

**PROCEEDINGS OF THE THIRTEENTH ANNUAL  
RUTGERS  
TURFGRASS SYMPOSIUM**

**Bruce B. Clarke, Director  
William A. Meyer, Associate Director**

**January 15-16, 2004  
Cook College**

## **Symposium Organizing Committee**

James Murphy, Chair  
Stacy A. Bonos  
Bruce B. Clarke  
Barbara Fitzgerald  
Bingru Huang  
James F. White, Jr.

## **Proceedings of the Thirteenth Annual Rutgers Turfgrass Symposium**

Stacy A. Bonos and Barbara Fitzgerald, Editors

*Rutgers Cooperative Extension educational programs are offered to all without regard to race, religion, color, age, national origin, gender, sexual orientation or disability.*

Welcome to the Thirteenth Annual Turfgrass Symposium at Cook College, Rutgers University/NJAES. This Symposium is organized each year for the Rutgers faculty, staff and students who share the results of ongoing turf research and exchange ideas on a wide range of topics in turfgrass science. Our keynote address will be given by Dr Fred Yelverton of North Carolina State University. The topic of transgenic turfgrasses is an important subject for discussion. Other outside speakers include Dr. Wayne Hanna, turfgrass breeder from the University of Georgia (formerly USDA, Tifton, GA), and Dr. Nathaniel Mitkowski of the University of Rhode Island. I would like to thank the Symposium planning committee, comprised of Jim Murphy (Chair), Stacy Bonos (editor), Bingru Huang, Bruce Clarke, Barbara Fitzgerald and Jim White.

The Center for Turfgrass Science continues to grow and improve with new members such as Brad Park who is working with Dr. Murphy on Sports Turf Management in New Jersey. They are conducting innovative research and outreach programs and have developed an excellent relationship with the Sports Turf Managers Association. They have also conducted extensive traffic studies at Hort Farm II on several important turfgrass species. Mr. T.J. Lawson has also contributed greatly to the success of these trials.

Last summer, there were many achievements in turfgrass breeding related to improved disease resistance. The improvements have focused on brown patch in tall fescue, dollar spot in creeping bentgrass, gray leaf spot in perennial ryegrass and stem rust in Kentucky bluegrass. Drs. Murphy and Clarke have developed a great deal of new information on the cultural and chemical control of anthracnose in annual bluegrass turf. Dr. Steve Hart and Darren Lycan have worked hard on the development of post emergent chemicals to control *Poa annua* and *Poa trivialis*. They are also assisting turfgrass breeders at Rutgers to evaluate the new Roundup Ready creeping bentgrasses. These are only some highlights, with many others contributing greatly to the Center.

It is also important to recognize the impact that Dr. Rich Hurley has made in expanding the undergraduate program in Turfgrass Science. He has recruited many excellent students, has placed students in many challenging summer internships, and has helped to allocate scholarship funds for our top students.

In 2003, the survey on the New Jersey Turfgrass Industry was finished. It is now in the editorial stage and will soon be released. It will highlight the major economic impact that the Turfgrass Industry has on our state economy.

Thank you for attending this year's symposium.

Sincerely,

William A. Meyer  
Associate Director, CTS

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# THIRTEENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

Cook College, Rutgers University  
January 15-16, 2004  
Foran Hall - Room 138

## Thursday, January 15, 2004

- 7:00 - 7:30 PM Registration
- 7:30 - 7:40 PM Welcome and Introduction: **Dr. Bruce Clarke, Director - Center for Turfgrass Science**
- 7:40 - 8:30 PM Keynote Address: **Dr. Fred Yelverton** (Department of Crop Science, North Carolina State University) *Transgenic Turfgrass: Issues and Opportunities*
- 8:30 - 10:00 PM Wine and Cheese Reception

## Friday, January 16, 2004

- 8:30 - 9:00 AM Registration, Coffee and Donuts**
- 9:00 - 10:00 AM SESSION I: PLANT IMPROVEMENT**  
(Moderator: Dr. William Meyer)
- 9:00 – 9:20 **Dr. C. Reed Funk** (Department of Plant Biology and Pathology, Rutgers University) *Germplasm Collection, Evaluation, and Recurrent Population Improvement of Turfgrasses and Underutilized Perennial Food Crops*
- 9:20 – 9:40 **Dr. Stacy Bonos** (Department of Plant Biology and Pathology, Rutgers University) *Progress Toward a Genetic Linkage Map of Creeping Bentgrass and QTL Analysis for Dollar Spot Resistance*
- 9:40 – 10:00 **Dr. Wayne Hanna** (Department of Crop and Soil Sciences, University of Georgia) *Developing a New Generation of Bermudagrass Turf Cultivars*
- 10:00 - 10:30 AM Discussion and Coffee Break**
- 10:30 - 11:30 AM SESSION II: TURFGRASS PATHOLOGY**  
(Moderator: Dr. Faith Belanger)
- 10:30 – 10:50 **Dr. Nathaniel Mitkowski** (Department of Plant Sciences, University of Rhode Island) *Biology and Control of Bacterial Wilt on Annual Bluegrass*

- 10:50 – 11:10 **Dr. Bradley Hillman** (*Department of Plant Biology and Pathology, Rutgers University*) *Variability of the Fungus that Causes Anthracnose Disease of Creeping Bentgrass and Annual Bluegrass*
- 11:10 – 11:30 **Dr. James White, Jr.** (*Department of Plant Biology and Pathology, Rutgers University*) *Dissemination of Endophytes in the Turfgrass Ecosystem*
- 11:30 - 12:00 PM Discussion and Poster Session**
- 12:00 - 1:30 PM Lunch and Poster Session**
- 1:30 – 2:30 PM SESSION III: TURFGRASS MANAGEMENT AND PHYSIOLOGY**  
(Moderator: Dr. Thomas Gianfagna)
- 1:30 – 1:50 **Dr. James Murphy** (*Department of Plant Biology and Pathology, Rutgers University*) *Field Infiltration and Saturated Conductivity of Creeping Bentgrass Root Zones*
- 1:50 – 2:10 **Dr. Daniel Giménez** (*Department of Environmental Sciences, Rutgers University*) *Soil Physics Research in Putting Green Root Zone Mixes*
- 2:10 – 2:30 **Dr. Bingru Huang** (*Department of Plant Biology and Pathology, Rutgers University*) *Exploring Heat Tolerance Mechanisms in Cool-season Turfgrasses*
- 2:30 - 3:00 PM Discussion and Coffee Break**
- 3:00 – 4:00 PM SESSION IV: TURFGRASS PHYSIOLOGY AND PEST MANAGEMENT** (Moderator: Brad Park)
- 3:00 – 3:20 **Dr. Faith Belanger** (*Department of Plant Biology and Pathology, Rutgers University*) *Efforts to Identify the Colonial Bentgrass Contribution to Dollar Spot Resistance in Colonial x Creeping Bentgrass Interspecific Hybrids*
- 3:20 – 3:40 **Dr. Stephen Hart** (*Department of Plant Biology and Pathology, Rutgers University*) *Bispyribac-Sodium Herbicide for Poa annua and Poa trivialis Control in Cool-Season Turfgrass*
- 3:40 – 4:00 **Dr. Albrecht Koppenhöfer** (*Department of Entomology, Rutgers University*) *White Grub Management with Nematodes and Pheromones*
- 4:00 - 4:30 PM Discussion/Closing Remarks**



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# **Plenary Presentations**

## Transgenic Turfgrasses: Issues and Opportunities

Fred Yelverton, Ph.D.  
 Professor and Extension Specialist  
 Co-Director, Center for Turfgrass Environmental  
 Research & Education  
*Department of Crop Science, North Carolina State University*

Biotechnology-derived creeping bentgrass that is tolerant to glyphosate appears to be the first transgenic turfgrass that will be commercially available. While approval is far from guaranteed, it is likely that approval will eventually be granted. If approved, this first transgenic turfgrass will be known as Roundup Ready® Creeping Bentgrass (RRCB). The approval of RRCB and future biotechnology-derived turfgrasses will depend on how well the scientific community can identify the risks associated with their release, and more importantly, the development of management strategies to deal with those risks.

To date, the discussion of the potential commercialization of RRCB has been healthy and generally lacking acrimony. However, as potential commercialization draws near over the next year or so, the entire issue of biotechnology-derived turfgrass (and other perennial grasses) will likely become more hotly debated. Many of the issues and much of the current debate related to RRCB are similar to the issues that faced traditional row-crop agriculture in the mid to late 1990s. By the end of 2002, acceptance of biotechnology-derived crops by US producers has been high (Table 1).

Table 1. Acceptance of biotechnology-derived soybean, cotton, canola, and corn in 2002.\*

	<u>United States</u>	<u>Global</u>
	<i>(% of planted acreage)</i>	
Soybean	84	51
Cotton	81	20
Canola	80	12
Corn	40	9

\*ISAAA and Monsanto estimates

Currently, 17 countries are planting biotechnology-derived crops and the percentage of planted acres is expected to continue to grow in the future.

While many of the issues surrounding RRCB are similar to traditional row-crop agriculture, there are important differences. None of the above-mentioned crops are grown as perennials. Potential hybridization (and frequency) with other creeping bentgrass species which are also perennials has, and will require extensive investigation. From a weed scientist's perspective, the following topics related to RRCB need to be thoroughly investigated and discussed: 1) If RRCB is planted on golf courses, will it escape to other areas on the golf course or beyond 2) If it does escape, will it become a

weed, 3) If it does escape, can it be successfully controlled with herbicides other than glyphosate, and 4) will weed populations be discovered that are resistant to glyphosate.

Will RRCB be found on other unintended areas on the golf course? I think the answer to this question is yes. It will be difficult to plant RRCB or any other turfgrass on the golf course and expect it not to be found in other areas. Seed movement with newly planted turfgrasses is quite common. Wind, water, and animals (including humans) are the most common culprits.

Will it become a weed? This issue will likely be the most hotly debated. There is no evidence of *Agrostis* spp. being major weeds in agricultural commodities or other natural ecosystems, with the exception of seed production areas in the Pacific Northwest. There is also no evidence to date that potential bentgrass hybrids will exhibit increased fitness (more weedy).

Can RRCB be controlled with herbicides other than glyphosate? Yes, herbicides other than glyphosate can be used to control RRCB. These include clethodim and fluazifop.

Will resistant weed populations be found? Nobody knows the answer to this question. However, it is safe to assume, given the genetic diversity of some turfgrass weeds such as *Poa annua*, that resistant biotypes do exist in nature and resistant populations may be found. Therefore, if RRCB is released, it will be important to initiate sound resistant management strategies that are effective in reducing the selection pressure for naturally-occurring resistant biotypes.

While much of the current debate centers on RRCB, there is little doubt that should this product be released, many transgenic turfgrasses will follow. In order to gain insight into what other biotechnology-derived turfgrasses may be, one should look at row-crop agriculture. It is likely that biotechnology-derived turfgrasses in the next 10 years will be similar technology to those discovered or commercialized in row-crop agriculture. In addition, corn and other grass crops such as wheat and rice will be the crops to watch. If we use these crops as indicators of what we may expect in turfgrasses over the next 10 years, the next biotechnology-derived turfgrasses will probably focus on three general areas: 1) improved drought tolerance, 2) improved root systems that will be more efficient in uptake and utilization of nutrients, and 3) turfgrasses that grow slowly.

In summary, the turfgrass community may be on the verge of substantial change. If RRCB is released, it will be a landmark event that will represent a new age of technology in turfgrass systems. There will be many challenges and opportunities with this new technology. The success or failure of this new technology will depend on how well we (the scientific community) identify and manage the risks.



## **Germplasm Collection, Evaluation, and Recurrent Population Improvement of Turfgrasses and Under-utilized Perennial Food Crops**

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Exceptionally rapid progress has and is being made (1) in the genetic improvement of recently domesticated plants and (2) in adapting old crop species to new environments, uses and production regimes. Adapting old crop species has been illustrated by the development of cultivars of wheat, rice, sorghum, maize, and millets to the high yield environments associated with the green revolution. Cultivars able to respond to and utilize high amounts of fertilizer and other production technologies have been responsible for most of the added food produced during the past 50 years. Improvements in stress tolerance, pest resistance, early maturity, harvest index, and growth profile have expanded regions where sorghum, winter cereals, and alfalfa are adapted. Exciting improvements have and are being made in recently domesticated rubber trees, oil palms, and many turf and forage grasses. Extremely dwarf cultivars of bermudagrass have been developed for sports turf in addition to exceptionally vigorous and productive forage grasses in the last seven decades. During this same period, dramatic advances were made in developing denser, lower-growing, darker-green, more stress tolerant, easier-to-mow, more attractive, persistent turf-type perennial ryegrasses with high seed yields and improved resistance to many important diseases and insect pests.

### **Genetic Improvement of Turf-type Perennial Ryegrasses**

Perennial ryegrass (*Lolium perenne L.*) is native to the eastern hemisphere. It was introduced to North America by early colonists from Europe and the British Isles in hay, bedding, and seed mixtures used to establish pastures and hay fields. During the past century, millions of pounds of perennial ryegrass were imported from New Zealand, Australia, Great Britain, and Europe primarily for turf use. Hundreds of millions of pounds of Oregon blue tag perennial and 'Linn' perennial ryegrass were also sold for turf use throughout the United States. Low-priced seed and rapid establishment made these ryegrasses a significant component of many turf seed mixtures. They were generally short-lived, difficult-to-mow, and susceptible to many of the disease, insect, and environmental stresses common to the mid-Atlantic region of the United States. Their origin and adaptation to the cool-summer, mild-winter, maritime climates of the British Isles, northwest Europe, southeast Australia, and New Zealand made them poorly suited as a long-lived attractive turf in regions with long, hot, humid summers, colder winters, and the many pests common to these stressful environments.

Seed growers, farmers, and research institutions in the British Isles and Europe, including the Welch Plant Breeding Station and major seed companies of the Netherlands, selected, developed, and distributed improved perennial ryegrass cultivars for their pastures and hay fields. Their lower-growing types with abundant tillers were

more persistent and better adapted to long-term pastures. They were also used extensively for sports turf in northwest Europe and the British Isles until the advent of 'Manhattan' perennial ryegrass released in 1967 (Funk, Engel, and Halisky, 1969) and subsequent improved turf-types. Improved pasture-type cultivars, such as 'S-21' and 'Pelo', found very limited use for turf in the United States because of higher seed costs, high susceptibility to *Rhizoctonia* brown patch, and poor mowing qualities under stresses of heat and drought.

Turfgrass scientists at Rutgers and Pennsylvania State University initiated programs to examine thousands of old lawns, parks, sports fields, cemeteries, and golf courses starting in 1962. They showed that of the trillions of ryegrass seeds used to establish these turfs only a few produced plants able to persist and grow to produce attractive individual plants that were at least one meter in diameter. The most attractive plants were found near the sheep meadow in Central Park in New York City (the parents of 'Manhattan'); in southeast Pennsylvania (the parents of 'Pennfine' and 'Birdie' perennial ryegrasses); in Patterson Park (one parent of 'Citation'); Riverside Park (3 parents of 'All-Star') and a school playground in Baltimore, MD; the campus lawn of the University of Maryland, College Park, MD (2 parents of 'Pennant'); Warinaco Park, Elizabeth, NJ, and the Colonia and Atlantic City golf courses near Colonia, NJ and Atlantic City, NJ. Genes for resistance to stem rust (*Puccinia graminis* Pers.) Were found in plants collected from old turfs in Missouri and Washington, D.C. They were incorporated into advanced breeding populations by population backcrossing leading to the development of 'Manhattan II' (Funk, Meyer, and Rose, 1984), 'Citation II', and 'Birdie II'.

Tillers obtained from these plants were collected and subsequently evaluated in frequently mowed turf trials. The best performing were subjected to progeny tests under turf maintenance and used to develop, 'Manhattan', 'Pennfine', and 'Birdie'. Plants obtained from crosses of the best performing selections were subsequently selected to initiate a long-term germplasm enhancement program using many cycles of phenotypic and genotypic recurrent selection, population backcrossing, and phenotypic assortive mating. Phenotypic selection involved (1) selection of darker green, more compact, disease-free, highly tillering seedlings during winter greenhouse tests; (2) inoculation and selecting for resistance to stem rust and crown rust caused by *P. coronada*; (3) selection of attractive, leafy, lower-growing, darker-green plants showing higher seed yield potential in spaced-plant nurseries; and (4) selecting attractive plants surviving in closely mowed turf trials subjected to stresses of heat, drought, disease, insects, and winter cold. Genotypic selection included extensive evaluation of single-plant progenies in closely mowed turf trials and spaced-plant nurseries. Population backcrossing was used to incorporate genes for resistance to crown rust, stem rust, gray leaf spot, and desirable endophytes into streams of continually improving breeding composites. Many cycles of phenotypic assortive mating was found to be very effective in selecting desirable recessive and quantitatively inherited genes in vigorous highly heterozygous plants. It was much more useful than severe types of inbreeding in that desirable recessive genes would immediately be evaluated in elite vigorous plants. Many separate breeding populations were developed with each subjected to continuing cycles of genetic

improvement to maintain genetic variability and reduce inbreeding. Intercrossing of selected populations led to a continuing stream of improved cultivars with each cycle showing marked improvement in national tests and commercial use. Cooperation with breeders and growers in western seed producing states resulted in concurrent and dramatic increases in seed yields. Discovery of beneficial effects on *Neotyphodium* endophytes in enhancing resistance to many insect pests, improving stress tolerance, and increasing persistence occurred during the early 1980's. This led to the identification of the most useful strains and their incorporation into the best breeding populations and cultivars.

A substantial expansion of germplasm collection into new regions of Europe and Asia started in 1996 when Dr. William A. Meyer was hired to direct an expanded turfgrass breeding program at Rutgers. This was very fortunate as new sources of genetic resistance to new challenges such as gray leafspot became available. Increasing hybrid vigor and persistence is also becoming evident with backcrossing much of this germplasm into the best breeding populations.

Improved turf-type perennial ryegrasses are currently widely used for sports and amenity turf in developed countries throughout all temperate areas of the world. They are also widely used for the winter overseeding of turfs in warmer regions. Seed usage has increased to over 200 million pounds annually. Seed prices have remained essentially stable since Manhattan was first marketed in the late 1960's due to genetic improvements in seed yield, disease resistance, early maturity, production technology, and competition. The best farmers of the late 1960's found it difficult to obtain over 700 pounds of seed of Manhattan. Its late maturity required irrigation and fungicides. Yields over 3,000 pounds per acre are currently being reported with many new cultivars showing average yields of over 2,000 pounds.

How long will it take to achieve similar genetic improvements in yield, nut characteristics, and acceptance of native American black walnuts, pecans, hickories, hazelnuts, nut pines, and edible oaks?

### **Genetic Improvement of Turf-type Tall Fescues**

An energetic program to improve tall fescue (*Festuca arundinacea* Schreb.) for turf use was initiated by the New Jersey Agricultural Experiment Station in 1962. Tall fescue was introduced into the USA from Europe. Its historical development and use was well documented by Buckner, Powell, and Frakes (Buckner et al., 1979). Introductions by private agriculturalists and government personnel were made and evaluated during the 1800's. Many introductions became naturalized in various locations. However, tall fescue was not given much prominence until the Oregon and Kentucky Agricultural Experiment Stations released 'Alta' and 'Kentucky 31' in the early 1940's. These cultivars had broad leaves and produced an open turf but became widely used, especially throughout the transition zone, due to their deep roots and greater heat tolerance than most other cool-season grasses.

As a result of natural selection, a few outstanding plants, of the trillions that were seeded, persisted and spread under stresses of frequent close mowing, heavy traffic, excessive shade, poor soils, and the diseases, harmful nematodes, and insect pests common to our very warm, humid summers and frequently cold winters. These conditions are very different from their areas of origin in Europe. These rare best adapted plants spread to produce dense, attractive turfs often exceeding one meter in diameter. A few hundred attractive, turf-type plants were selected from the thousands of hectares of old pastures and turfs examined. Tillers were established in spaced-plant nurseries and/or frequently mowed clonal evaluation tests at Rutgers University. All but a few dozen of the most promising plants were quickly discarded. The best selections were very different from any cultivar in existence at the time of the collection. They produced lower-growing turfs with finer leaves, greater density, darker color, and greater tolerance of close mowing. Latter studies showed that most contained a *Neotyphodium* endophyte which often enhances stress tolerance and resistance to many harmful insect pests. These elite plants most likely originated from the early introductions from Europe. However, a few could have been extremely rare segregates from Kentucky 31 or Alta.

After 18 years of germplasm collection and many cycles of population improvement, 'Rebel' tall fescue was released in 1980. The most promising parental plants were identified by their persistence and appearance in old turfs and performance in spaced-plant nurseries and mowed clonal tests. They also had to demonstrate their ability to transmit these characteristics to future cultivars in single-plant progeny trials conducted in closely mowed turfs. Intercrosses of the best performing plants were subjected to varying cycles of phenotypic and genotypic selection depending on their date of collection. Each cycle of selection showed continued progress in producing lower-growing, darker-green, attractive plants with improved turf performance scores. Selection was also effective in increasing seed yield and maintaining good stress tolerance. Substantial progress was and continues to be made in developing tall fescues with finer leaves, increased persistence under close mowing, and increased density.

Population backcrossing was also used to add useful genes from plant introductions and from trispecies hybrids of meadow fescue, perennial ryegrass, and tall fescue. These hybrids were obtained from the U.S. Regional Pasture Laboratory, University Park, PA. Many cycles of Phenotypic Assortive Mating were used to uncover and utilize desirable recessive genes in vigorous, highly heterozygous plants. These were especially useful in the development of lower-growing populations and cultivars. Many of these lower-growing tall fescues are demonstrating significant improvements in shade tolerance. Selection of plants able to survive intense interplant competition in closely mowed turf trials was also effective in the continued development of persistent, improved turf cultivars. Cooperation with plant breeders and seed production specialists have improved resistance to diseases such as stem rust and improved seed yield performance.

The success of this program is documented by the continuing stream of improved tall fescue cultivars, their performance in regional and national tests, and their rapid and widespread acceptance and use by the turfgrass industry.

## **Opportunities for Dramatic Genetic Improvements in Many Under-utilized Food Crops**

The success demonstrated in the genetic improvement of many turfgrass species during the past 42 years strongly suggest that similar achievements could be made in the genetic improvement of many nut trees and other under-utilized food crops. Superior new cultivars in these species would add greatly to future food security throughout the world in addition to environmental enhancement and improved lifestyles. The longer life cycles of most tree crops plus the greater cost of growing large populations could lengthen the time and cost. However, this should be largely offset by rapid increases in knowledge and technologies available and expected.

Plant breeders have and continue to demonstrate great and rapid success in (a) adapting old crops to new environments or production regimes and (b) domesticating and improving new crop species.

Black walnuts, shagbark hickories, shellbark hickories, American hazelnuts, far northern pecans, many nut pines, and edible oaks represent wild native American species of great promise. Genetically improved cultivars with substantially improved yield of nut meats, cracking qualities, consistency of yield, taste, nutritional quality, and range of adaptation could and should be developed. They will be exceptionally useful for organic gardeners, edible landscapes, commercial production, shade, beauty, wildlife, and environmental enhancement. Extensive but insufficient germplasm collection efforts have already been conducted mostly by members of the Northern Nut Growers Association (NNGA). A well organized, long-term effort is needed to expand, preserve, enhance, and utilize these valuable germplasm resources.

The native American black walnut is an extremely valuable tree for timber. It has much potential as an exceptionally productive nut tree of very high nutritional value and with a wide range of adaptation. Currently millions of pounds of nuts are gathered from wild trees. They yield less than 10 percent quality nut meats when processed by Hammond's Pantry in Missouri. The operation is profitable because of the high price and demand for nut meats and value of the shells. A few exceptionally rare trees selected from the wild contain over 30 percent nut meats with improved shelling and extraction characteristics. Genetic theory suggests that after a few cycles of population improvement, we should be able to obtain cultivars having 50 to 60% nut meats and the thin shells and superior cracking characteristics of the Persian (English) walnuts and pecans.

Many cycles of population improvement should give us hardy, well-adapted cultivars of American hazelnuts with large nutritious nuts. This species is cross-compatible with many other species of hazelnuts. Intercrosses of this immense germplasm resource combined with cycles of population improvement should produce a range of valuable cultivars adapted to all areas having temperate climates. Types range

from small to large bushes, small to large stately trees with excellent ornamental and timber values, and spreading bushes for erosion control and wildlife habitat. Each type could be developed to have large nutritious tasty nuts, high productivity, resistance to drought stress and colder climates, insect and disease resistance, and many highly ornamental characteristics. These include beautiful purple-red leaves, contorted stems, attractive bark and catkins, and cut leaves. Crosses of native American hazelnuts with European hazelnuts with larger nuts are also showing considerable promise in breeding programs conducted by members of the NNGA. Rutgers is currently collecting germplasm from the former USSR in search of new sources of quantitative and qualitative resistance to the Eastern Filbert Blight and other useful characteristics. Much of this work is being done in cooperation with scientists at Oregon State University, the Clonal Repository of the USDA Plant Germplasm System, and members of the NNGA in New York, Canada, and Minnesota.

We should also be able to achieve great success in adapting useful, widely grown nut trees to New Jersey and other new environments. Species include the Persian (English) walnut, the Japanese walnut including the heartnut, pistachio, almond, apricot, nut pines from Europe and Asia, and southern USA ecotypes of pecan. New germplasm resources becoming available throughout China and the former USSR should greatly enhance the success of these programs. We are currently devoting considerable effort to the genetic improvement of hazelnuts, pecans and Persian walnuts with some efforts on all of the above species. All offer great potential for long-term population improvement as resources become available and germplasm is assembled.

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## Progress Toward a Genetic Linkage Map of Creeping Bentgrass and QTL Analysis of Dollar Spot Resistance

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Genetic linkage maps and Quantitative Trait Loci (QTLs) associated with disease resistance have been developed for over 18 important crop species including corn, rice and wheat. Current genomic research on these important agronomic crops is at a stage where scientists can identify the organization of genes on chromosomes, specific metabolic pathways and individual gene functions. To date, very few genomic studies of turfgrasses have been conducted compared to the economically important food crops. But, once a genetic linkage map in creeping bentgrass is created, technologies, tools, and methods utilized in other crop species can be applied to creeping bentgrass. Keith Jones conducted the most extensive studies of the *Agrostis* genus almost 50 years ago. A more recent but limited molecular analysis of the genome was conducted by Warnke in 1998. Creeping bentgrass (*Agrostis stolonifera* L.) is a cross-pollinated, self-incompatible allotetraploid ( $2n=4x=28$ ) with an approximate DNA content of 5 pg and a haploid genome size similar to corn ( $3 \times 10^6$  kb).

The objectives of the project are to create a genetic linkage map of creeping bentgrass using genomic SSRs (microsatellites) and conserved grass EST-SSR markers, and subsequently identify DNA markers linked to dollar spot disease resistance. To do this, an intraspecific pseudo  $F_2$  mapping population of creeping bentgrass generated from a cross between a dollar spot resistant and a susceptible genotype was created. Approximately 100 microsatellite markers have been identified and characterized in the mapping population. Additionally, a field trial containing replicated plants of the 181  $F_2$  progeny and 200  $F_3$  and backcross progeny was planted in the fall of 2002. These plants were inoculated with the dollar spot pathogen (*Sclerotinia homoeocarpa*) and evaluated for disease in the summer of 2003. This phenotypic data will be compared to molecular marker data to identify QTLs associated with dollar spot resistance.

Initial analysis of the SSR loci indicates that several loci may be undergoing double reduction. Double reduction can occur when tetrads (quadravalents) form at meiosis. This indicates that at some loci, tetrasomic inheritance may be occurring. All previous literature has indicated strict disomic (bivalent) inheritance in this species. Phenotypic dollar spot data of the mapping population indicates a range in disease resistance among the progeny. The SSR markers are being compared to the phenotypic data to identify QTLs associated with dollar spot resistance. Once QTL's are identified, marker-assisted selection can be incorporated into the breeding program to screen quickly germplasm for resistant plants. Furthermore, the development of a genetic linkage map in tetraploid creeping bentgrass will be a major contribution to the advancement of turfgrass breeding and genetics.

## Developing a New Generation of Bermudagrass Turf Cultivars

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Tifdwarf and Tifway, two bermudagrass interspecific hybrids released in the early 1960s, are still making contributions to the turfgrass industry. TifSport and TifEagle, newer releases from the late 1990's, were developed through irradiation of existing cultivars. In the future, we will need turfgrass cultivars with more genetic diversity that use less water and require less fertilizer and pesticides to produce and maintain high quality turf. Improved management approaches can accomplish some of the future needs. However, management approaches are usually temporary. Genetic improvements can be more permanent.

In 1991, we began making triploid interspecific hybrids between new Cynodon transvaalensis x C. dactylon germplasm. In 1992, we planted over 27, 000 new interspecific hybrids and selected 448 for further testing. These selections have now been narrowed to 23 of the best that combine turf quality with new characteristics for more detailed testing. Significant progress has been made in selecting for genetic resistance to the mole cricket, a serious insect on bermudagrass. Bermudagrass likes sunlight, but we have selections that produce acceptable turf under 60% continuous shade. We have made some progress in selecting genotypes that produce acceptable turf for longer periods without water than Tifway and TifSport. Genetic differences have been observed for response to lower nitrogen levels. Newer hybrids, made in 2000 to 2002, should have improved cold resistance combined with desirable turf quality characteristics. It appears that the new hybrids will require fewer inputs to maintain desirable turf quality.

Triploid interspecific hybridization, as discussed above, is one of the best ways to develop bermudagrass cultivars that produce high quality uniform turf. However, progress is being made in developing and selecting dwarf genotypes that can be used to produce dwarf synthetics. We have two experimental synthetics that have performed well.

We are also conducting research in genetic transformation. We presently have glufosinate resistant genotypes in replicated tests. These herbicide resistant genotypes could become very important if methyl bromide is ever taken off the market. We are considering introducing genes for shade, insect, and disease resistance.



## Biology and Control of Bacterial Wilt on Annual Bluegrass

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For a long time, many superintendents and turfgrass professionals doubted the existence of bacterial wilt of annual bluegrass. Because this unique and troublesome disease is bacterial in origin, the pathogen responsible for the disease is more difficult to isolate and nearly impossible to control using traditional strategies. Most turf pathologists specialize in fungi; as a result, very little research has been directed at this pathogen until now.

While bacterial wilt on annual bluegrass has only really started to explode in the past five years or so, it has been known to exist since 1985, when it was first isolated in the lab of Dr. Joseph Vargas at Michigan State (Roberts et al., 1985). In the early 1990's there was a flurry of activity to try and use this bacterium as a biological control for annual bluegrass (Savage et al., 1993; Johnson, 1994). Investigators felt that a natural pathogen of annual bluegrass could be manipulated and ultimately be used to keep the weed out of bentgrass greens. After much research, a product called Xpo was finally marketed by EcoSoil Systems. Unfortunately, it only met with limited success. While bacterial wilt can be a difficult pathogen to eradicate, for reasons that are still unclear it is also a disease that is very difficult to successfully inoculate.

Bacterial wilt of annual bluegrass appears to exist anywhere there is *Poa annua* var. *annua*. The Japanese have observed it for almost a decade and have put a significant amount of time into researching its biology (Nishino et al., 1995; Nishino and Fujimori, 1998; Imaizumi and Fujimori, 1999). It is likely that the disease has the same causal agent worldwide. Originally thought to be *Xanthomonas campestris* pv. *poaannua* or pv. *graminis*, we now know that the disease is caused by, *Xanthomonas translucens* pv. *poae*.

Putting greens are primarily at risk for developing the disease. It appears to be only a weak pathogen and is exacerbated by physiological and disease related stresses. When greens are maintained below 1/8" cutting height, the disease spreads rapidly. It has been observed at higher cuts, such as fairways, but symptoms are mild and generally result in nothing more than sporadic etiolation. Symptoms on putting greens include rapid decline and small yellow spots or speckling. Sometimes spots will coalesce but often they remain distinct. The disease can mimic Anthracnose or early stages of *Pythium* Blight. The disease usually begins in the most stressed locations. Areas of high traffic, low light, poor drainage, poor air movement and high compaction are prime locations for the disease. In a number of instances we have observed it acting in concert with cool-season *Pythium*. In more than 80% of the observed Spring cases, winterkill was severe. We hypothesize that winterkill provides a nutrient source for bacteria living saprobially in soils or epiphytically on leaves. When bacterial inoculum reaches a certain threshold, disease develops. It has been demonstrated that inoculum levels of *X. translucens* must approach a level of approximately  $10^6$ - $10^8$  cfu before disease can be reliably induced (Kinkel, 1997; Nishino and Fujimori, 1996)

Our research has demonstrated that its preferred host is *Poa annua* var. *annua*. However, it will cause disease on *Poa attenuata* and can survive asymptotically on *Poa trivialis*. Because *P. attenuata* is not native to the United States, this particular host is of minimal consequence. The Japanese have observed limited disease on *Poa compressa* and have identified the bacteria surviving asymptotically on *Poa pratensis*. This suggests that the pathogen can survive for extended periods of time without its preferred host and may maintain itself indefinitely in natural grass populations of mixed *Poa*.

Controlling *X. t. pv. poae* is very difficult. Because it is a vascular bacterium, it cannot penetrate into a plant without the aid of a wound or an open hole. While stomates and hydathodes can provide an entry point, the most common method of inoculation on putting greens is through mower wounds. The very nature of the modern putting green necessitates daily cutting and thus constant wounding. Once bacteria have entered into a wound, they must pass into active xylem vessels before the plant seals the wound. If a significant number of bacteria are successful in entering the vascular column, they will reproduce and quickly move down into the crown of the plant. The bacteria will ultimately kill the plant by blocking xylem vessels and preventing the bulk of the water movement through the plant. Plants that become infected and express disease cannot be salvaged.

If a site has bacterial wilt, there is no easy solution to managing the disease. When dealing with fungal diseases, there is no end to "quick-fixes". Fungicides are in abundant supply and they are extremely effective. There are no "quick-fixes" for bacterial wilt. The available chemicals only slow disease spread, they do not cure it. Copper fungicides are a good example. They have a wide biocidal range and will kill most microorganisms they come into contact with (the key word being "contact"). But they will not cure diseased tissue because they do not penetrate into the plant where the bacteria are causing disease. And every mowing opens new wounds that have not been sealed with copper fungicide that can act as an entry point for the bacteria. Only antibiotics will cure the disease and there are none registered for bacterial wilt. Additionally, antibiotics are highly valuable for human health. It is generally considered irresponsible to use the same chemicals for crop protection. Additionally, antibiotics are very phytotoxic when applied to turf and will commonly do more damage than good.

The more difficult path to controlling this disease is to improve cultural management. Raising height of cut, potentially increasing fertilizer, reducing traffic and aggressively minimizing compaction will all help to alleviate the problem. The best solution is to remove any annual bluegrass, bentgrass is not a host for this disease.

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## Variability of the Fungus that Causes Anthracnose Disease of Creeping Bentgrass and Annual Bluegrass

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Anthracnose basal rot is a serious disease of annual bluegrass (*Poa annua*) and creeping bentgrass (*Agrostis stolonifera*) particularly in Poa-bent mix green in different geographic regions of the U.S. Historically, the disease had occurred in *P. annua* during the periods of high temperature and high humidity in summer, particularly in plants under nutritional and/or environmental stress. The problem is now becoming more evident in creeping bentgrass in Poa-bent mix greens. The disease has also been observed in pure bentgrass stands in certain regions of the U.S. and Canada, and has been diagnosed on turf during cold and wet periods in certain locations in the northeast U.S. Application of fungicide often does not provide satisfactory control of the disease. We have been investigating whether some of the problems controlling the fungus may be attributable to variability in the causal agents themselves.

Two fungal pathogens, *Colletotrichum graminicola* and *Microdochium bolleyi*, have been associated with anthracnose of turfgrass. Their biology and pathology remain poorly understood, but *C. graminicola* appears to be the more important pathogen. In our studies, *M. bolleyi* was confirmed to be consistently associated with anthracnose disease in some regions of the northeast, but it is still unclear whether the pathogen is a primary colonizer or a secondary colonizer in this region.

To begin characterizing *C. graminicola* isolates associated with turfgrass anthracnose, genomic DNA libraries were made from two different strains of the fungus. From these libraries, three transposons were identified and used as probes to investigate fungal population structure. Genomic Southern blots probed with DNA representing these three transposons indicated differential presence of the elements in 21 isolates of *C. graminicola* from different golf courses in Pennsylvania. Ribosomal DNA internal transcribed spacer (ITS) sequences from the 21 isolates suggested that the isolates could be divided into two distinct clades. When these studies were extended to *C. graminicola* isolates from other regions of the U.S. and Canada, it was found that not all isolates of *C. graminicola* contained sequences that hybridized to the transposon probes. To examine the relationships among the larger set of isolates, genes for superoxide dismutase,  $\beta$ -tubulin, and a conserved region of the Mat2 mating type locus were examined in addition to ITS sequences. Analysis of these sequences confirmed and extended previous results with the smaller subset of isolates.

Several approaches are being taken to examine biological properties of *C. graminicola* isolates representing different clades. To examine host/pathogen relationships, inoculations of different grass species and cultivars are being conducted. To

examine steps involved in fungal colonization, *C. graminicola* isolates have been transformed for green fluorescent protein (GFP) expression. Finally, *in vitro* studies using fungicides with different modes of action are being conducted to determine whether differential responses can be predicted based on phylogenetic relationships of the fungal isolates.

## Dissemination of Endophytes in the Turfgrass Ecosystem

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Infection of the embryo within the seed or vegetative production of the infected host has previously been believed to be the primary means of host infection of the endophytes from genus *Neotyphodium*. Recent studies of several *Neotyphodium* endophyte infected grasses have documented the presence of epiphyllous mycelium (mycelial nets), bearing conidiophores with conidia on leaf blades. We hypothesize that epiphyllous conidia may be another potentially important mechanism for endophytes to spread to uninfected grass individuals. In this respect it is important to develop a base of knowledge regarding this stage of development of endophytes. In this study, the ability of *Neotyphodium* sp. to grow and sporulate on the leaf surfaces of *Poa ampla* plants growing in greenhouse and growth chamber conditions was evaluated. Leaf washes from the host plate to agar media show that spores of *Neotyphodium* sp. are capable of germination and growth on agar medium. The slide-agar colonies of *Neotyphodium* sp., derived from leaf washes of *P. ampla*, were used to observe capability of spores to disseminate via wind and rain splash release and transport. Spores of *Neotyphodium* sp. could not be detached from the conidiophores by simulated wind, but were released in large numbers by simulated rain. The prints of slide-agar colonies of *Neotyphodium* sp. on the agar plates were used to assess ability of *Neotyphodium* sp. to survive under controlled conditions. The fungus was able to survive and the spores germinated after being dried for a minimum one week.

## Field Infiltration and Saturated Conductivity of Creeping Bentgrass Root Zones

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Laboratory assessment of saturated hydraulic conductivity ( $K_{\text{sat}}$ ) of sand root zones before construction of golf putting greens is often extrapolated to rather precise expectations of field water infiltration. This study compared pre-construction  $K_{\text{sat}}$  of mixes to water infiltration of 4-yr old creeping bentgrass putting greens in two microenvironments. Root zone mixes containing various sand size distributions were arranged in a randomized incomplete block design nested over two microenvironments. Double-ring infiltration through the 0- to 50-mm surface depth was measured. Pre-construction  $K_{\text{sat}}$  ranged from 407 to 937  $\text{mm h}^{-1}$ , with root zones ranked greatest to lowest: coarse, coarse-medium, medium = medium-fine 2, and medium-fine 1. As expected, water infiltration was dramatically lower than  $K_{\text{sat}}$ . Greater infiltration in the enclosed microenvironment was only observed on coarse sand (37  $\text{mm h}^{-1}$ ) compared to other root zones that had infiltration of 9 to 23  $\text{mm h}^{-1}$ . Infiltration rates of root zones in the open microenvironment ranged from 12 to 68  $\text{mm h}^{-1}$  and were ranked greatest to lowest: coarse-medium, medium, medium-fine 1 = medium-fine 2. Thus, expectations that pre-construction root zone  $K_{\text{sat}}$  serves as a precise index for water infiltration of putting greens are unreliable.

## Soil Physics Research in Putting Green Root Zone Mixes

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Root zones promoting the maximum expression of the genetic potential of golf course turf should provide adequate aeration and moisture, while resisting compaction by traffic. These properties are attained through manipulation of the physical composition of the root zone mixes, using their hydraulic properties as guide in material selection.

Saturated hydraulic conductivity,  $K_{sat}$ , and air-filled porosity at -3 kPa (-30 cm) are two criteria used more or less independently in the design of root zone mixes. An important concern with the use of  $K_{sat}$  as a design criterion is the high variability in its measurements and the difficulty in discerning whether the experimental results are in agreement with other measured properties. Presently, the definition of -3 kPa as the lower boundary of effective porosity (i.e., air-filled porosity) seems somewhat arbitrary and detached of the concept of water movement represented by  $K_{sat}$ . A more comprehensive approach to defining desirable hydraulic properties of putting green root-zone mixes is to develop a mathematical framework linking  $K_{sat}$  with predictor variables such as effective porosity, particle size distribution, and soil water retention properties. The objective of this research is to investigate and develop models of hydraulic properties that integrate information from several properties of root zone mixes as a way to reduce or eliminate the uncertainty in the determination of  $K_{sat}$ .

A data base with values of saturated hydraulic conductivity, water retention, particle size distribution, and bulk density for a range of turf putting green root zone mixes was collected combining measurements made at Rutgers with published information. The parameterization of particle size distribution and water retention curves will be discussed as a first step to incorporating these properties in the prediction of saturated hydraulic conductivity and soil water retention. Particular attention is given to the concept of effective porosity and to the impact of its definition on the prediction of  $K_{sat}$ .

Our results can be applied to validate test parameters used as criteria in the assessment of root zone mixes and, therefore, they have the potential of enhancing the process of construction of golf course putting greens by improving the selection of materials and mixture proportions and the testing practices used to guide selection.



## Exploring Heat Tolerance Mechanisms in Cool-season Turfgrasses

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High temperature is a primary factor limiting the growth for cool-season grasses. Supraoptimal temperature has been considered to be the primary environmental factor causing decline or death of cool-season grasses in the summer, although many other factors can be involved. Heat stress can cause damages to various growth and physiological processes. Photosynthesis is among the most sensitive physiological processes to high temperatures. Net photosynthetic rate decreases rapidly as temperature increases above the optimum level in cool-season turfgrasses whereas respiration rate of both shoots and roots increases with temperature. The imbalanced photosynthesis and respiration can result in higher daily total carbon consumption than carbon production under high temperature conditions for creeping bentgrass, which could lead to carbohydrate depletion and growth inhibition. High temperatures are known to induce oxidative injury in plants by inhibiting the antioxidant protection system, which can lead to leaf senescence. Shoot injury in creeping bentgrass under heat stress has been associated with oxidative damage induced by the suppression of activity of antioxidant enzymes and the induction of lipid peroxidation when both shoots and roots of creeping bentgrass were exposed to heat stress. Soil temperature has been found to be more critical than air temperature in controlling the growth of creeping bentgrass. Exposing roots of creeping bentgrass [*Agrostis stolonifera* var. *palustris* (syn. *A. palustris*)] to high soil temperature (35 °C) while maintaining shoots at the optimum air temperature (20 °C) significantly reduced shoot and root growth; lowering soil temperature from 35 °C to 20 °C at high air temperature (35 °C) had the opposite effects. Reducing soil temperature from the supraoptimal level (35 °C) while exposing shoots to high air temperatures significantly improved shoot and root growth of creeping bentgrass. Although soil temperatures clearly influence shoot growth of cool-season grasses, the physiological basis of shoot growth regulation are not well understood. It is not clear what are the major factors in roots that may sense high soil temperature and regulate shoot growth. Shoot injury and leaf senescence induced by high soil temperatures was associated with the inhibition of cytokinin production. Cytokinins are produced mainly in roots and may regulate shoot responses to high soil temperatures. Cytokinin content in both leaves and roots decreased when roots of creeping bentgrass were exposed to heat stress (35 °C); injection of cytokinin to the root zone alleviated leaf senescence. The adverse effects of high soil temperature could also be attributed to decreased nutrient uptake of roots in creeping bentgrass. Our recent study in creeping bentgrass found that the decline in cytokinin synthesis in roots preceded the decreases in water and nutrient uptake and leaf senescence during heat stress, suggesting that cytokinin may be the early root signal inducing changes in shoots, but this deserve further investigation

## **Efforts to Identify the Colonial Bentgrass Contribution to Dollar Spot Resistance in Colonial x Creeping Bentgrass Interspecific Hybrids**

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Creeping bentgrass (*Agrostis stolonifera* L.), an important turfgrass species, is highly susceptible to dollar spot disease caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett. Colonial bentgrass (*A. capillaries* L.) is a closely related species that generally has good dollar spot resistance. Interspecific hybrids between creeping and colonial bentgrass have been made and several hybrids exhibited excellent resistance to dollar spot. It may be possible to introgress the dollar spot resistance from colonial bentgrass into creeping bentgrass. We have generated a backcross population between one of the hybrids and creeping bentgrass that was field-tested in the summer of 2003. Approximately 25% of the backcross progeny exhibited resistance. These plants will be evaluated again in 2004.

We are taking multiple approaches to investigate the basis for the observed resistance, which is presumably originating from the colonial bentgrass parent of the hybrids. One approach is bulked segregant analysis and random amplified polymorphic DNA (RAPD) to identify DNA markers linked to the colonial bentgrass-derived genetic contribution to the dollar spot resistance in the progeny. PCR amplified DNA bands present only in the hybrid and absent from the creeping bentgrass parent were considered to be colonial bentgrass-derived markers. The markers were scored for presence or absence in the resistant and susceptible individuals. We have identified some markers that are found more often in the resistant individuals than the susceptible individuals, suggesting this approach may be promising. Identification of markers linked to dollar spot resistance would be useful in future marker-assisted selection for dollar spot resistance.

Another approach is suppression subtractive hybridization to identify genes overexpressed in the hybrid relative to its creeping bentgrass parent. Using the suppression subtractive hybridization method we have generated a library enriched for genes over-expressed or unique to one of the hybrids relative to the creeping bentgrass parent. Several of the differentially expressed genes are associated with disease resistance in other species. We are currently characterizing these genes to determine if they segregate with dollar spot resistance in the backcross progeny.

## **Bispyribac-Sodium Herbicide for *Poa annua* and *Poa trivialis* Control in Cool-Season Turfgrass**

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Bispyribac-sodium is a new herbicide for postemergence control of annual bluegrass (*Poa annua* L.) in cool-season turfgrass. Previous studies have shown postemergence applications of bispyribac reduced populations of both annual bluegrass and roughstalk bluegrass (*Poa trivialis* L.) in stands of creeping bentgrass (*Agrostis stolonifera* L.) and Kentucky bluegrass (*Poa pratensis* L.). However, the level of preemergence activity of bispyribac herbicide on cool-season turfgrass is not well understood. Field experiments were conducted in the fall of 2002 and 2003 at Adelphia, New Jersey to investigate the soil residual activity of bispyribac on three cool-season turfgrass species. Soil type was a Holmdel sandy-loam with a pH of 6.5 and 2% organic matter content. Bispyribac (0.15 and 0.3 kg ai/ha) was applied to an established stand of Kentucky bluegrass at 6, 4, 2, and 1 week before seeding (WBS). All applications were made with a CO<sub>2</sub> backpack sprayer delivering 374 L/ha. The existing turf was controlled with a non-selective herbicide sprayed one week before seeding to facilitate evaluations of desired seedlings. Creeping bentgrass 'L-93', perennial ryegrass (*Lolium perenne* L. 'Pizzazz'), and Kentucky bluegrass 'Kenblue' were verti-seeded into treated plots in early September. Ground coverage was evaluated 3 and 7 weeks after seeding (WAS) and once the following spring. Bispyribac did not reduce ground coverage of any species in 2002 but reduction of ground coverage was more prevalent in 2003. Bispyribac at 0.15 kg/ha applied 1 WBS reduced Kentucky bluegrass coverage by 69%. All bispyribac treatments made 1 WBS reduced perennial ryegrass cover. Bispyribac at 0.3 kg/ha reduced creeping bentgrass coverage when applied 1WBS.

Preemergence control of annual bluegrass was evaluated in a separate study. Bispyribac (0.07 to 0.15 kg/ha), dithiopyr (0.28 and 0.42 kg ai/ha), and bensulide (5.6 and 11.2 kg ai/ha) were applied to bare ground in early September of 2002 and 2003 at Adelphia, NJ. Bispyribac applications provided preemergence control of annual bluegrass that was comparable to or superior than both dithiopyr and bensulide in both years. This study suggests that establishment of Kentucky bluegrass, perennial ryegrass, and creeping bentgrass may be negatively affected by bispyribac applications made 1 to 2 weeks before seeding. In addition, bispyribac appears to have substantial preemergence activity on annual bluegrass when applied to bare ground.

## White Grub Management with Nematodes and Pheromones

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A complex of white grub species are the major turfgrass insect pest in the northeastern United States. The oriental beetle, *Exomala* (= *Anomala*) *orientalis*, is the most important white grub species in New Jersey and neighboring areas. Other species that reach damaging population levels include the Japanese beetle, *Popillia japonica*, the northern masked chafer, *Cyclocephala borealis*, the European chafer, *Rhizotrogus majalis*, and the Asiatic garden beetle, *Maladera castanea*.

### Wonderworm

In previous laboratory, greenhouse, and microplot field studies we had demonstrated the exceptional pathogenicity to a wide range of white grub species of *Steinernema scarabaei*, a new entomopathogenic nematode species isolated from epizootics in Japanese beetle and oriental beetle larvae in turfgrass areas in New Jersey. Laboratory studies indicated that this species is rather specialized to scarab larvae as hosts. To present, attempts at in vitro mass production of this nematode have been unsuccessful. It appears that more in depth studies of the particular nutritional/physiological requirements of this nematode and its symbiotic bacteria will be necessary to overcome problems with its in vitro production. However, this species is so exceptionally pathogenic to white grub, particularly to species that are not susceptible to presently commercially available nematodes (e.g. European chafer, May/June beetles, oriental beetle) that in vivo production should be a viable option. Thus, *S. scarabaei* reared in wax moth larvae are equally pathogenic to oriental beetle larvae as *S. scarabaei* reared in oriental beetle larvae, and variability in progeny production in wax moth larvae should not be difficult to overcome with some concerted effort.

Presently we are studying the potential of this species for long term white grub suppression, particularly with respect to low application rates. In September 2002, we treated 1.5-m<sup>2</sup> turfgrass enclosure containing 10 oriental beetle larvae per 0.1 m<sup>2</sup> with *S. scarabaei* at rates of 0, 0.4, 1.0, or 2.5 x 10<sup>9</sup> infective juvenile nematodes (IJs) per ha. At 31 DAT, every single larva recovered in the 3 nematode treatments had been killed by *S. scarabaei*. Based on our previous field studies, this extremely high efficacy could only have been achieved through additional infections caused by nematodes emerged from hosts infected by the originally applied nematodes. This hypothesis was also supported by the number of *S. scarabaei* that was baited out of soil samples taken from the plots. Compared to the levels observed in samples taken directly after application, the number of *S. scarabaei* baited had remained at the same level at the highest rate, and had increased 4-fold in the medium and lowest rate. Sampling in late April 2003 still showed 100% oriental beetle control, and *S. scarabaei* numbers baited from the soil had remained at the same levels as 31 DAT. Between late April and mid-August, *S. scarabaei* numbers

declined dramatically but were still similar to the numbers directly after application. In October 2003, oriental beetle populations in the control microplots were at  $8.1 \pm 3.1$  per  $0.1 \text{ m}^2$  but were 62-93% lower in the plots of the 3 *S. scarabaei* treatments. Surprisingly, *S. scarabaei* populations were very low.

A second long-term experiment was started in September 2003 using *S. scarabaei* rates of 0, 0.06, 0.12, 0.24, and  $0.6 \times 10^9$  IJs per ha. Probably due to cooler temperatures compared to the previous year, control rates at 34 DAT were only 10, 50, 77, and 87% from the lowest to the highest rate. However, *S. scarabaei* numbers baited from soil samples had increased 3.5- to 32.9-fold in the 4 nematode treatments compared to the levels observed in samples taken directly after application. If these nematode populations persist as well as in the first experiment, it can be expected that additional grub infection will occur in spring, giving the nematode populations another boost for improved persistence through summer.

### No Sex

Studies in blueberries and ornamental nurseries at Rutgers University (Drs. Polavarapu and Lashomb) have indicated the feasibility of mating disruption technology to manage oriental beetle populations. The sex pheromone of the oriental beetle consists of a 9:1 blend of (*Z*)-7-tetradecen-2-one and (*E*)-7-tetradecen-2-one. In 2002 we conducted the 1<sup>st</sup> mating disruption study in turfgrass. The plots measured 0.4 to 0.5 ha in size and were separated from each other by at least 90 m distance. Traps with rubber septa at 300  $\mu\text{g}$  concentration of (*Z*)-7-tetradecen-2-one were placed in each plot to monitor beetle flight and to determine whether pheromone application would reduce the number of male beetles finding the traps. The treatment plots were sprayed with sprayable pheromone twice at the rate of 50 g a.i./ha 1 wk and 3 wk after the first males were captured in traps. Male oriental beetle captures compared to the control plots were reduced by 87% and larval populations were reduced by 68%. Using the same methodology, in 2003 we compared the efficacy of a single pheromone application at 75 g a.i./ha applied 1 wk after first male capture to two applications at 12.5 g a.i./ha applied 1 wk and 3 wk after first male capture. Trap captures and larval populations were reduced by 88% and 74%, respectively, in the 2 x 12.5 g a.i./ha treatment, and by 74% and 71%, respectively, in the 1 x 75 g a.i./ha rate treatment.

To optimize the mating disruption technology we need to test the effect of typical turfgrass management practices on the pheromone efficacy. We found that at least 20% of sprayed pheromone remains on the grass foliage if no post-treatment irrigation is applied. This would reduce efficacy if the grass were to be mowed within a few days of application and the clippings removed as is practiced on many golf course fairways. However, 50% and 63% of the pheromone remaining on the foliage was washed into the thatch by 3 mm and 6 mm, respectively, of post-treatment irrigation.

Contamination of shoes or other surfaces that come into contact with pheromone-treated turf could result in male oriental beetles being attracted to those surfaces (i.e., 'bug' nuisances). Shoes that were walked for 30 min through turf areas treated with 75 g pheromone per ha were laid out in a non-treated turf area and the number of oriental

beetle males coming to them was counted during 45 min. Shoes walked 1 d after spraying attracted 49 to 81 males whereas shoes walked 8 d after spraying attracted 0 to 10 males. Lower pheromone application rates that have been shown to be effective for mating disruption should attract fewer males and will be tested in the future. However, some degree of male attraction to shoes coming into contact with the treated areas within the first few days after treatment can still be expected. We are presently also developing granular pheromone formulations and expect that these formulations should reduce the risk of pheromone contamination considerably.

## **Poster Presentations**

## **Molecular Identification of Texas x Kentucky Bluegrass Hybrids**

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Although Kentucky bluegrass (*Poa pratensis* L.) cultivars have been developed with good turfgrass quality, irrigation is often necessary to maintain quality during heat and drought stress conditions. Interspecific hybridization between Texas bluegrass (*Poa arachnifera* Torr.), a native to Texas and Oklahoma, and Kentucky bluegrass is being utilized to incorporate genes for heat and drought tolerance into Kentucky bluegrass. The breeding technique involves an initial inter-specific hybridization between Texas and Kentucky bluegrass, followed by modified backcrossing to Kentucky bluegrass (recurrent parent). DNA markers, such as SCAR (sequence characterized amplified region) markers, can be used to differentiate species and identify inter-specific hybrids between these two species. The objective of this study was to develop specific SCAR markers for Kentucky bluegrass and Texas bluegrass and use them to identify F<sub>1</sub> and BC generation hybrids between these two species. SCAR markers were developed by sequencing a single RAPD (Random Amplified Polymorphic DNA) band and designing primers to amplify the chosen band. Two SCAR primer pairs were designed to identify Kentucky bluegrass and Texas bluegrass. Eight Kentucky bluegrass cultivars, four Texas bluegrass plants and 12 hybrids from F<sub>1</sub> through BC<sub>3</sub> generations were analyzed with the SCAR primer pairs. The respective SCAR markers differentiated between Kentucky bluegrass and Texas bluegrass plants in all cases. All F<sub>1</sub> and BC<sub>2</sub> hybrids amplified both SCAR markers. Some BC<sub>3</sub> hybrids had lost the Texas bluegrass SCAR marker indicating introgression of the Kentucky bluegrass DNA in this generation. This breeding strategy of crossing Kentucky bluegrass with Texas bluegrass could expand the adaptation of Kentucky bluegrass through transition zone areas and into southern states where better heat and drought tolerance is needed for better performance.



## Evolutionary Descent of the Phytopathogenic Fungus *Colletotrichum graminicola*

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The evolutionary history of *Colletotrichum graminicola*, the causal agent of anthracnose in turfgrass and cereal crops, was reconstructed in the form of a phylogenetic tree using nucleotide sequences drawn from 80 fungal isolates of diverse geographic and host-plant origin. Three unlinked loci were included in the analysis: (1) the internal transcribed spacer region of the ribosomal DNA, (2) a portion of the superoxide dismutase gene, which included two introns, and (3) the conserved HMG-box found within the MAT-2 idiomorph of the fungal mating loci. In total, 1,428 nucleotides from the three genes were included in the analysis. To infer the evolutionary hypothesis from the aligned sequence data, maximum likelihood optimality criterion was employed because of its ability to efficiently estimate phylogenetic tree branching pathways based on an explicit model of evolution. Analysis of the nucleotide sequences demonstrated that each of the three genes had sufficient signal to consistently establish three main groups among the *C. graminicola* isolates. In order to test whether it was appropriate to combine the individual gene sequences into a single dataset, the statistical significance of topological congruence between the individual gene trees was evaluated by comparing optimal gene tree topologies to constrained trees using the Shimodaira-Hasegawa likelihood ratio test (LRT). The LRT established that none of the individual data sets significantly rejects the topology of the others. Concordance among the individual gene trees suggests that each reflects the species phylogeny and that it is appropriate to combine the characters into a single dataset.

Interpretation of *C. graminicola*'s evolutionary pathway as reconstructed by the combined dataset demonstrates a pattern of relative genetic homeostasis. The fungus is characterized by large, widespread populations which may have served to minimize significant adaptive change in ancestral populations of the fungus. As estimated by Bayesian posterior probabilities, support for the phylogenetic reconstruction is strong, with the recovery of three main groups. *C. graminicola* isolates from corn plants form a distinct monophyletic clade well removed from the turfgrass evolutionary path. The considerable level of diversification manifested between the grass and corn lineages suggests the occurrence of a speciation event between these two host-specific populations. Among the separate branch of the turfgrass lineages the tree bifurcates into two smaller groupings: clade A and B. The fungi included within clade A are derived from both *Poa annua* and *Agrostis spp.* turfgrass host plants; nevertheless they are quite closely related to one another. In contrast, clade B splits into two smaller sub-clades that appear to be evolving with some degree of host specificity: sub-clade B1 contains only fungi isolated from *Agrostis spp.*; sub-clade B2 isolates are uniquely derived from *Poa annua* host plants. Given the patterns of evolutionary descent suggested by the data, we discuss the potential development of habitat preference arising by sympatric population subdivision, with a concurrent reduction of gene flow and physiological isolation evolving as the lineages adapt to different ecological niches.

## Physiological Responses of Creeping and Velvet Bentgrasses to Drought Stress

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Bentgrass species are among the least studied cool-season grasses in terms of drought tolerance, and thus basic knowledge into water use requirements and relative drought tolerance of these increasingly utilized species is greatly needed. The objectives of this growth chamber study were to examine and compare drought tolerance and water use between creeping (*Agrostis palustris*) and velvet (*Agrostis canina*) bentgrasses in response to drought stress, and to determine major physiological factors associated with drought tolerance. Plants were subjected to two soil moisture treatments: (i) well-watered control: watered to field capacity 3x per week; (ii) drought stressed: irrigation completely withheld from entire soil profile. General drought performance under different soil moisture treatments was evaluated with visual turf quality ratings based on color, density, and leaf wilting. Turf water use was determined by measuring soil moisture depletion using time domain reflectometry (TDR) as well as leaf relative water content. Changes in photosynthesis, leaf osmotic adjustment, and rooting were examined to determine major physiological factors associated with drought tolerance.

Single leaf photosynthesis rates decreased similarly between creeping and velvet bentgrasses, and so did not seem to account for differences observed between non-stressed and stressed plants. Generally, velvet bentgrass plants were less sensitive to drought stress compared to creeping bentgrass, as manifested by maintaining better quality and prolonged leaf turgidity. Enhanced drought performance in velvet bentgrass was associated with higher soil moisture content and leaf water content, indicating that velvet bentgrasses utilized water more slowly. Different rates of water consumption could be related to root distribution: creeping bentgrasses had a greater number of roots in upper soil layers compared to velvet bentgrasses prior to initiation of drought, which could account for increased water use and faster depletion of available water supplies. Osmotic adjustment (OA) increased significantly with drought for both species, but the extent of OA was greater in velvet compared to creeping bentgrass. Greater leaf OA could account for enhanced maintenance of water content and leaf turgidity in velvet bentgrass plants, thus contributing to greater drought tolerance. Further work is in progress to determine the importance of specific compounds for the contribution of OA.

## The Possible Roles of Volatile Compounds in Insect Resistance of Turfgrasses

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Endophyte-infected turfgrasses often exhibit superior resistance to insects and disease and may be more tolerant of environmental stress. Turfgrasses that contain endophytes may require fewer pesticide treatments, and usually maintain their appearance longer during periods of drought and heat stress. Insect resistance of endophyte-infected grasses has been correlated with the production of alkaloids, however; in our previous research we were unable to correlate a particular alkaloid profile with insect resistance.

There may be other lines of defense against insects in addition to alkaloids. Some insects locate a suitable host by identifying and following the volatile organic compounds (VOCs) that are emitted by plants. Plants in turn produce jasmonic acid (JA) in response to insect feeding, and JA induces the synthesis of predator-attracting monoterpenes. In the grasses, several isomers of the monoterpene ocimene are the major volatiles produced by damaged plants. VOCs, however, may also play a role in the repulsion of insects or may be directly toxic to insect feeding. In addition to JA, a number of 5-10 carbon unsaturated aldehydes, ketones and alcohols are produced by damaged plants. In the following experiments we identified the VOCs from mechanically damaged ryegrass and determined the effects of individual compounds on insect feeding behavior.

Volatile compounds produced by intact plants and ground leaf tissue were collected by a purge-and-trap procedure and analyzed by gas chromatography/mass spectrometry. More than 40 volatiles were consistently identified and classified as hydrocarbons, aldehydes, alcohols, ketones, esters, terpenes and heterocycles. Similar to our results with tall fescue (Yue et al., *Phytochemistry* 58: 935-941, 2001), two compounds, 3-hexen-1-ol acetate (43%) and nonanal (40%) comprised most of the total volatile emission from intact leaves of ryegrass. When leaves were cut or macerated a large number of unsaturated C5-C10 aldehydes, ketones and alcohols were produced, but the levels of hydrocarbons and monoterpenes remained about the same.

Insect feeding studies were conducted with second instar fall armyworm larvae (*Spodoptera frugiperda*) in which the insects were fed an artificial diet spiked with each of the volatile compounds identified above. The results indicated that there were two classes of toxic compounds: , -unsaturated secondary alcohols or ketones, and , -unsaturated dienals. In the first group, 1-penten-3-one, 1-penten-3-ol, 1-hexen-3-ol and 1-octen-3-ol were all very toxic. 1-octen-3-ol has been reported to deter feeding of banana slugs, but this is the first report of its activity against an insect pest of turfgrass. It is also interesting to note that maceration of endophyte mycelia from liquid cultures produces large amounts of 1-octen-3-ol. (Z)-3-hexen-1-ol, (E)-3-hexen-1-ol and (E)-2-hexen-1-ol were moderately toxic, indicating that the unsaturated secondary alcohols were more toxic than their primary alcohol analogues. 3-pentanone and 1-hexanol were only slightly

toxic indicating the importance of unsaturation in the carbon chain. The most toxic aldehydes were the  $\alpha,\beta$ -unsaturated conjugated dienals such as (E,E)-2,4-hexadienal and (E,E)-2,4-decadienal. (*E*)-2-hexenal was moderately toxic to fall armyworm. (*E*)-2-hexenal is produced in large amounts by damaged leaves and has potent anti-microbial activity, but this is the first report of its activity against insects. In contrast, the major volatiles from intact leaf tissue (3-hexen-1-ol acetate and nonanal) and the ocimene isomers had no effect on insect feeding.

Plants may possess several lines of defense against insect predation including the production of toxic VOCs. Selection of grasses that produce high amounts of these compounds after insect feeding may be a useful strategy for defense against phytophagous insects.

## **Predicting Saturated Hydraulic Conductivity by Soil Particle- and Pore-Size Distributions**

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Saturated hydraulic conductivity,  $K_s$ , of root zone mixes is an essential parameter to predict infiltration, solute transport and other processes involved in the design and construction of golf course putting greens. Due to the high variability in  $K_s$  measurements, it is desirable to have predictive model based on available soil properties (texture, bulk density, particle size distribution and porosity). Models that are physically sound have the additional advantage of predicting properties in situations in which measurements are lacking.

Two of the most common models to predict  $K_s$  are: 1)  $K_s = Bd^x$ , and 2)  $K_s = C\Phi_e^n$  where  $d$  is a representative particle diameter obtained from a particle-size  $\Phi_e$  distribution, is an effective porosity obtained from water retention characteristic curves,  $B$  and  $x$ , and  $C$  and  $n$  are constants related to particle- and pore-size distributions, respectively. Our hypothesis is by combining information on particle and pore distributions we can reduce the number of empirical variables used in a model. Consequently, our objective is to develop a model to predict  $K_s$  that incorporates information on both properties. Particle-size distributions can be measured directly, whereas pore-size distributions are inferred from soil water retention curves.

Eight sandy materials were sampled and measured. Particle-size distributions were determined by sieving (sand size) and by the pipette method (clay content), with silt content determined as the difference between 1 and the sum of the sand and clay fractions; water retention curves were obtained by combination of the hanging column (-0.1 kPa to -5.0 kPa) and the pressure plate method (-5.0 kPa to -1500 kPa); and  $K_s$  was measured with the constant head method. Our data followed models 1 and 2. By defining  $n$  in model 2 as the slope of water retention curves,  $C$  was better related to  $d$  defined as the median of a particle-size distribution. This result underlines the potential for developing theoretical links integrating information on particle- and pore-size distribution in a single model.

## **Protein Alterations in Bentgrass in Response to Heat Acclimation and Direct Heat Stress**

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The acclimatization of plants to moderately high temperature plays an important role in inducing tolerance to subsequent lethal high temperatures. This study was performed to investigate the effects of heat acclimation and direct heat stress on protein degradation and synthesis in creeping bentgrass (*Agrostis palustris* Huds.). Plants were subjected to 5 different temperatures from 20 to 40 °C at 5 °C intervals (heat acclimation) for 1 week at each level of temperature or directly from 20 to 40 °C and maintained at this temperature for 1, 2, 3, 5, and 7 weeks (direct stress) in growth chambers. Heat acclimation induced expression of HSP60 as compared with direct heat stress. Direct heat stress induced HSP24 and HSP64 during 1-7 week of exposure. HSP36 was induced at 2 - 7 weeks of heat stress, and HSP95 at 5 and 7 weeks. Protein content decreased under both heat acclimation and heat stress. Soluble protein was more susceptible to high temperature with significant decline initiated at 25 °C while the total protein decreased when plants were exposed to 30 °C. The results indicated that heat stress caused protein degradation and also induced expression of HSPs. It is suggested that thermotolerance of creeping bentgrass induced by heat acclimation, as reported in previous studies, may be related to the induction of new protein, rather than protein degradation.

## **Anthracnose as Influenced by Nitrogen, Growth Regulators, Pre-emergence Herbicides, and Verticutting**

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The effect of nitrogen, the growth regulators Embark (mefluidide) and Primo MAXX (trinexapac-ethyl), and verticutting on the severity of anthracnose was assessed on *Poa annua* putting green turf at Rutgers University from April to October, 2003. The study was arranged as a 2 x 2 x 2 x 2 factorial design with four replications and turf was mowed at a minimum of ten times per week at 3.2 mm (0.125 in). Nitrogen was applied at 4.9 kg ha<sup>-1</sup> (0.1 lb. N/1000 ft<sup>2</sup>) every 7 days (~3.0 lbs/yr) or 4.9 kg ha<sup>-1</sup> (0.1 lb. N/1000 ft<sup>2</sup>) every 28 days (~1.5 lbs/yr). Embark 0.2 L treatments were applied twice at 2.2 L ha<sup>-1</sup> (0.7 fl. oz./1000 ft<sup>2</sup>) on 14 and 28 April. Primo MAXX 1MC was applied every two weeks throughout the season at 0.4 L ha<sup>-1</sup> (0.125 fl. oz./1000 ft<sup>2</sup>) beginning 14 April and continuing through September. Verticutting was conducted on plots at a 3 mm (0.12 in.) depth and 13 mm (0.5 in.) spacing every two weeks from 31 May to September. Curalan 50 DF 3.1 kg ha<sup>-1</sup> (1.0 oz/1000 ft<sup>2</sup>) was applied to the entire study on 13 and 31 May, 17 and 28 June, and 13 July to prevent dollar spot, since previous studies conducted on this site had confirmed that this product does not affect the development of anthracnose on *P. annua* greens.

Anthracnose developed from a natural infection in June and became well established on half of the trial by early July. The other half of the study was artificially inoculated with *Colletotrichum graminicola*, the causal agent of anthracnose, on 6 July with 7 x 10<sup>4</sup> conidia/mL to ensure uniform disease development. Turf receiving the high rate of nitrogen had 37 to 65% less disease than turf maintained with the low rate of nitrogen. Contrary to our original hypothesis that Embark would reduce anthracnose on *P. annua* greens by decreasing seedhead development and increasing turf vigor, Embark enhanced disease severity in June (six to eight weeks post-treatment), and had no effect on anthracnose from July through October, compared to turf not treated with this plant growth regulator. Embark did, however, reduce the number of seedheads per unit area and increased turf quality before the onset of disease. Repeat applications of Primo improved turf quality, increased turf density, and reduced anthracnose severity, compared to non-Primo treated turf.

The influence of verticutting on anthracnose was variable and dependent on the presence or absence of plant growth regulators and the nitrogen rate used. In the presence of Embark, Primo, or the sequential application of Embark and Primo, anthracnose was enhanced by verticutting at low nitrogen, but was unaffected at the high nitrogen rate. It would appear that stress related factors (e.g., low nitrogen fertility or growth regulation) may weaken turf or delay the healing of wounds created by verticutting and, therefore, may enhance the development of anthracnose in the presence of this cultivation treatment. Additional research, however, is needed to verify this hypothesis.

Finally, in a separate part of the study, the pre-emergence herbicides Dimension 1EC (dithiopyr) and Bensumec 4LF (bensulide) applied once on 28 April at 3.5 L ha<sup>-1</sup> (1.1 fl oz/1000 ft<sup>2</sup>) or 20.5 L ha<sup>-1</sup> (6.45 fl oz/1000 ft<sup>2</sup>), respectively, significantly increased disease, compared to similarly maintained turf without pre-emergence herbicides. The authors would like to thank the Tri-State Research Foundation, the Golf Course Superintendents Association of New Jersey, the New Jersey Turfgrass Foundation, and the Rutgers Center for Turfgrass Science for supporting this research.



## Identification of a Disease-Responsive Gene Present in Creeping Bentgrass But Not in Other *Agrostis* Species

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Creeping bentgrass (*Agrostis stolonifera* L.), an important turfgrass species, is highly susceptible to dollar spot disease caused by the fungus *Sclerotinia homoeocarpa* F. T. Bennett. Colonial bentgrass (*A. capillaris* L.), a closely related species, has good resistance. Several interspecific hybrids between creeping bentgrass and colonial bentgrass showed excellent dollar spot resistance in field tests (Belanger et al., 2004). In order to investigate the mechanism of dollar spot resistance in the interspecific hybrids, we are using suppression subtractive hybridization (SSH) to detect genes unique or overexpressed in either creeping bentgrass or an interspecific hybrid following fungal inoculation. RNA samples of a resistant hybrid (#15) and its susceptible creeping bentgrass parent were extracted from leaf tissues of dollar spot-inoculated plants. Two cDNA libraries were constructed using SSH to enrich for hybrid-specific clones and creeping bentgrass-specific clones. A disease-responsive gene was identified from two of the clones from the creeping bentgrass-specific library. The full-length sequence of the cDNA was obtained through RACE (rapid amplification of cDNA ends) PCR. The gene encoded a 319 amino acid protein of 34753 Daltons. The amino acid sequence of the protein was homologous to a wheat benzothiazol-induced protein, a wheat Hessian fly response protein, a maize beta-glucosidase aggregating factor, a barley jasmonate-regulated gene, and a wheat vernalization-related gene. CDART (conserved domain architecture retrieval tool) analysis revealed it had two conserved domains, a plant disease resistance response protein domain and a jacalin-like lectin domain. Northern blot analysis confirmed its expression was increased in creeping bentgrass upon dollar spot infection. No expression was detected in hybrid #15 or colonial bentgrass from either greenhouse or field samples. These results validated the effectiveness of the SSH method. Southern blot analysis indicated creeping bentgrass and another hybrid (#14) each had a single copy of this gene. Interestingly we didn't detect any homologs from related bentgrass species, such as colonial bentgrass, velvet bentgrass (*A. canina*), or redtop (*A. gigantea*). The apparent uniqueness of this gene to creeping bentgrass is very interesting regarding the evolution of creeping bentgrass.

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## Search for RAPD Markers Linked to Dollar Spot Resistance in Colonial x Creeping Bentgrass Hybrids

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Dollar spot, caused by *Sclerotinia homoeocarpa* F. T. Bennett, is among the most serious diseases of creeping bentgrass (*Agrostis stolonifera* L.), an outstanding cool season turfgrass species that is extensively used on golf courses. Currently, management of dollar spot relies heavily on the use of fungicides. Durable genetic resistance is much more desirable given the development of fungicide resistance and restrictions on fungicide usage. Colonial bentgrass (*A. capillaris* L.), a closely related species that is also occasionally used on golf courses, is recognized by breeders as having good resistance against dollar spot. Interspecific hybridization followed by backcrossing, which has been successfully used in breeding improved cultivars of numerous crop species, may be a useful approach to introduce disease resistance from colonial bentgrass into creeping bentgrass (Belanger et al., 2003; Belanger et al., 2004). In the summer of 2002, a dollar spot resistant colonial x creeping hybrid was crossed with a creeping bentgrass plant. In the spring of 2003, a population of 282 progeny individuals (four replicates of each individual) was planted into a field plot in a randomized complete block design. The field was inoculated with the dollar spot pathogen. The plants were rated throughout the summer for percent disease turf. We are using bulked segregant analysis and random amplified polymorphic DNA (RAPD) to identify DNA markers linked to the colonial bentgrass-derived genetic contribution to the dollar spot resistance in the progeny. PCR amplified DNA bands present only in the hybrid and absent from the creeping bentgrass parent were considered to be colonial bentgrass-derived markers. The markers were scored for presence or absence in the resistant and susceptible individuals. The markers highly associated with the resistant group will be converted to sequence-characterized amplified region (SCAR) markers. These DNA markers will be useful in future marker-assisted selection for dollar spot resistance.

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## Potential Use of Bispyribac for Annual Bluegrass Control in Cool-Season Turfgrass

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Previous studies have shown that bispyribac-sodium has activity on annual bluegrass (*Poa annua* L.) in creeping bentgrass (*Agrostis stolonifera* L.). However, the relative tolerance of other cool-season turfgrass is not well understood. Field experiments were conducted in the summer of 2002 and 2003 at Adelphia, New Jersey to evaluate the tolerance of four cool-season turfgrass species to varying rates of bispyribac. Bispyribac was applied at 37, 74, 111, 148, and 296 g ai/ha to mature stands of Kentucky bluegrass (*Poa pratensis* L. 'Gnome'), perennial ryegrass (*Lolium perenne* L. 'Jet'), tall fescue (*Festuca arundinacea* Schreb. 'Houndog 5'), and Chewings fine fescue (*Festuca rubra* L. ssp. *commutata* Gaudin 'Shadow II'). All applications were made to 0.9 x 3.0 m plots with a CO<sub>2</sub> backpack sprayer delivering 374 L/ha. Visual injury was evaluated and clippings were collected from the interior of each plot at 35 and 70 days after treatment (DAT) to determine the response of each species. Clippings were dried and weighed and expressed as percent of untreated check. Visual injury on all species at 35 DAT increased with increasing bispyribac rate. Kentucky bluegrass injury reached 27% when bispyribac was applied at 296 g/ha. Injury on other species did not reach 20%. Initial injury was primarily in the form of discoloration on perennial ryegrass, tall fescue, and fine fescue. Kentucky bluegrass exhibited more severe stunting and thinning symptoms. Bispyribac at 37 to 296 g/ha reduced Kentucky bluegrass clipping weights by 5 to 35%, respectively, as compared to the untreated check at 35 DAT in 2002. Initial perennial ryegrass, tall fescue, and fine fescue injury dissipated to 5% or less by 70 DAT. However, recovery of Kentucky bluegrass was less complete.

These studies suggest that bispyribac can severely injure Kentucky bluegrass. Kentucky bluegrass may not adequately tolerate bispyribac at rates necessary for annual bluegrass control. Perennial ryegrass, tall fescue, and fine fescue may show initial symptoms of injury, but levels are less severe and persistent than those exhibited by Kentucky bluegrass.

## **Traffic Tolerance of Cool-Season Turfgrasses**

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Cool season turfgrass species established to athletic fields and other turf areas are often subjected to intense traffic. In many cases, the quality of these turfgrass stands deteriorates significantly due to wear and compaction resulting from traffic. The objective of this research was to evaluate the traffic tolerance of Kentucky bluegrass, tall fescue, and perennial ryegrass cultivars and selections and determine individual cultivars and selections within each species that demonstrated improved tolerance to traffic. Turfgrass entries comprising the 2000 National Turfgrass Evaluation Program (NTEP) Kentucky bluegrass test and 2001 NTEP tall fescue test were evaluated. Varieties and selections as part of a 1999 Rutgers University perennial ryegrass test were also evaluated for traffic tolerance. Turfgrass wear was created using a Toro Groundsmaster unit fixed with a modified Sweepster equipped with rubber paddles. Wear was applied by operating the machine over approximately one-half of each plot two or more times per application. Soil compaction was created by utilizing a 1350 kg Wacker roller. Similar to wear applications, compaction was applied by passing over one-half of each plot two or more times per application. As a measure of traffic tolerance, turfgrass quality was visually assessed on a scale of 1-9 (9=highest quality) monthly during the growing season. The following Kentucky bluegrass entries showed the highest quality ratings when subjected to traffic in 2002: Princeton 105 (7.3), Tsunami (6.9), Midnight II (6.7), Award (6.7), Avalanche (6.7), Nu Destiny (6.5), Awesome (6.3), Total Eclipse (6.2), Barrister (6.2), Ginney (6.2), J-1838 (6.2), Cabernet (6.1), Impact (6.1), Moon Shadow (6.1). In 2002, traffic tolerance was highest among 42 tall fescue varieties and selections when rated for turfgrass quality. Of these statistically equivalent entries, the following displayed turfgrass quality ratings of 6.0 or greater in 2002: Titan Ltd. (6.8), GO-FL3 (6.4), Elisa (6.4), Bingo (6.3), Jaguar 3 (6.3), DLSD (6.2), 01-ORU1 (6.2), 01-RUTOR2 (6.2), Blackwatch (6.1), Millennium (6.1), F-4 (6.1), Masterpiece (6.0), Forte (6.0). Sixty-two perennial ryegrass varieties and selections comprising a 1999 Rutgers University perennial ryegrass test showed the highest turfgrass quality under traffic. Of these top performers, the following commercially available varieties were given mean quality ratings greater than 6.0: Prowler (7.3), Stellar (6.8), Courage (6.8), Kokomo (6.7), Grand slam (6.6), Gator 3 (6.4), Pacesetter (6.3), SR 4220 (6.3), Premier II (6.2), Manhattan 4 (6.2), Divine (6.1), Gallery (6.1), Jet (6.1), Paradigm (6.0), Citation fore (6.0), Summerset (6.0).

## **Accumulated Heat Thresholds for Bentgrasses**

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Bentgrasses often suffer from heat stress during hot summer months. This study was conducted to 1) determine cultivar and species differences in heat tolerance of bentgrasses, 2) examine the sensitivity of different physiological and morphological parameters to heat stress, and 3) determine heat accumulation units for summer bentgrass decline. A field study was conducted from May to October in 2002 and 2003. Nine cultivars of creeping bentgrass and two cultivars of velvet bentgrass were seeded in a randomized block design and mowed at two mowing heights. Turfgrass quality, photosynthesis rate, root morphology, and leaf chlorophyll, water, and protein content were measured bimonthly. Data from 2002 indicate that Penn A-4 and both velvet bentgrass cultivars were generally the most heat tolerant. Photosynthesis was the most sensitive parameter, while turfgrass quality was affected later in the summer than other physiological parameters. Heat accumulation units that induce physiological damages will be presented for the 2002 season.

## **Suppression Subtractive Hybridization to Identify Genes Overexpressed in Colonial x Creeping Bentgrass Hybrids Relative to Their Creeping Bentgrass Parent**

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Creeping bentgrass (*Agrostis stolonifera* L.), an important turfgrass species, is highly susceptible to dollar spot disease caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett. Colonial bentgrass (*A. capillaries* L.) is a closely related species that generally has good dollar spot resistance. Interspecific hybrids between creeping and colonial bentgrass have been made and several hybrids exhibited excellent resistance to dollar spot (Belanger et al., 2004). The molecular basis for this resistance can be investigated by using suppression subtractive hybridization (SSH) to detect low copy number transcripts that are unique or overexpressed in either the resistant hybrids or the creeping bentgrass parent following fungal inoculation. SSH is a method that allows one to subtract cDNAs from two samples, such as different physiological states or tissues, and then isolate genes that are transcribed at higher levels in one sample relative to the other. A benefit of this technique is that low and high copy number transcripts are normalized with respect to each other allowing for a higher chance of isolating rare transcripts. Two subtraction libraries were created from creeping and colonial bentgrass cDNAs, one is enriched for genes overexpressed in the hybrid relative to creeping parent, and the other is enriched for genes overexpressed in the creeping parent relative to the hybrid. 960 colonies from the colonial specific library and 480 colonies from the creeping specific library were screened for differential expression using reverse northern dot blot analysis. On average, 14% of the colonies screened from both libraries were differentially expressed. Further studies will utilize either Northern analysis or reverse transcriptase real time PCR to confirm the differential expression of these cDNAs. Plasmids from the differentially expressed colonies were sequenced. To date, 45 plasmids for the creeping subtraction library and 85 plasmids from the hybrid subtraction library have been sequenced. The average insert length ranged from 150 to 500 base pairs with the average length of 400 bp. The SSH technique has a restriction enzyme step, therefore none of the resulting clones will be full-length. Most of the clones sequenced are homologous to genes related to stress resistance in other plants, and so may be relevant to the disease response of the bentgrasses.

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## **Turfgrass Germplasm Collection from Central Asia**

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Rutgers University has had formal ties with the Republic of Uzbekistan and the Republic of Kyrgyzstan for several years. Reciprocal germplasm exchange agreements have been formulated with a number of prominent research institutions, including Tashkent State Agrarian University, the Uzbek Scientific Research Institute of Fruit Growing, Viticulture and Winemaking named after R. R. Shreder, the Uzbek Scientific Research Institute of Plant Industry (formerly The Vavilov Institute), the Uzbek Scientific Research Institute of Vegetable-Melon and Potato Growing, the Uzbek Scientific Research Institute of Forestry, the Botany Institute and the Kyrgyz Agrarian Academy, which includes five Scientific Institutes. The 2003 - 2005 joint turfgrass project between Rutgers University and Tashkent State Agrarian University was approved by both the Uzbek Ministry of Agriculture and Water Resources and the USDA. Through this project, Rutgers scientists have successfully collected and otherwise obtained potentially valuable turfgrass germplasm from Central Asia.

The genetic improvement of North American turfgrass species by the introduction, evaluation, and incorporation of desirable traits from unique accessions from around the world has proven to be a successful strategy. The focus of collection efforts continues to be on potentially shade tolerant grasses, grasses that appear productive on marginal, overgrazed lands, and grasses resistant to heat, drought, diseases and insects. The partnerships between Rutgers and Central Asian institutions and germplasm acquisition in the region have been due to efforts and contacts by Dr. David Zaurov. Currently, turfgrass germplasm from Central Asian is not well represented in U. S. collections. Through these efforts, Rutgers University currently possesses the largest and most diverse and unique collection of Central Asian turfgrass germplasm in the U. S.

During 2003 411 accessions of turfgrass from Central Asia were collected and brought back to Rutgers, (Table 1).

Table 1. Turfgrass species collected from Central Asia, 2003.

Country	Species	Number of Accessions
Kyrgyzstan	<i>Poa pratensis</i>	11
Kyrgyzstan	<i>Lolium perenne</i>	14
Kyrgyzstan	<i>Festuca rubra</i>	32
Kyrgyzstan	<i>Festuca pratensis</i>	14
Kyrgyzstan	<i>Poa angustifolia</i>	13
Kyrgyzstan	<i>Festuca sulcata</i>	24
Uzbekistan	<i>Poa Pratensis</i>	230
Uzbekistan	<i>Lolium perenne</i>	63
Uzbekistan	<i>Festuca arundinaceae</i>	10
<b>Total accessions collected in 2003:</b>		<b>411</b>

As part of a USDA grant, we also co-sponsored turfgrass field trials of North American cultivars in Uzbekistan. This is the first turfgrass breeding and evaluation program in Central Asia, and the field plots were well received. U. S. scientists were able to provide to our Uzbeki partners seeds of turfgrass cultivars for these trails and provided technical assistance in setting up the field plots. The preliminary results of this trial have been published in the International Agronomy Journal of Uzbekistan.

Drs. W. A. Meyer, C. R. Funk, J. A. Murphy, and D. E. Zaurov have hosted delegations from both Uzbekistan and Kyrgyzstan (in 1999, 2000, 2001, 2002, and 2003). During this period, a special mini-training program was developed and tours were set-up to show them how to establish a turfgrass collection, nursery, and breeding program. This visit also provided Rutgers with the opportunity to develop potential new programs in Uzbekistan and Kyrgyzstan.

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