

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

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

**January 10-11, 2002
Cook College**

Symposium Organizing Committee

Albrecht Koppenhöfer, Chair
Bruce B. Clarke
Barbara Fitzgerald
Stephen Hart
Bingru Huang
Jim White

Proceedings of the Eleventh Annual Rutgers Turfgrass Symposium

Bingru Huang and Barbara Fitzgerald, Editors

Associate Director's Opening Remarks:

Welcome to the 11th Annual Rutgers Turfgrass Symposium. The symposium planning committee, comprised of Albrecht Koppenhöfer (chairperson), Bingru Huang (Editor), Barbara Fitzgerald, Jim White, Stephen Hart and Bruce Clarke, has done a great job planning this year's program.

The topics at the symposium include a broad range of basic and applied research that has been conducted by the faculty of the Center for Turfgrass Science at Rutgers. We are honored to have Dr. Paul Rieke (Professor Emeritus, Michigan State University) as our keynote speaker. Dr. Rieke will be sharing the results of his distinguished career studying turfgrass soil management. Lane Tredway, who received a graduate degree from Rutgers and now is at the University of Georgia, will also be presenting some of his research.

The Center for Turfgrass Science continues to grow and mature as a center of excellence for turfgrass research, teaching and extension. We were pleased to have Dr. Stacy Bonos (Assistant Professor in Turfgrass Breeding) and Pedro Perdomo (Morris County Agricultural Agent) join the Center in 2001. Dr. Bonos' thesis work on the inheritance of resistance to dollar spot in creeping bentgrass recently earned her the Musser Foundation's Award of Excellence, an award given each year to the top turfgrass graduate student in North America.

The faculty of the Turfgrass Center continue to be engaged in cutting-edge turfgrass research. The Rutgers breeding program has developed many of the top performing varieties in the National Turfgrass Evaluation Trials. The most significant development in the breeding program in the last two years has been the identification of new sources of resistance to gray leaf spot. A number of new studies on drought and heat tolerance have been initiated by Dr. Huang and her students. Dr. Steve Hart has established an excellent research program in turfgrass weed management, while Dr. Jim Murphy continues to conduct excellent work on putting green soil mixtures. Dr. Murphy is also studying the ability of various bentgrass varieties to compete with annual bluegrass under wear and compaction stress. Dr. Koppenhöfer also is conducting many excellent studies concerning soil-inhabiting grubs that damage turfgrasses. These are just a few of the many research projects that are being conducted by Center faculty.

We hope that you find the 11th Annual Symposium an interesting and rewarding experience.

Sincerely,

Dr. William A. Meyer
Associate Director,
Center for Turfgrass Science

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ELEVENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

Cook College, Rutgers University
January 10-11, 2002
Foran Hall - Room 138

Thursday, January 10, 2002

- 7:00 - 7:30 PM Registration
- 7:30 - 7:40 PM Welcome: **Dr. Bruce Clarke, Director - Center for Turfgrass Science and Dr. Adesoji Adelaja – Director of Research / NJAES**
- 7:40 - 8:30 PM Keynote Address: **Dr. Paul Rieke** (Professor Emeritus, Department of Crops and Soils, Michigan State University) *A Historical Perspective of Turfgrass Soils*
- 8:30 - 10:00 PM Wine and Cheese Reception

Friday, January 11, 2002

- 8:30 - 9:00 AM Registration, Coffee and Donuts**
- 9:00 - 10:00 AM SESSION 1: TURFGRASS IMPROVEMENT**
(Moderator: Dr. C. Reed Funk)
- 9:00 - 9:20 **Dr. William Meyer** (Department of Plant Biology and Pathology, Rutgers University) *Breeding Progress for Resistance to Gray Leaf Spot in Perennial Ryegrass and Brown Patch in Tall Fescue*
- 9:20 - 9:40 **Dr. Stacy Bonos** (Department of Plant Biology and Pathology, Rutgers University) *The Effect of Sclerotinia homoeocarpa Isolate on Disease Progression and Inheritance of Dollar Spot Resistance in Creeping Bentgrass (Agrostis palustris Huds.)*
- 9:40 - 10:00 **Dr. Faith Belanger** (Department of Plant Biology and Pathology, Rutgers University) *Interspecific Hybridization as a Potential Method for Improving Dollar Spot Resistance of Creeping Bentgrass*
- 10:00 - 10:30 AM Discussion and Coffee Break**
- 10:30 - 11:30 AM SESSION 2: TURF PHYSIOLOGY AND ECOLOGY**
(Moderator: Dr. Bradley Hillman)
- 10:30 - 10:50 **Dr. Bingru Huang** (Department of Plant Biology and Pathology, Rutgers University) *Hormonal Regulation of Drought Tolerance in Cool-Season Turfgrass*

- 10:50 - 11:10 **Mr. Lane Tredway** (Department of Plant Pathology, University of Georgia) *Components of Resistance to Clonal Lineages of *Magnaporthe grisea* in Coronado and Coyote Tall Fescue*
- 11:10 - 11:30 **Dr. James White** (Department of Plant Biology and Pathology, Rutgers University) *Beneficial Effects of Endophytes on Grasses*
- 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: Dr. Donald Kobayashi)
- 1:30 - 1:50 **Dr. Albrecht Köppenhofer** (Department of Entomology, Rutgers University) *Integrated Control of White Grubs*
- 1:50 - 2:10 **Dr. Bruce B. Clarke** (Department of Plant Biology and Pathology, Rutgers University) *Chemical and Biological Control of Bentgrass Dead Spot*
- 2:10 - 2:30 **Dr. Steve Hart** (Department of Plant Biology and Pathology, Rutgers University) *Response of Glyphosate Resistant and Susceptible Bentgrass (*Agrostis* spp.) to Postemergence Herbicides*
- 2:30 - 3:00 PM Discussion and Coffee Break**
- 3:00 - 4:00 PM SESSION 4: TURF – SOIL MANAGEMENT**
(Moderator: Dr. Thomas Gianfagna)
- 3:00 - 3:20 **Dr. James Murphy** (Department of Plant Biology and Pathology, Rutgers University) *Root Zone Testing and Evaluation: Are New Standards Needed?*
- 3:20 - 3:40 **Dr. Daniel Giménez** (Department of Environmental Sciences, Rutgers University) *Estimating Water Content in Green Root Zone Mixes with TDR and a One-Parameter Model*
- 3:40 - 4:00 **Dr. Joseph Heckman** (Department of Plant Biology and Pathology, Rutgers University) *Role of Ammonium Nutrition in Control of Summer Patch Disease on Kentucky Bluegrass: A Review*
- 4:00 - 4:30 PM Discussion/Closing Remarks**

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Plenary Presentations

A Historical Perspective of Turfgrass Soils

Paul Rieke

Department of Crops and Soils, Michigan State University

The soil in which any plant grows has a significant impact on its growth and performance. Some of the early scientific pioneers of turf research such as Engel, Watson, and Daniel studied soil related problems. Several years later Ferguson and co-workers studied greens construction techniques and published the USGA Green Section specifications for greens. After a short time, Daniel and his students conducted studies on building greens and sports fields with sands. It was some time before the greens construction topic again became a hot topic for study due to new interest in soil amendments and to research funded by the USGA Green Section Research Committee. With the rapid growth in new golf courses and the current demand for highest quality putting green surfaces, soil conditions continue to be a major factor in the success of putting greens. As a result, cultivation and topdressing have received significant attention for the past 15 years. Drainage and irrigation have also been studied in greater detail because these practices are so important in dealing with soil problems. Of equal importance in soil management are chemical and biological aspects.

This presentation will cover some of the early research, but then will address more recent studies on cultivation, topdressing, and drainage conducted at Michigan State University and other institutions.

Breeding Progress for Resistance to Gray Leaf Spot in Perennial Ryegrass and Brown Patch in Tall Fescue

W. A. Meyer, S. A. Bonos, B. B. Clarke, R. F. Bara, D. A. Smith, M. M. Mohr,
E. Watkins and Y. Han

Department of Plant Biology and Pathology, Rutgers University

Since 1996, over 500 collections of perennial ryegrasses and their associated endophytes from Central and Eastern Europe have been evaluated at Rutgers University. Each year the most promising lines were topcrossed with the best Rutgers' germplasm available being used as the recurrent parent. The Rutgers germplasm originated from plants selected out of old turfs in the Northeastern US between 1962 and 1977. This material combined with new germplasm added from other US collections over the years has been cycled each year since the early 1970's as part of a population improvement program for perennial ryegrass. Continued improvements in turf quality, overall disease resistance and persistence have been made in the populations of perennial ryegrass released by Rutgers.

Gray leaf spot (caused by *Pyricularia grisea* (Cooke) Sacc) has become a new devastating disease of perennial ryegrass since the early 1990's in the Northeastern US and other parts of the humid USA. This disease never occurred on the Rutgers Adelpia Research Station in Freehold NJ until the fall of 2000. Without the presence of this disease breeding for improvements was not possible. Individual progenies of perennial ryegrass with improved resistance to gray leaf spot were identified in the fall of 2000. Fifteen of the 36 new sources of resistance trace their maternal origin to European collections. The other sources were from the original Rutgers germplasm sources. Most of the commercial varieties in this trial were devastated (up to 90%) by gray leaf spot while the most attractive gray leaf spot resistant progenies were damaged less than 5%.

In the spring of 2001 the maternal clonal parents of the most resistant progenies in the 2000 test were placed in isolated crossing blocks according to their plant type and maturity. The individual progenies and composites of the different crossing blocks were seeded on August 17, 2001 at the Adelpia farm. The three most recent populations had gray leaf spot ratings of over 7.7 out of 9 = no disease, while most of the commercial varieties rated from a 5.0 down to 1.5. These results were similar to the 2000 results for the commercial cultivars but progeny plots were improved because of the recombination of top resistant clonal lines.

It appears that it will be possible to make major improvements in the resistance to gray leaf spot in perennial ryegrass. The new populations are being increased at this time and additional cycles of selection are being initiated for the 2002 crop year.

Brown patch (caused by *Rhizoctonia soloni* Kuhn) is the most serious disease of tall fescue in most areas of the US. There has been gradual slow progress in the development of tall fescue varieties with improved resistance to brown patch. These

have resulted from a population improvement project on tall fescue that has been conducted at Rutgers for the past 40 years.

Many of the better performing widely adapted tall fescue varieties in recent years have been considered as semi-dwarf types such as Rembrant, Plantation, and Millenium which are moderately dense. Developing varieties with greater density in turf has resulted in higher levels of brown patch disease. At Rutgers, in recent years the focus on tall fescue breeding has shifted from selecting extremely dense types to selecting germplasm that exhibits a slightly more open canopy.

In the summer of 2001 the 1999 and 2000 tall fescue turf trials at Adelphia were inoculated with brown patch by spreading infected bluegrass seeds uniformly over these trials. Uniform epidemics occurred on these trials and some very good data was collected. Some of the moderately dense varieties in these two trials resulted in the highest levels of brown patch resistance. There were many new commercial cultivars and experimental selections with better performance compared to Kentucky 31 (a very poor open variety) and older commercial varieties. There were no varieties free from brown patch but many varieties showed improved resistance.

**The Effect of *Sclerotinia homoeocarpa* Isolate on Disease Progression
and Inheritance of Dollar Spot Resistance in Creeping Bentgrass
(*Agrostis palustris* Huds.)**

S.A. Bonos and W.A. Meyer

Department of Plant Biology and Pathology, Rutgers University

The dollar spot disease incited by *Sclerotinia homoeocarpa* F.T. Bennet. is an economically important disease of creeping bentgrass golf greens and fairways. As evidence of fungicide resistance and pesticide restrictions increase there will be fewer strategies to control dollar spot disease. Genetic resistance to dollar spot is a promising alternative to chemical, cultural and bio-control methods. Previous research in this area indicates that dollar spot may be quantitatively inherited with the possibility of two to five loci involved in resistance. It is unknown whether creeping bentgrass genotypes respond differently to different isolates or have major disease resistance genes in association with virulence genes in particular isolates of *S. homoeocarpa*. The objective of this study was to evaluate the response of creeping bentgrass crosses to three different isolates of *S. homoeocarpa*, evaluate disease progression and identify number of loci involved in resistance to individual fungal isolates.

In the spring of 1999, dollar spot resistant and dollar spot susceptible genotypes were crossed to develop full sib F₁ progeny. Seed was harvested, dried then treated with KNO₃ to induce germination. Seedlings were put in seedling flats described above and transplanted to the field on 17 Sep 2000. Five hundred progeny of each of two reciprocal crosses was established in a randomized complete block design. Plants were maintained as mowed tiller plots at approximately 5 cm with a rotary mower. One hundred progeny of each cross were inoculated with each of three isolates of *S. homoeocarpa* separately and with a mixture of the three isolates. Dollar spot disease was evaluated weekly throughout the growing season using a 1 to 9 scale, with 9 representing no disease and 1 representing completely brown turf. The determination of major genes was determined using the equation proposed by Fain (1978) and described by Lynch and Walsh (1998): $\text{Var}(o_i) = a + b_1\bar{o}_i + b_2\bar{o}_i^2$ where $\text{Var}(o_i)$ is the phenotypic variance within the *i*th sibship, and \bar{o}_i is the midparental value for this sibship. A significant value of b_2 is taken as an indication of a major gene (Lynch and Walsh, 1998). Minimum number of loci (genes) was determined using the Castle-Wright formula (Poehlman and Sleper, 1995) modified for environmental variation: $N = (P_1 - P_2)^2 / [8(\sigma_{F_1}^2 - \sigma_E^2)]$ where P_1 and P_2 are the means of the parent strains, σ_E^2 is the environmental variance among replicates of a parental clone pooled for all parental clones, and $\sigma_{F_1}^2$ is the variance of the F₁ progeny.

Susceptible parents responded differently to different isolates of *S. homoeocarpa* indicating that these two genotypes may contain different genes for susceptibility. Minimum loci calculations differed depending on the cross and the isolate further emphasizing the presence of different genes associated with resistance and susceptibility depending on grass genotype and fungal isolate. Major gene determinations were not significant indicating that a major gene may not be responsible for the variation observed in dollar spot resistance and that the variation may be due to environmental factors and other polygenes. This data supports previous research that dollar spot disease resistance is most likely quantitatively inherited.

Interspecific Hybridization as a Potential Method for Improving Dollar Spot Resistance of Creeping Bentgrass

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Interspecific hybridization has been used by breeders of many crops to introduce beneficial traits from related, often wild, species into crop species. Interspecific hybridization has not yet, however, been widely utilized by turfgrass breeders (Brilman, 2001) and may offer new opportunities for cultivar improvement. We previously investigated the frequency of interspecific hybridization between transgenic creeping bentgrass and four related *Agrostis* spp. (velvet bentgrass, colonial bentgrass, Highland bentgrass, and redtop bentgrass) using transmission of a herbicide resistance gene as a marker to identify the hybrids. Interspecific hybrids were recovered with all four *Agrostis* spp., although the frequency was low. The hybrids were found to be fertile.

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) susceptibility is currently one of the major management problems of creeping bentgrass. Colonial bentgrass has very good resistance to dollar spot but it is very susceptible to another fungal disease, brown patch (*Rhizoctonia solani* Kuhn). Interspecific hybridization between creeping bentgrass and colonial bentgrass and backcrossing to each parental species may be a way of improving the disease resistance of both species. These two species have the same chromosome number which may make introgression of the desirable genes from interspecific hybrids feasible.

To test this possibility we carried out a field test on our interspecific hybrids between creeping bentgrass and colonial bentgrass. In the field test we had 6 replicates of each of 35 colonial x creeping hybrids. The controls consisted of 6 replicates of the transgenic creeping bentgrass parents, 50 individuals of the creeping bentgrass cultivar Cobra, and 50 individuals of the colonial bentgrass cultivar SR7100. The field was inoculated with the dollar spot fungus and rated for disease. The transgenic creeping bentgrass pollen parent and the nontransgenic creeping bentgrass plants showed the expected high level of dollar spot susceptibility. The colonial bentgrass control plants showed the expected high level of dollar spot resistance. Four of the colonial x creeping hybrids in the test exhibited excellent dollar spot resistance throughout the season. Such dollar spot resistant hybrids may offer opportunities for cultivar improvement through gene introgression and for identification of the resistance genes involved.

Brilman LA (2001) Utilization of interspecific crosses for turfgrass improvement. Int Turfgrass Soc Res J 9:157-161

Hormonal Regulation of Drought Tolerance in Cool-Season Turfgrass

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Many physiological processes are involved in plant tolerance to drought stress. Hormone metabolisms play an important role in the regulation of plant growth and stress tolerance. Abscisic acid (ABA) is an essential constituent of higher plants. This hormone induces rapid stomatal closure and reduction in plant transpiration, thus lessening the decrease in leaf water potential and protecting leaf tissue from turgor loss during drought stress. ABA also induces the expression of several drought-resistant genes by reprogramming the cell to withstand dehydration stress.

Drought tolerance of turfgrass may be improved by manipulating endogenous ABA. To test this hypothesis, two Kentucky bluegrass cultivars, 'Midnight' (relatively drought resistant) and 'Brilliant' (relatively drought sensitive), were exposed to drought stress following foliar spray of ABA (100 μ mol). Turf quality, leaf water potential (Ψ), relative water content (RWC), net photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (Tr) declined whereas ABA content increased with drought in both cultivars. The decline in the physiological parameters was more severe in Brilliant than in Midnight. In contrast, the increase in ABA content was more dramatic in Midnight than in Brilliant. Exogenous application of ABA immediately prior to the exposure of plants to drought stress enhanced drought resistance for both cultivars. The enhancement of drought resistance during the early stage of drought could be related to induction of stomatal closure while improved growth during prolonged drought stress could be due to increased osmotic adjustment. These results suggest that it is possible to improve drought resistance in cool-season turfgrasses by manipulating ABA accumulation.

Components of Resistance to Clonal Lineages of *Magnaporthe grisea* in Coronado and Coyote Tall Fescue

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Sources of resistance to *M. grisea*, the causal agent of gray leaf spot, were recently discovered in Coyote and Coronado tall fescue by turfgrass breeders at Pure Seed Testing in North Carolina. The effectiveness of these resistance sources may be short-lived if multiple races of *M. grisea* exist in tall fescue populations. The objectives of this growth chamber study were to: (1) Determine which resistance components are responsible for reduced disease severity in Coronado and Coyote tall fescue; (2) Determine if clonal lineages of *M. grisea* in Georgia tall fescue populations correspond to pathogen races; and (3) Investigate the relationship between frequency of clonal lineages in tall fescue populations and isolate virulence.

Coronado and Coyote tall fescue were compared to the cultivars Rebel III (moderately susceptible) and Kentucky 31 (susceptible). Twelve weeks after seeding, each cultivar was inoculated with *M. grisea* isolates representing the five clonal lineages associated with tall fescue in Georgia. Inoculated plants were initially incubated at 24°C and 100% RH with no light for 24 hours. Subsequently, conditions were changed to 12 hr days at 30°C and 75% RH and 12 hr nights at 24°C and 100% RH. Disease incidence and average lesion length were recorded on a daily basis for seven days, and rates of disease progress and lesion expansion were estimated with regression analysis using the linear model. Incubation period and latent period were measured as the number of days from inoculation to the appearance of symptoms and conidia production, respectively. The number of conidia produced per unit lesion area was measured three days after the latent period had lapsed.

Compared to Kentucky 31 and Rebel III, Coyote and Coronado exhibited longer incubation and latent periods, reduced rate of disease progress, and lower final disease incidence. Incidence of foliar blighting, rate of lesion expansion, and final lesion length were similar in Coronado, Coyote, and Rebel III, but significantly less than in Kentucky 31. No significant difference in secondary inoculum production was detected among the cultivars tested.

Significant differences among isolates of *M. grisea* were detected for all parameters measured, but no significant interactions among cultivar and isolate were detected for any resistance component. Isolates belonging to the same clonal lineage tended to be similar in virulence, but some exceptions were observed. No relationship between virulence and the frequency of clonal lineages in field populations was evident.

Based on this study, the *M. grisea* resistance in Coyote and Coronado is the result of longer incubation and latent periods and a reduced rate of disease progress. Coronado and Coyote are resistant to all clonal lineages of *M. grisea* identified in Georgia tall fescue populations to date, therefore, no races of *M. grisea* could be differentiated in this study. These results are consistent with reports that multiple genes are responsible for *M. grisea* resistance in Coyote and Coronado, and indicates that these sources of resistance will be durable in the Southeastern United States.

Beneficial Effects of Endophytes on Grasses

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Endophytic microbes may colonize interior surfaces of plants without eliciting defense responses from host plants or causing disease symptoms. The benefits to plants of hosting such endosymbiotic microbes may be numerous. Diazotrophic bacterial endophytes in sugarcane have been shown to fix atmospheric nitrogen that enables hosts to grow indefinitely in soils low in available nitrogen. *Bacillus* spp. infected seedlings of many plants have been shown to have an enhanced resistance to diseases. Tall fescue seedlings infected by the endophyte *Neotyphodium coenophialum* show enhanced resistance to "damping off" disease caused by *Rhizoctonia solani*; while mature plants show increased drought tolerance and resistance to above ground and below ground insect and nematode pests. Similarly, several grasses infected by the endophytes *Epichloë typhina*, *E. festucae*, and *E. clarkii* were found to deter the feeding of migratory locusts; while endophyte-free plants were readily consumed by the locusts. It seems evident that plants benefit tremendously from the colonization of endosymbiotic microbes. The benefits to hosting mutualistic microbes likely outweigh losses in terms of nutrient use by the microbes. The notable effects in increasing resistance of grass hosts to herbivores has resulted in the proposal of the 'defensive mutualism hypothesis' for many of the endophytes in the family Clavicipitaceae; Ascomycetes (including *Balansia*, *Epichloë*, and *Neotyphodium*). The defensive mutualism hypothesis holds that a primary benefit to the host grass is defense against herbivory. Evidence has been accumulating that plants may gain additional benefits from hosting endophytic microbes. We have investigated the affect of *Epichloë festucae* on nutrient composition of several fine fescues. The results of this study suggest that endophytes increase nutrient content of hosts. This may explain observation by investigators of the generally improved appearance of many endophyte-infected grasses.

Integrated Control of White Grubs

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A complex of white grub species are the major turfgrass insect pest in the northeastern United States. The Oriental beetle, *Exomala (Anomala) orientalis*, has become the most important white grub species in New Jersey and some neighboring areas, whereas the Japanese beetle, *Popillia japonica*, until recently regarded as the key species, appears to be declining to insignificant densities. Other important species include the northern masked chafer, *Cyclocephala borealis*, and the primarily low-maintenance turf pests, European chafer, *Rhizotrogus majalis*, and Asiatic garden beetle, *Maladera castanea*. One major thrust of our program is to understand differences between these species as they related the optimizing measures to manage their populations in turfgrass.

Entomopathogenic nematodes

Entomopathogenic nematodes offer an environmentally safe alternative to chemical insecticides in the management of white grubs. Nematode efficacy in the field, however, has been variable. Some of this variability may be caused by the variability in nematode susceptibility among white grub species and their larval stages, and differences in pathogenicity to different white grub species among nematode species.

In laboratory and greenhouse studies we have compared the efficacy of different scarab-pathogenic *Steinernema* and *Heterorhabditis* spp. against the 3rd instar of 5 white grub species. Generally, the Japanese beetle was the most susceptible species. The northern masked chafer showed an intermediated level of susceptibility, whereas oriental beetle, European chafer, and Asiatic garden beetle were resistant to infection by all but one nematode species. *Steinernema* spec. (a putative new species isolated from Japanese and oriental beetle larvae in NJ) was highly pathogenic to Japanese beetle, oriental beetle, European chafer, and Asiatic garden beetle, but its performance against the northern masked chafer was not significantly better than that of the other nematode spp. However, in microplot field trials (at 2.5×10^9 nematodes/ha and 21 DAT), *Steinernema* sp. provided good to excellent (71-100%) control of all 5 white grub species. On the other hand, *H. bacteriophora* provided excellent control only against the Japanese beetle (90%) but no to mediocre (10-50%) control of the other white grub species.

We are investigating the effect of white grub (primarily oriental beetle) developmental stage (primarily 2nd vs 3rd instar grubs) on the efficacy of *Steinernema* sp. and *H. bacteriophora* under laboratory conditions. There appears to be no difference between 2nd and 3rd instar oriental beetle-susceptibility to *Steinernema* sp., whereas *H. bacteriophora* appears to be more pathogenic to 2nd instars. However, these observations have to be further clarified because recent observations have indicated the same trend for small (< 100 mg) vs large (> 175 mg) oriental beetle 3rd instars, i.e., no difference for *Steinernema* spp. but higher *H. bacteriophora* susceptibility of small 3rd instars. Our

observations also indicate that grubs that have emptied their intestines in preparation for pupation, prepupae, and pupae have low levels of nematode susceptibility.

Nematode-neonicotinoid interactions

In previous studies we had shown a synergistic interaction between nematodes and the neonicotinoid insecticide imidacloprid (Merit) in 5 white grub species and with 5 nematode species. Nematode interaction with the neonicotinoid thiamethoxam was weaker and only occasionally synergistic. Neonicotinoids and nematodes were also tankmix-compatible and there was no negative effect on nematode reproduction in white grubs infected after combined applications. This synergistic interaction has been confirmed for *Steinernema* sp. in oriental beetle and northern masked chafer grubs. No synergism, however, could be observed in the Asiatic garden beetle, likely due to the low susceptibility of this species to neonicotinoids.

Effect of turfgrass endophytes on white grubs and interaction with nematodes

Previous observations on the effect of turfgrass endophytes on white grubs have been variable but generally endophytes have had no or very little effect on white grubs. This is at least in part due to the low levels of endophyte alkaloids in turfgrass roots. We have observed the effect of endophyte status on white grub populations in various tall fescue breeding lines in the field. Over the last 3 years, white grub populations were generally higher in endophyte-infected (E+) vs endophyte-free (E-) tall fescue. However, the ratio E+/E- tended to decline from mid-August (1st and 2nd instar grubs) to mid-October (3rd instars). One possible explanation for this observation could be that E+ tall fescue is more attractive for egg-laying white grub females, but larval survival is reduced in the E+ tall fescue. Our greenhouse experiments, albeit variable, indicate reduced survival and weight gain of young white grubs in E+ vs E- tall fescue.

Our greenhouse experiments show a trend for increased *H. bacteriophora*-susceptibility in 2nd and 3rd instar oriental beetle when feeding on E+ tall fescue compared to E- tall fescue. However, the effect is weak and could not be confirmed in 2 field experiments.

Chemical and Biological Control of Bentgrass Dead Spot

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Bentgrass dead spot, incited by the fungus *Ophiosphaerella agrostis*, is a new disease of creeping bentgrass greens and tees that was first identified in Maryland, Pennsylvania, Ohio, and Virginia during the summer of 1998. It is favored by hot, dry weather and has only been observed on turf less than six years old. To date, this disease has been observed on creeping bentgrass, velvet bentgrass and bermudagrass. Symptoms of dead spot first appear as reddish brown spots 0.5 to 1 inch in diameter. Spots quickly fade to a tan color and are often confused with dollar spot, copper spot, cutworm damage or golf ball marks. When the disease is active, spots may have a bronzed outer margin, rarely coalesce, and are usually distributed randomly over the turf surface. Dead spot is most prevalent on high sand content sites. Little is known about the chemicals that suppress this disease and few fungicides are currently labeled for its control.

To identify fungicide classes that most effectively control dead spot, fungicides were evaluated in 2000 and 2001 at the Charleston Springs Golf Course in Millstone, New Jersey on a green naturally infested with *O. agrostis*. Fungicides representing ten different chemical classes were applied on a preventive basis each year at various rates and intervals from mid-July to mid-September. Chemicals were applied in water equivalent to 2 gal/1000 sq ft with a CO₂ powered sprayer. Data were collected for disease severity from late-July to mid-September.

The disease developed naturally on 28 July in 2000 and became very severe (i.e., 60% turfgrass infested) by late August. In 2001, disease development was delayed until 15 August and disease severity was moderate (39% on untreated turf area infested). In general, fungicides within the benzimidazole (Clearys 3336 50W at 4.0 and 8.0 oz/1000 sq ft), dithiocarbamate (Fore Rainshield 80W at 8.0 oz/1000 sq ft), nitrile (Daconil Ultrex 82.5SDG at 3.2 and 5.0 oz/1000 sq ft), phenylpyrrole (Medallion 50W at 0.33 and 0.5 oz/1000 sq ft) and the phosphonate (Chipco Aliette Signature 80WG at 4.0 oz/1000 sq ft; 2000 only) chemical classes provided the most effective control of dead spot (78-97% control), compared to untreated turf. Of the sterol-inhibiting fungicides, only propiconazole (Banner MAXX 1.3MC at 1.0 and 2.0 fl oz/1000 sq ft) adequately controlled the disease (95% control), whereas myclobutanil (Eagle 40W at 0.6 and 1.0 oz/1000 sq ft) provided moderate control (72-77% control in 2001 only) and triadimefon (Bayleton 50W at 2.0 oz/1000 sq ft) proved ineffective at the rates tested.

Similarly, two experimental strobilurin fungicides (BAS 500, 505, and 510) consistently suppressed the disease (82-98% control), while the strobilurins trifloxystrobin (Compass 50WG at 0.15 oz/1000 sq ft) and azoxystrobin (Heritage 50WG at 0.2 oz/1000 sq ft) provided poor to fair control (3 and 72% control, respectively). The carboximide (ProStar 70WG at 2.2 oz/1000 sq ft), dicarboximide (Chipco 26GT 2SC 4.0 fl. oz./1000 sq ft), and phenylamide (Subdue MAXX 2MC at 1.0 fl oz/1000 sq ft) fungicides and a strain of *Bacillus subtilis* (Companion I at 4.0 and 8.0 oz/1000 sq ft) did not significantly control dead spot, compared to untreated turf.

Response of Glyphosate Resistant and Susceptible Bentgrass (*Agrostis* spp.) to Postemergence Herbicides

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The development of glyphosate resistant creeping bentgrass (*Agrostis palustris* hud.) has led to the need to identify alternative postemergence herbicides for control of these and related *Agrostis* species. Field studies were conducted in 2001 in North Brunswick, NJ and Merion County, Oregon to evaluate the response of glyphosate resistant creeping bentgrass hybrids ('RR 365', 'RR 368', and 'RR 801'), glyphosate susceptible creeping bentgrass hybrids ('RS 365', 'RS 368'), colonial bentgrass (*Agrostis capillaris* L. 'SR 7100'), red top bentgrass (*Agrostis gigantea* With. 'Streaker') and dryland bentgrass (*Agrostis castellana* Boiss. and Reut. 'Trust'). Each plot contained four bentgrass plugs of each line planted at a spacing of two feet on May 9 in New Jersey and May 21 in Oregon. Glyphosate at 1.5 lbs ai/A, glufosinate at 1.5 lbs ai/A, fluazifop at 0.25 and 0.375 lbs ai/A, clethodim at 0.25 lbs ai/A, sethoxydim at 0.47 lbs ai/A, and a combination of fluazifop and glyphosate were applied 6 weeks after planting. Fluazifop, clethodim, and sethoxydim treatments included crop oil concentrate at 1.0% v/v. Plots were visually evaluated at 1, 2, 4, and 8 weeks after treatment (WAT) and plant diameters were taken prior to herbicide application and at 4 and 8 WAT. Glyphosate provided almost complete control of all susceptible bentgrass lines by 4 WAT and this level of control was maintained at 8 WAT. There was no evidence of injury or growth reduction on the glyphosate resistant lines. Glufosinate provided 95% or greater control of all bentgrass lines at 2 WAT. However, at 8 WAT regrowth was evident in Oregon. Fluazifop, clethodim, and sethoxydim provided slower control of bentgrass lines with control ranging from 50 to 90% at 4 WAT depending on species, herbicide, and research location. By 8 WAT fluazifop at 0.375 lbs ai/A applied alone or in combination with glyphosate showed the highest levels of control (>90%) across all bentgrass lines. This study is currently being repeated at both research locations with planting dates in August 2001.

Root-Zone Testing and Evaluation: Are New Standards Needed?

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Field and laboratory studies were initiated to identify factors that contribute to the success or failure of putting greens. Longer-term evaluation of the physical, chemical, and biological characteristics of the more than 30 root zone mixes in this project is critical to ensure that recommendations generated from the research represent what can be expected over the life span of a typical putting green. Root zone treatments were built in two microenvironments to assess 1) acceptable ranges of sand particle size distribution and depth of the root zone, 2) utility of various composts, peats and inorganic materials as amendments for mixes, 3) physical, chemical, and biological changes that occur as greens mature, and 4) the potential to reduce inputs.

Data was collected in 2001 for turf quality, brown patch disease incidence, algae incidence, soil and clipping tissue phosphorus, clipping yield, root mass distribution, irrigation requirement, soil hardness and strength, and physical properties of the 0 to 7.6-cm depth zone. Much of the data is currently being summarized.

Physical property changes have occurred in root zone mixes within two growing seasons after establishment; air-filled porosity of the field plots decreased, whereas capillary porosity increased compared to initial laboratory values. The presence of roots within the mixtures is likely contributing to the overall shift in pore size distribution. Greater water retention (greater capillary porosity) in a root zone has improved bentgrass performance during the first three growing seasons. The enclosed microenvironment reduced rooting of all root zone mixes within two growing seasons. The lower root mass in the enclosed microenvironment appeared congruent with the greater bulk density and lower total porosity of root zones in that microenvironment. Further evaluation of turf performance, root development, and root zone physical properties will establish the longer-term relationships between soil physical properties and turf responses.

Soil and clipping tissue phosphorus content is being analyzed to develop sufficiently level data that can be used to generate recommendations for phosphorus fertilization of sand based root zones. Work will be repeated in 2002 to assess veracity of sufficiency data.

Irrigation requirements of each plot were assessed from April through October 2001 through the metering of hand watering that was based on soil water content. Although data is still being summarized, it is apparent from a cursory review of the dataset that substantial differences in irrigation frequency exist among root zones. It is also likely that the amount of water required will vary among root zones. Root zones with a capillary porosity of 30% or more appear to have lower irrigation requirements under the climatic conditions of New Jersey. Data on irrigation requirement will be collected in 2002.

Data for turf quality, brown patch disease incidence, algae incidence, irrigation requirement, and soil hardness and strength has been collected in 2001 and is being summarized. Samples for soil and clipping tissue phosphorus, clipping yield, root mass distribution, and physical properties of the 0 to 7.6-cm depth zone have been collected and will be assessed during the winter of 2001/2002.

Assessment of ASTM F-1815

Variation in sample water content before any saturation event in the ASTM method (F-1815) results in different encapsulated air contents of the sample, which subsequently can affect K_{sat} , bulk density, and porosity of the material. The ASTM F-1815 method evaluates samples that are more accurately described as satiated (contain encapsulated air) rather than saturated. Variation in sample antecedent water content likely contributes to some of the variation in K_{sat} observed when the same material is tested at different laboratories. Procedures that ensure a consistent bias caused by antecedent sample water content are needed.

Investigations of the effect of air encapsulation as influenced by antecedent water content in commercial testing laboratories are needed. Vacuum saturation removes encapsulated air from sand root zone laboratory samples; however, vacuum saturation can dramatically impact pore size distribution of samples. A detailed comparison of methods to remove encapsulated air including sample drying, pre-saturation CO₂ flushing, and vacuum saturation are needed before further recommendations to improve the repeatability of the K_{sat} measurement can be made.

Estimating Water Content in Green Root Zone Mixes with TDR and a One-Parameter Model

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Fast and accurate methods to measure soil water content in situ are required to support turf management. Time Domain Reflectometry (TDR) is a very popular method to estimate soil-water content, but there are no calibration curves available for green mixes. The objectives of this study were to: 1) measure relationships between the dielectric constant, K_a , measured with TDR and soil-water content, W_v , for sandy soils mixed with various amounts of organic matter, and 2) test an existing model that uses two constant parameters to define the K_a - W_v relationship for soils. Five sands with variable size distribution, each mixed with 5%, 10% 20% and 40% organic matter content, were investigated. The air-dried mixes were packed in triplicate columns (0.10 m diameter and 0.35 m height), and TDR waveguides were placed from the top. Water was applied from the bottom of a column using a syringe pump. Our results show that soil organic matter, and to a lesser extent particle size distribution, influenced K_a - W_v relationships. Predictions with an existing (and commonly used) model were accurate within 3% in samples without or with low organic matter contents (5% and 10%). For soils with 20% to 40% organic matter contents, the model underestimated up to 16% the soil water content. We propose an alternative equation that uses one parameter that can be predicted from organic matter content and particle size distribution. In conclusion, accurate TDR estimates of water content in green mixes require site-specific calibration. This is particularly true if the material is coarse and/or it contains a significant amount of organic matter.

Role of Ammonium Nutrition in Control of Summer Patch Disease on *Kentucky Bluegrass: A Review*

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Summer patch is caused by the ectotrophic root-infecting fungus *Magnaporthe poae* Landschoot and Jackson. The disease, which often infects high maintenance turf, can be difficult to control because root infection occurs six to eight weeks before the appearance of foliar symptoms (Smiley et al., 1992). Studies conducted over the last decade have consistently demonstrated that the type of nitrogen (N) fertilizer applied to turf can strongly influence the severity of the disease (Thompson et al., 1993; Thompson et al., 1995; Hill et al., 2001 ab). The use of ammonium-based fertilizers such as $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{S}_2\text{O}_3$ or NH_4Cl reduces disease severity, whereas the use of nitrate sources of N, such as $\text{Ca}(\text{NO}_3)_2$, increases disease severity. Urea and N-SURE are N sources that may also increase disease severity.

The effect of different N sources on disease severity appears to be associated with changes in soil pH that accompany the use of a particular N fertilizer (Thompson et al., 1995). In general, ammonium sources of N acidify the soil, and nitrate sources of N may increase soil pH. Urea and N-SURE breakdown in the soil to supply N in the form of ammonia but the urea hydrolysis reactions that are associated with these N sources cause an elevation of soil pH. Such urease activity is especially high in the rhizosphere. Increases in rhizosphere pH that may result from the use of nitrate or urea sources of N may provide a pH environment that is conducive to the root-infecting fungus.

Ammonium form of N results in acidification of the bulk soil to the extent that nitrification of the applied N occurs before it is taken up by the plant. However, if most of the applied N is rapidly taken up as NH_4^+ before nitrification is allowed to occur, then the uptake of NH_4^+ would be expected to acidify the rhizosphere (Heckman and Strick, 1996). Studies (Bowman et al., 1989 ab) suggest that a Kentucky bluegrass turf may absorb 75% of a typical N application within 24 hours. Thus, a significant portion of N applied as $(\text{NH}_4)_2\text{SO}_4$ is probably taken up as NH_4^+ . Plant uptake of nitrate N, regardless of whether it was originally applied in the form of ammonium or nitrate, causes the rhizosphere pH to increase (Heckman and Strick, 1996).

In addition to the use of acidifying N fertilizers, soil acidification due to the application of elemental sulfur has also been shown to be effective in reducing the severity of summer patch (Hill et al., 2001a). However, regular applications of limestone, used in combination with $(\text{NH}_4)_2\text{SO}_4$ to maintain a favorable soil pH for optimum turfgrass growth and quality, do not decrease the effectiveness of $(\text{NH}_4)_2\text{SO}_4$ for the control of summer patch (Hill et al., 2001a). Thus, acidification of the bulk soil is not necessarily required for effective disease control. Rhizosphere acidification, as a result of a rapid rate of ammonium uptake in advance of nitrification may explain the effectiveness of $(\text{NH}_4)_2\text{SO}_4$ when the bulk soil pH is maintained at a higher level with limestone applications.

Thiosulfate is a nitrification inhibitor that has been shown to delay nitrification by 50% or more for a period of up to 22 days (Goos, 1985; Janzen and Bettany, 1986). The application of additional thiosulfate, as $K_2S_2O_3$, with an application of $(NH_4)_2S_2O_3$ further enhances rhizosphere acidification and increases the effectiveness of $(NH_4)_2S_2O_3$ for control of summer patch. The addition of thiosulfate to $(NH_4)_2SO_4$ enhances rhizosphere acidification, but does not necessarily increase disease control. Thus, thiosulfate applied in combination with an ammonium-based fertilizer can enhance rhizosphere acidification, but the influence of this nitrification inhibitor on disease control varies with the source of ammonium used.

In summary, ammonium-based fertilizers can effectively suppress summer patch disease as a result of rhizosphere acidification, bulk soil acidification, ammonium nutrition, and enhanced micronutrient availability. Because ammonium-based N is probably rapidly taken up by turfgrass largely as NH_4^+ , there may be limited value in the use of nitrification inhibitors for the purpose of further enhancing uptake in the ammonium form. A rapid rate of NH_4^+ uptake before nitrification is allowed to occur, will most likely ensure rhizosphere acidification even in soils that have been well limed. This may explain why a regular liming program does not decrease the effectiveness of $(NH_4)_2SO_4$ for control of summer patch in cool season turf.

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Poster Presentations

Heritability of Gray Leaf Spot Resistance in Perennial Ryegrass

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Gray leaf spot caused by *Pyricularia grisea* (Cooke) Sacc. [perfect stage *Magnaporthe grisea* (T.T. Herbert) Yaegashi & Udagawa] has become a devastating disease of perennial ryegrass (*Lolium perenne* L.) turfs since its first identification in 1986 by Dr. Peter Dernoeden (Vermeulen, 1999). Genetic resistance is the most promising of the control strategies for gray leaf spot. However, the germplasm base for perennial ryegrass cultivars is narrow and little resistance has been observed (Vaicunas and Clarke, 1998). The identification of gray leaf spot resistance can be used in recurrent selection techniques to incorporate genes for gray leaf spot resistance within the species improving the development of gray leaf spot resistant varieties. The objectives of the study was to 1) evaluate cultivars, experimental selections and single-plot progenies of perennial ryegrass for resistance to gray leaf spot, 2) identify the response to selection for gray leaf spot resistance in perennial ryegrass populations; 3) determine realized heritability of gray leaf spot resistance and 4) identify genetic relationships of resistant and susceptible populations of perennial ryegrass.

Two field trials were established in a randomized complete block design in August, 2000 and 2001, at the Plant Science Research Station, Adelphia, NJ. The outbreaks of gray leaf spot occurred naturally in both years on 17 Sep 2000 and 26 Sep 2001 on approximately four week old seedling turf. Gray leaf spot was rated using a 1-9 scale (9=no disease). Parents were selected for gray leaf spot resistance from data in 2000 and used to develop populations in 2001. Allelic data from 15 microsatellite loci was generated using PCR (Polymerase Chain Reaction) and sizes analyzed using ALF-express DNA sequencer (Pharmacia Biotech). All replicated data was subjected to analysis of variance. The selection differential, response from selection and realized heritability were calculated from the equation: $R = \sigma_p \cdot S$ or $R = \sigma_p \cdot S = (s_p - \bar{p}) = S$ described in Lynch and Walsh (1998) where σ_p is the mean phenotype of the offspring of the selected parents, s_p = the mean phenotypic value of selected parents, \bar{p} is the phenotypic value of the parent population before selection was made, and $R = R/S$ or h^2 or realized heritability.

Variation in gray leaf spot resistance among cultivars and selections of perennial ryegrass was observed. Thirty-six experimental selections had better leaf spot resistance than all commercially available perennial ryegrass cultivars. Fifteen of the 36 sources of resistance were germplasm collections from Europe. Selection of resistant parents in 2000 resulted in improved resistance in 2001. The selection differential (S) = 2.22, and the realized heritability = 0.98 indicate that selection for gray leaf spot resistance should be very effective in improving gray leaf spot resistance in subsequent generations. Most population distributions approach a normal distribution, which would indicate a quantitative type of inheritance, however, major genes may still be present.

Enhancement of *Steinernema Carpocapsae* Desiccation Tolerance by Genetic Improvement

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The entomopathogenic nematode (ENP) *Steinernema carpocapsae*, is an important biological control agent for a wide range of soil dwelling insect pests. However, the field efficacy of this ENP is limited by its sensitivity to high drought and salinity conditions. We report efforts to improve the desiccation tolerance of *S. carpocapsae* by transforming it with the trehalose-6-phosphate synthase (*tps1*) and glycogen synthase genes (*gsy1*). Trehalose-6-phosphate synthase and glycogen synthase are enzymes involved in the biosynthesis of trehalose, a disaccharide that accumulates to stabilize the lipid biomembranes in many organisms when in response to stress.

To increase desiccation tolerance by genetic modification, we have cloned gene *tps1* from yeast and *C. elegans* into expression vectors pJJ436 and pPD95.67, respectively. In addition, we also cloned *gsy1* from *Steinernema feltiae* into expression vector pJJ436. Vector pJJ436 contained the *Ce sq-1* promoter, whereas pPD95.67 contained the promoter of the *tps1 Ce* gene. Vectors contained *gfp* transformation gene which was used as a selection marker. Vector constructions (yeast: pJYe.1; *C. elegans*: pP67Ce.2; *S. feltiae*: pJTr.1) were microinjected independently into young *S. carpocapsae* females (48 h from infective juvenile stage). Injected females were mated with noninjected males for 2-4 days and progeny were screened for *gfp* expression. After selecting and retaining *gfp* expressing individuals for three generations, F3 progeny will be tested for desiccation tolerance.

Drought Resistance of Cool-Season Turfgrasses Associated with Endophyte Infection

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Endophytes have been found to promote drought resistance in forage grasses. Most studies of the effects of endophytes on host fitness have focused on endophyte-infected tall fescue and perennial ryegrass. This study was designed to examine drought resistance of four cool-season turfgrasses, tall fescue, perennial ryegrass, fine fescue, and Kentucky bluegrass, associated with endophytes, and to determine effects of endophytes on the recuperative ability of the four grasses. Endophyte-infected or endophyte free plants of each species were exposed to well-watered conditions or drought stress for 30 d in a growth chamber. Drought-stressed plants were then rewatered to allow recovery for 14 d. Drought stress reduced turf quality, relative water content, chlorophyll content, and increased electrolyte leakage for both endophyte-infected and endophyte-free plants in all four grasses. However, plants infected by endophytes exhibited better drought resistance relative to uninfected plants for all four species. The enhanced drought resistance was manifested by the increased turf quality, chlorophyll content, and relative water content, and decreased electrolyte leakage in endophyte-infected plants compared to endophyte-free plants. Endophyte infection increased the recuperative ability of plants from drought stress in all four species, particularly for tall fescue.

Use of Interspecific Hybrids to Identify Genes Responsible for Disease Resistance

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Some interspecific hybrids between creeping bentgrass and colonial bentgrass were found to have excellent dollar spot resistance in a field test in 2001 (Belanger et al., 2002). We are interested in pursuing this observation both as a means of developing improved cultivars and as a way of understanding the mechanism of resistance. The identification of hybrid plants with excellent dollar spot resistance offers us the possibility of isolating the genes involved in the resistance. We are constructing a subtraction library in an attempt to isolate genes expressed in the dollar spot resistant hybrids but not in the creeping bentgrass dollar spot susceptible pollen parent. In this method cDNAs which are expressed in both plants are selectively removed from the reaction, whereas those that are expressed only in the hybrid are retained. These cDNAs are then used to generate a subtracted library and the resulting clones sequenced to identify the genes. Hopefully some of the differentially expressed genes will be those responsible for the observed disease resistance. Ultimately, the identification of genes responsible for dollar spot resistance will be useful in enhancing the efficiency of bentgrass breeding programs.

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Identification of Annual and Perennial Ryegrass Using Reversed-Phase High-Performance Liquid Chromatography

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Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) of cereal grain proteins has received much attention in the literature in recent years as a quick and efficient means of cultivar identification. A study of perennial ryegrass (*Lolium perenne* L.) and annual ryegrass (*Lolium multiflorum* Lam.) was undertaken to determine if RP-HPLC could be adapted to the identification of these species. Chromatograms of the different cultivars of the seed kinds studied all had distinct peak patterns, which correlated with each of the two species. Cultivar differences within each species group were distinct enough so that all of the cultivars were distinguished from each other. RP-HPLC was shown to be a quick, repeatable and reliable method of ryegrass species and cultivar identification for a general screening of seed lots. Protein analysis of single seeds could differentiate annual ryegrass seeds from perennial ryegrass seeds. Mixtures and blends of ryegrass seeds were analyzed and compared. The sensitivity of the detector was such that the changes of area under the peaks of the chromatogram were able to detect a lower limit of 5% annual ryegrass in perennial ryegrass.

After-Ripening Dormancy in Cool-Season Grasses

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Freshly harvested seed of cool-season turfgrasses is normally harvested during June and July. The generally hot, dry weather following seed ripening is normally unfavorable for seedling establishment due to drought, disease, and excessive weed competition. As a result, most grass seed has evolved a system of after-ripening dormancy to delay germination until the cooler, more favorable conditions of early fall. This seed dormancy is a physiological process that is gradually overcome by time in storage following harvest. The expression of after-ripening dormancy is very dependent on the temperature of the imbibed seed during germination.

Research (Phaneendranath, 1977, Phaneendranath, Duell and Funk, 1978, Phaneendranath and Funk, 1978, and Phaneendranath and Funk, 1981) and experience at Rutgers show that establishment and performance of turf trials is frequently strongly influenced by the different degrees of after-ripening-dormancy present in various seed lots, especially when warm weather conditions occur immediately following seeding. One year old and older seed of good viability has overcome its dormancy and germinates immediately if moisture is adequate. Most new crop seed delays initiation of germination until cool weather arrives. However, depending on genotype and conditions of seed production and storage, some seed will germinate much better than other seed lots. Seed produced in New Jersey generally has less dormancy than seed produced and stored under conditions more favorable for the production of plump, bright seed in Idaho, Washington, and Oregon. The degree of low temperature required to overcome after-ripening dormancy during seed germination depends on the degree of dormancy remaining in a particular seed.

Conditions favorable to the reduction in and the speed of overcoming after-ripening dormancy include:

1. Delaying harvest until seed is fully ripe (Phaneendranath, Duell, and Funk, 1978).
2. Rain on freshly swathed seed while still drying in windows prior to threshing appears to leach some inhibitors of germination.
3. Seed storage under warm, humid conditions accelerates the physiological processes reducing and overcoming after-ripening dormancy. This is contrary to the recommendation to store seed under dry, cool conditions to prolong viability of seed and any contained endophyte in long-term storage. Freshly harvested seed stored in an open shed in New Jersey has shown less after-ripening dormancy than seed stored in a dry, air conditioned facility when planted six to ten weeks after harvest.

Extensive experience in New Jersey has failed to show any effect of *Neotyphodium* endophytes on the expression of after-ripening dormancy or seedling vigor of freshly harvested seed. The widespread, early perception that tall fescue seed free of endophytes had poor establishment was most likely due to the different conditions of seed production, and length and conditions of storage. Endophyte-containing tall fescue seed was generally produced in southwestern Missouri under hot, humid, often rainy conditions, harvested in early summer, and stored for at least three months under warm, humid conditions. These production and storage conditions are favorable to overcoming or reducing after-ripening dormancy. Endophyte-free seed was normally produced in Oregon. It ripened about one month later under generally dry, cooler conditions. It was usually stored for a shorter period under dry, cooler conditions.

After-ripening dormancy can be overcome by a number of laboratory techniques (Phaneendranath and Funk, 1978). These techniques are normally used only by seed laboratories and plant breeders in a rush to check seed viability or speed-up the breeding program. The most widely used technique is to imbibe seed with a dilute solution of KNO_3 and place it in a refrigerator for a few days prior to a germination test. This so called cold treatment is on moist seed. Unfortunately it has encouraged some to place dry seed in refrigerated storage prior to seeding. This merely delays overcoming of dormancy (Phaneendranath and Funk, 1981). Freshly harvested seed placed in a no-frost refrigerator at 2 to 5 degrees C maintained a high level of after-ripening dormancy for at least 12 months. The experiment was terminated at this time

4. Planting just prior to a period of cool nights and moderate day temperatures often allows successful seedings in late August or early September. Our tests at Rutgers includes solid-set irrigation immediately after seeding through emergence. Under these conditions seedling emergence of Kentucky bluegrass usually starts to become visible after 5 to 6 days. After-ripening dormancy is less of a problem in the establishment of perennial ryegrass. Kentucky bluegrass gives us the most problems with after-ripening dormancy. Tall fescue and fine fescues are intermediate. Seedings made after mid-September normally have few problems with seed properly produced and stored under conditions favorable to overcoming dormancy. Spring seeding should be free of these problems.

Seed growers and seed companies realize the costs of storage, insurance, interest, and unpredictable markets in keeping seed a full year after harvest. This would avoid problems of after-ripening dormancy. However, many knowledgeable turf growers realize the advantage of purchasing old crop seed months in advance of late summer seeding. This allows a better choice of high quality seed lots and cultivars, retesting to avoid problems, and elimination of any problems with after-ripening dormancy.

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Recycling Grass Clippings Sustains Soil Fertility

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Leaving clippings on the lawn recycles plant nutrients and enhances turfgrass quality. A recent study conducted at Rutgers concluded that when clippings are returned, an equivalent or better turf color can be achieved by using only 2 pounds of nitrogen per 1000 square feet per year instead of the usual rate of 4 pounds of nitrogen per 1000 square feet per year (Heckman et al., 2000). Leaving clippings was also found to reduce the population of weeds in turf.

In 1994, the first year the plots with the two different mowing practices were established, turf color improved throughout the growing season where clippings were returned when compared to where they were removed. A darker green, more luxuriant appearance was apparent within four months of initiating the practice of returning clippings. This difference in turf color continued during the following fall, winter, and spring months. In subsequent years of returning or removing clippings, a better turf color was consistently maintained when clippings were returned. These results suggest that the improved turf color was a result of nutrients being recycled within the turfgrass system.

When clippings are removed about 300 pounds of fresh clippings (58 pounds of dry matter) are collected per 1000 square feet of lawn in one year. Leaving these clippings on the turf would instead recycle an estimated (pounds per 1000 square feet per year) 2 pounds of nitrogen, 0.18 pounds of phosphorus (0.4 pounds P_2O_5) and 1.2 pounds of potassium (1.4 pounds K_2O). Thus, the recycling of clippings after a period of years may be expected to maintain soil fertility levels better than when clippings are removed.

After six years of comparing mowing practices soil test results confirm as predicted that higher levels of soil fertility are maintained when clippings are recycled (Table 2). Soil nutrient supplies to turfgrass were significantly greater for nitrogen, potassium, and magnesium where clippings were returned. The soil organic matter content was also increased by the return of clippings. These findings support the recommendation that fertilizer rates should be reduced when clippings are being recycled.

Based on the findings of the current study and previous research (Heckman et al., 2000) Rutgers Cooperative Extension recommendations for turf management when leaving clippings are as follows:

Use a slow release fertilizer to reduce surge growth and amount of clipping residue.

Apply less fertilizer. The nitrogen application rate should generally not exceed 2 pounds of nitrogen per 1000 square feet per year. Phosphorus and potassium application rates may also be reduced but the amounts to apply should be based on the results of regular soil sampling and testing.

Increase the frequency of mowing during periods of rapid growth.

Table 1. Influence of six years of mowing practice (clippings returned vs. clippings removed) on soil fertility (Mehlich-3 soil test method) of a Kentucky bluegrass turf at the Rutgers Hort Farm II. Soil sampling was performed on May 10, 2000 from the 0 to 2 inch depth.

Soil Test Item	Clippings Returned	Clippings Removed	Statistics [†]
Soil pH	6.3	6.3	NS
Exchange Capacity (meq/100g)	8.8	8.5	**
Soil Organic Matter %	3.3	3.0	**
Nitrate, NO ₃ -N (ppm)	2.3	1.7	*
Ammonium, NH ₄ -N (ppm)	8.7	5.3	**
Soluble Sulfur (ppm)	21	21	NS
Phosphorus (ppm)	245	244	NS
Potassium (ppm)	168	125	***
Calcium (ppm)	992	978	NS
Magnesium (ppm)	244	221	***

[†] *, **, *** Significant at the 0.05, 0.01, and 0.001 levels, respectively.

NS = not significant.

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Heckman, J.R., H. Liu, W.J. Hill, M. DeMillia, and W.L. Anastasia, 2000. Kentucky Bluegrass Responses To Mowing Practice and Nitrogen Fertility Management. *Journal of Sustainable Agriculture*. 15:25-35.

Take-All Patch Suppression on Creeping Bentgrass with Manganese Fertilization

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Take-all patch, caused by *Gaeumannomyces graminis* (Sacc.) Arx. & D. Olivier var. *avenae* (E.M. Turner) Dennis (*Gga*), is a disease of creeping bentgrass (*Agrostis stolonifera* L.). Previous research has shown that Mn fertilization can reduce take-all patch severity, but further research is needed. Because *Gga* has the capacity to oxidize Mn, the time and rate of Mn application may be important factors in the use of Mn as a management tool against this disease. The objective of the current study was to determine the best time and rate of Mn application for the suppression of take-all. Manganese (MnSO_4) treatments were applied to a golf course fairway in either October or April of 1998, 1999, and 2000 at 0, 2.25, 4.50, 6.75, and 9.00 kg Mn ha⁻¹. Severity of take-all patch was assessed each year during May and June. Throughout the study, Mn reduced the severity of take-all when applied either in October or in April. Moreover, the 2.25 kg Mn ha⁻¹ application rate was generally as effective in suppressing the disease as were higher application rates. Findings also suggest that over time previous applications of Mn fertilizer became less effective than new applications of Mn in suppressing take-all disease.

Impact of Liming and Nitrogen on the Severity of Summer Patch of Kentucky Bluegrass

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Although the application of acidifying fertilizers can reduce the severity of summer patch disease, this benefit must be balanced against the need to maintain a satisfactory soil pH level for plant growth. This field study was conducted from 1995 to 1998 to evaluate the application of lime (99, 198, and 396 kg CCE ha⁻¹) in combination with the acidifying fertilizer ammonium sulfate (196 kg N ha⁻¹ season) and the alkalizing fertilizer calcium nitrate (196 kg N ha⁻¹ season) on the severity of summer patch, caused by *Magnaporthe poae* Landschoot and Jackson in 'Georgetown' Kentucky Bluegrass. The two fertilizers were applied alone and in combination with lime or elemental sulfur (845 kg S ha⁻¹). On some rating dates, disease severity was greater on turf that received calcium nitrate versus ammonium sulfate. Compared to calcium nitrate alone, calcium nitrate plus lime (396 kg CCE ha⁻¹) enhanced symptom expression, while the addition of sulfur to calcium nitrate reduced symptom expression in 1998. Although turf treated with ammonium sulfate plus sulfur sustained low levels of summer patch in 1998, the combination also resulted in extremely poor turf quality. Compared to ammonium sulfate alone, the application of ammonium sulfate with lime raised soil pH but did not increase disease severity. Thus, lime may be applied along with ammonium sulfate, to maintain acceptable soil pH levels and turf quality, without reducing the effectiveness of ammonium sulfate for the control of summer patch in Kentucky bluegrass.

Variability of *Colletotrichum graminicola*, the Pathogen Causing Anthracnose Disease of Annual Bluegrass and Bentgrass

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Colletotrichum graminicola is an ascomycete fungus that causes anthracnose diseases of many grass hosts, including corn, sorghum, and turfgrass. Anthracnose is an important disease of annual bluegrass (*Poa annua*) and is increasingly seen as a problem on creeping bentgrass (*Agrostis* spp.). Although it has for years been a concern on golf course turf, anthracnose is now recognized as a major problem on turfgrass in the United States. *C. graminicola* can cause two distinct syndromes: a foliar blight evident as visible lesions on leaves, and a basal stem rot that can result in death of the plant. Of the two, the basal stem rot is more serious, representing a threat of growing proportions to *Poa*/bentgrass greens. Anthracnose basal stem rot remains poorly understood and is difficult to control. Using morphology, pathogenicity tests, and RAPD analysis, Browning et al. (1999) found variations in turf-infecting isolates of *C. graminicola* that appeared to be more geographically associated than host associated. However, results using RAPD markers were not definitive. In a recent project, P. Zhu examined anthracnose diseases of cranberry caused by different *Colletotrichum* isolates and species. Among the key findings of that study were that there was considerable variability in fungicide sensitivity of *Colletotrichum* isolates from different cranberry fields, and that this variability could be correlated with differences in fingerprint profiles of the isolates. The latter studies were done using a moderately repetitive fingerprint probe representing a retrotransposon of the fungus. One of the implications of the study was that genomic stress to the fungus in the form of specific fungicides may have resulted in retrotransposon activation and subsequent mutation/variation of the fungus.

This year, we initiated a project to examine *Colletotrichum graminicola* isolates from *Poa annua* and *Agrostis* greens from various locations in the Northeast that have been subjected over the last few years to different fungicide application regimes. This was done to determine whether a correlation similar to the one reported by Zhu and coworkers could be identified for this turfgrass pathogen. *C. graminicola* was isolated from creeping bentgrass and annual bluegrass greens exhibiting symptoms of the anthracnose disease. A pathogenic isolate of *C. graminicola* was used to generate a partial genomic library, and will be used to generate a complete library in 2002. Using the partial library, we have begun to identify moderately repetitive sequences that can be used as a fingerprint probe to examine the population of *C. graminicola* isolates using DNA hybridization methods. Several possibly useful clones have been identified, and will be examined further. Variation in morphology, pathology, and fingerprint pattern of *C. graminicola* isolates will next be examined and correlated with host species, geographic location, symptom type, fungicide regime, and time of epidemic. Results from this project should increase our understanding of the variation within *C. graminicola* populations in the Northeast and help in the development of suitable control measures, including breeding for resistance to the pathogen.

Cloning of a *Neotyphodium* sp. Chitinase Highly Expressed in Infected *Poa ampla*

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Endophyte-grass associations are naturally occurring mutualistic symbioses. Endophyte-infection can confer insect resistance, and in some cases disease resistance, to the infected plant. Because of these benefits, endophytes are often incorporated into turfgrass cultivars of several species. The factors that are involved in the establishment of these mutualistic interactions and the mechanisms underlying the endophyte-enhanced traits are the targets of considerable research interest. Apoplastic secreted proteins, both plant and fungal, are likely to be important components of the mutualistic interaction since they are located at the interface of the two species. We have therefore begun investigating some of the proteins secreted in culture and apoplastic proteins isolated from infected plants. Peptide sequencing of an abundant extracellular 52 kDa protein expressed in culture identified an endochitinase. Degenerate oligonucleotides were designed based on two of the peptides and used to amplify a fragment from a cDNA library constructed from endophyte-infected *P. ampla* leaf sheaths. The PCR clone was used to screen the cDNA library and a full length clone was obtained. The deduced amino acid sequence of the clone had 37-40% identity to other fungal chitinases, including those of several mycoparasitic and entomopathogenic fungi (Blaiseu and Lafay, 1992; Garcia et al., 1994; St. Leger et al., 1996). The clone had an open reading frame of 1377 bp, an untranslated 5' upstream sequence of 115 bp and a 3' end of 197 bp. Computer-aided protein analysis predicted there was a signal peptide of 17 amino acids and a catalytic domain of 400 amino acids. DNA gel blot analysis indicated there is a single copy of the chitinase gene in the fungal genome. RNA gel blot analysis revealed the fungal chitinase is highly expressed in infected *P. ampla* leaf sheaths. A 52 kDa apoplastic protein band isolated from infected leaf sheaths was subjected to peptide sequencing. The fungal chitinase was identified as a component of the band. Native activity gel analysis using the substrates 4-MU-(GlcNAc)₂ and 4-MU-(GlcNAc)₃ indicated the 52 kDa protein had endochitinase activity. The endochitinase has been partially purified by binding to colloidal chitin.

We now have evidence for expression of fungal endochitinase, endo- α -1,6-glucanase (Moy et al., 2002) and proteinase (Reddy et al., 1996) within the infected plant. In the biocontrol fungus *Trichoderma harzianum* the homologous enzymes are believed to function synergistically in the mycoparasitic activity of that fungus. We are investigating the hypothesis that these *Neotyphodium* sp. hydrolytic enzymes located in the apoplastic space of infected plants may function as a mycolytic system for the endophyte, perhaps contributing to the observed disease resistance seen in some endophyte-infected plants.

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Glucanase Expression by *Clavicipitaceous* Endophytes in Their Host Plants

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Some cool season grasses infected with Clavicipitaceous endophytes are known to have increased disease resistance when compared to uninfected grasses. To elucidate what endophyte derived factors might play a role in this observed disease resistance, we are attempting to clone and characterize products, such as cell wall degrading enzymes, that are secreted into the apoplastic spaces of endophyte infected grasses. Peptide sequencing of an apoplastic protein isolated from *Neotyphodium* sp. infected *Poa ampla* leaf sheaths revealed the presence of a glucanase. Degenerate oligonucleotides were used to amplify a fragment from a cDNA library constructed from endophyte-infected leaf sheaths. The PCR clone was used to screen the cDNA library and a full-length clone was obtained. The deduced amino acid sequence of the clone was 74% identical to an endo-1,6-glucanase from the mycoparasitic fungus *Trichoderma harzianum* (Lora et al., 1995). No other homologous sequences are in the database. Northern blot analysis demonstrated that several other endophyte-infected grasses, with increased resistance to diseases such as dollar spot, also express this glucanase. Southern blot analysis revealed that there may be a single copy of this glucanase in the *Neotyphodium* sp. genome. We will use a yeast expression system to characterize the activity of the protein. We have constructed a genomic library of the *Neotyphodium* sp. in the cosmid vector pWEB and have isolated a genomic clone for the glucanase. Sequencing of the genomic clone is currently underway.

Fungal cell walls contain chitin, -1,3-glucans, -1,6- glucans, and -1,3-glucans. In *T. harzianum*, the homologous endo-1,6-glucanase is believed to act synergistically with other hydrolytic enzymes in the mycoparasitic activity of that fungus. We are investigating the possibility that the endophytic apoplastic hydrolytic enzymes endo-1,6-glucanase, endochitinase (Li et al., 2002), and proteinase (Reddy et al., 1996) may contribute to the disease resistance observed in some endophyte-infected plants.

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***Agrobacterium tumefaciens*-mediated Transformation of Turfgrasses Using the Selectable Marker, Phosphomannose Isomerase**

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Genetically modified crops continue to face controversy and opposition, one reason for which is the use of bacterial antibiotic resistance genes as selectable markers and the still debated concern over the effect of these markers on the environment and human health. The combination of *E.coli*. phosphomannose isomerase (PMI) as a genetic marker with mannose as a selection agent has been suggested as being more environmentally friendly and socially acceptable, as well as possibly less injurious to the plant tissue to be transformed. A mannose selection system has been described for sugarbeet (Joersbo et al., 1998 and 1999) and maize (Wang et al., 2000) wherein the expression of bacterial PMI allowed the transformed plant cells to use mannose as a source of carbohydrate that would otherwise not be metabolized.

Here we report the transformation of three turfgrass species using the mannose selection system. Plasmid pNOV2804 (provided by Syngenta Seeds AG) contains the PMI structural gene (*manA*) of *E.coli* which converts mannose-6-P to fructose-6-P. This plasmid was modified to place the expression of PMI under the control of the strong, constitutive *Ubi-1* promoter from maize; the resultant plasmid pUBIMAN1 was transferred into *Agrobacterium tumefaciens* strain GV3101. Callus was derived from mature seeds of velvet bentgrass cv SR7200, creeping bentgrass cv Crenshaw, and tall fescue cv Plantation and Genesis. After co-cultivation of callus with *Agrobacterium* containing pUBIMAN, transformed callus was selected by its ability to grow on mannose. Callus, thus selected, was allowed to proliferate on mannose and then tested for increased PMI activity (relative to non-transformed callus) by enzymatic assay (Gracy and Noltmann, 1968). Analysis to date shows that there is much greater potential for this technique with bentgrasses than with tall fescue inasmuch as bentgrasses have a much lower basal PMI activity than does tall fescue, allowing for more certainty in selecting transformed tissue. Calli that proliferated on mannose and had higher PMI activity than non-transformed controls were placed on regeneration media; green shoots were allowed to grow in tissue culture before being transferred to soil. DNA from putative transformants will be subjected to PCR analysis and further confirmation will be by Southern analysis of DNA extracted from the resultant plants.

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Take-all Patch Resistance of Bentgrass Cultivars in Growth Chamber Experiments

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Take-all patch is a disease of bentgrasses caused by the ectotrophic root-infecting fungus *Gaeumannomyces graminis* var. *avenae* (*Gga*). This fungus infects and colonizes bentgrass roots in the fall and spring, when soil temperatures are between 10°C and 21°C. Take-all patch symptoms typically appear in late spring, when heat and drought stress induce foliar decline.

Because few variety trials have been exposed to take-all patch, little is known about the relative resistance of bentgrass cultivars to this disease. In order to fulfill the need for this information, we developed and implemented a growth chamber assay for measuring take-all patch resistance in bentgrass cultivars and selections.

In initial experiments, selected potting media (ProMix, 80:20 sand:peat, and calcined clay), inoculum sources (potato dextrose agar plugs, potato dextrose broth, sand/cornmeal, and infested rye grains), and *Gga* isolates (GgaMat1, GgaMat2, GgaMat3, GgaMat5, GgaO, and GgaWFO922) were tested for their potential use in a growth chamber assay. The calcined clay growth medium was more favorable for bentgrass growth and take-all patch development than ProMix and the sand-peat mixture. Infested rye grain inoculum was superior to other inoculum sources in the rate and consistency of take-all patch development. Three *Gga* isolates that ranged in virulence were selected: GgaMat5 was most virulent, GgaWFO922 was moderately virulent, and GgaO was least virulent.

Once developed, the growth chamber assay was applied to 16 creeping bentgrass cultivars, and one cultivar each of colonial bentgrass (SR7100) and velvet bentgrass (SR7200). Each cultivar was seeded at a rate of 20 g/m² in cone-tainers (20.3 cm deep x 3.8 cm diameter) containing a calcined clay growth medium. Six weeks after seeding, the cultivars were inoculated with one of the three selected *Gga* isolates by placing one infested rye grain just below the soil surface on two opposite edges of each pot. The inoculated cone-tainers were incubated at either 15°C or 20°C using a 12 hr light/dark cycle. Six weeks after inoculation, take-all patch resistance was determined by measuring the percent surface area exhibiting foliar symptoms. The experiment was repeated twice.

Overall, the velvet bentgrass cultivar SR7200 was the most resistant, and the colonial bentgrass cultivar SR7100 was moderately susceptible to take-all patch. Among the creeping bentgrass cultivars, Penn A-1, Seaside II, SR1020, Providence, Putter, and Penn G-2 were most resistant to the disease, whereas Penncross, Penneagle, and Penn A-

4 were most susceptible. All first- and second-order interactions among temperature, cultivar, and isolate were statistically significant in each experiment. The resistance of bentgrass cultivars was dependent upon *Gga* isolate and incubation temperature; therefore, take-all patch resistance may vary among locations according to climatic conditions and the resident pathogen population.

Influence of Nitrogen and Liming on the Incidence and Severity of Gray Leaf Spot in Perennial Ryegrass

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Gray leaf spot, caused by the fungus *Pyricularia grisea*, has emerged as one of the most devastating diseases of perennial ryegrass in the United States. Though recent studies have shown that increased nitrogen rate can result in greater disease severity, little is known about the impact of nitrogen source and the frequency of fertilizer applications on disease development. In two concurrent studies conducted in 2001, the effect of nitrogen sources alone and in combination with lime on the incidence and severity of gray leaf spot was assessed on three-month old 'Palmer II' perennial ryegrass maintained at North Brunswick, NJ.

In the first study, ammonium sulfate, calcium nitrate, urea, IBDU, and milorganite fertilizers were applied at weekly (12.2 kg N ha⁻¹), bi-weekly (24.4 kg N ha⁻¹), or monthly (48.8 kg N ha⁻¹) intervals from 24 Jun to 14 Sep. Plots treated with ammonium sulfate or urea sustained 7 to 65% more disease than turf fertilized with calcium nitrate. Although the total amount of nitrogen applied per growing season was the same, weekly applications of ammonium sulfate or urea yielded a greater incidence and severity of gray leaf spot than either bi-weekly or monthly applications of the same fertilizers. When applied on a monthly basis, disease severity was greater on turf that received 48.8 kg N ha⁻¹ versus the 24.4 kg N ha⁻¹ rate of ammonium sulfate, urea, or IBDU. No such response was observed with calcium nitrate and results with milorganite were inconclusive. Turf quality was not significantly different among treated plots.

In the second study, the acidifying fertilizer ammonium sulfate and the alkalizing fertilizer calcium nitrate were applied bi-weekly (24.4 kg N ha⁻¹) alone or in combination with lime (99, 198, and 396 kg CCE ha⁻¹) or elemental sulfur (845 kg S ha⁻¹) from 14 Jul to 14 Sep. On all rating dates, disease severity was reduced 50 to 76% on turf that received calcium nitrate versus ammonium sulfate. Compared to ammonium sulfate alone, ammonium sulfate plus lime (198 and 396 kg CCE ha⁻¹) reduced symptom expression 26 to 53%, respectively. Conversely, the combination of calcium nitrate and the same rates of lime increased disease severity 25 to 50%, respectively, when compared to calcium nitrate alone. Sulfur treated plots sustained very little disease, but sulfur significantly reduced soil pH resulting in very low turf quality. Similarly, although unfertilized turf sustained almost no disease (i.e., 1 to 2% turf area infested with *P. grisea*), turf quality was poor.

Response of Bentgrass Cultivars to Dollar Spot Under Different Cultural and Chemical Management Practices

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The susceptibility of bentgrass cultivars to dollar spot (*Sclerotinia homoeocarpa*) and brown patch (*Rhizoctonia solani*) was assessed to identify factors that can be used to reduce fungicide inputs while maintaining acceptable turf quality. Eight bentgrass cultivars were evaluated under field conditions. All cultivars were maintained at two cutting heights: 0.356 cm (greens height) and 0.953 cm (fairway height), and two nitrogen levels: 0.012 kg m⁻² and 0.031 kg m⁻² year⁻¹. Cultivar treatments were subdivided into six fungicide application schedules (untreated, 7, 14, 28, or 56 day intervals, or an economic threshold of 0.3% disease) using the contact fungicide chlorothalonil. For most cultivars, dollar spot was least severe on turf receiving the high rate of nitrogen. Cultivars Penn G2, SR 7200 and L-93 were least susceptible to dollar spot under most nitrogen and cutting height treatments, whereas Crenshaw, SR 1020, and Southshore sustained the greatest disease incidence and severity. Throughout the study, cultivars SR 7200, L93, and Penn G2 required the fewest number of fungicide applications to control dollar spot. In general, brown patch was most severe on turf maintained at greens height and high nitrogen.

Drought Tolerance of Kentucky Bluegrass in Relation to Abscisic Acid Accumulation

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Drought is one of most limiting factors for turfgrass growth. The experiment was designed to evaluate the relationship between ABA and drought tolerance in eight Kentucky bluegrass cultivars. Grasses were grown in well-watered control and drought stress condition for 25 d in growth chamber. Leaf ABA concentration showed significant linear increase with drought stress process in initial 11 d of treatment. Cultivars that differ in drought resistance varied in the amount of ABA accumulation and stomatal sensitivity to ABA accumulation. Drought sensitive cultivars had more rapid increases and greater amount of ABA in response to drought stress compare to drought resistant cultivars. However, stomatal conductance was more sensitive to ABA accumulation for drought resistant cultivars than sensitive ones. Leaf ABA concentration showed close correlation with leaf water potential and relative water content. ABA accumulation rate was significant negative related to the decline rate of turf quality and leaf RWC, but positive related with the increase rate of electrolyte leakage. These results suggest that ABA could be used as a metabolic indicator of drought resistance for cool-season turfgrasses and it is possible to improve drought resistance in cool-season turfgrasses by selecting for a low production and high sensitivity of ABA in response to drought.

Evaluation of Water Volume and Nozzle Type on the Efficacy of Trifloxystrobin and Selected Fungicides for the Control of Brown Patch in Colonial Bentgrass

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Trifloxystrobin (Compass 50WG) is a new fungicide recently marketed for the control of turfgrass diseases. It belongs to a new class of fungicides called strobilurins that are effective at extremely low rates (i.e., 2.1 to 5.7 g a.i. 90 m⁻²) and are considered by the EPA to represent a “reduced risk” to the environment. Although trifloxystrobin is effective against several important diseases of turf, little is known about the impact of application parameters on fungicide efficacy. Moreover, with the tendency of turfgrass managers to reduce the volume of water used to apply fungicides, and to employ a variety of application nozzles, it is important to ascertain the influence that these factors may have on the efficacy of this new fungicide chemistry. To do so, a study was conducted from 1999 to 2001 on colonial bentgrass cultivar ‘SR7100’ maintained at 0.95 cm at the Turf Research Farm in North Brunswick, NJ.

The efficacy of trifloxystrobin, azoxystrobin (Heritage 50WG), and chlorothalonil (Daconil WeatherStik 6F) were evaluated for the control of brown patch (*Rhizoctonia solani*), one of the most common and destructive diseases of golf course turf during the summer months. Trifloxystrobin (2.1 g a.i. 90 m⁻²) was incorporated in a factorial design using 1.9, 3.8, 7.6, 15.2, and 30.4 L H₂O 90 m⁻² and three nozzle types: flat fan (TeeJet 8003VS), rain drop (RA-10), and turbo flood jet (TF-VS5). Azoxystrobin (2.8 g a.i. 90 m⁻²) and chlorothalonil (127.7 g a.i. 90 m⁻²) were evaluated at the same water volumes, using a flat fan nozzle only. All fungicides were applied at 14 day intervals in 1999 and 21 day intervals in 2000 and 2001.

Throughout the three year study, the lowest water volume (1.9 L H₂O 90 m⁻²) generally provided the least effective disease control. However, disease control was generally independent of nozzle type. For each year, there were significant nozzle by water volume interactions. In 1999 for example, when applied with the flat fan nozzle, all fungicides were most efficacious at the 7.6, 15.2, and 30.4 L H₂O 90 m⁻² volumes. However, when using the rain drop nozzle, optimum disease control for trifloxystrobin was afforded at the 3.8, 7.6, and 15.2 L H₂O 90 m⁻² water volumes. In 2000, when nozzle type was a significant main factor, the rain drop nozzle did not provide as effective control of brown patch as either the flat fan or turbo flood jet. In the final year of the study, applying chlorothalonil at the highest water volume (30.4 L H₂O 90 m⁻²) reduced disease control in late-August and early-September. During the study, azoxystrobin was generally more effective in controlling brown patch than either trifloxystrobin or chlorothalonil.

Volatile Compounds of Endophyte-Free and Infected Tall Fescue (*Festuca arundinacea* Schreb.)

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Volatile compounds produced by intact plants and ground leaf tissue from endophyte-infected (E+) and endophyte-free (E-) tall fescue (*Festuca arundinacea* Schreb.) were collected by a purge-and-trap procedure and analyzed by gas chromatography/mass spectrometry. The volatile compound profile from ground leaf tissue was similar between E+ and E- clonal plants; however, the sheaths of E+ clonal plants produced higher levels of 1-octen-3-ol, a characteristic volatile compound derived from lipid peroxidation in fungi, which was absent in E- clonal plants. Intact plants produced fewer volatiles than macerated leaves. At 25 C, (Z)-3-hexen-1-ol acetate was the most abundant compound, accounting for 77 and 89% of the total volatile emission from E+ and E- plants respectively. Higher temperature (32 C) significantly reduced the production of (Z)-3-hexen-1-ol acetate. Nonanal was the most abundant compound at 32 C accounting for 52 and 45% of the total volatile emission from E+ and E- plants. Treatment of E+ and E- plants with jasmonic acid (JA) dramatically altered the volatile compound profile. The levels of (E)- α -ocimene increased more than 200-fold and accounted for at least 43% of the total volatile emission. Although the presence of endophyte resulted in some qualitative and quantitative differences in the production of volatile compounds, they are unlikely to account for the differences in insect resistance between E+ and E- plants. Nevertheless, the production of a unique spectrum of volatiles after JA treatment may represent a significant plant-based defense response in tall fescue that is independent of endophyte.

Turfgrass Germplasm Collection from Central Asia

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The genetic improvement of turfgrass species by the introduction, evaluation, and incorporation of desirable traits from unique accessions from around the world has proven a valuable strategy. The Turfgrass Center continues to support such collection efforts and this work will describe the current work in collecting turfgrass species from the former republics of Central Asia with a particular focus on finding those that may have potential as shade tolerant grasses, grasses that appear productive in otherwise marginal, overgrazed lands, and grasses from hot, humid areas, as well as resistance to diseases and insects. This poster presents the outcomes of two overseas collection trips to Central Asia; a trip to the Russian Federation to develop partnerships that could lead to procuring additional turfgrass accessions.

Anthocyanin Expression in Kentucky Bluegrass Transformed with the Maize LcR Gene: A Potential Method to Develop Colored Grasses

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Anthocyanins are the prominent red, purple and blue pigments in flowering plants. The production of anthocyanins is genetically regulated both temporally and spatially and is inducible. The maize LcR gene works as a trans-factor in maize and transformed *Arabidopsis*, activating the expression of biosynthetic genes in the anthocyanin pathway. We hypothesized that the introduction of this gene could induce the expression of anthocyanins in the leaves of other grasses, and if successful introduce new colors in the leaves of grasses. The maize LcR gene under the control of the 35S promoter, was co-transformed into Kentucky bluegrass with the hygromycin resistance gene. A total of 126 hygromycin-resistant transformants were obtained. At the callus stage, 21 transformants exhibited strong anthocyanin expression, showing red or purple calli, and the expression appeared to be light induced. However, we were not able to regenerate these 21 transformed lines. These results suggest that very strong anthocyanin expression at the callus stage impaired regeneration. Future studies will examine techniques to overcome this inhibition. From the remaining 105 transformants, we were able to regenerate 64 lines, and of this group more than half (36) expressed anthocyanins in the roots, with either red or purple roots. From these, we observed 10 regenerated lines that developed a deeper blue leaf color than the parent plants and which may be of interest for breeding; and three exhibited the desired purple leaf tips. These preliminary results indicated that we were successful in inserting the maize LcR gene into Kentucky bluegrass; and that the insertion of this gene caused a change in anthocyanins expression, possibly through the activation of related structural genes. The activation of anthocyanin biosynthesis in this species led to the observed induced red and purple leaf and root colors not present in the parent plants. Our results strongly suggest that this approach has potential for application in turfgrass morphological modification and in the development of new ornamental colored grasses.

Comment: Is this statement necessary? It does provide evidence of successful introduction of this gene in the hands of those involved in the current research, however, I would say it is unnecessary in the abstract.

Application of Somaclonal Variation in Triploid Bermudagrass Breeding

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Triploid bermudagrass are sterile F1 hybrids of *Cynodon dactylon* x *C. transvaalensis*. They provide the highest quality turf in warm southern regions. As triploid hybrids, they are sterile and have to be propagated vegetatively. Mutation breeding has been the only way to genetically improve high quality dwarf cultivars, either through natural mutant selection or through radiation induced mutant selection.

Somaclonal variation refers to variations occurred during the process of tissue culture and regeneration. Compared to traditional mutation breeding, there are some unique aspects of somaclonal variation: First, the frequency for genetic change can be significantly higher. Second, the regenerating procedure from tissue culture could act as a sieve that eliminates most of deleterious genetic changes. Third, since somaclonal variations are derived from one or a few cultured cells, such variation can be easily stabilized, especially for crops that reproduce vegetatively or by apomixis. Thus, a somaclonal variation with a new trait could be selected as a new cultivar that still retains all or most of the favorable qualities of an existing cultivar.

TifEagle is a newly released “ultradwarf” cultivar. Using its nodes as material, we successfully established an embryogenic callus culture and regeneration system. A wide range of variation in important turf traits, e.g. growth vigor, leaf length, leaf color, growth habit, length of internodes and texture, were observed among regenerated plants. With the criteria of traits mentioned, forty-eight of total 106 (45.3%) showed variation and further propagation indicated the variations were usually stable. A highly potential variant, designated showed more appealing color, higher density, shorter length of internodes was selected for further investigation in the laboratory and field.

Test plots established at Hort Farm II showed this somaclonal variant could withstand mowing at 5/32 inch during summer growing season. It also showed more appealing color than TifEagle, and its internode length was significantly shorter than TifEagle. Its leaf color retention in fall was about one week longer. In greenhouse tests, the difference of the internode length without mowing was also significant. Additionally, the somaclonal variant showed significantly better drought tolerance than TifEagle with a delay of severe desiccation of about 2 to 3 days. RAPD assay disclosed a slight difference at DNA level between the somaclonal variant and its original parent, TifEagle. Among 40 RAPD primers used and a total of 186 obviously amplified bands, a two bands difference with two different primers was identified.

Using nodes from the cultivar, ‘TifDwarf’, the tissue culture and regeneration system was also established and several regenerated plants with more appealing color and faster lateral growth rates were observed. These results indicate that somaclonal variation could be a practical method for triploid bermudagrass breeding.