2020 Research Summaries

TURFGRASS AND ENVIRONMENTAL RESEARCH PROGRAM

2020 Turfgrass and Environmental Research Program Summaries

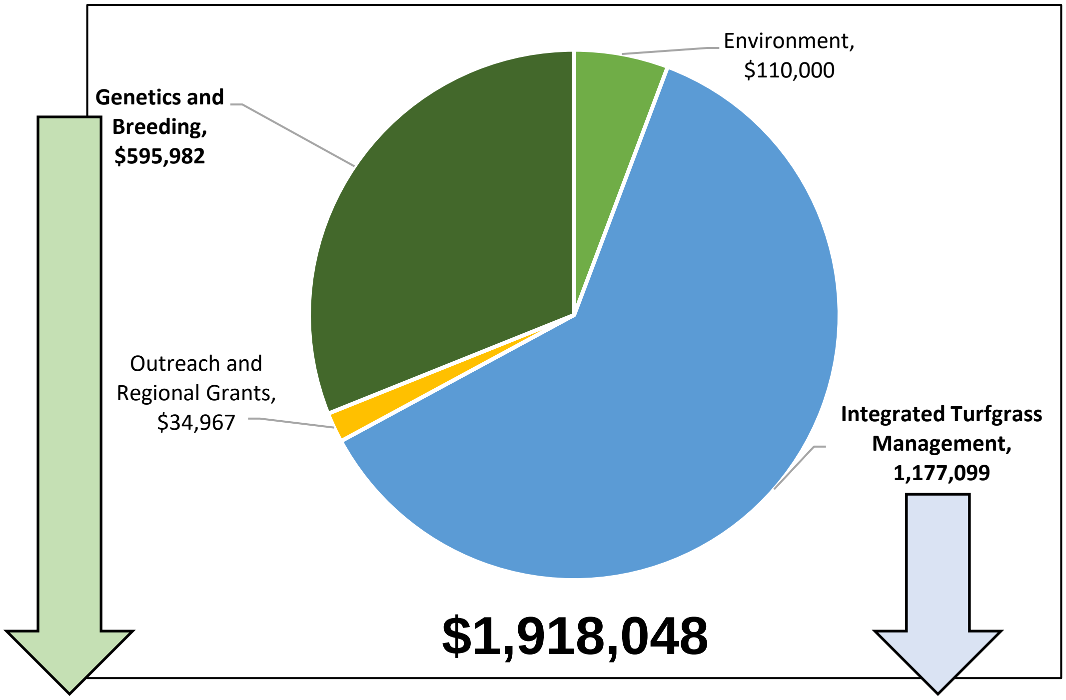
2020 Turfgrass and Environmental Research Program Summaries

2020 Turfgrass and Environmental Research Program Summaries

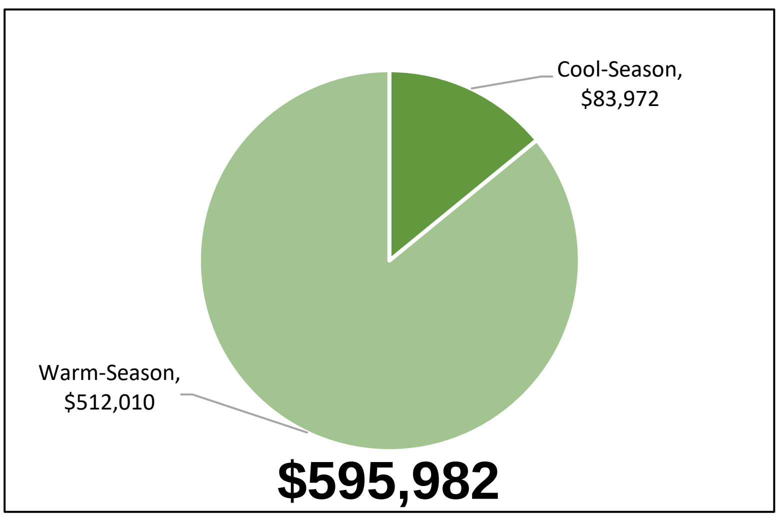
2020 Turfgrass and Environmental Research Program Summaries

2020 Turfgrass and Environmental Research Program Summaries

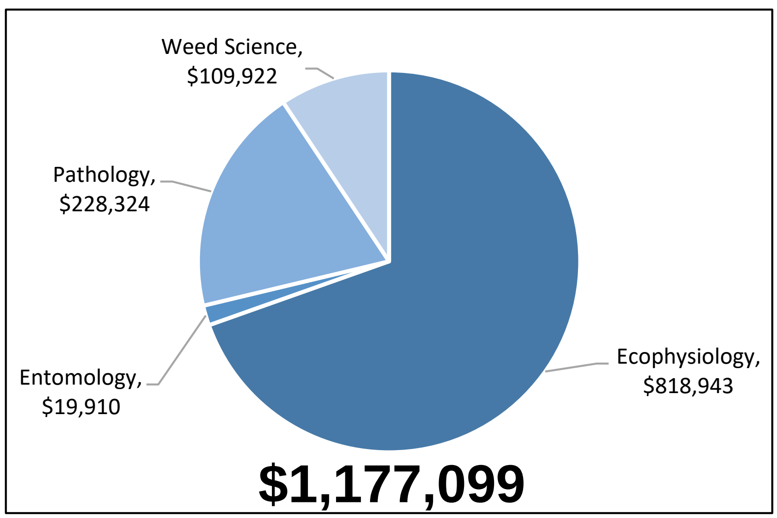
Turfgrass and Environmental Research Grants - 2020



Genetics and Breeding



Integrated Turfgrass Management



1. GENETICS AND BREEDING

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

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

1983-01-001

Annual Report – 2020

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 Slovenia, Croatia, Italy, Hungary, Serbia and Austria. These were evaluated in the spring of 2020 in the Netherlands and over 515 had seed produced in the summer of 2020 and were evaluated in New Jersey starting in fall 2020. Over 12,516 new turf evaluation plots, 169,119 spaced-plant nurseries plants, and 14,000 mowed single-clone selections were established in 2020 in New Jersey.

Over 7500 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 65,900 tall fescues, 12,722 Chewings fescues, 3,000 hard fescues, 4000 strong creeping red fescue 42,472 perennial ryegrasses, and13,596 bentgrasses were also screened during the winter in greenhouses, and superior plants were put in spaced-plant nurseries. Over 131 new intra- and inter-specific Kentucky bluegrasses were harvested in 2020.

The following crossing blocks were moved in the spring of 2020: four hard fescues (161 plants), three Chewings fescues (102 plants), three strong creeping red fescues (72 plants), fourteen perennial ryegrasses (850 plants), twentytwo tall fescues (850 plants), nine creeping bentgrasses (241 plants), three velvet bentgrasses (41 plants), and seven colonial bentgrasses (178 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 2020 were Spike GL, Signet, Sunburst, Pharoah and Furlong. The new tall fescues are Bullseye LTZ, Nano, Biddle, O'Keefe, Degas, Firehawk LTZ and Bonfire. Three Chewings fescues ,Woodall, Boltser and Momentum. Three creeping red fescues named Rev. Wisp and Leigh. There were four new creeping bentgrasses named 007XL, Matchplay, Tour Pro and Piranha. The new Kentucky bluegrasses were Isabel, Syrah ,Heidi, Powerplay, SR 2150, Starr, Bombay and Cloud.

Summary Points

- Continued progress was made in obtaining new sources of turfgrass germplasm. These sources are being used to enhance the Rutgers breeding program.
- Modified population backcrossing and continued cycles of phenotypic and genotypic selection combined with increasing sources of genetic diversity in turfgrass germplasm has resulted in the continued development and release of top performing varieties in the NTEP
- Five perennial ryegrasses, seven new tall fescues, three Chewings fescues, three creeping red fescues, eight Kentucky bluegrasses, four Creeping Bentgrasses were named and released in 2020
- Published 5 referred journal articles and 18 non-referred journal articles in 2020.
- Thirty- two Plant variety certificates issued and 2 PVP's applied for in 2020.

References

Publications 2020 (Peer Reviewed)

1. Vines, P.L., R.M. Daddio, J. Luo, R. Wang, N. Zhang, B.B. Clarke, W.A. Meyer, and S.A. Bonos. 2020. *Pyricularia oryzae* incites gray leaf spot disease on hard fescue (*Festuca brevipila*). Accepted to International Turfgrass Society Research Journal, October 9, 2020.

2. Bonos, S.A., E.N. Weibel, J. Honig, J. A. Murphy, L. H. Chappell and W. A. Meyer. 2020. Divot recovery of cool-season turfgrass species and mixtures in low maintenance fairways. Accepted to International Turfgrass Society Research Journal, September 1, 2020.

3. Qu, Y., E. J. Green, P. L. Vines, S. Wu, S. A. Bonos, and W. A. Meyer. 2020. Evaluation and genetic analysis of red thread (*Laetisaria fuciformis* Butdsall) disease incidence in tall fescue [*Festuca arundinacea* (Schreb.)]. Accepted to International Turfgrass Society Research Journal, August 1, 2020.

4. Yue, C., J. Wang, E. Watkins, S.A. Bonos, K.C. Nelson, J.A. Murphy, W.A. Meyer, and B.P. Horgan. 2019. Investigating the practices and challenges for turfgrass breeders and distributors. Hortscience 54:129-135. doi:10.21273/hortsci13343-18.

5. Qu, Y., R.M. Daddio, P.E. McCullough, S.A. Bonos and W.A. Meyer. 2019. Phytotoxicity of methiozolin on fine fescue. HortTechnology 29: 265-270.

Abstracts

1. 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).

2. Wu, D.S., A.L. Grimshaw, H.Y. Qu, P.L. Vines, E. N. Weibel, W.A. Meyer and S.A. Bonos. Inheritance of summer patch disease resistance in hard fescue. p. 53. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

3. Vines, P.L., R.M. Daddio, R. Wand, J. Luo, N. Zhang, B.B. Clarke, R.F. Bara, D. A. Smith, W.A. Meyer and S.A. Bonos. Koch's postulates for gray leaf spot disease in hard fescue (Festuca brevipilia). p. 51. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

4. Daddio, R.M., J.A. Honig, T.J. Gianfagna, B. S. Bushman, S.A. Bonos, and W.A. Meyer. p. 38. Effect of ethephon applications on levels of apomixis in Rockstar Kentucky bluegrass. p. 38. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

5. Qu, Y., E.J. Green, S.A. Bonos, and W.A. Meyer. 2020. Genetic analysis of tall fescue populations under rainout shelter. p. 34. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

6. Bonos, S.A. E.N. Weibel, P.L. Vines, T.J. Lawson, L. Hoffman, and W.A. Meyer. Breeding progress toward the perfect putting green. p. 25. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

7. Vines, P., R.M. Daddio, R.F. Bara, D.A. Smith, S.A. Bonos and W.A. Meyer. 2020. Breeding for gray leaf spot disease resistance in cool-season turfgrasses. p. 23. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

8. Zhang, N., G. Groben, H. Qu, S. Bonos, W. Meyer, J. Murphy, and B.B. Clarke. 2020. Microbiome studies: Drought resistance of tall fescue and dollar spot resistance of creeping bentgrass. p. 19. In Proceedings of the 29th Rutgers Turfgrass Symposium. January 10, 2020.

9. Qu, H.Y., E.J. Green, S.A. Bonos and W.A. Meyer. 2019. Genetic analysis on drought response of tall fescue [Festuca arundinacea (Schreb.)] in rain-out shelter using bayesian mixed linear models. In Agronomy Abstracts. ASA, Madison, WI.

10. Vines, P.L., R.M. Daddio, R. Wang, J. Luo, N. Zhang, B.B. Clarke, W.A. Meyer, and S.A. Bonos. 2019. Gray leaf spot disease of hard fescue (Festuca brevipila). In Agronomy Abstracts. ASA, Madison, WI.

11. Vines, P.L., J.A. Honig, J. Vaiciunas, C. Kubik, E.N. Weibel, W.A. Meyer, and S.A. Bonos. 2019. Quantitative trait locus mapping for growth habit morphology traits in perennial ryegrass (Lolium perenne L.). Crop Science Society of America annual meeting. San Antonio, TX.

12. Bonos, S.A., P.L. Vines, and W.A. Meyer. 2019. Turfgrass science: a future perspective on turfgrass breeding and related research. In Agronomy Abstracts. ASA, Madison, WI.

13. Meyer, W.A., S.A. Bonos, and P.L. Vines. May 15, 2019. Breeding cool-season turfgrass with improved seed production. International herbage seed group conference. Corvallis, OR.

Non-Refereed 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. 2020. Performance of bentgrass cultivars and selections in New Jersey turf trials. 2019 Rutgers Turfgrass Proceedings 51:1-31.

2. Wu, S. P. L. Vines, R. F. Bara, D. A. Smith, Y. Qu, R. M. Daddio, S. A. Bonos, and W. A. Meyer. 2020. Performance of fine fescue cultivars and selections in New Jersey turf trials, 2019. Rutgers Turfgrass Proceedings. 51: 33-64.

3. Daddio, R.M., P.L. Vines, R.F. Bara, D.A. Smith, E.N. Weibel, J.A. Murphy, S.A. Bonos, and W.A. Meyer. 2020. Performance of Kentucky bluegrass cultivars and selections in New Jersey turf trials, 2019. Rutgers Turfgrass Proceedings. 51: 65-129.

4. Halterman, J. R. F. Bara, D. A. Smith, R. M. Daddio, Y. Qu, P. L. Vines, S. A. Bonos, and W. A. Meyer. 2020. Performance of perennial ryegrass cultivars and selections in New Jersey turf trials, 2019. Rutgers Turfgrass Proceedings. 51:131-164.

5. Vines, P.L., R. F. Bara, D. A. Smith, R. M. Daddio, Y. Qu, S. A. Bonos, and W. A. Meyer. 2020. Performance of tall fescue cultivars and selections in New Jersey turf trials. 2019 Rutgers Turfgrass Proceedings 51: 165-186.

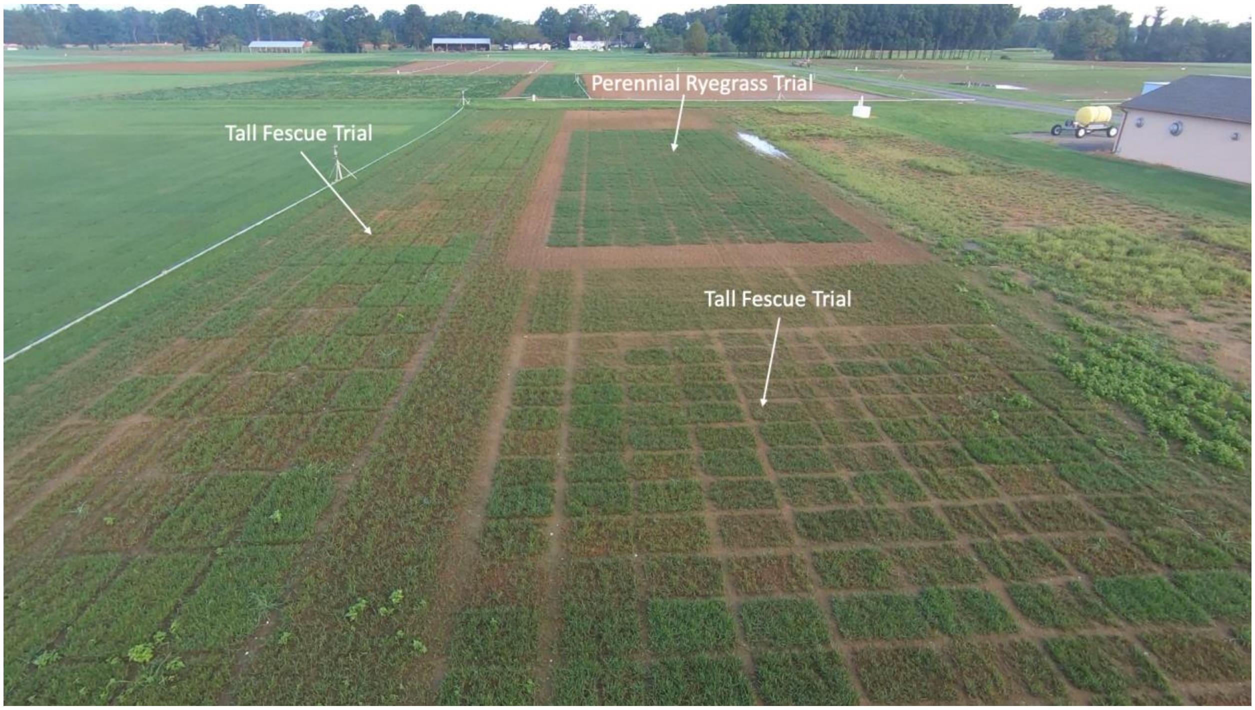


Figure 1. Tall fescue and perennial ryegrass trails seeded in July 2020 for gray leaf spot disease evaluations at the Plant Science Research and Extension Farm in Adelphia, NJ.

Figure 2. Gray leaf spot resistant (left) and susceptible breeding lines in trial seeded in July 2020 at the Plant Science Research and Extension Farm in Adelphia, NJ.

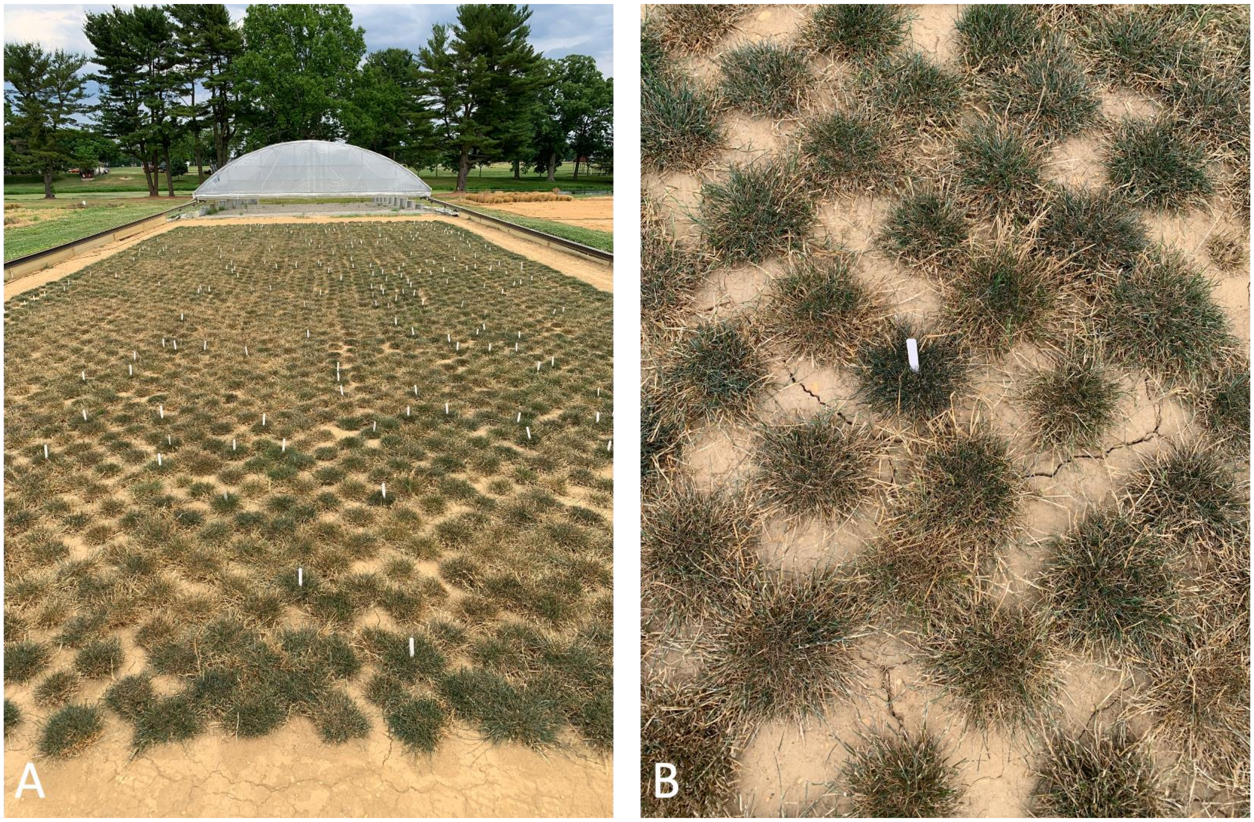


Figure 3. A: Selection of drought tolerant perennial ryegrass clones using a mobile rainout shelter at the Plant Science Research and Extension Farm in Adelphia, NJ, B: Selected clone maintaining green color and foliar growth characteristics after 90 days of drought stress at the Plant Science Research and Extension Farm in Adelphia, NJ.

USGA ID#: 2018-11-661

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

Project Leaders: Faith C. Belanger and Bruce B. Clarke

Affiliation: Rutgers University

Objectives of the project: The overall goal of this project is to determine the role of the *Epichloë festucae* antifungal protein in the well-established endophyte-mediated disease resistance seen in fine fescues. In one approach, we generated gene knockouts of the antifungal protein gene with the aim of determining if it is required for disease resistance. In another approach we have purified the antifungal protein using different expression systems to identify the best system for large-scale purification of the protein. An efficient expression system for purification of the *Epichloë festucae* antifungal protein will be required to test its activity in dollar spot infected creeping bentgrass and fine fescue. If the purified protein is effective, this could represent an additional method to control dollar spot and reduce fungicide inputs.

Start Date: 2018

Project Duration: 3 years

Total Funding: $120,000

Summary Points:

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

2. Gene knockouts of the antifungal protein gene were produced, but extensive efforts to inoculate the knockout isolates back into endophyte-free strong creeping red fescue were unsuccessful. However, the wild type isolate and complemented knockout isolates (transformed with plasmids containing the wild type antifungal protein gene) could be inoculated into plants. These results suggest the antifungal protein may be required for infection (the ability of a fungus to gain entry into the plant) and symbiosis once inside the host grass.

3. The activities of the *E. festucae* antifungal protein produced in yeast, bacteria, and in the fungus *Penicilllium chrysogenum* were assessed against the model fungus *Neurospora crassa*. The best activity of the *E. festucae* antifungal protein was obtained with the *Penicillium* expression system. Also, the *Penicillium* expression system was the most convenient for purification and produced the highest quantity of the *E. festucae*

antifungal protein. The *E. festucae* purified antifungal protein expressed in *Penicillium* had activity against the dollar spot fungus in culture.

4. Now that we have established a robust expression system for producing adequate quantities of the *E. festucae* antifungal protein we are ready to test application of the protein onto dollar spot infected plants to determine if this could be a new method for disease control.

Executive Summary:

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

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

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

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

In an attempt to determine the involvement of the antifungal protein in dollar spot resistance of the host grass, we generated two gene knockouts by using the CRISPR-Cas9 approach. The objective was to inoculate the knockout isolates back into endophyte-free strong creeping red fescue and assess the level of dollar spot resistance of the plants. If the antifungal protein is indeed a factor in the dollar spot resistance, as we suspect it is, then the plants harboring the knockout isolates would be expected to exhibit less or no dollar spot resistance. However, extensive attempts to inoculate strong creeping red fescue plants with the knockout isolates have been unsuccessful, although successful inoculations were obtained with the wild type isolate, which contained the antifungal protein gene. We also transformed the knockout isolates with plasmids containing the wild type antifungal protein gene and determined that the complemented isolates could be inoculated into endophyte free plants. This has raised questions about the possible importance of the *E. festucae* antifungal protein in the infection process and the symbiotic association with strong creeping red fescue. The inability of the knockout isolates to infect the host grass prevented an evaluation of the lack of the antifungal protein gene on disease resistance or susceptibility of the host, but we know it does have activity against the dollar spot fungus in lab tests. Although these results were frustrating, they lead to the unexpected possibility that the antifungal protein is a required factor in the infection process and symbiosis between *E. festucae* and strong creeping red fescue, in addition to having antifungal activity.

The interaction of fungal plant pathogens and symbionts with their hosts involves effector proteins, characterized as small-secreted proteins that can be important for colonization or for evasion of host defenses (Plett and Martin 2015; Uhse and Djamei 2018). The *E. festucae* antifungal protein has the characteristics of an effector protein in that it is a cysteine-rich small secreted protein and its expression is considerably higher in the infected plant tissue than in culture (Hassing et al., 2019; Sperschneider et al., 2016). Quantitative PCR analysis revealed that the expression level of the antifungal protein is more than 700-fold higher in the infected plant leaf sheath than in culture (Fig. 1). Additional research will be required to determine if the antifungal protein is indeed an effector protein. However, if *Efe*-AfpA is an effector protein it must be specific to certain interactions, since most *Epichloe* spp. do not have such a gene in their genomes

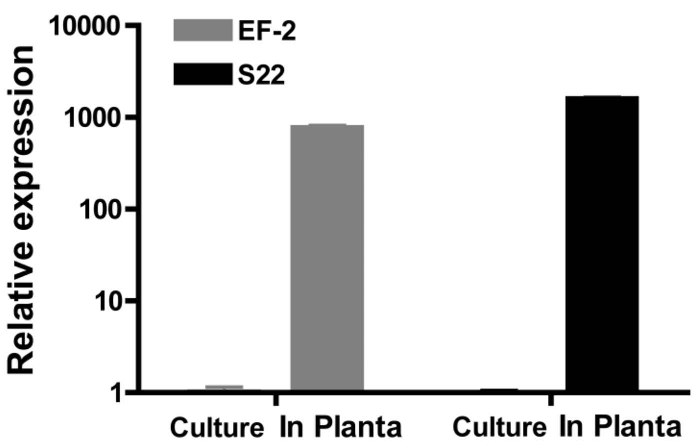


Fig. 1. Comparison of the expression level of the *E. festucae* antifungal protein gene, in culture with expression *in planta* relative to expression levels of two reference genes, elongation factor 2 (EF-2; gene model EfM3.021210) and 40S ribosomal protein S22 (S22; gene model EfM3.016650). The y-axis is a logarithmic scale.

E. festucae antifungal protein purification

Our hypothesis is that the *E. festucae* antifungal protein 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 the endophyte antifungal protein can protect creeping bentgrass plants from dollar spot disease so that it could be used as an alternative or supplement to synthetic fungicides. To do this requires a substantial amount, probably milligram levels, of purified antifungal protein, which is why we have been focused on testing different expression systems.

The *E. festucae* antifungal protein is highly expressed in infected strong creeping red fescue, as shown in Fig. 1 above; however, purification from plant tissue is not practical since that would require an excessive amount of starting plant material. Moreover, it is expressed at a very low level when the fungus is grown in culture, as seen in Fig. 1 above. We, therefore, explored generating the antifungal protein in several established protein expression systems. The objective was to identify a protein expression system that can generate a large amount of active antifungal protein in the simplest way. The antifungal protein was successfully expressed in the yeast *Pichia pastoris* (Tian et al., 2017) and in the bacterium *Escherichia coli (*Fardella et al., unpublished*)*. The *E. festucae* antifungal protein is similar to a protein from another fungus, *Penicillium chrysogenum,* which is designated PAF (*Penicillium* antifungal protein) and which also has antifungal activity (Marx, 2004). We obtained an engineered PAF overexpression strain of *P. chrysogenum* from Dr. Florentine Marx (Medical University of Innsbruck, Innsbruck, Austria) so that we could directly compare the production and activities of PAF and the *E. festucae* antifungal protein. Dr. Marx also provided a PAF knockout strain that we used to express the *E. festucae* antifungal protein in *Penicillium*. A comparison of antifungal activities of the *E. festucae* antifungal protein in the three expression systems, as well as the *Penicillium* antifungal protein PAF, against *Neurospora crassa* conidia, a model fungus used in such systems, is shown in Fig. 2. The *E. festucae* antifungal protein expressed in *Penicillium* had activity at the lowest concentration tested, whereas higher concentrations were required for activity of the protein produced in yeast and bacteria. Activity at low concentrations is important for it to be efficacious in future assays. The *E. festucae* antifungal protein expressed in bacteria had high activity at high concentrations of the protein. The requirement for high concentrations makes that system impractical for large-scale production of the protein. Also, the *Penicillium* expression system was the most convenient for purification and produced the highest quantity of the *E. festucae* antifungal protein.

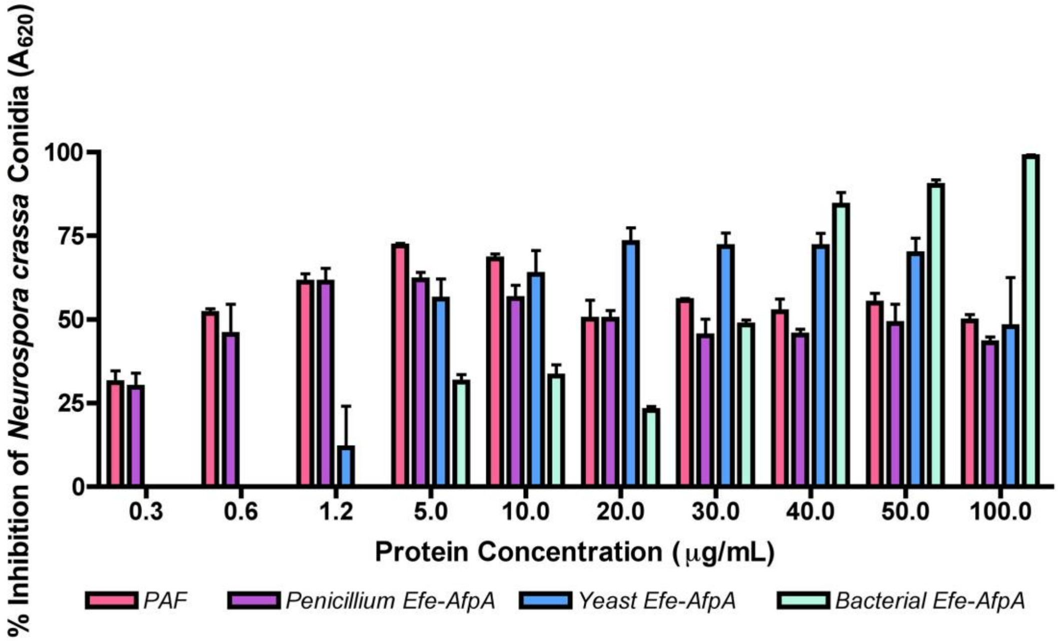


Figure 2. Comparative percent inhibition of growth of *Neurospora* conidia by the *E. festucae* antifungal protein expressed in *Penicillium*, yeast, and bacteria. Increasing concentrations of purified *E. festucae* antifungal protein (*Efe*-AfpA) expressed in *Penicillium chrysogenum*, yeast, and bacteria, and the *Penicillium chrysogenum* produced PAF were assayed in triplicate against *Neurospora* conidia and growth was measured after 24 hours. Percent inhibition was measured by absorbance at OD_{620}. Standard deviation is represented by error bars.

The purified *E. festucae* antifungal protein expressed in *Penicillium* inhibited growth of the dollar spot fungus (Fig. 3). Interestingly, in the same type of assay the *Penicillium* antifungal protein PAF did not have activity against the dollar spot fungus. The *E. festucae* and *Penicillium* antifungal proteins are 65% identical in their protein sequences. They both have activity against *Neurospora*, but only the *E. festucae* antifungal protein has activity against the dollar spot fungus. This is an important difference in activity between the two similar antifungal proteins that could be used in the future to identify the regions of the *E. festucae* antifungal protein critical for activity against the dollar spot fungus.

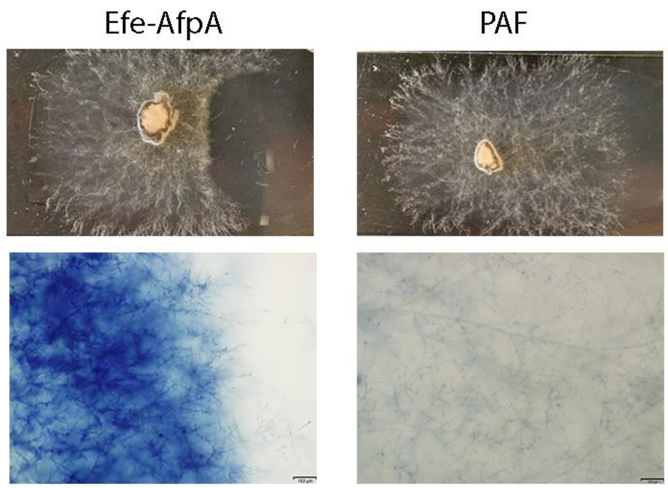


Fig. 3. Comparison of the activity of the *E. festucae* antifungal protein (*Efe*-AfpA) expressed in *Penicillium* with PAF, a similar antifungal protein from *Penicillium*, against the dollar spot fungus. In the upper panels purified *E. festucae* antifungal protein (left) or PAF (right) was placed on the right side of a plug of the dollar spot fungus. The *E. festucae* antifungal protein clearly inhibited the growth of the dollar spot fungus whereas PAF did not. The lower panels show dollar spot hyphae from the upper panels treated with Evans blue. Evans blue enters cells that have damaged cell membranes. Treatment with the *E. festucae* antifungal protein resulted in damage to the cell membranes of the dollar spot fungus whereas treatment with PAF did not.

References

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

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

Hassing, B., Winter, D., Becker, Y., Mesarich, C.H., Eaton, C.J., and Scott, B. (2019) Analysis of *Epichloë festucae* small secreted proteins in the interaction with *Lolium perenne*. PLoS ONE 14:e0209463

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

Plett, J.M., and Martin, F. (2015) Reconsidering mutualistic plant–fungal interactions through the lens of effector biology. Current Opinion in Plant Biol. 26:45-50

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

Uhse, S., and Djamei, A. (2018) Effectors of plant-colonizing fungi and beyond. PLoS Pathogens 14:e1006992

USGA ID#: 2019-15-685

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

Project Leader: David R. Huff and 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*.
- Objective 4 (Month 24-30): Align and map parental DNA sequences to the genome of allotetraploid *Poa annua*.
- 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*.
- 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.

Start Date: 2019

Project Duration: 3 Years

Total Funding: $91,824

Summary Points:

- Mowing increases global DNA methylation in *Poa annua*.
- *Poa annua* can pass the environmental effects of mowing to its unmowed offspring.
- Transgenerational plasticity in *Poa annua* is conferred, in part, by DNA methylation.
- The genome of *Poa infirma* Kunth, the annual female diploid parental species of *Poa annua*, was *de novo* sequenced to a level of 7 pseudomolecule chromosomes containing 1,125,464,162 bp (L90 = 7; $BUSCO_{(n=1614)}$ = 98.1%).
- The genome of *Poa supina* Schrader, the perennial male diploid parental species of *Poa annua*, was *de novo* sequenced to a level of 7 pseudomolecule chromosomes containing 84,729,999 bp (L90 = 7; $BUSCO_{(n=1614)}$ = 95.8%).

Summary Text:

In 2020, we published results from our Year-1 funding that mowing increases global DNA methylation in *Poa annua*, that *Poa annua* can pass the environmental effects of mowing to its unmowed offspring, and that transgenerational plasticity in *Poa annua* is conferred, in part, by DNA methylation (Benson, Mao, and Huff 2020). Furthermore, we speculate that *Poa annua's* observed epigenetic rewiring and patterns of non-Mendelian inheritance on golf course putting greens is largely driven by its status as a neo-allopolyploid between diploid species, *Poa infirma* and *Poa supina*. It is likely that, upon polyploidization and whole genome duplication, the *Poa supina* and *Poa infirma* sub-genomes interact in specific ways to confer onto *Poa annua* uncommon phenotypic plasticity and versatility to a wide range of environments. It is known that allopolyploidy may result in 'genome dominance' and 'biased fractionation' where one of the ancestral diploid subgenomes is repressed while the other subgenome retains higher gene expression levels and fewer DNA mutations. Genome dominance and biased fractionation is regulated in part by epigenetic signals such as cytosine methylation, histone modification, and small RNA silencing. Thus, it is highly likely that our observed methylation differences in *Poa annua* is related to its biased expression of subgenomes under mowing stress.

After completing the first year of this grant proposal's funded research, and after consulting with numerous colleagues and collaborators, we have come to realize that improving our resolution of methylation patterns in a non-model species such as *Poa annua* is fraught with difficulties and is unlikely to be the most fruitful avenue of investigation. However, these same colleagues and collaborators have encouraged an alternative approach for unraveling the phenotypic instability of *Poa annua* by utilizing *Poa annua*'s known subgenome architecture. Therefore, rather than continuing with our originally proposed methylome analyses, we believe that the USGA financial resources would be better utilized to unravel the downstream effect of epigenetic reprogramming on genome dominance and bias fractionation of *Poa annua's* subgenomes. We believe that studying the genomic architecture and transcriptional profiles of *Poa annua, Poa supina,* and *Poa infirma* is the most likely avenue to elucidate *Poa annua*'s phenotypic instability and thereby enhancing our efforts to develop commercial cultivars of elite and stable *Poa annua* for use on golf course putting greens. Thus, with permission given by the USGA Green Section, we have reallocated the remaining two years of funding towards the study of *Poa annua's* allopolyploid genomic architecture by sequencing the genomes of its diploid ancestors, *Poa infirma* and *Poa supina*. Budgetary details of our proposed changes have been submitted in a separate document.

Since mid-March, 2020, due to the COVID-19 pandemic, the entire Penn State University Park campus has been restricted to essential activities only by essential personnel. Currently, greenhouse plants are being maintained and field plots are being mowed but that is all we are able to do. The good news is that, just prior to the COVID-19 pandemic shutdown, and in collaboration with Drs. Scott Warnke and Shaun Bushman of the USDA, we were able to commence the de novo sequencing of the genomes of *Poa annua* and its diploid parental species *Poa infirma* and *Poa supina*.

In fact, our computational progress seems to have been enhanced by the pandemic shutdown because everyone involved was home-bound with access to High Performance Computation. Currently, all three of our Poa genomes are in various stages of the sequencing/bioinformatic analysis pipeline. The process of sequencing an organism's genome is complicated, arduous and requires hundreds of decision steps. Basically, it involves using computer programs to align the initial DNA sequencing reads into contigs, which are then scaffolded together into pseudomolecules. In our research, we used PacBio Hifi circular consensus sequencing (CCS) reads that were aligned into contigs with either HiCanu or Hifiasm assembly programs that were then scaffolded together using Dovetail Genomics' proprietary HiRise scaffolding assembly pipeline, which utilizes proximity ligation information along with the contig assembly information. To date, we have assembled the two parental species genomes down to the level of whole-chromosome pseudomolecules that represent approximately 95.8% to 98.1% complete genomes (Fig.1); which is excellent. From our sequencing efforts, and for the remainder of this specific project, the final genome size of *Poa infirma* is 1.125Gb and for *Poa supina* is 0.636Gb. The pre-scaffold genome size of *Poa annua* is currently measuring 1.8Gb with approximately 1K contigs and 98% BUSCO hits. Thus, the sizes of the three haploid (1n) Poa genomes are all within 8% of the estimated genome sizes we predicted from earlier flow cytometry measurements (Mao and Huff 2012).

The output of Dovetail Genomics' proprietary HiRise genome assembly pipeline, which utilizes proximity ligation information along with prior assembly information, was compared using two different input assembly methods, namely HiCanu versus Hifiasm. The final HiRise genome assembly of *Poa infirma*, a self-pollinated annual species, was found to be "best" with using the Hifiasm assembly prior to implementing HiRise scaffolding software; whereas, the genome assembly of *Poa supina*, a cross-pollinated perennial species, was found to be most complete using the HiCanu assembly process prior to implementing HiRise scaffolding software. The different results between these two assembly methodologies most likely resides in the level of heterozygosity of the two Poa species, i.e. cross-pollinated *Poa supina* being more heterozygous than the self-pollinating *Poa infirma*. In addition, dot-plot analyses indicated possible species-specific chromosomal inversions originating within Poa species (Fig 2) and several features of chromosome structure, including potential locations for ribosomal DNA and centromeres, were capable of being predicted based on characteristic patterns of GC-content (Fig. 3).

Finally, we anticipate completing the assembly of all three genomes in early 2021 after which, all three genomes will serve as the foundation for our differential subgenome expression analyses to be performed in the latter-half of 2021.

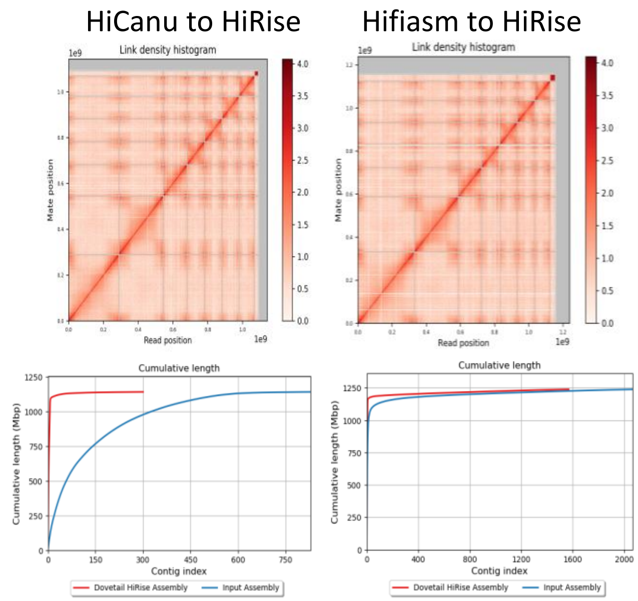
References:

1. Benson, C.W., Q. Mao, and D.R. Huff. 2020. Global DNA methylation predicts epigenetic reprogramming and transgenerational plasticity in *Poa annua* L. Crop Science *In Press*, doi: 10.1002/csc2.20337
2. Mao, Q. and D.R. Huff. 2012. The evolutionary origin of *Poa annua* L. Crop Science. 52:1910-1922. doi: 10.2135/cropsci2012.01.0016

A)

Poa infirma (self-pollination, annual)

Largest pseudomolecule	HiCanu to HiRise length (bp)	Hifiasm to HiRise length (bp)
1I	288,270,164	330,528,634
2I	256,214,653	256,709,825
3I	136,336,502	135,765,836
4I	101,404,149	106,899,081
5I	99,962,597	102,856,999
6I	97,972,374	102,618,788
7I	89,308,446	90,084,999
SUM	1,069,468,885	1,125,464,162
BUSCO (n=255)	99.61%	100.00%
BUSCO (n=1614)	92.10%	98.10%
N50	136,336,502	135,765,836
L90	7	7



B)

Poa supina (cross-pollinated, perennial)

Largest pseudomolecule	HiCanu to HiRise length (bp)	Hifiasm to HiRise length (bp)
2S	115,093,949	116,080,077
4S	114,460,630	115,329,687
1S	97,883,835	100,645,999
3S	84,729,999	80,429,451
7S	77,110,327	74,352,999
5S	74,292,368	72,790,999
6S	72,915,550	60,858,307
SUM	636,486,658	620,487,519
BUSCO (n=255)	99.22%	98.82%
BUSCO (n=1614)	95.80%	87.80%
N50	84,729,999	80,429,451
L90	7	12

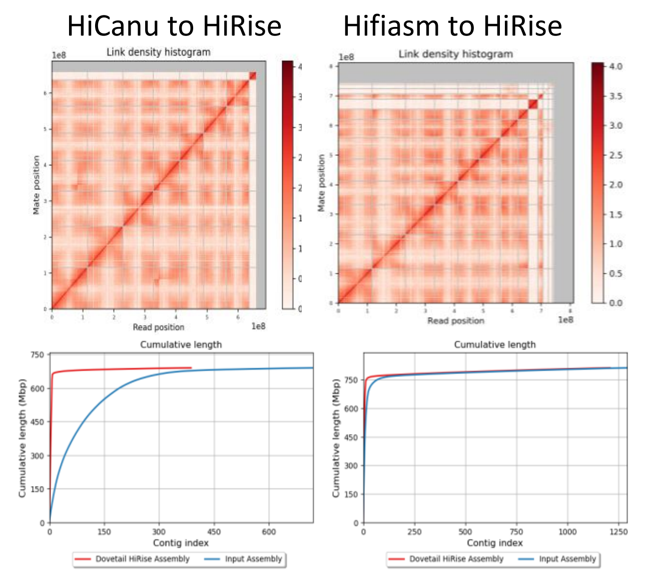
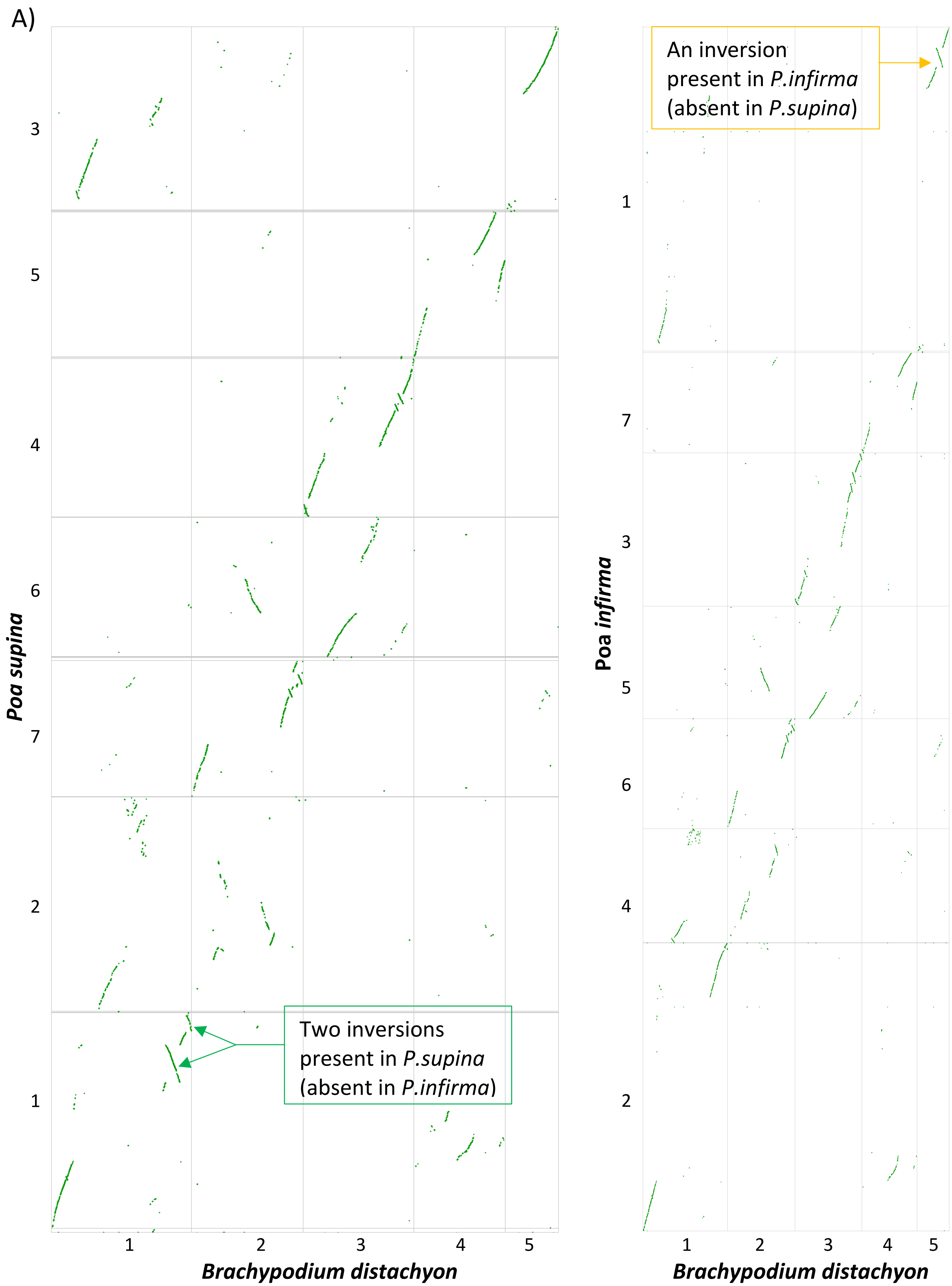


Fig. 1. Comparison of Dovetail HiRise genome assembly using different input assembly methods HiCanu versus Hifiasm for the two parental diploid species of *Poa annua*. For each parental species and method of assembly, cumulative length plots, continuity plots, pseudomolecule length, and genome assembly statistics are presented. A) The genome assembly of *Poa infirma*, a self-pollinated annual species, benefited from the Hifiasm assembly prior to proximity ligation and HiRise scaffolding (highlighted). B) The genome assembly of *Poa supina*, a cross-pollinated perennial species, however, exhibited the most complete genome assembly using the HiCanu assembly process prior to proximity ligation and HiRise scaffolding (highlighted).

A)



3
5
4
6
7
2
1
Poa supina
Two inversions present in P.supina (absent in P.infirma)
1
2
3
4
5
Brachypodium distachyon
An inversion present in P.infirma (absent in P.supina)
1
7
3
5
6
4
2
Poa infirma
1
2
3
4
5
Brachypodium distachyon

B)

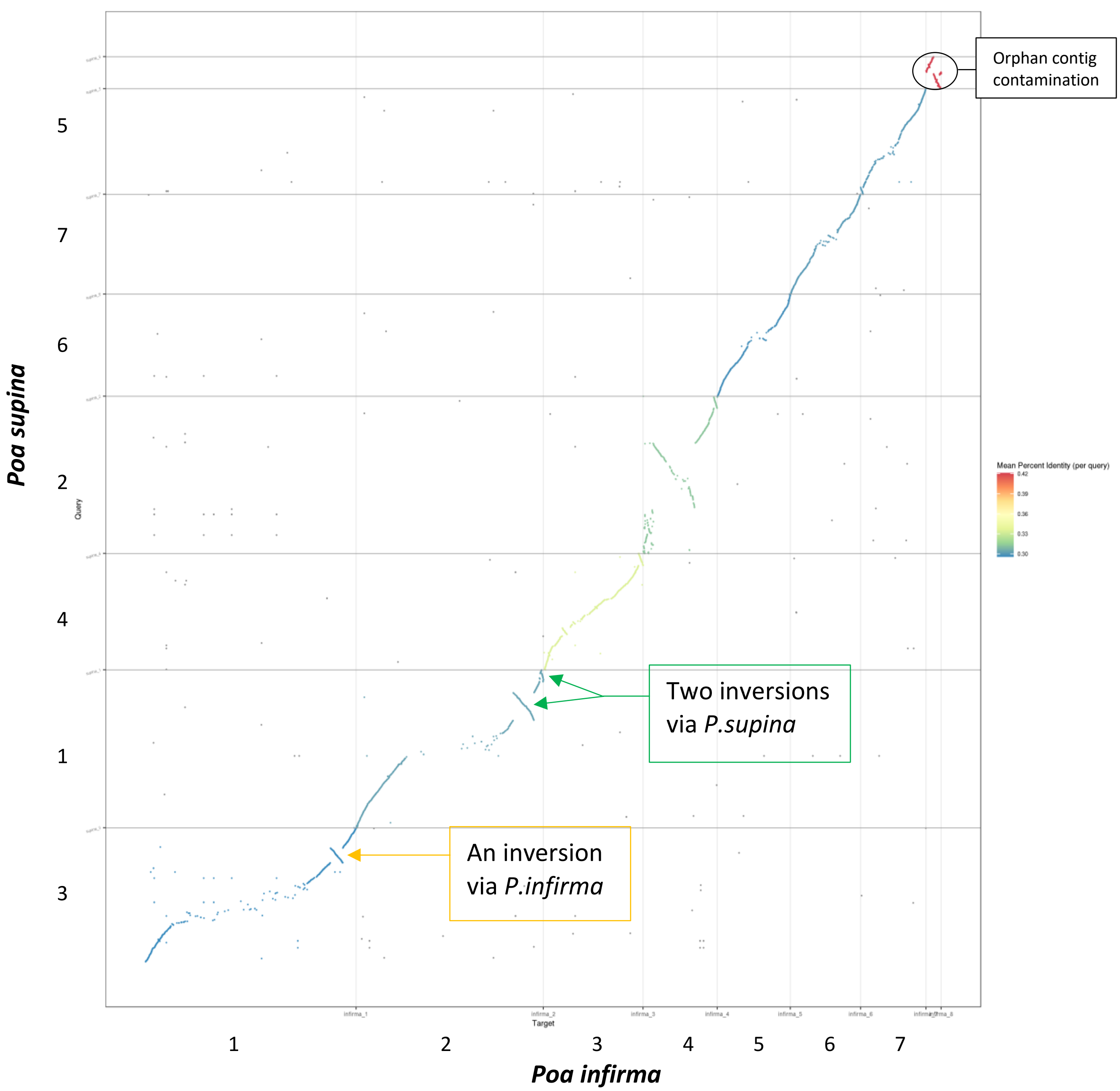


Fig. 2. Dot-plot analysis of Poa genomes. A) The seven whole-chromosome pseudomolecules of *Poa supina* and *Poa infirma* genomes aligned to the five chromosomes of the model grass *Brachypodium distachyon* indicate possible species-specific chromosomal inversions originating in the Poa species. B) The seven whole-chromosome pseudomolecules of *Poa supina* and *Poa infirma* genomes aligned with each other indicating their homologous relationships.

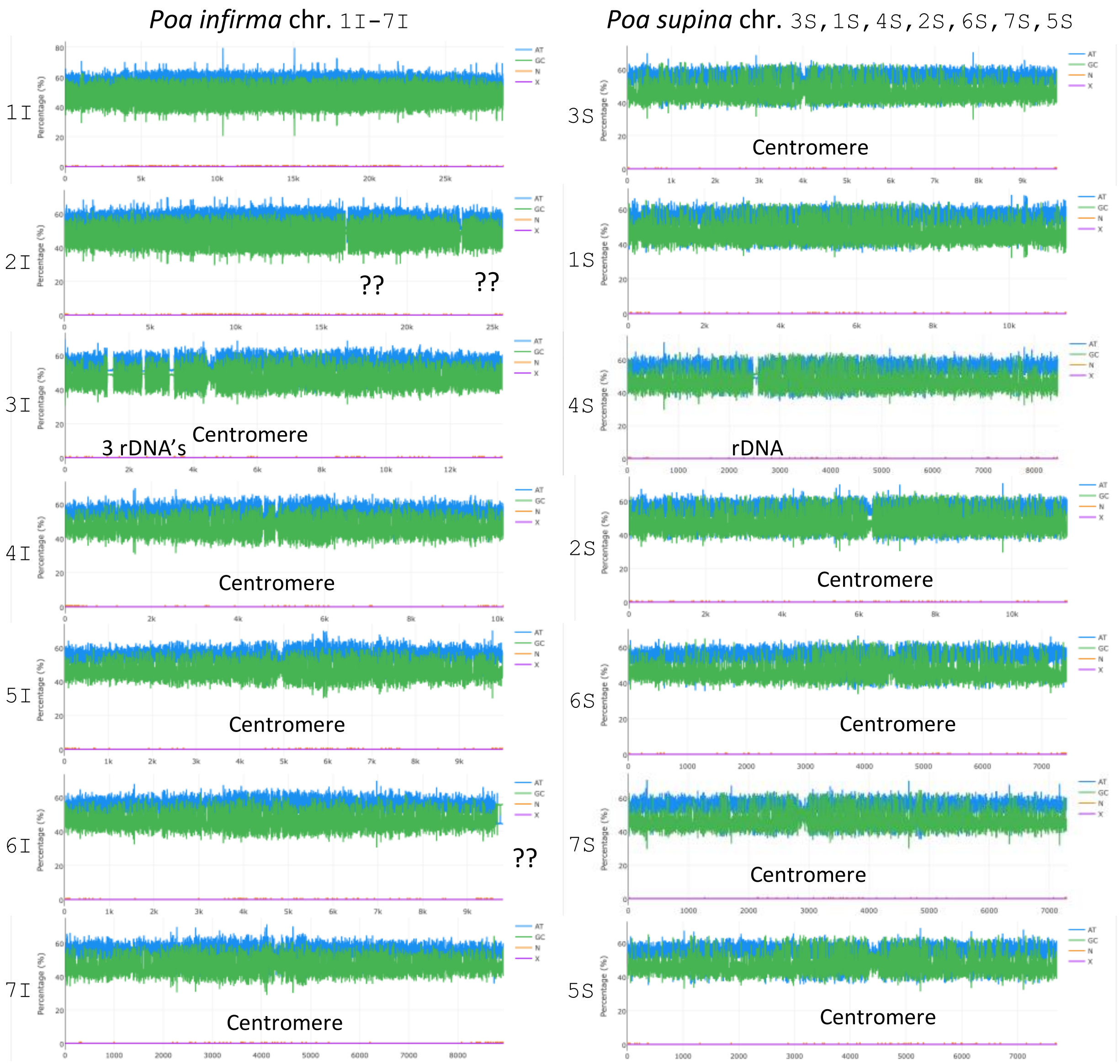


Fig. 3. Percent GC content (green) for a 10K bp sliding, non-overlapping window along each of the 7 whole-chromosome pseudomolecules of *Poa infirma* and *Poa supina*. Predicted chromosome features based on characteristic patterns of GC% content include potential locations for ribosomal DNA and centromeres. Chromosomes of each Poa species in the same row are homologous to one another, ex. 1I is homologous to 3S; 2I is homologous to 1S, 3I is homologous to 4S, etc..

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.

Background and Rationale

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

Methods

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

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

An additional set of 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 tolerance similar to the one conducted previously (Figures 1 and 2). Top performers from this second greenhouse trial will be planted in 2021 within the existing shade field trials.

Early Results

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

In the most recent greenhouse screening trial, variation in response to shade stress was again evident (Fig. 1). Data related to turf quality, leaf elongation rate, leaf angle, and biomass were collected in Oct 2019 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 (Table 1). 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 field study was kept under ambient conditions (no supplemental shade) as the nearby tree shade provided some degree of stress and first year winter kill prevented complete establishment of most entries. By late-summer 2020, most entries that had survived were deemed to be sufficiently established. A more thorough evaluation of these grasses will be completed in 2021.

Future Expectations

Tree shade in the heavily shaded treatment will be supplemented with artificial shade from neutral density fabric beginning in May 2021 unless the project undergoes further winter injury. Ratings of percent cover, normalized difference vegetation index, and turf quality will be collected monthly using digital images, a spectral reflectance meter, and visual ratings. The top 10 to 20 entries from the ongoing greenhouse screening trials will be planted in summer 2021 on an adjacent plot area and subjected to shade using a large polywoven fabric as early as fall 2021.

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

Fig. 1. Greenhouse plants prior to shade stress during the 2020 trial. Over 75 plants were screened for canopy coverage and productivity.

Fig. 2. Overview of selected grasses from 2020 greenhouse trial. Grasses showing variation in green coverage after 8 weeks of perpetual shade.

Table 1. Shoot dry weight for selected bermudagrasses after 8 weeks under artificial shade in the 2020 greenhouse trial.

Entry	Shoot Dry Weight (% control)
30x19	5.1s[Z]
14x19	6.5r-s
39x11	9.7q-s
Uganda	11.3q-s
#62	13.2p-s
4x19	13.9o-s
#72	14.7n-s
PI291591	15.5n-s
3048	15.6n-s
35x7	16.3m-s
TRC	16.4m-s
11x7	17.3m-s
21x20	17.5l-s
C. trans 2	17.7l-s
#34	18.0k-s
15x4	18.4j-s
7x20	18.9j-s
26x2	18.9j-s
30x20	19.1j-s
22x19	19.1j-s
OSU 1318	19.9j-s
43x2	20.0j-s
38x16	20.2j-s
5x3	21.6j-s
15x19	22.6i-s
#115	22.8i-s
40x20	23.3i-s
PI289922	23.4i-s
46x19	23.4i-s
Cardinal	24i-s
Celebration	24.8i-s
24x20	25.0i-s
3x16	25.3h-s
16x1	25.3h-s
#99	25.5h-s
7X8	26.1g-s
1x18	26.2g-s

18x15	26.8f-s
PI615161	26.9f-s
#111	27.0f-s
1115	27.3f-s
18x4	27.5f-s
29x6	27.6e-s
8x4	28.4d-s
PI291981	28.5d-s
OSU 1322	29.1d-s
19x9	29.6d-s
24x17	30.0d-s
22x20	30.2d-s
31x3	30.4d-s
12x10	30.4d-s
1x20	30.5d-s
12x3	31.2d-s
#4	31.4d-s
11x11	31.4d-s
2747	31.5d-s
22x20	32.2d-s
20x2	32.2d-s
4x4	32.4d-s
17x10	32.5d-s
TifGrand	33.0d-s
29x4	33.3d-s
PI291584	34.1d-s
38x9	34.3d-s
19x2	34.7d-s
Northbridge	34.7d-s
2x3	34.7d-s
39x20	34.8d-s
PI290905	35.0d-s
23x20	35.8d-s
C. trans 1	35.9d-s
3x7	36.0d-s
16x20	36.0d-s
36x19	36.8c-r
16x19	37.3c-r
OSU 1310	37.4c-r

15x4	37.7c-r
#61	37.8c-r
3x2	38.5c-q
3x7	39.9c-q
1x7	40.0c-q
21x14	40.3c-q
#29	40.7c-q
OSU1163	41.3c-q
23x17	44.1c-p
25x3	45.0c-p
PI291581	45.2c-o
N-TifEagle	46.1c-n
16x5	47.7c-m
37x3	47.9c-m
Patriot	49.4c-l
PI290812	49.7c-k
PI290894	49.8c-k
16x8	50.2c-j
20x20	54.2b-i
28x19	56.9b-h
OSU 1328	57.5b-g
16x11	57.9b-g
13x20	58.1b-f
PI286584	59.2b-e
Latitude 36	59.3b-d
27x2	67.3a-c
577	82.3ab
15x16	88.5a

[z]Means followed by the same letter are not significantly different (Duncan's multiple range test at $p \leq 0.05$).

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 affiliated with Delaware Valley University, Doylestown, Pennsylvania

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 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 this year.
- Research efforts were disrupted by the pandemic, critical training/learning opportunities for the student were cancelled.
- Two virtual bioinformatics workshops have been completed by the student, two more are in progress.
- RNA degradation and foreign contamination have been an issue but typical at the beginning of these types of studies.

Summary Text:

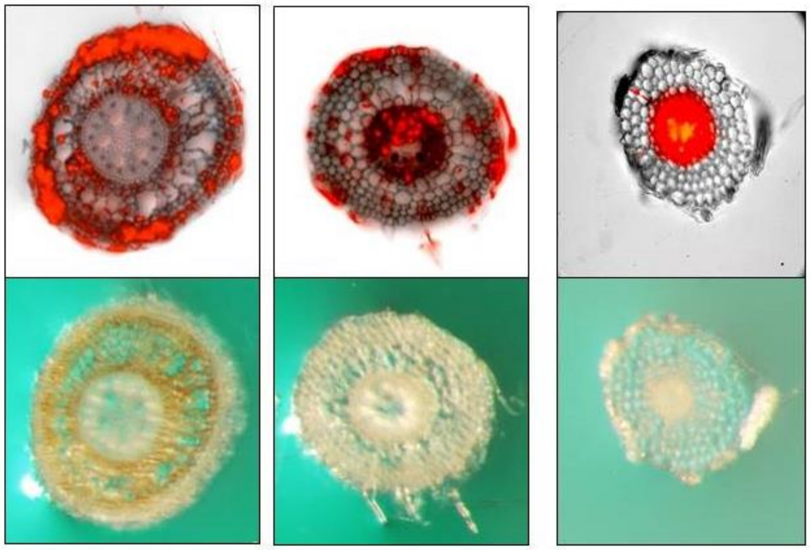
Bermudagrass (*Cynodon dactylon*) and interspecific hybrids of bermudagrass (*C. dactylon* × *C. transvaalensis*) are the predominant turfgrass used for athletic, commercial, and residential urban ground cover in the southern United States. In regions where bermudagrasses enter a cold temperature induced 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), a bioinformatics approach will be used 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 (Figure 3). 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 complement 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 similar to what was done previously.

A Ph.D. student was recruited in late spring and studies with bermudagrass, bentgrass, Kentucky bluegrass, wheat, and *Arabidopsis* species were started, but plagued with low quality RNA yields and often foreign fungal contamination. 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 will continue and will take some several years to complete.

Images:

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



.

Figure 2. 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.

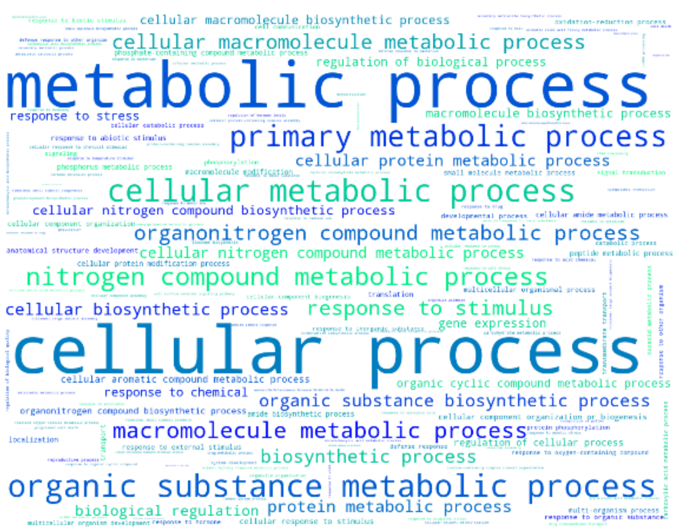


Figure 3. Word cloud representing all enriched Gene Ontology Biological Process terms up-regulated in 'U3' biotype during infection and colonization by *Ophiosphaerella herpotricha*. Font size represents the number of genes observed in each term.

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 still 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 grants funded by the US Golf Association and the Oklahoma Center for the Advancement of Science and Technology. Since 2015, six field trials have been established to test performance under golf putting green management conditions at the OSU Turf Research Center (TRC). In 2017, 2018, and 2019 replicated plots of several experimental selections and two standards (Champion Dwarf and TifEagle) were established for testing disease resistance. In 2018 the 2017-planting was infested with root-knot nematodes (*Meloidogyne marylandi*), leaf spot (*Bipolaris cynodontis*), and the 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.

The 2018 established putting green mowing trial, including 19 OSU experimental selections and three commercial cultivars (‘Champion Dwarf’, ‘TifEagle’, and ‘Sunday’), was continued this year. On the basis of data collection in the past three years, one genotype ‘11x2’ that had decent performance under low mowing heights was selected for further evaluation. This test is uncovered this winter to evaluate freeze tolerance and spring green up in the field and will be terminated in the spring of 2021. We established a 2019 putting green mowing test encompassing 16 OSU experimental selections and four cultivars (Champion Dwarf, TifEagle, ‘Mini Verde’, and Sunday). Unfortunately this new test was damaged by the flooding so we had to re-prepare plant materials in a greenhouse and replant the test in the summer of 2019. This test was continued in 2020 (Figure 1). In addition, 10 new experimental selections as local entries were included in the 2019 NTEP warm-season putting greens trial as field space was available at the OSU TRC. This group of experimental selections constitutes our 6th set of materials for putting green tests. This test was continued in 2020.

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 2020 onward, leaving the trial under ambient rainfall conditions. Optimally timed rainfall events as well as suitable amounts of rainfall prevented the on-set of visual symptoms of drought stress in 2020. This methodology will continue in 2021. Several experimental lines lacked suitable winter-hardiness under field conditions and have been eliminated by winter-kill since inception of the trial.

Another replicated trial established at the Oklahoma Panhandle Research and Extension Center, Goodwell, OK in the summer of 2017 was completed this year. As part of graduate student Mr. Shuhao Yu’s PhD dissertation research (Figure 2), this test included 78 OSU experimental selections and six commercial cultivars (Latitude 36 ™, NorthBridge ™, TifTuf®, ‘Tifway,’ Astro and ‘U3’). Data were collected for spring green up, turf quality, seedhead prolificacy, drought resistance, and fall color retention. The reliability estimates for drought response ranged from low to moderate ($0.20 \leq i^2 \leq 0.63$). The estimates for two spring greenup phenotypes were low (0.27 and 0.08), indicating low reliability of this response and suggesting that winter survivability and drought resistance should be evaluated in separate trials in the future.

Summary Points

- One green-type experimental genotype ‘11x2’ in the 2018 replicated test was selected for further evaluation.
- Three mowing tests were continued while one test was completed.
- Winter survivability and drought resistance in turf bermudagrass needs to be evaluated in separate trials due to the significant low reliability of winter survivability under drought stress.
- Disease resistance testing of green-type bermudagrass selections was continued.

Figure 1. Substantial variation for fall color retention exists in 16 OSU experimental genotypes along with four standard cultivars (Champion Dwarf, TifEagle, Mini Verde, and Sunday) in the 2019 putting green mowing trial (F16) at the OSU Turf Research Center, Stillwater, OK

Figure 2. Mr. Shuhao Yu, Ph.D. student was evaluating drought responses of experimental bermudagrass genotypes in a replicated test at the Oklahoma Panhandle Research and Extension Center, Goodwell, OK

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

Summary Text:

Bermudagrass and zoysiagrass are perennial warm season grasses commonly used as turfgrasses in subtropical and tropical regions of the US and other countries. Under hot, and often drier, conditions these grasses provide high quality turf with limited irrigation. Unfortunately, during cooler winter months in these regions, bermudagrass and zoysiagrass tend to lose their green color. In response, managers often overseed these species with annual or perennial ryegrass to maintain turf quality and quality on athletic fields. Ryegrasses serve to maintain green turfgrass during these cooler months, but also require substantial inputs of irrigation and fertilization, which makes this approach less economically and environmentally sustainable. An alternative to this approach is the identification of genetic variation within bermudagrass and zoysiagrass to retain green color and quality under cooler winter temperatures. Cultivars that possess increased winter color retention would serve as a tool for managers to increase profitability and decrease environmental impact.

With bermudagrass and zoysia grass germplasm from University of Florida, Oklahoma State University, and Texas A&M University, the USDA Forage and Range Research Unit (FRR) in Logan, UT initiated a greenhouse and growth chamber analysis of the cool temperature color retention of these two species. This research complements and supplements similar in field research at University of California at Riverside.

Beginning in fall 2017, the FRR received germplasm from the cooperating universities and initiated cloning to sufficiently replicate plants for the growth chamber evaluations. Plants were cloned to create replicates for the growth chamber cool temperature evaluations. Fifty cell (4.7 cm across × 6.4 cm deep) plant trays were used for the evaluation. Individual clonal ramets from each genotype were randomly assigned to individual cells in a tray. Each plant was replicated three times for the randomized complete block design. Each block was placed in a separate growth chamber. Once cloning was completed, the plants grew in the cells for one month prior to being placed in the growth chambers. After acclimating in the growth chamber for one week, temperatures were decreased by 20 °C to 5 °C over the course of eight weeks. Each week digital images were taken of each flat using a digital camera and a customized light box. While the bermudagrass clones were in the growth chamber, the zoysiagrass genotypes were clones and prepared, in the same manner, for the cool temperature evaluation. The evaluation continued with alternating bermudagrass and zoysiagrass runs until three runs were completed for each species. The 2019 US Government shutdown and the 2020 COVID-19 epidemic slowed and created some complications with the evaluations. However, as of January 2021 the research is on the final run. Digital images are processed weekly, including software to “cut” the image of each individual cell from the overall flat picture and then convert all images to ratings of dark green color index using Turf Analyzer software. Upon completion of the final run all data will be analyzed as a mixed model to determine the relative green color retention of each population.

Initial analyses identified differences among the populations for green color retention. Following the growth chamber studies, we will identify five high and low color retention genotypes from each species and complete a modified bulk segregant analysis using candidate genes to characterize potential genetic controls for this trait in both species. We will also compare the results obtained in the field studies in California to determine the relationship between growth chamber and field results. We hope the result will be a high-throughput methodology to characterize turfgrass species for cool-season color retention.

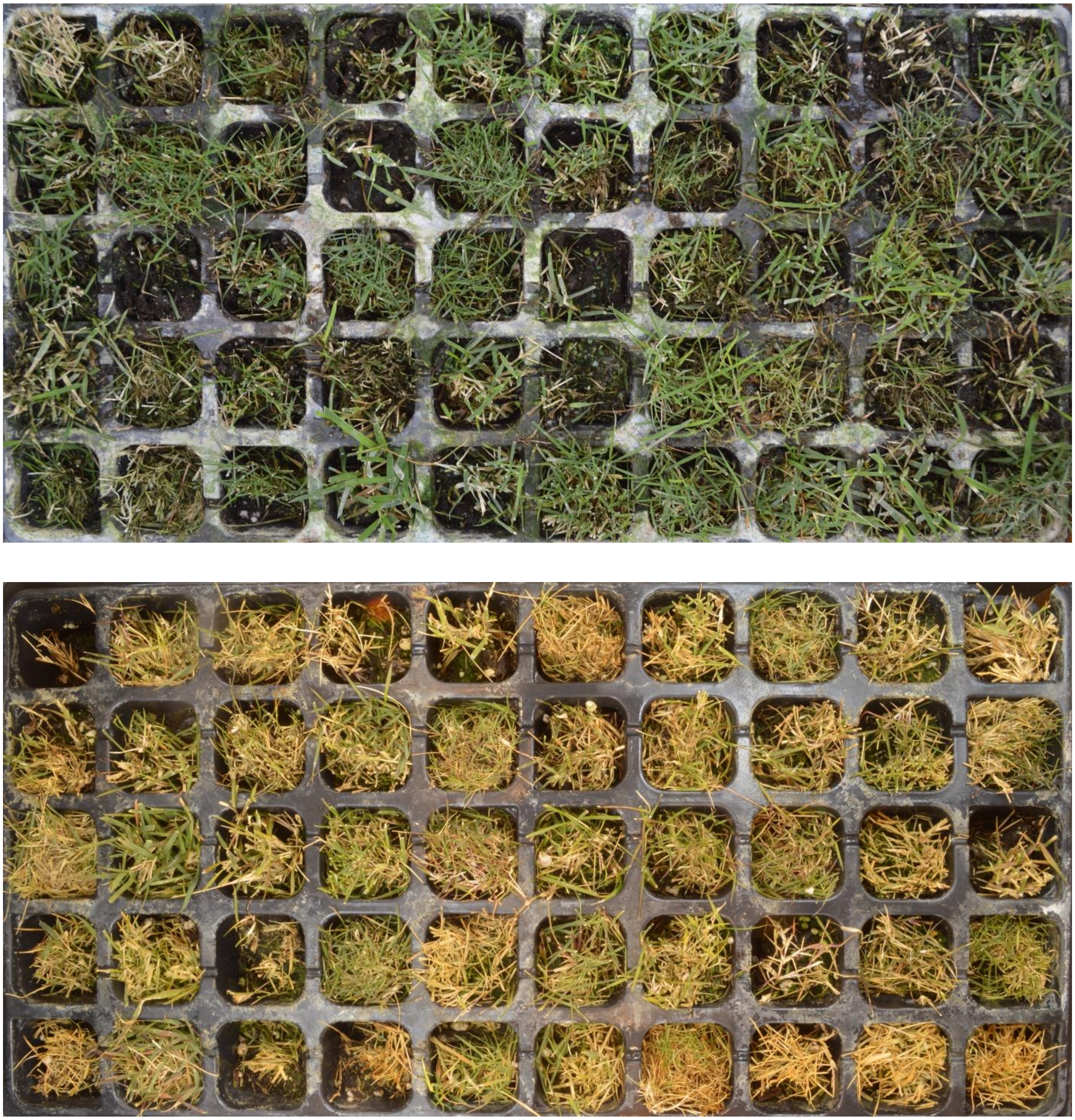


Figure 1. Digital images of trays containing 50 individual genotypes (clones) at different time points in the cool-season color retention evaluation.

USGA ID: 2016-38-608
Title: Breeding for Resistance to Winter Dormancy in Bermudagrass and Zoysiagrass
Project 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 commerical cultivars of bermdagrass and zoysiagrass show separation for fall wear tolerance and resistance to leaf tissue freeze damage.
- 100 lines of zoysiagrass were selected for winter performance and drought tolerance from spaced plant nurseries.
- 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 sting nematodes, drought and large patch resistance.

2017 trials of bermudagrass and zoysiagrass were terminated and the focus is now on 2019 planted trials of both species. The bermudagrass trial contains 27 entries; nine commercial cultivars and 18 experimental lines. The zoysiagrass trial contains 27 entries; 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. Through 2020, the trials completed establishment. During the fall of 2020 plots were split and traffic was applied as one pass of traffic per week for six weeks using a modified Baldree traffic simulator. The initial application of traffic was applied 6 October 2020, occurred weekly thereafter, and the final application occurring 10 November 2020. Visual ratings were initiated near the end of October for turf quality and percent green cover. Ratings continued through early December to capture both regrowth after ceasing traffic and the effects of freezing events. Two significant freeze events occurred for our location during the rating period. Both periods of freezing temperatures lasted for durations of five hours and occurred on the 2nd and 9th of December with lows of 28° F and 31° F respectively. Plots were watered to prevent moisture stress and mowed twice per week at 0.5". In the later half of 2020, fertilizer was applied as follows: 8 August, 10-0-20 was applied at 1/2 lb N per 1000 ft^2; 29 September, 12-0-0 was applied in liquid form at 1/8 lb N per 1000 ft^2; 23 October, 42-0-0 Polyon was applied at ½ lb N per 1000 ft^2.

Bermudagrass

The nine included commercial cultivars of bermudgrass were Tifway, TifTuf, Celebration, Latitude 36, NorthBridge, Tahoma 31, Landrun, Iron Cutter and Bimini. Visual ratings were initiated during the third week of traffic (Oct. 21) and continued for four weeks after the last application of traffic (Dec. 11). Table 1 contains the mean turf quality ratings of bermudagrass by date for each entry and its split treatment of no traffic (N) and traffic (Y). Entries are sorted by their Turf Performance Index (TPI) with traffic (last column). Therefore, the quality of an entry can be followed through fall with and without traffic. The last application of traffic occurred 10 November; therefore, any decrease in quality from 19 November to 11 December was due to the onset of dormancy caused decreasing photoperiod and exposure to low temperatures.

TifTuf and FB1630 were the best performing entries with and without traffic. Both grasses had the highest possible TPI values for TPI NT (no traffic) and TPI YT (yes traffic). Latitude 36 and Celebration were the next best commercial entries. Both were competitive in their maintanance of turf quality under traffic. Additional competitive experimental lines were FB1628, 481-2, 9-6-8 and 343-34.

In the absence of traffic, several entries, 481-2, Latitude 36, Celebration, 9-6-8, and FB1634 maintained acceptable quality until 19 November. However, FB1630 and TifTuf maintained acceptable quality without traffic until 30 November and 4 December respectively. In plots that received traffic, several entries maintained acceptable quality through 29 October (following four traffic applications); however, FB1630 maintained acceptable quality, with traffic, through 30 November.

Figure 1 illustrates the effects of traffic and low temperature stress on the visual percent green cover of bermudagrass. The ratings on 21 October were from non-trafficked plots. At this time, most entries had > 90% green cover. Applications of traffic stopped on 10 November; therefore, the ratings on 12 November provide an indication in the decline in cover following six weeks of traffic applications. Many of the most wear tolerant entries on 12 November were between 75 and 81% cover. This included TifTuf, FB1628, 481-2, 9-6-8, Celebration, Iron Cutter, Tahoma 31, 343-34, and 80V. FB1630 maintained the highest percent green cover after six weeks of traffic with a rating of 88%. The percent cover ratings from 19 November are uniformly higher and indicate the regrowth and recovery potential of each entry after the last application of traffic. Two entries increased above 90% green cover, 481-2 and FB1630, followed closely by Celebraton and TifTuf. The percent green cover ratings on 11 December occurred after two freeze events and additional daily lows in the the 30s. Therefore, the final rating provides an indication of an entrie's ability to maintain green cover through wear and low temperature stress. Many entries lost significant green cover following the freezing temperatures. For example, 19-12-2, went from 75% to 20% green cover between 19 November and 11 December. TifTuf maintained close to 70% green cover indicating that it is both wear tolerant and can maintain leaf tissue integrity through freezing temperatures (i.e. leaf tissue freeze resistance). FB1630, 481-2 and Celebration exhibited a significant loss in percent green cover following the two freeze events indicating that they are not resistant to leaf tissue freeze damage.

Back to TOC

Table 1. Turfgrass quality of 27 bermudagrass entries with and without wear through the fall of 2020 in Citra, FL

	10/21/20 Traffic		**10/29/20 Traffic**		**11/06/20 Traffic**		**11/12/20 Traffic**		**11/19/20 Traffic**		**11/30/20 Traffic**		**12/04/20 Traffic**		**12/11/20 Traffic**			
Entry	**N[§]**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**TPI NT**	**TPI YT**
TifTuf	**7.7[€]**	**6.0**	**8.0**	**6.0**	**8.0**	**5.7**	**7.0**	**5.3**	**6.7**	**5.3**	**7.0**	**5.3**	**6.3**	**5.0**	**5.3**	**5.0**	8.0	8.0
FB1628	**6.7**	**6.0**	6.3	**5.3**	6.3	**5.3**	**6.0**	**5.0**	5.7	**5.0**	5.3	**4.7**	**5.7**	**5.0**	**5.0**	**4.3**	5.0	8.0
FB1630	**7.7**	**7.0**	**7.3**	**6.3**	**6.7**	**6.3**	**6.7**	**6.0**	**7.0**	**6.0**	**6.3**	**6.0**	**5.7**	**5.3**	**4.7**	**4.7**	8.0	8.0
481-2	**7.3**	**6.3**	6.0	**5.3**	6.0	5.0	**6.0**	**5.7**	6.3	**5.7**	**5.7**	**5.3**	**5.7**	**5.0**	**4.3**	**4.7**	5.0	7.0
Latitude 36	**6.7**	5.3	**7.0**	**5.7**	6.3	**5.3**	**6.3**	**4.7**	**6.0**	4.3	**5.7**	**4.7**	4.7	4.0	**4.3**	**4.3**	6.0	7.0
Celebration	**7.7**	**6.0**	**7.3**	**6.0**	6.3	**5.3**	5.7	**5.0**	**6.0**	5.3	5.3	**5.0**	**5.3**	**4.3**	4.0	**4.0**	4.0	7.0
FB1903	**6.0**	**5.7**	5.7	**5.3**	5.3	**5.3**	**6.0**	**5.3**	4.7	4.3	5.0	**5.0**	4.3	4.0	3.7	3.3	2.0	5.0
9-6-8	**6.0**	5.0	**6.7**	**5.0**	**6.7**	5.0	**6.0**	4.3	**6.3**	**5.0**	**6.3**	4.3	**5.7**	**4.7**	**4.7**	**4.3**	8.0	4.0
343-34	**7.0**	**5.7**	**7.3**	**5.7**	**6.7**	5.0	**6.3**	**5.0**	5.7	**5.0**	**5.7**	4.3	5.0	4.0	**4.3**	3.3	6.0	4.0
80V	**6.3**	5.3	**7.0**	**5.7**	**7.0**	5.0	5.7	4.0	5.3	**4.7**	**5.7**	**5.0**	4.7	4.0	4.0	3.3	4.0	3.0
Landrun	**6.0**	5.3	6.0	**5.0**	6.3	4.7	5.0	3.7	5.3	4.3	4.7	3.7	4.7	**4.3**	3.3	**4.0**	1.0	3.0
FB1634	**6.0**	4.7	5.7	4.7	5.3	4.7	5.3	4.3	**6.0**	4.3	5.3	**5.0**	**5.3**	4.0	4.0	**4.0**	3.0	2.0
Iron Cutter	**6.3**	5.3	**7.3**	**6.0**	**7.0**	5.0	5.7	**4.7**	5.0	4.3	4.7	3.3	3.0	3.0	3.7	3.3	3.0	2.0
77V	5.3	5.0	5.0	4.7	5.3	4.3	5.0	3.7	5.3	4.3	4.7	4.0	4.7	4.0	**4.7**	**4.0**	1.0	1.0
343-44	5.7	4.7	6.0	**5.0**	6.3	4.7	5.3	4.0	5.0	3.7	4.7	3.3	4.3	3.0	**4.3**	3.7	1.0	1.0
Tifway	5.7	4.7	**6.7**	**5.3**	6.3	4.7	**6.0**	4.0	5.0	4.0	5.3	4.3	4.3	4.0	4.0	3.7	2.0	1.0
Tahoma 31	**6.0**	5.3	5.7	**5.0**	6.3	5.0	5.3	4.0	5.0	4.3	5.3	4.0	4.0	3.7	3.3	3.0	1.0	1.0
19-12-2	**6.0**	5.3	6.3	**5.0**	6.0	4.7	5.7	3.3	5.0	3.7	5.0	3.0	4.0	2.7	2.3	2.0	1.0	1.0
7-16-57	**6.3**	5.0	6.3	**5.3**	6.3	4.3	5.7	4.0	5.7	4.3	5.7	4.3	4.3	3.7	2.3	2.3	1.0	1.0
78V	5.7	4.7	5.3	4.3	5.7	4.0	5.0	4.0	4.7	3.7	4.7	3.7	4.0	2.7	3.7	3.0	0.0	0.0
NorthBridge	5.3	4.3	5.0	4.0	5.3	4.3	5.3	3.7	4.7	3.3	4.3	3.3	4.3	3.0	3.7	3.3	0.0	0.0
79V	5.7	4.3	5.7	4.3	5.3	3.7	4.3	3.3	4.7	3.7	4.3	3.7	3.7	3.0	3.3	3.3	0.0	0.0
9-6	5.0	4.7	5.0	4.7	5.3	4.7	5.0	4.0	5.0	4.3	4.7	4.3	4.0	3.7	3.3	3.3	0.0	0.0
Bimini	4.7	4.3	4.3	3.7	5.0	3.7	4.3	3.3	4.3	3.3	4.3	3.3	4.3	3.3	3.3	3.0	0.0	0.0
9-6-2	3.3	3.3	4.0	4.0	4.0	3.3	3.7	3.3	3.7	3.3	4.0	3.7	3.3	3.0	3.0	2.3	0.0	0.0
9-3-4	4.7	4.3	4.7	4.3	4.7	3.7	4.3	3.7	4.0	4.0	3.7	3.0	3.0	3.3	2.7	2.7	0.0	0.0
FB1902	3.7	3.3	4.0	3.7	4.0	3.0	4.3	3.0	4.0	2.7	3.7	2.7	3.7	2.3	2.0	2.0	0.0	0.0
lsd ($p < 0.05$)[±]	1.8	1.4	1.7	1.6	1.4	1.3	1.3	1.5	1.2	1.3	1.5	1.4	1.1	1.2	1.0	1.0		

[§]N = No Traffic; Y = Yes Traffic; TPI NT = Turf Performance Index No Traffic; TPI YT = Turf Performance Index Yes Traffic; Turf Performance Index = the number of times an entry fell into the top statistical catergory (bolded means) for a rating date.

[€]Visual ratings of turfgrass quality, where 1 = dead plot; 9 = extremely high quality; and 6 = acceptable quality.

[±]lsd = least significan difference. Turfgrass quality means with a column that differ by more than the lsd value are signficantly different ($p<0.05$).

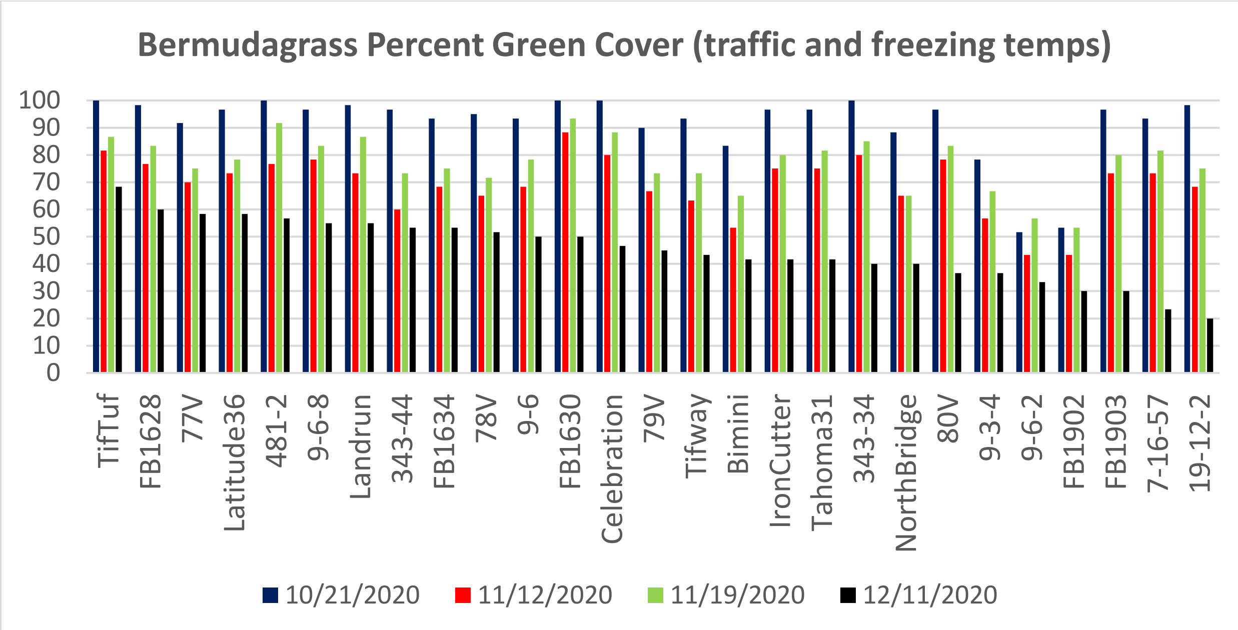


Figure 1. Visual Percent Green Cover ratings of 27 bermudagrass entries in the fall of 2020. In October plots were split between traffic and no traffic subplots. A single pass of traffic was applied using a Baldree traffic simulator on 6, 13. 20 and 27 October, and 3 and 10 November. Visual ratings of subplots were initiated 21 October and continue weekly until 11 December. Freezing temperatures occurred 2 and 9 December. The 21 October rating is from the subplot with no wear to provide a baseline for the effects of wear and freezing temperatures. The 12 November rating is from the trafficked subplot and provides an indication of the effects of weekly traffic treatments for the prior six weeks. The 19 November rating is from the trafficked subplot and provides an indication of recovery potential one weak after stopping traffic. The rating on 11 December is the combined effect of traffic and low temperature stress on percent green cover.

Zoysiagrass

Table 2 contains the mean turf quality ratings of zoysiagrass by date for each entry and its split treatment of no traffic (N) and traffic (Y). Visual ratings were initiated during the fourth week of traffic (Oct. 29) and continued for four weeks after the last application of traffic (Dec. 11). Entries are sorted by their Turf Performance Index with traffic (last column). Therefore, the quality of an entry can be followed through fall with and without traffic. The last application of traffic occurred 10 November; therefore, any decrease in quality from 19 November to 11 December was due to the onset of dormancy caused decreasing photoperiod and exposure to low temperatures.

CitraZoy, FAES1319, and FAES1335 were the best overall entries with and without traffic, achieving the highest possible TPI values (Table 2). These lines were closely followed by FAES1329, FZ1642 and FZ1683 which were equivalent without traffic and very similar with traffic. Entries with good quality without traffic and poor quality with traffic were FZ1440 and FZ1367.

Considering turfgrass quality with and without traffic, more than half of all entries had acceptable turfgrass quality without traffic on 19 November. For subsequent ratings, only five entries continued to consistently produce acceptable quality: CitraZoy, FAES1319, FAES1329, FZ1642 and FZ1683. FAES1319, FAES1329 and FZ1683 were the most consistent and able to maintain quality following the two freeze events. With traffic, 14 entries had acceptable quality on 29 October, following four weeks of traffic. After the fifth week of traffic only five entries had acceptable quality: CitraZoy, FAES1319, FAES1335, FZ1642, and FZ1683. No entries maintained acceptable quality after six weeks of traffic.

Figure 2 illustrates the effects of traffic and low temperature stress on the visual percent green cover of zoysiagrass. The rating on 29 October are from non-trafficked plots. At this time, all but three entries had > 90% green cover, with several near 100%. Applications of traffic stopped on 10 November; therefore, the ratings on 12 November provide an indication in the decline in cover following six weeks of traffic applications. At this point, eight entries maintained > 80% cover, with FAES1319 above 90%. The additional seven traffic tolerant entries were CitraZoy, FAES1329, FAES1335, FZ1642, FZ1680, FZ1682 and FZ1683. As observed with bermudagrass, several zoysiagrass entries exhibited an increase in percent green cover one week after the final application of traffic. Entries with the best regrowth were Zeon, FAES1306, FZ16106, and FZ1721. None of these were among the overall better performing entries. Entries with minimal decreases in green cover following the freeze events were FAES1319, CitraZoy, FZ1643, FZ1680, FZ1727, FZ1683, FZ1367, FAES1306, FZ1436, FZ1722 and FZ1368. These entries exhibit improved resistance to leaf freeze damage. Combining traffic tolerance and leaf tissue freeze resistance, three entries maintained their percent green cover at 80% or higher: CitraZoy, FAES1319, and FAES1329.

In conclusion, entries were identified within both bermudagrass and zoysiagrass that exhibit tolerance to fall applications of traffic and an ability to resist leaf damage from two freeze events. TifTuf, FB1628, FB1630, Latitude 36, 481-2 and Celebration were among the most wear tolerant bermudagrasses. TifTuf was more resistant to leaf tissue freeze damage; however, in the absence of freezing temperatures with traffic, FB1630 maintained acceptable turfgrass quality longer into the fall than any other entries in the study.

CitraZoy, FAES1319, FAES1335, FAES1329, FZ1642 and FZ1683 maintained better turf quality and tolerated traffic compared to other evaluated zoysiagrasses. Several entries appeared to have improved resistance to damage from freezing temperatures. However, those with the best combination of leaf tissue freeze damage resistance and traffic tolerance were CitraZoy and FAES1319.

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. A new 2020 zoysiagrass spaced plant nursery was planted with 1,000 accessions.

Seed was harvested from both bermudagrass and zoysiagrass crossing blocks. This seed will be germinated for new spaced plant nurseries in 2021. A new crossing block of bermudagrass was planted in 2020.

Table 2. Turfgrass Quality of 27 zoysiagrass entries with and without traffic through the fall of 2020 in Citra, FL

	10/29/20 Traffic		**11/06/20 Traffic**		**11/12/20 Traffic**		**11/19/20 Traffic**		**11/30/20 Traffic**		**12/04/20 Traffic**		**12/11/20 Traffic**			
Entry	**N**[§]	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**N**	**Y**	**TPI NT**	**TPI YT**
CitraZoy	**7.7**[€]	**7.0**	**7.3**	**6.3**	**6.7**	**5.3**	**6.7**	**5.3**	**6.0**	**4.7**	**6.3**	**5.0**	**5.3**	**5.0**	**7.0**	**7.0**
FAES1319	**8.7**	**8.0**	**8.0**	**6.7**	**6.3**	**5.3**	**6.3**	**5.3**	**6.3**	**5.0**	**6.3**	**5.7**	**6.3**	**4.7**	**7.0**	**7.0**
FAES1335	**7.3**	**7.0**	**7.0**	**6.3**	**6.3**	**5.3**	**6.7**	**5.3**	**5.7**	**4.7**	**5.7**	**5.0**	**5.7**	**5.3**	**7.0**	**7.0**
FAES1329	**8.0**	6.7	**6.7**	**5.7**	**6.0**	**5.0**	**6.3**	**5.3**	**6.0**	**4.3**	**6.3**	**5.0**	**6.0**	**4.7**	**7.0**	**6.0**
FZ1642	**8.7**	**8.0**	**8.0**	**6.3**	**6.7**	**5.0**	**6.7**	**5.0**	**6.0**	**4.7**	**5.7**	4.3	**6.0**	**5.0**	**7.0**	**6.0**
FZ1683	**8.0**	**7.0**	**7.3**	**6.0**	**6.7**	**4.7**	**6.7**	**5.3**	**6.0**	**5.3**	**6.0**	4.3	**6.0**	**5.3**	**7.0**	**6.0**
FZ1674	**7.7**	**7.3**	6.3	**5.7**	**5.3**	4.0	**5.3**	**4.7**	**5.3**	**4.3**	4.7	4.0	**5.3**	**4.7**	**5.0**	**5.0**
FZ1680	7.0	6.3	**6.7**	5.3	**6.3**	**5.0**	**6.0**	**5.3**	**5.7**	**5.0**	6.0	4.7	4.7	**4.3**	**4.0**	**4.0**
FZ1682	**8.0**	**7.0**	**7.3**	**5.7**	**5.7**	4.3	**5.7**	**4.7**	**5.7**	4.0	**5.3**	4.3	**5.3**	**4.7**	**7.0**	**4.0**
FZ1732	6.7	5.7	6.0	5.0	**5.3**	**4.7**	**6.0**	**5.0**	**5.7**	**4.3**	5.0	4.3	5.0	**4.3**	**3.0**	**4.0**
FZ1723	7.0	6.3	**6.7**	**5.7**	**6.3**	**4.7**	**6.3**	**4.7**	**5.7**	4.0	**5.7**	4.0	**5.3**	4.7	**6.0**	**3.0**
FZ1727	7.0	5.7	**6.7**	5.3	**5.7**	**4.7**	**5.7**	**5.0**	**5.3**	**4.3**	**5.3**	4.3	5.0	4.0	**5.0**	**3.0**
FZ1436	7.0	5.7	**6.7**	5.0	**5.3**	4.0	**6.0**	4.3	5.0	**4.3**	5.0	4.0	**5.7**	**4.3**	**4.0**	**2.0**
FZ1643	7.0	5.7	**7.3**	5.0	**5.7**	4.0	**6.0**	4.0	**5.3**	**4.3**	5.0	3.7	4.7	**4.3**	**4.0**	**2.0**
FZ1667	**8.3**	6.7	**7.3**	5.3	**5.3**	3.7	**6.0**	**4.7**	**6.0**	**4.7**	**5.3**	4.0	**5.7**	**4.7**	**4.0**	**2.0**
FZ1722	**7.3**	6.3	**7.0**	5.3	5.0	4.3	**5.7**	4.3	**5.3**	**4.3**	4.7	4.3	**5.7**	**5.0**	**5.0**	**2.0**
FZ1440	**7.3**	5.3	**7.0**	5.0	**6.0**	4.0	**6.0**	4.3	**5.7**	4.0	**5.7**	3.3	**5.7**	**4.3**	**7.0**	**1.0**
FZ1681	**7.7**	6.7	**6.7**	4.7	5.0	3.7	**5.3**	4.3	5.0	3.3	5.0	4.0	5.0	**4.7**	**3.0**	**1.0**
FAES1305	5.3	4.7	4.7	3.7	4.0	2.7	3.7	3.3	3.7	3.0	3.0	2.3	3.7	3.7	0.0	0.0
FAES1306	7.0	5.3	6.3	5.3	**5.3**	3.3	**6.0**	4.0	5.0	4.0	4.7	3.7	5.0	4.0	2.0	0.0
FZ1367	**7.3**	6.3	**7.0**	5.3	**6.3**	4.0	**5.7**	4.0	**5.3**	4.0	**5.3**	4.3	5.0	4.0	**6.0**	**0.0**
FZ1368	6.3	5.3	6.0	4.7	4.7	3.3	5.0	4.0	4.3	3.0	4.7	3.0	4.7	3.7	**0.0**	0.0
FZ16106	6.0	4.7	5.0	3.3	5.0	3.0	5.0	3.7	4.0	2.7	**4.3**	3.3	4.3	3.7	**1.0**	0.0
FZ1721	5.7	4.7	6.0	4.7	5.0	2.7	**6.0**	4.0	4.7	3.3	5.0	3.7	4.7	3.3	**1.0**	**0.0**
FZ1728	6.7	5.3	**6.7**	4.7	5.0	3.3	5.0	4.0	4.3	3.7	5.0	4.0	4.7	4.0	**1.0**	**0.0**
FZ1742	5.7	4.7	6.0	4.3	5.0	3.7	4.3	3.3	4.7	3.7	3.7	3.0	3.7	3.3	**0.0**	**0.0**
ZEON	6.0	4.3	5.0	4.0	4.0	2.3	4.0	3.3	4.0	3.3	3.3	2.3	4.0	3.0	**0.0**	**0.0**
lsd[±] **(p<0.05)**	1.5	1.1	1.6	1.1	1.5	1.0	1.3	0.8	1.3	1.1	1.3	0.9	1.2	1.2		

[§]N = No Traffic; Y = Yes Traffic; TPI NT = Turf Performance Index No Traffic; TPI YT = Turf Performance Index Yes Traffic; Turf Performance Index = the number of times an entry fell into the top statistical catergory (bolded means) for a rating date.

[€]Visual ratings of turfgrass quality, where 1 = dead plot; 9 = extremely high quality; and 6 = acceptable quality.

[±]lsd = least significan difference. Turfgrass quality means with a column that differ by more than the lsd value are signficantly different (p<0.05).

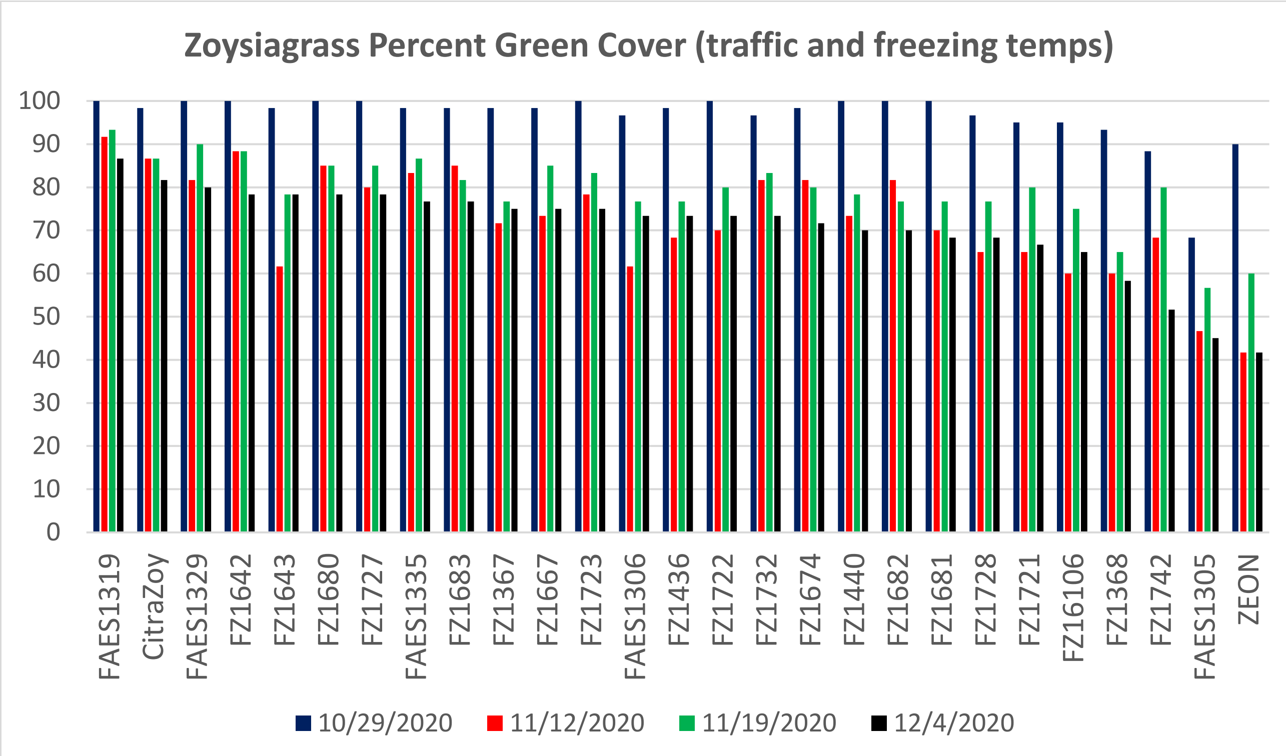


Figure 2. Visual Percent Green Cover ratings of 27 zoysiagrass entries in the fall of 2020. In October plots were split between traffic and no traffic subplots. A single pass of traffic was applied using a Baldree traffic simulator on 6, 13. 20 and 27 October, and 3 and 10 November. Visual ratings of subplots were initiated 29 October and continued weekly until 11 December. Freezing temperatures occurred 2 and 9 December. The 29 October rating is from the subplot with no wear to provide a baseline for the effects of wear and freezing temperatures. The 12 November rating is from the trafficked subplot and provides an indication of the effects of weekly traffic treatments for the prior six weeks. The 19 November rating is from the trafficked subplot and provides an indication of recovery potential one weak after stopping traffic. The rating on 11 December is the combined effect of traffic and low temperature stress on percent green cover.

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

- Evaluation process of Bermuda hybrids planted in 2018 and 2019 identified 73 entries suitable for different purposes and further tests. The selection process continues.
- Study including 21 UCR hybrids and 4 commercial cultivars at the Shadow Creek GC, Las Vegas, NV was established in July 2020 to evaluate performance in a harsher climate.
- 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. Cv. Latitude 36 and #17-8 are the best performers so far.
- Thirty-one of best performing kikuyugrass hybrids have been selected for further evaluation in larger test plots in 2021.
- Testing and selection of bermudagrass and kikuyugrass for drought tolerance was initiated in 2019 and continued in 2020.
- New cooperation with other warm-season grass breeding programs in the United States has been established under the Specialty Crop Research Initiative (SCRI) project.

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 will be on genetics and genomics in this project.

Bermudagrass

In addition to the existing collection of seven *Cynodon* species (over 100 accessions), a collection of bermudagrass genotypes from the University of Florida (195 accessions) and Oklahoma State University (350 accessions) was planted in 2016 and maintained in years 2017-2020. The collection is continuously supplemented with samples collected locally or donated to us by others. As in previous years, new hybrids are being produced; in 2020 864 new hybrids were planted. They are being screened for color retention and turf quality, together with collection accessions and 1634 hybrids planted in previous years. Based on this evaluation, 48 hybrids were selected for further testing in larger plots at a fairway mowing height, 19 hybrids for roughs/lawns and six for greens. These hybrids are currently propagated for establishment in test plots next year. Top performing collection accessions were again intercrossed in 2020 (detached tiller crosses).

Dry-down tests continued in 2020. This study includes 71 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. Two cycles of drought and recovery were performed in 2020. The results from 2019 and 2020 indicate high drought tolerance of several UCR hybrids. While high variation between the years was observed, many of our hybrids performed better in both years than the checks. The best UCR hybrids, ranking in top 10 in both years, were UCRC180040 and UCRC180229. The best commercial cultivar in both years was ‘Celebration’. Some hybrids appeared to be “primed” to respond to future stress, going dormant quicker in the second cycle.

The study of suitability of 12 UCR bermudagrass hybrids and three commercial cultivars for roughs and homeowner use, planted in 2019, continued in 2020. Entries were tested at the West Coast Turf in Coachella Valley (Thermal, low desert) and at the Santa Lucia Preserve in Carmel-by-the-Sea (Northern California, Monterey area). The highest quality at both locations was shown by ‘Bandera’ and BH 19-2, followed by BH 16-4 and BH 17-1. All evaluated bermudagrasses, except for ‘Midiron’ and BH 3-1, retained good color during both winters in Coachella Valley. At the Santa Lucia Preserve, winter dormancy was observed for all evaluated entries. As of November 2020, BH 30-9 showed the darkest green color, while ‘Bandera’ was the only entry almost completely dormant.

The fairway study planted in 2019 in Northern California at the Napa Golf Course in Napa and the Almaden Golf & Country Club in San Jose continued only at Napa GC, due to reasons beyond our control. 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. Both of them showed good quality throughout the year. In terms of winter color retention the best entries were 17-8, ‘TifTuf’, ‘Santa Ana’, TP 6-3, BF2 and ‘Latitude 36’, however ‘TifTuf’ was prone to flowering. ‘Celebration’ was the worst performing entry both in quality and winter color retention, followed by ‘Tahoma 31’.

The latest trial, planted in July 2020, is located at the Shadow Creek GC, Las Vegas, NV and is focused on winter color retention and spring green-up of the newest UCR hybrids showing good quality and color retention in Riverside. Twenty-one hybrids were selected for this study and will be compared to four cultivars: ‘Latitude 36’, ‘Santa Ana’, ‘Tahoma 31’ and ‘TifTuf’.

Based on performance in previous and ongoing studies, 17-8 and TP 6-3 were selected to be released as commercial cultivars. Additional data on their morphological and other characteristics are currently being collected.

Kikuyugrass

Evaluation of kikuyugrass hybrids planted in 2019 continued in 2020. Selection for quality, lower vigor, finer texture, less intensive flowering and good color retention identified 31 hybrids and accessions. These will be planted in 2021 in larger test plots for further evaluation. These hybrids will be also used for self-pollination to create lines with enhanced desirable traits for future crosses and selection.

The kikuyugrass dry-down study started in early summer of 2020. 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. Plots were established in June 2019 in three replicates, with two cycles of drought and recovery applied between June and October 2020. Kikuyugrass accessions entered dormancy faster than bermudagrass, however, their recovery was very fast. Reduction of green cover in the second drought period was much quicker than in the first one in all tested accessions. Several accessions showed better green cover retention than commercial checks in the first drought period, however during the second period only WT 19 and OPED 08 outperformed ‘AZ-1’.

Zoysiagrass

Evaluation of a large collection of zoysiagrass genotypes from the University of Florida (155 accessions) and Texas A&M (219 accessions) planted in 2016 continued. Color retention and the overall quality of these genotypes were evaluated visually. Considerable variation in both characters was observed among zoysiagrass genotypes with the average color values being higher than those of bermudagrass.

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 at the UCR Agricultural Operations field in Riverside, CA, and will be evaluated for the overall performance and tolerance to various stresses. UCR is the testing site for the overall performance, drought and salinity tolerance. In addition, 20 of UCR bermudagrass hybrids will be tested at six locations for the overall quality, and at UGA and UCR for drought tolerance.

Refereed Publications

Pudzianowska, M, Mock, TJ, Martin, PM, Lukaszewski, AJ, Baird, JH. Kikuyugrass germplasm collections in the United States and Australia show low levels of genetic diversity as revealed by DArTseq genotyping. Crop Science. 2020; 60: 2768– 2781. https://doi.org/10.1002/csc2.20231

Pudzianowska, M. and Baird, J.H. (2020), Genetic diversity and species-specific DNA markers of Cynodon Rich. Crop Sci.. Accepted Author Manuscript. https://doi.org/10.1002/csc2.20369

Figure 1. Bermudagrass hybrids and commercial cultivars at the Shadow Creek Golf Course, North Las Vegas, NV. Photo taken on 17 August 2020.

Figure 2. Bermudagrass genotypes from UCR and commercial cultivars on a fairway at the Napa Golf Course in Napa, CA. Plots were established on 22 May 2019. Photo taken on 17 January 2020. UCR 17-8 at bottom left, 'Santa Ana' at bottom right corner. Above them in adjacent plots 'Celebration' and UCR TP 6-3 (from left to right).

Figure 3. Kikuyugrass dry-down at UCR Agricultural Operations field in Riverside, CA, at the end of the first drought period. Photo taken on 23 July 2020.

Figure 4. Zoysiagrass lines in a single space planting nursery in SCRI-NIFA project at UCR Agricultural Operations field in Riverside, CA. Photo taken on 23 September 2020.

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 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 (650) could not be planted. They will instead be held over in the greenhouse until the spring of 2021. A total of 24 new selections (12 red and 12 yellow) were made from the 2017 Isolation Blocks and the 2015 SPN and planted in 2019 Isolation Blocks. Two of the red seed parents have not grown well in the isolation block and neighboring entries have overgrown those plots so the functional number of red seed parents is 10 while the yellow seed parents remains at 12. Seed from those isolation blocks will be harvested in spring 2021.
- 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 11 medium/coarse textured lines for recombination in an isolation block.

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. Lower level of maintenance requirements as well as shade tolerance compared to other turfgrasses makes zoysiagrass a desired option 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.

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 on 23 and 28 May 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 (Figure 1A) 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 (Figure 1B). 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 new plans are for field planting in the spring of 2021.

Percent germination for individual seed lots of the 26 families was evaluated by making three replications of 50 seed from each pedigree and germinating on moistened filter paper in a 100 x 20 mm petri dish. Percent germination results are presented in Table 1. Several of our top parental lines were comparable to 'Compadre'. Most notable are 6585-34 and 6596-05 which also had good traits such as seedhead exertion above the leaf canopy as well as good seedhead density contributing to seed yield potential.

In 2019 two new 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 have expanded to fill their plots (Figure 1C). Seed from the 2019 Red and Yellow Isolation Blocks are scheduled to be harvested in the spring of 2021.

In 2018, we entered a collaboration with Johnston Seed with the transfer of vegetative material from our most advanced synthetic parents. On 31 July 2018, one - 18 cell tray of each of 13 synthetic parents with intermediate texture were shipped to Johnston Seeds on MTA for evaluation for cold hardiness. Those parental lines were planted in Enid, OK on 2 Aug. 2018. In addition, 535 progeny from 16 medium coarse textured families were planted on 6 July 2018 for evaluation under their growing conditions. Data collected by Dr. Kevin Kenworthy at Johnston Seed Co. in 2019 and 2020 has allowed them 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 will be planted in Enid, OK and Dallas, TX in 2021 for evaluation of seed yields and other performance characteristics. Furthermore, an evaluation of the 535 progeny in the medium coarse textured spaced plant nursery led to the identification of 11 potential lines as seed parents with a texture classification of medium coarse to coarse. These 11 selected seed parents will be allowed to recombine in an isolation block starting 2021 in Enid OK and Dallas, TX.

Table 1. Percent seed germination of 26 seeded zoysiagrass families scarified with 30% NaOH.

Entry	Percent Germination[1]	
6585-34	78.0	A
6612-15	70.7	AB
6597-41	69.3	AB
Compadre	69.3	AB
6596-05	58.0	BC
6593-10	50.7	CD
6603-12	48.7	CDE
Red #1 Synthetic	48.7	CDE
Yellow Synthetic	48.0	CDEF
6595-18	45.3	CDEFG
6603-16	42.7	DEFGH
Zenith	41.3	DEFGHI
6611-18	38.7	DEFGHIJ
6600-10	37.3	DEFGHIJ
6610-36	35.3	EFGHIJK
6596-42	33.3	FGHIJKL
6618-31	31.3	GHIJKLM
6598-38	29.3	HIJKLM
6617-36	29.3	HIJKLM
6600-23	27.3	IJKLMN
6616-35	25.3	JKLMN
6599-05	20.7	KLMNO
6598-02	18.7	LMNOP
6594-09	16.7	MNOP
6596-34	12.7	NOP
6617-17	10.0	OP
6609-24	7.3	OP
Red #2 Synthetic	4.0	P
LSD[2]	15.2	
C.V.[3]	24.7	

[1]Percentage of germination was based on seedling growth of 50 seeds in each of 3 replications
[2]Means were separated using Fisher's Least Significance Difference Test ($P<0.05$). Means with an 'a' rating were in the top statistical group.
[3]C.V. (Coefficient of Variation) indicates the percent variation of the mean in each column.

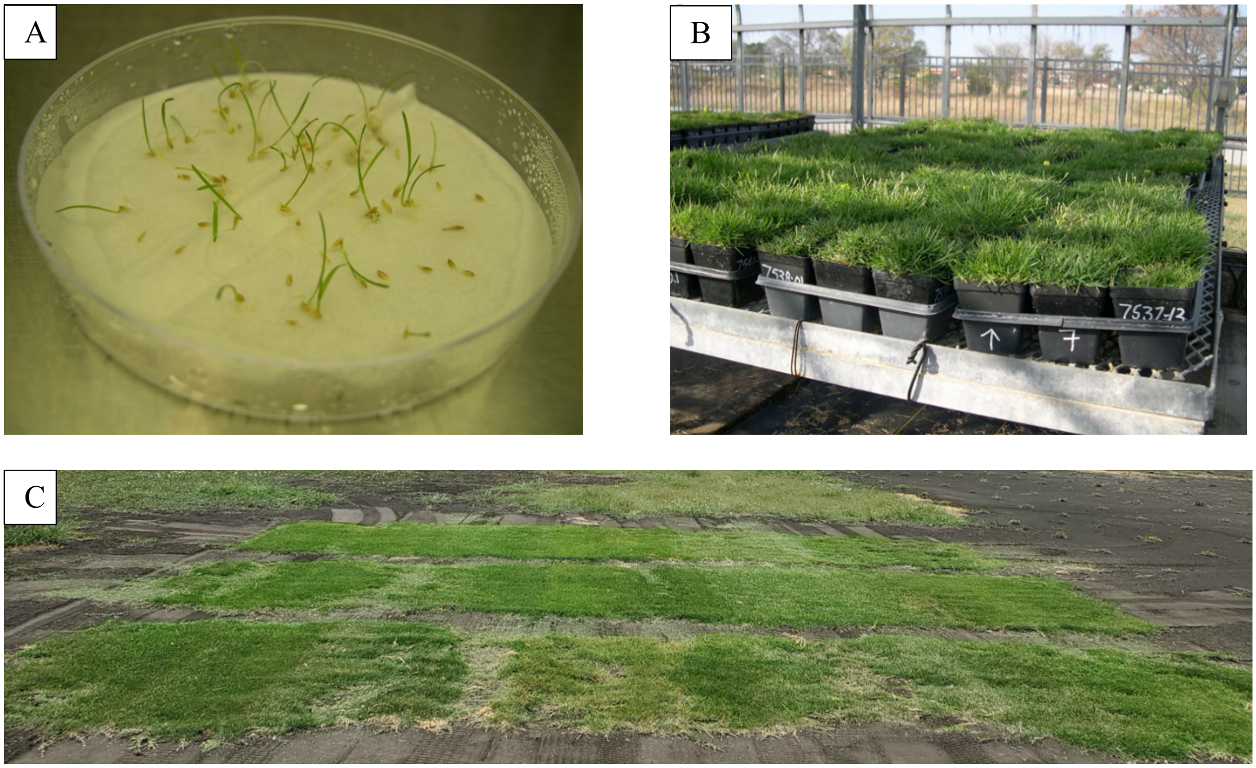


Figure 1. (A) Germination testing of seeded zoysias on moistened filter paper in a 100 x 20 mm Petri dish; (B) Seedlings resulting from the germination of 26 seed lots destined for planting in the 2021 Spaced Plant Nursery; (C) 2019 Yellow Isolation block containing three randomized replications of 12 parents photographed on 11 Apr 2020 at spring greenup (C).

References:

Patton, A. J., Reicher, Z. J., Zuk, A. J., Fry, J. D., Richardson, M. D., and Williams, D. W. 2006. A guide to establishing seeded zoysiagrass in the transition zone. Online. Applied Turfgrass Science doi:10.1094/ATS-2006-1004-01-MG.

Murray, J.J. and K. Morris. 1988. Establishing and maintaining zoysiagrass. Grounds Maint. 23:38-42.

Yeam, D.Y., Murray, J.J., Portz, H.L. and Joo, Y.K. 1985. Optimum seed coat scarification and light treatment for the germination of zoysiagrass (*Zoysia japonica* Steud.) seed. J. Kor. Soc. Hort. Sci. 26(2): 179-185.

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

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

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

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

Objectives:

1. **Phase I (year 1): 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. Notes were taken for those entries that exhibits no visible symptoms of large patch or billbug feeding because of the natural infestations, as well as any other stressors that were observed. (Tables 1, 2, 3a and b) Phase II has been completed.

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 will be propagated into 11 18-cell trays in Dallas during the winter of 2020/2021. This propagated material will be used for field evaluation in the form of replicated field trials (RFT) planted in summer 2021 at Dallas, Olathe, West Lafayette and seven additional environments. RFT in Dallas will be conducted under full sun as well as shade (63% PAR reduction). Among the many traits that are used to characterize turfgrasses (e.g. TQ, Spring Greenup, Genetic Color, etc.), they will also be evaluated for tolerance to large patch disease in Olathe, KS and to hunting billbug in West Lafayette, IN. RFTs planted with cooperators at seven additional locations in the transition zone will include these 65 elite hybrids and will be evaluated for three years 2021, 2022 and 2023.

Summary Points:
Phase II: Data were collected for progeny in spaced plant nurseries planted at three locations in 2018/2019/2020: Olathe, KS (where we planted 1,370 hybrids, 5.1% survived the winters and 20 were advanced); West Lafayette, IN (we planted 1,624 with 20.7% survived and 25 were advanced); and Dallas TX (where we planted 1,636 with 72.2% survived and 20 were advanced) (Table 1, 2, 3a and b). The objective of Phase II field testing was the selection of experimental lines that have comparable/superior cold tolerance to that of Meyer with tees to greens turfgrass quality for the golf industry in the transition zone. Particular attention was placed on the advancement of entries that exhibited no visible symptoms of large patch disease or hunting billbug susceptibility.

Summary Text:

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

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

TAM AgriLife, KSU and Purdue University have been working on a USGA-funded project since 2012 where the main objective is to incorporate large patch (*Rhizoctonia solani* AG 2-2LP) 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 with the identification of the top three entries as our goal.

For the current project, we have initiated 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 develop cold hardy zoysiagrasses with quality suitable for golf course fairways, tees, and putting greens. In addition to cold hardiness and turfgrass quality, experimental hybrids will also be evaluated for large patch, hunting billbug and shade tolerance.

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

Progeny populations were produced at TAM AgriLife-Dallas and shared with our collaborators located at Olathe, KS and West Lafayette, IN (Phase I). Phase II was begun when three spaced plant nurseries were planted: (1) summer 2018 in West Lafayette, IN by Aaron Patton and Ross Braun, (2) in 2017 and 2018 in Olathe, KS by Jack Fry and Manoj Chhetri and (3) in 2017 and 2018 in Dallas, TX by Ambika Chandra, Dennis Genovesi, Meghyn Meeks and Tianyi Wang. At West Lafayette, IN out of 1,624 progenies planted, 336 (20.7 %) survived (Figure 1A). Of the 336, there were a range of differences in leaf texture (8.0 to 3.5), genetic color (8.0 to 4.3), summer quality (7.5 to 4.9), fall turf cover (75.5 to 10%) and fall color retention (8.0 to 3.0) (data not shown). In Olathe, KS, out of 1,370 progenies planted only 70 (5.1%) survived with a range of different leaf textures (7.5 to 3.5) and vigor (8.0 to 2.0) (Figure 1B). In Dallas, TX, we planted 1,633 with 1,188 (72.7%) surviving since the climate is kinder to hybrids with less cold hardiness, we saw a range of ratings for survival (100 to 14.3%), spring greenup (4.3 to 2.0), establishment (5.2 to 2.3) and turf quality (8.0 to 2.0) (data not shown).

With the additional data taken at the three locations for 2019/2020 winter recovery and 2020 spring and summer growth and performance, dry-down (no supplemental irrigation) in Dallas during the summer 2020 (Figure 2), the top entries have been identified. From West Lafayette 30 hybrids have been identified from the 336 survivors (Table 1) and 25 will be tested in the 2021 RFT. Among the advanced lines are those with *Zoysia matrella*, *Z. pacifica* and *Z. minima* in their pedigrees. From Olathe 22 hybrids have been identified (Table 2) from 70 survivors with one *Z. pacifica* derivative. The top 20 will be field tested in 2021. Thirty-seven percent of the entries tested at West Lafayette were also tested at Olathe. Of those 37%, entries 6844-152 and 6844-154 both made the top entries list for the two locations. And from Dallas 20 hybrids have been identified for advanced testing (Table 3a and b) (15 from the 2017 SPN and 5 from the 2018 SPN) out of 1,188 survivors with 16 having *Z. pacifica* in their pedigrees. Note that complex hybrids were made in order to introgress desirable genes into a cold hardy background and are illustrated with the linages shown in Tables 1, 2 and 3a and b. Examples are shown in Table 1 where 6836-09 is a triple cross hybrid crossed by triple cross hybrid. 6844-xx in all three tables is an illustration of a single cross hybrid crossed by a triple cross hybrid. 6933-11 in Table 1 is an example of a double cross hybrid crossed by a double-crossed hybrid. Sod from each entry has been harvested and all 52 hybrids from Olathe, KS and West Lafayette, IN have been transferred to Dallas for propagation during the winter of 2020/2021. An 18-cell tray of each of the 65 entries will be shipped/delivered in the spring to each of the 10 locations for field testing in a replicated field trial with 6 plugs evenly spaced and planted per replication.

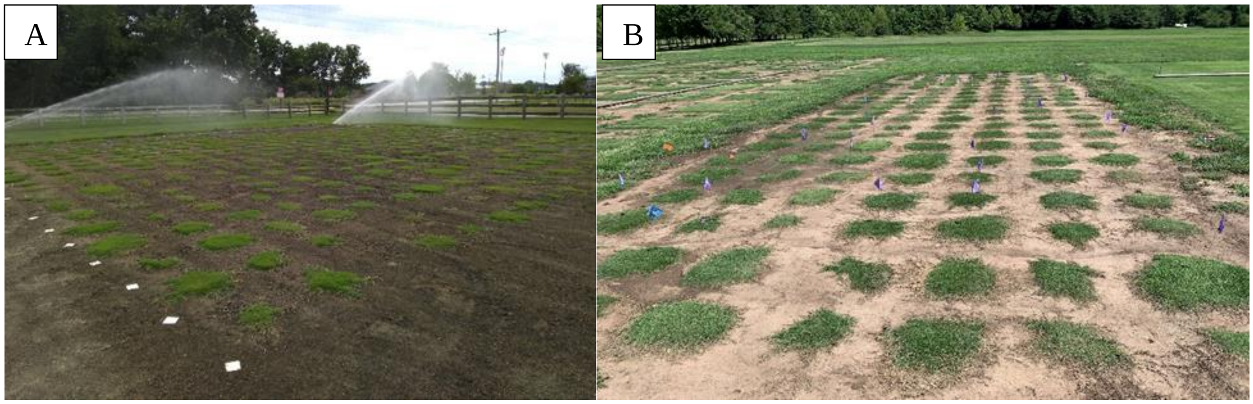


Figure 1. A. Tees to Greens Spaced Plant Nursery located in West Lafayette, IN taken July 2020. B Tees to Greens SPN located in Olathe, KS taken July 2020.

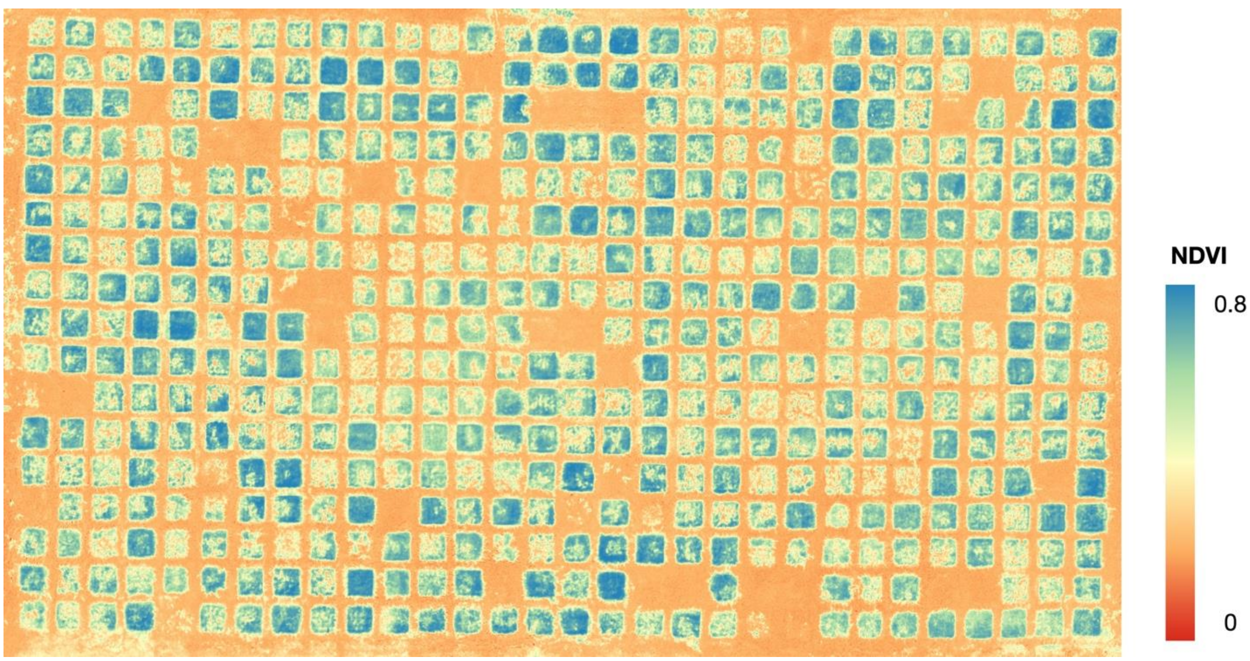


Figure 2. NDVI image derived from a multi-spectral sensor mounted on an unmanned aerial vehicle (UAV). The image was taken on 08/17/2020, 11 days into the 2nd dry-down of 2020 in Dallas, TX. NDVI is presented on -1 to +1 scale with values closer to 1 (more blue) represent higher vegetation and overall higher plant health. Plots with higher NDVI values were considered for advancement.

References:

Chandra, A., J. D. Fry, A. D. Genovesi, M. Meeks, M. C. Engelke, Q. Zhang, D. Okeyo, J. Q. Moss, E. Ervin, X. Xiong, S. Milla-Lewis, J.T. Brosnan, J. Griffin, and L. Parsons. 2017. Registration of 'KSUZ 0802' Zoysiagrass. J. Plant. Reg. 11:100-106. doi:10.3198/jpr2016.03.0010crc

Fry, J.D. and Dernoeden, P. 1987. Growth of zoysiagrass from vegetative plugs in response to fertilizers. J. Amer. Soc. Hort. Sci. 112:286–289.

Grau, F.V., and A.M. Radko. 1951. Meyer (Z-52) Zoysia. USGA J. Turf Manag. 4:30-31.

Okeyo, D., J. Fry, D. Bremer, C. Rajashekar, M. Kennelly, A. Chandra, D. Genovesi, and M. Engelke. 2011. Freezing tolerance and seasonal color of experimental zoysiagrasses. Crop Sci. 51:1-6.

Patton, A.J. 2009. Selecting zoysiagrass cultivars: Turfgrass quality, growth, pest and environmental stress tolerance. Appl. Turfgrass Sci. doi:10.1094/ATS-2009-1019-01-MG.

Table 1. Lineage and performance of top 30 interspecific hybrids selected from Purdue University from a spaced plant nursery planted with 336 genotypes in 2019 from the existing 1624 genotypes planted in 2018 in West Lafayette, IN.

		Mean TQ‡	Mean LT‡	Mean GC‡	Mean TD‡	Mean WK‡	SGU‡	FC‡
Genotype	Lineage†	2019-2020	2019-2020	2019-2020	2020	2019-2020	2020	2019
6836-09	[(Zmin x Zm)/Zm] x [(Zj x Zp)/Zj]	7.9 a	7.8 bc	8	9.0 a	5.3 c-h	5	7
6844-152	[Zm x Zj] x [(Zj x Zp)/Zj]	7.7 ab	6.0 h	6	8.5 ab	7.0 a-d	5	6
6844-42	[Zm x Zj] x [(Zj x Zp)/Zj]	7.7 ab	7.0 def	6.5	8.5 ab	6.5 a-e	5	6
6829-02	[(Zj x Zp)/Zj] x Zm	7.6 abc	7.3 cde	7.5	8.5 ab	4.3 e-i	5	6
6828-53	Zm x [(Zj x Zp)/Zj]	6.4 d-g	7.5 bcd	7	6.5 cd	3.5 ghi	4	7
6844-36	[Zm x Zj] x [(Zj x Zp)/Zj]	6.9 a-g	6.5 fgh	7	8.5 ab	6.0 b-f	5	6
6828-56	Zm x [(Zj x Zp)/Zj]	6.9 a-g	7.3 cde	7	7.0 bcd	5.0 d-i	5	6
6830-02	(Zm x Zj) x Zm	7.1 a-f	7.3 cde	7	7.0 bcd	2.8 i	3	6
6844-104	[Zm x Zj] x [(Zj x Zp)/Zj]	7.6 abc	7.0 def	6.5	8.5 ab	8.5 a	5	4
6835-33	(Zm x Zj) x Zp	6.0 g	7.5 bcd	7	7.0 bcd	2.8 i	2	5
6828-27	Zm x [(Zj x Zp)/Zj]	7.6 abc	6.8 efg	8.5	7.0 bcd	6.0 b-f	5	8
6828-77	Zm x [(Zj x Zp)/Zj]	6.9 a-g	7.0 def	6.5	7.5 a-d	4.0 f-i	4	8
6840-20	Zm x [(Zj x Zp)/Zj]	6.1 fg	7.3 cde	6.5	6.0 d	2.8 i	3	5
6933-11	(Zm x Zj) x (Zm x Zj)	7.3 a-e	7.0 def	6.5	8.0 abc	7.0 a-d	6	7
6830-39	(Zm x Zj) x Zm	6.4 d-g	7.8 bc	6	7.5 a-d	2.8 i	2	5
6844-04	[Zm x Zj] x [(Zj x Zp)/Zj]	7.3 a-e	6.0 h	7.5	8.5 ab	8.0 ab	6	6
6844-74	[Zm x Zj] x [(Zj x Zp)/Zj]	7.7 ab	6.8 efg	7.5	9.0 a	6.5 a-e	6	5
6844-202	[Zm x Zj] x [(Zj x Zp)/Zj]	7.6 abc	7.0 def	7	9.0 a	4.5 e-i	5	5
6829-69	[(Zj x Zp)/Zj] x Zm	7.1 a-f	7.3 cde	6	8.0 abc	4.0 f-i	3	6
6923-11	(Zm x Zj) x (Zj x Zj)	7.4 a-d	6.3 gh	6.5	8.5 ab	6.5 a-e	5	6
6844-53	[Zm x Zj] x [(Zj x Zp)/Zj]	7.1 a-f	7.3 cde	7.5	7.5 a-d	7.5 abc	6	5
6844-64	[Zm x Zj] x [(Zj x Zp)/Zj]	7.4 a-d	6.8 efg	6	8.5 ab	7.5 abc	6	5
6789-52	[(Zj x Zp)/Zj] x Zp	6.0 g	8.5 a	7.5	7.0 bcd	2.8 i	3	8
6844-203	[Zm x Zj] x [(Zj x Zp)/Zj]	7.4 a-d	6.5 fgh	6.5	9.0 a	5.0 d-i	6	5
6829-20	[(Zj x Zp)/Zj] x Zm	6.7 b-g	7.0 def	7	7.0 bcd	4.0 f-i	4	7
6844-150	[Zm x Zj] x [(Zj x Zp)/Zj]	7.6 abc	7.0 def	6.5	8.0 abc	5.5 c-g	5	5
6831-09	Zm x (Zm x Zj)	6.4 d-g	7.0 def	6.5	6.0 d	3.0 hi	2	5
6844-89	[Zm x Zj] x [(Zj x Zp)/Zj]	6.6 c-g	7.3 cde	6	7.0 bcd	4.0 f-i	5	6
6844-154	[Zm x Zj] x (Zj x Zp)/Zj]	7.0 a-g	7.0 def	6	7.5 a-d	6.0 b-f	4	5
6828-17	Zm x [(Zj x Zp)/Zj]	6.0 g	7.5 bcd	7	6.5 cd	2.8 i	3	6
Innovation	Zm x Zj	6.6 c-g	6.7 efg	6.2	6.8 cd	4.8 d-i	5.3	5.3
Meyer	Zj	3.9 h	6.0 h	5.3	3.7 e	4.1 e-i	3.7	6
Zeon	Zm	6.7 b-g	8.0 ab	7	7.0 bcd	4.3 e-i	5	6
Zorro	Zm	6.3 efg	7.9 abc	6	6.5 cd	3.3 ghi	4	8.5
LSD		*1.1*	*0.6*	*NS*	*1.5*	*2.5*	*NS*	*NS*

†Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Zmin; *Zoysia minima*; Complex crosses such as double and triple crosses to introgress desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
‡ TQ: turfgrass quality (*n* = 7 rating dates); LT: visual leaf texture (*n* = 4 rating dates); GC: genetic color (*n* = 2 rating dates); TD: turf density (*n* = 2 rating dates); WK: winterkill (*n* = 2 rating dates); SGU: spring greenup (*n* = 1 rating date); and FC: fall color retention (*n* = 1 rating date), all rated on were 1-9 scale similar to NTEP ratings collected on a 1-9 scale for various characteristics. For each trait a value of 9 is always equal to the most desirable performance and a value of 1 equal to the least desirable performance.

Table 2. Lineage and performance of best 22 interspecific hybrids selected from a set of 70 progeny from a Kansas State University spaced plant nursey located in Olathe, Kansas.

		SGU‡	Visual texture‡		TQ‡	Visual vigor‡		Wilting‡
Genotype	Lineage†	2020	2019	2020	2020	2019	2020	2020
6844-154	[Zm x Zj] x [(Zj x Zp)/Zj]	5.0§	6.0	6.5	6.5	3.5	4.5	5.0
6844-91	[Zm x Zj] x [(Zj x Zp)/Zj]	4.5	5.0	6.5	6.5	5.0	5.5	3.5
6830-56	(Zm x Zj) x Zm	4.0	6.5	7.5	7.5	6.0	6.0	2.5
6844-190	[Zm x Zj] x [(Zj x Zp)/Zj]	4.0	6.0	6.5	6.5	4.5	6.5	3.5
6940-15	Zm x Zj	4.0	5.0	7.0	7.0	5.0	4.5	6.5
6844-128	[Zm x Zj] x (Zj x Zp)/Zj]	4.0	5.0	4.5	4.5	4.0	4.5	7.0
6844-31	[Zm x Zj] x (Zj x Zp)/Zj]	3.5	4.5	8.0	8.0	7.0	7.0	5.0
6844-147	[Zm x Zj] x [(Zj x Zp)/Zj]	3.5	5.5	7.5	7.5	4.5	5.5	4.0
6829-36	[(Zj x Zp)/Zj] x Zm	3.5	5.5	5.5	5.5	8.0	5.0	3.5
6844-141	[Zm x Zj] x [(Zj x Zp)/Zj]	3.5	6.0	6.5	6.5	6.5	7.5	6.0
6924-47	(Zm x Zj) x (Zm x Zj)	3.5	5.0	6.5	6.5	6.5	7.0	4.5
6844-152	[Zm x Zj] x [(Zj x Zp)/Zj]	3.5	6.0	7.0	7.0	4.5	6.5	3.5
6924-66	(Zm x Zj) x (Zm x Zj)	3.5	5.0	6.0	6.0	7.0	6.5	4.0
6919-29	[(Zm x Zp)/Zj] x [Zm x Zj]	3.5	5.0	7.5	7.5	6.0	6.0	5.5
6844-34	[Zm x Zj] x [(Zj x Zp)/Zj]	3.5	6.0	6.5	6.5	4.5	5.0	3.0
6830-11	(Zm x Zj) x Zm	3.5	6.0	7.0	7.0	2.0	4.0	4.5
6924-44	(Zm x Zj) x (Zm x Zj)	3.5	5.0	7.0	7.0	8.0	6.0	4.5
6942-22	Zj x Zp	3.0	6.5	7.5	7.5	5.0	6.0	6.5
6839-08	Zm x [(Zj x Zp)/Zj]	3.0	7.5	7.0	7.0	5.0	5.0	4.5
6925-53	(Zm x Zj) x (Zm x Zj)	3.0	6.0	7.0	7.0	4.0	5.0	4.0
6831-08	Zm x (Zm x Zj)	3.0	6.0	7.0	7.0	3.5	5.0	4.5
6844-64	[Zm x Zj] x [(Zj x Zp)/Zj]	3.0	6.0	6.0	6.0	2.0	4.0	3.0
Innovation	Zm x Zj	3.5	5.5	6.0	6.0	5.0	6.0	2.0
KSUZ 1201	(Zj x Zp)/Zj	3.5	4.5	5.5	5.5	4.0	5.5	4.0
Meyer	Zj	4.5	4.5	6.0	6.0	7.0	6.5	4.0

†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.

‡SGU: Visual spring greenup ratings on 4/7/2020, visual texture ratings on 9/27/2019 and 6/10/2020, visual vigor ratings on 9/27/2019 and 6/10/2020, TQ: visual turf quality ratings on 6/10/2020, and visual wilting ratings on 8/27/2020. All data were rated on a scale 1-9 with 1=worst, 9=best.

§All data reported for each date are the average of two replications.

Table 3a. Lineage and performance of top 15 interspecific hybrids selected from Texas A&M AgriLife-Dallas spaced plant nursery planted with 831 genotypes in 2017.

		Mean SGU‡	Mean TQ‡	Turfgrass Quality‡ 2020			NDVI (UAV)§ 2020			Establishment‡ 2018				
Genotype	Lineage†	2019 & 2020	2018-2019	Normal TQ	Dry down	Reco very	7/30 (1st dry down)	8/6 (recov ery)	8/17 (2nd dry down)	06/ 25	07/ 25	09/ 10	GC‡	LT‡
6782-104	[(Zj x Zp)/Zj] x Zp	6.0a	7.5a	7.7a	7.0	7.0	0.73	0.77	0.69	1.0	2.0	3.0	9.0a	9.0
6782-120	[(Zj x Zp)/Zj] x Zp	5.5a	7.25a	7.0a	6.0	8.0	0.73	0.76	0.75	2.0	2.0	5.0	7.0	9.0
6782-42	[(Zj x Zp)/Zj] x Zp	5.0a	6.5	6.0a	7.0	8.0	0.73	0.75	0.71	1.0	2.0	3.0	6.0	9.0
6782-75	[(Zj x Zp)/Zj] x Zp	6.5a	7.0a	7.7a	6.0	7.0	0.69	0.74	0.73	2.0	2.0	5.0	9.0a	9.0
6782-79	[(Zj x Zp)/Zj] x Zp	4.0	6.3	7.0a	6.0	7.0	0.71	0.76	0.76	2.0	3.0	5.0	8.0a	9.0
6783-03	[(Zj x Zp)/Zj] x Zp	5.0a	8.5a	6.0a	5.0	7.0	0.73	0.76	0.73	2.0	3.0	6.0	7.0	9.0
6784-17	[(Zj x Zp)/Zj] x Zp	5.0a	6.8	7.3a	7.0	8.0	0.75	0.77	0.74	3.0	3.0	6.0	7.0	9.0
6785-19	[(Zj x Zp)/Zj] x Zp	4.5a	7.0a	6.3a	6.0	8.0	0.73	0.78	0.76	2.0	2.0	4.0	7.0	9.0
6785-22	[(Zj x Zp)/Zj] x Zp	4.5a	7.5a	6.0a	6.0	8.0	0.72	0.78	0.74	2.0	3.0	5.0	7.0	9.0
6786-02	[(Zj x Zp)/Zj] x Zp	5.5a	7.5a	7.7a	6.0	7.0	0.71	0.75	0.66	1.0	2.0	4.0	7.0	9.0
6787-18	[(Zj x Zp)/Zj] x Zp	6.5a	7.25a	7.0a	5.0	8.0	0.71	0.77	0.74	2.0	3.0	7.0	9.0a	9.0
6787-20	[(Zj x Zp)/Zj] x Zp	5.0a	7.75a	7.0a	5.0	7.0	0.74	0.77	0.77	3.0	3.0	6.0	9.0a	9.0
6789-23	[(Zj x Zp)/Zj] x Zp	6.0a	7.0a	6.7a	5.0	8.0	0.71	0.75	0.66	3.0	3.0	5.0	8.0a	9.0
6789-40	[(Zj x Zp)/Zj] x Zp	6.0a	7.5a	7.0a	5.0	8.0	0.71	0.76	0.71	1.0	2.0	3.0	8.0a	9.0
6791-06	[(Zj x Zp)/Zj] x Zp	6.5a	6.8	7.3a	4.0	8.0	0.69	0.73	0.71	2.0	3.0	6.0	.0a	9.0
Innovation	Zm x Zj	3.3	4.0	3.4	2.0	.	0.48	0.56	0.49	2.5	3.0	5.0	5.0	8.0
Meyer	Zj	3.0	4.5	1.8	2.0	.	0.29	0.38	0.35	1.5	1.5	2.5	5.5	7.0
Zeon	Zm	2.8	4.1	2.7	1.5	2.5	0.45	0.56	0.47	2.5	3.5	6.0	4.5	8.5
Zorro	Zm	2.8	4.1	2.5	1.0	2.0	0.3	0.42	0.37	1.5	2.5	6.0	5.0	8.5
LSD		*2.5*	*4.7*	*1.9*	*NS*	*NS*				*NS*	*1.3*	*NS*	*1.8*	*NS*

†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.
‡ SGU: spring greenup; TQ: turfgrass quality; establishment, GC: genetic color and LT: visual leaf texture, all rated on 1-9 scale with 1=worst and 9= best.
§NDVI (normalized difference vegetation index) obtained from Matrice 200 UAV equipped with Slant Range multi-spectral sensor. NDVI is presented on -1 to +1 scale with values closer to 1 represent higher vegetation and overall higher plant health.

Table 3b. Lineage and performance of top 5 interspecific hybrids selected from a Texas A&M AgriLife-Dallas spaced plant nursery planted with 805 genotypes in 2018.

		Establishment ‡ (1-9)	TQ‡	TQ‡	TQ‡ (Pre drydown)	TQ‡ (Dry down)	NDVI (UAV)§ 2020			SGU‡ 2020	LT‡ 2020
Genotype	Lineage†	8/9/19	10/3/19	6/15/20	7/3/20	7/20/20	7/30 (1st drydown)	8/6 (recovery)	8/17 (2nd drydown)	3/25	4/30
6792-44	[(Zj x Zp)/Zj] x Zp	7	5	6	7	6	0.68	0.74	0.69	7	9
6910-157	Zj x (Zj x Zm)	8	5	5	7	5	0.73	0.72	0.73	2	6
6910-172	Zj x (Zj x Zm)	6	6	7	8	5	0.70	0.72	0.70	5	6
6941-36	Zj x Zm	7	7	7	8	5	0.70	0.73	0.69	6	6
6829-34	Zm x (Zj x Zm)	5	4	6	9	4	0.68	0.72	0.69	7	7
Palisades	Zj	9	6	2	5	3	0.57	0.58	0.56	5	5
Zeon	Zm	8	4	2	4	3	0.46	0.50	0.47	4	8

†Zj: *Zoysia japonica*; Zm: *Zoysia matrella*; Zp: *Zoysia pacifica*; Complex crosses such as double and triple crosses to integrate desirable traits require the use of x, /, () and [] to indicate hybrid parentage.
‡ SGU: spring greenup; TQ: turfgrass quality; establishment, and LT: visual leaf texture, all rated on 1-9 scale with 1=worst and 9= best.
§NDVI (normalized difference vegetation index) obtained from Matrice 200 UAV equipped with Slant Range multi-spectral sensor. NDVI is presented on -1 to +1 scale with values closer to 1 represent higher vegetation and overall higher plant health.

USGA ID# 2018-16-666

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

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

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

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

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

Summary points
- In 2019 and 2020, zoysiagrass plots were maintained at a golf course rough mowing height (3.0 inches) under low-maintenance regimes receiving minimal-to-no pest control, nitrogen fertilization, and supplemental irrigation.
- Results from these evaluations indicate there are differences in performance due to regional climatic differences among sites.
- Overall, XZ14069, 09-TZ-54-9, and 16-TZ-12783, are entries that are in the top performing group, based on turf quality ratings, at three or more sites. Other entries such as 15-TZ-11766, 10-TZ-1254, XZ14070, 16-TZ-14114, and a few others also performed well, based on high TPI scores, at multiple sites.
- Golf ball lie data from two or more data collection events across Indiana, North Carolina, and Georgia locations revealed differences among entries and some of the top-performing entries above may provide a better golf ball lie to golfers.
- Data from this experiment will be used to assist in the selection of genotypes for future experiments and potential release of zoysiagrass genotypes that perform well under low-input conditions, which will assist in lowering golf course maintenance budgets.

Summary Text:

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

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

Data collection, from 2018 to 2020, was similar to typical NTEP trials in order to identify those that are best suited to for golf course roughs. In addition, golf ball lie was measured multiple times in 2019 and 2020 for each entry using the method developed by Richardson et al. (2010) at three sites. At the conclusion of the study, a cumulative turf performance index (TPI) score was generated for each entry and check within each location, representing the number of times it occurred in the top statistical grouping as determined by least significant difference across all parameters and all sampling dates. Golf course superintendents were unable to visit the experimental locations in 2020 for university field days due to Covid-19.

Data collection from each site has been analyzed and TPI and turf quality ratings, averaged across multiple ratings dates in 2019 and 2020 within each location, indicate several entries are performing consistently well at multiple sites (Tables 1 and 2, Fig. 1 and 2). These results indicate there are differences in performance due to climatic differences among sites. Overall, XZ14069, 09-TZ-54-9, and 16-TZ-12783, are entries that are in the top performing group, based on turf quality ratings, at three or more sites (Table 2). While other entries such as 15-TZ-11766, 10-TZ-1254, XZ14070, 16-TZ-14114, and others are also performing well, based on TPI scores, at multiple individual sites (Table 1). However, entries mentioned above are not in the top performing group at Indiana likely due to a colder climate and some winter damage from previous winters. Golf ball lie data from two or more data collection events across Indiana, North Carolina, and Georgia locations also revealed differences among entries and some of the top-performing entries also exhibited the ability to hold the golf ball higher in the canopy (i.e., high visible percentage of golf ball) (Table 3 and Fig. 3). We hoped to attain superintendent feedback on these entries in 2020; however, we were unable to due to restrictions from Covid-19. Further data analysis of the performance of these entries and golf ball lie data presented herein may indicate the need for recommendations of specific entries based on regional climatic differences.

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

References

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

Table 1. Cumulative 2-year turf performance index (TPI) for 24 coarse zoysiagrass phenotypes compared to five zoysiagrass checks, one bermudagrass check, and three cool-season turfgrass species checks) (entries, not checks, sorted by total TPI across five locations).

	TPI†					
Entries	IN	NC	GA	AZ	CA	TOTAL
XZ14069	6	13	12	10	7	48
09-TZ-54-9	6	10	11	10	8	45
10-TZ-1254	15	13	3	6	5	42
16-TZ-12783	0	11	17	7	7	42
XZ14070	11	10	10	5	2	38
16-TZ-14114	17	8	7	4	2	38
ZG09062	10	7	7	7	5	36
10-TZ-994	24	5	4	0	0	33
XZ14072	13	11	1	2	4	31
15-TZ-11766	0	5	12	5	8	30
16-TZ-13463	0	4	9	9	6	28
XZ14071	11	9	2	2	3	27
PURZ 1701	21	3	1	0	1	26
XZ14055	16	5	3	1	--‡	25
ZG09004	16	4	3	1	1	25
PURZ 1602	17	4	1	0	0	22
PURZ 1606	14	4	4	0	0	22
16-TZ-12036	2	7	7	3	3	22
09-TZ-89-73	1	3	6	5	5	20
ZG09055	12	4	3	--‡	0	19
XZ14074	0	0	3	7	5	15
PURZ 1603	9	3	2	0	0	14
PURZ 1702	3	2	3	0	0	8
XZ14092	3	1	1	2	0	7
'Chisholm' zoysiagrass	14	4	6	6	2	32
'Meyer' zoysiagrass	21	6	4	1	0	32
'Empire' zoysiagrass	18	6	7	4	4	39
'Jamur' zoysiagrass	20	6	7	4	4	41
'Zenith' zoysiagrass	0	4	0	0	1	5
'Riviera' bermudagrass	16	--‡	4	--‡	--‡	20
Fine fescue mixture	13	--	2	--	--	15
'Bluenote' Kentucky bluegrass	8	--	0	--	--	8
'Mustang 4' Tall fescue	17	--	1	--	--	18

† TPI is the turf performance index representing the number of times an entry occurred in the top statistical group across all parameters, highest possible TPI is 33 at Indiana, 15 at North Carolina, 17 at Georgia, 10 at Arizona, and 9 at California for a total TPI of 85.

‡ Entry or check either not planted at site or did not survive during establishment in 2018.

Table 2. Average 2-year turf quality ratings (1-to-9 scale, 9=maximum quality, 6=minimally acceptable quality; 1=lowest quality) across multiple rating dates in 2019 and 2020 for 24 coarse zoysiagrass phenotypes compared to five zoysiagrass checks, one bermudagrass check, and three cool-season turfgrass species checks) (entries, not checks, sorted by overall mean at North Carolina). Data in the top statistical group are indicated with **bolded** text.

	Overall Turf Quality				
Entries	IN	NC	GA	AZ	CA
XZ14069	4.0 jk†	**7.1 a**	**6.7 abc**	**6.8 a**	**7.7 a**
09-TZ-54-9	4.6 i-k	**7.0 ab**	**6.8 abc**	**6.9 a**	**7.3 ab**
16-TZ-12783	--‡	**6.8 abc**	**7.3 a**	**6.2 abc**	6.5 bcd
ZG09062	5.0 h-k	6.4 bcd	**6.4 a-d**	6.0 bcd	6.5 bcd
10-TZ-1254	6.2 c-h	6.4 bcd	4.4 ghi	6.0 bcd	6.0 def
XZ14072	6.8 b-f	6.2 cde	4.6 ghi	5.1 f-i	6.3 cde
16-TZ-12036	4.5 jk	6.1 cde	6.1 b-e	5.4 d-h	6.0 def
XZ14071	5.5 f-j	6.1 de	4.5 ghi	5.0 ghi	6.5 bcd
16-TZ-14114	7.0 b-e	5.9 def	6.0 b-e	5.7 b-g	5.5 e-h
PURZ 1602	6.8 b-f	5.9 d-g	4.7 f-i	4.9 hi	5.3 f-i
15-TZ-11766	--‡	5.9 def	**7.2 ab**	5.6 c-h	**7.2 abc**
XZ14070	5.8 e-i	5.8 d-g	5.8 c-f	5.8 b-f	5.0 g-j
16-TZ-13463	--‡	5.8 d-g	**6.2 a-e**	**6.3 ab**	6.7 bcd
XZ14055	6.0 d-h	5.7 e-h	4.3 ghi	5.3 e-h	--¶
09-TZ-89-73	3.8 k	5.7 e-h	6.0 b-e	5.9 b-e	6.2 def
10-TZ-994	**7.4 abc**	5.6 e-h	4.1 hi	3.8 jk	4.7 hij
PURZ 1702	5.3 g-j	5.5 e-i	3.8 i	3.9 jk	--§
XZ14092	4.1 jk	5.2 g-j	4.0 hi	5.6 c-h	5.3 f-i
ZG09055	6.9 b-f	5.1 hij	4.2 hi	--¶	4.2 j
PURZ 1603	6.5 b-g	4.9 ij	3.9 hi	3.8 jk	4.5 ij
PURZ 1701	**7.5 ab**	4.9 ij	4.2 hi	4.0 jk	4.2 j
PURZ 1606	6.8 b-f	4.8 j	4.7 f-i	3.6 k	3.0 k
ZG09004	7.0 b-e	4.8 j	5.4 d-g	4.4 ij	4.7 hij
XZ14074	--‡	--‡	3.8 i	**6.3 ab**	**7.7 a**
'Chisholm' zoysiagrass	6.4 b-h	5.7 e-h	5.8 c-f	5.9 b-e	5.8 d-g
'Meyer' zoysiagrass	**7.5 ab**	5.7 e-h	5.1 e-h	4.9 hi	5.5 e-h
'Empire' zoysiagrass	6.0 d-h	5.7 e-h	6.1 b-e	5.4 d-h	6.0 def
'Jamur' zoysiagrass	7.0 b-e	6.1 cde	6.0 b-e	5.7 b-g	6.0 def
'Zenith' zoysiagrass	3.7 k	5.3 f-j	3.8 i	5.1 ghi	4.9 hij
'Riviera' bermudagrass	6.0 d-i	--§	4.8 f-i	--¶	--¶
Fine fescue mixture	**7.6 ab**	--§	4.5 ghi	--¶	--¶
'Bluenote' Kentucky bluegrass	6.7 b-f	--§	2.3 j	--¶	--¶
'Mustang 4' Tall fescue	8.4 a	--§	2.3 j	--¶	--¶
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† Means within a column followed by the same letter are not statistically different at $\alpha \leq 0.05$ according to Fisher's protected least significant difference test.
‡ Entry did not survive 2018-2019 winter.
§ Entry or check did not survive during establishment in 2018.
¶ Entry or check not planted at site.

Table 3. Average 2-year percent visible golf ball lie data across multiple collection dates in 2019 and 2020 for 24 coarse zoysiagrass phenotypes compared to five zoysiagrass checks, one bermudagrass check, and three cool-season turfgrass species checks) (entries, not checks, sorted by overall mean at Indiana).

	% Visible Golf Ball					
Entries	IN		NC		GA	
XZ14069	52.3	a†	74.9	a	60.7	b-i
09-TZ-54-9	51.1	ab	47.8	a	63.7	a-g
XZ14055	49.2	ab	59.1	a	68.5	a-d
10-TZ-994	48.8	ab	44.2	a	68.7	a-d
XZ14070	47.5	ab	57.2	a	56.3	e-j
16-TZ-12036	46.7	ab	50.7	a	66.4	a-e
09-TZ-89-73	45.8	abc	60.5	a	54.3	g-k
XZ14071	41.5	a-d	52.6	a	64.2	a-g
ZG09062	39.4	a-e	52.4	a	58.1	d-j
PURZ 1602	38.9	b-e	59.2	a	55.3	e-k
PURZ 1702	38.4	b-e	59.7	a	62.8	a-h
XZ14092	37.6	b-f	49.3	a	72.0	ab
XZ14072	34.3	c-g	59.2	a	66.0	a-f
PURZ 1606	32.0	d-h	51.2	a	51.8	h-k
PURZ 1603	32.0	d-h	50.1	a	53.0	g-k
	30.2	d-h	60.3	a	54.9	f-k
10-TZ-1254	29.5	e-h	52.7	a	62.1	a-h
PURZ 1701	24.1	ghi	47.2	a	48.7	jk
ZG09055	22.0	hi	50.9	a	60.2	c-i
ZG09004	17.2	i	37.4	a	55.7	e-k
XZ14074	--‡		--‡		71.4	ab
15-TZ-11766	--‡		65.7	a	53.6	g-k
16-TZ-12783	--‡		57.1	a	73.5	a
16-TZ-13463	--‡		--‡		73.2	a
'Chisholm' zoysiagrass	28.4	e-h	42.3	a	60.2	c-i
'Meyer' zoysiagrass	36.8	b-f	54.0	a	56.1	e-j
'Empire' zoysiagrass	43.1	abc	54.6	a	49.4	ijk
'Jamur' zoysiagrass	38.3	b-e	51.3	a	53.5	g-k
'Zenith' zoysiagrass			52.7	a	59.0	d-j
'Riviera' bermudagrass	33.8	c-g	--‡		44.5	k
Fine fescue mixture	27.8	e-h	--‡		--‡	
'Bluenote' Kentucky bluegrass	26.7	f-i	--‡		--‡	
'Mustang 4' Tall fescue	25.0	ghi	--‡		--‡	
P-value	<0.0001		0.0602		<0.0001	

† Means within a column followed by the same letter are not statistically different at $\alpha \leq 0.05$ according to Fisher's protected least significant difference test.
‡ Entry or check either did not survive during establishment in 2018, winter of 2018-2019, or have enough turf cover to adequately measure ball lie.

Figure 1. Differences in summer turf quality among plots in Chandler, AZ on 21 July 2020.

Figure 2. Plot images of a few of the entries with high turf performance index scores or high overall turf quality and two standard checks in West Lafayette, IN on 13 July 2020.

Figure 3. Golf ball lie data collection at the West Lafayette, IN site on 14 Aug. 2020.

USGA ID: 2003-36-278
Title: Buffalograss Breeding and Genetics
Project leader Keenan Amundsen
Affiliation University of Nebraska-Lincoln
Objectives The primary objective of this study is to develop, through selection and plant breeding, buffalograss suitable for golf course fairways, tees, and roughs.

Start date 2018
Project duration Continuous
Total funding $30,000

Summary text

Buffalograss is native to the central Great Plains and is naturally adapted to the environmental conditions of the region including frequent and extended periods drought and heat stress. We are working to develop new buffalograss cultivars, taking advantage of its innate stress tolerance, with exceptional turfgrass visual and functional quality that are suitable for use on golf courses. A major focus of our program is on evaluating and identifying new sources of germplasm that contain desirable traits that we can move into elite buffalograss lines through conventional plant breeding. There is significant genotypic and phenotypic variability in our germplasm collection, and we have identified lines that differ in color, inflorescence numbers, stolon production, stolon internode length, pest resistance, shade tolerance, winter dormancy response, and turf quality (Figure 1).

Buffalograss is dioecious, which makes it interesting and challenging from a plant breeding perspective. Since buffalograss has separate male and female plants, we often develop new parent lines separately that are useful for creating future buffalograss hybrids suitable for seed or sod production. It is common to identify buffalograss lines with exceptional quality that do not flower (Figure 2). If those lines are able to form a dense sod with good sod strength, have good stolon production, and perform well in regional evaluation trials, then they are potentially suitable for sod or vegetative plug production. Subsequent crossing work is difficult with those lines and, for the most part, only possible if they can be forced to flower in the greenhouse so they often do not contribute further to the breeding pipeline. Without significant flower production, those lines are not useful for buffalograss seed producers. Instead, our program also identifies lines that produce a significant number of male or female flowers (Figure 3). Increased flower number is often associated with increased seed production.

A challenge for buffalograss breeding is to identify and develop the most suitable parents to create future buffalograss varieties. We have developed molecular tools to identify germplasm resistant to leaf spot disease and chinch bugs, two important buffalograss pests, without challenging buffalograss with the pests which is often laborious. The molecular tools have the potential to greatly accelerate our breeding pipeline. Much of our focus in 2020 has been to develop new genetic resources for studying buffalograss. Over the past decade, we have conducted at least five separate high throughput sequencing studies in buffalograss to identify genes that are associated with a condition such as chinch bug feeding, leaf spot disease, gender expression, or genotypic differences. Those studies have produced a wealth of data that continues to be useful. For example, genes that are differentially expressed between control and leaf spot challenged resistant and susceptible buffalograss lines have

already been reported. Using sequence data from that study, a comparative genetics study was done using the reference genome for foxtail millet [*Setaria italica* (L.) P. Beauv.] obtained from EnsemblPlants. The reference includes nine chromosomes, with a total length of 405 million base pairs, and 35,831 coding genes. Differentially expressed buffalograss transcripts in response to leaf spot disease were mapped to the foxtail millet genome (Figure 4). When zooming into specific genomic regions, several regions were identified on the foxtail millet genome that are associated with leaf spot susceptibility or resistance in buffalograss. In the absence of a buffalograss genome, this comparative genetics approach has allowed us to identify genomic regions that are important for leaf spot resistance and susceptibility. There is a great deal of synteny, conserved genes and gene order across genomes, among grasses, but more research is needed to determine to what extent the foxtail millet genome can be used as a reference for buffalograss.

We have also used information from those previous high throughput sequencing studies to develop simple sequence repeat, gene expression-based, and insertion/deletion molecular markers that can be used to identify buffalograss tolerant of chinch bug feeding and resistant to leaf spot disease. The sequence data was reanalyzed for single nucleotide polymorphisms (SNPs). By comparing 10 female to 10 male buffalograss genotypes from a linkage mapping population, we identified a single SNP that was associated with gender expression. The SNP is located in a gene that encodes a predicted receptor-like serine/threonine-protein kinase which is likely involved in regulating cellular expansion and differentiation. Interestingly similar genes are associated with flower development and timing. We also identified 3,520 SNPs that were conserved in 10 female buffalograss lines and 3,880 that were conserved in 10 male buffalograss lines. By challenging buffalograss with leaf spot disease and comparing buffalograss lines previously identified to be resistant or susceptible to leaf spot disease, we identified 724 SNPs associated with how buffalograss responds to leaf spot disease. A similar approach was taken to identify SNPs associated with how buffalograss responds to chinch bug feeding and 1,487 SNPs were found. By analyzing the data across studies, we found on average two SNPs that could uniquely identify each genotype. Together these SNPs and markers are useful for distinguishing lines and identifying germplasm that is likely resistant to important buffalograss pests. In the future we plan to develop cost effective ways for implementing these molecular markers as a step in our germplasm evaluation and buffalograss breeding pipeline. Molecular tools continue to be more cost effective for characterizing species like buffalograss, but their utility for cultivar development breeding efforts needs further research.

Summary points

- Several buffalograss lines were identified that have exceptional turfgrass quality, suitable for seed or sod production, and contribute to the development of future buffalograss cultivars.
- By comparing buffalograss to other grass genomes, genomic regions associated with leaf spot disease resistance or susceptibility were identified.
- More than 9,600 single nucleotide polymorphisms were identified, useful for future molecular marker studies.

Figure 1. Field evaluation of buffalograss lines segregating for color, canopy density, gender expression, and lateral spread.

Figure 2. Exceptional buffalograss line that does not produce flowers and is suitable for sod or vegetative plug production.

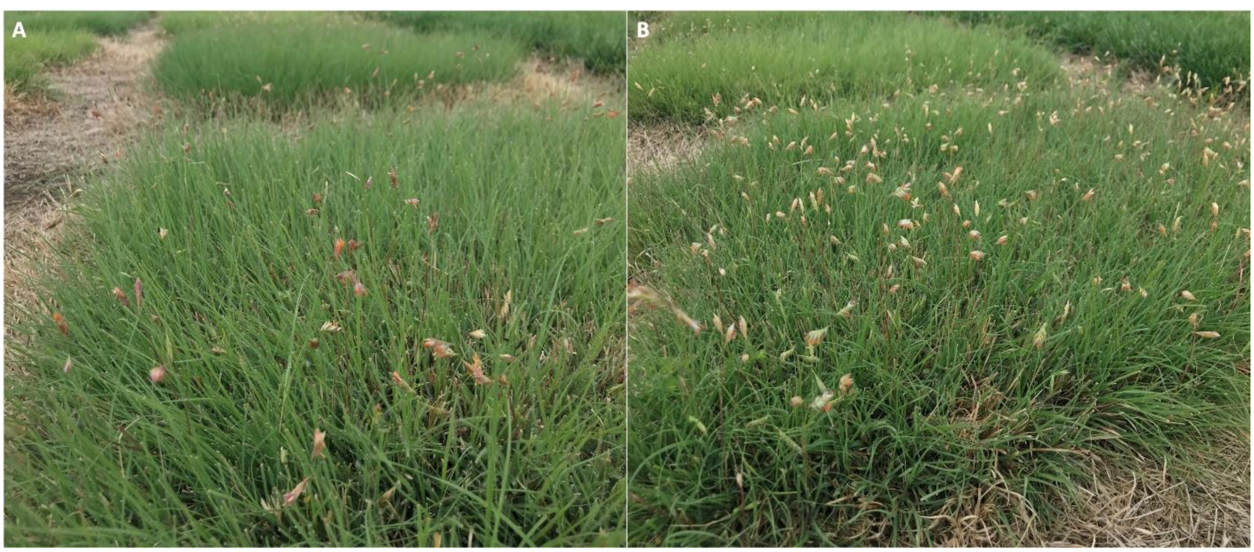


Figure 3. (A) A male buffalograss line producing a modest number of flowers. (B) A male buffalograss line producing several flowers.

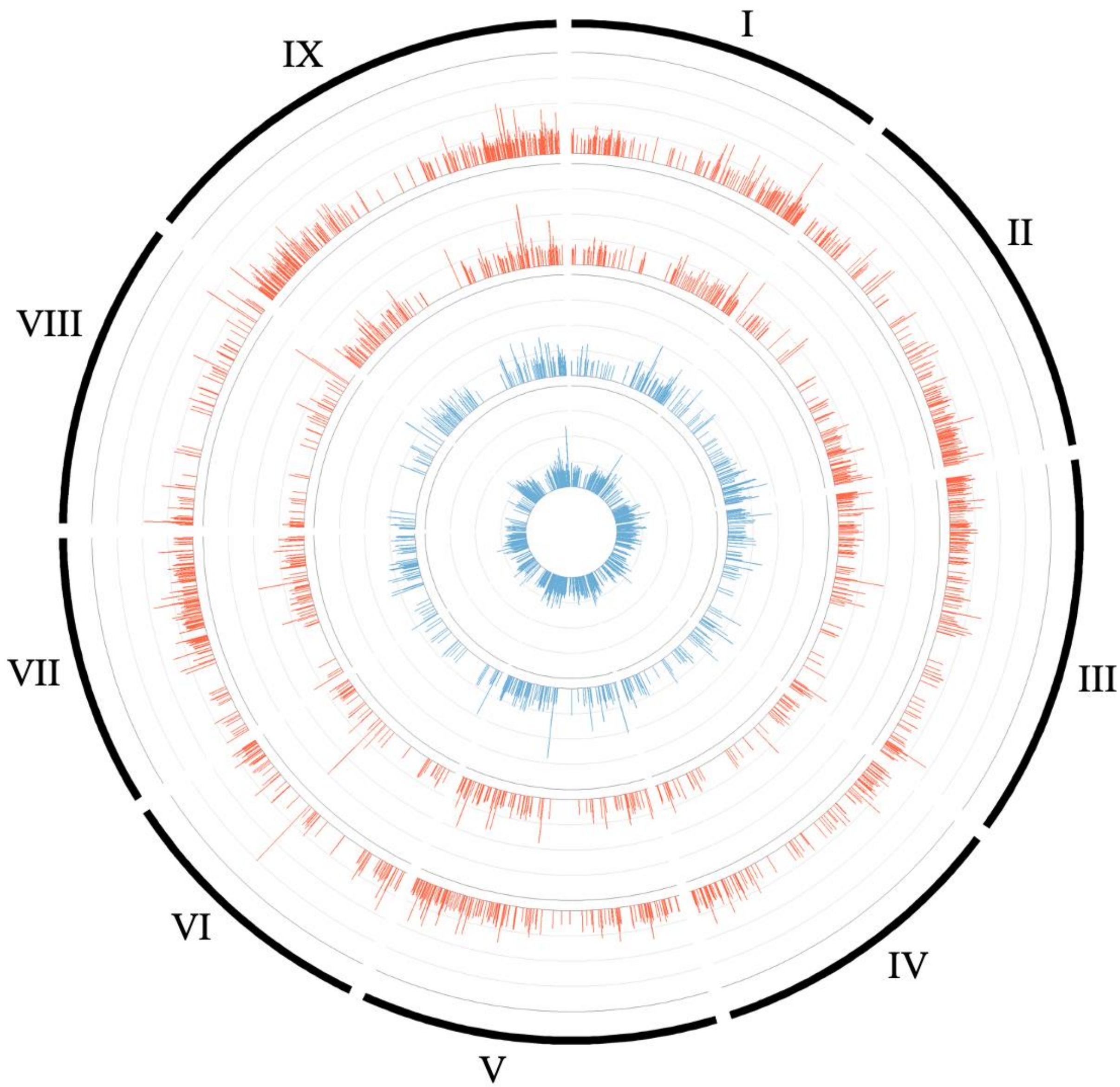


Figure 4. A circular diagram representing nine foxtail millet chromosomes shown in black. The red inner circles represent two buffalograss lines that are resistant to leaf spot disease and the inner blue circles represent two buffalograss lines susceptible to leaf spot disease. The magnitude of the red or blue bars show the relative increase in gene expression when challenged with leaf spot disease and the position of the bars on the circle depicts where the corresponding buffalograss genes map to the foxtail millet genome.

2. INTEGRATED TURFGRASS MANAGEMENT

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

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

USGA ID: 2017-04-614

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

Project leader: Maggie Reiter

Affiliation: University of California Cooperative Extension

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

Start date: 2017

Duration: 3 years

Total funding: $50,000

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

Objectives 1, 2, and 3
These objectives were addressed in previous annual reports. No additional research work was conducted in 2020.

Objective 4
Objective 4 was not accomplished due to a combination of COVID-19 pandemic limitations and the project leader's departure from the University of California in summer 2020.

Outreach and impacts
Results from objectives 1 and 2 were incorporated into Extension education about naturalized area management on golf courses. Outputs included a GCSAA Golf Industry Show half-day seminar in collaboration with J. Hoyle and J. McCurdy, a GCSAA Golf Course Management Magazine article in collaboration with K. Umeda and W. Burayu, presentations to local California GCSAA chapters, and a seminar at the Texas Turfgrass Association Summer Conference. Results from objective 3 were presented at the California Weed Science Society annual meeting, local California GCSAA chapter meetings, and other California integrated pest management clientele meetings. An article was published in the California Association of Pest Control Advisors trade magazine.

Research findings were used to provide local support to individual California golf courses converting anywhere from 2 to 20 acres of maintained turf to naturalized areas. Impacts can be inferred from published golf course case studies showing that labor, inputs, and water savings were approximately $2,000 for each converted acre (USGA, 2017a). Thus, our project expertise may have contributed to saving an estimated $4,000 to $40,000 annually for each of the aforementioned golf course facilities. Moreover, naturalized areas use just 22% of the water required to maintain traditional turfgrass, like bermudagrass (USGA, 2017b).

Summary points:

- Objective 4 was not accomplished due to COVID-19 pandemic and the project leader's departure from University of California
- Outreach was conducted in California and beyond to deliver the project findings to golf course superintendents

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 in the low desert southwest United States as a low input turfgrass replacement in non-play areas of golf courses.
2. Generate local research-based information 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: Annual report (2nd yr. 2020)

Summary Points: -

- Both white and pink varieties of Kurapia (*Lippia nodiflora* L.) remained green throughout the year with acceptable quality values of greater than 6 (1 = brown, 9 = dark green).
- Kurapia can be grown successfully at 40% drip irrigation rate (20% is insufficient & 80% is excessive) relative to bermudagrass.
- Nine out of the ten nativegrasses performed and maintained acceptable quality.
- *Aristida purpurea, Sporobolus aeroides, Eragrostis trichodes* and *Bouteloua gracilis* remained green throughout the year with acceptable quality values of greater than 5 for greenness.

Summary Text

Golf course superintendents and landscapers of residential and commercial properties are facing increased pressures including regulatory restrictions to reduce inputs of water, fertilizer, and pesticides 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, fertilizer and pesticides input 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 conclusive results and to apply specific recommendations for best management practices, our current research includes ten nativegrasses and two cultivars of groundcover (Table 1). The priority for the current project is to evaluate and determine the performance of nativegrasses under a nearly natural setting and the alternative groundcover at various rates of drip irrigation.

Two experiments, one for nativegrasses and one for groundcover were conducted 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 with four replicates. The second experiment consisting of two cultivars of a promising new groundcover, Kurapia, pink and white flower cultivars, was established with four replications in a RBCD. The irrigation requirements of the two cultivars were evaluated and compared under three levels of drip irrigation, 80, 40 and 20% relative to bermudagrass. The overall visual quality was evaluated for greenness and ground surface cover following the procedures developed by National Turfgrass Evaluation Program, where 1 is brown, 5 is light green, 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 low-input crop management practices as part of best management practices for the new groundcovers and nativegrass species.

Results:

This is the second-year report of three years investigation of the second phase of evaluating nativegrasses and Kurapia with emphasis on the overall plant quality, greenness, percent ground cover and growth uniformity under low inputs. This investigation revealed that when mowed twice during the year, the nativegrasses; alkali sacaton *(Sporobolus airoides)*, sand lovegrass (*Eragrostis trichodes*), purple threeawn (*Aristida purpurea)*, and blue grama (*Bouteloua gracilis* can be grown as year-round green grasses that have pleasant and attractive characters (Figure 1) compared to being unmowed. Kurapia, a new groundcover, exhibited the most vigorous growth and year-round greenness when grown from plugs under optimal irrigation and with low maintenance. 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 space allotted under optimum irrigation between May and October 2019. The white cultivar of Kurapia spread more rapidly across the surface area and covered the plot within a shorter time but shorter in height compared to the pink variety. In the second-year, during May to November 2020, further investigation on Kurapia's specific water requirements was conducted to determine the acceptable quality level (color, coverage, uniformity, etc.) at varying deficit irrigation regimes (20, 40 & 80% relative to bermudagrass). Results showed that irrigating at the 20% drip irrigation level negatively affected the greenness quality, 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 & 80% drip irrigation levels (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 (Figure 2). 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 a clay-loam or heavier textured soil. Visual estimates of greenness significantly correlated to Canopeo® digital estimates of percent greenness ($Y = -8.134 + 1.939*X$; $R^2 = 0.92$; $P < 0.01$) (Figure 4). This indicates that the use of digital estimates of percent greenness by means of a mobile phone Canopeo® app has practical utility and can be used to estimate greenness for Kurapia and grasses. Repeated visual estimates of greenness for Kurapia and grasses can be confirmed and validated when compared with Canopeo® digital estimates.

Table 1. List of native grasses and groundcovers evaluated in the low desert at Litchfield Park, Arizona in 2019/2020

	Common Name	Scientific Name
1	Alkali sacaton	*Sporobolus airoides*
2	Blue grama	*Bouteloua gracilis*
3	Bluestem, little "Cimarron"	*Schizachyritm scoparium*
4	Bluestem, Sand "Chet"	*Andropogon halii*
5	Buffalograss	*Bouteloua dactyloides*
6	Galleta, "Viva"	*Hilaria jamesii*
7	Grama, Sideoats "Vaughn"	*Bouteloua eurtipendula*
8	Lovegrass Sand, "Bend"	*Eragrostis trichodes*
9	Purple threeawn	*Aristida purpurea*
10	Sand dropseed	*Sporobolus cryptandrus*
11	Kurapia (two varieties)	*Lippia nodiflora (Pink & White flower)*

Figure 1. Nine of the ten nativegrasses established with optimal overhead irrigation and later grown under natural conditions in Litchfield Park, Arizona (seeded June 25, 2019). Note: the greenness of mowed plants as compared to unmowed once.

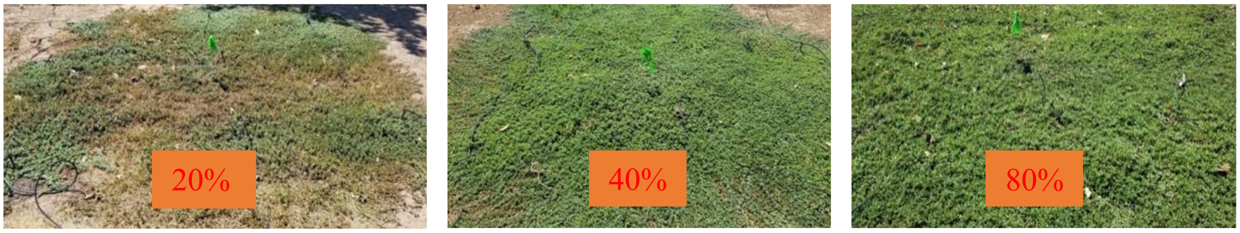


Figure 2. Greenness, surface area coverage, uniform growth and aesthetic value of Kurapia groundcover under three drip irrigation rates from May to October 2020. Note: the significant lower performance of Kurapia at 20% irrigation rate compared to 40% and 80% drip irrigation rates relative to bermudagrass. No significant differences in Kurapia growth and overall quality observed between the 40% and 80% rates.

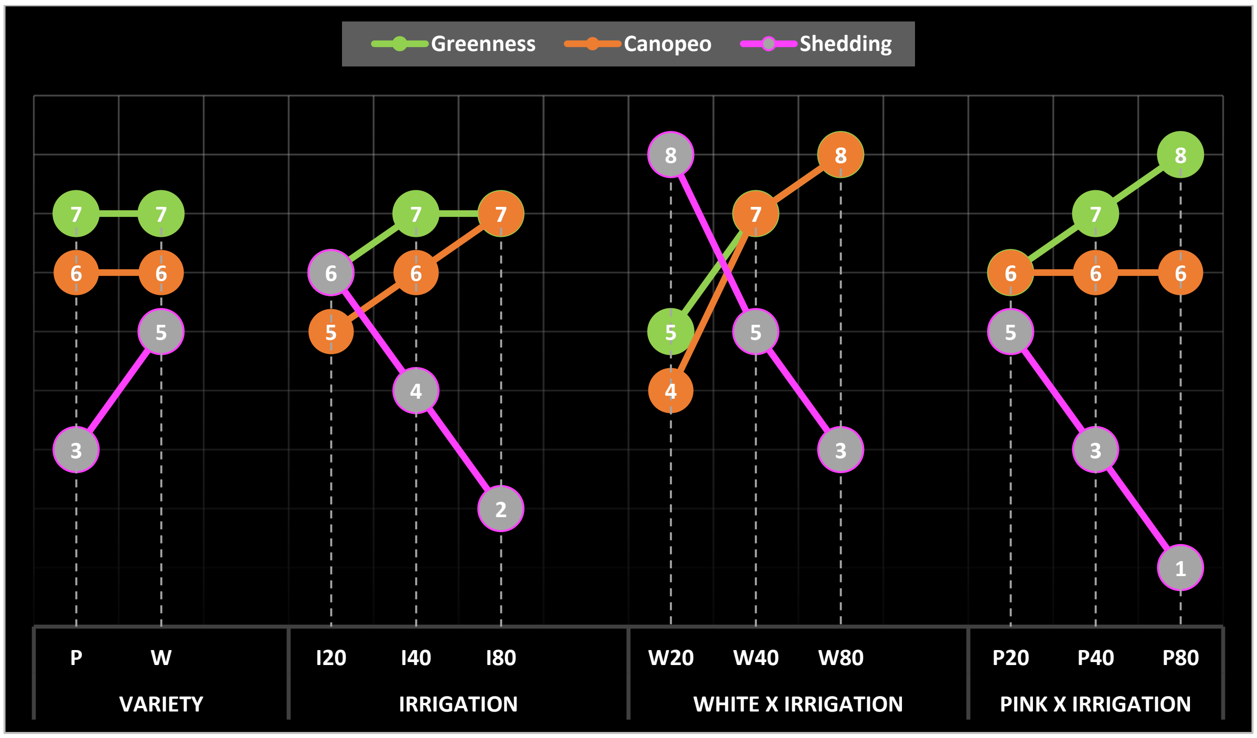


Figure 3. Greenness, quality, and flower shedding of each Kurapia variety at variable irrigation rates of 20, 40, 80%. Note: P-pink, W-white, I-irrigation. Greenness & Canopeo® (1 is brown or yellow, 5 is light green, and 9 is dark green), flower shedding (1 is 10% shedding, 5 is 50% shedding, and 8 is 80% shedding).

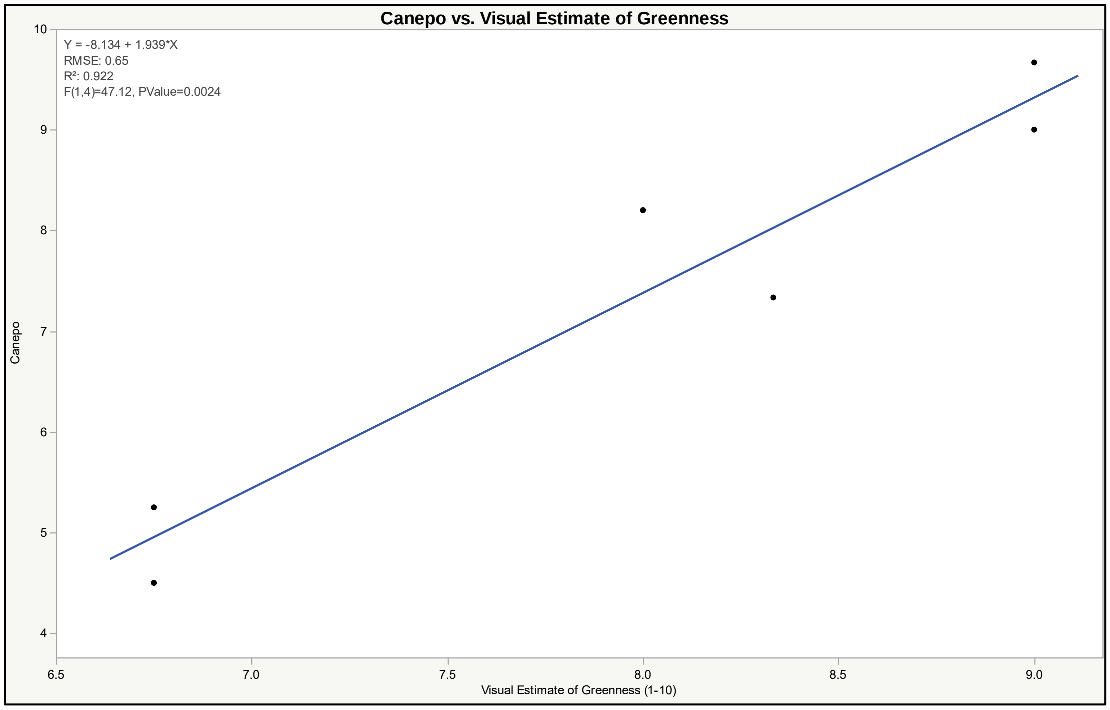


Figure 4. Visual estimates of greenness significantly correlated to Canopeo® app digital estimates of percent greenness (Y = -8.134 + 1.939*X; R^2 = 0.92; $P < 0.01$)

USGA ID: 2018-14-664

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

Project Leader(s): Brian Schwartz

Affiliation: The University of Georgia

Objectives:

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

Start Date: 2018

Project Duration: 3 years (2018 – 2020)

Total Funding: $28,500 to date

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

Summary Points:

1) **Country Club of Columbus**

August Rocco took over management of the renovated, old research green that was planted on July 15th, 2015 after William Smith's retirement this year. All three bermudagrasses (TifEagle, 12-TG-101, and 12-TG-143) established very quickly. A picture of the green during July of 2015 is below, as well as a summary of the 15 stimp measurements taken to-date.

C.C. of Columbus (15 Stimp Measurements)			
(2015 – 2020)		Fastest	Overall Avg.
Bermuda	12-TG-101	**12.1'**	**9.9'**
	TifEagle	**12.0'**	**9.7'**
	12-TG-143	**11.7'**	**9.3'**
	TifEagle Green	**12.0'**	**10.0'**

2) **Landings Club**

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

The Landings Club (10 Stimp Measurements)			
(2015 – 2020)		Fastest	Overall Avg.
Bermuda	TifEagle	**11.4'**	**9.7'**
	12-TG-39	**11.4'**	**9.6'**
	12-TG-101	**12.2'**	**9.5'**
	12-TG-143	**10.5'**	**9.3'**
	TifGrand	**12.5'**	**8.6'**
Paspalum	SeaStar	**11.5'**	**8.7'**

3) **Valdosta Country Club**

Barry Bennett renovated the old practice green prior to planting on May 25th, 2016. Tom Howard has been managing the green for the last three years. All five bermudagrasses (Tifdwarf, TifEagle, 12-TG-39, 12-TG-101, and 12-TG-143) established fairly quickly. A picture of the green during July 2020 is below, as well as a summary of the 14 stimp measurements taken to-date.

Valdosta C.C. (14 Stimp Measurements)			
(2016 – 2020)		Fastest	Overall Avg.
Bermuda	12-TG-39	**10.7'**	**9.2'**
	12-TG-101	**10.4'**	**9.1'**
	12-TG-143	**10.8'**	**9.1'**
	TifEagle	**11.5'**	**9.0'**
	Tifdwarf	**10.4'**	**8.5'**

4) **Atlanta Country Club**

Mark Esoda constructed a new research green adjacent to his bentgrass research green prior to planting on June 16th, 2016. Scott Lambert has been managing the green for the last three years. All bermudagrasses (TifEagle, 12-TG-101, and 12-TG-143) established quickly, but six large trees surrounding the green were removed September 6th, 2016 because the green was only getting 2 hours of sunlight. A picture of the green during July of 2020 is below, as well as a summary of the 13 stimp measurements taken to-date.

Atlanta Country Club (13 Stimp Measurements)			
(2016 – 2020)		Fastest	Overall Avg.
Bermuda	12-TG-101	**11.4’**	**8.4’**
	12-TG-143	**10.9’**	**8.0’**
	TifEagle	**10.4’**	**7.8’**
Bent	A-1	**10.5’**	**9.5’**

5) Big Canoe Golf Course

Lydell Mack converted a bentgrass nursery green to a research plot during 2017. This test site is divided in two equal areas, one treated as a “no-till” soil profile and the other “cored-out” and refilled with a new green’s mix. Two bermudagrasses (TifEagle and 12-TG-101) and two zoysiagrasses (Diamond and Primo) were planted in long strip-plots that span across both soil profiles on May 25th, 2017. Pictures of the green during 2020 are below, as well as a summary of the 7 stimp measurements taken to-date. The most important information to be gleaned from this trial will be the long-term survival potential of each genotype over several winters when covered, and where left unprotected during the winters.

Big Canoe Golf Course (7 Stimp Measurements)			
(2017 – 2020)		Fastest	Overall Avg.
New Rootzone	12-TG-101	**11.3’**	**9.9’**
	TifEagle	**11.0’**	**9.4’**
	Primo	**10.6’**	**9.4’**
	Diamond	**9.5’**	**8.4’**
No-Till	12-TG-101	**10.4’**	**9.3’**
	TifEagle	**10.7’**	**8.7’**
	Primo	**9.7’**	**7.7’**
	Diamond	**8.9’**	**7.6’**

6) TPC Sawgrass

Jeff Plotts constructed a large research site during the summer of 2017. Our experimental bermudagrass (12-TG-101) is being compared to four bermudagrass (TifEagle, Sunday, Imperial, and G12) and three zoysiagrass (Primo, Prizm, and Lazer) cultivars. A picture of the plots during February 2020 is below, as well as a summary of the 3 stimp measurements taken to-date.

TPC Sawgrass (3 Stimp Measurements)			
(2017 – 2020)		Fastest	Overall Avg.
Bermuda	G12	**10.3’**	**9.4’**
	Imperial	**10.8’**	**9.4’**
	12-TG-101	**9.7’**	**9.0’**
	TifEagle	**9.2’**	**8.8’**
	Sunday	**9.9’**	**8.7’**
Zoysia	Primo	**10.1’**	**8.7’**
	Prizm	**9.2’**	**8.5’**
	Lazer	**8.5’**	**8.0’**

7) Streamsong Golf Resort

Rusty Mercer constructed a new research site during 2018 to compare MiniVerde, TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. The goals of this research site are to test adaptation to long-season growing environments and very intense topdressing and growth regulator management. A picture of Mach 1 during January of 2020 is below, as well as a summary of the 2 stimp measurements taken to-date.

Streamsong (2 Stimp Measurement)			
(2018 – 2020)		Fastest	Overall Avg.
Bermuda	Mach 1	**10.1’**	**10.3’**
	12-TG-101	**10.1’**	**10.2’**
	MiniVerde	**9.0’**	**9.6’**
	TifEagle	**10.2’**	***8.8’**
	MiniVerde Green	**12.1’**	**11.9’**

8) East Lake Golf Club

Ralph Kepple constructed a new research site to compare MiniVerde, TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. Sprigs were planted during May of 2019 and established very quickly. A goal of this research site was to test grow-in time with Lexicon Intrinsic fungicide applications. Eight stimp measurements and a picture of the green in November of 2020 are below.

East Lake Golf Club (8 Stimp Measurements)			
(2019 – 2020)		Fastest	Overall Avg.
Bermuda	12-TG-101	**12.0’**	**10.5’**
	MiniVerde	**11.5’**	**10.1’**
	TifEagle	**12.0’**	**10.0’**
	Mach 1	**11.4’**	**9.9’**

9) Meadows Country Club

Pat Franklin constructed a new research site during 2019 to compare TifEagle and the UGA experimental variety 12-TG-101. Sprigs were planted during August of 2019 and established very quickly. The goals of this research site are to test adaptation to warm, long-season environments and a grow-in protocol that included Lexicon Intrinsic fungicide. Two stimp measurements and a picture of the green in January of 2020 are below.

Meadows C.C. (2 Stimp Measurements)			
(2019 – 2020)		Fastest	Overall Avg.
Bermuda	TifEagle	**8.3’**	**8.2’**
	12-TG-101	**8.1’**	**8.1’**

Olde Florida Golf Club

Darren Davis renovated his research site during 2019 to compare TifEagle, Mach 1, and the UGA experimental variety 12-TG-101. He will also test TifGrand and the UGA experimental variety 11-T-56. Sprigs were planted during September of 2019 and established very quickly. The goals of this research site are to test adaptation to the long-season growing environments, winter-time play and recovery, and very intense topdressing and growth regulator management. A picture of the plots during January 2020 is below, as well as a summary of the 2 stimp measurements taken to-date.

Olde Florida (2 Stimp Measurements)			
(2019 – 2020)		Fastest	Overall Avg.
Bermuda	Mach1	**10.7’**	**9.6’**
	TifEagle	**10.3’**	**9.2’**
	12-TG-101	**10.2’**	**9.1’**
	11-T-56	**9.6’**	**9.0’**

10) USGA Sponsored Students

Mr. Jonathon Fox successfully defended his M.S. thesis "Methods for Analyzing Shade Tolerance in Warm Season Turfgrasses" in December of 2018 and was hired in a full time position with the UGA Tifton turfgrass breeding program during the fall of 2019 to concentrate on developing grasses for golf course use. Mr. Matthew Mathis is currently pursuing a B.S. in Environmental Horticulture on the Turfgrass & Golf Course Management track. Matthew has taken ownership of several on-campus putting green trials and has been instrumental in propagating plant materials for the USGA sponsored research trials, as well as for those planted at the UGA Tifton Campus and at Pike Creek Turf.

USGA ID#: 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.

2019-2020 data is only just being received by NTEP, but first glance at information received to date indicates significant differences among not only species, but also among entries and cultivars within species. Surprisingly, establishment rate data did not show all bermudagrass entries as fastest to establish at all sites. In one case, the seeded bermudagrass entry ('ASC-117') did not germinate well, and with another, the entry was not adapted to a particular location (ie. 'Midiron' bermudagrass at Citra, FL). Also, the zoysiagrass entry 'FAES 1322', bred in Florida, struggled to establish in Dinuba (Fresno), CA and St. George, UT. Conversely, the native grass entries 'Habiturf' (a buffalograss, curly mesquite and blue grama mixture) and 'Cody' buffalograss demonstrated superior establishment at Dinuba, CA and St. George, UT, but struggled in Citra, FL.

Turfgrass quality ratings also varied by entry and geographical location. For some entries with establishment problems or poor adaptability at particular sites, the resulting quality ratings were expectedly low. We noted low quality ratings of 'Midiron' bermudagrass, 'Habiturf', 'ASC-117' bermudagrass and 'Cody' buffalograss at Citra, FL. 'ASC-117', due to low germination, did not perform well at most sites, but the native grass entries 'Habiturf' and 'Cody' buffalograss did perform well in the western states where they are adapted. As expected, the zoysia entries overall have consistently been the slowest to establish.

Within the first two years, significant differences among entries and species have been noted, mainly based on geography. The next three years will likely further separate these entries leading to better guidance on species and cultivars suited for low input golf course roughs.

SUMMARY POINTS

- Significant differences were noted in establishment rates among entries, with those differences often based on geographic location.
- As expected, native grass entries appear better adapted, and hence better suited for low input roughs in the drier western states. Thus far, these native grass entries have not been seen to be well suited for use in wetter, more humid sites in the southeast U.S.

- Bermudagrass and zoysiagrass entry performance varied based on adaptability across locations, as some experimental strains are better suited for the more humid eastern U.S. than the drier western U.S.

Figure 1. The zoysiagrass entry ‘FAES 1322’, bred in Florida, struggled to establish in Dinuba (Fresno), CA.

Figure 2. ‘Habiturf’ (a buffalograss, curly mesquite and blue grama mixture) in Citra, FL (*top-middle*). ‘Habiturf’ and ‘Cody’ buffalograss demonstrated superior establishment at Dinuba, CA and St. George, UT, but struggled in Citra, FL

USGA ID#: 2017-09-619

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

Project Leaders & Affiliations: Ning Wang (PI), Yanqi Wu, Justin Moss, Charles Fontanier, *Oklahoma State University*; Jack Fry and Dale Bremer, *Kansas State University*

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

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

SUMMARY POINTS:

1. A new remotely controlled ground-based turfgrass evaluation system was designed and fabricated. Preliminary field tests was conducted in a very limited way due to Covid-19 pandemic.
2. An NDVI camera was added to the drone-based turfgrass evaluation system.
3. The data acquisition control software for the ground-based turfgrass evaluation system was optimized to improve the efficiency of the system and the quality of the collected data.
4. Algorithms for data preprocessing and data analysis to extract turfgrass quality indices were modified and optimized based on the 2019 data.

SUMMARY TEXT:

Rational Bermudagrass (*Cynodon* spp.) is the most commonly used turfgrass for golf courses, lawns, parks, and sports fields in the southern USA and throughout tropical and warmer temperate regions in the world. Quality evaluation of turfgrass is an important task for turfgrass breeder and researchers. However, this task is dependent upon tedious, time-consuming, and subjective manual evaluations. *This research targets at developing a field evaluation tool with a goal of accelerating field data collection and data processing and analysis.*

Methodology and Project Progress: Figure 1 shows a project workflow map with percentages to show the up-to-date progress status.

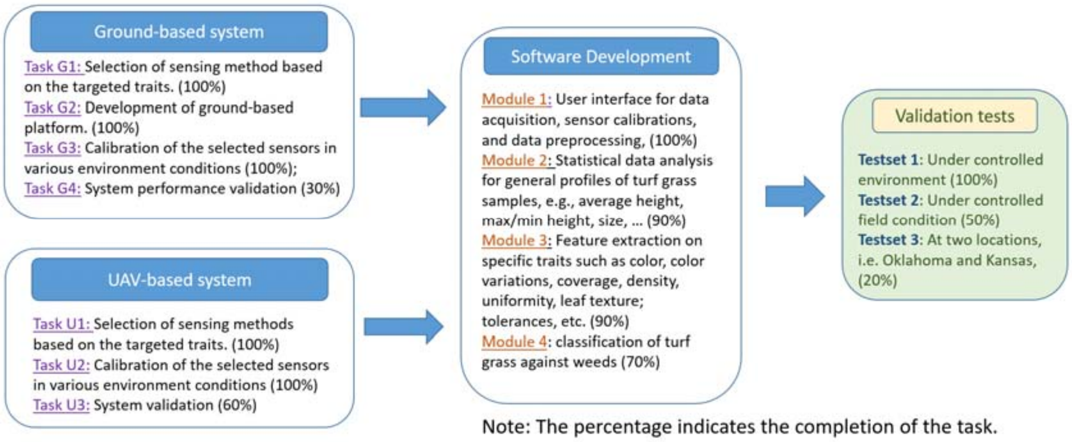


Figure 1. Updated project workflow map

Ground-based Platform

1. *Design and fabricate a new ground-based data acquisition system*
   Previously we used a golf cart as a mobile platform to carry sensors for turfgrass evaluation (Figure 2a). Due to its fixed width between wheels, the golf cart unavoidably ran over the samples which not only affected the collected data, but also created changes of the positions and angels of the sensors. Hence a new data acquisition platform was designed and fabricated. The new self-driven platform allows the changes of width based on the sample size in a nursery, maintains the previous sensor installation methods, and can be driven remotely with onboard motors and pulled with a golf cart (Figure2b). Shade panels are also added to avoid shadows which greatly affect the quality of collected images before.

   The new data acquisition platform still include an RTK GPS, an MS Kinect sensor, and a high resolution RGB camera. (Figure 2). The data acquisition control program and interface are also revised and optimized to make it more efficient and more convenient for users. The collected data are all GPS and time stamped respectively.

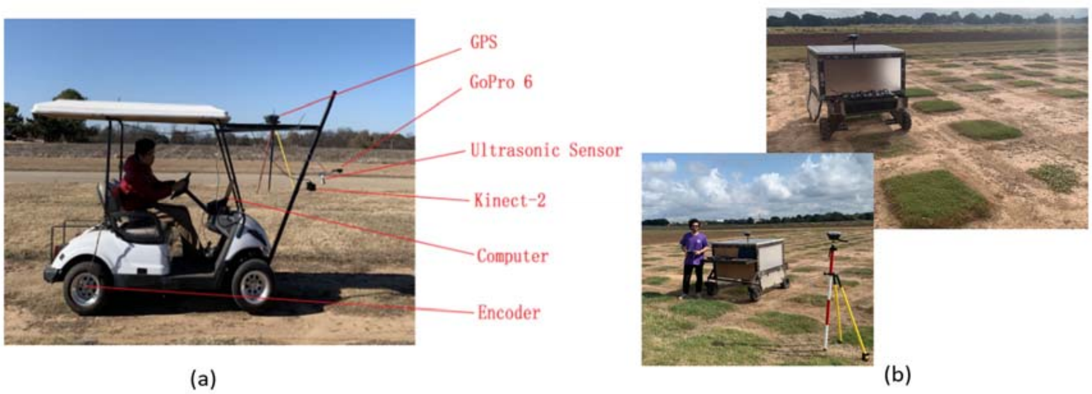


Figure 2. System platforms: (a) Golf-cart driven platform, (b) Remotely-controlled self-driven platform

2. *Sensor data preprocessing software and algorithms*
   In 2019 field tests, we collected images when the platform was driven with different speeds. We also tested various methods to collect sample images (e.g. video streams vs sampled images). All the image data were carefully analyzed and compared. The criteria used to determine the methods were the quality of the images and the complexity of the following data processing. Based on our analysis, the sampled images methods were selected. The sampling frequency can be chosen by users based on the size of the turfgrass sample.

   The most important procedure beside the normal image preprocessing procedures, such as normalization and filtering, etc., is image stitching to recover the actual field image. A fast algorithm was developed which could automatically locate a turfgrass sample, stitch multiple sample images when needed, identify missing turfgrass samples, and inserted processed image to a newly generated field map (Figure 3).

3. *Interpretation of the features extracted from collected images related to turfgrass rating*
   With the data collected in 2019, we refined the algorithm, developed in 2017-18, which analyzed the collected turfgrass images, extract features, and calculate turfgrass quality indices related to turfgrass quality rating based on NTEP evaluation criteria. However, due to the pandemic, we were not able to collaborate with Dr. Yanqi Wu to validate the algorithms with ground truth data.

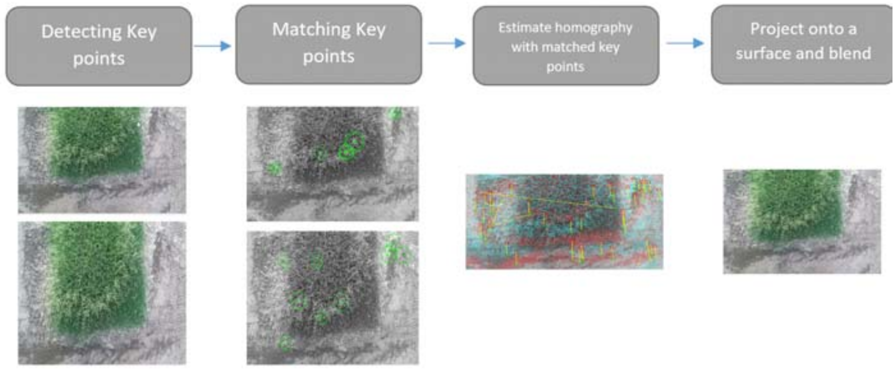


Figure 3 Data Preprocessing: Image stitching

UAV-based Platform

We maintained the DJI P3 drone with an RGB camera. Meanwhile, we designed and fabricated a new multi-rotor drone which gave a flexibility to carry combinations of a RGB camera, a thermal camera, and NDVI camera (Figure 4). Due to the pandemic, we were not able to collect the turfgrass data using drones on the OSU Agronomy farm in Stillwater. We were able to test the drones in another OSU Agronomy Farm on cotton and peanut fields. Figure 4 shows the drone platfoems. Currently we are developing algorithms to process the images collected by the drones.

Figure 4. The drone platforms: (a) The off-the-shelf DJI P3 drone with an RGB camera; (b) Newly developed multirotor drone with an RGB, a thermal camera, and an NDVI camera

Future Expectations

This granted project will end on December 30, 2020. The progress of the project was affected at the beginning stage (2017) when the designated graduate student left for another university and at the proposed testing stage due to the pandemic in 2020. We made the design and development of the platforms and develop the data acquisition software and data analysis algorithms, but conducted limited field tests. We will continue the field tests in summer 2021 and hopefully we will have an opportunity to demo the system to USGA then.

USGA ID#: 2019-10-680

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

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

Objectives: The objectives of this research are 1) to investigate the effects of weather variables, walking traffic, nitrogen application rate and soil moisture content on creeping bentgrass growth at putting green. 2) whether the potentially mineralized nitrogen (measured by the flush of CO_2 after rewetting of dried soil) determines bentgrass growth. 3) to develop a statistical growth model for creeping bentgrass that can be useful for making short-term nitrogen management decisions. 4) compare the statistical model with other nitrogen estimation model on nitrogen management

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

Summary Points:

1. Temperature, relative humidity and evapotranspiration are key weather factors determining bentgrass growth
2. Foot traffic, nitrogen rate and soil moisture are weakly correlated with bentgrass growth. However, model accuracy substantially increased when these variables were included.
3. Potentially mineralizable nitrogen in the top 5 cm of soil was positively correlated with bentgrass clipping production.
4. A data-driven statistical model using the random forest machine learning 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 should build a customized growth prediction models to manage nitrogen adaptively.

Summary Text:

Nitrogen is usually the most limiting nutrient for turfgrass growth. Therefore, a manager with a growth target could use the estimation between actual and desired growth to determine an appropriate nitrogen fertilizer rate. While the PACE Turf growth model has been used for this purpose, the model uses only temperature and is not specific to any particular species. Therefore, a more accurate method to help turf managers to make reasonable nitrogen application decisions would be valuable. To better understand turf growth and nitrogen demand, we need to identify the key factors influencing turfgrass growth and use the knowledge to build a growth model that could be used for bentgrass nitrogen management on golf course putting green.

METHODS

To investigate the interactions among soil, turfgrass, environment and management practices, this study was conducted on four ‘Focus’ bentgrass sand putting greens that vary in soil organic matter content and quality. The study was conducted in 2018-2020 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), we investigated the factors that influenced daily bentgrass growth and used the collected data to develop a growth prediction model. Generally, N fertilizer treatments were applied at 0, 0.1 and 0.2 lbs N/1000 square feet every two weeks, footwear traffic was applied by walking on the green wearing golf shoes. In 2018, traffic was maintained at high, medium and low level which represent the golf course received 3600 rounds/week, 1800 rounds/week and 0 rounds/week; In 2019, traffic levels were decreased and maintained at 1400 rounds/week, 700 rounds/week and 0 rounds/week. We additionally investigated the effect of soil moisture content on bentgrass growth on one of the greens. Treatments were maintained at high (25-27% volumetric water content), medium (18-20% volumetric water content), and low (8-13% volumetric water content) moisture levels in the top 3 inches during periods without precipitation. Turf visual quality was measured every two weeks and NDRE was measured before each clipping collection event. Soil samples were collected each month for estimating potentially mineralizable nitrogen -- a fraction of soil organic matter that can be easily converted to plant-available nitrogen. We measured the flush of CO_2 following rewetting dried soil after a 24-hour incubation at 25 °C.

All clipping data collected from 2018 and 2019 were used to build a statistical growth model. To build the bentgrass growth model, several different weather factors were selected as input variables, including air temperature, evapotranspiration, relative humidity, precipitation and wind speed. Weather data were 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” random forest package from Python.

In 2020, the growth prediction model built based on 2018 and 2019’s data 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 Growth Potential Model which estimates growth (and therefore N use) based on air temperature; 2) the experience method where 0.2 lbs N/1000 ft^2 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 only if the treatment fell below a proximal sensing measurement (NDRE) threshold. Clippings were collected three times a week, tissue was analyzed for N content, and soil moisture content and NDRE were collected before each mowing event.

PRELIMINARY RESULTS

Impact of management practices and soil potentially mineralizable nitrogen on bentgrass growth

Bentgrass growth rate overall was greatest on the plots contained the highest soil moisture content. Research plots receiving relatively high traffic levels produced significantly lower clipping yields than the plots receiving lower traffic. However, 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.

It is well known that soil N is a major source of N to the turf, yet soil N is rarely measured. In this study, potentially mineralizable nitrogen (PMN) of the soil at 0-5 cm had a strong positive relationship with bentgrass growth ($R^2 = 0.73$), while there was no correlation between PMN and growth at the 5-10 cm depth (Figure 1). Soil microbial biomass and labile organic nitrogen were more likely accumulated at the shallower depth of soil. Tracing and recording the potentially mineralizable nitrogen on the different putting greens could help turf managers adjust nitrogen rate and also be an important input for a growth prediction model.

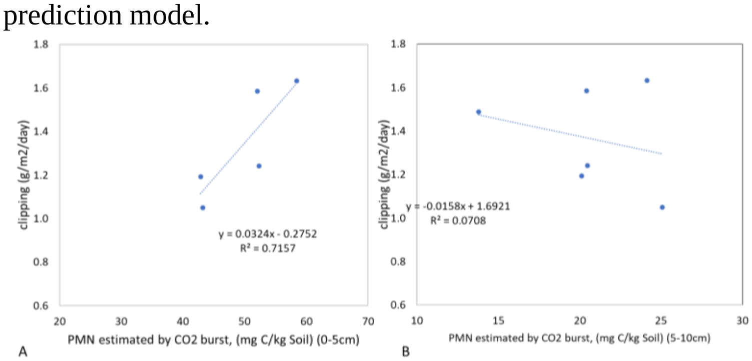


Figure 1. The correlation between potentially mineralizable nitrogen with bentgrass clipping production at top 5cm soil (A), at the soil depth of 5-10 cm (B)

Random Forest Model Performance

Both 2018 and 2019 data were used for building several random forest models, in search of one that was both simple and accurate. The full 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. without NDRE and soil moisture content input; 2. using only weekly weather data input. These reduced models focus on the variables that are most easily available or obtained by the end-user. Figure 2 shows the correlation between predicted clipping and actual clipping data for each model. These results suggest that excellent growth predictions can be made from readily available and easily obtained data, although using only weather data appears to be far less accurate than including management information and soil factors.

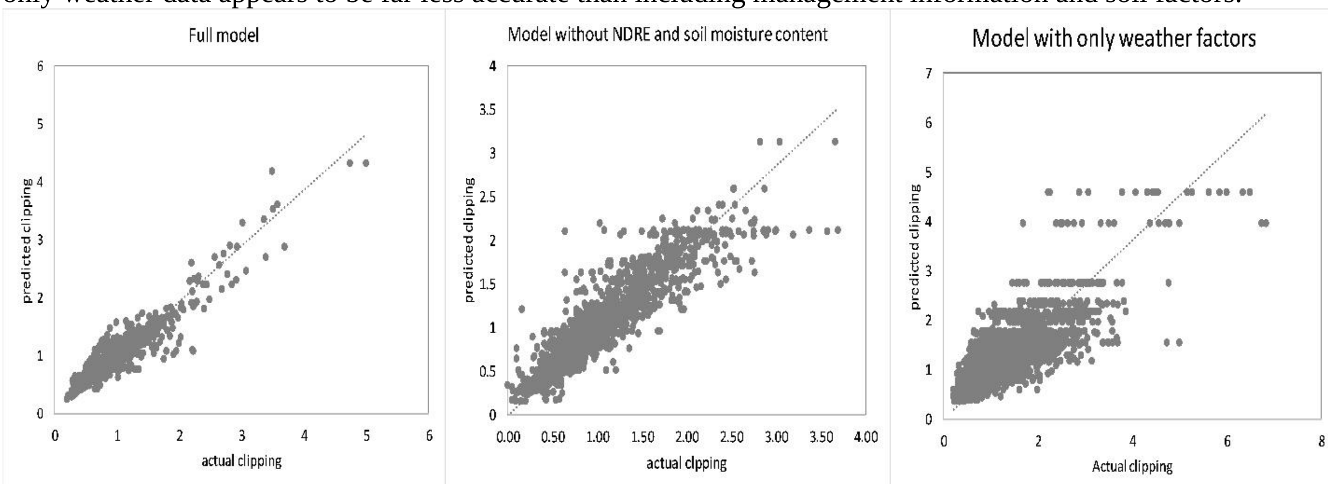


Figure 2. The scatter plot of predicted clipping and actual clipping from full model and reduced models.

Figure 3 shows the most important variables for each of the three random forest models. Generally, the most important management practices included N application rate, soil moisture content and traffic. The most important weather variables included relative humidity, evapotranspiration, and air temperature. Of the difficult to obtain measurements, NDRE and soil potentially mineralizable nitrogen increased model accuracy. We also observed that the frequency of mowing the grass influenced the bentgrass growth rate and grass that was mowed more frequently would be expected to have a higher daily clipping yield than infrequently mowed grass.

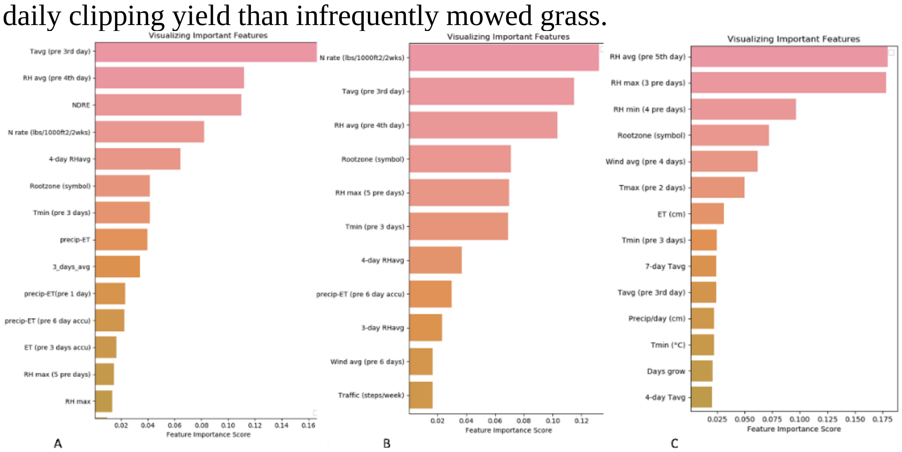


Figure 3 . Important features for two sets of prediction models (A) full model; (B) model without NDRE and soil moisture content; (C) model with only weather variables input.

We compared growth models built using weather and nitrogen application data from two golf courses and compared those predictions along with the predictions of the PACE Turf Growth Potential model to actual growth data. Figure 4 shows the Random Forest model improved upon the predictions of the Pace Turf model which uses average air temperature. This suggests that individual golf courses could build a customized growth prediction models. We are confident that with more variables added to the model (besides N and weather), the model accuracy would be greatly improved.

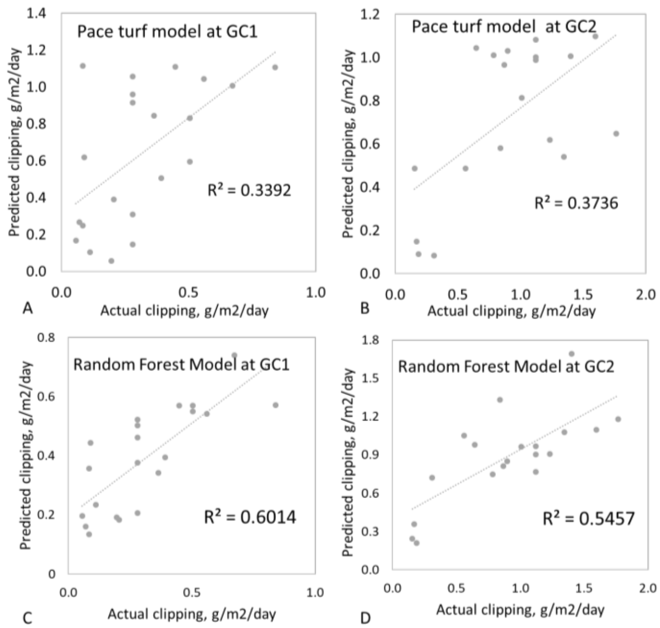


Figure 4. Model performance using different growth prediction models on two golf courses. (A) Pace Turf model performance on golf course 1. (B) Pace Turf model performance on golf course 2. (C) Random Forest model performance on golf course 1. (D) Random Forest model performance on golf course 2.

In 2020, we evaluated the use of various N management strategies on putting greens in Madison, WI (Figure 5). Overall, the PACE Turf model resulted in the greatest nitrogen fertilizer application, greatest overall clipping yield and highest visual quality. The experience method and the PACE Turf method were quite similar. The nitrogen program decided by our random forest model used only half of the nitrogen as the PACE Turf model; however, there were no significant differences in visual quality between these two models. The modified experience method resulted in the least N applied, but also showed lower turfgrass quality. In summary, it appears that growth models can be relied upon for helping turfgrass managers manage N more sustainably and adaptively.

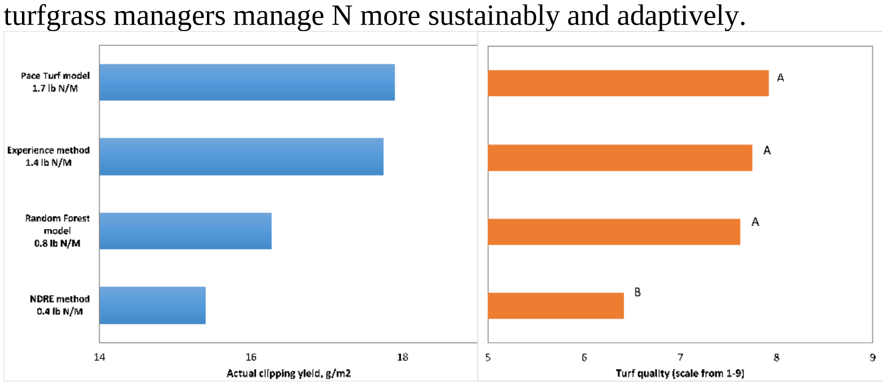


Figure 5. Three-month (May-August) bentgrass overall growth responses to nitrogen programs decided by different nitrogen prediction model (left) and its corresponding turfgrass average visual quality (right) (scale from 1-9, 1 represents bare soil, 9 represents best turf quality, 6 is acceptable).

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 otherwise was similar to the control in many parameters.
- Shade structure evaluation was delayed due to pandemic-related restrictions.

<u>Background and Rationale</u>

Management of shaded turfgrass systems can be complex. Understanding how turfgrasses respond to shaded environments is an ongoing research need for all turfgrass sites ranging from putting greens to home lawns. Commonly, neutral density shade fabric, typically made from a polywoven material, is used to screen for shade tolerance or provide a simulated shade treatment for management studies. Criticisms of these methods suggest they do not accurately simulate real-world shaded conditions that often reduce light quality (red:far red ratio), 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.

To characterize the energy balance of simulated shade structures, a uniform block of bermudagrass has been reserved at the OSU Turfgrass Research Center. Shade structures will be built and tested using varying heights (e.g., 1, 2, 3, 10-ft tall), shade densities (e.g., 50%, 80% shade), and shade materials (neutral or red selective). Sensors will be installed to measure net radiation, wind speed, ambient temperature, relative humidity, and surface temperature. Data will be collected under shaded and non-shaded conditions for each shade type to determine their effect on microclimatic conditions including long-wave radiation and evaporative demand. Similar measurements will be made under real-world shade conditions varying in tree species and shade severity.

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

Objective 3 of this project will test the effect of reduced red to far red light ratio on bermudagrass seedling growth. An 8-week study 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), blue polyester gel filter (LEE Filters, Burbank, CA), and a combination of the blue polyester gel filter and the black shade fabric. Evaluation of commercial lens filters was reported on in the prior research summary. The 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.

Early Results

Due to staff limitations associated with COVID-19 restrictions, we were unable to accomplish field evaluation of shade structure comparative performance. This objective (#1) will be pursued in summer 2021 assuming conditions improve.

In the greenhouse trial, each form of shade reduced overall productivity of bermudagrass, although shoot biomass was most reduced by black fabric (23% of control) and the combination treatment (7% of control). Black shade was also more detrimental to chlorophyll content and tillering. The blue shade lens resulted in the largest average leaf size/area (131% of control) and increased leaf length (136% of control) compared to the black fabric alone (114% of control). The results suggest bermudagrasses will respond to changes in light quality, but light quantity is a larger driver of productivity. These findings are being re-evaluated in a second run of the greenhouse experiment.

Future Expectations

Activities related to objective 1 will begin in Summer 2021 with measurement of wind, temperature, and light conditions under various man-made shade structures as well as various tree canopy types. Activities related to objective 3 were repeated in fall 2020 with data still being analyzed for final publication.

Fig. 1 Growth and development of 'Rio' bermudagrass (left) and 'Palmer's Pride' perennial ryegrass subjected to ambient conditions in the greenhouse.

Fig. 2 Growth and development of ‘Rio’ bermudagrass (left) and ‘Palmer’s Pride’ perennial ryegrass subjected to a blue shade lens.

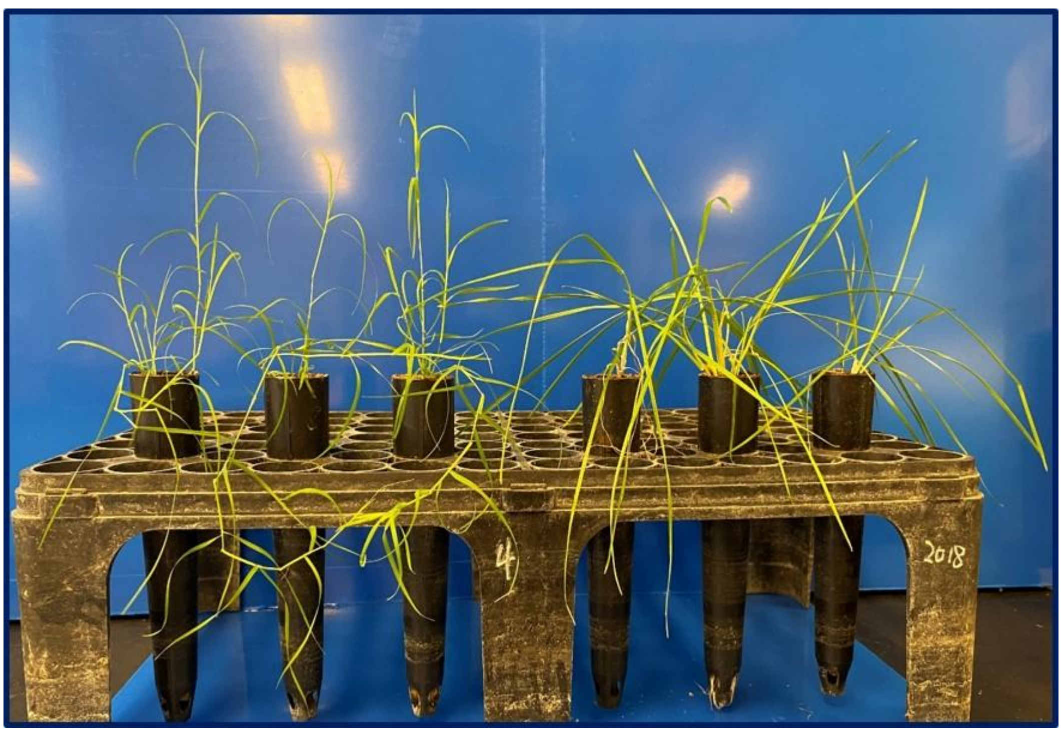


Fig. 3 Growth and development of ‘Rio’ bermudagrass (left) and ‘Palmer’s Pride’ perennial ryegrass subjected to a black shade fabric.

USGA ID#: 2019-08-678

Title: Alternate cover approaches to prevent winter injury on ultradwarf bermudagrass putting greens

Project Leaders: Michael D. Richardson and Douglas E. Karcher. Graduate student supervising the project is Thomas Walton, MS candidate.
Affiliation: University of Arkansas

Objectives:
- Examine the effects of various "air gaps" under winter protective covers on soil temperature and winter protection of ultradwarf bermudagrass putting greens.
- Assess differences in winter-injury and spring green-up between cover treatments and cultivars to determine if the inclusion of an airgap was beneficial

Start Date: Fall 2019
Project Duration: 2 winter seasons, ending in Spring 2021
Total Funding: $69,427

Summary Points:
- The winter of 2019-2020 was relatively mild and no winter injury or delayed spring greenup was observed on any of the cover treatments except the uncovered control
- The cover-only treatment increased the minimum 2.5 cm soil temperature by 2.3 °C over the uncovered treatment during the coldest period of the year
- The air gap treatments that included straw or foam batting increased the soil temperature the most (2.6-2.8 °C) over the uncovered control

Summary:
Winterkill is a constant concern on golf courses managing ultradwarf bermudagrass (*Cynodon dactylon x C. transvaalensis*) putting greens, especially as these grasses become more popular in colder regions. Although protective covers are a proven strategy to increase winter survival of putting greens (Goatley et al., 2007; DeBoer et al., 2019a), detrimental turfgrass loss has been observed in situations where rigorous covering protocols were followed (Fig. 1). Inclusion of materials like straw, batting fabric, or simply constructing a frame to create an air gap under protective covers appears to be an effective method of increasing soil temperatures compared to covers alone (Dionne, 2000; DeBoer et al., 2019b). However, there are no published studies exploring various strategies of creating an airgap to protect ultradwarf bermudagrass putting greens.

This trial was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR during the winter of 2019-2020. The trial was conducted across the three most popular ultradwarf bermudagrass cultivars, including 'Champion', 'MiniVerde', and 'TifEagle'. Each cultivar was replicated 4 times in plots that were 4 x 12.2 m (13 x 40 ft). Cover treatments (Table 1) were randomly assigned as strip plots (2.4 x 12 m or 8 x 39 ft) across all three cultivars. Covers were placed on plots when predicted low temperatures were -6.7 °C (20 °F). The experimental design was a strip-plot design, with cultivar and cover treatments randomly assigned across 4 replications. Soil temperature was continuously monitored at a 2.5 cm (1 inch) depth using soil temperature probes (Onset Company, Bourne, MA). Spring green-up was assessed as visual quality and percent green coverage using digital image analysis (Richardson, et al., 2001)

Table 1. Cover treatments and materials used in the study

Cover treatment	Material used for air gap
1) Uncovered	None
2) Cover alone - Black, woven polypropylene covers (Xton Inc. Florence, AL)	None
3) Cover plus straw (Fig. 1)	Erosion control mat with a thickness of 4 cm and a weight of 396 g m^{-2} (A.M. Leonard Horticultural Tool and Supply Co., Piqua, OH)
4) Cover plus batting (Fig. 1)	Polyester (polyethylene terephthalate) batting with a thickness of 2.5 cm and a weight of 311 g m^{-2} (Hendrix Batting, High Point, NC).
5) Cover plus pipe (Fig. 1)	15 cm diameter ABS drainage pipe split in half lengthways and placed 2 m apart (Fig. 2). This resulted in the cover resting ~7.5 cm above the turf surface. (Advanced Drainage Systems, Hilliard, OH)

The winter of 2019-2020 was relatively mild in Fayetteville AR. The lowest air temperature recorded was -9.4 °C (15.0 °F) on 14 Feb 2020 and only 3 days experienced a low temperature below -6.7 °C (20 °F). The lowest soil temperature (2.5 cm depth) recorded in the uncovered treatment was 0.90 °C (33.6 °F) on 21 Jan 2020. During the coldest week of the year (Jan 20-26, 2020), the straw plus cover treatment increased soil temperatures by 2.8 °C (5.1 °F) compared to the cover alone while all covered treatments were significantly warmer than the uncovered control (Fig. 3). The cultivar Champion was slower to greenup than other cultivars (Fig. 4), similar to earlier reports (De Boer et al., 2019a). All covered treatments contained more green cover throughout the spring compared to uncovered treatments (Fig. 4).

The first year of the present research, along with a preliminary study conducted at our location (De Boer et al 2019b), suggests that creating an air gap under covers can increase minimum soil temperatures by 2-3 °C compared to covers alone. However, we have not observed a significant effect of the air gaps on winter survival and spring greenup of any of the bermudagrass cultivars. As this project moves into the second season, one aspect that will be investigated more closely is the cost and labor expense associated with various air gap treatments. It is likely that including an air gap will not be practical on all putting green areas of a golf course, but they may be useful in areas that are more prone to low-temperature exposure such as shaded or north-sloping greens.

Literature Cited

DeBoer, E.J., M.D. Richardson, J.H. McCalla, and D.E. Karcher. 2019a. Reducing ultradwarf bermudagrass putting green winter injury with covers and wetting agents. Crop, Forage, and Turfgrass Management 5:190019. doi: 10.2134/cftm2019.03.0019.

DeBoer, E.J., M.D. Richardson, and J.H. McCalla. 2019b. Increasing winter soil temperatures with air gaps on ultradwarf bermudagrass putting greens. Poster presented at: C05 Turfgrass Science. ASA-CSSA-SSSA Annual Meeting, San Antonio, TX. 10-13 Nov. Poster 1573

Dionne, J., P.-A. Dubé, M. Laganiére, and Y. Desjardins. 1999. Golf green soil and crown-level temperatures under winter protective covers. Agron. J. 91:227-233. doi: 10.2134/agronj1999.00021962009100020009x

Goatley, J.M., Jr., J.P. Sneed, V.L. Maddox, B.R. Stewart, D.W. Wells, and H.W. Philley. 2007. Turf covers for winter protection of bermudagrass golf greens. Applied Turfgrass Science 4:1–9. doi:10.1094/ATS-2007-0423-01-RS.

Richardson, M.D., D.E. Karcher, and L.C. Purcell. 2001. Quantifying turfgrass cover using digital image analysis. Crop Sci. 41:1884-1888. doi:10.2135/cropsci2001.1884.

Figure 1 – Winterkill on a Champion bermudagrass putting green in Northwest Arkansas that was covered routinely during the winter. Note differences in survival in areas where seams in the cover were present. Photo taken May 2018.

Figure 2 – Cover treatments that were designed to create an air gap under the woven polypropylene covers.

Straw treatment

Foam batting treatment

ABS pipe treatment

Figure 3. Average minimum soil temperature (2.5 cm depth) during the coldest week (Jan. 20-26, 2020) of the 2019-2020 season as affected by various cover treatments.

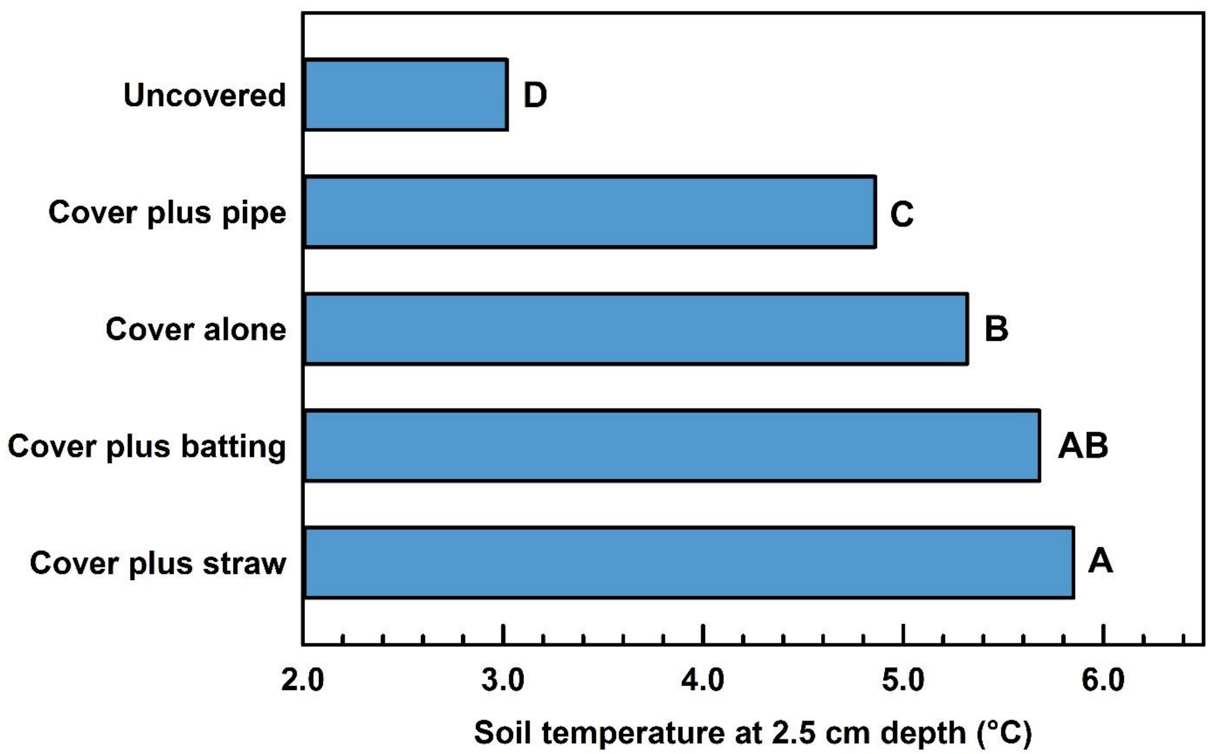
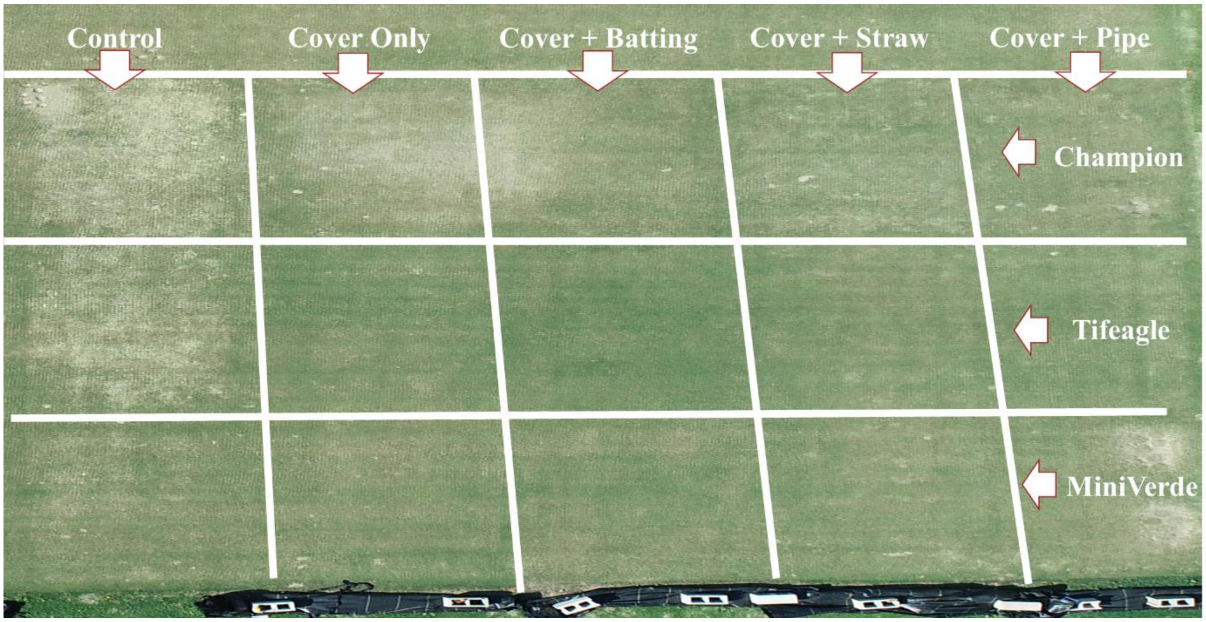


Figure 4. Aerial view (4/2/2020) of spring greenup in cultivar x cover treatments. Note improved spring greenup in all covered treatments and reduced greenup in Champion plots.



USGA ID#: 2019-16-686

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

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

Affiliation: Virginia Tech

Objectives:

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

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

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

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

Start Date: 2019

Project Duration: Three years

Total Funding: $90,000

Summary Points:

- Aeration treatments were conducted in August of 2020. Cold tolerance/spring green up data were collected in spring of 2019, aeration recovery data were collected following aeration and TruFirm and volumetric water data were collected monthly during the growing season.
- A second year of trinexapac-ethyl treatments were made during the fall/winter of 2019/20 on four different ultradwarf putting greens (‘G12’ and ‘TifEagle’) with four replications at each location.
- ‘Champion’ ultradwarf bermudagrass plugs were planted in the spring of 2020 in 6” pots with 80/20 greens mix over pea gravel to simulate USGA spec putting greens. Pots were maintained in temperature-regulated greenhouse to mimic summer conditions. Pots received urea (0.25#N/1000ft^2), trinexapac-ethyl (Primo Maxx 3 fl. oz/A), and chlorothalonil (Daconil Weatherstik, 3.6 fl. oz/1000ft2) every two weeks in the greenhouse. Pots were transferred into the field to acclimate in July of 2020, where pots were separated into two treatments; 1) trinexapac-ethyl (3oz/Acre every two weeks from July 23-October 8) and 2) nontreated control. Twenty-four randomized UDB pots underwent cold temperature acclimation to mimic fall conditions from July 23, – October

8, 2020. After undergoing fall temperature simulation, pots were treated to 15°F for 4, 6, 8, and 10 hours to mimic cold temperature exposure. Zero hours represented no damage and 14 hours represented lethal damage. After cold-temperature exposure, pots were allowed to warm to 75°F and rated for two weeks. Recovery/damage was rated, and pictures were subjected to digital image analysis to quantify green leaf tissue. Multiple runs of this project have been attempted as we refine our methods. Trials will be completed in the winter of 2020/21 to evaluate the impact of trinexapac-ethyl during winter dormancy and early spring green up.

- In early 2020 (Pre-Covid 19), Dr. Zhang and Jordan Booth evaluated a series of testing methods for rapid assessment of winter injury of bermudagrass using existing and improved methods.

Summary:

Work continued in 2020 on both the cover trials (obj. 2) and the core aeration trials (obj.1). Cover treatments were applied four times in 2020 and will be in 2021 whenever temperatures drop below 25°F. Air gap methods and heights are being evaluated as well as the use of double covers. Four ultradwarf bermudagrass aeration trials were once again aerated in 2020 with treatments ranging from zero surface disruption to 20% surface disruption. Results from these trials should be available in 2021.

Initial data indicate no significant temperature difference between air gap under covers and traditional cover methods (obj. 2). Air gap method and height had no impact as well. As expected, core aeration programs varied widely for recovery based on surface disruption, with the lowest percentage disruption recovering fastest. While initial differences in surface firmness (FieldScout TruFirm, Spectrum Technologies) were attributed to greens construction methods, after three years of treatments, surface firmness is now more closely correlated with % surface disruption. In winter of 2021, dormant plugs will be pulled to evaluate the impact of core aeration on cold tolerance.

Early greenhouse/growth chamber trials evaluating the impact of fall applications of trinexapac-ethyl on cold-tolerance of ultradwarf bermudagrass (Obj. 3) provided inconsistent results based on our methods. Percent green cover data were best modeled (JMP Pro 15) using non-linear Gompertz 3 parameter regression. This model was used to predict the 50% mortality rate of ultradwarf bermudagrass (time in hours/15°F). In the first run of the trial, fall applications of trinexapac-ethyl (TE) on ultradwarf bermudagrass (UDB) had a positive impact on both turf quality and turf color. Cold temperature exposure treatments included 4, 6, 8, and 10 hours. Ultradwarf bermudagrass pots survived all temperature x hour treatments. No damage was observed at 4 and 6 hours at 15°F but damage occurred above 6 hours at 15°F. Our model predicted 50% mortality of plants under trinexapac-ethyl treatments at 9.84 hours at 15°F vs. a 50% mortality rate for non-treated plants to be 11.38 hours

Methods were adjusted to provide a wider range of results, including a lethal treatment. Hours of cold temperature exposure were adjusted to 4, 7, 10, and 13 hours. Unfortunately, two runs of this trial resulted in varied results. The first run had no damage and the second run had lethal damage across all pots. Work continues to evaluate and improve our methods, using two Frigidaire Chest Freezers, modified with thermostat temperature controllers (Inkbird ITC308), grow lights, a small fan, and a small ceramic heater to serve as growth chambers. Methods continue to be evaluated so dormant and early green-up simulations can be run as well as future runs of fall simulation. These methods are critical to evaluating the cold tolerance impacts of our aeration studies as well.

Dr. Zhang and Mr. Booth were busy in the first three months of 2020 evaluating methods to rapidly assay winter-related injury (obj. 4). COVID-19 halted the ability to work together in the lab, but that work will continue in 2021.

Figure 1. Aeration and topdressing 2020.

Figure 2. Cold tolerance treatment results (10 hours at 15°F) Primo Left, Untreated Right.

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], Lindsey Hoffman[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 low temperature germination traits, and post-germination seedling vigor in response to low temperature and variable light intensities.
- Chlorophyll fluorescence imaging was used to screen for differences in seedling cold tolerance, indicating that cultivars such as Penn A-4, Penncross, and L-93 exhibited damage following simulated freezing events.
- Methods were optimized for testing the interactive effects of different light intensities and temperatures on seedling vigor and establishment in both growth chamber and field experiments.
- Based on controlled environment experiments, 50 to 90% reductions in light intensity increased photochemical efficiency when creeping bentgrass seedlings were challenged with a sudden decrease in air temperature.

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.

Twelve creeping bentgrass cultivars were selected based on a range of cold germination or spring green-up traits as identified in our previous research at UMass and UMN. In Year 2 experiments at UMass we confirmed baseline germination of creeping bentgrass at optimal (20°C, or 68°F) and low (10°C, or 50°F) temperatures, which were previously determined to serve as discriminatory temperatures for germination traits in this species. There were differences in total percent germination and germination rate among creeping bentgrass both at optimal and low temperatures (Figure 1, left panel). At low temperature (10°C), the cultivars Declaration, Luminary, and Penn A-4 exhibited the highest total percent germination. In contrast, the cultivars Independence and Piranha had the lowest total percent germination. These two cultivars also exhibited low percent germination at 20°C, which indicated these particular seed lots had low overall viability. However, in prior studies at UMN, the cultivar Independence also had poor germination in response to low temperature exposure. When evaluating the rate of germination at 10°C (Figure 1, right panel), the cultivars Declaration and Luminary exhibited the highest germination percentage at 10 d of low temperature exposure. Although Penncross had overall lower total percent germination at 10°C, this cultivar also was statistically similar to Declaration and Luminary. In contrast, L-93 and Memorial cultivars had the lowest germination rates following 10 d at 10°C. Interestingly, while L-93 also had slow germination rate at 20°C, the germination rate of Memorial seemed to be more negatively impacted at low temperature.

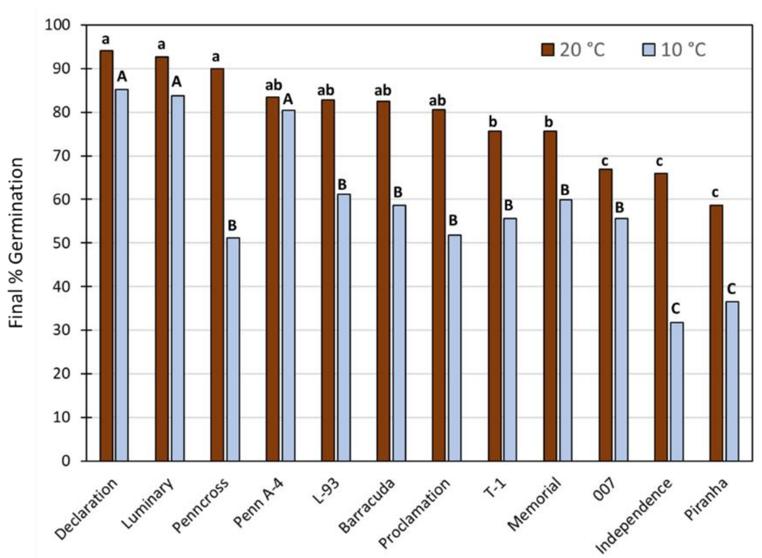
To better understand differences in post-germination seedling vigor, we tested the cold stress responses of 3 wk old seedlings in response to simulated overnight freezing events. Seedlings were grown at 15°C and then exposed to either -5°C (23°F) or -10°C (14°F) for 8 hours during the dark period. Chlorophyll fluorescence imaging was used to assess genetic differences among cultivars for seedling photosynthetic efficiency, and indirectly as a screening tool to detect potential leaf injury in response to freezing temperatures. Following a freezing event at -5°C, the cultivars with the lowest photochemical efficiency included Penn A-4, L-93 and Penncross (Figure 2). Similar results were also found when seedlings were exposed to -10°C, with Penn A-4 and Penncross exhibiting the lowest photochemical efficiency. In contrast, cultivars such as Barracuda and Proclamation maintained high photochemical efficiency following freezing, suggesting little injury to the photosynthetic apparatus. However, there were not many statistically significant differences compared to most other cultivars.

In addition to the effects of low temperature on seedling vigor and establishment, the interaction of temperature and light intensity can also play an important role in seedling stress tolerance. Specifically, the combination of high light intensity and low temperature can cause photoinhibition, a condition associated with stress-induced damages to the photosynthetic system and a decline in growth. Therefore, Year 2 experiments at UMN were specifically designed to test whether reductions in light intensity (e.g. through use of shade cloths) could minimize photoinhibition and improve the establishment of creeping bentgrass seedlings exposed to cold temperatures. The same 12 creeping bentgrass cultivars were for grown for 3 wk at 15˚C, under a light intensity of approximately 800 µmol m^{-2} s^{-1} PPFD. Plants were then exposed to combination treatments consisting of low or high temperature (10 or 20˚C) and 3 light intensity levels (800, 400, and 100 µmol m^{-2} s^{-1} PPFD) using shade cloths previously optimized in Year 1. Temperature and light treatments resulted in visible changes in creeping bentgrass growth and development (Figure 3). Under the highest light intensity (0% light reduction, 800 µmol m^{-2} s^{-1} PPFD), the leaves of most creeping bentgrass cultivars exposed to the low temperature of 10˚C exhibited a purple coloration, indicative of anthocyanin pigment accumulation. Based on visual estimates, the cultivars Memorial and L-93 had higher purple coloration of leaves; however, quantification of anthocyanins and chlorophylls are underway to confirm the visual estimates. Seedlings exposed to 10˚C for 7 d also exhibited lower vertical growth compared to seedlings at 20˚C, regardless of light intensity treatment. This suggested that the short-term shade cover, if implemented in the field, would not likely result in etiolated growth and scalping. Creeping bentgrass plants under 90% reduced light intensity (approximately 100 µmol m^{-2} s^{-1} PPFD) exhibited higher photochemical efficiency at both 20 and 10˚C. For cultivars such as Penncross and Memorial, photochemical efficiency levels were the same at 20 and 10˚C, regardless of light intensity. This suggests that covering creeping bentgrass seedlings with 50 to 90% shade cloth prior to a cold temperature event could decrease the potential for low-temperature photoinhibition.

A preliminary field trial was conducted this fall at UMN to test the effects of different shade cloth combinations to achieve 50 or 90% light intensity reductions while also having minimal impacts on canopy temperatures. The cultivars Memorial and T-1 were seeded into pots, and then the pots were placed into large plastic tubs filled with USGA sand to buffer soil temperature effects in the field. Similar to growth chamber results, a reduction in light intensity via shade treatments resulted in increased photochemical efficiency compared to seedlings exposed to ambient light intensity (Figure 4). Preliminary data based on digital image analysis also indicates that shade treatments may improve creeping bentgrass establishment in the field.

In the next year the goal will be to identify which key traits are most critical for early spring establishment (e.g. low temperature germination rate, seedling cold tolerance, sensitivity to photoinhibition). Field experiments are planned for spring 2021 to confirm results from growth chamber experiments at UMass and UMN. We are also selecting the top performing and bottom performing cultivars to test effects of chemical priming compounds for enhancing seed germination and seedling establishment.

Figure 1. Differences in seed germination traits among 12 creeping bentgrass cultivars. Seeds were germinated in petri dishes (50 seed per plate) at either 20°C (68°F) or 10°C (50°F) in controlled environment chambers. The left panel provides data for total percent germination at each temperature. The right panel represent cultivar statistical groupings for early germination rate at either 3 days at 20°C or 10 days at 10°C. Data were averaged using three petri dishes per cultivar in each experiment, and three experimental runs at each temperature.



Germination rate: 3 d @ 20°C	Germination rate: 10 d @ 10°C
Declaration (78.4%)	Declaration (86.1%)
Luminary (76.2%)	Luminary (84.4%)
Memorial (72.9%)	
Penncross (64.6%)	Penncross (85.8%)
Barracuda (65.0%)	Barracuda (72.3%)
T-1 (49.3%)	T-1 (77.4%)
Penn A-4 (41.2%)	Penn A-4 (74.8%)
Proclamation (50.1%)	Proclamation (70.4%)
L-93 (50.4%)	L-93 (57.6%)
	Memorial (46.4%)

Figure 2. Photochemical efficiency of creeping bentgrass seedlings in response to a simulated overnight freezing event at either -5°C (23°F) or -10°C (14°F). Left panels show the chlorophyll fluorescence responses under saturating and steady-state light conditions. The right panels are to cultivars selected for different photochemical efficiency responses under pre- and post-freezing conditions, including Barracuda and Penn A-4 creeping bentgrasses. A higher percentage of photochemical efficiency (represented by colors of red and orange) are a measure of a higher capacity for light harvesting and photosynthetic efficiency.

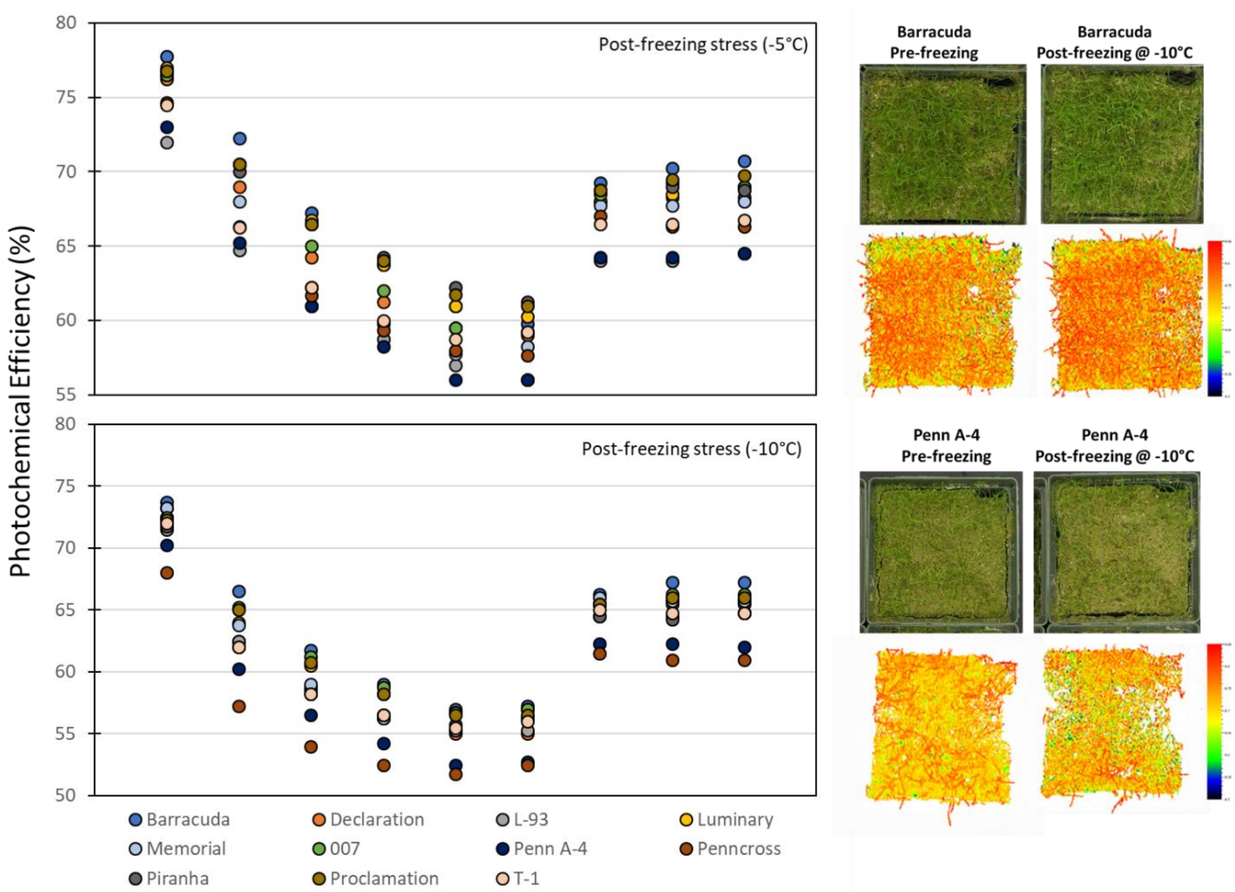


Figure 3. Creeping bentgrass seedlings exposed to combination treatments consisting of high or low temperature (20 or 10˚C) and 3 light intensity levels (800, 400, and 100 µmol m^{-2} s^{-1} PPFD). Different light intensity treatments were established with shade cloths to achieve from 0 to 90 % light reduction. Photos were taken following 7 days of treatment.

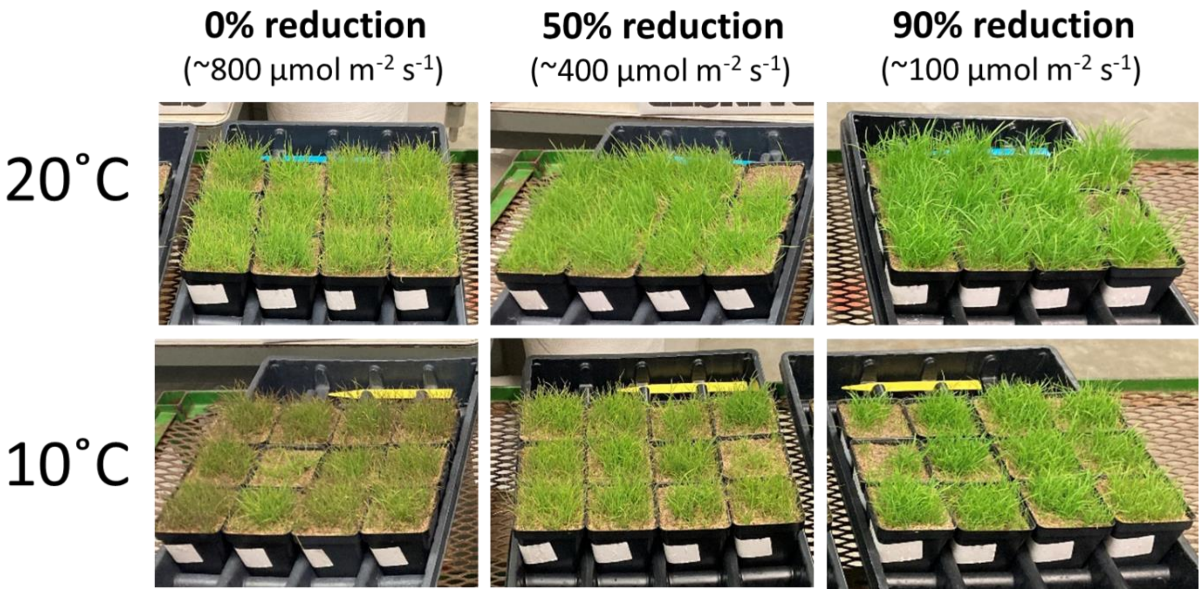
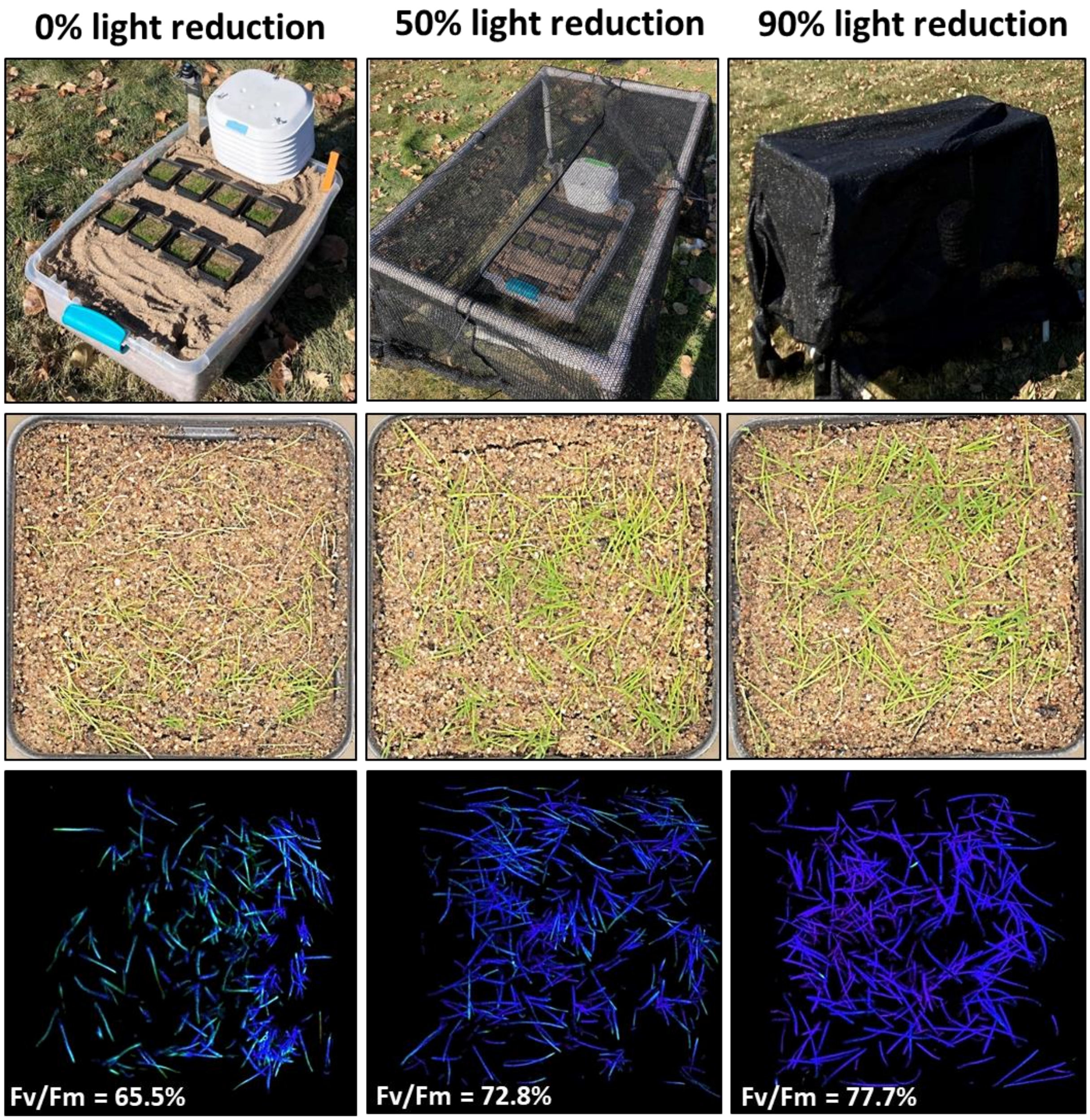


Figure 4. Differences in the establishment and percent photochemical efficiency (Fv/Fm) of T-1 creeping bentgrass exposed to different shade treatments in the field. Shade cloths were optimized to establish treatments of 0% (ambient sunlight), 50%, and 90% reduction in light intensity.



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 products and application rates for controlling *Poa* summer decline or improving heat tolerance.
3. Test the effectiveness of plant-health products for promoting summer performance of annual bluegrass on putting green conditions.

Start Date: 2019

Project Duration: three years

Total Funding: $118,552

This report describes the field study conducted in summer 2020, addressing the objective 3 to investigate effects of plant-health products on summer performance of annual bluegrass on putting green conditions.

Summary Points:

- Application of Daconil Action, Appear II, Daconil Action + Appear II, Daconil Action + Appear II + Primo, and Signature XTRA StressGard effectively enhanced Poa turf performance management under putting green conditions during summer months.
- The combined treatment of Daconil Action + Appear II or Daconil Action + Appear II + Primo was more effective in improving Poa summer turf growth and health than each individual treatment alone.
- Primo alone had no significant effects on Poa summer turf performance while the combination of Proxy and Primo suppressed turf growth.

Methodology

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

The field sites at the research farm (Filed #18 D North) 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 weekly application of Dollar Spot control (Emerald – Curalan), Brown Patch control (Prostar), Summer Patch control (Heritage TL), Growth Regulation (Primo Maxx 0.125 fl oz/1000 ft^2), 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 with a carrying volume of 2 gal/1000 ft^2.

Untreated control	Biotimulants	PGRs	Fungicides
Water (2 gal/1000 ft^2)	Amino acids (AAA, 60 mM /1000ft2)	Primo Maxx (trinexapac-ethyl, 0.1 fl oz / 1000 ft2)	Daconil Action (3.5 fl oz / 1000 ft^2) (DacAc)
	Hormone (AAB, 44 µM / 1000 ft2)	Proxy (ethephon) (2 fl oz / 1000 ft2)	Appear II (6 fl oz / 1000 ft^2) (AppII)
	Combined AAA and AAB (AAC)	Primo Maxx + Proxy (PP)	Daconil Action + Appear II (DAppII)
	Seaweed-based SWA 12 fl oz / 1000 ft^2		Daconil Action + Appear II + Primo (DAIIP)
	Seaweed-based SWB 15 fl oz / 1000 ft^2		Signature XTRA StressGard (4.0 fl oz / 1000 ft^2) (Sig),

All treatments were applied to 3’x 4’ field plots (4 replicates each) every 14 days between the time period of July 23^{rd} to September 1^{st}.

The following measurements were taken weekly based on weather conditions. Turf quality was visually rated. Normalized Difference Vegetation Index (NDVI), Stress Index (SI) and leaf area index (LAI) was evaluated using a multispectral radiometer (CropScan). 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. On September 8^{th} four root cores were taken at a depth of 5cm from each plot to analyze root characteristics.

Treatment effects on different parameters were determined by analysis of variance 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, Daconil Action + Appear II + Primo, and Signature XTRA StressGard had significantly higher turf quality (Fig. 1A). Applying Daconil Action + Appear II, Daconil Action + Appear II + Primo, Appear II, and Signature XTRA StressGard also maintained higher canopy cover (Fig. 2), and greener turf (DGCI) (Fig. 3) during most of the summer months from July to September.

Poa treated with Daconil Action + Appear II, Daconil Action + Appear II + Primo, Daconil Action, and Signature XTRA StressGard had significantly higher LAI (Fig. 4A), and NDVI (Fig. 5A) levels from late August throughout September. Poa with Daconil Action + Appear II, Daconil Action + Appear II + Primo, Daconil Action also had lower stress levels (Fig. 6A) compared to the control during late August and September.

Application of Primo alone had no significant effects on any of the parameters examined in this experiment during the summer months. *Poa* treated with the combination of Proxy and Primo had lower turf quality (Fig. 1B), DGCI levels (Fig. 3B), LAI (Fig. 4B), and NDVI (Fig. 6B) during July and into the middle of August. During this time, Proxy + Primo treated plots also had significantly higher stress levels (Fig. 5B).

Among the biostimulant treatments, SWA-treated plots appeared to have higher visual turf quality (Fig. 1C), DGCI (Fig. 3C), LAI (Fig. 4C), and NDVI (Fig. 5C), as well as lower SI (Fig. 6C) compared to the untreated control plots during the summer months, although the differences were not statistically significant ($p = 0.05$).

Plots treated with Daconil Action + Appear II and AAC amino acids had significantly lower canopy temperature compared to the control on September 10th. Other treatments had little to no significant effects on canopy temperature during most of the summer months (Fig. 7).

The biostimulant treatments examined in this trial did not have statistically ($p = 0.05$) significant effects on root growth, although there appeared to have greater length and dry weight (Fig. 8). The lack of statistically significant difference for root parameters between each treatment and the untreated control could be due to the variations between field plots, which could be overcome by increasing the number of root samples and replications in future experiment.

Despite implementing methods to control anthracnose disease the trial plots began showing signs of infection in mid-September. Plots with Daconil Action, Appear II, Daconil Action + Appear II, Daconil Action + Appear II + Primo, and Signature XTRA StressGard had lower anthracnose ratings than the control (Fig. 9).

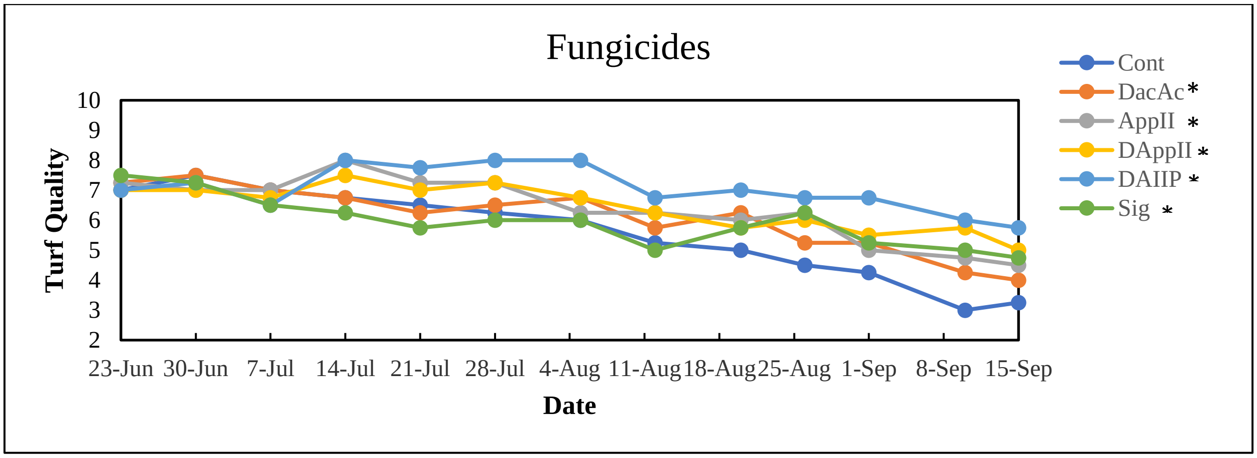
<u>Future expectations</u>

We will repeat the experiment in the research farm and on golf course putting greens in 2021. The future replication of the experiment with more affective anthracnose control over all plots may serve to confirm if improved performance is due specifically to improved heat tolerance. We will also increase the number of root sample collections from field plots and increasing replications to confirm effects of different treatment effects on root growth in future experiment.

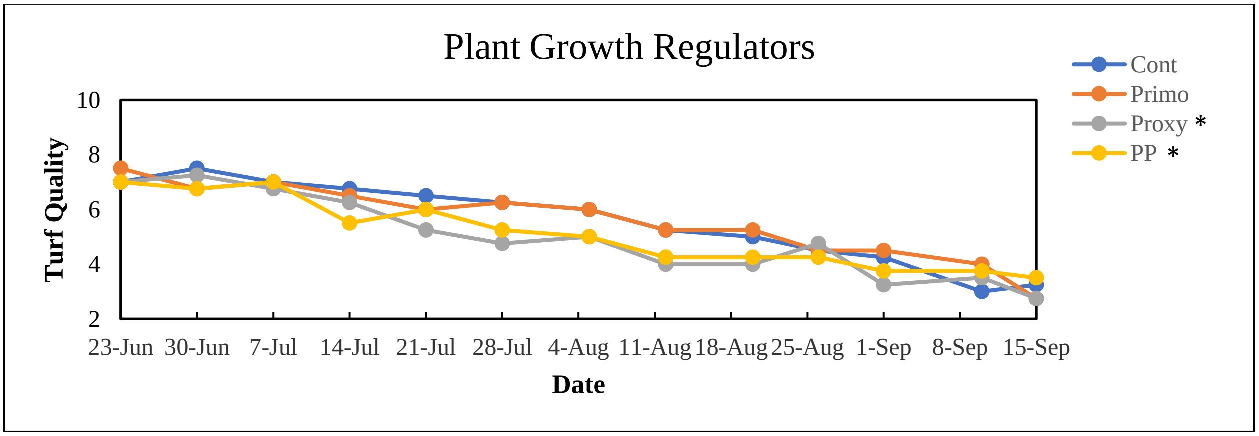
Fig. 1. Turf quality of Poa putting green as affected by different treatments.

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

A



B



C

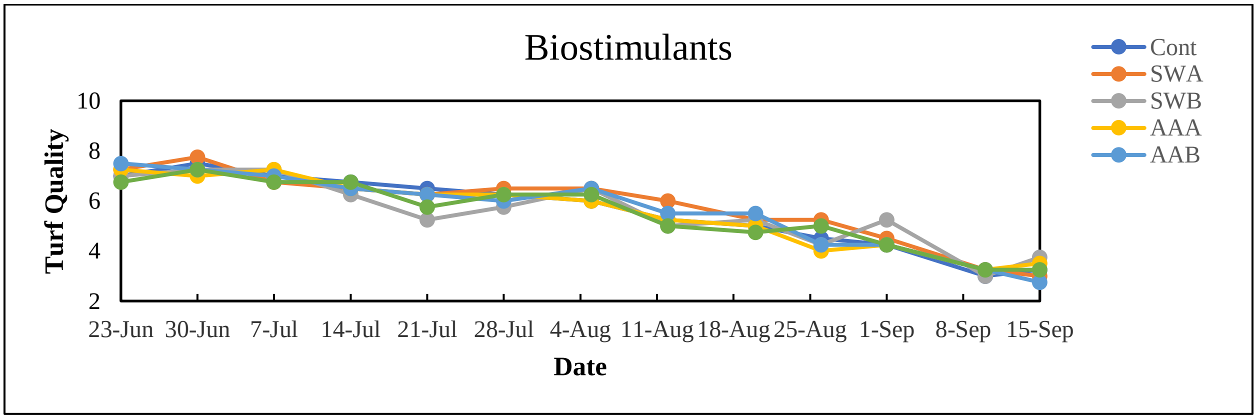


Fig. 2. Canopy cover of Poa putting green as affected by different treatments.
"*" indicate treatments significantly different from the control at p = 0.05.

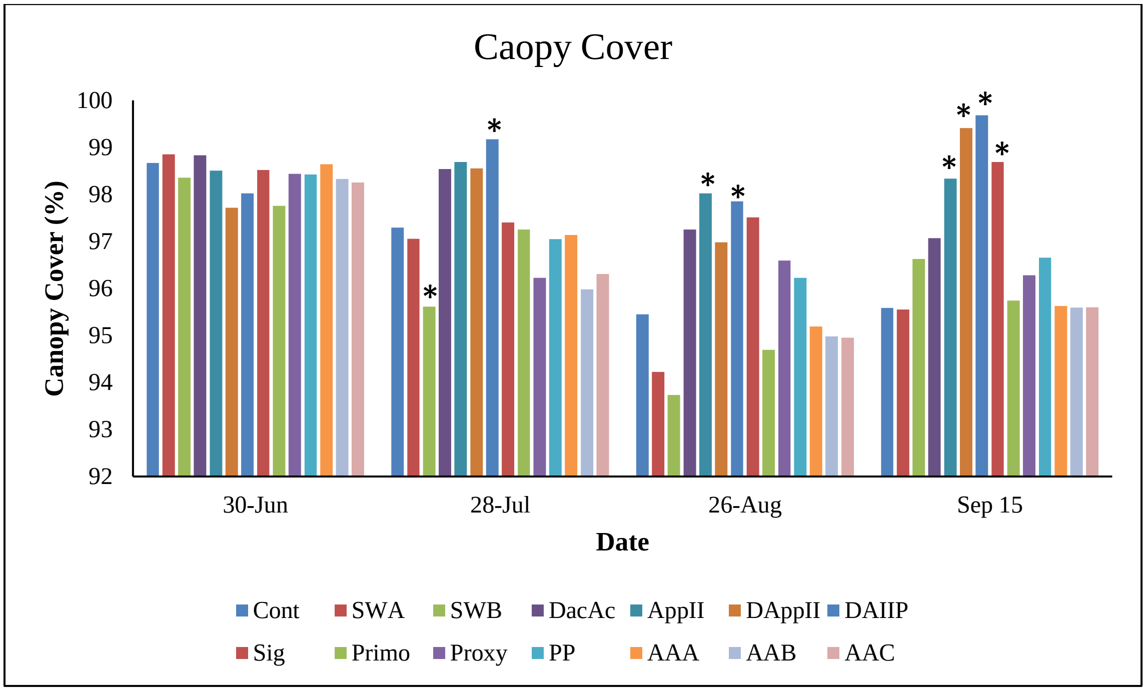


Fig. 3. Dark Green Color Index (DGCI) of Poa putting green as affected by different treatments. "*" indicate treatments significantly different from the control at p = 0.05.

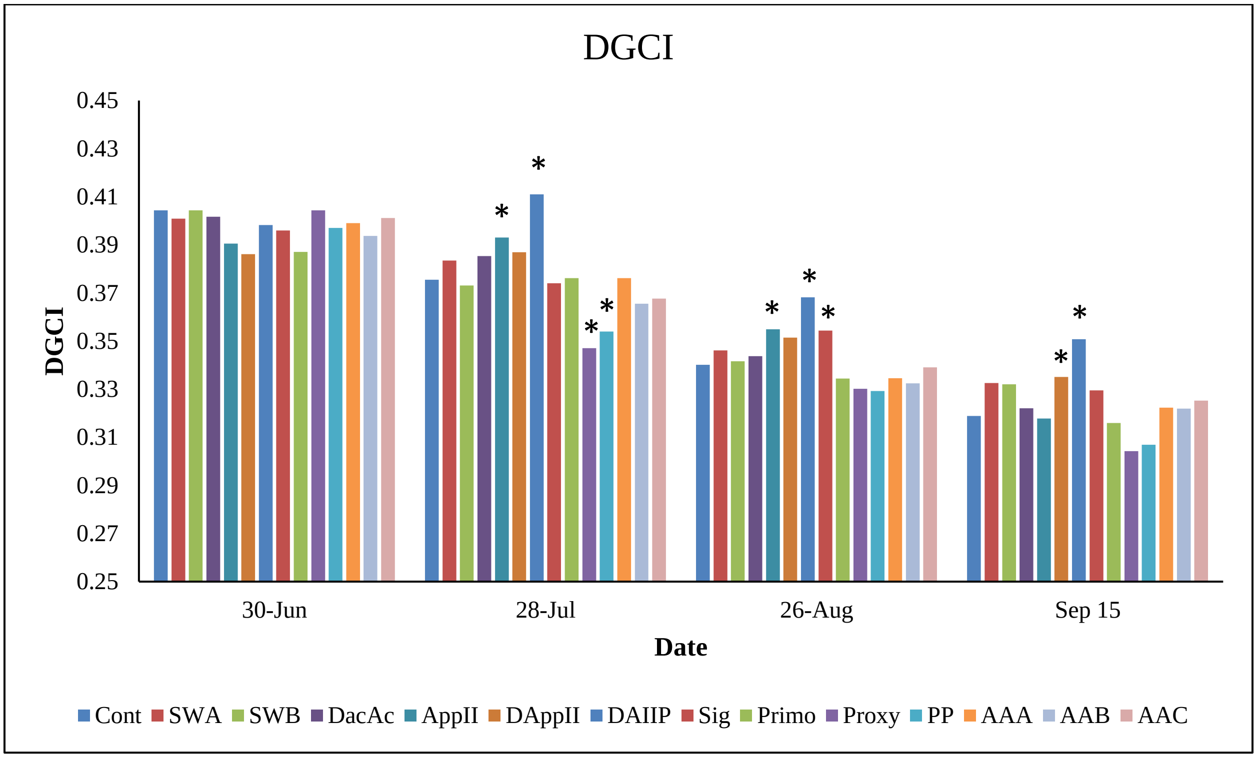


Fig. 4. Leaf Area Index (LAI) of Poa putting green as affected by different treatments.

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

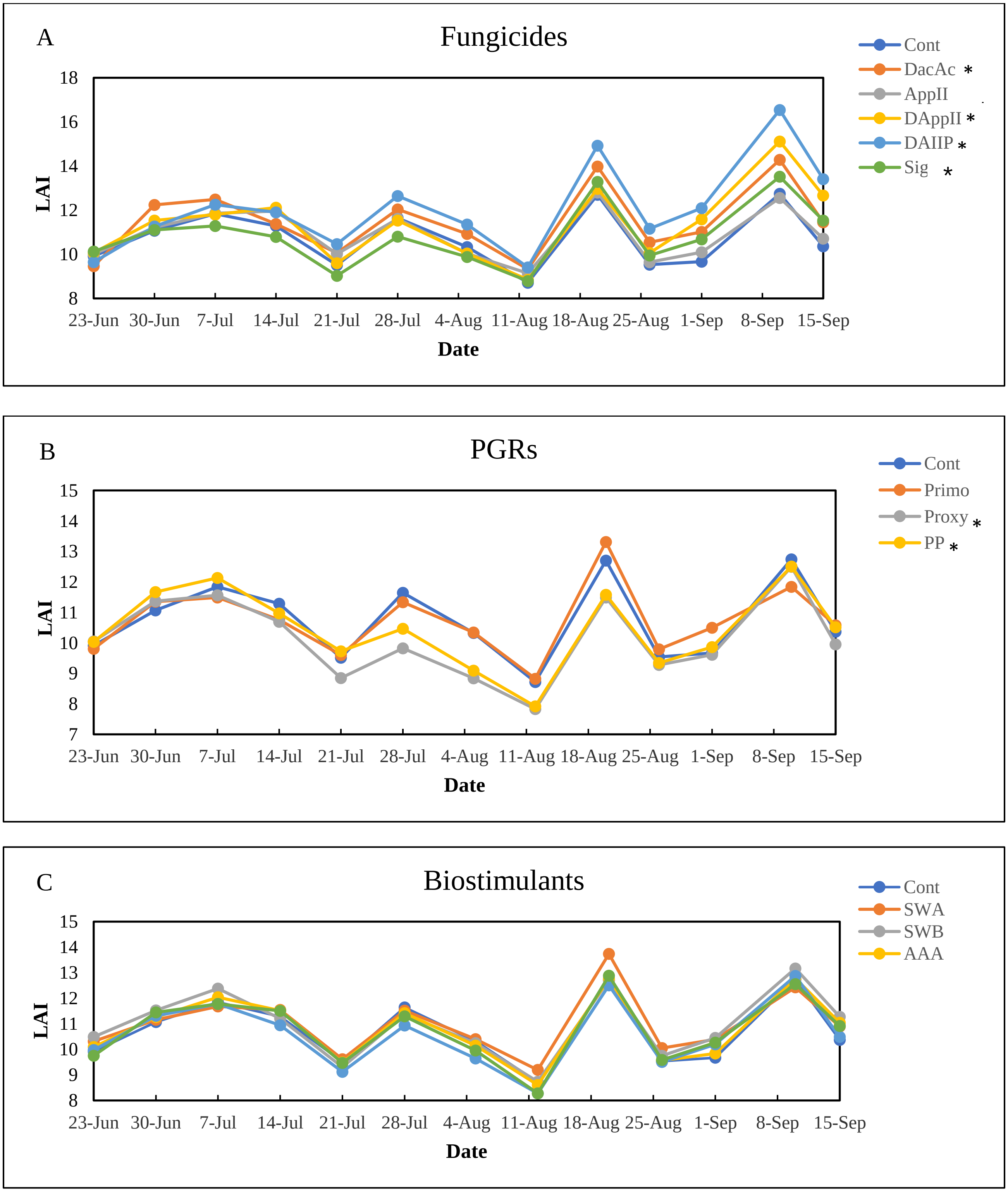
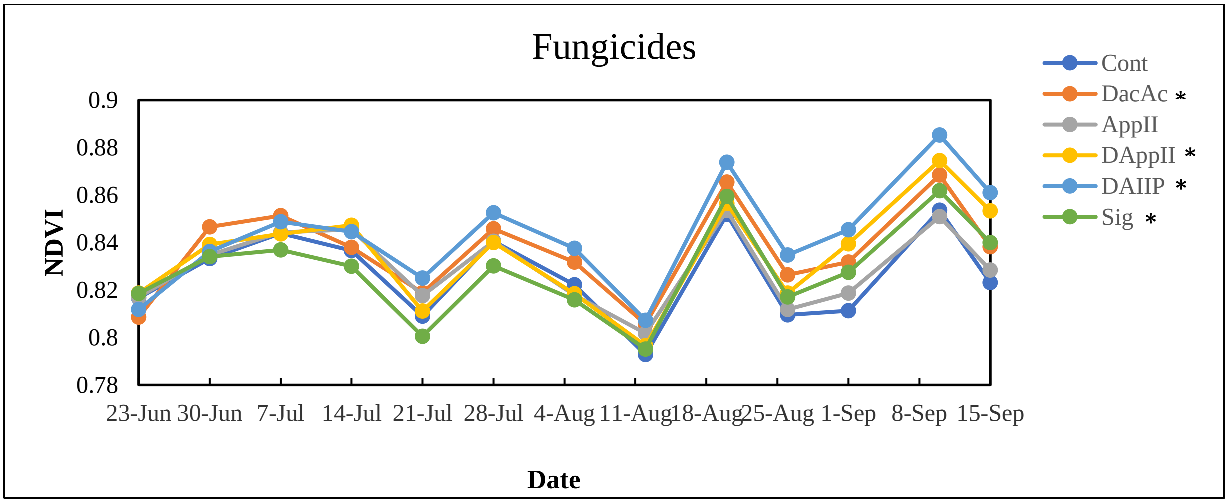
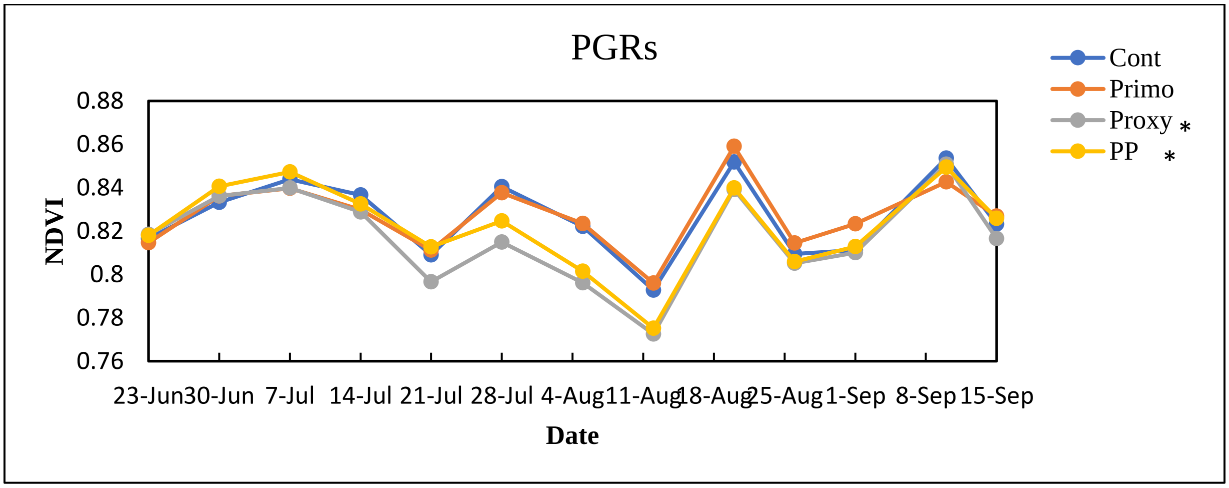


Fig. 5. Normalized Difference Vegetation Index (NDVI) of Poa putting green as affected by different treatments. "*" indicate treatments significantly different from the control at p = 0.05.





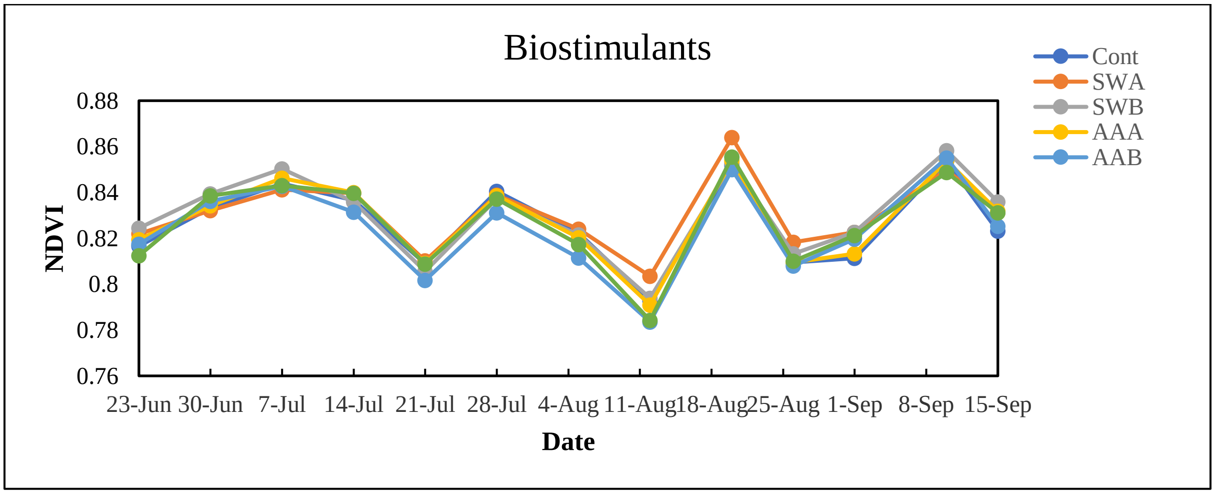
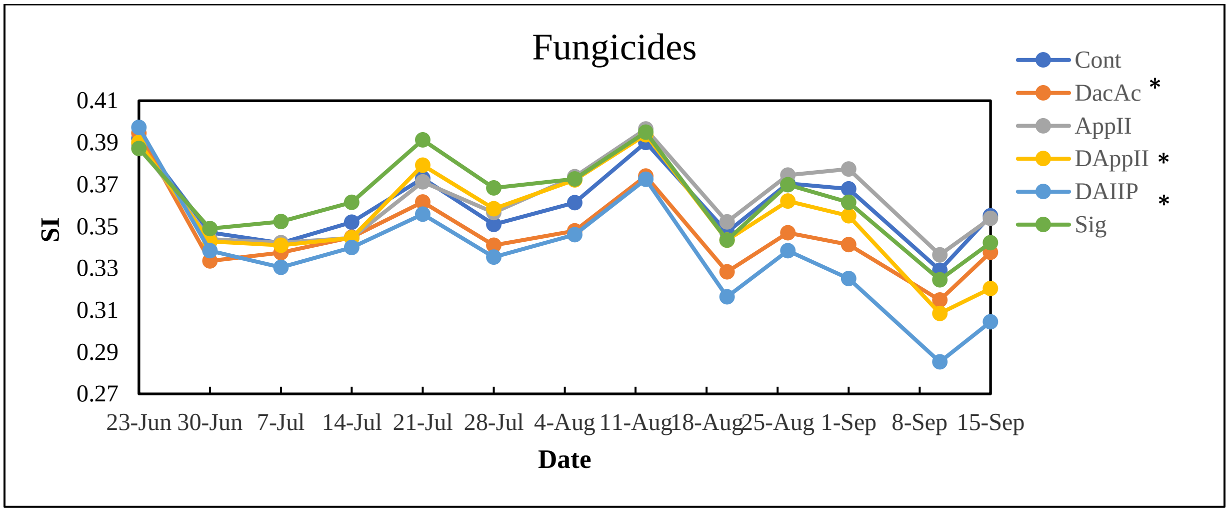
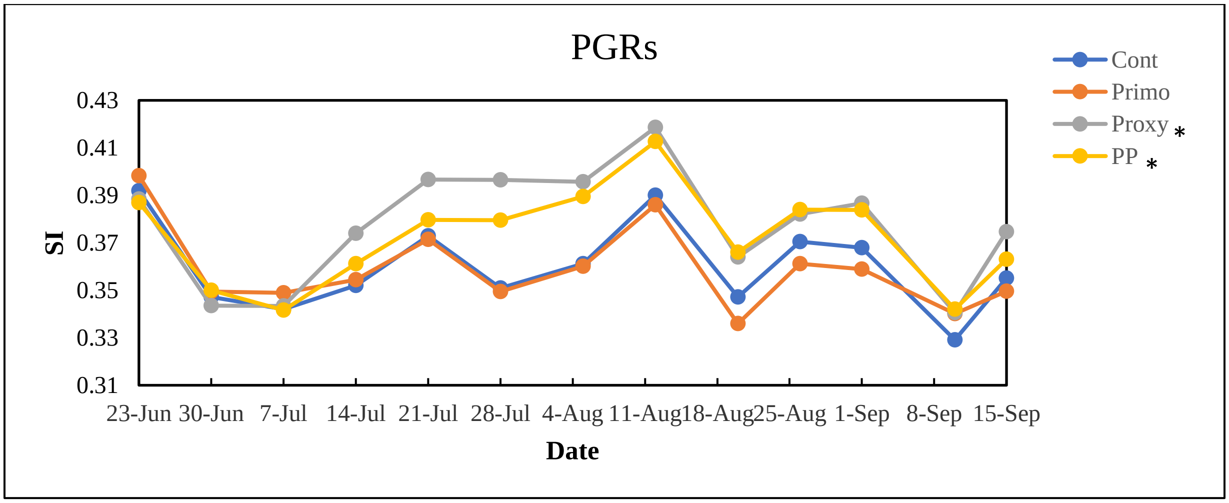


Fig. 6. Stress Index (SI) of Poa putting green as affected by different treatments. "*" indicate treatments significantly different from the control at p = 0.05.





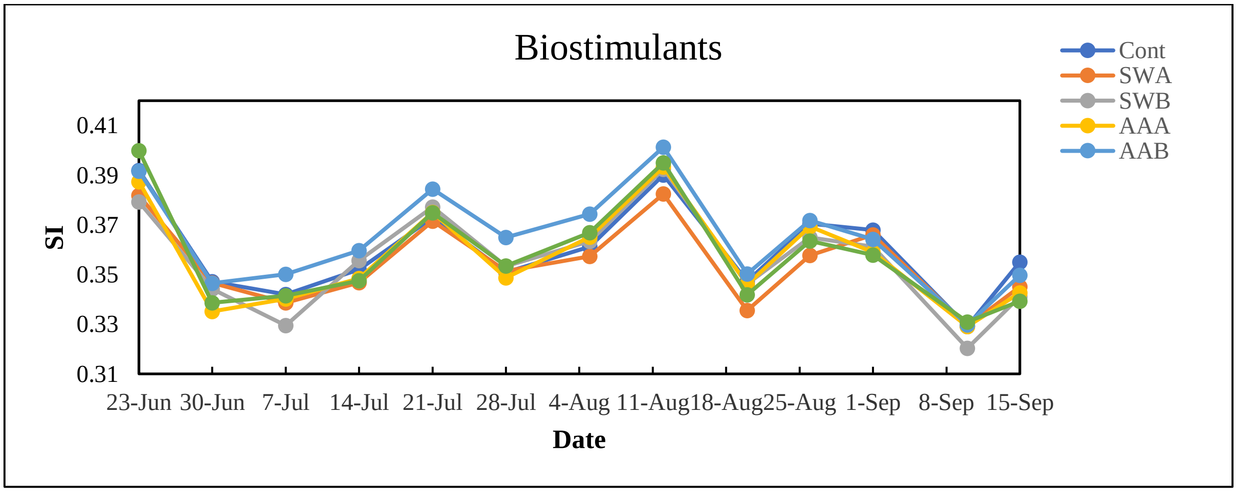


Fig 7. Canopy Temperature of Poa putting green as affected by different treatments. "*" indicate treatments significantly different from the control at p = 0.05.

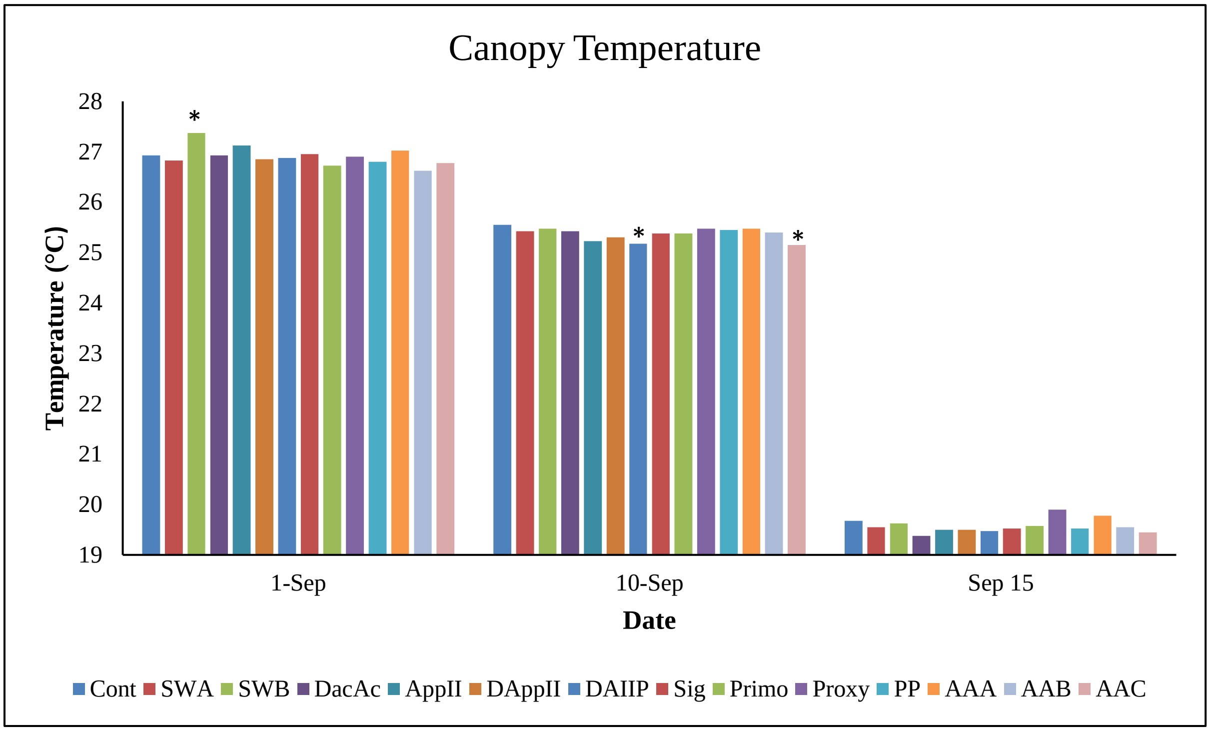
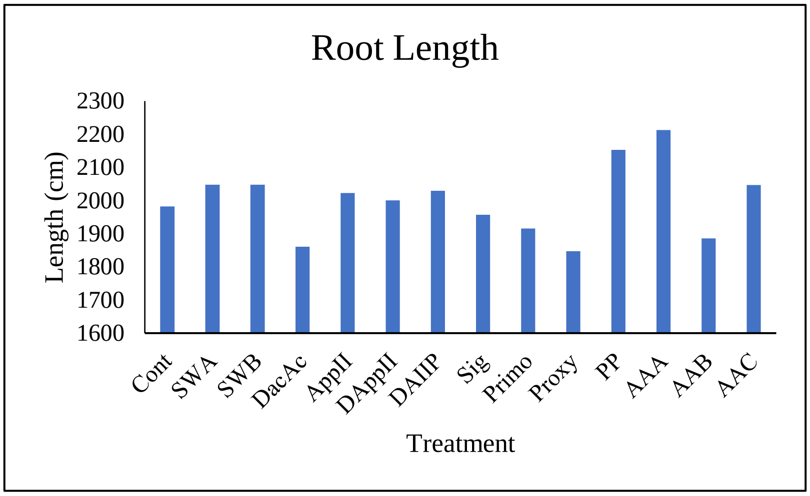
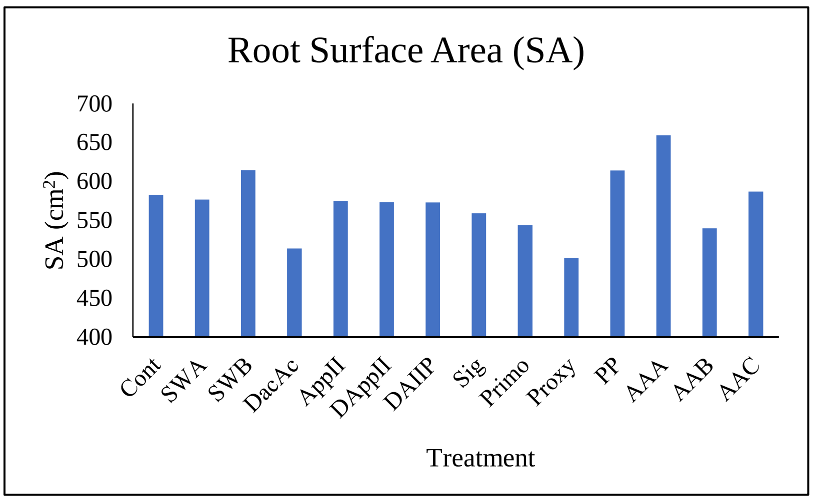


Fig. 8. Root Characteristics (length, surface area, average diameter, volume, and dry weight) collected from different treatment plots on September 8th.





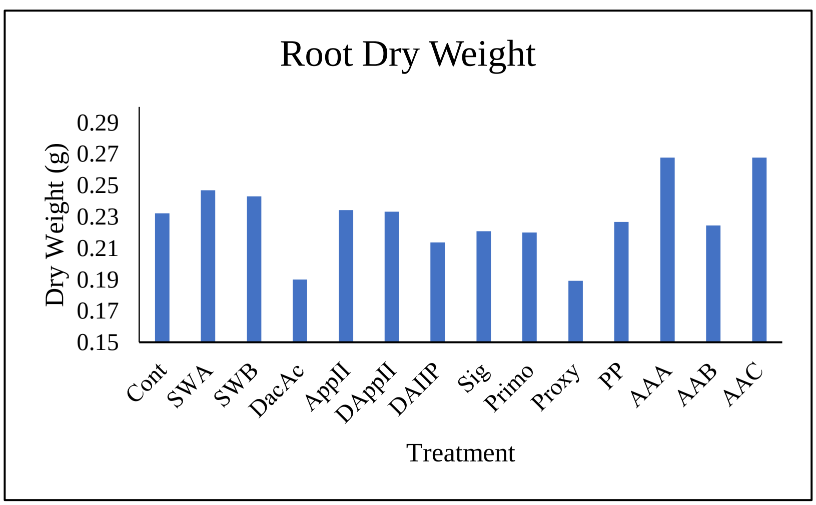
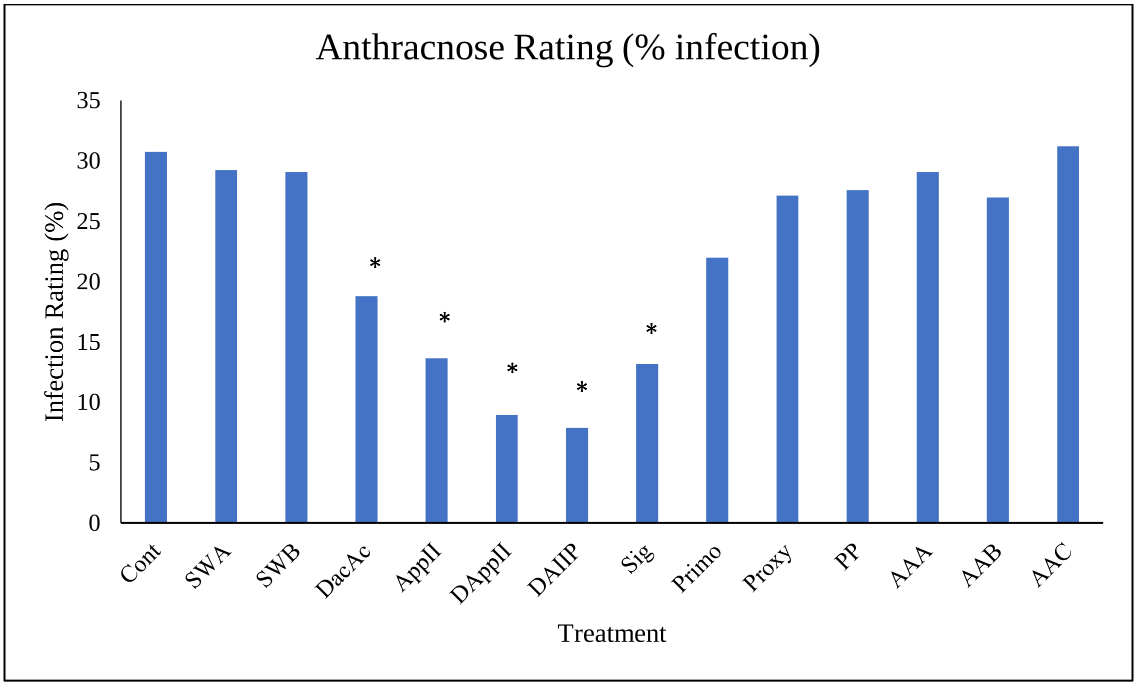


Fig. 9. Anthracnose Disease Rating on different treatment plots on September 15th. "*" indicate treatments significantly different from the control at $p = 0.05$.



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. 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.

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													

Image 1: Brian McDonald making the November Proxy applications to the annual bluegrass putting green at Lewis-Brown Farm in Corvallis Oregon, 2020.

USGA ID#: 2018-09-659

Title: Growing Degree Day Models to Guide PGR Application Rates

Project Leaders: William C. Kreuser

Affiliation: University of Nebraska-Lincoln

Objectives:

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

Start Date: January 1, 2018

Project Duration Three years

Total Funding: $120,000

Summary Points:

- Developed PGR GDD models for three cultivars of ultradwarf bermudagrass putting greens in NC, MS and TN with our collaborators. Peak suppression for prohexadione-calcium ranged from 50-45% with ideal re-application intervals of 120-160 GDD (base 10C). Peak suppression ranged from 49-62% with re-application intervals ranging from 216-300 GDD (base 10C) for trinexapac-ethyl. Warm-season results were published in Crop Science. Results have been added to GreenKeeper App.
- Developed PGR GDD models for paclobutrazol applications on creeping bentgrass putting greens. Clipping yield suppression ranged from 29-62% of the non-treated control depending on application rate. The ideal re-application interval ranged from 269-302 (base 0C) and model R^2 values ranged from 0.41 to 0.86. Results have been added to GreenKeeper App. Results were published in Crop Science during 2018.
- "Flipped" PGR models were tested to estimate the amount of PGR remaining in the plant when the PGRs were applied prior to their ideal re-application interval. A half-life approach model was used to schedule PGR application rate. The models tested resulted in an intensification of clipping yield suppression and increase phytotoxicity overtime. This indicates the models were too aggressive.

- Created a new PGR GDD model to account for the clipping yield suppression of multiple applications of PGR and DMI fungicides. This new model incorporates segmented regression with a linear clipping suppression response until a break point at 23.1% suppression and then switches to logarithmic decay regression as measured peak clipping yield suppression increased. The model was developed from a combination of various datasets and had an R^2 value of 0.763.
- The new PGR model was used to evaluate putting green performance when PGR ingredients were mixed in 2020. Mixing trinexapac-ethyl with paclobutrazol increased green speed by 8.4 inches compare to the non-treated control. This mixture in combination with higher levels of nitrogen fertilizer sustained high putting green stand density and acceptable color. This study will be replicated in 2021.

Development of a "Flipped" PGR Model

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

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

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

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

Refined PGR GDD Model

Clipping yield results from the flipped PGR study and a DMI fungicide PGR combination trial collected from a creeping bentgrass putting green during Fall 2019 were used to create an improved PGR model. This model accounts for the performance of individual PGR applications and generates a predicted "cumulative clipping yield suppression" value from the combination of the various PGR and DMI applications. The model uses segmented non-linear regression with a break point at 23.1% clipping yield suppression. It has an R^2 value of 0.763.

Tested the improved PGR GDD model on a bentgrass putting green

The goal of this trial was to evaluate the performance of this new PGR Model on a '007' creeping bentgrass putting green during the summer of 2020. This research green was built to USGA recommendations for putting green construction and was irrigated to prevent wilt. Treatments included a factorial of four PGR treatments (non-treated, trinexapac-ethyl at 5.5 oz/A, trinexapac-ethyl at 33 oz/A, and trinexapac-ethyl (5.5 oz/A) + paclobutrazol (16 oz/A) by two N fertilization rates (0.15 or 0.30 lbs N per 1000 ft^2) and at two different mowing heights (0.120 and 0.080 inches). The PGRs were applied at 200 GDD when trinexapac-ethyl was applied alone or 280 GDD when trinexapac-ethyl was mixed with paclobutrazol. Data were collected every two weeks from June through July. Measurements included green speed before and after clipping yield collection/quantification, turfgrass quality and color from a Holland Scientific Rapid Scan active sensor.

Turf maintained at the lower HOC and higher N rate had increased clipping yield. When averaged across the entire season, the grass mowed at 0.080 inches grow 45% faster than grass mowed at 0.120 inches. The accelerated would have growth rate increased nutrient removal during mowing and accelerate carbohydrate depletion. As a result, the higher N rate was required to sustain acceptable turfgrass quality at the lower height of cut.

The trinexapac-ethyl treatments (1x or 6x label rates) had the best turfgrass quality at each HOC and N treatment. There was never phytotoxicity from the high rate of trinexapac-ethyl applied alone. Total clipping yield suppression from that high rate of trinexapac-ethyl was greater than the 1x rate but was less than the combination of trinexapac-ethyl and paclobutrazol applied together at the maximum labeled rates for bentgrass putting greens (Fig. 3). Both the 6x rate of trinexapac-ethyl and combination treatment prevented enhanced clipping yield from the high N rate at both HOCs (Fig 4), while the 1x rate of trinexapac-ethyl was unable to offset the clipping yield enhancement that resulted from the higher N application rate.

Regarding ball roll distance, the PGR combination enhanced ball roll distance by 8.4 inches. The trinexapac-ethyl treatments applied alone only enhanced ball roll distance by 3 to 4 inches – a distance that golfers cannot perceive. Increasing N rate from 0.15 to 0.30 lbs N 1000 ft^{-2} did not influence ball roll distance and the lower HOC only enhanced ball roll distance by 4.8 inches.

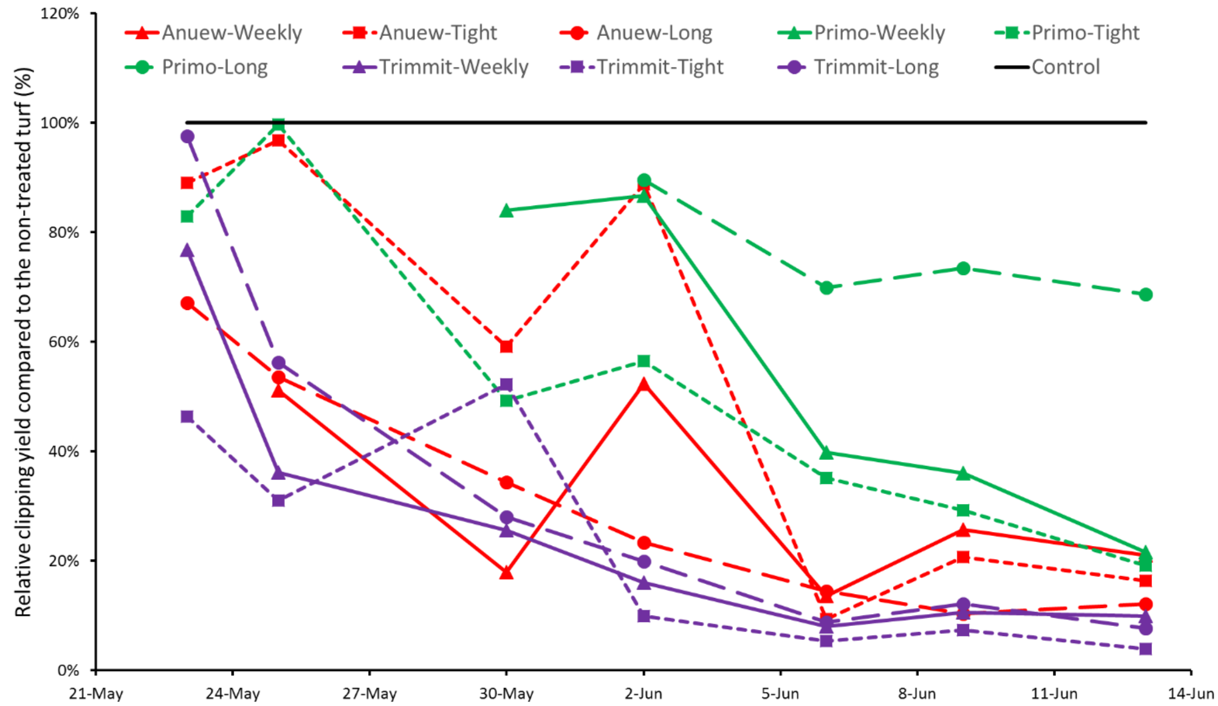


Figure 1. The impact of different PGR re-application models on the relative clipping yield suppression of a creeping bentgrass fairway mowed at 0.400 inches.

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

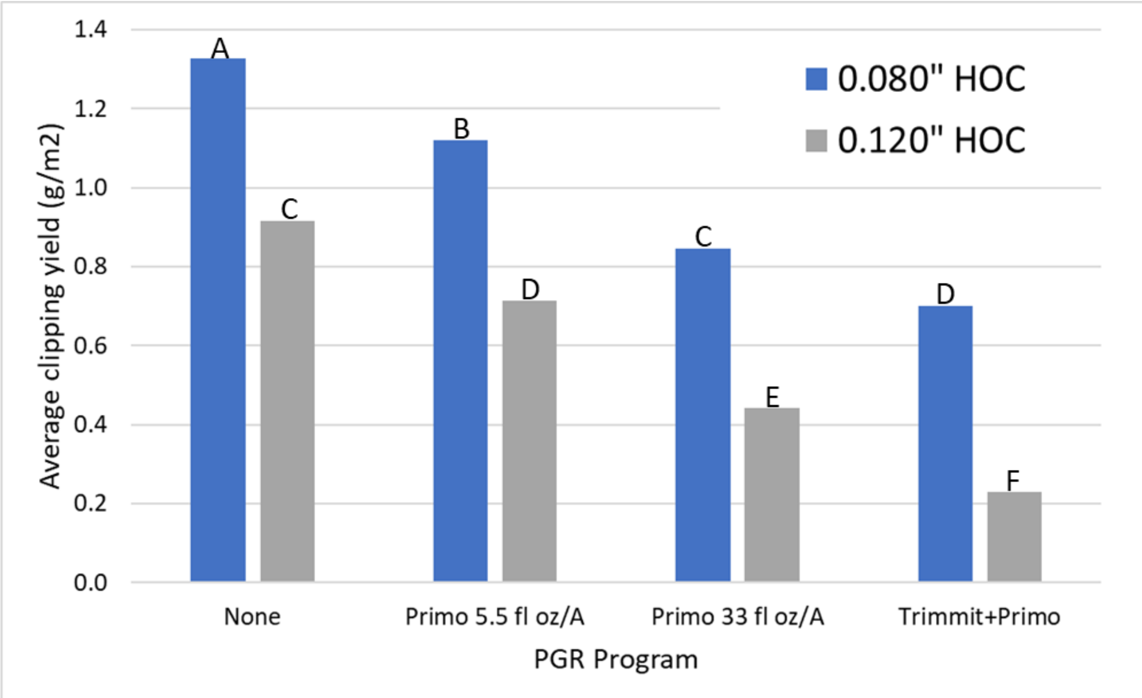


Figure 3. The influence of PGR program on average clipping yield production from a creeping bentgrass putting green at 0.120- or 0.080-inch height of cut.

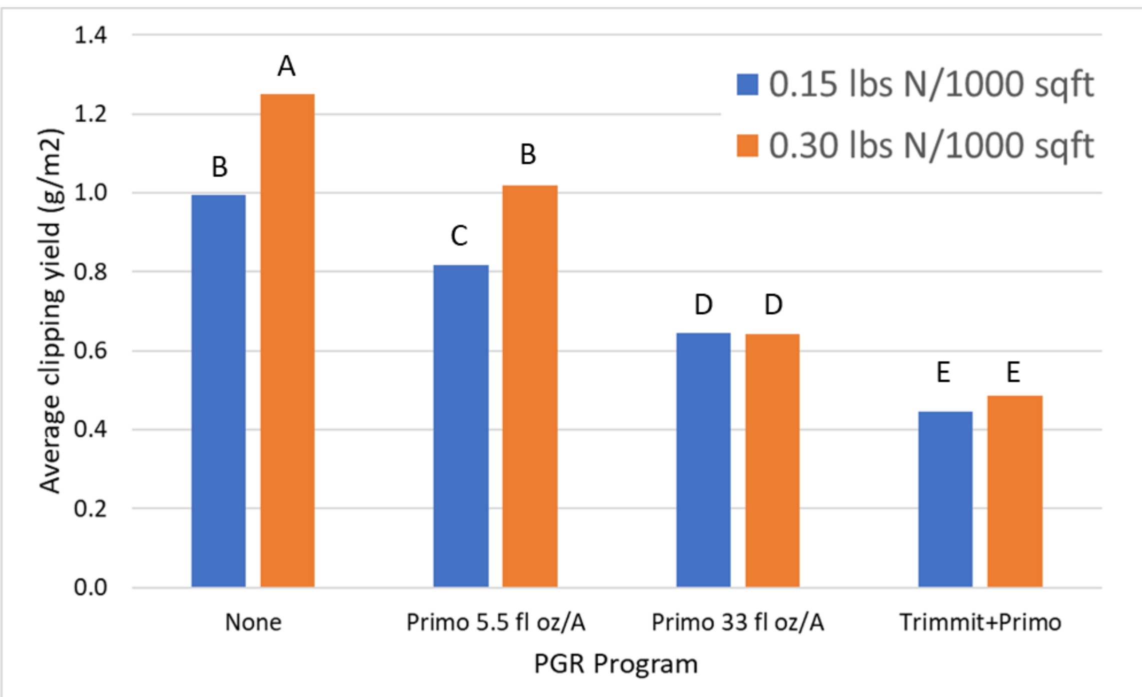


Figure 4. The influence of PGR program on average clipping yield production from a creeping bentgrass putting green receiving 0.15 or 0.30 lbs N ft^{-2} every two weeks.

USGA ID#: 2019-30-700

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

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

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

Start Date: August 1, 2019
Project Duration: Two years
Total Funding: $55,000

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

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

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

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

In 2016, we started validating the PGR stacking models developed from the 2016 and 2017 USGA grants: i) Modeling GA Production Improves Prediction of Turf Growth and PGR Performance, and ii) Growing Degree Day Models to Guide PGR Application Rates. Various rates of prohexadione-Ca, trinexapac-ethyl and paclobutrazol were applied in combination to a 'V-8' creeping bentgrass putting green during the fall of 2019 (Fig. 1). The clippings yield data were then normalized to the non-treated control and modeled with sinewave non-linear regression. The model coefficients of amplitude and period were then fitted to experimental PGR degradation models developed in past USGA grants (Fig. 2). The new PGR GDD model that was developed from the PGR data collected during fall 2019 and summer 2017 was used to create a segmented non-linear regression model. That model was added to GreenKeeper App in 2020. It allows managers can be alerted to potential problems and synergism when mixing different PGR active ingredients or when PGRs are applied too frequently. This dashboard will clearly warn managers of potential problems and guide lower application rates to minimize the risk of collar decline or PGR over-regulation. The team at GreenKeeper paid for all development costs associated with the addition of this new PGR model in GreenKeeper. A data analyst has been identified to analyze the use data generated by this new feature during spring 2021. This analysis will provide a metrics to show the value of USGA funded research for the turfgrass management community. It also will show the role of decision support tools in the industry.

Figure 1. The subtle color changes of the creeping bentgrass putting green following applications of PGR and DMI combinations at various rates during fall of 2019.

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 2020 results suggest that fairway creeping bentgrass soils can be categorized with Solivta tests as to their probability of equaling or exceeding the response of a standard N treatment.
- 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 offers two tests that have been 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 are correlated to soil microbial activity, and therefore, the Solvita soil tests should be able to estimate the mineralization potential of turfgrass soils. An estimate of the mineralization potential should help guide N fertilization.

Methods:
The study site is located in Storrs, CT, and was initiated in August, 2017. The experiment was set out as a split-block design with traffic (with/without) as the horizontal factor and compost (10 rates, in 0.25-lb increments from 0 to 2.25 lbs available N per $1000ft^2$) 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 and 2020 with fall applications of Suståne organic fertilizer.

In 2020, 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 that would be equal to or exceeding the responses from the standard N fertilization practice across the Solvita soil test values for each of the traffic treatments.

2020 Results:
Traffic effects were significant only for clipping yields (Table 1). In this case, the No-Traffic treatment yield was significantly greater than where traffic was applied. Fertilizer treatment effects were significant for all variables (Table 1). Averaged across traffic treatments, all responses were linear and significant in relation to fertilizer N rate ($P < 0.001$) (Fig. 1).

Compared with the Standard treatment, concentrations of SLAN were significantly lower at the non-fertilized 0 to 0.75 lbs N per 1,000ft^2 rates, but significantly greater once the N rate reached ≥ 1.5 lbs N per 1,000ft^2 (Table 1). Concentrations of CO2B were significantly lower at the non-fertilized 0 rate than the Standard, but significantly greater than the Standard treatment once the N rate reached ≥ 0.75lbs N per 1,000ft^2. NDVI was significantly less than the Standard treatment at the 0 and 0.25 rates, but not different from the Standard for the rest of the treatments. Response of DGCI from the Standard treatment was greater than the 0 and 0.25 lbs N per 1,000ft^2 treatments, but not different from rates ≥ 0.50 lbs N per 1,000ft^2. Visual quality, color, and density ratings were not different from the Standard treatments from rates ≥ 1.50 lbs N per 1,000ft^2. Percent green cover of the Standard treatment was significantly greater than the 0 and 0.25 lbs N per 1,000ft^2 treatments, but not different from rates ≥ 0.50 lbs N per 1,000ft^2. The 0 N rate was significantly lower than the Standard treatment for clippings yield, whereas the 1.75, 2.0, and 2.25 lbs N per 1,000ft^2 rates produced significantly greater clipping yields than the Standard treatment.

Correlations between fairway bentgrass responses in relation to SLAN and CO2B concentrations were all highly significantly with high *r* values across the traffic treatments (SLAN $r = 0.806$ to 0.966; CO2B $r = 0.807$ to 0.953) (Table 2). There was no difference in *r* values between traffic and non-traffic treatments. 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 organic compost and fertilizer plot responses producing responses that were equal or greater than the response of the Standard fertilizer treatment with respect to the SLAN and 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 ≥ 293 and ≥ 305 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 ≥ 148 and ≥ 152 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 we will be able to produce reliable tables 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 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 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 Table 5.

The results from 2020 suggest that the Solvita soil test results are well correlated to fairway creeping bentgrass responses. We have had two years of 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 fairway creeping bentgrass quality and growth responses, with analysis of variance *P* values for traffic and N rate treatment effects. 2020 results.

	SLAN	CO_2-Burst	NDVI	DIA† DGCI	Visual Quality	Visual Color	Visual Density	DIA Cover	Sum Monthly Clippings Yields
Traffic	mg kg^{-1}	mg L^{-1}			----------1-9; 9 best	--------		% green	g m^{-2}
No	255.8	124.1	0.656	0.429	6.4	6.4	6.2	65.3	29.2a
Yes	256.1	124.2	0.652	0.423	6.1	6.1	6.0	63.7	25.6b
Treatment‡									
0	212.1*	97.3*	0.591*	0.391*	4.6*	4.5*	4.6*	51.3*	17.6*
0.25	228.8*	107.8	0.614*	0.402*	5.0*	5.0*	4.9*	54.9*	19.7
0.5	239.3*	112.5	0.629*	0.417	5.5*	5.5*	5.4*	60.8	23.3
0.75	248.1*	122.2*	0.653	0.425	5.9*	5.9*	6.0*	63.2	25.0
1	256.4	122.8*	0.656	0.424	6.1*	6.1*	6.1	65.0	24.8
1.25	258.5	127.2*	0.661	0.425	6.1*	6.2*	6.0*	63.6	28.7
1.5	268.9*	131.0*	0.668	0.435	6.6	6.7	6.6	68.4	32.3
1.75	274.9*	137.1*	0.671	0.437	7.0	6.8	6.6	67.7	32.5*
2	280.6*	145.1*	0.687	0.445	7.3	7.2	7.2	73.6	35.3*
2.25	288.7*	150.9*	0.693*	0.446	7.6	7.4	7.3	73.4	36.7*
Standard	258.8	111.7	0.672	0.437	7.1	7.1	6.7	67.6	25.8
				AOV *P*-values					
Traffic	0.9462	0.8488	0.2035	0.3696	0.1009	0.3494	0.4266	0.3126	0.0487
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
T × T	0.0146	0.0687	0.1641	0.6947	0.1220	0.3093	0.1575	0.2852	0.3435

†DIA, digital image analysis

‡Compost and organic fertilizer rates (lbs N per 1,000ft^2); Standard treatment is liquid urea at approximately 0.2 lbs N per 1,000ft^2 every 21 days.

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

Table 2. Correlation coefficients (*r*) and *P* values for No-Traffic and Traffic plot responses in relation to Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO_2-Burst (CO2B) concentrations, and *P* values for the difference between traffic treatment *r* values for each variable. 2020 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	0.965	<.0001	0.959	<.0001	0.7953
DGCI	0.845	<.0001	0.921	<.0001	0.1878
Quality	0.966	<.0001	0.937	<.0001	0.2462
Color	0.944	<.0001	0.937	<.0001	0.8032
Density	0.917	<.0001	0.930	<.0001	0.7371
Cover	0.854	<.0001	0.806	<.0001	0.3934
Yield	0.921	<.0001	0.828	<.0001	0.1261
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	0.951	<.0001	0.953	<.0001	0.9271
DGCI	0.807	<.0001	0.895	<.0001	0.2286
Quality	0.921	<.0001	0.938	<.0001	0.6552
Color	0.890	<.0001	0.918	<.0001	0.5653
Density	0.879	<.0001	0.927	<.0001	0.3351
Cover	0.807	<.0001	0.890	<.0001	0.2653
Yield	0.928	<.0001	0.832	<.0001	0.1011

Table 3. Concentrations of Solvita Soil Labile Amino-Nitrogen (SLAN) and Soil CO_2-Burst (CO2B) concentrations of equaling or exceeding the response of the Standard fertilizer treatment at a selected probability of *P* = 0.67. 2020 results.

P = 0.67	SLAN-N, mg kg^{-1}	
Variable	No-Traffic	Traffic
NDVI	294	304
DGCI	324	342
Visual Quality	296	288
Visual Color	283	310
Visual Density	283	289
Percent Green Cover	312	327
Clipping Yields	267	275
All variables combined	293	305
P = 0.67	CO2B-C, mg L^{-1}	
Variable	No-Traffic	Traffic
NDVI	151	152
DGCI	159	163
Visual Quality	149	141
Visual Color	142	154
Visual Density	142	143
Percent Green Cover	158	166
Clipping Yields	134	141
All variables combined	148	152

Table 4. Estimated probabilities for Soil CO_2-Burst (CO2B) and Soil Labile Amino-Nitrogen (SLAN) test concentrations across both traffic treatments with all variables combined. 2020 results.

CO2B-C, mg L^{-1}	Probability of equaling or exceeding response from Standard fertilizer treatment	SLAN-N, mg kg^{-1}	Probability of equaling or exceeding response from Standard fertilizer treatment
50	0.02	150	0.03
75	0.05	175	0.07
100	0.15	200	0.12
125	0.38	225	0.22
150	0.67	250	0.35
175	0.87	275	0.52
200	0.96	300	0.68
225	0.99	325	0.81
250	1.00	350	0.89
275	1.00	375	0.94
300	1.00	400	0.97
		425	0.98
		450	0.99
		475	1.00
		500	1.00

Table 5. Recommended N rate based on SLAN or 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 2020.

Apply (1 – *P*) × Standard rate of N

For Example: Standard N rate = 0.2 lbs N/1,000ft^2 approx. every 21 d

SLAN	Probability	Apply, lbs N/1,000ft^2 approx. every 21 d	CO2B	Probability	Apply, lbs N/1,000ft^2 approx. every 21 d
≤ 150	0.035	0.20	≤ 50	0.016	0.20
175	0.066	0.19	75	0.052	0.19
200	0.123	0.18	100	0.155	0.17
225	0.217	0.16	125	0.378	0.12
250	0.354	0.13	150	0.669	0.07
275	0.520	0.10	175	0.871	0.03
300	0.682	0.06	200	0.957	0.01
325	0.809	0.04	≥ 225	0.987	0.00
350	0.894	0.02			
375	0.943	0.01			
≥ 400	0.971	0.00			

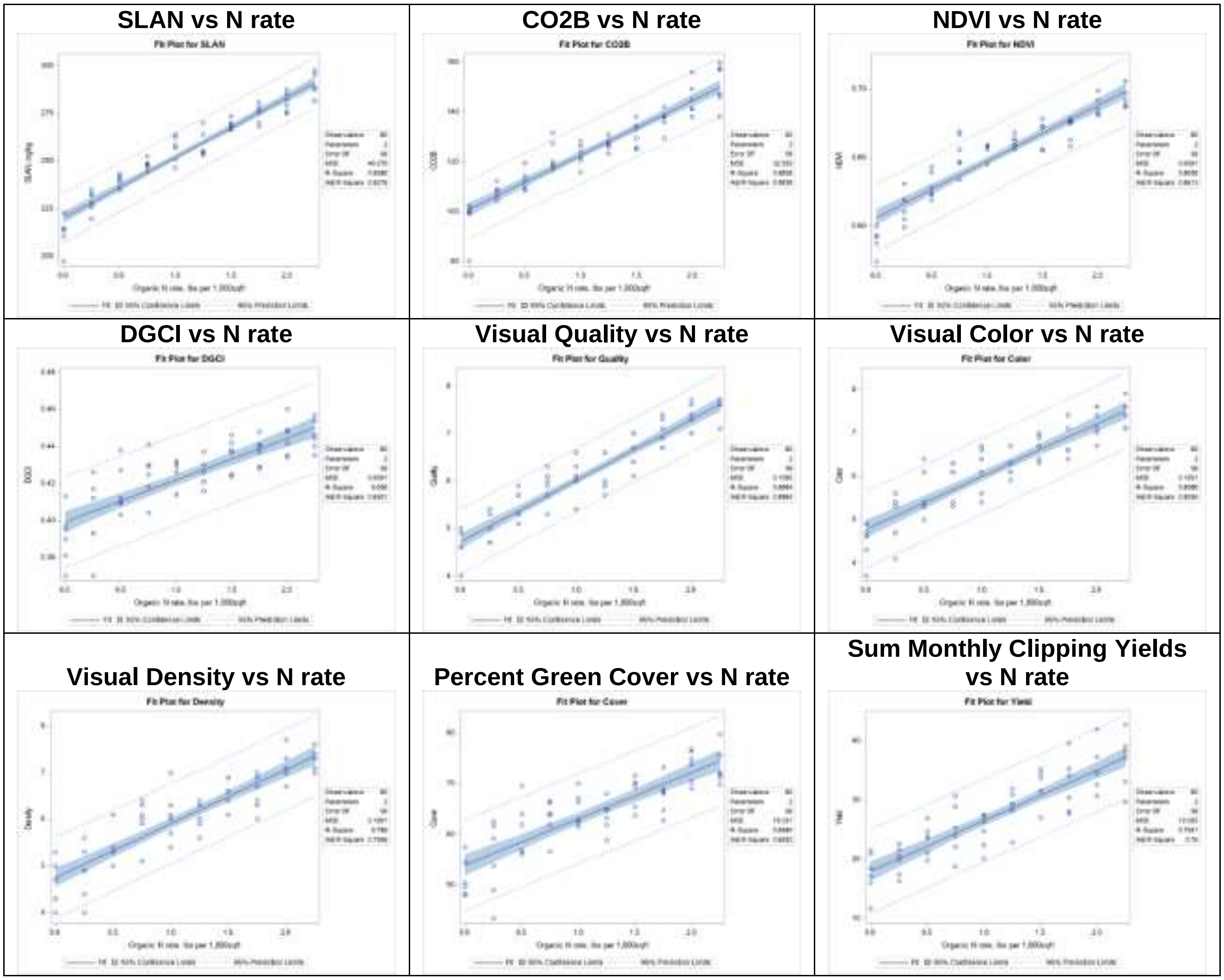
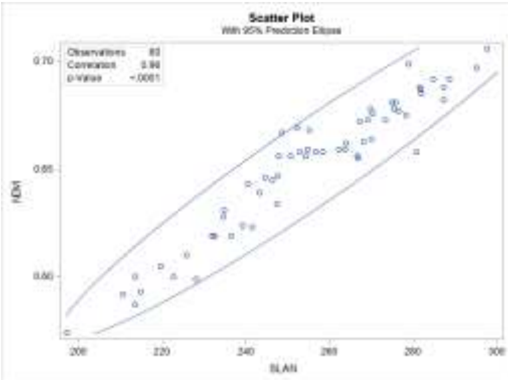
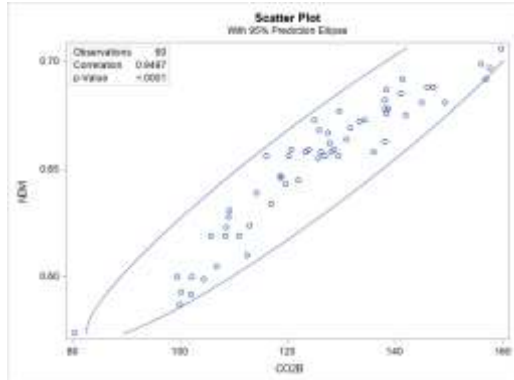
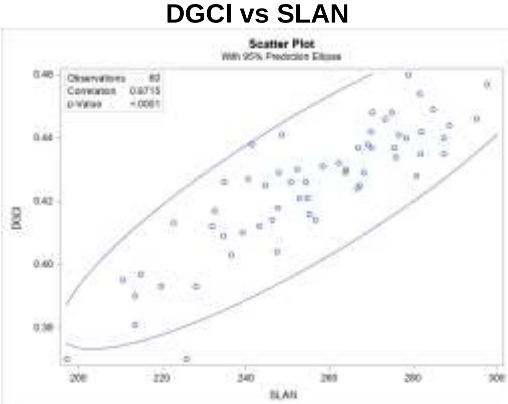
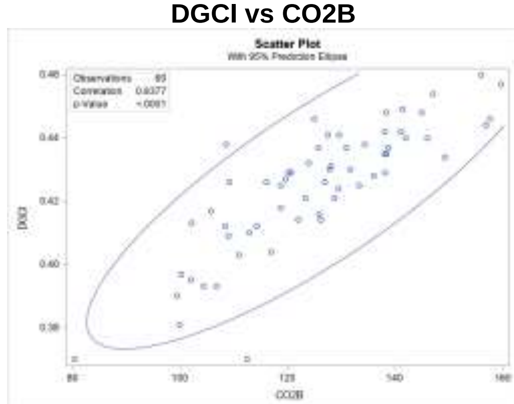
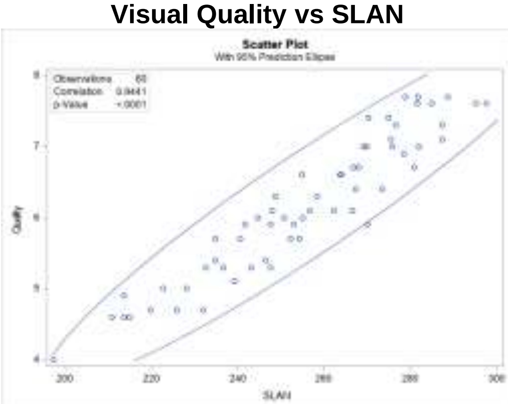
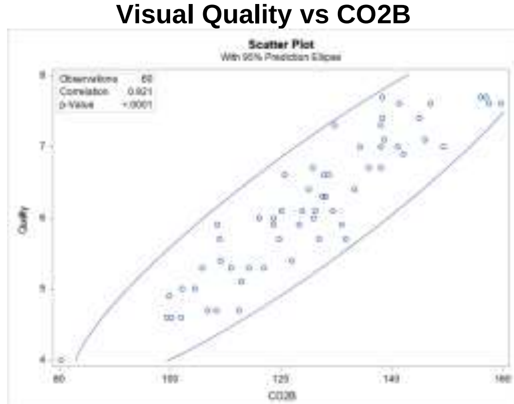
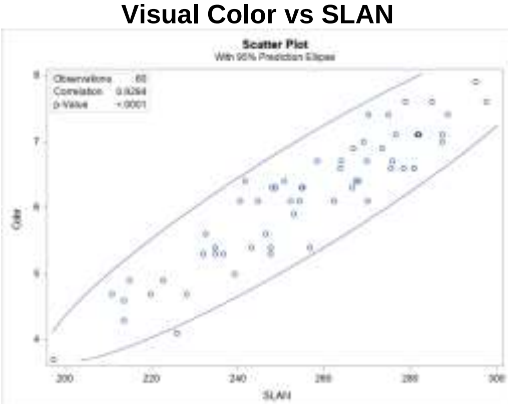
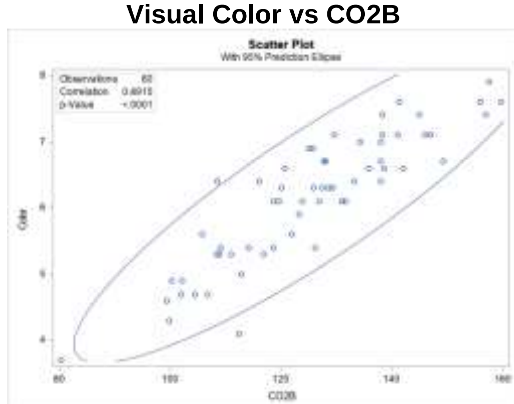


Figure 1. Fairway creeping bentgrass responses (Soil Labile Amino Nitrogen [SLAN], Soil CO_2-Burst [CO2B], Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and monthly clippings yields) in relation to organic fertilizer (initial compost followed by yearly Suståne applications) N rates for 2020. 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**
	
Continues next page	**Continues next page**

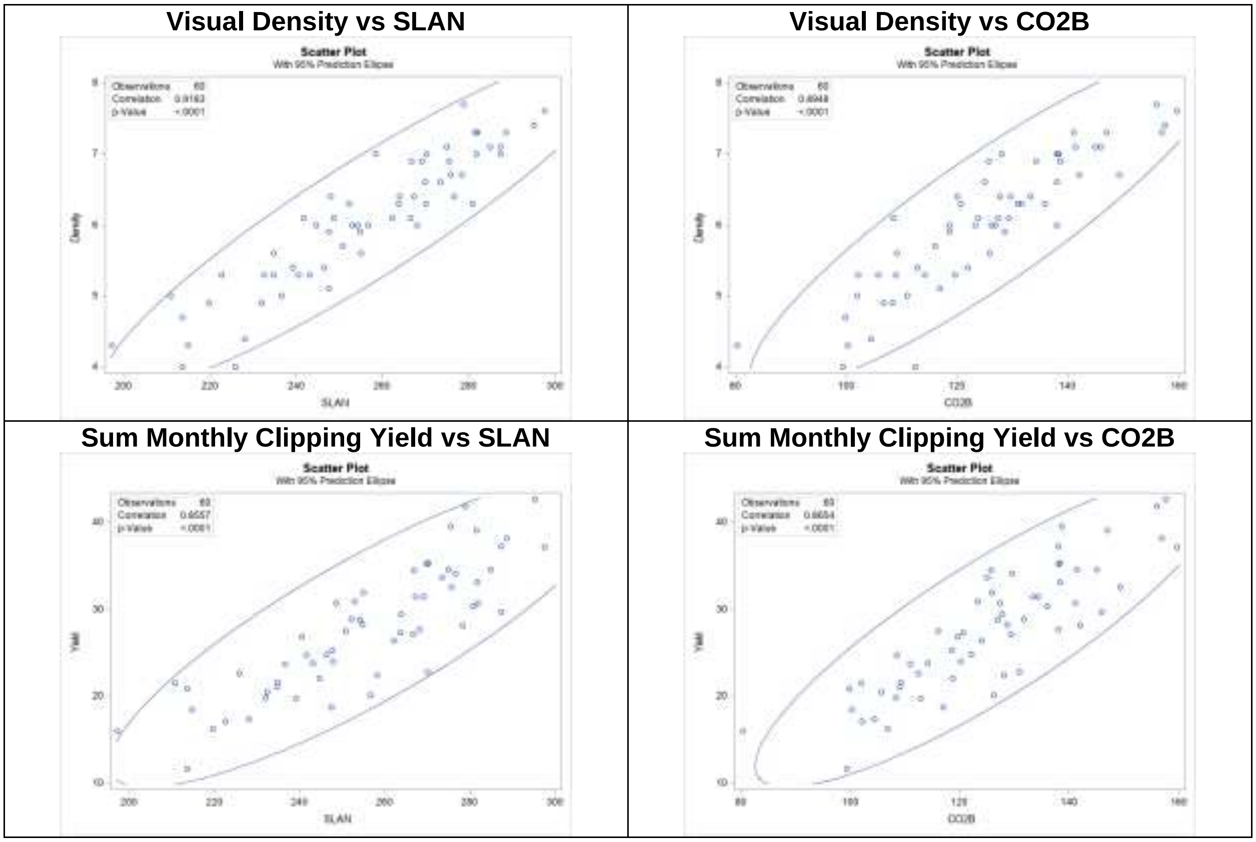


Figure 2. Correlations between fairway creeping bentgrass responses (Normalized Difference Vegetative Index [NDVI], Dark Green Color Index [DGCI], visual quality, visual color, visual density, percentage green cover, and monthly clippings yields) in relation to Soil Labile Amino Nitrogen (SLAN) and Soil CO_2-Burst (CO2B) concentrations averaged across sampling dates and traffic treatments plots for 2020. The ellipses represent the 95% prediction space.

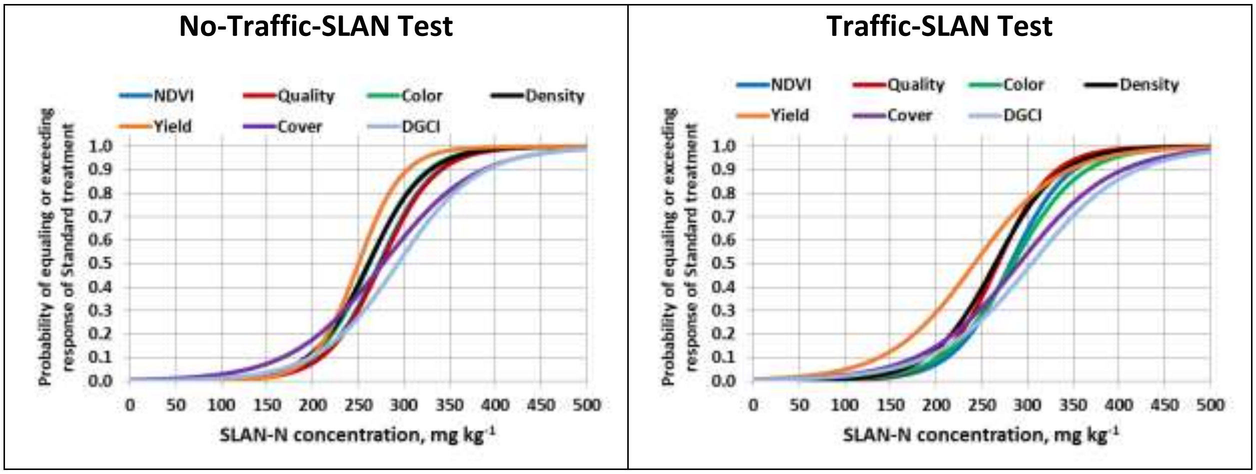


Figure 3. Probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft^2 every 21 days) in relation to the Solvita SLAN-N concentrations for the No-Traffic and Traffic plots. 2020 results pooled across all sampling dates.

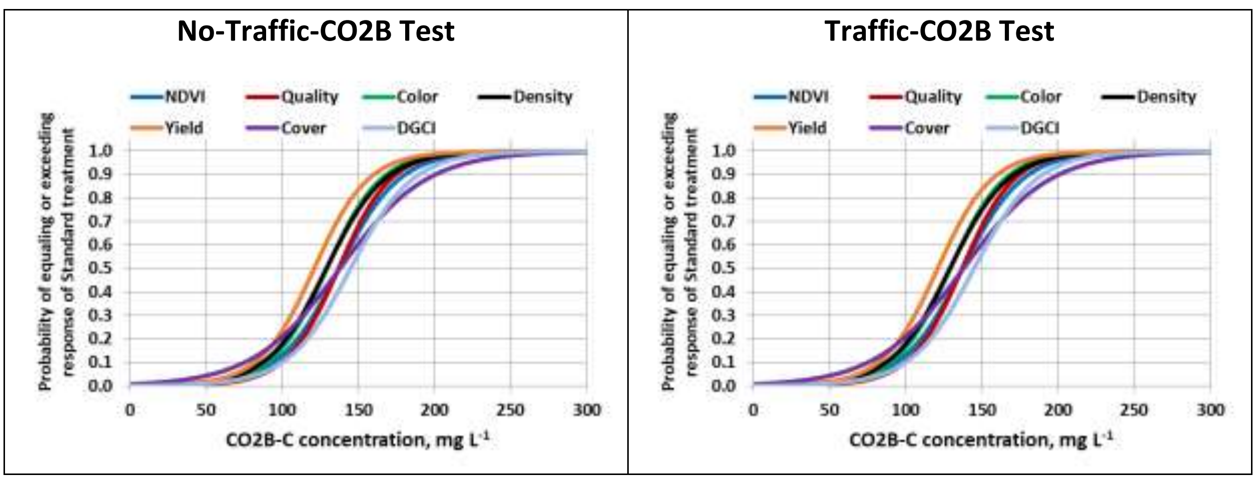


Figure 4. Probability curves of equaling or exceeding the NDVI, DGCI, visual color, visual color, visual density, percent green cover, and clippings yield response of the Standard fertilizer treatment (approximately 0.2 lbs N per 1000ft^2 every 21 days) in relation to the Solvita CO2B-C concentrations for the No-Traffic and Traffic plots. 2020 results pooled across all sampling dates.

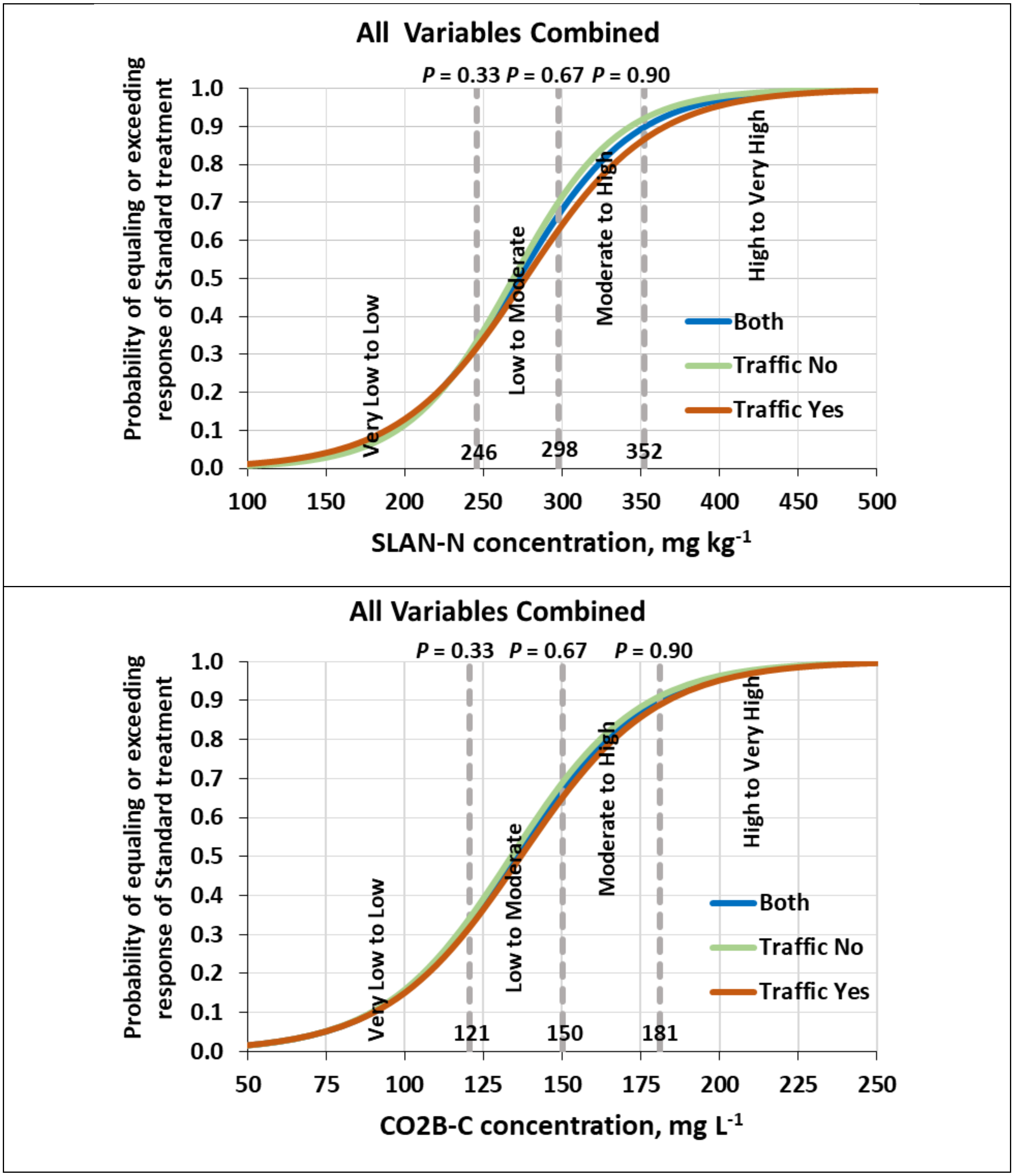


Figure 5. Probability curve representing all variables combined across both traffic treatments and categories of fairway creeping bentgrass responses that would be equal to or greater than the responses obtained from the Standard fertilizer treatment in relation to Solvita Soil Labile Amino-Nitrogen (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 Table 4. 2020 results.

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 is evaluating long-term (years 4-6) changes in turf performance, soil physical properties, and cultural management requirements of sand-capped fairway plots originally established in 2014. The project is being conducted at the Texas A&M Turfgrass Field Laboratory, College Station, TX, on 5-year old 'Tifway' bermudagrass sand-capped fairway research plots. Four replicated capping depth treatments have been constructed on both subsoils, including native soil topdressed at a depth of 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 is being utilized to assess sand-cap cultural management practices addressing surface organic matter accumulation, hydrophobicity, and subsoil sodicity issues arising from elevated Na and bicarbonates in the local water source.

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

The clay loam subsoil study focuses on surface organic matter management, specifically focusing on secondary cultural regimes for managing surface organic matter. Whole plots consist of sand-cap depth (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 are being monitored during the course of the season. Thatch depth and percent organic matter for the 0-2 inch sand-cap depth is being determined via combustion analysis and loss on ignition method at the end of each season.

In spring 2021, just prior to the conclusion of the study, saturated hydraulic conductivity and water release curve data (water to air-filled porosity) will also be developed for select treatments in order to better understand and characterize physical changes that have occurred over time with various treatments and cultural management inputs.

2020 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 continue to be associated with shallower sand-capping treatment depths of 2 and 4 inches (6 to 7 out of 9, respectively). The 8 inch capping depth continues to produce 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) continue to be associated with topdressed over time (TD 2 in.) treatments (30% VWC). The 2 and 4 inch capping depths exhibit intermediate soil moisture levels (~18-19% VWC), while the 8 inch capping depth supports the least moisture (~13%). These data are very similar to those observed during the 2019 season.

- 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 this 3rd year of the clay loam subsoil study, secondary cultural management treatments did not lead to any statistically significant differences for any of the measured parameters (turf quality, soil volumetric water content, organic matter levels, surface hardness). (Data not shown)

- As with the sandy loam study, capping depth was primary driver of treatment differences in the clay loam cultural management study. Turf quality trended lower for the 8" capping depth, but sustained minimally acceptable quality levels for most of the season atop the clay loam subsoil (Figure 5). This was presumably due to lower permeability and higher water holding capacity of the clay loam relative to sandy loam subsoil (data not shown). This was also reflected in soil moisture level differences (Figure 6).

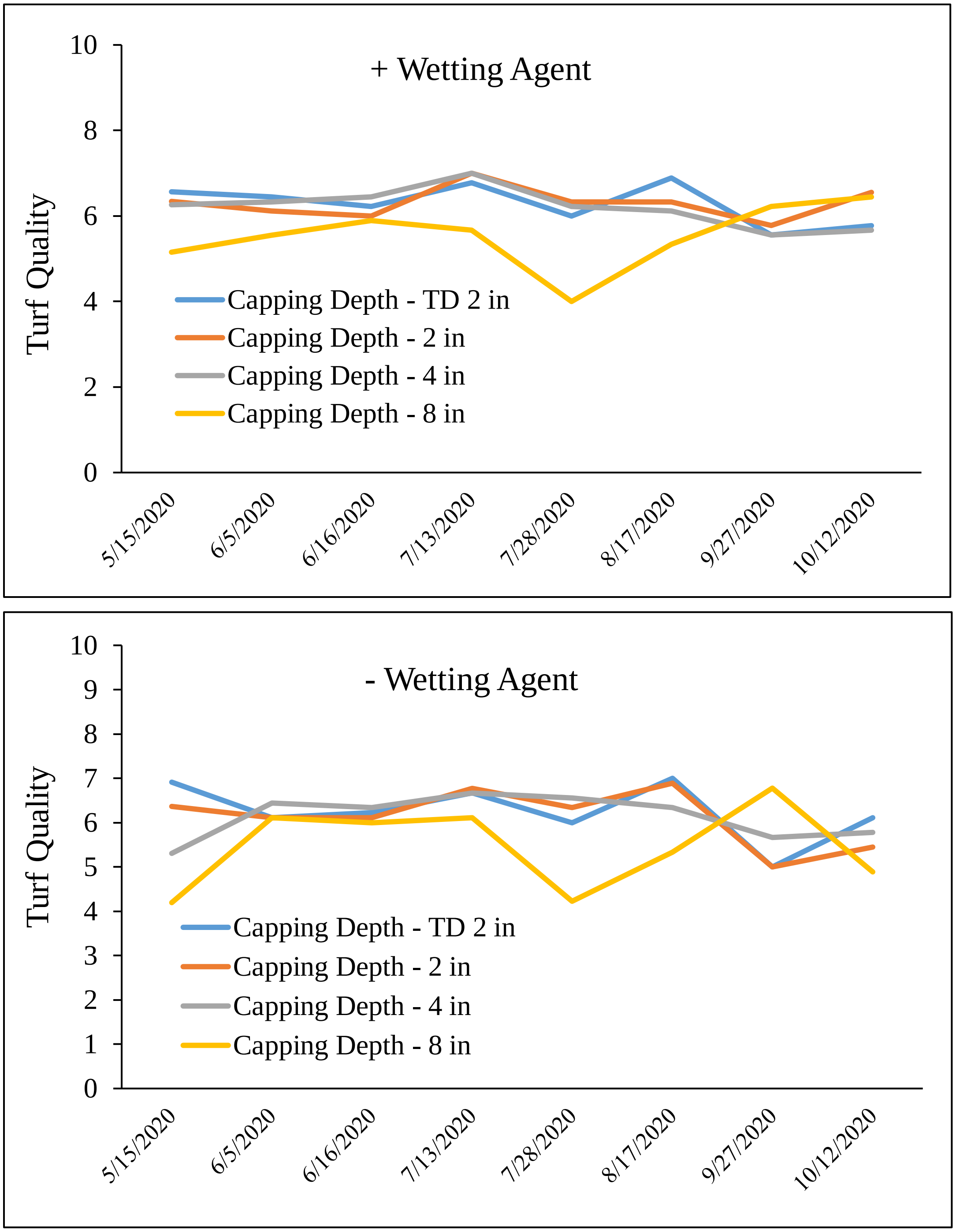


Figure 1. Visual Turf Quality for the sandy loam subsoil study for the 2020 season. Upper graph is for sand-cap depth treatments receiving Wetting Agent, while lower graph represents treatments not receiving Wetting Agent application. Means are pooled across gypsum application rate.

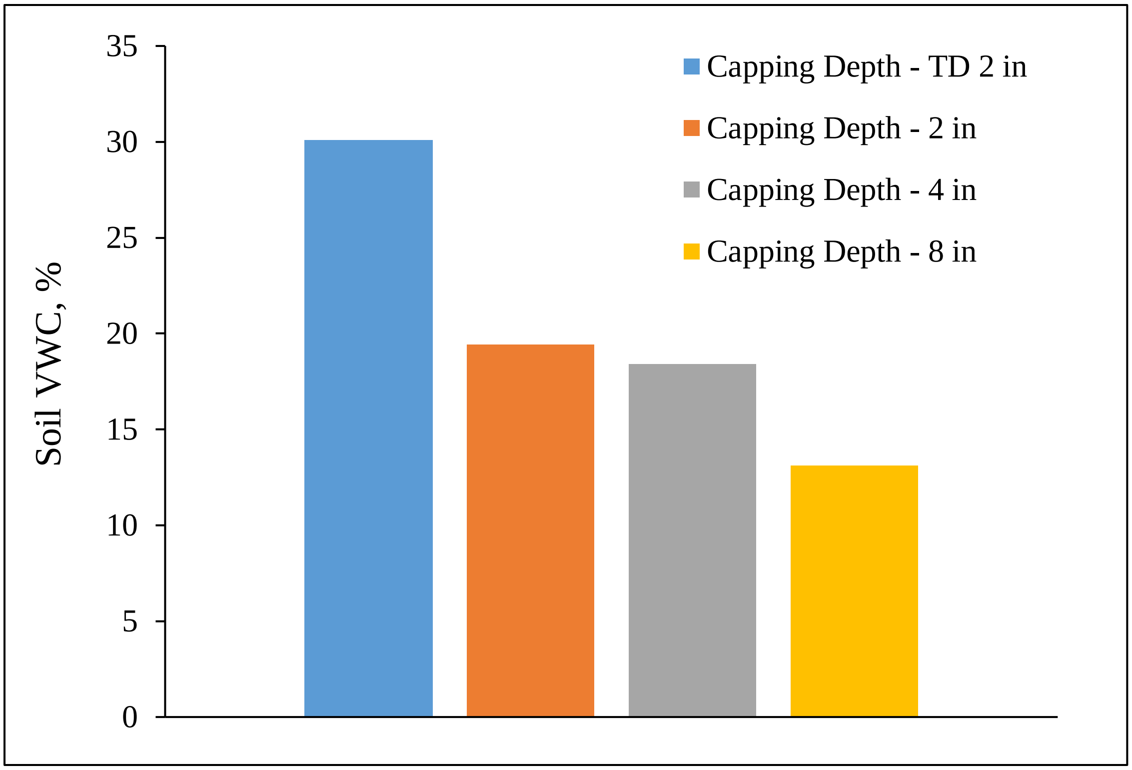


Figure 2. Effect of sand-capping depth on soil volumetric water content (0-3”) within upper sand-cap during the 2020 season for the sandy loam subsoil study. Data are pooled across gypsum and wetting agent application rates.

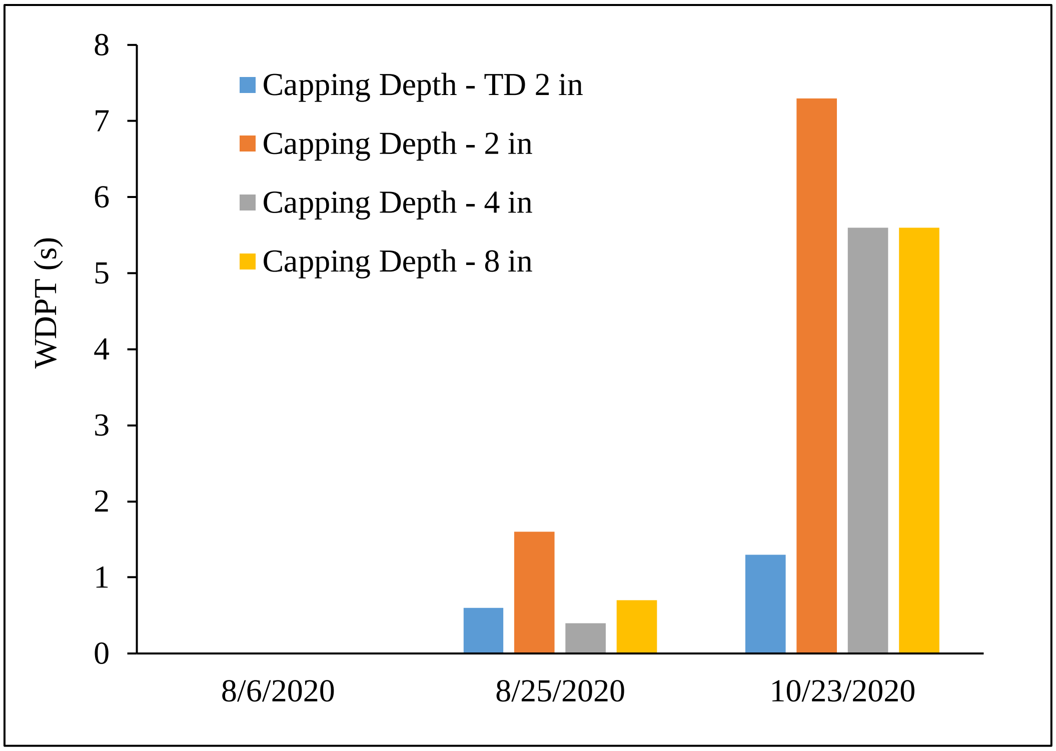


Figure 3. Effect of sand-capping depth on Water Droplet Penetration Time for 2020 testing dates in the sandy loam subsoil study. Data are for the non-wetting agent treatments, and are pooled over gypsum application rates.

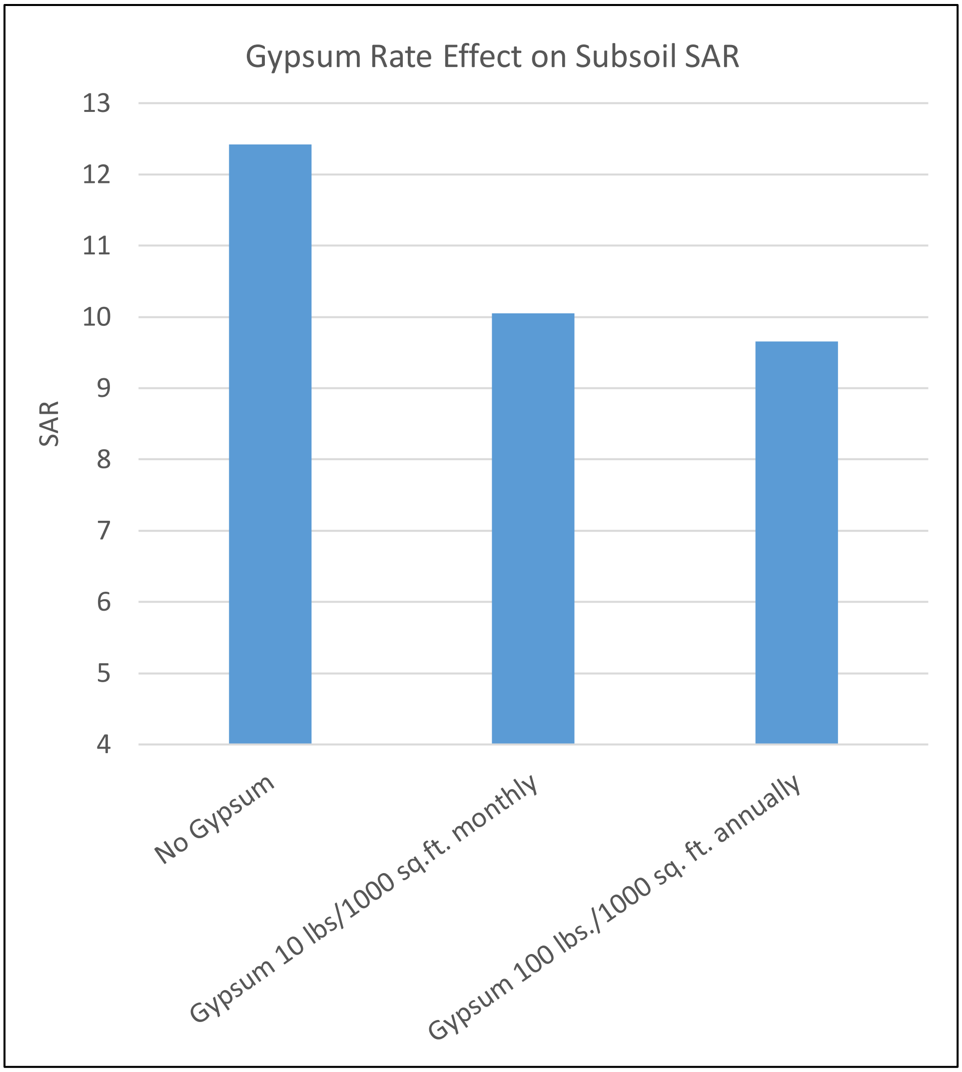


Figure 4. Effect of gypsum application rate on subsoil (0-1”) sodium adsorption ratio (SAR). Means are pooled across wetting agent and sand-capping depth treatments.

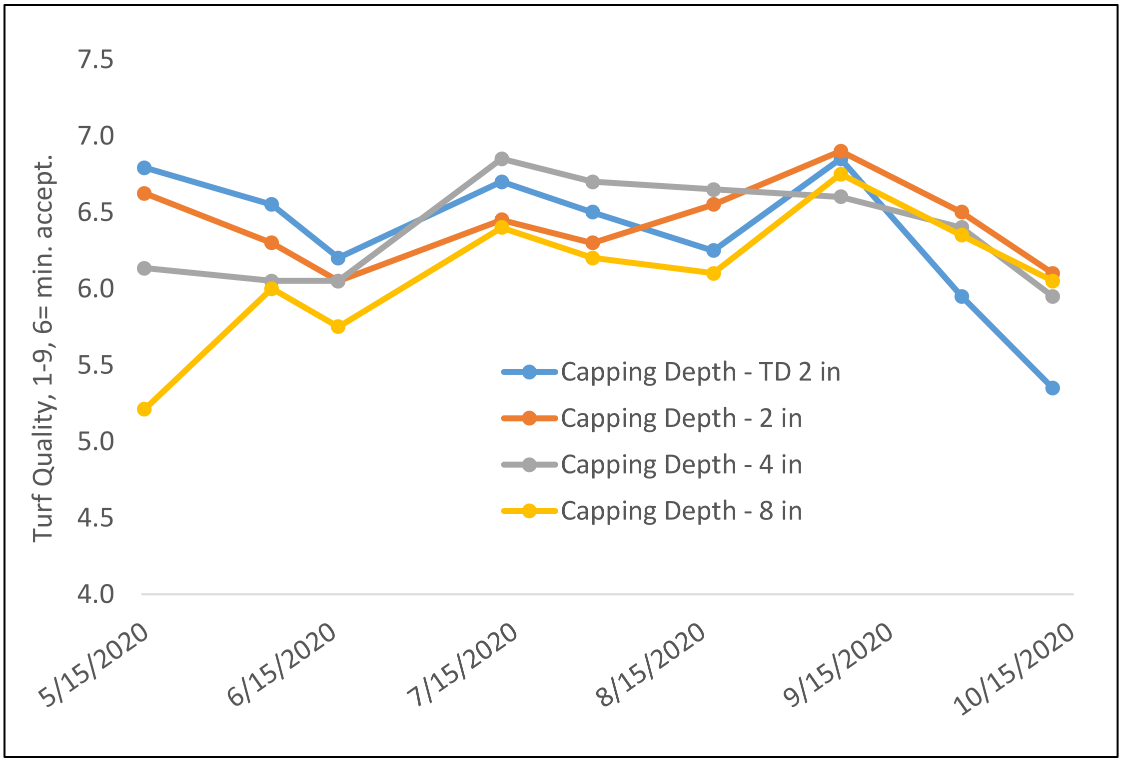


Figure 5. Sand-capping depth x date interaction on Turf Quality during 2020 for the clay loam subsoil study. Data are pooled across secondary cultural practice treatments.

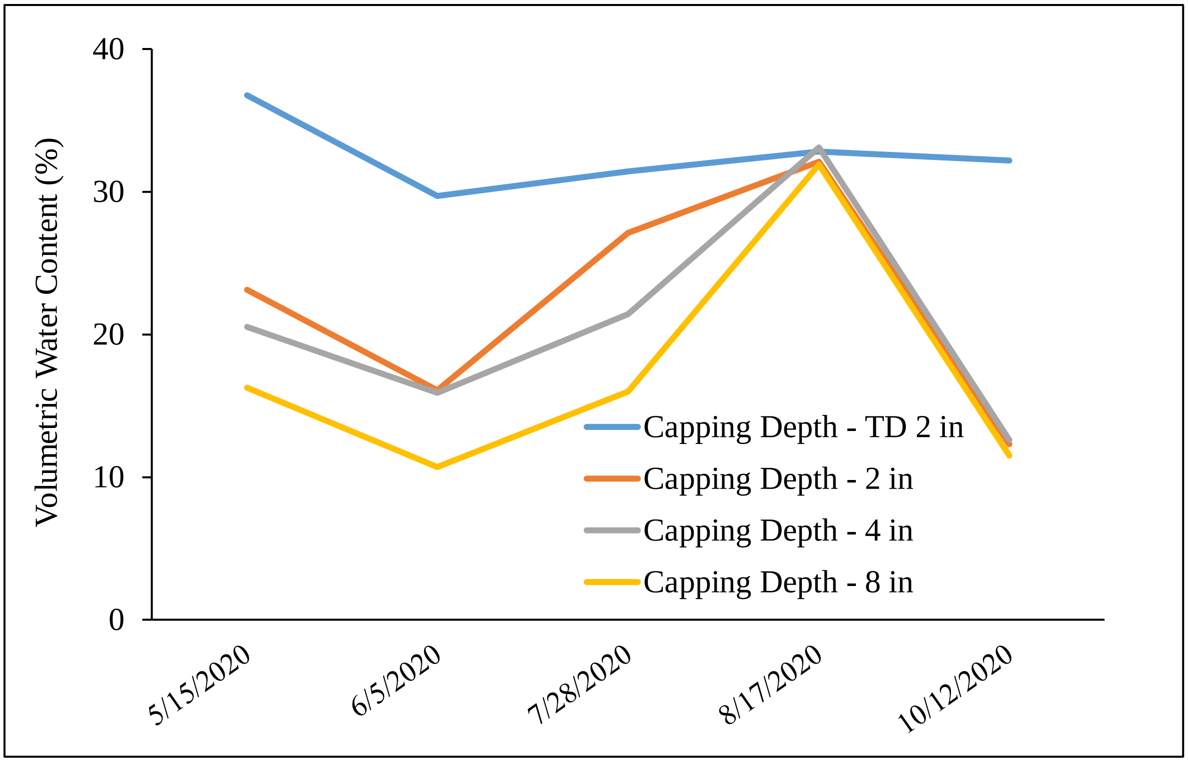


Figure 6. Sand-capping depth x date interaction for soil volumetric water content (0-3” depth) for the clay loam subsoil study. Data are pooled across secondary cultural practice treatments.

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, Hui Chen 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:

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 similarly to that of 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 are continuing treatment applications and monitoring turf and surface wetness responses for a 5th (2020), 6th and 7th 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.

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.

The trial was a 3 x 2 x 2 factorially arranged randomized complete block design with four blocks, which included the factors of 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. Controls (no mid-season topdressing) at each level of cultivation were also included for comparisons resulting in 14 total treatments (Table 1).

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

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

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

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

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

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

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

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

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

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

Data collection during 2020 included volumetric water content (VWC) of the surface 0- to 3-inch depth zone; visual ratings of turf quality, localized dry spot and residual sand after topdressing; normalized difference vegetation index (NDVI); and hand-watering quantity.

The 2021 plan for data collection includes field testing a dual head infiltrometer, which was recently acquired [SATURO | Automated Field Infiltrometer | METER Environment (metergroup.com), Pullman, WA]. This method offers the potential to improve data acquisition of field saturated hydraulic conductivity of treatments; our goal is to determine the time requirement for the device and thus how many treatments can be measured within a workday. Reminder: we anticipate that data collection in the 7th year of the project will include extensive measurements of physical properties and will be destructive of the plots.

Results

Analysis of data during 2020 has been hampered by the graduation and departure of experienced graduate students and slower training of new personnel due to COVID-19 work restrictions. Analysis of volumetric water content (VWC) of the surface 0- to 3-inch depth zone; residual sand after topdressing; normalized difference vegetation index (NDVI); and hand-watering quantity are in progress. Results for turf quality, wilt stress damage and localized dry spot are presented.

Visual turf quality

Visual ratings indicated that all plots within the trial generally had acceptable quality scores (≥ 5) throughout much of the growing season (Table 3). For plots that were not core cultivated, the pooled topdressing treatment effect improved turf quality compared to the control. However, on plots that were core cultivated, topdressing on average had no effect on turf quality compared to the control until late (October and November) in 2020. The limited effect of topdressing on turf quality compared to the control under core cultivated conditions was attributed to the benefit of heavy topdressing that the control received during backfill of the coring holes in October and April/May.

Visual ratings on the two controls in this trial indicated that core cultivation reduced turf quality very early and late in the growing season due to the presence of unhealed coring holes (Table 3). During the remainder of the growing season, the controls had similar turf quality except on 30 June and 14 October when the core cultivated control had better turf quality than the non-cultivated control.

Topdressing with all sand sizes produced acceptable turf quality; however, the sand size and cultivation factors frequently dominated the turf quality response among the factorial combinations of topdressing and core cultivation levels in the trial (Table 4). When differences were evident among the topdressing sand sizes, turf quality was typically better for the fine-medium (finest) topdressing sand compared the two coarser sands. Improved turf quality is likely due to the greater water availability in the plots topdressed with fine-medium sand (data not shown, analysis in progress). This trend for better turf quality on plots topdressed with fine-medium sand was reversed in November 2020, when the best turf quality was observed on plots topdressed with medium-fine sand. This quality response in November may be an indication that turf will decline on fine-medium plots in 2021.

As expected, turf quality was poorest on topdressed plots that were core cultivated in April. Turf quality was much better once coring holes had healed in May; however, the turf quality of cored plots remained lower than non-cored plots throughout the year. Better turf quality on non-cored plots corroborates the strong interest among golf course superintendents to reduce or eliminate core cultivation from their management programs.

Topdressing rate did not affect turf quality early in the growing season; when rate affected turf quality later in the year, better turf quality was observed on plots topdressed at 100 lb / 1000 sq ft compared to 50 lb. This response likely indicates that higher topdressing rate becomes more important as the season progresses, especially when heavier topdressing is done very early (April) in the growing season as well as very late (October) in the previous season.

Table 3. Turfgrass quality response to the pooled topdressing effect and controls on a 6-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.11- to 0.125-inch in New Brunswick NJ during 2020.

Orthogonal Contrasts	6-Apr	25-Apr	25-May	9-Jun	11-Jun	19-Jun	30-Jun	24-Jul	10-Aug	9-Sep	18-Sep	26-Sep	14-Oct	19-Nov
Non-cultivated	Turf quality (1 to 9 scale; 9 = best)													
Pooled topdressing	8.5 a[a]	7.0 b	9.0 a	8.6 a	7.4 a	8.5 a	8.5 a	8.3 a	8.1 a	8.3 a	8.0 a	7.5 a	8.2 a	6.9 a
versus control	7.8 b	8.0 a	8.0 b	7.5 b	7.3 a	6.8 b	7.0 b	7.0 b	7.5 b	6.8 b	6.8 b	6.0 b	5.0 b	7.0 a
Core cultivated														
Pooled topdressing	5.3 a	5.0 a	8.2 a	7.6 a	6.7 a	7.2 a	8.2 a	7.1 a	7.2 a	7.1 a	6.5 a	6.1 a	7.2 a	5.3 a
versus control[b]	5.3 a	5.3 a	8.0 a	7.8 a	7.5 a	7.0 a	8.0 a	7.0 a	7.5 a	7.0 a	6.3 a	5.8 a	6.3 b	4.8 b
Controls														
Non-cored control	7.8 a	8.0 a	8.0 a	7.5 a	7.3 a	6.8 a	7.0 b	7.0 a	7.5 a	6.8 a	6.8 a	6.0 a	5.0 b	7.0 a
versus cored control	5.3 b	5.3 b	8.0 a	7.8 a	7.5 a	7.0 a	8.0 a	7.0 a	7.5 a	7.0 a	6.3 a	5.8 a	6.3 a	4.8 b

[a] Means within an orthogonal contrast followed by a different letter indicate a significant difference at $P \leq 0.05$.

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

Table 4. Turfgrass quality response to topdressing sand size, rate and core cultivation on a 6-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.11- to 0.125 inch in New Brunswick NJ during 2020.

	06 Apr	25-Apr	25-May	09-Jun	19-Jun	30-Jun	24-Jul	10-Aug	09-Sep	18-Sep	26-Sep	14-Oct	11-Nov
Source	------ *P* of a significant *F* ------												
Sand Size (Size)	NS	***	NS	***	***	NS	***	***	***	NS	*	**	***
Sand Rate (Rate)	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	*	***	***
Size × Rate	NS	NS	NS	NS	NS	NS	**	NS	***	*	NS	NS	**
Core Cultivation (CC)	***	***	***	***	***	*	***	***	***	***	***	***	***
Size × CC	NS	***	NS	NS	NS	NS	**	NS	NS	NS	NS	*	***
Rate × CC	NS	NS	NS	*	*	NS	*	NS	NS	NS	NS	NS	NS
Size × Rate × CC	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	*
Size main effect	------ Turf quality (1 to 9 scale; 9 = best) ------												
Medium-coarse	6.9 a	5.7 b	8.4 a	7.8 b	7.3 b	8.3 a	7.4 b	7.2 b	7.4 b	7.2 a	6.6 b	7.4 b	6.1 b
Medium-fine	6.8 a	5.8 b	8.7 a	8.1 ab	7.7 b	8.4 a	7.6 b	7.5 b	7.6 b	7.2 a	6.8 ab	7.6 b	6.4 a
Fine-medium	7.0 a	6.6 a	8.6 a	8.4 a	8.4 a	8.5 a	8.2 a	8.3 a	8.2 a	7.4 a	6.9 a	7.9 a	5.9 b
Rate main effect													
50 lb/1000 ft^2	6.8 a[a]	6.1 a	8.6 a	8.2 a	7.9 a	8.2 b	7.7 a	7.8 a	7.6 a	7.4 a	6.6 b	7.5 b	5.8 b
100 lb/1000 ft^2	6.9 a	6.0 a	8.4 a	8.0 a	7.7 a	8.6 a	7.8 a	7.5 a	7.8 a	7.1 a	6.9 a	7.9 a	6.4 a
CC main effect													
None	8.5 a	7.0 a	9.0 a	8.6 a	8.5 a	8.5 a	8.3 a	8.1 a	8.3 a	8.0 a	7.5 a	8.2 a	6.9 a
Twice a year	5.3 b	5.0 b	8.2 b	7.6 b	7.2 b	8.2 b	7.1 b	7.2 b	7.1 b	6.5 b	6.1 b	7.2 b	5.3 b

[a] Means within a main effect followed by a different letter indicate a significant difference at $P \leq 0.05$.

Wilt stress and localized dry spot

Wilt stress first expressed in 2020 as topdressing was being completed on 9 June. Topdressed plots expressed greater wilt symptoms compared to the controls regardless of the cultivation level (Table 5). Core cultivation did not affect wilt on control plots. Most wilt stress damage healed within 10 days; however, many plots were prone to dryness throughout during 2020. Ratings for localized dry spots on 10 August were associated with topdressing regardless of the cultivation level (Table 5).

Table 5. Wilt, drought damage and localized dry spot response to the pooled topdressing effect and controls on a 6-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.125-inch in New Brunswick NJ during 2020.

Orthogonal Contrasts	Wilt 9-June	Damage 11-June	Damage 19-June	Localized Dry Spot 10-August
Non-cultivated	----- (% of plot area affected) -----			1 to 9, 9 = none
Pooled topdressing	4.8 a[a]	3.1 a	0.8 a	6.6 b
versus control[b]	0.0 b	0.0 b	0.0 a	8.8 a
Core cultivated				
Pooled topdressing	7.1 a	3.7 a	1.4 a	7.3 b
versus control	1.3 b	0.0 b	0.0 a	8.5 a
Controls				
Non-cored control	0.0 a	0.3 a	0.0 a	8.8 a
versus cored control	1.3 a	0.0 a	0.0 a	8.5 a

[a] Means within an orthogonal contrast followed by a different letter indicate a significant difference at $P \leq 0.05$.

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

Wilt stress and localized dry spots were affected by topdressing sand size and rate as well as cultivation (Table 6). Turf grown on the plots topdressed with medium-coarse and medium-fine sands exhibited greater wilt stress damage than plots topdressed with fine-medium sand.

Additionally, wilt stress damage was greater on plots topdressed at 100 lb 1000-ft^{-2} compared to 50 lb 1000-ft^{-2}; however, the rate response was only evident on plots topdressed with the medium-coarse and medium-fine sands (interaction data not shown). There was very little wilt stress damage on plots topdressed with fine-medium sand at either topdressing rate.

Interestingly, core cultivation also intensified wilt stress damage on topdressed plots. Previous reports have indicated that core cultivation was very effective at drying out the surface 0- to 3-inches of the turf.

Localized dry spot development on plots was affected by all factors in the trial (Table 6). Use of medium-coarse and medium-fine sands to topdress plots increased the incidence of localized dry spot compared to plots topdressed with fine-medium sand. And plots topdressed at 100 lb 1000-ft^{-2} with either medium-coarse or medium-fine sand had more localized dry spots than plots topdressed with those sand at 50-lb 1000-ft^{-2} (interaction means not shown). Rate had no effect on plots topdressed with fine-medium sand, which had nearly no localized dry spots.

Core cultivation reduced localized dry spots on plots topdressed with medium-coarse and medium-fine sands but mildly increased localized dry spot on plots topdressed with fine-medium sand (interaction means not shown).

Volumetric water content at the 0- to 3-inch depth zone is being analyzed and is expected to associated wilt stress damage and localized dry spots on plots is associated with treatment combination that produce the driest turf surfaces.

Table 6. Wilt, drought damage and localized dry spot response to topdressing sand size, rate and core cultivation on a 6-yr-old 'Shark' creeping bentgrass turf grown on sand-based root zone and mowed at 0.11- to 0.125 inch in New Brunswick NJ during 2020.

	Wilt 9-June	Damage 11-June	Damage 19-June	Localized Dry Spot 10-August
Source	-------------------- *P* of a significant *F* --------------------			
Sand Size (Size)	***	***	***	***
Sand Rate (Rate)	***	***	***	***
Size × Rate	***	***	***	**
Core Cultivation (CC)	**	*	NS	**
Size × CC	NS	NS	NS	***
Rate × CC	NS	NS	NS	NS
Size × Rate × CC	NS	NS	NS	NS
Size main effect	----- (% of plot area affected) -----			1 to 9, 9 = none
Medium-coarse	9.1 a[a]	4.8 a	2.3 a	5.8 c
Medium-fine	7.5 a	4.1 a	1.1 b	6.5 b
Fine-medium	1.3 b	0.4 b	0.0 c	8.5 a
Rate main effect				
50 lb/1000 ft^2	3.6 b	1.4 b	0.2 b	7.5 a
100 lb/1000 ft^2	8.3 a	4.8 a	2.1 a	6.3 b
CC main effect				
None	4.8 b	2.5 b	0.8 a	6.6 b
Twice a year	7.1 a	3.7 a	1.4 a	7.3 a

[a] Means within a main effect followed by a different letter indicate a significant difference at $P \leq 0.05$.

Summary Points:

- Work plans adjusted for COVID-19 restrictions were approved by Rutgers University in mid spring, which allowed the continuation of topdressing and cultivation treatments for a 5th growing season.
- The May cultivation treatment was re-scheduled for 6 April to avoid severe work restrictions. Re-scheduling to April reduced the time that coring holes were evident in late spring and early summer with cultivation in May; an April timing for core cultivation treatment will be employed in years 6 and 7 of the trial unless the TERC disagrees.
- Topdressing with all sand sizes produced acceptable turf quality; however, turf quality has typically been best on plots topdressed with fine-medium (finest) sand. To-date, improved turf quality has been attributed to the greater water availability in plots topdressed with fine-medium sand.
- Wilt stress and mild localized dry spot developed in some plots during 2020. Wilt stress damage was greater on plots topdressed with coarser sand and at the highest (100-lb) rate of topdressing. Plots that were core cultivated also experienced greater wilt stress. These treatments are also effective at maintaining drier conditions at the 0- to 3-inch depth zone.
- Due to a strong differential in dryness and wilt stress among plots, a procedure to differentially hand-watering and document the frequency and quantity water applied was developed in 2020. The data is being assessed to determine whether the method was effective at quantifying differences in irrigation requirement among treatments. Presuming the methodology is effective, it will be continued in years 6 and 7 of this trial.

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 1 report)

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

Summary Points:

- In general, turf quality ratings were greater for plots receiving spring cultivation compared to fall or no cultivation.
- All combinations of sand topdressing and cultivation treatments resulted in less yellow patch severity compared to the non-treated control.
- Soil infiltration rate sampling methods were able to detect difference between cultivation treatments, with spring cultivated plots resulting in less of a reduction in infiltration rate compared to fall cultivation treatments and topdressing only treatment.

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 fall cultivation treatments were applied 29 Sept 2020. Sand topdressing treatments were applied every 2-wks during the summer beginning 15 June 2020 and concluding 21 Sep 2020

Fungicides will be applied year-round to prevent diseases including anthracnose, yellow patch, and Microdochium patch. The plots are 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 being mowed no higher than 0.125 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 measure at the same time and location as surface firmness to determine if surface firmness differences were a result of a treatment response or soil moisture differences. Soil infiltration rates for each plot were collected on 26 May and 10 Aug 2020, 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 (data now shown). 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.

References:

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

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

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

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

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

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

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

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

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 percent change in infiltration rate over time as influenced by topdressing rate and cultivation treatment applied to annual bluegrass turf in Corvallis, OR during 2020.

	Δ Infiltration rate			VWC
Main effects	1st inch	2nd inch	3rd inch	
Topdressing rate (T)[†]		%		
50 lbs/M	-35	-26	-25	39
100 lbs/M	-28	-20	-20	38
Cultivation (C)[‡]				
HT[§] Spring	-12	-4	-4	39
HT Fall	-50	-41	-41	37
HT Spring & Fall	-12	2	-5	38
ST Spring	-24	-17	-16	37
ST Fall	-66	-57	-51	39
ST Spring & Fall	2	6	1	38
Topdress only	-60	-48	-44	38
Source of variation				
T	ns	ns	ns	ns
C	ns	ns	ns	ns
T x C	ns	ns	ns	ns
CV (%)	-202	-274	-231	11

*,**,*** 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.

Figure 1. Comparison of cultivation treatments, hollow tine cultivation (left) and solid tine cultivation (right), prior to backfilling with sand.

USGA ID#: 2020-12-717

Title: A simple, practical method for organic matter content determination by superintendents

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

Start Date: 2020
Project Duration: 2 years
Total Funding: $10,000

Summary Points:

- Samples containing pre-determined organic matter (OM) content (i.e. standards) were successfully created, as verified by loss on ignition (LOI).
- Methods adapted from Leifeld and Kogel-Knabner (2001) proved to be time-consuming and physically intensive for end user application.
- New methods are being developed to potentially provide a simpler, less time consuming protocol for use by golf course superintendents.

Summary Text:

The purpose of this study was to provide golf course superintendents a rapid, practical, inexpensive, and reliable method to test OM on golf courses using hydrogen peroxide (H_2O_2). If superintendents can test OM on-site, they can reduce time waiting for results, and potentially save money. Hydrogen peroxide has been used since the 1920’s to degrade organic matter in soil samples (Robinson, 1922). Others have successfully used H_2O_2 in a variety of modified methods (Mikutta et al., 2005). Our hypothesis is we can adapt highly technical methods to be usable for golf course superintendents. Due to low cost and availability, H_2O_2 as a reagent for degradation is a logical choice. Most laboratory protocols require a 30% solution of H_2O_2, we hypothesize that a 3% solution readily at retailers will be sufficient. We will also use testing materials, methods, temperatures, and equipment that are either already on premises at most golf course maintenance facilities or easily acquired.

Before sampling from putting greens with unknown OM content, our first task was to create samples of a known OM percentage to use as standards in our testing. Though this operation was time consuming, it was necessary to ensure procedures were working correctly before testing unknown samples. Soil cores were collected from a research putting green at the John Seaton Anderson Turfgrass Research Facility near Mead, NE. Cores were air dried for multiple days to facilitate separation of mineral from organic matter. Samples were placed in a water bath where dense soil particles sank to the bottom, leaving only OM floating at the top. OM was skimmed from the water and placed in an oven at 60°C for at least 24-h. Dry OM was then shredded in food processors so that it would pass through a 2 mm sieve and combined with pre-sieved (2 mm) and dried sand to create an OM content of 3%. Organic matter content of combined samples was determined by LOI. This samples were then used in method development and considered as a standard for OM determination.

The standard samples were analyzed for OM content using hydrogen peroxide (H_2O_2). Based on the review of H_2O_2 analysis methods performed by Mikutta et al. (2005), we chose to modify a method from Leifeld and Kogel-Knabner (2001), which itself was adapted from Kunze and Dixon (1986), that successfully extracted 84-93% OM from arable and grassland soils in Germany. As a first attempt, we used 30% H_2O_2, even though it is expensive and not easy to acquire. This was done to the verify method and after verification, less expensive and more available 3 and 6% H_2O_2 would replace the 30% H_2O_2. For this process, ~10 g of the mixed sample was placed into a beaker with 15 mL water and 10 mL H_2O_2 (30%). Immediately, samples began frothing and foaming (Figure 1), indicating the degradation of OM by H_2O_2. Additional H_2O_2 was added in 5 mL increments when foaming subsided, and this continued until there was no more foaming at room temperature. Samples were agitated at each addition of H_2O_2. This process lasted at least an hour and required multiple additions of H_2O_2. After sample foaming completely subsided, they were put into an oven at 65°C, where foaming reinitiated. During the time in the oven, samples dried rapidly to the point of complete evaporation and frequent reapplications of water and H_2O_2 were required. If samples dried out, results were highly variable and less than 55%, on average, of the standard 3% OM. When procedures were strictly adhered to, we were able to successfully degrade up to 90% of the standard 3% OM in the sample with minimal sample to sample variability, similar to results reported by Leifeld and Kogel-Knabner. The intensity and attention to detail that this procedure requires made it unrealistic for the purposes of this project. Golf course superintendents are not going to have the time nor staffing to execute these methods, and therefore it was deemed impractical for our purposes.

We need to modify our methods to adjust for the shortcomings of our initial attempt. We will discontinue the use of 30% H_2O_2 given any practical method would need to use 3 or 6% H_2O_2. We also concluded that any long term (overnight) heating method would not be practical for the purposes of our project due to sample drying overnight and the observed requirement to continually watch and add more H_2O_2 as needed. Additionally, overnight drying in a kitchen oven at a facility where no one is present presents a potential safety hazard. The modified method will use short term (1-3 hours) heating. We anticipate being able to successfully achieve a constant rate of degradation using a combination of H_2O_2 volume, agitation, and heating time. Once a consistent rate of OM loss is developed, we can correlate to the actual percentage of OM in the sample and give superintendents an accurate representation of the OM in their putting greens. Initial results indicate that the 3 or 6% H_2O_2, while less efficient at degrading OM (45-50% of the standard 3% OM), were relatively consistent in the level of degradation. Our hypothesis, based on these preliminary data, is that samples will be degraded to a constant degree and a conversion factor can be calculated by correlation to LOI. The modified methods are currently being tested in the lab using the standard samples. Unknown sample testing using cores from various putting greens will be initiated this spring. If our efforts are successful, we will collaborate with local superintendents to validate use.

Literature Cited

Kunze, G.W. and J.B. Dixon. 1986. Pretreatment for mineralogical analysis. In: Klute, A. (Ed.). Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. SSSA Book Series 5 American Society of Agronomy, Madison, WI, pp. 91-100.

Leifeld, J. and I. Kogel-Knabner. 2001. Organic carbon and nitrogen in fine soil fractions after treatment with hydrogen peroxide. Soil Biol. & Biochem. 33:2155-2158.

Mikutta, R., M. Kleber, K. Kaiser, and R. Jahn. 2005. Review: Organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. Soil Sco. Soc. Am. J. 69:120-135.

Robinson, G.W. 1922. Note on the mechanical analysis of humus soils. J. Agric. Sci. 12:287-291.

Figure 1. Bubbling due to destruction of organic matter by hydrogen peroxide.

USGA ID#: 2020-18-723

Title: Review of Procedures to Measure Organic Matter of Putting Green Surfaces

Project Team: Roch Gaussoin (PI), Doug Soldat, Jim Murphy, Doug Linde, Brian Whitlark, Eric Chestnut, Luqi Li

Affiliation: University of Nebraska–Lincoln, University of Wisconsin-Madison, Rutgers University, Delaware Valley University, USGA

Objectives:

1. Create a comprehensive literature review of organic matter (OM) sampling procedures, preparation, and analysis from golf course putting greens.
2. Identify important research objectives that will help to create a unified method for these processes.
3. Publish findings and identify potential research interests.

Start Date: 2020
Project Duration: One year
Total Funding: $12,992

Summary Points:

- Though similarities exist among putting green sampling procedures, there is no consensus on specific strategies.
- Loss-on-ignition (LOI) is the most common and practical method for analyzing OM in putting green samples.
- There are multiple studies that can be performed to answer questions identified in the review document.

Summary Text:

The purpose of this project was to review the strategies that have been, and are currently, used for OM analysis in putting greens, including: sample numbers and size, extraction from putting greens, storage and preparation, and laboratory analysis. Citations in the review included peer-reviewed literature, commercial laboratory procedures, first-hand experience from academics and professionals and other identified protocols. The draft document is appended to this summary.

As the literature was reviewed it was evident that few studies used the same protocol in the sampling process. Recommended sample quantity per green was inconsistent among sources. Most studies and laboratory protocols averaged five samples per green (Hannan, 2016; Lockyear, 2008; Schmid et al., 2014; Woods, 2020), yet Kauffman et al. (2013) reported that up to 67 samples were needed to determine a 0.25% OM difference in seashore paspalum putting greens. Han et al. (2016) used pre-treatment sampling in a variable forest soil in combination with a SAS bootstrapping method to accurately determine the number of samples required to determine bulk density. The group editing this literature review thought it would be useful to perform a similar study on putting greens to statistically determine how many samples are required per green.

Many studies took samples using a standard soil core sampler (19 mm) at a 100 mm depth and separated samples into sections at 20 mm depths for compositing. Samples can be composited at the same depths because OM concentration decreases with depth (Linde and Hannan, 2020). Considering golf course superintendents are most concerned with OM content closer to the playing surface, more accurate and practically useful OM analysis may result from dividing samples at predetermined depths.

As part of sample preparation, some individuals and laboratories prefer to remove verdure prior to OM analysis via LOI (Linde and Hannan, 2020; Schmid et al., 2014). Others do not recommend removal of verdure (Woods, 2020). There are legitimate arguments for and against this procedure. The removal of verdure could potentially be a source of error if removal is inconsistent. However, if verdure is not removed, the OM from the above-ground tissue would change the OM content as would verdure depth. Leaving verdure on samples could also simplify the pre-LOI process of analyzing OM. These conflicting and potentially confounding ideas led the authors to suggest a formal study be conducted comparing LOI results from putting green samples that have and have not had their verdure removed. Based on those results, an additional study could be conducted to determine the effect of grass height-of-cut on LOI results.

In soil characteristic investigations, it is common to grind soil samples and then sieve them to 2 mm prior to various analyses. This is typically done to ensure there are no large clods that would prevent inner particles from being included in the sample. Methods and laboratories were again split on the use of this practice for putting green OM analysis. Again, the authors thought that it would be useful to compare these practices side-by-side to determine what, if any, effect grinding, and sieving have on results.

Studies have shown that soil samples should be analyzed by LOI as soon as possible after extraction. Chapman (1997), Jones and Willett (2006), and Sun et at. (2013, 2015) all found changes in various chemical and physical structures when samples are analyzed more than 24 hours after being extracted from the soil. Lee et al. (2007) compared five storage methods for soil microbial activity and found that cooling samples at 20°C or freezing at -4°C were not different from each other and had little affect on results compared to air drying and rewetting. Based on these studies, samples should be analyzed as soon as possible after extraction from putting greens, ideally within 24 hours. However, due to the time it takes to extract, pack, ship, and finally get samples analyzed, this is most likely not possible for golf course superintendents. As a secondary option, samples should be cooled or frozen, and finally, if none of those options are available then air-drying is acceptable. In all cases, LOI should be performed as soon as possible after extraction.

Methods of measuring OM in soils have been modified throughout the years. In the 1920's, Robinson (1922) reported a successful method using hydrogen peroxide. Since then, hydrogen peroxide methods have been modified to be more accurate and efficient. Leifeld and Kogel-Knabner (2001) reported a method that was up to 90% effective at removing OM from soil samples, but it does take multiple applications of high-concentration hydrogen peroxide and between 1-3 days to complete the reaction. Other methods using various reagents have been used throughout the years, but most of them are no longer employed because of some combination of expensive and/or dangerous chemicals, time required to complete reactions, or environmentally hazardous byproducts. A method that does not need addition of chemicals is LOI. This process places samples under high heat for a specific amount of time until the OM has combusted and only non-combustible soil particles remain. There are different heating times and temperatures used for LOI analysis, but most agree that a temperature of 360°C and heating time of 2 hours, as proposed by Schulte and Hopkins (1996) is both accurate and time-efficient while avoiding overestimations that can occur under higher temperatures and longer times (Cambardella et al., 2001).

The literature review, in draft form, has identified historical procedures in sampling and analysis, identified current trends and described potential research to confirm and standardize procedures.

Literature Cited

Cambardella, C.A., A.M. Gajda, J.W. Doran., B.J. Wienhold, and T.A. Kettler. 2001. Estimation of particulate and total organic matter by weight loss-on-ignition. In Assessment Methods for Soil Carbon; Lal, R., Kimble, J.M., Follett, R.F., and Stewart, B.A., eds.; Advances in Soil Science, Lewis Publishers: Bocan Raton, FL. pp. 349–359.

Chapman, P.J., C.A. Shand, A.C. Edwards, and S. Smith. 1997. Effect of storage and sieving on the phosphorus composition of soil solution. Soil Science Society of America Journal 61, 315–321.

Han Y., J. Zhang, K.G. Mattson, W. Zhang, and T.A. Weber. 2016. Sample sizes to control error estimates in determining soil bulk density in California forest soils. Soil Sci. Soc. Am. J. 80:759-764. doi: 10.2136/sssaj2015.12.0422

Hannan, B. 2016. Golf greens organic matter content sampling procedure. New Zealand Sports Turf Institute.

Jones, D.L. and V.B. Willett. 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology & Biochemistry. 38:991-999.

Kauffman, J.M., J.C. Sorochan, and D.A. Kopsell. 2013. Field sampling warm-season putting greens for thatch-mat depth and organic matter content. HortTechnology 23(3):369-375.

Lee, Y.B., N. Lorenz, L.K. Dick, and R.P. Dick. 2007. Cold storage and pretreatment incubation effects on soil microbial properties. Soil Sci. Soc. Am. J. 71:1299–1305.

Leifeld, J. and I. Kogel-Knabner. 2001. Organic carbon and nitrogen in fine soil fractions after treatment with hydrogen peroxide. Soil Biology and Biochemistry. Vol. 33(15): 2155-2158.

Linde, D. and B. Hannan. 2020. Benchmarking putting green organic matter in the Philadelphia region. Bonnie Greensward. 2019-20 Phila. Assoc. of Golf Course Supts. Vol.4.

Lockyer, J. 2008. STRI – Testing for organic matter. Turfgrass Bulletin. Sports Turf Research Institute 242: 13-16.

Robinson, G.W. 1922. Note on the mechanical analysis of humus soils. J. Agric. Sci. 12:287.

Schmid, C.J., R.E. Gaussoin, and S.A. Gaussoin. 2014. Organic matter concentration of creeping bentgrass putting greens in the continental U.S. and resident management impact. Online. Applied Turfgrass Science. 11 (1) doi:10.2134/ATS-2014-0031-BR.

Schulte, E. E. and B. G. Hopkins. 1996. Estimation of soil organic matter by weight-loss-on ignition. Chap. 3. In F. R. Magdoff et al. (ed.). Soil Organic Matter: Analysis and Interpretation. SSSA Spec. Publ. 46, SSSA, Madison, Wis.

Sun, S.H., J. J. Liu, Y.F. Li, P.K. Jiang, and S.X. Chang. 2013. Similar quality and quantity of dissolved organic carbon under different land use systems in two Canadian and Chinese soils. J. Soil. Sediment. 13:34–42.

Sun, S.H., H. Y. Cai, S.X. Change, and J.S. Bhatti. 2015. Sample storage-induced changes in the quantity and quality of soil labile organic carbon. Scientific Reports.5:17496 doi. 10.1038/srep17496.

Walkley, A. and I.A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-37.

Woods, M. 2020. Soil organic matter: a bulletin list. via Twitter. February 17, 2020.

USGA ID#: 2017-01-611

Project Title: Effects of deficit irrigation and rootzone depth on water use and drought resistance of warm-season fairways.
Principal Investigators: Charles Fontanier and Justin Moss
Affiliation: Oklahoma State University

Objectives:

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

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

Summary Points:
- Field plots for a warm-season fairway deficit irrigation study show differences among cultivars regarding drought resistance and irrigation water requirements.
- Several cultivars of bermudagrass were able to maintain acceptable appearance in a relatively wet year with less than 25mm of supplemental irrigation and an effective K_c of 0.18.
- Restrictive rootzone depths increased drought severity for ‘Latitude 36’, ‘Meyer’, ‘OSU 1403’, ‘PremierPro’, and ‘Tifway’.
- ‘Celebration’, ‘U-3’, and ‘TifTuf’ did not appear to be affected by restrictive rootzones even under the most severe drought treatment.
- A novel cart-traffic simulator (RaGIS) was built allowing for efficient comparison of up to 8 cultivars under varying irrigation amounts simultaneously.
- Results using the RaGIS show ‘Meyer’ as having the poorest drought resistance but good recovery from traffic once rainfall occurs.

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

Modern irrigation practices typically rely on reference ET as calculated from meteorological data to estimate evaporative demand of the atmosphere. Warm-season turfgrass water use is then estimated as the product of reference ET and a crop coefficient of 0.6 to 0.7. Irrigation can then be scheduled to replace soil water lost through ET. Applying irrigation at volumes less than ETc is a common water conservation practice which attempts to maintain a target turf quality while reducing irrigation volumes. Many turfgrasses will demonstrate acceptable turf quality under deficit irrigation, although the severity of the program that sustains acceptable turf performance varies with species, cultivar, and soil/rooting properties (Feldhake et al., 1984; Poudel, 2010; Wherley et al., 2014). Research aimed at measuring the interactions of turf performance, plant water use rates, soil moisture content, rootzone depth, and traffic is warranted.

Methods

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

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

Experiment 2: A second experiment was conducted to study the effects of cart traffic on irrigation water requirements of common fairway turfgrasses. Small plots were established in summer 2018 from plugs (TifTuf, U-3, Latitude 36, Celebration, Tifway, OSU1403, and OKC1221) or sod (Meyer) as a randomized complete block design having three replications. The plots are unique in that they were planted as a pie wedge around a central point. A small–scale center pivot irrigation system was designed such that it created a radial gradient irrigation system (RaGIS) moving from near the center (wet) to the outer edge (dry). The pivoting arm was also designed to simulate golf cart traffic associated with the turning of wheels. Construction and installation of the RaGIS was completed in June 2019, and soil moisture sensors (TDR-315-L, Acclima) were installed to monitor volumetric water content to the 10-inch depth.

Initial irrigation and traffic treatments were applied briefly in August 2019 before rainfall halted the study. The study was resumed in 2020 with irrigation and traffic treatments initiating on June 8. Traffic was applied as 30 turns of the RaGIS five days per week for 3 weeks before allowing recovery for 5 weeks. A second traffic cycle was performed from July 6 through July 24. A third traffic period was performed from August

10 through August 25. Through all the traffic cycles and recovery periods, irrigation treatments remained constant applying a gradient ranging from 0 to 0.3 inches of water twice per week. Data were collected 2 to 3 times per week for visual turfgrass quality, NDVI, and green coverage using image analysis.

Results

Experiment 1:

Significant differences in drought resistance were apparent during late July 2018. Specifically, Meyer and OSU 1403 each showed drought stress at the 25% ET_c levels, while other cultivars showed no indication of stress at these levels (under unrestricted rooting). In 2019, minimal irrigation was required during May or June due to frequent rainfall. During July when soil moisture began to be depleted, NDVI was affected by the genotype by treatment interaction. Similar to the prior year, ‘Meyer’ and ‘OKC1403’ showed greater reductions in NDVI with diminishing soil moisture of the 25% ET_c treatment (Fig. 1).

In 2018, rootzone depth affected green coverage for ‘Latitude 36’, ‘Meyer’, ‘OSU 1403’, ‘PremierPro’, and ‘Tifway’ (Fig. 2). In general, the shortened rootzones resulted in less green coverage than the unrestricted rootzone for these bermudagrasses (Table 1). For Meyer, severe drought stress was typically observed across all rootzone depths, with the exception of the 25% ET_c treatment in which case the 8-inch rootzone performed worst. This suggests Meyer did not substantially benefit from an unrestricted rootzone which is indicative of short-rooted plants. Soil water extraction rates seemed to confirm the shorter roots for both ‘Meyer’ and ‘OSU 1403’. For ‘Celebration’, ‘TifTuf’, and ‘U-3’, rootzone did not have any visible effect on green coverage even in the 25% ET_c treatment. These findings reinforce the superior drought resistance of these grasses compared to others tested but also suggest drought resistance was not strictly related to rooting potential. Further research under more stressful conditions are required to better define water use rates and irrigation requirements of the more drought resistant grasses.

Experiment 2:

The RaGIS applied a diminishing amount of water from the center to the outer edge of the circular plot as designed. The time period for one trafficking rotation was ~33 seconds, resulting in the inner most wheels traveling 0.88 $m\ s^{-1}$ and the outer wheels 1.3 $m\ s^{-1}$. Over a 10-day period in August 2019, the RaGIS was used to apply traffic and differential irrigation resulting in measureable differences in soil surface moisture content (Fig. 3). However, the short duration of this cycle did not allow moisture in the lower rootzone to vary among treatments.

During the 2020 growing season, combined traffic and water stress were achieved in both the early cycle (June 8 – June 29) and the late cycle (August 10 – August 24). A theoretical soil water balance for each irrigation treatment shows development of water deficits during these periods, and fairly wet conditions during July (Fig. 4). Turfgrass quality for most bermudagrasses were similar to each other and not largely influenced by irrigation or traffic suggesting more severe stress is needed to test these grasses (Table 2). Meyer was substantially more sensitive to drought stress than bermudagrass but recovered rapidly once rainfall occurred even under routine cart traffic. On several occasions, surface soil moisture content was lower in Meyer than other grasses suggesting a higher ET rate. In conclusion, most bermudagrasses tolerated regular cart-traffic even at soil water deficits over 2-inches and a surface soil moisture of >20%. Meyer zoysiagrass fairways are more likely to show stress at deficits approaching 1-inch but can survive traffic under greater water deficits if seasonal discoloration is allowable.

Limitations of this project:

For each experiment, we proposed using ambient drought methods (as opposed to using a rainout shelter) which limited our ability to adapt to the fairly wet conditions seen the past three years in Stillwater. In particular, we struggled to calculate actual ET rates for each irrigation treatment due to the need for rain-free days. Future projects using a soil moisture sensing approach to estimating ET should be encouraged to use rainout shelters, although care must be taken to not artificially influence evaporative demand of the surface.

Accomplishments

This project funded one MS student project and several undergraduate research scholars. The project also led to development of the RaGIS which will allow OSU to pursue continued traffic and drought research in future years. In total, this project resulted in 4 research posters and one oral presentation at the Tri-Societies Meetings and Oklahoma Governor’s Water Conference. In the coming months, we hope to submit results from the MS thesis for publication in *Crop Science* and would welcome opportunities to present findings in trade publications as they arise.

Table 1. Effect of rootzone depth (RZD) and deficit irrigation level on green coverage of selected warm-season turfgrasses from Experiment 1 on 2018 July 26.

Grass	RZD	K25[z]	K50	K75	K100	Avg
		-----------------------	%	-----------------------		
Celebration	8-inch	59.9	53.5	66.8	65.4	61.4
	12-inch	53.1	61.7	72.5	71.2	64.6
	unrestricted	54.2	59.6	65.6	64.6	61.0
Latitude 36	8-inch	*12.2b*[y]	29.0	72.4	71.1	*46.2b*
	12-inch	*46.5a*	42.8	49.9	54.7	*48.5b*
	unrestricted	*53.2a*	62.3	64.0	70.4	*62.5a*
Meyer	8-inch	*11.2b*	41.1	66.3	54.8	*43.4b*
	12-inch	*44.3a*	54.0	57.0	59.8	*53.8a*
	unrestricted	*26.4ab*	45.2	53.8	54.4	*44.9b*
OSU 1403	8-inch	7.2	10.6	39.7	55.5	*28.2b*
	12-inch	10.2	21.7	49.7	43.8	*31.3b*
	unrestricted	30.5	39.7	53.8	52.8	*44.2a*
Premier	8-inch	*12.6b*	36.8	54.0	81.6	46.3
	12-inch	*38.4ab*	39.3	74.6	64.5	54.2
	unrestricted	*60.5a*	60.4	72.0	72.1	66.3
TifTuf	8-inch	42.5	38.7	54.8	60.1	49.0
	12-inch	45.8	54.4	50.3	64.0	53.6
	unrestricted	52.4	56.8	57.6	65.4	58.0
Tifway	8-inch	*14.0b*	22.6	45.0	46.3	*32.0b*
	12-inch	*16.3b*	47.8	51.3	53.2	*42.2a*
	unrestricted	*32.2a*	45.4	51.7	53.5	*45.7a*
U3	8-inch	26.2	30.6	60.8	67.3	46.2
	12-inch	32.1	59.3	64.6	67.1	55.8
	unrestricted	36.2	50.7	57.9	58.8	50.9

[z] K25, K50, K75, K100 represent 25, 50, 75, and 100% of plant water requirement.
[y] Means followed by the same letter within a column and genotype are not significantly different ($p \leq 0.05$).

Table 2. Turf quality scores for 8 turfgrasses under weekly cart traffic in the outer band of the RaGIS (Irr = 2% ET_c). Traffic was curtailed August 1 to allow recovery.

Grass	6/12	6/13	6/14	6/15	6/17	6/19	6/22	6/25	7/9	7/13	7/20	7/22
Celebration	7.0	7.0a [z]	6.5a	6.0a	5.5a	5.5a	6.0a	5.0a	6.5	6.5	7.0	7.0
Latitude36	7.0	7.0a	7.0a	6.0a	5.3a	5.3a	6.0a	5.3a	6.3	6.3	6.3	6.7
Meyer	5.7	5.0b	4.7b	3.3b	3.3b	3.3b	4.3b	3.3b	5.7	5.7	5.7	5.7
OKC1221	7.0	7.0a	6.5a	6.0a	5.0a	5.0a	6.0a	5.0a	6.0	6.0	7.0	6.5
OSU1403	7.0	7.0a	6.3ab	5.7a	5.0a	5.0a	6.0a	5.0a	6.0	6.0	6.7	6.3
TifTuf	7.3	7.3a	7.0a	6.0a	5.3a	5.3a	6.0a	5.3a	6.7	6.7	7.0	7.0
Tifway	6.7	6.7a	6.3ab	5.7a	5.0a	5.0a	6.3a	5.0a	5.3	5.7	6.3	6.3
U3	6.3	6.7a	6.0ab	6.0a	5.3a	5.3a	6.0a	5.0a	6.0	6.0	6.7	7.0

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

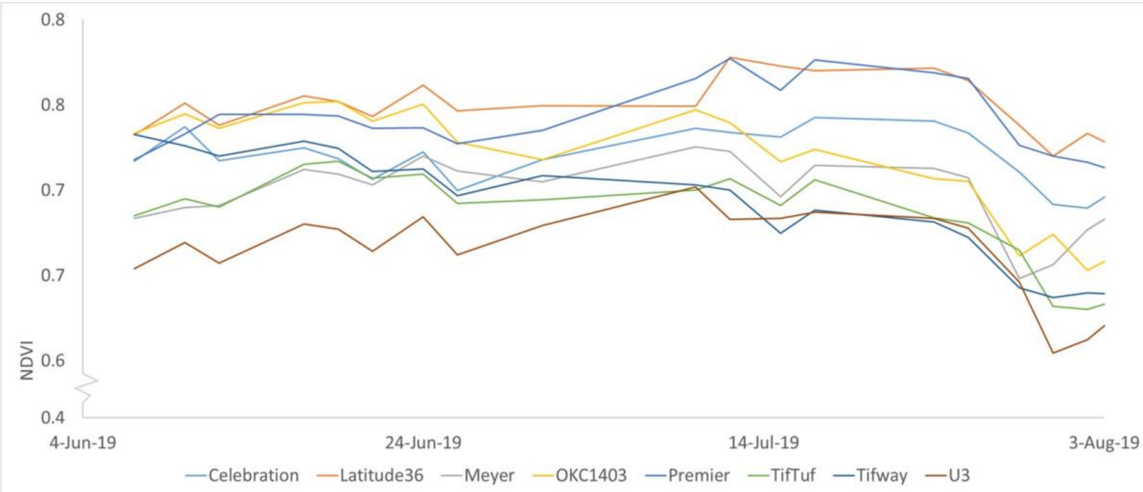


Fig. 1. Normalized difference vegetation index (NDVI) of 8 turfgrasses irrigated at 25% ET_c during the 2019 growing season.

Fig. 2. Effects of severe deficit irrigation on restricted rootzone areas of an experimental bermudagrass showing poor drought resistance: 8-inch rootzone (left), 12-inch rootzone (middle), and unrestricted rootzone (right).

Fig 3. UAS-derived image of the RaGIS after 2 weeks of operation (August 2019).

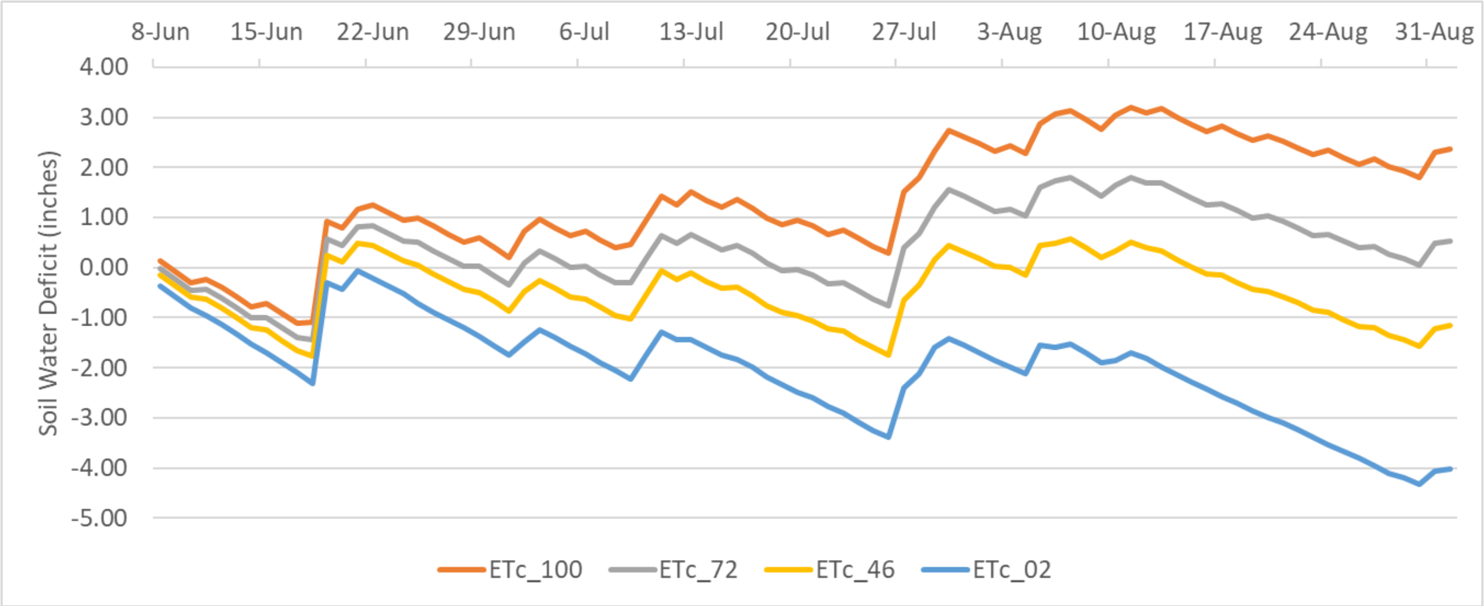


Fig. 4. Theoretical soil water deficit implemented across the RaGIS during 2020 growing season (June 8 – Sept 1). The method assumed a K_c of 0.7 and 100% of rainfall was effective.

USGA ID#: 2017-36-646

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

Project Leader: Priti Saxena, Robert Green, Eudell Vis and Valerie Mellano

Affiliation: California State Polytechnic University, Pomona, CA.

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

Start Date: November 2017

Project Duration: Three years

Total Funding: $45,000

Summary Points:

- Bermudagrass plots were maintained by the students and data was collected during the growing season (April – October) to estimate the effectiveness of the soil moisture sensors in conserving water in fairway condition.

- The soil moisture sensors treatments showed significant variations in reducing amount of water applied. In addition, the amount of water applied is lesser as compare to ET based irrigation during the study.

- The turfgrass quality and density ratings showed acceptable and healthy turfgrass in a fairway condition.

Summary Text:

To keep turfgrass healthy and playable without wasting water, automated soil sensor system manufacturers, water purveyors, and water managers have promoted soil moisture sensors based irrigation scheduling. Soil moisture sensors (SMS) provide an excellent irrigation management tool; with the potential to conserve water and reduce daily water use. Reducing a minute or two of irrigation application per valve on a golf course could significantly save on water and energy cost. Ability of turfgrass managers to control irrigation scheduling by assessing soil moisture accurately and quickly at the root zone, would help in water conservation and save thousands of dollars over a growing season (GCSAA, 2009).

In fine-medium texture soil, the accurate monitoring of moisture content and plant health is imperative along with the use of SMS to maintain adequate moisture levels. The goal of this study is to identify SMS systems that could reduce the number of irrigation cycles or amount of water applied while maintaining acceptable turfgrass quality as compared to traditional time-based irrigation scheduling on fairways.

In this study, hybrid bermudagrass [*C. dactylon* (L.) Pers. × *C. transvaalensis* (Burtt-Davy)] GN-1 plots (each 10 ft. × 10 ft. [3m × 3m]) are used, which were sodded in 2012 and separated by 3 ft. (0.91 m) in all directions at Center for Turf, Irrigation and Landscape Technology (CTILT), California State Polytechnic University, Pomona, CA. Three different soil moisture sensors (Toro, Rain Bird and tucor) were installed and compared to a control treatment (no SMS). The control plots were irrigated based on the ET value collected from CIMIS station #78. Irrigation amount is based on ET_{crop}, previous 2-d CIMIS ET_o (adjusted for precipitation), monthly warm-season turfgrass K_c, full run time multiplier (RTM) based on individual plot irrigation system distribution uniformity, low quarter (DULQ), and individual plot precipitation rate. Plots are individually scheduled once per week. Total weekly irrigation run time will be equally divided over five irrigation days per week.

Each plot is individually zoned and has a rotating nozzle sprinkler at each of the four corners. The experiment was laid in complete randomize design with three replications. Bermudagrass plots were maintained at the height of ½" and mowed twice a week during. The clippings were collected to measure the dry weight, weekly. The plots were double cut in opposing directions using a Tru-Cut walk behind reel mower. Glyphosate was spot-sprayed utilizing a Solo® 3-gallon backpack sprayer at a rate of 1 oz/ gallon to eradicate broadleaf weeds between rows. SpeedZone Southern® Boradleaf Herbicide was applied on the plots @ 1.5 oz /1000 sq. ft for the post emergent control of broadleaf weeds. The plots were verticutted and Urea was applied @ 1 lb N/100ft2 in fall season.

The data collection is conducted during April 1st – October 31st, 2018, 2019 and 2020). Each year, study starts with all plots at similar water content (field capacity) and data is collected in runtime (minutes) for each plot by week, month, season, irrigation applied for each plot, amount of saved applied irrigation (actual amount and as a percentage of no SMS control treatment), visual turfgrass quality, density and color ratings, clipping yield and soil salinity (bulk electrical conductivity). The results so far obtained showed that run times of soil moisture sensors were lesser than that of control plots. The results will provide irrigation applied for all the treatments, along with water savings for the SMS treatments during three years of study. The SMS response comparisons will also be reported. The bermudagrass turfgrass quality ratings are acceptable (6-7) for a fairway in Southern California based on NTEP rating (1-9).

Turfgrass plots showed acceptable turfgrass quality and density in a fairway condition. SMS are effective reducing amount of irrigation water used to maintain fairway turf quality and density under the conditions of the study. Comparison of research based SMS data with commercial grade SMS operation and set points should assist management of these systems. In summer 2020, the amount of water saved was more in Toro and Turcor sensor treatments, where as Rain Bird treatment applied more water than ET based irrigation. In Spring 2020, all the sensors showed reduction in water applications than control. More analysis still needs to be conducted on the available data.

Image 1: Twelve bermudagrass plots, CPP

Image 2: CPP students participating in the installation of SMS

USGA ID: 2017-37-647

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

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

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

Objectives:

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

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

Start Date: 2018 (three-year duration)

Total Funding: $204,876

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.

- A dry down was conducted to determine the appropriate soil moisture thresholds for the SMS-based treatment.

- Irrigation treatments were initiated, and total water use was recorded throughout two independent runs during summer and fall 2020.

- 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, golfer perceptions, and energy savings will be evaluated using data collected in 2020. The experiment will be repeated in 2021.

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 their use in implementing site-specific irrigation.

Progress to Date

Fairway Preparation

As described in the 2019 project update, nine fairways (six par 4's and three par 5's) 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 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 (wet, average, or dry) 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 a 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 that 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 of wilt 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 (Table 1).

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

Fairway	**Moisture Class**	**Lower Threshold Soil VWC (%)**	
		Run 1	Run 2
	Low	18	18
5	Moderate	30	29
	High	44	38
	Low	19	19
13	Moderate	26	26
	High	45	44
	Low	22	24
15	Moderate	21	21
	High	22	19

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. On 22 June, the position of the sensor for fairway 15 – low moisture class was changed due to initial installation being too close to a drainage line. The threshold for that sensor was subsequently reset based on visual observation after the treatments had begun. Irrigation treatments were started 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 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, and that water was included in the total volumes reported for each fairway. On 9 Aug., treatments were halted to allow the superintendent time for regular maintenance activities on the course. On 23 Aug., a second run of treatments began for the fall season, which ended on 16 Oct. During that time, the lower thresholds were reset, again based on the superintendent's input. Threshold values for the second run are shown in Table 1. The superintendent was again allowed to run individual irrigation heads, as needed.

Water use was recorded by the central controller for all applications across all treatments and total consumption was tracked over the course of the two treatment runs. A mobile application was created to record visual quality ratings on a scale of 1 – 3, corresponding to "poor," "fair," and "good" quality, that was used by both the superintendent and researchers during the treatment application runs. 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. A catch can audit of the irrigation system was conducted following the first run of treatments.

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 second run was one week longer than the first, 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 of the combined run data, including random effect terms for replicate within run. As no significant run × treatment interaction was present (Table 3), the data remained combined for examination of treatment effects. Treatment means were calculated and separated by Fisher's protected least significant difference using the *emmeans* package in R.

The analysis showed that 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 was 5.66 and 4.18 mm wk^{-1} for the first and second runs, 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.

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

Treatment	**Fairway**	**Run 1 Water Use (m^3)**	**Run 2 Water Use (m^3)**	**Irrigated Area (m^2)**	**Run 1 Irrigation Depth per Week (mm wk^{-1})**	**Run 2 Irrigation Depth per Week (mm wk^{-1})**	**Mean Irrigation Depth per Week (mm wk^{-1})**
ET	3	653.97	601.69	14410	6.48	5.22	6.98 a†
	6	1028.07	1037.96	18303	8.02	7.09	
	8	1473.54	1264.97	24471	8.60	6.46	
Traditional	9	469.59	354.82	11973	5.60	3.70	5.02 b
	10	1239.44	808.24	26439	6.70	3.82	
	14	659.35	523.78	15480	6.08	4.23	
SMS	5	446.66	385.92	16928	3.77	2.85	2.76 c
	13	452.40	347.37	21154	3.06	2.05	
	15	248.01	236.75	13467	2.63	2.20	

† 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 for combined treatment runs in 2020.

Source	Num dF	Den dF	MSE	F	*Pr*(>F)
Run	1	4	9.86	19.77	0.011
Treatment	2	8	26.76	53.66	< 0.001
Treatment x Run	2	8	0.76	1.53	0.274

Future Expectations

In December 2020, a survey will be given to the Edina Country Club Green Committee to gain a more thorough understanding of their perceptions of aesthetics and playability throughout the season. That information will be combined with the mobile app quality ratings, as well as the quality (NDVI) information from the PS6000 surveys, to analyze the performance of each fairway. Further analyses will be conducted to evaluate the relationship between golfer/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. Furthermore, we intend to conduct an energy analysis using data from the pump station electricity data to quantify the amount of energy saved, if any, using the various irrigation scheduling approaches.

Beginning in spring 2021, we will initiate another dry down and reset the SMS thresholds. Data will again be collected on total water consumption throughout the growing season and PS6000 surveys will be conducted on a routine basis. We expect that at the completion of 2021, we will have sufficient data for a full and comprehensive analysis of the water savings potential of various irrigation scheduling approaches. Our expectation, based on preliminary results from this year, is that this project will have defined and demonstrated a workflow for implementation of spatial mapping and SMS technology on a golf course, in addition to providing a case study that demonstrates the substantial water savings generated by using such an approach. This information will greatly benefit the golf industry and provide an important tool for superintendents and water managers.

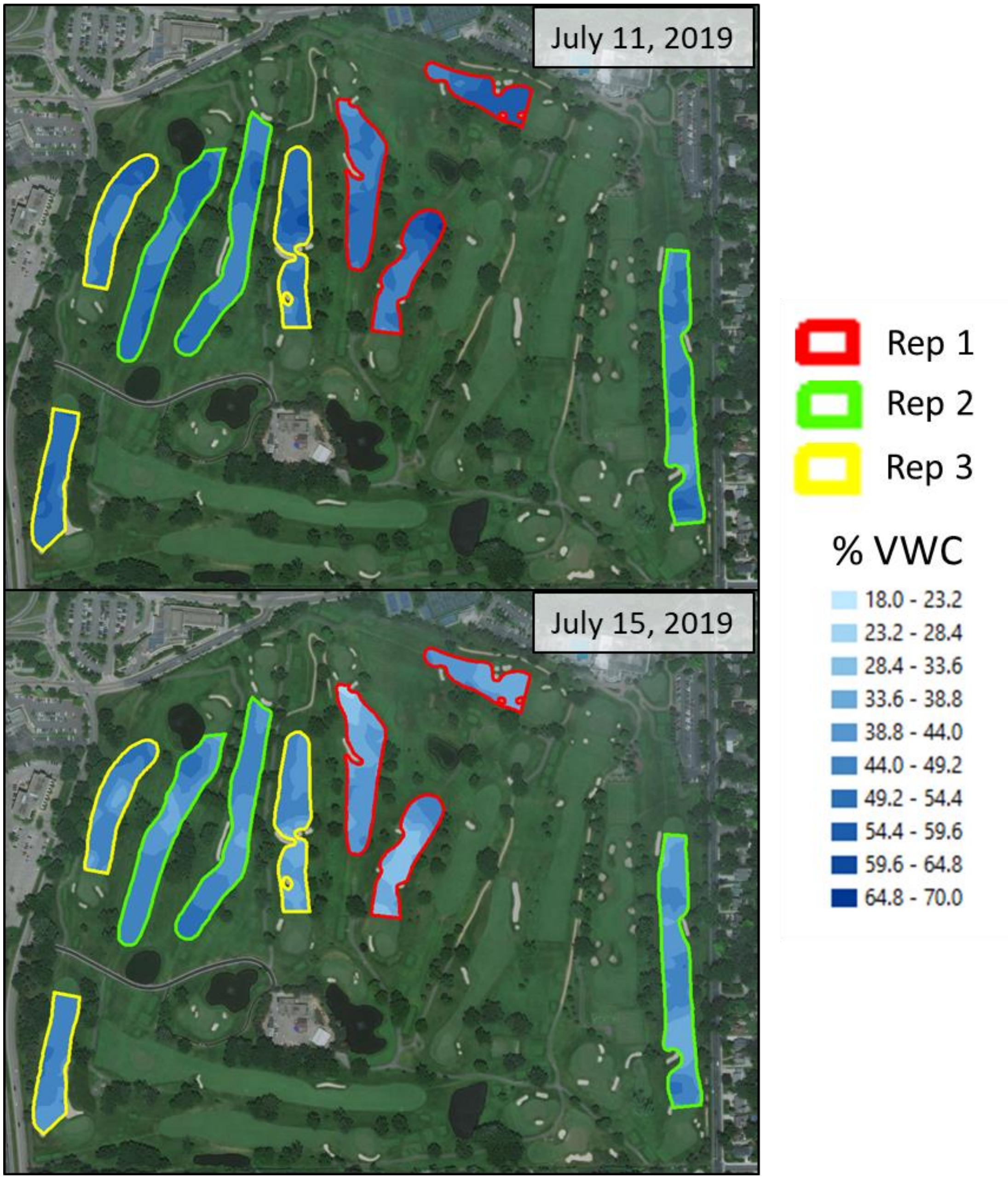


Figure 1. Soil moisture maps from July 11 and 15, 2019, where data were collected under saturated and approximate field capacity conditions, respectively. The nine fairways selected for use in the study were placed into similar groups of three based on their size, soil moisture (% volumetric water content; 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).

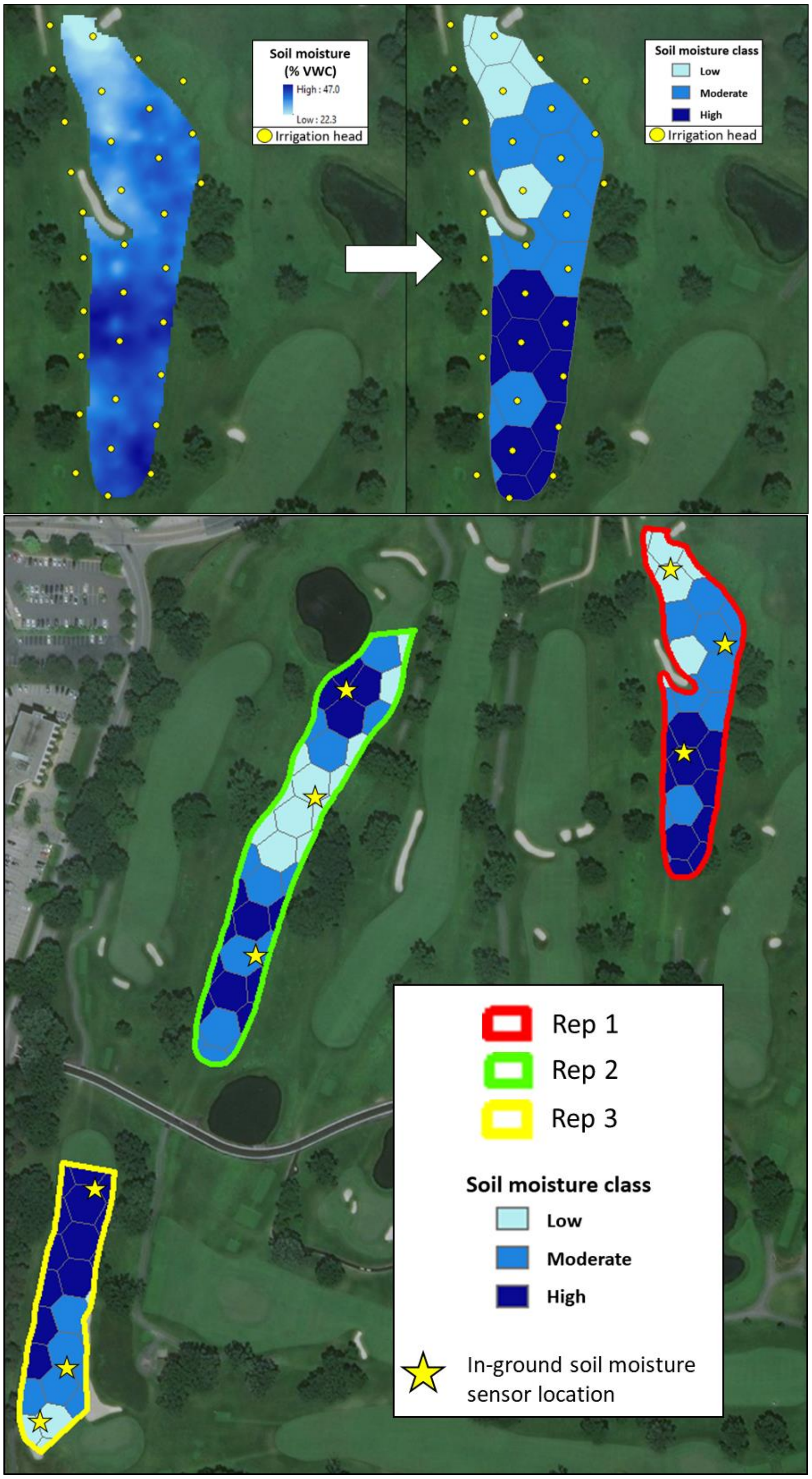


Figure 2. Top-left, percent volumetric water content (VWC) on one fairway in the soil moisture sensor treatment; top-right, soil moisture classes within delineated management zones on one fairway in the soil moisture sensor treatment; bottom, in-ground soil moisture sensor locations within each soil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

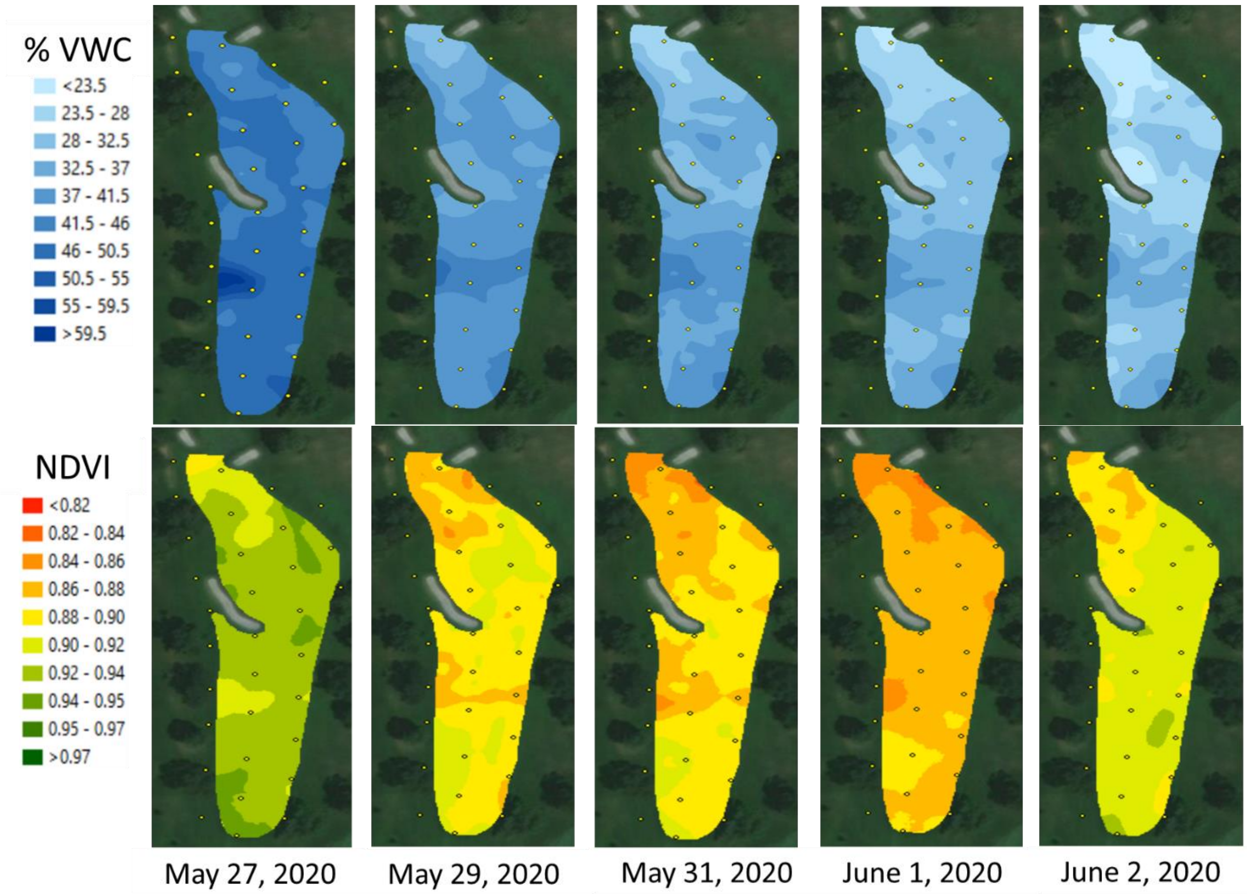


Figure 3. Kriged maps of volumetric water content (VWC) and normalized difference vegetation index (NDVI) on SMS-treatment fairway 5 over the course of a dry down event from May 27 – June 2, 2020 at Edina Country Club.

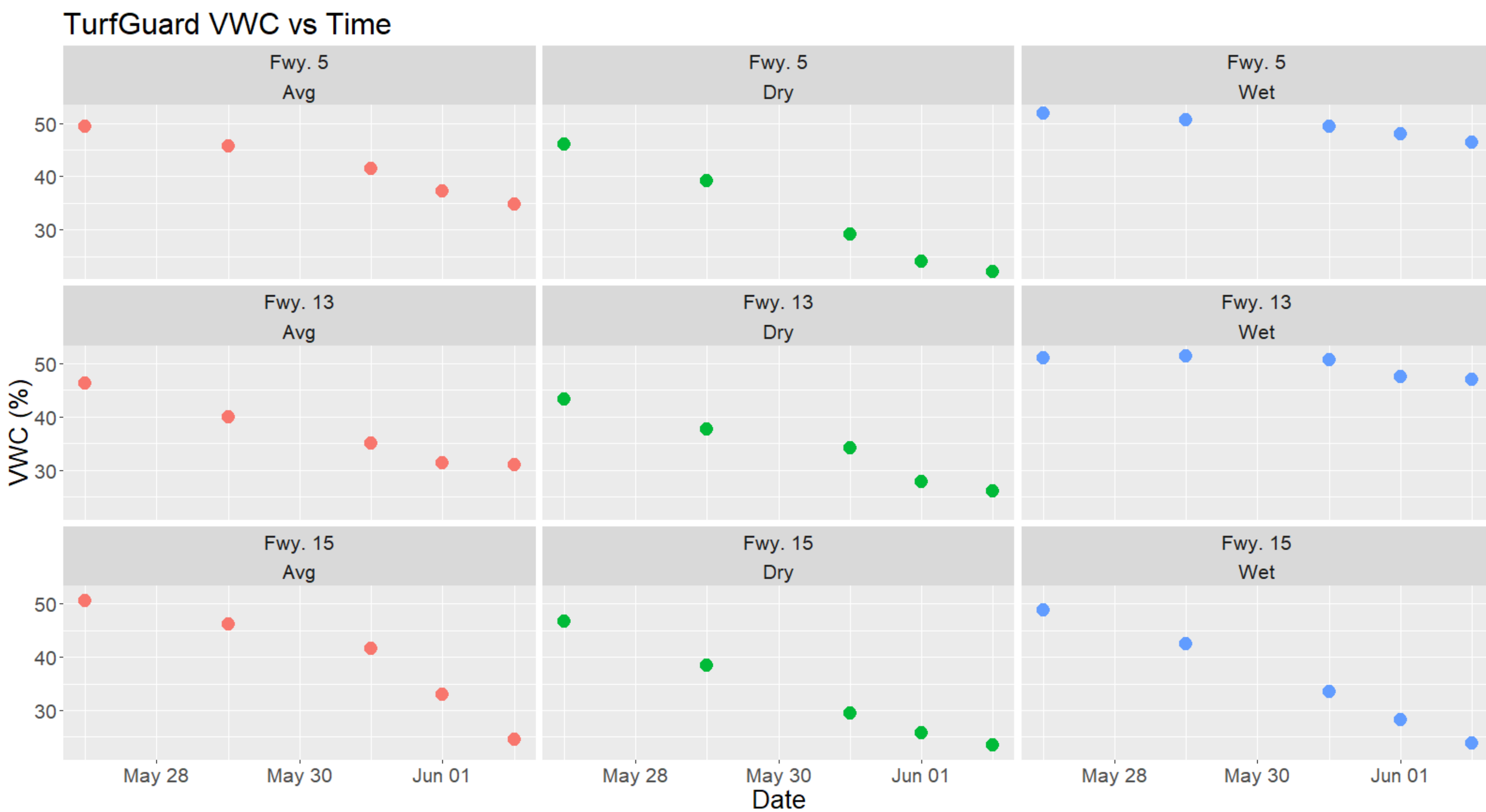


Figure 4. Mean, daily TurfGuard percent volumetric water content (VWC) value on fairways receiving the SMS-based irrigation treatment during a dry down event from May 27 – June 2, 2020 at Edina Country Club.

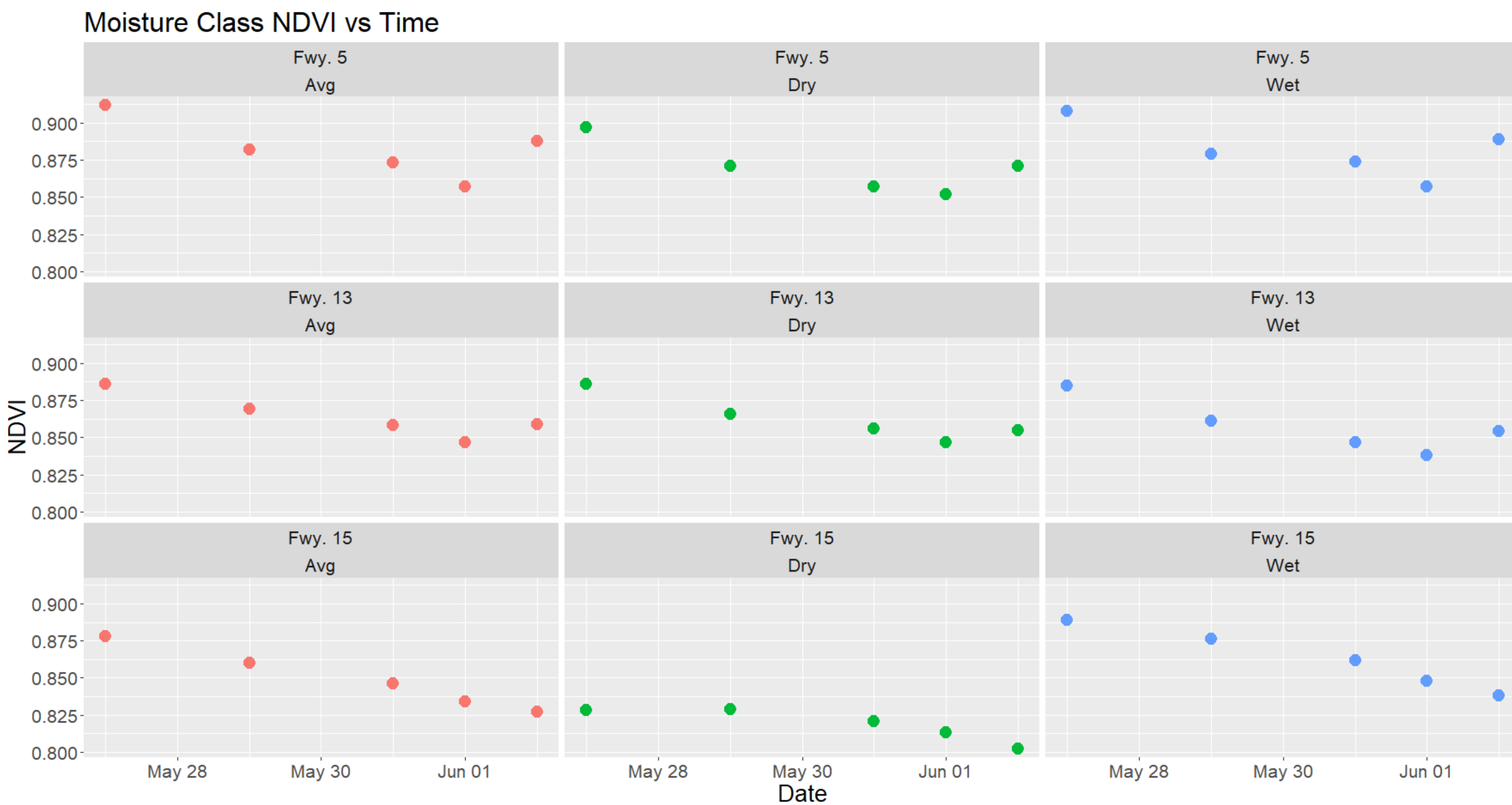


Figure 5. Mean normalized difference vegetation index (NDVI) as measured by the Precision Sense 6000 on fairways receiving the SMS-based irrigation treatment during a dry down event from May 27 – June 2, 2020 at Edina Country Club.

USGA ID #: 2017-38-648

Project Title: Integrating Canopy Dynamics, Soil Moisture, and Soil Physical Properties to Improve Irrigation Scheduling in Turfgrass

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

Start Date: 2018
Project Duration: 3 years
Total Funding: $129,733

Summary Points:

- Utilizing a decision tree for our decision-making process was an effective way to regulate irrigation scheduling.
- Soil moisture sensor-based irrigation yielded 84% water savings compared to traditional frequency-based irrigation and 72% savings compared to deficit irrigation (60% ET) approaches.

<u>Rationale</u>

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 components of the soil-plant-atmosphere continuum to generate better turfgrass irrigation decisions. We hypothesize that combining real-time soil moisture information, evapotranspiration (ET), and turfgrass canopy conditions will improve irrigation scheduling and reduce total water use relative to calendar schedules.

<u>Methodology</u>

Field research was conducted from June through September 2020 on 'Meyer' zoysiagrass (Zoysia japonica L.) at the Rocky Ford Turfgrass Research Center in Manhattan, KS. The objective was to integrate soil moisture and canopy information from in-situ sensors to determine turf canopy responses to soil water deficits and improve the timing and amount of irrigation events during the growing season. Treatment layout consisted of a Latin square design (8m x 8m plot sizes) comprising of four irrigation treatments: a traditional frequency-based irrigation (well-watered), a deficit irrigation based on reference ET (ETo), an irrigation based on SMS information, and a check treatment of zero irrigation (i.e., precipitation only). Soil moisture sensors (CS655, Campbell Sci. and TurfGuard, Toro) were installed at a 10 cm depth in the center of each plot. Eight normalized difference vegetation index (NDVI) sensors (SRS, Meter Group) were utilized to continuously monitor the turf canopy, and eight infrared radiometers (SI-111, Apogee) monitored canopy temperature of the turf. Aerial flights (eBee X) were conducted periodically to map the plot area with a multispectral sensor (MicaSense RedEdge MX). Various vegetation indices were explored for quantitative and qualitative evaluations of the turf canopy. Weather data were collected from an on-site Kansas Mesonet weather station to track air temperature, relative humidity, and precipitation throughout the growing season (https://mesonet.k-state.edu/).

Field Research (2020 Growing Season)

To carry out these objectives, we developed a simple irrigation decision tree for our decision-making process (Figure 1). A decision tree helps us determine a course of action by working through a list of questions. These questions centered around each of the soil-plant-atmosphere components. Triggering irrigation for the SMS-based irrigation treatments first depended on soil water content to determine if VWC reached a critical threshold range between 22% and 18%. This threshold range was based on estimated plant available water as determined from laboratory measurements of soil properties and greenhouse measurements of relationships between soil moisture and turf canopy performance. The second criteria centered around the health of the turfgrass canopy, to determine if green cover (GC) was within 5% of the traditional frequency-based irrigation treatments. Finally, we considered if the chance for rainfall was greater than 50% within 24 hours. As we processed the data along with these questions, we were then able to make our best-informed decision for irrigation.

Total precipitation for the duration of the 2020 study resulted in 334 mm. Supplemental irrigation for each of the treatments resulted in 330 mm for traditional frequency-based irrigation, 183 mm for 60% ET and 51 mm for the SMS-based irrigation plots (Figures 2 and 3). When comparing the SMS-based irrigation approach against ET-based irrigation scheduling, 72% water savings were achieved. When comparing the SMS-based irrigation approach against traditional calendar-based irrigation scheduling, 84% water savings were achieved. Utilizing various sensor technologies and a decision tree to guide our thought process yielded significant water savings. When comparing GC among treatments, the check (non-irrigated) resulted in 23 days when the GC fell more than 5% below the traditional frequency-based irrigation treatments, SMS-based irrigation treatments resulted in 2 days, and the 60% ET treatments resulted in zero days (Figure 3). This highlights how the turf canopy in the SMS-based irrigation treatments was not compromised throughout the season while achieving significant water savings.

During the 2020 growing season, two periods at end of June and August had a pronounced soil water deficit decline (Figure 4). These were the only periods the threshold for initiating irrigation in SMS plots was met, noted by the arrows in figure 4. Green cover slightly declined in SMS-based irrigation treatments when VWC reached the irrigation threshold but improved after irrigation, whereas GC continued to decline in the check treatments. NDVI measurements from aerial images on June-26 visually depict less green biomass in the check treatments (Figure 5). Other metrics such as forecasted ETo will be evaluated and considered to incorporate into the decision that may further irrigation savings and refine the decision-making process.

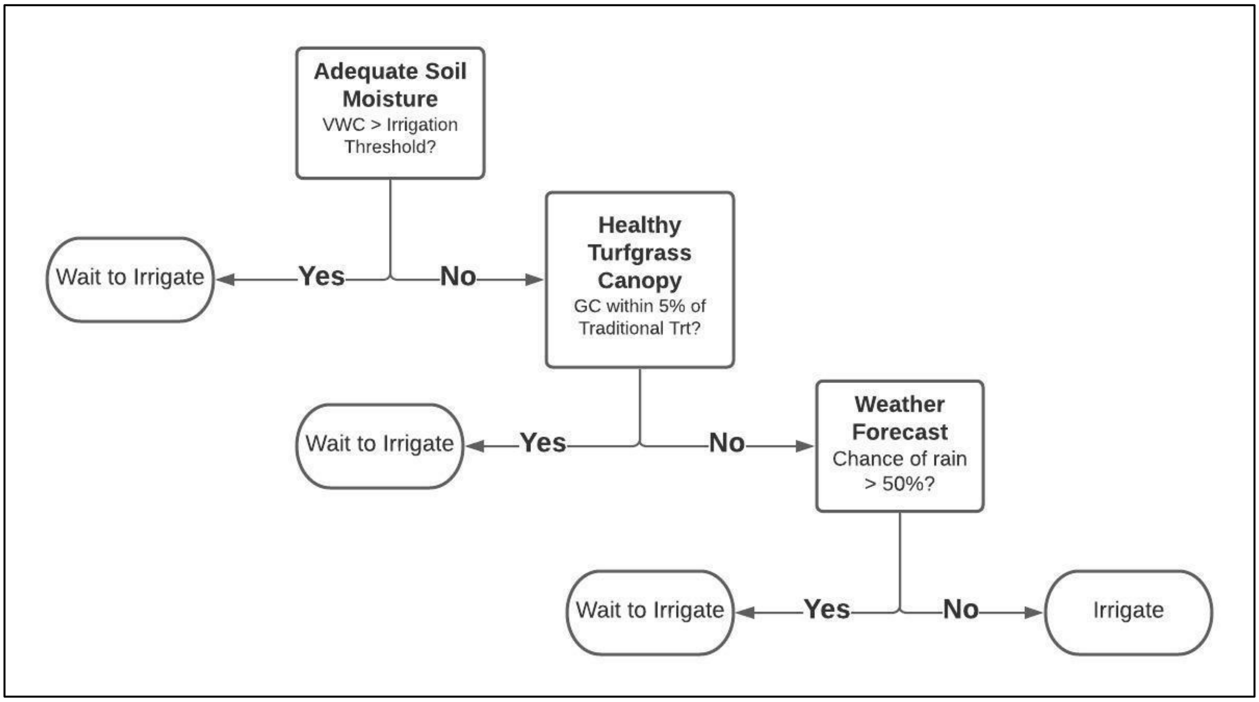


Figure 1. Decision tree sketch to determine irrigation scheduling.

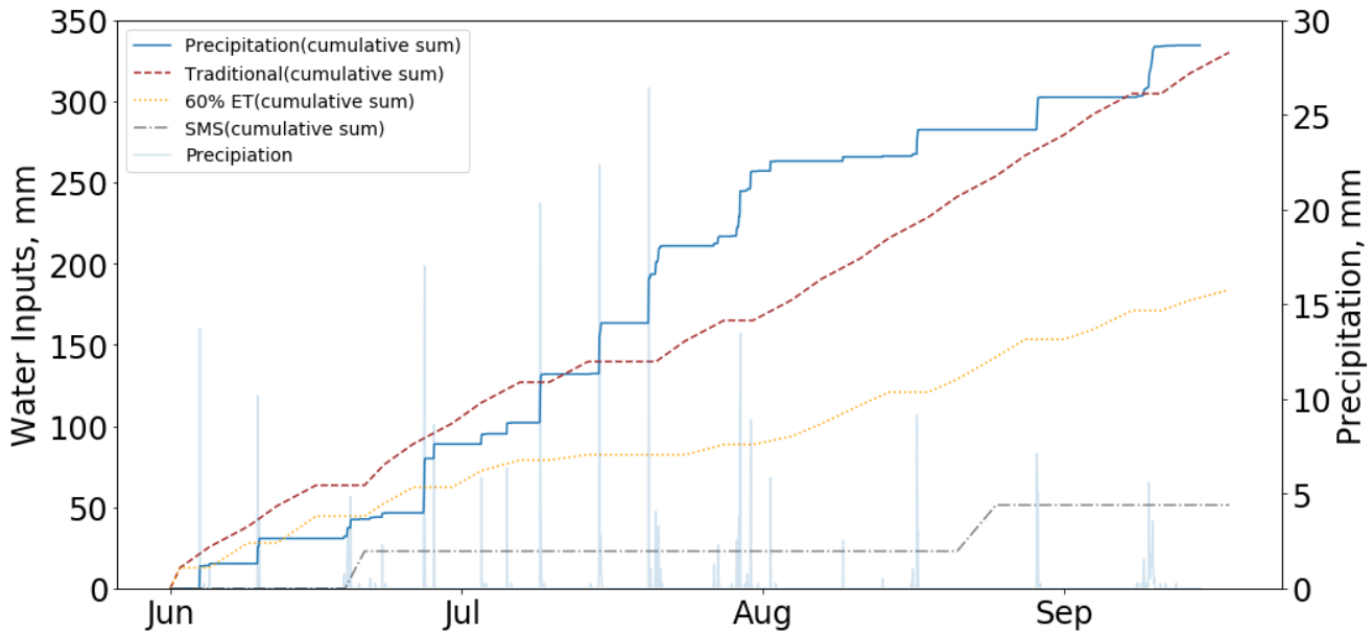


Figure 2. Precipitation and cumulative irrigation from the 2020 growing season.

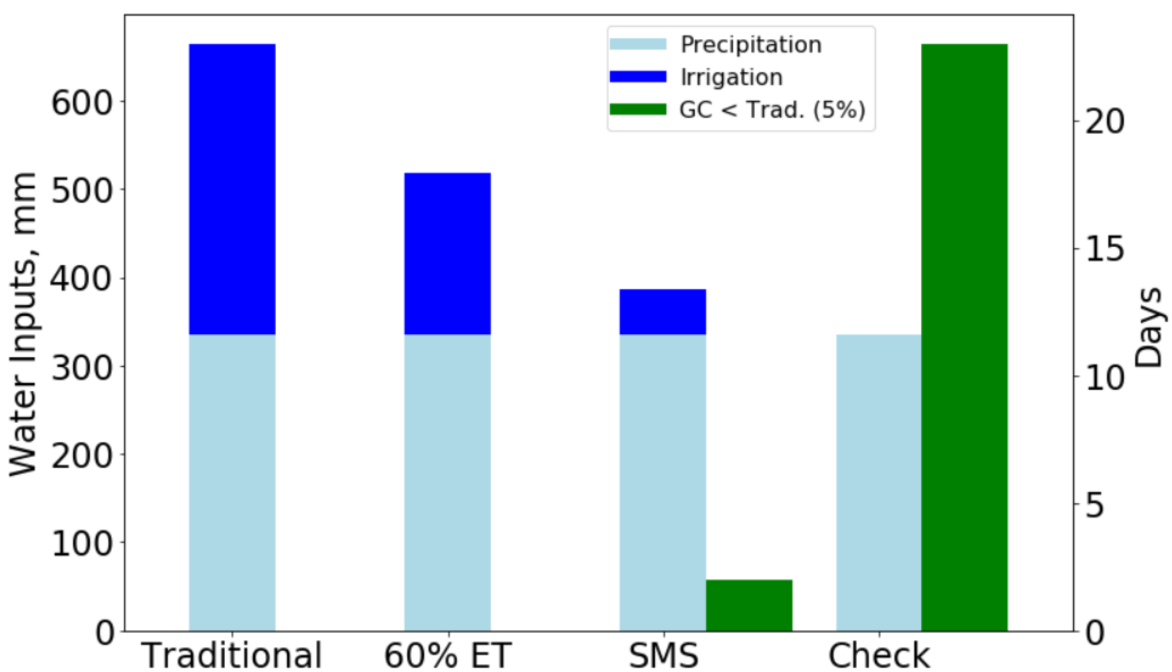


Figure 3. Total water inputs shown for each treatment from precipitation and irrigation. Total number of days shown where GC fell more than 5% below the traditional frequency-based irrigation treatments during the 2020 growing season.

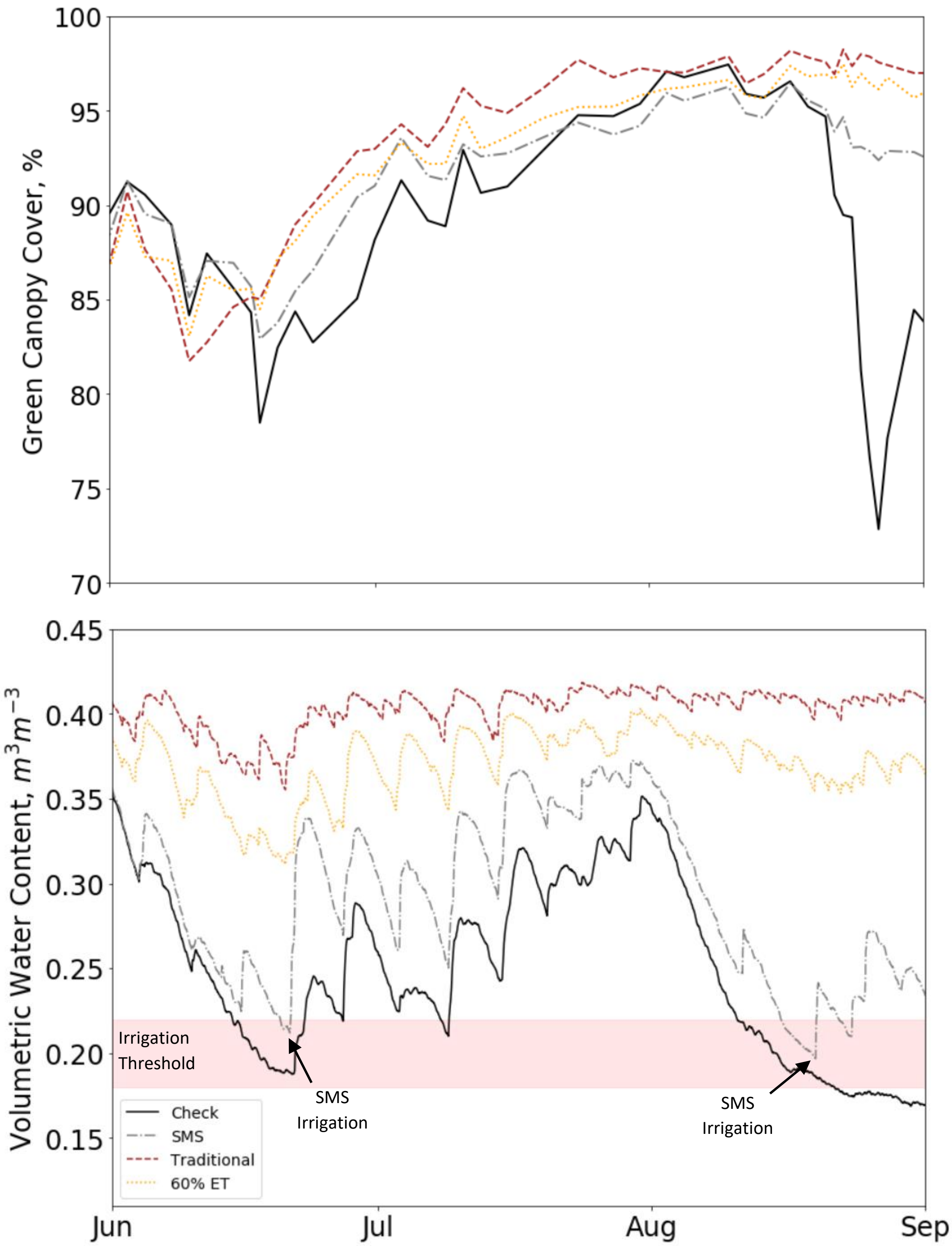


Figure 4. (Upper) Green cover percent throughout the 2020 growing season. (Lower) VWC at a 10-cm depth throughout the 2020 growing season showing irrigation threshold range. Arrows depict when irrigation was triggered.

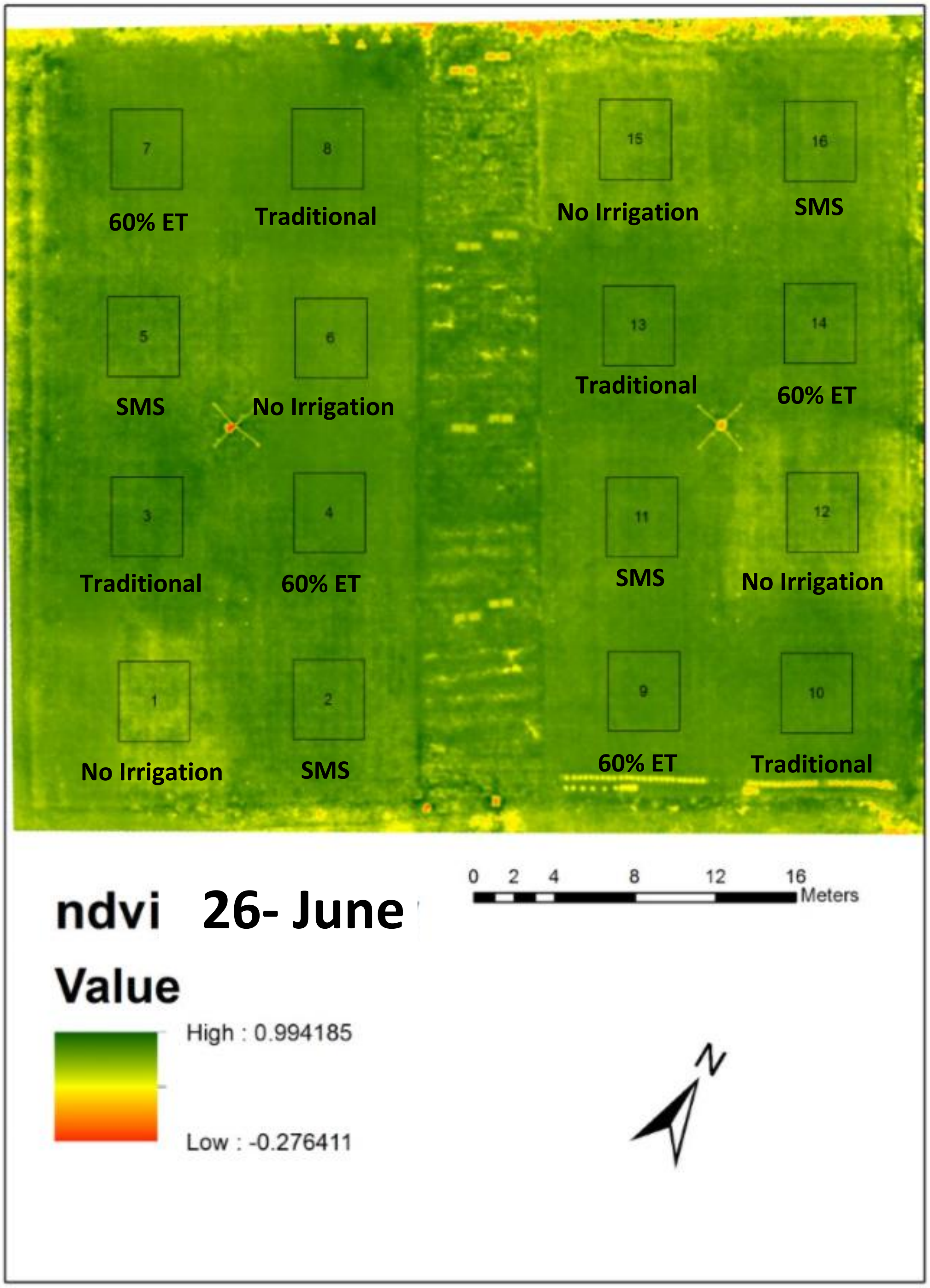


Figure 5. Aerial image of NDVI measurements 26 days after initiating treatments.

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:

- Results from 2020 support those of 2019 in that National Oceanic and Atmospheric Administration forecasted reference evapotranspiration (NOAA FRET) was a reliable predictor of onsite weather station Penman-Monteith reference evapotranspiration (ET_o).
- Similar to 2019, all irrigation scheduling treatments were able to maintain turf quality above acceptable thresholds throughout the 2020 season.
- No significant differences between irrigation scheduling treatments were observed for seasonal water use in 2020.
- No significant differences were detected for root development within the sand-cap or subsoil due to irrigation scheduling technique.

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 study were to evaluate turf performance and overall water use during the growing season using various irrigation scheduling techniques as well as determine whether the critical moisture threshold at which wilt occurs changes by season for application in SMS based systems.

Methodology

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

1) Wireless SMS

- Irrigation is applied based on 75% allowable depletion. Field capacity and permanent wilting was based on evaluation of wilt in relation to soil moisture in late May. (Fig. 1)

2) On-site Penman-Monteith ET_o

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

3) NOAA Forecasted ETo

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

4) Visual Wilt-based approach

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

Irrigation treatments are arranged in a randomized complete block design with 4 replicates and individual plot size of 21 ft x 21 ft. Plots are mowed 2-3 times per week at 0.5" height and fertilized with 0.75 lb N/ 1000 ft^2 every 3-4 weeks from May through September. Wetting agent (Aquatrols Revolution) is applied at the label rate every month during the growing season. Turf quality of plots is evaluated weekly using a 1-9 scale, with minimum quality = 5. Bi-weekly digital image analysis is performed using Turf Analyzer software (Green Research Services, LLC, Fayetteville, AR) (Karcher et al., 2017). Water usage is determined by utilizing a water meter installed at the valve of each plot. Toro Turf Guard ® Wireless SMS are placed in each plot to monitor volumetric water content (VWC). Each sensor has 2 sets of probes that monitor VWC

(%) at the upper portion of the sand-cap (3" depth) as well as the upper portion of the underlying subsoil (8" depth). In Visual Wilt-based plots, an additional sensor is positioned at a deeper depth for gaining greater spatial resolution, monitoring volumetric water content at the 6" sand-cap depth as well as deeper within the subsoil (11" 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 are subjected to ANOVA using the GLM procedure of SPSS (IBM, Inc.). Where appropriate, mean comparisons have been performed using Tukey's HSD ($P \leq 0.05$).

2020 Results

In the 2020 season, all irrigation scheduling techniques were successful in maintaining acceptable turf quality with no significant differences detected between scheduling techniques (Figure 1). Seasonal irrigation use ranged from 14.5" for FRET treatment to 16.4" for the SMS-based plots, however, no significant differences in water use were detected between scheduling techniques in the 2020 season (Figure 2). For root development, no significant differences were observed when comparing root mass within the sand-cap or within the subsoil between the various irrigation scheduling techniques (Figure 3).

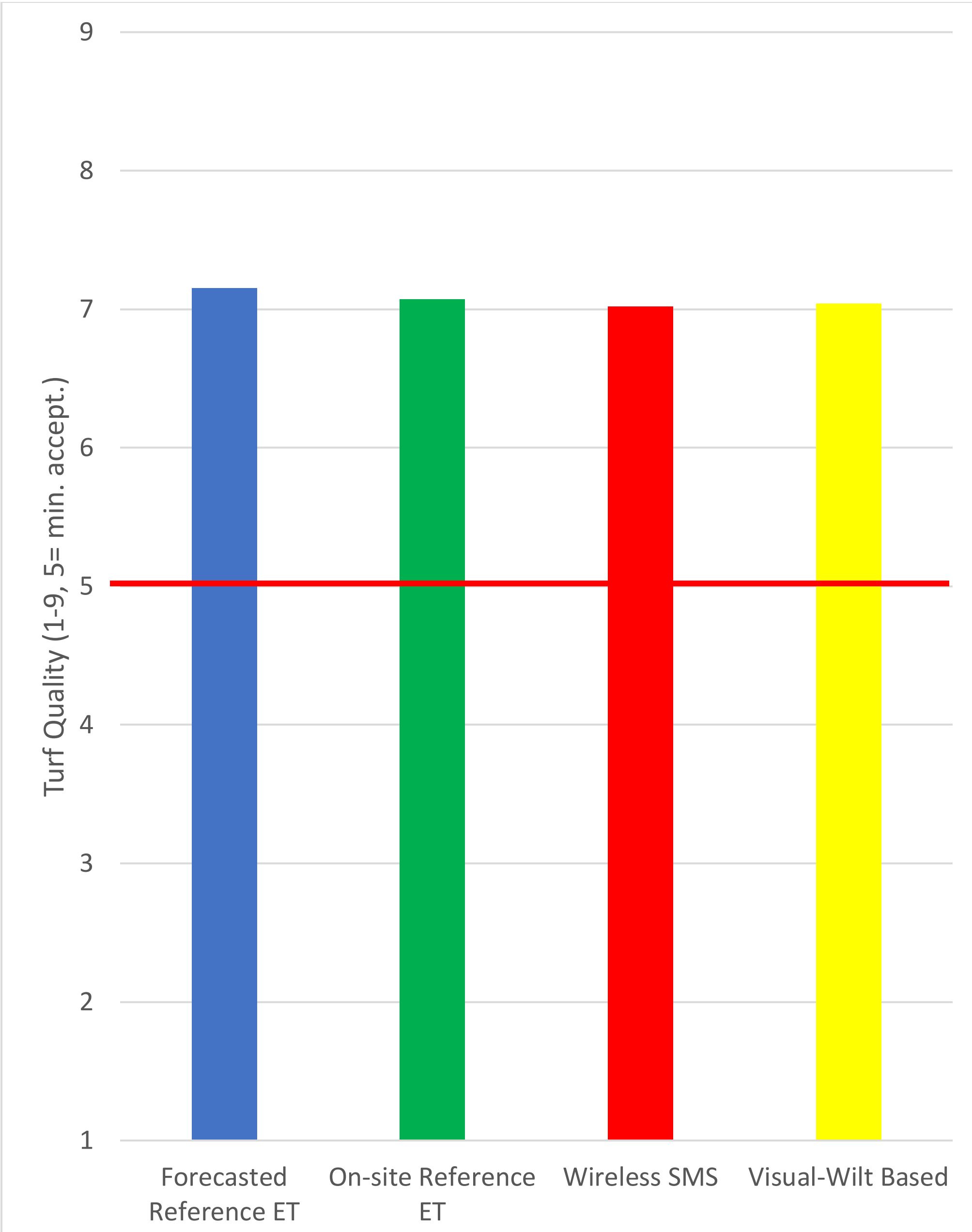


Figure 2. 2020 Visual turf quality as affected by irrigation scheduling treatment. Data were pooled across all rating dates. Horizontal red line indicates minimally acceptable turf quality.

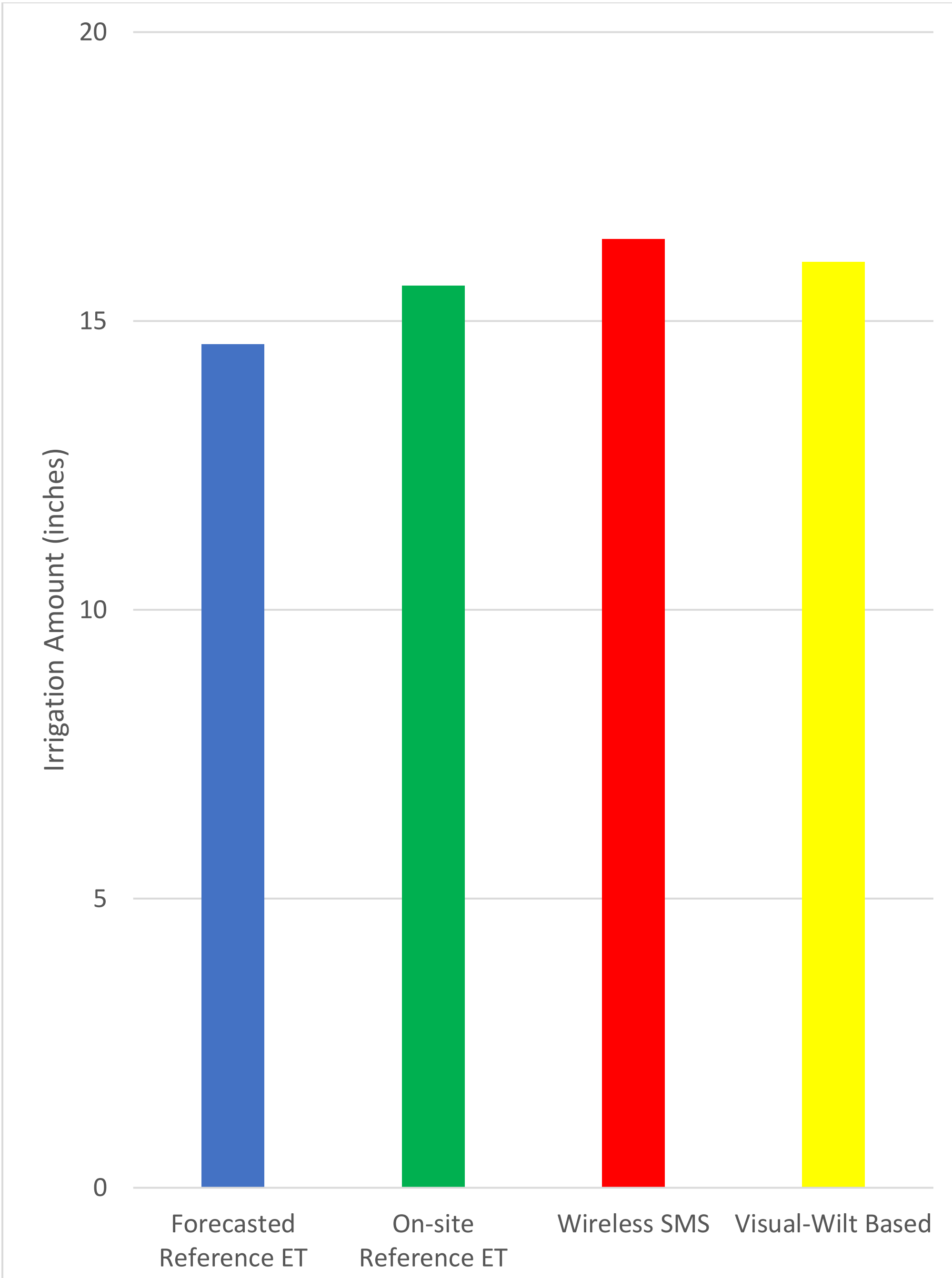


Figure 2. 2020 seasonal water use for each irrigation scheduling treatment. Data were derived from difference between water meter readings at trial initiation and end of season.

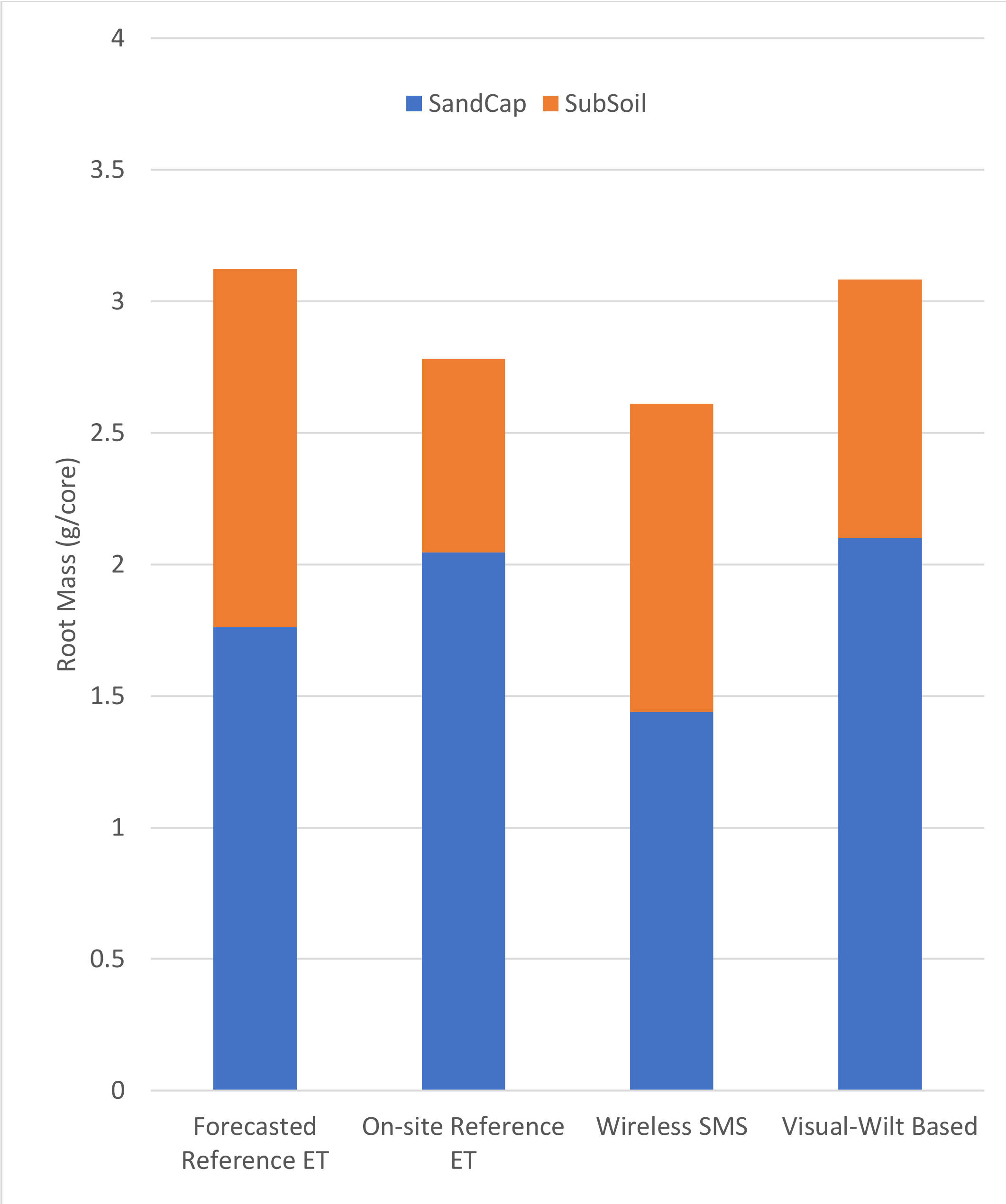


Figure 3. Root mass (grams per core) within sand-cap and subsoil fractions for each irrigation scheduling treatment. Root samples were harvested from plots in November 2020. There were no statistical differences in sand-cap, subsoil, or total root mass between irrigation treatments.

Figure 4. 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#: 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 a turfgrass recycled water irrigation field research trial and economic analysis.

Start Date: 2020
Project Duration: 3 years (year 1 of 3)
Total Funding: $120,000

SUMMARY POINTS:

- An irrigation research trial was conducted at the University of California South Coast Research and Extension Center to study the response of bermudagrass (*Cynodon dactylon*) turfgrass species to 12 recycled water irrigation treatments.
- We observed a good response from the smart controller to schedule irrigation based on the implemented soil moisture thresholds.
- Preliminary results suggest that the lower soil moisture threshold can be reduced to 65% of field capacity from the recommended 75% without much decline in the turf quality.

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 where rainfall is exactly opposite of the humid regions studied for best practices. Previous studies had focused on the implementation of smart landscape irrigation controllers when potable water, not recycled 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: An 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). To eliminate the plot edge effect and avoid interference between adjacent plots, adequate borders (2-3 feet) were considered, and measurements were taken at the center of each plot. Soil samples were collected twice from 4 different depths (0-24 inches with 6-inch increments) within and below the active turf root zone from all plots to study salt accumulation within the root zone due to the application of recycled water. The salinity of recycled water was monitored continuously to determine the degree of season-to-season variations. Turfgrass performance and quality subjected to 12 replicated treatments were monitored weekly using NDVI and canopy temperature handheld sensors from May to September 2020.

The field capacity of the soil was estimated as the soil moisture in the root zone ~12-24 hours after a heavy rainfall prior to the experiment. Two moisture level limits are very critical in making irrigation decisions using the controller used in this study. The soil moisture level where the smart controller will trigger irrigation if the moisture level in the root zone drops below that level (the lower level).The soil moisture level where the irrigation is shut off if the moisture level exceeds this limit (the upper level). The typically recommended levels are usually field capacity as the upper limit and 75% of field capacity as the lower limit.

Economic analysis: Our initial assessment revealed that a comprehensive literature review is missing from the academic work on the economies of water management in the golf course. To fill the gap, we performed a literature review of the studies implemented in the US and beyond, focusing on scientific knowledge, practical recommendations, and tools for efficient golf course irrigation and water management through turfgrass irrigation field research trials as well as economic analysis. A related strand of such literature takes a comprehensive approach starting from examining existing water conservation technologies and management strategies at golf courses to economic and statistical methodologies. Our findings indicate that we can explore multiple data sources to analyze the preferences and perceptions of water use according to weather and regional characteristics (see appendix A). Next, we have collected the necessary data on the type of technologies, water use, locations, and irrigation requirements for model calibration. We have also collected historical aggregate water use data to estimate the primary determinants of technology adoption and water use changes over time.

Results to date:

Irrigation uniformity test was performed on the plots following the ANSI/ASABE S626 standard method, which resulted in DU_{LH} and CU values of 0.85 and 85%, respectively. We observed a good response from the smart controller to schedule irrigation based on the implemented thresholds. The average daily EC of the irrigation water ranged from 0.92 to 1.74, with an average of 1.26 dS/m in 2020. The soil salinity ranged from 0.45 ds/m to 1.56 ds/m prior to imposing the treatments and from 0.52 to 3.90 ds/m after the experiment. The soil salinity increased over the study period, especially in the shallow soil depths indicating the accumulation of salts due to lack of leaching. It is expected that the leaching of salts from the soil root zone will occur during the rainy season, which will become more clear after analyzing the soil salinity data next year. The handheld data collect toward the end of the study in September showed that the lowest average NDVI value of 0.25 belonged to 55%-80%FC treatment (3 days/week frequency).

The highest NDVI values of 0.51 belonged to 65%-90%FC and 75%-100%FC treatments (3 days/week frequency).

Average infiltration rates were measured using SATURO automated infiltrometers. Sodium Adsorption Ration (SAR) of the soil samples was also measured in the lab. Canopy temperature data were also collected, which might give a good insight into the impact of turfgrass and different irrigation strategies on the urban heat island effect. These results are yet to be analyzed and since not presented in this report.

Future expectations of the project:

We will conduct the second year of the irrigation trial next year and focus more on the impact of irrigation treatments on soil infiltration rate and salinity in the root zone. We have developed a survey programmed in Qualtrics, and as the next step, we will be further developing the list of questions. We are also in the process of obtaining the IRB approvals from UCR. The survey is in the last steps of the development, and we are planning to run it in 2021 among golf courses.

Figure 1. Top: Automated infiltrometers are being used to measure the potential impact of varying irrigation rates using recycled water on infiltration rate (left). An overview of the research plots (right). **Bottom:** Graduate students are conducting a flow and catch can test (left). An overview of the smart soil moisture-based irrigation controller used in this study (right).

Table 1: Treatments imposed with the soil moisture sensors in the instrumented plots.

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.

Appendix A: Literature Review Summary (economic analysis)

Source	Purpose	Data/Method	Results
Throssell et al. (2009)	This study provides an accurate portrayal of golf course water use to guide industry agronomic and environmental initiatives and establish a baseline compared to data from future surveys to monitor industry change.	The data for 2003, 2004, 2005 on water-use and conservation survey is used to determine their number of irrigated turfgrass acres, water use, water cost, water sources, recycled water use, water quality, irrigation system characteristics, and water management and conservation strategies. This study takes cost-effectiveness analysis. A total of 2,548 completed surveys were returned from 16,797 golf facilities in the United States, yielding a 15% return rate.	Golf courses comprise an estimated 1,198,381 acres of irrigated turfgrass in the United States, and their total annual water use averaged over 2003, 2004, and 2005 is estimated at 2,312,701 acre-feet.
Baris et al. (2010)	This study evaluates available golf course water quality data and the extent of impacts, as determined by comparisons with toxicologic and ecologic reference points.	This study conducted a meta-analysis of the data with previously obtained water quality monitoring data from 17 studies of 36 golf courses throughout the United States and Canada. This study takes a statistical approach using the time-series analyses based on the pre-1998 data compared with the post-1997 time period.	The time-series analyses for nitrate-N (pre-and post-1997) showed fewer nitrate-N detections and fewer golf courses with nitrate-N detections pre-1998 (including 1997) compared with the post-1997 time period.
Watson and Thilmany (2011)	This study examined the water use and conservation practices on Colorado golf courses from 2000-2002, the economic impact of golf courses, alternative uses of water, water cost, and water-use policy in Colorado.	The data from the 2006 water-use survey is used to create a mathematical simulation model for golf course operations in Colorado to compare all golf courses' economic effects, adopting a marginal cost for water and the effects of imposing a water quota on Colorado's golf courses.	This study indicates that Colorado's golf courses are engaged in water conservation techniques and generate significant economic return per irrigated acre.

(continue)

Source	Purpose	Data/Method	Results
CSAA (2015)	This paper provides key information about management practices related to golf courses in light of regulations pertaining to water use, drought restrictions, and water quality protection.	This paper uses two survey data on the golf course in the United States: 1. The first characterizes water use and conservation practices for the typical 18-hole golf course; and 2. The second characterizes the survey information to make projections about water use and conservation practices for the nation's golf courses as a whole. This study takes a statistical and calibration approach to deliver specific information.	Regional water use related golf courses—variation in water use, median water volume used per 18-hole golf course—because of differences in climate, the number of facilities per region, the number of irrigated acres per golf facility, and length of the turf growing season (i.e., year-round turf growing seasons as Southeast and Southwest regions; and the shortest growing seasons as Northeast, North Central, and Northern portions of the Pacific region)
Gelernter et al. (2016)	This paper summarizes the results of the water use survey, trends in the use and conservation of water on golf courses over the past 8 years; thereby, identify where progress has been made and suggest areas needed for further research, education, and coordination in term of future efforts in conservation.	Data from both 2006 and 2014 water use surveys to the 15,386 golf facilities in the United States is used. This study takes a statistical and calibration approach and for the calculation of water use and acreage values.	Water is an increasingly limited resource that the golf course industry needs to manage and conserve effectively. Thus, they conclude the following measures: 1. using proven strategies for reducing water use; 2. adopting a metric for monitoring water use efficiency: 3. installing water meters; 4. Increasing golfer education, and 5. working with regulators to identify reasonable water conservation measures.
Scott, Rutty, and Peister (2018)	This paper examines golf course characteristics that influence water use variability (e.g., dominant soil type, ownership type, and age of course). This paper also explains the influence of climate variability on water use by comparing a climatically normal season (for the 1981–2010 period) with an anomalously dry and warm season to capture the potential impact of future climate change on water use (i.e., reduced precipitation and higher temperatures).	This paper uses a sample of 129 golf courses from Ontario (Canada) for the period 2007–2012 and optimization approach.	There were differences in water use between the regular and dry season varies substantially between individual courses. On a seasonal basis, average water use per course during the climatically regular season was 59.6 million liters, which increases 58% to 94.2 million liters during the exceptionally dry season. Notably, water use during the dry season increased over 200% at 17 courses, with an additional four courses that increased water use by over 400%.

References

Baris, Reuben D, Stuart Z Cohen, N LaJan Barnes, Juleen Lam, and Qingli Ma. 2010. "Quantitative analysis of over 20 years of golf course monitoring studies." *Environmental toxicology and chemistry* 29 (6):1224-1236.

CSAA, U., 2015. Water use and conservation practices on US golf courses. *Golf Course Environmental Profile*, *Phase*, *2*(1), pp.2-30.to

Gelernter, Wendy D, Larry J Stowell, Mark E Johnson, and Clark D Brown. 2016. "Documenting trends in nutrient use and conservation practices on US golf courses." *Crop, Forage & Turfgrass Management* 2 (1):1-10.

Scott, Daniel, Michelle Rutty, and Carly Peister. 2018. "Climate variability and water use on golf courses: optimization opportunities for a warmer future." *Journal of Sustainable Tourism* 26 (8):1453-1467.

Throssell, Clark S, Greg T Lyman, Mark E Johnson, Greg A Stacey, and Clark D Brown. 2009. "Golf course environmental profile measures water use, source, cost, quality, management and conservation strategies." *Applied Turfgrass Science* 6 (1):1-20.

Watson, Philip, and Dawn Thilmany. 2011. "Water-use efficiency in golf: An economic analysis of Colorado golf courses." *Golf Course Management*:88.

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

Project Objectives

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

Start date 2017 **Project duration** 4 Years **Total funding** $89,862

Summary of Activities

Measurements of ET

The research is conducted at the Eagle Lake Golf Course near Layton, UT, about 25 miles north of Salt Lake City. The general region around the course is a mixture of urban, residential, and some agriculture. Measurements include standard weather data, available radiation energy, heat flow into/out of soil, soil moisture and the fluxes of heat and water vapor (ET) from the surface. An image of the station and instruments is shown in Figure 1.

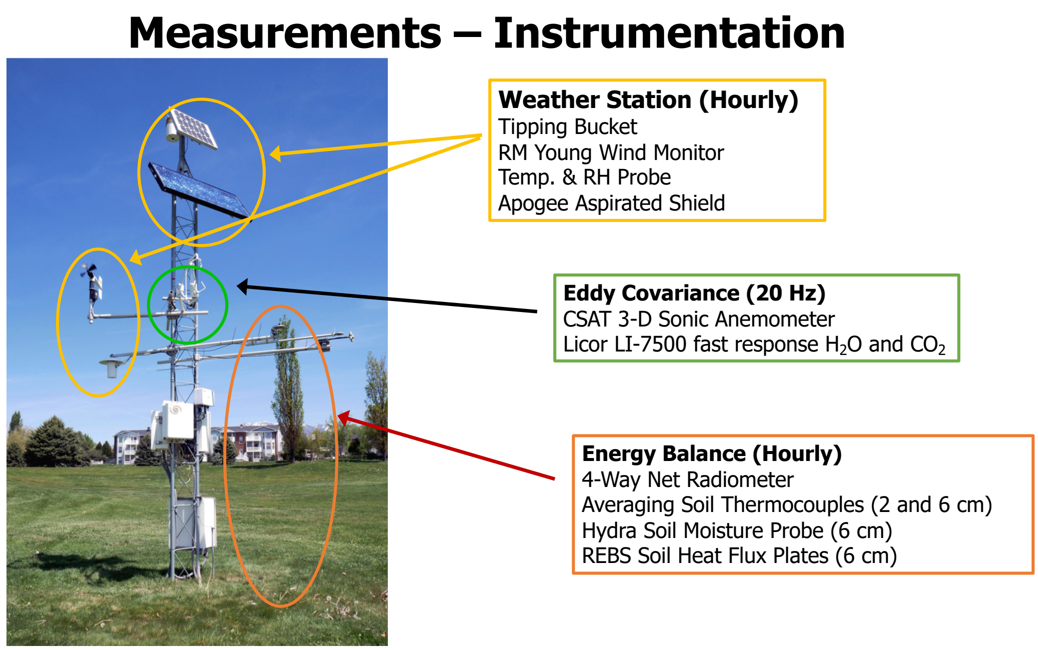


Figure 1. The eddy covariance and weather station.

ET From Eddy Covariance

Energy Balance Closure

The results from the eddy covariance and available energy measurements can be combined to check the internal consistency of the set of estimates. This is done by determining how well energy is conserved. The simple equation for the sources and uses of energy is:

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

Where R_{net} is net available radiation at the surface (radiation sources minus losses), G is soil heat flux, H is heat flow between the surface and air above, and LE is energy used in evaporating water (ET). In a perfect world where all measurements were ideal, the energy used would equal the energy available.

$$\frac{H+LE}{R_n-G} = 1 \quad (2)$$

Generally, eddy covariance values for H and LE are lower than available energy (R_{net} – G), since any errors reduce a covariance. The energy balance closure we obtained was rather good compared to most of the published literature. An example of two periods in 2018 is provided in Table 1.

Day of Year	Daily Closure
169	0.79
170	0.88
171	0.76
172	0.69
173	0.81
224	0.95
225	0.91
226	0.93
227	0.91
228	0.82

Table 1. Daily energy balance closure values for two sample periods.

The final daily ET values were obtained by "forcing the energy balance closure" as is commonly done if available energy measurements are of good quality. This means energy is added to the H and LE terms according to their ratio (H/LE), so that equation (2) is valid.

Daily ET Values

The daily ET values are shown for 2018 below in Figure 2. For a brief period in July, the winds came from an unusual direction so that data could not be analyzed directly for these periods. A published gap filling technique can be used to fill in these days.

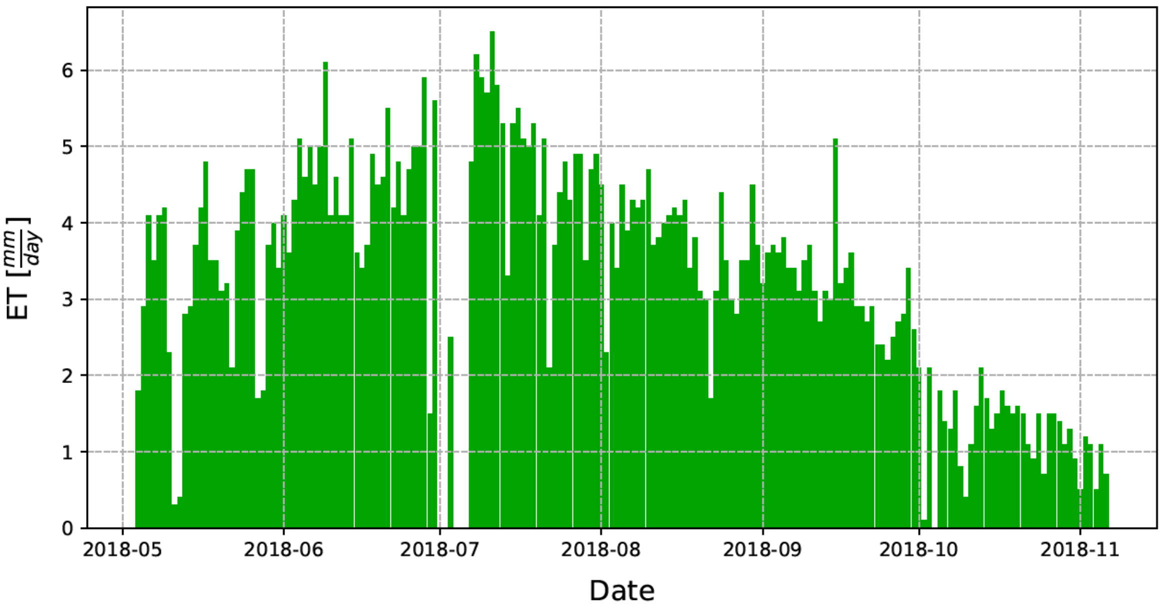


Figure 2. Daily ET values for 2018.

Note that the daily values range from about 4 mm (0.157 in) in late spring and late summer, to maximum values in mid-summer of about 6 mm (0.236 in). By autumn, they reduce to values of about 2.5 mm – 3 mm (0.985 in – 0.118 in).

Testing Reference ET Approach

A relatively simple and traditional approach to estimate ET is an empirical one based on reference ET. This is defined as the ET that would occur for an idealized surface of green vegetation, fully covering the ground, well-watered, and having a very high stomatal conductance. This can be estimated from weather data and available energy measurements using the Penman Monteith Equation. To get actual ET, this value is multiplied by a coefficient. A major problem of this approach is that the coefficient is not generally constant and varies with changes in the pants responses to environmental conditions.

It has been proposed that for irrigated turfgrass the value of about 0.8 be used, despite no rigorous measurements to back up the claim. We could test this approach with our results. Figure 3 shows the value of the reference ET coefficient each day for the growing season of 2018. The red line in the figure below denotes the 0.8 value.

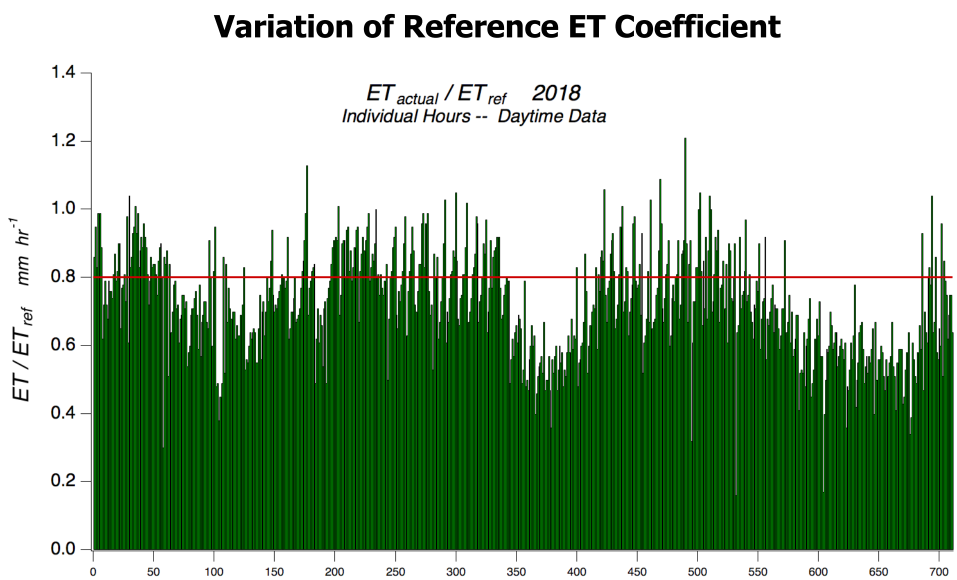


Figure 3. Daily values of coefficients for Reference ET

It is clear that the value of the coefficient is highly variable from day to day. It varies from about 0.5 to 1.0. Even if one chose to use the average of the daily values (questionable), the 0.8 value is not very accurate. The average of the daily values is roughly 0.65. It would seem this approach is not very trustworthy for use on irrigated turfgrass at this time, at least in the semi-arid environment of Utah.

Response of Turfgrass to Changes in Atmospheric Conditions

An example of the energy balance, aerodynamic and stomatal resistance and response to saturation deficit on a typical summer day is shown in Figure 4.

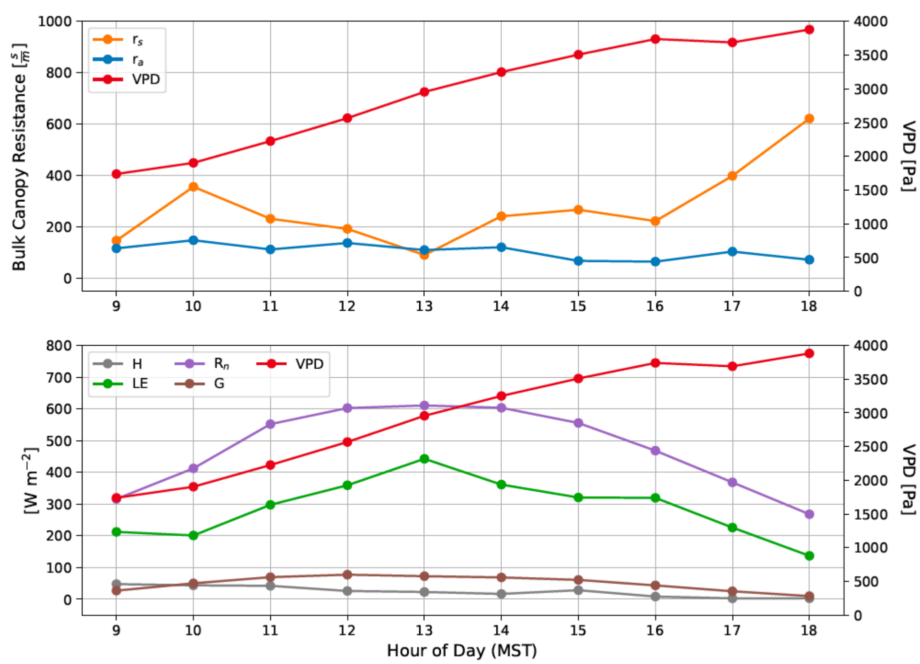


Figure 4. a. Changes in aerodynamic and stomatal resistance in response to vapor pressure deficit.
b. Energy balance terms during the day.

The aerodynamic resistance is a measure of the inability of turbulence motions to allow transport of heat and water vapor. It is the blue line on the upper graph. The stomatal resistance is the bulk value or collective average of the stomatal resistance of the foliage of the canopy, and is represented by orange line. The saturation deficit (VPD) is simply the saturation vapor pressure minus the actual vapor pressure. ET responds to VPD, and in most plants so does stomatal resistance. One can see that during the afternoon, when VPD grows large enough, the stomatal resistance increases. The turfgrass is partially closing the stomates in response to the high VPD. It does exhibit some response to the higher ET “demand”, by limiting the transpiration.

ET Estimates from Remote Sensing Models

The annual report from 2019 described the *triangle method* to estimate ET. Recall, this method looks at relationships between surface temperature and NDVI for a region of landscape that includes drier and wetter surfaces. The land cover classification of the region used is shown in Figure 5.

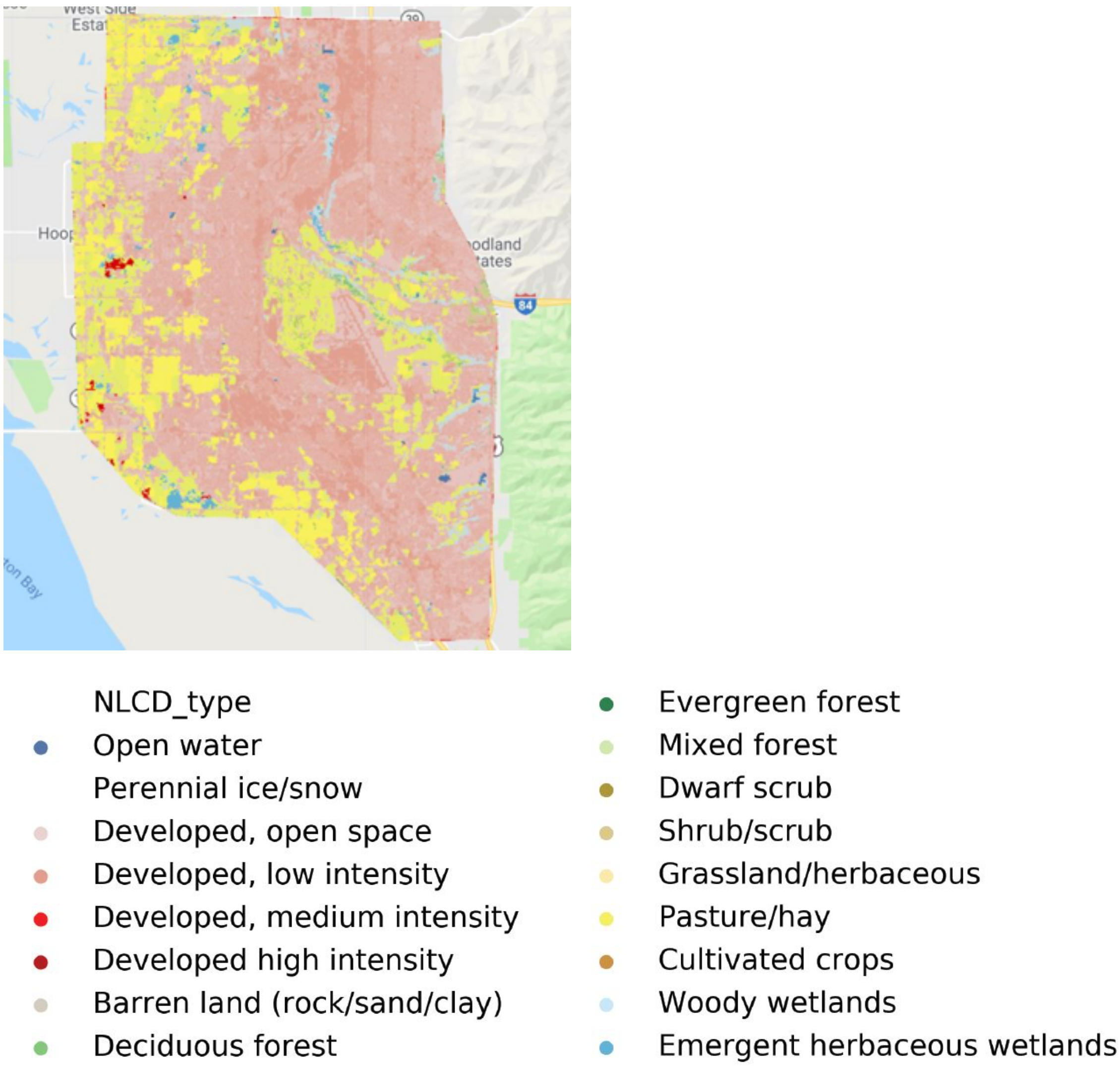


Figure 5. Land cover classification from NLCD Dataset

The triangle model outputs the Evaporative Fraction (EF), or ratio of ET to available energy for each element of the surface. The EF values for the region surrounding the golf course are shown below in Figure 6. The darker greens denote larger ET, while the lighter colors a low Et due to surfaces that have little vegetation.

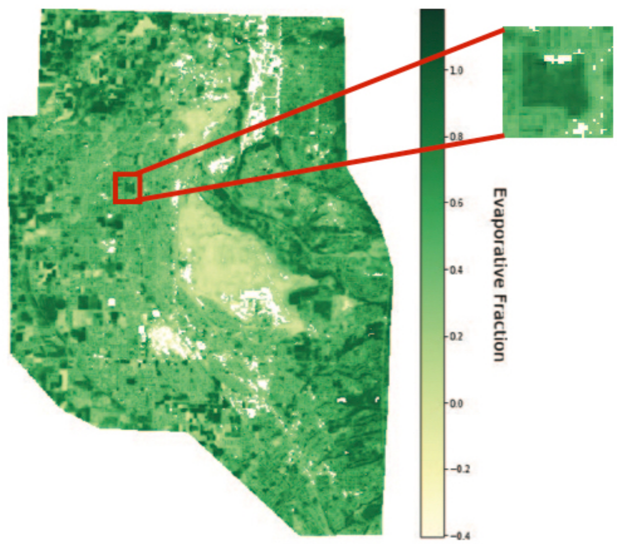


Figure 6. Example of evaporative fraction over region, with magnification of golf course

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

The performance of the triangle method was evaluated by comparing the estimates to the eddy covariance measurements of ET. The relationship is shown in Figure 7, and the agreement is reasonably good at the low to middle range of ET, where model estimates are within the uncertainty of the eddy covariance values. However, there is underestimation of the model at very high ET values.

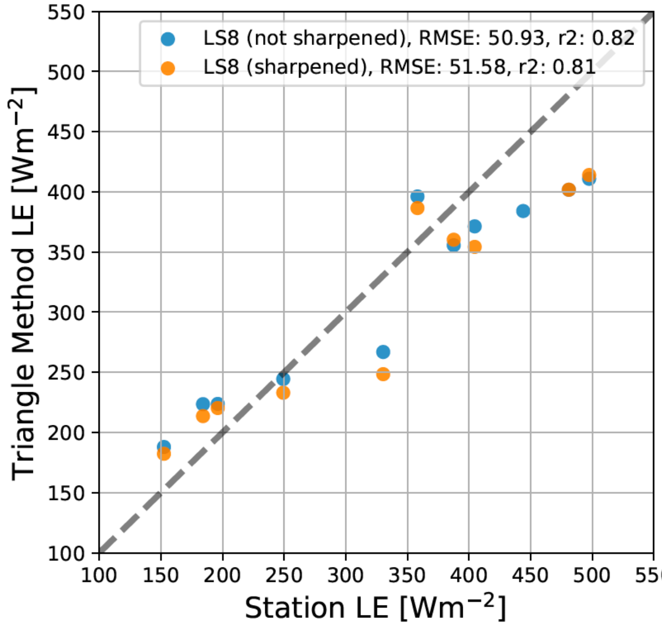


Figure 7. Model vs. measured ET values for original and sharpened surface temperatures

Plan of Work 2021

A new graduate student has arrived who will be co-advised by Lawrence Hipps and Alfonso Torres-Rua of Civil and Environmental Engineering. She has an M.S. from Texas A&M some background in urban water, and ET of irrigated turfgrass and this project will be a major part of her program. Another seasonal data set is planned for summer 2021.

Remote Sensing Models of ET

There is now the ability to use a number of remote sensing ET models in an easier way, thanks to a new platform called Open ET. It merges resources from NASA, Google Earth and other institutions. A number of modern remote sensing models are run by the platform, and users can get results for a given region. The golf course is relatively small, so we will have to request the high spatial resolution outputs.

https://openetdata.org

Evaluation of Models

We will use Open ET to acquire all ET estimates available for the growing season. Then the eddy covariance results can be used to evaluate the efficacy of the various models. Which one works best, and how well they estimate actual ET will be determined. Then by gap filling the values between the remote sensing results, weekly, monthly and seasonal ET estimates can be made and compared against the measured values at the site.

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. Optimize the best technology for ease of transfer to the golf industry and quantify water savings and implement precision turfgrass management practices

Start date 2018
Project duration 3 years
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, but there has been more research conducted demonstrating the benefits of site-specific management practices or pesticide applications. The overall goal of this project is to test various spectral sensors collecting images of golf course fairways to assess variability from aerial and ground truth measurements that can promote site-specific management to reduce water inputs on cool- and warm-season golf course fairways.

Methodology

UAV Flights and 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.

Ground-truth Data with Flight. 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).

Determining variations in plant available water in golf course fairways. Soil samples obtained as described above were placed in a pressure plate apparatus to identify water content at field capacity and permanent wilting point. Plant available water (PAW) determined from each sample was then used to develop spatial variability maps of PAW and correlate these data to other soil physical properties analyzed.

Results to Date

UAV Flights and Data Compilation. All flights for image capture were completed in 2018 and 2019. All images were stitched and compiled for analysis and conversion to NDVI using the various spectral bands from individual cameras. Preliminary results from these flights appear similar to previous reports with little apparent differences between sensors used for NDVI determination. We have overlayed the ground truth points over these flights to begin the comparison and correlations of passive aerial imagery calculations to those ground measurements (Figure 1). The comparison to soil parameters will be incredibly beneficial and a novel approach compared to current literature.

Ground-truth Data with Flight. Ground measurements were obtained in close proximity to later flights in 2018 and 2019 in both locations. Most soil analyses were completed prior to the COVID-19 shutdown, but there are still some outstanding data that has not been completed because of the forced closure of campus and labs. However, these missing data points are being systematically completed in order of least measurements remaining to maximize production as we complete this project. We have made great strides in streamlining the krigged mapping process to develop cleaner figures only showing fairways and reducing the color legend (Figure 2). There have been more recent publications describing drought recognition vegetation indices in cool- and warm-season turfgrass species, but we have not seen any information depicting variability in the soil physical properties and water characteristics measured in this study.

Determining variations in plant available water in golf course fairways. Our ability to map variability in PAW throughout these fairways and correlate those measurements to other ground-truth data and aerial imagery is an innovative technique. The novel attribute to these PAW measurements are the lack of influence to outside sources (climate, management, rainfall, etc.) typically affecting other ground and aerial data acquisition. Currently, there are krigged maps completed for PAW in the 0-5 and 5-10 cm depths of one fairway (Rawls GG #14; Figure 2). A Pearson correlation analysis was conducted to determine relationships of PAW to other ground-based measurements. Plant available water in the upper 5 cm depth was positively correlated (P-value < 0.05) with organic matter (0.526), clay (0.335), and bulk density (0.375). The positive correlation with bulk density was not expected, but reduced pore space from compaction may be increasing water held at field capacity. These same calculations will be made for the other fairways as all soil data analyses are completed.

Summary points

- NDVI calculations using these independent sensors do not seem effective at differentiating drought stress in golf course fairways
- Variability maps of laboratory measured plant available water were developed
- Moderate correlations between plant available water and soil physical properties (organic matter, clay, and bulk density) were documented
- Correlating soil physical properties to drone imagery NDVI will be a novel approach to assist with soil moisture variability and may enhance site-specific management designations
- Results from these analyses are expected to dictate site-specific management practices that can be targeted to specific zones to maximize frequency and benefit of management

Figure 1. Aerial NDVI calculated image with ground-truth GPS-coordinate points overlayed on the golf fairway. Buffers created around each point will be used for comparisons and correlations from aerial to ground-based data assessments.

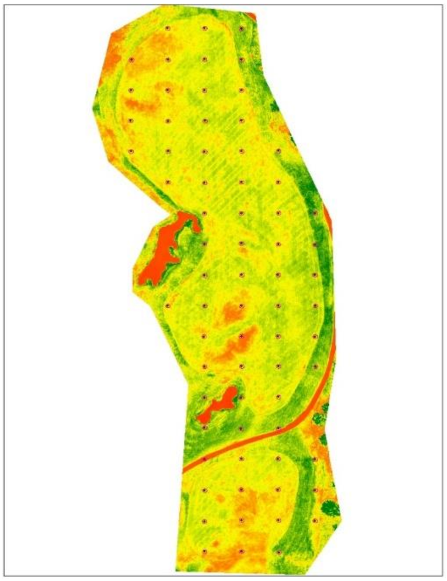
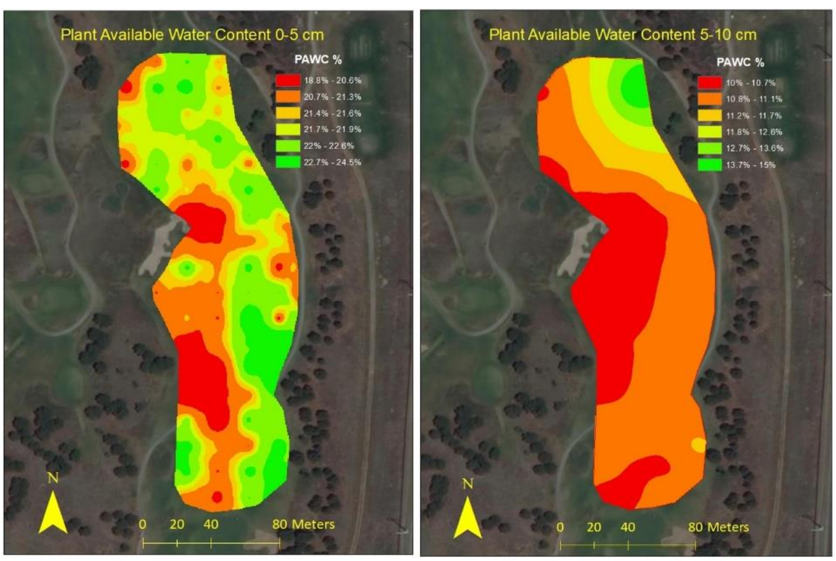


Figure 2. Spatial variability maps of plant available water for soil samples collected from 0-5 cm and 5-10 cm from Rawls Golf Course hole number 14.



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:
- One greenhouse experiment has been conducted to determine the efficacy of PGPMs on Kentucky bluegrass establishment under salinity. ‘Kenblue’ had a higher tissue biomass than ‘Moonlight’. Salinity inhibited turfgrass growth. Limited differences were observed among the PGPM products applied in the current study.
- Another greenhouse experiment has been conducted to determine the efficacy of PGPMs on Kentucky bluegrass establishment under drought. Similarly, ‘Kenblue’ had a higher tissue biomass than ‘Waterworks’. Drought adversely affected turfgrass growth. At least one growth index in BotaniGard 22WP, Companion, Molt-x, and Nortica 10WP-treated plants was higher than the control (i.e. no PGPM application) plants.
- Efficacy of PGPMs in Kentucky bluegrass establishment was evaluated in 2020. Remote sensing data (Normalized Difference Vegetation Index, Leaf Area Index, Relative Water Content, and Stress Index) showed that ‘Moonlight’ performed better than ‘Kenblue’ 60 days after seeding. Some differences were observed among PGPM products; however, most PGPM products performed similar or poorer than then the control (i.e. no PGPM application).
- A field trial to determine the effects of PGPMs on drought tolerance of Kentucky bluegrass had to be re-established in fall, 2020 due to a breakout of barnyard grass; thus, no drought conditions were induced. No differences in genotypes and PGPM products were detected in the remote sensing data.

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.

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

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

Greenhouse experiments – Two greenhouse experiments were conducted to determine the efficacy of PGPMs on Kentucky bluegrass establishment under salinity (Experiment 1) and drought (Experiment 2).

Experiment 1 – salinity. ‘Kenblue’ and ‘Moonlight’ were seeded in plastic pots in a greenhouse. After two weeks of germination under optimal conditions, half of the plants was hand-watered with tap water (i.e. non-saline) and the other half was watered with NaCl (6 dS m^{-1}) for four weeks (week 3 to 6). 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 ‘Moonlight’ in PH (12.7 vs. 11.4 cm), SDW (1.5 vs. 1.1 g), and RDW (0.8 vs. 0.6 g) (data not shown). RL and root to shoot dry weight ratio (RSR) were at a similar level between the two cultivars, averaged 14.3 cm and 52.0%. Higher specific root length (SRL, SRL = RL/RDW) of ‘Moonlight’ (26.8 cm/g) compared to ‘Kenblue’ (17.8 cm/g) is due to its lower RDW. Salinity resulted in reduced growth rate, ranging from 10.3% in PH to 31.5% in RDW. SRL increased from 19.7 cm/g under non-saline condition to 24.8 cm/g under saline condition due to higher reduction in RDW than RL. All PGPM treatments were similar as the control (i.e. no PGPM application), except Molt-x (PH and SDW) and Companion (SDW) that showed lower performance than the control.

Experiment 2 – drought. ‘Kenblue’ and ‘Waterworks’ were seeded and maintained in a greenhouse as described in Experiment 1. So were the PGPM applications. 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 experiment design and data collection and analysis were identical to Experiment 1.

‘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%). Higher reduction in RDW in ‘Waterworks’ than ‘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.

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						
BotaniGard 22WP	13.5a	1.22ab	0.69a	15.7a	54.7a	25.7b
Companion	13.7a	1.34a	0.67ab	17.2a	49.1ab	28.3ab
Molt-x	13.4a	1.19a-c	0.64ab	15.4a	52.3ab	26.4b
Nortica 10WP	13.5a	1.22ab	0.59a-c	15.4a	46.5b	31.5ab
RootShield PLUS^{+} WP	13.0a	1.13bc	0.61a-c	15.8a	51.9ab	31.8ab
Serenade	12.7a	1.05bc	0.57bc	15.2a	53.1ab	30.8ab
Control (no PGPM)	12.8a	1.01c	0.51c	15.4a	49.2ab	38.0a

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

Field experiments – Two field experiments were established in 2020 to determine the efficacy of PGPMs on Kentucky bluegrass establishment (Experiment 3) and drought tolerance (Experiment 4). Both field experiments were seeded in May 2020. A breakout of barnyard grass occurred in June. The overall turfgrass quality and coverage of both experiments were unacceptable by late June, although Quinclorac was applied. Therefore, Experiment 3 and 4 were re-established in July and August 2020, respectively. The experimental design of both experiments was split-plot, with the whole-plot treatment being the cultivar (‘Kenblue’ and ‘Moonlight’ in Experiment 3 and ‘Kenblue’, ‘Baserati’, and ‘Waterworks’ in Experiment 4). 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; 65 lbs/acre for the first two applications and 21.7 lbs/acre for later applications). Data were collected on two Normalized Difference Vegetation Index (NDVI) (NDVI1 = (R760-R710)/(R760+R710); NDVI2

= (*R*950-*R*660)/(*R*950+*R*660)), Leaf Area Index (LAI = *R*950/*R*660), Relative Water Content (RWC = *R1480*/*R1650*), and two Stress Index (SI1 = *R*710/*R*760; SI2 = *R*710/*R*810) with a MSR16 once monthly.

Experiment 3 – Establishment trial. Remote sensing data were collected in Aug., Sept., and Oct. 2020. In Aug., no differences were observed in any remote sensing indices between the cultivars. The only PGPM treatment differences were detected in RWC; however, no PGPM treatments outperformed the control (data not shown). Cultivar differences were detected in Sept. and Oct. (Table 3). 'Moonlight' had higher values in NDVI1 in Sept. and NDVI1 and 2, LAI, and RWC in Oct., but lower values in both SI indices in Sept. and Oct. 2020. PGPM differences were detected in NDVI1 and SI1 in Sept. and RWC in Oct. Only Molt-x outperformed the control treatment in NDVI1; while, PGPM treatments performed similar or poorer than the control in SI1 and RWC. This experiment will be repeated in 2021.

Experiment 4 – Drought tolerance trial. Drought condition was not induced due to late establishment. Remote sensing data were collected in Sept. and Oct. 2020. No differences were observed in any remote sensing indices among the cultivar or PGPM treatments (data not shown). This experiment will be continued in 2021.

Table 3. Remote sensing indices of Kentucky bluegrass as affected by cultivar and plant growth-promoting microorganism (PGPM) products in 2020. 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)), Leaf Area Index (LAI = *R*950/*R*660), Relative Water Content (RWC = *R1480*/*R1650*), and Stress Index (SI) 1 (SI1 = *R*710/*R*760) and 2 (SR2 = *R*710/*R*810). *R* means reflectance and the number indicates the wavelength (nm).

	September						October					
Treatment	NDVI1	NDVI2	LAI	RWC	SI1	SI2	NDVI1	NDVI2	LAI	RWC	SI1	SI2
Cultivar												
Kenblue	0.480b[z]	0.823a	10.591a	0.186a	0.352a	0.321a	0.487b	0.843b	11.991b	0.594b	0.346a	0.318a
Moonlight	0.512a	0.819a	10.415a	0.201a	0.324b	0.292b	0.558a	0.859a	13.577a	0.616a	0.284b	0.259b
PGPM products												
BotaniGard 22WP	0.504ab	0.830a	11.173a	0.167a	0.331ab	0.301a	0.526a	0.854a	13.261a	0.622a	0.313a	0.286a
Companion	0.507ab	0.827a	11.079a	0.189a	0.329ab	0.299a	0.514a	0.848a	12.534a	0.601a	0.322a	0.295a
Molt-x	0.520a	0.839a	11.619a	0.205a	0.317b	0.286a	0.546a	0.868a	14.232a	0.566c	0.295a	0.269a
Nortica 10WP	0.484b	0.806a	9.559a	0.220a	0.349a	0.317a	0.521a	0.849a	12.495a	0.580bc	0.317a	0.292a
RootShield PLUS^{+} WP	0.509ab	0.834a	11.347a	0.162a	0.326ab	0.296a	0.520a	0.850a	12.499a	0.622a	0.317a	0.289a
Serenade	0.477b	0.800a	9.087a	0.200a	0.354a	0.322a	0.505a	0.840a	11.770a	0.617ab	0.330a	0.302a
Control	0.471b	0.813a	9.657a	0.212a	0.360a	0.326a	0.526a	0.850a	12.697a	0.626a	0.312a	0.286a

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

USGA ID#: 2020-05-710

Title: Turfgrass under effluent water irrigation: long-term data collection

Investigator: Yaling Qian and Yao Zhang

Affiliation: Colorado State University

Objectives:

To collect and test soil properties and evaluate turfgrass on greens and fairways of golf courses irrigated with effluent water.

Start Date: 2020

Project Duration: 1 year

Total Funding: $10,000

Project Summary:

Rational: Increasing demand on fresh water supplies in the arid and semi-arid western US and more stringent wastewater discharge standards have made effluent water a common water source for irrigating golf courses. The future of turfgrass industry needs effective strategies to manage irrigation waters containing higher levels of soluble salts and sodium absorption ratio (SAR), particularly in arid and semi-arid regions. Long-term evaluation of soil properties through field research is essential for the development of effective management strategies.

Methodology: This study leverages historical soil samples from golf courses to examine long term impact of effluent water irrigation. The PI collected soil samples at the start of effluent water use for irrigation, then periodically thereafter. These soil samples were archived. The original plan was to sample several original sites in 2020 after 16 and 20 years of effluent water irrigation. However, due to a university travel restriction during pandemic, we delayed this task to 2021.

In a related study, the soil chemical properties of greens and fairways on two golf courses that use either effluent water or fresh water were compared. Golf course A transitioned to effluent water for irrigation while golf course B has always used fresh water (Figs 1-3). Two years after the start of effluent water irrigation, gypsum was applied granularly at 850 kg ha^{-1}. Five years after the start of effluent water irrigation, gypsum was injected through the irrigation system and applied granularly. The total amount of gypsum applied in year 5 was 2242 kg ha^{-1}.

Results: Soil analyses showed that many changes occurred over time due to the use of effluent water irrigation. Starting 6 years of using effluent water, soil electrical conductivity increased but remained well below the critical threshold levels for turf , whereas the average soil pH increased by 0.42 units 1 to 7 years of effluent water irrigation (Fig. 1). Compared to freshwater irrigation, effluent water irrigation increased soil pH. Sodium levels increased by 2 to 5 times despite the application of gypsum (Fig. 2).

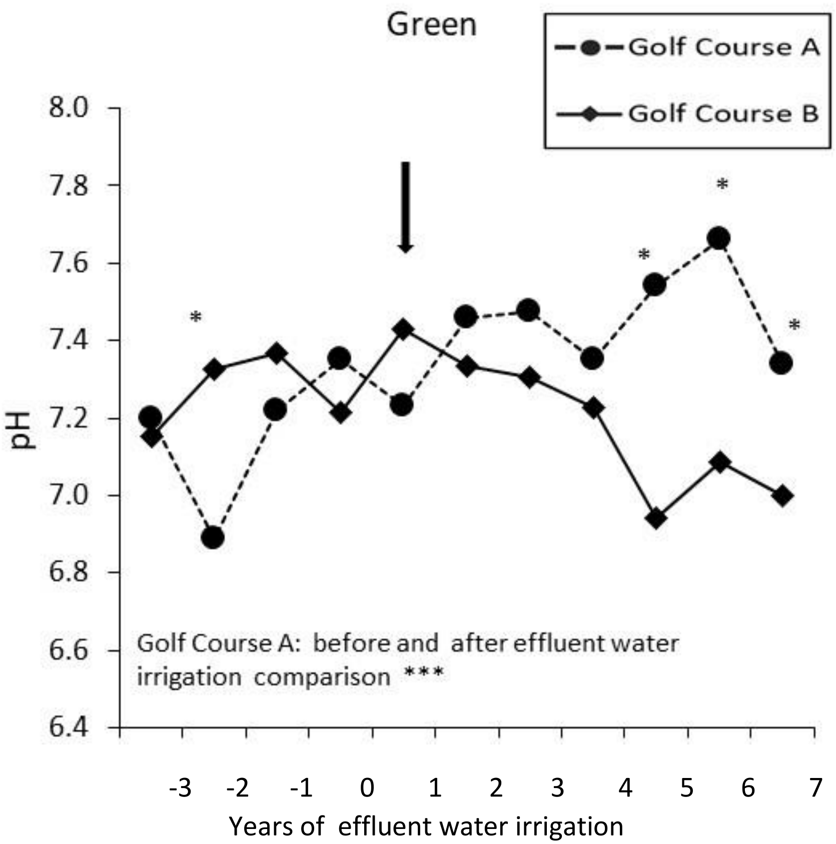
One unique finding is that the availability of micronutrients (Fe and Mn) decreased on greens irrigated with effluent water irrigation (Fig. 3). After the start of using effluent water irrigation, soil Fe concentration from greens of Golf Course A was significantly lower than greens of fresh water-irrigated Golf Course B in 2, 4, and 5 years after the start of effluent water irrigation, suggesting that iron availability is reduced under effluent water irrigation (Fig. 3). Soil Mn concentration from greens of Golf Course A was significantly lower than greens of fresh water-irrigated Golf Course B at 4 to 6 years after effluent water irrigation in Golf Course A (Fig. 3). There were significant reductions in both Fe and Mn availability of Golf Course A greens after the start of using effluent water (Fig. 3). It is possible that the observed reductions in Fe and Mn availability were associated with soil pH increase caused by effluent water irrigation (Fig. 1). Soil pH plays an essential role in micronutrient availability. The available Fe and Mn were observed to decrease on greens. Although no turfgrass tissue analysis has been done yet, iron chlorosis was observed at several locations.

On the positive side, effluent contains N, which allowed the turf manager to reduce annual N application by 60 – 70 kg ha^{-1}. Soil P and K levels increased in the soil after using effluent water, which would be beneficial for turfgrass and potentially lower the fertilizer cost (data not shown).

This study demonstrated that despite the benefits of effluent water irrigation, there are concerns relating to soil ESP and soil pH increases that could cause reductions in micronutrients availability in the soil. Golf course managers could apply proactive management practices, such as applications of soil amendments that provide Ca to replace Na, use of acidifying products could reduce soil alkalization, and application of micronutrients to lessen the negative impacts.

Summary Points:

- ✓ Effluent water contains nutrients which allowed the turf manager to reduce annual N application by 60 – 70 kg ha-1. .
- ✓ Effluent water irrigation resulted in increases in soil ESP and soil pH.
- ✓ The available micronutrients (Fe and Mn) decreased on greens irrigated with effluent water irrigation.



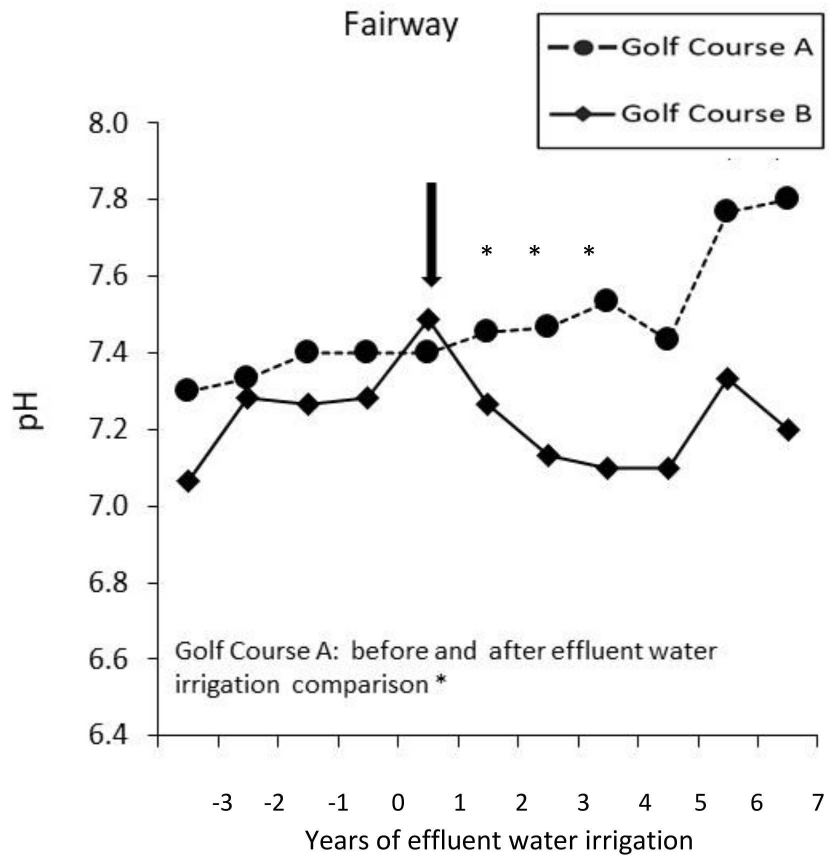


Fig. 1. Soil pH from putting greens and fairways of Golf Course A and Golf Course B.
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
Arrow indicates the year of beginning effluent water irrigation on Golf Course A.

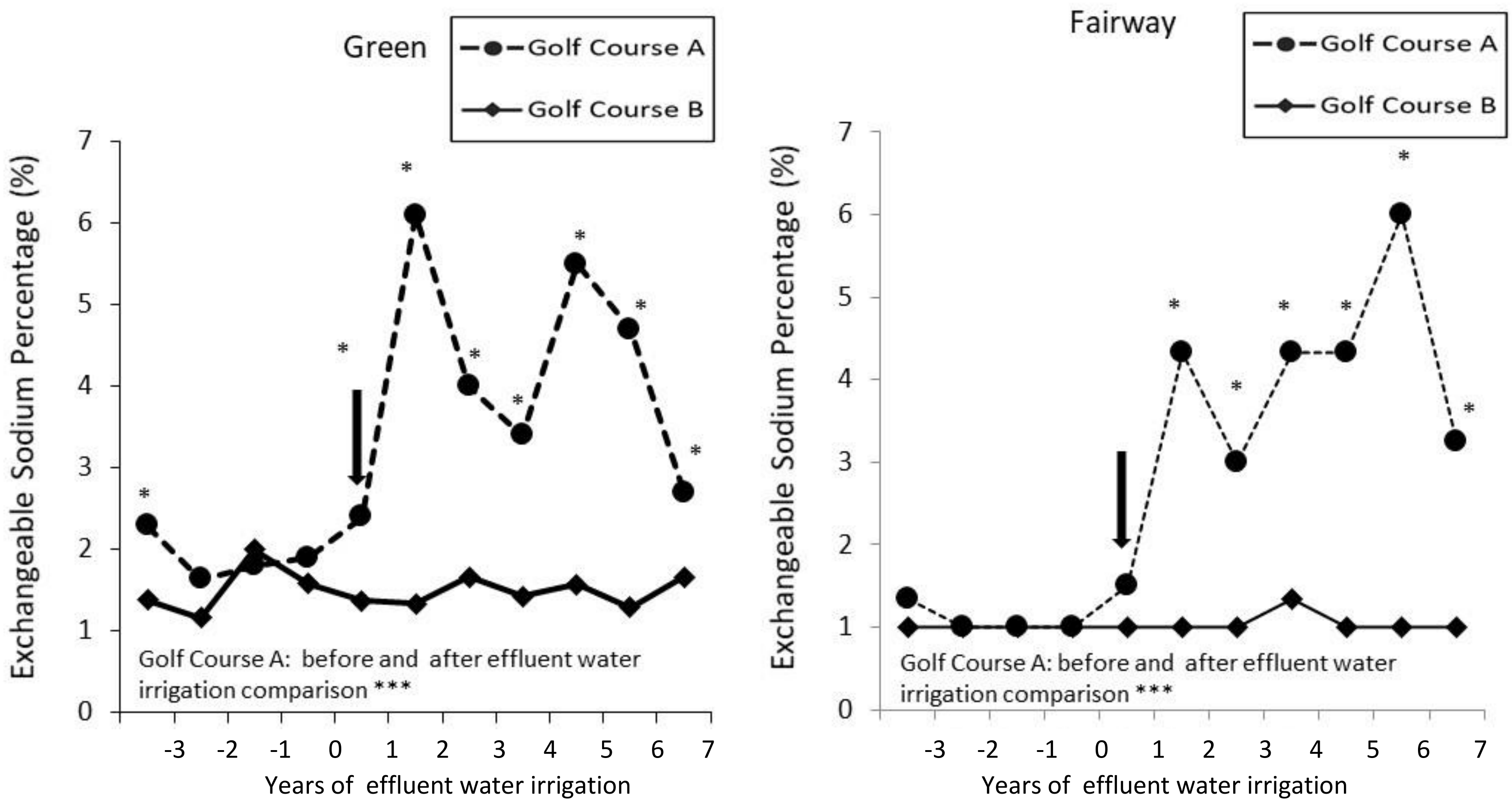
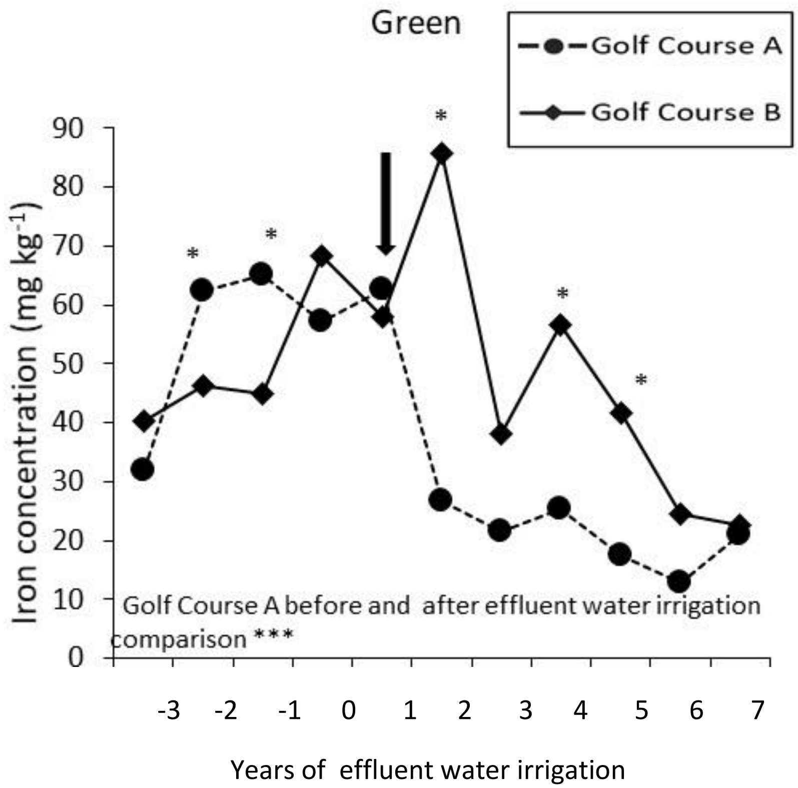


Fig. 2. Soil exchangeable Na percentage from Golf Course A and Golf Course B putting greens and fairways.
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
Arrow indicates the year of beginning effluent water irrigation on Golf Course A.



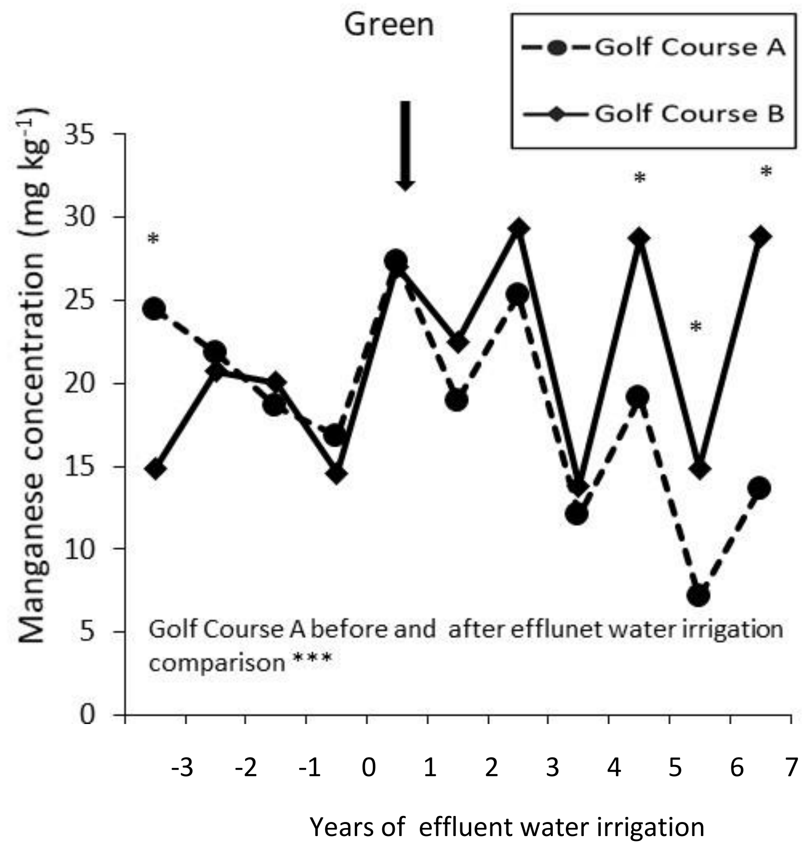


Fig. 3. Soil Fe and Mn concentration from Golf Course A and Golf Course B putting greens.
* Significant at the 0.05 probability level.*** Significant at the 0.001 probability level.
Arrow indicates the year of beginning effluent water irrigation on Golf Course A.

USGA ID#: 2018-20-670

Sclerotinia homoeocarpa epidemiology and resistance development as measured through improved molecular detection techniques.

Paul Koch[1], Bruce Clarke[2], Glen Groben[2], Jim Murphy[2], Geunhwa Jung[3], Ning Zhang[2]
[1]Department of Plant Pathology, University of Wisconsin – Madison
[2]Department of Plant Biology and Pathology, Rutgers University
[3]Stockbridge School of Agriculture, University of Massachusetts - Amherst

Year 3 Summary:

Two quantification assays for *Clarireedia* spp. (previously known as *Sclerotinia homoeocarpa*) were developed in 2018 and used in field studies in 2019 and 2020. Rutgers developed an effective qPCR-based assay for use in their studies, and the manuscript detailing the development of this method was recently published in the journal Plant Disease. Wisconsin developed an effective ddPCR-based assay for use in their studies, though analysis of field samples was slowed in 2019 due to staff turnover and slowed in 2020 due to a prolonged closure of the ddPCR facility for COVID-19 reasons. Massachusetts also developed a qPCR-based assay using different gene targets than Rutgers.

- Dollar spot sampling sites initiated in 2019 in New Jersey, Wisconsin, and Massachusetts were continued in 2020 and sampled throughout the growing season.
- Rutgers used their qPCR-based method to quantify *Clarireedia* spp in three different studies.
  - Determined the spatial distribution of the fungus in relation to dollar spot symptoms. The qPCR assay identified different pathogen populations across sampling dates for asymptomatic and symptomatic turf; the pathogen appeared to be randomly distributed throughout plots; and dollar spot was present at all dates in verdure (foliage) and crown tissue, albeit at a low level on some dates.
  - Determined the amount of the dollar spot fungus present at different depths in a turfgrass core. Verdure had the highest concentration on sampling dates when the pathogen was detected; the crown layer can have concentrations often similar to either the verdure or thatch layers; and the pathogen was only detected in the thatch when visual symptoms were present on the verdure. Thus, sampling of the verdure and crown layers appear to be the most useful layers for pathogen detection with the qPCR assay in field samples.
  - Determined the impact of both a dollar spot resistant and susceptible bentgrass cultivar on fungal development during the growing season. The qPCR assay could detect differences in the pathogen population among cultivars varying in susceptibility when symptoms were present. The amount of *Clarireedia* spp present on the resistant cultivar (Declaration) was typically lower than the susceptible cultivar (Independence) when symptoms were present. Evaluation of a better sampling strategy including increased number of subsamples and replication in future studies may allow the assay to detect cultivar differences before symptoms develop.
- Wisconsin used their droplet digital PCR assay to quantify *Clarireedia* spp in two different studies.

  - The spatial distribution study described above was replicated at Wisconsin in both year 2019 and 2020. Though results aren't currently available to present due to the slowdowns mentioned in bullet 1 above, significant progress has been made on the study and is detailed later in this update. We expect to complete ddPCR quantification and data analysis on these samples by the spring of 2021. A total of 216 samples were collected over four sampling dates (May 2019, August 2019, May 2020, July 2020). All 216 samples have been crushed using liquid nitrogen and will have DNA extracted and be prepped for ddPCR by the end of December 2020.
  - To evaluate the response of *Clarireedia* spp to two different fungicides (chlorothalonil and fluxapyroxad), samples were collected weekly from May through October in both 2019 and 2020. The ddPCR results are only available through August of 2019, and showed that *Clarireedia* spp on non-treated plots increased significantly in late July, shortly before an increase in symptoms in August. We expect to complete ddPCR quantification and data analysis on the remaining samples from both 2019 and 2020 by the spring of 2021.
- Massachusetts used multiple assays to both quantify *Clarireedia* spp. present in the turf and to detect fungicide resistant dollar spot isolates in the field.
  - Massachusetts developed a qPCR-based detection assay of *Clarireedia* spp targeting the EF1α and actin genes. The EF1α qPCR assay was effective in detecting and quantifying *Clarireedia* spp, and also found that 400 times more *Clarireedia* was detected in the leaves compared to the thatch.
  - The field competitiveness (fitness) of SDHI-resistant dollar spot isolates in the field was studied, and found that isolates with particular resistance mutations were highly competitive in the field in the absence of fungicides while other resistant isolates with different resistance mutations were not as competitive. This suggests that not all SDHI resistance is alike and the specific mutation will determine the competitiveness of the resistant isolate in the field.
  - Rapid loop-mediated isothermal amplification (LAMP) assays were developed to detect for fungicide-resistant dollar spot isolates in as little as 20 minutes. The two primer sets being used for this assay appear promising, but more work is still needed to make the results more consistent and reliable.
- Following analysis of the data collected at all three universities, we will begin to develop a plan for publishing the wealth of research conducted as part of this grant. One manuscript has already been published and we envision multiple additional manuscripts as a result of this collaborative work.
- The work presented here provides important information about the epidemiology of *Clarireedia* spp and the relationship between pathogen populations and the development of symptoms. It has also created additional questions that will require further study to enhance our knowledge of the dollar spot pathosystem and continue our advancement towards the development of novel control strategies.

Overall Project Objective 1: Develop one or more molecular methods to effectively quantify *Clarireedia* spp. in the field and assess the fungal response to host genotypes and cultural and chemical practices.

Year 3 Goal: Use the effective detection techniques developed in year 1 to conduct field studies in year 3.

<u>**Wisconsin Update**</u> **-**

1) Spatial Distribution of Dollar Spot in Asymptomatic and Symptomatic Turfgrass:

Objective: To determine distribution of the dollar spot pathogen on 'Penncross' creeping bentgrass before and after symptoms develop in the field.

Experimental Design: Groups at Rutgers University and the University of Wisconsin ran concurrent spatial distribution projects. At Wisconsin the project was conducted at the OJ Noer Turfgrass Research Facility in Madison, and the experimental design was a randomized complete block with 60- × 60-cm experimental units (plots) within a large stand of 'Penncross' creeping bentgrass. Three plots (blocks) were sampled each date in 2019 and 2020: May before dollar spot symptoms appeared and July or August when dollar spot symptoms were visible. Thirty-six subsamples were collected per plot in May 2019 and May 2020 before disease was visible and severely diseased plots were sampled on in August 2019 and July 2020. Subsample cores were collected from each 60- × 60-cm plot using a 50- × 50-cm grid with 36 evenly spaced intersects (10 cm apart). A 1-cm dia. × 2.5-cm deep core sample was collected at each intersect. A 5 cm non-sampled border was placed around the sampling grid to avoid sampling neighboring plots. DNA was isolated from tissue comprised of the verdure (foliage) and crown layers of individual subsample cores. Samples were frozen at -80°C until DNA can be extracted and ddPCR analysis conducted.

Results: A total of 216 samples were collected over four sampling dates (May 2019, August 2019, May 2020, July 2020). All 216 samples have been processed and await DNA extraction and ddPCR analysis, which we expect to complete by the end of December 2020. Analysis was slowed by two different events. In 2019, a postdoctoral researcher that was working on this project departed for another position and an MS student took over the project. In 2020, both our lab and the ddPCR facility was closed for a prolonged period of time due to the COVID-19 outbreak. Despite these slowdowns, full data collection and analysis is expected to be completed by spring of 2021.

2) Tracking Dollar Spot Pathogen Populations in Response to Fungicide Applications Over Time:

Objective: Determine whether fungicides with different modes of action and different recommended reapplication intervals impact the dollar spot pathogen population in different ways.

Experimental Design: A plot was established in May of 2019 at the OJ Noer Turfgrass Facility in Madison, WI on a 'Penncross' creeping bentgrass stand maintained under fairway conditions. This study researched the impact of chlorothalonil (Daconil WeatherStik), fluxapyroxad (Xzemplar), and a non-treated control on the dollar spot fungal population over the course of the growing season in both 2019 and 2020. The study design was a RCBD with 4 replications, and 8 subsamples were conducted from within each plot on a weekly basis, pooled (composited) using liquid nitrogen, and the DNA extracted using Qiagen DNA extraction kits. Samples were collected weekly from the first week of May through the last week of October, resulting in a total (post-pooling) of 311 samples in 2019 and 288 samples in 2020. Quantification of *Clarireedia* spp. is being conducted using ddPCR at the UW-Madison Biophysics Instrumentation Facility.

Results: A total of 311 samples were collected in 2019, and of those samples all 311 have been frozen with liquid nitrogen and had their DNA extracted, 252 have been prepped for ddPCR, and 192 have been analyzed using ddPCR. The ddPCR results are available through August and show that a large increase in *Clarireedia* gene copy on the non-treated plots occurred in late July (Figure 1), which shortly preceded a large outbreak in symptoms in August (Figure 2). There was a slight increase in *Clarireedia* gene copy on chlorothalonil-treated plots at the very end of our ddPCR analysis, though that hadn't yet corresponded with an increase in symptoms. *Clarireedia* gene copy stayed very low over the course of the entire study on fluxapyroxad-treated plots. Dollar spot development in 2020 occurred earlier in the season (Figure 3), in mid-July, and we will compare the ddPCR results from 2020 with 2019 to determine how the increase in fungal gene copy compared with the earlier increase in symptoms. A total of 288 samples were collected in 2020 and 24 samples have had their DNA extracted. We expect to complete DNA extraction and ddPCR analysis early in 2021 and will work with our collaborators to begin to develop manuscripts for publication in peer-reviewed journals.

Figure 1. Droplet digital PCR (ddPCR) results quantifying Clarireedia spp gene copy number from 2019 in Madison, WI in response to no fungicide, chlorothalonil (Daconil WeatherStik), and fluxapyroxad (Xzemplar). Gray shading represents 95% confidence intervals.

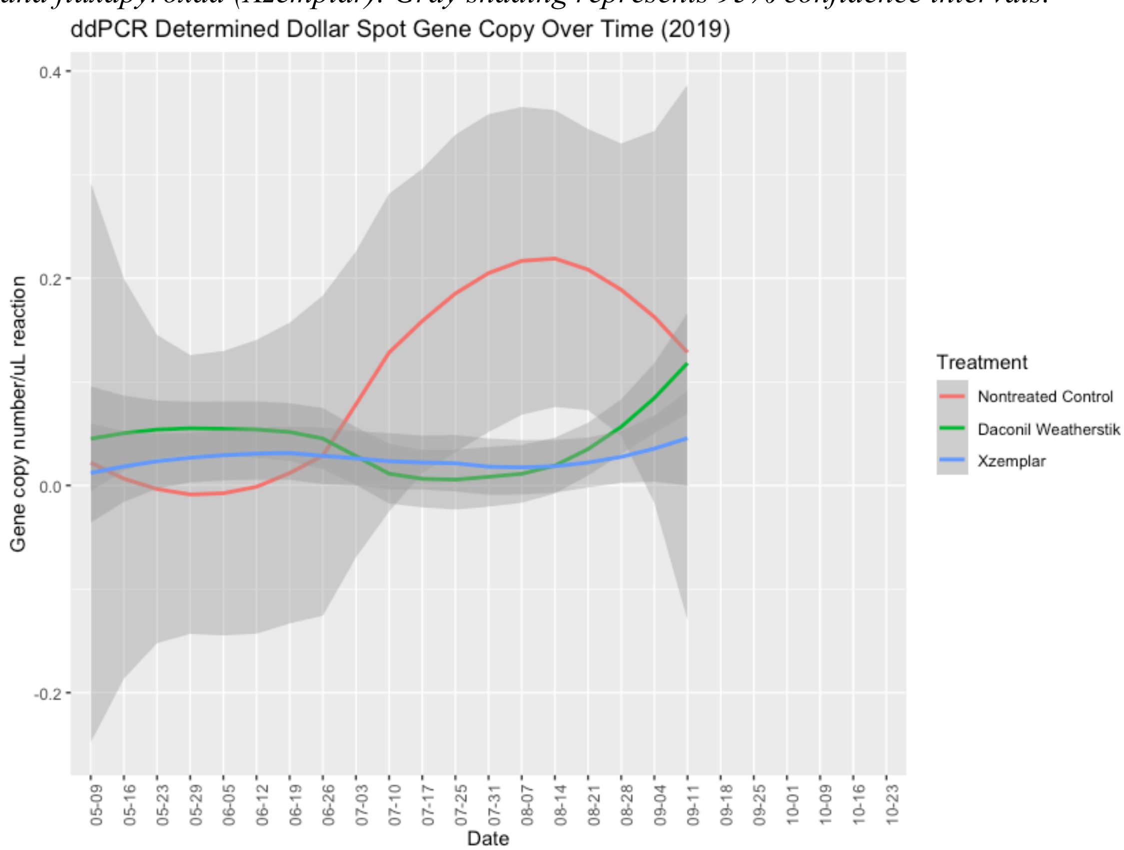


Figure 2. Dollar spot development at the OJ Noer Research Facility in Madison, WI in 2019 in response to no fungicide, chlorothalonil (Daconil WeatherStik), and fluxapyroxad (Xzemplar).

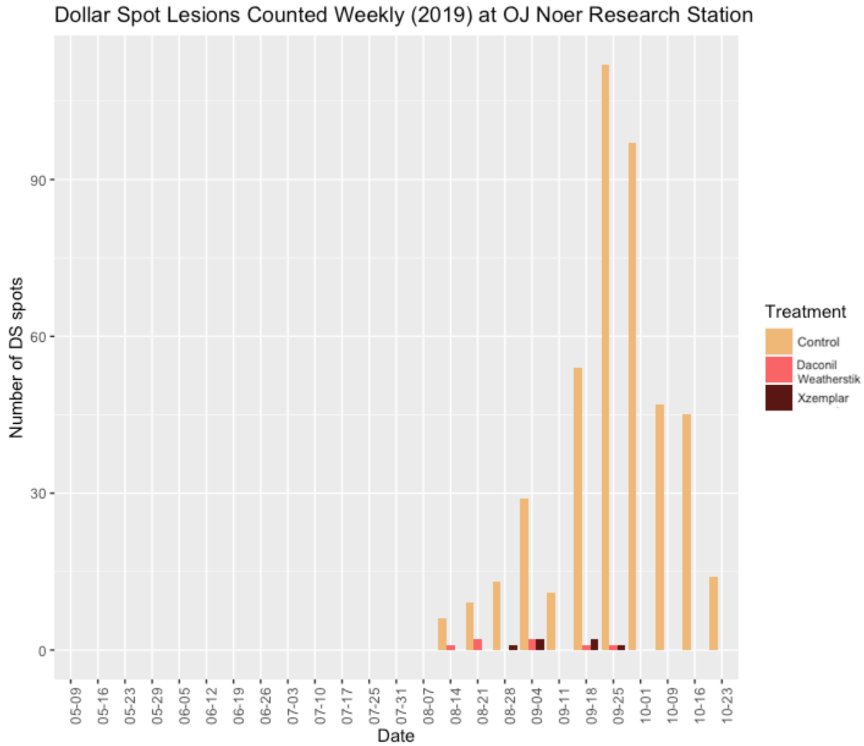
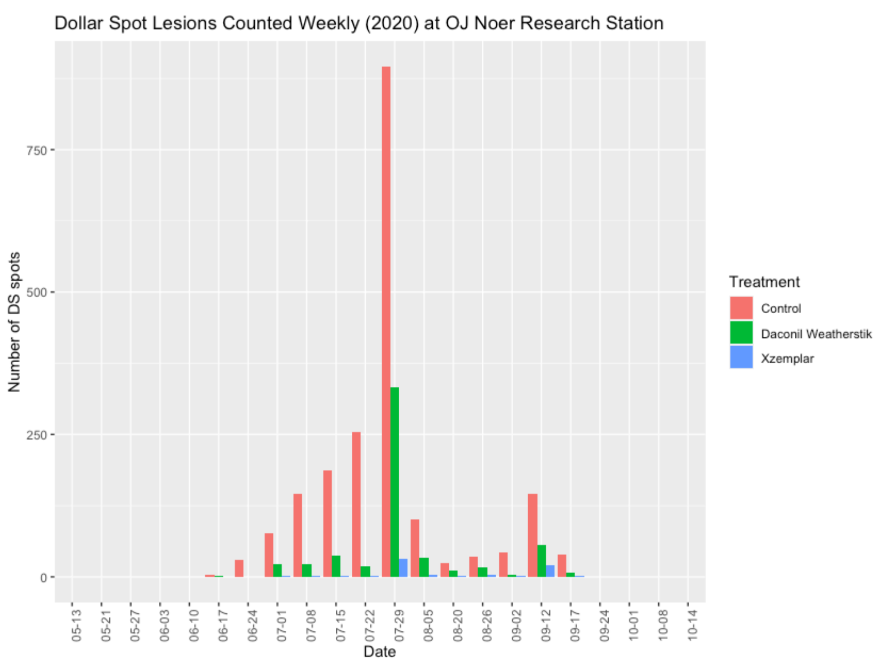


Figure 3. Dollar spot development at the OJ Noer Research Facility in Madison, WI in 2020 in response to no fungicide, chlorothalonil (Daconil WeatherStik), and fluxapyroxad (Xzemplar).



<u>Rutgers Update</u>:

1) Spatial Distribution of Dollar Spot in Asymptomatic and Symptomatic Turfgrass:

Objective: To determine distribution of the dollar spot pathogen in 'Independence' creeping bentgrass before and after symptoms develop in the field.

Experimental Design: Groups at Rutgers University and the University of Wisconsin ran concurrent spatial distribution projects. The experimental design was a randomized complete block with 60- × 60-cm experimental units (plots) within a large stand of 'Independence' creeping bentgrass maintained at 0.375 inch (9.5 mm). Three plots (blocks) were sampled each date in 2019 and 2020: May before dollar spot symptoms appeared and July or August when dollar spot symptoms were visible. Before sampling began in May 2019, the field received six preventive chlorothalonil (12.6 kg a.i. ha^{-1}) applications throughout 2018; however, only two chlorothalonil (12.6 kg a.i. ha^{-1}) applications were made in 2019 (21 October and 7 November) prior to the May 2020 sampling. Thirty-six subsamples were collected per plot on 1 May 2019 and 4 May 2020 before disease was visible and severely diseased plots were sampled on 24 August 2019 and 28 July 2020. Subsample cores were collected from each 60- × 60-cm plot using a 50- × 50-cm grid with 36 evenly spaced intersects (10 cm apart). A 1-cm dia. × 2.5-cm deep core sample was collected at each intersect. A 5 cm non-sampled border was placed around the sampling grid to avoid sampling neighboring plots. DNA was isolated from tissue comprised of the verdure and crown layers of individual subsample cores. The crown layer was ~5-mm deep below the verdure and accordingly included the surface thatch tissue. Only thirty of the thirty-six subsample cores per plot were used for DNA isolation. Each DNA isolation was run in triplicate using qPCR and the average cycle threshold (Ct) for each triplicated reaction was recorded. A Ct value less than 37 was considered positive for detection of dollar spot pathogen.

Results: The pathogen was detected in some cores at each sampling date and the percentage of positive detections for dollar spot among the 30 subsamples ranged from 37 % to 94 % (Table 1). The plots with no visual disease symptoms had a lower percentage of positive detections (37 % in May 2019 and 69 % in May 2020) compared to the visually diseased plots (94% in August 2019 and 77 % in July 2020; Table 1). Matching the Ct values of subsample core locations with disease symptoms within plots indicated that none of the cores had a Ct value lower than 25 on asymptomatic (1 May 2019 and 4 May 2020) sampling dates, but there were many cores with Ct values lower than 25 on the symptomatic sampling dates (24 August 2019 and 28 July 2020; Figure 4). Subsamples cores that were located adjacent to or within an infection center had a pathogen concentration threshold less than 25 Ct. The average Ct value of the thirty subsample cores (red crossbar; Figure 4) was 37.3 in May 2019, 30.9 in August 2019, 33.8 in May 2020, and 32.1 in July 2020. The change in pathogen concentration from May 2019 to August 2019 was a 37.5 fold increase (lower Ct value represents higher pathogen level on a log scale), followed by a 5.0 fold reduction from August 2019 to May 2020 and a 2.6 fold increase from May 2020 to July 2020. The difference in Ct values between May 2019 and May 2020 was likely attributed to the limited fungicide use during 2019; whereas the preventive fungicide applications during 2018 presumably reduced pathogen levels before sampling in May 2019. This suggests that a routine use of preventive fungicide applications can suppress the concentration of the pathogen in a turf compared to turf that receives limited fungicide use. Contour and semi-variogram plots generated using the geographical location of each core from one another indicated that there was no correlation of Ct values from neighboring samples and, therefore, the distribution of the pathogen was considered random (data not shown).

In summary, the qPCR assay identified different pathogen populations across sampling dates for asymptomatic and symptomatic turf; the pathogen appeared to be randomly distributed throughout plots; and dollar spot was present at all dates albeit at a low level on some dates.

Table 1. The number of positive and negative detections of Clarireedia in verdure and crown (~5-mm deep including surface thatch) layers of 90 subsamples (30 collected from three 60- 60-cm plots) before and after symptom development on 'Independence' creeping bentgrass managed as fairway turf in North Brunswick, NJ. [1]

	1 May 2019 [2]	24 August 2019	4 May 2020	28 July 2020
Positives	33	85	62	69
Negatives	57	5	28	21
Percent Positive	36.7 %	94.4 %	68.9 %	76.7 %
Average Ct	37.3	30.9	33.8	32.1

[1] Each DNA isolation was run in triplicate using qPCR and the average cycle threshold (Ct) for each triplicated reaction was recorded. A Ct value below 37 was considered positive for detection of *Clarireedia*.

[2] Samples were collected before (1 May 2019 and 4 May 2020) and after (24 August 2019 and 28 July 2020) dollar spot symptoms developed.

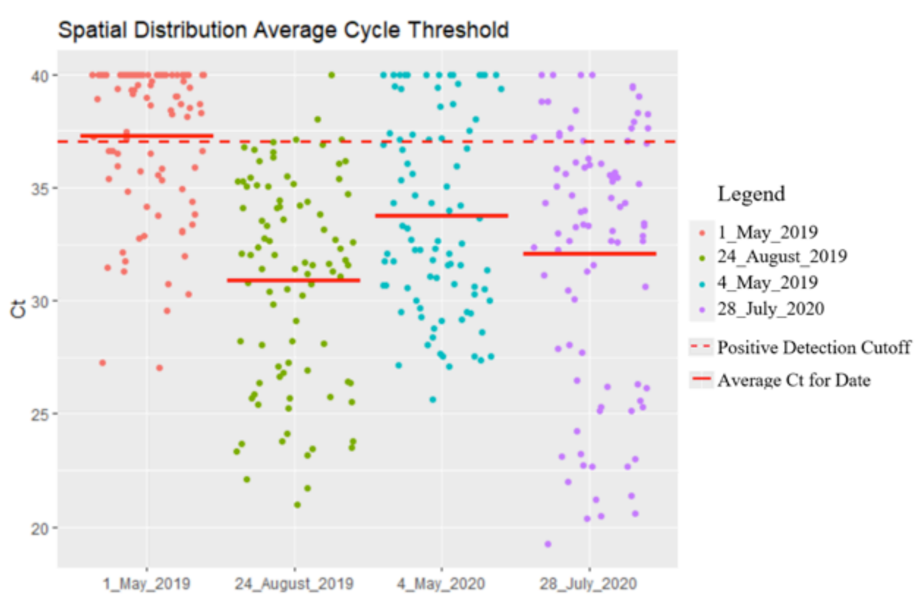


Figure 4. Average cycle threshold for each triplicate DNA amplification from the verdure and crown (~5 mm deep including surface thatch) layers of 'Independence' creeping bentgrass using the qPCR assay on each collection date (90 DNA samples per date). The red dotted line is the cycle threshold cutoff for positive detection of dollar spot. Ct value below 37 are considered positive for Clarireedia spp. The red solid line is the average Ct value for all 90 DNA samples on each collection date.

2) Quantifying *Clarireedia* at Different Depths within Core Samples Over Time:

Objective: To determine the dollar spot pathogen level at in different layers of turf cores throughout the year.

Experimental Design: Three 1-cm dia. × 2.5-cm deep cores were randomly collected from a large stand of 'Independence' creeping bentgrass (same field as the spatial study maintained at 0.375 inch [9.5 mm]) on 1 May 2019, 24 August 2019, 18 October 2019, 13 January 2020, 4 March 2020, 4 May 2020, 28 July 2020, 19 October 2020, and (anticipated) January 2021. Samples from May 2019, August 2019, October 2019, and January 2020 have been analyzed and we expect to analyze the remaining cores soon. Each core was sectioned into verdure and crown (~5 mm depth below the verdure and accordingly included the surface thatch) tissue, and four thatch layers; thatch below the crown layer was segmented into four 5-mm deep layers (Figure 5). A 0.05 gram sample from each layer was used for DNA isolation and then three separate 1 µl aliquots of the DNA was run using the qPCR assay.

Results: Dollar spot was not detected in any of the cores collected on 1 May 2019 (asymptomatic) at any layer, which could be due to the low number of subsamples (3) and the low pathogen level created by the routine preventive fungicide applications made during 2018 (Figure 6). On 24 August 2019 when disease pressure was low (few visual symptoms), the verdure had a low concentration of dollar spot (mean Ct = 34.6). The crown layer had only one replicate sample (out of 3) detected with dollar spot at a Ct value of 34.5 (similar to verdure). There was no detection of the pathogen in any of the thatch layers from August 2019. In October 2019 when disease symptoms were severe, the pathogen concentration in the verdure was very high (mean Ct = 22.8) while the concentration in the crown and thatch layers was much lower (mean Ct of 28.8 and 31.6, respectively). There was no statistical difference in the dollar spot concentration of the crown and thatch layers on this sampling date. Asymptomatic cores sampled from dormant turfgrass in January 2020 [represented in Figure 6 as January (-)] had a high pathogen concentration in the verdure (mean Ct = 26.4) but was lower than observed in October 2019. Pathogen concentration in the crown layer (mean Ct = 32.1) was less than the verdure, whereas dollar spot was not detected in the thatch layers in most cores. Cores sampled from turf with residual disease symptoms in January 2019 [January (+)] also had a high pathogen concentration in the verdure (mean Ct = 20.4) and a lower the concentration in the crown layer (mean Ct = 27.7). The pathogen was detected in thatch layers but at a lower pathogen concentration (mean Ct = 31.5) than the crown layer.

In summary, verdure had the highest concentration on sampling dates when the pathogen was detected; the crown layer can have concentrations often similar to either the verdure or thatch layers; and the pathogen was only detected in the thatch when visual symptoms were present on the leaves. Thus, sampling of the verdure and crown layers appeared to be the most useful layers for pathogen detection with the qPCR assay in field samples.

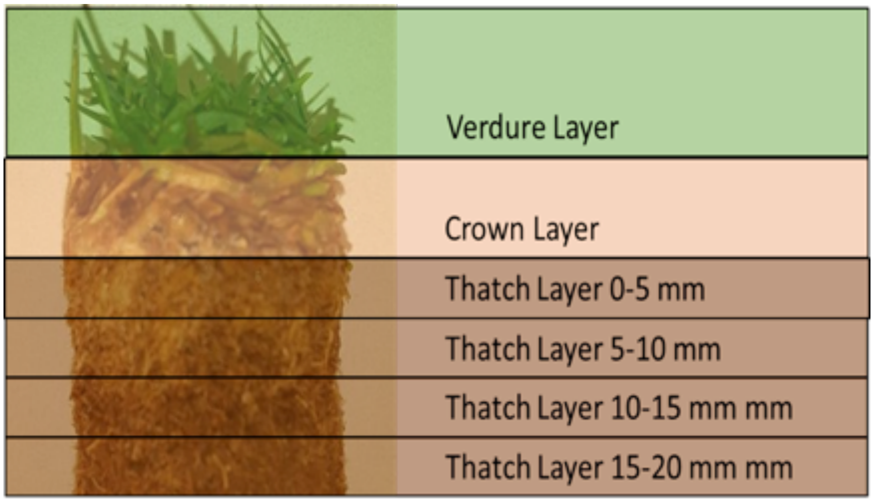


Figure 5. Picture depicting sectioning of each 1-cm diameter × 2.5-cm deep core into verdure, crown (~5 mm deep including surface thatch), and four 5-mm deep thatch layers.

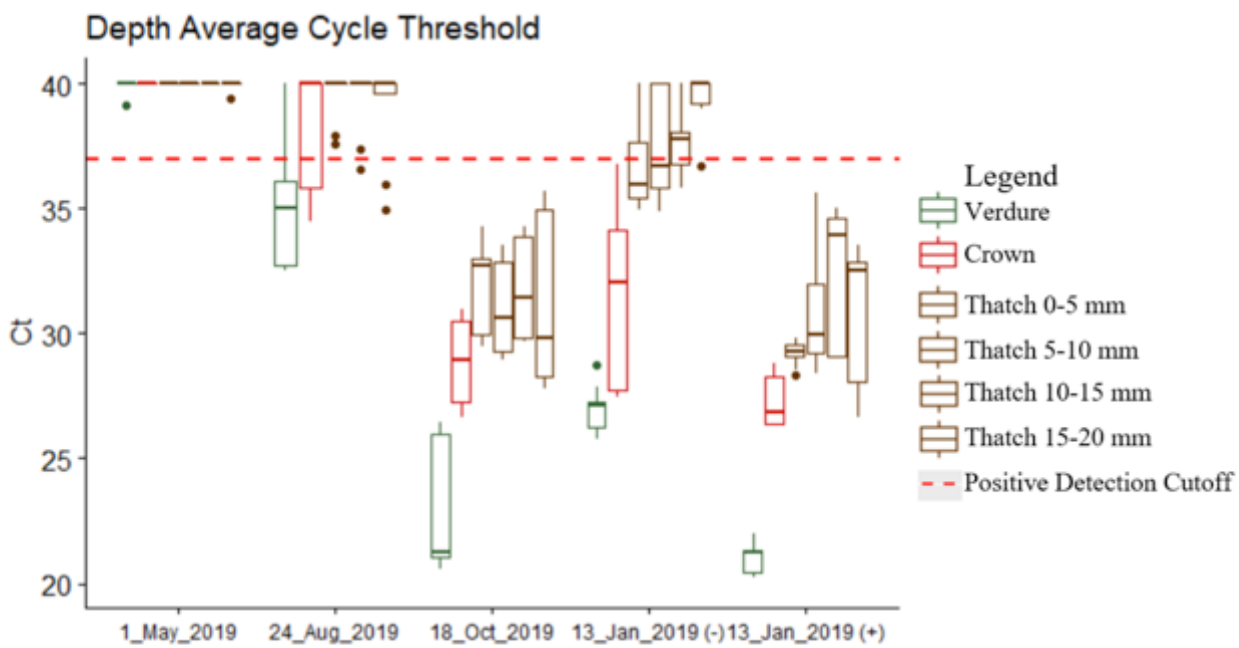


Figure 6. Average cycle threshold using the qPCR assay for verdure, crown (~5 mm deep including surface thatch) and thatch layers of 'Independence' creeping bentgrass sampled from 1 May 2019 through 13 January 2020. The red dotted line is the cycle threshold cutoff for positive detection of dollar spot. Ct value below 37 are considered positive for Clarireedia spp. The red solid line is the average Ct value for each layer sampled.

3) Tracking Dollar Spot Pathogen Populations in Bentgrass Cultivars Over Time:

Objective: Determine whether bentgrass cultivars have different populations of the dollar spot pathogen throughout the growing season.

Experimental Design: Six-year-old field plots of creeping bentgrass cultivars 'Independence' (dollar spot susceptible) and 'Declaration' (dollar spot tolerant) maintained at 0.375 inch (9.5 mm) were sampled weekly throughout the season. A completely randomized design with four replications and 0.9- × 1.5-m plots were used for this study. Ten, 1-cm dia. × 2.5-cm deep core samples were taken within a randomly selected 0.09 m^2 area inside each plot. Sample collection started on 1 May 2020 and continued weekly until 6 August 2020 for a total of 15 weeks. On each sampling date, disease severity ratings (number of lesions centers per plot) of the entire plot and the 0.09 m^2 sampling area were collected along with the ten cores. The verdure and crown (~5 mm depth below the verdure and accordingly included some surface thatch tissue) layers of the ten subsample cores were ground together with liquid nitrogen to make a composite sample for each plot. DNA was extracted from a 0.05-gram sample of each composite sample and then three separate 1 µl aliquots of the DNA was run using the qPCR assay. .

Results: The qPCR assay detected the pathogen in both cultivars for the duration of the fifteen-week study (Figure 7). The mean Ct values for the two bentgrass cultivars were not different at $p \leq 0.05$ until symptoms appeared on both cultivars on 4 June, although the pathogen level on Declaration (mean Ct = 33.6) was lower than the pathogen level on Independence (mean Ct = 32.0) at the $p \leq 0.1$ level on 28 May (Table 2). The appearance of visual symptoms occurred one week earlier on Independence than Declaration (28 May vs 4 June, respectively) and corresponded to an increase in the pathogen population identified with the qPCR assay. Once symptoms developed, the more susceptible cultivar Independence frequently (5 out of 10 rating dates) had a higher pathogen population (lower mean Ct value) than the more tolerant cultivar Declaration. Sampling from a different (random) location within plots each week may have increased the variability in the data set. New sampling strategies will be evaluated in subsequent studies (e.g., number of subsamples, random subsampling of the entire plot rather than a 0.09 m^2 area in each plot, increasing replication, and perhaps obtaining verdure and crown tissue using vertical mowing) to obtain a more representative assessment of the pathogen population.

In summary, the qPCR assay could detect differences in the pathogen population among cultivars varying in susceptibility when symptoms were present. Evaluation of a better sampling strategy including increased number of subsamples and replication in future studies may allow the assay to detect cultivar differences before symptoms develop.

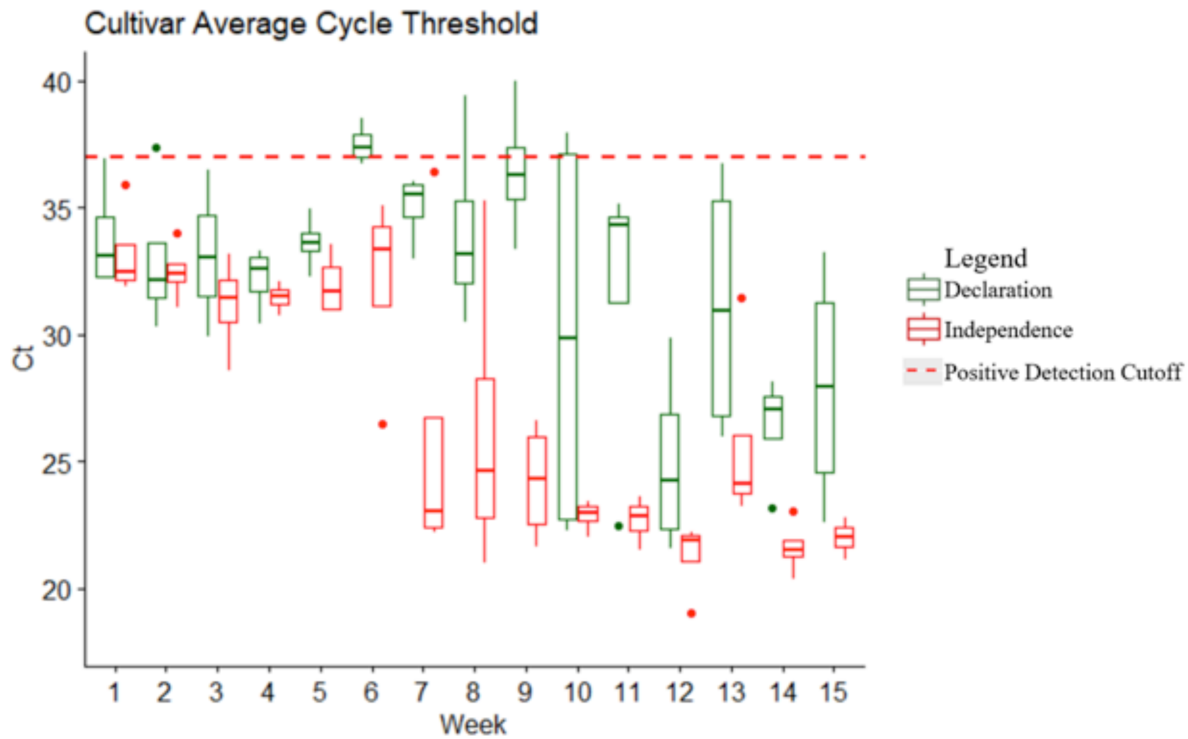


Figure 7. The average cycle threshold of a qPCR assay of verdure and crown (~5-mm deep including surface thatch) layers in 'Independence' and 'Declaration' creeping bentgrass turf sampled weekly from 1 May 2020 through 6 August 2020 for a total of 15 weeks. The red dotted line represents the cutoff for positive detections of dollar spot. Ct value below 37 are considered positive for Clarireedia. The red solid line is the average Ct value for each cultivar sampled.

Table 2. Average cycle threshold for a qPCR assay of the verdure and crown (~5 mm deep including surface thatch) layers and number of dollar spot lesion centers for 'Independence' and 'Declaration' creeping bentgrass turf sampled weekly from 1 May 2020 through 6 August 2020 for a total of 15 weeks.

Date	Week	Mean Ct value		p value[2]	Number lesion centers / plot	
		Declaration	Independence		Declaration	Independence
1 May	1	33.8 (2.19)[1]	33.2 (1.82)	0.670	0	0
8 May	2	33.0 (3.06)	32.5 (1.18)	0.765	0	0
14 May	3	33.1 (2.79)	31.2 1.92)	0.291	0	0
22 May	4	32.2 (1.31)	31.5 (0.58)	0.382	0	0
28 May	5	33.6 (1.09)	32.0 (1.25)	0.0938	0	1
4 June	6	37.5 (0.79)	32.1 (3.86)	0.0324*	1	12
12 Jun	7	35.0 (1.39)	26.2 (6.84)	0.0443*	1	17
19 June	8	34.1 (3.82)	26.4 (6.26)	0.0809	2	33
26 June	9	36.5 (2.73)	24.2 (2.35)	0.000495*	6	41
2 July	10	30.0 (8.59)	22.9 (0.60)	0.149	21	90
9 July	11	31.5 (6.07)	22.7 (0.90)	0.0279*	30	101
16 July	12	25.0 (3.74)	21.2 (1.48)	0.114	32	106
24 July	13	31.1 (5.39)	25.7 (3.83)	0.152	35	126
31 July	14	26.4 (2.18)	21.6 (1.09)	0.00802*	40	127
6 Aug	15	27.9 (4.88)	22.0 (0.71)	0.0542	46	146

[1] Column contains the average cycle threshold and standard deviation in parenthesis
[2] p value generated from analysis of variance comparing the means of the average cycle threshold of the cultivars
[3] The average number of dollar spot lesions in each plot

Massachusetts Update:

Quantifying *Clarireedia* at Different Depths within Core Samples Over Time:

Field sampling and storage of samples From June to September 2019, thatch samples from fairway turf mixed bentgrass and *Poa annua* were collected from ammonium sulfate treated/untreated area at the Joseph Troll Turf Research Center in S. Deerfield. Additionally, dollar spot infected (DSI) thatch and leaf samples were collected from putting greens. Collected samples were stored in liquid nitrogen for transport to our lab on the UMass Amherst campus. Upon arrival, the samples were stored at -80 degrees.

Two genes of interest, elongation factor alpha (EF1α) and actin gene (*Shact*), were selected for *C. homoeocarpa* based on the information provided in Allen-Perkin et al. (2018) and Hulvey et al. (2012). Primers for these genes were selected based on these references and the annealing temperature for both genes was determined to be 60°C. Total DNA was extracted from each sample using a DNeasy PowerSoil Kit and prepared for qPCR with QIAGEN QuantiTect SYBR Green RT-PCR Master Mix. All extracted DNA samples were analyzed in technical triplicates for each gene using a QIAGEN Rotor-Gene Q RT-PCR cycler. A nuclease-free water blank was used as a negative control. Cycler parameters for qPCR were are as follows: hold of 95 °C for 5 min, 40 cycles of 95 °C for 5 s, and 60 °C of combined annealing and extension for 10 s. For compassion with DNA, total RNA of leaf samples was extracted using QIAGEN RNeasy Plant Mini Kit. Total RNA of thatches was extracted from each sample using QIAGEN RNeasy PowerSoil Kit. All the RNA samples were reverse transcripted using QuantiTect Reverse Transcription Kit. Each cDNA sample was standardized by adjusting Ct value of *Sh*act is around 31. All extracted RNA samples were analyzed in technical triplicates for each gene using a QIAGEN Rotor-Gene Q RT-PCR cycler. A nuclease-free water blank was used as a negative control. Cycler parameters for qPCR were as follows: hold of 95 °C for 5 min, 40 cycles of 95 °C for 5 s, and 60 °C of combined annealing and extension for 10 s. Critical threshold (Ct) values were recorded at a threshold value set to 0.02.

Figures 8a and 8b show the fold change of *C. homoeocarpa* inoculum and expression between leaves and thatches. Error bars were used to represent the standard deviation of replicates.

Figure 8a

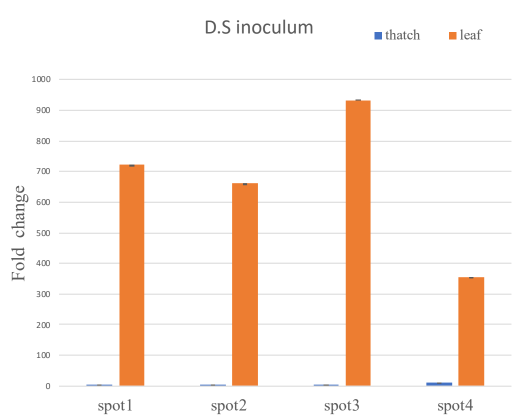


Figure 8b

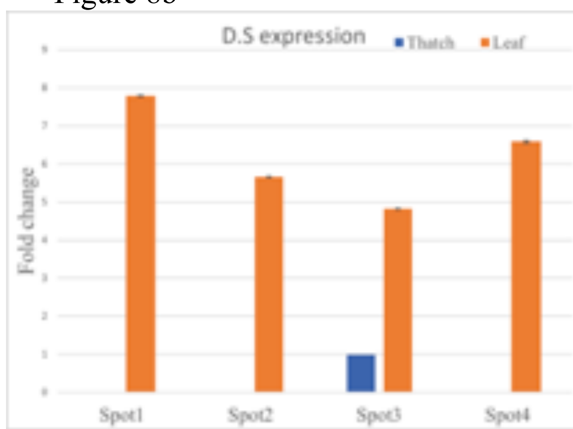


Figure 8. a) Graph of average Ct values obtained through ShEF1a gene analysis from dollar spot infected (DSI) thatch and leaf samples on four collection locations. b) Graph of average Ct values fold change obtained through ShEF1a and Shact genes analysis from dollar spot infected (DSI) thatch and leaf samples on four collection locations.

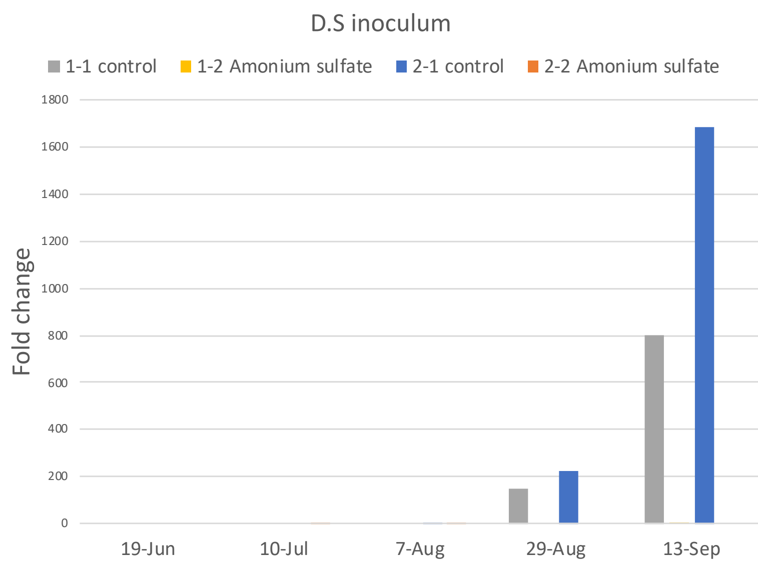


Figure 9. Graph of average Ct values obtained through ***ShEF1a gene*** *analysis from thatch samples on five collection dates.*

Results:

In general, there are more than four hundred times *C. homoeocarpa* inoculum in leaves than in thatches and the expression of EF1a is five times higher in leaves than in thatches. There is a significant correlation between *C. homoeocarpa* inoculum and expression. The variation between samples from different spots may have been due to sampling variations and leaf residue in thatches.

Additionally, according to Figure 9, dollar spot inoculum increased from the end of August. And the ammonium sulfate treatment inhibited the increase of dollar spot compared with untreated samples.

Overall Project Objective 2: Determine impact of fungicide class and number of applications on the development of fungicide resistance in *Clarireedia* spp. populations through *in vitro* assays and molecular techniques.

Year 3 Goals: For 2020, there were two main goals: (1) determine field competitiveness of previously described SDHI resistant dollar spot mutants and (2) develop a loop-mediated isothermal amplification (LAMP) approach for detecting *Clarireedia* spp. in the field with a future application in detecting resistance alleles from field samples more rapidly.

Massachusetts Update:

Field competitiveness of previously described SDHI resistant dollar spot mutants.
In the summer of 2020, a study was conducted on university research plots to identify the field competitiveness of the SDHI-resistant dollar spot mutant populations in response to fungicide treatment in the field. As described in Popko et al. 2018, Lee et al. 2020, and Lee et al. 2021(accepted) unique resistance patterns to different SDHI fungicides (boscalid, fluxapyroxad,

isofetamid, fluopyram, pydiflumetofen) are the result of several distinct point mutations that occur on the succinate dehydrogenase (SDH) enzyme, or Complex II involved in fungal respiration. As SDHI resistance of dollar spot becomes more widely observed in New England golf courses, it is important to understand the field competitiveness of SDHI resistant mutant isolates in the presence and absence of fungicides. The information gained in this study will allow for better understanding of epidemiology of SDHI resistant and sensitive populations and a more accurate and informed monitoring of SDHI resistant populations.

Three different dollar spot isolates were chosen for the study; a fungicide-sensitive field isolate (HRS10), a field isolate with B-H267Y mutation in SDH enzyme (J5) and a field isolate with a C-G91R mutation in SDH enzyme (M1). In previous studies, all resistant isolates were highly resistant to boscalid as compared to sensitive isolates, whereas all isolates harboring B-H267 mutations, including J5, retained high sensitivity to fluopyram in vitro and in-field. Based on these very significant and consistent results across multiple studies, these treatments were chosen for the current field competition study. Five fungal inoculum treatments were chosen as listed below, and all inoculated treatments were sprayed with either a water control, 0.18 fl oz/ 1000 sq ft boscalid (Emerald, BASF Corporation) or 0.15 fl oz/ 1000 sq ft of fluopyram (Bayer). This experiment was carried out in three replicates.

1. Treatment #1- Un-inoculated
2. Treatment #2- 100% of HRS10 inoculated
3. Treatment #3- 100% of J5 inoculated
4. Treatment #4- 100% of M1 inoculated
5. Treatment #5- 33.3% of each HRS10, J5 and M1 inoculated (Mix)

Depending on the dollar spot activity in the field, 10 samples per plot were collected as dollar spot infected leaf blades. The leaf blades were bleached with 10% Clorox and dried in sterile filter paper. Processed leaf blades were grown in acidic agar plates and agar plugs (5mm diameter) of mycelia were transferred into freshly prepared PDA plates. DNA was extracted from mycelia and detection of either mutation (B-H267Y or C-G91R) was conducted using the CAPS/dCAPS molecular marker system described in in Lee et al. 2020 (Year 2, Objective 2 from this grant). The sample collection and fungicide treatment days are listed in Table 3. Results observed in the 1.5% agarose gel following enzyme digestion were used to identify the genotype each sample.

Table 3: Summarized table of fungicide treatments and sample collection.

Date	**Activity**	**Sampling/Spraying Information**
Jun 12, 2020	Plot inoculation with fungal isolates	-
Jun 23, 2020	Initial sampling- S1 First Fungicides Spray	Collected 10 infected leaf blades from all 5 treatments- Total 450 samples
Jul 09, 2020	Second sampling- S2 Second Fungicides Spray	Collected infected leaf blades from treatment 1 (Un-inoculated) and 5 (Mix) treatments- Total 180 samples
Aug 28, 2020	Third Fungicides Spray	No sampling- Due to not much activity of dollar spot
Sept 04, 2020	Third Sampling- S3	Collected 10 infected leaf blades from all 5 treatments- Total 450 samples

Vegetative compatibility groups (VCG): The vegetative compatibility of each SDHI mutant dollar spot isolate were tested along with another fungicide sensitive isolate (Natural- identified from university research plots). According to the results, each of 4 fungal strains were vegetatively compatible as shown in Figure 10.

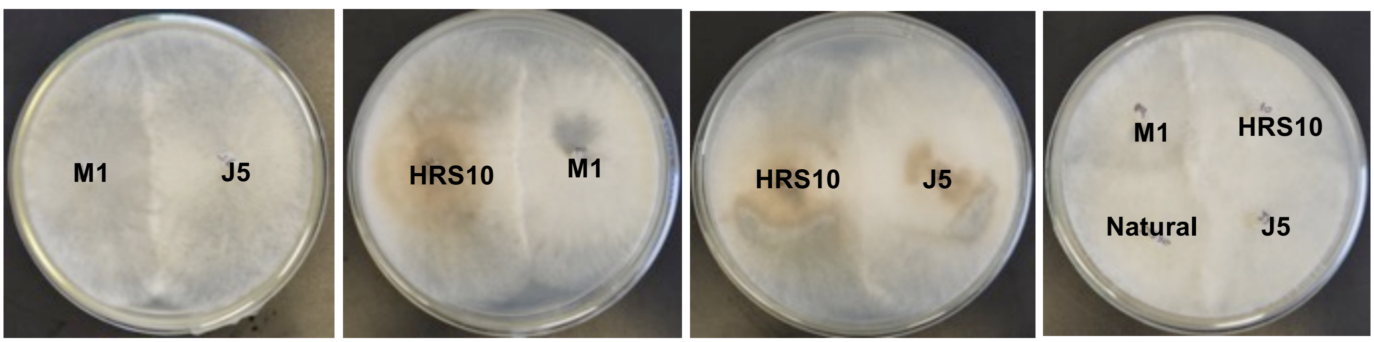


Figure 10: Vegetative compatibility of each selected isolates.

During the analysis of each DNA extractions and respective restriction enzyme digestion after the PCR, we observed that some purified DNA digestions had both digested and undigested bands in its gel images, and we hypothesized this happened due to the vegetative compatibility. This new genotype we identified as J5+M1 for the data analysis. The gel images were displayed like showed in Figure 11. However, further experiments will be required to validate the findings in the future.

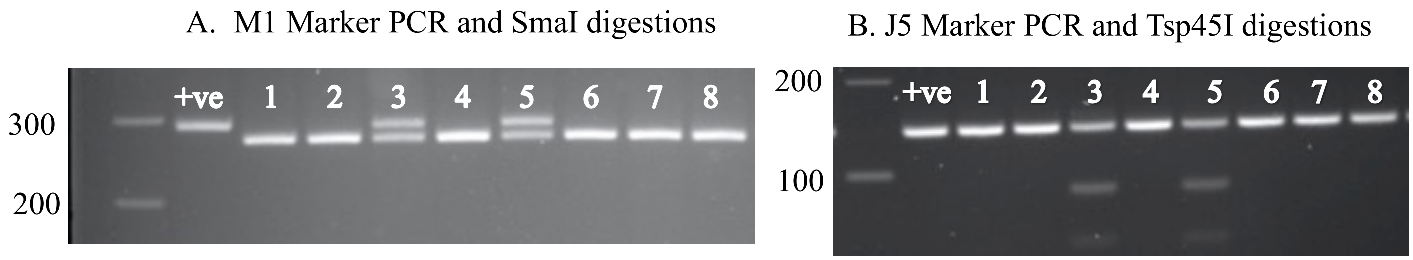


Figure 11: Gel images of restriction digestions of DNA purified from some selected samples. Lane 1 to 8 were shown cleaned DNA upon the PCR with respective dCAPs/CAPs marker and digested with respective restriction enzyme. Lanes 3 and 5 of A and B show both digested and undigested band patterns (two bands). In B, 1, 2, 4, 6, 7 and 8 are not digested with Tsp45I and in A, those lanes are digested with SmaI confirms that these isolates contains J5 mutation. Digested with the respective enzyme confirms that the mutation is not present in the DNA sample and undigested confirms that the mutation is present in the DNA sample (Lee et al. 2020).

Following the marker test, each of the samples were genotyped by their presence or absence of a mutation/genotype and Chi-square tests were used to calculate the p-values for interpreting the significance. According to the results, treatments #2, #3 and #4 had no significant changes in the population composition over the course of the study, meaning almost all samples collected from plots originally inoculated with HRS10, J5 or M1 were categorized as mostly same following enzyme digestion. In the treatment #1, J5 was abundant where no fungicides or boscalid was applied through the sampling 3, but M1, J5+M1 and HRS10 were abundant where treated with fluopyram showing significant difference of population changes in un-inoculated field plots. Interestingly, where no fungicides or boscalid were applied, dollar spot severity was very high on plots that were inoculated with J5 (Trts #3 and #5), as compared to other treatments, but much lower severity was observed on J5 plots treated with fluopyram. Further, on plots treated with a mix of all three isolates (Trt #5), a significant change in the population composition was observed where no fungicides, boscalid or fluopyram applied as compared to the expected population, with J5 occurring more frequently in the sampled populations than the other isolates. This suggests that J5 may be more competitive in the field even with no treatment of fungicides as compared to HRS10 and M1 isolates (Table 4).

Table 4: Chi-square analysis of Treatment #5, plots inoculated with 33.3% of each J5, M1 and HRS10.

			Average Frequency								
			Observed				Expected				Chi-Square test
Sampling	SDHI isolate/Fungicide treatment	Number of samples	J5	M1	J5+M1	HRS10+ Natural	J5	M1	J5+M1	HRS10+ Natural	
	Mix/Unsprayed	23	0.87	0.14	0	0	0.25	0.25	0.25	0.25	***
	Mix/Fluopyram	26	0.8	0.2	0	0	0.25	0.25	0.25	0.25	***
S1	Mix/Boscalid	24	0.69	0.28	0.05	0	0.25	0.25	0.25	0.25	***
	Mix/Unsprayed	27	0.66	0.04	0.22	0.1	0.25	0.25	0.25	0.25	***
	Mix/Fluopyram	26	0.35	0	0.65	0	0.25	0.25	0.25	0.25	***
S2	Mix/Boscalid	25	0.68	0	0.33	0	0.25	0.25	0.25	0.25	***
	Mix/Unsprayed	28	0.47	0.33	0.22	0	0.25	0.25	0.25	0.25	***
	Mix/Fluopyram	28	0.14	0.79	0.08	0	0.25	0.25	0.25	0.25	***
S3	Mix/Boscalid	25	0.41	0.48	0.13	0	0.25	0.25	0.25	0.25	***

S1- Sampling 1, S2- Sampling 2, S3- Sampling 3, ***- Significant at 0.1

Development of a LAMP detection technique for dollar spot in-field

Recent application of LAMP for COVID-19 detection inspired us to explore the method as a means for detecting dollar spot quickly in-field. Loop-mediated isothermal amplification requires a minimum of 4 ‘core’ primers (BIP, FIP, B3 and F3) and 2 optional ‘loop’ primers. Four sets of core primers were designed using a LAMP primer design tool developed by New England Biolabs in August 2020. Using a LAMP reaction mix (NEB #M1800) and known samples of dollar spot and other fungi, developed primer sets were tested for specificity and sensitivity under a wide variety of conditions (heating block vs thermocycler; incubation temperature; primer concentration; reaction preparation temperature, w/ and w/out loop primers, etc) to track and minimize the occurrence of false positives and false negatives. This LAMP reaction mix includes a pH dependent dye to allow for visualization of target DNA amplification in real time as the mix changes from pink to yellow following Bst polymerase activity. Color change has been quantified by measuring the absorbance ratio at 432 and 560 nm using a Cytation 3 Imaging Reader.

At present, two primer sets appear promising, though additional optimization experiments are necessary to better understand occasional variation in results. Figures 12 and 13 are two examples of variation seen in LAMP experiments. Best results with the fewest false positives have occurred where fresh primer stock solutions have been prepared just prior to reactions, so careful precautions must be taken to avoid carryover contamination. Thorough mixing of reagents prior to incubation is also highly important. Results using LAMP have consistently been visualized within 20 minutes of incubation at 65 degrees. At optimized conditions, this could be a very useful tool for detecting dollar spot quickly on-site. Future applications of this technique may include asymptomatic detection of dollar spot or detection of fungicide resistance alleles (particularly SDHI fungicide class) with proper primer sets targeting appropriate sequences.

For application in a true field setting, crude DNA extraction methods have also been explored as part of this project to find a consistent, reliable, and field-friendly alternative for extracting DNA directly from dollar spot infected leaf blades. So far, we have observed strong bands following PCR with DNA extracted using a method that involves boiling the tissues in extraction buffer for 10 minutes. However, we suspect multiple leaf blades (3-5) may be required for proper LAMP detection.

Figure 12: Preliminary sensitivity testing of core primer set P3 with three different loop primer set options (L3. L11 and L18) using DNA extracted from three field isolates.

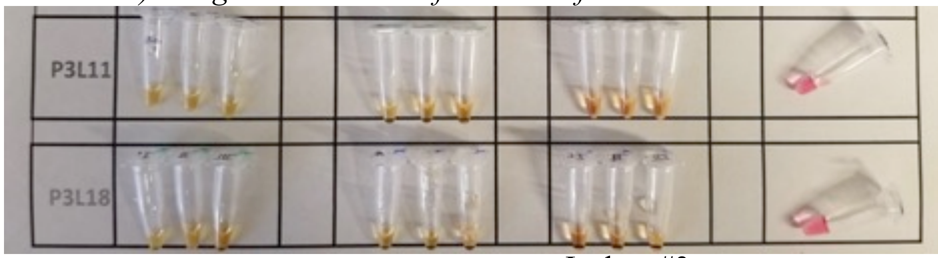
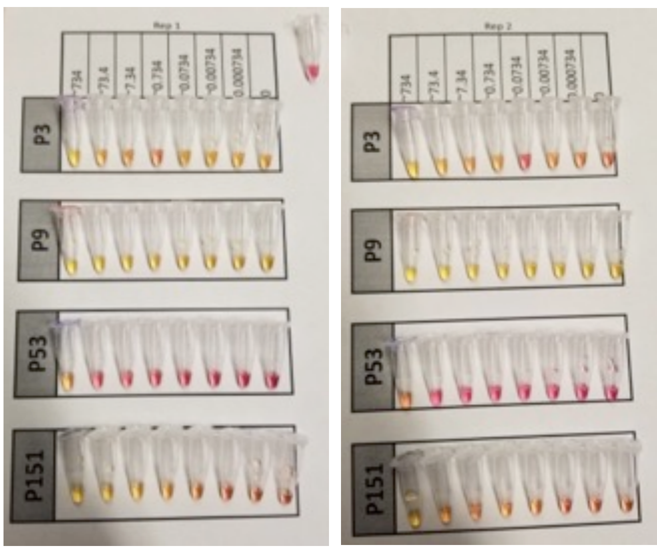


Figure 13: Sensitivity testing of four core primer sets (P3, P9, P53 and P151) using 10-fold dilutions of DNA extracted from Clarireedia spp.



USGA ID#: 2019-09-679

Using the Smith-Kerns Dollar Spot Model for precision management of dollar spot on golf course fairways

Paul Koch[1], Kurt Hockemeyer[1], Josh Friell[2], Walker Olson[2]
[1]Department of Plant Pathology, University of Wisconsin – Madison
[2]Toro Company, Bloomington, MN

Objective: Schedule dollar spot fungicide applications in different areas of a golf course based on site conditions using weather monitoring stations and the Smith-Kerns Dollar Spot Model.

Year 2 Summary: The trial consisted of three treatments in a randomized complete block design with 4 replications and an individual plot size of 6 x 10 feet. The treatments were a non-treated control, a traditional calendar-based fungicide program, and fungicide applications scheduled using the Smith-Kerns Dollar Spot Model. The trial was replicated at 3 nearby sites: the 7th hole at University Ridge GC in Madison, WI, the 18th hole at University Ridge, and the OJ Noer Turfgrass Research Facility located on land adjacent to University Ridge. The trial at the OJ Noer had a 4th treatment that was testing the impact of the slope of the dollar spot model probability on fungicide scheduling, which we refer to as the Clarke Correction based on research conducted by Dr. Bruce Clarke. This 'Clarke Correction' treatment at the OJ Noer was not related to the precision dollar spot research that was the focus of this project.

Coming into this experiment we expected dollar spot to be most severe on Hole 18 and lower on both Hole 7 and the OJ Noer. This is based on both the perceived microclimate and the experience of University Ridge Superintendent Phil Davidson. Hole 18 is buried in the woods (Figure 1) with limited air movement and Phil Davidson says that is always the first location on the course to develop dollar spot. Hole 7 is more open with ample air movement and Phil Davidson says that dollar spot pressure is normally less severe at that location (Figure 2). The OJ Noer environment is more similar to Hole 7 so we expect the conditions to mirror those on Hole 7.

To address the stated objective, we placed a weather sensor with the ability to calculate the Smith-Kerns dollar spot model adjacent to one of 3 research plots (Figure 3). These weather sensors then transmitted the data to the cloud and automatically calculated the Smith-Kerns Dollar Spot Probability and presented it on a web-based platform that we could access. We partnered with Josh Friell and Walker Olson of The Toro Company to develop beta versions of these weather sensors and used their platform to access the weather and Smith-Kerns data. Though the initial sensors tested in 2019 were not successful, the stations created in 2020 were successful until a piece of the sensor malfunctioned in mid-July. The malfunction was related to a 3rd party and by the time it was identified and a remedy developed it was too late in the season for the repair to make any meaningful difference.

Despite the late summer malfunction, the successful use of all 3 sensors for May, June, and most of July allowed us to collect important data. There were clearly differences in dollar spot pressure among the three locations that indicates that precision dollar spot management could be an effective way to more efficiently manage dollar spot. Hole 18 had the most dollar spot during the first part of the season and had comparatively

less later in the season (Table 1). On hole 7, dollar spot was much lower in the beginning of the season and gradually increased throughout the season (Table 2). The OJ Noer plot also had relatively low dollar spot numbers early in the season before increasing rapidly in mid-summer.

The Smith-Kerns probability at each site also showed differences between the sites at key times of the year (Figure 4). The probability on Hole 7 and the OJ Noer were largely similar throughout the year, which is what we expected given their surrounding environment. The probability on Hole 18 was similar to Hole 7 and the OJ Noer during some of the summer, but was markedly higher during periods in mid-June, early July, and mid-July. This corresponds with times of significant disease development on Hole 18. For example, Smith-Kerns probability on Hole 18 was nearly 20 points higher than Hole 7 and the OJ Noer for 4-5 days in early July and dollar spot severity exploded on Hole 18 (223 dollar spot foci on July 8th) and remained moderate on Hole 7 (49 dollar spot foci on July 8th). Somewhat confoundingly, the dollar spot severity at the OJ Noer also exploded on July 8th despite the Smith-Kerns probability being similar to Hole 7. Lastly, since the Smith-Kerns probabilities were largely over 20% at all 3 sites, we didn't observe any meaningful difference in the number of spray applications between the sites in our study.

Conclusions and Future Directions: The data in this 2-year preliminary study clearly indicates that dollar spot microclimates exist on golf courses and that severity within those microclimates can be predicted using small weather stations positioned strategically around a golf course. This has great potential to limit future fungicide usage by not only targeting WHEN a fungicide is required for dollar spot control, but WHERE on the course the environment is conducive for disease development.

Future advancements in this area of research should focus on both the technology to accurately measure and predict dollar spot pressure and the biology of the dollar spot system. The technology advancements will rely on the continued development of affordable and dependable weather stations that can be placed around a golf course and provide reliable measurements in real time of dollar spot probability. The biological advancements will be in determining how to identify different microclimates on a golf course and determining what differences in probability actually results in meaningfully different spray schedules.

Though there were several difficulties in conducting this research study, the study did successfully demonstrate that dollar spot develops on a site-specific basis (i.e microclimates) and that those sites can be predicted to at least some degree using the Smith-Kerns Dollar Spot Model and small, targeted weather stations. Continued advancement of this research will likely lead to the eventual implementation of precision disease management on golf courses, which will result in significant reductions in fungicide usage and significant financial savings to the golf course superintendent.

Table 1. Mean number of dollar spot infection centers per treatment at University Ridge 18 fairway in Madison, WI in 2020.

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jun 24	Jul 8	Aug 5
1		Non-treated control			78.3a	223.0a	5.5a
2	Standard Program	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 OZ/1000 FT2 2.0 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 0.5 OZ/1000 ft2 0.5 FL OZ/1000 FT2 0.26 FL OZ/1000 FT2 0.31 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 2.0 FL OZ/1000 FT2	May 29 Jun 25 Jul 16 Jul 30 Jul 30 Aug 13 Sep 10 Oct 8 Oct 22	15.0a	18.3a	0.0a
3	Smith-Kerns model: Standard	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 OZ/1000 FT2 2.0 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 0.5 OZ/1000 ft2 0.5 FL OZ/1000 FT2 0.26 FL OZ/1000 FT2 0.31 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 2.0 FL OZ/1000 FT2	28 day 21 day 14 day 14 day 28 day 28 day 14 day 14 day	76.0a	33.8a	0.0a
				LSD P=.05	101.59	175.93	10.99

[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Table 2. Mean number of dollar spot infection centers per treatment at University Ridge 7

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jun 24	Jul 8	Aug 5
1		Non-treated control			9.0a	49.0a	320.8a
2	Standard Program	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 OZ/1000 FT2 2.0 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 0.5 OZ/1000 ft2 0.5 FL OZ/1000 FT2 0.26 FL OZ/1000 FT2 0.31 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 2.0 FL OZ/1000 FT2	May 29 Jun 25 Jul 16 Jul 30 Jul 30 Aug 13 Sep 10 Oct 8 Oct 22	2.0a	5.5a	131.0a
3	Smith-Kerns model: Standard	Emerald Banner Maxx Interface Velista Secure Xzemplar Pinpoint 26 GT Banner Maxx	0.18 OZ/1000 FT2 2.0 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 0.5 OZ/1000 ft2 0.5 FL OZ/1000 FT2 0.26 FL OZ/1000 FT2 0.31 FL OZ/1000 FT2 4.0 FL OZ/1000 FT2 2.0 FL OZ/1000 FT2	28 day 21 day 14 day 14 day 28 day 28 day 14 day 14 day	17.5a	18.5a	160.8a
				LSD P=.05	20.23	66.88	266.31

[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Table 3. Mean number of dollar spot infection centers per treatment at the OJ Noer Turfgrass Research and Education Facility in Madison, WI in 2020.

		Treatment	Rate	Application Date/Interval	Dollar spot severity[a]		
					Jun 24	Jul 8	Aug 5
1		Non-treated control			28.0b	270.0a	485.0a
2	Standard Program	Emerald	0.18 OZ/1000 FT2	May 29	7.5c	27.3b	5.3b
		Banner Maxx	2.0 FL OZ/1000 FT2	Jun 25			
		Interface	4.0 FL OZ/1000 FT2	Jul 16			
		Velista	0.5 OZ/1000 ft2	Jul 30			
		Secure	0.5 FL OZ/1000 FT2	Jul 30			
		Xzemplar	0.26 FL OZ/1000 FT2	Aug 13			
		Pinpoint	0.31 FL OZ/1000 FT2	Sep 10			
		26 GT	4.0 FL OZ/1000 FT2	Oct 8			
		Banner Maxx	2.0 FL OZ/1000 FT2	Oct 22			
3	Smith-Kerns model: Standard	Emerald	0.18 OZ/1000 FT2	28 day	48.3a	75.3b	5.5b
		Banner Maxx	2.0 FL OZ/1000 FT2	21 day			
		Interface	4.0 FL OZ/1000 FT2	14 day			
		Velista	0.5 OZ/1000 ft2	14 day			
		Secure	0.5 FL OZ/1000 FT2				
		Xzemplar	0.26 FL OZ/1000 FT2	28 day			
		Pinpoint	0.31 FL OZ/1000 FT2	28 day			
		26 GT	4.0 FL OZ/1000 FT2	14 day			
		Banner Maxx	2.0 FL OZ/1000 FT2	14 day			
4	Smith-Kerns model: Clarke Correction	Emerald	0.18 OZ/1000 FT2	28 day	37.5a	57.8b	29.0b
		Banner Maxx	2.0 FL OZ/1000 FT2	21 day			
		Interface	4.0 FL OZ/1000 FT2	14 day			
		Velista	0.5 OZ/1000 ft2	14 day			
		Secure	0.5 FL OZ/1000 FT2				
		Xzemplar	0.26 FL OZ/1000 FT2	28 day			
		Pinpoint	0.31 FL OZ/1000 FT2	28 day			
		26 GT	4.0 FL OZ/1000 FT2	14 day			
		Banner Maxx	2.0 FL OZ/1000 FT2	14 day			
				LSD P=.05	16.88	54.31	115.31

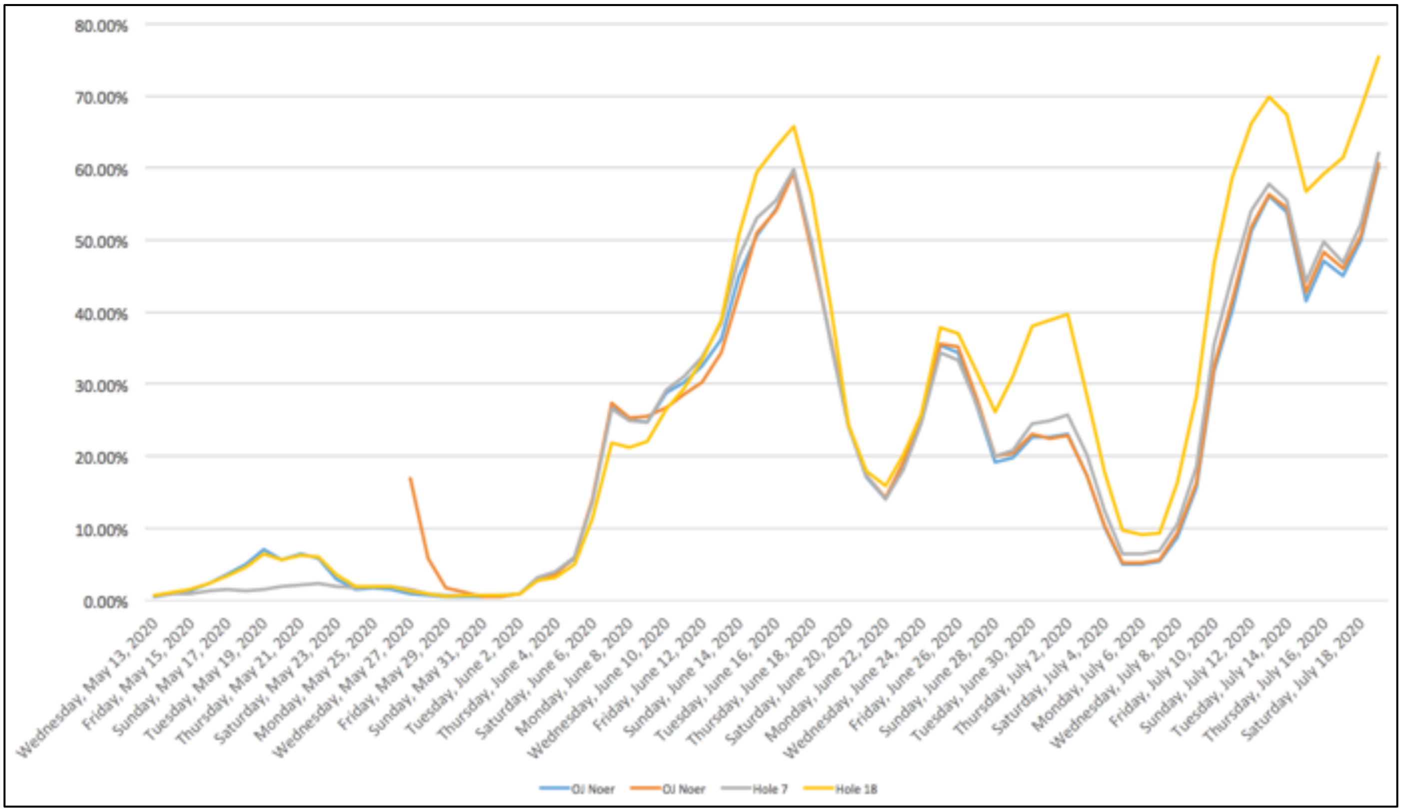
[a]Dollar spot was visually assessed as number of dollar spot infection centers per plot. Means followed by the same letter do not significantly differ (P=.05, Fisher's LSD). Means followed by dashes indicate no significant differences were observed among any of the treatments.

Figure 1. Hole 18 test site at University Ridge GC in Madison, WI in 2020.

Figure 2. Hole 7 test site at University Ridge GC in Madison, WI in 2020.

Figure 3. The weather station custom-built by Walker Olson at Toro at the Hole 18 research site. The same sensors were also placed at Hole 7 and the OJ Noer.

Figure 4. Smith-Kerns Dollar Spot Model Probabilities at the OJ Noer Center and Hole 7 and 18 at University Ridge GC in Madison, WI during the summer of 2020.



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: Include 3-6 bullet points that summarize the findings of your project to date
- 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
- Identify golf courses collaborating in this study

Summary Text:

Collection and isolation of *Gaeumannomyces* species from bermudagrass putting greens. The essential element for our program is to identify golf courses having take-all root rot problems in bermudagrass putting greens of Texas golf courses. 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 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 amended with mefenoxam, flutolanil, and iprodione). Plates were incubated at 25°C and were monitored for hyphae that curled back at the edges of fungal colonies, one of the typical cultural characteristics of *Gaeumannomyces* species. Hyphal tips were then transferred to PDA for isolation. Long-term storage of each isolate was achieved by keeping mycelial agar plugs in a 4-ml clear glass screw cap vial containing 1.5 ml of sterile distilled water. Vials were sealed with parafilm and kept in the dark at ambient temperature.

Development of molecular diagnosis of *Gaeumannomyces* species. Genomic DNA was extracted from each *Gaeumannomyces* isolate for the DNA-based diagnosis. Each isolate was grown on PDA at 25°C until petri dishes were entirely colonized. DNA isolation from harvested mycelium was conducted using ZYMO DNA Miniprep Kit. Internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using PCR with the previously-developed ITS1/ITS4 primer set. PCR was performed using Thermo Scientific Phire Plant Direct PCR Master Mix Kit. PCR amplicons were sent to Eton Biosciences for sequencing. In our initial screening, we found dominant isolates were determined as *Gaeumannomyces graminicola.*

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 four days 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 and un-inoculated control plants (Fig. 1). The fungus did not cause complete wilt of plants but inhibit plant growth.

Future approaches and expectations. Upon the confirmation of infection by *Gaeumannomyces* species, we try to implement a more integrated management approach for take-all root rot for bermudagrass putting greens and subsequently evaluate benefits of the new approach under field conditions. To achieve this goal, we will conduct surveys and implement a management program targeted for take-all root in bermudagrass putting greens for golf courses in collaboration. Golf course superintendents will participate in our survey to measure economic impact of take-all root rot management. The survey will collect information relevant to bermudagrass putting green management including rates and frequency of chemical applications (fertilizers, herbicides, fungicides, and insecticides) and labor cost for turf maintenance (mowing, irrigation, topdressing and aeration). Twenty golf courses in central and southern Texas will be identified and included in the second year of the project. Turf samples will be collected from these golf courses. Causal *Gaeumannomyces* species will be isolated, identified and characterized based on aforementioned methods developed in this study.

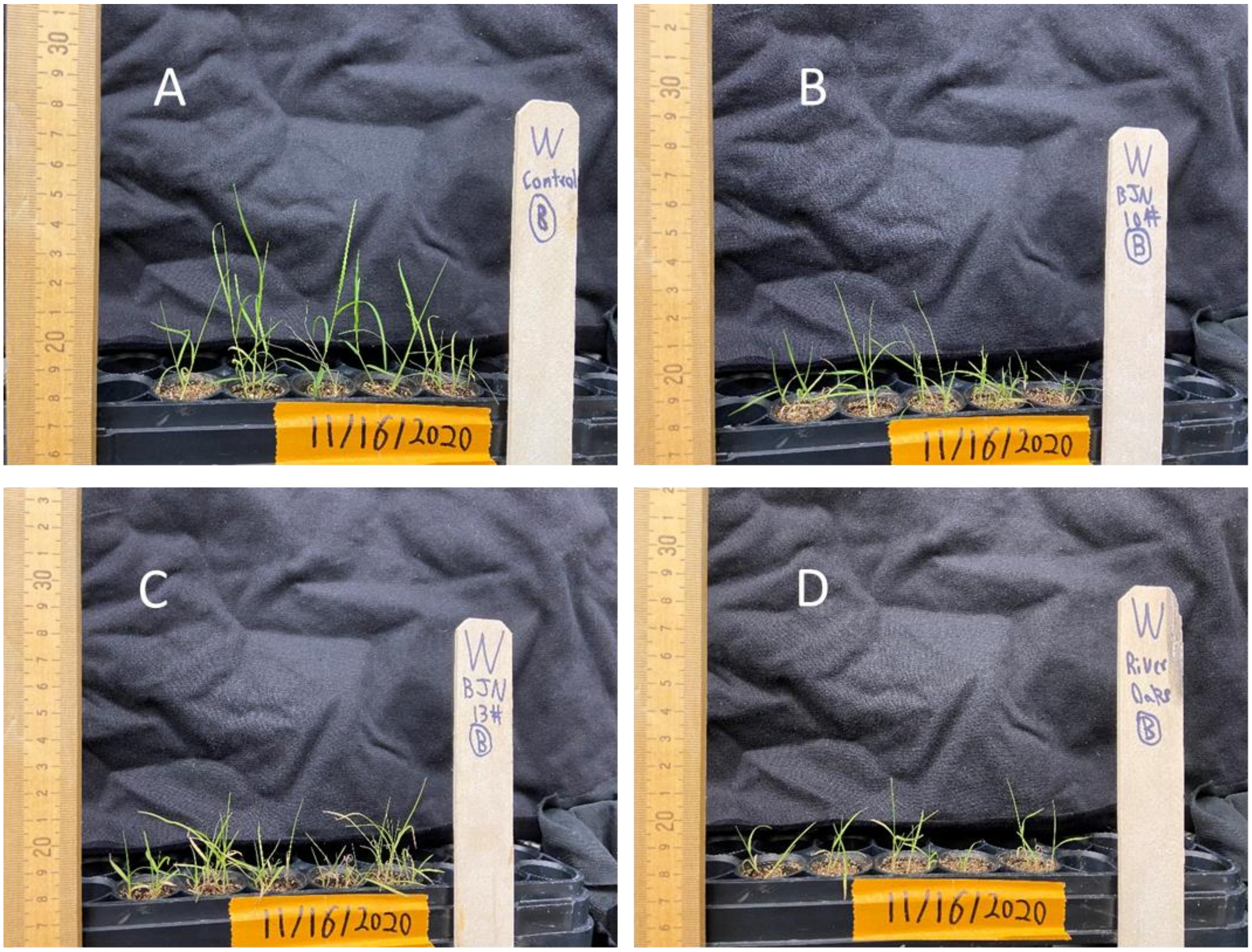


Figure 1. Pathogenicity assay for take-all root rot. Bermudagrass seedlings inoculated with three different isolates of *Gaeumannomyces* species, BJN10 (B), BJN13 (C) and RO17 (D) were compared with un-inoculated control plants (A).

USGA ID#: 2020-07-712

Title: Quantify the effects of iron sulfate on the growth rate of *Microdochium nivale in vitro* and develop a protocol for quantifying the pH of iron sulfate amended growth media

Lead Author: Clint Mattox
Project Leader: Alec Kowalewski
Affiliation: Oregon State University

Objectives: With the knowledge that iron sulfate changes the pH of growth media *in vitro*, a series of laboratory experiments were designed to quantify how iron sulfate affects the growth rate of *Microdochium nivale in vitro*. In addition, because pH is so important to this topic, another objective of this study was to develop a protocol for quantifying the pH of iron sulfate amended growth media.

Start Date: January 2020
Project Duration: 1 year
Total Funding: $17,400

Summary Points:

- The use of flat-tipped pH electrodes permitted a more robust method of quantifying the pH of growth media at ambient temperatures.
- Iron sulfate reduced the pH of the media over the course of the experiment, suggesting that it is important to note this change in trials of this type in order to avoid mis-interpreting results.
- Results indicate that pH changes caused by iron sulfate treatments is not the only mechanism behind suppression of *Microdochium nivale in vitro*.

Summary:

Multiple laboratory experiments were designed to quantify how iron sulfate suppresses *M. nivale in vitro*. Previous experiments at Oregon State University showed that the pH of the iron sulfate amended growth media was not stable, therefore the first task was to develop a protocol for quantifying the pH of the media that was modified with iron sulfate. After multiple iterations, it was decided that the most consistent method of obtaining a predictable growth media pH was to modify the pH of the media post-autoclave. In order to achieve this goal, we used a heated water bath to maintain the media post-autoclave in the liquid state. This method permitted us to calibrate our pH meter at the same temperature by placing the standards in the water bath for calibration. Once the media was modified with the experimental treatments, the media was poured into plates, and cooled. We then tested the pH of the surface of an extra plate (which we called the “witness plate”) using a flat-tipped electrode pH meter (Image 1). We maintained the witness plate under the same conditions of the other plates in the experiment with the exception that this plate was not inoculated with *M. nivale*. This technique provided a more robust means of quantifying the pH of the media at the ambient temperature that *M. nivale* was exposed to and permitted pH quantification of the witness plate throughout the experiment.

Image 1: Flat-tipped electrode.

Once the surface media pH was quantifiable, we designed an experiment to test the effects of iron sulfate on the growth of *M. nivale*, by testing iron sulfate at different concentrations as well as the effects of iron applied as DTPA chelated iron, and the effects of sulfate applied as magnesium sulfate. By quantifying the pH of the surface of the media at the beginning and the end of the experiments, we were able to make more robust conclusions. Table 1 provides the results for the first of two runs. The results indicated that *M. nivale* was suppressed compared to the non-treated control when exposed to media amended with $FeSO_4$ at 100 or 500 ppm Fe and when amended with chelated DTPA at 500 ppm Fe. While all of the treatments had an initial media surface pH of between 3.9 and 4.0, the pH of the witness plate for $FeSO_4$ at 100 and 500 ppm Fe decreased to 3.6 and 3.4 fourteen days after the trial began. This indicates that the pH change cannot be ruled out as a possible mechanism of *M. nivale* suppression.

Treatment	media pH Run 1 Start	*M. nivale* (mm^2)	media pH + 14 days
10 ppm Fe ($FeSO_4$)	3.99	4363 bc†	4.03
10 ppm Fe (DTPA)‡	3.93	5205 ab	4.06
SO_4 equivalent to 10 ppm Fe ($FeSO_4$)	3.88	4671 b	3.99
100 ppm Fe ($FeSO_4$)	3.94	603 d	3.59
100 ppm Fe (DTPA)	3.94	3608 c	4.02
SO_4 equivalent to 100 ppm Fe ($FeSO_4$)	3.91	5323 ab	3.99
500 ppm Fe ($FeSO_4$)	3.92	0 d	3.44
500 ppm Fe (DTPA)	3.96	999 d	3.99
SO_4 equivalent to 500 ppm Fe ($FeSO_4$)	3.92	5938 a	4.02
non-treated control	3.94	4592 bc	3.95

Table 1. The effects of iron sulfate heptahydrate, chelated iron, and magnesium sulfate heptahydrate on Microdochium nivale in vitro.
†Means in the same column followed by the same letter are not statically significant according to Tukey's Test at $P \leq 0.05$.
‡DTPA = ferrous chelate.

The second series of experiments looked at the effects of iron sulfate and lactic acid on pH and *M. nivale in vitro*. The intention of this step was to compare the suppression of *M. nivale* by iron sulfate to plates with a similar pH generated using lactic acid in order to quantify if pH, iron, or perhaps both pH and iron were causing the suppression of *M. nivale*. Witness plates for assessing pH changes throughout the trial were set aside as described for the previous experiment. The results of both experimental runs were similar and the results of run 2 are shown in Table 2. Similar to the first trial, the pH of the surface of the iron sulfate amended plates decreased over the course of the experiment. In this second experiment, it was possible to compare how the iron sulfate amended treatments compared to the media amended with lactic acid. The witness plate suggested that the pH of the media decreased as the concentration of both lactic acid and iron sulfate increased and also that the pH of the iron sulfate amended media decreased over time whereas the pH of the lactic acid amended was relatively stable. By quantifying the area of growth of *M. nivale* observed, it was shown that lactic acid concentrations greater than 999 ppm and iron concentrations greater than 100 ppm suppressed *M. nivale* compared to the control plates. Comparing the growth of *M. nivale* on plates with similar pH levels at the end of the experiment suggests that the drop in pH alone does not fully explain the suppression of *M. nivale* by the iron sulfate amended media. In particular, the plates amended with 200 and 300 ppm of iron had a pH at day 10 of the experiment of 3.6 and these iron concentrations suppressed *M. nivale* more than the 4975 ppm lactic acid amended plate with a final pH of 3.6.

Treatment	media pH Run 2 Start	*M. nivale* (mm^2)	media pH + 10 days
Control	5.2	5553 a†	5.2
500 ppm LA‡	4.6	5542 a	4.6
999 ppm LA	4.4	4464 b	4.4
4975 ppm LA	3.7	698 c	3.6
9898 ppm LA	3.4	139 d	3.4
14778 ppm LA	3.4	22 d	3.2
19608 ppm LA	3.2	0 d	3.2
10ppm Fe§	4.9	5476 a	4.7
100ppm Fe	4.3	514 c	3.8
200ppm Fe	4.1	138 d	3.6
300ppm Fe	4.0	66 d	3.6
400ppm Fe	4.0	39 d	3.5
500ppm Fe	3.9	7 d	3.5

Table 2. **The effects of pH amended media using iron sulfate heptahydrate and lactic acid on the expansion of *M. nivale in vitro*.**
†Means in the same column followed by the same letter are not statically significant according to Tukey's Test at P ≤ 0.05.
‡ LA = 1 Molar Lactic acid § Fe amended as $FeSO_4 \cdot 7H_2O$

Future expectations of the project:

The findings of this project will be submitted for a future manuscript publication and will also be included in future state, national, and international presentations.

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-two report is presented here)
Total Funding: $30,000

Summary Points:

- All treatments suppressed Microdochium patch compared to the not-treated control with the exception of 0.1 lbs. of iron applied every two weeks as DTPA iron in the first two years and 0.2 lbs. of iron applied as DTPA in the second year of the study.
- In the first two years of the study, less than 1.5% Microdochium patch was observed when any rate of iron regardless of source was applied in combination with phosphorous acid compared to the non-treated control with greater than 40% disease.
- Turfgrass quality was only considered acceptable throughout the trial for the fungicide control treatment. High rates of iron sulfate or iron sulfate plus phosphorous acid provided disease control, although these treatments darkened plots and produced some turfgrass thinning.

Summary:

The second year of this three-year study began on September 6th, 2019 and the last application was made on March 30th, 2020. The third-year applications began on September 5th, 2020 and will continue through March 2021. Table 1 provides the peak of disease for Microdochium patch for the first two years as well as turfgrass quality data on these dates. The results from the second-year data are similar to the first year. There was similar disease pressure with 45% and 50% disease on average for the not-treated control in both years respectfully. All treatments suppressed Microdochium patch compared to the not-treated control with the exception of 0.1 lbs. of

Figure 1. Overview of the Microdochium patch disease pressure on 11 February 2020 in Corvallis, OR.

iron applied every two weeks as DTPA iron in both years and 0.2 lbs. of iron applied at DTPA in the second year of the study. When iron was applied at 0.1 lbs. of iron per 1,000 square feet, iron sulfate did not suppress Microdochium patch more than DTPA iron, however at the 0.2 lbs. of iron per 1,000 square feet, iron sulfate did suppress Microdochium patch more than DTPA iron. The mechanisms behind the suppression of Microdochium patch by iron sulfate are not fully understood, however these field results and other laboratory studies suggest that the mechanism is not uniquely caused by the application of iron. This is most apparent when comparing the 0.2 lbs. of iron per 1,000 square feet rate in the absence of phosphorous acid, where iron sulfate consistently suppresses disease more than DTPA iron.

One of the objectives of this experiment is to test whether or not disease suppression is improved when phosphorous acid is added to the iron treatments. In the first two years of this study, adding phosphorous acid to the iron treatments improved disease suppression in all instances except for when 0.2 lbs. of iron was applied as iron sulfate because at this rate, iron sulfate applied alone already suppressed Microdochium patch to a high level. Indeed, the lower rate of iron sulfate, 0.1 lbs. of iron per 1,000 square feet, applied in combination with phosphorous acid suppressed disease as well as 0.2 lbs. of iron per 1,000 square feet in the absence of phosphorous acid. This finding suggests that iron sulfate levels can be combined with phosphorous acid in order to reduce iron inputs and achieve equivalent levels of Microdochium patch suppression as higher iron sulfate levels applied alone. When phosphorous acid was applied alone every two weeks, there was an average of 3.3% and 8.0% Microdochium patch observed in both years respectively.

	% Microdochium patch		Turf Quality	
	24 Jan. 19	11 Feb. 20	24 Jan. 19	11 Feb. 20
0.1 # Fe/M as $FeSO_4$	20.0% b[z]	26.3% bc[z]	5.0 abc[y]	4.3 ab[y]
0.1 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.4% c	1.1% d	5.0 abc	5.0 ab
0.2 # Fe/M as $FeSO_4$	1.8% c	0.4% d	5.0 abc	5.0 ab
0.2 # Fe/M as $FeSO_4$ 0.075 lbs. H_3PO_3 / M	0.0% c	0.1% d	5.8 ab	6.0 ab
0.1 # Fe / M as DTPA	32.5% ab	42.5% ab	4.0 bc	3.0 b
0.1 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.7% c	1.1% d	5.0 abc	5.0 ab
0.2 # Fe/M as DTPA	25.0% b	35.0% ab	4.0 bc	3.3 b
0.2 # Fe/M as DTPA 0.075 lbs. H_3PO_3 / M	0.1% c	0.3% d	5.3 abc	5.0 ab
0.075 lbs. H_3PO_3 / M	3.3% c	8.0% cd	5.0 abc	5.0 ab
Fungicide Control	0.0% c	0.0% d	7.3 a	8.0 a
Not-treated Control	45.0% a	50.0% a	3.3 c	2.8 b

Table 1. Letter diagram of effects of iron sources in the combination or the absence of phosphorous acid on percent Microdochium patch and turfgrass quality. [z] Means in the same column followed by the same letter are not significantly different according to Tukey's HSD (alpha ≤ 0.05). [y] Mean differences in the same column followed by the same letter are not significantly different according to Dunn's test (alpha ≤ 0.05).

Turfgrass quality was only considered acceptable throughout the trial for the fungicide control treatment because of the presence of disease to levels not considered acceptable for putting greens, the darkening of the plots by iron sulfate treatments, and some thinning of the plots from iron sulfate treatments (Figure 2). If these trials were to be performed on tees, fairways, or approaches, it is possible that similar results would yield turfgrass quality values considered to be acceptable to golf courses where traditional fungicide control options may not be available.

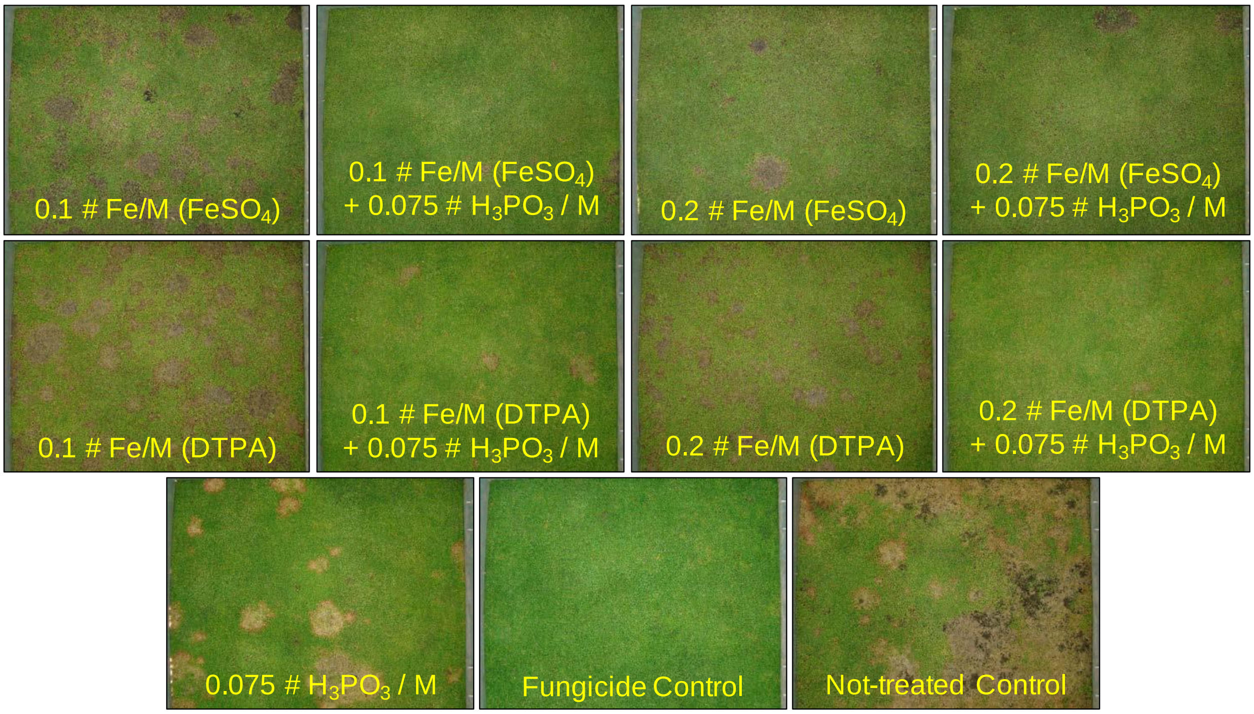


Figure 2. Light box photos collected at the peak of disease (11 February 2020) at the Oregon State University Lewis-Brown Horticulture Farm in Corvallis, OR.

Future expectations of the project:

This experiment is ongoing and will continue to assess Microdochium patch percentage and turfgrass quality. In addition, soil test analyses for all three years will be presented in the final report at the end of the three-year study. The results of this study will also be written as a manuscript and submitted to a scientific journal article and subsequent trade journal article. The results will also be presented at field days as well as at state, national, and international meetings.

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.

Start Date: September 2018
Project Duration: Three-year project (the year-two report is presented here)
Total Funding: $30,000

Summary Points:

- All treatments suppressed Microdochium patch severity to less than 2% compared to the control with the exception of phosphorous acid applied every two weeks.
- Plots that received either sulfur or iron sulfate in the winter months were in the group with the greatest anthracnose severity.
- There was no evidence that treatments affected putting green speeds or water infiltration.
- There was evidence that plots receiving phosphorous acid every two weeks resulted in an increased level of soil available phosphorus.

Summary:

This report focuses primarily on the second year of this ongoing long-term project. The second-year applications began on the 6th of September 2019 and ended on the 31st of March 2020. The third-year applications of this trial began on the 5th of September 2020 and are ongoing. In the second year of this study, Microdochium patch pressure was high with an average of 73% Microdochium patch on the 11th of February 2020 (Figure 1 and Table 1).

Figure 1. Overview of the Microdochium patch disease pressure on 11 February 2020 in Corvallis, OR.

Most treatments suppressed Microdochium patch to less than 2% compared to the non-treated control which was equivalent to the suppression provided by the standard traditional fungicide rotation. The one exception was the phosphorous acid application made every two weeks. This treatment resulted in significantly more disease than the fungicide rotation (12% Microdochium patch on the 11th of February 2020).

Treatment #	Description	Microdochium patch Feb 2020	Summer Anthracnose Response Aug. 2020
1	S[z] + PA[y]	0.1% c[x]	35.8%[w] a[x]
2	Sep, Oct, Nov, Apr = MO[v] + PA Dec, Jan, Feb, Mar = S + PA	0.5% c	24.8% abc
3	MO + PA rotated with S + PA	0.1% c	19.4% abcd
4	MO rotated with S + PA	0.7% c	28.6% ab
5	0.5 # $FeSO_4$ + PA	1.8% c	9.2% cde
6	1.0 # $FeSO_4$ + PA	0.0% c	9.5% cde
7	Sulfur (S)	0.7% c	10.8% bcde
8	Phosphorous Acid (PA)	11.8% b	4.9% ef
9	Fungicide Control	0.0% c	7.8% de
10	Non-treated control	72.5% a	1.0% f

Table 1. Letter diagram of effects of treatments applied every two weeks on percent Microdochium patch and anthracnose. [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. [v]MO = Mineral oil applied at 8.5 oz. 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] Means back-transformed from cube-root means.

One of the trial objectives is to observe the effects that the eight months of winter Microdochium patch treatments have on anthracnose severity the following summer. Anthracnose severity was very high in this trial, especially on treatments that received frequent sulfur or iron sulfate applications. Treatments listed on table 1 that received at least 2 lbs. of sulfur per 1,000 square feet per year included; treatments 1, 2, 3, 4, and 7. All of these treatments resulted in 10% or greater anthracnose in the summer compared to only 1 % anthracnose on the non-treated control plots that did not receive any treatments throughout the year. Plots receiving 0.5 lbs. or 1.0 lbs. of iron sulfate heptahydrate resulted in 9.2% and 9.5% anthracnose respectively, suggesting that iron sulfate applications may also be a concern regarding summer anthracnose severity.

Another objective of this trial is to quantify soil characteristics over time. Soil test results for samples collected from a 1" to 3" depth revealed that there was no clear treatment effect at this depth for soil pH, which is surprising considering the amount of sulfur and iron sulfate that was applied after two years (Table 2). Among the nutrients tested, one interesting observation was the change in phosphorus levels from 2019 to 2020. There was an increase in phosphorus levels in all plots that received phosphorous acid every two weeks except for plots that received 0.5 lbs. of iron sulfate heptahydrate in combination with phosphorous acid. It is unclear why this treatment did not also result in an increase compared to plots not receiving phosphorous acid. Iron in the soil is known to form iron-phosphate precipitates, which are not plant available. These results suggest that there may be some conversion of phosphorous acid to plant available forms of phosphorus over time.

An additional objective for this trial is to quantify changes in putting green characteristics. In order to quantify these characteristics in an area where disease pressure is otherwise high, half of the plots are sprayed with a preventative fungicide application. This side of the trial receives traffic equivalent to 73 golf rounds a day by walking over the plots 5 days a week with golf shoes. In 2020, water infiltration was recorded in May, after 8 months of treatments, in August after spring aerification and three months without treatments and in October after three applications had been made (Table 3). No significant differences were observed on any of the rating dates. Putting green speeds were quantified in July and October and there was no indication that treatments affect putting green speeds.

Treatment	May 2019 pH	May 2020 pH	Change in pH		May 2019 Phosphorus	May 2020 Phosphorus	Change in Phosphorus	
S[z] + PA[y]	6.42	6.15	-0.27	ns[x]	13.1	19.7	6.6	ab[w]
Sep, Oct, Nov, Apr = MO[v] + PA Dec, Jan, Feb, Mar = S + PA	6.50	6.15	-0.35	ns	12.5	19.2	6.7	ab
MO + PA rotated with S + PA	6.55	6.14	-0.42	ns	14.9	21.6	6.8	a
MO rotated with S + PA	6.48	6.10	-0.38	ns	15.4	15.8	0.4	d
0.5 # $FeSO_4$ + PA	6.61	6.36	-0.25	ns	14.8	17.6	2.9	cd
1.0 # $FeSO_4$ + PA	6.69	6.29	-0.40	ns	9.5	14.1	4.6	abc
Sulfur (S)	6.50	6.06	-0.44	ns	11.4	12.7	1.2	d
Phosphorous Acid (PA)	6.62	6.19	-0.43	ns	12.4	18.6	6.2	ab
Fungicide Control	6.56	6.12	-0.44	ns	9.9	13.4	3.5	bcd
Non-treated control	6.73	6.26	-0.47	ns	13.8	15.1	1.2	d

Table 2. Letter diagram of effects of two years of treatments applied every two weeks on the change of soil pH and soil phosphorus levels between May 2019 and May 2020. [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. [v]MO = Civitas Turf Defense applied at 8.5 oz. per 1,000 square feet. [x]ns = not significant. [w]Mean differences in the same column followed by the same letter are not significantly different according to Fisher's LSD test (alpha ≤ 0.05).

Treatment #	Description	Double Ring Infiltration (minutes at saturation) May 2020	Aug. 2020	Oct. 2020	Greenspeeds Jul. 2020	Oct. 2020
1	S[z] + PA[y]	20.2 ns[x]	6.9 ns[x]	9.3 ns[x]	9.7 ns[x]	11.1 ns[x]
2	Sep, Oct, Nov, Apr = MO[v] + PA Dec, Jan, Feb, Mar = S + PA	30.6 ns	8.1 ns	14.1 ns	10.1 ns	10.2 ns
3	MO + PA rotated with S + PA	35.7 ns	6.4 ns	16.1 ns	10.0 ns	10.8 ns
4	MO rotated with S + PA	37.9 ns	4.5 ns	17 ns	10.0 ns	10.8 ns
5	0.5 # $FeSO_4$ + PA	36 ns	7.3 ns	24.3 ns	9.9 ns	10.9 ns
6	1.0 # $FeSO_4$ + PA	31.9 ns	10.6 ns	18.9 ns	9.7 ns	11.1 ns
7	Sulfur (S)	37.8 ns	6.5 ns	14.4 ns	10.2 ns	11.3 ns
8	Phosphorous Acid (PA)	24.4 ns	7.5 ns	15.8 ns	9.9 ns	11.1 ns
9	Fungicide Control	46.6 ns	8.8 ns	23.2 ns	9.9 ns	10.8 ns
10	Non-treated control	49.5 ns	9 ns	22.4 ns	10.3 ns	10.8 ns

Table 3. Letter diagram of effects of treatments applied every two weeks on water infiltration and putting green speeds. [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. [v]MO = Civitas Turf Defense applied at 8.5 oz. per 1,000 square feet. [x]ns = not significant.

Future expectations of the project:

This long-term trial is ongoing and will continue at least through September 2021 and likely longer. Soil samples will be collected again in May 2021 following the Microdochium patch treatments and these will be analyzed to provide conclusive results regarding the effects of these treatments on soil nutrient levels and pH. In addition, soil samples that include the thatch and mat layer from the surface to 1" in depth are going to be ground using liquid nitrogen in order to ascertain if there are changes in the soil nutrition levels or pH near the green surface.

USGA ID#: 2019-04-674

Project Title: Biology and Management of Pythium Root Rot in Golf Course Putting Greens

Project Leaders: James P. Kerns
Affiliation: North Carolina State University

Objectives:

1. Determine the distribution and prevalence of pathogenic root-infecting *Pythium* species in golf course putting greens.
2. Assess aggressiveness towards mature turfgrass plants of *Pythium* species associated with Pythium root rot.
3. Determine *in vitro* sensitivity of *Pythium* species collected to various fungicides.
4. Develop a quantitative PCR assay to detect *Pythium* species in turfgrass roots.

Start Date: 2019
Project Duration: 3 years
Total Funding: $81,250

Summary Points:

- Isolate of *Pythium* species during summer months is challenging as most of the isolates recovered were non-pathogenic species such as *Pythium torulosum*.
- Based on the limited data we collected this year, we hypothesize that *Pythium* infection precedes symptom development in creeping bentgrass.
- *P. torulosum* growth was only inhibited by cyazofamid, fluazinam, and etridazole.
- *In vitro* sensitivity varied among *Pythium* species, but all were extremely sensitive to cyazofamid.

Summary Text:

Samples exhibiting symptoms of Pythium root rot that were submitted to the NC State Turfgrass Diagnostic Lab were selected for isolation of *Pythium* species. Affected roots were washed for at least 3 hours and plated on semi-selective and non-selective media. After 24 hours of incubation in the dark, candidate hyphae were transferred from the aforementioned plates to a fresh petri plate containing water agar to obtain a pure culture. Out of 125 isolates collected, 88 were identified as *Pythium torulosum* and the remaining were identified as either *Pythium vanterpoolii (8), P. irregulare (5), P. aphanidermatum (1), or P. volutum* (1). All of the isolates collected except for *P. torulosum* were extremely aggressive when placed on creeping bentgrass seedlings. The pathogenic *Pythium* species were primarily collected during May and June which is early in terms of symptom expression. Of the 125 isolates collected, 22 were collected from ultradwarf bermudagrass putting greens. Fifteen of the isolates collected were *P. vanterpoolii,* 4 were *P. torulosum,* and 4 were *P. arrhenomanes.* Identifications were conducted by extracting the ITS regions and amplifying using ITS 4 and 5 primers in PCR. Sequences were aligned and generated sequences were compared using GenBank's BLAST and the Oomycete Gene Table database. Sequence identification was corroborated with molecular characteristics such as oospore/oogonia dimensions and ornamentations, antheridia characteristics and colony morphology. We will continue isolation efforts and will construct a phylogenetic tree once we have collected isolates from next year.

Given the challenges associated with isolation, we plan to establish permanent plots at the Lake Wheeler Turfgrass Research and Education Lab in Raleigh, NC for sampling purposes. We will sample from 8 replicate plots that will remain untreated throughout the course of the spring and summer. We plan to commence sampling in February and sample every three weeks throughout 2020 to help establish a clear picture of the species associated with this disease. We will also bury soil temperature and soil moisture probes at this location to see if we can correlate these factors to Pythium root rot development. 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.

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^{o}F in order to prevent Pythium root effectively. Applications in June and July were not effective in preventing Pythium root rot. This further supports our hypothesis that infection precedes symptom development. In fact, infection could occur as creeping bentgrass rooting is at its height in March, April and May. 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).

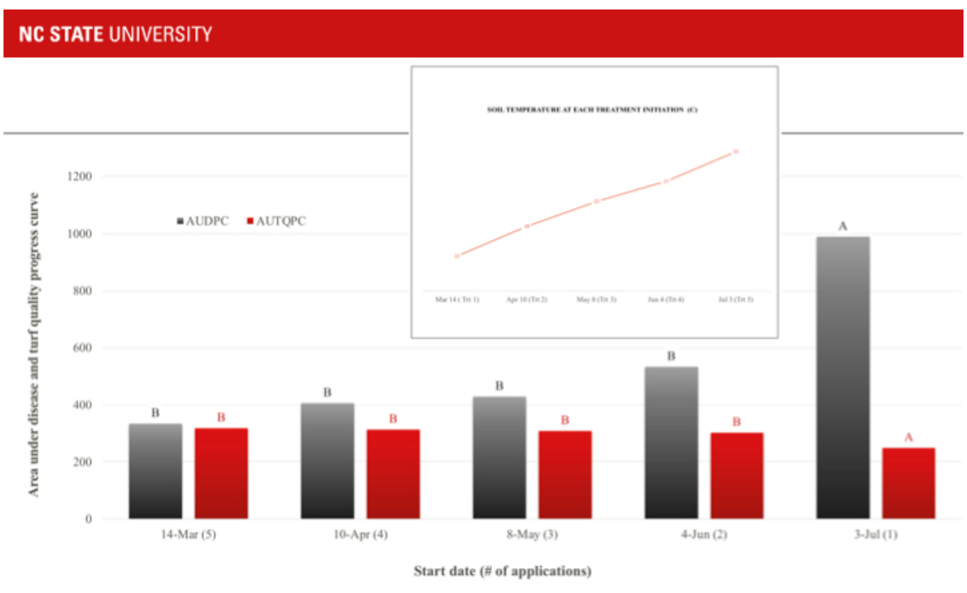


Figure 1. Efficacy of preventative cyazofamid applications for Pythium root rot in creeping bentgrass. Applications started in either March, April, May, June or July and were re-applied monthly until August. All applications were irrigated immediately with 1/8 inch of water and cyazofamid was applied at 0.45 floz/1000 ft^2.

Literature Cited

1. Abad, G., Shew, H. D., Grand, L. F., and Lucas, L. T. 1995. A new species of Pythium producing multiple oospores isolated from bentgrass in North Carolina. Mycologia 87 (6):896-901.
2. Andre Levesque, C., and De Cock, A. 2004. Molecular phylogeny and taxonomy of the genus Pythium. Mycological Research 108 (12):1363-1383.
3. Asano, T., Senda, M., Suga, H., and Kageyama, K. 2010. Development of multiplex PCR to detect five Pythium species related to turfgrass diseases. Journal of Phytopathology (158):609-615.
4. Bakker, M.G., T.B. Moorman, and T.C. Kasper. 2017. Isolation of Cultivation-Resistant Oomycetes, First Detected as Amplicon Sequences, from Roots of Herbicide-Terminated Winter Rye. Phytobiomes 1: 24-35.
5. Beard, J. B. 1992. Turf Management for Golf Courses. Burgess Publishing Company, Minneapolis, MN.
6. Hodges, C., and Coleman, L. 1985. Pythium-induced root dysfunction of secondary roots of Agrostis palustris. Plant Disease 69 (4):334-340.
7. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for Phytophthora and Pythium species. 70 (11).
8. Kerns, J., and Tredway, L. 2008. Influence of temperature on pathogenicity of Pythium volutum toward creeping bentgrass. Plant Disease 92 (12):1669-1673.
9. Kerns, J. P., Soika, M. D., and Tredway, L. P. 2009. Preventive control of Pythium root dysfunction in creeping bentgrass putting greens and sensitivity of *Pythium volutum* to fungicides. Plant Disease 93:1275-1280.
10. Kerns, J. P., and Tredway, L. P. 2007. First report of pythium root dysfunction of creeping bentgrass caused by *Pythium volutum* in North Carolina. Plant Dis 91 (5):632.
11. Miller, G. L., Grand, L. F., and Tredway, L. P. 2011. Identification and distribution of fungi associated with fairy rings on golf putting greens. Plant Disease 95 (9):1131-1138.
12. Smiley, R. M., Dernoeden, P. H., and Clarke, B. B. 2005. Compendium of Turfgrass Diseases. APS Press, St. Paul, MN.
13. Wang, P., Wang, Y., and White, J. 2003. Species specific PCR primers for Pythium developed from ribosomal ITS1 region. Letters in Applied Microbiology 37 (2):127-132.

USGA-ID: 2019-06-676

Title: Understanding and optimizing sampling methods for the Annual Bluegrass Weevil

Project leaders: Albrecht M. Koppenhöfer

Affiliation: Department of Entomology, Rutgers University, New Brunswick, NJ

Objectives: The goal is to optimize the use and predictive power of sampling/monitoring methods for ABW adults. Specifically, we will determine the effect of temperature and mowing height on the percentage of adults detected (1) in the clippings of a mower, (2) by vacuuming with a leaf blower, and (3) by soap flushing. In addition, (4) we will determine the effect of water volume applied and detergent concentration on the extraction efficiency of the soap flushing method.

Start date: 1-1-2019

Project duration: 2 years

Total funding: $19,910

The annual bluegrass weevil (ABW) is the most important and difficult to control insect pest of short-mown golf course turf in eastern North America. Golf course superintendents have relied primarily on synthetic insecticides for ABW management, but excessive insecticide use has led to widespread insecticide resistance to insecticides from several classes. Overuse of any remaining effective synthetic insecticides will likely desensitize ABW to these compounds as well. To delay resistance development, control products should only be applied when and where necessary. That requires effective monitoring and sampling methods for the adult or the larval stage.

The quickest and most likely to be used monitoring methods involve sampling adults by vacuuming, soap flushing, or clippings examination. However, various factors including temperature and mowing height are likely to influence the efficiency of these methods, particularly that of vacuuming and clippings examination. The method least likely to be affected by environmental conditions and mowing height is soap flushes where water mixed with liquid dish washing detergent is applied to a specified area which irritates the adult to the surface and up the grass blades where they can be counted. However, the effect of water volume and concentration of the detergent on extraction efficacy had not been examined previously.

In the first year of our study, using released color-marked adults and sampling under warm conditions to allow for the optimization of extraction methods, we had found that mowing height had a significant effect on adult recovery. Significantly more adults were recovered in mower clippings from a Toro flex 21”mower at greens height (15–24% recovery) than at fairway height (0.2%). Similarly, vacuuming recovered significantly more adults at greens height (29–33%) than at fairway height (4–5%). Soap-flushing with 0.5 Liter of water containing 0.4% dish washing detergent applied twice recovered 83% of adults from a fairway within 20 minutes.

To optimize the efficacy of the soap-flushing method, we extracted adults of a natural ABW population from fairway height turf using 0.2%, 0.4%, or 0.8% soap solution (lemon scented dish washing detergent). The solution was applied once at the beginning of the 20-minute observation period (0.5 or 1.0 Liter solution per square foot) or at the beginning and again 5 minutes later (both times 0.5 Liter). Adults were collected from the turf surface every 5 minutes.

Extraction efficacy increased with soap concentration, being highest at 0.8% whether applied once or twice at 0.5 Liter per square foot (Fig. 3). 0.4% tended to be more effective when applied twice at 0.5 Liter than applied once at 1.0 Liter; it was also all but impossible to apply the higher volume without significant run-off. In all treatments, at least 75% of the total recovery was reached after 15 minutes, but additional weevils were recovered by 20 min in all treatments. The most effective soap-flush protocol therefore is to apply 0.8% twice at 0.5 Liter after 0 and 5 min, and to observe for at least 15 minutes, better for 20 minutes.

In additional experiments we investigated the effect of environmental temperature on the recovery of natural populations of ABW adults from greens height turf by mowing and soaping (Fig. 1, 2, 4). As in previous experiments, much higher (10–45x) numbers of adults were recovered by soap-flushing than by mowing. Temperature (range 44–71 °F) had no effect on soap-flushing extraction efficacy. The number of adults picked up in mower clippings tended to increase with temperature, albeit not statistically significant due to low and highly variable adult counts.

Additional experiments in spring 2021 will attempt to solidify the trend in temperature effect on weevil extraction in mower clippings and investigate the effect of temperature on extraction efficacy by vacuuming.

- Soap-flushing is the most effective method for extracting adults (> 80% efficacy).
- Soap-flushing is most effective with 0.8% soap solution, applied twice at 0.5 Liter per square foot, and weevils collected for at least 15 minutes, better 20 minutes.
- Soap flushing is not significantly affected by environmental temperature.
- Mowing can recover a significant portion of adults in clippings from greens (15% without and 24% with brush attached in front of mower) but is ineffective on fairways (0.2% recovery).
- Weevil recovery by mowing tends to increase with environmental temperature.
- Vacuuming with a leaf blower recovers 4–5% of adults from fairways but 31% from greens.

Fig. 1. Mowing of greens height plots to pick up adult ABW.

Fig. 2. Soap extraction of adult ABW following mowing.

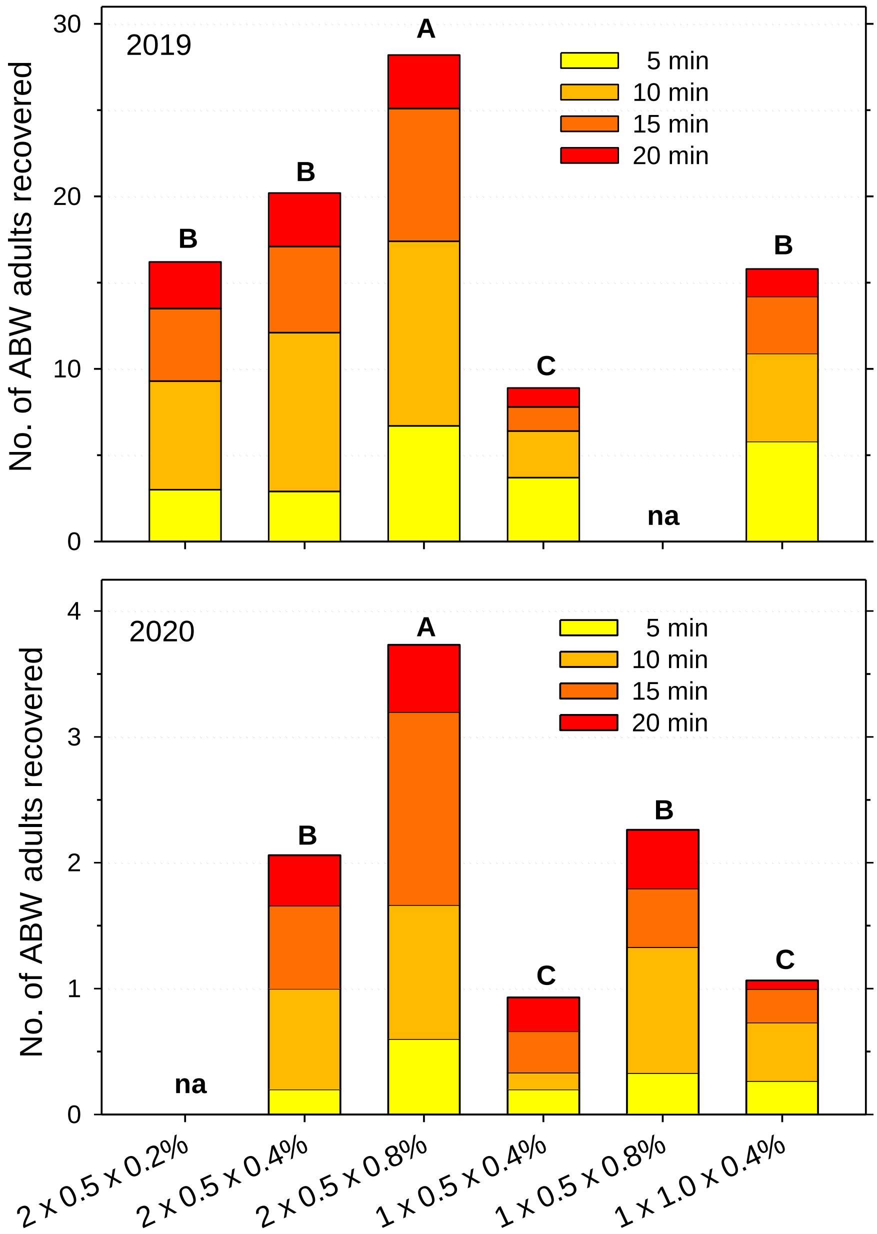


Fig. 3. Recovery of ABW adults by extraction with soap solution from an area mown at fairway height. Solution was applied at 0 minutes (1 x) or 0 and 5 minutes (2 x) with 0.5 or 1.0 Liters per application of 0.2, 0.4 or 0.8% soap solution. Adults were collected at 5, 10, 15, and 20 min after the first application of soap solution. Letters above bars indicate significant differences among the total numbers of adults recovered within 20 minutes. na = treatment not applied in experiment.

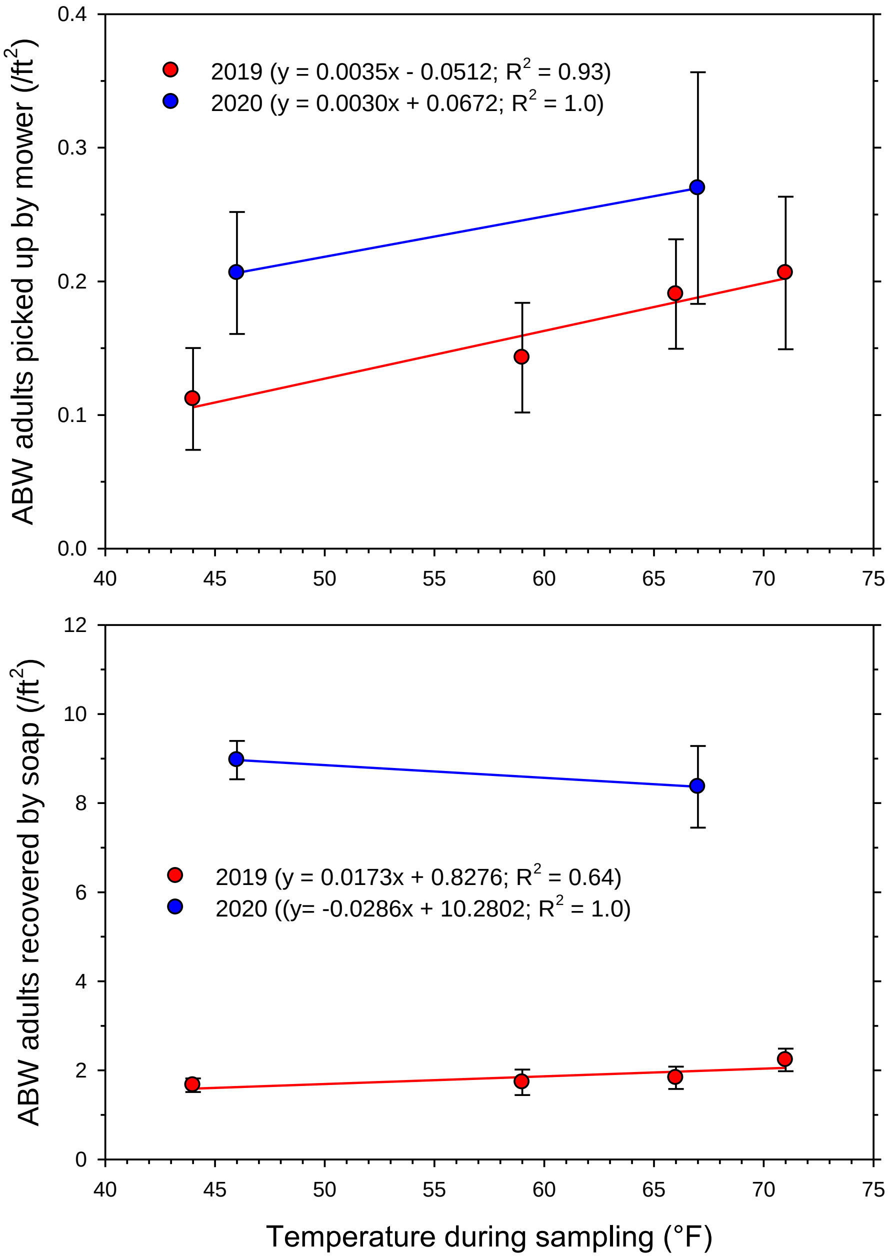


Fig. 4. Effect of temperature on recovery of ABW adults from an area mown at greens height by mowing followed by extraction with 0.8% soap solution (applied at 0 and 5 minutes and collected for 20 minutes) in four experiments in 2019 and two experiments in 2020.

USGA-ID: 2020-08-713

Title: Long-term suppression of turfgrass insect pests with native persistent entomopathogenic nematodes.

Project leaders: Albrecht M. Koppenhöfer, Ryan W. Geisert, Ana Luiza Viana de 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

Turfgrass is afflicted by many insect pests which are primarily controlled with synthetic insecticides, commonly in a preventive mode over large areas which can suppress many natural enemies of turf insect pests. Insecticides overuse leads to reduced insecticide efficacy through insecticide resistance and increased microbial insecticide degradation. There is a need for the development of alternative insect control methods, that, ideally, can provide long-lasting pest suppression.

Entomopathogenic nematodes (EPNs) have shown potential for the control of various turf insect pests including white grubs, caterpillars, weevils, and craneflies. EPN research has focused on using them as biopesticides applied inundatively with little concern for long-term effects. However, several studies in field crops have shown that inoculative applications of native EPN strains adapted to the local conditions and maintained to preserve their ability to persist in the environment, can effectively suppress pest populations for several years. In turfgrass, oriental beetle larvae were effectively suppressed for up to 4 years after a single application of the white grub-adapted species *Steinernema scarabaei*.

During 2019 we surveyed one fairway each at Pine Brook Golf Course (PB) and Howell Park Golf Course (HP), both in Monmouth County, central New Jersey for native EPNs. These fairways had received only limited insecticide applications for many years. Soil samples (7.5 cm deep × 2.5 cm diameter) were taken from the fairway and the adjacent rough. Samples were pooled within section on each side of the fairway, mixed thoroughly, and subsamples baited with wax moth larvae for EPN isolates. Single infected waxworm cadavers were placed onto emergence traps to recover any EPN infective juvenile nematodes (IJs). The IJs were stored in tap water in tissue flasks at 8°C. The majority of the EPN collected were either *Heterorhabditis bacteriophora* or *Steinernema carpocapsae*. Isolates of each species were mixed together to increase the genetic diversity of the populations to be used in the inoculative applications. The EPN populations were mass reared in waxworms and used to inoculate the field plots in early June 2020.

Field plots measuring 20 m × 10 m, located at PB and HP, were arranged along the fairway edge, with one half in the fairway, the other in the rough. Plots were separated by a minimum of 5 m. EPNs were applied at a rate of 1.25×10^9 IJ/ha. Treatments included *H. bacteriophora*, *S. carpocapsae*, a 1:1 mixture of both species, and an untreated control. There were two replicates per treatment at each golf course. Applications were made by dividing plots into 10 sections each measuring 2 m wide. Each strip received the appropriate number of IJs in 7 Liter water applied by watering can.

To minimize border effects when sampling for EPN and insect populations, samples were taken in each plot from a 4 m × 4 m area in the rough and one in the fairway that were 2 m distance from the fairway edge, and 3 m distance from the sides of the plot. For more uniform coverage, the sampling area was further divided into four 2 m × 2 m subplots.

EPN populations in the plots were determined 1 week before application to gauge the scale of resident populations and again 1 and 3 months after application. Ten soil cores (7.5 cm × 2.5 cm diameter) were taken per subplot, mixed thoroughly, and a subsample of 170 grams was placed into a plastic cup and baited with five waxworms for 9 days. Individual infected cadavers were treated as described above to collect any emerging IJs. The EPN species were determined by the color of the cadavers, which indicate the bacterial symbiont associated with the species, and by measuring the size of the recovered IJs under a microscope.

In mid-June 2020, plots were surveyed for ABW populations. Eight turf/soil cores (5.4 cm diameter × 3 cm depth) were taken per subplot and extracted in the laboratory by submersion in water saturated with table salt. The number of ABW life stages were recorded for each sample along with any other insects found in the samples.

In late July and early September, populations of surface-active insects were determined via soap flushes. In each subplot, one 30 cm × 30 cm area was treated with 500 ml of a soap solution (4% lemon scented Ultra Joy dishwashing liquid detergent) at the start of the sampling and again after 10 minutes. Any insects that became visible within 20 min were collected and brought to the lab for identification.

In late September 2020, plots were surveyed for white grub populations. Four turf/soil cores (10.5 cm diameter × 7.5 cm depth) were taken per subplot. The samples were taken apart by hand in the field and any white grub found were brought back to the lab for identification.

EPN detection showed a high level of variation. We expected detection to increase from the pre-application to post application phase. The number of EPN-infected waxworms had increased by 1 month after application at PB in fairway and rough (pre-application: 6 in fairway, 35 in rough; post-application: 24 in fairway, 137 in rough; Fig. 1) but at HP only in the fairway (pre-application: 9 in fairway, 49 in rough; post-application: 18 in fairway, 39 in rough; Fig. 2). The variation of nematodes collected did increase for both sites. At 3 months after application, EPN infections had increased at both sites (Fig 1, 2). It is possible this timing was preceded by the presence of suitable hosts that allowed the populations to flourish. It is important to note that not all of these increases matched with the treated plots, with nematode numbers increasing in untreated controls and red cadavers being found in plots not treated with *H. bacteriophora* (Fig. 1, 2). It is likely that pre-application wild type nematodes also increased during this timeframe.

ABW densities at 10 days after EPN application did not vary much between treatments except for the *H. bacteriophora*-treated rough at PB (Fig. 3). During the ABW survey, larvae of the black turfgrass ataenius (BTA) were recovered in similar numbers as ABW, also without any clear trends among treatments, likely due to the low densities. The very low densities of ABW and BTA in spring 2020 (around 10 per 0.1 m^2) likely did not allow for significant effects of EPN applications and certainly not for significant EPN recycling in these small host species. BTA adults were the only consistently recovered insect in the soap samples but did not show any significant trends among treatments. Very few larvae of annual white grubs were recovered during the white grub survey in September and were outnumbered by a scattering of BTA larvae across all treatments.

Additional sampling this year for EPNs and continued sampling of EPNs and potential hosts in 2021 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 continued 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 in 2020, 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.

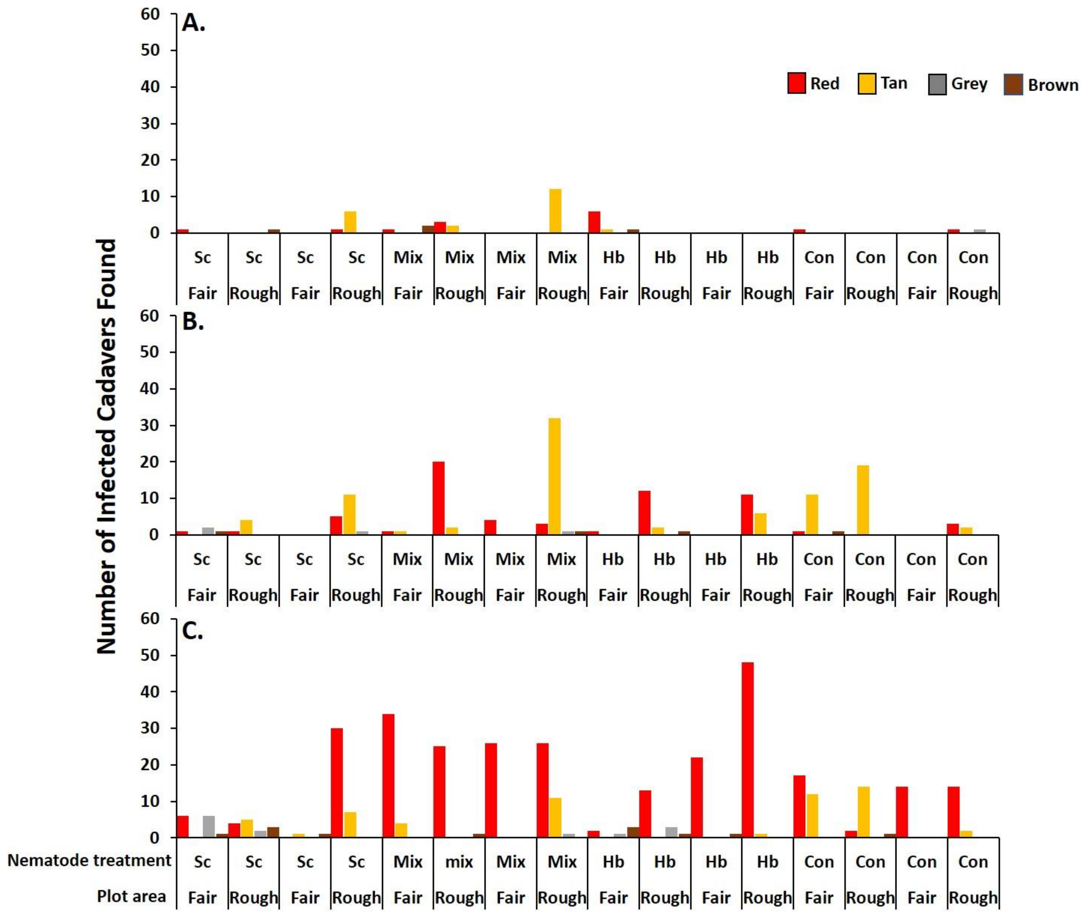


Figure 1. Numbers of EPN-infected waxworms recovered per plot by baiting soil samples taken at Pine Brook Golf Course. Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabidits bacteriophora* (Hb), both species (Mix), or were not treated (Con). Red cadavers were Hb-infeced and tan cadavers Sc-infected. The species causing grey and brown coloraton yet need to be identified. Soil samples were taken 1 week before (A) and 1 month (B) and 3 months (C) after applications.

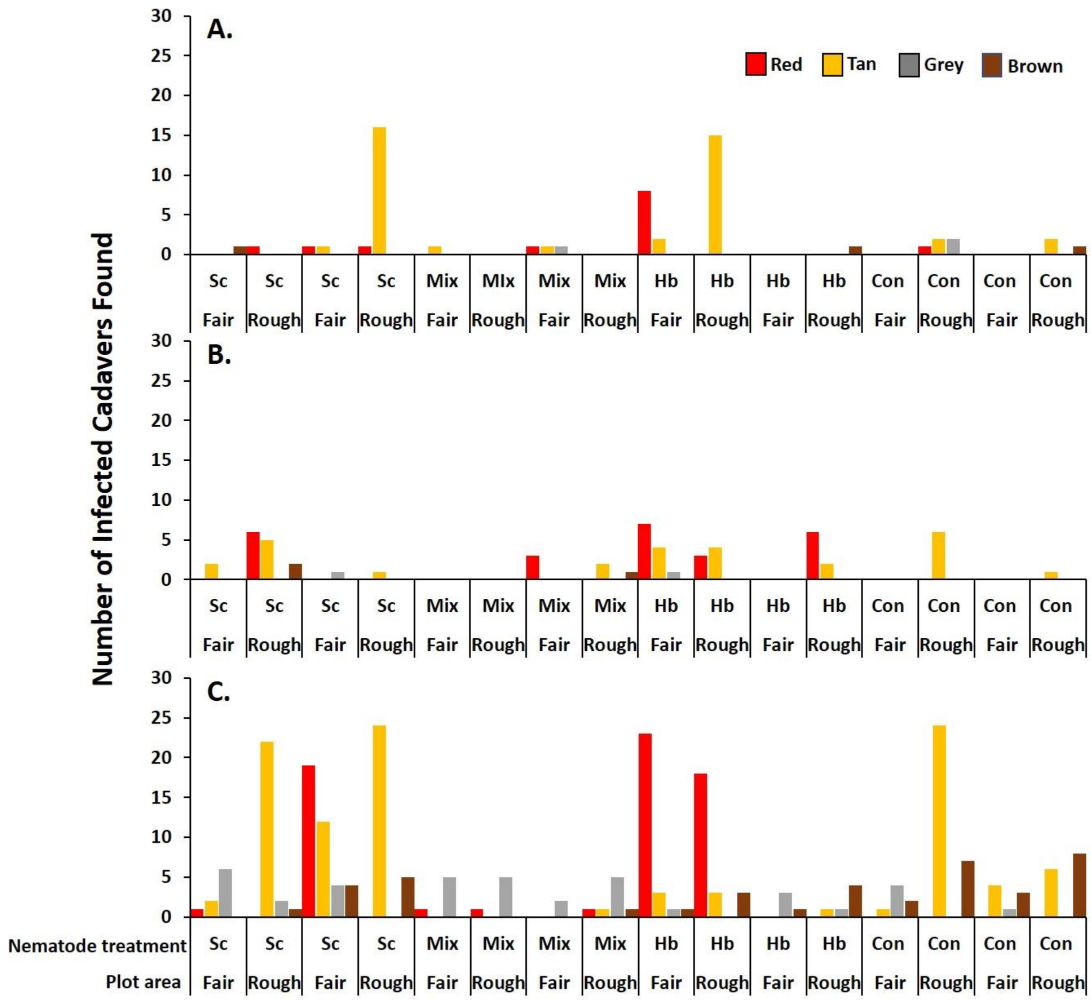


Figure 2. Numbers of EPN-infected waxworms recovered per plot by baiting soil samples taken at Howell Park Golf Course. Plots had been treated in early June 2020 with the EPN species *Steinernema carpocapsae* (Sc), *Heterorhabidits bacteriophora* (Hb), both species (Mix), or were not treated (Con). Red cadavers were Hb-infeced and tan cadavers Sc-infected. The species causing grey and brown coloraton yet need to be identified. Soil samples were taken 1 week before (A) and 1 month (B) and 3 months (C) after applications.

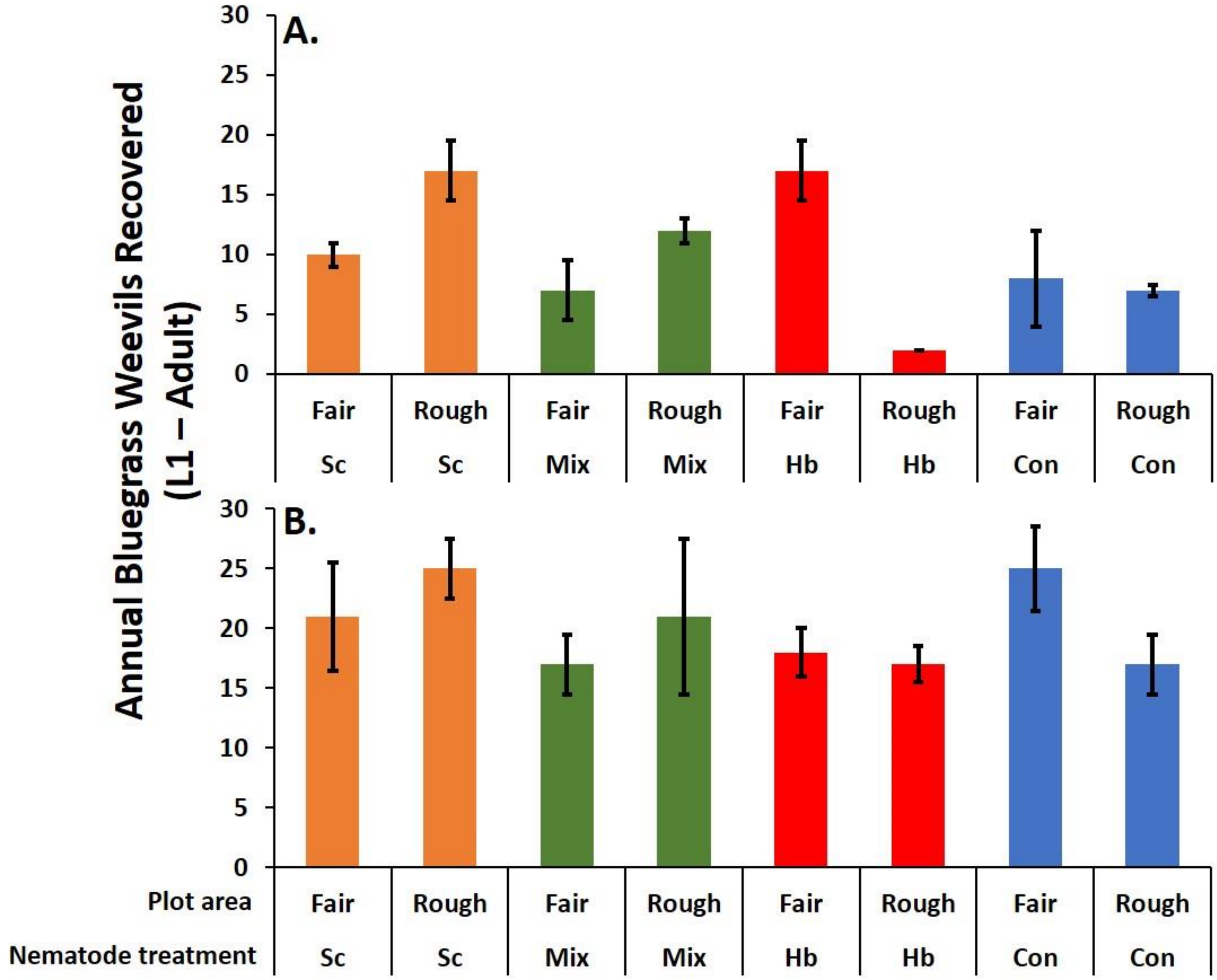


Figure 3. Counts of annual bluegrass weevil lifestages (L1 – Adults) taken from soil cores collected at (A) Pine Brook Golf Course and (B) Howell Park Golf Course at 10 days after EPN application in June 2020.

Figure 4. Sampling plots for EPNs through soil cores that will be baited with waxworms in the laboratory.

USGA ID#: 2019-07-677

Title: Progress toward solving the silvery-thread moss issue in cool-season putting greens

Project Leaders: Llo Stark, Zane Raudenbush, Matthew Johnson, Joshua Greenwood

Affiliation: University of Nevada Las Vegas, Ohio State University, Texas Tech University

Objectives:

1. Organize our laboratory experiment on the effects of carfentrazone 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. Silvery-thread moss (STM) from putting greens are more vigorous in regeneration and photosynthetic activity than mosses of the same species from native habitats. This finding extends to putting green mosses being more resistant to carfentrazone (CZ) applications in the lab than non-putting green mosses.

2. Carfentrazone applications are most effective when applied in full sunlight conditions, as simulated in the lab as 2000 PAR (μmol m^{-2} sec^{-1}).

3. Drench applications of common anionic surfactants suppressed silvery-thread moss growth in experimental golf course putting greens.

4. DNA was submitted from a male and a female clone of STM from Kentucky for long-read genome sequencing.

Summary Text:

Rationale

Silvery-Thread Moss (STM, *Bryum argenteum*) is an undesirable weedy species that has colonized golf greens across the USA and has proven difficult to erradicate. Our group of four researchers (Stark, Raudenbush, Johnson, and Greenwood) from three institutions (UNLV, Ohio State U., Texas Tech U.) initiated lab and field studies to (1) test the effectiveness of a surfactant-based product (*Dawn Ultra* dishsoap) and a moss suppressant on the market

(*Quicksilver*, known as Carfentrazone-ethyl, CZ here) on the growth response and photosynthetic health of STM; (2) determine the effect of CZ at different light intensities; (3) determine the effect of a single known surfactant (sodium dodecyl sulfate, SDS, ($CH_3(CH_2)_{11}SO_4Na$)) on moss growth in both putting green and laboratory settings; and (4) isolate high quality DNA for sequencing the genome of this moss.

Methodology

UNLV. During 2020, campus and laboratory access was limited due to pandemic concerns at all three of our institutions. As a result, although no new experiments were initiated at UNLV, we were able to focus on establishing a library of living cultures of STM reflecting a range of native and putting green sources. These cultures were subcultured repeatedly in order to yield 89 pure cultures of 37 genotypes (strains) of this species for future surfactant and genetic experiments. In addition, we grew cultures to send to Dr. Johnson at Texas Tech, to furnish sufficient biomass for genetic tests described below.

Ohio State. During 2020, Ohio State University suspended all nonessential research activities from March-June. In July, we received a research exemption to continue our field studies to evaluate the efficacy of surfactants to control STM. In August, several pilot studies were conducted on a creeping bentgrass research green at Hawks Nest Golf Course in Creston, Ohio to (1) evaluate the efficacy of a drench application of sodium dodecyl sulfate for STM control, and (2) observe any potential phytotoxic effects to ‘A1’ and ‘007’ creeping bentgrass following the application. In September, a replicated field experiment was conducted at Hawks Nest Golf Course in Creston, Ohio and Scioto Country Club in Columbus, Ohio. 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 (**Figure 1**). The four chemical control strategies were: (1) drench application of Dawn Ultra dish soap, (2) drench application of sodium dodecyl sulfate, (3) spray application of Quicksilver herbicide at 3.3 fl.oz./acre, and (4) 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. Treatments were applied on September 15 and October 9. Percent STM cover in each plot was measured using a rating grid at trial initiation and every two weeks thereafter until mid-November.

Texas Tech. During 2020, sequences from two clones, one male and one female, were submitted for long-read DNA sequencing, with the goal of assembling and annotating a new STM genome. Additional DNA and RNA extractions were also made for short-read sequencing to enhance the annotation of the genome. Received initial shipment of STM cultures from UNLV, which will be used for DNA extraction and characterization of genetic diversity.

Results to date

UNLV. Living cultures of 37 genotypes (strains) of STM are now established in growth chambers and are pure of contaminants (**Figure 2**). A manuscript on the assessment of carfentrazone (CZ) and light effects on regeneration and photosynthetic health of STM is progressing, with the major finding that CZ, while not killing the moss in laboratory applications even at higher than normal concentrations, is most effective at setting back moss regeneration when applied at light levels equivalent to full sunlight. A second manuscript on the effects of

SDS on STM is also in progress, with the major finding that remarkably low laboratory concentrations are effective at killing the moss.

Ohio State. Drench applications of Dawn Ultra dish soap and sodium dodecyl sulfate rapidly injured STM shoots at both locations (**Figure 3**). Minimal injury to creeping bentgrass (<5%) was observed from the initial application on September 15, but a slight increase phytotoxicity was observed from the October 9 application. Quicksilver also caused a significant amount of injury to the STM shoots, but the overall control was less than the drench applications of Dawn Ultra and sodium dodecyl sulfate (data not shown). A previous field experiment has demonstrated hollow-tine aerification can reduce the size of a STM infestation by providing "available sites" for the desirable turfgrasses to reestablish. In this study, an aerification hole created within a STM colony that was not treated with a chemical control typically healed within four days. Oppositely, aerification holes created in STM colonies treated with a chemical control strategy remained open for several weeks.

Texas Tech. Preliminary analysis of the RNA and DNA extracted from the two STM clones indicates a successful library preparation for short-read sequencing (**Figure 4**). High molecular weight DNA extraction from both clones was successful, but sequencing using the "PacBio" platform submitted in January 2020 did not generate enough data for genome assembly. Sequences were resubmitted to the Genomics core facility at the University of Connecticut using the "NanoPore" platform in October 2020.

Future expections for project

Future research will integrate our lab and putting green experimental findings on SDS concentrations at various application timings throughout the growing season and their effectiveness at controlling silvery-thread moss. In 2021, field research will be conducted to better understand the causes of phytotoxicity observed in the field experiments. Additionally, linear aerification will be added to the experimental design to encourage penetration of desirable turfgrass species into the injured STM colonies. Once the putting green data are available, we will design a lab experiment to compliment putting green trends. Data for genome assembly is expected in Spring 2021, and will provide context for accurate genotyping of wild and golf course populations. We will then proceed with low-depth resequencing of cultures established at UNLV to characterize genetic diversity of both on- and off-golf course genotypes.

Figure 1. Drench application of Dawn Ultra dish soap and sodium dodecyl sulfate to a creeping bentgrass research green infested with STM (*Bryum argenteum*) in Creston, Ohio.

Figure 2. Photo of growth chamber dedicated to propagating living, purified strains of STM) *Bryum argenteum*) mosses derived from putting greens and from native habitats in the USA.

Figure 3. Plot treated with drench application of sodium dodecyl sulfate and hollow tine aerification 1 day after treatment (left) and 3 days after treatment (right) in Creston, Ohio.

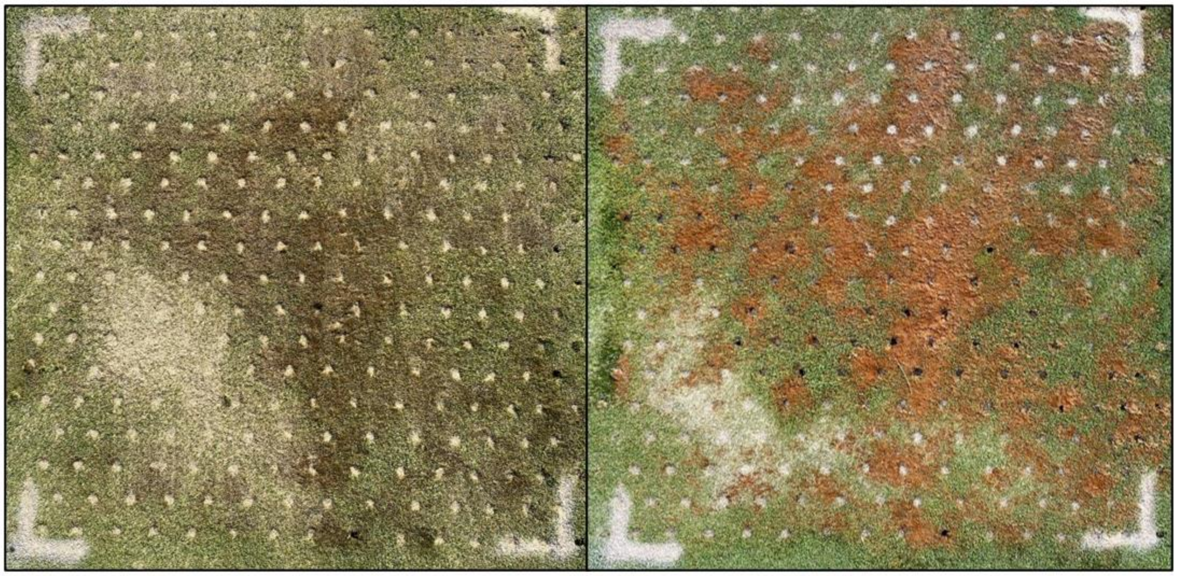
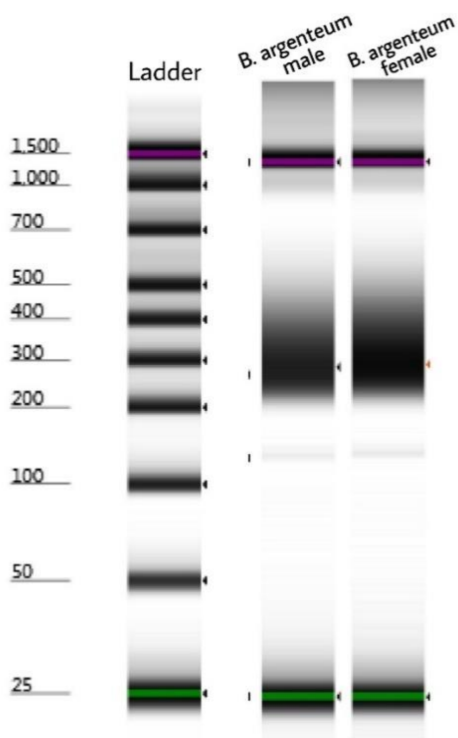


Figure 4. Agilent TapeStation fragment analyzer results for RNA libraries prepared from two STM (*Bryum argenteum*) clones. Library sizes (in base pairs) between 200 and 700 indicate successful library ready for short-read high-throughput sequencing.



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 herbicides as growth regulators in combination with two different fine fescue blends to determine the effect on characteristics associated with playability in naturalized areas

Start Date: 2020
Project Duration: 3 years
Total Funding: $89,842

Summary Points:
- Single glyphosate (560 g ha^{-1}) applications were most effective against deertongue when applied at 175 GDD and at flowering.
- July and September glyphosate applications also provided better control than April applications and all fluazifop (280 g ha^{-1}) treatments.
- Two fine fescue sites were established at Hort Farm for future experiments in 2021. Experiments will further evaluate herbicide efficacy in combination with various mowing treatments, and how herbicide programs affect the appearance and playability of various fine fescue cultivar mixtures.

Summary Text:

The first phase of this research was completed in 2020 to identify optimal timings for deertongue grass (*Dichanthelium clandestinum*) control in naturalized fine fescue areas. A field research experiment at Mendham Golf and Tennis Club located in Mendham, NJ was initiated in April 2020 to investigate the efficacy of fluazifop (280 g ha^{-1}) and glyphosate (560 g ha^{-1}) applied singly at five different application timings. Treatments were replicated five times and arranged in a two-by-five factorial alongside a non-treated control for comparison. Plots (2.0 by 3.0 m) were arranged in a RCBD with three blocks placed in an area where deertongue grass cover was >90% by mid summer and little fine fescue was present. Two blocks were placed in an area on the same golf hole where the deertongue grass infestation was more variable and mixed with fine fescue and forbs. Application timings were selected using a combination of growing degree-days (GDD; base 10° C), cooling degree-days (CDD; base 20°C), and weed developmental stages similar to Elmore et al. (2013). GDD accumulation was calculated beginning March 1 and CDDs were similarly calculated beginning August 15 using local weather data. Weed phenology was visually monitored on site. Herbicides were applied at approximately 75 and 175 GDD (which occurred on April 28 and May 26, 2020), during deertongue flowering in spring (June 18), in mid-July (July 22), and on 25 CDD for a final late-season application (September 22). Plots were not mowed until October, two weeks after the last herbicide had been applied. Treatments were applied using a CO_2-powered sprayer and four-nozzle boom equipped with 11003VS nozzles (TeeJet AIXR) nozzles on 19 inch spacings with a carrier volume of 410 L ha. Fluazifop was applied with NIS (0.25% v/v) per label instructions. To determine percent deertongue control, deertongue injury was visually assessed on a 0 (healthy) to 100 (necrotic) scale every two to three weeks from May through October. Deertongue coverage was also visually evaluated from 0 (none) to 100 (complete canopy cover) in each plot. The final rating of the 2020 growing season took place on October 22, one month after the last herbicide treatment was applied. All data were subject to ANOVA as a factorial using the GLIMMIX procedure in SAS (v. 9.4).

By the final rating date on October 22, the 175 GDD and flowering glyphosate applications resulted in 89 to 97% deertongue control (Table 2). Glyphosate applied at 175 GDD provided > 80% deertongue control from June 18 (7 WAT) until the last rating in October. Glyphosate applied at flowering similarly provided > 80% deertongue control from July 22 (9 WAT) onward. Deertongue injury from glyphosate applied at 75 GDD was greatest 3 WAT in May (79%) but < 60 % 51 WAT in June and for the rest of the season. Similarly, all application timings of fluazifop (280 g ha^{-1}) provided 11-60% deertongue control on the final rating date in October 2020.

To prepare for the next phases of research, two sites were seeded with fine fescue blends (*Festuca* L. spp.) on May 14, 2020 to establish on native sandy loam soil at Hort Farm 2 for experiments initiating in 2021. One site was split into four blocks that were split in half and seeded with two different fescue blends on either side. Each blend contained 50% v/v ‘Quatro’ sheep’s fescue (*F. ovina* L.) and 50% of either ‘Beudin’ or ‘Gladiator’ hard fescue (*F. Brevipila* Tracey) totaling 4 lb. PLS/1000 sq ft.

To further evaluate herbicide programs for deertongue control in fine fescue, another site was seeded with a 50% ‘Gladiator’ hard fescue and 50% ‘Quatro’ sheep’s fescue blend using the same at 4 lb. PLS/1000 sq ft rate. To establish uniform weed cover, deertongue plants were dug and removed from Mendham, NJ and transported to Hort Farm 2 to be processed. Within 24 hours, plants were washed free of soil and healthy stems and rhizomes were removed from whole plants and left to soak in water overnight. Green stem tissue was then cut after every third node, and the resulting 4 to 6-in fragments were planted 2 to 3-in deep throughout the site on June 11.

Future Research Expectations: These findings will be utilized to develop management programs from further suppressing deertongue grass in low maintenance areas. We hypothesize that combining a single herbicide application with a well-timed mowing treatment can slow deertongue recovery and enhance the efficacy of the herbicide programs.

Table 1. Herbicide treatments applied at Mendham Gold and Tennis Club in Mendham, NJ to native areas infested with deertongue grass.

Treatment	Active ingredient	Active ingredient rate (g ha^{-1})	Application code	Timing	Calendar date
1	Non-treated	-	-	-	-
2	Fluazifop[†]	280	A	75 GDD	April 28
3	Fluazifop	280	B	175 GDD	May 26
4	Fluazifop	280	C	Flowering	June 18
5	Fluazifop	280	D	Mid-July	July 22
6	Fluazifop	280	E	25 CDD	September 22
7	Glyphosate	560	A	75 GDD	April 28
8	Glyphosate	560	B	175 GDD	May 26
9	Glyphosate	560	C	Flowering	June 18
10	Glyphosate	560	D	Mid-July	July 22
11	Glyphosate	560	E	25 CDD	September 22

[†]Fluazifop was applied with NIS (0.25% v/v).

Table 2. Visual rating dates at Mendham Golf and Tennis Club in Mendham, NJ and corresponding weeks after each application was made. Treatments were applied on April 28, May 26, June 18, July 22, and September 22, 2020.

	Weeks after treatment (WAT)				
Rating date	A	B	C	D	E
May 26, 2020	3	*			
June 18, 2020	7	3	*		
July 9, 2020	10	7	3		
July 22, 2020	12	9	5	*	
August 11, 2020	15	12	8	3	
August 28, 2020	17	14	10	5	
September 22, 2020	21	18	14	9	*
October 22, 2020	25	21	18	13	4

Table 3. Effects of herbicide (fluazifop and glyphosate) and application timing (A, B, C, D, and E applied on April 28, May 26, June 18, July 22, and September 22 respectively) on visual evaluations of percent deertongue control at Mendham, NJ in 2020.

		Percent Deertongue Control (%)			
Herbicide	**App. code**	**3-4 WAT**	**9-10 WAT**	**14-15 WAT**	**17-18 WAT**
		Herbicide			
fluazifop		55 b[†]	46 b	50 b	40 b
glyphosate		72 a	76 a	75 a	80 a
Pr > F		0.0068	0.0001	0.0315	0.0001
		Application			
	A	68	37 c	43 b	51
	B	80	75 a	72 a	62
	C	65	76 a	74 a	67
	D	55	55 b	.	.
	E	48	.	.	.
Pr > F		NS	0.0001	0.0020	NS
		*Herbicide * Application*			
fluazifop	A	55	24	45 b	47 b
fluazifop	B	79	59	53 b	36 b
fluazifop	C	56	61	53 b	37 b
fluazifop	D	49	41	.	.
fluazifop	E	34	.	.	.
glyphosate	A	81	50	40 b	55 b
glyphosate	B	82	92	90 a	89 a
glyphosate	C	74	94	96 a	97 a
glyphosate	D	61	68	.	.
glyphosate	E	62	.	.	.
Pr > F		NS	NS	0.0058	0.0055

[†]Means followed by the same letter are not significant different according to Fisher's Protected LSD test; P=0.05.

Table 4. Deertongue grass (*Dichanthelium clandestinum*) injury in Mendham, NJ at the final rating 25, 21, 18, 13 and 4 weeks after herbicide applications on April 28, May 26, June 18, July 22, and September 22 respectively. Deertongue grass injury was evaluated visually on a 0 (no injury) to 100 (complete necrosis) percent scale relative to the non-treated control.

Percent Deertongue Control (%)

Treatment	Herbicide	App. code	Final rating (22 October 2020)	
1	Non-treated	-	0	f†
2	fluazifop	A	11	ef
3	fluazifop	B	29	de
4	fluazifop	C	37	cd
5	fluazifop	D	59	bc
6	fluazifop	E	34	cd
7	glyphosate	A	36	cd
8	glyphosate	B	88.8	a
9	glyphosate	C	96.8	ab
10	glyphosate	D	79	ab
11	glyphosate	E	62	b
	Pr > F		0.0174	

†Means followed by the same letter are not significant different according to Fisher's Protected LSD test; P=0.05.

Figure 1. May and June (B and C) applications of glyphosate provided greater deertongue control than other herbicide treatments at the conclusion of experiement on October 22, 2020 in Mendham, NJ.

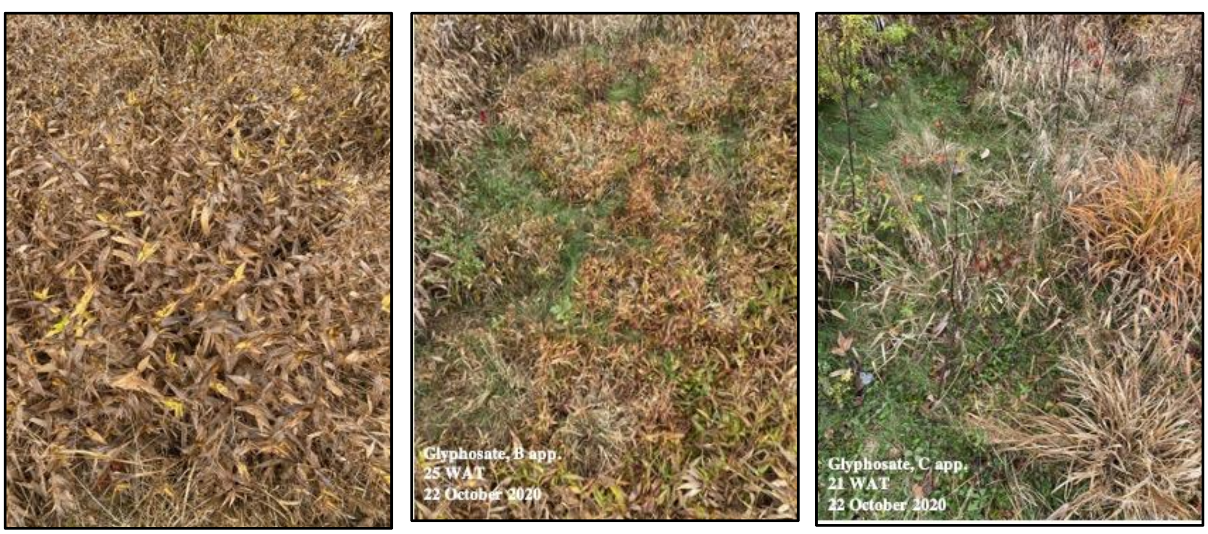


Figure 2. Deertongue rhizomes were uniformly planted June 11 on fine fescue seeded May 14, 2020. First leaves emerged from rhizomes 7 days later on June 17th (top left).

3. ENVIRONMENT

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: Five years; estimated conclusion is the summer of 2021
Total Funding: $44,200

Summary Points:
Our preliminary finding is that the irrigation system is often the key golf course component with the greatest potential for cost savings coupled with carbon footprint reduction.

Summary Text:

We anticipate that our deliverables will have a beneficial impact on golf course maintenance budgets. It will almost be the polar opposite of a narrowly focused research study. Rather, ours will be 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.

Currently, there is no single source of information related to the carbon footprint of a golf course, particularly one that focuses on e. 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 will be 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 being 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 at least 10 CO_2 molecules.) The questionnaire is being 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 may also consider the turfgrass species based on the recent work by Patton et al. (2015).

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_2 eq emissions and sequestration. The underlying equations are based on results in the published literature and on the US EPA's website. However, we are considering updating the current version of CarbonSave® to account for CH_4 and N_2O dynamics in turfgrass, which recent USGA-sponsored research has investigated.

Our work scope is producing an analysis of the CO_2 eq 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 one year of energy consumption data. Our analysis will also 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.

Results to Date.
The monthly energy analyses for the three golf courses are more than 90% complete. The carbon sequestration and the carbon emissions analyses are 30 to 50% complete. Figures 1 through 3 below illustrate the type of analyses we are doing for these golf courses.

Future Expectations.
We anticipate the project report will be completed this summer.

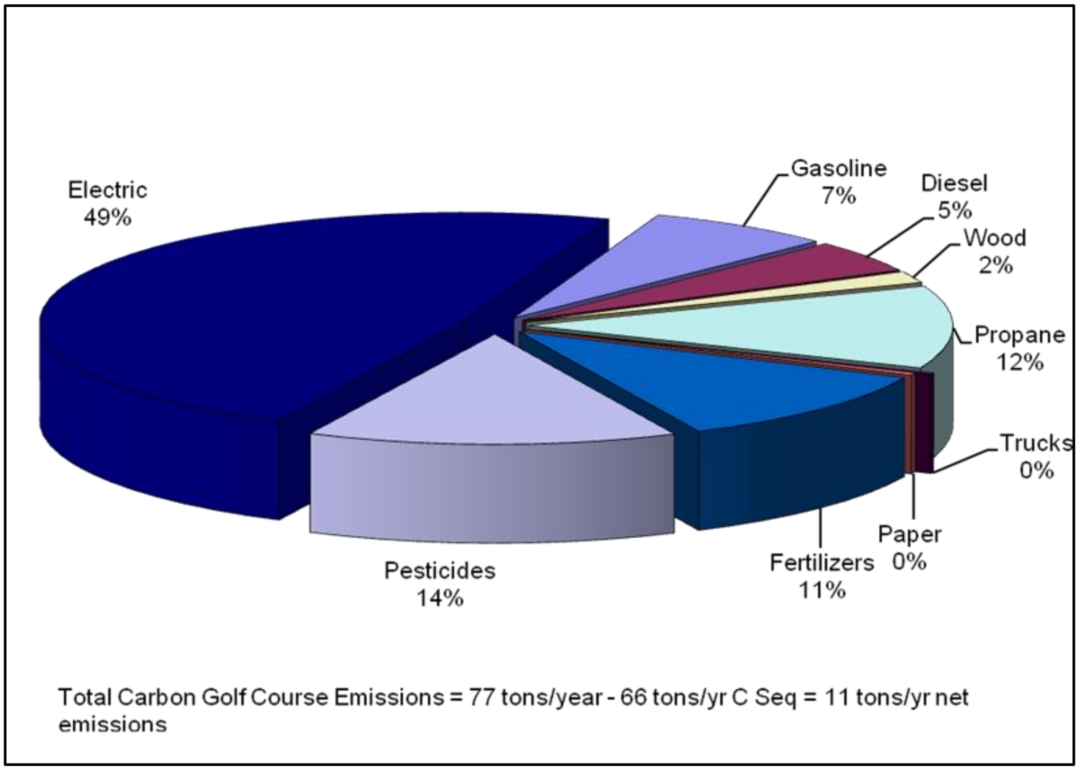


Figure 1. % Total Carbon Emissions for the Golf Course

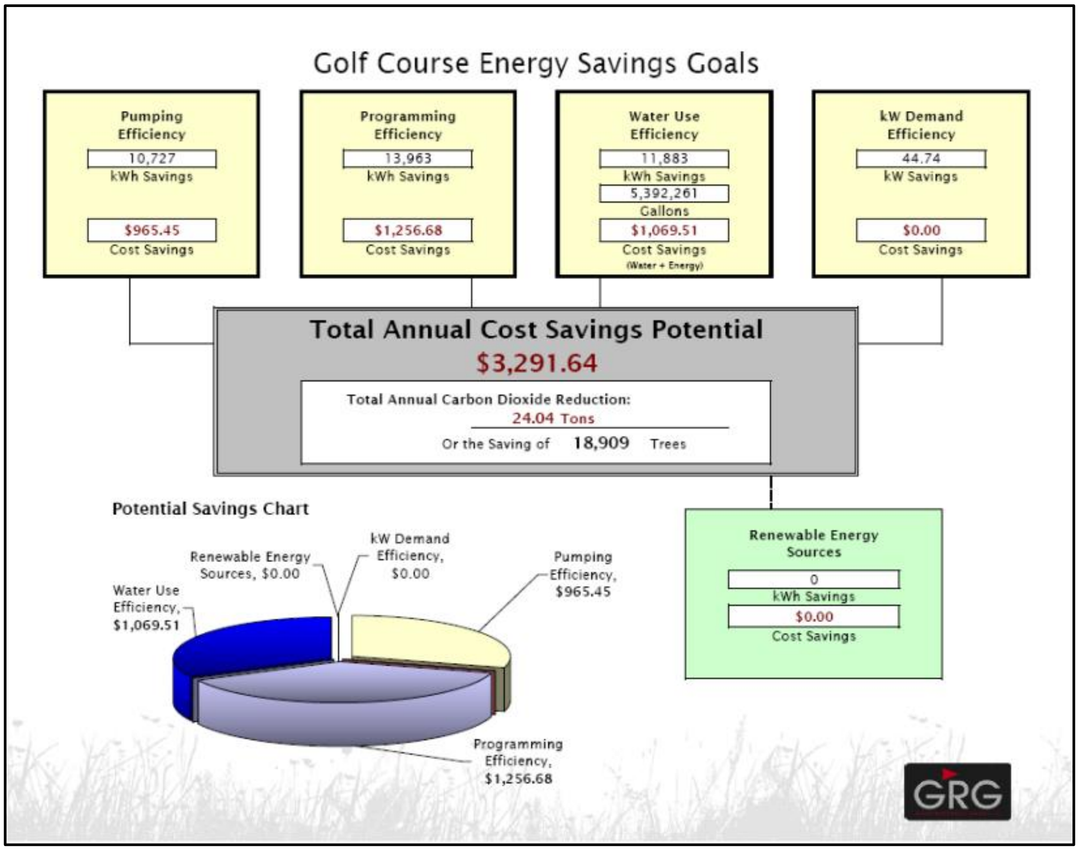


Figure 2. Golf Course Energy Savings Goals

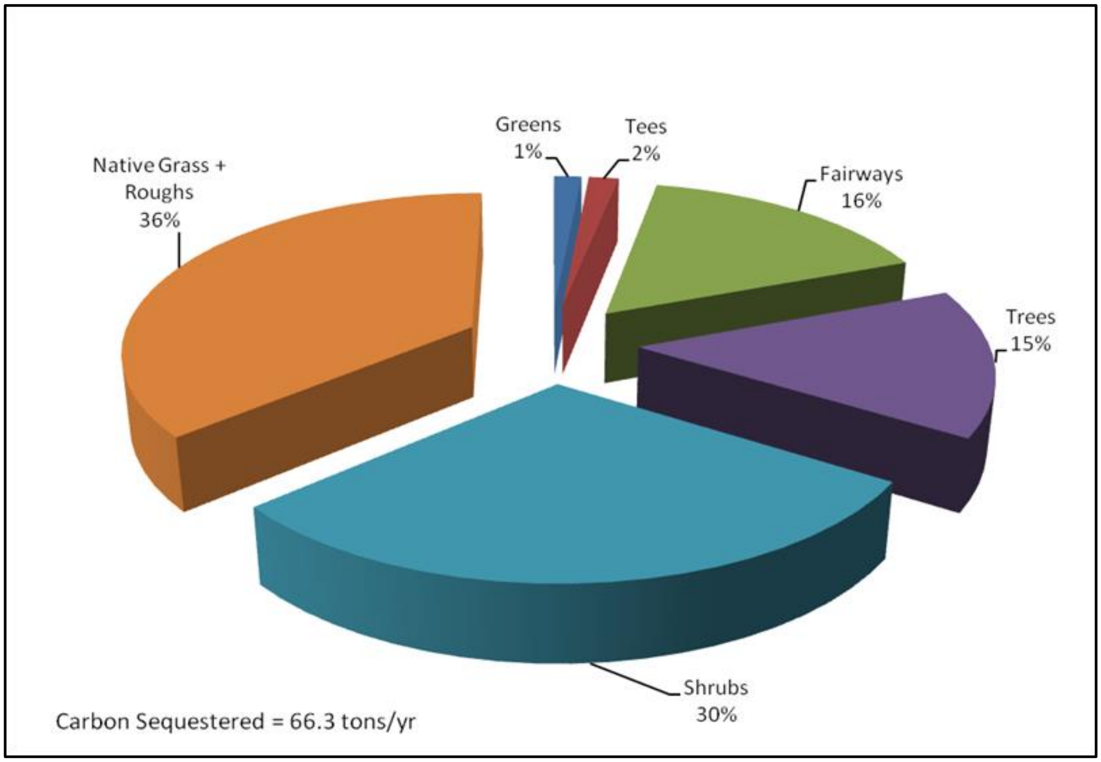


Figure 3. Golf Club (Complete Facility) % Carbon Sequestration

USGA ID# 2018-19-669

Title: Simulation of Nitrous Oxide Emissions and Carbon Sequestration in Zoysiagrass Fairway Turf Using the DAYCENT Model

Project Leaders: Mu Hong[1], Yao Zhang[2], Ross Braun[3], and Dale J. Bremer[1]
Affiliation: [1]Kansas State University; [2]Colorado State University; [3]Purdue University

Objectives:

1. Calibration and validation of the DAYCENT model 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: 2 years
Total Funding: $76,812

Summary Points:

- The DAYCENT model was parameterized and calibrated for zoysiagrass with field biomass and data from Braun and Bremer (2018a, 2019).
- N_2O flux measurements are well modelled by DAYCENT daily outputs.
- Sensitive parameters of DAYCENT model were identified using global sensitivity analysis.

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 provides a unique opportunity to calibrate the DAYCENT model for zoysiagrass turf; specifically, to predict long-term impacts of N fertilization and irrigation management on N_2O emissions and C sequestration. Such model development is important because continuous long-term measurements are expensive and time consuming. DAYCENT is a powerful model developed and used widely to predict GHG fluxes in agricultural lands. DAYCENT has been applied to C3 but not C4 turfgrasses such as zoysiagrass (Zhang et al., 2013a, 2013b, 2020), which must be calibrated separately because of specific physiological characteristics (Zhang et al., 2013a).

Materials and Methods

Information on weather, soil, and management practices was required for model simulations. Daily maximum/minimum temperatures and precipitation were obtained from an on-site weather station (http://mesonet.k-state.edu). Two soil cores from the upper 30-cm depth were taken at the study site to determine average soil texture (Table 1), from which field capacity and wilting point were calculated (Saxton et al., 2006). The soil pH is 7.0.

In the current project, the crop file of the DAYCENT model was parameterized for 'Meyer' zoysiagrass using the method of Zhang et al. (2013b, 2020). Belowground C allocation parameters (i.e., CFRTCW.2., CFRTCN.2. in Table 1), growth rate parameters (e.g., PRDX.1., FULCAN, PLTMRF), and shoot and root death rates (i.e., FSDETH.1., RDRJ, RDRM) were adjusted based on field biomass samples of 2019 and 2020 and research on the Meyer cultivar by Patton et al. (2007). Leaf lignin content was set at 6% (Hale et al.,2009; Hamido et al., 2016). Lignin content of roots was set at 24.3% based on lab analysis of zoysiagrass roots and rhizomes (10-cm depth) (Sluiter et al., 2008). To adjust C/N ratio parameters (Table 1), monthly model outputs of average leaf and root C/N ratios were compared with ranges of C/N ratios of field samples analyzed with the method of Braun and Bremer (2019).

Historical land use at the research site was simulated as Konza prairie for 4000 years to reach equilibrium, followed by cropland, then turfgrass with regular maintenance since the 1970s, and finally experimental plots since 2000. Turfgrass management practices of fertilization, irrigation, and mowing were scheduled for 2014-2016 according to Braun and Bremer (2018a). Urea was modeled as an input of NH_4^+ into the soil. Polymer-coated urea (90-day release) was modeled as 45 events of NH_4^+ inputs in 2-day intervals (Salman et al., 1989). Irrigation during the growing season outside the summer experimental periods (when N fertility and irrigation treatments were applied) were scheduled as weekly inputs of 2.5-cm water. Mowing was treated as a harvest event that returns harvested biomass (clippings) as litter (Zhang et al. 2013a), which was parameterized using field samples and research of Trappe et al. (2011) on Meyer.

The authors have already measured soil moisture, N_2O emissions, soil organic nitrogen, and soil organic carbon, for the simulated Meyer zoysiagrass fairway, in a field setting under an automated rainout shelter that allowed for precise control of irrigation at the Rocky Ford Turfgrass Research Center near Manhattan, KS (Braun and Bremer, 2018a, 2019). The scaling factor for potential evapotranspiration was set to 0.65.

Results

Results show measured and daily simulated N_2O fluxes are well matched under different irrigation and fertilization practices (Fig. 1). The Pearson's *r* across all management treatments is 0.72 ($P<10^{-5}$). The Pearson's *r* between measured and daily simulations for soil moisture, soil temperature, soil ammonium, and soil nitrate are 0.34, 0.98, 0.23, and 0.47, respectively.

Using the 'Sobol' method for global sensitivity analysis, parameters were identified by their total effect (total indices) and main effect (first order indices) in DAYCENT model simulations for soil ammonium, soil nitrate, volumetric water content, and nitrous oxide, respectively (Table 1; Fig. 2) (Zhang et al. 2020). Parameters in Table 2 (both total indices and first order indices $\geq$0.05) can be important in fine-tuning the simulations, and can be adjusted by Bayesian parameter uncertainty analysis in the next step.

Thereafter, the calibrated DAYCENT model will be validated by comparing simulation outputs with field measurements of soil moisture, N_2O emissions, and soil organic nitrogen measured from zoysiagrass in an earlier study at another site of the Rocky Ford Turfgrass Research Center (Lewis and Bremer, 2013). This validation will test and, hopefully, justify the model's long-term simulations of the impacts of irrigation and N-fertilization practices on N_2O emissions and carbon sequestration in zoysiagrass turf.

References

Braun, R.C., and D.J. Bremer. 2018a. Nitrous oxide emissions from turfgrass receiving different irrigation amounts and nitrogen fertilizer forms. Crop Sci. 58:1762-1775.

Braun, R.C., and D.J. Bremer. 2018b. Nitrous oxide emissions in turfgrass: A review. Agron. J. 2222-2232. doi:10.2134/agronj2018.02.0133

Braun, R.C., and D.J. Bremer. 2019. Carbon sequestration in zoysiagrass turf under different irrigation and fertilization management regimes. Agroecosystems, Geosciences, and Environment.

Hamido, S.A., E.A. Guertal, and C.W. Wood. 2016. Seasonal variation of carbon and nitrogen emissions from turfgrass. American Journal of Climate Change. 5:448–463. doi:10.4236/ajcc.2016.54033.

Hale, T.C., J.A. Reinert, and R.H. White. 2009. Resistance of zoysiagrasses (Zoysia spp.) to fall armyworm (Lepidoptera: Noctuidae): I. Leaf tensile strength and cell wall components. J. Int. Turfgrass Soc. 11: 639-648.

Lewis, J.D. and D.J. Bremer. 2013. Different nitrogen management regimes affect nitrous oxide emissions among one cool-season and two warm-season turfgrasses. Int. Turf. Soc. Res. J. 12:31-38.

Patton, A., J.J. Volenec, and Z.J. Reicher. 2007. Stolon growth and dry matter partitioning explain differences in zoysiagrass establishment rates. Crop Sci:1237–1245.

Saxton, K.E., and W.J. Rawls. 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Science Society of America Journal. 70:1569. doi:10.2136/sssaj2005.0117.

Sluiter, A, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker. 2008. Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure. 1617: 1-16.

Salman, O.A., G. Hovakeemian, and N. Khraishi. 1989. Polyethylene-coated urea. 2. Urea release as affected by coating material, soil type and temperature. Ind. Eng. Chem. Res. 28:633–638. doi:10.1021/ie00089a022

Trappe, J.M., D.E. Karcher, M.D. Richardson, and A.J. Patton. 2011. Bermudagrass and zoysiagrass cultivar selection: Part 1, Clipping Yield, Scalping Tendency, and Golf Ball Lie. ats. 8:0. doi:10.1094/ATS-2011-0630-01-RS.

Zhang, Y., Y. Qian, D.J. Bremer, and J.P. Kaye. 2013a. Simulation of nitrous oxide emissions and estimation of global warming potential in turfgrass systems using the DAYCENT model. J. Environ. Qual. 42:1100-1108.

Zhang, Y., Y. Qian, B. Mecham, and W.J. Parton. 2013b. Development of best turfgrass management practices using the DAYCENT model. Agron. J. 105:1151-1159.

Zhang, Y., M. Arabi, and K. Paustian. 2020. Analysis of parameter uncertainty in model simulations of irrigated and rainfed agroecosystems. Environmental Modelling & Software, 126, 104642.

Table 1. Definition and current value of parameters selected for sensitivity analysis.

Parameter	Definition	Current Value
FULCAN	Value of above ground live C at full canopy cover, above which there is no restriction on seedling growth (gC/m2)	200
PLTMRF	Planting month reduction factor to limit seedling growth (0.0-1.0)	0.2
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.	3.5
PPDF.1.	Optimum temperature for production for parameterization of a Poisson Density Function curve to simulate temperature effect on growth.	30
PPDF.2.	Maximum temperature for production for parameterization of a Poisson Density Function curve to simulate temperature effect on growth.	45
FALLRT	Fall rate (fraction of standing dead which falls each month)	0.5
FSDETH.1.	Maximum shoot death rate at very dry soil conditions (fraction/month)	0.1
RDRJ	Maximum juvenile fine root death rate at very dry soil conditions (fraction/month)	0.2
RDRM	Maximum mature fine root death rate at very dry soil conditions (fraction/month)	0.1
RDSRFC	Fraction of the fine roots that are transferred into the surface litter layer upon root death, the remainder of the roots will go to the soil litter layer	0.14*
WSCOEF.1.	Water Stress Coefficient used to calculate the water stress multiplier on potential growth based on the relative water content of the wettest soil layer in rooting zone	0.378*
WSCOEF.2.	Water Stress Coefficient used to calculate the water stress multiplier on potential growth based on the relative water content of the wettest soil layer in rooting zone	9*
PRBMX.1.1.	(N, intercept) parameter for computing maximum C/N ratio for belowground matter as a linear function of annual precipitation.	80
PRBMN.1.1.	(N, intercept) parameter for computing minimum C/N ratio for belowground matter as a linear function of annual precipitation.	40
PRAMX.1.2.	Maximum aboveground C/N ratio with biomass > biomax.	45
PRAMX.1.1.	Maximum aboveground C/N ratio with zero biomass.	30
PRAMN.1.2.	Minimum aboveground C/N ratio with biomass > biomax.	20
PRAMN.1.1.	Minimum aboveground C/N ratio with zero biomass.	9.6
BIOMAX	Aboveground biomass level above which the minimum and maximum C/E ratios of new shoot increments equal pramn(*,2) and pramx(*,2) respectively.	500
CFRTCW.2.	Minimum fraction of C allocated to roots with no water stress	0.2
CFRTCN.2.	Minimum fraction of C allocated to roots with no nutrient stress	0.2
FAVAIL.1.	fraction of N available per day to plants.	0.15*
MAXNIT	maximum daily nitrification amount (gN/m2/day)	1*
MINO3	fraction of new net mineralization that goes to NO3 (0.0- 1.0)	0.2*
NCOEFF	minimum water/temperature limitation coefficient for nitrify	0.03*
N2N2OA	N2/N2O ratio adjustment factor for computing the N2/N2O ratio during non-flooded conditions. Values > 1.0 increase this ratio, values (0.0-1.0) decrease this ratio.	1*
NADJFC	maximum proportion of nitrified N lost as N2O @ field capacity	0.012*
NADJWP	Minimum fraction nitrified N lost as N2O at wilting point	0.012*
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)	1*
Clay	Soil clay percent	23
Sand	Soil sand percent	14.4
BD	Soil bulk density	1.35

* Value that was kept the same as default value.

Table 2. Sensitive parameters for DAYCENT simulations.

Parameter	Main effect	Half 95% CI†	Total effect	Half 95% CI
		Soil VWC		
PLTMRF	0.0497	0.0616	0.1059	0.0132
FALLRT	0.0552	0.0616	0.1016	0.0127
Clay	0.5928	0.0388	0.6136	0.054
Sand	0.1277	0.0605	0.1364	0.0167
		Soil NH4		
PRDX.1.	0.1288	0.0605	0.472	0.0462
MAXNIT	0.1732	0.0595	0.3917	0.0406
		Soil NO3		
PLTMRF	0.2181	0.0583	0.4056	0.0416
PRDX.1.	0.3588	0.0528	0.4769	0.0465
		N2O_N		
NADJWP	0.0834	0.0612	0.0635	0.0081
N2N2OA	0.1198	0.0607	0.3247	0.0352
WFPSNIP	0.1518	0.06	0.3521	0.0375
BD	0.1606	0.0598	0.5655	0.0517

† Distance from mean value of the effect to 95% confidence interval (upward or downward).

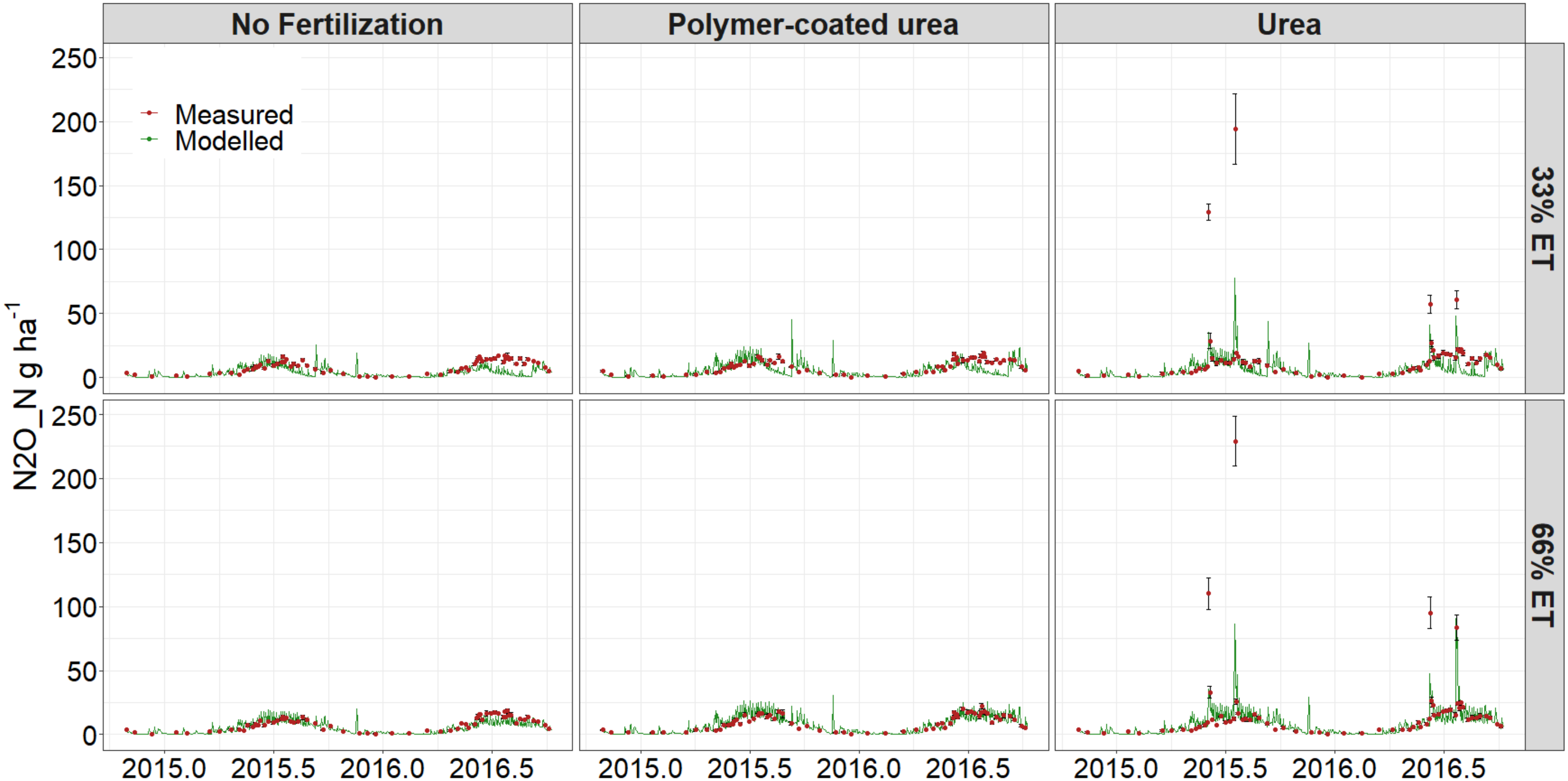
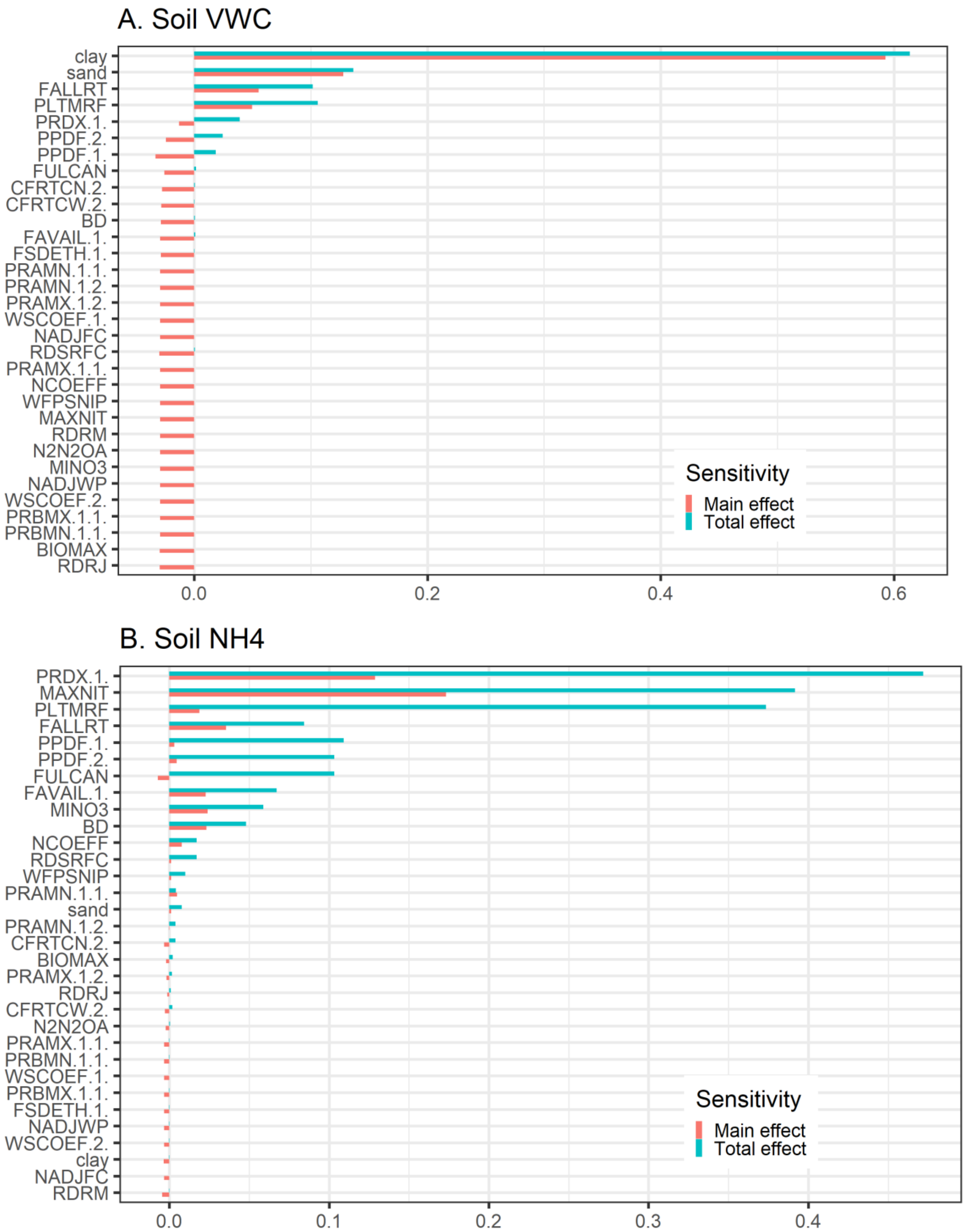


Figure 1. Measured and daily DAYCENT-simulated nitrous oxide (N2O) under low (33% ET)/medium (66% ET) irrigation and no/polymer-coated urea/urea fertilization.


A. Soil VWC
clay
sand
FALLRT
PLTMRF
PRDX.1.
PPDF.2.
PPDF.1.
FULCAN
CFRTCN.2.
CFRTCW.2.
BD
FAVAIL.1.
FSDETH.1.
PRAMN.1.1.
PRAMN.1.2.
PRAMX.1.2.
WSCOEF.1.
NADJFC
RDSRFC
PRAMX.1.1.
NCOEFF
WFPSNIP
MAXNIT
RDRM
N2N2OA
MINO3
NADJWP
WSCOEF.2.
PRBMX.1.1.
PRBMN.1.1.
BIOMAX
RDRJ
0.0
0.2
0.4
0.6
Sensitivity
Main effect
Total effect
B. Soil NH4
PRDX.1.
MAXNIT
PLTMRF
FALLRT
PPDF.1.
PPDF.2.
FULCAN
FAVAIL.1.
MINO3
BD
NCOEFF
RDSRFC
WFPSNIP
PRAMN.1.1.
sand
PRAMN.1.2.
CFRTCN.2.
BIOMAX
PRAMX.1.2.
RDRJ
CFRTCW.2.
N2N2OA
PRAMX.1.1.
PRBMN.1.1.
WSCOEF.1.
PRBMX.1.1.
FSDETH.1.
NADJWP
WSCOEF.2.
clay
NADJFC
RDRM
0.0
0.1
0.2
0.3
0.4
Sensitivity
Main effect
Total effect

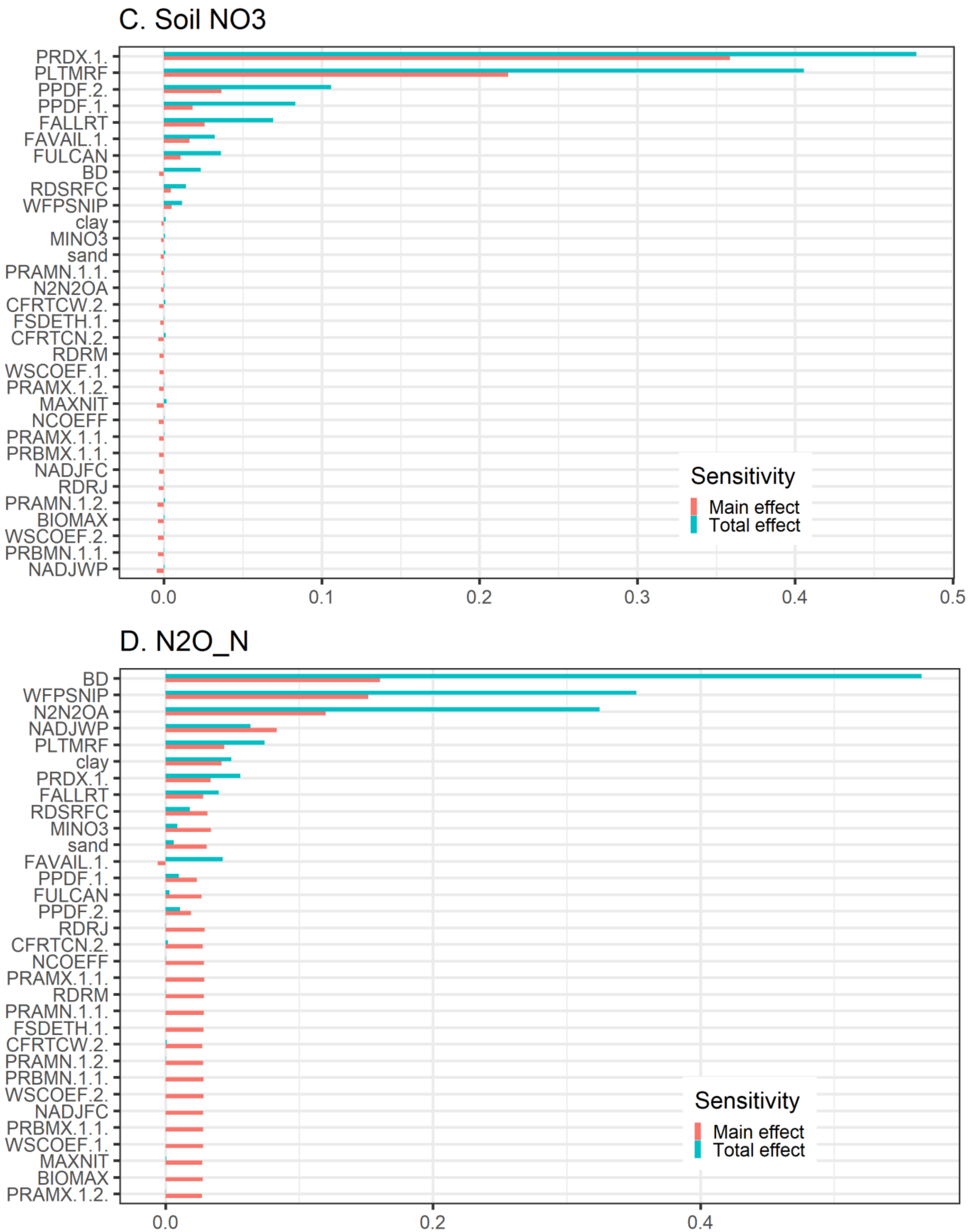


Figure 2. Relative ranking of sensitivity of DAYCENT model to selected parameters in simulating (A) soil volumetric water content (VWC), (B) soil ammonium (NH4), (C) soil nitrate (NO3), (D) nitrous oxide (N2O) in zoysiagrass, respectively. These parameters are defined in Table 1.

USGA ID#: 2020-01-706

Title: Community Values of Golf Courses: Building capacity to evaluate Natural Capital on Golf Courses throughout the United States

Project Leader: E. Lonsdorf[1], and B. Horgan[2]
Affiliation: [1]University of Minnesota; [2]Michigan St. University

Objectives:

1. Complete the ecosystem services provided by golf courses in multiple cities of the United States
2. Communicate our findings to scientific and USGA communities

Start Date: January 1, 2020
Project Duration: 1 year thus far
Total Funding: $90,000

Summary Points:

- We've analyzed five ecosystem services provided by each of over 1,000 golf courses across seven major US metro areas.
- Ecosystem service responses to land use change varies across cities and eco-regions - Philadelphia and Atlanta are most sensitive while Phoenix is least sensitive.
- We've incorporated flood modeling and integrated our land-use change tool with it.
- The team has submitted its work in two publications and presented its findings to numerous scientific and public audiences

Summary:

Nature's contributions to people, e.g. ecosystem services, support human systems around the world from agriculture to coastal resilience. With more than half the world's population living in urban areas (Elmqvist et al. 2019), most of people's potential to receive ecosystem service benefits occurs in cities. However, a straightforward, replicable approach to quantifying multiple urban ecosystem services has yet to emerge; and while there has been an increasing interest in integrating ecosystem services into urban planning decisions, uptake remains limited. Golf courses represent a substantial part of urban areas in the United States, comprising just over 9,300 km^2 from approximately 14,200 courses as of 2015.

Given their common occurrence in urban areas and the potential pressures to develop them, golf courses provide an important case study to evaluate urban ecosystem services in a changing environment. The goal of our work is to apply newly developed models of urban ecosystem services to address the question: how do golf courses support nature's benefits to the surrounding community in urban areas? And how do these benefits, relative to other land uses, vary across different cities in the United States? The overarching goals are to provide an approach that allows one to answer this question in any urban area in the United States, facilitate any city from

being able to answer this as easily as possible and communicate this information to scientists and potential stakeholders.

Completing Ecosystem Service Assessments-To address the contribution of golf courses to ecosystem services in urban areas, we used newly developed tools provided by the Natural Capital Project's Urban InVEST (Integrated Valuation of Ecosystem Services and Tradeoffs) modeling suite. We applied models of Urban Cooling, Stormwater Retention and Pollinator Habitat to seven metro areas in the United States that span a variety of social and ecological contexts: San Francisco Bay, Phoenix, Dallas-Ft. Worth, Minneapolis-St. Paul, Atlanta, Philadelphia and Detroit (Figure 1a). We identified golf courses in each metro area using Open Street Maps (Figure 1b).

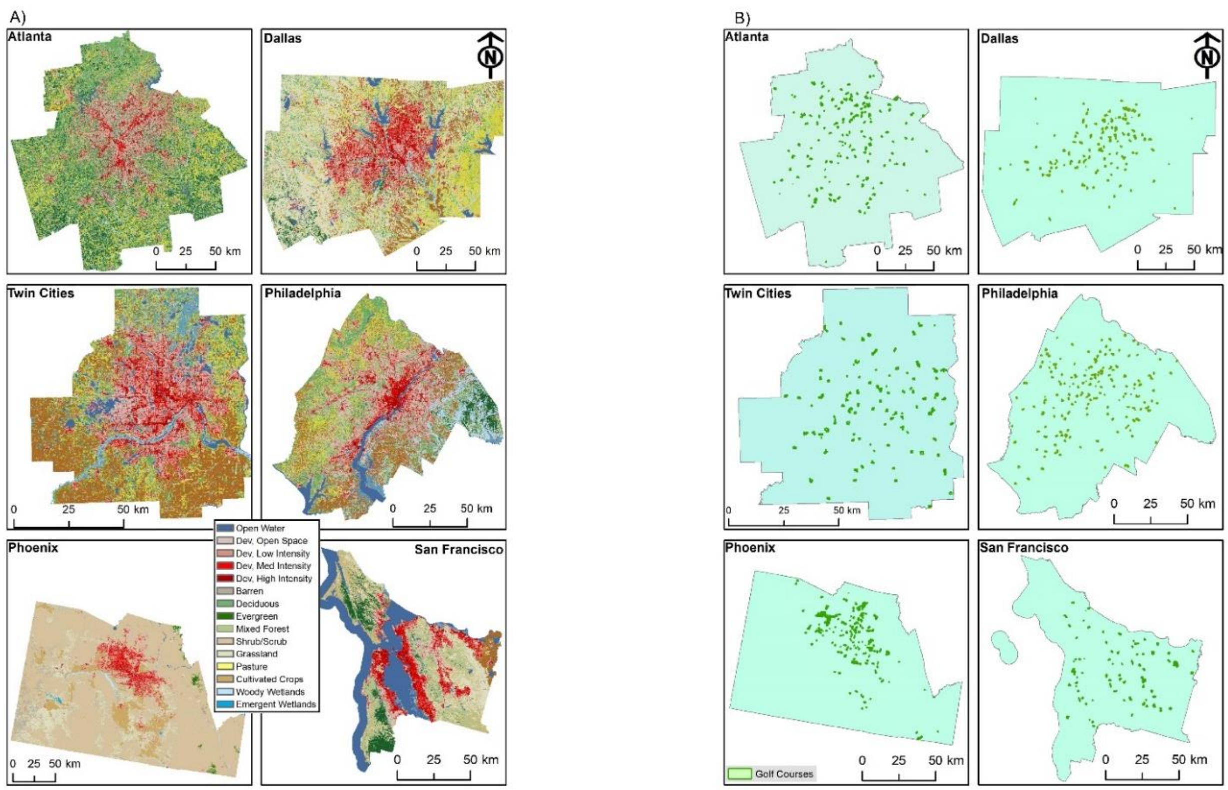


Figure 1. Land cover (a) and golf courses (b) maps for selected areas in six of the seven case study cities (Detroit not shown). Land cover data are 30 m resolution. Land cover Source: 2016 National Land Cover Database (https://www.mrlc.gov/data). Golf course source: Open Street Map (https://www.openstreetmap.org).

To evaluate the contribution of golf courses, we used a marginal value approach. We defined a golf course's marginal value as the change in a landscape's total ecosystem service value for a given service in response to a change in the golf course. We have developed an ArcGIS tool to automate this process, called the "wallpaper" approach as it identifies and replicates common urban land cover patterns, e.g. urban residential, suburban residential, city park. We replace a current golf course with one of these patterns and re-evaluate the ecosystem services. We've gathered necessary data to run models on all seven cities, including identifying golf courses described on Open Street Maps. We ran the marginal analyses for each city using the nutrient retention model, stormwater retention model, the urban cooling model and the pollinator habitat model. We've described each of these in previous reports so will not describe them here.

Our team has streamlined the analysis so that our approach could be applied easily in other cities. Chris Nootenboom addressed this "pick-a-city" challenge and worked to reduce the computational time it would take to analyze the baseline ecosystem services in any urban area. This requires identifying nationally-available data and appropriate ecoregionally-specific parameters identified during our seven-city analysis. We have developed a computational script that allows us to pick a city and develop a full analysis of these five services in about 5 minutes for any major metro area in the United States.

Finally, our team has added the capability to analyze the flood mitigation potential of individual courses. Team member Ben Janke developed a routine that allows us to use the wall-paper approach with CADDIES (Cellular Automata Dual-DraInagE Simulation[1]), a program designed to analyze floods. The model cannot be run on every course and for every scenario due to computation time needed to run finer time-scale and spatial scale assessments, so we've be selecting a subset of courses in each city. This work is continuing but the workflow has been completed and the application to the Twin Cities is nearly complete.

Results to date

We've completed a marginal value assessment of five different ecosystem services on each of 1,110 golf courses across seven metro areas in the United States. Overall, golf courses cool the air, retain more nutrients and stormwater, and support more biodiversity compared to residential or industrial land cover and are on par with more natural areas for these services. Our results indicate clear eco-regional differences in response to land use change, e.g. city parks increase pollination relative to golf courses in San Francisco, but decrease it in Phoenix. This is due to differences in dominant land cover within each of these cities. Also, sensitivity overall to land use change differs among cities and eco-regions with Atlanta and Philadelphia showing greatest change among land uses and Phoenix showing the least (Figure 2). Flood modeling indicates that some areas nearby a golf course may become more flood-prone when golf courses are converted to land uses with more impervious surface (residential and industrial; Figure 3).

Communication: As we near completing the analytical work, we have shifted efforts to communicating our approach and insights to science colleagues and potential stakeholders. Specifically, we have submitted two articles to peer-reviewed journals and both are in revision with expected acceptance for publication soon. We have also presented our work in conferences, workshops and training sessions throughout the world.

---

[1] http://emps.exeter.ac.uk/engineering/research/cws/resources/caddies-framework/caddies-2d/

Peer-reviewed papers in review:

Lonsdorf, E. V.**,** C. Nootenboom, B. Janke and B. Horgan. Ecosystem services provided by golf courses in urban landscapes. Landscape and Urban Planning

Hamel P., Guerry A., Polasky S., Hamann M., Lonsdorf, E., Nootenboom C., Janke B., Remme R., Han B., Liu H., Tardieu L., Viguie V., Sharp, R., Ouyang Z., Daily G. Mapping nature's benefits to co-create livable and sustainable cities. Nature Sustainability.

Presentations:

- October 2019: NorthWest Turfgrass Association
- October 2019: Ecosystem Services Partnership conference, Hannover, Germany
- November 2019: Gateway Golf Summit
- January 2020: Duluth, MN Parks and Recreation
- January 13-15, 2020: Workshop on Natural Capital Assessment, Chinese Academy of Science, Beijing, China
- Febuary 2020: Western Canada Turf Association
- February 2020: Seattle Golf Summit
- February 2020: Illinois Turfgrass Conference
- August 2020: International Urban InVEST Training
- October 2020: National Turfgrass Federation/Foundation For Food and Agriculture Research Stakeholder Summit

Future expectations

We've completed the marginal valuation work for each of our focal cities and are working to develop communication, outreach and visualization tools. We plan to use these materials to engage city planners to better understand how our approaches can support the inclusion of golf course's social and environmental benefits in planning. Our team is now collaborating with the World Bank on an urban planning project in China and is scoping engagements with the Inter-American Development Bank for urban planning in Latin America. Closer to home, we just started working with stakeholders on development planning for the Ponds at Battle Creek Golf Course that was recently slated to be sold by Ramsey County, MN.

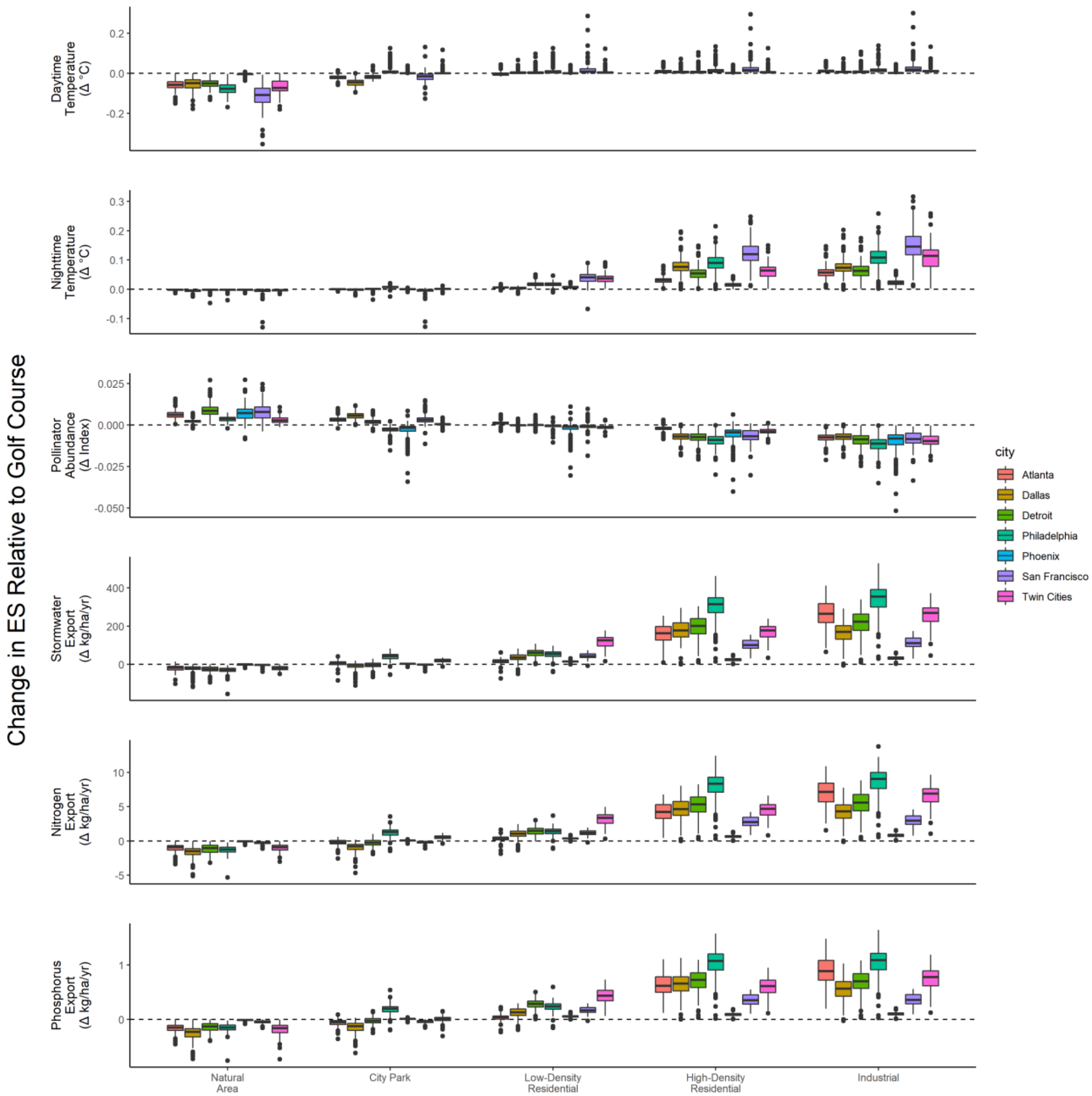


Figure 2. Effect of potential land use change on ecosystem services for each golf course across seven metro areas in the United States. For each scenario of land use change (e.g. Natural Area), change is represented by a box plot illustrating the 25th 50th and 75th quartiles with individual dots representing the extremes.

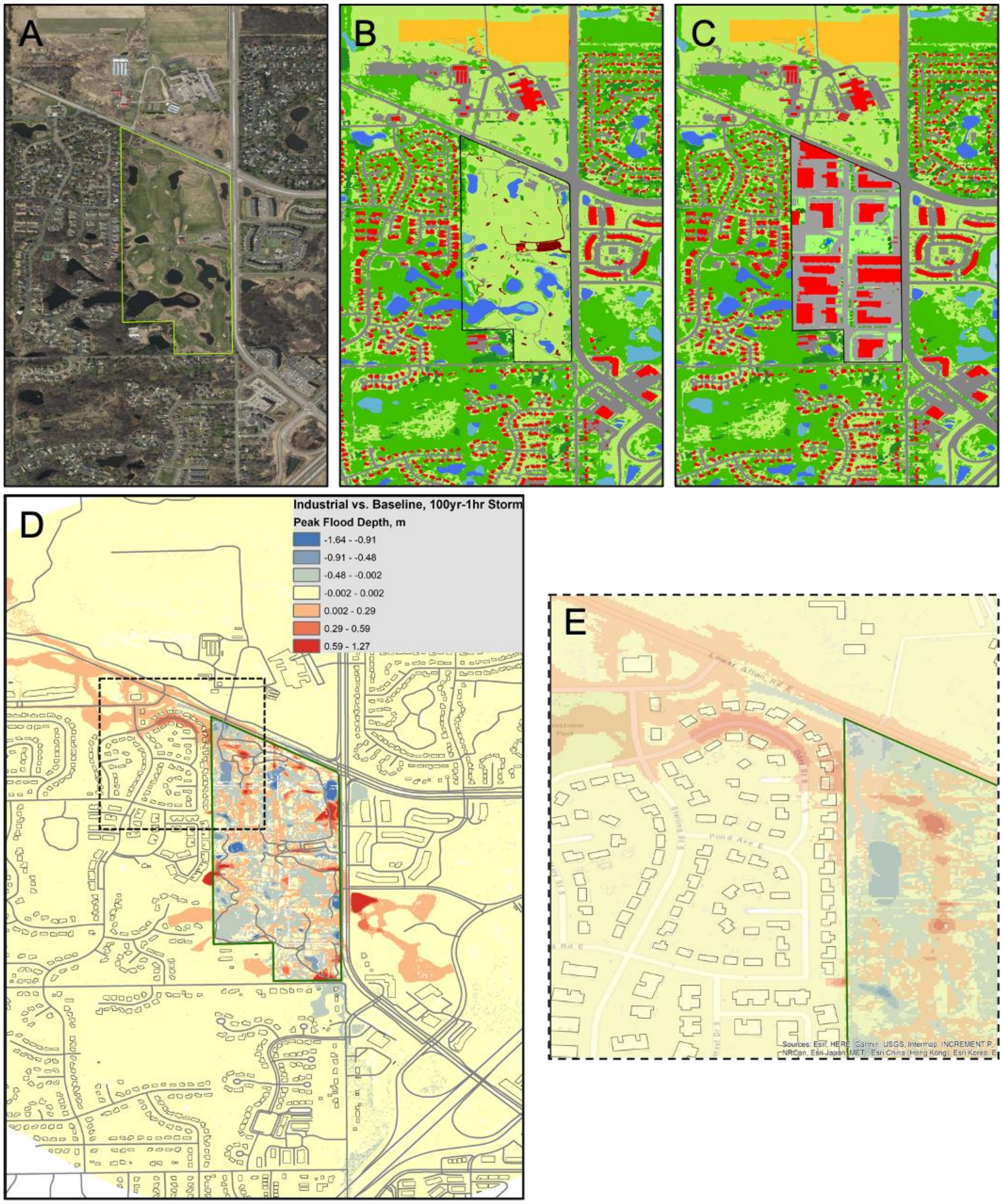


Figure 3. Application of the CADDIES flood model to a golf course for a 1-hour 100-year storm (94 mm rainfall depth). Aerial photo shown in (a), with existing land cover shown in (b). Modified land cover (c) is for an industrial-type land use with flattened elevation (i.e. ponds filled in). Resulting change in peak flood depth for a scenario of industrial land use on the course is shown in (d), and detailed inset at right (e) to illustrate street flooding, with red shading indicating areas of greater flooding for this scenario.

Literature Cited

Elmqvist, T., E. Andersson, N. Frantzeskaki, T. McPhearson, P. Olsson, O. Gaffney, K. Takeuchi, and C. Folke. 2019. Sustainability and resilience for transformation in the urban century. Nature Sustainability 2:267–273.

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.

Golf courses in the United States can be excellent habitats for maintaining and enhancing regional biodiversity and this benefit is best expressed in areas dominated by anthropogenic land-use. Much of the biodiversity on courses is found within non-turfgrass areas, which in many cases can be improved to provide more complex habitat for biodiversity. *Therefore, examining best management practices (BMPs) that can both facilitate biodiversity and engage patrons of the golf course community could benefit regional biodiversity and promotion of golf course environmental stewardship.* <u>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</u>. These data will provide a complete analysis of how three different ecologically-focused BMPs can influence biotic communities in golf course ecosystems and will provide much needed data on how the implementation of BMPs can make golf course ecosystems more environmentally valuable.

Construction of best management practices: To date, we constructed 5 pre-planned pollinator gardens (25 m^2) which equate to 192 individual plants (plugs) of 19 species of wildflowers and grasses native to the Midwest U.S. Adjacent to each of those gardens we placed a Bat Conservation Management Rocket Box Bat House elevated 4.25 m using a wooden post. At each of those 5 courses, we placed coarse woody debris (6 logs per

100 m of shoreline) into the shoreline of a pond which was studied in a 2016-2019 funded USGA study to assist with colonization of macroinvertebrates.

Diurnal and nocturnal pollinators: At each of the 10 sites (5 experimental and 5 control) we quantified 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 2020 by creating two plots per course and surveying each plot for two consecutive 10 min surveys – for a total of 160 total pollinator surveys. Plots located within experimental courses were placed within the pollinator garden and the next best available pollinator habitat at that course. Plots located within the control courses were placed within the two best areas of pollinator habitat at each course. During each 10 min survey we categorized pollinators into 9 functional groups and noted abundance of each group (citation). Nocturnal pollinators were sampled at each site at one of the plots (within the pollinator garden for experimental courses) once per month between July 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.

Macroinvertebrate assessment: Between June to September 2020, 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 (90 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.

Bat assessment: Between June 17 and July 1, 2020 , we deployed acoustic recorders (SM4BATFS; Wildlife Acoustics) equipped with ultrasonic microphones (SMM-U2) to all 10 golf courses to determine baseline bat activity prior to establishment of habitat modifications. Each golf course was surveyed for 7 nights from sunset to sunrise. Acoustic recorders were placed adjacent to areas designated for pollinator gardens at experimental sites. At control sites, we placed acoustic recorders in one of the two best areas of pollinator habitat identified at each course, as described for the pollinator sampling (see above). 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 2520 useable call files. We visually confirmed any SonoBat classifications of rare species by examining the call spectrogram for key characteristics known for that species (Figure 1).

Across sites we detected a diversity of species, including the big brown bat, silver-haired bat, Eastern red bat, hoary bat, evening bat, and tricolored bat, a species under conservation risk (Table 1). We did not detect activity by either the little brown bat or Northern long-eared bat, two species that are known to the area, but have been severely impacted by White-nose syndrome, a devastating fungal disease.

Future assessment: Between December 2020 and June 2021, we plan to continue to enumerate macroinvertebrate samples, and analyze bat acoustic and pollinator data. Starting in May/June 2021 we plan to begin collection of our second year of pollinator, bat and macroinvertebrate data, including surveys of bat boxes to determine occupancy.

Table 1. Number of bat calls acoustically recorded in June 2020 at each of 10 study sites, classified to species. 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*).

Site	Epfu	Labo	Laci	Lano	Nyhu	Pesu	Total
Biltmore	313		50	6	3		372
Bittersweet	49		116	6			171
Bobolink	300	25	85	22	25		457
BrynMawr	330	22	155	27	30		564
NorthShore	178	5	163	11	1		358
Renwood	80		22	4			106
SaltCreek	115		8			1	124
Schaumburg	76	2	27	4			109
VillageLinks	56	1	30	12			99
WhiteDeer	81		59	9			149
Total	**1578**	**55**	**715**	**101**	**59**	**1**	**2509**

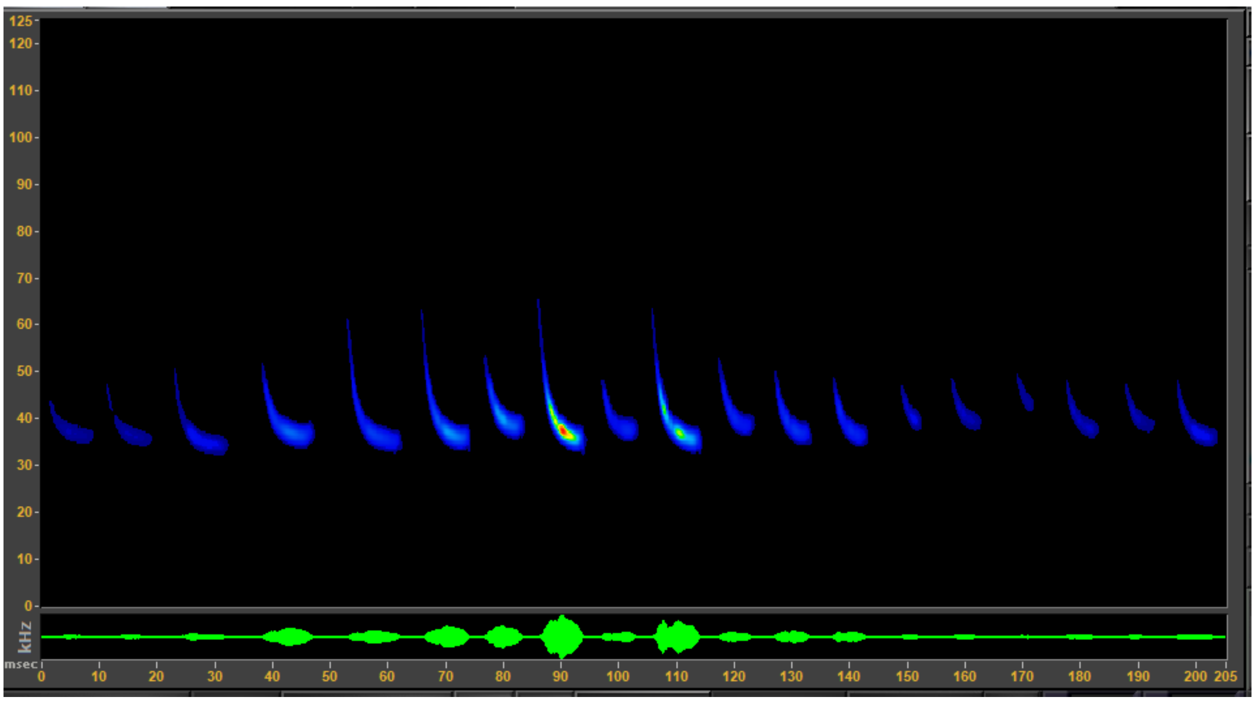


Figure 1. Spectrogram of an evening bat call (*Nycticeius humeralis*) recorded at the Biltmore Country Club on 23 June 2020. Spectrograms can be identified to species based on unique characteristics.

4. REGIONAL GRANTS

USGA ID#: 2019-21-691

Advancement of Five Elite Zoysiagrass Hybrids in the 2019 Zoysiagrass NTEP

Funds Requested by: Ambika Chandra, Associate Professor, Texas A&M AgriLife Research-Dallas, 17360 Coit Road, Dallas, TX 75252

Amount of funds requested: $1,000 per entry per year; $5,000 per year for five entries starting 2019; a total of $25,000 over a five-year period (2019 to 2023).

Background:
Cold Hardy/Large Patch Disease Tolerance: Since its initiation in 2012, significant progress has been made to develop cold hardy and large patch disease tolerant zoysiagrass hybrids as part of the collaborative project between Texas A&M AgriLife Research, Kansas State University and Purdue University, funded by the United States Golf Association. The Texas A&M AgriLife – Dallas breeding team has developed 2,858 new hybrids in 2011/2012 by crossing selected parental lines exhibiting large patch tolerance, fine or intermediate leaf texture, good turfgrass quality and cold hardiness. These hybrids were tested at three locations (Dallas, TX; Manhattan, KS and West Lafayette, IN) from 2012 to 2014 (2 yr. of turfgrass quality and winter recovery data). The 60 best hybrids underwent more extensive testing at nine locations across a wide range of environments for another 3 years. In 2018, the 10 best of the 60 hybrids were chosen based on their spring green up, winter injury, monthly turfgrass quality, large patch tolerance, and percentage establishment across all nine locations. The top three of these top 10 hybrids will be entered into 2019 Zoysiagrass NTEP.

Experimental # (tested as)	Advanced to 2019 NTEP as
TAES 6095-83	DALZ 1701
TAES 6099-145	DALZ 1808
TAES 6119-179	DALZ 1707

Winter color Retention and Performance in UC-Riverside: A total of 218 zoysiagrass hybrids were planted at UC-Riverside in the fall of 2016. Based on the data from 2016 to 2018, top performers for winter color retention and turfgrass quality are as follows:

Experimental # (tested as)	Advanced to 2019 NTEP as
TXZ 463	DALZ 1807
TXZ 488	DALZ 1802

Year 1 (2019-2020):
A total of 20 18-cell trays for each of the five elite hybrids were propagated at Texas A&M AgriLife-Dallas in 2018/2019. Propagated materials were delivered to NTEP headquarters in Beltsville, MD on June 5, 2019. These five elite hybrids will be tested as part of the 2019 National Zoysiagrass NTEP at 20 test locations including 11 standard and 9 ancillary locations. Hybrids will be tested for their overall performance at standard locations as well as for traits like drought, shade, large patch, billbug, traffic tolerance, divot recovery and sod strength at the ancillary locations. Out of these 20 test sites, 11 are in the transition zone or north of the transition zone [KS, MO, IN (x2), MD, VA, NC, TN, AR (x2), OK] and should provide a good platform to screen these hybrids for their cold hardiness. The trial was planted at all NTEP test sites in the of summer 2019 with either 6 ft x 6 ft or 7 ft x 7 ft plot size replicated three times. One tray of each of these hybrids were supplied to each test site where the 3 in x 3 in plugs were divided into four 1-1/2 in mini-plugs for planting on approximately one-foot centers making it a total of twenty-four (24) 1.5 in plugs per plot. Texas A&M-Dallas is one of the ancillary locations and will evaluate these hybrids under drought stress conditions. We anticipate receiving the first data set from the NTEP in 2021.

Year 2 (2020-2021):
NTEP (https://ntep.org/zg.htm) has not yet uploaded the data for the 2019 zoysiagrass test on their website. We anticipate the release of this data in spring 2021. This data will include the establishment rate of these advanced hybrids, turfgrass quality, spring greenup etc. from these 20 test sites.

USGA ID#: 2019-31-701

Title: On-course evaluation of new zoysiagrass hybrids

Project Leaders: James H. Baird, Marta Pudzianowska, and Pawel Petelewicz

Affiliation: University of California, Riverside (UCR)

Objective:

1. Evaluate advanced zoysiagrass lines from Texas A&M and commercial cultivars for adaptation and performance on golf course fairways in Northern California.

Start Date: 2019
Project Duration: 2 years
Total Funding: $4,000

Summary Points:

- Establishment of all zoysiagrass genotypes was slower than expected, especially in Fairfax. Most of the plots fully established by summer 2020. Cool temperatures, especially average low temperatures, were most likely a limiting factor for turf establishment.
- Several zoysiagrass genotypes retained acceptable color during the first winter of the study and good color retention in November 2020.
- There was considerable variation among the genotypes in terms of growth, quality, and color.

Summary Text:

Studies were initiated on July 24 and 25, 2019 at Meadow Club, Fairfax (Marin County) and Napa Golf Course, Napa (Napa County), respectively. Sod of existing cool-season turf (ryegrass, annual bluegrass) was removed from fairway areas on both golf courses in preparation for planting. Plant material arrived as plugs or was divided into plugs and planted in 5 x 5 ft plots (no alleys) with 3 replications per entry at each location. A total of 20 zoysiagrass genotypes were planted including 16 experimental lines from Texas A&M, 2 standard commercial cultivars (‘Innovation’ and ‘Diamond’), and 2 local standard commercial cultivars developed by UCR (‘El Toro’ and ‘De Anza’). Turf was evaluated visually for: ground cover (0-100%); quality (1-9, 9 = best); and green color (1-9, 9 = darkest) and uniformity (1-9, 9 = best). Normalized Difference Vegetation Index (NDVI) was measured using a GreenSeeker handheld crop sensor. The growth rate was expressed as days after planting (DAP) to reach 75% cover or higher. Experimental design was a randomized complete block. Data were subjected to analysis of variance and means separated using Tukey’s Least Significant Difference Test.

Average monthly temperatures for both locations are provided in Table 1. Cool temperatures, especially low temperatures, most likely were responsible for slow establishment of zoysiagrass (Table 2). The fastest growing entries reached 75% cover after 308 days in Napa and 352 days in Fairfax. Despite lower minimum temperatures, higher maximum temperatures, compared to Fairfax, allowed for faster establishment in Napa. Visual quality varied between two locations. Good quality, both in Fairfax and Napa, showed ‘Diamond’, DALZ 1802, DALZ 1807 and DALZ 1309 (Table 3). Although the first year of establishment is typically not the best predictor of fall/winter color retention, differences in green color were observed among the genotypes during the winter 2019/2020 (Table 4). Good color during the first winter at both locations retained DALZ 1802, DALZ 1807 and DALZ 1309. As of November 2020 low night temperatures in Fairfax and Napa induced dormancy already. Entries retaining good fall color were DALZ 1807, DALZ 1802, ‘Diamond’ and DALZ 1309 (data not shown). Differences in NDVI were low, and in Napa not statistically significant (Table 5). Some variation in uniformity among entries was observed in Napa, but in Fairfax differences were low, which probably resulted from large variation between individual plots (Table 6). Entries showing good uniformity were DALZ 1814, DALZ 1308 and ‘Diamond’. Evaluations will continue throughout upcoming winter and should provide better insight in winter color retention, and through 2021.

Table 1. Average monthly high and low temperatures (F) at Meadow Club, Fairfax, CA and Napa Golf Course, Napa, CA from July 2019 to November 2020.

Month (2019)	Meadow Club		Napa Golf Course	
	Avg.High (F)	Avg. Low (F)	Avg.High (F)	Avg. Low (F)
2019				
July	80.3	50.0	78.5	54.2
August	82.0	52.8	82.1	55.1
September	78.3	52.5	81.4	50.3
October	74.3	41.6	77.2	40.6
November	66.5	35.0	66.7	37.4
December	57.3	43.3	58.1	41.7
2020				
January	58.0	39.7	59.9	36.9
February	65.2	38.7	67.9	36.9
March	61.7	41.1	63.7	38.8
April	67.7	44.0	70.2	42.2
May	73.3	47.5	77.0	45.7
June	76.5	52.2	82.0	48.7
July	73.0	51.7	81.1	50.1
August	77.7	55.3	85.0	52.3
September	80.3	53.2	86.4	52.2
October	78.1	47.8	82.8	45.4
November	64.7	38.2	67.2	33.9

Table 2. Days after planting (DAP) to reach 75 % ground cover of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

	DAP to reach 75% cover	
Entry	Meadow Club, Fairfax CA	Napa Golf Course, Napa CA
DALZ 1308	383	352
DALZ 1309	383	352
DALZ 1701	352	383
DALZ 1702	383	383
DALZ 1703	383	352
DALZ 1707	397	352
DALZ 1802	383	352
DALZ 1807	383	352
DALZ 1808	383	352
DALZ 1809	383	383
DALZ 1810	383	383
DALZ 1811	383	383
DALZ 1812	383	397
DALZ 1813	352	352
DALZ 1814	397	352
DALZ 1815	352	352
De Anza	461	352
Diamond	383	308
El Toro	383	308
Innovation	397	352

Table 3. Turfgrass quality (1-9; 9= best) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

	Turfgrass Quality			
Entry	Meadow Club, Fairfax CA		Napa Golf Course, Napa CA	
DALZ 1308	7.0	abc	6.4	abcde
DALZ 1309	6.7	abcd	6.8	abc
DALZ 1701	6.6	abcde	5.2	fgh
DALZ 1702	6.7	abcd	5.9	cdefg
DALZ 1703	6.5	abcde	5.9	cdefg
DALZ 1707	6.0	bcde	6.0	bcdef
DALZ 1802	6.9	abc	7.2	a
DALZ 1807	7.1	a	6.9	abc
DALZ 1808	6.4	abcde	6.4	abcde
DALZ 1809	5.8	cde	4.6	h
DALZ 1810	7.0	ab	5.7	defg
DALZ 1811	6.0	abcde	5.0	gh
DALZ 1812	5.7	de	5.0	gh
DALZ 1813	6.5	abcde	5.5	efgh
DALZ 1814	6.6	abcde	6.3	abcde
DALZ 1815	6.2	abcde	6.6	abcd
De Anza	5.8	bcde	6.1	bcdef
Diamond	7.0	ab	7.0	ab
El Toro	6.0	bcde	5.9	cdefg
Innovation	5.5	e	5.1	fgh

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 4. Turfgrass visual color (1-9; 9= darkest green) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

Entry	Visual Color: Winter, Meadow Club, Fairfax CA		Visual Color: Winter, Napa Golf Course, Napa CA		Visual Color: Summer, Meadow Club, Fairfax CA		Visual Color: Summer, Napa Golf Course, Napa CA	
DALZ 1308	5.5	abcd	5.5	bcdef	8.8	a	8.0	ab
DALZ 1309	6.0	abc	6.7	ab	8.2	abcd	7.8	ab
DALZ 1701	5.6	abcd	4.8	defg	8.3	abcd	7.3	abc
DALZ 1702	5.9	abc	5.3	bcdefg	7.8	abcde	7.8	ab
DALZ 1703	5.3	abcd	5.3	bcdefg	7.8	abcde	7.6	abc
DALZ 1707	5.4	abcd	5.7	abcde	7.7	abcde	6.9	bc
DALZ 1802	6.8	a	7.2	a	8.0	abcde	8.4	a
DALZ 1807	6.8	a	6.8	ab	8.4	ab	8.2	ab
DALZ 1808	5.4	abcde	5.7	abcd	7.2	bcde	7.6	abc
DALZ 1809	4.5	cde	4.1	efg	6.8	de	7.0	abc
DALZ 1810	5.9	abc	5.1	cdefg	7.2	bcde	7.9	ab
DALZ 1811	4.5	cde	4.0	fg	7.3	abcde	7.6	abc
DALZ 1812	3.7	de	3.7	g	6.6	e	6.2	c
DALZ 1813	4.6	bcde	4.7	defg	8.0	abcde	7.2	abc
DALZ 1814	6.1	abc	5.9	abcd	8.0	abcde	6.9	bc
DALZ 1815	6.4	ab	6.3	abcd	8.3	abc	8.0	ab
De Anza	6.0	abc	6.5	abc	8.7	abcd	7.4	abc
Diamond	6.8	a	6.6	abc	8.4	ab	7.8	ab
El Toro	5.5	abcd	5.6	abcde	7.0	cde	7.6	abc
Innovation	3.4	e	4.1	efg	7.8	abcde	8.2	ab

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 5. Normalized difference vegetation index (NDVI) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

	NDVI			
Entry	Meadow Club, Fairfax CA		Napa Golf Course, Napa CA	
DALZ 1308	0.67	ab	0.74	a
DALZ 1309	0.69	a	0.75	a
DALZ 1701	0.64	ab	0.58	a
DALZ 1702	0.65	ab	0.64	a
DALZ 1703	0.56	ab	0.64	a
DALZ 1707	0.67	a	0.63	a
DALZ 1802	0.69	a	0.72	a
DALZ 1807	0.67	a	0.74	a
DALZ 1808	0.62	ab	0.67	a
DALZ 1809	0.57	ab	0.58	a
DALZ 1810	0.59	ab	0.64	a
DALZ 1811	0.56	ab	0.57	a
DALZ 1812	0.60	ab	0.56	a
DALZ 1813	0.57	ab	0.61	a
DALZ 1814	0.66	ab	0.68	a
DALZ 1815	0.64	ab	0.70	a
De Anza	0.62	ab	0.70	a
Diamond	0.69	a	0.75	a
El Toro	0.61	ab	0.68	a
Innovation	0.51	b	0.66	a

Means followed by the same letter in a column are not significantly different (P=0.05).

Table 6. Turfgrass uniformity (1-9; 9= best) of sixteen experimental zoysiagrasses and four commercial standards in Fairfax, CA and in Napa, CA.

	Turfgrass Uniformity			
Entry	Meadow Club, Fairfax CA		Napa Golf Course, Napa CA	
DALZ 1308	7.5	a	7.7	a
DALZ 1309	5.7	a	7.3	ab
DALZ 1701	7.5	a	6.0	abc
DALZ 1702	7.3	a	5.7	abc
DALZ 1703	6.7	a	6.0	abc
DALZ 1707	7.3	a	6.7	abc
DALZ 1802	6.5	a	7.7	a
DALZ 1807	6.7	a	8.0	a
DALZ 1808	7.0	a	7.3	ab
DALZ 1809	7.0	a	4.7	c
DALZ 1810	8.0	a	6.3	abc
DALZ 1811	6.5	a	5.0	bc
DALZ 1812	5.7	a	6.0	abc
DALZ 1813	7.5	a	5.7	abc
DALZ 1814	8.0	a	7.7	a
DALZ 1815	5.7	a	6.7	abc
De Anza	7.0	a	6.7	abc
Diamond	7.3	a	7.7	a
El Toro	6.0	a	6.0	abc
Innovation	5.7	a	6.7	abc

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. August 2020.

Figure 2. Zoysiagrass genotypes at Napa Golf Course, CA. August 2020.

Figure 3. Zoysiagrass genotypes at Meadow Club in Fairfax, CA. November 2020.

Figure 4. Zoysiagrass genotypes at Napa Golf Course, CA. November 2020.

USGA ID#: 2019-24-694

Title: Comparing Greenhouse Gas Emissions and Soil Microbial Populations from Turfgrass Fertilized with a Slow-Release Synthetic Fertilizer or an Organic Fertilizer

Project Leader: PI's Karl Guillard and John Inguagiato assisted by graduate student Kaitlyn Goodridge
Affiliation: University of Connecticut

Objectives: The objective of this study was to determine how slow-release synthetic and organic fertilizers influence greenhouse gas emissions and soil microbial community populations of turfgrass lawns.

Start Date: 2019
Project Duration: 2 years
Total Funding: $25,007 ($8,967 from the USGA; $16,040 from the New England Regional Turfgrass Foundation).

Summary Points:

- The addition of organic versus slow-release synthetic fertilizer to turfgrass lawn stands does not result in significantly different CO_2, N_2O, or CH_4 emissions compared to the non-fertilized control plots.
- Fertilizer rate did not have a significant effect on the greenhouse gas emissions compared the non-fertilized control plots.
- CO_2 gas emissions were greatest in June and July and dropped throughout the season.
- There was a net intake of CH_4 by the turfgrass environment in both years.
- Preliminary results suggest that fertilization has a significant effect on the turfgrass soil microbiome regardless of synthetic or organic N source.

Summary Text:

Need for the study:

The three most consequential greenhouse gases in turfgrass systems are carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). Managed turfgrass areas have shown potential for high soil C sequestration, but the emission of greenhouse gases from these fertilized landscapes may offset sequestration. The addition of N fertilizers to turfgrass has shown to increase the amount of N_2O emissions compared to non-fertilized turfgrass (Maggiotto et al., 2000). However, the emission of N_2O from fertilized turfgrass soils may vary by source of N. While several studies have evaluated greenhouse gas emissions from synthetic fertilizers, there are limited data that report on organic N fertilizer sources and no studies have compared greenhouse gas emissions between urban grassland lawns fertilized with slow-release synthetic and organic N sources.

The common perception that organic fertilizers are less harmful to the environment than synthetic fertilizers has led to an increasing use of organic amendments in turfgrass systems, including organic sources of N. Currently, synthetic fertilizers are the most commonly used N source for managed turfgrasses. As more people begin to switch to organic fertilizers, it is important to quantify and compare the greenhouse gas emissions from the soils of turf fertilized with organic and synthetic fertilizers.

Another common perception is that synthetic fertilizers reduce soil microbial populations. Moreover, organic N sources are routinely used as a means of increasing soil microbial activity and population diversity. Studying the differences in microbial populations between soils fertilized with organic and synthetic fertilizers will provide insight into how these inputs affect soil health. The 16S rRNA gene sequencing technology allows for the analysis of the microorganisms in these soil environments.

Methods:

Experimental and Study Design
This field study was conducted during June through October 2019 and 2020 at the Plant Science Research and Education Facility in Storrs, CT on an existing tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] turf that was established in September 2007. The experiment was set out as a randomized complete block design with three replications. Plot size was 1 x 1 m. Plots were fertilized once in June and October in 2019 and 2020 with either a poly coated urea 60% slow-release synthetic fertilizer (ProSeries 25-0-12) consisting of 24% urea nitrogen, 0.1% slowly available water soluble nitrogen, and 0.9% water insoluble nitrogen, or an all-natural organic fertilizer (Suståne 5-2-4). A non-fertilized control plot was also included in this study. Fertilizers were applied at four rates (50, 100, 150, and 200 kg N ha^{-1}) in equal split applications in June and October. Plots have received the same rate of synthetic or organic N yearly since 2008 (with the exception of 2010).

Greenhouse Gas Emissions
CO_2, N_2O, and CH_4 emissions were measured once a month (June through October) using the static chamber method (Livingston and Hutchinson, 1995) with a modification in chamber design as described by Morse et al. (2012). PVC collars were placed in the soil of each plot and remained there in order to minimize soil disturbance. On the day of sampling, PVC chambers were positioned into the soil collars and a gas sample was collected immediately, then again 30 and 60 minutes later. Gas samples were collected using a gas tight syringe through septa on the chambers. Gas samples were immediately injected into a 22 mL pre-evacuated gas vial after sampling. At the time of sampling, air temperature, barometric pressure, soil temperature and moisture at 10 cm, and chamber height were recorded.

Gas samples were taken to the laboratory and injected by a PerkinElmer TurboMatric 40 Trap headspace sampler into a PerkinElmer Clarus 580 gas chromatograph. The gas chromatograph uses a flame ionization detector and a Model Arnel methanizer to quantify CO_2 and CH_4 concentrations and an electron capture detector to measure N_2O. Concentrations of the gases were calculated in units of ppmv by comparing the chromatograph areas of samples to known standards.

Gas fluxes were calculated according to Helton et al. (2014) and Morse et al. (2012). Emissions for each gas were determined by calculating the slope of the regression between gas concentration and time over the one-hour incubation. Measurements of barometric pressure and temperature taken at the time of sampling were used with the ideal gas law to calculate in units of mass (mg m^{-3}) in R 3.6.1. The minimum detectable concentration difference (MDCD) was calculated for each gas (Yates et al., 2006) and all fluxes less than the MDCD were set equal to zero. The slope (mg m^{-3} hr^{-1}) was used to calculate emissions for any gas flux over the MDCD and had an $r^2 > 0.85$. Any non-linear slope with an $r^2 < 0.85$ was analyzed with the third time point dropped. Chamber heights were used to convert gas flux to units of mg m^{-2} hr^{-1}.

Greenhouse gas (CO_2, CH_4, and N_2O) flux (mg m^{-2} hr^{-1}) means were analyzed for treatment differences (treatment versus control, fertilizer source, fertilizer rate, and fertilizer source × fertilizer rate interaction) as a repeated measures design by using analysis of variance with Fisher’s LSD for mean separation in the GLIMMIX procedure of SAS 9.4.

Soil Microbiome
Soil samples were collected before and after fertilizer applications in June and October. Pre-fertilization samples were collected 1 to 5 days before fertilizer treatments were applied, and post-fertilization samples were collected 14 days after treatments. A total of 10 1.5-cm diameter soil cores were collected from each plot and trimmed to include the upper 10 cm of rootzone. Soil samples were stored on ice in the field, and then transferred to a −20˚C freezer until ready to process, which occurred within 2 weeks after sampling. After passing samples through a 1-mm sieve, DNA was extracted using the Qiagen DNeasy PowerSoil Kit following the manufacturer's protocol. DNA isolates were submitted to the Microbial Analysis, Resources, and Services lab at the University of Connecticut for quantification, PCR amplification, library preparation, and 16S sequencing. The V4 hypervariable region of the bacterial 16S rDNA genes in each sample were amplified by PCR and sequenced by the Illumina MiSeq platform.

Raw sequences were processed using the "DADA2" package in R 4.0.3 following the DADA2 Pipeline Tutorial (1.8) and adjusting parameters to our dataset. Forward and reverse reads were filtered and merged. After removing chimeras and sequences belonging to chloroplast, mitochondria, Eukaryota, and any unassigned sequences at the Kingdom level, an amplicon sequence variants (ASVs) table was created. Taxonomic levels of ASVs were assigned using the SILVA database (v.138). Downstream analysis was conducted using the "phyloseq" package unless otherwise specified and graphics were created using the "ggplot2" package in R.

Alpha diversity was evaluated through the Shannon diversity index. To compare the alpha diversity between samples, the non-parametric Kruskal-Wallis test was conducted with the Benajmini-Hochberg correction applied for multiple pairwise comparisons with the "dunn.test" package. Reads for each sample were normalized using variance stabilizing transformation with the "DeSeq2" package. Beta diversity was analyzed by calculating the Bray-Curtis dissimilarity matrices. To study the effect of fertilizer and rate on the microbiome community between samples, permutational multivariate analysis of variance (PERMANOVA) and homogeneity of dispersion were calculated with the "betadisp" and "adonis" functions in the "vegan" package. Pairwise PERMANOVA comparisons were calculated and adjusted with the Bonferroni correction.

Results:
Greenhouse Gas Emissions
In 2019, differences in greenhouse gas emissions for treatment versus control, fertilizer source, fertilizer rate, and fertilizer source × fertilizer rate interaction effects were not significant across the growing season ($P > 0.05$). CO_2 gas emissions were greatest in June and July and dropped throughout the season. Non-fertilized control plots showed high variability in CO_2 emissions relative to the treated plots between months (Fig. 1). The plots with organic fertilizer applications showed no statistical difference to plots that received slow-release synthetic fertilizer applications with respect to CO_2 emissions across all months, and neither were significantly different from the control plots. Most of the treatments had non-detectable concentrations of N_2O gas, with the exception of the first month of sampling (Fig. 1). CH_4 concentrations were also mostly non-detectable. The plots that did result in detectable concentrations showed a net intake of CH_4 by the turfgrass and soil environment (Fig. 1).

In 2020, there was a significant rate effect for the CO_2 gas emission ($P = 0.0242$). The plots that received the 50 kg N ha^{-1} rate of fertilizer had equal CO_2 emissions to the control plots that did not receive any fertilizer treatment. All other rates were significantly greater than the 50 kg N ha^{-1} rate, but they were also equal to the control plots. There was also a significant date effect ($P = <0.001$) for the repeated measures analysis where the CO_2 emissions were significantly greater in the gas samples collected during June and July compared to the samples collected in August, September, and October (Fig. 1). We saw similar trends to this in 2019. In 2020, N_2O and CH_4 concentrations were detectable in most treatments to analyze. However, there was no significant effect or interaction for these data ($P > 0.05$). As we saw in 2019, CH_4 concentrations showed a net intake of the gas (Fig. 1).

Soil Microbiome
Analysis of soil microbiome data are ongoing. To date, preliminary analysis from the spring 2019 fertilization event have been performed. Results suggest a significant difference among fertilizer sources (PERMANOVA $P = 0.003$) 14-d after fertilization occurred. Principle coordinate analysis shows clustering by fertilizer source with clusters corresponding to fertilized and non-fertilized treatments separating along the *x*-axis (PC1) (Fig. 2). Pairwise comparisons of the different fertilizer sources show that the soil microbial communities (beta-diversity) associated with organic and synthetic fertilization are different from the control ($P = 0.042$ and $P = 0.021$, respectively); however, they do not differ from each other ($P > 0.05$). These data suggest that the soil microbiome of fertilized turf is more similar than the non-fertilized turf 14 days after fertilization, regardless or organic or synthetic N-source. The preliminary data also suggest that differences in the alpha-diversity may also exist among fertilized and non-fertilized turf ($P = 0.06$). Greater overall bacterial diversity, and greater uniformity among representatives of those bacterial communities was observed in soil of fertilized turf, regardless of N source compared to non-fertilized turf. No soil microbiome differences were observed among fertilizer source or rates in pre-fertilization sampling during spring 2019.

Future Expectations:
It is anticipated with further analysis on more data of the soil microbial communities associated with perennial tall fescue, we will have a better understanding on how these treatments influence the soil microbiome. We also plan on analyzing our samples for fungal communities to determine how the application of various fertilizer sources and rates influence those populations in the soil as well.

Additionally, further analysis can be made to compare our greenhouse gas emissions and soil microbial communities to other environmental factors. At the time of sampling, measurements were taken such as soil moisture and temperature and additional soil analysis were completed after sampling such as the Solvita Soil CO_2-Burst and Soil Labile Amino Nitrogen tests, which measure soil labile C and N, respectively. By looking at how all of these different factors interact, we can have a better understanding of how organic and slow-release synthetic fertilizers influence the turfgrass and soil environment as an entire system.

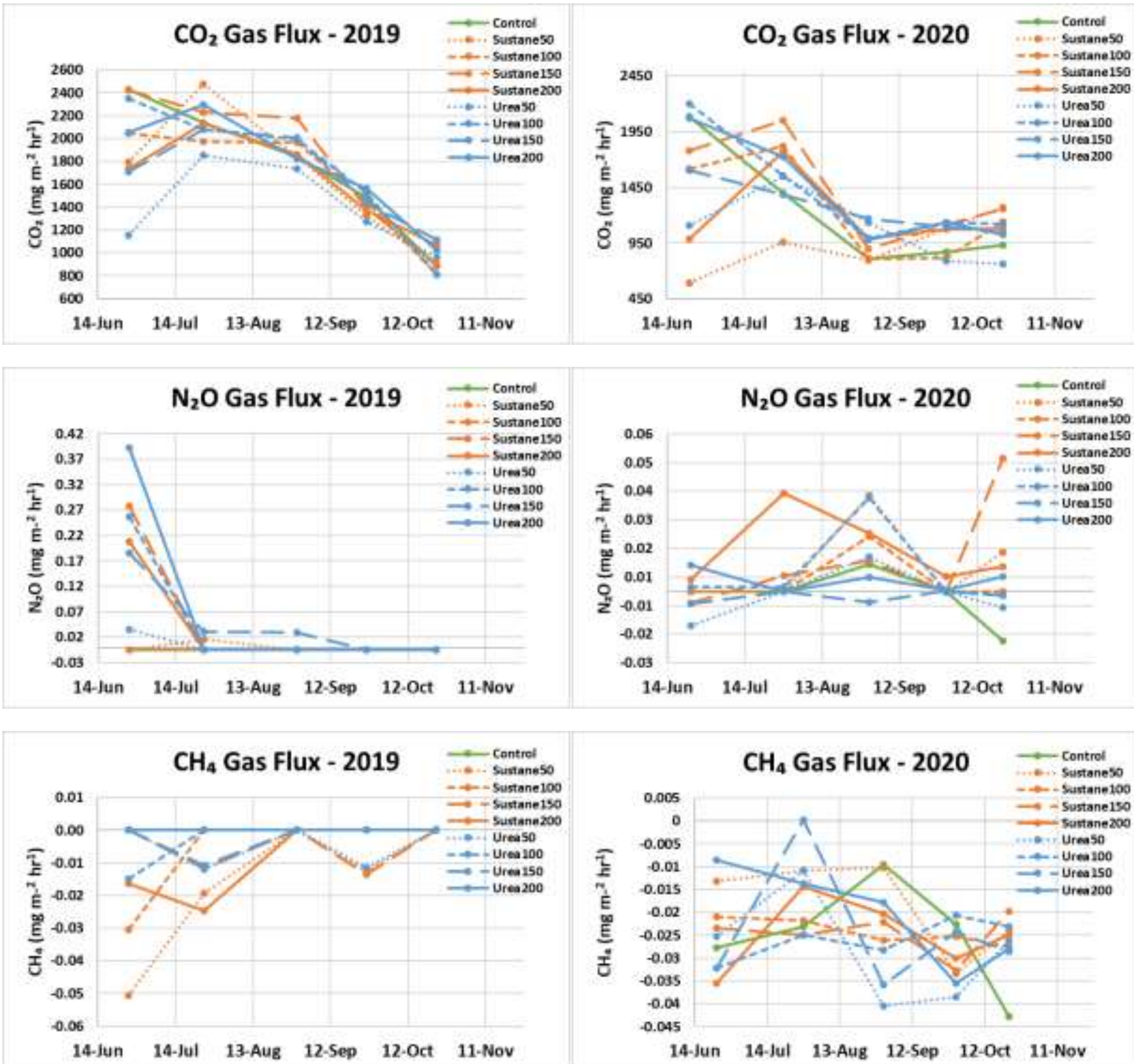


Figure 1. Responses of mean CO_2, N_2O, and CH_4 gas emissions (mg m^{-2} hr^{-1}) for each fertilizer source (non-fertiized control, Suståne, and Urea) and rate (0, 50, 100, 150, and 200 kg N ha^{-1}) across the monthly sampling dates in 2019 and 2020.

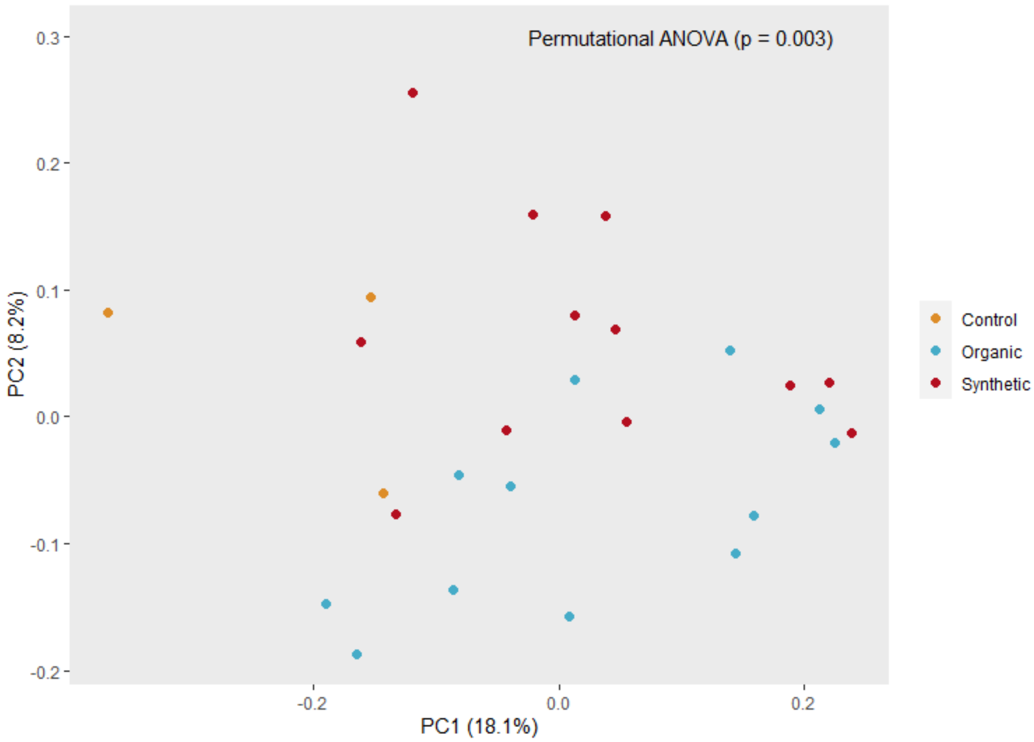


Figure 2. Principle coordinate analysis (PCoA) calculated as Bray-Curtis dissimilarity matrices of soil microbial communities associated with tall fescue turf after receiving applications of organic, slow-release synthetic, or no fertilizer. Permutational analysis of variance (PERMANOVA) *p* value = 0.003. Homogeneity of multivariate dispersions test *p* value = 0.8932.

References

Groffman, P.M., R.V. Pouyat, M.L. Cadenasso, W.C. Zipperer, K. Szlavecz., I.C. Yesilonis, L.E. Band, and G.S. Brush. 2006. Land use context and natural soil controls on plant community composition and soil nitrogen and carbon dynamics in urban and rural forests. For. Ecol. Manag. 236:177–92.

Helton, A.M., E.S. Bernhardt, and A. Fedders, 2014. Biogeochemical regime shifts in coastal landscapes: the contrasting effects of saltwater incursion and agricultural pollution on greenhouse gas emissions from a freshwater wetland. Biogeochemistry. 120:133–147.

Livingston G.P., and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: Applications and sources of error. In: P.A. Matson and R.C. Harriss (editors). Methods in Ecology. Biogenic trace gases: Measuring emissions from soil and water. Blackwell Science, Oxford. p. 14–51.

Maggiotto, S.R., J.A. Webb, C. Wagner-Riddle, and G.W. Thurtell. 2000. Nitrous and nitrogen oxide emissions from turfgrass receiving different forms of nitrogen fertilizer. J. Environ. Qual. 29:621–630.

Morse, J.L., M. Ardón, and E.S. Bernhardt. 2012. Greenhouse gas fluxes in southeastern U.S. coastal plain wetlands under contrasting land uses. Ecol. Appl. 22:264–280.

Yates, T.T., B.C. Si, R.E. Farrell, and D.J. Pennock. 2006. Probability distribution and spatial dependence of nitrous oxide emission: temporal change in hummocky terrain. Soil Sci. Soc. Am. J. 70:753–762.

USGA ID#: 2019-23-693

Title: Determining Precise Light Requirements of Warm-Season Putting Greens

Project Leader: Dr. Douglas Karcher, Dr. Michael Richardson, and Mr. Thomas Walton
Affiliation: University of Arkansas

Objectives: The objective of the project are to quantify the precise light requirements (in terms of daily light integral) for producing acceptable putting green quality as affected by species, cultivar, and management practices.

Start Date: Experimental area sprigged in June 2019. Treatments initiated July 2020.
Project Duration: Two growing seasons of treatment applications and evaluations.
Total Funding: $15,000

Summary Points:

- ‘Tifeagle’ ultradwarf bermudagrass reached full establishment several weeks faster than ‘Lazer’ zoysiagrass at equivalent sprigging rates.
- **‘**Lazer’ zoysiagrass demonstrated significantly improved shade tolerance compared to ‘Tifeagle’ ultradwarf bermudagrass.
- Although ‘Tifeagle’ had typically had higher Stimpmeter readings than ‘Lazer’, ‘Lazer’ averaged Stimpmeter values > 10 feet by the end of the growing season.
- ‘Lazer’ retained green color later into the growing season relative to ‘Tifeagle’.

Summary Text:

Throughout the southern and transition zones, there is an emerging alternative to ultradwarf bermudagrass for warm-season putting greens. There are several recently released fine-textured zoysiagrass varieties with potential for putting green use. Early research on greens-type zoysiagrass has shown promise regarding shade tolerance compared to bermudagrass, but there are concerns about whether zoysiagrass can produce high-quality putting surfaces. It would be beneficial to determine the precise amount of light (daily light integral) required by either an improved putting green zoysiagrass cultivar or an ultradwarf bermudagrass. Recent research at the University of Arkansas has demonstrated that it takes multiple experimental seasons to accurately determine the minimum daily light integral required by a turfgrass system. Therefore, our research objective is to compare ‘Lazer’ zoysiagrass to an industry standard ‘Tifeagle’ ultradwarf bermudagrass under various shade levels and management practices over two growing seasons. This work will result in us estimating the precise amount of light necessary to produce an acceptable quality putting green for each species, depending on mowing height and plant growth regulator (PGR) application.

Our experimental green, which was sprigged in 2019, is managed under five shade levels ranging from full-sun down to 80% shade, includes mowing heights of 0.100 or 0.125 inches, and either weekly or no PGR (trinexapac-ethyl) applications. Visual quality, green speed, and surface firmness. The experimental plots were fully established in June 2020 and were regularly assessed thereafter to compare species and treatment effects.

To date, 'Lazer' has shown significantly better shade tolerance than 'Tifeagle'. Regardless of mowing height and PGR treatment, 'Lazer' retained acceptable quality in shade levels up to 40% throughout the growing season (Fig. 1). Preliminary results indicate that the minimum light requirements, or daily light integral, for 'Lazer' is about 10 moles m^{-2} d^{-1} less than 'Tifeagle'. This trial will continue through 2021 and the differences in light requirement may change following two consecutive years of shade stress. The PGR treatment significantly improved turfgrass quality for 'Tifeagle' grown in the shade (Fig. 2), but had little impact on 'Lazer'. Although 'Tifeagle' had slightly faster green speeds, 'Lazer' produced Stimpmeter values > 10 feet late in 2020, was consistently firmer than the 'Tifeagle', and exhibited excellent fall-color retention (Fig. 3). These preliminary results suggest that 'Lazer' may be an acceptable putting green option in the transition zone, especially for golf courses with significant shade around their putting green complexes.

Figure 1. 'Lazer' (left) and 'Tifeagle' (right) under 40% shade, mowed at .100, and without PGR 9-weeks after initiating shade. Note significant thinning and reduced quality on 'Tifeagle' and high-quality and density of 'Lazer'. (8/31/2020)

Figure 2. Enhanced turfgrass quality of 'Tifeagle' with weekly PGR treatment (middle) under heavy shade-stress. Note severely reduced quality on the untreated plots left and right and dramatic color difference. The green strip on the far left of the picture is 'Lazer' under the same shade levels without PGR. (7/29/2020)

Figure 3. Fall color comparison, ‘Tifeagle’ (left) and ‘Lazer’ (right) (11/13/2020)

USGA ID#: 2019-25-695

Title: Investigation of Growth Regulators and Physiological Regulating Products on Winter Disease Incidence and Plant, Root Health

Project Leader: Joseph Roberts
Affiliation: Clemson University Pee Dee Research and Education Center

Objective: Understand the impact of plant growth regulators, fungicides with growth regulating potential, and products impacting hormone physiology for their impact on winter disease and overwintering in bermudagrass putting green turf

Start Date: November 2019
Project Duration: 1 year
Total Funding: $5,000

Summary Points:

- Fungicide applications to ultradwarf bermudagrass putting green turf reduce common overwintering diseases such as dollar spot and cream leaf blight
- Turf quality of ultradwarf bermudagrass can impacted by product applications due to phytotoxicity with specific products
    - In non-overseeded bermudagrass putting green turf, Anuew and Actigard reduced turfgrass quality compared to the non-treated control on 2 of 7 rating dates
    - In overseeded bermudagrass putting green turf, Anuew applications improved turfgrass quality compared to the non-treated control on 7 of 9 rating dates while Torque and Actigard applications reduced turfgrass quality compared to the non-treated on 4 and 6 of 9 rating dates, respectively.
- Spring rooting measurements did not show significant differences as a result of product applications

Trial Summary

Ultradwarf bermudagrass putting greens are impacted several fungal pathogens, particularly in the spring and fall seasons when there is minimal growth of turf. Preventive disease control is often warranted in order to maintain turfgrass density as turf is slow to recover during these periods. Spring dead spot (SDS), caused by *Ophiosphaerella* spp, is a frequent issue on bermudagrasses in the transition zone while take all root rot (TARR), caused by *Gaeumannomyces* spp., is an emerging disease with reports growing each year. These pathogens are known to infect bermudagrass roots in the late summer (i.e, *Gaeumannomyces* spp.) and fall months (i.e., *Ophiosphaerella* spp.) with detrimental symptoms that significantly impact both aesthetics and playability of golf course turf. Chemical management of these diseases includes the use of demethylation inhibitor fungicides, which have growth regulating potential and may be phytotoxic to turfgrass. In addition to these fungicides, superintendents are also known to apply plant growth regulators throughout the year in an effort to maintain putting surfaces. Applications of growth regulating products in addition to products that can alter hormone physiology warrants additional investigation as they may influence winter survival of ultradwarf bermudagrasses. A research trial was initiated in fall of 2019 to evaluate the impact of multiple products on turfgrass health and overwintering. The trial was performed over two separate locations: 1) a 'Tifeagle' bermudagrass green at the Pee Dee Research and Education Center (i.e., PDREC) in Florence, SC and 2) a 'Mini-Verde' bermudagrass green at World Tour Golf Links in Myrtle Beach, SC. Both sites were managed as golf course putting greens with the World Tour site receiving overseeding on 17 October, 2019. Mowing was performed at both sites using Toro triplex greens mowers with cutting heights between 0.120" and 0.145". Cutting heights were gradually increased during the winter months. The non-overseeded site was topdressed monthly over the course of the trial while the overseeded site received weekly light verticutting and light topdressing. Experimental areas at both locations received N fertilization applications totaling 5-7 lb N 1000 ft^{-2} in 2019. Treatment plots measured 3' by 10' and were arranged in a randomized complete block design with four replications. Treatments were applied to the plots as a foliar spray in water equivalent to 90 gal $acre^{-1}$ using a walk-behind CO_2 powered sprayer equipped with dual TeeJet 8004 nozzles. All plant growth regulators were applied to the foliage only while fungicides (i.e., Torque and Velista) received post-application irrigation totaling ~0.125". Treatments were initiated on 15 November 2019 and were reapplied at specified intervals through the spring to 31 March 2020. Foliar fungicides were applied to limit diseases like dollar spot, cream leaf blight, and Pythium blight, but all diseases were assessed when observed. Dollar spot was assessed as infection centers per plot and other diseases were rated as % turf area infested. Turf quality (scale of 1-9; 9=best), and turf color (NDVI; Fieldscout CM1000, Spectrum Technologies Inc., Aurora, IL) were assessed regularly throughout the trial period. In the spring of 2020, ball roll distance was assessed using a USGA Stimp meter and rooting was assessed at both locations by extracting three 1.5" soil cores from each plot. Cores were gently washed and evaluated for max rooting depth, average rooting depth, and root grade (scale 1-5; 5=best). All data was subjected to analysis of variance and means separations were performed using a least significant difference at 0.05 probability.

Unfortunately, symptoms of major root disease were minimal across the treatment period and into the spring of 2020. Take all root rot was not observed at either location, Spring dead spot symptoms were observed in the non-overseeded site at PDREC, but symptoms were not uniform and significant differences were not detected among treatment applications. Some foliar diseases were observed at the PDREC location and treatment differences were observed (Figure 1). On 31 January 2020, Velista and Torque applications reduced dollar spot disease compared to the non-treated control. On the same date, plots receiving Anuew applications had symptoms of cream leaf blight similar to areas receiving Actigard, but higher than all other treatments and the non-treated control. Both dollar spot and cream leaf blight epidemics were arrested with curative fungicide applications (i.e., Daconil Weatherstik or 26GT) applied at label recommendations. Product applications also impacted turfgrass quality across the trial period outside of disease development and results were dependent on overseeded vs. non-overseeded areas. In non-overseeded bermudagrass at PDREC, Actigard and Anuew reduced turfgrass quality compared to the non-treated control on 2 of 7 rating dates (data not shown). In overseeded bermudagrass at World Tour, Anuew applications consistently impacted turfgrass quality with significant improvement over the non-treated control observed on 7 of 9 rating dates (Figure 2). At the same location, plots treated with Torque and Actigard had lower turf quality than the non-treated control on 6 and 4 of 9 rating dates, respectively (Figure 2). Reduction in turf quality from Torque applications was not unexpected, as the active ingredient tebuconazole has been shown to cause phytotoxicity on ultradwarf bermudagrass. Phytotoxicty has not been previously examined with Actigard alone and warrants future investigation. Improvements in turf quality from Anuew treatments were observed through uniformity, color, and density (Figure 3). While differences in foliar disease and turf quality were detected over the course of the trial at 2 locations, differences in spring ball roll distance or rooting were not observed (data not shown).

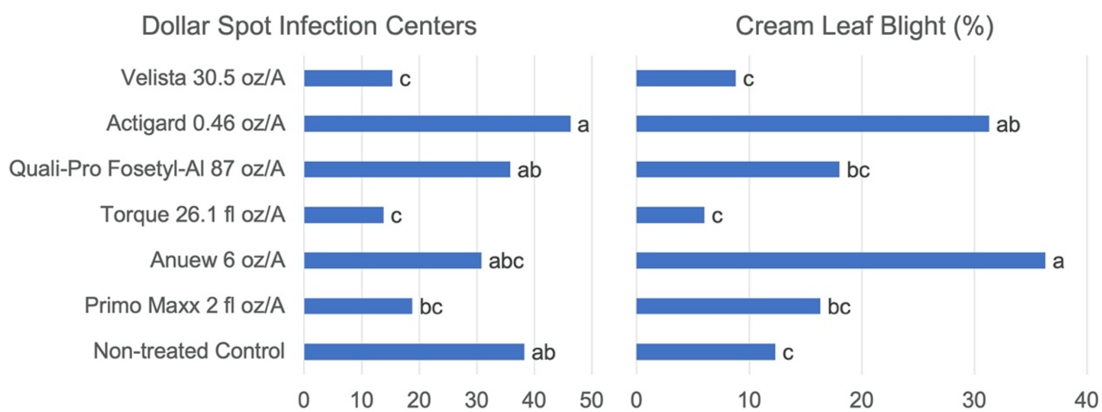


Figure 1. Impact of fungicide, plant growth regulator, and hormone physiology products on dollar spot and cream leaf blight in ultradwarf bermudagrass maintained as a putting green in Florence, SC. Actigard, Quali-Pro Fosetyl-AL, Anuew, and Primo Maxx were applied every 14 d from 15 Nov 2019 to 31 Mar 2020 while Torque and Velista were both applied on 15 Nov 2019 and 18 Feb 2020. Letters represent statistical differences using least significant differences by on probability <0.05.

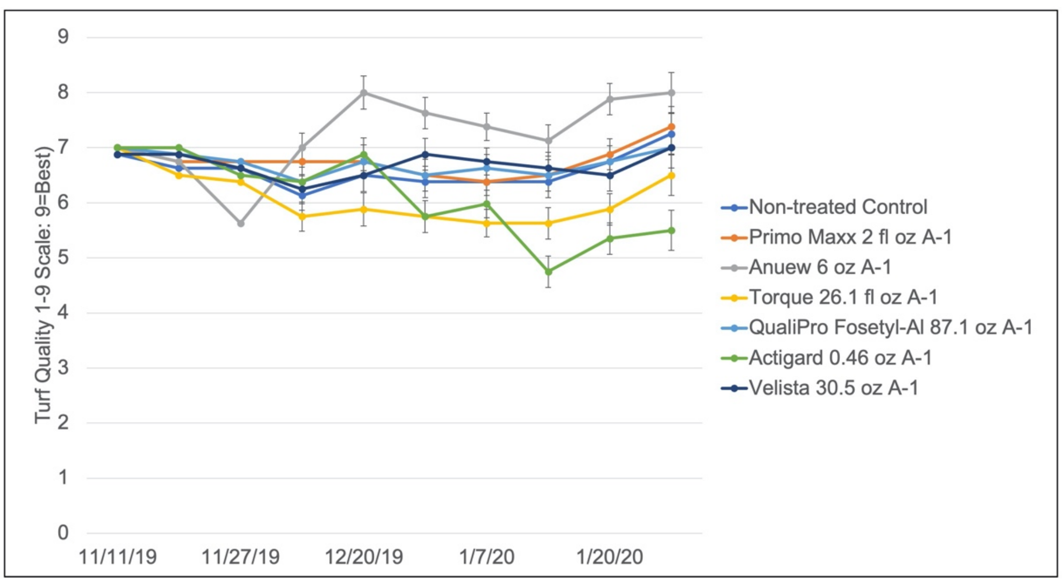


Figure 2. Turf quality impacts observed on overseeded ‘Mini-Verde’ bermudagrass putting green turf as a result of fungicide and plant growth regulator applications. Actigard, Quali-Pro Fosetyl-AL, Anuew, and Primo Maxx were applied every 14 d from 15 Nov 2019 to 31 Mar 2020 while Torque and Velista were both applied on 15 Nov 2019 and 18 Feb 2020. Error bars represent least significant difference at 0.05 probability

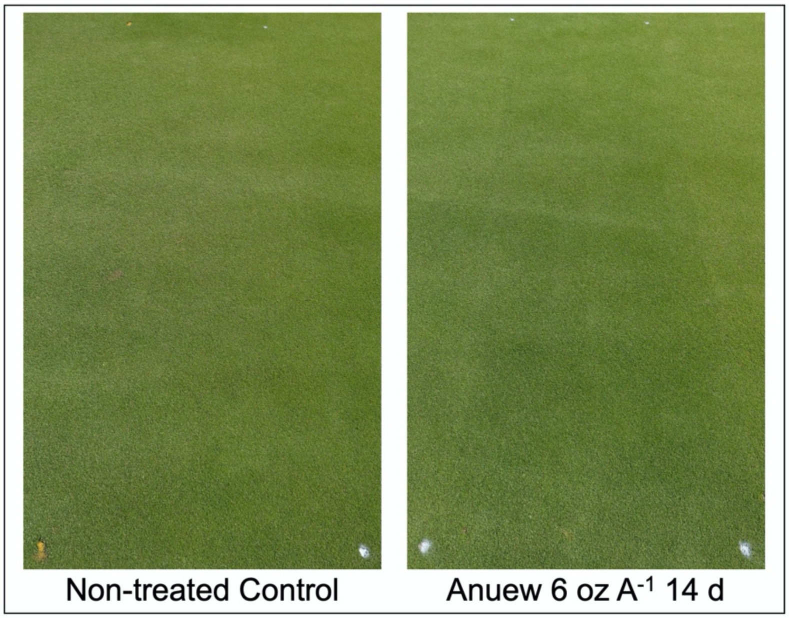


Figure 3. Comparison of turf quality of overseeding bermudagrass putting green turf receiving regular applications of Anuew beginning 15 Nov 2019. Photo taken on 4 April 2020.

Results from this 1-year trial show that products with plant growth regulating potential (i.e., DMI fungicides and PGRs) or altering hormone physiology (i.e., Actigard) can have variable effects on ultradwarf bermudagrass putting greens when applied in the off-season. Additional research is warranted to further confirm the trends observed, but funding is needed to carry this project forward for additional years. We appreciate support from the United States Golf Association in funding this trial and hope to use the preliminary data generated for future proposals.

Acknowledgements

This project was funded by the United States Golf Association. Special thanks to Laurie McBride, Albert Lynn, and Sara Rolfe for assistance in trial organization in addition to Scott Grumman at World Tour Golf Links for providing space to complete the trial.

USGA ID#: 2019-18-688

Title: Evaluating plant growth regulators and the soil surfactant Revolution® to alleviate drought stress in bermudagrass

Project Leader: Matteo Serena, Elena Sevostianova, Bernd Leinauer,
Affiliation: New Mexico State University

Objectives: To evaluate the effect of six plant growth regulators (PGRs) alone and in combination with soil surfactant Revolution® on visual turf quality, Normalized Difference Vegetation Index (NDVI), percent green turf cover, Dark Green Color Index (DGCI), soil moisture content and soil moisture uniformity of bermudagrass irrigated at two different evapotranspiration (ETo) replacement rates

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

Summary Points:

- The soil surfactant Revolution® had a positive effect on turfgrass quality, cover, DGCI and NDVI of drought-stressed plots during the months of September and October
- Plots treated with the PGRs Pac and Ful had lower turfgrass green cover, DGCI and NDVI during the months of September and October when compared to the plots treated with other PGRs and the control plots.
- Plots treated with the soil surfactant Revolution® had greater moisture content and uniformity compared to control plots.
- Bermudagrass treated with the PGRs TE and Pac had lower height compared to control plots, supporting the notion of PGRs reducing plant growth.

Summary Text:

The use of plant growth regulators (PGRs) and soil surfactants is common practice in the golf industry. Previous research demonstrated that trinexapac-ethyl (TE) resulted in higher bermudagrass turf quality compared to the control under drought conditions (Schiavon et al., 2014). Additionally, soil surfactants had proven the ability to increase turfgrass quality under reduced irrigation (Cisar et al., Leinauer et al., 2007, Schiavon et al., 2014, Alvarez 2016). More recently, research projects investigated if combination of both TE and the soil surfactant Revolution® increases bermudagrass quality under drought conditions to a greater extent than if both products were applied separately (Serena et al., 2018; Schiavon et al., 2019). However, results were inconsistent as there were several sampling periods with no differences for treatments of TE alone and TE+Revolution (Serena et al., 2018; Schiavon et al., 2019). Such findings might indicate a larger benefit of the PGR alone vs. the PGR+ soil surfactant in alleviating symptoms of drought related stress. To date, no research has investigated if active ingredients different from TE and a combination with a soil surfactant could play a beneficial role in drought mitigation. A research project was conducted at New Mexico State University to study the effects of several PGRs alone and in combination with Revolution® on turfgrass performance parameters and on soil moisture of drought stressed 'Princess 77' bermudagrass

Material and Methods

The study was conducted at the New Mexico State University Turfgrass Research Center in Las Cruces (NM) (arid, 1265-m elevation) from May to November 2020. Plots were mowed three times per week at a height of 1.2 cm (1/2”) by means of a reel mower with clippings returned. During the spring of 2020, prior to the research period, irrigation was applied at 100% ETo. From May to October plots were hand watered three times per week (Monday, Wednesday and Friday) using a hose with attached nozzle, that was previously calibrated. Irrigation treatments included 45% (drought stress) and 75% ETo (unstressed control). Chemical treatments were applied using a calibrated CO2 backpack boom sprayer (Bellspary Inc. Opelousas, LA) at 544 L ha^{-1} using three flat nozzles (XR1103VS Teejet) and operating at 482 KPa. The study was laid out in a completely randomized block with ETo as the whole block treatment. PGRs alone or tank mixed with Revolution® (6 oz/M) were applied on randomly assigned plots measuring 1.5 x 1.5 m.

The following plant growth regulators were included in the study:

1. Untreated Control (Control)
2. PrimoMaxx 11oz/A (TE)
3. Trimit 32oz/A (Pac)
4. Primo 11oz/A + Trimit 8oz/A (TE+Pac)
5. Anuew 15oz/A (ProHex)
6. Cutless 25oz/A (Ful)
7. Musketeer 20oz/A (TE+Pac+Ful)
8. Legacy 15oz/A (TE+Ful)

Visual turf quality was assessed on a scale of 1 to 9. A rating of 1 represented extremely poor, dead, or no turf and 9 indicated a perfect, exceptional green and uniform plot. A rating of 6 was considered minimally acceptable turfgrass growth. Digital image analyses (percent green cover and DGCI) was evaluated following methods described by Richardson et al. (2001). Dark green color index was calculated using the entire picture frame without excluding bare spots (Karcher and Richardson, 2003 and 2005). Normalized Difference Vegetation Indices (NDVI) was determined with a GreenSeeker Hand HeldTM Optical Sensor Unit Model 505 (NTech, Ukiah, CA). Soil moisture content was recorded twice per month starting 1 week after the first treatment application. Volumetric soil moisture content at depths of 0 to 7 cm was measured by means of a TDR 350 soil moisture meter (Field Scout TDR 350 Probe, Spectrum Technologies, Inc., Aurora IL). Irrigation was withheld for 24h before each measurement. Nine readings per plots were collected and averaged for moisture content. Moisture uniformity was calculated as standard deviation of the nine readings. Lower values indicate a uniform moisture distribution. Turf height of the turfgrass canopy was determined by means of a Prism Gauge (Turf-Tec International, Tallahassee, FL) and turf height was recorded as the difference between the measurement immediately after and three days after mowing. Data were collected every 14 days and then averaged over each month from May to October. To test the effects of surfactants on turfgrass quality, cover, NDVI, DGCI, soil moisture uniformity and turf height, data were subjected to an analysis of variance (ANOVA) using SAS PROC GLIMMIX followed by multiple comparisons of means using Fisher's LSD test at the 0.05 probability level.

Results to date

- Statistical analysis indicated a significant interaction between ETo, surfactant and month for quality (p=0.0369), cover (p <.0001), DGCI (p <.0001) and NDVI (p=0.0062). Results are shown in Figure 1. During the months of September and October, plots treated with Revolution® and irrigated at 45% of ETo maintained higher quality, cover, DGCI and NDVI compared to untreated plots. These results highlight the beneficial effects of using Revolution ® under extended periods of drought.
- There was a significant interaction between PGRs and sampling months for cover (p <.0001), DGCI (p <.0001) and NDVI (p=0.0046). Detailed results are presented in Figure 2. A prolonged use of Pac and Ful appear to have negative effect on turfgrass, as demonstrated by lower cover, DGCI and NDVI during the months of September and October compared to the other PGRs and the control (Fig. 2).
- Revolution® affected soil moisture content (p <.0001) and moisture uniformity (p <.0001). When plots were treated with the surfactant, moisture content reached 21.1% compared 17.4% on the untreated plots. Similarly, plots treated with Revolution® exhibited a more uniform moisture distribution (SD = 2.9) than the untreated plots (SD= 4.1).
- The PGRs had a significant effect on turf height (p=0.0163). In particular, plots treated with TE (11.8 mm) and Pac (11.7 mm) had significantly lower heights than the control plots (13.1 mm)

References

Alvarez, G., E. Sevostianova, M. Serena, R. Sallenave, and B. Leinauer. 2016. Surfactant and Polymer-Coated Sand Effects on Deficit Irrigated Bermudagrass Turf. Agron. J., 108:2245-2255.

Cisar, J.L., K.E. Williams, H.E. Vivas, and J.J. Haydu. 2000. The occurrence and alleviation by surfactants of soil-water repellency on sand-based turfgrass systems. J. Hydrol. 231–232:352–358. doi:10.1016/S0022-1694(00)00207-9

Karcher, D.E., and M.D. Richardson. 2003. Quantifying turfgrass color using digital image analysis. Crop Sci. 43:943–951. doi:10.2135/cropsci2003.9430

Leinauer, B., D. Karcher, T. Barrick, Y. Ikemura, H. Hubble, and J. Makk. 2007. Water repellency varies with depth and season in sandy rootzones treated with ten wetting agents. https://dl.sciencesocieties.org/publications/ats. Applied Turfgrass Sci. doi:10.1094/ATS-2007-0221-01-RS

Richardson, M.D., D.E. Karcher, and L.C. Purcell. 2001. Quantifying turfgrass cover using digital image analysis. Crop Sci. 41:1884–1888. doi:10.2135/cropsci2001.1884

Schiavon, M., B. Leinauer, M. Serena, B. Maier, and R. Sallenave. 2014. Plant growth regulator and soil surfactant's effects on saline and deficit irrigated warm-season grasses: I Turf quality and soil moisture. Crop Sci. 54:2815–2826. doi:10.2135/cropsci2013.10.0707

Schiavon, M., P. Orlinski, P. Petelewicz, M. Pudzianowska, and J. H. Baird. 2019. Effects of Trinexapac-Ethyl, Surfactant, and Nitrogen Fertilization on Bermudagrass Water Use. Agron. J. 111:1-10. doi:10.2134/agronj2019.03.0225

Serena, M., M. Sportelli, E. Sevostianova, R. Sallenave and B. Leinauer. 2018. Combining Trinecapac-Ethyl with a soil surfactant reduces bermudagrass irrigation requirements. Agon. J. 110:2180-2188 doi:10.2134/agronj2018.03.0148

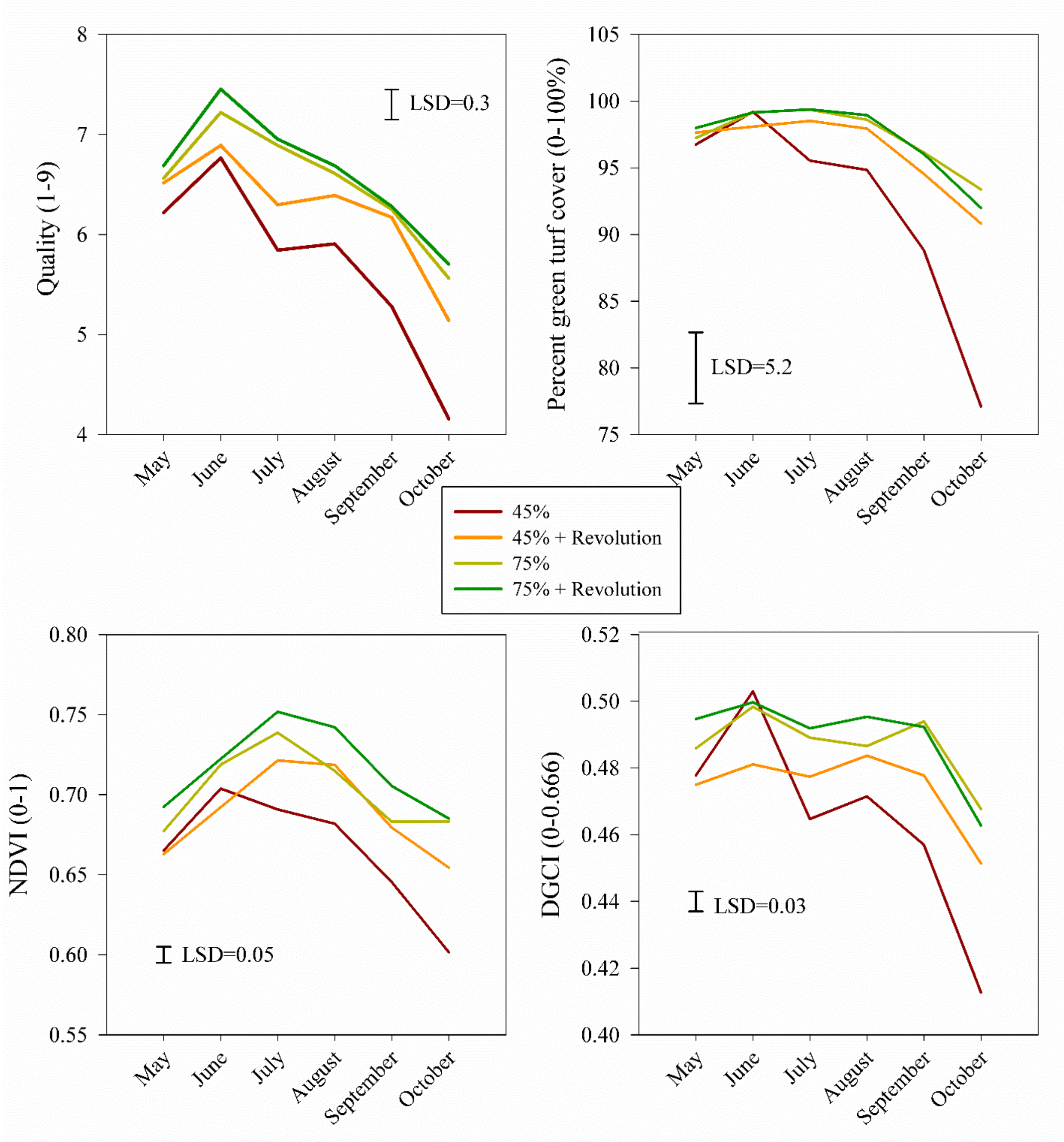


Figure 1. Bermudagrass Quality (top left), percent green turf cover (top right), Normalized Difference Vegetation Index (NDVI) (bottom left) and Dark Green Color Index (DGCI) (bottom right) affected by sampling month, irrigation replacement level (45% and 75%) and soil surfactant Revolution®. Datapoints represent an average of 32 values: eight PGR treatments [PrimoMaxx 11oz/A (TE), Trimit 32oz/A (Pac), Primo 11oz/A + Trimit 8oz/A (TE+Pac), Anuew 15oz/A (ProHex), Cutless 25oz/A (Ful), Musketeer 20oz/A (TE+Pac+Ful), Legacy 15oz/A (TE+Ful)], and four replications. Bars represent Fisher's LSD test at the 0.05 probability level.

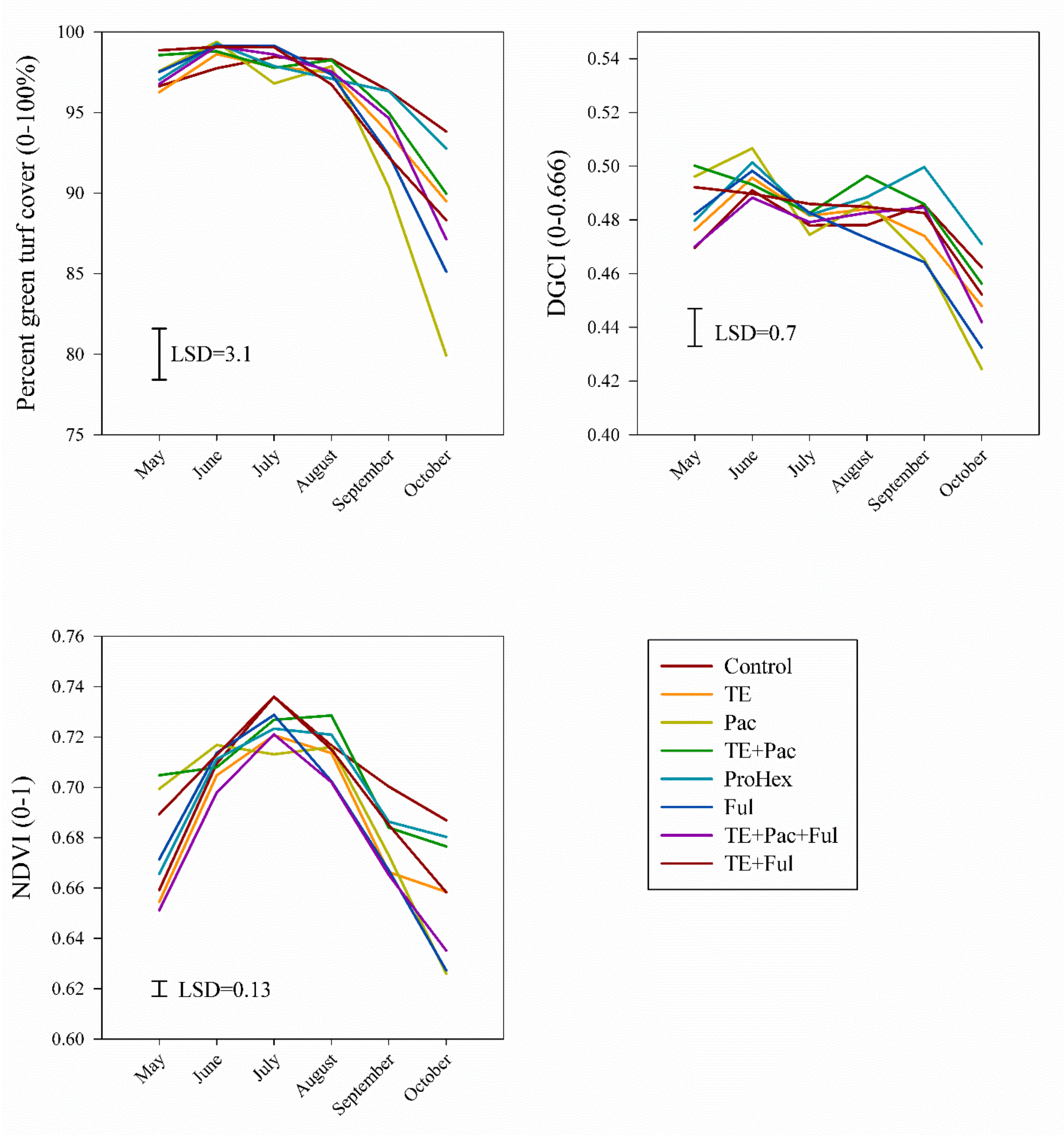


Figure 2. Bermudagrass percentage green cover (top left), normalized difference vegetation index (NDVI) (bottom left) and dark green color index (DGCI) (top right) as affected by sampling months and PGRs [PrimoMaxx 11oz/A (TE), Trimit 32oz/A (Pac), Primo 11oz/A + Trimit 8oz/A (TE+Pac), Anuew 15oz/A (ProHex), Cutless 25oz/A (Ful), Musketeer 20oz/A (TE+Pac+Ful), Legacy 15oz/A (TE+Ful)]. Datapoints represent an average of 16 values: ETo levels (45% and 75%) with or without Revolution® and four replications. Bars represent Fisher's LSD test at the 0.05 probability level.

Photo 1. Bermudagrass irrigated at 45% ETo and treated monthly with paclobutrazol (bottom - center, #4) or with trinexapac-ethyl, paclobutrazol and Revolution® (center – right, #16). Photo was taken on September 24, 2020.

Photo 2. Bermudagrass irrigated at 75% ETo and treated monthly with trinexapac-ethyl and paclobutrazol(bottom - center, #8) or with trinexapac-ethyl, paclobutrazol and Revolution® (center – right, #16). Photo was taken on September 24, 2020.

USGA ID#: 2019-12-682

Title: Surfactants for water conservation and their impact on soil health

Project Leader: Matteo Serena, Elena Sevostianova, Mohammed Omer, Omololu John Idowu, Bernd Leinauer, and Will Bosland
Affiliation: Extension Plant Sciences Department
New Mexico State University

Objectives:

i. To evaluate the effects of natural and market available chemical surfactants on the physical, chemical and biological soil health indicators in turfgrass under both sufficient and deficit irrigation in an arid environment
ii. To identify suitable minimum data set (a suite of soil measurements) that can be used for turfgrass soil health assessment and to develop soil health indexes from these measurements that will be related to turfgrass performance for bermudagrass and Kentucky bluegrass
iii. To assess the effects of natural and market available chemical surfactants on turfgrass quality under deficit irrigation
iv. Incorporate our findings into best turfgrass management practices

Start Date: 2019
Project Duration: 2 years (2019 – 2020)
Total Funding: $29,290.00

Summary Points:

1) Soil surfactants did not affect soil biological parameters Total Biomass, Arbuscular Mycorrhizal Biomass, Total Bacteria Biomass, Total Fungi Biomass, Fungi Bacteria Ratio, and Diversity Index for both Kentucky bluegrass and bermudagrass.
2) Soil biological parameters did not correlate with turfgrass performance parameters such as visual quality, percent coverage, or DGCI and NDVI.
3) Surfactants did not affect soil moisture, turfgrass quality, and soil moisture uniformity of Kentucky bluegrass.
4) When data were averaged over all sampling dates and ET replacement levels, Bermudagrass plots treated with Revolution had highest quality.
5) Bermudagrass plots treated with Dispatch or Revolution and irrigated at 45% ET replacement level showed similar quality than untreated plots irrigated at 75% ET.
6) Bermudagrass plots that received Revolution exhibited the most uniform soil moisture distribution.

Soil surfactants have been used in regular turfgrass maintenance programs to increase irrigation efficiency, because their use has been shown to increase uniformity and improve the moisture retention in the root zones (Alvarez et al., 2016; Kostka and Bially, 2005; Leinauer et al., 2001; Leinauer et al., 2010; Leinauer and Devitt, 2013; Mitra et al., 2005). Due to their ability to weaken the surface tension, wetting agents permit the penetration of water not only into repellent rootzone areas but also into the meso and micropores of soils. Thus, in addition to offering remediation of LDS and hydrophobic soil conditions, soil surfactants may also help to reduce irrigation requirements by improving water use efficiency. Several studies have documented improved turfgrass performance under drought or decreased irrigation when soil surfactants were applied (Alvarez et al., 2017; Cisar et al., 2000; Kostka, 2005; Kostka et al., 2007). Currently, 94% of golf courses in the United States have incorporated soil surfactants into their regular maintenance protocols (Gelernter et al., 2015).

While the benefits of surfactants in combating LDS and soil hydrophobicity have been well documented, the effects of long-term use of surfactants on overall soil health have not been studied. Golf course superintendents have started to report changes in the soil physical properties after long-term application of surfactants, such as decreased drainage, increasing anaerobic soil conditions, and lower turf quality.

Soil health or soil quality is “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (Doran & Parkin, 1996). To fulfill these requirements, healthy soils usually integrate physical, chemical, and biological attributes of the soil (Idowu et al., 2008). The interactions of soil chemical, physical, and biological properties often determine how effectively the soil functions in areas of nutrient retention and release, partitioning of rainfall into runoff and infiltration, moisture retention and release, resistance to environmental degradation, and buffering environmental pollutants (Karlen et al. 1997). The assessment of soil health is not based on the magnitude of any single soil parameter, but rather relies on selected soil measurements (soil health indicators) to quantify management-induced changes (Arshad & Coen, 1992; Doran & Parkin, 1994). A suite of soil measurements that best describe changes in response to management practices constitutes the minimum data set (MDS), and the MDS vary with soil health management goals (Andrews et al., 2002). Currently, there is no documentation on the MDS that can be used to holistically assess soil health of turfgrass in arid environments. Most of the previous studies have only focused on specific soil aspects, without any integration of the physical, chemical and biological attributes of the soil. Due to environmental concerns, chemicals applied to turfgrass systems are receiving increased scrutiny, and the impacts of these chemical need to be related to soil health.

Study

A study was conducted at New Mexico State University in Las Cruces, New Mexico from 2019 to 2020 to investigate the effects of repeated applications of commonly available chemical and natural soil surfactants on soil health, irrigation water requirement, and turfgrass quality (Figure 1). The study was initiated in 2018 and included four non-ionic wetting agents which were compared against an untreated control on ‘Princess 77’ bermudagrass (*Cynodon dactylon* L.) and ‘SR 2100’ Kentucky bluegrass (*Poa pratensis* L.).

Figure 1. Study area at New Mexico State University. Soil sampling on Kentucky bluegrass site (left), Bermudagrass plots (right).

The study included the following surfactants:
1) a modified methyl capped block co-polymer (trade name Revolution)
2) an alkyl polyglycoside (trade name Dispatch)
3) a natural wetting agent derived from *Yucca schidigera* (trade name Therm X-70) and
4) a rhamnolipid biosurfactant (trade name ZONIX). Rhamnolipids are glycolipids (two rhamnoses conjugated to fatty acid chains) produced by *Pseudomonas aeruginosa*. Their high surface activity has been reported not only for emulsifiers and detergents but also when applied to agricultural and horticultural soils (Ali et al., 2017; Renfro, 2013; Yang, 2008).

Turfgrass performance was evaluated twice per month by means of visual quality ratings, Digital Image Analysis (Coverage, Hue, Saturation, Brightness, and Dark Green Color Index), and Normalized Difference Vegetation Indices (NDVI) by means of a Greenseeker and subsequently averaged over sampling month. Soil biological indicators included the permanganate oxidizable carbon (Weil et al., 2003), soil organic matter using the Walkley-Black method (Nelson and Sommers, 1982) and the soil microbial community using the phospholipid fatty acid (PLFA) analysis (Buyer and Sasser, 2012). Phospholipid fatty acid analysis provides information on the amount of gram positive and gram negative bacteria; the amount of arbuscular mycorrhiza fungi and the total fungi; and the amount of anaerobes and actinomycetes. Soil physical measurements included saturated hydraulic conductivity, bulk density, dry aggregate size distribution, wet aggregate stability, and soil moisture retention characteristics.

Plots were mowed three times per week at a height of 1.2 cm (1/2”) by means of a reel mower with clippings returned. A pre-emergence herbicide, Barricade 4L (Prodiamine @ 21 oz/A) was applied in mid-March and in mid-June. The insecticide Acelepryin (Chorantraniliprole @ 12oz/A) was applied in mid-June for white grub control. Fertilization consisted of a total of 20 g N, 4 g P_2O_5, and 8 g K_2O m^{-2} for both grasses. Fertilizer was applied monthly from April to September on bermudagrass and in March, April, May, August, September, and October on Kentucky bluegrass. Iron fertilization was applied 3 times during the growing season by means of Six Iron™ (12-0-0) which was added to the spray tank at 6oz/1000sqft during July and August.

The field experiment was laid out in a completely randomized block design with two levels of irrigation (75% and 45% ETos on bermudagrass and 90% and 65% ETos on Kentucky bluegrass) as the block treatment and surfactants at the plot level. Each treatment combination was replicated four times.

Results

Bermudagrass

Analysis of variance revealed that the interaction between surfactants and sampling months had a significant effect on DGCI, soil moisture, NDVI, and quality (Table 1). Moreover, quality was also affected by the interaction between surfactants and irrigation level (Table 1). Surfactants as a main effect influenced soil moisture uniformity (Table 1). When data were averaged over both irrigation levels and all sampling months bermudagrass plots treated with Revolution had highest quality and highest soil moisture content. Bermudagrass plots treated with Revolution also exhibited the most uniform soil moisture distribution. However, surfactants did not affect soil biological parameters Total Biomass, Arbuscular Mycorrhizal Biomass, Total Bacteria Biomass, Total Fungi Biomass, Fungi Bacteria Ratio, and Diversity Index.

Kentucky Bluegrass

Analysis of variance revealed that the interaction between surfactants, ET, and sampling months had a significant effect on NDVI (Table 1). Similar to bermudagrass, ANOVA also revealed that the interaction between surfactants and sampling months had a significant effect on DGCI (Table 1). Soil surfactants did not affect cover, visual quality, soil moisture and moisture uniformity, neither as main effect nor as interactions with ET and sampling months. Similar to bermudagrass, surfactants also did not affect soil biological parameters.

Conclusion

Surfactant treatments had no effect on soil biological parameters measured for either Kentucky bluegrass or bermudagrass. Results indicate that surfactants do not positively or negatively influence the soil microbial community, regardless of the type of surfactant applied (organic or synthetic). However, longer term investigations should be conducted to verify if this trend holds if surfactants are applied over several years.

Table 1. Probability values obtained from ANOVA, testing the effects of surfactants, irrigation replacement based on evapotranspiration for short grass (ET), sampling month (Date), and their interactions on percent green cover (Cover), Dark Green Color Index (DGCI) (both parameters determined by means of digital image analysis), volumetric soil water content (moisture), Normalized Difference Vegetation Index (NDVI), visual turfgrass quality (Quality), and soil moisture uniformity of 'Princess 77' bermudagrass (*Cynodon dactylon* L.) and 'SR 2100' Kentucky Bluegrass (*Poa pratensis* L.).

Effect	Cover	DGCI	Moisture	NDVI	Quality	Uniformity
	Bermudagrass					
Surfactant	0.0850	0.0418	0.0027	0.0518	<.0001	0.0008
ET	0.0185	0.0157	0.0017	0.0039	0.0004	0.3771
ET*Surfactant	0.3483	0.3052	0.6677	0.7088	0.0286	0.0923
Month	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Surfactant*Month	0.0979	0.0112	0.0013	0.0223	0.0307	0.8578
ET*Month	0.0006	<.0001	<.0001	<.0001	<.0001	0.0004
ET*Surfactant*Month	0.5569	0.5860	0.1398	0.2820	0.9886	0.8952
	Kentucky Bluegrass					
Surfactant	0.3909	0.4988	0.2364	0.9810	0.0698	0.4749
ET	0.0053	0.0238	0.0662	0.0083	0.0027	0.5957
ET*Surfactant	0.2751	0.4403	0.7806	0.2388	0.1558	0.4436
Month	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Surfactant*Month	0.0554	0.0112	0.5959	0.0925	0.3804	0.6049
ET*Month	<.0001	<.0001	<.0001	<.0001	<.0001	0.0630
ET*Surfactant*Month	0.0798	0.4335	0.8815	0.0069	0.2971	0.8961

Table 2. Percent green cover (Cover), Dark Green Color Index (DGCI), soil moisture content (Moisture), Normalized Difference Vegetation Index (NDVI), and visual turfgrass quality (Quality) of 'Princess 77' bermudagrass as affected by different soil surfactants. Values are listed separately for each sampling month and averaged over two irrigation levels (45% and 75% of reference evapotranspiration for short grass).

Surfactant	May	June	July	August	September	October	November
				Cover			
Control	96.9A	99.6A	99.7A	98.4A	98.3A	96.9A	96.6AB
Dispatch	94.0A	99.6A	99.7A	95.6A	94.4A	93.0A	93.5AB
Revolution	93.5A	99.7A	99.7A	97.9A	98.8A	98.8A	98.7A
Rhamnolipids	93.7A	99.3A	98.9A	93.5A	93.9A	89.8A	92.8AB
Yucca	92.6A	99.3A	98.7A	96.6A	93.4A	87.7A	86.8B
				DGCI			
Control	0.4405A	0.5036A	0.5194A	0.4196A	0.4874AB	0.4826AB	0.4743AB
Dispatch	0.43A	0.5034A	0.5078A	0.4035A	0.4581B	0.4549AB	0.4509AB
Revolution	0.4286A	0.5012A	0.5179A	0.4332A	0.5056A	0.5049A	0.4921A
Rhamnolipids	0.429A	0.4992A	0.5154A	0.4082A	0.4637AB	0.4578AB	0.4637AB
Yucca	0.4254A	0.4937A	0.4946A	0.4092A	0.4509B	0.4404B	0.4363B
				Moisture			
Control		21.1A	15AB	13.3AB	13.9AB	11.4AB	10.2AB
Dispatch		19.9A	14.0B	11.0B	11.8B	8.6C	8.3B
Revolution		19.9A	16.8A	16.0A	15.8A	12.7A	12A
Rhamnolipids		19.7A	14.8AB	12.1B	12.1B	9.2BC	8.6B
Yucca		18.0A	13.8B	10.9B	12.1B	8.6C	8.7B
				NDVI			
Control	0.5994A	0.7434A	0.7471A	0.6546AB	0.6672AB	0.689AB	0.6515A
Dispatch	0.568AB	0.7375A	0.7346A	0.6327AB	0.6416AB	0.6822AB	0.6499A
Revolution	0.5689AB	0.7487A	0.7601A	0.6837A	0.7293A	0.7585A	0.7106A
Rhamnolipids	0.5552AB	0.723A	0.7418A	0.6269AB	0.6408AB	0.6895AB	0.6664A
Yucca	0.5347B	0.7135A	0.7153A	0.6186B	0.6363B	0.6546B	0.6284A
				Quality			
Control	5.3B	6.9C	6.6B	6.1B	5.0B	4.8B	4.6B
Dispatch	5.8B	8AB	7.1B	6.4B	5.6B	5.1B	4.8B
Revolution	6.6A	8.4A	8.2A	7.8A	7.1A	7.1A	6.2A
Rhamnolipids	5.6B	7.6BC	6.8B	6.1B	5.2B	5.1B	5.1B
Yucca	5.2B	7.3C	6.6B	6.2B	4.9B	4.5B	4.5B

¶Values followed by the same letter in each column (separately for each output variable) are not significantly different according to simulated adjustment (0.05).

Table 3. Percent green cover (Cover), Dark Green Color Index (DGCI), soil moisture content (Moisture), Normalized Difference Vegetation Index (NDVI), visual turfgrass quality (Quality), and soil moisture uniformity (Uniformity) of 'Princess 77' bermudagrass as affected by different soil surfactants. Values are averaged over seven sampling months and two irrigation levels (45% and 75% of reference evapotranspiration for short grass).

Surfactant	Cover	DGCI	Moisture	NDVI	Quality	Uniformity
Control	98.0A¶	0.4753AB	14.2AB	0.6789AB	5.6B	3.8A
Dispatch	95.7A	0.4584AB	12.3B	0.6638AB	6.1B	3.7A
Revolution	98.2A	0.4833A	15.5A	0.7086A	7.4A	2.5B
Rhamnolipids	94.6A	0.4624AB	12.8B	0.6634AB	5.9B	3.3AB
Yucca	93.6A	0.4501B	12.0B	0.643B	5.6B	3.6A

¶Values followed by the same letter in each column are not significantly different according to simulated adjustment (0.05)

Table 4. Visual turfgrass quality of 'Princess 77' bermudagrass as affected by different soil surfactants and reference evapotranspiration for short grass (ET). Values are averaged over seven sampling months.

Surfactant	45% ET	75% ET
Control	5.1D	6.1B
Dispatch	6.0BCD	6.2B
Revolution	6.7B	8.0A
Rhamnolipids	5.1CD	6.7B
Yucca	5.1CD	6.0BC

¶Values followed by the same letter are not significantly different according to simulated adjustment (0.05)

Table 5. Normalized Difference Vegetation Index (NDVI) of 'SR 2100' Kentucky bluegrass as affected by different soil surfactants, sampling months and irrigation levels based on 45% and 75% of reference evapotranspiration for short grass (ET).

Surfactant	May	June	July	August	September	October	November
				55% ET			
Control	0.7186A	0.7524A	0.7284A	0.6618A	0.6325BC	0.7234BC	0.7579C
Dispatch	0.6796AB	0.7325A	0.7411A	0.6711A	0.7006ABC	0.7714ABC	0.7962ABC
Revolution	0.6832AB	0.7178A	0.7161A	0.6702A	0.6796ABC	0.7479ABC	0.7849ABC
Rhamnolipids	0.7145A	0.7355A	0.7201A	0.6277A	0.6063C	0.6963C	0.7672BC
Yucca	0.6416B	0.73A	0.7363A	0.6675A	0.6939ABC	0.7799ABC	0.82AB
				85% ET			
Control	0.6854AB	0.7189A	0.7567A	0.7169A	0.7562AB	0.8157AB	0.8324A
Dispatch	0.6681AB	0.7316A	0.7512A	0.6802A	0.7224ABC	0.8041AB	0.838A
Revolution	0.6752AB	0.7078A	0.7332A	0.6984A	0.7496AB	0.8075AB	0.831A
Rhamnolipids	0.6932AB	0.7338A	0.7649A	0.7234A	0.7693A	0.8283A	0.8437A
Yucca	0.6734AB	0.7289A	0.7538A	0.7109A	0.7397AB	0.7972ABC	0.8209AB

¶Values followed by the same letter in each column (across both ET levels) are not significantly different according to simulated adjustment (0.05).

Table 6. Percent green cover (Cover) and Dark Green Color Index (DGCI) of 'SR 2100' Kentucky bluegrass as affected by different soil surfactants Values are listed separately for each sampling month and averaged over two irrigation levels (45% and 75% of reference evapotranspiration for short grass).

Surfactant	May	June	July	August	September	October	November
				Cover			
Control	97.4AB	98A	98.7A	95.4A	93.4A	97.2A	98.4A
Dispatch	97.2AB	98.3A	98.1A	94.2A	94.3A	95.9A	98.1A
Revolution	96.7AB	98.1A	97.3A	97.1A	97.3A	98.5A	98.9A
Rhamnolipids	97.8A	98.6A	98.8A	91.5A	91.3A	94.3A	97.3A
Yucca	95.8B	97.5A	98.4A	94.7A	95.7A	97.9A	99.1A
				DGCI			
Control	0.4843A	0.5028A	0.5281AB	0.4702A	0.5198A	0.5499A	0.5762A
Dispatch	0.4748A	0.5072A	0.5282AB	0.4693A	0.5141A	0.5467A	0.5777A
Revolution	0.4662A	0.4901A	0.5061B	0.4655A	0.5196A	0.5598A	0.5855A
Rhamnolipids	0.4856A	0.5092A	0.5293AB	0.4557A	0.5015A	0.5354A	0.5681A
Yucca	0.4671A	0.5095A	0.5357A	0.4777A	0.5293A	0.5712A	0.5981A

[¶]Values followed by the same letter in each column (separately for each output variable) are not significantly different according to simulated adjustment (0.05).

References

Alvarez, G., E. Sevostianova, M. Serena, R. Sallenave, and B. Leinauer. 2016. Polymer Coated Sand Effects on Deficit Irrigated Bermudagrass Turf. Agronomy Journal 108(6), 2245-2255. doi:10.2134/agronj2016.06.0329

Andrews, S. S., D. L. Karlen, and J. P. Mitchell. 2002. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agriculture, Ecosystems & Environment, 90:25-45.

Arshad, M. A., and G. M. Coen. 1992. Characterization of soil quality: Physical and chemical criteria. American Journal of Alternative Agriculture, 7:25-31.

Cisar, J. L., K. E. Williams, H. E. Vivas, and J. J. Haydu. 2000. The occurrence and alleviation by surfactants of soil-water repellency on sand-based turfgrass systems. J. Hydrol. 231,232:352-358.

Doran, J. W., and T. B. Parkin. 1994. Defining and assessing soil quality. SSSA special publication, 35, 3-3.

Gelernter, W.D., L.J. Stowell, M.E. Johnson, C.D. Brown, and J.F. Beditz. 2015. Documenting Trends in Water Use and Conservation Practices on U.S. Golf Courses.Crop, Forage & Turfgrass Management 1. doi:10.2134/cftm2015.

Idowu, O. J., H. M. van Es, G. S. Abawi, D. W. Wolfe, J. I. Ball, B. K. Gugino, B.N. Moebius, R.R. Schindelbeck, and A. V. Bilgili. 2008. Farmer-oriented assessment of soil quality using field, laboratory, and VNIR spectroscopy methods. Plant and Soil, 307:243-253.

Karlen, D. L., M. J. Mausbach, J. W. Doran, R. G. Cline, R. F. Harris, and G. E. Schuman. 1997. Soil quality: a concept, definition, and framework for evaluation (a guest editorial). Soil Science Society of America Journal 61:4-10.

Kostka, S. J. 2005. ACA 1820: A novel chemistry for rootzone water management in turfgrass systems. Int. Turfgrass Soc. Res. J. (Annexe) 10:89-90.

Kostka, S. J., and P. T. Bially. 2005. Synergistic surfactant interactions for enhancement of hydrophilicity in water repellent soils. Int. Turfgrass Soc. Res. J. 10:108-114.

Kostka, S. J., J. L. Cisar, S. Mitra, D. M. Park, C. J. Ritsema, L. W. Dekker, and M. A. Franklin. 2007. Irrigation efficiency – Surfactants can save water and help maintain turfgrass quality. Golf Course Industry 19:91-95.

Leinauer, B., and D. Devitt. 2013. Irrigation science and technology. In: B. Horgan, J. Stier, and S. Bonos, editors, Agron. Monogr. 56. ASA, CSSA, and SSSA, Madison, WI. p. 1075-1133.

Leinauer, B., E. Sevostianova, M. Serena, and M. Schiavon. 2010. Conservation of irrigation water for urban lawn areas. Acta Hortic. 881-487-492.

Leinauer, B., P. E. Rieke, D. VanLeeuwen, R. Sallenave, J. Makk, and E. Johnson. 2001. Effects of soil surfactants on water retention in turfgrass rootzones. Int. Turfgrass Soc. Res. J. 9:542-547.

Mitra, S., K. W. Kurtz, R. V. Plumb, A. Chavez, S. Kostka, and M. Franklin. 2005. Systematic injection of wetting agents can help retain higher moisture levels in the root zone. Intl. Turfgrass Soc. Res. J. (Annexe) 10:91-92.