figure 1). However, results were inconsistent, and a conversion/correction factor was not calculatable.

The inability to consistently degrade OM with H_2O_2 was likely due to insufficient reaction time when using 3 or 6 % instead of 30% H_2O_2 (Figure 2). With further testing we concluded that any reaction using these concentrations would require overnight heating at 60°C and additional H_2O_2 should be added at the 4th and 8th hour during digestion (Method 6, Table 1). An incubator shaker (New Brunswick Scientific, Edison, NJ 08817) (Figure 3) was used to evaluate if continuous agitation would decrease variability and increase reaction efficiency (Method 7, Table 1), as well as reducing sample size (Method 7.1, Table 1). We found a 12% H_2O_2 solution (Viva Dora, Redmond, WA) that is readily available for purchase online (Amazon.com) and used this concentration to further improve reaction efficiency (Method 7.2, Table 1). Agitation greatly improved the consistency of data collected and ensured a sufficient reaction that reduced sample weight close to the LOI confirmed amount. However, an incubator shaker is not normally available on a typical golf course, and thus the final method developed agitates samples by handshaking at the 4th and 8th hour after reaction initiation (Method 8):

1. Place 5 grams of sample into a beaker, replicate three times.
2. Add 20 ml of 12% H_2O_2 to beakers to start the reaction
3. Place samples and controls into an oven at 60°C
4. Agitate by handshaking the beaker while adding an additional 20ml of 12% H_2O_2 4 hours later
5. Repeat step 4 at 8 hours after reaction started
6. Continue in oven overnight.
7. Measure results 24hr after reaction started

Samples containing 1.5%, 3.0%, and 6.0% OM confirmed by LOI were evaluated using the described method (Table 2). Data were fitted with a linear regression model: $y = 0.5038x - 0.0677$, where y is percent OM from the H_2O_2 method, x is percent OM measured by LOI (Figure 4). The $R^2 = 0.6044$ indicating nearly 40% of the variability associated with the H_2O_2 method could not be accounted for or explained.

The R^2 suggests a high level of variation when using this method to quantify OM in a lab mixed sample with a pre-determined percentage OM. A reliable conversion factor using the H_2O_2 method that correlated to LOI could also not be obtained because of the inherent variability associated with the developed method. Soil samples from golf course greens are likely to introduce more variability compared to lab mixed samples. Therefore, we conclude that a rapid, practical, inexpensive, and reliable method to test OM content on golf courses cannot be achieved using hydrogen peroxide and equipment available on a typical golf course. To ensure the accuracy of soil OM content results, golf course superintendents should use reliable laboratories for testing for OM content.

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Table 1. Methods repeated to degrade organic matter using hydrogen peroxide.

Method 1	Adapted from Kunze & Dixon (1986) and Leifeld & Kogel-Knabner (2001) 1. Place ~10 mL of sample into a beaker 2. Add 30% H_2O_2 in a 1:1 ratio to the sample weight 3. Add H_2O in a 1:1 ratio to sample weight 4. Place in an oven at 65°C for 24-h
Method 2	Adapted from Kunze & Dixon (1986) and Leifeld & Kogel-Knabner (2001) 1. Place ~10 mL of sample into a beaker 2. Add 30% H_2O_2 in a 1:1 ratio to the sample weight 3. Add 10 mL H_2O into sample and stir with microspatula a. At this point significant frothing occurred for ~12 min 4. Add 5 mL H_2O_2, stir a. Bubbling occurred, but not much frothing 5. At 10 min add 5 mL H_2O_2, stir 6. At 10 min add 5 mL H_2O_2 , stir 7. Place sample in oven @ 65°C for 35 min 8. Remove from oven and sit at room temperature overnight 9. The next day, place back into the oven for 2 hours a. This was done because there was still liquid and sample in the beaker
Method 3	1. Place ~10 mL of sample into a beaker 2. Add 20 mL of 3% H_2O_2 to beaker a. 3% replaced 30% H_2O_2 at this point in order to see if it were a viable option 3. Agitate sample immediately with a microspatula for 10 min 4. Add another 20 mL of 3% H_2O_2 5. Agitate sample again for an additional 10 min 6. Place into oven at 60°C 7. Remove from oven 24-h later
Method 4	1. Place ~10 mL of sample into a beaker 2. Add 20 mL of 3% H_2O_2 to beaker 3. Agitate sample immediately with a microspatula for 10 min a. Use distilled water to remove particles from the side of the beaker and the microspatula 4. Place into oven at 60°C 5. At 1-hr check sample for frothing and reagitate a. Repeat this step 3 times 6. Add 10 mL H_2O_2 to the beaker and place back in the oven for 2 hours

Method 5 (Figure 2)	1. Place ~10 mL of sample into a beaker 2. Add 40 mL of 3% H_2O_2 to beaker 3. Agitate sample 4. Place into oven at 60°C overnight
Method 6	Adding incremental H2O2 (20ml) every 4 hours 1. Place 10 gram of sample into a beaker. Three treatments and a control. 2. Add 20ml of 3% H_2O_2 to beakers to start the reaction 3. Agitate samples 4. Place samples and the control into oven at 60°C 5. Agitate by hand every hour. Add 20ml of 3% H_2O_2 at the 4 and 8 hours after reaction starts (The use of microspatula was discontinued due to floating OM tend to attach to the microspatula) 6. Overnight after 8 hours 7. Measure results 4, 8, and 24hr after reaction starts
Method 7 (Figure 3)	An incubator shaker was used (New Brunswick Scientific, Edison, NJ 08817) 1. Place 10 gram of sample into a beaker, with three replications 2. Add 20ml of 3% H_2O_2 to beakers to start the reaction 3. Place samples and three controls into a heated shaker at 60°C and 125 RPM speed 4. Add 20ml of 3% H_2O_2 at the 4 and 8 hours after reaction starts 5. Overnight after 8 hours 6. Measure results 24hr after reaction starts
Method 7.1	Same as Method 7 except using 5 grams of sample in step 1
Method 7.2	Same as Method 7.1 except using 12% H_2O_2 solution (Viva Dora, Redmond, WA 98052) purchased from Amazon.com
Method 8	1. Place 5 grams of sample into a beaker, with three replications 2. Add 20 ml of 12% H_2O_2 to beakers to start the reaction 3. Place samples and controls into an oven at 60°C 4. Agitate by hand shaking the beaker while adding an additional 20ml of 12% H_2O_2 4 hours later 5. Repeat step 4 at 8 hours after reaction started 6. Overnight after 8 hours 7. Measure results 24hr after reaction started

Table 2. Data collected using method 8. Lab mixed samples with pre-determined OM content (1.5, 3, or 6%) treated with a total of 60 ml of 12% H_2O_2 added at 0, 4, and 8 hours after reaction started. Reactions were conducted in an oven at 60°C. Data were collected 24 hours after reaction started.

Treatment	$\%OM_{sample}$	Rep	$Mass_{Beaker}$ T_0	$Mass_{sample}$ T_0	$Mass_{Total}$ T_1	$Mass_{sample}$ T_1	$Mass_{sample}$ $(T_0\text{-}T_1)$	OM Lost to H_2O_2
			g					%
Control	1.5	1	49.334	5.015	54.334	5.000	0.015	0.30
Control	1.5	2	46.138	4.982	51.104	4.966	0.016	0.32
Control	1.5	3	49.749	5.042	54.777	5.028	0.014	0.28
H_2O_2 treated	1.5	1	46.905	4.990	51.838	4.933	0.057	1.14
H_2O_2 treated	1.5	2	47.072	4.993	52.021	4.949	0.044	0.88
H_2O_2 treated	1.5	3	47.706	4.900	52.704	4.998	-0.098	-2.00
H_2O_2 treated	1.5	4	46.099	4.996	51.044	4.945	0.051	1.02
H_2O_2 treated	1.5	5	48.899	4.994	53.842	4.943	0.051	1.02
H_2O_2 treated	1.5	6	48.456	4.907	53.381	4.925	-0.018	-0.37
H_2O_2 treated	1.5	7	47.845	5.003	52.793	4.948	0.055	1.10
H_2O_2 treated	1.5	8	50.119	5.005	55.088	4.969	0.036	0.72
H_2O_2 treated	1.5	9	45.405	4.990	50.346	4.941	0.049	0.98
H_2O_2 treated	1.5	10	48.403	5.005	53.354	4.951	0.054	1.08
Control	3.0	1	50.665	5.055	55.704	5.039	0.016	0.32
Control	3.0	2	49.368	5.005	54.354	4.986	0.019	0.38
Control	3.0	3	48.578	4.994	53.558	4.980	0.014	0.28
Control	3.0	4	50.661	5.028	55.672	5.011	0.017	0.34
Control	3.0	5	49.364	5.053	54.407	5.043	0.010	0.20
Control	3.0	6	48.574	5.006	53.560	4.986	0.020	0.40
H_2O_2 treated	3.0	1	49.331	4.963	54.167	4.836	0.127	2.56
H_2O_2 treated	3.0	2	49.745	4.991	54.621	4.876	0.115	2.30
H_2O_2 treated	3.0	3	49.376	4.962	54.266	4.890	0.072	1.45
H_2O_2 treated	3.0	4	49.334	5.053	54.288	4.954	0.099	1.96
H_2O_2 treated	3.0	5	46.138	5.009	51.126	4.988	0.021	0.42
H_2O_2 treated	3.0	6	49.749	5.024	54.668	4.919	0.105	2.09
H_2O_2 treated	3.0	7	46.905	5.006	51.820	4.915	0.091	1.82
H_2O_2 treated	3.0	8	47.072	4.997	51.982	4.910	0.087	1.74
H_2O_2 treated	3.0	9	47.706	5.009	52.708	5.002	0.007	0.14
H_2O_2 treated	3.0	10	46.099	5.040	51.057	4.958	0.082	1.63
H_2O_2 treated	3.0	11	48.899	5.054	53.866	4.967	0.087	1.72
Control	6.0	1	50.665	5.010	55.645	4.980	0.030	0.60
Control	6.0	2	49.366	5.041	54.383	5.017	0.024	0.48
Control	6.0	3	48.576	4.997	53.545	4.969	0.028	0.56
Control	6.0	4	50.661	5.075	55.705	5.044	0.031	0.61
Control	6.0	5	49.364	4.987	54.326	4.962	0.025	0.50

Control	6.0	6	48.574	5.007	53.557	4.983	0.024	0.48
H_2O_2 treated	6.0	1	49.316	5.025	54.139	4.823	0.202	4.02
H_2O_2 treated	6.0	2	49.731	5.008	54.617	4.886	0.122	2.44
H_2O_2 treated	6.0	3	49.369	5.001	54.204	4.835	0.166	3.32
H_2O_2 treated	6.0	4	48.456	5.061	53.402	4.946	0.115	2.27
H_2O_2 treated	6.0	5	47.845	5.043	52.708	4.863	0.180	3.57
H_2O_2 treated	6.0	6	50.119	4.958	54.958	4.839	0.119	2.40
H_2O_2 treated	6.0	7	45.405	5.035	50.311	4.906	0.129	2.56
H_2O_2 treated	6.0	8	48.403	5.008	53.270	4.867	0.141	2.82
H_2O_2 treated	6.0	9	48.114	4.978	52.937	4.823	0.155	3.11
H_2O_2 treated	6.0	10	48.410	5.029	53.280	4.870	0.159	3.16
H_2O_2 treated	6.0	11	50.828	4.995	55.714	4.886	0.109	2.18

$\%OM_{sample}$: pre-determined percentage of organic matter in the lab mixed sample

$Mass_{Beaker}\ T_0$: the mass of the beaker before reaction

$Mass_{sample}\ T_0$: the total mass of sample before reaction

$Mass_{total}\ T_1$: the total mass of the beaker and sample after completion of the reaction

$Mass_{sample}\ T_1$: the mass of sample after completion of the reaction

$Mass_{sample\ (T_0-T_1)}$: the difference in mass before and after the reaction

$$\text{OM Lost to } H_2O_2 = \frac{Mass_{sample}\ T_0}{Mass_{sample}\ T_0} \times 100\%$$

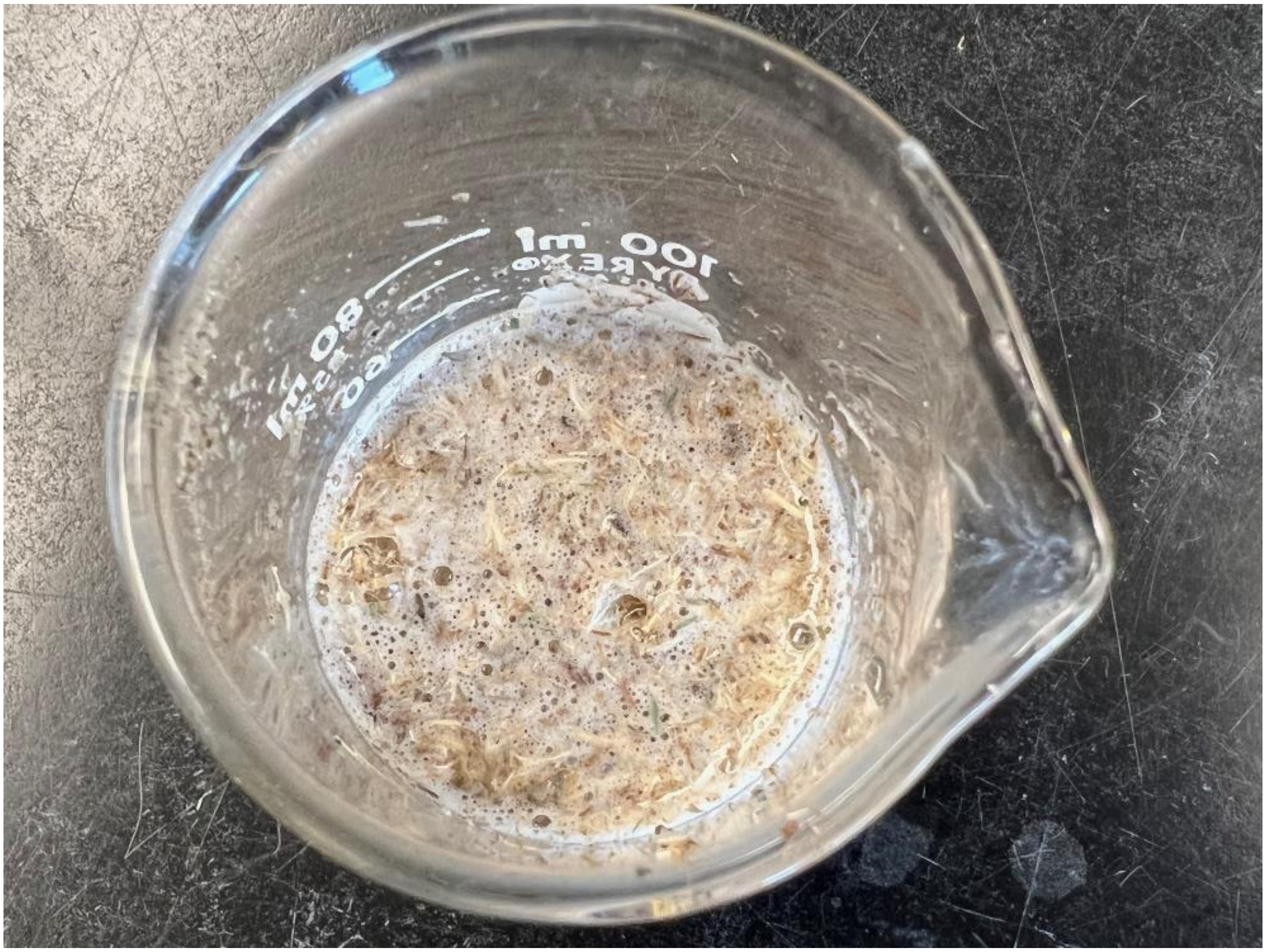

Figure 1. Bubbling due to degradation of organic matter by a 3% hydrogen peroxide solution. Lab mixed samples with a pre-determined 3% organic matter were tested.

Figure 2. Beaker contains lab mixed samples with a pre-determined 3% organic matter left overnight reaction with H_2O_2. A total of 40 ml of 3% H_2O_2 solution was added to the sample and heated at 60°C overnight. Note the quantity of undigested organic matter

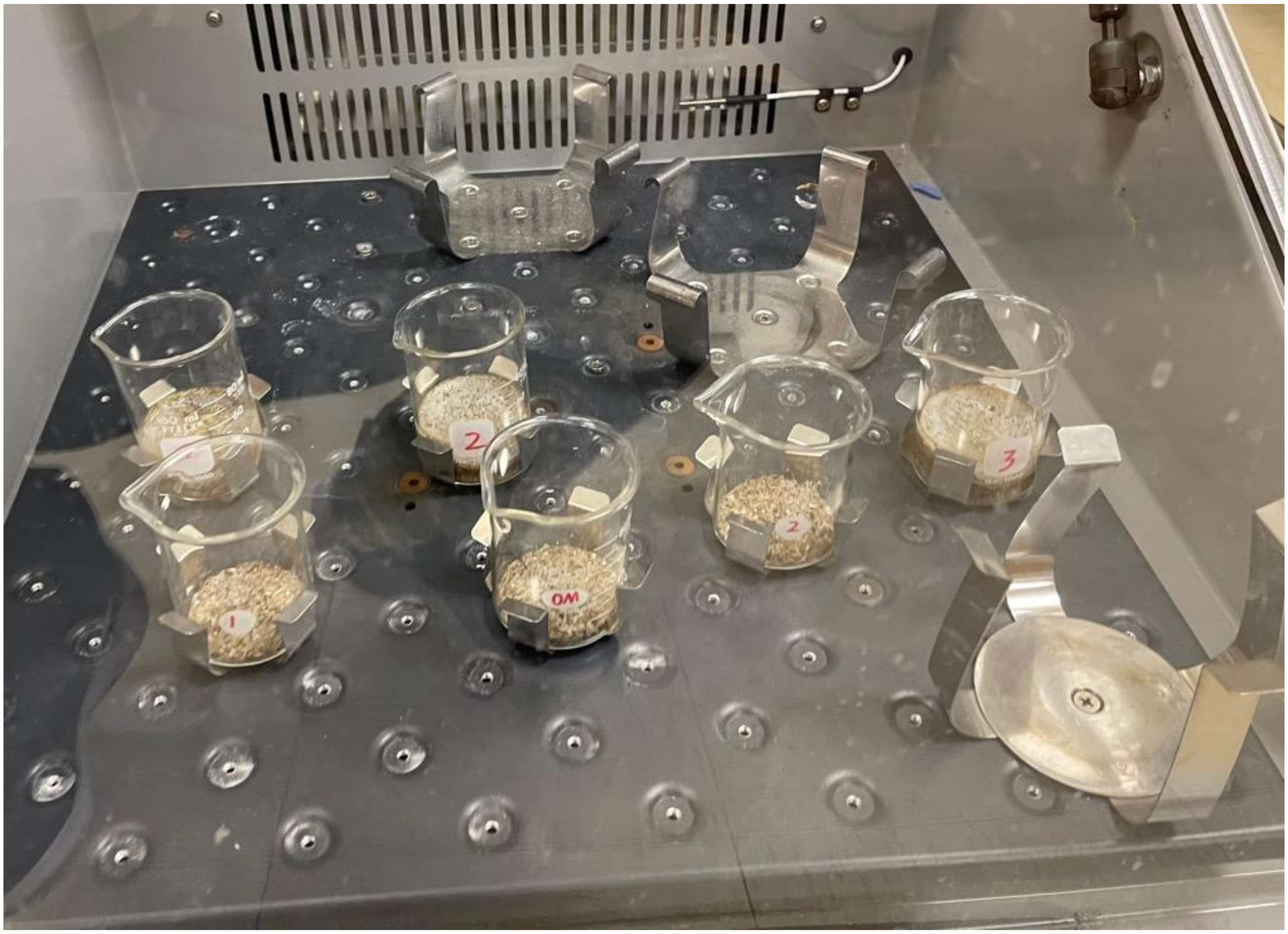

Figure 3. Lab mixed samples with pre-determined percentage organic matter on an incubator shaker (New Brunswick Scientific, Edison, NJ 08817)

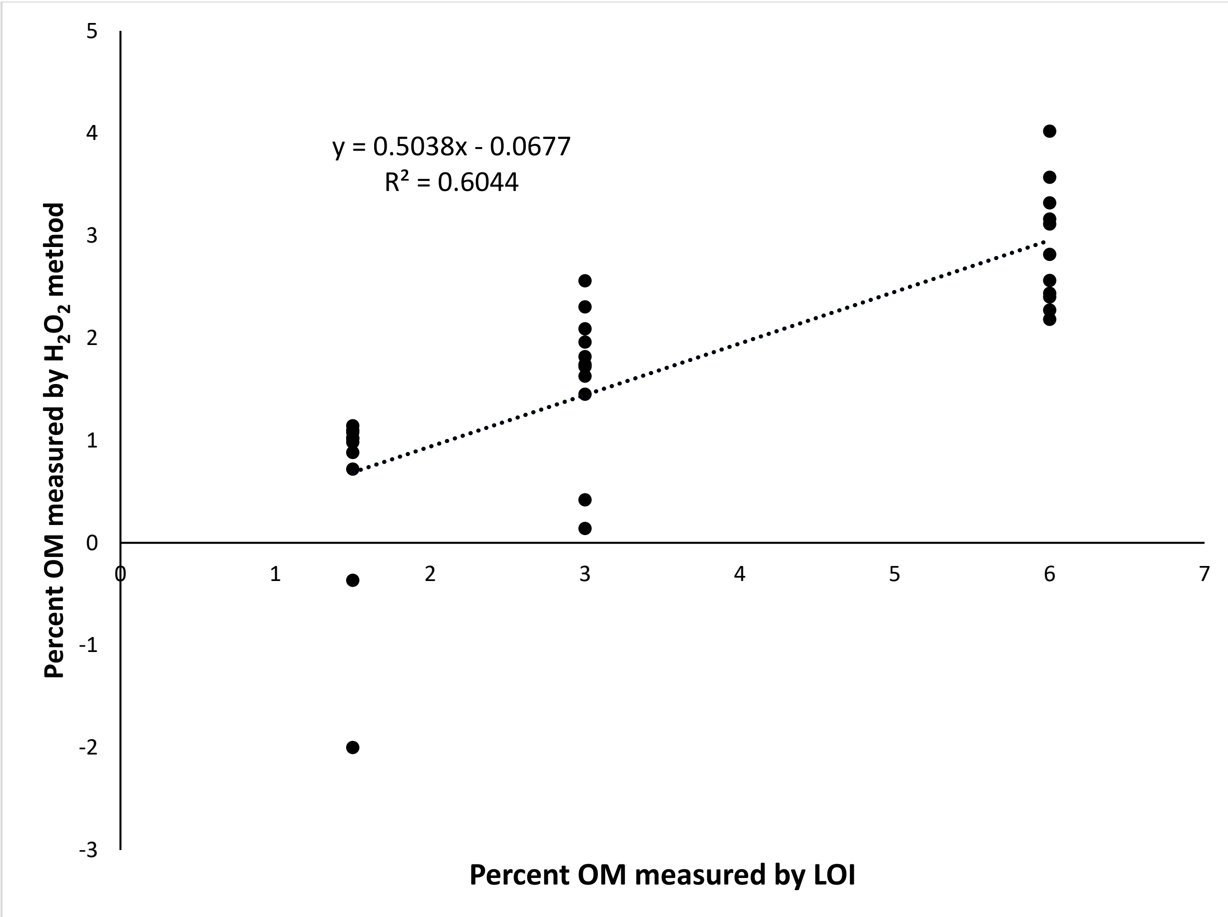

Figure 4. Linear regression of percent organic matter (OM) measured by H_2O_2 described (y) and percent OM measured by loss on ignition (LOI)(x).

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.*

Summary of Activities 2021

New Graduate Student

A new graduate student, Karem Meza Capcha, began work on the study in spring 2021. She helped install the sensors in the field, conducted all the leaf are measurements, coordinated with the golf course to clean the sensors, and is in charge of analyzing the ground-based data, getting the satellite data, and running the remote sensing ET models. She will expand her activities in 2022.

I. Measurements of ET and Energy Balance

Research was 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 2.6m. These instruments were installed on June 17, 2021, and were sampled at a rate of 20 Hz. Eddy covariance measurements were taken during the growing season (June 17 to October 27). An image of the station and instruments is shown in Figure 1.

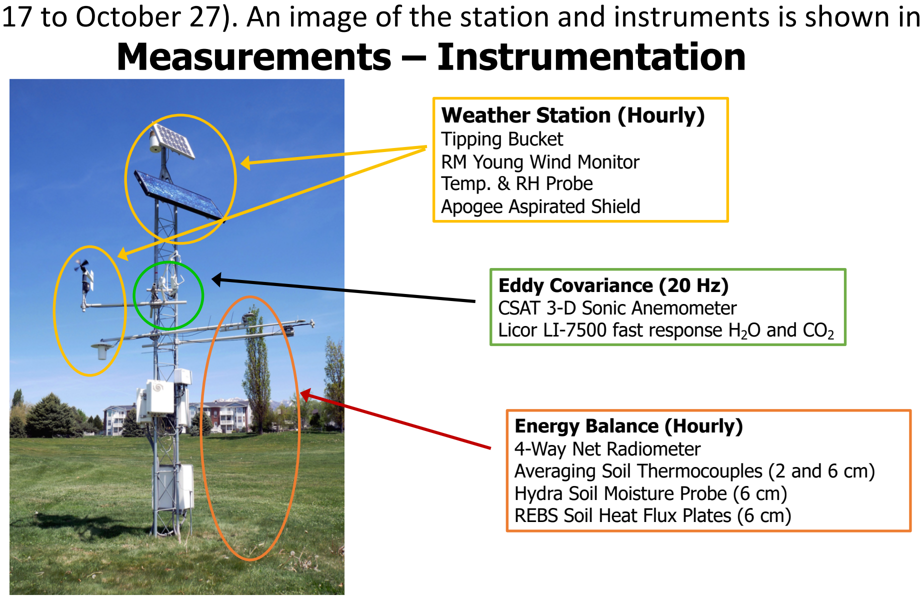

Figure 1. The eddy covariance and weather station.

Some extra time was required in 2021 to get sensors calibrated, purchase several new ones, and arrange some logistical details and changes in irrigation near our station with the golf course superintendent. We also have to move the location of some of the measurements, as described below. As a result, complete data collection began of 17 June 2021.

The location of the eddy covariance and energy balance measurements on the golf course are denoted in Figure 2. The most common wind direction is also represented.

Figure 2. Location of Measurement Station

Net Radiation and Soil Heat Flux

In 2021, the radiation and soil measurements were moved and reinstalled, due to irrigation issues at the actual tower. Net radiation measurements made with a SN-500 net radiometer (Apogee Instruments Inc., Logan, UT, USA) at a height of 1.45m (Figure 3). Due to irrigation issues around the EC tower, the sensor was set up approximately 20 m from the EC tower (Figure 4).

Figure 3: Net Radiometer

Figure 4. Locations of Eddy Covariance, and New Location for Radiation and Soil Sensors

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, and two TDR-310H soil water content (Acclima, Inc., Meridian, ID) at 0.04 m and 0.1 m (Figure 5). Due to irrigation issues around the EC tower, soil measurements were made at the same location as the net radiation (Figure 4).

Figure 5: Installation of soil moisture and temperature sensors

II. Irrigation Issues 2021

Northern Utah, like much of the western US, has experienced drought conditions, reaching a serious level during the summer of 2021. As a result, supplies of water were more limited for many users. The summer was also characterized by very hot temperatures, which created a large atmospheric demand for water used by plants. The combination of high demand and reduced supplies made it impossible for the golf course to maintain green turf everywhere. The fairways were kept green, but the driving range and some other places periodically has some "brown" patches during the middle of the summer. There can be seen by looking at the above Figure 4.

This provided a more complicated situation to interpret the results. However, it also offers an opportunity to examine the realism of future water limitation for golf courses.

II. Remote Sensing Data

USU Remote Sensing UAV

In addition to satellite information, we added data from research quality UAVs to allow very high spatial resolution of the golf course. The purpose for this is to quantify the spatial variations that lie underneath the coarser resolution 30 m or more) of the satellite data. This will allow us to make better interpretations of the combined results. It also will demonstrate the feasibility of using quality drone data and remote sensing models to estimate ET for a given location such as a golf course. USU has developed a drone fitted with high quality spectral cameras to acquire information of the surface at very high spatial resolution. The aircraft is a *Matrice 600 Pro*, with an *Altum* camera measuring six wavebands. The specifications of the camera are shown in Table 1.

Table 1. Specifications of the Altum multispectral sensor used in this study

Band Name	Center Wavelength (nm)	Bandwidth FWHM (nm)	Resolution
Blue	475	32	2064 x 1544
Green	560	27	2064 x 1544
Red	668	14	2064 x 1544
Red - Edge	717	12	2064 x 1544
Near - Infrared	842	57	2064 x 1544
LWIR Thermal Infrared	11,000	3,000	160 x 120

The system was flown two days (September 3 and October 21) during 2021. All spectral data are processed. A simple image combining reflectance of red, green, and blue is shown in Figure 6.

Figure 6. RGB image from drone images on September 3 (left) and October 21, 2021 (right)

Note that the hotter temperatures reduced after July, and some rain fell in August. By September 3, very little of the surface is brown due to lack of water. Soon after that, the entire course region was green as seen on the right image. Figure 7 shows the images for NDVI (related to green leaf area) and surface temperatures for 3 September 2021.

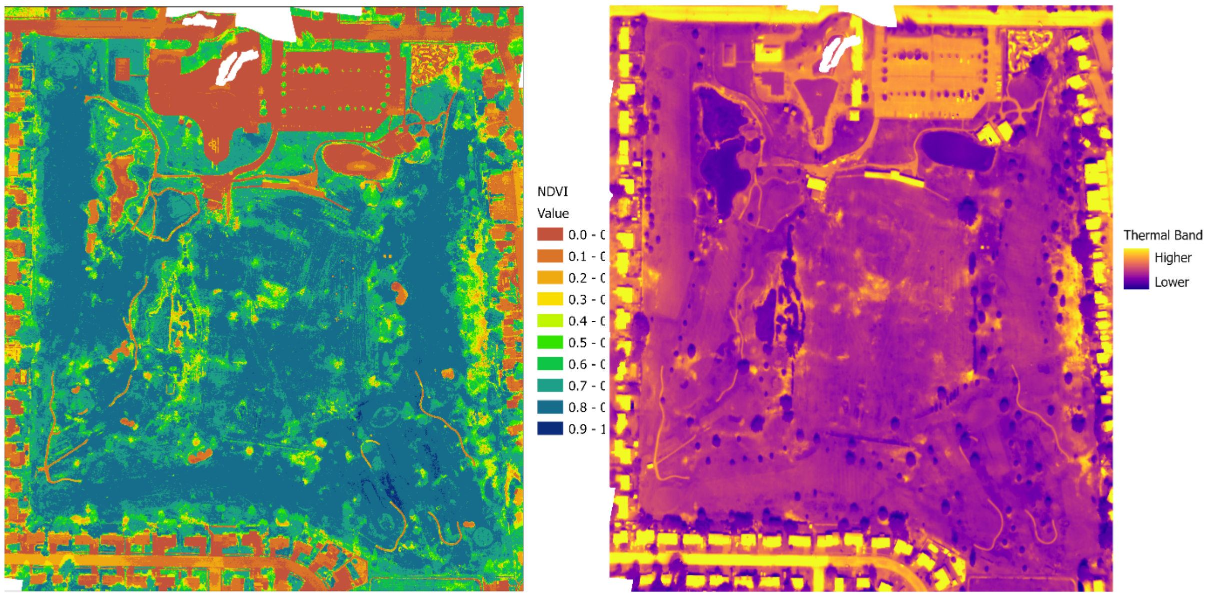

Figure 7. NDVI and Thermal Band from UAV images on September 3rd, 2021

Satellite Information

Data from several satellites were available during the summer. These included *Landsat 8*, (16-day coverage) *Sentinel II (2 – 3 day coverage)* and *Modis* (1-2 day coverage). These have been acquired and will be used to drive the remote sensing ET model. In addition to the Triangle Method, discussed in earlier reports, we will incorporate a more recent model which had already bee used by researchers for various surfaces. It is called the two-source model or TSEB, and based on the paper of Kustas and Norman (1999).

III. Estimation of Leaf Area Index

The leaf area index (area of green leaves per unit ground area) often referred as LAI, is a critical input into the TSEB model. Often, the value of LAI is estimated from remote sensing data, usually the NDVI. However, little research has been conducted for this approach on turfgrass. This requires actual measurements of LAI at various locations to fit a relationship with the remote sensing NDVI. Typical indirect measurements will not work due to the short height of a turfgrass canopy. So, destructive methods needed to be used here.

Ten samples with four replications were collected at Eagle Lake Golf Course. The turfgrass was sampled using a circular sampler (area of 78.54cm) and the sample coordinates were measured using an AeroPoint GCP (Propeller Aero, Sydney, NSW, Australia). Leaves were cut with scissors and leaves were ordered on white and transparent panels. Digital pictures were taken for digital image analysis using open-source software ImageJ and a ruler was used to calculate the total leaf within the sample. The results using the replications indicated the method has a high precision. We are currently analyzing the LAI and NDVI values to determine the relationship between them This will allow LAI to be determined from the remote sensing data, and then provided as input into the TSEB ET model.

Daily ET From Eddy Covariance Measurements
The daily ET values are shown for 2021 below in Figure 8. The data began on June 17, 2021.

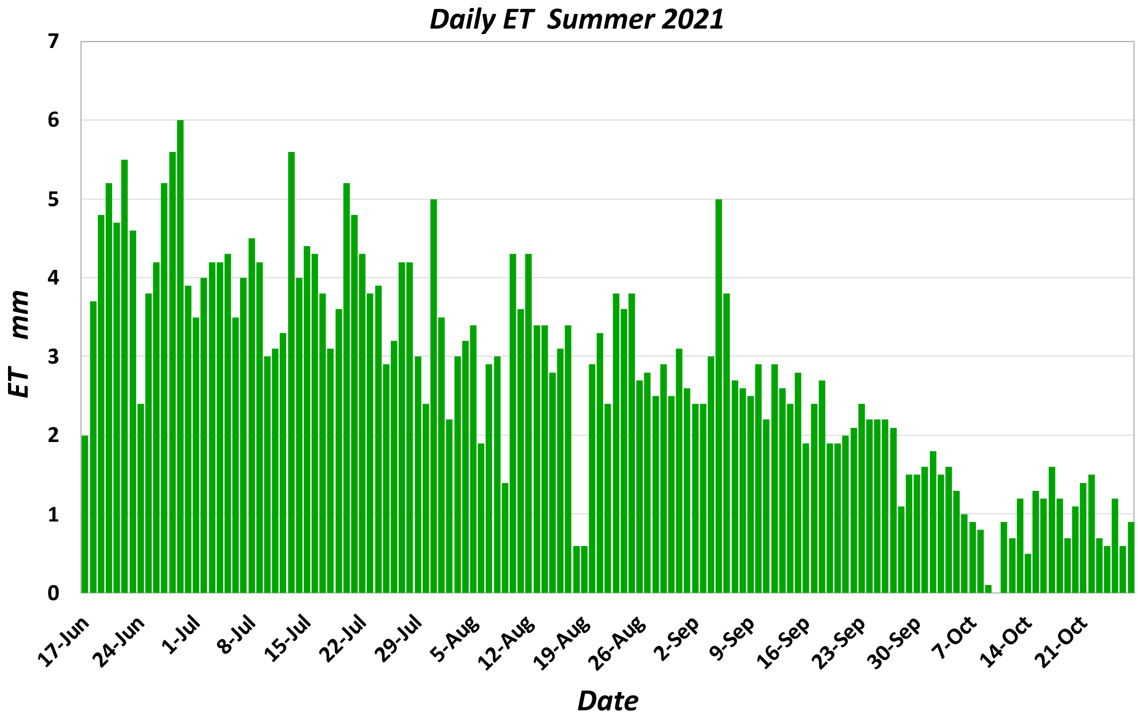

Figure 8. Daily ET values for 2021

Daily values averaged about 3.3 mm during the warmest period – mid-June to mid-September. But they ranged from under 2 mm to over 5 mm during those times. From mid-September to the end of October they gradually decreased from about 2 mm to less than or equal to 1 mm. The sum for the entire period of 132 days was about 374 mm or just under 15 inches.

Of course, these data only commenced on 17 June. We can make estimates for the earlier dates bases on weather conditions. At this point, a rough estimate for the entire season starting in mid-April would be about 539 mm or about 21 inches.

Plan of Work 2022

All eddy covariance and energy balance measurements will be finalized. The imagery from the USU UAV is initially processed and will be analyzed to calculate the spatial variation of the inputs to the remote sensing models.

Leaf Area Index
More measurements of this property will be made at various times in 2022. These will be combined with those made in 2021 to produce an adequate number of data points. A relationship will be fit to predict the leaf area index form key remote sensing reflectance values. These values then are input into the TSEB ET model.

Remote Sensing Models of ET
We will use the same Triangle Method reported last year, for the 2021 season. This will add more data points to verify its ability to predict the ET. Then, two sets of ET calculations will be done. One uses the *Open ET* (https://openetdata.org/) platform that several people at Utah State have contributed towards. These calculations are done by Open ET and provide monthly ET estimates. These will be validated with our data.

We will also run the two Source model, TSEB, referenced earlier, for every day we have either Landsat 8, MODIS, or Sentinel II images. The resulting ET values can be extrapolated to daily and will be validated with our eddy covariance estimates. We have a long professional relationship with the developers of TSEB at the USDA in Beltsville, MD. In fact, one of them is on the graduate committee of Ms. Mezza Capcha. They are interested in our study, as TSEB has never been tested for urban landscapes. Hence, we can get expert advice and help with using the model should we need such.

The result of the TSEB model will be a spatial distribution of ET over the entire golf course at 30 m resolution. The results lying withing the area sensed by the eddy covariance tower, can be compared with the

Evaluation of Models
We will then evaluate how the different remote sensing ET models perform. How do the weather and gold course conditions affect the performance of the models?

How to Deliver the Information to Users
Although this issue was not officially addressed in this research project, it is important to consider how such advances and information about water use 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.

References

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USGA ID#: 2017-38-648

Title: Integrating canopy dynamics, soil moisture, and soil physical properties to improve irrigation scheduling in turfgrass systems

Project Leaders: W. Dyer[1], D. Bremer[1], A. Patrignani[2], J. Fry[1], C. Lavis[1] and J. Friell[3]

Affiliation: [1]Dept. Horticulture and Natural Resources, Kansas State University; [2]Dept. Agronomy, Kansas State University, [3]Toro Company

Objectives:

1. Determine quantitative turf canopy responses to plant available water from in-situ soil moisture sensors (SMS)

2. Compare SMS-based irrigation scheduling to traditional irrigation and ET-based irrigation scheduling

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

Summary Points:

- During 2019, soil moisture thresholds ranging from 18.5 to 22.5 % volumetric water content (VWC) were determined based the onset of canopy stress relative to available water capacity in the silt-loam soils.
- Incorporation of multiple data-driven thresholds comprised of soil moisture (using thresholds derived in 2019), plant canopy conditions (percent greenness), and forecasted precipitation probability into a decision tree during the 2020 year, as opposed to a single variable (SMS) threshold in 2019, resulted in better irrigation timing and water savings.
- Specifically, compared with traditional irrigation, 81% less water was applied in 2020 when using multiple thresholds (VWC, canopy greenness, forecasted precipitation) compared to 66% less water applied in 2019 when using only VWC, despite 52% less precipitation in 2020 than 2019.

Summary Text:

Rationale

Current irrigation strategies used by golf courses and athletic fields often rely on calendar schedules or deficit irrigation strategies that completely ignore soil moisture conditions. Integrating information from soil moisture sensors (SMS) to existing irrigation techniques has the potential to substantially improve the timing and amount of each irrigation event. We are developing an innovative approach that integrates soil moisture along with additional components of the plant (canopy greenness) and atmosphere (forecasted precipitation) to generate better turfgrass irrigation decisions. We hypothesize that combining components from each of the soil-plant-atmosphere continuum will improve irrigation scheduling and reduce total water use relative to calendar schedules.

Comparing Treatments

- No irrigation treatment (Check)
- Traditional irrigation treatment, well-watered 0.5 inches 2x $week^{-1}$ (Traditional)
- Deficit irrigation treatment, 60% of reference evapotranspiration (ET_o) (60% ET)
- Developing a decision tree irrigation treatment, soil moisture-plant canopy greenness-forecasted precipitation [DT(SMS)]

Year 2021 overview

Field research was finalized at the end of the growing season in 2020. Year 2021 focused on data analysis and evaluating key concepts. One concept we sought to better define is the available water capacity sometimes defined as plant available water. Plant available water is defined by water held between field capacity and permanent wilting point. However, when characterizing plant available water there are limitations when defining field capacity and permanent wilting point. An article by Dr. Gaylon Campbell discusses these limitations/constraints {Field Capacity/Permanent Wilting Point: Do Standards Need to Changed? (environmentalbiophysics.org)}. Critical values for crop growth are associated with the soil moisture at field capacity (-10 or -33 kPa), however the concept of field capacity is flawed because there are no clear breakpoints for when gravitational drainage ceases (Ochsner, 2019). An often used standard that some labs will take for fine textured soils is -10 kPa; a fine textured silt loam soil was defined for our research plots. However, we explored the concept of the least limiting water range, which defines the region bounded by upper and lower soil water content in which water, oxygen, and mechanical resistance become major limiting factors for root growth (da Silva et al., 1994). In Figure 1, we set the upper limit at 10% air-filled porosity based on the least water limiting range and the lower limit at -1500 kPa. However, we realize this may not translate as well to those who use the traditional -10 or -33 kPa to define field capacity, which will skew the plant available water range. For example, we determined stress to occur within a range of 0.6 and 0.7 fraction of plant available water (fraction of available water capacity), which is slightly higher than the typical value of 0.5 used as an approximate threshold for vegetative moisture stress (Allen et al., 1998). If we were to use the more traditional -10 kPa value for our upper limit, we would see the turfgrass canopy stress occur closer to the 0.5 value. We will likely redefine the upper limit as -10 kPa, however the 10% air-filled porosity can be a good threshold target to fill the plant rootzone back to once irrigation is needed, which was our approach throughout this study.

Field Research Results Year 2020

Throughout this study we continuously looked to better define when irrigation should be triggered. Instead of relying on a single variable, (i.e., ET rates or soil moisture) we incorporated a simple irrigation decision tree to guide our irrigation management decisions by using one component each from the soil-plant-atmosphere continuum (VWC -Green canopy cover-Precipitation Forecast) (Figure 2). This approach was tested during the 2020 growing season, after gaining a more thorough understanding of the response of the plant canopy to soil moisture deficits in 2019. Total precipitation during the 2020 field study (i.e., 1 June to 31 August) was 305 mm, with highest rainfall events occurring in July (Figure 3). The longest consecutive period without rainfall was 11 days in late August. Supplemental irrigation for each irrigation treatment during 2020 was 268 mm in traditional, 153 mm in deficit 60% ET, and 51 mm in DT(SMS) plots. Thus, irrigation with the DT(SMS) irrigation approach saved 81% of water compared with traditional irrigation scheduling and 67% of water compared with the 60% ET-based irrigation scheduling. Interestingly, water savings were greater in 2020 than 2019, despite 52% less precipitation in 2020 than in 2019. This outcome is counterintuitive but can be attributed to the incorporation of the DT(SMS) method as described below. Thus, in 2019 the SMS treatment saved 66% of water compared to the traditional, whereas in 2020 the DT(SMS) treatment saved 81% compared to the traditional.

To illustrate how the decision tree guided our irrigation management decisions, Figure 4 depicts when irrigation was applied during the 2020 year. Pronounced soil drying occurred only during two periods in 2020, including at the end of June and again in late August (Figure 4B). During these periods, VWC in the DT(SMS) reached the VWC threshold for triggering irrigation in the DT(SMS) treatment, noted by the four arrows (Figure 4B). However, the two blue arrows were the only periods where all thresholds for triggering irrigation in the decision tree were met during the 2020 growing season. During these two periods, soil moisture in DT(SMS) reached the VWC threshold range, GCC had dropped below 5% of the traditional treatment, and a chance of rainfall was < 50%. However, the two black arrows denote when VWC for the DT(SMS) treatment reached the VWC threshold range, but GCC had not dropped below 5% of the traditional treatment, indicating canopy stress was not yet observed. Also, precipitation events shortly followed which allowed us to bypass the necessity for irrigating the DT(SMS) treatment. This demonstrates how delaying irrigation as feasible can improve the chance that rainfall will occur, which further delays the need for irrigation (Chabon et al., 2017).

Among treatments, the number of days in 2020 when GCC fell more than 5% below the traditional frequency-based irrigation treatment was 20 days in the check treatment, 2 days in DT(SMS), and 0 days in 60% ET (Figure 5). Therefore, by applying only 51 mm of irrigation in the DT(SMS) treatment, we were able to avoid 18 days of less than desired canopy cover.

The information gained from this research may provide turfgrass managers with a more meaningful way of interpreting soil moisture, plant canopy condition, and weather data to enable them to make meaningful changes in their irrigation practices. Future research should explore the best technologies that could add value if incorporated into data-driven irrigation management decisions.

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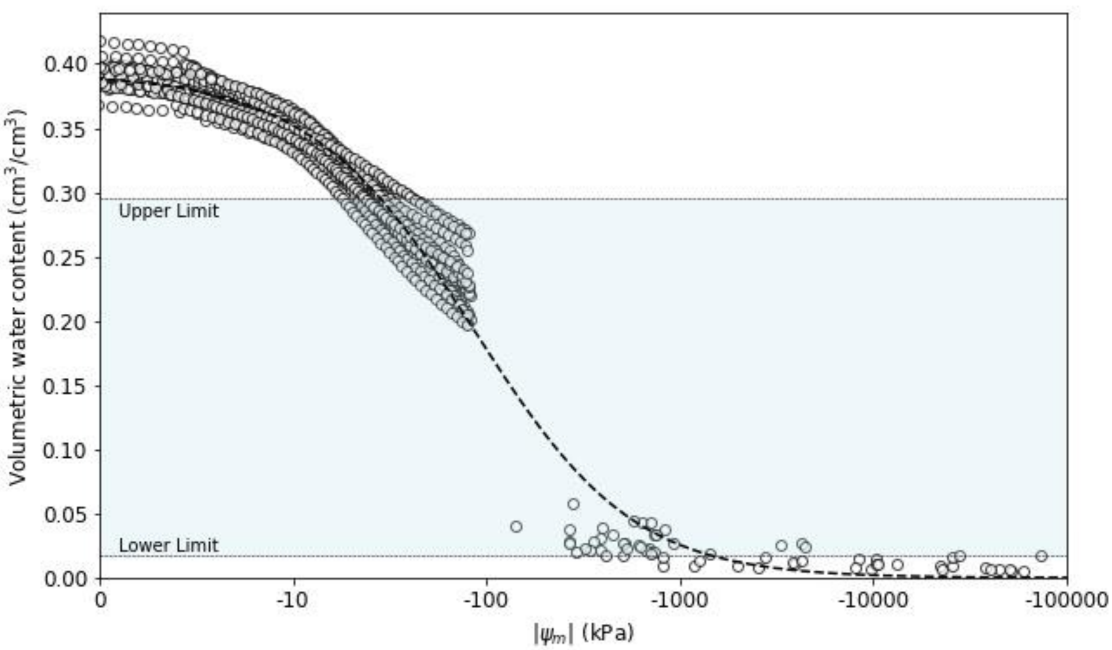


Figure 1. Soil water retention curve for a silt loam soil. Markers represent observations and the dashed line represents the van Genuchten model. The lower limit was estimated as the volumetric water content at -1500 kPa and the upper limit was estimated as the volumetric water content at which the soil has a 10% air-filled porosity. The plant available water capacity is indicated by the shaded area. Note that the typical definition of field capacity using a volumetric water content at -10 kPa would result in air-filled porosity <10% for this soil.

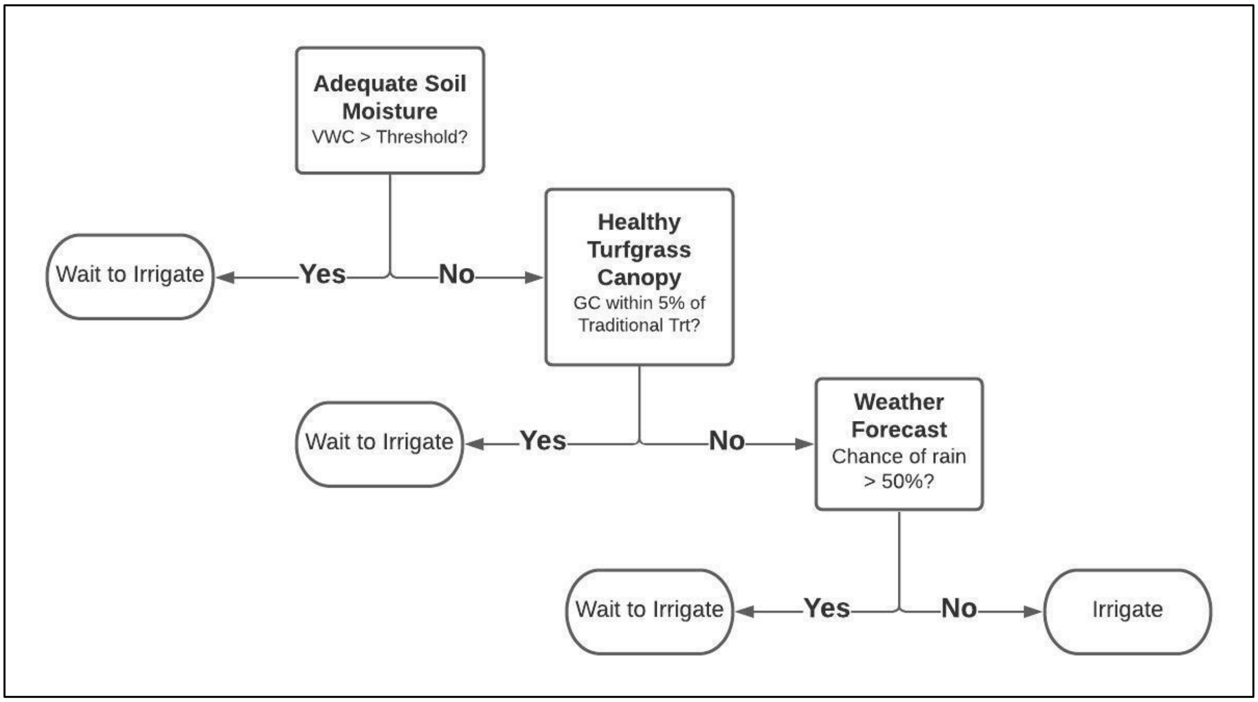


Figure 2. Decision tree sketch to determine irrigation scheduling. Questions centered around the soil-plant-atmosphere continuum.

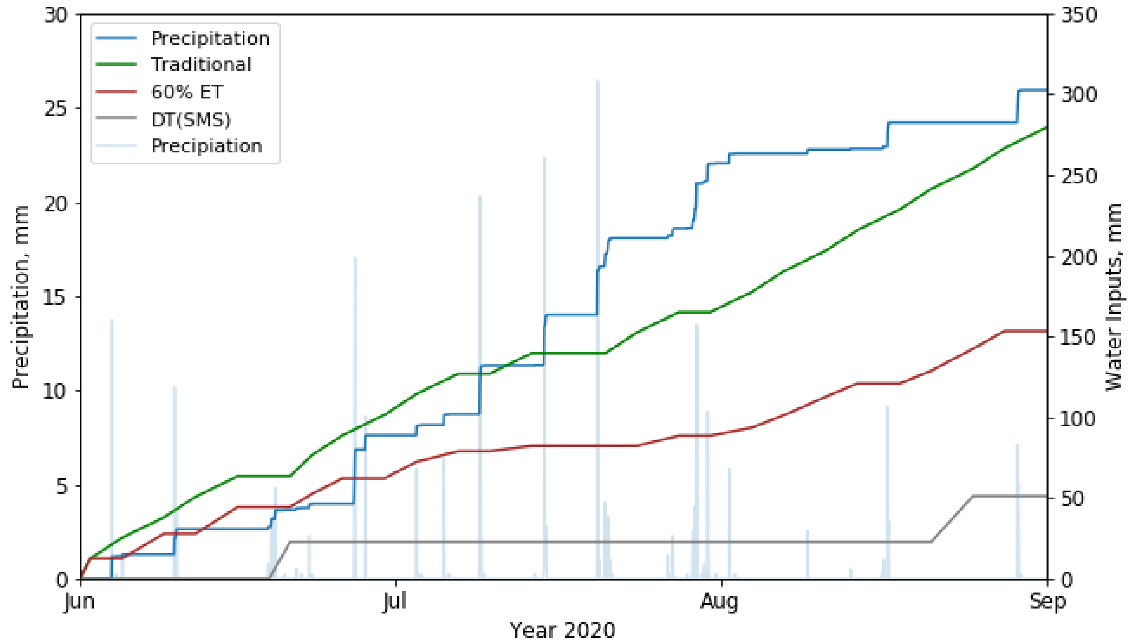


Figure 3. Precipitation and cumulative irrigation during the 2020 field study. Total precipitation was 289 mm, while water applications by irrigation was 268 mm in the traditional treatment, 153 mm in the 60% ET treatment, and 51 mm in the decision tree (SMS) treatment.

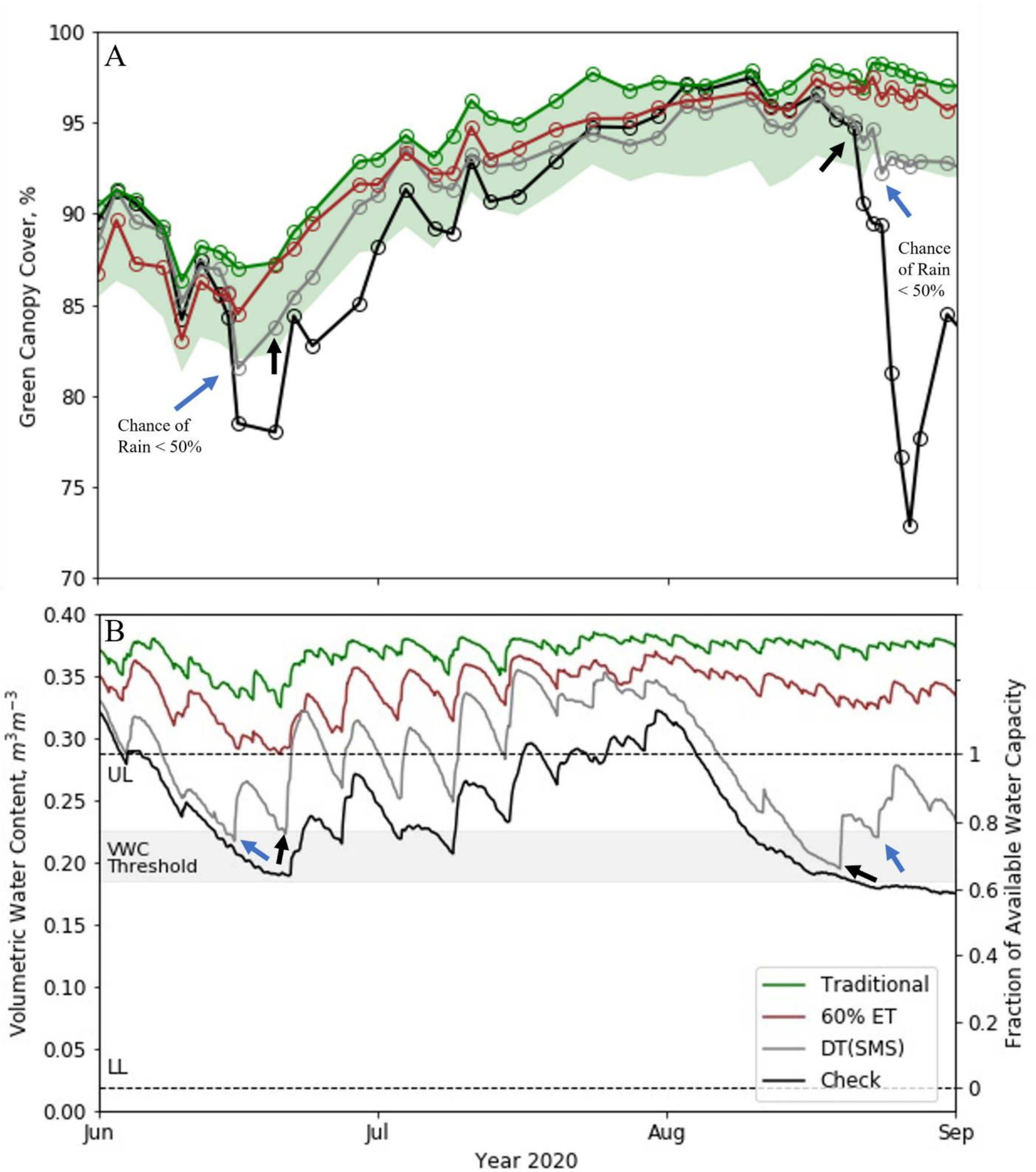


Figure 4. Components of soil-plant-atmosphere continuum through the 2020 growing season (volumetric soil water content, green canopy cover, forecast events). A) The shaded area in green canopy cover denotes the area 5% below the traditional irrigation treatment and the shaded area in volumetric soil water content denotes the threshold range for triggering irrigation. B) Four arrows depict dates when volumetric water content in the decision tree (DT [SMS]) treatment reached the irrigation threshold. Two blue arrows indicate when irrigation was triggered for the SMS treatment and two black arrows indicate GCC was still within the 5% range of the traditional treatment, bypassing the irrigation event.

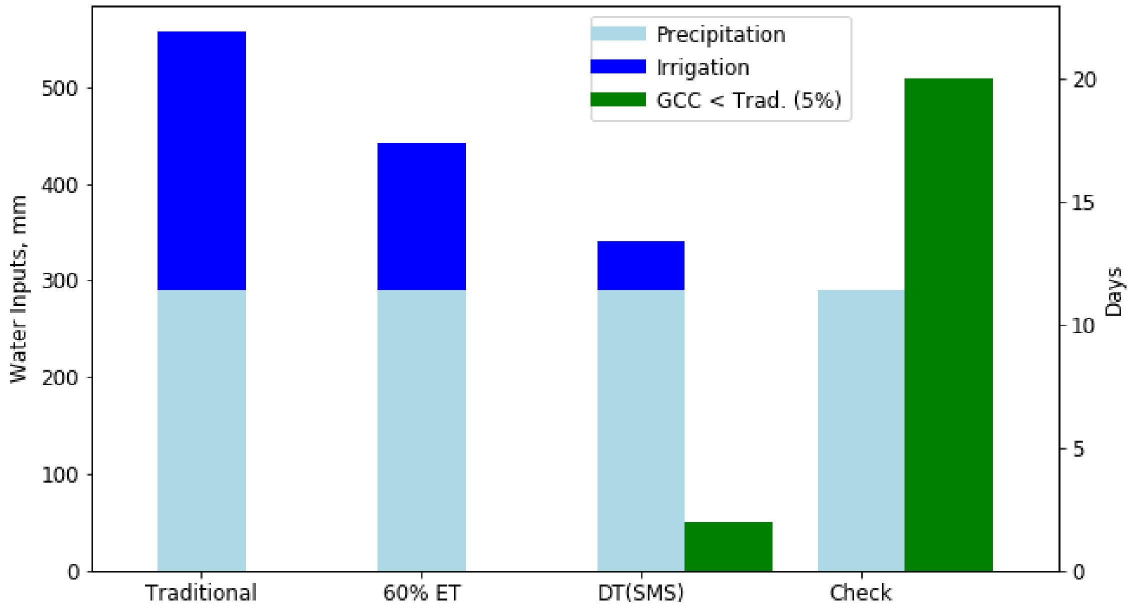


Figure 5. Total water inputs shown for each treatment from precipitation and irrigation. Green bars denote the total number of days when green canopy cover (GCC) in the DT(SMS) and check irrigation treatments was >5% below GCC in the traditional irrigation treatment during 2020; GCC in 60% ET never fell more than 5% below the traditional irrigation treatment.

USGA ID: 2021-06-730

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

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

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

Objectives:

1. Quantify response of turf and course conditions to changes in plant available water.

2. Quantify changes in water consumption between soil moisture sensor (SMS)-based, evapotranspiration (ET)-based, and traditional irrigation scheduling.

Start Date: 2018 (3-year duration + 1-year extension)

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

Summary Points:

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

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

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

- Precision Sense 6000 (PS6000) surveys were conducted twice weekly for each run.

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

- Turf quality data from 2020 and 2021 will be analyzed and a refereed journal article will be submitted in 2022.

Rationale

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

Progress to Date

Fairway Preparation

As described in the 2020 project update, nine fairways (six par 4s and three par 5s) at Edina Country Club in Minneapolis, MN were selected for use in the study and placed into similar groups of three based on size, soil moisture descriptive statistics, and spatial maps of soil moisture variability (Figure 1). Each grouping of three fairways is considered one replication in the study and each fairway within a grouping was assigned one of three irrigation treatments (i.e. randomized complete block design), which were initiated in 2020. Irrigation scheduling treatments for the study include:

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

Soil moisture sensor placement had been previously completed using two course surveys conducted with the Toro PS6000 in 2019. Those surveys provided field capacity-based segmentation and classification (low, moderate, or high soil moisture) of fairways that were assigned to the SMS-based treatment (Figure 2). Toro TurfGuard in-ground SMS were installed 22 Aug. 2019. One sensor was placed in each soil moisture class within each replication (Figure 2), for a total of nine sensors.

Threshold Determination

A dry down was conducted following 3.7 cm of precipitation from 27 May – 2 June 2020 on the SMS fairways, where irrigation was withheld to determine an initial lower threshold for triggering irrigation applications. During the dry down, routine PS6000 surveys were conducted to monitor soil moisture and normalized difference vegetation index (NDVI) across all fairways. The dry down was planned to continue until substantial change was no longer observed in the recorded NDVI values, or the superintendent was no longer comfortable or observed wilt. No additional precipitation occurred during the dry down and PS6000 surveys were conducted on a total of five days throughout the process. Examples of the volumetric water content (VWC) and NDVI maps generated during the dry down are shown for fairway 5 in Figure 3. The TurfGuard sensor VWC values for all nine sensors were also monitored throughout the dry down and values were averaged from midnight to midnight on days those surveys were conducted. Mean NDVI values for each moisture class on each SMS treatment fairway were also calculated on those days. For all soil moisture classes on all fairways, the comfort limit of the superintendent based on observation was reached before notable features were identified in the TurfGuard VWC or PS6000 NDVI data (Figure 4 and 5, respectively). Once the dry down process was stopped, values from each of the nine TurfGuard sensors were recorded and used as the lower threshold triggers for the irrigation zones. These initial values were used as a baseline moving forward in the study, but the dry down process was repeated prior to every run to confirm thresholds or make adjustments as needed (Table 1).

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

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

Irrigation Application & Data Collection

Prior to the initiation of treatments in 2020, the Toro Lynx central irrigation controller was configured to record water use for all irrigation heads, grouped by soil moisture class and fairway. Irrigation treatments were started for run 1 of 2020 on 22 June, following application of 2 mm of irrigation. For the SMS treatment, each time a sensor value dropped below the lower threshold, all irrigation heads assigned to that sensor (i.e. in that fairway and moisture class) were irrigated with 5.1 mm of water. The ET-based treatment irrigation depth was calculated using data from an on-site Campbell Scientific T-107 weather station (Campbell Scientific, Logan, UT) and applied 3 times wk^{-1}. The superintendent was responsible for running the traditional scheduling treatment. For all treatments, the superintendent was allowed to run individual irrigation heads in critical areas, as needed. On 9 Aug., treatments were halted to allow the superintendent time for regular maintenance activities on the course. A second run of treatments began for the fall season of 2020 on 23 Aug., which ended on 16 Oct. In 2021, single runs were conducted in both summer (13 June – 8 Aug.) and fall (16 Aug. – 10 Oct.) following similar procedures as 2020, except the SMS treatment fairways were irrigated with 7.6 mm of water when an irrigation event occurred.

Water use was recorded by the central controller for all applications across all treatments and total consumption was tracked over the course of each treatment run. A mobile application was created to record and georeferenced visual quality ratings of the fairways throughout each run by researchers and the superintendent. In 2020, a PS6000 survey was conducted at least 2 times wk^{-1}; one of which all nine fairways were surveyed and the other only the three SMS-based treatment fairways were surveyed. This was reduced to one PS6000 survey wk^{-1} of all nine fairways in 2021.

Results and Analysis to Date

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

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

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

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

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

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

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

Future Expectations

Mobile app quality ratings and NDVI information from the PS6000 surveys will be analyzed to determine the performance of each fairway. Further analyses will be conducted to evaluate the relationship between superintendent perception, water depletion, spatial variability, and turfgrass quality. The purpose of these analyses will be to determine whether acceptable and equal quality has been maintained for the SMS treatments, despite the significantly reduced water use. Findings will be written as a scientific article and submitted to a refereed journal in 2022.

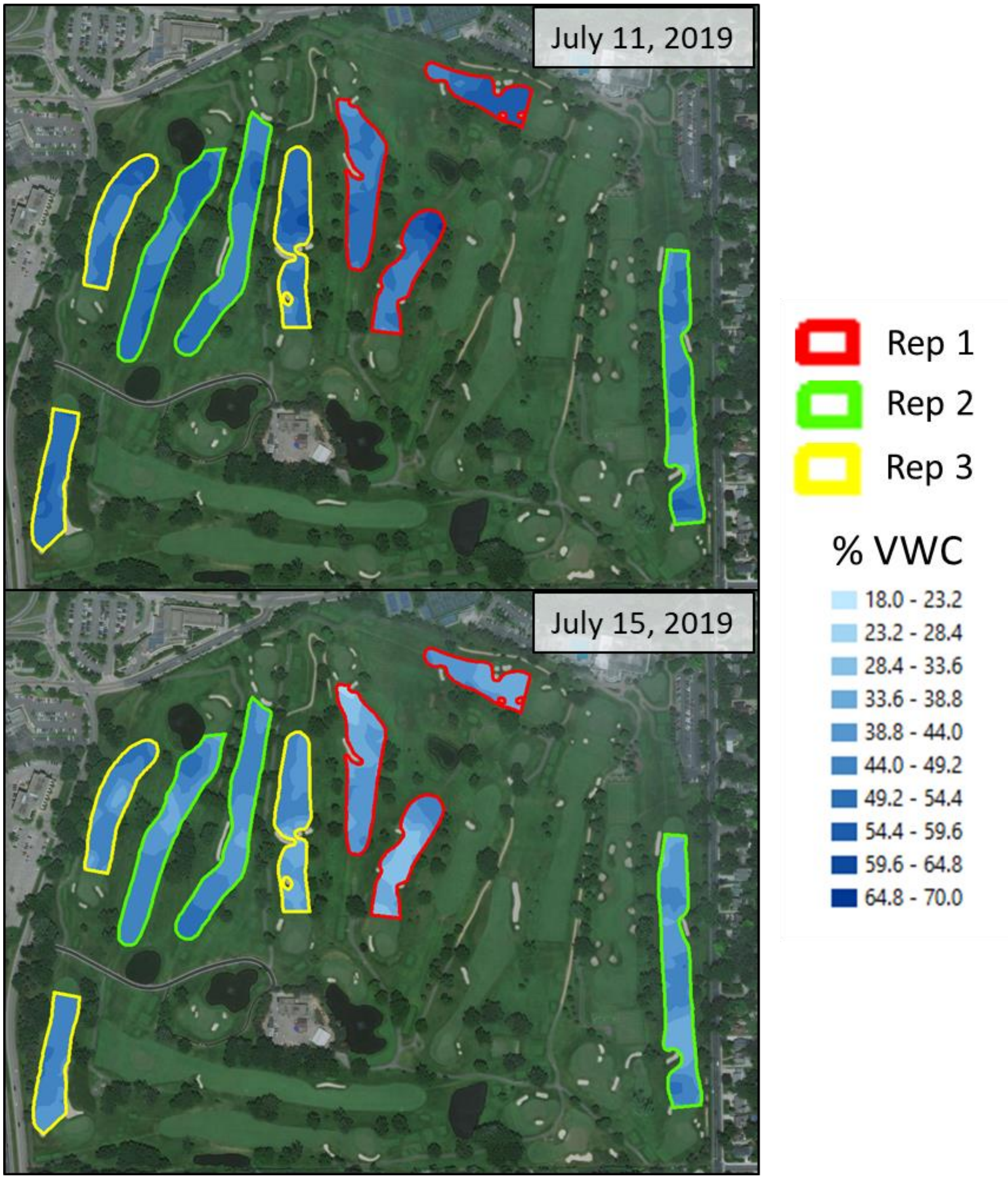


Figure 1. Soil moisture maps from July 11 and 15, 2019, where data were collected under saturated and approximate field capacity conditions, respectively. The nine fairways selected for use in the study were placed into similar groups of three based on their size, soil moisture (% volumetric water content;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

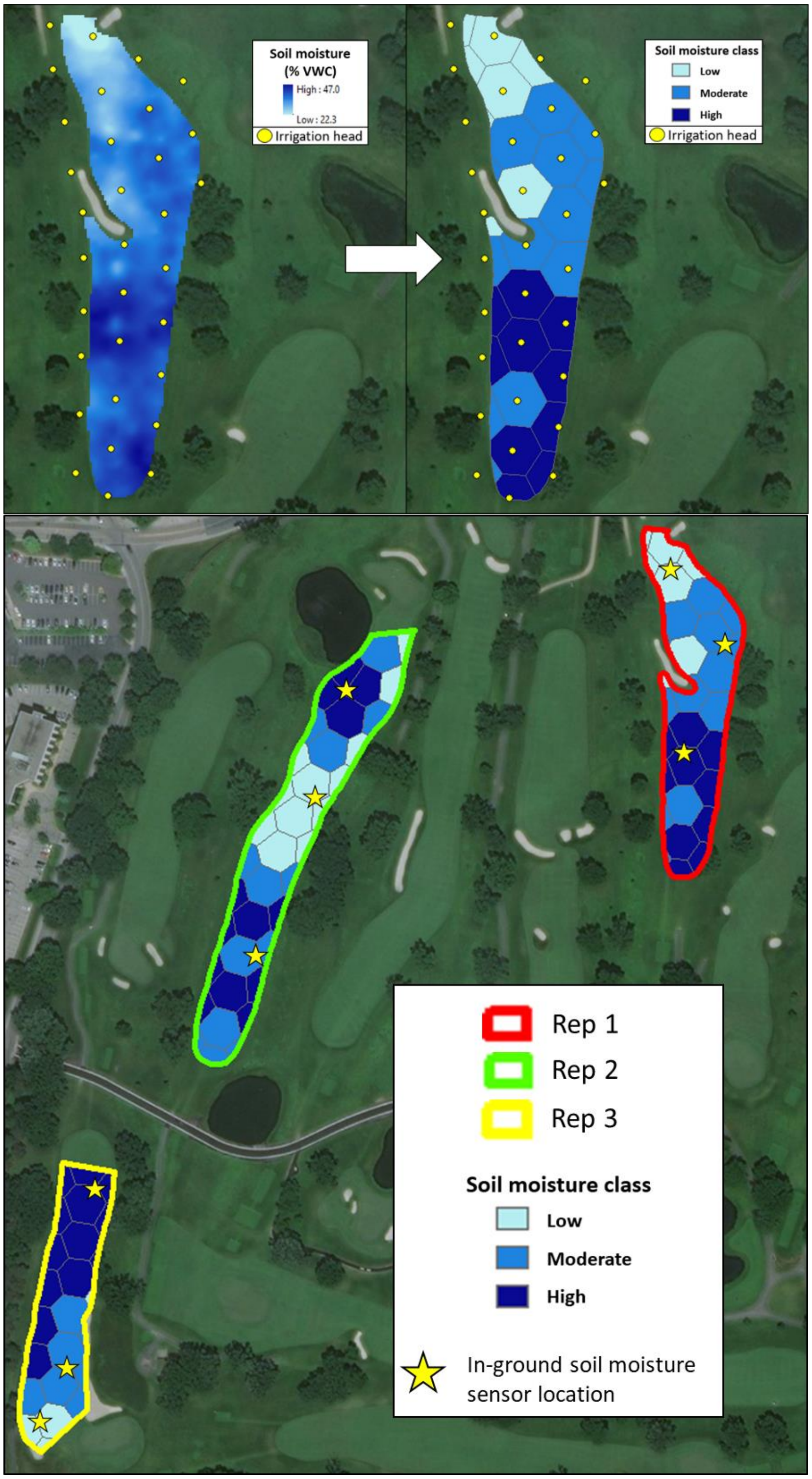


Figure 2. Top-left, percent volumetric water content (VWC) on one fairway in the soil moisture sensor treatment; top-right, soil moisture classes within delineated management zones on one fairway in the soil moisture sensor treatment; bottom, in-ground soil moisture sensor locations within each soil moisture class on the fairways receiving the SMS-based treatment.

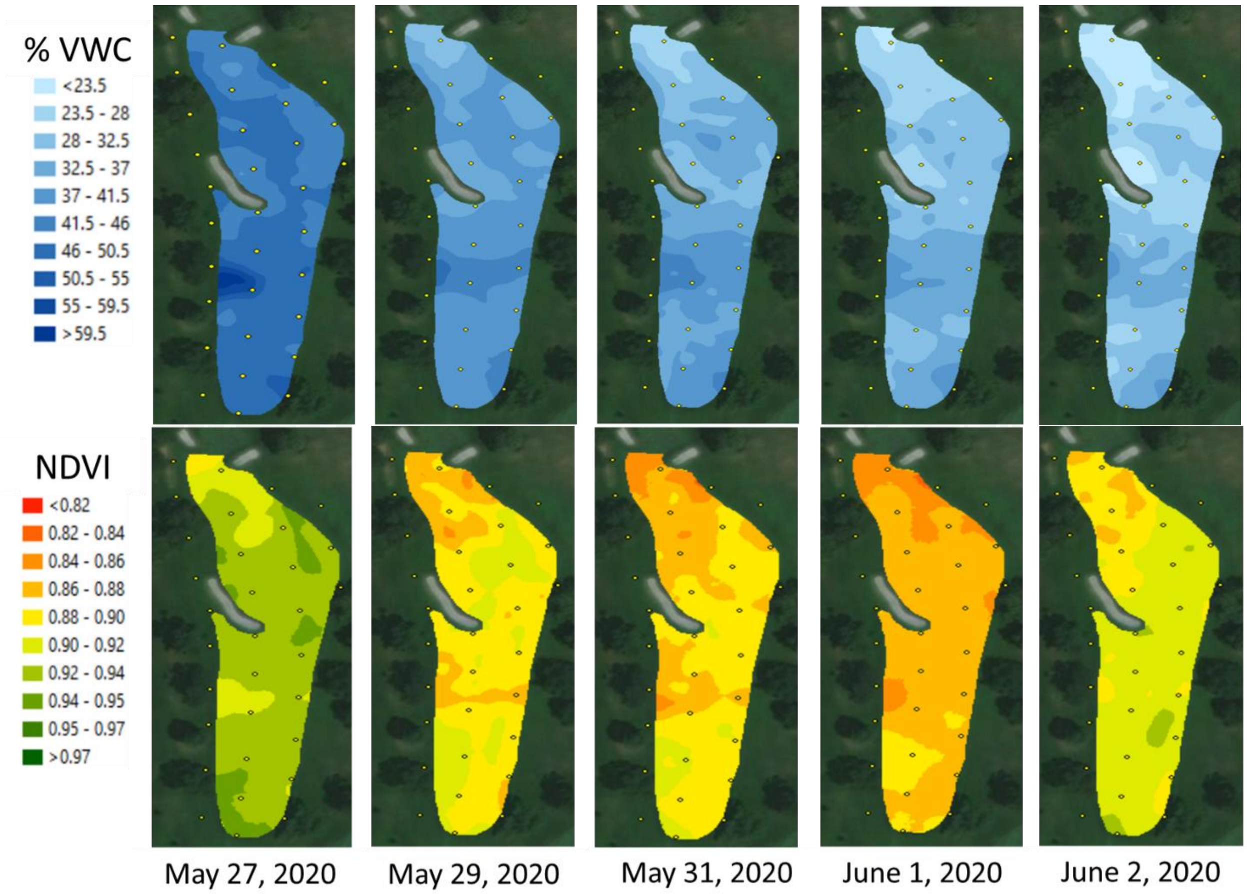


Figure 3. Kriged maps of volumetric water content (VWC) and normalized difference vegetation index (NDVI) on SMS-treatment fairway 5 over the course of a dry down event from May 27 – June 2, 2020 at Edina Country Club.

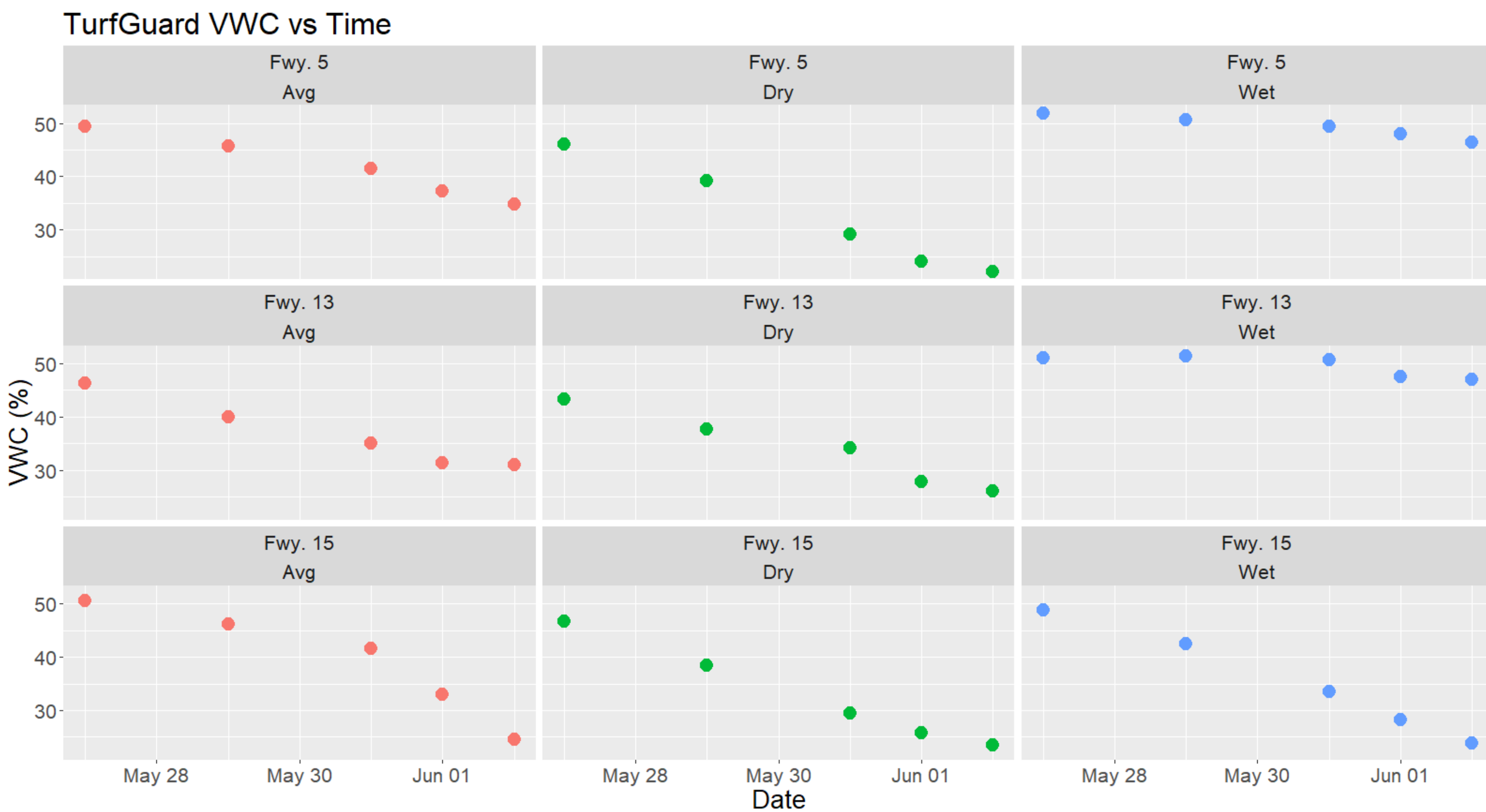


Figure 4. Mean, daily TurfGuard percent volumetric water content (VWC) of each moisture class (Avg = moderate, Dry = low, Wet = high) of fairways receiving the SMS-based irrigation treatment during a dry down event from May 27 – June 2, 2020 at Edina Country Club.

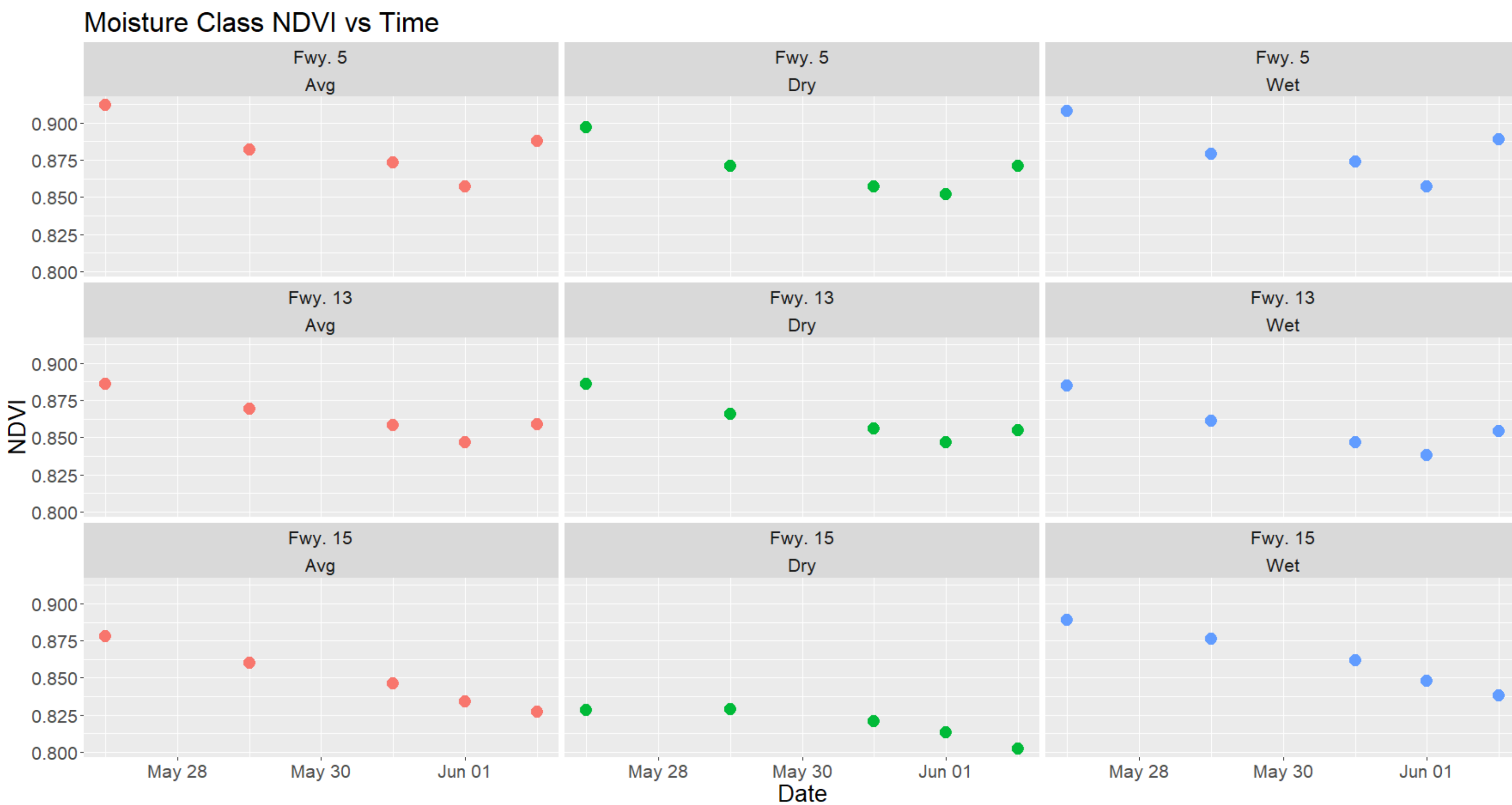


Figure 5. Mean normalized difference vegetation index (NDVI) as measured by the Precision Sense 6000 of each moisture class (Avg = moderate, Dry = low, Wet = high) of fairways receiving the SMS-based irrigation treatment during a dry down event from May 27 – June 2, 2020 at Edina Country Club.

USGA ID#: 2021-15-739

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

Project Leaders: Josh Friell[1], Ryan Schwab[1], Eric Watkins[1], Kurt Spokas[2], Dominic Petrella[3], Maggie Reiter[1]

Affiliation: [1] University of Minnesota, [2] USDA-ARS, [3]Ohio State ATI

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:

- Drought susceptible and tolerant cultivars of Kentucky bluegrass and creeping bentgrass were established under a rainout shelter and subjected to well-watered or non-irrigated treatments
- Study site soils were characterized for bulk density and a soil water retention properties, which showed a bimodal Seki model to provide the best soil water retention curve fit and a permanent wilt point (-1500 kPa) of 7.5% volumetric water content
- Canopy responses indicated that despite declines in some easily-measured variables like percent green cover and normalized difference vegetation index, plants may not be experiencing physiological stress as measured by photochemical efficiency, FvFm.
- Cores measuring 25 cm in diameter by 30 cm deep have been collected from each of the plots for further evaluation in a greenhouse during winter 2021. Flaws in the rain shelter design are being corrected to ensure proper, significant dry down in the second year of the experiment.

Summary Text:

Plot Establishment & Maintenance:

Two cultivars each of Kentucky bluegrass (*Poa pratensis* L.*)* and creeping bentgrass (*Agrostis stolonifera* L.) were established as individual plots (1.5 m x 1.5 m), under an automated rainout shelter at the Turfgrass Research, Outreach, and Education Center on the University of Minnesota's Saint Paul Campus. Cultivars of each species were chosen to have reasonably contrasting drought tolerance. Tolerant cultivars were 'Prosperity' and 'Piranha' and susceptible cultivars were 'Shamrock' and

'Penncross' for Kentucky bluegrass and creeping bentgrass, respectively. All plots were seeded on 10 Sep 2020 at a rate of 4.9 g m^{-2}. Harsh winter conditions, along with early drought, led to poor plot establishment; therefore, the Kentucky bluegrass plots were overseeded with an additional 9.8 g m^{-2} on 6 May 2021. During establishment, Kentucky bluegrass plots received 7.1 g N m^{-2}, 9.8 g P_2O_5 m^{-2}, and 2.5 g K_2O m^{-2}, and creeping bentgrass plots received 4.9 g N m^{-2}, 9.8 g P_2O_5 m^{-2}, and 3.3 g K_2O m^{-2}. To discourage lateral movement of moisture between plots, aluminum barriers were installed in the soil to a depth of 12.7 cm and plots were spaced at least 30.5 cm apart from each other. The research area was maintained as a golf fairway with little-to-no weed and disease tolerance. Weeds 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. A single application of trinexapac-ethyl (Primo Maxx, 438.5 mL ha^{-1}) was made on 30 June to encourage lateral spread of turf within plots. Plots were mown at 1.4 cm height of cut three times weekly and received 2.4 g N m^{-2} on 30 Jun, one week prior to the first run of irrigation treatments. One CS655 soil moisture sensor was installed in each plot (Campbell Scientific, Inc., Logan, UT) and wired to a CR300 data logger. Sod was removed and sensors were installed horizontally in the center of each plot at a 5.1 cm depth before soil was repacked surrounding the sensor body and sod was replaced.

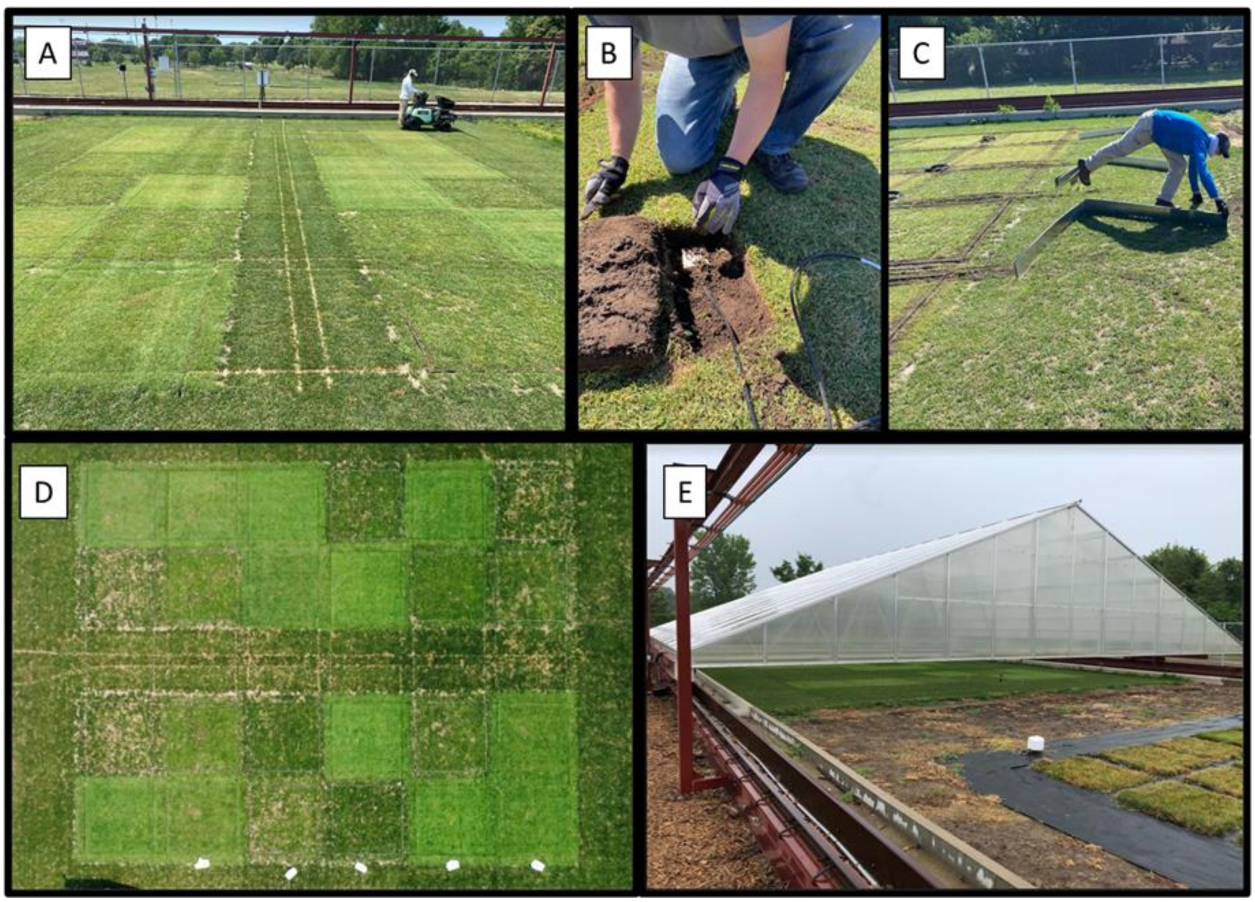


Figure 1. Plots in the dry down experiment in St. Paul, MN. A) following installation of soil moisture sensors; B) installing a CS655 soil moisture sensor; C) installing aluminum barriers between plots to discourage lateral water movement; D) overhead view of plots 15 d into first dry down; E) rainout shelter covering the dry down plots during a precipitation event.

Treatments & Data Collection:

Treatments consisted of two levels of irrigation: well-watered (100% ET_o replacement) and dry down (no irrigation). Three replications of each cultivar × irrigation treatment were arranged in a randomized complete block design. During the 2021 growing season, irrigation treatments were applied in two separate runs from 7 Jul - 5 Aug and 30 Aug - 18 Oct (30 and 50 days) with a 24-day recovery period in between. To generate rootzone conditions near field capacity at the beginning of each run, plots were supplied 2.5 cm irrigation and/or rainfall one day prior. Plots receiving the well-watered treatments were irrigated by hand three times per week using a hose-end flow meter (Model 825 Meter, Tuthill Corp., Burr Ridge, IL) and irrigation depth was equal to 100% reference evapotranspiration (ET_o, hereafter ET) as determined by an on-site weather station (WatchDog 2900ET, Spectrum Technologies, Inc., Aurora, IL). During dry down, the automated rainout shelter was moved over the plots during rain events and when rain was expected. Irrigation and/or rainfall were supplied at least 3 times weekly during the recovery period. Volumetric water content (VWC) was recorded every 15 minutes using the installed CS655 moisture sensors. The response of each plot to the irrigation treatments was quantified several ways (Table 1). Visual and physiological responses were measured three times per week and surface firmness was measured once per week in each plot. Point measurements of NDVI and NDRE, photosynthetically active radiation (PAR) absorptivity, and photochemical efficiency (a measure of internal physiological stress) were collected on the canopy directly above the tines of the buried soil moisture sensor.

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

Measured response	Tool
Turfgrass quality	-
Relative chlorophyll content	Field Scout CM 1000 (Spectrum Technologies, Inc., Aurora, IL)
NDVI (scan & point)	RapidSCAN CS-45 (Holland Scientific, Inc., Lincoln, NE)
NDRE (scan & point)	RapidSCAN CS-45 (Holland Scientific, Inc., Lincoln, NE)
Green canopy cover	Sony RX1000 III digital camera (Sony Corp., Tokyo, Japan); 60.3 x 90.8 x 64.8 cm LED lightbox emitting 90 μmol m^{-2} s^{-1}; Turf Analyzer 1.0.4 (Karcher et al., 2017)
Surface firmness	Clegg Impact Tester 0.5 kg model (Lafayette Instrument Co., Lafayette, IN)
PAR absorptivity	IMAGING-PAM M-Series Maxi Version (Heinz Walz Co., Effeltrich, Germany)
Photochemical efficiency	IMAGING-PAM M-Series Maxi Version (Heinz Walz Co., Effeltrich, Germany)

Soil Characterization:

On 11 June, six soil cores (5.1 cm diameter) were taken to a target depth of 15 cm with two samples being collected from arbitrary locations representing each species, and two collected from edges between the plots where no visible grass was growing. Two slices from each of the six cores were separated, weighed, and oven dried at 105 ^{o}C for 48 hours. Bulk density was calculated from the dry mass and calculated volume of the soil core.

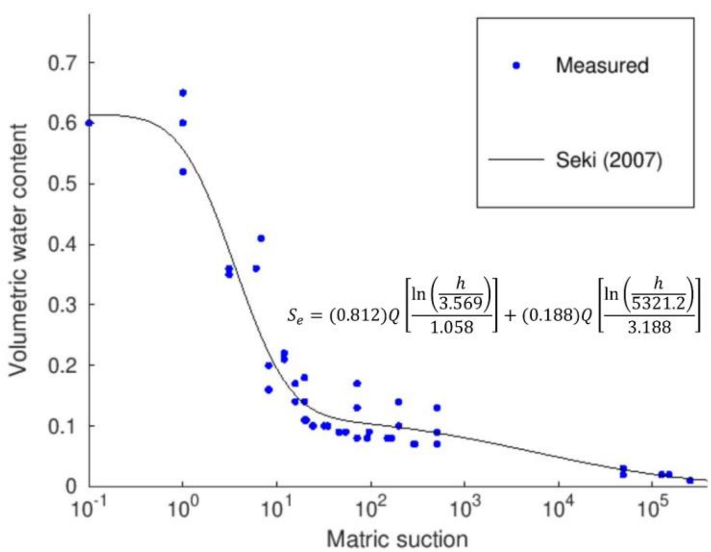
The centrifuge method (Khanzode et al., 2002; Lai et al., 2021; Rahardjo et al., 2017; Reatto et al., 2008; Reis et al., 2011) was utilized for the determination of the soil moisture characteristic curve for repacked subsamples of the various soil cores. The developed soil moisture potential, ψ, was calculated as in Smagin (2012) to be:

$$\psi \text{ (kPa)} = 0.0055 \text{ x } RPM^2 \text{ x } (R_2^2 - R_1^2)$$

Where RPM is the rotation per minute of the centrifuge setting, R_1 and R_2 are the distances from the rotation axis to the top (R_1) and bottom (R_2) of the soil sample, in meters**.**

Samples were placed in the Eppendorf 5810 centrifuge (The Eppendorf Co., Enfield, CT) and allowed 12 to 24 hours to equilibrate at the respective speed. Once the sample was at equilibrium, the sample was removed and the new mass was determined. The difference between the masses was allocated to the water drained from the soil sample. Following the lowest moisture potential (maximum rotation – 4000 RPM) the soil samples were placed into closed desiccators along with saturated salt solutions targeting 3 different relative humidity levels (5%, 33%, and 70% relative humidity) (Greenspan, 1977; Young, 1967) to achieve additional data points beyond the soil wilting point. These samples equilibrated for 2 weeks prior to moisture content determination.

The values acquired from the centrifuge and saturated salt equilibrium were then entered into the on-line version of the SWRC Fit program (Seki, 2007) for curve fitting various soil moisture curve models.



Model	Equation	Parameters	R^2	AIC
Brooks and Corey	$S_e = \begin{cases} \left(\frac{h}{h_b}\right)^{-\lambda} & (h > h_b) \\ 1 & (h \le h_b) \end{cases}$	$\theta_s = 0.60011$ $\theta_r = 0.027952$ $h_b = 0.95372$ $\lambda = 0.48181$	0.94631	-449.49
van Genuchten	$S_e = \left[\frac{1}{1+(\alpha h)^n}\right]^m \quad (m = 1 - 1/n)$	$\theta_s = 0.62792$ $\theta_r = 0.037338$ $\alpha = 0.67191$ $n = 1.6230$	0.93766	-439.02
Kosugi	$S_e = Q\left[\frac{\ln(h/h_m)}{\sigma}\right]$	$\theta_s = 0.62800$ $\theta_r = 0.055773$ $h_m = 4.1466$ $\sigma = 1.4798$	0.92183	-423.18
Fredlund and Xing	$S_e = \left[\frac{1}{\ln\left[e + (h/a)^n\right]}\right]^m$	$\theta_s = 0.61831$ $\theta_r = 0.000053088$ $a = 1.7486$ $m = 0.95381$ $n = 1.7325$	0.94669	-447.98
Durner	$S_e = w_1\left[\frac{1}{1+(\alpha_1 h)^{n_1}}\right]^{m_1} + (1-w_1)\left[\frac{1}{1+(\alpha_2 h)^{n_2}}\right]^{m_2}$	$\theta_s = 0.68980$ $\theta_r = 0.037559$ $w_1 = 0.094914$ $\alpha_1 = 72.189$ $n_1 = 20.938$ $\alpha_2 = 0.66949$ $n_2 = 1.6251$	0.93766	-433.02
Seki	$S_e = w_1 Q\left[\frac{\ln(h/h_{m1})}{\sigma_1}\right] + (1-w_1)Q\left[\frac{\ln(h/h_{m2})}{\sigma_2}\right]$	$\theta_s = 0.61426$ $\theta_r = 0.00010083$ $w_1 = 0.81253$ $h_{m1} = 3.5697$ $\sigma_1 = 1.0582$ $h_{m2} = 5321.2$ $\sigma_2 = 3.1888$	0.95420	-454.60

Figure 2. Combined soil water retention data for samples collected from plots in the dry down experiment. A Seki model provided the best fit of the data based on AIC and R^2 values where *h* is the matric potential, Q is the complementary cumulative normal distribution function, and S_e is the calculated effective saturation.

Analysis & Results to Date:

Soil Characterization:

Bulk density of the collected soil cores ranged from 1.03 - 1.71 g ml^{-1}. Analysis of variance of the bulk density values showed no differences between the turf covered samples and those with no turf or between the turf species.

Both the AIC and R^2 values showed a Seki model provided the best fit for the soil water retention curves based on the combined sample data (Fig. 2).

Soil Moisture & Canopy Response:

Total ET depths during the first and second runs were approximately 110 and 94 mm, respectively. At the commencement of the dry down periods, soil moisture values for all plots were 38.4 - 42.6% and 35.4-43.7% for the first and second dry downs, respectively. Following the dry down periods, VWC values of the dry down plots were 8.4 - 19.6% and 9.5 - 15.6% for the first and second dry downs, respectively.

Despite overseeding, the Kentucky bluegrass plots exhibited poor turf quality at the start of the first dry down period; however, the creeping bentgrass plots were of good turf quality. Although percent green canopy cover and normalized difference vegetation index declined with increasing soil matric suction, photochemical efficiency did not indicate that plots were experiencing physiological stress (Fig. 3). Only the tolerant Kentucky bluegrass cultivar exhibited a decline in photochemical efficiency in the first experiment, while other entries did not show much decline in photochemical efficiency compared to the start of the dry down.

Assuming a permanent wilt point of -1500 kPa soil water potential, the corresponding VWC was calculated from the to be 7.5%. This VWC value was not achieved at the 5 cm depth during either of the dry downs for any plot and visual indications of drought stress were not observed before the runs were stopped due to project scheduling and late-season weather.

Future Work:

Because significant drought stress was not achieved in our field plots this year, 25-cm diameter by 30-cm deep soil cores have been collected from each of the plots for further evaluation in a greenhouse during winter 2021. In addition, it was discovered that a flaw in the rain shelter design allowed sheet flow of water onto a few plots. This flaw is being addressed and additional runs will be performed beginning in spring 2022.

Plots for phase two of the trial are currently being established using the susceptible cultivars of each species. Irrigation treatments on these plots will be defined using soil water retention curves determined from core samples taken this fall and are on track to commence in summer 2022.

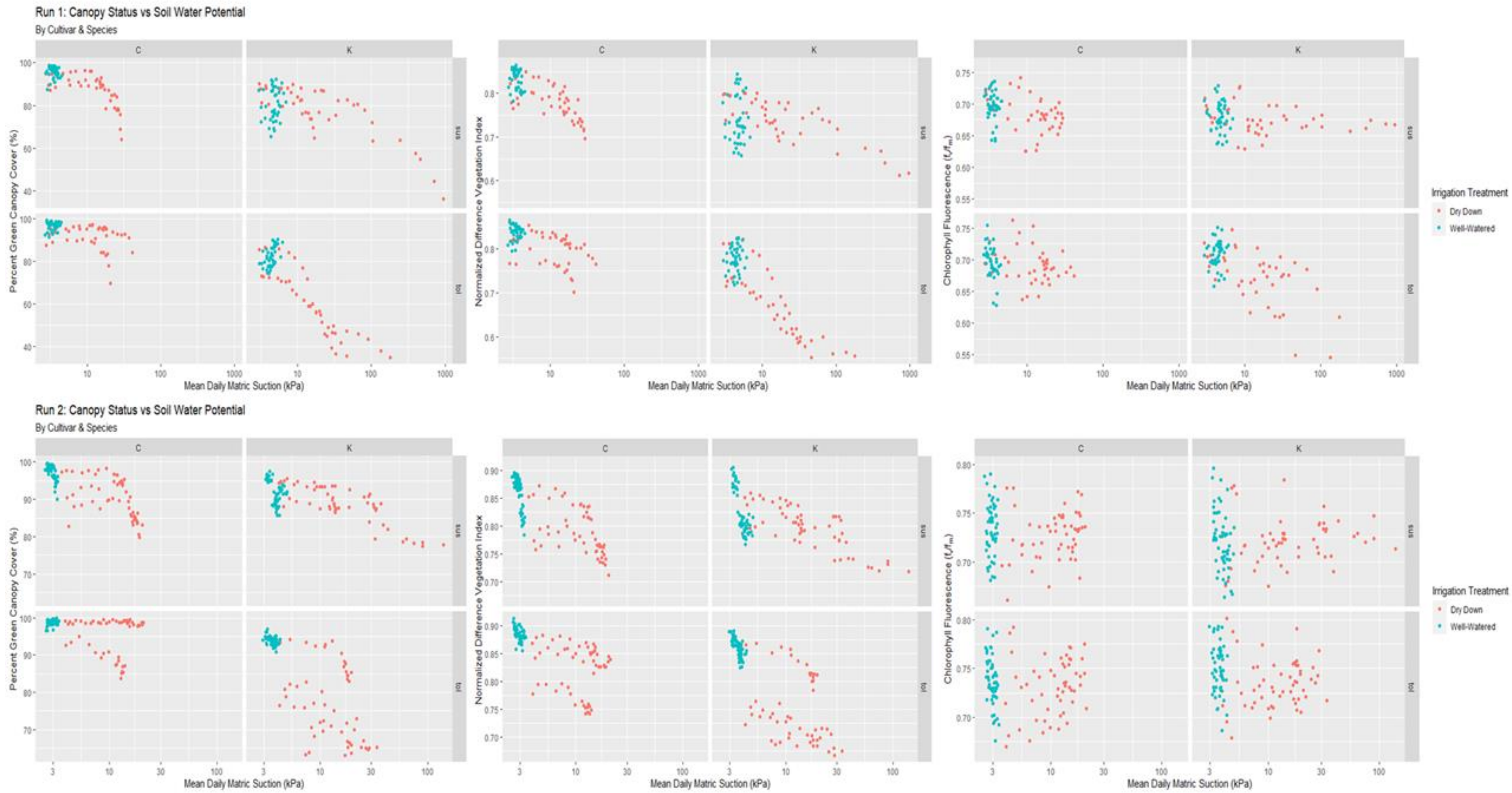


Figure 3. Percent green canopy cover, normalized difference vegetation index, and photochemical efficiency (chlorophyll fluorescence) versus soil water potential for drought susceptible (sus) and tolerant (tol) creeping bentgrass (C) and Kentucky bluegrass (K) for two runs of the dry down experiment at St. Paul, MN on well-watered and non-irrigated plots. Maximum photochemical efficiency is typically 0.800, and values below 0.500 indicate severe stress.

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USGA ID#: 2018-05-655

Title: Data-Driven Irrigation Scheduling Techniques for Managing Sand-Capped Fairways

Project Leader: Benjamin Wherley, Reagan Hejl, Kevin McInnes, and B. Grubbs

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

Objectives: Evaluate feasibility and determine best management practices for irrigation of sand-capped fairways when irrigating based on various data-driven scheduling techniques.

Start Date: 2018

Project Duration: 3 years (No Cost Extension Granted through 8/31/21)

Total Funding: $97,000

Summary Points:

- All irrigation scheduling approaches produced similar levels of acceptable turfgrass quality and percent green cover with no apparent differences in root development by the end of the project
- Over the two-year study, NOAA Forecasted Reference ET (FRET) was shown to be a reliable predictor of (R^2= 0.97) onsite weather station Penman-Monteith reference evapotranspiration (ET_o).
- Seasonal water use was 23% lower for the on-site ET_o based approach compared to SMS-based scheduling, although this did not result in elevated electrical conductivity within the sand-cap.
- Under wilt-based irrigation, the volumetric water content at which wilt occurred was highest mid-summer (4.4%), but declined during early and late season months (2.0%), suggesting different thresholds may be used throughout the season when using SMS-based irrigation scheduling

Summary Text:

Background

With current strains on water resources and with the increasing trend of capping degraded golf fairways with sand, research toward efficient methods for irrigation management on sand-capped soil is needed. Reference ET has proven to be an effective means of predicting irrigation requirements, however, access to locally representative weather station data is often a barrier for implementation. The recent availability of open-access NOAA FRET data provides ET data regardless of proximity to a weather station. This potentially offers superintendents another tool for scheduling irrigation. Unfortunately, research is lacking on how accurately FRET values predict actual on-site weather-station Penman-Monteith reference ET_o at a given location. In-ground soil moisture sensors (SMS) are another potential technology for aiding in golf course fairway irrigation scheduling, but these have been underutilized, largely due to soil heterogeneity present in most native soil systems. SMS may offer promise in sand-capped fairways due to the higher level of soil textural and depth uniformity across these systems. the objectives of this 2-year field study were to evaluate turfgrass performance, temporal and spatial soil moisture and salinity dynamics, and comparative water use associated with four irrigation scheduling approaches including 1) wireless SMS, 2) on-site ET_o, 3) NOAA FRET and 4) visual wilt-based.

Methodology

The 3-year study was initiated in 2018 with the construction of a 10,000 ft^2 sand-capped facility and establishment of Latitude 36 Bermudagrass (*Cynodon dactylon* L. Pers. x C. *Transvaalensis* Burtt-Davy). The 7" deep sand-cap was constructed from a medium coarse construction sand atop a fine sandy loam subsoil at the site. Various irrigation scheduling treatments were imposed during the 2019 and 2020 seasons, with the following scheduling approaches:

1) <u>Wireless SMS</u>

- Irrigation was applied based on 75% allowable depletion. Field capacity and permanent wilt point were based on evaluation of soil moisture at which wilt occurred in late May 2019.

2) <u>On-site Penman-Monteith ET_o</u>

- Plots were irrigated twice weekly based on the previous 3-day (Monday – Wednesday) or 4-day (Thursday – Sunday) on-site ET_o cumulative values multiplied by the warm-season turfgrass crop coefficient (0.6 x ET_o). Effective rainfall was accounted for in calculating irrigation requirements.

3) <u>NOAA Forecasted ET_o</u>

- Plots were irrigated twice weekly based on split applications of total weekly FRET values multiplied by the warm-season turfgrass crop coefficient (0.6 x ET_o). Effective rainfall was accounted for in calculating irrigation requirements.

4) <u>Visual Wilt-based approach</u>

- Wilt-based plots were irrigated back to field capacity with 2.3 cm of water once a given plot expressed 50% wilt.

Irrigation treatments were arranged in a randomized complete block design with 4 replicates and individual plot size of 20 ft x 20 ft. Plots were mowed 2-3 times per week at 0.5" height and fertilized with 0.75 lb N/ 1000 ft^2 every 3-4 weeks from May through September. Wetting agent (Aquatrols Revolution) was applied at the label rate every month during the growing season. Turf quality of plots was evaluated weekly using a 1-9 scale, with minimum quality = 5. Bi-weekly digital image analysis was performed using Turf Analyzer software (Green Research

Services, LLC, Fayetteville, AR) (Karcher et al., 2017). Water usage was determined by utilizing a water meter installed at the valve of each plot. Toro Turf Guard ® Wireless SMS were placed in each plot to monitor volumetric water content (VWC). Each sensor had 2 sets of probes that monitored VWC (%) at the upper portion of the sand-cap (3" depth) as well as the upper portion of the underlying subsoil (8" depth). In Visual Wilt-based plots, an additional sensor was positioned at a deeper depth for gaining greater spatial resolution, monitoring volumetric water content at the 6" sand-cap depth as well as deeper within the subsoil (11" depth). In November, a tractor-mounted Giddings Probe was used to remove two root/soil samples (2 inch diameter x 12 inch depth) from each plot and the sand-cap and subsoil were separated. To evaluate root development, the samples were rinsed and sieved to separate roots from soil. Roots were then oven-dried and weighed. Data were subjected to ANOVA using the GLM procedure of SPSS (IBM, Inc.). Where appropriate, mean comparisons were performed using Tukey's HSD ($P \leq 0.05$).

2020 Results

Across both seasons, all irrigation scheduling techniques were successful in maintaining acceptable turf quality (data not shown) and percent green cover at or around 80-90%, with no significant differences detected between scheduling techniques (Figure 1). Average seasonal irrigation use ranged from 37 cm in the On-Site ET_o treatment to 48 cm for the SMS-based plots, with significant differences ($P \leq 0.05$) only detected between the SMS and On-Site ET_o treatments (Figure 2). No significant differences were observed when comparing root mass between treatments within the sand-cap or within the subsoil (Figure 3). Within the Wilt-Based irrigation treatment, the 3" depth soil moisture content at which 50% wilt was observed was significantly lower during early and late season (~2% VWC) compared to mid-summer (~4.4% VWC) measurement periods.

A manuscript from the study has been prepared and is currently under consideration for publication in Agronomy Journal.

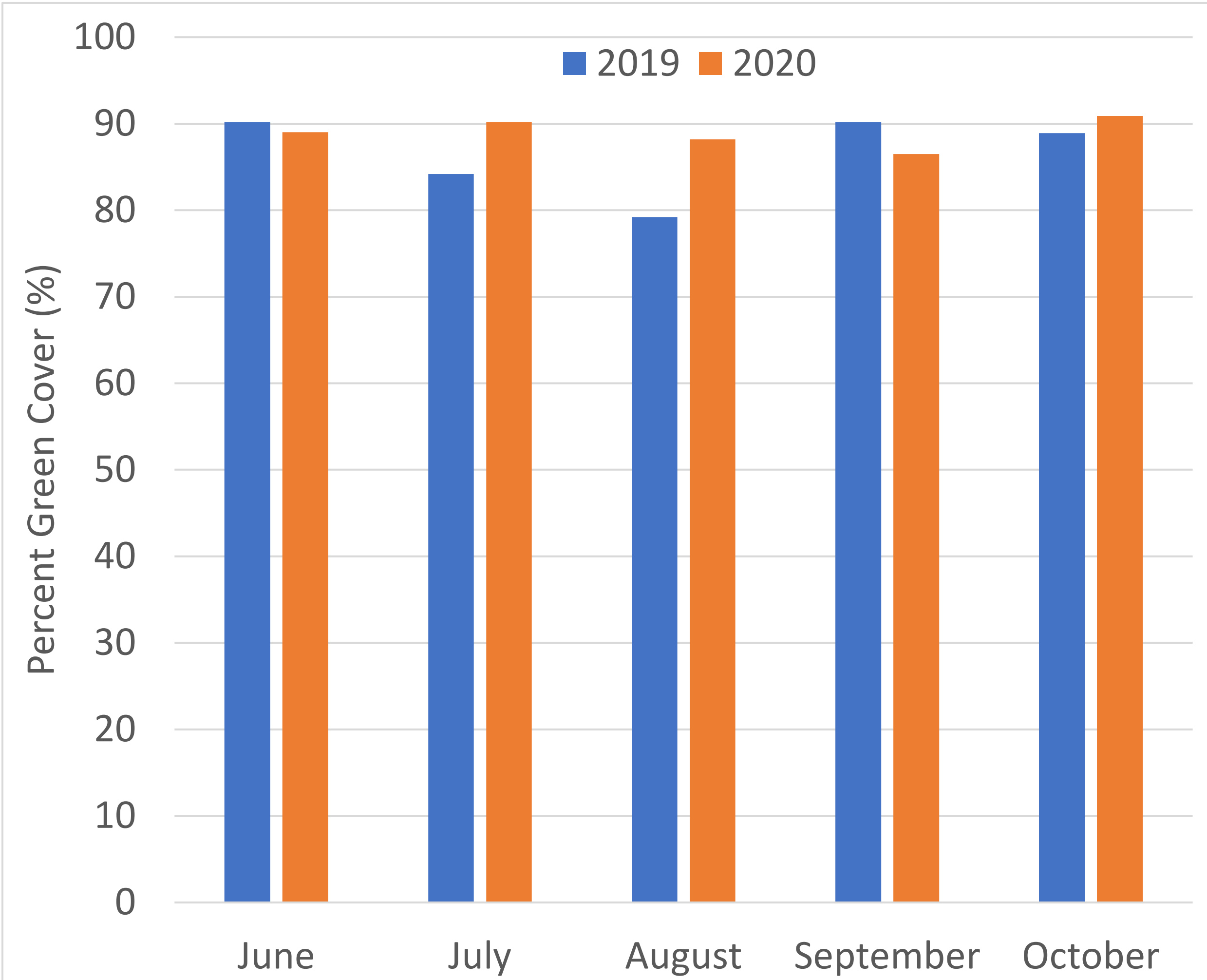


Figure 1. Monthly mean turfgrass percent green cover pooled across irrigation scheduling approach for the 2019 and 2020 seasons.

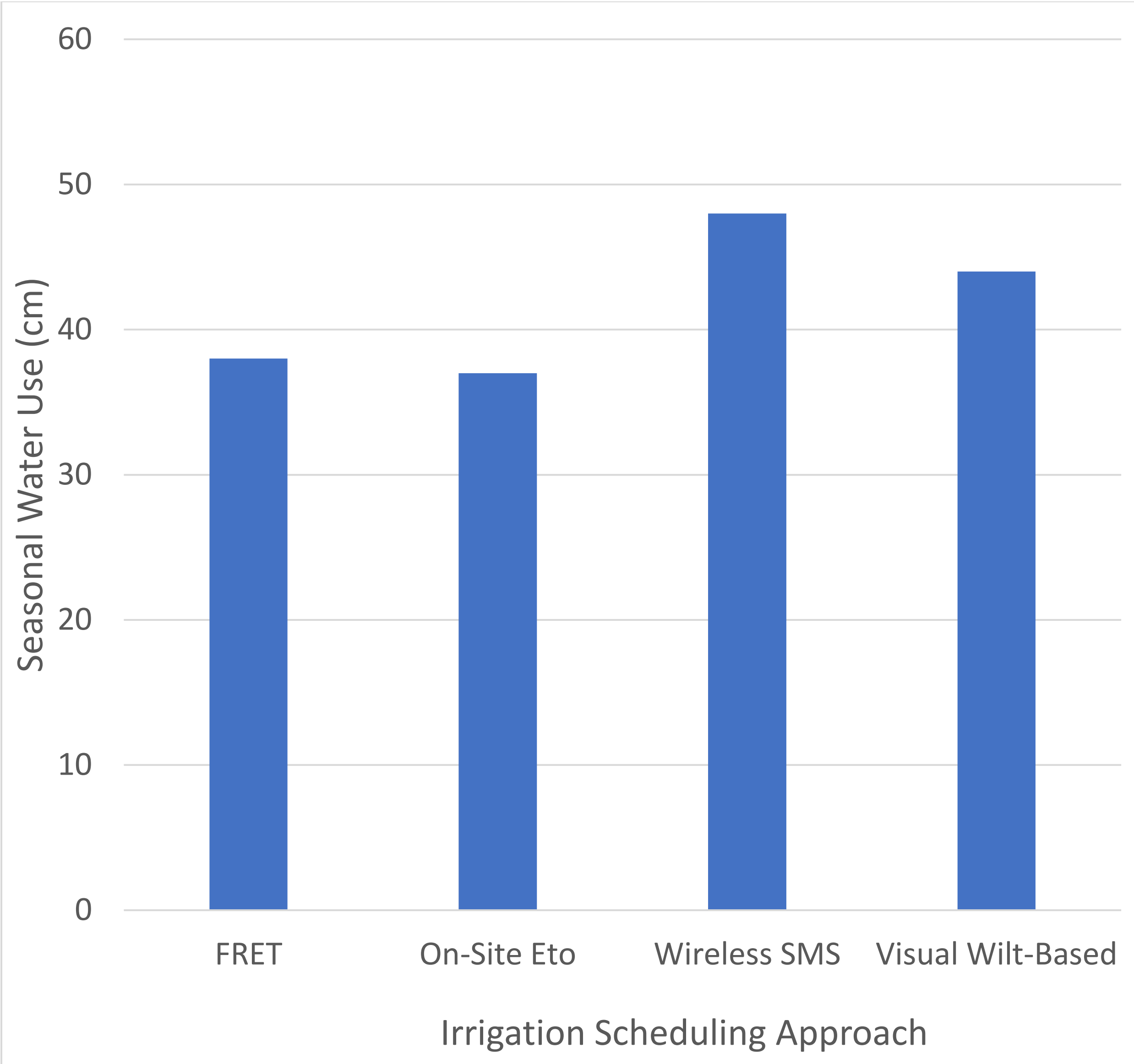


Figure 2. Mean seasonal water use for each irrigation scheduling treatment. Data were derived from difference between water meter readings at trial initiation and end of season, and are pooled across the 2019-2020 seasons.

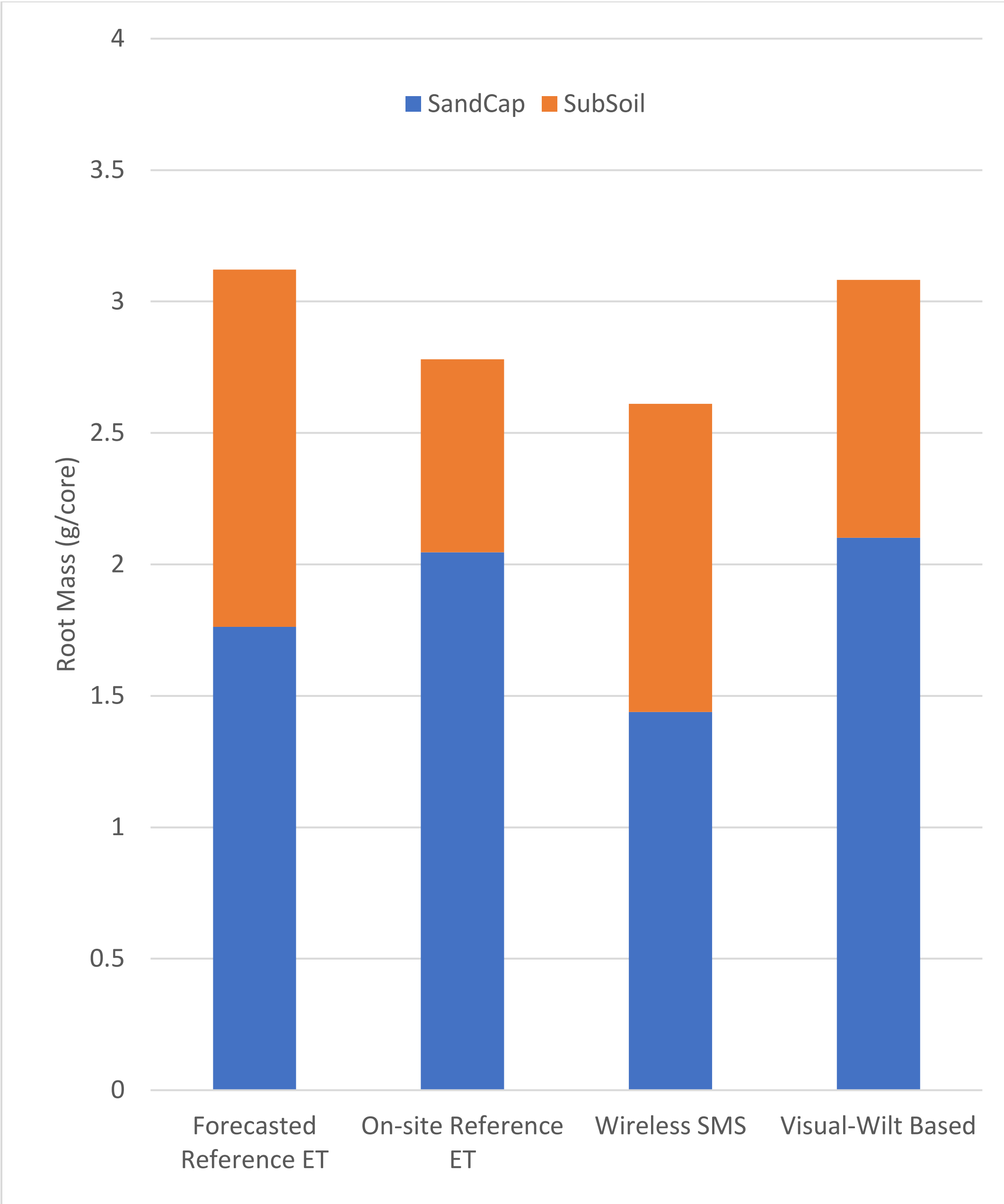


Figure 3. Root mass (grams per core) within sand-cap and subsoil fractions for each irrigation scheduling treatment. Root samples were harvested from plots in November 2020. There were no statistical differences in sand-cap, subsoil, or total root mass between irrigation treatments.

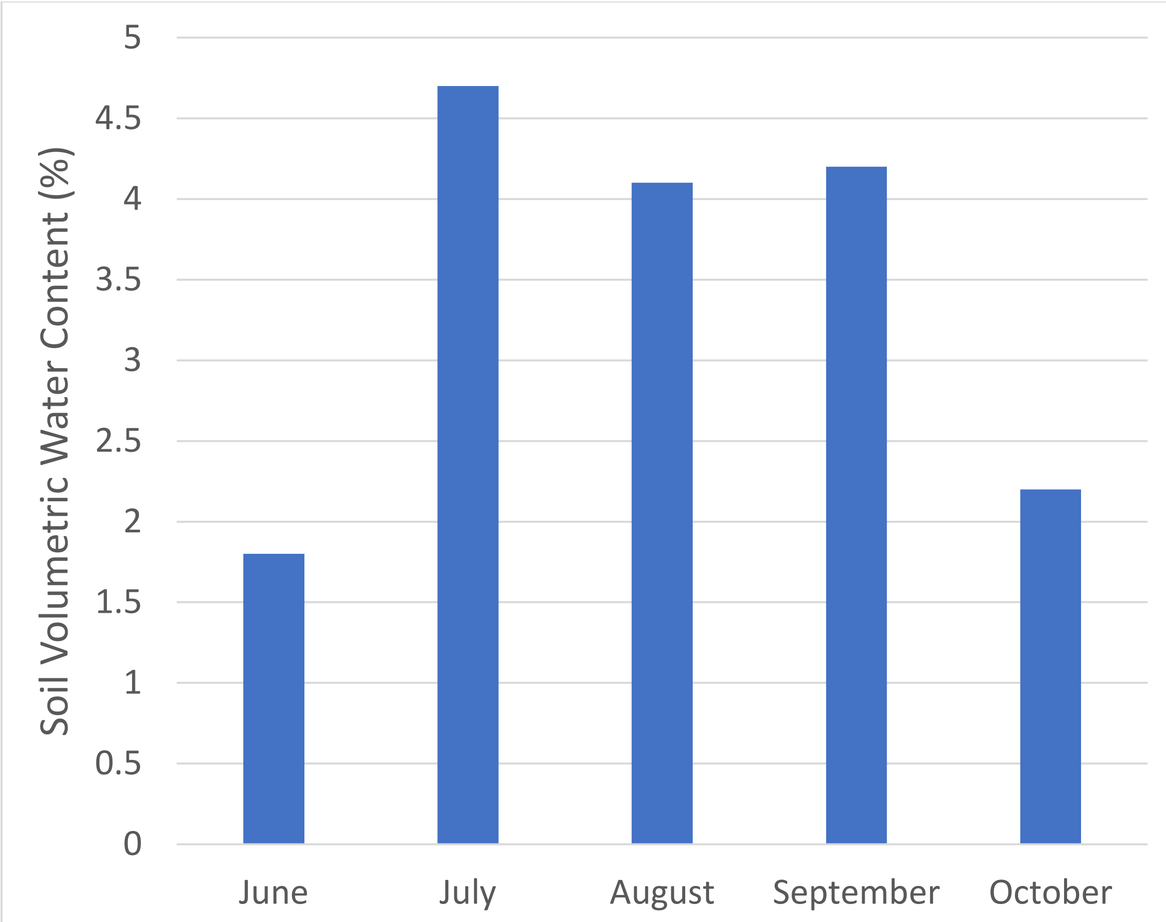


Figure 4. Soil volumetric water content (3" depth) at which 50% wilt was observed in the wilt-based treatment. Data are pooled across the 2019-2020 seasons.

Figure 5. Image of the sand-capping irrigation facility at Texas A&M University, College Station, TX. The facility contains 16 independently irrigated zones and was established in 2018 with 'Latitude 36' hybrid bermudagrass atop a 7" sand-cap.

USGA ID: 2018-08-658

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

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

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

Total Funding: $101,386

Dates: 2018-2020 (No Cost Extension Granted through 8/31/21)

Summary: As golf course irrigation water quality continues to decline, sand-capping of golf course fairways is increasing. This study evaluated long-term (years 4-6) changes in turf performance, soil physical properties, and cultural management requirements of sand-capped fairway plots originally established in 2014. The project was conducted at the Texas A&M Turfgrass Field Laboratory, College Station, TX, on 5-year old 'Tifway' bermudagrass sand-capped fairway research plots. Four replicated capping depth treatments have been constructed on both subsoils, including native soil topdressed at a depth of 1 inch of sand per year resulting in a 2 inch sand-cap at the initiation of this project (TD 2 in.), as well as capping depths of 2 inch, 4 inch, and 8 inch at construction. A split-plot design was utilized to assess sand-cap cultural management practices addressing surface organic matter accumulation, hydrophobicity, and subsoil sodicity issues arising from elevated Na and bicarbonates in the local water source.

The fine sandy loam subsoil study focused on subsoil sodicity and surface hydrophobicity management, specifically evaluating effects of wetting agent applications for mitigating surface hydrophobicity and gypsum application treatments for mitigating subsoil sodium accumulation, as well as the interaction of the two treatments on moving gypsum deeper into the profile. Within each capping depth, whole plots consist of wetting agent (Oars PS) applied at either 0 or 6 oz/ 1000 sq. ft., with gypsum (VerdeCal G applied at either 0 or 10 lbs./ 1000 sq. ft. monthly or as a single annual application at 100 lbs./1000 sq. ft) as the subplot treatment. Measurements including turf quality, soil volumetric water content, infiltration rates, water droplet penetration times, and subsoil (0-1 inch depth) sodium adsorption ratio were monitored within treatments across capping depths during the course of the 2018-2019 seasons. Root samples from sand and subsoil fractions were obtained in November 2020, were evaluated to determine differences in root biomass between treatments.

The clay loam subsoil study focused on surface organic matter management, specifically focusing on secondary cultural regimes for managing surface organic matter. Whole plots consisted of sand-capping depth (TD 2 inch, 2 inch, 4 inch, and 8 inch), with subplots consisting of either no secondary cultural management, verticutting, core aeration, verticutting + core aeration performed twice annually. Measurements including turf quality, percent green cover, surface firmness, and surface infiltration rates were monitored during the course of the season. Thatch depth and percent organic matter for the 0-2 inch sand-cap depth was also determined via combustion analysis and loss on ignition method at the end of the study.

Key Summary Points:

- Although traditional soil physical testing methods would suggest use of a 8 inch sand-cap based on the particle size distribution of this sand, the highest overall turf quality levels were associated with shallower sand-capping treatment depths of 2 and 4 inches (6 to 7 out of 9, respectively). The 8 inch capping depth generally produced lower turf quality levels, at times dropping to unacceptable levels (4 out of 9) (Fig. 1)

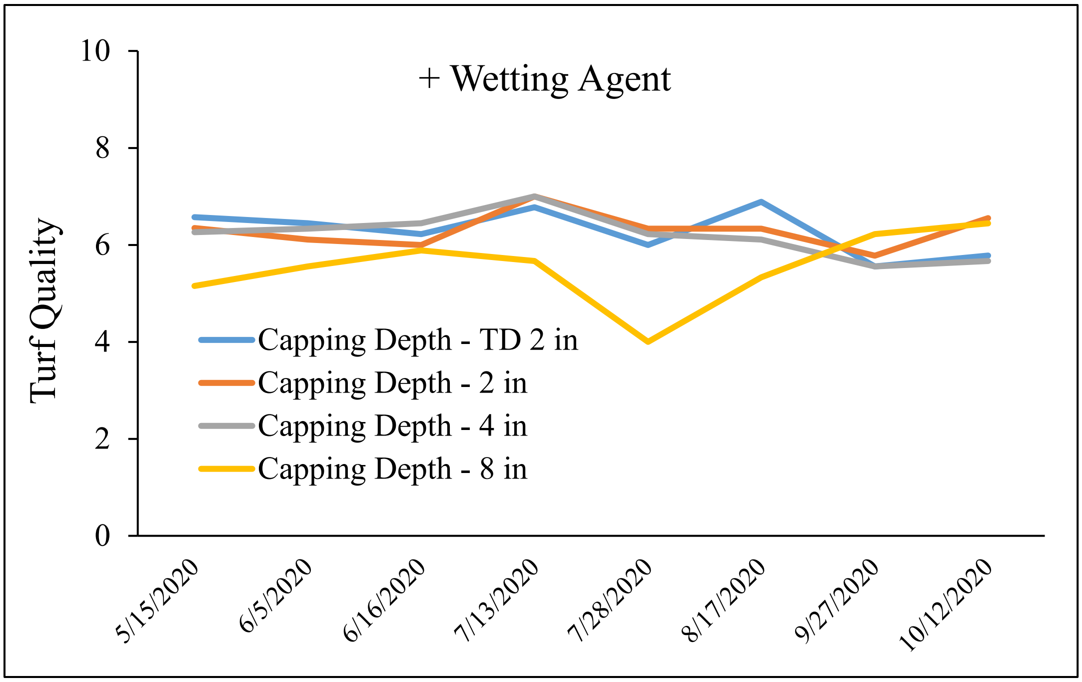
- The highest soil volumetric water contents within the upper sand-cap (0-3 in. depth) was associated with topdressed over time (TD 2 in.) treatments (30% VWC). The 2 and 4 inch capping depths exhibited intermediate soil moisture levels (~18-19% VWC), while the 8 inch capping depth supported the least moisture (~13%).

- Although water droplet penetration time (WDPT) tests performed in previous years had shown moderate to severe hydrophobicity (at 0.5" depth) within the 8-inch sand-capping depth treatments, the 2020 data showed more widespread, but minimal levels of hydrophobicity across all capping depths (Fig. 3).

- Based on data from subsoil SAR tests performed during October 2020, both gypsum treatments offered significant reductions in subsoil SAR (SAR = 10.1 and 9.6, respectively for 10 lbs. monthly and 100 lbs. annually) relative to the non-gypsum treatments (SAR= 12.5). While statistically significant, these reductions may not be considered to be agronomically beneficial, and highlight the importance of additional strategies for mitigating subsoil Na accumulation. (Fig. 4)

- In the clay loam subsoil study, secondary cultural management treatments did not lead to any statistically significant differences in visual turf quality during the 2020 season (data not shown). However, there was a significant effect of sand-capping depth on turf quality, with shallower (0-4") capping depths having generally higher TQ than the 8" capping depth. (Figure 5)

- Final surface organic matter levels (0-2"), measured during the 2020 season, generally declined with increasing secondary cultural intensity. The highest %OM (5.4%) was noted in the 8" sand-cap receiving no secondary cultural inputs, while the lowest %OM (4.3%) was associated with the 'verticutting + core aeration' treatment. (Figure 6)



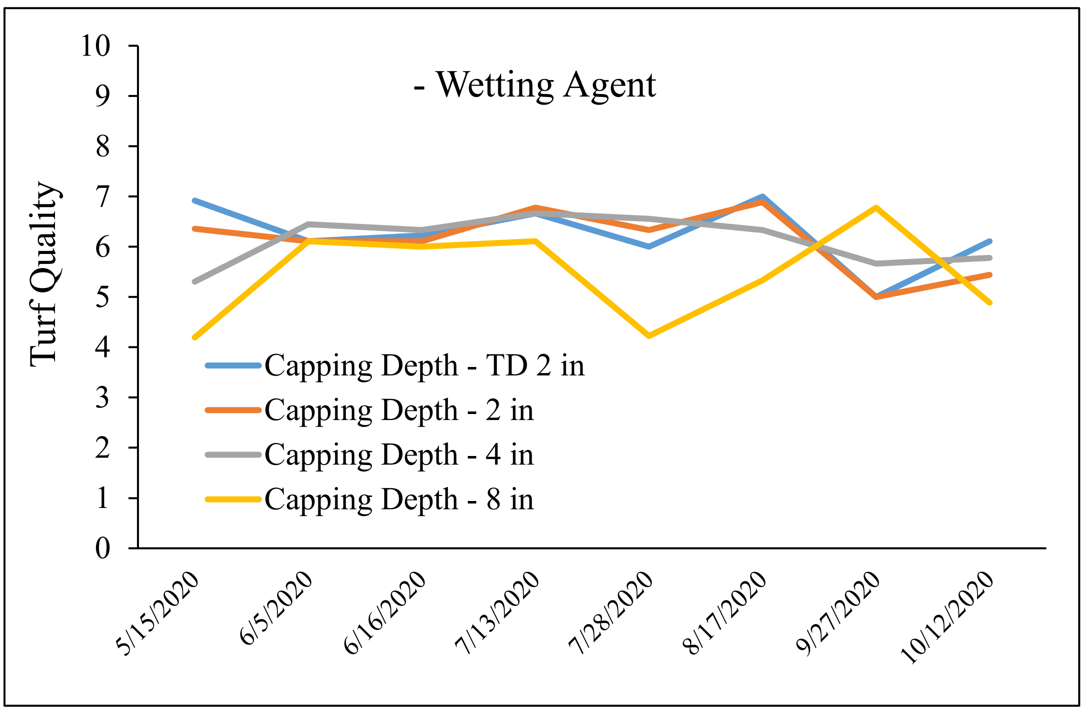


Figure 1. Visual Turf Quality for the sandy loam subsoil study for the 2020 season. Upper graph is for sand-cap depth treatments receiving Wetting Agent, while lower graph represents treatments not receiving Wetting Agent application. Means are pooled across gypsum application rate.

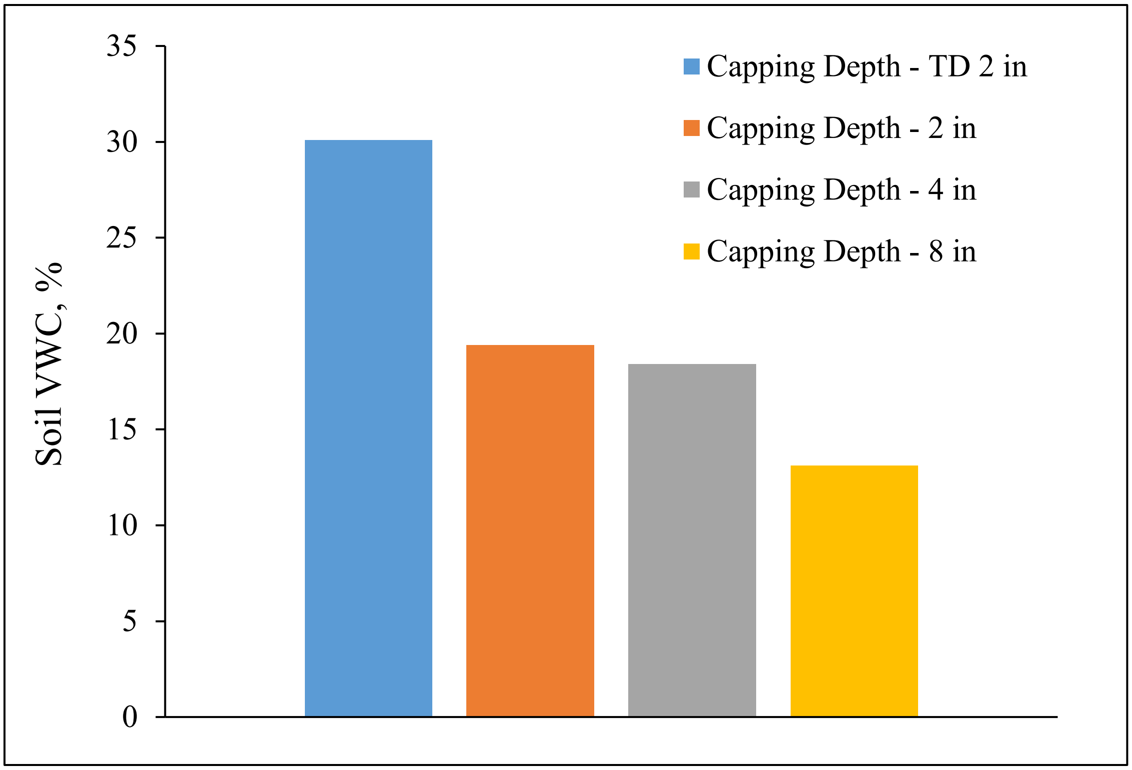


Figure 2. Effect of sand-capping depth on soil volumetric water content (0-3”) within upper sand-cap during the 2020 season for the sandy loam subsoil study. Data are pooled across gypsum and wetting agent application rates.

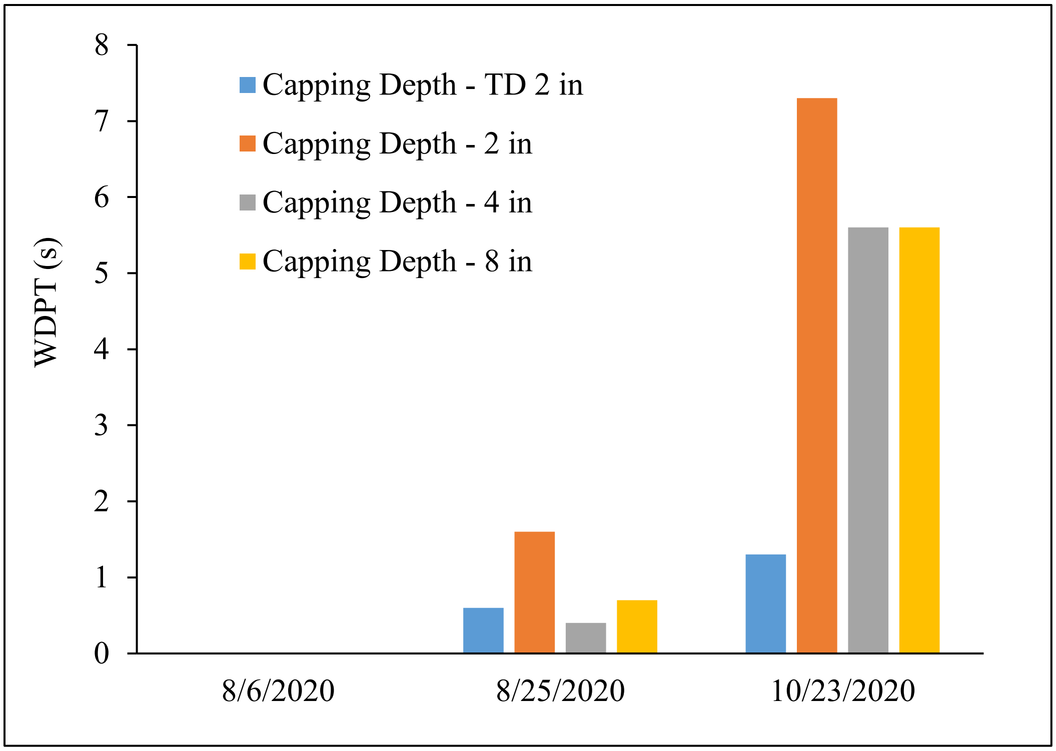


Figure 3. Effect of sand-capping depth on Water Droplet Penetration Time for 2020 testing dates in the sandy loam subsoil study. Data are for the non-wetting agent treatments, and are pooled over gypsum application rates.

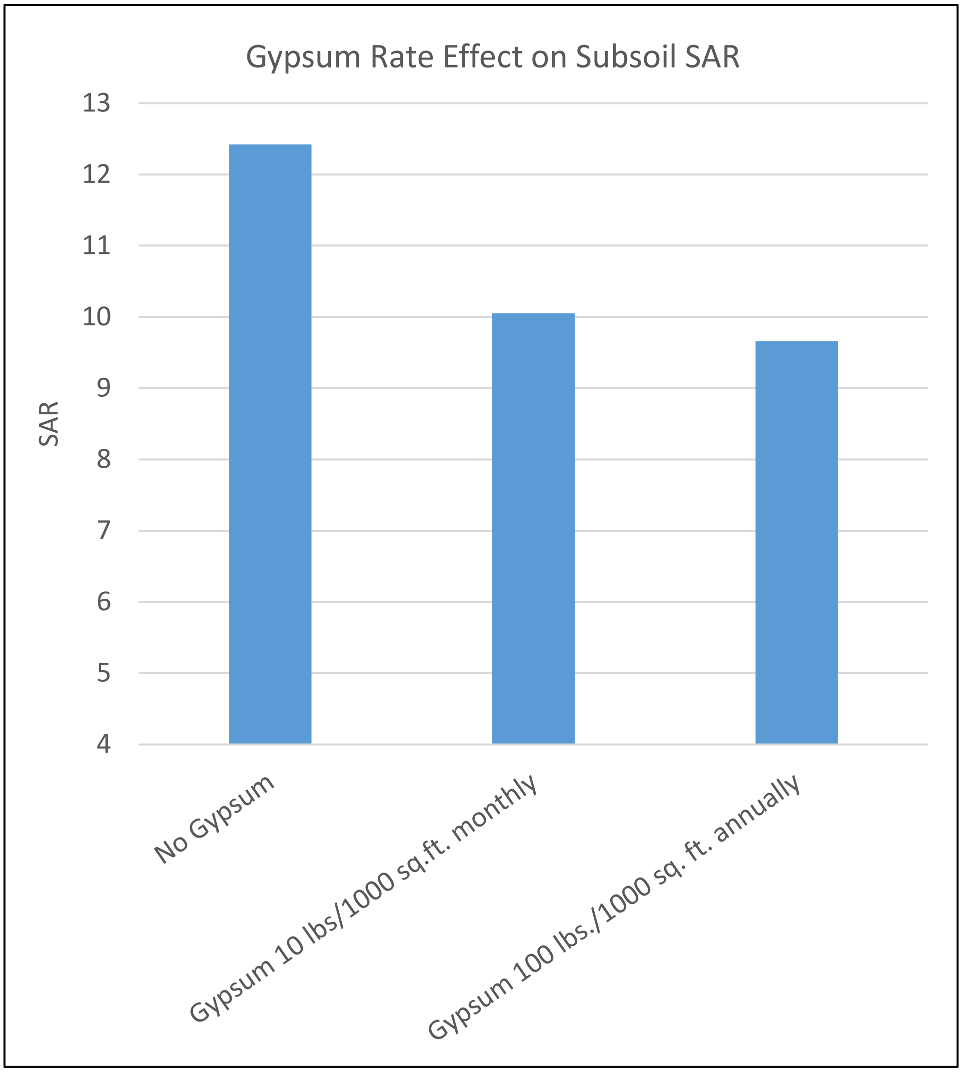


Figure 4. Effect of gypsum application rate on subsoil (0-1”) sodium adsorption ratio (SAR). Means are pooled across wetting agent and sand-capping depth treatments.

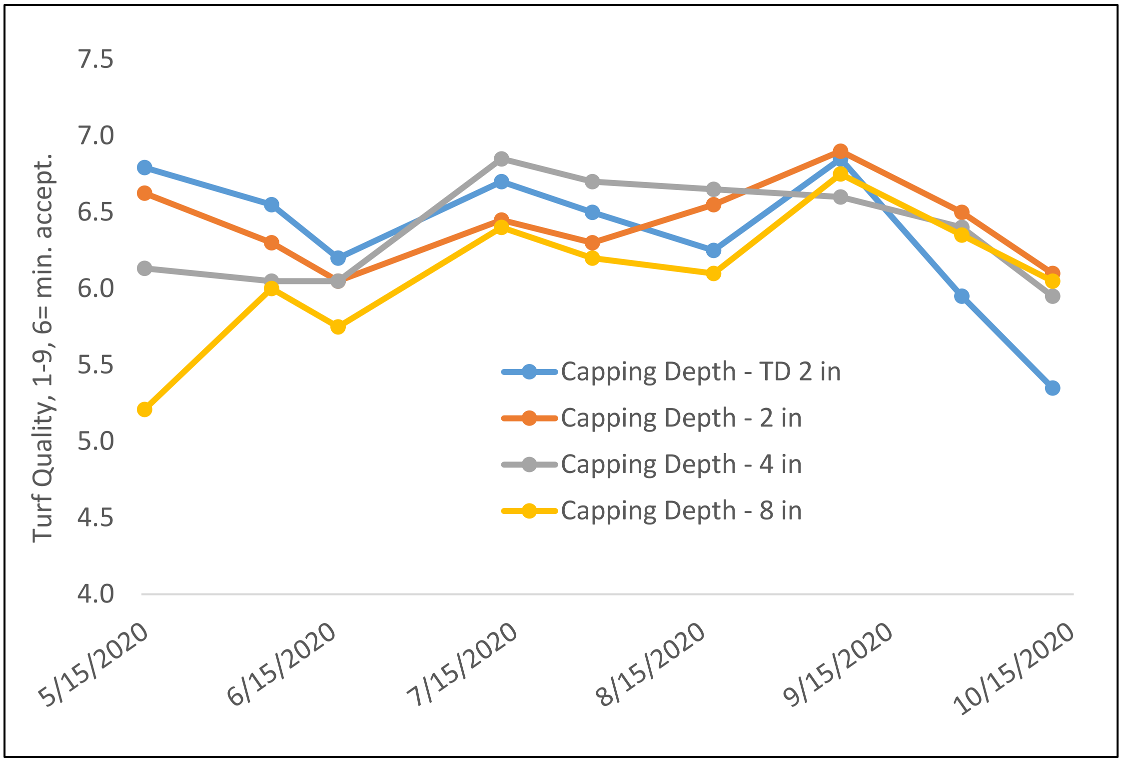


Figure 5. Sand-capping depth x date interaction on Turf Quality during the final (2020 season) for the clay loam subsoil study. Data are pooled across secondary cultural practice treatments.

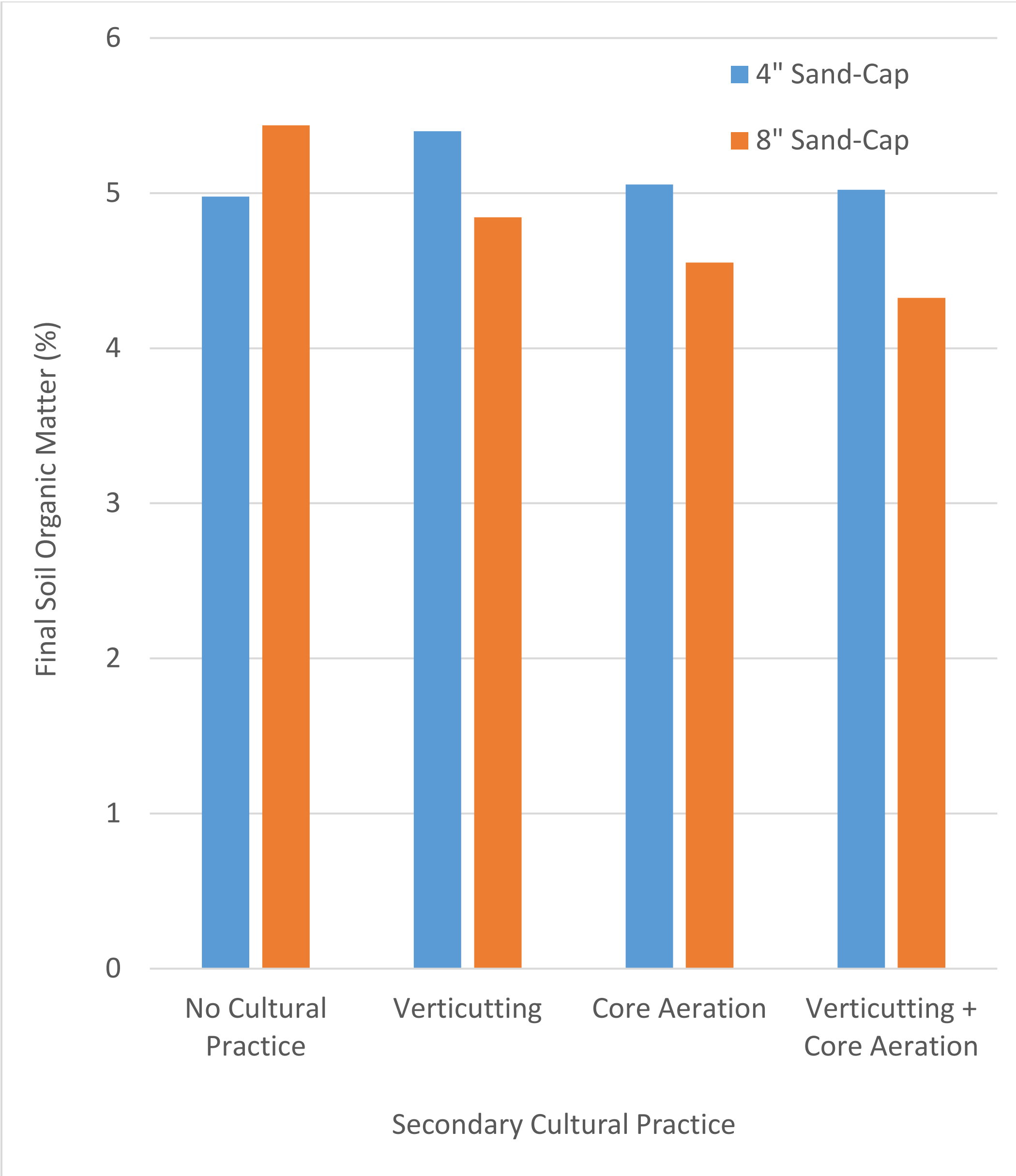


Figure 6. Final percent soil organic matter levels (0-2” depth) for the 4 and 8” sand-cap treatments at the conclusion of the 3-year secondary cultural management study.

USGA ID: 2018-04-654

Title: Enhancing Water Conservation through Remote Sensing Technology on Golf Courses

Project leader Dr. Joseph Young, Dr. Sanjit Deb, Dr. Glen Ritchie, Dr. Wenxuan Guo, Eduardo Escamilla, Juan Cantu, and Dr. David McCall[2]

Affiliation Texas Tech University and Virginia Tech University[2]

Objectives

1. Ground-truth spectral sensory data from a UAV to specifically recognize water-deficit stress
2. Determine soil physical properties that lead to high variability of plant available water within golf course fairways
3. Expand soil moisture mapping protocol to include salinity measurement from TDR instrument

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

Rationale

Water conservation strategies continue to be developed and tested throughout the golf industry. Agricultural producers have effectively incorporated remote sensing technology into maximizing yield while reducing inputs or targeting inputs to areas of greatest potential. Utilizing remote sensing data to improve turf management is a new area of study. However, research is needed to further elucidate the benefits of using the technology in golf course management. The overall goal of this project is to evaluate spectral sensors in specific bands and acquire ground-truthed data to better understand spatial variability patterns and determine accuracy of drone imagery.

Methodology

UAV Flights and Ground-Truth Data Compilation. Drone flights were completed in summer 2018 (Rawls GC n = 5; Amarillo CC n = 3) and 2019 (Rawls GC n = 3; Amarillo CC n = 3) over two holes at each location. A complete flight included collecting geo-referenced imagery from four sensors [Red/Green/Blue (RGB); Red Edge (RE); NIR850 nm; and NIR970 nm). All images were compiled and stitched into a single image per golf course fairway flown and analyzed in Blue Marble Global Mapper and ArcGIS software. Analysis consisted of NDVI calculations using RE/NIR850/NIR970 sensor with RGB images as a passive measurement. We enlisted assistance from Turf Scout (Dana Sullivan), who has been tremendously helpful with correcting some errors and analyzing imagery effectively. Soil samples and ground-based measurements were obtained from intersection points developed in Google Earth Path add-on within Google Earth. Soil samples (0-5 cm and 5-10 cm depths below turf thatch) were analyzed in the laboratory (texture, bulk density, organic matter, infiltration, plant available water, and thermal properties) along with measurements of soil compaction (0-5 cm and 5-10 cm), active NDVI (Turfscout Color Meter), and relative volumetric water content (VWC) with TDR at 3-inch (7.6 cm) depth (All instruments from Spectrum Technologies). Instrument data were obtained within 1-2 days of flight to overlay or correlate with analyzed drone images to validate any stress in fairways (Figure 1).

Expanding Soil Moisture Mapping Protocol for Salinity. With the extension of our funding availability, we were able to initiate work to expand TDR 350 mapping protocol developed by Dr. Chase Straw (Current Texas A&M University; former post-doc at University of Minnesota) to include variability in salinity. We obtained GPS-Coordinate data from three golf courses in Lubbock between September and October. The data will be included in spatial variability maps following the mapping protocol developed by Dr. Straw, but to assess variability in salinity measured from the TDR instrument. Soil samples (3-inch depth) were obtained for each 10th sample for laboratory soil EC determination to correlate the TDR measurement to actual soil EC values using a 1:2 ratio soil:water solution.

Results to Date

UAV Flights and Ground-Truth Data Compilation. All flights for image capture were completed in 2018 and 2019. Through guidance of Turf Scout, corrections to our images were performed along with NDVI determination over each flight. We will statistically determine variability within each fairway utilizing a 10 m square grid overlaying each fairway (Figure 1). Additionally, the 1 m boundary around each ground-truth GPS-coordinate has been extracted with NDVI data for those points on each flight (Fig. 1). These data will be correlated with ground-based assessments obtained at these same points to determine what factors (soil physical property, compaction, soil moisture, etc) relate to differences in NDVI spatial variability and what soil factors have greatest influence on plant available water (Fig. 2; Table 1). A correlation analysis of PAW from Rawls Course Hole 14 indicated a significant negative relationship with bulk density (-0.403) and unsaturated conductivity (-0.464). In contrast, significant positive relationships were determined for soil organic matter (0.564), porosity (0.403), and compaction (0.432).

Expanding Soil Moisture Mapping Protocol for Salinity. We completed GPS-coordinate TDR 350 data collection on three golf courses in Lubbock and have marked irrigation heads at one location. We are working through soil EC evaluations from soils samples at Lubbock Country Club where all data collection has been finalized. The remaining two course's soil samples will be evaluated in the coming weeks along with gathering GPS coordinates for irrigation heads at the other two locations. These data will allow us to determine if the soil moisture mapping protocol could be expanded to include salinity measurements from the same TDR instrument. There was high variability in salinity index across all fairways at each golf course with Lubbock Country Club ranging from 0.03-1.42 with a mean of 0.50. Hence, we are hopeful this research will guide golf course superintendents to a simple, easy-to-implement practice that can provide data support for site-specific management practices to precisely manage soil salinity concerns in golf course fairways.

Summary points

- The research expands our knowledge and understanding of NDVI variation within fairways and will identify ground-based data that leads to poorer NDVI.
- Variability maps of laboratory measured plant available water were developed
- Moderate negative and positive correlations between plant available water and soil physical properties were documented
- Wide accessibility to the TDR instruments and free soil mapping protocol provides a valuable tool for superintendents to implement site-specific management practices for irrigation or salinity management
- Results from these analyses are expected to dictate site-specific management practices that can be targeted to specific zones for maximum efficiency and to maximize frequency and benefit of management

Figure 1. Updated NDVI images from Rawls 14 demonstrating 10-m overlay grid, standard 850 nm NDVI, water-specific 970 nm NDVI, and 1-m NDVI from ground-truth data points for correlation analysis. All images shown were obtained in a flight on August 14, 2018 with image analysis assistance from Turf Scout, LLC Dana Sullivan.

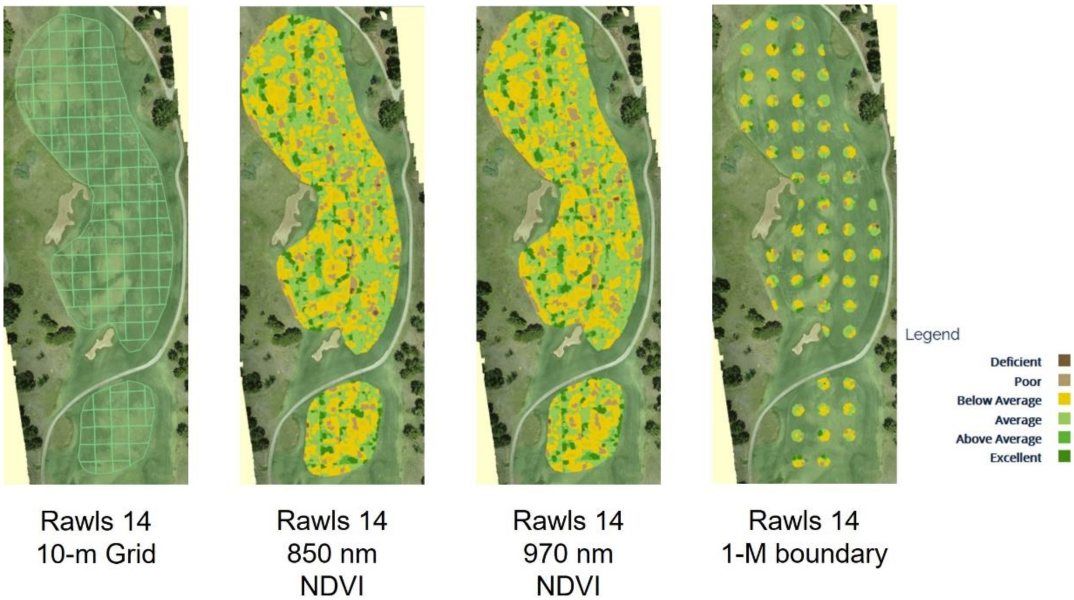
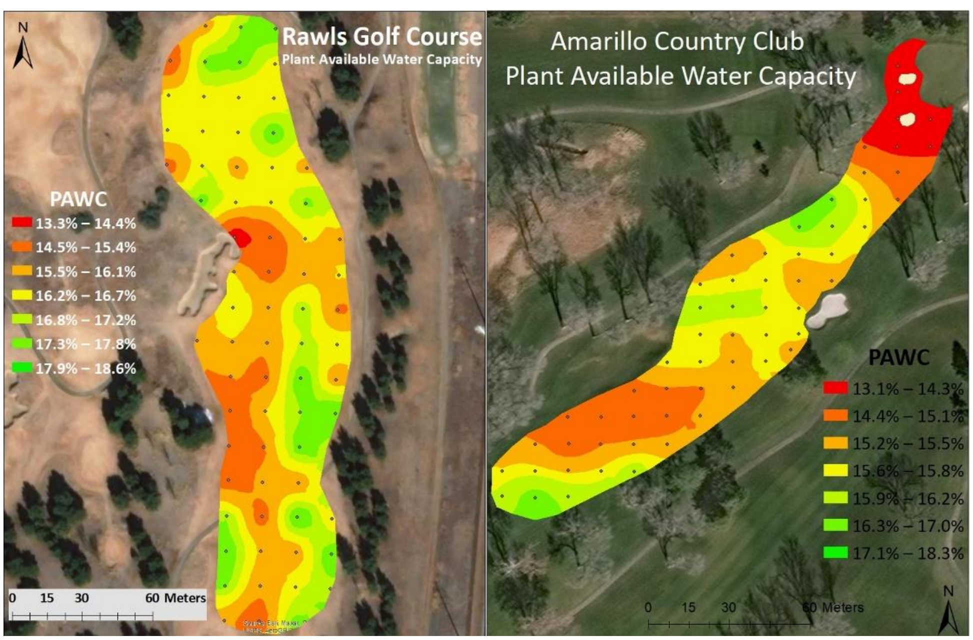


Figure 2. Spatial variability maps of plant available water averaged for soil samples collected from 0-5 cm and 5-10 cm from Rawls Golf Course hole #14 (Left) and Amarillo Country Club #16 (Right).



USGA ID#: 2020-13-718

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

Project Leader: Amir Haghverdi

Affiliation: University of California Riverside

Objectives: The overarching goal of this project is to develop and disseminate scientific knowledge, practical recommendations, and tools for efficient golf course irrigation and water management through field irrigation field research trials and economic analysis.

Start Date: 2020

Project Duration: 3 years (year 2 of 3)

Total Funding: $120,000

SUMMARY POINTS:

- Two irrigation research trials were conducted in southern California to investigate the response of hybrid bermudagrass (*Cynodon dactylon*) and buffaloograss (*Buchloe dactyloides*) to a wide range ET_o-based and soil moisture sensor (SMS)-based irrigation treatments.
- The smart SMS-based controller closely followed the programmed thresholds but the irrigation applications differed between years for the same treatments when converted to ET_o percentages.
- The hybrid bermudagrass NDVI values decreased across the treatments compared to 2020, which is (based on our preliminary assessment) attributed to salinity build-up in the root zone due to the application of recycled water.
- The smart ET-based controller showed an acceptable performance (4 to 10% overirrigation) compared to CIMIS ETo values.

SUMMARY TEXT

Rational:

Scientific research on the application and reliability of new landscape irrigation management approaches, including the use of smart controllers, has been mainly done in humid regions where the objective has been to avoid over-irrigation when rainfall is abundant. Currently, information is limited regarding the application of smart irrigation technologies to develop water conservation and deficit irrigation strategies in California. Previous studies focused on implementing smart landscape irrigation controllers when potable water was used for irrigation. Information is lacking on the accuracy and reliability of smart controllers to apply optimum water to fulfill the evapotranspiration plus the leaching requirements when recycled water is used for landscape irrigation, particularly when deficit irrigation is desired. Additionally, there is limited work on quantifying water savings from different new technologies/management strategies and returns to investment in the short- and long run.

Methodology:

Irrigation trial 1: A hybrid bermudagrass ('Tifgreen 328', *Cynodon dactylon*) irrigation research trial was implemented at the University of California South Coast Research and Extension Center (SCREC) in Irvine, California. The site was irrigated using recycled water and was managed using a CS3550 smart soil moisture-based irrigation controller plus TDT soil moisture sensors (Acclima Inc., Meridian, ID). A total of 48 research plots (12 irrigation treatments × 4 replications) were established to carefully investigate different combinations of lower and upper soil moisture thresholds to conserve water and sustain soil health (Table 1). Soil samples were collected twice per year from 4 different depths (0-24 inches with 6-inch increments) within and below the active turfgrass root zone from all plots to study salt accumulation within the root zone due to the application of recycled water. Turfgrass performance and quality subjected to 12 replicated treatments were monitored weekly using a handheld NDVI sensor from June 7 to October 27, 2021.

Irrigation trial 2: A buffalograss ('UC Verde', *Buchloe dactyloides*) field research trial was established at the University of California Riverside Agriculture Experiment Station (UCR AES) in Riverside, California. A total of 36 plots (sized 12 ft by 12ft) were planted using plugs in May 2020 and kept under non-limiting irrigation for one year to ensure proper root establishment and complete coverage of the plots. The trial started in June 2021 and twelve treatments were applied in a factorial completely randomized block design. There were six irrigation levels (80% ET_o, 70% ET_o, 60% ET_o, 50% ET_o, 40% ET_o, 30% ET_o), and two irrigation frequencies (3 days/week and 6 days/week) with three replicates. We used a Weathermatic SL4800 smart-controller to set the treatment settings and automate the irrigation throughout the study period.

Economic analysis: We have obtained and are in the process of cleaning monthly water consumption data from multiple water agencies for the industrial water sector that contains water meters associated with golf courses from 2011 to 2019. Water agency data will come from a maximum of 8 water agencies (Figure 1) for the current set of water agencies that have agreed to supply meter level data for the industrial sector. Once the above database is cleaned, we will match water-meter locations from the industrial water use sector using lat-long characteristics to lat-long data set to develop individual golf course level datasets. For each golf course in the dataset, we will evaluate how water use changed across different geographic regions and over time. In addition, we will highlight differences in trends and usage pre-drought, drought, and post-drought. Finally, we have also developed the survey, and our postdoc is creating an online version and obtaining IRB approval from UCR. The survey will be programmed in Qualtrics and implemented among golf courses identified in water use data (Figure 1) to obtain supplementary data. The survey data will provide us with the current water use efficiency technologies applied in the golf courses and help develop the cost-benefit analysis.

Results to date:

Trial 1 at UCANR SCREC: We observed a good response from the Acclima smart controller to schedule irrigation based on the implemented thresholds. However, when the applied water was converted to percentages of ET_o (25-81%) substantial differences were observed compared to 2020 values. Table 2 shows the results from the statistical analysis for the NDVI. The NDVI values ranged from 0.15 to 0.62 in 2021. The irrigation levels significantly affected NDVI values ($p < 0.001$). The irrigation frequency restrictions also showed a significant impact on NDVI ($p < 0.001$). The interaction of irrigation levels and frequency had a significant effect on NDVI values too in 2021 ($p<0.001$). Figure 2 shows the dynamics of NDVI values over time across the irrigation treatments for 2021. NDVI for most of the treatments stayed below the acceptable quality range except for 75-100FC on-demand treatment, which had NDVI≥0.5 for half of the data collection period. The decline in the NDVI values was more apparent in the 3-day irrigation frequency treatments as the summer progressed.

In 2021, soil salinity ranged from 0.73 ds/m to 2.37 ds/m in spring and 1.13 to 4.72 ds/m in fall. As shown in Figure 3, the soil salinity increased in the fall season, especially in the shallow soil depths (0-30cm), indicating the accumulation of salts due to high evapotranspiration demand over summer. Soil salinity distribution at different depths varied with the irrigation season as spring collected samples showed significantly higher accumulation at the 0-15 cm depth, while 15- 30 cm depth had substantially higher accumulation in the fall. The sodium adsorption ratio (SAR) ranged from 2.89 to 5.68 in spring and 3.26 to 7.76 in the fall of 2021. SAR distribution at different depths varied with the irrigation season as spring collected samples showed significantly higher values at the middle depths (30-45 cm), while shallow depths, particularly 0- 30 cm, had significantly higher values in the fall. Our preliminary assessment of the infiltration rate measurements revealed no clear pattern across the treatments.

Trial 2 at UCR AES: The statistical analysis of the NDVI data (Table 2) shows strong evidence that NDVI in 99% ET_o, 86% ET_o, and 74%ET_o differ from NDVI values for 62%ET_o, 49% ET_o, and 37% ET_o treatments. There is no evidence of differences among irrigation frequencies. In addition, the analysis of variance shows strong evidence that irrigation level and data collection date have a significant impact on NDVI. Figure 4 illustrates the changes in NDVI values over time across irrigation treatments for the buffalograss plots. The reduction in NDVI values is more pronounced for the severe deficit irrigation treatments. Overall, the Weathermatic controller showed an acceptable performance by overirrigation ranging from 4 to 10% compared with CIMIS ETo values.

Future expectations of the project:

We will run the second year of the buffalograss irrigation trial at UCR AES next year and finalize the analysis of the hybrid bermudagrass data collected over the last three years. We will distribute the survey among golf courses in Southern California. We will also use the hourly data to carry out our economic analysis.

Table 1: Treatments for the soil moisture-based irrigation trial conducted at SCREC in Irvine, California.

Treatment	Lower limit	Upper limit	Watering days
T1	75%FC	FC	3days/week
T2	65%FC	FC	
T3	65%FC	FC-10%	
T4	55%FC	FC	
T5	55%FC	FC-20%	
T6	75%FC	FC+10%	
T7	75%FC	FC	7days/week
T8	65%FC	FC	
T9	65%FC	FC-10%	
T10	55%FC	FC	
T11	55%FC	FC-20%	
T12	75%FC	FC+10%	

- FC denotes the field capacity of the soil.

Table 2: Statistical analysis of the bermudagrass and buffalograss response in terms of NDVI values to irrigation treatments imposed in 2021.

Irvine hybrid bermudagrass trial		**Riverside buffalograss trial**	
Treatment	**NDVI**	**Treatment**	NDVI
55-80FC	0.279 a	37% ETo	0.31d
55-100FC	0.266 a	49% ETo	0.33d
65-90FC	0.416 c	62% ETo	0.39c
65-100FC	na	74% ETo	0.46b
75-100FC	0.408 c	86% ETo	0.48ba
75-110FC	0.374 b	99% ETo	0.51a
Frequency		**Frequency**	
3 d week^{-1}	0.319 a	3 d week^{-1}	0.41a
On-demand	0.378 b	6 d week^{-1}	0.42a
Model effect		**Model effect**	
I	***	I	***
F	***	F	NS
I x F	***	I x F	NS
T	***	T	***
I x T	**	I x T	***
F x T	NS	F x T	NS
I x F x T	**	I x F x T	NS

NS, ***, **, and * are non-significant or significant at p ≤ 0.001, 0.01, and 0.05, respectively. Means sharing a similar letter are not significantly different, based on the Turkey's test at the significance level (α) = 0.05. I, F, and T in the table refer to irrigation levels, frequency, and time (i.e., repeated measures of visual rating each year over time), respectively.

Table 3. Summary of applied, targeted and programmed irrigation levels based on 81% irrigation efficiency for the buffalograss trial conducted at UCR AES in Riverside, California.

6 Days Irrigation Frequency						
Treatment ETo	80%	70%	60%	50%	40%	30%
Programed ETo	99%	86%	74%	62%	49%	37%
Applied ETo by Weathermatic	109%	94%	81%	67%	54%	41%
Overapplication	10%	8%	7%	5%	5%	4%
3 Days Irrigation Frequency						
Treatment ETo	80%	70%	60%	50%	40%	30%
Programed ETo	99%	86%	74%	62%	49%	37%
Applied ETo by Weathermatic	106%	92%	79%	66%	53%	41%
Overapplication	7%	6%	5%	4%	4%	4%

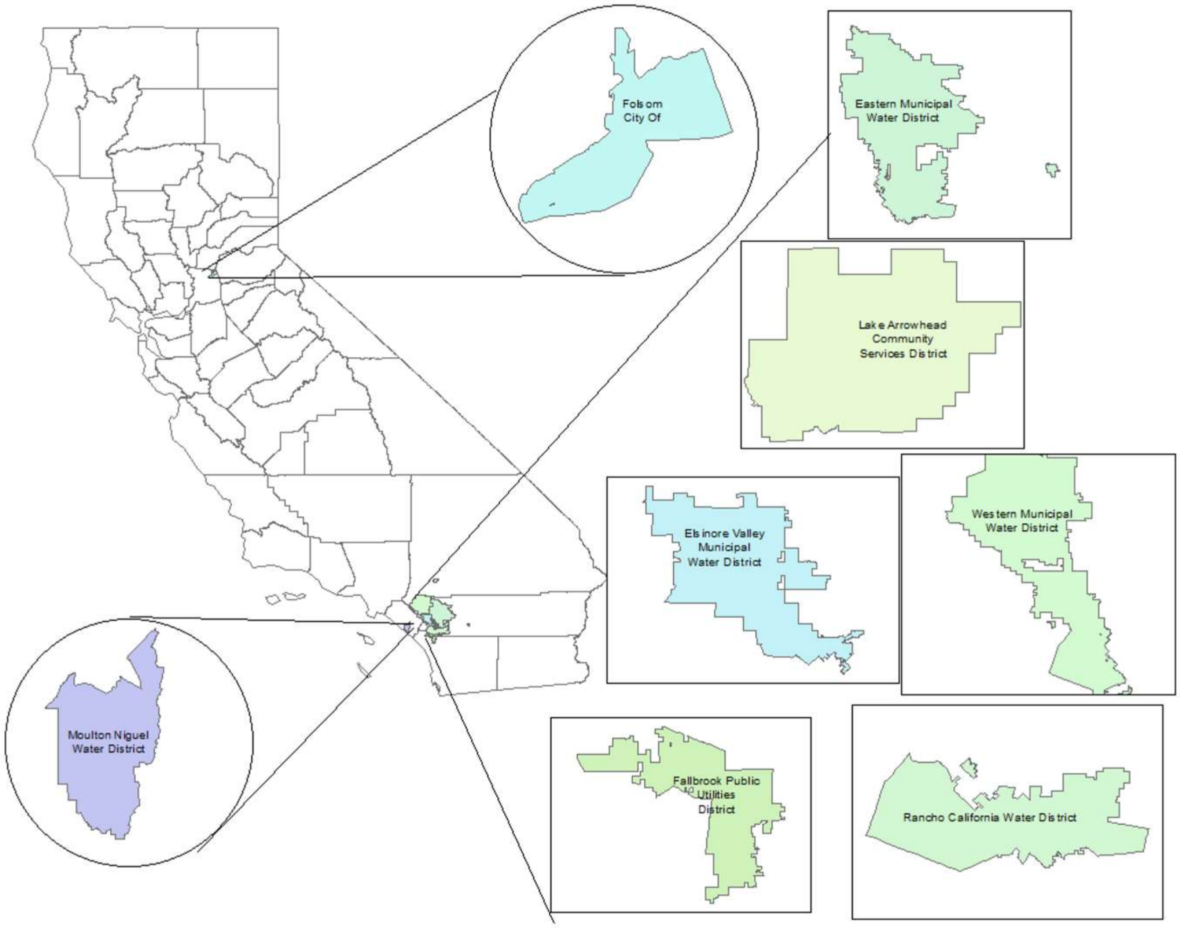


Figure 1. Map of Participating Water Agencies.

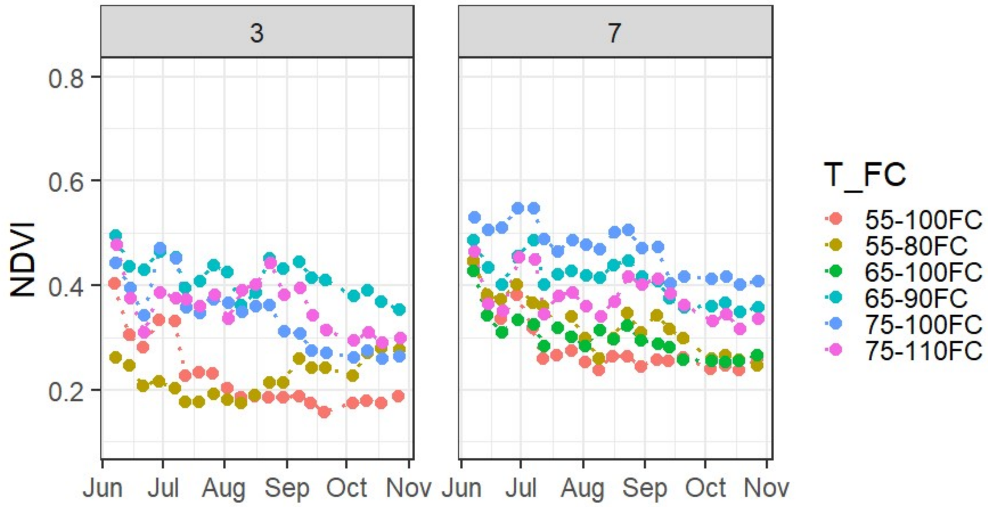
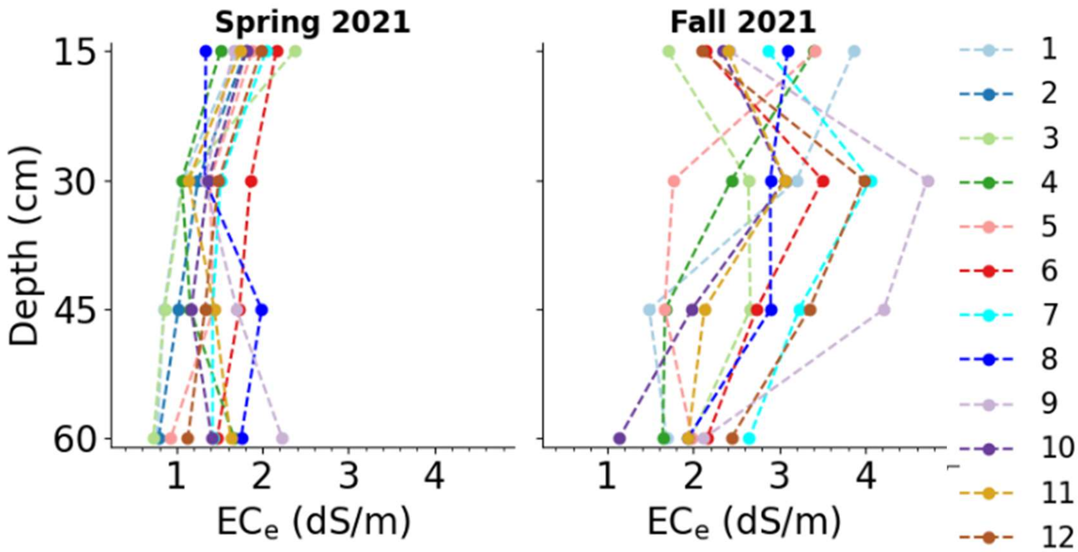


Figure 2. Changes in NDVI values of hybrid bermudagrass over time across the irrigation treatments for the restricted (3 d/week) and on-demand (7 day/week) irrigation treatments imposed in 2021.



Figure

Figure 3: Soil salinity (EC_e) distribution in the soil profile from soil samples collected before (Spring) and after (Fall) of the summer irrigation season in 2021 from hybrid bermudagrass plots located in Irvine, California. Legend at the right of the graphs represents the treatment number as shown in table 1.

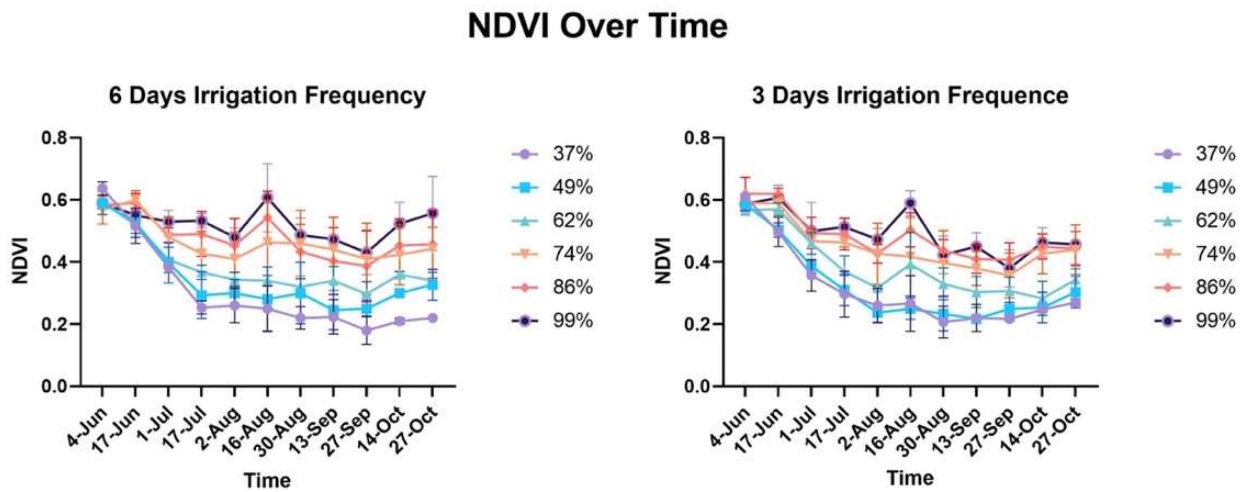


Figure 4. Changes in NDVI over time for the buffalograss irrigation trial conducted at UCR AES in Riverside, California.

USGA ID#: 2020-04-709

Project Title: Effect of acidification on soil bicarbonate concentration, infiltration rate, and Kentucky bluegrass performance.

Principal Leaders: Elena Sevostianova and Bernd Leinauer

Affiliation: New Mexico State University

Objectives:
The objectives of the field study are to:

1. Quantify changes of soil infiltration rates of rootzones irrigated with water high in bicarbonates and treated either with N-pHuric acid or with Curative.
2. Determine the effect of N-pHuric acid or Curative on the level of bicarbonates and other chemical parameters in soil.
3. Determine the effect of irrigation water treated with N-pHuric acid or Curative on the quality of Kentucky bluegrass

Start Date: 2021
Project Duration: 3
Total Funding: $116,580.00

Summary Points:
- Kentucky bluegrass irrigated with N-pHuric amended water had higher hydraulic conductivity compared to other irrigation treatments.
- Kentucky bluegrass irrigated with N-pHuric exhibited the best visual quality and the highest Dark Green Color Index DGCI.
- Infiltration rates measured by means of double-ring infiltrometer did not differ between irrigation treatments.

Summary Text
Background and Rationale
Many golf courses increasingly face the use poorer quality. Many of these sources, especially located in arid regions, contain high levels of dissolved bicarbonates. Bicarbonate levels in irrigation water are measured along with sodium, calcium, and magnesium content of both soil and water, as these are often believed to be the major cause of soil physical problems such as low infiltration rate and reduction of plant rooting. In arid climate zones, there is a concern that the deposit of calcium carbonate derived from irrigation water can seal soil pores over time, but this problem is separate from sodium-induced deterioration of soil physical conditions. While high levels of bicarbonates and sodium in irrigation water suggest that acidification is required, the question arises as to whether irrigation water acidification is necessary when ratios of Ca/Mg and HCO_3/CO_3 are high, but levels of sodium are low. Management practices that prevent the accumulation of calcite in soil have been described, however there is a lack of scientific evidence that such conditions occur in turf rootzones. While guidelines for interpretation of irrigation water quality consider many parameters and their interactions, such as EC and SAR, information about the effect on turfgrass performance of irrigation water high in bicarbonates is scarce.

Methods

The study was initiated in November 2020 at New Mexico State University's Turfgrass Salinity Research Center in Las Cruces, NM. The soil at the site consisted of a sandy loam, a skeletal mixed thermic Typic Torriorthents, a sandy entisol typical for arid regions. The research area consisted of sixteen 2m by 2m plots arranged in a block design. Kentucky bluegrass "Barserati" was seeded November 11, 2020 and established during spring 2021. During establishment plots were irrigated with potable water daily at 100% of ETo and mowed biweekly at a height of 7.5 cm by means of a rotary mower with clippings collected. During establishment, all plots received standard applications for fertilization and weed control.

All irrigation treatments started on 15th June 2021. Irrigation water was prepared in four 500-gallon tanks. To increase the level of bicarbonate to 500ppm, mix of sodium bicarbonate and potassium bicarbonate was used. All plots with established Kentucky bluegrass were irrigated 5 times a week with the corresponding irrigation waters from tanks by hand at 70% of ETo for the Las Cruces area. A detailed descriptions of ion concentrations in the irrigation waters are listed in Table 1. A control treatment received potable water only.

Four water treatments were used in the project:

Trt. 1. Potable water with low concentration of bicarbonates (200 ppm) was used as a control
Trt. 2. Potable water with high concentration of bicarbonates (450-500ppm)
Trt. 3. Potable water with high concentration of bicarbonates (450-500ppm) with N-pHuric acid was added in the amount needed to adjust the pH to 6.5
Trt. 4. Potable water with high concentration of bicarbonates (450-500ppm) with Curative was added following label recommendations

Table 1. Chemical analysis of water samples collected from four water tanks.

Constituents	Trt 1.	Trt 2.	Trt 3.	Trt. 4
pH	8.2	8.6	7.9	8.5
EC, mmho/cm	0.78	1.3	1.31	1.27
Sodium, Na, ppm	62	116	129	124
Potassium, K, ppm	6	93	92	95
Sulfate, SO_4-S, ppm	37	38	80	38
Carbonate, CO_3, ppm	<1.0	9.2	<1.0	9.1
Bicarbonate, HCO_3, ppm	205	451	306	463
Total alkalinity, $CaCO_3$, ppm	173	382	253	392
Nitrate, NO_3-N, ppm	0.1	0.1	0.1	<0.1
Total nitrogen, N, ppm	0.1	0.3	42.1	0.3
Ammonium, NH_4-N, ppm	0.1	0.2	0.6	0.2
Total Phosphorus, P, ppm	0.5	0.21	0.7	0.43

During the growing season, plots receiving treatments 1, 2, and 4 were fertilized with Urea in an amount to match the amount of nitrogen applied to treatment 3 with N-pHuric acid amended irrigation water. At the end of the growing period, each plot was supposed to having received the same amount of nitrogen (see Discussion).

Soil samples were collected at the 10, 20 and 30 cm depths and analyzed before and after the growing season for bicarbonates (titration with 0.01N sulfuric acid), pH, EC (conductivity bridge), and SAR (plasma emission spectroscopy). Infiltration rate of each plot was measured two times during growing season by means of double ring infiltrometer (Turf-Tec International, Tallahassee, FL) (Figure 3). Soil cores were collected twice during growing season (Figure 3) and the saturated hydraulic conductivity (Ksat) was measured in the lab conditions using the KSAT (METER Group, Inc. USA). One photograph per plot was taken every other week to determine Dark Green Color Index (DGCI) using digital image analysis. Visual quality of the turf was evaluated every other week. Plant tissue analysis was conducted at the end of growing season.

Each treatment was replicated four times. To test the effects of water treatments on Kentucky bluegrass quality, DGCI, Ksat, and infiltration rate, data were subjected to an analysis of variance (ANOVA) using SAS Proc Mixed followed by multiple comparisons of means using Fisher's LSD test at the 0.05 probability level.

2021 Results

Saturated hydraulic conductivity

The ANOVA revealed significant two-way interactions between water treatment and sampling date for Ksat, DGCI, and visual quality (Table 2).

Table 2. Results of ANOVA testing the effect of water quality on Dark Green Color Index (DGCI), saturated hydraulic conductivity (Ksat), and visual quality.

	DGCI	Ksat	Visual quality	Infiltration rate
Date	<0.0001	0.0002	0.0014	
Trt	<0.0001	0.0573	<0.0001	0.9481
Date*Trt	0.0052	0.0451	0.0146	

While saturated hydraulic conductivity values did not differ before the water treatments were started, at the end of the growing season they were higher for soil irrigated with N-pHuric acid. Values for soil irrigated with Curative, potable water, and water high in bicarbonates did not differ statistically (Figure 1).

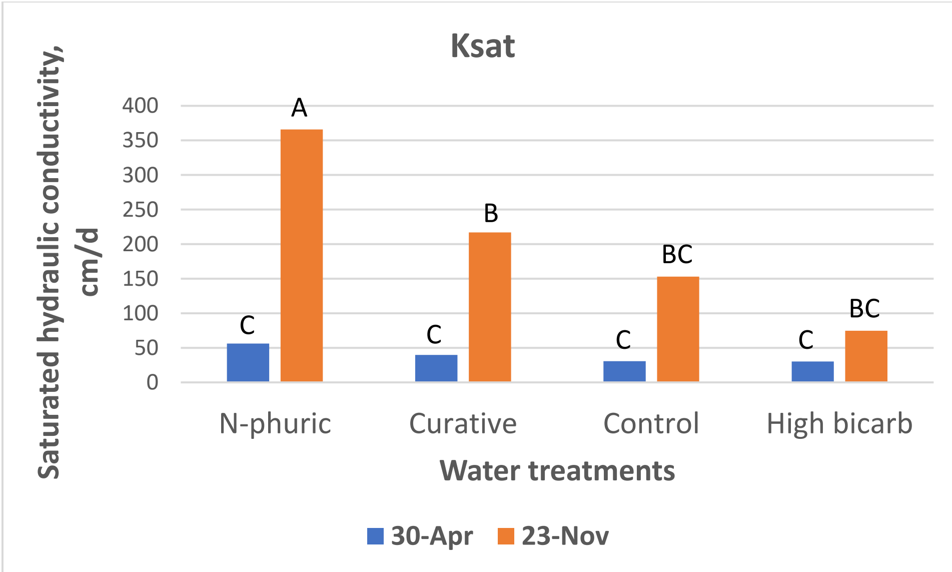


Figure 1. Saturated hydraulic conductivity of soil cores collected from the plots irrigated with four water treatments.

Visual rating and DGCI

ANOVA identified differences among visual quality and DGCI values of Kentucky bluegrass irrigated with N-pHuric acid and other treatments. Two weeks after irrigation water treatments were started and for the rest of the growing season, Kentucky bluegrass, irrigated N-pHuric acid exhibited higher visual quality (Figure 2).

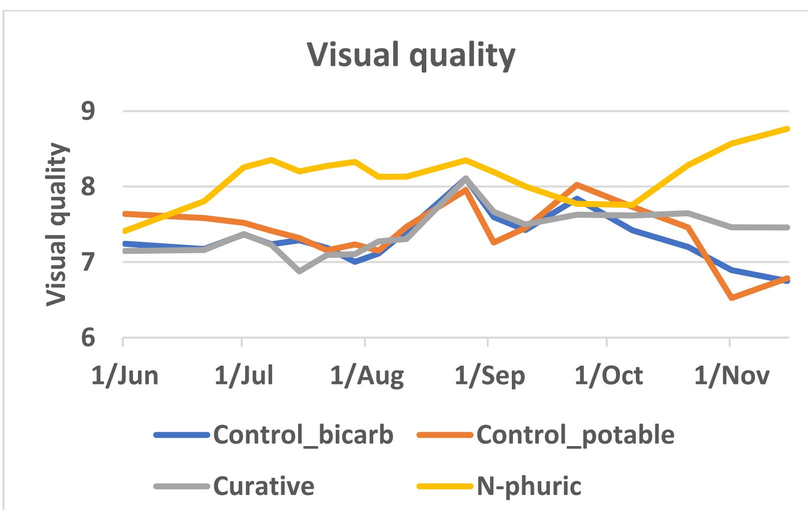


Figure 2. Visual quality of Kentucky bluegrass irrigated with four water treatments.

Infiltration rate using the double-ring infiltrometer.

There were no differences in infiltration rates on Kentucky bluegrass irrigated with four water treatments and measured by means of a double-ring infiltrometer (Figure 3).

Figure 3. Measuring of infiltration rate using double ring infiltrometers (left) and soil cores for the measurements of Ksat

Discussion:

Irrigation water sources were checked for study relevant parameters (bicarbonates, nitrogen content, pH, EC, etc.) periodically during the study by conducting a standard irrigation water test. Towards the end of the study, a significantly increase in color and quality was noticeable on the plots irrigated with N-PHuric amended water and we suspected that nitrogen may have been added in a greater amount than what was determined in the water test and matched by fertilization for other treatments. An additional test for irrigation water quality (hydroponic fertilizer test, Ward laboratories, Inc. NE) revealed that plots irrigated with N-pHuric acid amended water received an additional amount of nitrogen, ("total nitrogen, N"), which were not detected by the standard water test.

USGA ID#: 2019-02-672

Title: Comparing iron sulfate versus chelated iron for the suppression of Microdochium patch on annual bluegrass putting greens in the absence and presence of phosphorous acid

Lead Author: Clint Mattox
Project Leader: Alec Kowalewski
Collaborators: Brian McDonald, Emily Braithwaite, Alyssa Cain, Wrennie Wang, Chas Schmid
Affiliation: Oregon State University

Objectives: The objective of this experiment is to compare the effects of iron sulfate versus chelated iron in the presence or absence of phosphorous acid on the suppression of Microdochium patch and turfgrass quality.

Start Date: September 2018
Project Duration: Three-year project (the year-three report is presented here)
Total Funding: $30,000

Summary Points:

- In all three years, iron sulfate heptahydrate applications made in the presence or absence of phosphorous acid suppressed Microdochium patch compared to the non-treated control except for when 0.1 lbs. per 1,000 square feet of iron was applied as iron sulfate in the absence of phosphorous acid in the third year of the experiment.
- Chelated iron applied as DTPA in the absence of phosphorous acid did not suppress Microdochium patch compared to the non-treated control in any of the three years with the exception in the first year when 0.2 lbs. per 1,000 square feet of iron was applied.
- When phosphorous acid was applied in combination with either iron source, Microdochium patch was significantly suppressed compared to the non-treated control.
- Soil sample analyses suggest that phosphorous acid applications increase the amount of available phosphorus over time.

Figure 1. Overview of the Microdochium patch disease pressure on 20 January 2021 in Corvallis, OR.

Summary:

The third year of this three-year study began on September 5th, 2020, and the last application was made on March 31st, 2021. Disease pressure was high in all three years of the trial as indicated by the visual symptoms in Figure 1 and the levels of Microdochium patch in the non-treated control at the peak of disease in all three years of the experiment (Table 1). In all three years, iron sulfate heptahydrate applications made in the presence or absence of phosphorous acid suppressed Microdochium patch except for when 0.1 lbs. per 1,000 square feet of iron was applied as iron

sulfate in the absence of phosphorous acid in the third year of the experiment (Table 1). Chelated iron applied as DTPA in the absence of phosphorous acid did not suppress Microdochium patch compared to the non-treated control in any of the three years with the exception in the first year of the experiment when 0.2 lbs. per 1,000 square feet of iron was applied. In this occurrence, DTPA resulted in 25 percent Microdochium patch compared to 45 percent in the non-treated control, suggesting DTPA applied alone is not likely to provide acceptable suppression for putting greens. When phosphorous acid was applied in combination with either iron source, Microdochium patch was significantly suppressed compared to the non-treated control. Turfgrass quality data is not included in this report; however, no treatment other than the fungicide control were considered acceptable on all rating dates because of the lack of disease suppression. In addition, when iron sulfate was applied in the presence or absence of phosphorous acid, turfgrass thinning and darkening of the plots lead to unacceptable turfgrass quality.

	Percent Microdochium Patch at Peak at Disease		
	24 Jan. 2019	11 Feb. 2020	16 Feb. 2021
0.1 # Fe/M as $FeSO_4$	20.0% b[z]	26.3% bc[z]	25.0% bc[z]
0.1 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.4% c	1.1% d	9.0% cde
0.2 # Fe/M as $FeSO_4$	1.8% c	0.4% d	7.0% cde
0.2 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.0% c	0.1% d	6.3% de
0.1 # Fe / M as DTPA	32.5% ab	42.5% ab	47.5% a
0.1 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.7% c	1.1% d	11.8% cde
0.2 # Fe/M as DTPA	25.0% b	35.0% ab	38.8% ab
0.2 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.1% c	0.3% d	6.0% e
0.075 lbs. H_3PO_3 / M	3.3% c	8.0% cd	24.3% bcd
Fungicide Control	0.0% c	0.0% d	0.1% e
Non-treated Control	45.0% a	50.0% a	36.3% ab

Table 1. Letter diagram of effects of iron sources applied every two weeks in the combination or the absence of phosphorous acid from September through March in Corvallis, Oregon on percent Microdochium patch at the peak of disease over three years. [z]Means in the same column followed by the same letter are not significantly different according to Tukey's HSD (alpha ≤ 0.05).

In addition to Microdochium patch suppression, another objective of the experiment was to assess the effects on soil chemical analyses after three years of applications in the same location. To quantify these effects, soil samples were collected following the conclusion of treatments in early May of each year. Soil samples were collected to a depth of three inches and the verdure was removed. The samples were air dried and then passed through a 2mm screen. Material that did not pass through a 2mm screen was frozen using liquid nitrogen in a mortar and crushed using a pestle to include the mat layer in the soil analyses. Samples were analyzed at the Oregon State University Soil Health Laboratory for soil pH and nutrients were extracted using a Mehlich III extraction. Iron, phosphorus, potassium, and sulfur were included in the analyses. The results for soil pH and phosphorus are provided in Table 2.

It was hypothesized that iron sulfate applications in the same location over three years would decrease the soil pH over the course of the experiment; however, the soil analyses did not support this hypothesis. Regarding phosphorus, after three years of treatment applications, there was significantly more phosphorus in all treatments that included phosphorous acid compared to treatments lacking phosphorous acid. Phosphorous acid is not considered to be a plant available source of phosphorus and has been shown to decrease plant vigor when applied to soils that are deficient in phosphorus. These soil analyses support the hypothesis that the phosphorous acid applications will become plant available over time and future research may provide guidance on adjusting fertilizer recommendation when phosphorous acid is frequently used.

	pH			Phosphorus		
	2019	2020	2021	2019	2020	2021
0.1 # Fe/M as $FeSO_4$	6.1 ab^z	6.0 cdef	6.0 bcd	40 cd^z	19 ab	13 d
0.1 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	5.9 bc	6.0 def	6.0 cd	55 a	30 ab	30 b
0.2 # Fe/M as $FeSO_4$	5.9 bc	5.9 fg	5.9 de	42 cd	19 ab	13 d
0.2 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	5.8 c	5.8 g	5.8 e	48 abc	27 ab	23 c
0.1 # Fe / M as DTPA	6.0 abc	6.1 bcd	6.1 ab	42 bcd	19 ab	14 d
0.1 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	5.9 bc	6.1 bcde	6.0 bcd	52 ab	35 a	34 ab
0.2 # Fe/M as DTPA	6.1 ab	6.3 a	6.2 a	40 cd	13 b	14 d
0.2 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	6.0 abc	6.1 abc	6.2 ab	49 abc	26 ab	32 ab
0.075 lbs. H_3PO_3 / M	6.0 ab	6.0 cdef	6.0 bcd	44 bcd	28 ab	36 a
Fungicide Control	6.0 abc	5.9 ef	5.9 de	37 d	14 b	14 d
Non-treated Control	6.2 a	6.2 ab	6.1 abc	44 bcd	16 ab	17 d

Table 2. Letter diagram of effects of iron sources applied every two weeks in combination or absence of phosphorous acid from September through March in Corvallis, Oregon on soil pH and phosphorus concentrations quantified in parts per million (ppm) extracted using Mehlich III from soil samples collected to a 3-inch depth. ^{z}Means in the same column followed by the same letter are not significantly different according to Tukey's HSD (alpha ≤ 0.05).

Future expectations of the project:

This experiment concluded in May 2021. The results were presented at the 2021 Crop Science Meeting in Salt Lake City, UT on November 9th, 2021, and future stakeholder presentations in Oregon, nationally, and internationally will include these results. The full results of this study will be written as a manuscript and submitted to a scientific journal and subsequently proposed as a trade journal article.

USGA ID#: 2019-03-673

Title: Quantifying the long-term effects of alternative Microdochium patch management techniques on sand-based annual bluegrass putting green performance over multiple seasons

Lead Author: Clint Mattox
Project Leader: Alec Kowalewski
Collaborators: Brian McDonald, Emily Braithwaite, Alyssa Cain, Wrennie Wang, Chas Schmid
Affiliation: Oregon State University

Objectives: The objective of this experiment is to observe the long-term impacts of winter applications of alternatives to traditional fungicides on Microdochium patch suppression, summer putting green performance, and soil fertility on an annual bluegrass putting green.

Start Date: September 2018
Project Duration: Three-year project (the year-three report is presented here)
Total Funding: $30,000

Summary Points:

- In the third year, all treatments suppressed Microdochium patch compared to the non-treated control and were not statistically different from the fungicide control rotation.
- Anthracnose was most severe in late spring (14th of June 2021) on plots that had previously received treatments that included combinations of iron sulfate and phosphorous acid.
- As the summer progressed, highest anthracnose severity was observed on plots that received sulfur as elemental sulfur or as iron sulfate as a portion of the Microdochium patch treatments.

Summary:

This report focuses primarily on the third year of this ongoing long-term project, although with three years of data, more general conclusions can be made concerning treatment effects on the suppression of Microdochium patch and the effects of these treatments on the severity of anthracnose in subsequent summers. Third-year applications began on the 5th of September 2020 and ended on the 31st of March 2021. The fourth-year applications of this long-term trial began on the 7th of September 2021 and are ongoing. In the third year of this study, Microdochium patch pressure was high with an average of 54% Microdochium patch on the non-treated control plots on the 16th of February 2021 (Figure 1 and Table 1).

Figure 1. Overview of the Microdochium patch disease pressure on 16 February 2021 in Corvallis, OR.

In the third year of this experiment, all treatments suppressed Microdochium patch at the peak of disease compared to the non-treated control and were in the same statistical group as the fungicide control rotation (Table 1). When comparing data across the three trial years, all treatments suppressed Microdochium patch compared to the non-treated control in all three years; however, there were

exceptions to how the alternative to traditional fungicide treatments compared to the fungicide rotation in each year (Table 1). In the first year of the study, sulfur applied alone, phosphorous acid applied alone, and the combination of sulfur and phosphorous acid applied every two weeks did not suppress Microdochium patch as well as the fungicide rotation. In the second year of the study, phosphorous acid applied every two weeks did not suppress Microdochium patch as well as the fungicide control. The results from the first three years of this experiment suggest that sulfur and phosphorous acid, either applied alone or in combination do not consistently suppress Microdochium patch as well as the other treatments in this study. Turfgrass quality data is not included in this report; however, treatments including iron sulfate reduce turfgrass quality below acceptable levels because of darkening and thinning of the annual bluegrass stand.

Treatment		Percent Microdochium Patch at Peak of Disease		
#	Description	Jan 2019	Feb 2020	Feb 2021
1	S^z + PA^y	2.0% c[x]	0.1% c[x]	0.4% b[x]
2	Sep, Oct, Nov, Apr = MO^w + PA Dec, Jan, Feb, Mar = S + PA	0.3% d	0.5% c	1.0% b
3	MO + PA rotated with S + PA	0.3% d	0.1% c	0.8% b
4	MO rotated with S + PA	0.9% cd	0.7% c	2.6% b
5	0.5 # FeSO4 + PA	0.6% d	1.8% c	5.5% b
6	1.0 # FeSO4 + PA	0.5% d	0.0% c	1.3% b
7	Sulfur (S)	4.3% b	0.7% c	2.3% b
8	Phosphorous Acid (PA)	3.8% b	11.8% b	9.3% b
9	Fungicide Control	0.3% d	0.0% c	0.8% b
10	Non-treated control	40.0% a	72.5% a	53.8% a

Table 1. Letter diagram of effects of treatments applied every two weeks on percent Microdochium patch at the peak of disease over three years in Corvallis, Oregon on an annual bluegrass research green. [Z]S = Sulfur applied at 0.25 lbs. of S per 1,000 square feet. [Y]PA = Phosphorous acid applied at 0.075 lbs. of H_3PO_3 per 1,000 square feet. [x] Mean differences in the same column followed by the same letter are not significantly different according to Fisher's LSD test (alpha ≤ 0.05). [w]MO = Mineral oil applied at 8.5 oz. per 1,000 square feet.

In previous trials, it has been observed that treatments applied over multiple months from the fall through the spring to suppress Microdochium patch may influence the severity of anthracnose the following summer. The summer of 2021 in Corvallis, OR experienced extreme weather in the early summer, with temperatures reaching 110 degrees Fahrenheit in late June and anthracnose pressure was very high, reaching above 30% for certain treatments on the 17 July 2021 rating date (Table 2 and Figure 2).

Figure 2. Overview of the anthracnose disease pressure on 17 July 2021 in Corvallis, OR.

In June, the severity of anthracnose was highest on plots that had previously received treatments that included combinations of iron sulfate and phosphorous acid. By July, all treatments were in the group with the highest amount of anthracnose severity except for the non-treated control, the fungicide control, and the phosphorous acid treatment. The fungicide control had the least amount of anthracnose in July, with 0.6% anthracnose compared to seven other treatments with an average of 20% or more anthracnose. The fungicide control received the last application on March 3rd, 2021, with an application of Ascernity (2.24% Benzovindiflupyr + 7.48% Difenoconazole) at a rate of 1.0 oz. per 1,000 square feet and Heritage Action (50% Azoxystrobin + 1.18% Acibenzolar-S-methyl) at a rate of 0.4 wt. oz. per 1,000 square feet. Since fungicides are only effective when the fungus can actively absorb the fungicide, it is speculated that anthracnose was active in March or April, likely in the basal rot stage. The seven treatments in the group with the highest anthracnose severity all received sulfur as elemental sulfur or as iron sulfate as a portion of the Microdochium patch treatments. Previous research has suggested that sulfur applications increase the severity of anthracnose, providing a potential explanation why plots receiving only phosphorous acid and the non-treated control did not have as much anthracnose severity in July. By the month of August, all ten treatments resulted in high levels of anthracnose and significant differences were no longer observed.

Treatment		Percent Anthracnose by Month		
#	Description	14 Jun 2021	17 Jul 2021	17 Aug 2021
1	S[z] + PA[y]	0.0% c[x]	27.5% ab[x]	47.5% ns[w]
2	Sep, Oct, Nov, Apr = MO[v] + PA Dec, Jan, Feb, Mar = S + PA	0.4% c	26.3% ab	37.5% ns
3	MO + PA rotated with S + PA	0.1% c	20.0% ab	37.5% ns
4	MO rotated with S + PA	0.0% c	30.0% a	46.3% ns
5	0.5 # FeSO4 + PA	12.5% b	32.5% a	22.5% ns
6	1.0 # FeSO4 + PA	28.8% a	25.0% ab	17.5% ns
7	Sulfur (S)	0.6% c	32.5% a	38.8% ns
8	Phosphorous Acid (PA)	0.5% c	16.3% bc	28.8% ns
9	Fungicide Control	0.0% c	0.6% d	20.0% ns
10	Non-treated control	0.9% c	3.8% cd	28.0% ns

Table 2. Letter diagram of effects of treatments applied every two weeks from September 2020 through April 2021 on the severity of anthracnose in June, July, and August 2021 in Corvallis, Oregon on an annual bluegrass research green. [Z]S = Sulfur applied at 0.25 lbs. of S per 1,000 square feet. [Y]PA = Phosphorous acid applied at 0.075 lbs. of H_3PO_3 per 1,000 square feet. [x]Mean differences in the same column followed by the same letter are not significantly different according to Fisher's LSD test (alpha ≤ 0.05). [w]ns=not significant. [v]MO = Mineral oil applied at 8.5 oz. per 1,000 square feet.

Future expectations of the project:

The first three years of this long-term trial have concluded; however, this long-term experiment is ongoing. Microdochium patch and anthracnose severity will continue to be collected and soil samples will be collected in May of each year to assess long term changes in soil nutrition levels and pH. The 2021 soil samples are still be processed for laboratory analyses and the results will be published in the future. The authors intend to submit a manuscript for publication based on the first three year of results and to share these results with stakeholders in future local, national, and international meetings.

USGA ID#: 2021-02-726
Title: Bentgrass Cultivar and Autumn-applied Fungicide Timing Effects on Spring Suppression of Dollar Spot
Project Leaders: James A. Murphy, Bruce B. Clarke, Ning Zhang, Pingyuan Zhang, Glen Groben
Affiliation: Rutgers, The State University of New Jersey

Objectives:
Evaluate the 1) timing of autumn-applied fungicide, 2) disease tolerance in the host plant, and 3) antecedent inoculum load during autumn for effects on the onset, severity, and progress of dollar spot on bentgrass turf and the inoculum load during the subsequent growing season. 4) Based on results from preliminary trial 1, an additional objective was added to this research project: to evaluate the effect of fungicide chemistry on disease onset and progress.

Start Date: 2021
Project Duration: 2 years
Total Funding: $60,000

Summary Points:
1) The initial preliminary trial indicates that the onset and progress of dollar spot during the subsequent growing season can be affected by fungicide chemistry applied during the previous autumn (September, October, and November).
2) Tank mix applications of fluazinam + propiconazole in September and October had the greatest suppression of dollar spot during the subsequent growing season.
3) The effectiveness of a November timing of fluazinam + propiconazole appeared dependent on environmental conditions at the time of application. This suggests that a weather-based approach may be needed to optimize the efficacy of late-season application(s) for the control of dollar spot during the subsequent growing season.
4) Quantification of pathogen DNA indicated that the September fungicide timing was associated with a consistent reduction in the pathogen populations during the subsequent growing season.
5) Additionally, fungicide chemistries applied in autumn had different effects on the subsequent pathogen populations.
6) Trials to address objectives 2, 3, and 4 have been initiated and will be continued in 2022.

Summary:
Fairway turf represents a significant land area on golf courses, thus fungicide inputs on fairways to control dollar spot (caused by *Clarireedia* spp.) can be a major contributor to economic and environmental costs. Anecdotal observations and preliminary experimental evidence suggest that autumn-applied fungicide has the potential to reduce dollar spot on fairway turf during the subsequent growing season; however, the optimal timing is not clear. Results from a preliminary trial conducted 2019-2021 indicated that fungicide applied in September was associated with lower pathogen populations and treatments that included a September fungicide application were among treatments with the lowest disease severity. There was clearly a fungicide chemistry effect on pathogen population and disease severity. Chlorothalonil treatment had pathogen populations similar to the non-treated check during the subsequent growing season, both of which had populations greater than the plots treated with a fluazinam + propiconazole tank mix in September-October-November. Moreover, chlorothalonil treatment had greater disease severity (symptoms) in the subsequent growing season

compared to the fluazinam + propiconazole tank mix applied in September-October-November. Two additional trials were initiated during 2020-2021 to further explore the impact of autumn-applied fungicide (including weather-driven treatments); disease tolerance in the host plant (bentgrass cultivar); antecedent inoculum load during autumn; and fungicide chemistry. Each trial will be assessed for effects on the progress of dollar spot and pathogen populations on bentgrass turf during the subsequent growing season.

Materials and Methods

Trial 1 (objective 1): This trial was conducted on '007' creeping bentgrass (*Agrostis stolonifera*) managed as fairway turf at 0.9-cm on a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) in North Brunswick, NJ (40°28' N, 74°25' W). Nine treatments (Table 1) were applied from September to November 2019 and were arranged in a randomized complete block design with 4 replications. The trial was repeated on a different site in the same field during 2020-2021 using 6 blocks. Plot size was 0.91- by 1.5-m (3- by 5-ft).

Dollar spot developed naturally in the trial areas before the initiation of the study and was suppressed on 10 September 2019 and 2020 with fluazinam. Fungicide treatments were initiated on 24 September; seven treatments received a tank mix of fluazinam and propiconazole once (three timings), twice (three timings), or thrice (one timing) in September, October and/or November in 2019 and 2020 (Table 1). An eighth treatment received chlorothalonil thrice (September, October, and November) and the ninth treatment was a non-treated check.

Table 1. Fungicide and application timings to evaluate the effect of autumn fungicides applied in 2019 and 2020 on dollar spot onset and progress during the subsequent growing season on '007' creeping bentgrass in North Brunswick, NJ.

Treatments[1]	Number of Sprays	Fungicide Timing (Date)		
Non-treated check[2]	0			
Sep.[3]	1	Sep. 24		
Oct.[3]	1		Oct. 15	
Nov.[3]	1			Nov. 5
Sep.- Oct.[3]	2	Sep. 24	Oct. 15	
Sep. - Nov.[3]	2	Sep. 24		Nov. 5
Oct. - Nov.[3]	2		Oct. 15	Nov. 5
Sep. - Oct. -Nov.[3]	3	Sep. 24	Oct. 15	Nov. 5
Chlorothalonil[4]	3	Sep. 24	Oct. 15	Nov. 5

[1] Fungicide treatments initiated after the pre-treatment suppression of dollar spot on 10 September 2019 and 2020 with fluazinam (Secure) at 0.7 kg a.i. per ha.

[2] The non-treated control received no fungicide after the pre-treatment spray on 10 September.

[3] Seven of the treatment timings (three single, three double, and one triple) were applied in September, October and/or November 2019 (location 1) and 2020 (location 2) using a tank-mix of fluazinam (Secure) and propiconazole (Banner MAXX) at 0.7 kg a.i. and 1.5 kg a.i. per ha, respectively

[4] The eighth fungicide treatment applied chlorothalonil (Daconil Ultrex 82.5WG) at 15.3 kg a.i. per ha in September, October, and November 2019 (location 1) and 2020 (location 2).

The dollar spot infected area of each plot was measured every 1 to 7 days from September to November and May through the termination of trial 1. Disease severity data was $\log_{10}$ transformed to correct for heteroscedasticity and used to calculate the area under the disease progress curve (AUDPC). AUDPC was then analyzed using the mixed model in GLIMMIX, SAS version 9.4 (SAS Institute, Cary, NC) with fungicide treatment and year as main effects. Only significant main and interaction (fungicide treatment × year) effects were retained in the final model. Means of main effects and interactions was separated using Fisher's protected least significant difference at the 0.05 probability level.

For molecular quantification of *Clarireedia* spp, ten cores were collected from a randomly selected 0.09 m^2 area inside each 0.91 m by 1.5 m plot. Cores were 1 cm in diameter by 2.5 cm in depth. Samples were collected 1 wk after the last fungicide treatment on 11 November 2019 and 13 November 2020 and then again in the spring on 5 June 2020 and 28 May 2021. Dollar spot symptoms were present on all sampling dates except 28 May 2021. Each core was cut 5 mm into the thatch layer and the lower thatch was discarded. Ten cores from each plot were pooled before being ground in liquid nitrogen with a mortar and pestle. A composite sample of 0.25 g of the ground tissue was used for DNA isolation using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. qPCR reactions were conducted following a previously established protocol (Groben et al 2020). Each sample was run with three technical replicates and each qPCR reaction included a positive DNA control isolated from a pure culture of *C. jacksonii* and a negative control of PCR grade water (Sigma-Aldrich, WY). Cycle thresholds (Ct) below 37 was considered positive for dollar spot (Groben et al 2020). The average cycle threshold for each collection date was analyzed separately using analysis of variance in R. The means of fungicide treatment was separated using Tukey's honest significant difference.

Trial 2 (objectives 1 and 2): This trial was seeded on 8 September 2020 and managed as a fairway turf at 0.9 cm in North Brunswick, NJ. Prior to the initiation of fungicide treatments, the trial was inoculated on 30 March 2021 by uniformly distributing infested oats with a hand-shaker bottle at 1.34 g/m^2. Dollar spot was allowed to develop on plots and then suppressed by periodic applications of chlorothalonil (Daconil Ultrex 82.5WG at 15.3 kg a.i. per ha) during summer: 4 applications on 8 and 31 July, 14 August and 10 September. Dollar spot symptoms had not developed before treatments were initiated in trial 2.

A 3 × 12 factorially arranged randomized complete block design with 4 replications was used for this study. The cultivar factor has three levels (e.g., Coho, 007 and Independence) and represented low to high dollar spot susceptibility. The autumn-applied fungicide factor had twelve levels that included eight calendar-based treatments, two model-based treatments, one curative (threshold-based) treatment and a non-treated check. The eight calendar-based treatments were the same as in trial 1 (Table 1). The two model-based treatments used the Smith-Kerns dollar spot predictive model at action risk index thresholds of 20 or 40%. Fungicide was applied whenever the risk index output was equal or higher than action threshold and 21-d had elapsed since the previous application. The curative fungicide treatment was applied within 24 hours after a damage threshold of 314 mm^2 per 3-m^2 was observed; re-applications were made no sooner than 7 days after a previous application. All fungicides were applied from September through November. A tank mix of fluazinam (Secure 4.17SC) at 0.7 kg a.i. ha^{-1} (0.5 fl. oz. per 1,000 ft^2) and propiconazole (Banner MAXX 1.3MC) at 0.4 kg a.i. ha^{-1} (1.0 fl. oz. per 1,000 ft^2) was used for all treatments except the chlorothalonil (Daconil Ultrex 82.5WG) treatment, which was applied at 15.3 kg a.i. ha^{-1} (5 oz. per 1,000 ft^2). Data collection followed the same method as in trial 1. Data analysis is in progress.

For the molecular quantification of dollar spot, clippings were collected on 23 September 2021 for the pre-treatment population assessment and on 18 November 2021 at the end of the autumn-2021 treatments. Clippings were collected using a Toro Greensmaster Flex 21 mower (The Toro Company, MN) equipped with a verti-cut reel set to cut 23-mm into the turf canopy. Another sampling is planned during late May 2022.

Trail 2 will be continued for data collection in the subsequent growing season. This trial will also be repeated in 2022 on another field, which was seeded 7 September 2021.

Trial 3 (objectives 2, 3, and 4): This trial was seeded, maintained, pre-treatment inoculated and suppressed for dollar spot per the methods listed for trial 2, except that trial 3 did not receive a pre-treatment fungicide application on 10 September. Dollar spot symptoms developed on 24 September 2021 when fungicide treatments were initiated.

A 2 × 3 × 4 factorially arranged split-split plot design with 4 replications was used for this study. The first factor was arranged as the whole plot and had two levels of antecedent inoculum load created by either inoculating at 1.34 g/m^2 or not on 10 September 2021 (before initiation of autumn fungicide treatments). The second factor was cultivar, which had three sub-plot levels ('Coho', '007', and 'Independence') to represent a low to high range in disease susceptibility. The third factor was fungicide chemistry, which had four sub-sub-plot levels: fluazinam at 0.7 kg a.i. ha^{-1}, propiconazole at 1.4 kg a.i. ha^{-1}, a tank mix of fluazinam and propiconazole at 0.7 and 0.4 kg a.i. ha^{-1}, respectively, and a non-treated check which received no fungicide application during the treatment period. Fungicide applications were applied on 24 September, 15 October, and 5 November 2021. Disease data collection and analysis followed the same method as described in trial 1. The procedures for the molecular quantification of dollar spot in trial 3 were the same as those employed in trial 2. Autumn data and sample collection was completed for 2021 and the analysis is in progress.

Trail 3 will be continued for data collection in the subsequent growing season. This trial also will be repeated in 2022 on another field, which was seeded 7 September 2021

Results

Data analysis has only been completed for trail 1. Therefore, the results are only presented for trail 1.

Trial 1: The analysis of variance indicated that all fixed effects were significant in the final model as well as the fungicide treatment × year interaction effect for the dollar spot response during the fall and subsequent growing season (Table 2).

Table 2. The analysis of variance of dollar spot severity – measured as the area under the disease progress curve (AUDPC) during autumn and the subsequent growing season – during autumn and the subsequent growing as affected by nine autumn-applied fungicide treatments on '007' creeping bentgrass turf managed as a fairway in North Brunswick NJ.

Source[a]	df	*F*	P of significant *F*
Dollar spot progress during autumn fungicide treatment			
Fungicide treatment	8	30.36	<.0001
Year	1	24.71	0.0011
Fungicide treatment × Year	8	3.16	0.0045
Dollar spot progress during the subsequent growing season			
Fungicide treatment	8	25.45	<0.0001
Year	1	118.04	<0.0001
Fungicide treatment × Year	8	4.72	<0.0001

[a] Dollar spot severity from September to November (autumn) and May through the termination (subsequent season) of trial 1 for both years were analyzed with fungicide treatment and year as fixed effects; significant fixed and interaction (fungicide treatment × year) effects were retained in the final model.

The AUDPC response during autumn indicated that all fungicide treatments except the single November application, reduced disease symptoms compared to control (Fig. 1). The fungicide treatments applied in September and October provided the greatest disease control during both years of the trial.

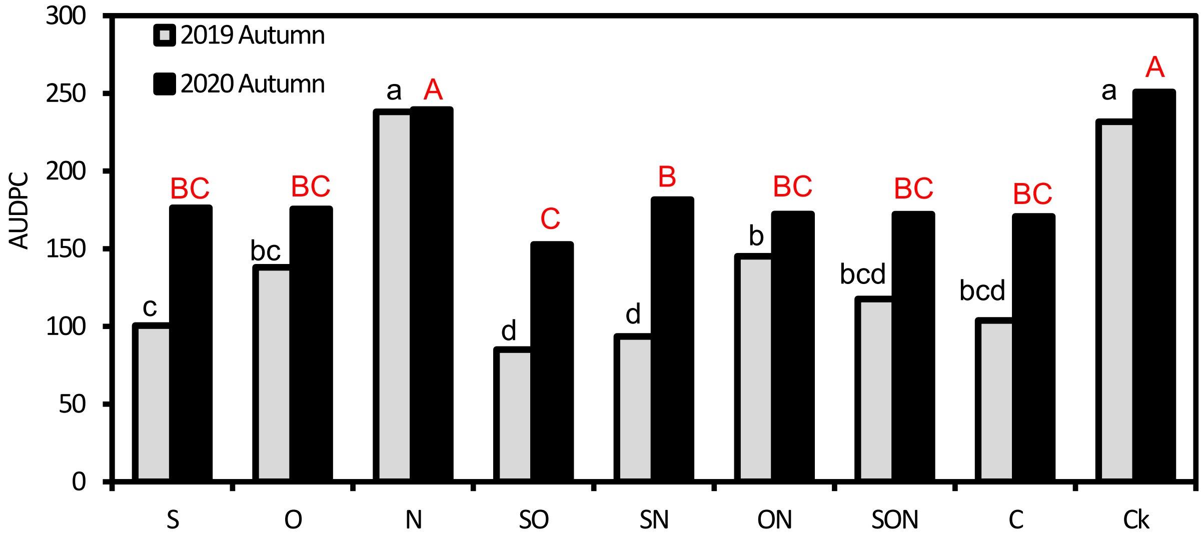


Figure 1. Autumn disease severity, measured as area under the disease progress curve (AUDPC), response for the fungicide treatment × year interaction. Lower case, black letters indicated treatment differences in 2019 and capitalized, red letters indicate treatment differences in 2020 according to Fisher's protected $LSD_{0.05}$. S = Sep., O = Oct., N = Nov., SO = Sep. - Oct., SN = Sep. - Nov., ON = Oct. - Nov., SON = Sep. - Oct. - Nov., C = chlorothalonil, Ck = non-treated check.

Except for the single November application in 2019, treatments that used the fluazinam + propiconazole tank mix suppressed dollar spot during the subsequent growing season in both years compared to the non-treated check (Fig. 2). However, the chlorothalonil treatment resulted in reduced (2020) or no (2021) suppression of dollar spot even though chlorothalonil suppressed disease symptoms the previous autumn in both trials (Fig. 1). The application of fluazinam + propiconazole in September-October or September-October-November provided the best suppression of dollar spot the following spring and early-summer in both trials. The potential of the November timing of fluazinam + propiconazole to reduce disease was probably dependent on environment conditions at the time of application (data not shown). Dollar spot was not active during colder weather in Nov. 2019, whereas warmer weather resulted in active dollar spot in Nov. 2020 (Figure 2).

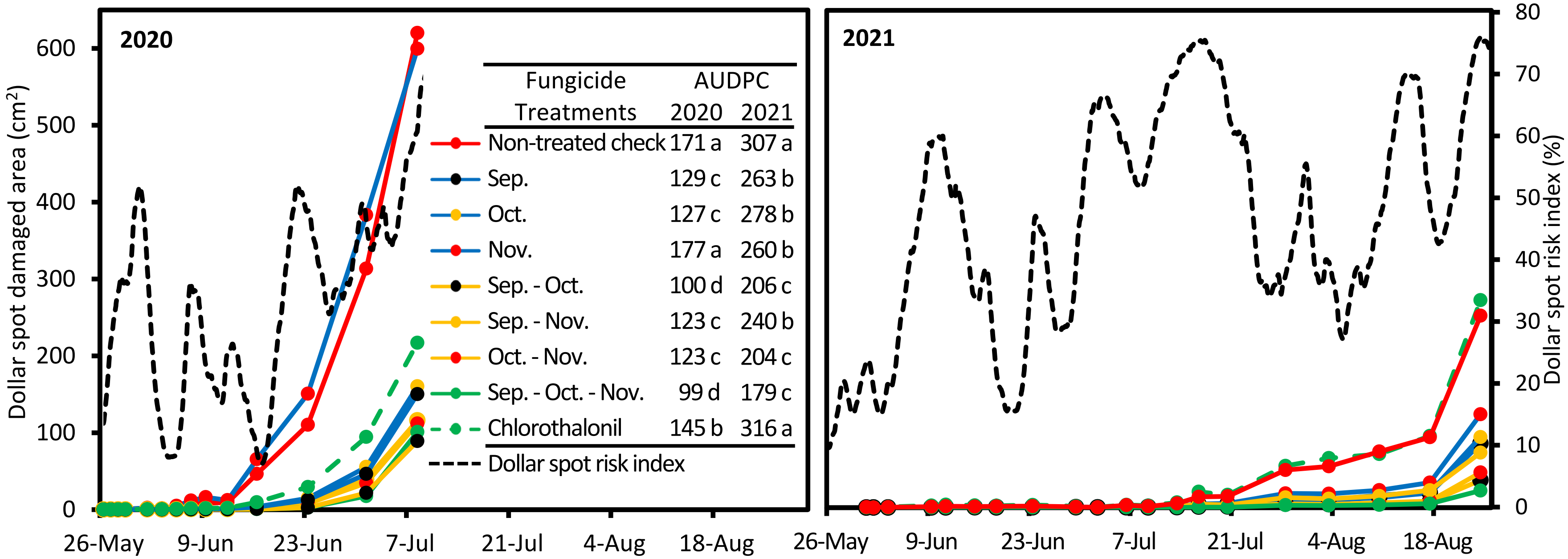


Figure 2. Disease severity during the subsequent growing season as affected by fungicide chemistry and late-season fungicide application on ‘007’ creeping bentgrass mowed at 9.5-mm in North Brunswick, NJ, during May to Aug of the subsequent year. For each year, different letters after AUDPC values indicate a significant difference between treatments. The dashed black lines depict the risk index based on the Smith-Kerns dollar spot predictive model.

6

For the molecular assessment of the pathogen population, there were statistical differences for the average cycle threshold (Ct) among the fungicide treatments on 11 November 2019 (Fig. 3). The treatments with the lowest Ct values (greatest pathogen population) were the non-treated check with a Ct value of 20.6. The two fungicide chemistries applied three times (September, October, November) had different Ct values; the fungicide tank mix had lower pathogen population (Ct=34.3) than chlorothalonil (Ct=27.3). All tank mix fungicide applications that included a September timing (S, SO, SN, SON) had had among the lowest pathogen populations (Ct above 31), while other timings had relatively greater populations (Ct above 29).

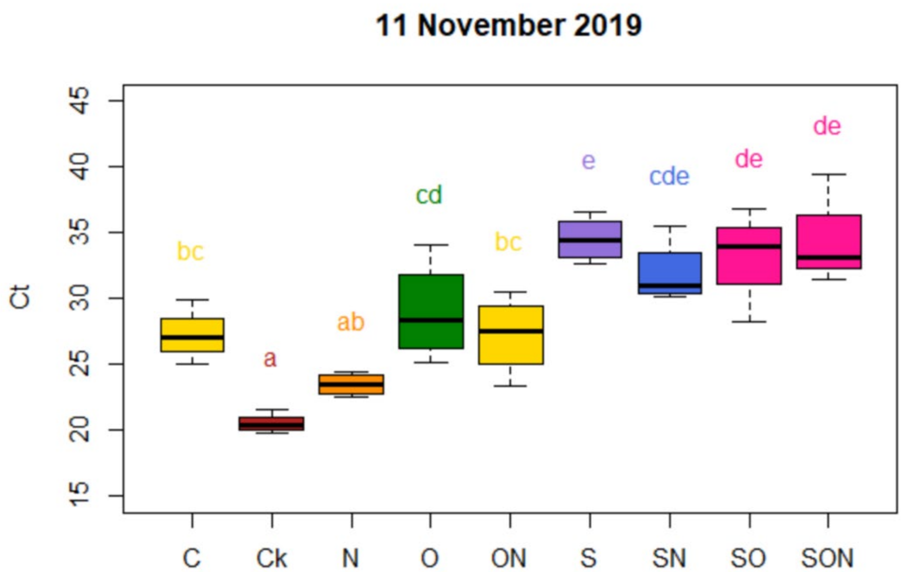


Figure 3. Boxplot of the average cycle threshold values (lower values represent a greater pathogen population) for fungicide treatments sampled on 11 Nov. 2019. The color of the box plot and the lower-case letters represent the statistical groupings from the Tukey honest significant difference test. Treatments with the same lowercase letters are not significantly different from one another. S = Sep., O = Oct., N = Nov., SO = Sep. - Oct., SN = Sep. - Nov., ON = Oct. - Nov., SON = Sep. - Oct. - Nov., C = chlorothalonil, Ck = non-treated check.

The statistical differences for the average cycle threshold among the fungicide treatments sampled on 13 November 2020 were not as distinct as observed in November 2019 (Fig. 4). The single tank mix fungicide applications did not differ from one another in 2020, nor were there differences among the double fungicide timings. However, the two fungicide chemistries applied three times (September, October, November) had different Ct values in 2020, which was a response similar to the observed in 2019; the tank mix had a lower pathogen population than chlorothalonil. All tank mix fungicide applications that included a September timing (S, SO, SN, SON) had relatively low pathogen populations (Ct above 27.2) while the other timings had greater populations (Ct below 26.6).

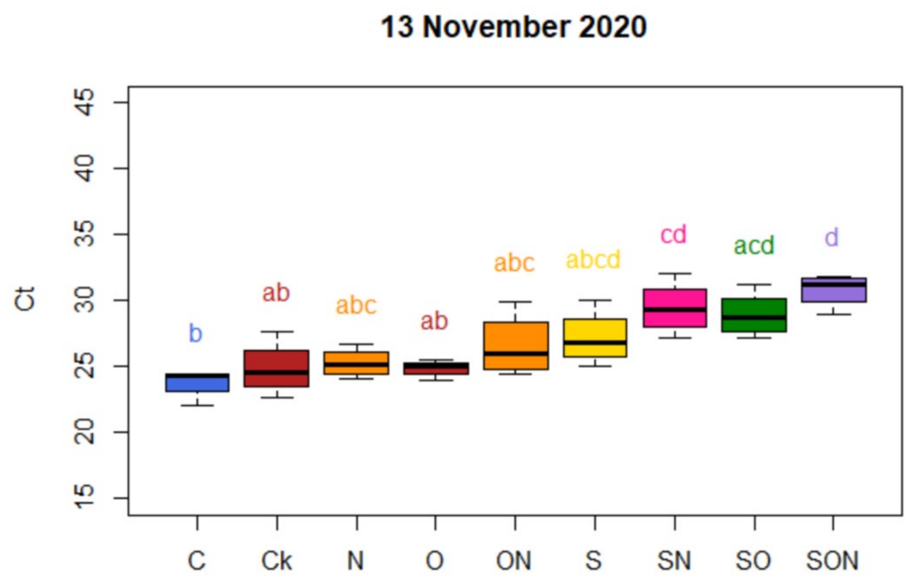


Figure 4. Boxplot of the average cycle threshold (lower values represent a greater pathogen population) for fungicide treatments sampled on 13 Nov. 2020. The color of the box plot and the lower-case letters represent the statistical groupings from the Tukey honest significant difference test. Treatments with the same lowercase letters are not significantly different from one another. S = Sep., O = Oct., N = Nov., SO = Sep. - Oct., SN = Sep. - Nov., ON = Oct. - Nov., SON = Sep. - Oct. - Nov., C = chlorothalonil, Ck = non-treated check.

The tank mix applied in November 2019 was among the treatments with the lowest Ct values (22.1; highest pathogen population) on 5 June 2020 and was statistically different from the Ct values for the chlorothalonil treatment and tank mix treatments applied in September, September-November, and September-October-November (Fig. 5). All the other treatments were not statistically different from one another.

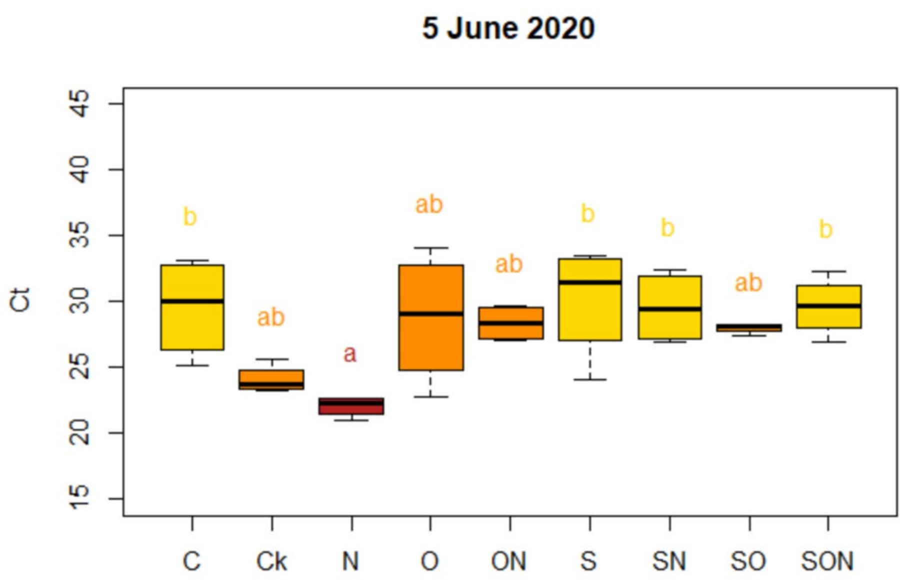


Figure 5. Boxplot of the average cycle threshold (lower values represent a greater pathogen population) for fungicide treatments sampled on 5 June 2020. The color of the box plot and the lower-case letters represent the statistical groupings from the Tukey honest significant difference test. Treatments with the same lowercase letters are not significantly different from one another. S = Sep., O = Oct., N = Nov., SO = Sep. - Oct., SN = Sep. - Nov., ON = Oct. - Nov., SON = Sep. - Oct. - Nov., C = chlorothalonil, Ck = non-treated check.

The 25 May 2021 sampling for pathogen population was performed when no disease symptoms were present; the initial disease lesions were observed on 31 May (Fig. 6). The chlorothalonil treatment applied in September-October-November 2020 was among the treatments with the lowest Ct values (29.3; highest pathogen population) on 25 May 2021, which was statistically different from the Ct values for the tank mix treatments applied in September-October and September-October-November. All the other treatments were not statistically different from one another. It is noteworthy, that the tank mix fungicide applied three times had among the highest Ct values (39.0) representing the lowest measured pathogen population.

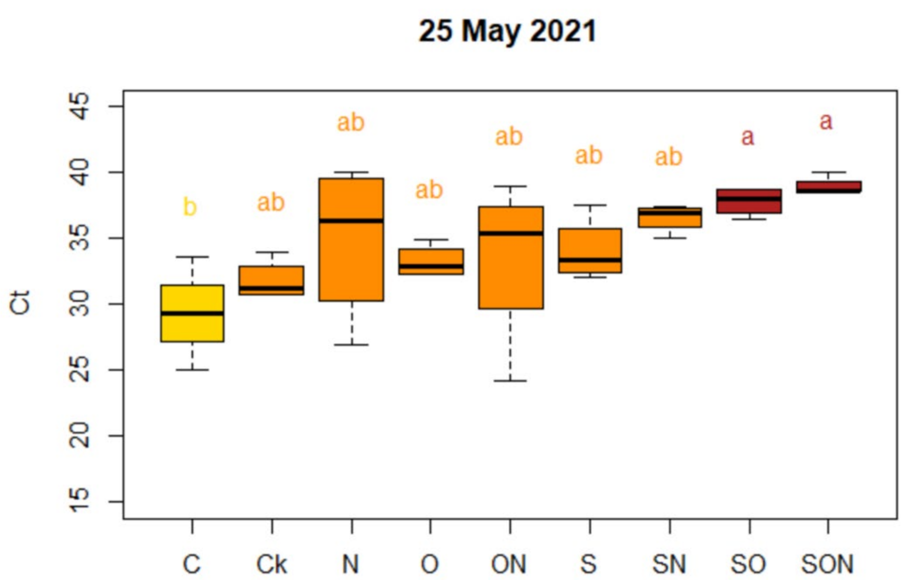


Figure 6. Boxplot of the average cycle threshold (lower values represent a greater pathogen population) for fungicide treatments sampled on 25 May 2021. The color of the box plot and the lower-case letters represent the statistical groupings from the Tukey honest significant difference test. Treatments with the same lowercase letters are not significantly different from one another. S = Sep., O = Oct., N = Nov., SO = Sep. - Oct., SN = Sep. - Nov., ON = Oct. - Nov., SON = Sep. - Oct. - Nov., C = chlorothalonil, Ck = non-treated check.

Preliminary Summary and Future Work

Preliminary results from trial 1 indicated that fungicide applied in September was associated with lower pathogen populations in the subsequent growing season. Additionally, treatments that included a September fungicide application were among treatments with the lowest disease severity in the subsequent growing season. There was clearly a fungicide chemistry effect on pathogen population and disease severity. The chlorothalonil treatment had pathogen populations (DNA quantification) similar to the check during the subsequent growing season, both of which were greater than the plots treated with fluazinam + propiconazole in September-October-November. Moreover, the chlorothalonil treatment had greater disease severity (symptoms) in the subsequent growing season compared to the fluazinam +

propiconazole applied in September-October-November. Future analysis will include a more thorough assessment of the pathogen population data related to the disease symptomology data.

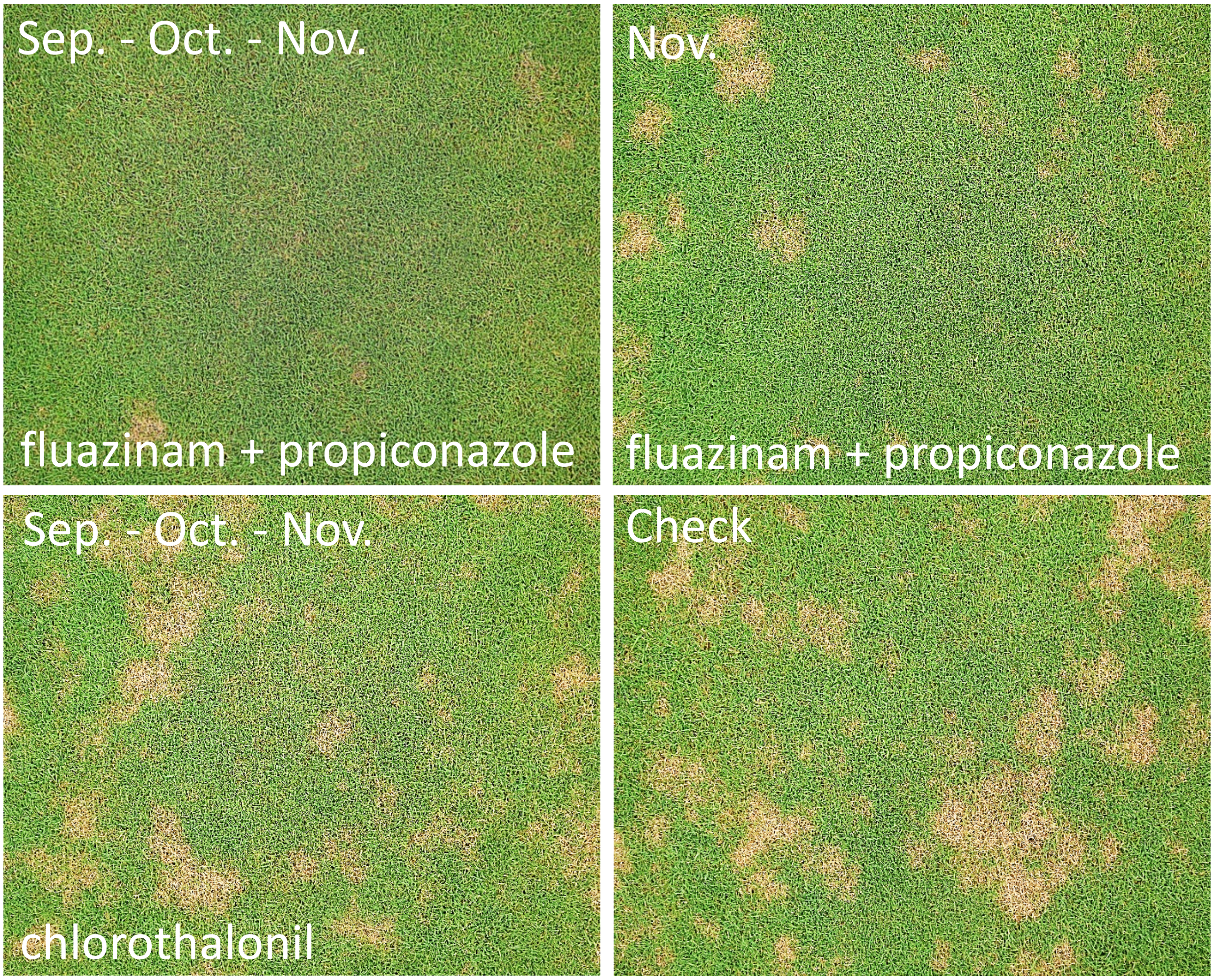


Figure 7. Low, moderate and high dollar spot severity on 13 Aug. 2021. The tank mix of fluazinam + propiconazole was applied at 0.7 and 1.5 kg a.i. per ha, respectively, during the months listed in the images. Chlorothalonil was applied at 15.3 kg a.i. per ha. The check did not receive fungicide application after the last pre-treatment spray on 10 September 2020.

Figure 8. Plot appearance after using a verti-cutter to collect clipping tissue for molecular quantification of dollar spot on 18 November 2021.

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:

- Of the four fungicides evaluated, only one, Velista (penthipyrad), showed a significant benefit from the addition of an adjuvant.
- Within trial disease variability can obscure potentially positive results.
- A better approach to screening adjuvant-fungicide combinations than brute force field testing is needed.

Introduction

Fungicides are an integral part of modern golf course turf management, particularly in the cool, humid region of the United States. Diseases can reduce turf quality at any time in the growing season and fungicides are applied frequently, and unlike other pest problems, throughout the entire calendar year. In the cool regions of the US, the last fungicide application is often made in November to prevent snow mold.

Pesticide uptake is key to the activity of systemic pesticides. Systemic fungicides must be absorbed into the plant and move within the plant in order to exert fungicidal action. Simply, if a commercial fungicide formulation typically results in approximately 25% of the active ingredient absorbed into the plant, then the application rate could be reduced by 50% if a method can be found to double fungicide absorption. Adjuvants have long been used in row crop agriculture to increase herbicide uptake, but there has been little research on this approach with fungicides.

Fungicide uptake into turfgrass foliage is a complex process involving environmental factors; including air temperature and relative humidity, plant factors; such as leaf wax composition and coverage, and fungicide factors, such as lipid solubility and uptake kinetics. Each of these factors can interact to increase or decrease fungicide activity. Some pesticides are rapidly absorbed by plants and adjuvants would be of little value. However, some pesticides are slowly absorbed by plants, and these products may be especially likely to benefit from an adjuvant addition. Pesticide uptake into foliage occurs in the liquid phase, once a spray droplet dries completely, uptake stops. Thus, factors such as spray gallonage, relative humidity (vapor pressure deficit is the preferred measurement), and air temperature can interact to affect uptake. This area of research is not widely studied because there is little economic benefit to make a product work better if it already is effective at a certain rate.

Adjuvants come in many different categories, but most share the same property, they are amphiphilic molecules, that is, they are large molecules with a polar head and hydrophobic tail. Surfactants have many uses including wetting agents, emulsifiers, dispersants, and anti-foaming agents. An adjuvant is any additive that improves pesticide performance and almost pesticides have some type of adjuvant to improve solubility, handling, dispersion, etc. Activator adjuvants are those products designed to specifically improve pesticide performance and are classified chemically as methylated seed oils (MSO), crop oil concentrates (COC), non-ionic surfactants (NIS), and organosilicone (OS) adjuvants. Ideally, a representative compound from each category would be tested to determine the best activity. However, most commercially available adjuvants may have more than one adjuvant type in a product, for example, organosilcone adjuvants, which are excellent at reducing the surface tension of water, allowing droplets to spread thoroughly on the leaf surface, are often combined with a non-ionic surfactant in commercially available products.

Our research examined four different activator adjuvants alone or in combination with an inorganic fertilizer, either urea ammonium nitrate (UAN) or ammonium sulfate (AMS). While the mechanism is not well understood, adding these inorganic salts has been shown to improve the activity of a variety of pesticides. We selected four fungicides to include in this trial, Velista, Tourney (metaconazole), Banner (propiconazole), and Secure (fluazinam). The activator adjuvants are Dyne-Amic, Induce, MSO, and Cohere (Helena Chemical Company, Memphis, TN). Cohere is a sticker, that is, an adjuvant that will adhere the pesticide to the foliage even during rain or irrigation events. Cohere was only included in the evaluation of Secure, a contact fungicide. Each pesticide was tested in a factorial combination of treatments. Adjuvants were a factor and inorganic fertilizer was a second factor. Fungicide applications were initiated on June 8, 2021. Each fungicide was applied at the lowest label rate and maximum interval between applications. Velista, Banner, or Tourney were applied every 3 weeks at 13, 44, or 8 oz product/A. Secure was applied every 2 weeks at 21.8 ounces product/A. These treatments served as standard treatments. In order to determine if the adjuvants were enhancing control, each fungicide was applied at 2/3 of the standard treatment rate with the adjuvants and inorganic salts as shown in table 1.

Results in 2021 were disappointing. Disease variability was high making comparisons challenging. For example, Velista showed some positive responses with adjuvants, but the standard rate of Velista, 13 oz product/A performed equally well as the reduced rate of Velista, 8.7 oz product/A. We expected the 13 oz rate to provide superior control to the 8.7 oz rate, and then adjuvant enhancement would be clear, that is if the 8.7 oz rate with an adjuvant provided comparable or superior control compared to the 13 oz rate, then the adjuvants are clearly providing improved control. However, with no difference between the 13 and 8.7 oz rate, the adjuvants did not significantly improve control (Table 1). When analyzing the Velista data, the effects of adjuvants were significant on the August 17th rating when each adjuvant gave significantly better control than not using an adjuvant. The inorganic fertilizers did not improve activity in the Velista trial, although AMS showed less control than either UAN or no fertilizer on the August 17th rating (Table 1). A common technique for quantifying disease development is known as the area under the disease progress curve (AUDPC) which essentially integrates the disease data over an entire growing season. Our data for Velista AUDPC was significant, but only at the 0.1 level of probability. Again, this data showed that all three adjuvants provided slightly better disease control than not using an adjuvant (Table 2).

Research conducted in 2019 with Banner showed significant increases in activity with the adjuvant Dyne-Amic and even better results with Dyne-Amic plus UAN. However, this enhanced activity was not observed in 2021 with Banner. Additionally, no benefits from adjuvants were seen with Tourney or Secure.

Lastly, we have been using a drone-mounted camera to collect images of our research trials. We believe that drones can change the way turf is managed in the future and are looking at the potential for disease prediction using aerial drone images. In this trial, our drone images are being used to accurately collect data on the percent of diseased area within a treatment (Figure 1).

While the results achieved in 2021 are disappointing, we look forward to repeating and expanding these trials in 2022.

Table 1. Effects of adjuvants and inorganic fertilizers in combination with reduced rates of Velista on dollar spot activity.

Treatment	Rate (oz prod/A)	7/13	7/20	8/3	8/17	AUDPC
Control		0.7	19 a	40 a	56 a	1147 a
Velista	13	0.2	7 bcd	13 bcd	33 bcd	484 bcde
Velista	8.7	0.2	9 bcd	10 cd	31 cd	450 cde
Velista + Dyne-Amic	8.7 +0.25 % v/v	0.2	9 bcd	11 cd	22 d	400 de
Velista + Induce	8.7 + 0.5 % v/v	0.5	11 bc	21 b	29 cd	622 bcd
Velista +MSO	8.7 + 0.5% v/v	0.3	6 cd	8 d	24 d	345 e
Velista + UAN	8.7 + 2.5 % v/v	0.3	9 bcd	14 bcd	38 bc	558 bcde
Velista + AMS	8.7 + 20 gm/L	0.3	9 bcd	21 b	46 ab	714 b
Velista + Dyne-Amic + UAN	8.7 + 0.25 % + 2.5 %v/v	0.0	8 bcd	6 d	26 cd	348 e
Velista + Induce + UAN	8.7 + 0.5 % + 2.5 % v/v	0.3	10 bcd	13 bcd	27 cd	481 bcde
Velista +MSO + UAN	8.7 + 0.5% + 2.5 % v/v	0.2	7 bcd	11 cd	22 d	376 de
Velista + Dyne-Amic + AMS	8.7 + 0.25 % v/v + 20 gm/L	0.7	13 ab	20 bc	38 bc	683 bc
Velista + Induce + AMS	8.7 + 0.5 % v/v + 20 gm/L	0.2	3 d	11 bcd	26 cd	379 de
Velista +MSO + AMS	8.7 + 0.5 % v/v + 20 gm/L	0.2	6 bcd	13 bcd	28 cd	450 cde
	LSD P=0.05	NS	7	10	13	251

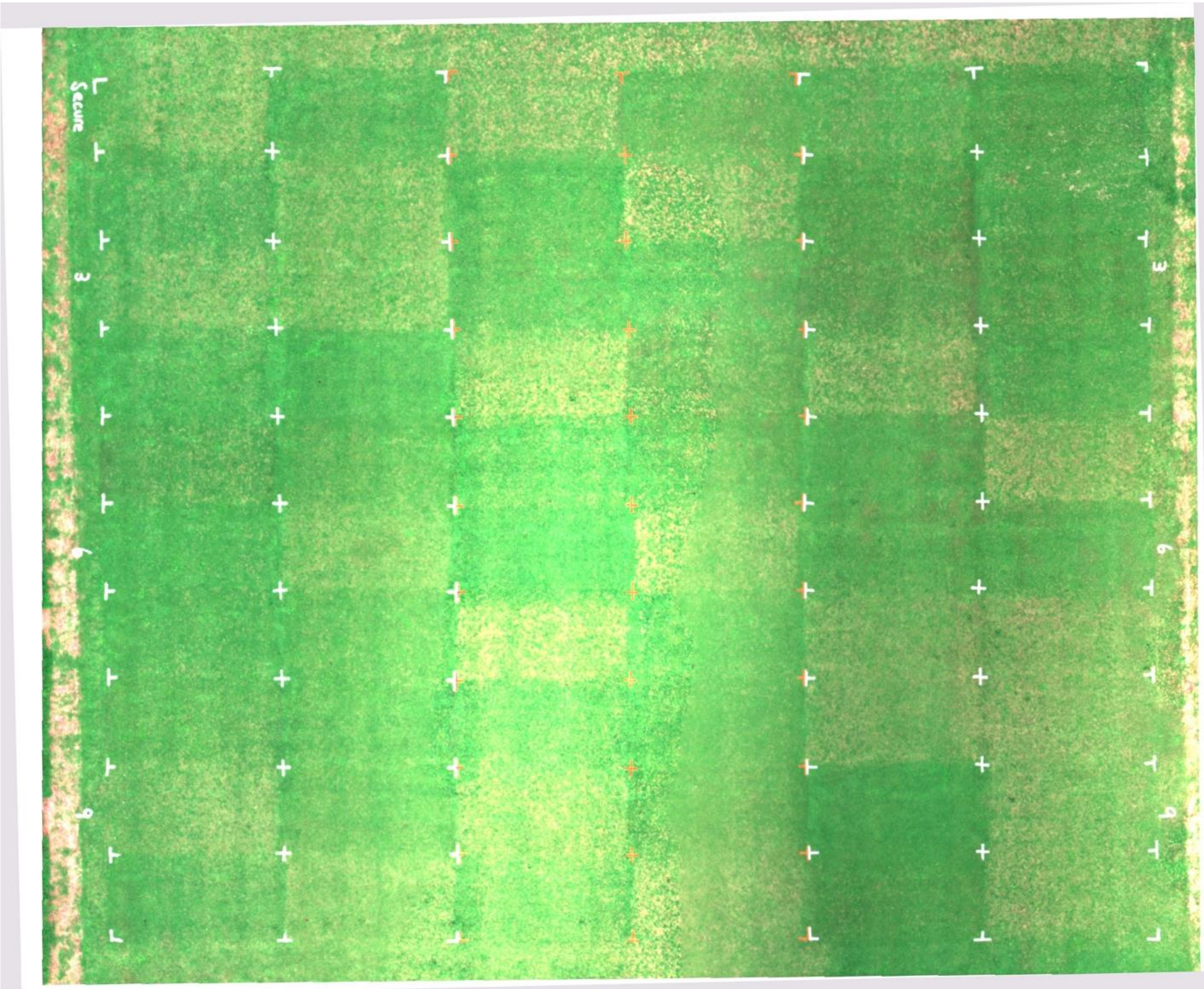
Velista applied every 21 days.

Table 2. Main effect of adjuvant on Dollar spot coverage and on the cumulative dollar spot as denoted by the area under the disease progress curve (AUDPC).

Adjuvant	Dollar Spot cover, 8/17/21	AUDPC
None	38 a	574 a
Dyne-Amic	29 b	477 ab
Induce	28 b	494 ab
MSO	25 b	390 b
LSP (P=0.05)	8.1	NS*

*Significant at P=0.1

Figure 1. Drone RGB image of Secure Fungicide dollar spot trial collected on August 4th, 2021.



USGA ID#: 2019-04-674

Project Title: Biology and Management of Pythium Root Rot in Golf Course Putting Greens

Project Leaders: James P. Kerns
Affiliation: North Carolina State University

Objectives:
1. Determine the distribution and prevalence of pathogenic root-infecting *Pythium* species in golf course putting greens.
2. Assess aggressiveness towards mature turfgrass plants of *Pythium* species associated with Pythium root rot.
3. Determine *in vitro* sensitivity of *Pythium* species collected to various fungicides.
4. Develop a quantitative PCR assay to detect *Pythium* species in turfgrass roots.

Start Date: 2019
Project Duration: 3 years
Total Funding: $81,250

Summary Points:
- Isolate of *Pythium* species during summer months is challenging as most of the isolates recovered were non-pathogenic species such as *Pythium torulosum*.
- Based on the limited data we collected in 2020, we hypothesize that *Pythium* infection precedes symptom development in creeping bentgrass.
- *P. torulosum* growth was only inhibited by cyazofamid, fluazinam, and etridazole.
- *In vitro* sensitivity varied among *Pythium* species, but all were extremely sensitive to cyazofamid.

Summary Text:

Samples exhibiting symptoms of Pythium root rot that were submitted to the NC State Turfgrass Diagnostic Lab were selected for isolation of *Pythium* species. Affected roots were washed for at least 3 hours and plated on semi-selective and non-selective media. After 24 hours of incubation in the dark, candidate hyphae were transferred from the aforementioned plates to a fresh petri plate containing water agar to obtain a pure culture. Out of 125 isolates collected, 88 were identified as *Pythium torulosum* and the remaining were identified as either *Pythium vanterpoolii (8), P. irregulare (5), P. aphanidermatum (1), or P. volutum* (1). All of the isolates collected except for *P. torulosum* were extremely aggressive when placed on creeping bentgrass seedlings. The pathogenic *Pythium* species were primarily collected during May and June which is early in terms of symptom expression. Of the 125 isolates collected, 22 were collected from ultradwarf bermudagrass putting greens. Fifteen of the isolates collected were *P. vanterpoolii,* 4 were *P. torulosum,* and 4 were *P. arrhenomanes.* Identifications were conducted by extracting the ITS regions and amplifying using ITS 4 and 5 primers in PCR. Sequences were aligned and generated sequences were compared using GenBank's BLAST and the Oomycete Gene Table database. Sequence identification was corroborated with molecular characteristics such as oospore/oogonia dimensions and ornamentations, antheridia characteristics and colony morphology. We will continue isolation efforts and will construct a phylogenetic tree once we have collected isolates from next year.

Given the challenges associated with isolation, we plan to establish permanent plots at the Lake Wheeler Turfgrass Research and Education Lab in Raleigh, NC for sampling purposes. We will sample from 8 replicate plots that will remain untreated throughout the course of the spring and summer. We plan to commence sampling in February and sample every three weeks throughout 2020 to help establish a clear picture of the species associated with this disease. We will also bury soil temperature and soil moisture probes at this location to see if we can correlate these factors to Pythium root rot development. In 2020, we established the permanent plots and only were able to sample in February, June-November. We have over 100 *Pythium* isolates collected and we are currently conducting identifications. Currently, we have collected *P. vanterpoolii, P. rhizo-oryzae,* and *P. graminicola*. There are many more that will be identified using molecular and morphological techniques soon. This work is ongoing, but the figure below explains what we collected from these plots at Lake Wheeler. It is interesting that the most diversity we collected in terms of pathogenic Pythium species is in April and May and little diversity is recovered during the summer months. This summer in August and September we did not collect any Pythium species at all. This indicates that infection is occurring in late spring and early summer and symptoms are not expressed until creeping bentgrass is under physiological stress from heat and humidity during the summer months.

Sensitivity of *Pythium* isolates vary dramatically to fungicides. All isolates tested were extremely sensitive to cyazofamid and etridiazole. Most the isolates we collected were insensitive to propamocarb, which is the first report of insensitivity to this chemistry. The non-pathogenic speices, *P. torulosum*, was only sensitive to cyazofamid, fluazinam and etridiazole. It grew readily on the other fungicides we tested, which may explain why it is so prevalent during our summer sampling strategy. Certain species like *P. vanterpoolii,* were highly sensitive to QoIs. This is similar to what Kerns and Tredway document with *P. volutum*. We will continue to screen isolates for sensitivity as we collect them. This portion will continue into 2021.

All of this work was delayed due to the Covid-19 pandemic. We are working through pathogenicity experiments and the quantitative PCR assay. We have completed pathogenicity work with the isolates we have collected, but that will finish April of 2021. The quantitative PCR assay development will start in Fall of 2020 and will be completed in summer of 2021. In addition to this work, we started a preventive fungicide trial in spring of 2019. Applications of cyazofamid were made starting in March, April, May, June or July and subsequent monthly applications were applied after the initial application. The product was applied at 0.45 floz/1000 sq ft and applications were irrigated immediately after application with 1/8 inch of water. We found that preventive applications have to start no later than May 1 in order to be effective. In other words, soil temperatures could not exceed 72°F in order to prevent Pythium root effectively. Applications in June and July were not effective in preventing Pythium root rot. This further supports our hypothesis that infection precedes symptom development. In fact, infection could occur as creeping bentgrass rooting is at its height in March, April and May. We were able to get a partial year to validate the preventative treatments in 2020. We will conduct another trial in 2021 and will also include another location in Florence, SC.

Table 1. *In vitro* sensitivity of *Pythium* species (number of isolates) to commercially available fungicides.

NC STATE UNIVERSITY

Pythium species	Fungicides[x]									
	EC_{50} Concentrations µg ml^{-1}									
	cyazofamid	fluazinam	etridiazole	azoxystrobin[y]	fluoxastrobin[y]	pyraclostrobin[y]	mefenoxam	chlorothalonil	propamocarb	fluopicolide
***P. aphanidermatum* (2)**										
P. aph	9.895 a[z]	0.380 de	0.439 def	>10 a	>10 a	>10 a	0.074 e	3.390 de	>10 a	6.640 b
P. aph2	0.035 d	0.559 de	2.310 a	>10 a	>10 a	>10 a	0.226 e	3.094 def	>10 a	>10 a
***P. irregulare* (1)**										
P. irr	4.098 b	>10 a	0.755 d	0.9354 b	3.336 b	0.643 b	0.202 e	>10 a	>10 a	>10 a
***P. arrhenomanes* (2)**										
WRGC5	0.039 d	0.198 de	1.368 c	>10 a	>10 a	>10 a	3.116 b	8.907 ab	1.141 c	0.956 d
Sedgefield	0.004 d	0.110 e	0.518 def	>10 a	>10 a	>10 a	0.204 e	1.212 efg	>10 a	>10 a
***P. vanterpoolii* (6)**										
RBR	0.012 d	0.237 de	0.241 ef	0.0608 c	0.06 c	0.271 b	1.965 bcd	9.137 ab	>10 a	>10 a
P1	0.058 d	0.267 de	0.799 d	>10 a	>10 a	>10 a	>10 a	0.997 fg	>10 a	>10 a
Lambert	0.031 d	0.292 de	1.945 ab	0.1637 c	0.116 c	0.06 b	0.485 e	3.501 d	>10 a	>10 a
DMC15	0.044 d	0.241 de	0.775 d	.0733 c	0.116 c	0.047 b	2.547 bc	7.615 bc	>10 a	>10 a
DMC22	0.026 d	0.212 de	0.642 de	.0904 c	0.113 c	0.047 b	0.618 e	>10 a	6.468 b	3.341 c
Pinehurst	0.074 d	0.432 de	1.287 c	>10 a	>10 a	>10 a	>10 a	6.276 c	>10 a	>10 a
***P. ultimum* var. *ultimum* (1)**										
P. ult	0.367 d	3.01 c	0.383 def	0.1284 c	0.163 c	0.139 b	>10 a	7.491 bc	>10 a	>10 a
***P. volutum* (1)**										
OC6	0.002 d	0.058 e	1.341 c	0.0431 c	0.095 c	0.041 b	1.833 cd	0.678 g	>10 a	>10 a
***P. torulosum* (4)**										
LW1	0.098 d	0.819 d	1.532 bc	>10 a	>10 a	>10 a	>10 a	7.173 bc	>10 a	>10 a
LW5	0.042 d	0.195 de	0.223 ef	>10 a	>10 a	>10 a	>10 a	1.935 defg	>10 a	>10 a
LW10	0.056 d	0.210 de	0.532 def	>10 a	>10 a	>10 a	>10 a	1.729 defg	>10 a	>10 a
LW12	0.045 d	0.212 de	0.257 ef	>10 a	>10 a	>10 a	>10 a	2.08 defg	>10 a	>10 a
***P. vexans* (1)**										
Ed-mum-27	>10 a	5.229 b	0.526 def	0.1649 c	0.263 c	0.92 b	0.3576 e	0.127 g	>10 a	3.966 c
***P. myriotylum* (1)**										
Ed-mum-22	1.078 c	0.591 de	0.148 f	0.076 c	0.058 c	0.403 b	0.168 e	>10 a	6.407 b	>10 a

[x] Commercial formulations of fungicides.
[y] SHAM (50 µg ml^{-1}) was added with fungicides to reduce alternative oxidase pathway.
[z] Values followed by the same letter within a column are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

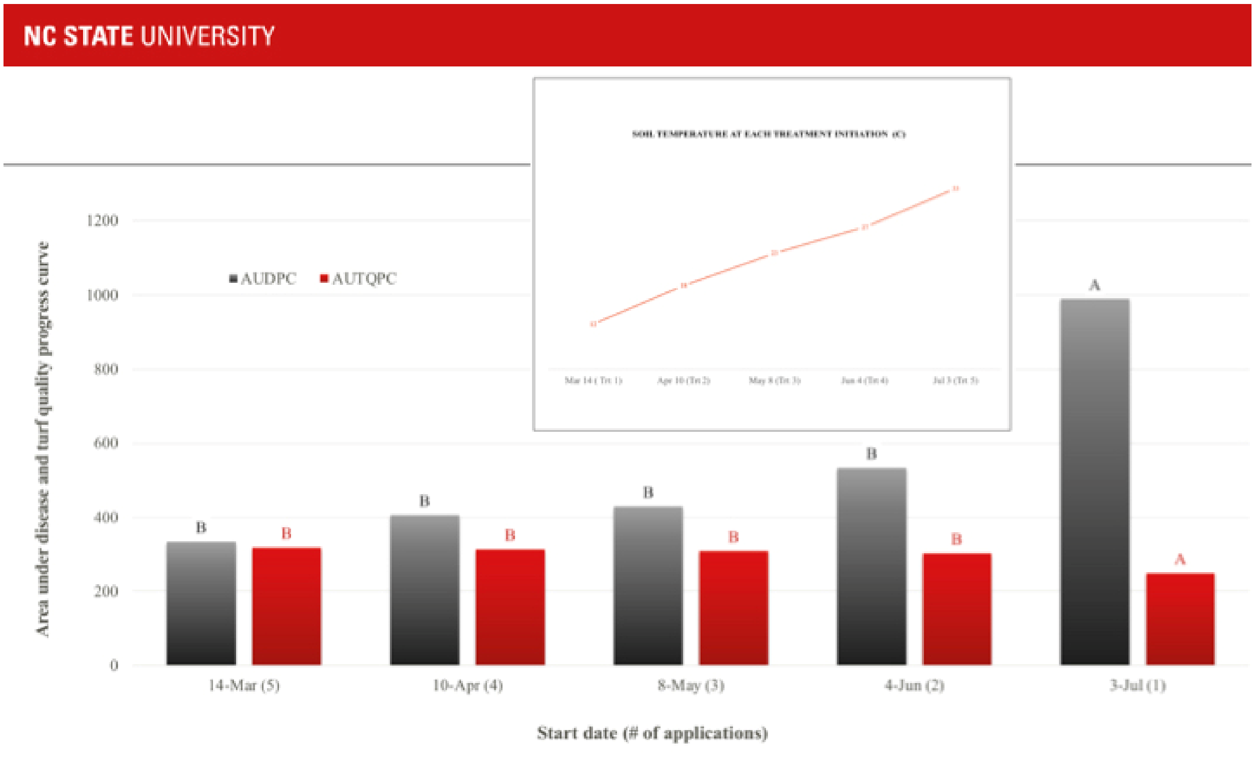


Figure 1. Efficacy of preventative cyazofamid applications for Pythium root rot in creeping bentgrass. Applications started in either March, April, May, June or July and were re-applied monthly until August. All applications were irrigated immediately with 1/8 inch of water and cyazofamid was applied at 0.45 floz/1000 ft^2.

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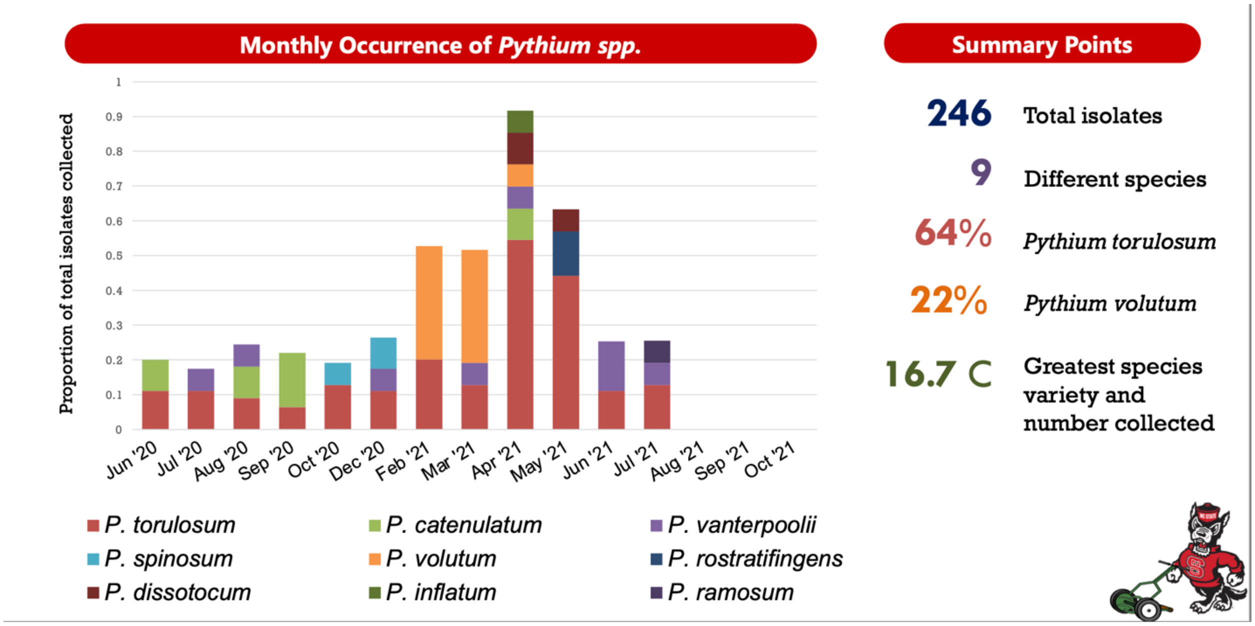


Figure 2. Prevalence of *Pythium* species isolated from a 'Dominant Plus' creeping bentgrass putting green in Raleigh, NC. Note that there are multiple species associated with Pythium root rot and most of the pathogenic species are present in March, April and May.

USGA ID#: 2020-16-721

Title: Economic impact of take-all root rot on bermudagrass putting green management

Project Leader: Young-Ki Jo
Affiliation: Texas A&M University

Objectives: The objectives of the project is to develop a diagnostic protocol for *Gaeumannomyces* species associated with take-all root rot in bermudagrass and to remediate economic losses from the disease in bermudagrass putting greens.

Start Date: 2020
Project Duration: 3 years
Total Funding: $80,296

Summary Points:

- Set up the protocol of *Gaeumannomyces* species isolation from bermudagrass
- Set up the protocol of DNA-based identification of *Gaeumannomyces* species
- Set up the pathogenicity assay to determine virulence of *Gaeumannomyces* species

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. Given the difficulty of traveling during the recent Covid-19 pandemic, three golf courses in the Houston metropolitan area and two in Brazos County 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 (Fig. 1). Long-term storage of each isolate was achieved by keeping mycelial agar plugs in a 4-ml clear glass screw cap vial containing 1.5 ml of sterile distilled water. Vials were sealed with parafilm and kept in the dark at ambient temperature.

Development of molecular diagnosis of *Gaeumannomyces* species. Genomic DNA was extracted from each *Gaeumannomyces* isolate for the DNA-based diagnosis. Each isolate was grown on PDA at 25°C until petri dishes were entirely colonized. DNA isolation from harvested mycelium was conducted using ZYMO DNA Miniprep Kit. Internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using PCR with the previously-developed ITS1/ITS4 primer set. PCR was performed using Thermo Scientific Phire Plant Direct PCR Master Mix Kit. PCR amplicons were sent to Eton Biosciences for sequencing. In our initial screening, we found dominant isolates were determined as *Gaeumannomyces graminis* and *G. arxii.*

Establishment of in-planta assay for virulence of *Gaeumannomyces* species. For evaluating virulence of *Gaeumannomyces* species, the bermudagrass seedling pathogenicity assay has been developed. Bermudagrass seeds were surface sterilized in 1.2% NaClO for 10 minutes and rinsed 10 times with sterile distilled water. Seeds were pre-germinated in a petri dish containing two 7.5-cm filter papers moistened with 3 ml of sterile distilled water. 66-ml plastic cone-tainers were subsequently filled with sterile moistened vermiculite. At the depth of 3 cm from the top of each cone-tainer, five plugs (5-mm diameter) of actively-growing mycelium from each isolate were placed. Germinating seeds were added at the top of this layer, which were then covered with a final layer of vermiculite to fill to capacity. Prepared units were placed at a constant temperature of 25°C with a photoperiod of 16 h. All cone-tainers were watered daily to soil field capacity for the first week of incubation and once every other day thereafter. In 3 weeks after inoculation, take-all root rot symptoms could be observed.

Significant differences of plant health phenotypes were noticed between inoculated plants with *Gaeumannomyces* species and uninoculated control plants. Certain fungal isolates of *G. graminis* caused wilts of bermudagrass seedlings (Fig. 2). Most *G. graminis* isolates significantly inhibited plant growth compared with uninoculated plants or plants inoculated with *G. arxii* (Fig. 2).

Future approaches and expectations. Upon the confirmation of infection by *Gaeumannomyces* species and their pathogenicity, we try to develop and implement a more integrated management approach for golf course superintendents. Based on accurate diagnostics and detection of more aggressive *Gaeumannomyces* species, proper management practices of take-all root rot will be able to be incorporated into current bermudagrass putting green maintenance.

We have experienced the difficulty in travels, field site visits and in-person meetings due to the Covid-19 pandemic in past 2 years. In the third year of this project, we will invite at least 20 Texas golf course superintendents to participate in the survey and the implementation of an improved management program targeted for take-all root in bermudagrass putting greens. Turf samples will be collected from these golf courses. Causal *Gaeumannomyces* species will be isolated and identified. Also, their pathogenicity will be determined using the diagnostic methods developed in this study. The survey for Texas golf courses will measure economic impact of take-all root rot in the golf course management. Information we are interested in relevant to bermudagrass putting green management includes the rates and frequency of chemical applications (fertilizers, herbicides, fungicides, and insecticides) and labor cost for turf maintenance (mowing, irrigation, topdressing, and aeration). Finally, we will estimate potential economic impact of take-all root rot and its management on golf courses.

Figure 1. Fungal cultures of Isolates *Gaeumannomyces graminis* and *G. arxii* show phenotypical differences on potato dextrose agar medium after 10 days incubation at 25°C.

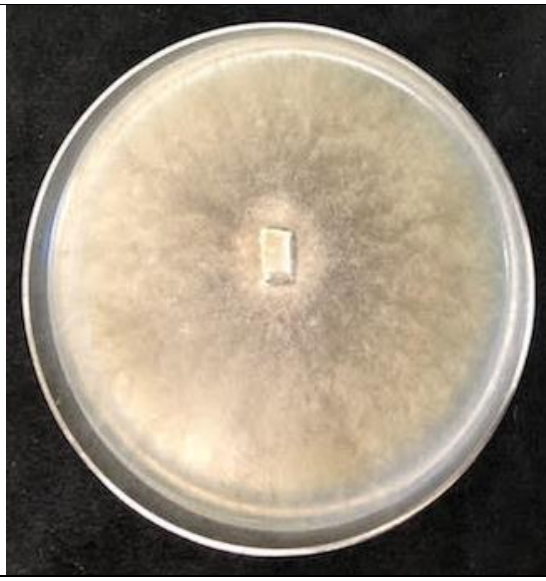
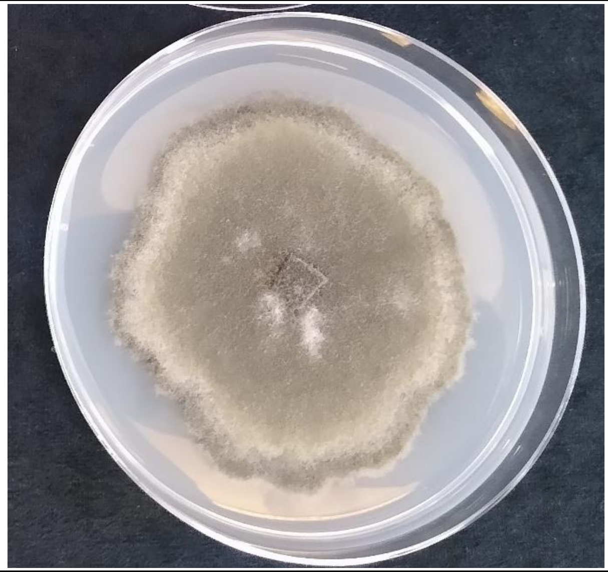
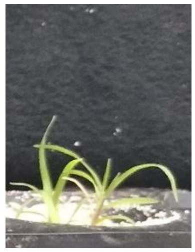
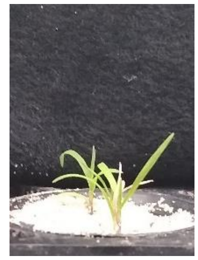
G. graminis	*G. arxii*
	

Figure 2. Pathogenicity of *Gaeumannomyces* species with bermudagrass seedlings. Uninoculated control plants are compared with three *G. graminis* isolates and one *G. arxii* isolate at 21 and 35 days after inoculation (DAI).

DAI	Uninoculated	*G. graminis* (A)	*G. graminis* (B)	*G. graminis* (C)	*G. arxii*
21					
35					

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: 2 years
Total Funding: $107,604
Summary Points:

- The trial started in 2021 and data has been collected.
- Results are too preliminary to draw any conclusions at this time

Summary Text:

Enhanced degradation:

Bioassay method - Intact 2-inch-diamter and 6-inch-deep turf profiles will be removed from the field and placed into a greenhouse for bioassay. Turf profiles will be 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 will be removed from the profiles, the soil will be mixed and placed into small clay pots. Then tomato plants will be 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 will be 5 replications of every treatment.

Small plot experiment - Field plots were treated with 4 applications of SDHI nematicide (Indemnify) and 4 applications of SDHI fungicide (Velista) annually for four years, while other plots had no exposure to SDHI pesticides. Turf profiles will be removed for the bioassay procedure described above to determine if SDHI nematicide is as effective when applied to soil that was been regularly treated than to soil that has not.

Golf course experiment – Ten golf courses will be selected for this experiment, five that had never used SDHI nematicide and five that had used it regularly and reported that it didn't work as well as it once did. Five turf profiles will be collected from 1 to 3 different green from each golf course. The profiles will be subjected to the bioassay described above above to determine if SDHI nematicide is as effective when applied to soil that was been regularly treated than to soil that has not.

Progress to-date – The 1 and 2 month bioassays from the small field plot experiment are completed and the 6-month bioassay is scheduled for late December 2021. The experiment is being repeated and the 1 month bioassay is due to be taken down in January. The profiles have been collected from the golf courses and are due for the 1 month bioassay in December 2021.

Resistance:

In-vitro screening method – Sting and grass root-knot nematodes will be extracted from soil or turf plugs by modified Baerman or mist extraction method, respectively. Either 25 sting nematodes or 50 root-knot nematode juveniles in 1 ml solution will be added to microwell plates. Then 1 ml of nematicide solution, containing twice the target concentration of SDHI nematicide (Indemnify), will be added to the nematode solution, bringing the final nematicide concentration to the target amount. The nematicide concentrations are based on our calculations of the maximum concentration of a.i. (fluopyram) that the nematodes would be exposed to from a field application followed by irrigation with 1/8-inch of water. The concentrations tested will be 0, ½X, and 1X of this amount. At 1 hr, 24 hr, and 72 hr after nematicide solution is added the number of nematodes moving or not moving will be recorded. After nematode movement is recorded, asodium hydroxide will be added to the solution to stimulate nematode movement and nematode movement will be recoded again. There will be five replications of each treatment and time interval combination.

Small plot experiment - Field plots were treated with 4 applications of SDHI nematicide (Indemnify) and 4 applications of SDHI fungicide (Velista) annually for four years, while other plots had no exposure to SDHI pesticides. These plots will be sampled and nematodes extracted will be subjected to the in-vitro test described above to determine if nematodes with prolonged exposure to SDHI nematicide behave differently to exposure than those that have not.

Golf course experiment – Golf courses that use SDHI nematicide regularly but still have high numbers of either sting or root-knot nematodes will be identified and sampled. Our target is to have 5 for sting nematode and 5 for grass root-knot nematode. Additionally, 5 locations with populations of sting nematode and 5 of grass root-knot nematode that have neve been exposed to SDHI nematicide will be sampled. The nematodes collected from each site will be subjected to the in-vitro screening described above to determine if nematodes with prolonged exposure to SDHI nematicide behave differently to exposure than those that have not.

Progress to-date – A preliminary study was conducted over the summer to work out methodologies and rates. The nematodes are currently being collected and bioassay will start next week.

Metabarcoding:

Soil will be collected from the small plot and golf course experiments described above. Microbial and fungal DNA will be extracted from soil, amplified, and sequenced. This will be used to compare the microbial and fungal composition of soil regularly treated with SDHI fungicide with those that have not.

Progress to-date – Small samples of soil from each location are being stored in a -60 C freezer, and DNA will be extracted for analysis at the same time (likely March 2022).

Figure 1. Graduate student collecting soil profile cores for degradation experiments.

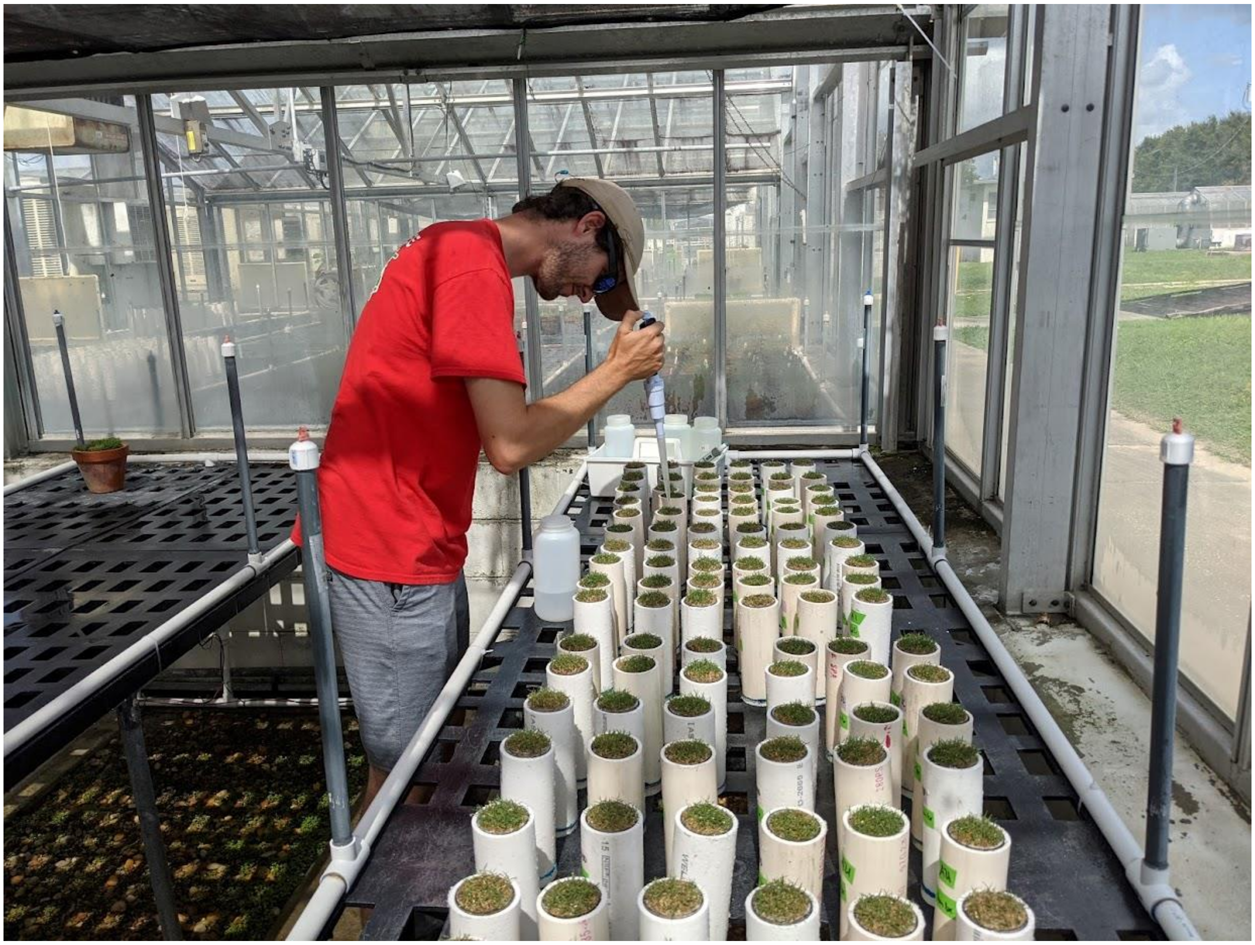


Figure 2. Graduate student applying treatments to soil profiles in the greenhouse.

Figure 3. Graduate student staining nematode egg masses on tomato root bioassay.

Figure 4. Golf course sample location where sting nematodes are present at high numbers despite regular applications of SDHI nematicide.

USGA-ID: 2020-08-713

Title: Long-term suppression of turfgrass insect pests with native persistent entomopathogenic nematodes.

Project leaders: Albrecht M. Koppenhöfer, Ana Luiza Sousa

Affiliation: Department of Entomology, Rutgers University, New Brunswick, NJ

Objectives: Isolate, characterize, and develop native persistent entomopathogenic nematodes for long-term insect pest suppression in golf course fairways and roughs.

Start date: 4-1-2019

Project duration: 3 years

Total funding: $29,865

Entomopathogenic nematodes (EPNs) have shown potential for the control of white grubs, caterpillars, weevils, mole crickets, and crane flies, but most research has focused on inundative applications and short-term effects. In field crops, several studies have shown that inoculative applications of native EPN strains adapted to the local conditions and maintained to preserve their ability to persist in the environment, can effectively suppress pest populations for several years.

During 2019 we surveyed one fairway each at Pine Brook Golf Course (PB) and Howell Park Golf Course (HP), both in Monmouth County, New Jersey for native EPNs. The majority of EPNs collected were *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. Mixes of isolates of each species were used to inoculate the field plots on one fairway each at PB and HP in early June 2020. These plots (20 m × 10 m) were half in the fairway, the other in the rough. Plots were separated by ≥ 10 m. Treatments were *H. bacteriophora*, *S. carpocapsae*, a 1:1 mixture of both species, all applied at a total of 1.25×10^9 infective juveniles/ha. Control plots were left untreated. There were two replicates per treatment at each golf course. Samples of each kind were taken in each plot from a central 4 m × 4 m area in the rough and one in the fairway.

EPN populations in the plots were determined 1 week before application and again 1, 4, 6, 13, and 15 months after application. Forty soil cores (7.5 cm × 2.5 cm diameter) were taken from each plot side (Fig. 1), mixed thoroughly, and a subsample of 120 grams placed into a plastic cup and baited with five waxworms for three consecutive 3-day baiting rounds. EPN-infected waxworms were collected and incubated to determine EPN species by the color of the cadavers and the size and behavior of the infective juveniles emerging from the cadavers. EPN detection (i.e., number of infected waxworms) was highly variable. Generally, higher EPN numbers were detected in the rough vs. the fairway. Numbers tended to be higher in the treated plots than the untreated plots for the species the plots were treated with (Fig. 2).

A third EPN species, *Steinernema cubanum* or closely related to it, was also found regularly in many plots in both the fairway and the rough side, generally in higher numbers late in the season (Fig. 2). Isolates of this species recently found elsewhere in New Jersey have shown high virulence to white grubs. It is possible that this species uses primarily larger white grub instars as hosts in late summer early fall but not in spring when soil temperature might be too cool.

ABW populations were determined in mid-June 2020 and 2021. Thirty-two turf/soil cores (5.4 cm diameter × 3 cm depth) were taken from each plot side and extracted. The number of ABW life stages and any other insects were recorded. ABW densities were generally very low, but in both years significantly higher in the untreated fairway than in the untreated rough. In both years, numbers in the fairway were significantly lower in the plots treated with both EPN species (47% lower in 2020, 89% lower in 2021) than in the untreated plots and the ones treated with *S. carpocapsae* only (Fig. 3). No differences were detected in the rough. The only other insects found in significant numbers were larvae of the black turfgrass ataenius (BTA), numbers of which were not significantly affected by treatments.

Surface-active insect populations were determined in July and early September of 2020 and 2021 via soap flushes (Fig. 4). In each plot side, two 30 cm × 30 cm areas were treated with 1,000 ml of a 8% soap solution. Any insects found within 20 minutes were identified in the lab. Soap extraction revealed many insects types but only adults of ABW and BTA were found in number high enough for meaningful analysis. ABW numbers were higher in the fairway than the rough, and in the fairway they were significantly lower in the plots treated with *S. carpocapsae* and the species combination than in the untreated plots. BTA numbers were higher in the fairway than the rough, and in the fairway, they were significantly lower in all EPN treatments than in the untreated plots.

White grub populations were determined in late September 2020 and 2021. Sixteen turf/soil cores (10.5 cm diameter × 7.5 cm depth) were taken per plot side (Figure 5). Any soil insects found were identified in the lab. Sampling for white grubs showed a mix of oriental beetle followed by Japanese beetle and a few northern masked chafers. Due to the low densities, the three species were pooled. White grubs were more common in the rough than in the fairway. In the rough, densities were significantly lower in the plots treated with *H. bacteriophora* than in the untreated plots; no treatment effect could be detected in the fairway (Fig. 6). BTA larvae were more common in the fairway than in the rough but were not affected by EPN treatment in either rough or fairway. Numbers of other insect types detected were too low and variable for meaningful analysis.

Continued sampling in 2022 should help us determine if the to date observed patterns are consistent and if the applied EPNs persist and have any long-term effects on insect pest densities.

- EPN numbers have increased across the field plots following applications of lab reared mixes of native isolates.
- The increase in EPN numbers into the fall suggest that they are recycling from hosts present in the test plots.
- ABW, white grubs, and BTA, albeit all in low densities, are present in the plots and could contribute to EPN recycling.
- We will continue to observe the densities of EPN as well as the insect species present in the fields for an additional year.

Fig. 1. Sampling of soil cores to determine populations of entomopathogenic nematodes.

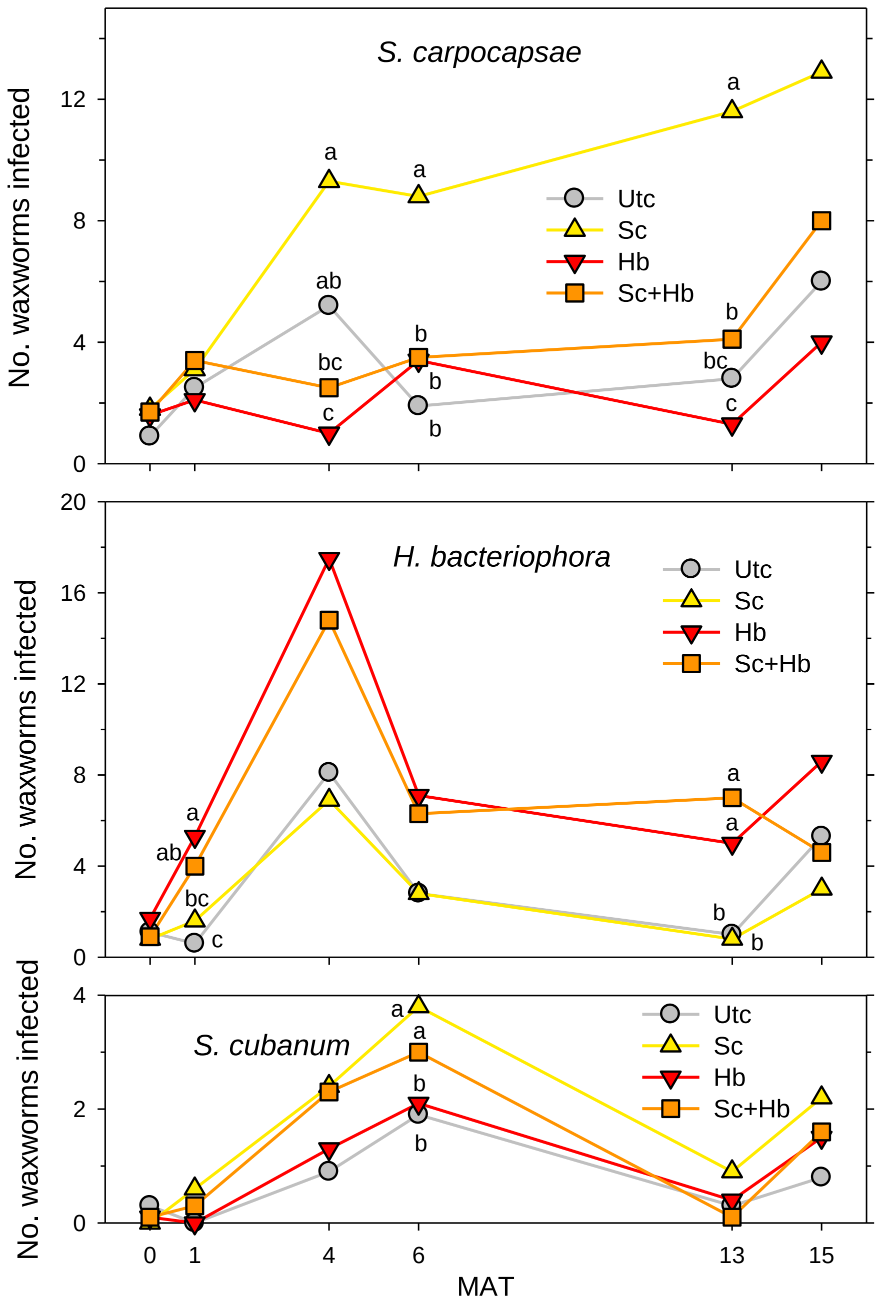


Fig. 2. Numbers of EPN-infected waxworms recovered per plot by baiting soil samples taken from plots extending from the fairway into the rough. Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc). A third species was regularly recovered that was identified as *Steinernema cubanum* or very closely related to it. Soil samples were taken 1 week before (0) and 1 to 15 months after application (MAT).

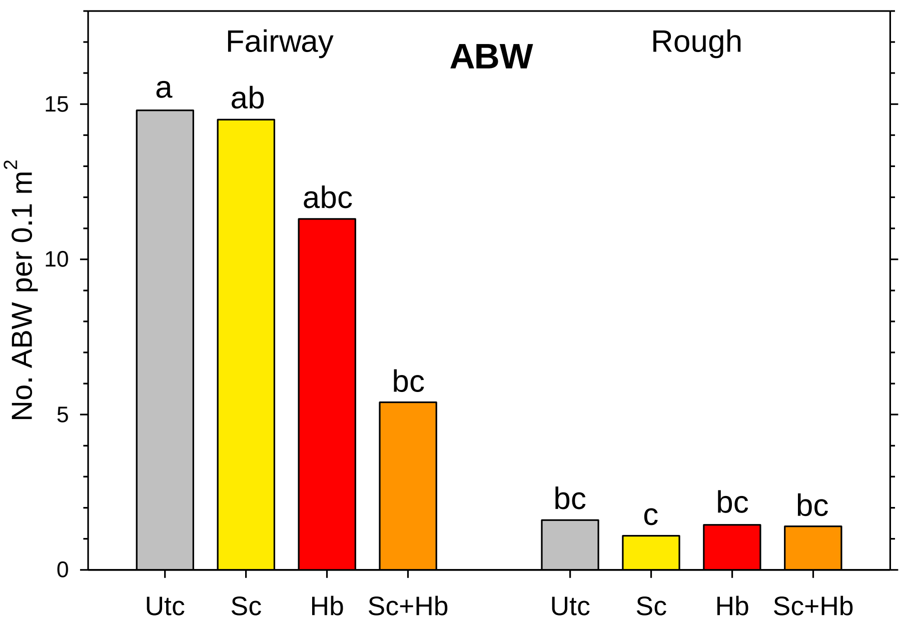


Fig. 3. Densities of annual bluegrass weevil developmental stages (L1 – teneral adults) in soil cores collected in mid-June 2020 and 2021 in plots extending from the fairway into the rough (data combined for both years). Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc).

Figure 4. Extraction of surface-active insects with soap solution.

Fig. 5. Taking cup cutter cores to determine populations of white grubs and other soil insects.

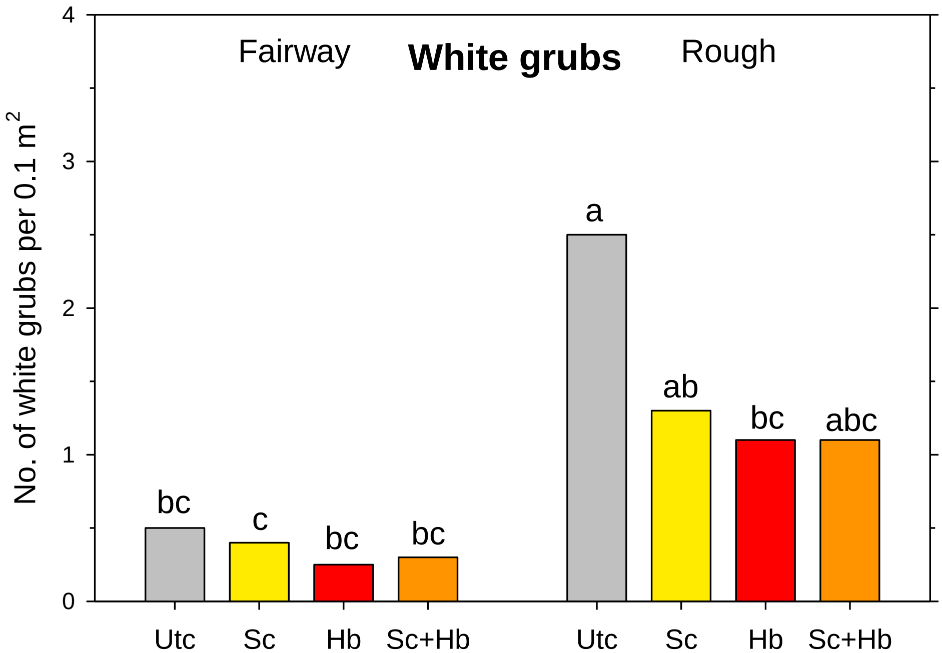


Fig. 6. Densities of annual white grub larvae in soil cores collected in late September 2020 and 2021 in plots extending from the fairway into the rough (data combined for both years). Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabditis bacteriophora* (Hb), both species (Sc+Hb), or were untreated control (Utc).

Project ID: 2020-09-714
Project Title: Characterization of turfgrass plant induced defenses in response to annual bluegrass weevil feeding

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Objectives:

(1) Characterize creeping bentgrass and annual bluegrass tolerance to annual bluegrass weevil herbivory
(2) Identify plant response mechanisms associated with ABW feeding
(3) Quantify nutritional composition of potential cool- and warm-season turfgrass hosts

Start Date: 2021 (*delayed one year due to COVID; original start 2020)
Project Duration: 2 years
Total Funding: $51,444

Summary Points:

1. Creeping bentgrass (*Agrostis stolonifera, CBG*) has been hypothesized to be more tolerant to annual bluegrass weevil (ABW) herbivory compared to annual bluegrass (*Poa annua,* ABG)
2. CBG and ABG plugs were infested with ABW and sampled every 5-7 days from early to late instar development. CBG harbored fewer larvae (3.55 vs. 6.8 per plug) over the course of the assay, suggesting CBG negatively influences ABW development
3. Turfgrass foliar, stem and root tissue samples were collected in response to ABW herbivory at three different life stages (adult, early-instar, and late instar) for both creeping bentgrass and annual bluegrass (*currently awaiting defense phytohormone analysis*)
4. CBG and perennial ryegrass possessed higher concentrations of chlorophyll and carotenoids than ABG and bermudagrass. The nutritional and ecological significance of these findings may assist in determining potential novel hosts for ABW

Summary:

The annual bluegrass weevil (ABW) has long been believed to be a specialist of annual bluegrass (*Poa annua,* ABG) though several studies over the last decade combined with observations of populations in recently invaded areas suggest that the insect may complete its lifecycle in other species. Creeping bentgrass (*Agrostis stolonifera*) (CBG) is a commonly encountered species that ABW can develop within, though damage is rare and usually less severe than in ABG.

Furthermore, individual insects incur fitness costs (e.g. longer development times, lower weight gain) when developing within CBG. These findings suggest that CBG may possess inducible defense mechanisms to counter ABW herbivory.

This project seeks to characterize differential responses of CBG and ABG phytohormone and protein production in response to ABW feeding at different life stages and in all plant parts to better understand the defense response in turfgrasses to insect herbivory. The identification and characterization of these mechanisms may lead to the development of improved (either resistant or tolerant) turfgrass cultivars or management strategies for inducing existing turfgrass stands to better defend themselves from insect attack.

Objective 1: Characterize creeping bentgrass and annual bluegrass tolerance to annual bluegrass weevil herbivory

We screened two CBG cultivars (Penncross and A4) and ABG for potential differences in tolerance to ABW.

Methodology:

***Insects**:* ABW adults were vacuum collected from overwintering habitat on a golf course (Harrisburg Country Club and Golf Course, Harrisburg, PA, USA). Adults were assessed for viability, sexed, and kept separately in 840 ml plastic containers filled with sandy loam soil and sand (3:1 ratio) in an incubator (10 h light at 6°C:14 h dark at 4°C). Prior to bioassays, adults were placed in clear plastic containers (~50 ABW) with mesh lids and placed into an incubator (14 h light at 21°C:10 h dark at 14°C). Equal number of male and female ABW adults (5:5) were infested and remained in turfgrass plugs for 7 days. Adult weevils were manually extracted from each of the treatment plugs over a 3-day period to avoid continuous egg oviposition and standardized larval development and age.

Turfgrass Growth and Maintenance: Plant materials were established from seed (*Agrostis stolonifera cv.* Penncross and wild-type *Poa annua*) in plastic growth containers (30 cm x 15 cm x 4 cm) in a greenhouse. Seedlings were grown on a pasteurized mixture of sandy loam soil and sand (3:1 ratio), watered as necessary, and clipped twice a week to maintain fairway-height conditions (13 mm).

Results:

Both CBG and ABG supported similar densities of early instar larvae (average = 5.6 per plug) early in the assays when the majority of the ABW were approximately 2nd instar. As ABW larval development progressed (~ 3rd-5th instars), we observed a shift in survivorship between treatments. CBG harbored fewer larvae (3.55 vs. 6.8 per plug) compared to ABG. ABW development was also observed to be slower when developing in CBG.

Future direction:

In 2022, we will continue to replicate tolerance assays comparing CBG and ABG at various ABW developmental stages. Phytohormone extraction data collected under Objective 2 will provide information as to whether the production of defensive compounds can explain the differences in development and survivorship of ABW within turfgrass treatments. These findings may influence CBG cultivars selections for the 2022 assays.

Objective 2: Identify plant response mechanisms associated with ABW feeding

The characterization of inducible plant defense phytohormones to ABW feeding in CBG and ABG was examined during three discrete lifestages.

Methodology:

ABW were caged on CBG (Penncross) and ABG arenas in the greenhouse according to the methodologies described in Objective 1. Since the lifestage or the feeding location (stem borer vs. crown feeding stages) may influence host plant defense responses, phytohormone extractions were performed at three distinct larval stages or when the larval instar average was 2.5, 3.2, and 4.0, corresponding to stem borers/early instars (~10 d after adult removal), early crown feeders (~15 d), and larger larvae/late crown feeders (~ 20 d). Before each extraction, eight randomly selected untreated control pots were sampled for larvae, and age was determined based on head capsule width. Turfgrass foliar and stem tissues were manually plucked by hand until approximately 200mg dry weight was acquired. Roots were separated at the crown of the plant and rinsed in water at room temperature to remove soil, silt and sand material. Root tissue was dried on an absorbent paper towel to remove excess water from the washing step. Tissue samples were collected from four biological replicates per tissue type from 8 technical replicates per grass type per larval development stage. During the collection process tissue samples were stored in 5ml centrifuge vials on dry ice, then flash frozen using liquid nitrogen, and transferred into a -80°C freezer for future phytohormone extractions. Hormone extraction and analysis were conducted using a modified crude extraction procedure reported by Liu et al. (2012).

Results:

All tissues have been ground and are being run through ultra high-performance liquid chromatography instrumentation. Results are currently being analyzed for treatment effects and are expected to be available by the end of December of 2021.

Future Directions

We will replicate the defense phytohormone collection, extraction and analysis of CBG and ABG in response to ABW feeding injury in Spring of 2022.

Objective 3: Quantify nutritional composition of potential cool- and warm-season turfgrass hosts

We assessed the nutritional value of known susceptible, tolerant and candidate turfgrass species to identify essential nutritional traits for ABW development and to help predict potential host plant expansion.Visual stimuli and cues such as host plant pigment which are derived from carotenoids and chlorophyll may not be a primary nutrition source of a host plant but play a role in host plant identification.

Methodology:

Extracts from four turfgrass grass species (ABG, Perennial ryegrass (*Lolium perenne*), CBG (*Agrostis stolonifera cv.* A4 and Penn Cross) and bermudagrass (*Cynodon dactylon*)) were assessed for baseline total carotenoid and chlorophyll (a & b) content in the absence of ABW.

Plant material was seeded and maintained using the procedures described above. Plants were maintained for 3 weeks after maturity (~ 5 to 6 weeks after seeding) before extractions were made. The turfgrass extract was obtained by cutting 2 cm above the crown, placing clipped material in a mortar, and grinding with a pestle. Turfgrass extract was placed in amber vials and stored at 4˚C until analysis.

Roughly 20ml of turfgrass extract from each species and cultivar was mixed with 1ml of ethyl acetate. The solution underwent centrifugation at 5000 rpm for approximately 10min. Supernatant was removed and pipetted onto a well plate. Absorbance was measured between 400 and 700 nm using a BioTek Synergy™ THX multi-mode reader. Chlorophyll and carotenoid concentration was calculated using the formula provided by Lichtentaler and Wellburn (1985) to calculate intensity of pigment.

Results:

Both CBG cultivars and perennial ryegrass chlorophyll concentrations were significantly higher (7.9-8.3 mg/100ml) than ABG (3.25mg/100ml) and bermudagrass (3.1mg/100ml). Similarly, carotenoid concentrations were higher for CBG cultivars and ryegrass (790-1080mg/100ml) than ABG (594 mg/100ml) and bermudagrass (489 mg/100ml).

Future Direction:

The ecological and physiological significance of these findings will be assessed in behavioral bioassays. Additional baseline measurements for protein content, flavonoids, DPPH radical activity and other macro nutritional elements are currently being assessed.

Figure 1:

Annual bluegrass (*Poa annua*, ABG; left) and Creeping bentgrass (*Agrostis stolonifera*, CBG; right) infested with 4th instar annual bluegrass weevil larvae.

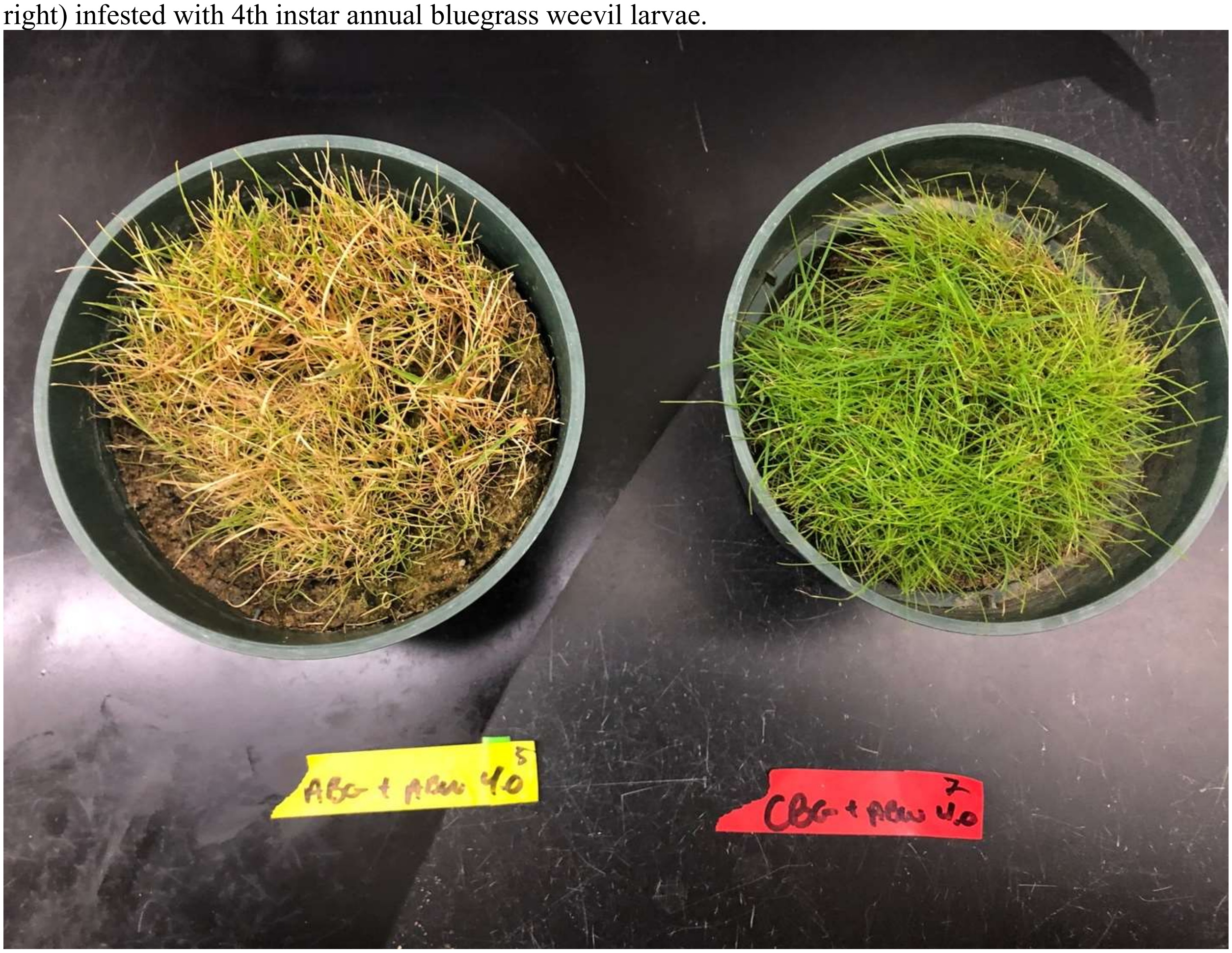
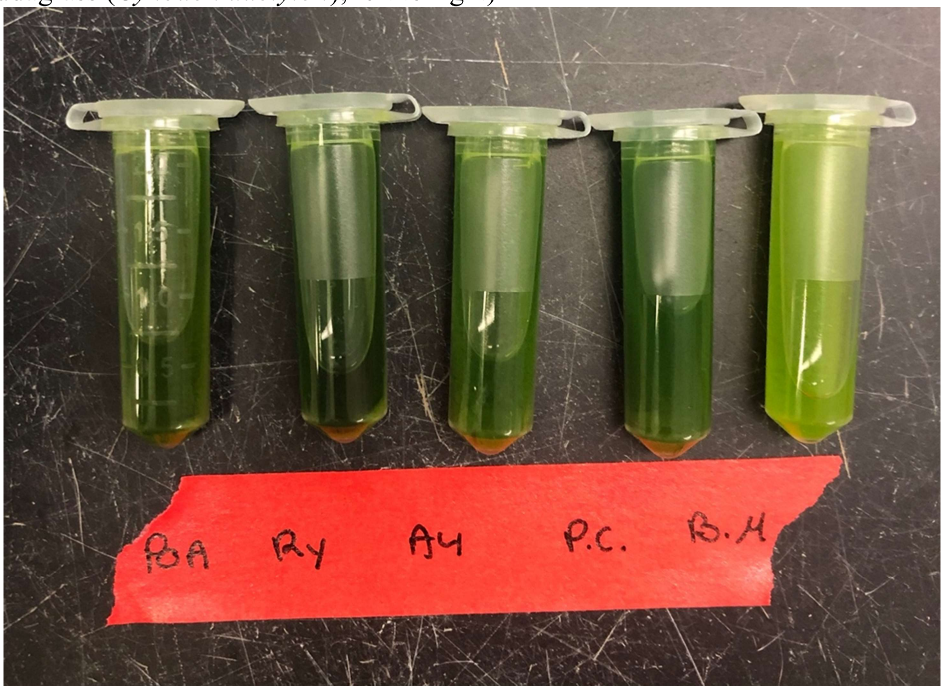


Figure 2:
Turfgrass chlorophyll and carotenoid extract (Annual bluegrass (*Poa annua*), Perennial ryegrass (*Lolium perenne*), creeping bentgrass (*Agrostis stolonifera cv.* A4 and Penn Cross) and bermudagrass (*Cynodon dactylon*); left to right)



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Lichtenthaler, Hartmut K., and Alan R. Wellburn. "Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents." *Biochemical Society Transactions* 11, no. 5 (October 1, 1983): 591–92. https://doi.org/10.1042/bst0110591.

Liu, H., Li, X., Xiao, J., & Wang, S. (2012). A convenient method for simultaneous quantification of multiple phytohormones and metabolites: application in study of rice-bacterium interaction. *Plant methods*, *8*(1), 1-12.

USGA ID#: 2019-07-677

Title: Progress toward solving the silvery-thread moss issue in cool-season putting greens

Project Leaders: Llo Stark, Zane Raudenbush, Matthew Johnson, Joshua Greenwood

Affiliation: University of Nevada Las Vegas, Texas Tech University, The Davey Tree Expert Company

Objectives: (unchanged from 2020 report)

1. Organize our laboratory experiment on the effects of carfentrazone-ethyl (carfentrazone or CZ here) and light intensity on silvery-thread moss (STM, also *Bryum argenteum*) into a manuscript suitable for publication.
2. Initiate and complete field experiments on surfactants on the inhibition silvery-thread moss in experimental and working putting greens in Ohio.
3. Characterize the genetic diversity of silvery-thread moss in the United States using specimens from on- and off-golf courses by comparing to a newly assembled and annotated genome sequence.

Start date: January 1, 2019

Project duration: 3 years

Total funding: $119,991

Summary Points:

1. In order to understand (a) how golf greens become contaminated with STM (local adaptation vs. dispersal), and (b) how diverse the strains (genotypes) of STM are when compared against off-green (native) strains, we initiated a genetic approach consisting of sequencing the *Bryum argenteum* genome (RNA and DNA). Two genotypes were sequenced over the last year.

2. An experimental analysis of the effects of carfentrazone and SDS (sodium dodecyl sulfide) applications to STM continued, and two manuscripts are in preparation.

3. Effective suppression of STM by carfentrazone was observed at a high dosage (12.5 × recommended dosage), and only when followed by a 10-hour exposure to a full sunlight equivalent (2000 µmol m^{-2} sec^{-1} PAR, photosynthetic active radiation).

4. SDS was superior to carfentrazone in suppressing STM, with a dosage of 0.5% concentration for one minute reducing the photosynthetic capacity to levels associated with cell death (Fv/Fm <0.2). Shoot regeneration was suppressed to ~90% of controls, unlike the carfentrazone treatments. The treatment (0.5%) used in the lab is much less than the SDS concentration of Dawn® dishsoap (~20%).

5. Drench applications of Dawn dishsoap and SDS resulted in greater long-term reduction of STM compared to applications of carfentrazone in a research putting green at Hawks Nest Golf Course in Creston, OH. At Scioto Country Club in Upper Arlington, OH, overall STM control was reduced (<20% of control) for all treatments.

Summary Text:

Rationale (unchanged from 2020 report)
Silvery-Thread Moss (STM, *Bryum argenteum*) is an undesirable weedy species that has colonized golf greens across the USA and has proven difficult to erradicate. Our group of four researchers (Stark, Raudenbush, Johnson, and Greenwood) from three institutions/companies (UNLV, Texas Tech U., Ohio State / Davey Tree) initiated lab and field studies to (1) test the effectiveness of a surfactant-based product (*Dawn Ultra* dishsoap) and a moss suppressant on the market (*Quicksilver*, known as carfentrazone-ethyl) on the growth response and photosynthetic health of STM; (2) determine the effect of carfentrazone at different light intensities; (3) determine the effect of a single known surfactant (sodium dodecyl sulfate, SDS, ($CH_3(CH_2)_{11}SO_4Na$)) on moss growth in both putting green and laboratory settings; and (4) isolate high quality DNA for sequencing the genome of this moss.

Methodology

UNLV. During 2021, we developed a manuscript on the effects of dosing STM with carfentrazone at different light intensities, and are currently developing a second manuscript on the effects of dosing STM with the surfactant SDS (sodium dodecyl sulfide). Currently we are deciding on a third experiment to conduct. We also continued to maintain a library of living cultures of STM reflecting a range of native and putting green sources (37 genotypes/strains). Two genotypes became a genetic focus from native sites (a mating pair from the University of Kentucky campus) and were transferred by clone to Texas Tech.

Texas Tech. During 2021, efforts continued to produce sequences from two clones of STM, one male and one female, with the goal of assembling and annotating a new (to science) STM genome. We sequenced the RNA and DNA of both genotypes using "short-read" Illumina technology (NovaSeqSP6000), resulting in 84.3 Gbp (gigabase pair) of DNA and 12.6 Gbp of RNA sequences. To produce a more contiguous DNA assembly, we also used single-molecule sequencing of the female genotype. We used the Oxford NanoporePromethlON sequencer at the University of Connecticut to generate 66.9 Gbp of additional long-read data. We filtered the long-read data to remove contaminant sequences from fungi, bacteria, and viruses, and assembled using Flye to generate an initial assembly of 556 Mbp (megabase pairs) across 5,109 contiguous sequences (contigs). We used the short-read sequence data to filter and further remove overlapping contigs belonging to fungal genomes, and arrived at a final assembly of 350 Mbp across 2,735 contigs.

Davey Tree. We transferred the subcontract from Ohio State to Texas Tech in 2021. This allowed Dr. Raudenbush to continue working on a volunteer basis with employer permission, and increased the effort from Dr. Johnson's lab to expand the genetic testing for STM. In April 2021, a replicated field experiment at Hawks Nest Golf Course and Scioto Country Club was completed. The study evaluated the effectiveness of different chemical control strategies in conjunction with hollow tine aerification. A 4 × 2 factorial treatment structure with a completely randomized design was used at both locations to evaluate four chemical control strategies in combination with hollow-tine aerification: (1) drench application of Dawn Ultra dish soap, (2) drench application of sodium dodecyl sulfate (SDS), (3) spray application of Quicksilver herbicide (carfentrazone) at 3.3 fl.oz/acre, and (4) an untreated control. Two levels of hollow-tine aerification (with or without aerification) were applied one day following the chemical applications. Plugs were removed from the 3' × 3' aerified plots and holes backfilled with dry sand. Percent STM cover in each plot was measured using a rating grid containing 256 intersects at trial initiation and every two weeks thereafter until mid-November (2020). Two additional ratings were completed the following spring to evaluate STM recovery.

Results to date

UNLV. A summary of the major effects of carfentrazone and a surfactant (SDS) on STM health and regeneration is given in **Figure 1**, derived from two manuscripts in preparation. Detrimental health effects occurred for STM at doses of 12.5× the recommended dose in full sunlight (**Fig. 1A**). Both native (off-green) and green (on-green) STM were killed or nearly killed using a short exposure to 0.5% SDS (**Fig. 1B**). Shoot regeneration following carfentrazone treatments was delayed by ~10-15 days when compared to controls (no carfentrazone treatment), indicating that carfentrazone can retard new shoot formation but not prevent regeneration, even if applied under full sunlight conditions (**Fig. 1C**). Nearly complete suppression of new shoot formation occurred following exposure to STM shoots to SDS (**Fig. 1D**).

Texas Tech. The STM genome assembly contains 81.5% of all "universal" genes found in all land plant genes, and 95.7% of universal genes found in all green plants. Our initial genome assembly for STM suggests a large amount of genome evolutionary reorganization since the last common ancestor with the mosses *Ceratodon purpureus* and *Physcomitrium patens*, the closest relatives of STM with published genomes (**Figure 2**). This finding is surprising because previous results had indicated conserved genome structure between *C. purpureus* and *P. patens* despite 250 million years of evolution.

Davey Tree. Overall, the long-term efficacy of the chemical control treatments differed between locations. At Hawks Nest Golf Course, applications of Dawn Ultra dish soap and SDS provided greater STM control compared to carfentrazone when evaluated in March 2021. At Scioto Country Club, no differences in STM control were observed among the chemical control treatments in March 2021, and the overall control was reduced compared to Hawks Nest. Hollow tine aerification did not affect STM control at either location. Interestingly, several plots treated with drench applications of dish soap at Hawks Nest had complete control of STM (**Figure 3**), while plots at Scioto had <20% control of STM; it is unclear if genetic or environmental differences are responsible for the inconsistent field control.

Future expections for project

For the final year of the project, we are currently assessing potential experimental designs for a lab experiment that will better inform USGA on practices for suppression of STM on golf courses using carfentrazone and/or SDS. We seek to understand, through genetic, ecophysiological, and field (golf course) analyses, the colonization and evolutionary processes responsible for STM presence on golf greens. Our approach will consist of (1) A lab experiment (still under discussion) investigating the action of SDS on life history phases responsible for the spread of STM, specifically rhizoids and protonema; (2) An additional survey of superintendents in the USA to include requests of additional cores of STM from greens across the country, and which will include data or observations on mowing height, root zone construction, irrigation source, grass species on green, infestation status, control strategies, date of green opening and date of infection with STM, and the size of the STM patches; (3) An investigation into the genetic diversity of STM strains (distinct genotypes of *Bryum argenteum*) both on and off (but nearby) of putting greens in order to approach the question of, how is STM colonizing or actively evolving on the putting green habitat?; and (4) A genetic approach to the question of, why are all of the known putting green genotypes female?

Images:

Figure 1. Effects of carfentrazone, Light, and a surfactant (SDS) on the health and shoot regeneration capacity of Silvery-Thread Moss (STM). Chlorophyll fluorescence following exposure to carfentrazone and three light intensities (**panel A**) and following exposure to SDS (**panel B**). Shoot regeneration following exposure to carfentrazone and three light intensities (**panel C**) and following exposure to SDS (**panel D**). CZ=carfentrazone-ethyl, PAR=photosynthetically active radiation, Golf=genotype from a putting green, Field=genotype from native habitat.

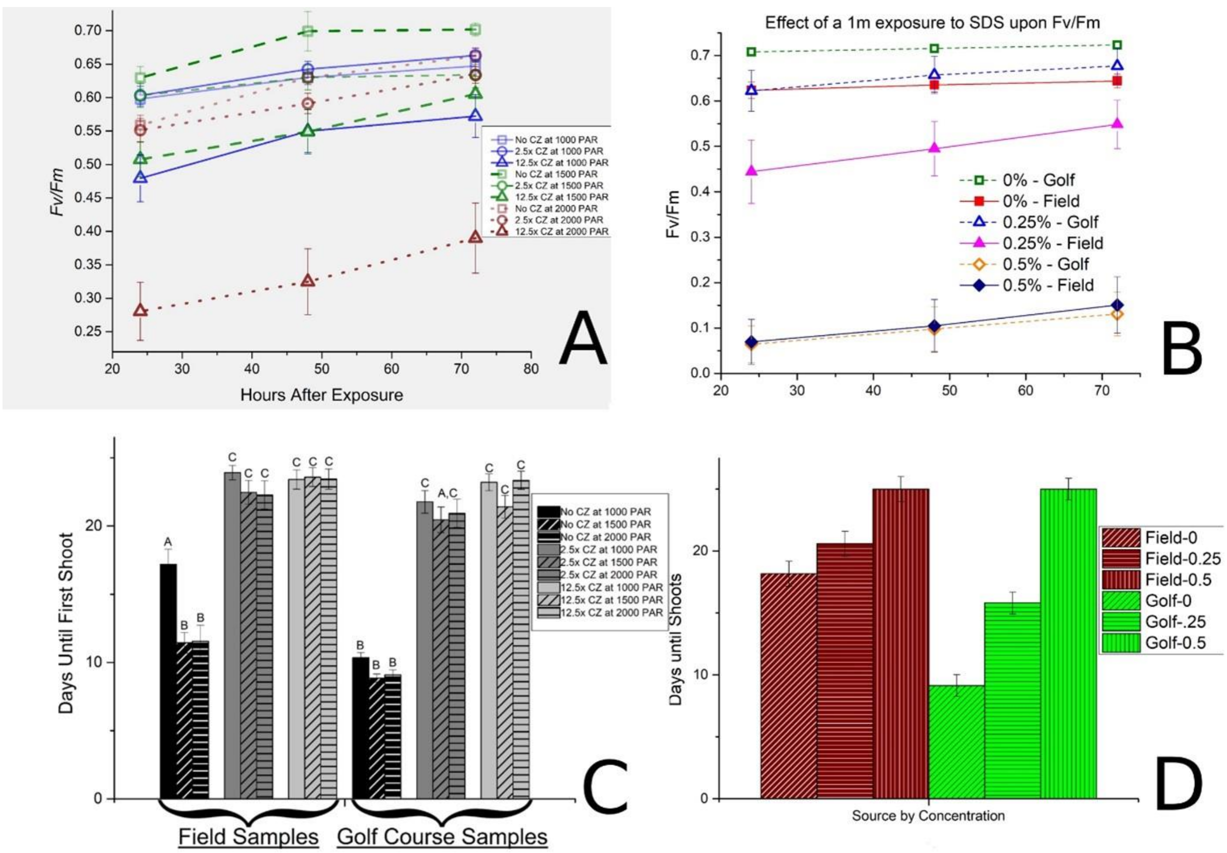


Figure 2. Genome synteny (chromosomal position) analysis between *Bryum argenteum* (STM, black portion of the circle, top middle as chr353) and the assembled chromosomes of the moss *Ceratodon purpureus* (other colored parts of the circle). Black lines indicate connections of sequence similarity between the largest *B. argenteum* scaffold (a genomic sequence separated by gaps of known length; total length 5.5 Mbp) and *C. purpureus*. Each portion of the *B. argenteum* scaffold has a different position on the *C. purpureus* genome, indicating large scale genome (evolutionary) reorganization since the common ancestor of the two species.

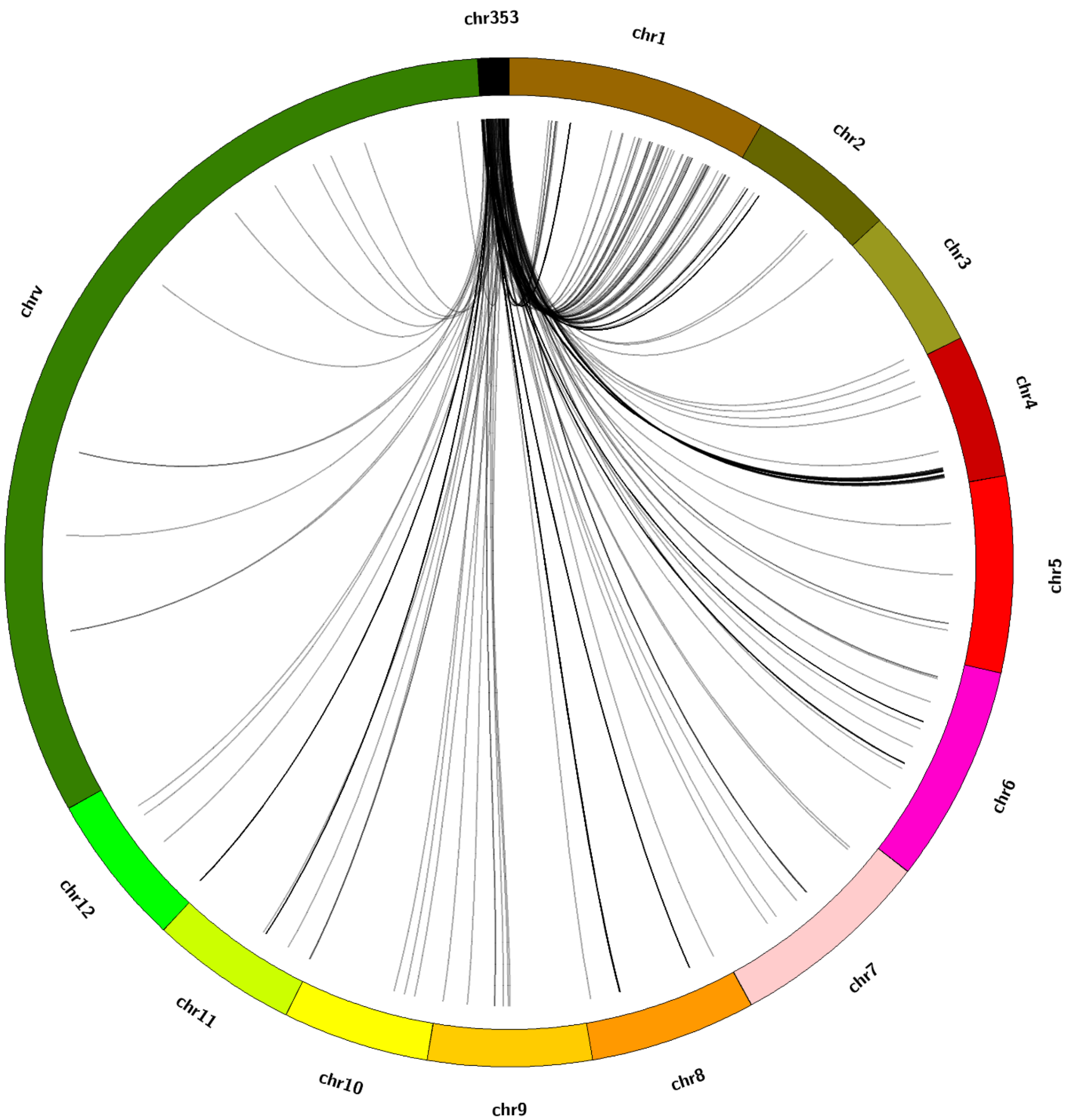
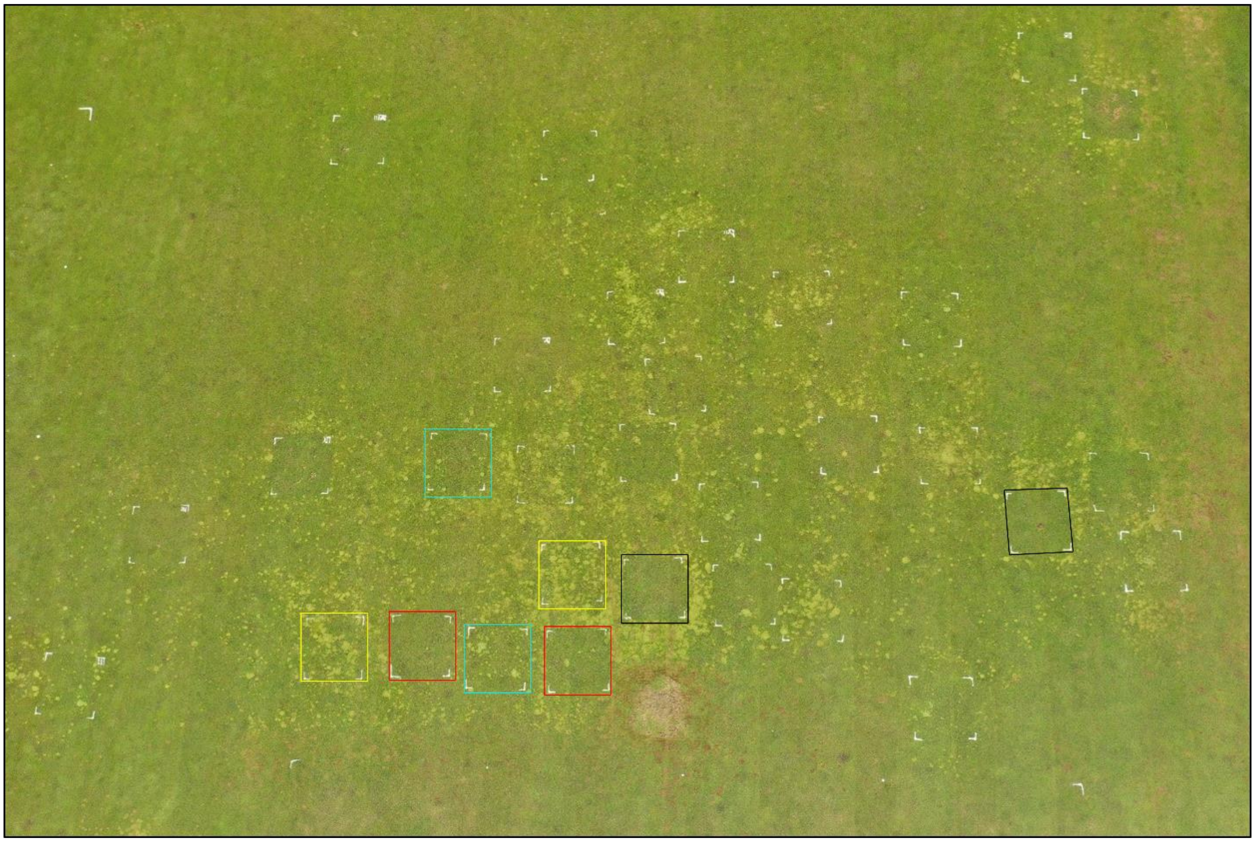


Figure 3. *Bryum argenteum* (STM) experiment in a research green at Hawks Nest Golf Course in Creston, Ohio. Plots treated with sequential applications of carfentrazone in September and October 2020 highlighted with blue box. Plots treated with drench applications of Dawn dishsoap and sodium dodecyl sulfate (SDS) highlighted with black and red boxes, respectively. Nontreated plots with yellow box. Drone image captured March 2021.



USGA ID#: 2020-15-720

Title: Improving weed control and playability in naturalized fine fescue areas

Project Leader: Matthew T. Elmore, Phillip L. Vines, and Katherine H. Diehl

Affiliation: Rutgers, The State University of New Jersey

Objectives:

1. Identify the ideal herbicide application timing for deertongue grass control
2. Determine if frequent mowing provides deertongue grass control alone or in combination with an herbicide application
3. Evaluate various plant growth regulators in combination with two different fine fescue blends to determine effects on characteristics associated with playability in naturalized areas

Start Date: 2020
Project Duration: 3 years
Total Funding: $89,842

Summary Points (Preliminary):

- According to 2020 and 2021 deertongue grass efficacy trials, glyphosate was extremely effective when applied anytime at or after 175 GDD. No fine fescue injury was observed at any time in either trial. Fluazifop was less effective than glyphosate. Dluazifop should be applied at 175 GDD or 25 CDD, but not in mid-summer for best efficacy.
- Monthly mowing reduced deertongue grass cover in 2021, but it is unknown how it will affect cover when mowing is suspended in 2022.
- 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.
- Ethephon + trinexepac-ethyl was more effective than other PGRs evaluated in improving playability of fine fescue areas. No PGR treatments reduced fine fescue seedhead (culm) production, but this may be desireable from an aesthetic perspective.

Summary Text: 800-1200 words

2020 Efficacy Trial

The first phase of this research was completed in 2020 at Mendham Golf and Tennis Club (Mendham, NJ) to identify optimal timings for deertongue grass (*Dichanthelium clandestinum*) control in naturalized fine fescue areas. The conclusions of that experiment were largely presented in the 2020 report. A final rating after greenup in 2021 confirmed that glyphosate applied at or after 175 GDD were the most effective treatments, providing 81 to 94% control (Figure 1). All fluazifop treatments were less effective (20 to 58% control) than the most effective glyphosate treatments. Fluazifop was least effective when applied in mid-July. All other application timings demonstrated similar efficacy. This experiment determined that glyphosate is much more effective than fluazifop for deertongue grass control. Fine fescue injury was not observed at any time during the experiment (data not presented).

2021 Efficacy Trial

In 2021, a trial building on the result of the aforementioned 2020 trial was intiated. The trial investigated glyphosate and fluazifop efficacy at two different application timings in a factorial with monthly mowing. Application timings were 175 GDD and 25 CDD, based on the 2020 experiment which found these to be effective timings. They are also practical in that equipment can be driven through naturalized areas at these times without concern for tire track disrupting aesthetics. A more comprehensive explanation of methods can be found in the project proposal. The 175 GDD application was made on May 13, 2021. The 25 CDD application was made on September 27, 2021. The 175 GDD fluazifop treatment was also treated with fluazifop at 25 CDD. As the trial progressed through the summer it was apparent that a single application of fluazifop, even combined with mowing, was not going to be effective. In an effort to develop practical weed control programs with both herbicides, we made the decision to make another fluazifop application. Mowing was initated three weeks after the 175 GDD application on June 9 and concluded on October 5. A walk-behind push mower bench set to 6" was used. A separate area for the 2022 experiment was established in autumn 2021 in a similar fashion to the 2021 trial site.

The results from the 2021 trial will be best evaluated in May 2022 after spring greenup when a final rating and grid intersect count will be conducted. Glyphosate was again more effective against deertongue grass than fluazifop (Table 1). No fine fescue injury from glyphosate was observed at any time (data not presented). Mowing improved deertongue grass control, but did not appear to reduce the number of plants present. Mowing improved the efficacy of fluazifop on one rating date in August. Effects of mowing alone and in combination with herbicides will be best evaluated in early 2022 upon a final rating at spring greenup.

2021 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. The less competitive hard fescue cultivar Beudin was compared to the more competitive cultivar Gladiator. Herbicides and traditional plant growth regulators (herafter 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}) + ethophon (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 12, 2021 at the first emergence of fine fescue seedheads.

Various measurements and observations were made to understand how treatments affected fine fescue playability and seedhead production. A thin stand of fine fescue that allows the golfer to find and play the golf ball along with with seedhead production that contributes to the aesthetic is important. A playability rating was taken on a 1 (extremely difficult to find and play golf ball) to 10 (extremely playable, similar to a mowed rough) scale in late June and October. At the same time, a red golf ball was dropped into each plot from shoulder height and a digital image of the ball taken. These images will be subject to digital image analysis to determine the amount of fine fescue obscuring the golf ball. With golf balls still in the plots, drone images were collected to correlate with digital images collected on the ground. In late June, a seedhead count was conducted by measuring the number of fine fescue culms within a 1' by 1' frame. Two counts were conducted per plot and the sum of these two counts were analyzed. Biomass was collected at the end of the season in October. Biomass was collected using a rotary push mower bench set to 6" height with a bag attachment. A single pass (0.5-m wide) was made down the center of each plot and the clippings were collected, dried and weighed.

According to the visual assessment of playability, the main effect of Beudin + Quatro blend had better playability than the Gladiator + Quatro blend in October (Table 2). Beudin + Quatro also tended ($P = 0.09$) to produce less biomass. PGR treatments did not affect biomass, but did affect playability. The main effect of PGR affected playability at both the June and October rating. Ethephon + trinexapac ethyl improved playability compared to the non-treated. Triclopyr and ethofumesate improved playability in June but not October. Interestingly, indaziflam did not affect playability or any other measured factor. This was suprising given our preliminary research and an additional recently published project (Braun et al. 2021; **doi.org/10.1002/cft2.20134**) which we were involved. Turfgrass injury from indaziflam or any other PGR treatment was not observed at any time during the experiment (data not presented). While ethephon + trinexapac-ethyl improved playability more than other treatments, it did not affect the number of seedheads produced. This suggests that the aesthetic of the fine fescue area can be maintained, but playability improved with a single application of ethephon + trinexapac-ethyl in May. We are still processing the digital and drone images which will provide a more quantitative assessment of playability. This trial will be repeated in 2022 on the same site.

Future Research Expectations:

Deertongue Efficacy Research

2022 research will confirm if glyphosate alone again demonstrated excellent efficacy against deertongue grass without fine fescue injury. Fluazifop is less effective, but 2022 observations will determine if a two-application program alone or in combination with mowing is an effective strategy for control. This experiment will be repeated in 2022 as described in the original proposal.

Fine Fescue Playability Research

We will repeat the experiment in 2022 to confirm that ethephon + trinexepac-ethyl is more effective than other PGRs, and that Beudin + Quatro is a better cultivar blend to improve the playability of high grass fine fescue areas. In the 2021 research we did not observe as much biomass production in the plots overall as we typically observe at golf courses that struggle with keeping these high grass fine fescue areas playable (Figure 3). We did not expect this limited biomass production and tried to ameliorate it by making an application of urea at 25 kg ha^{-1} in mid June. While it had some effect, it still did not result in sufficient biomass production. While it is not typical for superintendents to apply fertilizer to these areas, we plan to be more aggressive with N fertilization in 2022 to help separate the PGR treatments and produce a fine fescue stand akin to what we typically see on golf courses that desire improved playability of these high grass fine fescue areas.

Table 1. Effects of monthly mowing and herbicide applications on deertongue grass control in 2021 in North Brunswick, NJ. Glyphosate (560 g ha^{-1}) and fluazifop (280 g ha^{-1}) were applied at 175 growing degree-days on May 13, 2021 and at 25 cooling degree-days on September 27, 2021.

Mowing	Herbicide	4 WAT (June)	8 WAT (July)	12 WAT (August)	16 WAT (October)
		Mowing			
		----------deertongue grass control (%)----------			
Mowing		- †	68		81
No mowing		-	31		32
	Pr > F	-	<0.001	<0.001	0.002
		Herbicide			
	Glyphosate 175 GDD	74 a‡	91 a	84 a	95 a
	Fluazifop 175 GDD	59 b	68 b	60 b	74 b
	Glyphosate 25 CDD	-	-	-	39 c
	Fluazifop 25 CDD	-	-	-	41 c
	None	0 c	34 c	36 c	34 c
	Pr > F	<0.001	<0.001	<0.001	<0.001
		*Herbicide * mowing*			
Mowing	Glyphosate 175 GDD	-	97 a	78 a	95 a
	Fluazifop 175 GDD	-	65 cde	75 a	83 ab
	Glyphosate 25 CDD	-	-	-	79 bc
	Fluazifop 25 CDD	-	-	-	83 ab
	None	-	68 cd	73 a	69 c
No mowing	Glyphosate 175 GDD	-	85 b	91 a	95 a
	Fluazifop 175 GDD	-	71 c	45 b	65 c
	Glyphosate 25 CDD	-	-	-	0 d
	Fluazifop 25 CDD	-	-	-	0 d
	None	-	0 f	0 c	0 d
	Pr > F	-	<0.001	<0.001	<0.001

†The first mowing treatment was conducted 4 weeks after the 175GDD herbicide application and were not bourne out at the June rating. Thus they are not presented.
‡Means followed by the same letter are not significant different according to Fisher's Protected LSD test; P=0.05.

Table 2. Effects of plant growth regulators and two cultivar blends on fine fescue seedhead production, playability, and biomass production in 2021 in North Brunswick, NJ.

Cultivar	PGR	Playability June	Playability October	Seedheads	Biomass
		1 to 9†		#‡	g
Cultivar blend					
Beudin + Quatro		5.5	5.7 a§	93	128
Gladiator + Quatro		5.4	5.0 b	70	193
	Pr > F	*0.60*	*< 0.001*	*0.25*	*0.09*
Plant growth regulator (PGR)					
	ethofumesate	5.4 bc	5.6 ab	69	160
	indaziflam	5.1 bc	5.1 b	98	156
	ethephon + trinexapac-ethyl	6.3 a	5.9 a	75	152
	triclopyr	5.6 b	5.3 b	83	172
	None	4.9 c	5.1 b	83	161
	Pr > F	*0.02*	*0.04*	*0.34*	*0.75*
*Cultivar * PGR*					
Beudin + Quatro	ethofumesate	5.5	6.1	78	110
	indaziflam	5.3	5.5	114	134
	ethephon + trinexapac-ethyl	6.3	6.3	79	117
	triclopyr	5.5	5.5	91	143
	None	5.0	5.5	103	134
Gladiator + Quatro	ethofumesate	5.3	5.0	60	211
	indaziflam	5.0	4.8	81	178
	ethephon + trinexapac-ethyl	6.3	5.7	71	187
	triclopyr	5.8	5.0	75	200
	None	4.8	4.8	63	188
	Pr > F	*0.76*	*0.86*	*0.74*	*0.39*

†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.
‡The number of fine fescue culms per two square feet in late June.
§Means followed by the same letter are not significant different according to Fisher's Protected LSD test; P=0.05.

Figure 1. Deertongue grass control on April 27, 2021 in Mendham, NJ following applications of fluazifop or glyphosate in 2020 at various application timings. Control was determined by using grid intersect counts. Bars with the same letter are not signifciantly different according to Fisher's Protected LSD test (P = 0.05)

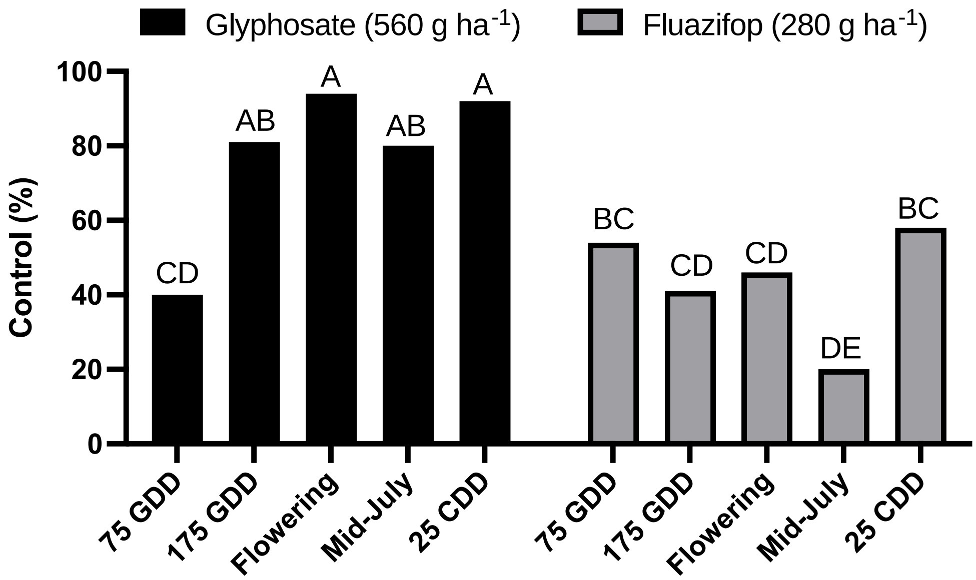


Figure 2. A plot treated with glyphosate at 175 GDD, photographed on July 2, 2021 showing excellent deertongue grass control compared to a non-treated plot at right.

Figure 3. Fine fescue playability trial on July 2, 2021 showing the fine fescue culms.

Figure 4. Fine fescue playability trial on July 2, 2021 showing two red golf balls amongst the fine fescue.

USGA ID# 2018-19-669

Title: Simulation of Nitrous Oxide Emissions in Zoysia Turfgrass Using DAYCENT and DNDC

Project Leaders: Mu Hong[1], Yao Zhang[2], Ross Braun[3], and Dale J. Bremer[1]
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Objectives:
1. Calibration and validation of the DAYCENT and DNDC models for emissions of nitrous oxide (N_2O), a greenhouse gas (GHG) implicated in climate change, from fairway zoysiagrass;
2. Prediction of long-term impacts of N fertilization and irrigation management practices on N_2O emissions and C sequestration in fairway zoysiagrass; and
3. Estimation of long-term impacts of N fertilization and irrigation management on GHG inventories by estimating energy expenses associated with turfgrass maintenance (e.g. mowing and irrigation).

Start Date: 2018
Project Duration: Continuing
Total Funding: $76,812

Summary Points:
- The DAYCENT and DNDC models were parameterized, calibrated, and validated for zoysiagrass with field data from Braun and Bremer (2018a, 2019) and Lewis and Bremer (2013).
- Daily N_2O fluxes and annual cumulative N_2O emissions were well modelled and adequately validated by DAYCENT for different irrigation and fertilization practices, whereas performance of DNDC was poor.
- In C4 turfgrass such as zoysiagrass, DAYCENT can well simulate the impacts of irrigation and N-fertilization practices on N_2O emissions in daily and cumulative N_2O fluxes, better than DNDC.

Summary Text:

Nitrous oxide (N_2O) is an important greenhouse gas that has been implicated in global climate change and therefore should be monitored in turfgrass systems, which cover large land areas and are typically fertilized with nitrogen (N) and irrigation (Braun and Bremer, 2018b). A previous USGA-funded study at K-State revealed significant effects of N fertilizer type and irrigation management on N_2O emissions in zoysiagrass over two years and C sequestration over three years (Braun and Bremer, 2018a, 2019). The acquisition of these data provided a unique opportunity to calibrate the process-based models such as DAYCENT (daily time-step version of the CENTURY biogeochemical model) and DNDC (i.e., DeNitrification-DeComposition model) for zoysiagrass turf; specifically, to predict long-term impacts of N fertilization and irrigation management on N_2O emissions and C sequestration. Such model development is important because continuous long-term measurements are expensive and time consuming. DAYCENT and DNDC were developed to predict crop production, soil carbon and nitrogen cycles, and

GHG fluxes in agricultural systems. Both models have been used widely in agricultural crops. DAYCENT has been applied to C3 but not C4 turfgrasses such as zoysiagrass (Zhang et al., 2013a, 2013b). DNDC predicted lawn-care practices of either minimal, moderate, or intensive management archetypes generated from a survey sample in Nashville, TN (Gu et al., 2015). DAYCENT and DNDC need to be calibrated to C4 turfgrasses such as zoysiagrass because of their specific physiological characteristics that have not been parameterized (Zhang et al., 2013a, 2020). Recently, the Bayesian theorem coupled with certain Monte Carlo techniques to quantify uncertainties in the outputs of process-based models has been a popular approach to calibrate many parameters of process-based models (Gurung et al., 2020; Zhang et al., 2020). Therefore, we evaluated those approaches in this study.

Materials and Methods

Measurements of N_2O emissions from Braun and Bremer (2018a) were used to calibrate two ecosystem models, DAYCENT and DNDC. In Braun and Bremer (2018a), six treatments of N fertilizer (at 0 or 98 kg ha^{-1} yr^{-1}) and irrigation combinations were included: granular urea irrigated at 66% ET (Urea_66% ET), granular urea irrigated at 33% ET (Urea_33% ET), poly-coated urea irrigated at 66% ET (PCU_66% ET), poly coated urea irrigated at 33% ET (PCU_33% ET), no N fertilizer irrigated at 66% ET (None_66% ET), and no N fertilization irrigated at 33% ET (None_33% ET).

DAYCENT and DNDC models were calibrated and validated using the process described by Gurung et al. (2020). Specifically, nine sensitive DAYCENT parameters (selected by Sobol total indices ≥0.025; Table 1) and seven DNDC parameters (parameters that are relevant and available; Table 2) were calibrated by Bayesian calibration with a sampling importance resampling (SIR) method. SIR is a direct Monte Carlo method, and the coupling of SIR and Bayes' theorem has been a popular approach (Gurung et al., 2020). In brief, after exhaustive simulations of a million parameter sets (each parameter was randomly drawn from a uniform distribution with pre-defined ranges), each parameter set (including all parameters in Table 1 or 2) was ranked by weights of summed likelihoods using the model outputs and field measurement. Hyperparameters, the sigma for Gaussian likelihood calculation, were set by the averages of standard deviations of the field measurements. The best parameter set was determined by the maximum sum of likelihood for simulations of daily VWC, NH4, NO3, and biweekly N_2O emissions in the Urea_66% ET treatment (Braun and Bremer, 2018b).

After linearly interpolation of measured and simulated data (Braun and Bremer, 2018b; Lewis and Bremer, 2013), biweekly N_2O averages and annual N_2O cumulative emissions were calculated. Given the temporally heterogeneous nature of N_2O, the biweekly N_2O averages were used for calibration and validation instead of daily N_2O values from simulations and measurements. The daily emissions of N_2O simulated by process-based models may be lagged or led (Brilli et al., 2017; Smith et al., 2008; Yue et al., 2019). The best parameter set determined by the maximum sum of likelihood, by using biweekly N_2O averages, which resulted in better model performance in fitting both biweekly and daily N_2O than when using daily N_2O (data not shown).

Results

The calibrated DAYCENT model described the biweekly N_2O data well with strong coefficient of determinations (R^2), weakly moderate relative root of mean square errors (RRMSE), and minimal relative mean deviations (RMD) (Study 1, Table 3). The DAYCENT annual cumulative N_2O simulations of Urea_66% ET was only 3% higher than the estimate linearly interpolated from measurements, which is revealed by (mathematically equals to) the RMD of biweekly N_2O average = 0.0320 (Study 1, Table 3). In contrast, the calibrated DNDC model inadequately described the biweekly N_2O data with weakly moderate R^2 and very high RRMSE (Study 1, Table 4). DNDC's annual N_2O simulation of Urea_66% ET underestimated the interpolated measurements by 37% on average (Biweekly N_2O RMD = -0.3709; Study 1, Table 4).

In general, N_2O validation of the calibrated DAYCENT model, using an independent dataset from Lewis and Bremer (2013), performed well with strong R^2, low to moderate RRMSE, and low RMD, especially in treatments of Urea_66% ET, Urea_33% ET, PCU_66% ET, and None_66% ET (Study 1, Table 3). Their averaged annual cumulative N_2O simulations were within -27 to +16% of the interpolated measurements (Study 1, Table 3). Although validation of daily and biweekly N_2O was generally poor for the independent study (Lewis and Bremer, 2013), the average annual cumulative N_2O simulated values were acceptable (within +10% of the interpolated measurements) (Study 2 Biweekly N_2O, Table 3).

Similar to results from the calibration phase, the N_2O validation phase of the DNDC model was also much poorer than DAYCENT, especially under PCU and no N (None) fertilizations (Study 1, Table 4). The DNDC average annual cumulative N_2O simulations underestimated the interpolated measurements by 44 to 84%. Notably, DNDC's validation for the independent study yielded slightly lower R^2, higher RRMSE, but larger RMD compared to DAYCENT (Study 2 Biweekly N_2O, Tables 3 and 4). However, average annual cumulative N_2O simulation underestimated the interpolated measurement by 27% (Study 2 Biweekly N_2O, Table 4).

Results show biweekly N_2O fluxes and annual cumulative emissions under different irrigation and fertilization practices were adequately validated by DAYCENT, although validation using data from the independent study was relatively poor. Notably, daily flux simulations of DAYCENT also performed adequately (e.g., R^2=0.25-0.71), close to the simulations of biweekly N_2O flux (e.g., R^2=0.25-0.89). In contrast, the daily and biweekly simulations were generally poor in DNDC. Although having great fit in soil temperature, both models need improvements in daily simulations (estimates) of soil VWC, NH4, NO3, which will be critical to substantially optimize N_2O simulations. In C4 turfgrass such as zoysiagrass, DAYCENT can well simulate the impacts of irrigation and N-fertilization practices on N_2O emissions in daily and cumulative N_2O fluxes, better than DNDC. In the next phase, which has already began, the calibrated DAYCENT model will be utilized to predict long-term impacts of different irrigation and N-fertilization practices on N_2O emissions and carbon sequestration in zoysiagrass turf.

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Table 1. Prior uniform distribution of DAYCENT sensitive parameters and calibrated values after the SIR analysis.

Parameter	Definition	Lower bound	Upper bound	Input file	Calibrated Value
PRDX(1)	Coefficient for calculating total monthly potential production as a function of solar radiation outside the atmosphere. It functions as a radiation use efficiency scalar on potential production.	1	4	crop.100	3.826
FALLRT	Fall rate (fraction of standing dead which falls each month)	0.01	0.9	crop.100	0.808
MINO3	Fraction of new net mineralization that goes to NO3 (0.0- 1.0).	0.05	0.4	site.100	0.140
MAXNIT	maximum daily nitrification amount (gN/m2/day)	0.1	4	site.100	3.769
NADJWP	Minimum fraction nitrified N lost as N_2O at wilting point	0.001	0.012	site.100	0.012
N2N2OA	N2/N_2O ratio adjustment factor for computing the N2/N_2O ratio during non-flooded conditions. Values > 1.0 increase this ratio, values (0.0-1.0) decrease this ratio.	0.01	2	site.100	0.813
WFPSNIP	Adjustment on inflection point for the water filled pore space effect on denitrification curve (< 1.0 allow denitrification to occur at lower soil water content; > 1.0 require wetter conditions for denitrification)	0.85	1.15	site.100	0.955
FAVAIL(1)	Fraction of N available per day to plants.	0.01	0.5	fix.100	0.163
FWLOSS(4)	Scaling factor for potential evapotranspiration.	0.6	0.9	fix.100	0.602

Table 2. Prior uniform distribution of DNDC parameters and calibrated values after the SIR analysis.

Parameter	Definition	Lower bound	Upper bound	Calibrated Value
Maximum_yield	The maximum biomass productions for grain (0.1% of total biomass) under optimum growing conditions (kg C/ha)	2	15	14.482
Accumulative_temperature	Accumulative air temperature from seeding till maturity of the crop (0 °C base)	3441	4434	3838.292
Water_requirement	Amount of water needed for the crop to produce a unit of dry matter of biomass (g water/g dry matter)	120	320	164.621
Leaf_ratio	Leaf biomass / (stem biomass + leaf biomass)	0.4	0.99	0.689
Root_fraction	Root fraction of a plant	0.1	0.4	0.344
Leaf_C/N	Leaf C/N ratio	15	45	44.759
Root_C/N	Root C/N ratio	39	90	39.739

Table 3. Model evaluation of DAYCENT simulations of daily SoilT, VWC, NH4, NO3, N_2O (daily and biweekly). Coefficient of determination (R^2), relative root mean square error (RRMSE), and relative mean difference (RMD).

Study	Variable	Treatment	RRMSE	RMD	R^2
1	SoilT	Urea_66% ET	0.0999	0.0134	0.9572
		Urea_33% ET	0.1017	0.0146	0.9565
		Polymer-coated urea_66% ET	0.1026	0.0115	0.9546
		Polymer-coated urea_33% ET	0.1019	0.0139	0.9564
		None_66% ET	0.1029	0.0227	0.9569
		None_33% ET	0.1022	0.0237	0.9587
	Soil VWC	Urea_66% ET	0.2602	-0.2091	0.0484
		Urea_33% ET	0.2738	-0.1947	0.1518
		Polymer-coated urea_66% ET	0.2684	-0.2165	0.0089
		Polymer-coated urea_33% ET	0.2794	-0.2049	0.1391
		None_66% ET	0.2748	-0.2245	0.0081
		None_33% ET	0.3062	-0.2440	0.0623
	Soil NH4	Urea_66% ET	0.6915	-0.1293	0.0603
		Urea_33% ET	0.6594	-0.2040	0.1263
		Polymer-coated urea_66% ET	0.4287	-0.3352	0.0044
		Polymer-coated urea_33% ET	0.4552	-0.3572	0.0046
		None_66% ET	0.5742	-0.5101	0.0506
		None_33% ET	0.6075	-0.5417	0.0356
	Soil NO3	Urea_66% ET	0.6971	-0.0463	0.2929
		Urea_33% ET	0.5863	-0.0329	0.1732
		Polymer-coated urea_66% ET	0.4579	0.0349	0.0053
		Polymer-coated urea_33% ET	0.5208	0.0493	0.0027
		None_66% ET	0.4096	-0.1468	0.0025
		None_33% ET	0.4961	-0.1595	0.0125
	N_2O	Urea_66% ET	1.3582	-0.1927	0.5911
		Urea_33% ET	1.2704	-0.3519	0.6832
		Polymer-coated urea_66% ET	0.3653	0.0996	0.7125
		Polymer-coated urea_33% ET	0.5104	-0.1461	0.3694
		None_66% ET	0.3629	-0.1114	0.6710
		None_33% ET	0.6055	-0.3509	0.3137
	Biweekly N_2O	Urea_66% ET	0.4469	0.0320	0.8483
		Urea_33% ET	0.5482	-0.1919	0.7958
		Polymer-coated urea_66% ET	0.3597	0.1599	0.8906
		Polymer-coated urea_33% ET	0.5547	-0.0391	0.5763
		None_66% ET	0.3677	-0.0625	0.8178
		None_33% ET	0.6606	-0.2714	0.4734
2	SoilT	Urea_Regular irrigation	0.1365	-0.0694	0.9739

	Soil VWC	Urea_Regular irrigation	0.2591	-0.1221	0.0532
	Soil NH4	Urea_Regular irrigation	1.0233	0.3117	0.4165
	Soil NO3	Urea_Regular irrigation	0.9545	-0.6990	0.0405
	N_2O	Urea_Regular irrigation	3.1283	0.0748	0.2473
	Biweekly N_2O	Urea_Regular irrigation	1.7096	0.0917	0.2248

Table 4. Model evaluation of DNDC simulations of daily SoilT, VWC, NH4, NO3, N_2O (daily and biweekly). Coefficient of determination (R^2), relative root mean square error (RRMSE), and relative mean difference (RMD).

Study	Variable	Treatment	RRMSE	RMD	R^2
1	SoilT	Urea_66% ET	0.1701	0.0611	0.9359
		Urea_33% ET	0.1684	0.0624	0.9336
		Polymer-coated urea_66% ET	0.1670	0.0591	0.9357
		Polymer-coated urea_33% ET	0.1667	0.0617	0.9350
		None_66% ET	0.1734	0.0686	0.9329
		None_33% ET	0.1712	0.0701	0.9334
	Soil VWC	Urea_66% ET	0.2000	-0.1397	0.0783
		Urea_33% ET	0.1894	-0.0144	0.0039
		Polymer-coated urea_66% ET	0.2126	-0.1607	0.0740
		Polymer-coated urea_33% ET	0.1830	-0.0388	0.0224
		None_66% ET	0.2209	-0.1726	0.0894
		None_33% ET	0.1720	-0.0885	0.0486
	Soil NH4	Urea_66% ET	0.9699	-0.9053	0.0222
		Urea_33% ET	0.9644	-0.9090	0.0600
		Polymer-coated urea_66% ET	1.0206	-0.9881	0.0373
		Polymer-coated urea_33% ET	1.0241	-0.9882	0.0409
		None_66% ET	1.0235	-0.9877	0.0218
		None_33% ET	1.0239	-0.9878	0.0200
	Soil NO3	Urea_66% ET	1.1815	-0.7005	0.0278
		Urea_33% ET	1.0583	-0.6890	0.0162
		Polymer-coated urea_66% ET	1.0193	-0.9087	0.0141
		Polymer-coated urea_33% ET	0.9985	-0.8961	0.0003
		None_66% ET	0.9827	-0.8911	0.0157
		None_33% ET	0.9903	-0.8798	0.0000
	N_2O	Urea_66% ET	3.0227	-0.0305	0.0590
		Urea_33% ET	2.6730	-0.0803	0.0729
		Polymer-coated urea_66% ET	1.1405	-0.9031	0.0600
		Polymer-coated urea_33% ET	1.1134	-0.8939	0.0464
		None_66% ET	1.1412	-0.8918	0.0553
		None_33% ET	1.1320	-0.8928	0.0568
	Biweekly N_2O	Urea_66% ET	1.4009	-0.3709	0.4130
		Urea_33% ET	1.2757	-0.4360	0.3735
		Polymer-coated urea_66% ET	1.2400	-0.8402	0.0210
		Polymer-coated urea_33% ET	1.1942	-0.8236	0.0096
		None_66% ET	1.2308	-0.8168	0.0158
		None_33% ET	1.2141	-0.8181	0.0130

2	SoilT	Urea_Regular irrigation	0.1537	0.0957	0.9624
	Soil VWC	Urea_Regular irrigation	0.2385	-0.1046	0.0418
	Soil NH4	Urea_Regular irrigation	1.3173	-0.8775	0.5963
	Soil NO3	Urea_Regular irrigation	1.1720	-0.9568	0.0110
	N_2O	Urea_Regular irrigation	3.7976	-0.3300	0.0007
	Biweekly N_2O	Urea_Regular irrigation	1.8968	-0.2702	0.1627

USGA ID#: 2020-10-715

Project Title: Golf course biodiversity project: Facilitating abundance and biodiversity of at-risk taxa on golf course ecosystems

Project Leaders: [1]Joe Milanovich, Ph.D, [1]Martin Berg, Ph.D, [2]Seth Magle, Ph.D., [2]Liza Lehrer, M.S.

Affiliation: [1]Loyola University Chicago, [2]Lincoln Park Zoo

Objective: The objectives of this research are to: (1) conduct an analysis of the effectiveness of newly constructed pollinator gardens within golf courses to increase abundance and diversity of diurnal and nocturnal invertebrate pollinators, (2) examine the usefulness of placing bat boxes within golf courses to facilitate the abundance and diversity of bats, and (3) quantify whether additions of coarse woody debris in golf course ponds can measurably increase abundance and biodiversity of macroinvertebrates.

Start Date: 2020
Duration: 3 years
Total Funding: $60,000

Summary:

- Best management practices including 25 m^2 pollinator gardens, Rocket booster bat boxes, and addition of coarse woody debris were constructed at 5 golf courses in Cook, DuPage, McHenry, and Lake Counties, IL.
- Sampling of bats, macroinvertebrates, and diurnal and nocturnal pollinators was conducted between June to September within golf courses with BMPs and 5 courses without.
- Identification, enumeration, and analysis of data is currently ongoing.

Diurnal and nocturnal pollinators: At 9 sites (5 experimental and 4 control) we continued to quantify abundance and diversity of diurnal pollinators within the non-turfgrass areas (control sites) or within the planted pollinator gardens (experimental sites) once per month between June to September 2021. We did this by creating two plots per course and surveying each plot for two consecutive 10 min surveys – for a total of 160 total pollinator surveys. Similar to 2020, during each 10 min survey we categorized pollinators into 9 functional groups and noted abundance of each group (Fukase and Simons, 2016). Nocturnal pollinators were sampled at each site at one of the plots (within the pollinator garden for experimental courses) once per month between May to September using a 32 Watt light trap with a 365 Quantum Black Light from Bioquip, Inc. Light traps were turned on at 1030 pm and operated until 0630 am. Contents were collected the following morning and stored in 70% ETOH.

Our result to date show diurnal pollinators were not significantly different between experimental (garden) and control (no garden) sites in 2020 and 2021 (Two-way ANOVAS, $P < 0.05$; Fig. 1); however, several taxa varied across years. For example, large bees, large fly's, and other taxa were found in greater numbers across all sites in 2021 compared to 2020 (Fig. 1). In 2020, wasps and butterflies were found in greater abundance compared to 2021 (Fig. 1). Sampling was altered in 2021 at two sites unexpectedly, as one course removed and moved a pollinator garden (and subsequent bat box) in June 2021 and pollinator sampling at one site did not occur due to restricted access of project personnel. To date, we are in the process of completing nocturnal pollinator sample identification.

Macroinvertebrate assessment: Between May to September 2021, we collected 3 macroinvertebrate samples at each site (experimental and control) once per month using a 20 cm diameter 80 µm mesh plankton net attached to a 74 µm mesh bucket and a D-frame dip net (500 µm mesh) across a 0.3 m (linear) area. Samples (150 total) were immediately stored in 70% ETOH and are currently being analyzed. In addition, at each site we collected a suite of water quality variables using a YSI multiprobe. All of these samples were taken from the same locations examined during the 2016-2019 USGA study, and from 2020.

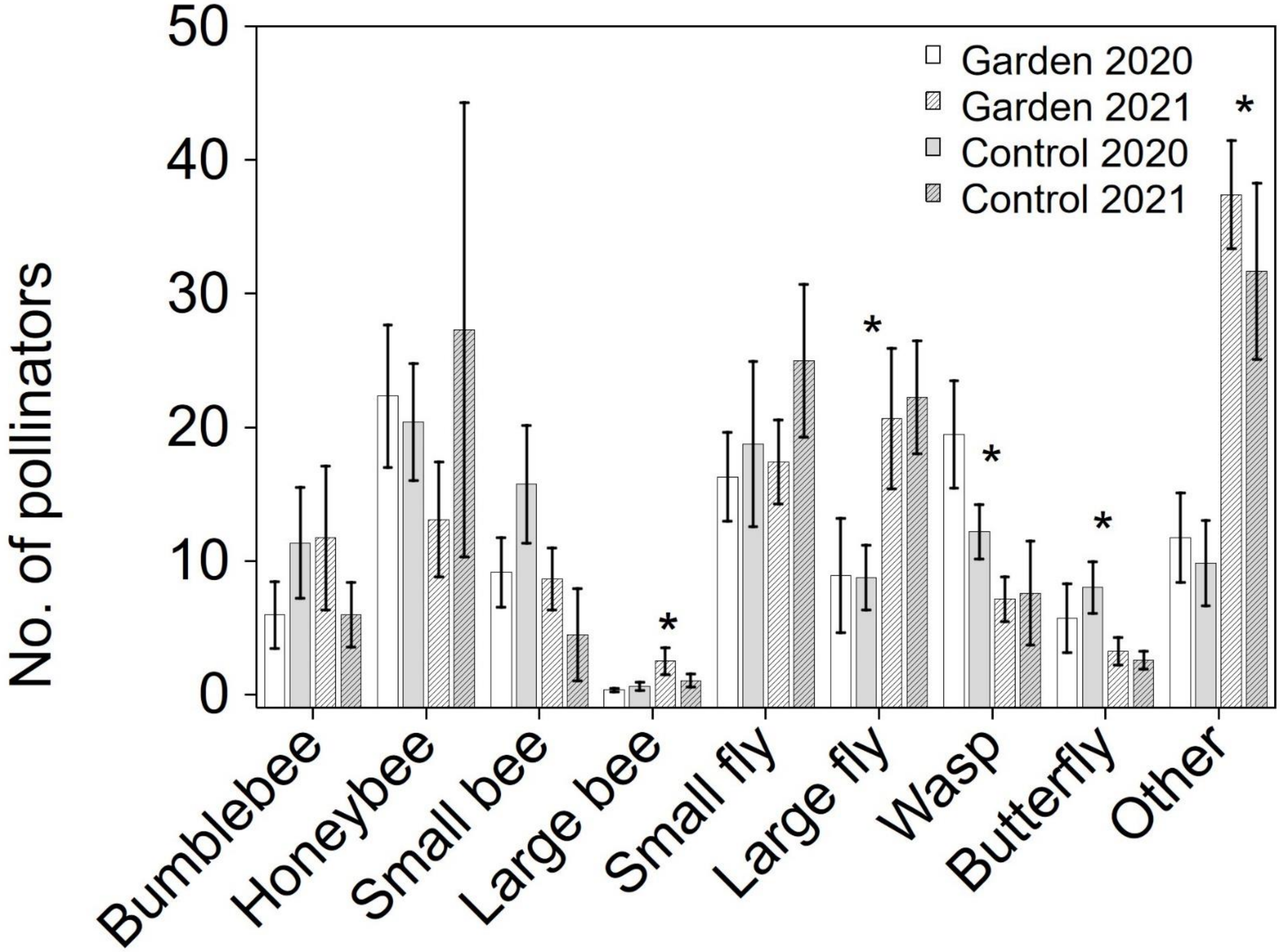

Figure 1. Mean±SE of the number of pollinator individuals per taxa within months, sampling plots, and surveys for sites with or without gardens in 2020 and 2021.

Bat assessment:

Bat boxes at 4 experimental sites (see above for details on altered sampling) were inspected approximately once per month May - August. We did not yet observe any evidence of bat occupancy during these visits.

Between June 14 and June 30, 2021, we deployed acoustic recorders (SM4BATFS; Wildlife Acoustics) equipped with ultrasonic microphones (SMM-U2) 8 of 10 courses (4 experimental and 4 control). Each golf course was surveyed for 7 nights from sunset to sunrise. Acoustic recorders were placed adjacent to pollinator gardens at experimental sites. At control sites, we placed acoustic recorders in one of the sampled pollinator habitats identified at each course, as described for the pollinator sampling. Recorders were programmed to record when triggered by ultrasonic noise in the environment surrounding the detector. After deployment, call files were scrubbed and processed using a bat call processing software (SonoBat v. 4.45 Midwest; Arcata, CA). Calls considered in the summary were based on those that met rigorous quality standards, resulting in 3140 useable call files. We visually confirmed any SonoBat classifications of rare species by examining the call spectrogram for key characteristics known for that species (see Fig. 2 for example).

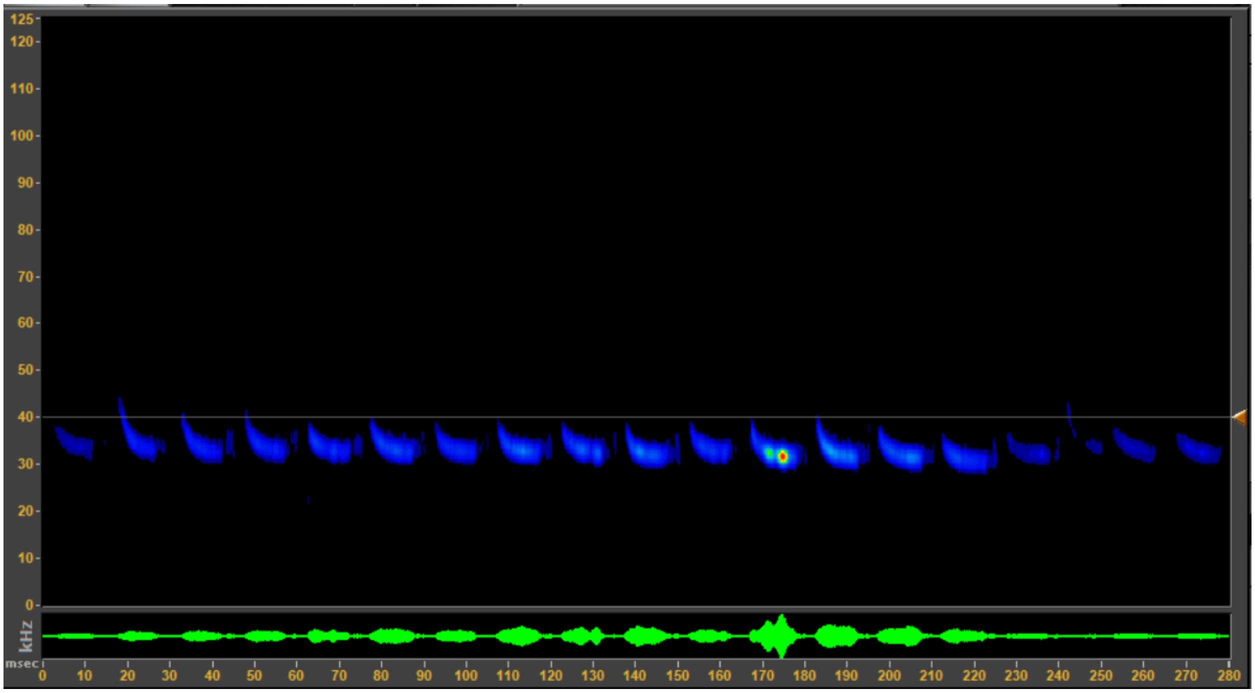

Figure 2. Spectrogram of an Eastern red bat call (*Lasiurus borealis*) recorded at Bryn Mawr Country Club on 28 June 2021 at 9:37 PM. Spectrograms can be identified to species based on unique characteristics.

Across sites we detected a diversity of species, including the big brown bat, silver-haired bat, Eastern red bat, hoary bat, and evening bat (Fig. 3). This year, we did not detect activity by any of three species known to our area that are considered to be under conservation risk (little brown bat, Northern long-eared bat, and the tricolored bat). We detected no differences to date in bat activity compared to the previous year, or between the experimental and control courses (Two-way ANOVAS, $P < 0.05$; Fig. 3).

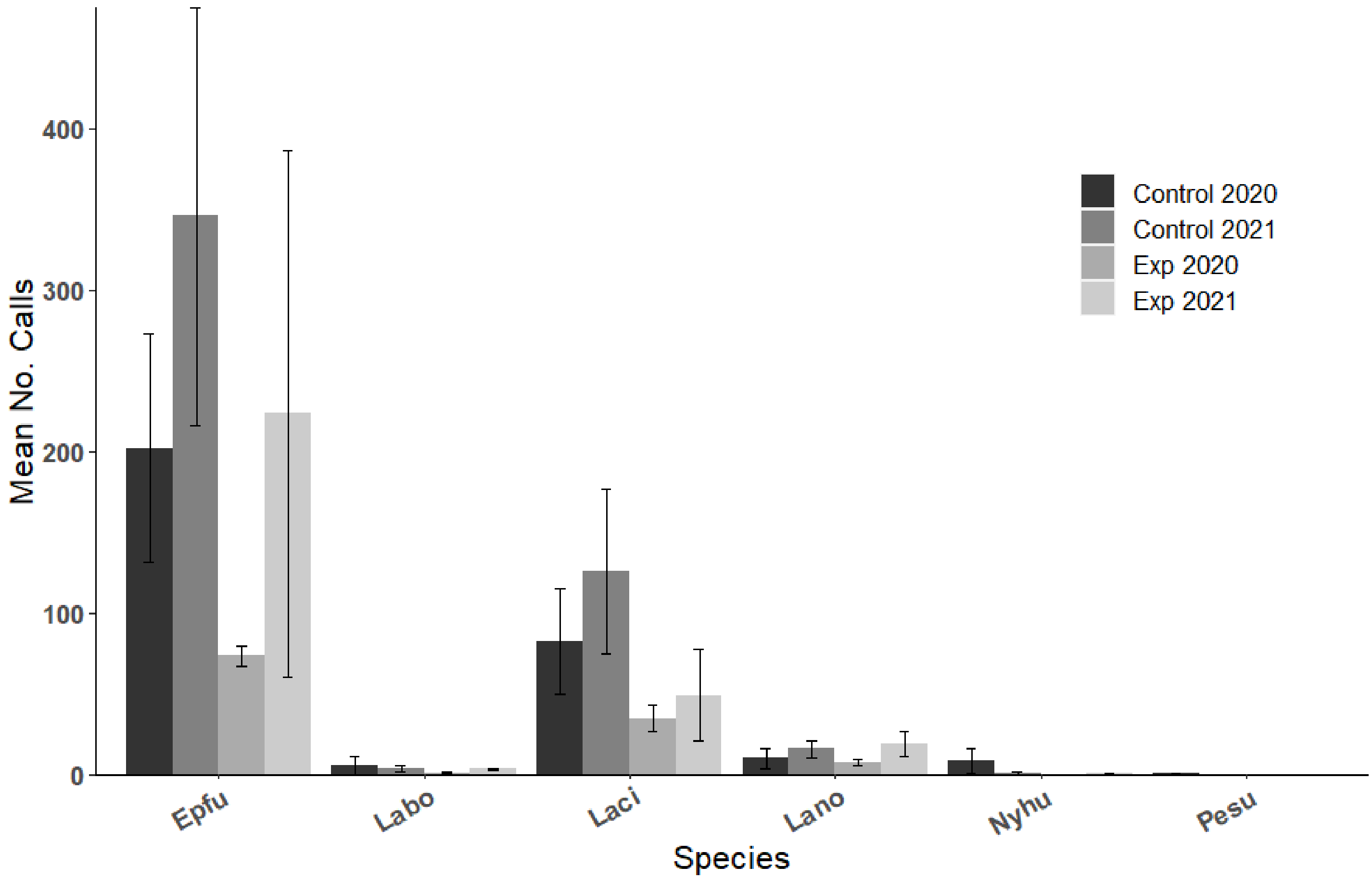

Figure 3. Mean±SE of the number of bat calls recorded per species across sampling plots and years for sites with (Exp) or without gardens (Control) in 2020 and 2021. Species classifications were determined using a bat call analysis software (SonoBat) and rare species were visually confirmed. Species are as follows: Epfu: Big brown bat (*Eptesicus fuscus*); Labo: Eastern red bat (*Lasiurus borealis*); Laci: Hoary bat (*Lasiurus cinereus*); Lano: Silver-haired bat (*Lasionycteris noctivagans*); Nyhu: Evening bat (*Nycticeius humeralis*); Pesu: Tricolored bat (*Perimyotis subflavus*).

Future assessment: Between December 2021 and December 2022, we plan to continue to enumerate macroinvertebrate samples, and analyze bat acoustic and pollinator data. Starting in May/June 2022 we plan to begin collection of our third year of pollinator, bat and macroinvertebrate data, including surveys of bat boxes to determine occupancy.

USGA ID#: 2019-21-691

Advancement of Five Elite Zoysiagrass Hybrids in the 2019 Zoysiagrass NTEP

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Background: The USGA has sponsored 5 experimental entries from Texas A&M. Namely, DALZ 1701, 1707 and 1808 selected for cold-hardiness and large patch resistance in the transition zone, and DALZ 1802 and 1807 selected for winter color retention in Riverside, California. These genotypes have also rated highly for establishment and turfgrass quality in previous multilocation and environment experiments. Due to their overall performance in earlier trials, these were advanced to the 2019 Zoysiagrass NTEP (further referred to as 2019 NTEP in this report).

Cold Hardy Zoysiagrasses: Cold Hardy/Large Patch Disease Tolerance: Since its initiation in 2012, significant progress has been made to develop cold hardy and large patch disease tolerant zoysiagrass hybrids as part of the collaborative project between Texas A&M AgriLife Research, Kansas State University and Purdue University, funded by the United States Golf Association. The Texas A&M AgriLife – Dallas breeding team has developed 2,858 new hybrids in 2011/2012 by crossing selected parental lines exhibiting large patch tolerance, fine or intermediate leaf texture, good turfgrass quality and cold hardiness. These hybrids were tested at three locations (Dallas, TX; Manhattan, KS and West Lafayette, IN) from 2012 to 2014 (2 yr. of turfgrass quality and winter recovery data). The 60 best hybrids underwent more extensive testing at nine locations across a wide range of environments for another 3 years. In 2018, the 10 best of the 60 hybrids were chosen based on their spring green up, winter injury, monthly turfgrass quality, large patch tolerance, and percentage establishment across all nine locations. The top three of these top 10 hybrids will be entered into 2019 Zoysiagrass NTEP.

Experimental #	Advanced to 2019 NTEP as	Lineage
TAES 6095-83	DALZ 1701	[(*Z. matrella* × *Z. matrella*) × *Z. japonica*] × *Z. japonica*
TAES 6099-145	DALZ 1808	*Z. japonica* × *Z. japonica*
TAES 6119-179	DALZ 1707	*Z. japonica* × [(*Z. matrella* × *Z. matrella*) × *Z. japonica*]

Winter color Retention and Performance in UC-Riverside: A total of 218 zoysiagrass hybrids were planted at UC-Riverside in the fall of 2016. Based on the data from 2016 to 2018, top performers for winter color retention, very fine leaf texture (greens types) and turfgrass quality are as follows:

Experimental #	Advanced to 2019 NTEP as	Lineage
TXZ 463	DALZ 1807	(*Z. minima* × *Z. matrella*) × *Z. pacifica*
TXZ 488	DALZ 1802	*Z. matrella* × *Z. pacifica*

Data was reported for 15 of the 20 locations in the 2019 NTEP Report. This included data from standard locations in the west (Riverside, CA), south (Auburn, AL; Gainesville, FL; and Ft. Lauderdale, FL) and transition zone (Olathe, KS; West Lafayette, IN; Fayetteville, AR; Stillwater, OK). Data was also provided for ancillary locations [West Lafayette, IN (billbug); Columbia, MO and Jay, FL (large patch); Raleigh, NC and Knoxville, TN (traffic); Griffin, GA (sod strength); Fayetteville, AR (divot recovery); Dallas, TX (drought); and College Station, TX (shade)], although most were too premature for ancillary testing since years 2019 and 2020 were establishment years. Therefore, data provided in this report has combined standard and ancillary trial data for traits such as establishment, spring greenup, winter survival, genetic color, leaf texture, fall color, and seedhead density. Establishment will be presented by region (west, south, and transition zones). Turfgrass quality will be reported separately for standard trials, traffic (Raleigh, NC) and drought (Dallas, TX) tolerance tests. Minimal data was collected for resistance to large patch and mite damage. To rank overall performance, a turfgrass performance index was tallied for each event an entry placed in the top statistical group among all 39 entries and commercial cultivars as determined by Fisher's LSD.

Establishment – Data for each entry was extracted from the 2020 NTEP report to represent establishment at the end of 2019 or beginning of 2020 as a starting point as well as early or late summer as an ending point. In western and southern locations, Empire was the quickest to establish followed by DALZ 1701 (Table 1). DALZ 1707 was ranked in the top group in Texas and Jay, FL. DALZ 1802 was only ranked in the top group in California and Alabama. DALZ 1808 was also in the top group in California, Texas, and Alabama. In the transition zone, Empire was also the quickest to establish, followed by DALZ 1808 and Zeon (Table 2). DALZ 1701 and 1707 also had high establishment though not ranked in the top groups more than once or twice out of 7 collection dates. In contrast, Meyer and DALZ 1807 were not in the top grouping for any location and the cold-hardy experimental entries had significantly faster establishment than Meyer in Oklahoma, Kansas, and North Carolina. For the experimental entries, total TPI for establishment was highest for DALZ 1701 (8) followed by DALZ 1808 (6), DALZ 1707 (4), DALZ 1802 (2), and DALZ 1807 (0).

Table 1. Percent establishment of experimental entries and commercial cultivars in the western and southern United States.

	Percent Establishment												
	Western		Southern										
	Riverside, CA		Dallas, TX		Auburn, AL		Griffin, GA		Jay, FL		Gainesville, FL		
Entry	Feb-20	Jun-20	Mar-20	Jun-20	2019	Jun-20	Sep-19	Nov-19	Oct-19	Jun-20	Sep-19	Dec-19	TPI
DALZ 1701	50.0	86.7 a	43.3	96.7 a	43.3 a	96.0 a	2.3	4.7	41.7	91.7 a	53.3 a	93.0 a	7
DALZ 1707	50.0	80.0	46.7	96.7 a	23.3	80.0	2.0	3.3	31.7	81.7 a	40.0	90.0	2
DALZ 1802	45.0	93.3 a	20.0	73.3	13.3	83.3 a	2.3	2.3	20.0	63.3	33.3	76.7	2
DALZ 1807	33.3	73.3	13.3	48.3	5.0	73.0	2.0	2.3	11.7	36.7	33.3	76.7	0
DALZ 1808	63.3	97.7 a	46.7	100.0 a	33.3	96.0 a	2.7	3.0	28.3	61.7	43.3	90.0	3
Emerald	50.0	94.7 a	33.3	88.3 a	30.0	96.0 a	2.3	3.3	26.7	65.0	40.0	83.3	3
Empire	68.3 a	96.3 a	70.0 a	100.0 a	15.0	80.0	3.3 a	6.0 a	66.7 a	96.3 a	60.0 a	96.0 a	10
Meyer	36.7	60.0	20.0	55.0	15.0	70.0	2.0	2.7	30.0	41.7	40.0	70.0	0
Zeon	70.0 a	99.0 a	36.7	96.7 a	23.3	92.7 a	2.0	3.7	28.3	75.0	40.0	90.0	4
LSD	12.6	17.1	14.9	12.1	28.1	17.2	1.2	1.1	12.3	20.0	10.2	9.0	
CV	13.4	10.0	22.2	8.3	45.7	10.5	24.3	18.1	25.4	15.5	13.6	6.2	

Table 2. Percent establishment of experimental entries and commercial cultivars in the transition zone.

	Transition Zone							
	Stillwater, OK 2020		Olathe, KS		Lafayette, IN	Raleigh, NC		
Entry	Spring 2020	Summer 2020	Sep-19	Jun-20	Oct-19	15-May	14-Aug	TPI
DALZ 1701	80.7	89.7	30.0	83.3	65.0	35.0	91.7 a	1
DALZ 1707	94.7 a	94.7 a	40.0	85.0	75.0	30.0	81.7	2
DALZ 1802	60.0	80.0	10.7	1.0	55.0	11.3	70.0	0
DALZ 1807	55.0	67.5	10.0	0.0	46.7	8.7	56.7	0
DALZ 1808	87.0 a	94.7 a	26.7	85.0	75.0	31.7	89.7 a	3
Emerald	72.7	89.0	17.7	76.7	70.0	26.7	78.3	0
Empire	95.7 a	99.0 a	75.0 a	96.0 a	91.7 a	61.7 a	97.7 a	7
Meyer	55.0	82.3	16.7	65.0	63.3	15.7	63.3	0
Zeon	88.7 a	97.7 a	24.3	86.0 a	75.0	35.0	81.7	3
LSD	14.2	7.8	13.0	10.1	7.9	11.4	9.6	
CV	10.7	5.2	31.3	12.4	7.1	24.5	9.6	

Turfgrass Quality – In California, DALZ 1802 was the only entry to have above acceptable turfgrass quality in 2019 and 2020 (Table 3). DALZ 1807 was also formerly selected in California but did not have acceptable quality compared to other entries in 2020. In College Station, TX, both DALZ 1802 and 1807 had the highest turfgrass quality ratings among the top 5 experimental entries and cultivars presented. Across other southern locations, DALZ 1802 continued to place in the top statistical group while DALZ 1807 did not perform well. Among the cold-hardy entries, DALZ 1701 and 1808 were top performers next to Emerald in southern and transition zone locations (Tables 3 and 4). In the northernmost location of West Lafayette, IN, DALZ 1707 and 1808 had quality like Empire and Zeon which was higher than DALZ 1701. Meyer was not a top performer in any location.

Table 3. Turfgrass quality of experimental entries and commercial cultivars in western and southern United States.

Entry	Turfgrass Quality								TPI
	Western		Southern						
	Riverside, CA		College Station, TX †	Auburn, AL	Griffin, GA	Jay, FL†	Gainesville, FL	Ft. Lauderdale, FL	
	2019	---2020-----------------------------------							
DALZ 1701	4.7	6.0 a	6.1	5.5 a	5.4 a	6.0 a	6.5 a	7.5 a	6
DALZ 1707	4.0	5.4	6.3	4.8	5.6 a	6.2 a	6.4 a	7.2	3
DALZ 1802	6.3 a	6.5 a	7.2 a	5.0 a	5.5 a	6.2 a	6.4 a	8.1 a	8
DALZ 1807	6.0 a	5.4	7.3 a	4.6	4.4	5.0	5.4	6.9	2
DALZ 1808	4.3	5.6	6.1	5.1 a	5.5 a	6.1 a	6.4 a	7.3 a	5
Emerald	4.3	5.8 a	6.7	5.5 a	5.5 a	6.1 a	6.5 a	7.4 a	6
Empire	4.0	5.5	6.2	4.8	5.7 a	6.4 a	6.4 a	7.4 a	4
Meyer	3.7	4.8	4.9	3.9	4.8	5.4	5.5	6.6	0
Zeon	5.0	5.7	6.6	5.4 a	5.5 a	6.1	6.5 a	7.4 a	4
LSD	0.9	1.0	0.4	1.0	1.0	1.0	1.0	1.0	
C.V	11.0	10.5	3.8	12.5	11.3	10.0	9.7	8.2	

† Shade not imposed in College Station, TX; and large patch not imposed in Jay, FL

Table 4. Turfgrass quality of experimental entries and commercial cultivars in the transition zone.

Entry	Turfgrass Quality						TPI
	Transition Zone						
	Stillwater, OK	Olathe, KS	Fayetteville, AR	Columbia, MO†	West Lafayette, IN	Knoxville, TN†	
DALZ 1701	6.1 a	7.4	7.2 a	5.3 a	5.3	5.3 a	4
DALZ 1707	6.1 a	7.3	6.4	4.5	7.5 a	5.7 a	3
DALZ 1802	5.7 a	1.7	7.5 a	1.8	0.8	5.0	2
DALZ 1807	4.9	3.1	6.5	2.4	1.3	4.0	0
DALZ 1808	6.1 a	7.7 a	6.7	5.0 a	7.1 a	5.6 a	5
Emerald	6.2 a	8.4 a	7.0 a	5.8 a	6.7	5.5 a	5
Empire	6.1 a	6.6	6.5	4.0	7.4 a	5.8 a	3
Meyer	5.1	4.6	5.8	2.6	5.1	4.8	0
Zeon	6.2 a	8.6 a	6.9	5.8 a	7.1 a	5.6 a	5
LSD	1.0	1.0	1.0	1.0	1.0	1.0	
C.V	10.4	11.7	9.0	17.1	12.7	11.5	

† Traffic not imposed in Knoxville, TN; large patch not imposed in Columbia, MO.

Ancillary Testing: Under traffic, Empire was the only entry with acceptable quality among the presented entries although the cold-hardy entries had relatively higher ratings than 1802, 1807 and other cultivars (Table 5). After rating normal quality in early 2020, two drought and recovery cycles were imposed in Dallas, TX. Under normal conditions, DALZ 1701, 1707, 1802, and 1808 had higher than acceptable quality. After withholding water for 18 days in the first drydown cycle, all entries were below acceptable, but DALZ 1701 and Empire had higher ratings. Following 19 days of recovery, all experimental entries recovered above acceptable quality, but DALZ 1701 was the only entry in the top statistical group next to Empire. Though not statistically different from other experimental entries (excluding DALZ 1807), DALZ 1701 also had the highest quality in the second drydown cycle after another 18 days without water. The second recovery cycle was initiated with heavy rainfall, and after only 4 days DALZ 1701 was the only experimental entry in the top ranked group. For these experimental entries, total TPI for quality across all locations and trials was highest for DALZ 1701 (12), followed by DALZ 1802 and 1808 (10), DALZ 1707 (6), and DALZ 1807 (2).

Table 5. Turfgrass quality of experimental entries and commercial cultivars under traffic an drought stresses.

	Turfgrass Quality (ancillary trials)						
		Dallas, TX (drought)					
Entry	Raleigh, NC (Traffic)	Normal	Drydown cycle 1	Recovery cycle 1	Drydown Cycle 2	Recovery cycle 2	TPI
DALZ 1701	5.1	7.3	5.0	8.0 a	5.7	6.3 a	2
DALZ 1707	5.0	6.9	4.7	6.7	5.0	6.0	0
DALZ 1802	5.4	7.2	4.0	6.3	5.0	6.0	0
DALZ 1807	2.6	5.5	2.7	6.3	3.3	4.3	0
DALZ 1808	4.9	6.5	4.0	7.3	5.0	5.7	0
Emerald	4.3	6.6	4.0	6.7	5.0	5.3	0
Empire	6.5 a	7.9 a	5.3	9.0 a	5.0	6.3 a	4
Meyer	3.9	3.5	2.3	3.3	2.3	2.3	0
Zeon	4.5	6.5	4.0	6.7	5.3	5.3	0
LSD	0.8	0.8	1.2	1.1	1.4	1.2	
C.V	10.2	17.2	16.4	9.7	17.1	13.1	

Spring greenup and winter survival – In Dallas, TX, most entries including commercial cultivars were slow to greenup in 2020 such that very little difference was observed when rated (Table 6). However, in Alabama, DALZ 1701 was quicker than other entries except Zeon. In the transition zone, DALZ 1701, 1707, and 1808 had variable performance. All three entries did well in Arkansas and Kansas relative to Meyer, but in Indiana, DALZ 1707 and 1808 were more cold-hardy than 1701 and Meyer. In Oklahoma, 1701, 1807, and 1808 were similar to Zeon which had higher greenup than other cultivars. In Tennessee, greenup was lower for 1808 and Meyer compared to 1701, 1707, Emerald, Empire, and Zeon. Contrastingly, 1808 was the only top ranked entry in North Carolina. DALZ 1802 and 1807 were very poorly ranked across all locations. DALZ 1701 and 1707 ranked in the top group 4 out of 8 times, like Emerald but less than Zeon. In Dallas, TX, a late freeze occurred after spring greenup in 2020. Empire suffered the most damage while other entries sustained little to no damage (Table 7). Other observations of winterkill were rated in the transition zone as percent living cover in spring (Table 7). Emerald and Zeon had the highest percent survival across locations where as DALZ 1802 and 1807 suffered greatly from winterkill across all transition zone locations. Other entries had high survival in Arkansas, which indicates relatively mild conditions compared to Indiana, Missouri, and Tennessee where more winterkill occurred. In Indiana, DALZ 1707 and 1808 had higher survival than DALZ 1701 and Meyer, but DALZ 1701 was higher than 1707, 1808, and Meyer in Missouri. In Tennessee, survival was higher for all three entries compared to Meyer.

Table 6. Spring greenup of experimental entries and commercial cultivars in the southern region and transition zone of the United States.

	Spring Greenup								
	Southern		Transition Zone						TPI
Entry	Dallas, TX	Auburn, AL	Stillwater, OK	Olathe, KS	Fayetteville, AR	West Lafayette, IN	Knoxville, TN	Raleigh, NC	
DALZ 1701	1.0	6.7 a	2.3 a	7.3	8.3 a	3.7	5.7 a	2.7	4
DALZ 1707	1.0	5.7	1.0	8.7 a	8.7 a	6.0 a	6.0 a	3.7	4
DALZ 1802	2.7	5.0	1.0	1.0	4.0	1.0	3.0	3.0	0
DALZ 1807	2.3	5.0	2.0 a	1.0	2.3	1.0	2.3	2.7	1
DALZ 1808	3.0	4.7	2.0 a	7.7	8.3 a	5.0	4.3	4.0 a	3
Emerald	2.0	5.0	1.3	8.0 a	8.3 a	6.0 a	5.7 a	3.0	4
Empire	1.0	4.3	1.3	7.7	8.0 a	4.3	5.7 a	3.0	2
Meyer	1.0	2.3	1.3	8.0 a	8.3 a	5.3	4.0	2.3	2
Zeon	2.0	6.0 a	2.0 a	8.7 a	7.7 a	5.3	5.7 a	3.0	5
LSD	0.7	1.4	1.3	1.0	1.5	0.8	2.5	0.9	
C.V	19.3	18.5	50.6	10.9	13.4	15.7	33.8	17.9	

Table 7. Frost tolerance and winter survival of experimental entries and commercial cultivars.

	Frost Tolerance	Percent living cover in spring				
Entry	Dallas, TX	Fayetteville, AR	Columbia, MO	West Lafayette, IN	Knoxville, TN	TPI
DALZ 1701	9.0 a	90.0 a	41.7 a	46.7	45.3 a	4
DALZ 1707	9.0 a	88.3 a	20.0	81.7 a	50.7 a	4
DALZ 1802	7.7 a	66.7	11.7	0.0	18.7	1
DALZ 1807	8.3 a	43.3	15.0	0.0	17.0	1
DALZ 1808	9.0 a	86.7 a	33.3	75.0	29.7	2
Emerald	9.0 a	90.0 a	56.7 a	81.7 a	45.7 a	5
Empire	5.7	83.3 a	40.0	91.7 a	48.3 a	3
Meyer	7.3 a	86.7 a	10.0	51.7	31.0	2
Zeon	8.0 a	83.3 a	56.7 a	78.3	45.3 a	4
LSD	2.2	12.1	22.2	10.6	24.7	
C.V	13.7	9.7	57.1	17.5	41.6	

Genetic Color - Twelve locations recorded genetic color. In general, the color ratings of the cold-hardy experimental entries varied across locations but were most often similar in color to each other and cultivars, but sometimes darker than Zeon (Table 8). DALZ 1808 was rated having a lighter green color than 1701 and 1707 in some locations. DALZ 1802 has a darker green color compared to all entries ranking in the top group in 10 out of 12 locations. DALZ 1701 had the next highest ranking in eight locations. DALZ 1707 and 1807 had a dark geen color in 6 locations each, followed by DALZ 1808 in 5 locations.

Leaf texture - Eleven locations rated leaf texture. In general, the cold-hardy experimental entries were coarser than Emerald and Zeon, finer than Empire, but similar to or finer than Meyer (Table 9). All of these entries ranked in the top group less than three times. DALZ 1802 and 1807 were very fine (*Z. mnima* and *Z. pacifica* derivatives) compared to all other entries and Zeon, ranking in the top group 8 out of 11 locations.

Fall color retention – Fall color retention was rated twelve times across eight locations. Three of these locations were in the western or southern regions and five locations were in the transition zone (Table 10). In California, DALZ 1802 and 1807 retained their color longer than other entries and checks. However, across all locations represented, DALZ 1802 was superior to 1807 and other entries with a top ranking 9 out of 12 times. Two of these ratings were in Indiana where both DALZ 1802 and 1807 suffered from winterkill the previous season and thus had low fall color retention. DALZ 1807 also had low fall color retention in other locations. In general, the three cold-hardy experimental entries retained their color similarly to Emerald, Empire, and Zeon which was longer than Meyer. DALZ 1701 ranked in the top group 6 times which was more than other entries except DALZ 1802.

Seedhead density – Seedhead density was rated once in each California, Florida, and North Carolina. DALZ 1807 and 1808 produced the fewest seedheads ranking in the top group all three times (Table 11). Seedhead density for DALZ 1701 and 1707 was like Empire and Meyer in California. In Gainesville, FL and Raleigh, NC heavy seedhead production was observed by DALZ 1701, which was greater than 1707, Emerald, Empire, Meyer, and Zeon.

Wilting – Although Olathe, KS was not an ancillary location for drought, wilting was observed on at least one occasion. At this time, Meyer was the worst performer showing high wilt compared to Emerald, Empire, Zeon and all 5 experimental entries which were similar with high resistance to wilt under the unknown duration of drought (Table 11).

Table 8. Genetic color of experimental entries and commercial cultivars.

	Genetic Color												
	Western	Southern						Transition Zone					
Entry	Riverside, CA	Dallas, TX	College Station, TX	Auburn, AL	Griffin, GA	Gainesville, FL	Jay, FL	Stillwater, OK	Fayetteville, AR	Knoxville, TN	West Lafayette, IN	Raleigh, NC	TPI
DALZ 1701	8.3 a	7.3 a	6.7	7.0	7.0 a	8.7 a	8.0 a	7.0	6.7 a	7.7 a	7.3	7.0 a	8
DALZ 1707	7.0 a	5.3	7.0 a	6.0	6.7 a	7.0	6.3	6.7	6.0	7.7 a	7.7 a	6.3 a	6
DALZ 1802	7.7 a	7.3 a	8.0 a	8.7 a	6.7 a	8.0	7.7 a	8.0 a	7.7 a	7.0 a	1.0	6.7 a	10
DALZ 1807	6.0	6.3	7.5 a	7.7 a	5.7	7.0	8.0 a	8.0 a	7.7 a	6.0	1.0	6.7 a	6
DALZ 1808	7.0 a	5.0	7.0 a	4.7	6.7 a	6.7	5.3	5.3	5.0	8.0 a	6.0	5.7 a	5
Emerald	6.7	7.7 a	7.0 a	6.0	6.7 a	7.7	7.0	7.0	7.0 a	7.3 a	7.0	6.0 a	6
Empire	7.0 a	7.0 a	6.7	7.3 a	6.7 a	8.7 a	7.0	6.7	5.7	7.7 a	6.7	6.7 a	7
Meyer	7.0 a	6.3	6.0	6.0	6.0	7.7	7.0	7.0	6.7 a	7.7 a	7.0	6.7 a	3
Zeon	5.0	5.3	7.3 a	6.3	6.7 a	8.0	6.0	6.3	6.7 a	7.7 a	6.0	5.7 a	5
LSD	1.6	1.2	1.3	1.7	0.8	1.0	1.1	0.9	1.2	2.1	1.6	2.0	
C.V	13.9	12.2	8.5	17.5	7.5	8.0	10.0	7.3	11.3	18.7	14.2	10.1	

Table 9. Leaf texture of experimental entries and commercial cultivars.

	Leaf Texture											
Entry	Riverside, CA	Dallas, TX	College Station, TX	Auburn, AL	Jay, FL	Stillwater, OK	Olathe, KS	Fayetteville, AR	Knoxville, TN	West Lafayette, IN	Raleigh, NC	TPI
DALZ 1701	5.7	6.3	6.0	4.0	4.7	6.7	6.7	5.7	7.7 a	7.0	6.7	1
DALZ 1707	5.7	6.3	5.7	2.7	4.7	7.0	7.0	5.0	8.0 a	6.7	7.0	1
DALZ 1802	9.0 a	9.0 a	7.7 a	9.0 a	7.0	8.0 a	9.0 a	9.0 a	3.0	1.0	9.0 a	8
DALZ 1807	9.0 a	9.0 a	8.0 a	9.0 a	9.0 a	8.0 a	.	9.0 a	0.0	1.0	9.0 a	8
DALZ 1808	5.0	6.3	5.3	3.3	3.0	6.3	6.7	5.3	8.0 a	7.0	6.7	1
Emerald	6.3	7.3	6.3	5.3	7.7	7.7 a	8.0	6.3	9.0 a	7.7 a	7.7	3
Empire	3.7	4.7	5.3	8.3 a	1.3	5.7	4.0	4.0	6.3	6.0	6.0	1
Meyer	6.0	6.3	5.3	3.0	4.3	7.0	6.0	5.0	7.7 a	7.0	6.7	1
Zeon	6.7	7.7	6.3	6.7	7.7	7.3	8.0	6.3	9.0 a	8.0 a	7.7	2
LSD	0.8	0.6	0.6	1.0	1.2	0.6	0.7	1.0	2.2	1.6	0.6	
C.V	7.9	6.1	6.9	12.0	13.1	5.5	6.5	9.5	17.2	14.7	5.5	

Table 10. Fall color retention of experimental entries and commercial cultivars.

	Fall Color Retention												
	Riverside, CA		Auburn, AL	Griffin, GA		Stillwater, OK		Olathe, KS	Fayetteville, AR	West Lafayette, IN		Raleigh, NC	
Entry	Nov-20	Dec-20	Nov-20	Sep-20	Oct-20	Nov-20	Dec-20	Nov-20	Oct-20	Oct-20	Nov-20	Nov-20	TPI
DALZ 1701	8.3 a	5.7	4.7	6.3 a	6.0 a	8.0	2.7	5.7	6.7	8.0 a	8.0 a	7.0 a	6
DALZ 1707	7.0	5.0	4.3	6.3 a	6.3 a	7.3	2.7	6.0	7.0	7.0 a	7.3 a	6.0	4
DALZ 1802	9.0 a	8.7 a	6.3 a	6.3 a	6.3 a	9.0 a	5.5 a	7.0 a	8.3 a	1.0	1.0	6.3	9
DALZ 1807	8.7 a	7.3	5.3 a	5.3	5.3	7.0	5.5 a	.	7.3	1.0	1.0	5.7	3
DALZ 1808	7.3	4.7	4.0	6.3 a	6.3 a	6.7	2.3	5.3	6.0	6.3	7.0 a	7.7 a	3
Emerald	7.3	5.7	6.0 a	6.0	5.7 a	7.3	3.0	6.3	7.0	6.0	5.3	6.0	2
Empire	7.7	5.0	5.0	6.7 a	6.7 a	7.3	1.3	4.7	5.3	7.0 a	6.7 a	5.7	4
Meyer	4.0	2.7	2.7	5.7	5.7 a	5.7	1.7	4.0	4.7	6.0	4.0	5.0	1
Zeon	8.0	5.0	6.3 a	6.3 a	6.3 a	8.0	2.3	5.7	7.7	7.0 a	6.3 a	5.7	5
LSD	1.0	1.1	1.9	1.0	1.1	0.9	1.3	0.9	1.3	1.9	2.0	1.0	
C.V	7.6	11.4	22.9	10.1	10.9	8.2	27.9	10.1	11.7	18.3	20.1	9.2	

Table 11. Seedhead density, wilting under drought and resistance to large patch and mite damage of experimental entries and commercial cultivars.

	Seedhead Density			Drought wilting	Large Patch	Mite Damage	
Entry	Riverside, CA	Gainesville, FL	Raleigh, NC	Olathe, KS	Dallas, TX	Riverside, CA	TPI
DALZ 1701	3.0	1.0	4.0	8.7 a	7.0 a	7.7 a	2
DALZ 1707	3.7	4.0	6.3	8.3 a	6.0 a	5.7	1
DALZ 1802	9.0 a	5.0	6.3	7.0	8.7 a	7.0	1
DALZ 1807	8.3 a	8.0 a	9.0 a	.	8.7 a	6.3	3
DALZ 1808	8.7 a	6.3 a	8.3 a	8.3 a	8.7 a	6.3	4
Emerald	8.7 a	3.7	7.0	8.0 a	8.0 a	6.3	2
Empire	4.0	7.7	6.0	9.0 a	7.0 a	7.0	1
Meyer	4.3	6.0	8.0 a	3.3	8.7 a	4.7	1
Zeon	8.7 a	4.7	5.7	7.0	6.7 a	8.3 a	2
LSD	2.0	2.5	1.6	1.6	3.3	1.5	
C.V	22.3	26.5	15.8	12.9	24.4	13.4	

Resistance to large patch and mites – Large patch was only rated once in one location (Dallas, TX) where all entries presented were similar in resistance (Table 11). Similarly, resistance to mite damage was only rated once in one location (Riverside, CA). At this time, DALZ 1701 had higher resistance and less damage like other entries except Meyer and 1707 which had higher damage and less resistance (Table 11).

Traffic tolerance – Traffic tolerance was rated as a percentage of green cover five times from 30 Sept 2020 to 2 Nov 2020 in Raleigh, NC. DALZ 1802 and 1701 retained the highest amount of green cover across all five dates which was significantly higher than Emerald, Empire, Meyer, and Zeon (Table 12). Interestingly, and as previously mentioned, DALZ 1802 and 1701 did not have acceptable turfgrass quality ratings under traffic though DALZ 1701 was rated higher than 1802 but less than Empire.

Table 12. Percent green cover of experimental entries and commercial cultivars as a result of mechanical traffic in Raleigh, NC.

	Percent Green Cover					
Entry	30-Sep	9-Oct	16-Oct	26-Oct	2-Nov	TPI
DALZ 1701	93.3 a	92.3 a	89.0 a	83.0	75.7 a	4
DALZ 1707	90.0 a	87.3	81.7	76.7	68.0	1
DALZ 1802	95.0 a	95.0 a	93.3 a	89.7 a	81.7 a	5
DALZ 1807	86.7	83.3	79.3	73.3	65.0	0
DALZ 1808	88.3	84.0	80.0	74.0	65.0	0
Emerald	90.0 a	85.7	81.0	75.0	68.3	1
Empire	91.7 a	88.0	83.0	77.3	67.3	1
Meyer	85.0	81.7	77.0	70.0	60.0	0
Zeon	85.0	80.0	76.0	69.0	61.7	0
LSD	5.2	6.0	5.2	5.7	6.1	
C.V	3.3	4.1	4.0	4.8	5.7	

Overall turfgrass performance – All traits considered, DALZ 1701 was a top ranked entry 50 times out of a potential 98, which was higher than all other entries presented. This was followed by Empire (48), DALZ 1802 (47), Zeon (40), Emerald (38), DALZ 1808 (35), DALZ 1707 (32), DALZ 1807 (25), and Meyer (11). Comparing the cold-tolerant experimental hybrids, DALZ 1701 offers fast establishment, high turfgrass quality, good greenup, a dark green genetic color, intermediate leaf texture, high fall color retention, tolerance to large patch and zoysiagrass mite, and high tolerance to drought and traffic. The cold hardiness of DALZ 1701 is limited in Indiana, but DALZ 1707 and 1808 have a higher survival there. For the two entries selected in California, DALZ 1802 and 1807 both have very fine leaf textures. DALZ 1807 has low performance across multiple locations and is sensitive to colder climates. However, DALZ 1802 has a high turfgrass quality, dark green genetic color, and tolerance to traffic, but exhibits limited cold tolerance.

Table 13. Total turfgrass performance index across all traits evaluated in 2019 and 2020 for experimental entries and commercial cultivars.

Entry	Establishment	Turfgrass Quality	Spring Greenup	Frost Tolerance	Living Cover in Spring	Genetic Color	Leaf Texture	Fall Color Retention	Seedhead Density	Drought Wilting	Large Patch Resistance	Mite Resistance	Traffic Tolerance	Total TPI
DALZ 1701	8	12	4	1	3	8	1	6	0	1	1	1	4	50
DALZ 1707	4	6	4	1	3	6	1	4	0	1	1	0	1	32
DALZ 1802	2	10	0	1	0	10	8	9	1	0	1	0	5	47
DALZ 1807	0	2	1	1	0	6	8	3	3	.	1	0	0	25
DALZ 1808	6	10	3	1	1	5	1	3	3	1	1	0	0	35
Emerald	3	11	4	1	4	6	3	2	1	1	1	0	1	38
Empire	17	11	2	0	3	7	1	4	0	1	1	0	1	48
Meyer	0	0	2	1	1	3	1	1	1	0	1	0	0	11
Zeon	7	9	5	1	3	5	2	5	1	0	1	1	0	40

USGA ID#: 2019-31-701

Title: On-course evaluation of new zoysiagrass hybrids

Project Leaders: James H. Baird, Marta Pudzianowska, and Pawel Petelewicz

Affiliation: University of California, Riverside (UCR)

Objective:

1. Evaluate advanced zoysiagrass lines from Texas A&M and commercial cultivars for adaptation and performance on golf course fairways in Northern California.

Start Date: 2019
Project Duration: 2 years, extended one year
Total Funding: $4,000
2021 Funding: ?

Summary Points:

- Several zoysiagrass genotypes retained acceptable color during the winter at both locations.
- There was considerable variation among the genotypes in quality, genetic color and winter color retention.
- Due to severe drought in the spring and summer 2021, irrigation was turned off at Meadow Club in certain areas of the golf course; therefore, the study at this location was terminated. Additional data on turfgrass quality, seedhead production and green cover retention were collected.

Summary Text:

Studies were initiated on July 24 and 25, 2019 at Meadow Club, Fairfax (Marin County) and Napa Golf Course, Napa (Napa County), respectively. Sod of existing cool-season turf (ryegrass, annual bluegrass) was removed from fairway areas on both golf courses in preparation for planting. Plant material arrived as plugs or was divided into plugs and planted in 5 x 5 ft plots (no alleys) with 3 replications per entry at each location. A total of 20 zoysiagrass genotypes were planted including 16 experimental lines from Texas A&M, 2 standard commercial cultivars (‘Innovation’ and ‘Diamond’), and 2 local standard commercial cultivars developed by UCR (‘El Toro’ and ‘De Anza’). In 2020 and 2021 turf was evaluated visually for: quality (1-9, 9 = best); seedhead production (1-9, 9 = highest); winter green color (1-9, 9 = darkest) and genetic color (1-9, 9 = darkest); density (1-9, 9 = densest) and uniformity (1-9, 9 = best). Normalized Difference Vegetation Index (NDVI) was measured using a GreenSeeker handheld crop sensor.

Due to slow establishment of zoysiagrasses in Northern California the trial was extended by one year. Low precipitation in the winter and spring of 2020/2021 resulted in severe drought in Northern California. Many golf courses in this area were forced to significantly reduce irrigation. Meadow Club had to turn off irrigation on most of the golf course, which unfortunately resulted in termination of the zoysiagrass trial there in early April 2021. Some of the traits presented in this report were therefore collected only at Napa Golf Course. Additional data of turfgrass quality (1-9, 9 = best), seedhead production (1-9, 9 = highest) and % green cover retention under drought (evaluated visually) were collected at Meadow Club. Experimental design was a randomized complete block. Data were subjected to analysis of variance and means separated using Tukey's Least Significant Difference Test.

Several zoysiagrass entries had high quality at both locations, paired with good winter color retention, DALZ 1309, DALZ 1807 and 'Diamond' were the best performers among them (Table 1 and 2). DALZ 1309 and DALZ 1807 also had high NDVI readings, density, uniformity and low seedhead production (Tables 1 and 3). DALZ 1807 was also characterized by dark genetic color (Table 2). Both entries are fine-textured. 'Diamond' was coarser and had slightly lower density, but high uniformity. 'Diamond' exhibited more intensive flowering compared to DALZ 1309 and DALZ 1807. Other entries retaining green color in the winter at both locations were DALZ 1802, DALZ 1814 and DALZ 1815. DALZ 1802 had high density at both locations, and very good quality at Napa GC, however its quality at Meadow Club was slightly lower. Some entries with low uniformity, such as DALZ 1809, DALZ 1811, DALZ 1812 and 'El Toro', had poor turfgrass quality. DALZ 1809, DALZ 1811 and DALZ 1812 also showed poor winter color retention.

The data for zoysiagrass entries performance after ca. two months of drought at Meadow Club are presented in Table 4. No entries retained acceptable quality. The highest quality and green cover retention were exhibited by DALZ 1702 and 'Diamond', while 'Innovation' had the lowest ratings for these traits. Variation in seedhead production under drought was observed, with DALZ 1702 and DALZ 1802 not producing seedheads, and DALZ 1308 producing them intensively.

Table 1. Turfgrass quality (1-9; 9= best) and seedhead production (1-9, 9 = highest) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

Entry	Turfgrass Quality				Seedhead production	
	Meadow Club		Napa Golf Course		Napa Golf Course	
DALZ 1308	6.4	abcd	6.0	bcde	1.0	a
DALZ 1309	6.4	abcd	6.7	ab	1.0	a
DALZ 1701	6.2	abcde	4.6	ghi	2.8	a
DALZ 1702	6.1	abcde	5.4	defg	1.1	a
DALZ 1703	5.7	abcdef	5.2	fgh	2.3	a
DALZ 1707	5.5	cdef	5.2	efg	5.9	b
DALZ 1802	6.3	abcde	7.0	a	1.0	a
DALZ 1807	6.7	a	6.8	ab	1.1	a
DALZ 1808	5.6	abcdef	5.7	cdef	1.0	a
DALZ 1809	5.1	ef	4.3	hi	3.3	ab
DALZ 1810	6.6	abc	5.3	efg	1.6	a
DALZ 1811	5.5	bcdef	4.3	i	2.1	a
DALZ 1812	4.8	f	4.1	i	3.2	a
DALZ 1813	5.8	abcdef	4.6	ghi	3.0	a
DALZ 1814	6.3	abcde	6.2	abcd	1.1	a
DALZ 1815	5.7	abcdef	6.4	abc	1.0	a
De Anza	5.1	def	5.6	cdef	3.0	a
Diamond	6.6	ab	6.8	ab	3.3	ab
El Toro	5.2	ef	5.2	efg	3.6	ab
Innovation	4.7	f	4.7	ghi	1.6	a

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 2. Turfgrass winter visual color (1-9; 9= darkest green), genetic color (1-9; 9= darkest green) and Normalized difference vegetation index (NDVI) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

Entry	Visual Color (winter)				Genetic Color				NDVI			
	Meadow Club		Napa Golf Course		Meadow Club		Napa Golf Course		Meadow Club		Napa Golf Course	
DALZ 1308	5.7	abcd	6.1	abcde	8.8	a	8.0	ab	0.58	abcd	0.65	abc
DALZ 1309	6.3	ab	7.1	abc	8.2	abcd	7.8	ab	0.63	a	0.69	ab
DALZ 1701	5.6	abcd	5.2	defg	8.3	abcd	7.3	abc	0.53	abcd	0.53	def
DALZ 1702	5.8	abc	5.7	abcdef	7.8	abcde	7.8	ab	0.56	abcd	0.60	abcdef
DALZ 1703	5.1	abcd	5.2	defg	7.8	abcde	7.6	abc	0.44	cd	0.57	cdef
DALZ 1707	5.3	abcd	5.5	cdefg	7.7	abcde	6.9	bc	0.55	abcd	0.58	bcdef
DALZ 1802	6.0	abc	7.2	ab	8.0	abcde	8.4	a	0.58	abcd	0.65	abc
DALZ 1807	6.6	a	7.3	a	8.4	ab	8.2	ab	0.61	abc	0.70	a
DALZ 1808	5.5	abcd	6.1	abcdef	7.2	bcde	7.6	abc	0.56	abcd	0.63	abcde
DALZ 1809	4.9	abcd	4.8	efg	6.8	de	7.0	abc	0.44	bcd	0.54	cdef
DALZ 1810	5.6	abcd	5.6	bcdefg	7.2	bcde	7.9	ab	0.48	abcd	0.58	bcdef
DALZ 1811	4.4	bcd	4.4	fg	7.3	abcde	7.6	abc	0.43	cd	0.52	ef
DALZ 1812	3.7	d	4.0	g	6.6	e	6.2	c	0.39	d	0.48	f
DALZ 1813	4.7	abcd	4.8	efg	8.0	abcde	7.2	abc	0.44	bcd	0.56	cdef
DALZ 1814	6.1	abc	6.3	abcde	8.0	abcde	6.9	bc	0.58	abcd	0.64	abcd
DALZ 1815	6.1	abc	6.2	abcde	8.3	abc	8.0	ab	0.56	abcd	0.63	abcde
De Anza	5.7	abcd	6.6	abcd	8.7	abcd	7.4	abc	0.55	abcd	0.65	abc
Diamond	6.7	a	7.1	abc	8.4	ab	7.8	ab	0.62	ab	0.69	ab
El Toro	5.0	abcd	5.7	abcdef	7.0	cde	7.6	abc	0.48	abcd	0.60	abcde
Innovation	4.2	cd	4.9	efg	7.8	abcde	8.2	ab	0.40	d	0.57	cdef

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 3. Turfgrass (1-9; 9= densest) density and uniformity (1-9; 9= best) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

Entry	Density				Uniformity	
	Meadow Club		Napa Golf Course		Napa Golf Course	
DALZ 1308	8.5	ab	7.8	ab	8.0	a
DALZ 1309	8.8	a	8.5	a	7.8	ab
DALZ 1701	6.5	cde	7.1	bcd	5.5	def
DALZ 1702	5.7	e	6.6	cd	5.8	cdef
DALZ 1703	5.8	e	6.1	de	6.0	bcdef
DALZ 1707	6.3	cde	6.3	de	6.0	bcdef
DALZ 1802	8.5	ab	8.5	a	7.8	ab
DALZ 1807	8.8	a	8.7	a	8.0	a
DALZ 1808	6.0	de	6.1	de	7.0	abcd
DALZ 1809	4.8	ef	6.3	de	5.0	ef
DALZ 1810	6.3	cde	6.5	d	6.2	abcdef
DALZ 1811	5.8	de	6.2	de	4.7	f
DALZ 1812	6.0	de	6.6	cd	4.8	ef
DALZ 1813	6.3	cde	6.5	d	5.8	cdef
DALZ 1814	8.0	abc	7.8	ab	8.0	a
DALZ 1815	8.3	ab	8.6	a	7.5	abc
De Anza	6.5	bcde	6.7	bcd	6.7	abcde
Diamond	7.5	abcd	7.7	abc	7.8	ab
El Toro	3.8	f	5.1	e	5.5	def
Innovation	6.2	de	6.6	cd	6.2	abcdef

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 4. Turfgrass quality (1-9; 9= best), seedhead production (1-9, 9 = highest) and green cover (%) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA, after ca. two months without irrigation.

Restricted irrigation - Meadow Club (06/24/2021)						
Entry	Turfgrass Quality		Seedhead production		Green Cover %	
DALZ 1308	4.0	ab	9.0	e	37.5	ab
DALZ 1309	4.3	ab	1.7	ac	33.3	ab
DALZ 1701	4.5	ab	5.0	abcde	35.0	ab
DALZ 1702	5.5	a	1.0	a	55.0	a
DALZ 1703	3.3	ab	5.7	bcde	36.7	ab
DALZ 1707	4.3	ab	7.3	de	45.0	ab
DALZ 1802	5.0	ab	1.0	a	47.5	ab
DALZ 1807	4.7	ab	1.0	a	46.7	ab
DALZ 1808	4.0	ab	1.0	abc	35.0	ab
DALZ 1809	3.0	ab	2.0	abcd	40.0	ab
DALZ 1810	4.5	ab	1.5	abc	40.0	ab
DALZ 1811	3.5	ab	6.5	bde	42.5	ab
DALZ 1812	4.3	ab	4.7	abcde	53.3	a
DALZ 1813	3.5	ab	2.0	abc	30.0	ab
DALZ 1814	4.5	ab	7.5	de	40.0	ab
DALZ 1815	3.3	ab	1.0	a	23.3	ab
Diamond	5.3	a	2.0	abc	43.3	ab
El Toro	3.3	ab	7.3	de	36.7	ab
Innovation	2.0	b	2.3	abc	13.3	b

Means followed by the same letter in a column are not significantly different (P=0.05).

Figure 1. Zoysiagrass genotypes at Meadow Club in Fairfax, CA after ca. 2 months without irrigation. June 2021.

Figure 2. Winter color retention of zoysiagrass genotypes at Napa Golf Course, CA. December 2020.

USGA ID#: 2019-32-702 (Purdue), 2019-34-704 (KSU)

Title: On-course Evaluations of New Zoysiagrass Hybrids

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

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

Cooperators: Scott Johnson, Shadow Glen Golf Club, Olathe, KS; Brad Pugh, Country Club of Terre Haute, Terre Haute, IN, and Randy Brehmer, The Fort Golf Course, Indianapolis, IN.

Objectives: Evaluate replicated field trials comprised of elite zoysiagrass hybrids at multiple environments in the transition zone with the objective to select experimental hybrids that have comparable/superior cold tolerance to Meyer, but higher quality, improved density, and improved large patch tolerance.

Start Date: 2019
Project Duration: 2 years
Total Funding: $8,000 ($4,000 KSU) ($4,000 Purdue)

Summary Points:

- There were differences in rate of establishment among genotypes.
- DALZ 1701, 1702, 1707, and 1810 had moderate-to-good performance across all sites.
- DALZ 1808, 1813, 1703 usually had similar or slightly lower performance than the four top performing genotypes.
- There are multiple genotype options for potential zoysiagrass release in the future that will have better turf quality, finer leaf texture, improved density, and darker green color than Meyer in a variety of climates across both northern and southern areas of the transition zone.

Summary Text:

Expansion studies that included the top ten experimental hybrids and two zoysiagrass cultivar checks were established on driving ranges or nursery areas at Shadow Glen Golf Club, Olathe, KS on 17 June 2019, The Fort Golf Course, Indianapolis, IN on 2 July 2019, and The Country Club of Terre Haute, Terre Haute, IN on 2 July 2019 (Figure 1). The study was set up as randomized complete block with four replicates in Olathe, KS and three replicates at IN sites (Figure 1).

From 2019 to 2021, data collected at each site included ratings of quality, density, and uniformity, seasonal/genetic turf color, spring green-up, leaf texture, and percent visual turf cover (i.e., establishment rate) in accordance to National Turfgrass Evaluation Program guidelines (Morris & Shearman, 1999) (Table 1). In addition, naturally occurring large patch cover was visually rated on a 0 to 100%. Due to the COVID-19 pandemic and travel restrictions, less data collection events occurred at the Terre Haute, IN site. Data for each parameter were analyzed for each location separately with SAS and means were separated with Fisher's

Protected LSD test (α = .05) (Table 1). After analysis, a cumulative turf performance index (TPI) score was generated for each treatment across all parameters, except establishment rate, at all sites.

When visual turf cover was evaluated from two to 15 months after planting (MAP), there were differences in establishment rates among genotypes and cultivar checks at each site (Table 2). There were multiple instances when DALZ genotypes exhibited greater turf cover (i.e., faster establishment) between two and 15 MAP compared to either of the cultivar checks, Innovation and Meyer, at each respective site. DALZ 1703, 1808, 1811, and 1812 often exhibited faster turf establishment (i.e., greater turf cover) across multiple sites compared to the other genotypes and cultivar checks. Overall, the majority of these DALZ genotypes exhibited a faster or similar establishment compared to Innovation, a recently released cultivar, or Meyer.

After multiple years of data collection at each site (Table 1), there were both consistencies and differences in performance across sites, particularly due to climate (Figure 2). Genotype entries such as DALZ 1701, 1702, 1707, and 1810 had moderate-to-good performance across all sites; and DALZ 1808, 1813, 1703 usually had slightly lower performance than previous four mentioned genotypes (Figure 2). Performance of DALZ 1811 was more variable across sites, for example receiving both high-to-low TPI scores across sites, and DALZ 1809 and 1812 were generally the poorest performing genotypes across sites. There were six to nine genotypes that had a higher TPI score than the best performing cultivar check at each respective site. In comparison to the DALZ genotypes, Meyer consistently performed poorly, which illustrates the improvements achieved in zoysiagrass breeding since Meyer's release in 1951. Moreover, Innovation had poor-to-moderate performance in comparison to these DALZ genotypes, which illustrates the advancements in breeding in the last ten years. Overall, results indicate there are multiple genotype options for potential zoysiagrass release in the future that will have better turf performance, finer leaf texture, and darker green color in a variety of climates across both northern and southern areas of the transition zone.

Three of these DALZ genotype entries have been selected and planted at sod farms in Indiana, Missouri, Oklahoma, and Texas in 2021 for further evaluation, and to obtain feedback from sod producers. In combination with data from other research farms and past USGA-sponsored research related to these genotypes, results from this experiment is currently being written to be published in a peer-reviewed scientific journal in 2022.

References:

Morris, K. N., & Shearman, R. C. (1999). NTEP turfgrass evaluation guidelines. National Turfgrass Evaluation Program, Beltsville, MD.

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

Acknowledgements:

We would like to thank Scott Johnson, CGCS at Shadow Glen Golf Club, Olathe, KS; Brad Pugh, CGCS at the Country Club of Terre Haute, Terre Haute, IN, and Randy Brehmer, CGCS at The Fort Golf Course, Indianapolis, IN for hosting and managing a zoysiagrass expansion site.

Table 1. Statistically significant data collection events out of the total collection events for each data parameter for each site during the duration of the experiment.

Site	Code[a]	Turf quality[b]	Leaf texture[c]	Spring color[d]	Summer color[d]	Fall color[d]	Density[e]	Dark green color index[f]	Ball lie percentage[g]	Other	Max. TPI[h]
Shadow Glen Golf Club, Olathe, KS	KS1	2 of 6	2 of 2	2 of 3	3 of 3	3 of 3	--[i]	1 of 1	--[i]	--	13
The Fort Golf Course, Indianapolis, IN	IN1	4 of 6	2 of 2	--[i]	3 of 6	1 of 1	3 of 3	1 of 1	0 of 1	Mowing quality (1 of 1)	15
The Country Club of Terre Haute, Terre Haute, IN	IN2	3 of 5	2 of 2	--[i]	1 of 2	0 of 1	1 of 1	--[i]	--[i]	Mowing quality (1 of 1)	8

[a] Site code corresponds to Figure 2.
[b] Turf quality: 9 = maximum quality; 6 = minimum acceptable quality; 1 = lowest quality.
[c] Leaf texture: 9 = fine and 1 = coarse.
[d] Seasonal color/genetic color/color retention ratings: 9 = darkest green; 6 = minimally acceptable color; 1 = straw brown turf.
[e] Density: 9 = maximum density; 6 = minimally acceptable density; 1 = lowest density.
[f] Dark green color index: digital images calculated on a 0 to 1 scale with higher values corresponding to darker green color.
[g] Ball lie: Percentage visible golf ball within the turf canopy measured three times within each plot from using the method developed by Richardson et al. (2010).
[h] Maximum turf performance index (TPI) is the maximum TPI number for each site based on number of statistically significant data parameters.
[i] Data not collected at site.

Table 2. Establishment differences among treatments based on visual turf cover (0-100%) at two to 15 months after planting (MAP) in 2019 to 2020 for each site.

DALZ genotype or cultivar	Shadow Glen Golf Club[a]			The Fort Golf Course[a]			The Country Club of Terre Haute[a]		
	3 MAP	11 MAP	13 MAP	2 MAP	12 MAP	15 MAP	2 MAP	12 MAP	15 MAP
1701	75 bcd[b]	89 ab	100	28 abc	63 cd	91	31	65 b-e	91 bcd
1702	74 bcd	88 ab	98	25 cde	72 abc	95	37	75 ab	97 ab
1703	93 a	95 a	100	29 abc	82 ab	99	33	87 a	100 a
1707	65 d	77 bc	98	24 cde	72 abc	97	28	55 def	87 cd
1808	82 abc	93 a	100	34 ab	82 ab	95	38	73 abc	94 abc
1809	63 d	65 c	97	25 cde	70 bc	94	30	68 bcd	90 bcd
1810	70 cd	92 a	100	28 abc	68 bc	95	33	62 b-e	89 bcd
1811	77 bcd	85 ab	100	27 bcd	77 abc	99	25	53 ef	86 cde
1812	89 ab	97 a	100	35 a	88 a	100	33	75 ab	95 ab
1813	72 cd	85 ab	95	23 cde	62 cd	89	30	57 de	83 de
Innovation	38[c]	50[c]	80[c]	21 de	48 de	91	29	60 cde	90 bcd
Meyer	53[c]	62[c]	90[c]	19 e	43 e	83	29	42 f	80 e
P-value	.0120	.0039	.6326	.0046	.0004	.0901	.0779	<.0001	.0008

[a] Sites were planted with vegetative plugs on 17 June 2019 at Shadow Glen Golf Club, Olathe, KS; 2 July 2019 at The Fort Golf Course, Indianapolis, IN; and 2 July 2019 at The Country Club of Terre Haute, Terre Haute, IN.

[b] Means within each column with a common letter or no letters are not significantly different according to Fisher's protected LSD (α = .05).

[c] Innovation and Meyer were removed from establishment data analysis because fewer vegetative plugs per plot were used during planting compared to the top ten experimental hybrids at Shadow Glen Golf Club in Olathe KS.

Figure 1: Ten experimental zoysiagrasses, and Meyer and Innovation at The Fort Golf Course, Indianapolis, IN on September 23, 2021.

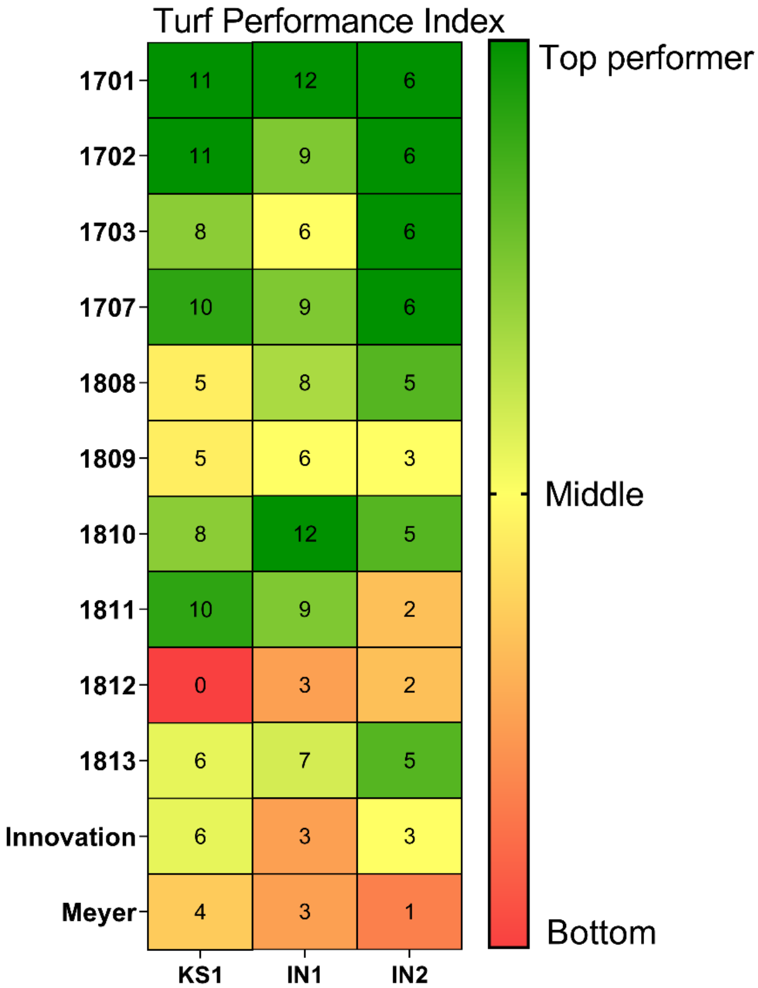


Figure 2. Cumulative turf performance index score at each location, which is the number of times a treatment occurred in the top statistical group across all parameters (Table 1) except establishment rate. Treatments are sorted numerically by DALZ code and two cultivar checks are listed at the bottom. The site abbreviation and maximum possible turf performance index number is the following: Shadow Glen Golf Club, Olathe, KS (KS1, 13); The Fort Golf Course, Indianapolis, IN (IN1, 15); and The Country Club of Terre Haute, Terre Haute, IN (IN2, 8).

USGA ID#: 2019-33-703

Title: On-course evaluation of new zoysiagrass hybrids

Project Leader: Kai Umeda and Worku Burayu
Affiliation: University of Arizona

Objectives: Evaluate and compare the adaptation and performance of zoysiagrass cultivars in the low desert southwest United States

Start Date: 2019
Project Duration: 3 years
Total Funding: $6,000
Report type: Final report 2021

Summary Points:
1) For the attributes of winter green color retention and the ability to establish a stand and cover the soil surface, DALZ 1308 and 1309 performed well;
2) During the second winter after establishment, DALZ 1308, 1309, 1802, 1807, and Diamond retained greenness;
3) Establishment and spreading of zoysiagrasses was very slow during the first year after planting plugs;
4) Zoysiagrasses mowed at a rough height of cut with a rotary mower were scalped and recovery was slow.

Summary Text:
Maintaining green turfgrass year around is a goal for the low desert southwest U.S. where warm season dormant bermudagrass is overseeded with a cool season annual grass in the winter. In recent years, winter survival of warm season zoysiagrasses improved such that green color retention was observed for some cultivars. A field study of 16 new zoysiagrass cultivars, along with 2 commercial cultivars, and 2 bermudagrass hybrids was established at the Wigwam Golf Club in Litchfield Park, AZ. The experimental plots for each turfgrass cultivar measured 8 ft by 8 ft and were arranged in a randomized complete block design with 3 replicates. Zoysiagrass plugs and bermudagrass sod strips were planted on two dates: 11 July and 02 August 2019 (Table 1). The site of the plots was irrigated with overhead sprinklers and water was applied multiple times per day until the plugs were adequately established. No mowing was done until summer 2021 and only starter fertilizer was applied after 2019 planting. The grasses were regularly evaluated during the first summer 2019 for plug survival, establishment, and overall performance and appearance that included color, density, and vigor (Table 2 and annual report 2019). Two cultivars, DALZ 1814 and 1815, did not survive in year 1 and were replanted in 2020. Turfgrass greenness was evaluated during the late winter to early spring season when turfgrasses were typically dormant.
In January 2020, during the first winter after planting, the zoysiagrass cultivars, DALZ 1308, 1309, 1802, 1807, and Diamond remained relatively green compared to other cultivars and bermudagrasses (Table 3). Again, in early February 2021, cultivars DALZ 1308, 1309, 1802, 1807, and Diamond retained greenness during the winter (Figure 3). With mild winter temperatures (Figures 4 and 5), the four DALZ cultivars appeared to remain slightly greener than Diamond

zoysiagrass. The spreading of zoysiagrasses was very slow during the first year of establishment (Figure 1). In year 2, DALZ 1308 and 1309 appeared to spread more than the other cultivars to cover the soil surface (Figure 2). The zoysiagrasses tended to grow “clumpy” when plugged but also did spread laterally on the soil surface very slowly. During the summer 2021, “clumpy” zoysiagrasses were mowed at a rough height cut (~3 inches) with a rotary mower and were scalped. The recovery was extremely slow. (No data collected).

For attributes of winter green color retention and the ability to establish a stand and cover the soil surface, DALZ 1308 and 1309 performed well under the low desert southwest U.S conditions. For green color retention under relatively mild winter temperatures, DALZ 1308, 1309, 1802, 1807, and Diamond exhibited more green than other cultivars.

Table 1. Zoysiagrasses and bermudagrasses planted and evaluated at Wigwam Golf Club in the low desert Arizona, 2019-21.

Cultivar	Planting date 2019	Plant material
DALZ 1308	11 July	48 plugs
DALZ 1309	11 July	48 plugs
DALZ 1701	11 July	24 plugs
DALZ 1702	11 July	24 plugs
DALZ 1703	11 July	24 plugs
DALZ 1707	11 July	24 plugs
DALZ 1802	02 August	48 plugs
DALZ 1807	02 August	48 plugs
DALZ 1808	11 July	24 plugs
DALZ 1809	11 July	24 plugs
DALZ 1810	11 July	24 plugs
DALZ 1811	11 July	24 plugs
DALZ 1812	11 July	24 plugs
DALZ 1813	11 July	24 plugs
DALZ 1814	02 August replant 18 June 2020	48 plugs
DALZ 1815	02 August replant 18 June 2020	48 plugs
Innovation	11 July	24 plugs
Diamond	02 August	48 plugs
Tifway 419	02 August	sod strip
TifTuf	02 August	sod strip

Table 2. Comparison of zoysiagrass cultivars after planting, 2019, Litchfield Park, AZ

Turfgrass cultivar	Turf survival (%)	Turf performance
	23 Sep	15 Oct
DALZ 1308	97 ab	6.8 abcd
DALZ 1309	100 a	7.0 abcd
DALZ 1701	93 ab	5.7 bcd
DALZ 1702	98 ab	6.3 bcd
DALZ 1703	100 a	7.8 abc
DALZ 1707	79 b	5.7 bcd
DALZ 1802	53 c	4.8 d
DALZ 1807	97 ab	5.3 cd
DALZ 1808	100 a	7.7 abc
DALZ 1809	100 a	7.8 abc
DALZ 1810	98 ab	8.2 ab
DALZ 1811	100 a	6.8 abcd
DALZ 1812	100 a	7.8 abc
DALZ 1813	100 a	8.0 ab
DALZ 1814	6 d	1.0 e
DALZ 1815	6 d	1.3 e
Innovation	100 a	6.5 abcd
Diamond	93 ab	6.2 bcd
Tifway 419	100 a	9.0 a
TifTuf	100 a	9.0 a

Performance ratings 1-9; 1=poor, 9=best
Means within a column with that same letter are not significantly different by Tukey-Kramer HSD at *p*=0.05.

Table 3. Comparison of zoysiagrass cultivars for greenness and surface coverage, 2020 - 2021, Litchfield Park, AZ

Turfgrass cultivar	Turf Greenness							Surface Coverage	
	28 Jan 20	24 Apr 20	19 May 20	09 Jun 20	15 Dec 20	01 Feb 21	14 Apr 21	09 Jun 20	01 Feb 21
DALZ 1308	5.3 ab	5.7 abc	6.3 abc	6.0 abc	6.0 a	6.3 a	5.3 a	4.0 abcd	7.0 abc
DALZ 1309	6.0 a	6.3 ab	6.3 abc	6.3 abc	6.0 a	6.0 ab	5.3 a	4.3 abcd	7.0 abc
DALZ 1701	4.0 bcd	4.8 abc	4.0 d	5.7 abc	5.0 abc	3.7 abc	6.0 a	3.0 bcde	4.7 abc
DALZ 1702	3.7 cd	3.7 bc	3.6 d	4.0 c	2.3 d	2.3 c	1.7 b	1.3 de	2.7 c
DALZ 1703	4.2 bcd	5.0 abc	5.0 bcd	5.3 abc	4.3 abcd	3.3 abc	6.3 a	2.7 bcde	5.0 abc
DALZ 1707	3.7 cd	4.2 abc	5.0 bcd	5.3 abc	5.0 abc	3.3 abc	5.7 a	2.3 bcde	3.3 bc
DALZ 1802	6.3 a	6.7 ab	6.7 abc	7.7 a	6.0 a	5.0 abc	6.3 a	5.0 abc	5.3 abc
DALZ 1807	6.0 a	6.2 ab	6.3 abc	7.3 ab	6.0 a	6.0 ab	6.7 a	4.3 abcd	6.3 abc
DALZ 1808	4.0 bcd	4.7 abc	5.0 bcd	5.3 abc	4.7 abc	3.0 bc	6.7 a	2.7 bcde	5.3 ab
DALZ 1809	4.0 bcd	4.3 abc	4.8 cd	5.0 bc	4.7 abc	3.3 abc	5.0 ab	3.0 bcde	6.0 abc
DALZ 1810	5.0 abc	6.3 ab	6.7 abc	6.3 abc	5.7 ab	3.7 abc	6.0 a	4.0 abcd	6.0 abc
DALZ 1811	4.2 bcd	5.0 abc	5.0 bcd	5.3 abc	5.0 abc	3.0 bc	4.7 ab	1.7 cde	4.3 abc
DALZ 1812	4.0 bcd	4.3 abc	5.3 bcd	6.3 abc	5.0 abc	3.0 bc	5.7 a	3.7 bcd	6.3 abc
DALZ 1813	4.0 bcd	4.0 abc	5.0 bcd	5.0 bc	4.7 abc	3.3 abc	5.0 ab	2.7 bcde	5.0 abc
DALZ 1814	-	1.0 d	1.0 e	1.0 d	3.7 bcd	3.7 abc	3.7 ab	1.0 e	6.0 abc
DALZ 1815	-	2.3 cd	1.0 e	1.0 d	3.7 bcd	4.0 abc	5.3 a	1.0 e	3.7 abc
Innovation	3.3 d	4.2 abc	4.0 d	4.3 c	4.0 abcd	3.0 bc	5.0 ab	1.7 cde	3.7 abc
Diamond	6.3 a	7.3 a	7.7 a	7.7 a	6.0 a	5.7 ab	5.0 ab	5.7 ab	6.0 abc
Tifway 419	3.7 cd	6.0 ab	7.0 ab	6.3 abc	3.3 cd	3.0 bc	5.7 a	7.3 a	8.0 a
TifTuf	4.0 bcd	6.0 ab	7.0 ab	7.3 ab	4.0 abcd	3.0 bc	6.0 a	7.3 a	7.7 ab

Ratings 1-9; 1=poor, 9=best

Means within a column with that same letter are not significantly different by Tukey-Kramer HSD at *p*=0.05.

Figure 1. Turf surface coverage in June 2020, Litchfield Park, AZ

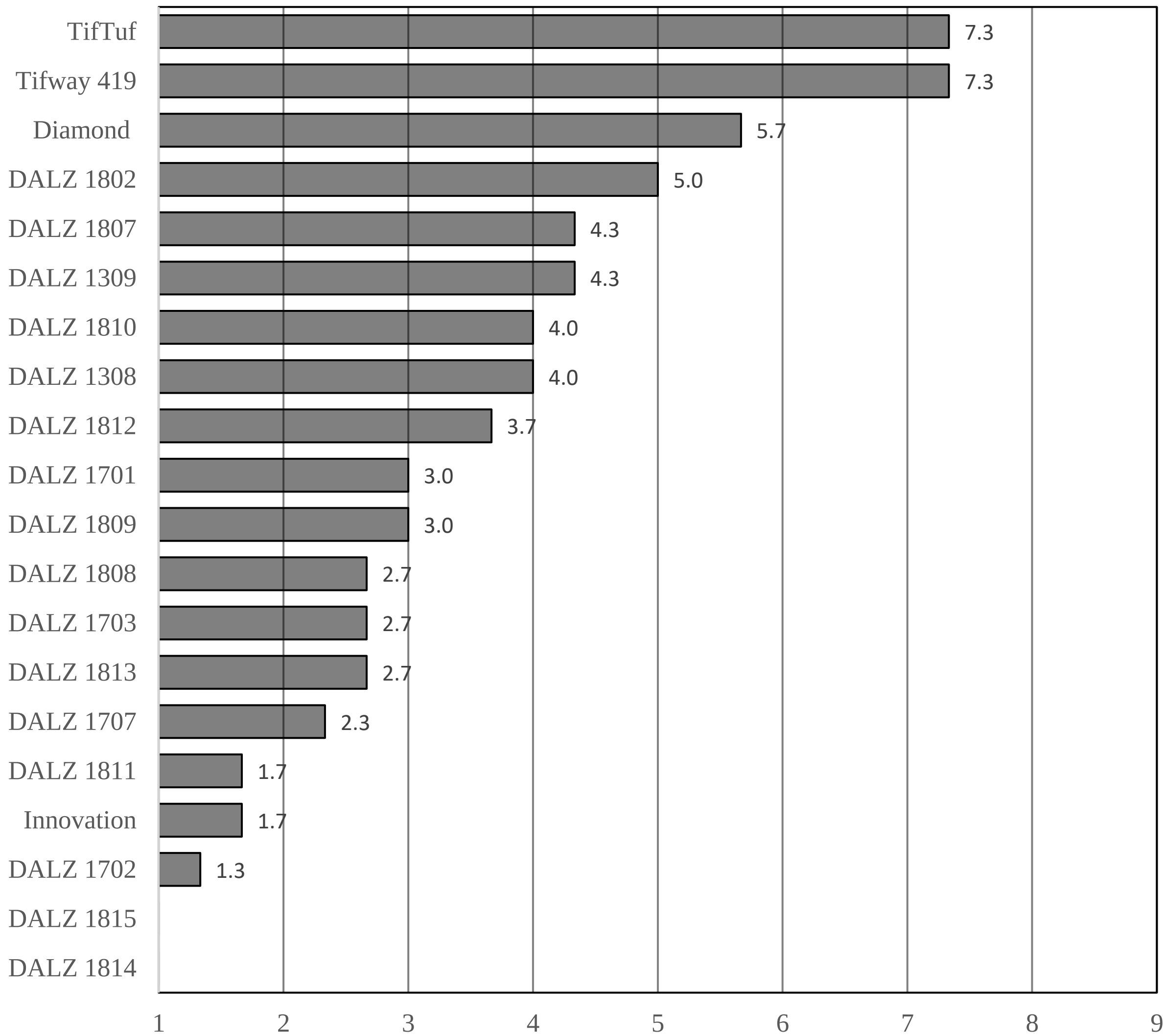


Figure 2. Turf surface coverage in February 2021, Litchfield Park, AZ

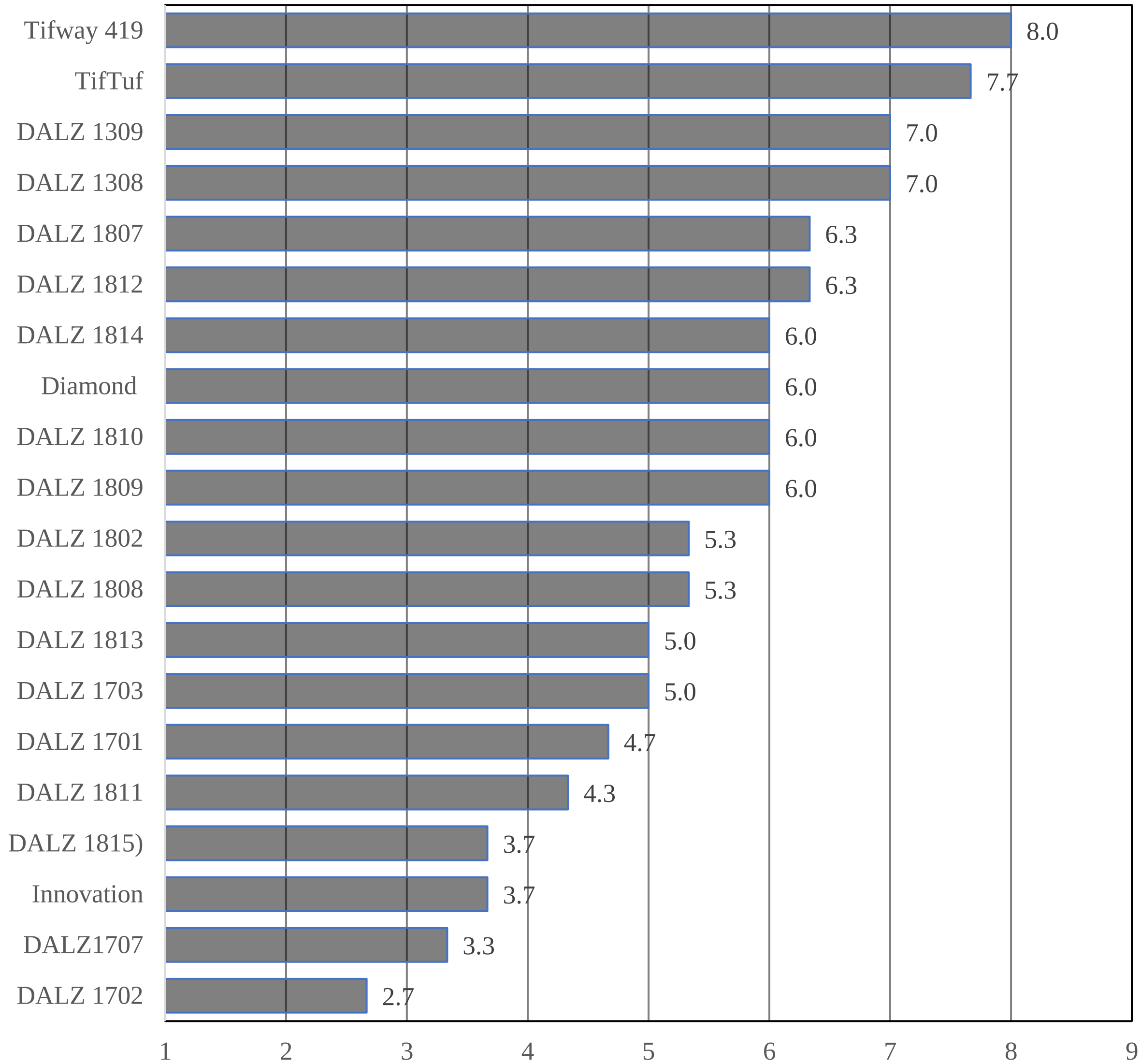


Figure 3. Comparative turf greenness in February 2021, Litchfield Park, AZ

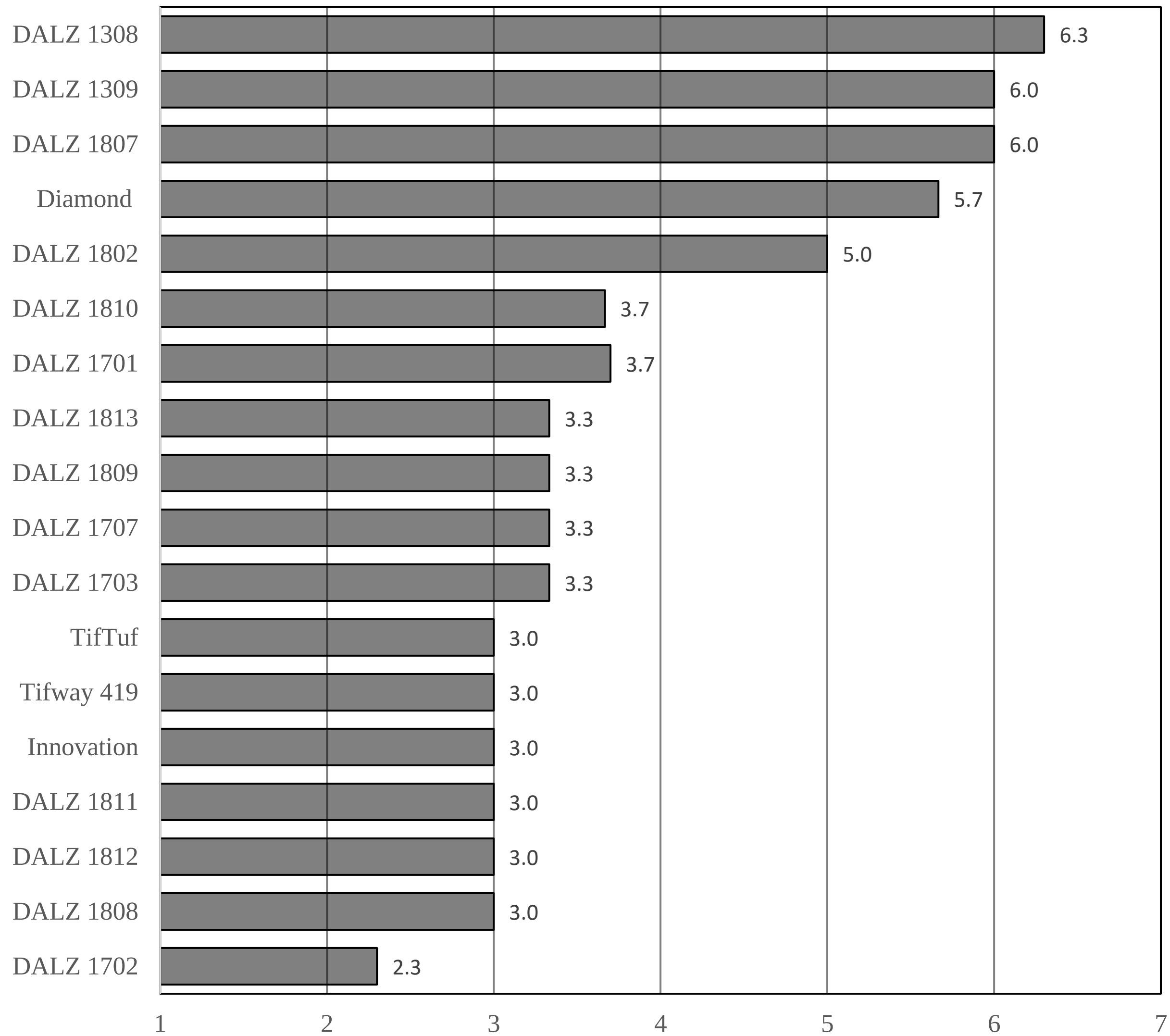


Figure 4. Winter temperatures in Litchfield Park, AZ in 2019-20 (National Weather Service).

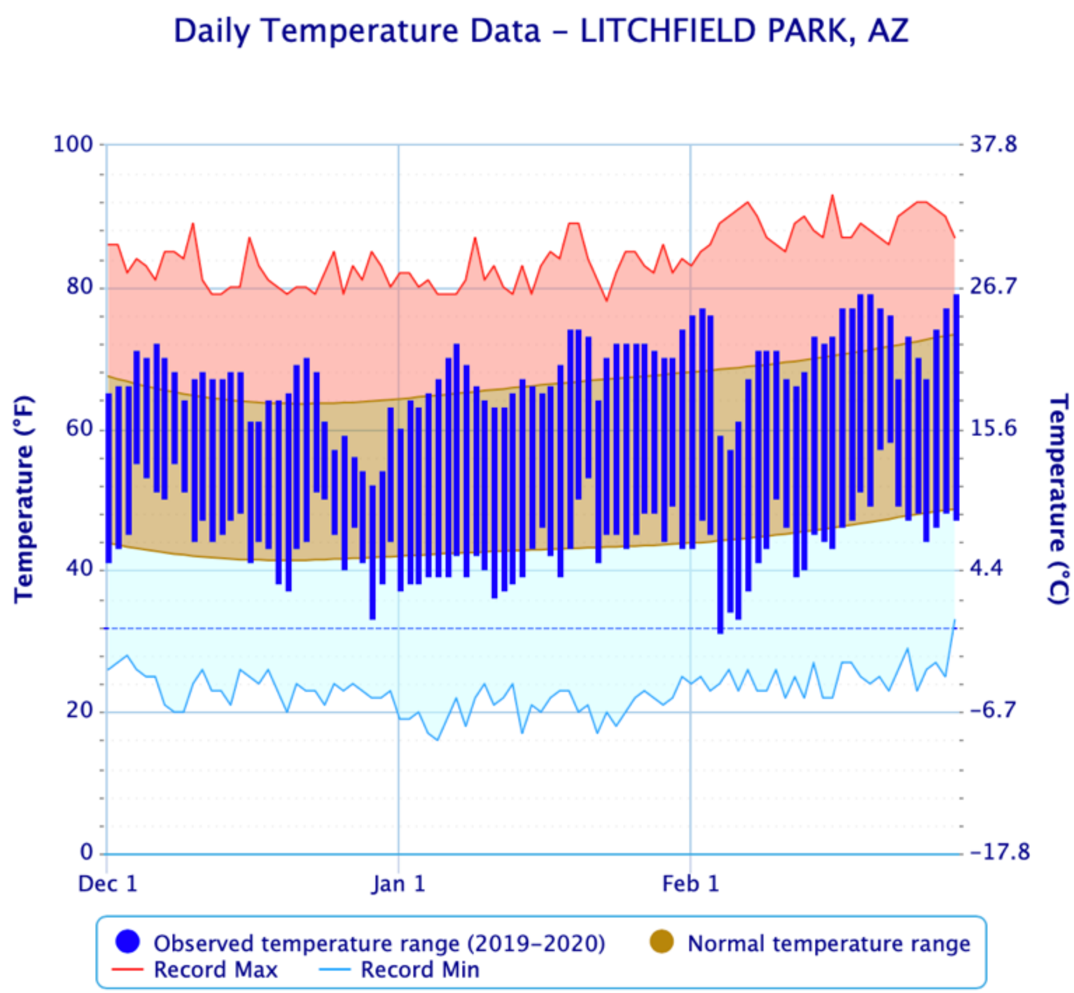
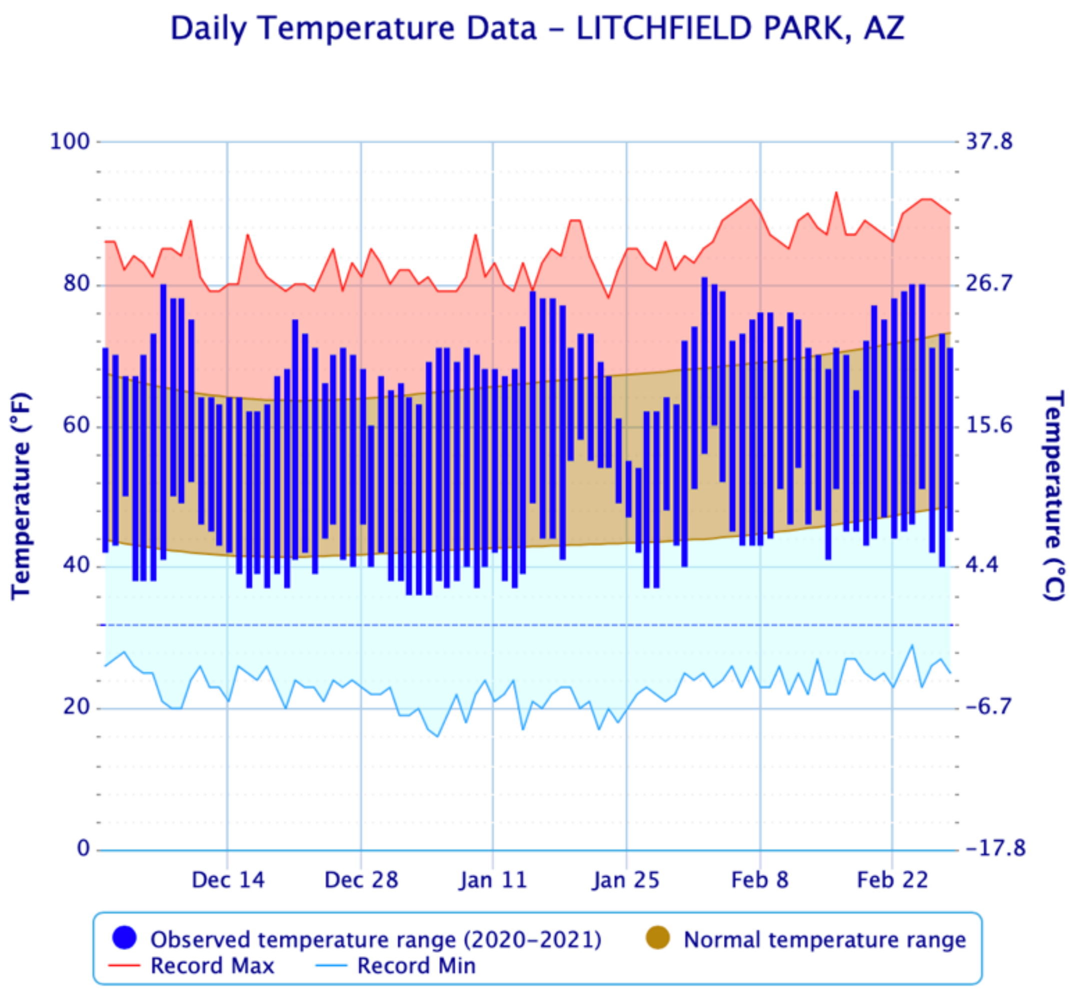


Figure 5. Winter temperatures in Litchfield Park, AZ in 2012-21(National Weather Service).



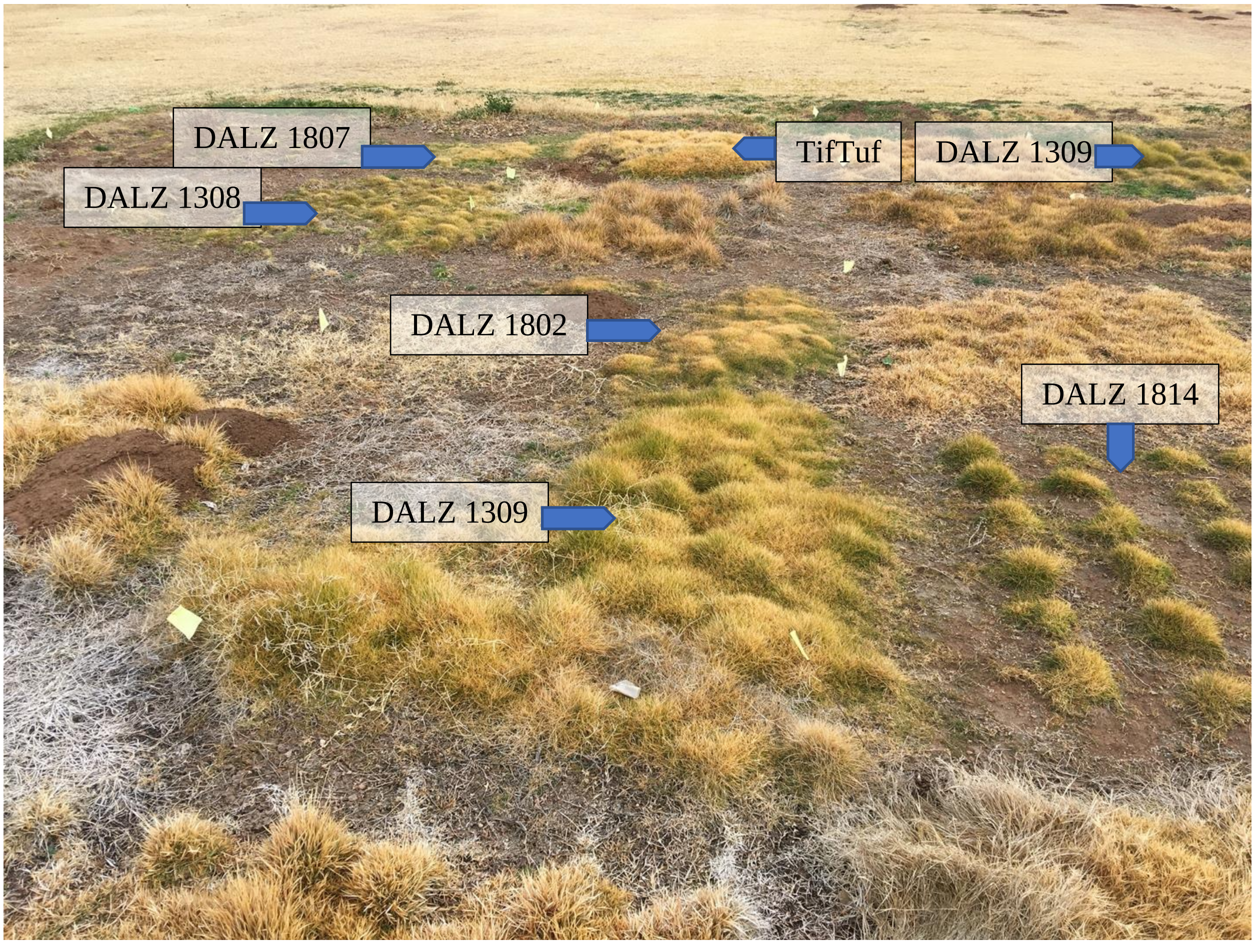


Figure 6. Green color retention of turfgrasses January 2021, Litchfield Park, AZ.

USGA ID#: 2020-19-724

Title: Evaluation of imazapic applications on fairway height cut Celebration bermudagrass for mowing reduction and turf quality effects

Project Leader: L.B. (Bert) McCarty

Affiliation: Clemson University, Clemson, SC

Objectives:
Plateau® herbicide is labelled for golf course turf and its use has potential benefit as an economical treatment for reducing mowing of bermudagrass roughs and fairways. Golf courses typically spend many labor hours mowing roughs and fairways. While trinexapac-ethyl is effective and its qualitative benefits are well researched, imazapic traditionally has not been promoted or sold by distributors except for treatment of native areas. Imazapic also lends additional benefit in regulating and reducing growth of weeds. Celebration bermudagrass is planted second to Tifway (419) in acreage and evaluation of this grass's tolerance to Plateau is warranted.

1) Qualitative effects, tolerance, of Plateau applications on Celebration bermudagrass
2) The rate range for tolerance
3) The extent and duration of regulation, reduction in clippings, reduction in mowing frequency
4) General weed effects whether growth regulation or control/decreased populations

Start Date: June 2021
Project Duration: 1 year
Total Funding: $6,000

Summary Points:
- Clipping weights were reduced similarly (~≥60%), regardless of rate with suppression duration being rate dependent (**Table 2**). This lasted for ~10 days at 1 oz/ac, 24 days for 2 oz/ac, and over 28 days for 4 oz/ac. Primo Maxx at 16 oz/ac provided similar reduction for ~24 days.
- Repeat applications 28 days after the initial extended clipping growth reduction through 21 days after the sequential one (**Table 2**).
- Turf color response to PGR applications was measured in two ways: (1) visual phytotoxicity (%); and (2) electronically via Normalized Difference Vegetation Index (NDVI) (0 to 1) ratings.
- Visual phytotoxicity for any treatment never exceeded the maximum level deemed acceptable (30%) (**Table 3**). Greatest phytotoxicity (~10%) followed the highest rate of Plateau examined (4 oz/ac) and lasted for 24 days. Approximately 8% phytotoxicity followed Primo Maxx at 16 oz/ac and lasted for ~10 days.
- Repeat applications caused turf phytotoxicity for the 4 oz/ac Plateau from 14 days (10%), to ~8% at day 21 and ~4% at day 28 following the sequential application.
- No differences in NDVI ratings occurred throughout the study for any product at any rate (**Table 4**).

Trial Summary
Plateau can be safely used on Celebration bermudagrass at rates up to 4 oz/ac. At the 4 oz/ac rate, some short-term phytotoxicity can be expected but appears transitory (**Figure 1**). Plateau applications reduced clipping weights by over 60% for all rates and the duration of reduction was highly rate dependent. Reduction lasted for 10 days at 1 oz/ac, 24 days for 2 oz/ac and >28 days for 4 oz/ac. Without a sequential application, a rebound shoot growth effect occurred for the 1 oz/ac at day 28. This research confirms field observations where Plateau is a very active PGR, with a general rule-of-thumb of ceasing shoot growth

for 7 to 10 days for each ounce of product applied per acre.

Table 1. Treatments, rates, and timings of Plateau (imazapic) and Primo MAXX (trinexapac-ethyl) on growth and turf tolerance of Celebration Bermudagrasses.

Treatments	Rate (Acre)	Remarks
untreated	---	---
Plateau 2L	1 oz	two applications, 28 days apart (June 28 fb July 26, 2021)
Plateau 2L	2 oz	two applications, 28 days apart (June 28 fb July 26, 2021)
Plateau 2L	4 oz	two applications, 28 days apart (June 28 fb July 26, 2021)
Primo MAXX 1L	16 oz	two applications, 28 days apart (June 28 fb July 26, 2021)

No weeds were in the trial plot area, thus objective 4 was not evaluated. Additional research is warranted to further confirm the trends observed. We appreciate support from the United States Golf Association in funding this trial and hope to use the data generated for future proposals.

Acknowledgements

This project was funded by the United States Golf Association. Special thanks to Timothy (Tee) Stoudemayer for assistance in trial organization and to Mike Echols at Clemson University Athletics for providing space to complete the trial.

Table 2. Celebration bermudagrass clipping weights (g/1.5 m^2) following Plateau and Primo MAXX use.

		Clipping Weights (g/1.5 m^2)			
Rating Date		Jul-8-2021	Jul-15-2021	Jul-22-2021	Jul-26-2021
Days After 1st/2nd Application		10, 10	17, 17	24, 24	28, 28
Treatment	**Rate**				
Untreated	---	12.460 a	5.145 a	4.115 a	2.785 b
Plateau	1 oz/a	5.463 b	2.970 ab	5.013 a	5.000 a
Plateau	2 oz/a	4.433 b	1.255 bc	2.350 b	3.000 b
Plateau	4 oz/a	2.395 b	0.408 c	1.610 b	2.463 b
Primo Maxx	16 oz/a	2.850 b	0.755 bc	1.698 b	1.790 b
LSD P=.05		4.682	2.321	1.710	1.626

Table 2 (cont).

Clipping Weights (g/1.5 m^2)				
Aug-2-2021	Aug-9-2021	Aug-16-2021	Aug-23-2021	Aug-30-2021
35, 7	42, 14	49, 21	56, 28	63, 35
2.468 a	4.338 a	3.148 ab	5.320 ns	4.795 ns
2.353 a	2.340 ab	3.638 a	8.305	8.950
0.950 b	0.943 b	1.198 bc	5.665	7.943
0.765 b	0.998 b	0.440 c	6.663	9.833
0.348 b	0.600 b	0.318 c	1.370	3.055
1.392	2.347	2.140	8.372	9.074

Table 3. Celebration bermudagrass Phytotoxicity (%) following Plateau and Primo MAXX use.

		Celebration Bermudagrass Phytotoxicity (%)			
Rating Date Days After 1st/2nd Application		Jul-8-2021 10, 10	Jul-15-2021 17, 17	Jul-22-2021 24, 24	Jul-26-2021 28, 28
Treatment	**Rate**				
Untreated	---	0.0 b	0.0 b	0.0 b	0.0 ns
Plateau	1 oz/a	0.0 b	0.0 b	0.0 b	0.0
Plateau	2 oz/a	2.5 b	2.5 b	2.5 b	0.0
Plateau	4 oz/a	8.8 a	8.8 a	8.8 a	2.5
Primo Maxx	16 oz/a	7.5 a	3.8 b	3.8 b	1.3
LSD P=.05		4.682	3.30	4.04	4.04

Table 3 (cont).

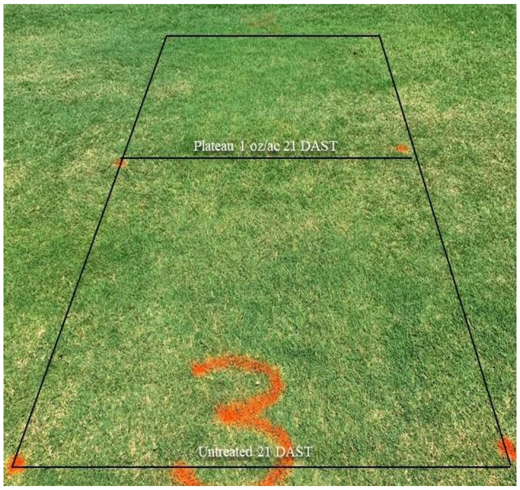
Celebration Bermudagrass Phytotoxicity (%)				
Aug-2-2021	Aug-9-2021	Aug-16-2021	Aug-23-2021	Aug-30-2021
35, 7	42, 14	49, 21	56, 28	63, 35
0.0 ns	0.0 b	0.0 b	0.0 b	0.0 ns
0.0	1.3 b	1.3 b	0.0 b	0.0
0.0	1.3 b	1.3 b	0.0 b	0.0
2.5	10.0 a	7.5 a	3.8 a	0.0
0.0	0.0 b	0.0 b	0.0 b	0.0
3.45	2.11	3.22	1.72	0.00

Table 4. Celebration bermudagrass Normalized Difference Vegetation Index (NDVI) ratings following Plateau and Primo MAXX use.

		NDVI (0 to 1)			
Rating Date Days After 1st/2nd Application		Jul-8-2021 10, 10	Jul-15-2021 17, 17	Jul-22-2021 24, 24	Jul-26-2021 28, 28
Treatment	**Rate**				
Untreated	---	0.5898 ns	0.6890 ns	0.6558 ns	0.6385 ns
Plateau	1 oz/a	0.6175	0.6833	0.7003	0.6850
Plateau	2 oz/a	0.5693	0.6895	0.6935	0.6730
Plateau	4 oz/a	0.6050	0.6640	0.6725	0.6628
Primo Maxx	16 oz/a	0.6283	0.6505	0.6578	0.6833
LSD P=.05		0.1029	0.0330	0.0498	0.0414

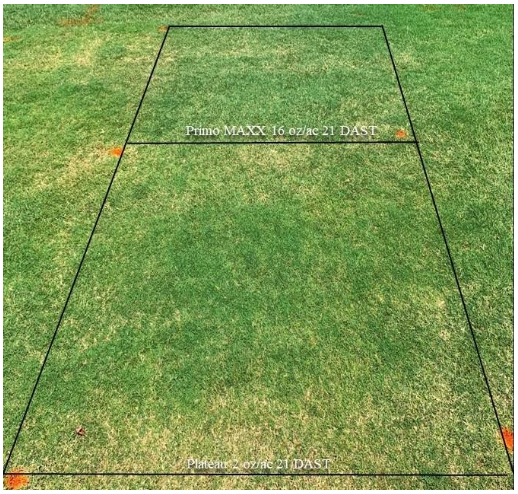
Table 4 (cont).

NDVI (0 to 1)				
Aug-2-2021	Aug-9-2021	Aug-16-2021	Aug-23-2021	Aug-30-2021
35, 7	42, 14	49, 21	56, 28	63, 35
0.6488 ns	0.6345 ns	0.6235 ns	0.6223 ns	0.6568 ns
0.6380	0.6640	0.6670	0.6953	0.6898
0.6280	0.6438	0.6810	0.6883	0.6963
0.6328	0.6088	0.6323	0.6918	0.7115
0.6140	0.6395	0.6238	0.6715	0.7020
0.0339	0.0498	0.0504	0.0744	0.0509



Untreated (bottom) vs. Plateau 1 oz/ac 21 DAST

Plateau 1 (bottom) vs. 4 oz/ac 21 DAST



Plateau 2 (bottom) vs. Primo 16 oz/ac 21 DAST

Plateau 4 (bottom) vs. 2 oz/ac 21 DAST

Primo 16 oz-ac (bottom) vs. Untreated 21 DAST

Figure 1. Celebration bermudagrass tolerance to various Plateau rates and Primo (16 oz/ac) 21 days after a sequential treatment (DAST).

USGA ID#: 2021-19-743

Title: NC1208 Dollar Spot Cultivar Project

Project Leaders: Paul Koch, Stacy Bonos, Nancy Dykema, Geunhwa Jung, John Kaminski, Megan Kennelly, Richard Latin, James Murphy, Joe Vargas

Affiliation: University of Wisconsin – Madison, Kansas State University, Michigan State University, Penn State University, Rutgers University, University of Massachusetts - Amherst

Objective: The primary goal of this project is to combine multiple cultural practices (dew removal and biocontrol) with varying levels of host resistance to determine the level of dollar spot suppression that can be achieved in the absence of fungicides.

Start Date: 2021
Project Duration: 3 years
Total Funding: $36,000

Summary Points:

- 'Coho' was selected as the highly dollar spot resistant cultivar, 'Shark' was selected as the moderately resistant cultivar, and 'Penncross' was selected as the susceptible cultivar
- The cultivars were planted in late summer or fall at the following institutions:
  - Kansas State University
  - Michigan State University
  - Penn State University
  - Rutgers University
  - University of Massachusetts – Amherst
  - University of Wisconsin – Madison
  - USGA Pinehurst Research Facility
- A mixture of host resistance, dew removal, and biocontrol will be tested using a split-split plot design for their combined control of dollar spot beginning in 2022.

Summary Text:
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.

In this study we will compare 3 levels of host resistance (high, medium, low) with daily dew removal and the use of biocontrols for their ability to suppress dollar spot at seven different institutions around the country. The experimental design will be a split-split plot with cultivar as the main plot and dew removal and biocontrol as the subplots. 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. All research sites were seeded in the late summer or early fall of 2021, and the expectation is that treatments will be initiated in the spring of 2022.

Figure 1. Research plot shortly after seeding on August 5th, 2021 at the OJ Noer Turfgrass Research Facility in Madison, WI.

Figure 2. Research plot demonstrating good establishment on September 30th, 2021 in Pinehurst, NC.

USGA ID#: 2021-17-741

Title: Golf ball reaction and soil strength study

Project Leader: John N. Rogers III, Thomas O. Green, James R. Crum, Jackie Guevara

Affiliation: Michigan State University

Objectives: Determine golf ball reaction from simulated inbound golf shots on various rootzone mixtures, turfgrass species, and mowing heights

Start Date: 2021

Project Duration: 2-year potential

Total Funding: $17,400 - year 1

Summary Points:

- In both trial 1 and 2, the 9% silt + clay was observed to have the lowest (firmer) TruFirm response when compared to TDS 2150 and 15% silt + clay.
- The golf ball bounced and rolled the furthest on the control (#28 sand) and 7% silt +clay plots when compared to TDS 2150 and 15% silt + clay.
- The deepest pitch marks were observed on the TDS 2150 and 15% silt + clay plots.
- The 15% silt + clay rootzone plots had the highest soil moisture compared to other rootzone mixtures.

Summary Text: Golf course playability and the golfer experience improves when golfers are able to bounce their golf shots up onto putting greens and when golf shots bounce and roll from tee shots. While soil moisture and surface organic matter levels influence inbound golf ball reaction, the rootzone components also play a role; one that is not well understood in the golf industry. For over half a century golf courses have enjoyed the benefits of sand topdressing fairways/approaches or even placing a sand “cap” over poorly drained soils to yield drier conditions. However, field observations have determined that such a scenario does not necessarily (and even unlikely) yield a firm surface that delivers good golf ball reaction (bounce and roll). We would like to offer courses better guidance on how to improve fairway/approach drainage characteristics, offer a rootzone with adequate aeration porosity and yet still deliver a firm, stable surface that yields good ball reaction. More so this study may give insight on improving golf shots by allowing golfers the option of bouncing shots to gain distance while placing them nearer target putting green. The best soil and turfgrass combinations will be assessed with a golf shot simulator and TruFirm apparatus provided by the United States Golf Association.

The materials and methods of the study were the following: seven rootzone mixtures (Table. 1), two turf species (80/20 *Poa pratensis*, Kentucky bluegrass and *Lolium perenne*, perennial ryegrass mix in 2021; *Agrostis stolonifera*, creeping bentgrass in year two, 2022), and two

heights of cut (Kentucky bluegrass/perennial ryegrass mix at 0.600 inches in 2021; creeping bentgrass at ~0.500 inches in 2022), and three replications. The experimental site was constructed in the summer of 2000 to assess the effects of natural and artificial soil enhancements on strength of an athletic field rootzone when subjected to simulated traffic. To prepare for the golf ball reaction study, the site was renovated in the summer of 2020 and reseeded with an 80/20 Kentucky bluegrass and perennial ryegrass mix and has since been maintained as a simulated golf course fairway. Treatment plot dimensions were 10 feet by 16 feet.

Surface firmness was assessed with a USGA TruFirm apparatus while ball roll/bounce reaction was assessed using a modified pitching machine at setting #12 to simulate launch conditions for a 6-iron (Fig. 1). The simulated 6-iron launches were achieved by setting the machine to test parameter #12 with the nominal approach angle of 46°, speed of 84, spin of 100 where the top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5. More so the ball pitch mark depth was measured after each ball launch series (3 balls) while a time domain reflectometry device TDR 300 (Spectrum Technologies, Aurora, IL) was used to collect volumetric water content in each treatment plot. The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline assessed on control plot with #28 sand rootzone).

More so when analyzing ball reaction response, data results show significant differences among rootzone mixtures and collection dates (Table 2). The procedure was to record the aggregate bounce and roll (feet) of a simulated 6-iron approach shot from a modified pitching machine with three ball launches per plot**.** In both trial 1 and 2, the aggregate ball reaction response increased toward the end of the data collection period as soil moisture levels decreased (Table 3). The golf ball bounced and rolled the furthest when launched on to the control (#28 sand) and 7% silt +clay plots when compared to TDS 2150 and 15% silt + clay (Table 4).

Data results also show significant differences among the rootzones and collection dates when analyzing pitch mark depth response (Table 2). When analyzing the effect of collection date, pitch marks became less evident as soil moisture levels decreased as the trial progressed (Table 3). The deepest pitch marks were observed on the TDS 2150 and 15% silt + clay plots (Table 4). Furthermore, the trial period significantly affected the pitch mark depth response where data suggests that trial 1 had deeper pitch marks compared to trial 2 (Table 5).

When analyzing volumetric water content response, results show significant differences among the rootzones, collection date, and trial period (Table 2). The dry-down period significantly affected volumetric water content (Table 3). The 15% silt + clay rootzone plots had the highest soil moisture compared to other rootzone mixtures (Table 4). More so trial period significantly affected volumetric water content response where data suggests that trial 1 was observed to have higher soil moisture compared to trial 2 (Table 5).

The data results show significant differences among the rootzone mixtures when observing TruFirm response (Table 6). The testing procedure included recording the average index, based on a 0.000 to 1.000 scale, produced by four missile drops per treatment plot where higher numbers are indicative of less firm surfaces. In both trial 1 and 2, the rootzone with 9% silt + clay was observed to have the firmest surface response when compared to TDS 2150 and 15% silt + clay (Table 7).

Future expectations: The project should study golf ball reaction and soil strength in a creeping bentgrass fairway. It has been proposed that the site at East Lansing, MI will be renovated into a creeping bentgrass surface in 2022.

Table 1. A list of different rootzone mixtures used in the treatment plots to study golf ball reaction and soil strength in 2021 at East Lansing, MI.

Treatment	Percent retained [z]							
	Size class (mm)							
	FG (2.0)	VCoS (1.0)	CS (0.5)	MS (0.25)	FS (0.1)	VFS (0.05)	Silt (0.002)	Clay (<0.002)
TDS 2150 sand	0.0	0.0	2.4	64.0	32.9	0.1	0.5	0.0
#28 sand	1.4	13.7	26.0	42.5	14.1	0.4	1.0	0.9
7% silt + clay [y]	1.5	13.0	22.4	38.5	15.9	1.7	3.9	3.1
9% silt + clay	1.5	12.2	21.6	36.6	16.5	2.3	5.7	3.6
15% silt + clay	1.1	10.2	18.9	33.5	17.5	3.7	10.6	4.4
Profile [x]	1.0	11.2	24.8	44.8	14.1	0.8	1.6	1.8
ZeoPro [w]	0.5	9.6	25.0	43.4	17.8	0.8	2.0	0.9

[z] The retained percentage is the weight of soil particles in each size class defined by the United States Department of Agriculture (USDA) as being fine gravel (FG), very coarse sand (VCoS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS), silt and clay.

[y] Well-graded sand (#28) was used as a base and mixed on a volume basis with a sandy loam soil to produce the sand-soil mixes.

[x] Profile rootzone mixture consisted of 75% well-graded sand (#28), 20% porous ceramic product manufactured from illite clay and amorphous silica, and 5% sphagnum peat by volume.

[w] ZeoPro rootzone mixture consisted of 80% well-graded sand (#28), 10% clinoptilolite, and 10% sphagnum peat by volume.

The treatment plots were irrigated to field capacity and allowed to dry down to near wilting point; soil moisture thresholds were assessed using the control plot with #28 sand rootzone.

The study site at East Lansing, MI was first constructed in the summer of 2000 as a simulated athletic field plotted with different rootzone mixtures. It was renovated in the summer of 2020 as a simulated golf fairway plotted with different rootzone mixtures.

Table 2. Analysis of variance results for ball reaction, pitch mark depth, and volumetric water content in trial 1 and 2 in 2021 at East Lansing, MI.

Source of variation	df	Ball reaction (feet) [z]		Pitch mark depth (inch) [y]		Volumetric water content (%) [x]	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
		P > F		*P > F*		*P > F*	
Days after irrigation (D)	4	0.0012*	0.0008	0.0012*	0.0005	0.0009*	<0.0001
Treatment (T)	6	<0.0001	<0.0001	0.0016	<0.0001	<0.0001	<0.0001
D*T	24	0.97	0.98	0.77	0.87	0.99	0.99
Trial (TR)	1	0.19		0.0024		0.04	
T*TR	6	0.14		0.51		0.83	
D*T*TR	54	0.99		0.88		0.99	

[z] Ball reaction test response was the recorded measurement (feet) of the aggregate bounce and roll of a simulated 6-iron approach shot from a modified pitching machine; the test procedure included three ball launches per plot.

[y] Pitch mark depth was the recorded measurement (inch) of the average depth created by simulated 6-iron approach shots from a modified pitching machine; the test procedure included three ball launches per plot.

[x] Soil moisture response was the recorded measurement (%) of the average volumetric water content of the treatment plot; the test procedure included using a time domain reflectometry apparatus TDR 300 from Spectrum Technologies, Aurora, IL.

The study site was a simulated golf fairway plotted with different rootzone mixtures.

The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).

The golf shots were simulated 6-iron launches from a USGA modified pitching machine at setting #12 with a nominal approach angle of 46°, speed of 84, spin of 100. The top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5.

* Mixed factor effects ANOVA (analysis of variance) model in SAS (version 9.4; SAS Institute, Cary, NC) at $P \leq 0.05$).

Table 3. Effect of soil moisture dry-down period on ball reaction, pitch mark depth, and volumetric water content in 2021 at East Lansing, MI.

Days after irrigation	Ball reaction (feet) [z]		Pitch mark depth (inch) [y]		Volumetric water content (%) [x]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	5.8 c	9.4 c	0.60 a	0.49 a	40.3 a	36.1 a
1	10.0 b	10.4 c	0.63 a	0.48 ab	37.3 ab	32.9 ab
2	7.7 bc	12.4 bc	0.60 a	0.43 ab	35.9 bc	32.0 b
3	11.6 b	16.4 ab	0.55 a	0.41 b	33.3 cd	26.9 c
4	15.9 a	18.0 a	0.46 b	0.29 c	32.0 d	24.5 c
LSD	4.0	4.4	0.08	0.07	3.5	3.7

[z] Ball reaction test response was the recorded measurement (feet) of the aggregate bounce and roll of a simulated 6-iron approach shot from a modified pitching machine; the test procedure included three ball launches per plot.

[y] Pitch mark depth was the recorded measurement (inch) of the average depth created by simulated 6-iron approach shots from a modified pitching machine; the test procedure included three ball launches per plot.

[x] Soil moisture response was the recorded measurement (%) of the average volumetric water content of the treatment plot; the test procedure included using a time domain reflectometry apparatus TDR 300 from Spectrum Technologies, Aurora, IL.

Golf shots were simulated 6-iron launches from a USGA modified pitching machine at setting #12 with a nominal approach angle of 46°, speed of 84, spin of 100. The top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5.

The study site was a simulated golf fairway plotted with different rootzone mixtures.

The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).

Mean values within columns separated according to Fisher's least significant difference (LSD) at P = 0.05.

Table 4. Effect of various rootzone mixtures on ball reaction, pitch mark depth, and volumetric water content in 2021 at East Lansing, MI.

Treatment	Ball reaction (feet) [z]		Pitch mark depth (inch) [y]		Volumetric water content (%) [x]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TDS 2150 sand	3.8 d	4.3 c	0.62 a	0.54 a	38.6 b	35.3 b
Control (#28 sand)	19.6 a	19.0 a	0.43 c	0.31 d	25.5 d	21.5 d
7% silt + clay	12.2 b	16.5 a	0.55 ab	0.38 cd	33.3 c	27.8 c
9% silt + clay	11.0 bc	13.8 ab	0.51 bc	0.39 cd	34.5 bc	27.9 c
15% silt + clay	6.4 cd	10.5 b	0.65 a	0.51 ab	47.4 a	40.5 a
Profile	12.0 b	13.9 ab	0.60 ab	0.44 bc	35.3 bc	30.6 c
ZeoPro	6.5 cd	15.4 ab	0.61 a	0.38 cd	35.9 bc	29.9 c
LSD	4.7	5.3	0.18	0.08	4.1	4.4

[z] Ball reaction test response was the recorded measurement (feet) of the aggregate bounce and roll of a simulated 6-iron approach shot from a modified pitching machine; the test procedure included three ball launches per plot.
[y] Pitch mark depth was the recorded measurement (inch) of the average depth created by simulated 6-iron approach shots from a modified pitching machine; the test procedure included three ball launches per plot.
[x] Soil moisture response was the recorded measurement (%) of the average volumetric water content of the treatment plot; the test procedure included using a time domain reflectometry apparatus TDR 300 from Spectrum Technologies, Aurora, IL.
Golf shots were simulated 6-iron launches from a USGA modified pitching machine at setting #12 with a nominal approach angle of 46°, speed of 84, spin of 100. The top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5.
The study site was a simulated golf fairway plotted with different rootzone mixtures.
The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).
Mean values within columns separated according to Fisher's least significant difference (LSD) at P = 0.05.

Table 5. Effect of trial on pitch mark depth and volumetric water content in 2021 at East Lansing, MI.

Trial	Pitch mark depth (inch) [z]	Volumetric water content (%) [x]
1	0.57	35.8
2	0.42	30.5
LSD	0.03	1.6

[z] Pitch mark depth was the recorded measurement (inch) of the average depth created by simulated 6-iron approach shots from a modified pitching machine; the test procedure included three ball launches per plot.
[x] Soil moisture response was the recorded measurement (%) of the average volumetric water content of the treatment plot; the test procedure included using a time domain reflectometry apparatus TDR 300 from Spectrum Technologies, Aurora, IL.
Golf shots were simulated 6-iron launches from a USGA modified pitching machine at setting #12 with a nominal approach angle of 46°, speed of 84, spin of 100. The top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5.
The study site was a simulated golf fairway plotted with different rootzone mixtures.
The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).
Mean values within columns separated according to Fisher's least significant difference (LSD) at P = 0.05.

Table 6. Analysis of variance results for TruFirm response in trial 1 and 2 in 2021 at East Lansing, MI.

		Trial 1	Trial 2
Source of variation	**df**	***P > F***	
Days after irrigation (D)	4	0.06*	0.83
Treatment (T)	6	<0.0001	<0.0001
D*T	24	0.87	0.93
Trial (TR)	1	0.07	
T*TR	6	0.29	
D*T*TR	54	0.97	

The study site was a simulated golf fairway plotted with different rootzone mixtures.
The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).
The TruFirm apparatus was provided by the USGA; the test procedure included 4 missile drops per plot and recorded on a 0.000 to 1.000 index.
*** Mixed factor effects ANOVA (analysis of variance) model in SAS (version 9.4; SAS Institute, Cary, NC) at $P \leq 0.05$).**

Table 7. Effect of various rootzone mixtures on TruFirm response in 2021 at East Lansing, MI.

Treatment	TruFirm [z]	
	Trial 1	Trial 2
TDS 2150 sand	0.627 a	0.629 a
Control (#28 sand)	0.569 bc	0.544 cd
7% silt + clay	0.569 bc	0.574 bc
9% silt + clay	0.546 c	0.524 d
15% silt + clay	0.617 a	0.584 b
Profile	0.558 bc	0.561 bc
ZeoPro	0.581 b	0.557 bc
LSD	0.03	0.03

[z] TruFirm response based on a 0.000 to 1.000 index; the test procedure included recording the average index of four missile drops per treatment plot.
The study site was a simulated golf fairway plotted with different rootzone mixtures.
The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).
Mean values within columns separated according to Fisher's least significant difference (LSD) at P = 0.05.

Figure 1. A photograph showing the test apparatus that was used in the golf ball reaction and soil strength study in 2021 at East Lansing, MI Golf shots were simulated 6-iron launches from a USGA modified pitching machine at setting #12 with a nominal approach angle of 46°, speed of 84, spin of 100. The top wheel control dial was set at 2.5, and the bottom wheel control dial at 8.5. The ball reaction test response was the recorded measurement (feet) of the aggregate bounce and roll of a simulated 6-iron approach shot from a modified pitching machine; the test procedure included three ball launches per plot.
The study site was a simulated golf fairway plotted with different rootzone mixtures.
The study was initiated on 3 September (trial 1) and replicated 15 September (trial 2) with five collection dates per trial where treatment plots were irrigated to field capacity and allowed to dry down to near wilting point (baseline was assessed on control plot with #28 sand rootzone).

USGA ID#: 2021-01-725

TITLE: Revision, promotion and funding of the National Turfgrass Research Initiative (NTRI)

PROJECT LEADER:

Kevin Morris, President
National Turfgrass Federation (NTF)
P. O. Box 106
Beltsville, MD 20704

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 TEXT:

Turfgrass is an estimated $60 billion, 60 million-acre industry in the U.S., making turfgrass the third largest agricultural crop in the U.S. by acreage. However, the turfgrass industry faces serious challenges such as water shortages, concerns about pesticide use, fertilizer restrictions and economic issues. Research is needed to help golf overcome these challenges and thrive over the next 25-30 years, but *recurring* federal government turfgrass research funding falls far below research funding for other comparably sized agricultural industries, averaging less than $1,000,000 annually. The National Turfgrass Research Initiative (NTRI) was developed in 2004 as a joint strategic plan between turfgrass industry stakeholders and USDA-ARS to document research needs and help secure funding. NTRI now needs updating, refocusing and subsequently, additional funding.

National Turfgrass Federation (NTF) efforts have led to a recurring Congressional increase of $3,000,000 for turfgrass research within USDA-ARS (enacted in December 2019 for Fiscal Year 2020), the first step in confronting the golf industry's needs. The ARS funding will build on the existing ARS turfgrass effort by adding federal scientists and staff, focusing on long-term genomics, water conservation and ecosystem services studies.

To further these efforts and build on our success, this project addresses turfgrass' biggest challenges by 1) surveying the entire turfgrass industry to document its size and scope, 2) identifying, prioritizing and documenting national research needs, 3) seeking more federal recognition and support of turfgrass research to address prioritized needs, 4) seeking non-traditional federal and non-governmental organization (NGO) funding sources for research, and 5) publicizing this effort and successes to engage stakeholders and inform the public. These steps, taken in conjunction with the research conducted by USDA-ARS over the next several years, will have a significant positive impact on solving the challenges faced by the turfgrass industry.

Progress to Date

A 2017 turfgrass stakeholder workshop developed a priority list of research and resulted in USDA-ARS committing new funding in FY19 ($225,000) to sequence the genomes of turfgrasses at several locations. The ARS researchers have now organized into a 'Turfgrass Consortium', which allows them to collaborate and develop their research plans. Work on several turfgrass species will likely result in new genomes sequenced within the next 12-18 months. This funding will contribute foundational information to aid the development of improved heat, cold, drought, disease and insect resistant grasses.

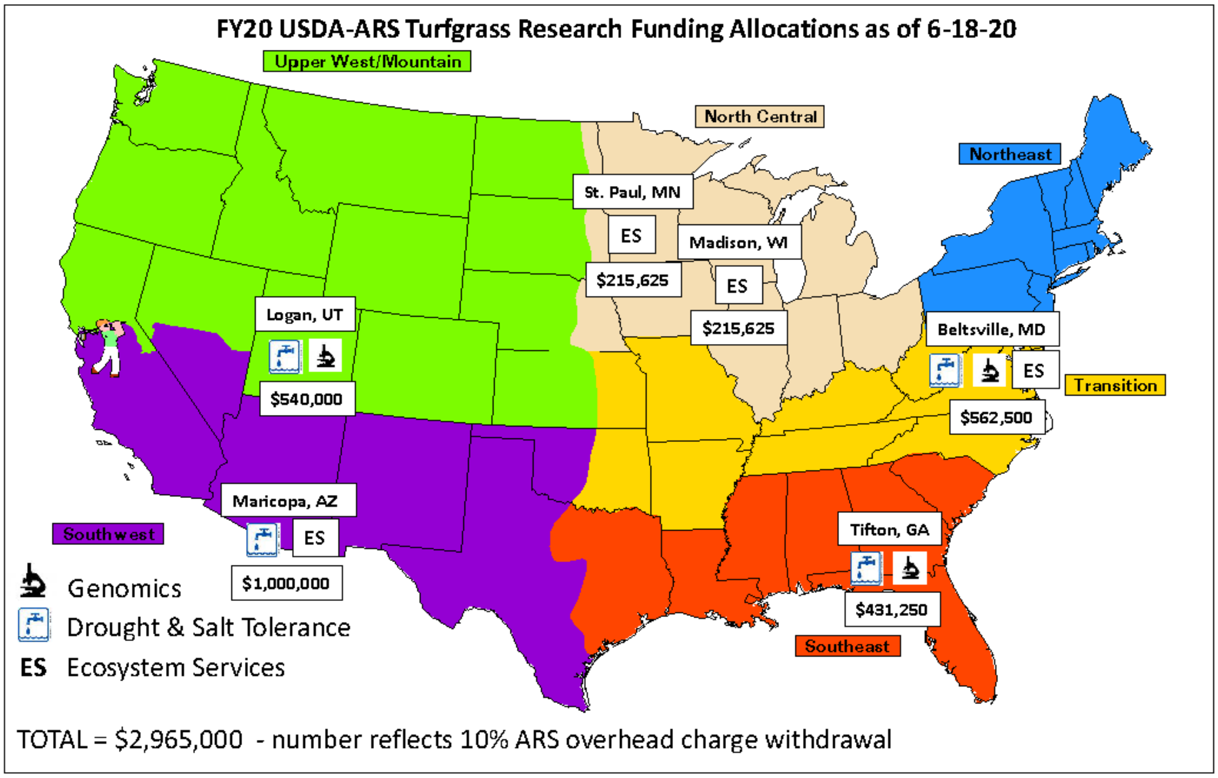


Figure 1. Fiscal USDA-ARS turfgrass research funding allocations as of June 18, 2020.

The ARS federal funding initiated in December 2019 was discussed with ARS National Program Staff in early 2020 and allocated to six locations (see above map). Due to COVID, these processes were delayed, however, NTF worked with ARS to develop position descriptions, advertise candidates, conduct interviews online and fill four new, permanent turfgrass scientist positions in 2020/2021. The hired researchers include a geneticist and an agronomist at Maricopa, AZ working on developing improved water conserving germplasm as well as management systems for the desert southwest U.S., a bioinformaticist at Logan, UT to analyze genetic information generated by ARS geneticists, and a geneticist at Beltsville, MD working on water saving and sustainable germplasm for the eastern U.S. These scientists, as well as researchers at St. Paul, MN, Madison, WI and Tifton, GA will conduct extensive, long-term research on turfgrass genomics, water conservation and ecosystem service maximization.

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 Turfgrass Stakeholder Summit II. Due to COVID, the in-person event was rescheduled from March 2020 and delivered virtually in October. Approximately seventy summit participants represented various segments of the turf industry, including golf, lawn care, seed/sod, plant protectants, irrigation, equipment, research and others. Leaders from these various segments delivered updates on their research needs. Participants then broke into groups to identify priority research needs, and finally came back together as a virtual group to finalize those needs.

The presentations, needs prioritization and a summary can be found here:
https://www.nationalturfgrassresearchinitiative.info/.

The summit also served as a ‘Convening Event’ for FFAR, which is charged by Congress to develop and fund innovative research programs utilizing a 1:1 match of dollars from Congress and industry. The ‘Convening Event’ identified potential research topics, of which FFAR and the turfgrass industry can develop into cooperative, unique programs using the 1:1 funding match.

The number one priority item determined by Stakeholder Summit II participants was the need for a National Turfgrass Survey, which will not only document acreage, scope and economic value of this crop, but also justify the need for increased federal research funding. We recently 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. If this project is funded, we will develop baseline figures on the size, scope, value and impact of the turfgrass industry in the U.S. Also, if this funding is awarded, we will ask Congress to direct the USDA, National Agricultural Statistics Service (NASS) to conduct follow-up surveys of the industry every five years.

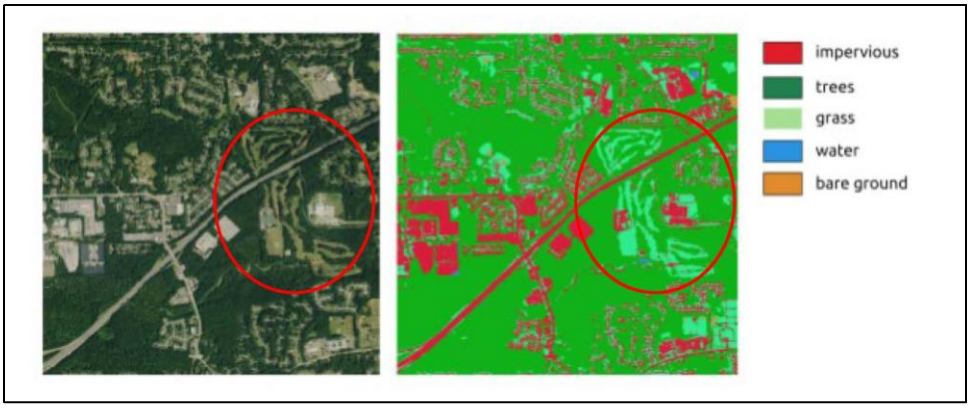


Figure 2. West Atlanta suburb NAIP orthoimage and labels at 60cm resolution. buildings, roads, lawns and sports fields are easily distinguished. The light green areas in the red ovals (Figure 1 above) are a golf course. Note how the labeled image on the right side of figure 1 identifies the grass of the golf course.

Due to the funding allocated to USDA-ARS in late 2019, we spent considerable time in 2020 and 2021 selecting and interviewing scientists, while other ARS turfgrass research was initiated. We therefore decided not to request additional ARS funding until we can show results from the initial $3,000,000. Thus, we focused our efforts on the National Park Service (NPS) for funding.

NPS is a well-known, well respected federal agency that most Americans recognize and are now encountering regularly (national park visitation is at record levels during the pandemic). We feel that encouraging and aiding turfgrass improvement at highly visible NPS sites, especially those at crowded, damaged locations will raise the awareness of functional turf’s importance among the general public. To that end, after finally locating the proper NPS official, we developed a draft proposal for consideration. The proposal includes funding for improvement of NPS turfgrass sites with training, education, equipment, resources and research. Since we missed the 2021 appropriation request deadline by the time we finalized this proposal, we plan on submitting this effort for funding in FY22.

In addition to contacting the National Park Service, we have been in contact with Department of Defense research program leaders, as well as the American Association of State Highway Transportation

Officials (AASHTO). These groups and others may be possible sources for future turfgrass research funding.

Future Plans

The promotion of NTRI via social media, web site and other means will start in 2022. The purpose of this effort is to educate turfgrass industry members on the success and importance of federal turfgrass research. Industry involvement is essential for future advocacy and funding, particularly when millions of taxpayer dollars are being requested. To further support this effort, NTRI will updated with the new priorities identified in Turfgrass Stakeholder Summit II and the NTRI web site (and possibly NTF web site) will be updated to provide this new information.

In 2022, we will further discussions with House and Senate Agriculture staff concerning the 2023 Farm Bill. At this point, we are considering two major requests: first, recognition of the need, and recurring funding for a regular national turfgrass survey conducted by USDA-NASS. This will hopefully follow a successful survey conducted via the USDA-AMS project funding and will allow these surveys to be conducted in perpetuity. Secondly, funding authorization for a significant competitive turfgrass research program in the Farm Bill is essential. *Authorization* is needed before an *appropriation* request can be submitted, and subsequently funded. Federal appropriation of competitive research dollars specific for turfgrass is the ultimate goal of this project.

SUMMARY POINTS

- With the help of NTF and new Congressional funding, four new, permanent turfgrass scientists were hired by the USDA-ARS in 2020 and 2021, working on turfgrass genomics, water conservation and ecosystem service maximization.
- A National Turfgrass Survey grant proposal was developed and submitted to the USDA-AMS for funding consideration.
- Turfgrass Stakeholder Summit II was held in fall 2020 and resulted in potential funding opportunities between the turfgrass industry and the Foundation for Food and Agriculture Research (FFAR).
- Contacts were made, and a proposal developed to improve National Park Service turf sites with equipment, resources, training and research. NPS is a visible and well-respected federal agency that can champion the need for quality, functional turfgrass in high traffic areas.
- Plans are being made for Congressional support of turfgrass specific research and survey needs in the next Farm Bill.

USGA ID#: 2019-05-675

Title: Native Grasses and Alternative Groundcovers for the Southwest

Project Leader: Kai Umeda and Worku Burayu

Affiliation: University of Arizona

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

Start Date: 2019
Project Duration: 3 years
Total Funding: $45,000
Report Type: final report (3rd yr. 2021)

Summary Points: -
- Both white and pink cultivars of Kurapia (*Lippia nodiflora* L.) remained green throughout the year exhibiting acceptable quality.
- Kurapia grew successfully with greater than 40% of the irrigation rate needed for bermudagrass.
- Nine of the ten nativegrasses performed and demonstrated acceptable quality. *Schizachyrium scoparium* (little bluestem) did not establish an adequate stand.
- ➢ *Aristida purpurea* (purple threeawn), *Sporobolus aeroides* (alkali sacaton), *Eragrostis trichodes* (sand lovegrass), and *Bouteloua gracilis* (blue grama) remained green throughout the year and exhibited acceptable quality and maintained greenness.

Summary Text
Golf course superintendents and landscapers of residential and commercial properties are facing increased pressures from regulatory restrictions to reduce water use while maintaining turfgrass quality. Hence, the necessity and demand for seeking appropriate alternative plant materials to satisfy the landscaping needs of the southwest United States is increasing. To address these demands, many superintendents are interested in using alternative plant species that perform well under low-input management. However, there are significant factors associated with the adaptation and establishment of alternative grass species. To alleviate these concerns, The University of Arizona Cooperative Extension Turfgrass Science program in Maricopa County initiated an evaluation for adaptation and performance of nativegrasses and alternative groundcovers under low water use conditions. Our initial three years (2016-2018) of research identified prospective nativegrasses and a promising new groundcover for establishment and adaptation under irrigated

low desert Arizona conditions. To obtain more conclusive results and to apply specific recommendations for best management practices, our current research includes ten nativegrasses and two cultivars of a groundcover, Kurapia. The priority for the current project is to evaluate and determine the performance of nativegrasses under a nearly natural water-use setting and the alternative groundcover at various rates of water applied by surface drip irrigation.

Two experiments, one for nativegrasses and one for the groundcover were initiated in 2019 at the Wigwam Golf Club in Litchfield Park, AZ. In the first field experiment, each nativegrass species was seeded into 8 ft by 8 ft plots arranged in a randomized complete block design (RCBD) with four replicates (Table 1 and Figure 1). The nativegrasses were regularly irrigated with the existing overhead irrigation system during the first two years and then limited to the natural rainfall in the final year, 2021. The second experiment consisted of two Kurapia cultivars, white and pink flowered, established with four replications in a RBCD. The surface drip irrigation applications of water for the two cultivars were evaluated and compared under three levels of 80, 40, and 20% relative to bermudagrass irrigation. Nine emitters were spaced approximately 2 ft apart per plot area. The overall visual quality was evaluated for greenness and spreading to provide ground surface cover using the procedures developed by National Turfgrass Evaluation Program, where 1 is brown and 9 is dark green. Digital estimates of percent greenness were taken using a mobile phone app, Canopeo®. A single-lens mobile-phone camera (focal length equivalence ~26 mm) was held ~1 m (3 feet) above the canopy and pictures were taken straight down between 8 am and 11 am on clear days. Percentage of green canopy cover values obtained using the Canopeo® app were also compared with visual estimates. Data were analyzed using JMP ver. 14.3 statistical software and means compared using Student's t-test. The project's ultimate goal is to provide the professionals of the Arizona green industry and golf courses with specific recommendations for best management practices for Kurapia and nativegrass species.

Results

This investigation revealed that when mowed twice during Year 2, the nativegrasses; *Sporobolus airoides* (alkali sacaton), *Eragrostis trichodes* (sand lovegrass), *Aristida purpurea* (purple threeawn), and *Bouteloua gracilis* (blue grama) grew as year-round green grasses under moderate winter temperatures when there was no hard frost occurrence (Figures 4 & 5). During the summer 2021, all the species of grasses demonstrated full growth from tillering to flowering when natural rainfall occurred during the previous winter and during the summer monsoon. Rainfall was 1.74 inches from December 2020 to March 2021 and 5.76 inches during July to September 2021.

In the first year (2019 report), both white and pink flowered cultivars of Kurapia established very well and covered 98% (white flower variety) and 72% (pink flower variety) of the soil surface of the plots under optimum regular overhead sprinkler irrigation from May to October 2019. The white cultivar Kurapia spread more rapidly across the surface area and covered the plot within a shorter time but exhibited shorter height compared to the pink variety. In Year 2 and 3, from May through summer, Kurapia's specific water requirements was determined for acceptable quality (color, surface coverage, uniformity, and flowering) at varying irrigation regimes (20, 40, and 80% relative to bermudagrass). Results showed that irrigating at the 20% drip irrigation level negatively affected the greenness color, the surface area coverage, and uniform growth of Kurapia during the May to October 2020 growing period (Figure 2). The percentage difference in these ratings was

significant among treatments and compromised the overall health, vigor, and appearance of Kurapia at the low 20% rate of application. Flower shedding was also significantly greater and more rapid at a 20% deficit irrigation level compared to 40 and 80% drip irrigation levels (Table 2 and Figure 3). Flower shedding is usually caused by stress such as insufficient or irregular/infrequent irrigation. Kurapia has a characteristic white or pink flower that look like clover flowers, only more globular-shaped. It's very attractive to pollinators and beneficial insects, that contribute to better environmental health and local ecology without requiring much water. If flowers and bees are not desirable, mowing and reducing water can suppress flowering. Both cultivars of Kurapia showed adaptability to varied irrigation levels. No significant difference was observed between the two cultivars of Kurapia for greenness, surface area coverage, and uniformity under 40 and 80% rates of drip irrigation. After establishment, Kurapia could be irrigated at an equivalent 40% level, as additional water does not contribute a significant gain in appearance or rate of growth. The drip irrigation used at the site was in a lighter textured soil and less than an equivalent rate of 40% may work in heavier textured soil. Visual estimates of greenness significantly correlated to Canopeo® digital estimates of percent greenness ($Y = -8.134 + 1.939*X$; $R^2 = 0.92$; $P < 0.01$) (Table 2 and Figure 3). This indicates that the use of digital estimates of percent greenness by means of a mobile phone Canopeo® application has practical utility and can be used to estimate greenness and surface area coverage for Kurapia and grasses.
The white flowered Kurapia tended to visually demonstrate better qualities than the pink flowered cultivar, especially with respect to flowering and the ability to provide uniform ground surface coverage. In June 2021, generally the hottest and driest month of the year, the irrigation levels of 40 and 80% of water applied to bermudagrass were similar and significantly better than when only 20% was applied. The pink flowered Kurapia demonstrated more stress symptoms with less water as it was less green, did not spread uniformly, and shed flowers more readily. In later July and August 2021, when monsoon rains of more than 5 inches occurred, all of the Kurapia recovered and exhibited good quality color and uniformity in the plots (observed with no data collected). A final casual observation in February 2022 following slightly colder hard frosts, the pink cultivar appeared to remain more green compared to the white cultivar.

Table 1. Nativegrasses and groundcovers evaluated in the low desert at Litchfield Park, AZ in 2019 - 2021

Common Name	**Scientific Name**
Alkali sacaton	*Sporobolus airoides*
Blue grama	*Bouteloua gracilis*
Buffalograss, "Texoka"	*Bouteloua dactyloides*
Galleta, "Viva"	*Hilaria jamesii*
Little bluestem, "Cimarron"	*Schizachyrium scoparium*
Purple threeawn	*Aristida purpurea*
Sand bluestem, "Chet"	*Andropogon halli*
Sand dropseed	*Sporobolus cryptandrus*
Sand lovegrass, "Bend"	*Eragrostis trichodes*
Sideoats grama, "Vaughn"	*Bouteloua eurtipendula*
Kurapia (two varieties)	*Lippia nodiflora (pink & white flower)*

Table 2. Kurapia performance comparing 2 cultivars under 3 irrigation regimes, Litchfield Park, AZ, June, 2021.

	Greenness	Spreading	Uniformity	Flower	Canopeo
Variety					
Pink	6.3	6.8	6.3	4.7	4.9
White	6.5	7.6	6.8	6.8	5.0
Irrigation					
20%	5.5	5.9	5.5	4.5	2.5
40%	7.0	8.1	7.4	6.1	6.0
80%	7.3	8.6	7.5	7.1	6.5
White@20	6.0	6.5	6.3	6.3	2.3
White@40	6.0	7.0	6.3	6.3	6.0
White@80	7.2	8.8	7.4	7.6	6.6
Pink@20	5.0	5.3	4.8	2.8	2.8
Pink@40	6.6	7.2	6.8	5.2	5.8
Pink@80	7.3	8.3	7.7	6.3	6.3

Quality ratings 1-9, 1= poor, 9=best

Figure 1. Performance of nativegrasses in September 2021at Wigwam Golf Club, Litchfield Park, AZ demonstrated for turf managers.

Figure 2. Kurapia greenness and overall aesthetic value; surface area coverage and uniform growth under three drip irrigation rates relative to bermudagrass from May to October 2020. Note the significant lesser performance of Kurapia at 20% irrigation rate compared to 40% and 80% drip irrigation rates. No significant differences in Kurapia growth and overall quality observed between the 40% and 80% rates.

Figure 3. Kurapia evaluation for greenness; uniformity over surface area and Canopeo rating; and flower shedding at irrigation rates of 20, 40, and 80%.
Cultivars – P=pink, W=white; Irrigation levels – I20=20%, I40=40%, I80=80%.
Greenness color ratings – 1 = brown and 9 = dark green
Flower shedding – 1 = least flower shedding, 9 = most flower shedding.

Figure 4. Winter temperatures in Litchfield Park, AZ in 2019-20 (National Weather Service).

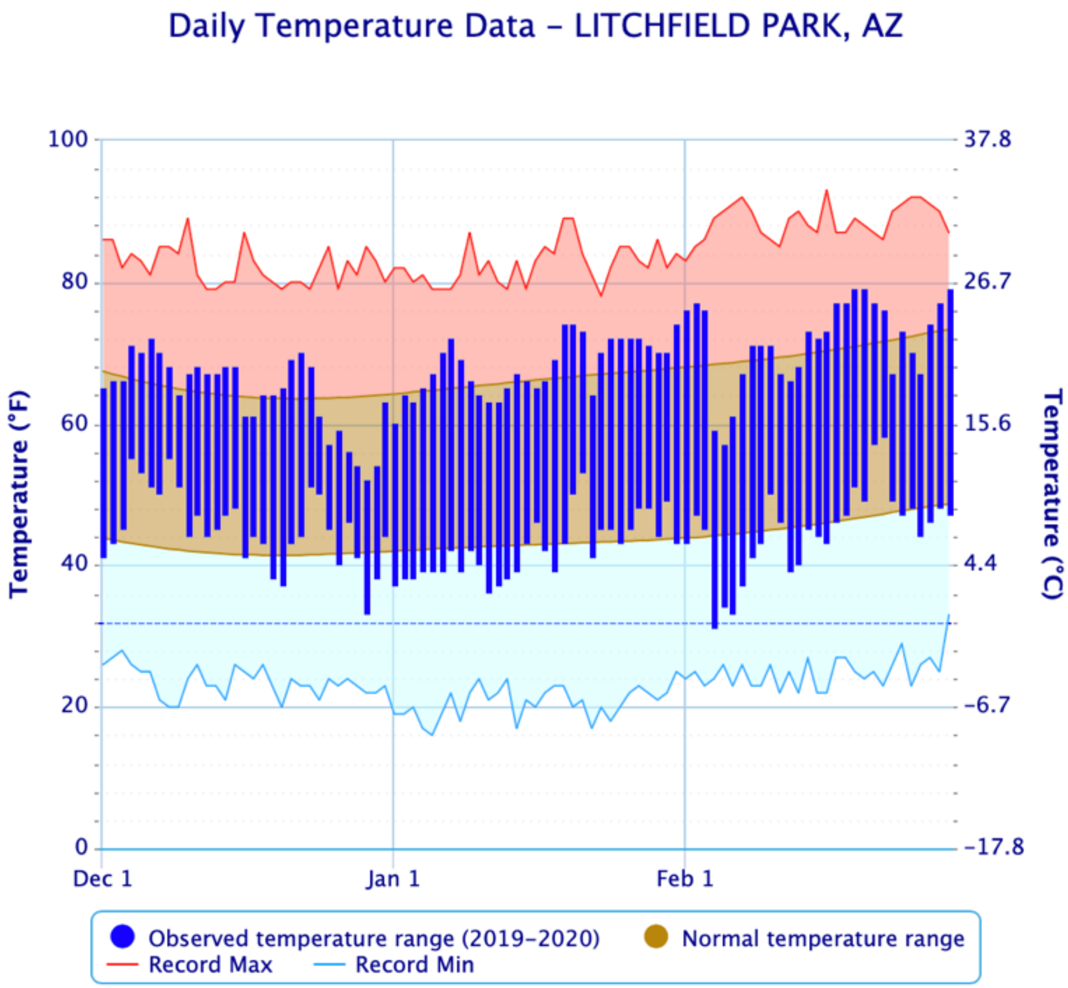
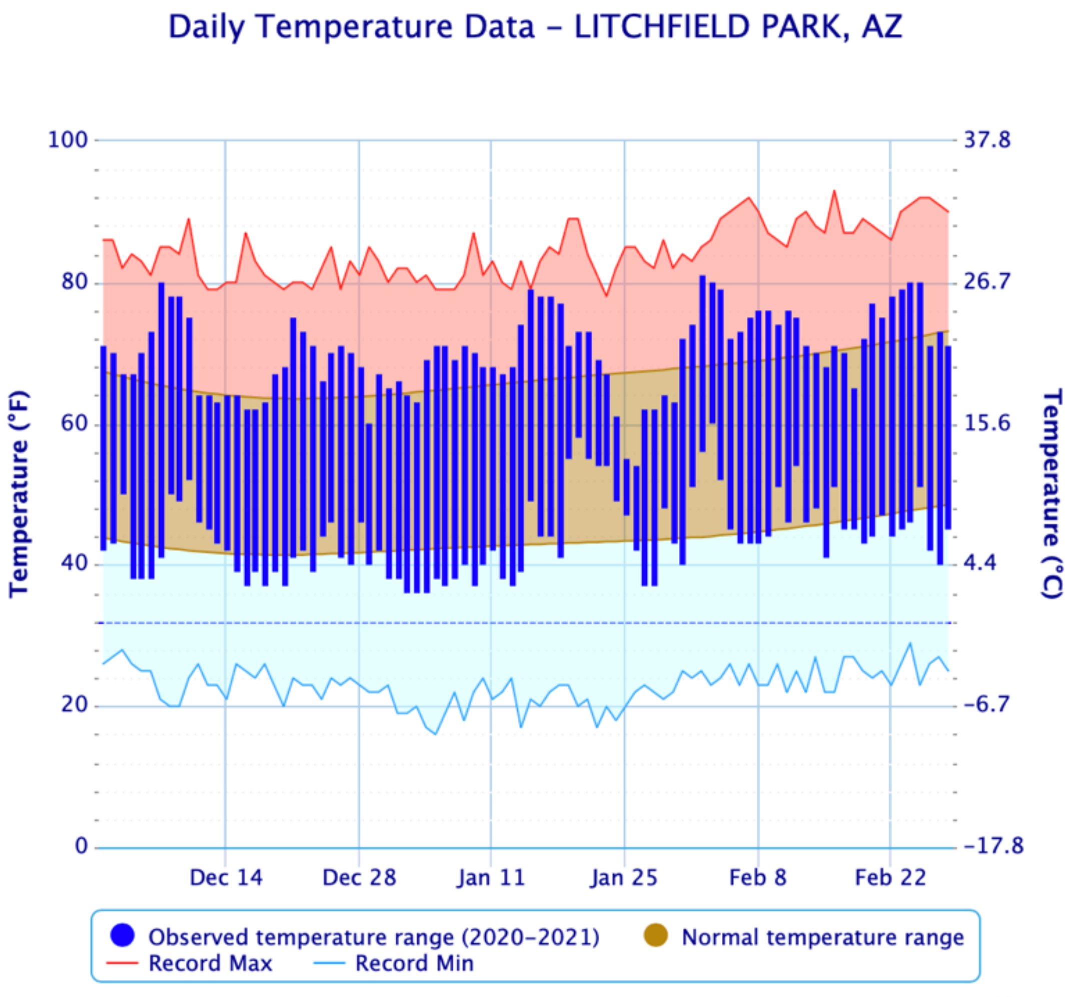


Figure 5. Winter temperatures in Litchfield Park, AZ in 2010-21(National Weather Service).



USGA ID#: 2016-03-550

Title: Creating Cost Savings Opportunities based on a Carbon Footprint Analysis using CarbonSave®: Demonstration and Analysis for Three Diverse Golf Courses

Project Co-Leaders: Stuart Cohen, Ph.D., CGWP and Andy Staples, ASLA, ASGCA
Affiliation: Environmental & Turf Services, Inc. and Staples Golf Design

Objective: Demonstrate how superintendents can save costs by reducing their carbon footprint.

Start Date: 2016
Project Duration: Six years; estimated conclusion is the summer of 2022
Total Funding: $44,200

Summary Points:
We found that electricity usage, with and without the irrigation system, is usually the key golf course component with the greatest potential for cost savings coupled with carbon footprint reduction. We also found that trees on a golf course are excellent sequesterers of CO_2. Unfortunately, this goes against the current trend of removing trees from golf courses.

Summary Text:
We anticipate that our deliverables will have a beneficial impact on golf course maintenance budgets concurrent with carbon-equivalent emissions. It will almost be the polar opposite of a narrowly focused research study. Rather, ours is a broad synthesis of 'the old and the new': the old well-established conclusions about fuel and electricity-related emissions coupled with relatively-recently-funded-USGA research into the influences of turfgrass varieties, fertilizer types, and irrigation on the carbon footprint, particularly with regard to the unintentional production of nitrous oxide following applications of N-fertilizer.

Currently, there is no single source of information related to the carbon footprint of a golf course, particularly one that focuses on electricity. This study will be positioned as a leading source of information for any golf course interested in finding ways to reduce costs and their carbon footprint. And, since the cost reduction is a primary driver of our research, this study will also maintain its relevance long after its completion in the event interest in carbon footprints increases, which is likely during this new administration.

This project has endured a delay of approximately two years, due to the failure of the original four golf courses to fully cooperate during the information-gathering phase. Those golf courses have been replaced by three golf courses located in northern California, southwestern Ohio, and northwestern Arkansas.

Methodology: Calculation of Greenhouse Gas (GHG) Emissions and Sequestration. The initial set of data needed for both the CO_2 eq and cost savings calculations is obtained using a 50+ item questionnaire, and the process includes the review of monthly energy bills. (CO_2 eq = carbon dioxide equivalent for global warming potential; thus one molecule of methane or nitrous oxide are each equivalent to 25 and 300 CO_2 molecules, respectively.) The questionnaire was updated/revised to reflect the latest USGA-sponsored research results. For example, we tentatively determined that questions should be added regarding specific types of fertilizers or irrigation practices based on recent research on N_2O (nitrous oxide) emissions by Qian et al. (2015), Nannenga and Walker (2015), and Bremer et al. (2015).

We also consider the turfgrass species based on the recent work by Patton et al. (2015). Trees do an excellent job of C sequestration. For example, a 35-year-old hardwood that grows at an average rate sequesters C at a rate of 48 lb C/tree/yr, whereas a typical conifer sequesters at the rate of 38 lb C/tree/yr (EIA/US DOE, 1998). We are trying to derive regression equations to estimate the rates of C sequestration by turfgrass.

Following is an example question (#9) from our questionnaire.

9. How much electricity (in kWh) do you use monthly for:
   a. total Irrigation?
   b. maintenance facility?
   c. cart charging?
   d. other?

The information is then entered into the spreadsheet component of CarbonSave®. The spreadsheet is used to estimate CO_2eq emissions and sequestration. The underlying equations are based on results in the published literature and on the US EPA's website.

Our work scope is producing an analysis of the CO_2eq emissions, sequestration and net footprint of each golf course. Equally important, we are also summarizing total electricity and other energy costs and savings opportunities, as follows.

Our energy analysis is encompassing at least one year of energy consumption, preferably three years, allocated by month, in order to understand the annual trends of use, the current efficiency levels and the opportunity for upgrading to more efficient technology. More important, our analysis will include several 'what if?' scenarios.

Cost Savings. Cost savings are being identified using the industry standard energy engineering principals and are being prioritized based on the most advantageous return on investment. No projects with payback longer than five years will be considered. Projects with less than two year paybacks will be our primary focus. Recommendations will be broken out by "in house" projects where management can make the improvements with very little to no financial investment. We will flag high priority "must do" projects where a financial commitment must be made, and moderate priority "should plan for" projects that can/should be budgeted for the future.

Figure 1 depicts the various amounts of the U.S. electrical grid. Please note that the C cost of electricity is highly variable. It can range from 254 lb CO_2e/MWh to 1,690 lb CO_2e/MWh (Table 3).

Results to Date. The monthly energy analyses for the three golf courses are complete. The carbon sequestration and the carbon emissions analyses are 30% and 100% complete, respectively. The table and the figure illustrate the type of analyses we are doing for these golf courses. These numbers are PRELIMINARY.

Future Expectations. We anticipate the project report - - which will be a paper to be submitted to a journal - - will be completed this spring.

Table 1. Preliminary Results

Golf Course	Turf type	CO_2e Emission (metric tons)	CO_2e sequestration (metric tons)	Net Sequestration (C_{seq}-$C_{emission}$) (metric tons)

#1 (Ohio)	Cool season	487	1,619	1131
#2 (California)	Cool season	519	702	-111
#3 (Arkansas)	Warm season	374	2,182	1,808

Table 2. Carbon Dioxide (Equivalents) Emission Ranks: Carbon Footprint Activities that are at Least in Partial Control of the Superintendent

ACTIVITY OR MATERIAL	OHIO GC	CALIFORNIA GC	ARKANSAS GC	AVERAGE RANK
ELECTRICITY USE FOR EVERYTHING BUT IRRIGATION*	1	1	1	1.0
GASOLINE	2	3	4	3
DIESEL	3	4	3	3.3
FERTILIZER PRODUCTION, ETC.	4	2	6	4.0
ELECTRICITY FOR IRRIGATION*	5	5	2	4.0
NITROUS OXIDE GENERATED BY FERT USE	6	6	5	5.7
TOTAL PESTICIDES	7	7	7	7
NATURAL GAS	NA	8	8	8

*These two impact (emission) parameters are dependent on the regional variability in utilities.

Figure 1. A Carbon Footptrint Factor Beyond the Control of the Superintendent: eGRID

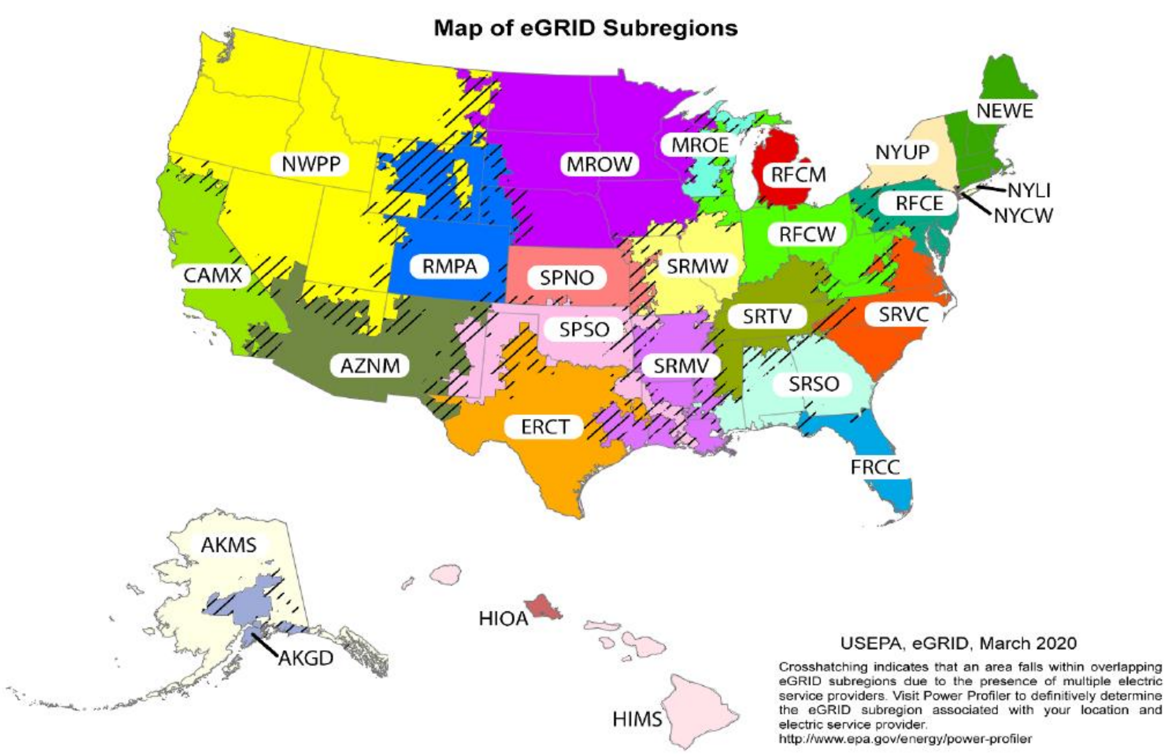
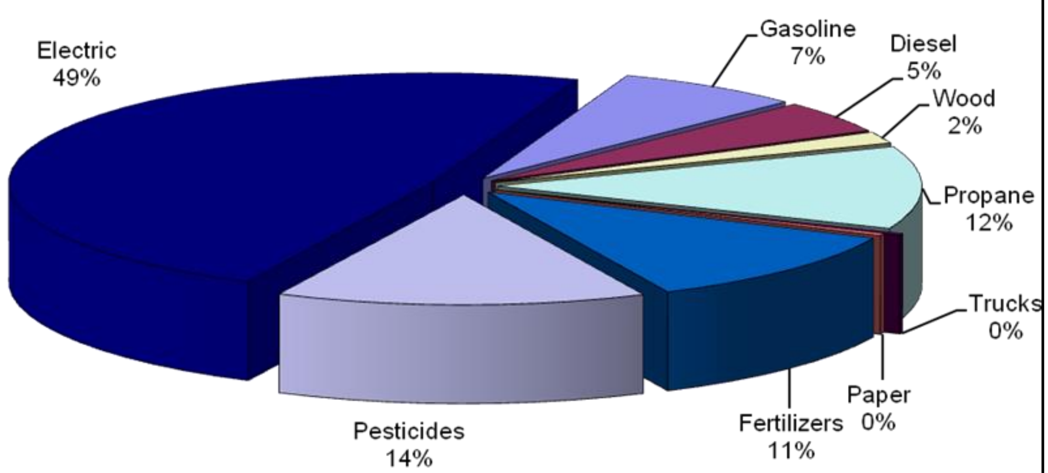


Table 3. Electronic Grid

Top 3 Emitters (US EPA, 2020)			
eGRID Subregion Acronym	eGRID Subregion Name	Location	Pounds CO_2e/MWh
MROE	MRO East	Wisconsin (mostly)	1,689.7
HIOA	HICC Oahu	Oahu	1,682.6
SRMW	SERC Midwest	Missouri, Illinois	1,676.8

Top 3 for Lowest Emissions (US EPA, 2020)			
eGRID Subregion Acronym	eGRID Subregion Name	Location	Pounds CO_2e/MWh
NYUP	NPCC Upstate NY	Most of New York (upstate only)	253.9
CAMX	WECC California	Most of California	498.7
AKMS	ASCC Miscellaneous	More than ½ of Alaska	527.0

Thus there is high variability in CO2 emissions per utility: the highest subregion emits 6.6 times the amount of the lowest impact subregion.



Total Carbon Golf Course Emissions = 77 tons/year - 66 tons/yr C Seq = 11 tons/yr net emissions

Figure 2. % Total Carbon Emissions for Golf Course X

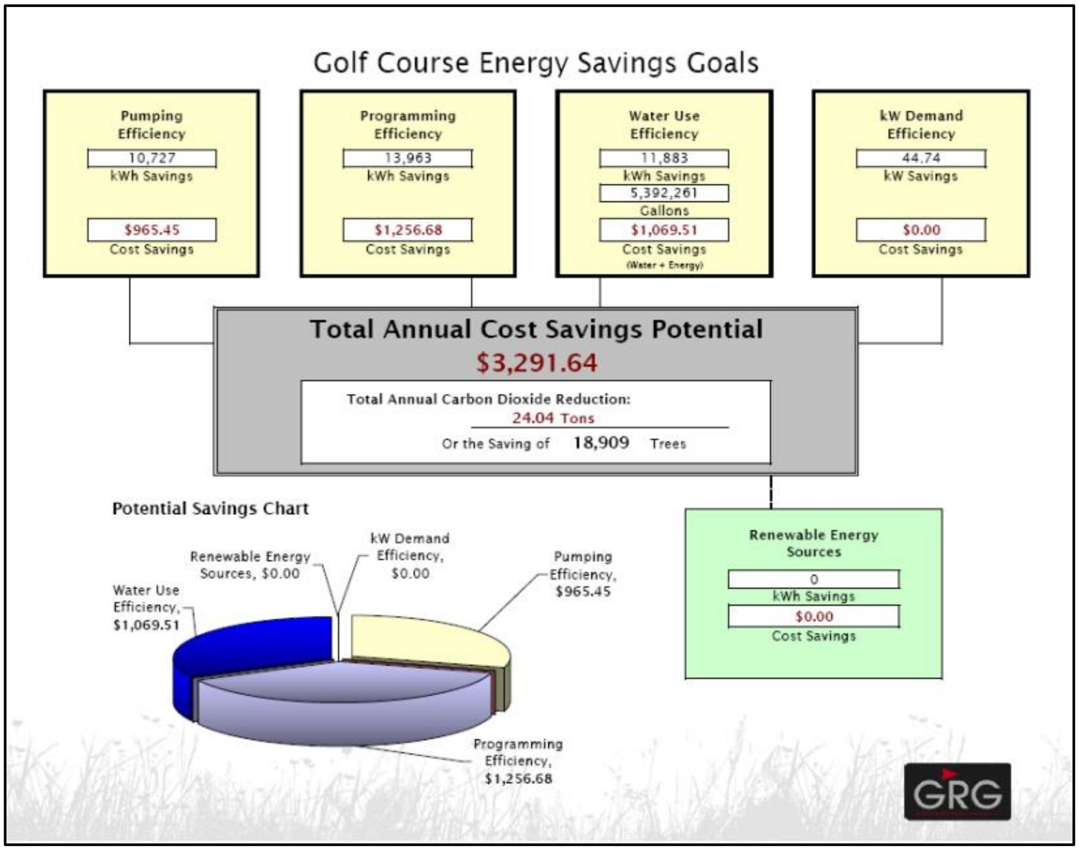


Figure 3. Golf Course Energy Savings Goals

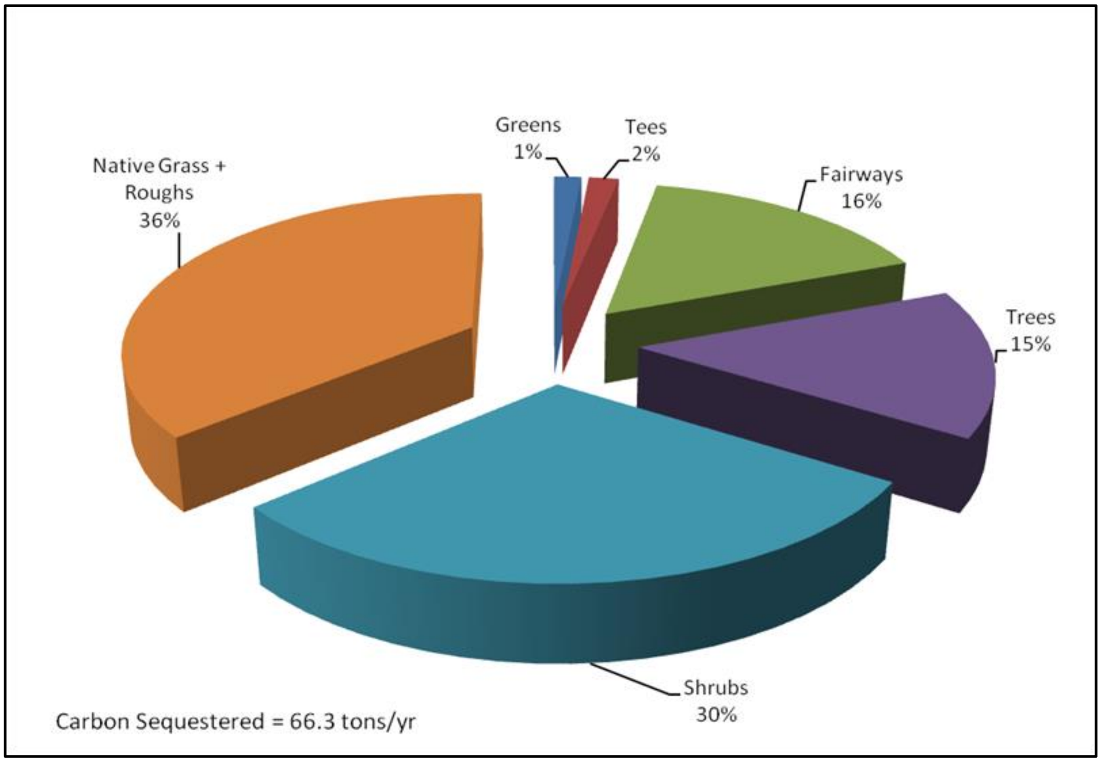


Figure 4. Golf Club (Complete Facility) % Carbon Sequestration

CARBON FOOTPRINT PARAMETERS THAT CAN BE INFLUENCED BY THE SUPERINTENDENT

1. Electricity
   This has, by far, the single most important impact on the golf course's carbon footprint.
2. Gasoline and Diesel Fuel
   These are obvious candidates for savings.
3. Fertilizer
   A little known factor is the production of nitrous oxide during fertilizer metabolism: nitrous oxide has 298 times the global warming potential of carbon dioxide.
4. Trees
   Trees typically sequester 2-100 lb C/tree/yr, depending on the ages of the trees (older [more] vs younger [less], and hardwoods [more] vs conifers [less]). Thus, 50-100 planted acres of trees could offset typical golf course carbon emissions.

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