Third Annual Turfgrass Symposium

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Director's Opening Remarks:

I would like to take this opportunity to welcome you to the Third Annual Rutgers Turfgrass Symposium at Cook College/NJAES. The Symposium was established 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.

From its inception in 1991, the Symposium has helped to foster a better understanding of the basic and applied research projects conducted at the Center. It has also encouraged interaction, collaboration, and common interests among researchers from within the Rutgers Community. I can think of no better way to bring together individuals with such diverse expertise and backgrounds for the purpose of enhancing collegiality and scientific advancement than through a vehicle such as the Annual Turfgrass Symposium. I hope that you will take full advantage of the many opportunities that this Symposium has to offer and feel confident that the Center has laid the framework for a long and valued tradition.

Finally, I would like to give special recognition to the Symposium Planning Committee comprised of Deena Amont, Faith Belanger, Christina Hartman, Brad Hillman, Don Kobayashi, and Lisa Lee. They spent many hours arranging the program and did a superb job. Without their hard work and attention to detail, the Symposium would not have been possible. I would also like to thank Gerri Muenzer, my administrative assistant, and all of the researchers who agreed to give presentations or posters at this year's meeting. Their cooperation and strong commitment to the Center is most appreciated.

Bruce B. Clarke

Interim Director,

Center for Interdisciplinary

Studies in Turfgrass Science

January 21, 1994

THE THIRD ANNUAL RUTGERS TURFGRASS SYMPOSIUM

Cook College Campus Center
Cook College, Rutgers University
January 21-22, 1994

Program Schedule

Friday, January 21, 1994

6:30 - 10:00 PM

6:30 - 7:00 Registration

7:00 - 7:05 Welcome and Introduction -- **Dr. Bruce Clarke**, Interim Director, Center for Interdisciplinary Studies in Turfgrass Science

7:05 - 7:15 Opening Remarks, Comments from the Deans

Dr. Zane Helsel , Dean of Cooperative Extension, Rutgers University

Dr. Rod Sharp , Dean of Research, Director of NJAES

7:15 - 8:00 Keynote Address -- **Dr. Bob Carrow** (Department of Crop and Soil Sciences, Univ. of Georgia): *Research Strategies for Turfgrass Water Conservation*

8:00 - 10:00 Wine and Cheese Mixer, Poster Session

Saturday, January 22, 1994

8:00 AM - 5:00 PM

8:00 - 9:00 Registration, Coffee and Donuts

9:00 - 10:00 Session #1 (Turfgrass Physiology)

Dr. Bruce Clarke, Moderator

9:00 - 9:30 **Dr. Richard Hull** (Department of Plant Sciences, University of Rhode Island): *Increasing Nitrogen Use Efficiency by Turfgrasses*

9:30 - 9:45 **Dr. Jim Murphy** (Department of Plant Science, Rutgers University): *Near-Infrared Reflectance Spectroscopy for Monitoring Nitrogen Status of Turfgrass*

9:45 - 10:00 **Dr. Joe Heckman** (Department of Plant Science, Rutgers University): *Results from Commercial and Experimental Fertilizer Trials on Turfgrass*

10:00 - 10:30 Discussion and Coffee

10:30 - 11:30 Session #2 (Turfgrass Improvement)

Dr. David Huff, Moderator

10:30 - 11:00 **Dr. Richard Hurley**, (Lofts Seed, Inc.): *Cool Season Grass Breeding Progress, Future Objectives For Breeding Priorities, and Interest In Initiating Breeding Programs in New Species*

11:00 - 11:15 **Dr. Reed Funk** (Department of Plant Science, Rutgers University): *Opportunities and Challenges In Turfgrass Breeding*

11:15 - 11:30 **Dr. Lisa Lee** (Center for Agricultural Biotechnology, Rutgers University): *Creeping Bentgrass Transformation*

11:30 - 1:30 Discussion/Poster Session/Lunch

1:30 - 2:30 Session #3 (Turfgrass Endophytes)

Dr. Nilgun Tumer, Moderator

1:30 - 2:00 **Dr. Keith Clay** (Dept. of Biology, Indiana University): *Comparative Ecology of Endophyte Infected and Uninfected Grasses*

2:00 - 2:15 **Dr. Faith Belanger** (Dept. of Plant Science, Rutgers University): *Characterization of an Endophytic Fungal Protease which is expressed in the Infection of* Poa ampla

2:15 - 2:30 **Dr. Brad Hillman** (Dept. of Plant Pathology, Rutgers University): *Double stranded RNAs and Antimicrobial Activity Associated with* Atkinsonella hypoxylon

2:30 - 3:00 Discussion/coffee

3:00 - 4:00 Session #4 (Turfgrass Pathology and Stress)

Dr. Donald Kobayashi, Moderator

3:00 - 3:30 **Dr. Monica Elliott** (Fort Lauderdale Research and Educational Center; University of Florida): *Will Biological Control Agents Control Turfgrass Diseases?*

3:30 - 3:45 **Dr. Barbara Zilinskas** (Dept. of Plant Science, Rutgers University): *Antioxidant Enzymes and Environmental Stress Tolerance*

3:45 - 4:00 **Dr. Carlos Neyra** (Dept. of Plant Science, Rutgers University): *Development of Bacterial Inoculants for the Use On Turf, Range and Pasture Grasses*

4:00 - 5:00 Discussion/Closing Remarks/Refreshments, Social

Preregistered Participants

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Research Strategies For Turfgrass Water Conservation *Robert N. Carrow, Crop and Soil Science, Georgia Experiment Station, University of Georgia*

Increasing Nitrogen Use Efficiency By Turfgrasses *Richard J. Hull, Department of Plant Sciences, University of Rhode Island*

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Results from 1993 Turfgrass Fertilizer Trials *J. R. Heckman, Department of Plant Science, Rutgers University*

Cool Season Grass Breeding Progress, Future Objectives For Breeding Priorities, and Interest In Initiating Breeding Programs In New Species *Richard Hurley, Lofts Seed, Inc.*

Breeding and Evaluation of Kentucky Bluegrass, Tall Fescue, Fine Fescue, Perennial Ryegrass, and Bentgrass for Turf *C. Reed Funk, James Murphy, Bruce Clarke, Karen Plumley, James White, William K, Dickson, Ronald Bara, Suichang Sun, Dirk Smith, Randy Prostak, and Pedro Perdomo, Department of Plant Science, Rutgers University*

Creeping Bentgrass Transformation *Lisa Lee, Christina Hartman, Cynthia Laramore, Peter Day, and Nilgun Tumer, Center for Agricultural Molecular Biology, Rutgers University*

Ecological Consequences Of Endophyte-Infection In Grasses *Keith Clay, Department of Biology, Indiana University*

Purification And Characterization Of An Endophytic Fungal Protease Which Is Induced On Infection Of Its Host Grass *Jon T. Lindstrom and Faith C. Belanger, Plant Science Department, Rutgers University*

Double-stranded RNAs and Antimicrobial Activity Associated with *Atkinsonella hypoxylon* *Chan-Seok Oh and Bradley I. Hillman, Department of Plant Pathology, Rutgers University*

Will Biological Control Agents Control Turfgrass Diseases? *Monica L. Elliott, University of Florida - IFAS*

Antioxidant Enzymes and Environmental Stress Tolerance *Lynne H. Pitcher1, Christine L. Hartman2, Lisa Lee2, Nilgun Tumer2, and Barbara A. Zilinskas1, Plant Science Department1, and AgBiotech Center2, Rutgers University*

Development of Bacterial Inoculants for the Use On Turf, Range and Pasture Grasses *Carlos A. Neyra, O. Olubayi, D. Zaurov and R. W. Duell, Plant Science Department, Rutgers University*

Poster Presentations

Isolation of Bacteria With Suppressiveness to Summer Patch Disease Using Enrichment Cultures *Nour El-Barrad, David Thompson, Bruce Clarke and Donald Kobayashi, Department of Plant Pathology, Rutgers University*

Utilization of the Polymerase Chain Reaction to Identify*Magnaporthe poae* and Its Phylogenic Characterization Among Various Ascomycetes *Tracy E. Bunting, Karen A. Plumley, Bruce B. Clarke, and B. Hillman, Department of Plant Pathology, Rutgers University*

Esterase Differences In Seedlings And Seedling Mixtures Of Hard And Red Fescues *G. W. Freeman and F. A. Yoder, Jr., New Jersey Department of Agriculture*

Esterase Isoenzyme Electrophoresis as a Method of Separating Colonial and Creeping Bentgrass Mixtures *G. W. Freeman and F. A. Yoder, Jr., New Jersey Department of Agriculture*

Particle Gun Transformation of Creeping Bentgrass *Agrostis palustris*) Using the *bar* Gene *Christina L. Hartman1, Lisa Lee1, Nilgun Tumer1, Lynne Pitcher2, Barbara Zilinskas2, AgBiotech Center1 and Department of Plant Science2, Rutgers University*

Evaluation of N-Viro Aglime as a Soil Amendment for Turfgrass Establishment *W. Hill and J. R. Heckman, Department of Plant Science, Rutgers University*

Isolation and Characterization of Carbendazim-Degrading Bacteria *M. Andrew Holtman and Donald Kobayashi, Department of Plant Pathology, Rutgers University*

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Is Expression Of Fungal Invertase A Factor In Choke Disease? *Cuong Lam1, Faith Belanger1, James White2, and Jaleh Daie3, Plant Science Department, Rutgers University1, Department of Biology, Auburn University2, and Department of Botany, University of Wisconsin3*

Development of Herbicide Resistant Turfgrass through Mutant Selection and Protoplast Transformation *Lisa Lee, Cynthia Laramore, Peter Day, And Nilgun Tumer, Center for Agricultural Molecular Biology, Rutgers University*

Development Of An Endogenous Plasmid Into A Cloning Vector In *Clavibacter xyli* Subsp. *cynodontis* For Expressing Insecticidal Genes *T. Y. Li, Y. P. Zhang and T. A. Chen, Department of Plant Pathology, Rutgers University*

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Influence of Liming, N-form, Sulfur, and Application Method on Soil pH and Summer Patch *James A. Murphy1, David C. Thompson2, and Bruce B. Clarke2, Department of Plant Science1 and Department of Plant Pathology2, Rutgers University*

Selection of Wear Resistant Tall Fescue (*Festuca arundinacea*) *M. W. Ventola and J. A. Murphy, Department of Plant Science, Rutgers University*

A Study on the Chromosomal Homologs of the Native Plasmid pCXC100 In *Clavibacter xyli* subsp. *cynodontis* *Y. P. Zhang, T. Y. Li, and T. A. Chen, Department of Plant Pathology, Rutgers University*

Research Strategies For Turfgrass Water Conservation

Robert N. Carrow

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The goal is direct and simple: to minimize water use on turfgrass sites. Sometimes this goal is expressed in other terms, such as "turfgrass water conservation" or "efficient use of turfgrass water". Achievement, however, is complex with each specific turfgrass site offering a unique set of challenges. Strategy is the science and art of devising and implementing plans or methods toward a goal. Nine methods can be incorporated into an overall turfgrass water conservation strategy and each has the potential for research input. Optimum water use efficiency cannot be achieved by any one approach but can with appropriate consideration of all nine methods. These nine components are: (1) develop grasses with high drought resistance, (2) develop grasses with high salt resistance, (3) refine the use of effluent or wastewater, (4) improve management practices to efficiently use water, (5) improved irrigation system design, (6) improved irrigation scheduling, (7) water harvesting, (8) translating research into educational formats, and (9) development of science-based waster conservation and drought contingency plans.

The emphasis of this presentation will be on research challenges for development of drought and salt-resistant grasses. Both stresses are complex with a host of plant morphological, anatomical, and physiological characteristics that contribute to resistance. The ultimate goals of plant breeders and physiologists are a) to identify within a species the most important mechanisms contributing to resistance, and b) develop rapid, reliable techniques to screen germplasm for these characteristics.

One important area that has often been neglected by breeders is the aspect of root tolerance to soil environmental stresses. Just as rooting depth/extent is genetically controlled, so is root tolerance. In most field situations, turfgrasses do not reach their genetic potential for rooting depth/extent because of intolerance to an adverse soil environment. In addition to soil-borne pests (diseases, insects, nematodes), there are only five major edaphic factors that limit rooting: high soil strength, low soil oxygen, high soil temperature, Al/Mn toxicities, and salinity toxicity.

The first hypothesis discussed will be: that the most rapid advancement in development of drought-resistant turfgrasses can be achieved by improvement of root tolerance to the five major edaphic stresses. "Drought stress" in most field situations is really a secondary stress - the primary stress being whatever limits maximum rooting potential.

The second hypothesis to be presented is that: identification of salt-resistant germplasm is best accomplished in a soil-based screening procedure. Such a procedure must allow for: osmotic adjustments; creation of salt toxicity; and sufficient dry-down conditions for tissue dehydration. Only when the full complement of stress mechanisms are imposed will true salt resistance be identified.

Increasing Nitrogen Use Efficiency By Turfgrasses

Richard J. Hull

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As with most terrestrial plants, turfgrasses obtain their nitrogen from the soil solution in the form of nitrate or ammonium. Under soil conditions suitable for turf growth, ammonium is normally oxidized by chemotrophic bacteria to nitrate (nitrification) making it the nitrogen form generally available to the grass roots.

Plant roots absorb most nutrient ions by harnessing the energy contained in the electrochemical gradient generated across the plasmid membranes of epidermal and cortical cells. This potential is created by the activity of a proton (H+) transporting ATPase which spans the plasma membrane. The reaction catalyzed by this enzyme hydrolyzes ATP to transport proton from the cytoplasm into the cell walls creating an electrical potential across the plasma membrane of between 75-125 mv (outside negative) and a pH gradient of about 1.5-2.0 units. Ion specific transport proteins in the plasma membrane carry ions into the cytosol of root cells, frequently against a concentration gradient, by utilizing the energy of these transmembrane gradients.

Nitrate absorption by roots is invariably an active process because it occurs against both a concentration and electrical gradient. This is achieved by chemically linking the influx of nitrate with that of protons, which is energetically favored. A proton:nitrate cotransporter, which carries two protons with each nitrate delivered into a cell, catalyzes this reaction. It is currently believed that three transporters are involved in the absorption of nitrate. A constitutive high affinity transporter (CHAT) operates at low capacity even when roots had been growing in a nitrate-free medium. A high affinity transporter (HAT) operates at high capacity but is induced by external nitrate and is responsible for most uptake when nitrate concentrations are low (<300 µM). Both the CHAT and HAT systems exhibit saturation kinetics and operate within a defined concentration range. At higher nitrate concentrations (>500 µM), a third transporter becomes involved in nitrate absorption. This is a high capacity but low affinity transporter (LAT) that responds linearly to increasing nitrate concentration. The LAT system enables plants to absorb nitrate rapidly during short periods when concentrations may be high (following fertilizer applications).

Once nitrate enters root cells it must be reduced to ammonium before it can be assimilated into organic nitrogen (amino acids, proteins, nucleic acids, etc.). These are energy demanding processes and most roots have a limited capacity for them. If nitrate absorption exceeds the capacity of roots for reduction and assimilation, nitrate will be translocated to the leaves where these processes are fueled directly by photosynthetic energy. The presence of nitrate or it metabolites in leaves channels the flow of photosynthetic products from sucrose synthesis and transport to roots to amino acid synthesis and shoot growth. This in turn can negatively influence the efficiency of nitrate uptake and metabolism by roots.

It appears, from the outline presented above, that nitrate use efficiency by turfgrasses can be increased by maximizing its uptake from the soil solution by grass roots. Two conditions will accomplish this. First, increasing the affinity of the HAT system for nitrate (low Km value) and its capacity for transport (high Vmax value) will enable roots to lower the nitrate concentration of the soil solution to levels which will make roots more competitive with the soil microflora and minimize the potential for nitrate leaching. Second, increasing the allocation of photosynthetic product to roots, thereby increasing their growth rate, will provide more root surface for nutrient absorption and permit the plants to invade a greater and deeper soil volume. Increasing the metabolic efficiency of N use (reduction and assimilation) will make turfgrasses more competitive and permit them to produce more leaf surface per unit of nitrogen expended. While significant differences in nitrogen use efficiency have been observed among turfgrasses, e.g. 26.6 mg leaf tissue/mg N for tall fescue vs. 23 for perennial ryegrass, it is not clear if that level of efficiency will increase nitrate recovery from the soil and retention within the turf-soil ecosystem.

We have demonstrated significant differences in kinetic parameters of the HAT system of nitrate absorption among turfgrass species. Both Km and Vmax values were found to exhibit even greater variation among cultivars of a turfgrass species, When these kinetic parameters were paired with field observations of nitrogen recovery of clippings, reasonable correlations were obtained during times of vigorous turf growth. This is encouraging because it indicates that a simple laboratory assay for kinetic parameters of nitrate absorption could be used to screen turfgrass genotypes for superior nitrogen recovery in the field.

We have quantified nitrogen losses from intensively managed turf including nitrate leaching, denitrification, ammonia volatilization following fertilizer application, and clipping removal. Runoff was not a factor in the plot area used. If clippings were retained on the turf, all other nitrogen losses collectively accounted for less than 15% of the 17.25 g N m-2 (3.5 lbs/1000 sq ft) applied each year. Consequently most fertilizer nitrogen was retained within the turf-soil system. Analysis of this system revealed that on average 240 g N m-2 were present, of which 85% was in the soil fractions. Turfgrass plants and thatch accounted for 15% of total nitrogen present. This large pool of soil organic nitrogen (>200 g m-2) likely is subject to sufficient mineralization to meet the annual needs of most turfgrass stands if the grass is efficient enough in its absorption of nitrate and ammonium to recover it. Our research has indicated that turfgrasses exhibit much genetic variability in their capacity for nitrate absorption. This may be exploited to develop cultivars highly efficient in nitrogen recovery thereby permitting significant reductions in fertilizer application and further minimizing the potential for other nitrogen losses.

Near-Infrared Reflectance Spectroscopy for Monitoring Nitrogen Status of Turfgrass

James A. Murphy, Gregory Castano, James Henry, and Gary Gentilucci

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Nitrogen fertilization programs for turfgrasses are typically based on subjective criteria such as visual estimation of turf color, density, and growth. A more objective approach to evaluating the N requirement of turfgrass would improve management of fertility and associated stresses such as disease and subsequently reduce the potential need for pesticide applications. Acceptance by turf managers and consultants of a quantitative approach would be based on the reliability and rapidity of the method. Non-infrared reflectance spectroscopy (NIRS) is a relatively new method which can rapidly determine the N concentration in turfgrass clippings. Objectives of this research were to: (i) compare clipping N concentration as determined by a commercially available NIRS scanner to Kjeldahl determination of N and (ii) evaluate the relationship of clipping N concentration and dollar spot (*Lanzia* and *Moellerodiscus* spp.) disease incidence as a means of developing optimum N levels for creeping bentgrass (*Agrostis palustris* L.) turf. Linear regression of NIRS-N on total Kjeldahl N for clippings collected from both creeping bentgrass and perennial ryegrass (*Lolium perenne* L.) turf during the 1992 growing season were highly significant (r2=0.892 and 0.937, respectively). The slopes of both regressions were close to 1, but NIRS tended to overestimate clippping N concentration as compared to total Kjeldahl N.

This strong linear relationship was demonstrated over a range of N concentrations as well as throughout the growing season, indicating NIRS is a reliable analysis tool. Preliminary analysis of clipping N concentration and dollar spot severity data of bentgrass turf indicates that disease decreases with increasing N concentration, but disease suppression diminishes as N concentration approaches 5% (NIRS). Further research will identify how applicable this "Threshold" clipping N concentration is throughout the growing season and across differing environments. Speed and ease of operation make NIRS a potentially attractive tool where N analysis needs to be performed quickly, permitting timely and efficient N fertility decisions while proper remediation is still possible. Furthermore, NIRS shows promise as a research tool where rapid N analysis for a large number of samples is necessary. Screening for enhanced N uptake in turfgrass germplasm, and studying relationships between plant N and disease are two possible areas of research where such a tool could provide benefit.

Results from 1993 Turfgrass Fertilizer Trials

J. R. Heckman

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A field trial was conducted on newly established Kentucky bluegrass turf at Rutgers Hort Farm III to evaluate commercial fertilizers on turf color and clipping yield. The trial included 14 fertilizers and a control arranged in a randomized complete block design. The fertilizer treatments represented quick and slow release nitrogen products currently on the market and experimental polymer coated ureas. Plots were split to include two different mowing management systems: clippings removed with a bagging mower versus clippings returned with a mulching mower. Mowing management resulted in no difference in turf color ratings in May, June, and July but by August there was a visibly darker turf color on the plots mowed with the mulching mower. The superior turf color ratings where clippings were returned persisted from August through December. The color ratings from the control plots indicated that the field site was well suited for the purposes of evaluating differences among fertilizers because nitrogen deficiency was exhibited and turf color was very responsive to the addition of nitrogen. Comparison of fertilizers with different nitrogen release characteristics applied once at the same nitrogen rate showed that fertilizers with a high proportion of quick release nitrogen provided for better turf color ratings early in the season at the expense of poorer color ratings late in the season. Comparison of fertilizers that were identical except for the potassium source (potassium chloride versus potassium sulfate) exhibited no difference in turf color or quality. Additional findings from fertilizer product comparisons will be presented at the symposium.

Cool Season Grass Breeding Progress, Future Objectives For Breeding Priorities, and Interest In Initiating Breeding Programs In New Species

Richard Hurley

Lofts Seed, Inc.,
Chimney Rock Rd., Bound Brook, NJ 08805

During the last thirty years dramatic advances have been made in breeding perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) for improved turf qualities. For both ryegrass and tall fescue, highly significant improvements have already been achieved for increased persistence, darker green leaf color, resistance to net blotch (caused by *Drechalera* spp.), finer leaves, lower growth profile, improved mowing quality, greater plant density, and overall turf performance.

Fungal endophytes (*Acremonium* spp.) have been incorporated into some seeded varieties of *Lolium* and *Festuca* spp. to enhance overall turf performance and resistance to a number of harmful turfgrass insects, including Billbugs (*Sphenophorus* spp.) and many lepidopterous species of sod webworms. Presently, turfgrass breeders have a better understanding of fungal endophytes and their ability to modify turf performance. This has provided greater efficiency in selecting germplasm sources of *Lolium* and *Fescue* spp.

Although moderate progress has been made in improving resistance to brown patch disease (caused by *Rhizoctonia solani*) in perennial ryegrass and tall fescue, greater progress may be anticipated if large populations of breeding material are screened in the mid-south where extreme summer disease pressure is found. The greatest challenge for breeding tall fescue is in improved summer performance that includes tolerance to high summer temperatures, resistance to brown patch, Pythium blight, and tolerance of acid soils of the southeastern U.S. where large quantities of tall fescue is used.

To date, slow progress has been made with breeding both perennial ryegrass and tall fescue for stable rust resistance (caused by *Puccinia* spp.) which is important for continued high yields in the major seed production states of the Pacific Northwest and for improved performance in reduced maintenance turf.

Over the last ten years, there has been increased interest in breeding creeping bentgrass (*Agrostis palustris*) for improved performance on golf course putting greens, tees, and fairways. Recently, a few new varieties have been released that show improved performance compared to the standard variety Penncross. Encouraging results from modest breeding efforts show that significant improvements may be anticipated with continued breeding of this species. To date, the improvements have been made towards a more upright growth with fine leaves and a denser growth.

As improvements are made with traditional cool season turfgrasses, we need to consider possible use of other species for low maintenance sites. For new breeding project considerations, the following species should be explored: Nimblewill (*Muhlenbergia schreberi*), Broomsedge (*Andropogon virginious*), Purpletop (*Tridens flavus*), Hairgrass (*Deschampsia flesuosa*), Switchgrass (*Panicum virgatum*), Indiangrass (*Sorghstrum nutans*), Sweet Vernalgrass (*Anthoxanthum odoratum*), Junegrass (*Koeleria cristata*), Redtop (*Agrostis alba*), and Bulbous bluegrass (*Poa bulbosa*). In addition to grasses, the use of Pine Barren Sedge (*Carex pennsylvanica*), Blackgrass (*Juncus gerardi*) or Path Rush (*Juncus tenuis*) may have potentials as a low maintenance cover on selected sites.

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

C. Reed Funk, James Murphy, Bruce Clarke, Karen Plumley, James White, William K, Dickson, Ronald Bara, Suichang Sun, Dirk Smith, Randy Prostak, and Pedro Perdomo

Department of Plant Science
Rutgers University, New Brunswick, NJ 08903

1. Promising turfgrass germplasm and associated endophytes were collected from old turfs in New Jersey, Colorado, France, and Spain. New sources of endophytes were found in *Poa* species native to Colorado and the mountains between France and Spain.

2. Over 6,900 new turf evaluation plots and over 7 acres of spaced-plant nurseries were established in 1993.

3. Field and greenhouse research is continuing on a herbicide management program to selectively remove rough bluegrass from Kentucky bluegrass turfs.

4. An additional 175 turf-type tall fescue progenies were sent to Dr. Ronny Duncan at the Georgia Agricultural Experiment Station at Griffin, GA for evaluation on very acid soils (pH 4.0) in an area which also receives severe stress from summer heat, drought, and many diseases, insects, and nematodes. A few attractive, dark green, turf-type plants survived the severe summer of 1993 in tests established in 1991 and 1992. They are being selected for additional evaluation and breeding studies. This study was also designed to assess the role of various *Acremonium* endophytes on various aspects of turf performance under these conditions.

5. Severe turf loss was observed on all endophyte-free fine fescues in the 1989 National Fine Fescue Tests at both Adelphia and North Brunswick, NJ. Due to the extensive colonization of turf roots by dark ectotrophic mycelium, symptoms were initially associated with the disease summer patch. High populations of chinch bugs subsequently increased turf damage and slowed recovery. Studies are in progress to determine whether some endophytes in fine fescue might be associated with enhanced resistance to the summer patch disease.

6. *Acremonium* endophyte enhanced resistance to the dollar spot disease was again observed in field trials of fine fescue. Both mycelial growth and damage by the dollar spot fungus was greatly reduced on fine fescues containing an endophyte.

7. Kentucky bluegrass cultivars and selections showed significant differences in degree of wilting under heat and drought stress. Striking differences were also noted in amount of turf loss following summer stress in a hot, dry environment receiving limited air circulation. Bluegrass showing reduced wilting and better fall recovery generally showed an ability to remove moisture at a greater depth during heat stress.

8. A few experimental selections of Kentucky bluegrass are performing well in low-maintenance turf trials receiving limited fertilizer, no irrigation, and no fungicides or insecticides. Most of the entries showing the best recovery from severe summer stress have been classified as mid Atlantic ecotypes. They have deep extensive rhizomes, an ability to develop a deep root system during hot weather, medium broad leaves, and a growth habit intermediate to the tall narrow-leaved midwest common types and the lower-growing turf types. They are much more vigorous in spaced-plant nurseries than most turf-types. They generally show improved resistance to billbugs and better tolerance of some other insect pests.

9. BVMG (Baron, Victa, Merit, Gnome) types of Kentucky bluegrass are showing increasing

damage from stripe smut and other turfgrass maladies. The widespread use of these similar and probably closely related bluegrasses appears to promote an increasing abundance of pathogens adapted to these host genotypes.

10. Seed production was initiated on Elf and APM perennial ryegrasses, Proformer Chewings fescue, and Ecostar hard fescue. Germplasm developed at the New Jersey Agricultural Experiment Station was used in the breeding of these varieties

11. Damage by four species of billbugs (bluegrass, little, uneven, and hunting) continues to be the greatest cause of turf loss in older, low-maintenance Kentucky bluegrass trials at both Adelphia and North Brunswick, New Jersey. Genetic resistance to these insect pests is being stressed in our turfgrass breeding program.

Creeping Bentgrass Transformation

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Our goal is to improve turfgrasses through implementation of biotechnology. Our objective is to develop turfgrass transformation systems for the incorporation of agronomically important genes into turfgrasses. We have focused on three approaches: 1) particle gun transformation; 2) direct DNA uptake through protoplasts; and 3) mutant selection through tissue culture somaclonal variation.

We have developed tissue culture and regeneration systems for creeping bentgrass. Embryogenic callus lines with high regeneration potentials were established from eight commercially important creeping bentgrass varieties. The embryogenic callus lines and suspension cultures derived from callus cultures provide target cells for development of stable transformation systems and mutant selection. The *E. coli* ß-glucuronidase (GUS) gene was used as a scorable marker and the *bar* gene, which confers resistance to the herbicide bialaphos (BastaTM), was used as a selectable marker. Particle bombardment transformation method was developed for creeping bentgrass. Stable transformants have been obtained from three varieties (Emerald, Pitter, and Southshore). A total of 237 plants have shown herbicide resistance in greenhouse tests. They are from 12 independent bombardment filters. Transgenics have also been confirmed by polymerase chain reaction (PCR) assay and Southern blot hybridization to show the presence of the transgene. We have also developed a protoplast regeneration system for creeping bentgrass. A feeder layer was used for turfgrass protoplast regeneration. Protoplast derived calli were obtained from 6 creeping bentgrass varieties. Plants were regenerated from SR1020, Southshore, and Pennlinks. We are using direct DNA uptake with either electroporation or Polyethylene glycol (PEG) to optimize stable transformation with mutant AHAS (acetohydroxyacid synthase) genes which confer resistance to the herbicide Pursuit. Regenerants from selection with Pursuit are being tested for herbicide tolerance. We are also using mutant selection to generate Pursuit resistant creeping bentgrass with callus and suspension cultures. Plants were regenerated from several tissue sectors of our first experiment. Herbicide spray tests of these regenerants are in progress.

Ecological Consequences Of Endophyte-Infection In Grasses

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Many grasses are infected by clavicipitaceous fungal endophytes that have significant effects on the ecology of their hosts. Research in our lab has focused on the ecological interaction of infected and uninfected grasses with other grasses (competition) and with insects (herbivory). Greenhouse and field studies have examined the ecological performance of infected plants over a range of environmental conditions while laboratory feeding studies have examined the effect of endophyte- infection on a range of insect and vertebrate herbivores. The interaction of endophyte-infection and insect herbivory on grass competitive interactions has been examined in experiments where levels of herbivory, density and identify of competitors, and the infection status of grass species have been manipulated simultaneously. Results indicate that infected plants are generally more vigorous and more competitive than uninfected grasses and this advantage is increased by insect herbivory. The importance of the genetic identity of the endophyte on plant performance is currently being investigated by inoculating plants with endophytes from different host species and populations and then following their demographic performance in natural habitats.

Purification And Characterization

Of An Endophytic Fungal Protease Which Is Induced On Infection Of Its Host Grass

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Recently we reported the detection of a novel *Acremonium typhinum* protease which is expressed in the endophytic infection of the grass *Poa ampla*. This protease is a cell wall and membrane-associated serine endoprotease with an essential thiol group and it is active in high concentrations of detergent (4.6% SDS). Similar protease activity was detected in other endophyte-infected *Poa* spp.

We have purified the *A. typhinum* protease and completed some characterization of its properties. Similar amounts of the enzyme were presented intracellularly, possibly within membrane vesicles, and ionically bound in the cell wall. The intracellular enzyme was purified 176-fold to near homogeneity as assessed by SDS-PAGE. High specific activity cell wall enzyme was obtained by extraction of the walls with 1 M NaCl. The electrophoretic mobility and activity of the two forms were indistinguishable. The *A. typhinum* protease was extremely abundant in endophyte- infected leaf sheath tissue. The total amount of protease protein is probably over 2% of the total leaf sheath protein. Since the fungal hyphae constitute a small fraction of the total leaf sheath material, the high recovery of the protease indicates it is a surprisingly abundant fungal protein, further underscoring its probable biological significance.

The protease was not denatured by high SDS concentrations and migrated at a high apparent molecular mass in SDS-gelatin activity gels. The protease was denatured by high temperature and the thermally denatured form of the protease migrated in SDS-PAGE as a 32 kDa protein. Labelling with [3H] diisopropylfluorophosphate confirmed that the 32 kDa protein band contained the catalytic site of the enzyme.

We have prepared a polyclonal antibody to the 32 kDa protein band using protein in a gel slice as antigen. Expression of an antigenically similar protease was detected in 5 other endophyte-infected *Poa* spp.

The requirement for a reductant, such as ß-mercaptoethanol or DTT, for activity suggested the presence of an essential thiol group. The 32 kDa band was labelled with fluorescein-5-maleimide and 5-iodoacetamidofluorescein confirming the presence of at least one thiol group.

The protease was active over a wide pH range, from 7 to 11, with highest activity at pH 9-11, indicating it is an alkaline protease. The protease was active between 27 C and 42 C with a maximum at 37 C. At 55 C the activity was less than 10% of that at 37 C.

The *A. typhinum* protease was recognized by biotinylated lectins indicating it is a glycoprotein. Lectins of three different classes reacted with the 32 kDa band of the protease. Lectins binding the 32 kDa protease band were: *Pisum agglutinin* which binds -linked mannose and -linked glucose, wheat germ agglutinin which binds N- acetylglucosamine, and peanut agglutinin which binds galactosyl end-groups.

The *A. typhinum* protease was detected in extracts of the cultured fungus, but only in primary cultures, i.e. those established directly from surface-sterilized leaf sheath tissue. On subsequent subculture of the fungus this activity was no longer detected. The protease activity was inducible, however, on reinfection of endophyte free *P. ampla* seedlings. We have developed a method whereby 50-80% of inoculated seedlings became endophyte-infected. All of the newly infected plants had detectable protease activity.

Double-stranded RNAs and Antimicrobial Activity Associated with *Atkinsonella hypoxylon*

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Three years ago, we initiated a project to examine fungal endophytes of turfgrass species for the presence of extrachromosomal elements with two main ideas in mind. Presence of extrachromosomal elements such as double-stranded RNAs or plasmids may indicate the presence of a subcellular pathogen that has a deleterious effect on the endophyte, rendering it less fit than an uninfected counterpart. In this instance, eliminating the extrachromosomal elements may have a positive effect on colonization potential of the fungus. Alternatively, extrachromosomal elements may not be deleterious to the fungus, but may be useful for expression of foreign genes, either as vectors themselves or by exploiting their regulatory elements. We screened a total of 36 endophytic or closely related epiphytic fungi for dsRNA elements or plasmids. Of these, five of six isolates of *Atkinsonella hypoxylon*, an epiphyte that causes choke disease on poverty grass and related grasses (*Danthonia spp.*) contained dsRNAs in high concentrations. The dsRNAs appeared to fall within two categories based on their mobilities through agarose or acrylamide gels. Complementary DNA (cDNA) libraries of dsRNAs isolated from one of strains were used to confirm the apparent difference in the two types of dsRNAs: clones representing one type {strain 2H} cross-hybridized on northern blots with co-migrating segments of the strain with similar sized dsRNAs (A3), but not with any segments from the other three strains (1L, A1, and A4). Efforts to transfer dsRNAs by hyphal anastomosis from one strain to another resulted in an unexpected finding. Strains 2H and 3A could grow together on the same culture plate, and strains 1L, A1, and A4 could grow together on the same plate. However, when either strain 2H or strain 3A was co-cultured with strains 1L, A1, and A4, each of those three were inhibited or killed. The antifungal activity associated with strains 2H and 3A extended to other species as well.

For possible future exploitation of these dsRNAs or their regulatory elements, we characterized all three dsRNAs associated with strain 2H by mapping and sequencing overlapping clones, and we have begun examining gene expression. The three segments had no significant sequence similarity to each other, confirming results from the northern blot experiment that the three segments are genetically unique. Substantial coding regions were deduced from the sequences of segments 1 and 2, but not from segment 3. Although no homology was identified between the amino acid sequence deduced from segment 2 and other sequences in the database, segment 1 bore landmarks associated with RNA-dependent RNA polymerase, an enzyme that would be expected to be encoded by a replicating RNA species.

Will Biological Control Agents Control Turfgrass Diseases?

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There are basically two difference tactics that will be used for biological control of plant diseases - native and genetically engineered microorganisms. However, in both cases, there is a tendency to put more emphasis on the biological control agent than on the pathogen and/or the disease process. Without this information, we are constantly working in a "black box" resulting in microbes that work well in a controlled environment, but fail when they are finally tested in the field under realistic parameters. The result is thousands of microbes that have had their one shot at fame in a research article, but never see the light of day as practical, economically viable products.

I think it would be useful to focus on a small number of host-pathogen systems or models. After gaining a better understanding of the actual disease process, then the most successful biological control agents can be added to the model and studied in details also. In some ways it could be compared to the human genome project or the corn genome project, in that certain labs within a consortium would be responsible for specific aspects of each model system to be studied.

Turfgrass would be a good model system because it has a number of diseases, especially root rots, that are not easily controlled with chemical fungicides. Furthermore, turfgrass receives the greatest majority of fungicide applications in the United States. Because turfgrass is essentially a perennial crop, it would be an ideal crop for development of the classic biological control agent - a microbe that is applied once and need never be applied again. However, who will develop the biological control agents to be used on turfgrass? It is important to remember that it is not enough to isolate microbes and screen them for disease control. Someone must develop the commercial formulation which means the company will want to be assured of financial profits. In other words, is solving disease problems with biological control agents compatible with economic gain? There are no economically successful biological disease control agents on the market to date.

We also need to re-educate the public concerning biological control agents. Let me emphasize the word "re-educate". In order to obtain grant funds, scientists have greatly publicized biological control. The journalists have been successful at their job in passing this information on to the general public. Now, this public believes we can eliminate chemicals and still maintain quality and quantity of agricultural products, including turfgrass. However, they are not aware of the cost or time involved in reaching that goal. They are also not fully aware that scientists had no intention of eliminating chemicals all together. Our goal was simply to reduce chemical use in the environment.

Antioxidant Enzymes and Environmental Stress Tolerance

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In our laboratory, we are particularly interested in how plants cope with oxidative stress, i.e., collectively several reactive, highly toxic forms of oxygen which are generated as a by-product of normal metabolism and whose production is enhanced under environmentally adverse conditions. If these reactive oxygen species are not efficiently and rapidly removed from the cell, biological damage ensues, ultimately leading to cell death. We contend that oxidative stress is a common denominator of most abiotic stress conditions. Moreover, there is evidence that oxyradicals mediate biotic stress as well.

The inevitable production of reactive oxygen species in all aerobic organisms and the damage caused if they are left unchecked underscore the importance of antioxidant defense systems. This defense involves both enzymatic and non-enzymic mechanisms. We have focussed on the former, in particular the two major enzymatic antioxidants in plants, namely superoxide dismutase (SOD) which scavenges superoxide radical and ascorbate peroxidase (APX) which eliminates hydrogen peroxide, both toxic forms of oxygen.

We have purified and characterized SOD and APX from pea, and have cloned the cDNAs encoding these enzymes. We used these cDNAs to construct transgenic tobacco plants which overproduce these enzymes in order to test the hypothesis that plants with higher levels of the enzymatic antioxidants might better tolerate environmental stress. As tobacco tissue is readily transformed via *Agrobacterium tumefaciens* and is easily regenerated into plants, our efforts to date have relied on this transformation system while an efficient turfgrass transformation and regeneration system was being developed. The results obtained thus far with tobacco are indeed promising. Transgenic tobacco overexpressing ascorbate peroxidase is better equipped to deal with oxidative stress conditions, suffering less injury than nontransformed controls.

Very recently, creeping bentgrass callus cultures have been transformed by particle bombardment with plasmids containing either a pea SOD or APX plant expression cassette and the *bar* gene, enabling selection of transformants with the herbicide bialaphos. Regenerates obtained from calli selected on 8 mg/L bialaphos tested positive for the presence of the transgenes (BAR, APX and SOD) by polymerase chain reaction. Experiments are presently underway to see if the pea APX and SOD cDNAs are stably expressed in these plants.

Development of Bacterial Inoculants for the Use On Turf, Range and Pasture Grasses

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Azospirillum bacteria have received a great deal of attention in recent years following their isolation from a number of plant species including turf, forage and cereal grasses. These bacteria are known to fix atmospheric nitrogen in culture media and in association with plant roots. They are also known to produce a variety of plant growth hormones including giberellins, auxins and cytokinins and to promote root growth, water and mineral nutrient uptake.

This report deals with the response of several grass species to inoculation with *Azospirillum brasilense* strain Cd. Bacterial cells were delivered as seed coating using a "dry" vermiculite-based inoculant or as a "liquid" inoculant containing methyl cellulose as an adhesive. Both systems, "dry" and "liquid", were structured to deliver 108 CFU per gram of seed. Experiments were conducted under controlled environmental conditions in a greenhouse (16 h day/8 h night cycle). Results of experiments conducted in 1992 indicated significant positive responses to inoculation (using either "dry" or "liquid" forms) by most grass species tested (Olubayi et.al., 1992). The best responses were obtained on several festucoid species, wheatgrass, Russian wildrye and perennial ryegrass. Overall, there was an average increase in growth of between 60% and 100%. These results have been confirmed and expanded further in experiments conducted in 1993. A similar trend has been observed in field trials conducted at the Adelphia Research Center during the summer and fall of 1993.

Root colonization studies have been conducted using scanning electron microscopy (SEM) on tall fescue (*Festuca arundinacea* Schreb.) inoculated with *Azospirillum brasilense* Cd. Our observation indicate that *Azospirillum* colonizes the entire root length and forms microcolonies composed of bacterial aggregates connected by fibrillar material. No evidence of attachment to root hairs was observed. The production of bacterial aggregates and fibrillar structures have been reproduced in culture media in response to a high C/N ratio. These findings should help further our knowledge of plant-*Azospirillum* interactions and the possibility of developing inoculants of higher quality and effectiveness. Growth media with a C/N ratio of between 50:1 and 100:1 induces overproduction of exopolysaccharides, cell aggregation and production of encysted forms with enhanced survivability under environmental stress.

References:

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Isolation of Bacteria With Suppressiveness to Summer Patch Disease Using Enrichment Cultures

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A method was developed to isolate bacteria that were parasitic to *Magnaporthe poae,* the causal agent of summer patch disease, for their potential use as biocontrol agents. The method was based on enrichment cultures originally designed to isolate bacteria with degradative potentials of complex compounds. Bacterial inocula that were utilized to initiate enrichment cultures originated from several different soils collected from various sites. Initially, *M. poae* mycelia were buried as bait in the soils for extended periods. Buried mycelia were then recovered and rigorously washed in distilled water to remove loosely adhering particles. Any bacteria that remained adhered to the *M. poae* mycelia were suspected as parasitic organisms and were then used to inoculate enrichment cultures composed of a minimal medium broth supplemented with 0.1% *M. poae* mycelium as a sole carbon source. Cultures were incubated for periods of 1 to 2 weeks to allow turbid bacterial growth, at which time a small aliquot was transferred to new cultures containing fresh media. Transfers were repeated for several weeks to enrich for bacteria that could grow on *M. poae* mycelium as a sole carbon source. With each transfer, enrichment cultures were serially diluted and plated onto agar media to randomly select bacterial isolates. Single isolates were screened for chitinase activity on a minimal medium supplemented with chitin, the major cell wall component of *M. poae*. Bacterial isolates that expressed chitinase activity were then tested in growth chamber studies for the suppression of summer patch disease. Isolates from all soil sources were identified that suppressed summer patch symptom development in growth chamber studies at levels of 50% or greater compared to untreated control plants. These results suggest that the enrichment culture procedure may prove useful as a primary screen in the selection of bacteria for biocontrol purposes.

Utilization of the Polymerase Chain Reaction to Identify *Magnaporthe poae* and Its Phylogenic Characterization Among Various Ascomycetes

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Magnaporthe poae Landschoot & Jackson is a heterothallic, ascomycetous fungus which causes summer patch, a devastating disease of cool season turf. Definitive identification of the disease has been impractical, due to the length of time needed for the production of the sexual phase. This requires pairing the unknown isolate with the two mating types across a sterile wheat seedling root and waiting an average of three months for the formation of perithecia. Because no distinctive structures are present in nature, our objective was to create a DNA-based method of detection, using DNA probes or PCR amplification.

A three kilobase *Bam*HI fragment of DNA from a virulent isolate of *M. poae* was subcloned into the plasmid vector pGEM3Zf+ (Promega). In Southern analysis, this subcloned hybridized to *Bam*HI digested DNA from all *M. poae* isolates tested and DNA from *Glomerella* (ana. *Colletotrichum*) *graminicola*. Following sequencing of 600 bases of this clone, we designed primers that specifically amplify a 450 nucleotide fragment from the DNA of all known *M. poae* isolates tested (17). In addition, 40 isolates of various other fungi have been tested which do not produce the 450 nucleotide fragment. During the summer of 1993, this technique was used to identify 16 isolates of *M. poae* from turf samples from New Jersey and Kentucky. Currently the estimated time required to definitively identify *M. poae* on the roots of a turf sample is one to two weeks.

In order to clarify the phylogenic relationship of *M. poae* to *M. grisea*, *M. salvinii, M. rhizophila*, *Gaeumannomyces graminis* var. *graminis, Glomerella* (ana. *Colletotrichum*) *graminicola*, *Cryphonectria parasitica*, and *Leptosphaeria korrae* the sequence of the first intragenic spacer region of the genomic ribosomal DNA is being determined. These sequences can be aligned to analyze the differences and describe the evolutionary distance between species.

Esterase Differences In Seedlings And Seedling Mixtures Of Hard And Red Fescues

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Rapid and efficient methods are needed to differentiate between hard fescue (*Festuca longifolia* Thuill.), creeping red fescue (*Festuca rubra* L. subsp. *rubra*) and chewings fescue (*Festuca rubra* L. subsp. *commutata*). Visual evaluation of seeds and the ammonia root fluorescence test often fail to separate species and subspecies of fescue. This study was initiated to determine if distinct esterase banding patterns provide consistent indicators of the species or subspecies and if the components of mixtures of hard and red fescue seedlings could be detected using polyacrylamide gel electrophoresis (PAGE). Hard and red fescue seedling extracts had distinct esterase isoenzyme banding patterns. The staining intensity of bands at Rf 0.62 to 0.64, indicative of the hard fescues, was used to detect hard fescues in hard fescue-red fescue mixtures. Hard fescue was detected by visual examination of PAGE gels when the proportion of hard fescue seedlings in the mixture was as low as 25%. A band at Rf 0.50 which the creeping red fescue cultivars did not exhibit was useful in differentiating chewings fescues from creeping red fescues.

Esterase Isoenzyme Electrophoresis as a Method of Separating Colonial and Creeping Bentgrass Mixtures

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Rapid and efficient methods are needed to differentiate between creeping bentgrass (*Agrostis palustris* Huds.) and Colonial bentgrass (*Agrostis tenuis* Sibth.) species and to complement both visual evaluation of seeds and growth chamber tests. This study was initiated to determine if varying proportions of colonial bentgrass seeds could be detected in creeping bentgrass seeds using esterase isoenzyme polyacrylamide gel electrophoresis (PAGE) and to determine at what lower proportion level esterase isoenzyme PAGE was no longer capable of resolving the creeping bentgrass admixture. The PAGE procedures revealed that colonial and creeping bentgrass seed extracts had distinct esterase isoenzyme banding patterns and the staining

intensity of bands at Rf 0.55 to 0.70 could be used to detect mixtures of creeping and colonial bentgrass. Proportions as low as 10% colonial bentgrass seeds in creeping bentgrass seeds were detected by visual examination of PAGE gels and proportions as low as 5% were detected using densitometric scans of the gels. The study demonstrated that PAGE esterase isoenzyme analysis was an effective method to detect colonial bentgrass contamination in creeping bentgrass and can provide the seed analyst with another test to supplement visual evaluation of seeds and growth chamber tests.

Particle Gun Transformation of Creeping Bentgrass (*Agrostis palustris*) Using the *bar* Gene

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We have developed regeneration and particle gun transformation methods for creeping bentgrass, a type of turfgrass used for golf greens. Embryogenic callus lines of Emerald, Putter, and Southshore have been transformed using the BioRad Helium gun. Transgenic plants have been obtained from the varieties Emerald, Putter, and Southshore. Plants were recovered from both plate and liquid selection. Four experiments of 12-14 filters yielded a total of 105 plants that survived the initial spray concentration. Of these, 26 survived the higher spray rate. Transgenics were further confirmed by PCR and Southerns. This transformation system has been used to introduce the ascorbate peroxidase (AP) and superoxide dismutase (SOD) genes into bentgrass. These genes are intended to improve stress tolerance.

Evaluation of N-Viro Aglime as a Soil Amendment for Turfgrass Establishment

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A green house pot experiment evaluated N-Viro Aglime as a soil amendment for the establishment of Kentucky bluegrass on an acid infertile sandy loam soil. N-Viro Aglime is a new product that is derived by chemically reacting dewatered sewage sludge with various alkaline reagents. The N-Viro process produces an exothermic reaction and a high pH that kills pathogens and increases the solids content above 50%. N-Viro Aglime is marketed as an alternative for agricultural limestone for crop production. Various rates of N-Viro Aglime and dolomitic lime were incubated with the soil which had an initial pH of 4.5. After a period of 16 weeks of incubation, it was found that 3 times as much N-Viro Aglime was required to achieve the same soil pH as with dolomite lime. The phosphorus replacement value of N-Viro Aglime was evaluated with the following treatments: 1) Unlimed soil pH 4.5, 2) unlimed soil pH 4.5 plus phosphorous fertilizer, 3) dolomite limed soil pH 6.3, 4) dolomite limed soil pH 6.3 plus phosphorous fertilizer, 5) N-Viro Aglime amended soil pH 6.3, 6) N-Viro Aglime amended soil pH 6.3 plus phosphorous fertilizer. All treatments were adequately fertilized with nitrogen and potassium. Total clipping dry matter yields after 70 days of growth were 0.02, 1.15, 0.09, 2.22, 1.28, 2.60 grams per pot for the above treatments, respectively. Results indicate that turfgrass growth was very limited on unlimed and dolomite limed soil when not fertilized with phosphorous. Turfgrass growth was greater on N-Viro Aglime amended soil than on dolomite limed soil both when phosphorous fertilizer was added and when phosphorous was not added. Although N-Viro Aglime is needed in greater quantity to raise the pH of an acid soil, phosphorous fertilizer is needed in less quantity due to the added benefit of the phosphorous that is present in the N-Viro product.

Isolation and Characterization of Carbendazim-Degrading Bacteria

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Enrichment cultures were used to isolate several bacteria with the ability to degrade the heterocyclic compound carbendazim. The bacteria originated from soil obtained from sites known to have been subjected to repeated treatments of the structurally-related compound, benomyl, a fungicide used extensively for the control of turfgrass diseases. Each isolate obtained from enrichment cultures were spot- plated onto a minimal agarose medium supplemented with 0-0.1% carbendazim as a sole carbon source. Due to its low solubility in aqueous solutions, the addition of carbendazim resulted in suspension of particulate matter with the agarose-based medium. Potential degraders were considered to be those bacteria that grew on the medium, and displayed a zone of clearing of carbendazim around the bacterial colony. Growth kinetics of the bacteria, measured in a minimal medium broth supplemented with carbendazim as a sole carbon source, separated the isolates into two distinct classes based upon growth rates. HPLC analysis of culture filtrates indicated loss of carbendazim over time. Characterization of the pathways for degradation at the chemical and genetic levels are currently under investigation.

Detection and Analysis of Genetic Diversity in Magnaporthe poae Using Silver-Stained Random Amplified Polymorphic DNA (ssRAPD) Fingerprints

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Genetic variability among 41 fungal isolates was surveyed using silver-stained RAPD markers (ssRAPD). DNA fingerprints for 23 out of 35 *Magnaporthe poae* isolates were phenotypically unique, while all six isolates comprising an outgroup of additional ectotrophic, dermatiaceous fungi were unique. Silver-stained RAPD markers were found to be reproducible and valuable as a means of isolate identification. Analysis of Molecular Variance (AMOVA) showed that the *M. poae* isolates were significantly different (P=0.001) from the outgroup isolates indicating the utility of ssRAPD fingerprints as an accurate means of identifying individuals within a species. AMOVA further demonstrated that *M. poae* isolates were significantly different (P=0.001) among 12 locations. This result is important information for research programs concerned with obtaining genetically representative isolates of *M. poae*. From a subset of 12 isolates, pathogenicity (13) was found to decrease as genetic distance from the most pathogenic isolate increased. Using ssRAPD markers to obtain measurements of genetic relatedness and variation within and between fungal populations aided our understanding of the ecology and biology of the species.

Is Expression Of Fungal Invertase A Factor In Choke Disease?

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We are interested in the physiology of the endophyte-grass relationship in *Festuca rubra* L. subsp., some of which are important turfgrass species. Many naturally occurring and cultivated strains of *F. rubra* have been found to be infected with *Epichloe typhina* (Pers.:Fr) Tul. For most of the plant life cycle the *E. typhina* endophytes are intercellular and asymptomatic. At the time of flowering, however, some grass-endophyte combinations can result in choke disease. Choke disease results from the normally intercellular mycelia becoming external and forming a reproductive structure called a stroma on the floral-producing culms and preventing their growth. The dense external fungal tissue causes interference in the further development of the floral tissue resulting in elimination or reduction in the amount of seed produced. The potential for choke disease limits the usefulness of turfgrass varieties infected with the disease-causing fungal endophytes because commercial seed production is not possible.

The ability of the fungus to utilize available sugars in the flowering meristem has been proposed as a factor in the rapid growth of the fungi and subsequent induction of choke disease (White et al., Mycologia 83:601-610, 1991). Proceeding from this hypothesis, we found that choke stroma tissue contains high levels of cell wall and soluble invertase activities. This finding raised the possibility that invertase expression may be an important factor in the expression of choke disease. We therefore have undertaken an investigation of invertase expression in fungal endophytes.

Our results to date indicate that:

1) Invertase activity in choke stroma tissue is 12-28 fold higher than in the stem tissue just below the stroma. Stroma tissue is a mixture of plant and fungal tissue. Cell wall invertase activity was approximately twice as high in that portion of the stroma tissue that was visibly enriched in fungal tissue. We are currently trying to determine if the stroma invertase activity originates from the plant or the fungus, or both.

2) The level of invertase expression in endophyte isolates grown in culture was quite

variable.

3) Fungal invertase activity was ß-fructosidase activity.

4) Invertase expression in many isolates was substrate inducible. In other isolates there was little effect of carbohydrate source on the invertase level.

We are speculating that a high level of invertase activity in the stroma could result in the stroma being a strong sink for phloem transport of sucrose. The increased level of sucrose could provide the carbohydrate necessary for the fungal growth which must occur prior to production of asexual and sexual spores.

Development of Herbicide Resistant Turfgrass through Mutant Selection and Protoplast Transformation

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We have developed a protoplast regeneration system for creeping bentgrasses. A feeder layer was used for turfgrass protoplast regeneration. Protoplasts were isolated from embryogenic suspension cultures. High osmolarity with 5% mannitol was required for the isolations and initial divisions and regrowth of protoplasts. Turfgrass suspension cultures were necessary as feeders for the formation of protoplast derived callus. Six creeping bentgrass varieties produced protoplast derived calluses. Plants were regenerated from SR1020, Southshore, and Pennlinks. *E. coli* B-glucuronidase (GUS) gene constructs were used to quantitate the direct DNA uptake with protoplasts. Good transient expressions of GUS genes were obtained using either polyethylene glycol (PEG) or electroporation. Stable transformations with mutant AHAS (acetohydroxyacid synthase) genes which confer resistance to herbicide Pursuit were in progress. Regenerants from Pursuit resistant protoplast derived colonies are being tested for herbicide tolerance in greenhouse.

Mutant selection provides the advantage of producing herbicide resistant varieties from non-recombinant DNA technology, so there are no regulatory barriers to commercialization. Dilution series were tested to determine the toxic concentrations of Pursuit to embryogenic callus and suspension cultures. We used 20 µM Pursuit for mutant selection with a number of callus cultures. Cultures with good growth were periodically transferred to fresh media with the same concentration of herbicide. Tissue sectors with vigorous growth are tested for regeneration at various time intervals. Plants were regenerated from several tissue sectors of our first attempt. Herbicide spray tests of these regenerants are in progress.

Development Of An Endogenous Plasmid Into A Cloning Vector In *Clavibacter xyli* Subsp. *cynodontis* For Expressing Insecticidal Genes

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A 51 kb endogenous plasmid, pCXC100, from *Clavibacter xyli* subsp. *cynodontis* (CXC) has been mapped. Seven DNA fragments which covered the entire pCXC100 with 3-10 kb overlapping between two adjacent fragments have been obtained with suitable restriction enzyme digestions. They were subcloned into *E. coli* plasmid pBR325. The plasmid containing each fragment was then transferred into CXC by electroporation and three subclones survived. In order to determine the exact region of replication and stability, the subclones were further deleted and the stability of the deletion clones in the CXC transformants was confirmed. A stable shuttle vector pINC suitable for CXC and *E. coli* was constructed which contained Tcr and Ampr genes from PBR325 and several single restriction enzyme cloning sites. The insecticidal protein gene [CryIA(c)] from *Bacillus thuringiensis* has been cloned into the shuttle vector. The plasmid pIB which contained Bt gene in the vector pINC was transferred into CXC by electroporation. The expression of the Bt gene in *E. coli* and CXC was confirmed by western blot analyses. Using antibodies specific to Bt toxin the expression of Bt in *E. coli* was strongly demonstrated but the signals in CXC was very weak. Study on the improvement of Bt expression in CXC is now being carried out.

Molecular Analysis of Antifungal Production in *Pseudomonas cepacia* M53

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Pseudomonas cepacia M53 produces an antifungal compound that inhibits the growth of several fungal plant pathogens, including *Magnaporthe poae*, the causal agent of summer patch disease. The antifungal compound was partially purified and characterized from culture filtrates of *P. cepacia* M53. Many physical properties of the compound were similar to previously characterized phenylpyrrole compounds such as pyrrolnitrin, produced by biocontrol strains of *Pseudmononas*. These compounds are known to inhibit the growth of several fungal plant pathogens, and are thought to be the compound(s) by which *P. cepacia* M53 inhibits the growth of *M. poae*. Transposon mutagenesis was used to study the genetic pathway involved with the production of the antifungal compound in strain M53. Eight Tn*5* mutants were isolated that were either greatly reduced or no longer inhibited the growth of the indicator fungus, *Botrytis cinerea*, in agar plate assays. Seven of the eight mutants differed according to the specific *Eco*RI DNA fragment where Tn*5* had inserted. DNA sequences that flanked the Tn*5* insertions were used as probes in colony hybridization experiments to recover corresponding wild-type DNA clones. Several clones were isolated and identified from a genomic cosmid library for seven of the eight mutants. Southern blot and restriction enzyme pattern analyses of the recovered cosmid clones indicated that the genetic location for five of the eight mutants resided at genomic sites that were not closely linked to each other. Antifungal activity using plate assays against *B. cinerea* were restored by genetic complementation studies in four of the eight mutants. The antifungal phenotype, however, was not restored with corresponding clones for the remaining four mutants. These data suggest that production of pyrrolnitrin in *P. cepacia* M53 is genetically complex.

Influence of Liming, N-form, Sulfur, and Application Method on Soil pH and Summer Patch

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Summer Patch caused by the root-infecting fungus *Magnaporthe poae* is less severe when Kentucky bluegrass (*Poa pratensis* L.) is fertilized with ammonium (NH_4^+) forms of N. Associated with the use of NH_4^+ fertilizer is an increase in soil acidity. This study was initiated in 1992 to 1) examine the influence of soil pH, N-form, and S (acidifier) addition on the

development of summer patch on 'Baron' Kentucky bluegrass turf grown on a sandy loam and 2) determine the ability of surface and subsurface application of N fertilizers and S to alter soil pH and summer patch development. A 2X2X2X2 treatment factorial was used in a split-plot, randomized complete block design with 5 replications. Low and high soil pH levels were established by liming before turf establishment (main plot). The three subplot factors were: i) N-form (NH4+ and NO3-), ii) S addition (none and 14.6 g m-2), and iii) surface or subsurface application of factors i and ii. Injection of N fertilizers and S was achieved with a high pressure water injection cultivator (Hydrojet 3000; Minneapolis, MN). As expected, liming increased soil pHs, while S and NH4+ application decreased soil pH; however, limed soil inhibited the acidification of soil with NH4+ fertilization. The application method did not influence pH changes near the soil surface (0 to 7.5 cm depth), but, injection of NH4+ fertilizer lowered soil pH in the 7.5 to 15 cm zone, whereas surface application had no effect at that depth. A moderate level of disease activity occurred during August 1993. Disease severity data indicated that N-form had the greatest effect on disease expression, with the NH4+ form of fertilizer decreasing summer patch symptoms. Neither liming nor S application had a significant effect on the disease, but this may be a result of the highly variable (C.V.= 148%) and low level of disease expression. Research will continue to define the cultural and environmental factor which influence summer patch severity.

Selection of Wear Resistant Tall Fescue (festuca arundinacea)

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Tall fescue *(Festuca arundinacea* Schreb.) is excellent for development of wear resistant cultivars in sports turfs because of its lower maintenance requirements. Its drought tolerance also makes it a logical choice in a water conservation situation. Research on the tolerance of tall fescue to traffic was conducted at Rutgers Plant Science Research Station in Adelphia, NJ on a Freehold sandy loam that is resistant to compaction. Traffic was applied to field plots in the spring and fall using 75 and 55 passes, respectively, of the Meyers traffic simulator. Visual estimates of turf density were taken four times during wear treatment, and weekly during wear recovery periods. Several plant criteria were used to evaluate resistance to wear including measurements of verdure, tiller density, and root weights. Under high maintenance conditions, the improved low-growing turf-type tall fescue cultivars from a dense turf that is more resistant to wear than some of the dual-purpose cultivars first used for turf. All tall fescues recovered much faster from spring wear than from fall wear. The shorter day lengths of fall reduce the vegetative growth more dramatically in the improved low-growing cultivars than the dual-purpose cultivars. Our hope is that these studies will help breeders more effectively select the wear tolerant cultivars of the future.

A Study on the Chromosomal Homologs of the Native Plasmid pCXC100 In *Clavibacter xyli* subsp. *cynodontis*

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Two repeated elements, N3 and X2, were identified on the 51-kb native plasmid, pCXC100, of *Clavibacter xyli* subsp. *cynodontis* (Cxc), which is a non- pathogenic, Gram-positive bacterium inhabiting the xylem of Bermudagrass. Their chromosomal homologs, CH3 and CX2, were cloned from the genome of Cxc, respectively. Each of H3 and X2, only one copy was observed on pCXC100 when analyzing the entire plasmid DNA with the repeated element probes. However, five CX2 copies and more than fifteen CH3 copies were observed on the Cxc genome. The H3, X2 and CH3, CX2 were two independent repeated elements or homologs because no cross-hybridizations were detected between H3 and X2, H3 and CX2, X2 and CH3, and CH3 and CX2. According to DNA deletion analyses, a 0.6-kb fragment of H3 and a 1.0-kb fragment of X2 on the plasmid were responsible for the hybridization with Cxc chromosome. H3 was then sequenced and a pair of inverted repeat was found by comparing with the DNA database. DNA sequencing of CH3, X2 and CX2 is now in process.