PROCEEDINGS OF THE NINTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

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

January 13-14, 2000
Cook College

Symposium Organizing Committee

Thomas Gianfagna, Chair
Faith Belanger
Bruce B. Clarke

Barbara Fitzgerald
Stephen E. Hart
Jim White

Proceedings of the Ninth Annual Rutgers Turfgrass Symposium

Stephen E. Hart, Barbara Fitzgerald, and Sarah Lycan, Editors

Director's Opening Remarks:

Welcome to the Ninth Annual Rutgers Turfgrass Symposium at Cook College/NJAES. This Symposium was established in 1991 to provide Rutgers faculty, students, and staff with an annual forum for the exchange of ideas on a wide range of topics in turfgrass science. In recent years, the format has been expanded to include presentations from colleagues at other institutions. I would like to thank the participants from outside the Rutgers Community as well as the Center Faculty who have agreed to present their research at this year's meeting. Their expertise and strong commitment to the advancement of turfgrass science is most appreciated.

I would also like to thank the Symposium Planning Committee, comprised of Tom Gianfagna (Chair), Steve Hart, Faith Belanger, Jim White, and Barbara Fitzgerald, for their hard work in the preparation of this year's program. They have spent many hours arranging the oral portion of the program as well as the poster session and tonight's social. Without their diligence and attention to detail, this year's Symposium would not have been possible. I am sure that I speak for everyone in attendance when I extend my congratulations for a job well done.

The new millenium is indeed an exciting time for the Rutgers Turfgrass Program. The Center Faculty have developed internationally recognized research, undergraduate, graduate, and continuing professional education, and service programs at Cook College. As these efforts have expanded, so too has the level of support from the turfgrass industry.

During the past two decades, the Turfgrass Industry has donated over two million dollars in grants, scholarships and gifts to our turfgrass program. This includes the construction of a 5,000 sq. ft. storage facility in 1996 and a new $750,000 Turfgrass Education Complex at Hort Farm II. This Education Complex will be named after Ralph Geiger, an ardent supporter of the Rutgers Turfgrass Program, and is scheduled for completion in the summer of 2000. We are indeed fortunate to have such committed and generous partners.

In 1995, the Center embarked on an ambitious plan to hire six new faculty in support of our mission at Cook College/NJAES. To date, we have attracted four outstanding scientists (Bill Meyer, Jim White, Steve Hart, and Albrecht Koppenhofer). This year, in collaboration with the Plant Science Department, we are in the process of hiring a turfgrass physiologist. It is my hope that these scientists will work closely with the current Center Faculty to meet the growing needs of the turfgrass industry.

Thank you again for coming to this year's symposium. I hope that you will find it an interesting and worthwhile experience.

Sincerely,

Bruce B. Clarke, Director
Center for Turfgrass Science

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January 13-14, 2000
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7:40 - 8:30 PM	Keynote Address: **Mr. Joseph Yoder** (Novartis Crop Protection, Inc.) *Food Quality Protection Act of 1996 and Its Impact on the Turfgrass*

Industry

8:30 - 10:00 PM Wine and Cheese Reception

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(Moderator: Dr. Thomas Gianfagna)

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A Microsatellite-based PCR Assay to Identify Epichloë Endophytes In Planta

9:20 - 9:40 **Dr. James White** (Department of Plant Pathology, Rutgers University)
Epiphyllous Stages of Endophytes: Mechanisms for Genetic Change of Endophytes and Enhanced Fungus Disease Resistance in Endophyte-Infected Turfs

9:40 - 10:00 **Dr. Robert Tate, III** (Department of Environmental Sciences, Rutgers University) *Microbial Community Development in Greens Mixes and Its Role in Manageability of Golf Greens*

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Impact of Cultural Management Practices on the Development of Gray Leaf Spot in Cool-Season Turfgrasses

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Plenary Presentations

Food Quality Protection Act of 1996 and Its Impact on the Turfgrass Industry

Joseph F. Yoder
Novartis Crop Protection, Greensboro, NC

The Food Quality Protection Act of 1996 (FQPA) was passed by unanimous vote in the US congress on August 3rd, 1996. The bill provided for no transition period and was effective immediately upon signing by President Clinton. FQPA includes major new provisions that present substantial challenges to the regulatory and registrant communities. Among these are: the "reasonable certainty of no harm" safety standard, aggregate and cumulative risk assessments, an additional 10 fold margin of safety for children, inclusion of Section 18s tolerances in the overall risk assessment process, requirements of testing for endocrine disruption testing requirements, and that all of the 9000+tolerances be reviewed by Aug. 3rd, 2006.

EPA has substantial freedom in their implementation of the law and is allowing an increased politicization of the regulatory process to occur. The new provisions require that new regulatory policies, testing protocols and methods of risk assessment be developed. I believe that we currently have neither the scientific consensus nor the regulatory structure to adequately review a large portion of the tolerances as required by this law.

Unrealistically high risk assessment driven by the use of worst case "default" assumptions will likely lead to the loss of many uses currently important to the turfgrass industry. In many cases the data bases needed to support turf uses are weak, the companies have historical and emotional ties to their agricultural uses, and the economic base for the active ingredient is in the main agricultural markets. When faced with jeopardizing primary agricultural uses or dropping / seriously restricting turf uses, I believe that many providers of agricultural pesticides will choose the latter.

An overview of FQPA, reasons why turfgrass pesticide uses are at risk, examples of potential losses, and options for action will be covered in the presentation.

A Microsatellite-based PCR Assay to Identify Epichloë Endophytes in Planta

Christina Moon, *Department of Plant Pathology, University of Kentucky, Lexington, KY*

Epichloë endophytes are a group of filamentous fungi that include both sexual (*Epichloë*) and asexual (*Neotyphodium*) species. As a group they are genetically diverse, and form associations with cool season grasses (subfamily Pooideae) that range from mutualistic to antagonistic. A microsatellite-based PCR assay is described for in planta fingerprinting this group of fungi is described. Partial genomic libraries in M13mp19 were constructed with size-fractionated genomic DNA from endophyte isolates *E. typhina* E8 and *Neotyphodium* sp. Lp1. These libraries were screened at low stringency with a mixture of digoxygenin-labeled dinucleotide and trinucleotide repeat oligonucleotide probes. Positive clones were sequenced, and ten unique microsatellite loci were identified. Additional microsatellite loci were identified from *E. bromicola* (Groppe et al., 1995), and serendipitously from *N. lolii* isolate Lp19 in the 3' untranscribed region of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase gene. Primers were designed for each locus and a panel of endophytes, representing a range of *Neotyphodium* spp. and strains and all *Epichloë* spp., were screened to determine the degree of microsatellite length polymorphism. Eight loci were particularly informative for strain identification, and two multiplex PCR primer sets were developed to amplify these loci. Primers were fluorescently labeled to enable the size of the amplified alleles to be precisely determined by automated analysis, and an allele profile for each strain was readily generated. A reference database of allele sizes has been established for the Epichloë endophyte isolates examined, and this will be expanded as new strains are analyzed. The assay was shown to resolve endophyte groups to the level of known isozyme phenotype groups. Template DNA prepared from either culture or infected plant material can be amplified, and in a blind test the assay was used successfully to identify a set of endophytes in planta. In addition to endophyte identification, microsatellite fingerprints were shown to support known evolutionary relationships within the Epichloë group, particularly of known interspecific hybrid endophytes where multiple alleles were often amplified at a single microsatellite locus.

Epiphyllous Stages of Endophytes: Mechanisms for Genetic Change of Endophytes and Enhanced Fungus Disease Resistance in Endophyte-Infected Turfs

James White, Marshall Bergen, Adam Freehoff, Bill Meyer, Melinda Moy, Raymond Sullivan, and Faith Belanger, *Department of Plant Pathology, Rutgers University*

Species of Epichloë have given rise to the asexual Neotyphodium endophytes. These species perennate in the leaves, rhizomes, and culms of infected grasses and grow into the seed when it is produced, infecting the embryo within the seed. It has long been assumed that Neotyphodium endophytes lack the capacity for external spread or genetic interaction and that they remain endophytic at all times. We have observed that many grasses infected by endophytes commonly demonstrate an epiphyllous network of mycelium on which conidia are produced. In a recent study Moy et al (see poster) have shown that epiphyllous mycelium and endophytic mycelium are part of the same life cycle. It is important to evaluate what conditions stimulate development of the epiphyllous mycelium on grass leaves. These studies are important because they may demonstrate that Neotyphodium endophytes possess an external potentially spreading stage and may help explain the variability seen among Neotyphodium endophytes. An epiphyllous stage may provide the site for interactions between individuals of Neotyphodium endophytes. Parasexual interactions on leaf surfaces could explain the polymorphisms in many genes shown to occur in high frequency among Neotyphodium endophytes.

Recent data suggests that in the absence of sexual reproduction, genomes of Neotyphodium endophytes are subject to expansion through addition of chromosomes of other endophytes. It is proposed that such fusions are a function of the epiphyllous stages of endophytes. Recently Rutgers researchers have demonstrated that many endophyte-infected plants show enhanced fungus disease resistance in endophyte-infected fine fescue grasses. This effect underscores the potential of endophytes in enhancing hardiness of host plants. Previously, we have believed that fungus-produced compounds may be involved in defending plants against fungal predators. However, a correlation between the occurrence of the epiphyllous mycelial state and resistance to fungal infection was demonstrated. Fungus disease resistance is proposed to be the result of a niche exclusion phenomenon whereby endophytes already occupy the surfaces of leaves thereby excluding colonization by potential pathogenic fungi.

Microbial Community Development in Greens Mixes and Its Role in Manageability of Golf Greens

E. Gaulin and R. L. Tate III, *Department of Environmental Sciences, Rutgers University*

A clear relationship between soil physical and chemical properties and turfgrass quality and resilience are easily seen. Less obvious is the fact that the microbial community serves as a intermediary between the physical environment and higher plants. That is, the soil microbes are not only affected by the nature of the plant community and the physical properties of the soils but the traits of each of these entities are altered (and under appropriate conditions) improved by the microbes. Thus, occurrence of a stable, functional microbial communities in any soil system, but especially in quasi soil systems, such as golf greens, is essential for establishment and sustenance of the aboveground community. Traditional means of assessing soil microbial community development have included determination of microbial population densities, assessment of the occurrence of specific populations (e.g., nitrifiers, denitrifiers, pseudomonads, actinomycetes, bacilli), and the quantification of individual enzyme activities (e.g., dehydrogenase, urease). These data, although interesting, have not proven to be adequate indicators of long-term stability of the golf greens. Measures of microbial diversity (metabolic and phospholipid fatty acid diversity), although reasonably non-specific, have been shown to reveal community evolution and stability in a variety of soil ecosystems. For this study, rootzone mixtures containing 10% (v/v) zeolite, Profile, sphagnum peat, or Irish peat were evaluated using these diversity measures and the more traditional assessors of microbial activity. Incubated samples of field and greenhouse mixes were grouped by metabolic diversity according to similarity of microbial community structure. Microbial diversity assessments were more useful in separating sample based on community structure than were dehydrogenase and viable bacterial population density data alone. For example, these studies suggest that mixes containing 10% Zeopro zeolite have similar community structure to that of mixes containing a traditional 10% by volume sphagnum peat.

Selective Annual Bluegrass Control - Where is Roundup-Ready Turfgrass?

Bruce Branham, *University of Illinois*, *Department of Natural Resources and Environmental Sciences, Urbana, IL*

The selective control of *Poa annua* L. (annual bluegrass) in intensively maintained turfgrass has been a goal of turf managers for the last 40 years. However, finding a herbicide that will remove annual bluegrass from other turfgrasses has proven an elusive and difficult task. Many herbicides have shown promise only to fail in actual use. Ethofumesate (trade name - PROGRASS) has shown considerable promise yet has largely failed to achieve widespread use or consistent results. The failure of ethofumesate to achieve consistent results is due to two factors. First, because of the limited use of this herbicide in agriculture, few resources were expended to understand the mode of action and factors necessary for this herbicide to achieve consistent results. While primarily used as a preemergence herbicide, ethofumesate works as a postemergence herbicide against annual bluegrass. Addition of urea or ammonium sulfate enhances the postemergence action of ethofumesate. However even if consistent results with ethofumesate could be obtained, the second factor that few fully grasp is that while turf managers despise annual bluegrass, they dislike bare ground much more. Thus, an application of an herbicide which controls 80% of the annual bluegrass in polystand containing greater than 15% annual bluegrass is unacceptable to most turf managers because it results in bare ground, requiring overseeding and a reduction in the playability of the turf. This unique situation requires that any herbicide must be able to gradually reduce the annual bluegrass without quickly controlling it.

This same problem will confront turf managers with Roundup-Ready turfgrass. A golf course renovated with Roundup-Ready turfgrass may not solve the annual bluegrass problem particularly on older turfs with a large annual bluegrass seed bank. Populations of annual bluegrass may be high enough after renovation that a turf manager who controls annual bluegrass with Roundup will be faced with a costly reseeding effort. Soil sterilization to control annual bluegrass seed in the soil may be necessary to successfully used Roundup-Ready turfgrass.

Research Directions in Turfgrass Weed Management

Stephen E. Hart and Darren W. Lycan, *Department of Plant Science, Rutgers University*

Weed management research in turfgrass represents a wide variety of challenges and opportunities for areas of study, especially for a new turfgrass weed scientist. Some of these research areas include management of problem weeds, evaluation of new weed management technologies such as biological control agents and new herbicides, environmental fate of pesticides, and evaluation and integration of the products of biotechnology (herbicide resistant turfgrass varieties) into turfgrass weed management.

In the past year, I have had an opportunity to attend a wide variety of scientific meetings in turfgrass science and interact with the turfgrass science faculty both here at Rutgers and other Universities. Several extension talks on turfgrass weed management have been presented and feedback received from landscape contractors, golf course superintendents, and sod producers. In addition, I have been able to conduct an initial year of field research during which I learned a great deal concerning experimental design and execution of turfgrass weed management trials. These experiences have allowed for the development of a turfgrass weed mangement program focused in the following areas: broadleaf weed and summer annual grass management, *Poa annua* (annual bluegrass management), tolerance of cool season turfgrasses to herbicides, and integrating herbicide resistant turfgrass varieties into turfgrass weed management.

Research and demonstration trials on broadleaf weed and summer annual grass weed (primarily crabgrass and goosegrass) management will be an integral part of the turfgrass weed management program. This research will be utilized to support extension programs geared primarily to landscape contractors and homeowners. These programs will also provide some benefit to golf course superintendents and sod producers. This area will also provide some opportunity for more focused research on problem weeds and new herbicide products. Of particular interest is quinclorac herbicide, which has potential for use on newly seeded turfgrass. In addition, little is known in the area of turfgrass variety response to this herbicide. Research studies will also be conducted to find potential new herbicides for weed management in turfgrass.

Poa annua (annual bluegrass) is a serious weed problem found in highly managed low cut turf on golf courses and athletic fields. *Poa annua* is also a problem weed in sod production. Most people find *Poa annua* aesthetically objectionable in highly maintained turf due to its pale green color. In addition, *Poa annua* will produce seed heads in the spring even under very close mowing conditions impacting playability on golf course putting greens. However, the largest problem with *Poa annua* is that a higher degree of maintenance and inputs in terms of water and fungicides are required to keep *Poa annua* alive during the summer months as compared with cool season turfgrasses. In sod production, sod cannot be certified if infested with *Poa annua*.

There are few herbicides available for control of established infestations of *Poa annua*. Therefore, management of *Poa annua* will require an integrated approach combining cultural and chemical practices. This approach will establish a synergism between the turfgrass management research program and the turfgrass weed science program. The turfgrass management research program has demonstrated the importance of planting dates when restoring bentgrass greens previously infested with *Poa annua*. In addition, it has also been observed that newer bentgrass varieties may compete more aggressively with *Poa annua* compared with older bentgrass varieties. This Spring, several research projects will be initiated to evaluate chemical management approaches, such as the effectiveness and application timing of ethofumesate and plant growth regulators, combined with cultural management practices. Summer seeding of bentgrass also presents the challenge of developing weed management programs for summer annual grass control on newly seeded bentgrass. Research is currently underway to evaluate the effectiveness of preemergence herbicides as a preventative treatment to exclude *Poa annua* from newly constructed golf course greens. Research efforts will also be directed toward finding new herbicide options that can be safely used on cool season turfgrasses for *Poa annua* control.

Research in the area of herbicide tolerance of cool season turfgrasses will also be a major focus of the weed science program. This area has not been extensively studied and is not well understood. This area of research will establish a synergism between the turfgrass plant breeding program and the turfgrass weed science program. Research on the response of cool season turfgrasses will provide immediate and practical information to clientele as well as establish a potential area for long term basic research. Studies will be conducted to evaluate the interspecific variability between creeping, colonial and velvet bentgrass as well as intraspecific variability between bentgrass varieties within species. This spring, 27 different Kentucky bluegrass varieties will be evaluated for their response to preemergence and postemergence herbicides. If interspecific and intraspecific genetic variability in the response of cool season turfgrasses to herbicides is evident basic research will be conducted to elucidate the physiological mechanisms for herbicide tolerance. This information may be useful for turfgrass breeders to select for herbicide tolerance and help us understand and possibly mitigate cultural and environmental factors contributing to the increased risk for herbicide injury to cool season turfgrasses.

The biotechnology revolution, which has had a dramatic impact on weed management systems in agronomic crops, will undoubtedly impact turfgrass weed management. However, the development and commercialization of glyphosate resistant turfgrass will present several research and management challenges. Some of these challenges include the efficacy of glyphosate on key weed species and the tolerance of genetically modified turfgrasses to glyphosate. Other key issues include development of alternative herbicide programs for renovation of glyphosate resistant turfgrass, weed resistance management to glyphosate and decreasing the risk of glyphosate injury to non-target species.

Annual Bluegrass Invasion as Affected by Seeding Date and Bentgrass Cultivars

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A number of cultivars of creeping (*Agrostis stolonifera*) and velvet (*A. canina*) bentgrass have been released that exhibit greater phenotypic variation than was previously available. This affords the opportunity to assess the potential for genetic differences in the competitive ability of these bentgrasses against annual bluegrass. The goals of this research project are to identify bentgrass cultivars that exhibit an improved genetic competitive ability against annual bluegrass invasion under the influence of traffic, and to determine if the time of year for establishment affects the competitive posture of bentgrasses against annual bluegrass invasion.

Trials were initiated in 1998 and 1999 on sandy loam to evaluate the influence of seeding date and bentgrass (creeping and velvet bentgrass) cultivar on the amount of bentgrass that will establish in competition with emerging annual bluegrass plants. First-year results are available for the 1998 trial. The least invasion of *Poa annua* during establishment was observed for the June seeding date compared to all other dates. The second best establishment of bentgrass occurred with an August seeding, whereas seeding in September and October resulted in the lowest bentgrass populations. A seeding date by cultivar interaction was found on all observations dates. **'**Penncross' had consistently lower bentgrass population than other cultivars. 'Providence' had similar bentgrass population compared to 'Penn A4', 'L-93' and 'SR 7200' for the June, August and September seeding dates, but was less than these cultivars for the May and October seeding bentgrass. Penn A4 and L-93 had consistently high bentgrass population for all seeding dates. SR 7200 (velvet bentgrass) had similar bentgrass populations to Penn A4 and L-93 in the May, June and August seedings; however, SR 7200 had lower bentgrass population than these cultivars in the September and October seedings. It is apparent that seeding date and cultivar selection impacts the success of renovating *Poa annua* infested turf. Further evaluation is needed to understand the importance of the interaction between seeding date and cultivar.

Other field trials have been initiated to evaluate *Poa annua* encroachment into creeping and velvet bentgrass cultivars maintained as putting green and fairway turf. These studies are assessing the bentgrass cultivars under the traffic stresses: wear, compacted soil, and wear plus compacted soil. Fairway and putting green trials were established on sandy loam with *Poa annua* population ranging from 10 to 19% in the fairway trial and 5 to 16% in the putting green trial. Traffic treatments were initiated in August 1999 and have affected turf quality. The traffic that combines wear and compaction has produced the lowest quality turf in the fairway trial. Ratings in August and October 1999 indicate that wear has been more detrimental to turf quality than compaction treatment. There has not been a significant interaction between cultivar and traffic treatment, indicating that cultivar ranking under the non-trafficked conditions was statistically similar to the cultivar ranking under traffic. *Poa annua* and bentgrass populations will be assessed in November 1999 and in spring, summer and fall of 2000.

A putting green trial was seeded 28 May 1999 on a sand-based (85:15 sand:peat by volume) root zone conforming to USGA guidelines. A mowing height of 3.2-mm ($^{1}/_{8}$–inch) was achieved on 9 September 1999. The entire experimental was overseeded in September and November 1999 with Petersen's creeping bluegrass (perennial *Poa annua*) at ≤ 0.5 g m^{-2} (0.1 lb / 1000 ft^2) and will be overseeded periodically throughout the study to simulate the gradual introduction of weed seed commonly experienced on golf turf. Traffic treatments were initiated in October 1999. Evaluation of turf performance in response to traffic has been initiated and will continue through the duration of the project, along with assessemtent of *Poa annua* encroachment.

The Use of Laser Flow Cytometry for Species Determination in Agrostis

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The taxonomic classification of the genus *Agrostis* has proven to be one of the most complicated of the grass genera. Classification based upon morphological and anatomical characters is difficult and complicated by the misapplication of names, both common and scientific. Species of *Agrostis* differ in ploidy levels and these differences can by used as a diagnostic tool to assist in species determination. The objective of this study was to: 1) evaluate the use of laser flow cytometry as a quick, reliable tool to aid in species determination of Agrostis, based on ploidy level and 2) identify which morphological characters were associated with ploidy level. The 6 *Agrostis* species evaluated were velvet bentgrass, *A. canina* L. subspecies *canina* (2n=2x=14), brown velvet *A. canina* L. subspecies *montana* (Hartm.) Hartm. (2n=4x=28), creeping bentgrass *A. stolonifiera* L. (2n=4x=28), colonial bentgrass *A. tenius* Sibth. (2n=4x=28), dryland bentgrass, *A. castellana* Boiss. & Reut. (2n=6x=42), and redtop *A. alba* L. (2n=6x=42). Plant material was prepared for flow cytometry analysis following a modified technique reported by Huff and Palazzo (1998). Significant differences in 2C DNA content were found between species ($P < 0.01$). Reported chromosome numbers were positively correlated with observed 2C DNA content ($r = 0.94$, $P < 0.01$ (run1), $r = 0.92$, $P < 0.01$ (run2)). Flag leaf length was associated with 2C DNA content ($r = 0.84$, $P < 0.01$ (run1), $r = 0.80$, $P < 0.01$ (run2)). These results indicate that laser flow cytometry is a quick, reliable tool to distinguish certain species of *Agrostis*. This technique is currently being utilized in the Rutgers Turfgrass breeding program.

Update on the Rutgers Creeping Bentgrass Biotechnology Program

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The goals of the Rutgers Turfgrass Biotechnology program are to produce improved creeping bentgrass cultivars through a combination of genetic engineering and breeding methods. The development of a cultivar of creeping bentgrass resistant to the nonselective herbicide Round-Up would be of great benefit to the golf course industry. This would provide a simple, safe, and effective method of controlling *Poa annua*, a persistent weed on greens, tees and fairways. Through the Rutgers-Scotts-Monsanto partnership, development of such a cultivar is actively underway.

We are also testing the potential of genetic engineering to improve disease resistance of creeping bentgrass. A number of genes have been reported to improve disease resistance when transformed into other plant species. We are testing some of these genes in creeping bentgrass. Currently, transgenic lines expressing five potential disease resistance genes have been planted in field tests. From ratings of a field test established in 1998, two lines show enhanced dollar spot resistance relative to the average disease response of the nontransgenic controls. We have also established a new field test containing these and other transgenic lines. They will be evaluated in the summer of 2000 to determine if the resistance observed in 1999 is repeatable.

In addition to the potential benefits of transgenic cultivars of creeping bentgrass, there are questions regarding the potential risk of outcrossing to related species. We have two studies underway addressing this risk. Both studies are using the bar gene which confers resistance to the herbicide bialaphos as a marker to determine pollen flow from transgenic bentgrass to the related species. One study is designed to determine the biological capability of creeping bentgrass outcrossing to four related *Agrostis* species. For this study, plants were vernalized in the field and then brought into the greenhouse for bagged crossing. We obtained a low number of herbicide resistant hybrids. These hybrids are currently in a nursery plot. In the spring 2000 they will be assessed for fertility. Often interspecies hybrids are sterile. The second study is a field test which will assess the risk of outcrossing in a more natural situation.

Collecting, Evaluating and Integrating New Turfgrass Germplasm into the Rutgers Breeding Program

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In the past 3 years the Turfgrass Breeding Project has made extensive collections from old turf areas in central and eastern Europe, Inner Mongolia and the northeastern USA. Seed of all these collections were first put into turf trials to evaluate their turf quality and disease and insect resistance. The primary species collected have been Kentucky bluegrass (*Poa pratensis*), narrow leaved bluegrass (*P. angustifolia*), perennial ryegrass (*Lolium perenne*), fine fescue (*Festuca* species), tall fescue (*F. arnundinacea*), bentgrass (*Agrostis* species), *Koeleria* species, and tufted hairgrass *(Deschampsia caespitosa).*

In the last three years, we have seeded over 500 of these new Kentucky bluegrass collections in turf trials. They have exhibited a wide range of growth characteristics with a few from each year's collections showing promising turf performance. Some of these are being increased for further testing. In the 1998 collection, over 40 Kentucky bluegrass lines showed excellent stripe rust (*Puccinia striiformis*) resistance in the seed increase field in southwestern Holland. These collections are showing a higher percentage of improved turf performance compared to the earlier collections.

Four hundred perennial ryegrass collections (with over 100 containing endophytes) are being integrated into the Rutgers germplasm pool using a backcrossing technique with the most advanced germplasm from Rutgers as the recurrent parent material. The collections from 1998 have resulted in the highest percentage of plants with a turf-type growth habit.

In the fine fescue collections from 1997, 8 sources of strong creeping fescue have been identified as having improved turf performance and were used in crosses with the most advanced Rutgers germplasm. Seven of these contained an enodphyte. Six Chewings fescues (with 4 containing endophytes) were also identified as improved and used in crosses with Rutgers best performing breeding material.

Over 100 new sources of *Deschampsia caespitosa* have been included in turf trials at Rutgers. There is a need to find improved resistance to bill bugs and better heat tolerance in this species.

Some of the *Koeleria* collections from eastern Europe have resulted in germplasm with better floret fertility and a turf type growth habit. These were harvested and included in low maintenance turf trials in the fall of 1999 at Adelphia.

A few colonial bentgrasses (*A. tenuis*) from eastern Europe have been included in developing populations with improved tolerance to brown patch. These are being increased for the harvest in 2000. These are being combined with 5 US collections from the northeastern US and Oregon.

Over 600 velvet bentgrasses *A. canina* and 800 creeping bentgrasses (*A stolonifera*) have been collected in the northeastern US. In the past 3 years, these are being evaluated for turf performance and seed production in New Jersey. To date, the most promising materials have been from Long Island, NY. A collaborative project with Pickseed West, Inc. has resulted in a national test entry "Pick MVB" velvet bentgrass. It has performed well to date in the 1998 NTEP greens trial at Hort Farm II.

The greatest challenge in creeping bentgrass breeding for New Jersey is the identification of sources with dollar spot resistance. In the thesis work of Stacy Bonos, 14 sources out of 500 have been identified as having improved resistance to this serious disease.

New Technology for Insecticidal Nematodes That Attack White Grubs

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Disposable micro-fermenter technology could permit local production of biopesticides eliminating costly formulation, storage, transport, waste disposal, and capital. Because only "fresh" bioinsecticides would be applied, improved field performance would be expected. A kit is envisioned that contains a micro-fermenter with dehydrated media, seed inoculum, air pump, and simple instructions. The project is attempting to devise this technology using entomopathogenic nematodes.

A new nematode strain has been identified possessing the dual attributes of ease-of-culture coupled with superior field efficacy against white grubs. An enriched culture medium has been developed that provides good growth of the nematode and its bacterial symbiont. By examining salt from media for insect cell culture, salt concentrations were identified for our growth medium. Optimal medium concentration of 5 to 8% (w/v) provided the highest yield and recovery (i.e., initial molt), and largest adult hermaphrodites. We further identified optimal media pH, temperature, and inoculum density (4,000 infective juveniles per ml).

The above strain, media, and culture conditions combine to provide high yields (up to 274,800 nematodes per ml), productivity (up to 119 x 104/liter/hr), proportion of infective stages (>95%), and quality in shaker flasks. When we applied the lessons obtained from liquid culture in flasks to our disposable bag fermenter, results were less favorable. Here the key obstacle was inadequate recovery. The reasons for low recovery are unclear but presumably are physical (e.g., high culture viscosity, inadequate mixing). Additional studies will be needed on bag design; in particular, we need to test more complex designs. We must also resurrect our earlier studies on understanding the dynamics of what influences infective juvenile recovery.

Enhancing Biological Control of Turfgrass Insect Pests: White Grubs, Entomopathogenic Nematodes and Imidacloprid

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Entomopathogenic nematodes offer an environmentally safe alternative to chemical insecticides in the management of white grubs. Several studies have shown that the efficacy of entomopathogenic nematodes to curatively control white grubs can be improved if they are integrated with other pathogens but these combinations have limitations. For example, the combination of nematodes and *Paenibacillus* (*Bacillus*) *popilliae* is feasible only for long-term control in high economic threshold situations whereas the combination of nematodes and *Bacillus thuringiensis* Buibui strain is feasible only for scarab species that are sufficiently susceptible to this bacterium.

A more efficient combination with wider applicability should be that of nematodes and the chloronicotinyl insecticide, imidacloprid. Currently, imidacloprid is one of the most widely used insecticides for white grub control because of its high efficacy, relatively low vertebrate toxicity, low application rates, long systemic persistence, and relatively small effect on beneficial invertebrates. However, the decline of its efficacy with advancing white grub development combined with the difficulty to predict white grub outbreaks, requires its application in a preventative approach over large areas that often would require only partial or no grub control. The combination of imidacloprid and nematodes would allow curative treatments against older white grub stages, and because these stages are easier to detect, treatments could be limited to infested areas, reducing cost and environmental impact.

Efficacy of imidacloprid-nematode combinations for white grub control

In a series of greenhouse and field experiments, we tested the effect of application timing, imidacloprid rate, white grub species, and nematode species on the efficacy of imidacloprid-nematode combination for white grub control.

We showed that imidacloprid interacted synergistically with the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema glaseri* in 3rd instars of the Japanese beetle, *Popillia japonica*, the Oriental beetle, *Exomala orientalis*, and the masked chafers, *Cyclocephala borealis*, *C. pasadenae*, and *C. hirta*. The degree of interaction varied with nematode species. The strongest and most consistent synergism occurred between imidacloprid and *S. glaseri*. Synergism between imidacloprid and *H. bacteriophora* was weaker and less consistent. Combinations of imidacloprid and *Steinernema kushidai* only resulted in additive mortality. We observed synergistic interaction whether nematode were applied at the same time as imidacloprid or 14 days later. Although our study suggested that the synergistic interaction could occur at imidacloprid rates as low as 10 – 25% of the recommended field rate, the extent to which the chemical can be reduced seems to depend on a number of conditions including timing of application (earlier better) and white grub species.

The combination of nematodes and imidacloprid could be used for effective curative treatments of white grub infestations. The compatibility of nematodes and imidacloprid in tank mixes increases the feasibility of this approach. In addition, imidacloprid has no negative effects on nematode reproduction in white grubs and fitness of the emerging progeny. Rather, the increased number of hosts succumbing to nematode infection in combinations with imidacloprid should improve nematode recycling that may lead to additional white grub mortality and longer persistence of nematode populations.

Laboratory study on the mechanism of imidacloprid-nematode interaction

In order to successfully infect a host, the infective juvenile stage nematodes have to locate a potential host, attach to its cuticle, penetrate, and establish in the host's body cavity. However, during their coevolution with soil pathogens such as entomopathogenic nematodes, white grubs have developed a series of behavioral, morphological, and physiological barriers to infection. To elucidate the mechanism of interaction between entomopathogenic nematodes and imidacloprid, we conducted a series of experiments testing the effect of imidacloprid exposure on various white grub defensive mechanisms.

In vials with soil and grass, mortality, speed of kill, and nematode establishment were negatively affected by imidacloprid with *S. kushidai* but positively affected with *S. glaseri* and *H. bacteriophora.* In all other experiments, imidacloprid had a similar effect for all three nematode species on various factors important for the successful nematode infection in white grubs. Nematode attraction to grubs was not affected by imidacloprid treatment of the grubs. Establishment of intra-hemocoelically injected nematodes was always higher in imidacloprid-treated grubs but the differences were small and in most cases not significant. The major factor responsible for synergistic interactions between imidacloprid and entomopathogenic nematodes appears to be the general disruption of normal nerve function due to imidacloprid resulting in drastically reduced activity of the grubs. This sluggishness facilitates host attachment of infective juvenile nematodes. Grooming and evasive behavior in response to nematode attack was also reduced in imidacloprid-treated grubs. The degree to which different white grub species responded to entomopathogenic nematode attack varied considerably. Untreated *P. japonica* grubs were the most responsive to nematode attack among the species tested. Untreated *C. borealis* grubs showed a weaker grooming and no evasion response, and untreated *C. hirta* grubs showed no significant response. Chewing/biting behavior was significantly increased in the presence of nematodes in untreated *P. japonica* and *C. borealis* but not in *C. hirta* and imidacloprid-treated *P. japonica* and *C. borealis*. Our observations, however, did not provide an explanation for the lack of synergism between imidacloprid and *S. kushidai*.

Impact of Cultural Management Practices on the Development of Gray Leaf Spot in Cool-Season Turfgrasses

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Although known as a pathogen of St. Augustinegrass (*Stenotaphrum secundatum*) in the southern United States since 1950, gray leaf spot has recently become an extremely destructive disease of perennial ryegrass (*Lolium perenne*) and, to a lesser extent, tall fescue (*Festuca arundinacea*) in the Northeastern and Mid-Atlantic States. The disease, caused by the fungus *Pyricularia grisea* (Cooke) Sacc., is favored by prolonged periods of high humidity (>95%) and warm air temperatures (>25^0C). Major epiphytotics of gray leaf spot occurred with increasing frequency in 1991, 1995, and 1998. To date, however, little is known about the influence of management practices on the development of gray leaf spot in cool-season turf.

The goal of the current study was to evaluate the impact of nitrogen rate (0, 0.7, 1.4, and 2.7 kg N/90 m^2), cutting height (1.3, 1.9, 3.8, 6.4, and 8.9 cm), and clipping removal on the incidence and severity of gray leaf in perennial ryegrass and tall fescue turf in the field over a three year period. Nitrogen was applied as Urea. Three perennial ryegrass cultivars (Manhattan III, Palmer II, and SR 4200) and two cultivars of tall fescue (Jaguar II and K-31) were selected for evaluation. Turf was established on a Norton loam with a pH of 6.1 to 6.4 in three separate locations at the Turf Research Farm, North Brunswick, NJ. The sites were inoculated each year with either a conidial suspension of *P. grisea* (250,000 conidia per 1 m^2), infested clippings (7g/sq. ft.), or infested transplants. Data was collected for turf quality and severity of gray leaf spot.

The effect of nitrogen rate on gray leaf spot was both cultivar and year dependent. In 1997 when disease intensity was low to moderate, disease severity decreased (22 to 69%) with increasing nitrogen rate; however, in 1998 and 1999 when disease intensity was high, disease severity was positively correlated with nitrogen rate. Throughout the three-year study, lower cutting height consistently resulted in a lower incidence of gray leaf spot. Compared to the 8.9 cm mowing treatment, disease severity decreased 52%, 66% and 45% for the lowest cutting heights used in 1997 (3.8 cm), 1998 (1.3 cm), and 1999 (1.3 cm), respectively.

The removal of clippings reduced the incidence of gray leaf spot up to 41% in 1997, when disease intensity was low. At high disease intensity (1998 and 1999), however, the influence of clipping removal on disease development was negligible. In general, the tall fescue cultivars were significantly less susceptible to gray leaf spot than the perennial ryegrass cultivars evaluated in this study.

Poster Presentations

Evaluation of Disease Resistance of New Bentgrasses Under Various Management Practices

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Over the past decade, several improved bentgrass cultivars have been developed for use on golf course greens and fairways. Many of these cultivars have been reported to have excellent disease resistance. Although it has been suggested that these cultivars may help reduce fungicide usage, to date, little research has been conducted to support these claims. To address this issue, a study was designed to evaluate the disease response of several new bentgrass cultivars maintained under different nitrogen fertility, mowing height, and fungicide regimes.

Eight bentgrass cultivars (Crenshaw, Southshore, Penncross, L-93, SR 1020, SR 7200, A-4 and G-2) representing a wide range in disease susceptibility were selected for evaluation. All cultivars included in the study were creeping (*Agrostis stolonifera*) bentgrasses, except SR 7200, which is a velvet (*A. canina*) bentgrass reported to have good tolerance to dollar spot (*Sclerotinia homoeocarpa*) and brown patch (*Rhizoctonia solani*). L-93, G-2 and A-4 were selected for their improved resistance to both dollar spot and brown patch, whereas Crenshaw and SR 1020 were chosen for their susceptibility to dollar spot and copper spot. Penncross is the current industry standard.

Cultivars were established on 29 Sep 1998 at a seeding rate of 1.5 lb./1000 sq. ft. on a Nixon loam with a pH of 6.1. Each cultivar was maintained at two cutting heights: 0.141 in. (greens height; mowed daily) and 0.375 in. (fairway height; mowed three times per week), and two nitrogen levels: 2 lb N / 1000 sq ft (a low N fertility) and approximately 5 lb N / 1000 sq ft (a high N fertility). Fertilizer was applied once every two weeks from 14 Apr through 10 Nov. Each cultivar x fertilizer x cutting height treatment was subdivided into six fungicide application schedules (untreated, 7, 14, 28, or 56 day intervals, or an economic threshold of 0.3% disease) using the contact fungicide chlorothalonil (Daconil Ultrex 82.5WDG) at a rate of 1.9 oz /1000 sq ft. Chlorothalonil is a commonly used contact fungicide that was selected for its short residual activity, thus providing the opportunity to identify host/management treatments that provide enhanced disease suppression with reduced fungicide usage. Fungicide was applied in water equivalent to 2 gal. per 1000 sq. ft. with a CO_2 powered sprayer. The fungicide rate for all regimes was increased from 1.9 oz to 2.85 oz/1000 sq ft during the month of Aug due to increased disease pressure. The site was inoculated with isolates of *S. homoeocarpa* and *R. solani* on 16 Jun and 8 Sep, respectively. Data was collected for turf quality, color, density, and dollar spot severity. Other foliar diseases (e.g. brown patch and copper spot) were also evaluated when they developed.

Dollar spot was first seen on 25 Jun. Disease quickly became uniform throughout the study and peaked in late Jul. Dollar spot was least severe on turf receiving the high (5 lb N/1000 sq ft) rate of nitrogen. Cultivars G2, SR 7200 and L-93 were less susceptible to dollar spot under most nitrogen and cutting height treatments. Brown patch was most severe on turf maintained at greens height and high nitrogen. By continuing this study in 2000, we hope to identify cultivars that require less fungicide input while maintaining acceptable turf quality. Results from this research should help superintendents make informed choices when selecting new bentgrass cultivars and should improve their ability to manage these cultivars once they are established.

Determination of Optimal Phosphorus Recommendations for Turfgrass Early Establishment and Soil Coverage

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Runoff from non-point sources has been implicated as an uncontrolled source of phosphorus (P) in surface waters. By evaluating P response on early establishment and soil coverage of turfgrass, reasonable P application recommendations can be made to minimize water quality deterioration. Since rapid turfgrass establishment also plays a role in the prevention of nitrate leaching and soil erosion, P fertilization may be used to enhance turfgrass establishment and thus limit subsequent environmental degradation.

Phosphorus has a critical soil test level below which crop response to the nutrient may be expected, and above which crop response is not expected. For most crops, this delineation using the Mehlich-3 soil test P is 36 mg/kg. The critical level of P in turfgrass has not yet been determined, and may not be the same value. During establishment, turfgrasses often benefit from P fertilization. Turfgrass establishment may require higher soil levels of P than do many other crops, since turfgrass is generally seeded at cold soil temperatures, when P availability is limited.

Three studies were performed on turfgrass to assess the role of P on early establishment and soil coverage. The objective was to determine how different turfgrasses respond to P fertilization at different soil test levels.

Study I: **To monitor establishment and growth of turfgrass species and cultivars.**

Methods: In the greenhouse, in August 1998, the following turfgrasses were studied: Kentucky bluegrass (Classic, SR2000, America), perennial rye (Wind Dancer, B6-Prelude III, Manhattan III), and tall fescue (Tarheel R5DR, Gazelle, TMI-RBR Millennium). They were seeded on a soil that had near optimum (for most crops) Mehlich-3 soil test P level of 34 mg/kg. Plant height data, collected for turfgrass grown in pots on unamended soils and P-amended soils, were compared to determine relative growth. Clipping dry weight was similarly compared.

Results: By the second week after seeding, the height of all species and varieties were increased by P fertilization. Forty-two days post-seeding, P fertilized turfgrass yielded more clipping dry weight than turfgrass grown on corresponding non-P treated soils, for all species and cultivars. In general, the cultivars of Kentucky bluegrass exhibited the greatest response to P fertilization, and the perennial rye cultivars had the least response.

Study II: To determine the critical level of P needed for three species of turfgrass.

Methods: Early spring (11-13 hr. sunlight, 60-70^{o}F) was simulated in an Environmental Growth Chamber. Turfgrass was seeded on 19 soils collected from various sites, with Mehlich-3 soil test P ranging from 11 mg/kg to 1160 mg/kg. Plant height data for turfgrass grown in conetainers on unamended soils and P-amended soils were compared to determine relative growth.

Results: By Week 5, all three species exhibited growth responses to P fertilization. The soils with lowest soil test P levels were, as expected, most responsive to P fertilization. To achieve 90% relative growth at Week 6, Kentucky bluegrass (Midnight) required P fertilization when it was grown on soils with less than Mehlich-3 soil test P level of 475 mg/kg. Tall fescue (Coronado) and perennial rye (BFP) exhibited much less response to P. Soils with Mehlich-3 soil test P levels less than 100 mg/kg, for tall fescue (Week 5), and less than 75 mg/kg for perennial rye (Week 8), required P fertilization for 90% relative growth.

As these results may be unique to these conditions, further research is being conducted to better define the critical soil test P levels.

Study III: **To monitor response of turfgrass grown in the field under different soil test P levels.**

Study IIIa. Mehlich-3 soil test P of 59 mg/kg.

Methods: A field study was undertaken on September 9, 1999 at Rutgers Plant Science Experiment Station near Adelphia, NJ. Kentucky bluegrass (Midnight), tall fescue (Coronado), and perennial rye (BFP) were studied at various P treatments. Plant height data and density ratings for turfgrass grown on unamended soils and P-amended soils were compared to determine relative growth and density.

Preliminary Results: The density of the three species responded to P treatment, in agreement with the data from Studies I and II. Kentucky bluegrass (Midnight) exhibited the greatest turfgrass density response to P, on this soil, followed by tall fescue (Coronado). Perennial rye (BFP) exhibited the least response to P fertilization.

Study IIIb. Mehlich-3 soil test P of 259 mg/kg.

Methods: A field study was undertaken on September 28, 1999, at Rutgers Plant Science Experiment Station near Adelphia, NJ. Plant height data and density ratings for turfgrass grown on unamended soils and P-amended soils were compared to determine relative growth and density.

Results: A density response to P fertilization for tall fescue (Coronado) resulted, but only at Week 6. Perennial rye (BFP) had only a slight response during Weeks 4-6. The stand of Kentucky bluegrass (Midnight) was poor and revealed no useful density data.

Summary: The establishment and early growth of all three species responded to P fertilization, except at very high soil test levels. Kentucky bluegrass (Midnight) exhibited the greatest response, followed by tall fescue (Coronado), then perennial rye (BFP). The soil test level of P played a role in response. Since different species of turfgrass require varying critical P levels for adequate establishment and coverage, consideration for soil test P level and the turfgrass species should be incorporated into the decision-making process regarding P fertilization. Research to address the appropriate P rates of fertilization is in progress.

Extracellular Enzyme Production and Biological Control Activity is Regulated by a Catabolite Activator Protein (CAP) Homolog in the Bacterium, C3, a Taxonomically-Related Strain to *Lytobacter mycophilus* (proposed Gen. nov., sp. nov) Strain N4-7.

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Bacterial strain C3 was previously isolated from a leaf of Kentucky bluegrass in Nebraska, and demonstrated biocontrol efficacy against brown patch disease caused by *Rhizoctonia solani* (Giesler and Yuen, 1998. Crop Prot. 17:509-513). Bacterial strain N4-7 was isolated in New Jersey and was demonstrated to have biocontrol activity against summer patch disease caused by *Magnaporthe poae* (Kobayashi and El-Barrad, 1996. Cur. Microbiol. 32:106-110). 16s rDNA sequence analysis, fatty acid methyl ester analysis and phenotypic comparisons indicated that strain C3 is taxonomically related to strain N4-7, but is significantly different from other described bacteria to warrant taxonomic positioning as a new genus and species.

In an effort to understand the mechanisms involved in biocontrol activity, strain C3 was subjected to random transposon mutagenesis. A single mutant of strain C3, designated 5E4, was isolated that is devoid of chitinase, glucanase, and esterase activity, and is reduced in extracellular protease activity. Molecular characterization of mutant 5E4 indicated a transposon insertion in a gene that has significant nucleotide sequence identity to the catabolite activator protein (CAP) of *Escherichia coli*, *Erwinia chrysanthemi* and *Xanthomonas campestris*. Biological assays indicated mutant 5E4 is affected in in vitro antifungal activity against *M. poae* and *R. solani*, is no longer able to colonize *M. poae* mycelia, and is reduced in biocontrol activity of summer patch on Kentucky bluegrass. These studies provide the first step towards understanding the antifungal traits expressed by strain C3, and the regulatory pathway that controls biocontrol activity.

DNA Fingerprinting in Perennial Ryegrass (*Lolium perenne*) Using Simple Sequence Repeats (SSRs): an Update on Progress and Findings

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Perennial ryegrass is a valuable grass species due to its agronomic importance as a forage crop and its economic importance as a lawn, athletic field, and golf turf. The release of new cultivars has steadily increased making cultivar identification necessary to maintain varietal purity and to protect both consumer and breeder rights. However, cultivar identification has become increasingly difficult due to the breeding nature of this species (cross-pollinated) and to the limited amount of germplasm involved in population improvement. Our previous work (Kubik et al. 1999) has demonstrated that SSRs are sufficiently abundant and sufficiently polymorphic to be useful genetic tools in perennial ryegrass. Since this work was last reported, an additional 21 SSR loci have been isolated from a DNA library, enriched for (GA)n and (GT)n SSRs. The library was constructed from a single endophyte free experimental clone named SNM 56. We are currently assessing the genetic diversity of 7 perennial ryegrass cultivars which represent closely and distantly related plant material (Manhattan II, Linn, Pennfine, Loretta, Palmer III, BFP(Jet), and Affinity). We sampled thirty individual plants per cultivar, therefore a total of 210 plants were genotyped in this study.

The goals of this research were to:

i) Assess SSR allele frequency at all 25 SSR loci for the seven perennial ryegrass cultivars in the study (each cultivar is represented with 30 individual randomly sampled plants).
ii) Determine the optimum sample size for a synthetic cultivar (i.e. are 30 plants too many or too few to represent a cultivar?)
iii) Explore genetic diversity within and among cultivars to better understand the boundaries of a cultivar.
iv) Determine if a single unknown clone can be correctly assigned to the proper cultivar.

This is currently a work in progress. Generation of genotypes for each cultivar is complete. However, the data needs to be re-scored with updated software.

Identification of Epiphyllous Mycelial Nets on Leaves of Grasses Infected by Clavicipitaceous Endophytes

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Fungal endophytes of genus *Neotyphodium* have long been known to inhabit the internal tissues of plants without producing external reproductive structures on plants. For these endophytes, infection of the embryo within the seed has been the accepted mode of transmission. In this study, we examined several species of endophyte-infected grasses, including *Bromus setifolius*, *Festuca ovina, F. rubra,* and *Poa ampla,* and documented the presence of epiphyllous mycelial nets on their leaves. To evaluate whether the epiphyllous nets belong to the endophytes, we isolated the epiphyllous mycelium from the surfaces of *Poa ampla* leaves and analyzed it by DNA sequencing and microscopy. DNA isolates from wax peels showed that the epiphyllous net belonged to the endophyte, *Neotyphodium typhinum*. The mycelial nets produced by the clavicipitaceous endophytes may play a role in defense of host plants from potential pathogens through "niche exclusion". The production of conidia on epiphyllous mycelia suggests that the endophyte may also have the opportunity for contagious spread to uninfected plants through epiphyllously-produced conidia.

Transformation of Tall Fescue and Velvet Bentgrass Using Agrobacterium tumefaciens

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Monocots, including turfgrasses, have been difficult to transform via the *Agrobacterium*-mediated method which works well with dicots. This has been a roadblock to production of transgenic monocots inasmuch as other methods frequently introduce large numbers of gene copies into the nuclear genome, leading to the possibility of sense suppression and unstable inheritance. Our laboratory has been working to overcome the difficulties inherent in Agrobacterium-mediated transformation of monocots to circumvent the above-described problems of direct gene transfer. We previously reported the successful transformation of creeping bentgrass (*Agrostis palustris*). Herein we report the successful transformation of two additional species: tall fescue (*Festuca arundinacea* Schreb) and velvet bentgrass (*Agrostis canina*).

Callus cultures were initiated from mature seeds of velvet bentgrass and endophyte-free tall fescue. Pieces of calli were co-cultivated with a suspension of supervirulent *Agrobacterium tumefaciens* which contains a suitable selectable marker (for hygromycin resistance) and the easily scorable GUS reporter gene within the T-DNA border of its co-integrate vector. Each of these transgenes is driven by promoters for strong, constitutive expression in monocots, respectively the promoters from the rice actin and maize ubiquitin genes. Transformed calli were selected on hygromycin-containing medium, and transformation was confirmed by assay of the callus for GUS activity. Further verification was achieved by Southern hybridization analysis using probes for the GUS gene and the hygromycin resistance gene. Of seventeen transformants tested, half had one or two insertion sites, and the remainder had several. This work demonstrates the suitability of using *Agrobacterium tumefaciens* for transformation of turfgrass species, as also shown previously in our work with creeping bentgrass.

Developing a Transformation System for *Neotyphodium typhinum*

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Turfgrass benefits from symbiotic fungal colonization. Increased plant vigor and tolerance to abiotic stress are examples of synergistic effects that have been documented. However, not all commercial turfgrass varieties harbor endophytes. e.g. Kentucky bluegrass (*Poa pratensis*). Understanding the physiological basis for endophyte-grass interactions is important to generate novel grass/fungus combinations with improved agronomic traits.

We are investigating the *Neotyphodium typhinum /Poa ampla* interaction. The endophyte *Neotyphodium typhinum* like in other fungal/grass endophytic associations, produces a serine protease during its mutualistic interaction with its host *Poa ampla* (Lindstrom et al. 1993; Lindstrom and Belanger, 1994). The abundance and cell wall location suggests that this protease may be important for infection. This enzyme was characterized, cloned and sequenced (Reddy et al. 1996). Sequence analysis of the cDNA clone showed that proteinase At1 is homologous to subtilisin-like serine proteases produced by entomopathogenic, nematophagous, and mycoparasitic fungi, (St. Leger, 1995; Bonants et al.1995). In those systems, expression of the homologous proteases is considered to be important for infection. For instance, protease-deficient mutants of *Beauveria bassiana*, an entomopathogen, had reduced virulence against the migratory grasshopper, *Melanoplus sanguinipes* (Bidochka and Khachatourians, 1990). Although studies to date suggest that this proteinase may be important for infection and subsequent colonization, more concrete evidence is needed. We are attempting to provide this by targeted gene inactivation whereby the expression of the proteinase would be disrupted. For this we will need a fungal transformation system. Here we report successful transformation of the endophyte fungus *N. typhinum* with the marker genes, GUS and EGFP.

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Identification of Fine Fescue Selections Using Ribosomal DNA Sequences

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Sequences of the Internal Transcribed Spacer (ITS) regions of the ribosomal DNA locus were evaluated as a tool for identification of fine fescue selections. Sequences were obtained from 44 selections belonging to 11 different species and subspecies, collected from wild populations or old turf sites in North America, Europe, and Asia. Phylogenetic analysis divided the fine fescues into two major clades. These clades corresponded to the division of the fine fescues into the *F. rubra* and *F. ovina* aggregates, which is based on morphological characteristics. Within the *ovina* and *rubra* aggregates, the maximum number of differences between selections were 18 and 19, respectively. As a result, the phylogenies provided little resolution and bootstrap support values were generally low. Subclades within the *ovina* aggregate closely corresonded to species, but there was no apparent relationship between subspecies and ITS clades within the *rubra* aggregate. Based on these results, ITS sequences appear to be a reliable tool for placing fine fescue selections in the *rubra* or *ovina* aggregates. ITS sequences may also be useful for resolving taxonomic issues and identifying species within the *ovina* aggregate, but not within the *rubra* aggregate.

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Volatiles May Play a Role in the Chemistry of Pest Resistance in Endophyte-Infected Grasses

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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. Peramine, ergovaline, and lolitrem B reduce insect feeding and survival and these compounds are not produced by endophyte-free grasses.

There are other lines of defense against insects that may also be enhanced in endophyte-infected grasses. Insects locate a suitable host by identifying and following the volatile compounds that are emitted by plants. The purpose of this investigation was to determine if there are differences in the nature or amount of volatile compounds produced in the presence of the fungal endophyte. More than 30 volatile organic compounds were identified from the sheath and blades of tall and fine fescue. Both endophyte-infected and endophyte-free plants produced saturated hydrocarbons including pentane (C5), hexane (C6), octane (C8), nonane (C9), decane (C10), dodecane (C12), tridecane (13) and tetradecane (C14). Straight short-chain hydrocarbons are derived from free radicals formed by lipid peroxidation in *in vitro* systems and living organisms. The lower levels of these hydrocarbons in the leaf sheath may indicate that endophyte-infected grasses have a lower level of lipid peroxidation.

Lipoxygenases found in plant tissues can form fatty acid hydroperoxides, including 9-OOH, 13-OOH and 15-OOH, which could be split by hydroperoxide lyase into two parts with aldehyde at one side and oxo-acid at the other side. Some of the aldehydes can be isomerized into ketones. Aldehydes can also be converted into alcohols by alcohol dehydrogenase. Endophyte-infected leaf sheaths produced less lipid peroxidation products than the endophyte-free sheath, including *cis*-3-hexenal, *trans*-2-hexenal, *cis*-3-hexen-1-ol, 2,4-hexadienal, nonanal, 6-nonenal, 2,6-nonadienal and decanal.

Endophyte-infected sheaths produced lower amounts of aromatic compounds such as the C8, C9 and C10 aromatic alkane isomers, monoterpenes such as cymene and limonene, as well as benzene acetaldehyde and acetophenone. Both the aliphatic aldehydes and alcohols, and the monoterpenes are known to attract insect pests in plants. The lower levels produced by endophyte-infected grasses may allow the plant to effectively hide from insects seeking suitable hosts.

Endophyte-infected leaf sheaths also produced some unique compounds that are not present in the endophyte-free sheath such as 1-octen-3-ol, 2-octen-3-ol, and cyclohexanone. The formation of C8 aldehydes and alcohols are known to occur in basidiomycetes and other fungi. 1-octen-3-ol was found as attractant for tabanid flies, tsetse flies and mosquitoes. More research needs to be done to determine if, for example, this compound plays a role in attracting beneficial insects to plants or repelling insect pests.

In summary, the lower levels of lipid peroxidation products and the presence of unique compounds such as 1-octen-3-ol may act as the first line of defense against insect attack in turfgrasses. By incorporating endophytes derived from different sources it may be possible to alter the volatile organic compound environment above the turf to hide the plants or repel insect pests.

Transformation of Kentucky Bluegrass (*Poa pratensis* L) with Betaine Aldehyde Dehydrogenase Gene

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The characteristic of apomixis makes Kentucky bluegrass an ideal target for genetic improvement by biotechnology. Efficient transformation of Kentucky bluegrass will not only make it easy to intensify some specific traits of current varieties, such as environmental stress tolerance, but also will help to integrate new germplasm into the plants. We have successfully grown bluegrass callus tissue *in vitro* and subsequently regenerated plants from the calli. Particle bombardment was used to transform BADH gene into seed-derived callus from the Kentucky bluegrass variety, Langara. Cotransformation method was used to transform Hyg resistance gene and BADH gene into the callus, which were driven by rice actin promoter and maize ubiquitin promoter, respectively. After screening and regeneration on 200mg/L Hyg added medium, 65 Hyg resistant transgenic plants, which came from 81 different Hyg resistant callus lines, were obtained. PCR assay identified 43 transgenic lines contained BADH gene. While most of the transgenic plants grew normally on the media and in the green house, some lines showed weakened roots and growth vigor. BADH gene expression and the degree of salt and drought tolerance of these transgenic plants are being studied at present.