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# Turfgrass and Environmental Research Program

# 2016 Research Summaries

**CONFIDENTIAL – NOT FOR PUBLICATION**

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# 2016 Turfgrass and Environmental Research Summaries

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1983-01-001

# Annual Report - 2016

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

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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:** $10,000 per year

As of October 30, 2016 over 1,682, promising turfgrasses and associated endophytes were collected in Italy ,Greece, Romania and Mallorca These are having seed produced in the Netherlands and will be evaluated in New Jersey starting in fall 2016. Over 9,855 new turf evaluation plots, 136,681 spaced-plant nurseries and 9276 mowed single-clone selections were established in 2015.

Over 200,000 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 38,770 tall fescues, 8,000 Chewings fescues, 4,000 hard fescues, 27,000 perennial ryegrasses and 8,000 bentgrasses were also screened during the winter in greenhouses and superior plants were put in spaced-plant nurseries. Over 95 new inter- and intra-specific Kentucky bluegrasses were harvested in 2016.

The following crossing blocks were moved in the spring of 2016: 7 hard fescues (253 plants), 2 Chewings fescues (61 plants), 7 perennial ryegrasses (243 plants), 6strong creeping red fescues(134 plants), 10 tall fescues (313 plants), 7 creeping bentgrasses (96 plants) ,5 velvet bentgrasses (82 plants) and 9 colonial bentgrasses (181 plants).

To enhance our breeding for resistance to gray leaf spot, a August 25 planting of 2400 perennial ryegrasses were seeded. Excellent *Pythium* blight control was attained and a good gray leaf spot epidemic occurred. This data will be used to select future varieties of perennial ryegrass. Over 27,000 perennial ryegrasses were planted in the spring of 2016 as spaced-plants. They were allowed to develop seed heads in the late spring and selections were made for stem and crown rust resistance and heat tolerance.

The breeding program continues to make progress breeding for disease resistance and improved turf performance. New Promising varieties named and released in 2016 were Intense,Spark and Ruckus perennial ryegrasses, new tall fescues GTO, Amity, SuperSonic, Maestro, Avenger II ,Titanium SLS,
Selkirk, Firecracker SLS, and Raptor III. There was also one creeping red fescue fescue named Xeric and two hard fescues Jetty and Gladiator. New Kentucky blugrasses Zinger and Mazama. There was one new creeping bentgrasses named Piranha and three new colonial bentgrasses Puritan, Musket, and Heritage. .

## 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. This has resulted in the continued development and release of top performing varieties in the NTEP

- Two perennial ryegrasses, 9 new tall fescues, 2 Kentucky bluegrasses and 3 fine fescue, and 1 creeping bentgrass and three new colonial bentgrasses were released in 2016.

- Published or have in press over 4 referred journal articles in 2016

- 27 Plant variety certificates issued and 20 PVP's applied for

## References

**Refereed Research Publications:**

Honig, J.A., E. Zelzion, N. E. Wagner, C. Kubik, V. Averello, J. Vaiciunas, D. Bhattacharya, S.A. Bonos, and W. A. Meyer. 2017. Microsatellite (SSR) identification in perennial ryegrass (Lolium perenne L.) using next generation sequencing. Accepted to Crop Science August 21, 2016.

Koch, E. D. J. Honig, J. Vaiciunas, and S. A. Bonos. 2017. Endophyte effect on salinity tolerance in perennial ryegrass. Tentatively Accepted to International Turfgrass Society Research Journal August 21, 2016.

Yue, C., J. Wang, E. Watkins, S.A. Bonos, K.C. Nelson, J.A. Murphy, W.A. Meyer, and B.P. Horgan. 2016. Heterogeneous U.S. and Canada consumer preference for turfgrass attributes. Submitted to Canadian Journal of Agricultural Economics, Nov. 25, 2015. Tentatively accepted for publication July 8, 2016.

Jespersen, D., E. Merewitz, Y. Xu, J. Honig, S. Bonos, W. Meyer, B. Huang. 2016. Quantitative trait loci associated with physiological traits for heat tolerance in creeping bentgrass. Crop Science Vol. 56 No. 3, p. 1314-1329.

**Abstracts:**

William A. Meyer and Stacy A. Bonos. 2016. Major advances from the Rutgers turfgrass breeding program. p. 18. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

David Jespersen, Stacy A. Bonos, Faith C. Belanger, Paul Raymer and Bingru Huang. 2016. Characterization and validation of molecular markers linked to heat and drought tolerance for marker-assisted selection of stress tolerant creeping bentgrass. p.22. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Vincenzo Averello, Christine Kubik, Jennifer Vaiciunas, Stacy A. Bonos, William A. Meyer and Josh Honig. 2016. Classification of tall fescue (*Festuca arundinacea* Schreb.) cultivars and collections using chloroplast microsatellite (cpSSR) markers. p.45. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Vincenzo Averello, Christine Kubik, Jennifer Vaiciunas, Stacy A. Bonos, William A. Meyer and Josh Honig. 2016. Classification of tall fescue (Festuca arundinacea Schreb.) cultivars and collections using nuclear microsatellite (nuSSR) markers. p.46. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Stacy A. Bonos, Eric Koch, Jennifer Vaiciunas, Josh Honig, William A. Meyer, Udi Zelzion and Debashish Battacharya. Differential gene expression of salt-stressed perennial ryegrass. p. 47. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Austin L. Grimshaw, Trent M. Tate, William A. Meyer, Eric Watkins and Stacy Bonos. 2016. Evaluation of hard fescue (Festuca brevipila Tracy) for summer patch (Magnaporthiopsis poae J. Luo & N. Zhang) resistance. p. 54. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Trent M. Tate, William A. Meyer, Stacy A. Bonos, Patrick E. McCullough. 2016. Evaluation of nine tenacity selected fine fescues: Quantifying the tolerance levels and determination of the absorption and translocation. p. 71. In Proceedings of the 25th Rutgers Turfgrass Symposium. March 18, 2016.

Averello, V. C. Kubik, J. Vaiciunas, W. Meyer, S. Bonos and J. Honig. 2016. Genetic diversity of tall fescue (Lolium arundinaceum (Schreb.) Darbysh.) cultivars and collections using chloroplast microsatellite (cpSSR) markers. Plant Animal and Genome Conference. San Diego, CA. January 8, 2016.

Eric Watkins, Stacy A. Bonos, Chengyan Yue, Kristen Nelson, Brian Horgan, Paul Koch, James A. Murphy, Bingru Huang, William A. Meyer and Bruce B. Clarke. 2015. Germplasm improvement of low-input fine fescues in response to consumer attitudes and behaviors. In Agronomy Abstracts, Madison, WI.

Stacy A. Bonos, Jennifer Vaiciunas, Udi Zelzion, Debashish Bhattacharya, William A. Meyer, Eric Koch and Joshua Honig. 2015. Transcriptome analysis of salt-stressed perennial ryegrass. In Agronomy Abstracts, Madison, WI.

David Jespersen, Joshua Honig, Stacy A Bonos, William A. Meyer and Bingru Huang. 2015. Quantitative trait loci associated with delayed heat-induced senescence in creeping bentgrass. In Agronomy Abstracts, Madison, WI.

Trent Matthew Tate, William A. Meyer, Stacy A Bonos, Patrick E. McCullough and Carrie Mansue. 2015. Evaluation of tenacity selected fine fescues to eleven rates of tenacity from 0-8966 grams a.I. ha-1. In Agronomy Abstracts, Madison, WI.

Vincenzo Averello, Christine Kubik, Jennifer Vaiciunas, William A. Meyer, Stacy A Bonos and Joshua Honig. 2015. Genetic diversity of tall fescue (Lolium arundinaceum (Screb.) Darbysh.) cultivars using microsatellite (SSR) markers. In Agronomy Abstracts, Madison, WI.

**Non-referred Publications:**

Weibel, E.N., T.J. Lawson, J.B. Clark, J.A. Murphy, B.B Clarke, W.A. Meyer and S.A. Bonos. 2016. Performance of bentgrass cultivars and selection in New Jersey turf trials. 2015 Rutgers Turfgrass Proceedings 47:1-40.

Tate, T.M., A.L. Grimshaw, D.A. Smith, R.F. Bara, M.M. Mohr, E.N. Weibel, S.A. Bonos and W.A. Meyer. 2016. Performance of fine fescue cultivars and selections in New Jersey turf trials. 2015 Rutgers Turfgrass Proceedings 47:41-58.

Grimshaw, A.L., T.M. Tate, M.M. Mohr, R.F. Bara, D.A. Smith, E.N. Weibel, J.A. Murphy, S.A. Bonos and W.A. Meyer. 2016. Performance of Kentucky bluegrass cultivars and selections in New Jersey turf trials. 2015 Rutgers Turfgrass Proceedings 47:59-124.

Qu, Y., M.M. Mohr, R.F. Bara, D.A. Smith, E. Szerszen, S.A. Bonos and W.A. Meyer. 2016. Performance of perennial ryegrass cultivars and selections in New Jersey turf trials. 2016 Rutgers Turfgrass Proceedings 47:125-148.

Trent Tate, Ronald F. Bara, Dirk A. Smith, Melissa M. Mohr, Stacy A. Bonos, and William A. Meyer. 2016. Performance of Tall Fescue Cultivars and Selections in New Jersey Turf Trials. 2015 Rutgers Turfgrass Proceedings 47:149-176.

2013-02-463

**Project Title:** Germplasm Improvement of Low-Input Fine Fescues in Response to Consumer Attitudes and Behaviors

**Project leader**: Eric Watkins

**Affiliation**: University of Minnesota

**Objective:**

The long-term goal of this project if the development of improved, low-input fine fescue cultivars that provide economic and environmental benefits for the public.

**Start Date:** 2012
**Duration:** four years
**Total Funding:** $40,000

*This project provides matching funds for a five-year USDA-NIFA project funded by the Specialty Crops Research Initiative (grant number 2012-51181-19932). The project involves 10 scientists, along with graduate students and support staff, from three Universities (University of Minnesota, Rutgers University, and the University of Wisconsin). The project has four objectives: Objectives 1 and 2 involve social science research that will determine what consumers desire in new low-input fine fescue varieties; Objective 3 is focusing on identifying breeding material that is tolerant of stresses common to low-input turf environments; Objective 4 is addressing the challenges of educating end-users about the use of fine fescues in parks, lawns, and golf courses.*

The fine fescue species have great potential to be functional grasses in sustainable landscapes including lawns, parks, and golf courses. In 2016, much of our research focused on three areas important to golf course superintendents: traffic tolerance, heat stress tolerance, and disease resistance, in particular resistance to snow mold diseases.

*Traffic (led by Jim Murphy, Rutgers)*: A major concern for turfgrass managers considering increasing their use of fine fescues is the ability of these grasses to withstand wear and traffic; this is especially of concern to golf course superintendents who might want to use fine fescues on fairways. In a seasonal traffic study, turf uniformity and fullness of cover were reduced by abrasive wear during all seasons (spring, summer, autumn). Turf uniformity was strongly influenced by fine fescue entry but the effect depended on the season during which wear was applied. Greater differences in turf uniformity were observed among fine fescues when wear was applied during summer compared to spring and autumn. This information will be useful for breeders wanting to select traffic tolerant genotypes using traffic simulators. We also evaluated the effect of nitrogen on fiber content on three fine fescues (Fig. 1). Significant differences were observed on fiber contents among three fine fescue cultivars regardless of N level. 'Beacon' hard fescue had the highest levels of total cell wall, lignocellulose, and lignin while 'Garnet' Chewings fescue exhibited the lowest levels.

*Heat Stress (led by Bingru Huang, Rutgers)*: We screened a large number of fine fescues and identified heat stress tolerant and heat stress susceptible cultivars within each fine fescue species (Fig. 2). We then continued our work on heat stress tolerance by studying proteins associated with leaf senescence during heat stress. Cultivars of fine fescue that had previously shown different heat tolerance were exposed to heat stress temperature at 38/33 $^{o}$C (day/night temperatures) and optimal temperature at 22/18 $^{o}$C in a controlled environment growth chamber. Membrane and soluble proteins showed altered abundance during heat stress and different responses between cultivars. Comparison of differential protein expression between cultivars differing in the level of heat-induced leaf senescence or heat tolerance will allow for a greater understanding of metabolic pathways regulating heat tolerance which can be used to develop more effective selection protocol for this important trait.

*Snow Mold (led by Paul Koch, UW-Madison)*: We have continued to learn more about snow mold resistance in the fine fescues. Abnormally warm temperatures throughout much of the winter of 2015-2016 resulted in lower than average snow mold development at some sites, however, the Timber Ridge Golf Club site in Minocqua, WI did develop significant snow mold, with most fine fescue plots exhibiting between 5 and 15% disease. Hard fescues had the lowest amount of disease sheep and Chewings fescue had the highest (Fig. 3). Additionally, a growth chamber screening was conducted during the winter of 2015-2016 two cultivars from each the 5 fine fescue species for their resistance to *Microdochium nivale*. We found that cultivars of hard fescue and sheep fescue were the most resistant to pink snow mold, cultivars of Chewings fescue were the most susceptible, and cultivars of slender and strong had intermediate levels of resistance. Turfgrass managers in areas where snow mold are a concern should utilize hard and sheep fescues, while breeders should work to make improvements in diseases resistance in the other fine fescues species.

Summary Points

- Turfgrass breeders should screen new germplasm for traffic tolerance in autumn
- Differences in heat stress tolerance have been identified and we are now working on ways to use that information as we develop new fin fescue cultivars
- Hard and sheep fescue have superior snow mold resistance compared to other fine fescues and should be utilized in northern climates where snow mold is a concern.

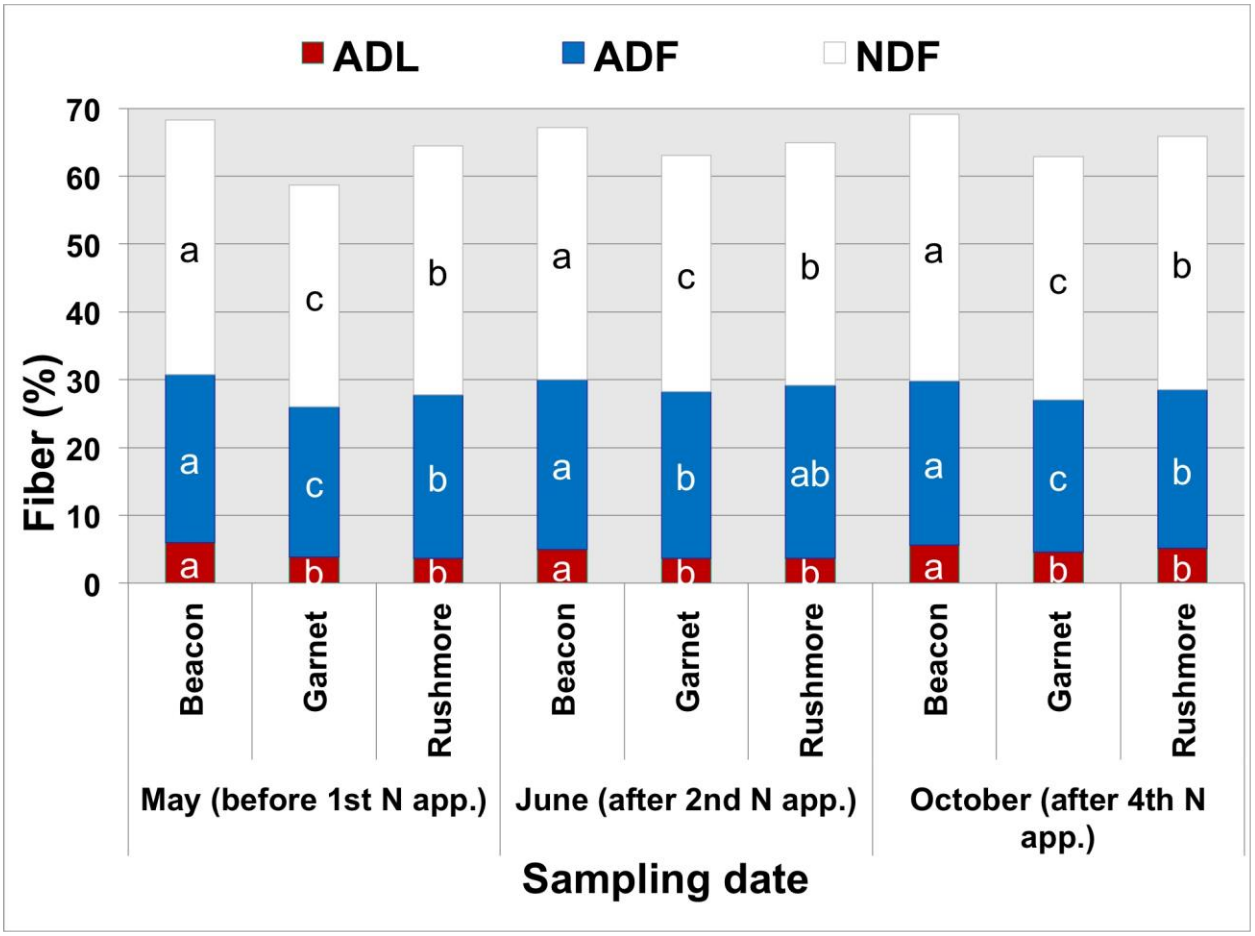



Figure 1. The total cell wall (NDF), lignocellulose (ADF) and lignin (ADL) content of fine fescue cultivars in May (before 1st nitrogen application), June (after 2nd nitrogen application) and October (after 4th nitrogen application) during 2015. Tested cultivars were 'Beacon' hard fescue, 'Garnett' strong creeping red fescue, and 'Rushmore' Chewings fescue.

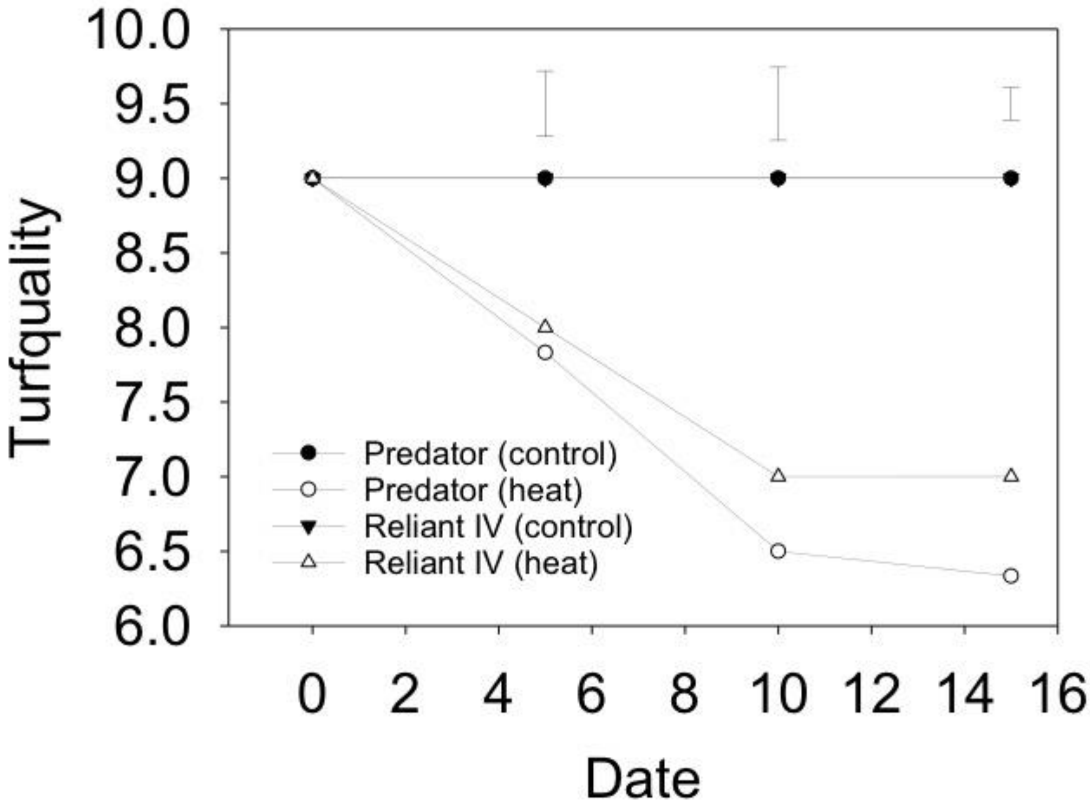



Figure 2. Turf quality for two hard fescue cultivars with or without heat stress. Both cultivars maintained the same turf quality during stress, while Reliant IV was more tolerant of heat stress than Predator.

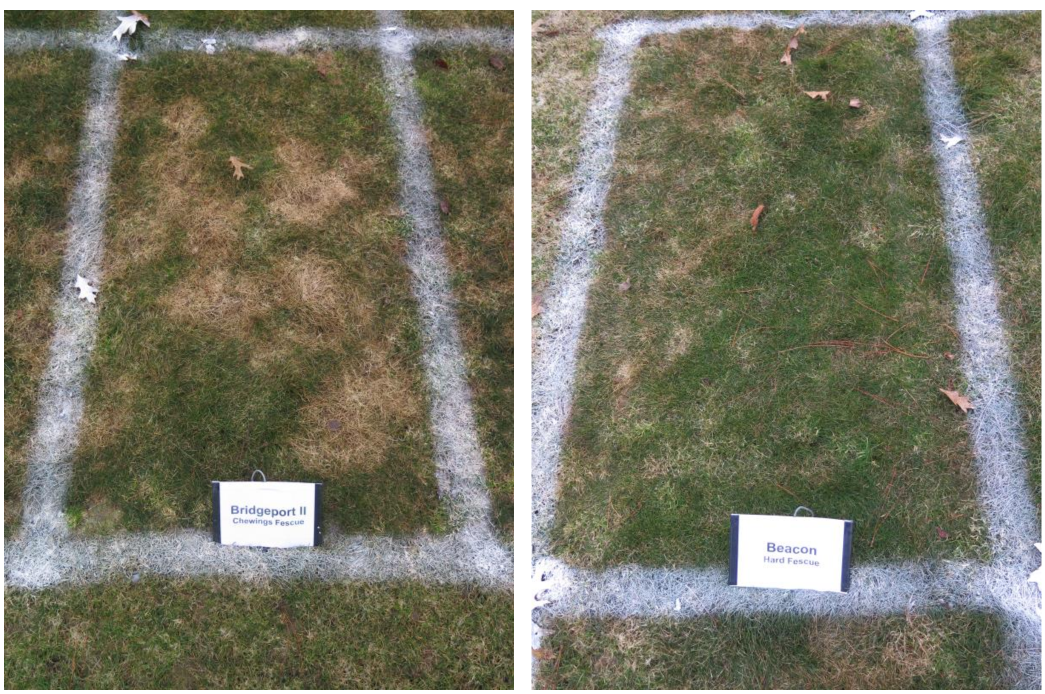



Figure 3. Differences in T. ishikariensis severity on Bridgeport II (Chewings) and Beacon (Hard) fine fescue on March 30th, 2016 at Timber Ridge GC in Minocqua, WI.

2014-02-491

**The evaluation of novel hybrid bluegrass in northwest Oklahoma as low-input turf**

**Objectives:**

1. Obtain second year performance data on hybrids derived from Texas x Kentucky crosses that were seeded in late September 2014 at USDA-ARS in Woodward Oklahoma.

2. Determine seed yield and viability from seeds harvested in 2015 & 16.

As water use restrictions continue to increase, there is a need for turf-grass with improved performance under hot and dry conditions. Seed producing hybrids derived from crosses between Texas and Kentucky bluegrass were selected in an effort to develop low-input turf with these traits. After establishment, turf quality ratings in the low input trial on 6-17-15 (Table 1; Fig 1) indicated hybrids #57 and #67 contained turf quality ratings 6.3 and 6.0 respectively and were not significantly different from the Kentucky or hybrid checks. From 6-17-15 to 8-25-15 weather conditions became much hotter, drier, and windier. Three consecutive days in early August (8-6 to 8-8) were particularly stressful with daily highs ranging from 100 - 104°, constant wind speeds 20 – 30 mph, and no rain. Hybrids #67, TK24 SPS, and TK43xTrenton-35:24 were not significantly different from SolarGreen (Table 1; Fig 1) (rating = 4.5). Most of the other hybrids or checks either entered dormancy or started to die during this period. By mid-November 2015, in the low input trial, many of the hybrids and Kentucky checks did not persist through the summer. Hybrid #67 continued to appear to contain greater heat/drought tolerance than most of the other hybrids and Kentucky checks. The gusty sandblasting conditions created erosion that made mowing difficult. By late May 2016, hybrid #67 contained the highest turf quality rating of all the experimental hybrids (Fig 2). In the second year as conditions became hotter and drier, pure Texas bluegrass D4 began to display the ability to remain green and productive longer than many of the other entries (Fig 2). In late June the ability to irrigate the medium trial was lost due to a technical problem. The hot, dry and windy conditions gradually caused the majority of the entries in both the low and medium input trials to turn brown and eventually die (Fig 2). Seed yield varied for harvests in 2015 and 16 (Fig 3). Some of the entries including #57 severely declined from 2015 to 2016 resulting in a reduced seed yield. Seeds from 2015&16 harvests and additional hybrids are currently being re-evaluated at Woodward in 2x2 plots that were seeded on 10-19-16 (Fig 3). Ten hybrids that germinated fair to good in two weeks are scheduled to be tested in larger plots in Stillwater OK (OSU).

**Summary:**

>After two years of evaluation a limited number of the hybrids appeared to have greater heat tolerance than the Kentucky checks.

>Seed production varied by hybrid and harvest year.

>Based on seed production and germination, six hybrids in the current trial and four recently identified hybrids are in the process or scheduled for further evaluation at Woodward USDA-ARS and at Stillwater (OSU).

Table 1. Characteristics and performance of hybrids and checks in the low and medium input seeded turf trial

| ID Medium | | 11-20-14 | 2-13-15 | 4-29-15 | 6-17-15 | 8-25-15 | 9-17-15 | 11-4-15 | 3-1-16 | 4-4-16 | 5-25-16 | 7-15-16 | 8-29-16 | 9-27-16 | 10-9-15 | 4-29-15 | 10-9-15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Seeded 9-24-14*** | N | Germ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | Spread (cm) | Density | Texture | Color |
| #125 Tkiso | 3 | 7.0 a | 7.3 a | 5.0 bc | 5.0 def | 3.0 bcdef | 4.0 bcdef | 4.3 abc | 4.3 abc | 3.7 bcd | 4.7 bcd | 3.7 abcd | 3.0 abcd | 61.7 abcd | 5.0 abcde | 4.0 cde | 5.3 abcd |
| #21 D4-10xPoland | 3 | 5.3 abc | 6.3 abc | 5.0 bc | 4.0 fg | 1.0 f | 1.3 gh | 1.7 de | 2.3 de | 2.0 efg | 3.0 def | 2.0 def | 1.7 de | 30.0 ef | 3.0 ef | 6.0 a | 4.7 cd |
| #50 TK43xTrenton | 3 | 4.3 bcde | 3.7 de | 3.3 d | 3.3 gh | 1.3 ef | 2.0 fgh | 1.7 de | 1.7 de | 1.7 fg | 2.3 ef | 1.7 ef | 1.7 de | 30.0 ef | 3.0 ef | 3.0 efg | 5.5 abcd |
| #56 WL63xRussian | 3 | 2.0 efg | 2.0 e | 1.7 e | 2.3 h | 1.0 f | 1.0 h | 1.0 e | 1.3 e | 1.3 g | 2.0 f | 1.0 f | 0.7 e | 15.0 f | 1.3 f | 4.0 cde | 5.0 bcd |
| #57 TK43xTrenton | 3 | 2.3 defg | 2.0 e | 6.3 ab | 6.3 abc | 3.3 abcde | 5.0 abcde | 5.0 abc | 3.3 bcd | 4.3 bc | 4.0 cde | 2.0 def | 2.0 cde | 38.7 cdef | 6.0 abc | 4.3 bcd | 4.7 cd |
| #67 TK24xHuntsville | 3 | 3.0 cdefg | 4.0 cde | 5.0 bc | 5.3 cde | 3.3 abcde | 3.3 defg | 3.3 bcde | 2.0 de | 2.0 efg | 3.0 def | 2.0 def | 1.7 de | 33.3 ef | 4.0 cde | 3.3 def | 6.7 a |
| #71 TK24xHuntsville | 2 | 4.0 bcdef | 4.5 bcde | 3.0 de | 3.5 gh | 2.5 cdef | 3.5 cdef | 3.0 cde | 2.5 de | 3.0 cdef | 4.0 cde | 2.5 cdef | 1.5 de | 28.0 ef | 4.5 bcde | 4.0 cde | 6.5 ab |
| #87 WL63xRussian | 2 | 1.5 fg | 2.0 e | 3.0 de | 4.5 efg | 3.5 abcd | 4.0 bcdef | 4.5 abc | 3.0 cde | 2.0 efg | 4.0 cde | 2.0 def | 2.0 cde | 35.0 ef | 6.0 abc | 3.5 def | 6.0 abc |
| (TK43xTrenton)xRussian | 3 | 2.7 defg | 2.7 e | 3.0 de | 4.0 fg | 4.0 abcd | 4.7 abcde | 3.3 bcde | 5.3 a | 4.0 bc | 4.0 cde | 3.7 abcd | 4.0 ab | 66.7 ab | 5.3 abcd | 3.7 def | 6.0 abc |
| TK24 SPS | 2 | 1.0 g | 2.0 e | 3.0 de | 4.0 fg | 5.0 ab | 4.0 bcdef | 4.0 abcd | 6.0 a | 4.5 bc | 4.5 bcd | 2.5 cdef | 2.0 cde | 37.5 def | 7.0 a | 2.5 fg | 4.0 d |
| TK43xTrenton WT35:24 | 2 | 2.5 defg | 3.0 e | 4.0 cd | 4.5 efg | 4.5 abc | 5.5 abc | 4.5 abc | 4.5 abc | 4.0 bc | 4.5 bcd | 4.5 a | 4.5 a | 65.0 abc | 6.5 ab | 3.0 efg | 5.0 bcd |
| Texas-D4 | 3 | 2.3 defg | 2.7 e | 2.7 de | 3.3 gh | 3.0 bcdef | 4.0 bcdef | 4.7 abc | 6.0 a | 6.7 a | 5.0 abc | 4.0 abc | 4.3 a | 66.7 ab | 5.7 abc | 3.3 def | 4.0 d |
| Absolute | 3 | 4.3 bcde | 3.7 de | 5.7 ab | 6.0 abcd | 4.3 abc | 4.3 abcde | 4.7 abc | 3.3 bcd | 4.0 bc | 5.0 abc | 3.0 abcde | 2.0 cde | 33.3 ef | 6.3 ab | 5.3 ab | 6.7 a |
| Bandera | 3 | 4.7 abcd | 5.7 abcd | 6.0 ab | 5.0 def | 2.0 def | 3.0 efgh | 2.7 cde | 2.0 de | 2.3 defg | 4.0 cde | 2.0 def | 1.7 de | 13.3 f | 3.3 def | 4.0 cde | 4.7 cd |
| Midnight | 3 | 6.3 ab | 5.7 abcd | 6.3 ab | 7.0 a | 4.7 ab | 5.7 ab | 6.3 a | 5.0 ab | 4.7 b | 6.7 a | 2.7 bcdef | 2.0 cde | 43.3 bcde | 6.7 ab | 5.3 ab | 6.7 a |
| SolarGreen | 3 | 6.0 ab | 6.7 ab | 6.7 a | 6.7 ab | 5.3 a | 6.3 a | 5.7 ab | 4.7 abc | 4.7 b | 6.0 ab | 4.7 a | 3.7 abc | 70.0 a | 7.0 a | 2.0 g | 5.0 bcd |
| ThermalBlue | 3 | 6.3 ab | 5.7 abcd | 6.3 ab | 5.7 bcde | 4.0 abcd | 5.3 abcd | 4.3 abc | 4.3 abc | 4.0 bc | 5.7 abc | 4.3 ab | 3.7 abc | 70.0 a | 6.0 abc | 2.7 fg | 5.0 bcd |
| Tsunami | 2 | 4.5 abcde | 4.0 cde | 6.5 a | 6.5 abc | 4.0 abcd | 4.5 abcde | 5.0 abc | 3.0 cde | 3.5 bcde | 6.0 ab | 3.0 abcde | 2.5 bcd | 50.0 abcde | 6.0 abc | 5.0 abc | 6.5 ab |

| ID Low | | 11-20-14 | 2-13-15 | 4-29-15 | 6-17-15 | 8-25-15 | 9-17-15 | 11-4-15 | 3-1-16 | 4-4-16 | 5-25-16 | 7-15-16 | 8-29-16 | 9-27-16 | 10-9-15 | 4-29-15 | 10-9-15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Seeded 9-25-14*** | N | Germ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | TQ | Spread (cm) | Density | Texture | Color |
| #125 Tkiso | 3 | 9.0 a | 8.7 a | 5.7 abcd | 4.0 cd | 1.7 ef | 2.0 cde | 1.3 cde | 2.7 de | 2.7 defg | 3.3 efg | 2.7 def | 2.0 de | 36.7 cde | 2.7 def | 6.0 bc | 4.3 de |
| #21 D4-10xPoland | 3 | 6.3 cde | 7.3 abc | 4.7 cdef | 3.7 d | 1.3 f | 1.0 e | 1.0 de | 1.0 f | 1.3 fg | 2.3 fg | 1.7 efg | 1.0 efg | 20.0 efg | 1.3 f | 7.3 a | 5.0 bcde |
| #50 TK43xTrenton | 3 | 6.7 bcde | 6.3 abcd | 3.0 g | 4.0 cd | 1.0 f | 1.0 e | 0.7 e | 0.7 f | 1.0 g | 2.0 g | 0.7 g | 0.0 g | 10.0 fg | 1.0 f | 3.7 fgh | 4.7 cde |
| #56 WL63xRussian | 2 | 5.5 def | 6.5 abcd | 3.5 fg | 4.0 cd | 1.5 ef | 2.0 cde | 1.0 de | 1.0 f | 2.0 efg | 3.0 efg | 2.0 defg | 1.5 def | 27.5 def | 2.0 ef | 3.0 hi | 5.0 bcde |
| #57 TK43xTrenton | 3 | 3.7 fg | 4.7 defg | 6.0 abc | 6.3 a | 1.0 f | 1.0 e | 1.0 de | 0.7 f | 1.0 g | 2.3 fg | 1.0 g | 1.3 def | 16.7 efg | 1.0 f | 5.0 cde | 4.0 e |
| #67 TK24xHuntsville | 3 | 7.7 abc | 7.3 abc | 6.3 ab | 6.0 a | 4.0 abc | 3.7 abc | 2.3 abcd | 3.7 cd | 4.3 bcd | 5.3 abcd | 3.0 de | 2.3 cd | 46.7 bcd | 4.3 bcd | 4.3 efg | 6.7 a |
| #71 TK24xHuntsville | 2 | 5.5 def | 6.0 bcde | 4.5 def | 4.0 cd | 2.0 def | 2.5 cde | 1.5 cde | 2.0 ef | 2.0 efg | 3.5 defg | 2.5 def | 2.0 de | 28.5 def | 3.0 cdef | 3.5 gh | 6.0 abc |
| #87 WL63xRussian | 2 | 2.0 g | 2.5 gh | 3.0 g | 3.5 d | 2.0 def | 2.0 cde | 1.0 de | 0.5 f | 1.0 g | 2.0 g | 1.5 fg | 1.0 efg | 15.0 efg | 3.0 cdef | 3.5 gh | 4.5 cde |
| (TK43xTrenton)xRussian | 3 | 5.0 ef | 5.0 cdef | 5.0 bcde | 4.3 cd | 3.7 abc | 4.3 ab | 3.3 ab | 5.0 abc | 3.3 cde | 4.3 bcde | 3.3 cd | 3.7 b | 55.0 abc | 5.3 ab | 4.0 efgh | 5.7 abcd |
| TK24 SPS | 3 | 1.7 g | 2.0 h | 4.3 defg | 4.7 bc | 4.7 a | 5.0 a | 2.7 abc | 4.3 bc | 5.0 abc | 4.3 bcde | 1.7 efg | 2.0 de | 30.0 def | 6.7 a | 2.3 i | 6.3 ab |
| TK43xTrenton WT35:24 | 2 | 2.0 g | 3.0 fgh | 3.5 fg | 4.0 cd | 4.0 abc | 5.0 a | 2.5 abc | 5.5 ab | 4.0 bcd | 4.0 cdef | 4.5 bc | 4.5 b | 62.5 ab | 5.0 abc | 4.0 efgh | 6.0 abc |
| Texas-D4 | 3 | 3.7 fg | 3.7 efgh | 4.0 efg | 4.0 cd | 3.3 abcd | 5.3 a | 3.7 a | 6.0 a | 6.3 a | 6.0 ab | 5.0 b | 6.0 a | 71.7 a | 5.7 ab | 3.3 ghi | 5.3 abcde |
| Absolute | 3 | 8.0 abc | 8.3 ab | 7.0 a | 5.7 a | 1.0 f | 1.3 de | 1.0 de | 1.3 ef | 2.7 defg | 3.3 efg | 1.7 efg | 0.3 fg | 13.3 fg | 1.3 f | 4.7 def | 5.0 bcde |
| Bandera | 3 | 7.3 abcd | 6.3 abcd | 6.7 a | 6.0 a | 1.0 f | 1.0 e | 1.0 de | 1.0 f | 1.0 g | 2.7 efg | 1.0 g | 0.3 fg | 0.0 g | 1.0 f | 3.7 fgh | 4.3 de |
| Midnight | 3 | 7.0 abcde | 5.0 cdef | 6.3 ab | 6.0 a | 1.3 f | 1.0 e | 0.7 e | 0.7 f | 2.0 efg | 2.7 efg | 1.3 fg | 0.0 g | 13.3 fg | 1.0 f | 5.5 bcd | 5.0 bcde |
| SolarGreen | 2 | 8.5 ab | 8.5 a | 7.0 a | 6.0 a | 4.5 ab | 5.0 a | 3.5 a | 5.5 ab | 5.5 ab | 7.0 a | 6.5 a | 3.5 bc | 65.0 ab | 5.5 abc | 3.0 hi | 5.0 bcde |
| ThermalBlue | 3 | 8.7 ab | 7.0 abcd | 7.0 a | 6.0 a | 3.0 bcde | 3.0 bcd | 2.0 bcde | 4.0 bcd | 4.3 bcd | 5.7 abc | 5.0 b | 4.7 b | 60.0 ab | 4.0 bcde | 3.7 fgh | 5.0 bcde |
| Tsunami | 2 | 7.0 abcde | 6.5 abcd | 6.0 abc | 5.5 ab | 2.5 cdef | 2.0 cde | 1.5 cde | 2.0 ef | 3.0 def | 5.5 abc | 2.5 def | 1.5 def | 32.5 cdef | 3.0 cdef | 6.5 ab | 5.5 abcd |

Medium-input = 2.5” mowing height; 2 lb N/1000 $ft^2$ Spring and Fall; irrigation to prevent stress or dormancy.

Low-input = 3.5” mowing height; 0.75 lb N/1000 $ft^2$/ Spring & Fall; no irrigation after establishment.

Germ 1-9 (9 best); TQ=Turf-Quality 0-9 (9 best, 0 dead); Density 1-9 (9 most dense); Texture 1-9 (9 fine); Color 1-9 (9 dark). Means within the same column within a treatment (low or med) containing the same letter are not significantly different (α =0.05).

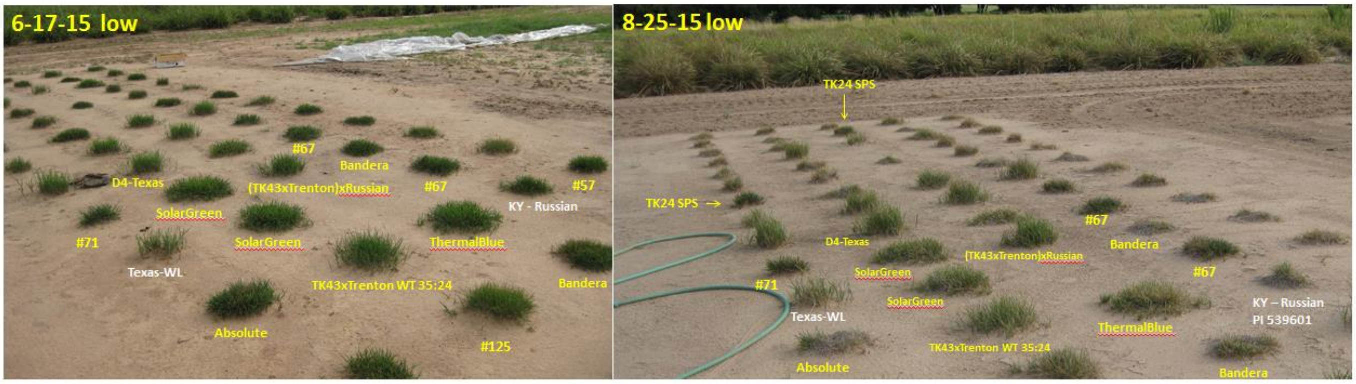



Figure 1. The low input trial photographed on 6-17-15 and in late August when many of the entries started to decline in quality.

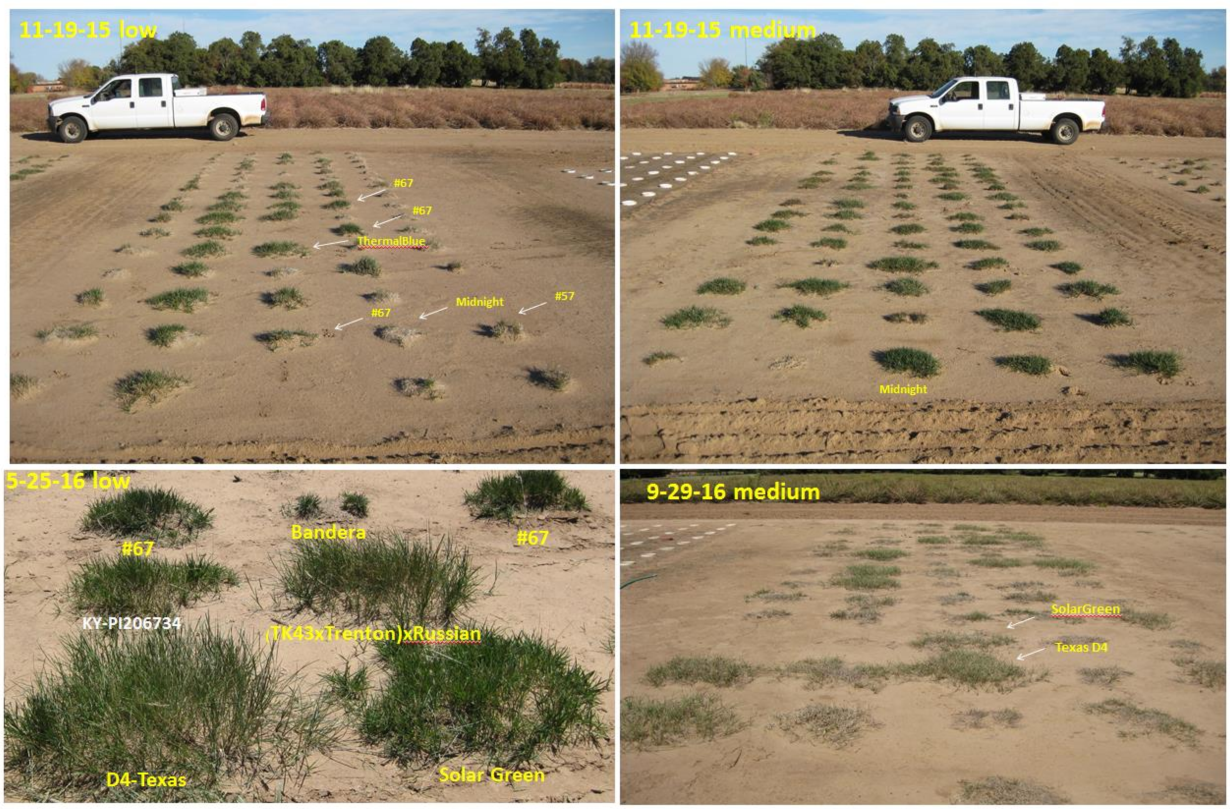
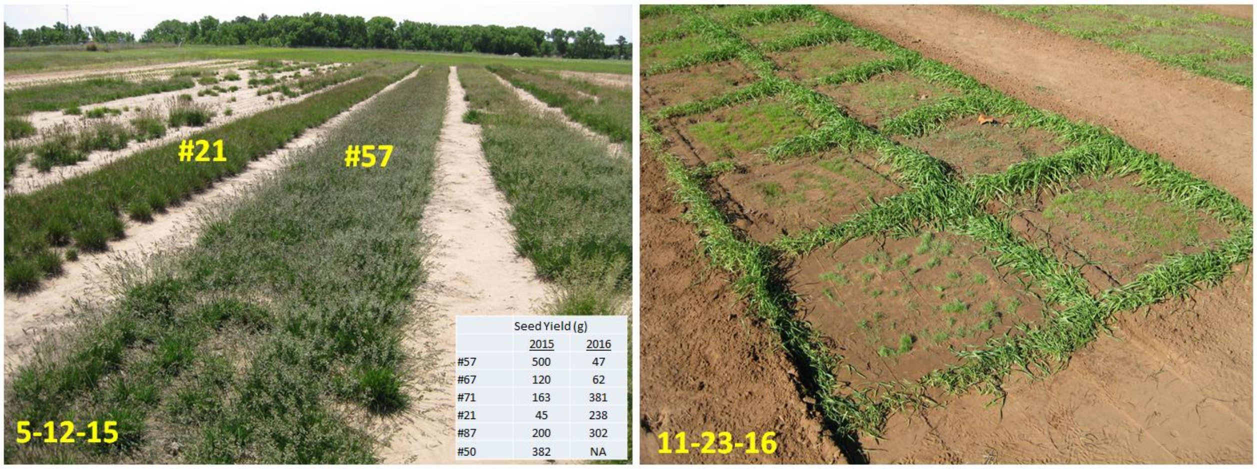



Figure 2. By mid-November 2015, in the low input trial, many of the hybrids and Kentucky checks did not persist through the summer (Top left). In the medium input trial, the addition of irrigation enabled many of the Kentucky checks and hybrids to persist as seen with the difference between Kentucky check Midnight (Top right).




| | Seed Yield (g) | |
|---|---|---|
| | 2015 | 2016 |
| #57 | 500 | 47 |
| #67 | 120 | 62 |
| #71 | 163 | 381 |
| #21 | 45 | 238 |
| #87 | 200 | 302 |
| #50 | 382 | NA |

Figure 3. A portion of the seed increase nursery and seed yields obtained in 2015 & 16 and a newly seeded (10-19-16) trial (2 x 2) plots at Woodward ARS.

# Evaluation of Crown Membrane Health and Gas Accumulation in Response to Ice Stress and Management Practices of Creeping Bentgrass and Poa Annua

Emily Merewitz and Kevin Frank
Michigan State University

Start date: 2015
Project duration 2 years
Total funding: $20,000

**Objectives:**
1. *Investigate whether crown membrane fatty acid ratios and composition may correlate to toxic gas accumulation and are they differentially accumulated between creeping bentgrass and poa annua under ice cover stress*
2. *Evaluate how lipid profiles and FFA change over a time course of ice cover*
3. *Use a simulated ice cover experiment to determine whether membrane health changes due to incubation of turf with specific ice cover*
4. *Evaluate whether chemical treatments commonly used in the turf industry reduce turf loss due to ice cover, particularly related to membrane disruption or FFA accumulation*

Creeping bentgrass and annual bluegrass are two important putting green species that are sensitive to ice cover damage. Creeping bentgrass is typically more tolerant to ice stress than annual bluegrass. A major cause of damage under prolonged ice cover is the accumulation of toxic gases and damage to grass crown tissue. Several management practices have been reported to improve turf survival of winter, but have not been investigated in controlled studies. This project aimed to determine whether commonly used plant growth regulators (PGRs) and an oil based product, Civitas, have an effect on turf survival of ice cover and is that survival related to membrane or crown health.

Separate creeping bentgrass and annual bluegrass fields were maintained at the Hancock Turfgrass Research Center at Michigan State University. Plots of both species were treated in late summer through fall of 2014 and 2015 every two weeks with: Civitas, mefluidide, propiconazole, or trinexapac ethyl at label recommended rates. Turfgrass plots underwent natural acclimation to cold conditions in fall of 2014 and 2015. Turfgrass plugs were then taken on 11 Nov. 2014 and 25 Nov. 2015 from each plot, planted in 4 inch plastic pots in native soil, and then transferred to an environmentally controlled low temperature growth chamber (-4$^{o}$C) where they underwent 1) no ice or 2) ice cover (0.5” thick) treatments. Turfgrass plugs were taken out of the low temperature growth chamber at 0, 20, 40, and 60 days after temperature treatments. Plants were then destructively sampled by cutting the plants in half. Half of the plant went to gas chromatography mass spectroscopy (GC/MS) for analysis of free fatty acids while the other half went towards a percent regrowth assay in a greenhouse.

In 2016, ice covered plugs treated with Civitas had the greatest regrowth after 40 days in the low temperature growth chamber. Plugs that were treated with mefluidide, propiconazole, or not treated had the same amount of regrowth after 40 days in the low temperature growth chamber. Trinexapac-ethyl treated plugs had less regrowth than untreated plugs. After 60 days in the growth chamber, civitas and mefluidide treated plugs had greater regrowth than the untreated plugs, while trinexapac-ethyl treated plugs had less regrowth than the untreated control (Figure 2). Similar results were found in 2015 on all sampling dates. Civitas, mefulidide, and propiconazole treated plugs had greater regrowth than trinexapac-ethyl and untreated plugs.

After 60 days in the low temperature growth chamber under non-ice covered conditions, plugs treated with mefluidide or propiconazole had greater regrowth when compared to the untreated plugs. No differences were detected between non-ice covered treatments after the 20 and 40 day sampling times. In 2015, Civitas had greater regrowth than untreated plugs on all sampling days while mefluidide had more regrowth than the control after 20 and 60 days and propiconazole had greater regrowth after 20 and 40 days in the low temperature growth chamber (Figure 3).

For both years, at 20 (Table 1), 40 (Table 2) and 60 days (Table 3) under ice cover, the majority of the fatty acids detected within annual bluegrass were linolenic acid, linoleic acid, oleic acid, stearic acid, palmitoleic acid, and palmitic acid. Plants that were treated with mefluidide, propiconazole, and Civitas have a greater percentage of polyunsaturated fatty acids, with linoleic acid being the most increased due to these chemical treatments, compared with trinexapac-ethyl and untreated samples (Table 1). The unsaturated fatty acid linoleic acid is a precursor to the plant hormone jasmonic acid, a hormone involved in stress responses and the induced systemic resistance pathway. Further evaluation of these profiles is needed and is being performed.

Summary Points

- Annual bluegrass regrowth after simulated ice cover in a growth chamber was significantly affected by plant growth regulator or Civitas treatments.
- Enhanced survival of annual bluegrass after treatment with plant growth regulators or Civitas could be related to shifts in fatty acid accumulation.
- Trinexapac ethyl treated plugs had less survival than the untreated plugs under ice cover on two sampling dates.

Figure 1 – Annual bluegrass plants treated with Civitas, mefluidide, propiconazole, trinexapac-ethyl, or untreated under ice (0.5” thick) in a low temperature growth chamber (-4°C)

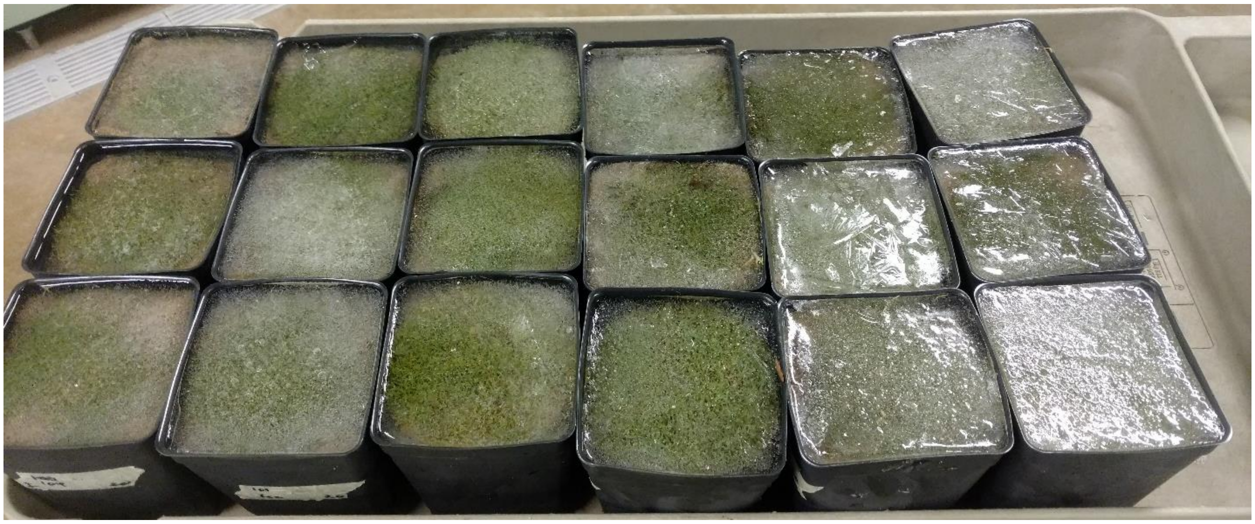


Figure 2 – Regrowth (%) of annual bluegrass plugs maintained under ice cover in 2016 (A) and 2015 (B) a low temperature growth chamber (-4 °C) that were treated with different plant growth regulating compounds. Different letters indicate statistically significant differences within a sampling day (P ≤ 0.05). NS represents sampling days with no significance (P≤ 0.05).

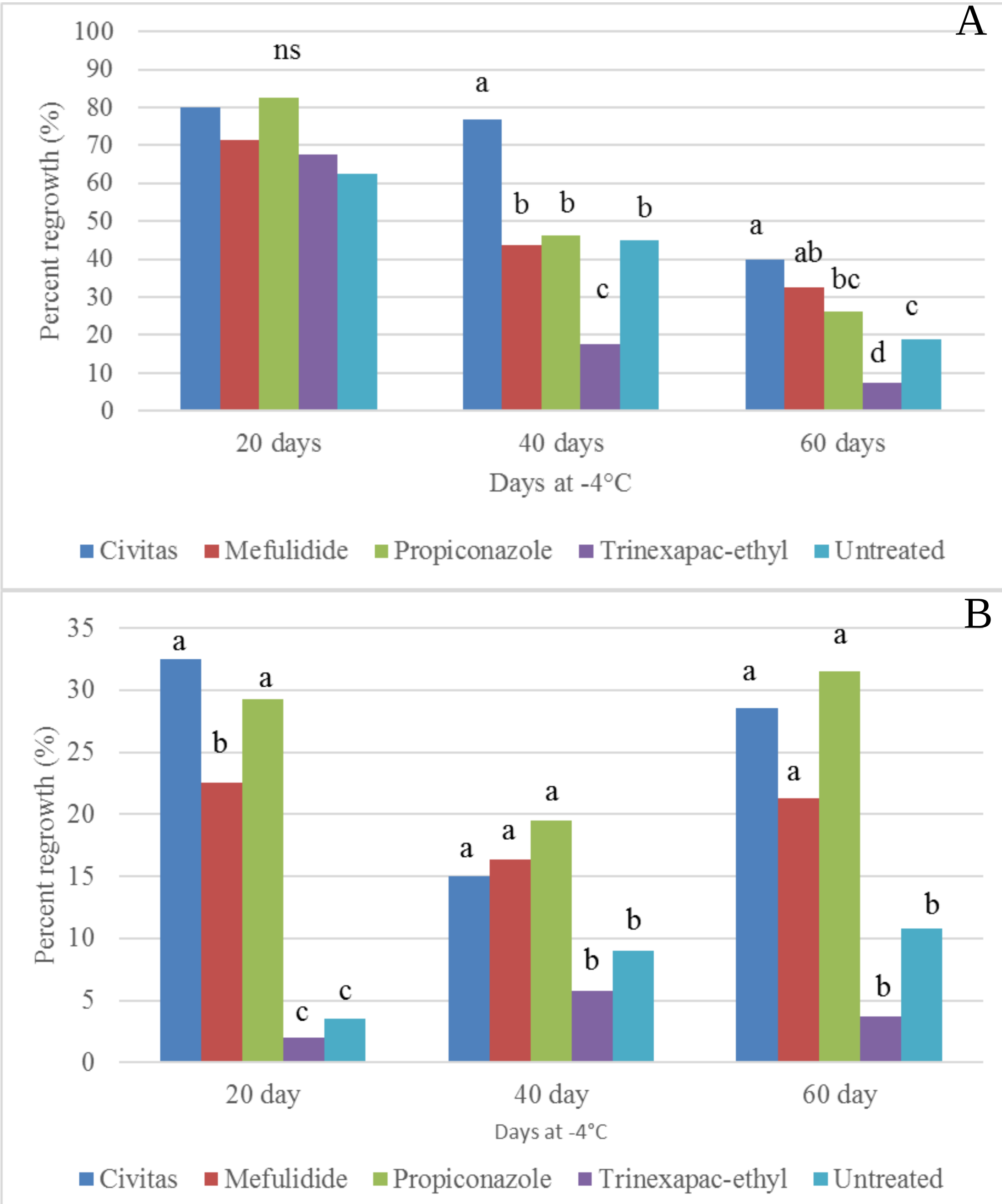


Figure 3. – Regrowth (%) of annual bluegrass plugs maintained with no ice cover in 2016 (A) and 2015 (B) in a low temperature growth chamber (-4 °C) that were treated with different plant growth regulating compounds. Different letters indicate statistically significant differences within a sampling day (P ≤ 0.05). NS represents sampling days with no significance (P≤ 0.05).

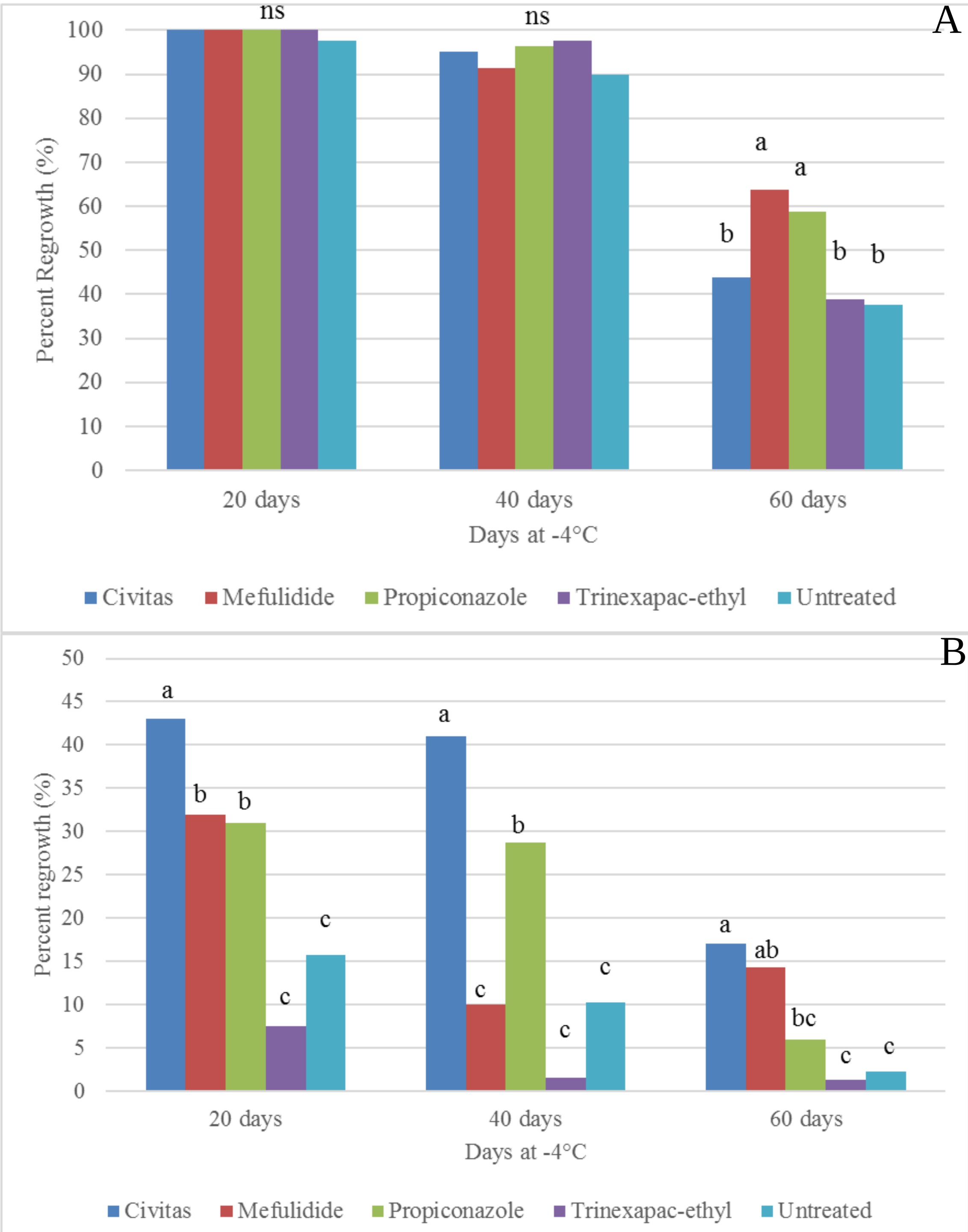


Table 1. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 20 days of ice coverin 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column (P ≤ 0.05).

| | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fatty Acids | | | | | | Fatty Acids | | | | | |
| | Molar percentage (mol %) | | | | | | Molar percentage (mol %) | | | | | |
| | saturated | | unsaturated | | | | saturated | | unsaturated | | | |
| | 16:0 | 18:0 | 16:1 | 18:1 | 18:2 | 18:3 | 16:0 | 18:0 | 16:1 | 18:1 | 18:2 | 18:3 |
| | Palmitic acid | Stearic acid | Palmitoleic acid | Oleic acid | Linoleic acid | Linolenic acid | Palmitic acid | Stearic acid | Palmitoleic acid | Oleic acid | Linoleic acid | Linolenic acid |
| Civitas | 30.5 bc | 29.1 b | 6.6 ab | 6.7a | 13.5 b | 12.9 ab | 29.27 bc | 28.4 b | 6.65 a | 8.45 a | 14.05 b | 12.35 ab |
| Propiconazole | 26.9 c | 26.2 b | 4.7 c | 5.3a | 21.6 a | 15.3 a | 28.87 bc | 26 b | 6.3 a | 6.77 ab | 18.6 a | 11.25 abc |
| Mefluidide | 29.3 c | 26.2 b | 6.3 ab | 7.1a | 19.0 a | 11.7 ab | 25.32 c | 26.2 b | 4.7 c | 6.57 bc | 20.42 a | 15.17 a |
| Trinexapac-ethyl | 33.4 ab | 35.4 a | 7.2 a | 5.8a | 9.0 bc | 9.3 b | 33.25 ab | 35.5 a | 6.2 ab | 4.85 c | 8.65 c | 8.7 bc |
| Untreated | 35.0 a | 37.0 a | 5.5 bc | 5.6a | 8.0 c | 8.1 b | 34.52 a | 37.47 a | 4.97 bc | 6.5 bc | 7.95 c | 6.87 c |
| LSD | 3.83 | 4.73 | 1.33 | 2.22 | 4.83 | 5.73 | 4.49 | 4.01 | 1.25 | 1.84 | 3.44 | 5.02 |

Table 2. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 40 days of ice cover in 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column ($P \leq 0.05$).

| | **2015** | | | | | | **2016** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fatty Acids** | | | | | | **Fatty Acids** | | | | | |
| | **Molar percentage (mol %)** | | | | | | **Molar percentage (mol %)** | | | | | |
| | **saturated** | | **unsaturated** | | | | **saturated** | | **unsaturated** | | | |
| | **16:0** | **18:0** | **16:1** | **18:1** | **18:2** | **18:3** | **16:0** | **18:0** | **16:1** | **18:1** | **18:2** | **18:3** |
| | **Palmitic acid** | **Stearic acid** | **Palmitoleic acid** | **Oleic acid** | **Linoleic acid** | **Linolenic acid** | **Palmitic acid** | **Stearic acid** | **Palmitoleic acid** | **Oleic acid** | **Linoleic acid** | **Linolenic acid** |
| Civitas | 29.25 bc | 28.83 b | 6.28 a | 7.08 | 12.93 bc | 13.43 ab | 27.63 b | 26.7 b | 6.60 | 7.85 a | 12.9 b | 16.1 a |
| Propiconazole | 27.38 bc | 25.9 b | 6.65 a | 6.33 | 19.30 a | 10.33 ab | 25.68 b | 26.48 b | 4.98 | 5.13 b | 23.53 a | 10.45 b |
| Mefluidide | 24.25 c | 26.18 b | 4.15 b | 5.35 | 22.03 a | 15.93 a | 26.05 b | 26.8 b | 4.78 | 5.85 b | 24.28 a | 9.78 b |
| Trinexapac-ethyl | 31.98 ab | 36.43 a | 7.05 a | 6.08 | 9.33 bc | 9.65 b | 33.35 a | 36.98 a | 5.83 | 5.43 b | 8.4 c | 9.43 b |
| Untreated | 34.88 a | 36.90 a | 4.78 b | 5.73 | 7.63 c | 8.15 b | 33.15 a | 29.05 b | 5.28 | 5.9 b | 15.5 b | 9.63 b |
| LSD | 5.53 | 4.43 | 1.14 | NS | 4.79 | 6.00 | 3.35 | 3.02 | NS | 1.62 | 3.35 | 2.39 |

Table 3. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 60 days of ice cover in 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column ($P \leq 0.05$).

| | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fatty Acids | | | | | | Fatty Acids | | | | | |
| | Molar percentage (mol %) | | | | | | Molar percentage (mol %) | | | | | |
| | saturated | | unsaturated | | | | saturated | | unsaturated | | | |
| | 16:0 | 18:0 | 16:1 | 18:1 | 18:2 | 18:3 | 16:0 | 18:0 | 16:1 | 18:1 | 18:2 | 18:3 |
| | Palmitic acid | Stearic acid | Palmitoleic acid | Oleic acid | Linoleic acid | Linolenic acid | Palmitic acid | Stearic acid | Palmitoleic acid | Oleic acid | Linoleic acid | Linolenic acid |
| Civitas | 26.95 c | 29.03 b | 6.08 a | 6.93 a | 13.53 bc | 13.38 a | 26.63 b | 27.55 b | 6.10 | 6.75 | 17 b | 13.85 a |
| Propiconazole | 26.95 c | 26.25 b | 4.00 b | 4.40 b | 17.25 ab | 10.60 ab | 28.5 b | 25.18 b | 6.50 | 6.20 | 24.03 ab | 10.53 ab |
| Mefluidide | 29.58 bc | 25.88 b | 6.60 a | 6.60 ab | 22.00 a | 10.25 ab | 27.75 b | 26.48 b | 5.20 | 4.78 | 21.2 a | 12.35 bc |
| Trinexapac-ethyl | 34.18 a | 35.43 a | 6.33 a | 5.90 ab | 9.73 cd | 9.18 b | 35.03 a | 36.8 a | 4.93 | 5.65 | 8.8 c | 6.3 d |
| Untreated | 32.63 ab | 36.38 a | 4.33 b | 5.73 ab | 7.13 d | 7.08 b | 34.3 a | 35.38 a | 4.95 | 7.08 | 9.13 c | 8.65 cd |
| LSD | 4.22 | 5.28 | 1.26 | 2.25 | 5.79 | 3.90 | 3.49 | 1.38 | NS | NS | 4.61 | 3.18 |

Understanding endophyte-mediated dollar spot resistance in red fescue as a new approach to improving management of dollar spot in creeping bentgrass

Zipeng Tian and Faith C. Belanger

Dollar spot is one of the most problematic diseases for many golf courses, particularly on creeping bentgrass, often requiring repeated applications of fungicides. In addition to creeping bentgrass, dollar spot can also be a problem on strong creeping red fescue. However, when strong creeping red fescue is infected with the symbiotic fungal endophyte *Epichloë festucae*, the plants exhibit resistance to dollar spot (Clarke et al., 2006). How infection by the endophytic fungus confers disease resistance to the host red fescue is not known. Resistance to fungal pathogens is not an established effect of endophyte infection of other grass species, and may therefore be unique to the fine fescues.

We are characterizing an *E. festucae* antifungal protein that we first identified through a large scale transcriptome study comparing endophyte-free and endophyte-infected red fescue plants, with the goal of identifying plant or fungal genes that may be involved in the observed disease resistance (Ambrose and Belanger, 2012). The *E. festucae* antifungal protein is a secreted protein and is highly expressed in the infected plant tissues. Most *Epichloë* species do not have a gene for a similar antifungal protein. These features make it a good candidate for involvement in the unique resistance to fungal pathogens observed in endophyte-infected red fescue. Understanding the mechanism behind the endophyte-mediated disease resistance in the fine fescues may lead to new approaches for dollar spot management in other grass species, such as creeping bentgrass. The objective of this project is therefore to characterize the endophyte antifungal protein and determine if it does play a role in the disease resistance.

We partially purified the endophyte antifungal protein from infected plant tissue and confirmed that it did have activity against the dollar spot fungus in a plate assay. However, it is difficult to obtain enough of the protein directly from the infected plants to test its activity on a larger scale. We therefore have expressed the protein in the yeast *Pichia pastoris* to generate larger amounts of the protein. *P. pastoris* has become a highly successful system for the expression of secreted proteins (Ahmad et al., 2014). The *E. festucae* antifungal protein was expressed in *P. pastoris* and proteins from the culture filtrate were analyzed on an SDS polyacrylamide gel (Fig. 1). A band at the expected size was the major protein. Sequence analysis of the protein band indicated it was indeed the antifungal protein. The antifungal protein was partially purified from the yeast culture filtrate and found to have activity against the dollar spot fungus in several different assays. The results from one assay are shown in Fig. 2.

In summary, the results we have obtained from the *E. festucae* antifungal protein support the hypothesis that it may be a component of the disease resistance seen in endophyte-infected strong creeping red fescue.

References

Ahmad, M., Hirz, M., Pichler, H., Schwab, H. (2014) Protein expression in *Pichia pastoris*: recent achievements and perspectives for heterologous protein production. App. Microbiol. Biotechnol. 98:5301-5317

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

**Bullet Points**

1. The fungal endophyte *Epichloë festucae* produces an abundant antifungal protein that is not found in most *Epichloë* species.

2. We have expressed the *E. festucae* antifungal protein in yeast and partially purified it from the culture filtrate.

3. The partially purified *E. festucae* protein had antifungal activity against the dollar spot fungus. It may therefore be a component of the well-established unique endophyte-mediated disease resistance seen in strong creeping red fescue.

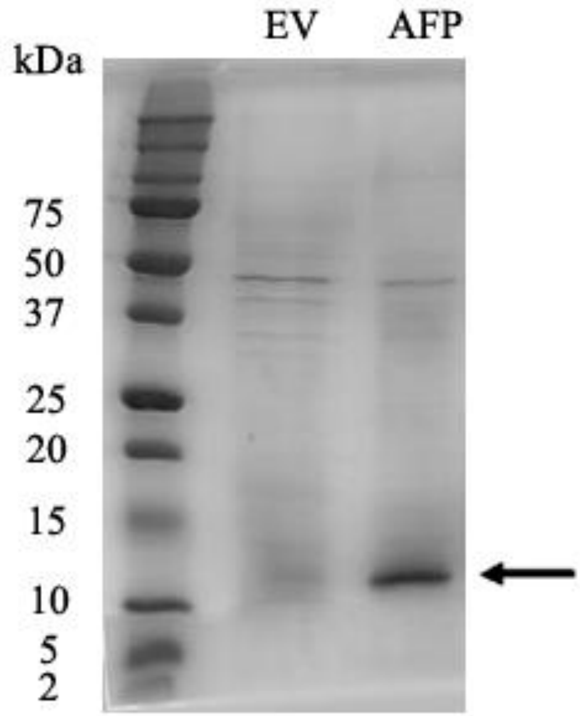



Fig. 1. SDS-polyacrylamide gel of proteins from the culture filtrate of the yeast *P. pastoris* transformed with the empty vector (EV) or with the vector containing the antifungal protein coding sequence (AFP). The arrow indicates the band confirmed by sequence analysis as being the antifungal protein.

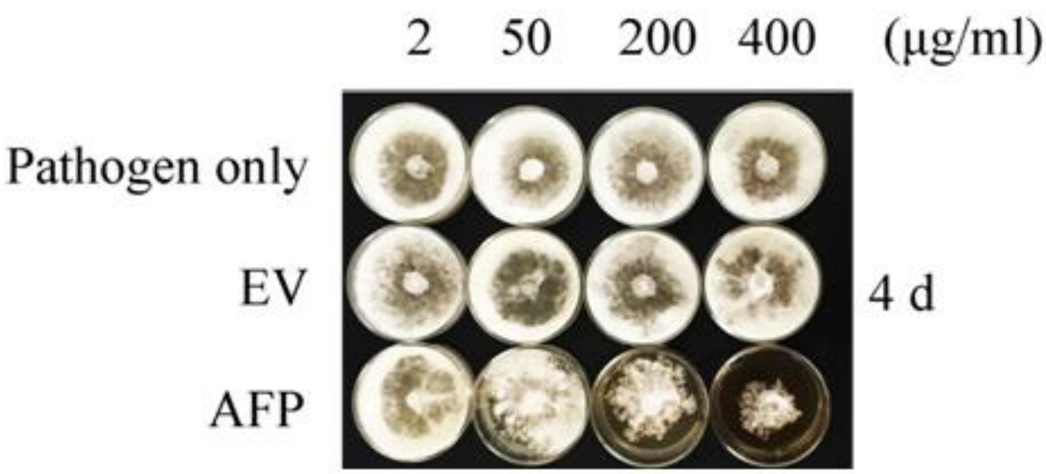



Fig. 2. Inhibition of growth of the dollar spot fungus by the partially purified antifungal protein from the P. pastoris culture filtrate. Different concentrations of the antifungal protein or proteins from the culture filtrate of the empty vector control were incorporated into the agar and a small piece of the dollar spot fungus was placed in the center of the plate. The results shown are after 4 days.

Summary of research progress and results

Title: **Genetic engineering of turfgrass for enhanced multi-stress resistance**

Hong Luo (PI), Department of Genetics and Biochemistry, Clemson University

In the face of a global scarcity of water resources and the increased salinization of soil and water, abiotic stress is the big challenge of modern agriculture practice. The major objective of this research is to genetically engineer enhanced tolerance to various adverse environmental conditions, such as drought, salt, heat and nutrient deficiency in turfgrass plants using transgenic technologies. Specifically, we propose to develop methodology to evaluate and demonstrate the feasibility of genetically engineering multi-stress tolerance in transgenic turfgrass through simultaneous overexpression of three genes encoding an *Arabidopsis* vacuolar $H^+$-pyrophosphatase, *AVP1*, a rice SUMOylation E3 ligase, *OsSIZ1*, and a cyanobacterial flavodoxin, *Fld*. To this end, we have prepared a chimeric gene construct containing expressing cassettes overexpressing *AVP1*, *OsSIZ1* and *Fld* genes together with a selectable marker gene for plant transformation, *bar*, for herbicide resistance (**Fig. 1**). In this chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, the *AVP1* gene driven by the cauliflower mosaic virus 35S (CaMV35S) promoter, the *OsSIZ1* gene driven by the corn ubiqintin (Ubi) promoter, and the *FNR:Fld* gene (the Fld gene translationally fused to the pea FNR chloroplast-targeting transit signal peptide for chloroplast targeting of the Fld protein) driven by the Ubi promoter were linked to the herbicide glufosinate (*phosphinothricin*) resistance gene, *bar*, driven by the cauliflower mosaic virus 35S (CaMV35S) promoter. This construct was then introduced into *Agrobacterium* strain, LBA4404 for plant transformation. We have simultaneously prepared creeping bentgrass embryogenic callus from mature seeds and used as targets for gene transfer by *Agrobacterium* infection using the LBA4404 strain harboring the chimeric gene construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*. Potentially transformed plant cells were selected in the presence of herbicide, glufosinate (*phosphinothricin*). Transformed cells surviving herbicide selection were regenerated into plants and grown in greenhouse for further analysis (**Fig. 2**). So far, we have successfully generated about 20 independent transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*. Next step would be to conduct experiments analyzing transgene expression in theses transgenic plants. Representative lines will be propagated in greenhouse and evaluated for their performance under various environmental stresses. In summary, we have:

- Prepared chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, containing expression cassettes to overexpress three stress-related genes, *AVP1*, *OsSIZ1* and *Fld* as well as a selectable marker gene for plant transformation, *bar*, for herbicide resistance.
- Prepared embryogenic callus of creeping bentgrass from mature seeds as targets for gene delivery.
- Conducted creeping bentgrass transformation using *Agrobacterium*-mediated transformation of embryogenic callus with the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*.
- Produced around 20 independent transgenic lines harboring p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar* for further analysis.

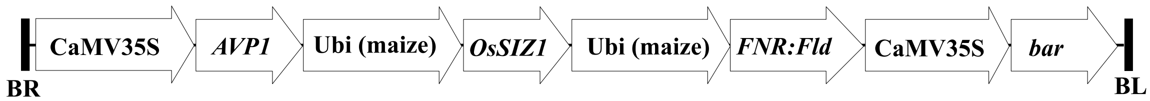



**Figure 1.** Schematic diagram of the chimeric gene expression construct, p35S-*AVP1*/Ubi-*OsSIZ1*/Ubi-*FNR:Fld*/p35S-*bar*, in which the *AVP1* gene driven by the cauliflower mosaic virus 35S (CaMV35S) promoter, *OsSIZ1* gene driven by the corn ubiqintin (Ubi) promoter, and *FNR:Fld* gene (the Fld gene translationally fused to the pea FNR chloroplast-targeting transit signal peptide) driven by the Ubi promoter were linked to the herbicide glufosinate (*phosphinothricin*) resistance gene, *bar*, driven by the cauliflower mosaic virus 35S (CaMV35S) promoter. The right border (BR) and the left border (BL) of the T-DNA in the binary vector were labeled.

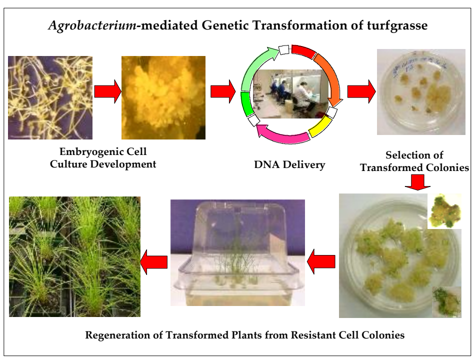



**Figure 2.** *Agrobacterium*-mediated transformation of turfgrass. Embryogentic callus was induced from mature seeds of the creeping bentgrass and used as target for gene transfer by *Agrobacterium* infection. Potentially transformed plant cells were selected in the presence of herbicide, glufosinate (*phosphinothricin*). Transformed cells surviving herbicide selection were regenerated into plants and grown in greenhouse for further analysis.

# Development of New Bermudagrass Varieties with Improved Turf 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 turfgrass in the southern USA and throughout tropical and warmer temperate regions of the world. The long-term goal of the Oklahoma State University (OSU) turf bermudagrass breeding program is to develop seeded and clonally propagated cultivars with high turf quality and improved resistance to abiotic and biotic stresses. Turf bermudagrass breeding and evaluation research activities performed by the OSU team in 2016 are summarized as follows.

A nursery of 98 cold hardy plants was evaluated for turf performance, seed yield and related traits as part of Ph.D. graduate student Yuanwen Guo's thesis project. Results of this experiment indicated that large genetic variability existed for 12 of 13 tested adaptive, morphological and reproductive traits within this cold hardy germplasm. Spring greenup was found to be highly and positively correlated with turf density and fall color retention. Leaf spot disease had negative correlations with greenup and inflorescence prolificacy. Percent seed set was negatively associated with raceme length. Broad-sense heritability estimates were 0.03-0.25 for first internode length and fourth leaf blade width, 0.36 for first internode diameter, 0.64 for fourth leaf blade length, and 0.72-0.80 for inflorescence prolificacy, raceme length and percent seed set. The large genetic variability within the cold hardy germplasm has potential value in selecting superior parental plants with desirable traits for producing interspecific hybrids and forming synthetic cultivars, in addition to developing new breeding populations.

Spring Dead Spot (SDS) disease is a major biotic threat to turf bermudagrass in the transition zone. Thirty-five official and four local entries in the 2013 NTEP bermudagrass trial established at the OSU Turf Research Center (TRC) are under study to test SDS disease resistance. Experimental entries developed in the OSU program, OKC 1131, OKC 1163, OKC 1302, OKS 2009-3, OKS 2011-1, and OKS 2011-4 are included in the NTEP trial. Each plot of the replicated experiment was inoculated on two sites with a blend of *Ophiosphaerella herpotricha* fungal pathogen on November 11, 2016 (Figure 1). We anticipate SDS symptoms in the nursery will appear in two or three years and continue for an additional two to four years.

Developing greens-type bermudagrass cultivars is an important component of the current project funded by the US Golf Association. Sixteen experimental selections and four commercial cultivars (Champion Dwarf, Mini Verde, Sunday, and Tifdwarf) were tested for putting green turf performance in a replicated field trial established at the OSU TRC in 2015 (Figure 2). The OSU experimental entries were selected from more than 11,000 clonal plants derived from new interspecific crosses between selected *Cynodon dactylon* and *C. transvaalensis* parents. The trial will continue in 2017 and 2018.

**Summary Points**

- A common bermudagrass experiment indicated large variability for seed yield, morphology and adaptation related traits within 98 cold hardy plants.
- The 2013 NTEP bermudagrass trial entries plus local standards are being evaluated for resistance to Spring Dead Spot caused by *Ophiosphaerella herpotricha* at Stillwater, OK.
- Sixteen fine-textured bermudagrass selections and four greens-type commercial cultivars were tested in a replicated field trial for turf performance under greens management conditions.

**Figure Captions**

Figure 1. The 2013 NTEP bermudagrass trial at Stillwater, OK was inoculated with the fungal pathogen *Ophiosphaerella herpotricha* to test for Spring Dead Spot disease resistance.

Figure 2. A mowing trial of 16 fine textured clonal bermudagrass selections and four commercial cultivars was established to test turf quality under putting green management conditions at the OSU Turf Research Center, Stillwater, OK.

**Annual Report on USGA ID#2014-03-492**

Developing and Validating a New Method to Improve Breeding for Cold-tolerant Bermudagrass

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Objectives: To develop a new technique that simplifies evaluation of bermudagrass for cold tolerance, and thereby improves breeding efficiency and facilitate the process.

In 2016, we performed a number of experiments to further evaluate the correlation and/or association of bermudagrass (*Cynodon dactylon* (L.) Pers.) in response to aryloxyphenoxypropionate (AOPP) herbicide and cold temperature stresses. The first experiment we conducted is to prove and validate the concept that responses of bermudagrass to the two stresses are truly linked somewhere in the pathway, rather than just correlated and individual events. The underlying logic is if the two pathways are truly linked, then subjecting the plant to Stress 1 at lower intensity and/or duration would enable the plant to "acclimate" so when Stress 2 occurs, subsequent plants that are susceptible will be better adapted. This concept is just like acclimating plants to chilling stress in fall before the winter freezing temperature occurs. We conducted a preliminary study in the past, and in 2016 we performed a complete experiment. We chose the bermudagrass cultivars "Riviera" and "Celebration" with known differences in cold-tolerance as the experimental materials and subjected the plants to chilling temperature at 4 $^{o}$C for 0, 1, 2, 4, 8, and 11 days. Plants were then moved to the greenhouse for 2 hours before subjecting them to a fenoxaprop-ethyl (Acclaim Extra®) application at 0.2 kg ai/ha with NIS at 0.25% v/v. Bermudagrass responses to AOPP herbicide without pre-chilling temperature treatment or with pre-chilling for different lengths were objectively evaluated by digital image analysis using WinRhizo (Regent Instruments Inc., Quebec, Canada) every week up to 4 weeks for percent discoloration. The experimental design was a 2 × 6 factorial combination of bermudagrass cultivar and pre-chilling treatment arranged in a CRD with 3 replications. To be concise, we only report data collected at 2 weeks after herbicide application (WAT) when maximal herbicide injury is visible. Results indicated that as expected, without herbicide application "Riviera" showed better tolerance to chilling stress, evidenced by reduced leaf discoloration (Fig 1). Two weeks after AOPP herbicide application, without pre-chilling treatment both "Riviera" and "Celebration" showed injury symptoms and "Celebration" showed more than twice the discoloration compared to "Riviera" (Fig 2). With pre-chilling treatment, both "Riviera" and "Celebration" showed reduced amount of herbicide injury as the length under chilling temperature increased. The most interesting part is after 4 days under chilling temperature, both "Riviera" and "Celebration" showed minimal herbicide injury at 15% discoloration or less, and the cultivars showed no differences in herbicide damage (Fig 2). When days under pre-chilling temperature increased to 8 or 11 days, the two cultivars segregated again. This finding was very intriguing as it confirms that the mechanism of bermudagrass in response to the two stresses is truly linked. To confirm the difference of cold-tolerance of these two cultivars, we also conducted a freezing test and determined that the $LT_{50}$ of "Riviera" is -10.6 $^{o}$C, significantly lower than "Celebration" (Fig 3).

In 2016, we have also performed experiments to profile the lipids of the two bermudagrass cultivars at normal conditions, under chilling stress, and after AOPP herbicide application. We are in the process of analyzing the data and will likely reach a conclusion next year.

In 2016, we have also continued working on the segregating population created by co-PI Wu by crossing “A12935” and “A12936”. The entry “A12396” is a breeding line selected from the Oklahoma State University (OSU) bermudagrass germplasm known for cold hardiness. The entry “A12395” is a collection from Puerto Rico which is susceptible to low temperature stress. We performed a freezing test of the two genotypes and determined their $LT_{50}$ to be -11.1 $^{o}$C and -8.2 $^{o}$C for cold-hard “A12396” and cold-sensitive “A12935”, respectively (Fig 4). We have previously established the tolerance of the two genotypes to AOPP herbicides; built upon it we continued the screening of the segregating progenies for AOPP herbicide tolerance (Fig 5). After screening 116 progenies (n=4), we were able to determine their distribution of tolerance to AOPP herbicide (Fig 6). The nature of the heritability of the tolerance trait is yet to be determined. Currently, selected progenies in each category have been propagated in the greenhouse; their tolerance to cold temperature will be determined in the laboratory and under field conditions in 2017. Additionally, our future plan also includes evaluating the expression of *ACCase*, and evaluate possible downstream genes in the fatty acid biosynthesis pathway.

Summary:

- We generated evidence that further indicates bermudagrasses’ tolerance to AOPP herbicide and cold temperature is likely linked;
- Utilizing a segregating population, we were able to further prove the usage of AOPP herbicide as a method to differentiate bermudagrass based on their cold-tolerance;
- We are still working on the mechanism, and we expect an in-depth discovery sometime in the next year or beyond.

**Fig 1.** Bermudagrass “Riviera” and “Celebration” percent leaf discoloration (%) evaluated by image scanning system (WinRhizo, Regent Instruments Inc.) following 0, 1, 2, 4, 8 or 11 days at chilling temperature of 4 $^{o}$C. Error bars represent standard error for each mean (n=3) at $P$<0.05. Means for the same pre-chilling treatment labeled with the same cap letter were not significantly different based on Fishers’ Protected LSD at $P$<0.05; Means for the same cultivar labeled with the same small letter were not significantly different based on Fishers’ Protected LSD at $P$<0.05.

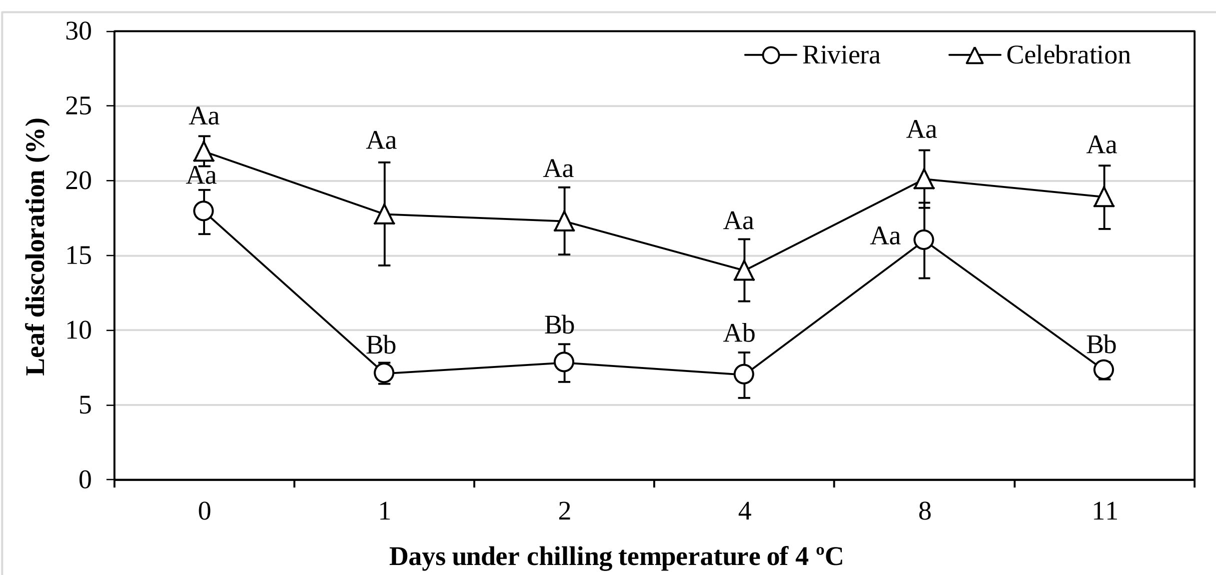


**Fig 2.** Bermudagrass "Riviera" and "Celebration" percent leaf discoloration (%) evaluated by image scanning system (WinRhizo, Regent Instruments Inc.) at 2 weeks after fenoxaprop-ethyl application with or without pre-chilling treatment at 4 °C for 0, 1, 2, 4, 8 or 11 days. Error bars represent standard error for each mean (n=3) at *P*<0.05. Means for the same pre-chilling treatment labeled with the same cap letter were not significantly different based on Fishers' Protected LSD at *P*<0.05; Means for the same cultivar labeled with the same small letter were not significantly different based on Fishers' Protected LSD at *P*<0.05.

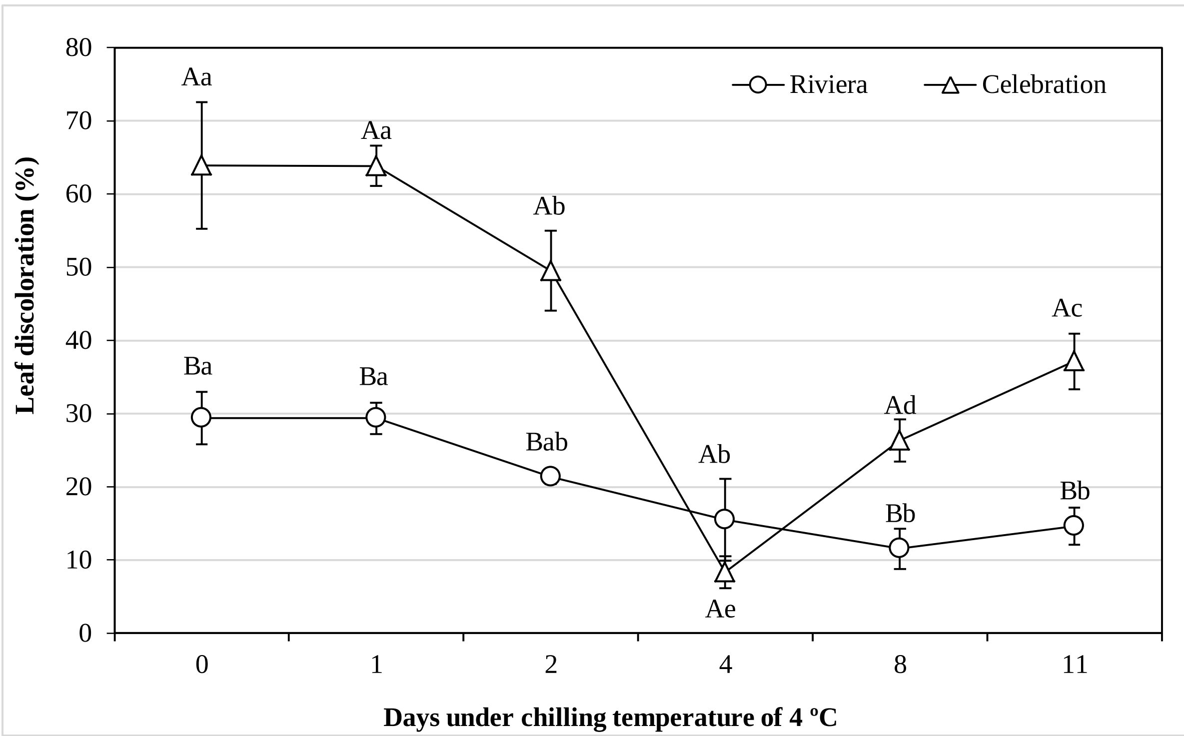



**Fig 3.** Electrolyte leakage (%) of bermudagrass "Riviera" and "Celebration" stolons at various temperatures ranging from 0 to -28 °C after acclimation at 4 °C for 48 h (n=6).

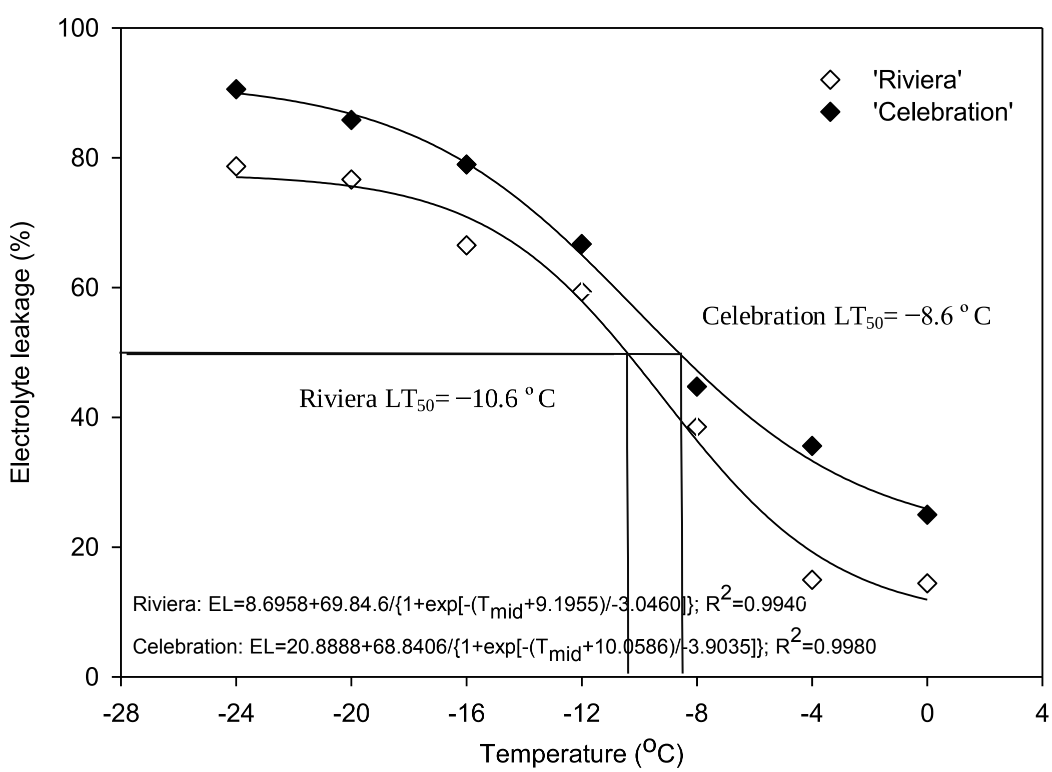


**Fig 4.** Electrolyte leakage (%) of bermudagrass genotype “A12935” and “A12936” stolons at various temperatures ranging from 0 to -28 °C after acclimation at 4 °C for 48 h (n=6). Genotype “A12935” is a cold-sensitive entry collected from Puerto Rico, and “A12936” is a breeding line selection for cold-tolerance. Both genotypes were provided by Co-PI Wu at the Oklahoma State University.

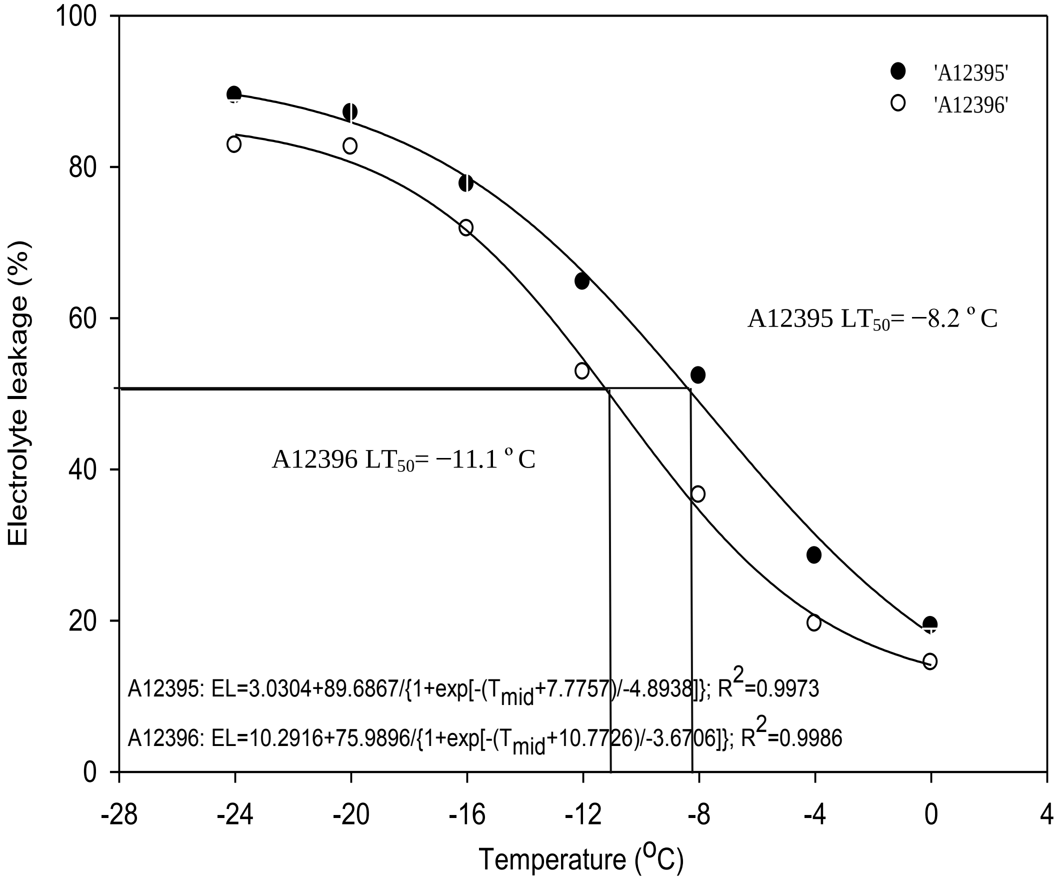


**Fig 5.** Representative images of bermudagrass progeny plants from the segregating population of "A12935" × "A12936" in response to fenoxaprop-ethyl (Acclaim Extra®) application at 2 or 3 weeks after herbicide application treatment (WAT). Herbicide-tolerant progenies at 2 (A) or 3 (B) WAT; Herbicide-sensitive progenies at 2 (C) or 3 (D) WAT.

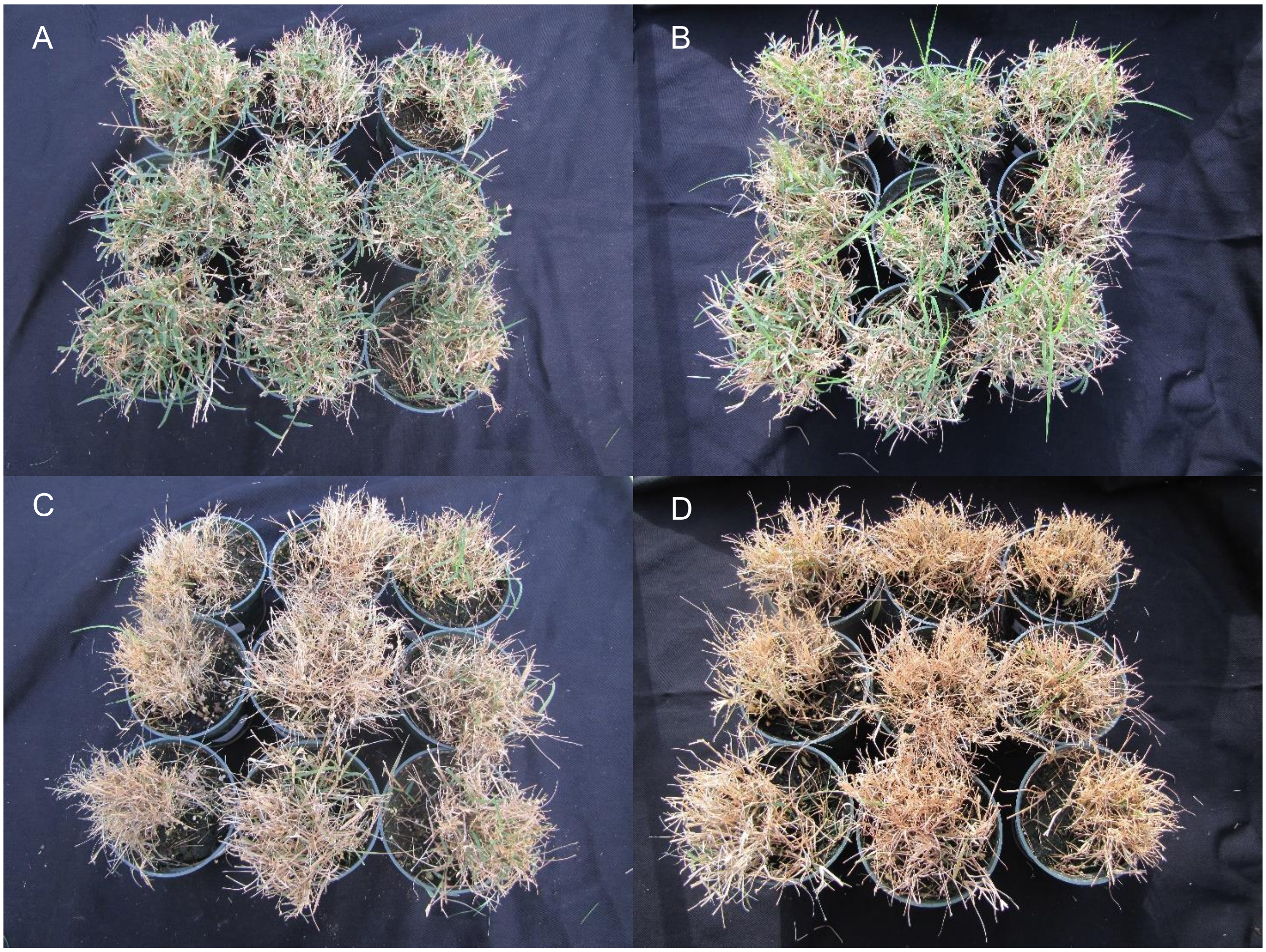
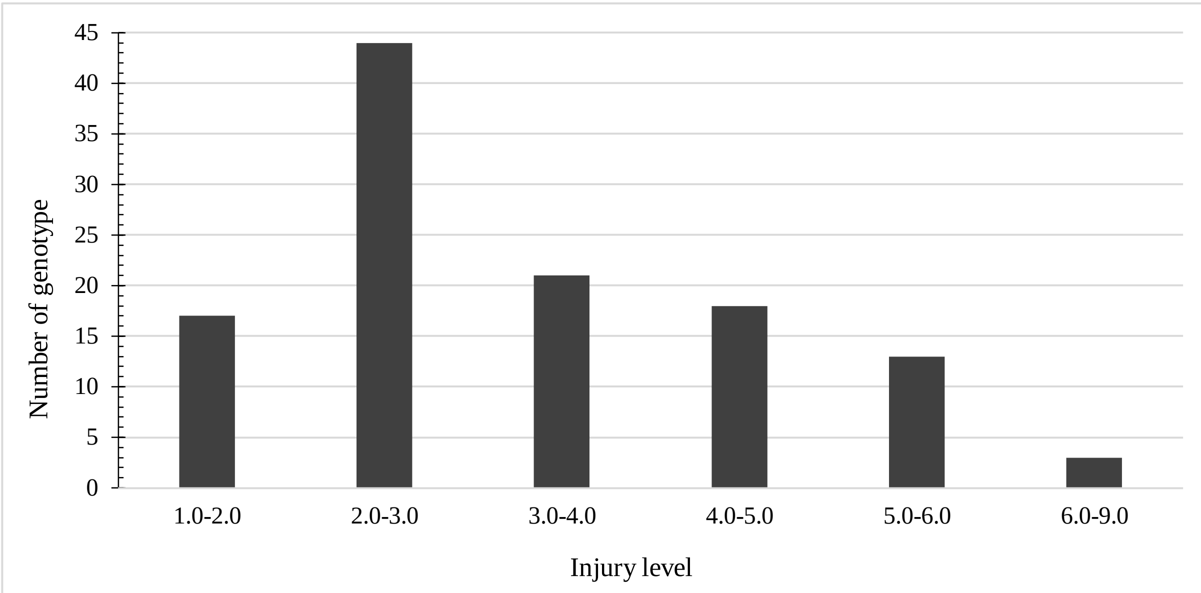


**Fig 6.** Segregation of 116 bermudagrass progenies (n=4) from population of “A12935” × “A12936” following fenoxaprop-ethyl application at 2 weeks after treatment. Segregation was based on visual injury assessment from 1-9 with 9 being no injury and 1 being plant death.



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

A. Dennis Genovesi and Ambika Chandra
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 fine textured vegetative types with the potential for rapid turf establishment.
2. Breed to improve characteristics such as turf quality, competitive ability and persistence under biotic and abiotic stresses.

**Start Date:** 2014 (continued from 2010)
**Project Duration:** 3 years
**Total Funding:** $ 89,317

**Summary Text** –

Zoysiagrass (*Zoysia* spp.) is a warm season, perennial grass used on sports fields and home lawns that is increasing in popularity due the need for low inputs such as fertilizer, water and less frequent mowing. Most cultivars are vegetatively propagated however an alternative, relatively inexpensive, way is to propagate zoysiagrass is by seed (Patton et al 2006). Availability of seeded varieties is limited to Japonica types such as 'Zenith' and 'Compadre' being the most popular. The focus of this research project is the development of a multi-clone synthetic variety which exhibits a texture that is finer than Zenith and seed yields that meet the production goals needed to make it profitable to produce. Since the initiation of the project in 2010, our breeding strategy has been the utilization of the classical plant breeding method known as phenotypic recurrent selection. The approach involves alternating between Spaced Plant Nurseries (SPN) and isolation crossing blocks. This strategy should allow for the gradual increase over multiple generations of desirable alleles affecting seed yields combined with finer leaf texture in the population.

In 2015 we began our third cycle of recurrent selection with the germination of seed harvested from four isolation blocks planted in 2013. The isolation blocks were grouped based on seed head color (red vs green) and flowering date (early vs late). Seed from these blocks were collected in mid-summer of 2014, cleaned during the winter and processed in early spring of 2015. Seed was scarified with 30% NaOH for 35 min. (Yeam, et. al. 1985). A total of 50 of the strongest seedlings from each family were planted in the field 7/23/15 to establish a Spaced Plant Nursery (SPN) of 1,750 progeny with Zenith and Compadre as checks. During 2016 notes were collected as shown in Table 1. Out of 1,750 progeny, 191 were identified for potential advancement to isolation blocks based on seed head color, density, height of exertion and leaf texture (Figure 1). This number is still too large so further evaluation for turf quality will need to be considered. Additional notes for flowering date will be taken in the spring of 2017 to help with the identification of the best experimental seed parents and assignment to the appropriate isolation and synthetic blocks in the summer of 2017.

In addition, seed that had been harvested from three synthetics in the summer of 2014 (1) early flowering / red seed head, (2) late flowering / red head and (3) late flowering / green seed head were cleaned and scarified as before. Seed from these three synthetics were used to plant a replicated field trial (RFT) 7/14/15 at the Research Center – Dallas at a rate of 2 lbs./1000 sq. ft. (Figure 2). In addition a second RFT was planted by Johnston Seeds 6/10/15 in Enid, OK. Seed from DALZ 1512 and DALZ 1513 synthetics were also transferred to Patten Seeds for evaluation.

Data was collected from the Dallas RFT in 2016 and is shown in Table 2. The turfgrass quality of

DALZ 1512 and 1513 were better than the seeded checks, Zenith and Compadre, on one rating date (5/26/2016), and TAES 6619 was better than seeded checks on 9/8/2016. For all other analyses, no significant differences were observed for turfgrass quality between the seeded checks and the experimentals. As expected, the turf quality for the vegetative checks (Palisades and Zorro) was generally better than that of the seeded entries. Even, about one year after planting, the establishment rates for the seeded entries were significantly better compared to the vegetative checks. There was no difference between the establishment rate of seeded experimentals and the seeded checks. In 2016, fall color for TAES 6619 was better than the seeded checks; whereas, the fall color of DALZ 1512 and 1513 was similar to the seeded checks. All seeded entries had a fall color rating lower than the vegetative checks. Spring greenup of DALZ 1512 and TAES 6619 was similar to the seeded checks. Seed head production was significantly higher on the experimental entries over that of the checks. While this does impact turf quality, it could be viewed as beneficial for planting seed production fields. In 2017 parental lines will be identified for the creation of new three clone synthetics for seed production purposes and RFT testing.

## Summary Points

1. The third cycle of recurrent selection continued with the planting of a spaced plant nursery consisting of 1,750 progeny on 7/23/15. Data were collected from the nursery in 2016 scoring for seed head color, density, height of exertion and leaf texture as well as turf quality. Additional data will be collected for flowering date (early vs late) in the spring of 2017 to enable the identification of our best new seed parents for advancement to isolation blocks in the summer of 2017.
2. Along side the recurrent selection breeding strategy, three sets of three clone synthetics were identified for evaluation in RFT and potential commercial product development. Seed harvested in 2014 was treated and planted in replicated field trials on 7/14/15 and data collected in 2016. Parental lines will be selected from the SPN for new synthetics to be planted in the summer of 2017.

**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.

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.

**Figure 1**. 2015 Seeded Zoysia SPN. **A:** Yellow seeded zoysia experimental, B: Red seeded zoysia experimental. C-D: Variability in the leaf texture of seeded zoysia experimentals.
**Figure 2**. Differences in rate of establishment four weeks after seeding/planting between seeded synthetic entries and a vegetative check, Zorro.
**Figure 3**. Pictures of the RFT plots one year after seeding/planting (8/1/2016) showing differences in establishment rate and turfgrass quality.

**Table 1.** Progeny evaluation in 2016 from the 2015 Seeded Zoysia SPN. Initially, individuals within each family with ≥ 5 visual turfgrass quality were selected and compared for differences in seedhead color, height, and density. Some individuals with turfgrass quality ≤ 5 were later included in the analysis due to their high seedhead density. Of the 1,750 individuals in this nursery, a total of 191 from 35 families were identified as potential seed parents.

| Family No. | No. selected progeny | Visual Color† | | Visual Height (cm)‡ | | | Measured Height (cm)‡ | | Mean Quality§ | | Mean Density¶ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Red | Yellow | Low | Medium | High | | | | | | |
| 1 | 5 | 5 | 0 | 4 | 0 | 1 | 13.0 | a* | 5.6 | a | 1.4 | a |
| 2 | 2 | 2 | 0 | 1 | 1 | 0 | - | | 6.0 | a | 1.0 | a |
| 3 | 2 | 2 | 0 | 1 | 1 | 0 | - | | 6.5 | a | 1.0 | a |
| 4 | 9 | 5 | 4 | 3 | 5 | 1 | 20.0 | a | 5.6 | a | 1.4 | |
| 5 | 4 | 4 | 0 | 4 | 0 | 0 | - | | 6.0 | a | 1.0 | |
| 6 | 5 | 5 | 0 | 2 | 1 | 2 | 12.0 | a | 6.0 | a | 1.2 | |
| 7 | 4 | 4 | 0 | 1 | 3 | 0 | - | | 6.3 | a | 1.0 | |
| 8 | 4 | 3 | 1 | 3 | 0 | 1 | 18.0 | a | 6.8 | a | 1.0 | |
| 9 | 8 | 6 | 2 | 0 | 1 | 7 | 14.7 | a | 4.8 | a | 2.5 | a |
| 10 | 10 | 10 | 0 | 1 | 1 | 8 | 14.0 | a | 4.3 | a | 2.9 | a |
| 11 | 8 | 7 | 1 | 1 | 4 | 3 | 15.3 | a | 5.0 | a | 2.0 | a |
| 12 | 4 | 4 | 0 | 0 | 1 | 3 | 13.7 | a | 4.0 | a | 2.8 | a |
| 13 | 3 | 2 | 1 | 0 | 1 | 2 | 17.5 | a | 4.3 | a | 2.3 | a |
| 14 | 6 | 6 | 0 | 1 | 2 | 3 | 12.7 | a | 6.0 | a | 1.3 | |
| 15 | 5 | 4 | 1 | 0 | 1 | 4 | 14.5 | a | 4.0 | a | 2.6 | a |
| 16 | 10 | 8 | 2 | 0 | 2 | 8 | 12.5 | a | 3.9 | a | 2.8 | a |
| 17 | 4 | 3 | 1 | 1 | 0 | 3 | 15.7 | a | 3.8 | a | 2.5 | a |
| 18 | 2 | 1 | 1 | 1 | 1 | 0 | - | | 6.0 | a | 1.0 | a |
| 19 | 5 | 1 | 4 | 1 | 1 | 3 | 12.3 | a | 5.4 | a | 1.8 | a |
| 20 | 5 | 0 | 5 | 3 | 2 | 0 | - | | 6.0 | a | 1.4 | a |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | - | | - | | - | |
| 22 | 10 | 1 | 9 | 0 | 0 | 10 | 14.4 | a | 4.4 | a | 2.5 | a |
| 23 | 3 | 0 | 3 | 0 | 0 | 3 | 15.0 | a | 3.7 | a | 3.0 | a |
| 24 | 2 | 0 | 2 | 1 | 1 | 0 | - | | 6.5 | a | 1.0 | a |
| 25 | 14 | 8 | 6 | 2 | 1 | 11 | 14.2 | a | 4.4 | a | 2.7 | a |
| 26 | 6 | 0 | 6 | 1 | 0 | 5 | 13.4 | a | 3.8 | a | 2.7 | a |
| 27 | 5 | 0 | 5 | 2 | 1 | 2 | 10.5 | | 5.0 | a | 2.2 | a |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | - | | - | | - | |
| 29 | 2 | 0 | 2 | 0 | 0 | 2 | 14.5 | a | 4.0 | a | 2.0 | a |
| 30 | 3 | 0 | 3 | 0 | 0 | 3 | 13.7 | a | 3.3 | a | 3.0 | a |
| 31 | 3 | 3 | 0 | 1 | 0 | 2 | 13.0 | a | 4.7 | a | 2.3 | a |
| 32 | 7 | 1 | 6 | 4 | 1 | 2 | 10.5 | | 5.6 | a | 1.7 | a |
| 33 | 12 | 10 | 2 | 1 | 0 | 11 | 12.1 | | 3.3 | | 2.8 | a |
| 34 | 8 | 1 | 7 | 0 | 0 | 8 | 13.9 | a | 3.1 | | 3.0 | a |
| 35 | 11 | 8 | 3 | 1 | 2 | 8 | 12.1 | | 3.9 | a | 2.6 | a |
| Total | 191 | 114 | 77 | 41 | 34 | 116 | - | - | - | - | - | - |
| C.V.$_{(\%,\#)}$ | - | - | - | - | - | - | 14.0 | | 29.5 | | 12.1 | |
| Family# | - | - | - | - | - | - | 0.0003 | | < 0.0001 | | < 0.0001 | |

*Significant at the P ≤ 0.05 level.

† Seedhead color was visually rated for selected progeny as either red or yellow/green.

‡ Selected progeny were initially rated categorically for seedhead height as low (< 5cm), medium (5-10 cm), or high (> 10 cm) on 7 Sept 2016.

§ Quality was visually rated on a 1-9 scale (1= poor; 6=minimum; 9=excellent) on 24 Aug 2016. The quality ratings from all selected progeny in each family were averaged and statistically compared using Tukey's HSD.

¶ Seedhead density was visually rated on a 1-3 scale ( 1= <30%; 2 = 30-60%; 3 = >60%) on 7 Sept 2016, and was averaged from all selected progeny for statistical comparison using Tukey's HSD.

# Coefficient of variation was calculated from the ANOVA by dividing the root mean square error by the grand means for each trait and multiplied by 100.

**Tables 2. A and B**. Performance of advanced synthetic seeded zoysia lines, DALZ 1512, DALZ 1513 and TAES 6619 compared to seeded checks Compadre and Zenith, and vegetative checks Palisades and Zorro.

**A.**

| | **Turfgrass Quality‡** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Entry** | 10/28/15 | 04/13/16 | 05/11/16 | 05/26/16 | 06/16/16 | 07/12/16 | 08/01/16 | 09/08/16 |
| DALZ 1512 | 5.0 ab | 5.0 abc | 6.0 a | 5.0 ab | 7.0 a | 5.3 ab | 3.7 bc | 2.0 c |
| DALZ 1513 | 5.3 a | 4.7 abc | 5.3 a | 4.3 bc | 7.0 a | 4.7 b | 2.3 d | 2.0 c |
| TAES 6619 | 4.0 b | 3.7 bc | 4.7 a | 4.0 cd | 6.0 a | 5.0 b | 3.7 bc | 3.0 b |
| Compadre¶ | 5.0 ab | 5.3 ab | 5.3 a | 3.3 d | 7.0 a | 5.3 ab | 3.3 bcd | 2.0 c |
| Palisades# | 5.3 a | 3.3 c | 4.7 a | 5.3 a | 5.7 a | 5.0 b | 4.3 ab | 4.0 a |
| Zenith¶ | 5.3 a | 5.0 abc | 5.7 a | 3.3 d | 7.0 a | 5.0 b | 3.0 cd | 2.0 c |
| Zorro# | 5.7 a | 5.9 a | 5.7 a | 5.7 a | 6.7 a | 6.3 a | 5.0 a | 3.7 ab |
| $LSD_{(0.05)}$†† | 1.3 | 1.9 | 1.7 | 0.9 | 1.2 | 1.2 | 1.1 | 0.7 |
| C.V. $_{(\%)}$‡‡ | 14.5 | 22.2 | 17.8 | 11.9 | 10.0 | 12.5 | 17.2 | 15.3 |

**B.**

| | **Establishment (%)†** | | | **Fall Color‡** | | **Greenup‡** | **Seedheads (%)§** |
|---|---|---|---|---|---|---|---|
| **Entry** | 11/23/15 | 04/13/16 | 08/01/16 | 11/23/15 | 11/11/16 | 03/15/16 | 05/26/16 |
| DALZ 1512 | 95.0 a* | 73.3 a | 100.0 a | 2.3 b | 2.3 c | 4.7 bc | 88.3 a |
| DALZ 1513 | 93.3 a | 80.0 a | 96.7 a | 3.3 ab | 2.0 c | 4.0 c | 75.0 b |
| TAES 6619 | 71.7 b | 63.3 a | 95.0 a | 2.3 b | 3.0 b | 4.7 bc | 75.0 b |
| Compadre¶ | 88.3 a | 76.7 a | 98.3 a | 2.0 b | 2.0 c | 5.3 ab | 10.0 c |
| Palisades# | 28.3 c | 21.7 b | 76.7 b | 3.3 ab | 3.7 a | 4.0 c | 6.7 cd |
| Zenith¶ | 91.7 a | 68.3 a | 95.0 a | 2.7 b | 2.0 c | 5.3 ab | 15.0 c |
| Zorro# | 21.7 c | 25.6 b | 76.7 b | 4.3 a | 3.7 a | 6.3 a | 0.0 d |
| $LSD_{(0.05)}$†† | 10.9 | 18.0 | 7.8 | 1.4 | 0.6 | 1.3 | 8.8 |
| C.V. $_{(\%)}$‡‡ | 8.7 | 16.8 | 4.8 | 27.9 | 12.5 | 15.2 | 12.8 |

* Significant at the 0.05 probability level.

† Seeded entries were sown on July 14, 2015 in three replications of 122 cm x 122 cm plots. Establishment data was collected before entering dormancy.

‡ Fall color, spring green-up, and turfgrass quality were collected on a 1-9 scale (1 = brown/dormant, 9 = completely green/ excellent; 5 = minimum acceptable green color).

§ Seedhead percentages were collected as a visual estimation of plot coverage.

¶ Compadre and Zenith were seeded checks sown at a rate of 2 lbs. /1000 sq. ft.

# Palisades and Zorro were planted as vegetative plugs with four 10 cm plugs per plot.

†† Means were separated using the student's t-test (LSD) at a 0.05 significance level.

‡‡ Coefficients of variation (C.V.) were determined from analysis of variance by dividing the root mean square error by the grand mean and multiplying by 100.

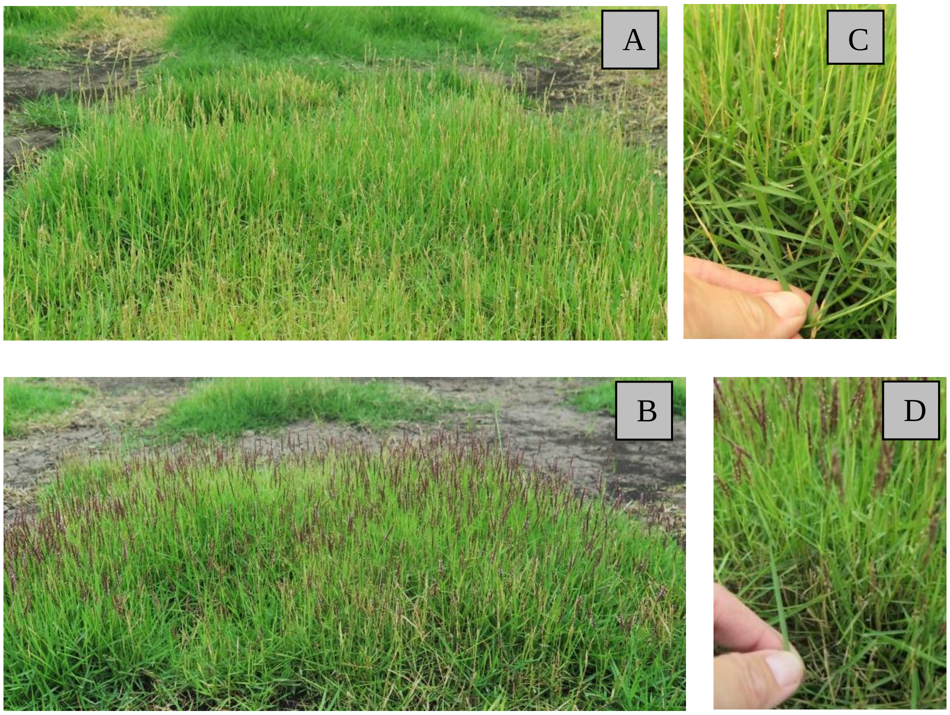



**Figure 1**. 2015 Seeded Zoysia SPN**. A:** Yellow seeded zoysia experimental, B: Red seeded zoysia experimental. C-D: Variability in the leaf texture of seeded zoysia experimentals.


Figure 2. Differences in rate of establishment four weeks after seeding/planting between seeded entries and a vegetative check, Zorro.

Figure 3. Pictures of the plots one year after seeding/planting (8/1/2016) showing differences in establishment rate and turfgrass quality.

# Development of Large Patch Resistant and Cold Hardy Zoysiagrass Cultivars for the Transition Zone

## 2016 Update

**Investigators**: Jack Fry[1], Ambika Chandra[2], Megan Kennelly[1], Aaron Patton[3], Dennis Genovesi[2], Mingying Xiang[1], and Meghyn Meeks[2]

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

**Cooperators:** Erik Ervin, Virginia Tech; Grady Miller, North Carolina State Univ.; Justin Moss, Oklahoma State Univ.; Mike Richardson, Univ. of Arkansas; John Sorochan, Univ. of Tennessee; Xi Xiong, Univ. of Missouri

**Objective**: Phase III (year 3-6) of the evaluation process is focused on field testing in the form of replicated spaced plant nurseries comprised of the newly generated progeny population. The objective of Phase III field test is the selection of experimental lines that have comparable/superior cold tolerance to Meyer, fine texture, and large patch tolerance.

**Update on Progress**

This was the second year of field evaluation for 60 zoysiagrass experimental lines after they were selected from 2,858 progeny. These progeny were developed at Texas A&M AgriLife Research in Dallas, Texas by crossing 22 cold-hardy zoysiagrasses with TAES 5645 (*Z. japonica*), which has demonstrated reduced susceptibility to large patch in growth chamber studies.

In September 2014, twenty top-performing progeny were selected from space plantings in Manhattan, West Lafayette, IN, and Dallas, TX. These sixty progeny were returned to Dallas for propagation. In June 2015, vegetative plugs of the 60 progeny along with the standard cultivars Meyer, Zorro, El Toro, Zeon, and Chisholm, were shipped from Dallas, TX and planted in three replicate plots (25 or 36 sq. ft.) in Manhattan, KS, West Lafayette, IN and Dallas, TX. In 2015, the same progeny were also distributed to research cooperators in Blacksburg, VA; Chicago, IL; Columbia, MO; Fayetteville, AR; Knoxville, TN; Raleigh, NC; and Stillwater, OK for evaluation in replicated plots.

**Data Collection and Results**

In 2016, data were submitted from all locations except Chicago where a personnel change recently took place. For presentation in Tables 1 to 9, the top-performing seven progeny are shown along with the controls (standards). Data summaries below refer to an evaluation of all 60 experimental progeny (and not all are shown in tables). In this progress report, for brevity, comparisons are made to Meyer, which is the standard zoysiagrass cultivar used in the transition zone. Data presented are averages from the locations submitting data for a given parameter, and were analyzed using PROC GLM.

- **Winterkill.** Winterkill was rated at IN in May, 2016 as percent of the plot exhibiting symptoms. Meyer had 12% winterkill; 20 progeny had winter injury levels statistically similar to Meyer, and 40 had more winter injury (Table 1).
- **Green up.** Spring green up was rated visually on a 1-9 scale as 1 = brown and 9 = fully green at AR, MO, NC, OK, TX, and KS. Green-up ratings ranged from 3.1 to 5.7 (Table 2). Twelve progeny had higher green up ratings than Meyer (3.6); all others were statistically similar.
- **Percent Cover.** Percent cover was rated visually (0 to 100 % scale) at MO, NC, OK, TN, VA, TX, KS, and IN between May and November. Average coverage ranged from 17 to 61%. Three progeny had greater coverage than Meyer (34%); all others were not statistically different from Meyer (Table 3).

- **Summer Color.** Color was rated visually on a 1 to 9 scale (9 = darkest green) at AR, MO, NC, OK, TX, and KS. Color ranged from 5.0 to 7.0; none of the progeny had genetic color that was better or worse than Meyer (6.0) (Table 4).
- **Leaf texture.** Leaf texture was rated visually between May and August on a 1 to 9 scale (1 = coarsest and 9 = finest) at AR, MO, NC, OK, TX, and KS. Texture ratings ranged from 4.2 to 7.9; three progeny had a finer leaf texture than Meyer (5.8) (Table 5).
- **Quality.** Turfgrass quality was rated on a 1-9 scale (1 = dead; 6 = minimally acceptable; and 9 = ideal) between May and September at AR, IN, KS, MO, NC, OK, TX, and VA. Average quality ranged from 5.0 to 7.1; four progeny had quality that was better than Meyer (6.0) (Table 6).
- **Fall color.** Fall color was rated visually between October and December on a 1 to 9 scale (1 = brown and 9 = dark green) at MO, NC, OK, TX, VA, and KS. Fall color ranged from 2.9 to 5.5; none of the progeny differed from Meyer (3.9) (Table 7).
- **Large patch.** Large patch was evaluated at KS, where plots were inoculated in September, 2016, and in OK, where a natural infestation occurred. In Kansas, Meyer (42% of plot area affected) had more large patch than all zoysiagrass progeny (0 to 23%) (Table 8). The top performing zoysiagrass progeny had little or no large patch present. In Oklahoma, Meyer had 77% of plot area affected by large patch, which was significantly higher than all but one of the progeny (0 to 58%) (Table 9). Plots were also inoculated in AR and data will be taken in 2017.

**Summary highlights**:

- Sixty zoysiagrass progeny, each arising from a cross between a parent with reduced susceptibility to large-patch and a cold-hardy parent, are under evaluation after initially screening > 2,800 progeny for quality and cold hardiness.
- Progeny are being evaluated under golf course management conditions at locations throughout the transition zone for turf quality characteristics and reduced susceptibility to large patch
- The fungus causing large patch was inoculated in plots in Manhattan, KS and a natural infestation occurred in Stillwater, OK. Meyer had the largest percentage of plot area affected by the disease at both locations; many of the progeny exhibited no symptoms.
- Progeny showed a wide range of variability in turf quality characteristics including winter injury/hardy, spring green up, establishment rate, genetic color, leaf texture, turfgrass quality, and fall color.
- Among this group of experimental zoysiagrasses, there appear to be promising progeny that have good winter hardiness, resistance to large patch, and improved turf quality characteristics.

Fig. 1. Large patch symptoms in Meyer zoysiagrass (left) compared to an experimental progeny in November, 2016 after inoculating in September at Manhattan, KS.

**Table 1. Winterkill of top-performing zoysiagrass progeny and standard cultivars in late spring 2016 in IN.**

| Entry | **Winter kill (%)**† |
|---|---|
| 6101-9 | 16.7 |
| 6121-5 | 18.3 |
| 6099-8 | 18.3 |
| 6100-13 | 23.3 |
| 6099-151 | 23.3 |
| 6096-36 | 23.3 |
| 6101-52 | 25.0 |
| Zorro | 73.3 |
| El Toro | 65.0 |
| Zeon | 66.7 |
| Chisholm | 20.0 |
| Meyer | 11.7 |
| LSD | 24.5* |

†Winter injury was rated on a 0 to 100% scale; n =3.

*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 2. Spring green up of top-performing zoysiagrass progeny and standard cultivars in spring 2016 in AR, MO, NC, OK, TX, and KS.**

| Entry | Spring green up† |
|---|---|
| 6119-179 | 5.7 |
| 6095-73 | 5.5 |
| 6099-447 | 5.4 |
| 6099-145 | 5.4 |
| 6097-74 | 5.2 |
| 6126-71 | 5.1 |
| 6119-14 | 5.1 |
| Zorro | 4.4 |
| El Toro | 5.3 |
| Zeon | 4.3 |
| Chisholm | 4.7 |
| Meyer | 3.6 |
| LSD | 1.6* |

†Spring green up was rated on a 1-9 scale (1 = brown; 9 = fully green); n = 18.

*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 3. Percent cover of top-performing zoysiagrass progeny and standard cultivars in summer 2016 in IN, MO, NC, OK, TN, TX, VA, and KS.**

| Entry | Cover (%)† |
|---|---|
| 6099-69 | 57.1 |
| 6119-179 | 55.6 |
| 6099-359 | 55.2 |
| 6095-73 | 50.9 |
| 6096-36 | 50.7 |
| 6101-52 | 50.5 |
| 6121-5 | 50.3 |
| Zorro | 34.6 |
| El Toro | 60.1 |
| Zeon | 43.0 |
| Chisholm | 60.0 |
| Meyer | 34.2 |
| LSD | 22.0* |

† Percentage cover was rated on 0 to 100 % scale; n = 24. Grasses were planted in the previous summer (2015) as vegetative plugs (2 inch diam., 12 inches apart).

*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 4. Summer color of top-performing zoysiagrass progeny and standard cultivars in mid summer 2016 in AR, MO, NC, OK, TX, and KS.**

| Entry | **Genetic color**[†] |
|---|---|
| 6102-289 | 7.0 |
| 6096-117 | 6.8 |
| 6095-83 | 6.8 |
| 6119-14 | 6.7 |
| 6126-71 | 6.7 |
| 6101-71 | 6.7 |
| 6100-86 | 6.7 |
| Zorro | 6.9 |
| El Toro | 5.2 |
| Zeon | 6.9 |
| Chisholm | 5.0 |
| Meyer | 6.1 |
| LSD | 1.1* |

[†]Genetic color was rated on a 1-9 scale (1 = brown/straw/dead; 9 = dark green); n = 18.

*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 5. Leaf texture of top-performing zoysiagrass progeny and standard cultivars in mid summer 2016 in AR, MO, NC, OK, TX, and KS.**

| Entry | **Leaf texture**[†] |
|---|---|
| 6096-137 | 7.8 |
| 6119-155 | 7.3 |
| 6102-289 | 7.1 |
| 6101-154 | 6.9 |
| 6101-32 | 6.8 |
| 6101-52 | 6.8 |
| 6100-26 | 6.8 |
| Zorro | 7.8 |
| El Toro | 4.4 |
| Zeon | 7.9 |
| Chisholm | 4.2 |
| Meyer | 5.8 |
| LSD | 1.2* |

[†]Leaf texture was rated on a 1-9 scale (1 = coarsest; 9 = finest); n = 18.

*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 6. Turfgrass quality of top-performing zoysiagrass progeny and standard cultivars in summer 2016 in AR, IN, KS, MO, NC, OK, TX, and VA.**

| Entry | **Turfgrass quality**† |
|---|---|
| 6095-73 | 7.1 |
| 6099-69 | 7.0 |
| 6101-26 | 7.0 |
| 6101-52 | 7.0 |
| 6101-154 | 7.0 |
| 6101-32 | 6.9 |
| 6119-179 | 6.9 |
| Zorro | 6.7 |
| El Toro | 7.0 |
| Zeon | 6.5 |
| Chisholm | 6.7 |
| Meyer | 6.1 |
| LSD | 0.9* |

†Turfgrass quality was rated on a scale of 1-9 (1 =dead; 6 = minimally acceptable; 9 = ideal); n = 24.
*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 7. Fall color of top-performing zoysiagrass progeny and standard cultivars in late fall 2016 in MO, NC, OK, TX, VA, and KS.**

| Entry | **Fall color**† |
|---|---|
| 6126-71 | 5.5 |
| 6095-83 | 5.2 |
| 6102-289 | 5.2 |
| 6119-87 | 4.9 |
| 6095-101 | 4.9 |
| 6095-117 | 4.9 |
| 6119-155 | 4.9 |
| Zorro | 4.3 |
| El Toro | 4.2 |
| Zeon | 4.1 |
| Chisholm | 4.1 |
| Meyer | 3.9 |
| LSD | 1.5* |

†Fall color was rated on a 1-9 scale (1 = brown; 9 = dark green); n = 18.
*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 8. Large patch infestation in top-performing zoysiagrass progeny and standard cultivars in Nov. 2016 in KS.**

| Entry | **Large patch (%)†** |
|---|---|
| 6099-447 | 0.0 |
| 6095-101 | 0.0 |
| 6101-26 | 0.0 |
| 6104-150 | 0.0 |
| 6099-359 | 0.0 |
| 6100-146 | 0.0 |
| 6102-62 | 0.3 |
| Zorro | 6.7 |
| El Toro | 1.7 |
| Zeon | 7.3 |
| Chisholm | 1.3 |
| Meyer | 41.7 |
| LSD | 15.8* |

†Large patch was rated as a percentage of the plot area affected on a 0 to 100% scale; n = 3.
*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Table 9. Large patch infestation in top-performing zoysiagrass progeny and standard cultivars in Nov. 2016 in OK.**

| Entry | **Large patch (%)†** |
|---|---|
| 6102-47 | 0.0 |
| 6095-101 | 0.0 |
| 6101-26 | 0.0 |
| 6104-150 | 0.0 |
| 6097-74 | 0.0 |
| 6096-137 | 0.0 |
| 6102-196 | 0.0 |
| Zorro | 26.7 |
| El Toro | 48.3 |
| Zeon | 36.7 |
| Chisholm | 0.0 |
| Meyer | 76.7 |
| LSD | 15.8* |

†Large patch was rated as a percentage of the plot area affected on a 0 to 100% scale; n = 3.
*To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD value ($P < 0.05$).

**Project Title:** Utilizing Molecular Technologies to Develop Zoysiagrass Cultivars with Improved Cold Tolerance
**USGA ID#:** 2015-03-518
**Investigators:** S.R. Milla-Lewis, Aaron Patton, and Brian Schwartz

**Objectives:** The overall objective of this project is to improve the efficiency of selecting for cold tolerance in zoysiagrass breeding by identifying genomic regions controlling this trait and associated molecular markers that can be used for selection. Phase I of the project focused on evaluation of a mapping population for i) field winter survival at Laurel Springs, NC, and West Lafayette, IN and ii) DNA markers useful for creating a map of the zoysiagrass genome. In Phase II, genotypic marker data was used with field data collected 2014-2016 to create a high-density DNA marker map and to identify genomic regions controlling cold tolerance in zoysiagrass and associated molecular markers.

**Progress Update and Results:** A mapping population of 175 individuals derived from the cross of cold-tolerant 'Meyer' and cold-susceptible 'Victoria' was developed. In June 2014, three replications of each single individual and nine controls including the two parents were planted in a randomized complete block design (RCBD) in 3 x 3 ft plots at the Upper Mountain Research Station in Laurel Springs, NC, and the William H. Daniel Turfgrass Research and Diagnostic Center in West Lafayette, IN. Additionally, the population was replanted at these two locations in June 2015 for secondary evaluations during the 2015-2016 winter season These four copies of the population and two years of evaluation created six unique environments for winter injury testing. Digital imaging was used to evaluate establishment and winter injury. Winter survival data was taken in spring 2015 and 2016 and significant variation in winter injury was observed within the population, including ten lines that performed as well as or better than Meyer across all environments. This variability in winter injury seen in the progenies was integral for the identification of markers associated with cold tolerance in zoysiagrass.
DNA marker data was collected on the population and used to generate a linkage map of the zoysiagrass genome. One-hundred-twelve simple sequence repeat (SSR) markers and 2,306 sequencing-derived single nucleotide polymorphism (SNP) markers were used to construct a high-density genetic map of the zoysiagrass genomes. The map covers 323 mega basepairs (Mbp) and 1973.1 centimorgans (cM) as well as all 20 chromosomes of the zoysiagrass allotetraploid genome. It is the first SNP-based map of the *Zoysia japonica* genome. This map was used in conjunction with winter injury data from six environments to identify genomic regions associated with winter injury. A total of ninety-three genomic regions associated with winter injury were identified across six environments and on all 20 chromosomes. Nine of these genomic regions had major effects and were observed in two or more environments. These genomic regions and markers linked with them could be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

**Progress Update and Results:**

- A mapping population of 175 individuals has been developed crossing cold-tolerant cultivar 'Meyer' and cold susceptible cultivar 'Victoria'.
- The mapping population was established in June 2014 in Laurel Springs, NC, and West Lafayette, IN in 3'x3' plots in three replications in randomized complete block design (RCBD). Additionally, the population was replanted at these locations in June 2015 for secondary evaluations during the 2015-2016 winter season.
- The mapping population was evaluated for winter injury and survival using digital image analysis in 2014-2016. Significant variation in winter injury was observed including ten lines that performed as well as or better than Meyer across all environments.
- A total of 2,418 DNA markers were used to construct a high-density genetic map of the zoysiagrass genome. The map all 20 chromosomes and is the first high density genetic map of the *Zoysia japonica* genome.
- Using the genetic map and field data, ninety-three genomic regions associated with winter injury were identified. Nine of these genomic regions had major effects and were observed in multiple environments. These genomic regions and markers linked with them will be valuable in implementing marker assisted selection for winter hardiness in a zoysiagrass breeding program.

| | NC,A, 2015 | NC, A, 2016 | NC, B, 2016 | IN, A, 2015 | IN, A, 2016 | IN, B, 2016 | Average Overall |
|---|---|---|---|---|---|---|---|
| **11-TZ-4720** | ✓ | ✓ | | ✓ | ✓ | | ✓ |
| **11-TZ-4738** | ✓ | ✓ | | ✓ | ✓ | | ✓ |
| **11-TZ-4755** | | ✓ | ✓ | ✓ | ✓ | | ✓ |
| **11-TZ-4778** | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| **11-TZ-4826** | ✓ | ✓ | | ✓ | ✓ | | ✓ |
| **11-TZ-4836** | ✓ | ✓ | ✓ | | | | ✓ |
| **11-TZ-4851** | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| **11-TZ-4877** | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| **11-TZ-4890** | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| **11-TZ-4891** | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| **MEYER** | ✓ | ✓ | | ✓ | ✓ | | ✓ |

**Table 1:** Zoysiagrass mapping population lines that suffered less than 25% winter injury in four or more environments. These ten lines performed as well as or better than Meyer in the winters of 2014-2015 and 2015-2016 at the Upper Mountains Research Station (Laurel Springs, NC) and the William H. Daniel Turfgrass Research and Diagnostic Center (West Lafayette, IN)

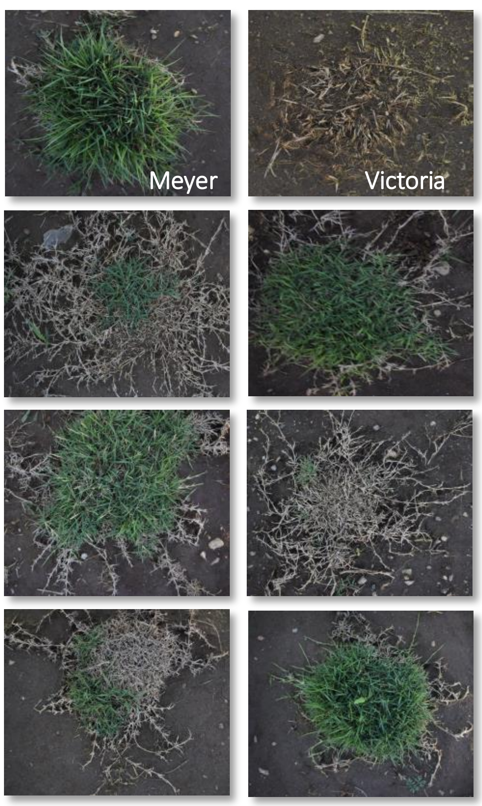



**Figure 1:** The mapping population and parents showed variation in winter injury levels in the winters of 2014-2015 and 2015-2016.

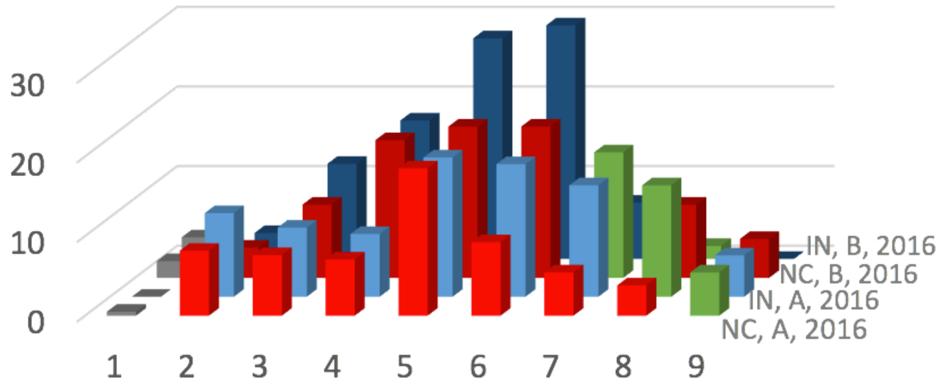



**Figure 2:** Winterkill distribution of lines for 175 progenies, Meyer, and Victoria for both copies of the population at Laurel Springs, NC and West Lafayette, IN in 2016. Winterkill is a measure of winter injury on a scale of 1 (completely dead) to 9 (no winterkill). Bars in grey and green indicate where Victoria and Meyer fell, respectively.

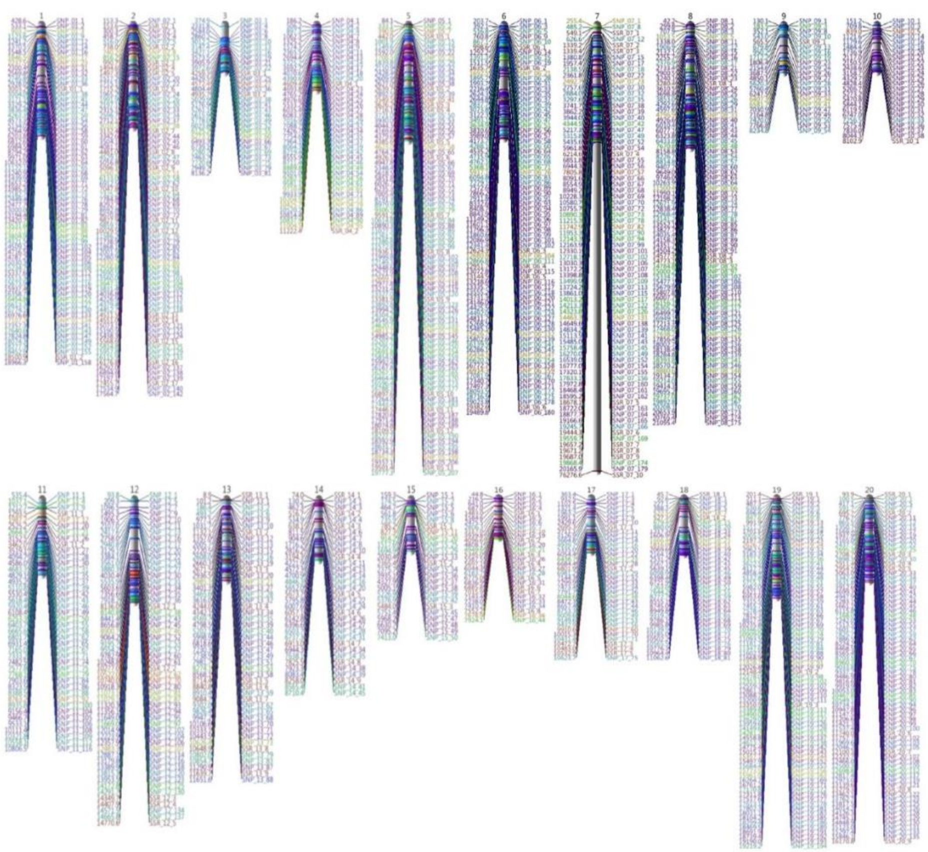


**Figure 3:** Genetic map of the zoysiagrass genome with 2,418 DNA markers. This high-density map was used to identify genomic regions controlling freeze tolerance.

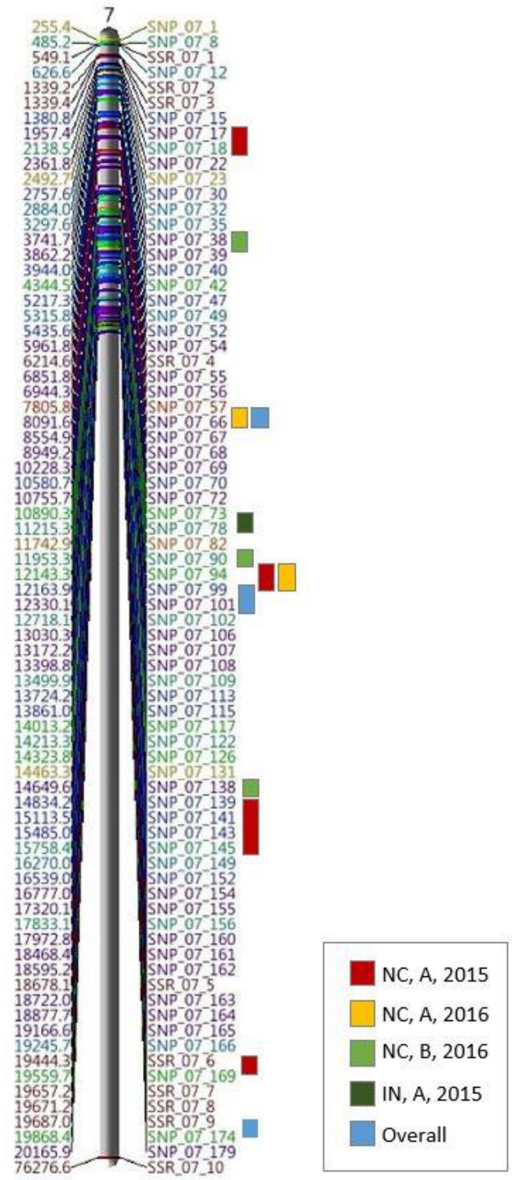



**Figure 4:** A marker map of chromosome 7 with genomic areas of interest that were identified in individual field environments and across all environments highlighted.

2016-35-605

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

li a eth Kellogg
anforth Plant Science Center

Seashore paspalum is becoming a prominent turfgrass for salt affected areas. A driving force in the development of paspalum has been the set of resources generated by breeders such as the world-wide germplasm collection gathered by Dr. Ron Duncan. To maintain this rapid pace of development, we seek to build upon and update these resources in three key ways:

First, while the current germplasm collection is widespread geographically, it may not represent the full breadth of phenotypic and genotypic variation present in the species due to its focus on golf courses and "turf-type" ecotypes. To increase available diversity, we have collected 20 phenotypically diverse individuals from wild populations during a June 2016 collection trip along the southeast coast of the United States (Image 1). Over the next year, we plan to extend our collection efforts both within the US and internationally.

Second, we are developing genomic resources for seashore paspalum. An early draft of the seashore paspalum reference genome has been completed and made available to us. As a pilot study we have resequenced the genomes of six genetically diverse accessions available from the USDA GRIN collection, and have mapped our new sequences to the reference genome sequence for SNP calling and further downstream analyses. These bioinformatic steps are currently ongoing, but preliminary observations suggest that read quality is high and that there is sufficient nucleotide diversity for downstream analyses including population genetics and marker-assisted breeding. A long-term goal is to expand our sequencing efforts to more accessions to identify genomic regions that may contain genes relevant for turfgrass improvement. These sequences and results will be of immense and immediate use to breeders attempting quantitative genetics studies in this system.

Third, as sequencing costs continue to plummet, phenotyping methods are quickly becoming the bottleneck for large-scale quantitative genetics studies. With this in mind we are developing a high-throughput system for scoring salt-tolerance and other phenotypes in seashore paspalum. We combine measurements of the concentration of 20 ions ("Ionomics") with analysis of images from plants grown at varying salinity levels (Image 2). We have completed a small pilot project of this method in which we collected tissue from each plant for ionomics analysis on two separate weeks. The macronutrients (Na, K, Ca, Mg) show large treatment effects (Figure 1), but genotypic differences remain unclear among the small sample of accessions studied. We see potential genotypic variation in tissue $Na^+$ concentration in the second week of our middle salt treatment (ECw = 30); however, whether this is due to genotypic differences or chance is uncertain. In contrast, we notice strong genotypic differences in heavy metal accumulation that are consistent across treatments and time points (Figure 2). The patterns of accumulation are also consistent across heavy metal ions (i.e., genotypes that have high Cd concentrations tend to also have high Co, Zn, and Cu concentrations). Image analysis is ongoing. A second pilot is currently underway using a modified methodology aimed to improve our ability to detect genotypic differences in salt tolerance.

By increasing diversity in the germplasm collection, improving the genomic resources for the species, and developing high throughput phenotyping methodologies, we have taken the first vital steps toward developing seashore paspalum as a tractable system for large-scale genetic studies.

**Summary of outcomes to date:**

- We have added 20 wild accessions to our collection with further collection trips planned.
- We performed whole genome re-sequencing on six accessions at 30x coverage. Bioinformatic analysis is ongoing. Preliminary results indicate abundant nucleotide diversity for further analysis. More accessions will be sequenced in the future.
- A high throughput phenotyping system is in development. A second pilot with improved methods is underway.
- Ionomics analyses suggest that salt tolerance is heavily influenced by the environment (treatment) for this small set of accessions.
- Heavy metal accumulation appears to be under genetic control and to vary among genotypes.

Image 1. A patch of wild seashore paspalum in Georgia. Individuals were collected from this site and others like it along the coast of the South Eastern United States during our June 2016collection trip.

Image 2. Seashore paspalum accessions being subjected to three salinity treatments in the first pilot of our phenotyping methods.

Figure 1. Changes in macronutrient concentration when plants are subjected to salt stress. Data shown are from the first week of tissue collection

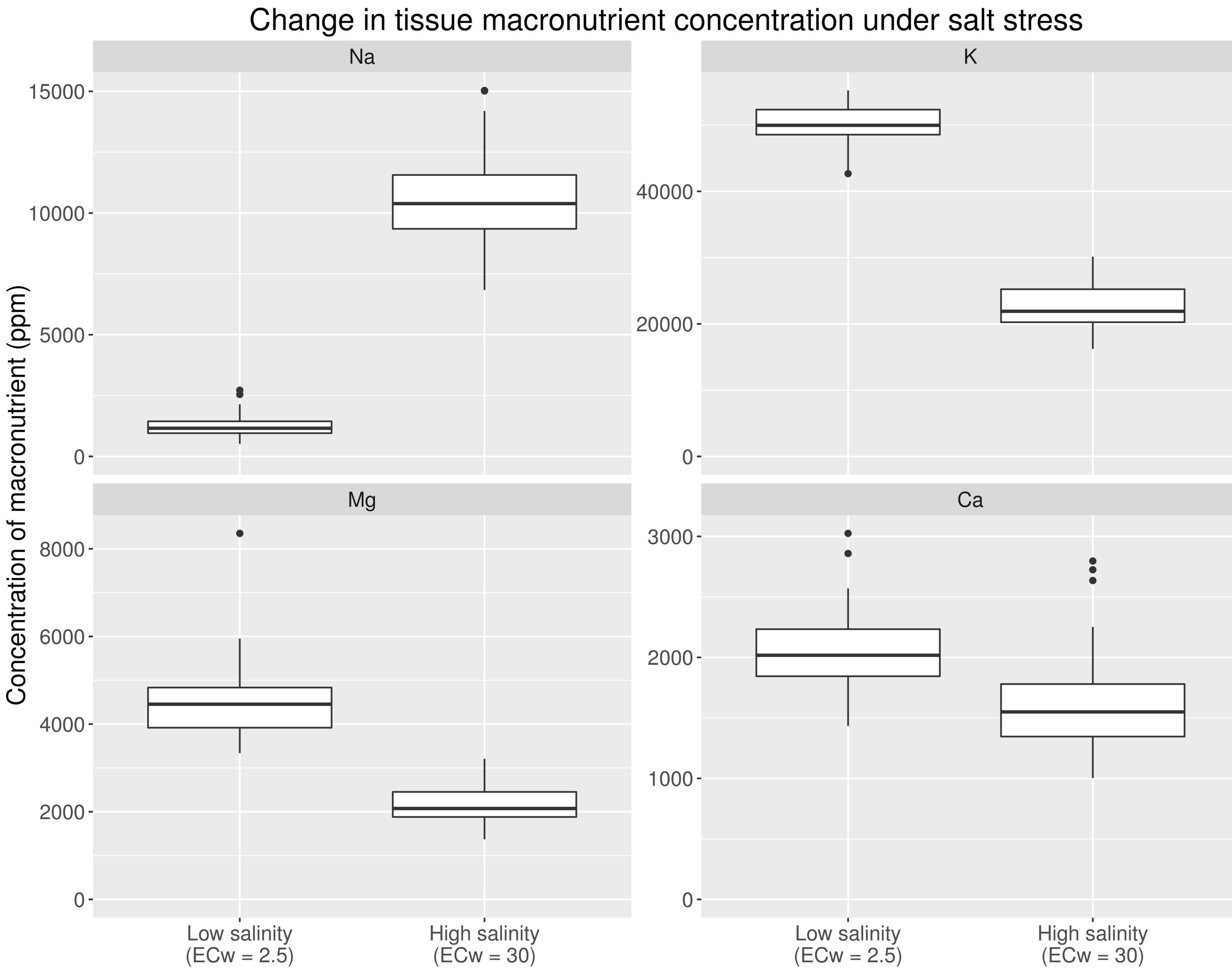
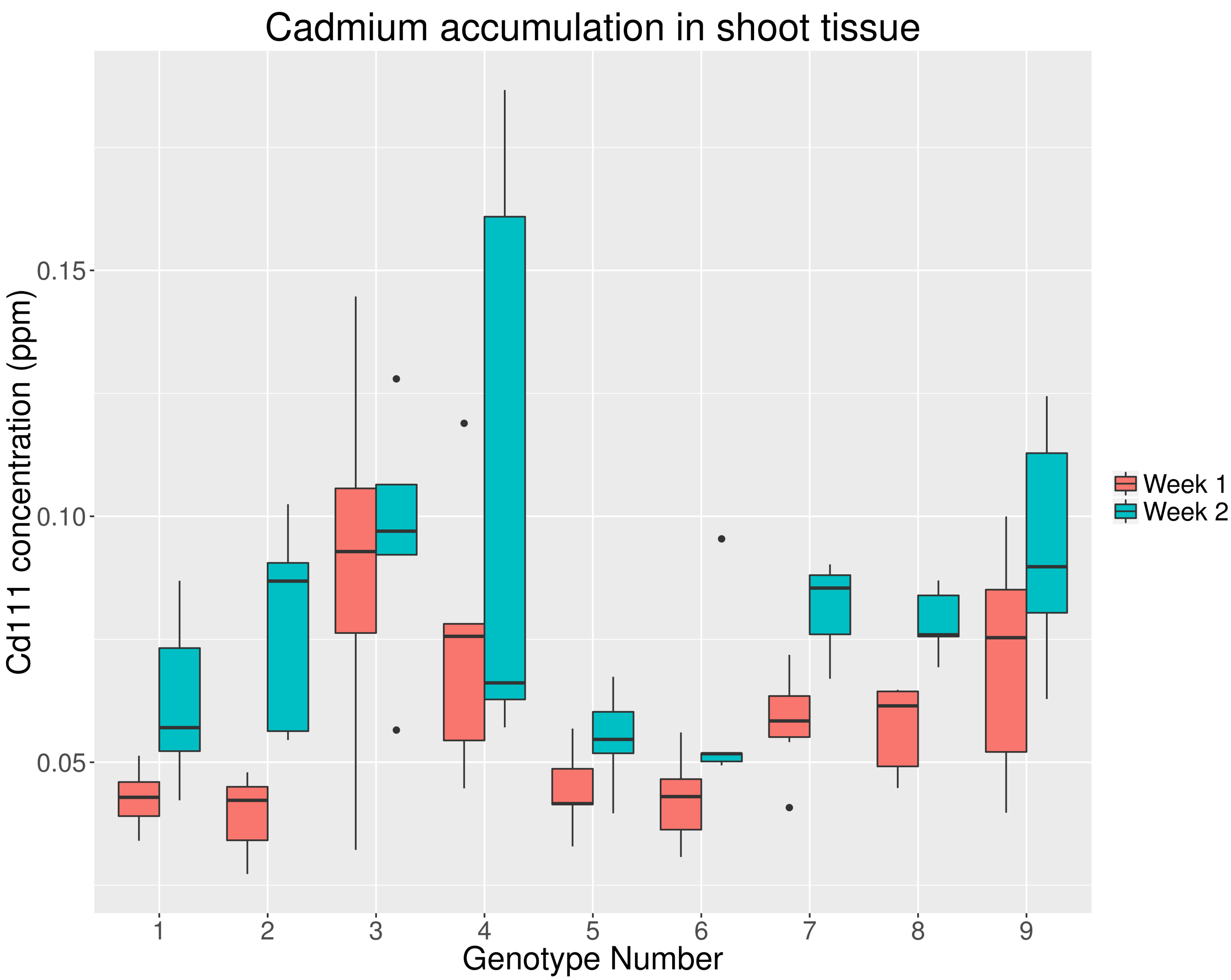


Figure 2. Differences in Cadmium accumulation between genotypes. Tissue was collected from plants in the low salt (ECw = 2.5) treatment on two occasions. A similar pattern is seen in the concentration of other heavy metals.



2003-36-278

Buffalograss Breeding and Genetics

Keenan Amundsen

USGA ID#: 2003-36-278

1. Buffalograss can tolerate moderate shade and traffic stress
2. Buffalograss can grow in a broad range of soil types
3. Forty elite buffalograss accessions were evaluated and top performers advanced

Demand for management input reductions is drawing golf course superintendents to consider native and naturally adapted turf species such as buffalograss. Buffalograss is native to the short grass prairies of the Great Plains and has exceptional heat, drought, and low temperature tolerance. It is a stoloniferous, warm season species that forms a dense sod. Cultivar development of native and reduced management input species such as buffalograss is the focus of the University of Nebraska-Lincoln (UNL) turfgrass breeding program. The buffalograss collection at UNL consists of nearly 2,000 buffalograss clones derived from vegetative selections and progeny from pairwise crosses. The breeding program consistently establishes crossing blocks to test combining ability between select male and female accessions and to fix desirable turf traits in buffalograss populations. Crossing block accessions are grouped in an attempt to fix stand persistence, high seed yields, leaf spot and false smut resistance, shade tolerance, chinch bug resistance, and turfgrass quality (Figure 1). Female plots are harvested separately and progeny evaluated for two to three years. An advanced lines evaluation trial was established in May of 2016 to 40 buffalograss accessions and maintained at 0.5" mowing height (Figure 2). Establishment rate and quality data was collected, and significant variability was observed among the clones. Top performing individuals will be selected and evaluated for potential use as vegetative plugs or sod and or used in the next crossing blocks. Buffalograss populations from crossing blocks are separately evaluated for turf quality and seed production potential (yield, seed weight, seed quality), and recurrent phenotypic selection is imposed to further improve the populations. Four populations are currently being evaluated from the 2013 crossing blocks and populations from the 2015 crossing blocks will be established during the 2017 growing season.

Another focus of our program is to address common misperceptions about buffalograss. There are observational-based reports suggesting that buffalograss is intolerant of shade, lacks traffic tolerance, and is only adapted to lighter soils. Many of these reports are based on observations from natural buffalograss stands or from research conducted on common buffalograss or early cultivars. These reports are misleading since newer cultivars of buffalograss don't share the same characteristics as early types and since most of these concerns can be addressed through common turfgrass management practices (Amundsen et al. Int. Turf. Soc. Res. J. 2017). As an example, in the sandhills region of the Great Plains, buffalograss is often found in the low areas between sand hills and is not commonly found on the hills. Heavier soils tend to accumulate in the low areas, leading to the observation that buffalograss prefers heavier soils. Buffalograss performance when grown in different soil types has not been tested. To address this misperception, mean turfgrass quality data and soil types for each site was obtained from the National Turfgrass Evaluation Program (NTEP) 1991, 1996, and 2002 buffalograss tests.  Turfgrass quality was grouped based on soil types and plotted.  No significant differences were observed for buffalograss quality among the different soil textural classes (Figure 3). Multi-year experiments were also completed that were designed to test buffalograss response to traffic (Figure 4) or shade (Figure 5).  In each study (soil type, traffic, and shade), variability was found among buffalograss entries for turfgrass quality, suggesting that prior observational-based reports on buffalograss performance should be re-visited.


Figure 1. Isolated buffalograss crossing block at the UNL turfgrass research center near Mead, NE.

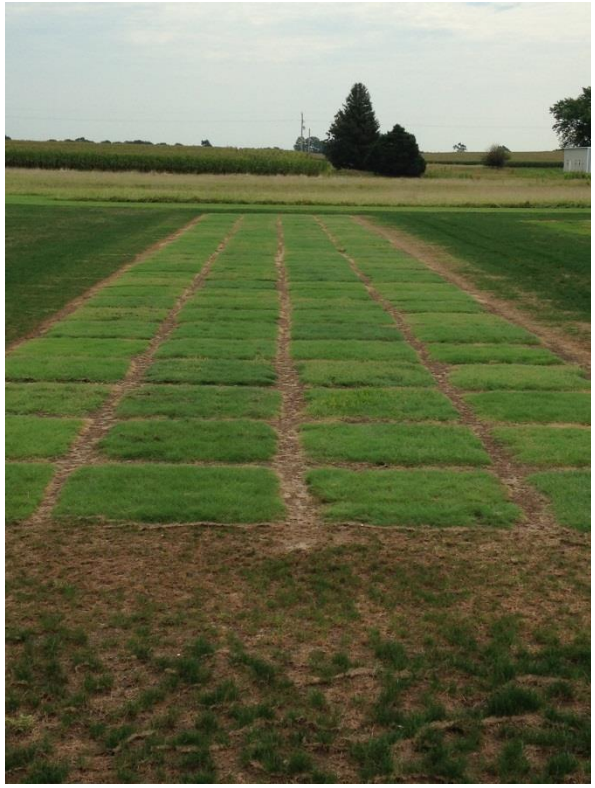


Figure 2. Performance of 40 buffalograss accessions from an advanced evaluation trial at the UNL turfgrass research center near Mead, NE.

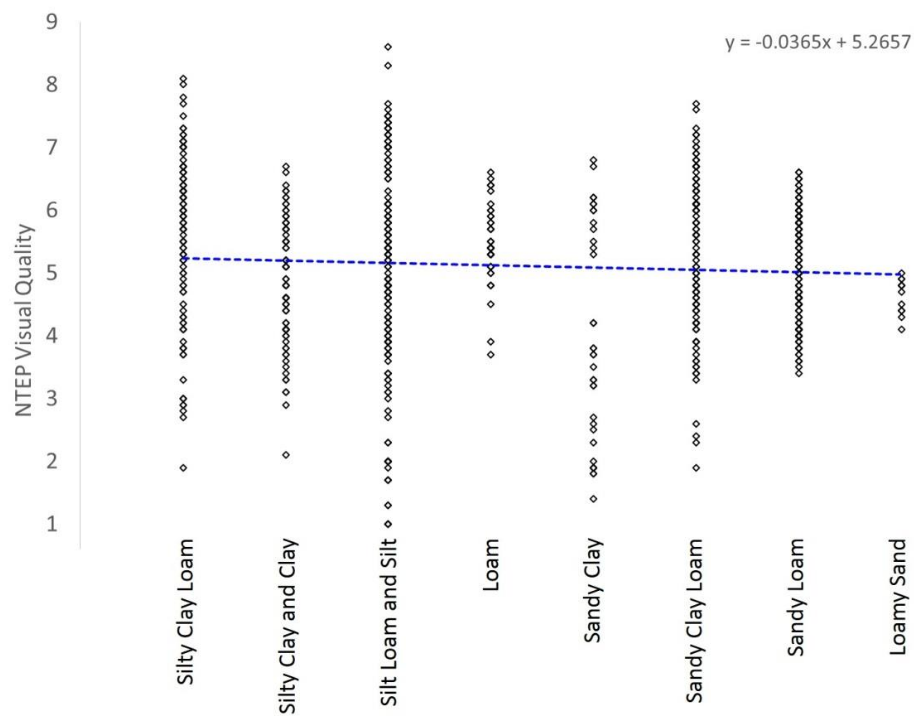



Figure 3. Mean turfgrass quality of buffalograss entries from the NTEP 1991, 1996, and 2002 buffalograss trials grouped by soil type. A best fit line, having near zero slope, is shown by the dashed line.

Figure 4. Traffic tolerance variability of 104 buffalograss accessions tested at the UNL turfgrass research center near Mead, NE.


Figure 5. Prestige buffalograss performance following two years of light treatments.  Prestige was grown in full sun or under black shade cloths that block 30% or 60% natural light.

# Development and Release of Turf-Type Saltgrass Variety

Yaling Qian and Tony Koski
Colorado State University

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

Objectives:

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

Inland saltgrass is indigenous to western North America where it has adapted to grow in specific niches of wet, alkaline, and saline soils, but is also found on drier and less salty sites. The planting of saltgrass on roughs and possibly even on fairways could help golf courses conserve potable water because of its tolerance to lesser quality (reclaimed water, saline ground and surface waters) water resources while maintaining acceptable turf and providing playing surface. On a broader scale, inland saltgrass has value for use in re-vegetation projects and in areas that commonly have high salinity levels.

Two breeding cycles took place from 2001 to 2010 at Colorado State University. However, due to project down time, some of the plant materials have been lost. Saltgrass germplasms from the breeding cycles remain for further evaluation and selection. After data and material evaluation, we have started plant propagation for selected lines in the greenhouse since summer of 2016. Efforts are in progress in rescuing, increasing and taking inventory of plant materials. So far, we have rescued 13 males and 13 female selected from the second-generation nursery. These saltgrass accessions represent the best turf-type individuals produced. They were clones. After material increase, the lines will be used to establish field plots. Data on spread, establishment, growth, general turf characteristics, and seed production will be collected to provide information for future development.

Two inland saltgrass accessions that have potential for turf and revegetation use on saline sites have been included in a field study to evaluate for sprigging establishment (Picture 1). Results indicated that sprig storage time (up to 2 days) did not affect establishment as long as the sprig materials were kept in shade (temperature under 30 °C) and in a closed bag to prevent desiccation. Saltgrass sprigged in May established adequate coverage (≥ 75%) in September with springing rates at > 270 bush/acre. For plots sprigged in June, only sprigging rates > 400 bushels/acre established adequate coverage (≥ 75%) by September. The accumulated growth degree day (GDD) to achieve adequate coverage was 1531 and 1703 for 800 and 400 bushels/acre sprigging rates, respectively. The accumulated GDD assessment provided in our study suggests that sprigged saltgrass has higher establishment GDD requirements than seeded saltgrass. Among the two line evaluated, the male line had better quality and stronger rhizomes than the female line. After full establishment, the plots have been rated for turf quality and disease incidence and measured for growth. Saltgrass maintained an average turf quality rating between 6 and 8 with 6 as the minimal acceptable rating for the quality and color. Rust was seen in July to September. Two lines differ dramatically in the severity of rust infection with one line almost immune to rust infection. Without mowing, the plants had a 18 cm maximal height.

Saltgrass seeds were harvested from a female saltgrass line with composite saltgrass lines to provide pollen sources. The harvested saltgrass seeds were stratified to establish field plots. Minirhizotron tubes were installed on the seed-established saltgrass plots to monitor root growth over time (Picture 2). Saltgrass had two flushes of root growth. The first occurred earlier in the season than expected, months before shoot growth. This may be a result of wound stimulation from the installation of observation tube. The second and larger flush of root growth coincided with the onset of shoot growth above ground, and began when soil temperature reached 15ºC. When soil temperatures were above 15 ºC, saltgrass roots continued to grow at a slow but steady rate during the summer months.

**Summary Points:**

- Effort is in progress to increase saltgrass materials for distribution for evaluation by interested partners;
- Saltgrass sprigged in May established adequate coverage (≥ 75%) in September with springing rates at > 270 bush/acre;
- The accumulated GDD assessment provided in our study suggests that sprigged saltgrass has higher establishment GDD requirements than seeded saltgrass;
- Saltgrass root growth coincided with the onset of shoot growth above ground, and began when soil temperature reached 15ºC.

Picture 1: Established saltgrass.

Picture 2a: Install minirhizotron observation tubes

Picture 2b: Mini-rhizotron observation tubes installed on seed-established plots.

**Project Title:** Genetic Improvement of Prairie Junegrass

**Project leader**: Eric Watkins

**Affiliation**: University of Minnesota

**Objective:**

1. Determine the genetic potential of native prairie junegrass germplasm for use as low-input turfgrass.

**Start Date:** 2007
**Duration:** ten years
**Total Funding:** $100,000

Prairie junegrass (*Koeleria macrantha*) has shown the potential to be successfully used as a turfgrass in lower-input environments. The species is widely distributed throughout much of the western United States and can also be found throughout much of Europe and Asia. The species has several attributes that would make it a useful low-input turfgrass including drought tolerance, survival of low and high temperature extremes, and reduced vertical growth rate. We have evaluated material from our collection (consisting primarily of germplasm collected in the Great Plains of the U.S.) and material from the USDA National Plant Germplasm Resources Network (NPGS) and used those evaluations to assemble breeding nurseries.

We have identified several challenges facing turfgrass breeders: a) this species lacks high levels of seed production, which results in low availability and high costs for end users; b) mowing quality is often poor; c) slow growth rate can lead to poor establishment (Fig. 1); and d) leaf rust disease is prevalent in many unimproved accessions (Fig. 2). Our breeding program is continuing to select genotypes that are show improvement in these areas. We are beginning projects aimed to determine the environmental cues that are necessary for flowering of this grass, which may help us better understand how seed production might be increased. We are also interested in investigating how to make crosses between tetraploid and diploid types as a way to move traits across the reproductive barrier of differing ploidy levels.

In recent years, we have been attempting to learn more about the microbial communities that develop near the roots of low-input turfgrasses, including prairie junegrass. Research in other cropping systems has suggested that plant genotype can affect the makeup up soil microbial communities. In order to determine how the genotype of a prairie junegrass might affect the soil microbial community, we grew plants representing several accessions that been previously evaluated for seed production and turfgrass quality in our breeding program. Plants were grown in the greenhouse and maintained under typical growth conditions. Rhizoplane soil was collected form the root surface of individual plants representing each accession. DNA was isolated using MoBio Power Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) and quantified using the

Qubit dsDNA HS kit (Thermo Fisher Scientific, Waltham, MA, USA). Amplicon preparation and sequencing were performed by the University of Minneosta Genomics Center. The V5-V6 hypervariable regions of the 16S rDNA were PRC amplified using the BSF784/1046R primer set. Sequence data was then analyzed using appropriate statistical techniques. We found that there were significant differences among the accessions (Fig. 3). Combined with results we have found in related projects, we may pursue more of this research in hope of developing low-input turfgrass cultivars that can positively influence soil microbial communities, resulting in more resilient turfgrass systems.

We will continue to investigate this species with the hopes of eventually releasing a cultivar that provides a slow growing, drought-tolerant turf that can withstand the stresses common to cold climates.

**Summary Points:**

- Prairie junegrass has shown potential as a low-input turf for cold climates due to its tolerance of extreme temperatures, slow growth rate, drought tolerance.
- There are a number of barriers to widespread use of this species, including susceptibility to rust disease, slow establishment, and poor seed production.
- We have conducted research on the soil microbial communities associated with prairie junegrass and found that significant differences in these microbial communities.

Figure 1. Slow establishment of prairie junegrass can lead to high levels of weed invasion.

Figure 2. Leaf rust is a common problem on native accessions when grown as turf.

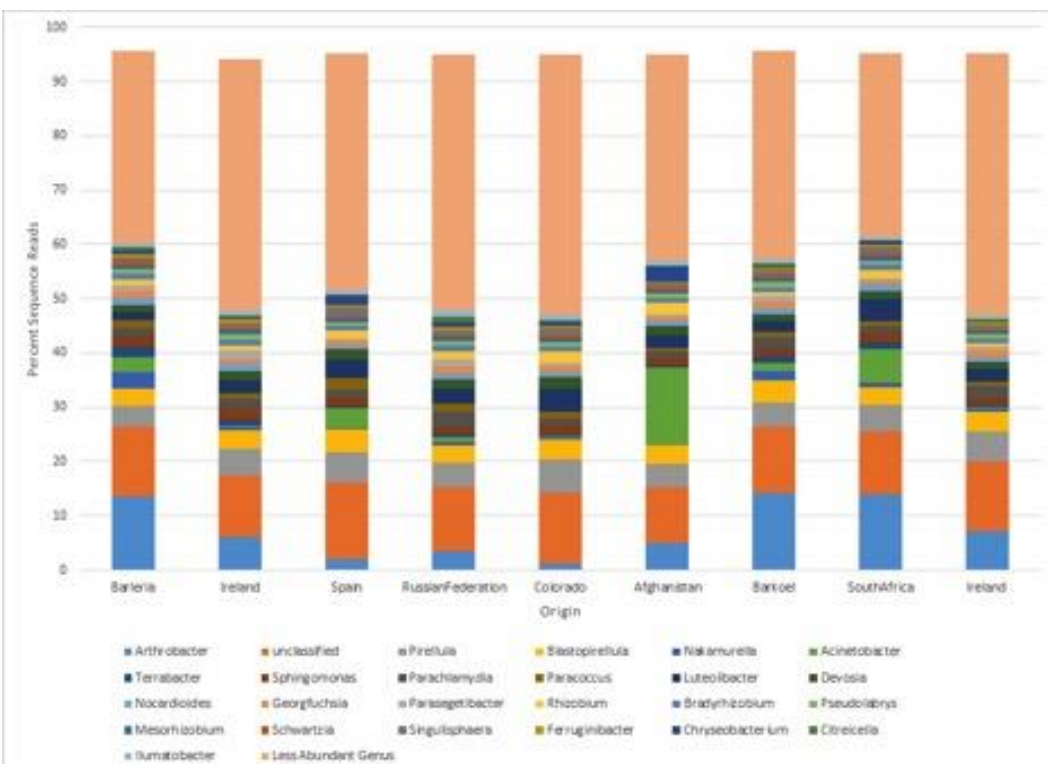



Figure 3. Distribution of taxonomic genus of Operational Taxonomic Units (OTUs) found to differ significantly by origin with Koeleria macrantha. The OTUs were found to vary significantly by using the Kruskal–Wallis test.

2016-05-555

# Improved Wheatgrass Turf for Limited Irrigation Golf Course Roughs

Joseph Robins and Shaun Bushman,

The severity of recent droughts and the long-term impact of climate change have heightened the need for water conservation in the U.S. Golf courses are now actively pursuing methods to limit areas that require supplemental irrigation. One approach employed by golf courses is the conversion of out-of-play areas from traditional turfgrasses to xeriscapes or to low-maintenance turfgrasses. In the northern semi-desert regions of the western U.S. the wheatgrasses provide a source of turfgrass that survive without supplemental irrigation. Wheatgrass turfgrass quality is less than that that of traditional species, but their use allows for substantial water savings without the need to xeriscape areas of golf courses.

The USDA Forage and Range Research wheatgrass breeding program has focused on the development of four wheatgrass species: crested wheatgrass (*Agropyron* cristatum), intermediate wheatgrass (*Thinopyrum intermedium*), thickspike wheatgrass (*Elymus lanceolatus*), and western wheatgrass (*Pascopyrum smithii*). Crested wheatgrass and intermediate wheatgrass are native to Eurasia. Thickspike wheatgrass and western wheatgrass are native to North America. Intermediate, thickspike, and western wheatgrass are highly rhizomatous. The crested wheatgrass breeding populations exhibit some rhizome development. To determine the turfgrass potential of these wheatgrass populations, we designed a study to compare elite breeding populations against standard cultivars. Populations will be grown alone and in two- and three-way combination with other species (Table 1) and will be managed under high (3 in) and low mowing (2 in) treatments and limited (50 % evapotranspiration replacement) and no supplemental irrigation.

**Table 1. List of species and populations included in the turfgrass quality evaluation.**

| Species | Population |
|---|---|
| Crested wheatgrass | RoadCrest |
| Crested wheatgrass | CWG-Select |
| Intermediate wheatgrass | Tegmar |
| Intermediate wheatgrass | IWG-Select |
| Thickspike wheatgrass | Sodar |
| Thickspike wheatgrass | TWG-Select |
| Western wheatgrass | Rosana |
| Western wheatgrass | WWG-Select |
| Kentucky bluegrass | Park |
| Hard fescue | Durar |

We seeded experimental plots of the 45 treatments at the Utah State University Evans Farm (Millville, UT) in late May 2016. Plots were hand seeded at a rate of 6 pounds 1000 $ft^{-2}$ for all entries but Park Kentucky bluegrass, which was seeded at a rate of 2 pounds 1000 $ft^{-2}$. Component ratios of mixed treatments corresponded to the percentage of the mixture multiplied by the stand alone seeding rate. The experimental design was a split plot modification of a randomized complete block design. Whole plots corresponded to mowing heights and sub-plots corresponded to treatment. Irrigation levels were separated spatially and will be treated as an additional factor in the experimental analysis. Following seeding, plots were established with the use of germination fabric and uniform irrigation. Uniform irrigation was maintained weekly throughout the establishment year at a 75 % evapotranspiration replacement rate. This ensured uniform establishment prior to initiating mowing and irrigation treatments in 2017 (Figure 1). Substantial warm-season grass weed pressure occurred during the summer of 2016. Due to sensitivities of different treatment species to various herbicides at the seedling stage, weed control consisted of mowing the warm-season grasses at a moderate height. Following full plot establishment, we used pre-emergent herbicide to control wee establishment.

**Figure 1. Photo of established wheatgrass plots at Millville, UT in summer 2016.**

**Conclusions**

1) **Plots successfully established, despite warm-season grass weed pressure.**
2) **Data collection begins in 2017 and will include quality, color, and ground cover.**
3) **Comparisons to fescue and bluegrass species will allow the potential of wheatgrasses in out-of-play areas to be determined.**

2015-12-527

A New Sodicity Index for Improving Risk Assessment and Management of Saline and Sodic Soils

Doug Soldat, Ph.D.

Will Bleam, Ph.D.

University of Wisconsin-Madison

- A new SAR equation is being tested and compared to three existing SAR equations
- The new equation appears to be much more robust (stable) as water evapo-concentrates than the existing equations
- Soil type is having a larger than expected impact on composition of soil solution following eight months of incubation

There is no bigger challenge facing the golf industry than water use. We believe that one day, use of potable water for golf course (and landscape) irrigation will be considered indefensible, even in water-rich parts of the world. This means that alternative sources of potentially poor water quality will become the norm. Alternative sources of irrigation include primary, secondary or tertiary effluent and harvested water from surface runoff. These sources have considerable spatial and temporal variation in their chemical composition. A plethora of management guides can be found for using poor-quality water for turfgrass irrigation; however, very little research has been conducted as to how the specific water quality parameters affect golf course soils. We recently discovered flaws in the SAR and SARadj equations which lead to inaccurate assessments of sodic hazard and faulty recommendations. For this project, we will test a new equation that corrects the flaws of the previous equations using an experimental approach in the laboratory that allows us to observe exactly what happens to saturated hydraulic conductivity as sodium and salinity levels change during evapo-concentration. The results of this research are expected to demonstrate a more accurate way of estimating sodic hazard and will improve our understanding of and ability to assess poor irrigation water quality in golf course soils.

In 2016, we identified golf courses that had soil types that would be ideal for testing. We sampled soils and waters and tested the waters for ion composition and calculated SAR using three established methods and a new method which we feel addresses serious flaws in the established methods (Table 1). We the concentrated the waters to 4 dS/m via evaporation and re-tested them for ion composition and SAR (Table 2). The evaporation simulates the concentration that happens in a drying soil. Finally, we have been incubating the waters and soils for eight months and the extracting the soil water and analyzing the ionic composition of the solution for calculation of SAR (Table 3).

The results suggest that the new equation is more stable than the established equations as the water concentrates, suggesting it is better able to accurately predict the precipitation of ions from solution. However, we are surprised at the large differences in SAR found when the waters are incubated in soils for eight weeks. This suggests that soil type has a large and controlling impact on sodium hazard and soil factors must be considered when attempting to estimate the impact of poor quality irrigation water. We plan to continue investigating the dynamics and interactions of the waters and soils in 2017.

Table 1. Comparison of the SAR of several irrigation waters based on three established calculation methods and a new method of calculation

| Water | Original SAR | Bower Equation | Suarez Equation | Soldat/Bleam Equation |
|---|---|---|---|---|
| Olive Grove | 4.31 | 6.60 | 11.49 | 8.57 |
| Gila | 8.82 | 18.97 | 28.24 | 14.42 |
| Pecos | 4.06 | 6.67 | 18.26 | 9.43 |
| Grand | 10.71 | 18.11 | 40.20 | 38.43 |
| Sevier | 5.82 | 12.94 | 16.14 | 10.78 |
| Lee Park | 6.38 | 8.56 | 24.33 | 18.90 |
| Britton | 1.95 | 3.12 | 7.76 | 5.87 |

Table 2. Comparison of the SAR of the same irrigation waters in Table 1, but concentrated to 4 dS/m.

| Water | Original SAR | Bower Equation | Suarez Equation | Soldat/Bleam Equation |
|---|---|---|---|---|
| Olive Grove | 7.92 | 12.93 | 19.91 | 8.90 |
| Gila | 12.06 | 13.31 | 32.65 | 14.61 |
| Pecos | 4.55 | 6.59 | 20.46 | 9.16 |
| Grand | 60.10 | 63.54 | 168.71 | 75.46 |
| Sevier | 11.89 | 23.63 | 26.85 | 12.01 |
| Lee Park | 13.51 | 17.14 | 45.06 | 20.06 |
| Britton | 3.51 | 5.81 | 13.75 | 6.15 |

Table 3. SAR (Soldat/Bleam Equation) of soil solution after eight weeks of incubation using three soil types and two irrigation waters. The waters were evapo-concentrated to 4 dS/m prior to incubation.

| Irrigation Water | Troxel Soil (WI) | Barnes Soil (SD) | Cecil Soil (VA) |
|---|---|---|---|
| Britton | 3.07 | 6.83 | 4.37 |
| Grand | 12.18 | 9.40 | 21.12 |

2015-13-528

## Incorporating Cultivation Practices and Products to Reduce Salinity Parameters from Poor Quality Irrigation Water on Golf Course Fairways

Texas Tech University - Joseph Young

The second year of field research was completed in October 2016 with minor lab work remaining. The research was initiated at both Rawls Golf Course and Meadowbrook in June 2016 applying cultivation treatments and products to the same strip-split plot randomized complete block design that was established in 2015 (Table 1; Images 1 and 2). Last fall and winter provided leaching rainfalls and some heavy snow storms early that facilitated movement of salts deeper into the rootzone. However, the summer months were extremely dry, which increased reliance on supplemental irrigation to maintain adequate playing conditions at both locations. The injury from cultivation and time to full recovery at each golf course was the primary factor for statistical differences in percent green cover, spectral analyses (Fig. 1), and soil moisture content as observed in 2015. Core-aerified plots had significantly less green cover, color, and moisture than sliced or control treatment strips at both locations initially, but the recovery process was lengthened at the Rawls Golf Course (Fig. 1). Soil texture analysis was conducted on samples from each course, and the Rawls Golf Course location contains 5% greater clay content than soil from Meadowbrook on average. Hence, the additional clay content keeps the soil from being as pliable to encompass the voids left from cultural practices applied.

There is limited evidence that the salinity levels reached in this region with our high ET demand and water quality results in significant above ground effects on the turf. It is rare to see wide spread areas of bare ground with saline or sodic soils as I have observed from other regions around Texas with heavier clay soils. Our typical sandy clay loam type soils and variability in water quality may not result in high salinity/sodicity levels to see above ground effects; however, increased soil EC levels have been documented in irrigated areas on golf courses in this region. The increased irrigation demand and limited leaching type rainfall events this year were thought to make great conditions to separate salinity levels based on soil electrical conductivity (EC). The primary differences in soil EC observed were from Meadowbrook Golf Club where significant differences in products were observed at the August and October soil sampling dates (no differences in June) (Fig. 2). Similar to 2015, granular products (gypsum and Verde-Cal G) significantly increased measured EC level in both months. This increase in EC level was observed for many products as the untreated control treatments remained in the lowest group. Both golf courses exhibited significant differences in soil pH in October following the full season of applications (Fig. 3). We are currently scanning all soil with the PXRF gun to determine potential correlations with large chemical elements and salinity measurements, and we will do further water quality analysis from both locations to estimate the greatest salinity hazard at each course (saline vs sodic or both).

## Summary bullet points

- Increased clay content (5% greater) at Rawls Course significantly increased recovery time from cultivation practices, especially core aerification, which resulted in poor above ground characteristics.
- Relative soil moisture at 1.5 inch (3.8 cm) depth was significantly reduced throughout the summer 2015 and 2016 at Meadowbrook with core aerification.
- Core aerification decreased ratio vegetation index (RVI) throughout summer at Rawls Course, but cultivated practices provided significantly better RVI at Meadowbrook late in the summer.
- The granular gypsum and verde-cal G applications reduced soil pH in October at both golf courses with Meadowbrook maintaining significantly lower pH than other treatments.
- The same granular treatments (gypsum and verde-cal G) significantly increased soil EC levels at Meadowbrook in August and October, but no significant differences were observed at Rawls Golf Course in 2016.

### Tables, Figures, and Images

| Trt # | Product trt | Rate/1,000 $ft^2$ | Application timing |
|---|---|---|---|
| **1** | Untreated control | None | None |
| **2** | Kelly's gypsum | 10 lbs | Applied once a month |
| **3** | ACA 2994 | 8 fl oz | Applied once per two months |
| **4** | ACA 2786 | 4.5 fl oz | Every two weeks |
| **5** | ACA 1900<br>ACA 2786 | 8 fl oz<br>4.5 fl oz | Initial application and 6 wks after<br>Two aps two weeks apart between ACA 1900 |
| **6** | Oars PS | 5 fl oz | Applied once a month |
| **7** | Vertical G | 12 lbs | Applied once a month |
| **8** | Oars PS<br>Vertical G | 5 fl oz<br>12 lbs | Applied once a month with liquid applied over the top of granular |
| **9** | DG Gypsum | 12 lbs | Applied once a month |
| **10** | Cal-Pull | 6 fl oz | Applied once a month |

Table 1. Products and rates applied to three replicates of each cultivation treatment [non-cultivated, AerWay Slicer, and core-cultivated (3/4 inch diam. tine on 2 inch spacing)]. Initial applications were made on 10 June 2016 with subsequent applications made on manufacture recommendation.

Image 1. Fairway at Meadowbrook following cultivation practices on 10 June 2016.

Image 2. Application of dry granular products to appropriate treatments at Rawls Golf Course.

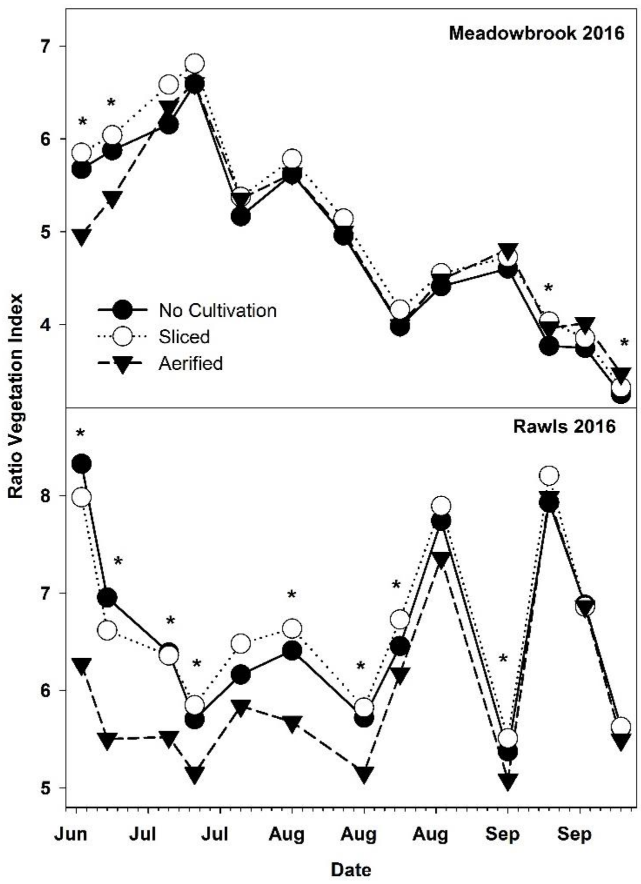



Figure 1. Ratio vegetation index (RVI) from Meadowbrook Golf Course (GC) and Rawls GC in 2016. Asterisks are placed above rating dates that were significantly different. Core aerified treatments had poorer RVI on every rating date at Rawls GC with differences, but cultivated treatments at Meadowbrook were better than control treatments late in the summer.

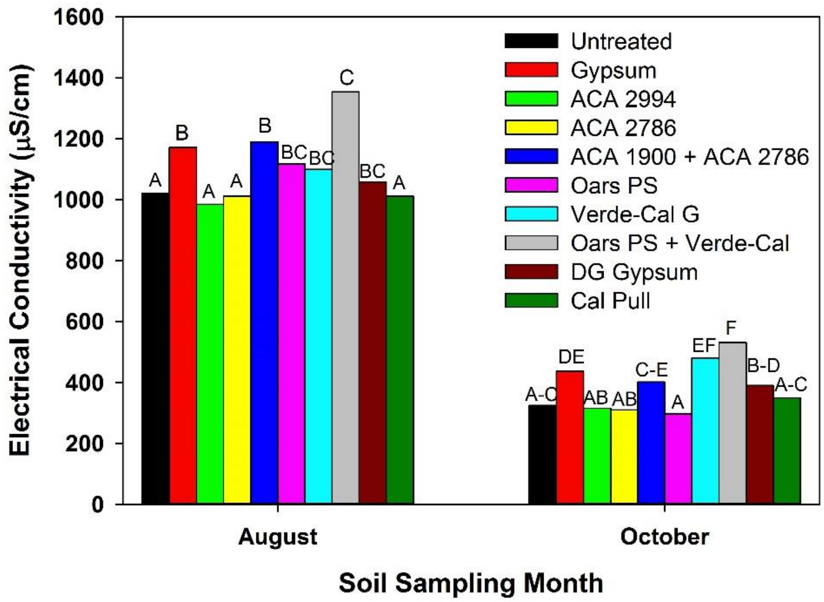



Figure 2. Soil electrical conductivity from August and October samples collected at Meadowbrook Golf Club. Bars sharing the same letter within sampling dates are statistically similar at $\alpha = 0.05$.

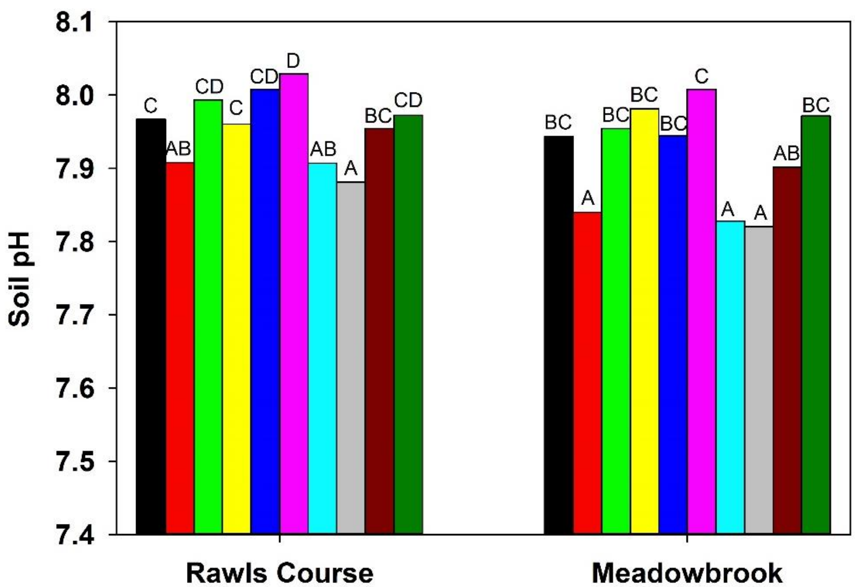



Figure 3. Soil pH from October soil samples from both Rawls Golf Course (GC) and Meadowbrook GC in 2016. Bars within a golf course sharing the same letter are statistically similar at $\alpha = 0.05$.

# Accuracy of FieldScout TDR 300 Soil Moisture Meter in Saline Soils

Bernd Leinauer, Matteo Serena, Dawn VanLeeuwen, and Elena Sevostianova
New Mexico State University

Objectives:

1. To evaluate the accuracy of a FieldScout TDR 300 hand held soil moisture sensor in a USGA sand at salinity levels ranging from 0.46 to 20 dS $m^{-1}$
2. To compare the accuracy of a TDR 300 to a permanently installed Decagon 5TE soil sensor

**Start Date:** 2015
**Project Duration:** 2 years
**Total funding:** $6,000

Measuring soil moisture with Time Domain Reflectometry (TDR) sensors can aid in turfgrass water conservation efforts, help improve playing conditions, green speed, and irrigation efficiency, and can assist in rootzone salinity management. However, information is lacking on the accuracy and reliability of newly introduced hand-held electromagnetic moisture sensors in saline soils. A laboratory study was conducted at New Mexico State University during 2015 to investigate the accuracy and reliability of a FieldScout TDR soil moisture sensor and a Decagon 5TE soil sensor at different salinity levels (expressed as electrical conductivity of the saturated soil paste extract ECe).

Columns measuring 14 cm in height and 20 cm in diameter were filled with a sand meeting USGA specifications for particle size distribution. Columns were subsequently saturated for 24 hours with either distilled (ECw = 0 dS $m^{-1}$), tap (ECw = 0.7 dS $m^{-1}$), or saline water (ECw = 2, 4, 6, 8, 10, and 15.5 dS $m^{-1}$) which resulted in ECe of 0.46 (distilled water), 1.08 (tap water), and 3.68, 5.40, 5.78, 7.68, 9.38, and 19.84 dS $m^{-1}$, respectively. Two FieldScout TDR 100 (Spectrum Technologies, Inc. Aurora, IL) (rod length 7.6 cm) and two Decagon 5TE (Decagon Devices Inc., Pullman, WA) (rod length 5 cm) sensors were used in this study. The soil sensors were inserted into the columns and subsequently placed onto a pressure plate inside a pressure chamber to record sensor readings at different soil moisture levels. For the purpose of this study we used the Spectrum Technologies' TDR 100 sensor instead of the TDR 300 as it uses the same measurement technology as the TDR 300 (Spectrum Technologies, pers. communication) but does not have a long handle attached to the body that holds the rods. Columns and sensors were then exposed to increasing air pressures which initiated soil drying by removing water from the soil. At the end of the dry-down period, columns were dried at 105 °C. Volumetric soil moisture was subsequently determined for each moisture level and data comparisons were based on either fitting linear regressions or quadratic polynomials to all salinities. Results are presented for the TDR 300 only.

There were no differences between values from the two sensor replicates therefore data were pooled over both sensors. Overall, sensor values increased with increasing soil moisture as slopes differ significantly from 0 for every soil salinity. Slopes for ECe ≥ 5 dS $m^{-1}$ were greater than for salinities of ECe < 4 dS $m^{-1}$. The slope at ECe = 19.8 dS $m^{-1}$ was 4 times higher than the slope at ECe = 0.5 dS $m^{-1}$. These results suggest that different salinity levels need separate calibration if the absolute soil moisture value is of interest. When separate quadratic polynomials were fit for each salinity the models differed significantly from 0 for salinities of ECe =3.7 and greater. Only for the salinities of ECe = 0.5 and 1.1 dS $m^{-1}$ data did not suggest to fit a quadratic term. However, caution needs to be used when fitting a quadratic polynomial as some of the moisture ranges included only 4 data points.

Table: Intercept and slope for the linear regression models to measure soil moisture with a Spectrum TDR 300 at different soil salinities compared to moisture determined gravimetrically (SE = Standard Error).

| Soil salinity (ECe) | Intercept | SE | Slope | SE |
|---|---|---|---|---|
| 0.5 | 3.13 | 7.61 | 1.07d* | 0.43 |
| 1.1 | 5.06 | 8.11 | 1.02d | 0.37 |
| 3.7 | 5.76 | 6.07 | 0.95d | 0.32 |
| 5.4 | 8.67 | 7.22 | 1.46cd | 0.35 |
| 5.8 | 3.74 | 5.77 | 2.20bc | 0.34 |
| 7.7 | 7.32 | 6.36 | 2.51b | 0.34 |
| 9.4 | 9.79 | 6.44 | 2.99b | 0.34 |
| 19.8 | 5.67 | 5.93 | 4.28a | 0.36 |

* Values followed by the same letter are not significantly different from one another (Fisher's protected LSD, $\alpha = 0.05$)

**Summary Points**

- Hand held TDR sensors can help in turfgrass water conservation efforts and in improving playing conditions
- Soil moisture readings between two replicate TDR 300 sensors with a rod length of 7.5 cm (3") did not differ from one another across a wide range of soil salinities.
- Soil sensors estimated moisture in a USGA sand accurately at salinity levels of ECe < 5 dS $m^{-1}$
- Different salinity levels need separate calibration for ECe > 5 dS $m^{-1}$ if the absolute soil moisture value is of interest rather than the relative difference.

**Evaluating small unmanned aircraft systems for detecting turfgrass stress with an emphasis on drought**

Dale Bremer and Deon van der Merwe, Kansas State University

Our objectives were to: 1) evaluate the utility of using ultra-high resolution remote sensing sensors mounted on small unmanned aircraft systems (sUAS) to detect early drought stress in turfgrass, before it is visible to the naked eye; and 2) compare sUAS remote sensing measurements with traditional techniques conducted at ground level.

The second year of a field study was conducted from 1 July to 29 August, 2016, on creeping bentgrass mown at 16 mm under a rainout shelter (Fig. 1). Six irrigation treatments were applied to create a gradient of irrigation regimes from well-watered to severe deficit irrigation, including 100, 80, 65, 50, 30, and 15% evapotranspiration (ET) replacement (well-watered to severely stressed). Measurements were taken weekly with a Canon S100 digital camera, modified to include near infrared (NIR), green, and blue bands. The camera was mounted on a S800 EVO hexacopter flown at 25 m above ground level within 3 hours of local solar noon. Images were processed using Agisoft PhotoScan Pro (Fig. 2) and AgVISR. Eight vegetation indices (VI; combinations of NIR, green, and blue bands) and each of the three individual bands were evaluated for their ability to detect early drought stress. Additional measurements included soil moisture (7.5 cm depth; FieldScout TDR 300), visual quality, percentage green cover (digital image analysis); and NDVI (handheld FieldScout 1000 and handheld Holland Scientific RapidSCAN CS-45).

Results from the first year (2015) indicated the near infrared (NIR) band and the GreenBlue VI [(Green-Blue)/(Green+Blue)] detected drought stress in turfgrass before it was visible (data not shown). On 7 July, 2016, 7 days after initiation of irrigation treatments, soil moisture had already declined and was significantly less at 30 and 15% ET than at 100% ET (Fig. 3A). However, on the same day turfgrass quality remained acceptable ($\geq 6$) and similar among all ET treatments (Fig. 3B). Percentage green cover was also similar among ET treatments (Fig. 3C). No differences in NDVI were detected among ET treatments with the handheld FieldScout 1000 (data not shown), but the RapidSCAN CS-45 indicated lower NDVI at 15% than at 100% ET (Fig. 3D). Among the 8 vegetation indices and 3 individual bands utilized with the sUAS, the NIR band, GreenBlue VI, Enhanced 1 VI [(NIR+Green-2Blue)/(NIR+Green+2Blue)], and Enhanced 2 VI [(NIR+Green-Blue)/(NIR+Green+Blue)] detected differences between 100% ET and the two lowest irrigation levels on 7 July, which was the same trend as soil moisture (Figs. 3E to 3H, 3A). Interestingly, the trends in visual quality and percentage green cover one week later (15 July) were similar to soil moisture and the aforementioned VIs on 7 July. Namely, visual quality and percentage green cover were lower at 15 and 30% than at 100% ET (Figs. 3 and 4). This indicates ultra-high remote sensing mounted on sUAS detected drought stress in the turfgrass canopy at least 8 days before it was visible to the naked eye.

**Bullet Points:**

- Seven days after initiation of ET treatments (7 July), no differences in turf quality or percentage green cover were evident among ET treatments (Fig. 3B and 3C).
- However, soil moisture was less at 15 and 30% than 100% ET (Fig. 3A).

- On the same day (7 July), the NIR band and the GreenBlue, Enhanced 1, and Enhanced 2 vegetation indices detected differences in vegetative properties between 100 and 15/30% ET treatments (Fig. 3E to 3H) (same trend as soil moisture, Fig. 3A).
- Trends observed on 7 July in these vegetation indices and the NIR band were predictive of visual quality and percentage green cover 8 days later, on 15 July (Figs. 3 and 4).
- Ultra-high resolution remote sensing with small UAS detected drought stress at least 8 days before it was visible to the human eye.


**Figure 1**. Aerial view of creeping bentgrass plots (top, highlighted with black border). Precipitation was excluded from plots by an automated rainout shelter (inset A), which covered plots during rainfall. This allowed precise irrigation amounts to be applied to individual plots (inset B).

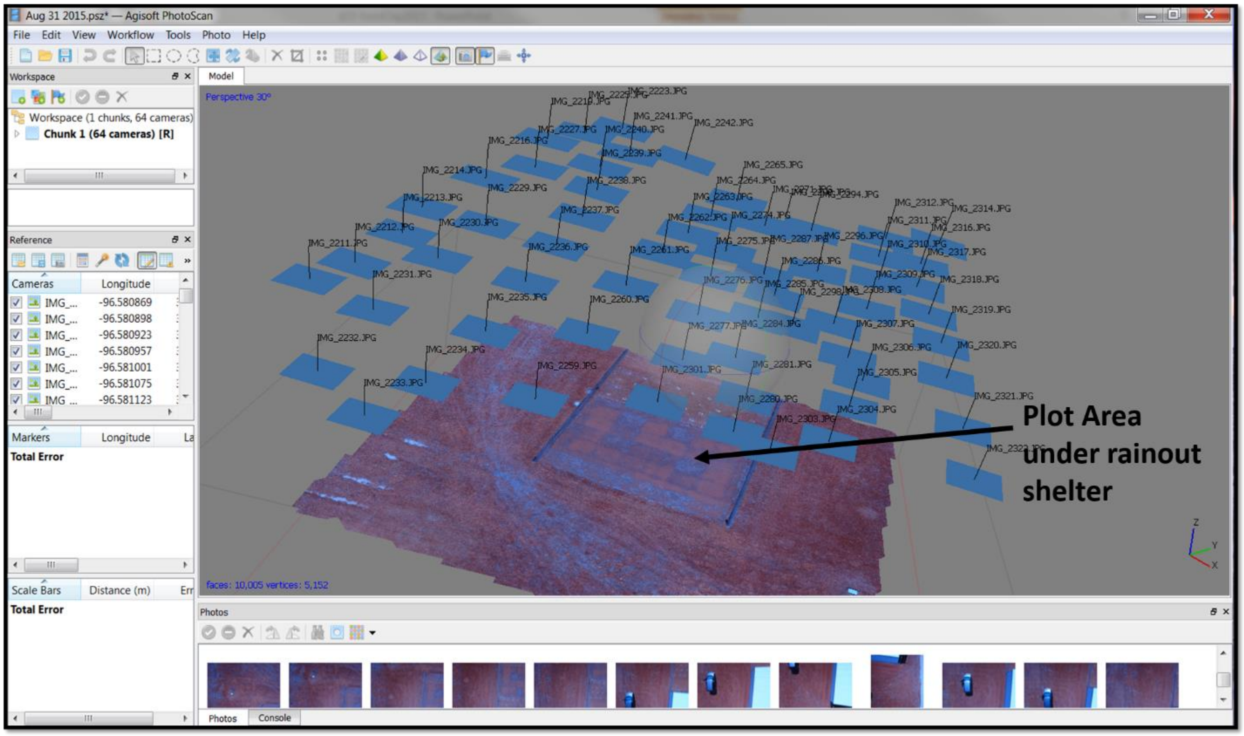



**Figure 2**. Building a model of the plot surface area using digital images taken from sUAS. Blue squares indicate position of camera during flyover of plots. Multiple images were “stitched” together to minimize angle effects and create an orthomosaic image, from which vegetation indices were developed.

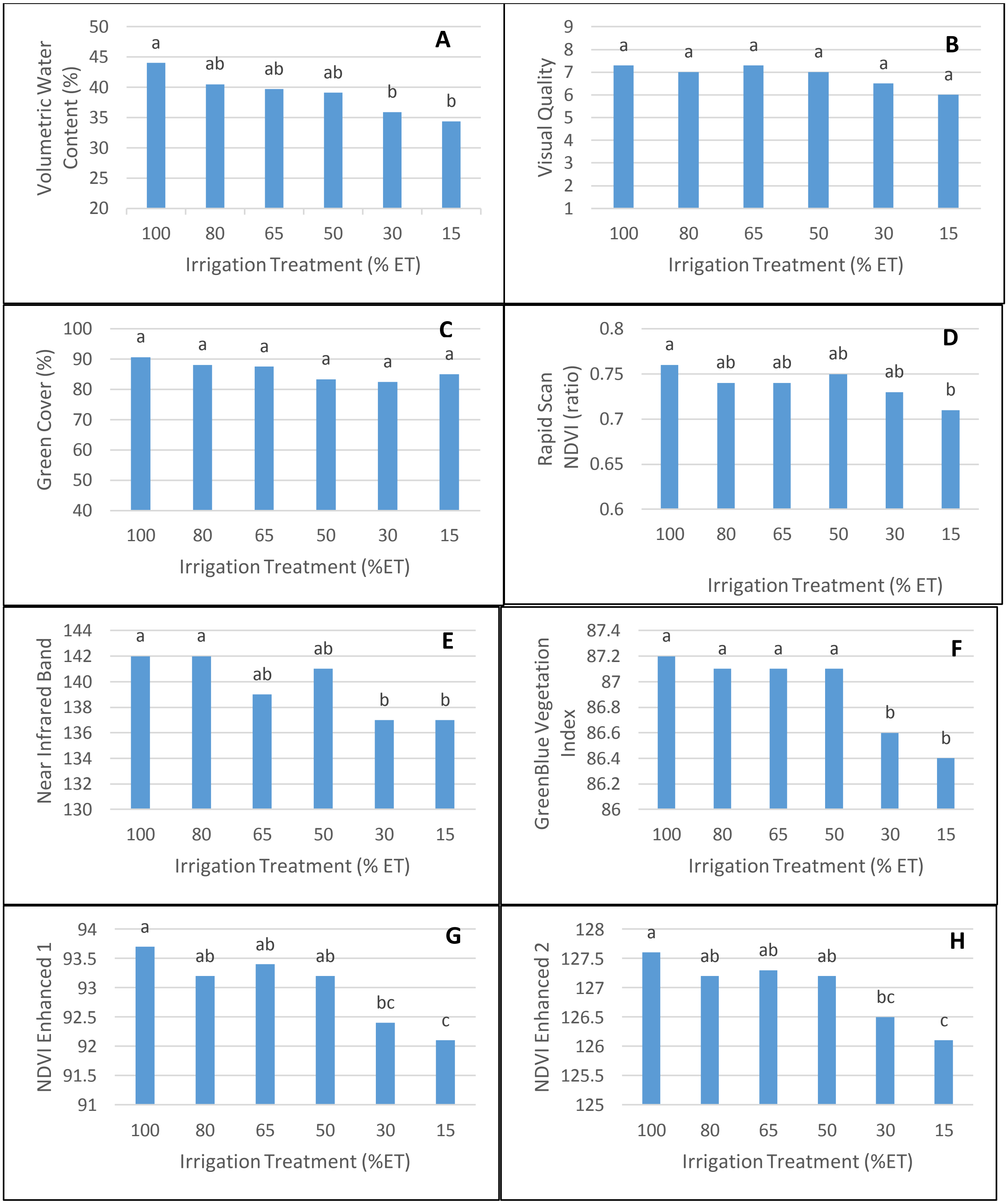
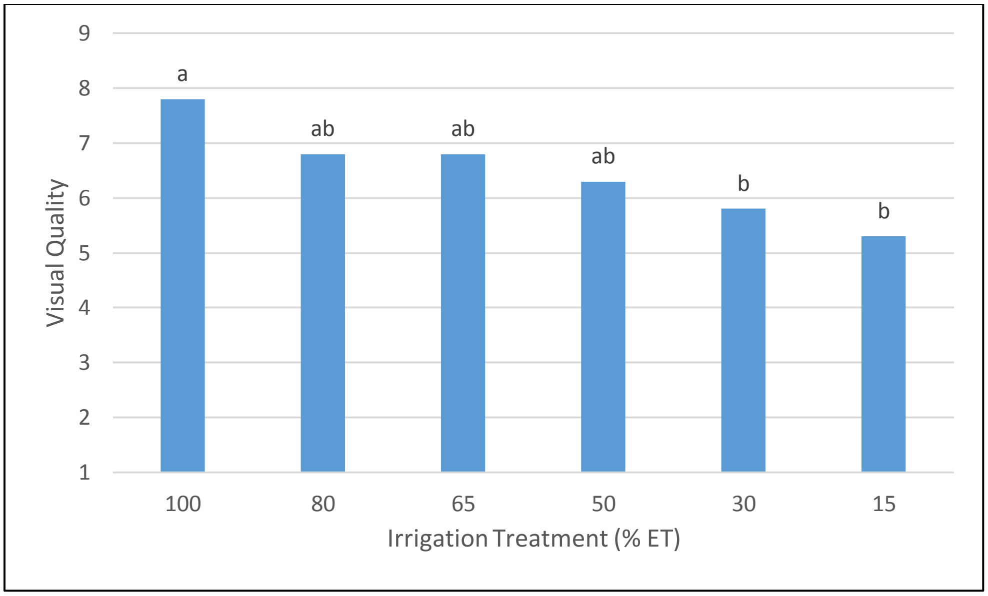



Figure 3. Measurements on 7 July (7 days after initiation of irrigation treatments) of volumetric soil water content (A); visual quality (B); percentage green cover (C); NDVI with handheld instrument (D); near infrared (E); GreenBlue vegetation index (F), Enhanced 1 vegetation index (G); and Enhanced 2 vegetation index (H); D through E were obtained from modified digital camera mounted on the sUAS.



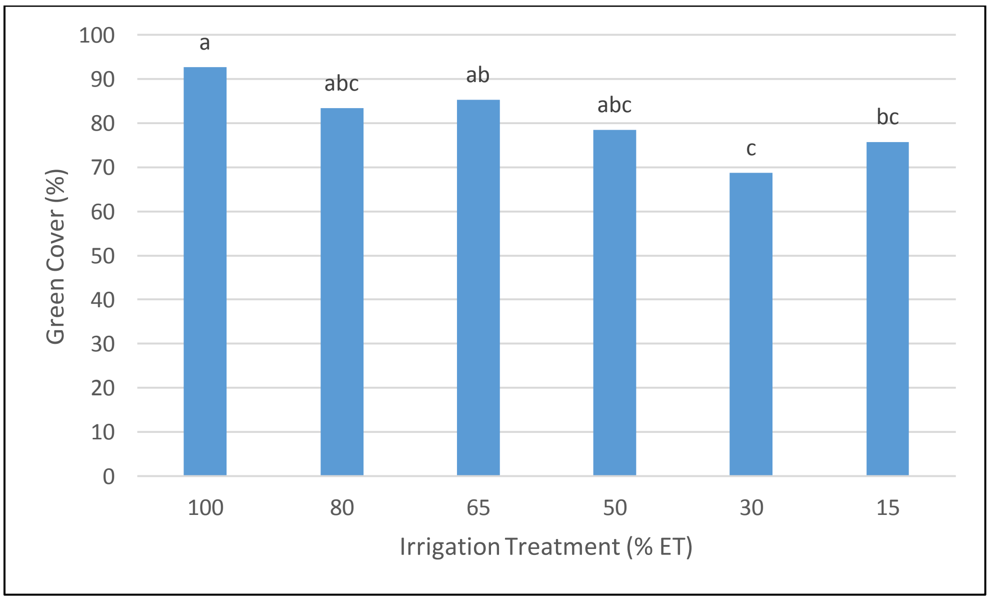



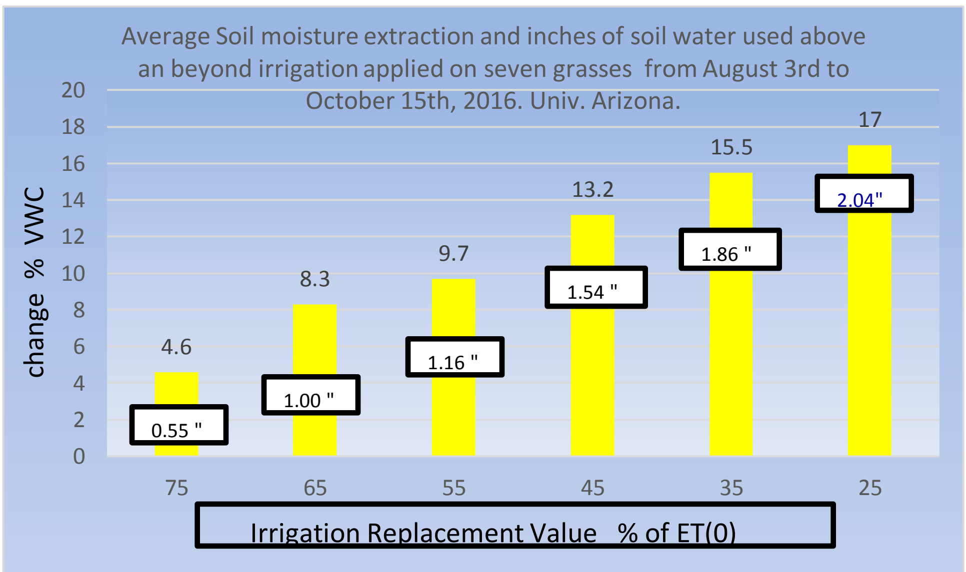

Figure 4. Measurements on 15 July (15 days after initiation of irrigation treatments) of visual quality (top); and percentage green cover (bottom). Trends were similar to soil moisture, near infrared band, and GreenBlue vegetation index 8 days earlier (7 July), indicating they were good predictors of visual quality and percentage green cover.

# "Low Maintenance Grasses for Water Conservation"

D.M .Kopec, J.J. Gilbert, N .Leitner and M .Pessarakli. University of Arizona.

Golf courses in the southwest remain under continuous pressure to reduce water use, even though significant reductions in applied irrigation have been realized through the use of ET based irrigation, soil moisture sensor based irrigation scheduling, and improved sprinkler head nozzle technology. There are areas on both large acreage golf and target-style courses which may be targets for reduced water use. Such areas include (1) wide landing zones and (2) "off rough" areas on smaller sized target courses which are either desert, gravel or other non-playable surfaces. This project addresses the water use of seven low maintenance warm season grasses which may have the potential to be used on such areas. Three buffalograss cultivars representing three generations of turf-type development and four low maintenance bermudagrasses were established in 2015 in a Linear irrigation gradient (LIGA)design with 50 foot long plots, replicated 4 times. Irrigation ET replacement plots were 75%, 65%, 55%, 45%, 35% and 25% of on-site Reference ET using the standard Penman-Monteith equation. Turfs were mowed weekly at 3.0 inches and irrigated nightly. Plots were scored for color, quality, density and percent plot cover using the NTEP visual rating system. Chloropyll Index (C.I,.) values were taken using a Spectrum Technology Field Scout CM 1000 Chlorophyll meter, and digital estimates of percent plot cover was taking using the '*Canopeo*' smart phone application developed by Oklahoma State University. Volumetric water content was determined for the 12 inch soil depth on August 3$^{rd}$ and again on Oct 22$^{nd}$.
By the end of the trial in late October, all 3 seeded buffalograsses and 3 of the seeded bermudagrasses required 45% ET replacement to maintain quality (at least 5.0 minimum at all times), while seeded Wrangler bermudagrass did so at 35% ET replacement. In order to maintain a fully acceptable turfgrass (at least 6.0 or greater at all times) all 3 buffalograsses required 55% ET replacement, as did Cheyenne II and Wrangler bermudagrass. Jackpot required 65 ET replacement and Nu-Mex Sahara required 75% ET replacement. The amount of soil water extracted ranged from 2.04 inches for turfs irrigated at 25% of ET, to 0.55 inches for turfs irrigated at 75% of ET. Only at the 25% ET replacement level, was the grass effect significant for the amount of water extracted and total water use (irrigation applied" + soil water extracted" ). At 25% ET replacement, Wrangler and Jackpot bermudagrass used the least amount of water statistically (5.5 -5.7 inches), and also extracted the least amount of soil water (1.7-1.9 inches). At all other ET replacement levels, there was no

significant difference between grasses for total water use or amount soil moisture extracted ( from August 3rd to October 22nd). Since there were differences in quality among grasses within ET replacement levels, other plant factors would be involved in determining over all turf appearance (quality) in response to differential amounts of applied irrigation. C.I. values were positively correlated with visual turfgrass estimates of percent plot green, and the turfgrass INDEX (sum of the NTEP scores for color, quality and density) as simple Pearson Product correlations = ranged from 0.79 -0.86, but *Canopeo* percent plot cover values were not strongly correlated with (C.I) values, or any visual parameters at all.




Summary of Environmental condition for LIGA implementation phase, August 3rd to Oct 15th, 2016. Linear irrigation plots , Karsten Turf Center, University of Arizona.

| Parameter | August | September | October |
|---|---|---|---|
| # Days in trial period | 28 | 30 | 15 |
| Ref ET(o) (inch) | 6.89 | 6.4 | 15.75 |
| Air Tmax / Tmin (F) | 96 / 71 | 92 / 65 | 91 / 50 |
| Soil avg. temp@ 4 inch (F) | 82 | 75 | 68 |
| Rain (inch) | 3.79 | 1.08 | 0.31 |
| # Days 100 F or more | 7 | 4 | 0 |
| # Days 95 F or more | 20 | 12 | 3 |
| # Days 90 F or more | 28 | 22 | 10 |
| # Days 85 F or more | 28 | 28 | 15 |

Ref ET(0) = Penamn - Monteith equation, on site station. Total Ref ET(0) fro 73 day trial = 15.78 inch.

Rain = rainfall in inches. Rainfall subtracted from Reference ET(0).

Table 10. ET replacement irrigation level which maintained 7 seeded low maintenance grasses at **marginal** turfgrass quality (5.0 or greater) after 70 days of differential irrigation using the Linear irrigation gradient (LIGA), August 3rd to August Oct 15th, 2016, Univ. Arizona.

| | | ET replacement Level | | | | | |
|---|---|---|---|---|---|---|---|
| | | 25% | 35% | 45% | 55% | 65% | 75% |
| Grass | Cultivar | | | | | | |
| *Buffalograss* | Bison | | | [5.5] | | | |
| | TopGun | | | [5.5] | | | |
| | SunDancer | | | [5.5] | | | |
| *Bermudagrass* | Nu-Mex Sahara | | | [5.0] | | | |
| | Jackpot | | | [5.5] | | | |
| | Cheyenne II | | | [5.0] | | | |
| | Wrangler | | [5.3] | | | | |

ET replacement value = Percentage of Reference ET(0) from on site weather station using standardized Penman Monteith equation. Total ET (0) from August 3rd to Oct 22nd = 16.04 inches

Quality value =(1-9) 1= dead, 4=poor, 5=marginal, 6= fully acceptable, 9= best possible.
Values are the mean of replications per each grass/ET replacement level combination.
Value in parenthesis is the grass mean turfgrass quality score at end of trial on October 15th, 2016.

Table 11. ET replacement irrigation level which maintained 7 seeded low maintenance grasses at **fully acceptable** turfgrass quality (6.0 or greater) after 70 days of differential irrigation using the Linear irrigation gradient (LIGA), August 3rd to August Oct 15th, 2016, Univ. Arizona.

| | | ET replacement Level | | | | | |
|---|---|---|---|---|---|---|---|
| | | 25% | 35% | 45% | 55% | 65% | 75% |
| Grass | Cultivar | | | | | | |
| *Buffalograss* | Bison | | | | [6.3] | | |
| | TopGun | | | | [6.0] | | |
| | SunDancer | | | | [6.3] | | |
| *Bermudagrass* | Nu-Mex Sahara | | | | | | [6.5] |
| | Jackpot | | | | | [6.5] | |
| | Cheyenne II | | | | [6.3] | | |
| | Wrangler | | | | [6.3] | | |

ET replacement value = Percentage of Reference ET(0) from on site weather station using standardized Penman Monteith equation. Total ET (0) from August 3rd to Oct 22nd = 16.04 inches

Quality value =(1-9) 1= dead, 4=poor, 5=marginal, 6= fully acceptable, 9= best possible.
Values are the mean of replications per each grass/ET replacement level combination.
Value in parenthesis is the grass mean turfgrass quality score at end of trial on October 15th, 2016.

JPEGS

DSCN0568

Outside edge of multiple cultivar entries within the LIGA.  Note droughted / low quality turf towards end of plots which receive reduced irrigation.

DSCN0569

Side view of plot with outside edges exhibiting poor quality from low ET replacement  treatments.

DSCN0570:

Side view of plot with outside edge of plot producing lower quality turfs from mid to low ET replacement treatments.

# Low-Input New Groundcover and Native Grass Species for Turfgrass Replacement in the Low Desert

Kai Umeda and Worku Burayu
University of Arizona
December 2016

Objectives:
Evaluate and compare the adaptation and performance of native grass species and a new groundcover, Kurapia (*Lippia nodiflora*), in the southwest United States low desert arid region as a low input turfgrass replacement in non-play areas of golf courses.

Generate local research-based information on the feasibility of growing Kurapia and the native grass species by properly assessing their interactions with insect pests and weeds, water and fertility requirements.

Increase the awareness of stakeholders about the characteristics of Kurapia and native grass species for low water use requirements and potential water saving capacity.

Start Date: 2016
Project Duration: 3 years
Total Funding: $45,000

Golf courses in the southwest United States are affected by poor water quality and quantity for managing turfgrasses. To reduce water use on golf courses, the use of lesser input plant species in golf course landscapes is gaining interest. This project investigates native grass species and a new groundcover as a low input turfgrass replacement in non-play areas of golf courses. Eleven plant species were evaluated - nine grass species, a native forb, and an introduced horticultural groundcover (Table 1). Eight grasses are native to the southwest and one is an introduced forage grain from Africa. A laboratory experiment was conducted to determine the germination rate of the seeds of plant species. Two field trials were conducted and plant species seeded in 6 ft by 6 ft plots arranged in a randomized complete block design with three replicates in non-play areas of two golf courses: Camelback Golf Club in Scottsdale, AZ planted on 31 May 2016 and Whirlwind Golf Club planted on 1 June 2016 in Chandler, AZ. Field plots were installed with overhead sprinkler irrigation to deliver optimal daily water to germinate and establish the crop stands. Data were collected for: laboratory germination; weekly field plant emergence; plant height, percent ground cover; and overall plant quality for color and vigor.

In the laboratory, *Eragrostis tef* (teff), *Eragrostis intermedia* (plains lovegrass), and *Hilaria rigida* (big galleta) began to germinate within 24 hours (Figure 2). *Sporobolus cryptandrus* (sand dropseed), *Sporobolus airoides* (alkali sacaton) and *Muhlenbergia asperifolia* (alkali muhly) began to germinate within 48 hours. Teff and plains lovegrass had germination rates of over 76%; *Bouteloua gracilis* (blue grama) exhibited 40% germination; and sand dropseed, big galleta, alkali sacaton and alkali muhly showed less than 10% germination within 7 days. *Bouteloua dactyloides* (buffalograss), *Sporobolus contractus* (spike dropseed), and the forb *Zinnia acerosa* (desert zinnia) failed to germinate within 21 days under room temperature conditions.

Field experiments showed all plant species except desert zinnia establishing a stand at Camelback Golf Club. At Whirlwind Golf Club, all species, except teff, failed to emerge or to establish. At Camelback, teff emerged within 3-5 days and there was better than 60% stand establishment at 1 week after seeding (WAS) (Figure 3). Big galleta emerged over a 3-week period to establish a 40% stand. Buffalograss and the dropseeds were very slow to establish a stand. Most of the grasses and Kurapia, that was plugged, covered the surface area of the plots within 5 to 8 WAS (Figure 4). Sand dropseed, plains lovegrass, and teff grew to a height of more than 25 inches at 8 WAS (Figure 5). Kurapia and buffalograss grew no more than 2 and 5 inches in height, respectively. Before first mowing in early July, all plant species exhibited good quality and vigor. Late summer observations showed that Kurapia was very aggressive and vigorous as a groundcover. All of the native grasses performed at varying and acceptable levels to establish and provide surface area coverage throughout the late summer (Figure 6).

Summary Points:

- Nine native and two non-native plant species were evaluated to provide ground cover under sprinkler irrigation.

- All plant species, except desert zinnia, emerged and established well under field conditions.

- Grasses and groundcovers germinated, established, and provided varied surface area coverage.

- Kurapia exhibited the most vigorous and aggressive growth when grown from plugs under optimal irrigation.

Table 1. List of native grasses and groundcovers evaluated in the low desert Arizona at Camelback Golf Club, Scottsdale, AZ, 2016.

| Common name | Scientific name | Seed rate/Acre |
|---|---|---|
| Alkali sacaton | *Sporobolus airoides* | 3.0 lb |
| Alkali muhly | *Muhlenbergia asperifolia* | 1.2 lb |
| Blue grama | *Bouteloua gracilis* | 4.0 lb |
| Buffalograss | *Bouteloua dactyloides* | 218 lb |
| Teff | *Eragrostis tef* | 5.0 lb |
| Plains lovegrass | *Eragrostis intermedia* | 1.00 lb |
| Big galleta | *Hilaria rigida* | 174 lb |
| Sand dropseed | *Sporobolus cryptandrus* | 1.0 lb |
| Spike dropseed | *Sporobolus contractus* | 1.0 lb |
| Desert zinnia | *Zinnia acerosa* | 2.2 lb |
| Kurapia | *Lippia nodifora* | 43,560 plugs* |

*Kurapia plugs planted on 12-inch spacing in 6 ft by 6 ft plots.

Figure 1. Performance of native grasses and groundcovers under sprinkler irrigation at 7 weeks after seeding (WAS) in Scottsdale, Arizona, 2016.

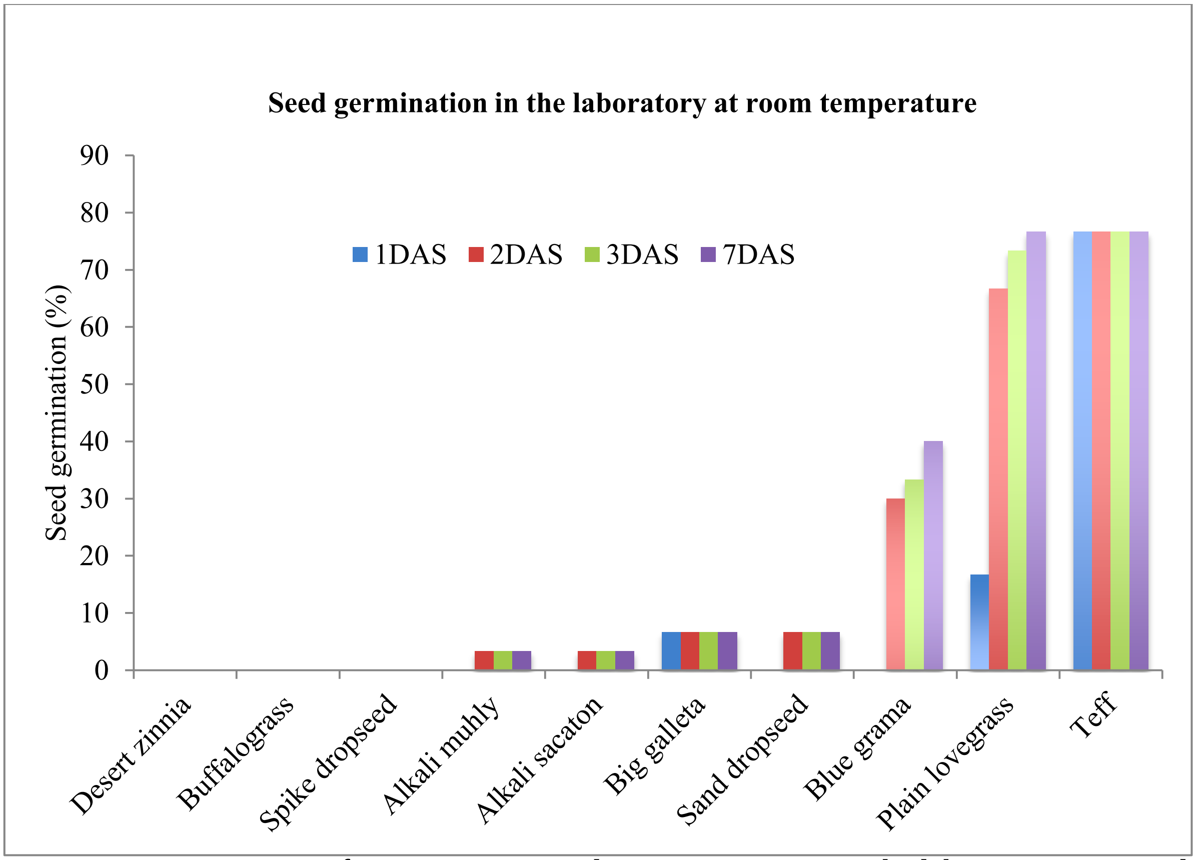



Figure 2. Determination of native grasses seed germination rates in the laboratory at intervals of days after seeding (DAS), June 2016.

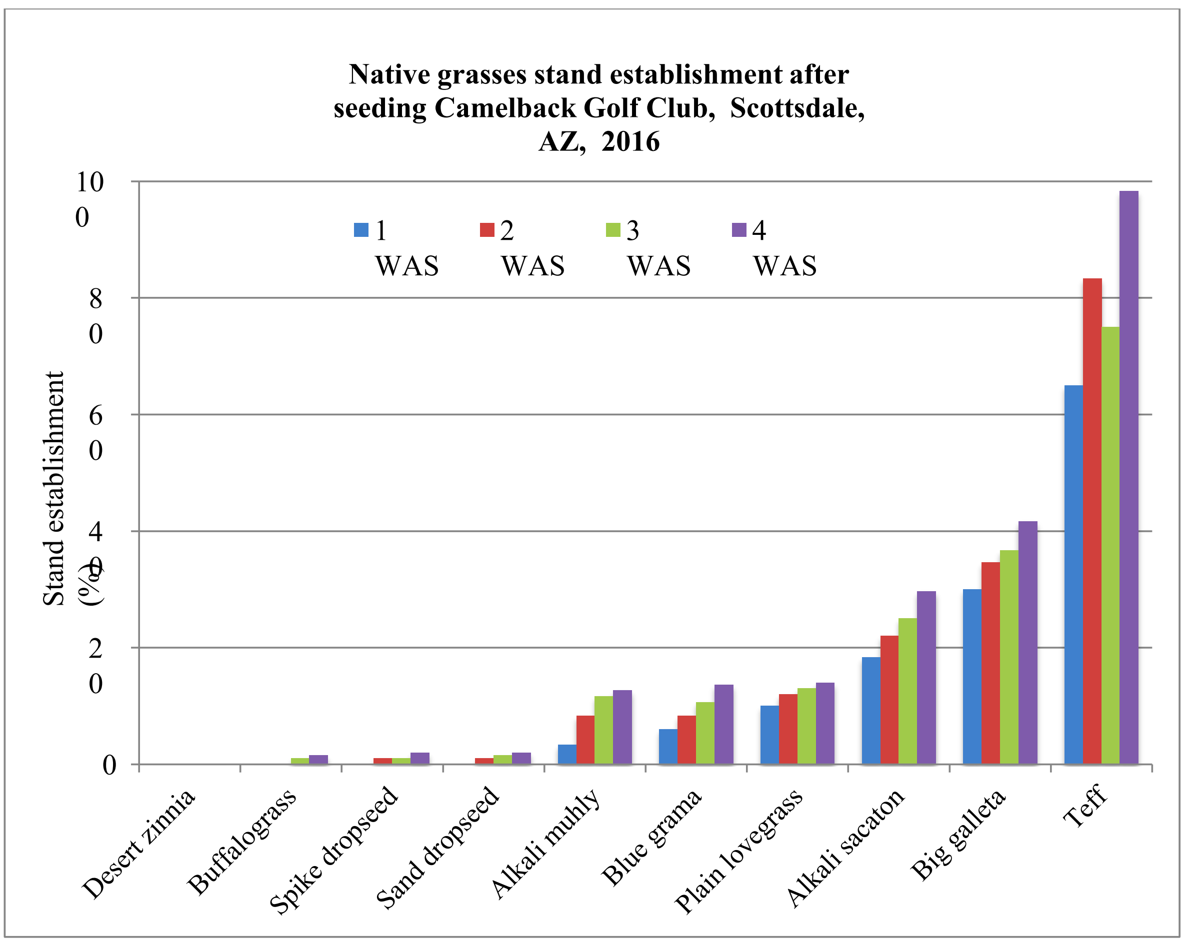



Figure 3. Germination and emergence of 10 plant species in the field for 4 weeks after seeding (WAS) to establish a stand.

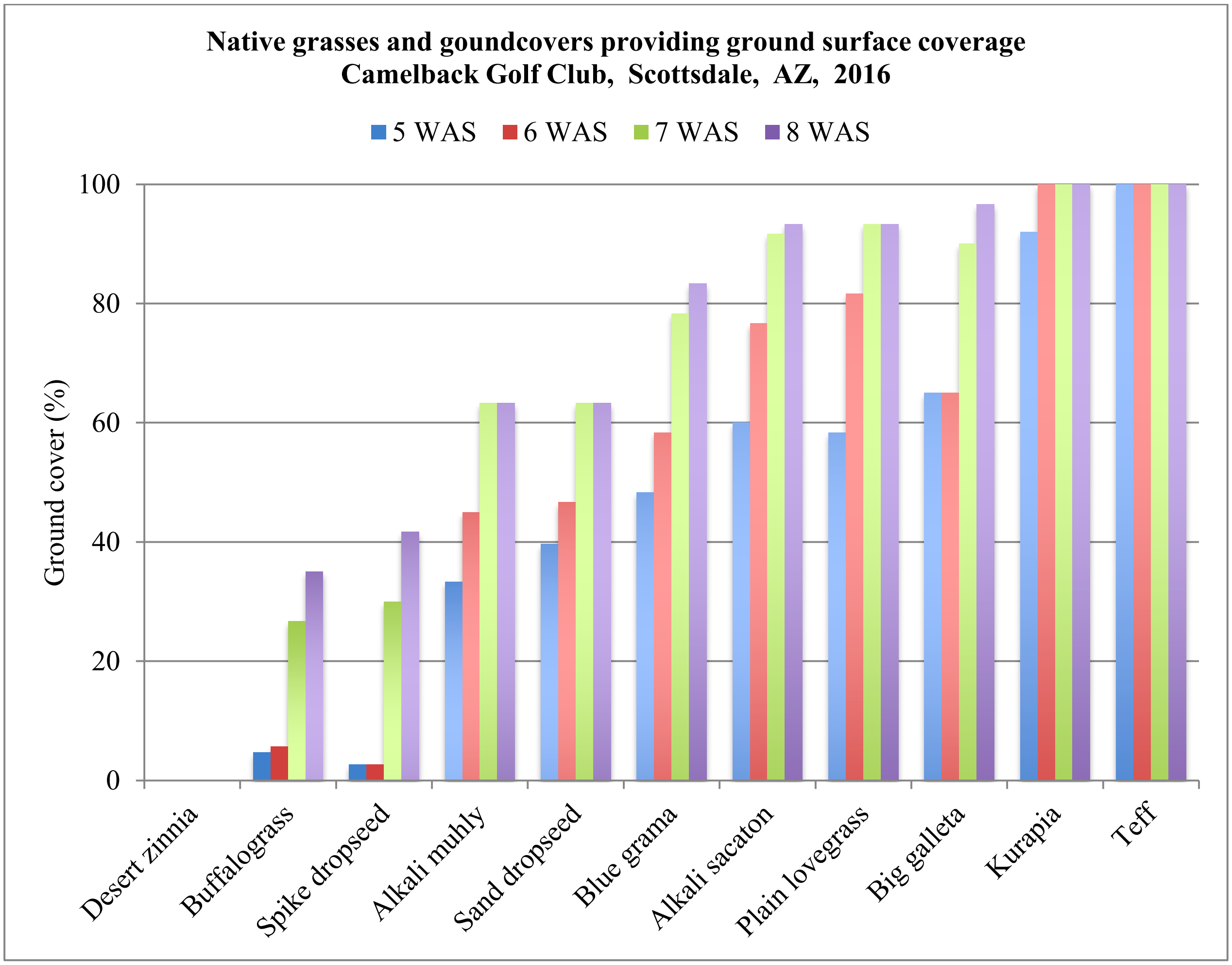



Figure 4. Performance of 11 plant species to provide ground surface coverage at intervals of weeks after seeding (WAS). Kurapia was planted as plugs.

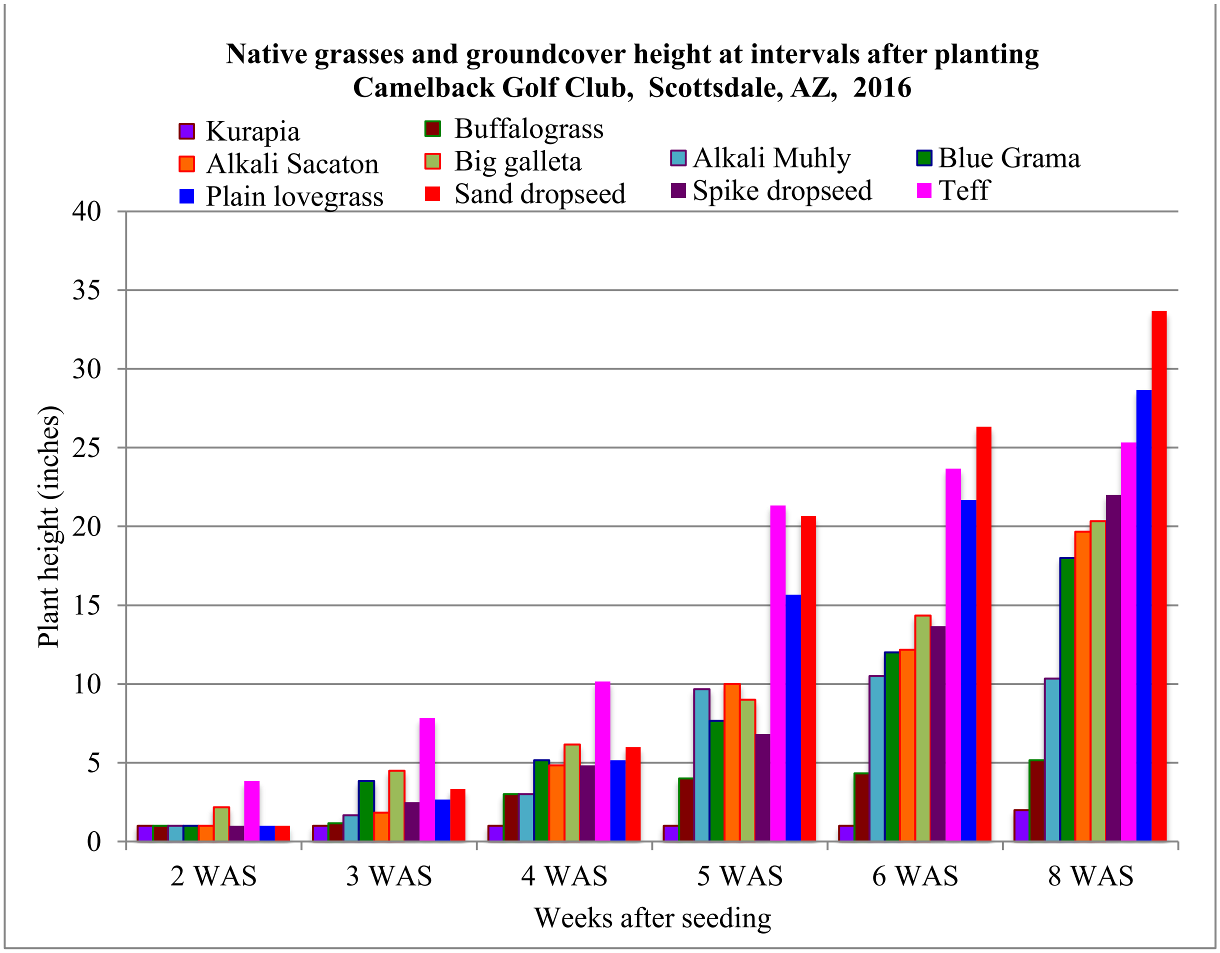



Figure 5. Native grasses and kurapia groundcover heights before first mowing.

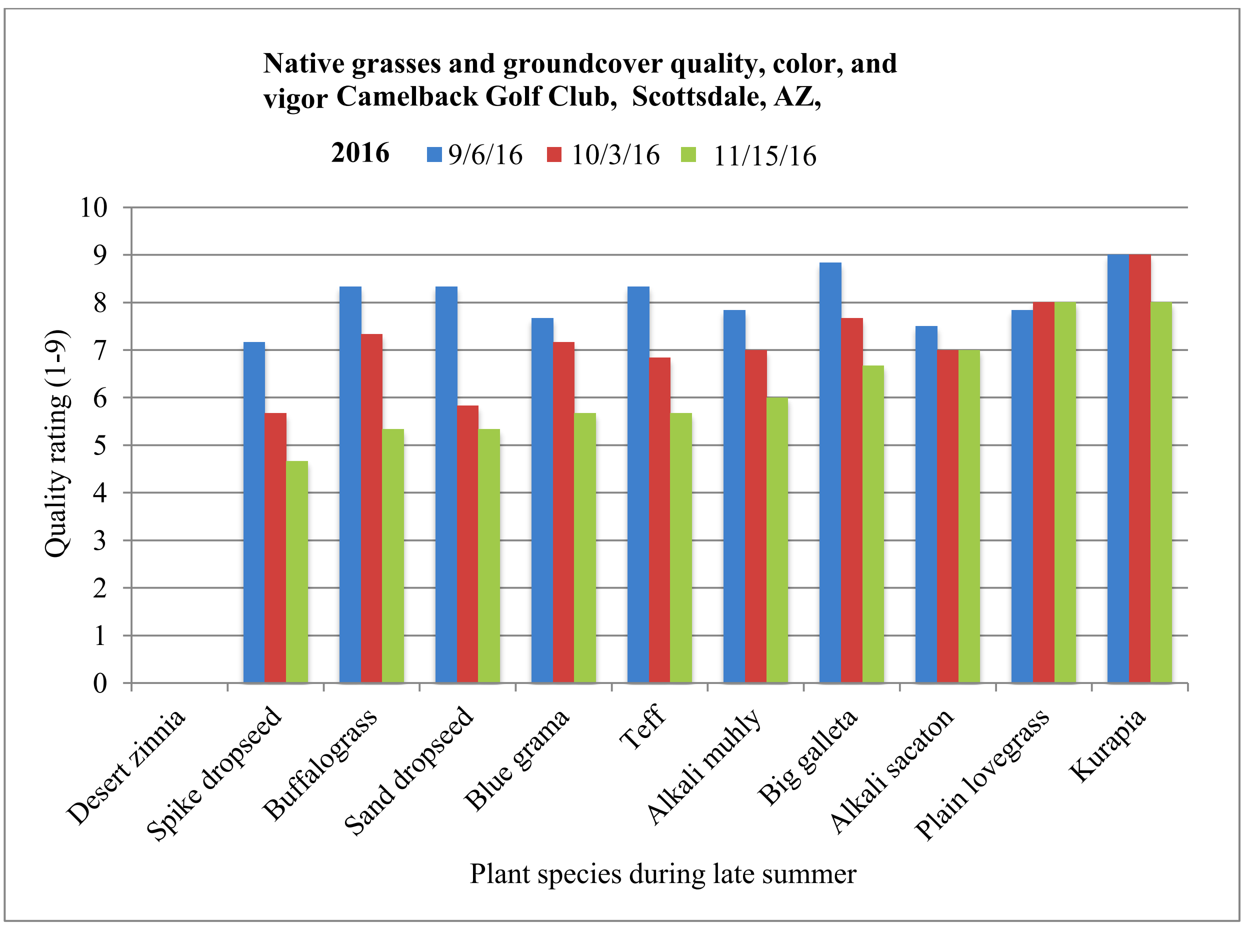



Figure 6. Native grasses and groundcover appearance at intervals during the late summer following three mowing events.

Figure 7. Native grasses and groundcover performance under sprinkler irrigation at 4 weeks after a third mowing on 12 October 2016 and under deficit irrigation at once per week.

USGA Semi Annual Research Report

# Assessing Tree to Grass Water Use Ratios: Significance to the Golf Course Industry

Dr. Dale Devitt, School of Life Sciences, Univ. Nevada Las Vegas

Since receiving funding from the USGA in support of our study entitled “Assessing Tree to Grass Water Use Ratios: Significance to the Golf Course Industry” we have made significant progress in initiating the research and addressing the research objectives stated in the proposal. First and foremost we selected a qualified student (Ms. Tamara Wynn) to undertake this project as part of her M.S degree in Biology at UNLV. We were fortunate to have a well- established tree research experimental orchard (10 species all planted approximately 20 years ago) that we could incorporate into the study and 12 non weighing lysimeters to use in estimating evapotranspiration of four turfgrass species.

## Site Development

Although we had an established orchard of landscape trees we had to trench to a depth of 120 cm in both a N-S and E-W direction to isolate each tree to make sure no roots from a given tree were accessing water from an area below a neighboring tree. Many roots were cut but very few were found below a depth of 30 cm. After the trenches were cut, a tractor was used to refill the trenches and smooth out the surface around the trees. Basins were then built up around each tree that could hold the weekly irrigation volumes. We cored into the soil of each basin and installed a theta probe access tube which would allow us the ability to lower a theta probe to a depth of 100 cm. In one of the three replicates of each species we also installed a deep time domain reflectometry probe at 150 cm to assess deep soil moisture that might reflect a drainage component. All trees received a single yearly application of mixed fertilizers during early spring and they will continue to receive this yearly amount throughout the duration of the study.

## Sensor Installation

Each of the 30 trees selected for the study were equipped with 10 mm Granier probes to assess transpiration velocity within the xylem. Bark was gently removed with sand paper and holes were drilled for the combo sensors. All probes were then sealed with putty, surrounded with foam and insulated with a foil wrap. Cables from the 30 trees were run through flexible plastic tubing to protect the cables from rodents. All of the cables were routed to a data logger where they were wired into a multi-plexer. A program was written to collect and parse the data into a meaningful table. To convert the velocity measurements into a flux we conducted a dye experiment in which we drilled a ¼ inch hole to the center of selected trees, mounted a stopper funnel system that allowed red dye (sanfranin) to slowly move into the horizontal hole. We added additional dye on a daily basis and continued the dye application for a one week period. At the end of one week, we removed the stopper funnel system and cored a parallel hole 2 cm above where the dye entered. Intact cores were dried mounted on wood and sanded to assess dye movement. We will measure the distance the dye penetrated and use this length to estimate the conductive tissue area in each tree, which will allow us to convert velocity to flux measurements.

## Lysimeters

We have 44 non weighing lysimeters installed at our center for use in quantifying evapotranspiration. The lysimeters are 60 cm in diameter and 120 cm in depth with a theta probe access tube inserted at the center position. We selected 12 of these for use in quantifying evapotranspiration of turfgrass. In a large tall fescue plot we selected three lysimeters to remain as tall fescue. Three of the Isyimeters in this area were converted to perennial ryegrass. In another research plot planted to bermudagrass we selected three Isyimeters to remain as bermudagrass and three were converted to bentgrass. All grasses receive 0.5 pounds of Nitrogen per 1000 square feet on a monthly basis

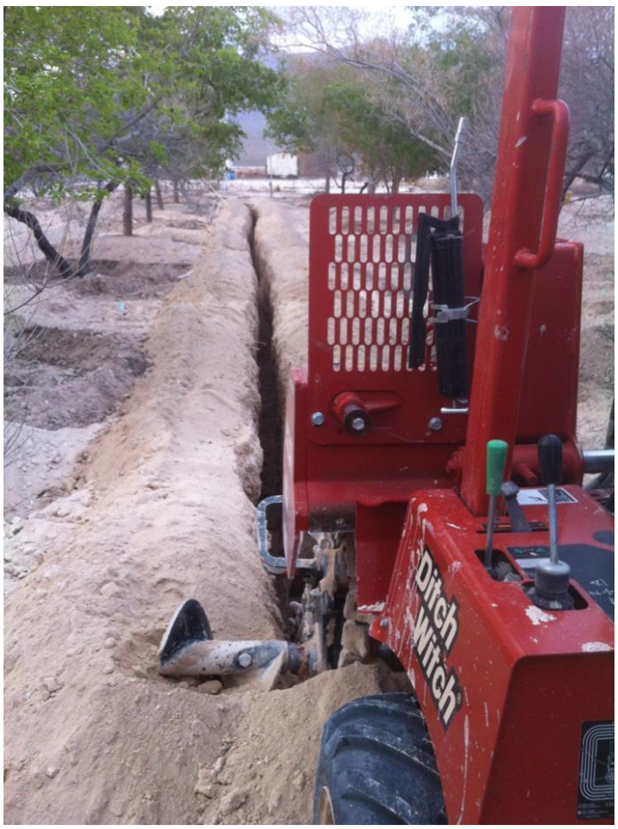
Irrigations and water balance

We initiated the study in May of 2016 and began applying irrigations to the tree basins and lysimeters planted to turfgrass. The goal has been to irrigate in a way that does not contribute to a drainage component that would have to be included in the hydrologic balance. As such we irrigate based on the previous weeks evapotranspiration rate, where ET = Input-Output-Change in Storage. In the tree basins we make weekly soil volumetric water content estimates at 10, 20,30,40,60 and 100 cm, enabling a weekly soil water in storage estimate. We also take soil volumetric water content estimates at 150 cm to monitor any deep soil water movement (no movement has been observed as of this report). Irrigation water is applied to the tree basins from an irrigation hose that is equipped with an accurate flow meter to estimate irrigation volume in liters. Lysimeters containing turfgrass are hand watered by measuring irrigation volumes with a graduate cylinder and then transferring that water to a watering can where the water is applied in a uniform fashion to each lysimeter. In the case of the ryegrass and bentgrass we had difficulty establishing and maintaining 100 % cover in all of the lysimeters, especially during the heat of July in which temperatures remained above 115 F for a one week period. Because of these problem we had to abandon the standard water balance approach and give additional water to help maintain the two grass species. This led to over irrigation and drainage build up. We are currently evacuating drainage via a drainage system that is based on ceramic extraction cups placed at 110 cm depth that are hooked to a vacuum extraction system. We are hopeful with cooler temperatures in fall that we can get good establishment of the ryegrass and bentgrass and get the drainage volumes back to very low values.

Evapotranspiration

We now have 19 weeks of ET estimates for the 30 trees based on the hydrologic balance approach. There is currently a significant amount of variability in the ET estimates when comparing replicates. This variability was expected since no two trees are identical. We will begin to normalize the ET estimates based on canopy volumes, basal canopy areas, trunk diameters, tree heights and leaf area index measurements to see if we can minimize this variability. Most of the trees show an increase in ET from late spring to summer with higher values holding throughout the summer period. We are looking forward to the fall measurements to see how the tree ET adjusts back down under lower environmental demand and how this will relate to the response observed with the turfgrass. Turfgrass ET has also revealed a similar temporal response. Once we normalize the tree ET values we will begin to compare the ET between the trees and turfgrass. The raw data suggest a 20 fold difference in the weekly ET when comparing the trees (higher) with the turfgrass (lower). The basal canopy area of the trees is not 20 fold larger than the turfgrass area which would suggest tree to grass water use ratios that would clearly favor the trees. We will finalize the normalization process during the next few months, enabling us to report on actual tree to grass water use ratios for each tree grass combination. We will also finalize our estimates of conductive xylem tissue area to convert the transpiration velocities into flux estimates. This process will also allow us to compare the sap flow approach with the hydrologic balance approach.

Surveying 100 Tree Site

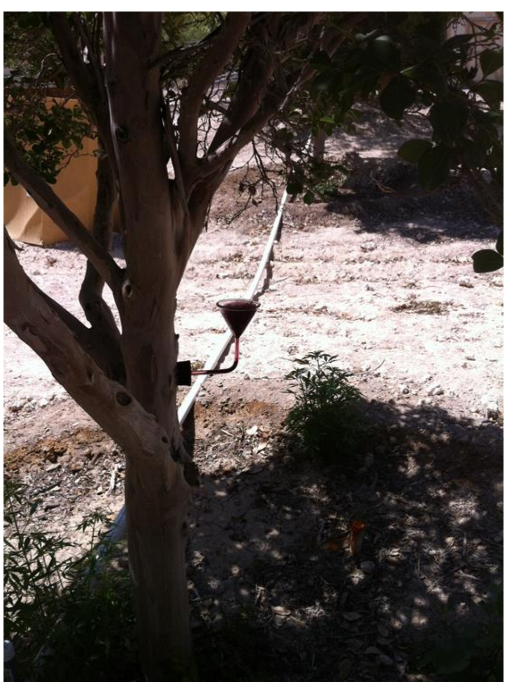


Trench dug to 120 cm to cut roots, isolating each tree

Roots observed between plots, almost entirely in the upper 30 cm

Basins built around each tree to hold irrigation water

Granier probes installed and covered with protective insulation.



Dye (sanfranin) injected into the xylem to assess the area of conductive tissue.

All irrigations applied with an accurate recording meter


Data logger with cables from 30 granier probes installed in the 10 species (3 replicates).

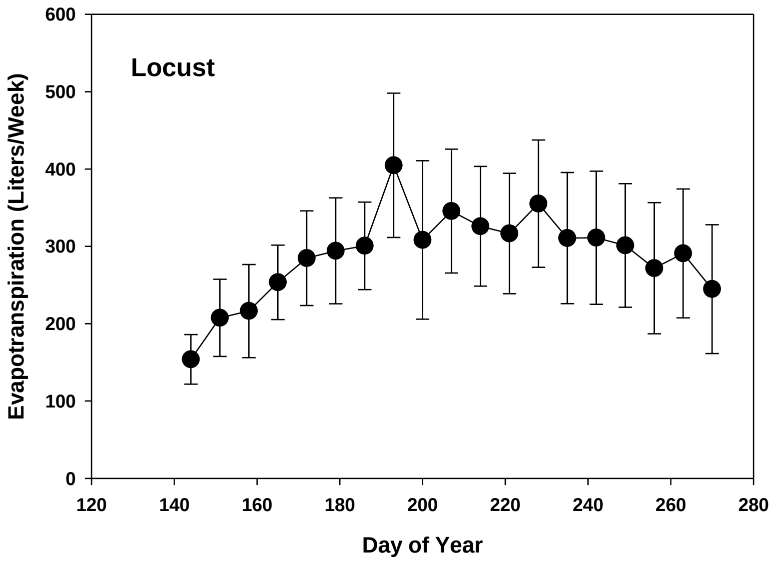

Cores taken above the point of dye injection to assess the area of conductive tissue.

Leaves of each species harvested for leaf area.

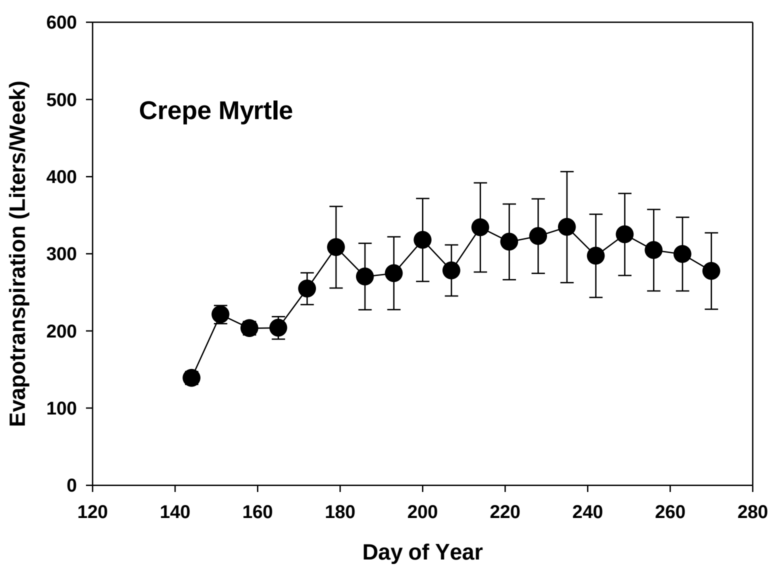
Tree heights, canopy volumes, basal canopy areas measured on each tree.

Tall Fescue

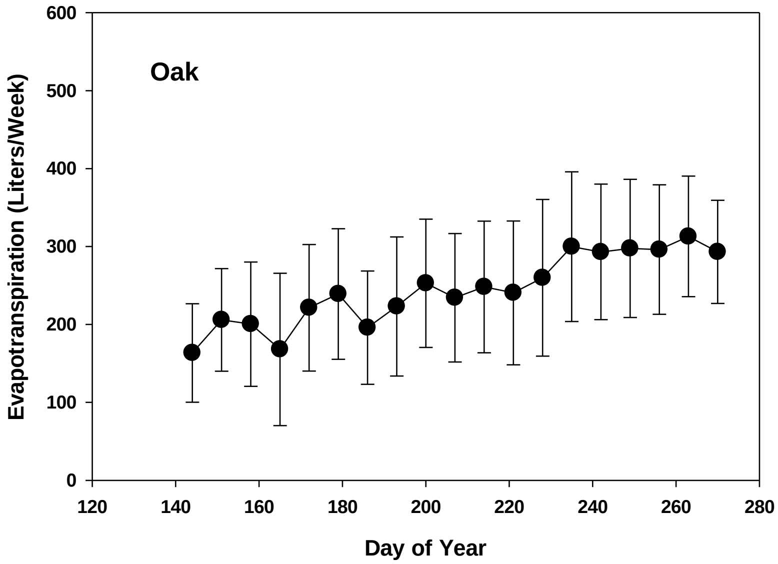
Ryegrass



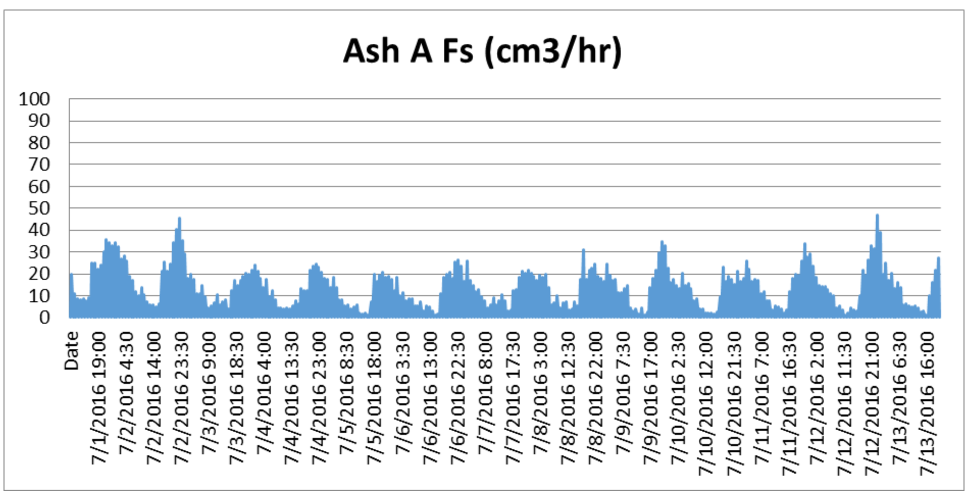

Evapotranspiration of Locust measured on a weekly basis with the hydrologic balance approach.




Evapotranspiration of Crepe Myrtle measured on a weekly basis with the hydrologic balance approach.




Evapotranspiration of Oak measured on a weekly basis with the hydrologic balance approach.




$cm^3$/hr

Sap flow (Granier probe) of Ash on a daily basis in mid-July of 2016.

# NATIONAL EVALUATION OF COOL-SEASON TURFGRASS WATER USE AND DROUGHT RESISTANCE

Progress Report, Feb. 21, 2017, The Lawn Institute

## ABSTRACT

This is an exciting new project that will evaluate, through a nationwide trial, Kentucky bluegrass and tall fescue for their water use and drought resistance. Data generated from this project will be used to identify, label and certify low-water using cool-season grass cultivars for use on lawns, parks, athletic fields and golf courses. We have been allocated $5,000 per year for three years ($15,000 total) to help with the funding of this project.

Kevin Morris, NTEP and Michael Kenna, USGA

# ANNUAL PROGRESS REPORT – FEBRUARY 2017

As discussed at last year's TPI Lawn Institute Research Committee meeting, the United States Golf Association (USGA) budgeted considerable funding to conduct a national water use and drought tolerance trial, utilizing the National Turfgrass Evaluation program (NTEP) as its evaluation organization. USGA is funding the building of rainout shelters and irrigation infrastructure at several locations, and is working with NTEP in determining testing protocols, data collection methods, etc. Besides data collection on water use and drought resistance parameters, the goal of this effort is for the EPA Water Sense® program to adopt these (or similar methods) and to agree to certify the first plant species with the Water Sense® label. USGA has become a Water Sense® partner and we have talked to the Water Sense® staff about certifying grasses and there is interest in this idea. EPA is very interested in the concept (they have never certified a plant or plants as water saving) as USGA Green Section Research Director Dr. Mike Kenna and I have met with them to discuss collaborative efforts. However, EPA needs to see more about the methods and tests, as well as we believe, some successful trials. Also, they will need our help in solving some legal requirements when certifying a product (could be unique for plants, however). Attachment A is our proposal that was submitted to WTSC last year and chosen for funding.

Since last year we have made considerable progress with this trial. We have assembled an advisory committee consisting of researchers, Turfgrass Water Conservation Alliance (TWCA), A-List representatives and other seed companies. The advisory committee has met via nine conference calls and has developed extensive protocols, including two approaches to evaluate drought (see Attachment D). The committee has also developed budgets for each approach. As it appeared that we would have sufficient entries and overall funding, we decided to go ahead with the establishment of a cool-season grass trial at 10 locations in fall 2016. One half of those locations use rainout shelters (Approach 1) and the other half utilize in-ground irrigation that will use Approach 2 (see Attachment B).

Because of space limitations (only around 30-35 entries can be accommodated), we limited the trial to only Kentucky bluegrass and tall fescue. We chose tall fescue over perennial ryegrass because of more interest from seed companies in submitting entries (than perennial ryegrass). Fortunately, when our deadline passed, we had received 32 entries (14 bluegrass, 18 tall fescue). We added three standards to the trial (one each of Ky. Bluegrass, tall fescue and perennial ryegrass). See Attachment C for the list of entries and sponsors.

Most locations planted the trial in fall 2016, while a few had to wait on infrastructure improvements (mostly irrigation), and therefore will plant in spring 2017. Planting plans were developed for both Approach 1 and 2 sites (see Attachment E). We intend to initiate drought treatments in 2017 on those locations with mature plots.

Rainout shelters have been ordered and these will be delivered this spring to each of the five Approach 1 sites. Installation will be performed by staff at each site with potential help from NTEP. As a part of the grant agreement, NTEP will return a portion of the funding allocated for rainout shelter purchases back to each researcher to help with installation and other initial expenses. Approach 2 sites will receive $15,000 initial set-up costs for irrigation installation and/or other expenses.

The cost to run each trial location is high, and only a portion of that will be covered by USGA's donation ($250,000). Entry fee levels were set at $8,000 for the cool-season trial, which netted us just past the 30 paid entries we could accommodate. However, we still needed other donations and to that end, we secured funding from the Washington Turfgrass Seed Commission ($105,000) and the Lawn Institute ($15,000). Our budget for this trial can be found in Attachment F. We sincerely appreciate the support received from The Lawn Institute for the initiation of this trial.

**ATTACHMENT A**

# *The Lawn Institute*
## *GENERAL RESEARCH GRANT APPLICATION*

(Applications must be submitted on this form or exact duplicate **(2 page max)**. Email copies accepted in Microsoft Word only. Use no smaller than 10-point type.)

**Project Title: USGA/NTEP National Water Use Trial – Cool-Season Species**


**Principal Investigator(s): Kevin N. Morris, Michael P. Kenna**

**Institution: National Turfgrass Evaluation Program, United States Golf Association Green Section**

**Address: (NTEP) BARC-West, Bldg. 005, Rm. 307, Beltsville, MD 20705**
**(USGA) P. O. Box 2227, Stillwater, OK 74076**

**Phone: 301-504-5125 (KM), 405-743-3900 (MK) Email: kmorris@ntep.org, mkenna@usga.org**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**Statement of problem or issues to be addressed by this project:** Turfgrass is being scrutinized for its water use, leading to restriction or replacement of turf in some areas, most notably California. Water utilities, looking to reduce outdoor water use, have led the charge to encourage homeowners, municipalities and businesses to reduce or replace turf, often by offering financial incentives. The turfgrass industry has been working to develop cultivars that use less water, but there is a need to evaluate, on a national scale, actual water use rates of these new cultivars. We propose establishing such a trial at multiple locations, using cool-season grass species to document water savings of new cultivars under rainout shelters and zone-level irrigation systems. In addition, parameters will be established in conjunction with EPA Water Sense®, or a similar organization to certify (or label) those grasses that meet the water saving/efficiency criteria. This EPA Water Sense® (or other organization) certification will allow breeders, seed companies, sod growers and others in the industry to market their products as 'water saving', hence not all grasses are equal in their water use. We feel this labeling effort will help to 'destigmatize' turf and allow for the use and promotion of water saving turfgrasses.

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**Proposed project completion date** (when results will be publicly available):

Results will be published as per normal NTEP policies; trial is completed in 2020, therefore, certification will be applied most likely in 2021

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**Descriptive summary of proposed research plan/project:** Research program will start in fall 2016 for *cool-season grasses*. Six to eight (or potentially more) locations will be utilized (depends on funding we receive from Lawn Inst, sponsor entry fees and other groups we solicit), with the locations split between Approach 1 (individual plot watering) and Approach 2 (zone level irrigation) - see following protocols. *Please note that the following protocols are a draft and may be modified somewhat, based on input and feedback from stakeholders.*

Rainout shelters will be built at 3-4 locations, particularly where summer rains are prevalent. Also, 3-4 sites will be built in drier locations, with four different irrigation zones, replicated twice, such that four irrigation levels (based on ET) can be implemented. Then, after plots are well established, Approach 1 (plot level watering) and Approach 2 (zone level irrigation) will be implemented at 3-4 sites each.

Approach 1 will measure the amount of water used by each plot for a set drought 'season' of approximately 100 days. Drying down to a prescribed level of green/brown cover will be the trigger to hand water a plot. Watering amounts will be recorded for each plot over the dry-down 'season'. Approach 2 will be ET based and measure quality attributes over time, as well as amount of water needed to achieve a desired level of quality. Both approaches will be repeated for three growing seasons.

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**Current knowledge about project topic/area** (include citations where appropriate)**:** This project is based on research conducted and published by Turfgrass Water Conservation Alliance members, Kansas State University, University of Arkansas, Texas A&M University and others (see references on page 2 of water use protocols below)

## ATTACHMENT A (page two)

**Significant potential benefits of project findings:** This project will be the first to document, at multiple sites, water use of cool-season cultivars, in inches per year needed to achieve a particular level of quality or green cover, compared to noted standard cultivars. This is important because: 1) new drought tolerant cultivars need to be compared with older standard cultivars for actual water use and ability to maintain quality and green cover, 2) we need to show municipalities, water utilities, regulators and others that drought tolerant cultivars are available with documented water use data and 3) having a certification program that identifies and labels drought tolerant cultivars is essential to the continued use of turfgrass on lawns and other areas where water use is a major concern.

In addition, it is important the turf industry come together to cooperate on a project such as this, demonstrating that reducing water use is important to this industry, as well as to show that we have been successful in reducing water use. For the Lawn Institute, it is highly beneficial to join forces with the USGA, NTEP and other groups we are talking with (Washington Turfgrass Seed Commission, Irrigation Assoc., etc.) concerning the importance of this project. This will put the Lawn Institute in a leadership position, aligning with other national groups to focus on turfgrass water use and conservation.

**Deliverables** (list form and date available of information or technical advancements resulting from this project, i.e., peer-reviewed journal article(s); trade magazine article(s); educational talk(s); technical manual(s);

Data from each of three years collected, summarized and published on the NTEP web site, educational talks and articles for USGA, TPI and other organizations, successful certification of at least some entries by the chosen certifying organization or agency (EPA Water Sense® or other)

**Technical qualifications and expertise of investigators necessary to accomplish this project:**

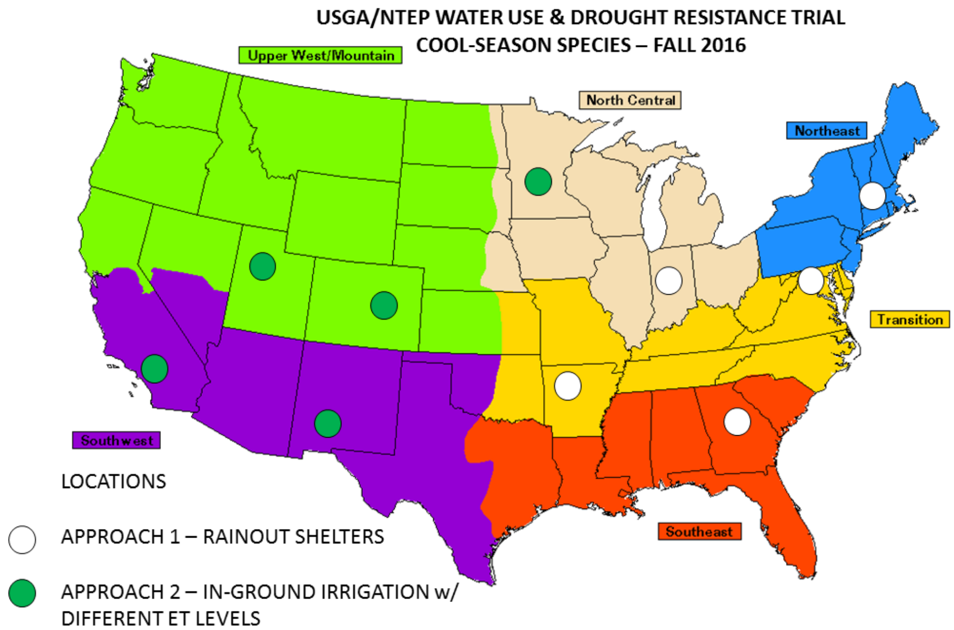
Kevin Morris, Executive Director, NTEP
Michael Kenna, Director of Research, USGA Green Section

**Budget Requirements (U.S. Dollars) (based on 8 total sites – 4 each of Approach 1 and 2))**

| | (Year) | | (Total Budget) | (Funds Requested from The Lawn Institute) |
|---|---|---|---|---|
| 1st | 2017 | | $ 145,426 | $ 10,000 |
| 2nd | 2018 | | $ 145,427 | $ 10,000 |
| 3rd | 2019 | | $ 145,427 | $ 10,000 |
| | | **Total** | **$ 436,280** | **$ 30,000** |

**Current or Pending Source(s) of Other or Additional Project Funds or Partners:** Source USGA (committed) Project Support $ 250,000
Source Wash. Turf. Seed Comm.Project Support $ 105,000
Source NTEP Project Support $ 51,280
**Total of "Other Support" $ 406,280**

# ATTACHMENT B




## USGA/NTEP Water Use & Drought Resistance Trial
## Cool-season grass locations

Approach 1
(restrict water
For 100 days)

Approach 2
(reduced
$ET_o$ levels)

Amherst, Massachusetts
College Park, Maryland
Griffin, Georgia
W. Lafayette, Indiana
Fayetteville, Arkansas

St. Paul, Minnesota
Las Cruces, New Mexico
Riverside, California
Logan, Utah
Ft. Collins, Colorado

# ATTACHMENT C

2016 National Cool-Season Water Use/Drought Resistance Test

Entries and Sponsors

| Entry No. | Name | Species | Sponsor |
|---|---|---|---|
| 1 | BAR PP 110358 | Kentucky Bluegrass | Barenbrug USA |
| 2 | Barrari | Kentucky bluegrass | Barenbrug USA |
| 3 | Everest | Kentucky bluegrass | Jacklin Seed by Simplot® |
| 4 | Blue Note | Kentucky bluegrass | Mountain View Seeds |
| 5 | Babe | Kentucky bluegrass | Seeds, Inc. |
| 6 | NAI-13-132 | Kentucky bluegrass | Columbia River Seed |
| 7 | NAI-13-14 | Kentucky bluegrass | Columbia River Seed |
| 8 | Blue Devil | Kentucky bluegrass | Columbia River Seed |
| 9 | Dauntless | Kentucky bluegrass | Columbia River Seed |
| 10 | PST-K13-137 | Kentucky bluegrass | Pure-Seed Testing, Inc. |
| 11 | PST-K13-143 | Kentucky bluegrass | Pure-Seed Testing, Inc. |
| 12 | PST-K15-169 | Kentucky bluegrass | Pure-Seed Testing, Inc. |
| 13 | PST-K11-118 | Kentucky bluegrass | Pure-Seed Testing, Inc. |
| 14 | PST-K13-141 | Kentucky bluegrass | Pure-Seed Testing, Inc. |
| 15 | Midnight | Kentucky bluegrass | Standard entry |
| 16 | SR 4650 | perennial ryegrass | Standard entry |
| 17 | BarRobusto | tall fescue | Barenbrug USA |
| 18 | BAR FA 121095 | tall fescue | Barenbrug USA |
| 19 | DLFPS 321/3677 | tall fescue | DLF Pickseed USA |
| 20 | DLFPS 321/3679 | tall fescue | DLF Pickseed USA |
| 21 | DLFPS 321/3678 | tall fescue | DLF Pickseed USA |
| 22 | Nonet | tall fescue | Jacklin Seed by Simplot® |
| 23 | GO-AOMK | tall fescue | Grassland Oregon |
| 24 | Supersonic | tall fescue | Mountain View Seeds |
| 25 | Titanium 2LS | tall fescue | Mountain View Seeds |
| 26 | Thor | tall fescue | Columbia Seeds |
| 27 | Thunderstruck | tall fescue | Columbia Seeds |
| 28 | RS4 | tall fescue | Landmark Turf & Native Seed |
| 29 | Kingdom | tall fescue | Site One Landscape Supply |
| 30 | MRSL TF15 | tall fescue | Site One Turf & Landscape Supply |
| 31 | Catalyst | tall fescue | Standard entry |
| 32 | Stetson II | tall fescue | Site One Landscape Supply |
| 33 | PST-5SDS | tall fescue | Pure-Seed Testing, Inc. |
| 34 | PST-R511 | tall fescue | Pure-Seed Testing, Inc. |
| 35 | LTP-SYN-A3 | tall fescue | Lebanon Seaboard Corp. |

## ATTACHMENT D

# July 25, 2016 version

**Trial details:**

1. Cool-season grass trials (two species) will be established in 10 locations for each species in fall 2016.

2. Data will be collected for three growing seasons: 2017, 2018 and 2019

3. Two approaches will be used:

   Approach 1 – individual plot watering and
   Approach 2 – zone level irrigation (see pages two and three for a description of each approach).

4. An equal number of rainout shelters and zone level irrigation plots will be built (see attached map and locations list). The rainout shelters will be utilized where summer rainfall is possible (and needs to be restricted).

5. Since plot space will be limited, the first priority for entries will include only Kentucky bluegrass and tall fescue. If space is not filled with those two species, some perennial ryegrass entries can be included in the trial.

6. Trial locations will be managed using a mowing height of 2 – 2.5" and fertilization of 0.25 – 0.33 lbs. of N/1000 sq. ft./growing month.

7. Digital image technology will be used to measure percent green cover on plots. Training will be provided to cooperators so that images are collected properly.

8. NTEP will hire additional staff to monitor the performance of trials, data and image collection, and to perform site visits.

9. Since the plot areas will be costly to build and the trial will require considerable labor to manage, each species trial will be limited to 30 total paid entries (plus 3 standards), 3 reps of each for a total of 100 plots at each test site.

10. USGA and NTEP will pursue certification/qualification and/or branding of drought tolerant or low-water using cultivars. Therefore, we anticipate that at the end of the trial period, the system will be in place to apply this certification (or brand) to those entries that qualify (qualification requirements will be in place before entry submission).

**Rainout shelters similar to this will be built and installed at five locations (see map)**

## ATTACHMENT D (page two)

**Here are more details on the two proposed water use/drought approaches. These approaches are based on similar protocols reported by Kansas State University, University of Arkansas and others (see selected references below):**

1) **<u>Approach 1- Individual Plot Level Irrigation:</u>**. The amount of plant material per entry would need to be sufficient to establish to a final area of approximately 32.28 sq. ft per entry per site. (*10.76 sq. ft./plot x 3 reps*)
   a. Year 1- Plots are fully established under full irrigation levels (plot size is *1 meter x 1 meter or 10.76 sq. ft.)*
   b. Years 2, 3, 4, etc.- Following uniform irrigation of all plots to initiate the study, full scale, automated irrigation is terminated, and individual plots are thereafter monitored on a regular basis (*could be daily, bi-weekly, or weekly to correspond to particular watering frequencies allotted by the region or budget provided the cooperator*) during the morning hours of the dry-down 'season'.
   c. When quality attributes (*wilt/firing/% green cover, etc.*) of a specific plot or plots are noted to have fallen below a defined threshold (*i.e. 50% green cover or another prescribed level*), it is hand-irrigated with an amount of water necessary to recharge the root zone to field capacity (between ½" to 1"). Irrigation events are recorded on a per plot basis, so that total irrigation applied over the season can be calculated on a plot basis and statistics applied.
   d. A dry-down 'season' would last around 100 days, then plots would be fully irrigated to assess recovery. Turf quality ratings will be collected as well during dry down and recovery.
   e. A rain-out shelter will be employed for this approach. Data produced through the work would document 1) 'water quantity required (inches) per entry' for each location, 2) turfgrass quality before and during dry-down, during and after recovery, and a 3) ranking of the entries used.

Selected References:


Lewis, J.D. et al. 2012. Wilt-Based Irrigation in Kentucky Bluegrass: Effects on Visual Quality and Irrigation Amounts Among Cultivars. Crop Sci. 52:1881–1890. doi: 10.2135/cropsci2012.01.0033

Richardson, M. D. el al. 2009. Drought Tolerance of Kentucky Bluegrass and Hybrid Bluegrass Cultivars. Online. Applied Turfgrass Science. doi:10.1094/ATS-2009-0112-01-RS.

Richardson, M.D. et al. 2012. Irrigation Requirements of Tall Fescue and Kentucky Bluegrass Cultivars Selected Under Acute Drought Stress. Online. Applied Turfgrass Science doi:10.1094/ATS-2012-0514-01-RS.

Steinke, K. et al. 2010. Drought Response and Recovery Characteristics of St. Augustinegrass Cultivars. Crop Sci. 50:2076-2083. doi:10.2135/cropsci2009.10.0635. Published online 16 June 2010.

USGA Turfgrass and Environmental Research Online. Vol. 11, No. 6, June 1, 2012, p. 1-12.
http://www.lib.msu.edu/cgi-bin/flink.pl/?recno=205406

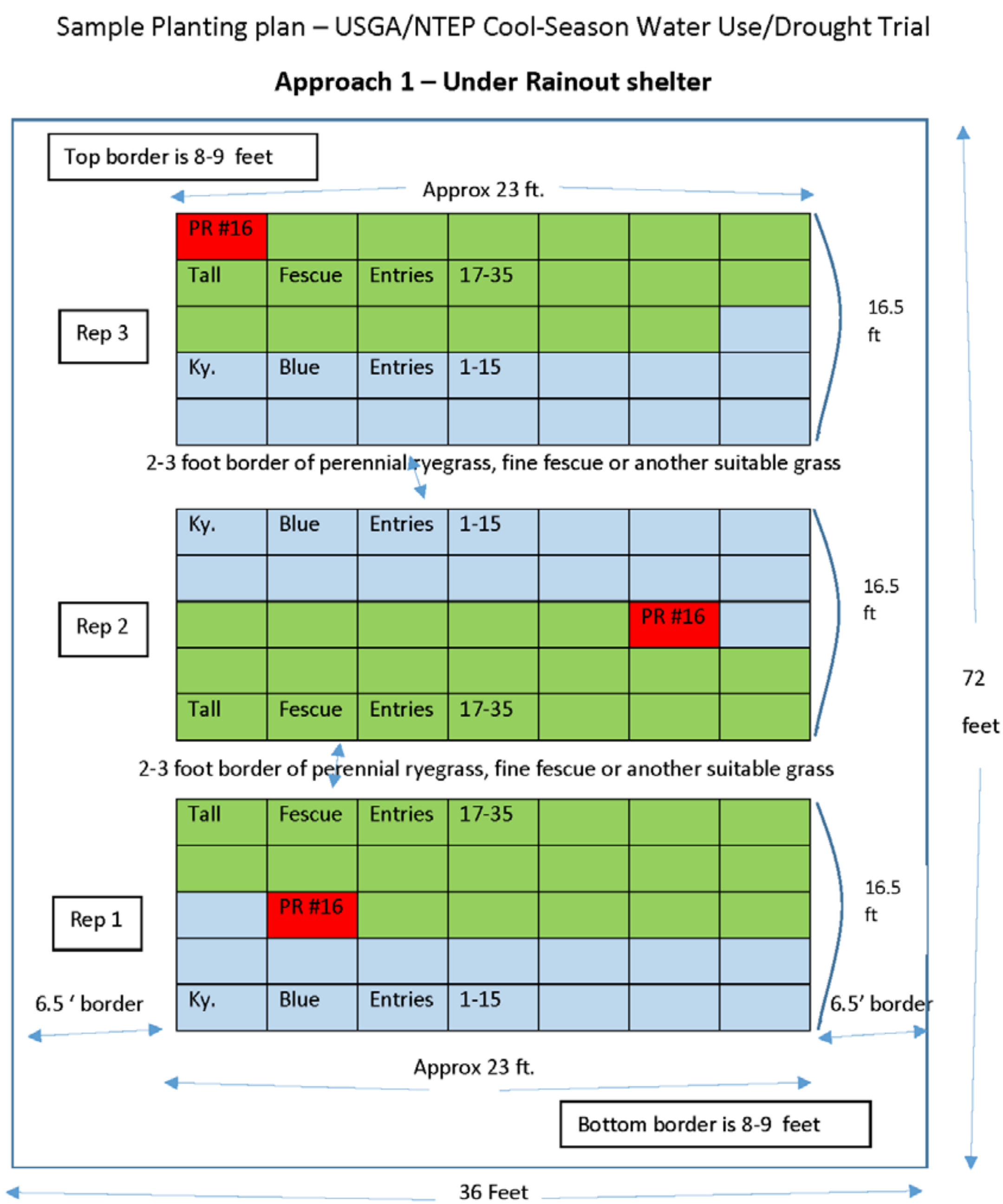

**Plots would be individually watered after they reach the desired drought stress threshold.**

## ATTACHMENT D (page three)

**<u>Approach 2- Zone Level Irrigation:</u>** Larger study area size (~3 to 4 times more area and plant material) would be needed for accommodating multiple studies or 'zones' of irrigation. The amount of plant material per entry would need to be sufficient to establish to a final area of approximately (3 ET levels x 3-6 entry reps/ET level x 10.76 sq. ft) ~200 sq. ft. per location *(depends on location irrigation design and availability)*. This trial would not be conducted under rainout shelter due to size constraints.

a. Year 1- Similar to Approach 1, a full set of replicated entries would be established, but within each of 3 target irrigation ET levels (zones). Plots (*1 m x 1 m or similar size*) will be fully established under full irrigation levels.
b. Years 2-4- Irrigation treatments imposed. ET levels will correspond to 3 levels of historical reference evapotranspiration ($ET_o$) for the location, the maximum of which should be near full water requirement (~0.75 x $ET_o$ for cool-season) and lowest of which should be ~1/4 of this maximum level. Alternatively, if $ET_o$ data are unavailable, one could arbitrarily apply defined amounts (i.e. ¾" per week, ½" per week, and ¼" per week to the respective zones.
    i. Cool-season: 0.75 x $ET_o$, 0.5 x $ET_o$, 0.25 x $ET_o$ applied 2x weekly
c. Frequency of irrigation to plots would also be a constant 1 or 2 day per week irrigation schedule *(a single frequency should be decided on for all locations)*.
d. Irrigation scheduling to account for rainfall
    i. Approach 1- Let system run regardless of rainfall, do not adjust irrigation
    ii. Approach 2- Do not adjust schedule for any events <0.25". Account for 50% effective rainfall for all other events in adjusting irrigation applied for each zone. (For instance, if a 1" rainfall is received; all plots are turned off for one event. If ½" is received, only the low irrigation level may be turned off, but others receive appropriate % adjustments to account for ¼" effective rainfall.
    iii. Ultimately the key will be accurate accounting of total water received within each zone on a weekly basis.
e. Quality attributes (*wilt/firing/% green cover, etc.*) of all plots within each irrigation level will be noted regularly during the study, just prior to an irrigation day during the morning hours.
f. At the conclusion of the study, irrigation + rainfall for each zone would be totaled by week (~10-14 weeks in duration). Quality (>6) or other parameter (>75% green cover) of interest in determining acceptability would also be noted on a per plot basis for each week. Finally, the particular amount of water needed to sustain acceptable quality each week would be determined on a plot by plot basis and totaled for the study. This amount might fluctuate by week or month. For example, bluegrass may maintain acceptable quality with only 0.5 x $ET_o$ in June, but in July or August, may require 0.75 to maintain acceptability. This method will account for weekly or monthly changes in minimal irrigation levels required.
g. This approach is best suited for areas of the US that likely see visible drought stress arise in summer months where irrigation is not applied, i.e. (New Mexico, California, Colorado, etc.).
h. Repeating the studies over three years will allow for upper and lower end seasonal requirements to be determined for each location.
i. Data produced through the work would also document 1) 'water quantity required (inches)' per entry for each location, 2) turfgrass quality ratings at regular intervals, and a 3) ranking of the entries used.

# ATTACHMENT E



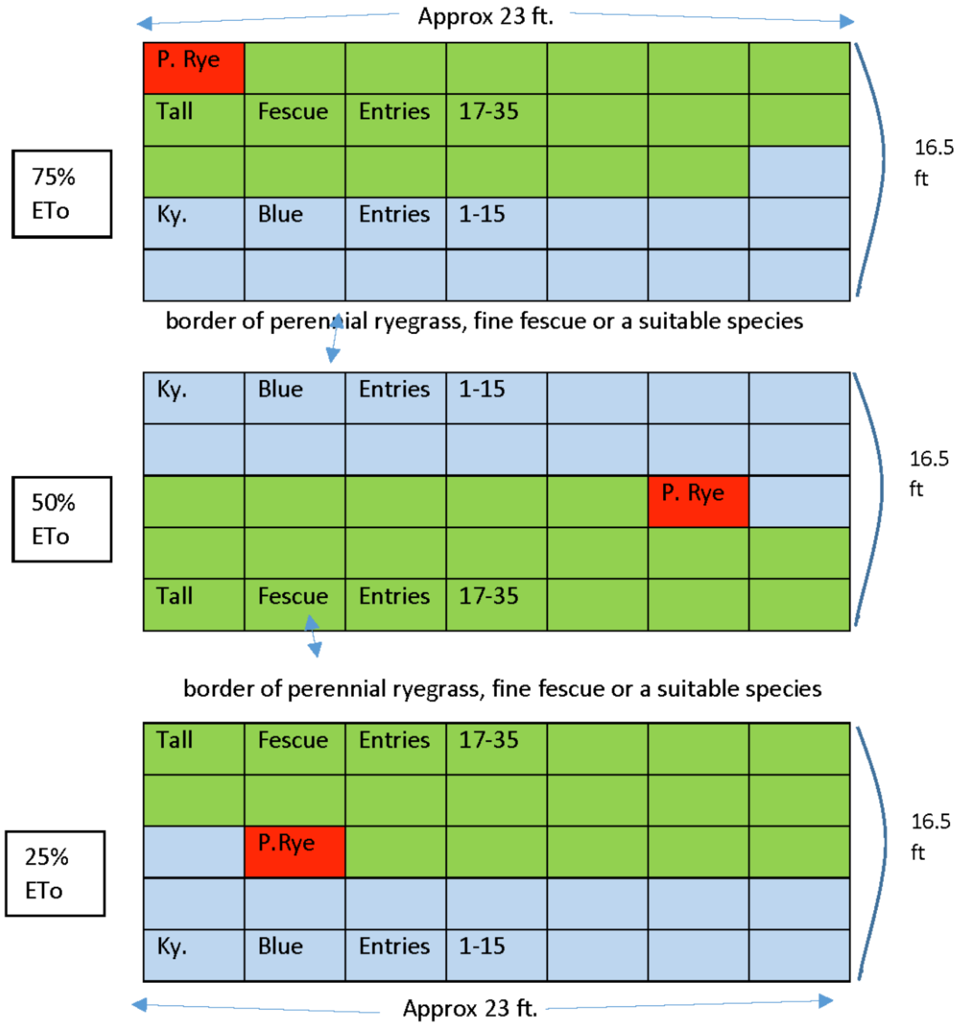

Rainout shelter is 36 x 72 feet

Plots are 1 meter x 1 meter (3.28 ft x 3.28 ft) (10.76 sq. ft.)

Sample Planting plan – USGA/NTEP Cool-Season Water Use/Drought Trial

**Approach 2 – $ET_o$ Based**

REPLICATION 1




Plots are 1 meter x 1 meter (3.28 ft x 3.28 ft) (10.76 sq. ft.)

ATTACHMENT F

USGA NTEP WATER USE/DROUGHT TRIAL COST AND REVENUE ESTIMATES

| | Approach 1 - plot watering | | | | | Approach 2 - zone level irrigation | | | | | | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | per location | | | | | per location | | | | | |
| | 2016 | 2017 | 2018 | 2019 | | 2016 | 2017 | 2018 | 2019 | | | |
| | establishment | Yr 1 data | Yr 2 data | Yr 3 data | | establishment | Yr 1 data | Yr 2 data | Yr 3 data | | | |
| | | collection | collection | collection | TOTAL | | collection | collection | collection | TOTAL | | |
| Build shelter | 15,000 | | | | 15,000 | | | | | | | |
| site prep/irrigation install | | | | | | 15,000 | | | | 15,000 | | |
| plot maintenance | 5,000 | 5,000 | 5,000 | 5,000 | 20,000 | 5,000 | 5,000 | 5,000 | 5,000 | 20,000 | | |
| watering individual plots | | 9,000 | 9,000 | 9,000 | 27,000 | | | | | | | |
| DIA pictures and processing | | 900 | 900 | 900 | 2,700 | | 900 | 900 | 900 | 2,700 | | |
| processing of data | | 450 | 450 | 450 | 1,350 | | 1,080 | 1,080 | 1,080 | 3,240 | | |
| miscellaneous | | | | | | 520 | 520 | 520 | 520 | 2,080 | | |
| TOTALS -per trial site | | | | | 66,050 | | | | | 43,020 | | |
| Number of sites | | | | | 5 | | | | | 5 | | |
| TOTAL COSTS | | | | | 330,250 | | | | | 215,100 | | 545,350 |
| NTEP OVERHEAD (12.5%) | | | | | | | | | | | | 68,169 |
| USGA support | | | | | | | | | | | | -250,000 |
| WA Turfgrass Seed Commission | | | | | | | | | | | | -105,000 |
| Lawn Institute | | | | | | | | | | | | -22,500 |
| Other Grants?? | | | | | | | | | | | | |
| DEFICIT (to be covered by entry fees) | | | | | | | | | | | | 236,019 |
| PAID ENTRIES | entry fee | | | | | | | | | | | |
| 32 | 8,000 | | | | | | | | | | | 256,000 |
| Scenario 1 - build five shelters | | | | | | | | | | | | |
| and install five zone level | | | | | | | | | | | | |
| irrigation sites - 10 total sites | | | | | | | | | | | | |
| **for one cool-season trial only** | | | | | | | | | | | | |
| zone level costs are for three | | | | | | | | | | | | |
| reps of three irrigation levels | | | | | | | | | | | | |

SCENARIO 5 - COOL-SEASON

$8000 entry fee, 5 locations of each Approach

**Annual report on USGA ID#: 2014-09-498**

Is It True That Certain Wetting Agents Remove Organic Coatings from Water-Repellent Sand Particles?

Xi Xiong[1], Stephen H. Anderson[2], Keith W. Goyne[2], and Robert J. Kremer[2]

University of Missouri, [1]Division of Plant Sciences and [2]Department of Soil, Environmental and Atmospheric Sciences.

Objectives: To evaluate various wetting agents for effects of removing organic coatings from hydrophobic sand surface.

Soil hydrophobicity is caused by the accumulation of organic coatings on the surface of soil particles. On sand-based putting greens, hydrophobic soil repels water, and consequently leads to localized dry spot (LDS) development. Wetting agent, amphiphilic molecules function as a “bridge” between the hydrophobic sand surface and water molecules, is the primary tool superintendents use to battle with LDS. There are a few wetting agents in the turf market, however, acclaim the property of removing organic coatings from the sand surface, and potentially solve the problem of hydrophobicity.

Results from the previous year’s laboratory experiment showed that Matador and OARS were able to reduce the hydrophobicity of sand to minimal level after one-time application following three washes. This was confirmed by removal of dissolved organic carbons in the leachate. Results from field experiment in 2015 also corroborated this effect. In 2016, we continued our field and laboratory experiments to gain a better understanding towards this group of wetting agents.

In 2016, the second year field experiment was carried out on a USGA green where LDS has been historically observed (Fig 1). Treatments, including Matador, OARS, and pHAcid, in addition to Hydro-Wet, Tournament-Ready, and Cascade Plus, were applied monthly (from May to September), to plots arranged as a RCBD with 4 replications. Hydrophobicity, measured as molarity of ethanol droplet (MED) test at 0-5 months after the initial treatment application (MAIT) showed reduced hydrophobicity following applications of all wetting agents to various extents, with the only exception of pHAcid (Table 1). No differences were found between plots treated with OARS or Matador for MED over the 5 months period. During the experiment, dollar spot (*Sclerotinia homoeocarpa* F.T. Benn.) was observed in the plot area, and plots received pHAcid, Matador and Cascade Plus showed 5-8 times greater dollar spot incidence compared to the untreated control at 5 weeks after the initial treatment (WAIT) (Table 2). The higher water content found in plots received pHAcid, Matador and Cascade Plus (Fig 2) likely contributed to the greater dollar spot incidence observed. Turf quality, measured by normalized difference vegetation index (NDVI), also showed that plots received these three wetting agents maintained greater overall turf quality compared to control and other wetting agents (Fig 3), likely attributed to the greater soil moistures they maintained.

In the laboratory, we utilized the same sand-column systems containing naturally occurred hydrophobic sand explained in previous report. The objective in 2016 laboratory experiment was to evaluate the influence of repeat wetting agents application which simulates field practices where monthly applications were typically performed. Similar to the experiment conducted in 2015, hydrophobic sand collected from the field were homogenized before packed uniformly to the same bulk density (1.66 $g/cm^3$). Wetting agents, at a higher volume (70 ml) than the pore volume (58ml), were applied, before three washes using water at pore volume 24h after wetting agent application. All leachates were collected for determining volume, dissolved (DOC) and particulate organic carbon (POC), and sand columns were dissembled for hydrophobicity test after oven dried to constant weight at 50 $^{o}$ C at the end of the experiment. Treatments were arranged in a CRD with 3 replications, and the entire experiment was repeated.

Compared to untreated sand, sand columns treated with water resulted in a 50% increase in hydrophobicity (Fig 4), likely due to the changes in orientation of the organic coatings during the dry-wet cycle. Application of Matador, despite the number of applications, reduced hydrophobicity to none. OARS treated sand columns, however, showed minimal hydrophobicity following one-time application, but approximately doubled the hydrophobicity following 3-time applications. It is yet to be determined the underline mechanism that explains this desperation between Matador and OARS; nevertheless, it is likely related to how the organic coatings on hydrophobic sand been removed and/or replaced. We are currently in the process of analyzing DOC and POC results for possible answers for this question. It is also worthy to note that repeat application of OARS under field conditions did not negatively impact soil hydrophobicity and hence turf quality. This is likely due to the differences in the amount of organic carbon introduced through treatment under field and laboratory conditions, and also likely related to the microbe activity under field conditions which was absent in the laboratory experiments. Analysis is undergoing for determining the possible influences of these factors.

Summary


- Under field conditions, both Matador and OARS applied monthly reduced soil hydrophobicity, compared to the untreated controls.
- Laboratory experiment revealed that the mechanism of these two wetting agents in organic coating removal/replacing could be different.
- Analysis that are still ongoing include DOC and POC for laboratory experiment, PLFA for microbial analysis from field plots, and sand particle analysis by Scanning Electron Microscope for both field and laboratory experiments.

Table 1. Treatment effect on soil hydrophobicity measured by molarity of ethanol droplet test (MED; molar) at 1 inch soil depth from 0 to 5 months (May to October, respectively) after initial treatment application (MAIT) in 2016.

| Compound | 0 MAIT | 1 MAIT | 2 MAIT | 3 MAIT | 4 MAIT | 5 MAIT |
|---|---|---|---|---|---|---|
| | ------------------------------MED (molar) --------------------------------- | | | | | |
| Control | 2.63 a2[†] | 3.33 a1 | 3.18 a1 | 3.08 a1 | 3.03 a1 | 3.05 a1 |
| pHAcid | 2.53 ab2 | 3.13 a1 | 2.95 ab1 | 2.85 a1 | 3.00 a1 | 3.03 a1 |
| Hydro-Wet | 2.43 ab12 | 2.50 b12 | 2.63 cd1 | 2.30 bc2 | 2.23 b23 | 1.95 c3 |
| Tournament Ready | 2.40 ab1 | 2.53 b1 | 2.55 cd1 | 2.40 b1 | 2.45 b1 | 2.35 b1 |
| OARS | 2.28 b2 | 2.63 b1 | 2.68 bc1 | 2.25 bc2 | 2.25 b2 | 2.13 bc2 |
| Matador | 2.45 ab12 | 2.65 b1 | 2.53 cd1 | 2.05 c3 | 2.45 b12 | 2.18 bc23 |
| Cascade Plus | 2.50 ab12 | 2.60 b1 | 2.35 d123 | 2.23 bc23 | 2.43 b123 | 2.13 bc3 |

[†]Means followed by the same letters in each column were not significantly different based on Fisher's protected LSD at *P*<0.05; Means followed by the same numbers in each row were not significantly different based on Fisher's protected LSD at *P*<0.05.

Table 2. Treatment effect on percent disease cover (%) evaluated at 1, 5, 9, 13, and 17 weeks after the initial treatment application (WAIT) in 2016.

| Compound | 1 WAIT | 5 WAIT | 9 WAIT | 13 WAIT | 17 WAIT |
|---|---|---|---|---|---|
| | ------------------------------------% disease ------------------------------------ | | | | |
| Control | 1.0 a23 | 0.8 b23 | 0.0 a3 | 4.0 a1 | 0.8 ab23 |
| pHAcid | 1.0 a5 | 4.5 a23 | 0.8 a5 | 8.0 a1 | 1.0 ab5 |
| Hydro-Wet | 0.3 a23 | 1.3 b23 | 0.0 a3 | 4.0 a1 | 0.0 b3 |
| Tournament Ready | 0.8 a34 | 0.8 b34 | 0.0 a4 | 5.8 a1 | 1.0 ab34 |
| OARS | 1.3 a34 | 6.5 a1 | 0.8 a34 | 6.8 a1 | 2.0 ab234 |
| Matador | 0.5 a34 | 1.5 b34 | 0.8 a34 | 7.0 a1 | 2.8 a23 |
| Cascade Plus | 1.3 a34 | 5.0 a12 | 0.3 a4 | 7.3 a1 | 0.8 ab4 |

[†]Means followed by the same letters in each column were not significantly different based on Fisher's protected LSD at *P*<0.05; Means followed by the same numbers in each row were not significantly different based on Fisher's protected LSD at *P*<0.05.

Fig 1. Field plots overall view. Picture were taken at 7 weeks after the initial treatment application (WAIT) on July 11, 2016.

Fig 2. Volumetric water content (VWC; %) influenced by wetting agents applied. Data were collected every other week from 1 to 19 weeks after the initial treatment application (WAIT). There were no wetting agent by evaluation timing interaction; hence, wetting agent main effect was presented. Bars labeled with the same letters were not significantly different based on Fisher's Protected LSD ($P$<0.05).

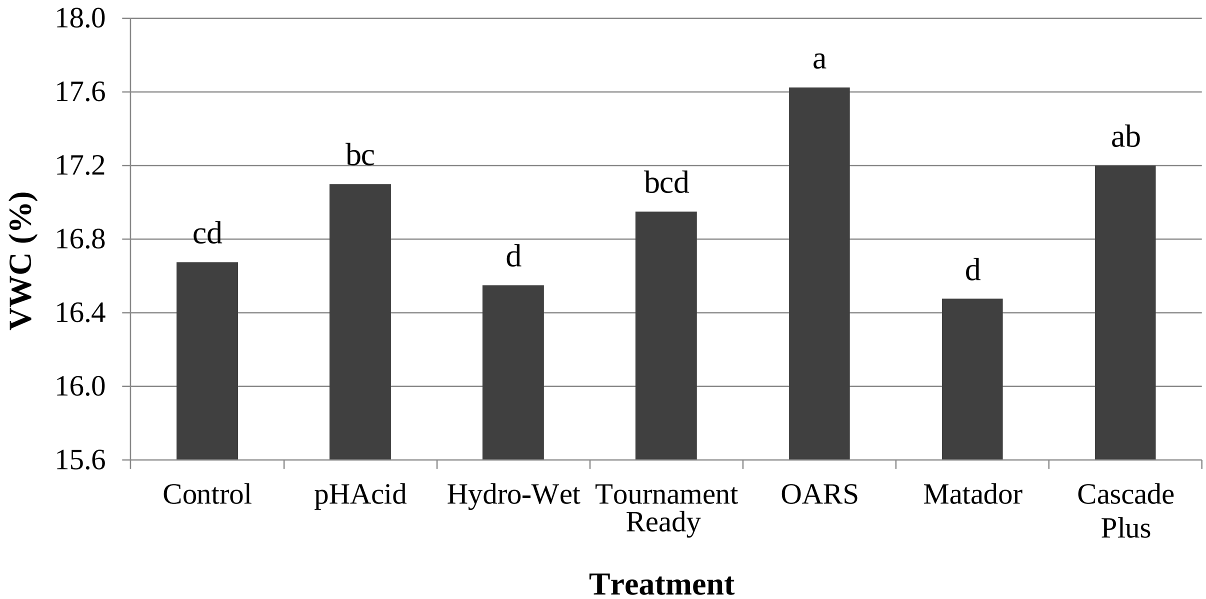



Fig 3. Normalized difference vegetation index (NDVI) influenced by wetting agents applied. Data were collected every other week from 1 to 19 weeks after the initial treatment application (WAIT). There were no wetting agent by evaluation timing interaction; hence, wetting agent main effect was presented. Bars labeled with the same letters were not significantly different based on Fisher's Protected LSD ($P$<0.05).

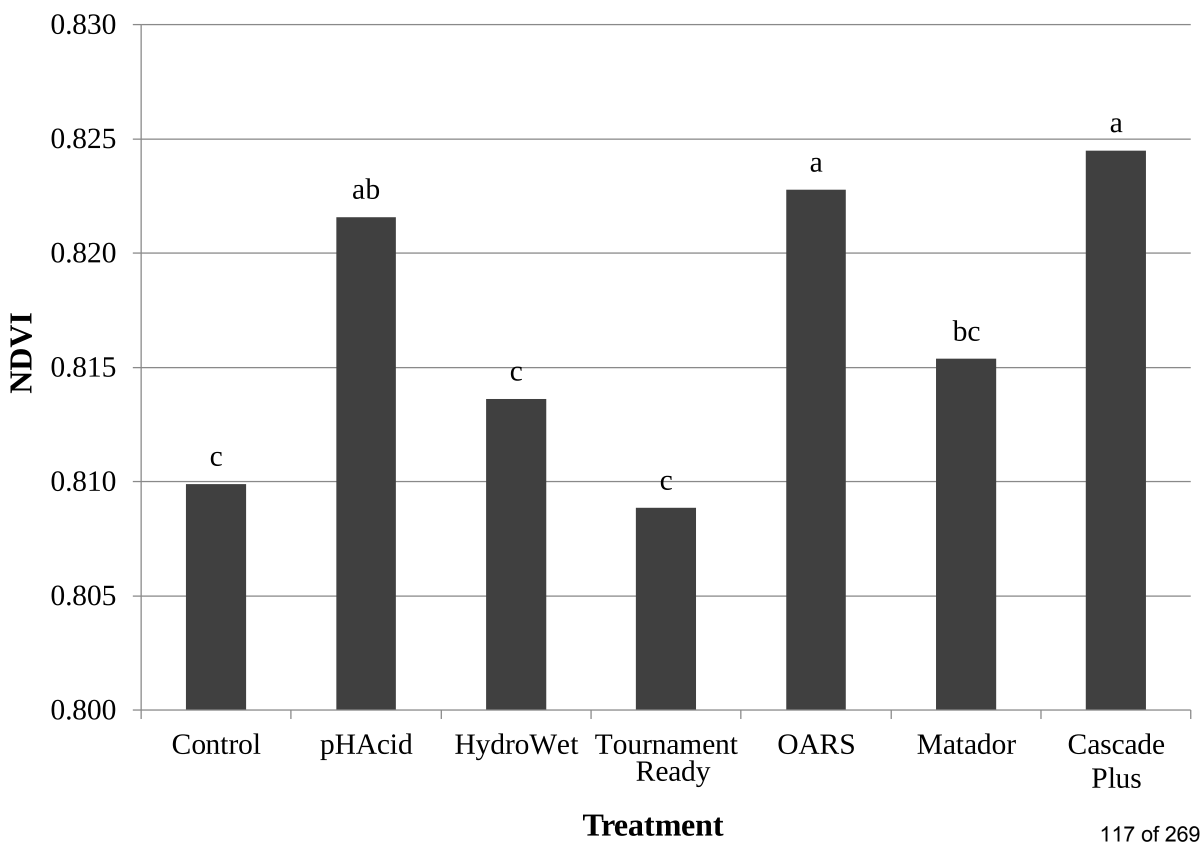
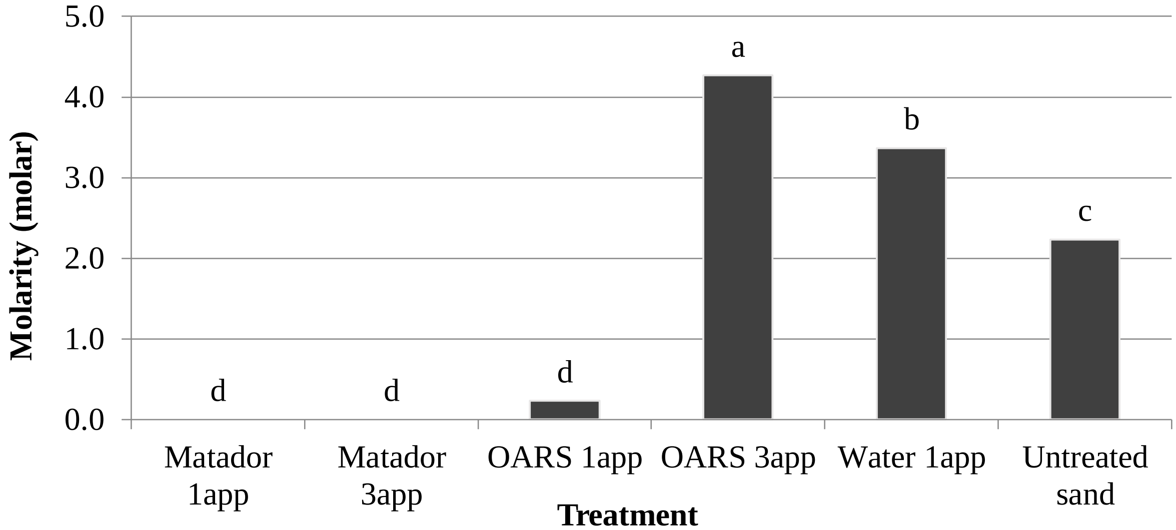


Fig 4. Soil hydrophobicity influenced by wetting agents that were applied to sand columns in the laboratory. Hydrophobicity was determined by using molarity of ethanol droplet (MED; molar) test after 1 or 3 times wetting agent applications (1 or 3 app, respectively), compared to sand columns received no or water only treatment. Bars labeled with the same letters were not significantly different based on Fisher's Protected LSD (*P*<0.05).



**Establishment of a Standard Screening Method for Drought Tolerance in Creeping Bentgrass**

Principal Investigator(s): Dr. Qi (Chee) Zhang
University: North Dakota State University

- Drought tolerance of creeping bentgrass varied in genotypes and growth stages.
- 'Bengal' had high root and shoot growth under drought conditions at the germination and seedling growth stage. 'Penn A-4', 'L-93', 'T-1', 'Pinup', 'Cobra 2', 'Putter', 'Pennlinks II', and 'Kingpin' had the same level of root growth as 'Bengal' but lower shoot biomass.
- 'SR 1150', '007', 'Bengal', 'Declaration', 'T-1', 'Focus', 'Cobra 2', 'Pennlinks II', and 'V8' showed high root length and clipping dry weight under drought conditions during the vegetative growth, in which 'SR 1150' and '007' also show high visual quality.

The objective of this project is to determine the reliability of selecting drought-tolerant creeping bentgrass in a polyethylene glycol (PEG) - hydroponic culture.

Twenty-three creeping bentgrass cultivars were germinated in PEG solutions (0.0, -0.3, and -0.6 MPa) for four weeks (W). The experiment was setup as a split-plot design, with the whole-plot factor being PEG concentration and the sub-plot factor being bentgrass cultivar. Data were collected on root length (RL), root dry weight (RDW), and shoot dry weight (SDW). RL decreased with an increase of drought severity, while SDW was not affected by drought (Table 1). RL ranged from 7.9 cm in 'Penn A-4' to 4.6 cm in 'Independence' at W4. 'Bengal' had a SDW of 218.0 mg, higher than all other grasses. The cultivar x drought interaction (Table 1) observed in RDW was mostly due to the close ranking of RDW in the large number of cultivars evaluated in the present study. Overall, 'Penn A-4' and 'Independence' had the highest and lowest RDW, respectively (Table 1). Drought tolerance was also evaluated during the vegetative growth on RL, clipping dry weight (CDW), and quality. RL and CDW decreased with increasing drought levels (Table 2). The longest and shortest roots were observed in '007' and 'Penn A-1', respectively, at W5. 'SR 1150' had a total CDW of 74.0 mg, 90% higher than 'MaCkenzie'. Most grasses showed acceptable quality ($\geq 6$) at 0.0 MPa (Table 3). The number of cultivars which had acceptable quality at -0.3 MPa decreased from six ('SR 1150', '007', 'T-1', 'Pennlinks II', 'Focus', and 'Alpha') at W1 to three ('SR 1150', '007', and 'Independence') at

W3, and no cultivars performed adequately at W5. At – 0.6 MPa, only ‘Alpha’ showed acceptable quality at W1.

Creeping bentgrass drought tolerance was evaluated under the putting green and fairway conditions in 2015 (July 7 – Sept. 29) and 2016 (July 1 – Sept. 8) (Figures 1 and 2). Drought stress was simulated by withholding irrigation during the study period and canopy reflectance and visual quality were recorded. The experimental setup was a RCBD with three replicates. Significant differences were only observed in the stress index ($R_{710}/R_{810}$) on Aug. 12 and 24, 2015 and Aug. 22 and 30, 2016 in the putting green and on Aug. 24, 2015 and Aug. 24 and 30, 2016 in the fairway (Table 4). However, all grasses provided acceptable quality on all the evaluation dates (data not shown). It was probably due to a combination of frequent precipitation, high water holding capacity of clay soil at the research site, and cool climate of the upper Northern region. No comparisons were made between the results from the PEG-hydroponic system and the field study due to limited variations observed in bentgrass performance under the field conditions.

Table 1. Root length (cm), root dry weight (mg), and shoot dry weight (mg) of creeping bentgrass seedlings grown under drought.

| | Root length (cm) | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Root dry weight (mg) | Shoot dry weight (mg) |
| Drought (MPa) | | | | | | |
| 0.0 | 3.0 | 4.9 | 6.2 | 7.4 | 12.2 | 108.1 |
| -0.3 | 1.7 | 3.8 | 5.8 | 7.4 | 36.7 | 122.3 |
| -0.6 | 1.3 | 2.3 | 3.7 | 4.9 | 19.9 | 103.7 |
| LSD ($P \leq 0.05$) | 0.2 | 0.2 | 0.3 | 0.4 | 0.3 | ns |
| Cultivar | | | | | | |
| Penn A-4 | 2.5 | 4.7 | 6.3 | 7.9 | 32.7 | 123.5 |
| L-93 | 2.1 | 4.0 | 6.4 | 7.6 | 26.7 | 97.0 |
| T-1 | 2.1 | 3.9 | 5.5 | 7.5 | 30.7 | 106.1 |
| Pinup[†] | 2.3 | 3.8 | 6.0 | 7.4 | 26.1 | 153.4 |
| Cobra 2[†] | 2.3 | 3.9 | 5.8 | 7.4 | 27.3 | 107.1 |
| Memorial | 1.8 | 3.7 | 5.5 | 7.3 | 18.8 | 69.8 |
| Putter | 1.9 | 3.9 | 5.5 | 7.2 | 24.4 | 102.1 |
| South shore | 2.2 | 4.2 | 5.5 | 7.1 | 24.0 | 105.6 |
| Kingpin | 2.2 | 4.0 | 5.5 | 7.1 | 32.7 | 154.6 |
| Pennlinks II | 2.0 | 3.6 | 5.0 | 7.1 | 25.0 | 103.2 |
| Bengal | 2.2 | 3.7 | 5.4 | 7.0 | 24.9 | 218.0 |
| Crystal Bluelinks | 2.2 | 3.7 | 5.4 | 7.0 | 23.2 | 142.6 |
| V8[†] | 2.4 | 4.1 | 5.4 | 6.6 | 21.2 | 98.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Penn A-1 | 1.9 | 4.0 | 5.5 | 6.6 | 18.3 | 105.9 |
| Focus[†] | 2.1 | 3.7 | 5.4 | 6.4 | 22.4 | 113.5 |
| Declaration | 2.3 | 3.7 | 5.2 | 6.2 | 24.4 | 140.3 |
| Alpha | 1.7 | 3.1 | 4.5 | 6.0 | 31.0 | 107.0 |
| Mackenzie | 2.0 | 3.5 | 5.0 | 6.0 | 19.9 | 92.1 |
| Tyee | 2.0 | 3.5 | 4.7 | 5.9 | 22.5 | 99.3 |
| Penncross | 1.4 | 2.7 | 4.3 | 5.4 | 13.1 | 61.3 |
| SR 1150 | 1.6 | 3.0 | 4.2 | 5.2 | 13.1 | 94.8 |
| 007 | 1.3 | 2.7 | 3.9 | 5.1 | 13.8 | 75.8 |
| Independence | 1.0 | 2.5 | 3.7 | 4.6 | 12.2 | 90.2 |
| LSD ($P \leq 0.05$) | 0.5 | 0.7 | 0.9 | 1.2 | 8.6 | 60.6 |
| Drought x cultivar | ns | ns | ns | ns | * | ns |

ns and * mean not significantly different and significantly different at $P \leq 0.05$ level, respectively.

[†]Creeping bentgrass cultivar not included in the field study.

Table 2. Root length (cm) and clipping dry weight (mg) of mature creeping bentgrass grown under drought.

| | Root length (cm) | | | Clipping dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|
| | Week 1 | Week 3 | Week 5 | Week 1 | Week 3 | Week 5 | Total |
| Drought (MPa) | | | | | | | |
| 0.0 | 1.9 | 4.3 | 6.1 | 28.8 | 44.7 | 20.5 | 94.0 |
| -0.3 | 1.8 | 2.7 | 4.0 | 24.7 | 11.5 | 14.8 | 51.1 |
| -0.6 | 1.2 | 2.3 | 2.5 | 17.9 | 5.4 | 2.8 | 26.2 |
| LSD ($P \leq 0.05$) | 0.5 | 0.6 | 0.8 | 4.0 | 2.8 | 2.4 | 5.9 |
| Cultivar | | | | | | | |
| 007 | 3.3 | 5.0 | 6.5 | 24.7 | 31.2 | 15.3 | 71.1 |
| Penn A-4 | 1.8 | 3.5 | 6.1 | 23.4 | 15.7 | 16.2 | 55.4 |
| Pinup[†] | 1.6 | 3.9 | 5.6 | 29.3 | 19.2 | 7.9 | 56.3 |
| Tyee | 2.4 | 3.8 | 5.4 | 24.6 | 16.5 | 10.7 | 51.8 |
| Independence | 1.8 | 3.7 | 5.4 | 20.2 | 20.4 | 15.3 | 55.8 |
| Pennlinks II | 2.4 | 4.0 | 5.4 | 23.1 | 24.3 | 14.1 | 61.4 |
| Bengal | 0.5 | 3.3 | 5.2 | 35.1 | 20.5 | 15.6 | 71.2 |
| Cobra 2[†] | 0.4 | 2.9 | 5.0 | 20.6 | 27.5 | 16.0 | 64.1 |
| SR 1150 | 1.4 | 3.5 | 4.8 | 31.7 | 24.6 | 17.7 | 74.0 |
| Focus[†] | 2.0 | 3.6 | 4.7 | 23.6 | 24.9 | 16.0 | 64.5 |
| V8[†] | 1.6 | 3.1 | 4.7 | 22.6 | 26.0 | 12.7 | 61.3 |
| Declaration | 2.4 | 3.7 | 4.3 | 30.1 | 23.8 | 14.5 | 68.5 |
| T-1 | 1.8 | 3.3 | 4.3 | 27.6 | 23.2 | 14.7 | 65.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kingpin | 2.1 | 3.3 | 4.1 | 28.4 | 27.5 | 12.2 | 68.1 |
| Putter | 1.7 | 2.9 | 4.1 | 25.8 | 19.5 | 10.0 | 55.2 |
| Memorial | 2.1 | 3.3 | 4.0 | 20.5 | 18.3 | 10.5 | 49.3 |
| South shore | 1.4 | 2.6 | 3.7 | 29.8 | 18.7 | 9.4 | 57.9 |
| L-93 | 1.4 | 2.1 | 3.2 | 21.2 | 17.4 | 10.9 | 49.5 |
| Crystal Bluelinks | 1.2 | 2.1 | 3.2 | 20.6 | 12.1 | 8.7 | 41.4 |
| Mackenzie | 0.9 | 2.4 | 3.1 | 17.0 | 12.6 | 9.3 | 39.0 |
| Alpha | 1.1 | 1.8 | 3.0 | 18.5 | 20.2 | 14.6 | 53.3 |
| Penncross | 0.6 | 1.7 | 2.8 | 15.4 | 13.2 | 10.5 | 39.1 |
| Penn A-1 | 0.6 | 1.6 | 2.3 | 14.3 | 15.5 | 10.1 | 39.9 |
| LSD ($P \leq 0.05$) | 1.5 | 1.8 | 2.2 | 11.1 | 7.7 | 6.6 | 16.2 |
| Drought x cultivar | ns | ns | ns | ns | ns | ns | ns |

ns means not significantly different at $P \leq 0.05$ level.

†Creeping bentgrass cultivar not included in the field study.

Table 3. Visual quality of mature creeping bentgrass grown under drought. Visual quality was rated with a 1 – 9 scale, in which 9 = optimal quality, 6 = acceptable quality, and 1 = dead grass.

| | Week 1 | | | Week 3 | | | Week 5 | | |
|---|---|---|---|---|---|---|---|---|---|
| Cultivar | 0.0 MPa | -0.3 MPa | -0.6 MPa | 0.0 MPa | -0.3 MPa | -0.6 MPa | 0.0 MPa | -0.3 MPa | -0.6 MPa |
| SR 1150 | 7.0 | 6.3 | 5.0 | 7.0 | 6.3 | 4.7 | 6.7 | 5.3 | 3.3 |
| Focus† | 7.0 | 6.0 | 4.0 | 7.0 | 5.7 | 3.7 | 5.7 | 4.3 | 2.0 |
| 007 | 7.0 | 6.3 | 4.0 | 6.0 | 6.0 | 4.0 | 5.0 | 4.3 | 3.7 |
| Pennlinks II | 7.0 | 6.0 | 5.3 | 6.7 | 5.7 | 4.7 | 5.0 | 4.3 | 3.7 |
| T-1 | 7.0 | 6.0 | 4.0 | 6.3 | 5.7 | 3.3 | 5.7 | 4.3 | 2.3 |
| Alpha | 7.0 | 6.0 | 6.0 | 7.0 | 5.7 | 5.3 | 6.0 | 4.7 | 5.3 |
| Independence | 7.0 | 5.7 | 5.0 | 7.0 | 6.0 | 4.3 | 6.7 | 4.7 | 4.0 |
| Mackenzie | 7.0 | 5.0 | 4.0 | 6.7 | 4.0 | 2.7 | 6.0 | 3.0 | 1.3 |
| Cobra 2† | 7.0 | 5.0 | 5.3 | 7.0 | 5.0 | 5.0 | 6.3 | 3.7 | 3.3 |
| Tyee | 7.0 | 5.3 | 4.0 | 6.7 | 4.7 | 3.0 | 5.3 | 3.3 | 2.3 |
| Declaration | 7.0 | 5.7 | 5.0 | 5.7 | 5.7 | 4.7 | 5.3 | 4.0 | 3.3 |
| Bengal | 7.0 | 4.7 | 4.3 | 7.0 | 4.7 | 4.0 | 6.0 | 3.0 | 3.7 |
| V8† | 7.0 | 5.0 | 5.0 | 6.7 | 4.7 | 4.7 | 4.7 | 3.7 | 3.7 |
| L-93 | 7.0 | 5.3 | 3.7 | 6.7 | 4.3 | 2.7 | 5.7 | 2.7 | 2.0 |
| Putter | 7.0 | 5.0 | 3.7 | 6.3 | 5.3 | 2.7 | 6.0 | 2.7 | 1.3 |
| Penncross | 7.0 | 5.7 | 3.3 | 7.0 | 5.0 | 2.0 | 6.7 | 4.3 | 1.3 |
| South shore | 7.0 | 5.0 | 3.7 | 5.7 | 5.3 | 2.3 | 3.3 | 3.7 | 2.0 |
| Crystal Bluelinks | 7.0 | 4.7 | 3.3 | 6.3 | 3.7 | 2.7 | 5.7 | 2.7 | 1.7 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Penn A-4 | 7.0 | 5.0 | 4.7 | 6.7 | 4.7 | 4.7 | 5.7 | 3.3 | 4.0 |
| Penn A-1 | 7.0 | 5.3 | 3.3 | 6.3 | 5.0 | 2.0 | 5.0 | 3.0 | 1.7 |
| Memorial | 7.0 | 5.0 | 4.3 | 6.7 | 5.7 | 3.3 | 5.0 | 3.7 | 2.0 |
| Pinup[†] | 7.0 | 5.3 | 3.3 | 5.7 | 5.0 | 2.3 | 5.3 | 4.3 | 1.7 |
| Kingpin | 7.0 | 5.0 | 4.0 | 6.3 | 5.0 | 4.0 | 4.7 | 3.7 | 2.3 |
| LSD ($P \leq 0.05$) | ns | ns | 1.5 | ns | ns | 1.6 | 1.6 | 1.6 | 1.8 |

ns means not significantly different at $P \leq 0.05$ level.

[†]Creeping bentgrass cultivar not included in the field study.

Table 4. Stress index [$R_{710}/R_{810}$, R and the subscript numbers indicate the light reflectance at the specific wavelength (nm)] of creeping bentgrass managed under the putting green and the fairway conditions.

| | Putting green | | | | Fairway | | |
|---|---|---|---|---|---|---|---|
| | 2015 | | 2016 | | 2015 | 2016 | |
| Cultivar | Aug. 12 | Aug. 24 | Aug. 22 | Aug. 30 | Aug. 24 | Aug. 22 | Aug. 30 |
| L-93 | 0.29 | 0.35 | 0.39 | 0.39 | 0.36 | 0.35 | 0.34 |
| T-1 | 0.30 | 0.31 | 0.36 | 0.36 | 0.31 | 0.32 | 0.31 |
| Alpha | 0.30 | 0.34 | 0.39 | 0.38 | 0.30 | 0.31 | 0.29 |
| Putter | 0.31 | 0.35 | 0.43 | 0.42 | 0.34 | 0.33 | 0.32 |
| Southshore | 0.29 | 0.36 | 0.40 | 0.40 | 0.34 | 0.32 | 0.32 |
| Kingpin | 0.32 | 0.36 | 0.43 | 0.42 | 0.30 | 0.32 | 0.31 |
| Crenshaw† | 0.31 | 0.35 | 0.39 | 0.39 | 0.37 | 0.30 | 0.30 |
| Imperial† | 0.31 | 0.36 | 0.42 | 0.42 | 0.33 | 0.36 | 0.34 |
| Century† | 0.32 | 0.36 | 0.42 | 0.41 | 0.35 | 0.36 | 0.34 |
| Penncross | 0.32 | 0.36 | 0.42 | 0.43 | 0.34 | 0.35 | 0.34 |
| A-4 | 0.30 | 0.35 | 0.39 | 0.39 | 0.35 | 0.32 | 0.33 |
| Crystal bluelinks | 0.31 | 0.36 | 0.39 | 0.38 | 0.33 | 0.35 | 0.34 |
| Alister† | 0.30 | 0.36 | 0.43 | 0.46 | 0.35 | 0.35 | 0.32 |
| Pennlinks II | 0.31 | 0.35 | 0.39 | 0.40 | 0.35 | 0.34 | 0.33 |
| Penn A-1 | 0.32 | 0.35 | 0.42 | 0.42 | 0.37 | 0.34 | 0.33 |
| Penn G-6† | 0.31 | 0.35 | 0.42 | 0.42 | 0.34 | 0.33 | 0.32 |
| 007 | 0.30 | 0.33 | 0.44 | 0.46 | 0.31 | 0.34 | 0.34 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MacKenzie | 0.30 | 0.33 | 0.47 | 0.45 | 0.34 | 0.34 | 0.33 |
| Tyee | 0.30 | 0.34 | 0.41 | 0.42 | 0.32 | 0.32 | 0.32 |
| SR 1150 | 0.31 | 0.36 | 0.43 | 0.45 | 0.33 | 0.38 | 0.37 |
| Memorial | 0.30 | 0.35 | 0.39 | 0.38 | 0.34 | 0.33 | 0.34 |
| Independence | 0.30 | 0.34 | 0.39 | 0.37 | 0.30 | 0.32 | 0.30 |
| Declaration | 0.31 | 0.36 | 0.46 | 0.46 | 0.33 | 0.36 | 0.36 |
| LS – 44[†] | 0.29 | 0.34 | 0.39 | 0.39 | 0.37 | 0.32 | 0.32 |
| Bengal | 0.28 | 0.35 | 0.37 | 0.37 | 0.33 | 0.35 | 0.33 |
| LSD ($P \leq 0.05$) | 0.02 | 0.03 | 0.06 | 0.05 | 0.04 | 0.04 | 0.03 |

[†]Creeping bentgrass cultivar not included in the hydroponic experiments as it is no longer commercially produced.

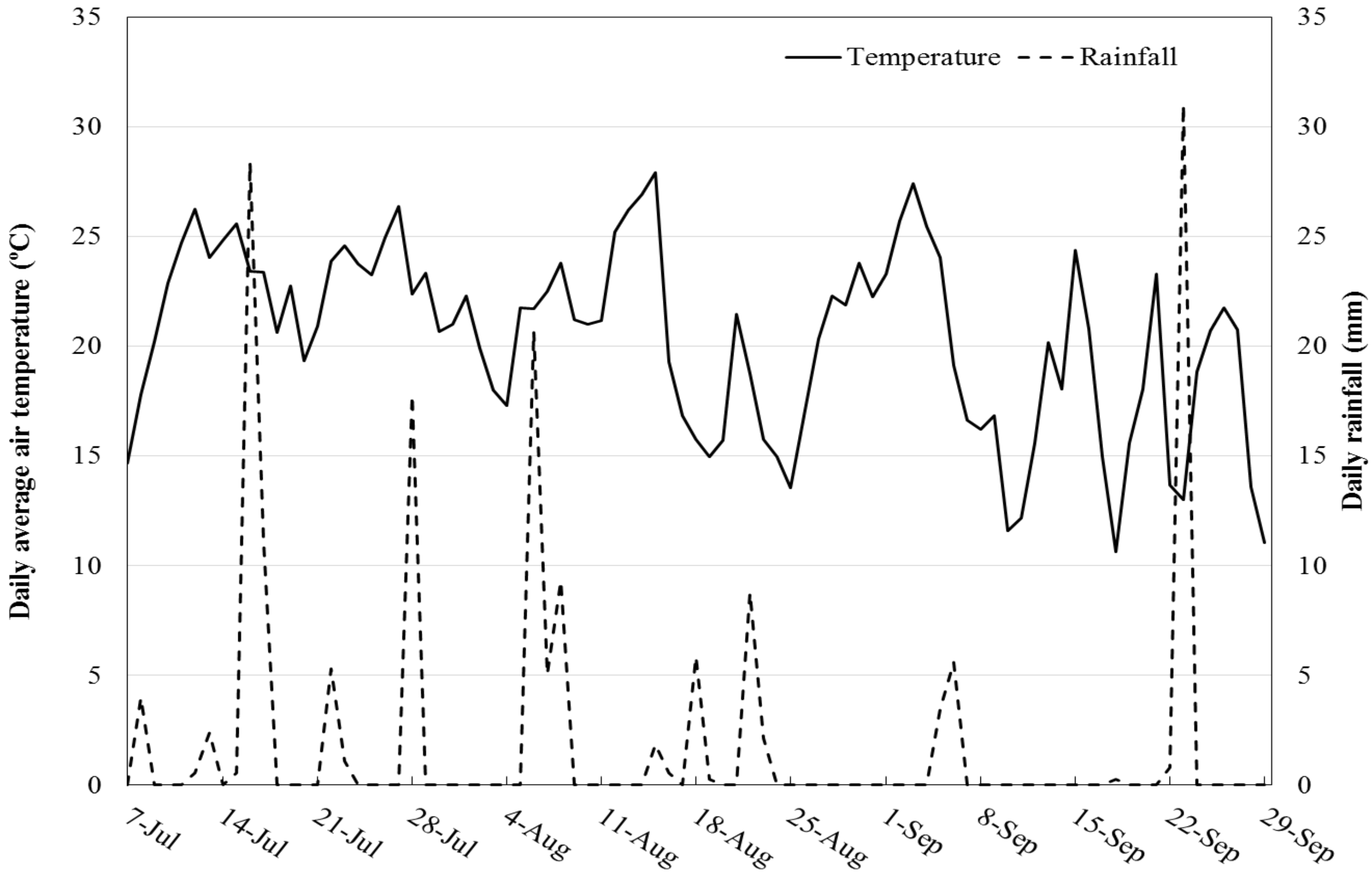



Figure 1. Daily average air temperature (ºC) and rainfall (mm) from July 8 to Sept. 29, 2015.

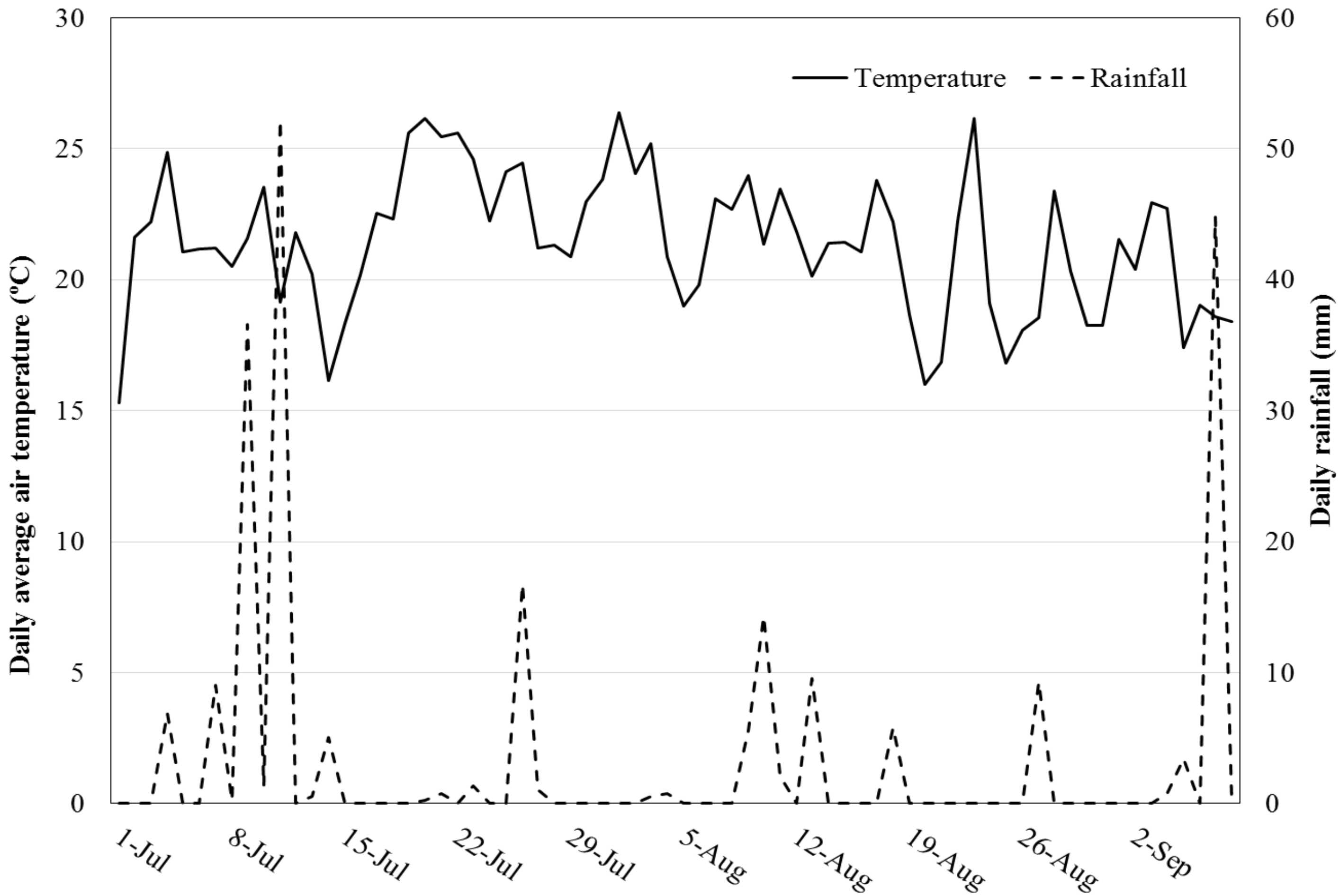



Figure 2. Daily average air temperature (ºC) and rainfall (mm) from July 1 to Sept. 8, 2016.

**Excessive Winter Crown Dehydration Affects Creeping Bentgrass Cold Hardiness**
**USGA ID#: 2015-15-530**
**Darrell J. Michael and William C. Kreuser, Ph.D.**

- A two-year field study was conducted during the winter of 2014-15 and 2015-16 to evaluate the effectiveness of commonly used desiccation prevention treatments.
- Heavy sand topdressing and covers provided a physical protective barrier from the environment which reduced desiccation, sustained crown moisture content, and accelerated spring green-up.
- The relationship between crown moisture and cold hardiness is currently being evaluated in a winter desiccation growth chamber.

The below abstract has been submitted for publication and is currently in the process of review:

Winter desiccation injury can severely impact golf courses in the northern Great Plains; however, little is known about its prevention. While anecdotal evidence suggests numerous prevention options are available to practitioners, the impacts these prevention practices have on turfgrass survival remain unclear. The objective of this two-year study was to evaluate the effectiveness of winter desiccation prevention treatments on turfgrass survival across multiple locations in Nebraska. To assess treatment effectiveness, crown moisture content was measured monthly from December to March at Mead, NE and in March at Axtell, NE. Visual turf quality was rated in the field at Mead, and in the greenhouse from Axtell to monitor survival and rate of spring green-up. Late fall treatments included heavy sand topdressing, a permeable or impermeable cover, anti-transpirant, turf colorant, horticultural spray oil, and wetting agent. The results from this study indicate that both protective cover treatments and sand topdressing were the best performing treatments at both Mead and Axtell (Figs. 1, 3). The crown moisture content under these treatments was as high as 0.764 g $H_2O$ $g^{-1}$ FW at Mead, compared to the lethally low 0.251 g $H_2O$ $g^{-1}$ FW observed in the control at Axtell (Figs. 1, 3). Treatments that sustained crown moisture content levels throughout the winter resulted in a higher turf quality in the spring and recovered faster at both sites (Figs. 2, 4). Sprayable products performed less consistently and rarely provided any added benefit. These results suggest that heavy sand topdressing and protective covers applied late in the fall can reduce desiccation in high-risk areas by sustaining crown moisture contents and improving turf survival, reducing the likelihood of turf death from winter desiccation.

The crown moisture/cold hardiness evaluation study conducted in the growth chamber is still in the preliminary phases. Preliminary results have shown promise but need further refinement before conclusions can be made (Fig 5). The study is still on going and expected to be completed by the summer of 2017.

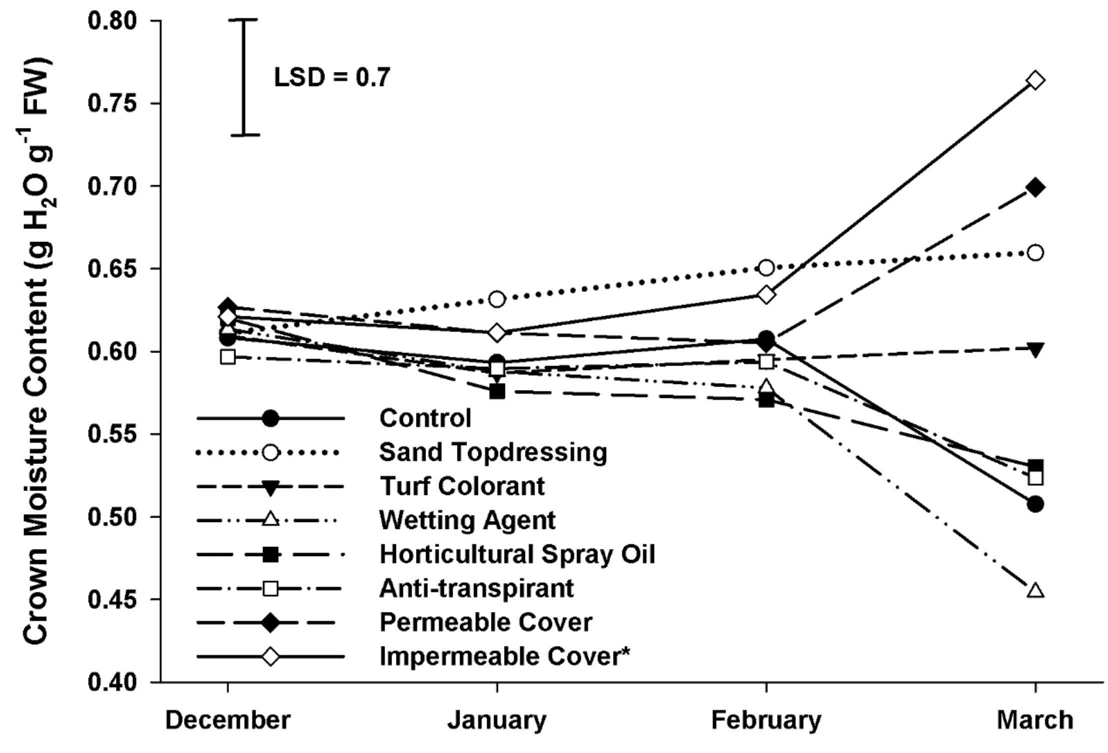



Figure 1. Crown moisture content as affected by spray-applied and cover treatments in Mead, NE. Data were pooled across 2014-15 and 2015-16 studies ($p<0.001$). *3.5 mil clear impermeable cover

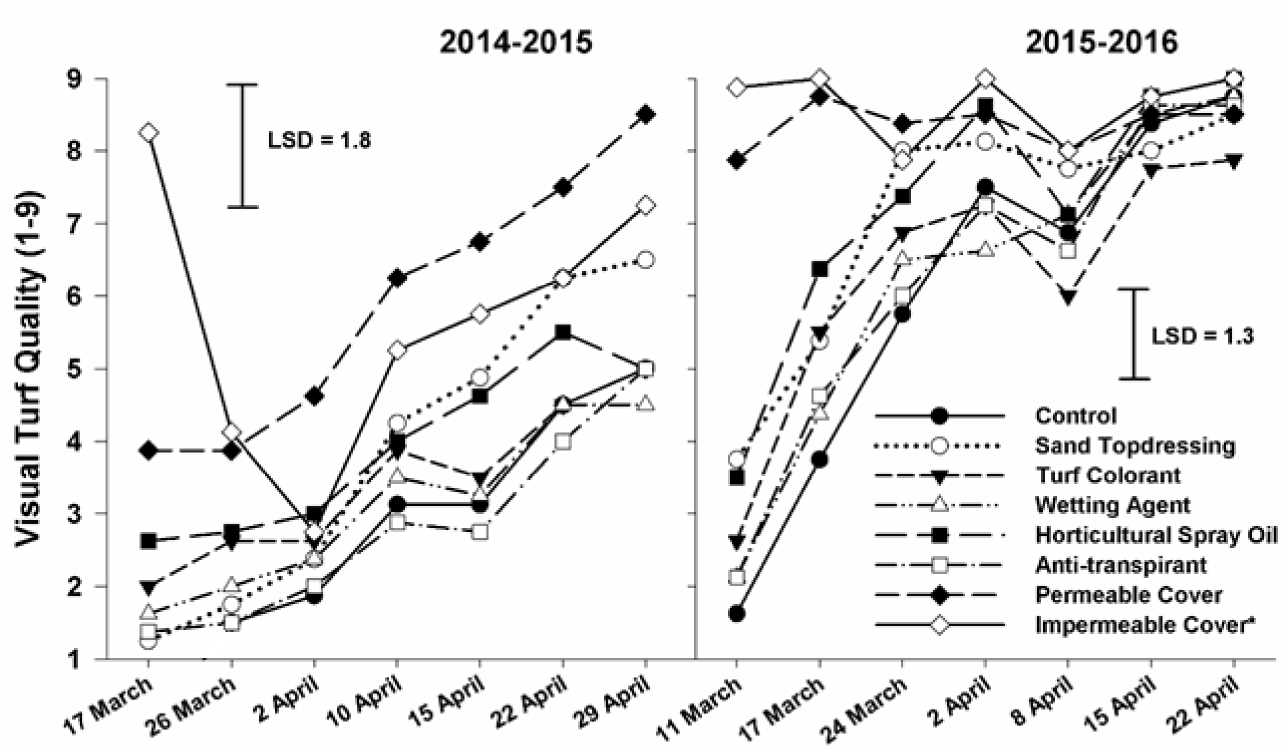
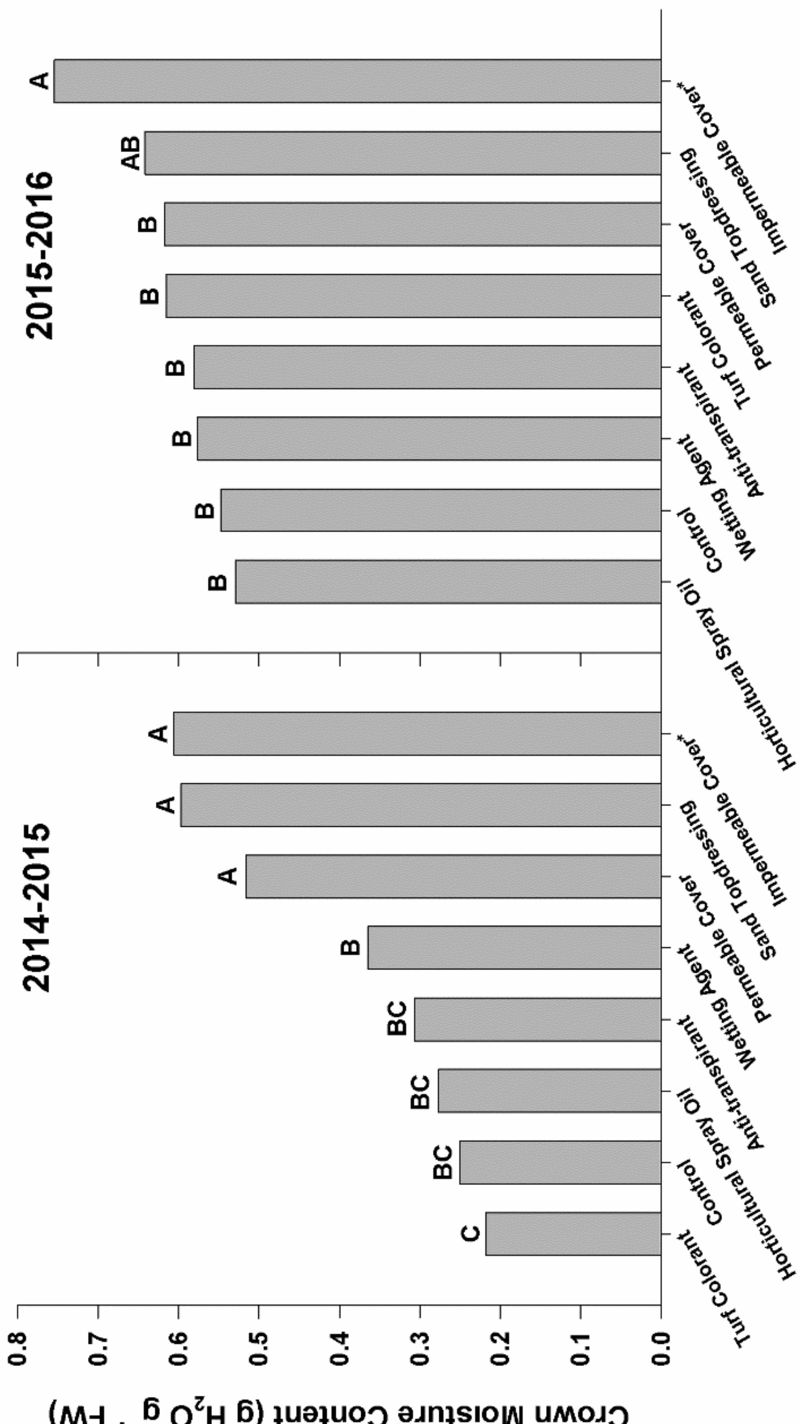
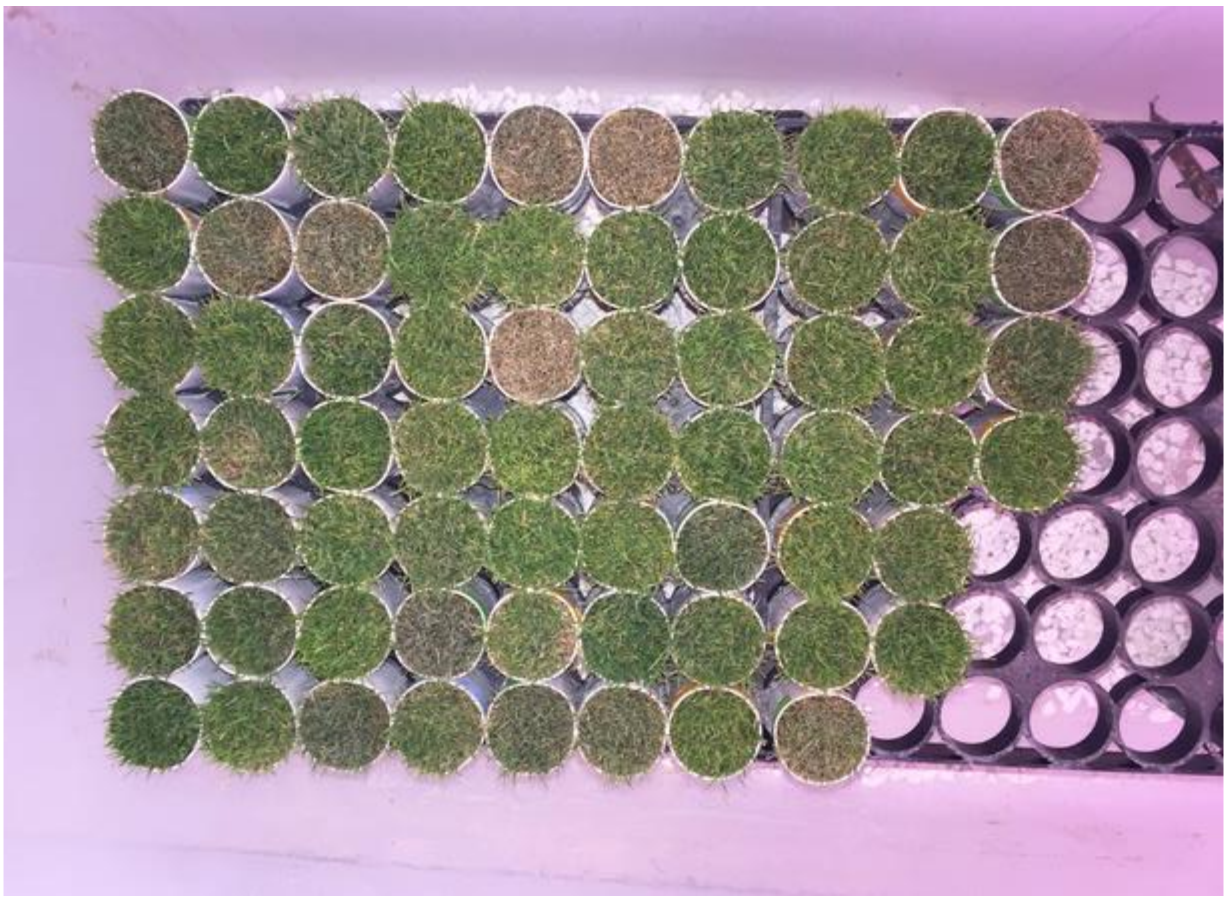



Figure 2. Visual turf quality as affected by spray-applied and cover treatments after removal of covers in Mead, NE (p<0.001). *3.5 mil clear impermeable cover



2014-2015
2015-2016
Crown Moisture Content (g H2O g-1 FW)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Turf Colorant
Control
Horticultural Spray Oil
Anti-transpirant
Wetting Agent
Permeable Cover
Sand Topdressing
Impermeable Cover*
C
BC
BC
BC
B
A
A
A
B
B
B
B
B
B
AB
A

Figure 5. Deficit irrigation treatments are being applied to achieve various crown moisture content ranges. After prolonged deficit irrigation, cones are subjected to decreasing freezing temperatures to evaluate which crown moisture content range provides the greatest level of cold hardiness. Visual water stress and photooxidative stress can be observed in deficit irrigated cones.



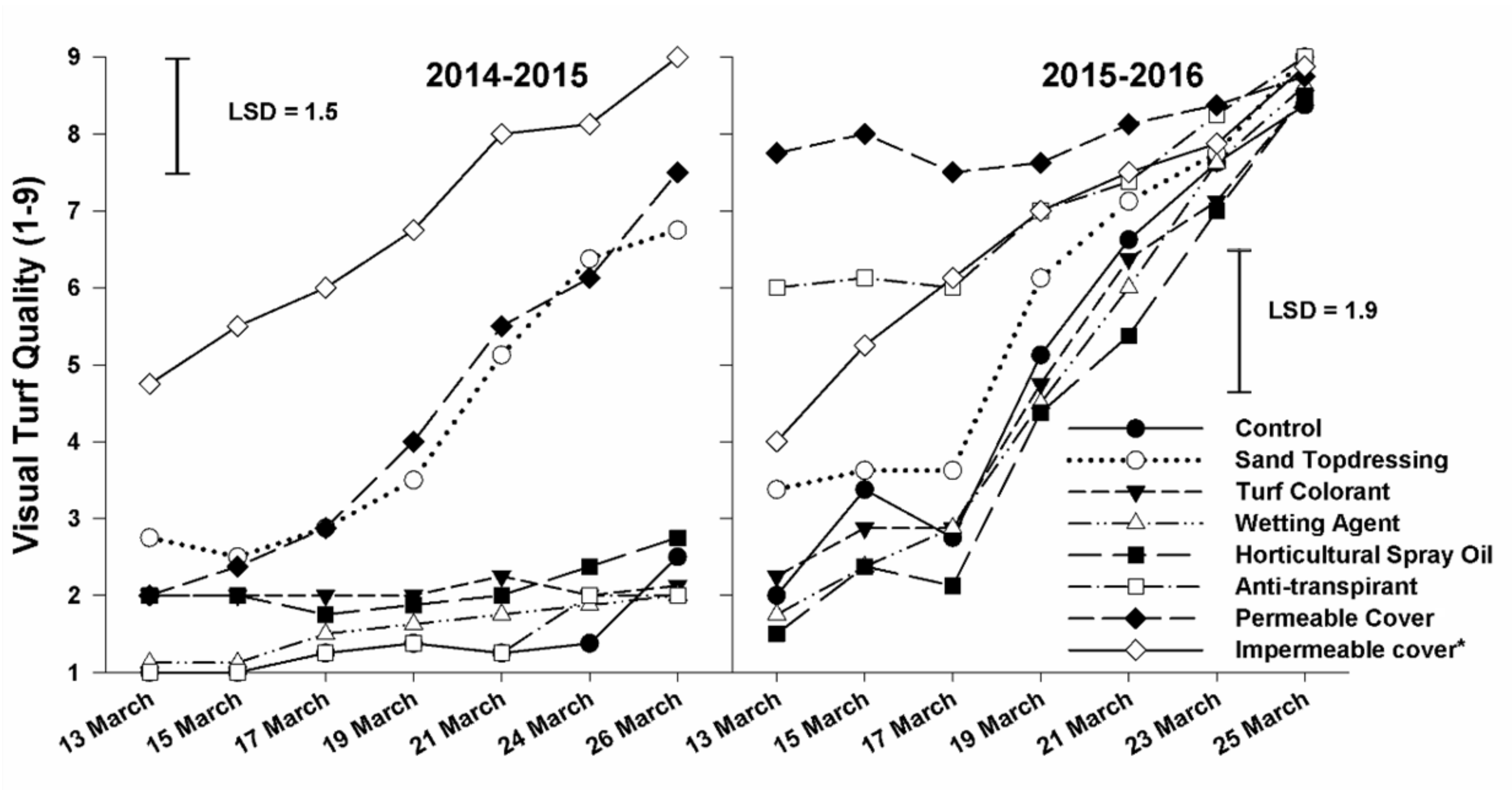



Figure 4. Visual turf quality as affected by spray-applied and cover treatments in Axtell, NE after removal of covers. Samples were evaluated in the greenhouse for recuperative capacity and rate of green-up (p<0.001). *White impermeable cover

# The Effects of Mowing Delay on Proxy Efficacy for Poa annua Seed Head Suppression

## USGA ID#: 2015-19-534

Alec Kowalewski, Brian McDonald, and Micah Gould
Oregon State University
December 1, 2016

**Research Summary (Year 2)**

- Eliminated treatments after proxy application due to non-significant results from year 1.
- Spread out treatments before the Proxy app to 12, 9, 6, 3, and 0 days (including one untreated at 0 days before mowing).
- Regardless of the number of days mowing is delayed prior to application, Proxy decreased seed head production in comparison to the control.
- No differences were observed when mowing was continued 0, 3, 6, 9, or 12 days before Proxy application.

**Introduction**

Annual bluegrass seed head production on putting greens results in a number of detrimental effects including, but not limited to, reduced putting green speed and consistency, and reduced aesthetics. As a result, herbicides and plant growth regulators are often used to suppress seed head flushes. Research and practical field applications have shown that Embark (mefluidide) and Proxy (ethephon) provide the best reduction in seed head production. However, because of the phytotoxicity that often occurs with Embark (and it was recently removed from the market), Proxy has become the product of choice for suppression of annual bluegrass seed heads, however, its effectiveness - especially in the Midwest - is often inconsistent. Recent research conducted in the greenhouse has shown that Proxy absorption and translocation from the flag leaf substantially improves seed head suppression. However, daily mowing removes the flag leaf.

**Year 1 Objective and Findings (Spring 2015):**

Objective

- The initial objective was to determine if mowing delays prior to and following the application of Proxy will affect the seed head suppression of annual bluegrass during the spring flush.

Findings

- Results obtained in 2015 suggest mowing should be delayed the day of Proxy application until after the product is applied (data not shown).

**Year 2 Objective, Materials and Methods, and Findings (Spring 2016):**

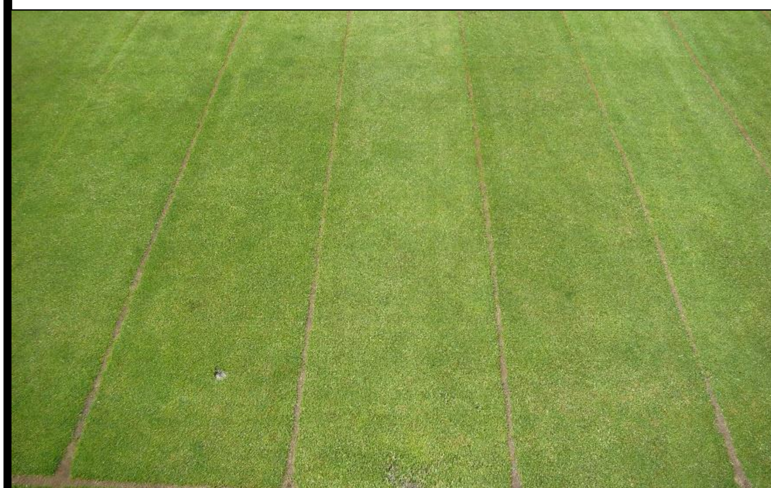
Objective

- The objective of year two research was to determine if increasing the mowing delays, allowing for the development of a larger flag leaf, prior to the application of Proxy will affect the seed head suppression of annual bluegrass during the spring flush (Figure 1).

Materials and Methods

Application of Proxy PGR significantly reduced seed head production as of June 7th at a 0.01 level of probability. The prior week, May 31st was not significant (Table 1). The main effect of mowing delay **before Proxy application** was not significant on any date. The only significant differences between treatments was that of the control that received no proxy and the rest of the treatments that received Proxy and mowing (Image 1).

**Image 1: Effects of Proxy on Poa seed head suppression 2 weeks after application in Corvallis, OR.**



Poa spring seed head flush exhibited by treatment without Proxy (middle) versus plots with Proxy and mowing before application (left and right plots).

This year, Proxy was applied on 3/4/16 at 5 fl. oz./1,000 ft.$^2$ on the entire green to control seed heads early in the year before the trial started. The idea was to look at the Poa seeding later in the spring to take advantage of faster turf growth from warmer temperatures, however, seed heads did not develop to a great degree, which limited results.

Results obtained in 2016 findings suggest putting greens can be mowed the day of Proxy application without an effect on seed head suppression. However, the question remains how soon (how many hours) after Proxy application can the putting green be mowed without effect seed head suppression.

**Year 3 Research (Spring 2017):**

Objective

- The objective of future research (spring 2017) will explore how soon mowing can be done after Proxy application.

Mowing treatments after application in 2017 will include 1, 2, 3, 4 and 6 hours after Proxy application (Table 2). Proxy will be applied at 5 oz./1,000 ft$^2$ and may include up to 3 separate applications during the spring seed head flush, which will begin in March of 2017. The mowing timings will occur according to the date of Proxy application.

**Figure 1:** Mowing timing – days before Proxy application (5.0 fl. oz. per 1,000ft$^2$) in 2016 in Corvallis, OR, days mowed before Proxy application (shaded in blue).

| Proxy? | Last Mowing Date Before App | | **- 12 Days** | **- 9 Days** | **- 6 Days** | **- 3 Day** | **0** |
|---|---|---|---|---|---|---|---|
| Proxy @ 5.0 fl. oz./M | 12 | | | | | | |
| Proxy @ 5.0 fl. oz./M | 9 | | | | | | |
| Proxy @ 5.0 fl. oz./M | 6 | | | | | | |
| Proxy @ 5.0 fl. oz./M | 3 | | | | | | |
| Proxy @ 5.0 fl. oz./M | 0 | | | | | | |
| Untreated | 0 | | | | | | |

Note: After this schedule, the plots were mowed 7 days a week until the end of the trial.

**Table 1:** Effects of mowing date prior to Proxy application on analysis of variance and mean for separation for seed head counts observed on 31 May and 7 June, 2016 in Corvallis, OR.

| **Source of Variation** | Num DF | Den DF | 31-May *Pr* > F | 7-Jun *Pr* > F |
|---|---|---|---|---|
| Mowing prior to Proxy application | 5 | 15 | ns | ** |

| **Mowing [¥] prior to Proxy application[¥]** | 31-May Seed Heads per 12.6 inch$^2$[‡] | | 7-Jun Seed Heads per 12.6 inch$^2$[‡] | |
|---|---|---|---|---|
| 0 days before app | 14.5 | a[±] | 9.4 | b |
| 3 days before app | 14.9 | a | 9.3 | b |
| 6 days before app | 18.8 | a | 13.0 | b |
| 9 days before app | 14.7 | a | 9.0 | b |
| 12 days before app | 16.9 | a | 10.7 | b |
| No proxy | 24.9 | a | 18.9 | a |

ns = Not significant; ** Significant at a 0.01 level of probability; [‡] Surface area of a 4 inch diameter cup cutter;

¥ Proxy applied at 5 oz./1,000 $ft^2$ on 17 May 2016; ¥ Mowing applied beginning May 5th; ± lower case letters represent a significant difference at a 0.05 level of probability. Mean separations were obtained using Fisher's LSD.

**Table 2:** Expanded mowing treatments after Proxy application, research to be conducted in spring of 2017 in Corvallis, OR.

| **Mowing after Proxyƚ application** |
| --- |
| 1 hour after application |
| 2 hours after application |
| 3 hours after application |
| 4 hours after application |
| 6 hours after application |
| No Proxy |

ƚProxy will be applied in March 2017 at 5 oz./1,000 $ft^2$, with possibly up to 3 apps through the growing season.

2016-09-559

Breaking Seed Dormancy in Buffalograss

Keenan Amundsen and Katie Kreuser

USGA ID#: 2016-09-559

1. Buffalograss seed dormancy can be overcome with a potassium nitrate treatment
2. Water may be as effective as potassium nitrate for breaking seed dormancy
3. Potassium nitrate seed treatment alters the seed coat allowing for rapid water uptake

Buffalograss seed is different compared to seed of other grass species commonly used for turf. Typically 1-5 caryopses are encapsulated in a bur-like structure, and these burs are sold as seed (Figure 1). Buffalograss burs have a strong seed dormancy response, common for many native grass species. Following harvest and seed cleaning, seed germination is often less than 30% even when viability is near 100%. Seeds are soaked in a potassium nitrate solution followed by wet storage at low temperature to break seed dormancy. The germination percentage of treated seeds increases to near 90%. A green dye is used by seed producers to indicate the seed has been treated to break dormancy (Figure 1). Another method for overcoming seed dormancy in buffalograss is to remove the caryopses from the bur. The caryopses readily germinate in the absence of a seed treatment. This method is not practical since isolated caryopses have poor shelf life and isolating them adds to post-harvest seed production costs. Since the caryopses readily germinate, the mechanisms conferring dormancy are believed to be in the seed coat, or the bur structure, but no research has been done to understand these mechanisms in buffalograss. This project is focused on understanding mechanisms of seed dormancy in buffalograss, characterizing molecular and physiological changes at germination, and testing alternative seed treatments to break dormancy.

Dormancy in many species is controlled by the endogenous hormones gibberellic acid (GA) and abscisic acid (ABA), where increased levels or sensitivity to ABA promotes dormancy and increased GA signaling or sensitivity promotes germination. A preliminary hormone profiling study was done to compare changes in ABA and GA among untreated, water soaked, and potassium nitrate treated buffalograss seeds. Concentrations of ABA did not change but levels of GA increased, supporting the idea that increased GA levels promote buffalograss seed germination. Subsequent experiments had variable results and GA levels in treated seeds were not consistently greater than in untreated seeds. For the above treatments, percent germination was also collected for 28 days following treatments (Figure 2). Germination percentage was the same between the water soaked and potassium nitrate treatments, suggesting water soaking may be as effective for breaking dormancy as the potassium nitrate treatment. Long term studies are needed to determine if the promotion of germination by the water treatment is short-lived or sustained.

In addition to the germination tests, water imbibition rates were measured. Similar to a soil hydrophobicity test, 10 seeds from each treatment were affixed to paper towel and a drop of water was placed on each seed (Figure 3). The length of time for the water droplet to be absorbed by the seed was measured. The potassium nitrate treatment caused more rapid water absorption by the seed than either the untreated or water soaked seeds. The potassium nitrate treatment is altering the seed coat and allowing for the water to be taken up, impacting imbibition and ultimately promoting germination. The mechanisms for seed dormancy are still not understood and additional seed hormone profiling, genetic studies, and plant breeding approaches to remove seed dormancy are underway.

Figure 1. Buffalograss exposed caryopses (left), bur-like structure (middle), green-dyed potassium nitrate treated burs (right).

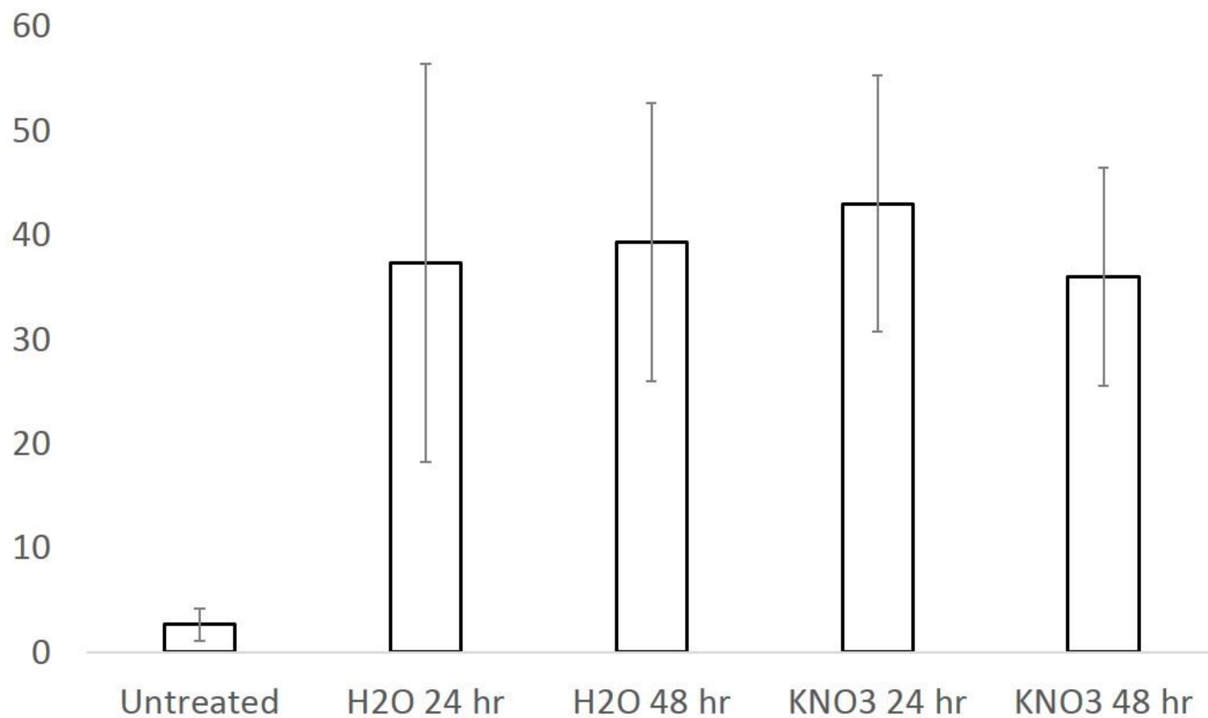



Figure 2. Twenty-eight day germination percentage of untreated, 24 h or 48 h water treated, or 24 h or 48 h potassium nitrate treated buffalograss burs.

Figure 3. Test for water droplet infiltration of buffalograss burs.

**Reducing ultradwarf bermudagrass putting green winter injury with covers and wetting agents**

Michael D. Richardson, Douglas E. Karcher and Eric J. DeBoer. University of Arkansas, Department of Horticulture, Fayetteville, AR 72701

As ultradwarf bermudagrass (*Cynodon dactylon x Cynodon transvaalensis*) putting greens move further north in the transition zone, there is an increased risk of sustaining winter injury from low temperature exposure and crown desiccation. The benefits of utilizing covers for winter protection are well documented but there are significant labor costs associated with covering and uncovering greens during the winter to allow for play during favorable weather. While the current recommendation is to cover bermudagrass greens when the low temperature is forecasted to drop to -4 °C (O'Brien and Hartwiger, 2013), it may be possible to lower this forecasted temperature, resulting in fewer covering events, reduced labor costs and more days open for play.

Localized dry spot (LDS) is a common problem on sand-based putting greens and can lead to desiccation of the turfgrass crown and even death of the plant. Symptoms of LDS are easily recognized when turf is actively growing but may not be apparent while the turf is dormant. Wetting agents are commonly applied during the growing season to combat effects of LDS but little information exists on the effects of a late-fall/early-winter wetting agent application on winter survival and spring green-up of ultradwarf bermudagrass.

Objectives:

- Examine predicted low-temperature thresholds for covering Tifeagle, Champion, and Mini-Verde ultradwarf bermudagrass putting greens
- Investigate the effects of a late-fall wetting agent application on soil moisture and winter survival of ultradwarf bermudagrass

Materials and Methods:

This trial was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. The treatments included three ultradwarf bermudagrass cultivars ('Champion', 'Mini-Verde', and 'Tifeagle'), five cover treatments based on forecasted low temperatures (-9.4, -7.8, -5.6 or -4.0 °C and an uncovered control), and two wetting agent treatments (Revolution applied at 1.9 ml $m^{-2}$ on Dec. 7, 2015 and an untreated control) The experimental design was a strip split plot, where cover treatments were applied as strip plots across cultivars and cover x cultivar plots were further split with the wetting agent treatments (Photo 1). Data to be discussed includes % turfgrass coverage (collected using digital image analysis) during spring greenup.

Results:

It should be noted that Fayetteville, AR experienced an unseasonably warm winter for 2015-2016, so the extent of winter injury was atypical for this location.

- 'Tifeagle' and 'Mini-Verde' experienced less winter injury and better spring greenup than 'Champion' (Figure 1, Photo 2)
- The use of protective covers enhanced spring green-up and recovery for all cultivars (Figure 2)
- A late season wetting agent application improved spring green-up of ultradwarf greens (Figure 3, Photo 3)

Photo 1. Overview of the trial site, showing the various cover treatments stripped across the three cultivars of ultradwarf bermudagrass

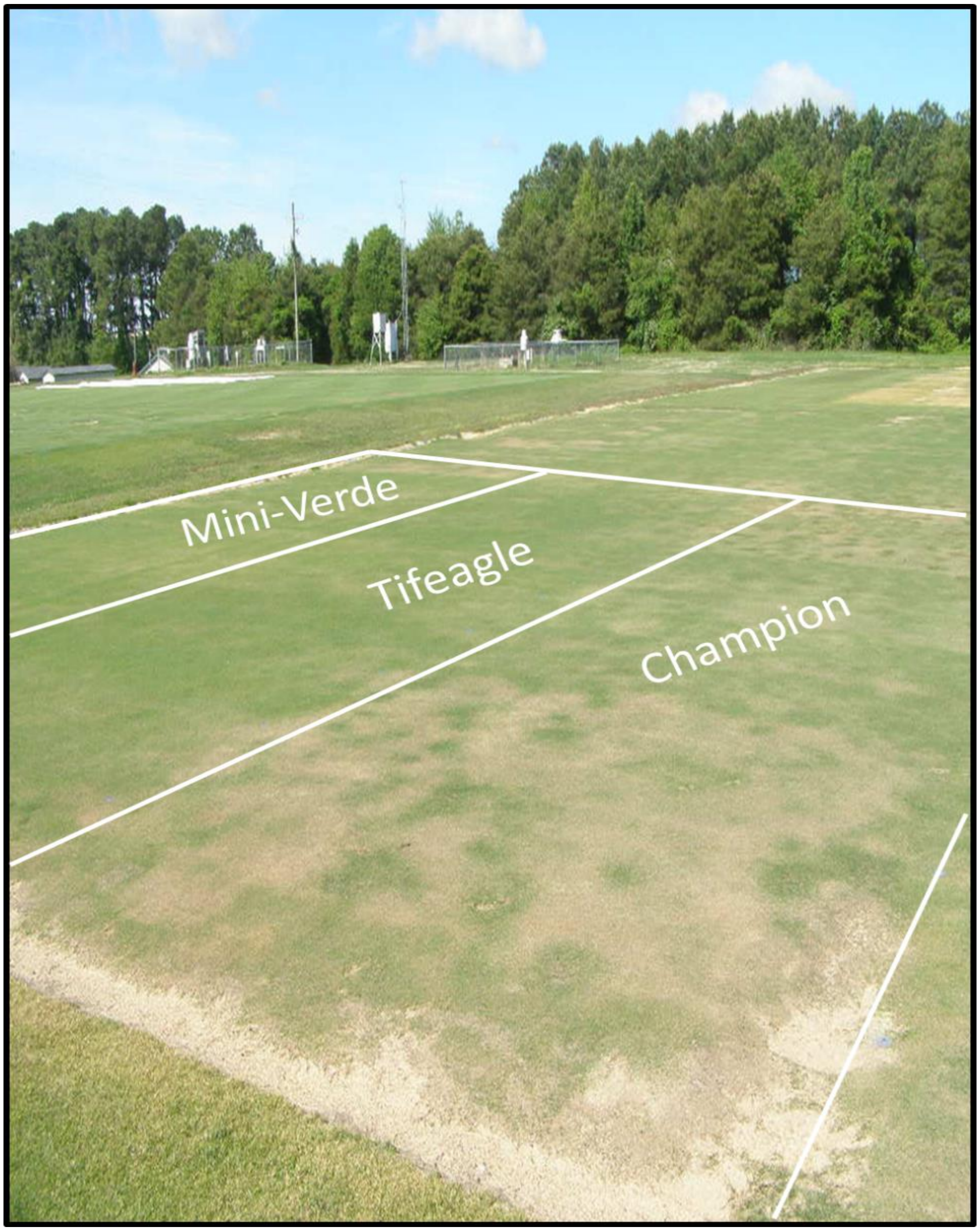



Photo 2 – Spring greenup of three ultradwarf bermudagrass cultivars – Photo taken on 21 April 2016

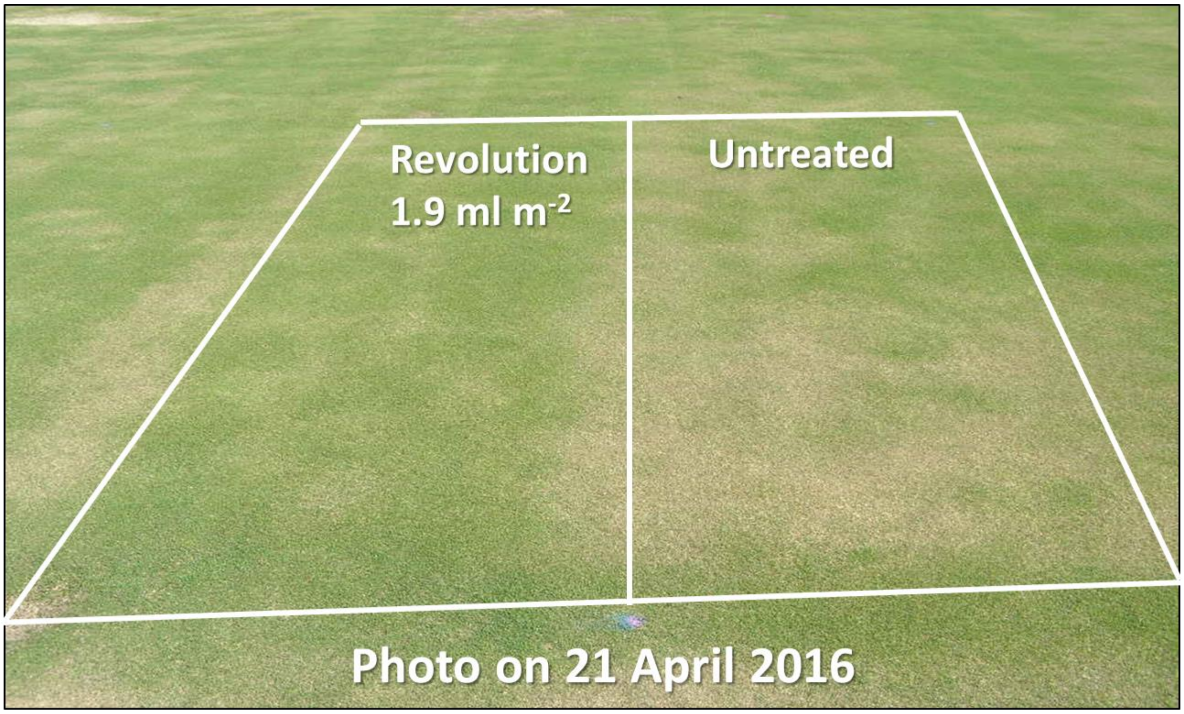



Photo 3 – Spring greenup of ultradwarf bermudagrass, as affected by a late-season wetting agent application.

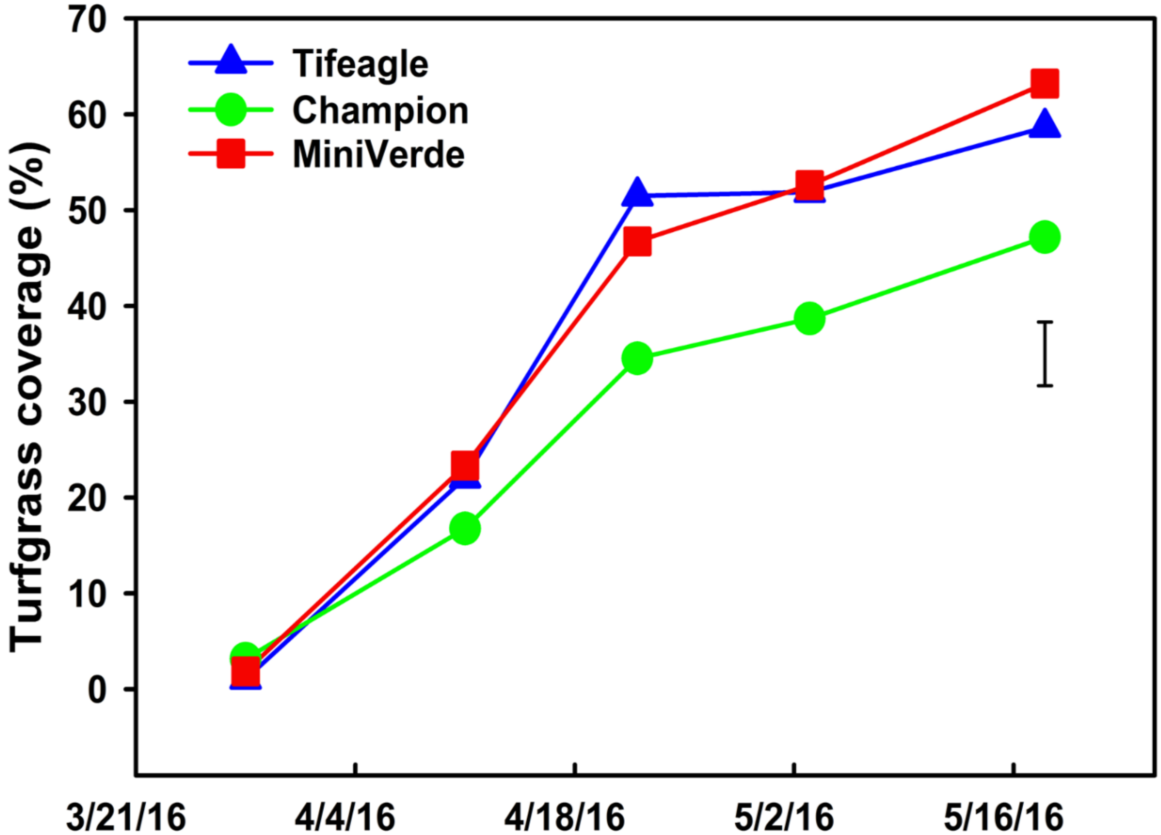



Figure 1. Effect of cultivar on spring greenup of ultradwarf greens. Error bar represents the least significant difference (P=0.05) for comparing treatments.

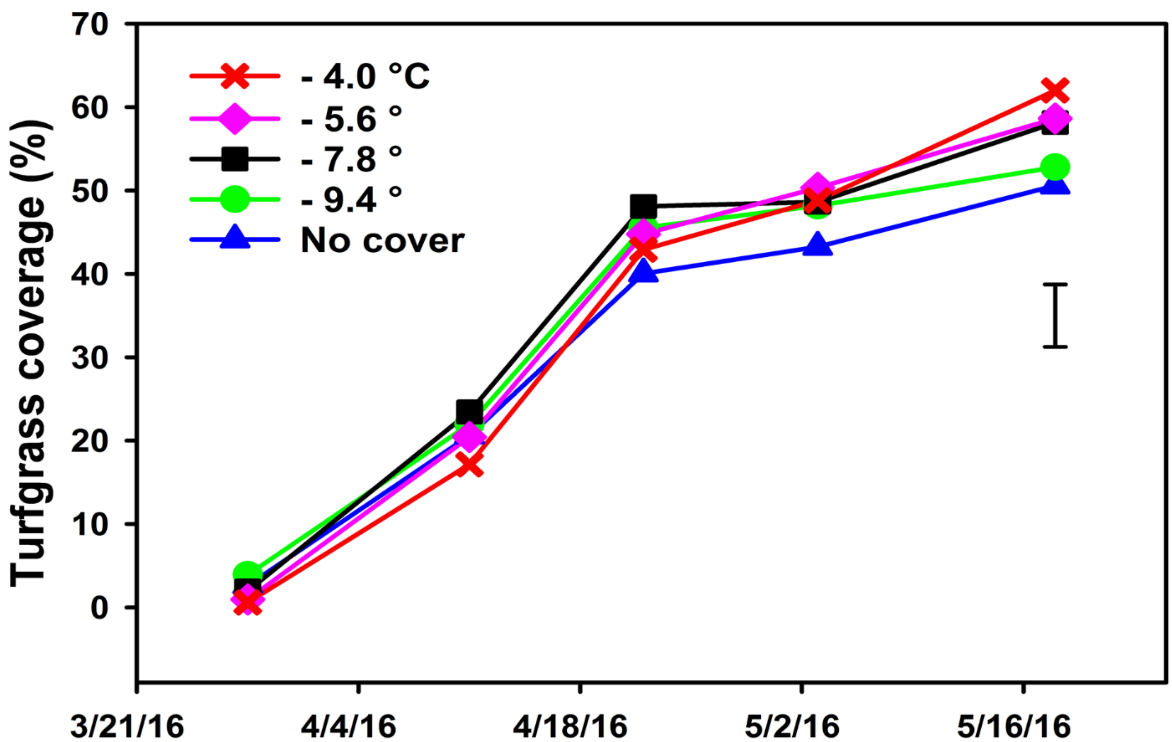



Figure 2. Effect of cover treatments on spring greenup of ultradwarf greens. Error bar represents the least significant difference (P=0.05) for comparing treatments.

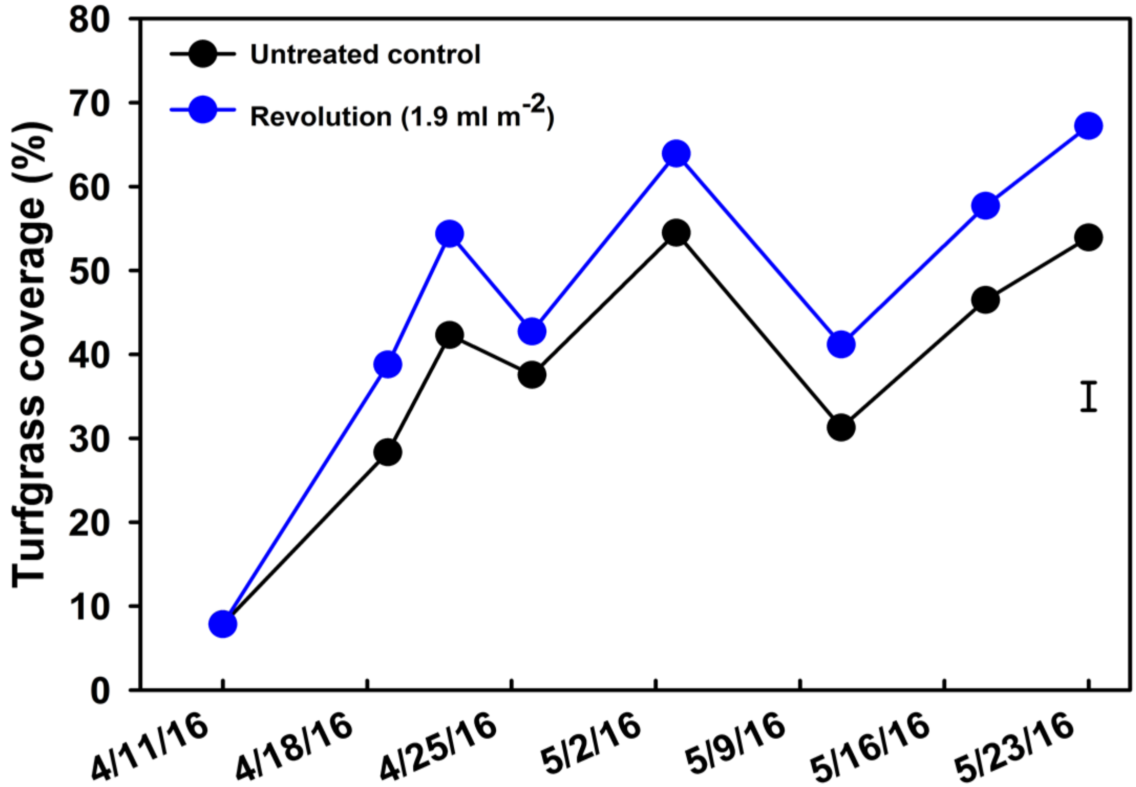



Figure 3. Effect of a late-season wetting agent application on spring greenup of ultradwarf greens. Error bar represents the least significant difference (P=0.05) for comparing treatments.

**Progress Report:** December 2, 2016

**Project Title:** Evaluation of Putting Green Bermudagrass for Shade Tolerance and Evaluation of Fairway Bermudagrasses for Water Use Rates

**Principal Investigators:** Justin Q. Moss

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Start Date: 2014
**Number of Years:** 3
**Total Funding:** $60,000

**Project #1:** Evaluation of Putting Green Bermudagrass for Shade Tolerance

**Objectives:**

- *Evaluate experimental and commercialized putting green bermudagrasses against Diamond zoysiagrass under full sun and shaded conditions.*

**Research Progress:**
- Plots were planted from sprigs on a sand based putting green in July 2013 and fully established in 2014.

**Preliminary Results:**
- TifEagle, 264, MiniVerde, and Champion performed best in 2014 while Diamond, MiniVerde, TifEagle, and Champion were the best cultivars in 2015.
- 16-13-8 and 1-75-2 were consistently ranked lowest in 2014 and 2015.
- Diamond zoysiagrass exceptionally performed well in 2015 compared to all bermudagrass cultivars.

A research site was planted using greenhouse-grown grass sprigs on June 7, 2013. A row of fully mature *Platanus occidentalis* runs parallel to the east side of the study area and provides natural, tree shade in the morning and early afternoon, depending on season. The site was blocked to provide six replications of each cultivar. Photosynthetically active radiation was collected throughout the study period. The plots were covered with a geo-textile to protect against low temperature injury.

The standard entries, ‘Champion’, ‘Mini Verde’, ‘TifEagle’ Bermudagrass, and ‘Diamond’ Zoysiagrass and experimental entries were planted on 5 x 5 ft. plots. Plots were mowed 6 times per week at a 0.155 height and nitrogen was applied at 49 kg/ha monthly. Irrigation was applied at rates and frequencies necessary to maintain acceptable green turf. Trinexapac–ethyl was applied as a standard treatment to all plots during the growing season.

Turfgrass visual quality was assessed monthly based on 1-9 scale. In addition, Normalized difference vegetative index (NDVI) was collected monthly using a GreenSeeker sensor to assess shade stress through changes in turfgrass color and cover.

In 2014, TifEagle, 264, MiniVerde, and Champion significantly performed better than Diamond zoysiagrass (Table 1). Experimental entries, 13-78-5, 16-13-8, and 1-75-2 including Diamond zoysiagrass showed unacceptable TQ in 2014. In 2015, all bermudagrass cultivars poorly performed compared to Diamond zoysiagrass. In 2016, Diamond zoysiagrass and Champion bermudagrass were not statistically different, but Diamond was significantly better than the other commercial standards for bermudagrass. However, the area covered by Diamond zoysiagrass has been reduced through competition in plot perimeters. All experimental lines were ranked lower than industrial standards, MiniVerde, TifEagle, and Champion.

Table 1. The turf visual quality means of bermudagrass collected monthly in 2014, 2015 and 2016.

| 2014 | | | 2015 | | | 2016 | | |
|---|---|---|---|---|---|---|---|---|
| Cultivar | TQ | NDVI** | Cultivar | TQ | NDVI | Cultivar | TQ | NDVI |
| TifEagle | 6.6 a* | 0.739 | Diamond | 7.0 a | 0.679 | Diamond | 7.4 a | 0.697 |
| 264 | 6.1 a | 0.708 | MiniVerde | 5.8 b | 0.611 | Champion | 6.8 ab | 0.688 |
| MiniVerde | 6.1 a | 0.737 | TifEagle | 5.4 bc | 0.630 | MiniVerde | 6.7 b | 0.669 |
| Champion | 6.0 a | 0.743 | Champion | 5.0 c | 0.607 | TifEagle | 6.7 b | 0.681 |
| 13-78-5 | 4.8 b | 0.656 | 13-78-5 | 4.7 cd | 0.599 | 264 | 5.7 c | 0.659 |
| Diamond | 4.8 b | 0.596 | 264 | 4.6 cd | 0.590 | 13-78-5 | 3.6 d | 0.631 |
| 16-13-8 | 4.3 bc | 0.617 | 16-13-8 | 4.1 d | 0.570 | 16-13-8 | 3.3 de | 0.595 |
| 1-75-2 | 3.9 c | 0.563 | 1-75-2 | 2.9 e | 0.493 | 1-75-2 | 2.8 e | 0.554 |

* Treatments within column with same letters are not significantly different at $p$=0.05.
**Normalized difference vegetation index (near infrared reflectance - red reflectance) / (near infrared reflectance + red reflectance)

For irradiance data, PAR sensors were equally spaced in distance from one another creating a light gradient as the sensors increased in proximity to the tree line. The significance of the difference can most easily be noted in summer months as defoliation in winter months significantly increases the relative amount of light to the highest shade treatment (Table 2).

Table 2. Photosynthetically Active Radiation (PAR) average measurements reported for February, July, and November of 2016 by sensor location in uM/m²s/day.

| | Average Daily PAR by Sensor Location (uM/m^2s) | | |
|---|---|---|---|
| Month-Year | East | Center | West |
| Feb-16 | 13044.2 | 7313.5 | 7898.9 |
| Jul-16 | 13342.7 | 18419.2 | 21500.6 |
| Nov-16 | 6266.7 | 7533.5 | 10010.4 |

**Project #2:** Evaluation of Fairway Bermudagrasses for Water Use Rates

**Objectives:** *Evaluate, measure, and explain any differences in water use rates among several industry standard bermudagrass cultivars vs OSU experimental bermudagrasses.*

**Research Progress:**

- Lysimeters were maintained in the greenhouse from December to April each year to prevent winter injury.

**Preliminary Results:**

- ET rates in 2014 ranged from 4.93 mm $d^{-1}$ to 6.19 mm $d^{-1}$ and ranged from 3.88 mm $d^{-1}$ to 6.03 mm $d^{-1}$ in 2015.

- DT-1 was the most water use cultivar in 2014 and 2015 with ET of 6.19 mm $d^{-1}$ and 6.03 mm $d^{-1}$, respectively.
- OKC 1163 and OKC 1131 were the low water use cultivars in both 2014 and 2015, respectively.

The research site was a former 2002-2006 NTEP (National Turfgrass Evaluation Program) bermudagrass trial with 2.4 x 2.4 m plots. Six genotypes out of ten were new which were not included in the original NTEP trial. These were ‘Latitude 36’, ‘NorthBridge’, DT-1, OKC 1302, OKC 1131, and OKC 1163. The new genotypes were planted in the plots by removing the former grass using sod cutter in May, 2013.

The lysimeters were constructed using polyvinyl chloride (PVC) tube, 15.2 cm inside diameter and 35.6 cm long with a root zone depth of 30.48 cm and an extra 5.1 cm of length below the root zone to accommodate a drain valve on the bottom (Photo 1). A threaded ball valve was installed onto the bottom inside of each lysimeter. The inner side of the lysimeter was filled with a geo-textile porous sheet to prevent any loss of the rooting medium. The rooting medium is a calcined clay product.

Field ET rate was collected from May to September in 2014 and August to September in 2015 (Photo 3).

OKC 1131, NorthBridge, and OKC 1163 were the low water use cultivars in both 2014 and 2015 whereas DT-1 was the higher water use cultivar (Table 2). ‘Celebration’ and ‘Tifway’ were ranked as the second most water use group in 2014 and 2015. OKC 1302 was one of the higher water use cultivars in 2014, but it was among the lower water use cultivars in 2015.

Table 2. Mean daily ET rate (mm d-1) of ten bermudagrass entries under non-limiting soil moisture conditions in 2014 and 2015.

| 2014 | | 2015 | |
|---|---|---|---|
| Entries | ET* | Entries | ET |
| DT-1 | 6.19 a** | DT-1 | 6.03 a |
| Celebration | 6.08 ab | Tifway | 5.36 ab |
| OKC 1302 | 6.07 ab | Celebration | 5.19 ab |
| Tifway | 6.00 b | Premier | 5.15 ab |
| Premier | 5.71 c | Latitude36 | 5.10 b |
| Latitude 36 | 5.70 c | TGS_U3 | 4.99 bc |
| TGS_U3 | 5.51 d | OKC1302 | 4.59 bcd |
| OKC 1131 | 5.18 e | NorthBridge | 4.19 cd |
| NorthBridge | 5.17 e | OKC1163 | 4.01 d |
| OKC 1163 | 4.93 f | OKC1131 | 3.88 e |

* Water use in mm $d^{-1}$. Values are the mean of 14 ET rates and 6 ET rates in 2014 and 2015, respectively.

** Treatments within column with same letters are not significantly different at *p*=0.05.

Photo 1. A row of fully mature *Platanus occidentalis* on the east side of the study area.

Photo 2. A lysimeter in a field plot.

Photo 3. ET rate collection from filed plots.

**Title:** Developing a Rapid Method for Diagnosing Herbicide Resistance in Annual Bluegrass

**Project Leader:** James T. Brosnan

**Affiliation**: University of Tennessee

**Objectives**: Determine if agar-based rapid diagnostic tests can be used to diagnose herbicide resistance in annual bluegrass populations harvested from golf course turf.

**Start Date**: 2016

**Project Duration:** Two years

**Total Funding**: $57,156

**Summary Text**: Reports of herbicide resistance in annual bluegrass (*Poa annua* L.) are greater than any other weed species commonly found in turf. Annual bluegrass phenotypes resistant to mitotic inhibitors (e.g., prodiamine), acetolacatate synthase inhibitors (ALS; e.g., foramsulfuron, trifloxysulfuron, etc.), photosystem II inhibitors (PSII; e.g., simazine), and enolpyruvylshikimate-3-phosphate (EPSP) synthase (e.g., glyphosate) inhibitors have been identified on golf courses following continued use of the same pre- or postemergence herbicides in lieu of diversified weed management programs.

Traditional means of testing annual bluegrass for herbicide resistance can be labor intensive, costly, and time consuming. Rapid diagnostic tests have been developed to confirm herbicide resistance in weeds of agronomic cropping systems that correlate well with traditional whole plant bioassays. These tests involve transplanting weed seedlings of resistant and susceptible populations into petri plates filled with agar and discriminatory rates of herbicide. This technique has successfully been used to provide farmers confirmation of rigid ryegrass (*Lolium rigidum*) populations resistant to both ALS and acetyl co-A carboxylase inhibiting herbicides, as well as Italian ryegrass (*Lolium multiflorum*), goosegrass (*Eleusine indica*), horseweed (*Conzya canadensis*), and common waterhemp (*Amaranthus rudis*) populations resistant to glyphosate.

Research was conducted at the University of Tennessee in 2016 to determine if agar-based rapid diagnostic tests could be used to confirm herbicide resistance in annual bluegrass harvested from golf course turf. Separate experiments were conducted using annual bluegrass phenotypes resistant to ALS inhibiting herbicides and glyphosate via target site mutation; an herbicide susceptible control was included in each for comparison. Single tiller plants were washed free of growing media and transplanted into autoclavable polycarbonate plant culture boxes filled with 65 mL of murashigee-skoog media amended with glyphosate (0, 6, 12, 25, 50, 100, 200, or 400 µM) or trifloxysulfuron (6.25, 12.5, 25, 50, 75, 100, or 150 µM). Treatments were arranged in a completely randomized design with 50 replications and repeated in time. Mortality in agar was assessed 7 to 12 days after treatment (depending on herbicide) and compared to responses observed after treating 98 individual plants of each phenotype with glyphosate (560 g $ha^{-1}$) or trifloxysulfuron (27.8 g $ha^{-1}$) in an enclosed spray chamber. Fisher's exact test ($\alpha = 0.05$) determined that mortality in agar with 100 µM glyphosate was not significantly different than treating whole plants via traditional spray application. Similarly, mortality in agar with 12.5 µM trifloxysulfuron was not significantly different than spraying whole plants with herbicide. While work is on-going to determine if this agar-based test can be used to assess resistance to photosystem II inhibiting herbicides and other modes of action, current findings indicate that this method can reliably diagnose annual bluegrass resistance (or susceptibility) to glyphosate or trifloxysulfuron in 12 days or less.

**Summary points**:

- Herbicide-resistant annual bluegrass is becoming increasingly problematic on golf courses throughout the transition zone and southern United States.
- Traditional means of confirming herbicide resistance in annual bluegrass can be labor intensive, costly, and time consuming leaving superintendents with little guidance regarding proper management in-season.
- A rapid diagnostic assay in agar culture can now reliably diagnose annual bluegrass resistance (or susceptibility) to glyphosate or trifloxysulfuron in 12 days or less.

Picture 1- Herbicide-resistant annual bluegrass (*Poa annua*) following two broadcast applications of glyphosate at 1120 g $ha^{-1}$ during winter dormancy in Rockford, TN.

Picture 2- Autoclavable polycarbonate plant culture box used to diagnose annual bluegrass (*Poa annua*) resistance to herbicides in agar culture.

Picture 3- Response of three annual bluegrass (*Poa annua*) phenotypes to 12.5 µM trifloxysulfuron in agar culture. Note that the two resistant phenotypes on the left are not affected by trifloxysulfuron while the susceptible phenotype on the right shows severe tissue discoloration 7 days after treatment.

**Minimum Daily Light Integral Requirements for Warm-Season Fairway/Tee and Rough Cultivars: Mowing Height and Growth Regulator Interactions**

Ben Wherley, Casey Reynolds, Zhaoxin Chen, and Russell Jessup
Texas A&M University Department of Soil & Crop Sciences

Maintaining turf in shade is a significant management challenge for golf course superintendents. Environmental differences with regard to month of the year, shade sources, intensity, duration of shade, or hours of direct sunlight make it difficult to specify a minimum light requirement in terms of hours/day that can extend across various situations. Rather than responding to a number of hours of direct sunlight or percent shade, plants respond to the cumulative daily total number of photons (measured in moles/sq. meter/day) received within the photosynthetically active wavelengths (400-700 nm), termed daily light integral (DLI). The limited amount of turfgrass DLI research that has been conducted previously has involved greenhouse experiments or focused on ultradwarf bermudagrass.

This field study is being conducted over multiple seasons under replicated treatments offering 0 to 90% reductions in photosynthetic photon flux (PPF). Objectives are to 1) determine minimal DLI requirements for 10 zoysiagrass and bermudagrass cultivars commonly used on golf courses, 2) determine how minimal DLI requirements change seasonally (spring, summer, and fall months), 3) determine effects of fairway & rough cutting height (0.75” vs. 2”) on minimal DLI, and 4) determine impacts of trinexapac-ethyl (TE) on minimal DLI requirements.

A 15,000 sq. ft. irrigated shade research facility has been constructed in 2015 at the Texas A&M Turfgrass Field Laboratory. The turfgrasses utilized in this project are shown in Table 1. Parallel studies are being conducted: a ‘rough study’ conducted at 2” mowing heights, and a fairway study managed at 0.75” mowing heights. Both studies are arranged in a completely randomized design with 4 replicate plots per treatment and 6 density-neutral shade levels (0, 30, 50, 70, 80, 90% photosynthetic photon flux reduction) as the whole plot factor. Shade structures cover plots throughout the year, but are removed for short periods for mowing, fertilization, and collecting data. After sodding plots in July 2015, grasses were given 6 weeks to establish under full sun conditions before shade structures were moved onto plots. Turf quality, digital image analysis of percent green cover, NDVI, and rooting data are being measured monthly and will be regressed against shade level to identify critical DLI thresholds for each entry at the end of the project. For reference, shade treatments of 0, 30, 50, 70, 80, and 90% shade correspond to summer (June to August) mean DLI of ~48, 27, 22, 12, 9, and 6 mol/m2/d and autumn (November) mean DLI of ~19, 11, 8, 5, 4, and 2 mol/m2/d. Thus, time of year will be an important consideration when interpreting and applying DLI data for specific situations. Some preliminary findings through the first year of the study include the following:

- Zoysiagrass cultivars show better shade tolerance than bermudagrass at both mowing heights, maintaining >50% cover until exposed to ~55-70% shade levels. JaMur shows good shade tolerance relative to other cultivars, maintaining >50% cover up until 70% shade exposure. (Figs. 1 & 2)
- At the fairway mowing height, most bermudagrass cultivars fall below 50% green cover when receiving more than ~25% shade. However, Tifgrand does not fall below 50% green cover until exposed to >50% shade. (Fig. 1)
- At the rough mowing height, JaMur, Zeon, and Zorro zoysiagrass show greater than 50% green cover at shade levels up to ~60%. Tifgrand exhibits superior shade tolerance to other bermudagrass cultivars, maintaining >50% cover up to 40% shade. Latitude 36 and Celebration exhibit similar shade responses, falling below 50% cover at >20% shading. Tifway exhibits the poorest shade tolerance at fairway height, requiring almost 85% full sun in order to maintain 50% green cover (Fig. 2)

- Preliminary data are showing considerable benefit of trinexapac-ethyl on fairway height turf for most cultivars at 30, 50, and 70% shade levels, but little to no benefit in full sun, 80%, or 90% shade. (Tables 2 & 3)
- Turf quality, digital image analysis of percent green cover, NDVI, and rooting data are being measured monthly and will be regressed against shade level to identify critical DLI thresholds for each entry at the end of the project.

Table 1. Species, cultivars, and origin of entries included in the Texas A&M shade study. St. Augustinegrass has been included in the rough height study as a shade tolerant check.

| **Species** | **Cultivar** | **Origin** |
|---|---|---|
| Bermudagrass | Tifway | University of Georgia |
| | TifGrand | University of Georgia |
| | Latitude 36 | Oklahoma State University |
| | Celebration | Sod Solutions |
| Zoysiagrass | Zeon | BladeRunner Farms, Inc. |
| | Zorro | Texas AgriLIFE Research |
| | Palisades | Texas AgriLIFE Research |
| | JaMur | BladeRunner Farms, Inc. |
| | Geo | Sod Solutions |
| St. Augustinegrass | Palmetto | Sod Solutions |

Table 2. Percent green cover of bermudagrass cultivars in the fairway study as affected by trinexapac ethyl (TE) application at 30, 50, and 70% shade levels during July 2016. Percent increase or decrease in green cover due to TE application is also provided.

| | **TE** | **30% shade** | **50% shade** | **70% shade** |
|---|---|---|---|---|
| Celebration | - | 23 | 23 | 22 |
| | + | 43 | 38 | 22 |
| | | +87% | +65% | 0% |
| Tifway | - | 26 | 27 | 26 |
| | + | 30 | 26 | 26 |
| | | +15% | +0% | +0% |
| Tifgrand | - | 48 | 38 | 28 |
| | + | 57 | 50 | 42 |
| | | +19% | +32% | +50% |
| Latitude 36 | - | 24 | 27 | 11 |
| | + | 30 | 38 | 36 |
| | | +25% | +41% | +227% |

Table 3. Percent green cover of zoysiagrass cultivars in the fairway study as affected by trinexapac ethyl (TE) application at 30, 50, and 70% shade levels during July 2016. Percent increase or decrease in green cover due to TE application is also provided.

| | TE | 30% shade | 50% shade | 70% shade |
|---|---|---|---|---|
| Palisades | - | 34 | 42 | 42 |
| | + | 63 | 62 | 66 |
| | | +85% | +48% | 57% |
| JaMur | - | 45 | 43 | 46 |
| | + | 51 | 57 | 61 |
| | | +13% | +33% | 33% |
| Zeon | - | 26 | 24 | 39 |
| | + | 34 | 41 | 31 |
| | | +31% | +71% | -21% |
| Zorro | - | 42 | 28 | 31 |
| | + | 47 | 43 | 53 |
| | | +12% | +54% | 71% |
| Geo | - | 30 | 41 | 28 |
| | + | 45 | 54 | 41 |
| | | +50% | +32% | 46% |

Figure 1. Percent green cover for the turfgrass cultivars in the fairway turf shade study (mowing height 0.75”) during June 2016, nine months after imposing shade treatments.

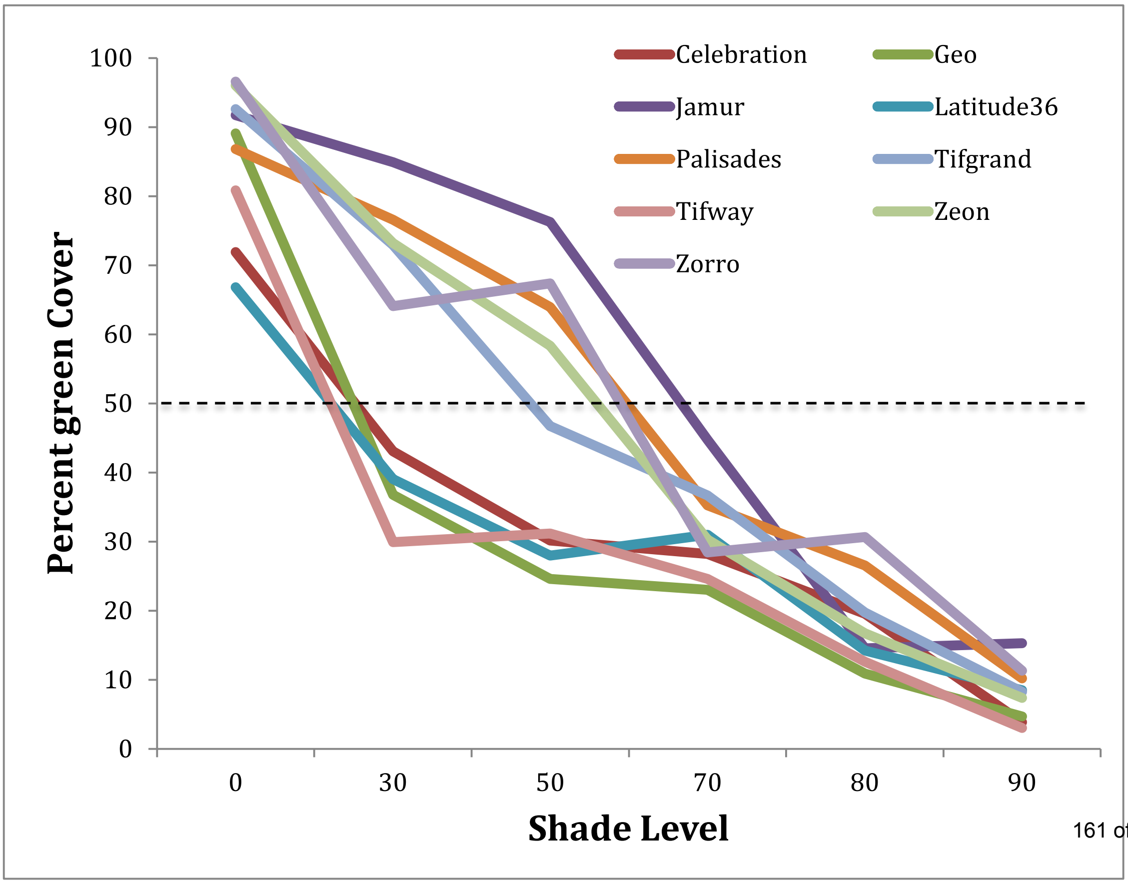

Figure 2. Percent green cover for the turfgrass cultivars in the rough shade study (mowing height 2”) as of June 2016, nine months after imposing shade treatments.

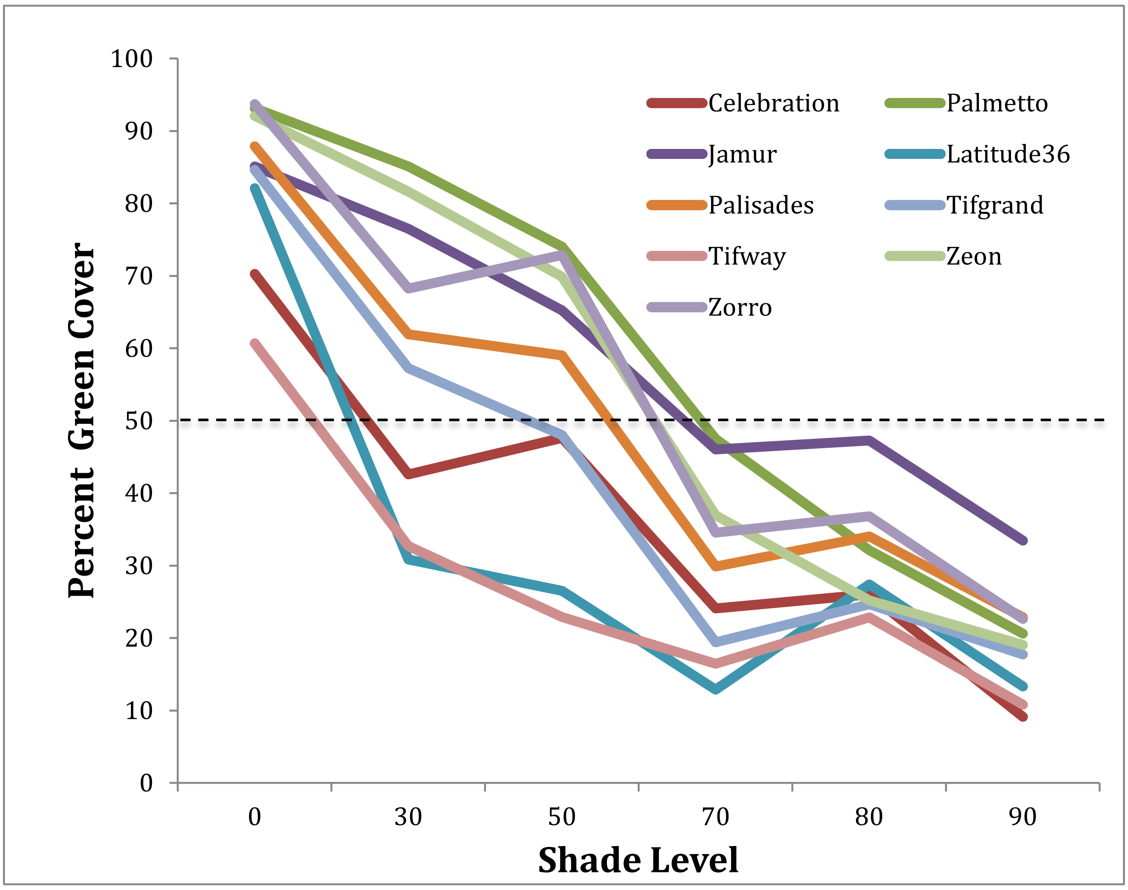


Figure 3. Image of Texas A&M Research Field Laboratory shade study area with shade structures in place.

# *Determining Base Temperature for Warm- and Cool-Season Turfgrasses*

**Ethan Flournoy, Christian M. Baldwin, Barry R. Stewart, H. Wayne Philley, K. Raja Reddy, and James D. McCurdy**

Mississippi State University

**William C. Kreuser**

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Growing degree days are used to describe the amount of heat energy received by a plant over a given time period. Growing degree days are used extensively in agriculture to predict plant growth stages, such as, time from emergence to flowering or emergence to maturity. Using a growing degree day model a turfgrass manager can better time pesticide and growth regulator applications compared to a calendar day approach.

Growing degree days are calculated by averaging the daily high and low air temperature and subtracting a base temperature. Base temperature is defined as the temperature at which a plant species becomes physiologically inactive causing shoot growth to cease. Calculating growing degree days with an inaccurate base temperature can equate to a difference of up to two or three calendar weeks. The objective of this research was to determine the base temperature of warm- and cool-season turfgrass species/cultivars.

A study was conducted using the Soil-Plant-Atmosphere-Research (SPAR) units at the R.R. Foil Plant Science Research Center at Mississippi State University to determine the base temperatures for five cool-season and five warm-season turfgrasses. The SPAR unit facility is comprised of ten naturally-lit chambers that have the ability to simulate and monitor several environmental aspects, such as temperature, humidity, UV-B light exposure, soil moisture, and $CO_2$ levels (Figure 1). The SPAR units are controlled by a dedicated computer system located inside the Environmental Plant Physiology Laboratory. Cool-season turfgrasses included 'Penn A1/A4' and 'Penncross' creeping bentgrass (*Agrostis stolonifera* L.), 'Midnight' Kentucky bluegrass (*Poa pratensis* L.), 'Fiesta 4' perennial ryegrass (*Lolium perenne* L.), and 'Falcon V' tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.). Warm-season turfgrasses included 'MSB-285', 'Latitude 36', 'TifEagle', and 'Tifway' hybrid bermudagrasses (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt-Davy) and 'Meyer' zoysiagrass (*Zoysia japonica* Steud.). MSB-285 is an experimental cultivar from the Mississippi State University breeding program.

This cultivar was a top performer in the 2013 National Turfgrass Evaluation Program/USGA warm-season putting green test (www.ntep.org) and has unique alleles compared to other ultradwarf bermudagrass cultivars when amplified with the simple-sequence repeat marker ES295668 (Harris-Schultz, personal communication, 2013).

Day/night temperature regimes for the cool-season grasses were 18/10, 22/14, 26/18, 30/22, 34/26 °C. Day/night temperature regimes for the warm-season grasses were 20/12, 25/17, 30/22, 35/27, 40/32 °C. Each unit included six replications for each of the five turfgrasses used for a total of 30 randomized pots for each temperature regime. Turfgrasses were grown in a 3:1 sand to native top soil mix in lysimeters measuring 41 cm in depth and 10 cm in diameter. Nutrients and moisture were maintained at optimum conditions using drip irrigation containing half-strength Hoagland's nutrient solution three times per day. Clipping yield was collected every three days using scissors and a PVC guard cut to the desired height (Figure 2). Additional parameters measured included chlorophyll index, root dry weight, and tissue nutrient content. Clipping yield was subjected to quadratic regression analysis as a function of temperature in order to determine base temperature.

Base temperatures for Penn A1/A4, Penncross, Midnight, Fiesta 4, and Falcon V are –2.23, -0.44, 4.69, 3.81, and 4.25 °C, respectively. Base temperatures for Latitude 36, MSB-285, Tifway, and TifEagle are 13.21, 12.51, 12.67, and 12.58 °C, respectively. Base temperature for Meyer zoysiagrass was unable to be determined due to lack of performance during the trial. Using these base temperatures to calculate growing degree days can more accurately represent total accumulated growing degree days. Accurately calculating growing degree days can be the difference between a timely and mistimed application.

## Summary Points

- Base temperature for the bermudagrasses cultivars ranged from 12.51 to 13.21°C
- Base temperature for the cool-season species ranged from -2.23 to 4.69 °C
- Using these base temperatures to calculate growing degree days can more accurately represent total accumulated growing degree days.
- Future research is needed to validate base temperatures in a field setting.

**Figure 1:** The Soil-Plant-Atmosphere-Research (SPAR) facility is located at Mississippi State University in Starkville, Mississippi. The facility is composed of ten naturally-lit chambers on a 20 × 30 m concrete pad. Each unit is composed of a Plexiglas chamber measuring 2.5 m high, a steel soil bin measuring 1.0 m deep × 2.0 m long × 0.5 m wide, and an air handling unit.

**Figure 2:** Grasses for the base temperature study were grown in lysimeters measuring 10 cm in diameter and 41 cm deep. Clippings were collected every 3 days using scissors and a PVC guard cut to desired mowing height. Clippings were oven dried for at least 48 hours and then weighed.

# Evaluating Sand-Capping Depth and Subsoil Influence on Fairway Performance, Irrigation Requirements and Drought Resistance

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Sand-capping golf course fairways is a trend driven by the need for improved turfgrass growing and playing conditions, especially on areas where low-quality irrigation water and fine-textured soils exist. Due to the significant cost sand-capping can add to a construction/renovation budget, less than optimal depths of sand are often placed atop the existing soil. The ideal placement depth ultimately depends on physical properties of the sand, environmental conditions, and providing a balance of water to air-filled porosity for optimal growing conditions. However, no specifications currently exist for sand-based construction atop an existing soil. This study seeks to develop science-based information that can contribute to development of such recommendations.

During 2016, data on the temporal dynamics of water movement within and through sand-cap treatments following summer irrigation/rain events have been closely monitored. Time domain reflectometry (TDR) probes were placed horizontally at various depths in both the 20 cm depth sand-cap plots (5 cm and 15 cm) and in the 10 cm sand-cap plots (5 cm). Figures 1 and 2 depict temporal volumetric water content changes between irrigation cycles during June 2016, both atop clay loam and sandy loam subsoils. When comparing soil moisture at the 5 cm depth between the 10 and 20 cm sand-cap treatments, moisture content decreased to a much greater extent between irrigation events within the 20 cm sand-cap. At the 15 cm depth, soil moisture fluctuated less between irrigation events, regardless of sand-cap depth. Sand-cap moisture content at the 15 cm depth remained higher atop clay loam as compared to sandy loam subsoils between irrigation cycles.

Point-in-time measurements were also obtained prior to irrigation events on multiple dates during the 2016 summer within 1x per week irrigation treatments to characterize available soil moisture at different depths within the sand-cap treatments (Fig 3). Moisture content was obtained using a handheld soil moisture meter with different probe lengths six days after plots were irrigated. Data spatially depict water retention across the sand profile for various sand-cap treatments. Although significantly less water is available within the 0-4 cm depth for the 20 cm deep sand-cap, a substantial amount of water is still available to plants deeper within the sand profile. This likely explains the few differences that have been observed in turfgrass coverage between caps of varying depths throughout the study.

Sodium adsorption ratio (SAR) of fairway subsoils (upper 10 cm) increased sharply within the initial 12 months of the study due to the high Na concentration (~270 ppm) of irrigation water, but declined over the winter months due to an abundance of natural rainfall (Figl 4). SAR has been delayed/mitigated to some extent by sand-capping.

Measurements of thatch development were made during December 2015 (15 months into the study). Data showed that the 20 cm sand-cap had significantly less thatch development compared to the 0, 5, and 10 cm sand-caps.

During 2017, a 60-day drought will be imposed across the study in order to determine treatment effects on drought resistance, with recovery evaluated after the summer drought has ended.

**Summary Points**

- The different subsoils, clay loam and sandy loam, are having an effect on moisture content in the overlying sand-cap, which may have implications on the ideal sand-cap placement depth.

- Although few differences were observed in year 1 due to either irrigation frequency or capping depth, some turfgrass drought stress was observed during 2016 on the 20 cm sand-cap as compared to the shallower capping treatments under 1x/week irrigation. This was most pronounced atop the sandy loam as compared to the clay loam subsoil, likely due to the different physical and hydraulic properties of the underlying subsoil.

- The Sodium Adsorption Ratio (SAR) is likely to gradually increase from growing season to growing season, which may have a negative effect soil structure and possibly root development within the subsoil as these systems age.

- Differences in the rate of thatch accumulation suggest that management of organic matter should not be overlooked when managing a sand-cap system, however, a delayed rate of thatch accumulation has been observed with the deepest (20 cm) sand-cap.

- A 60-day drought will be imposed during summer of 2017 in order to better understand sand-cap x subsoil treatment effects on drought resistance, recovery, and/or survival.

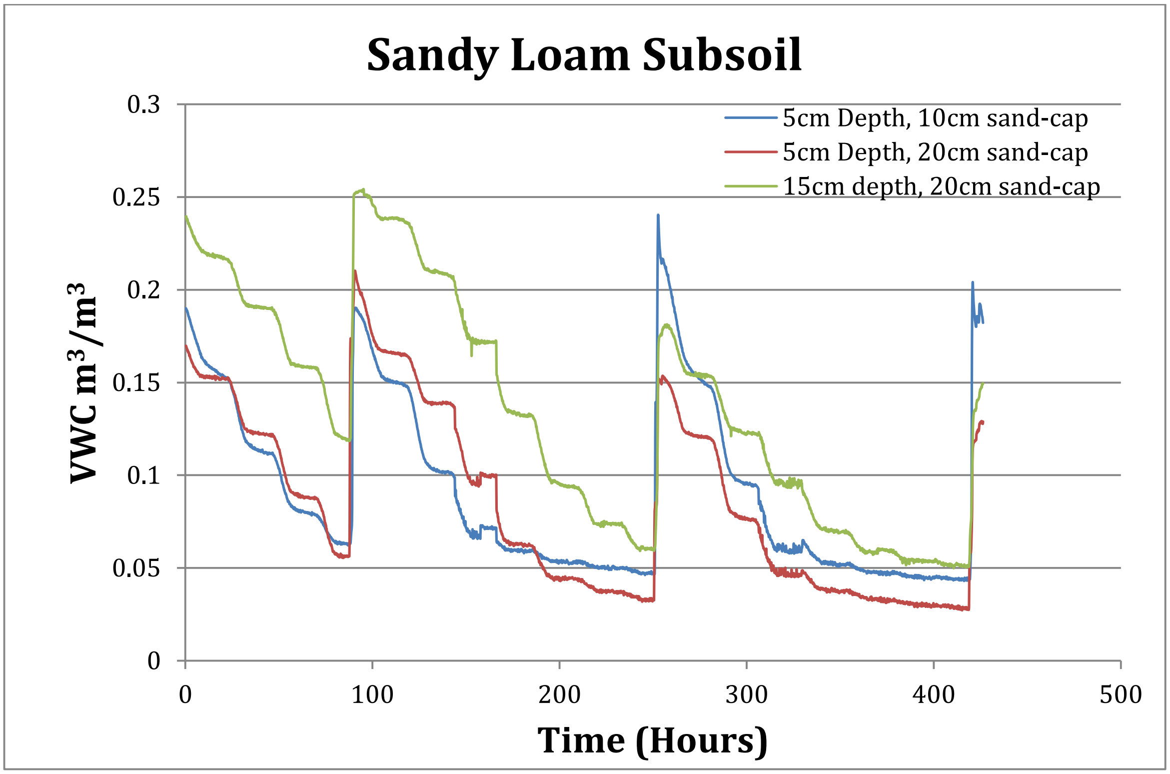


**Figure 1.** Temporal soil moisture dynamics between irrigation events at both 5 and 15 cm depths within 10 cm depth sand-cap (5 cm moisture only) and 20 cm depth (5 and 15 cm depth moisture) sand-cap treatments atop the sandy loam subsoil. Data are for 1x/week irrigation during the month of June 2016.

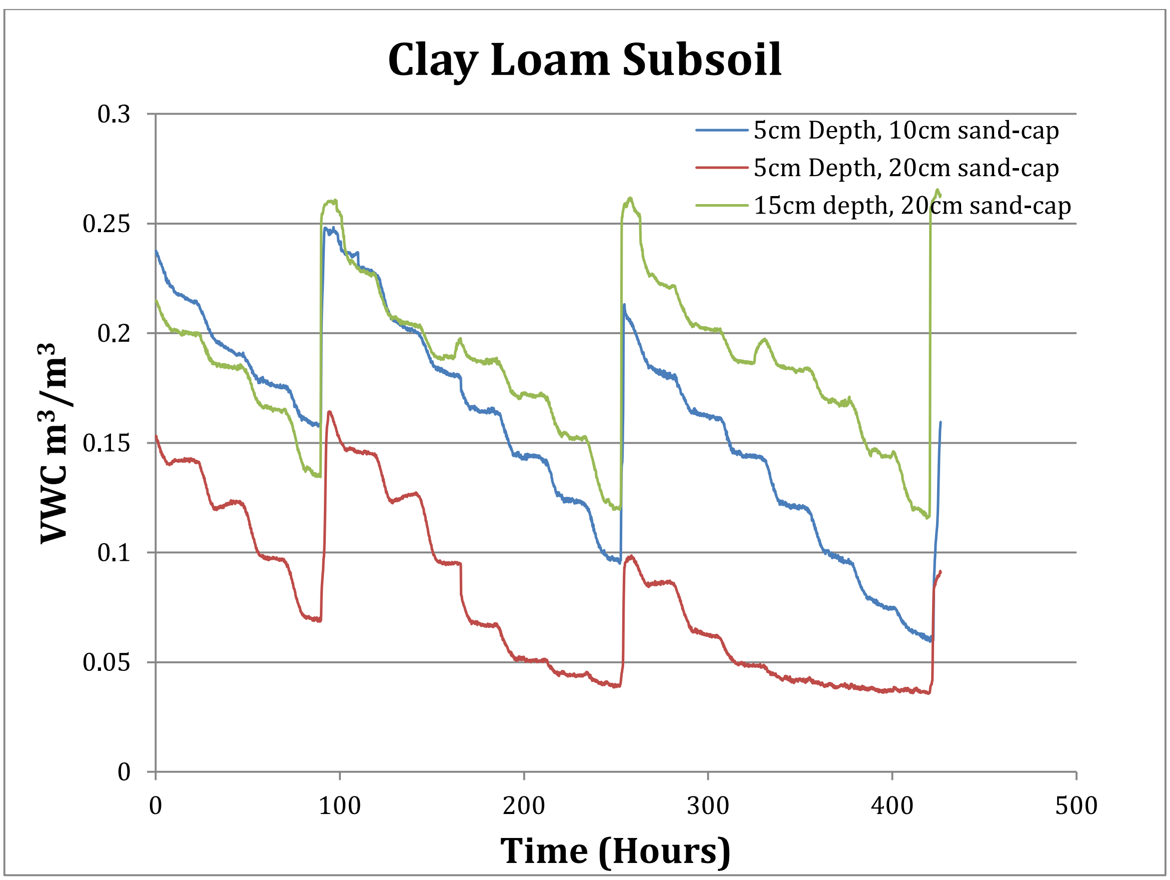


**Figure 2.** Temporal soil moisture dynamics between irrigation events at both 5 and 15 cm depths within 10 cm depth sand-cap (5 cm moisture only) and 20 cm depth (5 and 15 cm depth moisture) sand-cap treatments atop the clay loam subsoil. Data are for 1x/week irrigation during the month of June 2016.

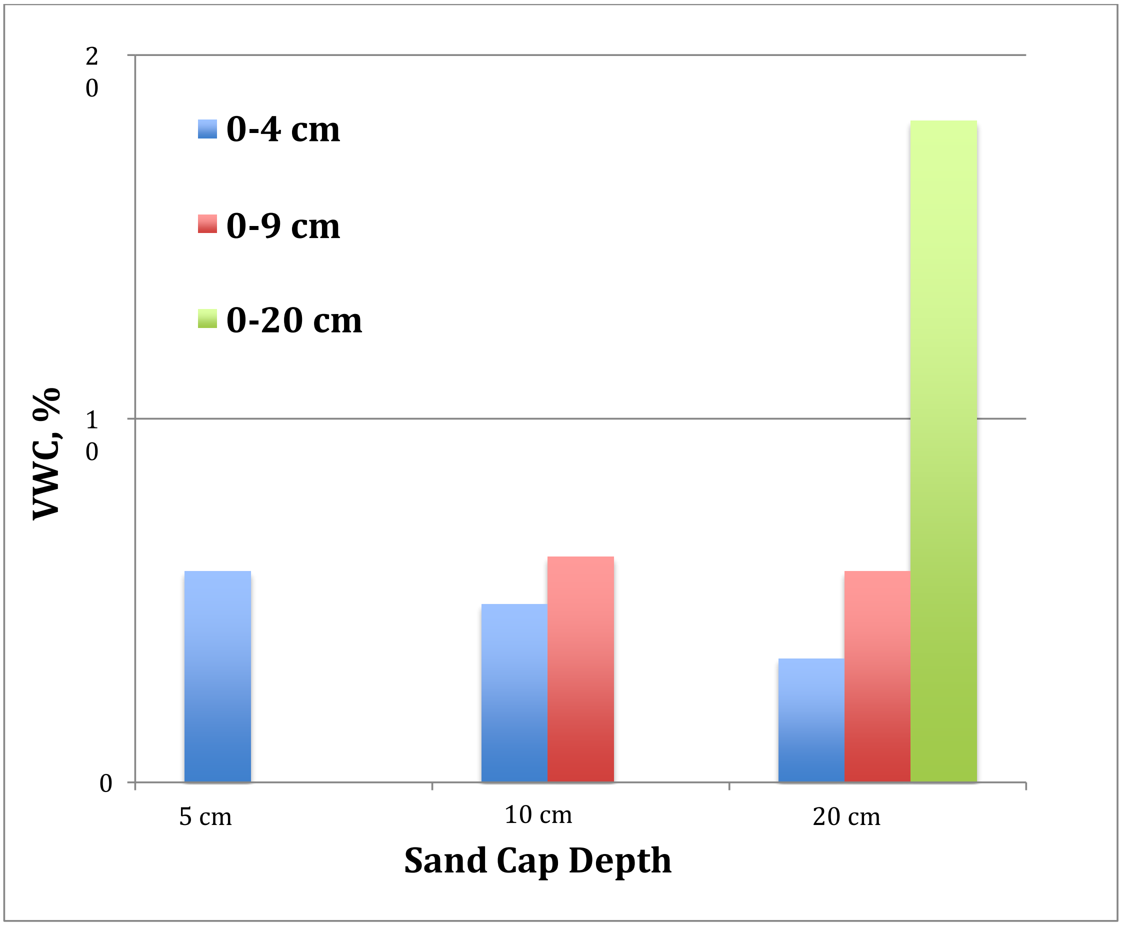


**Figure 3.** Volumetric water content across various depths within each of the three sand-cap treatments atop sandy loam subsoil. Data were taken six days after previous irrigation in 1x/ weekly irrigation treatments. Data are an average of two measurement dates during June 2016, and were obtained using a Field Scout Soil Moisture Probe with 4, 9, and 20 cm depth tines.

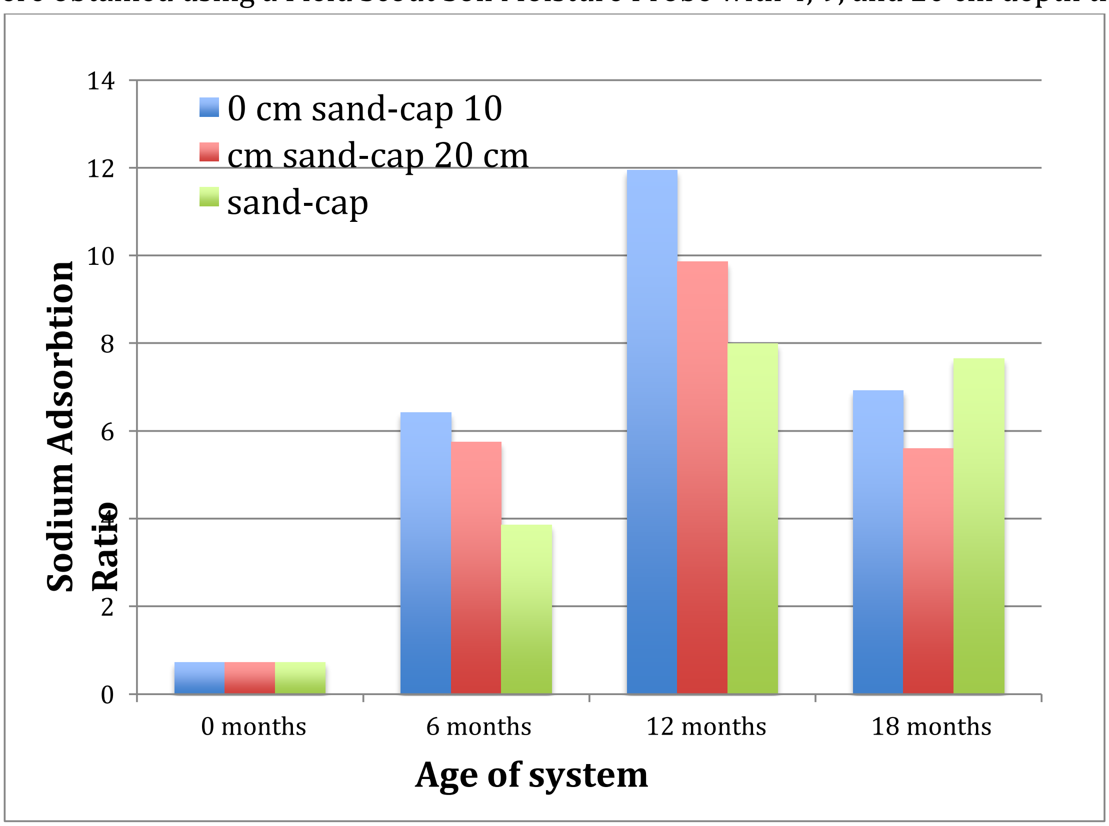


**Figure 4.** Sodium adsorption ratio of sandy loam subsoil underlying sand-caps at 0, 6, 12, and 18 months (study initiation, spring 2015, fall 2015, and spring 2016, respectively).

2016 Progress Report: USGA ID#: 2016-06-556

**Effects of Finer-Textured Topdressing Sand on Creeping Bentgrass Putting Green Turf**

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- Core cultivation treatments were initiated in May 2016 on a 20-month-old 'Shark' creeping bentgrass (*Agrostis stolonifera*) turf maintained as a putting green turf; topdressing treatments were initiated in June.
- Turf responses to topdressing and cultivation were evident during the first year of this trial.
- Turf quality was better when topdressing was applied, regardless of sand size and rate, compared to non-topdressed controls.
- Turf quality was acceptable on all plots but slightly poorer on cored plots compared to non-cored plots throughout most of 2016.
- Volumetric water content at the 0- to 38-mm surface depth zone was reduced by both topdressing and cultivation, with cultivation having a more pronounced effect.

This project is evaluating the effect of topdressing sand size on the playability and physical properties of a sand-based putting green turf. Specific objectives include determining the effects of (i) eliminating coarse particles from topdressing sand (subsequently increasing the quantities of medium, fine and very fine particles) and (ii) core cultivation (plus backfilling holes with medium-coarse sand) on turf performance and the physical properties at the surface of a 'Shark' creeping bentgrass (*Agrostis stolonifera*) turf.

This trial used a 3 x 2 x 2 factorially arranged randomized complete block design with four replications and was initiated on a ~20-month-old turf in May 2016. The factors include sand size (medium-coarse, medium-fine, fine-medium), rate of midseason topdressing (50 and 100 lbs. per 1,000 sq. ft. every 2 weeks), and cultivation (cored plus backfill or non-cored). A non-topdressed control at both levels of cultivation were included for orthogonal comparisons resulting in 14 total treatments (Table 1). The medium-coarse sand meet USGA recommendations for putting green construction; whereas the medium-fine and fine-medium sands contain little to no coarse particles and the fraction of fine sand exceeded USGA recommendations (Table 2).

Forty-five core samples (25-mm diam.) were collected in May to characterize the initial thatch-mat depth and organic matter concentration before treatment initiation. Turf quality was visually rated June through October. Additionally, a mild algae outbreak in late September and residual sand on the surface of plots after topdressing were rated. Sand and clippings collected in the mower basket were sampled from each plot three times during 2016 to determine sand weight and particle size distribution. Initial surface firmness and hardness were assessed along with volumetric water content of the surface 0- to 38-mm depth zone.

Analysis and interpretation of this first-year data is ongoing; however, initial assessments indicate that turf performance and surface wetness were affected by topdressing and core cultivation treatments. Topdressing (pooled over all treatments) improved turf quality on 13 out of 15 rating dates compared to non-topdressed plots (Fig. 1). Core cultivation in May 2016 subtly reduced turf quality compared to non-cored plots throughout most of 2016; however, turf quality of core cultivated plots was acceptable (Fig. 2). Similarly, turf density was greater in topdressed plots compared to non-topdressed plots on 14 of 15 rating dates during 2016 (data not shown). Topdressing applied at 100 lbs.

per 1,000 sq. ft. every 2 weeks increased turf density compared to 50 lbs. per 1,000 sq. ft. on 6 of 15 rating dates in 2016. Core cultivation reduced turf density compared to non-cored plots throughout much of 2016.

Topdressing (pooled over all treatments) reduced volumetric water content at the 0- to 38-mm depth zone compared to non-topdressed plots on 13 out of 31 dates during 2016 (Fig 3.). Moreover, volumetric water content in this zone was greater in plots topdressed with fine-medium sand compared to medium-fine and medium-coarse sands (data not shown). Core cultivation in the spring of 2016 decreased volumetric water content throughout the year (31 dates) compared to non-cultivated plots during 2016 (Fig. 4).

Table 1. Summary of the individual treatment combinations of topdressing (sand size and rate) and cultivation as well as two controls (no topdressing during the growing season) being evaluated on 'Shark' creeping bentgrass grown on a sand-based rootzone.

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

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

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

¶, Core cultivation to the 1 ½-in depth was performed twice a year (10 May and 2 November) using ½-inch diameter hollow tines spaced to remove 10% of the plot surface area annually. Coring holes were backfilled with 600 lbs. per 1,000 sq. ft. of medium-coarse sand. Non-cored plots were topdressed with the respective sand size at 400 and 300 lbs. per 1,000 sq. ft. in May and October, respectively, to fill the surface thatch and verdure layers to the same extent as backfilled, cored plots.

Table 2. Particle size distribution of sands used to topdress plots on a 'Shark' creeping bentgrass grown on a sand-based rootzone.

| Sand | 1000 µm Very Coarse | 500 µm Coarse | 250 µm Medium | 150 µm Fine | 53 µm Very Fine |
|---|---|---|---|---|---|
| | ------------------------------ | --------- % (by weight) | retained --------- | ----------------------------- | |
| Medium-coarse | 0 | 33.8 | 57.7 | 8.4 | 0.1 |
| Medium-fine | 0 | 0.1 | 76.7 | 22.7 | 0.5 |
| Fine-medium | 0 | 5.7 | 25.8 | 66.8 | 1.7 |

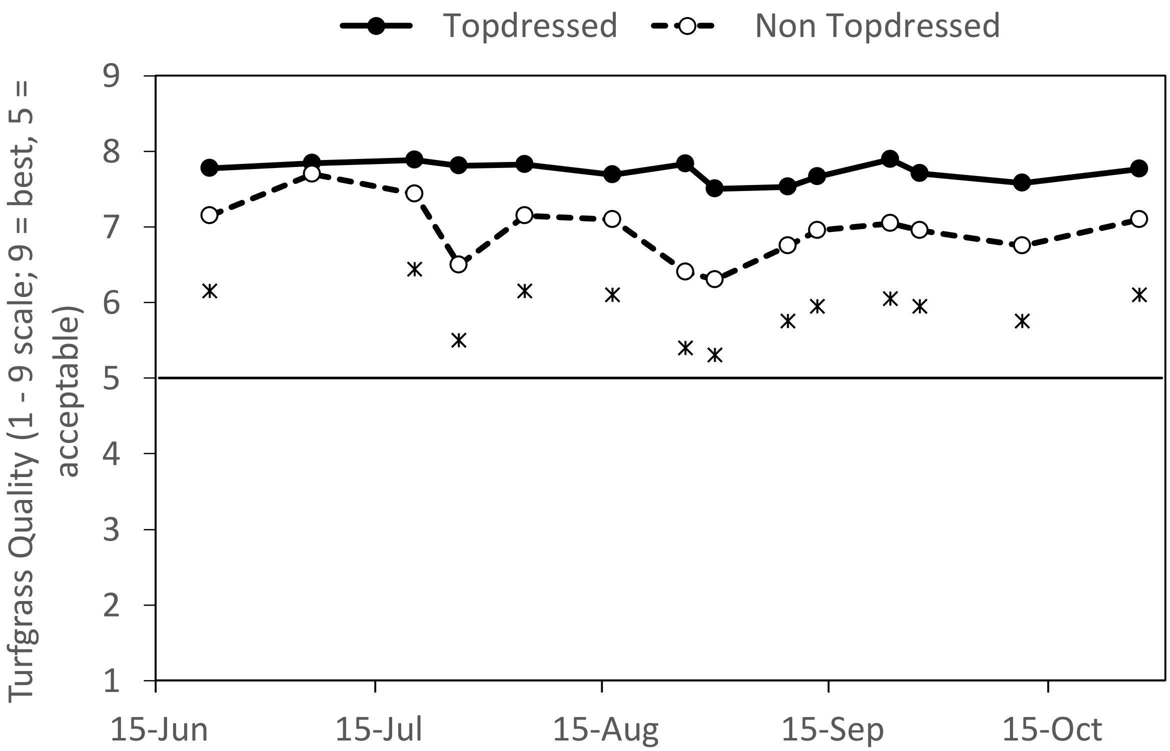


Figure 1. Effect of topdressing (pooled over all treatment levels) compared to non-topdressed plots on turf quality of a 'Shark' creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2016.

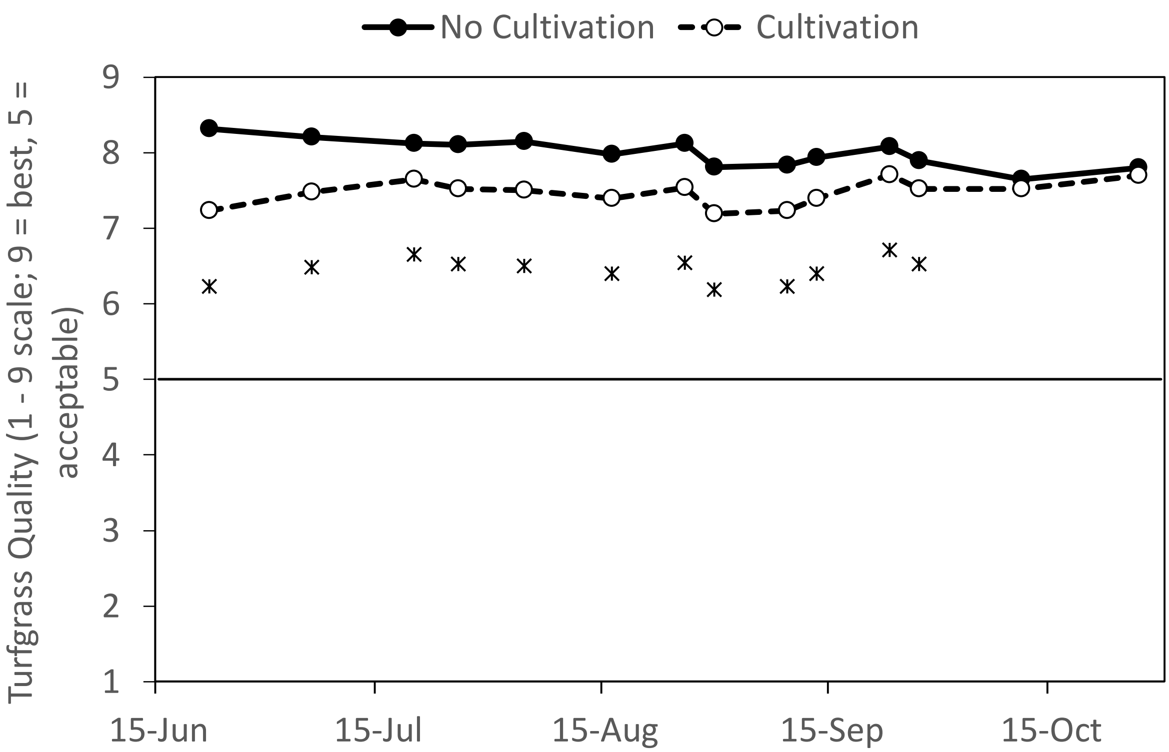


Figure 2. Effect of core cultivation on turfgrass quality of a ‘Shark’ creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2016.

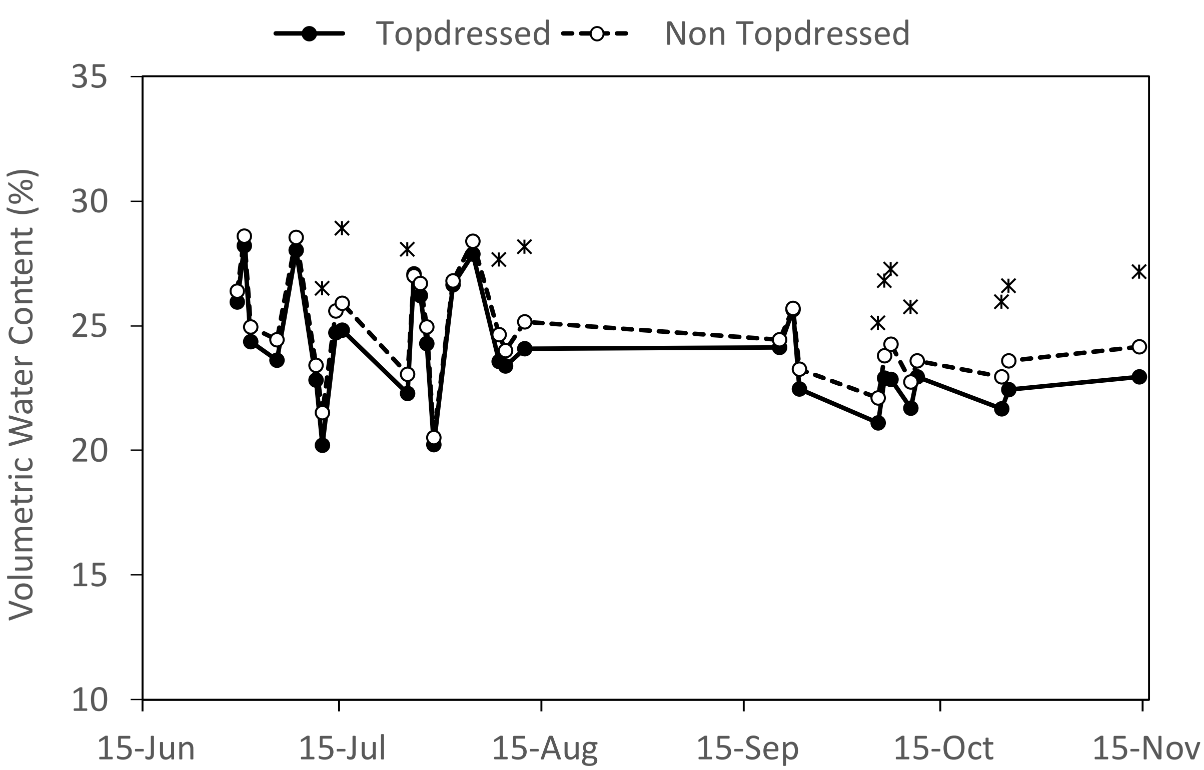


Figure 3. Effect of topdressing (pooled over all treatment levels) compared to non-topdressed plots on volumetric water content of the 0- to 38-mm surface depth zone of a ‘Shark’ creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2016.

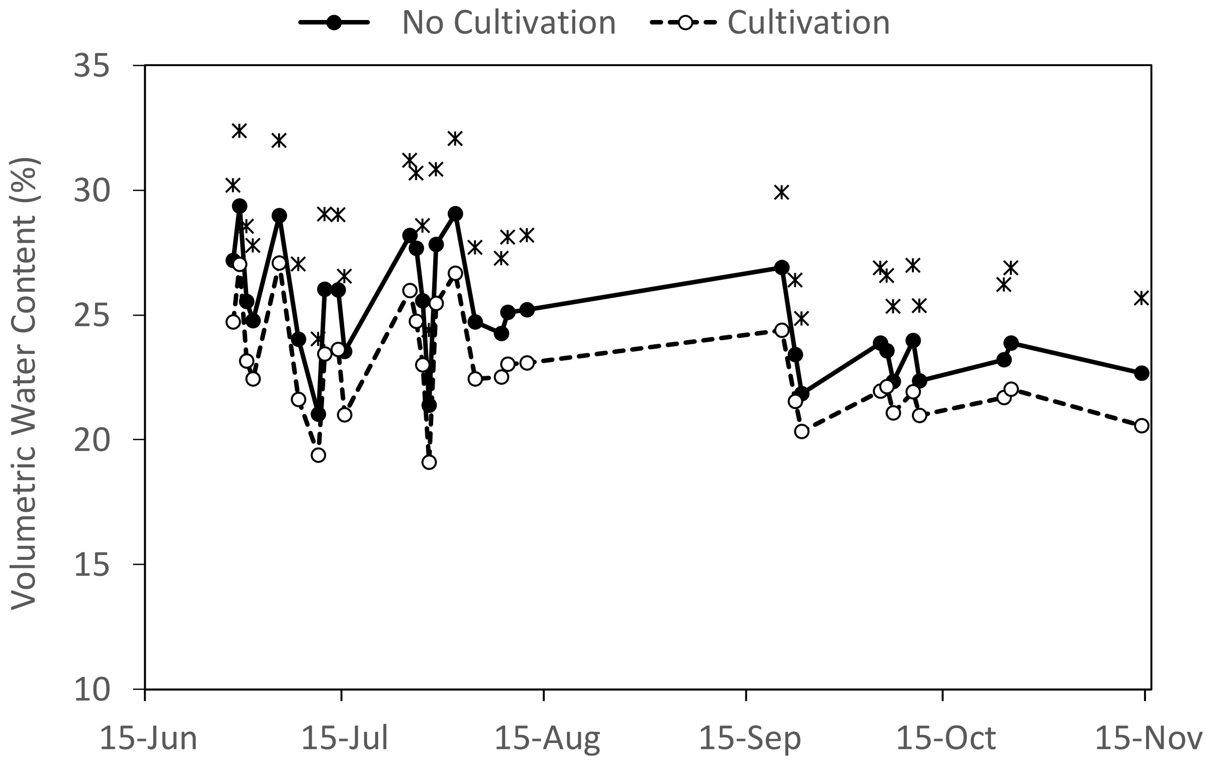


Figure 4. Effect of core cultivation on volumetric water content at the 0- to 38-mm surface depth zone of a ‘Shark’ creeping bentgrass turf maintained at 2.8-mm in North Brunswick, NJ during 2016.

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

K. McInnes, C. Reynolds, B. Wherley, and T. Baker - Texas A&M University

Ultradwarf bermudagrasses on golf greens produce a dense canopy that traps some topdressed sand particles. Larger sand grains appear to be more resistant to falling or being worked through the canopy than are finer grains. Sand trapped in the canopy can produce a less desirable playing surface and can be picked up by and damage mowers. Given such, it is becoming common practice to topdress ultradwarf bermudagrass greens with sand having finer particles (i.e., with less fraction of large particles) than those of the sand that the green was originally constructed. Finer sand grains can produce favorable playing properties of the surface, but they can also produce unfavorable properties with excessive use, such as those problems associated with excess water retention and reduced aeration. To develop recommendations for acceptable properties and application rates of topdressing sand, this project seeks to characterize the interaction of physical properties of finer topdressing sand with sand used to backfill after aeration, and the consequential effects on putting green surfaces. A range of golf courses with ultradwarf bermudagrass greens is currently being identified. For each course, topdressing sand, sand picked up by mowers, and sand used to manage organic matter and to aerate will be examined. Cumulative annual amounts of sands used and geometries of placement will be estimated from measurements and management records. The information below was developed during the first 6 months of the 3-year project.

To develop sampling protocols for sands that we might collect from courses, we requested samples of ultradwarf topdressing sands from some of the major suppliers in the Southeast. These sands are being analyzed for particle size distribution and water retention as would be produced at the surface of a putting green. Two sands received from one supplier, one reported to be widely used on a routine basis and the other on occasions of a major events in Georgia and the Carolinas were analyzed for particle size distribution using a standard sieve stack on a Ro-Tap shaker. Both showed particle size distributions that were appreciably finer than the fine side of the USGA recommendation for sands used for greens construction (Fig. 1). Both sands had no particles larger than 1 mm and less than 4% between 0.5 and 1 mm diameter. Water retentions (capillary porosities) of both sands were greater and air-filled porosities were less than that recommended by the USGA for greens construction (Fig. 2).

Topdressing with finer sand than used in construction of a green will likely reduce infiltration rate and increase surface water content. With the expectation of being able to measure these effects, a 15-cm diameter permeameter was constructed to test in situ infiltration rates and near-surface water retentions in putting greens (Fig. 3). The permeameter setup includes a water depth sensor to measure infiltration rate (Fig. 4) and dielectric-based water content sensors to measure near-surface water retention (0 to 5-cm volumetric water content) with time after irrigation.

**Summary Points**

- Topdressing sands tested have been appreciably finer than that recommended by the USGA for putting greens construction.

- Capillary porosity of topdressing sands tested have been appreciably greater than that recommended for putting greens construction.

- Air-filled porosity of topdressing sands tested have been appreciably less than that recommended for putting greens construction.

- A device to quantify in situ saturated hydraulic conductivities and surface water contents of putting greens has been constructed and tested.

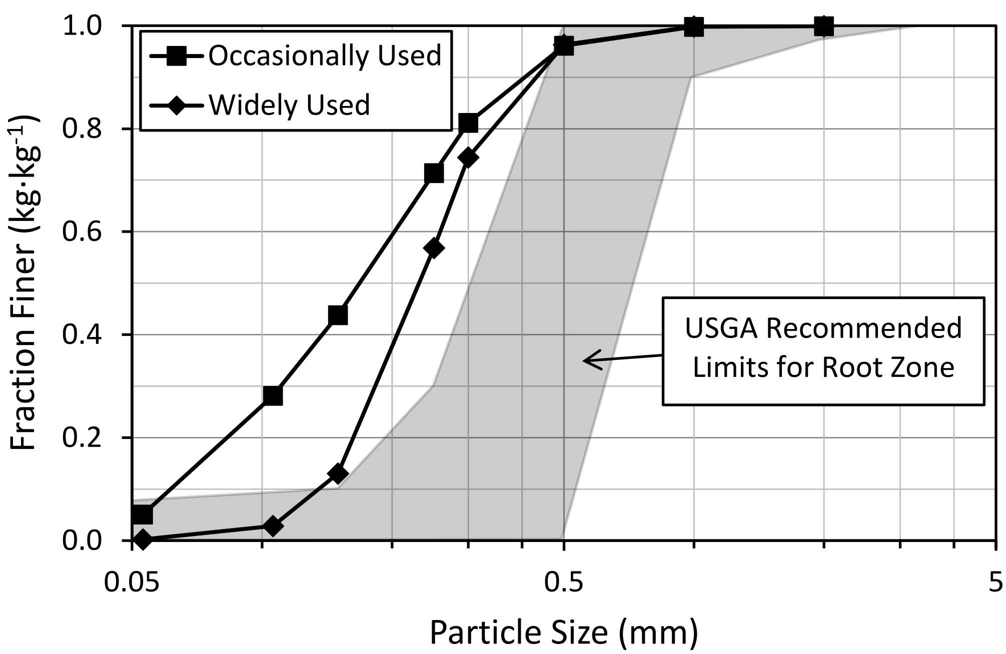


Figure 1. Particle size distribution of sand recommended to be used for putting green construction (shaded area) along with the distributions of sand grains in a widely used and occasionally used topdressing sand on ultradwarf putting greens.

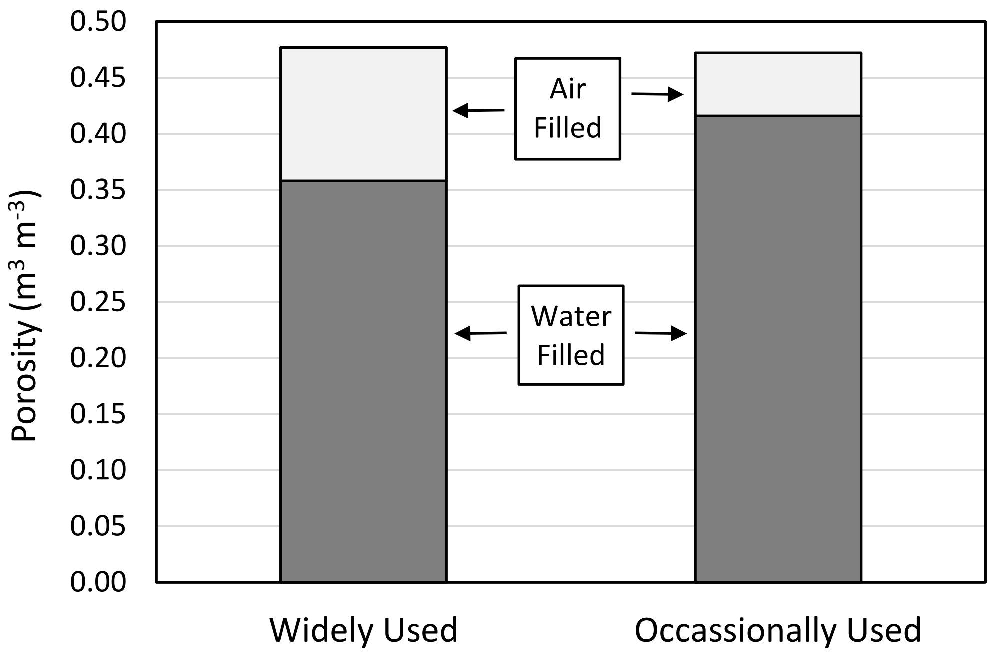


Figure 2. Water retentions in 5-cm deep columns of sand where the surface was at 30 cm water tension. Sands are the same as those whose particle size distributions are shown in Fig. 1.

Figure 3. Permeameter to test in situ infiltration rates and near-surface water retention in putting greens.

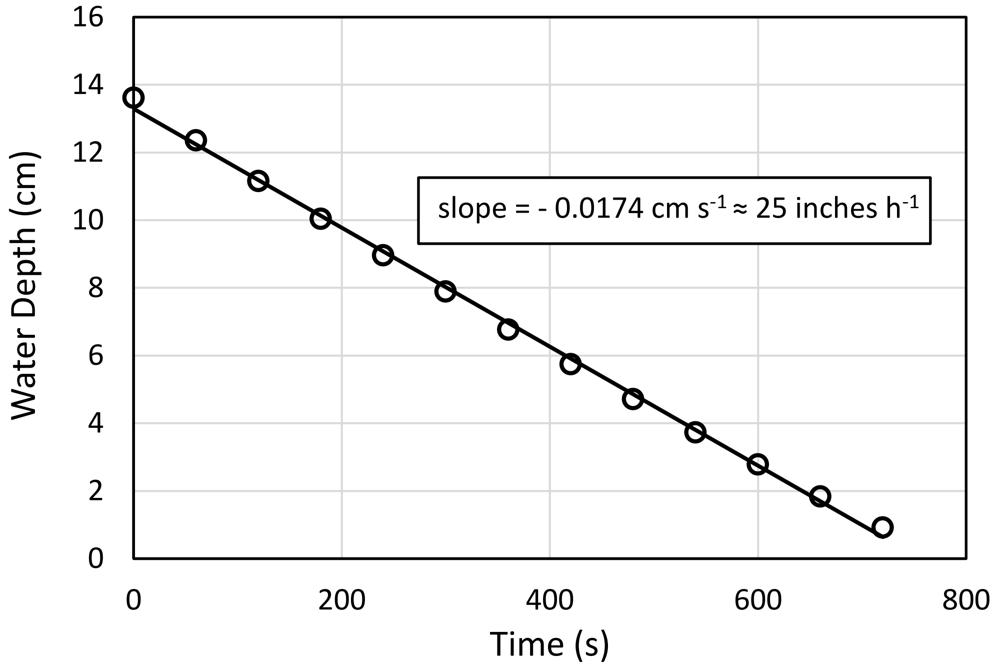


Figure 4. Water depth within the permeameter on a test putting green vs. time. Circles indicate measured data and the line represents a linear regression of depth with time. Slope indicates infiltration rate or saturated hydraulic conductivity of the putting green surface.

**Genesis and prevention of iron-cemented layers in sand putting green soil profiles**
**USGA ID#: 2015-14-529**

**December, 2016**

**Glen R. Obear and William C. Kreuser, Ph.D.**

- A column study was initiated in early 2016 and will be completed in 2017. The objectives of the study are to determine how root zone chemistry and Fe rate affect layer formation.
- Preliminary data show that putting greens with high pH gravel layers may be more prone to formation of cemented layers when high rates of iron are applied. Root zones with high pH sand may be less likely to form layers at the sand/gravel interface due to immobilization of Fe near the surface.
- In 2017-2018, soil samples will be collected from at least 50 golf courses to determine the distribution of Fe-cemented layers, and to create and validate a model to explain their formation.

Iron-cemented layers form in putting greens, leading to decreased water infiltration and anaerobic conditions in the soil. These layers have been documented, but the factors that lead to their formation have not been studied. The objectives of this study are to determine 1) how root zone chemistry affects iron accumulation at different application rates, and 2) the distribution of these layers across the US, which will provide a dataset against which a model can be created and validated.

A column study (Fig. 1) was established as a 2x2x3 factorial design with three replications. The root zone was comprised of a silica sand from Florida (pH 5.5) or a calcareous sand from Wisconsin (pH 8.2); both met USGA particle size recommendations. The gravel layer was comprised of either limestone (pH 8.8) or granite (pH 5.4). After establishment of creeping bentgrass, columns have received weekly applications of ferrous sulfate at a rate of 10 or 50 kg $ha^{-2}$, and these are being compared to untreated columns. All columns are irrigated to replace 200% of water lost through evapotranspiration. Air permeability is measured every 14 to 28 days to track changes in pore space resulting from iron accumulation.

After each iron application, x-ray fluorescence (XRF) is being used to measure the concentration of Fe inside columns in 2.5 cm depth increments. To take these measurements, we constructed an autosampler stand to position the columns for automated XRF analysis (see this video for more details: https://www.youtube.com/watch?v=iJzYzuITz44). The stand allows for scanning while columns are rotating, producing an extremely accurate way to measure average soil Fe at different depth increments inside columns (Fig. 2). This stand, which took over 10 months to construct, is the first of its kind and offers a new way to study soil formation. This provides an exciting opportunity for engineered turf soils and the USGA to revolutionize the way we study all soils of the world.

Data from a preliminary trial show that after eight applications at a rate of 200 kg $FeSO_4$ $ha^{-2}$, a marked accumulation was observable at the interface of sand and gravel in a column with low-pH sand and high-pH gravel (Fig. 3). Iron oxidized above the gravel layer in columns with low pH sand and high pH gravel. In columns with high pH sand, the Fe became immobilized near the surface and never reached the gravel layer. These findings suggest that iron-cemented layers are more likely to form in root zones with high pH gravel. However, these layers may be less likely to form in putting greens with high pH sand, since the iron is immobilized before it reaches the gravel. The full-scale study will provide a wealth of information about how root zone chemistry affects iron accumulation.

Figure 1. Columns were constructed to meet the recommendations of the USGA for putting green construction.

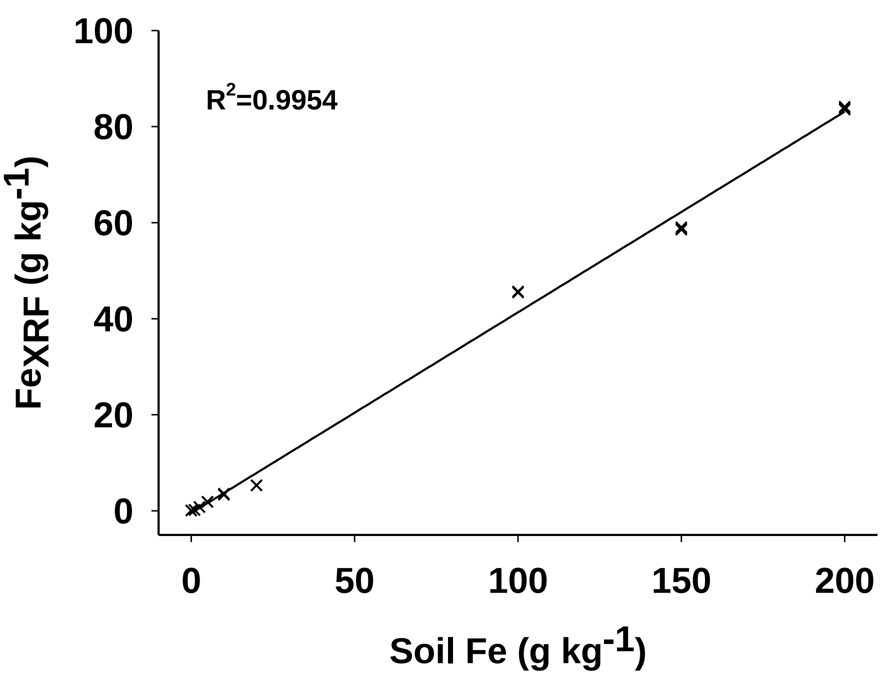


Figure 2. Calibration of an automated XRF stand with soil standards of known Fe concentration. Each sampling cluster on the graph above actually contains data points from 10 separate scans. This robust method will allow us to track Fe accumulation very accurately.

Figure 3. Iron-cemented layer at the sand/gravel interface of a root zone with a low-pH sand and a high-pH gravel. The column received eight total applications of Fe at a rate of 200 kg $FeSO_4$ $ha^{-1}$.

**How does clay move and accumulate in sand root zones?**

**December, 2016**

**Glen R. Obear and William C. Kreuser, Ph.D.**

- A column study was initiated in October 2016 and will be completed in mid-2017. The objectives of the study are to determine how water chemistry and construction practices influence clay movement in two-tiered sand putting greens.
- Preliminary baseline data are currently being collected, and leaching of the columns will begin in early 2017.
- The results from this study will improve our understanding of how soil and water chemistry interact to influence performance of engineered turf soils.

In 2014, we documented thin layers of clay that had formed in 9-year old putting greens in a Mississippi golf course (paper available here: Catena- Clay Lamellae Paper ). This observation led us to study how clay moves and accumulates in two-tiered sand putting greens. We constructed columns to the recommendations of the USGA (2004), and amended them to contain 0, 1, 3, or 5% clay by weight. These ranges were selected to be above and below the recommended cutoff of <3% clay-sized particles for new putting green construction. Columns will be leached with either 0.1 or 1 pore volume of water for a series of repeated leaching events. The entire study will be replicated using two different water sources (CaCl-based or NaCl-based) to study how water chemistry influences clay movement.

After each leaching event, x-ray fluorescence (XRF) is being used to measure the clay content (using Fe as a tracer) inside columns in 2.5 cm depth increments. To take these measurements, we constructed an autosampler stand to position the columns for automated XRF analysis (see this video for more details: https://www.youtube.com/watch?v=iJzYzuITz44). The stand allows for scanning while columns are rotating, producing an extremely accurate way to measure average clay content at different depth increments inside columns (Fig. 1). This stand, which took over 10 months to construct, is the first of its kind and offers a new way to study soil formation. This provides an exciting opportunity for engineered turf soils and the USGA to revolutionize the way we study all soils of the world.

In addition to XRF measurements, air permeability will be measured to document changes in pore space resulting from clay movement. The columns will be photographed regularly to visually document clay accumulation, and at the end of the study, the columns will be split vertically and dissected to measure clay concentrations in 2.5 cm depth increments to further validate XRF clay measurements. A subset of columns with accumulations of clay will be analyzed using a micro-CT x-ray scanner, which produces 3-D models of soils and allows for calculation of pore space in 50 µm depth increments. Leachate will be collected from each column throughout the study period, and a mass balance of clay will be produced to document how clay responded to leaching treatments.

The results of this research could help aid future construction recommendations for putting greens. The findings will also improve our understanding of how soil and water chemistry interact to influence performance of engineered turf soils.

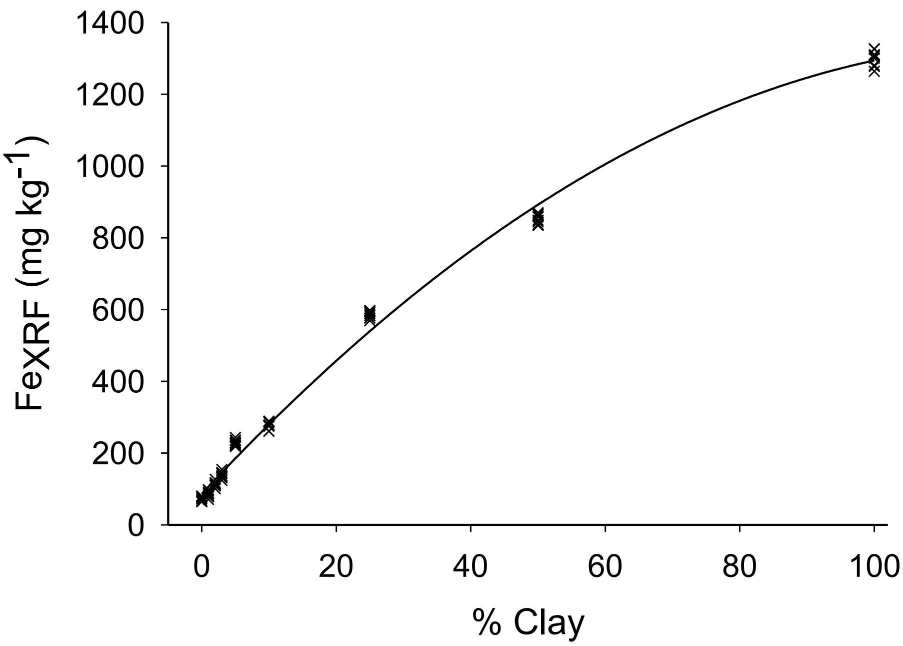


Figure 1. Calibration of an automated XRF stand with soil standards of known clay concentration. Iron is used as a tracer for clay. Each sampling cluster on the graph above actually contains data points from 10 separate scans. This robust method will allow us to track clay movement very accurately.

# Effects of Sulfur, Calcium Source and pH on Microdochium Patch
## USGA ID#: 2014-10-499

Alec Kowalewski, Brian McDonald and Clint Mattox
Oregon State University

Research Summary (Year 3)

- Sulfur applications reduced Microdochium patch on an annual bluegrass putting green.
- Sulfur applications resulted in fewer curative fungicide applications when using the development of infection centers as an action threshold to control Microdochium patch.
- Sulfur applications decreased turf color and increased Anthracnose activity when summer fungicides were not applied.

**Introduction**

Historically, more money is spent on fungicides to combat Microdochium patch (*Microdochium nivale*) in the Pacific Northwest and Western Canada than any other turfgrass disease. As a result of the financial burden and the potential for development of fungicide resistance associated with frequent fungicide applications, as well as growing pesticide bans and restrictions, turf managers as a whole are looking for methods to mitigate pesticide applications. Therefore, the primary objective of this research is to determine if sulfur applied with and without various calcium sources can reduce the number of annual fungicide applications necessary to manage Microdochium patch on annual bluegrass.

**Year Three Findings**

The results in 2016 were very similar to those in 2014. As a reminder, the 2015 results followed the same trends but were muted by the unusually dry winter. In comparison to the control which required 4.1 applications over an 8 month period, plots treated with 3.0 and 6.0 lbs. sulfur per 1,000 $ft^2$ annually required 2.9 and 1.6 fungicides applications, respectively (Table 1). Medium and high rates of sulfur did reduce the number of infection centers in February, but the differences were small.

Sulfur applications of 3 lbs. per 1,000 $ft^2$ annually reduced turf color ratings by 0.5 points in July 2016 as compared to the control, but surprisingly, the 6 lb. annual rate of sulfur was statistically the same as 0 lbs. of Sulfur (Table 2). Percent anthracnose disease was higher in August of 2016 with the medium and high rates of sulfur averaging 4.6 and 5.0 percent disease, respectively, compared to the control which averaged 0.4 percent disease. There were no differences in anthracnose disease from the calcium products. No fungicides were applied for anthracnose, but it is possible the fungicide applications made to control Microdochium patch may have affected the anthracnose disease the following summer.

**Table 1:** Effects of sulfur rate and calcium type on Microdochium patch infections centers observed in March 2016, and the number of fungicide applications made to control Microdochium patch from Oct 1, 2015 to May 31, 2016, Corvallis, OR.

| **Sulfur rate**[z] | Microdochium patch infection centers (per 25 $ft^2$) | | Number of Microdochium patch fungicide applications[y] | |
|---|---|---|---|---|
| 0 lbs | 2.2 | a[x] | 4.1 | a |
| 3 lbs | 1.5 | a | 2.9 | b |
| 6 lbs | 0.8 | a | 1.6 | c |
| **Calcium source**[w] | | | | |
| None | 0.7 | a | 2.6 | a |
| Calcium carbonate | 3.2 | a | 3.0 | a |
| Calcium sulfate | 0.3 | a | 2.7 | a |
| Calcium phosphate | 1.7 | a | 3.1 | a |

[z] 0.0, 3.0 and 6.0 lbs. sulfur/1,000 $ft^2$ annually, applied at 0.25 and 0.5 lbs. sulfur/1,000 $ft^2$ per month x 12 months, respectively from Jan 2009 to Dec 2015. From Mar 2005 to Dec 2008, 0.0, 1.5 and 3.0 lbs. sulfur/1,000 $ft^2$ annually, applied at 0.125 and 0.25 lbs. sulfur/1,000 $ft^2$ per month x 12 months, respectively.

[y] Fungicide applications of propiconazole plus PCNB (2.0 fl. oz + 6.0 fl. oz/1,000 $ft^2$) were made on a per plot basis using the following infection threshold, 5 small spots or one spot exceeding 1 inch in diameter, from Oct 1, 2015 to May 31, 2016.

[x] Means followed by the same letter within each factor of S rate and calcium source are not significantly different according to Fishers' Protected LSD ($\alpha$=0.05).

[w] All calcium sources were applied after core cultivation in May and Sep from 2005 to 2015 at a rate of 12.5 lbs product/1,000 $ft^2$, totaling 25.0 lbs. product/1,000 $ft^2$ annually.

**Table 2:** Effects of sulfur rate and calcium source on turf color observed in July 2016 and percent Anthracnose cover (0-100%) observed in August 2016 in Corvallis, OR.

| **Sulfur rate**[z] | Turf color (1-9) | Percent Anthracnose cover (0-100%)[y] |
|---|---|---|
| 0 lbs | 7.6a[x] | 0.4a |
| 3 lbs | 7.1b | 4.6b |
| 6 lbs | 7.3ab | 5.0b |
| **Calcium source**[w] | | |
| None | 7.3a | 3.5a |
| Calcium carbonate | 7.4a | 3.0a |
| Calcium sulfate | 7.3a | 3.7a |
| Calcium phosphate | 7.2a | 3.1a |

[z]0.0, 3.0 and 6.0 lbs. sulfur/1,000 ft2 annually, applied at 0.25 and 0.5 lbs. sulfur/1,000 ft2 per month x 12 months, respectively from Jan 2009 to Dec 2015. From Mar 2005 to Dec 2008, 0.0, 1.5 and 3.0 lbs. sulfur/1,000 ft2 annually, applied at 0.125 and 0.25 lbs. sulfur/1,000 ft2 per month x 12 months, respectively.

[y]No fungicides were applied to these plots after the conclusion of the 1 Oct 2014 to 31 May 2015 Microdochium patch scouting cycle.

[x]Means followed by the same letter within each factor of S rate and calcium source are not significantly different according to Fishers' Protected LSD ($\alpha$=0.05).

[w]All calcium sources were applied after core cultivation in May and Sep from 2005 to 2015 at a rate of 12.5 lbs product/1,000 $ft^2$, totaling 25.0 lbs. product/1,000 $ft^2$ annually.

# Detection and disruption of virulence factors associated with *Ophiosphaerella* spp., the causal agents of spring dead spot of bermudagrass

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Project duration: 2015-2017

- Spring dead spot is the most devastating disease of bermudagrass in the transition zone where it goes dormant in the winter.
- The genomes of three *Ophiosphaerella herpotricha,* five *O. korrae,* and three *O. narmari* isolates were sequenced.
- The transcriptomes from cultures of the same eleven *Ophiosphaerella* isolates were obtained.
- Current efforts are attempting to identify products secreted by the fungi when infecting and colonizing resistant and susceptible hosts.

Bermudagrass is among the predominant turfgrasses used for commercial and residential urban ground cover in the southern United States. Spring dead spot (SDS) is the most devastating disease of bermudagrass in the transition zone where it goes dormant in the winter and is caused by a complex of three species of *Ophiosphaerella*. To develop effective, durable resistance to SDS in bermudagrass cultivars, a thorough understanding of how the pathogen induces necrosis of host tissues is necessary. Based on our recent insights into the spring dead spot host/pathogen interaction and how they differ for resistant and susceptible cultivars (Figure 1), we are now using a bioinformatics approach to identify the fungal gene(s) encoding the necrosis-inducing effectors. The first objective of this study is: to produce a genome sequence of *O. korrae*. In a previous study, a preliminary draft genome sequence of *Ophiosphaerella herpotricha* was generated using one 2$^{nd}$ generation sequencing approach. Since producing that previous genome sequence, higher throughput 3$^{rd}$ generation sequencing approaches have been commercialized. This latter technology is less expensive than previous approaches and can produce extremely long reads i.e. tens of thousands bases versus the hundreds generated by 2$^{nd}$ generation sequencing approaches.

We sent total genomic DNA samples from three *Ophiosphaerella herpotricha,* five *O. korrae,* and three *O. narmari* to Novogene Corp., Hong Kong, for Illumina sequencing (Table 1) Total genomic DNA of one isolate of each species was sent to Research and Testing Laboratory, Lubbock, TX, for Pacific Biosciences Technology (PacBio) sequencing to generate very long DNA sequence reads that can serve as the scaffolds for the genome assemblies. We have received back the PacBio sequences for *O. korrae* and *O. narmari* and are still awaiting the *O. herpotricha*; however, the Novogene sequences were of such high quality the PacBio data only slightly improved the *O. korrae* and *O. narmari* assemblies. Currently, analysis of the genomes and gene function is ongoing. Most genes identified thus far appear to be orthologous (genes in different species that evolved from a common ancestral gene, and retained their original function) and are shared between *Ophiosphaerella* spp. (figure 2).

We also used Novogene Corp to sequence total RNA (RNA transcripts of expressed genes, or transcriptome) from cultures of the eleven *Ophiosphaerella* isolates. This will be compared to the fungal RNA expressed when the fungi are infecting the plants and will be used in the next two objectives of the study. Current efforts are directed at identifying compounds secreted by the fungi when infecting and colonizing resistant (U3) or susceptible hosts (Tifway 419). To do this, secreted products will be extracted and dialyzed against pure water with dialysis cassettes of different molecular weight cutoff sizes. Products in the dialyzed water will be digested and identified by tandem mass spectrometry. The secreted products will be analyzed in the Orbitrap Fusion Tribid mass spectrometer coupled with an electrospray ion source detector and compared to customized databases using Mascot (Matrix Science, Inc. Boston, MA). The databases will be generated from the data obtained in objective one from other plant pathogenic fungi characterized in the literature.

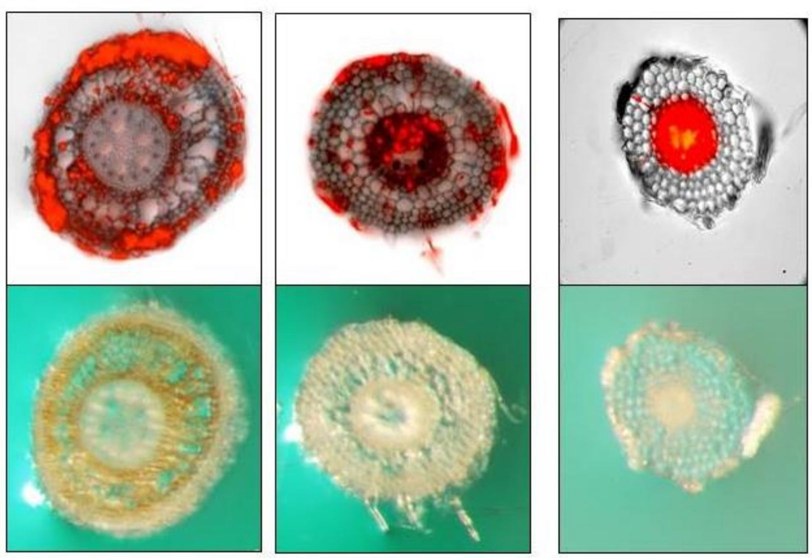

Figure 1. Colonization of a spring dead spot susceptible bermudagrass and cortical necrosis by *Ophiosphaerella korrae* (left), a tolerant bermudagrass (Center) exhibiting vascular colonization by *O. korrae* and no necrosis, and *O. korrae* colonization of a grass which does not produce disease (right). Pictures by F. Flores.

| Genome Metric | *Ophiosphaerella herpotricha* | | | *Ophiosphaerella korrae* | | | | | *Ophiosphaerella narmari* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **KS28** | **TX2.5A** | **16FISCC** | **14BISCC** | **OW11** | **TX1.4** | **KY162** | **HCW2** | **BCGCC2** | **AUS58** | **ATCC202719** |
| Genome Assembly Size | 66,125,271 | 67,214,859 | 63,855,078 | 68,185,353 | 67,389,091 | 72,180,653 | 71,419,005 | 71,157,136 | 47,063,809 | 47,000,963 | 47,756,967 |
| n50 | 61,683 | 54,308 | 50,289 | 37,869 | 40,856 | 31,766 | 51,429 | 47,026 | 213,028 | 221,777 | 1,524,584 |
| Largest Contig | 914,685 | 1,067,401 | 686,546 | 458,646 | 516,330 | 423,522 | 484,841 | 479,782 | 1,060,903 | 1,885,537 | 1,378,950 |
| Number of Contig | 27,846 | 29,099 | 20,526 | 26,403 | 14,402 | 34,418 | 13,696 | 12,155 | 7,233 | 7,094 | 5,309 |
| GC% | 41.4 | 41.1 | 40.1 | 39.2 | 38.1 | 41.5 | 39.2 | 38.6 | 46.5 | 46.4 | 45.8 |
| Numer of Protein Coding Genes | 14,588 | 14,511 | 13,364 | 14,156 | 12,701 | 14,468 | 12,988 | 12,720 | 12,115 | 14,215 | 13,462 |
| Number of Complete Genes (AUG+Stop) | 13,901 | 14,001 | 13,285 | 13,460 | 12,576 | 13,880 | 12,602 | 12,615 | 12,006 | 14,091 | 13,384 |
| Number of Introns | 23,959 | 23,220 | 23,812 | 22,492 | 21,039 | 23,577 | 21,071 | 12,263 | 21,426 | 23,711 | 23,069 |
| Number of Exons | 38,182 | 37,455 | 37,108 | 36,203 | 33,606 | 37,660 | 33,841 | 33,875 | 33,684 | 37,913 | 36,448 |
| Number of Genes without Introns | 3,452 | 3,739 | 3,118 | 3,791 | 3,393 | 3,623 | 3,446 | 3,367 | 3,139 | 3,599 | 3,374 |
| Intergenic Bases | 45,295,214 | 45,658,788 | 42,898,131 | 46,297,742 | 47,072,483 | 50,543,721 | 50,791,964 | 50,737,579 | 26,776,106 | 25,855,809 | 26,602,284 |
| Bases Non Coding | 47,152,037 | 47,648,742 | 44,763,120 | 48,214,060 | 48,622,005 | 52,409,605 | 52,594,436 | 51,716,166 | 28,404,482 | 27,633,830 | 28,450,111 |
| Intragenic Bases | 20,830,057 | 21,556,071 | 20,956,947 | 21,887,611 | 20,316,608 | 21,636,932 | 20,627,041 | 20,419,557 | 20,287,703 | 21,145,154 | 21,154,683 |
| Average Gene Size | 1,471 | 1,485 | 1,568 | 1,546 | 1,600 | 1,495 | 1,543 | 1,377 | 1,657 | 1,488 | 1,571 |
| Exons per gene | 2.6 | 2.6 | 2.8 | 2.6 | 2.6 | 2.6 | 2.6 | 2.7 | 2.8 | 2.7 | 2.7 |
| Introns Per Gene | 1.6 | 1.6 | 1.8 | 1.6 | 1.7 | 1.6 | 1.6 | 1.0 | 1.8 | 1.7 | 1.7 |
| Average Exon Size | 506.0 | 521.1 | 516.8 | 540.3 | 558.9 | 523.9 | 558.6 | 551.5 | 557.0 | 502.1 | 528.4 |
| Average Intron Size | 77.5 | 85.7 | 78.3 | 85.2 | 73.7 | 79.1 | 85.5 | 79.8 | 76.0 | 75.0 | 80.1 |

Table 1. Genomic statistics for three isolates of *Ophiosphaerella herpotricha*, five isolates of *O. korrae* and three isolates of *O. narmari*.

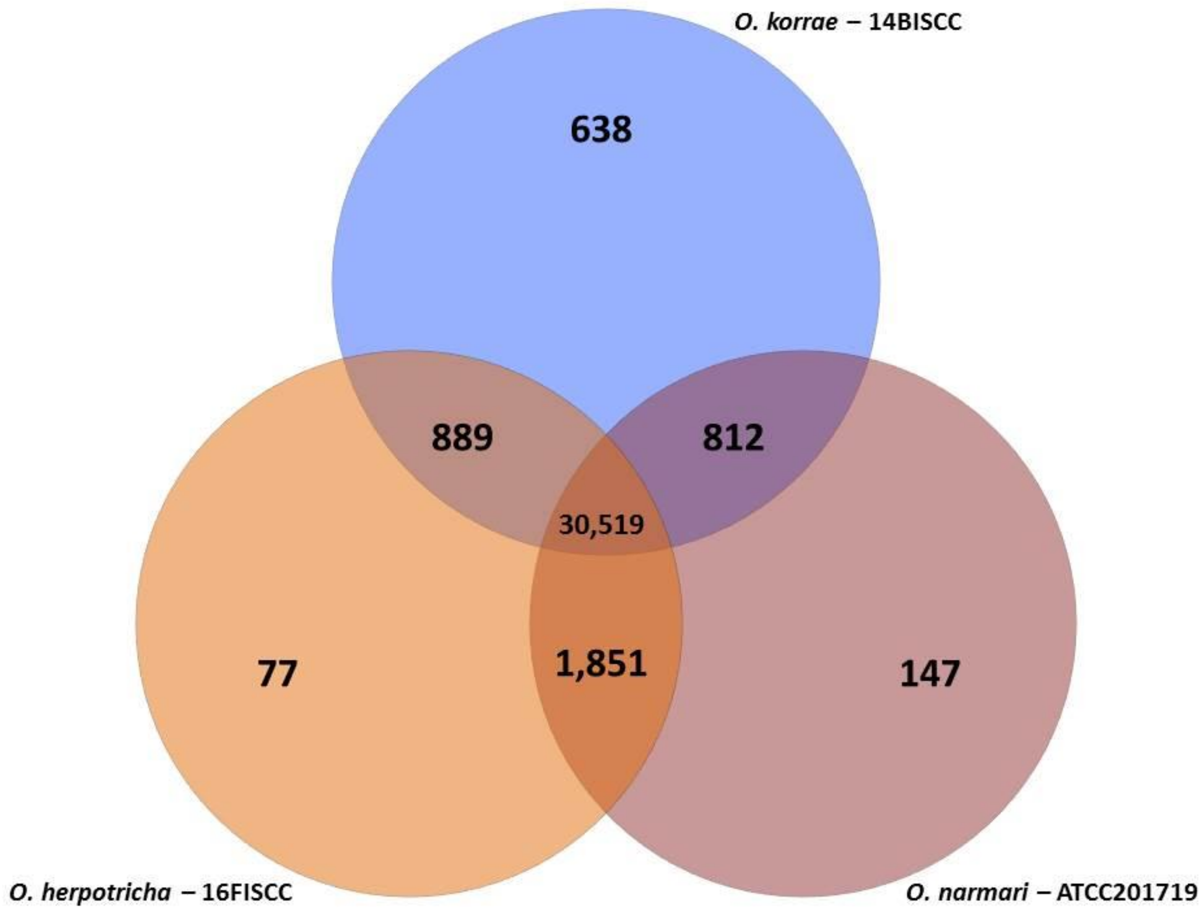


Figure 3. . Venn diagram of the orthologous genes (genes in different species that evolved from a common ancestral gene, and retained function) of *Ophiosphaerella korrae*, *O. herpotricha*, *O. narmari*. The three species share the majority of protein coding genes (~70%) (where three circles overlap). Fewer genes are shared between two species (where two circles overlap). Each species has a lower number of unique genes (where circles do not overlap). Figure areas are not to scale.

**Title:** Influence of Spray Rate Volume and Adjuvant Additives on Fungicidal Control of Large Patch.

**Objective:** Determine optimal spray rate volumes and adjuvant combinations for enhanced control of large patch.

**Authors:** J.J. Benelli and B.J. Horvath

**Body:**

Large patch, *Rhizoctonia solani* AG 2-2 LP, epidemics are common on Japanese lawngrass (JLG; *Zoysia japonica*) landscapes in the transition zone US (Fig. 1). Large patch is primarily managed using fungicide applications on intensively managed turfgrass such as golf course fairways. However, despite the use of fungicides, difficulties in controlling these epidemics have been observed. Experiments were conducted to: (I) identify the optimal fungicide deposition site for large patch control; (II) determine spray application methods that result in optimal deposition; and (III) determine if the optimal spray application methods result in reduced large patch severity under field and greenhouse conditions.

The first experiment evaluated large patch control using four fungicides (Heritage, Torque, Prostar, and Daconil Ultrex) applied on the stem, sheath, or leaf of JLG. Applications were made using a pipette to dispense single droplets of fungicide solution onto the target sites. Results of this experiment suggest that improved large patch control occurs when fungicides are deposited lower in the plant canopy (Fig. 2). All xylem mobile fungicides resulted in a significant reduction of large patch severity when applied on the sheath or stem compared to applications on the leaf.

The second experiment identified spray application methods that result in greater fungicide penetration in JLG canopies. Four spray rate volumes (0.23, 0.92, 1.83, and 3.67 gal/M) were applied with and without an organosilicone surfactant onto JLG maintained in a greenhouse. A fluorescent tracer was included in all treatment combinations and the use of black light illumination aided in identification of spray deposits (Fig. 3). Results suggest that higher spray rate volumes, with or without an organosilicone surfactant, increased the percentage of stems and sheaths that contained spray deposits by as much as 35% compared to the lowest spray rate volume (Fig. 4 . The spray rate volumes of 0.92 and 1.83 gal/M resulted in the most leaf surface coverage.

The third experiment evaluated various spray rate volumes and adjuvants additives on fungicidal control of large patch under field and growth chamber conditions. The four spray rate volumes (0.23, 0.92, 1.83, and 3.67 gal/M) and two adjuvants (organosilicone surfactant and a modified vegetable oil adjuvant) were applied with three fungicides (Heritage, Torque, and Prostar).

Increased spray rate volume resulted in significant decreases in large patch severity under field and growth chamber conditions (Fig. 5). The highest spray rate volume (3.67 gal/M) resulted in a 20% reduction in large patch severity compared to the lowest spray rate volume (0.23 gal/M). Large patch development was less affected by the use of adjuvants compared to spray rate volume.

In conclusion, this research demonstrated that enhanced fungicidal control of large patch occurs when fungicides are deposited lower in the plant canopy. The use of higher spray rate volumes, with or without adjuvant additives, resulted in greater penetration of the spray solution and improved fungicidal control of large patch. Future research is needed on the combination of higher spray rate volumes and adjuvants on turfgrass safety and large patch control under variable environmental conditions.

**Bullet points:**

- Fungicides applied on the sheath or stem provided greater large patch protection compared to fungicide applied on the leaf
- Increases in spray rate volume resulted in greater penetration of the solution in Japanese lawngrass canopies and improved fungicidal control of large patch.
- Future research is needed on the safeness of adjuvant additives on Japanese lawngrass under variable environmental conditions.

**Fig. 1.** Large patch, caused by *Rhizoctonia solani* AG 2-2 LP, is a severe disease of Japanese lawngrass in the transition zone United States.

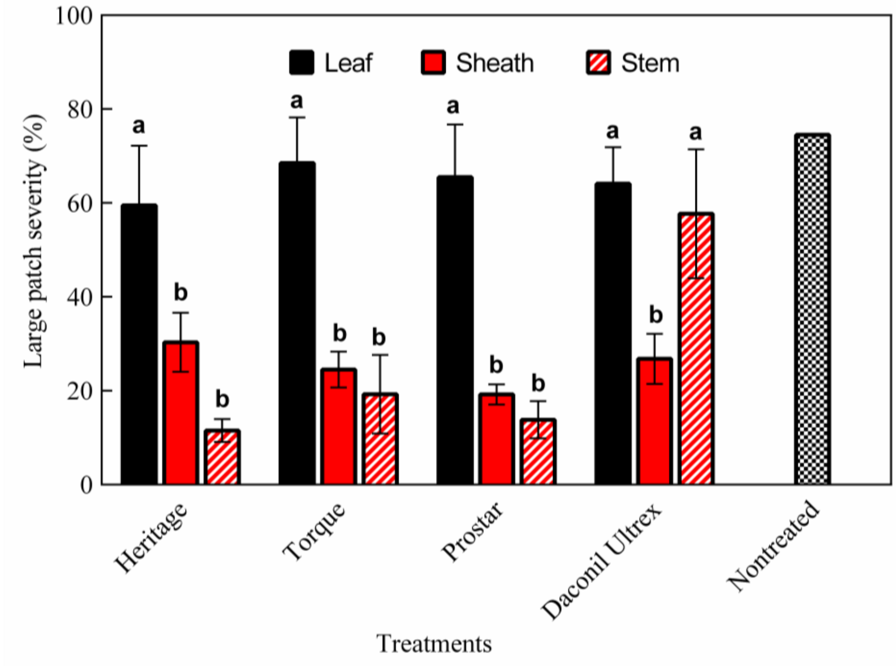


Fig 2. Effect of fungicide application deposition site on large patch severity.

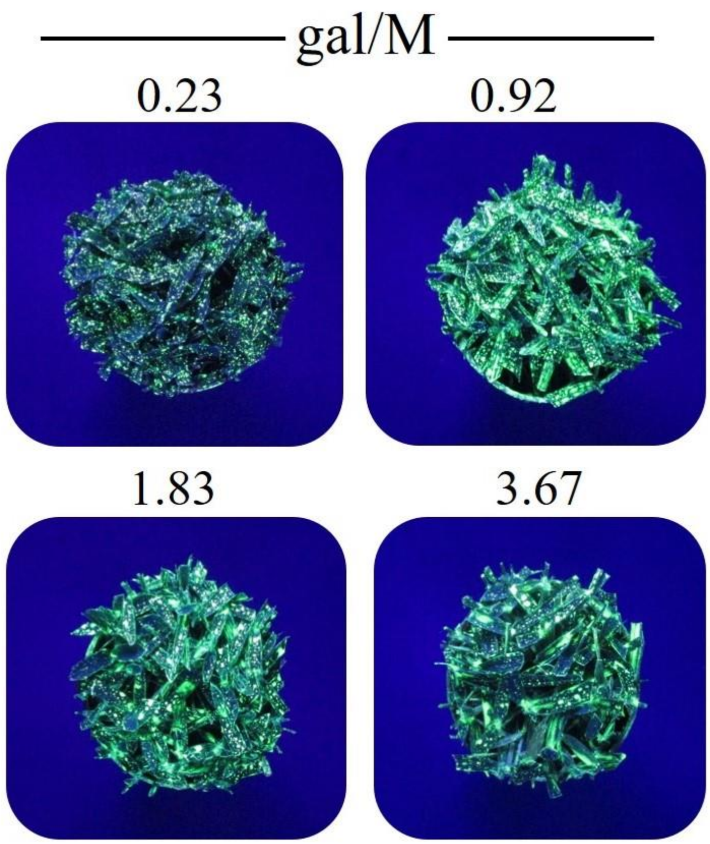


Fig. 3. A fluorescent tracer was used to assess spray deposition characteristics with the aid of black light illumination.

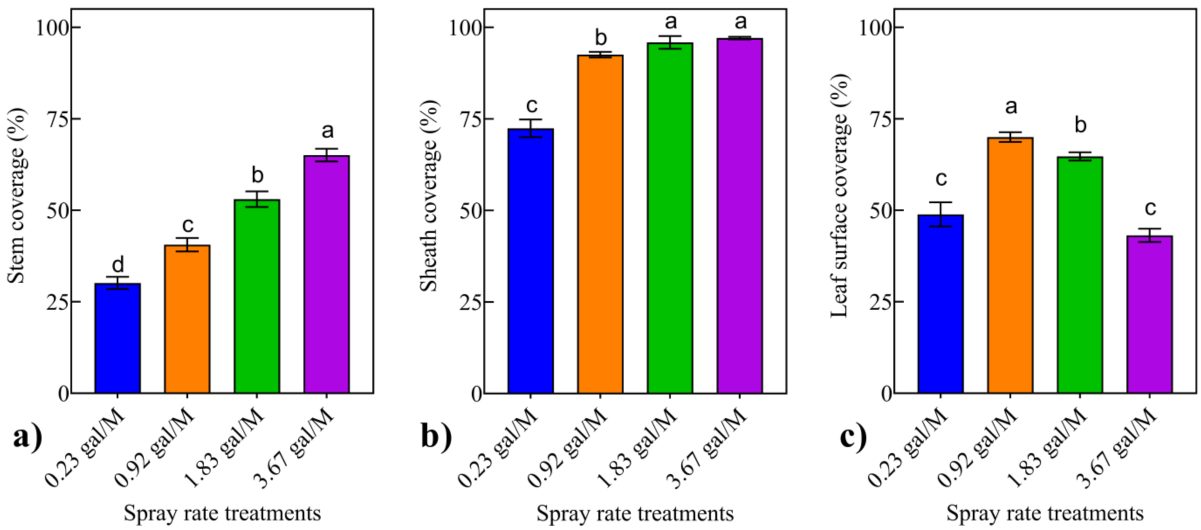


Fig. 4. Spray deposition measurements on Japanese lawngrass stems (a), sheaths (b), and leaf surfaces (c) in response to various spray rate volumes.

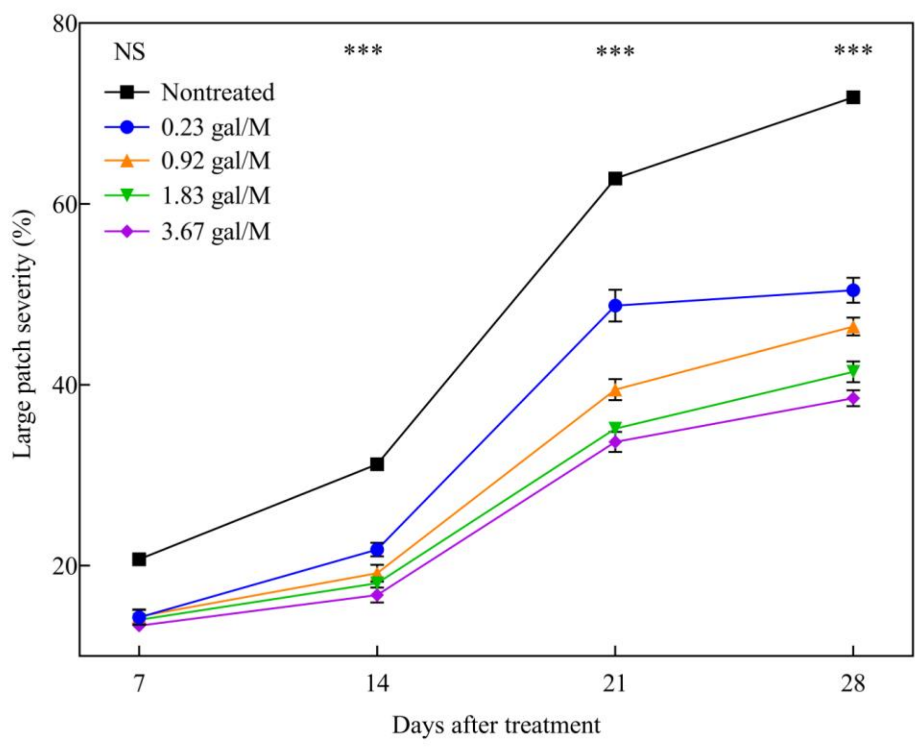


Fig. 5. Large patch severity (pooled across fungicides) in response to various spray rate volumes under growth chamber conditions.

USGA ID#: 2016-18-568

**Management Strategies of a *Sclerotinia homoeocarpa* Population with Multiple Fungicide Resistance and Multidrug Resistance**

**Hyunkyu Sang, James T. Popko, and Geunhwa Jung**
Stockbridge School of Agriculture
University of Massachusetts, Amherst

Objectives:
1. To assess field efficacy of dicarboximide, DMI and SDHI fungicides in a dicarboximide-resistant *S. homoeocarpa* population.
2. To develop the best fungicide options for controlling a *S. homoeocarpa* population with multiple fungicide resistance.
3. To understand how many applications of non-dicarboximides are required in order to revert a dicarboximide-resistant population into sensitive by monitoring the population shift.
4. To determine how persistent the reverted dicarboximide-sensitive population will be after reversion.

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is one of the most significant diseases of cool-season turfgrass on golf courses. Resistance to the benzimidazole and dicarboximide classes and reduced sensitivity to the sterol demethylation inhibitor (DMI) fungicide class in *S. homoeocarpa* populations has been reported, moreover, a select number of golf courses also contain *S. homoeocarpa* populations with high levels of reduced dicarboximide (iprodione and vinclozolin) sensitivity. In order to better understand the practical implications of dicarboximide fungicide resistance, we conducted a field trial in 2015 and 2016 on a golf course fairway with a dicarboximide-resistant *S. homoeocarpa* population to develop fungicide options for dollar spot control.

During the 2015 and 2016 field season, fungicide efficacy was tested on two different fairway locations at Wethersfield Country Club (WCC) in Connecticut and the population exhibited a combination of four different isolate genotypes with differing resistance profiles to the benzimidazole, dicarboximide and DMI fungicide classes. Field efficacy data in 2015 and 2016 showed a fairly similar trend. Reduced field efficacy was observed using the following fungicides: iprodione (Chipco 26GT), vinclozolin (Curalan), and low rate of propiconazole (Banner MAXX II). On the other hand, good control was observed with high rate of boscalid (Emerald), fluxapyroxad (Xzemplar), fluazinam (Secure), and Enclave (Fig. 1).

*In vitro* fungicide sensitivity assays of *S. homoeocarpa* isolates sampled before and after fungicide applications were conducted to monitor changes of dicarboximide-resistant isolates in the population. All *S. homoeocarpa* isolates in the population were insensitive to the DMI fungicide, propiconazole. Increased number of dicarboximide-resistant isolates was observed in plots treated with propiconazole and iprodione in 2015 and 2016 (Fig. 2A and 2B). Number of dicarboximide-resistant isolates was decreased 21 days after fungicide application in 2015 (Final sampling) and in the 2016 overwintered sampling. However, number of dicarboximide-resistant isolates was still high for iprodione and propiconazole applied plots in 2016 Final sampling (Fig. 2A and 2B). The higher application rate of boscalid displayed excellent control of the dicarboximide-resistant population in 2015, however, the dicarboximide-resistant population increased 20% after overwintering. In 2016, boscalid provided good control of the dicarboximide-resistant population (Fig. 2A and 2B). Propiconazole and iprodione applications reduced the percentage of thiophanate-methyl (TM; benzimidazole fungicide) resistant isolates following sampling 7 days after treatment in 2015 and 2016. Due to the high level of control with applications of boscalid, the TM-resistant population was not detected 7 days after treatment in 2015, but the resistant population increased at the Final sampling and decreased after overwintering (Fig. 3A and 3B). The 4$^{th}$ application of boscalid increased the percentage of TM-resistant population by 2-fold in 2016 (Fig. 3A and 3B). In 2017, we will continue to monitor dicarboximide-resistant population by collecting *S. homoeocarpa* from infected turfgrass after overwintering and test the sensitivity of isolates to different fungicides to validate the previous year's results are repeatable.

**Summary**

- Non-DMI and non-dicarboximide fungicides (Xzemplar, Emerald, Secure), and Enclave provided better control of the multiple-fungicide resistant *S. homoeocarpa* population than Banner MAXX, Chipco 26GT, or Curalan.
- Dicarboximide-resistant isolates in the population was increased by applications of propiconazole and iprodione, however, they decreased after fungicide applications ended in 2015.
- 2-4 applications of boscalid are required to shift a bimodal dicarboximide-resistant/sensitive population to a unimodal dicarboximide-sensitive population. However, the reverted sensitive population shifted back to bimodal population after overwintering in 2015 and 21 days after treatment in 2016.
- Propiconazole and iprodione provided good control of the thiophanate-methyl-resistant population.

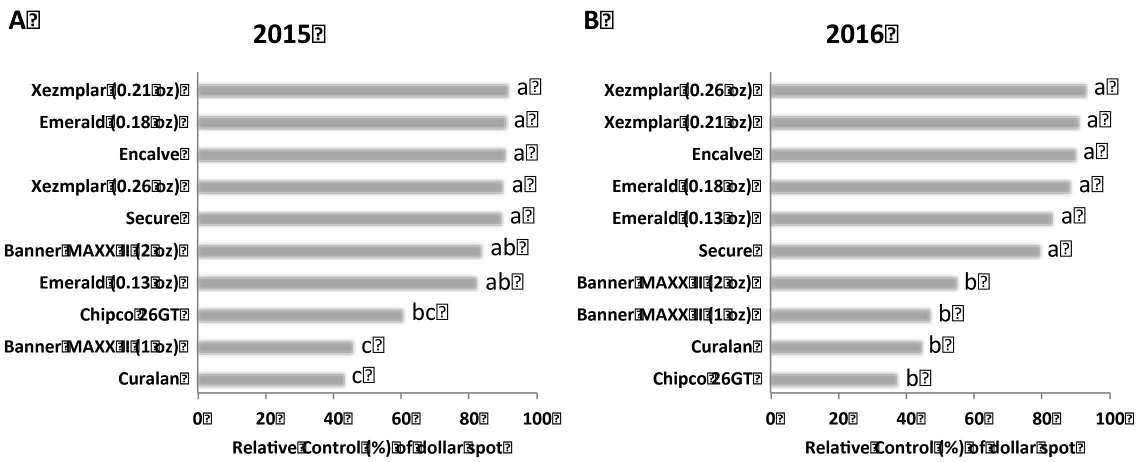


Fig. 1. Relative control percent (%) of dollar spot of fungicide treatments on two different fairway locations at Wethersfield Country Club, CT in 2015 (A) and 2016 (B). Relative control percentage (RC%) data were collected by counting number of individual infection centers and calculating area under (AUDPC) values for all rating dates among all treatments. Rating began on the first date of the first fungicide application and concluded 21 days after the final application. RC% was calculated with the following formula: [(untreated-fungicide treated)/untreated] × 100 = RC%. Different letters on top of bar indicated significantly different ($p<0.05$) according to Fisher's protected least significant difference.

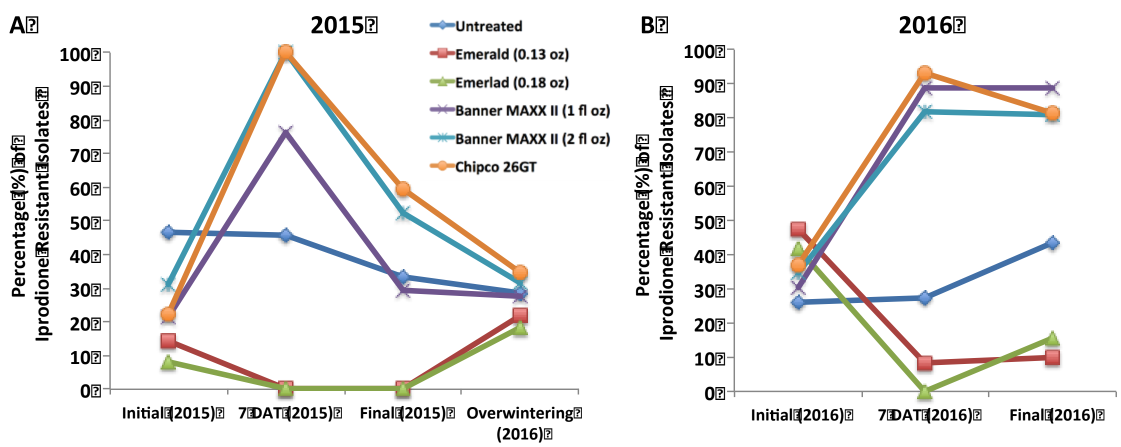


Fig. 2. Percentage of dicarboximide-resistant isolates on untreated and different fungicides treated plots in 2015 (A) and in 2016 (B). Initial, 7-DAT, Final, and Overwinter indicate initial sampling before fungicide application, 7 days after treatment of fungicide, 21 days after final treatment of fungicide, and after overwintering, respectively.

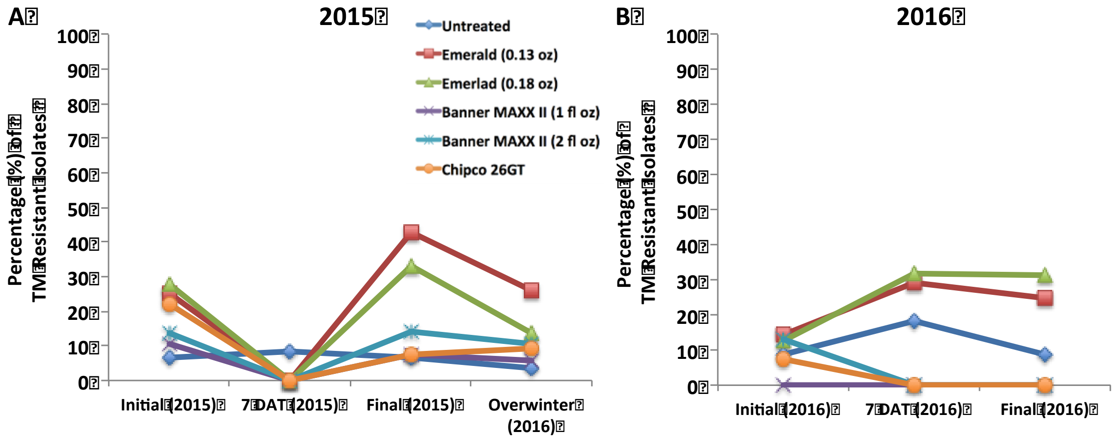


Fig. 3. Percentage of benzimidazole-(TM: thiophanate-methyl) resistant isolates on untreated and different fungicides treated plots in 2015 (A) and in 2016 (B). Initial, 7-DAT, Final, and Overwinter indicate initial sampling before fungicide application, 7 days after treatment of fungicide, 21 days after final treatment of fungicide, and after overwintering, respectively.

2016 Progress Report: USGA ID#: 2016-21-571

**Bentgrass Tolerance, Disease Predictive Models and Fungicide Timing to Control Dollar Spot on Fairway Turf**

James A. Murphy, James Hempfling, Bruce B. Clarke
Dept. of Plant Biology and Pathology, Rutgers University

- Dollar spot forecasting by a logistic regression model had good accuracy for highly susceptible cultivars during 2015, but over-predicted during 2016.
- Good to excellent, season-long disease control was achieved when subsequent fungicide timing was based on a threshold program, but total fungicide inputs and the level of disease control depended on the cultivar and, to a lesser extent, the initial fungicide timing.
- Fungicide applications on 'Declaration' creeping bentgrass that were threshold-based produced excellent disease control and resulted in only three and one fungicide applications during 2015 and 2016, respectively, regardless of the initial fungicide application date.
- In contrast, threshold-based fungicide applications on 'Independence' creeping bentgrass resulted in a total of six or seven applications during 2015 and four or five applications during 2016, depending on the initial fungicide timing.

This research project is organized into two field trials. The objectives of the first trial include evaluating dollar spot (caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett) incidence and disease progress on six bentgrasses that vary in tolerance to dollar spot disease; and assessing the reliability of two existing weather-based models for predicting dollar spot epidemics on those cultivars and species. Six bentgrass cultivars ['Independence', 'Penncross', 'Shark', '007' and 'Declaration' creeping bentgrass (*Agrostis stolonifera*), and 'Capri' colonial bentgrass (*A. capillaris*) (Figure 1)] that vary in tolerance to dollar spot were evaluated for disease incidence every two to five days and compared to a growing degree day (GDD) model for predicting the onset of disease symptoms and a logistic regression model for predicting season-long disease activity. An accurate prediction of the onset of disease symptoms in highly susceptible cultivars occurred with the GDD model during 2015 but not 2016. A high risk of dollar spot was forecast by the logistic regression model one week before symptoms first appeared in highly susceptible cultivars during both years. Throughout the rest of the growing season, disease forecasting by the logistic regression model had good accuracy for highly susceptible cultivars during 2015, but over-predicted during 2016 (Figures 2 and 3). Disease forecasting on tolerant cultivars has not been accurate with either model in both years of this study.

The objectives of the second trial include evaluating the effect of pre-symptomatic (initial) timings for fungicide application on dollar spot incidence and disease progress on a susceptible and a more tolerant bentgrass cultivar; and determining the extent that pre-symptomatic fungicide application(s) on these cultivars may affect the total fungicide usage over a growing season when subsequent fungicide applications are based on either a disease-threshold or a predictive-model. Treatments in this trial were arranged as factorial combinations of bentgrass tolerance to dollar spot, initial fungicide application timing, and subsequent fungicide timing. Declaration (more tolerant) and Independence (susceptible) were the cultivars used for the bentgrass tolerance factor. Initial fungicide application timings occurred (1) at the first appearance of disease symptoms (threshold-based; < 2 infection centers/8 square feet); (2) on May 20 (calendar-based); (3) when the logistic regression model reached a 20% risk index; or (4) at a GDD range of 20-30, 30-40, 40-50, 50-60, or 60-70 (base temperature15 C [60 F] starting April 1). Subsequent fungicide timings were based on the logistic regression model, or on a disease threshold, or were withheld completely to assess long-term effects of initial fungicide timings. All possible combinations of initial and subsequent fungicide timings were applied on both cultivars and all fungicide applications used Emerald 70WG (boscalid, BASF) at 0.18 ounce/1,000 square feet. Threshold-based plots were monitored as often as daily for dollar spot incidence. The number of applications to threshold- and model-based plots were recorded.

The initial fungicide application factor had minimal impact on long-term (May through November) control of dollar spot during 2015. Conversely, the factors of subsequent fungicide timing and bentgrass cultivar had a much greater impact on disease control. Excellent (< 1 infection center/8 square feet) long-term control of dollar spot was achieved for both cultivars when subsequent fungicide timing was based on either the logistic regression model or the calendar-based program. The logistic regression model reduced fungicide inputs by zero or one application during 2015 and one or two applications during 2016, depending on the initial fungicide timing, compared to the calendar-based program (nine applications) (Table 1). Good to excellent, long-term disease control was also achieved when subsequent fungicide timing was based on a threshold program, but the total fungicide input and the level of disease control depended on the cultivar and, to a lesser extent, the initial fungicide timing. Subsequent fungicide applications on Declaration plots that were threshold-based produced excellent disease control and resulted in only three and one fungicide applications during 2015 and 2016, respectively, regardless of the initial fungicide application date. In contrast, the threshold schedule for subsequent applications on Independence plots resulted in a total of six or seven applications during 2015 and four or five applications during 2016, depending on the initial fungicide timing (Table 1). Moreover, disease incidence occasionally surpassed the target threshold value on Independence plots and reached levels (up to 2.5 infection centers per 8 square feet) during the growing season that may not be acceptable at some golf courses (Figure 4). This research will be continued during 2017.

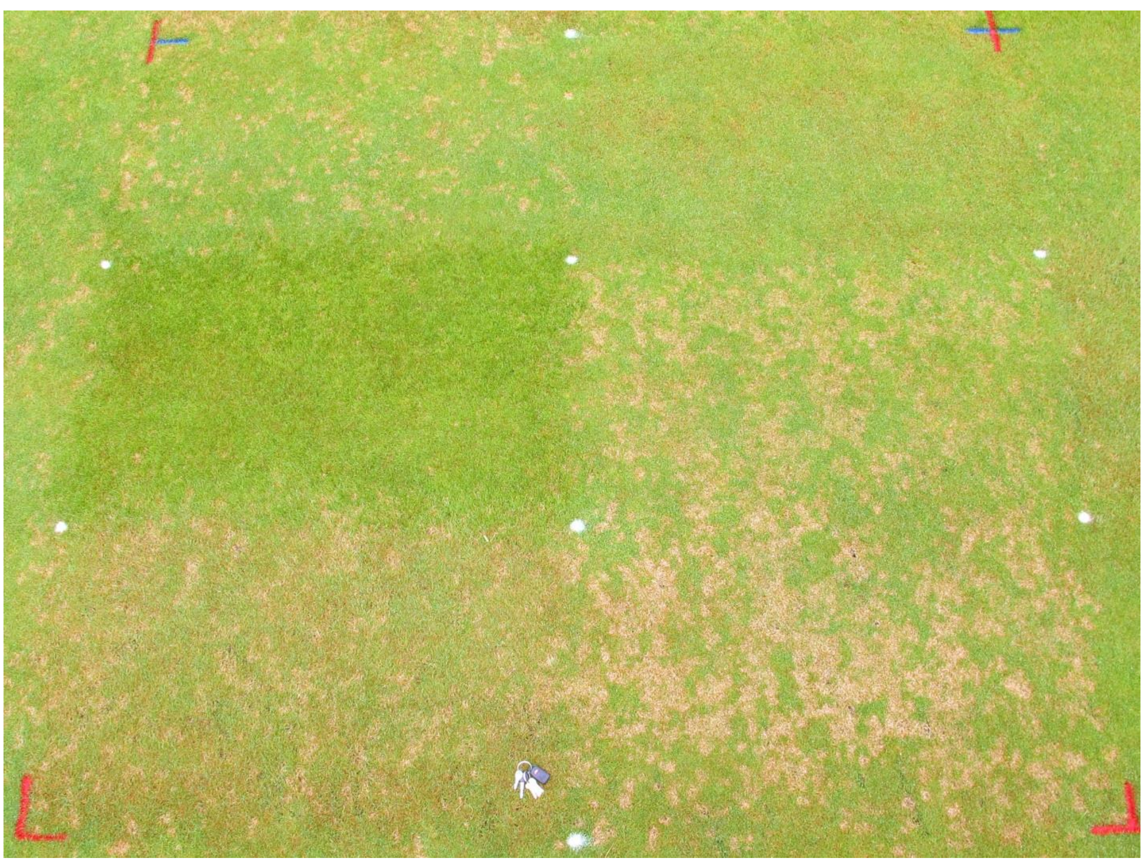

Figure 1. Bentgrass cultivars vary in their tolerance to dollar spot (clockwise from top left): 007, Declaration, Shark, Independence, Penncross and Capri. Photo by J. Hempfling

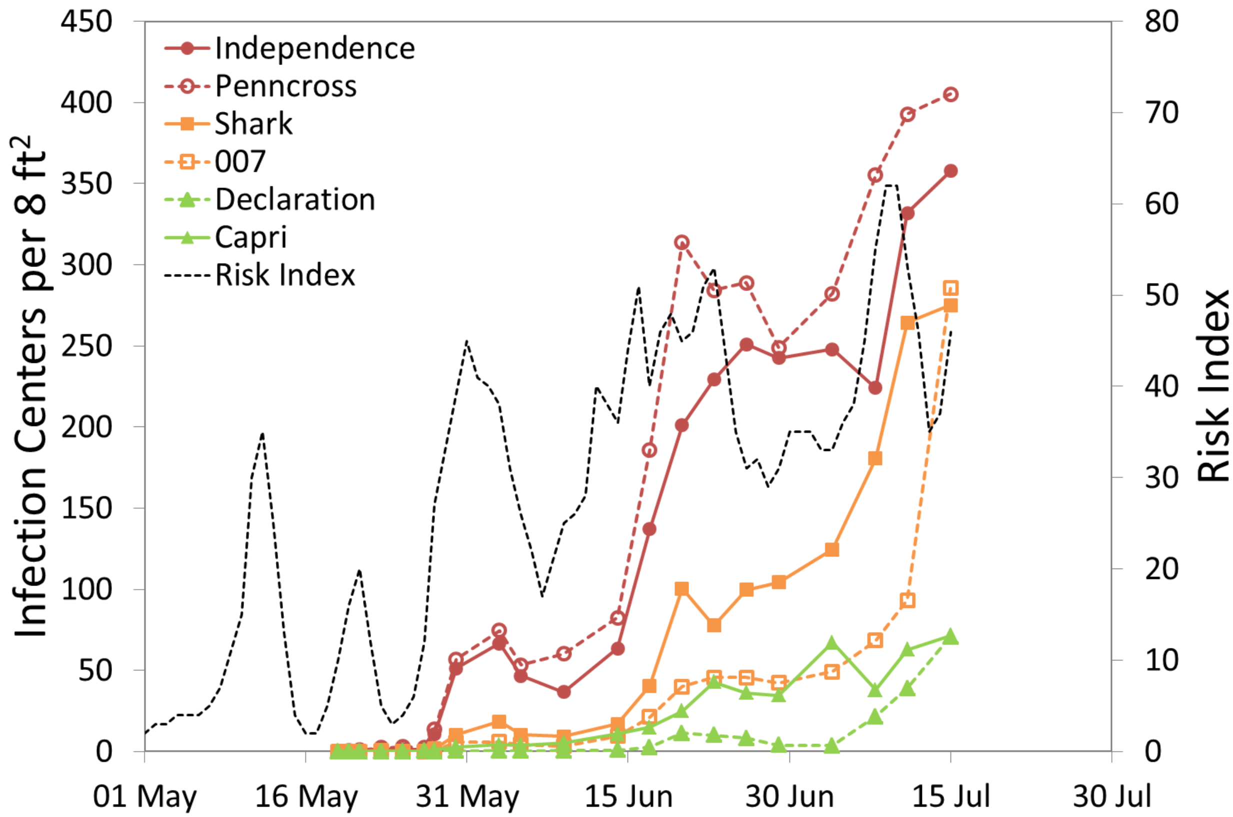

Figure 2. Number of dollar spot infection centers in highly susceptible (red lines), moderately susceptible (orange lines), and more tolerant (green lines) bentgrass cultivars and dollar spot risk index (black line) calculated using a logistic regression model during 2015.

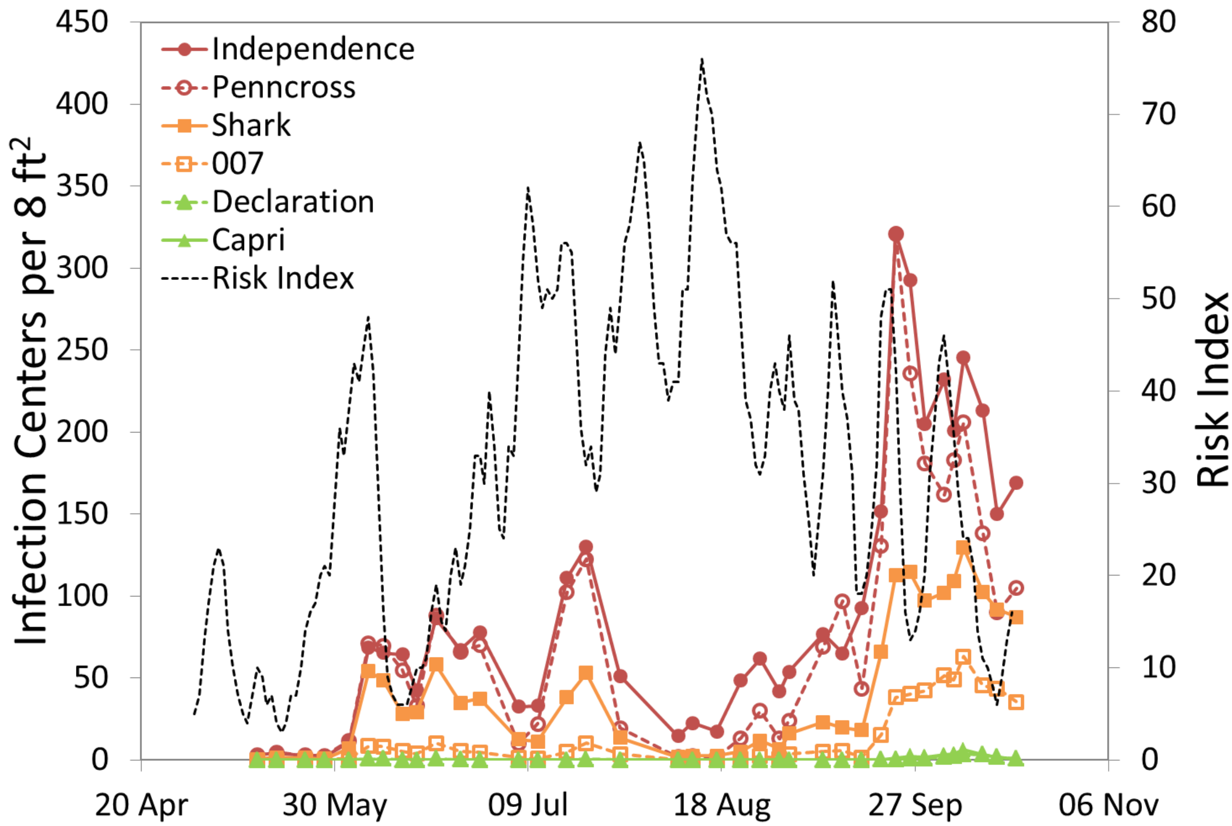

Figure 3. Number of dollar spot infection centers in highly susceptible (red lines), moderately susceptible (orange lines), and more tolerant (green lines) bentgrass cultivars and dollar spot risk index (black line) calculated using a logistic regression model during 2016.

Table 1. Total number of fungicide applications used to control dollar spot based on bentgrass cultivar and initial and subsequent fungicide timings during 2015 and 2016.

| | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Declaration | | | Independence | | | Declaration | | | Independence | | |
| | Subsequent Fungicide Timing | | | | | | Subsequent Fungicide Timing | | | | | |
| Initial Fungicide Timing | Calendar | Logistic Model | Threshold | Calendar | Logistic Model | Threshold | Calendar | Logistic Model | Threshold | Calendar | Logistic Model | Threshold |
| | Total Number of Fungicide Applications | | | | | | | | | | | |
| 20-30 GDD | - | 9 | 3 | - | 9 | 7 | - | 8 | 1 | - | 8 | 5 |
| 30-40 GDD | - | 9 | 3 | - | 9 | 7 | - | 7 | 1 | - | 7 | 4 |
| 40-50 GDD | - | 8 | 3 | - | 9 | 7 | - | 7 | 1 | - | 7 | 5 |
| 50-60 GDD | - | 8 | 3 | - | 8 | 6 | - | 7 | 1 | - | 7 | 4 |
| 60-70 GDD | - | 8 | 3 | - | 8 | 6 | - | 7 | 1 | - | 7 | 5 |
| Logistic | - | 8 | 3 | - | 9 | 7 | - | 8 | 1 | - | 8 | 4 |
| Threshold | - | 8 | 3 | - | 8 | 6 | - | 0 | 1 | - | 8 | 5 |
| Calendar | 9 | 8 | 3 | 9 | 8 | 6 | 9 | 7 | 1 | 9 | 7 | 4 |

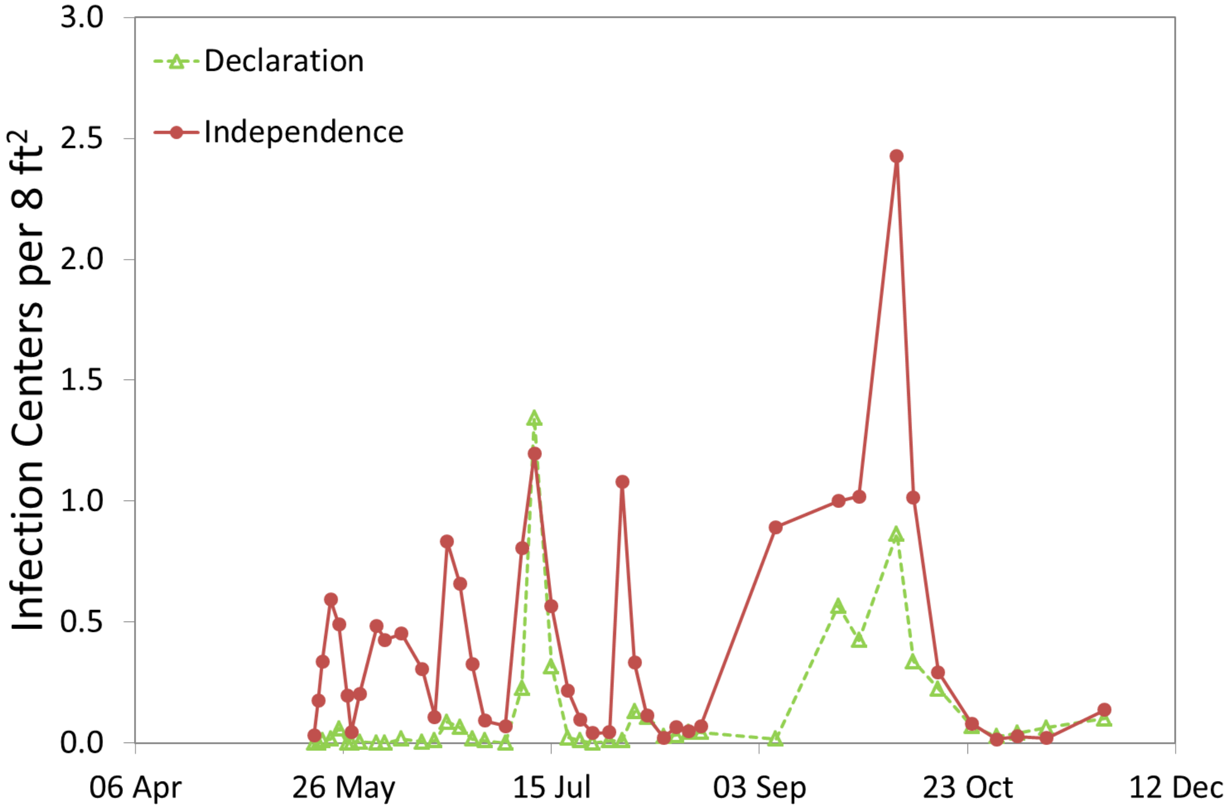


Figure 4. Number of dollar spot infection centers in highly susceptible ('Independence') and more tolerant ('Declaration') creeping bentgrass cultivars ~~having~~ using a subsequent fungicide timing based on a disease target threshold (< 2 infection centers/8 square feet) during 2015.

Project Title: **Characterizing growth and life history of silvery-thread moss in cool-season putting greens: assessing vulnerability to stress in the life cycle**

Principal Investigator(s): **Lloyd Stark, Steve Keeley, Zane Raudenbush**

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**Summary**

During 2016, we accumulated material of the invasive putting green moss, *Bryum argenteum*, from 15 golf courses in the USA, and material of the same species from natural habitats from 17 localities throughout the USA. This material was collected and shipped to us (at UNLV) in dried condition, placed into culture on sieved sand media, and subcultured until the culture of moss was free from algal contaminants. Our culture technique produces single clonal lines (genotypes) of each moss collection. When we had produced 7 such genotypes from putting greens and 7 such genotypes from non-putting green habitats, we initiated an experiment comparing putting green genotypes with non-putting green genotypes in terms of the growth dynamics of each group. Our preliminary results indicate that the Silvery-Thread Moss (STM, also *Bryum argenteum*) has evolved a suite of traits that allow it to successfully compete with bent grass in putting greens. These traits include the ability to germinate rapidly, accelerated growth rates, the production of denser and taller shoots, the tendency to produce large mats of rhizoids (the "rooting" function of mosses), and to avoid sexual reproduction in favor of vegetative growth. Such accelerated growth rates could leave this species vulnerable to stress at a particular phase in the life cycle, and this will be the subject of research in Year 2 (2017)

**Overview**

The purpose of this project is to address a biological concern in golf course greens, the Silvery-Thread Moss (STM), known scientifically as *Bryum argenteum*. This moss has infested golf course putting greens across the USA, and golf course superintendents have expressed concerns regarding effective eradication approaches. We initiated this project with these goals:

1. Accumulate representative collections of STM from a variety of golf courses and representative collections of STM from non-golf course habitats, place these genotypes into pure culture, and compare their life history and stress responses. In essence, we wish to determine how different the golf course strains of this species are compared to populations not in golfing greens. Understanding these differences will help us formulate better treatment plans for eradication.

2. Evaluate the effectiveness of administering specific stresses, including the application of carfentrazone, at various points in the life cycle of STM. These life cycle stages include (in sequence from juvenile to adult) protonema, rhizoid, juvenile shoots, adult shoots, and asexual reproductive structures.

**Progress to Date for Year 1 (2016)**

Establishing genotypes of STM at the University of Nevada, Las Vegas. Dr. Zane Raudenbush (now at The College of Wooster, Ohio) distributed information slips with instructions on how to collect this moss from greens at two USGA national conferences. The response was good, with superintendents sending core samples of the moss to Dr. Llo Stark at UNLV. In addition, Dr. Raudenbush made collections from golf courses in Ohio, and Raudenbush and Stark (along with potential postdoctoral student Joshua Greenwood) visited and collected this moss from a rural Nevada golf course. We have received at least one sample of STM from a total of 15 golf courses. These golf course samples include courses from Alberta (Canada), California, Colorado, Illinois, Minnesota, Nevada, Ohio, Oregon, and South Dakota. Of these 15 genotypes (a specific genetic strain from the golf course in question), 12 have been purified in culture and the remaining 3 are in the process of purification. The purification is accomplished by placing shoots of STM into culture, allowing the moss to proliferate (with contaminants), and then after a few weeks of growth subculturing the shoot apices of the moss. Such an approach is successful in freeing the collection from algal and bacterial contaminants because the aerial portions of the moss are normally free of contaminants. This subculturing was repeated (usually two or three times) until a pure culture was obtained. Contaminants usually included assorted green algae and cyanobacteria. From receipt of sample to a pure culture of a specific genotype takes about 3 months in the lab.

In order to compare the responses of STM from golf course greens with STM genotypes collected from non-golf course habitats, we received and collected specimens from a total of 17 localities from a variety of urban and natural “off course” habitats. These “off course” collections are from Arizona, California, Georgia, Kentucky, Massachusetts, New Mexico, Nevada, Oregon, Pennsylvania, and Washington. The habitats range from sidewalk cracks, along streets, in parks, or in native habitats on soil. Of these 17 genotypes, we have purfied 10 in culture, and expect to have all 17 genotypes in pure culture in a few months. We are shooting for 15 genotypes of STM from golf course greens, and 15 genotypes of STM from “off course” habitats, and should reach that number in a few months.

2. Experiment 1: Comparing the Growth Dynamics of Golf Course STM to “Off Course” STM. We initiated an experiment using 7 golf course genotyes and 7 “off-course” genotypes of STM, following the progression of life stages as follows: protonema, shoots, bulbils (asexual reproductive structures), and gametangia (sperm or egg clusters). We are interested in detecting differences in growth dynamics and the expression of life history stages between mosses from golfing greens vs. mosses of the same species not inhabiting golfing greens. The experiment is in its 12$^{th}$ week as of this writing (October 2016), and some interesting trends merit mention:

A. Moss from the putting greens germinate faster.

B. Moss from the putting greens grow faster laterally as measured by protonemal proliferation.

C. Moss from the putting greens produce shoots sooner, as assessed by the day of shoot induction.

D. Moss from the putting greens grow faster in height as measured by shoot height.

E. Moss from the putting greens produce a higher number of shoots per unit area as measured by shoot counts.

F. Moss from the putting greens produce greater cover of rhizoids (the rootlike filaments extending from shoots) as measured by rhizoid cover.

G. Moss from the putting greens produce fewer specialized asexual reproductive structures as measured by bulbil counts and protonemal gemma counts.

H. Moss from the putting greens express sex later, as assessed by the number of perigonia (sperm containing structures) and perichaetia (egg containing structures), and when they express sex, it is typically higher on the shoot.

I. Moss from the putting greens, to date, are exclusively female.

J. Moss from the putting greens appears to have a higher nutrient need.

These life history and growth dynamic differences between strains from golf course greens and strains from habitats excluding golf courses are exciting because they indicate recent evolution pressure on golf course putting green habitats. Our "common garden" approach to comparing the growth dynamics of specific genotypes represents one of the strongest approaches to discern between differences related to genetic vs. environmental causes. Because of common culture conditions, any differences we see between green and off-green genotype cultures can be attributed to genetic differences (as opposed to a plastic response). The above differences are many, and indicate strong selective pressures acting on golf course greens over the last few hundred years, a very short evolutionary timeframe. These are likely to be among the life history features that give this moss a competitive advantage in putting greens, and relate to the ability to colonize rapidly, spread laterally rapidly, produce shoots quickly that grow tall and dense, extend rhizoids into the sand and laterally, require lots of nutrients, and not be reliant on sexual reproduction.

**Plans for Year 2 (2017)**

1. Complete Experiment 1. We currently are comparing 7 genotypes of golf course STM with 7 genotypes of "off course" STM. We need to increase the number of genotypes (strains) tested to about 15 in order to be statistically sound in experimental design, and we should be able to complete this experiment early in Year 2.

2. Initiate Desiccation Tests on STM. We will begin stressing the STM mosses with drying (desiccation) stresses at key phases of their life history, in an attempt to pinpoint weak links in the life history of this moss weed.

**Note**

Because the project was funded at 50% of budget, we have delayed hiring a graduate student or laboratory tech person until the second year. In 2017 we anticipate hiring a half-time graduate student, lab tech, or postdoctoral student (probably Mr. Joshua Greenwood of UNLV) who will assist Dr. Stark in conducting sets of experiments on environmental stress effects on STM strains from golf course greens.

2016-19-570

Comparative Control Methodology of Belonalaimus longicaudatus and Meloidogyne Species on Golf Course Putting Greens - Glenn Galle and Jim Kerns, North Carolina State University

- Sting nematode has been observed deeper in the soil column during the summer months than expected
- Sting nematode numbers increase earlier in the spring on creeping bentgrass putting greens than bermudagrass greens
- Root-knot nematode sampling has been delayed, and results are unavailable
- Cultivar screening is currently in the planning stage, and the project is expected to start in the spring of 2017

Progress Report and Research Summary

Trial one from the research project is progressing as anticipated, with the sampling for *Belonolaimus longicaudatus* entering its third year at Raleigh Golf Association ('L-93' creeping bentgrass) and Benvenue Country Club ('Champion' bermudagrass), and is about eighteen months along at Wilson Country Club ('A-1/A-4' creeping bentgrass). The data shows that sting nematode reaches its highest numbers in late summer on both bermudagrass and creeping bentgrass courses with numbers dropping significantly during the winter months. However, differences in start timing of nematode population growth has been observed with numbers increasing in March or April on creeping bentgrass putting greens whereas they increase in late April and May on bermudagrass putting greens. This is significant and indicates that earlier nematicide application is necessary depending upon the turfgrass species used.

An unanticipated result is the dramatic shift of nematode populations from the top ten centimeters of the soil column during the spring months to the middle ten centimeters during the summer months on creeping bentgrass in 2015 as seen in figure two. A similar trend was observed in creeping bentgrass in 2016, although sting nematode was more evenly distributed throughout the entire thirty-centimeter soil column during the summer. From figure one, the bermudagrass course also showed an even nematode population distribution throughout the entire thirty-centimeter soil column during the summer months of both years. This is beyond the rooting depth of both turfgrasses and we are further investigating why the nematodes are this deep in the soil column. In both turfgrass species, the winter months show a majority of the nematodes in the top ten centimeters of the soil column and at significantly high population levels, indicating that feeding may be occurring during the winter months when bermudagrass is dormant and creeping bentgrass is slow to produce new roots. This indicates the potential addition of a fall nematicide application to a current spray program may be necessary to protect turfgrass roots from nematode feeding when they are highly vulnerable.

The final year of sampling for sting nematode will help to provide stability to trends currently present, and potentially will clear up any differences observed between the two years of current data.

The *Meloidogyne spp.* sampling portion of the project at Sedgefield Country Club ('Champion' bermudagrass) has just finished its first year, and data is inconclusive and erratic. Unfortunately, we were asked to vacate the course for a year due to winter damage and therefore had to start sampling a year after we started the sting nematode portion. Sampling for three years is still planned.

Trial two from this project is currently in the planning phase. We are collecting nematodes and establishing populations in the greenhouse for use in the cultivar screen. Cultivar selection is also occurring at this time, and seed or sprig collection will begin in the winter. The planned start of the project is spring of 2017 and it is expected to take approximately one year.

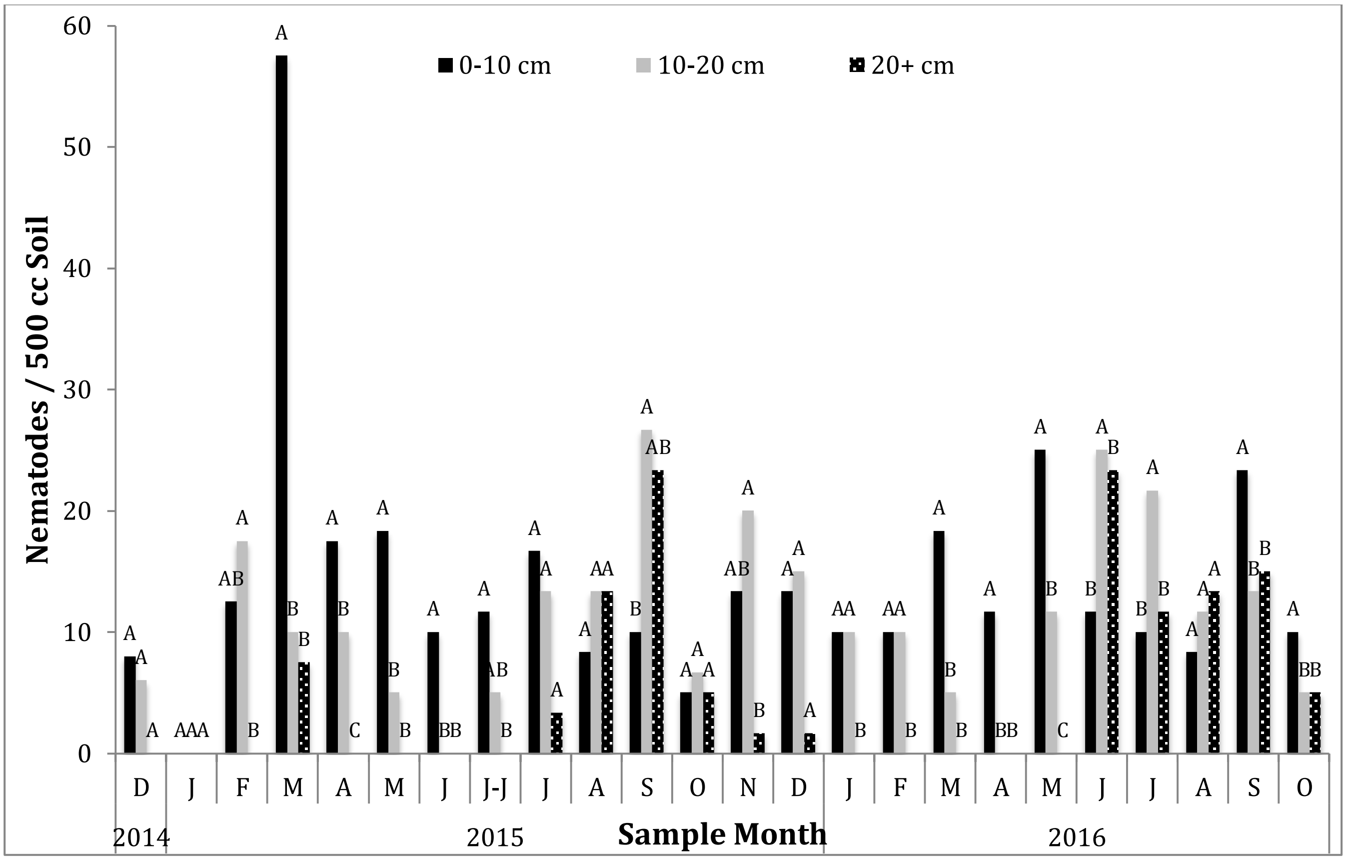


Figure One. Sting nematode population sampling distribution on bermudagrass from Benvenue Country Club, Rocky Mount, NC.

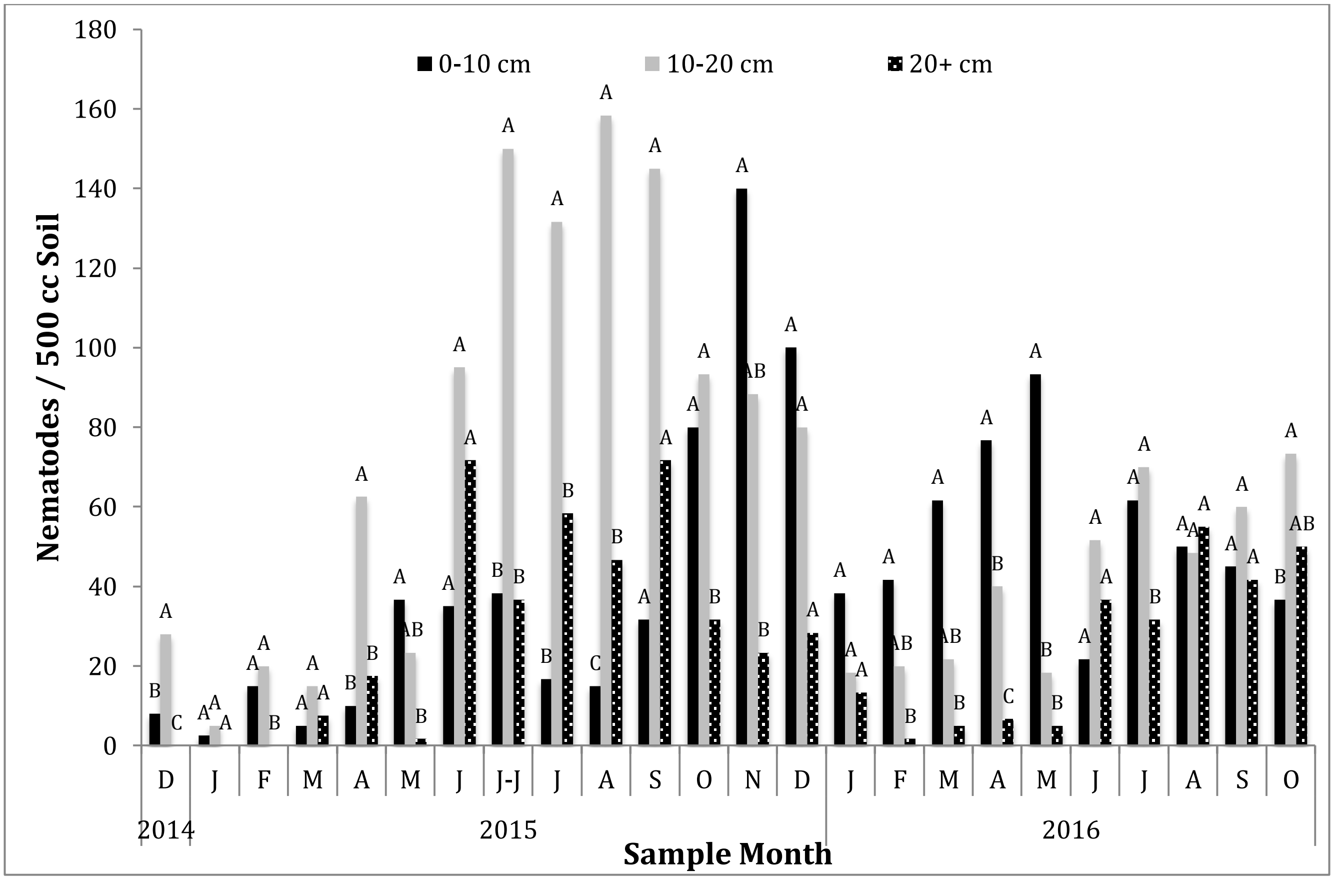


Figure Two. Sting nematode population sampling distribution on creeping bentgrass from Raleigh Golf Association, Raleigh, NC.

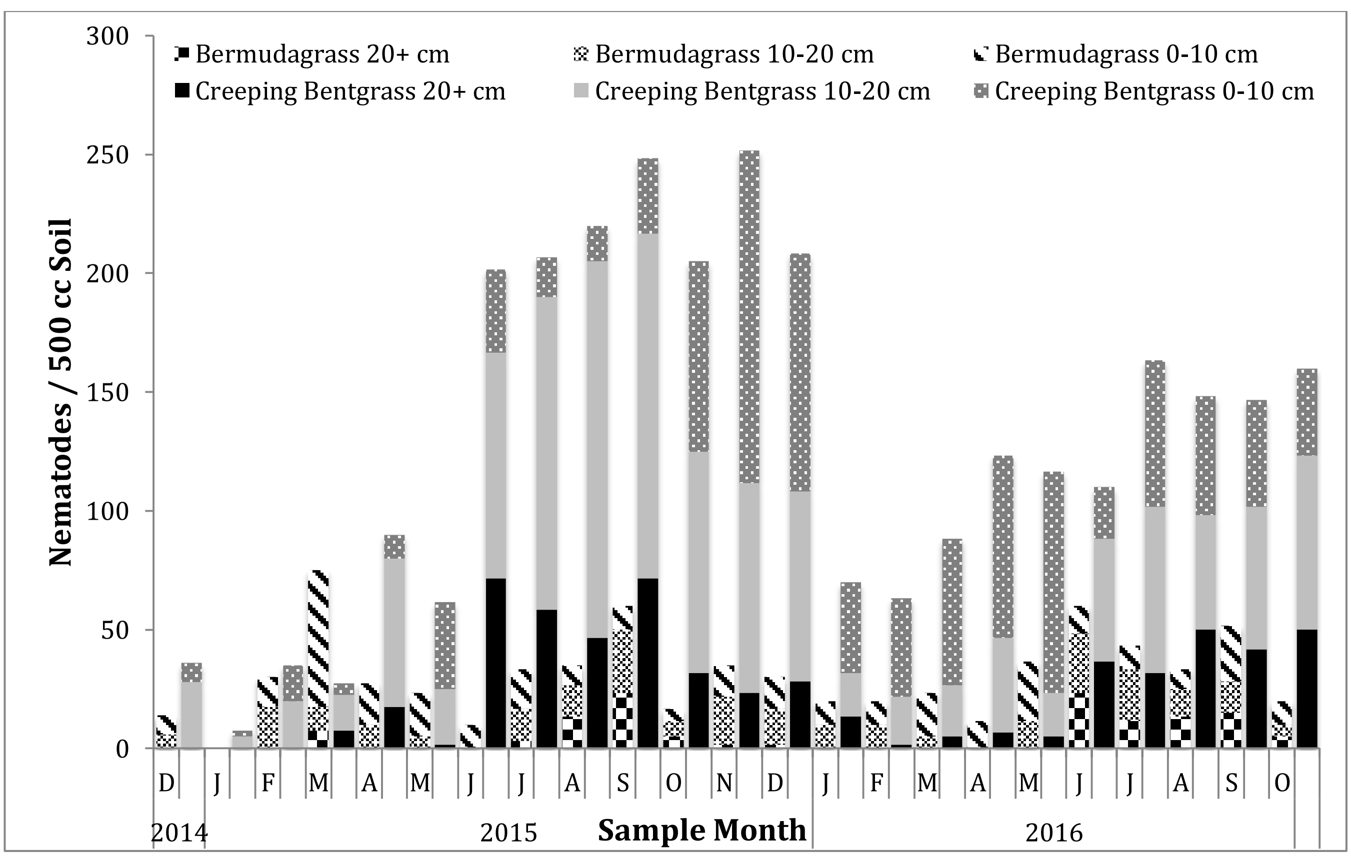


Figure Two. Comparison between creeping bentgrass and bermudagrass population dynamics.

**Title:** Biorational control of important golf turf insect pests

**Project leader (**or co-leaders): Albrecht M. Koppenhöfer, Olga S. Kostromytska, Shaohui Wu

**Affiliation:** Department of Entomology, Rutgers University, New Brunswick, NJ

**Objectives:** The overall goal is to develop a better understanding of the role that biorational insecticides can play in the management of important golf turf insect pests with particular emphasis on the annual bluegrass weevil to facilitate insecticide resistance management in this difficult-to-control pest.

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a serious and expanding golf course pest with demonstrated ability to develop resistance to a range of insecticides. The primary purpose of this project is to develop biorational alternatives for the management of ABW as safer and more sustainable alternatives to traditional synthetic insecticides that will also facilitate better insecticide resistance management. Biorational materials are being tested on golf course fairways against insecticide-susceptible and -resistant ABW adults and larvae. They are also being tested against larvae of white grubs and black cutworm because treatments against these pests could be done at the same time as for ABW, increasing chances of adoption of the biorational controls and further decreasing insecticide applications.

Biorationals tested include the products Grandevo (*Chromobacterium subtsugae* strain PRAA4-1 and its fermentation products), Venerate (heat-killed *Burkholderia* spp. strain A396 bacteria and their fermentation products), BotaniGard ES (entomopathogenic fungus *Beauveria bassiana* GHA strain), and Molt-X (botanical azadirachtin). They were chosen because they have reasonable costs, product stability, and relatively long shelf lives at room temperature, and have very different modes of action unlikely to be affected by the broad insecticide resistance observed with ABW.

Experiments presented here were conducted on fairways of golf courses with ABW populations that were considered fully susceptible to pyrethroid insecticides or pyrethroid-resistant with a resistance ratio ($RR_{50}$) of around 2x and 70x, respectively, compared to the most pyrethroid-susceptible population determined by us. Two application of Molt-X provided quite consistently around 40% control irrespective of resistance level when applied when eggs and first to second instar larvae peaked but was ineffective when applied against third and fourth instars (Fig. 1). Two application applied when third and fourth instar larvae peaked gave around 35% control with Grandevo, irrespective of resistance level, but only 23 to 30% with Venerate (not statistically significant against the resistant population). When targeting the overwintered adults around peak densities before the start of egg-laying, two application provided 36 to 47% control of susceptible and resistant populations with Grandevo and 49 to 55% control with Venerate.

Previously, we had observed a synergistic effect on ABW control of combinations of BotaniGard with the Talstar targeting overwintered adults in around peak densities in a pyrethroid-resistant population. Laboratory tests indicated that the synergistic interaction was due to the oil carrier in the BotaniGard formulation with combination of the oil alone with Talstar causing similar mortality as the BotaniGard-Talstar combination. Hence, overuse of these combination could still select for pyrethroid resistance, and the combinations have to be used carefully.

The control rates observed with the above biorationals tend to be lower than those observed with the more effective synthetic insecticides against pyrethroid-susceptible ABW populations. However, against resistant ABW populations the biorationals are as effective or more effective (depending on resistance level) than most synthetic insecticides. They thus offer new options for the management of resistant ABW populations. And when rotated with still effective synthetic insecticides, they can be used in resistance management in resistant and susceptible populations.

- The pyrethroid bifenthrin (Talstar) and the organophosphate chlorpyrifos (Dursban) were ineffective against insecticide-resistant ABW adults.
- BotaniGard (AI: *Beauveria bassiana*) was ineffective against ABW larvae and adults.
- Talstar and BotaniGard interacted synergistically providing effective control of ABW adults.
- Molt-X, Grandevo, and Venerate (currently not labeled for turfgrass) can provide acceptable control of pyrethroid-susceptible and -resistant ABW and may be useful in the management resistant populations and in resistance management.

**Table 1**. Effect of pyrethroid resistance on densities (± SE) and percent control of annual bluegrass weevil developmental stages in early June (peak 4th to 5th instar) in golf course fairways treated with Talstar and sequential applications (about 1 week apart) of Grandevo and Venerate applied around the peak in densities of overwintered adults in late April/early May.[a]

| Treatment | Rate (lb product/acre)[b] | No. of stages / ft$^2$ (% control) | |
|---|---|---|---|
| | | susceptible | resistant |
| Untreated Control | --- | 148.9.1 ± 21.1 a | 45.8 ± 6.1 a |
| Talstar P | 0.1[c] | 28.8 ± 3.7 d (80) | 34.1 ± 4.0 ab (25) |
| Grandevo | 2 x 4.0 | 88.9 ± 11.8 b (39) | --- |
| Grandevo | 2 x 8.0 | 79.1 ± 11.9 bc (47) | 28.9 ± 4.1 b (36) |
| Venerate | 2 x 8.0 | 69.0 ± 13.3 c (55) | 23.3 ± 4.2 b (49) |
| Venerate | 2 x 16.0 | 75.4 ± 12.9 bc (50) | --- |

Means within resistance level with same letter are not signficantly different ($P$ > 0.05).

[a] Data are combined from two experiments for each resistance level.

[b] Rate for each application

[c] lbs A.I./acre for Talstar

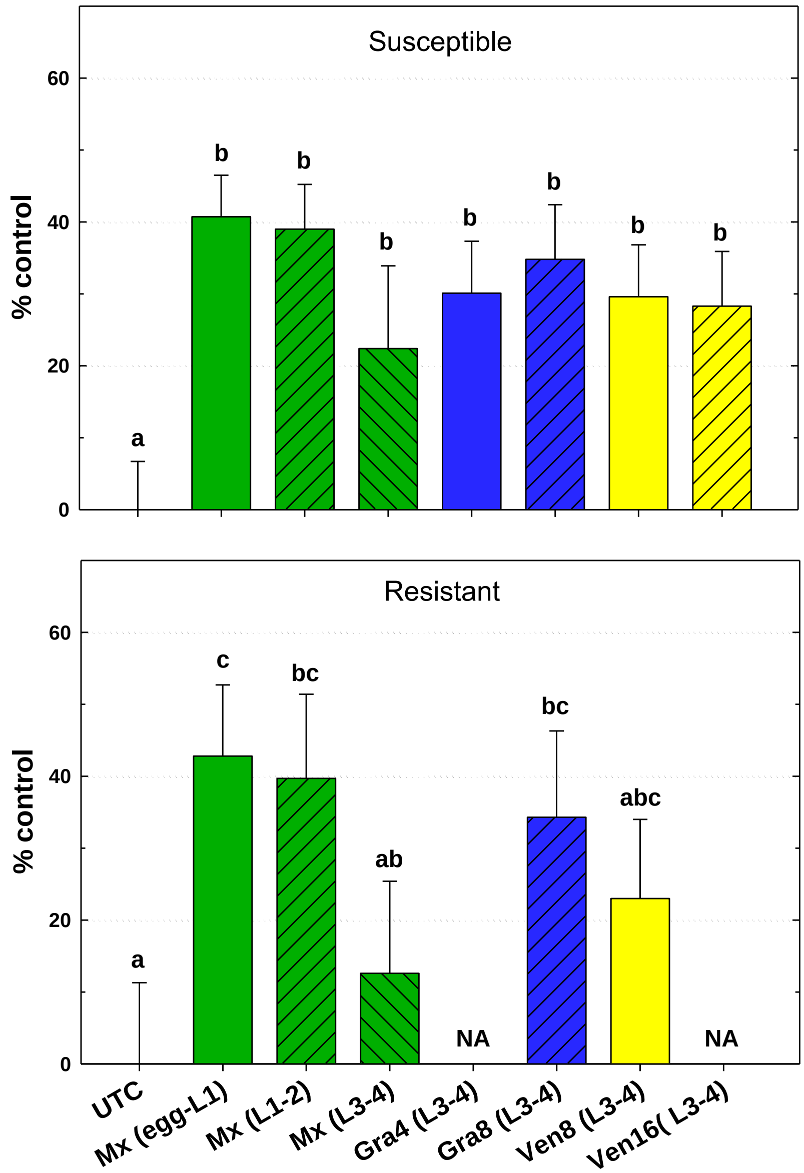


Fig. 1. Effect of pyrethroid resistance on control of annual bluegrass weevil developmental stages in early June (peak 4th to 5th instar) in golf course fairways treated with sequential applications of Molt-X (Mx; 1.44 lb product/acre per application), Grandevo (Gra; 4 and 8 lbs product/acre per application), and Venerate (Ven; 8 and 16 lb product/acre per application). Molt-X was applied at peak eggs and peak 1st instar larvae (egg-L1), at peak 1st instar and peak 2nd instar (L1-2), and at peak 3rd instar and peak 4th instar (L3-4). Grandevo and Venerate were applied at peak 3rd instar and peak 4th instar. Data are combined from two experiments for each resistance level. Means within each resistance level with the same letter did not differ significantly (*P* > 0.05).

**Title:** Developing optimal management programs for annual bluegrass weevil populations with different insecticide resistance levels

**Project leader (**or co-leaders): Albrecht M. Koppenhöfer, Olga S. Kostromytska, Shaohui Wu

**Affiliation:** Department of Entomology, Rutgers University, New Brunswick, NJ

**Objectives:** The overall goal is to develop a better understanding of the degree and scope of insecticide resistance in ABW populations as a basis for the development of recommendations on resistance management. For this project in particular the objective is: Compare field efficacy of typical insecticides used against ABW adults and larvae against 4 ABW populations representing the full scope of insecticide resistance levels observed to date.

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a serious and expanding golf course pest with demonstrated ability to develop resistance to a range of insecticides. Previous and ongoing in depth studies (incl. our own) on ABW insecticide resistance have been restricted to laboratory and greenhouse studies. Any to-date field observations on resistance originate from product efficacy testing trials that are generally poorly designed to truly understand how resistance affects product efficacy and that have, if at all, characterized resistance in simple yes/no Petri dish assays. Only by studying the individual tools separately (different products applied only once at specific times) will it be possible to understand how to put together optimal management programs for different resistance levels. Our ongoing research is specifically designed to test the efficacy of individual applications of the commonly used adulticides and larvicides on fairways at four golf courses representing the full spectrum of pyrethroid-resistance as clearly characterized in our lab studies. Resistance ratios ($RR_{50}$s) to the pyrethroid bifenthrin at the four courses were 2, 30, 95, and 343.

To keep the size of experiments manageable, insecticide applications targeting adults were tested in separate experiments from those targeting larvae. Adulticides (Table 1) were applied at the optimal timing to control overwintered adults, i.e., when most adults have moved onto the short mown areas in spring but before females start laying eggs. Timing was determined by vacuum sampling of adults, degree day accumulation (base 50 °F) (120 $GDD_{50}$), and indicator plant phenology (forsythias half gold : half green). Larvicides (Table 1) were applied to target young larvae around late bloom of flowering dogwood (200 $GDD_{50}$) and mid-size larvae around full bloom of hybrid Catwba rhododendron, 400 $GDD_{50}$). Treatments were evaluated at around 700 $GDD_{50}$ when most developmental stages were around 5th instar.

For adulticides, we observed no interaction between resistance level and insecticides. Control at 2x and 30x resistance was higher than at 95x, and control was the lowest at 342x. All insecticides caused significant control but there were no differences among insecticides. At 2x and 30x, all insecticides except Dursban significantly reduced ABW populations; at 100x only Talstar caused significant reduction; and at 343x none of the insecticides caused significant reduction (Fig. 1, left).

For larvicides (Fig. 1, right), timing of application did not affect efficacy of Acelepryn, Ference, and Arena. Resistance level and insecticide interacted significantly. Ference was not affected by resistance level; Conserve only showed a difference between 30x and 343x, but not between any other timings; and all other insecticides were significantly affected by resistance, although, due to high variability in the data, not always consistently. Repeating these experiments should further clarify the effects of resistance on the various insecticides. However, the findings already indicate that Ference might be the most effective insecticide against highly resistant ABW population followed by Conserve. Provaunt seems to be the only other insecticides that does not seem to be affected up to the 95x level, but it is completely ineffective at the 343x level.

- Talstar, Dursban, Provaunt, and Conserve efficacy against ABW adults declines with pyrethroid-resistance level, starting around the 95x resistance level, and they are completely ineffective against highly resistant populations.
- Dursban is not an effective replacement for pyrethroids.
- Ference and Conserve as larvicides appear to be unaffected by resistance to date.
- Provaunt is effective up to the 95x level but completely ineffective against highly resistant ABW larvae.
- Acelepryn, Arena, and Dylox are strongly affected by resistance starting around the 95x level.

**Table 1**. Insecticides tested against adults (Ad) and young (L1-2) and older (L2-4) ABW larvae.

| Insecticide class | Active ingredient | Trade name | Rate (lb ai/ac) | Targets |
|---|---|---|---|---|
| Pyrethroid | Bifenthrin | Talstar | 0.100 | Ad |
| Organophosphate | Chlorpyrifos | Dursban | 1.000 | Ad |
| | Trichlorfon | Dylox | 6.000 | L3-4 |
| Spinosyn | Spinosad | Conserve | 0.400 | Ad, L2-4 |
| Oxadiazine | Indoxacarb | Provaunt | 0.225 | Ad, L2-4 |
| Anthranilic diamide | Chlorantraniliprole | Acelepryn | 0.156 | L1-2, L2-4 |
| | Cyantraniliprole | Ference | 0.156 | L1-2, L2-4 |
| Neonicotinoid | Clothianidin | Arena | 0.247 | L1-2, L2-4 |

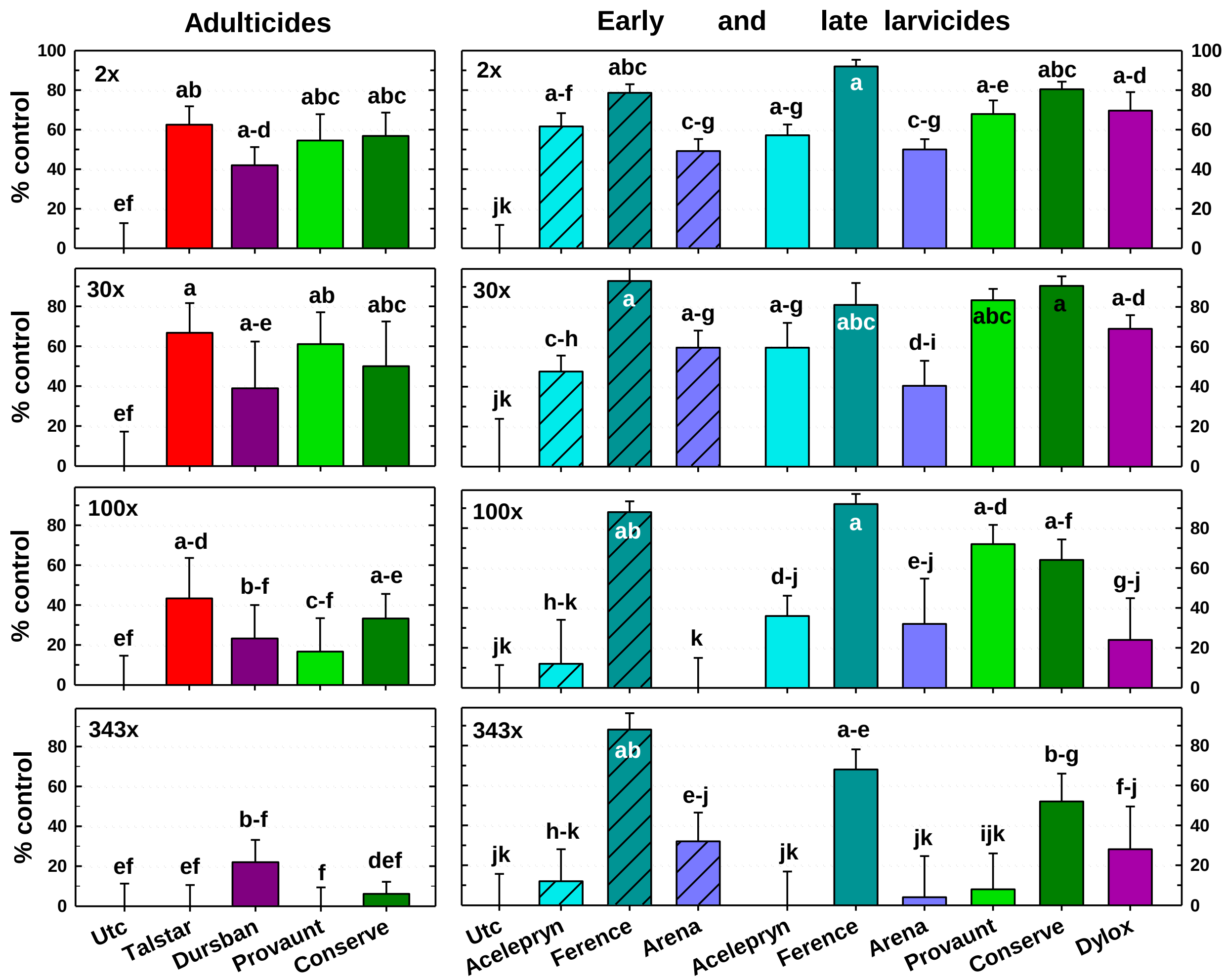


Fig. 1. Effect of pyrethroid resistance level (2x, 30x, 100x, 343x) on control of annual bluegrass weevil developmental stages in early June (peak 4th to 5th instar) in golf course fairways treated in spring with adulticides at peak densities of overwintered adults (forsythias half gold : half green), with early larvicide targeting young larvae (late bloom dogwood), or with late larvicides targeting mid-size larvae (full bloom rhododendron). Means within the four left panels combined and the four right panels combined, respectively, with the same letter did not differ significantly ($P > 0.05$).

**USGA Turfgrass and Environmental Research Grant**
**2016 Summary – Year-End Report (1st year)**

**Project Title**: Effects of mowing height and nitrogen fertilization on annual bluegrass weevil oviposition, larval development, and turfgrass damage

**Principal Investigator**: Dr. Benjamin A. McGraw

**University**: Pennsylvania State University

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**Objective 1**: Determine the effects that putting green mowing heights have on ABW adult survival, diel activity, larval growth and development, and turfgrass damage

**Objective 2**: Determine the impacts that early-season N fertility regimes have on adult preference and larval development; characterize the interactions between mowing height and fertility on larval abundance and turfgrass damage expression

**Start Date:** 2016
**Project Duration:** Three years
**Total Funding:** $ 45,000

**Introduction:**

Putting green mowing height had a significant effect on adult removal in greenhouse trials. The lowest treatment (2.5 mm or 0.100") removed approximately 40% of the adults in both years of the study (Fig. 1). However, adult removal greatly diminished as mowing height increased. Removal was minimal at medium (3.2 mm/0.125" = 7- 13%) and high (3.8 mm/0.150" = 2- 7%) green heights, as well as collar- and fairway-treatments (< 1%). In both years of the study, most of the adults (96% +) survived mowing. This suggests that attention must be given to where clippings are disposed to minimize the potential for weevils to reinvade high-valued turf areas.

Laboratory and field experiments determined that ABW females readily lay eggs in green-height turf and larvae are capable of developing to damaging stages. In 2015, significantly more eggs were laid in the low- and medium-height treatments in no-choice assays (Fig. 2). However, we observed a deviation from normal egg laying behavior. Most eggs were deposited loosely, rather than under the leaf sheath or in the stem of the plant (Fig. 3). Significantly more loose eggs were observed as mowing heights decreased. Despite ovipositional differences, mowing height did not have a significant effect on either larval abundance (4th instar – pupa) or larval fitness (5th instar weight).

Observational studies were conducted to determine periods when adults were most active on top of the turfgrass canopy and therefore most susceptible to removal. Time-lapse (laboratory) or modified-still photography (field) was used to describe adult vertical activity with UV- or fluorescent-marked weevils. No distinct patterns were detected in laboratory experiments when holding weevils at constant temperatures. However, activity was significantly increased when temperatures were between 15 and 25° C (59 ° – 77° F). Adult vertical movement in the field was largely influenced by temperature (Fig. 4.). Activity on top of the canopy was linearly correlated with temperatures in April and May. However, June observations showed that weevil activity was negatively correlated with temperatures, as weevils moved below the canopy when temperatures exceeded 20° C (69° F). A second-order polynomial model developed from the combined data predicts that adult activity on top of the canopy is greatest between 14 and 18° C (57 and 64° F).

***Objective 2**: Determine the impacts that early-season N fertility regimes have on adult preference and larval development; characterize the interactions between mowing height and fertility on larval abundance and turfgrass damage expression*

ABW ovipositional preference and larval development was assessed for three early-season N-fertility regimes. In choice-assays, significantly more adults were found in high-N plots (48.8 kg N $ha^{-1}$ $mo^{-1}$ or 1 lb N $M^{-1}$ $mo^{-1}$) in 2015, but not in 2016 studies. However, we found significantly more eggs in the medium-N treatments (19.5 kg N $ha^{-1}$ $mo^{-1}$ or 0.4 lb N $M^{-1}$ $mo^{-1}$) in both years. This is the rate currently recommended for managing anthracnose (*Colletotrichum cereale*) in *P. annua* greens in the Northeast.

No significant differences were detected between N fertility treatments in the field for either late-instar larval (4th and 5th instars) or pupal densities. Although statistical differences were not detected, more larvae were recovered from the low-N treatment (4.9 kg N $ha^{-1}$ $mo^{-1}$ or 0.1 lb N $M^{-1}$ $mo^{-1}$). Additionally, larval fitness (as measured by 5th instar weight) was not affected by N-fertility treatment.

**<u>Future research (2017):</u>**

We have completed mowing studies relative to Objective 1. However, our laboratory will continue to conduct research in this area to validate the temperature model and investigate variables (e.g. double-cutting, brushing) to improve adult removal. Nitrogen fertilization and growth regulation studies will be conducted in 2017 to complete Objective 2.

<u>Bullet Points</u>:

1. Moderate percentages of ABW adults (~ 40%) were removed with a single, low mown treatment (2.5 mm or 0.100"). The effect of mowing on adult removal diminished with increasing mowing heights.

2. Most adults (> 96%) survived the act of mowing. No significant differences were detected between mowing treatments in the number of adults killed by the mower.

3. Females were capable of ovipositing into the lowest putting green heights, though more eggs were placed outside the turfgrass stem or sheath as mowing height decreased.

4. Adult activity on top of the turfgrass canopy was greatest when temperatures were between 14 and 18° C (57 and 64° F).

5. Significantly more eggs were collected from moderate fertility (0.1 lb N $M^{-1}$ $wk^{-1}$) treatments than low- or high-N treatments.

6. Larvae were capable of developing in all mowing height and fertility treatments. No significant differences in larval fitness were detected between treatments.

**Figure 1.** Effect of putting green mowing height on the removal of *L. maculicollis* adults in greenhouse studies using a bench-mounted reel mower (2015-16). Columns with the same letters (capital or lowercase) are not significantly different from one another at the α = 0.05 level).

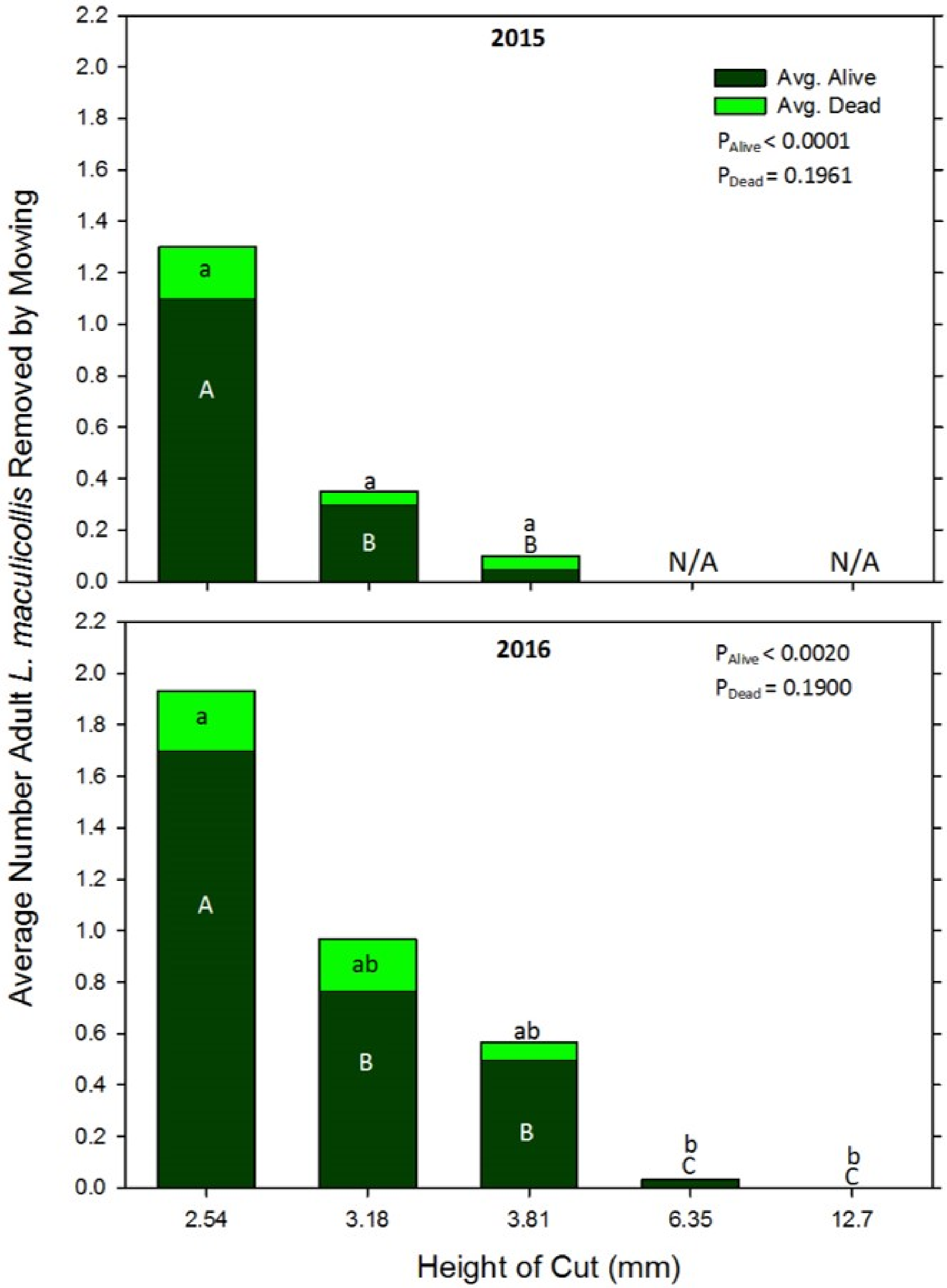


**Figure 2.** Number of *L. maculicollis* eggs oviposited by mowing height treatment in no-choice assays (2015-16). Columns within years with the same letters (capital or lowercase) are not significantly different from one another at the α = 0.05 level).

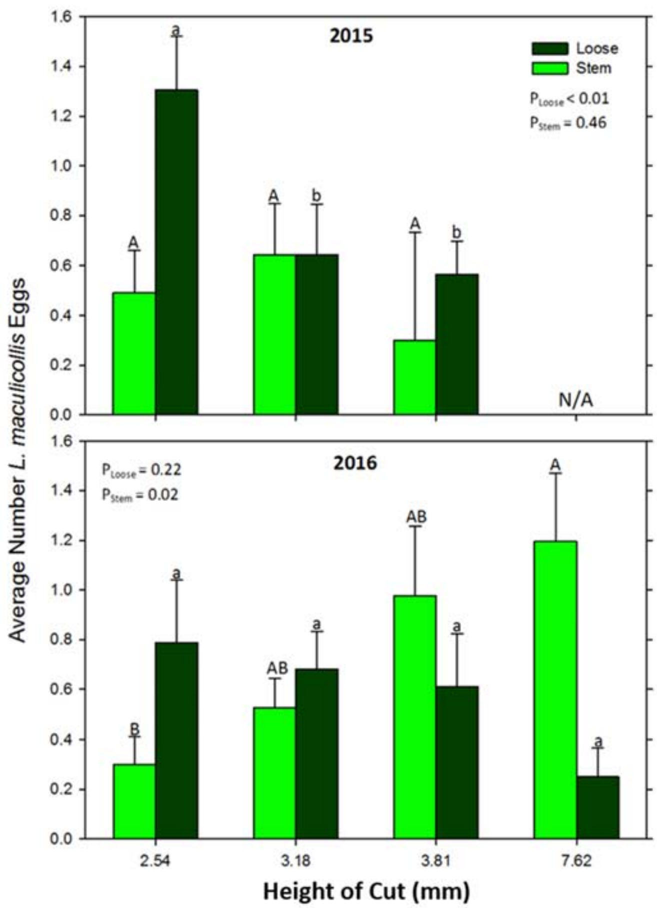
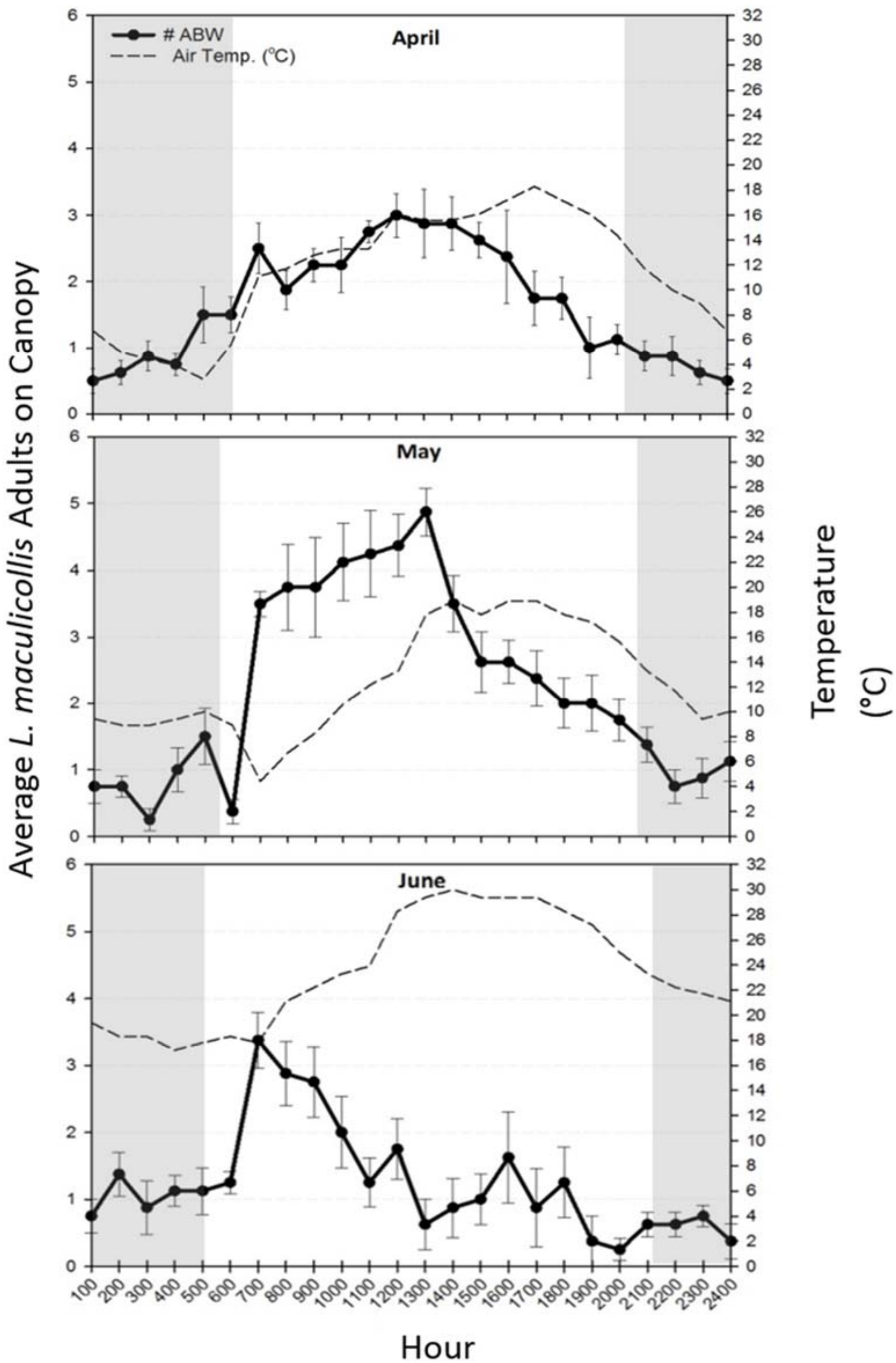



**Figure 3.** Differences in adult oviposition behavior was noted between putting green-height and higher turfgrasses. Left: eggs are deposited inside the stem of the plant at fairway-height (12.5 mm/0.500”). Right: Many of the eggs deposited in putting-green heights were loose or outside of the plant.

**Figure 4.** Average number of *L. maculicollis* adults on top of the canopy and temperature over three 24-hr field observations during spring. Still photographs of marked weevils were made hourly. Hours shaded in grey represent periods of darkness.



Annual report on USGA ID# 2015-08-523

Selection of Insecticides Applied at Different Timings for Control of Billbug Species on Zoysiagrass Fairways

Bruce A. Barrett and Xi Xiong, University of Missouri

Objectives: The overall objective is to evaluate insecticides for efficacy and appropriate application timing for the control of billbug damage on zoysiagrass fairways.

Billbug (*Sphenophorus* spp.), especially hunting billbug (*S. venatus vestitus* Chittenden), is an emerging problem in Missouri and the surrounding states in recent years that left untreated, causes severe damages to zoysiagrass (*Zoysia japonica* Steud.), a dominant grass species for golf course fairways in this region. Billbugs are small weevils with adults ranging 7-8 mm long (< 0.3 inches), and larvae are legless. The females lay eggs in the grass stems and once hatched, the larvae feed inside of the stem before they grow larger and then burrow down to the soil to feed on roots and crowns (Fig 1), sometimes as deep as 15 cm (6 inches) below the surface. The larvae are believed responsible for the major damages of the turf, although evidence shows that adult feeding leads to severe turf quality reductions as well. Presence of billbug easily goes undetected due to their smaller size, hiding/feeding site, and the adults' nocturnal nature. Without pitfall traps, damages caused by billbugs are often misdiagnosed as winter-kill, drought, white grubs or other stresses.

Field plots, established on a zoysiagrass fairway where billbug damages have been previously documented, were treated with insecticides included bifenthrin (Talstar®), deltamethrin (DeltaGard®), lambda-cyhalothrin (Scimitar®), and imidacloprid (Merit®) for control of adults, and clothianidin (Arena®) and thiamethoxam (Meridian®) for control of larvae, and chlorantraniliprole (Acelepryn®) for controlling both stages. Insecticides, alone or in combination, were applied in split-plot as single (May 5, 2016), or sequential (June 2, 2016) at the highest label suggested rates arranged in a RCBD with four replications. The whole-plot measured 5 × 10 ft with 10 ft boarder, and the sub-plot measuring 5 × 5 ft. Pitfall traps (total 128) were installed at the center of each sub-plot (Fig 2) and monitored each week. Weekly evaluations included turf quality and normalized difference vegetation index (NDVI), and billbugs counts to the species. All data were subjected to ANOVA using Proc Mixed in SAS 9.4.

In 2016, analysis of weekly turf quality evaluation revealed no significant interactions, and insecticide main effect revealed a similar trend compared to 2015 where all treated plots maintained the same or greater overall turf quality compared to the untreated control (Fig 3). Among treatments, no clear trend was found between application of pyrethroids, or neonicotinoids, or combinations of the two chemicals in turf quality responses. The top three treatments that resulted in greater overall turf qualities, however, all contained a pyrethroid which targets on adult billbugs. When data from both 2015 and 2016 were combined (data not shown), it appeared that treatments contained deltamethrin + thiamethoxam or bifenthrin alone resulted in greater turf quality consistently.

In 2016, hunting billbug population increased approximately 8 times compared to the population trapped in 2015. The total hunting billbugs trapped per pitfall trap over the growing season of 2016 were up to 42, compared to a maximal of 5 in the year of 2015. When weekly hunting billbug counts per trap was plotted over time (Fig 4), it clearly showed a peak adult activity in fall starting from late July to early September. Spring insecticide application in this study might have suppressed the spring adult peak, as evidence from other experiments where insecticides were withdrawn. However, the second peak in fall likely indicates hunting billbugs have two generations per year in our region, compared to bluegrass billbugs which appear to be one generation per year. This result also indicates that application in spring might not be sufficient to control the fall hunting billbug generation in our region.

When analyzed by each week, insecticide treatment effect in hunting billbug counts were only effective on May 23, July 29, and August 10, 2016 (Fig 5). Plots maintained relatively greater turf quality did not appear to show reductions in billbugs counts. Since adult billbug crawls over a significant distance, it is likely that the billbugs trapped in a particular pitfall trap could be from distant plots. Additionally, plots that showed a relatively lower turf quality, such as control and plots received imidacloprid + Meridian, also showed a relatively reduced number of billbug counts.

Summary:

- Hunting billbugs in Missouri likely have 2 generations per year with the second adult activity peak in August;
- Insecticide treatment containing pyrethroid, such as deltamethrin+ thiamethoxam and bifenthrin alone, appeared to provide better turf quality over the two-year period;
- Future research will focus on determination of optimal insecticide application timing likely involves fall applications targeting on adults.

**Fig 1.** Billbug larvae feeding at the thatch/soil interface (left) and the affected zoysiagrass shoots which can be easily pulled off from the ground (right). Photograph was taken on Oct 26, 2016.

**Fig 2.** Field plots established on a ‘Meyer’ zoysiagrass (*Z. japonica*) fairway at a local Golf Course, Columbia, Missouri. The pitfall traps were established in the center of each sub-plot and remained underground year round.

**Fig 3.** Turf quality (1-9) influenced by insecticide main effect in 2016. There were no interactions between insecticide and application timing, or between insecticide and evaluation timing; hence data were pooled over a 17-week period. Bars labeled with the same letters were not significantly different based on Fishers' Protected LSD at 0.05 level.

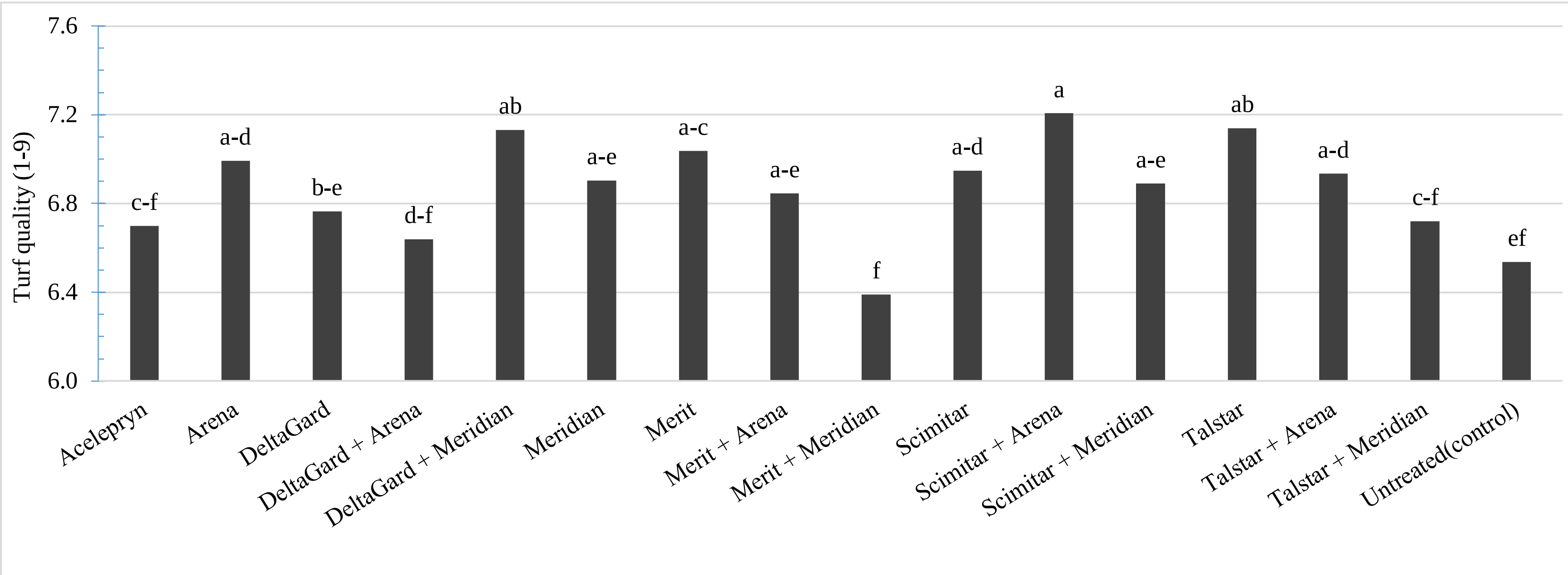


**Fig 4.** Average hunting billbug counts per pitfall trap fluctuated over the growing season in 2016. No significant interaction of weekly counts by other factors were found; hence hunting billbug count at each week were pooled across all treatments.

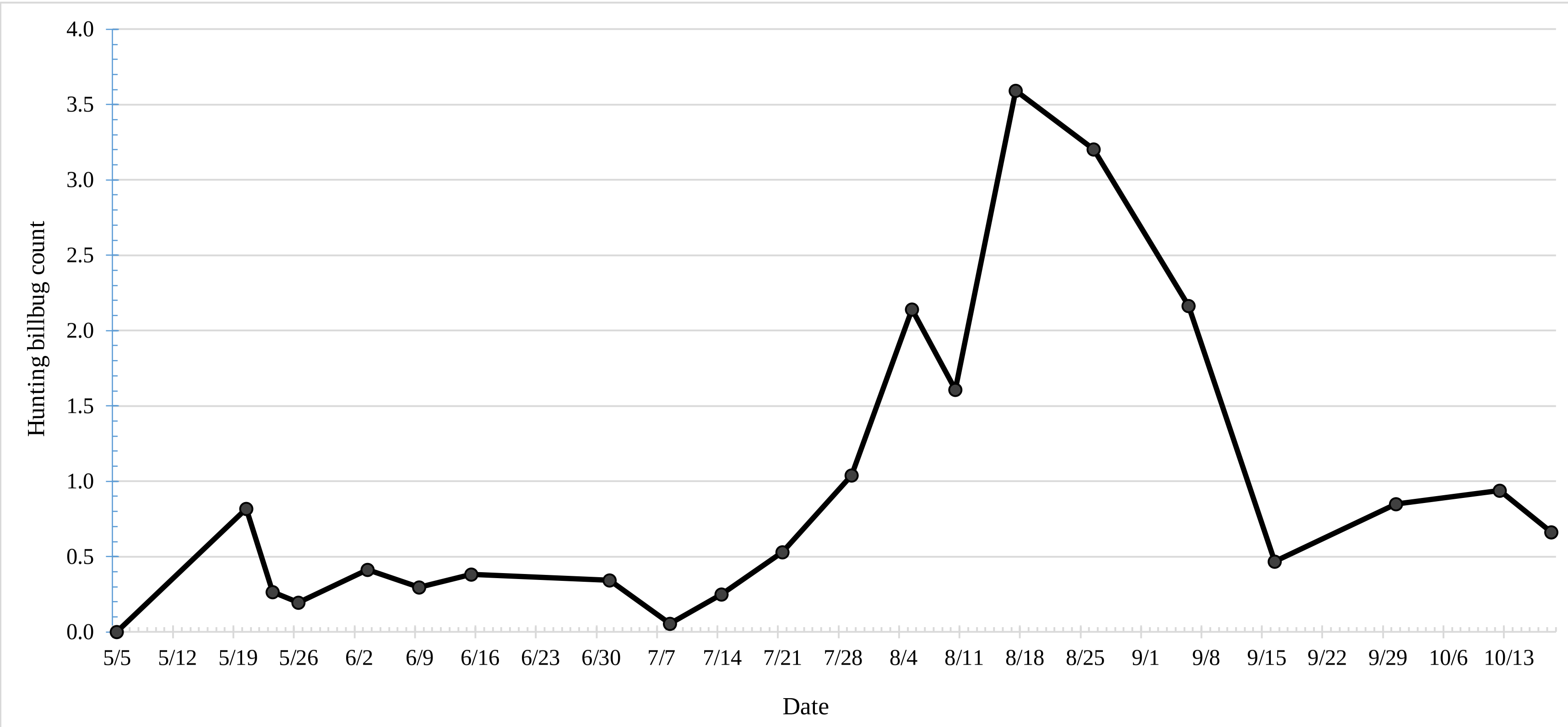
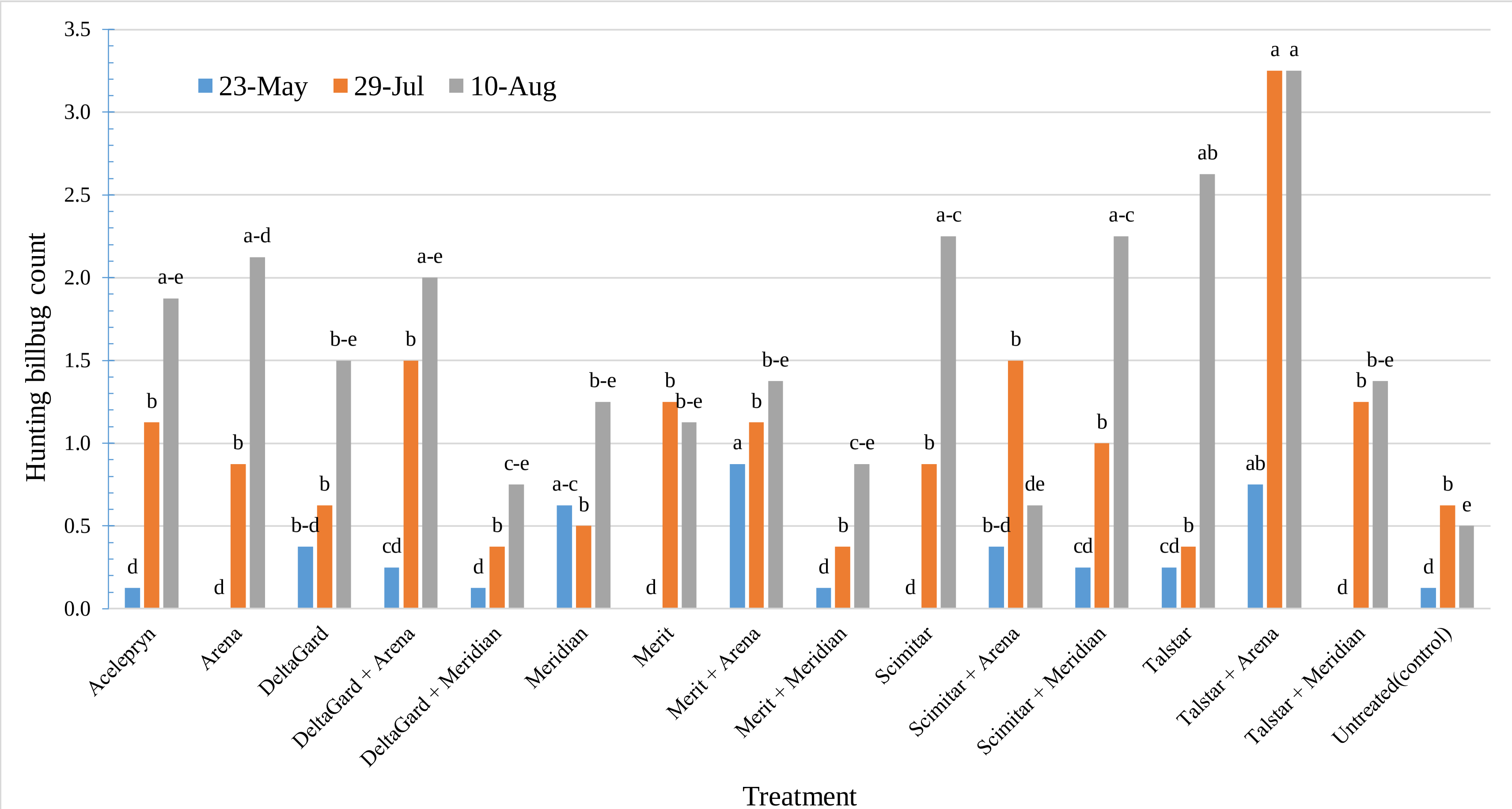


**Fig 5.** Treatment effect on hunting billbug counts evaluated on May 23, July 29, and August 10, 2016, which corresponded to 7, 12 and 14 weeks after the initial treatment application, respectively. Number of application did not influence hunting billbugs; hence insecticide main effect were presented at each evaluation time. Bars at each evaluation timing labeled with the same letters were not significantly different based on Fishers' Protected LSD at 0.05 level.



**Understanding Billbug Chemical Communication to Improve Management**

Douglas S. Richmond, Matthew D. Ginzel and Alexandra G. Duffy
Department of Entomology, Purdue University
USGA ID#: 2015-10-525

**Objectives:**

1) Characterize the response of bluegrass billbug and the hunting billbug to a known billbug aggregation pheromone ((S)-2-methyl-4-octanol).
2) Identifying species-specific volatile attractants that may be useful for monitoring and management.
3) Clarify the role of cuticular hydrocarbons in close-range mate recognition.

**Start Date:** 2015
**Project Duration:** 2 Years
**Total Funding:** $59,202

**Summary Points**

- Although neither hunting billbug nor bluegrass billbug adults were attracted to the putative aggregation pheromone (S)-2-methyl-4-octanol under field conditions, Y-tube olfactometry assays revealed that hunting billbug males are attracted to grass host volatiles while females responded to volatiles from male billbugs.

- Findings suggests that female billbugs may be responding to a male-produced volatile sex pheromone, the structure of which has not yet been determined.

- The cuticles of hunting and bluegrass billbug are coated with a series of aliphatic hydrocarbons that differ both between species and between sexes of a given species.

- Differences in cuticular hydrocarbon profiles could provide the basis for mate recognition between billbug species.

- Once the absolute configuration of both volatile and tactile chemical cues has been clarified, it may be possible to incorporate this knowledge into monitoring and management programs designed to manipulate the host- and mate-finding behavior of billbugs associated with turfgrass.

**Summary**

Behavioral chemicals, called semiochemicals, mediate insect behavior at multiple spatial scales. Semiochemicals that are active over longer distances include host-plant volatiles, sex pheromones or aggregation pheromones that are typically olfactory-perceived. These volatile stimuli are used by insects to find hosts, potential mates, or other insects of the same species. Close-range semiochemicals associated with mating behavior are typically non-volatile, long-chain cuticular hydrocarbons on the insect surface (Thornhill & Alcock 1983) and are usually perceived through antennal contact. Despite the fact that sequential use of both volatile and contact semiochemicals for reproductive success has been documented in several insect species (Guarina et al. 2008, Hughes et al. 2015, Eliyahu 2008), the role of semiochemicals in directing the host- and mate-finding behavior of the billbug species complex associated with turfgrass has never been examined.

The long-term goal of this research is to understand the role of semiochemicals in orchestrating the host- and mate-finding behavior of billbugs associated with golf course turf. Our research efforts focused on characterizing the response of bluegrass and hunting billbugs to a known weevil aggregation pheromone (2-methyl-4-octanol) and determining the potential for semiochmicals to influence billbug dispersal and mate finding behavior. Efforts to understand these cues could lead to the development of novel, biologically-based monitoring and management strategies designed to manipulate billbug behavior.

To determine the extent to which hunting and bluegrass billbugs were attracted to the putative synthetic aggregation pheromone (2-methyl-4-octanol) (Zarbin et. al 2003), paired pitfall traps were monitored at several sites in West Lafayette, Indiana. One pitfall trap in each pair was baited with the pheromone lure. The number of males and females of each species captured in each trap was recorded. Because our results indicated that the number of captured billbugs of either species or sex did not differ between baited and un-baited traps at any time during the experiment, a binary choice y-tube olfactometry assay was designed to examine the attractiveness of several other biologically relevant odor sources.

Y-tube treatments were comprised of five combinations of hunting billbugs and above-ground Bermudagrass host-plant material that were all compared to a purified air control (Fig. 1). The response of hunting billbugs to the different odor sources varied depending on sex. Males positively responded to treatments containing Bermudagrass, but not to males or females of the same species (Fig. 2). In contrast, females positively responded to treatments containing males of the same species, but did not orient toward treatments containing only host-plant material or other females.

To examine the potential for cuticular hydrocarbons to mediate close-range mating behavior, we performed a short series of laboratory mating assays, and analyzed the cuticular hydrocarbons of male and female hunting and bluegrass billbugs. Because our mating assays with hunting billbug revealed behaviors consistent with the presence of behaviorally active cuticular compounds (Fig. 3), whole body hexane extracts were characterized using gas chromatography/mass spectrometry. Results confirmed qualitative and quantitative chemical differences in the hydrocarbon profiles between hunting and bluegrass billbugs, and quantitative differences between the sexes of both species (Table 1).

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**Figure 1.** Y-tube olfactometry bioassays were used to characterize the response of hunting billbugs to plant and insect volatile organic compounds that may help billbugs locate both host-plants and mates.

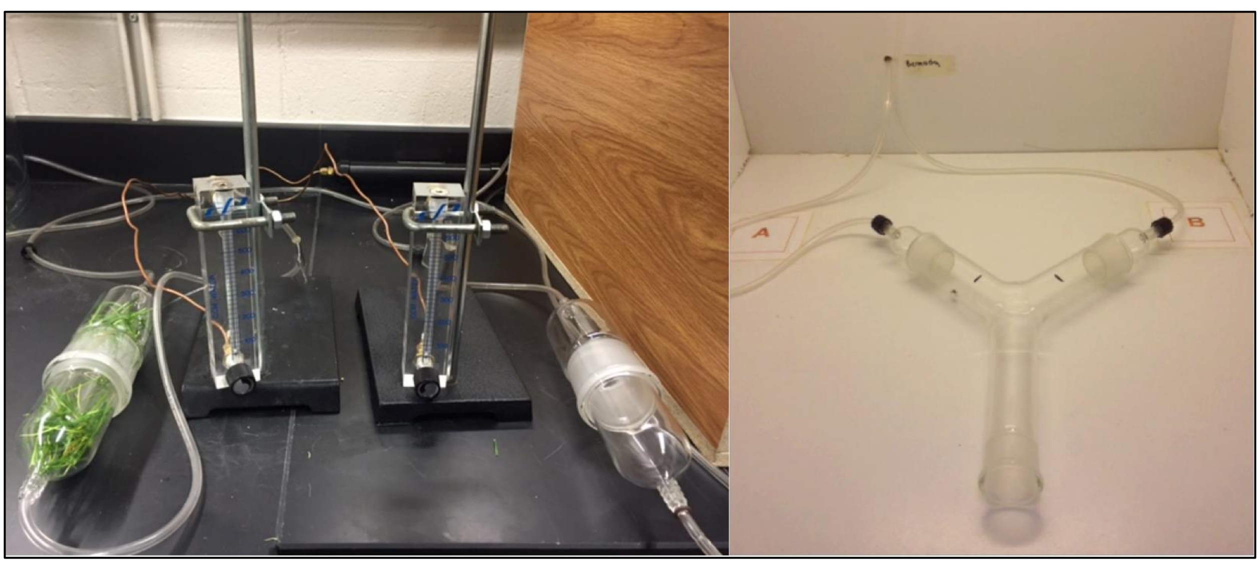


**Figure 2.** Percentage of hunting billbug adults responding to five combinations of other billbugs of the same species and/or host-plant material vs. purified air in a y-tube olfactometer bioassay. *P<0.05, **P<0.01 (Observed vs. Expected Chi-Square).

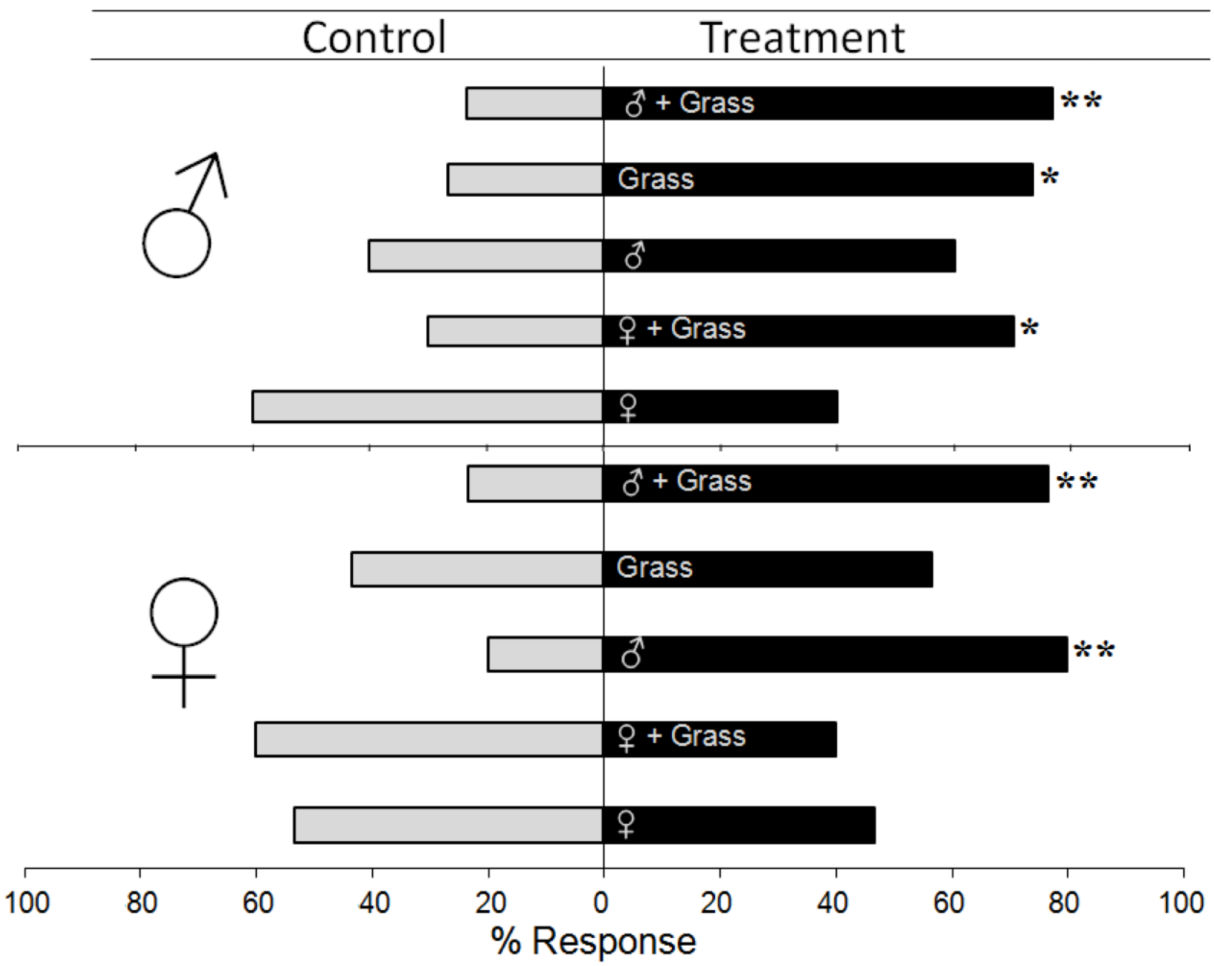


**Figure 2.** Because billbugs frequently occur as a complex of several closely-related species that overlap geographically and temporally, tactile chemical signals may be used to facilitate close-range mate recognition.

**Table 1.** Relative differences in the cuticular hydrocarbon profiles of hunting and bluegrass billbugs.

| Peak #[a] | Retention Time (min) | Relative Proportion of Total Hydrocarbons[b] | | | |
|---|---|---|---|---|---|
| | | Hunting Billbug | | Bluegrass Billbug | |
| | | Female | Male | Female | Male |
| 1 | 22.35 | a | a | a | a |
| 2 | 22.91 | ab | ab | b | a |
| 3 | 23.11 | a | a | a | a |
| 4 | 23.63 | ND | ND | b | a |
| 5 | 23.85 | ab | b | a | ab |
| 6 | 24.48 | b | a | c | b |
| 7 | 24.60 | a | a | a | a |
| 8 | 25.18 | a | a | a | a |
| 9 | 25.26 | ND | ND | a | b |
| 10 | 25.33 | ND | ND | a | b |
| 11 | 25.45 | a | a | a | a |
| 12 | 27.3 | a | a | b | b |
| 13 | 27.36 | a | a | ND | ND |
| 14 | 27.40 | ND | ND | a | a |
| 15 | 27.53 | ND | ND | a | a |
| 16 | 27.66 | a | a | a | a |

[a] Peaks correspond with individual compounds identified through GC/MS.
[b] Differences in the relative abundances of cuticular hydrocarbons of hunting billbug males (n=10) vs. females (n=10) and bluegrass billbug males (n=8) vs. females (n=5) were tested with MANOVA followed by mean separation using Tukey's HSD test. Cells in the same row that contain the same letter are not significantly different (α<0.05). ND=not detected.

2015-09-524

**Title:** Biological control of black cutworm in turf with baculovirus - 2016

**Project Leader:** Robert Behle (USDA-ARS-NCAUR, 1815 N. University Ave., Peoria, IL)

**Co-Investigator**: Doug Richmond (Purdue University, Department of Entomology, West Lafayette, IN)

**Objectives**:

1) Determine effective application rates and formulations of the virus required for control of larvae,
2) Compare baculovirus treatments with alternative control treatments when applied under field conditions,
3) Evaluate compatibility of virus applications with integrated management strategies for pest control within the golf-turf environment.

**Start Date:** Spring 2015

**Project Duration:** 3

**Total Funding:** $60,000

**Bullet Points**

Young black cutworm larvae remain susceptible to infection by baculovirus for five days after hatching (prior to reaching the fourth instar of development), suggesting that recurring weekly applications may provide effective control of this pest. (Figure 2)

Applications of baculovirus occlusion bodies (OBs) to field grown bentgrass degrade rapidly, especially when applied at low rates (<$1.6 \times 10^{11}$ OBs/A), thus limiting pest control efficacy. (Figure 3)

Weekly treatments of field grown bentgrass managed at putting green height provided consistent control of small and medium sized larvae when the baculovirus was applied at high "label" recommended rates ($1.6 \times 10^{11}$ OBs/A), but did not provide improved efficacy after three weekly applications. (Table 1)

After artificial infestations of small and medium sized larvae, virus treatments at the higher rates significantly reduced feeding damage to bentgrass, although the slow speed of kill allowed some damage to occur.

**Summary text:**

Research continues to hone in on the requirements needed to develop the baculovirus, *Agip*MNPV, as a biological insecticide for control of black cutworm (BCW) larvae in turf. This year, field and laboratory experiments evaluated application rates, multiple applications, caterpillar susceptibility, and residual insecticidal activity. The unifying premise of the experiments was to determine if multiple applications of virus would be capable of providing season long control of cutworm larvae infesting managed turfgrass.

*Larval susceptibility to virus infection*

For most insects, susceptibility to infection by pathogens decreases as development proceeds through the larval stages (instars). In the laboratory, larvae completed three instars in 5 days and remained highly susceptible to virus infection. Beginning at 6 days, larvae entered the fourth instar and required significantly more virus to initiate infection (Figure 1). The high susceptibility of small larvae suggests that applications at 5-day intervals could effectively control larvae hatching from a continuous infestation.

*Evaluations for residual insecticidal activity*

Treatment intervals could be extended if the virus remains active for an extended time period after application. Unfortunately, insecticidal activity decreased rapidly for low and medium application rates ($\geq$1.6 x $10^6$ OBs/A) to field grown bentgrass, which lost about 50 % of the original activity by two days after application (Figure 2). The high application rate (1.12 x $10^{12}$ OBs/A) maintained insecticidal activity with over 90% larval mortality for two days after application.

*Virus source – commercial samples vs laboratory samples*

Andermatt Biocontrol (Grossdietwil, Switzerland) is in the process of developing an *Agip*MNPV biopesticide for control of black cutworm and provided a test sample of product (Exilon) for evaluation. In preliminary laboratory experiments, the commercial sample provided equivalent insecticidal activity with freshly produced virus (NCAUR). This was the first indication to support the application of either commercial or laboratory produced samples in the following experiments.

*Field efficacy experiments – Purdue University*

Three weekly application of virus treatments were sprayed on field plots of creeping bentgrass that was maintained at 3/16 inch height and managed as a golf green to determine if repeated applications would build up residue of virus and improve control of cutworms. All treatments (low and medium rates; Exilon and NCAUR virus sources) were applied as aqueous sprays at 2 gallons/1000 $ft^2$. After applications dried, PVC cages (8 inch diameter) were installed in plots and artificially infested with neonates or five $2^{nd}$- $3^{rd}$ instar BCW larvae. Larvae were allowed to feed within the cages for seven days before being flushed from the turf using a standard soapy water solution. Larval survival in treated plots was compared with larval survival in untreated control plots and plots treated with a chemical standard, Acelepryn SC (chlorantraniliprole). Larvae counts and plant damage estimates are reported in Tables 1 and 2, respectively.

Exilon and NCAUR sources of virus provided similar levels of control and medium application rates provided better control than lower application rates. None of the virus treatments matched the level of control provided by the chemical insecticide. Lower levels of control by virus applications were expected because medium and low rates of virus were applied in an effort to observe improvements in pest control due to repeated applications to the same plots. Unfortunately this benefit was not observed.

**Table 1.** Mean (±SE) number and percent control of black cutworm neonates and 2nd and 3rd instar larvae in plots of creeping bentgrass treated with different formulations (NCAUR and Exilon) and rates of baculovirus (*Agip*MNPV) or Acelepryn SC (chlorantraniliprole). Two hundred eggs and five 2nd and 3rd instar larvae were placed on plots at 8d intervals with treatments being applied at the beginning of each interval. The number of surviving larvae was recorded 7d after each application. Three applications were made and infestations were created on August 2, August 10, and August 18, 2016. Black cutworm larval populations were assessed on August 9, August 17, and August 25. 2016.

| Treatment | Rate (ml or g/A) | Survival Interval 1 | % Control Interval 1 | Survival Interval 2 | % Control Interval 2 | Survival Interval 3 | % Control Interval 3 |
|---|---|---|---|---|---|---|---|
| Neonates | | | | | | | |
| Untreated | | 130.5± 9.7ab | 0.0 | 197.5± 1.7a | 0.0 | 147.8±32.8a | 0.0 |
| Exilon | 320.0 ml | 81.0±16.4b | 37.9 | 148.3±11.2b | 24.9 | 48.3± 8.3cd | 67.3 |
| Exilon | 44.0 ml | 103.5±17.3ab | 20.7 | 193.0±14.3a | 2.3 | 88.8±17.4b | 39.9 |
| NCAUR | 72.7 g | 72.6± 8.9b | 44.4 | 104.8± 8.2c | 46.9 | 27.0±11.0de | 81.7 |
| NCAUR | 10.0 g | 94.5± 5.0ab | 27.6 | 144.8±12.5b | 26.7 | 76.5± 4.9bc | 48.2 |
| Acelepryn | 354.0 ml | 0.0± 0.0c | 100.0 | 0.0± 0.0d | 100.0 | 0.0± 0.0e | 100.0 |
| 2nd & 3rd Instar | | | | | | | |
| Untreated | | 4.5±0.3ab | 0.0 | 4.0±1.3a | 0.0 | 2.8±0.6ab | 0.0 |
| Exilon | 320.0 ml | 1.3±0.8cd | 71.1 | 1.3±0.bc | 67.5 | 2.3±0.9ab | 17.9 |
| Exilon | 44.0 ml | 4.8±0.5a | 0.0 | 2.8±0.8ab | 30.0 | 3.8±0.5a | 0.0 |
| NCAUR | 72.7 g | 2.0±0.9c | 55.6 | 0.5±0.5c | 87.5 | 1.3±1.3bc | 53.6 |
| NCAUR | 10.0 g | 4.0±0.4ab | 11.1 | 1.0±0.7c | 75.0 | 2.5±0.9a | 10.7 |
| Acelepryn | 354.0 ml | 0.0±0.0d | 100.0 | 0.0±0.0c | 100.0 | 0.0±0.0c | 100.0 |

*Numbers in same column followed by different letters are significantly different at α=0.05

**Table 2.** Mean (±SE) percent defoliation by black cutworm neonates and 2nd and 3rd instar larvae in plots of creeping bentgrass treated with different formulations (NCAUR and Exilon) and rates of baculovirus (*Agip*MNPV) or Acelepryn SC (chlorantraniliprole). Two hundred eggs and five 2nd and 3rd instar larvae were placed on plots at 8d intervals with treatments being applied at the beginning of each interval. Three applications were made and infestations were created on August 2, August 10, and August 18, 2016. Defoliation by black cutworm larvae was assessed on August 9, August 17, and August 25. 2016.

| Treatment | Rate (ml or g/A) | % Defoliation Interval 1 | % Defoliation Interval 2 | % Defoliation Interval 3 |
|---|---|---|---|---|
| | | Neonates | | |
| Untreated | | 23.8±4.7b | 57.5±11.1ab | 27.5±4.3a |
| Exilon | 320.0 ml | 17.5±4.3bc | 30.0± 7.1b | 16.3±3.1ab |
| Exilon | 44.0 ml | 32.5±8.5a | 73.8±14.6a | 32.5±8.3a |
| NCAUR | 72.7 g | 22.5±4.3b | 46.3±12.8ab | 21.3±4.3ab |
| NCAUR | 10.0 g | 25.0±3.5b | 46.3±14.0ab | 22.5±1.4a |
| Acelepryn | 354.0 ml | 0.0±0.0c | 3.8± 1.3c | 0.0±0.0b |
| | | 2nd & 3rd Instar | | |
| Untreated | | 46.3±7.5a | 40.0±17.3a | 51.3±5.5a |
| Exilon | 320.0 ml | 21.3±4.7b | 11.3± 3.8b | 23.8±3.8c |
| Exilon | 44.0 ml | 48.8±5.2a | 17.5± 1.4b | 51.3±5.2a |
| NCAUR | 72.7 g | 32.5±7.5ab | 12.5± 6.0b | 30.0±4.6bc |
| NCAUR | 10.0 g | 42.5±7.5a | 15.0± 8.4b | 42.5±4.8ab |
| Acelepryn | 354.0 ml | 0.0±0.0c | 2.5± 1.4b | 0.0±0.0d |

*Numbers in same column followed by different letters are significantly different at α=0.05

**Figure 1.** Plots of creeping bentgrass with pvc cylinders used to create artificial infestations of black cutworm neonates and 2nd & 3rd instar larvae for the experiment.

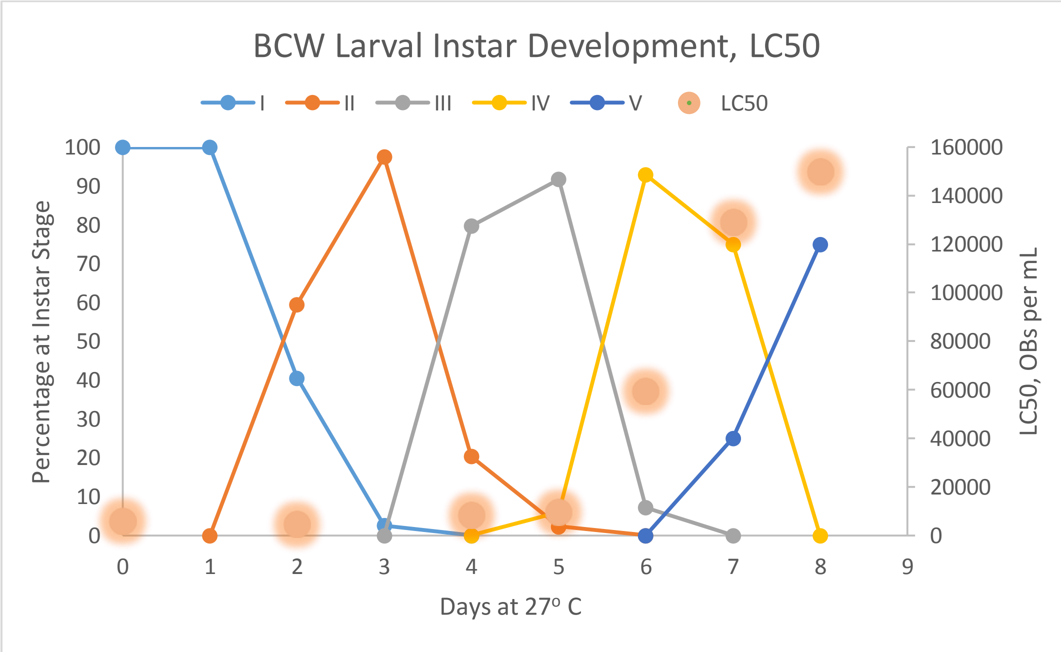



Figure 2. Development for black cutworm larvae and their susceptibility to infection by baculovirus (LC50) showing high susceptibility (low LC50 values) to virus through 5 days of growth (instars I, II, and III).

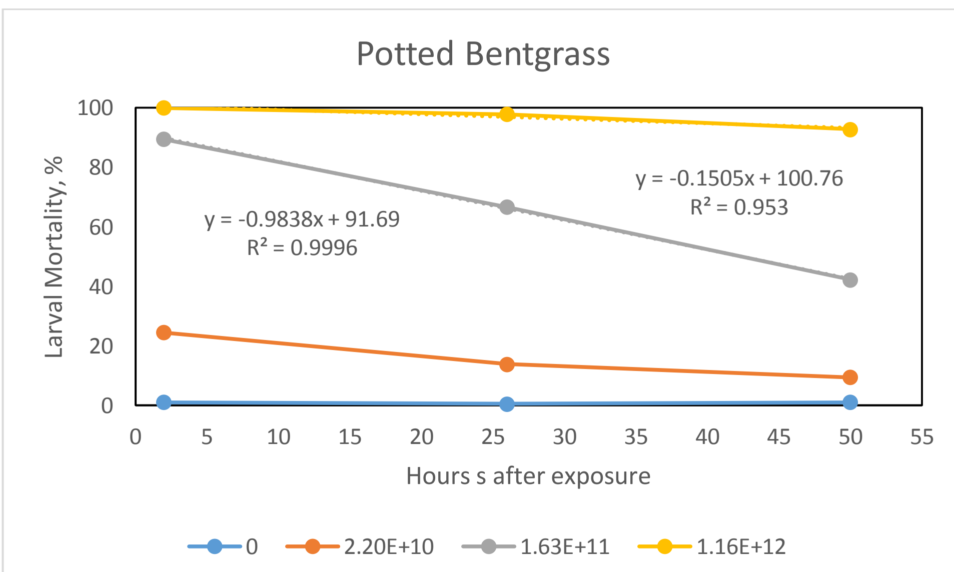



Figure 3. Loss of insecticidal activity based on larval mortality for four rates of *Agip*MNPV (0, $2.20 \times 10^{10}$, $1.63\times 10^{11}$, $1.16\times 10^{12}$ OBs/A) applied to bentgrass after exposure to 2, 26, or 50 hours of natural weather conditions, measured as decreasing mortality of neonate black cutworm when exposed to treated bentgrass growing in pots.

## Sand Topdressing Effects on Earthworm Activity in Warm-Season Golf Course Turfgrass

Mary C. Savin, Michael D. Richardson, Douglas E. Karcher and Paige E. Boyle

As earthworms burrow, they ingest soil and organic matter and excrete casts within the soil profile or on the soil surface. Typically, earthworms are viewed as beneficial; however earthworm surface casting in low-cut turfgrass systems can create muddy, inconsistent playing surfaces, increase water retention, result in disease, weed and pest invasion, and reduce photosynthesis and overall aesthetics. Pesticide use for earthworm control is illegal in the United States, so turfgrass managers must implement cultural practices to reduce surface casting. Sand topdressing is a cultural practice used on golf courses to reduce organic matter buildup and to provide a smooth, consistent playing surface. Since sand is abrasive against the soft-bodied earthworms, sand topdressing has been suggested as one method of earthworm casting control.

In an ongoing research trial at our location, significantly more earthworm casts have been observed in zoysiagrass and bermudagrass plots receiving heavy sand topdressing compared to light topdressing, regardless of turf establishment method, use or absence of core aeration, or root zone. The objective of this research is to quantify the effect of rootzone soil texture and long-term sand topdressing rates on earthworm casting activity in simulated golf course tee boxes.

### Methods

This experiment was conducted at the University of Arkansas Agricultural Research and Extension Station, Fayetteville, AR, and consisted of simulated golf course tee boxes established in 2008 with *Cynodon dactylon* L. 'Patriot' maintained at a height of 1.3 cm. The treatments included two rootzone treatments, including the native Captina silt loam soil (Typic Fragiudult) and a sand-capped system (15 cm of USGA sand) over the native silt loam soil. In addition, two topdressing treatments were also imposed over the rootzones, including light topdressing (0.64 cm $yr^{-1}$) and heavy topdressing (2.54 cm $yr^{-1}$) treatments.

Cast counts (no. m-2) were conducted weekly throughout the growing season. Volumetric moisture content and temperature (data not shown) were measured weekly at a 7.5 cm depth using a TDR-300 (Spectrum Technologies, Aurora, IL) and a handheld Checktemp probe (Hanna Instruments, Woonsocket, RI), respectively. The experimental design of the study was a two-factor (rootzone and topdressing) randomized complete block. Month was included as a factor in a repeated measures analysis model in PROC MIXED (SAS v. 9.4, SAS Institute, Inc., Cary, NC).

### Results

There was a significant three way interaction between rootzone, topdressing, and month on earthworm casting activity (Table 1). Peak casting activity, regardless of rootzone, occurred in the fall and spring (Fig. 1). The sand rootzone had significantly greater casting activity compared to the soil rootzone (Fig. 1). Heavy topdressing resulted in significantly greater casting activity compared to light topdressing in the soil rootzone, but there was no significant difference in casting activity between topdressing treatments in the sand rootzone (Fig. 2). Preliminary data suggest that the predominant earthworm species present in the experimental area are likely native *Diplocardia* species. In addition, a survey of golf courses in the Arkansas-Oklahoma region is ongoing to determine earthworm species composition and Shannon-Weaver density, and diversity.

**Table 1.** Analysis of variance showing main effects and interaction of rootzone, topdressing, and month on casting activity at the University of Arkansas Agricultural Research and Extension Station, Fayetteville, AR

| Effect | DF | F value | *Pr* > F |
|---|---|---|---|
| Rep | 3 | 0.32 | 0.8141 |
| Rootzone | 1 | 3.23 | 0.1702 |
| Topdressing | 1 | 10.64 | 0.0172 |
| Rootzone*Topdressing | 1 | 3.49 | 0.1109 |
| Month | 36 | 32.57 | <.0001 |
| Rootzone*Month | 36 | 8.05 | <.0001 |
| Topdressing*Month | 36 | 7.09 | <.0001 |
| Rootzone*Topdressing*Month | 36 | 5.57 | <.0001 |

**Fig. 1**. Monthly average of weekly cast counts by rootzone between November 2015 and June 2016. Error bar represents the least significant difference ($P$=0.05) and can be used to compare treatment means.

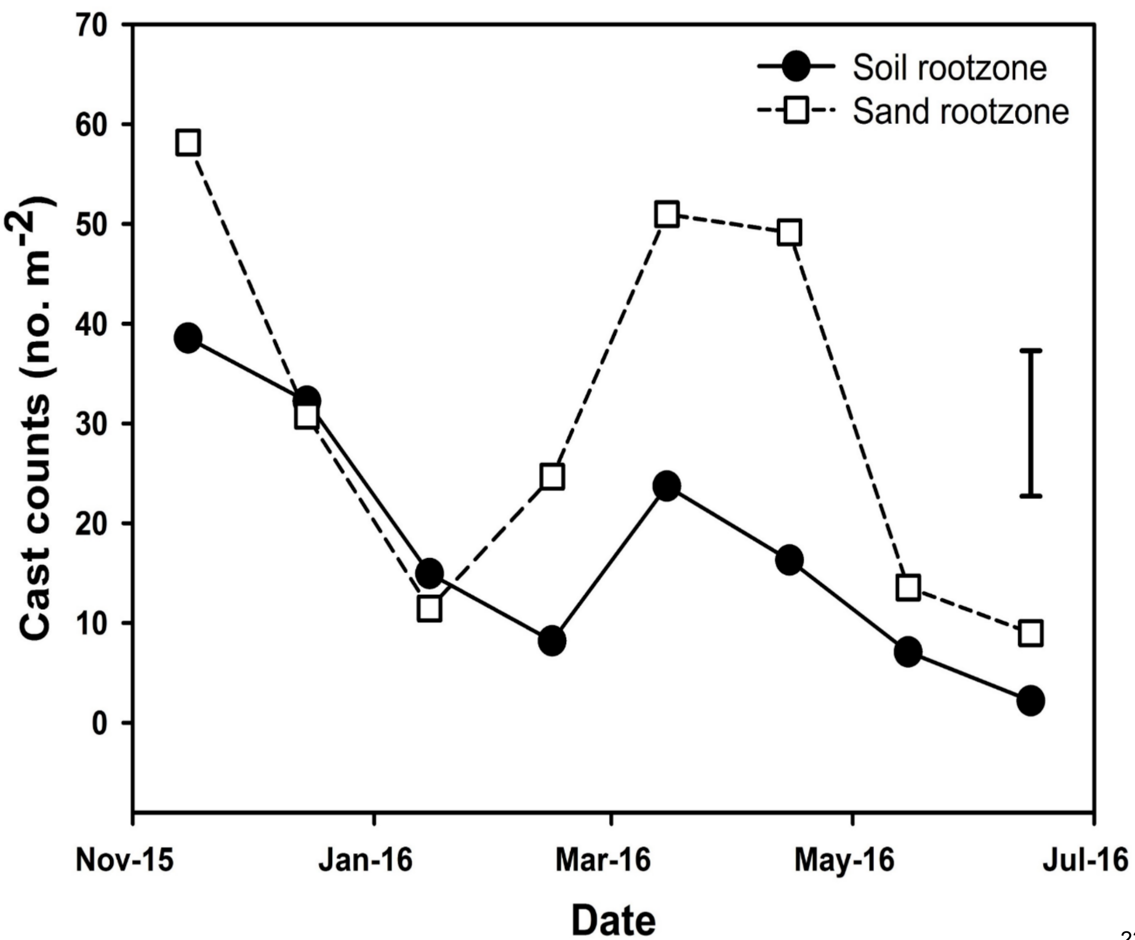
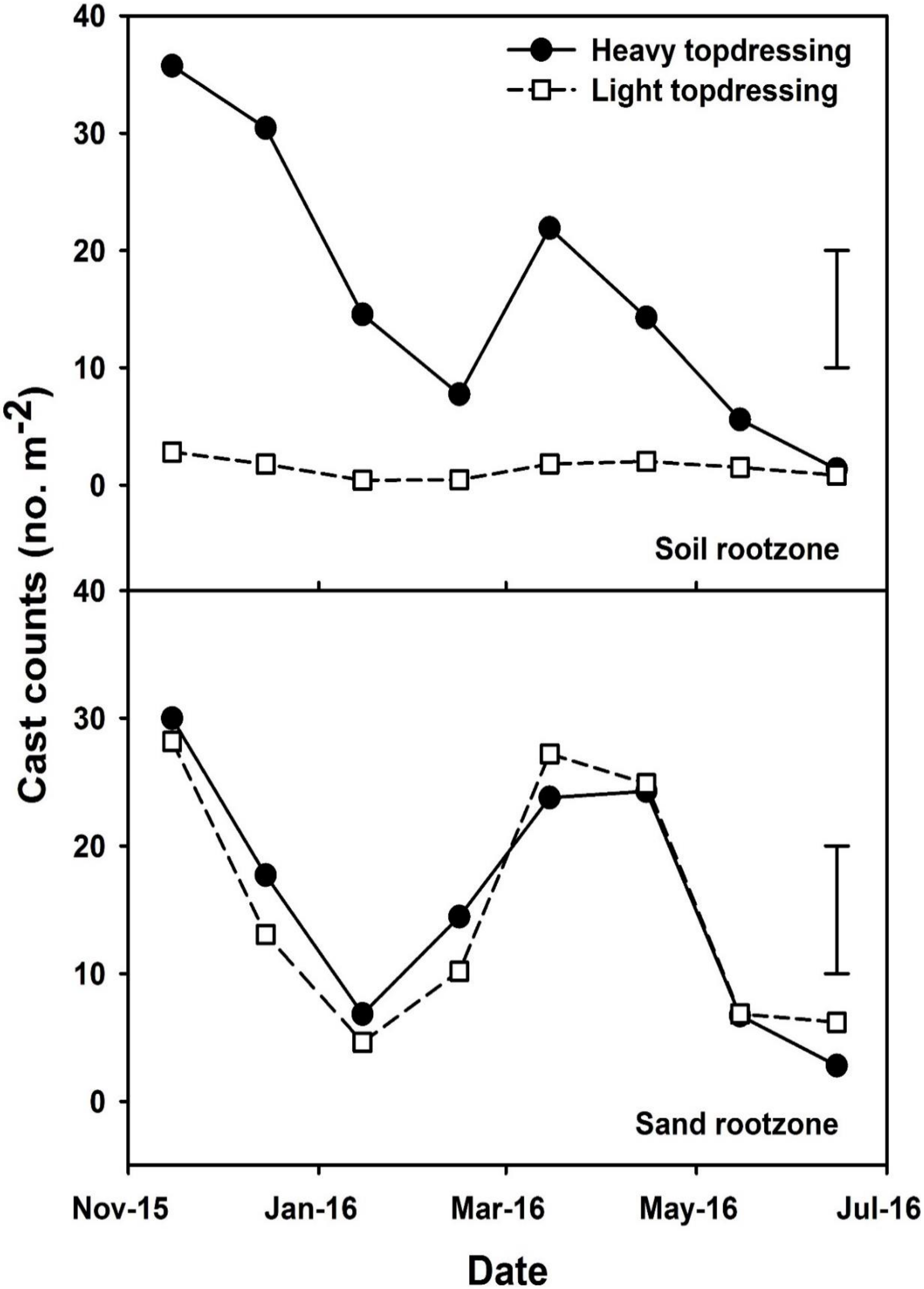


**Fig. 2**. Monthly average of weekly cast counts by soil (top) and sand (bottom) rootzone and topdressing treatment between November 2015 and June 2016. Error bar represents the least significant difference (*P*=0.05) and can be used to compare treatment means.



# USGA GREEN SECTION RESEARCH PROGRAM

ANNUAL REPORT

DECEMBER 2, 2016

PROJECT TITLE:

Evaluation of Warm-Season Grasses for Putting Greens
USGA ID#: 2013-17-478

PROJECT LEADER

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

START DATE

2013

PROJECT DURATION

Five years

TOTAL FUNDING

$90,000

SUMMARY TEXT

With the increased interest in the use of bermudagrass on greens, a project was developed to evaluate three warm-season grass species on USGA specification putting greens at eleven locations across the southern and mid-western U.S. Trial sites include university locations (7) and golf courses (4). The trial parameters dictate a higher mowing height and a less intensive management regime as compared to typical ultradwarf bermudagrass management, while targeting green speeds of 9-10 feet.

The trial consists of twenty-eight total entries, with fourteen bermudagrass, eleven zoysiagrass and two seashore paspalum entries. Trials were planted anywhere from mid-June to mid-August 2013. As explained last year, winter injury from 2013-14 was significant at some locations. This winter injury caused NTEP to replant some or all entries at four locations in summer 2014. The winter of 2014-15 was also colder than normal in some locations, which delayed some entry development and hence, collection of some of the more advanced data parameters. Also, various issues led to the unfortunate abandonment of the trial at Tequesta, FL.

In 2015, several experimental bermudagrass entries performed equal to, or better than our standard entries 'Tifdwarf', 'Tifeagle' and 'Mini-Verde' at some locations. 'MSB-264' and 'MSB-285' have performed very well thus far at several of the more southern locations. Other experimentals such as '08-T-18', '11-T-861', 'FAES 1302' and 'OKC 16-13-8' have shown good turf quality at several locations. And the new commercial cultivar 'Sunday' has finished in the top turf quality statistical group at eight of the ten locations. Significant differences in genetic color, density, leaf texture and fall color retention were noted among entries, which largely led to the quality ratings separation.

For zoysia in 2015, several experimental entries produced turf quality that rivaled many of the bermudagrasses. 'DALZ 1308' was one of the best zoysia entries in 2015, finishing in the top statistical group at every location, including the most northern location (Bloomington, IN). Other entries, such as 'DALZ 1306', 'DALZ 1307' and 'DALZ 1309', were consistent performers. Commercially available 'L1F' was a top performer at a few southern locations. It is possible that several of these entries may end up as suitable alternatives for bermuda on lower input greens in the southern to mid-central U.S.

The two seashore paspalum entries demonstrated excellent establishment and reasonable quality thus far. 'UGA 1743' and the standard entry 'SeaDwarf' performed very similarly at almost locations in 2015. Also, as expected, both seashore paspalum entries died at the northern locations of Lexington, KY and Bloomington, IN.

Ball roll measurements were collected at most locations in 2015. However, only two locations (Jay, FL and Mississippi State, MS) recorded 100 inches of roll using the stimpmeter on at least one rating date. Only the bermuda entries 'Tifeagle', 'FAES 1302' and 'CTF-B10' achieved ball roll distances of at least 100 inches at both locations. None of the zoysia or seashore paspalum entries rolled at least 100 inches, however, that could be due to differences in management needs.

## SUMMARY POINTS

- At some locations in 2015, several new or experimental bermudagrasses provided turf quality equal to or better than established standards 'Tifdwarf', 'Tifeagle' and 'Mini-Verde'.
- Several zoysia entries produced turf quality rivaling the best bermuda entries. The two seashore paspalum entries performed similarly in 2015, and as expected, both entries did

not survive winter in the most northern sites.

- Ball roll data showed only a few bermuda entries with distances of least 100 inches under this medium maintenance regime.
- No seashore paspalum or zoysia entries produced a ball roll measurement of 100 inches on at least one rating date at any location. The maintenance practices for these two species may require adjustment for the 100-inch threshold to be achieved.

USGA GREEN SECTION RESEARCH PROGRAM

ANNUAL REPORT

DECEMBER 2, 2016

PROJECT TITLE:

On-Site Testing of Grasses for Overseeding of Bermudagrass Fairways
USGA ID#: 2016-24-574

PROJECT LEADER

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

START DATE

2016

PROJECT DURATION

Three years

TOTAL FUNDING

$90,000

SUMMARY TEXT

Even though golf course overseeding usage is declining, resort courses and some private and public facilities will continue the practice into the future. Therefore, this project was developed to address issues related to overseeding of bermudagrass fairways. A focus of this project is the use of saline/low quality water or sites that reduce water use by irrigating with lower evapotranspiration (ET) replacement rates.

An advisory committee consisting of representatives from the USGA, NTEP, universities and seed companies selected ten golf courses, in key areas of the southern and southwestern U.S. to evaluate this trial. The advisory committee recommended that not only single cultivars, but also blends and mixtures of various species could be included in the trial. Therefore, twenty-

two entries were submitted that consist of ten ryegrass blends, nine single perennial ryegrass cultivars, one intermediate ryegrass, one annual ryegrass and one *poa trivialis*. Three standard entries were also added to the trial (one each of perennial ryegrass, intermediate ryegrass and *poa trivialis*).

Trial locations were selected in important use areas and/or locations with challenging environments/unique characteristics. Unfortunately, a central California golf course that was chosen rescinded its participation just prior to establishment. As we were unable to locate a suitable replacement course in California, the following is a list of nine selected trial locations established in fall 2016:

| Golf Course | Location | Cooperator | University |
|---|---|---|---|
| Jekyll Island Golf Club # | Jekyll Island, GA | Dr. Clint Waltz | Georgia |
| Lonnie Poole @ NC State # | Raleigh, NC | Dr. Grady Miller | N.C. State |
| The Rawls @ Texas Tech # | Lubbock, TX | Dr. Joey Young | Texas Tech |
| Lakeside # | Stillwater, OK | Dr. Charles Fontanier | Oklahoma State |
| New Mexico State Univ. # | Las Cruces, NM | Dr. Bernd Leinauer | New Mexico State |
| Tucson Country Club ^ | Tucson, AZ | Dr. David Kopec | Arizona |
| Lost Key ^ | Pensacola, FL | Dr. Bryan Unruh | Florida |
| Texas A&M Univ. Campus ^ | College Station, TX | Dr. Casey Reynolds | Texas A&M |
| Mississippi State Univ. | Starkville, MS | Dr. Wayne Philley | Mississippi State |

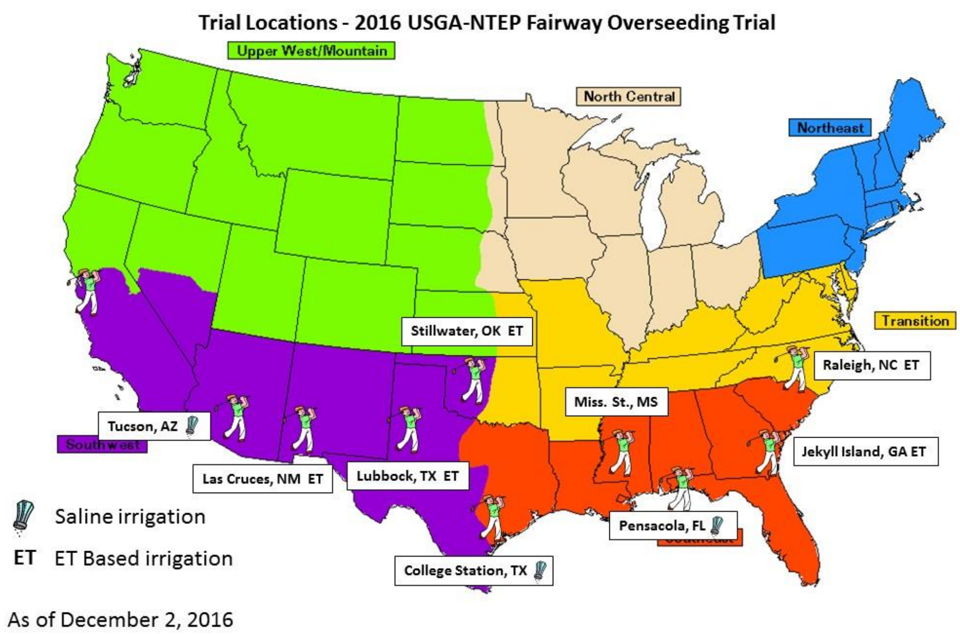
*# Uses reduced water rates via ET replacement*
*^ Utilizes saline irrigation water*

Entries were established in 100 sq. foot plots, replicated three times where fairway traffic is evident, but also outside of landing zones. In fall 2016, trials are rated for establishment rate, color and quality. Winter ratings will focus on percent cover of overseeding grass, color, quality, texture and growth rate. Spring and summer 2017 ratings also consist of color, quality, texture and growth rate, with additional ratings of density and percent green cover of bermuda and overseeding grass during the transition back to 100% bermuda. In fall 2017, each trial location will be reseeded with the same entries at the same physical location, with the same data collection protocols as in 2016.

Year one data will be published on the NTEP web site (and via CD) in late summer 2017, with year two data being published in late summer 2018. Data is for use by cooperators, extension personnel, seed companies and golf course superintendents in making recommendations or purchasing decisions.

SUMMARY POINTS

- This trial focuses on cultivar, blend and mixture performance of twenty-five entries, primarily under reduced (ET based) water rates or the use of saline (low quality) irrigation water.
- Nine golf course sites, chosen based on geographic location and maintenance characteristics, were established in fall 2016 via large plots on fairways.
- Data collection of establishment, color, quality, texture, growth rate and transition commenced in fall 2016 and will continue through spring 2017.
- Plots will be reestablished in fall 2017, in the same physical location and with the same entries, for year two of data collection.
- Year one data will be available via NTEP in late summer 2017, with year two data availability in late summer 2018.




**The impact of putting green management on visible wear caused by golf cleat/sole designs**
**USGA ID#: 2016-26-576**

Thomas A. Nikolai, Ph.D. Michigan State University
Douglas Karcher, Ph.D. The University of Arkansas

The objectives of the research are to: 1) identify particular components of golf cleat sole designs that result in the least to greatest perceived differences in regard to green friendliness 2) identify putting green management practices that negate the visible damage caused by the most intrusive and/or destructive of the current golf cleat/sole designs and 3) search for correlations between Tru-Firm and TDR measurements when collecting data regarding turfgrass management practices including sand-topdressing, irrigation, vertical mowing, rolling and long-term thatch thickness.

To address Objective 1 golf cleat/sole traffic studies were performed at 11 locations in three states (Michigan, Arkansas, Florida) with over 20 cleat/sole designs and a non-trafficked check plot. The studies were conducted on various cultivars and ages of creeping bentgrass, annual bluegrass, ultradwarf bermudagrass, and seashore paspalum putting greens. At each location plots were trafficked with different cleat/sole designs and golfers rated the putting surface on a scale of 1-5 for putting green smoothness. Specifically, the rating scale was:

1 = Excellent; no visible traffic
2 = Very good; I think I see foot traffic
3 = Good; some visible foot traffic but I would not mind putting on the surface
4 = Fair; visible foot traffic that would most likely deflect my putt and
5 = Poor; terrible putting conditions recommend banning the cleat/sole from our golf course.

Researchers will avoid reporting manufacture golf shoe brand names because the objective of the study is to identify “components of golf cleat sole designs that result in the least to greatest perceived differences in regard to green friendliness”, not which current design is most damaging. One necessary exception to that rule comes from a study conducted at Field Day at The Hancock Turfgrass Research Center in 2016. The Field Day data are different because the research team made an evaluation comparing among the most aggressive golf cleat/sole designs from the 1990’s to the more recent Adizero golf cleat/sole that has been perceived as being among the most destructive on putting greens the past several years. A result of that study was raters perceived the poorest performing cleat/sole design of our current time period as being more green friendly than among the most aggressive products from the past (Fig. 1).

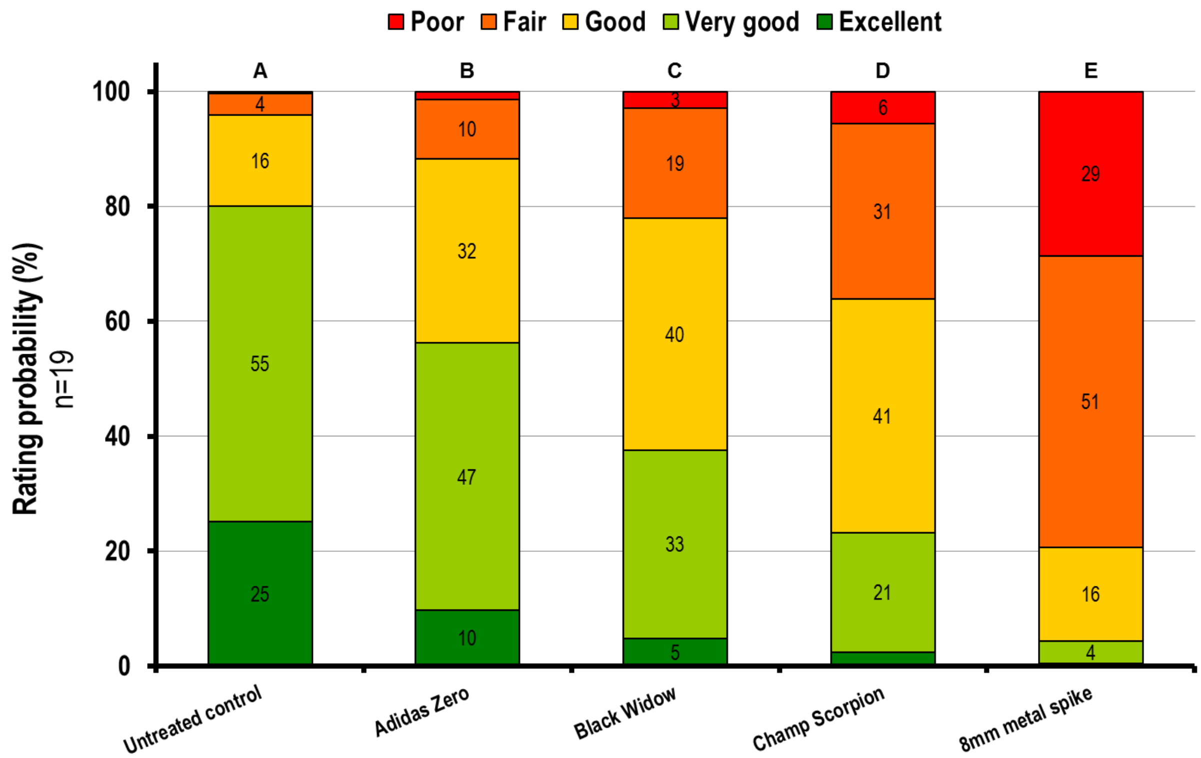



**Figure 1.** **Predicted probabilities of spike treatments to be rated as either "Excellent", "Very Good", "Good", "Fair", or "Poor". Probabilities were estimated using logistic regression analysis of data collected from 3 replicate plots evaluated at the Hancock Turfgrass Research Center, East Lansing, MI on a 1-year old Pure Distinction creeping bentgrass putting green on August 17, 2016. Data is generated from 285 observations and bars that do not share a letter are significantly different ($\alpha = 0.05$).**

With respect to observations regarding components of golf cleat/sole design and green friendliness made at the other 10 sites in 2016, results include:

1. Metal spikes (both 8 and 6 mm) were perceived to be the most destructive at 10 of 11 sites. The single anomaly was that the 6mm metal spike was the second poorest treatment.
2. Spikeless/rubber outsole golf shoes result in the least amount of apparent foot traffic on all putting surfaces (bentgrass, annual bluegrass, bermudagrass, and paspalum).
3. In general cleated/rubber outsole shoes result in the most perceived foot traffic, however, the range is wide and to date researchers have not quantified variables that result in some designs having more visible wear than others.
4. The spikeless/TPU (thermoplastic polyurethane) outsole is often perceived as creating more damage than most of the cleated/rubber outsole golf shoes. Again, to date researchers have not quantified why but have collected or are collecting manufacturer data regarding type of plastic, number of cleats and protrusions, etc. regarding each golf cleat/ sole design.

To address Objectives 2 and 3 Pure Distinction creeping bentgrass and an annual bluegrass putting greens at Michigan State University (MSU) and Pure Distinction creeping bentgrass and Tifeagle ultradwarf bermudagrass putting greens at the University of Arkansas were treated with different levels of grooming (none vs. 3x weekly), lightweight rolling (none vs. 3x weekly), sand-topdressing (none vs. every other week), and fertility (low vs. high N). Treatments were evaluated for foot traffic tolerance on three occasions during the season. Other data collection included green speed measurements, total biomass to the surface to a one inch depth, surface firmness as measured with the Tru-Firm, and volumetric water content from the surface to a 1.5 inch depth.

Results from Objective 2 indicate:

1. Creeping bentgrass had more visible damage following 20 simulated rounds of golf at both sites compared to annual bluegrass (MSU) and ultradwarf bermudagrass (UA).
2. At UA and MSU, fertility had the biggest impact of visible damage on creeping bentgrass (Fig. 2) and at UA the impact was similar on the ultradwarf bermudagrass areas. Higher nitrogen rates resulted in greater visible damage from foot traffic, which was more pronounced on the creeping bentgrass relative to the ultradwarf bermudagrass but was not significant on the annual bluegrass.

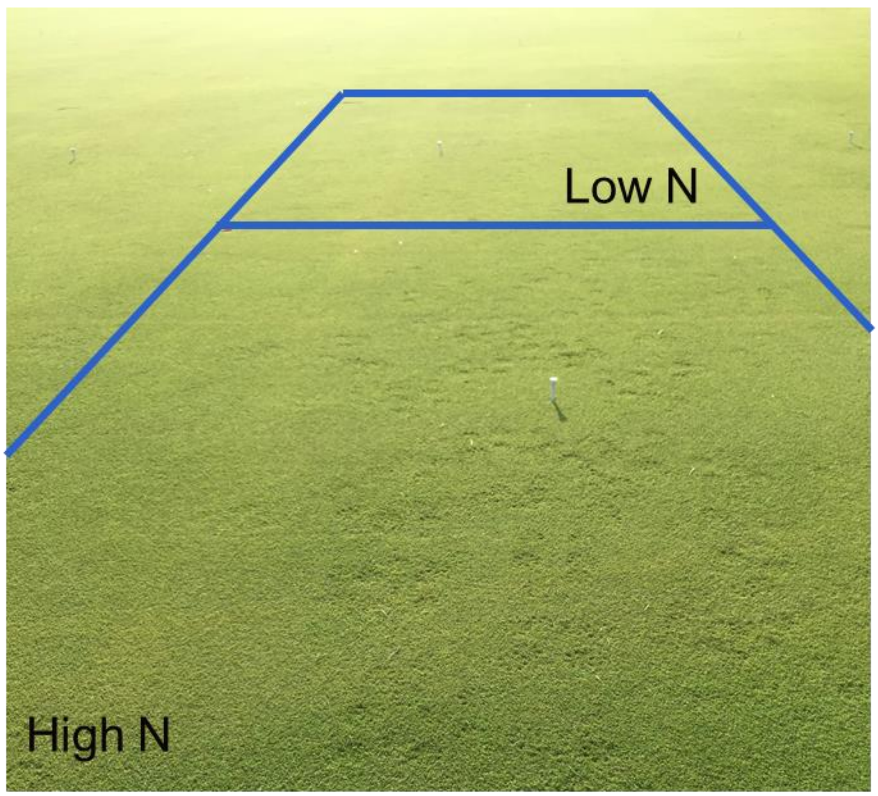



**Figure 2. Fertility effects on visible damage on a 'Pure Distinction' creeping bentgrass putting green following 20 simulated rounds of golf (photo taken July 28, 2016; Fayetteville, AR). The "High N" plot (foreground) received 0.5 lb. N / M monthly whereas the "Low N" plot (background) received half that amount.**

To date, data have not yet been fully analyzed for Objective 3. These studies will be repeated in 2017 at MSU and UA.

# ‘Grass Roots’ Initiative Summary- 2016

The 2016 season was the second full season of the ‘Grass Roots’ exhibit at the US National Arboretum. Geoff Rinehart continues to work as ‘Grass Roots’ Initiative Coordinator and he was assisted this year by one full-time and three part-time interns (about 2.0 FTE of intern/seasonal labor). This season presented additional management challenges as we experienced one of the most challenging summers over the last few decades in the mid-Atlantic region with 60 days above 90 degrees at the Arboretum. We continue to make adjustments and improvements to the exhibit to enhance the aesthetic presentation and visitor experience and education on the exhibit. Two of the most encouraging aspects as the landscaping of ‘Grass Roots’ continues to develop have been the maturing of the turfgrass swards, which provides a good opportunity for visitors to compare their growth habits and features; and the growth in height and girth of the ornamental grasses which were planted to provide a contrast to the horizontal aspect of the turfgrass swards and provide both boundaries between the displays and a mode of consistent connection among the displays. As the exhibit’s plant material has matured, this has enhanced both the aesthetic attractiveness of the space and the educational experience for visitors. In addition, the opening of a new ‘Grass Roots’ exhibit the Maryland SoccerPlex in Boyds, MD this autumn and the addition of another display to the Arboretum ‘Grass Roots’ exhibit in Spring, 2017 will provide the opportunity to expand ‘Grass Roots’ public outreach.

~~.~~ During both 2015 and 2016, based upon spot counts throughout the year on the exhibit, we estimate that ‘Grass Roots’ has hosted approximately 30,000 visitors annually. Typical visitor numbers throughout the year can range from practically no visitors on weekdays from December-February to 1000+/day during peak weekends in mid-Spring when temperatures are in the 60’s and 70’s and many plants at the Arboretum are in flower.

Visitors have engaged with the exhibit in a variety of ways ranging from the interactive exhibits; putting a ball on the golf green or kicking a soccer ball on the sports field during one of our ’Grass Roots’ Family Fun Day” events; participating in one of our guided tours……or just relaxing with a picnic under a tree in the lawn areas.

Typical ‘Grass Roots’ exhibit visitors include many families with school-age children, new homeowners seeking information about properly caring for their first lawn, tourist visitors from all over the country, volunteers from local businesses and organizations who have helped with ‘Grass Roots’ maintenance and also learned a lot about turfgrass during the course of their visit, master gardeners seeking to enhance their knowledge and understanding of turfgrass management, and landscape designers seeking to learn more about different grass varieties.

## Public and Professional Outreach and Education

In 2016 we conducted three homeowner lawn care workshops in the spring (April 9, April 23, and May 21) two in the autumn (September 17 and October 22). Attendance for these events ranged from 12 to 30 people. ‘Grass Roots’ general lawn care workshops include a 75-90-minute PowerPoint presentation covering turfgrass identification, site evaluation, and the five major cultural practices of turfgrass management, followed by a 20-30-minute tour of the 'Grass Roots' exhibit. The exhibit has been an excellent resource to "field illustrate" the different turfgrass species and management concepts discussed during the classroom presentation.

Geoff Rinehart and Arboretum native plant collection curator Joan Feeley co-hosted a workshop with about 20 attendees for the Chesapeake Conservation Landscape Council on Saturday, April 23. This workshop included a 60-minute lawn care presentation by Geoff Rinehart followed by a tour of 'Grass Roots' and a tour of the native plant collection.

On May 15, 'Grass Roots' co-hosted the National Cherry Blossom Festival "Petal to the Parks" bike ride which included stops at the National Mall and other NPS parks in DC and culminated with a tour of 'Grass Roots' for participants.

Other visiting groups to which 'Grass Roots' exhibit tours were provided this year include National Golf Day event attendees (May 17), Park Trust School Group (June 3), horticulture staff from the Biltmore Estate (July 19), the Professional Grounds Maintenance Society- DC chapter (August 4), Fairfax Co. Master Gardeners (October 10), and a delegation from the Chinese Ministry of Agriculture (October 19). 'Grass Roots' also hosted over 150 5$^{th}$ graders participating in various sports/lawn games and athletic events as part of a Department of Education-sponsored "Let's Read/Let's Move" event on August 5.

In addition to these on-site presentations, Geoff Rinehart presented about the 'Grass Roots' Initiative at the Mid-Atlantic Turfgrass Expo in January, provided lawn care training to the Fairfax County (Virginia) and Montgomery County (Maryland) Master Gardeners during their winter training series in February and an evening lawn care seminar to the Takoma Park (MD) Garden Club in March; tabled a booth offering lawn care information at the Silver Spring (MD) Garden Show in April; and helped to staff a Montgomery Co. Master Gardeners Farmer's Market booth in August.

**Government Outreach**

Grass Roots' location at the US National Arboretum (which is administered by the US Department of Agriculture) and its proximity to the Capitol, Executive Office Buildings and departmental agencies provide a unique opportunity to outreach to federal government officials. In early 2016, NTF Executive Director Kevin Morris and Coordinator Geoff Rinehart had "one-on-one" meetings with science policy analysts Drs. Elizabeth Stulberg and Richard Pouyat in the Office of Science and Technology Policy on and USDA-ARS Administrator Dr. Chavonda Jacobs-Young, Dr. Jose Costa, and Dr. Simon Liu to advocate for the significance of the turfgrass industry and the need for increased federal funding of turfgrass science research. 'Grass Roots' was featured in the USDA-ARS "Scientific Discoveries" publication in May, 2016 as one of three programs highlighted in the "Education and Science Literacy" section. Dr. Chavonda Jacobs-Young and ARS program leadership staff visited and toured the 'Grass Roots' exhibit as part of a larger Arboretum tour on June 22. On May 18, 'Grass Roots' participated in National Golf Day by tabling an educational display at the US Capitol.

**Maryland SoccerPlex 'Grass Roots' Exhibit**

In 2016, we expanded the 'Grass Roots' Initiative and installing a 1-acre 'Grass Roots' exhibit at the Maryland Soccerplex, located approximately 25 miles NW of Washington, DC in Boyds, MD. The NTF was able to collaborate with Maryland SoccerPlex to apply for and receive a USDA-NIFA Specialty Crop block grant to fund the materials cost of exhibit construction. The exhibit was built adjacent the complex's stadium and features 10 interactive educational displays while using a similar format to the Arboretum's 'Grass Roots' exhibit. In addition to a large amount of league participation, the Maryland Soccerplex hosts many regional and national youth soccer tournaments, is the home of the Washington Spirit professional women's soccer team, and has over 600,000 visitors/year. Funding for the construction and initial maintenance for the Soccerplex exhibit was provided by the Maryland Department of Agriculture. 'Grass Roots' Coordinator Geoff Rinehart cooperated with the Maryland SoccerPlex leadership and staff to help guide and participate in the construction and coordinate regional sponsors who contributed in-kind donations of materials and labor. Geoff Rinehart will continue to collaborate with the Maryland SoccerPlex next year to assist with minor adjustments and survey work to be conducted as per the grant.

**Looking Forward-2017**

In addition to providing modest support and conducting survey research at the Maryland SoccerPlex exhibit, we have plans for improving and enhancing the outreach of 'Grass Roots' in 2017, as well. Over this winter and into 2017, we look forward to reaching out to invite more school groups (possibly using a First Green model curriculum); youth groups (including First Tee- DC); professional landscape architect/design organizations; other horticultural interest groups; and, of course, government officials and policymakers. Next spring, we anticipate expanding the Arboretum exhibit and adding a display which includes ongoing *Danthonia spicata* field research by USDA turf scientist Dr. Scott Warnke. The 'Grass Roots' exhibit will be a tour stop for the International Turfgrass Research Conference Pre-Conference Tour in July, 2017. Also, plans are currently being developed with Arboretum leadership to host a 2-day Turfgrass Summit in fall, 2017.

# ‘Grass Roots’ Initiative Summary-2016

- The Maryland SoccerPlex 1-acre ‘Grass Roots’ exhibit in Boyds, MD was built in 2016 and will be maintained by SoccerPlex staff. A Maryland Department of Agriculture NIFA grant helped fund the construction.

- The Arboretum ‘Grass Roots’ exhibit hosted an estimated 30,000 visitors/year in 2015 and 2016. The ‘Grass Roots’ website (www.usna.usda.gov/Education/turfgrass.html) complements the educational concepts in the exhibit and contains information about the benefits of turrfgrass, basic lawn care information, and links to each state’s turfgrass extension education website

- In 2016, ‘Grass Roots’ coordinator Geoff Rinehart conducted 6 lawn care workshops and hosted tours for several groups visiting the exhibit, including the Chesapeake Conservation Landscape Council, Biltmore Estate staff, master gardeners, the Professional Grounds Maintenance Society, and USDA-ARS administration staff.

- Plans for 2017 include adding another display at the Arboretum featuring USDA turf research (Spring), host part of the ITRC pre-conference tour (July), hosting a Turfgrass Summit (Fall), and additional outreach to school/youth and horticulture professional groups.

The ‘Grass Roots’ golf display includes a 1000 sq. ft. bentgrass putting green Zoysia “fairway”, and a bentgrass tee.

Children enjoy the putting green at a Family Fun Day” event this fall.

The Maryland SoccerPlex ‘Grass Roots’ exhibit opened in November, 2016 and features 10 interactive displays. The sports field display includes “mini-fields” of ‘HGT’ Kentucky bluegrass, ‘Latitude 36’ bermudagrass, ‘Zeon’ zoysiagrass, and a synthetic infill surface.

The ‘Grass Roots’ lawn displays include 7 different warm-season and 5 different cool-season swards for visitors to compare and contrast.

Operation Monarch: Developing Protocols for Monarch Butterfly Conservation Plantings in Golf Course Naturalized Roughs, Parks, and other Urbanized Areas

Adam Baker, Daniel McNamara, Carl Redmond, Gregg Munshaw and Daniel Potter
University of Kentucky

USGA ID#: 2016-36-606

**Objectives:**

1) Evaluate methodology for establishing native milkweed (common, swamp, and butterfly) in golf course naturalized roughs

2) Document effectiveness of golf course milkweed plantings, with or without wildflower strips, for attracting and sustaining monarchs and other pollinators

3) Support and promote golf courses for monarch butterfly conservation through outreach education, webinars, conferences, trade journal articles, and media releases

This research project establishes a new conservation initiative, Operation Monarch Butterfly for Golf Courses [OMBGC]. It evaluates protocols for establishing monarch butterfly habitat and food resources in golf course naturalized roughs, and promotes the program's implementation and visibility through extension education and outreach. Populations of this charismatic beloved butterfly, renowned for its long-distance migration, are threatened by loss of wild milkweeds (*Aclepias* species) which are its only larval hosts. Monarchs require stands of native milkweeds for caterpillar development, accompanied by wildflowers to provide nectar for the butterflies during egg-laying and to sustain them along their migration routes. Milkweeds have been decimated by urbanization and by herbicide use in fields of genetically-modified herbicide-resistant crops. Increasing monarch butterfly populations through domestic/international actions and public-private partnerships is one of the three overarching goals of the 2015 National Strategy to Promote the Health of Honey Bees and Other Pollinators.

This is an opportunity for the Golf Industry to provide leadership in helping to reverse monarch decline by establishing milkweeds and nectar plants in naturalized roughs. Stands of milkweed and nectar plants adjacent to cart paths and tees, accompanied by interpretative signage, offer opportunities for conservation education while providing interest during play. Such areas can be mowed annually, in late autumn, after the monarch migrations. OMBGC complements and extends other golf-related environmental initiatives including the Audubon Cooperative Sanctuary Program, and Operation Pollinator for Golf Courses.

For Objective 1, we are evaluating establishment methods for three native milkweeds, common (*Asclepias syriaca*), swamp (*A. incarnata*), and butterfly (*A. tuberosa*), all of which are commercially available and suitable for naturalized golf course settings. Treatments include three preparation methods 1) vertical mower 2) fraze mower, and 3) scalp (with herbicides applied in spring as needed); and two seeding methods 1) drill 2) hand sown. Plots were prepared and seeded in November 2016 and will be evaluated in 2017 and 2018.

For Objective 2, we cooperated with superintendents in autumn 2016 to establish two larger plots in naturalized roughs of each of three golf courses. The planting schemes being compared include 1) common milkweed, 2) common milkweed strip-cropped with native wildflowers, 3) a 50:50 mix of swamp/butterfly milkweed, 4) swamp/butterfly milkweed mix strip-cropped with native wildflowers, and 5) a commercial monarch conservation seed mix containing swamp and butterfly milkweeds plus native wildflowers, with six replicates of each planting scheme. Plots were prepared by scalping to weaken existing vegetation, scarifying with a vertical mower, raking out debris, and hand seeding. For the milkweed plots strip-cropped with nectar plants, the wildflower strips border the milkweeds on the side most visible from play.

Beginning in spring 2017 we will compare effectiveness of the planting schemes by evaluating the number, height, and developmental stage of the milkweed plants monthly for two growing seasons. Usage by monarchs will be assessed by examining a subset of plants in each plot for eggs and larvae biweekly from 10 June through 10 October in each year. We will also document usage by non-monarch butterflies and bees monthly during the first and second growing seasons. Butterflies will be counted and identified on the wing during timed walk-arounds of each plot. Bees will be surveyed using 50-bee collections from milkweed flowers and from the accompanying wildflowers. Effectiveness of the golf course plantings for attracting and sustaining monarchs will be compared to Monarch Waystations (small gardens with milkweed and nectar plants), some 11,000 of which have been planted at schools, businesses, parks, zoos, nature centers, and by backyard gardeners. Our hope is to demonstrate that golf courses can also play a valuable role in conservation of monarch butterflies and other pollinators.

Outreach education is an important component of this project. We will publish articles in Golf Course Management, USGA Green Section Record, and horticultural trade journals to disseminate the findings. We will publicize OMBGC to national environmental conservation and advocacy organizations (e.g., monarchwatch.org; monarchjointventure.org, wildones.org), facilitate the certification of participating golf courses as Monarch Waystations and work with industry sponsors to develop educational materials for courses committing to OMBGC.

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# Golf Course Habitat Restoration Pilot Program: A Pilot Project between the U.S. Golf Association and The Nature Conservancy

Technical Interim Report:
November, 2016



Submitted by:
Andrew J. Peck, Ph.D.
Freshwater Project Manager
The Nature Conservancy

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**Background**

The Delaware River, the jewel of Mid-Atlantic river systems, is a largely intact and highly productive watershed that supports abundant and diverse freshwater mussel populations, annual shad and eel runs, and naturally reproducing populations of brook, rainbow and brown trout. The Neversink River, a major tributary to the Delaware, also supports similar species diversity because of its high water quality and largely intact floodplains. The Lynx at Riverbend Golf Club is located in the floodplain of the Neversink River, just upstream of the confluence with the Delaware River in Port Jervis, New York (Exhibit I). Though much of the Neversink floodplain is intact, floodplain development does increase in the vicinity of Port Jervis. The golf club and the area high school, both are located in floodplain and across the river from each other, are suseptible to inundation by floodwaters. The latest innundation event was in the fall of 2011 when Tropical Storm Irene was quickly followed by Tropical Depression Lee.

**Exhibit I.** Location of The Lynx at Riverbend Golf Club relative to the Village of Port Jervis, the Delaware River and the Neversink River.

The combination of these two storms caused catastrophic damage across the region. The Lynx at Riverbend Golf Club, also suffered extensive damages due to 6 -10 feet of inundation for more than a week. The flooding deposited fine sediment across all of the fairways and in the water features of the course. The sediment suffocated fairway turf, buckled cart paths, decimated actively managed out-of-play turf areas, and deposited unwanted and otherwise "weedy" seed. The damage caused by this single flooding event caused the course to close for much of the Fall of 2011, and it still has not fully recovered. The exent of damage to the course caused by this single flooding event was not avoidable (e.g. sediment deposition in fairways, bunkers and ponds, buckled cart paths, etc.). While the damage caused by such an extreme weather event may not be entirely preventable, the magnitude of impacts could be reduced in future events with the incorporaton of nature-based solutions. For example, generally unused, out-of-play areas can be restored to native plant communities, or "naturalized," as nature is often a first line of defense in extreme weather situations.

**Project Overview**

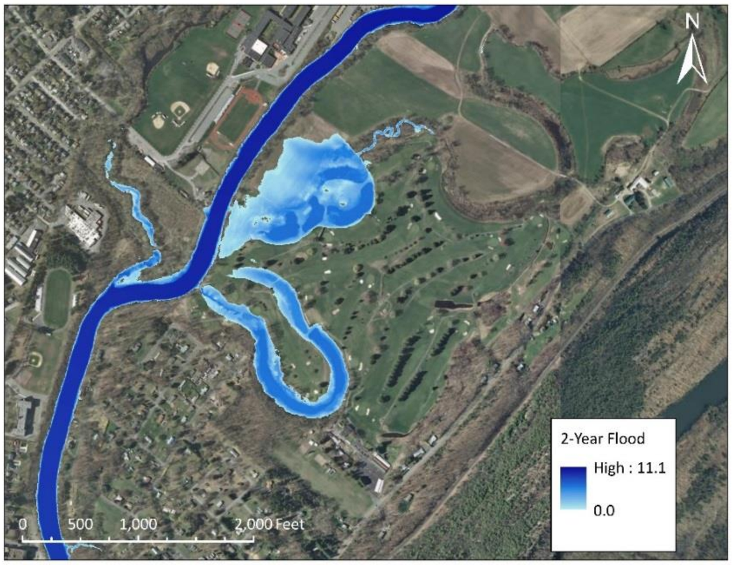
The U.S. Golf Association (USGA), The Nature Conservancy and The Lynx at Riverbend Golf Club are evaluating future management options by developing an understanding not only how to improve day-to-day operations and how course patrons use the course, but also how, when, and where floodwaters are likely to impact the course infrastructure. The objective is to reduce of the acreage of managed areas to save resources in both day-to-day operations and also reduce the amount of effort needed to restore the course to pre-event conditions following the next flood event; naturalized areas are generally better suited to recover with little to no intervention.

**Hydrologic Evaluation**

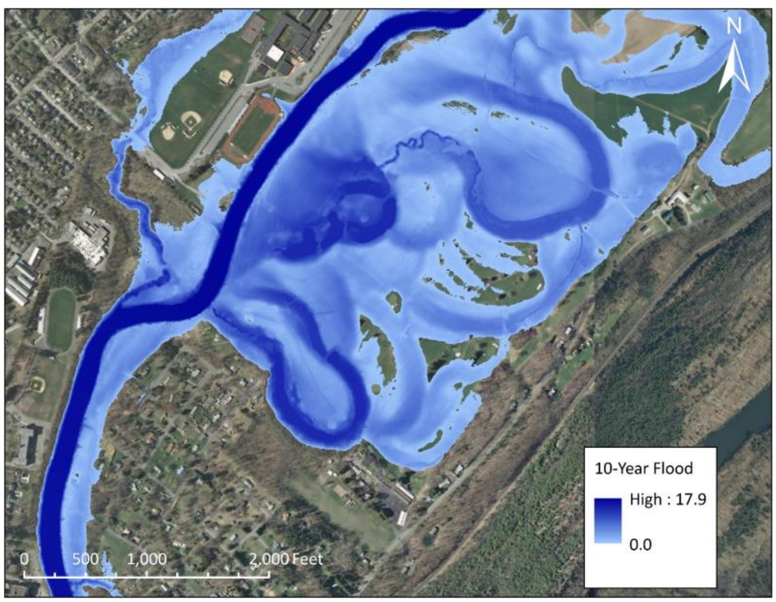
During the Spring of 2015, Milone and McBroom, Inc. a hydrologic consultant, conducted inundation modeling of the course to help determine the progression of inundation from river flooding at various stages. It was quickly determined that the entire course is likely to become inundated at relatively low river stage heights due to the design of the drainage system (Exhibit II). Because all of the stormwater drainage systems ultimately discharge into the Neversink River, the reverse is also true. As the water levels in the Neversink rise and overtop the drainage pipes, water then backs up throughout the entire system, ultimately flooding the entire course before the Neversink actually overtops it banks. This is exacerbated by run-off which drains off the ridge to the south of the course. Major investments into stormwater management strategies would ultimately prove ineffective, not mention cost-prohibitive. The decision was made to focus efforts more on being able to recover more quickly from flooding events rather than trying to stop flooding from occurring in the first place.

**Exhhibit II.** Inundation mapping of The Lynx at Riverbend Golf Club.Darker areas represent the deepest points of inundation and lighter shading represents shallower levels of inundation. The flood event of 2011 produced a discharge of 28,800 cubic feet per second (cfs) which closely equates to a 30 year storm with a three percent chance of being experienced or exceeded in any given year. Precipitation and hydrologic analysis indicates that storms of this magnitude could be expected to increase in frequency/likelihood with climate change.

1. Two-year flood frequency (conditions with a 50% likelihood of exceeded in a given year)



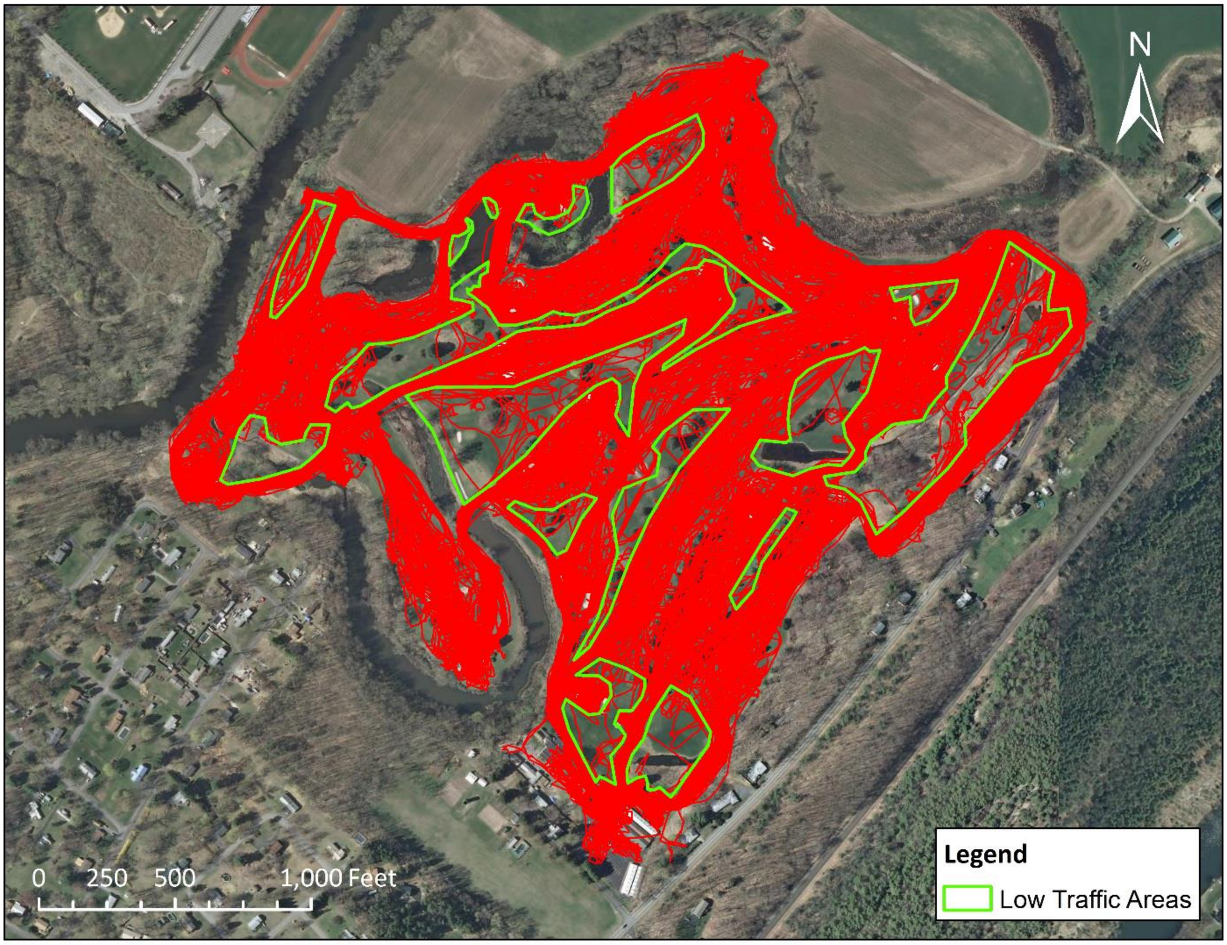

2. 10-year flood frequency (conditions with a 5% chance of being exceeded in a given year)




**Course User Mapping and Analysis**

Using USGA's user tracking tool, we were able to track more than 120 rounds of golf across the entire course (Exhibit III). The mapping clearly showed usage patterns associated with cart paths and fairways as well as preferred pathways into out-of-bounds areas. 12 zones of little to no use by golfers were identified and these areas were further evaluated for feasibility of naturalization using course operations, liklihood of inundation, and liklihood of success as our selection criteria.

**Exhibit III.** Individual user track (red lines) mapping, using GPS technology, identified several areas of low-use by patrons, which are candidates for naturalization (area inside green lines).




**Restoration Plan Development**

Initial restoration plans included the excavation of two new pond areas and an extension of another existing water feature (see accompanying project design plans). Around these new water features and in the selected naturalization areas, we would plan to remove remaining turf grasses and plant a wide variety of native grasses, sedges, shrubs, and trees in these areas. An additional three acres, across three separate low-use locations, would also be prepped and planted with native vegetation. In total, approximately five acres would be improved to reduce the necessary day-to-day management and flood event recovery costs, while providing improved aesthetics for course patrons and both resident and migratory wildlife such as turtles, salamanders, pollunators, rabbits and birds. Deer and other mammals are omnipresent in the region and will not be a focus of restoration efforts.

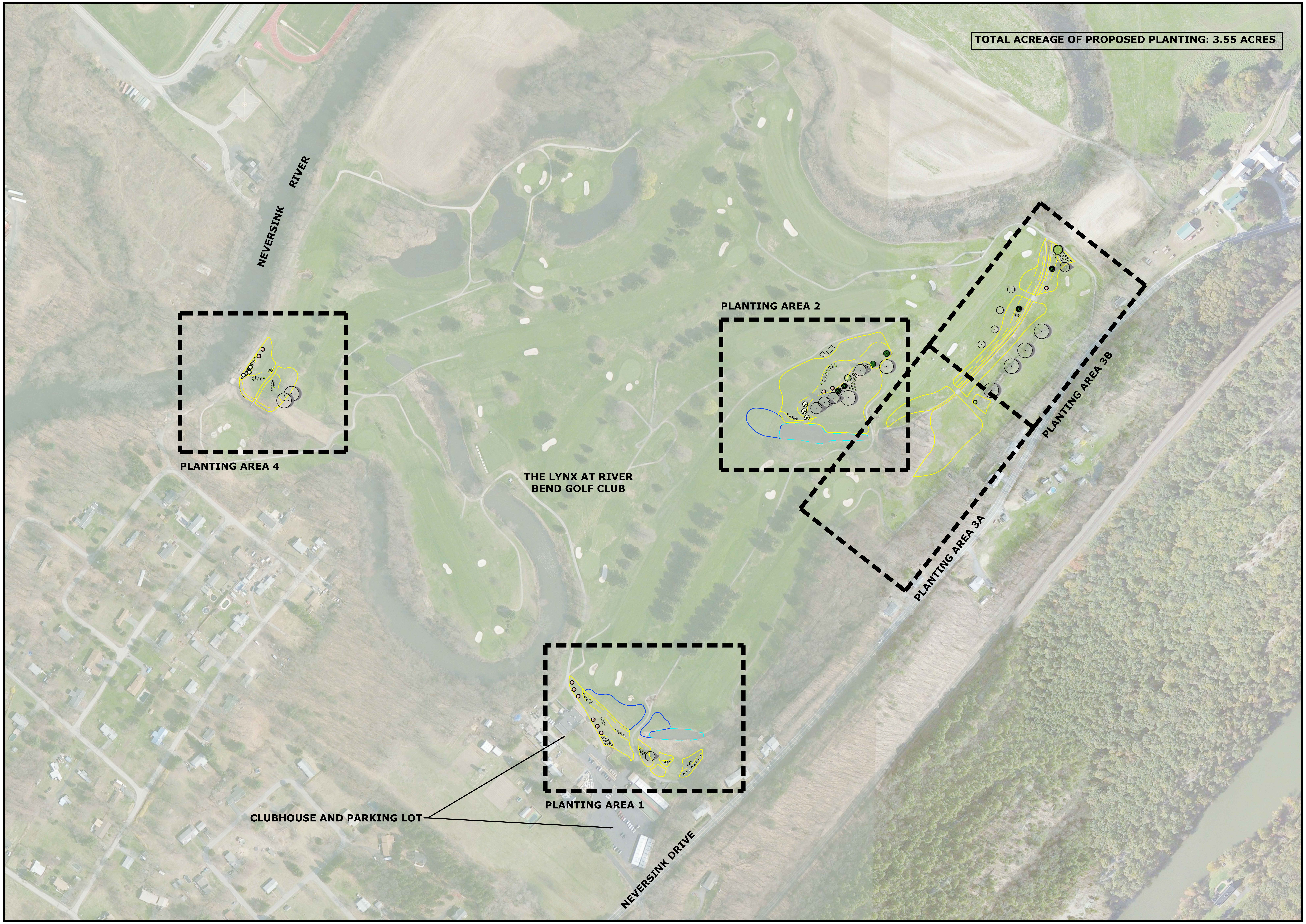
**Permitting Considerations**

The full project was required to be evaluated through a state and federal permit review process because the course is located in a floodplain and USGA funds are being leveraged with funds from the New York Governor's Office of Storm Recovery (NYS GOSR) funds (mix of federal and state resources). This process accounts for necessary Clean Water Act and Endangered Species Act reviews as well as New York's State Environmental Quality Review (SEQR), which includes an assessment of historical significance. The Upper Delaware is also renowned for artifacts pre-dating European settlement. On or near the footprint of the The Lynx at Riverbend, a conflict between Native Americans and European Settlers is known to have occurred. Under regulations of the New York State Historic Preservation Office, the creation of new water bodies on the course would require the execution of extensive artifact assessments, because of the likely presence of artifacts and burial grounds. These required assessments, totaling more than $80,000 and coupled with the likelihood of additional high costs if artifacts were discovered, have recently caused us to re-evaluate our restoration plan.

Rather than create and excavate new pond structures, we will leave them as super-saturated areas and simply plant appropriate low-growing, inundation-tolerant plant and shrub species. Opting not to excavate the proposed ponds releases more than $80,000 dollars of state funding that can now be invested in plant species to naturalize additional acreage throughout the course. We will be reformulating our planting scheme to most effectively utilize these additional funds and naturalize low-use, out-of-bounds areas from active management and reduce the implications of future flooding events.

**Revised Timeline**

We are in the process of reformulating our planting design and our permit application to showcase the new scope of work. We expect this process to be completed in the January/February timeframe which will allow for a staggered implementation schedule. The revised schedule calls for an intial planting of super-saturated areas in late-May or June 2017, well after the first frost, followed by more intensive site preparation and planting in currently managed areas in September or October, 2017.

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CONCEPTUAL PLANTING PLAN - OVERALL

USGA NATURALIZATION PROJECT
THE LYNX AT RIVER BEND GOLF CLUB
PORT JERVIS
ORANGE COUNTY, NEW YORK

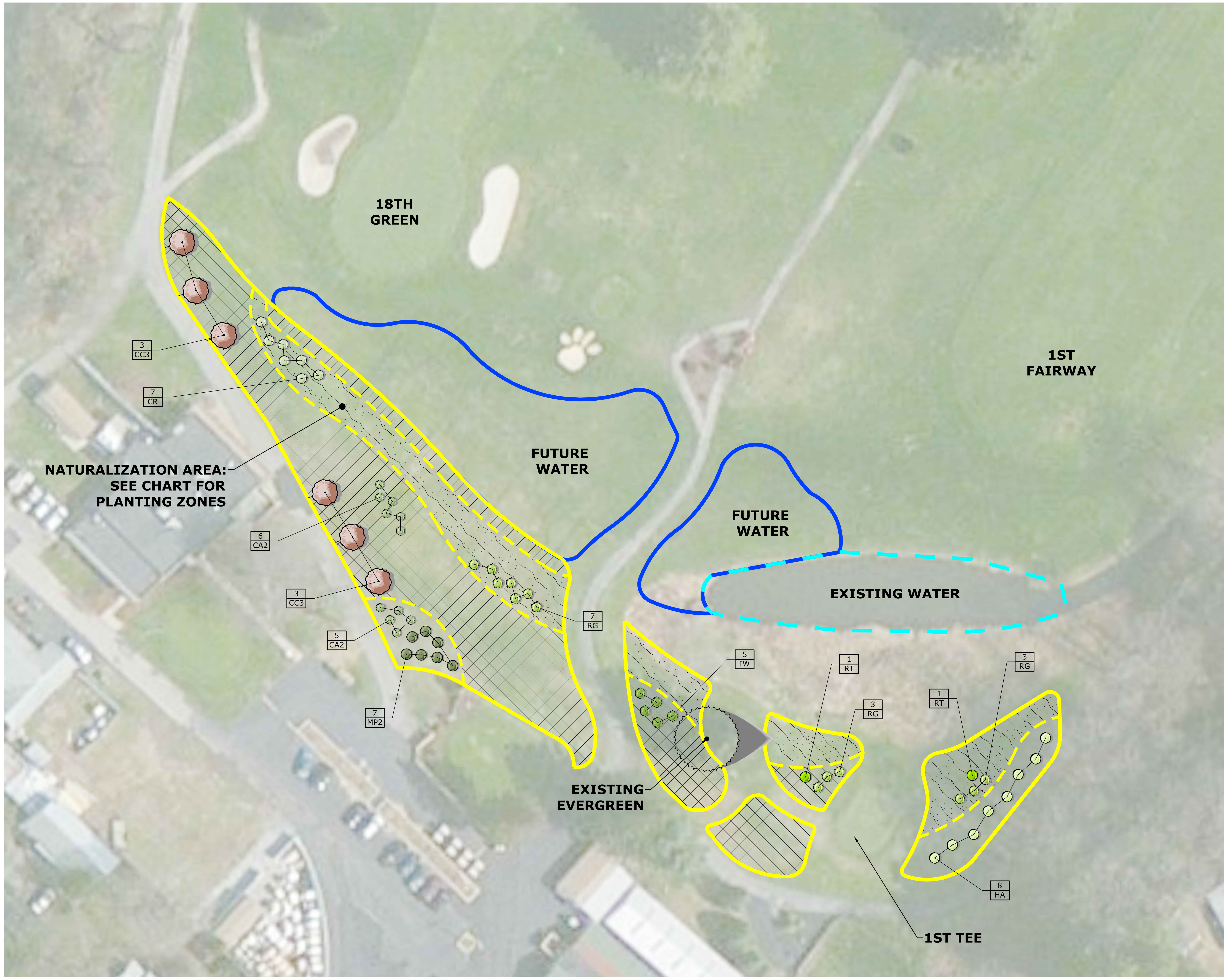
| DESIGNED | DRAWN | CHECKED |
|---|---|---|
| MTD | BDK | MTD |

SCALE: 1"=150'

DATE: JULY 18, 2016

PROJECT NO. 1967-21

SHEET NAME: SP




## SITE PREPARATION / CONSTRUCTION SCHEDULE

1. HERBICIDE APPLICATION TO REMOVE DIFFICULT SPECIES.
2. MECHANICALLY REMOVE UNDESIRABLE LARGER WOODY SPECIES.
3. DEEP TINE AERATION AND LAWN SCALPING.
4. SMOTHER TURF WITH CLEAN TOPSOIL AND MULCH FOR PLANTS.

**TOTAL ACREAGE OF PROPOSED PLANTING:**
**0.68 ACRES *(THIS SHEET ONLY)***

## PLANT SCHEDULE AREA 1

| TREES | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CC3 | 6 | Cornus florida `Cherokee Brave` | Cherokee Brave Dogwood | 2.5" Cal. | B & B | SINGLESTEM |

| SHRUBS | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CA2 | 11 | Clethra alnifolia | Summersweet Clethra | --- | #3 | FULL & DENSE |
| CR | 7 | Cornus sericea | Red Twig Dogwood | --- | #3 | FULL & DENSE |
| HA | 8 | Hamamelis virginiana | Common Witch Hazel | --- | #3 | FULL & DENSE |
| IW | 5 | Ilex verticillata | Winterberry | --- | #3 | FULL & DENSE |
| MP2 | 7 | Myrica pensylvanica | Northern Bayberry | --- | #3 | FULL & DENSE |
| RG | 13 | Rhus aromatica `Gro-Low` | Gro-Low Fragrant Sumac | --- | #3 | FULL & DENSE |
| RT | 2 | Rhus typhina `Tiger Eyes` | Tiger Eyes Sumac | --- | #3 | FULL & DENSE |

## NATURALIZED PLANT SCHEDULE AREA 1

WET EDGE — 1,769 sf
- Carex crinita / CaterpillarSedge
- Eupatorium dubium / Joe-Pye Weed
- Iris versicolor / Blue Flag
- Juncus effusus / Soft Rush
- Lobelia cardinalis / Cardinal Flower
- Matteuccia struthiopteris / Ostrich Fern
- Onoclea sensibilis / Sensitive Fern
- Osmunda cinnamomea / Cinnamon Fern
- Senecio aureus / Golden Ragwort
- Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
- Vernonia noveboracensis / Comon Ironweed

TRANSITION — 9,384 sf
- Asclepias tuberosa / Butterfly Milkweed
- Carex pensylvanica / Pennsylvania Sedge
- Dennstaedtia punctilobula / Hay-scented Fern
- Echinacea purpurea / Purple Coneflower
- Lobelia cardinalis / Cardinal Flower
- Onoclea sensibilis / Sensitive Fern
- Osmunda cinnamomea / Cinnamon Fern
- Schizachyrium scoparium / Little Bluestem Grass
- Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
- Vernonia noveboracensis / Common Ironweed

MEADOW — 18,557 sf
- Andropogon gerardii / Big Blue Stem
- Carex pensylvanica / Pennsylvania Sedge
- Chasmanthium latifolium / Wood Oats
- Deschampsia flexuosa / Wavy Hair Grass
- Echinacea purpurea / Purple Coneflower
- Erigeron philadelphicus / Philadelphia Fleabane
- Geranium maculatum / Spotted Geranium
- Liatris spicata / Spike Gayfeather
- Lupinus perennis / Wild Lupine
- Panicum virgatum / Switch Grass
- Phlox paniculata / Garden Phlox
- Rudbeckia laciniata / Cutleaf Coneflower
- Schizachyrium scoparium / Little Bluestem Grass
- Sorghastrum nutans / Indian Grass
- Sporobolus heterolepis / Prairie Dropseed
- Symphyotrichum novae-angliae / New England Aster
- Waldsteinia fragarioides / Appalachian Barren Strawberry

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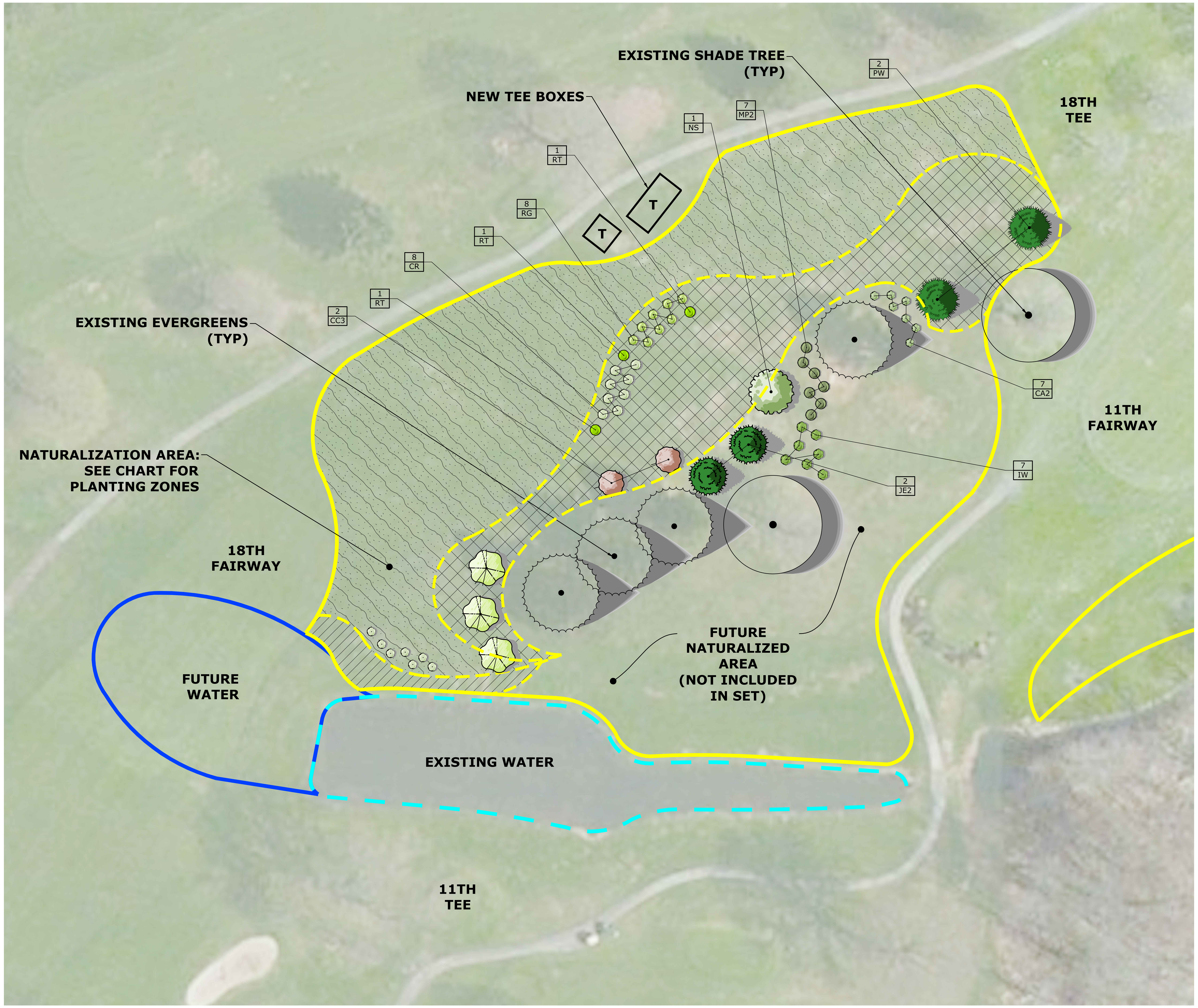
| DESCRIPTION | DATE | BY |
|---|---|---|

CONCEPTUAL PLANTING PLAN - AREA 1
USGA NATURALIZATION PROJECT
THE LYNX AT RIVER BEND GOLF CLUB
PORT JERVIS
ORANGE COUNTY, NEW YORK

| DESIGNED | DRAWN | CHECKED |
|---|---|---|
| MTD | BDK | MTD |

SCALE 1"=30'
DATE JUNE 9, 2016
PROJECT NO. 1967-21
SHEET NAME 1




## SITE PREPARATION / CONSTRUCTION SCHEDULE

1. HERBICIDE APPLICATION TO REMOVE DIFFICULT SPECIES.
2. MECHANICALLY REMOVE UNDESIRABLE LARGER WOODY SPECIES.
3. DEEP TINE AERATION AND LAWN SCALPING.
4. SMOTHER TURF WITH CLEAN TOPSOIL AND MULCH FOR PLANTS.

**TOTAL ACREAGE OF PROPOSED PLANTING: 1.35 ACRES *(THIS SHEET ONLY)***

## PLANT SCHEDULE AREA 2

| TREES | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| BR | 3 | Betula nigra | River Birch | 2.5" Cal. | B & B | FULL & DENSE |
| CC3 | 2 | Cornus florida `Cherokee Brave` | Cherokee Brave Dogwood | 2.5" Cal. | B & B | SINGLESTEM |
| JE2 | 2 | Juniperus virginiana | Eastern Red Cedar | 7`/8` HT. | B & B | FULL & DENSE |
| NS | 1 | Nyssa sylvatica | Sour Gum | 2.5" Cal. | B & B | FULL & DENSE |
| PW | 2 | Pinus alba | White Pine | 7`/8` HT. | B & B | FULL & DENSE |

| SHRUBS | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CO | 7 | Cephalanthus occidentalis | Buttonbush | --- | #3 | FULL & DENSE |
| CA2 | 7 | Clethra alnifolia | Summersweet Clethra | --- | #3 | FULL & DENSE |
| CR | 8 | Cornus sericea | Red Twig Dogwood | --- | #3 | FULL & DENSE |
| IW | 7 | Ilex verticillata | Winterberry | --- | #3 | FULL & DENSE |
| MP2 | 7 | Myrica pensylvanica | Northern Bayberry | --- | #3 | FULL & DENSE |
| RG | 8 | Rhus aromatica `Gro-Low` | Gro-Low Fragrant Sumac | --- | #3 | FULL & DENSE |
| RT | 3 | Rhus typhina `Tiger Eyes` | Tiger Eyes Sumac | --- | #3 | FULL & DENSE |

## NATURALIZED PLANT SCHEDULE AREA 2

WET EDGE — 1,738 sf
Carex crinita / CaterpillarSedge
Eupatorium dubium / Joe-Pye Weed
Iris versicolor / Blue Flag
Juncus effusus / Soft Rush
Lobelia cardinalis / Cardinal Flower
Matteuccia struthiopteris / Ostrich Fern
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Senecio aureus / Golden Ragwort
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Comon Ironweed

TRANSITION — 35,569 sf
Asclepias tuberosa / Butterfly Milkweed
Carex pensylvanica / Pennsylvania Sedge
Dennstaedtia punctilobula / Hay-scented Fern
Echinacea purpurea / Purple Coneflower
Lobelia cardinalis / Cardinal Flower
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Schizachyrium scoparium / Little Bluestem Grass
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Common Ironweed

MEADOW — 21,522 sf
Andropogon gerardii / Big Blue Stem
Carex pensylvanica / Pennsylvania Sedge
Chasmanthium latifolium / Wood Oats
Deschampsia flexuosa / Wavy Hair Grass
Echinacea purpurea / Purple Coneflower
Erigeron philadelphicus / Philadelphia Fleabane
Geranium maculatum / Spotted Geranium
Liatris spicata / Spike Gayfeather
Lupinus perennis / Wild Lupine
Panicum virgatum / Switch Grass
Phlox paniculata / Garden Phlox
Rudbeckia laciniata / Cutleaf Coneflower
Schizachyrium scoparium / Little Bluestem Grass
Sorghastrum nutans / Indian Grass
Sporobolus heterolepis / Prairie Dropseed
Symphyotrichum novae-angliae / New England Aster
Waldsteinia fragarioides / Appalachian Barren Strawberry

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DESCRIPTION | DATE | BY

**CONCEPTUAL PLANTING PLAN - AREA 2**
USGA NATURALIZATION PROJECT
THE LYNX AT RIVER BEND GOLF CLUB
PORT JERVIS
ORANGE COUNTY, NEW YORK

DESIGNED: MTD | DRAWN: BDK | CHECKED: MTD
SCALE: 1"=30'
DATE: JUNE 9, 2016
PROJECT NO.: 1967-21
SHEET NAME: 2


## SITE PREPARATION / CONSTRUCTION SCHEDULE

1. HERBICIDE APPLICATION TO REMOVE DIFFICULT SPECIES.
2. MECHANICALLY REMOVE UNDESIRABLE LARGER WOODY SPECIES.
3. DEEP TINE AERATION AND LAWN SCALPING.
4. SMOTHER TURF WITH CLEAN TOPSOIL AND MULCH FOR PLANTS.

**TOTAL ACREAGE OF PROPOSED PLANTING:**
**0.07 ACRES *(THIS SHEET ONLY)***

## PLANT SCHEDULE AREA 3A

| TREES | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| AL | 1 | Amelanchier laevis | Allegheny Serviceberry | 2.5" Cal. | B & B | FULL & DENSE |

## NATURALIZED PLANT SCHEDULE AREA 3A

MEADOW — 2,750 sf

Andropogon gerardii / Big Blue Stem
Carex pensylvanica / Pennsylvania Sedge
Chasmanthium latifolium / Wood Oats
Deschampsia flexuosa / Wavy Hair Grass
Echinacea purpurea / Purple Coneflower
Erigeron philadelphicus / Philadelphia Fleabane
Geranium maculatum / Spotted Geranium
Liatris spicata / Spike Gayfeather
Lupinus perennis / Wild Lupine
Panicum virgatum / Switch Grass
Phlox paniculata / Garden Phlox
Rudbeckia laciniata / Cutleaf Coneflower
Schizachyrium scoparium / Little Bluestem Grass
Sorghastrum nutans / Indian Grass
Sporobolus heterolepis / Prairie Dropseed
Symphyotrichum novae-angliae / New England Aster
Waldsteinia fragarioides / Appalachian Barren Strawberry

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| DESCRIPTION | DATE | BY |
|---|---|---|
| | | |

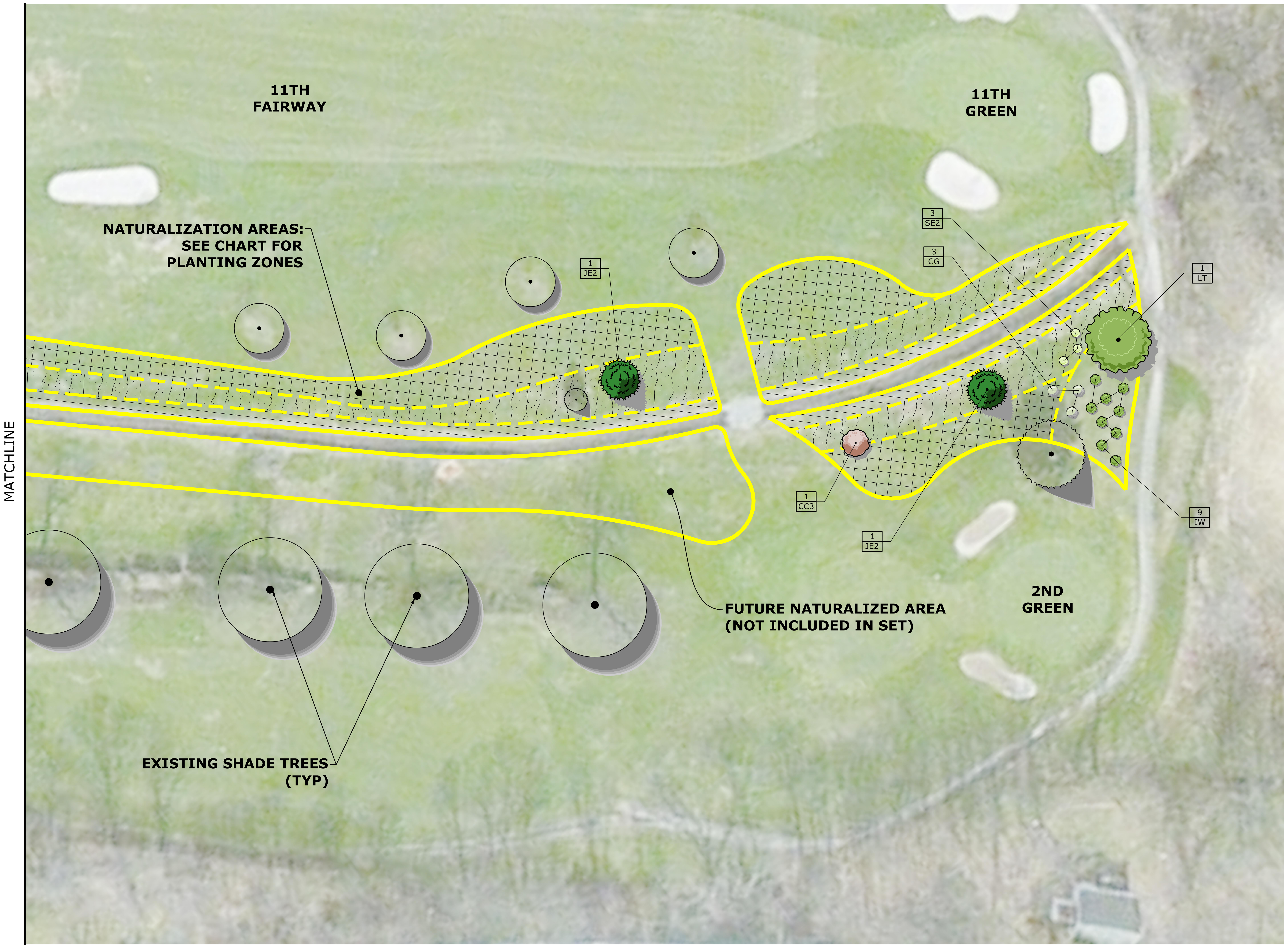
**CONCEPTUAL PLANTING PLAN - AREA 3A**

**USGA NATURALIZATION PROJECT**
**THE LYNX AT RIVER BEND GOLF CLUB**

**PORT JERVIS**
**ORANGE COUNTY, NEW YORK**

| DESIGNED | DRAWN | CHECKED |
|---|---|---|
| MTD | BDK | MTD |

SCALE: 1"=30'
DATE: JUNE 9, 2016
PROJECT NO.: 1967-21
SHEET NAME: **3A**




## SITE PREPARATION / CONSTRUCTION SCHEDULE

1. HERBICIDE APPLICATION TO REMOVE DIFFICULT SPECIES.
2. MECHANICALLY REMOVE UNDESIRABLE LARGER WOODY SPECIES.
3. DEEP TINE AERATION AND LAWN SCALPING.
4. SMOTHER TURF WITH CLEAN TOPSOIL AND MULCH FOR PLANTS.

**TOTAL ACREAGE OF PROPOSED PLANTING:**
**0.77 ACRES *(THIS SHEET ONLY)***

## PLANT SCHEDULE AREA 3B

| TREES | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CC3 | 1 | Cornus florida `Cherokee Brave` | Cherokee Brave Dogwood | 2.5" Cal. | B & B | SINGLESTEM |
| JE2 | 2 | Juniperus virginiana | Eastern Red Cedar | 7`/8` HT. | B & B | FULL & DENSE |
| LT | 1 | Liriodendron tulipifera | Tulip Tree | 2.5" Cal. | B & B | FULL & DENSE |

| SHRUBS | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CG | 3 | Cornus racemosa | Gray Dogwood | --- | #3 | FULL & DENSE |
| IW | 9 | Ilex verticillata | Winterberry | --- | #3 | FULL & DENSE |
| SE2 | 3 | Sambucus canadensis | Elderberry | --- | #3 | FULL & DENSE |

## NATURALIZED PLANT SCHEDULE AREA 3B

WET EDGE — 6,825 sf
Carex crinita / CaterpillarSedge
Eupatorium dubium / Joe-Pye Weed
Iris versicolor / Blue Flag
Juncus effusus / Soft Rush
Lobelia cardinalis / Cardinal Flower
Matteuccia struthiopteris / Ostrich Fern
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Senecio aureus / Golden Ragwort
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Comon Ironweed

TRANSITION — 12,957 sf
Asclepias tuberosa / Butterfly Milkweed
Carex pensylvanica / Pennsylvania Sedge
Dennstaedtia punctilobula / Hay-scented Fern
Echinacea purpurea / Purple Coneflower
Lobelia cardinalis / Cardinal Flower
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Schizachyrium scoparium / Little Bluestem Grass
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Common Ironweed

MEADOW — 13,727 sf
Andropogon gerardii / Big Blue Stem
Carex pensylvanica / Pennsylvania Sedge
Chasmanthium latifolium / Wood Oats
Deschampsia flexuosa / Wavy Hair Grass
Echinacea purpurea / Purple Coneflower
Erigeron philadelphicus / Philadelphia Fleabane
Geranium maculatum / Spotted Geranium
Liatris spicata / Spike Gayfeather
Lupinus perennis / Wild Lupine
Panicum virgatum / Switch Grass
Phlox paniculata / Garden Phlox
Rudbeckia laciniata / Cutleaf Coneflower
Schizachyrium scoparium / Little Bluestem Grass
Sorghastrum nutans / Indian Grass
Sporobolus heterolepis / Prairie Dropseed
Symphyotrichum novae-angliae / New England Aster
Waldsteinia fragarioides / Appalachian Barren Strawberry

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DESCRIPTION | DATE | BY

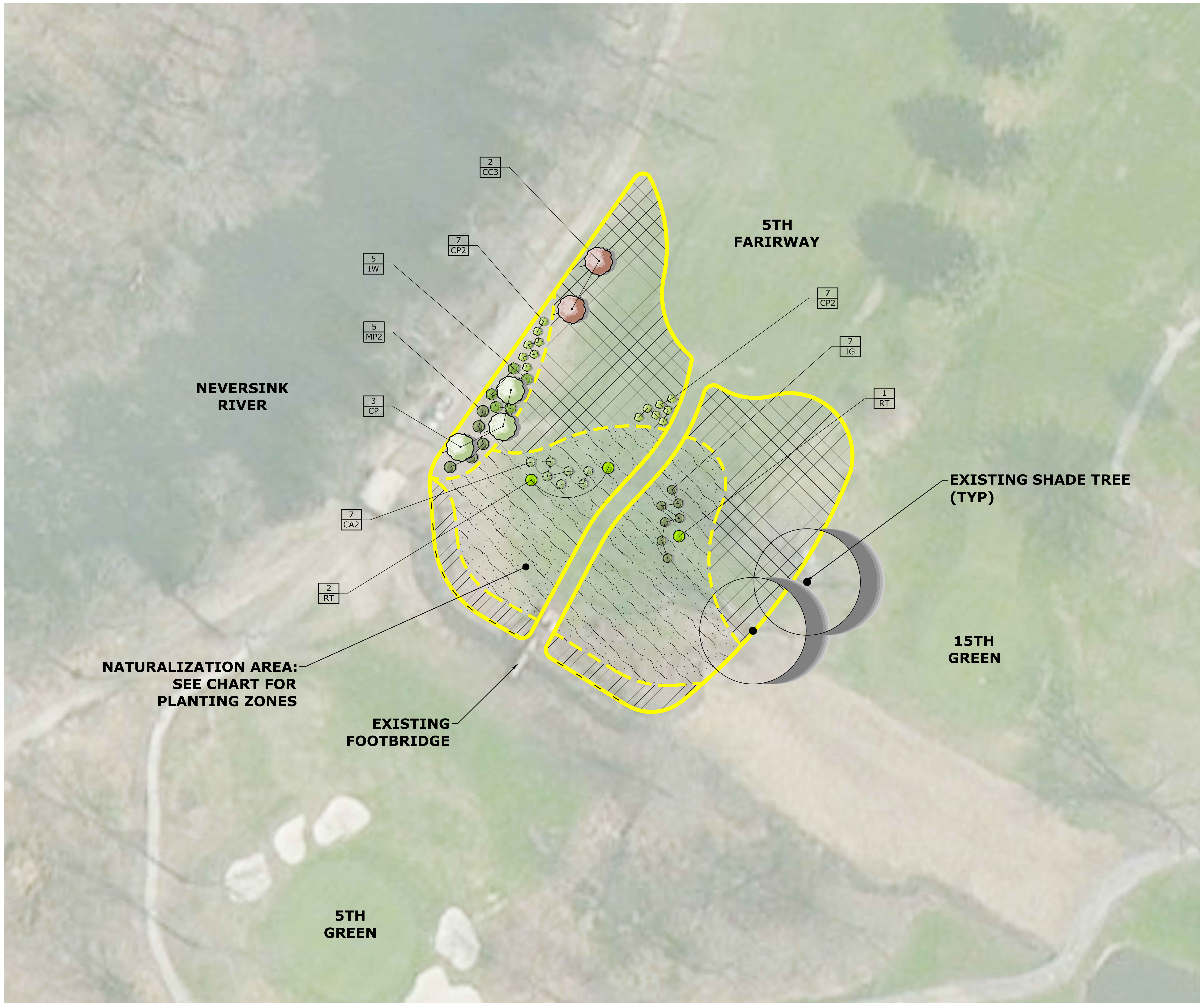
**CONCEPTUAL PLANTING PLAN - AREA 3B**
USGA NATURALIZATION PROJECT
THE LYNX AT RIVER BEND GOLF CLUB
PORT JERVIS
ORANGE COUNTY, NEW YORK

MTD DESIGNED | BDK DRAWN | MTD CHECKED
SCALE 1"=30'
DATE JUNE 9, 2016
PROJECT NO. 1967-21
SHEET NAME 3B

## SITE PREPARATION / CONSTRUCTION SCHEDULE

1. HERBICIDE APPLICATION TO REMOVE DIFFICULT SPECIES.
2. MECHANICALLY REMOVE UNDESIRABLE LARGER WOODY SPECIES.
3. DEEP TINE AERATION AND LAWN SCALPING.
4. SMOTHER TURF WITH CLEAN TOPSOIL AND MULCH FOR PLANTS.

**TOTAL ACREAGE OF PROPOSED PLANTING:**
**0.68 ACRES *(THIS SHEET ONLY)***




## PLANT SCHEDULE AREA 4

| TREES | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CC3 | 2 | Cornus florida `Cherokee Brave` | Cherokee Brave Dogwood | 2.5" Cal. | B & B | SINGLESTEM |
| CP | 3 | Cornus florida `Cherokee Princess` | Cherokee Princess Dogwood | 2.5" Cal. | B & B | SINGLESTEM |

| SHRUBS | QTY | BOTANICAL NAME | COMMON NAME | SIZE | CONT. | COMMENTS |
|---|---|---|---|---|---|---|
| CA2 | 7 | Clethra alnifolia | Summersweet Clethra | --- | #3 | FULL & DENSE |
| CP2 | 14 | Comptonia peregrina | Sweet Fern | --- | #3 | FULL & DENSE |
| IG | 7 | Ilex glabra | Inkberry Holly | --- | #3 | FULL & DENSE |
| IW | 5 | Ilex verticillata | Winterberry | --- | #3 | FULL & DENSE |
| MP2 | 5 | Myrica pensylvanica | Northern Bayberry | --- | #3 | FULL & DENSE |
| RT | 3 | Rhus typhina `Tiger Eyes` | Tiger Eyes Sumac | --- | #3 | FULL & DENSE |

## NATURALIZED PLANT SCHEDULE AREA 4

WET EDGE — 2,127 sf
Carex crinita / CaterpillarSedge
Eupatorium dubium / Joe-Pye Weed
Iris versicolor / Blue Flag
Juncus effusus / Soft Rush
Lobelia cardinalis / Cardinal Flower
Matteuccia struthiopteris / Ostrich Fern
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Senecio aureus / Golden Ragwort
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Comon Ironweed

TRANSITION — 13,020 sf
Asclepias tuberosa / Butterfly Milkweed
Carex pensylvanica / Pennsylvania Sedge
Dennstaedtia punctilobula / Hay-scented Fern
Echinacea purpurea / Purple Coneflower
Lobelia cardinalis / Cardinal Flower
Onoclea sensibilis / Sensitive Fern
Osmunda cinnamomea / Cinnamon Fern
Schizachyrium scoparium / Little Bluestem Grass
Vernonia lettermannii `Iron Butterfly` / Iron Butterfly Ironweed
Vernonia noveboracensis / Common Ironweed

MEADOW — 14,304 sf
Andropogon gerardii / Big Blue Stem
Carex pensylvanica / Pennsylvania Sedge
Chasmanthium latifolium / Wood Oats
Deschampsia flexuosa / Wavy Hair Grass
Echinacea purpurea / Purple Coneflower
Erigeron philadelphicus / Philadelphia Fleabane
Geranium maculatum / Spotted Geranium
Liatris spicata / Spike Gayfeather
Lupinus perennis / Wild Lupine
Panicum virgatum / Switch Grass
Phlox paniculata / Garden Phlox
Rudbeckia laciniata / Cutleaf Coneflower
Schizachyrium scoparium / Little Bluestem Grass
Sorghastrum nutans / Indian Grass
Sporobolus heterolepis / Prairie Dropseed
Symphyotrichum novae-angliae / New England Aster
Waldsteinia fragarioides / Appalachian Barren Strawberry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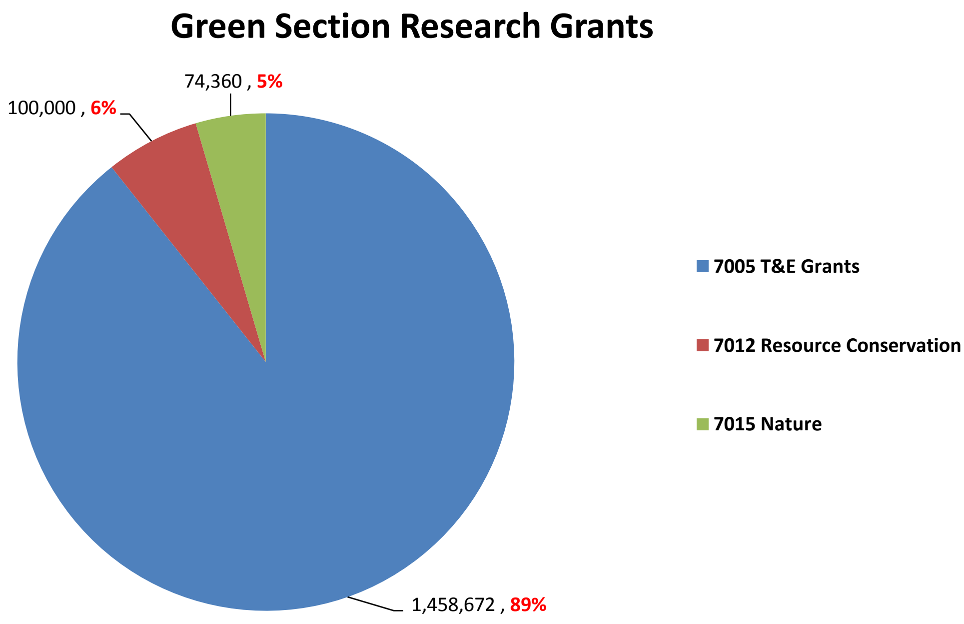
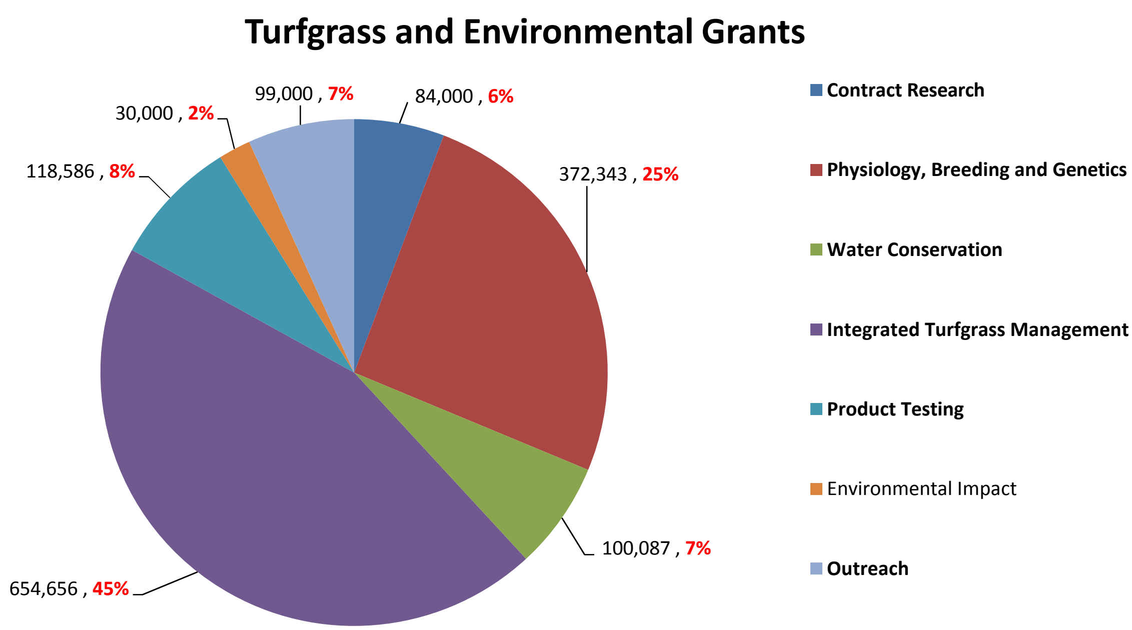
| DESCRIPTION | DATE | BY |
|---|---|---|

CONCEPTUAL PLANTING PLAN - AREA 4
USGA NATURALIZATION PROJECT
THE LYNX AT RIVER BEND GOLF CLUB
PORT JERVIS
ORANGE COUNTY, NEW YORK

| DESIGNED | DRAWN | CHECKED |
|---|---|---|
| MTD | BDK | MTD |

SCALE 1"=30'
DATE JUNE 9, 2016
PROJECT NO. 1967-21
SHEET NAME 4




| **Turfgrass & Environmental Research** | **2016** | **2017** |
|---|---|---|
| Contract Research | 84,000 | 159,800 |
| Physiology, Breeding and Genetics | 372,343 | 366,179 |
| Water Conservation | 100,087 | 169,196 |
| Integrated Turfgrass Management | 654,656 | 632,261 |
| Product Testing | 118,586 | 112,451 |
| Environmental Impact | 30,000 | 12,600 |
| Outreach | 99,000 | 74,500 |
| **7005 T&E Grants** | **1,458,672** | **1,526,987** |
| **7012 Resource Conservation** | **100,000** | **100,000** |
| **7015 Nature** | **74,360** | **54,329** |
| **7000 Grant Accounts** | **1,558,672** | **1,626,987** |