SEVENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

Table of Contents

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Cook College, Rutgers University
January 15-16, 1998
Foran Hall - Room 138

Thursday, January 15, 1998

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. Frank Rossi,** Cornell University. *Innovative Approaches for Plant Growth Regulator Use in Turf*

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

Friday January 16, 1998

8:00 - 8:50 AM Registration, coffee and donuts

8:50 - 10:00 AM SESSION 1: TURF MANAGEMENT
Moderator: **Dr. Donald Kobayashi**

8:50 - 9:20 AM **Dr. Peter Landschoot** , Pennsylvania State University
Evaluation of Fiber and Inclusion Amendments for Athletic Field Soils

9:20 - 9:40 AM **Dr. Bruce Clarke,** Department of Plant Pathology
Fungicide Injection Technology for Improved Root Disease Control

9:40 - 10:00 AM **Dr. James Murphy,** Department of Plant Science
Assessing Root Zone Mixes for Putting Greens Over Time Under Two Environmental Conditions

10:00 - 10:30 AM Discussion and Coffee Break

10:30 - 11:30 AM SESSION 2: TURF PHYSIOLOGY/BIOTECHNOLOGY
Moderator: **Dr. Barbara Zilinskas**

10:30 - 10:50 AM **Dr. Martin Petrovic,** Cornell University. *Fate of Nitrogen in Turfgrass Systems*

10:50 - 11:30 AM **Dr. Chee-kok Chin,** Department of Plant Science. *Do Lipids Play a Role in Turfgrass Disease Resistance?*

11:30 - 12:00 PM Discussion and Poster Session

12:00 - 1:30 PM Lunch and Poster Session

1:30 - 2:30 PM SESSION 3: PEST MANAGEMENT
Moderator: **Jim Willmott**

1:30 - 1:50 PM **Dr. Randy Gaugler,** Department of Entomology. *Recent Advances With Entomopathogenic Netamodes*

1:50 - 2:10 PM **Dr. Jennifer Johnson-Cicalese,** Department of Plant Science. *Evaluating Endophyte/Fine Fescue Associations with Chinchbug Bioassays*

2:10 - 2:30 PM **Dr. Paula Shrewsbury,** Department of Entomology. *Biological Control of White Grubs (Coleoptera: Scarabaeidae) in Turfgrass*

2:30 - 3:00 PM Discussion and Coffee

3:00 - 4:00 PM SESSION 4: TURF GERMPLASM ENHANCEMENT
Moderator: **Dr. Faith Belanger**

3:00 - 3:20 PM **Dr. C. Reed Funk,** Department of Plant Science. *Opportunities for the Genetic Improvement of Underutilized Plants for Turf*

3:20 - 3:40 PM **Dr. Ponaka Reddy,** Department of Plant Pathology. *A Study of Genes Involved in Development of Endophytes*

3:40 - 4:00 PM **Christine Kubik,** Department of Plant Science. *Characterization of Microsatellite Loci in Perennial Ryegrass (Lolium perenne)*

4:00 - 4:30 PM Discussion/Closing Remarks

PROCEEDINGS OF THE SEVENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

Bruce B. Clarke, Director

William A. Meyer, Associate Director

January 15-16, 1998

Cook College

Symposium Organizing Committee

Faith Belanger, Chair
Bruce B. Clarke
Gerri Muenzer
Paula M. Shrewsbury

Proceedings of the Seventh Annual Rutgers Turfgrass Symposium

Paula M. Shrewsbury, Editor

Director's Opening Remarks:

Welcome to the Seventh Annual Rutgers Turfgrass Symposium. The Symposium Planning Committee, comprised of Faith Belanger, Paula Shrewsbury, Jim White, and Gerri Muenzer, has worked hard to make this year's program a success. 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 the entire Center Faculty when I extend my congratulations for a job well done.

The topics presented at this Symposium represent a broad range of basic and applied research conducted at Rutgers and 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.

The Center Faculty have worked hard to develop excellent research, undergraduate and graduate teaching, continuing professional education, and outreach programs at Cook College. To support these efforts, the Turfgrass Industry has donated more than one million dollars during the past 15 years in the form of research grants, student scholarships, equipment, gifts, and the construction of a Turfgrass Conference and Laboratory Building (1984) and the C. Reed Funk Equipment Storage Facility (1996). Last year alone, the Turfgrass Industry provided more than $50,000 in student scholarships, and $20,000 in research grants. They also raised more than $40,000 for turf research via the Second Annual Rutgers Turf Research Golf Classic and embarked on a $500,000 fund raising campaign for the construction of a new Turfgrass Education Complex at Hort Farm II. This unprecedented level of support has and will continue to greatly enhance our research, teaching, and outreach efforts at Cook College/NJAES. We are indeed fortunate to have such committed and generous partners.

I look forward to the continued expansion of the turfgrass program at Cook College and the exciting opportunities that lie ahead. In 1998, I hope that efforts to hire a Turfgrass Biotechnologist and an Extension Specialist in Turf and Ornamental Weed Science will come to a successful conclusion. Moreover, the opportunity to fill the Turf Physiology position recently vacated by Dr. Mike Richardson will be actively pursued.

I hope that you will enjoy the 1998 Turfgrass Symposium and will take advantage of the many benefits that it provides. Your active and enthusiastic participation will help make this year's Symposium a resounding success.

Sincerely,

Bruce B. Clarke, Director Center for Turfgrass Science

Plenary Presentations

Innovative Approaches for Plant Growth Regulator Use in Turf

Frank S. Rossi, Ph.D., Assistant Professor of Turfgrass Science, *Cornell University*

The concept of plant growth regulation dates back to the early 1940's with discovery of 2,4-D for use as an herbicide followed by the development of maleic hydrazide in the 1950's. Through the next three decades, plant growth regulators (PGRs) were widely used for application to utility turfs to reduce their mowing requirements by inhibiting turfgrass shoot growth. With improved technology and the introduction of newer materials, the effectiveness and potential utility in turfgrass management has increased. Today plant growth regulators are used to improve turf color, allow for quicker establishment, reduce clippings, suppress seedheads, control broadleaf weeds, and help to maintain larger carbohydrate reserves in the turf through root enhancement.

Plant growth regulators can be classified into one of three distinctly different groups. Class A PGRs interfere with the production of gibberellins late in their biosynthetic pathway. Class B PGRs interfere with the production of gibberellins early in their biosynthetic pathway and class C PGRs are mitotic inhibitors, disrupting cell division.

Trinexapac-ethyl is a class A plant growth regulator labeled for use in turfgrass management for reducing shoot growth without causing significant injury. Trinexapac-ethyl inhibits the hydroxylation of GA20 to GA1, a biosynthetic step that occurs late in the gibberellin biosynthesis pathway. It may increase abscisic acid (ABA) levels and lead to decreases in shoot growth, and a possible increase in carbohydrate storage that may improve freezing stress tolerance.

Triazole plant growth regulators such as paclobutrazol are class B PGRs that act as anti-gibberellin compounds much earlier in the biosynthetic pathway by inhibiting the cytochrome P-450-mediated monoxygenase reactions involved in the isoprenoid pathway. Triazoles also induce a variety of other responses in plants including reduced or altered sterol biosynthesis, increased proline content, increased chlorophyll content, delayed senescence and increased stress tolerance. It has also been reported that ABA levels are increased in plants grown under triazole regulation. It has been suggested that the lowered gibberellic acid and increased ABA levels increase stress tolerance during chilling or freezing.

Paclobutrazol is one of the triazoles that best enhances stress tolerance. The cytochrome P-450 dependent oxidation of ent-kaurene to ent-kaurenoic acid is specifically inhibited in the gibberellin pathway by paclobutrazol. It has also been shown to stimulate root dry matter accumulation and increase turf density. In apple wood (*Malus* x *domestica* Borkh.), paclobutrazol increased the levels of nonstructural carbohydrates, an effect that was shown to persist throughout the winter and spring following application.

Theoretically, late fall applications of a plant growth regulator could improve the winter hardiness of plants by altering their carbohydrate status during acclimation when energy is being produced and used for storage rather than for top growth. This treatment could coincide with the gradual cessation of shoot growth, the initiation of the hardening process, membrane alteration, and accumulation of photosynthate. This could lead to a plant with enhanced cryoprotective features and an increased energy source, allowing it to withstand the incipient freeze-thaw periods. In addition, while plant growth regulators reduce shoot growth, they have been shown to maintain or slightly enhance root formation, thereby changing the root-shoot ratio in favor of the root, which could enhance overwintering.

The interaction of the rate and timing of application of PGRs on turfgrass may effect the storage carbohydrates and subsequently the tolerance of the crown tissue to freezing temperatures and the maintenance of dormancy through freeze-thaw cycles. Determining the relative freezing tolerance of annual bluegrass at various stages of acclimation and deacclimation for these plants for each PGR application could illustrate the effectiveness of these treatments for freezing stress survival. The relative freezing tolerance takes into account any pre-existing injury to the plant such as PGR treatment or insect injury before determining the temperature at which 50% of the remaining uninjured plant material is killed. The investigation of these relationships may lead to the development of a practical management tool for turfgrass managers to use to lessen winter injury.

Evaluation of Fiber and Inclusion Amendments for Athletic Field Soils

A.S. McNitt and P.J. Landschoot, *Department of Agronomy, Penn State University*

Many new athletic fields are constructed with sand root zones to reduce the effects of compaction and poor drainage associated with soil root zones. However, the cohesive force between sand particles is low and the sand can easily shift. This can result in increased divoting and poor traction. We evaluated six fiber and soil inclusion products for their effects on the soil physical properties and playing surface qualities (including traction and divoting) of a sand/peat root-zone mix under three wear levels. The treatments included products manufactured specifically for athletic field sand root-zones and included Netlon, Sportgrass, and Turfgrids. We also evaluated recycled inclusion amendments including recycled shredded carpet from DuPont Nylon_ and two shredded shoe products from Nike Soil physical properties and playing surface qualities including bulk density, soil water content, surface hardness, traction, and turf quality and density were measured through two growing seasons. Water infiltration rates were measured near the end of each growing season. The recycled inclusion amendments (shredded carpet and shoes) tended to reduce soil bulk density and reduce surface hardness, while the manufactured products (Netlon, Sportgrass, and Turfgrids) often had bulk densities and surface hardness values that were higher than the control. The treatments that lowered soil bulk density and reduced surface hardness values generally exhibited greater turfgrass wear resistance. The lower soil bulk density and surface hardness occurred without a corresponding lowering of traction values.

Assessing root zone mixes for putting greens over time under two environmental conditions

J. Murphy, S. Murphy, J. Honig, H. Motto, B. Clarke, R. Tate and E. Gaulin

This project is designed to i) improve recommendations for sand particle size distribution and the depth of the root zone by consideration of the microenvironment, ii) evaluate composts as organic additives and inorganic products for root zone mixes compared to peat sources, iii) assess the potential of various root zone mixes to reduce management and resource inputs, and iv) monitor the physical, chemical, and biological changes that occur in root zones as greens mature for understanding factors that contribute to the success or failure of greens. Packed cores of various sands, differing in particle size distribution but falling within USGA specifications for greens mixes, have been characterized in terms of physical properties in the laboratory. A large number of organic and inorganic amendments added to sand mixes at various rates have been tested as to their effects on the physical properties of the sands. Organic amendments evaluated in the laboratory included Dakota peat, sphagnum peat, Irish peat, AllGro compost, Fertl-soil, sewage sludge compost, and leaf compost. Inorganic amendments evaluated in the laboratory included Greenschoice, Profile, Isolite, Axis, Zeopro, and soil. Physical properties were frequently affected by the interaction between amendment and amendment rate. Total porosity was usually increased by amendment, and the sand size distribution and the type of amendment determined whether the increase occurred primarily in the air-filled porosity or the capillary porosity. Increases in air filled porosity did not always correspond with increased saturated hydraulic conductivity.

Root zone mixes having a range of characteristics were identified for study in the two microenvironments of the field research facility at North Brunswick, NJ. Putting green construction was begun in the spring of 1997 and completed in the late fall 1997. Organic amendments being evaluated in field plots include Dakota peat, sphagnum peat, Irish peat, AllGro compost, and Fertl-soil. Inorganic amendments being evaluated in fields trials include Axis, Greenschoice, Isolite, Kaofin, Profile, Soil, and Zeopro. Seven sands differing in particle size distribution were established in fields trials; three of the sands fell within USGA guidelines and the remaining four sands varied in the fine or very fine sand size ranges. Plots of various root zone depths were constructed using the three sands meeting USGA guidelines. The construction depths selected for each sand were based on water release curves in the laboratory. It is anticipated that the field plots will be seeded in the early spring of 1998 as weather permits.

The microbial ecology aspect of the project has progressed in a lab study and in a survey of golf greens of different ages. Differences in various microbial paramters were characterized in soil and sand amended with sphagnum moss peat, and the changes were monitored over time. The parameters used were dehydrogenase activity (which is associated with microbial respiration), estimation of viable bacteria, and metabolic diversity of the microbial population. Sand had much less microbial activity than soil. The effect of sphagnum moss peat added to soil was generally negative in terms of microbial activity, but sometimes increased activity in sand. Field samples taken from greens of different maturity and from neighboring fairways are being assessed in terms of the microbiological parameters. In 1998, the development of the microbiological community in the constructed root zone plots will be monitored.

Fate of Nitrogen in Turfgrass Systems

A. Martin Petrovic,
Department of Floriculture and Ornamental Horticulture,
Cornell University, Ithaca, NY

There is much concern in the general public, government regulators and environmentalists as to the impact of fertilizers and pesticides applied to golf courses on water quality. The results from early studies in this area suggest that fertilizers and pesticides applied to turf had little or no impact on groundwater quality. However, these studies were of limited scope (few or one soil, limited number of pesticides and climatic factors). The objective of our work was to develop a better understanding of the factors and conditions that lead to pesticide and fertilizer leaching from golf type turf (fairways and greens).

The leaching of NO3-N, NH4-N and PO4-P was evaluated under well-maintained fairway conditions having three soils types (sand, Arkport sandy loam and Hudson silt loam). The study was conducted at the ARESTS Facility (Automated Rainfall Fall Exclusion System for Turfgrass Studies) which contains 27 draining lysimeters (3.2 m X 3.2 m, 9 of each soil type), a rainout shelter, and individual irrigation and drainage monitoring for each lysimeter. The site was established in May of 1991 with seeded creeping bentgrass (Pencross). Normal and extremely wet precipitation conditions were also evaluated. From May to November, the rainout shelter excluded natural precipitation and irrigation was provided based on historic weather data for a normal year and for a very wet year (wettest in 101 years). All plots received at least one inch of water per week. A urea-methylene urea fertilizer (Scotts Hi Maintenance Fertilizer, 32-3-10) was applied in May, September and October at a rate of 1 lb. N/1000 sq. ft. Plots were mowed 3 times weekly at 1.2 cm height, a sample collected for nutrient analysis and clipping were not returned to the site.

We found that the leaching of phosphorus from fertilization was zero, even from the sand lysimeters. Nitrate leaching was limited and only influence by soil type where 9 % of the applied N fertilizer leached from the sand, 3% from the silt loam and 1.5% from the sandy loam 1.5%. The amount of precipitation-irrigation did not affect leaching. From half (sandy loam) to over 90 % (silt loam) of the applied N in the fertilizer was recovered in the clippings of creeping bentgrass from lysimeters with soil, while only 9 % was recovered in the clipping from the sand lysimeters. Most of the remaining fertilizer N was found in the soil (as roots, organic matter or fertilizer). The total estimated N recovery was slightly larger than the amount applied. Generally, there was good agreement in the data between the traditional N source and analytical methods and the enriched N15 fertilizer and mass spec analysis. Therefor, we recommend the use of the traditional methods and fertilizers over the N15/mass spec approach because of a lower cost unless detailed soil and atmosphere N fate is needed. The table below summarizes the fate of the N fertilizer in our studies.

Total amount of N recovered in clippings, soil and leachate as a per cent of applied nutrient; creeping bentgrass fairway turf.

Soil	Total N in clippings	NO3 -N +NH4 -N in leachate	Range in total N in soil	Total N Recovered
	-------------------------	% of applied	----------------------------	
Arkport sandy loam	52*	1.5	< 0	53.5
Hudson silt loam	91	3.1	< 0	94.1
Sand	8	9.1	106.4 - 116.7	124 - 134

* Corrected for the amount recovered in unfertilized plots and amount in irrigation water.

Do Lipids Play a Role in Turfgrass Disease Resistance?

Chun-lin Wang, Jing-song Xing, and Chee-kok Chin,
Department of Plant Science, Rutgers University

Lipids and their metabolites have now been established as crucial regulators of cellular functions in many biological systems. In our work on tomato and eggplant, we observe that a sequence of events involving lipid metabolism is initiated when the plants are challenged by pathogens. A number of intermediates, including certain active oxygen species are formed in the process. Our data indicate that some of these intermediates are involved in disease resistance.

To demonstrate that lipids are involved in disease resistance we have transformed tomato and eggplant with the yeast D-9 desaturase gene. These transgenic plants display enhanced resistance to a number of pathogens and possess higher levels of 16:1, 16:2 and 18:1 fatty acids. Lipid peroxides also increase. On the basis of our data, we suggest that lipids play a general role in plant, including turfgrass, disease resistance. A model on how lipids regulate disease resistance will be proposed and discussed.

Recent Advances With Entomopathogenic Nematodes

Randy Gaugler,
Department of Entomology, Rutgers University

A strain of the entomopathogenic nematode, Heterorhabditis bacteriophora, genetically enhanced for thermotolerance by introduction of a heat-shock protein gene from the free-living nematode Caenorhabditis elegans, was released in turfgrass field microplots in spring, summer, and fall 1996. As predicted, transgenic and wildtype strains did not differ in their ability to persist. We document the regulatory procedures at the federal, state, university, or local levels needed before field release, none of which posed any significant difficulties. Our risk assessment study supports the regulatory view that the transgenic nematode strain is an unlikely environmental threat. Subsequent regulatory reviews in the U.S. appear likely to continue to be decided on a case-by-case basis according to organism phenotype rather than the techniques used to generate them. This is the first report of a non-microbial, genetically-engineered insect natural enemy being released into the environment.

Evaluating Endophyte/Fine Fescue Associations with Chinch Bug Bioassays

Jennifer Johnson-Cicalese, Margaret Secks, William Meyer,
Department of Plant Science, Rutgers University

Considerable diversity exists among grass genotypes and strains of the *Neotyphodium* endophyte, and the interactions between the two result in an even greater range of plant reactions. For example, differences in amount of hairy chinch bug (*Blissus leucopterus hirtus* Montandon) damage were found in field trials among several endophyte/fescue associations. To gain a better understanding of these interactions, laboratory trials were conducted. Petri-dish preference tests, using grass tillers and first-instar chinch bugs, compared the number of chinch bugs on a fescue/endophyte combination with its endophyte-free counterpart. Non-choice 'cup' trials evaluated first-instar survival on endophyte-infected (E+) and endophyte-free (E-) fine fescues.

In most preference tests, chinch bugs preferred the E-Tillers. However, some endophytes, such as the Cambridge endophyte in a Chewings fescue selection and Spring Lake endophyte in a blue fescue, did not act as a deterrent to feeding. When chinch bug survival on several species was compared, the E+ hard and strong creeping red fescues were the most toxic, E+ Chewings fescues Intermediate, and no differences in chinch bug survival were found between an E- blue fescue and 2 E+ blue fescues. A strong creeping red fescue infected with the Rose City endophyte had the least chinch bug damage in the field, but when evaluated in the lab, no differences were detected between this endophyte and three other endophytes. Conflicting results between trials were also obtained with the Chewings fescues.

In order to reduce variability and evaluate one endophyte in several hosts, a trial was conducted with a set of artificially-inoculated clones developed by F. Belanger. When two strong creeping red and three Chewings fescue clones inoculated with the Rose City and Delaware endophytes were evaluated, no patterns in chinch bug survival emerged. In some cases, survival was worse on Rose City and in others, on Delaware, depending on its host. This work illustrates the complexity of the endophyte/plant relationship. Our next step will be to evaluate the compounds produced by these endophyte/fescue associations.

Biological Control of White Grubs (Coleoptera: Scarabaeidae) in Turfgrass

Paula M. Shrewsbury,
Department of Entomology, Rutgers University

Japanese and other scarab beetle larvae, commonly referred to as white grubs, are the most serious and damaging insect pest of turfgrass in the United States (Tashiro 1987, Potter and Braman 1991). Endophytic fungi, *Neotyphodium* (= *Acremonium*) spp., form a mutualistic symbiosis with perennial ryegrass, tall fescue, and fine fescue grasses. Endophytic infection of these grasses has led to varying degrees of resistance to several leaf, stem, and phloem feeding insects (Siegel et al. 1987, Dahlman 1991). Endophyte infected turfgrass has shown variable effects on white grub survival, growth, weight, and density in field, laboratory, and greenhouse studies (Potter et al. 1992, Murphy et al. 1993). Studies have found that some endophyte infected turfgrasses have sub-lethal effects on white grubs (reduction in weight gain) rather than lethal effects (reduction in survival). *Beauvaria bassiana* is an entomopathogenic fungi that occurs in nature and has activity against a wide range of insect hosts. *B. bassiana* is now commercially available to pest managers and is labeled to control a wide range of ornamental and turfgrass pests, including white grubs. However, repeated applications of *B. bassiana* are often required to reduce a pest population. This can lead to impractical control costs. In this study, I test the hypothesis that Japanese beetle white grubs feeding on endophyte infected turfgrass are stressed and therefore more susceptible to *B. bassiana*. If true, the combination of these two biological controls may reduce the number of *B. bassiana* applications required for grub population reduction. The overall objective of this study is to determine if there is a synergistic effect on white grubs when both "biological controls" are used in unison. This study was conducted at Rutgers University greenhouses. Treatments (12) included three fine fescue varieties from Rutgers 1991 National Trials, both with (E+) and without (E-) endophytes (Jamestown II E+, Jamestown II E-, Reliant E+, Reliant E-, SR 5100 E+, and SR 5100 E-), where one half of each turf type / endophyte combination received an application of *B. bassiana* (B+) and one half received water (B-, control). Turf plugs were taken from the field, potted, and placed in the greenhouse. Japanese beetle white grubs (first and second instar) were field collected from Kentucky bluegrass sod and implanted into each of the turf pots. Treatments (12 replicates) were arranged in a randomized complete block. Data were taken on grub survival and weight.

The effect of *B. bassiana* on grub survival varied with turf type. *B. bassiana* reduced survival of grubs on Jametown II, but was not influenced by endophyte infection. There was no endophyte effect on survival. The effect of *B. bassiana* on grub weight also varied with turf type. Both endophyte infection and *B. bassiana* reduced grub weight on Reliant. In addition, when both treatments were applied there was a synergistic effect in grub weight reduction. These studies suggest a need for further examination of the lethal (survival) and sub-lethal (weight) effects of *B. bassiana* on grubs as they are influenced by number of *B. bassiana* applications, turf type, and endophyte infection.

Opportunities for the Genetic Improvement of Underutilized Plants for Turf

Reed Funk, *Department of Plant Science,*
New Jersey Agricultural Experiment Station, Rutgers University

Exciting progress has been made during recent decades in the genetic improvement of many turfgrasses including perennial ryegrass, tall fescue, hard fescue, Chewings fescue, and creeping bentgrass. The best new varieties show little resemblance to varieties available in 1960. Population improvement programs in these species were based on collection of elite plants surviving in old turfs. This was followed by many cycles of phenotypic assortive mating followed by phenotypic and genotypic recurrent selection combined with a modified population backcrossing program. This enabled breeders to make dramatic changes in many characteristics of value to the turfgrass industry. Each cycle of improvement built on all previous cycles in these highly heterozygous, heterogeneous, cross-pollinated species. The discovery, study, and utilization of superior strains of useful *Neotyphodium* endophytes added to the value of many species. The substantial and continued genetic improvement made in these species and associated symbionts suggests that we will not be able to predict the true usefulness of unimproved species until they have been subjected to an extensive collection and evaluation of their useful genetic variation. This must be followed by many cycles of effective population improvement.

Kentucky bluegrass (*Poa pratensis* L.) continues to be the premier lawn-type turfgrass for temperate regions of the United States and Canada. It is hardy, attractive and widely adapted. Extensive rhizomes enable it to spread and recover from stresses of heat, drought, excess wear, insect injury and disease damage. Kentucky bluegrass has an immense range of genetic diversity including nearly every characteristic needed in an ideal lawn grass. Its apomictic reproduction allows us to utilize a rare, outstanding, highly heterozygous plant as the foundation of a uniform, true-breeding variety with the advantages of both hybrid vigor and seed propagation.

Single-plants selected from old turfs and controlled hybridization have produced many good, but not truly outstanding varieties. Our inadequate control of apomictic versus sexual reproduction has limited our ability to effectively use many of the population improvement programs that have been so successful in the rapid genetic improvements of many sexual, cross-pollinated species. New ideas and increased efforts are needed to improve breeding methods in apomictic species.

Most breeding efforts in Kentucky bluegrass have been directed to developing lower-growing, disease resistant turf-type varieties. These efforts should continue. However, considerable emphasis should be made to develop mid-Atlantic types with increased tolerance of heat and drought, improved resistance to, and recovery from insects and diseases, and high seed yields. This would greatly increase the value of Kentucky bluegrass in the transition zone.

Genetic improvements are also needed in common-type Kentucky bluegrasses able to produce economical seed yields under dryland production and thrive in low-maintenance turfs. Improved varieties of *P. angustifolia* should also be developed and evaluated for this purpose.

Opportunities exist in the selection and improvement of low-growing, fine textured varieties of bulbous bluegrass (*P. bulbosa*), low-growing perennial types of *Poa annua* and *P. supina*

Interspecific crosses between Kentucky bluegrass x Canada bluegrass (*P. compressa*), Texas bluegrass (*P. arachinifera*) x Kentucky bluegrass, and *P. angustifolia* x Kentucky bluegrass should be of considerable interest and value.

The genus, *Agrostis*, contains a number of species with considerable potential for genetic improvement for turf. Creeping bentgrass (*A. palustris*) is the premier turfgrass for closely mowed golf course putting greens. Penncross, released by the Pennsylvania Agricultural

Experiment Station in 1954, dominated the market for improved bentgrasses for over three decades. Following the release of Pennlinks (1986), SR-1020 (1987), and Providence (1988), a number of other improved creeping bentgrasses have been developed. Many were specifically adapted to various areas of the country or management regimes. Considerable potential exists for additional improvements in this species.

Velvet bentgrass (*A. canina* L. subsp. *canina*) can form an attractive, low-growing, compact, soft turf with very fine leaves spreading by creeping, leafy stolons. Dr. Howard B. Sprague, a noted Rutgers agronomist, studied turfgrasses during the 1930's. He felt that velvet bentgrass had great potential. It required little or no fertilizer, grew well in sun or shade, and performed well as either a putting-green or lawn-type turf.

His variety, Raritan, was released by the New Jersey Agricultural Experiment Station in 1940. Due to disruptions caused by World War II, this variety was lost. Recent research by the Rhode Island Agricultural Experiment Station, Seed Research of Oregon, and the New Jersey Agricultural Experiment Station indicate great potential for additional genetic improvement of this species.

Brown bentgrass (*A. canina* L. subsp. *montana*) is a drought-resistant lawn grass, spreading by rhizomes to form a fine compact turf. It is widespread in the British Isles and found in temperate regions of Europe and Asia. We are not aware of any efforts to collect, evaluate, or improve this grass for turf use in the United States.

Colonial bentgrass (*A. tenius*) is extensively used as a lawn grass in the British Isles, northern Europe, and New Zealand, often in mixtures with Chewings fescue. It is also preferred for putting greens and fairways in these countries. High susceptibility to Rhizoctonia brown patch makes it less useful in areas with hot, humid summers. An extensive search for colonial bentgrasses surviving in old turfs of the mid-Atlantic region could well provide germplasm for the genetic improvements needed in this attractive turfgrass.

Dryland or Highland bentgrass (*A. castellana*) is a hardy, densely to loosely-tufted perennial turfgrass, vigorously spreading from stout, short rhizomes. The variety, Highland, was selected in Oregon from naturalized stands. Its characteristics are strongly suggestive of a Mediterranean origin. Recent collections from old, closely mowed turfs in the mid-Atlantic region of the United States indicate that substantial improvement can be made in attractiveness and turf performance.

Redtop (*A. gigantea*) has been used for pastures, hay, and erosion control throughout the northeastern and north central parts of the United States, especially on infertile, poorly drained, acid soils. It was widely used as a temporary grass in lawn seed mixtures prior to the development of improved turf-type perennial ryegrasses. Redtop is more robust and less aggressive than dryland or colonial bentgrasses. Germplasm collection, evaluation and enhancement programs should be effective in developing better adapted varieties with improved performance for low-maintenance turfs on wet, acid, and infertile soils.

Significant advances have been made in the genetic improvement of Chewings fescue and hard fescue. Efforts to obtain additional improvements should continue. In addition many opportunities exist for substantial enhancement of turf performance of strong creeping red fescue, slender creeping red fescue, sheeps fescue, blue fescue, and hybrids between hard and blue fescue.

Selections of *Puccinellia* spp. are highly salt tolerant and can produce attractive, fine textured turfs for saline soils and for roadsides where salt is frequently used for removal of snow and ice.

Barkoel an improved variety of *Koeleria* has produced a surprisingly dense, attractive, low-growing turf under low maintenance conditions but has not performed well when fertilized. Are we discarding many useful species and germplasm sources because of inadequate evaluation techniques? Recent selections of *Deschampsia* spp. also show promise for low maintenance turfs.

Finer textured, lower growing, darker green selections of orchardgrass are occasionally found growing on poor soils and in moderate to heavy shade. An extensive germplasm collection effort followed by a few cycles of population improvement might make orchardgrass a more useful species for turf.

Zoysiagrass has a tremendous amount of genetic variation and many characteristics needed in an excellent low-maintenance turfgrass. Its native range extends from southern Australia through Indonesia and the Phillippines to islands north of Japan. Types exist with a very low growth habit and fine leaves; others have excellent salt-tolerance. It has good resistance to heat, drought, infertile soils, many diseases, and insect pests. Eastern Asia, where many ecotypes evolved, has climate and soils similar to those of eastern North America. If New Jersey and other parts of the eastern United States had been colonized by settlers from Korea and northeast China, it is likely that Zoysia would be our dominant turfgrass.

Dramatic advances have been made in the genetic improvement of bermudagrass for warmer regions. These range from exceptionally fine-textured, low-growing varieties (Tifdwarf), and wear-tolerant types for sports turf, to very productive, robust forage grasses. Germplasm collection from the mountains of Africa, Europe, and southern Asia, followed by cycles of population improvement could extend the use of this exceptionally wear tolerant grass into more northern areas.

White dutch clover, strawberry clover, birdsfoot trefoil, turf-type alfalfa, crown vetch, and perennial sweet pea are examples of legumes which may well play an increasingly important role in many lower maintenance turfs. Symbiotic nitrogen fixation and deep tap roots aids in enhancing soil fertility and structure and promotes growth of associated grasses and other plants. Attractive flowers add to the beauty and diversity of turfs containing legumes.

A Study of Genes Involved in Development of Endophytes

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Clavicipitaceous symbionts of grasses include species of *Epichloë* and *Atkinsonella*. The species of these two genera show several common features. Among common features is that both are symbionts of cool-season grasses, possess *Neotyphodium* conidial states, and form stromata on inflorescence primordia. These fungi infect primordial tillers growing on, or within, primordial leaves in the expanding tillers. As the grass inflorescence primordium differentiates on the tiller, mycelium of the symbiont proliferates to fully enclose the living meristems within a mycelial stroma, trapping the meristem in an arrested state of development. Fungal reproductive structures differentiate on the surface of the mycelial stroma. Because of the potential to manipulate endophyte genomes by making crosses using stromata, we have initiated studies to understand factors affecting development of stromata of *Atkinsonella* and *Epichloë*. When *Atkinsonella hypoxylon* is isolated from plant tissues and grown on standard agar media, the colonies do not contain the same structures and appearance of the stroma on host tissues. Instead, the mycelium is undifferentiated (non-branching, non-pigmented, and without any conidial structures). Acting on the hypothesis that compounds produced by the host plant may be necessary to induce proper differentiation of the fungus, we screened numerous compounds to identify those that are active in induction of differentiation.

Kinetin, a plant hormone belonging to the cytokinins, was found to induce the *Atkinsonella* to form colonies comparable to stromata as seen on plant tissues. We applied differential display RT-PCR for the analysis of gene expression in *Atkinsonella* with and without kinetin in the growth medium. The comparative gene expression analysis performed has yielded several fungal cDNAs representing genes whose expression may be necessary during the differentiation of the fungus on the plant. Characterization of those genes is under investigation This research may lead to the development of cultural procedures to breed and select endophytes for specific traits of importance in turf, such as enhanced drought tolerance, insect resistance, and fungus-disease resistance.

Characterization of Microsatellite Loci in Perennial Ryegrass (*Lolium perenne*)

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Perennial ryegrass (*Lolium perenne*) cultivars are usually distinguished by morphological characteristics, but morphological criteria can be ambiguous. Genetic markers may improve cultivar identification and may also advance understanding of relationships among taxa. One promising type of molecular marker are microsatellites. Microsatellites are DNA sequences that contain short tandem dinucleotide or trinucleotide repeats, and are randomly dispersed throughout the genome in most organisms making them a valuable tool for marker assisted breeding. We have isolated six microsatellite loci from perennial ryegrass, and our studies indicate these markers are abundant in this grass with potentially 11,600 loci in the genome. In addition to being abundant, microsatellite loci are highly polymorphic. For example, we were easily able to distinguish between half-sib progeny with six markers. Finally, we have used microsatellite data to build a phylogenetic tree suggesting genetic relationships among several perennial ryegrass cultivars and other *Lolium* species. These markers appear to provide reasonable insights into genetic relationships, but on the basis of these studies, characterization of more loci can help make fingerprinting and establishing relationships among taxa more concrete. We have only scratched the surface with this preliminary work.

Poster Presentations

Induction of Differentiation in the Clavicipitaceae

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Biotrophic symbionts of the Clavicipitaceae are of interest because of the impact they have been shown to have on the ecology of the host plants and health of animals that consume them. Some species, such as *EpichloN baconii,* are endosymbiotic, while others, such as *Atkinsonella hypoxylon,* are episymbiotic. Our knowledge of the interactions between mycobiont and phytobiont in the symbiosis is rudimentary to nonexistent. Like many symbionts, these fungi may be isolated from host tissue and grown in pure culture. These cultures, however, never develop the structures that are seen when the fungus forms its reproductive structures (called stromata) on host tissues. As explanation of this observation, it is reasonable to propose that development of the mycobiont is controlled to some degree by compounds produced by the phytobiont. To gain clues as to what these compounds may be, we have examined responses of Clavicipitaceae in culture to a broad range of compounds.

EpichloN baconii was grown in shaker culture and resultant mycelial balls were pipetted into wells of SF-N and SF-P Micro- Plates (Biolog Inc., Hayward, CA). Each MicroPlate well contained a different carbon source. After incubation for 10 days at 21oC, the wells were examined. The wells showed that numerous carbon substrates had been utilized. Mycelium from *E. baconii* grown in the wells was examined under a microscope, and it was determined that the *Neotyphodium* anamorph formed when salicin was a carbon source.

For the *Atkinsonella hypoxylon* studies, 100 ppm cytokinin was added to a medium consisting of 1% sucrose, MS basal medium, 0.3% yeast extract, and 1.5% agar. The growth and development of *A. hypoxylon* were examined over a 4-6 week period. Over this period of time, the mycelium of *A. hypoxylon* turned yellow-gray and formed the *Neotyphodium* synanamorph and cupulate ephelidial conidiomata. Exudate from the conidiomata contained ephelidial conidia. Without cytokinin, the mycelial was white to off-white without the development of conidiomata.

Growth Characteristics Associated with the Classification Types of *Poa Pratensis*

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The apomictic breeding behavior of *Poa pratensis* L. provides the opportunity to study many unique genotypes. There are over 100 cultivars of Kentucky bluegrass which can vary dramatically in performance characteristics such as disease resistance, stress tolerance and growth habit. Major classification groups of Kentucky bluegrass have been presented based on common growth and stress performance characteristics gathered from field data. The major classification types of *Poa pratensis* are Compact, Bellevue, Mid-Atlantic, BVMG, Common, and Aggressive types. The objective of this study was to quantify morphological and growth characteristics of the major classification types of Kentucky bluegrass. A nursery evaluation trial was established in May 1996 at the Plant Science Research Station, Adelphia, New Jersey on a Freehold sandy loam (fine, mixed, mesic Typic Hapludult). The study was a randomized complete block design of 45 cultivars and selections and nine replications. Measurements included: plant height; panicle height; flag leaf height, length and width; subtending leaf length and width; rhizome spread; and rhizome internode length and width. Compact type cultivars generally had a lower, more prostrate growth habit. The Mid-Atlantic and Common types had an upright, erect growth habit. Mid-Atlantic type cultivars had a wider rhizome spread than the compact types. Further measurements and development of this classification system are needed as more cultivars are developed with exceptional characteristics.

Susceptibility of Fine Fescue Species, Cultivars, and Selections to *Magnaporthe poae*

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Summer patch, caused by *Magnaporthe poae*, is a serious disease of bluegrass and fine fescue turfs in the United States. *M. poae* and *Gaeumannomyces incrustans* were isolated from fine fescue turfs exhibiting patch disease symptoms at two locations in New Jersey during the summers of 1988 to 1990. Isolates of *M. poae* were highly pathogenic on cultivars and selections representing five fine fescue species in growth chamber and field experiments, whereas the *G. incrustans* isolates were only mildly pathogenic. As a group, strong creeping red fescues were least susceptible to summer patch and Chewings fescues were variable in their response to *M. poae*. The intensity of summer patch symptoms observed on Chewings fescues was less severe in natural infestations than in artificial inoculation studies. Hard fescues were highly susceptible to the disease in natural infestations, but demonstrated less damage in field inoculations and growth chamber tests. Little variability in disease susceptibility was observed among the hard fescues tested. Slender creeping red fescues were severely damaged in all tests.

Separation of Fine Fescues using Reversed-Phase High-Performance Liquid Chromatography

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Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) techniques were applied

to the purity analysis of hard fescue (*Festuca ovina* var. *duriuscula* (L.) Koch), chewings fescue (*Festuca rubra* L. subsp. *commutata)* and creeping red fescue (*Festuca rubra* L. subsp. *rubra*) seeds based on protein separations. Graphs of the retention times of the proteins eluted from the column showed differences in the species of hard fescue and red fescue. Differences were also demonstrated among the varieties of the fescues studied. RP-HPLC analysis of seed proteins was used to determine the proportion of components in a hard and chewings fescue mixture. Liquid chromatography appears to be a rapid and efficient method of analysis for hard and red fescue seeds.

Ergosterol as an Indicator of Endophyte Biomass in Grass Seeds

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Endophytic fungi of the Tribe Balansiae form an important symbiosis with cool-season forage and turf grasses. These seed-borne fungi can manifest severe livestock disorders in grazing animals, but enhance stress and pest resistance in the host grass. The methods currently used to detect endophytes in grass seeds require a large input of time and labor. Ergosterol, a tri-terpene sterol, is highly specific to the Kingdom Fungi and has been used to estimate fungal biomass in soil and other plant-fungal systems. A series of experiments was conducted to assess the application of ergosterol analysis to endophyte determinations in grass seeds. Ergosterol was present in all endophytic fungi tested, ranging from 185.2 μg g-1 in *Epichloë typhina* to 1225.5 μg g-1 in *Neotyphodium lolii*. Significant differences in ergosterol content occurred both between and within species of endophytic fungi, and significant correlations were observed between ergosterol and fungal biomass. Ergosterol content of grass seeds was highly correlated (r2 = 0.99) to the endophyte content of the seeds, suggesting that the technique could be used to estimate concentration of endophyte mycelium in an unknown sample. The endophyte content of a group of fine fescue seed samples of diverse origin was predicted from the ergosterol analysis and was found to be correlated with microscopic analysis of those seed lots. It is concluded that ergosterol analysis can be used in both diagnostic and research laboratories to predict endophyte content in seed samples.

Bioactivity of *Epichloë festuceae* Against *Sclerotinia homoeocarpa.*

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Endophytic fungi of the Tribe Balansiae form a mutualistic, symbiotic relationship with many C3

grasses, including the economically important *Festuca spp.* Recent results from long-term field trials in New Jersey have indicated that endophytes of the fine fescue grasses (i.e. red fescue and chewings fescue) provide almost absolute host resistance against common dollar spot (causal agent *Sclerotinia homoeocarpa).* A series of laboratory studies was initiated to assess if the resistance observed in field trials was associated with the production of antibiotic chemicals by the endophytes. A dual culture assay system was used to test inhibitory activity of several isolates of *Epichloë festuceae* against many common grass pathogens. Biological activity was greatest against *S. homoeocarpa* and other leaf-infecting pathogens, but limited against root pathogens. In a second set of experiments, endophyte broth cultures were partitioned against organic solvents of varying polarity. Organic extracts were tested in agar diffusion assays against the same pathogenic fungi. Organic extracts again had the highest activity against *S. Homoeocarpa* and the highest antifungal activity was observed in ethyl acetate fractions followed by chloroform and hexane. Current work is being directed towards identifying those compounds.

Amplified Fragment Length Polymorphisms (AFLP) in *Epichloë festucae* and Related Grass Endophytes

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Epichloë festucae is an endophytic fungus that forms symbiotic relationships with fine fescues, a group of fine-textured grass species in the genus *Festuca*. The presence of this endophyte confers host resistance to herbivores, insects, fungal pathogens, and abiotic stresses due to alkaloids or other metabolites that are produced in endophyte infected grasses. However, several problems have prevented *E. festucae* from realizing its full potential as a biological control agent in turfgrass management, and scientists are interested in manipulating the endophyte/host relationship so that this can be achieved. This study was conducted to evaluate a new technique for revealing molecular markers, called Amplified Fragment Length Polymorphisms (AFLP), in *E. festucae* and related grass endophytes. AFLP fingerprinting of 24 isolates of *E. festucae* and 15 other endophytes with 10 primer pairs resulted in the amplification of 942 unique restriction fragments. Neighbor-joining analysis of polymorphisms created a phylogeny similar to those obtained from ribosomal DNA sequences, although some significant differences were observed. Within *E. festucae*, four clades of closely related isolates were identified that correspond to the host species from which they were isolated. Isolates within each clade were also shown to have similar growth rates *in vitro*, which may be a determinant of host compatibility. AFLP fingerprinting appears to be a powerful technique for revealing polymorphic molecular markers

in closely related organisms and for identifying groups of individuals that are biologically similar. Future studies will concentrate on additional phenotypic characteristics that may be correlated to variation in AFLP markers.

Environmentally Sensitive/Sustainable Turfgrass Management Extension Outreach

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Situation: Public concern regarding environmental quality has raised questions about the risks associated with turfgrass fertilizers, pesticides, water use and clipping disposal. However, turfgrasses largely enhance environmental quality - especially when proper species/cultivar selection, establishment and maintenance practices are employed. This program seeks to address environmental and health concerns by increasing awareness and adoption of sustainable turfgrass management practices by the public and commercial horticulturists.

Goal: To increase the adoption and practice of environmentally sensitive, sustainable turfgrass management practices through applied research and demonstration projects and extension outreach to commercial turf managers and the public.

Key Projects:

Low maintenance turf demonstration. Seventeen top performing cultivars, in New Jersey trials, were established, September 1997, in 3x5 ft(with 4 replicates in randomized complete block design. The following were planted: Koelaria macrantha; 3 cultivars of hard, chewings and tall fescue; 1 cultivar of buffalograss; 3 cultivars of perennial ryegrass(added to demonstrate higher mowing needs) and 3 cultivars of Kentucky bluegrass. Each will be evaluated for performance under low maintenance and for mowing requirements. Demonstration is located on the property of Rutgers Cooperative Extension of Camden County and will be used for commercial and public educational meetings.

Evaluation of mowing practices on the occurrence of beneficial arthropods and earthworms in tall fescue turf. In cooperation with Shrewsbury. Predatory arthropods help prevent pest outbreaks in turf. Additionally earthworms promote thatch breakdown, enhance soil physical properties and improve turf growth and ecology. Mowing practices alter turf microclimate including temperature and humidity. Also, clipping return should increase biological activity associated with decomposition and greater numbers of predatory arthropods. Tall fescue will be evaluated at a 1 inch and 3 inch mowing height and with and without clippings returned.

Populations of ants, spiders, ground and rove beetles and earthworms will be measured. Evaluation will be conducted 1998 through 2000.

Evaluation of Turf for Orchard Floor Managment. Turfgrasses reduce weeds and pests when established in commercial orchards. This study is evaluating Koelaria macrantha and three cultivars of each: hard, chewing and tall fescues. Established, in a peach orchard, in October, 1996.

Future projects: Evaluation of seedbed modification for improved establishment of Koelaria macrantha including phosphorous and nitrogen fertility and organic amendments.

Antifungal Compounds from *Epichlo* æ *Festucae*

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Epichloë endophytes commonly form a mutualistic, symbiotic association with many cool-season grasses (Bacon, et al.,1990). These nonpathogenic fungi have been identified as an ecological advantage to the grass by enhancing resistance of the host plant against specific insect pests (Siegel, et al., 1990; Latch, 1993) and drought (West,1994). In recent years, the endemic endophyte of fine fescue has been shown to enhance resistance of the host against dollar spot (*Sclerotinia homoeocarpa*) (Clarke, et al., 1997), a common pathogen of many cool-season turfgrasses. The basis of insect resistance in endophyte-infected grasses lies predominantly in several alkaloid mycotoxins that are produced in the symbiotic association (Porter, 1995). However, very little information is available regarding chemicals that might be related to the resistance to fungal disease pathogens such as *S. homoeocarpa*.

The objective of this research was to screen liquid cultures of *Epichloe festucae* for anti-fungal compounds. The activity of crude culture extracts was monitored using bioautography with *Chryphonectria parasitica* as the test organism. Cultures were grown for 6 weeks in MIO4T medium, with the first 2 weeks on a rotary shaker, and the final 4 weeks as a stationary culture. The crude extract was initially separated by liquid / liquid partition. A bioassay-monitored chromatography of extract resulted in five 3-substituted indole derivatives (**1**~**5**), a sesquiterpene (**6**) and a simple diamide (**7**) with anti-*C. parasitica* activity. To our own knowledge, it is the first time a sesquiterpene has been identified from in vitro cultures of the endophyte. Compounds **1** and **2** were also tested against *Magnaporthe poae*, *Rhizoctonia solani* and *Lactisaria fusiformis*. The greatest activity of **1** and **2** was against *L. fusiformis* with 66.9% and 99.3%

inhibition, respectively, at 100 ppm. Further analysis of the bioactive fractions by GC-MS showed the existence of five trace indole derivatives (**8~12**).

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