



Turfgrass and Environmental Research Online

...Using Science to Benefit Golf



The *2008 USGA Turfgrass and Environmental Research Summary* is an annual compilation of projects currently funded by USGA's Turfgrass and Environmental Research Program. The summary contains research summaries of 75 projects funded in 2008.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 350 projects at a cost of \$29 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf.***

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2008 USGA Turfgrass and Environmental Research Summary

Jeff L. Nus

Since 1983, the United States Golf Association has funded more than 350 university research projects at a cost of \$29 million. The Turfgrass and Environmental Research Program provides direction to these intuitions and employs **science as the foundation to benefit golf** in the areas of turfgrass and resource management, sustainable development, and environmental protection. At the end of each year, the USGA provides a summary of the research conducted under this important national program and this report summarizes the results from 2008.

There are two primary goals of the research program. The first is to develop turfgrasses and cultural systems with better stress tolerance and reduced water requirements and pesticide use. To address the USGA's first research goal, 49 research projects were funded in integrated turfgrass management, physiology, breeding, genetics, and course construction practices. The second goal is to investigate environmental issues and sustainable resource management for golf courses. Five research projects that investigate the environmental impact of golf courses are reported.

The research program actively coordinates and supports research, associated educational programs, and other partnerships to benefit golf, the environment, and people. For example, the USGA, GCSAA, and National Turfgrass Evaluation Program (NTEP) together have developed turfgrass variety testing programs conducted on golf courses throughout the United States. In addition, the USGA works with state research foundations and superintendent chapters to fund applied research through the Grant-in-Aid Research Program. Ten research projects are included in this report under the Grant-in-Aid Research Program. USGA's Turfgrass and Environmental Research Program also provides funding to the National Fish and Wildlife Foundation to support the Wildlife Links Research Program. Seven projects are currently being funded and summaries of their progress are included in this report.



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The United States Golf Association Turfgrass and Environmental Research Program

Vision

Use science as the foundation to benefit golf in the areas of turfgrass and resource management, sustainable development and environmental protection.

Mission

Coordinate and support research, associated educational programs, and partnerships to benefit golf, the environment, and people.

Goals

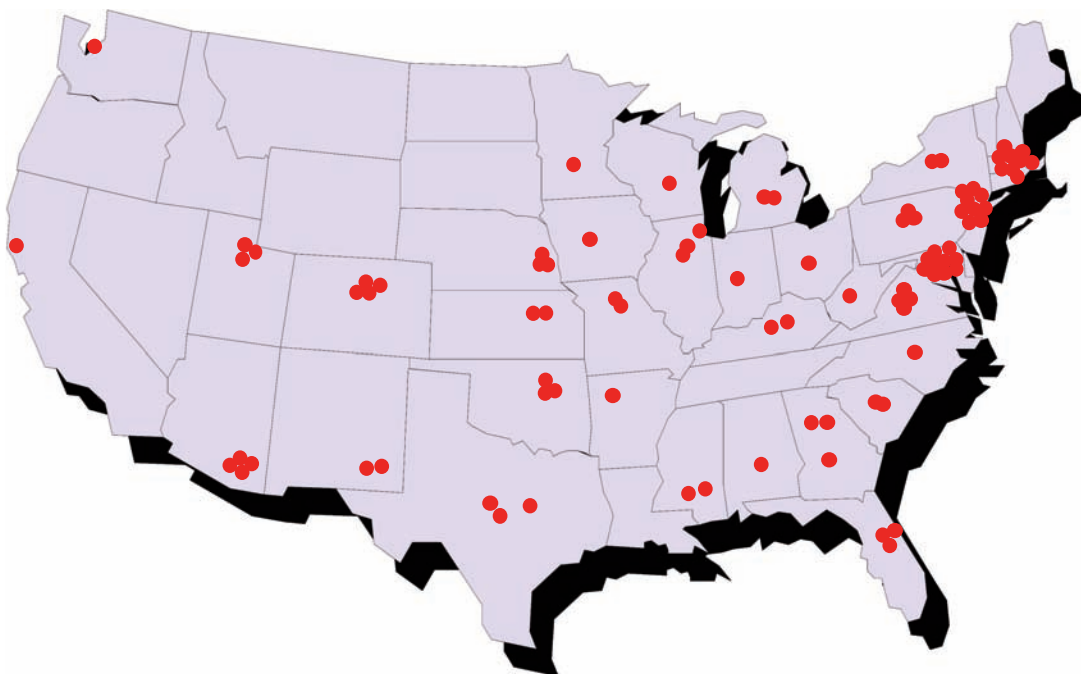
Develop turfgrasses and cultural systems with enhanced stress tolerance and reduced supplemental water requirements, pesticide use and costs.

- *Course Construction Practices*
- *Integrated Turfgrass Management*
- *Breeding, Genetics, and Physiology*

Investigate environmental issues and sustainable resource management for golf courses.

- *Environmental Impact of Golf Courses*
- *Wildlife and Habitat Management*

**Location of 2008 projects funded by the USGA
Turfgrass and Environmental Research Program**



**USGA Green Section Turfgrass and Environmental Research
Project Grants in 2008**

Project Area	Number	Grant \$	% of Total
Course Construction Practices	2	60,000	3.8 %
Integrated Turfgrass Management	22	444,438	28.1 %
Sustainable Management	7	121,605	7.7 %
Pathology	8	181,667	11.5 %
Entomology	7	141,166	8.9 %
Breeding, Genetics, and Physiology	30	640,599	40.4 %
Cool-season Grasses	17	379,921	24.0 %
Warm-season Grasses	6	141,049	8.9 %
New or Native Grasses	7	119,629	7.5 %
Environmental Impact	12	311,668	19.6 %
Fate and Transport	5	111,668	7.0 %
Wildlife Links Program	7	200,000	12.6 %
Outreach Programs	27	127,000	8.0 %
Grant-in-Aid Research Program	17	58,000	3.7 %
Product Testing	7	34,000	2.1 %
Cooperative Research-Allied Assoc.s	3	35,000	2.2 %
Total	93	1,584,205	100.0%

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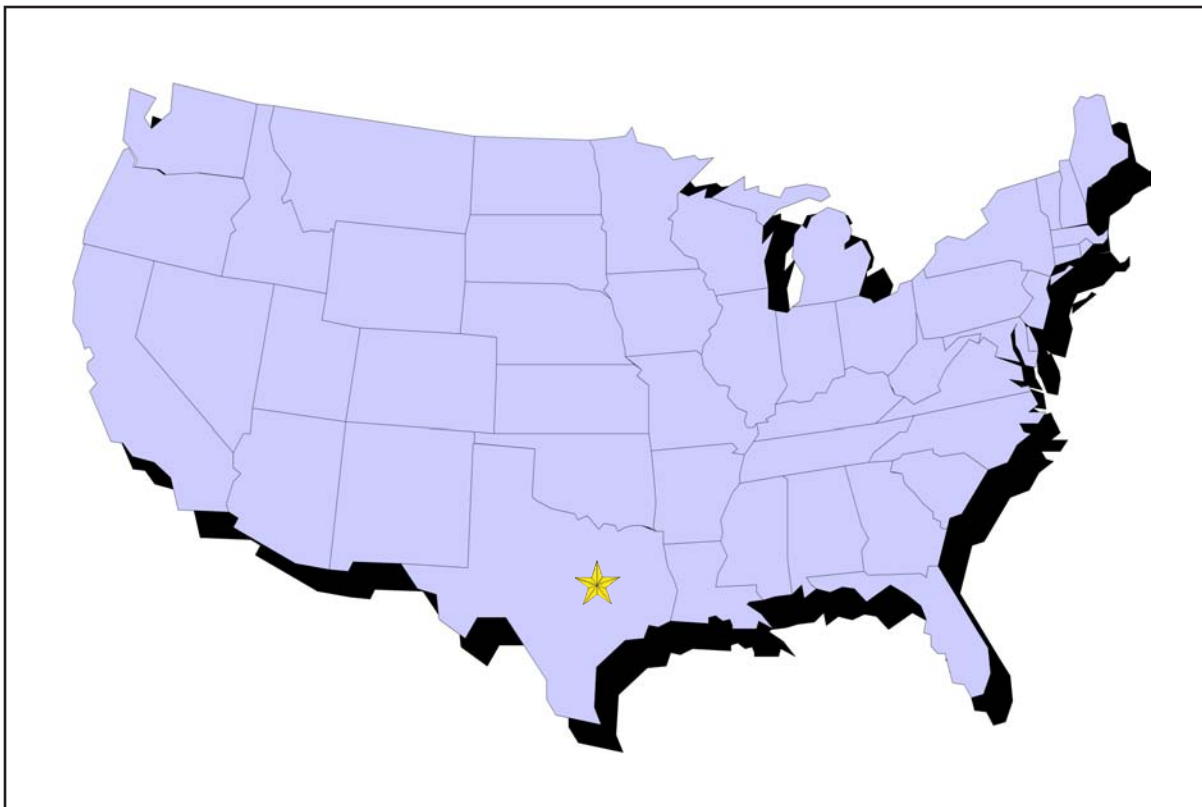
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Course Construction Practices

To take advantage of new ideas and technologies and to address the environmental and economic challenges of the coming decades, USGA sponsors research studies on the construction and establishment of golf courses. Preference is given to research studies that address issues related to:

1. Materials testing procedures for putting green rootzones and bunker sands
2. Alternative construction methods and materials
3. Effects of irrigation water quality on the selection of construction materials
4. Moisture-related problems in putting green rootzones
5. Calcareous sands
6. Various organic matter sources
7. Greens/surrounds interface construction and management
8. Alternative irrigation methods
9. How to amend various-textured native soils for use as tees (and possibly low-cost greens).

Interdisciplinary approaches are expected including the disciplines of soil physics, soil chemistry, soil microbiology, physiology, management, and pathology. Studies should give due consideration to environmental issues and to the use of alternative water sources for golf course irrigation.



Locations of the following projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the category of Course Construction Practices

A Comparison of Water Drainage and Storage in Putting Greens Built Using Airfield Systems and USGA Methods of Construction

Kevin McInnes and James Thomas
Texas A&M University

Objectives:

1. Investigate potential for clogging of geotextile pores from fines migrating out of the rootzone mixture.
2. Investigate the changes in the temporal distribution of drainage and spatial distribution of water holding capacity of a green constructed with a geotextile compared to a green constructed with gravel.
3. Investigate construction methods designed to reduce down-slope movement of water in sloping greens.
4. Develop criteria for selecting appropriate rootzone mixtures for putting greens constructed with geotextiles.

Start Date: 2007

Project Duration: three years

Total Funding: \$90,000

This research investigates the dynamics of water movement through, and storage within, the rootzone of putting greens constructed using a geotextile atop a plastic support acting as the drainage structure (Airfield System's design) compared to a green constructed with a gravel-based drainage structure (USGA recommended).

Constructed to USGA specifications with a 100-mm layer of gravel, sand-based rootzone mixture placed over gravel holds water at the rootzone-gravel interface at tensions between 0 and 100 mm water when watered to such a degree that drainage occurs. The Airfield Systems' design replaces the gravel with a geotextile atop a 25-mm deep porous plastic support for drainage. Our laboratory observations have shown that the bottom of the rootzone in the Airfield Systems design is typically at about 50 mm less tension after drainage.

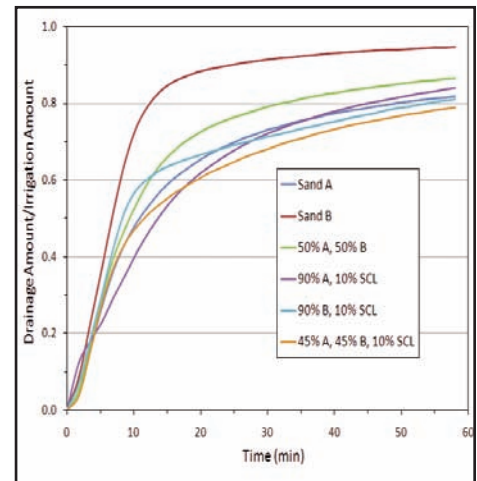
One hundred eighty permeameters were constructed from 150-mm inner diameter by 350-mm tall PVC pipe and end caps. Each permeameter was filled with 300-mm deep rootzone mixture over

a geotextile supported on a 25-mm deep plastic geogrid (Airfield Systems' AirDrain). A tensiometer was placed on top of the geotextile in each test cell before it was filled with the rootzone mixture.

Ten geotextiles were evaluated: two woven, three spunbond, five needle punch. The woven materials were WM104F (Propex) and FW404 (Tencate). The spunbond fabrics were Typar 3301L (Fiberweb), Typar 3341G (Fiberweb), and Lutradur 130g (Freudenberg). The needle punch materials were NW351 (Propex), NW401 (Propex), NW1001 (Propex), NW10 (Gundle/SLT Environmental), and NW16 (Gundle/SLT Environmental). Apparent opening sizes (AOS) of the ten geotextiles were 212, 425, 300, 250, 122, 300, 212, 150, 150, 150 micrometers, respectively.

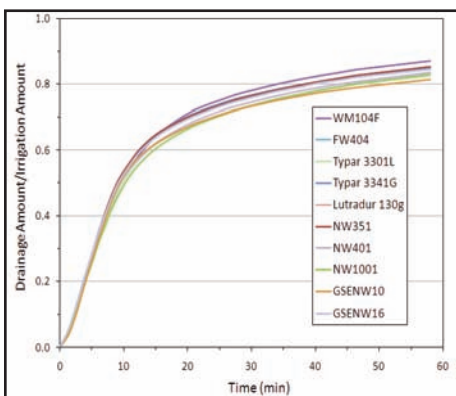
Six rootzone mixtures with differing distributions of fines were created by blending three parent materials with differing particle size distributions, two sands and a sandy clay loam (SCL). One parent-material sand's particle size distribution fell in the middle of the USGA specifications (Sand A). The other parent-material sand exceeded the specified limits for fine and very fine sand (Sand B). Total fines <150 micrometer ranged from 6% to 39% for the six rootzone mixtures. Each geotextile was tested in combination with all six rootzone mixtures.

Test cells were irrigated at rates representative of cultural practices for establishment and maintenance of a putting green. Greater amounts of water were applied periodically to simulate storm events and to observe changes in whole-system permeability and drainage. Changes in whole-system permeability and drainage were assessed by measuring the temporal distributions of drainage rate from an instantaneous application of 25 mm (1 inch) of water to the top of the test cell.



Permeability and drainage of tests cells were affected by rootzone mixtures (above), but not through clogging of geotextiles.

We did not observe positive pressure atop the geotextiles for any appreciable time. When drainage rates from the test runs were averaged across rootzone mixtures for each of the geotextiles, no significant differences in drainage rates were observed. We conclude that geotextiles in the test cells have not clogged. When drainage rates from the test runs were averaged across geotextiles for each of the rootzone mixtures, significant differences in drainage rates were observed. We conclude that the particle size distribution of the rootzone mixtures affects drainage rate, as expected, but not through clogging of the geotextiles.



Ten geotextiles were evaluated: two woven, three spunbond, five needle punch. Permeameter tests showed that none of the geotextiles clogged during the test period using 1 inch (25 mm) of irrigation water.

Summary Points

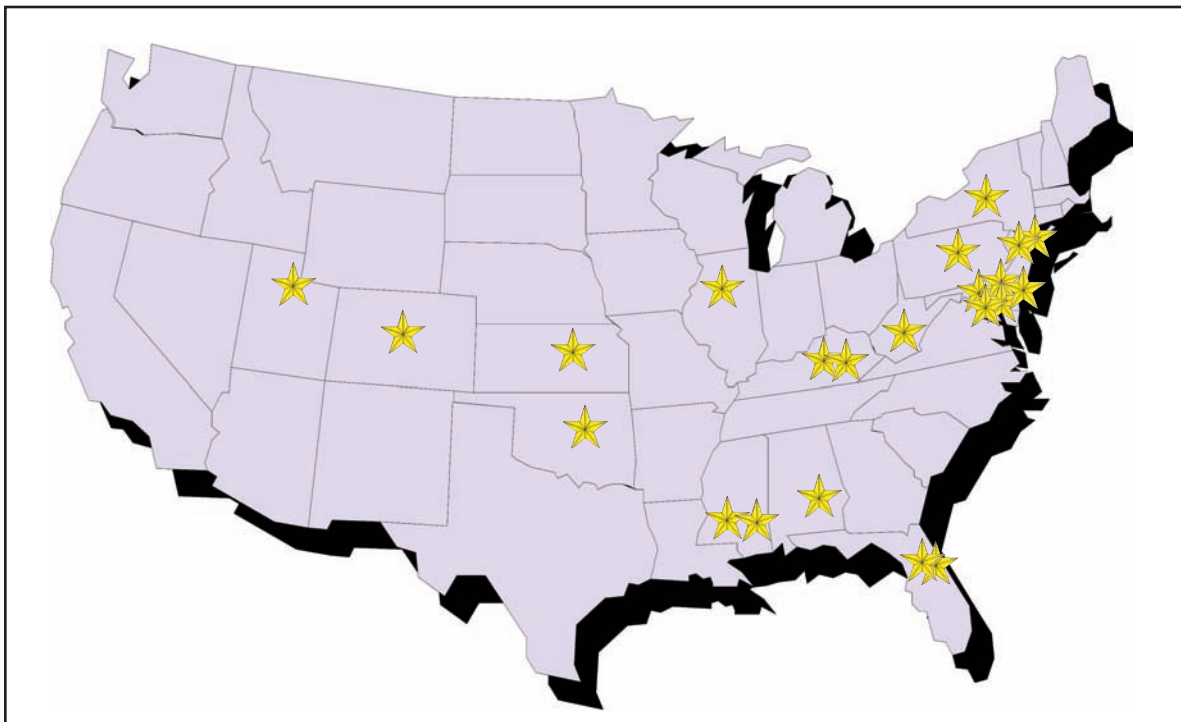
- Clogging of geotextiles was not observed
- Permeability and drainage of tests cells containing rootzone mixtures with total fines <150 micrometers ranging between 6 and 39% were not affected by geotextiles whose apparent opening size ranged from 150 to 400 micrometers.
- Permeability and drainage of tests cells were affected by rootzone mixtures, but not through clogging of geotextiles.

Integrated Turfgrass Management

Improved turfgrasses developed for use on golf courses require management practices that provide quality playing surfaces while conserving natural resources and protecting the environment. A series of research projects are being funded with the aim of conserving natural resources by reducing the use of water, pesticides, and fertilizers. The objectives of these studies will focus on the following:

1. Develop cultural practices that allow efficient turfgrass management under unique conditions, such as poor quality soils, shade, and marginal quality water.
2. Determine the range of adaptability and stress tolerance of turfgrasses.
3. Evaluate direct and interacting effects of two or three cultural practices like mowing, irrigation, fertilization, cultivation, compost utilization, and develop programs to control pests and organic matter accumulation (thatch).
4. Investigate pest management practices such as biological, cultural, and mechanical controls, application of turf management practices utilizing IPM and reduced inputs, and pest modeling and forecasting.

The results of these studies should lead to the development of turfgrass management programs that conserve our natural resources and reduce costs, with minimal impairment of playing quality conditions or aesthetic appeal. We encourage regional cooperation among researchers where similar climactic and soil conditions exist.



Location of the following projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the category of Integrated Turfgrass Management

Biological and Biorational Management Options for the Annual Bluegrass Weevil on Golf Courses

Benjamin A. McGraw and Albrecht M. Koppenhöfer
Rutgers University

Objectives:

1. Conduct surveys for entomopathogenic nematodes in annual bluegrass weevil (ABW) infested areas and adult ABW hibernation sites on golf courses.
2. Determine the virulence to annual bluegrass weevil of entomopathogenic nematodes, *Bacillus thuringiensis* (Bt) strains, and several biorational compounds.
3. Determine the field efficacy of promising entomopathogenic nematodes, *Bacillus thuringiensis*, and several biorational compounds.

Start Date: 2006

Project Duration: three years

Total Funding: \$69,532

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, formerly 'Hyperodes weevil', is a serious and expanding pest of close-cut annual bluegrass on golf courses through much of the Northeast. Adult ABW emerge from overwintering sites in leaf litter and tall rough in early April and migrate to short mowed turfgrasses (greens, tees, fairways) to feed and mate. Females lay eggs directly into the stem of the turfgrass plant from late April through May in New Jersey.

The young larvae are initially stem borers, feeding internally on the plant, ultimately tunneling through the crown and destroying the turfgrass plant. Later instars feed externally on crowns and roots which leads to the most extensive turf loss typically around early to mid June (Figure 1). Damage caused by the 2nd and 3rd generations is usually less severe and more localized as peak larval densities tend to be lower than in the 1st generation. In previous project years we found that



Figure 1. First generation larval feeding damage to an edge of a fairway (June 23, 2008).

entomopathogenic nematodes (EPNs) naturally occurring in golf course fairways not treated for ABW control can cause up to 50% generational ABW mortality. Additionally, EPNs have provided high levels of larval control (65-100%) when applied to field-infested turf cores in the laboratory.

We examined the efficacy of commercial EPN products against 1st generation ABW immature stages in field trials in 2008. Products based on *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis bacteriophora* were chosen for closer examination in field trials since they have provided at least 70% control in past field trials (Figure 2). Since lower control levels in 2007 than in 2006 were believed to be linked to high ABW larval populations in the treated areas, we included higher EPN rates [0.5, 1.0, and 2 billion infective juveniles (IJs) per acre] in the 2008 trials. However, despite lower larval densities, the higher EPN rates did not provide greater or even equivalent control than in 2007. Only the high rate of *S. carpocapsae* provided statistically significant control (86%).

In the 2007 trials, treatments consisting of combinations of species and applications split into half doses applied in consecutive weeks provided higher levels of control than the 1 billion IJs per acre rate alone. In 2008, the three combinations of two species treatments failed to improve upon the levels of control observed at the full rate (1 billion IJs/acre) of either of the species applied alone. Similarly, splitting applications into two consecutive half-rate treatments (0.5 billion IJs/acre) did not provide better control than the full or half rate applied once. The inconsistent control of ABW by EPNs could be due to numerous abiotic (e.g., weather, soil type) and biotic (e.g., EPN formulation, persistence,

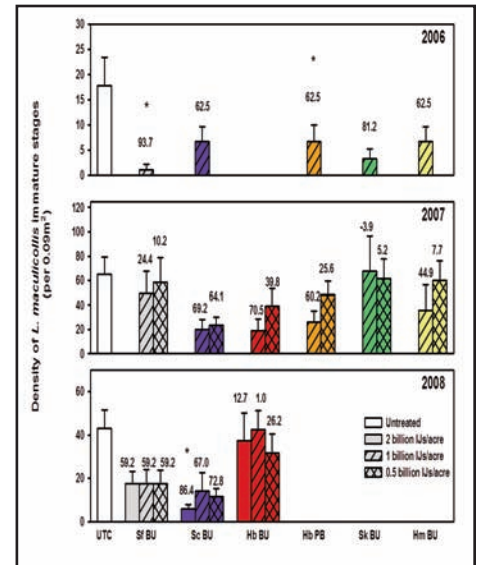


Figure 2. Density of ABW stages (average ± SE) in a field experiment 2 weeks after application of the nematodes *S. carpocapsae* (Sc), *H. bacteriophora* (Hb), *H. megidis* (Hm), *S. feltiae* (Sf), and *S. kraussei* (Sk) for field trials conducted in 2006, 2007, and 2008. Nematodes were commercial strains (BU = Becker Underwood) except for one field isolated (Hb PB). Figures above bars are percent reduction compared to untreated control (UTC). Asterisks (*) indicate significant mean separation from the UTC ($P < 0.05$).

ABW density) factors. Another year of field trials will be conducted to solve some of the inconsistencies in control of ABW with EPNs.

Summary Points

- Entomopathogenic nematodes can provide significant control of ABW larvae under laboratory conditions. However, in field trials, control was inconsistent between years.
- The level of suppression achieved in the field is likely affected by factors such as EPN concentration, ABW larval densities, and timing of application.
- An additional field season should help clarify the effects of application timing and concentration, and ABW density on the ability of EPNs to suppress ABW below damaging thresholds.

Developing Best Management Practices for Anthracnose Disease on Annual Bluegrass Putting Green Turf

James A. Murphy, Bruce B. Clarke, and Joseph A. Roberts
Rutgers University

Objectives:

1. The multiple objectives of this research were organized into four field studies on annual bluegrass putting green turf that were designed to evaluate the main effect and interactions of : 1) irrigation quantity; 2) lightweight rollers and mowing equipment; 3) topdressing and foot traffic, and 4) nitrogen fertilization on anthracnose disease.

Start Date: 2006

Project Duration: two years

Total Funding: \$60,000

Anthracnose is a destructive disease of annual bluegrass and bentgrass putting green turf throughout the United States. The disease, caused by the fungus *Colletotrichum cereale*, begins as small areas of yellowed turf (1 to 2 inches in diameter) with individual leaf blades eventually senescing. The frequency and severity of anthracnose outbreaks on golf course putting greens has increased over the past decade perhaps due to practices employed to improve playability and increase ball roll distances on putting greens.

An evaluation of how irrigation management practices influence anthracnose was initiated in 2006. Three consecutive years indicate that deficit irrigation (40% daily ET_0 replacement) generally had the greatest amount of disease compared to plots with higher soil water content. Disease tended to decrease as irrigation increased; that is, 60% ET_0 replacement had less disease than 40% ET_0 , and 80% ET_0 had less disease than 60% ET_0 . Replacing 100% ET_0 daily (excessive soil water) had levels of disease similar to 40% ET_0 treatments in August 2006 and 2008. While this relationship was not observed in 2007, turf quality of 100% ET_0 plots was

greatly reduced by August in all three years of study. Irrigation at 80% ET_0 often resulted in the least disease and best turf quality. An additional study was initiated in 2008 to evaluate the effect of mowing timing relative to daily irrigation at 100% ET_0 . Disease severity was the same regardless of whether irrigation was applied before or after mowing.

A study to follow up on previous work was initiated in 2006 and compared the effect of lightweight roller type on anthracnose. Both roller types (i.e., sidewinder and triplex-mounted vibratory) reduced disease compared to non-rolled turf under moderate disease pressure in 2007 and 2008. The heavier sidewinder roller had less disease than the triplex mounted vibratory roller on 4 of 13 rating dates over two years. Turf areas where rollers changed direction of travel and received a "clean-up" mowing at the edge of a putting green had less disease compared to the area where rollers traversed with no clean-up mowing on 6 of 13 rating dates.

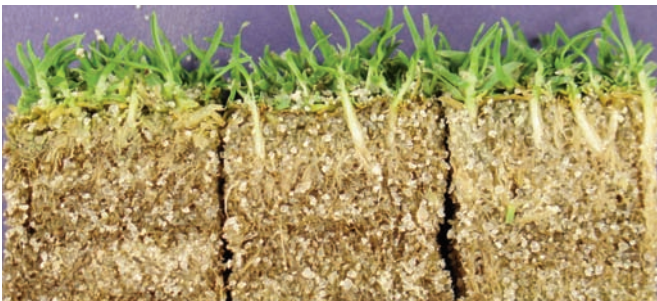
A study was initiated in 2007 to determine the effect of variable rate and frequency of summer soluble N fertility on anthracnose severity. Nitrogen applied at 0.1 lb per 1000 ft^2 every 7 days or 0.2 lb per 1000 ft^2 every 14 days had the greatest reduction in anthracnose severity throughout both years. Nitrogen applied at 0.1 lb per 1000 ft^2 every 14 days was the lowest N treatment to reduce disease severity. Summer soluble N fertilization at 0.1 lb per 1000 ft^2 every 28 or 56 days had the greatest anthracnose severity.

A study was initiated in 2007 to evaluate the effects of sand topdressing and foot traffic

(golf shoes) on anthracnose severity. Sand topdressing initially increased anthracnose in 2007; however, continued weekly applications of sand reduced anthracnose severity by August 2007 and throughout 2008. Sand topdressing reduced anthracnose severity regardless of foot traffic, and foot traffic decreased anthracnose in the presence or absence of sand topdressing. The combination of daily foot traffic with weekly sand topdressing resulted in the lowest disease severity and best turf quality in both 2007 and 2008.

Summary Points

- Deficit irrigation (40% ET_0) induced wilt stress and intensified anthracnose severity. Irrigation at 80% ET_0 often resulted in the lowest anthracnose severity and best turf quality.
- Lightweight rolling every other day with either roller type (i.e., sidewinder or triplex-mounted vibratory) effectively increased ball-roll distance and decreased anthracnose severity under moderate disease pressure.
- Adequate nitrogen fertility to sustain moderate growth was needed to reduce anthracnose severity. Nitrogen applied every 7 (0.1 lb per 1000 ft^2) or 14 days (0.2 lb per 1000 ft^2) provided the greatest reduction in anthracnose severity. Applications every 14 days at 0.1 lb per 1000 ft^2 also reduced disease, but to a lesser extent.
- Sand topdressing initially increased anthracnose in 2007. Continued weekly applications of sand reduced anthracnose severity by August 2007 and throughout 2008. Sand topdressing reduced anthracnose severity regardless of foot traffic. Moreover, daily foot traffic decreased anthracnose severity. The combination of daily foot traffic with weekly sand topdressing resulted in the lowest disease severity and best turf quality in both 2007 and 2008.



Light frequent sand topdressing buries and protects crowns and leaf sheaths. Note depth of crowns in the middle (1 ft^3 per 1000 ft^2 per week) and right (2 ft^3 per 1000 ft^2 per week) profile samples are deeper than the profile sample on the left (no topdressing).

Relative Pathogenicity and Fungicide Sensitivity of Isolates of *Rhizoctonia* and Other Fungal Pathogens and the Disease Responses of Seashore Paspalum and Zoysiagrass Cultivars

Carol M. Stiles, Philip F. Harmon, and Kevin E. Kenworthy
University of Florida

Objectives:

1. To confirm pathogenicity of *Rhizoctonia zeae* on seashore paspalum and screen isolates of *R. solani* and *R. zeae* to different fungicides.
2. To confirm whether isolates of *Fusarium* or *Microdochium spp.* obtained from diseased seashore paspalum samples are pathogenic. If so, identify and characterize these isolates and develop diagnostic responses and management recommendations.
3. Screen existing seashore paspalum and zoysiagrass cultivars for resistance to various pathogens.
4. Screen zoysiagrass germplasm accessions to select for resistance to *Rhizoctonia spp.*

Start Date: 2006

Project Duration: three years

Total Funding: \$82,000

A diverse group of *Rhizoctonia* pathogens cause disease on warm-season turfgrasses in Florida. Diagnosing these related diseases can be difficult because they produce very similar symptoms on closely-mown turf during overlapping periods of disease-favorable conditions.

Seashore paspalum isolates collected during sampling efforts have proven to be very diverse. Isolates that fall within the *Waitea circinata* species complex that includes *R. zeae*, *R. oryzae*, *W. circinata* var. *circinata* and others are especially interesting. Additional research needs to be done to further characterize this group of pathogens.

Rhizoctonia zeae isolates have been used to inoculate seashore paspalum cultivars and symptoms similar to those observed in the field (and similar to leaf and sheath spot on bermudagrass) resulted. The pathogens were re-isolated from the symptomatic turf confirming pathogenicity.

Some broad-spectrum fungicide products may be management tools for all of the *Rhizoctonia* diseases, but our fungicide-amended media assays indicate that species of the pathogens differ in sensitivities to most products. These differences may help to explain reports by superintendents of varying management results and efficacy windows for the same product used to treat similar disease symptoms.

Isolates of *R. zeae* were compared to isolates of *R. solani* that cause large patch, those that cause brown patch, *R.*



Isolates of several *Rhizoctonia spp.* have been characterized from warm-season turf in Florida and initial results suggest a more diverse group than expected.

cerealis, and some related species. Isolates of *R. zeae* were less sensitive to all fungicides tested (azoxystrobin, flutolanil, iprodione, propiconazole, pyraclostrobin, tebuconazole, thiophanate methyl, and triadimefon) than *R. solani* isolates. Our results agreed with previously published findings in that *R. zeae* was completely insensitive to thiophanate methyl, but we also discovered that even with fungicides that are effective for both large patch and leaf and sheath spot, leaf and sheath spot isolates are less sensitive as a group than large patch isolates.

Additional differences between isolates were identified that may have management implications. Yellow patch isolates were sensitive as a group to iprodione, but some *R. zeae* and *Marasmius spp.* isolates were moderately to completely insensitive according to our test.

Fungicide evaluation trials on seashore paspalum for control of large patch, dollar spot, and fairy ring have shown that DMI fungicides such as triadimefon, propiconazole, triticonazole,

metconazole, and combinations of strobilurins with these fungicides are safe and effective as disease management tools on seashore paspalum. The products Headway and Tartan have been among the best treatments evaluated and offer broad-spectrum management options from single products.

Progress has been made toward identifying *Fusarium* isolates that affect seashore paspalum. Some of the isolates have been identified as previously described *Fusarium* species and others may be previously undescribed. Considerable work and additional collections will need to go into investigating these apparently new species prior to publishing descriptions and names; however, pathogenicity trials have been completed and management trials are beginning to provide insight. Research is ongoing.

An inoculation of Dr. Kenworthy's zoysiagrass evaluation trial resulted in little disease. Additional isolates are also being tested on a small number of cultivars to find the most pathogenic and consistent isolate to use in further screening and selection research.

Summary Points

- Isolates of several *Rhizoctonia spp.* have been characterized from warm-season turf in Florida and initial results suggest a more diverse group than expected.
- Amended-media studies indicate that *Rhizoctonia spp.* vary in sensitivity to many different fungicides and isolates often vary within species, as well.
- A diverse group of pathogens have been isolated from seashore paspalum turf in Florida. We suspect at least two of the species we have confirmed pathogenicity with are previously undescribed.

Natural Enemies and Site Characteristics Affecting Distribution and Abundance of Native and Invasive White Grubs on Golf Courses

Daniel A. Potter and Carl T. Redmond
University of Kentucky

Objectives:

1. Determine identity and incidence of microbial pathogens and parasitoids of Japanese beetle and masked chafer grubs on golf courses across Kentucky, the first such study in the transitional turfgrass zone. Quantify site characteristics associated with particular grub species and natural enemy incidence.
2. Evaluate how grass species, mowing height, and golf course pesticides affect incidence of pathogen infection and parasitism of naturally occurring white grubs in the field and if impact of naturally-occurring pathogens can be enhanced.
3. Test the hypothesis that natural enemy load on Japanese beetle grub populations on golf courses is greater in geographical regions where populations have stabilized than in regions into which the pest has more recently spread.

Start Date: 2007

Project Duration: three years

Total Funding: \$60,000

Biological insecticides and natural enemy conservation can reduce the need for chemical inputs on golf courses. This work is the first survey of white grubs and their natural enemies on golf courses in the transitional climactic zone, and the first anywhere for masked chafers (*Cyclocephala spp.*), the most important native grub pests. We seek new pathogens having promise as bio-insecticides and to clarify how site characteristics might be altered to enhance natural suppression of grub populations.

Grub survey kits were sent to 34 golf superintendents throughout Kentucky in late summer asking them to collect 30 grubs and a soil sample from their worst non-treated grub site. Samples were returned via overnight mail. Six additional Lexington-area golf courses were intensively sampled in late August, mid-September, and early October to track natural enemy incidence over time.

Grubs were identified, incubated for 30 days, and dissected to assess mortality from bacterial, fungal, or protozoan pathogens. Masked chafers (MC) and Japanese beetles (JB) accounted for 64 and 30%, respectively, of grubs sent in by superintendents. MC also predominated on Lexington courses. Grub populations declined from about 18 per 0.1 m² in late August to about 5 per 0.1 m² in October and 2 per 0.1 m² the following spring owing to natural mortality agents. *Tiphia* wasps, *Metarhizium* fungus, *Serratia* (amber disease) and *Paenibacillus* (milky disease) bacteria, and entomopathogenic



Grubs were identified, incubated for 30 days, and dissected to assess mortality from bacterial, fungal, or protozoan pathogens.

nematodes (EPN) infected 2, 5, 8, 20, and 18% of the MC grubs, and 0, 3, 5, 12, and 19% of the JB grubs sent in by superintendents.

Ovavesicula, a protozoan that causes debilitating disease in adult JB, was uncommon in KY, but gregarines (*Stichospora*) infected 26% of JB grubs in the spring. The latter two pathogens were absent or uncommon in MC grubs. Additional *Stichospora*-infected grubs were reared to determine their fate which has not previously been studied. Such grubs emerged as adults although their maturation was slightly delayed. Eighty EPN strains, 40 each from MC and JB grubs, are being processed for PCR identification.

Replicated stands of irrigated turfs used in fairways or roughs of transition zone golf courses were sampled for grub species preference and incidence of parasitoids and pathogens. Plots maintained at fairway height (5/8") consisted of creeping bentgrass (CB), perennial ryegrass (PR), zoysiagrass (Z), and bermudagrass (B). Plots at rough height (2.5 in)

consisted of turf-type tall fescue (TF), Kentucky bluegrass (KB), PR, or a TF/KB mix. Of the fairway-height grasses, Z and B had the highest incidence of MC grubs. MC predominated in CB, whereas JB favored PR. JB populations were highest in rough-height grasses, outnumbering MC 2-4 fold. Skunk damage was greatest in CB and PR at fairway height. There was little skunk digging in fairway-height Z or B, or in rough-height grasses. Parasitism by *Tiphia* occurred in all grasses but was greatest in zoysiagrass.

Grub collection kits were sent to cooperators in 22 states to survey natural enemy load in JB populations throughout the species range in the USA. Collected grubs are being analyzed for pathogens to test whether impact of particular agents is associated with how long JB has been established in a given area.

Summary Points

- Grub species and associated pathogens and parasitoids were surveyed on 27 Kentucky golf courses, the first such study in the transitional climactic zone. Masked chafers and Japanese beetle grubs accounted for about 66 and 30% of the grub infestations, respectively. Insect-pathogenic nematodes, *Tiphia* wasps, milky disease, and other pathogens account for moderate to high natural mortality at some sites.
- Nematodes isolated from MC and JB grubs are currently being identified.
- Turfgrass species and mowing height influence incidence of grubs and natural enemies.
- Pathogens of JB grubs are being surveyed across the eastern and central United States to determine if natural enemy load can predict area-wide cycles of decline of grub populations.

Interpreting and Forecasting Phenology of the Annual Bluegrass Weevil in Golf Course Landscapes

Daniel C. Peck, Masanori Seto, and Maria Diaz
Cornell University

Objectives:

1. Describe patterns of variation in population fluctuations and phenology.
2. Describe the overwintering strategy by establishing the factors that affect site selection and success.
3. Document the relationship between overwintering sites and developmental sites.
4. Develop and validate a degree-day model to forecast phenology.

Start Date: 2006

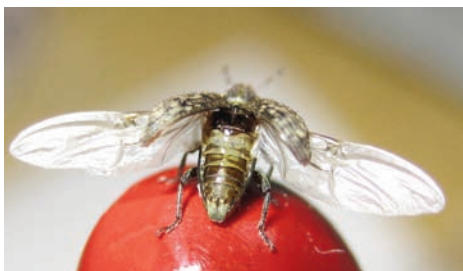
Project Duration: three years

Total Funding: \$90,000

The annual bluegrass weevil (ABW) is a burgeoning pest throughout the Northeast and Mid-Atlantic regions. The stem-boring and crown-feeding larvae cause highly visible damage to short-cut *Poa annua*, a major component of golf course playing surfaces. Management options are largely limited to pyrethroids, and applications may be made 2-5 times a season. Poor targeting often leads to control failures.

Detailed population surveys were conducted at two sites in Upstate NY to describe patterns of variation in population ecology across year, site, and habitat. Population data were collected from 2004-2006. Based on extractions of larvae from soil cores and collections of adults flushed by a disclosing solution, 3,838 larvae and 8,576 adults were sampled. Five larval instars were confirmed based on head capsule width. Because there is no overlap, instar can be determined with a simple measurement. Adults were also identified as male and female, callow and mature. Because there is no divergence in male and female population curves, assessing gender does not help to interpret adult fluctuations.

Most population parameters (e.g. fluctuation curves, abundance, synchrony, number and timing of generations) varied more between years than between sites.



Annual bluegrass weevil launching into flight from the top of a stick

Sex ratio and abundance varied between management habitats. For instance, males were more abundant in the rough where the mean sex ratio (male:female) was 1.7:1, versus 1:1 in the fairway. In terms of insect load, larvae and adults were 8-9 times more abundant on the fairway than the rough. Across the fairway itself, abundance was consistently greater near the edge at one site, but insects were evenly distributed across the fairway at the other site.

An analysis of three years of population data shows that degree-day may be a better fit than Julian date at predicting occurrence of the first generation. Given low variation in R^2 values, using the most convenient base-temperature model may be feasible. In 2008, we partnered with collaborators across NY to validate this model through focused population surveys.

Field surveys showed that overwintering adults tend to settle along the tree line adjacent to the fairway, establishing up to 60 meters from the fairway and 10 meters into the woods. Little or no overwintering occurs on the fairway or adjacent rough. In a choice experiment, we showed that white pine litter is not a preferred overwintering substrate. Adults preferred to settle in rough-mown grass and a combination of pine and deciduous litter over fairway-mown grass and pine litter alone. Captures in linear pitfall traps were greatest in spring. At one site, directional movement toward the fairway in spring was confirmed, but there was no evidence for reverse movement in the fall.

Results lead us to propose a new conceptual model of flux between habitats and overwintering site selection. Our theory is that spring immigration occurs mostly by walking, with orientation to low-mown turf. Fall emigration occurs through flight with orientation to defined tree lines. Through a "snow-fence" effect, adults stop flying at the tree line, drop to the ground,



Annual bluegrass weevil eggs laid in a stem of annual bluegrass

and settle into overwintering substrates according to preferences. In 2008, laboratory and field observations confirmed that ABW is capable of vigorous flights. Better targeting of ABW will depend on future studies that emphasize dispersal behavior, host plant associations, and habitat preferences.

Summary Points

- Most population parameters (e.g. shape of the fluctuation curve, number of generations, and generation time) vary more between years than between sites.
- Sex ratio and abundance vary dramatically between rough and fairway habitats. Across the fairway, insect distribution does not explain the prevalence of damage along the edge.
- Degree-day accumulation may be better than calendar date at predicting phenology, and a preliminary model has high predictive power for timing of the first generation.
- Overwintering adults tend to settle along tree lines adjacent to the fairway, as far as 10 m into the woods and 60 m from the edge of the fairway. Little to no overwintering occurs on the fairway and bordering rough. Pine litter is not preferred over other overwintering substrates.
- It is hypothesized that adults immigrate to fairways in spring largely by walking with orientation to low cut turf, but they emigrate in fall largely by flying with orientation to defined tree lines where they settle into overwintering substrates.

Seasonal Life History and Suitability of Horticultural Plants as Nectar Sources for *Larra bicolor*, a Parasitoid of Mole Crickets in the Northern Gulf Coast

David W. Held

Mississippi State University

Objectives:

1. Determine the seasonal life history of *Larra bicolor* in the northern Gulf Coast.
2. Determine the suitability of flowering plants as nectar sources for *Larra bicolor*.
3. Determine if incorporating wildflowers on golf courses will facilitate the establishment of *Larra* wasps on that site.

Start Date: 2006

Project Duration: three years

Total Funding: \$29,232

Larra bicolor, an introduced ectoparasitoid of mole crickets, has spread into the northern Gulf region (Mississippi and Alabama) from where it was originally introduced (Florida). This creates an opportunity for golf course superintendents in this area to utilize this biological control agent.

We are monitoring wasp activity monthly on four sites on the Mississippi Gulf Coast where where *L. bicolor* is known to occur. Across all three years, wasps were active beginning in June and generally ending in November. Comparison of activity of foraging wasps on turf is comparable to foraging wasps on nectar sources. This suggests that the presence of wasps on nectar sources will indicate wasps foraging in turf. Wasps were not active at St. Andrews in 2008. This site was one of the first along the Mississippi coast where *L. bicolor* was detected.

In 2007 and 2008, we established a replicated garden of flowering herbaceous plants at the Coastal REC. The garden consists of four replicates of the 15 plant taxa arranged in 0.5 × 0.5 m plots. Between 1100-1300 h during peak flight, numbers of *L. bicolor* on flowering plants were recorded. Of all the species present and flowering, only two, *Pentas* and



Common garden experiment conducted in 2007 and 2008 to assess preference of *L. bicolor* for flowering ornamental plants

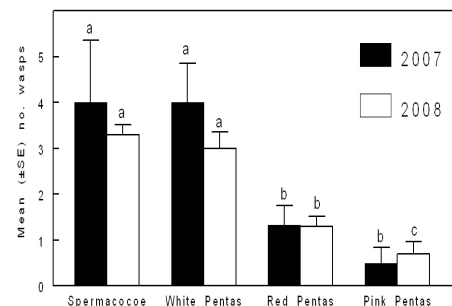
Spermacoce, were visited by *L. bicolor*. White pentas was readily foraged upon by *L. bicolor* and numbers of wasps were comparable to *Spermacoce*. Among pentas, white-flowered pentas was significantly different from either pink or red-flowered pentas.

In 2007, both red and pink flowered pentas were comparable, however, they were significantly different in 2008. There may be volatiles or ultraviolet visual cues recruiting wasps to these flowers. In 2008, attempts to observe recruitment to flowers or host mole crickets under controlled conditions in a wind tunnel were unsuccessful.

In 2007 and 2008, diel activity of *L. bicolor* was studied by monitoring wasp activity on flowers of blooming plots of *Spermacoce verticillata* and white-flowered *Pentas lanceolata*. These plots were part of the garden experiment previously described. This study was conducted from August 7-10, 2007 and October 9-11, 2008 when wasp populations were abundant enough to collect data. The number and sex of wasps on flowering plants in 8 plots in the garden were taken hourly from 0500-2000 h each day.

In both years, wasps begin actively foraging about 2-3 hours after sunrise to near dusk. Sunrise, and not temperature, likely triggers activity. Air temperatures in August when wasps became active were 27-28 C, whereas wasps were active when air temperatures were 18.3 C in Oct. Males and females are present throughout the day with females being more transient. Populations were more clearly male-biased in October than in August samples. It is generally thought that *L. bicolor* flowers are male-biased, but these data suggest that may depend on time of year. At certain times during the August 2007 observations, numbers of male and females were almost equal.

In May and June, plots were



Numbers of wasps recorded on plants in the common garden experiment. Data from 6 days in 2007 and 10 days in 2008 during the peak of activity were selected and analyzed. Only four taxa, two species, were observed with wasps. There were significantly more wasps on *Spermacoce* and the white-flowered pentas than either of the other two flowering pentas in both years.

established on a local golf course (Great Southern Golf Course) and at the Coastal REC. The objective was to determine if proximity to pentas could increase parasitism of mole crickets. Plots of flowering plants were established in the center of 11-m transects. Once wasps were active on the flowers, containers of mole crickets would be buried in the ground at 1, 3, and 5 meters from the center. Unfortunately, wasps were not present in the flowering plots during July, August, or September.

Summary Points

- *Larra bicolor* have a 4-5 month activity in the northern Gulf region to parasitize mole crickets. Wasps are active mostly during daylight hours. Although the impact of insecticides on *L. bicolor* have not been evaluated, turf managers wanting to conserve these biological controls should consider making insecticide applications later in the day as numbers of foraging wasps wane.

- In two trials, pentas, particularly white pentas, recruit equivalent numbers of *L. bicolor* as flowering *Spermacoce verticillata*. Attempts to assess the impact of nectar sources on parasitism were not successful in 2008 and will be repeated in 2009.

Rooting and Carbohydrate Metabolism in Creeping Bentgrass Putting Greens in Response to Summer Irrigation and Aeration

Jinmin Fu and Peter H. Dernoeden
University of Maryland

Objectives:

1. Evaluate physiological processes and rooting of putting green-height creeping bentgrass in response to two irrigation management and three aeration regimes.
2. Determine the influence of aeration and irrigation frequency on creeping bentgrass summer performance and root longevity during periods of supraoptimal temperature stress.
3. Provide information on the effects of soil temperature and soil water content on carbohydrate metabolism and its relationship to summer bentgrass decline.

Start Date: 2006

Project Duration: three years

Total Funding: \$90,000

There has been little study on the impact of irrigation and aeration management on rooting in creeping bentgrass grown in a sand-based rootzone under field conditions.

'Providence' creeping bentgrass was grown on a sand-based rootzone meeting USGA recommendations. Plots were subjected to two irrigation programs: light and frequent versus deep and infrequent. Light, frequent plots were irrigated daily on rain-free days to maintain a moist condition in the upper 4-6 cm (1.6-2.4"); whereas, deep, infrequent irrigated plots were irrigated at leaf wilt to a depth > 24 cm (>9.5").

A majority of roots (55%) were found in the upper 2.4" (6 cm) of soil at the end of the summer, regardless of irrigation regime. Deep, infrequent irrigated bentgrass produced a greater number of roots, longer roots, and a larger root surface area and a smaller root diameter (2007) vs. light, frequent irrigated bentgrass.

Soil temperatures were on average < 0.7 C (< 1.4 F) greater in light, frequent irrigated bentgrass. Deep, infrequent irrigated bentgrass had lower canopy photosynthetic rates, but respiration was similar to light, frequent irrigated bentgrass. Canopy temperatures were 2.2 C (4.0 F) higher in deep, infrequent vs. light, frequent irrigated bentgrass.

Deep, infrequent irrigated bentgrass had lower color and quality and lower chlorophyll levels in 2006 and most of 2007. By late summer, however, color and quality and higher chlorophyll levels were detected in deep, infrequent vs. light, frequent irrigated bentgrass. Deep, infrequent irrigated bentgrass developed a less



Soil temperature was measured by installing temperature sensors about 0.8 inches below the soil surface.

thick thatch-mat layer, which contained less organic matter versus light, frequent irrigated bentgrass.

Deep, infrequent irrigated bentgrass leaves had higher water soluble carbohydrate and total non-structural carbohydrate levels in 2006, but higher storage carbohydrate levels in both years. Deep, infrequent irrigated bentgrass had higher storage carbohydrate and non-structural root carbohydrate levels than light, frequent irrigated bentgrass in both years. Deep, infrequent irrigated bentgrass accumulated proportionately more non-structural carbohydrate in roots versus leaves. Nearly twice as much water was applied to light, frequent versus deep, infrequent plots in both years.

Regarding aeration, three regimes were assessed: spring only, spring plus three summer corings, and a non-cored check. Spring core aeration holes were filled to the surface with topdressing, but in summer, aerated plots cores were brushed to re-incorporate soil and no additional topdressing was applied.

In 2005, total root counts and total root length were increased by summer coring vs. spring coring. Total root counts and total root length generally were greater in the entire profile in spring plus summer-cored versus spring or non-cored

bentgrass in 2007.

Data indicated that summer core aeration should be avoided the first summer of establishment. If necessary, only core aerate to the depth of the thatch-mat layer. The % total root counts in the 0-6 cm (0-2.4") of soil ranged 61 to 74%, 58 to 59%, and 62 to 77% among all three coring treatments in late summer of 2005, 2006, and 2007, respectively.

Spring and spring plus summer-cored plots developed a thicker thatch layer than non-cored bentgrass. The amount of organic matter (loss on ignition) in the thatch-mat layer increased in all three regimes, but the levels remained the same among regimes. However, the organic matter concentration (gravimetric organic: dry wt. of cores) was lower in cored plots. Organic matter concentration less than 110 g kg⁻¹ was associated with better turf performance.

Spring and spring plus summer coring reduced quality for about two weeks, but generally had higher color ratings than non-cored bentgrass. Late summer quality was better in cored plots. Chlorophyll a and a+b levels were higher for spring and spring plus summer cored bentgrass in both years.

Summary Points

- Deep, infrequent irrigation produced a greater number of roots, longer roots, a larger root surface, lower soil temperature, less thatch, higher water soluble and total non-structural carbohydrates than light, frequent irrigation.
- Data indicated that summer core aeration should be avoided the first summer of establishment.
- Spring and spring plus summer-cored plots exhibited reduced quality for about two weeks, but generally had higher color ratings and chlorophyll a and b levels than non-cored plots.

Infection and Colonization of Bermudagrass by *Ophiosphaerella herpotricha*, the Causal Agent of Spring Dead Spot

Nathan R Walker
Oklahoma State University

Objectives:

1. To transform *O. herpotricha* to express fluorescent protein genes.
2. Evaluate infection and colonization of bermudagrass cultivars at different temperatures.
3. Evaluate differences in infection and colonization between bermudagrass cultivars that vary in disease susceptibility.

Start Date: 2006

Project Duration: three years

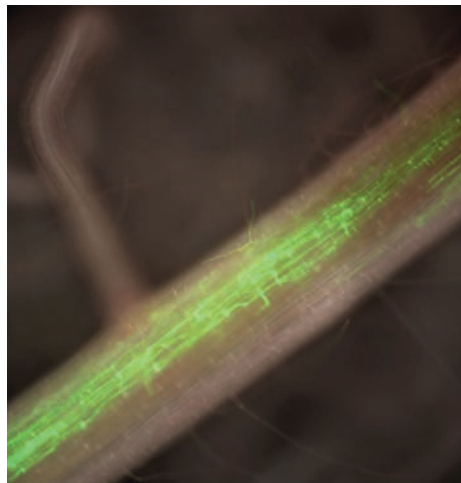
Total Funding: \$59,684

Spring dead spot is the most devastating and important disease of bermudagrass that undergoes winter dormancy. The disease is caused by one or more of three fungal species in the genus *Ophiosphaerella* (*O. herpotricha*, *O. korrae*, or *O. narmari*). The disease results in unsightly dead patches on fairways, tees, and bermudagrass greens, giving way to the encroachment of weeds and costly management efforts to eliminate weeds and reestablish grass in the affected area.

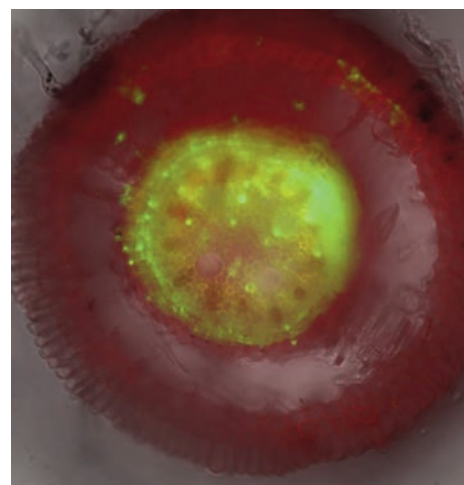
A critical limitation to the study of turfgrass root diseases is the inability of researchers to rapidly and easily study the plant-fungus disease interaction because it happens below ground and often inside of roots. The overall goal of this study is to enhance our understanding of the interaction between *O. herpotricha* and its bermudagrass host and how environmental and host factors influence this interaction.

We have inserted two different fluorescent reporter genes (red and green) into the fungus and examined root and

stolon infection of various bermudagrasses. For the interspecific hybrid (*Cynodon dactylon* × *C. transvaalensis*) cultivars 'Tifway' and 'Midlawn', root cortical cells were rapidly colonized. However, the root's stele remained uninfected 10 days after inoculation. For a *C. transvaalensis* accession, both the root cortex and stele were colonized by eight days after inoculation. In general, 'Tifway' roots exhibited greater colonization and necrosis than the more tolerant cultivar 'Midlawn' and *C. transvaalensis*, which, though its roots were heavily colonized, exhibited very little necrosis.



Root cortical cells were rapidly colonized in the interspecific hybrids 'Tifway' and 'Midlawn'.



For a *C. transvaalensis* accession, both the root cortex and stele were colonized by eight days after inoculation.

transvaalensis, stolon tissues were colonized without any apparent necrosis.

Ongoing studies using the confocal scanning laser microscope will optically 'section' infected roots and produce 3-dimensional images of the fungus as it moves in and on bermudagrass roots. This basic information on how the cultivars react to the fungus will improve our ability to enhance and deploy host-plant resistance through traditional breeding efforts at Oklahoma State University.

Summary Points

- Fungal colonization of root tissues varied among the bermudagrass cultivars examined.
- Colonization and necrosis of roots was extensive for 'Tifway', less for 'Midlawn', however, limited necrosis occurred for the *C. transvaalensis* accession even though it was extensively colonized.
- Internal colonization of stolon resulted in severe necrosis for 'Tifway' but not for the *C. transvaalensis* accession.
- This information will be used to enhance host-plant resistance through traditional breeding efforts at Oklahoma State University.



Spring dead spot is the most devastating and important disease of bermudagrass.

Additional studies have been conducted on the stolons of these bermudagrasses. When stolons were inoculated on unwounded internodes, very limited epidermal and cortical infections were observed after 28 days for all three bermudagrasses. However, stolons that were root-inoculated six weeks earlier became infected through cut end of the stolon.

For 'Tifway' stolons, infection resulted in internal necrosis and cavities, similar, but less severe response was observed for 'Midlawn', however in *C.*

Silicon Amendment: A Component of an Integrated Gray Leaf Spot Management Strategy

Wakar Uddin

Pennsylvania State University

Objectives:

1. To evaluate accumulation of silicon in perennial ryegrass plants.
2. To determine the effects of soil type, source of silicon, and rate of silicon amendment on gray leaf spot severity and incidence.
3. To devise a management strategy for gray leaf spot through integration of silicon into a fungicide program.

Start Date: 2006

Project Duration: three years

Total Funding: \$45,000

Gray leaf spot, caused by *Magnaporthe oryzae*, is a serious disease of perennial ryegrass turf that can cause extensive damage to turf in golf course fairways. The disease is effectively managed by fungicide applications. However, turf managers often explore the possibilities of various cultural practices that can be relatively easily integrated along with fungicide into a broader disease management strategy.

Soil silicon amendment have been proven effective in controlling both soilborne and foliar fungal diseases of several plants including some turfgrass species. However, the effects of silicon on gray leaf spot development of perennial ryegrass turf were not known. This study was undertaken to investigate the effects of silicon on gray leaf spot incidence and severity in perennial ryegrass.

The experiments were set up in Willow Hollow Golf Course and Lulu Temple Country Club in Pennsylvania in a split-plot design with rate of silicon as the main-plot factor and rate of fungicides as the split-plot factor. Silicon (calcium silicate) was applied at six different rates: 0,

0.5, 1, 2, 5, and 10 metric ton/ha. The fungicides selected for the study were: a systemic fungicide, azoxystrobin, and a contact fungicide, chlorothalonil. Each fungicide was sprayed at low, medium, and high label rates - 0.31, 0.46 and 0.61 a.i. kg/ha azoxystrobin, and 4.53, 6.29 and 8.18 a.i. kg/ha chlorothalonil, respectively. Calcium silicate was broadcast as a top dressing in each treatment plot (76.2 x 45.7 cm) and incorporated by core aeration using a Toro procore 660 open-tine. The cores were 1.9 cm in diameter and 7.6 cm in depth at 7.6 cm x 7.6 cm spacing.

The grass in the plots was maintained at a 3.5 cm height and mowed twice a week. The grass was fertilized with (5% N, 5% P₂O₅ and 20% K₂O) at 1.22 x 10⁻³ kg/m² with Team for crabgrass control before the experiments was initiated. Fungicides were applied six weeks after application of silicon and repeated after 14 days to deliver two applications prior to inoculation with *Magnaporthe oryzae* (60 x 10³ conidia/ml H₂O with 0.1% Tween 20).

Fungicides were applied in water equivalent of 814 L/ha (2 gallons/1000 sq ft). Disease severity (index 0-10; 0=turf area asymptomatic, 10=91-100% of turf area necrotic) was assessed weekly for six weeks. Disease progress over time was evaluated using the parameter estimates, rate *r*, and AUDPC, where *r*=rate of disease progress and AUDPC = area under the disease progress curve. Data were analyzed using regression and ANOVA procedures.

The results showed that effects silicon and fungicide on gray leaf spot severity were significant (P=0.05). Assessment of the disease showed that gray leaf spot severity (weekly), rate parameter, *r*, and the area under disease progress curve (AUDPC; severity-weeks) were significantly reduced by application of silicon and fungicides.

Although all three rates of each of

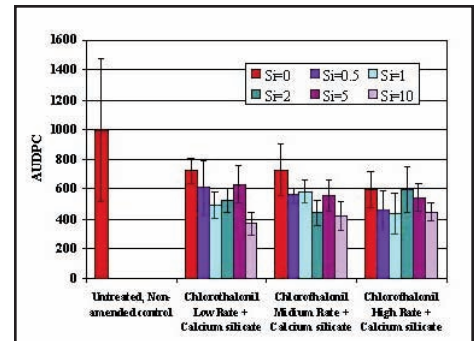


Figure 2. Comparison of AUDPC for the non-fungicide-treated and non-amended control, low, medium and high rates of chlorothalonil, and all treatments of chlorothalonil combined with calcium silicate treatments in Site 1.

the systemic or contact fungicide reduced gray leaf spot severity similarly, combination of silicon and the fungicide provided further reduction of the disease. Increased rate of silicon application in combination with fungicide generally provided greater reduction of gray leaf spot.

This study demonstrates that control of gray leaf spot in perennial ryegrass by fungicide application may be augmented by amendment of soil with silicon. This study suggests that silicon may be used as an important component of an integrated gray leaf spot management strategy that includes cultural and chemical approaches.

Future studies are warranted to determine the residual effects of silicon on gray leaf spot development in silicon-amended soil over time and interactions between silicon and various commonly used fungicides for gray leaf spot control.

Summary Points

- Gray leaf spot severity was significantly reduced by amendment of soil with silicon.
- Effects of fungicides on gray leaf spot were augmented by application of silicon.
- Increased rate of silicon application generally provided greater suppression of the disease within the fungicide treatments.

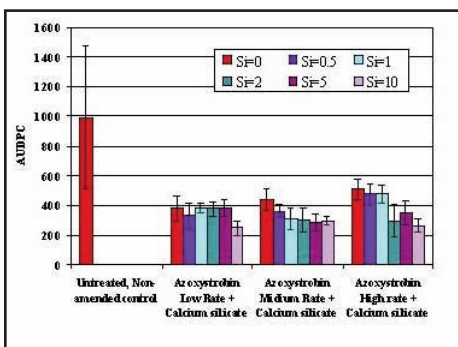


Figure 1. Comparison of AUDPC for the non-fungicide-treated and non-amended control, low, medium and high rates of azoxystrobin, and all treatments of azoxystrobin combined with calcium silicate treatments at Site 1.

Optimization of Foliar Nitrogen Nutrition to Improve Turf Performance Under Energy Stress

S. Henning, B. E. Branham, and R. L. Mulvaney
University of Illinois

Objectives:

1. To determine the efficiency of ammoniacal-N uptake in the field from foliar feeding, particularly when done in combination with other turf care products.
2. To determine the positive or negative benefits of foliar ammoniacal-N applications on highly managed turfgrass
3. To determine fertilizer use efficiency through foliar feeding versus conventional fertilization.
4. To determine the effect of foliar ammoniacal-N applications on root and shoot growth, development, and performance in the field
5. To determine if the practice of foliar application of nitrogen fertilizer to a crop that has a portion of its foliage removed frequently is useful.

Start Date: 2007

Project Duration: three years

Total Funding: \$88,810

Plants primarily assimilate nitrogen through root uptake of nitrate from the soil solution. Plants must convert nitrate to ammonia before it can be incorporated into nucleic acids, proteins, and other nitrogen containing compounds. It is estimated that plants can use almost 25% of their photosynthetically derived energy in this conversion process.

One possible way to reduce this energy-intensive process is to provide nitrogen in the ammonium form, typically as urea or ammonium sulfate. Our research seeks to understand the practice of foliar fertilization and determine the positive or negative impacts of this process. By applying fertilizers enriched with a stable, non-radioactive isotope of nitrogen (^{15}N) as a tracer, nitrogen use can be directly tracked throughout the plant.

Beginning in early 2008, we analyzed the tissue from several experiments conducted in the summer of 2007 that compared urea and ammonium sulfate applied to creeping bentgrass maintained

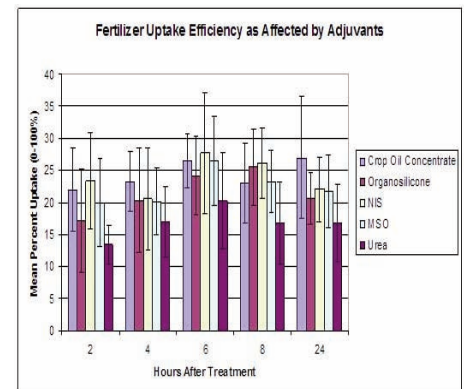
at a 0.5" height of cut. The application rate of each fertilizer was 0.2 lbs N/1000 ft² with a spray volume of 40 GPA. Studies on the time course of ^{15}N absorption suggest that nitrogen applied to the foliage is absorbed by the plant within 4-6 hours. We have measured uptake efficiencies from 10-35% under field conditions, suggesting that uptake efficiency in the field can be quite variable.

Our studies to determine the effect of spray gallonage on uptake efficiency indicate that lower spray gallonages increase uptake of foliar applied N. Results suggest that applying fertilizer at gallonages above 20 GPA does not result in significant foliar feeding.

Studies to determine if tank-mixing adjuvants with fertilizer would increase uptake efficiency indicated that the addition of an adjuvant to foliar-applied fertilizer significantly increased uptake efficiency although results did not indicate that a particular class of adjuvant is most effective. Results of other tank-mixing studies suggest that tank-mixing of fertilizer with other leaf-applied chemicals may affect uptake efficiency.

In the summer of 2008, we initiated a field study to determine whether foliar applications of $\text{NH}_4\text{-N}$ have a positive effect on plant metabolism. Plots of creeping bentgrass (*Agrostis stolonifera* L. 'Penn A-4') were maintained at three heights of cut, 0.125, 0.1 and 0.085", on a native soil putting green topdressed (10-day intervals) with sand and treated with foliar nitrogen. Foliar urea (as an $\text{NH}_3\text{-N}$ source), and calcium nitrate (as a $\text{NO}_3\text{-}$ source), are being applied weekly at annual rates of application of 2.4 lbs N/1000 ft²/year.

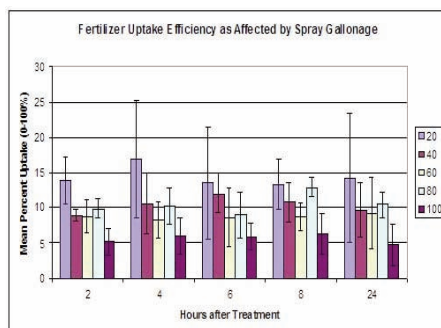
Nitrogen applications are divided



Mean fertilizer uptake over a 24-hour period as affected by adjuvants. Error bars indicate one standard deviation.

evenly into weekly applications applied in April through October. Clippings are collected once per week during the study, and the plot area is mowed six days per week. For evaluation and comparison of traditional soil applied N-fertilization, both forms of N fertilizer are applied weekly as a soil drench at the same annual N rates as the foliar applications.

Data collected include leaf tissue N concentration, clipping production, percent coverage, turf quality, turf color, chlorophyll concentration, root mass, and disease incidence.



Mean fertilizer uptake over a 24-hour period as affected by spray gallonage (20, 40, 60, 80, and 100 GPA). Error bars indicate one standard deviation.

Summary Points

- Foliar applied N enters the leaf within 4-6 hours of application.
- Lower spray gallonages are more effective at promoting N-uptake through the leaf.
- Addition of an adjuvant for foliar fertilization is recommended for optimizing foliar N-uptake of urea.
- Tank-mixing fertilizer with commonly leaf-applied chemicals has limited impact on N-uptake through the leaf.

Use of a Baculovirus for Season-long Control of Black Cutworms on Golf Courses and Compatibility with Soil Insecticides and Insect-resistant Turfgrasses

Daniel A. Potter and Andrea Bixby
University of Kentucky

Objectives:

1. Evaluate AgipMNPV, a naturally occurring baculovirus, as a bio-insecticide for season-long and multi-year preventive control of black cutworms (BCW) on golf courses.
2. Compare infectivity and persistence of AgipMNPV to BCW in sand-based and soil-based putting green and fairway height creeping bentgrass habitats.
3. Investigate compatibility and possible synergism of AgipMNPV with soil insecticides used for grub control on golf courses.
4. Investigate compatibility of endophytic and other insect-resistant turfgrasses with biological control of black cutworms by AgipMNPV.

Start Date: 2007

Project Duration: three years

Total Funding: \$60,000

Biological controls, once established, have the potential to provide prolonged suppression of insect pests on golf courses. In 2003, a former graduate student discovered a caterpillar-specific virus decimating populations of black cutworms (BCW) on Kentucky golf courses. The virus, identified as *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV), was amplified by feeding it to healthy BCW, harvesting their cadavers, and mixing the concentrated virus particles with water.

This crude biological insecticide was applied to turf to see if it would infect resident larvae. Initial testing showed this AgipMNPV suspension to quickly kill young larvae, but that larger ones require higher dosages and continue to feed for several days before being killed. Virus-infected BCW rupture in death and spread millions of infective virus particles onto foliage and thatch. Virus sprays gave good control of BCW in small-plot field trials in fairway- and collar-height creeping bentgrass.

Thousands of laboratory-reared BCW were infected with AgipMNPV during the winter, harvested, and stockpiled to prepare enough virus suspension for a realistic field trial. Six whole tees at each of two central Kentucky golf courses, plus 2 meters into the surrounds, were sprayed with virus suspension in spring 2008, with six untreated tees on each course used for comparison. Efficacy was determined at monthly intervals by sampling natural den-



Once infected by the virus, black cutworm larvae can be used to prepare virus suspensions and applied to the field to further infect resident larvae.

sities of BCW and by implanting sentinel larvae and eggs, using irritant (soap) drenches to recover short-term survivors, and rearing them to determine how many ultimately died from virus.

The virus provided 78% control of young larvae hatched from eggs and 45-56% control of third instars placed in the turf one week after application. We are currently examining hundreds of BCW blood smears on microscope slides to determine how many weeks of suppression the virus application provided.

Studies were initiated in 2008 to investigate the virus' compatibility with host-plant resistance. BCW that develop on endophytic grasses or Kentucky bluegrass show delayed growth and development. Such stress may increase their susceptibility to virus residues encountered in the turf.

BCW were allowed to feed in the greenhouse for 3 days on 'Rosalin' perennial ryegrass with (E+) or without (E-) endophyte. Four rates of virus were then applied to the grass blades. Larvae were

left to feed on grass for 10 days and then survival, growth, and incidence of virus were assessed. Main effects of endophyte and virus were highly significant. Larvae feeding on E+ grass were stunted, yet the virus caused similarly high (85-97%) mortality of BCW irrespective of grass type. This shows that AgipMNPV is not deactivated by alkaloids in the E+ grasses.

Inactivation of microbes by solar radiation can be a major hurdle to biological insecticides under field conditions. Optical brighteners have been used as adjuvants for insect pathogens in other systems because they absorb UV light and can protect original virus activity for weeks after application.

In summer 2008 we evaluated two optical brighteners for enhancing efficacy and persistence of AgipMNPV against BCW in fairway-height creeping bentgrass field plots. The virus provided an average of 89, 44, and 18% mortality after 1, 3 and 5 weeks, respectively, but the brighteners did not significantly synergize or prolong infectivity.

Summary Points

- AgipMNPV gave good short-term control of BCW on golf course tees under actual play conditions. Extent to which it gave season-long control is still being evaluated.
- AgipMNPV is compatible with insect-resistant endophytic turfgrasses.
- AgipMNPV also gave good control in fairway-cut creeping bentgrass, but addition of optical brighteners to the virus from UV degradation did not detectably prolong infectivity or synergize its efficacy at lower rates.

Accurate Identification and Gene Expression in Relation to Virulence of *Rhizoctonia* Isolates Infecting Turfgrasses

Dilip K. Lakshman
USDA-ARS

Objectives:

1. Molecular identification of *Rhizoctonia solani* isolates pathogenic to turfgrasses using Universally Primed-Polymerase Chain Reaction (UP-PCR) and nucleic acid hybridization analysis.
2. Expressed Sequence Tag (EST) analysis for surveying genes and creating a gene database for *R. solani* with emphasis on genes affecting virulence and pathogenicity.

Start Date: 2007

Project Duration: three years

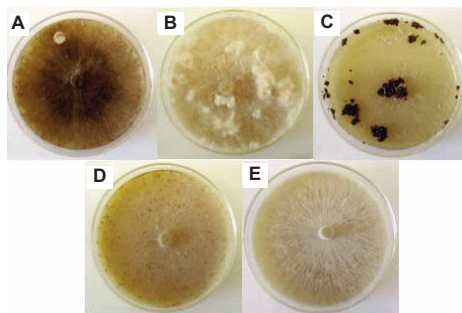
Total Funding: \$90,000

Rhizoctonia blight (brown patch), caused by *R. solani* is a disease of cool-season grasses, including bentgrasses, fescues, and ryegrasses. Anastomosis groups (AG) 1, 2, 4 and 5 have been previously isolated from blighted grasses. *Rhizoctonia* leaf and sheath spot of both warm and cool season grasses are caused by both *R. zeae* and *R. oryzae*.

Rhizoctonia species and AGs are reported to differ in sensitivity to common fungicides. Also, the prevalence and severity of *Rhizoctonia* diseases on turfgrasses depends, among other factors, on infection by a particular species and AG of *Rhizoctonia*. Thus, minimization of chemical use as well as consistent and reliable management of *Rhizoctonia* diseases with genetic and biological methods will largely depend on identification of *Rhizoctonia* isolates to species and subspecies level and knowledge of its virulence-regulating genes.

During the summer of 2008, we collected more *Rhizoctonia* samples from Northern Virginia and Beltsville area of Maryland. We were able to collect 136 isolates from Leesburg and Woodbridge of VA, and 83 isolates from Beltsville, MD. Dr. Brandon Horvath and his research team also helped in collecting additional 22 samples from Richmond and Virginia Beach. The total *Rhizoctonia* isolates we have at Beltsville USDA-ARS, so far, amounts to 448. Out of that, around 7% of isolates belong to either *R. zeae* or *R. oryzae*. The nuclear staining of suspected *R. cerealis* isolates from Blacksburg (collected in 2007) proved to be *R. solani* since all of them were multi-nucleate.

The molecular identification of *Rhizoctonia* isolates will be carried out by



Differences in colony morphology of a few isolates after 10 days incubation on PDA. Isolates A (*R. solani* AG2-1), B (*R. oryzae*), C (*R. solani* AG1-1A), D (*R. zeae*) and E (*R. solani* AG4) were collected either from Richmond, Beltsville, or Leesburg area.

employing AFLP, UP-PCR, and sequencing of rDNA-ITS regions. Sequencing ITS regions will be utilized to group sample isolates together with tester strains in order to come up with their phylogenetic relationships. The banding patterns of UP-PCR and AFLP will be used to construct phenograms of the tested isolates. Similar to the analysis of ITS regions, the bands generated will be analyzed with the results of tester strains to find differences at molecular level.

Out of the total isolate collection, we selected a random sample of approximately 10% encompassing different geographic locations. The colony morphology of these isolates was recorded after 10 days incubation on PDA. The color of mycelial mat and color and size of sclerotia of the isolates differed according to their species and anastomosis group (AG). The average hyphal diameter of all the isolates were within 5-11 μ m which agrees with the previous published data for *R. solani*, *R. zeae*, and *R. oryzae*.

Presently, we are extracting DNA from the selected isolates for carrying out molecular detection techniques. Simultaneously, we are planning to perform hyphal fusion experiments with tester strains on water agar plates in order to

determine AGs of the isolates. The research work carried out previously has recorded the AGs of 1, 2, 3, 4, 5 and 6 from diseased turfgrasses. However AG1, AG2-2 and AG4 are the most common. The data generated from molecular techniques will be considered for accurate identification of brown patch causal agents into their species and intraspecific level.

We are also investigating the pathogenicity of *R. solani* through Expressed Sequenced Tag (EST) analyses. Approximately 1,000 clones each from two virulence-differentiated EST libraries have been sequenced and analyzed. The number of unigenes identified from virulent and avirulent libraries are 214 and 590, respectively. We are conducting a rudimentary analysis of genes under the two virulence differentiable conditions. However, as in any gene expression project, we need to sequence more ESTs to make a comprehensive bioinformatics analysis of the two EST libraries to identify up- or down-regulated genes related to virulence.

Summary Points

● We have collected a total of 448 *Rhizoctonia* isolates from golf courses and lawns of Virginia and Maryland. Out of them, around 7% of isolates belong to either *R. zeae* or *R. oryzae*. The colony morphology, color of mycelium, color and size of sclerotia of a random sample of 10% of those isolates have been characterized so far.

● We have conducted initial experiments with UP-PCR technique and amplification of ITS regions and selected the primers for future full-scale experiments.

● From the initial analysis of the two EST libraries thus far, about 214 and 590 unigenes have been identified from the virulent and avirulent EST libraries, respectively.

Mole Cricket Sensory Perception of Insecticides

Olga Kostromytska and Eileen A. Buss

University of Florida

Objectives:

1. Determine the type, location, and abundance of different sensilla on the antennae and mouthparts of *S. vicinus* and *S. borellii*.
2. Demonstrate the physiological effect of insecticides on the mole cricket nervous system and/or ability of mole crickets to detect chemical stimuli.
3. Demonstrate the behavioral response of mole crickets to sub-lethal insecticide doses.

Start Date: 2007

Project Duration: three years

Total Funding: \$84,208

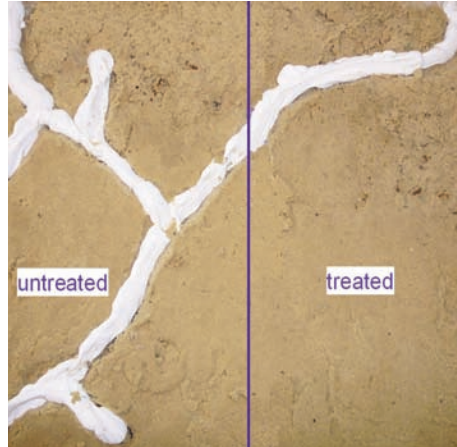
Mole crickets (*Scapteriscus spp.*) are mobile subterranean insects that damage grass by their subsurface tunneling and root feeding. Mole crickets avoid areas treated with insect pathogens, but their response to insecticide-treated soil is poorly understood.

The sensitivity of insects to chemical stimuli is affected by the presence and abundance of chemosensory structures (sensilla) on their bodies. We collected 10 adult males and females each of tawny and southern mole crickets from sound and linear pitfall traps in spring 2007, examined their antennae and mouthparts with a scanning electron microscope (SEM), and identified and counted the sensilla present. Southern mole cricket adults had ~80 flagellomeres (antennal segments), and tawny mole crickets had ~70. Females had more flagellomeres than males for both species.

Two-dimensional laboratory assays were conducted to demonstrate if tawny and southern adult mole crickets could detect acephate, bifenthrin, fipronil, and indoxacarb. Behavioral observations occurred for the first 90 minutes in the dark under red light, and the amount and length of tunneling within 24, 48, and 72 hours were recorded.

Tunneling initiation points were chosen randomly by either mole cricket species in the acephate experiment. The amount of tunneling in the acephate treated arenas did not significantly differ from the control for tawny mole crickets. However, southern mole cricket tunneling after 72 hours was significantly reduced by the high rate of acephate. At the lowest rates, southern mole crickets tunneled less in the treated areas.

Tunneling initiation was random



Tawny mole cricket nymphs tunneled less in areas treated with bifenthrin and fipronil (¼ of labeled rate), than in untreated sand.

in arenas with all rates of bifenthrin and the control. During the first 90 minutes, mole crickets in bifenthrin-treated sand made more tunnel branches, closed more tunnels, and had rapid, erratic movements compared to the controls. However, by 72 hours, tunneling activity was greater in untreated compared to treated sand. Avoidance of treated areas was not observed, and the overall length of tunneling in treated areas did not differ from the length of tunnels in untreated areas.

In arenas with the full rate of fipronil, 80% of mole crickets started tunneling into the untreated area, which indicated a potential repellent effect. Tunneling initiation was random at the two lower rates. The total amount of tunneling by 72 hours in the control was greater than in arenas treated with any of the three rates of fipronil. Most mole crickets (about 70%) that tunneled through fipronil-treated sand and then entered untreated sand did not return to the treated sand. After exposure to the full fipronil rate, adults made tunneling leg movements, but remained in one place, making existing tunnels wider. Exposure to fipronil did not result in increased tunneling.

Responses of tawny and southern mole crickets to indoxacarb were similar.

Tawny and southern mole crickets closed ~30% and ~40% of their tunnels during the first 90 minutes, respectively. After 90 minutes of exposure, mole crickets tremored and had erratic leg and wing movements. After ~48 hrs of exposure, mole crickets were motionless, but responded with kicks and tremors if disturbed. Total amount of tunneling by 72 hours was significantly reduced in arenas with the highest rate of indoxacarb for both species. Mole crickets started tunneling predominantly in the untreated area and most (~90%) egg laying occurred in untreated sand. However, mole crickets tunneled equally on both treated and untreated halves of the arenas.

All mole crickets survived in control arenas. Mortality was 90% after 48 hours for the full rates of acephate, bifenthrin, and fipronil. Mortality of both species was 60% in the arenas treated with the highest rate of indoxacarb. In general, southern mole crickets tend to move faster in tunnels than tawny mole crickets and make more tunnels during the first hour of a test.

Summary Points

- Southern and tawny mole cricket antennae and palps have similar numbers and types of sensilla, but southern mole crickets have more flagellomeres, and thus may be more sensitive to chemical stimuli.
- Females of both species have more flagellomeres than males, possibly because males are more aggressive and clip off antennae.
- All of the insecticides tested reduced overall tunneling activity if evaluated after 3 days. However, females preferred to lay eggs on untreated areas within arenas, not in treated sand.
- Tawny mole cricket nymphs tunneled less in areas treated with bifenthrin and fipronil (¼ of labeled rate), than in untreated sand. Avoidance to other insecticides was not observed.

The Efficacy of Spring Fungicide Applications Plus Organic Fertilizer for Controlling Spring Dead Spot of Bermudagrass

Maria Tomaso-Peterson
Mississippi State University

Objectives:

1. Determine the efficacy of spring and fall fungicide applications for reduction of spring dead spot incidence and severity.
2. Determine the effect of organic fertilizer for the reduction of spring dead spot incidence and severity and overall improvement of turf quality.

Start Date: 2007

Project Duration: three years

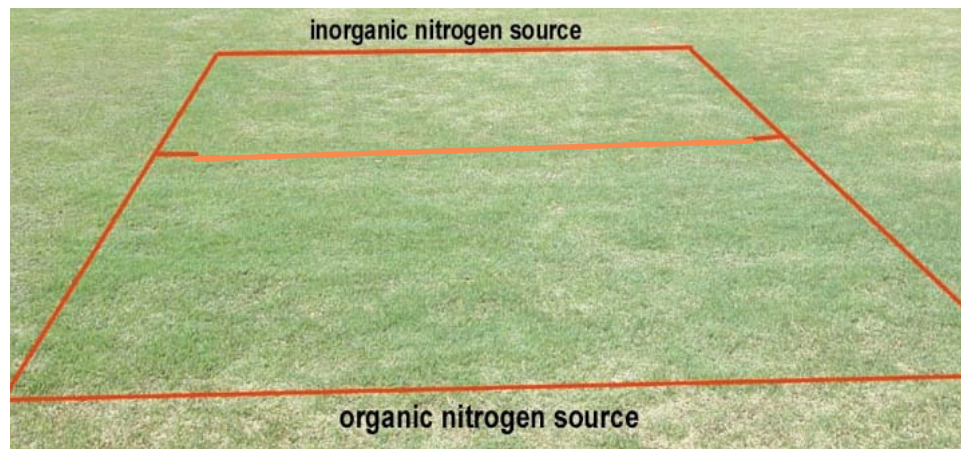
Total Funding: \$30,000

Spring dead spot is a serious root-rot disease of bermudagrass and is the most important disease of hybrid bermudagrasses managed as putting green and fairway turf. Aesthetically undesirable necrotic patches ranging from a few inches to several feet in diameter are evident in the spring and early summer in bermudagrass swards that experience a dormant period.

Three fungal species in the genus *Ophiosphaerella* (*O. korrae*, *O. herpotricha*, and *O. narmari*) are identified as the causal organisms throughout the United States and Australia. In Mississippi, *O. korrae* has been identified as the causal organism of spring dead spot.

Results of a previous study conducted at Mississippi State University suggests the frequency of *O. korrae* in bermudagrass roots was greatest during spring transition compared to summer and fall transition growth periods. As a result of the observed fungal activity in bermudagrass roots during spring transition, this study was initiated in the spring of 2007 in a 'Tifway' bermudagrass fairway with a history of spring dead spot. Symptoms of spring dead spot were observed throughout the study area in the spring of 2007. The treatment plots (15 ft × 10 ft) were arranged in a split-plot randomized complete block design replicated four times.

Fungicide treatments were the whole-plot factor and nitrogen (N) source is the sub-plot factor (7.5 × 10 ft subplots). Fungicide treatments were applied during the spring and fall transitions. The N sources include 12-2-12 organic fertilizer and 12-2-12 blend of inorganic fertilizer including ammonium sulfate (21-0-0), triple super phosphate (0-46-0) and muriate of potash (0-0-60) applied at 1.0 lb of



Untreated control plot, March 2008. Half the plot was treated with an organic N source (foreground) and the other half with an inorganic N source (background) throughout the 2007 growing season.

N per 1000 ft² per month (May-October).

Spring dead spot severity was visually rated (1 to 9; 9 = no disease) in the spring of each year and quantified using digital image analysis. Recovery of symptomatic patches was monitored throughout spring transition. Turfgrass quality was recorded each month of the growing season.

Fungicide applications had a significant effect on spring dead spot ratings in the spring of 2008. Neither the N source (organic vs inorganic) nor the interaction of fungicide and N source had an effect on spring dead spot severity. Rubigan treatments applied in April, September, October, or September and October; September application of Rubigan tank-mixed with thiophanate-methyl; October and November applications of propaconazole and myclobutanil, respectively, were all similar with spring dead spot ratings of > 8.5. Rubigan applied in March, April, May or March, April, September, October, and the untreated control were similar with an average spring dead spot rating of 7.3.

Spring green-up was similar for all treatments. However, some plots receiving organic N initially appeared greener than the inorganic N plots. Turfgrass quality was similar for all treat-

ments each month and averaged 6.0 (scale 1 to 9; 9=best) for the growing season. In the spring and summer of 2008, the soil pH in plots receiving organic N (pH=5.63) was significantly higher than the soil pH of plots receiving inorganic N (pH=5.21).

We anticipate the results of this study will identify a fungicide/fertility disease management program efficacious for controlling spring dead spot of bermudagrass managed as fairway turf. These results will also allow us to determine whether there is an added benefit of using an organic nitrogen source that includes biostimulants and microbes as compared to an inorganic, acidifying fertilizer for reducing spring dead spot incidence and severity.

Summary Points

- Fungicide treatments, including fenarimol (4.0 fl oz/1000 ft²) applied April, September, and October (2007), significantly reduced spring dead spot in 2008
- N source had a significant effect on soil pH.
- Spring green-up and turfgrass quality were similar for all treatments.
- Turf recovery from spring dead spot symptoms was evident in late June 2008.

Developing Best Management Practices for Bermudagrass Control in Zoysiagrass Fairways

Scott McElroy and Mark Doroh
Auburn University

Objectives:

1. Evaluate the best integrated practices for fairway conversion of bermudagrass (*Cynodon dactylon*) to 'Zorro' zoysiagrass (*Zoysia matrella*) turf through various cultural and chemical methods.
2. Evaluate the influence of various cultural practices on zoysiagrass competitiveness with bermudagrass.
3. Evaluate the competitive effects of various weed species on seeded 'Zenith' zoysiagrass (*Z. japonica*).
4. Evaluate new aryloxyphenoxypropionate (AOPP) herbicides for control of bermudagrass in zoysiagrass turf.

Start Date: 2007

Project Duration: two years

Total Funding: \$20,000

Zoysiagrasses (*Zoysia spp.*) are becoming a popular choice of turf for golf courses in the transition zone. Their relatively slow growth rate, high density, and tolerance to shaded areas make them a good choice for a golf course setting. However, one of the most difficult weeds to control in zoysiagrass is bermudagrass (*Cynodon dactylon*). Using herbicides to control bermudagrass in zoysiagrass has traditionally been ineffective due to their physiological similarities as C₄, warm-season grasses. Therefore, zoysiagrass is typically susceptible to the same herbicides used to control bermudagrass.

Recent research has been conducted to evaluate new aryloxyphenoxypropionate (AOPP) herbicides for their efficacy on bermudagrass control. Favorable results have been observed for bermudagrass control and zoysiagrass safety when AOPP herbicides are tank-mixed with triclopyr. Additional research is now being conducted to evaluate other AOPP herbicides for bermudagrass control.

The fairway conversion study was initiated in May 2008. Plots measured 5' X 10' and were arranged in a randomized complete block design. 'Zorro' zoysiagrass was sprigged at a rate of 10 bushels/1000 ft². Siduron applied at establishment provided the poorest bermudagrass control. Treatments with EPTC and dazomet applied before zoysiagrass establishment yielded the lowest bermudagrass groundcover. Treatments with good bermudagrass control yielded higher zoysiagrass cover than those with poor control.

To evaluate the influence of cul-



Research has been conducted at Auburn University to evaluate new aryloxyphenoxypropionate (AOPP) herbicides for their efficacy on bermudagrass control.

tural practices on zoysiagrass competitiveness with bermudagrass, a standard cup cutter was used to transplant 'Common' and 'Tifway' bermudagrass plugs into 'Zorro' zoysiagrass plots. Treatments included increasing rates of nitrogen with and without trinexpac-ethyl. Plugs were rated monthly for maximum diameter spread.

At one and two months after initial treatment, there was no significant difference among treatments. This is designed as a long-term study and it is reasonable to assume that greater separation in treatments will be observed in time. We will be initiating this study in a japonica and matrella type next year to evaluate this effect.

A greenhouse study was initiated in October 2008 to evaluate the competitive effects of various weed species on seeded 'Zenith' zoysiagrass (*Zoysia japonica*). It has been our observation that *Digitaria spp.* are the most detrimental to zoysiagrass development, while goosegrass and sedge species are not as competitive. The study involves an additive design in which zoysiagrass seeding rate is held constant while weed seeding rate is increased. Ratings of percent weed and turf

cover will be measured over an 8-week period.

The evaluation of new AOPP herbicides for bermudagrass control study was initiated in June 2008. Plots were visually rated for bermudagrass control and zoysiagrass injury. Digital images utilizing a light box were also taken and analyzed for percent green cover using Sigma Scan Pro.

When applied at 3-week intervals, clodinafop and fenoxaprop are not safe to apply to zoysiagrass turf and do not effectively control bermudagrass. Metamifop tank-mixed with triclopyr is safe to apply to zoysiagrass and effectively controls bermudagrass.

Summary Points

- EPTC and dazomet in mixtures provided the best management strategy for fairway conversion of bermudagrass to zoysiagrass.
- Studies have been initiated to evaluate the competitive effects of weed species on 'Zenith' zoysiagrass.
- Metamifop tank-mixed with triclopyr is safe to apply to zoysiagrass and effectively controls bermudagrass.

Salinity Management in Effluent Water Irrigated Turfgrass Systems

Yaling Qian

Colorado State University

Objectives:

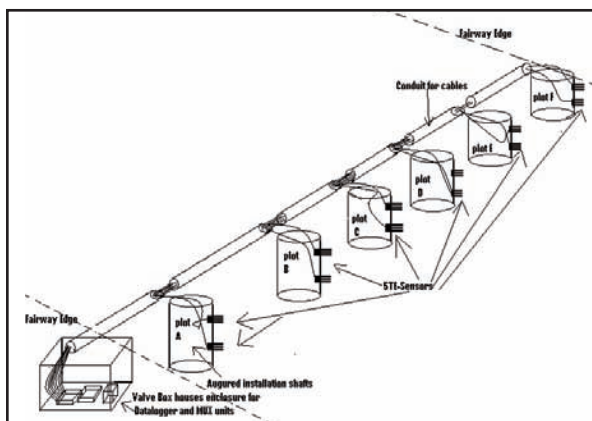
1. To determine spatial and temporal salinity accumulation patterns in soil profiles on a golf course fairway site with effluent water irrigation.
2. To evaluate different management practices for reducing sodium and salt accumulation in the soil.

Start Date: 2008

Project Duration: three years

Total Funding: \$82,459

In many large urban areas in the arid and semiarid regions of the U.S., the population increase has not only increased the fresh water demand but also increased the volume of wastewater generated. Effluent water appears to be the only water resource that is increasing as other sources are depleted. Effluent water has emerged as a reliable and consistently available source of water for golf course irrigation. The very nature of effluent water warrants special management steps to be taken to minimize the possible drawbacks associated with its constituents. The constituents are dependent on the source of effluent water. Some of the main constituents include: salts of different types, nutrient elements, and organic compounds. Effluent water has relatively high levels of sodium concentration relative to calcium and magnesium. Golf course managers are often concerned about salinity and sodicity issues associated with effluent water irrigation. Long-term and continued use of effluent water may lead to increased soil sodicity, with a resulting reduction of soil infiltration, permeability, and aeration, especially in clayey soils, exacerbating salinity problems.



In 2008, a series of in-ground salinity and soil water content sensors on two fairways at the Heritage at Westmoor Golf Course in Westminster, Colorado were installed.

We sampled and tested soil from fairways of 10 golf courses that were near metropolitan Denver and Fort Collins, CO. Among these courses, five had been irrigated exclusively with effluent water for 5-33 years. The other five with similar turf species, age ranges, and soil textures had used surface water for irrigation. Our results indicated that soils from fairways where effluent water was used for at least 5 years exhibited slightly higher soil pH and higher concentrations of extractable Na, B, and P. Compared to sites irrigated with surface water, sites irrigated with effluent water exhibited higher EC and a higher sodium adsorption ratio (SAR) of saturated paste extracts.

In 2008, we installed a series of in-ground salinity and soil water content sensors on two fairways at the Heritage at Westmoor Golf Course in Westminster, Colorado. At each fairway, 6 plots were established. On each plot, two 5TE sensors were installed at 15 cm and 30 cm below soil surface. A total of 12 sensors were installed on each fairway. Wire leads from each sensor were buried and connected to a data logger located at the edge of the fairway. These sensors measure three parameters: temperature, volumetric water content, and the electric conductivity of the soil.

The installation process was performed over the course of several weeks followed by technical testing and calibration. Prior to automatic data collection, sensor-measured soil electrical conductivity (EC) was compared to conventional saturated paste extracted soil EC to assess data accuracy. Significant linear correlation was observed between sensor-measured soil salinity vs. saturated paste extracted soil salinity ($r = 0.77$).

After calibration, data loggers have been programmed to collect data once a day to



A total of 12 sensors were installed on each fairway to measure temperature, volumetric water content, and electrical conductivity of the soil.

provide information on spatial and temporal salt accumulation patterns.

Data collected thus far showed that in early August when the weather was relatively dry, a well-drained fairway had an average soil salinity of 1.8 and 1.3 dS/m at 15 and 30 cm below soil surface, respectively, whereas the poorly drained fairway had 2.95 and 2.43 soil EC at 15 and 30 cm below soil surface, respectively. A significant thunderstorm in mid-August effectively leached soil salts. Our preliminary data indicated that the accumulation of salts appears to relate to precipitation patterns, soil texture, and drainage effectiveness.

Summary Points

- Effluent water is a reliable and consistently available source of water for golf course irrigation.
- Salinity and sodicity are major concerns associated with effluent water irrigation.
- Compared to sites irrigated with surface water, sites irrigated with effluent water exhibited higher EC and higher sodium adsorption ratio (SAR) of saturated paste extract.
- Significant linear correlation was observed between 5TE salinity sensor-measured soil salinity vs. saturated paste extracted soil salinity
- Accumulation of salts appears to relate to precipitation patterns, soil texture, and drainage effectiveness.

Cultural Practices, Environment, and Pathogen Biology: Studies for Improved Management of Large Patch of Zoysiagrass

Megan Kennelly, Jack Fry, Rodney St. John, and Dale Bremer
Kansas State University

Objectives:

1. Determine the effects of aeration, verticutting, and sand topdressing on large patch and investigate the biology of the interaction of cultural practices and disease.
2. Determine the effects of nitrogen source and time of application on disease development.
3. Study the environmental conditions associated with disease development in the field.
4. Compare large patch susceptibility of 34 new freeze-tolerant zoysiagrass genotypes.
5. Study the effects of different preventative fungicide application timing and correlate with weather conditions to develop better guidelines for fungicide deployment.

Start Date: 2008

Project Duration: three years

Total Funding: \$46,806

Large patch, caused by *Rhizoctonia solani* AG 2-2, is the most common and severe disease of zoysiagrass in the transition zone. Knowledge is lacking about the interaction of cultural techniques, weather, and disease development. In this project, we are conducting field experiments at several sites to investigate these interactions.

In preliminary experiments, spring aeration, verticutting, and sand topdressing surprisingly led to higher levels of large patch. As work progresses, we will better elucidate the influence of cultivation practices on large patch, and we will monitor the effects of weather on disease development. We will also investigate fungicide application timing and correlate it with environmental data to develop a model for optimal fungicide deployment if fungicides are used.

In spring and fall 2008, we started to build our collection of large patch isolates to use in further studies. We currently have 60 isolates, stored on oat seeds, for long-term stability.

We carried out the first set of cultural and fertility practices for Objectives 1 and 2. Plots were established at 3 sites (Manhattan, Olathe, and Haysville, KS). At all 3 sites, plots are set up as a split-plot with 4 replications. The main treatment plots are 12 x 20 feet. Treatments are cultivation (aeration + verticutting + topdressing) versus non-cultivated. The subplot is fertility, either spring + fall or summer fertilization. For the spring + fall treatment, plots were treated with 1 pound N/1,000 ft² as urea (46-0-0), in both spring and fall.



Large patch, caused by *Rhizoctonia solani* AG 2-2, is the most common and severe disease of zoysiagrass in the transition zone.

The summer treatment was 2 lbs N/1,000 ft² as polymer coated urea.

To induce disease development, all plot areas were inoculated in September 2008 by taking out small cores, inserting large-patch-infested oats, and replacing the cores. Disease will be assessed in spring 2009.

We deployed temperature and wetness sensors in several experimental plots in fall 2008. The data will be analyzed this winter, and the same type of measurements will be taken in the coming seasons.

We have started to propagate the 34 zoysiagrass lines in the greenhouse. The zoysiagrass is propagated by taking 2-3 node sections of stolons and placing in a soilless potting mix under mist for several weeks, then transferring to the greenhouse.

In addition, field plantings of the zoysiagrass progeny were inoculated in both Manhattan and Olathe. Disease symptoms were apparent in some plots this fall. Disease will be assessed in the spring, along with time-to-recovery.

We established fungicide timing trials at two sites, an inoculated site at our research facility and a naturally-infested area at a local golf course.

Summary Points

- First set of cultural practices was carried out and plots inoculated for disease ratings in spring 2010.
- Zoysiagrass progeny were inoculated in the field, and plant material is being multiplied in the greenhouse.
- Fungicides were applied for first timing experiments, and disease will be rated next spring.

Optimizing Oriental Beetle Mating Disruption Through a Better Understanding of Dispersal Behavior

Albrecht M. Koppenhöfer & Valerie Fournier
Rutgers University

Objectives:

1. Examine the dispersal pattern of non-mated and mated oriental beetle females.
2. Explore the dispersal behaviors of male oriental beetle in pheromone-treated and non-treated areas.
3. Determine the distance male oriental beetles travel in response to different pheromone rates and/or sources.

Start Date: 2008

Project Duration: two years

Total Funding: \$19,986

The oriental beetle (OB) is the most important turfgrass insect pest in New Jersey, Connecticut, Rhode Island, and southeastern New York. Our overall objective is to investigate the dispersal biology of oriental beetle adults in order to improve the efficacy of mating disruption to control oriental beetle grubs in turfgrass.

Before effective application parameters for oriental beetle mating disruption using sex pheromone can be recommended, we need to understand how far oriental beetle females move. Since females should not be affected by their sex pheromone, they may mate outside pheromone-treated areas and migrate into treated areas to deposit eggs.

Female dispersal studies are hampered by the lack of female attractants and the fact that females are active around dusk. Females were placed into the turf-



Males were attracted to females from as far as 100 feet distance in controlled studies.

grass between 5 and 7 p.m. in early July. Most females either dug into the soil or only crawled short distances. Few females alighted but only flew 3-12 feet, after which they either did not alight again or could not be found. We will test fluorescent markers in future observations and look for other improved tracing methods.

Oriental beetle collected from multiple black light traps placed at multiple locations during early to mid-July contained on average $52.0 \pm 2.4\%$ females. These trap captures suggest that female may fly significant distances. Females trapped from selected traps were immediately frozen and will be dissected to determine their mating status.

Oriental beetle male attraction to different pheromone sources was investigated in release and recapture field studies.

Trécé Japanese beetle traps were placed in the ground with only the funnel part above ground in areas that had been treated with Merit in the previous year. The traps were lured with a virgin female placed in a metal mesh cage, red rubber septa with 10 or 30 μg sex pheromone, or one pellet (approx. weight 28 mg) of a dispersible pheromone formulation containing 5% pheromone. This pellet formulation effectively disrupted oriental beetle mating in previous field studies.

Color-marked males were released 12.5 to 200 feet downwind from the traps (100 males per distance). Recapture rates were determined after 24 hours. For each treatment, one replicate was tested on each of three consecutive days with trap locations switched daily. Analysis of variance showed that recapture was higher for the pellet and the septa than the virgin females. Recapture rates declined logarithmically with distance for all pheromone sources. The low recapture rates with virgin females are in part due to the fact that females produce less



The oriental beetle (OB) is the most important turfgrass insect pest in New Jersey, Connecticut, Rhode Island, and southeastern New York.

pheromone and call only for a few hours around dusk. Pheromone production may also have been affected by the unnatural female placement in cages.

Males were attracted to females from as far as 100 feet distance. Recapture of unmarked males from the background population was the highest for the pellet ($1,388 \pm 295$ per day), intermediate for the septa ($30 \mu\text{g}$; 682 ± 261 ; $10 \mu\text{g}$: 231 ± 172), and the lowest for the virgin female (96 ± 41). Future studies will examine how pheromone treatment of an area affects oriental beetle dispersal.

Summary Points

- OB males are attracted to pheromone lures and formulation pellets from at least 200 feet distance, but attraction to females is weaker and over shorter distances.
- A 1:1 sex ratio in black light trap captures suggest that female OB may disperse similarly as males. Limited direct observation suggest more limited dispersal.

Improvement of Water Management Strategies and Practices

Kevin Morris

National Turfgrass Federation, Inc.

Douglas Johnson

Shaun Bushman

Paul Johnson

Utah State University

Objectives:

1. Collect, evaluate, and improve grass germplasm to reduce water use and other inputs on turfgrass stands.
2. Develop an understanding of the ecology and physiology of promising plant materials for the western U.S.
3. Evaluate promising North American and Asian plant materials for their use on public and private lands.
4. Facilitate germplasm exchange with Asian scientists.

Start Date: 2007

Project Duration: two years

Total Funding: \$35,000

This research involves the collection of seeds of promising reduced-input turfgrasses in Eurasia and their subsequent evaluation in representative field environments in Utah. The objective of this research is to identify germplasm that may be used for reduced-input turf in the western U.S.

Dr. Douglas Johnson from the FRRL joined staff from the N.I. Vavilov Institute of Plant Industry (VIR) in St. Petersburg, Russia to collect seeds of reduced-input turfgrass species in Kyrgyzstan (2006), northwestern Russia (2007), and the southern Ural Mountains of Russia (2008). Seeds were collected in the field, cleaned, and cataloged, and then exported to the U.S.

During 2006, 92 collections of *Agrostis*, *Festuca*, *Trisetum*, *Poa*,

Puccinellia, and other species used in the U.S. turf industry were made in Kyrgyzstan. These were planted in replicated field trials at Evans Farm at Logan in June 2007. During June 2008, Dr. Rob Soreng from the Smithsonian Institution obtained vouchers and taxonomically verified these collections. These collections will be evaluated for their drought tolerance and turf quality characteristics under 50% ET_o irrigation in 2009 and 2010.

During 2007, 49 collections of *Agropyron*, *Agrostis*, *Festuca*, *Koeleria*, and *Poa* were made in northwestern Russia that may have potential for reduced-input applications in the western U.S. In 2008, 42 collections in the genera *Agrostis*, *Festuca*, *Koeleria*, and *Poa* were also made in the South Ural Mountains of Russia.

These 91 collections from Russia will be evaluated in replicated field trials at the Greenville Farm at Logan and the Wasatch Front at the Utah Botanical Center near Kaysville. Transplants of these

Russian collections will be established in June 2009 and maintained under 50% ET_o irrigation. During 2010 and 2011, the most drought-tolerant materials with favorable turf quality characteristics will be identified under water-limited conditions.

A greenhouse salt screening experiment was completed, evaluating primarily accessions of *Poa pratensis* and some other *Poa* species. The experiment was conducted twice with sufficient replicates, and results are nearing publication.

Stimulated by a grant from the United States Golf Association, we brought on a Ph.D. student (Shyam Shridhar) to identify genes that respond to salt stress in *Poa*. Shyam completed the RNA-based differential gene expression technique and is currently cloning and sequencing the candidate genes. *Poa* collections, intended for water deficit stress response evaluations, are established in a replicated trial in the field.

A field study of fine fescue collections from central Asia was initiated to evaluate turf potential under low maintenance conditions characteristic of the Intermountain West.

Summary Points

- Many grasses of various species, have been collected in natural settings in Kyrgyzstan (2006), northwestern Russia (2007), and the southern Ural Mountains of Russia (2008). Many of these grasses have been planted in field trials to evaluate their turf potential and low water use efficiency.
- USDA germplasm and other *Poa* germplasm was screened in the greenhouse for salt tolerance.
- A Ph.D. student is using biotechnology to identify genes that confer salt tolerance in *Poa*.
- A collection of fine fescues were established in a field trial to evaluate their performance under low maintenance conditions.



Dr. Douglas Johnson joined staff from the N.I. Vavilov Institute of Plant Industry (VIR) in St. Petersburg, Russia to collect seeds of reduced-input turfgrass species in Kyrgyzstan (2006), northwestern Russia (2007), and the southern Ural Mountains of Russia (2008).

Enhancement of Soil and Soil Management Practices

Kevin Morris

National Turfgrass Federation, Inc.

Richard Zobel

Appalachian Farming Systems Research Center

Objectives:

1. The purpose of this research is to develop improved soils and or soil management practices for turfgrass.

Start Date: 2007

Project Duration: three years

Total Funding: \$35,000

Topsoil is often unavailable in sufficient amounts during urban soil constructions or restoration of degraded soils. Therefore, there is an increasing use of mineral and organic by-products from agricultural and manufacturing industries as topsoil replacements, or as amendments in constructed soils.

A selected suite of municipal and agricultural organic by-products (anaerobically digested poultry litter, poultry litter compost, yard waste compost, and turkey litter compost), and quarry industry products were evaluated as topsoil replacements in a growth chamber experiment. The objective was to determine which by-product mix(es) provided the most desirable soil properties for turfgrass growth compared to a reference commercial topsoil mix.

Plant biomass was similar in most treatments, however it was consistently and statistically significantly lower in treatments where yard waste compost was used. Microbial activity varied among treatments. We found that the substrates present in the constructed soils promoted bacterial dominance compared to the fungal dominance in the reference commercial mix. It is also likely that the higher pH of the quarry mixes promoted the observed bacterial dominance in those treatments.

Similarly, soil enzyme activities also varied among treatments. Organic amendments increased soil β -glucosidase activity in all treatments over their non-organic amended controls. A strong correlation was found between β -glucosidase and available phosphorus. Results of this study suggest that selected waste streams can be effectively used in constructed topsoil.

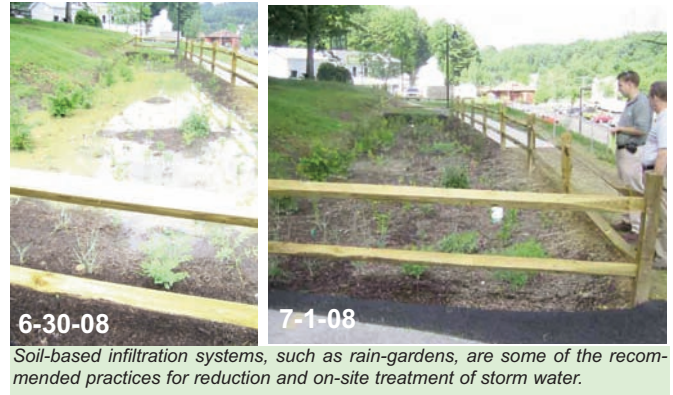
The growing residential and commercial development of urban and rural areas results in an increased demand for borrowed topsoil for landscaping purposes. Yet, topsoil definition itself varies

among authoritative sources and include the A horizon surface layer, the A master horizon (A_m), or a mixture of A and E master horizons. This study was aimed at evaluating how different topsoil definitions affect these properties of the borrowed topsoil. The hypothesis was that a mixture of A and E horizons will result in larger salvaged soil volume while having minor, if any, adverse effects on the borrowed topsoil characteristics.

Of the over 100,000 entries of the USDA-NRCS National Soil Survey Center (NSSC) database, 59,300 entries of different soil orders (excluding histosols, oxisols, and andisols) from the 48 contiguous states were used. AE-mix topsoil resulted in an average reduction of 38% in organic carbon and negligible changes in average sand, silt, and clay content compare to its respective A_m topsoil (i.e. among A_m and AE-mix constructed from the same pedon). Yet, average thickness of AE-mix topsoil was over 2.5 times that of its respective A_m topsoil.

The study provides average topsoil characteristics on a soil order basis that can serve as a reference in developing and/or refining guidelines for topsoil characteristics and specifications for borrowed topsoil.

Recent implementation of phase II of USEPA National Pollutant Discharge Elimination System (NPDES) requires the participation of urban municipalities of less than 100,000 people in the program. This means that most municipalities in urbanized areas will be required to capture and treat storm water. Soil-based infiltration systems, such as rain-gardens, are some of the recommended practices for reduction and for on-site treatment of storm water. Hence, at minimum, rain garden soil needs high infiltration rates and the ability to promote and sustain cover



Soil-based infiltration systems, such as rain-gardens, are some of the recommended practices for reduction and on-site treatment of storm water.

vegetation.

Research was conducted to identify local materials, and mixes thereof, in developing recipes for rain garden planting soil from locally available materials. Local topsoil, three quarry sands (<1/4" sandstone, washed <1/4" limestone, and non washed <1/4" limestone), USGA sand, and two commercially available composts (coarse and fine texture) were evaluated.

The mixture of sandstone:topsoil: yard waste (55:25:20) with saturated hydraulic conductivity of 2.5 inches/hour and pH of 7.5 (6.8 in 10mM $CaCl_2$) was selected for further use. The above mix recipe was used in a large-scale operation to create a planting soil for the construction of a rain garden at a nearby municipality (Beckley, WV).

The vegetation development and soil chemical and physical properties are monitored and evaluated at different times from inception. In addition, a water quality monitoring system is expected to be installed in the near future for further performance evaluations.

Summary Points

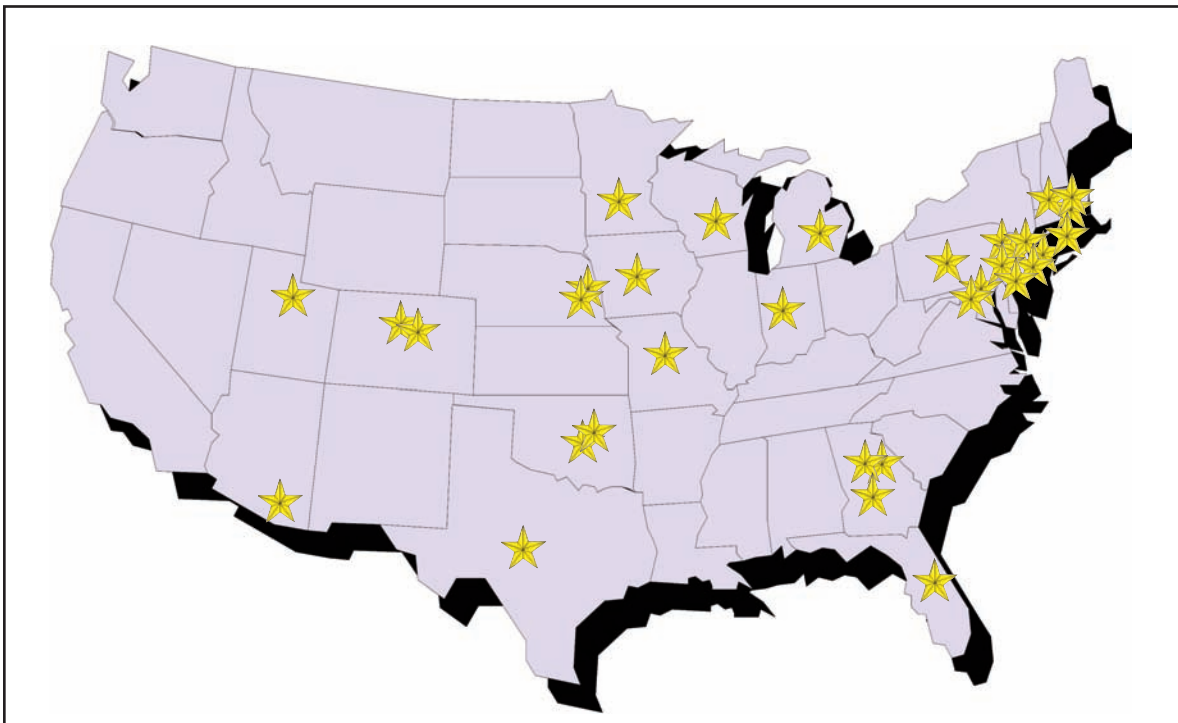
- Good turfgrass growth can result from soil constructed from organic wastes and quarry industry by-products
- Mixing A and E horizon soil profiles from a construction site can create a "topsoil" that performs much like a natural topsoil
- Several soil mixes are being evaluated for use in rain gardens (i.e., on-site treatment of storm water).

Breeding, Genetics, and Physiology

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

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

Research in the fields of biotechnology, genetics, cytogenetics, cytology, entomology, genetics, microbiology, nematology, pathology, physiology, and other sciences that support the project objectives and provide improved techniques for improving golf turf species will be considered.



Locations of the following projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the category of Breeding, Genetics, and Physiology

Identification of the Colonial Bentgrass Contribution to Dollar Spot Resistance in Colonial x Creeping Bentgrass Interspecific Hybrids

Faith C. Belanger
Rutgers University

Objectives:

1. To construct a genetic linkage map for colonial bentgrass.
2. To identify the regions of the colonial bentgrass genome that confer dollar spot resistance to the colonial x creeping bentgrass interspecific hybrids.

Start Date: 2006

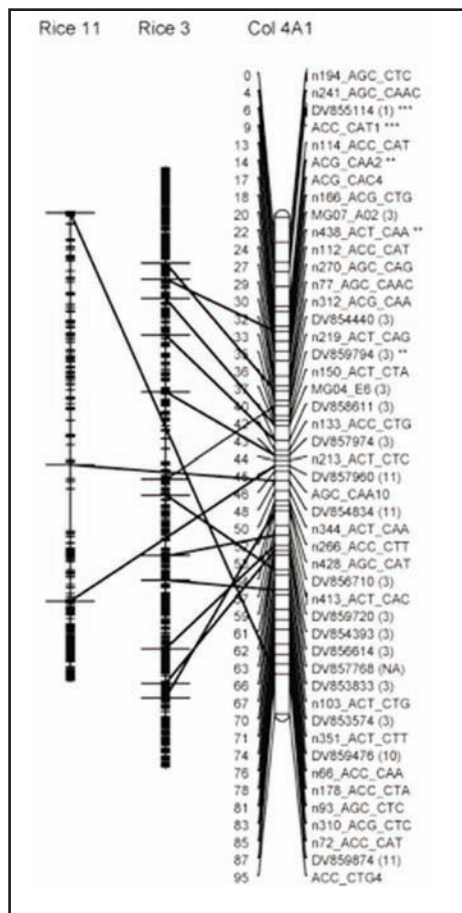
Project Duration: three years

Total Funding: \$90,000

Dollar spot disease is one of the major management problems encountered with creeping bentgrass. The related species, colonial bentgrass, has good resistance to dollar spot and may be a source of novel genes or alleles that could be used in the improvement of creeping bentgrass. Despite the dollar spot resistance of colonial bentgrass, creeping bentgrass is generally preferred because colonial bentgrass does not have the desirable aggressive stoloniferous growth habit of creeping bentgrass, which aids in repair of the turf from the damage incurred during play.

Interspecific hybridization has been used by breeders of many crops to introduce beneficial traits from related species into crop species. However, it has not yet been used in bentgrass breeding and so presents a great opportunity for creeping bentgrass improvement. The ultimate goal of such an approach would be to develop bentgrass cultivars with the stoloniferous growth habit of creeping bentgrass combined with the dollar spot resistance of colonial bentgrass.

For the past few years we have been investigating the possibility of using interspecific hybridization between colonial bentgrass and creeping bentgrass to introduce the dollar spot resistance of colonial bentgrass into creeping bentgrass. We have generated dollar spot resistant colonial bentgrass x creeping bentgrass interspecific hybrids. One of the hybrids was crossed with a creeping bentgrass plant and progeny were evaluated over two years for dollar spot resistance to assess the feasibility of introgression of dollar spot resistance from colonial bentgrass into creeping bentgrass. Of the 271 backcross genotypes in the test, 31 (11%) exhibited 20% or less disease, similar to the parental interspecific hybrid, in both



Colonial bentgrass linkage group 4A1. Most of the genes mapped to this linkage group are similar to genes on rice chromosomes 3 and 11.

years of testing. From this we can estimate that three colonial bentgrass genes may be required for the observed resistance.

One approach to eventual identification of genes conferring important phenotypic traits is genetic linkage mapping of a segregating population. We used 93 backcross individuals as the mapping population for genetic linkage mapping of the colonial bentgrass genome. This is the first genetic linkage map of colonial bentgrass. As part of the project, we generated colonial bentgrass expressed sequence tag (EST) resources that were used for mapping genes, and we developed a new method of marker identification called dideoxy polymorphism scanning. We used

the established rice-wheat chromosomal relationships to make the colonial bentgrass linkage group assignments. Most (81%) of the mapped ESTs conformed to the expectation of chromosomal location based on the location of the most similar genes in rice. The linkage map covers 1156 cM and it consists of 212 amplified fragment length polymorphic markers and 110 gene-based markers. Colonial bentgrass is an allotetraploid species ($2n = 4X = 28$, A1 and A2 subgenomes). The map consisted of the expected 14 linkage groups, which could be assigned to either the A1 or A2 homoeologous subgenomes.

To identify colonial bentgrass genomic regions conferring the dollar spot resistant phenotype, we searched for colonial bentgrass markers found in all of the resistant plants. A cluster of 14 markers on linkage group 2A1 and a single marker on group 3A1 were found in the resistant plants suggesting that these regions may be the chromosomal locations of the colonial bentgrass genes required for dollar spot resistance.

In the future it may be possible to develop molecular markers linked to the colonial bentgrass dollar spot resistance genes that could be used in marker-assisted selection.

Summary Points

- Colonial bentgrass is resistant to dollar spot disease. Hybridization with creeping bentgrass may be a way to improve the dollar spot resistance of creeping bentgrass.
- Field evaluations of the backcross progeny from a cross of an interspecific hybrid with creeping bentgrass suggest that three colonial bentgrass genes may be required for the observed dollar spot resistance.
- We developed the first genetic linkage map of colonial bentgrass. Our data suggests the colonial bentgrass dollar spot resistance genes may reside on linkage groups 2A1 and 3A1.

A Bentgrass Breeding Consortium to Support the Golf Industry

Michael Casler **Geungwha Jung** **Scott Warnke** **Stacy Bonos and Faith Belanger** **Suleiman Bughrara**
University of Wisconsin University of Massachusetts USDA-ARS Rutgers University Michigan State University

Objectives:

1. To develop elite clones of creeping bentgrass with multiple pest resistances and stress tolerances that can be delivered to the seed industry for use in synthesizing new creeping bentgrass cultivars broadly adapted to a range of ecological and environmental conditions including reduced pesticide application.

Start Date: 2003

Project Duration: three years

Total Funding: \$170,817
(three institutions)

Crosses have been made between creeping bentgrass and creeping x colonial bentgrass hybrid plants selected during Phase I of this project, creating new progeny populations. Progeny populations will be screened for resistance to dollar spot and snow mold pathogens and will also be evaluated for DNA markers previously associated with resistance to these two diseases. Plants will be selected based on both DNA markers and disease evaluations to test the effectiveness of DNA markers as a selection tool. Selected plants will be crossed with additional elite plants to create additional disease-resistant populations.

This research will build upon previous results of Phase I of this project and will tie into previous results that have mapped putative genes for disease resistance in creeping bentgrass. Elite clones of creeping bentgrass and creeping x colonial hybrids will be released to private breeders



Selection of the most disease resistant clones at the O.J. Noer Turfgrass Research and Education Center, Verona, WI.

for use in developing new varieties for the golf industry.

Activities for 2008 focused on the establishment of replicated clonal evaluation plots of progeny from elite crosses, extraction of DNA from leaves, and the initial assay of DNA markers. Replicated field evaluations were established in

Massachusetts, New Jersey, and Maryland in 2007 and 2008.

In 2009, data will be collected on disease resistances of field-grown plants and DNA markers. Selected plants will be used to create new hybrid plants, progeny populations, and releases of individual clones or genotypes to bentgrass breeders who are developing new varieties.



Creeping bentgrass clones originating from Illinois, Michigan, or Wisconsin tended to have better resistance ratings to both dollar spot and snow mold when evaluated in their state of origin compared to clones from outside that state. Four creeping bentgrass clones demonstrating various levels of resistance to the dollar spot fungus, *Sclerotinia homeocarpa*, at Gateway Golf Club, Land 'O Lakes, WI., are shown above.

Summary Points

- Creeping bentgrass plants with improved resistance to both dollar spot and snow mold fungi have been identified and propagated.
- These plants will be released to private turf breeders for use in developing new and more disease-resistant creeping bentgrass varieties.
- This research has contributed to the identification of specific genes for resistance to snow mold and dollar spot diseases, which can be used to design more efficient and effective breeding methods based using DNA marker technologies. These methods will be tested in the current phase of this project.

Development of Seeded Turf-type Saltgrass Varieties

Dana Christensen and Yaling Qian
Colorado State University

Objectives:

1. Evaluate new collections and Cycle 2 population. Select parents from these and intercross for the Cycle 3 population.
2. Screen the collection for rhizome depth.
3. Evaluate selection's potential for commercial seed harvest.
4. Correlate meristems to plant size.

Start Date: 2006

Project Duration: three years

Total Funding: \$78,822

The native grass, desert saltgrass, has potential in western US golf courses because of its traffic resistance and tolerance of salty soils and water. Under these conditions it outperforms other drought resistant species such as buffalograss, bermudagrass, and blue grama.

The 3000 Cycle 2 plugs, planted August 2007, grew into their 3 by 3 foot plots this year. Each plug represents a different phenotype. We took measurements on percent grow-in, flowering dates, spike numbers, height, verdure, and leaf shred from mowing.

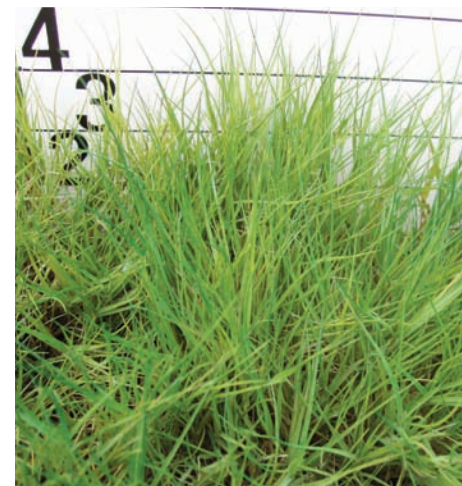
Resistance to leaf rust (*Puccinia subnitens*) has been an important trait for selection, as the disease can render saltgrass leaves brown the first week of August. This past year, natural infection along the Front Range of Colorado has been spotty and we have resorted to making collections of uredia (spores) throughout northeast Colorado. About 50 pounds of infected leaves are washed in 100 gallons of water with a commercial spray



Wild population behind chairs, Cycle 2 turf in front of chairs

adjuvant/surfactant. The material is screened through cheese cloth, the cloth is washed, and all supernatant is put into a sprayer and sprayed onto nursery plants immediately.

Natural disease pressure can usually be quantified in June, so that collecting and artificial inoculation can be planned, if necessary, for the first week of August. We speculate the daily morning dews in August aid infection, since this is the time of year of natural re-infection by urediospores. Susceptible check phenotypes showed telia development in September. Ratings on telia on all plants can be made in October, using a modified wheat leaf rust rating. Inheritance of resistance is complex, and progeny testing is used to track resistance genes.



Mature turf from seedling with smaller shoot diameter

It is established that the size of an organ is proportional to the size of the meristem from which it develops. Based on this, large shoot meristems should give rise to large shoots, and small shoot meristems should give rise to small shoots. Correlations also exist between small shoots and high shoot density and large shoots and low shoot density. Between 1960 and 1980, there was a flurry of activ-



Harvesting 2 cm long shoots to measure diameter

ity in measuring correlations and correlated responses in many species. Some of the success in breeding for turf traits can be attributed to positive correlations among turf traits.

Much of the effort in a breeding program is planting and maintaining the nurseries. If the material going into the outdoor nursery were higher caliber, it would present increased efficiency in selection. We set up an experiment in which we measured the diameter of 2-cm long shoots of random new progeny seedlings in the greenhouse. We transplanted this experiment into the field and, after a year, measured height and shoot density. Seedlings with small diameter shoots matured into turf with shorter height and higher shoot density.

Summary Points

- Phenotypes planted in August 2007 were evaluated for percent fill-in, flowering date, height, and mowing quality.
- Artificially inoculate the nursery with leaf rust and rate response.
- Seedlings with small diameter shoots mature into turf-type plants.

Perennial Ryegrass Anti-freeze Protein Genes Enhances Freezing Tolerance in Plants

Shui-zhang Fei
Iowa State University

Objectives:

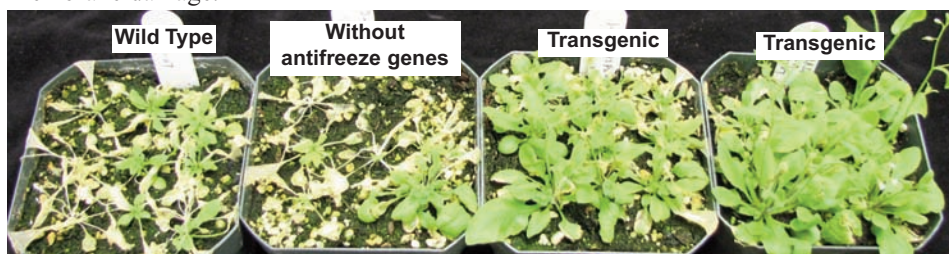
1. Measure and compare freezing tolerance of perennial ryegrass cultivars with annual ryegrass cultivars before and after cold acclimation.
2. Characterize functions of the antifreeze protein genes IRIP1 and IRIP2 by overexpressing them in the model species *Arabidopsis* and assess membrane damages and overall survival rate of the transgenic plants under both cold-acclimated and non-acclimated conditions.

Start Date: 2006

Project Duration: three years

Total Funding: \$89,784

Cold acclimation or cold hardening is an effective mechanism to increase freezing tolerance in temperate plants. Seedlings of two annual ryegrasses cultivars, 'Gulf' (early flowering) and 'Tur' (late flowering), and two perennial ryegrass cultivars, 'Caddyshack' (cold tolerant) and 'Lafayette' (cold sensitive) were used for measuring leakage induced by freezing. Five-week-old seedlings were placed into growth chambers at 23 °C for two weeks and then acclimated at 4 °C for various periods before they were used for ion leakage test, a measurement of cell membrane damage.



Transgenic *Arabidopsis* lines ines showed improved survival rates after being subjected to -4, -6, and -8 °C for 70 minutes compared to a wild type or seedlings carrying the empty vector alone without the antifreeze genes.

Freezing tolerance tests showed that after 14 days of cold acclimation at 4 °C, freezing tolerance greatly increased in both cultivars of perennial ryegrass, but it did not change in the two annual ryegrass cultivars. This suggests that perennial ryegrass can successfully cold acclimate, whereas annual ryegrass cannot. Increasing the length of cold acclimation beyond 14 days, however, did not increase freezing tolerance in perennial ryegrass.

Genes that are highly responsive to cold are detected in higher copy numbers in cold-acclimated perennial ryegrass than in non-cold-acclimated controls. Many genes are highly up-regulated by cold, while some genes are down-regulat-

ed. Among the highly upregulated genes, two ice recrystallization inhibition protein (IRIP) genes, LpIRIP1 and LpIRIP2 were identified from a cDNA library constructed from cold-acclimated perennial ryegrass 'Caddyshack'. Reverse transcriptase PCR (RT-PCR) results showed that both genes were highly up-regulated after one day or seven days of acclimation at 4 °C.

The DNA sequences of both genes suggest that they probably share similar functions with known antifreeze proteins from other plant species. Both LpIRIP1 and LpIRIP2 were transformed into *Arabidopsis* ecotype 'Columbia' through *Agrobacterium*-mediated transformation for overexpression. Transgenic plants are allowed to advance to the T₂ generation. We randomly selected six T₂

(EV-6).

The survival rates of the same transgenic lines were also evaluated after cold acclimation. One-month-old seedlings from the selected T₂ lines were acclimated at 4 °C for eight days before being subjected to freezing temperatures. All seedlings, including wild type (Col-0) and seedlings carrying an empty vector (EV-6) showed improved survival rates after being subjected to -10, -12, and -14 °C for 70 minutes. The selected LpIRIP1 and LpIRIP2 transgenic lines showed much higher survival rates than exhibited by either the wild type control or the plants carrying the empty vector alone after cold acclimation.

Ion leakage test of these transgenic lines indicated that under non-cold-acclimated condition, only seedlings from LpIRIP1-7 and LpIRIP2-4 showed reduced ion leakage than the control plants Col-0 or EV-6 after freezing at -4 or -5 °C. However, after cold acclimation, all selected transgenic lines showed reduced ion leakage under either -8, -9 or -10 °C of freezing comparing to the control plants, although the ion leakage for the control plants also decreased.

Summary Points

- Ion leakage test indicated that perennial ryegrass can successfully cold acclimate, while annual ryegrass is unable to cold acclimate. Two weeks of cold acclimation is sufficient for perennial ryegrass to achieve freezing tolerance, and lengthening the cold acclimation beyond two weeks did not increase freezing tolerance further.

- Overexpressing the two perennial ryegrass antifreeze protein genes, LpIRIP1 and LpIRIP2, in the model species *Arabidopsis* enhanced freezing tolerance as evidenced by the increased survival rate and reduced ion leakage after the transgenic lines were subjected to freezing temperatures.

Breeding and Evaluation of Kentucky Bluegrass, Tall Fescue, Perennial Ryegrass, and Bentgrass for Turf

C. Reed Funk, William A. Meyer, and Stacy A. Bonos
Rutgers University

Objectives:

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

Start Date: 1982

Project Duration: Continuous

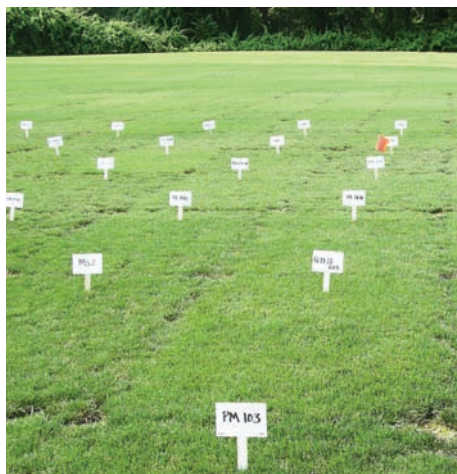
Total Funding: \$10,000 per year

As of October 30, 2008, over 1,150 promising turfgrasses and associated endophytes were collected in France, Italy, and Islands of Finland. Many of these associated endophytes should be new and unique and should have properties to enhance turfgrass performance. Over 9,434 new turf evaluation plots, 92,000 plants in spaced-plant nurseries, and 30,000 mowed single-clone selections were established in 2008.

Over 200,000 seedlings from intra- and inter-specific crosses of Kentucky bluegrass were screened for promising hybrids under winter greenhouse conditions of short daylengths and cool temperatures. Over 19,500 tall fescues, 34,000 perennial ryegrasses, 9,000 bentgrasses and 8,200 fine fescues were also screened during the winter in greenhouses, and the superior plants put into spaced-plant nurseries. The progenies of 150 new hybrid Kentucky bluegrasses were screened in spaced-plant nurseries to determine apomixis levels and other important turf and seed production characteristics.



Thirteen new perennial ryegrasses were released during 2008 with improved gray leaf spot resistance.



Continued developments of turf-type tall fescue are being released with improved brown patch resistance.

The following crossing blocks were moved in the spring of 2008: 5 hard fescues, 2 strong creeping red fescues, 2 Chewings fescues, 16 perennial ryegrasses, 20 tall fescues, 4 velvet bentgrasses, 4 colonial bentgrass, and 3 creeping bentgrass crossing blocks.

The 30 perennial ryegrasses identified in two different locations of the 2004 National Turfgrass Evaluation Trial in New Jersey have continued to display resistance to gray leaf spot (*Pyricularia grisea*) through 2008. These were developed in collaboration with other organizations since the fall of 2000 when the first severe epidemic occurred at Adelphia, New Jersey.

We are making continuous progress with annual cycles of recurrent selection in perennial ryegrass for gray leaf spot, dollar spot (*Sclerotinia homocarpa*), red thread (*Latisaria fusiformis*), and crown rust (*Puccinia coronata*). Some of the newly released perennial ryegrasses released this year are 'Defender', '1G Squared', 'Derby Extreme', 'Amazing GS', 'Manhattan 5 GLR', 'Gray Star', 'Kokomo II', 'Primary', 'Uno', 'Transformer', 'Gray Goose', 'Gray Fox', and 'Palmer GLS'.

New promising Kentucky blue-

grasses and Texas x Kentucky bluegrass hybrids are 'Ridgeline', 'Aries', 'Aura', 'Delight', 'Futurity', 'Katie', 'Rhapsody', 'Hampton', 'Juliet', and 'Gaelic'.

Continued developments of turf-type tall fescue are being released with improved brown patch resistance. They include 'Falcon NG', 'Jamboree', 'Terrier', 'Rocket', 'Essential', 'Fat Cat', 'Bullseye', 'Virtuosa', 'Mustang 4', 'Firecracker LS', 'Titanium LS', and 'Cochise IV'.

'Foxfire' strong creeping red fescue and 'Intrigue II' Chewings fescue were released in 2008.

Summary Points

- Continued progress was made in obtaining new sources of turfgrass germplasm from old turf areas in Europe. 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 and beneficial endophytes enables significant improvements in the performance of new cultivars. Thirteen new perennial ryegrasses were released during 2008 with improved gray leaf spot resistance.
- Twelve new improved tall fescues were released in 2008.
- Substantial progress was made in developing intra- and inter-specific hybrids of Kentucky bluegrass. Ten new promising Kentucky bluegrass cultivars were released in 2008.
- Two new fine fescue for low maintenance turf were released.
- One creeping bentgrass was released called 13M with dollar spot resistance. Colonial bentgrass clones with improved brown patch resistance were used to develop 4 populations. Clones of velvet bentgrass found with resistance to copper spot, dollar spot, brown patch, and pythium were used in velvet population increases.

Production, Maintenance, and Evaluation of Triploid Interspecific Bermudagrass Hybrids for QTL Analysis

Wayne Hanna and Andrew Paterson
University of Georgia

Objectives:

1. Increase the size of the T574 x T89 mapping population by 100 or more triploid interspecific hybrids.
2. Evaluate the hybrids for characteristics important in turf improvement and provide the information to Dr. Paterson for association to the molecular map.

Start Date: 1999

Project Duration: ongoing

Total Funding: \$90,000

Hand pollinations of the *Cynodon transvaalensis*-T574 x *C. dactylon*-T89 cross were made in the spring of 2007. Crosses were harvested and the products of this cross were planted in the greenhouse in the spring of 2008.

Progeny were transplanted to 5-cm pots, and single plants were established in 2m x 2m plots (methyl bromide fumigated soil) in the field in 2008. We were able to produce 20 new hybrids (confirmed with flow cytometry). Plant samples have been made available to A. Paterson for the molecular study.

The maintenance nursery for the triploid hybrids was re-established in 2008 in newly fumigated soil to maintain the purity of the stocks.

After analyzing the data on seed



The products of the cross between *C. tranvaalensis*-T574 and *C. dactylon*-T89 are being propagated and established in field plots on 2-meter by 2-meter centers at the University of Georgia research facilities.



The interspecific triploid hybrids ranged in color from 1 to 3 ($LSD_{0.05}=1.5$), in turf quality from 4 to 8.5 ($LSD_{0.05}=1.8$)

head formation, longest stolon, plant radius, texture, and vigor which we collected on the hybrids during the past three years and provided to Dr. Paterson, he found 105 (however, he indicated that this may be a little over-estimated) statistically significant QTL marker trait associations.

Summary Points

- QTL molecular marker associations (105) were found for seed head formation, longest stolon, plant radius, plant texture, and vigor in the bermudagrass hybrids.
- New hybrids have been developed to add to the population for use in the genetic map.

Resistant Turfgrasses for Improved Chinch Bug Management on Golf Courses

Tiffany Heng-Moss, Fred Baxendale, Robert Shearman, Gautam Sarath, and Paul Twigg
University of Nebraska

Objectives:

1. Evaluate selected cool- and warm-season turfgrasses for resistance to chinch bugs in the *Blissus* complex.
2. Investigate the biochemical and physiological responses of buffalograss to chinch bug feeding.
3. Identify genes conferring resistance to chinch bugs.

Start Date: 2003

Project Duration: six years

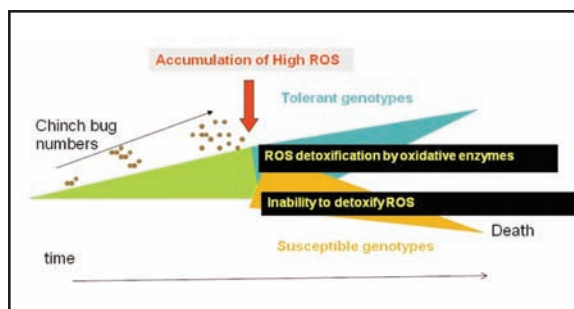
Total Funding: \$60,000

The overall goal of this research is to identify chinch bug-resistant turfgrasses, investigate the mechanisms of this resistance, and identify specific genes contributing to the resistance. Knowledge of specific resistance mechanisms is valuable for identifying biochemical and physiological markers for use in germplasm enhancement programs, and for characterizing plant defense strategies to insect feeding.

We continue to evaluate selected warm-season turfgrasses for resistance to chinch bugs. Of the 100 buffalograss genotypes evaluated in greenhouse and field studies, four have been categorized as highly resistant ('Prestige', NE 184, NE 196, and NE PX 3-5-1) and 24 as moderately resistant. Of the resistant buffalograsses studied, 'Prestige' exhibited the highest level of resistance even though it often became heavily infested with chinch bugs. Subsequent choice and no-choice studies characterized 'Prestige' as tolerant.

A second component of this research investigated the underlying biochemical and physiological mechanisms responsible for chinch bug resistance. The impact of chinch bug feeding on the physiological responses of resistant and susceptible buffalograss has been evaluated through gas exchange and chlorophyll fluorescence measurements at specific time intervals using established procedures. These studies have demonstrated that resistant plants can generate energy for recovery from chinch bug feeding. Susceptible plants appear unable to maintain compensatory photosynthesis and, as a consequence, suffer substantially more tissue damage from chinch bug feeding.

Our research also focused on characterizing protein changes, specifically oxidative enzymes, in resistant and sus-



To protect themselves from the effects of ROS accumulation, plants have developed oxidative enzymes that break down ROS.

ceptible turfgrasses challenged by chinch bugs and explored the value of these changes as protein-mediated markers to screen for insect-resistant turfgrasses. Reactive oxygen species (ROS), such as hydrogen peroxide, are known to be important early signals for altering gene expression patterns in plant cells in response to abiotic and biotic stressors.

Despite the benefits gained from reactive oxygen species molecules like hydrogen peroxide as defense signals, accumulation of these molecules can be toxic to cells. To protect themselves from the effects of ROS accumulation, plants have developed oxidative enzymes that break down ROS. Peroxidase, catalase, and superoxide dismutase have all been documented as ROS scavengers in plants stressed by insects and pathogens.

Research by our group has documented increased levels of peroxidases following chinch bug feeding in the resistant buffalograss, 'Prestige', and a loss of catalase activity in the susceptible buffalograss, '378'. These findings support our working hypothesis that an initial plant defense response to chinch bug feeding is to elevate the levels of specific oxidative enzymes, such as peroxidase, to help detoxify peroxides that accumulate as a result of plant stress (see figure).

Native gels stained for peroxidase have identified differences in the isozyme profiles of resistant and susceptible buffalograsses. Studies are currently under-

way to identify these specific peroxidases and measure their expression over time in buffalograsses challenged by chinch bugs using qRT-PCR.

Our group has successfully constructed and characterized two subtracted cDNA libraries. These libraries were prepared from the resistant buffalograss 'Prestige' and the susceptible '378' buffalograss 5 days after initiation of chinch bug feeding. Several transcripts showed significant levels of change in 'Prestige' (resistant) when compared to the chinch bug susceptible buffalograss '378'.

These transcripts were cloned, sequenced, and categorized according to putative function. These assays indicated up-regulation of three defense-related transcripts in the resistant 'Prestige' buffalograss, but not in the susceptible buffalograss '378'.

Summary Points

- Warm-season grasses with resistance to chinch bugs in the *Blissus* complex have been identified.
- Commercial production of 'Prestige' provides consumers with a high quality buffalograss with improved chinch bug resistance.
- We hypothesize that peroxidases and other oxidative stress enzymes are playing multiple roles in the resistant plant's defense response, including the downstream signaling of plant defense reactions to chinch bug injury and/or efficient removal of reactive oxygen species.
- This research identified genes differentially expressed in response to chinch bug feeding and will serve to increase the genomic resources available for buffalograss and facilitate development of improved turfgrasses with resistance to chinch bugs.

Development and Application of Molecular Markers Linked to Heat Tolerance in *Agrostis* Species

Bingru Huang, Faith Belanger, Stacy Bonos, and William Meyer
Rutgers University

Objectives:

1. To identify molecular markers linked to heat tolerance for heat tolerance genes from a thermal *Agrostis* species.
2. To develop a marker-assisted selection system for improving heat tolerance in creeping bentgrass.

Start Date: 2003

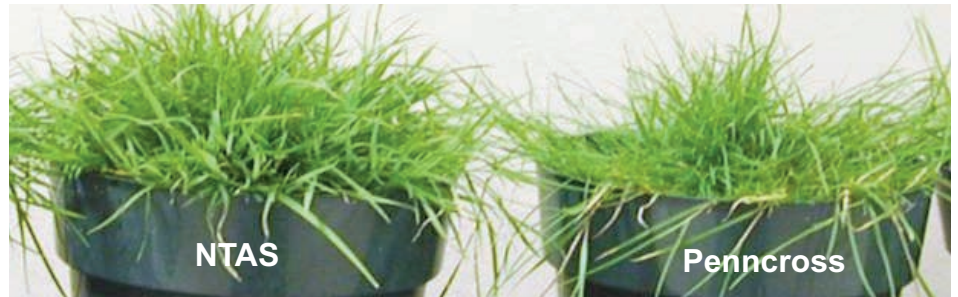
Project Duration: three years

Total Funding: \$90,000

Two independent studies, suppression subtractive hybridization (SSH) and proteomic profiling, were conducted to explore the molecular mechanisms of higher heat tolerance of *A. scabra* vs. creeping bentgrass at both RNA and protein levels. A total of 143 unique genes with higher expression level in heat-stressed thermal *A. scabra* were identified using differential display (DD-PCR) or suppression subtractive hybridization (SSH) approaches.

An ongoing study on proteomic profiling of heat-stressed vs. un-stressed *A. scabra* and creeping bentgrass has revealed 70 differentially expressed proteins. A large portion of identified genes have functions involved in protein and carbon metabolism, signaling / transcription, and stress defense. Therefore, these genes and their protein products are highly likely to be associated with the increased heat tolerance observed in thermal *A. scabra* compared to creeping bentgrass.

Several common genes with known functions in heat tolerance were identified from both gene expression and proteomic profiling, including those encoding for proteins involved in carbon metabolism (fructose 1,6-bisphosphate aldolase), stress and defense responses (phenylalanine ammonia-lyase, disulfide isomerase, and glutathione S-transferase). All of these genes code for products that are suggested to function in heat stress tolerance by either increasing glucose utilization or amplify signaling molecule biosynthesis. In addition, four heat-responsive genes encoding heat shock protein 70 (HSP70), heat shock protein 16 (HSP16), cysteine protease (AsCP1), and expansion (AsEXP1) were selected based on their heat-inducible patterns in both gene expression (northern blot) and protein



Agrostis scabra (NTAS) and creeping bentgrass (Cv. Penncross) exposed to 35°C for 12 days in a growth chamber.

(western blot) analyses. These genes may play the critical roles in the heat tolerance and could be used as potential candidate genes for marker development.

Eight heat-responsive genes were selected based on results from previous studies of differential gene expression analysis and proteomics. The apparent conservation of gene function across species provides a unique opportunity to translate discoveries in model species to creeping bentgrass. Availability of genome sequences from closely related species (rice, maize, wheat, and barley) in the public domain will dramatically shorten the effort to obtain the full length cDNA fragments of gene orthologs and increase the efficiency of creeping bentgrass marker development. Orthologous regions for the chosen candidate genes were identified from public databases.

SSR primer pairs were designed from the *A. scabra* EST sequences or the identified orthologous genes of related species. SSRs within each of the selected genes were found using the SSR identification tool Primer3 based on the *A. scabra* full-length cDNA. The same software was used to design primers amplifying fragments that contained the SSR region following main parameters and all other parameters in default settings.

Screening of two candidate gene primers (cysteine protease and expansin) indicated that one of the expansin primers showed polymorphisms. This primer pair amplifies a 166 bp, and a 188 bp fragment in 7418-3 and L93-10 genomes, respec-

tively, which harbor the tetra-nucleotide repeat motif "AGCT".

Summary Points

- Suppression subtraction hybridization (SSH) cDNA libraries from the heat-tolerant *A. scabra* identified several important 'heat tolerance' genes or molecular markers from thermal *A. scabra*. Heat-responsive genes in thermal bentgrass were categorized into five functional groups: protein metabolism, signaling/transcription, carbon metabolism, stress defense, and other metabolism. Some genes have unknown functions. The largest group of heat-responsive genes is involved in stress/defense, followed by the group of genes related to protein metabolism.

- Proteomic profiling revealed that the up-regulation of sucrose synthase, glutathione S-transferase, superoxide dismutase, and heat shock protein St1 (stress inducible protein) may contribute to the superior root thermotolerance of *A. scabra*. Phospho-proteomic analysis indicated that two isoforms of fructose-bisphosphate aldolase were highly phosphorylated under heat stress, and thermal *A. scabra* had greater phosphorylation than *A. stolonifera*, suggesting that the aldolase phosphorylation might be involved in root thermotolerance.

- Several PCR-based SSR markers from heat-responsive genes were developed, which could be used in marker-assisted selection of heat-tolerant bentgrass and other cool-season turfgrass species.

Quantitative Trait Loci (QTL) Mapping of Resistance to Gray Leaf Spot in *Lolium*

Geunhwa Jung
University of Massachusetts

Objectives:

1. To generate populations by crosses of resistant clones (from the MFA x MFB and L4B-5 x MF-8 populations) with resistant plants to be selected from commercial perennial ryegrass cultivars and breeding lines including 'Paragon GLR', 'Gray Star', 'Gray Fox', 'Grey Goose', 'Manhattan-5', 2COL-07, and 2NKM-1.
2. To develop perennial ryegrass plants having a broad spectrum of GLS resistance by pyramiding multiple resistant genes originated from various sources of *Lolium* species and cultivars.

Start Date: 2003

Project Duration: three years

Total Funding: \$87,883

Despite the fact that perennial ryegrass (*Lolium perenne*) is a valuable cool-season turfgrass, gray leaf spot caused by *Magnaporthe grisea* has become a serious problem on perennial ryegrass fairways on golf courses. Under favorable environmental conditions, gray leaf spot (GLS) can completely destroy ryegrass stands in a short period of time.

The use of host resistance is an environmentally sound method to control gray leaf spot which has been well studied and utilized in other hosts (mainly rice) of *M. grisea*. With the use of recently improved perennial ryegrass cultivars ('Paragon GLR', 'Palmer GLS', 'Panther GLS', 'SR 4600', 'Protégé', 'Gray Star', 'Gray Fox', 'Gray Goose', and 'Manhattan 5') with resistance to gray leaf spot, there has been some concern that those resistant cultivars might break down due to selection of pathogen isolates that can overcome the resistance genes. Research on interaction between pathogen variability and host resistance in perennial ryegrass needs immediate attention.

Previously detected GLS-resist-



QTL mapping for gray leaf spot resistance is essential in developing resistant (left) versus susceptible (right) perennial ryegrass cultivars.

ant QTLs in MFA and MFB plants are related with partial resistance. The combination of partial resistance has proven to be more durable to attacks by different GLS pathogen races and so is likely to last longer than race-specific resistance. To evaluate interaction between pathogen variability and host resistance in GLS disease, two ryegrass mapping parent clones (MFA, MFB), five commercial resistant cultivars, 'Paragon GLR' (two selected GLS-resistant, Paragon-1R, Paragon-2R and susceptible plants, Paragon-3S and Paragon-4S based on the previous inoculation experiment), 'Gray Star', 'Gray Fox', 'Grey Goose', 'Manhattan 5' (obtained from Dr. Stacy Bonos, Rutgers University and commercial turfgrass seed companies), and two breeding lines, 2COL-07 and 2NKM-1 (from seed company) were inoculated using twelve perennial ryegrass and one rice isolates. The isolates were: GG9, GG11, GG12 (Dr. M. Farman, Univ. of Kentucky), BL00, LP97, Lin00 (Dr. A. Hamblin, Univ. of Illinois), 6082 (Dr. S. Leong, Univ. of Wisconsin), 05T-04, 02V-23.1, 04S-01, 06T-02, 11W-03, and 11W-07 (Dr. W. Uddin, Penn. State Univ.).

The ryegrass plants were grown in French Hall greenhouse at University of Massachusetts-Amherst and then inoculated with the isolates under growth chamber conditions. As previously evaluated, MFA and MFB were resistant to all those isolates and Paragon-3S and Paragon-4S were very susceptible as expected. All commercial cultivars and experimental lines tested were highly resistant to the isolates but 2NKM-1 and 'Gray Star' were moderately resistant. A marginal significant interaction between isolates and genotypes was observed, but more inoculation experiments are required to confirm the result.

The results encourage further investigation. Some individual plants (5-10) will be randomly selected from those

resistant cultivars and breeding lines in order to test whether there is any significant interaction between pathogen variability and host resistance of different sources. Further inoculations using clonally replicated plants and 13 geographically diverse isolates will be performed to check whether the resistance in commercial cultivars differs from ones in MFA and MFB.

If pathogen variability does have a significant interaction with host resistance, crosses will combine different sources of resistance genes so that the genetic basis of gray leaf spot resistance can be improved in perennial ryegrass. Further, DNA markers significantly associated with QTLs for GLS resistances of various sources will be developed for marker-assisted selection. Multiple disease resistance genes will be incorporated into an elite perennial ryegrass cultivar which will increase the use of host resistance as an integrated pest management strategy for turfgrass managers.

Summary Points

- Significant difference in pathogenicity among 13 gray leaf spot (GG9, GG11, GG12, BL00, LP97, Lin00, 05T-04, 02V-23.1, 04S-01, 06T-02, 11W-03, and 11W-07) and one rice (6082) isolates under the controlled greenhouse conditions.

- Seven perennial ryegrass cultivars and breeding lines with improvement of GLS resistance showed a high level of resistance to the 13 geographically diverse isolates, which might indicate race non-specific resistance in perennial ryegrass.

- Preliminary results indicated a marginally significant interaction between gray leaf spot isolates and ryegrass germplasms under controlled greenhouse conditions. However, more inoculation experiments at single individual plant rather than a cultivar of mixed genotypes are needed to confirm the finding.

Breeding and Evaluation of Turf Bermudagrass Varieties

Yanqi Wu, Dennis L. Martin, and Charles M. Taliaferro
Oklahoma State University

Objectives:

1. Assemble, evaluate, and maintain *Cynodon* germplasm with potential for contributing to the breeding of improved turf cultivars.
2. Improve bermudagrass germplasm for seed production potential, cold tolerance, leaf firing resistance, and other traits that influence turf performance.
3. Develop, evaluate, and release seed- and vegetatively-propagated turf bermudagrass varieties.

Start Date: 2006

Project Duration: three years

Total Funding: \$90,000

Bermudagrass is the most widely used turfgrass in the southern USA and throughout tropical and warmer temperate regions of the world. With USGA financial support, the OSU turf bermudagrass genetic improvement program made new progress in the enhancement of turf bermudagrass germplasm and the development of experimental cultivars in 2008.

Initial screening of 1,080 putative F1 progeny plants (*C. dactylon* x *C. transvaalensis*), field established in 2006, was continued in 2008 by evaluating winter color retention, spring green-up, winterkill, foliage color, texture, sod density, seedhead abundance, and overall turf quality. Large variations were observed for turf performance and adaptation trait descriptors. We will select the best 1-2% of the plants for advancement to a replicated performance trial under fairway management to begin in 2009.

A turf bermudagrass germplasm nursery, field planted in summer 2007, was fully established on the OSU Agronomy Farm in 2008. The germplasm nursery contained 298 genotypes consisting of original accessions from geographical regions in the world, selected promising



A turf bermudagrass germplasm nursery, planted in summer 2007, was fully established on the OSU Agronomy Farm in 2008.

breeding lines, and commercial standard cultivars representing *C. dactylon* and *C. transvaalensis* and inter-specific hybrids of the two taxa. A progeny selection nursery of a tetraploid bermudagrass population in initial cycle (C0) was planted in the summer 2008.

The broad-based breeding population was formed by polycrossing tetraploid and desirable Chinese *Cynodon* germplasm accessions. A second selection nursery consisting of approximately 1,500 clonal progeny plants was planted in the summer, as well. The putative F₁ hybrids plants were derived from inter-specific hybridizations of Chinese *C. dactylon* accessions (4x=36 and 6x=54) with elite OSU *C. transvaalensis* (2x=18) breeding lines. Plants in each of these two populations will be evaluated over the next 2 to 3 years for turf performance traits and for seed yield and seed quality traits for the Chinese tetraploid population.

A field trial was continued to comprehensively evaluate eight OSU experimental synthetics for turf performance traits against clonal and seeded standard cultivars at the Turfgrass Research Center in 2008. Standard field performance parameters for fairway-type bermudagrass were assessed in this trial. In addition to the trial, a 2007-2012 NTEP

ancillary bermudagrass trial was inoculated with *Ophiosphaerella herpotricha*, one of the casual agents of spring dead spot in fall 2008. A second 2007 NTEP ancillary bermudagrass trial was also inoculated with *O. korrae*, an additional causal agent of the disease, in 2008.

Tolerance to the disease will be assessed over the next four years in these trials as well as in a 2004-2012 comprehensive evaluation of 32 clonal selections. Preliminary testing of an in-field minilysimeter technique for water use rate was initiated on 20 bermudagrass varieties in fall 2008. These trials are managed under golf course fairway conditions.

Summary Points

- A clonal bermudagrass selection nursery was evaluated and screened for turf performance and adaptation traits.
- Two new selection nurseries were field planted in 2008.
- A new turf bermudagrass germplasm nursery was fully established in 2008.
- A field trial to comprehensively evaluate eight new experimental synthetics was continued.
- Spring dead spot disease tolerance evaluation has been expanded in 2008.
- Water use rate testing of selection began in fall 2008.



A field trial was continued to comprehensively evaluate eight OSU experimental synthetics for turf performance traits against clonal and seeded standard cultivars at the Turfgrass Research Center in 2008.

Accelerated Discovery of *Cynodon* Genes and DNA Markers by cDNA Sequencing

Andrew H. Paterson
University of Georgia

Objectives:

1. We will more than double the Internet-accessible 'gene encyclopedia' for *Cynodon* by sequencing 12,000 *Cynodon* expressed sequence tags (ESTs).
2. To nurture application of EST resources to many questions in *Cynodon* biology and improvement, we will develop a freely-available online resource of 'conserved intron scanning primers'.

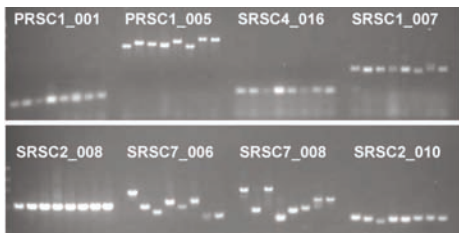
Start Date: 2006

Project Duration: three years

Total Funding: \$90,000

As a fringe benefit of the Human Genome Project, it has become possible to discover most of the genes that comprise the genetic blueprint of a plant or animal at manageable cost and in a short timeframe. Such 'gene encyclopedias' empower researchers to determine the role(s) of each gene in the life-cycle of an organism, identify hereditary differences among individuals, and engineer genotypes that better suit human needs. This provides an alternative to controversial biotechnology approaches using genetically modified organisms (GMOs) and may permit society to reap the potential benefits of many research goals in a publicly acceptable and environmentally safe manner.

While most genes have been identified for some crops such as maize, rice, and sorghum, *Cynodon* (bermudagrass) lags far behind. Our recently completed USGA project yielded sequences for portions of nearly 5,000 *Cynodon* genes, and a partner project (A. Guenzi) has yielded nearly 4,000 more for a total of about 9,000. While seemingly large, these numbers are tiny in comparison to 868,456 known for wheat, 472,163 for barley, and similarly high numbers for many other



Examples of eight conserved-intron-scanning primer PCR products using the same primers for (from left in each set) rice, maize, sorghum, pearl millet, tef, bermudagrass, wheat, and barley.

crops (per GenBank, as of this writing). Studies in other organisms suggest that EST sequencing is the most cost-effective gene discovery method up to at least 100,000 sequences. In other words, there is much more to learn about *Cynodon* genes from this efficient approach.

We have more than doubled knowledge of the *Cynodon* transcriptome (set of genes that encode an mRNA) to about 20,000 ESTs (sequences for portions of genes). While still far smaller than the numbers available for many other crops, this will considerably improve our knowledge of the *Cynodon* gene set, identifying many additional genes for which functions can be deduced based on analogy to genes with similar sequence ('spelling') in other plants.

The resulting increased number of genes will be sufficient to begin pursuing important applications with a reasonable expectation of success, some of which we elaborate on below. Further, the proposed tools are central to establishing sufficient 'critical mass' to warrant a 'turf genomics initiative' of national scope, and will immediately leverage two NSF awards in making the results web-accessible and user-friendly.

A *Cynodon* 'gene encyclopedia' will provide the community with the complete set of tools needed to locate and isolate important and unique *Cynodon* genes, setting the stage for a new era in breeding and genetics research in *Cynodon* and other turfgrasses.

In addition to enriching knowledge of the *Cynodon* gene set, we have also provided on-line resources to foster development of large numbers of DNA markers suitable for a wide range of studies. Conserved intron scanning primers (CISP) provide large numbers of PCR-based 'pan-grass' tools suitable for linking genomics research in many crops of criti-

cal economic importance but which lack appreciable sequence information (such as *Cynodon*), to burgeoning knowledge in botanical models and better-studied crops. Because CISPs are based on PCR, they require little DNA. This makes them suitable to varying levels of technology and cost associated with practice in a wide range of scenarios ranging from high-throughput application in breeding programs to targeted study in molecular biology labs.

Because CISPs are designed within genes, this approach permits us to target variation in genes directly, rather than indirectly via a proxy DNA marker. This increases the likelihood of finding the specific mutation responsible for a trait, rather than merely a diagnostic tool.

Summary Points

- This project increased the number of *Cynodon* gene sequences to about 20,000, more than doubling our knowledge of its genes and their functions.
- Identification of corresponding rice/sorghum genes will permit scientists to deduce the probable functions of many *Cynodon* genes and also reveal features that are present in the genomic DNA that surrounds *Cynodon* genes but are absent from the ESTs, such as the 'promoters' (on/off switches) that regulate their expression.
- Large numbers of 'conserved intron scanning primers,' (PCR-based markers) are ideal for many applications in *Cynodon* research and improvement.
- While a complete sequence of the *Cynodon* genome remains far in the future, our work will build the framework for efficient progress by linking this project, the most extensive *Cynodon* sequencing effort, to the first detailed *Cynodon* genetic map.

Multiple Stress Tolerance, Seed Dormancy Breaking, and Establishment of Seeded Saltgrass

Yaling Qian and Dana Christensen
Colorado State University

Objectives:

1. Quantify cold hardiness of potential varietal releases and advanced seeded lines.
2. Continue to determine the level of salinity tolerance during germination (seeded type only) and as mature turf for potential new cultivars.
3. Evaluate different seed treatments to break seed dormancy.
4. Evaluate saltgrass seeding establishment in the field with high and moderate levels of soil salinity.
5. Determine saltgrass rooting characteristics and soil moisture extraction patterns under two irrigation regimes.

Start Date: 2006

Project Duration: three years

Total Funding: \$72,036

Saltgrass seeds have a low germination rate due to their seed dormancy. We evaluated different seed treatments and found that stratification and machine scarification improve germination and establishment of seeded saltgrass. However, information is lacking concerning the effects of seeding date and seeding rate on establishment of saltgrass.

Growing degree days (GDD) or heat accumulation units are useful in predicting suitable seeding time for warm-season grasses. We have completed a study to 1) determine the effect of seeding rate, seeding date, and two different seed treatments on saltgrass germination and establishment, and 2) determine the required accumulative GDD for saltgrass to establish adequate coverage (80%) after seeding. Seeding dates tested were May 15, June 15, and July 15 at two locations (Horticulture Research Center and a golf course in Denver).

This field study showed that machine scarification and stratification broke saltgrass seed dormancy equally well. Saltgrass seeded in May established adequate coverage (80%) in September even using the lowest seeding rate (74 kg ha⁻¹). For plots seeded in June, only the higher seeding rates (123 and 170 kg ha⁻¹) established adequate coverage by the end of growing season. For plots seeded in July, however, even the highest seeding rate failed to establish adequate coverage in September.

The accumulated GDD to achieve adequate coverage was 1,748, 1,663, and 1,435 for 74, 123, and 172 kg ha⁻¹ seeding rates, respectively. When seeded on July 15, total accumulated GDD toward the first



The May seeding date is best for saltgrass establishment in Colorado, with seeding rates as low as 74 kg ha⁻¹ achieving optimal establishment of saltgrass in one season.

frost was 889 and 1,220 at our two experimental sites. Therefore, it was unlikely to achieve adequate coverage when saltgrass was seeded in July in Northern Colorado. However, a higher seeding rate may be used to compensate for the June seeding date to achieve adequate coverage by the end of the growing season.

The accumulated GDD assessment provided in our study suggests that saltgrass has higher establishment GDD requirements than bermudagrass and buffalograss, but lower or similar GDD requirements as zoysiagrass. Our results will aid in selecting appropriate seeding dates for different climates for successful establishment of saltgrass.

Saltgrass has been classified as a halophyte, however, saltgrass is less salinity tolerant during germination than established turf stands. Our lab and growth chamber tests showed that Proxy solution (at 5-10 mM a.i.) and thiourea (30 mM) increased saltgrass germination under a range of salinity treatments (0-30 dS/m). Experiments have been conducted in 2008 to scale up growth chamber studies to field conditions in order to confirm the effectiveness of seed pretreatments with Proxy and thiourea in enhancing saltgrass seed germination under saline conditions. Water (control), Proxy (5-10 mM a.i.), and thiourea (30 mM) were used as pretreatment agents to soak scarified saltgrass

seeds for 24 to 48 hours.

After pre-treatment, saltgrass was seeded under four different salinity conditions in the field. Data were collected weekly on soil salinity, the number of seeds germinated, and plot coverage. Saltgrass germination percentage was 51% under non-saline condition, which was reduced to 14% when soil salinity increased to 18 dS m⁻¹. Soaking saltgrass seeds in Proxy solution (5-10 mM a.i.) increased saltgrass germination percentage under all salinity levels. However, thiourea pre-treatment did not increase saltgrass germination in the field conditions.

Summary Points

- The May seeding date is best for saltgrass establishment in Colorado, with seeding rates as low as 74 kg ha⁻¹ achieving optimal establishment of saltgrass in one season.
- Saltgrass has higher establishment GDD requirements than bermudagrass and buffalograss but lower or similar GDD requirements as zoysiagrass.
- Soaking scarified saltgrass seeds with 5-10 mM Proxy solution increased the subsequent germination of saltgrass at 5 - 20 dS m⁻¹ soil salinity.
- Thiourea pre-treatment did not increase saltgrass germination in the field conditions.

Breeding Seashore Paspalum for Recreational Turf Use

Paul Raymer
University of Georgia

Objectives:

1. Develop superior quality turf cultivars suitable for use on golf courses and recreational venues.
2. Document and improve disease resistance.
3. Further improve salt tolerance.
4. Develop improved weed management strategies.
5. Develop molecular tools to support breeding.

Start Date: 2006

Project Duration: three years

Total Funding: \$90,000

The University of Georgia has an established seashore paspalum breeding effort for the development of improved cultivars suitable for use by the golf course industry. Thus far, our cultivars have been well accepted by the turf industry both domestically and internationally. The University of Georgia turfgrass breeding program has the largest and most diverse collection of seashore paspalum ecotypes in the world. We are now utilizing this growing germplasm collection to generate new genetic variation through recombination.

This approach allows us to generate thousands of unique individuals each year. Individual plants are hand-trimmed in the greenhouse and undesirable plants eliminated. In 2008, more than 6,000 individuals were also screened for salt tolerance in the greenhouse and approximately 2,000 salt-tolerant individuals were later transplanted to field plots for further eval-



Dr. Paul Raymer develops superior seashore paspalum cultivars for use on golf courses.



Automated rain-out-shelter covers field plots during natural rainfall events allowing us to impose drought conditions.

uation of turf quality and resistance to dollar spot. This approach allows our breeding program to efficiently evaluate large numbers of individuals for important traits and should insure continued improvement in turf quality, disease resistance, and salt tolerance in our future cultivar releases.

Over the past two years, a number of turf trials were established including a Vegetative Preliminary Trial, a Fairway Variety Trial, and a Green Variety Trial at Griffin, as well as a Fairway Variety Trial at Tifton. In addition, in 2007, three advanced breeding lines were entered into the first National Turfgrass Evaluation Program (NTEP) multi-state trials for seashore paspalum. All of these trials are now generating valuable data on the performance of several advanced lines in our program.

Based on the data that we have accumulated thus far, it appears that several of these lines represent significant improvements over any existing cultivars and therefore are being positioned for release as our next cultivar. Vegetative increase of these lines is now underway and plans are to begin more broad-scale evaluation during 2009.

In 2007, Dr. Bob Carrow and his staff initiated a replicated field study with 24 entries under our automated rain out shelter. These plots were subjected to three

dry-down cycles during the 2008 growing season. During these dry down events, individual plots were rated twice weekly for turf quality, leaf firing, and normalized difference vegetative index (NDVI).

When the data were averaged over all dry-down periods, one of our advanced lines topped the lists for NDVI, turf quality, and color under drought stress.

The top group for turf quality also included 'SeaIsle 2000', 'Sea Dwarf', 'SeaIsle 1', and several other advanced lines. The bottom group for turf quality included 'Aloha', 'Salam', 'Tifway' bermudagrass, and a number of our advanced lines.

During the summer of 2009, we plan to continue this evaluation for drought resistance as well to establish a new drought tolerance experiment with our newest advanced lines. Data generated from such studies will be essential in developing the next generation of drought tolerant seashore pasaplum cultivars.

Summary Points

- Extensive greenhouse and field testing for salt tolerance, turf quality, and disease resistance is used to identify new cultivars with a combination of improved traits.

- Drought studies conducted under a rain-out shelter help us to identify potential cultivars that can maintain turf quality even during periods of prolonged water stress.

Germplasm Development and Management of Buffalograss Varieties

Robert Shearman
University of Nebraska

Objectives:

1. Breed, select, and evaluate seeded and vegetative genotypes with improved turfgrass quality, pest resistance, and stress tolerance.
2. Improve our basic knowledge of the genetics of buffalograss through modern molecular marker technologies.
3. Expand understanding and use of efficient management practices for best genotypic performance.
4. Develop protocols for best turfgrass establishment.

Start Date: 2006

Project Duration: three years

Total Funding: \$90,000

During 2008, several trials were conducted to address these objectives. Twenty-one single crosses were constructed to assess seed yield potential and continue to create variation in our germplasm. Significant yield differences were observed among the crosses, and high yielding compatible parents were identified. Approximately 1,300 individual plants were selected from these progeny. In addition, 25 collections were made from five states. These selections and collections were established in the greenhouse and transplanted to the field in June for turfgrass performance evaluations. Hybridization and collection have enriched the germplasm as a major source of new genotypes for cultivar development.

A new set of buffalograss advanced lines (Series IV), consisting of 104 genotypes was established in replicated plots at the John Seaton Anderson Turfgrass Research Facility located near Mead, NE. The genotypes in the Advanced Lines IV trial were from previous advanced line trials, selection trials, and recent collected materials. These genotypes will be evaluated for turfgrass per-



Buffalograss performance is best met through genetic improvement and development for optimum management practices.

formance characteristics and seed yield potential for three or more years. Outstanding genotypes will be identified and further evaluated for possible release.

An experimental line and cultivar evaluation trial, consisting of 20 genotypes was initiated in 2007 at five locations to assess the performance of these elite genotypes over a wide range of environments. Some genotypes did not establish well in some locations in 2007, so those sites were re-established in 2008 along with four additional locations, providing nine locations in eight states. Data from each location were analyzed separately for seeded and vegetative genotypes.

Buffalograss performance is best met through genetic improvement and development for optimum management practices. Even though buffalograss is an ideal low-input turfgrass, it is still important to know its response to different mowing heights and fertilizer application rates. Management studies involving nitrogen rates, mowing heights, and cultivars were conducted. Results from these studies indicated significant differences among N rates, mowing heights, and genotypes. Interactions were mostly non-significant. Buffalograss genotypes responded differently to the management practices.

The effects of growing degree days (GDD) of harvested sprigs, environment, and their interaction on buffalograss vegetative establishment from sprigs were studied. Though genotype and environment do play a role, GDD proved to be the major contributor in successful establishment suggesting that sprigging after 1,050 GDD will not be successful. Further study is needed to better understand the GDD influence on pre- and post-harvest physiology of sprigs.

Developing a buffalograss genome map will be helpful to better understand the function, location, and inheritance of genes involved in pest resistance, stress tolerance, and improved



USGA's research committee members view improved buffalograss selections at the University of Nebraska.

turfgrass quality. Crosses were made between two diploid (NE 3297 x NE 2871) parents. An F₁ mapping population was generated. Sequence Related Amplified Polymorphism (SRAP) markers were used to evaluate the pattern of polymorphism in the parents and their progenies. Based on marker segregation, F₂ and backcross types of segregations were observed among the progenies.

Summary Points

- Germplasm was enriched through collection and hybridization. New selections and advanced lines were established.
- Elite genotypes were evaluated under a wide range of environments. Significant differences were observed among genotypes tested for most traits. The trial will continue and seeds of some elite genotypes will be increased.
- Results from management studies showed significant differences among N rates, mowing heights, and genotypes, but not for their interactions. The trials will continue for conclusive results.
- Growing degree-day accumulation is a major contributor to successful establishment. Further study is needed to further understand the nature of the influence.
- Framework mapping of diploid population of buffalograss will be developed to better understand this grass.

Genetic Improvement of Prairie Junegrass

Eric Watkins

University of Minnesota

Objectives:

1. Determine the genetic potential of native prairie junegrass (*Koeleria macrantha*) germplasm for use as low-input turfgrass.

Start Date: 2007

Project Duration: three years

Total Funding: \$30,000

Grass species that are native to North America should be better able to cope with our environment and could lead to overall reductions in inputs such as fertilizers, pesticides, and water. Prairie junegrass (*Koeleria macrantha*), which is native to the Great Plains of the United States, 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 it can also be found throughout much of Europe and Asia. Based on data that has been collected in recent years, this species appears to perform well in Minnesota under low-input conditions (no irrigation, limited nitrogen application, and no fungicide or insecticide applications).

Prairie junegrass has several attributes that make it a useful low-input turfgrass in Minnesota including tolerance of droughty and alkaline soils, tolerance of sandy areas, survival of low and high temperature extremes, and reduced growth rate. 'Barkoel' was the first cultivar of this species specifically developed for use as a turfgrass. However, this cultivar was developed with ecotypes from Europe. We are proposing the development of a cultivar using germplasm native to North



Variation in seed production characteristics of three *Koeleria macrantha* accessions.

America.

Developing a high quality turfgrass is not, by itself, adequate. In order to be used by consumers, an economically viable turfgrass cultivar must be able to produce sufficient quantities of seed. Non-selected populations of the species can produce seed for 4-5 years. Collections of natural ecotypes made in 2005 suggest that individual genotypes may possess the ability to be highly productive. However, it is unknown if it can produce economically adequate amounts of seed.

In order for a cultivar of this species to be used on a wide scale, two criteria must be met: 1) the cultivar must pos-



Collecting prairie junegrass in southeastern Minnesota

sess adequate turfgrass quality in a medium to low maintenance management situation, and 2) the cultivar must possess adequate seed production traits so that a sufficient supply of seed can be produced at a reasonable cost.

We have collected native prairie junegrass germplasm from Minnesota, South Dakota, North Dakota, Colorado, and Nebraska. These germplasm collections have been established in breeding nurseries, and in some cases, experienced one cycle of selection. We have established several spaced-plant evaluations that will be used to determine the genetic variation present in our populations for various turfgrass and seed production characteristics.

In 2007, two experiments were established in both St. Paul and Becker, MN. The first experiment evaluated the



Poor mowing quality of a *Koeleria macrantha* genotype

USDA collection of *Koeleria macrantha* for seed production potential in Minnesota. The second experiment evaluated the same collections for turf potential as mowed spaced plants.

The seed production study is now complete and we found significant variation among accessions. Collections that showed high levels of seed production potential included germplasm collected in Iowa. Collections with low levels of seed production were generally from areas of southwestern Asia. Based on these results, we will place additional focus on the inclusion of local collections in our breeding program in order to improve seed production potential of the species.

Preliminary results from the mowing study indicate that sufficient variation for many important turf traits exists in the USDA collection. Of particular interest to our program are differences in mowing quality and the ability to maintain green color through summer stress periods. The mowing study will continue through 2009 and top-performing accessions will be integrated into our breeding program.

Summary Points

- Great diversity exists in public collections of *Koeleria macrantha*.
- Local collections will result in improved seed production characteristics.
- Integration of traits from diverse germplasm should be effective in the development of a low-input cultivar.

Evaluating Poverty Grass (*Danthonia spicata* L.) for Use in Tees, Fairways, or Rough Areas in Golf Courses in the Midwest

Nadia E. Navarrete-Tindall, Brad Fresenburg, and J.W. Van Sambeek
University of Missouri

Objectives:

1. Identify best practices to optimize seed germination, seed production, and field seeding rates.
2. Determine tolerance to shade and herbicides.

Start Date: 2007

Project Duration: two years

Total Funding: \$20,000

Poverty grass (*Danthonia spicata* L.), a native-cool season perennial grass with wide distribution in the United States, is being evaluated for its suitability for use on golf courses. Potential benefits of using poverty grass include reduced dependency on fertilizers and irrigation and less frequent mowing. The goal is to identify practices to improve seed germination and successfully establish field plots as monocultures or with other native species.

This grass is found in most prairies, savannas, and open woods throughout Missouri. However, seed commercially available is very limited. Seed has been collected every year since 2005 from different locales in Missouri in cooperation with seed producer Mervin Wallace of Missouri Wildflowers nursery. This seed is being used for trials and is also available for private seed producers in Missouri.

Seed averages 400,000 PLS/pound and is highly dormant. Untreated seed collected in Missouri in 2006 was 89% dormant with less than 5% germination under 25°C. Seed collected in 2008 was also mostly dormant.

Different combinations of stratification (moist storage), scarification (mechanical disruption of seed coat), light, and temperature were evaluated to break



Plots established with scarified seed at three seeding rates (1000, 2000, and 3000 PLS/sq ft) in fall 2007 had 24, 25, and 30% poverty grass ground cover respectively in May 2008.

seed dormancy and improve germination.

Light improved germination of scarified seed (63 % vs. 46% in dark) and non-scarified seed (30 % vs. 4 % in dark). Germination after 40 days at 25°C for scarified seed exposed to cold, moist stratification (5°C) for 10, 20, 30, and 40 days averaged 64, 66, 62, and 59%, respectively, in contrast to 35, 36, 53, and 59%, respectively, for non-scarified seed. The best seed treatment combinations will be evaluated on existing seed collections to determine if dormancy varies among seed sources and length of storage.

Seedlings were started in June 2006 from scarified seed sown in trays filled with a commercial soil mix or a silt loam soil for seed production plots. Seedlings from Minnesota, but not Missouri seed sources, were infected with *Dreschlera* leaf spot. Seedlings established 3-4 inches apart on Mexico silt loam soil at the University of Missouri-Bradford Research and Extension Center in June 2007 produced an average of 146 lb/acre of seed collected manually in June 2008. Plots established with scarified seed at three seeding rates (1000, 2000, and 3000 PLS/sq. ft.) in fall 2007 had 24, 25, and 30% poverty grass ground cover respectively in May 2008. Bare ground was 60% average for all rates.

Because these plots were infected with white clover and a few other broadleaves (5-15% cover), 2,4 D and Remedy herbicides were sprayed at a rate of 0.5 or 1 pint/acre. Remedy reduced clover and



Four shade levels (0, 30, 45, and 80%) are being evaluated on the growth rate and seed production of poverty grass using structures covered with shade cloth.

2,4 D did not have much effect. Poverty grass did not seem to be affected by either herbicide. Additional weed control was done by hand. Ground cover will again be evaluated in 2009.

Shade tolerance studies were initiated in summer 2008 in pots filled with a soil mix at Bradford Farm and will be replicated at Carver Farm at Lincoln University in 2009. Four shade levels (0, 30, 45, and 80%) are being evaluated on the growth rate and seed production of poverty grass using structures covered with shade cloth.

Summary Points

- Natural prairies or savannas in Missouri are a good source of seed of poverty grass.
- Mechanical scarification and light increased seed germination of poverty grass.
- Poverty grass grown with complete weed control produced 146 lb. seed per acre. However, more work is needed to reduce weed competition.
- Broadleaf herbicides 2,4-D and Remedy do not seem to affect poverty grass growth when applied during the growing season in May-June.

Evaluation of Perennial Ryegrass, Creeping Bentgrass, and Kentucky Bluegrass Cultivars for Salt Tolerance

Stacy A. Bonos, Josh A. Honig, Thomas Gianfagna, and Bingru Huang

Rutgers University

Objectives:

1. Evaluate salinity stress tolerance of cultivars of several turfgrass species, including perennial ryegrass, creeping bentgrass, and Kentucky bluegrass using a combination of greenhouse and field screening techniques.
2. Begin studies to understand physiological basis for salt tolerance among these cool-season turfgrass cultivars.
3. Develop cultivar recommendations of salt-tolerant cultivars for turfgrass managers.
4. Initiate inheritance studies of salt tolerance.

Start Date: 2007

Project Duration: three years

Total Funding: \$75,000

Water conservation is a necessary and responsible practice, especially in high water using urban landscapes and golf courses. As water conservation efforts increase, the need for identifying turfgrasses with salt tolerance is necessary.

The goal of this project is to evaluate and screen commercial cultivars of perennial ryegrass, creeping bentgrass, and Kentucky bluegrass cultivars for salt tolerance and initiate inheritance and physiological studies. A greenhouse and a field screening technique for salt tolerance developed at Rutgers will be compared for their ability to evaluate salt tolerance in cool-season turfgrasses.

Cultivars of perennial ryegrass, creeping bentgrass, and Kentucky bluegrass are being tested under both greenhouse and field conditions. Two greenhouse screening runs were conducted on clones from five perennial ryegrass cultivars at four salinity levels (0, 5, 10, and 15 dS/m). Significant differences were observed between salinity treatments with the highest salinity treatments causing the most injury to perennial ryegrass plants. Significant differences were also observed between clones. Clones of 'Palmer III' exhibited the highest percent green ratings compared to other cultivars.

Two greenhouse screening runs were conducted on 21 Kentucky bluegrass cultivars at four salinity levels (0, 3, 6, and 9 dS/m). The cultivars exhibiting the highest percent green ratings were 'Eagleton', 'Liberator', and 'Cabernet'. The cultivars and selections with the lowest percent green were a Texas x Kentucky bluegrass selection, A03TB-246, 'Baron', and the Kentucky bluegrass selection A03-84.

One greenhouse run was conduct-



Salt injury on cool season turfgrasses evaluated under field conditions.

ed on clones from eight bentgrass cultivars at four salinity levels (0, 3, 6, and 9 dS/m). The second run is scheduled for the spring of 2009.

A field study to evaluate cultivars of perennial ryegrass, Kentucky bluegrass, and creeping bentgrass was established in the fall of 2006. Twenty-one Kentucky bluegrass cultivars, 22 perennial ryegrass cultivars, and 15 bentgrass cultivars were established. They were evaluated for salt tolerance in the summer of 2007 and 2008 by treating with a salt solution (EC=10 dS/m) three times per week throughout the summer.

By the end of the season in 2007, the soil EC reached levels above 3 dS/m which caused significant stress on the turfgrass plants. Significant differences were observed among cultivars and selections under field conditions. 'Bewitched', the experimental selection, A03-84, 'Langara', 'Bedazzled', 'Jefferson', 'Diva', P105, 'Rhythm', and 'Liberator' had the highest percent green leaf tissue under these conditions while 'Julia' had the least.

The field results were not strongly correlated to greenhouse salt chamber results. We hope to identify the critical

factors influencing salinity tolerance under field conditions in order to develop efficient selection techniques for improving salinity tolerance in cool-season turfgrasses. Clones of perennial ryegrass were established in the fall of 2007 and treated with a salt solution with an EC of 10 dS/m. Initial broad-sense heritability was estimated to be 0.78, indicating a large proportion of the variation could be contributed to genetic effects. Narrow-sense heritability studies have also been established and will be calculated after the data has been analyzed.

Summary Points

- Significant differences were observed in five perennial ryegrass cultivars treated with 4 different salinity levels (0, 5, 10, and 15 dS/m) under greenhouse conditions.
- Clones of 'Palmer III' exhibited the percent green ratings compared to other cultivars.
- Significant differences were observed in 21 Kentucky bluegrass cultivars treated with 4 different salinity levels (0, 3, 6, and 9 dS/m) under greenhouse conditions. The cultivars exhibiting the highest percent green ratings were 'Eagleton', 'Liberator', and 'Cabernet'. The cultivars and selections with the lowest percent green were a Texas x Kentucky bluegrass selection, A03TB-246, 'Baron' and the Kentucky bluegrass selection A03-84.
- Twenty-one Kentucky bluegrass cultivars, 22 perennial ryegrass cultivars, and 15 bentgrass cultivars were established in a field trial in the fall of 2006 to evaluate for salinity tolerance under field conditions. Data was collected in 2007, 2008, and will continue through 2009.
- Initial broad-sense heritability of salinity tolerance in perennial ryegrass was estimated to be 0.78 from replicated clones.

Confirmation of QTL Markers for Dollar Spot Resistance in Creeping Bentgrass

Stacy A. Bonos, Joshua Honig, and Christine Kubik

Rutgers University

Objectives:

1. Genotype backcross and pseudo F₃ generations for the 180 SSR markers identified to date.
2. Evaluate the populations for dollar spot resistance to a different isolate of *Sclerotinia homoeocarpa* than previously evaluated.
3. Confirm QTLs in the backcross and pseudo F₃ generations to provide definitive SSR QTL markers that can be directly utilized for marker-assisted selection in turfgrass breeding programs.

Start Date: 2007

Project Duration: two years

Total Funding: \$50,000

Previous research projects have identified QTL (Quantitative Trait Loci) for important traits in mapping populations of creeping bentgrass. However, all initial QTL experiments have several limitations. They may detect ghost QTLs, fail to detect a real QTL, and may over- or underestimate the true effects of QTLs. Furthermore, QTLs can vary in different environments and different genetic backgrounds, and QTLs may not be linked to the quantitative trait after further rounds of recombination.

The confirmation of initial QTLs is necessary to successfully utilize QTL markers in marker-assisted selection breeding programs. Almost every QTL confirmation study finds some discrepancy with initial QTL experiments. The goal of this project is to confirm putative QTL for dollar spot resistance identified in a previous study (funded by the USGA, OJ Noer Foundation, and USDA) by evaluating the QTL markers in three subsequent mapping populations (two backcross populations and a second generation [pseudo F₃] population) developed from the initial mapping population.



A replicated, mowed spaced-plant trial of the pseudo F₂, pseudo F₃ and backcross populations was planted in the spring of 2008 to reevaluate the population to different virulent isolate of *S. homoeocarpa*.



Host resistance for plant diseases is an important part of improving turfgrass cultivars. Researchers at Rutgers University are using molecular techniques to identify genetic components that are responsible for resistance to dollar spot caused by *Sclerotinia homoeocarpa*, prevalent on golf course turf.

This confirmation step is necessary because it is important to determine if the QTLs remain linked to dollar spot resistance after further rounds of recombination. This project will result in easily useable, reproducible, SSR QTL markers for dollar spot resistance that will be publicly available to turfgrass breeding programs interested in using them for marker-assisted selection.

We have identified approximately 200 unique SSR markers. Approximately two-thirds of the markers have been genotyped in the backcross and pseudo F₃ generations. Initial genetic linkage maps of each original parents based on approximately 200 SSR markers were developed. Three putative QTL markers have been identified for the 'Crenshaw' isolate and one different putative QTL marker was identified for the perennial ryegrass isolate.

We analyzed the backcross and second generation populations for these putative QTLs and are in the process of scoring the markers and genotyping the progeny for presence or absence of the QTLs. This data will be compared to the phenotype data to determine whether the QTL remains linked to dollar spot resistance in these populations.

A replicated, mowed spaced-plant

trial of the pseudo F₂, pseudo F₃ and backcross populations was planted in the spring of 2008 to reevaluate the population to a different virulent isolate of *S. homoeocarpa* than was previously evaluated. We will inoculate the plants next spring and evaluate the populations for two years for dollar spot resistance.

Summary Points

- Three putative SSR QTL markers were identified in the initial population for the 'Crenshaw' isolate. One was identified in the susceptible parent and three were identified in the resistant parent. One QTL has been identified in the resistant parent for the perennial ryegrass isolate.
- Two of the three QTLs for the 'Crenshaw' isolate show evidence of dominance for susceptibility and only one exhibits dominance for resistance. This is consistent with gene number estimations (2-5 genes).
- Selection for a combination of markers identified genotypes with good dollar spot resistance. These markers may be useful in marker-assisted selection breeding programs to develop new cultivars with improved dollar spot resistance.
- Classical genetics and molecular markers support evidence for dominant genes for susceptibility.

Linking Drought Tolerance Traits and Candidate Genes in Perennial Ryegrass through Association Mapping

Yiwei Jiang
Purdue University

Objectives:

1. Evaluate the natural diversity of drought tolerance traits in perennial ryegrass.
2. Estimate the genetic population structure of perennial ryegrass.
3. Choose candidate genes involved in drought tolerance and genotyping the population for candidate genes.
4. Identify the correlation between the candidate gene and plant performance for target traits.

Start Date: 2007

Project Duration: three years

Total Funding: \$30,000

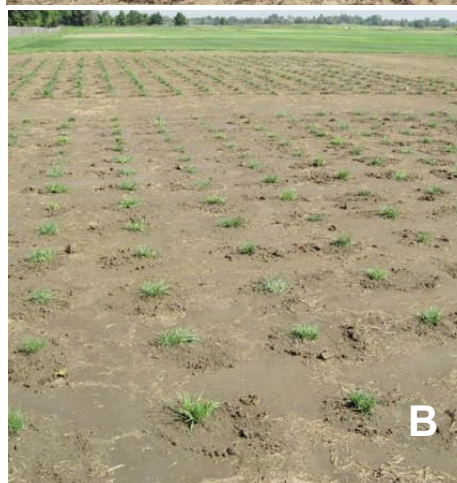
Production and utilization of drought tolerant turfgrass materials is an important approach for water conservation and for improving turfgrass performance in water-limited environments. However, the progress of breeding drought tolerant turfgrass can be restricted due to complex nature of drought stress, the large genotype by environment interactions, and the limited knowledge of the genes or traits linked to drought tolerance.

In recent years, association mapping has been developed as a novel and more powerful mapping technique. It uses a natural plant population to provide molecular markers associated with a phenotypic trait of interest and serves as an alternative method for mapping quantitative traits loci (QTL). Compared to linkage mapping in traditional bioparental populations, association mapping offers three main advantages: increased mapping resolution, reduced research time, and greater allele numbers.

A mapping population of 192 diploid perennial ryegrass accessions (*Lolium perenne* L.) was established. These grasses were selected from 43 countries and regions to maximize genetic diversity. The populations were planted in three different locations in Indiana: West Lafayette, Wanatah, and Vincennes.

Each accession was propagated by tillers from the mother plant so that plants for each individual accession used in different locations are genetically identical. Screening of perennial ryegrass populations in three locations over multiple years will generate more reliable data for evaluating drought response and tolerance.

A total of 105 simple sequence



Perennial ryegrass accessions were planted in the field and drought tolerant traits will be evaluated at Vincennes (A), West Lafayette (B), and Wanatah (C).

repeat (SSR) markers were used to screen 192 populations of diploid perennial ryegrass for assessing genetic diversity.

The cluster analysis by unweighted pair-group methods using arithmetic averages (UPGMA) and principal component analysis (PCA) showed that perennial ryegrass accessions chosen for this study was diverse without forming the major population groups, except for 5 commercial cultivars that clustered together, suggesting that genetic diversity in commercial cultivars is limited.

The first three principle components only explained 11.4 % (7.0 %, 2.3 %, and 2.1 %) of total variation. The pair-wise plots of first three principle components exhibited some levels of separation, but not clearly identifiable groups. The result indicated that this perennial ryegrass collection would be an ideal population for further association mapping of candidate genes for drought tolerance.

Perennial ryegrass is a self-incompatible species that can potentially provide high-resolution association mapping. The project will sample natural diverse populations to evaluate the physiological adaptations of perennial ryegrass to drought stress and to identify, through the association mapping approach, genes that play an important role in drought tolerance and adaptation.

Summary Points

- The diploid perennial ryegrass was planted and drought tolerance traits will be evaluated.
- The mapping population showed a large genetic diversity.
- Genetic population structure will be estimated and candidate genes will be continuously chosen for future association analysis.

Selection of Bermudagrass Germplasm that Exhibits Potential Shade Tolerance and Identification of Techniques for Rapid Selection of Potential Shade Tolerant Cultivars

Gregory E. Bell and Yanqi Wu
Oklahoma State University

Objectives:

1. Screen bermudagrass germplasm collections and selections for their performance in shaded environments.
2. Determine turfgrass characteristics that may be useful for screening future selections for potential shade tolerance.
3. Create 1 or 2 genetic populations by physiological and molecular selections of shade tolerant and susceptible parents for future research.

Start Date: 2008

Project Duration: three years

Total Funding: \$90,000

A research site was assigned and planted using greenhouse-grown bermudagrass plugs on June 22, 2007 at the Oklahoma State University Turfgrass Research Center, Stillwater, OK. The site was specifically constructed to host this and future shade selection projects. Funds for development of the site were obtained from the Oklahoma Agricultural Experiment Station, Stillwater, OK. Additional research funding was secured for this project through the Oklahoma Turfgrass Research Foundation and from the Huffine Endowed Professorship. Additional sources of funding are under consideration.

The research site receives mid- to late afternoon shade, depending on season, from a dense, mature evergreen canopy on the west side of the site. The site meets the most important parameters for effective shade research. Late afternoon vegetative shade is provided by conifers on the west side of the plots. These conifers also provide root competition and reduce the predominantly westerly airflow.

Maple trees have been planted along the south side of the site and redbud trees along the east side. We attempted to increase the duration of shade at the site by planting vines along a hoop structure in 2008, but we had limited success. Consequently, in the next few years, as the deciduous shade trees mature, we will design neutral shade canopies that limit photosynthetic efficiency and adjust to the duration that we desire. Using canopies, we can increase the shade duration to increase photosynthetic stress or decrease duration to limit the loss of potential selections.

Bermudagrass	Sun			Shade			Shade/sun Decline***
	VisualTQ	Visual Rank*	NDVI**	VisualTQ	Visual Rank	NDVI	
	1-9=best	--- LSD ---		1-9=best	--- LSD ---		--- % ---
Patriot	7.6	A	0.7646	7.1	A	0.7365	-3.7
C19	7.2	B	0.7681	6.7	BC	0.7479	-2.6
C23	7.0	BC	0.7772	6.3	EFHGHIJKL	0.7333	-5.6
Tifton10	7.0	BCD	0.7300	6.5	BCDEFG	0.7261	-0.5
Tifton4	7.0	BCDE	0.7579	5.8	QRSTU	0.6796	-10.3
C17	6.9	BCDEF	0.7515	6.6	BCD	0.7462	-0.7
C20	6.9	BCDEFG	0.7703	6.3	DEFGHIJK	0.7460	-3.2
C29	6.8	BCDEFGH	0.7463	6.4	CDEFGHI	0.7099	-4.5
C28	6.8	CDEFGHI	0.7443	6.7	B	0.7183	-3.5
C116	6.8	CDEFGHI	0.7532	6.1	JKLMNOP	0.7154	-5.0
C34	6.8	CDEFGHIJ	0.7329	6.0	KLMNOPQ	0.6775	-7.6
C35	6.7	CDEFGHIJK	0.7419	6.3	DEFGHIJ	0.7022	-5.4
C12	6.7	CDEFGHIJK	0.7338	5.8	PQRST	0.7168	-2.3
C13	6.7	DEFGHIJKL	0.7582	6.6	BCD	0.7464	-1.6
C27	6.7	DEFGHIJKL	0.7466	6.4	BCDEFGH	0.7295	-2.3
PRC-06-5	6.6	EFHGHIJKL	0.7394	6.4	CDEFGHI	0.7140	-3.4
Celebration	6.6	EFHGHIJKL	0.7537	6.3	DEFGHIJ	0.7387	-2.0
C72	6.6	EFHGHIJKL	0.7402	6.3	EFHGHIJKL	0.7182	-3.0
C16	6.6	EFHGHIJKL	0.7401	6.2	HIJKLMNO	0.7114	-3.9
C4	6.6	FGHIJKLMN	0.7554	6.5	BCDE	0.7524	-0.4
C73	6.6	FGHIJKLMN	0.7270	6.0	KLMNOPQ	0.7055	-3.0

Top 24 bermudagrass selections determined by visual quality means collected on six rating dates from May 19 to August 15, 2008.

The study consists of 45 bermudagrass selections and four standards ('Celebration', 'Patriot', 'Tifton 4', and 'Tifton 10'), replicated five times on the shade site and on an adjacent site that is in full sun for about 90% of each day. Visual turf quality (TQ) and NDVI (Normalized Difference Vegetation Index) were assessed every two weeks in 2008 and results are reported for six rating dates from May 19 to August 15, 2008.

In 2008, shade stress occurred on the shade site for 12% longer each day than on the sun site. However, this short duration of shade stress was presumably sufficient to cause an average 4.9% decline in TQ and a 3.4% decline in NDVI when selections were individually compared between the shade site and the sun site.

The top performing selections ranked by LSD mean separation ($P=0.05$) of TQ ratings indicate that there is significant variation among selections in both sun and shade. Visual TQ ratings change with rating date. They are accurate for ranking selections on any given day but are not

accurate for comparisons among rating dates. The NDVI is consistent and can be accurately compared among dates. Consequently, it is a good tool for accurately evaluating seasonal changes or for individual comparisons over multiple rating dates.

According to NDVI, the selections that had the best TQ in sun declined from 0.4% to 10.3% in shade suggesting that some of these selections tolerate shade better than others. As we increase shade stress, we are confident that we can determine selections with potential for shade tolerance.

Summary Points

- In 2008, the shade site received 88% of the solar irradiance received on the sun site.
- Turfgrass quality ratings and NDVI indicated significant diversity among selections.
- The mean visual turf quality decline between like selections in full sun to shade was 4.9% and the mean decline in NDVI quality was 3.4%.

Identification of Quantitative Trait Loci (QTL) Associated with Drought and Heat Tolerance in Creeping Bentgrass

Bingru Huang, Stacy Bonos, and Faith Belanger
Rutgers University

Objectives:

1. To evaluate variations in drought and heat tolerance for two mapping populations of bentgrass segregating for disease resistance.
2. To identify phenotypic traits associated with drought and heat tolerance.
3. To identify QTL markers associated with drought and heat tolerance utilizing the available linkage maps.

Start Date: 2008

Project Duration: three years

Total Funding: \$89,912

A creeping bentgrass mapping population (L93-10 x 7418-3) segregating for dollar spot resistance was evaluated for variation in drought tolerance in this study. We examined both parents and 100 F₂ progenies. An intra-specific pseudo F₂ mapping population (180 individuals) was generated from a cross between a dollar spot resistant (L93-10) and a susceptible (7418-3) genotype in the spring of 2003. All individual clones (104 from L93-10 x 7418-3) were planted in one large plastic container (60 cm x 120 cm x 30 cm deep) filled with a mixture of sand and soil and replicated five times.

Plants were exposed to drought stress by withholding irrigation. During the dry-down period, several drought-stress indicators were evaluated to determine the genotypic variation and phenotypic traits for drought tolerance in these two mapping populations. Turf quality was rated visually based on the scale of 1-9 (9 = best) as the indicator of general turf performance. Several most commonly used parameters for phenotyping drought tolerance, including relative water content, osmotic adjustment, cell membrane stabil-

ity, and water use efficiency were analyzed.

The phenotypic data with the 'L-93' x 7418 population demonstrated that turf quality for two parents and 102 progenies exhibited large variation, with visual rating ranging from 3 to 6 following 7 days of drought stress. Leaf relative water content (RWC) and osmotic adjustment (OA), two commonly used stress indicators, are significantly correlated to turf quality under drought stress, with correlation coefficients being 0.73 and 0.68, respectively. Both traits exhibited a wide range of variation, with RWC varying from 33% to 94% and OA ranging from 0 to 0.50.

A field study was conducted in summer and fall 2008 in a fully automated mobile shelter (35' x 60') at Rutgers University in North Brunswick. The L93-10 x 7418-3 mapping population was established in a mowed spaced-plant evaluation trial in the rainout shelter in April 2008. The individual clones were arranged in a randomized complete block design with 3 replications.

From July to September, all plants were maintained under well-watered conditions and examined for turf quality, canopy green leaf biomass, leaf area index, and cell membrane stability to analyze for variation and phenotypic traits for heat tolerance. During October and November,

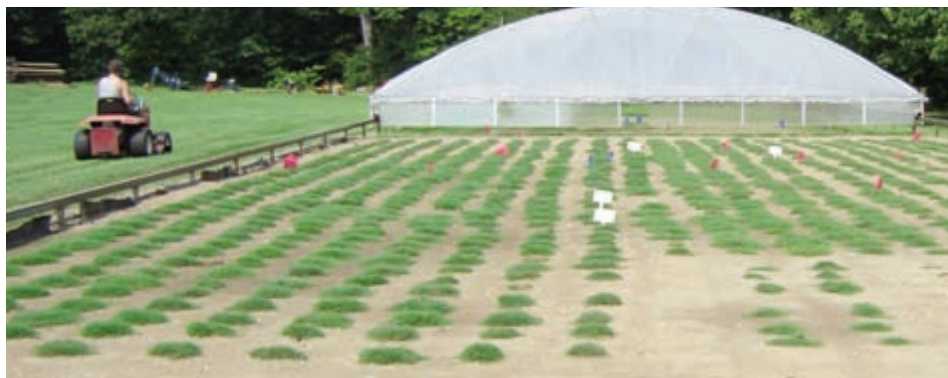
plants were exposed to drought stress by withholding irrigation to examine genetic variation in drought tolerance. Data for the field study will be analyzed and summarized in December.

QTL analysis was conducted on several phenotypic traits including electrolyte leakage (EL), relative water content (RWC), and osmotic adjustment in the 'L93' x 7418-3 mapping population. No significant QTLs were found on the more drought sensitive parent ('L-93'). Seven QTLs were found on the more drought tolerant parent (7418-3). Four significant QTLs (1 RWC; 2 EL; 1 TQ) and three suggestive QTLs (1 RWC; 1 WUE; 1 OA) were detected. These QTLs were found in four chromosome regions, with co-location of RWC and TQ, RWC and EL, and WUE and OA.

QTLs markers for TQ, RWC, and EL were highly correlated to drought tolerance. These initial QTLs from the greenhouse study will be compared to phenotypic traits associated with drought tolerance evaluated under field conditions.

Summary Points

- Phenotypic variations in drought tolerance exist in the creeping bentgrass mapping population developed for dollar spot resistance.
- Significant QTLs were identified for TQ, RWC, and EL, and those QTL markers are highly correlated to drought tolerance.
- QTL markers for drought tolerance were located in four regions of the genetic linkage map for the drought tolerant parent.
- Major QTLs located in the same chromosomal region for turf quality, relative water content, cell membrane stability, osmotic adjustment, and water use efficiency may be useful as molecular markers for marker-assisted selection.



A field study was conducted in summer and fall 2008 in a fully automated mobile shelter (35' x 60') at Rutgers University in North Brunswick, NJ.

Evaluation of the New England Velvet Bentgrass Collection

Rebecca Nelson Brown and Cynthia Percivalle
University of Rhode Island

Geunhwa Jung and Jason M Dowgiewicz
University of Massachusetts

Objectives:

1. Collect velvet bentgrass (*Agrostis canina* L.) germplasm accessions.
2. Identify and group accessions based on their genetic similarities for analysis and breeding.
3. Evaluate accessions for improved resistance to biotic and abiotic stresses.

Start Date: 2007

Project Duration: three years

Total Funding: \$90,217

The New England Velvet Bentgrass germplasm collection is a collaborative effort by researchers at the University of Rhode Island and the University of Massachusetts. More than 250 accessions have been collected from old golf courses throughout New England. In 2008, more than 750 individuals were evaluated in the field at UMass for turf quality and resistance to brown patch and dollar spot. The entire collection was screened for resistance to copper spot in the greenhouse at URI.

Velvet bentgrass (*Agrostis canina*) has excellent tolerance to lower levels of sunlight, nitrogen, and water. Velvet bentgrass is considered native to New England and coastal regions as far south as Maryland. Many of the old velvet greens have been regrassed with modern creeping bentgrass varieties due to over management, high thatch layer production, and lack of available velvet bentgrass commercial seed. The stress tolerance genes found in velvet bentgrass need to be preserved as potentially irreplaceable genetic resources.

The identification and genetic diversity of the collection are being measured with molecular markers and flow cytometry to confirm that accessions are



Planting plugs of velvet bentgrass.



More than 250 accessions have been collected from old golf courses throughout New England.

diploid *Agrostis canina*. Fingerprinting the accessions will permit them to be grouped based on genetic identity, subdividing the collection into groups which can be converted to seed populations for genebank storage. The groups can be represented by a subset of the clones for more efficient screening.

Screening for copper spot resistance was done in the greenhouse using clones grown in a sand-peat mix, clipped to fairway height, and fertilized with excess nitrogen. The plants were inoculated using copper spot (*Gloeocercospora sorghi*) isolates and maintained under appropriate conditions to promote disease. Disease severity was evaluated on a 0-10 scale with 0 indicating complete loss of foliage and 10 indicating no damage. The varieties 'SR7200' and 'Greenwich' were used as standards for comparison.

Sixty-two percent of the accessions were effectively defoliated by copper spot. Both 'SR7200' and 'Greenwich' show some resistance to copper spot; 30% of the accessions tested were at least as resistant. The germplasm collection is being evaluated in the field for turfgrass quality and genetic color. Velvet bentgrass has a tendency to be lighter green than other bentgrasses. The identification of velvet bent-

grass which is genetically darker green may make turfgrass managers more comfortable applying less nitrogen.

The core collection is being evaluated for wear tolerance, a common abiotic stress on highly utilized turfgrass. Golf course putting greens, tees, and fairways are particularly susceptible to wear injury due to the funneling of intense and frequent traffic onto a relatively small surface area.



The velvet bentgrass collection at the University of Rhode Island.

Summary Points

- Collaborative studies continue with several researchers in New England.
- Significant progress has been made to identify germplasm that have enhanced resistance to biotic and abiotic stresses.

Evaluating Methods for Vegetative Propagation and Enhancement of Seed Production of Greens-type *Poa annua* Cultivars

David R. Huff

Pennsylvania State University

Objectives:

1. Establish efficient vegetative propagation methods of greens-type *Poa annua* for sod production and for establishing/renovating golf course putting greens.
2. To scale-up our results for greens-type cultivar production to a larger commercial level by collaborating with sod producers.
3. To release a genetically stable, vegetative greens-type cultivar exhibiting superior putting green quality and stress tolerance.

Start Date: 2008

Project Duration: two years

Total Funding: \$20,000

Poa annua is widely recognized to provide a large portion of high quality putting surfaces in many regions of the U.S., Canada, Australasia, and Scandinavia. However, despite repeated attempts to develop improved cultivars of greens-type *Poa annua* for the golf course industry, there currently are no commercially available sources suitable for use in new construction or renovation. Our previous genetic research suggests that the perennial greens-type phenotypes result from the action of mowing which causes a repression of the plant growth hormone gibberellic acid (GA) signaling pathway through a non-Mendelian epigenetic mechanism.

A somewhat analogous situation occurs in the wild, weedy annual-types of *Poa annua* from applications of plant growth regulators, such as Primo. However, in the absence of the mowing stimulus, the GA pathway progressively becomes unsilenced resulting in reversion of the greens-type plants back to the annu-



Poa annua is widely recognized to provide a large portion of high quality putting surfaces in many regions of the U.S., Canada, Australasia, and Scandinavia.

al type. The annual type is undesirable as a putting surface and requires years of mowing in order to develop a perennial greens-type form. We believe that the genetic stability of perennial greens-type *Poa annua* is capable of being maintained through vegetative propagation in combination with mowing.

We established vegetative plots using different source plant materials including aerification cores, solid sod, and shredded sod/plugs. Planting densities of these materials were varied in order to maximize the limited amount of our planting stock while, at the same time, attempting to achieve full coverage in a reasonable amount of time.

We also began evaluating vegetative establishment in the greenhouse in combination with the exogenous applications of gibberellic acid in an attempt to further reduce the time required to achieve full coverage. All greenhouse and field plots are being mowed to a height of 1 cm (approx. 3/8 inch) as soon as possible after plant materials have rooted in order to maintain the greens-type phenotype.

This year, we were fortunate enough to supply vegetative planting material of greens-type *Poa annua* to turfgrass researchers at 5 universities participating in the Northeast 1025 Project titled: "Biology, Ecology, and Management of Emerging Pests of Annual Bluegrass on Golf Courses".

Future expectations of this research project will be to extend the most successful techniques identified for the vegetative propagation of greens-type *Poa annua* to a larger scale approaching that of commercial sod production. Vegetative material from the previous vegetative propagation studies will provide the necessary planting material for us to work directly with interested sod producers for



vegetative plots were established using different source plant materials including aerification cores, solid sod and shredded sod/plugs.

an evaluation of scaled-up production. Ultimately, our research will enable the commercial availability of greens-type *Poa annua* cultivars that possess high turf quality, disease resistance, and stress tolerance which will significantly reduce inputs for the management of *Poa* greens and will increase the supply of greens-type *Poa annua* for turfgrass scientists throughout the USA to conduct valuable collaborative research in other areas of *Poa annua* golf course management.

Summary Points

- Our research continues to indicate that the dwarf nature of perennial greens-type *Poa annua* results from an epigenetic inhibition of the biosynthetic pathway for the plant growth hormone gibberellic acid (GA).
- We demonstrated that greens-type *Poa annua* is responsive to exogenous applications of gibberellic acid which may aid in its vegetative establishment.
- This year, we supplied vegetative planting material of greens-type *Poa annua* to turfgrass researchers at 5 universities participating in the Northeast 1025 Project titled: "Biology, Ecology, and Management of Emerging Pests of Annual Bluegrass on Golf Courses".

Evaluation and Development of *Poa* Germplasm for Salt Tolerance

Paul Johnson
Utah State University

Joseph Robins and B. Shaun Bushman
USDA-ARS

Objectives:

1. To identify salt-tolerant *Poa* germplasm that can be incorporated into breeding and genetics efforts.
2. To identify genes whose RNA transcript levels vary between control and high salinity treatments.

Start Date: 2007

Project Duration: three years

Total Funding: \$75,237

It is widely known that one of the greatest challenges confronting the turf industry today is water. Golf course superintendents and other landscape managers are being asked to use less irrigation water, use lower quality water sources, and allow more use of the turfgrass areas. Turfgrass with high turfgrass quality and greater salt tolerance is essential.

Beginning in 2005, the USDA-ARS Forage and Range Research Lab (FRRL) in Logan, Utah and Utah State University started work in the area of salt and drought tolerance evaluation in *Poa*. A cooperative agreement has also been established with Utah State University. As part of this cooperative effort, we are evaluating a large number of National Plant Germplasm accessions, collections from around the world, somaclonal lines of *P. pratensis* from Dr. Nancy Ehlke at the University of Minnesota, and numerous currently available varieties.

These lines are being evaluated in field conditions for turf and seed production characteristics, drought tolerance, and other traits and will be used to combine stress tolerance and turf quality traits in germplasm that can be used by the seed industry.



Seedlings were grown in sand and when at the 3-tiller stage, were subjected to increasing salt concentrations (increased by 3.0 dS m⁻¹ increments every 2 weeks) and evaluated until plant death.

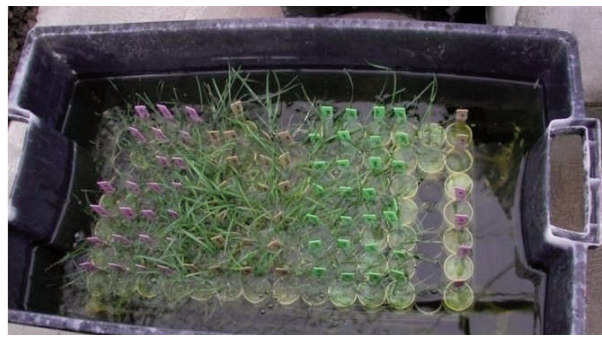
Many *Poa* species, and most of the available germplasm within *Poa pratensis*, has not been rigorously evaluated for salt tolerance and turf characteristics in the arid West climate. Similarly, there is not a good understanding of the genetic control of salt tolerance in *Poa*. We are expanding a breeding and genetics program to develop improved salt-tolerant bluegrass (*Poa*) germplasm.

Using our large and growing collection of germplasm from a variety of sources, we have and will continue to conduct a rigorous program of screening for salt tolerance and will identify candidate genes associated with salt tolerance in *Poa* using suppressive-subtractive hybridization methods.

We completed an initial evaluation of salinity tolerance of *Poa* germplasm with a proven method developed at the FRRL. In a greenhouse, seedlings were grown in sand and when at the 3-tiller stage, were subjected to increasing salt concentrations (increased by 3.0 dS m⁻¹ increments every 2 weeks) and evaluated until plant death.

Based on two runs of the experiment, several germplasm sources were identified with consistently high salinity tolerance during both runs of the study. The salinity tolerance of some of the germplasm sources was similar to that of the tall fescue and perennial ryegrass check entries. Results suggest that selection and hybridization among the better germplasm sources will potentially result in bluegrass cultivars with increased salinity tolerance. The results of this study will be submitted for publication in a refereed journal in the coming months.

A Ph.D. graduate student, Mr. Shyam Shridhar, is currently conducting the gene expression work, specifically to identify candidate genes turned on during. This process involves imposing salt stress



Poa germplasm was emersed in increasingly saline water and evaluated in the greenhouse for salinity tolerance.

on salt-tolerant and salt-sensitive Kentucky bluegrass accessions. These accessions were identified in the screening procedure described above. RNA, the results of gene expression, was extracted from roots and shoots, purified, and subjected to suppression-subtractive hybridization (SSH) to detect differentially expressed sequences of DNA.

This procedure will allow us to identify genes expressed at a higher level in the salt-tolerant germplasm shoots and roots than sensitive germplasm shoots and roots. Currently the SSH procedure is completed, and the differentially-expressed genes are being detected and sequenced. In the coming months, we will be comparing the expressed gene sequences to a publicly available plant sequence database to assign possible gene function.

Summary Points

- We have observed significant variation in salt tolerance among the National Plant Germplasm accessions.
- The most salt-tolerant accessions exceeded the tolerance of Kentucky bluegrass check varieties and approached or exceeded the tolerance of perennial ryegrass and tall fescue check varieties.
- These encouraging results will result in the evaluation of a wider selection of *Poa pratensis* and *Poa spp.* germplasm.
- Genes expressed at higher levels in salt-tolerant germplasm will be identified and considered for future DNA markers.

Genetic Enhancement of Turfgrass Germplasm for Reduced-input Sustainability

Kevin Morris
National Turfgrass Federation, Inc.

Scott Warnke
USDA-ARS

Objectives:

1. The objective of this research, conducted at the USDA, ARS Beltsville Agricultural Research Center in Beltsville, MD, is to use genetic and biotechnology approaches to identify and develop turfgrass germplasm with improved biotic and abiotic stress resistance. Efforts will be made to identify molecular markers associated with desirable traits and to combine useful traits into germplasm able to grow with reduced inputs.

Start Date: 2007

Project Duration: three years

Total Funding: \$50,000

In order to improve the stress tolerance of turfgrass, we have undertaken the following five projects:

Development of *Danthonia spicata* as a Low Maintenance, Native Turfgrass Species

A key aspect to the development of *Danthonia spicata* (Poverty grass) as a low maintenance turfgrass species is obtaining a better understanding of the biology of the species. Therefore, we have spent time collecting plant material and observing the growth and reproductive characteristics of the species. Initial observations indicate that there is variation present in the species, and there may be more than one species present in natural stands.

Several new locations for the collection of germplasm were identified. A seeding rate trial was established at the University of Maryland turfgrass center using three different seeding rates. The results of the first year indicate no significant difference in turf quality between the three seeding rates.

Field Screening of Bentgrass Germplasm for Resistance to Important Turfgrass Diseases

A field trial containing clonally propagated plants from a bentgrass mapping population, developed by Dr. Geunhwa Jung at the University of Massachusetts, was established at the University of Maryland turfgrass center. The study involves approximately 300 entries replicated three times, plugged into a ryegrass turf.

The first rating of this material for disease resistance was conducted in the summer of 2008. The artificial inoculation with *Sclerotinia homoeocarpa* was not as effective as we would have liked. Slow fungal growth during inoculum development resulted in a late inoculation of the field trial. However, disease development did occur, and our first rating of the material was conducted with differences clearly present.

Development of *Koeleria macrantha* as a Low-input Turfgrass

The goal of this research is to increase the genetic potential of prairie junegrass (*Koeleria macrantha* Ledeb.) for use as a low-input turfgrass. Research on *Koeleria* is all being conducted by the University of Minnesota and has initially been focused on germplasm collection. Future research may involve the establishment of research plots in Maryland to determine the tolerance of *Koeleria* germplasm to the stressful growing conditions often present during Maryland summers.

Identification of Brown Patch Resistant Tall Fescue

The objective of this study was to use digital image analysis (DIA) to evaluate tall fescue plant introductions (PIs) for resistance to both *Rhizoctonia solani* and *R. zae*. This study included 15 PIs selected from the USDA germplasm database and three commercial cultivars with varying brown patch resistance.

The commercial cultivars had the lowest mean disease severity in each experiment. Mean disease severity ranged from 59-93% for *R. solani* and from 32-64% for *R. zae*. Current work involves screening the best PIs and using 20 seeds

sampled from each PI to evaluate the diversity of resistance to both *R. solani* and *R. zae* that may be present in each PI.

Bentgrass Breeding Consortium: Molecular Breeding for Dollar Spot and Snow Mold Resistances

The objective of this study was to identify candidate Miniature Inverted-repeat Transposable Elements (MITEs), a class of transposable elements that has not been previously described in turfgrasses, from *Agrostis* and assess their value as a molecular-marker tool.

DNA sequences were screened using the FindMITE program to identify candidate MITE sequences. FindMITE identified 202 MITE-like sequences, or 1.26% of the 16,064 sequences. The MITE display markers had a significantly higher polymorphism rate (0.42) between the mapping population parents than Amplified Fragment Length Polymorphism (AFLP) markers at 0.28. The MITE display protocol would be an effective tool for diversity analyses and mapping in *Agrostis*.

Summary Points

- There is variation present in naturalized stands of the low maintenance species *Danthonia spicata* (Poverty grass), allowing for potential selection of superior plant types.
- A field trial of bentgrass germplasm, planted in Maryland, exhibited differences in response to dollar spot (*Sclerotinia homoeocarpa*) infection.
- Tall fescue plant introductions (PIs) inoculated with *Rhizoctonia solani* and *R. zae* showed varying degrees of tolerance to both diseases.
- Miniature Inverted-repeat Transposable Elements (MITEs) show promise as an effective tool for diversity analyses and mapping in *Agrostis* species.

Breeding Turf-type Annual Ryegrass for Salinity Tolerance

L. R. Nelson
Texas AgriLife Research

Objectives:

1. To identify improved salt-tolerant genotypes and introgress the tolerance into adapted turf-type genotypes of annual ryegrass and increase seed of these populations for experimental testing.
2. To select plants of turf-type annual ryegrass which are infected with a fungal endophyte and determine if presence of the endophyte provides this population with increased tolerance to high salt concentrations.
3. To test the experimental lines of annual ryegrass under high salinity growing conditions in hydroponic and field conditions and release the line as a salt-tolerant variety.

Start Date: 2008

Project Duration: three years

Total Funding: \$88,276

Research findings from this project are a continuation of earlier research from past years. Screening germplasm and cultivars for salt tolerance is an important aspect of the ryegrass turf breeding program in Texas. Results indicate a good correlation between field screening and greenhouse screening. Methods studied were planting seed in the field in a high salinity soil and irrigating with high salinity water at Pecos, Texas.

Each entry was planted in 5 ft rows at a seeding rate of 2 g/5 ft. Plants were grown in sand in cone-tainers (4-cm wide and 20-cm tall) which were placed in a salt tank where salt concentration was gradually increased over time. Plants were



Methods studied were planting seed in the field in a high salinity soil and irrigating with high salinity water at Pecos, Texas.

immersed every 3 to 4 days in salt water. Seed were planted in rows in sand in flats on December 14, 2007. The first immersion (4000 ppm salt water, or 6.4 dS m⁻¹) of flats occurred on January 25 when plants were in 3-leaf stage. Thereafter, salt water concentration was gradually increased as flats were immersed for 2 minutes every 3 days.

Each entry was rated on several dates on a 1 to 9 scale, where 9 = dead plants. Results from the first year of experimentation indicated that the field trial and the greenhouse immersion technique are correlated and are a good indication of salinity tolerance of genotypes tested.

We will continue to screen genotypes using both of these techniques in the future. We will also screen segregating populations for salt tolerance with the goal of developing and releasing a salt-tolerant cultivar in the future. We will also increase seed from the latest and hopefully salt-tolerant populations for further testing.



Screening germplasm and cultivars for salt tolerance is an important aspect of the ryegrass turf breeding program in Texas. Results indicate a good correlation between field screening and greenhouse screening.

Summary Points

- Field and greenhouse screening of genotypes for salinity tolerance ratings were correlated.
- A greenhouse screening technique where plants in flats were immersed in salt water for 2 minutes every 3 days holds promise for future research.

Bermudagrass (Cynodon spp.) and Seashore Paspalum (Paspalum vaginatum) Cultivar Response to the Sting Nematode (Belonolaimus longicaudatus)

Wenjing Pang, William T. (Billy) Crow, and Kevin E. Kenworthy
University of Florida

Objectives:

1. Determine the range of response (resistance or tolerance) of bermudagrass and seashore paspalum cultivars to the sting nematode and identify the best performing cultivars.
2. Investigate if a proposed alternative method for assessing sting nematode response is as effective or more efficient than traditional methods.

Start Date: 2008

Project Duration: three years

Total Funding: \$31,407

While sting nematodes are found most commonly in sandy coastal areas, the University of Florida Nematode Assay Lab has diagnosed this nematode on bermudagrass from inland areas of Alabama, Mississippi, Arkansas, Louisiana, Georgia, Tennessee, Texas, Oklahoma, Kansas, and California.

Recent cancellation of fenamiphos (Nemacur, Bayer CropScience) has resulted in the need of better nematode management tactics. Currently, Curfew Soil Fumigant (Dow Agrosciences) is the most effective management for sting nematodes. However, Curfew is not cost effective and environmental restrictions highlight the need for alternative options.

Utilization of resistant or tolerant cultivars is the most efficient, least costly practice for nematode management on turf. Although a range in responses of bermudagrass to sting nematodes has been found, most cultivars were reported as susceptible. Information about the responses of several newer bermudagrass and seashore paspalum cultivars is lacking.

Separate glasshouse experiments for bermudagrass and seashore paspalum will be conducted to assess the range of these species for response to sting nematodes. In 2008, two experiments were conducted using six cultivars of bermudagrass in one experiment and three cultivars of seashore paspalum in the second.

All cultivars were propagated by means of nematode free aerial stolons into clay pots (1,500 cm³) with a USGA sand. Once established, inoculations were done on June 21, 2008 using 200 nematodes per

pot. The experiments were maintained at a temperature range of 24° C to 34° C under natural daylight. They were trimmed at 1.0-cm mowing height and fertilized once every other week using 18-3-6 (N-P-K) at a rate of 2 kg / 100 m² (4 lb / 1000 sq. ft) per year.

Experiments were harvested 90 days after inoculation by removing a 5-cm diameter core from the center of each pot. Nematodes were extracted from the soil cores and counted under a microscope. Roots were analyzed using WinRhizo root scanning software to determine root lengths and surface areas.

Reproduction and mean nematode counts were greatest on 'Champion' bermudagrass with no differences between the remaining cultivars. Significant differences were not found between the seashore paspalum cultivars. However; biologically, 'SeaDwarf' was the best host for sting nematodes.

Mean total root length comparisons were made within a given cultivar for its uninoculated and inoculated treatments. For all cultivars except 'TifEagle', the inoculated treatments led to reductions in total root length. Differences between uninoculated controls and inoculated treatments were significant only for 'Champion' bermudagrass, and 'Aloha' and 'Sea Isle 1' seashore paspalum.

The percent reduction of 'Champion' was similar to all bermudagrass cultivars except 'TifEagle'. However, the uninoculated root length of 'Champion' was approximately one-third of the other greens-type uninoculated root lengths. This may be the basis for the difference observed for its inoculated treatment.



In 2008, two experiments were conducted using six cultivars of bermudagrass in one experiment and three cultivars of seashore paspalum in the second.

Similar reductions between 'Champion' and other cultivars will be more detrimental to 'Champion' due to its smaller root system. Because 'Champion' was a better host for sting nematode reproduction (least resistant) and its root system was more heavily damaged (poor tolerance), it was considered the most susceptible cultivar to sting nematode damage in this study. 'SeaDwarf' supported higher nematode reproduction than other seashore paspalum cultivars.

Summary Points

- The sting nematode caused damage to 'Champion', 'Sea Isle 1', and 'Aloha'.
- Sting nematode damage was not as significant to 'TifSport', 'Celebration', 'Floradwarf', 'TifEagle', 'Tifgreen', or 'SeaDwarf'.
- 'Champion' was the most susceptible bermudagrass cultivar exhibiting poor resistance and tolerance.
- 'SeaDwarf' exhibited better tolerance than other seashore paspalum cultivars studied.

Collection and Evaluation of Native Grasses from Grazed Arid Environments for Turfgrass Development

D.M. Kopec, S.E. Smith, and M. Pessarakli
University of Arizona

Objectives:

1. Collect native grasses from rangelands with long history of livestock grazing in southern Arizona.
2. Evaluate primary growth habit-related characteristics of collected clones.
3. Evaluate relationships among characteristics in clones evaluated and select clones for field evaluation based on a simple index involving independent culling levels.

Start Date: 2008

Project Duration: three years

Total Funding: \$29,993

There is a nationwide effort to use native grass species in turf systems, as most species used now are typically non-native. This study involves initial collection and description of growth characters of native range grasses as mowed and unmowed plants. The research may result in populations of native grasses that are suitable for use in turf plantings in the southwestern U.S. Procedures we perfect in this research may be used in projects to evaluate native plants for potential turf development.

From July to September 2007, almost 300 clones from seven species of perennial range grasses were collected from a 150-mile radius of Tucson, Arizona from rangelands with a known history of intensive livestock grazing. Collections included the sod-forming grasses, curly mesquite, and false grama, and the bunch-grasses sprucetop grama, wolftail, blue grama, black grama, and hairy grama. These clones were propagated and grown under near-optimal conditions in a greenhouse.

In early January 2008, and again six weeks after a "grazing" (severe defoliation) event, data were collected from plants actively growing in the greenhouse. These included (1) plant height (2) plant diameter in two directions, and visual scores for (3) innate plant density, and (4) overall turf quality (1-5, 5 = best for the latter traits). During this phase of our research, clones noted by mere subjective observations were identified as "flagged" clones.

Height/width ratios (lower values suggest a more "wider" than "taller" growth habit) before and after defoliation were relatively consistent up to a value of



From July to September 2007, almost 300 clones from seven species of perennial range grasses were collected from a 150-mile radius of Tucson. Species included curly mesquite, false grama, sprucetop grama, wolftail blue grama, black grama, and hairy grama.

5, for pre- and post-defoliation measurements, respectively. Interestingly, subjectively "flagged" clones were very close to the 1:1 line of best fit.

Stem and box plot data show that there may be considerable useful variation for growth habit in curly mesquite, wolftail, and sprucetop grama. These species had a low H/W ratio before defoliation.

After a single defoliation event, most clones exhibited increased height, with some variation remaining for low H/W ratio growth response for curly mesquite, wolftail, and sprucetop grama. Other species showed very little variation for H/W ratios following defoliation.

When plotted against the average of turfgrass quality and density scores after defoliation, clones with the lower height/width ratio values had the greatest numerical mean quality-density averages. In this case again, the "flagged" clones were again close in fit to the 1:1 line of H/W vs. the mean quality and density scores. This data set shows that that plant height alone is not related to turf quality or density, but the height/width ratio is a better predictor of turf quality.

The next phase of research includes the establishment of clonally replicated spaced-plant nurseries (accomplished in August 2008) that include clones selected based on greenhouse performance. Field evaluations will involve the same measurements taken in the greenhouse, but also the response of these clones to regular mowing events. In addition to the identification of clones appropriate for use in turf plantings, results from this component of the projects should allow for determination of the utility of greenhouse-based measurements for predicting actual turf quality in the field.

Summary Points

- From July to September 2007, almost 300 clones from seven species of perennial range grasses were collected from a 150-mile radius of Tucson.
- There may be considerable useful variation for growth habit in curly mesquite, wolftail, and sprucetop grama.
- Plant height alone is not related to turf quality or density. The height/width ratio is a better predictor of turf quality.

Environmental Impact

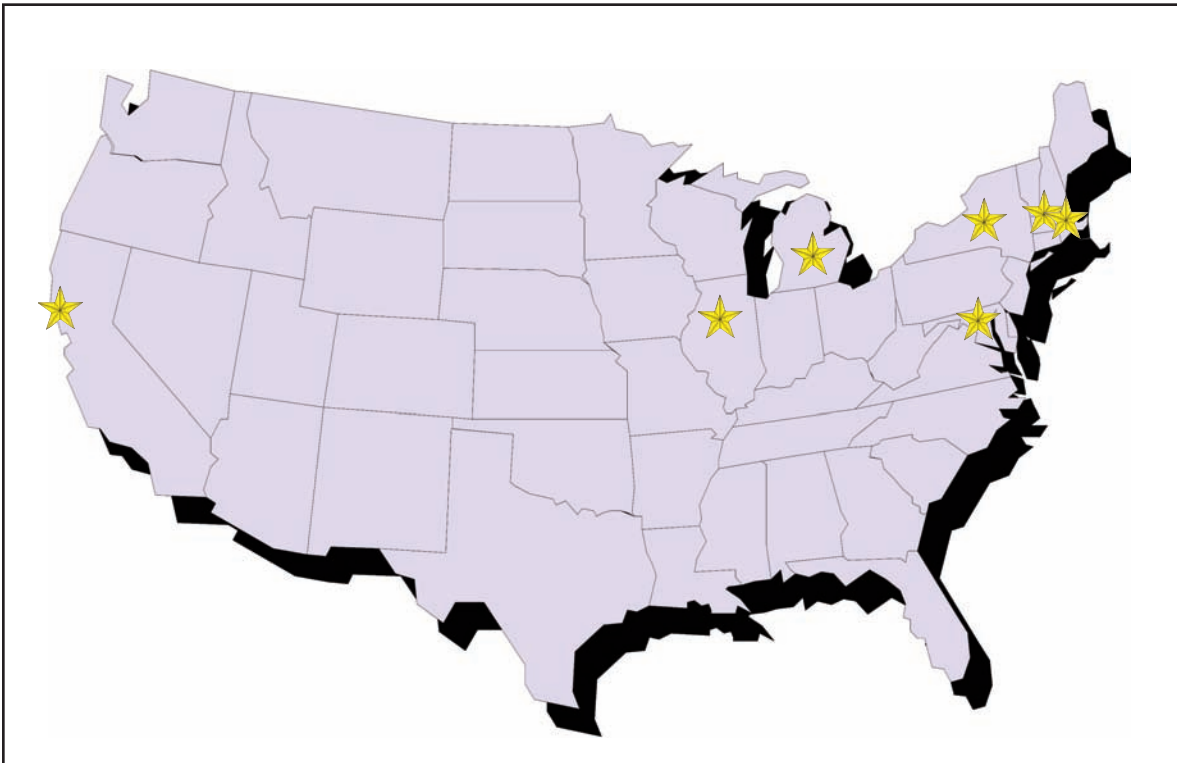
The public is concerned about the effects of golf courses on the environment. In response to this concern, the USGA has conducted research examining the fate of pesticides and fertilizers since 1991. The USGA continues to support scientifically based investigations on the environmental impact of golf courses. The focus remains on research to understand the effects of turfgrass pest management and fertilization on water quality and the environment.

Research on best management practices evaluates pesticide and fertilizer programs for golf courses in order to make turfgrass management recommendations that protect environmental quality. The research is conducted on university experiment stations and golf courses. The projects evaluate pesticides or nutrients that pose an environmental risk, and identify cultural practices that minimize volatilization, surface runoff, and groundwater contamination.

Pesticide and nutrient fate models are used to predict the environmental impact of turfgrass pesticides and fertilizers. From 1991 through 1997, research sponsored by the USGA demonstrated:

1. Measured nitrogen and pesticide leaching was minimal and that surface transport (runoff) posed a greater problem for golf courses, especially on heavy textured soils in high rainfall areas of the country.
2. The turf/soil ecosystem enhances pesticide adsorption and degradation that greatly reduces the amount of chemical that moves below the rootzone.
3. Current agricultural fate models need modification to predict the fate of pesticides and fertilizers applied to turfgrasses grown under golf course conditions.

The results of USGA-sponsored pesticide and fertilizer fate research is being used to calibrate and validate existing pesticide fate models for turfgrasses managed under golf course conditions.



Locations of the following projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the category of Environmental Impact

The Pesticide Matrix Project: Developing a Data Base Tool to Guide Environmentally Responsible Pesticide Selection

Bruce Branham and Thomas Fermanian
University of Illinois

Stuart Cohen
Environmental and Turf Services

Jennifer Grant
Cornell University

Objectives:

1. To create a resource that is available electronically to help superintendents better understand the environmental characteristics of golf course pesticides and optimize their environmental stewardship.

Start Date: 2006

Project Duration: two years

Total Funding: \$90,000

Golf course superintendents consider many factors when selecting a pesticide for a specific use, including cost, efficacy, and turf safety. However, it is currently much more difficult for a superintendent to assess environmental risk and its relevance to their golf course. What is the risk to groundwater supplies when a particular pesticide is applied? What is the risk to surface water, fish, or bees?

These are complex questions requiring not only data, but also a method to integrate the data into a form that allows meaningful conclusions. The first step of this project is to collect relevant data on environmental fate, toxicology, and environmental endpoints from publicly available databases. To date, we have collected the majority of the data needed.

The second part is to create a model or software program that calculates the relative risk to specific environmental features from the application of a specific pesticide active ingredient. Consider a golf course with a stream flowing through the

property. The golf course superintendent may want to know the probability of a pesticide intended for use to reach the stream, and if it does, what is the likelihood that it will cause problems for fish in the stream? A rudimentary risk assessment determines the likely concentration of the pesticide in the stream and whether this concentration is high enough for concern.

Integral to our process of building this resource has been the solicitation of feedback from the following groups:

- End-users of the resource, golf course superintendents;
- EPA staff with expertise in pesticide fate assessment and modeling, regulatory enforcement and economic impact;
- The scientific community via presentations at scientific meetings; and
- Scientists from pesticide manufacturers represented by Crop Life America and Responsible Industry for a Sound



Categories of risk factors included in the pesticide matrix model include risks to ground water, surface water, birds, and non-target invertebrates.

Environment.

The challenge of this project is to develop a tool that is easy to use, while retaining a sound scientific basis for estimating potential environmental risks of using a particular pesticide. At this point, several components of the final model have been selected. We expect the model to yield information on risk to ground water, surface water, birds, and non-target invertebrates for each of the over 100 pesticide active ingredients in our database. The risk determination will be based on risk ratios and presented in a format that is easy to interpret.

Recommendations for best management practices to minimize environmental risk of an application and maximize environmental stewardship will be provided. The resource will help superintendents make more informed environmental decisions on the pesticides they choose to use.

Summary Points

- A database of pesticide properties needed for risk assessment is being compiled.
- The database will serve as the foundation to predict potential environmental risk of a pesticide's active ingredient.
- The end result of this research will be a resource that will help superintendents make more informed environmental decisions regarding the pesticides they apply.

Data Obtained/to be Obtained		Numbers to be Calculated
Environmental Fate Properties	Toxicological Endpoints	Toxicological Reference Points
Koc	Ref. dose (RfD), cancer slope factor	HAL (drinking water Health Advisory Level) (calculated when MCLs or HALs unavailable)
Aerobic soil metabolism $t_{1/2}$	LC ₅₀ aquatic invertebrates	Acute MACs (.1 x LC ₅₀ or EC ₅₀ , or NOEC) for invertebrates
Turf dissipation $t_{1/2}$	LC ₅₀ aquatic vertebrates	Acute MACs (.1 x LC ₅₀ or NOEC) for vertebrates
Vapor pressure	LC ₅₀ and/or LD ₅₀ for birds	Avian exposure to "short range grass" (Pfleeger et al., 1996 modification of Kenaga approach)
Water solubility	LC ₅₀ earthworms	Amphibian MACs based on Lam & Cohen (2006).

The development of a pesticide matrix involves obtaining pesticide environmental fate and toxicological endpoints and calculating toxicological reference points when necessary.

Utilizing Reduced-risk Pesticides and IPM Strategies to Mitigate Golfer Exposure and Hazard

J. Marshall Clark, Raymond Putnam, and Jeffery Doherty
University of Massachusetts

Objectives:

1. Determine the level of hazard of volatile and foliar dislodgeable residues of the reduced-risk pesticides carfentrazone, halofenozide, and azoxystrobin following total course and full-rate applications.
2. Determine the effect of partial course application strategies (e.g. tees and greens only) and post application irrigation on volatile and foliar dislodgeable pesticide residues following full-rate applications of carfentrazone, halofenozide, and azoxystrobin.
3. Model the relationship of volatile and dislodgeable foliar residues vs. actual golfer exposure using urinary biological monitoring techniques or, for pesticides that are not amenable to biomonitoring, dosimetry techniques.

Start Date: 2007

Project Duration: three years

Total Funding: \$90,000

This study seeks to determine actual levels of golfer exposure to "reduced-risk" pesticides following application to turfgrass. The fate of pesticides after application largely determines how much of it is available for potential human exposure.

We have analyzed pesticide residues in the air and on turfgrass (dislodgeable foliar residues, DFR) in 43 pesticide applications using either chlorpyrifos, carbaryl, cyfluthrin, chlorothalonil, 2,4-D, MCPP-p, dicamba, imidacloprid, and carfentrazone. This season, three applications of the reduced-risk fungicide azoxystrobin were made. Analyses of these samples are in progress.

This study also evaluates best management practices for reducing golfer exposure to reduced-risk turfgrass pesticides. While many "standard" pesticides have been removed from use, new reduced-risk pesticides have been added to the IPM practitioner's toolbox. To date, there is no dosimetry or biomonitoring data on these reduced-risk pesticides, which exhibit low mammalian and environmental toxicity, low potential for ground water contamination, low pest resistance potential, and are compatible with IPM.

To determine precisely how much of the environmental residues is actually transferred to golfers during a round of golf, we measure exposure to volunteer golfers using dosimetry (measuring pesticide residues on full-body cotton suits and personal air samplers) and biomonitoring (measuring urinary metabolites). This work is being done in cooperation with the



To determine precisely how much of the environmental residues is actually transferred to golfers during a round of golf, volunteers are used for dosimetry (measuring pesticide residues on full-body cotton suits and personal air samplers) and biomonitoring (measuring urinary metabolites).

New England Regional Turfgrass Foundation.

Dosimetry and biomonitoring, together with concurrently collected dislodgeable foliar and airborne residue data, provides a unique database on golfer exposure and has allowed us to develop a golfer exposure model. The central predictor of exposure in the model is the transfer factor (TF), which is ratio between the pesticide residues measured in the environment versus the amount that actually ends up transferring to the golfer (as measured by dosimetry).

A transfer factor of 1,800 has been calculated for carfentrazone. We will compare the biomonitoring and dosimetry results for these reduced risk compounds with those previously determined for chlorpyrifos, carbaryl, cyfluthrin, 2,4-D, MCPP, dicamba, chlorothalonil and imidacloprid. This season (2008) we determined exposure in 24 rounds of golf following application of azoxystrobin without post-application irrigation.

Hazard quotients (HQs) less than or equal to 1.0 indicate that the exposure resulted in a pesticide dose at which adverse effects are unlikely. To date, HQs determined for chlorpyrifos, carbaryl,

cyfluthrin, 2,4-D, dicamba, MCPP, chlorothalonil, imidacloprid, and carfentrazone have been 20- to 1.25 million-fold below 1.0, indicating safe exposure levels using the EPA Hazard Quotient criteria.

The hazard quotient calculated for carfentrazone was 0.0000008, 1.25-million fold lower than a hazard quotient of 1.0. This HQ was significantly lower than those calculated for 2,4-D, MCPP, and dicamba (0.001 - 0.01). It is important to note that carfentrazone is considered a reduced-risk replacement for these phenoxy acid herbicides. The TF model will still allow us to calculate a hazard quotient in pesticides that are not amenable to biomonitoring.

Summary Points

- Researchers have evaluated exposure in 24 rounds of golf following the application of azoxystrobin (2008) and will compare this with future results from halofenozide (2009) and with those results of previous experiments on chlorpyrifos, cyfluthrin, carbaryl, chlorothalonil, 2,4-D, MCPP-p, dicamba, imidacloprid, and carfentrazone.
- Determination of golfer exposure to reduced-risk pesticides will provide a novel dataset for these IPM-friendly compounds.

Optimization of Vegetative Filter Strips for Mitigation of Runoff from Golf Course Turf

J. Marshall Clark, Jeffery J. Doherty, Guy R. Lanza, and Om Parkash
University of Massachusetts

Objectives:

1. Use selected plant species in a field study to evaluate the efficacy of vegetative filter strips (VFS) and their most effective arrangement.
2. Determine the fate of pesticides retained in VFS and the major mechanisms of degradation.

Start Date: 2008

Project Duration: three years

Total Funding: \$90,000

The loss of pesticides and nutrients into surrounding bodies of water and the resulting decreases in water quality has led to the use of best management practices on golf courses. One such practice is the use of vegetative filter strips (VFS) to intercept runoff water and thus prevent its loss and the loss of any associated pesticides and nutrients to surrounding water bodies.

Joint greenhouse and field studies have been implemented to evaluate selected plants for their effectiveness in removing pesticides and nutrients from turfgrass runoff waters that enter vegetative filter strips (VFS). Ten plant species were evaluated in a greenhouse pot study to determine which species most effectively remove six pesticides (2 fungicides, 2 herbicides, and 2 insecticides) from a silt loam soil. Five species (big blue stem, blue flag iris, eastern gama grass, prairie cord grass, and woolgrass) were determined to be most effective.

A run-on plot, consisting of 12 VFS, was established. Each VFS had a 5% slope and was lined with an impermeable liner. A manifold was placed on the front (top) edge of each VFS to evenly apply run-on water using a solvent pump deliv-

ery system. At the bottom of each VFS, runoff water was collected. Lysimeters near the bottom of each strip sampled subsurface water. A bromide tracer study determined any hydraulic differences between VFS prior to planting. VFS were then established in replicates of three (unvegetated, random mixture of plants, succession of plants, and turfgrass cut to three heights).

The six pesticides used in the greenhouse study, plus cyfluthrin, will be used in the VFS run-on field trials (growing seasons 2009 and 2010). Pesticides will be applied with a water volume that would be generated for both a 1-year and a 5-year rain event. Bromide will also be added to the pesticide containing water at 4 g/L as a tracer.

Several storm/run-on scenarios on the bare (pre-planted) VFS were evaluated. The volume of runoff water applied as run-on to each VFS was based on a 1-year rain event. The runoff water generated during a 1-year rain event was calculated to be 25.4 gallons over the course of 24 hours from an turfgrass area 3 feet by 20 feet with a 5% slope. This water volume is then applied to the top of the VFS as run-on water. Soil presaturation was achieved prior to the initiation of the storm event.

An artificial rainfall system has been constructed similar to those used in previous USGA-funded runoff studies in

Minnesota. This system provides uniform droplet size with low kinetic energy. The storm scenario selected for the 1-year rain event and the initial bromide tracer studies was as follows: Artificial rain for 6 hours total (6 am - 12 noon, for ~2.4 inches total rainfall, 0.4 inches/hr) and run-on for 2 hours (11 am - 1 pm @ 12.7 gal/hour). In the case of the 5-year rain event, this would involve adding 3.8 inches of water as rain over 24 hours and 62.1 gallons of water as runoff over 24 hours.

The mass of pesticide lost will be evaluated using the concentration of the pesticide and the volume of water collected during runoff. In addition to pesticides, runoff water will be monitored for losses of nitrogen and phosphorus from fertilizer inputs. Soil, soil water, and plants within the VFS will be analyzed to determine if the pesticides lost from the runoff water are sorbing to the soil, being degraded in the soil, taken up by the plants, or lost to leaching or subsurface flow. Values will be compared against the bromide tracer, which will move freely with the run-on water.

Soil sampling will be conducted at three different depths at 3 locations within the VFS (0.3 m, 1.83 m, and 3.35 m from the top of each strip). Even chemicals that sorb tightly to the soil can be found deeper in the soil profile than would be expected based on their physical and chemical properties because of preferential flow pathways.

Summary Points

- A pump-driven delivery system has been fabricated to deliver run-on water onto the VFS.
- Nine VFS have been planted (3 VFS will remain unvegetated) with either turfgrass or the five plant species selected from the greenhouse study and allowed to mature.
- An artificial rainfall simulator system has been fabricated and installed at the run-on plot.



Soil, soil water, and plants within the VFS will be analyzed to determine if the pesticides lost from the runoff water are sorbing to the soil, being degraded in the soil, taken up by the plants, or lost to leaching or subsurface flow.

Nitrogen and Phosphorus Fate in a 10+ Year Old Kentucky Bluegrass Turf

Kevin W. Frank
Michigan State University

Objectives:

1. Determine nitrate-nitrogen and phosphorus leaching from a turfgrass stand that has been continually fertilized for 18 years.
2. Continue data collection from the Long Term Nutrient Fate Research Area at MSU; currently we have data collection for 10 years consecutively.

Start Date: 2003

Project Duration: five years

Total Funding: \$68,886

USGA initially funded research at Michigan State University to determine nitrogen fate and leaching from a Kentucky bluegrass turf in 1991. Similar to previous research, the initial research at MSU conducted from 1991 through 1993 indicated that there was minimal risk of nitrate-nitrogen leaching from Kentucky bluegrass (*Poa pratensis* L.) turfgrass.

Since the summer of 1998, percolate samples have been collected from the same monolith lysimeters and analyzed for nitrate-nitrogen ($\text{NO}_3\text{-N}$). As of 2008, the turfgrass area has now been under continual fertilization practices for 18 years with percolate collection for the last 10 years consecutively.

From July 1998 through 2002, lysimeters were treated annually with urea at a low N rate 98 kg N ha^{-1} (4 applications) and a high N rate of 245 kg N ha^{-1} (4 applications). From 1998-2002 for the high N rate, there was a dramatic increase in $\text{NO}_3\text{-N}$ leaching from 5 mg L^{-1} in 1998



Since the summer of 1998, percolate samples have been collected from the same monolith lysimeters and analyzed for nitrate-nitrogen ($\text{NO}_3\text{-N}$).



Dr. Kevin Frank (above right) explains to members of USGA's Research Committee the efficiency of nitrogen use by mature Kentucky bluegrass when fertilized at a low ($2 \text{ lb./1000 ft}^2/\text{year}$) and a high rate ($5 \text{ lb./1000 ft}^2/\text{year}$). Results indicate that the high rate of nitrogen fertilization is much more than the turf needs and can result in unacceptable levels of nitrate-nitrogen in leachate.

to 25 mg L^{-1} in 2002. During the same time frame, there was a modest increase in $\text{NO}_3\text{-N}$ leaching from 3 mg L^{-1} in 1998 to 5 mg L^{-1} in 2002. In 2003, the N rate was reduced to 196 kg N ha^{-1} for the high N rate, while the low N rate remained at 98 kg N ha^{-1} .

Since 2003, phosphorus from triple superphosphate (20% P) has been applied at two rates, 49 and 98 kg P ha^{-1} split over two applications. The phosphorus application dates coincide with nitrogen application dates in the spring and autumn.

In 2003, the concentration of $\text{NO}_3\text{-N}$ leaching from the high N rate treatment did not decline from the previous years. The average $\text{NO}_3\text{-N}$ concentration leached from the low and high N rate treatments was 6.3 and 31.6 mg L^{-1} . In 2004, the concentration of $\text{NO}_3\text{-N}$ leaching from the high N rate treatment declined drasti-

cally from previous years. The average concentration of $\text{NO}_3\text{-N}$ in leachate for the high N rate was 8.5 mg L^{-1} . This was a decrease in $\text{NO}_3\text{-N}$ concentration of 23.1 mg L^{-1} from 2003. For the low N rate the average concentration of $\text{NO}_3\text{-N}$ in leachate for the low N rate was 1.2 mg L^{-1} . From 2004 through 2008, the mean $\text{NO}_3\text{-N}$ concentration for the low and high N rates was 2.9 and 9.4 mg L^{-1} .

The concentration of phosphorus detected in leachate remains very low regardless of treatment. The mean concentration of phosphorus detected in leachate since initiating phosphorus treatments in 2003 has been less than 0.02 mg L^{-1} .

Summary Points

- The mean $\text{NO}_3\text{-N}$ concentration from 2004 through 2008 is less than 10 mg L^{-1} .
- Results continue to indicate low amounts of phosphorus leaching.

Attenuation of Pharmaceuticals, Personal Care Products, and Endocrine-disrupting Compounds by Golf Courses Using Reuse Water

Mike McCullough

Northern California Golf Association

Objectives:

1. To understand the fate and transport of PPCP/EDCs in golf courses when reuse water is used as the sole source of irrigation water.
2. To evaluate the capacity of turfgrass as a biofilter to remove PPCP/EDCs in reuse water.
3. To extend knowledge to stakeholders and reduce the uncertainty of how long-term use of reuse water for irrigation may impact groundwater quality.

Start Date: 2008

Project Duration: two years

Total Funding: \$60,000

Little is known about the fate and transport of emerging contaminants such as pharmaceuticals, personal care products, and endocrine-disrupting compounds (PPCP/EDCs) from reuse water in landscape systems receiving reuse water through irrigation. This project will investigate the fate and transport of PPCP/EDCs in turf with the goal to evaluate the effectiveness of turf in mitigating their vertical movement (i.e., leaching).

Studies to determine the stability of the target compounds in wastewater were initiated. Samples were taken at $t=0$ and regular intervals thereafter to be analyzed for the target compounds. Simvastatin hydroxy acid and estrone were removed from the target list due to their

historically low concentrations in post-UV wastewater effluent. In addition, sulfamethoxazole, phenytoin, primidone, TCP, and TCEP were added due to their high concentrations in post-UV wastewater effluent.

It was found that all compounds were stable for the first 10 days. However, by day 20, atenolol, fluoxetine, diclofenac,

Compound	Concentration
Sulfamethoxazole	1,500
Atenolol	500
Trimethoprim	16
Fluoxetine	16
Meprobamate	480
Phenytoin	120
Carbamazepine	170
Diazepam	2.3
Atorvastatin	7.7
Primidone	130
TCP	1,900
TCEP	470
Gemfibrozil	2.3
Bisphenol A	<5.0
Diclofenac	34
Naproxen	14
Triclosan	16

Analyte concentrations (ng/L) at $t=0$ in post-UV wastewater effluent

and naproxen began to show decreases in concentration, indicating degradation had begun to occur. Therefore, it is recommended that the wastewater be sampled during the initial filling of the wastewater storage containers to determine analyte concentrations and then replaced every 2 to 3 weeks to avoid changes in analyte loading.

Studies were also undertaken to identify any effects that the lysimeter components would have on the concentration of the target analytes. Deionized water that had been spiked with the target analytes was poured into a beaker containing ~400

g of diatomaceous earth and allowed to stand for three days. Samples were taken daily and analyzed to determine the degree to which the target analytes adsorbed onto the diatomaceous earth. Only fluoxetine was found to be affected by the diatomaceous earth, decreasing from 52 ng/L to 3.1 ng/L over the course of three days. Therefore, any fluoxetine degradation observed in the study should be viewed accordingly.

Summary Points

- We have purchased non-labeled and ^{13}C -labeled standards for all the candidate compounds listed in the proposal and are close to establishing analytical conditions for these compounds.
- We are developing sample preparation methods and evaluating method performance.
- We have received two soils that will be used for the laboratory-scale experiments. We plan to develop and finalize the experimental design for these laboratory-scale experiments in the second quarter.
- Efforts were devoted to assisting in the collection of soil in Eldorado Valley for the meso-scale experiments.
- The lysimeter plots at UCR are currently being renovated. New sod will be installed before spring of 2009. We plan to check leachate collection equipment from each plot and identify 8 plots for use in the research commencing in the summer of 2009.
- Meters were ordered from the vendor and shipped to Las Vegas. Equipment checked out correctly and installation at Wildhorse Golf Course is scheduled for October 2008. Other flux meters are being shipped in mid-October to golf courses in San Jose and southern California with installation to follow.



Setting the lysimeters into the holes at UCR. Researchers will check leachate collection equipment from each plot and identify 8 plots for use in the research commencing in the summer of 2009.

Audubon International

Audubon International strives to educate, assist, and inspire millions of people from all walks of life to protect and sustain the land, water, wildlife, and natural resources around them. In 1991, Audubon International launched the Audubon Cooperative Sanctuary Program for Golf Courses (ACSP), an environmental education program designed to help golf courses play a significant role in enhancing and protecting wildlife habitat and natural resources. The ACSP provides an advisory information service to help golf courses conduct environmental projects and achieve positive recognition for their efforts.

In addition, the Audubon Signature Program works closely with planners, architects, managers, and key stakeholders of new golf course developments to merge wildlife conservation, habitat enhancement, resource conservation, and environmental improvement with the economic agenda associated with the development. Involvement in the development stages of a project enables Audubon International to ensure that biodiversity conservation, environmental quality, and sustainable management are built into the project and continue after construction is completed.



The redesigned and greatly improved wash pad at Prairie Dunes Country Club provides adequate collection and filtering of wash water. Identifying problems, choosing the most environmentally responsible treatment, and implementing a solution are a key focus area for Audubon Cooperative Sanctuary Program for Golf Course members.



The Green Golfer Challenge gave the staff at Pecan Hollow in Plano TX an opportunity to introduce golfers to our efforts to become a Certified Audubon Cooperative Sanctuary and to encourage them to be good stewards of the environment as they played golf. Outreach projects like this are simple way to get golfers involved and build support for the game, the environment, and the program.



Signage provides students and the general public with education about the inhabitants of the golf course. Signs like this one at Washington Reservation in Ohio, when placed strategically around the nine holes, provide information about birds and other wildlife that may be seen. Washington Reservation is the first Certified Golf Signature Sanctuary in Ohio.



Jennifer and Philip Nola were the 2007 Grand Prize winners of the Green Golfer Challenge, receiving 2 tickets to the 2008 US Open at Torrey Pines Golf Course in LaJolla, California. Their home base is Farmingdale NY and home course is Bethpage State Park.

The Audubon Cooperative Sanctuary Program for Golf Courses

Ron Dodson

Audubon International

Objectives:

1. Enhance wildlife habitats on existing golf courses by working with golf course superintendents and providing advice for ecologically sound course management.
2. Encourage active participation in conservation programs by golfers, golf course superintendents, golf officials, and the general public.
3. Recognize the people who are actively participating in environmentally responsible projects.
4. Educate the public and golfing community about the benefits of golf courses and the role they play relative to the environment and wildlife.

Start Date: 1991

Project Duration: ongoing

Total Funding: \$100,000 per year

Audubon International is a non-profit environmental organization that envisions communities becoming more sustainable through good stewardship of the natural environment where people live, work, and recreate through responsible management of land, water, wildlife, and natural resources. Since 1991, it has worked in partnership with the USGA to offer the Audubon Cooperative Sanctuary Program for Golf Courses (ACSP), an award-winning education and certification program that promotes ecologically-sound land management and the conservation of natural resources on golf courses. Audubon Signature Programs provide comprehensive environmental education and planning assistance to new developments.

Today, more than 2,200 golf courses in 30 countries participate in the ACSP for Golf Courses. More than half of those enrolled have developed an environmental plan to guide management of the golf course and 723 have achieved certification for their outstanding best practices. We also awarded 664 "Certificates of Achievement" to recognize golf courses for outstanding accomplishments to improve wildlife habitat, save water, conserve energy, and reduce waste.

Audubon International provided environmental planning services to 161 projects (126 golf-related) development projects in 36 U.S. states and 10 countries, covering 75,000 acres of land, through the Audubon Signature Programs in 2007/08. Seventeen projects achieved certification, bringing the total number of golf courses that have been designated as Certified Audubon Signature Sanctuaries to 70 in 22 US states and 3 countries.

An analysis of environmental



Southern Chorus Frog. Out of Golf Digest America's 100 Greatest Golf Courses, 56% are members of the Audubon Cooperative Sanctuary Program for Golf Courses and undertaking voluntary action to protect and enhance wildlife habitats. Photo Credit: Marvin Bouknight, Oldfield Naturalist, SC

improvements on certified golf courses compared with golf courses that are enrolled but not yet certified revealed significant differences. Certified courses are 2-4 times more likely to be involved in environmental projects, such as exotic plant removal; prairie, wetland, and stream restoration; and endangered or threatened species reintroduction.

Efforts to enhance wildlife habitats on golf courses are done on a regional and site-specific basis. In addition to participation in the program's environmental management and certification activities, golf courses are invited to participate in wildlife monitoring events and activities. Birdwatching teams from 72 golf courses each identified an average of 46 bird species during the 2008 North American Birdwatching Open, a 24-hour bird count conducted at the height of bird migration in March, April, and May. (Start date based on location.)

Audubon International encourages active participation in conservation programs by golfers, golf course superintendents, golf officials, and the general public through a variety of outreach and education activities. In 2005, Audubon International, the United States Golf

Association, and The PGA of America launched the Golf and the Environment Initiative to foster environmental awareness, action, and positive results throughout the golf industry. A Web site, www.golfandenvironment.org serves as a clearinghouse of golf and environment information. In addition, a Green Golfer pledge drive to engage golfers has been launched. Currently there are over 6,400 Green Golfers who have taken the pledge and over 105 Golf Courses involved in the Green Golf Challenge.

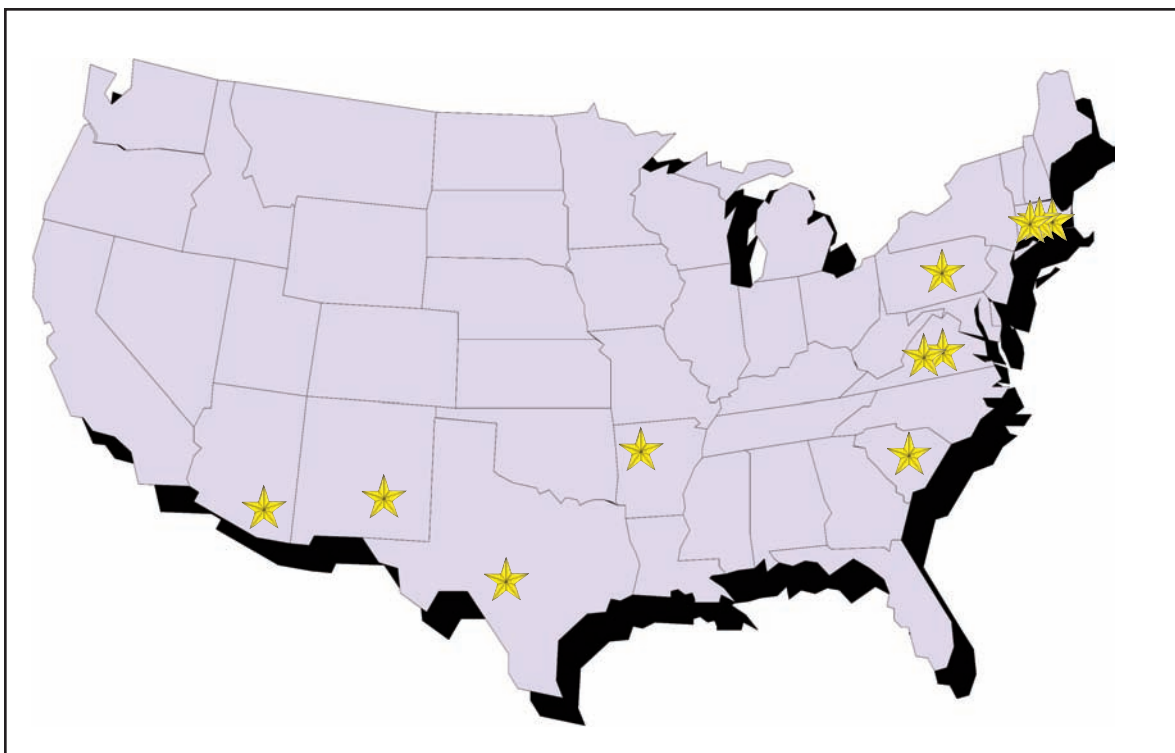
Over 2000 people took part in seminars and field training conducted by Audubon International staff in 2007/08. More than 500 conservation organizations are directly involved with golf courses as a result of their participation in the ACSP.

Summary Points

- Number of golf courses enrolled in the Audubon Cooperative Sanctuary Program (ACSP): 2,205
- Number of golf courses enrolled in the Audubon Signature Program: 237
- Total number of acres registered in ACSP for Golf Courses: 519,474
- Number of conservation organizations directly involved with golf courses as a result of their participation in the ACSP: >500
- Average number of bird species sighted on 72 golf courses participating in the 2008 North American Birdwatching Open: 46
- Number of Certified Audubon Cooperative Sanctuary Golf Courses: 80
- Total number of golf courses certified in Environmental Planning: 1,780
- Number of golf course personnel and others educated by Audubon International in 2007/08 through seminars, conference presentations, and site visits: >2,000

Grant-in-Aid Research Program

USGA's Turfgrass and Environmental Research Program relies on science to attain answers that will help ensure the long-term success of the golf course management industry. Frequently projects may span several years. Many times, however, golf course superintendents need answers to very applied problems to help them meet the many challenges of properly managing golf courses. The Grant-in-Aid Research Program was created to address this need. This program allows directors of all eight USGA Green Section regions to identify applied problems and the appropriate researchers in their regions to solve those problems. Research projects funded under this program most often include cultural aspects of golf course management. Examples include what fungicides work best on a particular disease, or the management of new turfgrass cultivars, renovation techniques, safe and effective use of herbicides, insecticides, or fertilizers. These projects are usually short duration (one to three years), but can offer golf course superintendents answers to practical, management-oriented challenges that they can put into use quickly.



Location of current projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the Grant-in-Aid Program

Assessing the Usefulness of Physical Water Conditioning Products to Improve Turfgrass Quality and Reduce Irrigation Water Use

Bernd Leinauer, Ty Barrick, and Cody Robertson
New Mexico State University

Objectives:

1. To assess the effect of three water conditioning systems on perennial ryegrass establishment, overall turfgrass performance, and root distribution under potable and saline irrigation.
2. To study the impact of water conditioning units on salinity build-up in the rootzone.

Start Date: 2005

Project Duration: two years

Total Funding: \$6,000

Physical water conditioning units such as AquaPhyd, Carefree, Fre-Flo, Magnawet, and Zeta Core have been used in the golf course industry because of manufacturers' claims that they improve turf quality and reduce scaling, compaction, the effect of salts on soil physical and chemical characteristics and plant growth, and reduce the amount of irrigation water necessary for adequate turfgrass growth and quality. These products are gaining popularity, particularly in the Southwest, despite the lack of scientific evidence to support the manufacturers' claims.

A study conducted at New Mexico State University in spring and summer of 2008 investigated the effect of physical conditioning systems on turf color, quality, and cover, and chemical rootzone composition of a perennial ryegrass stand irrigated with either saline or potable water. The soil at the site is a Torriorthent, a sandy entisol typical of arid regions.

Treatments consisted of 2 levels of irrigation water quality and 5 conditioning products. During 2008, irrigation was



Conditioning treatments included two catalytic water conditioners (Zetacore and FreFlo), a magnetic water conditioner (Magnawet), a hydro-electrical conditioner (Aqua-PhyD), and a control treatment.



A study conducted at New Mexico State University in spring and summer of 2008 investigated the effect of physical conditioning systems on turf color, quality, and cover, and chemical rootzone composition of a perennial ryegrass stand irrigated with either saline or potable water.

applied daily with either potable water (TDS 397 ppm, SAR 1.55, pH 8) at approximately 90% ET_0 or saline water (TDS 3,110 ppm, SAR 8.94, pH 7.5) at approximately 125% ET_0 . Conditioning treatments included two catalytic water conditioners (Zetacore and FreFlo), a magnetic water conditioner (Magnawet), a hydro-electrical conditioner (Aqua-PhyD), and a control treatment.

The area was established in 2007 with 'IG2' perennial ryegrass. Data collection included turfgrass color and cover (measured by digital image analysis), visual assessment of turfgrass quality, salinity build-up in the rootzone, and turfgrass stress as measured by Normalized Difference Vegetation Index (NDVI).

The water conditioning units had no effect on turfgrass quality and only saline water reduced quality significantly. When plot coverage and turf color data were averaged for water qualities, Magnawet and Zetacore plots had lower coverage and lighter green color than control plots or plots irrigated with Fre Flo-treated water at the end of the growing

period (November 2008). Aqua-PhyD plots did not differ from control plots for coverage or color.

There was an obvious and expected difference in soil salinity between plots irrigated with potable water and those irrigated with saline water. All the treatments that received saline water showed higher levels of salts compared to treatments receiving potable water. However, water conditioning had no effect on the chemical composition of the rootzone.

Summary Points

- Studies were conducted at New Mexico State University to assess physical water conditioning products under potable and saline irrigation.
- Products tested had no effect on the quality of a perennial ryegrass stand.
- At the end of the 2008 growing period, Zetacore- and Magnawet- treated plots had lower coverage and lighter green color than control plots.
- Physical water conditioning had no effect on the chemical composition of either saline or potable irrigated rootzones.

Preventive and Curative Control of Algae on Putting Greens Using Products Other than Daconil

John E. Kaminski
University of Connecticut

Objectives:

1. To determine the influence of various products on algae control of golf course putting greens.

Start Date: 2007

Project Duration: two years

Total Funding: \$6,000

Turfgrass quality on golf course putting greens receiving few hours of sunlight or exposed to prolonged periods of excessive moisture is often reduced for a number of reasons. Shaded environments generally are associated with poor air movement, excessive soil moisture, and extended periods of leaf wetness. Even greens located in excellent growing environments, but built with no or poor internal drainage, are susceptible to thinning during the stressful summer months. Stress-related injury to the turfgrass often leads to an increased susceptibility to other turfgrass disorders including algae and moss invasion, as well as infection from various turfgrass pathogens.

Algae infestation on closely mown putting greens continues to be difficult to manage. While applications of various fungicides such as chlorothalonil, maneb, and mancozeb have been shown to suppress algae, these products must be applied on relatively short intervals prior to the appearance of symptoms and their efficacy varies. Further complicating the use of fungicides is the exclusion of chlorothalonil from use in specific regions of New England. In areas where chlorothalonil use remains legal, new label restrictions on the total amount of product that can be applied and increased time between application intervals may drastically limit the ability to control algae over the course of an entire season.

Previous studies revealed a reduction in algae growth from phosphite-based fungicides and select wetting agents. Although the main purpose of these studies did not involve an investigation of algae, products suppressed algae between 83 and 100% when compared to the untreated control. Little information is available regarding the influence of phosphite products and wetting agents for controlling



The influence of fungicides on algae suppression may be influenced by other tank-mixed partners such as wetting agents.

algae. The objective of this study will be to determine the ability of various phosphites and wetting agents to suppress algae; and 2) assess the interaction among effective algae control products and fungicides commonly used on golf course putting greens.

Studies were initiated at the University of Connecticut Plant Science Research and Education Facility located in Storrs, CT. When applied preventively, most phosphite treatments, as well as Daconil, reduced algae populations when compared to the untreated control. When single applications of phosphites and fungicides were applied as a curative treatment, algae development was not reduced or slowed and no differences were observed among treatments.

In addition to the impact of phosphites, the interaction among fungicides and wetting agents was assessed. The least amount of algae was observed within plots treated with Insignia + Magnus, Daconil + Revolution, and Daconil alone. Moderate suppression of algae was exhibited within plots treated with Insignia + Revolution, Protect + Dispatch, Trinity + Magnus, Daconil + Duplex, and Protect + Duplex. No other treatment provided a reduction of algae compared to the untreated control plots.

Although various treatments provided suppression of algae, it appeared that

applications of each product alone provided varying results than when the products were tank-mixed with different wetting agents. A final greenhouse study investigated the influence of nitrogen source on algae development. In this study, only pots received ammonium sulfate (21-0-0), calcium nitrate (15.5-0-0), and ammonium nitrate (34-0-0) significantly reduced algae when compared to the untreated control or other nitrogen sources.

Additional field and greenhouse studies are planned for 2009 to further investigate the preventive and curative suppression of algae, as well as any interactions with other commonly applied products. Laboratory studies will also be developed to assess the direct inhibition of algae from phosphorous acid and various commercially available phosphite products.

Summary Points

- Algae growth is a chronic problem on thinning golf course putting greens during the summer months.
- Current data suggest that most phosphite products can reduce algae when applied preventively, but curative control may be more difficult.
- The influence of fungicides on algae suppression may be influenced by other tank-mixed partners such as wetting agents.

Bermudagrass Control Programs in Kentucky Bluegrass

J. B. Willis and S. D. Askew
Virginia Polytechnic and State University

Objectives:

1. Develop effective bermudagrass control programs utilizing multiple active ingredients, avoiding summer stress, and targeting bermudagrass throughout its growing season.

Start Date: 2008

Project Duration: one year

Total Funding: \$3,000

Common bermudagrass is a troublesome weed in cool-season roughs and fairways. Several programs have been recommended for common bermudagrass suppression in cool-season turfgrass, however complete control is rarely achieved and highly variable in field situations.

Four treatments of fenoxaprop-P plus triclopyr applied monthly provided acceptable suppression of common bermudagrass in trials in North Carolina and California. Researchers in Georgia suppressed bermudagrass in tall fescue with fenoxaprop and ethofumesate combinations. None of these programs resulted in complete control, only suppression.

Research at Virginia Tech found that concentrating herbicide applications in spring and fall at three-week intervals controlled bermudagrass and avoided herbicide treatment during summer when injury to cool-season turf is more likely. This



Treatment combinations containing ethofumesate and triclopyr applied in summer significantly injured Kentucky bluegrass.

research also incorporated mesotrione as a tank mix partner to fenoxaprop-P and triclopyr. The addition of mesotrione improved bermudagrass control over the products alone, and all three products tank-mixed controlled bermudagrass 98%.

The limitation of Virginia Tech's findings is that better performing programs exceed labeled maximum annual product use restrictions. Combinations that maximize bermudagrass control and do not exceed these annual use limits are needed. There are only five herbicides that can selectively suppress bermudagrass in Kentucky bluegrass. These include

fenoxaprop (Acclaim Extra), ethofumesate (Progress), siduron (Tupersan), triclopyr (Turflon), and mesotrione (Tenacity).

The experimental location is Kentucky bluegrass rough that was infested with 'Vamont' common bermudagrass sod 4 years ago. Experimental design is randomized complete block with 3 replications and 6 by 10 ft. plots. Data collected will include bermudagrass and other weed control and cover, turfgrass injury, color, and quality. The duration of this trial will be from spring 2008 to summer 2009 to evaluate treatment effects 1.5 years after treatment initiation.

Preliminary results indicate that several treatment scenarios have significantly reduced bermudagrass cover while increasing Kentucky bluegrass cover. Very few treatments significantly injured Kentucky bluegrass. However, treatment 3 controlled bermudagrass greater than 97%; this treatment exceeds label restrictions for annual usage rates. The modified Virginia Tech programs (Treatments 6 and 7) have also controlled bermudagrass equivalent to treatment 5. Treatments 5, 6, and 7 all control bermudagrass significantly better than the industry standard (treatment 4).

Herbicide Treatments

- 1) Nontreated control no herbicides applied.
- 2) Negative control for bermudagrass industry standard treatments for broadleaf and summer annual grass weed control, three-way plus crabgrass preemergence control product both at labeled rates and timings.
- 3) Industry Standard Renovation fall renovation using glyphosate spot-treatment according to label recommendations (2 apps. at 5 qt/A each at 3 wk interval and reseed).
- 4) North and South Carolina recommendation 4 applications of fenoxaprop-P at 28 fl oz/A plus triclopyr at 32 fl oz/A applied at monthly intervals starting June.
- 5) Virginia Tech top 3-Way 3 applications of mesotrione at 4 fl oz/A plus triclopyr at 32 fl oz/A plus fenoxaprop-P at 28 fl oz/A at 3 week intervals both spring and fall.
- 6) VT modified 1 2 applications of fenoxaprop-P at 28 fl oz/A plus triclopyr at 32 fl oz/A applied at 3 week intervals both spring and fall, with mesotrione at 4 fl oz/A at 3 week intervals throughout summer (not to overlap with fenoxaprop-P plus triclopyr applications)
- 7) VT modified 2 2 applications of fenoxaprop-P at 28 fl oz/A applied at 3 week intervals both spring and fall, with mesotrione at 4 fl oz/A plus triclopyr at 16 fl oz/A at 3 week intervals throughout summer (not to overlap spring and fall applications).
- 8) 5 herbicide combo fenoxaprop-P at 28 fl oz/A plus triclopyr at 32 fl oz/A plus ethofumesate at 64 fl oz/A plus siduron at 24 lbs/A plus mesotrione at 4 fl oz/A applied 2 times spring and fall at three week intervals.
- 9) 5 herbicide sequence 1 fenoxaprop-P at 28 fl oz/A plus ethofumesate at 64 fl oz/A plus siduron at 24 lbs/A applied 2 times spring and fall at three week intervals, and triclopyr at 32 fl oz/A plus mesotrione at 4 fl oz/A applied 4 times in summer at 3 week intervals (not to overlap spring and fall applications).
- 10) 5 herbicide sequence 2 fenoxaprop-P at 28 fl oz/A plus ethofumesate at 64 fl oz/A plus siduron at 24 lbs/A applied 4 times in summer at three week intervals, and triclopyr at 32 fl oz/A plus mesotrione at 4 fl oz/A applied 2 times spring and fall at 3 week intervals.
- 11) 4 herbicide combo same as #6 but drop siduron to save costs.
- 12) 4 herbicide sequence 1 same as #7 but drop siduron.
- 13) 4 herbicide sequence 2 same as #8 but drop siduron.

Herbicide treatments used in the bermudagrass control study at Virginia Tech.

Summary Points

- Treatment combinations containing ethofumesate and triclopyr applied in summer significantly injured Kentucky bluegrass.
- Several treatment combinations were shown to control bermudagrass better than the current industry standard.

Organic Matter Dilution Programs for Sand-based Putting Greens

Erik Ervin and Brandon Horvath

Virginia Polytechnic Institute and State University

Jason Henderson

University of Connecticut

Objectives:

To compare various cultivation approaches that remove from 10 to 27% surface area and determine treatment effects on agronomic performance of a mature putting green in east-central Virginia. The ultimate goal is to determine which organic matter dilution program maintains mat layer organic matter at less than 4% while providing the fewest days of putting quality disruption each year.

Start Date: 2008

Project Duration: three years

Total Funding: \$9,000

Aggressive organic matter dilution programs are intended to slow loss of aeration porosity and subsequent infiltration rates thereby allowing superintendents to more easily manage their putting greens and lessen the effects of summer bentgrass decline. Our research compared various cultivation approaches that removed from 10 to 27% surface area to determine the effects on agronomic performance of a mature putting green in east-central Virginia.

We used a mix of tine sizes and verticutting to achieve a range of surface removal. Our research was done on mature (8-yr-old) 'Penn A4' greens that are in play. Our treatments were imposed on two practice putting greens at the Independence Golf Club, the home course of the Virginia State Golf Association near Richmond.

Prior to initiation of the study, analysis of 4 randomly-selected cup cutter cores revealed a mat layer with 5.8% organic matter and an infiltration rate of 11 inches/hr. Various combinations of small tines (0.25"), big tines (0.50"), and verticutting (3-mm blade) were imposed in late March and early September to provide a

range of seasonal surface removal from 0% to 26.6% (Table 1). Verticut blade spacing and depth was 1 inch, while tine spacing was 1.33" X 1.5", with a coring depth of 2".

Heavy sand topdressing of approximately 12 ft³ (1,200 lbs/1000 sq. ft.) was applied on both days of cultivation, supplemented by six light topdressings of 2 ft³ (200 lbs/1000 sq. ft.) every 3 weeks between cultivations for a seasonal total of about 36 ft³. Cultural management of these greens were identical to all others on the golf course, receiving preventive pesticide applications, daily mowing at 0.115", and April through September fertilization of 1.6 lbs N/1000 sq. ft.

Cultivation treatment had no effect on soil temperature, soil moisture, or ball roll distance throughout the summer season. Close-up pictures were taken for digital image analysis of recovery rate, but analysis is still in process. Visual quality observations indicated almost complete recovery of cover at 2 weeks after coring in the spring for treatment 2, where 0.25" tines were used in 2 passes to remove 5% surface area. All other spring treatments were more aggressive, removing 10 to 14%; these treatments required 8 to 10 weeks for recovery.

Fastest fall recovery was again seen with the small tines (0.25"). Interestingly, fill-in was just as fast when 2 passes were made with the ¼-inch tines as when only 1 pass was made. This observation agrees with others who observed that recovery was only affected by tine diameter and not by tine spacing. Cup-cutter plugs for quantification of mat layer percent organic matter were taken in late October and have yet to be processed.



Cultivation treatments being applied on Sept 4. Left lane shows a 0.25" tine treatment; middle lane shows a 0.5" treatment; right lane shows a 0.25" coring + verticutting treatment.

Summary Points

- Previous transition zone research at the University of Arkansas on a relatively young (2-yr-old) 'Penn G2' sand-based green indicated that aggressive annual removal of 15-23% surface area via verticutting was required to keep mat layer organic matter below 4%. However, recovery of putting green uniformity took at least twice as long as coring.
- Our transition zone study in Richmond, Virginia is being conducted on 8-yr-old 'Penn A4' sand-based putting greens that are in-play, and began the trial at 5.8% organic matter (above the USGA recommended mat layer limit of 4%).
- Closely spaced core aeration, with or without verticutting treatments, were imposed spring and fall so that annual surface area removal of plots ranged from 10% to 27%.
- No overall bentgrass decline was noted during the 2008 growing season and none of the treatments affected soil temperature, soil moisture, or ball roll distance.
- Faster recovery was seen on those plots where surface area removal was the least. That is, where ¼-inch tines were used at close spacing (1.33" x 1.5"), and the green was either cored once or twice.

Treatment	Surface Area Removed (%)		
	March 28	Sept 4	Total
1. Untreated check	0	0	0
2. 0.25" tine core aerations X2	5	5	10
3. 3-mm blade verticutting	11.8	11.8	23.6
4. 0.25" tine core aeration + 3-mm blade verticutting	2.5	2.5	16.8
5. 0.5" tine core aeration + 0.25" tine core aeration X2	9.8	5	14.8
6. 0.5" tine core aeration	9.8	9.8	19.6
7. 0.5" tine core aeration + 3-mm blade verticutting + 0.25" tine core aerations X2	9.8	11.8	26.6

Table 1. Cultivation treatments and percent surface area removed.

Impact of Sand Type and Application Rate of Fairway Topdressing on Soil Physical Properties, Turfgrass Quality, Disease Severity, and Earthworm Castings

Jason J. Henderson
University of Connecticut

Objectives:

1. Determine whether particle size distribution and/or application rate will affect turfgrass quality, disease incidence and earthworm activity.
2. Quantify the effects of particle size distribution and topdressing layer depth on soil physical properties.
3. Use the resultant data to make recommendations to improve the practice of fairway topdressing.

Start Date: 2007

Project Duration: two years

Total Funding: \$6,000

Fairway topdressing is a relatively new cultural practice that is being adopted by several golf course superintendents throughout the United States to improve playing conditions. Some of the benefits reported have been improved drainage, less disease, and firmer fairways. The benefits to fairway topdressing seem unanimous, but the practice requires a significant budget, considerable labor, time, and commitment to implement properly. Additionally, many questions remain unanswered with regards to topdressing material selection, application rates, and the turfgrass management implications as the topdressing layer accumulates.

Sands used in USGA putting green construction and subsequent topdressing have been thoroughly researched to optimize macroporosity while maintaining sufficient water-holding capacity. However, due to the strict specifications, these sands are prohibitively expensive when considered for use on larger fairway acreage. Therefore, recommendations for fairway sands are often very general.

The cost of a USGA sand does not improve the practicality of implementing this program for many golf courses. The impact of using sand that does not meet USGA specifications, however, has not been thoroughly investigated. Particle size distribution will likely affect infiltration and water retention at the playing surface. Topdressing materials that are too fine may retain excess moisture, whereas, a sand that is too coarse may predispose a large portion of the course to moisture stress. The short- and long-term impact of topdressing native soils is unknown.

This study was initiated on an 'L-93' creeping bentgrass (*Agrostis stolonifera*) stand managed as a golf course fairway at the University of Connecticut Plant Science Education and Research Facility in the summer of 2007. The first factor is sand type; fine, medium, and coarse. The second factor is application rate; 4 ft³/1000 ft², 8 ft³/1000 ft², and 12 ft³/1000 ft². A control is included that receives no topdressing applications.

Topdressing applications are kept constant and are applied once per month starting in May and ending in November. This design allows the comparisons of each sand type applied at each of the three rates. The three different rates will also

enable the development of three different depths of topdressing over time.

Weekly data collection includes volumetric soil moisture, soil penetration resistance, turfgrass cover, turfgrass color, and turfgrass quality. Soil physical properties will be determined on an annual basis.

Summary Points

- Topdressed plots showed a faster green-up response than the untreated control plots in mid-April regardless of sand type. Plots that received higher rates of application exhibited a greater greening response than plots receiving lighter rates of application.
- Topdressed plots exhibited less dollar spot incidence than untreated plots. Plots that received higher rates of topdressing had less dollar spots than plots that received lower rates of topdressing, regardless of sand type.
- Earthworm pressure was not sufficient to determine treatment differences.
- Topdressed treatments had higher resistance to penetration than the untreated control plots demonstrating a firmer surface than the untreated controls. The fine sand had the greatest resistance to penetration, followed by the medium sand and the coarse sand, respectively. Plots receiving higher rates of topdressing exhibited greater firmness than plots receiving the lower rates.
- Untreated controls had the highest volumetric soil moisture content in the top 2" of the playing surface compared to all topdressing treatments. The fine and medium sand treatments hold more water than the coarse sand treatments. Regardless of sand type, the higher the rates of application the less water is held in the top 2" of the playing surface.



Topdressing applications are kept constant and are applied once per month starting in May and ending in November. This design allows the comparisons of each sand type applied at each of the three rates.

Best Management Practices for the Conversion of Established Bermudagrass to Buffalograss

Gerald Henry
Texas Tech University

Objectives:

1. Examine the effect of seeding rate on the establishment of seeded buffalograss.
2. Examine the effect of various seedbed preparation techniques (verticutting, aeration, and topdressing) on the establishment of seeded buffalograss.

Start Date: 2008

Project Duration: two years

Total Funding: \$6,000

Growing concern over the sufficiency and variability of present water supplies in western Texas and other areas of the arid southwest led to the examination of several reduced-input turfgrass species for water conservation. Buffalograss [*Buchloe dactyloides* (Nutt) Engelm.] is a warm-season turfgrass that shows excellent drought, cold, and salinity tolerance. Increasing awareness and acceptance of buffalograss as a viable turfgrass option in arid environments requires investigation into conversion techniques for its establishment.

Research was conducted at the Texas Tech Turfgrass Research Station during the summer of 2008 on an established bermudagrass rough. The parameters evaluated included four seedbed preparation treatments and two buffalograss seeding rates. Bermudagrass was

sprayed with glyphosate at 2.4 kg ai/ ha using small-plot spray equipment two weeks prior to seedbed preparation. All plots were scalped following herbicide application and subsequent desiccation of bermudagrass.

Seedbed preparation treatments consisted of verticutting in two directions, verticutting plus topdressing (0.6-cm layer), verticutting plus topdressing plus aeration (hollow-tine), or no seedbed preparation. 'Texoka' buffalograss was examined at seeding rates of 101 or 146 kg/ha and was planted on June 17, 2008. A starter fertilizer was applied at seeding and all plots were lightly brushed to ensure good seed-to-soil contact. Plots were irrigated daily by an automated irrigation system that applied approximately 3.8 cm of water per week. Plots were mowed once a week to a height of 5.1 cm with a rotary mower.

Treatments were arranged in a randomized complete block design with four replications of treatments.

Buffalograss conversion was visually evaluated weekly for the first 2 months and monthly thereafter using a scale of 0 (no cover) to 100% (complete cover). Buffalograss turfgrass quality was assessed on a similar time frame using a scale of 0 to 9, where 9 is considered to be optimal turf quality and 6.5 the minimum acceptable level.

Buffalograss germination was minimal regardless of treatment and competition from bermudagrass regrowth became evident one

month after seeding. Bermudagrass had recovered fully from glyphosate applications by the conclusion of the trial (October 15, 2008) and occupied a majority of each plot. Differences in buffalograss establishment did not exist between the two seeding rates regardless of seedbed preparation treatment. However, verticutting, aeration, and topdressing treatments increased buffalograss cover by 10% 2 months after seeding compared to no seedbed preparation.

Percent buffalograss cover never exceeded 15% regardless of seedbed preparation or seeding rate treatments at the conclusion of the trial. Due to low buffalograss conversion, turfgrass quality ratings were not taken. Further research is necessary to identify chemical treatments to reduce the regrowth potential of bermudagrass and increase the establishment of buffalograss from seed.

Summary Points

- Bermudagrass was difficult to control chemically and grew back from glyphosate treatments 3 to 4 weeks after buffalograss seeding.
- Bermudagrass germination was minimal regardless of treatment and competition from bermudagrass regrowth became evident one month after seeding.
- Differences in buffalograss establishment did not exist between the two seeding rates regardless of seedbed preparation treatment.
- Verticutting, aeration, and topdressing treatments increased buffalograss cover by 10% 2 months after seeding compared to no seedbed preparation.
- Percent buffalograss cover never exceeded 15% regardless of seedbed preparation or seeding rate treatments at the conclusion of the trial (October 15, 2008).
- Due to low buffalograss conversion, turfgrass quality ratings were not taken.



Seedbed preparation treatments consisted of verticutting in two directions, verticutting plus topdressing (0.6-cm layer), verticutting plus topdressing plus aeration (hollow-tine), or no seedbed preparation.

Comparison of Chlorothalonil, Propiconazole, and Iprodione Products for Control of Dollar Spot and Brown Patch Diseases

Peter Landschoot and Michael Fidanza
Pennsylvania State University

Objectives:

1. To determine if different fungicide products containing chlorothalonil, propiconazole, or iprodione provide differences in control of dollar spot and brown patch diseases of bentgrass maintained as a golf course fairway.

Start Date: 2008

Project Duration: two years

Total Funding: \$6,000

Chlorothalonil, propiconazole, and iprodione are among the most popular and effective fungicides for controlling foliar diseases of turfgrasses. Three fungicide trials were conducted on bentgrass maintained as a golf course fairway at two different locations in Pennsylvania (a golf course in the southeastern portion of the state and a research facility in central Pennsylvania). Treatments included three or four different products applied at the same rate and timing for each active ingredient (chlorothalonil, propiconazole, and iprodione).

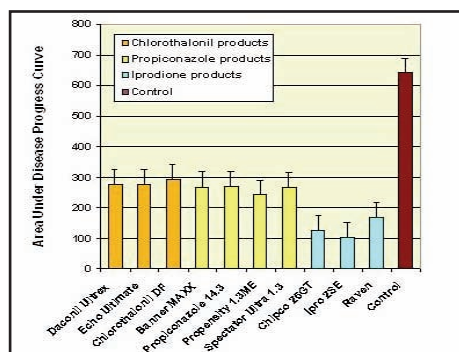
Results show differences in disease control among active ingredients in two of the three trials and a few differences among products with the same active ingredient in all three trials. At the southeastern Pennsylvania site, all fungicide treatments provided good to excellent control of dollar spot. No differences in dollar spot incidence were observed between chlorothalonil and propiconazole fungicides, or among the three chlorothalonil products (Daconil Ultrex, Echo Ultimate, and Chlorothalonil DF), or the four propiconazole products (Banner MAXX, Propiconazole 14.3, Propensity 1.3ME, and Spectator Ultra 1.3).

Two of the three iprodione products (Chipco 26GT and Ipro 2SE) performed similarly to the chlorothalonil and propiconazole products with respect to dollar spot control (no differences occurring on any rating date). Only one iprodione product, Raven, showed reduced efficacy compared to Chipco 26GT and Ipro 2SE on two of the seven rating dates.

Area under the disease progress curve (AUDPC) is a calculated value used to assess disease epidemics for an entire test period. The only fungicide treatment with a higher AUDPC value (than all oth-

ers) was the Raven treatment.

Two tests were conducted at the central Pennsylvania site. Differences in dollar spot severity were observed among chlorothalonil, propiconazole, and iprodione treatments, with iprodione generally showing better control than chlorothalonil and propiconazole over the test period. No differences in control were observed among the three chlorothalonil products (Daconil Ultrex, Echo Ultimate, and



Comparative area under the disease progress curve (AUDPC) to control dollar spot for various fungicides

Chlorothalonil DF) or the four propiconazole products (Banner MAXX, Propiconazole 14.3, Propensity 1.3ME, and Spectator Ultra 1.3).

As in the southeastern Pennsylvania trial, two of the three iprodione products (Chipco 26GT and Ipro 2SE) performed similarly with respect to dollar spot control. However, Raven showed reduced dollar spot control compared to Chipco 26GT and Ipro 2SE on three of the nine rating dates. Area under the disease progress curve values indicated the best dollar spot control was achieved with the iprodione products, followed by propiconazole and chlorothalonil products.

In the colonial bentgrass trial, all fungicide treatments were applied in June prior to brown patch symptom development. By mid-summer, differences in brown patch severity were observed among chlorothalonil, propiconazole, and iprodione fungicides, with chlorothalonil and iprodione generally showing better

control than propiconazole. No differences in brown patch control were observed among the three chlorothalonil products (Daconil Ultrex, Echo Ultimate, and Chlorothalonil DF) or three iprodione products (Chipco 26GT, Ipro 2SE, and Raven) on any rating date or among AUDPC values. Area under the disease progress curve values indicate the best brown patch control was achieved with the chlorothalonil and iprodione treatments, followed by propiconazole treatments in 2008.

Summary Points

- With respect to dollar spot control, iprodione products generally showed better control than chlorothalonil and propiconazole products at the central Pennsylvania location. However, in the southeastern Pennsylvania trial, the three different active ingredients were very similar in controlling dollar spot.
- Generally, chlorothalonil and iprodione products provided better brown patch control than propiconazole products.
- All chlorothalonil products included in this study (Daconil Ultrex, Echo Ultimate, and Chlorothalonil DF) performed similarly in both dollar spot trials and the brown patch trial.
- No differences among propiconazole-containing products (Banner MAXX, Propiconazole 14.3, Propensity 1.3ME, and Spectator Ultra 1.3) were observed with respect to dollar spot control in either dollar spot trial. However, AUDPC values indicated better brown patch efficacy for Propensity 1.3ME compared with Spectator Ultra 1.3 when evaluated over the entire 2008 test period.
- Of the three iprodione products (Chipco 26GT, Ipro 2SE, and Raven) Chipco 26GT and Ipro 2SE provided the most consistent dollar spot control. Raven showed less dollar spot control compared with Chipco 26GT and Ipro 2SE on several rating dates and in AUDPC values in both trials.

Identification, Pathogenicity, and Control of Leaf and Sheath Blight of Bermudagrass Putting Greens

Bruce Martin and Dara Park
Clemson University

Objectives:

1. Collect isolates of *R. zeae* from symptomatic bermudagrass and complete Koch's postulates in greenhouse tests.
2. Determine the influence of Primo and thiophanate-methyl and Heritage treatments on disease severity.
3. Determine the effects of various rates of N and K on disease severity and control.

Start Date: 2007

Project Duration: two years

Total Funding: \$6,000

In recent years there has been an increasing frequency of occurrence of 'leaf and sheath blight' caused by *Rhizoctonia zeae* (or related fungi) on bermudagrass putting greens. In South Carolina, *R. zeae* is a well documented pathogen of creeping bentgrass putting greens, causing brown-patch like symptoms in the heat of summer. The fungus has been identified and pathogenicity documented as well on St. Augustinegrass, centipedegrass, and seashore paspalum. In all cases, the disease has not been controlled with benzimidazole fungicides, as *R. zeae* is essentially immune to that chemistry.

Recent outbreaks have occurred on all of the common ultradwarf bermudagrass cultivars on putting greens ('TifEagle', 'Champion', and 'MiniVerde') as well as 'TifDwarf' and even 'TifGreen' in the southeastern United States. Generally, the disease is first noticed in late August to early fall months. Initial symptoms are bronze patches of a few cm up to 100 or more cm in diameter. If not controlled quickly with fungicides, the pathogen blights and bleaches the lower leaves that results in a persistent distinct patch symptom. Disease has been



Generally, the disease is first noticed in late August to early fall months. Initial symptoms are bronze patches of a few cm up to 100 or more cm in diameter.

Fungicide	Exp No.	Rate	Spray Dates	Disease Severity (%)
Heritage TL	1	1.0 fl oz/1000 ft ²	July 15, 29 and August 12	66.8 a
Endorse	1	4 oz/1000 ft ²	July 15, 29 and August 12	62.8 a
Tartan	1	2 fl oz/1000 ft ²	July 15, 29 and August 12	51.8 ab
Trinity	2	2 fl oz/1000 ft ²	July 15, 29 and August 12	35 b
DisArm	1	0.27 fl oz/1000 ft ²	July 15, 29 and August 12	55.8 ab
Banner Maxx 1.3 ME	1	1 fl oz/1000 ft ²	July 15, 29 and August 12	45.5 ab
Insignia	1	0.9 oz/1000 ft ²	July 15, 29 and August 12	60.5 ab
Fore + Chipco Signature	1	8 + 4 oz/1000 ft ²	July 15, 29 and August 12	59.5 ab
Untreated	1			61.3 a

Table 1. Single component fungicides, rates and timings for early curative control of *Rhizoctonia* leaf and sheath spot in bermudagrass putting greens.

known to recur in spring months after turf emerges from dormancy and presumably can detrimentally affect spring transition in overseeded systems.

In several instances, stress induced by aggressive verticutting has induced severe outbreaks of persistent disease symptoms. Low nutrition or reduced recuperative potential by any of the reasons outlined would be expected to increase disease severity. In the summer and fall of 2008, the disease again occurred on several golf courses in South Carolina and throughout portions of the southeastern United States. Cultivars were 'TifEagle', 'Champion', 'TifDwarf', 'MiniVerde', and 'TifGreen'.

Identification of *R. zeae* has been based solely on cultural characteristics, based primarily on sclerotia size and abundance. Recently, however, molecular methods have been used to distinguish among *R. zeae*, *R. oryzae*, and *R. circinata*. Cultures have been recovered from bermuda patches that resemble (culturally) all three of these fungi. Molecular identification of these isolates will be conducted during the winter of 2008/09.

In 2007, three trials were conducted but went out curatively after symptoms became well established. Trials were placed in late September (Experiment 1) and late October (Experiment 2) when symptoms were severe and day length and temperatures were not favorable for bermudagrass growth. In 2008, trials were conducted on a chipping green in

Columbia, SC and a putting green near Florence. Intentions were to initiate the trials in July prior to symptom expression, but symptoms already were present when the trials were established.

Only Heritage gave some control in the single component fungicide trial. In another experiment, however, we looked at a 'program' approach of rotation of fungicides and fungicide mixtures with one set of treatments watered-in before sprays dried, and the other set left on the leaf. We did observe that watering-in the fungicides made a positive difference, and these trials gave us some evidence that preventive treatments have to be initiated much earlier than we first supposed.

Summary Points

- Leaf and sheath blight caused by *Rhizoctonia zeae*, and related fungi, on bermudagrass putting greens has not been controlled with benzimidazole fungicides.
- Molecular methods have been used to distinguish among *R. zeae*, *R. oryzae*, and *R. circinata* and will be conducted during the winter of 2008.
- Fungicide trials were conducted in 2007 and 2008. Only Heritage gave some control in the single component fungicide trial.
- Watering-in fungicides made a positive difference and suggested preventive treatments have to be initiated much earlier than first supposed.

Nitrogen Nutrition of *Distichlis* (Saltgrass) under Normal and Salinity Stress Conditions Using ^{15}N

Mohammad Pessaraki
University of Arizona

Objectives:

1. To determine nitrogen uptake of saltgrass under salt stress conditions using ^{15}N .

Start Date: 2008

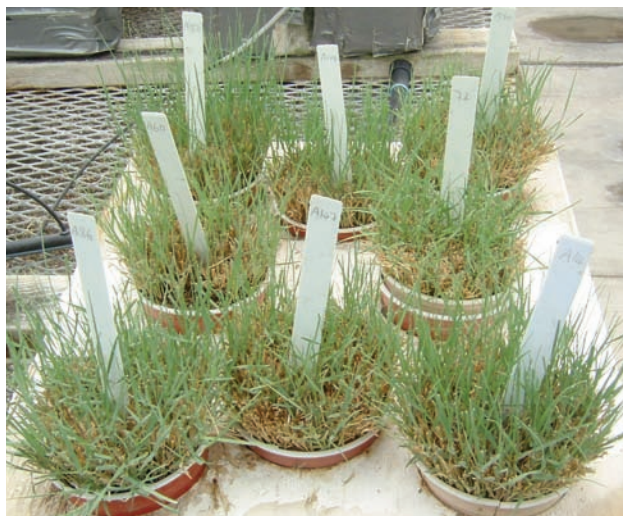
Project Duration: two years

Total Funding: \$6,000

Saltgrass (*Distichlis spicata*) is a warm-season potential turfgrass species that has the ability to grow under highly saline, poor soil conditions, and with limited available water and nutrient (i.e. nitrogen) sources. This characteristic could prove to be beneficial in certain turfgrass areas requiring low maintenance in arid regions with saline soils and limited water or low fertility/limited nutrient contents and poor soil conditions. Growth of this species could be important to aid in conservation of water and nutrients and use of salt-affected soils. Test of the species' ability to grow under highly saline conditions and limited nutrients, as well as low available water sources, are needed before it can be applied to a turfgrass system.

Twelve saltgrass genotypes (A37, A49, A50, A60, 72, A86, A107, A126, A136, A138, 239 and 240) collected from several southwestern states of the United States were studied in a greenhouse to evaluate their growth in terms of shoot and root lengths and dry matter (DM) weights (shoot clipping weight) and nitrogen (^{15}N) uptake under salt stress condition. The grasses were grown under saline (NaCl) conditions at EC of 20 dSm^{-1} using a hydroponic technique using Hoagland solution. A randomized complete block (RCB) design trial was used with four replications of each genotype and each treatment. Nitrogen (^{15}N) was used to determine the exact amount of N taken up by the plants.

Each week, the plant shoots (clippings) were harvested for the evaluation of the dry matter (DM) production. At each harvest, both the shoot and root lengths were measured and recorded. The harvested plant materials were oven-dried at 60°C and DM weights were measured and recorded. Six harvests were included in the experiment. The ^{15}N samples for only one



Grasses were grown under saline (NaCl) conditions at EC of 20 dSm^{-1} using a hydroponic technique using Hoagland solution.

harvest were analyzed. At the termination of the experiment, plant roots were also harvested, oven-dried at 60°C and DM weights were measured and recorded.

The results of the average shoot length per week showed a decrease in all varieties of plants grown under saline condition compared to the control. The root length for the duration of the experiment showed increased root growth of eight of the twelve varieties grown under saline condition when compared to the control. These eight varieties include A60, A86, A107, A126, A136, A138, 239 and 240.

All varieties, except A86, produced a higher weekly average fresh weight for control plants than those grown under saline conditions. The fresh weight of the roots showed an increase in six of ten of the varieties (A49, A86, A107, A126, A136 and 240) for the plants grown under saline conditions. Two of the varieties, A138 and 239, did not produce recordable root fresh weight.

The grass DM weight showed an increase in ten of the twelve varieties (A37, A50, A60, 72, A107, A126, A136, A138, 239, 240) and in the control plants in comparison to those grown under saline conditions. However, the percent DM weight of the shoots (average dry weight/average fresh weight) showed an

increase in nine out of the twelve varieties (A49, A50, A60, 72, A86, A107, A126, 239 and 240) in the plants grown under saline conditions compared to the control, with variety A136 being equal under the both salt conditions.

Root DM weight showed an increase in eight of the ten varieties (A49, A50, 72, A86, A107, A126, A136 and 240) grown under saline conditions. The percent root DM (average dry weight/average fresh weight) showed

increased DM production in seven of the ten varieties (A37, A50, A60, 72, A86, A126 and 240) grown under saline conditions.

The partial results of the ^{15}N analysis show a substantial increase in nitrogen concentration of the plant tissues. This indicates that saltgrass is a true halophyte and accumulates the extra N in its tissues which can be gradually available to the growing parts of the grass.

Summary Points

- The results of this experiment confirmed that saltgrass has a high salt tolerance and is a true halophyte.
- Root length, root fresh wt., root DM wt., percent shoot, and root DM wts. in plants grown under saline condition increased.
- The amount of DM produced is the most significant result as it is a direct representation of saltgrass' ability to grow under highly saline conditions.
- Based on the results of this experiment, each of the tested varieties had a high degree of salt tolerance.
- Based on the results of the ^{15}N analysis, all the clones accumulated significant amounts of nitrogen in their tissues under salinity stress condition.

Leaf Cuticle Characteristics and Foliar Nutrient Uptake by a Cool-season and Warm-season Turfgrass

Michael D. Richardson, James C. Stiegler, Douglas E. Karcher, and Aaron J. Patton
University of Arkansas

Objectives:

1. Determine the seasonal dynamics of the turfgrass cuticle in a cool-season and warm-season turfgrass species managed under putting green conditions.
2. Compare the seasonal uptake of foliar nitrogen by a cool-season and warm-season turfgrass species managed under putting green conditions.

Start Date: 2008

Project Duration: two years

Total Funding: \$6,000

Foliar fertilization refers to the process of nutrient uptake through the foliage or other aerial plant parts. As a supplement to traditional root-feeding programs, foliar fertilization has been observed to be an increasingly common practice in today's golf course management. Recent surveys of Arkansas golf course superintendents indicate that nearly all golf course superintendents use foliar fertilization on some area of their golf course, and this method of nutrient application often comprises a major portion of annual nitrogen (N) inputs to putting greens.

Although previous research has documented uptake of N by turfgrass leaves in controlled-environment studies, there have been no studies which demonstrate its potential effectiveness in a field environment. It is known from previous agricultural research that environmental factors and seasonal dynamics of leaf cuticle characteristics can influence the foliar absorption of N solutions. Therefore, the aim of this project is to increase scientific understanding of the turfgrass leaf cuticle, while assessing foliar uptake of N during a two-year putting green research trial.

Experimental areas of 'Penn A1'



Golf course superintendents often use foliar fertilization as a primary means of feeding putting greens.



Foliar nitrogen is applied to the putting green turf and an ammonia volatilization trap is installed after application.

creeping bentgrass and 'Tifeagle' ultra-dwarf bermudagrass were developed on an established sand-based putting green in Fayetteville, Arkansas. The greens were maintained according to typical management practices for the region. Foliar uptake of N was studied each month from May to September to determine if foliar uptake was consistent across the growing season.

An isotopic tracer technique that allows for positive identification and direct measurement of fertilizer N in the plant tissue was used in the study. ¹⁵N-labeled urea solutions were sprayed at two different rates to represent a low and high rate (0.10 or 0.25 lb. N / 1000 ft²) common to foliar fertilization rates of golf course superintendents. For a 24-hour period after treatment, plots received no irrigation or rainfall in order to track only foliar absorption of N. Plant tissues were sampled at 1, 4, 8, and 24 hours after application in order to develop a time-course analysis of foliar N uptake. In addition, an enclosed chamber with an internal acid trap was installed within the plots after treatment to estimate the volatilization of N as ammonia from the plots.

Results from the first year of monthly foliar urea-N applications indicate that both species proved receptive to foliar uptake and ammonia volatilization losses were minimal (averaged < 2% of applied N). A range of 24-57% of the fertilizer N applied was recovered in leaves/shoots at 1 hour after treatment, while peak foliar

absorption was normally observed around 4 hours after treatment.

Foliar uptake (measured as a % of N applied) was significantly reduced at higher application rates. The highest maximum absorption of N applied observed over 24 hours, was in the month of May on bentgrass (76%). Absorption of N by bentgrass leaves was affected by month of year, while ultradwarf bermudagrass was not affected.

On bentgrass, the significant decrease in N recovered within plant tissue as the season progressed (May averaged 59% of applied N across all sampling times, while September averaged 37%), is currently believed to be attributed to leaf cuticle changes that made leaves more hydrophobic and possibly less receptive to nutrient absorption. Continued laboratory investigations are underway to better understand this observed trend.

Summary Points

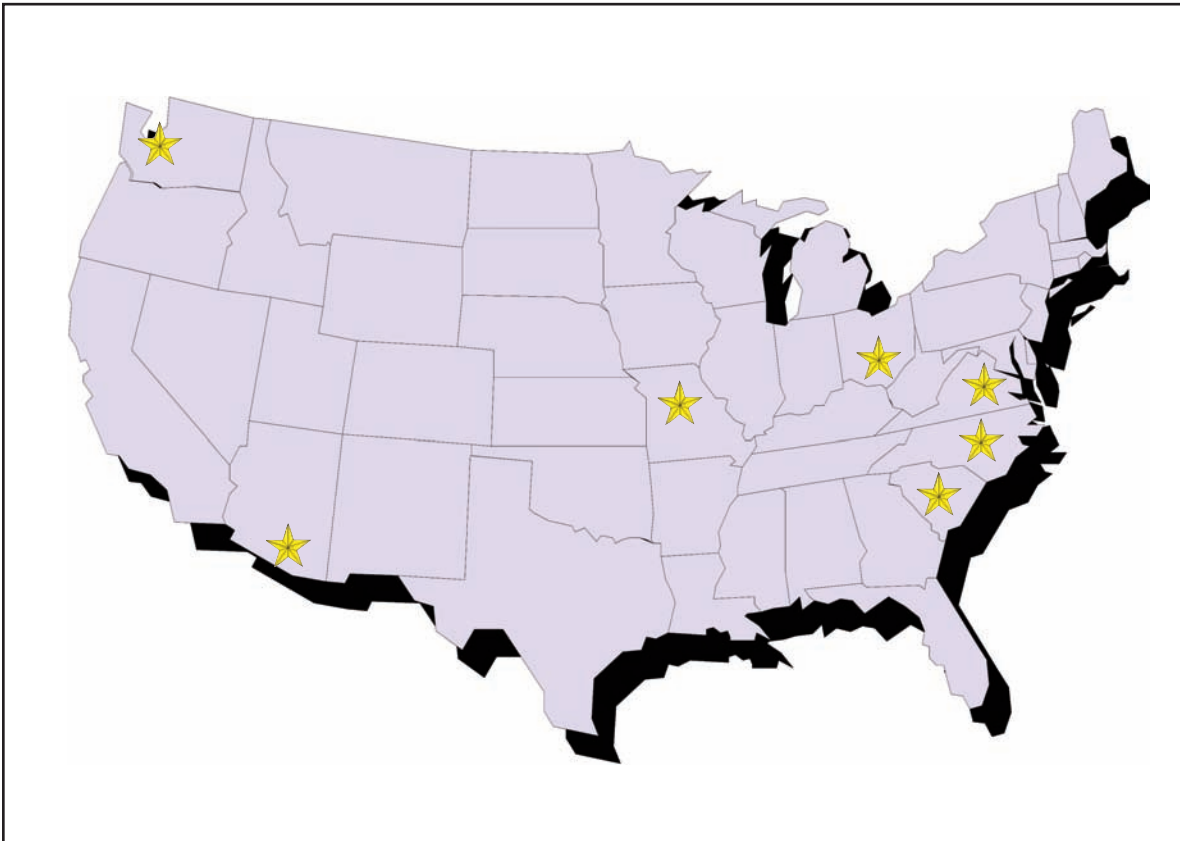
- Both creeping bentgrass and ultradwarf bermudagrass greens are receptive to foliar uptake of urea nitrogen.
- Most of the nitrogen applied to putting green turfgrass foliage is absorbed in the first 4 hours after application.
- Foliar uptake in creeping bentgrass was reduced during warmer months, suggesting a change in the composition of the leaf cuticle.
- Loss of foliar-applied nitrogen to ammonia volatilization appears to be minimal.

Wildlife Links Program

Golf courses offer excellent opportunities to provide important wildlife habitat in urban areas. With more than 17,000 golf courses in the United States comprising in excess of two million acres, great potential exists for golf courses to become an important part of the conservation landscape.

Wildlife Links is a cooperative program with the National Fish and Wildlife Foundation that funds innovative research, management, and education projects that help golf courses become an important part of the conservation landscape. The United States Golf Association is providing through the Wildlife Links Program which began in 1996. The objectives of Wildlife Links are to:

1. Facilitate research on wildlife issues of importance to the golf industry.
2. Provide scientifically credible information on wildlife management to the golf industry.
3. Develop wildlife conservation education materials for the golf industry and golfers.
4. Implement wildlife monitoring programs that will improve management on golf courses.



Location of the following project funded in 2008 through the Wildlife Links Program administered by the National Fish and Wildlife Federation

Reproductive Success and Habitat Use of Painted Buntings on Golf Courses in Coastal South Carolina

Patrick G.R. Jodice, Mark Freeman, and Jessica Gorzo
Clemson University

Chris Marsh
Low Country Institute

Objectives:

1. Locate nests of Painted Buntings on study courses and relate various metrics of reproductive success (clutch size, daily nest survival, hatching success, brood size at fledging) to characteristics of nesting habitat at the nest, patch, and territory scale .
2. Locate fledgling groups of Painted Buntings on study courses and determine home ranges and habitat use.
3. Determine use of grass, out of play areas for foraging by Painted Buntings and compare its availability among courses.
4. Compare reproductive success and nesting habitat to previously measured values in the southeastern US.

Start Date: 2008

Project Duration: three years

Total Funding: \$60,000

The eastern population of the Painted Bunting (*Passerina ciris*) breeds in a restricted range of the Atlantic Coastal Plain, from North Carolina to Florida. The Painted Bunting is considered a species at risk by various conservation organizations due in part to its restricted distribution and low population size.

Within the coastal counties of South Carolina, golf courses and golf course communities are common and becoming more prevalent. Recent research funded by the Wildlife Links Program showed that while Painted Buntings were found on numerous courses in Horry County, SC, they did not occur on courses with highly altered landscapes.

We are assessing the conservation value of golf courses in the Beaufort County area of SC for Painted Buntings. The Beaufort County region represents an area of high risk for the species due to ongoing development, but also potentially high conservation reward. If courses in this area can provide habitat that will support successful breeding populations of Painted Buntings, then a substantial portion of the breeding range can be managed in such a way as to take advantage of the extensive private land holdings in the region.

A pilot season of field research was initiated in May 2008. Searching for Painted Bunting nests is a pain-staking and tedious endeavor, and, for that reason, we chose to focus our efforts on only two golf courses. The field research team searched the Chechessee Creek Club and Old Tabby Links on Spring Island in Beaufort County, South Carolina, throughout May, June, and July 2008.



By locating individual buntings over the course of several days and carefully plotted their locations and activities, a home range map and a better understanding of the habitats being used during the nesting period was achieved.

Researchers located individual buntings during the day and over the course of several days and carefully plotted their locations and activities. This technique often resulted not only in the location of a nest, but also in a home range map and a better understanding of the habitats being used during the nesting period. Locations were plotted with a handheld GPS and developed into a GIS data layer. This data layer can be overlaid with GIS coverage of the courses to examine habitat use at the nest, patch, and territory scale.

Using the territory-mapping approach, we located 14 nests of Painted Bunting on the two study courses. Five nests were located on the Old Tabby Links on Spring Island. Three of these nests were located in wax myrtle bushes, while the other two nests were located in thickets of mixed shrubs. Nine nests were located at the Chechessee Creek Club. Four of these were located in wax myrtles, while the remaining nests were located in saplings or large trees.

We also were able to locate and

track six groups of Painted Bunting fledglings on Spring Island and eight groups on Chechessee Creek. These data will be used to develop habitat use models for fledglings and hence improve our ability to develop habitat management guidelines.

Field research will be renewed in May of 2009. We will focus our efforts on the same two courses and begin mapping territories as soon as possible during the breeding season. An early start to territory mapping will improve our ability to find nests and track fledgling groups. During the 2009 breeding season, we also will begin to investigate the value of mowed versus unmowed grass plots for Painted Bunting foraging activity.

Summary Points

- Searches for nests of Painted Buntings were conducted on the Chechessee Creek Club and Old Tabby Links in Beaufort County, South Carolina, from May - July 2008. Areas searched included roughs and shrub/scrub habitat throughout the courses.

- We located 14 nests on the two courses. Most nests were found in wax myrtles or shrub/scrub habitat, although a few nests also were located in large hardwood trees. We also located and mapped the habitat use of 14 groups of Painted Bunting fledglings. Data from both the nest locations and fledgling groups are being incorporated into a GIS data layer that will be used to assess habitat use.

- Data collection in 2009 will focus on additional nest searching and territory mapping and may use the assistance of volunteers in an effort to increase the sample size of nests and territories that can be monitored. We also will initiate research to examine the use of mowed versus unmowed grass plots as feeding areas for Painted Buntings.

Abundance and Diversity of Stream Salamanders on Golf Courses

Raymond D. Semlitsch

University of Missouri

Objectives:

1. Measure stream salamander abundance and diversity on 10 golf courses in western North Carolina to make biologically relevant management suggestions to improve the quality of golf courses for amphibian biodiversity.

Start Date: 2008

Project Duration: two years

Total Funding: \$65,800

The southern Appalachian Mountain region harbors an exceptional amphibian diversity which is dominated by salamanders. These salamanders are integral ecological components of the headwater ecosystems they inhabit where they often account for the majority of vertebrate biomass. The majority of these salamanders are found in close association with headwater stream habitats, forming communities that are comprised of five to nine species from the genera *Desmognathus*, *Eurycea*, *Gyrinophilus*, and *Pseudotriton*. These salamanders are stream salamanders of the family Plethodontidae and all have biphasic life cycles consisting of an aquatic larval stage that is followed by a terrestrial adult stage.

Stream salamanders are dependent upon both the stream and the surrounding riparian habitat for foraging, breeding, and dispersal. Though less conspicuous and least studied, the larval stage is essential to the persistence of or the reestablishment of adult salamanders in the surrounding riparian habitat.

Managing landscapes with an eye for both human use and preservation of biodiversity can create a win-win situation for stakeholders and wildlife. Considering that the average golf course consist of more than 150 acres of green space (70% is rough, non-play areas) and there are



Salamanders are integral ecological components of the headwater ecosystems they inhabit.

more than 17,000 golf courses in the U.S. that total over 2.2 million acres, we suggest there is great potential for golf courses to serve as sanctuaries for many wildlife species if the habitat needs of species are present.

Our project will measure stream salamander abundance and diversity in order to make biologically relevant management suggestions to improve the quality of golf courses for stream salamanders. We will sample 10 golf courses in the montane region of the southern Appalachians within a 25-mile radius of Highlands, NC to compare to adjacent (upstream, downstream) control areas and to previous work we have conducted on national forest land.

During the 2008 summer field



Stream salamanders are dependent upon both the stream and the surrounding riparian habitat for foraging, breeding, and dispersal.

season, Mark Mackey (M.S. Graduate Student) and Grant Connette (Field Research Technician) were able to establish contact and participation agreements with 10 golf courses in the Highlands, NC area. They were also able to delineate streams on each course to establish six 25-meter transects (2 upstream, 2 in-course, 2 down stream) for intensive sampling during 2009. Further, they conducted a pilot project to determine the effectiveness of sampling protocols and leaf-bag sampling in different habitat types.

They found that leaf bags were highly effective for larval salamanders and that the four primary species found in



Four primary species of salamanders found in national forest streams were also present in some streams on golf courses.

national forest streams were also present in some streams on golf courses. Intensive sampling of larval and adult salamanders on all 10 golf courses will begin next year in May through August 2009.

By comparing the abundance and diversity of larval salamanders in streams and adult salamanders in the adjacent terrestrial habitat on golf courses relative to upstream and downstream areas, we will be able to assess the adequacy of current course management. We will then develop recommendations on the management of in-stream and riparian habitats on each golf course to maintain or improve habitat for salamanders.

Summary Points

- Agreements of participation were obtained for 10 different golf courses in the Highlands-Cashiers area of North Carolina.
- Six different 25-meter stream transects on each course were delineated for intensive sampling and comparison, yielding a total of 60 different stream segments to be sampled across all 10 golf courses.
- Preliminary sampling of larval salamanders was conducted to obtain the best sampling methods and a preview of species' presence on golf courses.
- A pilot project was conducted examining the efficiency of the leaf-litter bag technique as a method for sampling larval salamanders in different stream habitats.

Brown-headed Nuthatch Enhancement Study

Mark Stanback
Davidson College

Objectives:

1. Assess the importance of pine density and competition with eastern bluebirds on the spatial distribution of brown-headed nuthatch nests.
2. Assess the numerical response of brown-headed nuthatches to the experimental exclusion of eastern bluebirds.
3. Assess the ability of eastern bluebirds to usurp nest sites from brown-headed nuthatches.
4. Provide golf course managers in the Southeast with recommendations to increase the numbers of brown-headed nuthatches on their golf courses.

Start Date: 2007

Project Duration: two years

Total Funding: \$7,400

The brown-headed nuthatch (*Sitta pusilla*) is a cooperatively breeding bird endemic to the southeastern United States. But for nearly half a century its numbers have been in decline. Habitat degradation is usually blamed. Brown-headed nuthatches are said to be habitat specialists - dependent on old growth pine forests. As development overtakes more of the Southeast, there are fewer old pine stands usable by these birds.

Another cavity-nesting species has increased dramatically in number in the same region - the Eastern Bluebird (*Sialia sialis*). Bluebirds tend to fare well in a variety of human-altered habitats. Moreover, bluebirds have been the beneficiaries of nest box programs throughout their range. We hypothesized that the burgeoning bluebird population in the Southeast is negatively impacting brown-headed nuthatches.

My students and I have been monitoring nest boxes on six golf courses near Davidson, North Carolina since 2001. For each nest box, we measured the distance to the nearest three pine trees (*Pinus echinata*). The distance to the third was our measure of pine density. Boxes for



1.5" entrance holes (left) accommodate both bluebirds and nuthatches; smaller holes (right) exclude the larger bluebirds.

which the third closest pine was less than 50 meters away were considered to be in "pine-rich" habitat; boxes for which the third closest pine was > 50 meters away were considered to be in "pine-poor" habitat.

Standard 1.5" entrance holes accommodate both bluebirds and nuthatches; 1.25" holes accommodate nuthatches, but are too small for bluebirds. We randomly assigned boxes on three golf courses to the 1.25" treatment. Pine density had no significant effect on nesting by nuthatches, contrary to the predictions of the conventional wisdom. Hole size, however, had a highly significant effect on nuthatch settlement: nuthatches settle where competition with bluebirds is minimized, regardless of local pine density.

We recorded the number of nuthatch nests on three courses with a subset of 1.25" entrance holes vs. the number on three other courses on which all boxes had standard "bluebird-friendly" 1.5" entrance holes. We monitored boxes in 2004 (prior to the addition of "nuthatch-friendly" holes on our experimental courses) to ensure that all six courses were similar in their lack of nuthatches. For the 2005, 2006, and 2007 breeding season, we monitored the numbers of nuthatch nests on both experimental and control courses. Numbers of nuthatches increased in each year of the study, suggesting that bluebirds competitively exclude nuthatches from available habitat.

These results clearly demonstrate that nuthatches flourish only where bluebirds are excluded. Prior to the 2008 breeding season, we reversed the treatments on our six golf courses: our three "bluebird-friendly" courses became "nuthatch-friendly" and vice-versa. Of the 32 boxes containing nuthatch nests in 2007, 31 contained bluebird nests in 2008.

The competitive superiority of bluebirds is unquestionable.



Brown-headed nuthatches breed in cooperative groups in the southeastern US. (photo by Austin Mercadante)

Summary Points

- Brown-headed nuthatch numbers have declined throughout the Southeast. Their supposed dependence on old growth pine forests - and susceptibility to habitat alteration - is usually blamed for this decline. We offer an alternative hypothesis - that nest site competition with a burgeoning eastern bluebird population is responsible.
- We monitored nest box use by bluebirds and nuthatches on golf courses and found that pine density had little effect on nest box use by nuthatches. Instead, the exclusion of bluebirds (via smaller entrance holes) was the best predictor of nest box occupation by nuthatches.
- Brown-headed nuthatch numbers increased dramatically on three golf courses where bluebirds were excluded from one-third of nest boxes. Control courses had few, if any, breeding nuthatches over the same period.
- When bluebird-friendly holes were returned to experimental boxes, bluebirds quickly evicted resident nuthatches.
- To prevent monopolization of nest boxes by bluebirds, golf courses in the Southeast should provide smaller entrance holes on a subset of their nest boxes.

Avian Pesticide Exposure on Golf Courses

Daniel A. Cristol and Ryan B. Burdge

The College of William and Mary

Objectives:

1. To determine the extent of pesticide exposure in nestling bluebirds and to determine if these insectivorous songbirds are able to obtain sufficient invertebrate food on golf courses treated with insecticides.

Start Date: 2007

Project Duration: one year

Total Funding: \$27,500

Several studies have illustrated the abundance and diversity of birds that can be found at golf courses, but less is known about nesting success. In addition, the chemicals often employed by golf course managers are known to negatively affect wildlife, including reproductive and behavioral impairments, yet the extent of avian exposure on golf courses remains largely unknown.

Studies indicate that eastern bluebirds can breed successfully in nest boxes installed on golf courses, but there is also evidence that the nestling birds weigh less than same-aged counterparts at suburban sites with no pesticide inputs. Our research objectives were to determine the extent of pesticide exposure in nestling bluebirds and to determine if these insectivorous songbirds are able to obtain sufficient invertebrate food on golf courses treated with insecticides.

Experimental design consisted of monitoring and comparing breeding activity in more than 500 nest boxes at 8 golf courses and 7 non-golf course reference sites. Our reference sites consisted of suburban locales with some level of human activity (e.g. local parks), but which were confirmed to not apply pesticides. Participating golf courses shared their



During the 2007 and 2008 breeding seasons, we collected and analyzed 290 blood samples from nestling birds.

detailed chemical application logs, allowing us to know specifically which type, where, and when pesticides were applied.

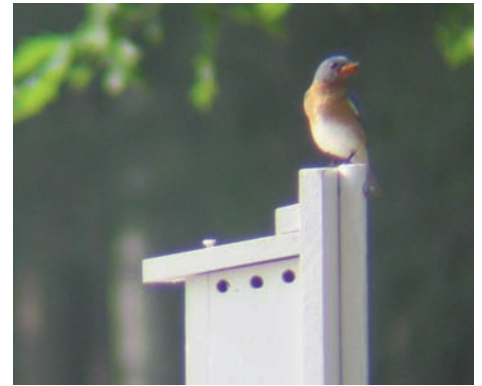
During the 2007 and 2008 breeding seasons, we collected and analyzed 290 blood samples from nestling birds to assess cholinesterase enzyme activities, the standard method for determining exposure to many common insecticides. All nestlings were measured and banded during sampling efforts. We collected more than 450 prey items directly from nests through use of esophageal ligatures and an additional 20 insects were collected from turf areas 24 hours after insecticide application. These 20 insects and 30 insects from nests after pesticide applications were analyzed for pesticide residues.



Physical measurements of nestlings revealed that, as previously reported, golf course chicks weighed less than birds on reference sites of the same developmental age.

Lab analyses found no evidence of enzyme inhibition in golf course nestlings, suggesting they had not been exposed to enough pesticide to detect any direct effects. No pesticide residues were detected in 50 prey items insects. Nestling diet and parental feeding rates were the same at golf courses and reference sites. However, physical measurements of nestlings revealed that, as previously reported, golf course chicks weighed less than birds on reference sites of the same developmental age.

Much of the application of pesti-



Experimental design consisted of monitoring and comparing breeding activity in more than 500 nest boxes at 8 golf courses and 7 non-golf course reference sites.

cides occurred after nestlings fledged from nests. Because the nestlings we sampled appear not to have been exposed to pesticides, additional research efforts are underway to track survival of fledgling bluebirds after they leave the nest.

These young, inexperienced birds may be more likely to be exposed to pesticides through ingestion of dead or moribund insects. If these young birds have a low survival rate, it may be that the successful nesting efforts observed for adult birds overestimate the habitat quality of golf courses. Only by measuring condition and survivorship at every stage of the life cycle can we gain confidence that golf courses are not ecological traps.

Summary Points

- Nestling eastern bluebirds are at low risk of pesticide exposure on golf courses.
- Eastern bluebirds are able to forage comparable type and amount of insect prey on golf courses compared to other suburban environments.
- Nestling bluebirds tend to be lower weight than reference birds, potentially affecting post-fledging survivorship.
- Continued research will track survival and pesticide exposure in juvenile birds.
- Additional work is needed to assess pesticide exposure and associated effects in other golf course wildlife and at other stages of the life cycle.

Puget Sound Salmon-Safe Golf Course Program

David Burger
Stewardship Partners

Objectives:

1. Conduct independent evaluations of area golf courses based on an adapted version of the Salmon-Safe Parks and Natural Areas Certification Guidelines to determine how management addresses water quality and habitat conservation.
2. Identify habitat restoration opportunities with participating golf courses and connect them with local resources to conduct on-the-ground improvements.
3. Develop an education and outreach campaign to build support for Salmon-Safe golf course management.
4. Test the opportunities for wide-scale implementation of Salmon-Safe golf course certification.
5. Demonstrate leadership within the golf industry in voluntary restoration and conservation efforts to recover native salmon and other fish and wildlife.

Start Date: 2007

Project Duration: one year

Total Funding: \$12,500

Over the past several years, Stewardship Partners has collaborated with the Oregon based Salmon-Safe Inc. to develop a Salmon-Safe certification and labeling program for Puget Sound area farmers and other landowners who adopt fish-friendly farming practices and participate in salmon habitat restoration projects. The project is proving to be successful, with over 40 farms certified to date, in-store retail campaigns with Seattle area grocers, extensive media coverage, and a variety of promotional materials produced for use by farmers in their direct marketing efforts.

As a follow-up to this successful program, we are considering additional opportunities to use the Salmon-Safe label and evaluation tool to advance environmental management practices of other types of landowners and businesses. For example, the Salmon-Safe program has been adapted for municipal park systems as well as corporate and university campuses.



Salmon-Safe guidelines represent an important opportunity because golf courses can have a large environmental impact from use of water, application of chemicals, conversion of wetlands, and alteration of stream and riparian habitat.

As a result of our promotion and outreach in Puget Sound, we have been approached by the Western Washington Golf Course Superintendents Association to explore opportunities to utilize the Salmon-Safe program as an evaluation tool to assess environmental performance and a public education tool to promote exemplary golf course management.

This represents an important opportunity because golf courses, many of which are located along productive stream systems, can have a large environmental impact from use of water, application of chemicals, conversion of wetlands, and alteration of stream and riparian habitat. Golf courses are increasingly being required to address these issues as several species of salmon, such as Puget Sound Chinook, have been listed under the federal Endangered Species Act.

Due to the increases in awareness of these issues among the public and golf course managers themselves, there have been some initial efforts within the industry to improve management practices. For example, the Northwest Golf Course Environmental Alliance is a newly formed effort to pro-actively demonstrate positive trends within the industry through their commitment to a set of Environmental Stewardship Guidelines.

Another industry effort is the Cooperative Sanctuary Program developed by the US Golf Association and Audubon International. Stewardship Partners, in conjunction with Salmon-Safe, Inc. will build on this momentum to establish a meaningful and regionally-based third-party certification program under the auspices of Salmon-Safe. We will adapt the Salmon-Safe Parks and Natural Areas Certification Guidelines to be more specific for golf course management and conduct



Golf courses are increasingly being required to address these issues as several species of salmon, such as Puget Sound Chinook, have been listed under the federal Endangered Species Act.

independent pilot evaluations of three golf courses based on these guidelines. We will also help evaluate restoration opportunities and connect these golf courses with local organizations to conduct on-the-ground restoration work.

We have identified several golf courses which would be good candidates for piloting such a program based on their reputation for environmental performance, participation in habitat restoration along salmon bearing streams, and the interest of the managers. We will pilot the program with these initial candidates to determine the feasibility of more widescale implementation of a Salmon-Safe golf certification program.

Summary Points

- Stewardship Partners, in conjunction with Salmon-Safe, Inc. is establishing a meaningful and regionally-based third-party certification program under the auspices of Salmon-Safe for golf courses in the Pacific Northwest.

- Several golf courses have been identified which would be good candidates for piloting such a program based on their reputation for environmental performance, participation in habitat restoration along salmon bearing streams, and the interest of the managers.

Using Buffer Zones to Promote Amphibian Populations

Michelle D. Boone

Miami University

Objectives:

1. To determine the effects of a 1-meter buffer zone around ponds to bolster cricket frog populations.
2. To determine the effects of the presence or absence of a potential predator (two grass carp, two bluegill sunfish, two crayfish) and to the presence or absence of a sublethal level of the insecticide imidacloprid.

Start Date: 2007

Project Duration: two years

Total Funding: \$46,653

Research was conducted to investigate how the use of buffer zones around water resources could help bolster amphibian populations. We made contact with three golf courses [Twin Run Golf Course (Hamilton, OH), Hueston Woods Golf Course (College Corner, OH), and Oxford Country Club (Oxford, OH)] that each had two ponds: one that they agreed to leave a 1-meter terrestrial grass buffer zone, and another that they mowed to the edge. Additionally, we used two "reference" ponds at the Ecology Research Center (Oxford, OH).

We spent approximately 2 months collecting cricket frog pairs for the golf course and related projects. Eight enclosures were added to each golf course pond. To half of the enclosures 40 cricket frog tadpoles were added, and to the other half green frog tadpoles were added (so that we could examine effects on two species).

Golf courses were checked 2-3 times per week for signs of metamorphosed frogs, and water quality measurements were taken weekly to every other week. The aquatic experiments are still underway and will be terminated by the end of October, 2008.



Cricket frogs were used both at the golf course ponds and reference ponds to test for the effects of imidacloprid.

Cricket frogs that reached metamorphosis were given individual marks and released at the golf course ponds so that subsequent terrestrial survival can be assessed. We will search for these cricket frogs next spring when the breeding season ensues.

In another study, we exposed cricket frog tadpoles and green frog tadpoles reared in cattle tank mesocosm ponds at Miami University's Ecology Research Center (Oxford, OH) to presence or absence of a potential predator (two grass carp, two bluegill sunfish, two crayfish) and to the presence or absence of a



Green frogs were also used to test for the effects presence or absence of a potential predator (two grass carp, two bluegill sunfish, two crayfish) and to the presence or absence of a sublethal level of the insecticide imidacloprid.

sublethal level of the insecticide imidacloprid. This experiment was conducted over the summer and completed at the beginning of September. Currently, we are analyzing the data and preparing the manuscript for publication.

We also conducted a laboratory study with cricket frogs exposed to water from each golf course pond and the presence or absence of a sublethal exposure to the insecticide imidacloprid (which at least one of the golf courses was using). This study allowed us to see how individual tadpoles were affected by the golf course water relative to controls (ponds from the



Eliminating fish from ponds is a critical step because ponds without fish allow for greater abundance of amphibians and more diverse communities.

reference site with aged tap water) in the presence or absence of the insecticide.

This study will give us insight into the effects that we observe in Project 1 located on the golf course. The student collected water from each of the six golf course ponds and one reference site each week, and then raised the tadpoles in this water. In this study, survival was measured daily, and at the end of the study, we determined mass and developmental stage.

Additionally, in a separate study, the student also examined whether the presence of the insecticide and the golf course water affected susceptibility to a crayfish predator. Both studies have been completed and a manuscript is currently being prepared from this work.

Summary Points

- Research is underway to test for the effects of a 1-meter grassy buffer around golf course ponds on the population of cricket frogs.
- A laboratory study was also conducted with cricket frogs exposed to water from each golf course pond and the presence or absence of a sublethal exposure to the insecticide imidacloprid.
- In a separate study, it was also examined whether the presence of the imidacloprid in the golf course water affected susceptibility to a crayfish predator.

Population and Community Responses of Reptiles to Golf Courses

Matt Goode
University of Arizona

Objectives:

1. Examine diversity, distribution, and relative abundance of reptiles living on three golf courses varying in age, location, and surrounding housing density.
2. Implant radio transmitters into Gila monsters to examine movement patterns, habitat use, and survival in response to varying features of golf courses.
3. Inform residents, golfers, and golf course personnel about local reptiles via interactions, presentations, and the development and distribution of a field guide booklet.
4. Engage golfers in monitoring reptiles by writing down wildlife observations in a logbook available in each clubhouse.
5. Develop recommendations for golf course designers and managers that can be used to retrofit existing courses and design new courses to maximize benefits to reptiles.

Start Date: 2008

Project Duration: two years

Total Funding: \$59,994

The demand for golf course communities in Arizona has steadily increased as baby boomers transition into retirement. Presently, there is a lack of information on the potential role that golf courses can play in maintaining native reptile populations and communities, especially in arid environments like the southwestern United States. The more information we have on how to design and manage golf course communities, the better we will be at maximizing the benefits that golf courses can provide for wildlife.

During the past year, we used mark-recapture to determine relative abundance and distribution of reptiles using golf courses in Arizona. To date, we have documented literally thousands of tortoises, snakes, and lizards using various features of golf courses. Using high-resolution imagery, we are analyzing distribution and abundance data in the context of landscape structure. We are finding that certain aspects of the golf course are used by cer-



The more information we have on how to design and manage golf course communities, the better we will be at maximizing the benefits that golf courses can provide for wildlife.

tain species, while others are avoided. For example, irrigated vegetation along fairways and surrounding greens and tee boxes are used well out of proportion to their availability, while open fairways appear to be avoided, especially by snakes. Areas of the golf course where natural desert has been incorporated into the design of the course correspond to areas of greater relative abundance of most species.

We have been radio tracking Gila monsters to gain a better understanding of how they are responding to the golf course and its surroundings. We have observed a clear pattern of use by Gila monsters characterized by general avoidance of open fairways and other areas of turf. However, Gila monsters tend to spend more time on the edges of fairways and greens, presumably searching for prey in the dense, irrigated vegetation.

We have observed individual Gila monsters changing the location and use of their home ranges as newly constructed homes become more common. Interestingly, the Gila monsters have started to concentrate more of their activity in areas immediately adjacent to the golf course that are off limits to development.

Although radiotelemetry is an excellent tool for tracking the movements of Gila monsters, you can only obtain information on the animal's location by physically following its signal. In 2008, we began using a new technique that allows us to quantify the exact movement path taken by a Gila monster. The technique involves the use of florescent powder that we apply to the Gila monster by gluing rabbit fur onto its belly and loading up the fur with the powder. After allowing the Gila monsters some time to move about, we come back after dark with a UV light and follow its exact track. This tech-



University of Arizona scientists have been radio tracking Gila monsters to gain a better understanding of how they are responding to the golf course and its surroundings.

nique allows us to determine if Gila monsters are avoiding certain features of their habitat, such as fairways and roads.

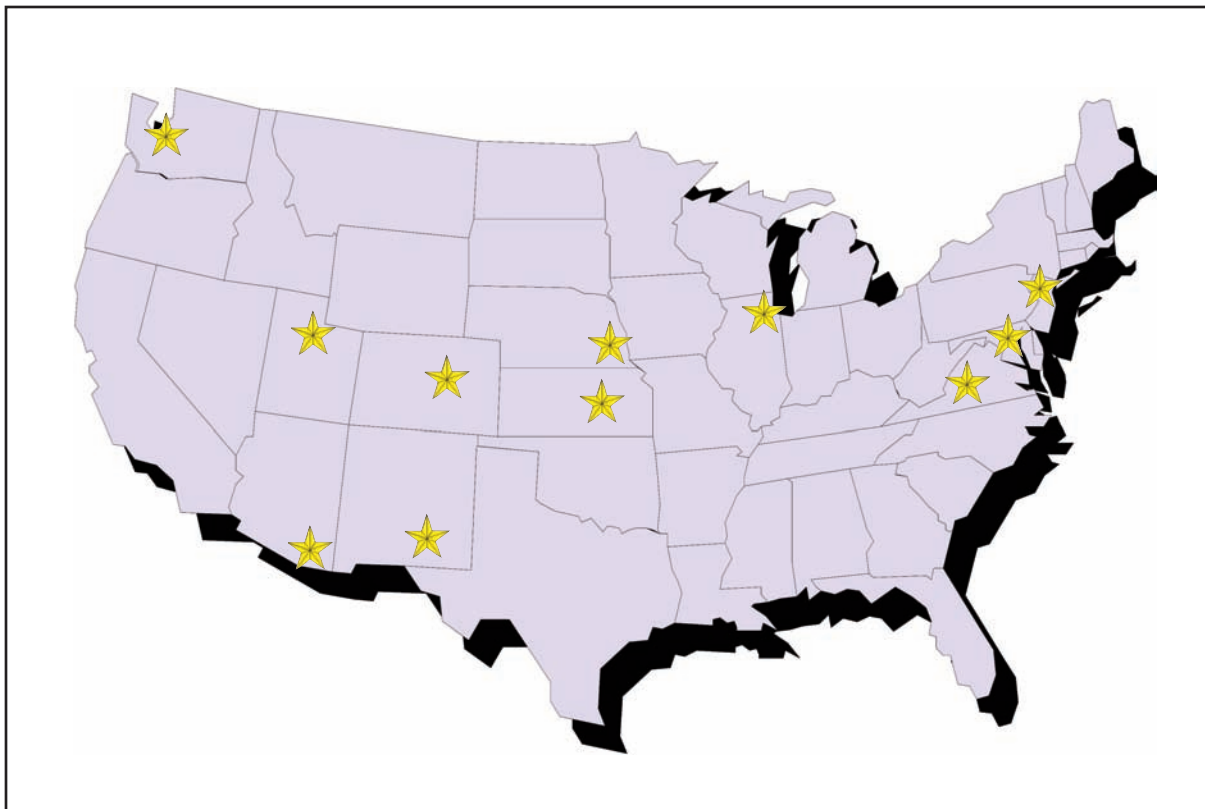
Using radiotelemetry, we only get the straight-line distance between successive fixes, but florescent powder allows us to determine the exact distance moved. So far, we have found that Gila monsters typically move two to three times farther than their straight-line distances would indicate.

Summary Points

- Reptile species vary in their use of golf course features, but most species tend to avoid open fairways.
- We have observed a greater abundance of reptiles using thick, irrigated vegetation adjacent to fairways and greens.
- The placement and layout of the golf course helps to determine the distribution of reptiles, and certain species alter their distributions to take advantage of irrigated vegetation and natural areas that are incorporated into the golf course as hazards or rough.
- Using radiotelemetry and florescent powder tracking, we have determined that Gila monsters tend to avoid open fairways, and tend to utilize irrigated areas along the edges of the golf course out of proportion to their availability.

Product Testing Program

Every year golf course superintendents are introduced to new products in the marketplace. Without results from properly designed, objective research, superintendents are asked to make buying decisions based on word-of-mouth, previous experience from colleagues, or the representative of the product. Several surveys indicate that golf course superintendents desire side-by-side product evaluations to assist them in making product purchases. The result of this desire for this type of information is the Product Testing Program section of USGA's Turfgrass and Environmental Research Program. Currently, USGA is funding three projects that fall into this category of USGA-supported research, including cooperators at several sites across the country (see map below).



Locations of the following projects funded in 2008 by the USGA Turfgrass and Environmental Research Program under the category of Product Testing

Evaluation of Plant Growth Regulators and Biostimulants for Use in Managing Summer Bentgrass Decline

Bingru Huang and Yan Xu
Rutgers University

Objectives:

1. To investigate whether the application of selected PGRs and biostimulants to a bentgrass putting green would enhance turf quality during summer months.
2. To determine whether these products would help alleviate summer bentgrass decline by delaying leaf senescence and promoting root growth.

Start Date: 2007

Project Duration: two years

Total Funding: \$20,000

Summer bentgrass decline is a major concern of superintendents growing creeping bentgrass on putting greens across the country, especially in the southern states and the transition zone. Some golf courses apply various compounds on bentgrass, hoping to improve turf growth during summer months.

Numerous organic products, claimed to have the functions as plant growth regulators or biostimulants, have emerged in recent years. Some organic materials such as seaweed extracts and amino acids are being used as fertilizer supplements in commercial turfgrass management. Among them, seaweed extracts are widely used in various biostimulant product formulations. Many of biostimulants promise better turf quality and stress tolerance.

The use of PGRs has recently been expanded as a means to promote turfgrass performance under stressful conditions. We propose to investigate whether the application of selected PGRs and biostimulants to a bentgrass putting green would enhance turf quality during summer months and to determine whether these



Summer bentgrass decline is a major concern of superintendents growing creeping bentgrass on putting greens across the country, especially in the southern states and the transitional zone.

products would help alleviate summer bentgrass decline by delaying leaf senescence and promoting root growth.

The study was conducted in 2007 and 2008. This report summarizes the 2007 results. We tested the following selected products that are extensively used in the golf course maintenance or pure chemicals with great potential to be marketed as stress reducers:

Primo Max (from Syngenta, 0.125 oz. / 1000 sq. ft.): Inhibitor of gibberellic acid synthesis and vertical shoot growth.

TurfVigor (from Novazymes, 15 oz. / 1000 sq. ft.): Combines patented high impact microbial strains, and macro and micronutrients.

CPR (from Emeraled Isle Solutions, 6 oz. / 1000 sq. ft.): a blend of natural sea plant extracts, micronutrients, and a surfactant.

Aminoplex (from Grigg Brothers, 2 oz. / 1000 sq. ft.): A proprietary mixture of 15 plant based L-amino and organic acids, complex polysaccharides, and natural hormones.

Proxy and StressGard (from Bayer, 5 oz. + 0.38 oz. / 1000 sq.ft.): Combined treatment with a Type II plant growth regulator that suppresses seedhead of *Poa annua* and white clover while promoting lateral growth of cool-season turf and a fungicide product that provides long-lasting broad-spectrum disease control and improved turf quality.

Aminoethoxyvinylglycine (AVG) (from Sigma, 25 μ M): an ethylene synthesis inhibitor that suppresses leaf senescence and helped to maintain greener turf for an extended period in a recent growth chamber study when creeping bentgrass was exposed to 35^o C.

6-benzylamine (BA) (from Fisher, 25 μ M): a synthetic cytokinin that demonstrated functions in delaying leaf senescence and improving heat tolerance in creeping bentgrass in controlled-environment conditions.

We also included two control treatments:

Nutrient control (Hoagland's solution): complete nutrient solution including all kinds of macronutrients and micronutrients to sustain plant growth.

Water control: regular ground water.

All chemical treatments were applied following the manufacture recommended rate (carried in 2 gal. of water). We evaluated turf quality, chlorophyll content, photosynthesis rate, leaf area index (indicator of canopy density), green leaf biomass (indicator of canopy color), and root growth.

Summary Points

● TurfVigor- and CPR-treated plots consistently maintained significantly higher turf quality than both nutrient and water control plots during the whole treatment period. Turf quality of Primo Max-treated plots was also higher than that of the nutrient and water control plots starting from mid-August. Other products, such as BA, AVG, and Aminoplex had some positive effects in August.

● Shoot growth and physiological activity of creeping bentgrass was promoted when treated with certain biostimulants or PGRs. The decline of canopy photosynthetic rate was less severe in TurfVigor-, Primo Max-, and BA-treated plots during mid-July and/or early August. Canopy density and color were maintained higher in CPR-, TurfVigor-, and Primo Max-treated plots in July and August.

● The only positive effects on root length and surface area were observed in the Proxy- and StressGard-treated plots in early September, indicating this product may affect the recovery of creeping bentgrass from summer stress.

● Our results suggested that proper use of selected biostimulants and PGRs could promote turf growth and alleviate summer bentgrass decline in warm climates.

Evaluation of Cytokinin Plant Extract Biostimulants, Iron and Nitrogen Products For Their Effect On Creeping Bentgrass Summer Quality

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Objectives:

1. To determine if products containing biostimulants, iron and/or nitrogen would affect dollar spot (*Sclerotinia homoeocarpa*) severity and summer quality of creeping bentgrass (*Agrostis stolonifera*) putting green turf in Illinois and Maryland.
2. To evaluate the impact of products containing biostimulants, iron and/or nitrogen on summer quality of creeping bentgrass putting green turf in Illinois and Maryland in the absence of disease.

Start Date: 2007

Project Duration: two years

Total Funding: \$40,000

Golf course superintendents often limit nitrogen (N) fertility on greens for the purpose of improving green speed. To maintain acceptable turf color and plant health under conditions of limited N input, superintendents often apply liquid biostimulants in the summer. Numerous companies market products containing cytokinins (usually from seaweed) and other plant extracts in liquid forms for use on golf greens.

Cytokinins are considered biostimulants, which have been shown to improve shoot and root health of creeping bentgrass in controlled environment studies. Biostimulant is a broad term and refers to products that may contain one or more active ingredients including: cytokinins, nutrients, humates, organic acids, hormones, vitamins, microbial inoculants, and other plant extracts.

Often biostimulant products contain either iron (Fe) or N or both. Manufacturer labels state that these prod-

ucts improve turf color, root growth, plant development, and environmental stress tolerance. While N and Fe are known to improve turf color and quality, it remains unclear if products containing cytokinins and/or other biostimulants provide any additional benefits in the field.

Lessons learned in 2007 resulted in a change in the treatment structure in 2008. In 2007, IronRoots, Knife, Lesco's 12-0-0 Chelated Iron Plus Micronutrients (hereafter Lesco 12-0-0), PanaSea Plus, Ultraplex, urea and Daconil Ultex were evaluated for their ability to suppress dollar spot. Except for Daconil Ultrex, none of the other products claim disease suppression on their labels.



Iron darkened the turfgrass at both sites immediately after application and appeared as a checkerboard pattern across the study area where applied, but the effect was transient unlike urea.

As expected, plots treated with Daconil Ultrex exhibited the best summer quality in both IL and MD since it effectively controlled the disease. Urea was the next best treatment, but urea-treated plots eventually were severely damaged by dollar spot. Except as noted below, none of the other treatments reduced dollar spot and plots treated with those products were damaged severely, resulting in poor turf quality in both IL and MD. In MD, however, some slight reduction in dollar spot



NDVI measurements were taken to supplement visual color ratings at both sites by use of a small handheld meter.

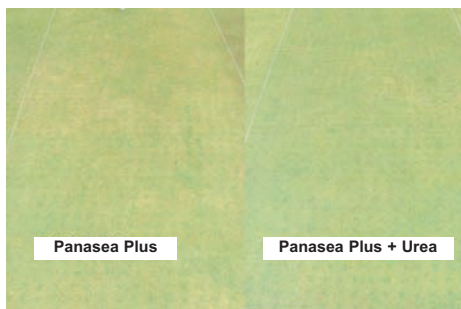
was observed in plots treated with Knife, but the effect was temporary and did not result in acceptable quality at any time.

Products containing Fe and N improved turf color, but due to the presence of disease, the plots could not be evaluated for environmental stress impact on summer quality. Hence, Daconil Ultrex was replaced by Roots Concentrate in 2008, and both sites were treated with fungicides to prevent blighting by pathogens.

Since PanaSea Plus, Roots Concentrate, and IronRoots contain no N, they were compared with and without N from urea. Therefore, urea was applied alone or tank-mixed with IronRoots, Roots Concentrate, and PanaSea Plus at 0.15 lb N/ 1000 ft². All treatments were applied on a 14-day interval, except Knife which was evaluated at two rates applied on either a 14-day or 28-day interval as specified on the label. All products were tested at label rates and application intervals as specified on product labels. Both study sites were treated with fungicides in 2008 throughout the study period to control dollar spot and brown patch (*Rhizoctonia solani*). (continued on next page)



Both studies were conducted on USGA constructed bentgrass greens that were either a 'G-2' / 'L-93' blend (IL) or 'Providence' (MD).



Panasea Plus and Roots Concentrate, which do not contain Fe or N, did not improve visual quality over untreated control at either site.

The studies were conducted on mature stands of either 'G-2' + 'L-93' blend (IL) or 'Providence' (MD) creeping bentgrass grown on a sand-based rootzone constructed to USGA recommendations. The research greens were mowed five to six times weekly to a height of 0.156 inches. The MD site received 1.75 lb N/ 1000 ft² between April 19 and May 30 2008, and an additional 0.25 lb N/1000 ft² was applied on August 6, 2008. The IL site received 0.5 lb N/1000 ft² during May 2008. Otherwise, both sites did not receive any supplemental N. All treatments were applied in 50-87 gal. water/A using a CO₂ pressurized backpack sprayer equipped with an 8004 Tee Jet nozzle. Plots were arranged in a randomized complete block with four replications.

Turfgrass color and quality were assessed visually on a 0 to 10 scale where 0 = entire plot area brown or dead; 7 = minimum acceptable color and quality and 10 = optimum greenness and uniformity. Chlorophyll levels influence turf color and normalized difference vegetation index (NDVI) readings also were obtained using a Field Scout TCM 500 Color Meter. Scalping injury was evaluated in MD on a 0 to 5 visual scale where 0 = no scalping; 2.5 = objectionable levels of scalping; and 5.0 = severe injury.

The performance of treatments in



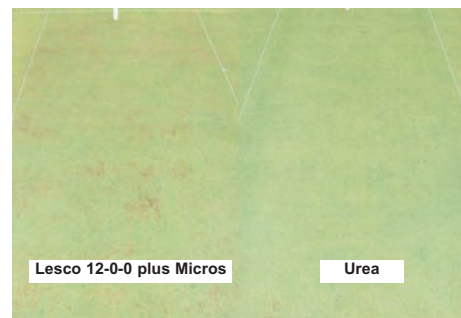
The first year, products were evaluated for ability to suppress dollar spot disease but were unable to do so at both sites.

IL and MD were very similar. When data were averaged over the season, treatments containing urea provided the best quality at both sites. There were no significant differences in color or quality in plots treated with urea alone compared to plots treated with IronRoots + urea, Roots Concentrate + urea, or PanaSea Plus + urea at either site. Hence, little or no benefit was observed by tank-mixing any of the biostimulant products with urea.

Furthermore, urea alone provided equal or better color and quality when compared to all other treatments on most rating dates in late summer at both locations. Only treatments containing urea mitigated the injurious effects of scalping in MD. When data were averaged over the season, Ultraplex (IL and MD) and Lesco 12-0-0 (IL) improved quality compared to the control. Knife, IronRoots, Roots Concentrate and PanaSea Plus improved quality in early summer in MD.

In IL, PanaSea Plus consistently improved quality over the control from midsummer on, whereas plots treated with Knife, IronRoots, and Roots Concentrate had a level of quality equal to the control. When data were averaged over the season, the aforementioned biostimulants had provided a level of quality equivalent to the control at both locations.

Visual color ratings often were improved by products containing Fe and N. PanaSea Plus and Roots Concentrate



Urea alone and treatments containing urea helped mitigate injury from scalping on a 'Providence' bentgrass green in MD, 2008

sometimes improved color, but they did not improve color to an acceptable level on most rating dates. Color ratings, as determined by NDVI, were similar between sites. In both IL and MD, highest NDVI ratings were observed in plots treated with urea alone or mixed with IronRoots, Roots Concentrate, or PanaSea Plus.

In MD, there were no other treatments that consistently improved color as measured by NDVI. In IL, plots treated with Ultraplex and Lesco 12-0-0 registered NDVI ratings equivalent to urea-treated plots when monitored 3 days, but not 7 days, after application. Since NDVI measures color as influenced largely by chlorophyll, it can be concluded that the Fe applied did not impact chlorophyll levels. Applications of Fe improved turf color by eliciting a darkening of foliar color. That is to say, the canopy does not appear greener, but darker.

Summary Points

- Early in the season in MD, IronRoots, Knife, Lesco 12-0-0, Ultraplex, and treatments containing urea improved turf color and quality compared to the control and often provided for enhancement of color and quality to a high level. In IL, differences among treatments became evident in July. Both Ultraplex and Lesco 12-0-0 consistently improved turf quality and color compared to the control, whereas Knife had provided no apparent beneficial effect in IL.
- When data were averaged over the season in both IL and MD, urea alone and treatments containing urea generally provided for best summer quality, but there were no differences among plots treated with urea alone or mixed with biostimulants.
- Urea alone and treatments containing urea helped to mitigate scalping injury.
- When data were averaged over the 2008 season, Lesco 12-0-0 (IL) and Ultraplex (IL and MD) improved quality compared to the control; whereas, IronRoots, Knife, PanaSea Plus, and Roots Concentrate did not improve quality at either location.
- Any potential visual benefits from the biostimulants were masked by the presence of Fe or N in Knife and IronRoots or by the N in urea when tank-mixed with Roots Concentrate or PanaSea Plus.
- PanaSea Plus and Roots Concentrate, which do not contain Fe or N, had only a small impact on turf color and quality, and did not improve quality above the minimum acceptable level on most rating dates after early July in MD and mid-August in IL.
- NDVI color ratings were consistently highest in plots treated with urea, IronRoots + urea, Roots Concentrate + urea, and PanaSea Plus + urea in IL (2007 and 2008) and MD (2008). Iron generally had little or no effect on color as measured by NDVI.

2007 Buffalograss Experimental Line and Cultivar Evaluation

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Anthony Koski	Colorado State University
Michael J. Goatley	Virginia Tech
Rodney St. John	Kansas State University
Gwen Stahnke	Washington State University

Objectives:

1. Assess the turfgrass performance of elite buffalograss genotypes across several locations involving a wide range of environmental conditions and possibly make recommendations.

Start Date: 2008

Project Duration: three years

Total Funding: \$12,000

Buffalograss [*Buchole dactyloides* (Nutt.) Engelm] is a low-growing, warm-season turfgrass native to the Great Plains of the US. It has an outstanding combination of drought, heat, and cold tolerance and is ideally suited for turfgrass use where low or minimum inputs of water, nutrients, pesticides, and energy are required. Breeding and developing genotypes that meet the future need of the turfgrass industry requires the testing of elite genotypes across a wide range of environmental conditions. For this reason, a trial consisting of 20 turf-type buffalograss genotypes was initiated in 2007.

In 2008, a second trial consisting of nine seeded and seven vegetative genotypes was established at nine locations in eight states. The experimental design was a randomized complete block design with three replications. The plots were 5 ft by 5 ft. Data collected at each location were reported except the two locations in Kansas.

At Mead, NE, data were collected both on 20 genotypes established in 2007 and 17 genotypes re-established in 2008. Significant differences were observed among genotypes tested for establishment rate, seedling vigor, lateral spread, and color in 2007; for turfgrass cover, spring green up, and color in 2008, and turfgrass density and quality in both years.

The 2008 trial also had significant differences among vegetative genotypes for percent cover at 2, 4, and 8 weeks after plantings, density, and quality. Seeded genotypes differed for percent cover two



Breeding and developing genotypes that meet the future need of the turfgrass industry requires the testing of elite genotypes across a wide range of environmental conditions.

weeks after planting and for summer density. Some new experimental lines were superior to the best commercial cultivar check.

In Colorado, data were reported only for vegetative genotypes in 2007. Results indicated significant differences among genotypes tested for turfgrass cover at 2, 4, 8, and 12 weeks after planting, as well as turfgrass leaf texture, spring green-up, density, and quality. Two genotypes consistently outperformed the best check at this location.

At Logan, Utah, 2007 data analysis indicated significant differences among genotypes tested for each group for turfgrass cover, quality, color, and leaf texture. The result indicated significant difference among genotypes for turfgrass color and quality. Some experimental lines demonstrated excellent performance.

At Las Cruces, NM, significant differences were observed among genotypes evaluated for turfgrass cover at different times and fall discoloration. Some experimental lines performed similar to or better than the commercially available

cultivars.

At Tucson, AZ, significant differences were observed among seeded genotypes tested for turfgrass cover, color, and quality. Vegetative genotypes differed significantly only for percent cover. Two experimental lines performed similar to or better than the best commercially available cultivar.

At Blacksburg, VA, significant differences were found among vegetative genotypes for turfgrass cover, texture, color, and quality, but seeded genotypes differed only in turfgrass cover. Some experimental lines were as good as the best commercially available check.

Summary Points

- Significant differences were observed among genotypes tested for most traits.
- A few genotypes performed similar to or more than the best check in most locations.
- Quality data were slightly low.
- Genotypes performed differently to the range of environments at which they were tested.